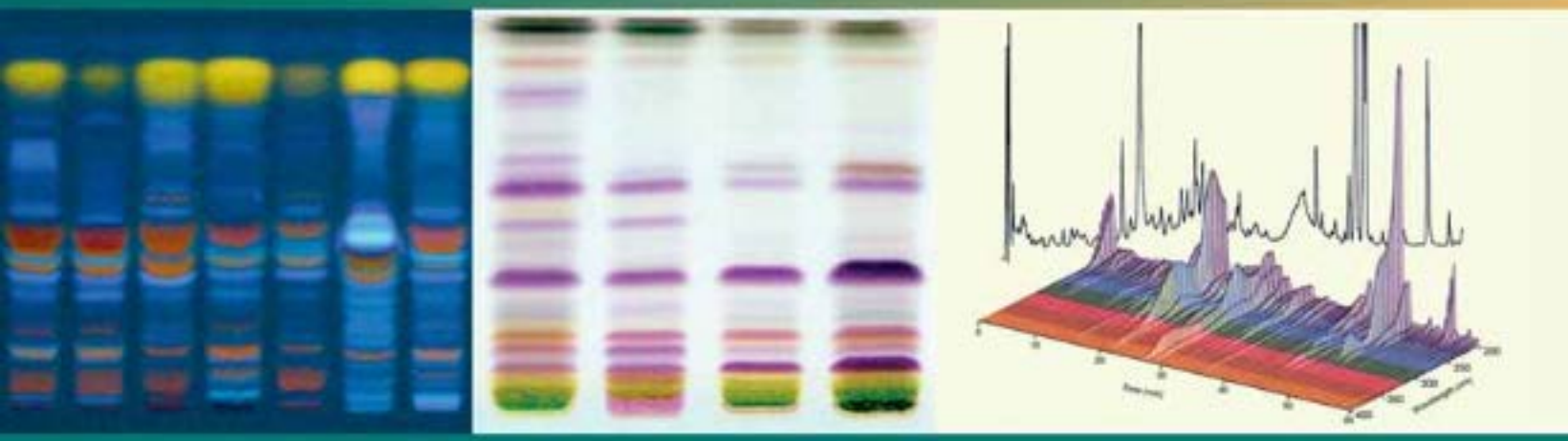
Hildebert Wagner · Rudolf Bauer · Dieter Melchart
Pei-Gen Xiao · Anton Staudinger *Editors*

Chromatographic Fingerprint Analysis of Herbal Medicines

Thin-Layer and High Performance Liquid Chromatography of Chinese Drugs



Volume 1

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TCM-KLINIK BAD KÖTZTING

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Hildebert Wagner · Rudolf Bauer · Dieter Melchart
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Second, Revised and Enlarged Edition

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Editorial

On the occasion of the twentieth anniversary of the establishment of the first hospital of Traditional Chinese Medicine (TCM) in Germany, the editors are pleased to present a new analytical manual for the quality proof of Chinese herbal drugs that meets the high standards of the European Drug Regulatory Authority.

The chromatographic TLC-, HPLC- and GC-fingerprint analytical technique described in the monographs has never been used in any Pharmacopoeia, although it is the most comprehensive, non-sophisticated chromatographic method for a science-based identity and stability proof of Chinese Herbal Drugs and includes the detection of possible falsifications or adulterations. This fingerprint analysis enables, for the first time, the detection of the complex entities of all main low-molecular constituents of a plant drug with the advantage that the single constituents can be made visible in coloured TLC photographs and HPLC-peak profiles. Using online recordable UV-spectra with the Diode Array technique, it is also possible to gain information about the chemical structure of single constituents.

Each new monograph also contains a description of the macroscopic descriptions, an updated list of all the main bioactive constituents of a drug identified to date, and the pharmacological and biological activities of the single herbal drugs and their therapeutic application. A comprehensive reference list informs the reader about new analytical topics and trends.
The eighty individual herbal drug monographs were first published by Dr. Wühr Publishers, Bad Kötzting and are now offered in an updated and corrected form in this new, two-volume manual published by Springer Publishing Company. A third volume containing further 40 herbal drug monographs will be completed by the end of 2012.

Scientific experts from the Universities of Munich (Germany) and Graz (Austria), along with around 25 scientific co-workers and technicians, contributed to this comprehensive work. All participants in the project are most grateful to the owner of the TCM-hospital Bad Kötzting who has supported the project from its very beginning. In the later phases of the project, we also received financial support from the AiF-program of the German Ministry of Economics in Berlin (Germany).

The editors: H. Wagner, R. Bauer, D. Melchart, Xiao Pei-Gen, A. Staudinger
April 2011

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- We are deeply indebted our many scientific coworkers and colleagues at the University of Munich (Germany) and the University of Graz (Austria), as well as to the Technical Assistants of the Department of Pharmacy, Munich named following:
Department of Pharmacy Munich, **Scientific Assistants**: Bauer Michael, Fischer Mathilde, Liu Yong, Michler Hans, Müller-Jakic Barbara, Nenninger Anneli, Nörr Heidrun, Offermann Franziska, Plößl Florian, Pröbstle Andrea, Wild Bernice, Zschocke Sibylle. **Technical Assistants:** Bächer Silvia, Grieblinger Elisabeth, Koch Stefanie, Marotel Dana, Mayr Christine.
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Introduction

Facts and Perspectives on Chinese Herbal Drugs

When we began our work on the new analytical monographs 15 years ago, we faced the challenge of how the quality proof should be performed in order to meet both the requirements of a science-based authenticity proof of the Chinese drugs and the high standards of the European Drug Regulatory Authority. Based on the experience we had gained from our first TLC-fingerprinting of herbal drugs (Wagner and Bladt 2001), we decided to use the chromatographic TLC and HPLC fingerprint analytical technique. This method enables the researcher, for the first time, to detect the complex entities of all main low molecular constituents of a plant drug, with the advantage that the single constituents can be made visible in coloured TLC photographs and in a quantifiable HPLC-peak profiling. At the same time, for safety reasons, these new techniques can be used to exclude possible falsifications and adulterations of herbal drugs. These criteria and advantages have also persuaded the Chinese scientific experts who advocated this analytical method as the best, presently available, non-sophisticated and feasible method for quality proof of herbal drugs (Liang et al. 2010). The fingerprint technology for identification of herbal drugs is also the favored method in the framework of the international ISO-Standardisation* of the "Quality and Safety of TCM". If the barcode DNA-analysis of all frequently used Chinese drugs becomes available in the near future, we can supplement and correlate the chromatographic analyses with those of the DNA-fingerprint analyses and thereby optimize the quality proof of the drugs in general (Heubl 2010).

- **Authenticity of TCM-drugs not definitely assessed**
  Many TCM herbal drugs are not yet produced under controlled cultivations, but originate from wild collections. Even if the drugs are derived from cultivations, it must be taken into account that they can originate from quite varied climate zones and that they may be harvested under altered conditions. Therefore, in the past, the botanical authenticity and homogeneity within a defined plant species could not be guaranteed. We have thus investigated as many herbal drug samples of one plant species as we were able to acquire from different districts and markets in China, along with reference drugs from German herbal drug firms (Wagner et al. 2011).

- **Uncertain botanical nomenclature**
  The non-uniform nomenclature for the same plant in various regions of China is a significant problem. This uncertainty can cause impermissible substitutions or falsifications, as occurred 15 years ago when the root of *Stephania tetrandra* (Hanfangji) was mistaken for the root of *Aristolochia fangji* (Guanfangji) and administered to women

*Resolution 18 of the 2nd plenary meeting of ISO/TC 249 held in The Haque, Netherlands on May 2-4th 2011 [Establishment of the working group "Quality and Safety of TCM products" under german convenorship] www.iso.org and www.din.de

as tea medication that produced severe nephrotoxic side effects. The *Aristolochia* herbal drug contains the carcinogenic aristolochic acid. After the detection of this falsification, the drug was banned from the Chinese Pharmacopoeia in 2002. Meanwhile, special TLC- and HPLC-fingerprint methods were developed which allow the detection of even micrograms of these acids in an herbal drug or drug mixtures: see Radix Stephaniae p. 311 Mo. No. 29, Radix Clematidis p. 355 Mo. No. 33 and Caulis Sinomenii p. 369 Mo. No. 34. A similar example is the Chinese tetraploid *Acorus calamus*/*tatarinowii* drug, Mo. No. 65 p. 777, which differs in its very high content of carcinogenic ß-asarone from the diploid *Acorus calamus* drug known officially in most western countries.

- **Great variability of plant species**
  A further difficulty in the identification of TCM-drugs is the fact that, in many Chinese monographs, more than 2 species or subspecies (sometimes up to 9 species) are listed and are often labelled as synonyms, subspecies or subvarieties. For example in Fritillariae bulbus Mo. No. 2 p. 13, nine species are listed, and the monographs for Epimedii herba- Mo. No. 43 p. 485, Dioscoreae rhizoma Mo. No. 53 p. 615 and Uncariae ramulus c. uncis Mo. No. 32 p. 343 list five species each without any evidence that the chemical composition of the various "species" are qualitatively and/or quantitatively equivalent and can be substituted for one another. As a result of our fingerprinting investigations, we could show that in many cases considerable differences were detectable between the single species and the main official herbal drug. Correspondingly it may be suggested that a great number of these "subspecies" do not possess pharmacological and therapeutic equivalence.

- **Conclusion:** What have we learned from the authenticity proof of Chinese herbal drugs? In addition to a continuation of further pharmacological and molecular-biological investigations, we must immediately initiate comprehensive bar-code DNA-fingerprint analyses of the most frequently used official Chinese plant drugs. The first priority should be given to those Chinese plants within taxa that are frequently substituted or adulterated with other species and could be nearly indistinguishable morphologically or chemically (see herbal drugs of the Apiaceae familiy Mo. No. 9, 14, 15, 16, 44).

- **Processing of TCM-drugs**
  Apart from the simple cutting and cleaning of the raw drugs, the Chinese Pharmacopoeia describes many other types of pre-treatment or processing unknown to western Pharmacopoeias. In the Chinese Pharmacopoeia 2005 (People's Republic of China, English Edition Vol I Appendix II A – 24) the processing is to be defined "to fulfil the requirements of drugs", whatever that may mean for each single drug. In one recent publication, the purpose of processing is explained as "to alter the appearance, the physical characteristics and chemical constituents of a herbal drug" (see Zhao Z et al. 2010). In none of the monographs, however those crude drugs containing toxic constituents, the necessity of the various processing is rationalized and clearly substantiated. According to the Chinese Pharmacopoeia, processing can be achieved primarily through the following methods: roasting and broiling, scalding, calcining, carbonizing, steaming, boiling, stewing, processing with wine, vinegar, or salt water, and different kinds of stir baking. Some chemicals or herbal drugs may also be used for the processing.

  In the Monograph No. 79 p. 977, we describe a TLC- and HPLC-fingerprint analysis of two unprocessed (non-pretreated) and processed *Aconitum spp.*, *Aconitum carmichaeli*

and *Aconitum kusnezoffii*. Processing was performed, according to the "Heishunpian" and "Baifupian" instructions of the Chinese Pharmacopoeia, with salted water and Radix Glycyrrhizae, black beans and water or after scalding by heating at high temperature with sand (clamshell or talc). The TLC- and HPLC-fingerprint analyses showed that in the processed roots, the alkaloids Aconitine and Mesaconitine were degraded to a great extent and detectable only in a very small amount as compared with the content of these alkaloids in the raw unprocessed roots. Another herbal drug which requires processing is Rhizoma Pinelliae (Mo. No. 7 p. 71) which is not permitted to be prescribed in unprocessed form for oral therapy.

Conclusion: Modern analytical techniques using the HPLC-quantitation should replace the classical methods of processing described in the Chinese Pharmacopoeia. Recent publications demand a safe limit to be stipulated for the Aconitine content in processed *Aconitum* drugs (Singhuber et al. 2009).

- **Endo (Phyto) Fungi in Chinese Herbs**
  During the development of the new monographs, we discovered a conspicuous occurrence of very lipophilic acetylenic compounds of the Falcarin(di)ol type in the roots of three *Angelica* spp. (Mo. No. 9, 14 and 15 p. 99, 161 and 171), in the root of *Ligusticum chuanxiong* (Mo. No. 16 p. 181) and in three *Panax* spp. (Mo. No. 70, 72 p. 843, 875). Initially, we considered them to be constituents biosynthesized from the plants. Meanwhile, however, several publications appeared in which the original production of these compounds from endo(phyto)fungi in Chinese plants could be assessed (Strobel and Daisy 2003; Li et al. 2007). The most famous example of the production of a long-known terpene alkaloid. by an endo(phyto) fungus is the *Taxus brevifolia* tree, the bark of which contains the symbiotic living fungus *Taxomyces andreanae*. This fungus is able to biosynthesize the same terpene alkaloid, paclitaxel, as the *Taxus* tree (Stierle et al. 1993). Which organism, the fungus or the plant, first produced paclitaxel and was the gene supplier for the other organism is not known. The acetylene compounds falcarinols possess antibiotic and antitumoral activity. They are very lipophilic and can be easily detected because of their very characteristic UV-spectra. Therefore they are of interest for the "identity proof" of a plant and it can also be suggested that they contribute to the pharmacological and therapeutic effect of some Chinese plants containing these compounds. It can be expected that in the future, additional metabolites produced by phytofungi will be detected. There is no doubt that this surprising new knowledge will initiate a promising new area of research.

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Guidelines for the experimental work

Source of the herbal drugs

As described above, the herbal drugs must originate from clearly identified botanical species. Additionally, it must be taken into consideration that differences in cultivations, climatic conditions, time of harvest, drying and storing conditions can cause slight chromatographic deviations which cannot be avoided and are normal. Therefore it is worthwhile to investigate as many herbal drug samples of one species as possible from different geographic and ecological areas.

Extraction conditions

The chosen extraction procedures should be rapid, but efficient according to present scientific knowledge and should include of the total entity of the low molecular constituents of a herbal drug. This can be achieved in most cases using alcohol (MeOH or EtOH). Additional fingerprints can be obtained by extraction using petroleum ether/hexane or chloroform (for lipophilic compounds) or water/water-acetone mixtures (for tannins, high polymeric procyanidines, and amino acids) as solvents. Polysaccharides and proteins can be characterized using their sugar- or amino acid-fingerprints after enrichment and acidic or enzymatic hydrolysis.

Chromatographic conditions

Plates/columns:

- For the chromatography TLC- or HPTLC-standardized Silica Gel F 254 (Merck) plates, in some specific cases also aluminum oxide- or cellulose coated plates (Merck) are used. HPTLC-plates are precoated with Silica Gel of an average particle size and a narrow size distribution of 5 µm as opposed to TLC material of 15 µm average particle size and a broader size distribution.

- For all HPLC-analyses reversed phase C-18 or C-8 columns (LiChroCART® 125-4/250-4 LiChrospher® 100 RP-18 (5 µm), Merck or LiChroCART® 125-4/250-4 LiChrospher® 60 RP select B (5 µm), Merck), can be used with a Merck HITACHI L-4500 A Diode Array Detector.

- A GC-analysis is shown e.g. for Monograph No. 65 Rhizoma Acori. Apparatus: Varian GC 3800, Varian Saturn 2200 (El/Cl, ms^n) ion trap-mass spectrometer, Autosampler: CTC CombiPal, Separation column: Varian VF-5ms with 10 m precolumn (deactivated methyl-polysiloxan), Carrier gas: Helium.

Detection/Solvent system:

The Appendix lists the reagents and basic solvent systems used most frequently in TLC and HPLC for the detection of main structure types of drug constituents in herbal drugs.

Reference compounds:

The availability of reference compounds for the identification of characteristic constituents of any plant facilitates the identity (quality) proof of a herbal drug and their compounds are requirements for quantitative determination. If they cannot be isolated in the researcher's own laboratory, some

can be purchased from special firms. In Germany the firm Phytolab in Vestenbergsgreuth (www.phytolab.com) offers many reference compounds which are listed as “marker compounds” in the Chinese Pharmacopoeia.

Reproducibility of the fingerprint analysis

If the same technical conditions described are used, it can be expected that even with the use of instruments from other firms, very similar TLC- and HPLC-fingerprints can be obtained. If, however, for any reason, the grade of separation and/or the R*f*- and Rt-values deviate from those stipulated in the Monographs, the sequence and the overall TLC-zone- and HPLC-peak profiles must still be in agreement with those documented in our Monographs.

Photography

The TL-chromatograms were developed by a Canon PowerShot G2 digital camera in a CAMAG Reprostar 3 cabinet using WinCats software (www.camag.com).

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Radix Bupleuri – *Chaihu*

Pharmacopoeias: Pharmacopoeia of the People's Republic of China
English Edition, 1992/2005[1]

Japanese Pharmacopoeia 1986 (Jap. XI).

Official drugs: In Chinese Pharmacopoeia: the roots of *Bupleurum chinense* DC. (= *B. falcatum* auct. Sin. non L.) and *Bupleurum scorzonerifolium* Willd. *(= B. falcatum var. scorzonerifolium* (Willd.) Ledeb.). - Apiaceae - The drugs differ both in their morphology and in their origin. *Beichaihu (B. chinense)* originates from northern China (north of the Yellow River), while *Nanchaihu (B. scorzonerifolium)* is indigenous to the southern provinces[1]. The Japanese Pharmacopoeia requires *Bupleurum falcatum* L. (= *B. scorzonerifolium* Willd. var. *stenophyllum* Nakai) or varieties of this species[3, 4].

Adulterations: *B. longiradiatum* Turcz. (toxic!)[1], occasionally contaminations with roots of *Aconitum* spec.[3].

Description of the drugs:[1]

B. chinense: frequently branched roots, 6-15 cm long, 3-8 cm in diameter, externally blackish-brown or light brown, texture hard and tenacious, not easy to break

B. scorzonerifolium: relatively thin roots, non or slightly branched, externally reddish-brown or black-brown, texture slightly soft

Pretreatment of the raw drug:

Stalk-remnants are removed, the drug is washed and moistened, cut into thick slices and dried (Chaihu). The sliced drug is then soaked in vinegar and dried under mild heat (Cuchaihu).

Medicinal use: Often in combination with other drugs as antihepatotoxic, antipyretic, analgesic, sedative, and antidepressive agents, in cases of menstrual complaints, uterine and anal prolaps, sudden loss of hearing and malaria[1, 4, 5].

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,2,4,6]	
Taste:	bitter, slightly acrid
Temperature:	cool
Channels entered:	liver, gall bladder
Effects:	resolves Yang Heat patterns, relaxes constrained Liver Qi, raises the Yang Qi, diaphoretic, gastrointestine-regulative, liver function-restorative, spleen-invigorative
Symptoms and indications:	fever in common cold, alternating chills and fever, epigastric, chest and flank pain, nausea, vomiting, vertigo, indigestion, menstrual disorders, hemorrhoids, prolapse of the uterus and rectum, diarrhea due to collapse of Spleen Qi

Main constituents (see Fig. 1):

- **triterpene saponins of the oleanan-type:**
  saikosaponin a, c, d, in addition, also saikosaponins b1 – b4, e and f [7]. Saikosaponin b1 – b4 are artefacts of saponins a and d, which arise during extraction of the plant juices in acid medium by splitting off the 13ß, 28-epoxy group[3, 8], monoacetylsaikosaponins and acidic saponins, which are derived from oleanolic acid[7].
- **sapogenins:** the saikogenins E, F and G are recognized as being genuine, while the saikogenins A, B, C and D are regarded as artefacts of the latter [4, 9].
- **polyacetylenes:** saikodiyne A, B, C[10] and further C15-compounds[11].*)
- neutral phytosterols such as α-spinasterol and stigmasterol.
- fatty acids such as palmitic, oleic, linoleic and stearic acid[5, 7], polyhydroxysterols.
- the lignan saikochromon A[9], amino acids, sugar, e.g. the sugar alcohol adonitol[12], and the furanocoumarin angelicin (isolated from *B. falcatum*)[13].
  *) Probably of phytofungi origin

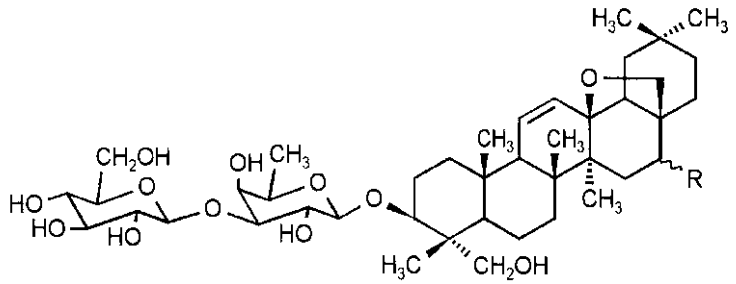
Pharmacology:

In vitro effects:

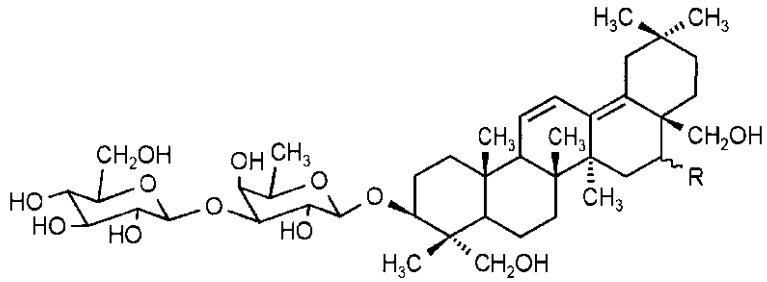
- hemolytic[3,8]
- local anesthetic (decoction)[3]
- antiviral (polysaccharides)[14]
- effects on liver enzymes: Inhibition of glucose-6-phosphatase and NADPH-cytochrome-C-reductase, stimulation of 5'-nucleotidase[3,15].

In vivo effects[3, 4, 15]

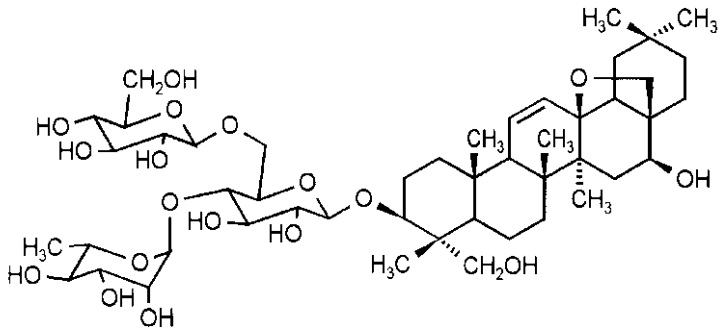
- antihepatotoxic (rats, humans)
- antipyretic (rabbits, mice, rats)
- analgesic (saponins and saikogenin A) (mice)
- anti-inflammatory (rat-paw edema model)
- antigranulomatotic in rats
- sedative (saponins and saikogenin A) (mice)
- cholagogue and choleretic (whole-plant extract) (dog)
- anticholesterolemic (saikosaponin a, d and genin A, D (rats and rabbits)
- anti-ulcerogenic (rats)
- antihypertensive



Saikosaponin a: R = β-OH Saikosaponin d: R = α-OH



Saikosaponin b_1: R = β-OH Saikosaponin b_2: R = α-OH



Saikosaponin c

Fig. 1: Formulae of the main saponins

TLC fingerprint analysis:

1) Extraction:

A: 10 g of coarsly-ground drug are Soxhlet-extracted with 120 ml methanol p.a. for 4 hrs. The clear extract is concentrated to approx. 10 ml under vacuum at 40 - 60° C, and then filled up to 10,0 ml with MeOH p.a.

The following extraction procedures are suitable for quick TLC identification of the drug:

B: 5 g coarsly-ground drug are treated with 50 ml methanol p.a. for 1 hr in the ultrasonic bath and the sediment is filtered off. The filtrate is then concentrated to approx. 5 ml as described above, and filled up to 5,0 ml with methanol p.a.

C: 5 g coarsly-ground drug are treated three times in an ultrastirrer for 2 min. with 25 ml methanol p.a. each. The total filtrate is then evaporated to 5,0 ml as described under B.

2) Standards: Saikosaponins a, d, and c, dissolved in MeOH p.a. (5mg/ml)

3) Separation parameters:

Applied amount:	10 µl extract, 10 µl standard
Plates:	Silica gel 60 F_{254}, Merck
Solvent system:	ethyl acetate-ethanol-water (80 + 20 + 10)
Direct evaluation:	UV 254 nm and UV 365 nm
Spray reagents (16):	a) Vanillin-sulphuric acid reagent (solution I: 1 % ethanolic vanillin solution. solution II: 5 % ethanolic sulphuric acid). The TLC plate is sprayed vigorously with 10 ml solution I, and thereafter with 5 – 10 ml solution II. It is then heated at 110° C for 5 – 10 min. under observation, and evaluated in vis (Fig. 2).
	b) Blood-reagent (10 ml of a 3,65 % sodium citrate solution are transferred to 90 ml fresh cattle-blood, and 2 ml of this mixture mixed with 30 ml phosphate buffer solution pH 7,4 (Phosphate buffer pH 7,4: 0,682 g potassium hydrogen phosphate and 39,34 ml 0,1 N sodium hydroxide are filled up to 100,0 ml with distilled water). The TLC plate is vigorously sprayed in the horizontal position.
	c) Natural products – polyethyleneglycol reagent (solution I: 1% methanolic diphenylboric acid-β-ethylamine ester, solution II: 5% ethanolic polyethyleneglycol-4000). The TLC plate is sprayed vigorously with 10 ml solution I and thereafter with 8–10 ml solution II and evaluated in UV 365 nm.

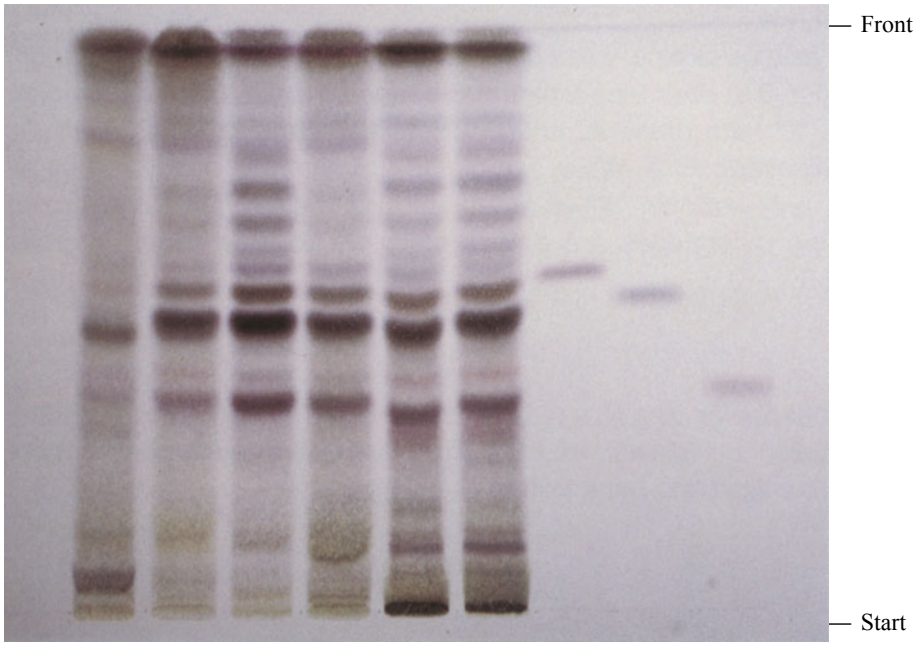


Fig. 2:
Thin layer chromatogram of Bupleurum samples

Drug samples:

1,2	Commercial drugs from China
3	Radix Bupleuri from China, Hebei Province (north of the Yellow River)
4	Radix Bupleuri from China, Hubei Province (south of the Yellow River)
5,6	Japanese drug samples

Test substances:

T1	saikosaponin d	R*f* = 0,55
T2	saikosaponin a	R*f* = 0,50
T3	saikosaponin c	R*f* = 0,35

4) Description of the chromatograms

UV 254 nm:
Direct evaluation in UV 254 nm shows zones, quenching fluorescence at the solvent front and at the start. A distinct quenching spot occurs at R*f* 0,48. Further weakly quenching zones are distributed over the entire R*f* range.

UV 365 nm:
At the solvent front, at R*f* 0,9, and at R*f* 0,4 – 0,6, bright blue fluorescent spots are visible. They do not show any enhancement of the fluorescence with natural products polyethyleneglycol reagent.

Vanillin-sulphuric acid reagent, vis (**Fig. 2**):
The major saponins are visible as blue to blue-violet coloured spots: Saikosaponin d (R*f* 0,55) brown-violet, saikosaponin a (R*f* 0,50) dark blue-violet, saikosaponin c (R*f* 0,35) dark violet. A brown-violet saponin zone "X" with diene-structure which quenches at UV 254 nm, is detectable directly beneath saikosaponin a. While samples 2 – 6 all show nearly the same saponin pattern, in sample 1 only saponin "X" (see HPLC Fig. 6) is detectable. At the solvent front, brown coloured spots indicative of polyacetylenes are detectable, next to blue zones (sterols, sapogenins) in the R*f* range of 0,7 – 0,9.

Blood reagent:
Hemolysis is caused by saikosaponins d, a and c, by substances at the solvent front (fatty acids, sterols) and by substances in the R*f*-region 0,6 – 0,8 (saponins). They appear as more or less white zones on a red-brownish plate background.

Distinction of the drug-types:

Since the characteristic saikosaponins are mainly present in the bark of the root, [7, 17] it can be suggested, that drug samples which contain less bark portions, as found in sample 1, have a lower saponin content and are of inferior quality. A high proportion of the material consisted of large pieces of root, so that the amount of root bark, is considerably less than in the samples which consisted of fine material.

Chinese drug-samples from Hubei differ from both the Hebei and Japanese origin by lacking the saponins in the R*f*-range of R*f* 0,6 – 0,8. *B. scorzonerifolium* can be distinguished from *B. chinense* by that way. *B. chinense* may be differentiated from the Japanese species only by the two characteristic violet-blue spots at R*f* 0,15.

HPLC fingerprint analysis

1) Sample preparation:

2 ml extract (10 g drug/10 ml MeOH) are concentrated to dryness at 40 – 60°C under vacuum, and the residue dissolved in 1 ml distilled H_2O. The aqueous suspension is then treated 1 – 2 minutes in a ultrasonic bath to dissolve any residue adhering to the flask. The suspension is then filtered through a Millipore® filtration unit, type HV 0,45 mm, into a Sepac C18 cartridge (classic, short body) which has been pre-conditioned with 5 ml methanol p.a., followed by 10 ml distilled water. The flask is washed with 5 ml distilled water and the water also filtered through the filtration unit into the Sepac cartridge. The cartridge is washed with a further 5 ml distilled water and 15 ml MeOH p.a. 30%. Elution of substances still absorbed on the cartridge is carried out with 10 ml MeOH p.a.. Methanol is then evaporated off under vacuum, and the residue dissolved in 0,5 ml methanol p.a.

2) Injection volume: 10 µl

3) HPLC data:

Apparatus:	Liquid Chromatograph HP 1090 Photodiode array detector HP 1040 A
Column:	LiChroCART 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART 4-4 with LiChrospher® 100 RP 18, Merck
Solvent sytem:	A: Water B: Acetonitrile
Gradient:	isocratic, 30% B (5 min.), linear 30 – 50% B in 20 min., 50 – 90% B in 20 min., isocratic, 90% B (5 min.).
Flow:	1,0 ml/min.
Detection:	200 nm

4) Description of the chromatograms:

Retention times of the main peaks:

Peak	**Rt (min.)**	**Compounds**
1	10,7	saikosaponin c
2	12,6	saponin (non identified)
3	13,1	saponin (non identified)
4	15,1	saikosaponin a
5	20,9	saikosaponin d
6	34,4	polyacetylene
7	35,5	polyacetylene
8	43,3	sterol

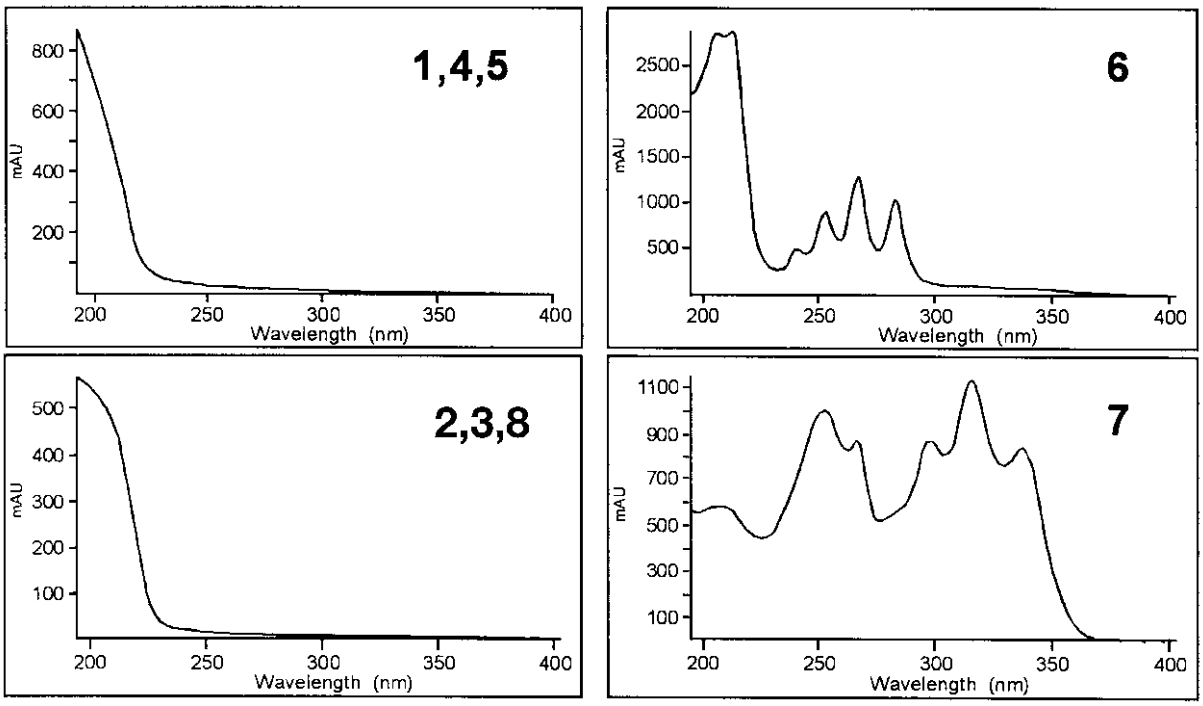


Fig. 3: UV-spectra of the major compounds

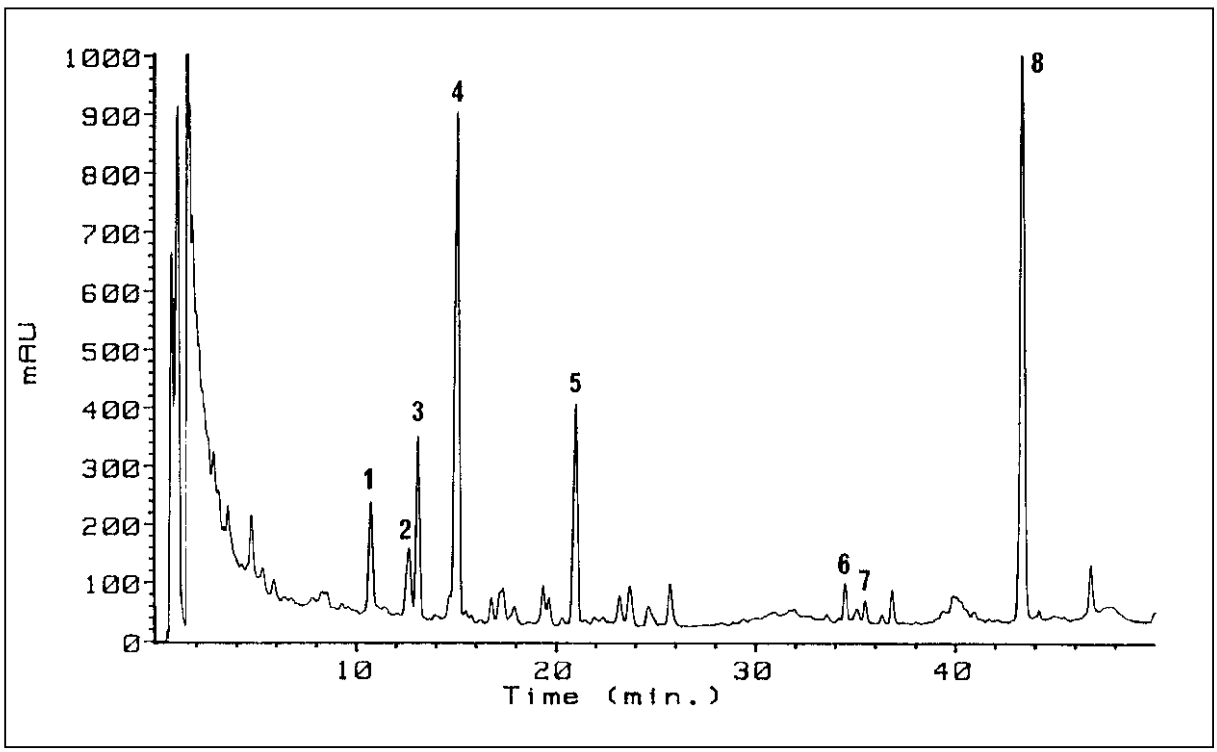


Fig. 4: HPLC fingerprint chromatogram of a drug sample of Chinese origin, Hebei Province (North) (sample 3).

Apart from the saikosaponins c (**1**), a (**4**) and d (**5**), further saponins (?) at Rt 12,6, 13,1 (**2,3**) are present in detectable quantities. The polyacetylene compounds at Rt 34,4 (**6**) and Rt 35,5 (**7**) are present at low concentrations. The sterol appears at Rt 43,5 (**8**) as characteristic peak. The investigated drug sample from Hubei Province (South) exhibited an almost identical chromatogram.

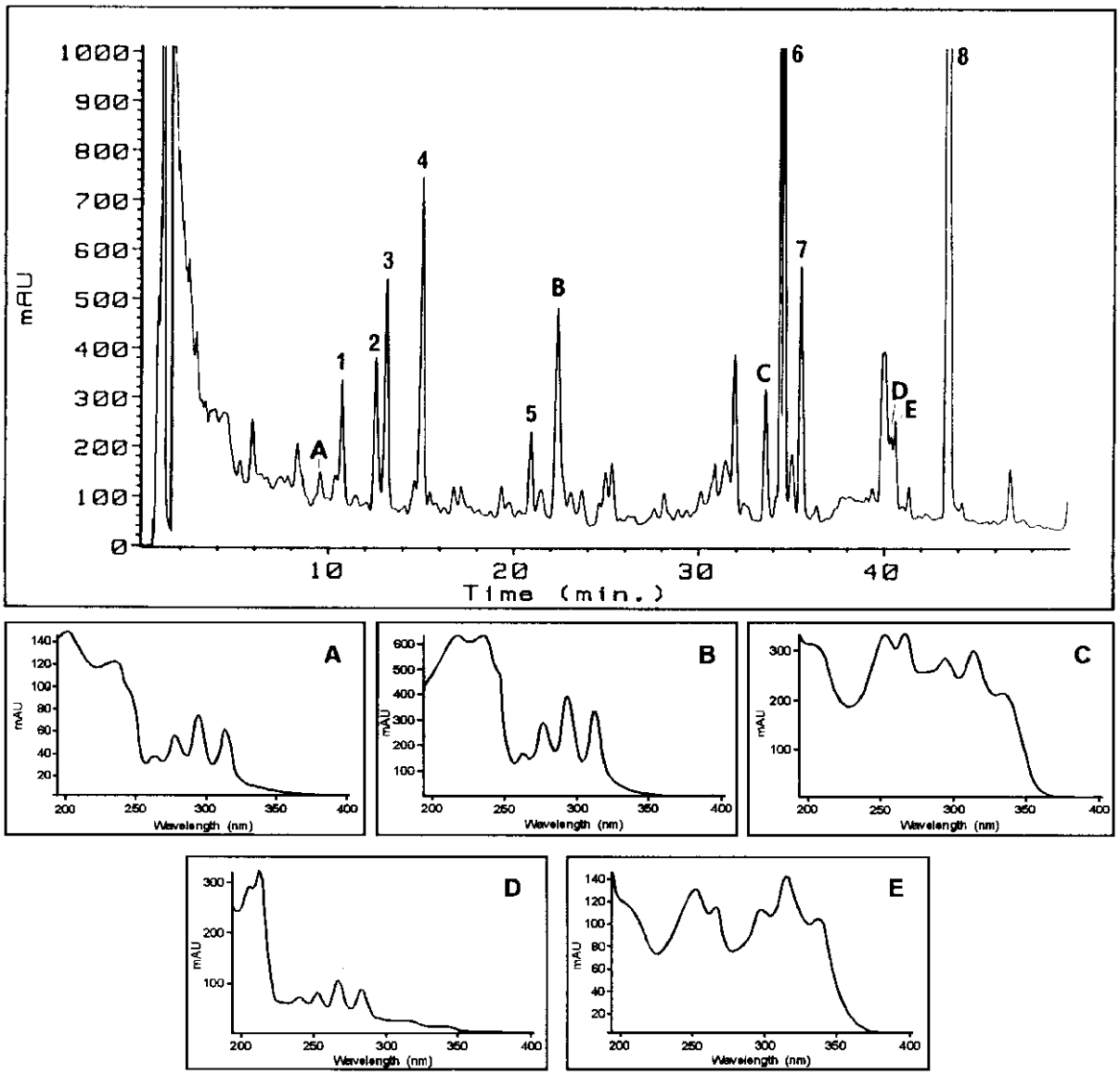


Fig. 5: HPLC fingerprint analysis of a drug sample of Japanese origin (sample 5), with UV-spectra of A–E

The chromatogram shows all characteristic saponin peaks **1 – 5**, the polyacetylenes **6** and **7** and the sterol (peak **8**) in high concentration. Further polyacetylenes are detectable at
Rt 9,5 (**A**); 22,3 (**B**); 33,5 (**C**); 40,5 (**D**) and 41,3 (**E**).

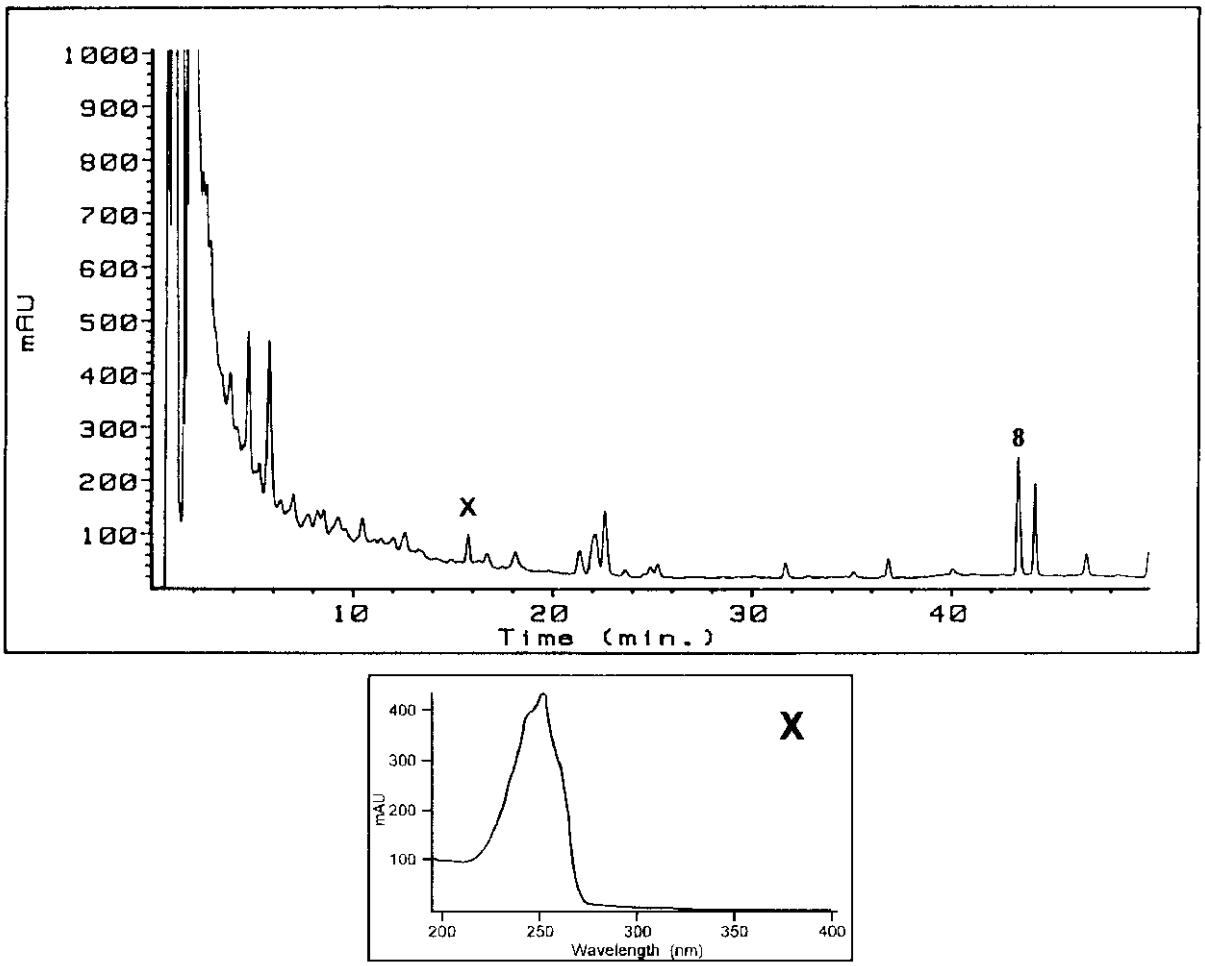


Fig. 6: HPLC fingerprint analysis of a commercial drug imported from China (sample 1), with UV-spectrum of X.

Whereas in all of the other imported drug samples investigated, the peaks **1 – 5** and **8** were all detectable, in this sample the characteristic saponins (**1 – 5**) and the acetylene compounds are lacking. A substance at Rt 15,8 (**X**) shows a UV-spectrum typical of saikosaponins with diene-structure. This HPLC-pattern corresponds with that of the TLC. Therefore sample 1 does not meet the requirement of quality.

Discussion:

Distinct differences between the Chinese drugs collected in Northern and Southern parts of China are not discernable, neither macroscopically nor analytically. In contrast, Japanese drugs obviously differ from the Chinese types in their considerably more diverse polyacetylene content, as well as macroscopically (lighter-coloured, fine roots without much branching).

The saikosaponins, particularly a and d, (see peaks 4 and 5 in the HPLC-fingerprints) might be the relevant components for establishing the drug quality, as they are suggested to be responsible for most of the

pharmacological effects of the drugs. The detection of these saponines is required for a drug of good quality.

Bupleurum species as e.g. *Bupleurum falcatum* which contain the saikosaponins a and d, might be acceptable as substitutes to the Chinese species. In contrast, *Bupleurum longiradiatum* Turcz, which contains these saponins, is designated as toxic [7].

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Bulbus Fritillariae – *Beimu*

Pharmacopoeia: Pharmacopoeia of the People's Republic of China
English Edition, 1992/2005[1]

Official drugs: The Chinese Pharmacopoeia 2005 contains five monographs:

(a) Fritillariae thunbergii bulbus *(Zhebeimu):*
the dried bulbs of *F. thunbergii* Miq. (= *F. verticillata* Willd. var. *thunbergii* (Miq.)) - Liliaceae

(b) Fritillariae cirrhosae bulbus *(Chuanbeimu):*
the dried bulbs of *F. cirrhosa* D. Don, *F. unibracteata* Hsiao et K. C. Hsia, *F. przewalskii* Maxim. (the first three species are also known as "Songbei" or "Qingbei" according to the different characters of plant material) and *F. delavay* Franch. ("Lubei")

(c) Fritillariae pallidiflorae bulbus *(Yibeimu):*
the dried bulbs of *F. walujewii* and *F. pallidiflora* Schrenk[1]

(d) Fritillariae hupehensis bulbus (*Hubeibeimu*):
the dried bulbs of *F. hupehensis* Hsiao et K. C. Hsia

(e) Fritillariae ussuriensis bulbus (*Pingbeimu*):
the dried bulbs of *F. ussuriensis* Maxim.

Description of the drug[1]:

(a) *Zhebeimu:*	Dabei:	the outer single scale leaves of bulb, slightly crescent in shape, 1-2 cm high, 2-3 cm in diameter, outer surface whitish to pale yellow, inner surface white
	Zubei:	whole whitish bulb, flattened cylindrical, 1-1,5 cm high, 1-2,5 cm in diameter
	Zhebeipan:	slices cutted from the outer single scaly leaves of bulb, 1-2 cm in diameter, surface pale yellow
(b) *Chuanbeimu:*	Songbei:	subconical or subspherical, externally whitish bulbs, 0,3-0,8 cm high, 0,3-0,9 cm in diameter
	Qingbei:	nearly oblate, 0,4-1,4 cm high, 0,4-1,6 cm in diameter
	Lubei:	long conical, externally whitish or pale brownish yellow, 0,7-2,5 cm high, 0,5-2,5 cm in diameter
(c) *Yibeimu:*	*F. walujewii:*	externally whitish and smooth, oblate, 0-5-1,5 cm high
	F. pallidiflora:	conical, relatively large, varying in size, pale yellowish-white
(d) *Hubeibeimu:*		oblate, whitish or brownish, 0,8-2,2 cm high, 0,8-3,5 cm in diameter
(e) *Pingbeimu:*		oblate, milk white or pale yellowish-white, 0,5-1 cm high, 0,6-2 cm in diameter

Pretreatment of the raw drug:

Chuanbeimu and *Yibeimu:* Cleansed and dried in the sun or in warmth.

Zhebeimu: Cleansed and sorted according to size. "Dabei" = large bulbs without buds, "Zubei" = small bulbs without buds. The bulbs are threshed, and the plant juices absorbed by mixed-in shell limestone. The fresh bulbs which have been cut into thick slices, washed, and finally dried, are denoted "Zhebeipan".

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Medicinal Use:

In Traditional Chinese Medicine Bulbus Fritillariae is used as antitussive, mucolytic, expecto-rant, and against inflammatory swellings and knots in the throat and lungs. *F. thunbergii* is used also in cases of mastitis and depression[2,3,4].

Effects and indications according to Traditional Chinese Medicine:[1,2,3,4]

	F. thunberghii (a) *Zhebeimu*	*F. cirrhosa* (b) *Chuanbeimu*
Taste:	bitter	bitter, sweet
Temperature:	cold	cold
Channels entered:	Lung, Triple Burner, Stomach, Liver	Lung, Heart
Effects:	clears Heat, transforms Hot Phlegm, dissipates Nodules, mucolytic, expectorant, calming	clears Heat, transforms Phlegm, dissipates Nodules, mucolytic, antitussive, antipyretic
Symptoms and Indications:	acute Lung Heat patterns with productive cough, swellings and nodules of neck, Mastitis, Lung abscess, depression	dry cough, chronic cough, cough with bloody sputum, consumptive cough; in cases of nodules, sores, swellings, scrofula; Lung or Breast abscesses due to Phlegm Fire

Main constituents (see Fig. 1):

- **steroid alkaloids with cevane structure:**
  in (a): verticine (= peimine), verticinone (= peiminine), isoverticine[5]
  in (b): imperialine (= sipeimine)[5]; delavine, delavinone from *F. delavay*[6]
  in (c): imperialine[5,7]
- **alkaloids with jervin structure:**
- steroids: ß-sitosterol, stigmasterol, cholesterol derivatives
- fatty acids: stearinic, palmitic acids *(F. unibracteata)*[8]
- diterpenes: kaurane derivatives *(F. thunbergii)*[9]

Pharmacology:

In vitro effects:

- inhibition of PAF-induced thrombocyte aggregation: verticine exhibits pronounced, verticinone weaker effect. The methanol extract is hardly effective, imperialine not at all[10].
- inhibition of ADP-induced thrombocyte aggregation: verticinone and verticine show moderate activity, imperialine has no effect[10].

- inhibition of c-AMP phosphodiesterase: cevane alkaloids of *F. persica* have shown effects[11].
- anticholinergic activity: ebeinone, a cevane alkaloid of *F. imperialis* inhibits acetylcholin-induced contractions in isolated guinea pig ileum; verticine and isoverticine are inactive[12].
- spasmolytic activity: the effects of imperialine are similar to those of papaverine[3].
- antiviral effects: the ethanol extract of *F. imperialis* has shown activity against herpes simplex type 1 and vesicular stomatitis virus[13].
- ACE-inhibition: fatty acids from *F. verticillata* were active[14].

In vivo effects:

- antitussive effects: verticine and verticinone in mice, guinea-pigs and cats[5].
- expectorant effects: alkaloid fraction and Fritillaria-saponins in mice[3].
- hypotensive effect: verticine and verticinone in high doses; at low doses the contrary effect becomes evident[5].
- sedative effect: verticine and verticinone antagonize the stimulatory effect of caffeine and potentiate the sedative effect of chlorpromazine in mice[5].

In humans, preparations of Fritillariae bulbus exhibit antitussive and expectorant effects[3].

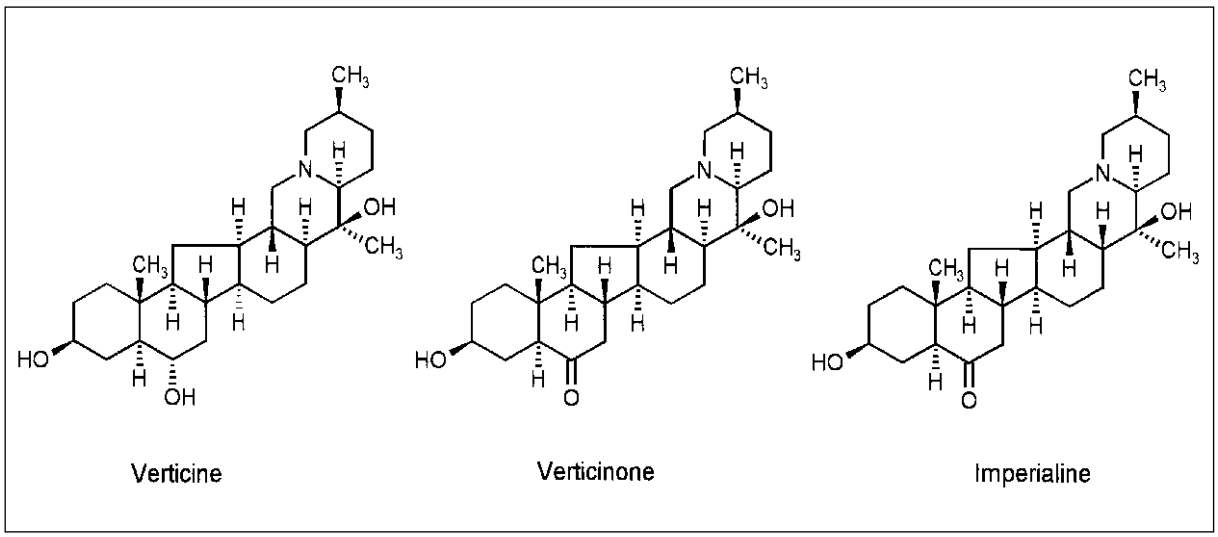


Fig. 1: Formulae of the main compounds

TLC fingerprint analysis:

1) Extraction:

   10 g coarsely-ground drug are treated for 1 hr with 50 ml 0,1 N sulphuric acid in an ultrasonic bath under occasional stirring. The suspension is centrifuged, the supernatant decanted off and then alkalized with 5 ml conc. ammonia (pH ~ 11). The aqueous phase is then distributed three times with 50 - 60 ml diethylether, and the combined ether phases are dried over sodium sulphate. The sample is evaporated to dryness and the residue dissolved in 1,0 ml methanol p.a.

2) Standards: verticine, verticinone, imperialine, emetine, each dissolved in MeOH p.a. (5 mg/ml).

3) Separation parameters:

Applied amount: 20 µl extract, 10 µl standard

Plates: Silica gel 60 F_{254}, Merck

Solvent system: Ethyl acetate - methanol - conc. ammonia (85 + 10 + 5)

Direct evaluation: UV 254 nm and UV 365 nm.

Spray reagents: [15]

a) Dragendorff reagent according to Munier and Macheboeuf:

Solution A: 0,85 g basic bismuth nitrate dissolved in 10 ml glacial acetic acid and 40 ml water.

Solution B: 8 g potassium iodide dissolved in 30 ml water.

Stock solution: Equal volumes of A and B are mixed.

Spray solution: Before use, mix 1 ml stock solution with 2 ml glacial acetic acid and 10 ml water.

The plate is sprayed vigorously, dried in a moderately warm air-stream and then treated with a 10 % aqueous sodium nitrite solution.

b) Vanillin-sulphuric acid reagent (VS):

The plate is sprayed with 5 - 10 ml 1 % ethanolic vanillin solution, and then immediately sprayed with 10 ml 5 % ethanolic sulphuric acid. It is heated at 110 °C for 5 - 10 min. and evaluated in vis.

Drug samples:

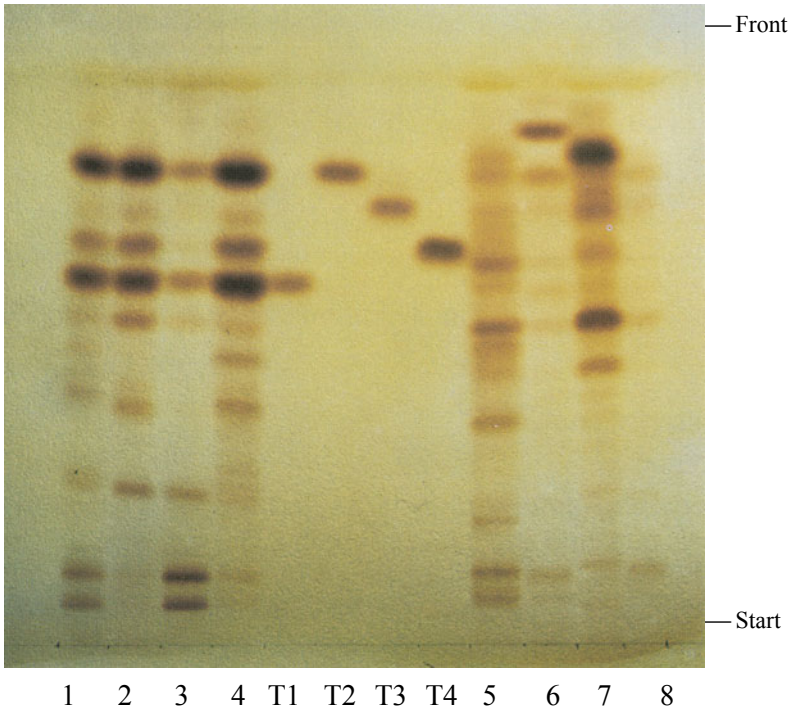
1,2 Commercial drugs from China „Zhebeimu“
3 Drug sample from *F. verticillata* var. *thunbergii*
4 Japanese drug sample „Zhebeimu“
5 Chinese drug sample „Pingbeimu“ *(F. ussuriensis?)* Jilin Province
6, 7 Chinese drug sample „Chuanbeimu“, Sichuan Province
8 Chinese drug sample „Chuanbeimu“ *(F. unibracteata?),* Sichuan Province.

4) Description of the chromatograms:

UV 254 nm:
Direct evaluation in UV 254 nm indicates for all drug samples a fluorescence quenching spot at the solvent front. Sample 6 exhibits an additional quenching zone at R*f* 0.9.

UV 365 nm:
Sample 7 shows two strongly fluorescent bright blue zones at R*f* 0,1 and 0,9. Samples 5 and 8 show similar fluorescent spots at R*f* 0,8.

Fig. 2:
Thin layer chromatogram of the drug samples (Dragendorff reagent, vis)



Test substances:

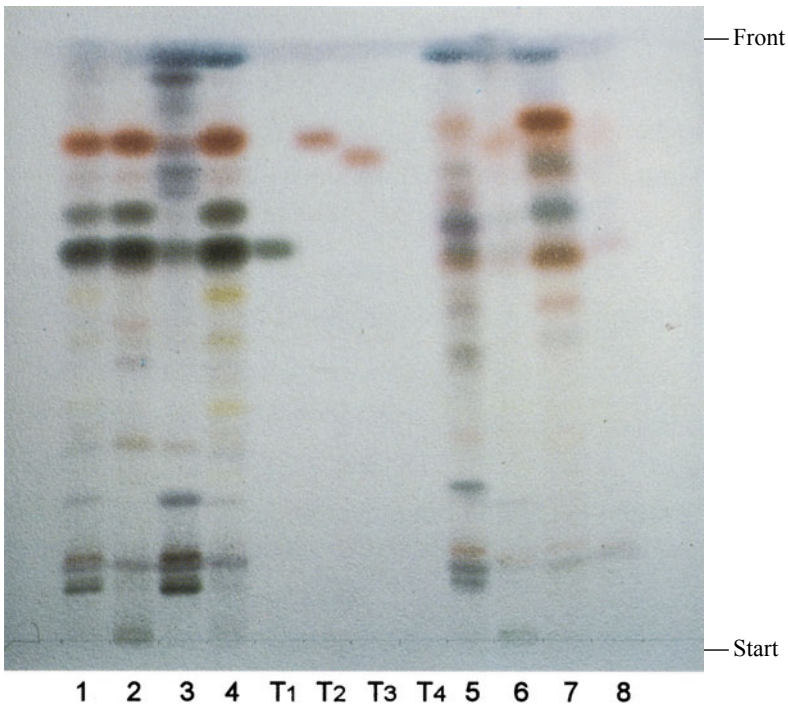
T1 verticine*, R*f*=0,65
T2 verticinone*, R*f*=0,85
T3 imperialine*, R*f*=0,79
T4 emetine, R*f*=0,71

* Test substances were kindly provided by Prof. Koh Kaneko, Japan.

Dragendorff-reagent, vis (**Fig. 2**):

The drug-samples exhibit several orange-brown zones in the R*f* region 0,05 - 0.9. Samples 1-4 are characterized by the alkaloids verticine (R*f* 0,65) and verticinone (R*f* 0,85). They are further characterized by an additional alkaloid at the same R*f*-value as emetine (R*f* 0,71). Sample 5 deviates from the above in that it shows its main compounds at R*f* 0,55 and 0,7. Sample 7 contains a large quantity of an unidentified alkaloid at R*f* 0,9. Samples 3, 6 and 8 are characterized by their lower alkaloid content. Samples 1, 3 and 5 contain polar alkaloids at R*f* 0,05 and 0,15 (alkaloid glycosides?).

Fig. 3:
Thin layer chromatogram of the drug samples (VS-reagent, vis)



Vanillin-sulphuric acid reagent, vis (**Fig. 3**):

The various drug samples produce practically the same component profile as after detection with Dragendorff reagent. However, characteristic colours are produced. Verticine appears green-blue, verticinone and imperialine red. The alkaloid glycosides stain green-blue or red, and blue-violet sterols are visible at the solvent front. However, emetine and some other Dragendorff positive spots give no colour reaction with VS-reagent.

Distinction of the drug types:

Drug samples designated *Zhebeimu* obviously possess higher alkaloid contents than those from *Chuanbeimu*. In spite of macroscopic differences (large bulbs or cut) samples 1-4 *(Zhebeimu)* exhibited similar chromatograms with the characteristic alkaloids verticine and verticinone. The samples 5 - 8 *(Chuanbeimu),* however, show an inconsistent alkaloid pattern although they do not differ macroscopically (uniform white bulbs of approx. 0,5 cm diameter). This may be explained by the fact that the Chinese Pharmacopoeia allows four species of the genus *Fritillaria* as *Chuanbeimu.*

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extracts used for TLC over a Millipore® filtration unit type HV 0,45 mm
2) Injection volume: 20 µl extract (conc. = 1 g drug/100 ml)
3) HPLC data:
   - Apparatus: Liquid Chromatograph HP 1090; Photodiode Array Detector HP 1040 A, Hewlett Packard
   - Column: Aluspher® RP 18 select B (5 µm), Merck*)
   - Solvent system: A: Phosphate buffer 0,005 M, pH 9 (= 0,68 g KH_2PO_4 dissolved in ca. 900 ml H_2O dist., adjusted to pH 9 with 1N-KOH, filled up to 1000 ml, pH-control).
     B: Acetonitrile
   - Gradient: isocratic, 35 % B (5 min.), linear 35 - 60 % B in 15 min.
   - Flow: 1,0 ml/min.
   - Detection: 210 nm
   - Note: Attempts to optimize the separation of alkaloids by using ion pair chromatography and detection at wavelength 200 nm were not successful.
     *)Alternative column: LiChroCart® 250-4 Li Chrospher® 100 RP-18 (5 µm), Merck

4) Description of the HPLC-chromatograms:

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	5,5	imperialine
2	7,3	verticinone
3	8,4	verticine
4	11,4	alkaloid X
5	14,8	compound Y

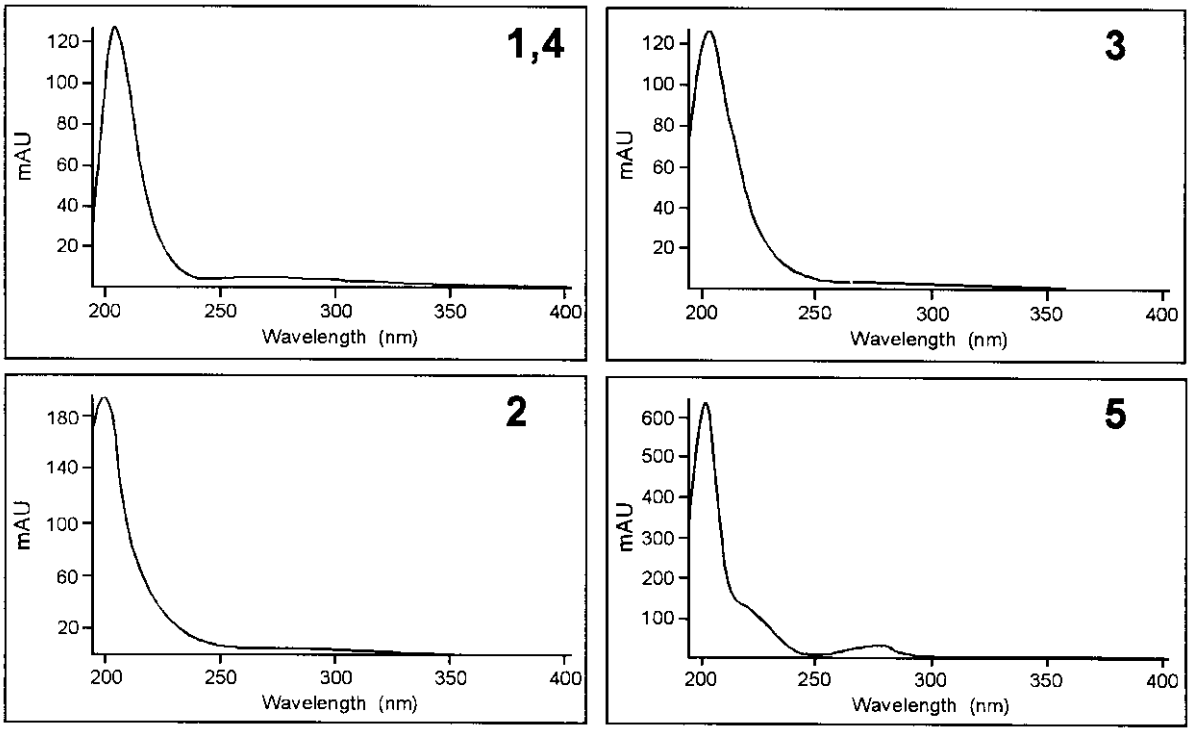


Fig. 4: UV-spectra of the major compounds

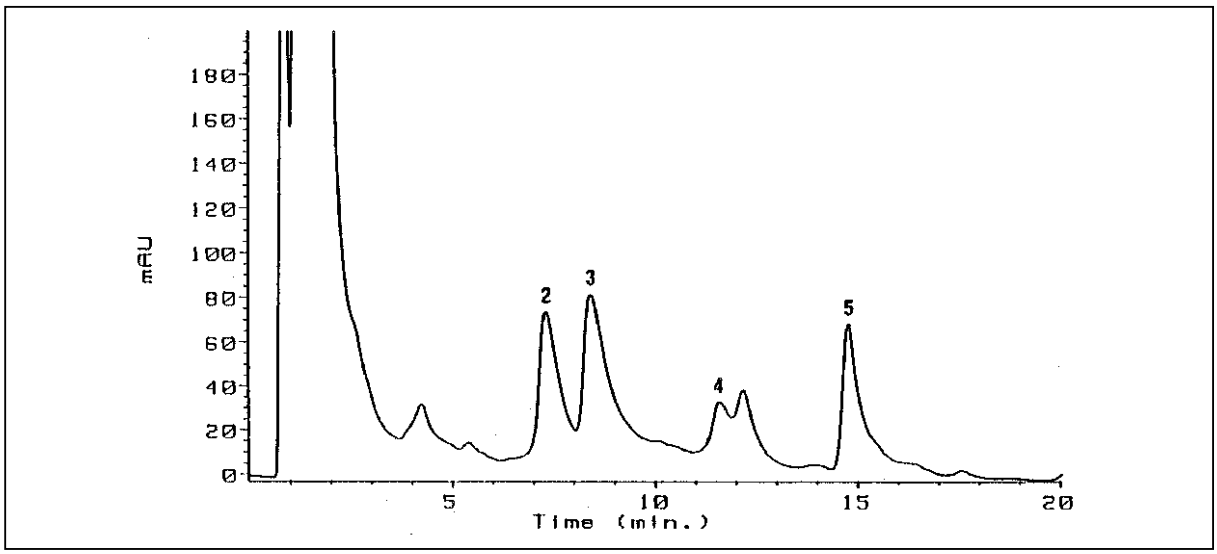


Fig. 5: HPLC fingerprint chromatogram of Zhebeimu (sample 2)

The HPLC fingerprints as in sample 2 are characterized as follows: in addition to verticinone (**2**) and verticine (**3**) at Rt 7,3 and 8,4 min., respectively, another „alkaloid X“ (**4**) is detectable at Rt 11,4, (TLC: R*f* 0,7), and occurs in the drug samples 1, 2, and 4. Peak **5** at Rt 14,8 seems to be a non alkaloid compound. It is found on TLC at the solvent front, and does not react with Dragendorff reagent.

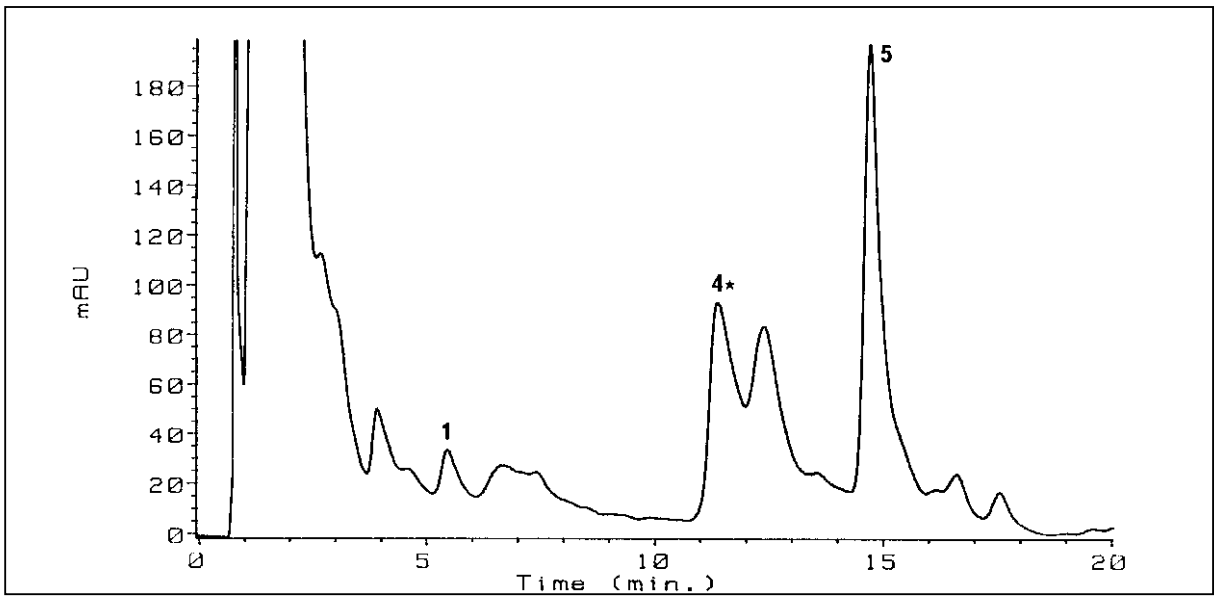


Fig. 6: HPLC fingerprint chromatogram of Chuanbeimu (sample 7)

The alkaloids verticinone (**2**) and verticine (**3**) are missing in the HPLC fingerprints (e. g. sample 7). The alkaloid imperialine (**1**) is detectable only in small quantities. At Rt 11,4 the chromatogram shows a peak which might indicate alkaloid „X“ (**4***) in high concentration. This does not correspond to the relative amounts shown by the spots in TLC. Compound **5** is more concentrated.

Discussion:

The alkaloids are particularly suited for proving the identity and quality of the drug Bulbus Fritillariae, since the main pharmacological effects of the drug may be derived from these compounds.

The differences between the alkaloid-patterns of both types of drugs, which can already be detected by TLC, are also distinct in HPLC. Thus, in *Chuanbeimu* the compounds verticine and verticinone are almost totally absent.

On account of the diversity of species which are officially accepted as *Chuanbeimu,* the latter cannot be identified by the presence of one or two leading substances only. However, the drug is characterized by the presence of cevane alkaloids (in TLC between R*f* 0,5 and 0,9) which can be stained with Dragendorff **and** vanillin-sulphuric acid reagents.

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Additional references (chromatographic analysis):

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Li SL, Lin G, Chan SW, Li P, Determination of the major isosteroidal alkaloids in bulbs of Fritillaria by high-performance liquid chromatography coupled with evaporative light scattering detection, J. Chromatogr. A 909(2), 207-214 (2001)

Radix Rehmanniae – *Dihuang*

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 1992/2005[1]

Official drugs:

– *Rehmannia glutinosa* (Gaertn.) Libosch

– *Rehmannia glutinosa* var. *hueichingensis* (Chao et Shih) Hsiao

– *Rehmannia glutinosa* var. *purpurea* Makino

– *Rehmannia glutinosa* var. *lutea* Makino

– Scrophulariaceae –

Description of the drug[1]:

Fresh: The brownish-yellow root is 8-24 cm long and 2-9 cm in diameter.

The outer bark is thin and has longitudinal wrinkles and irregular scars.

Dried: The brownish-black root is 6-12 cm long and 3-6 cm in diameter.

The root appears in mostly irregular shrunken masses.

Pretreatment of the raw drug:

Dried, steamed or steamed with wine.

Medicinal use: In Traditional Chinese and Japanese Medicine as an antidiabetic, antipyretic, antirheumatic, diuretic, hemostatic, laxative and spasmolytic drug.

Topically for the treatment of eczema and burns.

The pretreated drug is also used as a sedative, tonic, antihypertonic and gynecological drug.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,2,3,4]

State:	xian, fresh	sheng, dried untreated	shu, cooked in wine
Taste:	slightly sweet, bitter	sweet, slightly bitter	sweet
Temperature:	cold	cold	warm
Channels entered:	liver, kidneys, heart	liver, kidneys, heart	liver, kidneys, heart
Effects:	cools heat and blood, promotes production of body fluid and arrests bleeding	reduces the heat in the blood, nourishes the yin	strengthens, revitalizes, nourishes the yin, tonifies and supplements the blood
Symptoms/ Indications:	impairment of yin in febrile diseases, excessive thirst, red tongue, exanthemes, skin eruptions, bloody vomiting, nose bleeding, blood in stools, sore throat	deficiency of yin with internal heat, febrile diseases, excessive thirst, red tongue, exanthemes, skin eruptions, bloody vomiting, nose bleeding, blood in stools, sore throat, diabetes caused by internal heat, consumptive fever	deficiency of yin of liver and kidneys, pain and weekness of the loins and knees, menstrual disorder, anemia, anxiousness, buzzing in the ears, sleeplessness, cardiac palpitation, dizziness, greying of beard and hair, diabetes caused by internal heat consumptive fever

Main Constituents (see Fig. 1):

- **iridoids** (aucubin, catalpol, 6-O-ß-feruloyl-ajugol, geniposide, glutinoside, jioglutinoside, leonuride, melittoside, monomelittoside, rehmannioside A/B/C/D) [5]
- **phenethylalcohol glycosides** (acteoside, martynoside)[6]
- **norcarotenoides** (jiocarotenoside)[7]

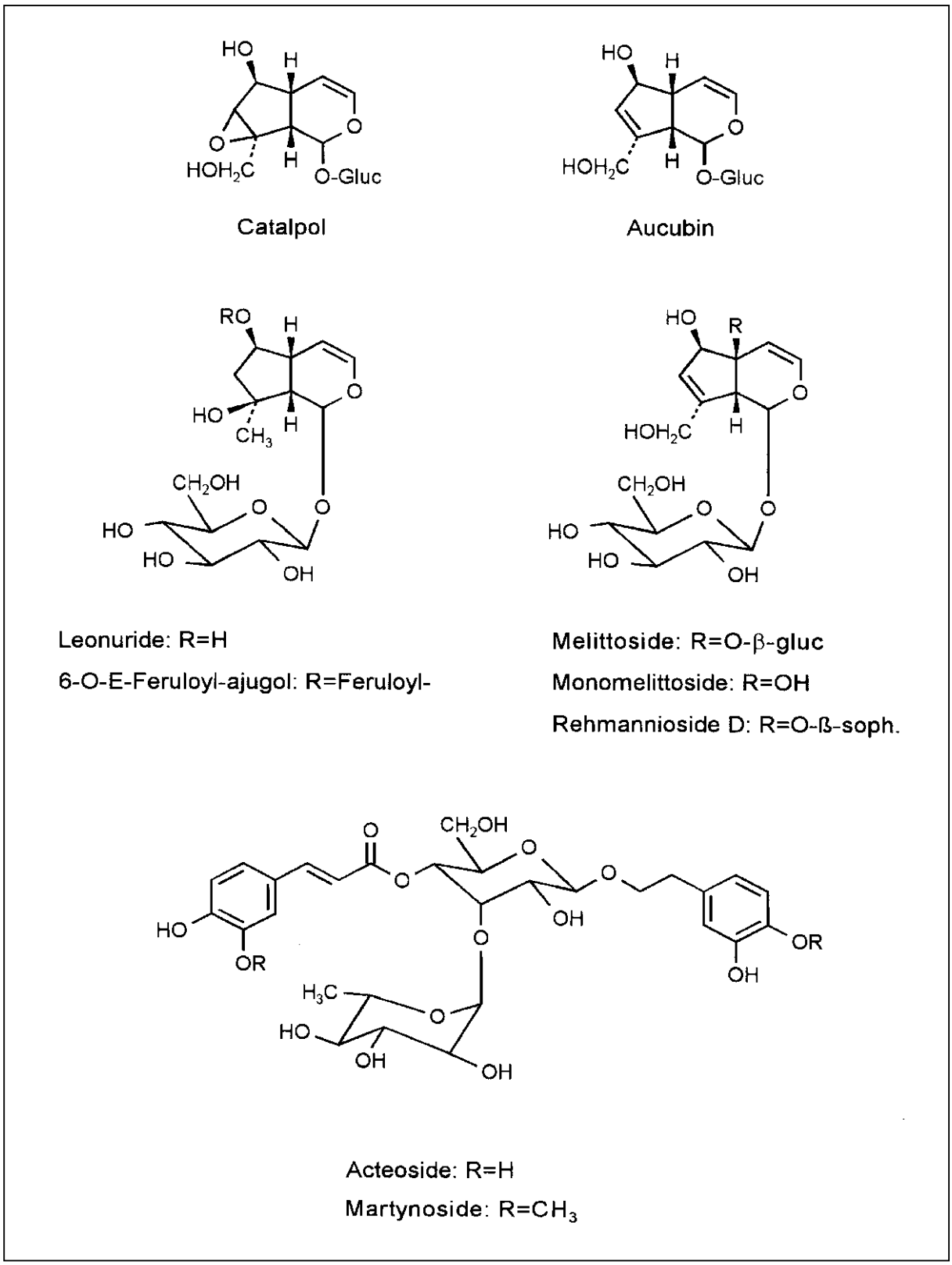

Fig. 1: Formulae of the main compounds

Pharmacology[2,3,9]:

In vitro effects
– inhibition of 5-lipoxygenase[8]
– antifungal effect[2]
– liver protection[2]

In vivo effects:
– antirheumatic[2]
– cardiotonic[2]
– antihypertensive[2]
– diuretic[2]
– hypoglycemic[2]
– anti-eczematic[2]

TLC fingerprint analysis

1) Extraction:
5 g coarsely ground drug are extracted with 150 ml methanol p.a. for 4 hrs in a Soxhlet apparatus. The extract is then filtered, the filtrate concentrated to approx. 15,0 ml and the solution filled up to 15,0 ml with methanol p.a.

2) Standards:
Martynoside, feruloyl-ajugol, leonuride, aucubin, catalpol, glucose, melittoside
(1 mg dissolved in 1 ml methanol p.a.)

3) Separation parameters:

Applied amount:	40 µl extract
Plates:	Silicagel 60 F_{254}, Merck
Solvent system:	toluene-chloroform-methanol-water (20+50+40+3) (iridoids, phenethyl-alcohol glycosides, norcarotenoides)
Direct evaluation:	– VIS – UV 254 nm and UV 365 nm
Spray reagents:	Vanillin-sulphuric acid reagent (VS) 1 % ethanolic vanillin solution (I) 5 % ethanolic sulphuric acid (II)
	The TLC plates are sprayed successively with reagents I and II and then heated at 100 °C for 5-10 min.

Drug samples:

1	Commercial drug	from Korea
2		– from China (Sichuan Province)
3		– from China (Hunan Province)
4		– from China (Hebei Province)

Test substances:

T1 martynoside, R*f* = 0,70
T2 6-O-ß-feruloyl-ajugol/leonuride, R*f* = 0,65/0,45
T3 aucubin, R*f* = 0,40
T4 catalpol, R*f* = 0,35
T5 glucose, R*f* = 0,30
T6 melittoside, R*f* = 0,20

4) Description of the chromatograms:

Vis:
When evaluated directly in visible light, several yellow spots of norcarotenoid compounds are visible in the upper R*f* region (0,8 - 1,0).

UV 254 nm:
A few spots of slightly quenching fluorescence appear distributed over the entire R*f* range.

UV 365 nm (**Fig. 2**):
The norcarotinoid compounds in the upper R*f* region exhibit red and yellow fluorescences. Martynoside (R*f* 0,7) and feruloyl-ajugol (R*f* 0,65) show bright blue fluorescence. In the lower R*f* region, several bright blue fluorescent spots are visible.

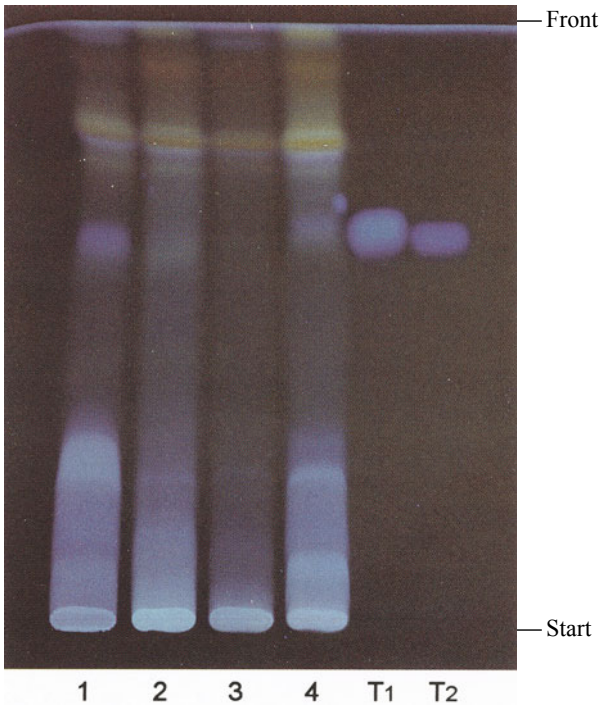

Fig. 2:
Thin layer chromatogram of *Rehmannia* samples (UV 365)

Vanillin-sulphuric acid reagent (VS) vis. (**Fig. 3**):

– The iridoids and phenethylalcohol glycosides generate yellow-orange, violet and brown coloured spots, the norcarotinoides violet and dark-brown zones.

– Martynoside (R*f* 0,7 = **T1**) gives a yellow-orange colour.
It is located directly adjacent to the red-brown zone of feruloyl-ajugol (R*f* 0,65 = **T2**).

– In the middle R*f* region, the iridoids leonuride (R*f* 0,45 = **T2**) and catalpol (R*f* 0,35 = **T4**) can be clearly distinguished as violet and brown zones, respectively.

– The red-brown zone of aucubin (R*f* 0,4 = **T3**) is overlapped by leonuride in most of the drug samples.

– The lower R*f* region is characterized by glucose (R*f* 0,3 = **T5**) as a dark-brown zone, melittoside (R*f* 0,2 = **T6**) as an orange-brown zone, and rehmannioside D (R*f* 0,1) as a dark-brown zone.

– The phenethylalcohol glycoside martynoside can be found only in samples 1 and 2.

– Leonuride can be detected in all samples, sample 4 shows the highest amount.

– Aucubin is not detectable in all samples.

– The iridoid catalpol shows its highest concentration in sample 4; in sample 2 the concentration is very low and not well detectable.

– Sample 1 from Korea is a good standard sample.

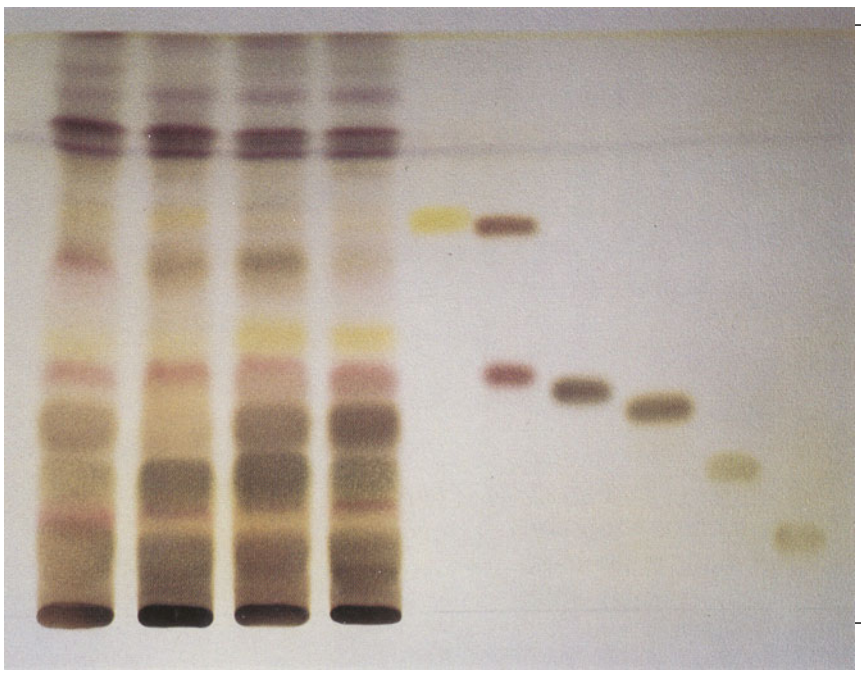

Fig. 3: Thin layer chromatogram of *Rehmannia* samples (VS/VIS)

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract used for TLC over Millipore® filter type HV 0,45 mm

2) Injection volume: 5 µl of the methanol extract (conc. = 5 g drug/15 ml)

3) HPLC data:

Apparatus: Liquid Chromatograph HP 1090,
Photodiode-array-detector HP 1040 A (Hewlett Packard)

Column: LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5µm), Merck

Pre-column: LiChroCART® 4-4 with Li Chrospher®, 100 RP 18, Merck

Solvent system: A: distilled water (+1 % (v/v), 0,1 N-H_3PO_4)
B: acetonitrile (+1 % (v/v), 0,1 N-H_3PO_4)

Gradient: linear
0 – 10 % B in 20 min.
10 – 25 % B in 20 min.
25 – 50 % B in 20 min.

Flow: 1,0 ml/min.

Detection: 200 nm

4) Description of the HPL-chromatograms: (**Fig. 4 – 7**)
Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	4.5	rehmannioside A
2	5.3	catalpol
3	8.7	aucubin
4	9.0	monomelittoside
5	10.7	rehmannioside D
6	11.0	melittoside
7	12.2	leonuride
8	33.6	acteoside
9	38.8	feruloyl-ajugol
10	41.2	martynoside

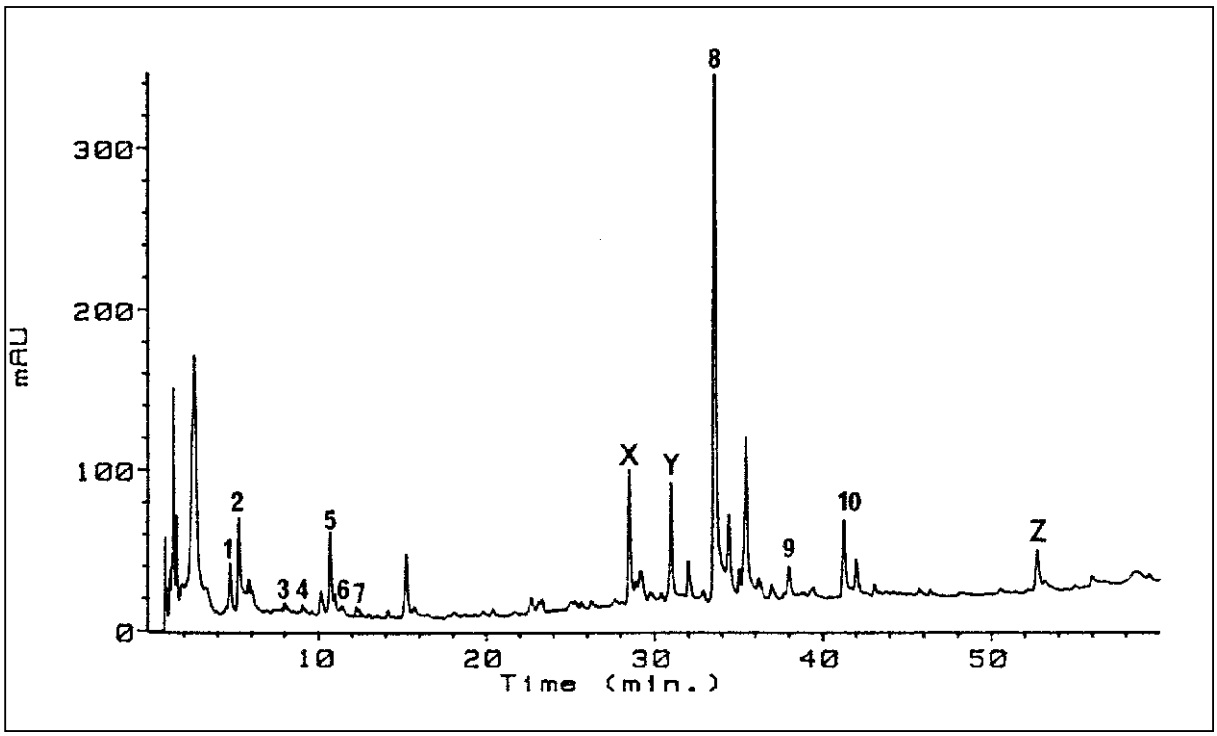

Fig. 4: HPLC fingerprint chromatogram of a drug sample of Chinese origin (Sichuan Province) (sample 2)

Fig. 4:

The HPLC fingerprint is characterized by the peaks of rehmannioside A (peak **1**), catalpol 2 (peak **2**), rehmannioside D (peak **5**), acteoside (peak **8**), feruloyl-ajugol (peak **9**) and martynoside (peak **10**).

Aucubin, monomelittoside, melittoside and leonuride (peak **3**, **4**, **6**, **7**) are only detectable in minor concentrations.

In the Rt-range 28-42 min., several phenethylalcohol glycosides with characteristic UV spectra (e. g. acteoside) are detectable.

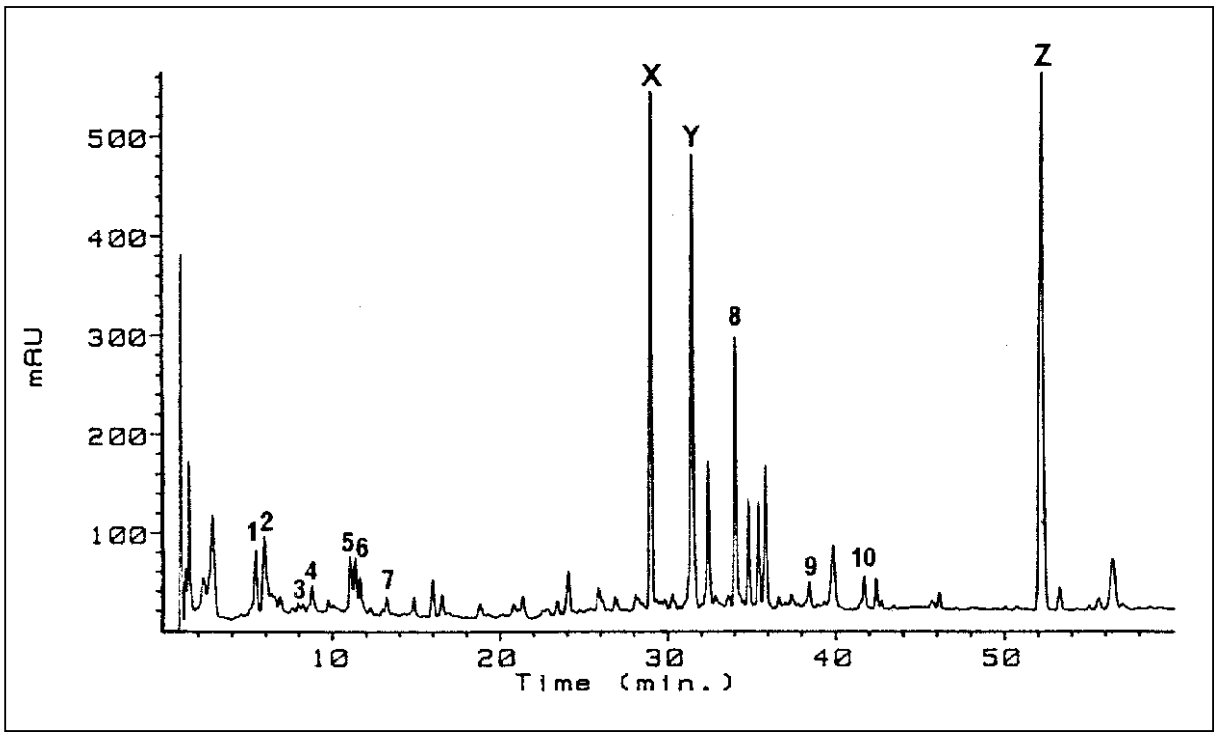

Fig. 5: HPLC fingerprint chromatogram of a drug sample from Korea (sample 1)

Fig. 5:

– The peak pattern of the iridoids is very similar to that of the Chinese sample (No. 2).

– In the phenethylalcohol glycoside region, distinct quantitative differences are noticeable.

– The most prominent compounds of the phenethylalcohol glycosides (**X** and **Y**) are found in the Rt-region from 28-33 min., prior to the acteoside peak **8** (Rt 34,0).

– At Rt 52 min. a characteristic phenolic compound (**Z**) is detectable.

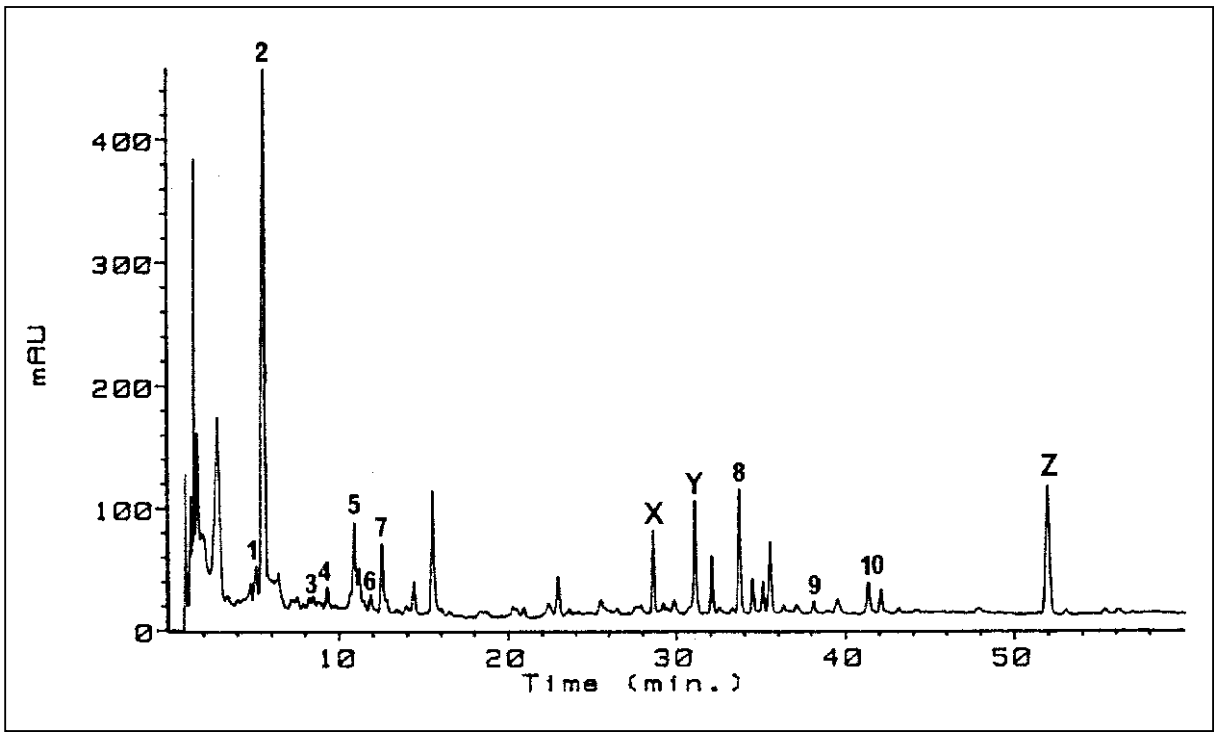

Fig. 6: HPLC fingerprint chromatogram of a Chinese drug sample (Hunan Province) (sample 3)

Fig. 6:

– The main component is catalpol (peak **2**).

– From the other iridoid compounds leonuride (peak **7**) is present in higher concentrations than in the first two drug samples, whereas the phenethylalcohol glycosides are present in considerably lower concentrations.

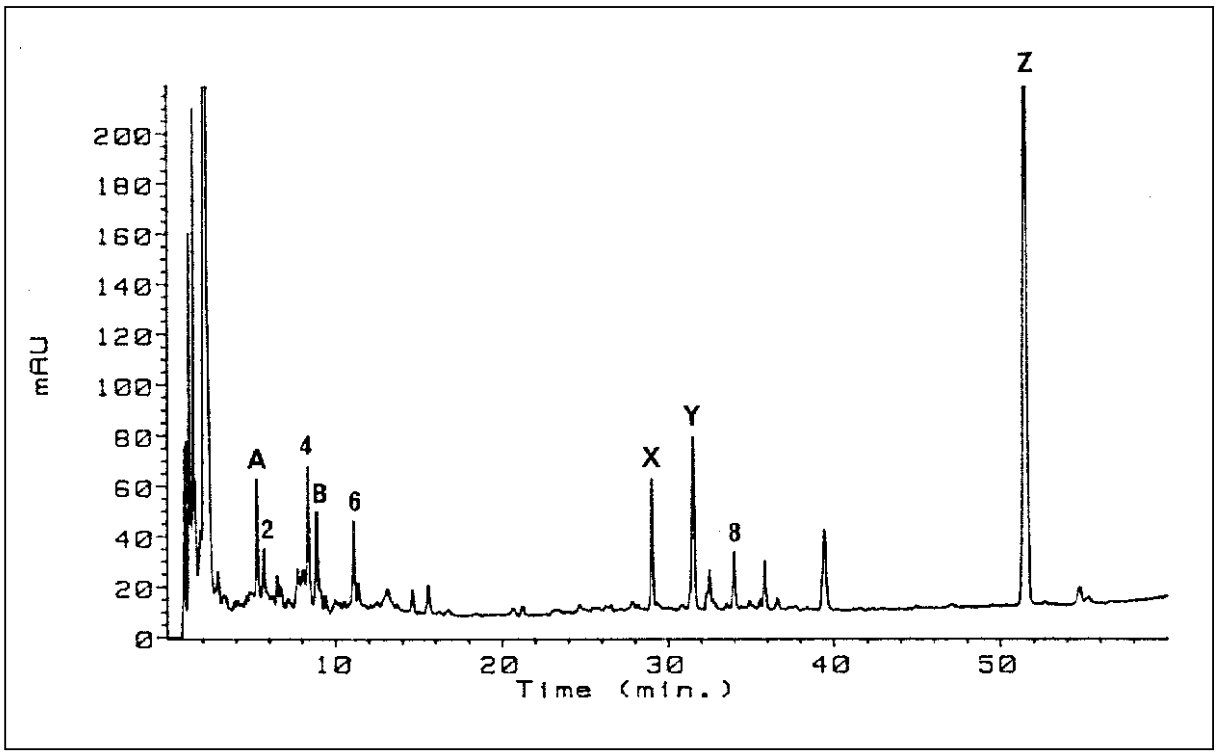

Fig. 7: HPLC fingerprint chromatogram of a pretreated *Rehmannia* drug sample

Fig. 7:

– The pretreated Rehmannia roots exhibit a distinctly different fingerprint chromatogram.

– In the iridoid fingerprint region different new compounds (A and B) are detectable.

– The iridoids appear only at lower concentrations.

– The phenethylalcohol glycosides appear at much minor concentrations.

Discussion:

The fingerprint chromatograms of the untreated Rehmannia samples show a very uniform composition of constituents but vary quantitatively.

The iridoid catalpol and the phenethylalcohol glycoside acteoside are best suited as marker compounds.

Pretreated drugs are clearly distinguishable from untreated Rehmannia roots by their HPLC fingerprint chromatogram, because the compounds A and B are detectable in the iridoid region.

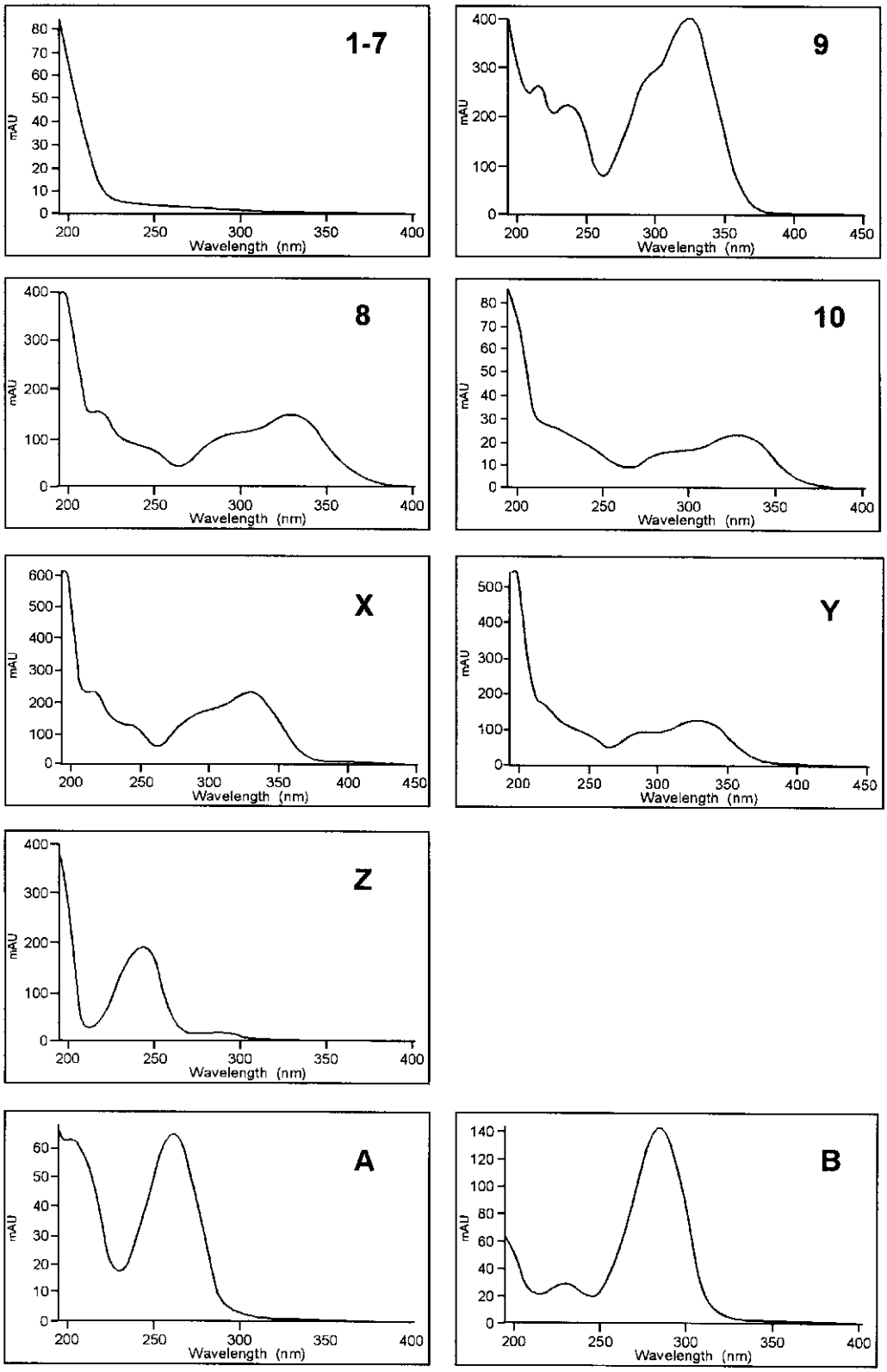

Fig. 8: UV spectra of the main constituents

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Fructus Schisandrae – *Wuweizi*

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 1992/2005[1]

Official drugs: *Schisandra chinensis* (Turcz.) Baill.

Schisandra sphenanthera (Rehd & Wils)

– Magnoliaceae –

Description of the drug[1]: Chinese Pharmacopoeia distinguishes the fruits of *Schisandra chinensis* (Beiwuweizi) and *Schisandra sphenanthera* (Nanwuweizi) which differ in their morphology and in their origin.
Beiwuweizi originates from North China, while Nanwuweizi is indigenous to southern provinces.

Schisandra chinensis: The berries are 5-8 mm in diameter, the outer surface is red or violet-red, wrinkled and oily with soft pulp. The 1-2 seeds are brownish-yellow and reniform.

Schisandra sphenanthera: The fruits are smaller and have brownish-red or dark-brown colour.

Pretreatment of raw drug: Dessicated and crushed before use (wuweizi), steamed with vinegar (cuwuweizi).

Medicinal use: In Traditional Medicine in China and Japan as antihepatotoxic antiasthmatic, antitussive, antidiabetic, sedative and tonic drug and also in cases of cholera.

Effects and indications according to Traditional Chinese Medicine[1,2,3,4]

Taste:	sour
Temperature:	warm
Channels entered:	lungs, kidneys, heart, liver
Effects:	adstringent, calming, fortifying the qi and the kidneys
Symptoms, Indications:	chronic cough, shortness of breath, perspiration, night sweating, enuresis, loss of semen, chronic diarrhea, anxiety accompainied by heart palpitations, sleeplessness, diabetes caused by internal heat

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

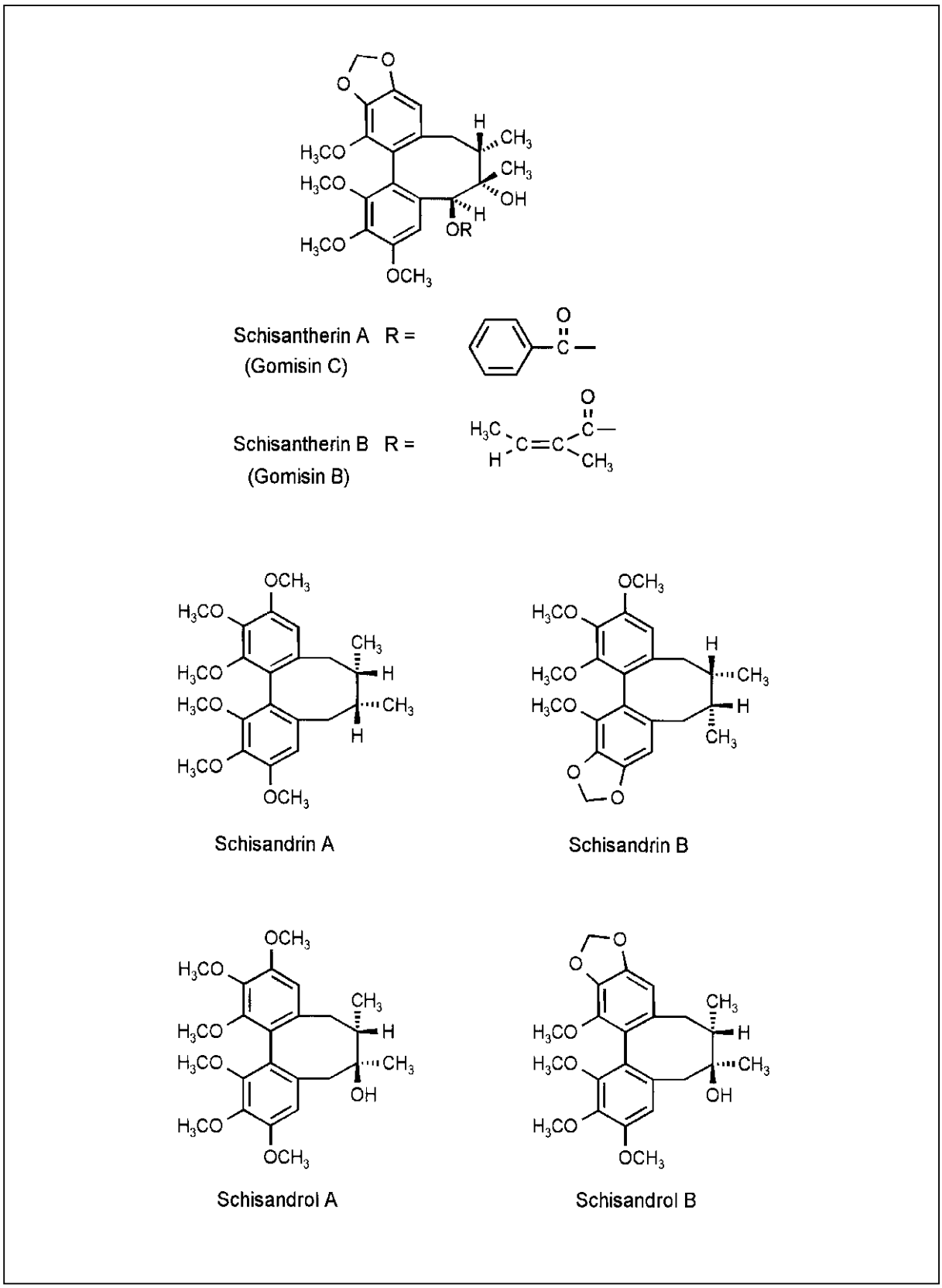

Fig. 1: Formulae of the main compounds

Main constituents (see Fig. 1):

- **dibenzo-[a,c]-cyclooctene lignans** (schisandrol A/B, schisandrin A/B/C, schisantherin A-E, gomisin, angeloylgomisin, anwulignan, wulignan, epiwulignan, epischisandron)
- **monoterpenes** (borneol, 1,8 cineol, citral, p-cymol, α,ß-pinene)
- **sesquiterpenes** (sesquicarene, (+) α-ylangene, chamigrenal, α- and ß-chamigrene, ß-bisabolene)[3,6]

In addition: vitamins C and E, fumaric acid, stigmasterol[2,7]

Pharmacology:

In vitro effects:
- antioxidative activity[5]
- antibacterial[6]

In vivo effects:
- liver-protective effects, SGPT-lowering effects[2]
- antiinflammatory effects on the stomach and intestinal tract[7]
- stimulating lung function[2]
- adaptogenic effect[2]
- cardiovascular effects[2]
- stimulation of the uterus[2]
- anticonvulsant[7]
- neuroleptic[7]

TLC fingerprint analysis

1) Extraction:
   5 g of coarsely ground drug are soxhlet-extracted for 2 hrs with 150 ml methanol p.a.
   The extract is filtered and the filtrate concentrated to approx. 15,0 ml. The solution is filled up to 15,0 ml with methanol p.a.

2) Standards:
   Schisandrin A and B, schisantherin A, schisandrol A and B, stigmasterol
   (1 mg dissolved in 1 ml methanol p.a.)

3) Separation parameters:

Applied amount:	20 µl extract
Plates:	Silicagel 60 F_{254}, Merck
Solvent system:	toluene – ethyl acetate – glacial acetic acid (70 + 33 + 3) [lignans, terpenes]

Spray reagents:

Anisaldehyde-sulphuric acid reagent (0,5 ml anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol + 5 ml conc. sulphuric acid are mixed in this order).

The plate is sprayed (approx. 10 ml) and heated for 5 – 10 min. at 100 °C. The evaluation is carried out in vis.

Drug samples:

1	Commercial drug from China	*(Schisandra sphenanthera)*
2	Commercial drug from China (Sichuan Province)	*(Schisandra sphenanthera)*
3	Commercial drug from China (Hebei Province)	*(Schisandra chinensis)*
4	Commercial drug from Japan	*(Schisandra chinensis)*

Test substances:

T1	Schisandrin B, R*f* = 0.80
T2	Schisandrin A, R*f* = 0.66
T3	Stigmasterol, R*f* = 0.57
T4	Schisantherin A, R*f* = 0.47
T5	Schisandrol A/schisandrol B, R*f* = 0.39/44

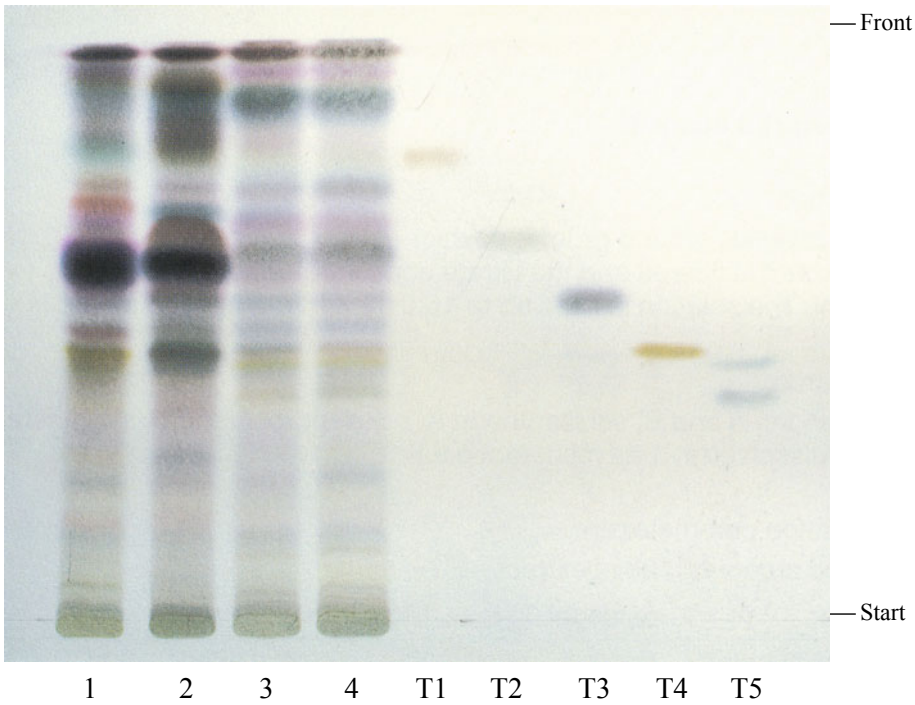

Fig. 2: Thin layer chromatogram of *Schisandra* samples

4) Description of the chromatogram:

Anisaldehyde-sulphuric acid reagent, vis (**Fig. 2**):

- The *Schisandra chinensis* and *Schisandra sphenanthera* show a very similar qualitative but a different quantitative pattern of lignan compounds.
- The sample 3 and 4 of *Schisandra chinensis* are characterized by a minor concentration of Schisandrin B (**T1**; R*f* = 0.80), higher concentration of Schisandrin A (**T2**; R*f* = 0.66) and again lower concentrations of Schisandrol A and B (**T5**; R*f* = 0.39 and 0.44).
- In contrast to *Schisandra chinensis* the samples 1 and 2 of *Schisandra sphenanthera* show in the R*f* – range of 0.75 – 0.95 two strong dark blue zones of non identified lignans. The compound at R*f* 0.81 is overlapped by Schisandrin B. The lignan might be identical with Schisandrin A. Schisantherin A with light brown colour is overlapped by another compound. Schisandrol A and B are absent.

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract used for TLC over Millipore® filtration unit, type HV 0,45 mm.

2) Injection volume: 2 µl methanolic total extract (conc. = 5 g drug/15 ml)

3) HPLC data:

Apparatus:	Liquid chromatograph HP 1090, Photodiode array detector HP 1040 A, Hewlett Packard
Column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
Solvent system:	A: water distilled (+ 1 % (V/V) 0,1 N-H_3PO_4) B: acetonitrile (+ 1 % (V/V) 0,1 N-H_3PO_4)
Gradient:	linear 40-80 % B in 20 min.
Flow:	1,0 ml/min.
Detection:	210 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	7.9	schisandrol A
2	8.4	schisandrol B
3	11.5	schisantherin A
4	11.8	schisantherin B
5	15.5	schisandrin A
6	17.3	schisandrin B

Description of the HPL chromatograms:

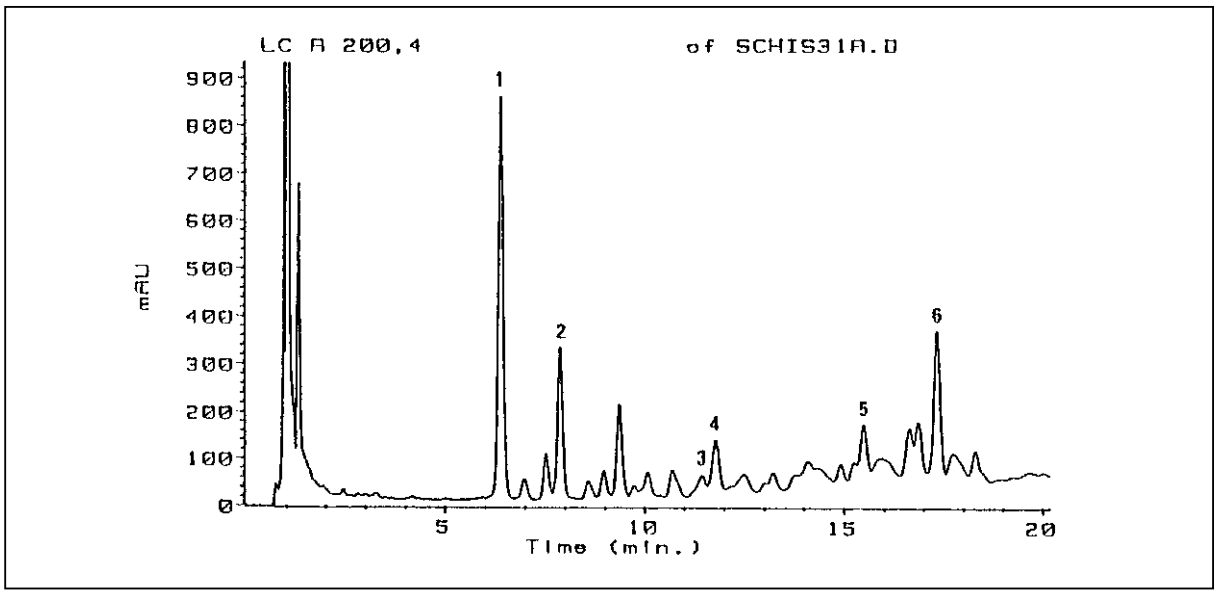

Fig. 3: HPLC fingerprint analysis of a commercial drug sample from Japan *(Schisandra chinensis,* sample 4)

The HPLC-chromatogram of *Schisandra chinensis* (sample 4) is characterized by Schisandrol A (**1**, Rt = 7.9), Schisandrol B (**2**), schisandrin A (**5**) and schisandrin B (**6**). Schisantherin A (**3**) and schisantherin B (**4**) are present in minor concentrations.

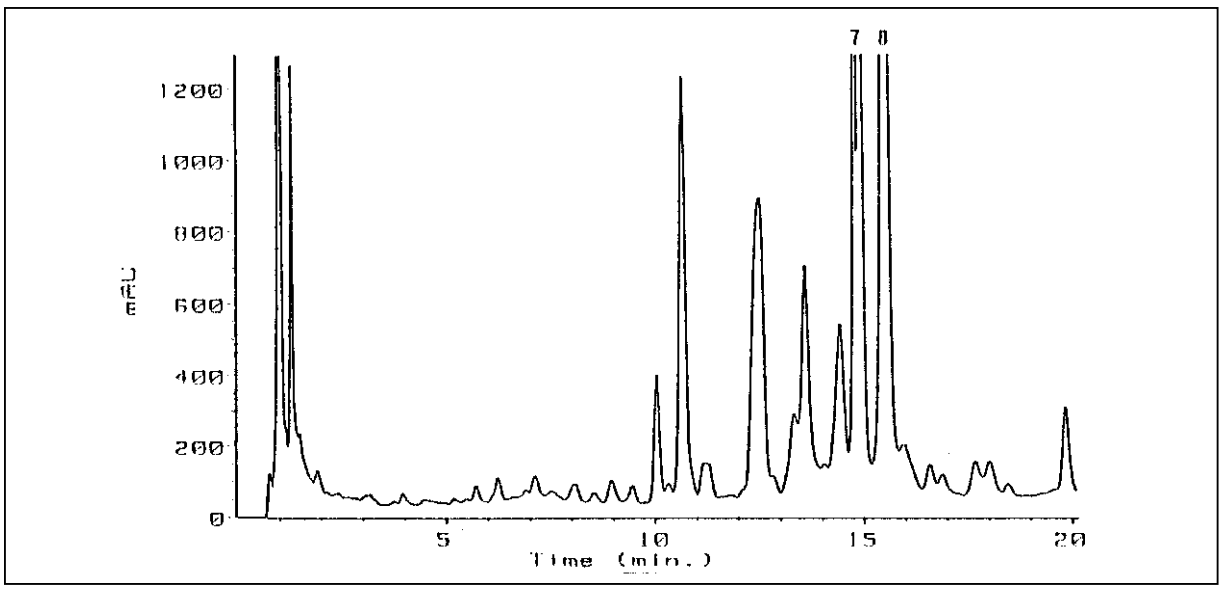

Fig. 4: HPLC fingerprint analysis of a drug sample of Chinese origin (Sichuan Province); (*Schisandra sphenanthera,* sample 2)

In drug sample 2 of *Schisandra sphenanthera* the main peaks are in the Rt-range 10-17 min. An exact assignment of the following peaks was difficult because of the overlapping of the peaks by other lignans. One of the peaks 7 and 8 at Rt = 14.7 and 15.5 respectively could be assigned to schisandrin B, the peak at Rt = 12.5 to schisantherin B.

Note: The Chinese Pharmacopoeia 2005 demands for *Schisandra chinensis* not less than 0.40 % of schisandrin whereas for *Schisandra sphenanthera* not less than 0.12 % schisantherin A. This is consistent with Schisandrin as selected marker compound for *Schisandra chinensis* and Schisantherin A for *Schisandra sphenanthera*.

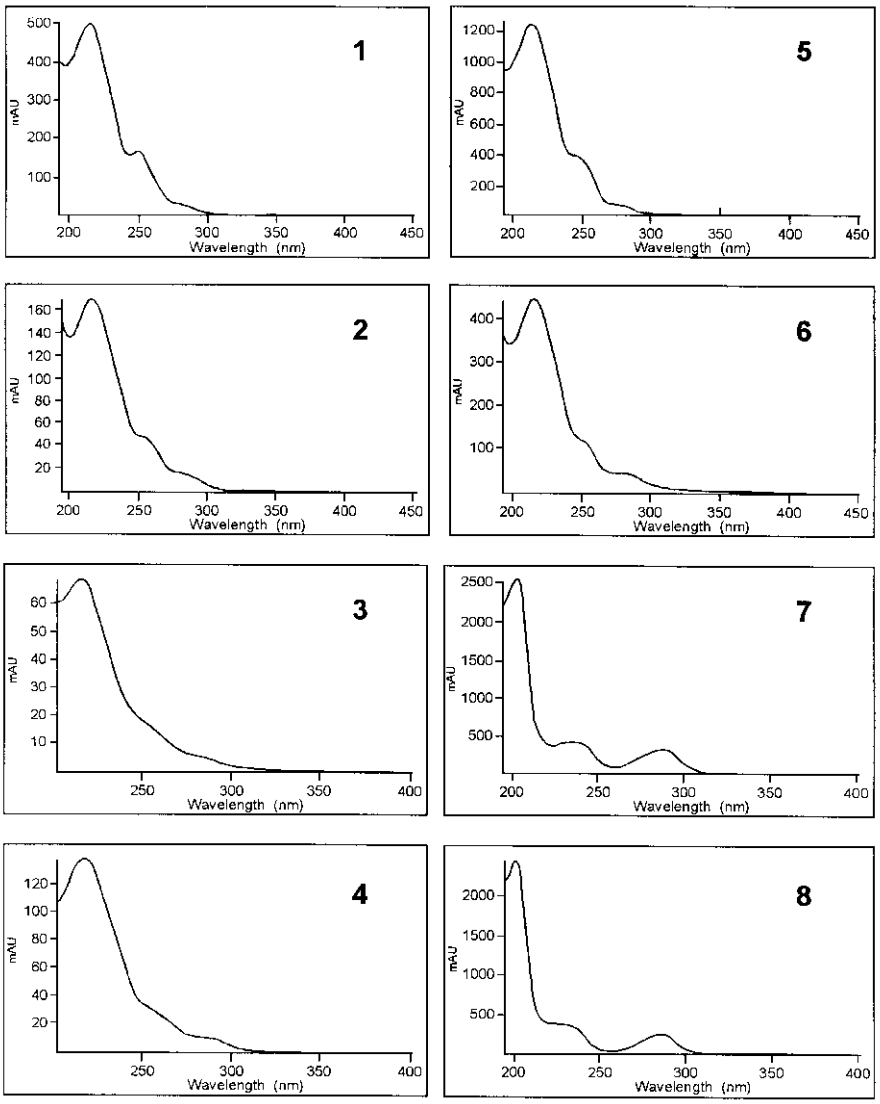

Fig. 5: UV spectra of the main compounds

Discussion:

For the HPLC fingerprint analysis of *Schisandra chinensis* (Beiwuweizi) the lignans schisandrol A and B as well as schisandrin A and B are best suited for identification, whereas schisantherin A and B are less typical because of their very low concentrations.

Schisandra sphenanthera (Nanwuweizi) can be differentiated from *Schisandra chinensis* by the deficiency of schisandrol A/B, as seen in the HPL– and TL – chromatograms respectively.

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Radix et Rhizoma Asari – *Xixin*

Pharmacopoeias: Chin. Ph. IX
Pharmacopoeia of the People's Republic of China,
English Edition 1992/2005[1]

Official drugs: Manchurian Wildginger root is the dried root and rhizome of: *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag. (= *Asarum heteropoides*)*Asarum sieboldii* Miq. (= *Asarum sieboldii*) *Asarum sieboldii* Miq. var. *seoulense* Nakai – Aristolochiaceae –

The former two are known as "Liaoxixin".

The drug is collected at the fruiting stage in summer or in early autumn, removed from adhering soil and dried in the shade.

Origin:[3] *A. heterotropoides* var. *mandshuricum* and A. *sieboldii var. seoulense* originate Origin:[3] Manchuria (China), Amurland (Russia) and Korea, while *A. sieboldii* is indigenous to China and Honshu (Japan).

Description of the drug:[1,3]

A. heterotropoides var. *mandshuricum*: Cylindrical, short-branched rhizomes, 1–10 cm long, 2–4 mm in diameter; externally greyish brown, rough with ringed nodes, internodes 2–3 mm long; longpetioled leaves with glabrous surface, lamina tapered, margins entire, acute at the apex and deeply cordate at the base, 4–10 cm long, 6–12 cm wide, pale green; pungent aromatic odour, pungent and tongue-numbing taste.

A. sieboldii: Rhizomes 5–20 cm long, 1–2 mm in diameter, internodes 0.2–1 cm long; lamina only weakly tapered and thin; odour and taste relatively weak.

A. sieboldii var. *seoulense*: Rhizomes 1–5 mm in diameter, internodes 0.1–1 cm long; petioles hairy, lamina thicker than *A. sieboldii*; taste and odour like *A. sieboldii.*

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Medicinal use: In Traditional Medicine in China (and Japan) as analgesic, antitussive, sedative, diaphoretic and antiasthmatic drug to treat common cold, headache, toothache, sinusitis with nasal obstruction, rheumatic arthralgia, cough and dyspnoea due to retention of phlegm and fluid. Incompatible with Radix et Rhizoma Veratri[1,4,5].

Effects and indications according to Traditional Chinese Medicine[2,5,6,7]

Taste:	pungent, acrid
Temperature:	warm
Channels entered:	heart, lungs, liver, kidneys
Effects:	diaphoretic, expectorant, sedative, analgesic
Symptoms and indications:	all types of colds, fevers, chills, headaches, acute toothaches, sinusitis, cough and dyspnoea due to retention of phlegm, pharyngitis, chronic gastritis, rheumatoid arthritis

Main constituents (see Fig. 1)[3,6,7]**:**

- **Alkamides:** dodeca-2*E*,4*E*,8*Z*,10*E/Z*-tetraenoic acid-isobutylamide, deca-2E,4E-dienoic acid-isobutylamide (= pellitorine)[8,9]
- **Tetrahydrofurofurano lignans:** (-)-asarinin, (-)-sesamin[9,10]
- **Benzylisoquinolinalkaloid:** higenamine[3,11,12,13]
- **Essential oil** (2.5 – 5.5 %): monoterpenoids (asarinol A,B,C,D; α-,β-pinene; 1,8-cineol; camphene; eucarvone)[14,15]; phenylpropanoids (methyleugenol, croweacin, elemicin, safrole, asaricine, estragole, kakuol)[16,17,18,19,20,21]
- Feruloyl and coumaroyl glycosides[22]

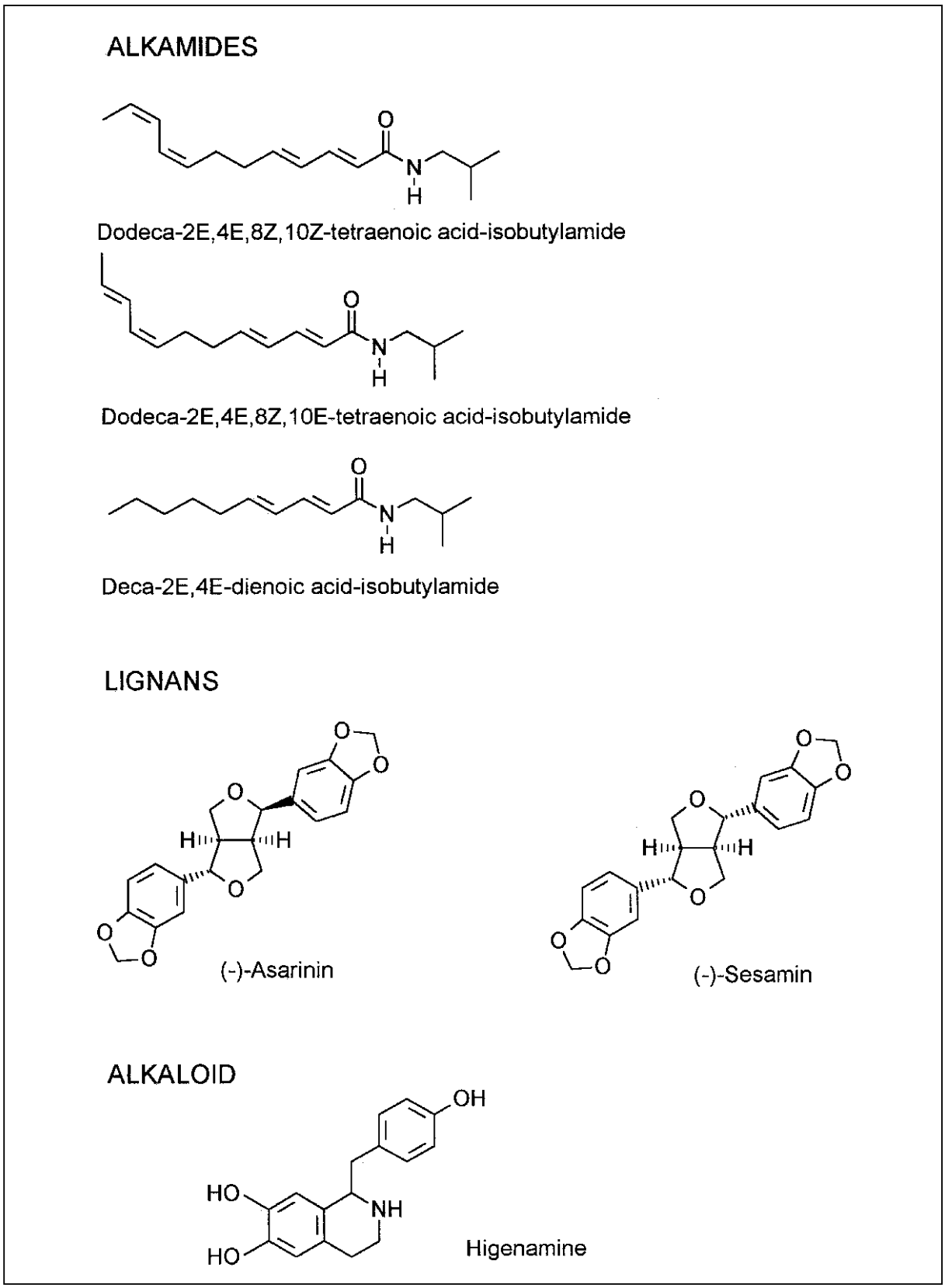


Fig. 1a: Formulae of the main constituents

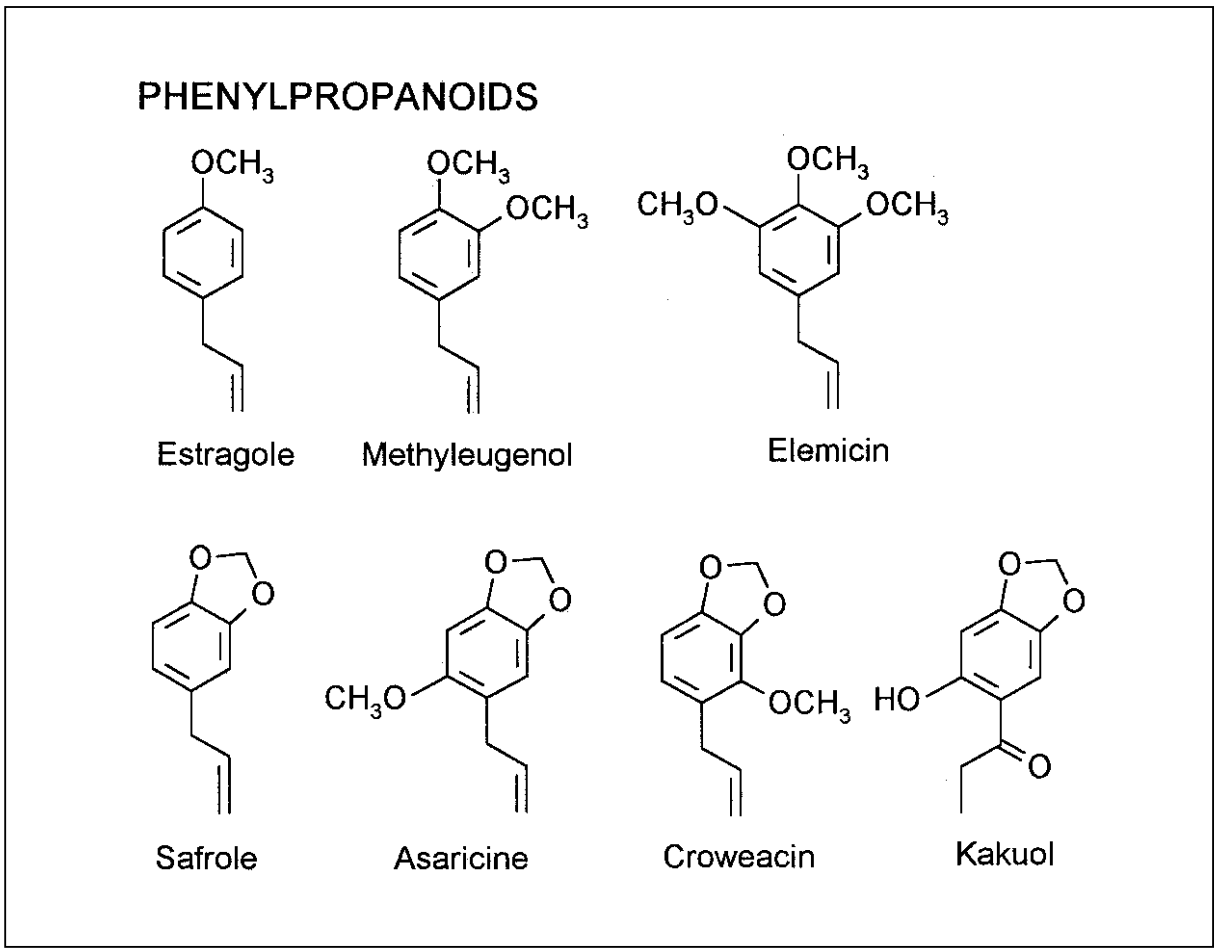


Fig. 1b: Formulae of the main constituents

Pharmacology:

In vitro effects:

- immunosuppressive (antimitogenic activity)[23]
- inhibition of cAMP phosphodiesterase[24]
- antiphlogistic[25,26]
- antiallergic[25, 27, 28, 29, 30]
- antitussive[31]
- antihistaminic[31]
- antibacterial[6,32]
- inhibition of Δ5-desaturase in polyunsaturated fatty acid biosynthesis[10]
- antimycotic[33,34]

In vivo effects:

- cardiac output stimulating effects[35, 36, 37]
- insecticide[9,38,39]
- sedative[32]
- analgesic[32,40]
- antipyretic[6,32,40]
- local anesthetic[6,32]
- anti-inflammatory[32]
- β-adrenergic effects (including cardiotonic, vasodilatatory, smooth muscle relaxant, lipid metabolism enhancing, hyperglycemic effects)[32]
- antiallergic[27,32]
- effects on the respiratory system (antagonization of respiratory depression caused by morphine, relaxation of pig bronchies)[32]
- affects turnover of receptor molecules[41]
- anticonvulsant[40]

TLC-fingerprint analysis:

1) Extraction:

   5 g powdered drug are Soxhlet-extracted for 2 hrs with 50 ml *n*-hexane. The extract is evaporated to dryness and redissolved to a concentration of 10 mg/ml *n*-hexane (or ethanol for the use in HPLC).

2) Standards:

   (-)-Sesamin, (-)-asarinin, methyleugenol, dodeca-2E,4E,8Z,10E/Z-tetraenoic acid-isobutylamide, safrole (1 mg dissolved in 1 ml *n*-hexane for TLC or 1 ml ethanol for HPLC)

3) Separation parameters:

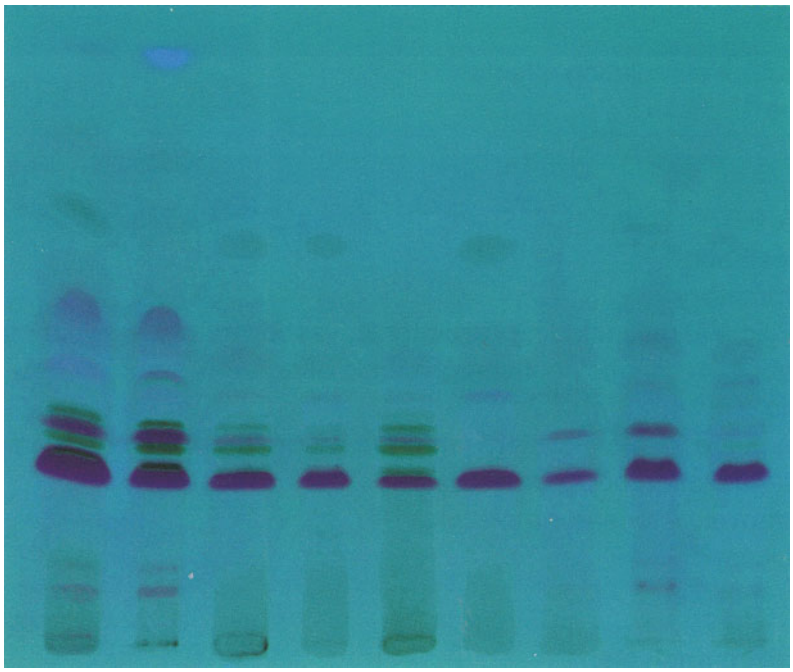
Applied amount:	20 µl extract, 5 µl standard solution
Plates:	Silica gel 60 F_{254} (Merck)
Solvent system:	toluene-ethyl acetate-glacial acetic acid (95+5+5) (lignans, terpenes, phenylpropanes, alkamides)

4) Detection:

Direct evaluation:	UV 254 nm and UV 365 nm
Spray reagent [42]:	Vanillin/sulphuric acid reagent (solution I: 1 % ethanolic solution of vanillin; solution II: 5 % ethanolic sulphuric acid) The TLC plate is intensively sprayed with 10 ml of solution I and then with 5-10 ml of solution II; then heated for 5-10 min. at 110 °C. The evaluation is carried out in VIS.

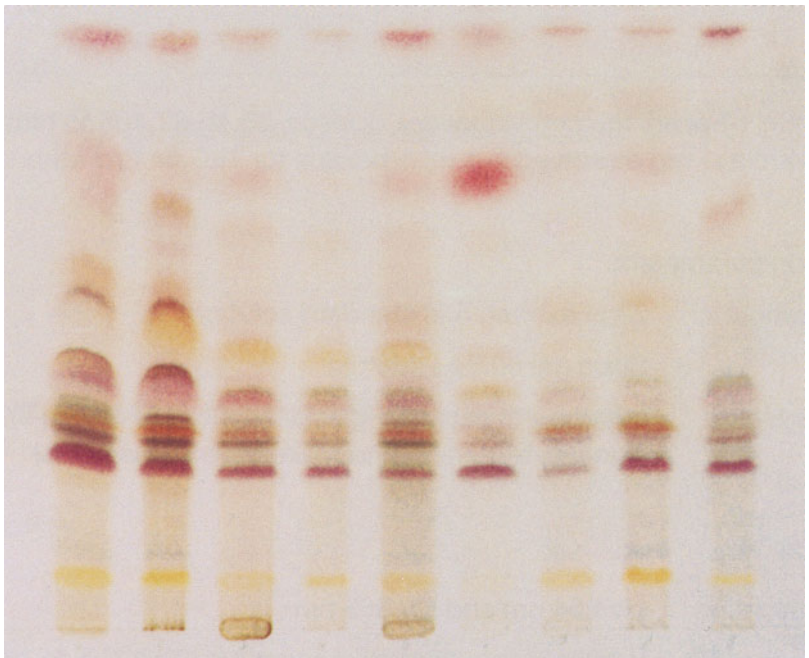
Fig. 2: Thin layer chromatogram of the *n*-hexane extracts of drug batches 1–9.
a) UV 254 nm
b) detection with VS-reagent, VIS

Fig. 2a) UV 254 nm



1 2 3 4 5 6 7 8 9

2b) after detection with vanillin/ sulphuric acid reagent, VIS



1 2 3 4 5 6 7 8 9

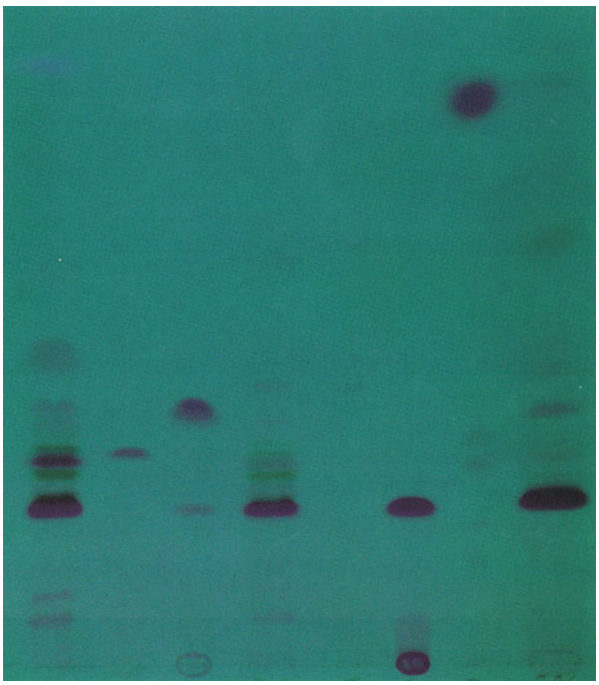
Drug samples:

1 Herba Asari
(TCM-Klinik Kötzting)
2 *Asarum sieboldii*
(Seoul, Korea)
3 *Asarum sieboldii*
(TCM-Klinik, Kötzting)
4 *Asarum heterotropoides* var.
mandshuricum (China)
5 Radix Asari
(Markt-Apotheke Teisendorf)
6 *Asarum heterotropoides* var.
mandshuricum (China), cultivated
7 Asari sieboldii radix (Japan)
8 Asari heterotropoidis radix
(Japan)
9 Herba Asari (Phytopet, Andorra)

Test substances:

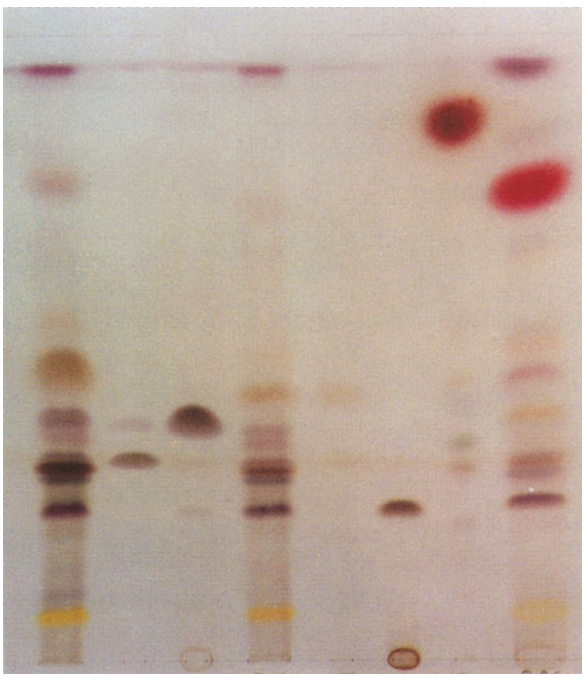
T1 (-)-sesamin, R*f* = 0.33
T2 (-)-asarinin, R*f* = 0.41
T3 methyleugenol, Rf = 0.44
T4 dodeca-2E,4E,8Z,10E/Z-
tetraenoic acid-isobutylamide, R*f* = 0.26
T5 safrole, R*f* = 0.90

Fig. 3 a) UV 254 nm



2 T1 T2 3 T3 T4 T5 6

Fig.3 b) Detection with VS-reagent, VIS



2 T1 T2 3 T3 T4 T5 6

Fig. 3: Thin layer chromatogram of 3 selected drug samples and 5 reference compounds
a) under UV 254 nm
b) detection with VS-reagent, VIS

5) Description of the chromatograms:

Fig. 2a: UV 254 nm:

The different drug batches appear nearly similar. The main components differ in their concentrations. Alkamides, lignans and phenylpropanoids show intensively quenching spots.

Fig. 2b: VS-reagent, VIS:

Alkamides turn to brown-black, lignans to violet-brown colour.

Fig. 3a: UV 254 nm:

Alkamides, lignans and phenylpropanoids show quenching spots.

Fig. 3b: VS-reagent, VIS:

- The dominating alkamide dodeca-2E,4E,8Z,10E/Z-tetraenoic acid-isobutylamide appears at R*f* 0.26 (**T4**). It turns to a dark brown-black colour with VS-reagent.
- The major lignans (-)-asarinin (R*f* 0.41 (**T2**)) and (-)-sesamin (R*f* 0.33 (**T1**)) appear with violet-brown colour.
- All batches show a high concentration of essential oil with constituents in the upper R*f*-range: methyleugenol (R*f* 0.44 (**T3**)) gives a light red-brown colour, whereas safrole (R*f* 0.90 (**T5**)) turns slowly to intensive yellow-brown (after some hours, safrole could be detected also in batch 6). The content of methyleugenol can be up to 50 %[(3)] high in every batch, according to literature.
- Other essential oil components appear with a varying number of pink-violet zones (R*f* 0.5–1.0).

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract used for TLC over millipore filtration unit, type HV 0,45 µm.

2) Injection volume: 5 µl ethanolic solution (10 mg/ml)

3) HPLC parameter:

Apparatus:	Liquid chromatograph HP 1050 Photodiode array detector HP 1040 M
Column:	LiChroCART 125-4 with LiChrospher 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART 4-4 with LiChrospher 100 RP 18, Merck
Solvent system:	A: water B: acetonitrile

Gradient:	37 % MeCN for 10 min. (isocratic); 37-55 % MeCN in 10 min. (linear)
Flow rate:	2,0 ml/min.
Detection:	210 nm

Retention times of the main peaks:

Peak	**Rt (min.)**	**Compounds**
1	5,1	essential oil component
2	6,3	essential oil component
2*	7,1	not identified
2**	7,4	not identified
3	8,3	essential oil component
4	8,7	essential oil component
5	9,1	methyleugenol
6	12,3	(-)-sesamin
7	13,3	safrole
8	13,7	not identified
9*	14,9	not identified
9	15,2	(-)-asarinin
10/11	17,6-17,8	dodeca-2E,4E,8Z,10E/Z-tetraenoic acid-isobutylamides

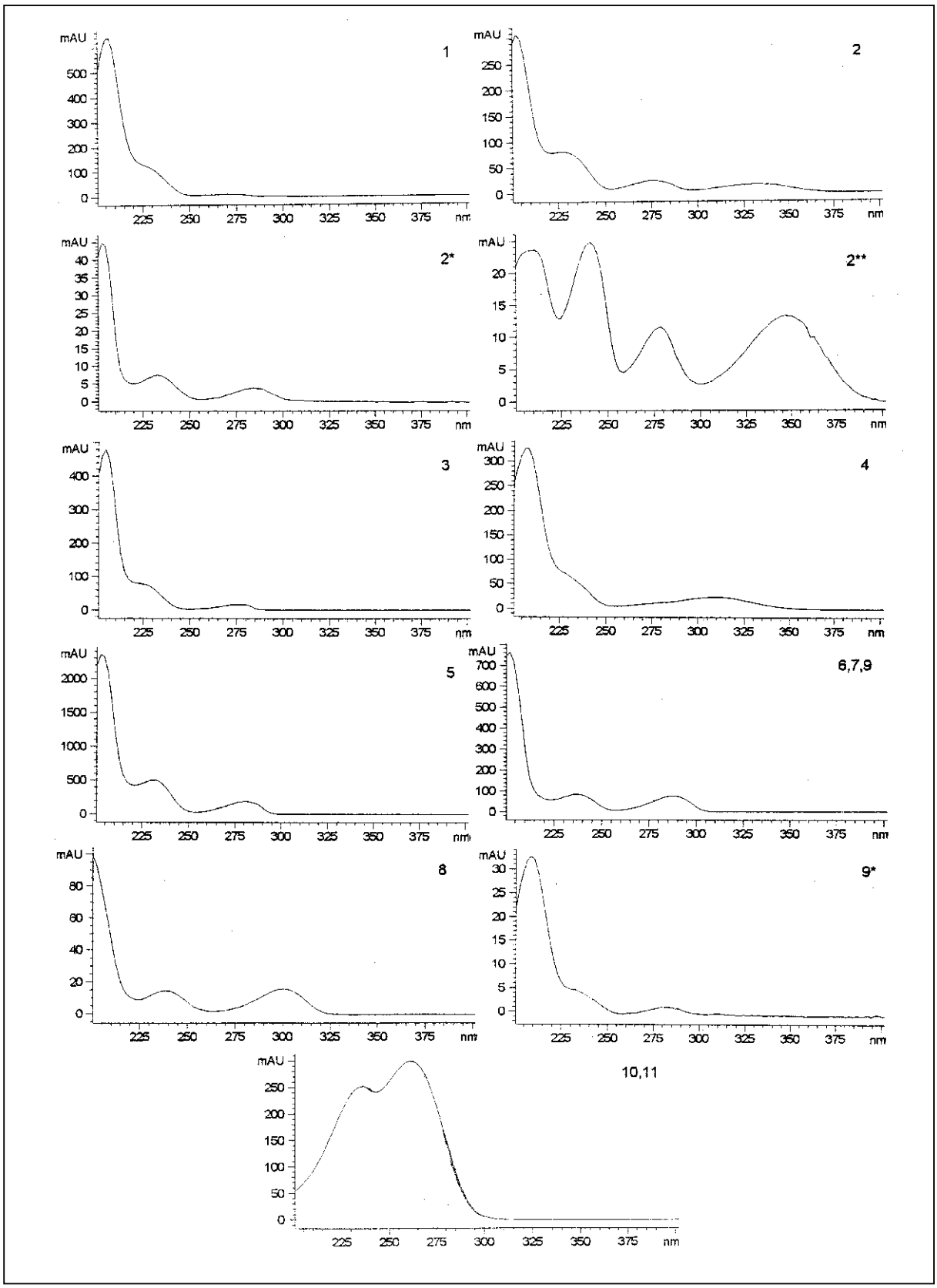


Fig. 4: UV-spectra of the main constituents

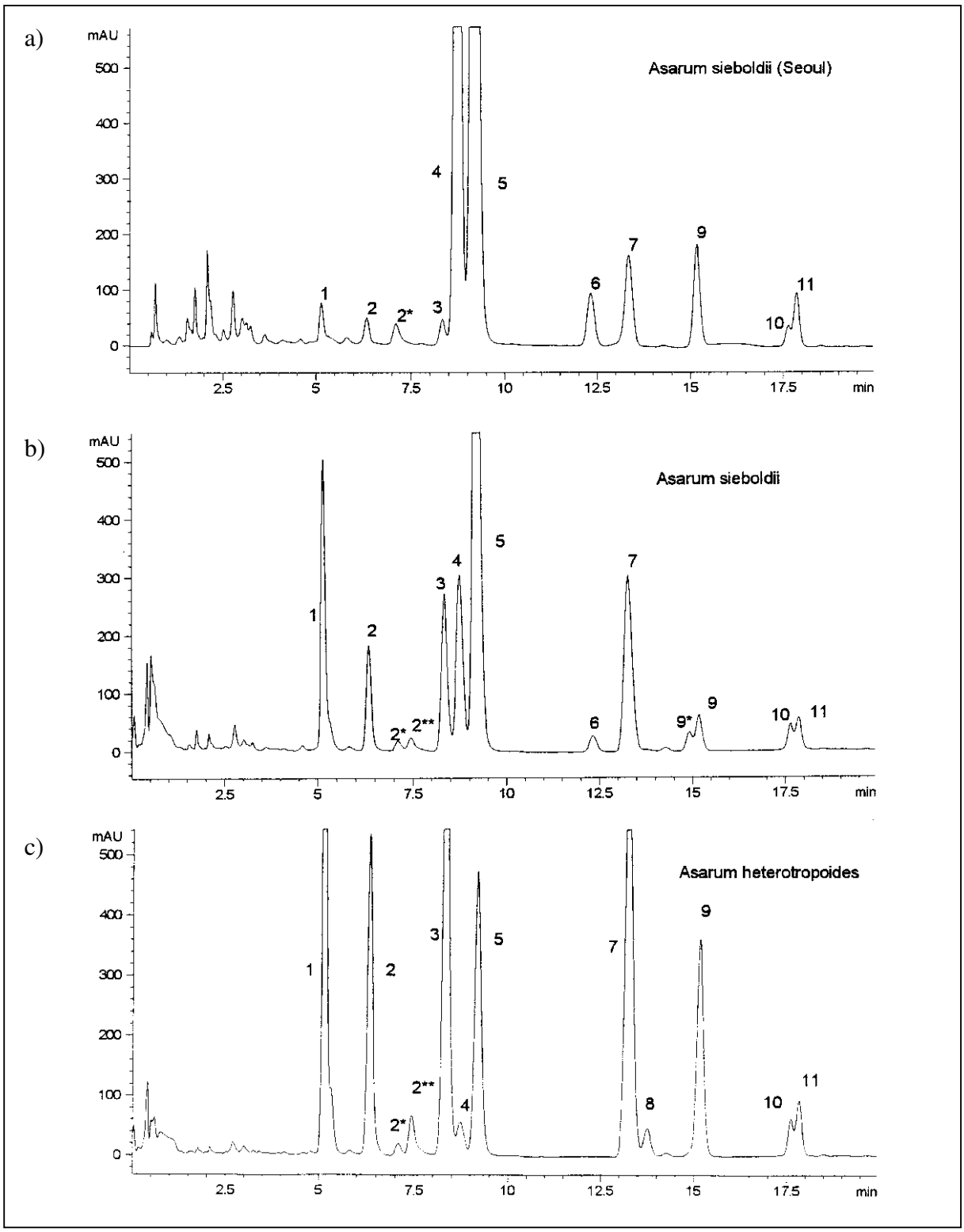


Fig. 5: HPLC fingerprint analysis of the *n*-hexane extracts of 3 selected drug samples
a) *Asarum sieboldii* (Seoul) (= TLC sample 2)
b) *Asarum sieboldii* (= TLC sample 3)
c) *Asarum heterotropoides* var. *mandshuricum*. (= TLC sample 6)

4) Description and Discussion of Fig 5 a, b,c

The appearence of the essential oil components **1, 2, 3, 4, 5** and **7** is characteristic for the HPLC fingerprint of the *n*-hexane extracts of Radix et Rhizoma Asari (Xixin). In most of the tested batches (drug samples 1–9, see TLC) methyleugenol (peak **5**, Rt 9.1 min.) was dominating. According to the literature [3], safrole should be the dominating compound in *Asarum heterotropoides*, as demonstrated by the selected drug sample 6. However, it was lacking in other *Asarum heterotropoides* batches. This is indicative for a great variation in quality.

The lignan (-)-asarinin (peak **9**, Rt 15,2) seems to be typical for Xixin, while (-)-sesamin sometimes occurs only in very low concentrations. Every batch contained dodeca-2E, 4E,8Z,10E/Z-tetraenoic acid-isobutylamides, a mixture of isomers, which can not be separated. Their concentration was very similar in all batches.

Asarum sieboldii can be distinguished from *Asarum heterotropoides* by the lacking of the benzylisoquinoline alkaloid higenamine in *Asarum sieboldii*. For its analysis another polar solvent system has to be be used as described in[11] and[12].

Since *Asarum* spec. belong to the *Aristolochia* family the presence of the cancerogenic aristolochic acid in the root is likely as reported in the reference[43]. For its TLC- and HPLC-determination see also Monograph Radix Clematidis and Caulis Sinomenii.

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Herba Houttuyniae cordatae – *Yuxingcao*

Pharmacopoeias: Chin. Ph. IX
Pharmacopoeia of the People´s Republic of China, English Edition 1992/2005[1]

Official drug: Aerial parts of *Houttuynia cordata* THUNB., Saururaceae, collected in summer and dried in the sun

Origin: China, Japan, Korea, Vietnam

Description of the drug:[1,3] Stems flattend cylindrical, twisted, 20-35 cm long, 2-3 mm in diameter; externally brownish-yellow, with several longitudinal ridges and distinct nodes, the nodes of the lower parts bearing remains of fibrous roots; leaves alternate, lamina rolled and crumpled, when whole, cordate, 3-5 cm long, 3-4.5 cm wide; apex acuminate, margins entire; the upper surface dark yellowish-green to dark brown; petioles slender, accreted with a stipule at the base, forming a sheath; spike terminal yellowish-brown; odour fishy on rubbing; taste slightly adstringent.

Medicinal use: In Traditional Chinese Medicine against inflammation of the respiratory tract, for the treatment of virulent carbuncles and sores, lung abscess, cough with thick yellow-green sputum, edema, acute dysentery, acute urinary infections[1,2].

Effects and indications according to Traditional Chinese Medicine[2,4,5]

Taste:	pungent and slightly cold
Temperature:	acrid, cool
Channels entered:	large intestine, lung
Effects:	removes toxic heat; promotes the drainage of pus; relieves dysuria
Symptoms and indications:	reduces swellings and abscesses: for Lung abscesses or Lung heat cough with expectoration of thick, yellow-green sputum; for toxic sores, internally and topically; drains damp-heat and promotes urination: for Large Intestine damp-heat diarrhea or damp-heat in Lower Burner with painful urinary dysfunction

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents (see Fig. 1):

– **essential oil** (3-oxo-dodecanal, methyl-n-nonylketone, myrcene, lauric aldehyde, α-pinene, camphene, limonene, linalool, bornyl acetate, caryophyllene)[6,7]

– **aporphine alkaloids** (cepharanone B (**1**), aristolactam AII (**2**), aristolactam BII, piperolactam A (**3**), norcepharadione B (**4**), cepharadione B (**5**), 7-chloro-6-demethyl-cepharadione B)[8,9,10]

– **pyridine derivatives** (2-nonyl-5-decanoyl-pyridine, 3,5-didecanoyl-pyridine (**6**), 3,5-didecanoyl-4-nonyl-1,4-dihydropyridine (**7**))[8,9,10,11]

– **flavonoids** (quercetin, afzelin, quercitrin, isoquercitrin, hyperoside, rutoside, quercetin-rhamnoside) [11,12,13]

– **phenols** (chlorogenic acid, protocatechu acid, vanillic acid, p-hydroxy-benzoic acid methyl ester) [9,12,14]

– fatty acids (stearic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid)[7, 12, 15]

– sterols (β-sitosterol, stigmasterol, spinasterol, stigmast-4-en-3,6-dione)[9,12]

– triacylbenzene (**8**), vomifoliol[9,11]

– lignans (sesamin)[10,11]

Pharmacology:

In vitro effects:

– antibacterial activity (grampositive and gramnegative bacteria) of essential oil, flavonoids, aporphine alkaloids and phenols[9,16]

– inhibition of platelet aggregation (N(4-hydroxystyryl)benzamide)[17]

– inhibition of cyclooxygenase and lipoxygenase (fatty acids, sterols and quercetin)[9,15]

– stimulation of phagocytosis[9]

– antiviral activity[18,19,20]

In vivo effects:

– diuretic effect[21]

– anti-inflammatory effect (rat paw edema)[21]

– inhibition of histamine release (rat)[22]

– moderate cytotoxic activity[23, 24]

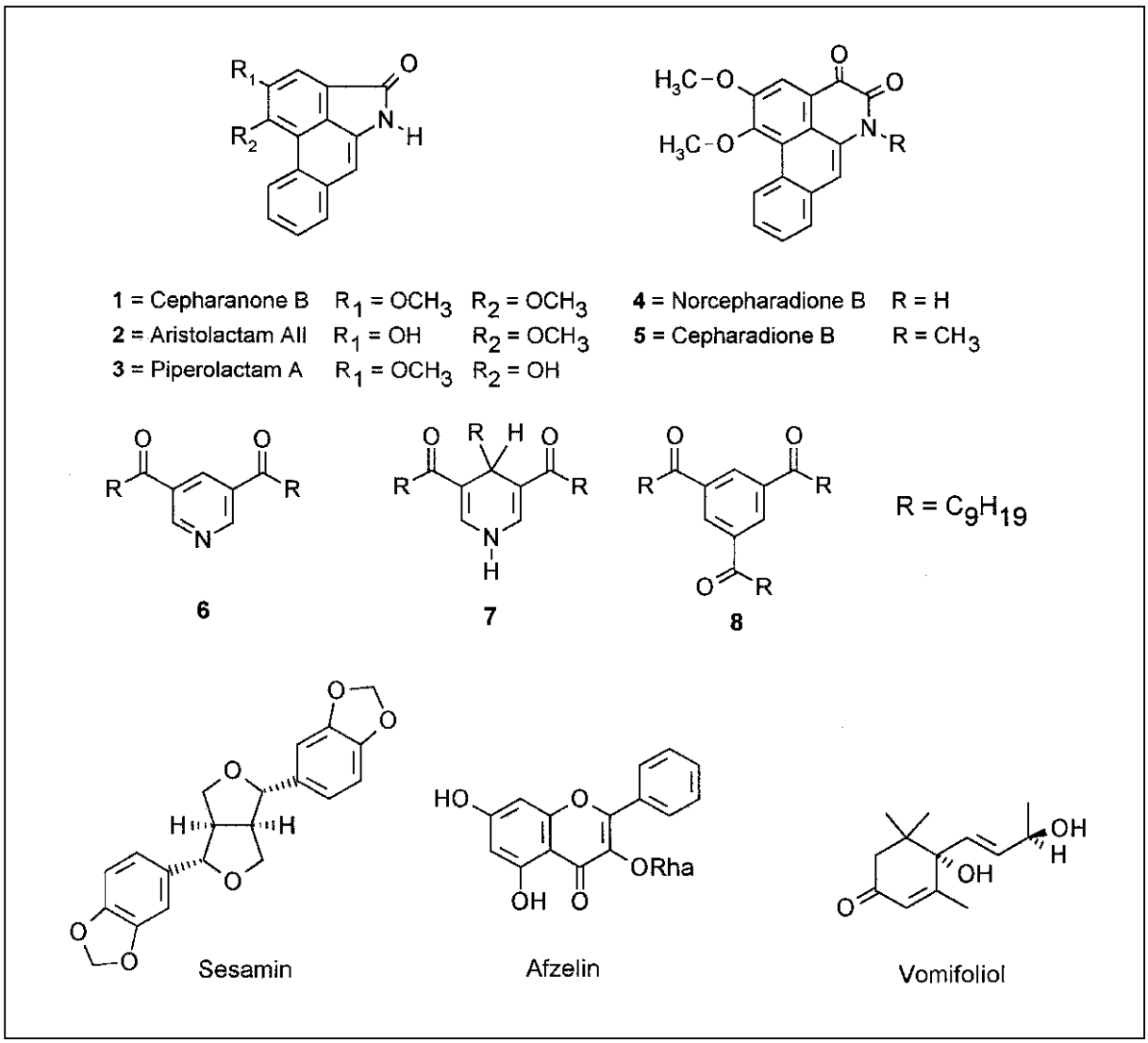

Fig. 1: Formulae of the main constituents

TLC analysis:

1) Preparation of extracts:

   5 g powdered drug are Soxhlet extracted with 100 ml methanol for 1 h. The extract is filtered and evaporated to 10 ml.

2) Reference compounds:

   β-sitosterol, phytol, linoleic acid (Sigma), 1,3,5-tridecanoylbenzene, hyperoside, rutoside, chlorogenic acid (Roth) (1 mg each dissolved in 1 ml *n*-hexane)

3) Separation parameters:

Applied amount:	30 µl extract, 10 µl standard
Plates:	Silica gel 60 F_{254} (Merck ck)
Solvent system:	*n*-hexane - ethyl acetate (5+4) (LM I: pyridine derivatives, phytol, fatty acids, sterols, aporphin alkaloids)
	ethyl acetate – formic acid – glacial acetic acid – water (100+11+11+26) (LM II: phenolics, flavonoids)

4) Detection:

Direct evaluation:	UV 254 nm and UV 365 nm
Spray reagents[25]:	Vanillin/sulphuric acid reagent (for lipophilic constituents) Solution 1: 1 % ethanolic solution of vanillin Solution 2: 5 % ethanolic sulphuric acid
	The plate is intensively sprayed with 10 ml of solution 1 and then with 5-10 ml of solution 2; then heated for 5–10 min. at 110 °C.
	The evaluation is carried out in VIS.
	Natural products - polyethyleneglycol (NP/PEG) reagent (for polar constituents)
	(Solution 1: 1 % methanolic solution of diphenylboric acid-β-ethylamine ester; solution 2: 5 % ethanolic solution of polyethyleneglycol-4000)
	The TLC plate is vigorously sprayed with 10 ml of solution 1 and thereafter with 8–10 ml of solution 2.
	The evaluation is carried out under UV 365 nm.

Drug samples:

A, C, D, E:	from China
B:	from Korea
F:	from Vietnam
G:	from Germany (Botanical Garden, Düsseldorf)

Test substances:

T1	phytol, R*f* = 0.76
T2	β-sitosterol, R*f* = 0.73
T3	linoleic acid, R*f* = 0.76
T4	1,3,5-tridecanoylbenzene, R*f* = 0.99
T5	hyperoside, rutin, chlorogenic acid, R*f* = 0.22, 0.05, 0.15/0.52

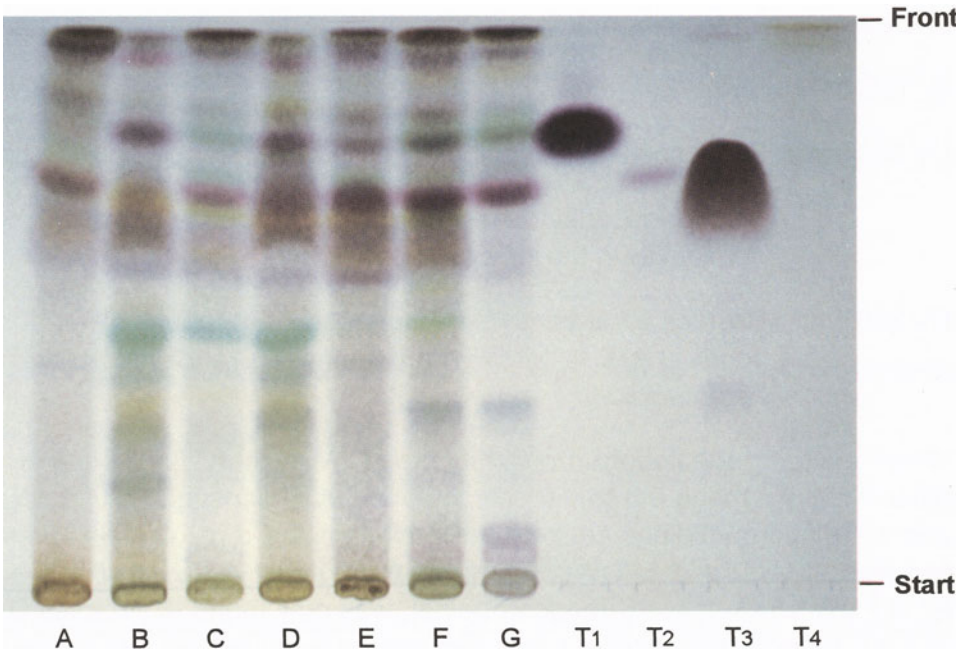

Fig. 2:

TLC separation of *H. cordata* methanolic extracts in LM I after spraying with vanillin-sulphuric acid reagent (VIS)

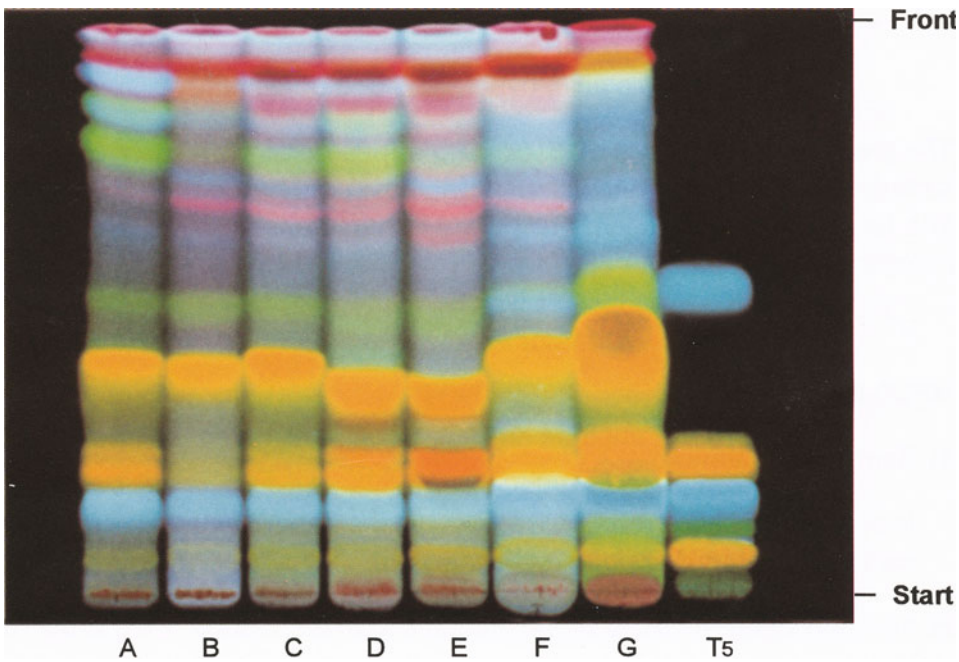

Fig. 3:

Thin layer chromatogram of *H. cordata* methanolic extracts in LM II after spraying with NP/PEG (UV 365 nm)

5) Description of the chromatograms:

a) TLC of *H. cordata* extracts in LM I (Fig. 2)

UV 254 nm: Fluorescence quenching zones in the R*f*-range 0.6 - 0.9 and 0 - 0.15.

1,3,5-Tridecanoylbenzene (**T4**) can be found just below the front, and pyridine derivatives in the R*f*-range 0.7 - 0.9.

UV 365 nm: Besides other faintly blue fluorescent compounds, two strong blue fluorescent zones at R*f* 0.4, 1,4-dihydropyridines and aristolactam BII at R*f* 0.1 can be detected.

When analyzing the lipophilic constituents in LM 1, the various samples of *H. cordata* (A-G) differ in the R*f* range 0.3 to 0.6 only in quantitative aspects. Fatty acids, phytol, β-sitosterol and 1,4-dihydropyridines are present in all samples. Aristolactams were only detectable in samples A, C, D and E (UV 365 nm: blue). Stigmast-4-en-3,6-dione, a fluorescent zone at R*f* 0.7 (UV 254 nm +, VS-reagent: beige), was only found in samples D, E and G. The zone of 1,3,5-tridecanoylbenzene was detectable in every extract just below the front (UV 254 +, VS-reagent: violet). After detection with vanillin-sulphuric acid reagent, violet spots of linoleic acid (R*f* 0.76, **T3**), β-sitosterol (R*f* 0.73, **T2**) and phytol (R*f* 0.76, **T1**) could be detected in all samples.

b) TLC of *H. cordata* extracts in LM II (Fig. 3)

UV 254 nm: various zones from R*f* 0.1 to 0.95

UV 365 nm: Faintly blue fluorescent zones and intensive blue and orange fluorescent zones at R*f* 0.9 were identified as aristolactams and dioxoaporhines. After spraying with NP/PEG, these spots loose their fluorescence whereas the flavonoids and phenols appear as intensive orange or blue fluorescent zones (rutin, chlorogenic acid, hyperoside and quercitrin R*f* = 0.30).

The phenolic constituents of *H. cordata* appeared as fairly identical patterns in all samples examined. Aristolactams and dioxoaporphines could be identified in samples A, C, D and E only. The flavonoid pattern seems to be very characteristic for *H. cordata* and can be used for identification.

HPLC fingerprint analysis:

1) Sample preparation:

In order to remove chlorophyll, 1 ml of the methanolic extract is passed over a Sep-Pak RP18 cartridge (Waters-Millipore) and eluted with methanol. The eluate is evaporated to 1.0 ml.

2) Injection volume: 10 µl of the methanolic extract (conc. = 5 g drug/10 ml)

3) HPLC parameter:

Apparatus: Liquid Chromatograph HP 1090 with photodiode array detector HP 1040 A (Hewlett-Packard)

Column: LiChroCART 125-4 with LiChrospher 100 RP18, 5 µm (Merck)

Precolumn: LiChroCART 4-4 RP18 (Merck)

Solvent: A: water + 1 % 0,1 N H_3PO_4
B: acetonitrile + 1 % 0,1 N H_3PO_4

Gradient: 5–25 % B in 20 min., 25–95 % B in 30 min., 95–100 % B in 10 min., linearly.

Flow: 1,0 ml/min.

Detection: 210 nm and 235 nm

4) Description of the chromatograms:

Retention times of the main peaks (Fig. 4a and b)

Peak	**Rt (min.)**	**Rt_{rel}**	**Compound**
1	5,0	0,27	protocatechuic acid
2	5,7	0,31	caffeic acid derivative
3	8,6	0,46	chlorogenic acid
4	8,9	0,48	caffeic acid derivative
5	11,2	0,60	rutin
6	16,2	0,87	hyperoside
7	16,6	0,89	isoquercitrin
8	18,6	1,00	quercitrin
9	21,0	1,13	afzelin
10	27,6	1,48	aristolactam AII
11	28,8	1,55	piperolactam
12	29,0	1,56	phenolic compound
13	29,7	1,60	norcepharadione B
14	32,2	1,73	aristolactam BII
15	32,6	1,75	cepharadione B

Since quercitrin is the characteristic major flavonoid of all *H. cordata* samples, it can be used as a reference compound.

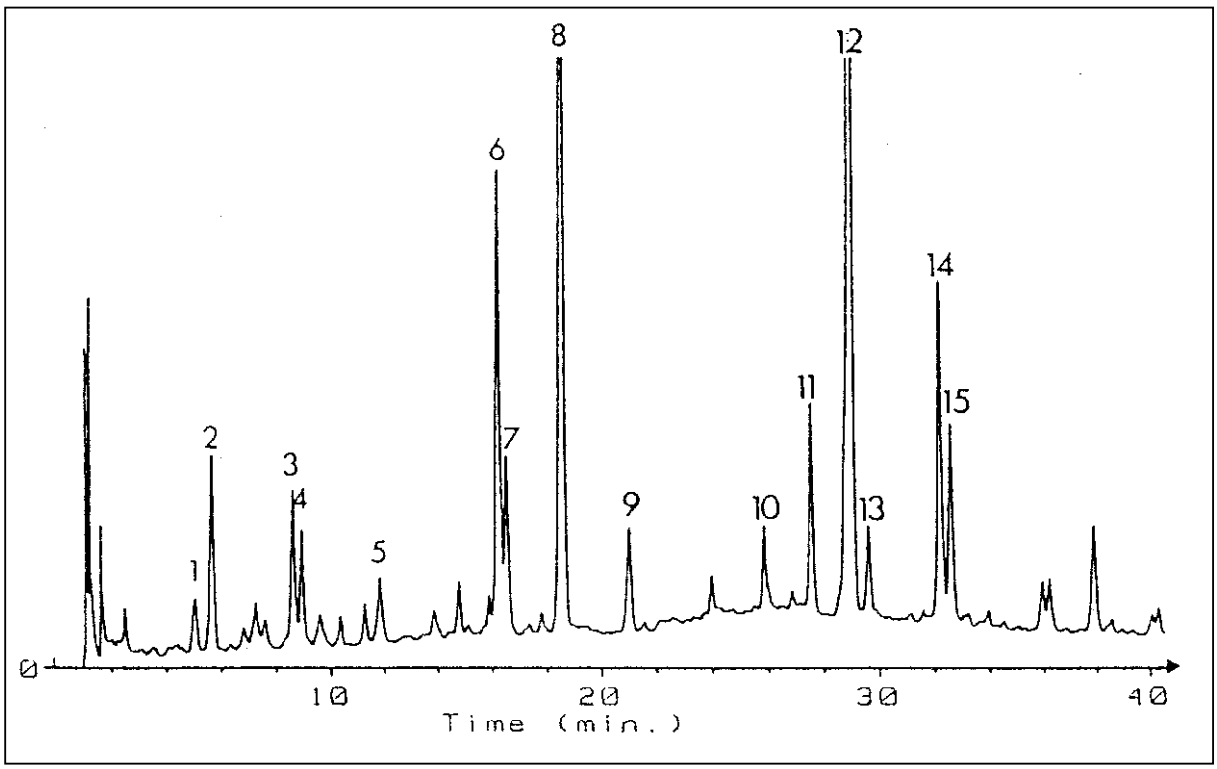

Fig. 4a: HPLC fingerprint chromatogram of *Houttuynia cordata* methanolic extract (sample A)

H. cordata extracts are characterized by the peaks of quercitrin, hyperoside and isoquercitrin (Fig. 4a). The phenolic compounds (peaks **1**,**2**,**3**,**4**), aristolactams (peaks **10**,**11**,**14**) and dioxoaporphines (peaks **13** and **15**) could be identified only in the zoomed chromatogram. The only differences between the various extracts refer to the concentration of aristolactams and dioxoaporphines. Extract G was devoid of any alkaloids. In extracts B, E, and F, only aristolactam BII could be detected. The differences might be caused by different origin, different time of harvesting or storing.

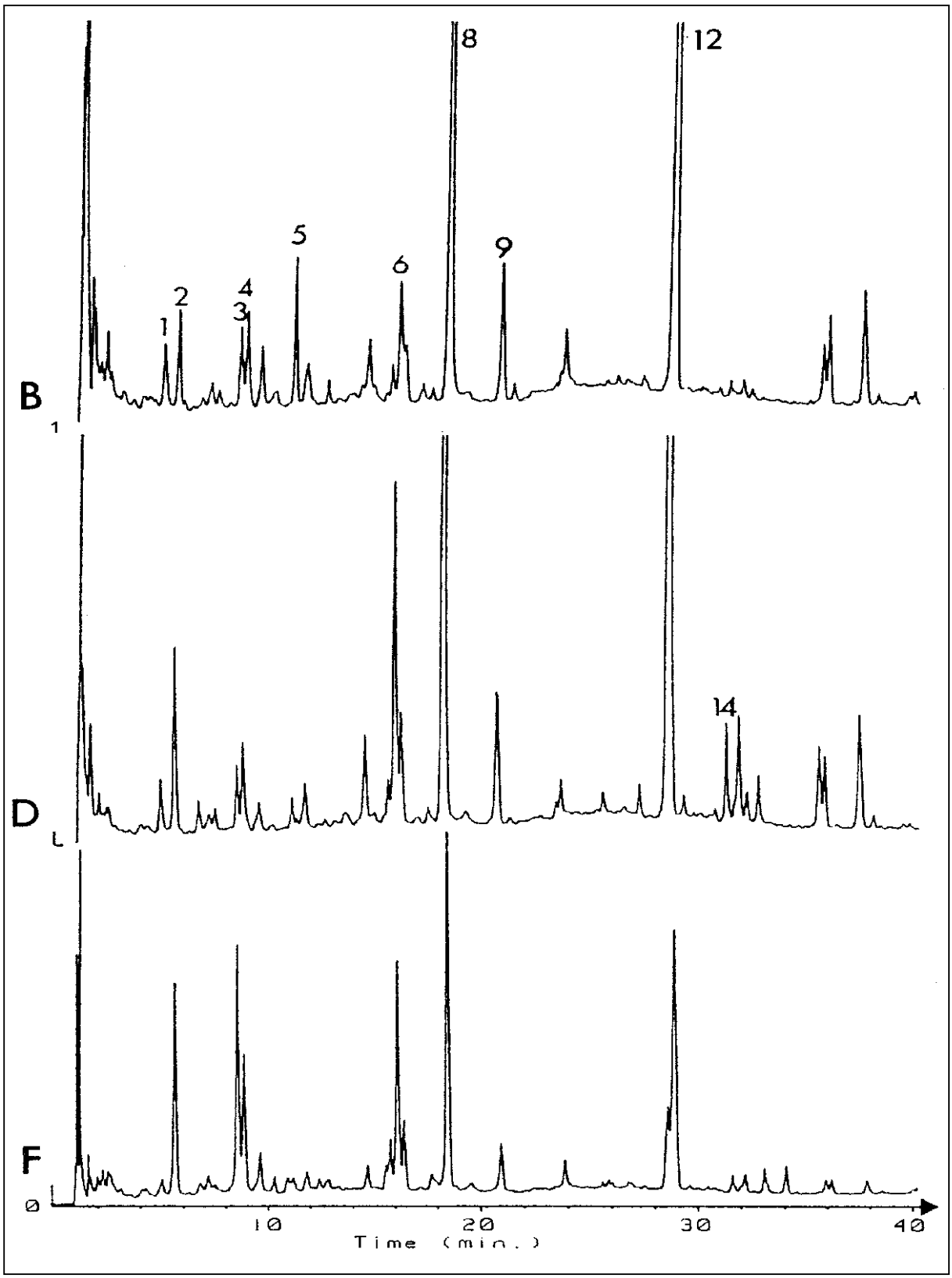

Fig. 4b: HPLC fingerprints of *Houttuynia cordata* extracts (samples B, D and F)

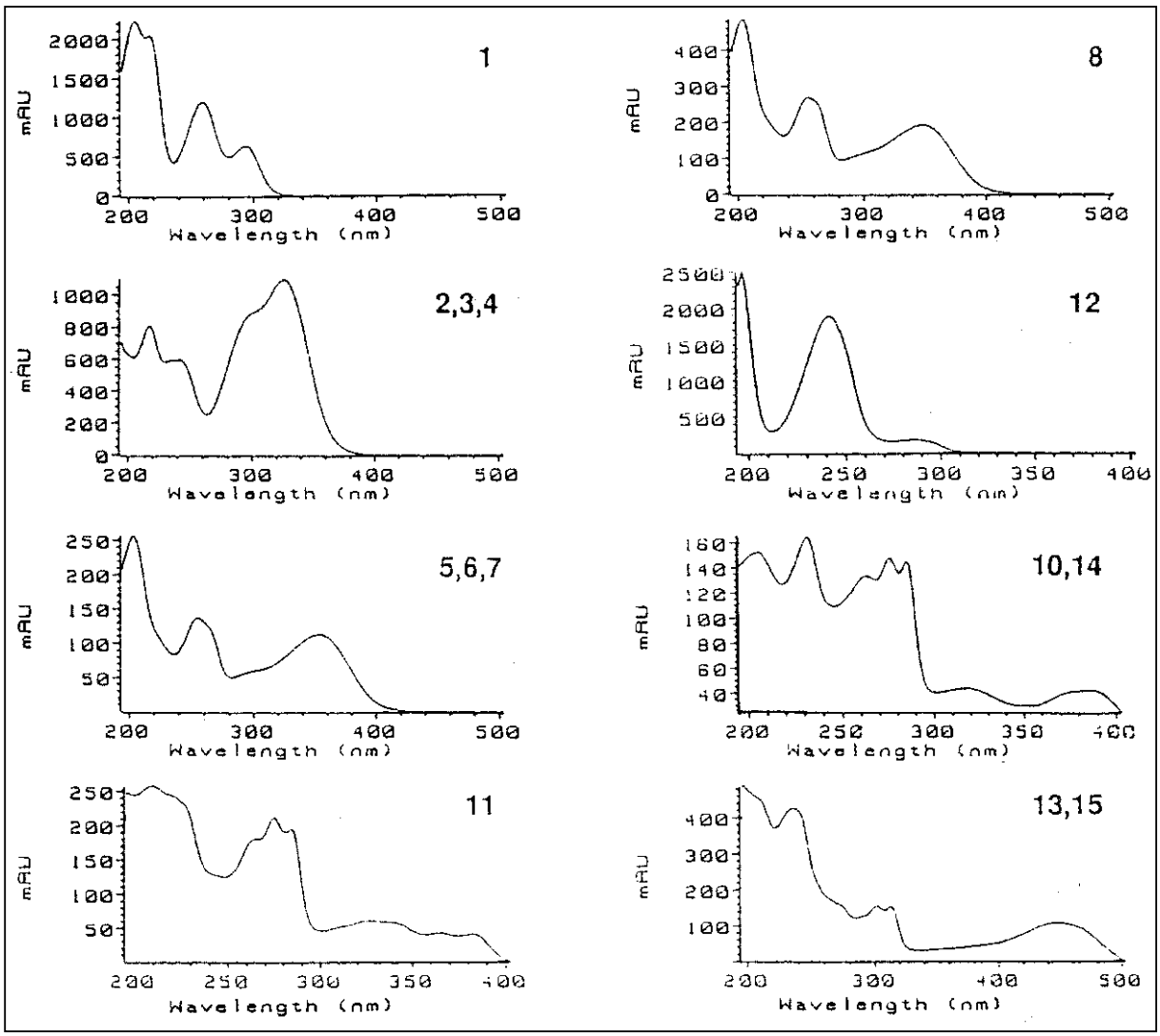

Fig. 5: Online recorded UV-spectra of the main peaks in the HPLC separation of *Houttuynia* extracts

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Meng J, Leung KS, Jiang Z, Dong X, Zhao Z, Xu LJ, Establishment of HPLC-DAD-MS fingerprint of fresh *Houttuynia cordata*, Chem Pharm Bull (Tokyo) 53(12):1604-1609 (2005)

Rhizoma Pinelliae – *Banxia*

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 1992/2005[1]

Official drug: Pinellia Tuber is the dried tuber of *Pinellia ternata* (Thunb.) Breit., (Fam. Araceae). The drug is collected in summer and autumn, washed clean, removed from the outer bark and fibrous root and dried in the sun[1,2]

Description of the drug: Subspheroidal rhizomes, some slightly oblique, 1–1.5 cm in diameter. Externally white or yellowish, apex marked with a dented stem scar, surrounded by close and dotted root scars, base obtuse and rounded, relatively smooth; texture hard, fracture white, starchy; taste pungent; whit numbing and irritating sensation, odourless[1,2]

Substitute drugs: *Pinellia pedatisecta* Schott (only aerial parts are used)[3,4]

Falsification: Since Rhizoma Arisaematis from *Arisaema amurense* and other *Arisaema* spp. has a separate monograph the presence of this drug has to be recognized as falsification.

Pretreatment of the raw drug: Pretreatment with alumen solution (Qingbanxia) or Radix Glycyrrhizae and lime water (Fabanxia)[1,2] (protein precipitating effect!) is reported to reduce or eliminate the "toxicity". Cooking and drying[5] destroys the skin- and mucous membrane irritating phenols. Processing with ginger (Jiangbanxia)[2] also reduces toxicity.

Medicinal use: In Traditional Chinese Medicine internally as antiasthmatic, antiemetic, expectorant[1] and cytostatic drug[6]:

Cough and asthma with much *phlegm*; dizziness and palpitation due to retention of *phlegm* and fluid; vertigo caused by *wind-phlegm*; headache with cold extremities due to attack of *phlegm*; stuffiness in the chest and the epigastrium; globus hystericus. *Rhizoma Pinelliae (processed with ginger)* is often used for relieving nausea and vomiting, while *Rhizoma Pinelliae (processed with alumen)* for removing *damp* and *phlegm*. *Rhizoma Pinelliae (unprocessed)* is used externally for boils, sores, lymphadenitis [1] and ulcers[2].

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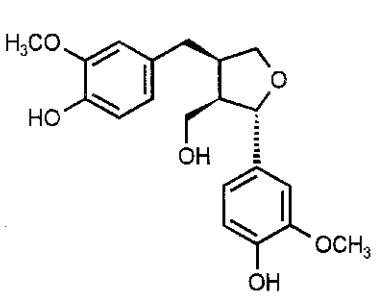
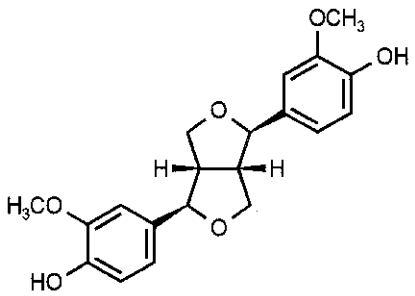
Effects and indications according to Traditional Chinese Medicine[1,2,4,7]

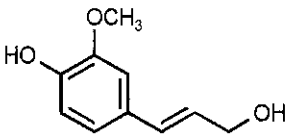
Taste:	acrid
Temperature:	warm
Channels entered:	spleen, stomach
Effects:	removes damp and phlegm, harmonizes the stomach, stops vomiting and relieves nausea, dissipates nodules and reduces distension
Symptoms and indications:	antiasthmatic, antitussive, antiemetic



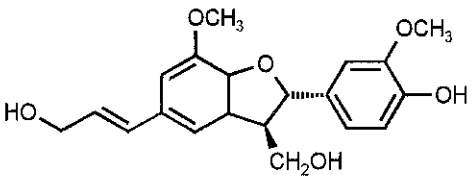
(+)-Lariciresinol



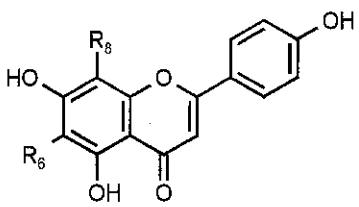
(-)-Pinoresinol



Coniferylalcohol



Dehydrodiconiferylalcohol



Vicenin-1	R_6 = xylose,	R_8 = glucose
Corymboside	R_6 = arabinose,	R_8 = galactose

Fig. 1: Formulae of the main constituents

Main constituents[9] **(see Fig. 1):**

- **lignans** ((+)-lariciresinol, (-)-pinoresinol)[9]
- **phenylpropanoides** (coniferylalcohol, dehydrodiconiferylalcohol)[9]
- **flavone-C-glycosides** (corymboside, vicenin-1)[9]
- **phenols** (homogentisic acid and its 2-monoglucoside[10] protocatechualdehyde and its 3,4-diglucoside[11] (in the fresh harvested, non-pretreated drug only))
- **fatty acids** (linoleic acid)[9]
- **polymers** (polysaccharide[12], proteins (lectin "pinellin"))[13].

Miscellaneous compounds:

- sugars[9], amino acids[14], guanosine[15], nucleosides (adenosine)[9], cholin[16], *l*-ephedrin[17], β-sitosterol[16], β-sitosterol-glucoside[18].

Pharmacology:

In vitro-effects:

- PAF-antagonism: probably attributable to the lignans (e.g. lariciresinol, pinoresinol[9]).
- Inhibition of platelet-aggregation: found for the methanol extract[9].
- cAMP-phosphodiesterase inhibition: found for the decoction[19].
- Cell-agglutinating and mitogenic activity: reported for the protein "pinellin"[20] cited in[8].
- Relaxing and antihistamine-like effects, probably caused by *l*-ephedrin[17].

In vivo effects:

- The decoction of *Pinellia ternata* is used prophylactically against vomiting[8].

Toxicology:

The protein "pinellin", which has abortive properties[15] is destroyed by pretreatment with alum solution, ginger, lime water or long decoction. The phenols (e.g. homogentisic acid, its glucoside[10] and 3,4 dihydroxybenzaldehyde[11]) possess skin and mucous membranes irritating properties and can be destroyed by cooking and drying[5]. The raw drug is used for external treatment only, whereas for internal use the raw drug has to be pretreated. The LD_{50} of unprepared Rhiz. *Pinelliae ternatae* injected *i.p.* into mice is approx. 13 g/kg.

TLC-fingerprint analysis (see Fig. 2 and 3)

1) Preparation of extracts:

5 g coarsely ground drug are soxhlet extracted for 1 hr with 100 ml methanol. The extract is then filtered, concentrated and filled up to 10,0 ml with methanol. 2 ml are used for HPLC-analysis.

7 ml of the same solution are filtered and evaporated at 40 ˚C to dryness. The residue, dissolved in 2,0 ml of 80 % methanol, is used for TLC-analysis.

2) Reference compounds:

β-sitosterol, linoleic acid, coniferyl alcohol (Sigma), pinoresinol, lariciresinol, dehydrodiconiferyl alcohol (Inst. of Pharm. Biology, Univ. of Munich, Germany).

3) Separation parameters:

Applied amount:	30 µl extract
Plates:	Silica gel 60 F_{254}, Merck
Solvent system:	Chloroform – distilled methanol – water (80 + 25 + 4) (β-sitosterol, phenyl propanes, lignans)

4) Detection:

Direct evaluation:	UV 365 nm
Spray reagent [21]:	vanillin-phosphoric acid reagent (1 g vanillin dissolved in minor quantity of ethanol filled up to 100 ml with o-phosphoric acid 50 %). The plate is intensively sprayed with 10 ml solution and then heated for 5 – 10 min. at 110 ˚C. The evaluation is carried out in VIS.

Drug samples:

1 authentic Pinelliae rhizoma sample (Korea)

2 Pinelliae rhizoma commercial drug sample (China)

3 Pinelliae rhizoma sample (Qingbanxia) pretreated with alum

4 Pinelliae rhizoma sample (Jiangbanxia) pretreated with ginger

5 Pinelliae rhizoma sample (Fabanxia) pretreated with licorice and lime

6 Arisaematis rhizoma sample (spez. unknown) (China)

7 Arisaematis amurensis rhizoma sample (Korea)

Testsubstances:

T 1	ß-sitosterol	10 µl	=	20 µg, R*f* = 0.95
T 2	pinoresinol	3 µl	=	6 µg, R*f* = 0.91
T 3	lariciresinol	3 µl	=	6 µg, R*f* = 0.71
T 4	coniferyl alcohol	3 µl	=	3 µg, R*f* = 0.74
T 5	dehydrodiconiferyl alcohol	3 µl	=	6 µg, R*f* = 0.68
T 6	linoleic acid	6 µl	=	12 µg, R*f* = 0.8

(1–2 mg each dissolved in 1 ml methanol and the listed amounts applied for TLC).

Description of the chromatograms:

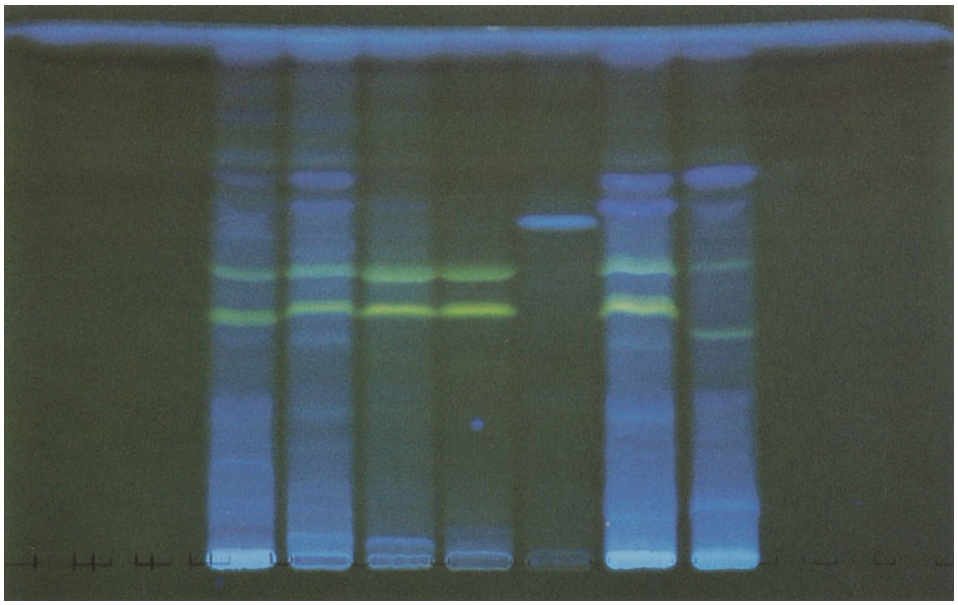


Fig. 2: Thin layer chromatogram of methanolic extracts of *P. ternata* and *Arisaema* species in UV 365 nm

– In the samples 1–4, 6 and 7 a pair of yellow green fluorescent spots of the flavon-C-glycosides vicenin-1 (R*f* = 0.54) and corymboside (R*f* = 0.46) are visible. Both are lacking in sample 5, which might be due to the pretreatment with licorice and lime. All samples show a blue fluorescent spot at the solvent front and further spots in the upper and lower region which are partly due to plant acids. In samples 1,2,6 and 7 one prominent dark blue spot can be detected at R*f* = 0.78. The sample 5 is characterized by a bright blue spot at R*f* = 0,75 only. The reference compounds T 1 – T 6 do not show any reaction under UV 365 nm.

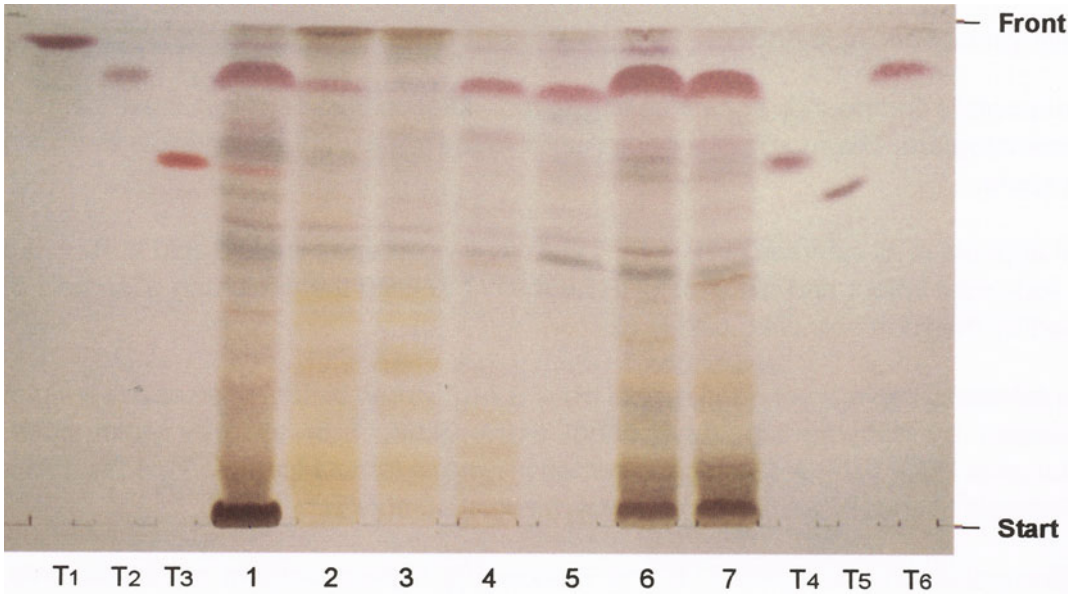


Fig. 3: Thin layer chromatogram of methanolic extracts of *P. ternata* and *Arisaema* species after spraying with vanillin-phosphoric acid-reagent (VIS)

– The more lipophilic compounds appear as blue to red-violet coloured spots in the upper part of the chromatogram. The more polar compounds in the lower part show yellow to brown coloured spots.

– Sitosterol appears in all samples at R*f* = 0.95 (**T 1**) as a violet spot, linoleic acid at R*f* = 0.8 (**T6**) with red-violet colour and pinoresinol (R*f* = 0.91, **T 2**) (with the exception of sample 3) overlapped by the zone of linoleic acid (**T 6**).
– In *Pinellia ternata* (sample 1) only lariciresinol (R*f* = 0.71, **T 3**) can be identified as a red-violet spot, followed by dehydrodiconiferylalcohol (R*f* = 0.68, **T 5**) as grey violet zone. Coniferylalcohol (R*f* = 0.74, **T 4**) can be detected in *Pinellia ternata* (sample 1), Arisaematis rhizoma (sample 6) and Arisaematis amurensis rhizoma (sample 7).
– It is likely that the "irritating polymeric phenols" on the TLC-start of the non-pretreated samples 1 and 6+7 with brown color were degraded through the pretreatment with ginger, licorice and lime.
– A clear TLC-distinction of the *Pinellia ternata* and *Arisaema* – drugs is hardly possible.

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract over Millipore® filtration unit, type HV 0,45 µm

2) Injection volume: 10 µl methanolic total extract (conc. = 5 g drug/10 ml)

3) HPLC parameter:

Apparatus:	Liquid chromatograph HP 1090 Photodiode array detector HP 1040 A, Hewlett Packard
Separation column:	LiChroCART® 125–4 with LiChrospher® 100 RP 18 (5 µm), Merck
Precolumn:	LiChroCART® 4–4 with LiChrospher® 100 RP 18, Merck
Solvent:	A: distilled water adjusted to pH 3.0 with o-phosphoric acid B: acetonitrile + 1 % (V/V) 1 N-H_3PO_4
Gradient:	0 – 20% B in 25 min., 20 – 95% B in 25 min., linearly, 95% B in 5 min., isocratic
Flow:	1,0 ml/min.,
Detection:	210 nm

Retention times of the main peaks (Fig. 4)

Peak	Rt (min.)	Compound
1	8,5	adenosine
2	19,2	coniferyl alcohol
3	21,2	vicenin-1
4	22,2	corymboside
5	29,1	lariciresinol
6	29,6	dehydrodiconiferylalcohol
7	31,4	pinoresinol
8	49,2	linoleic acid

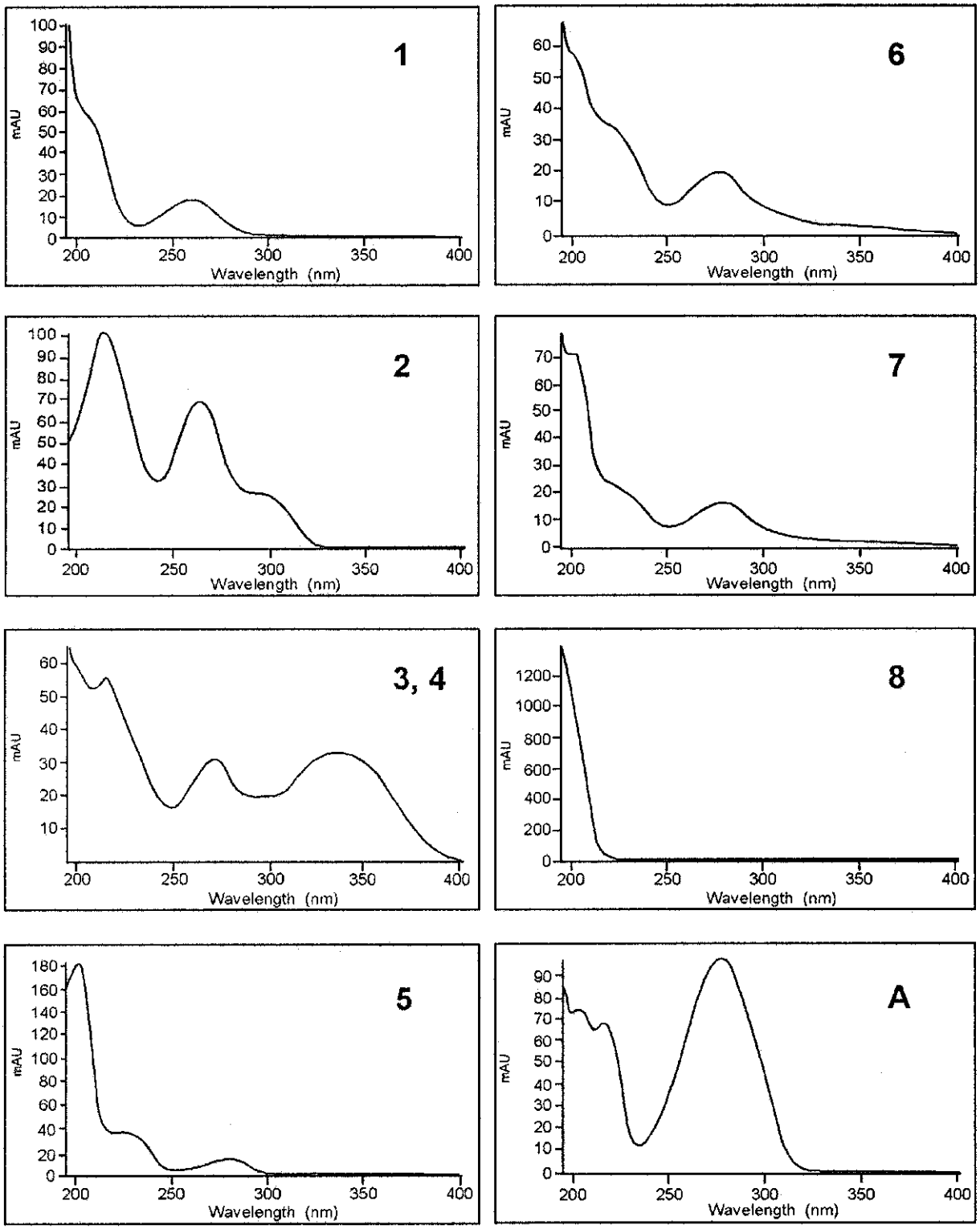


Fig. 4: UV-spectra of the main compounds of *P. ternatae* extract and extracts of *Arisaema* species

Description of the chromatograms:

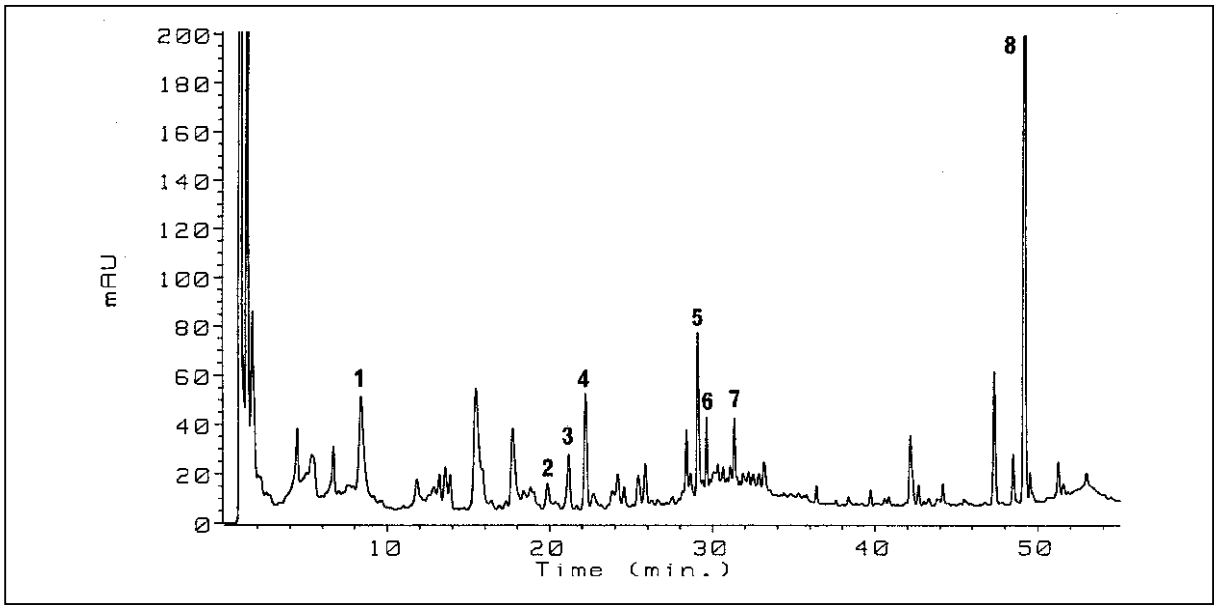


Fig. 5a: HPLC fingerprint chromatogram of non pretreated *Pinellia ternata* extract (sample 1)

The main characteristic constituents of this sample are located in the Rt-range of 20 – 32 min. Vicenin-1 (**3**) and coniferyl alcohol (**2**) can be detected in small amounts, adenosine (**1**), corymboside (**4**), lariciresinol (**5**), dehydrodiconiferylalcohol (**6**) and pinoresinol (**7**) appear in relative high concentration. Linoleic acid (**8**), eluted with Rt 49,2 min., is by far the most dominant and characteristic compound.

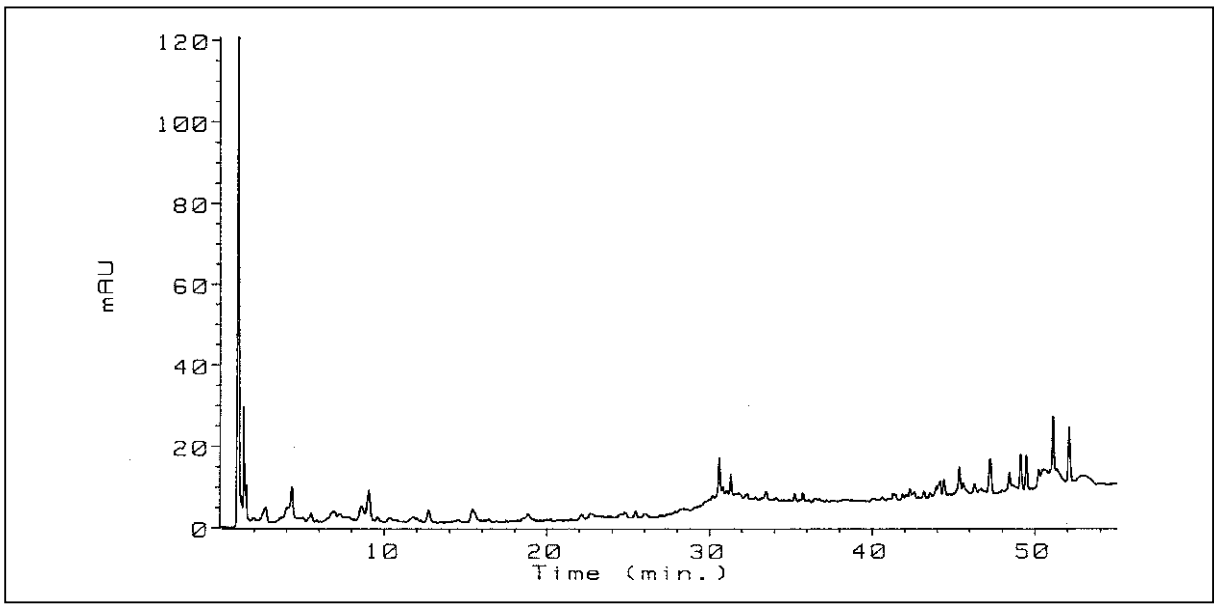


Fig. 5b: HPLC fingerprint chromatogram (Qingbanxia) of pretreated *P. ternata* extract (sample 3)

None of the main constituents of sample 1, in the range of 9 – 22 min., 30 – 32 min. and 45 – 55 min. could be assigned or identified respectively.

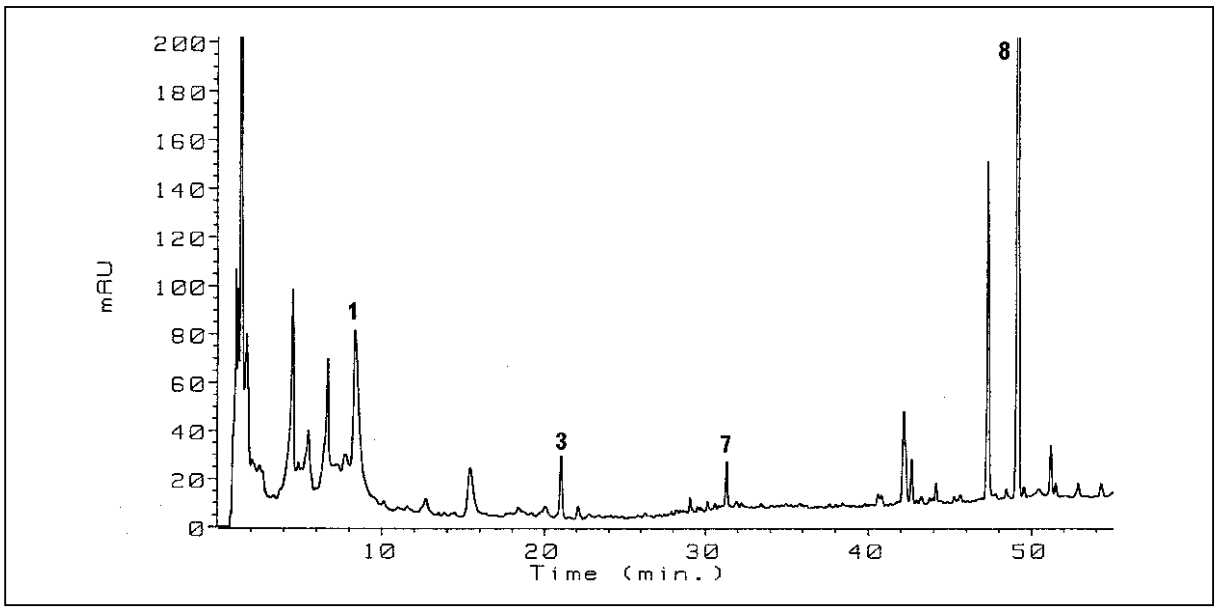


Fig. 6a: HPLC-fingerprint chromatogram of Arisaematis rhizoma extract (sample 6).

The fingerprint analysis is similar to that of sample 1. Detectable are adenosine (**1**), vicenin-1 (**3**), dehydrodiconiferyl alcohol (**7**) and linoleic acid (**8**). The compounds 4, 5 and 6 are lacking or present in very small amounts only.

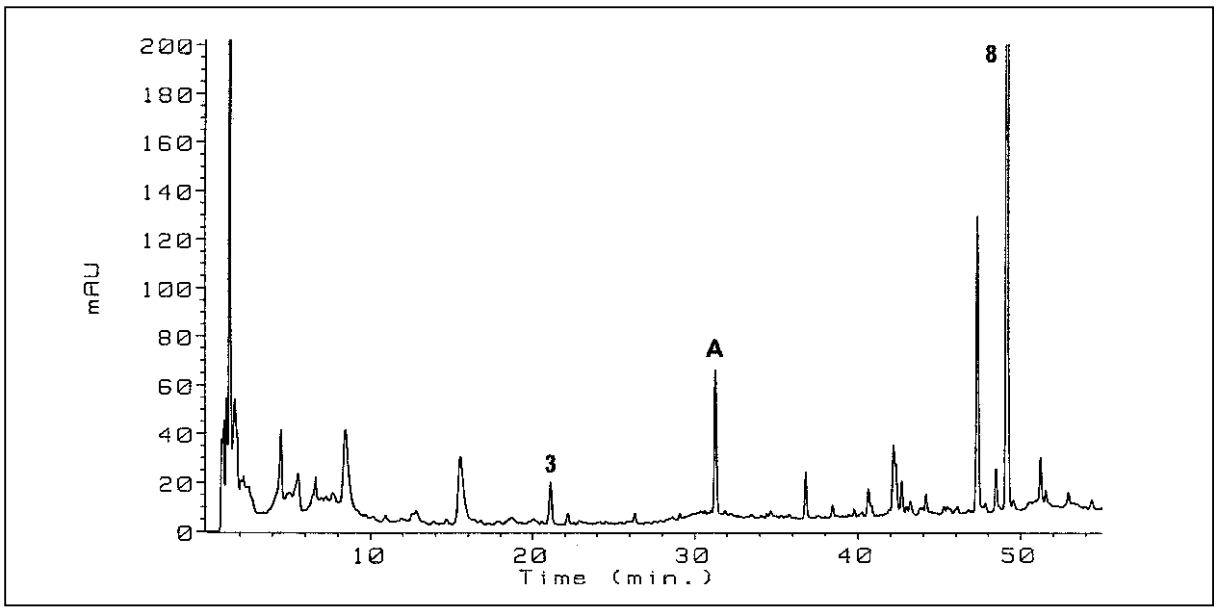


Fig. 6b: HPLC-fingerprint chromatogram of *Arisaematis amur.* extract (sample 7).

The fingerprint analysis shows adenosine (**1**), vicenin-1 (**3**) and linoleic acid (**8**) and resembles as such in part the chromatogram pattern of *Pinellia ternata* (sample 1). A prominent peak (**A**) at Rt 31.25 min. shows a UV-spectrum (max. 280 nm!) which is different from those of all other spectra.

Discussion

For the identification of *Pinellia*-drugs the lignan compounds, the phenylpropane derivatives, flavon-C-glycosides, sterols and linoleic acid can be used. For unambiguous identification of the drug TLC- and HPLC-methods are necessary. Since the fingerprint and the use of the *Arisaema amurense* drug are very similar to those of *Pinellia ternata*, the use of Rhizoma Arisaematis in combination with *Pinellia* Tuber seems to be justified. The same might be true for *Pinellia pedatisecta*. A clear discrimination of *Pinellia ternata* and Arisaema drugs is possible macroscopically only.

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Radix Astragali – *Huang Qi*

Pharmacopoeias:

Chin. Ph. IX
Pharmacopoeia of the People's Republic of China,
Engl. ed. 1992 /2005[1], Jap. Ph. XI
Japanese Pharmacopoeia 1986, English edition 1987[2]

Official drugs:

Astragalus membranaceus (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao[1,2], *Astragalus membranaceus* (Fisch.) Bge.

– Fabaceae –

Description of the official drugs[1, 2]:

The root is cylindrical, usually not branched, 30–90 cm long, 1–3.5 cm in diameter. The bark is brownish-yellow (but not red), with irregular, longitudinal furrows and horizontal lenticellike patterns. The ligneous texture is hard and difficult to break, after fracture fibrous. The cortex is yellowish-white in cross-section. The xylem is pale-yellow, with radiate striations. At older roots the centre part is blackish-brown and occasionally rotten or hollowed. The odour is weak. The taste is slightly sweet and slightly bean-like on chewing.

Falsification of drugs:

Hedysarum polybotrys Handel-Mazzetti as a red adulteration of[6,7,8] and other *Hedysarum* species (*Hedysarum mongholicum* Turczaninov, *Hedysarum vicioides* Turcz.).

Differentiation: *Astragalus* root reveals in a vertical section under the microscope no solitary calcium oxalate crystals outside the fiber bundle[2].

Pretreatment of the raw drug:

The drug is moistened so that it may be cut into slices and then dried, occasionally roasted with honey[1, 9]. For a better storage the bark can be impregnated with the black decoction of *Koelreuteria paniculata* Lax. (Sapindaceae)[8].

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Medicinal use:

In Traditional Medicine in China and Japan as an immunostimulant, tonic (adaptogen), hepatoprotective, diuretic, antihypertensive, antidiabetic, antipyretic and expectorant drug [1,5,6,8].

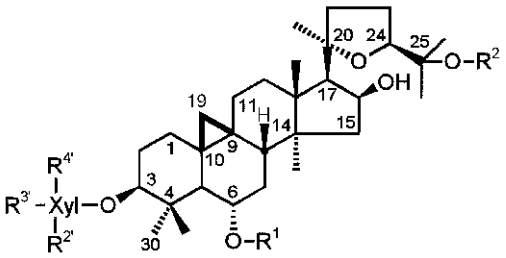
Effects and indications according to Traditional Chinese Medicine:[1,3,9,13]	
Taste:	sweet
Temperature:	warm
Channels entered:	lungs, spleen, heart
Effects:	fortifies and stimulates the immune system
Symptoms and indications:	Qi-deficiency and yang-weakness with: lack of strength (profuse perspiration, deficiency of blood, disturbances in the healing of ulcers), digestive disorders (loss of appetite, diarrhea), prolapse of the rectum, prolapse of the uterus, uterine hemorrhage, inflammation of the kidneys (edema), diabetes (mellitus).
Contraindications:	Yin deficiency, Yang hyperactivity, Qi blockage[1,3,9].

Main constituents (see Fig. 1):[12–23]

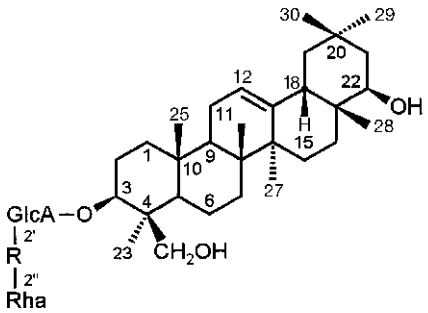
- **Triterpene saponins:** Astragalosides I–VIII, acetyl-astragaloside, soyasaponin[12–16]
- **Flavonoids:** Isoflavones (calycosin, formononetin)[17]
  Isoflavanes (isomucronulatol)[16–18]
  Pterocarpanes (9-methoxy-nissolin)[17,18]
- **Polysaccharides:** Astragalans I, II, III (α-1-4-gluc.: MW 36.300, 12.300, 34.600)[19]
  Astraglucans 1, 2, 3 (α-1-4-, α-1-6-gluc.)[20–22]
  Astraheterosaccharides 1, 2 (galacturonic/glucuronic acid, rha, gluc, ara)[20]
  Astramem bramin Mem - P - (galacturonic acid, ara, gala, rha)[23]
- **Biogenic amines:** Betaine, choline, γ-amino-butyric acid (GABA)[8]

Triterpene saponins:

Aglycone: Cycloastragenol (9,19-cyclo lanostane type)



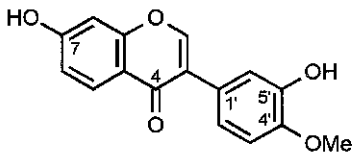
		R1	R2	R2'	R3'	R4'
Acetyl-Astragaloside	I	Glc	H	Ac	Ac	Ac
Astragaloside	I	Glc	H	Ac	Ac	H
Isoastragaloside	I	Glc	H	Ac	H	Ac
Astragaloside	II	Glc	H	Ac	H	H
Isoastragaloside	II	Glc	H	H	Ac	H
Astragaloside	III	H	H	Glc	H	H
Astragaloside	IV	Glc	H	H	H	H
Isoastragaloside	IV	H	Glc	H	H	H
Astragaloside	V	H	Glc	Glc	H	H
Astragaloside	VI	Glc	H	Glc	H	H
Astragaloside	VII	Glc	Glc	H	H	H



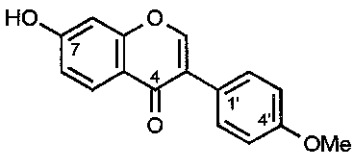
Aglycone: Soyasapogenoi B (Δ^{12} oleanene type)

Astragaloside	VIII	R= Xyl
Soyasaponin	I	R= Gal

Isoflavones:

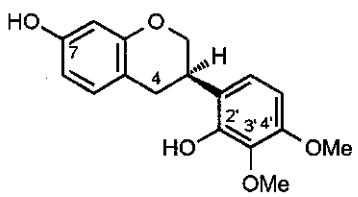


Calycosin



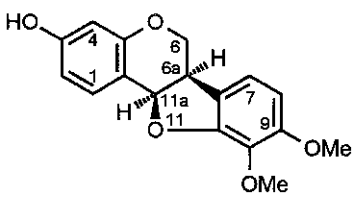
Formononetin

Isoflavans:



Isomucronulatol

Pterocarpans:



9-Methoxy-nissolin

Fig. 1 Formulae of the main constituents

Pharmacology:

in vitro effects:

– immunostimulatory (enhancement of phagocytosis and T-killer cell activity and of IgA, IgM, IgE concentration)[24–27]

– cardiotonic[29]

– sperm motility enhancing[28]

in vivo effects:

– immunostimulatory (activation of RES, induction of α- and γ-interferon, elevation of T-helper cell activity, elevation of chemotactic activity of macrophages, inhibition of retroviral reverse transcriptase and DNA polymerase)[23,30–33,35]

– adaptogenic[36–39]

– antiinflammatory[40]

– antihypertensive[40]

– liver protective[41,42]

– cardiotonic (positive inotropic)[43]

Toxicology:

– Peroral doses of 100 g drug/kg in rats given within 2 days by a drink solution of the crude extract (prepared by reflux of 100 g coarsely ground drug for 6 hrs with 1000 ml distilled water and concentrated to 100 ml by rotary evaporation) showed no adverse side effects[4,10].

– Intraperitoneal injection of the crude extract in rats determined the acute toxicity (LD_{50}) by 40 g/kg[4,10].

– The drug showed no mutagenic effects[44].

TLC fingerprint analysis (see Figs. 2+3):

1) Extraction:

20 g coarsely ground drug are soxhlet extracted with 200 ml methanol p.a. for 1 hr, the methanol raw extract filtered and the filtrate evaporated in vacuum to dryness. The viscous residue is dissolved in 25 ml hot water and the suspension extracted in a separation funnel twice with 10 ml and 5 ml water saturated n-butanol. The (saponin-containing) butanol upper phases are combined (~15 ml), while the (sugar-containing) aqueous lower phase is discarded.

2) Standards:
   1 mg each dissolved in 1 ml methanol p.a.

3) Separation parameters:
   Plates: Silica gel 60 F 254 (Merck)
   Applied amounts: 50 µl butanol phase, 20 µl standard solution
   Solvent system 1[1,13]: chloroform – methanol – water 65 + 35 + 10 (lower phase) (Fig. 2 and 4)
   Solvent system 2 [45]: ethyl acetate – methanol – water 100 + 13,5 + 10 (Fig. 3)
   Both solvent systems are used for the TLC of flavonoids and saponins.

4) Detection:

   Spray reagents[45]:

   a) Komarowsky reagent (KOM): Fig. 2 and 3
   1 ml methanolic 50 % sulphuric acid solution and 10 ml methanolic 2 % p-hydroxy-benzaldehyde solution are prepared shortly before use.
   The TLC plate is intensively sprayed, heated for 10 min. at 110 ˚C and evaluated in VIS.

   b) Blood reagent (BL): Fig. 4

   10 ml of a 3,65% sodium citrate solution is added to 90 ml fresh bovine blood. 2 ml of this mixture is combined with 30 ml phosphate buffer solution pH 7,4 (0,682 g potassium dihydrogen phosphate and 0,157 g sodium hydroxide are dissolved in 100 ml distilled CO_2-free water).
   The TLC plate is sprayed with ca. 10 ml reagent and evaluated in VIS.

Drug samples:

1 Radix Astragali Commercial drug (China, Shanxi Province)
2 Radix Astragali Commercial drug (China, Heilongjiang Province)
3 Radix Astragali Commercial drug (China, Gansu Province)
4 Radix Astragali Commercial drug (Korea)
5 Radix Astragali Commercial drug (Mongolia)

Standards:	Fig. 2	Fig. 3
T_1 astragaloside I	R*f* = 0,65	R*f* = 0,50
T_2 astragaloside II	R*f* = 0,45	R*f* = 0,40
T_3 astragaloside III	R*f* = 0,35	R*f* = 0,30
T_4 astragaloside IV	R*f* = 0,30	R*f* = 0,25
T_5 astragaloside V	R*f* = 0,20	R*f* = 0,15
T_6 astragaloside VI	R*f* = 0,20	R*f* = 0,10
T_7 9-methoxy-nissolin-3-O-β-D-glucoside	R*f* = 0,60	R*f* = 0,55
T_8 9-methoxy-nissolin	R*f* = 0,90	R*f* = 0,90
T_9 calycosin-7-O-β-D-glucoside	-	R*f* = 0,52
T_{10} calycosin	-	R*f* = 0,88
T_{11} isomucronulatol-7-O-β-D-glucoside	R*f* = 0,55	R*f* = 0,60
T_{12} isomucronulatol	R*f* = 0,90	R*f* = 0,95

Standards:

T_1 isomucronulatol-7-O-β-D-glucoside
T_2 isomucronulatol
T_3 astragaloside III
T_4 astragaloside IV
T_5 astragaloside V
T_6 astragaloside VI
T_7 9-methoxy-nissolin-3-O-β-D-glucoside
T_8 9-methoxy-nissolin
T_9 calycosin-7-O-β-D-glucoside
T_{10}calycosin

5) Description of the TLC chromatograms:

Fig. 2

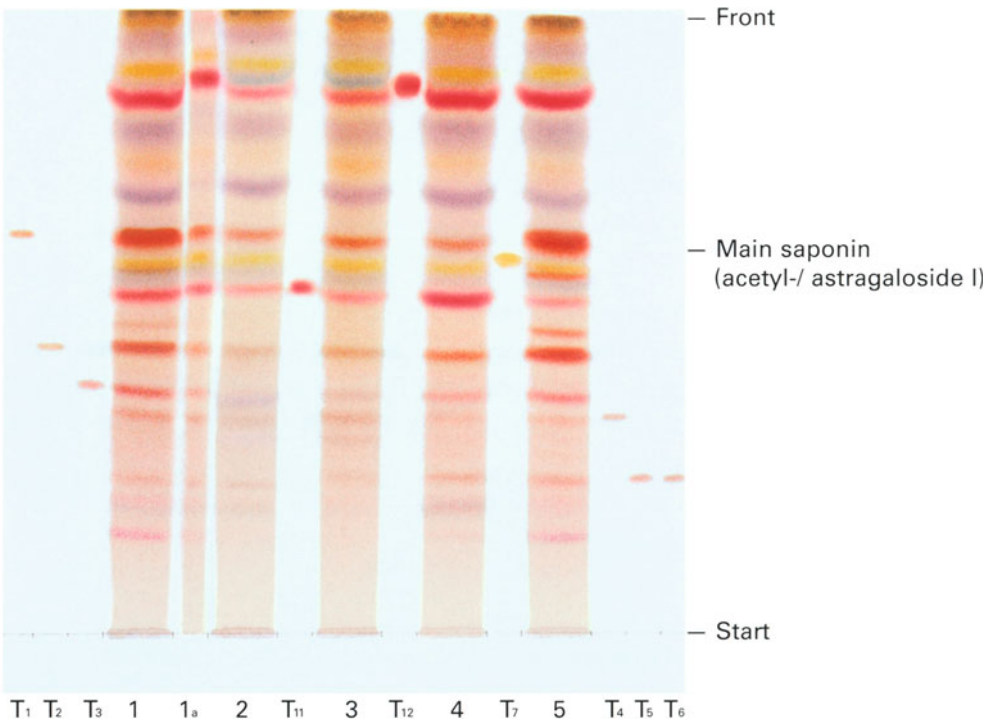


Fig. 2: Thin layer chromatogram of *Astragalus* samples 1–5 in solvent 1 with KOM/VIS

- The saponins (astragalosides) appear in the middle R*f* region 0,25–0,65 initially as brown red and later as olive green zones (significant change of colour).
- The main saponin astragaloside I (T1) is a dominating brown red zone at R*f* 0,65 and is mostly overlapping the (tri-)acetyl astragaloside at R*f* 0,67.
- Next prominent saponins are the brown red zones of astragaloside II (T2) at R*f* 0,45 and of the non-acetylated (less lipophilic) astragaloside III (T3) at R*f* 0,35.
- The astragaloside IV (T4) at R*f* 0,30 and astragaloside V (T5) and VI (T6) at R*f* 0,20 are visible as light brown zones.
- The isoflavane glycoside isomucronulatol-7-O-β-D-glucoside (T11) is a dominating red zone at R*f* 0,55 and also the accompanying isoflavane aglycone isomucronulatol, (T12) at R*f* 0,90.
- The pterocarpane glycoside 9-methoxy-nissolin-3-O-β-D-glucoside (T7) is visible at R*f* 0,60 and the accompanying pterocarpan aglycone 9-methoxy-nissolin at R*f* 0,90 as yellow orange zones.
- Blue violett brown zones (probably isoflavanes) appear at R*f* 0,75 and R*f* 0,80.
- The sapogenins cycloastragenol and soyasapogenol give violet brown zones at the solvent front.
- In sample 1a the sapogenins were enriched by purchine, a SepPak-C18-column with hot water.

Fig. 3: KOM/VIS:

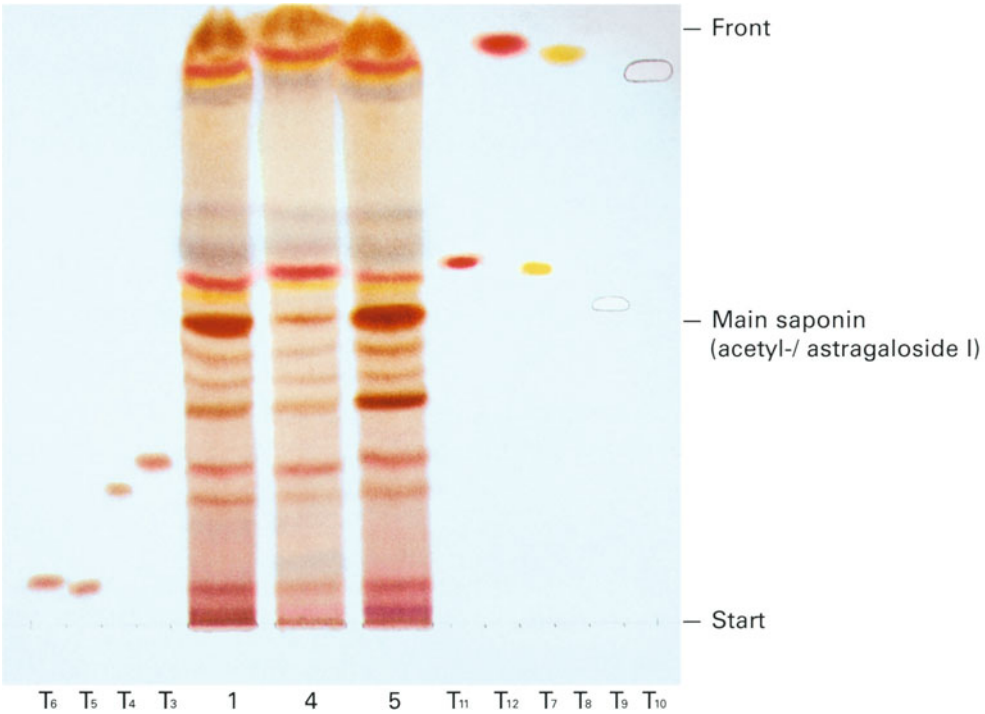


Fig. 3: Thin layer chromatogram of *Astragalus* samples 1, 4, 5 in solvent 2 with KOM/VIS

Description of Fig. 3

- The astragalosides appear in the lower R*f* region (0,10–0,50) as initially brown violet and later olive green zones (significant change of colour).
- The main saponin astragaloside I (T1) and the (tri-)acetyl astragaloside, overlapping each other, produce the most intensive brown violett zone at R*f* 0,50.
- Next prominent saponins are the brown violet zones of the astragaloside II (T2) at R*f* 0,40 and of the non-acetylated astragaloside III (T3) at R*f* 0,30.
- Other acetylated saponins, iso-astragaloside I and iso-astragaloside II, are visible as brown violet zones between the main saponin at R*f* 0.50 and the next prominent saponin zone at R*f* 0,40.
- The non-acetylated astragaloside IV (T4) at R*f* 0,25, astragaloside V (T5) at R*f* 0,15 and astragaloside VI (T6) at R*f* 0,10 are visible as brown violet zones beneath astragaloside III (T3).
- The isoflavane glycoside isomucronulatol-7-O-β-D-glucoside (T11), appears as red zone at R*f* 0,60 above the yellow orange zone of the pterocarpane-glycoside 9-methoxy-nissolin-3-O-β-D-glucoside (T7) at R*f* 0,55.
- The accompanying isoflavane aglycone isomucronulatol (T12) appears as red zone at R*f* 0,95 above the pterocarpane aglycone 9-O-methoxy-nissolin (T8) which appears as a yellow orange zone at R*f* 0,90.
- The *Astragalus* drug samples 1, 4 + 5 exhibit in the TLC fingerprint analysis a homogeneous flavonoid profile varying only in the content of isomucronulatol glucoside/aglycone and 9-methoxy-nissolin glucoside/aglycone. Some differences are visible in the astragaloside pattern.
- T9 and T10 could not be detected in the samples 1, 4 and 5.

Fig. 4: BL/VIS:
In order to achieve a better visualization of the astragaloside pattern, 5 ml of the (sugar free) n-butanol-phase is prepared with 5 ml ice cooled 0,1% sodium hydrogen carbonate to remove most of the 3, 7- or 4´-OH flavonoids as water soluble phenolates (lower phase), whereas the acylglycosyl saponins do not undergo alkaline hydrolysis.

The alkali free washed n-butanol upper phase is concentrated in rotary vacuum heat to 0,5 ml and subjected to a methanol (1 ml) presaturated SepPak-RP-C18-column, which is eluted by 0,5 ml hot distilled water to get a nearly aglycone free solution for the following TLC.

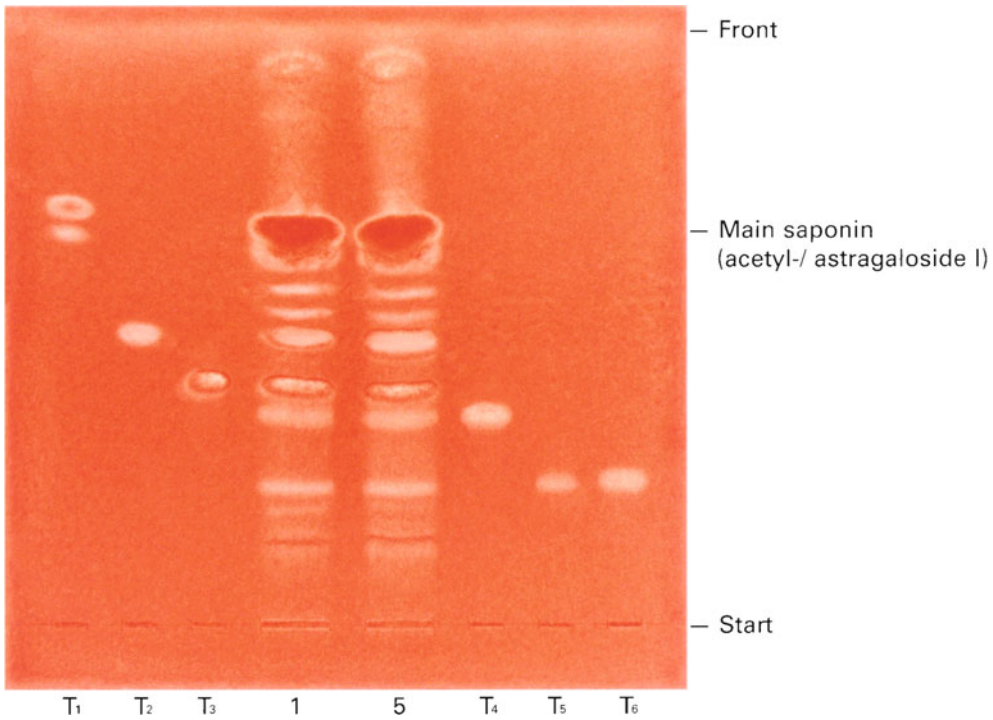


Fig. 4: Thin layer chromatogram of *Astragalus* samples 1 and 5 in solvent 1 with BL/VIS

Description of Fig. 4

– The saponin glycosides (astragalosides) in the R*f*-range 0,15-0,65 and the corresponding aglycones at R*f* 0,90 are visible immediately or after 10 min slight warming as white hemolytic bands against the red background of the plate.

HPLC fingerprint analysis (see Figs 5–6d):

1) Sample preparation:

a) for flavonoid – HPLC:

250 µl of the extract-n-butanol phase (20 g drug/15 ml n-BuOH) are applied to a SepPak C18–cartridge, which has been pre equilibrated and eluted before with 1 ml methanol p.a.

Elution with 0,5 ml fresh distilled water gives the butanol-water-eluate (= solution for HPLC analysis) and retains the sapogenins on the RP-C18 column. The eluate is filtered through

a Millipore filtration unit type HV 0,45 µm before HPLC injection.

b) for saponin – HPLC

The n-BuOH-phase (20 g drug/15 ml) is evaporated and dissolved in a minimal volume of MeOH (~3 ml) and the solution dropped in 50 ml icecooled ether-acetone (1:1)-mixture. The precipitate (containing the major part of saponins) is separated by centrifugation, dissolved in 1 ml hot MeOH and injected into the HPLC apparatus.

2) Injection volume: 25 µl butanol-water eluate (conc.= 20 g drug/15 ml n-BuOH) (solution 1a)
25 µl precipitate solution (conc. = 100 mg/1 ml MeOH) (solution 1b)
25 µl reference solution (conc.= 1 mg/1 ml MeOH = 0,1%)

3) HPLC data:

Apparatus:	Liquid chromatograph HP 1090
	Photodiode array detector HP 1040 A, Hewlett Packard
Pre-column:	LiChroCART 4-4 with LiChrospher 100 RP 18 (5 µm), Merck
Separation-column:	LiChroCART 125-4 with LiChrospher 100 RP 18 (5 µm), Merck
Solvent system:	A: distilled water (+1 % 0,1N-H_3PO_4)
	B: acetonitrile (+1 % 0,1N-H_3PO_4)
Gradient:	10% B in 5 min. (isocratic)
	10% – 20% B in 10 min. (linear)
	20% – 25% B in 10 min. (linear)
	25% – 33% B in 10 min. (linear)
	33% – 35% B in 10 min. (linear)
	35% – 60% B in 10 min. (linear)
Flow:	1,0 ml/min.
Detection:	200 nm

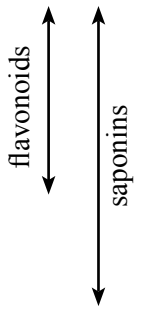


Table 1: Retention times of the main peaks (flavonoids): (Fig. 6a–6c)

Peak	Rt (min.)	Compound
1	12,0	calycosin-7-O-β-D-glucoside
2	20,2	9-methoxy-nissolin-3-O-β-D-glucoside
3	22,2	isomucronulatol-7-O-β-D-glucoside
4	22,8	calycosin
5	33,5	formononetin
6	34,1	9-methoxy-nissolin
7	36,1	isomucronulatol

Table 2: Retention times of the main peaks (saponins): (Fig. 6d)

Peak	Rt (min.)	Compound
1′	27,5	astragaloside VI
2′	26,8	astragaloside V
3′	32,5	astragaloside IV
4′	33,5	astragaloside III
5′	43,6	isoastragaloside II
6′	45,8	astragaloside II
7′	46,6	astragaloside I
8′	48,0	acetyl-astragaloside I

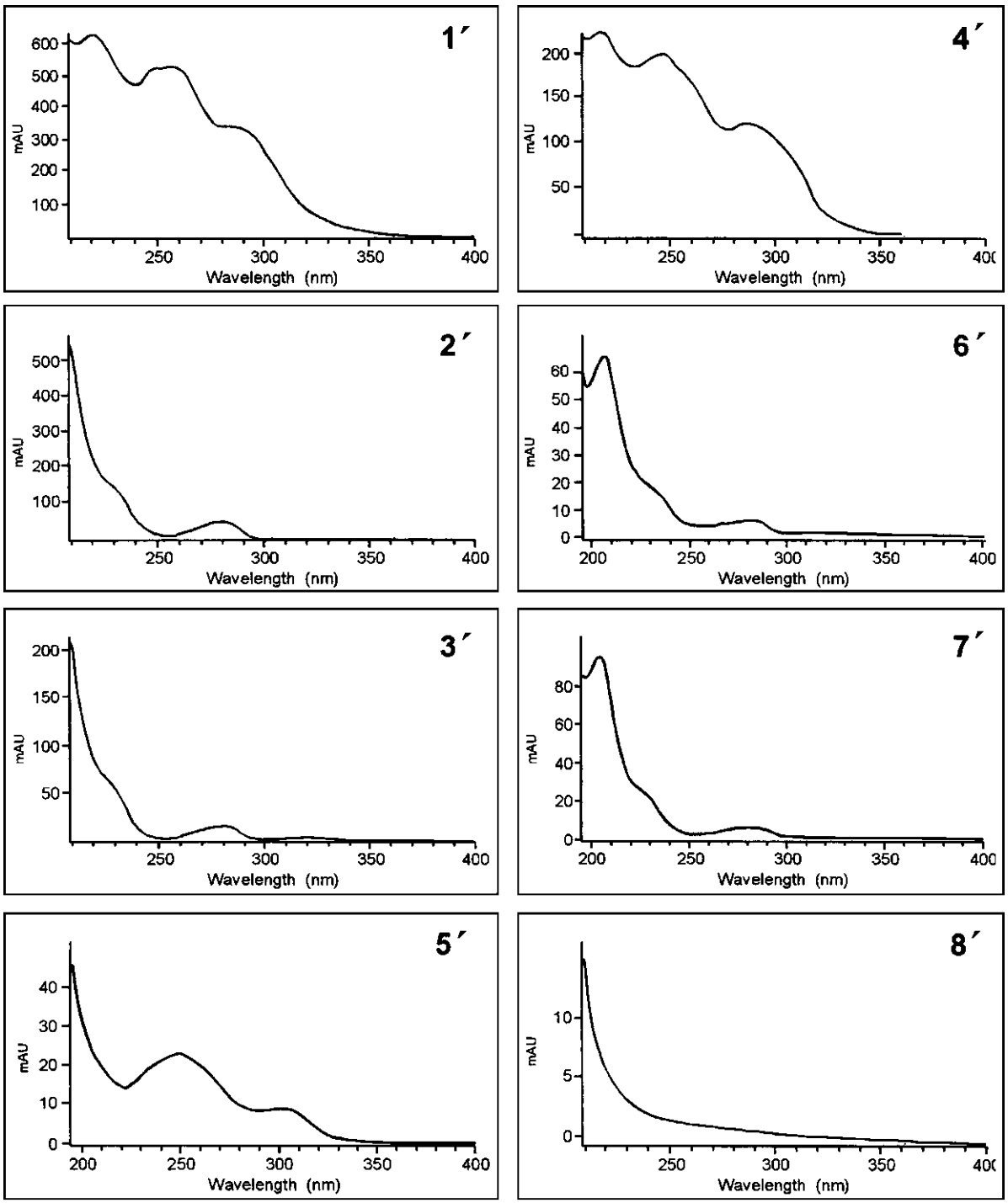


Fig. 5: UV spectra of the main compounds (Table 2)

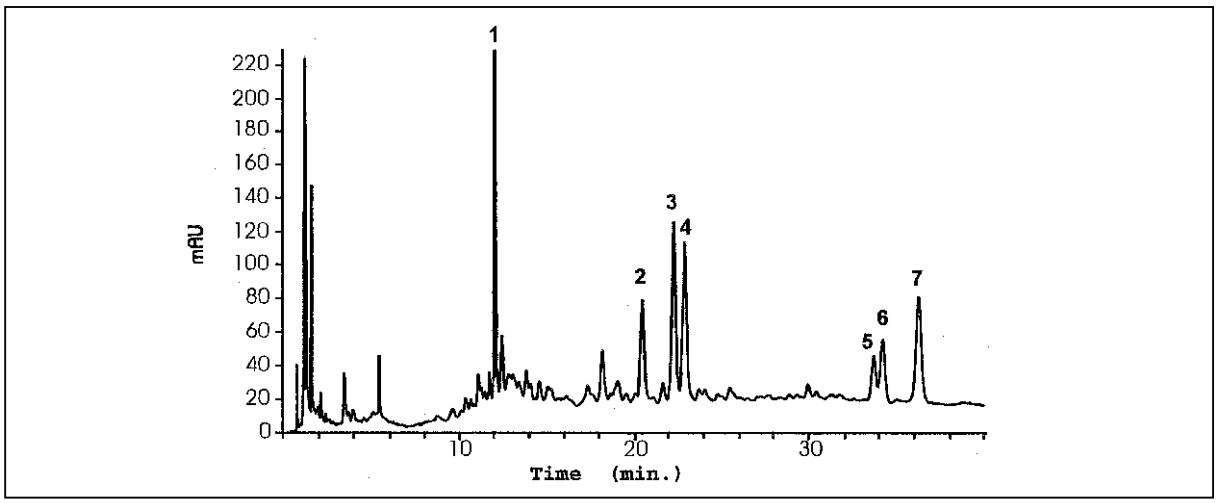


Fig. 6a: HPLC flavonoid – fingerprint of *Astragalus membranaceus* sample 1 from China (Shanxi)

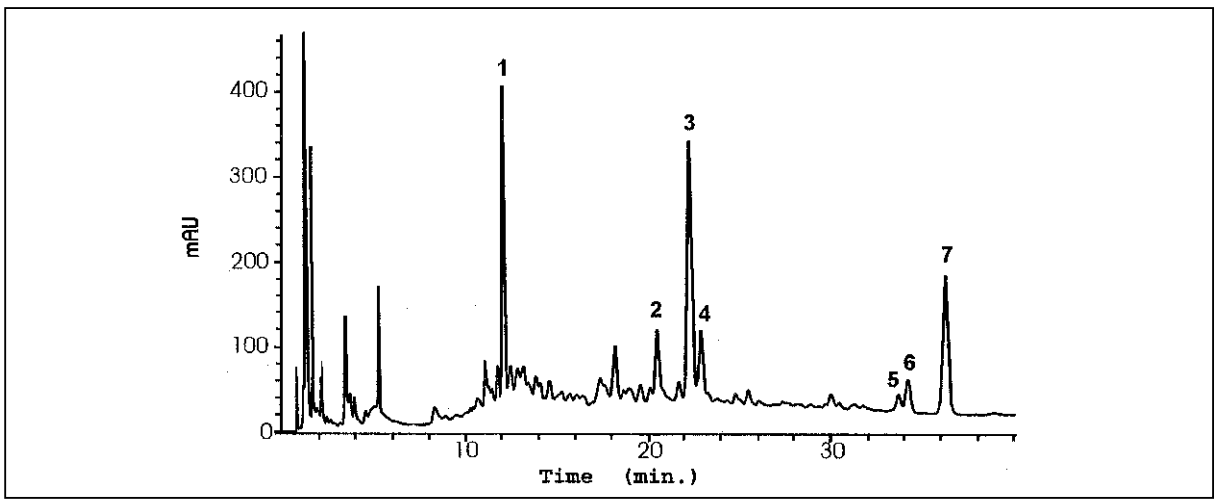


Fig. 6b: HPLC flavonoid – fingerprint of *Astragalus membranaceus* sample 4 from Korea

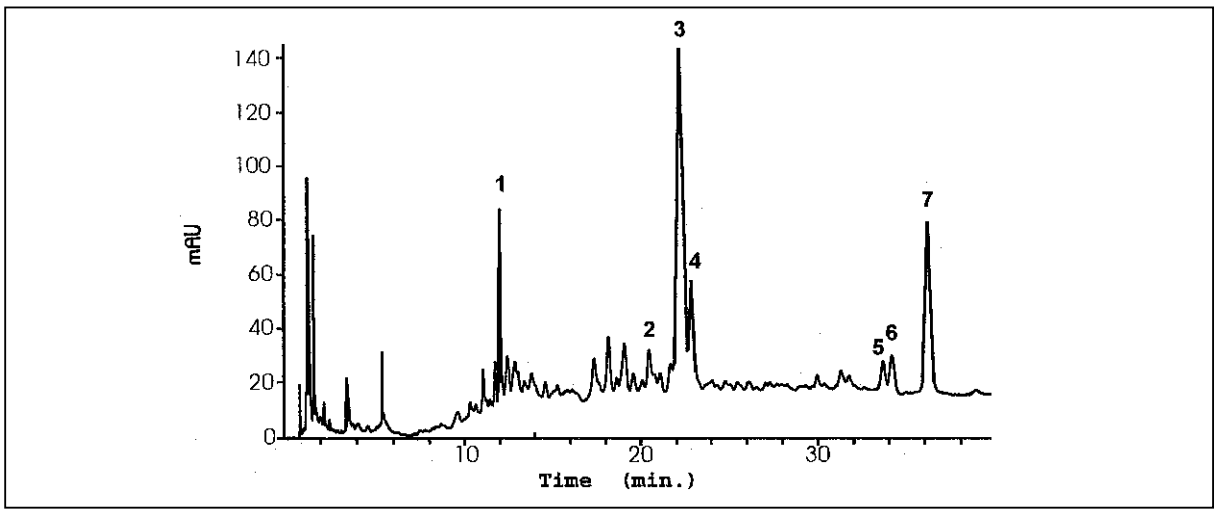


Fig. 6c: HPLC flavonoid – fingerprint of *Astragalus mongholicus* sample 5 from China (Mongolia)

Description of Fig. 6a, 6b and 6c:

The HPLC flavonoid fingerprints of the *Astragalus* samples are characterized by the isoflavones calycosin-7-O -β-D-glucoside (**P1**) and calycosin (**P4**), the isoflavans isomucronulatol-7-O-β- D-glucoside (**P3**) and isomucronulatol (**P7**), the pterocarpans 9-methoxy-nissolin-3-O-β-D-glucoside (**P2**) and 9-methoxy-nissolin (**P6**), and the iso-flavone formononetin (**P5**). The drug samples show qualitatively almost identical flavonoid profiles, and differ only quantitatively in the peaks **P3** and **P4**.

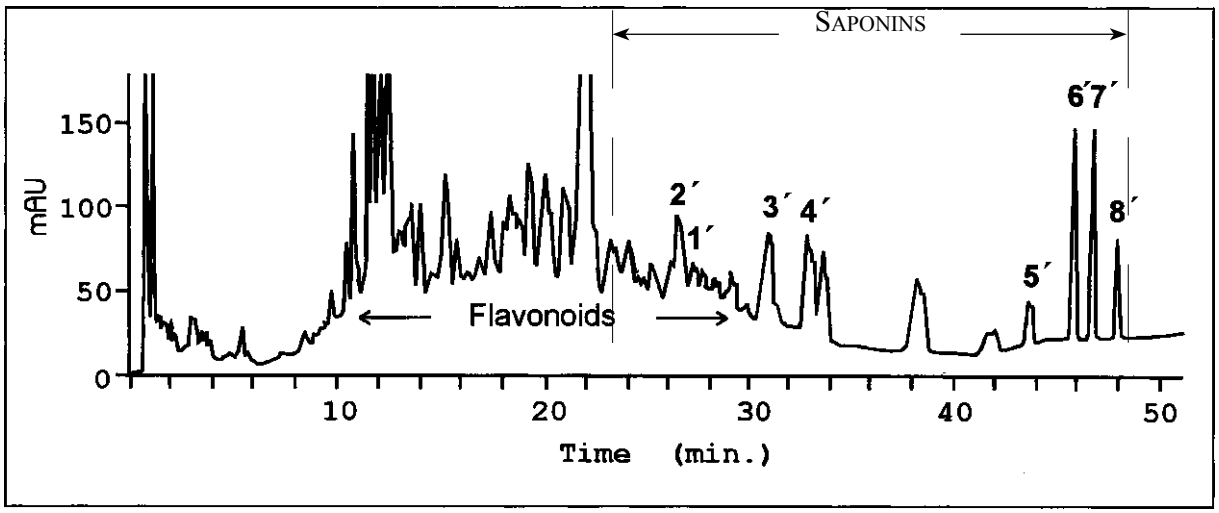


Fig. 6d: HPLC-saponin-fingerprint of *Astragalus mongholicus* (sample 5)

Discription of Fig. 6d

The astragalosides I – VI appear in the Rt range of 25 to 50 min., whereas the non completely removed flavonoids are eluted between 10 and 25 minutes. The HPLC fingerprint spectra of the ether-acetone-precipitated n-BuOH-phases of several *Astragalus* roots exhibit different astragaloside patterns depending on their provenance, harvest and storage time.

The drug sample 1 (from Shanxi province) and drug sample 5 (from Peking market) had a very high astragaloside I content, whereas the other drug samples showed a low astagaloside I and II content. Overstored drugs or drugs of less quality are identifiable by a lesser content of (acetyl)-astragalosides I and astragaloside II.

Discussion:

Since the pharmacological and therapeutic effects of the *Astragalus* drug might be due to a synergism of saponins and flavonoids, the TLC- and the HPLC- fingerprint analysis of both classes of compounds is necessary for an unambigious identification and standardization of *Astragalus* root.

Because of the low saponine content of the raw drug it is necessary, to enrich the concentration of saponins for the HPLC-fingerprint-analysis by n-BuOH-funneling and ether-aceton-precipitation.

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Radix Angelicae pubescentis – *Duhuo*

Pharmacopoeias: Pharmacopoeia of the People's Republic of China English Edition, 1992/2005[1]

Official drug: The dried roots of *Angelica pubescens* Maxim *f. biserrata* Shan *et* Yuan. – Apiaceae[1]

Substitutes: More than 30 species in two families are used as Duhuo in different areas of China, which mainly belong to the genus of *Angelica, Heracleum* (Apiaceae) and *Aralia* (Araliaceae).

Description of the drug:[1]

Somewhat cylindrical, with 2–3 or more branches at the lower part, 10–30 cm long. Root stock enlarged, conical, with abundant transverse wrinkles, 1.5–3 cm in diameter, apex exhibiting remains of stems and leaves or sunken spots. Externally greyish-brown or dark brown, longitudinally wrinkled, with prominent, transverse lenticels and slightly prominent scars of rootlets. Texture relatively hard, soft when moistened; fracture showing bark greyish-white, scattered with abundant brown oil cavities, wood greyish-yellow to yellowish-brown, cambium ring brown. Odour, characteristic and aromatic.

Pretreatment of the raw drug:

Foreign matters are eliminated, washed clean, then baked to half-dryness, piled up for 2–3 days to soften, then dried by baking, cut into thin slices, and dried in the sun or at a low temperature[1].

Medicinal use: Antirheumatic and analgesic agent for the treatment of rheumatic pain, especially that of the lower back[2].

Effects and indications according to Traditional Chinese Medicine[1,2]

Taste:	pungent, slightly bitter
Temperature:	warm
Channels entered:	kidney and urinary bladder
Effects:	expels wind and dampness, stops pain, and releases the exterior and disperse cold
Symptoms and indications:	rheumatic arthritis with pain in the lower back and knees; headache due to attack of cold on the ashaoyin channel characterized by association of headache with precordial and cold legs

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents (see Fig. 1):

– essential oil[3,4]

– **coumarins** and **their glycosides**[5–16]:

angular-dihydrofuranocoumarins:	columbianedin, columbianetin acetate, columbianetin propionate, columbianetin, columbianin, angelidiol, columbianetin-β-D-glucopyranoside;
linear-dihydrofuranocoumarins:	nodakenetin, nodakenin, marmesinin;
furanocoumarins:	bergapten, xanthotoxin, imperatorin, isoimperatorin, oxypeucedanol hydrate, byakangelicin, isopimpinellin, psoralen;
angelol-type prenylcoumarins:	angelols A, B, C, D, E, F, G, H, J, K, L;
other simple coumarins:	osthol, osthenol, 7-methoxy-8-senecioylcoumarin, 8-(3-hydroxy-isovaleroyl)-5,7-dimethoxycoumarin, angelin, glabra-lactone, scopoletin, coumurrayin, umbelliferone, angelitriol, 6-[1(R),2(R)-1,2,3-trihydroxy-3-methylbutyl]-7-methoxycoumarin, meranzinehydrate, peucedanol, ulopterol, apiosylskimmin
– organic acids:	caffeic acid, isoferulic acid[17], amino-butyric acid[18] and 2,3,4,9-tetrahydro-pyrido[3.4-b]indole-3-carboxylic acid[9]
– polyacetylenes:	falcarindiol, 11(S),16(R)-dihydroxy-9(Z),17-dien-12,14-diyn-octadecyl acetate[19]
– sesquiterpenes:	bisabolangelone[19]

– sugars, e.g. sucrose[9]

– β-sitosterol and daucosterol[9]

– adenosine[9]

Pharmacology:

In vitro effects:

– cyclooxygenase and 5-lipoxygenase inhibitory effect[20]

– anti-platelet aggregation and thromboxane formation[21]

– anti-proliferation effect[22]

– cardiovascular effect[18,23]

– antibacterial[23]

In vivo effects:

– anti-platelet aggregation and thromboxane formation (mice, rat)[24]

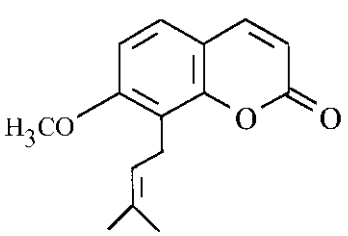
– anti-inflammation (mice, rat)[17]

– analgesic effect (mice, rat)[17]

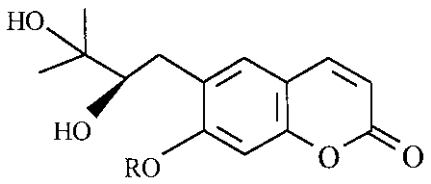
– cardiovascular effect (rat, cat)[22,25]

– trachea relaxation (guinea-pig)[26]

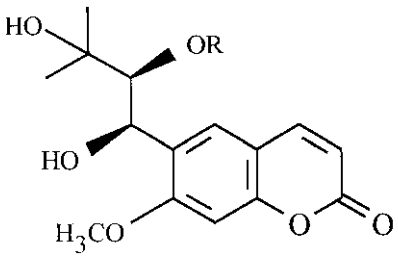
– photo sensitive[23]



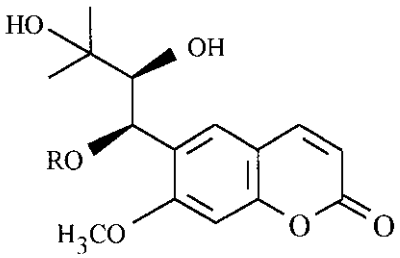
osthol



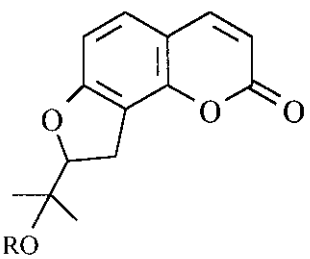
R=H, peucedanol
R=CH_3, ulopterol



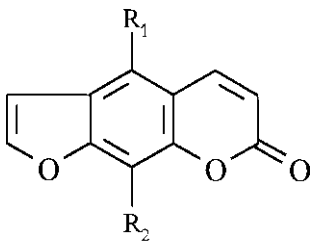
R=angeloyl, angelol-A
R=tigloyl, angelol-B
R=2-methlbutyryl, angelol-C



R=angeloyl, angelol-K
R=tigloyl, angelol-D
R=isovaleryl, angelol-L



R=angeloyl, columbianedin
R=acetyl, columbianetin acetate
R=H, columbianetin
R=glu-O-glu, columbianin



R_1=OCH_3, R_2=H, bergapten
R_1=O  , R_2=H, isoimperatorin
R_1=H, R_2=O  , imperatorin
R_1=H, R_2=OCH_3, xanthotoxin

Fig. 1: Formulae of the main compounds in Angelicae pubescentis radix

TLC fingerprint analysis:

1) Extraction: 5 g of powdered drug are Soxhlet-extracted with 50 ml dichloromethane (plate A) or *n*-hexane (plate B) for three hrs. The extract is evaporated to dryness. 100 mg of the residue are dissolved in 10.0 ml EtOH p.a.

2) Standards: Columbianedin, columbianetin, columbianetin acetate, osthol, umbelliferone, bergapten, xanthotoxin, imperatorin, isoimperatorin are dissolved in EtOH p.a. at a conc. of 1 mg/ml.

3) Separation parameters:

Applied amounts:	5 µl of extract (10 mg/ml) and standard (1 mg/ml) solutions
Plates:	Silica gel 60 F_{254}, Merck
Solvent system:	Toluene-ethyl acetate (80 + 20)
Direct evaluation:	UV 254 nm and UV 365 nm
Spray reagent:	Anisaldehyde-sulphuric acid reagent (0.5 ml anisaldehyde, 10 ml glacial acetic acid, 85 ml MeOH, 5 ml conc. sulphuric acid, mixed in this order) The plate is sprayed with ca. 10 ml reagent and then heated under observation for 5–10 min. at 100 °C; evaluation in VIS.

Plate A (Fig. 2):

Drug samples (origin and date):
1. Radix Angelicae pubescentis (Shenyang, China, 4/96)
2. Radix Angelicae pubescentis (Shenyang, China, 8/96)
3. Radix Angelicae pubescentis (Hangzhou, China, 9/94)
4. Radix Angelicae pubescentis (Guangzhou, China, 10/94)
5. Radix Angelicae pubescentis (Xian, China, 10/94)
6. Radix Angelicae pubescentis (Hongkong, 10/94)

Reference substances:
T1 columbianedin + columbianetin, R*f* = 0.59/0.15
T2 osthol + columbianetin acetate, R*f* = 0.58/0.47
T3 umbelliferone + angelol-B, R*f* = 0.21/0.05
T4 isoimperatorin + imperatorin, R*f* = 0.66/0.53
T5 bergapten + xanthotoxin, R*f* = 0.50/0.49
T6 angelol-A + angelol-K, R*f* = 0.07/0.02

Plate B (Fig. 3 + 4):

Drug samples (grown in):
7. *Angelica pubescens f. biserrata* (Hubei)
8. *Angelica dahurica* (Liaoning)
9. *Angelica apaensis* (Sichuan)
10. *Heracleum rapula* (Yunnan)
11. *Heracleum moellendorffii* (Sichuan)
12. *Heracleum candicans* (Yunnan)
13. *Heracleum stenopterum* (Yunnan)
14. *Aralia cordata* (Sichuan)

4) Descriptions of chromatograms

Plate A:

UV 365 nm (see Fig. 2):

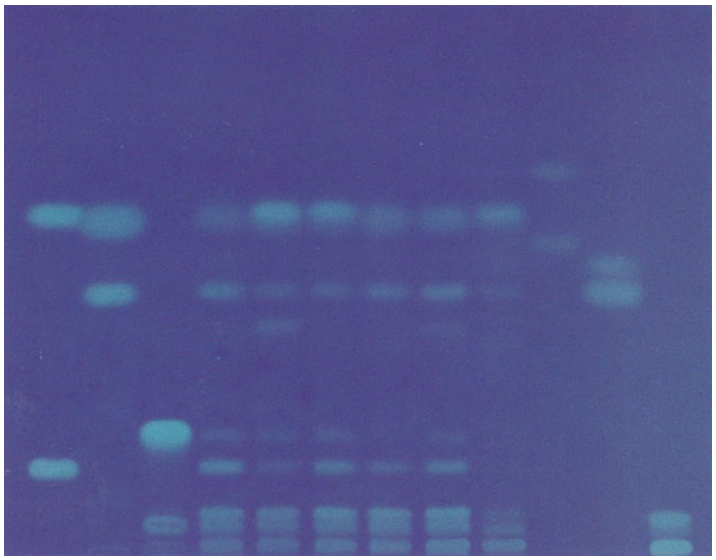


Fig. 2: Thin layer chromatogram of Angelicae pubescentis radix extracts (UV 365 nm)

Violet-blue fluorescent spots at R*f* 0.59 (columbianedin), 0.47 (columbianetin acetate), 0.15 (columbianetin) and a blue spot at R*f* 0.58 (osthol) are typical for *Angelica pubescens*; yellow spots at R*f* 0.66 (isoimperatorin), 0.53 (imperatorin) and 0.50 (bergapten) can also be observed. Spots with violet-blue colour above the start (angelol-A: R*f* 0.07; angelol-B: R*f* 0.05 and angelol-K: R*f* 0.02) are conspicuous. A green-yellow spot at R*f* 0.21 (umbelliferone) is prominent. Further weak fluorescent spots are detectable in the range of R*f* 0.15–0.47.

UV 254 nm:

The fluorescence quenching of spots under 254 nm is generally weaker than fluorescence under 365 nm.

After spraying with anisaldehyde sulphuric acid:

Except two spots at R*f* 0.58 (osthol) and 0.16 (a mixture of free unsaturated fatty acids), only few other spots can be observed (R*f* 0.39 and 0.18).

Plate B:

UV 254 nm (see Fig. 3):

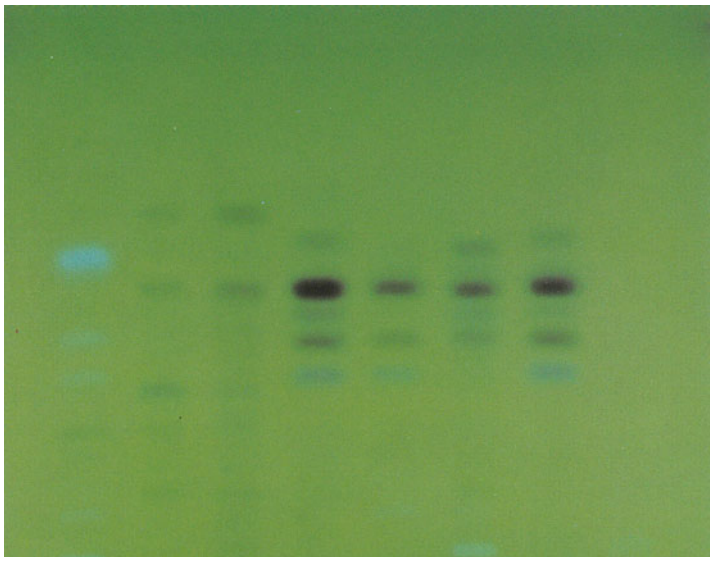


Fig. 3: Thin layer chromatogram of some substitutes of Angelicae pubescentis radix (UV 254 nm)

The difference between *Angelica pubescens* and the substitutes is obvious, because spots in the former exhibit mainly blue fluorescence while the substitutes possess mainly purple or violet spots on the light background. Isoimperatorin (R*f* 0.66), imperatorin (R*f* 0.52) and phellopterin (R*f* 0.52) are the main compounds in *Angelica dahurica* and *Angelica apaensis*. Spots at R*f* 0.52 (imperatorin or pimpinellin), 0.50 (bergapten), 0.49 (xanthotoxin or isopimpinellin) seem to be characteristic of *Heracleum* species. The spot at R*f* 0.39 in *H. rapula, H. moellendorffii* and *H. stenopterum* is sphonedin and at R*f* 0.56 in *H. rapula* and *H. stenopterum* is isobergapten. *Aralia cordata* supplies only one blue spot at R*f* 0.06.

UV 365 nm:

The difference between *Angelica pubescens* and its substitutes is striking because in the extracts of the substitutes, many light- (imperatorin, isoimperatorin, bergapten and isobergapten), orange- (xanthotoxin, pimpinellin and isopimpinellin) or green-yellow (sphondin) fluorescent spots are visible which all represent furanocoumarins.

After spraying with anisaldehyde sulphuric acid (see Fig. 4):

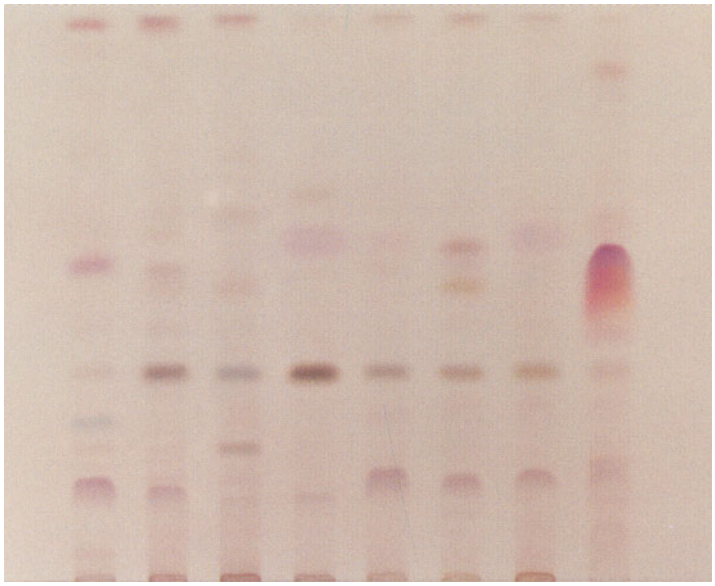


Fig. 4: Thin layer chromatogram of some substitutes of Angelicae pubescentis radix after spraying with anisaldehyde sulphuric acid reagent; VIS

Besides the spot at R*f* 0.90, a general characteristic spot in the substitutes appears at R*f* 0.35 (falcarindiol). The distinct pink spots in the range of R*f* 0.18–0.20 represent free unsaturated fatty acids. Few other zones can be seen in the different substitutes. *Aralia cordata* shows at least 6 zones at R*f* 0.56, 0.50, 0.42, 0.35 and 0.17 in different colour.

Distinction of the drug-types:

Angelica pubescens contains mainly dihydrofuranocoumarins and 6- or 8- prenylcoumarin derivatives, which show prominent blue or violet fluorescence under UV 365 nm and blue under UV 254 nm. The substitutes can be easily distinguished from it because their main constituents in *Angelica* and *Heracleum* represent furanocoumarins which show less blue or violet fluorescence but mainly light-, orange- or green-yellow fluorescence under 365 nm, less blue quenching spots but mainly purple or violet dark spots under 254 nm. *Aralia cordata* shows only one fluorescent spot at R*f* 0.06. The main composition of different *Angelica pubescens* samples seems similar, but the content of each compound varies.

HPLC fingerprint analysis:

1) Sample preparation:
   The extracts prepared for TLC analysis can be used also for HPLC analysis.

2) Injection volume: 5µl

3) HPLC data:

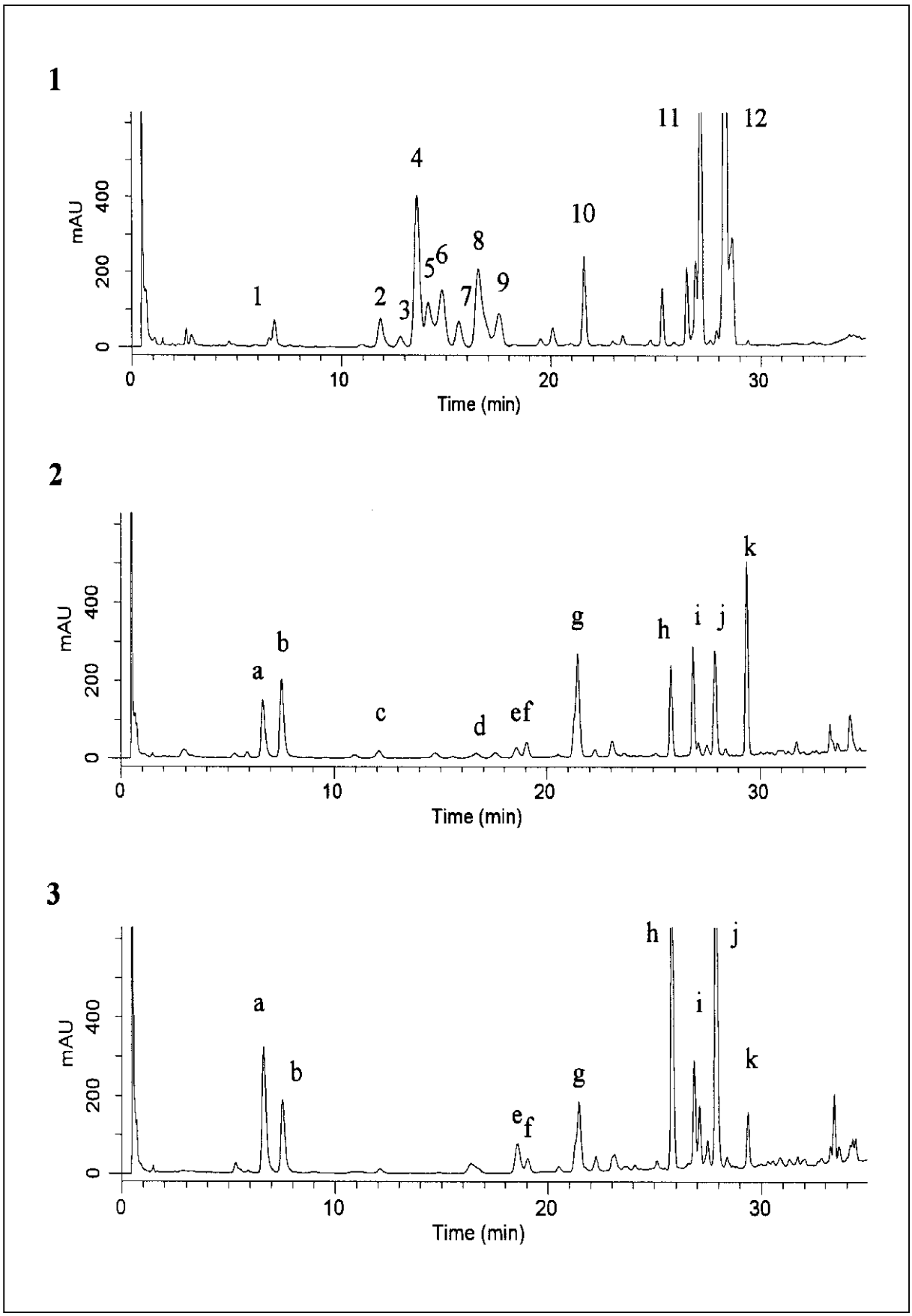
Apparatus:	Liquid Chromatograph HP 1050 with DAD HP 1040M (Hewlett Packard)
Column:	LiChroCART 125–4 with LiChrospher® 100 RP 18 (5 µm), Merck.
Precolumn:	LiChroCART 4–4 with LiChrospher® 100 RP 18 (5 µm), Merck.
Solvent system:	A: water B: acetonitrile

Gradient: linear 25–30 % B in 15 min, 30–70 % B in 15 min, 70–95 % B in 5 min.
Flow: 1.0 ml/min.
Detection: 210 nm

Drug samples (grown in):
1. *Angelica pubescens f. biserrata* (Hubei)
2. *Angelica dahurica* (Liaoning)
3. *Angelica apaensis* (Sichuan)
4. *Heracleum moellendorffii* (Sichuan)
5. *Heracleum candicans* (Yunnan)
6. *Aralia cordata* (Sichuan)

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	6.9	columbianetin
2	12.0	angelol-B
3	12.9	angelol-D
4	13.8	angelol-A
5	14.3	angelol type coumarin
6	15.0	angelol-K
7	15.8	angelol type coumarin
8	16.7	angelol type coumarin
9	17.7	angelol type coumarin
10	21.7	columbianetin acetate
11	27.2	osthol
12	28.3	columbianedin
a	6.6	furanocoumarin
b	7.5	furanocoumarin
c	12.1	xanthotoxin
d	16.7	bergapten
e	18.6	furanocoumarin
f	19.0	furanocoumarin
g	21.5	furanocoumarin
h	25.8	imperatorin
i	26.9	phellopterin
j	27.9	isoimperatorin
k	29.4	falcarindiol
l	16.4	isopimpinellin
m	18.9	pimpinellin
n	19.5	isobergapten
o	11.0	furanocoumarin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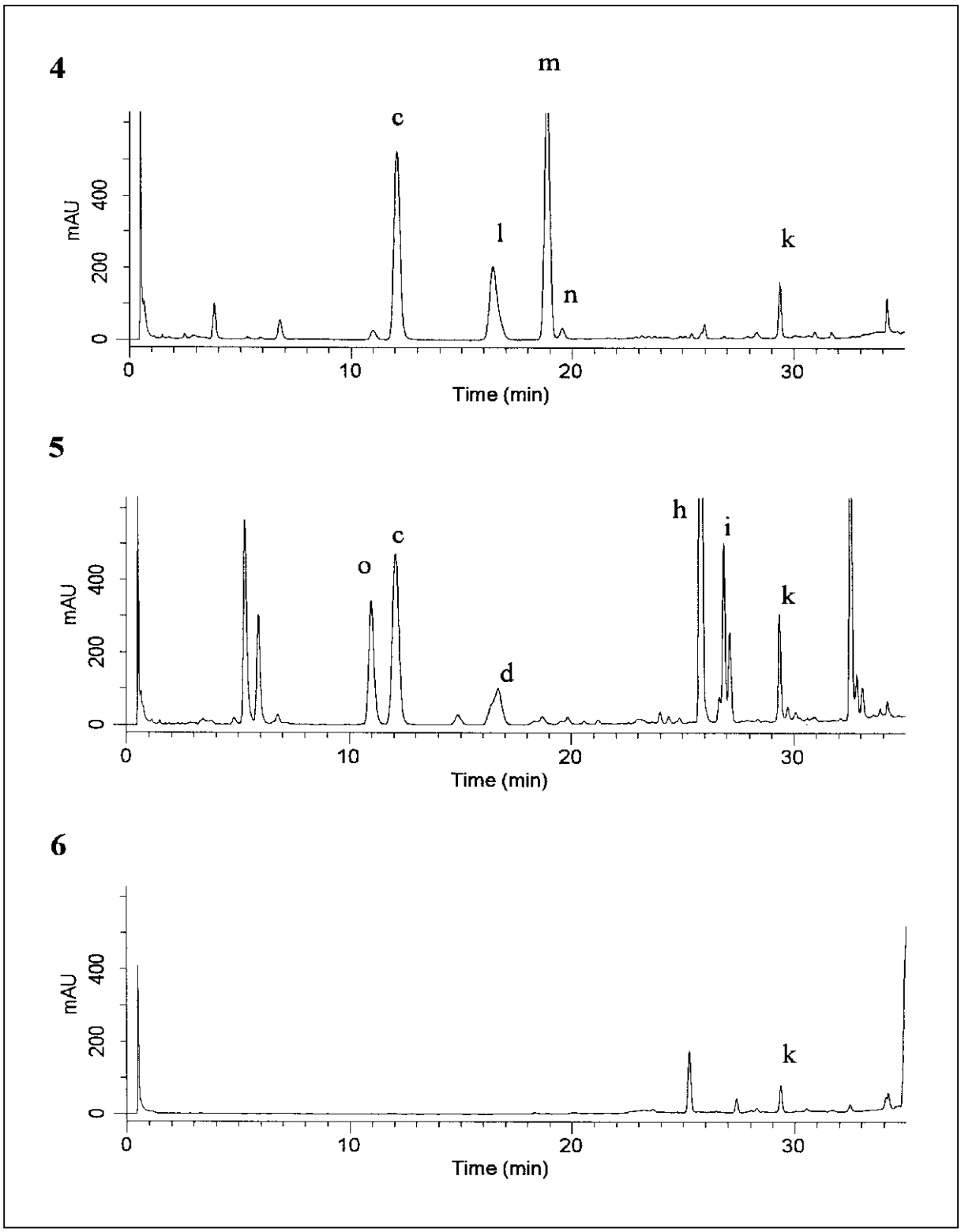

Fig. 5: HPLC fingerprint analysis of extracts from Angelicae pubescentis radix and some substitutes

1. *Angelica pubescens f. biserrata*; 2. *Angelica dahurica*; 3. *Angelica apaensis*;
4. *Heracleum moellendorffii*; 5. *Heracleum candicans*; 6. *Aralia cordata*

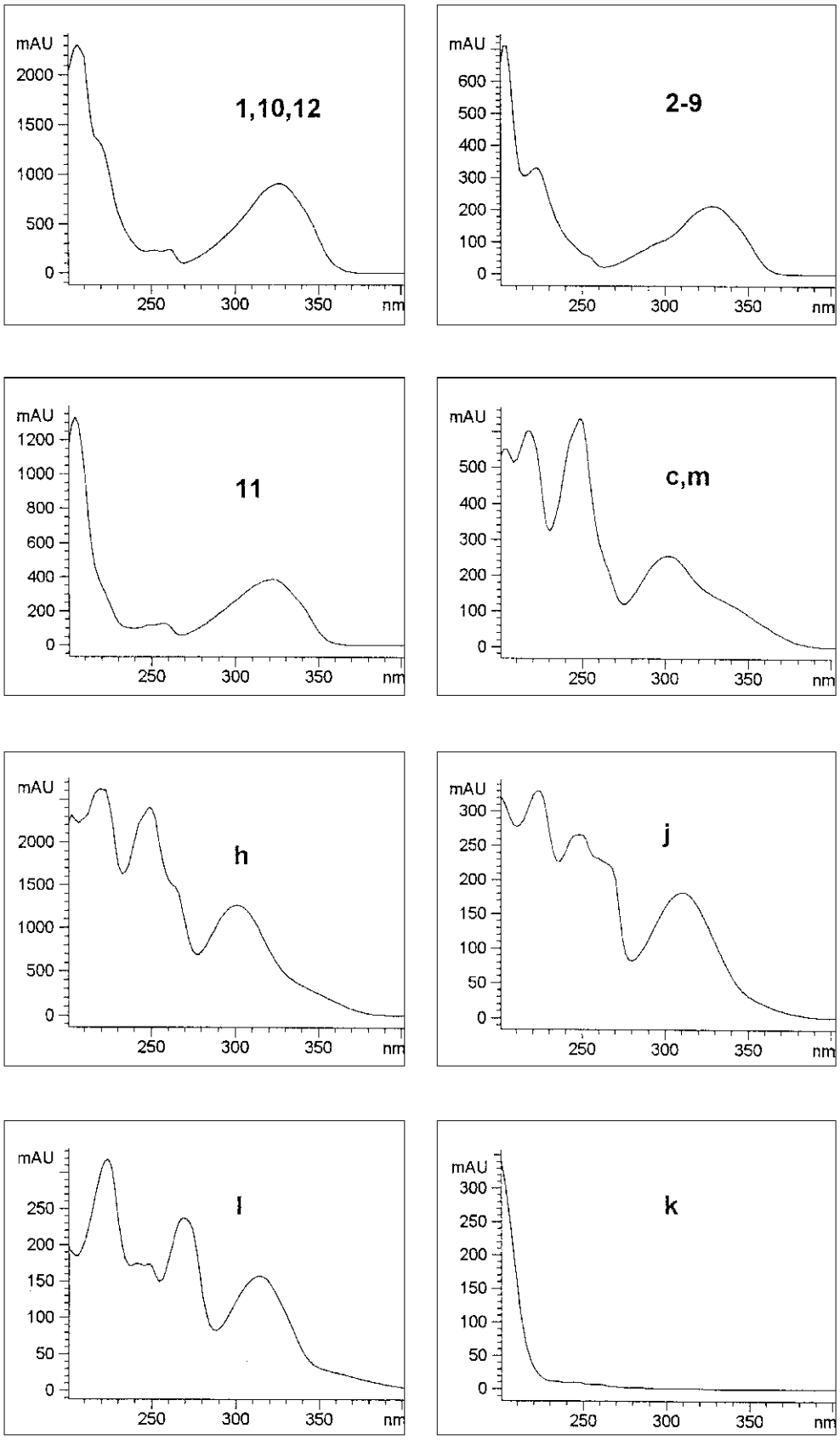

Fig. 6: UV-spectra of the major compounds in *Angelica pubescens* and some substitutes

4) Description of the chromatograms: (Fig. 5,1–6)

Besides osthol (Rt 27.1), columbianetin (Rt 6.9), columbianetin acetate (Rt 21.6) and columbianedin (Rt 28.3), angelol-type coumarins (2–9) present characteristic peaks in extracts from *Angelicae pubescentis* radix (see Fig. 5). Distinct different constituents can be seen in the substitutes. Besides a peak (k) of a polyacetylene at Rt 29.4 (falcarindiol), most of the detectable compounds are furanocoumarins (a-o), which can be easily distinguished by DAD recorded UV spectra (see Fig. 6). Imperatorin (Rt 25.8), phellopterin (Rt 26.9) and isoimperatorin (Rt 27.9) are major constituents in *Angelica dahurica* and *Angelica apaesis*. Xanthotoxin (Rt 12.1), isopimpinellin (Rt 16.4), pimpinellin (Rt 18.9) are characteristic of *Heracleum moellendorffii, H. rapula* and *H. stenopterum*. *Heracleum candicans* is dominated by imperatorin and xanthotoxin. No coumarin peak appears in the chromatogram of the extract from *Aralia cordata*.

Discussion:

In Chinese medicinal market, especially in Southwest of China, many *Heracleum* species (called niuwei duhuo) and some *Aralia* species (called jiuyan duhuo) are sold as substitutes of duhuo. They can be easily distinguished from *Angelica pubescens* by TLC, because *Angelica pubescens* contains mainly dihydrofuranocoumarins and 6- or 8-prenylcoumarin derivatives, which show prominent blue or violet-blue fluorescence under UV 365 nm. In contrast, the constituents in substitutes of *Angelica* and *Heracleum* species are mainly composed of furanocoumarins which show light-, orange- and green-yellow fluorescence instead of blue or violet-blue. *Aralia cordata* shows only one fluorescent spot under both 254 nm and 365 nm. The content of osthol and columbianedin which are major constituents in *Angelica pubescens* were found to be different in various samples, which indicates varying quality of the drugs on the market. They can be analyzed easily by HPLC. The retention times of the main peaks in HPLC fingerprints of *Angelica pubescens* also differ from those in the drug substitutes. Therefore, they can easily be distiguished by TLC and HPLC.

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Rhizoma Atractylodes macrocephalae
Baizhu

Pharmacopoeias: Pharmacopoeia of the People's Republic of China, English Edition 1992/2005[1], Japanese Pharmacopoeia, English Edition 1996 (Jap. XIII)

Official drugs: In Chinese Pharmacopoeia: the rhizomes of *Atractylodes macrocephala* Koidz. – Asteraceae – (the title of the monograph is "Rhizoma Atractylodis macrocephalae", the second monograph "Rhizoma Atractylodis" deals with another *Atractylodes*-species, *Atractylodes lancea*, Chinese: *Cangzhu*).

The Japanese Pharmacopoeia accepts *Atractylodes ovata* (= *Atractylodes macrocephala*) together with *Atractylodes japonica* (Japanese: *wabyakujutsu*) in one monograph as "Atractylodes Rhizome" whereas *Atractylodes lancea* is listed in the monograph "Atractylodes lanceae Rhizome".

Therefore "Atractylodes rhizomes" are derived from *Atractylodes lancea* in China and from *Atractylodes macrocephala* or *Atractylodes japonica* in Japan.

Synonyma: *Atractylodes ovata* DC. rhizome, largehead Atractylodes rhizome, white Atractylodes rhizome, *yu zhu,* Japanese: *kara-byakujutsu*

Description of the drug[1,3]:

In irregularly plump masses. 3–13 cm long, 1.5–7 cm in diameter. Externally greyish-yellow or greyish-brown, with warty protrudings, interrupted longitudinal wrinkles and glooves, and scars of fibrous rootlets, remains of stems and bud scars attached to the apex. Texture hard, uneasily broken, fracture uneven, yellowish-white to brownish, scattered with brownish-yellow dotted oil cavities. The material dried by baking appearing horny and relatively deep coloured or cracked.

Allied drug: Dried rhizomes of *Atractylodes japonica* Koidz. ex Kitam. (Japan), see "Official drugs".

Pretreatment of raw drug:

After elimination of the foreign matter the drug is washed, softened thoroughly, cut into thick slices and dried (*Baizhu*), the slices are stir-fried with fine powders of terra flava usta until the outer surface ashens, then sifted (*Tubaizhu*). In a hot pot, the slices are added to bran (stir-fried with honey before) and heated until burnt yellow, taken out and sifted (*Chaobaizhu*).

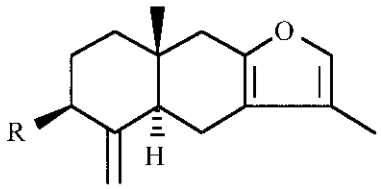
Medicinal use[4,5]: In Traditional Chinese Medicine recommended as digestive, diuretic and antihydrotic. In combination with other aromatics used for the treatment of anemia, bronchitis, cough, diarrhea, dysuria, eczema, edema, gasping, gastroenteritis, jaundice, nausea, nightsweats, vertigo, anorexia, dyspepsia and as a sedative during pregnancy.

Effects and indications according to Traditional Chinese Medicine[4,7]

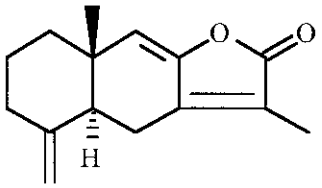
Taste:	bitter, sweet
Temperature:	warm
Channels entered:	spleen and stomach
Effects:	1. replenishes *qi* and strengthens the *spleen*, 2. resolves dampness and promotes water metabolism, 3. stops sweating and calms the fetus. *Rhizoma Atractylodis macrocephalae processed with terra:* invigorates the function of the *spleen*, regulates the function of the *stomach* and prevents miscarriage
Symptoms, Indications:	Hypofunction of the *spleen* with anorexia, abdominal distension and diarrhea; dizziness and palpitation due to retention of *phlegm* and fluid; edema; spontaneous sweating; threatened abortion. *Rhizoma Atractylodis macrocephalae processed with terra:* Hypofunction of the spleen with anorexia and diarrhea; threatened abortion

Main constituents (see Fig. 1):

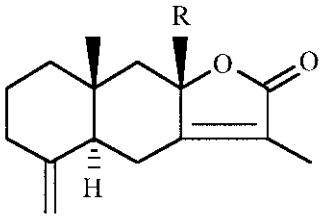
- **Sesquiterpene hydrocarbons** (β-maaliene, eremophila-1[9],11-diene, cyperene, *trans*-caryophyllene, γ-elemene, α-humulene, acoradiene, γ-patchoulene, aromadendrene), furyl sesquiterpenes (atractylon, 3β-hydroxyatractylon, 3β-acetoxyatractylon), and sesquiterpene lactones (atractylenolides I, II, III, IV; 8-ethoxyasterolide)[7–10]
- a sesquiterpene lactame: atractylenolactame[15]
- **polyacetylenes** (tetradeca-4*E*,6*E*,12*E*-triene-8,10-diyne-1,3,14-triol and derivatives)[8,11]
- **polysaccharides** AM-1, AM-2, AM-3[12,13]
- the coumarin scopoletin[8]
- **In addition:** Sitosterol, triterpenoid esters and palmitic acid[9,14]



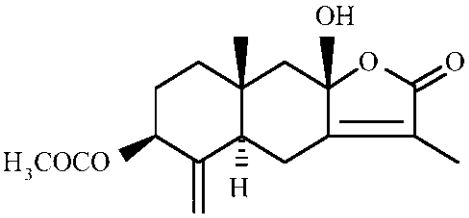
R = H : Atractylon
R = OH : 3ß-Hydroxyatractylon
R = OAc : 3ß-Acetoxyatractylon



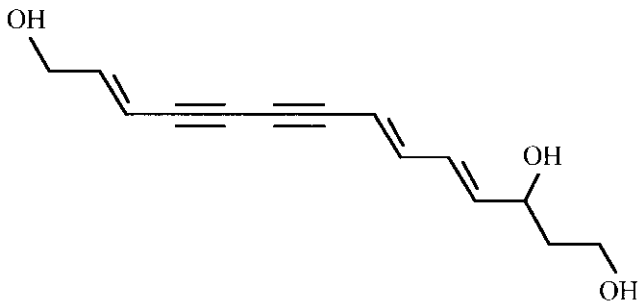
Atractylenolide I (Dehydroasterolide)



R = H : Atractylenolide II (Asterolide)
R = OH: Atractylenolide III (8ß-Hydroxyasterolide)



Atractylenolide IV



Tetradeca-4*E*,6*E*,12*E*-triene-8,10-diyne-1,3,14-triol and derivatives
(e.g. acetoxyl-, senecioyl-, methylbutyrylesters; *E/Z*-isomers)

Fig. 1: Formulae of the main compounds

Pharmacology[2,16]:

In vitro effects

– antineoplastic activity[17]

– phototoxic for *Saccharomyces cerevisiae*[18]

In vivo effects:

– inhibition of stress-induced ulcera in rats (50 % methanol extract, atractylon)[19]

– increase of the body weight and swimming endurance of mice after intragastric administration of *Atractylodes macrocephala* decoction[20]

– stimulation of the phagocytic function of the reticuloendothelial system[21]
– increase of leukocytes in patients with leukopenia[22]
– diuretic effects (rats, rabbits, dogs) and hypoglycemic action (rats) – both could not yet be confirmed due to the limited number of cases studied[23,24]
– anticoagulant activity (rats)[25]
– actions on the cardiovascular system (vasodilatory and cardiac depressant action, reduction of the blood pressure)[23,26]
– liver-protective effects (mice)[27]

TLC fingerprint analysis:

1) Extraction:

   5 g coarsely ground drug are soxhlet-extracted with 120 ml *n*-hexane p.a. for 2 hours. The extract is evaporated to dryness and the residue is redissolved in 5.0 ml ethanol p.a.

2) Standards: Atractylon, atractylodin, 3-β-acetoxyatractylon, atractylenolide I, dissolved in ethanol p.a. (1 mg/ml).

3) Separation parameters:

Applied amount:	15 µl extract, 10 µl standard
Plates:	Silica gel 60 F_{254}, Merck
Solvent system:	*n*-hexane - ethyl acetate (95 + 5), no saturation in the TLC chamber
Direct evaluation:	UV 254
Spray reagents:	Anisaldehyde-sulphuric acid reagent (0.5 ml anisaldehyde + 85 ml methanol + 10 ml glacial acetic acid + 5 ml conc. sulphuric acid are mixed in this order). The plate is sprayed with approx. 10 ml of the reagent and heated for 5–10 min. at 100 °C. The evaluation is carried out in VIS.

4) Thin layer chromatograms and descriptions:

Drug samples of Fig. 2a and 2b

1 Atractylodis macrocephalae rhizoma, TCM-Hospital Kötzting, Germany
2 Atractylodis japonicae rhizoma, Botanical Garden of the University of Düsseldorf
3 Atractylis koreanae rhizoma, Kang Weon Province, South Korea
4 Atractylodis lanceae rhizoma, TCM-Hospital Kötzting, Germany
5 Atractylodis chinensis rhizoma, Kunming

Reference compounds:

T1 atractylon, R*f* = 0.90
T2 atractylodin, R*f* = 0.70
T3 β-acetoxyatractylon, R*f* = 0.33
T4 atractylenolide I, R*f* = 0.28

Description of the chromatograms

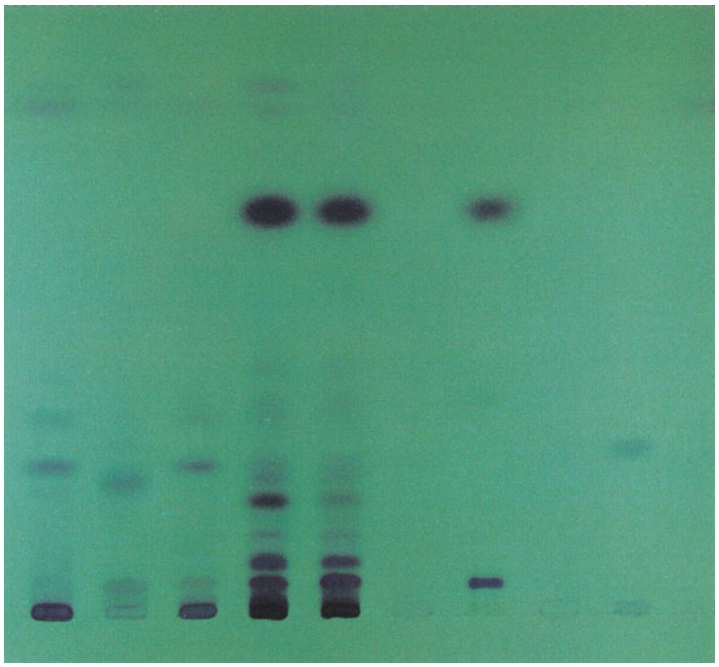


Fig. 2a: Thin layer chromatogram of different *Atractylodes* species and reference compounds (UV 254 nm)

UV 254 nm (**Fig. 2a**):

Atractylodes macrocephala rhizomes (**1**): Direct evaluation in UV 254 nm shows weak quenching spots at R*f* 0.9 (atractylon, **T1**) and 0.28 (atractylenolide I, **T4**). Under these conditions, few more quenching zones are distributed over the entire R*f* range in comparison to samples **4** and **5**, *Atractylodes lancea* and *Atractylodes chinensis* rhizomes respectively, in which the extracts are dominated by the quenching spot of the polyacetylene atractylodin (**T2**, R*f* 0.7) and some spots in the start region. *Atractylodes japonica* rhizomes (**2**) can be distinguished from *Atractylodes macrocephala* rhizomes (**1**) (in this concentration) by the lack of atractylenolide I at R*f* 0.28. However, there is another quenching zone at R*f* 0.24.

Anisaldehyde-sulphuric acid reagent, VIS (**Fig. 2b**):

In all *Atractylodes* species, atractylon (**T1**) appears as a yellow-orange zone at R*f* 0.9. Heated more strongly, the colour changes to dark orange, finally to brown-purple (see also the colour of its derivative β-acetoxyatractylon (**T3**) at R*f* 0.33 in extracts **4** and **5**). Again, the main compound of **4** and **5** – atractylodin (**T2**), occuring as a characteristic dark green spot at R*f* 0.7 – is not visible in samples **1**, **2** and **3** under these conditions. **T4** (atractylenolide I) cannot be detected with that spray reagent (there is just a very weak purple zone at R*f* 0.28). A distinction between herbal drugs **1**, **2** and **3** which appear very similar in TLC is better performed by HPLC.

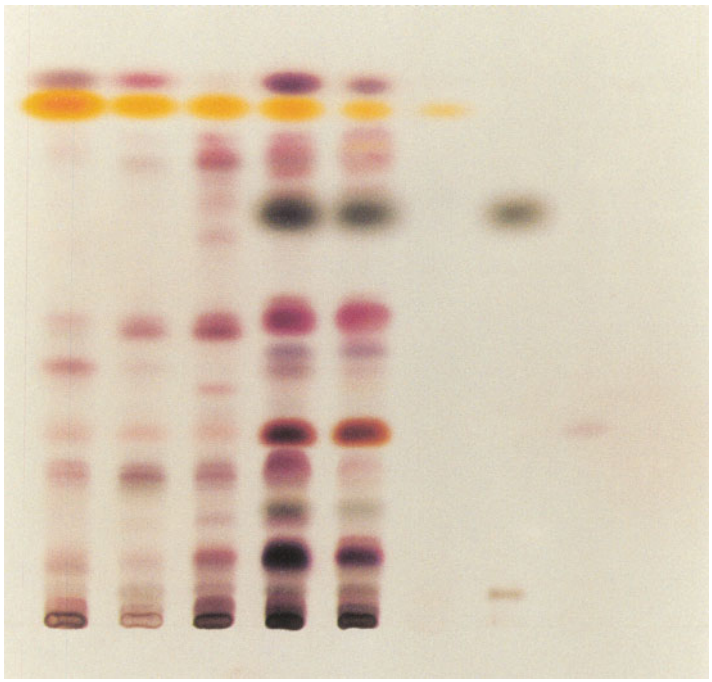


Fig. 2b: Thin layer chromatogram of different *Atractylodes* species and reference compounds (anisaldehyde-sulfuric acid reagent, VIS)

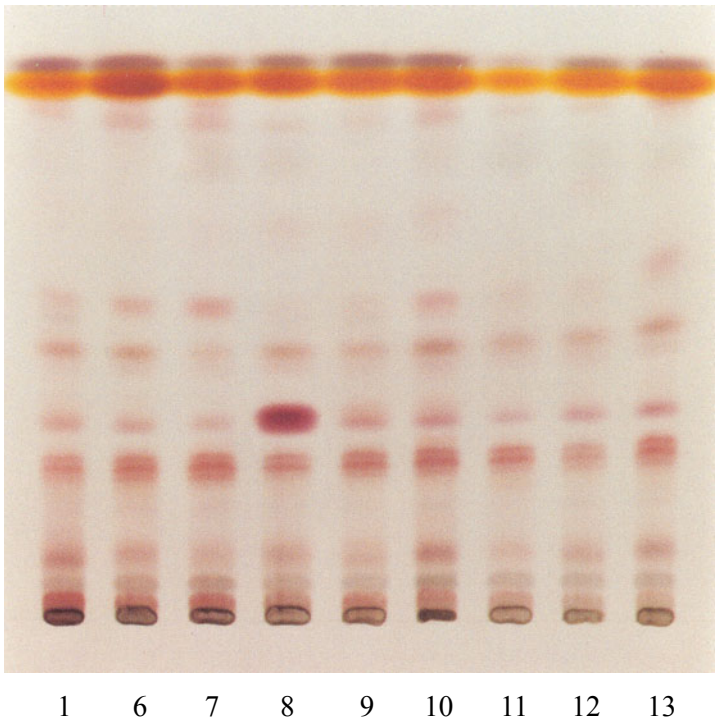


Fig. 3: Thin layer chromatogram of *Atractylodes macrocephala* rhizomes from various origins after detection with anisaldehyde-sulphuric acid reagent, VIS

Drug samples of Fig. 3

1 Atractylodis macrocephalae rhizoma, TCM-hospital Kötzting, Germany
6 Atractylodis macrocephalae rhizoma, Kunming
7 Atractylodis macrocephalae rhizoma, Dong-zhimen hospital, Beijing
8 Atractylodis macrocephalae rhizoma, Shanghai
9 Atractylodis macrocephalae rhizoma, Hong-Kong
10 Atractylodis macrocephalae rhizoma, Shaanxi Province
11 Atractylodis macrocephalae rhizoma, Beijing
12 Atractylodis macrocephalae rhizoma, Guang-zhou
13 Atractylodis macrocephalae rhizoma processed with bran (*chaobaizhu*), Phytopet, Andorra

Description of the chromatogram (**Fig. 3**):

Anisaldehyde-sulphuric acid reagent, VIS:

Comparison of the different *Atractylodes macrocephala* samples shows a very similar pattern for all samples. A violet zone at R*f* 0.95 (mainly generated by compounds from the essential oil) is followed by the characteristic zone of atractylon (see Fig. 2b). More or less intensive brown-red zones can be seen at R*f* 0.53, 0.46, 0.34 (3β-acetoxyatractylon, **T3**), between R*f* 0.23–0.3 and near the start zone. The sample from Shanghai (**8**) differs from the other samples by its high content of 3β-acetoxyatractylon at R*f* 0.34.

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract used for TLC over Millipore® filtration unit, type HV 0.45/µm.

2) Injection volume: 2 µl ethanolic total extract (conc. = 5 g drug/5ml)

3) HPLC data:

Apparatus:	Liquid Chromatograph HP 1050 (Hewlett Packard)	
Column:	LiChroCART® 125-4 with LiChroSpher® 100 RP18 (5 µm), Merck	
Pre column:	LiChroCART® with 4-4 LiChroSpher® 100 RP18 (5 µm), Merck	
Solvent system:	A: water B: acetonitrile	
Gradient:	linear:	62–70 % B in 10 min. 70–95 % B in 2 min.
	isocratic:	95 % B (8 min.)
Flow:	1.0 ml/min.	
Detection:	215 nm, 255 nm, 365 nm	

Description of the HPLC – chromatograms:
Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	2.6	Atractylenolide III
2	3.5	Polyacetylene with ene-diyne-diene-chromophore
3	3.9	Polyacetylene with ene-diyne-diene-chromophore
4	4.4	Atractylenolide II
5	6.1	Atractylenolide I
6	8.2	Atractylodin
7	13.6	Atractylon
8	15.4	Sesquiterpene(s)

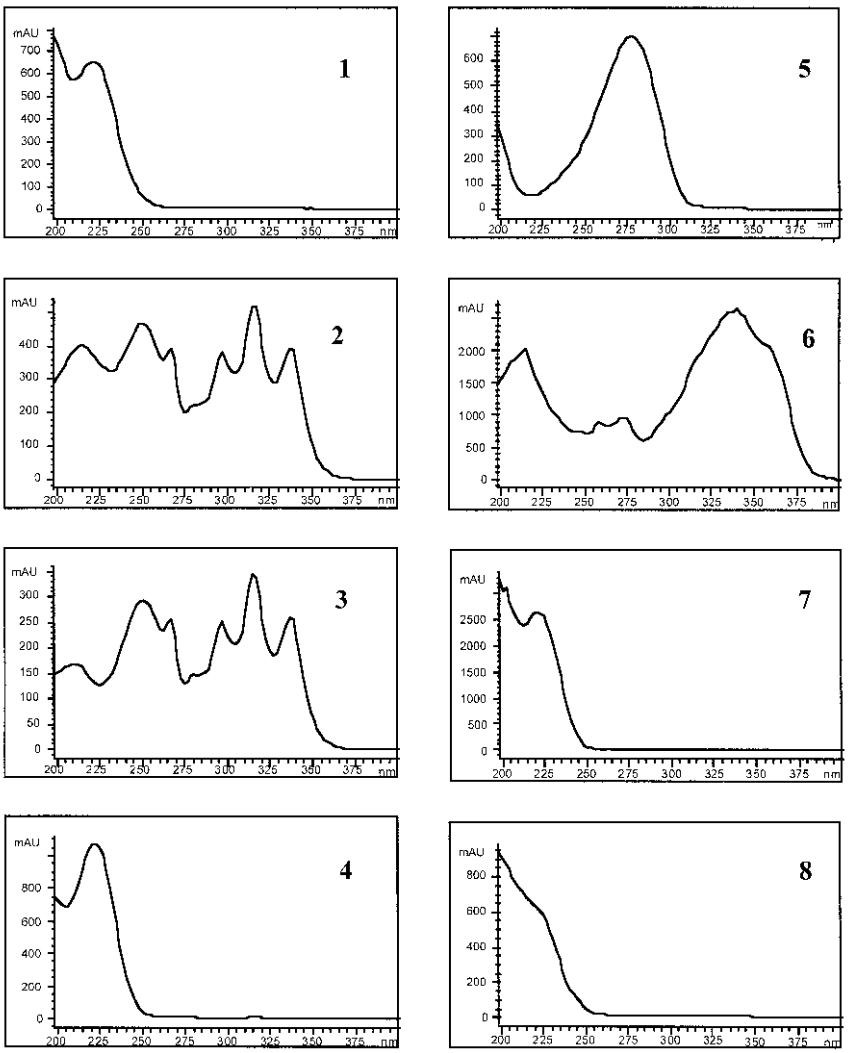


Fig. 4: UV spectra of the main compounds

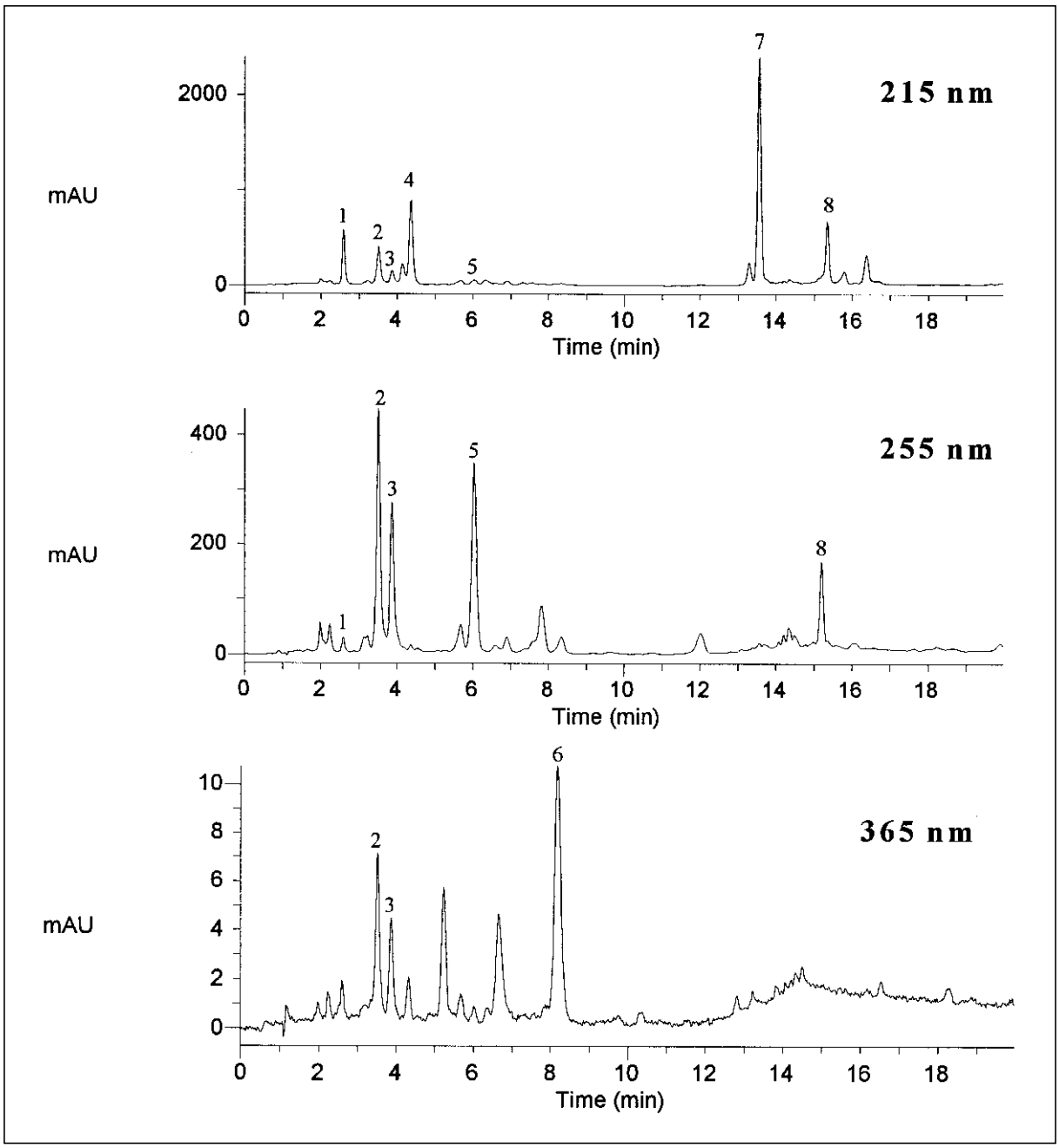


Fig. 5: HPLC fingerprint chromatograms of the *n*-hexane extract of *Atractylodes macrocephala* rhizomes (drug sample **1**, TCM-Hospital Kötzting, Germany)

The chromatogram detected at UV 215 nm is characterized by the main compound atractylon (**7**) and by the peaks of atractylenolides II (**4**) and III (**1**). The polyacetylenes (**2** and **3**) and atractylenolide I (**5**) are predominant peaks of *Atractylodes macrocephala* rhizomes when detected at UV 255 nm. Peak **8** (the UV spectrum and other investigations indicate a mixture of sesquiterpenes) is essential in both chromatograms. By detection at UV 365 nm traces (see mAu-values) of the polyacetylene atractylodin can be detected in most of the investigated *Atractylodes macrocephala* samples. Interestingly, atractylodin (**6**) is the principal compound of the *n*-hexane extract of *Cangzhu* (*Atractylodes lancea* rhizomes) and was never described as a compound of *Atractylodes macrocephala* in the literature before.

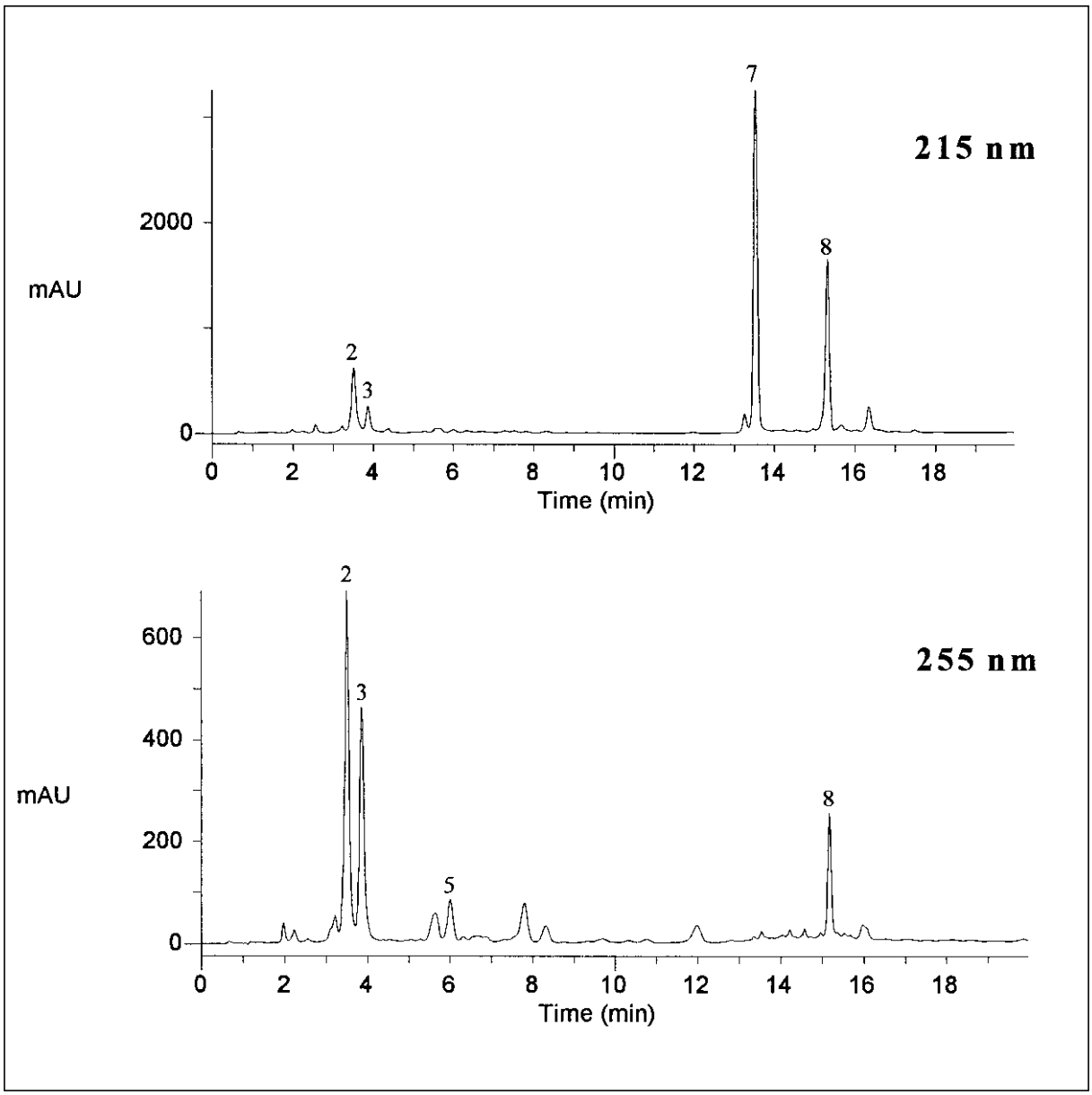


Fig. 6: HPLC fingerprint chromatograms of the *n*-hexane extract of *Atractylodes macrocephala* rhizomes from Kunming (drug sample **6**)

The chromatograms of *Atractylodes macrocephala* rhizomes from Kunming demonstrate another extreme of *Atractylodes macrocephala* samples: there is a lack of peaks compared to sample **1** (Fig. 5) but the drug contains higher amounts of atractylon (**7**). Besides just the polyacetylenes (**2**,**3**) – dependent from the detection wavelength – and the presumed sesquiterpene(s) (**8**) exhibit intensive signals. The atractylenolides (except atractylenolide I, **5**) are missing.

Detection at UV 365 nm gave no significant atractylodin signal.

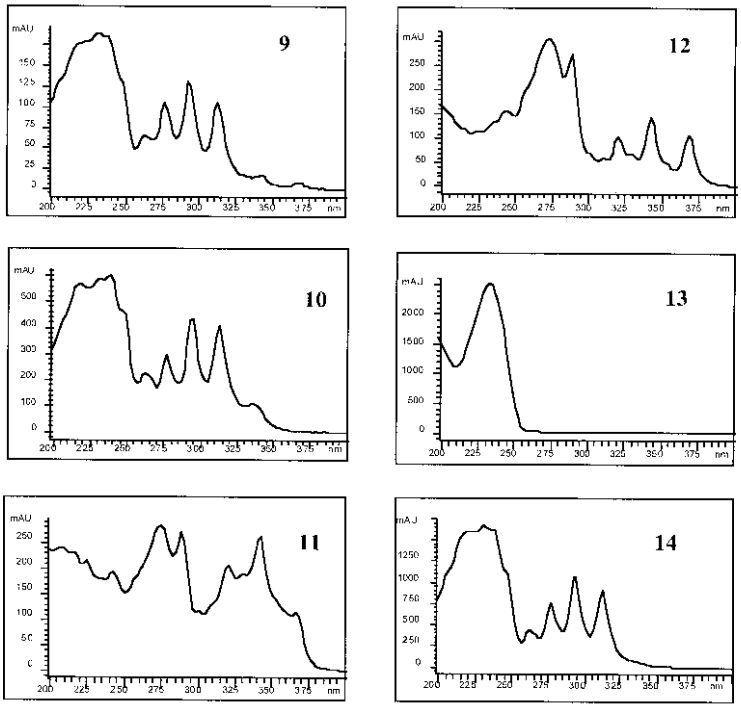


Fig. 7: UV spectra of compounds **9-14** from *Atractylodes japonica* or *Atractylis koreana* rhizomes

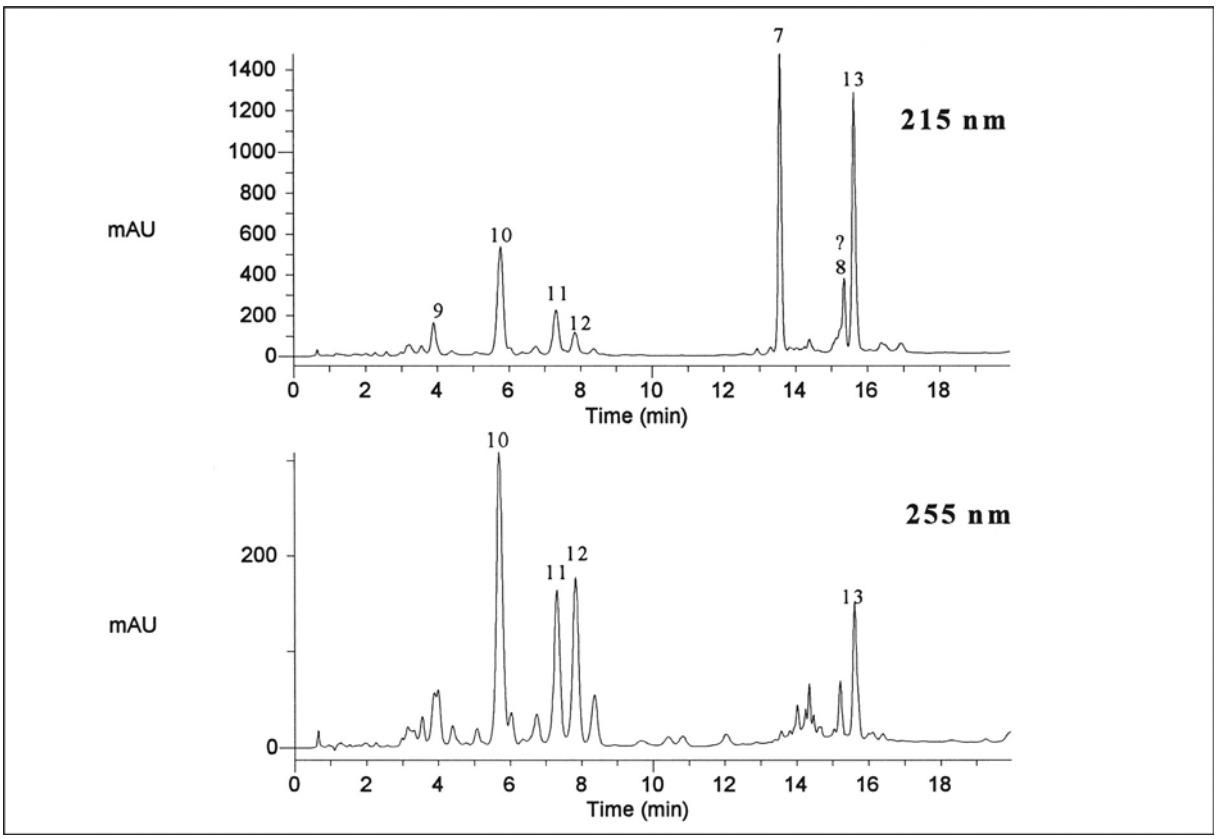


Fig. 8: HPLC fingerprint chromatograms of *n*-hexane extracts of *Atractylodes japonica* rhizomes grown in the Botanical Garden of the University of Düsseldorf (drug sample **2**)

Although there is some similarity on the TLC plate, HPLC offers a good possibility to distinguish *Atractylodes macrocephala* and *Atractylodes japonica* rhizomes: apart from atractylon, the main peak at UV 215 nm (**7**) – which is common to both – their fingerprint chromatograms are completely different. Except peak **13** at 15,6 min. (which shows one absorption maximum at 234 nm) the other major peaks at UV 215 nm are polyacetylenes with UV spectra different from those of **2** and **3** (see Figures 4 and 7). Peaks No. **9** at 3,9 min. No. **10** at 5,8 min. and No. **14** at 2,0 min. gave the UV spectrum of an ene-diyne-ene chromophore (all maxima shifted to lower wavelength), while **11** and **12** reveal UV spectra with a bathochromic shift. So detection at UV 215 nm is sufficient to distinguish between these species and detection at UV 255 nm (or 365 nm) provides no further information.

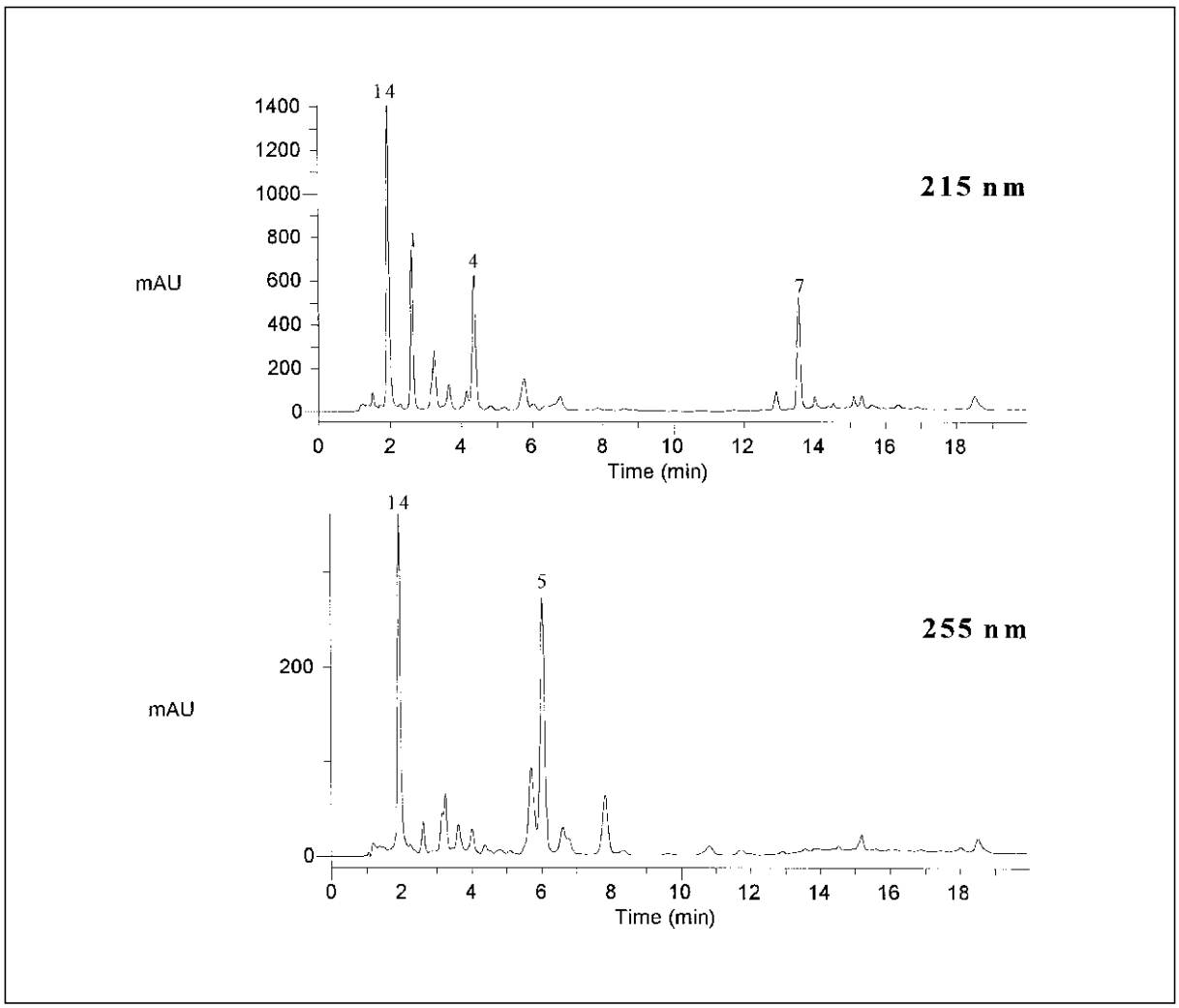


Fig. 9: HPLC fingerprint chromatograms of the *n*-hexane extract of *Atractylis koreana* rhizomes, Kang Weon Province (drug sample **3**)

In the HPLC fingerprint chromatograms, *Atractylis koreana* rhizomes are completely different from *Atractylodes macrocephala* rhizomes. Although *Atractylis koreana* shows atractylenolides I (**5**), II (**4**) and III (**1**) and atractylon (**7**), the latter is not the major compound in UV 215 nm: *Atractylis koreana* rhizomes mainly contain of an ene-diyne-ene-chromophore polyacetylene (Fig. 7) at Rt 13,6 min. which – together with **5** – dominates the chromatogram at 255 nm. The intensive peak **8** is missing.

Discussion:

By TLC analysis, *Atractylodes macrocephala* rhizomes strongly differ from *Atractylodes lancea* and *Atractylodes chinensis* rhizomes by the lack of the polyacetylene atractylodin (an intensive green spot) in the former. HPLC at UV 215 nm is the best method to distinguish *Atractylodes macrocephala* rhizomes from *Atractylodes japonica* and *Atractylis koreana* rhizomes. As the HPLC pattern for all examined *Atractylodes macrocephala* samples was very uniform and just differs in quantitative aspects, drugs that show the presented HPLC fingerprint of *Atractylodes japonica* or *Atractylis koreana* (or other significantly different chromatograms) should not be accepted as *Baizhu* as long as it is not pharmacologically proven that the three related species are equal in their potency.

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Rhizoma Belamcandae sinensis
Shegan

Pharmacopoeia: Pharmacopoeia of the People`s Republic of China, English edition 1992/2005[1]

Official drugs: The rhizomes of
– *Belamcanda sinensis* (L.) DC.
– Iridaceae

Places of origin: China (Anhui, Guangdong, Guangxi, Henan, Hubei, Jiangsu, Zhejiang Provinces)
Japan
Korea

Description of the drug[3]: Yellow-brown and black-brown wrinkled sections which are 3–10 cm long and 1–2 cm in diameter, and whose surface exhibits closely spaced rings, stem scars and secondary roots.

Adulterants: *Iris tectorum* Maxim, *Iris dichotoma*, *Iris japonica* Thunb.

Pre-treatment of the raw drug[3]:
Cleaned, moistened, cut into thin slices and dried.

Medicinal use[2,4]: In Traditional Chinese Medicine as anti-asthmatic, anti-allergic, antiphlogistic, antipyretic, expectorant, mucolytic, tuberculostatic, gynecologic, hepatic, carminative, purgative, cytostatic drug and also in cases of malaria.

Effects and indications according to Traditional Chinese Medicine[2–6]

Taste:	bitter, slightly sharp
Temperature:	cold
Channels entered:	lungs, liver, spleen
Effects:	reduces heat, balances out temperature, detoxifies, dissolves mucous, drains mucous and saliva
Symptoms and indications:	coughing, shortness of breath, asthma; painful, swollen throat and larynx; congested mucous with coughing; tuberculosis; irregularity and pain during menstruation; stomach pains and digestive problems; through healing the liver the eyes are made clear; furuncles and skin ulcers; intestinal disorders; malaria and intermittent fever; abundance of heat.
Counter-indications:	pregnancy, weakness of the spleen with diarrhoea

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents: (see Fig. 1)

- **isoflavonoids** (belamcandinin, **iridin, irigenin**, irisolidinon, irisflorentin, iristectorigenin A, munginin, **tectoridin, tectorigenin**)[7–11]
- **flavonoids** (Rhamnocitrin)[9]
- **acetophenones** (apocynin, androsin, picein, acetoveratron, tectoruside)[12,13]
- **ketones** (iriphlophenone glycoside, sheganon)[12,13]
- **triterpenes** and spirobicycloiridals[14]
- **xanthones** (mangiferin)[15]
- **stilbene compounds** (resveratrol, dihydroresveratrol)[13]

In addition: flavanones, protocatechuic acids, vanillin acid, coumaric acid, p-hydroxy-benzoic acid, adenin, sitosterin[2,13]

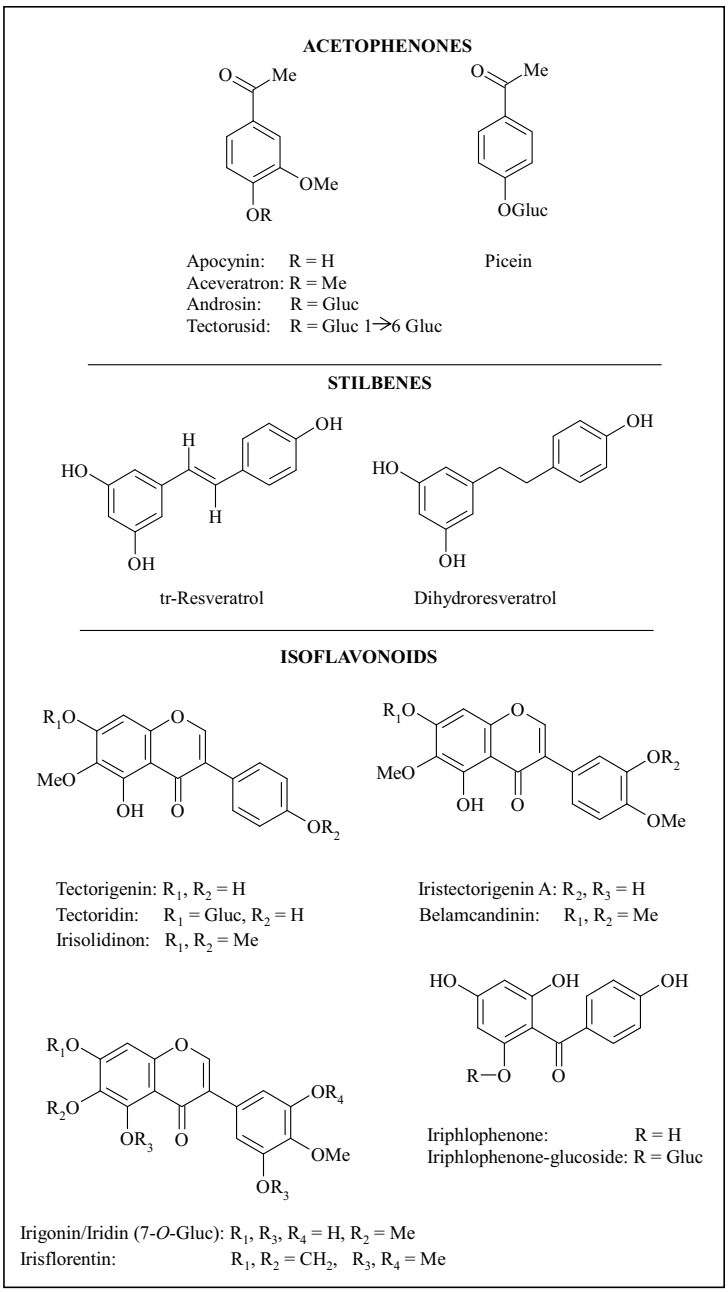


Fig. 1: Formulae of the main compounds

Pharmacology:

in vitro effects:

- antifungal[2]
- antiviral[16]
- inhibition of aldose reductase[17]
- inhibition of 5-lipoxygenase[18]
- phytoestrogen activity[21,22]

in vivo effects:

- antitumoral (tectorigenin)[23]
- antifungal[2]
- antiviral[2]
- increases the secretion of mucous and saliva[2]
- acts on leukemia-P388 tumor cells[19]
- uterus-relaxing effect[20]
- barbiturate-potentiating effect[17]

TLC fingerprint analysis:

1) Extraction:

   10 g coarsely ground drug are extracted for 5 hours with 150 ml methanol p.a. in a Soxhlet apparatus. The drug extract is then filtered, the filtrate concentrated to approximately 15 ml and the solution filled up to 15 ml with methanol p.a. (room temperature approximately 20 °C).

2) Standards:

   Apocynin, androsin, iridin, irisflorentin, iriphlophenone glycoside, mangiferin, resveratrol, tectoridin, tectorigenin, tectoruside dissolved in ethanol p.a. (1 mg/ml).

3) Separation parameters:

Applied amount:	30 µl extract, 10 µl standard solution
Plates:	Silica gel 60 F_{254}, Merck
Solvent systems:	Solvent I: toluene-chloroform-methanol-formic acid (10+60+20+10) Solvent II: toluene-chloroform-methanol-formic acid (10+70+10+10) (acetophenones, benzophenones, flavones, isoflavones, stilbenes, xanthones)
Direct evaluation:	– VIS – UV 254 nm and UV 365 nm
Spray reagent:	Vanillin-sulphuric acid reagent (VS) 1 % ethanolic vanillin solution (I) 5 % ethanolic sulphuric acid (II) The TLC plates are sprayed vigorously with reagent I and II and heated at 100 °C for 5-10 minutes. They are evaluated in VIS.
Drug samples:	1. Commercial drug imported from China 2. Commercial drug from China (Sichuan Province) 3. *Iris tectorum*, commercial drug from Germany

4. Commercial drug from Japan.
5. Commercial drug from China (Shaanxi Province)
6. Commercial drug from China (Beijing)

Test substances: Solvent I:

T1 belamcandinin (R*f* 0,9), irisflorentin (R*f* 0,68), apocynin (R*f* 0,65)
T2 resveratrol (R*f* 0,38), androsin (R*f* 0,15)
T3 iridin, tectoridin (R*f* 0,1)
T4 mangiferin (R*f* 0,02)
T5 tectoruside (R*f* 0,01)

Solvent II:

T6 irisflorentin (R*f* 0,82), tectorigenin (R*f* 0,65), resveratrol (R*f* 0,61) T7 androsin (R*f* 0,4), tectoridin (R*f* 0,35), iriphlophenone glycoside (R*f* 0,17)

The TLC chromatograms of drug samples 1, 2 and 3 in solvent I/II toluene-chloroform-methanol-formic acid (10+60+20+10) and (10+70+10+10) in UV 365 nm, and after detection with VS reagent, are illustrated in Figures 2, 3 and 4.

4) Description of the chromatograms:

VIS: When evaluated directly in visible light, several yellow spots due to isoflavones, flavones and xanthones are visible in the middle and upper R*f* range (0,5–0,8).

UV 254 nm: Spots of quenching fluorescence can be found distributed over the entire R*f* range.

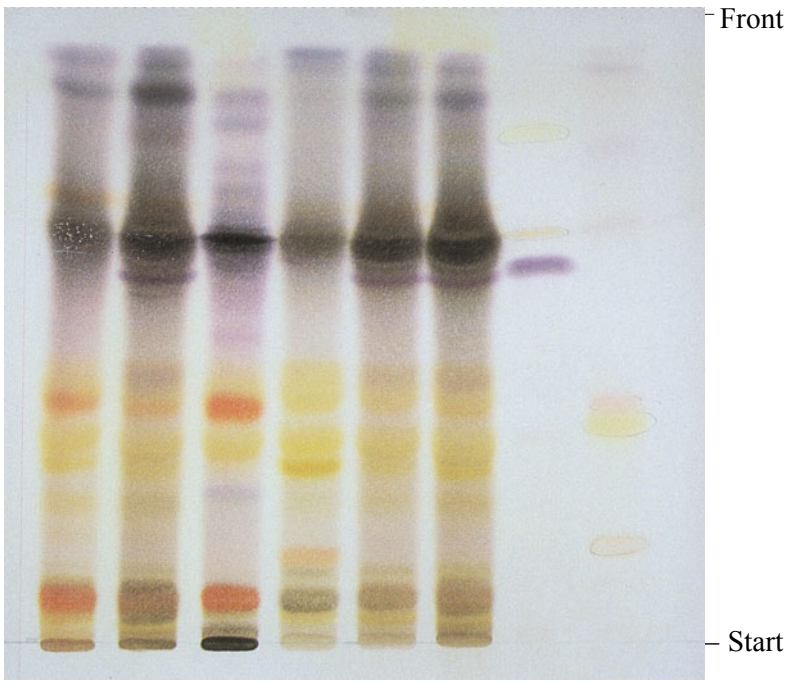


Fig. 2: Thin layer chromatogram of the drug samples 1-6 in the solvent I toluene-chloroform-methanol-formic acid (10+60+20+10) after detection with VS reagent (in VIS).

Terpene and stilbene compounds generate violet-black spots in the upper R*f* range. For most *Belamcanda* extracts the violet spot due to resveratrol is clearly visible at R*f* 0,61 (**T6**). The iriphlophenone glycoside (R*f* 0,17; **T7**) exhibits an orange colour. The isoflavonoid aglycones can be found in the upper (R*f* 0,8–0,9) and the yellow-brown isoflavonoid glycosides in the middle R*f*-range. The flavones appear as light yellow spots in the lower and middle R*f* range.

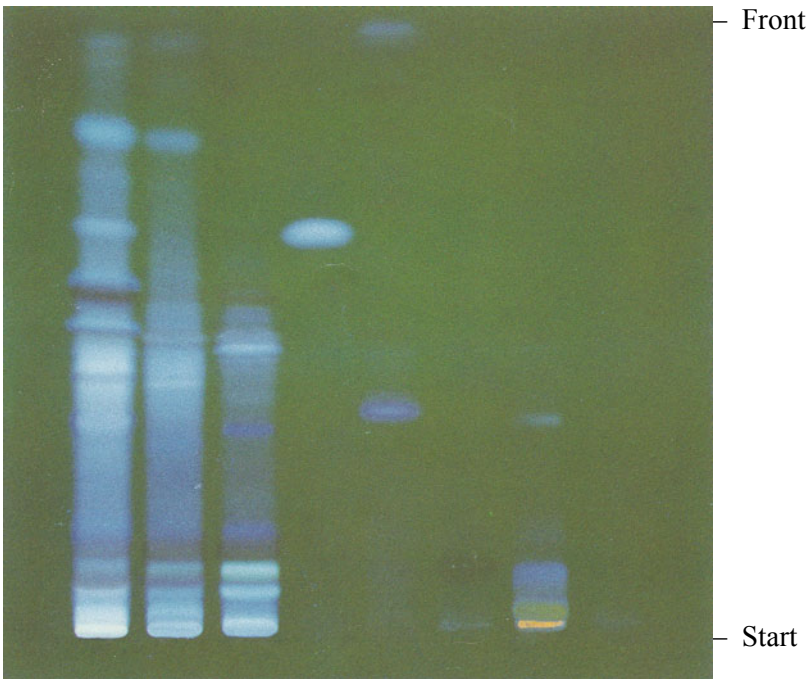


Fig. 3: Thin layer chromatogram of the drug samples 1, 2, 3 and reference compounds in the solvent II toluene-chloroform-methanol-formic acid (10+70+10+10) in UV 365 nm.

The blue fluorescent isoflavonoid aglycones and stilbenes (e.g. irisflorentin R*f* 0,68; **T1** and resveratrol R*f* 0,38; **T2**) appear in the upper and middle R*f* range. A grey spot due to the isoflavonoid glycosides iridin and tectoridin (R*f* 0,1; **T3**) appears in the lower range.

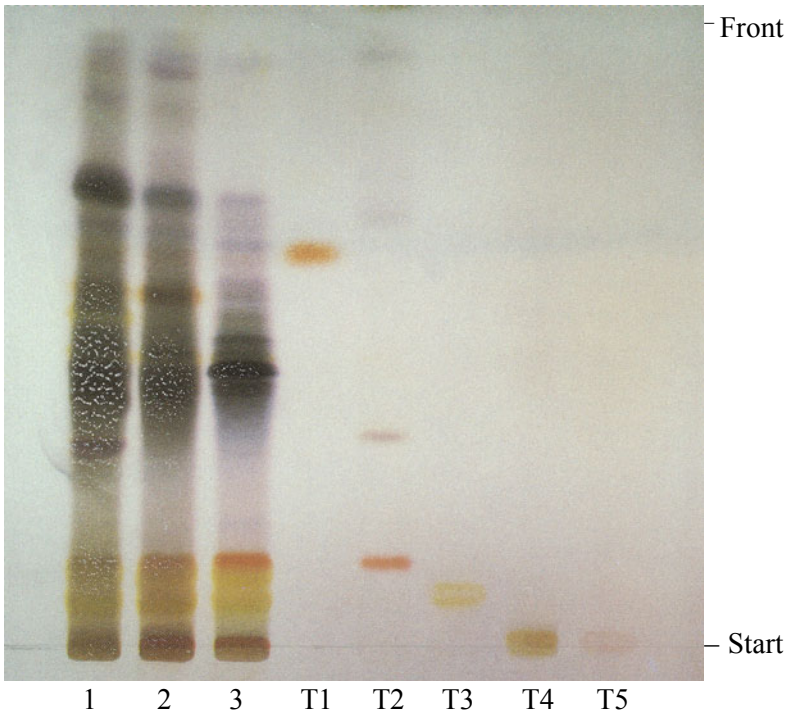


Fig. 4: Thin layer chromatogram of the drug samples 1, 2, 3 and reference compounds in the solvent II toluene-chloroform-methanol-formic acid (10+70+10+10) after detection with VS reagent in VIS.

The acetophenones apocynin (R*f* 0,65; **T1**) and androsin (R*f* 0,15; **T2**), and the apocynindiglycoside tecturoside (R*f* 0,01; **T5**) give orange spots, whereas the xanthone glycoside mangiferin (R*f* 0,02; **T4**) shows a light brown spot.

Drug sample 1 is suitable as a standard sample for the drug Rhizoma Belamcandae sinensis. The spots generated by resveratrol (R*f* 0,65; violet), androsin (R*f* 0,4; red), the isoflavonoid glycosides tectoridin and iridin (R*f* 0,35; yellow-brown), mangiferin (R*f* 0,08; yellow-brown), and tectoruside (R*f* 0,08; red) are characteristic for identification of *Belamcanda sins* (see Fig. 2).

Drug sample 2 may be from *Iris tectorum* should be regarded as a substitute drug on account of the high acetophenone content and because the stilbene derivative resveratrol is lacking. *Iris tectorum* can be distinguished from *Belamcanda sinensis* only by its high acetophenone content and the absence of resveratrol.

Drug sample 3, with the two well-defined red spots of androsin (R*f* 0,4) and tectoruside (R*f* 0,08), is the substitute drug *Iris tectorum* (see Fig. 2).

Drug sample 4 from Japan is characterized by the absence of resveratrol and the acetophenone glycosides. Therefore this sample might be derived from a different *Belamcanda* species. The presence of iriphlophenone glycoside (R*f* 0,15; orange) seems to be characteristic of the Japanese *Belamcanda* species.

Resveratrol appears in drug samples 5 and 6, along with the yellow spots of the isoflavonoid glycosides and the xanthone mangiferin. Resveratrol fluoresces light blue on the reverse side of the TLC plate after detection with VS reagent in UV 365 nm. The aceto- and benzophenones are absent.

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract used for TLC over Millipore® filter type HV 0,45 µm.

2) Injection volume: 3 µl of the methanol extract (concentration = 10 g drug/15 ml)

3) HPLC data:

Apparatus:	Liquid Chromatograph HP 1090 Photodiode-array-detector HP 1040 A (Hewlett-Packard)
Column:	LiChroCart® 125-4 with LiChroSpher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCart® 4-4 with LiChroSpher® 100 RP 18 (5 µm), Merck
Solvent system:	A: distilled water (+1 % 0,1 N H_3PO_4) B: acetonitrile (+1 % 0,1 N H_3PO_4)
Gradient:	0–20 % B in 25 min. 20–30 % B in 15 min. 30–60 % B in 20 min.
Flow:	1,0 ml/min.
Detection:	210 nm

Peak	Rt (min.)	Compound
1	9,8	protocatechuic acid
2	11,4	picein
3	13,5	tectoruside
4	13,8	iriphlophenone glycoside
5	14,5	androsin
6	19,6	mangiferin
7	21,6	apocynin
8	27,9	tectoridin
9	28,3	iridin
10	32,9	resveratrol
11	34,5	dihydroresveratrol
12	41,3	tectorigenin
13	41,5	irigenin
14	43,2	iristectorigenin A
15	48,8	irisflorentin

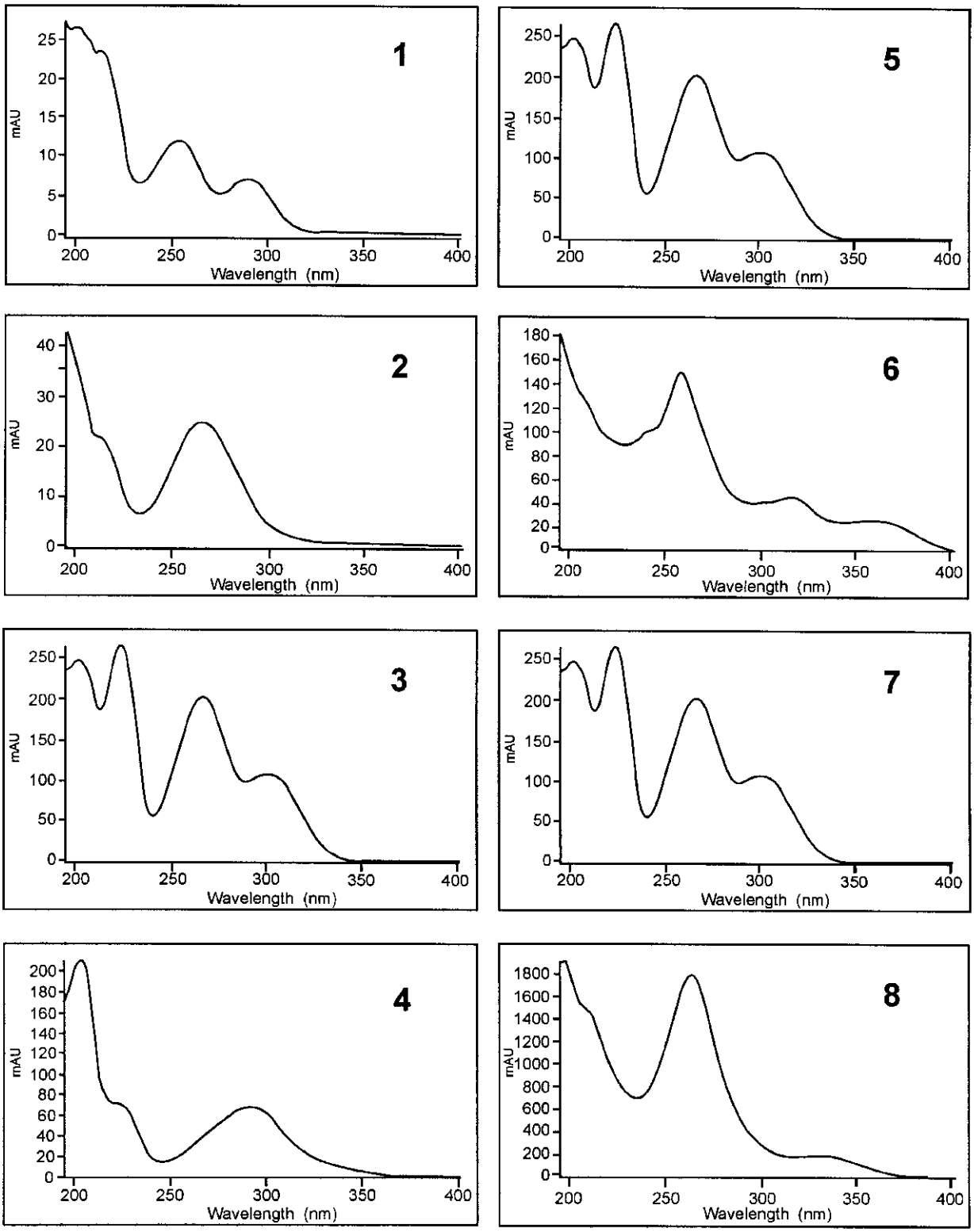
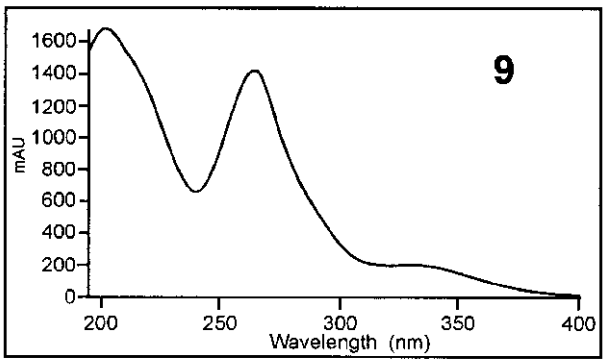


Fig. 5: UV spectra of the main compounds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1200
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800
600
400
200
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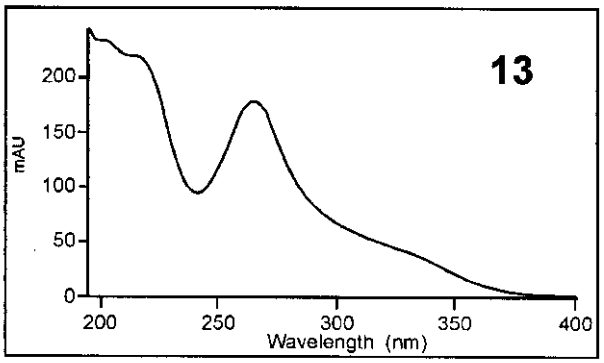
200
250
300
350
400
Wavelength (nm)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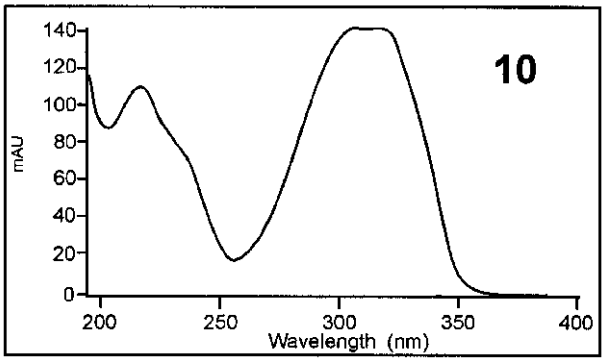
Wavelength (nm)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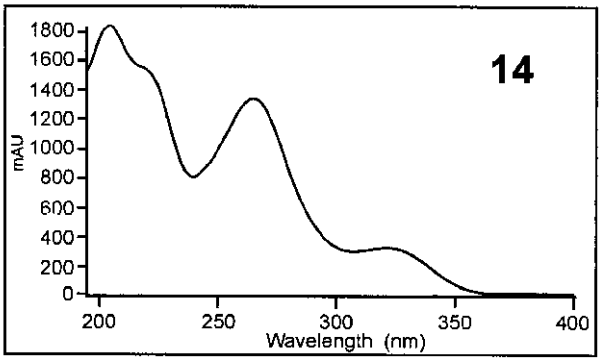
Wavelength (nm)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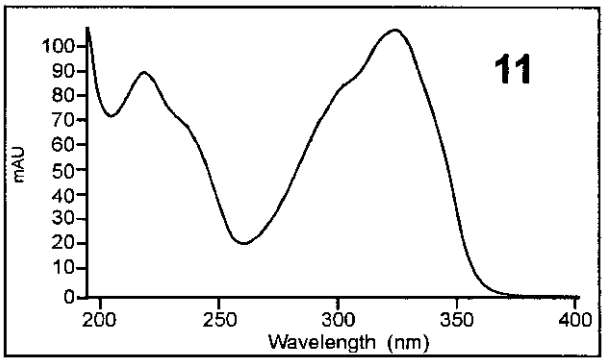
Wavelength (nm)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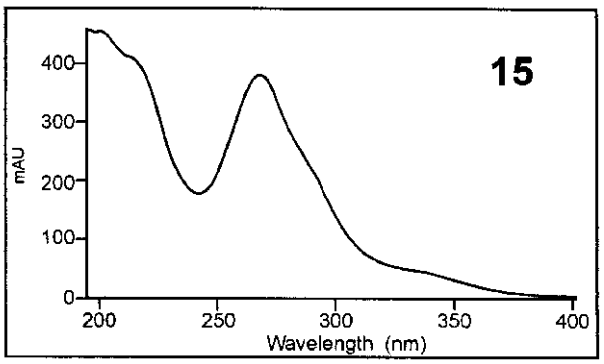
Wavelength (nm)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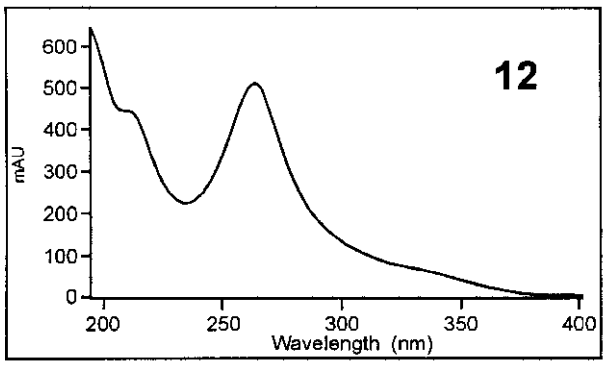
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Wavelength (nm)

Comparative HPLC investigations of different drug samples

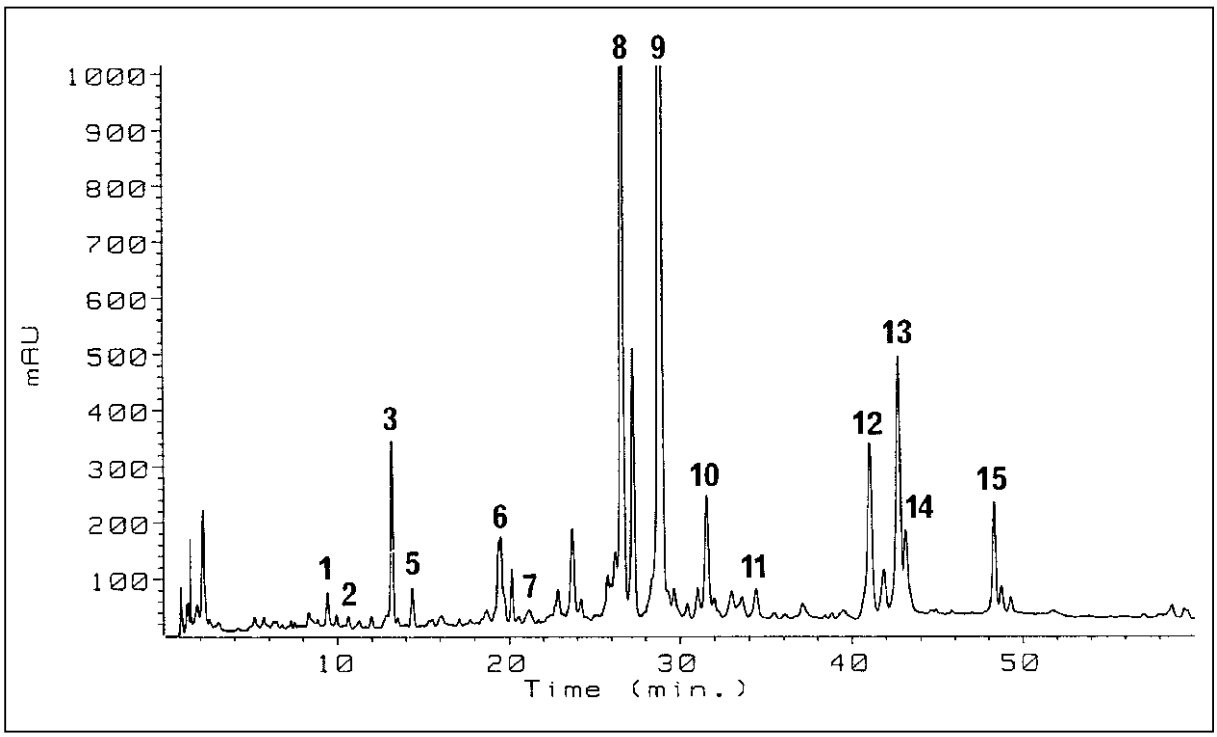

Fig. 6: HPLC fingerprint chromatogram of a commercial drug imported from China (No. 1, standard sample).

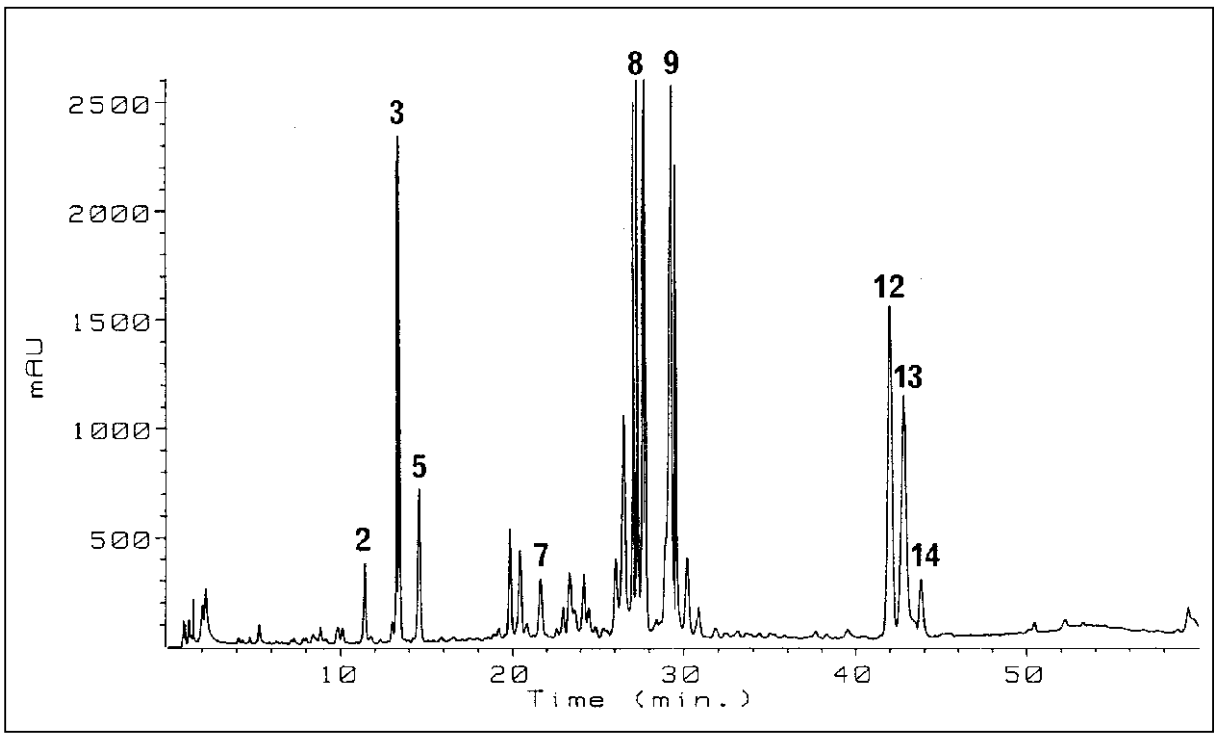

Fig. 7: HPLC fingerprint chromatogram of a drug sample from China (Sichuan Province) (No. 2).

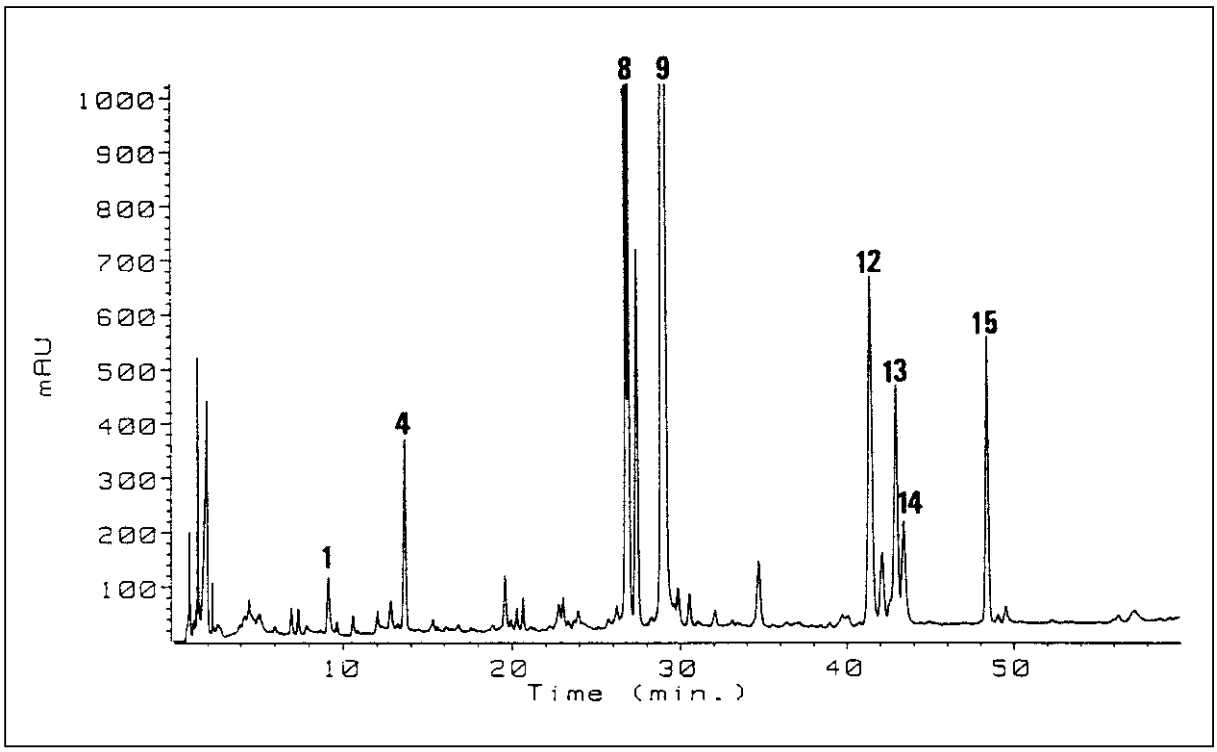

Fig. 8: HPLC fingerprint chromatogram of a commercial drug from Japan (No. 4).

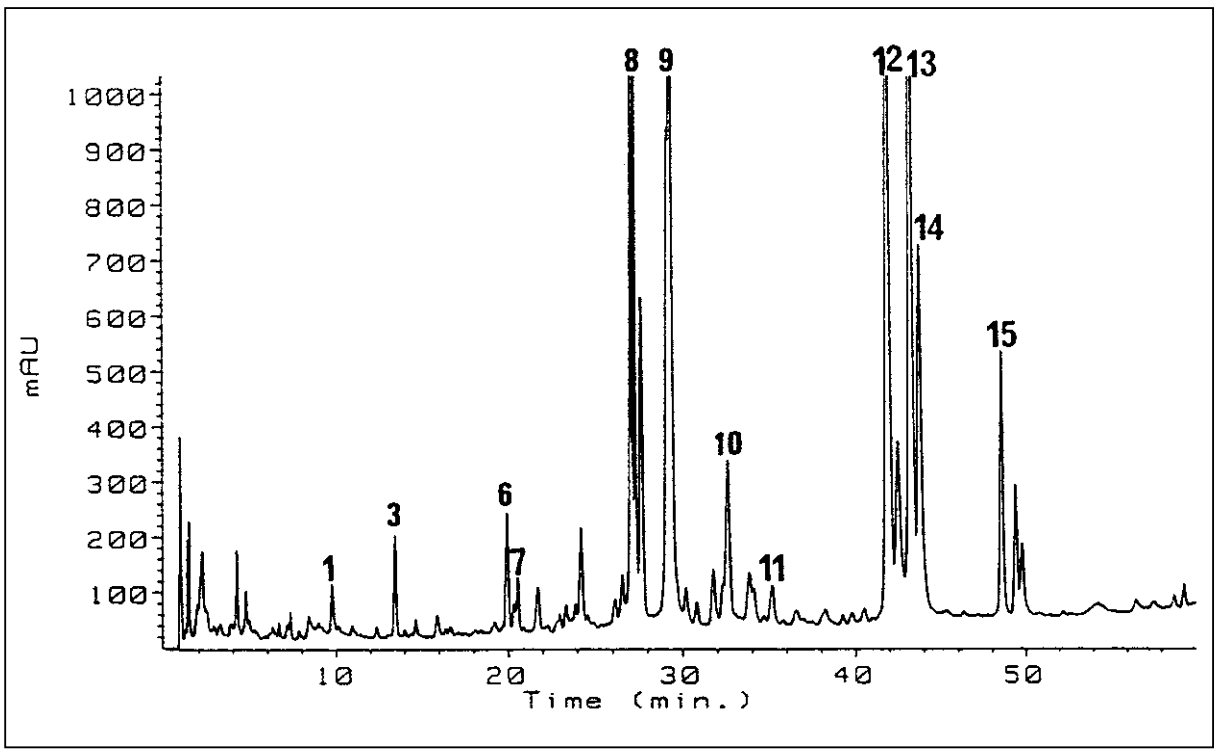

Fig. 9: HPLC fingerprint chromatogram of a drug sample from China (Shaanxi Province) (No. 5).

Description and discussion of the chromatograms

Fig. 6: HPLC fingerprint chromatogram of a commercial drug imported from China (Sample No. 1).

This fingerprint chromatogram is suitable as a drug standard.

It exhibits the isoflavonoid glycosides tectoridin (**8**) and iridin (**9**) as main constituents. Protocatechuic acid (**1**), the acetophenones picein (**2**), tecturoside (**3**), androsin (**5**) and apocynin (**7**), the xanthone glycoside mangiferin (**6**), and the stilbene derivatives resveratrol (**10**) and dihydroresveratrol (**11**) can be detected at minor concentrations. The isoflavonoid aglyca tectorigenin (**12**), irigenin (**13**), iristectorigenin (**14**) and irisflorentin (**15**) are eluted in the upper Rt range of the chromatogram.

Fig. 7: HPLC fingerprint chromatogram of a drug sample from China (Sichuan Province) (Sample No. 2).

This chromatogram is distinguishable from drug sample 1 mainly because of the quantities of the different compounds present. Tectoridin (**8**) and iridin (**9**) are the main constituents. The extract is also rich in the acetophenone glycosides picein (**2**), tectoruside (**3**) and androsin (**5**). The xanthone mangiferin (6) and the stilbene derivatives (**10**+**11**) are not detectable. This drug sample could be the adulterant *Iris tectorum*, on account of the high content of acetophenone glycosides and the absence of xanthones.

Fig. 8: HPLC fingerprint chromatogram of a commercial drug from Japan (Sample No. 4).

This fingerprint chromatogram is distinguishable from 1 and 2 mainly by the absence of acetophenones, xanthones and stilbene derivatives. The isoflavonoid glycosides (**8**+**9**) remain the main constituents. This Japanese drug batch contains the benzophenone derivative iriphlophenone glycoside (**4**), which was also present in other Belamcanda batches from Japan, and, to a lesser extent, in Chinese drug batches. Important constituents are protocatechuic acid (**1**) and the isoflavoneaglyca (**12**-**15**). Particularly noticeable is the large amount of the isoflavone aglycon irisflorentin (**15**). The Japanese drug samples always exhibited these characteristic patterns of constituents. A different *Belamcanda* species could be involved here.

Fig. 9: HPLC fingerprint chromatogram of a drug sample from China (Shaanxi Province) (Sample No. 5).

This drug sample contains large quantities of isoflavonoid aglycones (**12**–**15**). The main constituents, however, are tectoridin (**8**) and iridin (**9**). Higher concentrations of stilbene compounds (**10**+**11**) may be detected in this sample. Among the acetophenones, only tectoruside (**3**) and its aglycon, apocynin (**7**), are detectable. Likewise, mangiferin (**6**) can be easily detected.

Discussion

The HPLC batches of all the drug samples are characterized by the main constituents tectoridin (**8**) and iridin (**9**). Further important characteristics of the methanol extract of *Belamcanda sinensis* are the acetophenones picein (**2**), tectoruside (**3**), androsin (**5**), and apocynin (**7**), which can be present in different amounts, as well as the xanthone glycoside mangiferin (**6**) and the stilbene derivatives resveratrol (**10**) and dihydroresveratrol (**11**).

Japanese drug batches contain no or only very few acetophenones, xanthones and stilbenes. They contain however the benzophenone iriphlophenone glycoside. *Iris tectorum* is charactersized by a large amount of the acetophenone glycosides tectoruside (**3**) and androsin (**5**), as well as by the absence of xanthones and stilbene derivatives.

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Herba Lycopi lucidi – *Zelan*

Phamacopoeia: Chin. Ph. IX
Pharmacopoeia of the People's Republic of China, English Edition 1992/2005[1]

Official drug: *Lycopus lucidus* Turcz. var. *hirtus* Regel
– Lamiaceae –

The drug is collected between summer and autumn when the stems and the leaves are growing luxuriantly, and is dried in the sun.

Description of the drug:[1]

Stems square, infrequently branched, shallowly furrowed longitudinally on four sides, 50–100 cm long, 2–6 mm in diameter; externally yellowish-green or purplish, nodes apparently purple, white-tomentose; texture fragile, fracture yellowish-white, pith hollowed. Leaves opposite, short petiole; lamina mostly crumpled, when whole, lanceolate or oblong, 5–10 cm long; the upper surface blackish-green, the lower surface greyish-green and densely glandular-dotted, pubescent on both surfaces; apex acute, margins serrate. Flowers yellowish-brown, aggregated in leaf axils in verticillate cymes, corolla mostly fallen off, bracts and calyx persistent. Odourless; taste, weak.

Pretreatment of the raw drug:[1]

Foreign matters are eliminated, washed briefly, softened thoroughly, cut into sections and dried.

Medicinal use: In Traditional Chinese Medicine for activation of blood circulation, elimination of *blood stasis* and induction of diuresis for the treatment of menstrual disorder, amenorrhea, dysmenorrhea, postpartum abdominal pain due to *blood stasis* and edema.

Effects and indications according to Traditional Chinese Medicine[2,3]

Taste:	bitter, pungent
Temperature	slightly warm
Channels entered:	liver and spleen
Effects:	activation of blood circulation, elimination of *blood stasis* and diuresis
Symptoms and indications:	menstrual disorder, amenorrhea, dysmenorrhea, postpatum abdominal pain due to *blood stasis* and Edema

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Main constituents (see Fig. 1):

- **Flavonoides:** luteolin-7-O-β-D-glucoside[4]
- **Plant acids:** rosmarinic acid, caffeic acid[4]
- **Terpenoids:** betulinic acid, oleanolic acid, ursolic acid and 2β-hydroxy-ursolic acid[4]
- **Essential oil:** *trans*-caryophyllene, humulene, α-pinene, β-pinene, myrcene, *trans*-cymene, limonene, thymol, farnesene, caryophyllene-epoxide and phytol[2,4]
- Carbohydrate: oligosaccharide lycopose[2]
- Tannin.

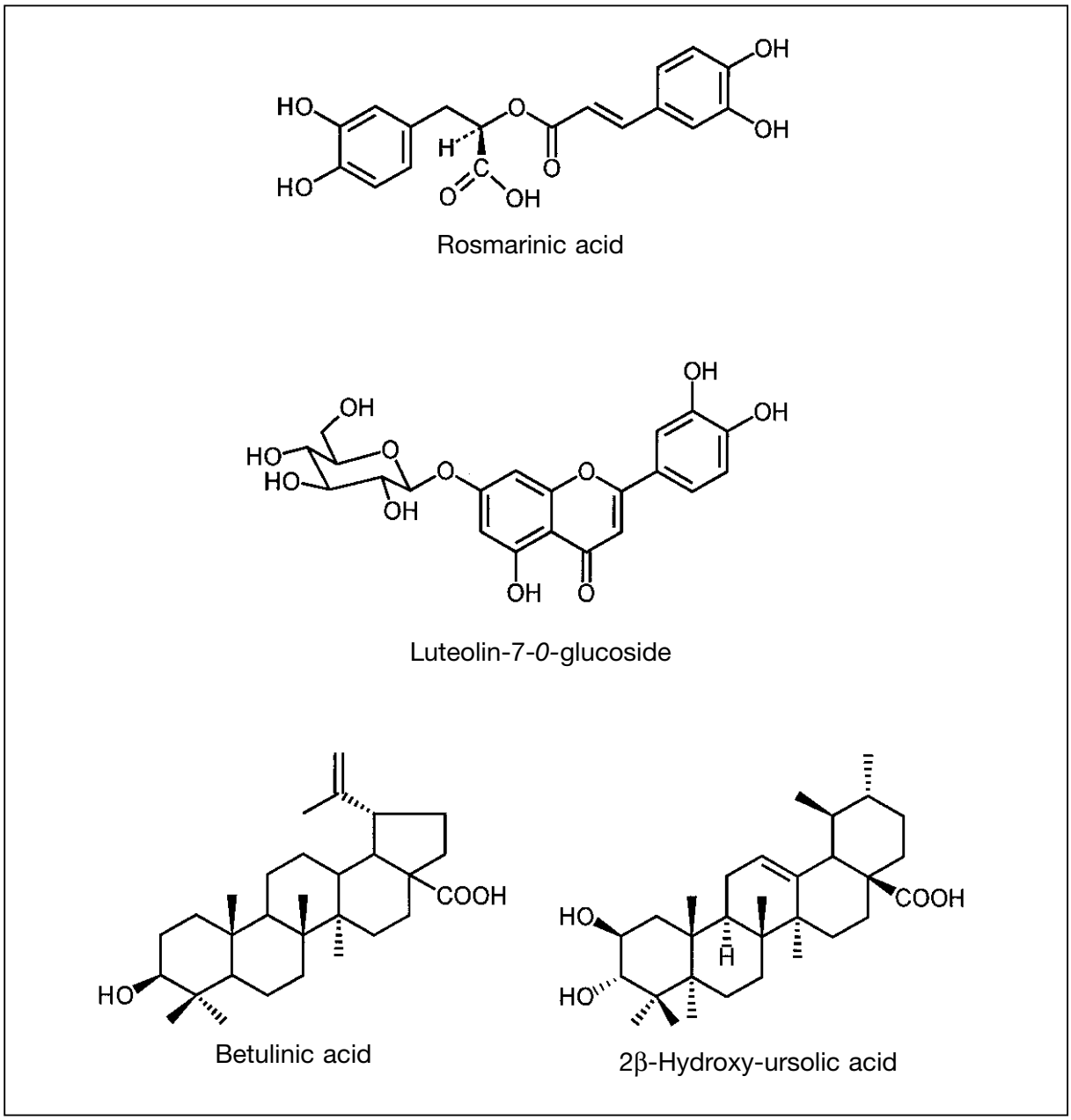

Fig. 1: Formulae of main constituents

Pharmacology:

In vitro effect:

– Inhibition of cyclooxygenase and lipoxygenase[4]

In vivo effects:

– activation of blood microcirculation and reduction of blood viscosity[5]
– diuretic effect[6]

TLC analysis:

1) Preparation of extracts:

   5 g powdered drug are extracted in a Soxhlet apparatus with 100 ml methanol for 1 hr. The extract is filtered and evaporated to 10 ml.

2) Reference compounds:

   Betulinic acid and 2β-hydroxy-ursolic acid (1 mg dissolved in 1 ml methanol respectively).

3) Separation parameters:

Applied amount:	20 µl extract, 5 µl standard
Plates:	Silica gel 60 F_{254} (Merck)
Solvent system:	chloroform – methanol (98 + 2)

4) Detection

   Spray reagent[7]: Vanillin/sulphuric acid reagent (VS):

   Solution I: 1 % ethanolic solution of vanillin)
   Solution II: 5 % ethanolic sulphuric acid

   The TLC plate is intensively sprayed with 10 ml of solution I and then with 5-10 ml of solution II, then heated for 5-10 min. at 110 °C. The evaluation is carried out in Vis.

Drug samples:

1 Herba Lycopi lucidi (China)
2 Herba Lycopi (Hongzhou)
3 Herba Lycopi (Shaanxi)
4 Herba Lycopi (Tong Ren Tang, Beijing)

Test substances:

T1 2β-hydroxy-ursolic acid + betulinic acid, R*f* = 0.67/0.54
T2 betulinic acid, R*f* = 0.54

5) Description of the chromatogram:

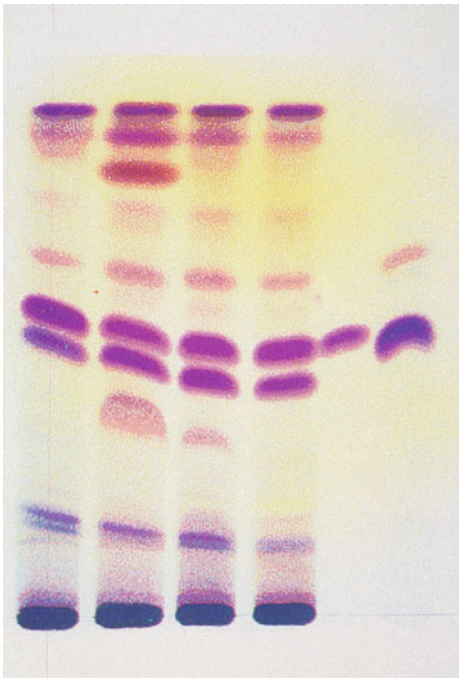

Fig. 2: TLC Chromatogram of *L. lucidus* methanolic extracts, detection with VS-reagent, VIS

The various drug samples showed very similar component profiles after detecting with vanillin-sulphuric acid reagent. The drug samples exhibit a series of deep red to violet zones in the region of R*f* 0.4 –0.95 and are characterized by betulinic acid (R*f* 0.54) and 2β-hydroxy-ursolic acid (R*f* 0.67). An intensive deep red zone below the zone of betulinic acid can be detected in all of the samples. In the front region, several zones are observed, which might be due to lipophilic essential oil components. A red brown spot at R*f* 0.8 is detected in sample 3 only. All these spots show no quenching under UV 254 nm before spraying with vanillin-sulphuric acid reagent.

HPLC fingerprint analysis:

1) Sample preparation: Filtration of extract used for TLC over Millipore filtration unit, type HV 0.45 µm.

2) Injection volume: 5 µl methanolic extract (conc. = 5g drug/10 ml)

3) HPLC data:

Apparatus:	Liquid Chromatograph HP 1090 with photodiode array detector HP 1040 A (Hewlett-Packard)
Column:	LiChroCART 125-4 with Lichrospher 100 RP 18 (5 µm), Merck
Precolumn:	LiChroCART 4-4 with Lichrospher 100 RP 18 (5 µm), Merck
Solvent:	A: Water + 1 % 0.1N H_3PO_4
	B: Acetonitrile + 1 % 0.1N H_3PO_4
Gradient:	5–20 % B in 15 min., 20–100 % B in 30 min. linearly
Flow:	1.0 ml/min.
Detection:	210 nm

Retention times of the main peaks:

Peak	Rt (min)	Compound
1	4.8	not identified
2	8.5	caffeic acid
3	11.6	luteolin-7-O-β-D-glucoside
4	13.4	rosmarinic acid
5	15.3	caffeic acid derivative
6	19.0	caffeic acid derivative
7	21.6	2β-hydroxy-ursolic acid
8	23.9	not identified
9	24.8	not identified
10	29.2	betulinic acid
11	29.9	triterpenoid
12	32.1	not identified

4) Description of the chromatograms:

Betulinic acid **10**, the characteristic major compound, can be found in all samples. An additional triterpene, 2β-hydroxy-ursolic acid **7**, is found in all samples but low concentrated in sample 2. Further characteristic peaks originate from luteolin-7-O-β-D-glucoside **3**, rosmarinic acid **4**, caffeic acid **2** and other caffeic acid derivatives. All caffeic acid derivatives show very similar UV spectra. The concentration of these compounds in the extracts varies. Luteolin-7-O-β-D-glucoside appears in sample 2 and sample 4 in concentrated form whereas they are just detectable in the extracts of sample 1 and sample 3.

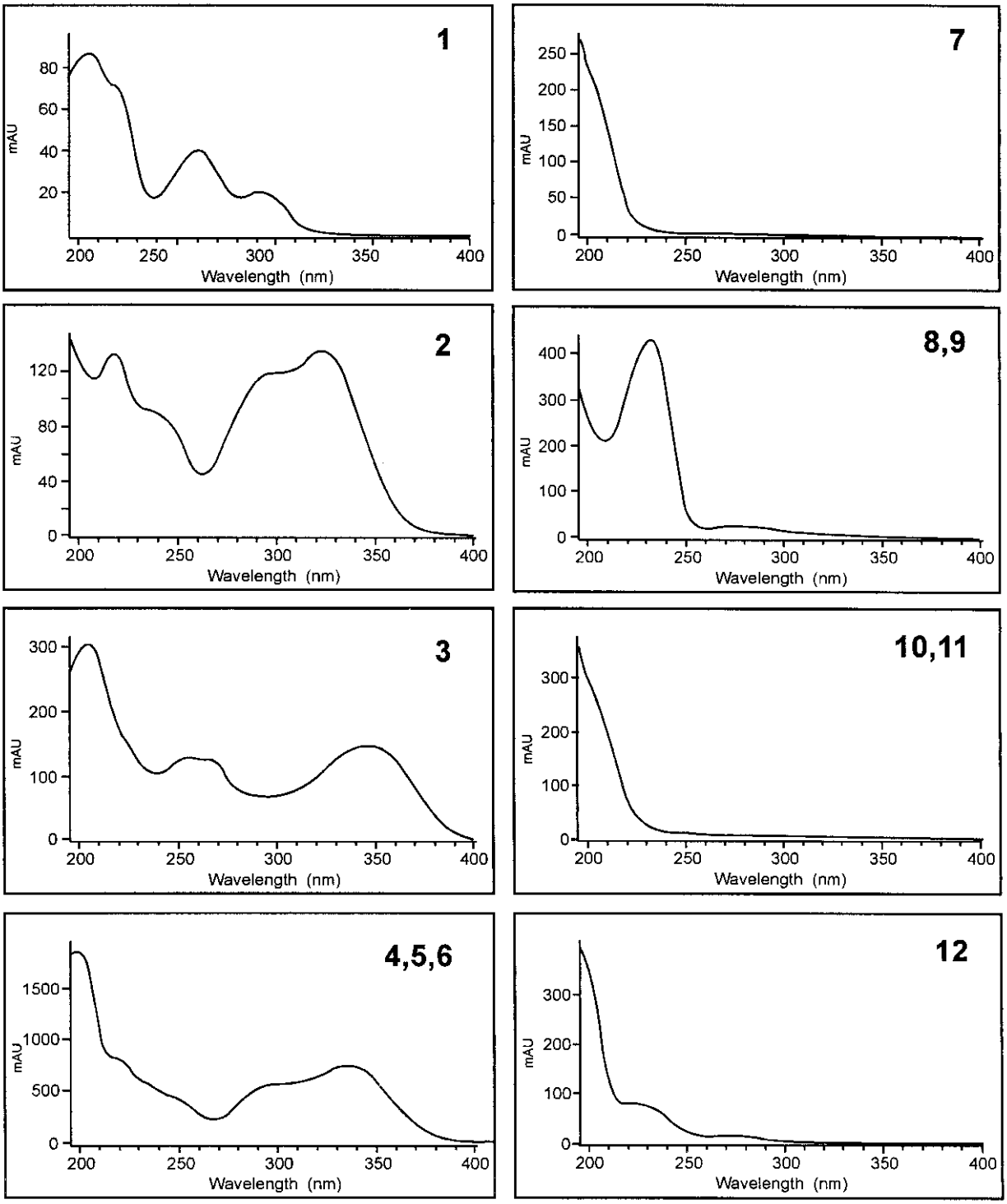

Fig. 3: UV spectra of the main compounds

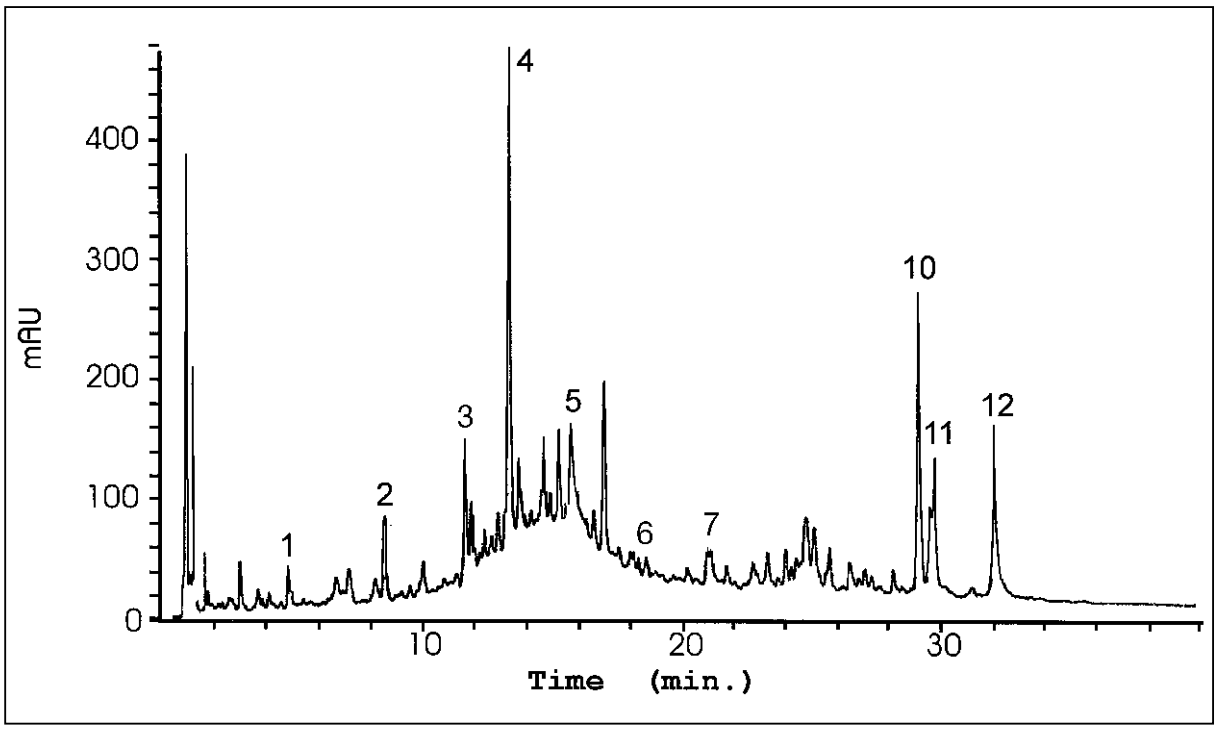

Fig. 4: HPLC fingerprint analysis of a drug sample of Chinese origin (Beijing); (*Lycopus lucidus*, sample 4)

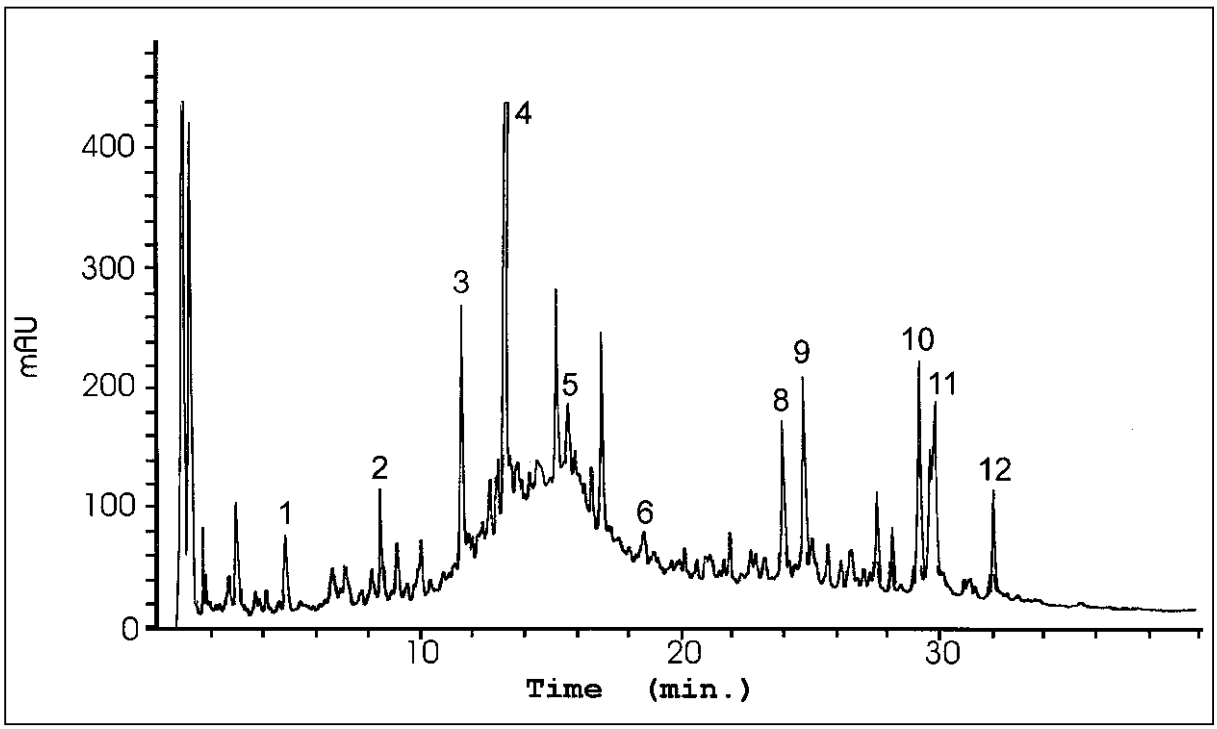

Fig. 5: HPLC fingerprint analysis of a drug sample of Chinese origin (Shaanxi Province); (*Lycopus lucidus*, sample 2)

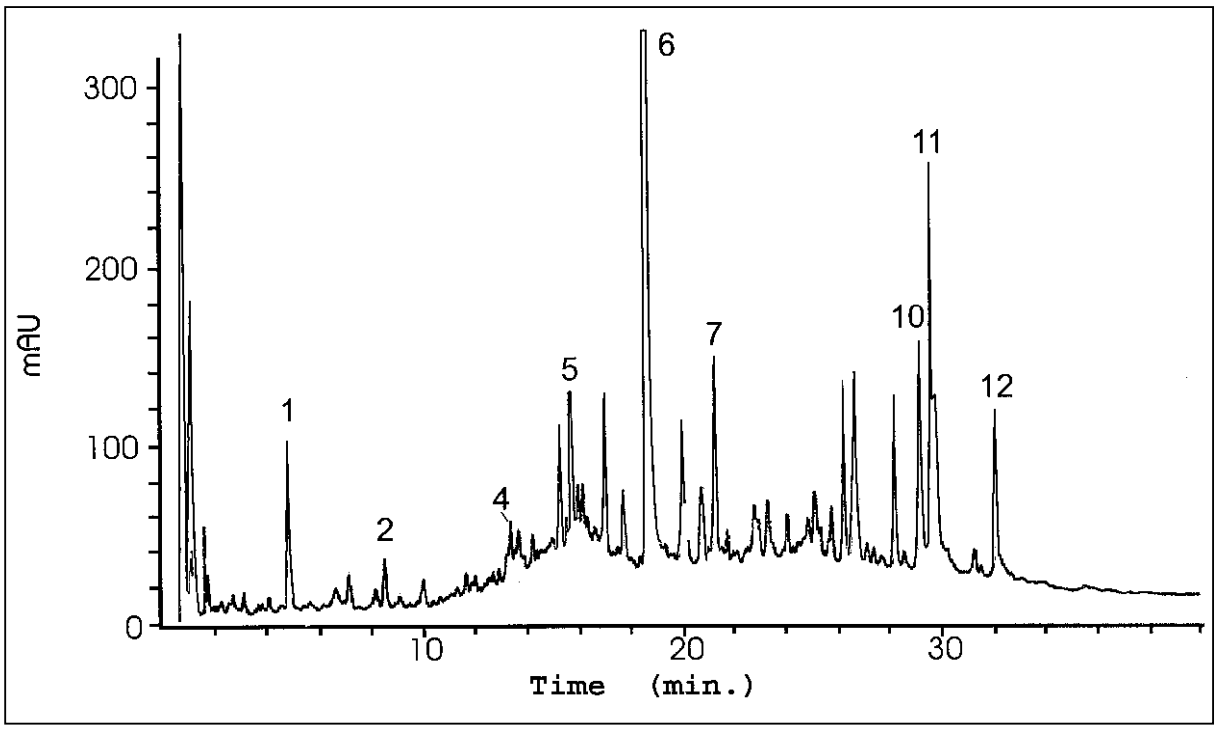

Fig. 6: HPLC fingerprint analysis of a drug sample of Chinese origin (Zhejiang Province); (*Lycopus lucidus*, sample 3)

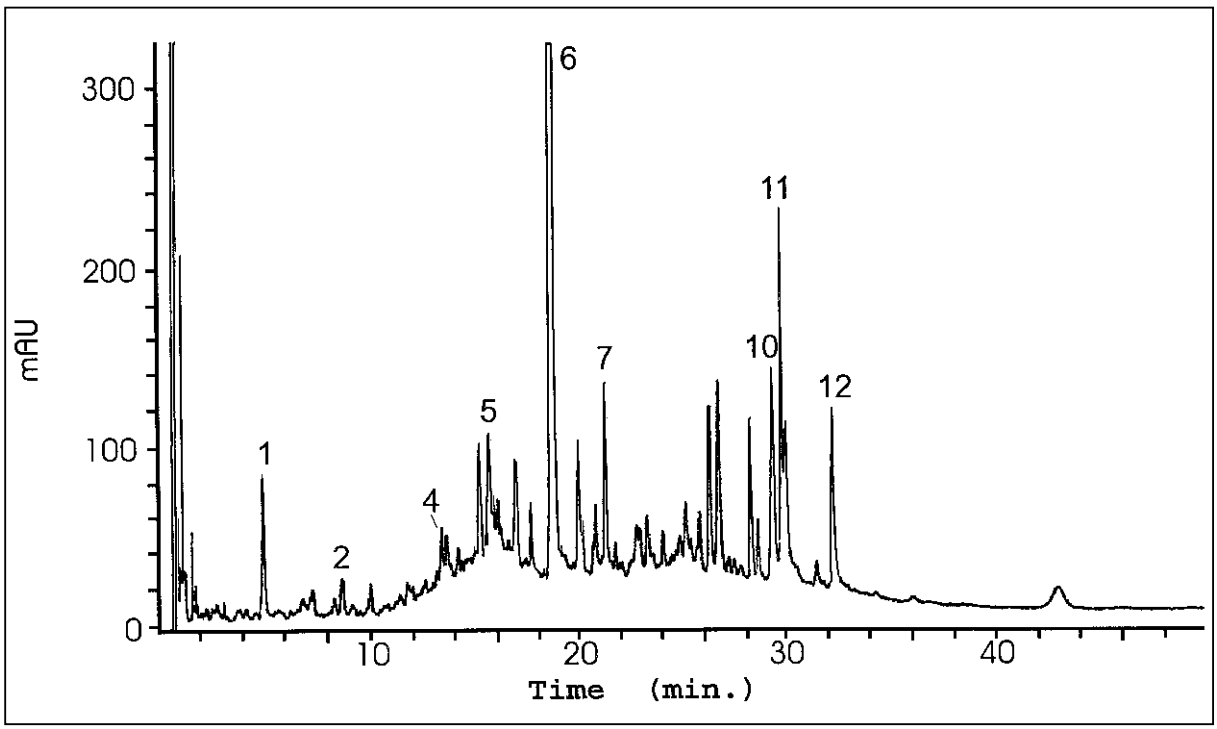

Fig. 7: HPLC fingerprint analysis of a drug sample of Chinese origin (*Lycopus lucidus*, sample 1)

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Rhizoma seu Radix Notopterygii
Qianghuo

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 1992/2005[1]

Official drugs: Underground parts of *Notopterygium incisum* Ting ex H.T. Chang and *Notopterygium forbesii* Boiss. – Apiaceae –

Description of the drug[1]:

Notopterygium incisum: Cylindrical and slightly curved rhizomes, 4–13 cm long, 0.6–2.5 cm in diameter, externally brown to blackish brown, wood yellowish-white with distinct rays, texture light and fragile, easily broken; odour aromatic, taste slightly bitter and pungent;

Canqiang: short internodes, dense, raised annulations, silkworm-shaped; *Zhujieqiang*: elongated internodes, bamboo-shaped

Notopterygium forbesii: Bark pale brown, wood yellowish white, texture lax and fragile, easily broken, odour and taste relatively slight;

Tiaoqiang: subcylindrical rhizomes, bearing stems and remains of leaf sheats, subconical roots, longitudinally wrinkled and with dense annulations near rhizome, 8–15 cm long, 1–3 cm in diameter;

Datouqiang: rhizomes sometimes large and irregular nodiform, roots relatively thin, 0.6–1.8 cm in diameter

For a macroscopic and microscopic distinction between the two species, see[3].

Pretreatment of the raw drugs: Cleansed, softened thoroughly, cut into thick slices and dried in the sun.

Medicinal use: In Traditional Chinese Medicine as an antirheumatic and analgesic drug; used against headache in common cold, rheumatic arthralgia (rheumatism) and aching of back and shoulders[1]; the treatment of laryngitis is also reported[6]

Effects and indications according to Traditional Chinese Medicine[1,2,4,6–8]

Taste:	acrid, bitter, aromatic
Temperature:	warm
Channels entered:	bladder, kidney
Effects:	releases the exterior, disperses cold, expels wind-dampness, unblocks painful obstruction and alleviates pain, guides Qi to the greater Yang channel and governing vessel
Symptoms and indications:	exterior cold with chills, fever, headache and body pains, stiffness of the neck, joint pains, feeling of heaviness, sleepiness, pain in the occipital region, painful obstruction especially in the upper limbs and back

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Main constituents (see Fig. 1):

- **coumarins**, mainly **furanocoumarins:** e.g. isoimperatorin, bergapten, cnidilin, notopterol, notoptol, anhydronotoptol, ethylnotopterol[9–14]
- **coumaringlycosides:** nodakenin[9], bergaptol-O-*β*-D-glucopyranoside, 6′-O-*trans*-feruloylnodakenin[10]
- **aromatic esters:** phenethyl ferulate, *p*-hydroxyphenethyl anisate[9], (-)-bornyl ferulate, coniferyl ferulate[15] and free *trans*-ferulic acid
- **steroids:** pregnenolone[16], *β*-sitosterol[13] and *β*-sitosterol glucoside[10]
- polyacetylenes: falcarindiol[9]
- **fatty acids** such as palmitic acid, linoleic acid, oleic acid, and sugars like galactose, glucose, rhamnose and saccharose[17]
- **essential oil**[18,19]

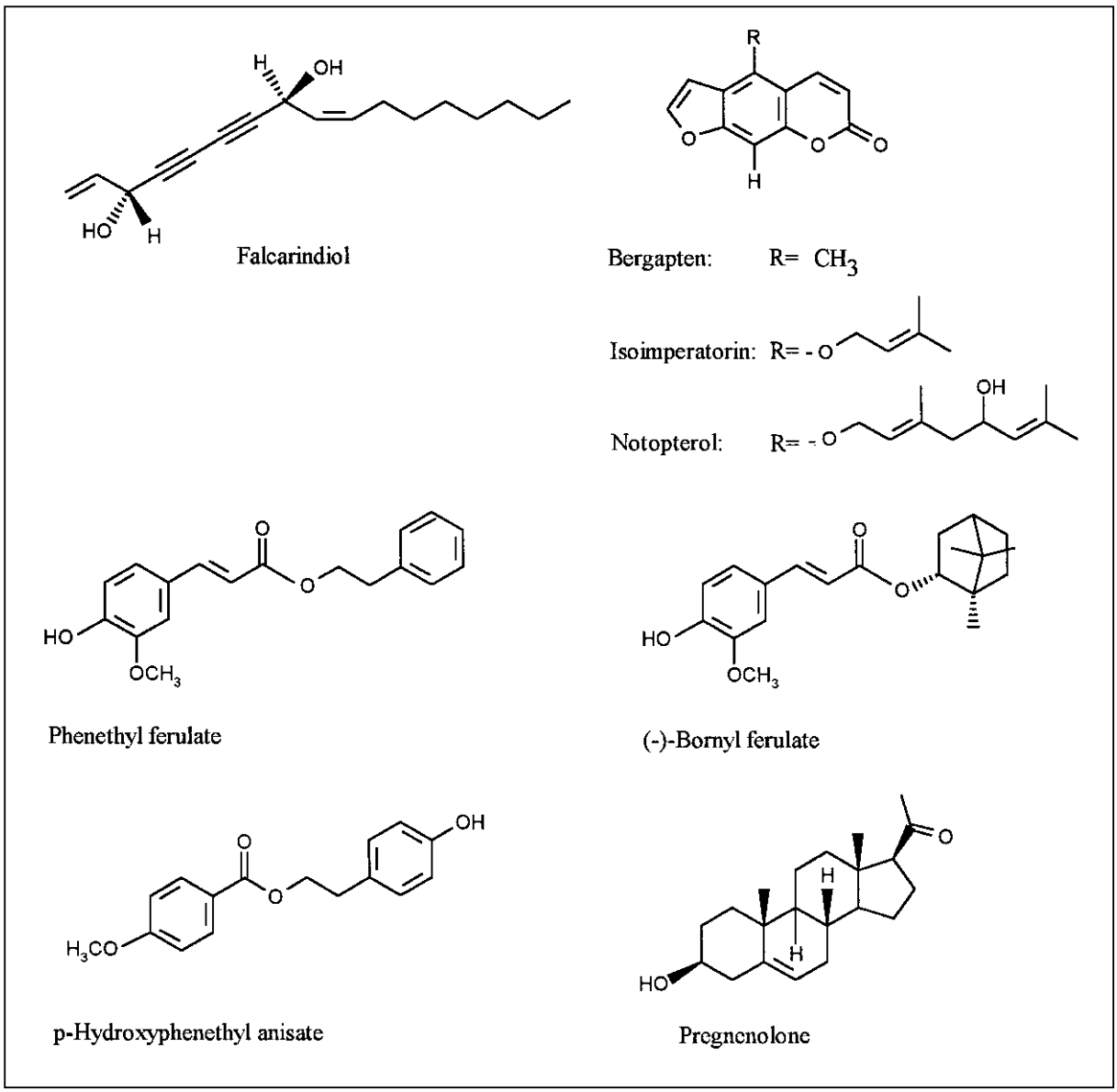

Fig. 1: Formulae of the main compounds

Pharmacology:

In vitro effects:

- inhibition of 5-lipoxygenase (phenethyl ferulate, (-)-bornyl ferulate, falcarindiol, linoleic acid)[20]
- inhibition of cyclooxygenase 1 (phenethyl ferulate, (-)-bornyl ferulate, linoleic acid)[20]
- antioxidative activity (phenethyl ferulate, falcarindiol)[20,21]

In vivo effects:

- analgesic (acetic acid-induced writhing test in mice: notopterol)[22]
- anti-inflammatory (vascular permeability test in mice: notopterol)[22]
- inhibition of lipid peroxidation in carbontetrachloride treated mice (methanol extract)[23]
- prolongation of pentobarbital-induced hypnosis in mice (notopterol)[22]
- prevention of pituitrin-induced acute myocardial ischemia in rats and stimulation of myocardial circulation in mice (volatile acid preparation of *N. incisum*)[24]

TLC fingerprint analysis:

1) Extraction:

   3 g of powdered drug are extracted with 50 ml *n*-hexane for 2 hrs in a Soxhlet apparatus. The extract is then evaporated to dryness and the residue dissolved in 10 ml ethanol p.a..

2) Standards:

   Isoimperatorin, (-)-bornyl ferulate, phenethyl ferulate, linoleic acid, falcarindiol, notopterol (1 mg each dissolved in 1 ml ethanol p.a.).

3) Separation parameters:

Applied amount:	10 µl extract, 5µl standard
Plates:	Silicagel 60 F_{254}, Merck
Solvent system:	toluene-ethyl acetate-glacial acetic acid (90+10+1)
Direct evaluation:	UV 254 nm and UV 365 nm
Spray reagent:	Anisaldehyde-sulphuric acid reagent[25] (0,5 ml anisaldehyde, 10 ml glacial acetic acid, 85 ml MeOH, 5 ml conc. sulphuric acid, mixed in this order) The plate is sprayed with ca. 10 ml reagent and then heated under observation for 5–10 min. at 100 °C; evaluation in VIS.

Drug samples:

1 *N. incisum*, TCM-Academy, Hangzhou
2 *N. incisum*, Toyama Medical and Pharmaceutical University, Japan
3 *"N. incisum"*, drug market Shaanxi (possibly mixture with *N. forbesii*)
4 *N. incisum*, Institute for Medicinal Plant Development, Beijing
5 *N. incisum*, Phytopet, Andorra
6 *"N. forbesii"*, drugmarket Yunnan, possibly *N. incisum*
7 *N. forbesii*, Toyama Medical and Pharmaceutical University, Japan

Test substances:

T1 isoimperatorin, R*f* = 0,50
T2 (-)-bornyl ferulate, R*f* = 0,40
T3 phenethyl ferulate, R*f* = 0,35
T4 linoleic acid, R*f* = 0,30
T5 falcarindiol, R*f* = 0,20
T6 notopterol, R*f* = 0,10

4) Description of the chromatograms:

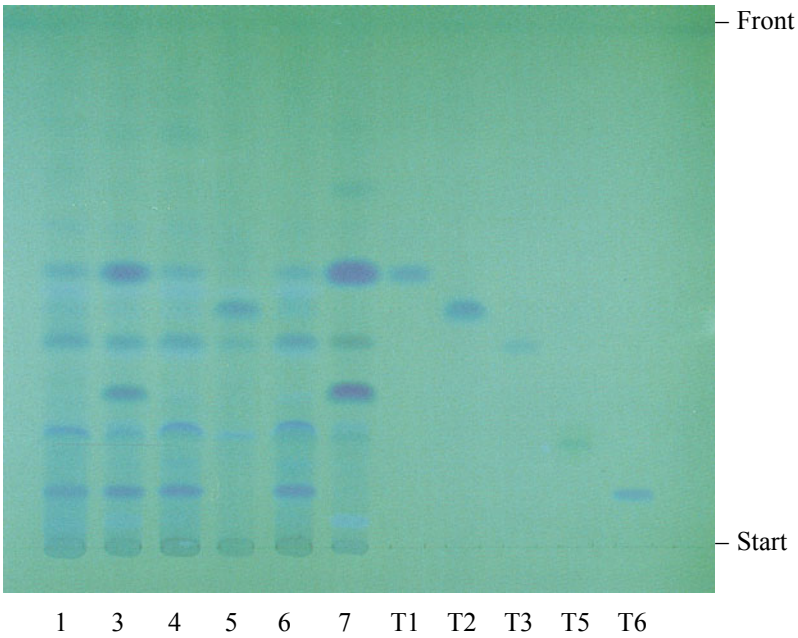

Fig. 2: Thin layer chromatogram of *Notopterygium* samples (UV 254 nm)

In UV 254 nm, isoimperatorin (R*f* 0,5: **T1**), (-)-bornyl ferulate (R*f* 0,4: **T2**), phenethyl ferulate (R*f* 0,35: **T3**) and notopterol (R*f* 0,1: **T6**) can be detected as zones which strongly quench fluorescence. In sample 3 and sample 7, *p*-hydroxyphenethyl anisate occurs as a strong additional zone at R*f* 0,25. Besides, there are some weakly absorbing zones which are distributed over the entire R*f* range. Notopterol was lacking in samples 5 and 7.

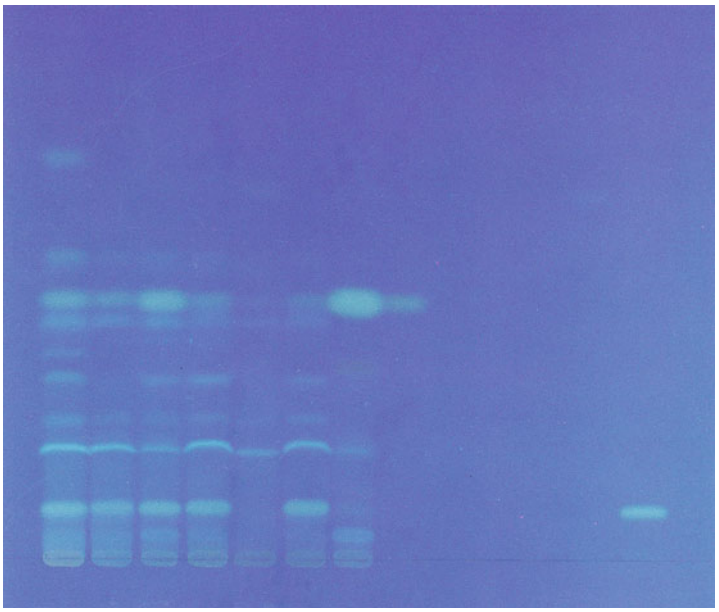

Fig. 3: Thin layer chromatogram of *Notopterygium* samples (UV 365 nm)

The two furanocoumarins isoimperatorin (R*f* 0,5: **T1**) and notopterol (R*f* 0,1: **T6**) appear as bright white fluorescent spots. Some blueish fluorescent zones are visible in the range of R*f* 0,1-0,55. The thin, bright blue flourescent zone at R*f* 0,2 shows an enhancement of fluorescence with natural products polyethylenglycol reagent or 5 % ethanolic KOH. (-)-Bornyl ferulate (**T2**) and phenethyl ferulate (**T3**) can be seen as weak dark zones.

Comparing the different drug samples, sample 1, 2, 3, 4 and 6 showed nearly the same pattern. In sample 5 and 7 notopterol was missing, and sample 7 contained an extraordinary high amount of isoimperatorin.

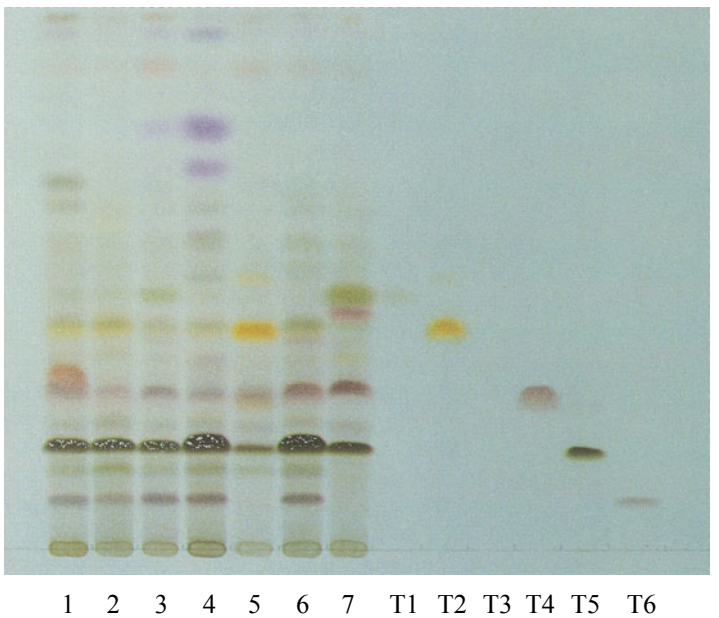

Fig. 4: Thin layer chromatogram of *Notopterygium* samples (anisaldehyde-sulphuric acid/VIS)

Falcarindiol (**T5**) occurs as a characteristic brown coloured spot (R*f* 0,2), linoleic acid (**T4**) as purple zone (R*f* ca. 0,3), sterols and triglycerides as purple zones at the front. Isoimperatorin (**T1**) is detected as a greenish spot, (-)-bornylferulate gives a yellow-orange colour, and notopterol generates a purple spot.

The comparison of the different drug samples gave a very similar TLC pattern for all samples. The main difference are two conspicious violet zones (R*f* 0,8 and 0,75) in samples 3 and 4, which possibly derive from volatile oil compounds. Samples 5 and 7 differ from the others, because notopterol was missing, and sample 7 showed an additional purple zone right below the isoimperatorin spot.

The distinction between the two *Notopterygium* species by TLC analysis could be based on the lack of notopterol, combined with a high content of isoimperatorin and the strong fluorescent quenching zone of *p*-hydroxyphenethyl anisate at R*f* 0,25 (UV 254 nm) for *N. forbesii* (sample 7). Therefore sample 6, which looks very much like the samples of *N. incisum* (sample 1, 2, 4), possibly represents bamboo-shaped *N. incisum* purchased as *N. forbesii*, and sample 3 is likely to be a mixture of both drugs. Sample 5 seems to be a variety of *N. incisum,* which contains a high amount of (-)-bornyl ferulate, and no notopterol.

HPLC fingerprint analysis:

1) Sample preparation: 1:5 dilution of the extract used for TLC with ethanol p.a., then filtration over Millipore® filter type HV 0,45 µm

2) Injection volume: 10 µl extract (conc. = 3 g drug/50 ml)

3) HPLC data:

Apparatus:	Liquid Chromatograph HP 1050,	
	Photodiode-array-detector HP 1040 M,	
	HP Chemstation (Hewlett Packard)	
Column:	LiChroCART® 125-4 with LiChroSpher® 100 RP-18 (5 µm), Merck	
Pre-column:	LiChroCART® 4-4 with LiChroSpher® 100 RP-18 (5 µm), Merck	
Solvent system:	A: distilled water	
	B: methanol	
Gradient:	linear:	60–70 % B in 15 min.
		70–95 % B in 10 min.
	isocratic:	95 % B for 5 min.
Flow:	linear: 0,7–1,0 ml/min. in 15 min. (The flow gradient is necessary to separate phenethyl ferulate and notopterol!)	
	isocratic:	1,0 ml/min. for 15 min.
Detection:	210 nm	

4) Description of the chromatograms:

Retention times of identified compounds:

Peak	Rt (min.)	Compounds
1	5,8	bergapten
2	8,5	*p*-hydroxyphenethyl anisate
3	12,3	phenethyl ferulate
4	13,0	notopterol
5	13,9	isoimperatorin
6	18,6	falcarindiol
7	22,1	(-)-bornyl ferulate

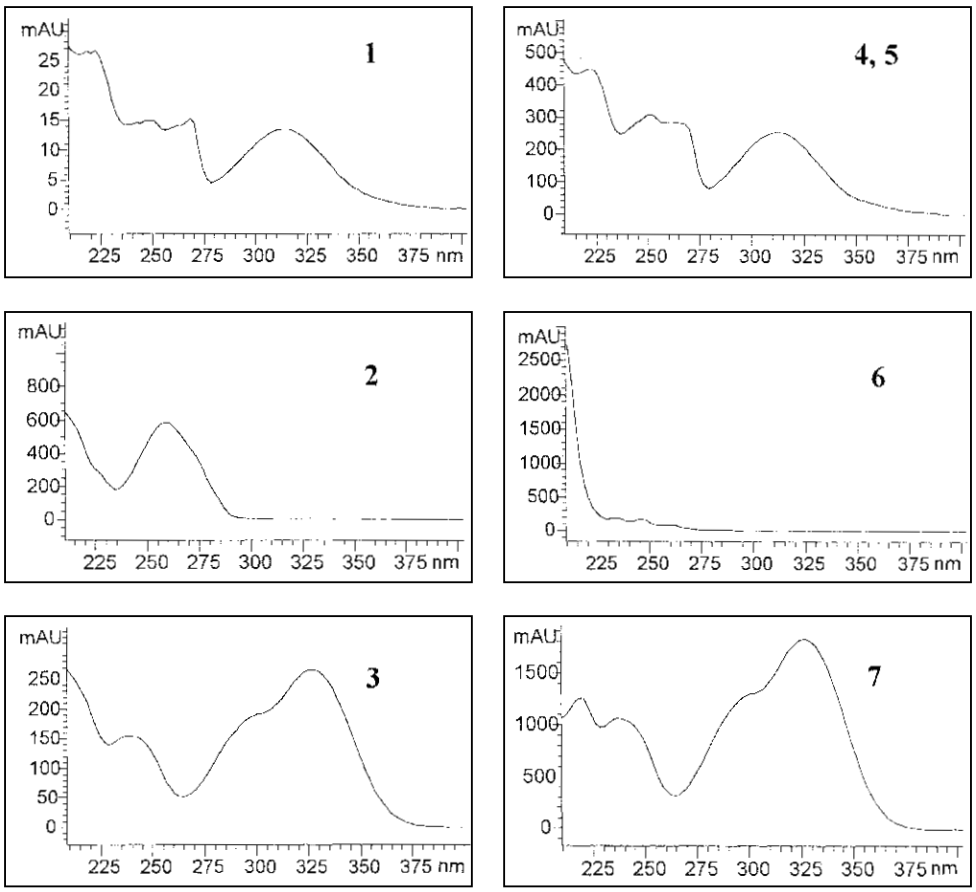

Fig. 5: UV-spectra of the identified compounds

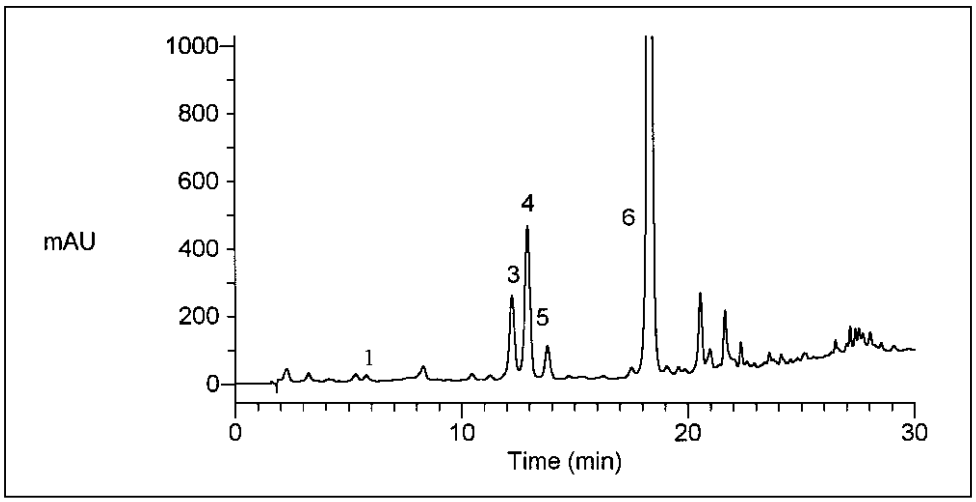

Fig. 6 HPLC fingerprint of the *n*-hexane extract of the underground parts of *N. incisum* (sample 4)

The chromatogram of the *n*-hexane extract of most of the commercial drug samples of *N. incisum* is characterized by phenethyl ferulate (peak **3**), notopterol (peak **4**), isoimperatorin (peak **5**) and falcarindiol (peak **6**), with falcarindiol as the main compound (see Fig. 6). Besides, some small peaks with mainly coumarin spectra, e.g. bergapten (peak **1**), or spectra of aromatic acid esters can be observed. The minor compound (-)-bornyl ferulate was not detected.

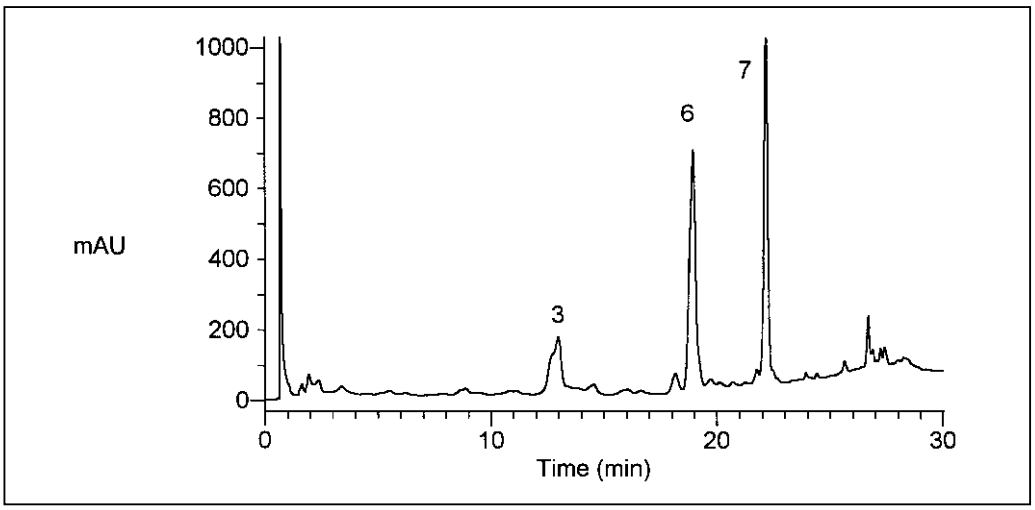

Fig. 7: HPLC fingerprint of the *n*-hexane extract of the underground parts of *N. incisum* (sample 5)

In some drug samples, falcarindiol (peak **6**) and (-)-bornyl ferulate (peak **7**) are the main compounds. Phenethyl ferulate (peak **3**) is low concentrated, notopterol is missing (see Fig. **7**).

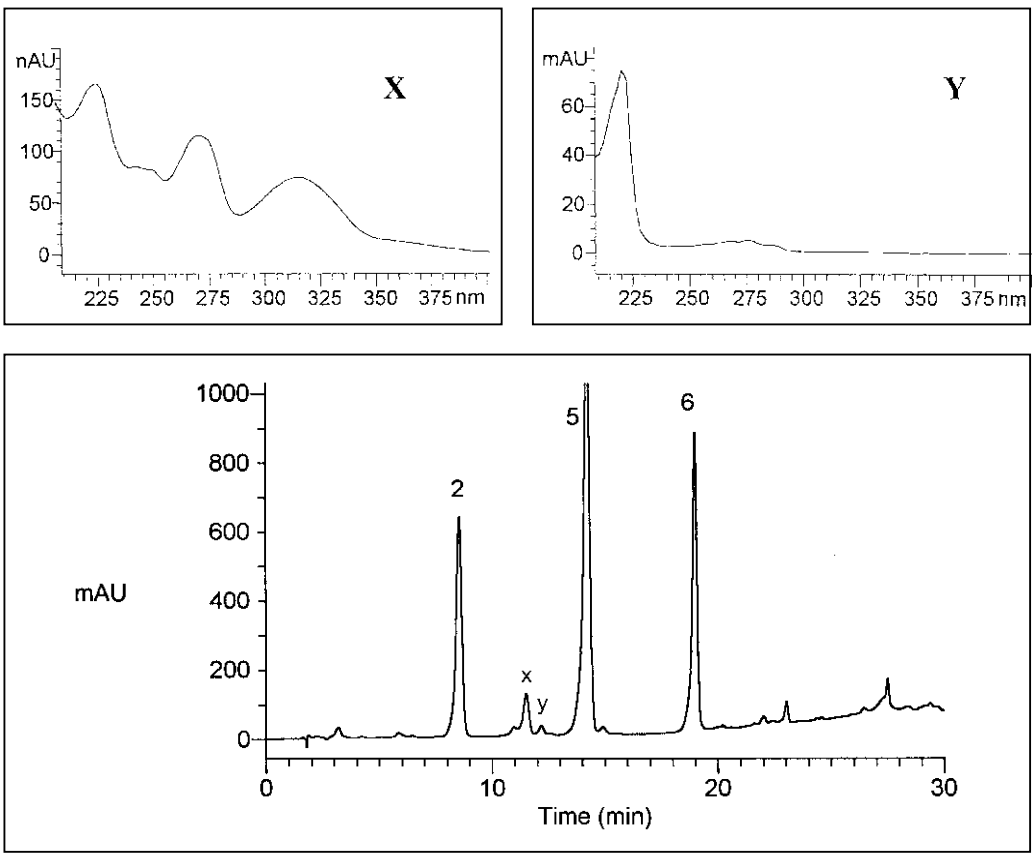

Fig. 8: HPLC fingerprint and UV-spectra of the *n*-hexane extract of the underground parts of *N. forbesii* (sample 7)

In extracts from the roots of *N. forbesii*, the main compounds are isoimperatorin (peak **5**), falcarindiol (peak **6**) and *p*-hydroxyphenethyl anisate (peak **2**). Instead of phenethyl ferulate and notopterol, there are two further peaks in the same Rt region: peak X with a furanocoumarin spectrum and additionally peak Y (see Fig. 8).

Discussion:

Most of the commercial Chinese samples of *Qianghuo* consist mainly of silkworm-shaped *N. incisum*, sometimes mixed with *N. forbesii* or bamboo-shaped *N. incisum*. The typical HPLC fingerprint of these drug samples is characterized by phenethyl ferulate, notopterol, isoimperatorin, and, as main compound, falcarindiol (Fig. 6). (-)-Bornyl ferulate is a minor compound and can only occasionally be detected. The content of the unstable polyacetylene falcarindiol can be taken as indicator for the freshness of the drug.

A special variety of *N. incisum*, externally also silkworm-shaped, possesses (-)-bornyl ferulate as main compound apart from falcarindiol (Fig. 7).

N. forbesii, the second officinal *Notopterygium* species, which is very difficult to distinguish from bamboo-shaped *N. incisum* by external morphology, is often mixed with *N. incisum*. The HPLC pattern of this species seems to be characterized by *p*-hydroxyphenethyl anisate at Rt 8,5 min, a high amount of isoimperatorin, phenethyl ferulate and notopterol either lacking completely or present only in minor concentrations (Fig. 8).

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Radix Angelicae sinensis
Danggui

Pharmacopoeias:

Chinese Pharmacopoeia X
Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[1]

Official drugs:

Angelica sinensis (Oliv.) Diels (syn. *Angelica polymorpha* Maxim var. *sinensis* Oliver)
– Apiaceae –

The drug is known as Toki (Japanese) and Chinese Angelica Root (English).

The following substitute drugs are not official:
– *Angelica acutiloba* (Sieb. & Zucc.) Kitagawa (Japan);
– *Angelica acutiloba* Kitagawa var. *sugiyamae* (Japan);
– *Angelica gigas* Nakai (Taiwan);
– *Angelica uchiyamana* Yabe (Taiwan)[5]

Description of the drug: [1,4]

The almost cylindrical root is 15–25 cm long and 3–5 or more branched at the lower part. The colour is yellowish-brown to brown, the surface is longitudinally wrinkled and transversely lenticillate. The Chinese Pharmacopoeia distinguishes between the root stock (Guitou) which is 1.5–4 cm in diameter, annulated, with purple or yellowish-green remains of stems and leaf sheaths, the main roots (Guishen) which are lumpy on the surface, and the branching roots (Guiwei) which are 0.3–1 cm in diameter with thick upper portions and thin lower portions, showing few rootlet scars. The texture is flexible, the fracture yellowish-white or yellowish-brown. The wood is paler in colour than the thick bark. The bark also shows brown spotted secretory cavities. The odour is strongly aromatic, the taste sweet, pungent and slightly bitter.

Pretreatment of raw drug:

The drug is collected in late autumn. After removing soil and rootlets it is slightly dried and then tied up into a small bundle, placed on a shelf and smoke dried.

After elimination of foreign matter the root is softened thoroughly by washing, cut into slices and dried in the sun or at low temperature.

Sometimes the slices of Radix Ancelicae sinensis are stir-fried with wine to dryness (according to (1): Appendix 3.2 "stir frying with wine").

Angelicae Extractum Liquidum (extraction medium: 40–70 % ethanol)

Medicinal use: [1,2,3,4,6]

In Traditional Chinese Medicine it is recommended as a tonic, hemopoietic, spasmolytic, analgesic and anti-inflammatory. It is used for the treatment of menstrual disorders, amenorrhea, dysmenorrhea, anemia, constipation, rheumatic arthralgia, traumatic injuries, carbuncles, boils and sores.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,2,3,4,6]

Taste:	sweet, acrid, bitter
Temperature:	warm
Channels entered:	*heart, liver, spleen*
Effects:	strengthens and invigorates the *xue*; tonifies the blood and regulates the menses; activates blood circulation and disperses cold; moistens the intestines and relaxes the bowels; reduces swelling, expels pus, generates flesh and alleviates pain.
Symptoms and indications:	blood deficiency with symptoms like dizziness and palpitations, menstrual disorders such as irregular menstruation, amenorrhea and dysmenorrhea; constipation; abdominal pain; rheumatic arthralgia, traumatic injuries; carbuncles, sores and abscesses. *Radix Angelicae sinensis stir-fried with wine*: amenorrhea, dysmenorrhea, rheumatic arthralgia and traumatic injuries.

Main constituents (see Fig. 1)[7–16]

- **essential oil:**
  mainly monomeric phthalides: ligustilide (*E* and *Z*), butylidenephthalide (*E* and *Z*), butylphthalide, senkyunolide A[8,9]
  besides: carvacrol, isoeugenol, vanillin, α-pinene, β-bisabolone, myrcene, cuparene, etc.[10]
- **phthalide dimers:** riligustilide, E-232, levistolide A, senkyunolide O[11,12,13]
- **organic acids and their esters:** ferulic acid, coniferyl ferulate, succinic acid, nicotinic acid, folic acid, valerophenone-*O*-carboxylic acid, vanillic acid, linoleic acid, palmitic acid, oleic acid[9,13,14]
- **vitamins:** vitamins A, B1, B12 and E, biotin[15]
- **polyacetylenes:** falcarindiol, falcarinol[16]
- **sterols** (β-stigmasterol, stigmasterol), adenine, uracil, saccharose, polysaccharides, brefeldine A[9]

Pharmacology (see also[17]):

In vitro effects:

- inhibition of uterus contraction and relaxation of the isolated uterus (volatile oil)
- anti-inflammatory: inhibitory effect on cyclooxygenase-1 and 5-lipoxygenase[13]
- inhibition of platelet aggregation and inhibition of serotonin release from thrombocytes (ferulic acid)[18]
- anti-microbial activity (brefeldine A)[19]

In vivo effects:

- anti-asthmatic, spasmolytic on tracheal muscles (ligustilide, butylidenephthalide, butylphthalide)[20,21]
- anti-asthmatic in clinical studies[17]
- effects on blood circulation in anesthetized dogs[17]
- inhibition of platelet aggregation and reduction of vascular permeability (water extract, ferulic acid) [17,18]

– increase of phagocytosis activity of macrophages (ferulic acid)[22]
– activation of hematopoesis (polysaccharides)[23]
– liver protective effects (amino acids)[24]
– gastrointestinal protective effects (polysaccharides)[30]

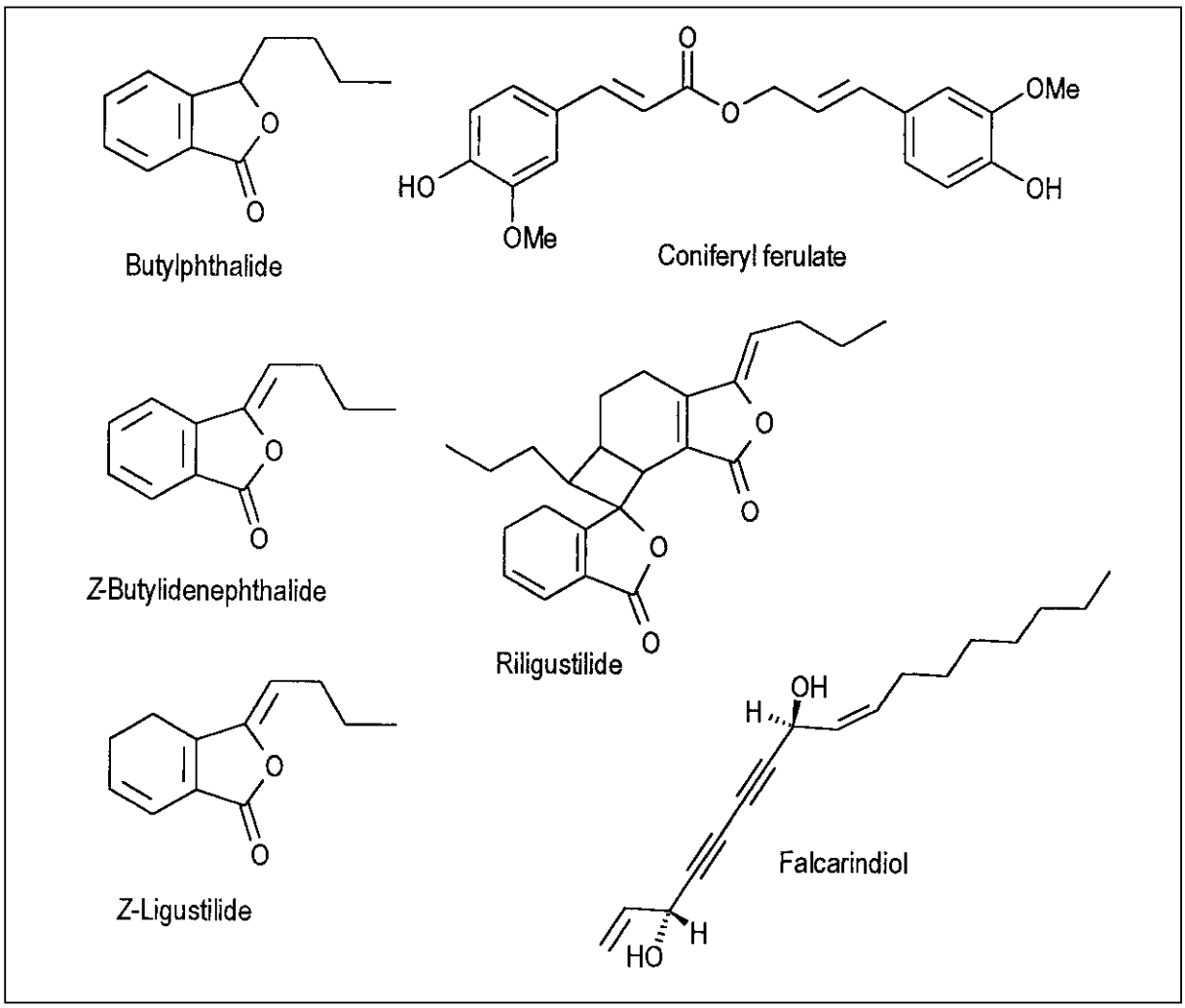

Fig. 1: Formulae of the main constituents

TLC-fingerprint analysis[13,25]

1) Extraction: 5 g coarsely ground drug are soxhlet-extracted with 50 ml *n*-hexane p.a. for one hour. The extract is evaporated to dryness and redissolved in 2.5 ml ethanol and filtered over Millipore® 0.45 µm filtres.

2) Reference compounds: *Z*-Ligustilide (R*f* 0.80), linoleic acid (R*f* 0.35), falcarindiol (R*f* 0.19), dissolved in ethanol (1mg/ml).

3) Separation parameters:

Applied amount:	20 µl extract, 5 µl standard solution
Plates:	Silica gel 60 F254 (Merck)
Solvent system:	Toluene – ethyl acetate – acetic acid (90+10+1), chamber saturation, 15 cm

4) Detection:

Direct evaluation:	$UV_{254\ nm}$ and $UV_{365\ nm}$
Spray reagent:	Anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde, 10 ml glacial acetic acid, 85 ml methanol and 5 ml conc. sulphuric acid, mixed in this order). The TLC plate is intensively sprayed with 10 ml of the reagent and heated for 5–10 min. at 100 °C under observation. The evaluation is carried out in VIS.

Drug samples	Origin	Species
1	Koetzting 08.07.93	*Angelica sinensis*
2	Singapore	*Angelica sinensis*
3	East Earth Herb Inc.	*Angelica sinensis*
4	Kanton 12.03.96	*Angelica sinensis*
5	Kun Ming 12.03.96	*Angelica sinensis*
R	Reference compounds	
6	China, authentic	*Angelica acutiloba*
7	China, authentic	*Angelica dahurica*
8	China, authentic	*Angelica pubescens*

5) Description of the chromatograms:

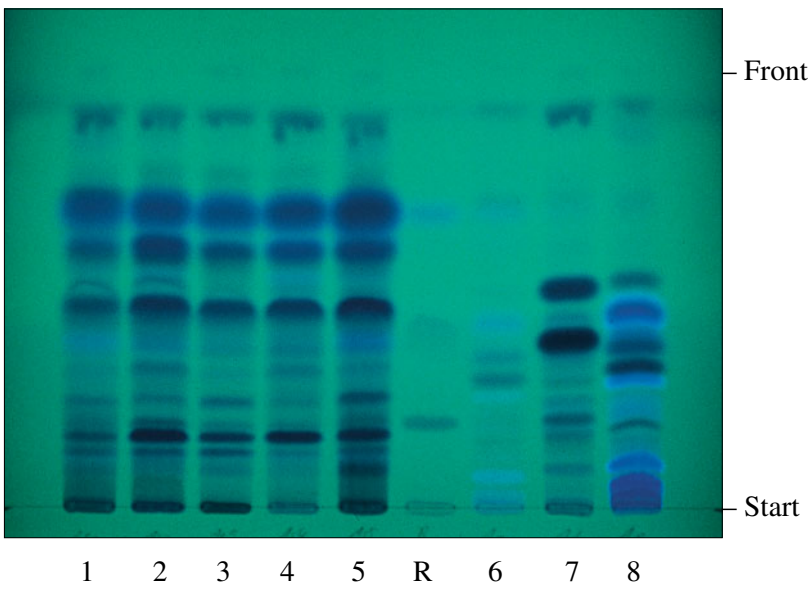

Fig. 2a:
TLC separation of *Angelica sinensis* *n*-hexane extracts (UV_{254} nm)

Fig. 2a: $UV_{254\ nm}$:

Under $UV_{254\ nm}$ four major absorbing zones at R*f* 0.17 (coniferyl ferulate), R*f* 0.47 (levistolide A), R*f* 0.61 (tokinolide B) and R*f* 0.71 (*Z*-butylidenephthalide) are visible in the five *Angelica sinensis* drug samples examined. Furthermore *Z*-ligustilide appears as a dark blue fluorescent zone at R*f* 0.68, coeluting with *E*-butylidenephthalide at this zone. *E*-ligustilide gives a weak blue fluorescent band at R*f* 0.64.

Although the Japanese substitute *Angelica acutiloba* (trace 6) is known to contain the same phthalides like *A. sinensis*, the TLC-pattern of the drug sample examined looks quite different. The zones of *Z*-ligustilide and butylidenepthalide are only very weak, indicating that this species contains much less of these compounds. Instead, two absorbing zones, probably furanocoumarins, are visible at R*f* 0.35 and R*f* 0.30.

The TLC-fingerprints of the two other Chinese Angelica species which are used in Traditional Chinese Medicine differ markedly from the fingerprint of *Angelica sinensis*. The TLC-chromatogram of *A. dahurica* (trace 7) is characterized by two main absorbing zones derived from furanocoumarins at R*f* 0.37 and R*f* 0.5 (also see [27]), whereas the coumarins in *A. pubescens* (trace 8) appear as blueish fluorescent zones in the R*f*-range of 0 – 0.5 (also see[28]).

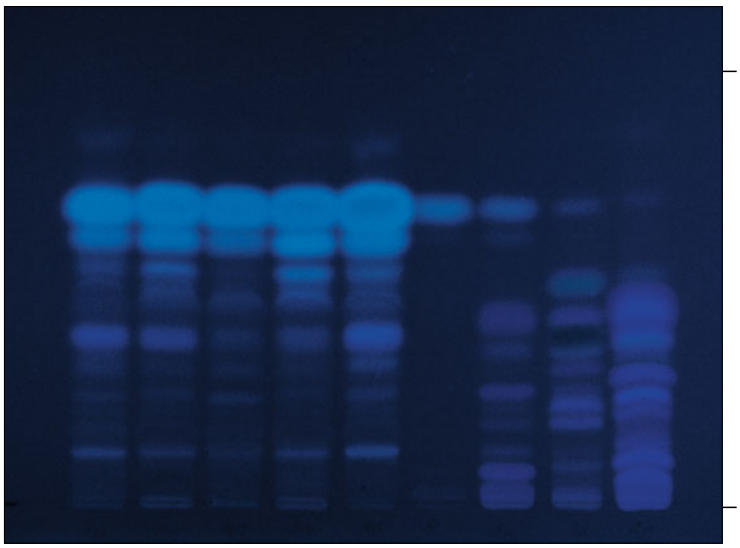

Fig. 2b:

TLC separation of *Angelica sinensis n*-hexane extracts (UV_{365} nm)

Fig. 2b: UV_{365nm}:

Under UV_{365nm} the TLC-chromatogram of *A. sinensis* is dominated by the bright blue fluorescent zone of *Z*-ligustilide at R*f* 0.80. Further blue fluorescent zones are detected at R*f* 0.7 (*E*-ligustilide), R*f* 0.46 and R*f* 0.14.

The TLC-chromatogram of *A. acutiloba* (trace 6) looks quite similar to that *of A. sinensis*, but with marked quantitative differences with respect to the content of *Z*-ligustilide.

The coumarins of *A. dahurica* (trace 7) and *A. pubescens* (trace 8) are visible as yellowish or blue flourescent bands between R*f* 0 and R*f* 0.82.

Fig. 2c: AS-reagent, VIS:

After detection with AS-reagent, linoleic acid becomes visible as a prominent violet spot at R*f* 0.3–0.45. Obviously the roots of the species investigated contain this unsaturated fatty acid in large amounts. In addition another violet band at R*f* 0.23 is detected in all of the drug samples

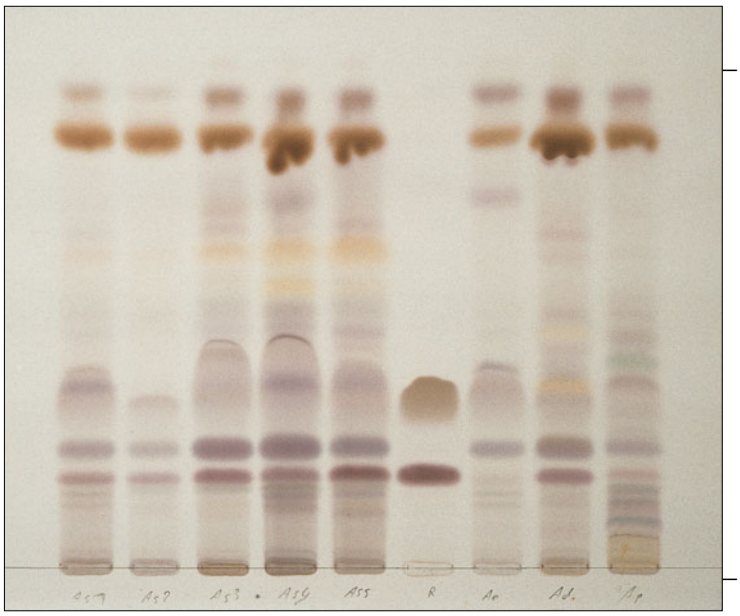

Fig. 2c:
TLC separation of *Angelica sinensis n*-hexane extracts after spraying with anisaldehyde sulphuric acid reagent (VIS)

investigated. Falcarindiol which was invisible under UV-light is detected as greenish-brown band at R*f* 0.19.

After detection with AS-reagent the chromatograms of the four different *Angelica* species show no marked differences.

Discussion:

When analyzed by TLC the drug samples of *Angelica sinensis* showed a very constant pattern of constituents with the main compound *Z*-ligustilide giving a dominant bright blue fluorescent zone.

The TLC-fingerprint of the Japanese species *Angelica acutiloba* is very similar to that of *A. sinensis* and shows mainly quantitative differences. Depending on the phthalide content in *A. acutiloba* samples of different quality, it can be difficult to distinguish these two drugs by TLC-analysis. Another official Chinese drug, *Ligusticum chuanxiong*, also contains phthalides as main compounds and therefore also shows a similar TLC-chromatogram to *A. sinensis*. Please refer to[25,29].

On the other hand, the two Chinese *Angelica* species *A. dahurica* and *A. pubescens* can easily be distinguished by their TLC-fingerprints which are dominated by the coumarins[(27,28)].

HPLC fingerprint analysis[22]

1) Sample preparation: The same extracts are used as for TLC

2) Injection volume: 10 µl ethanolic solution of the extracts

3) HPLC parameters:

Apparatus:	Liquid chromatograph HP 1050 with photodiode array detector HP 1050
Column:	LiChroCART® 125–4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART® 4–4 with LiChrospher® 100 RP 18 (5 µm), Merck
Solvent system:	A: water; B: acetonitrile
Gradient:	40–55 % B in 15 min. (linear), 55–95 % B in 18 min. (linear), 95 % B for 2 min. (isocratic)
Flow rate:	1.0 ml/min.
Detection:	210 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compounds
1	6.7	coniferyl ferulate
2	8.7	senkyunolide A
3	9.4	butylphthalide
4	11.0	*E*-ligustilide
5	11.7	*E*-butylidenephthalide
6	12.3	*Z*-ligustilide
7	12.8	*Z*-butylidenephthalide
8	17.3	falcarindiol
9	21.2	tokinolide B
10	22.0	riligustulide
11	22.1	levistolide A
12	26.2	α-linolenic acid
13	28.8	linoleic acid

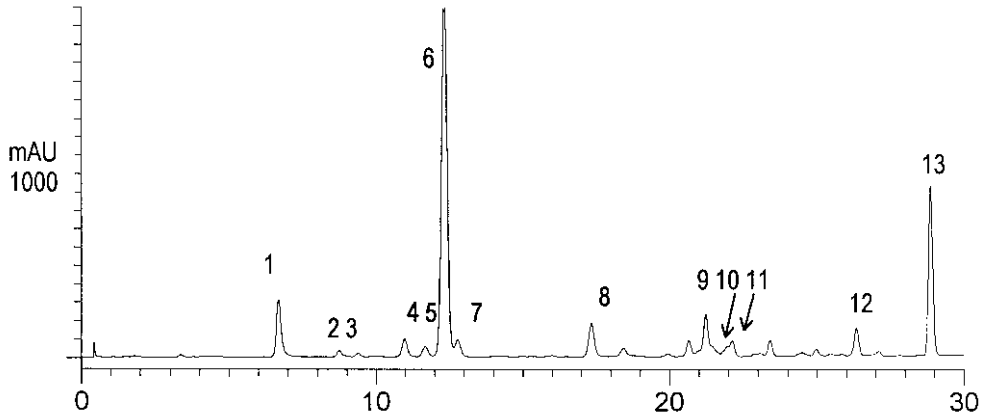

Fig. 3a: HPLC-fingerprint chromatogram of *A. sinensis* *n*-hexane extract (detection at 210 nm)

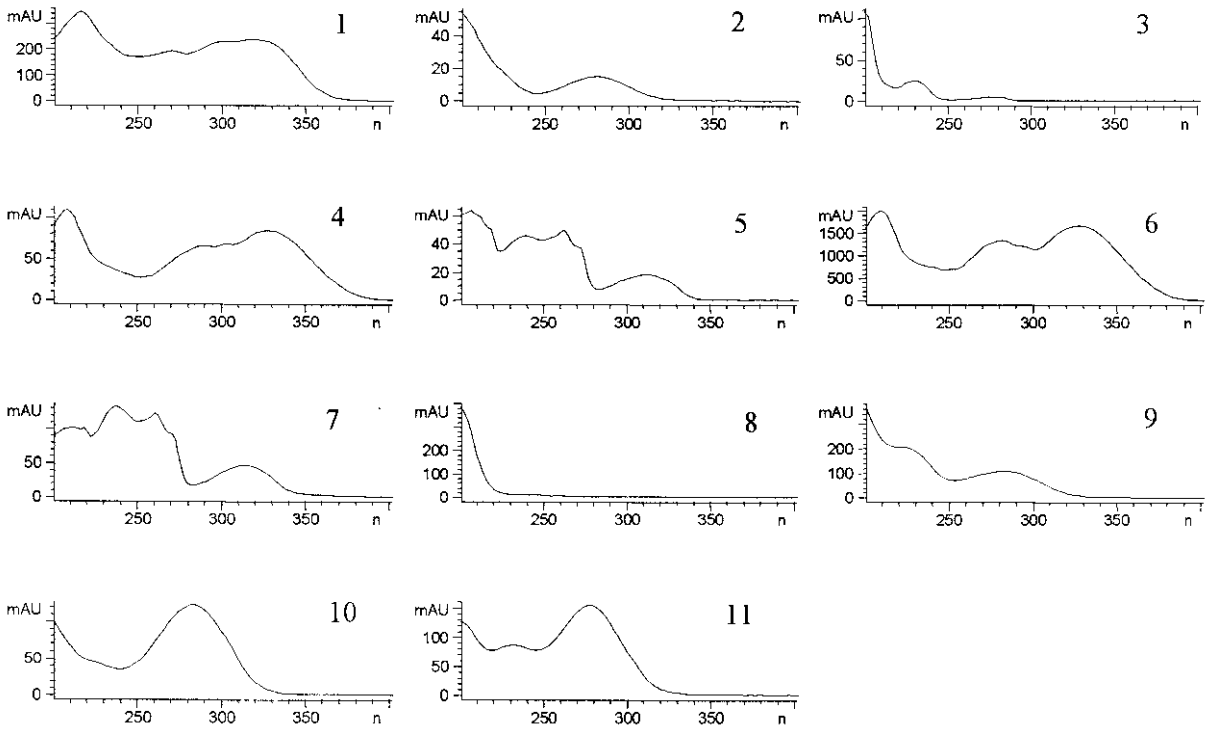

Fig. 3b: Online recorded UV-spectra of the main peaks in the HPLC-separation of *A. sinensis* extracts.

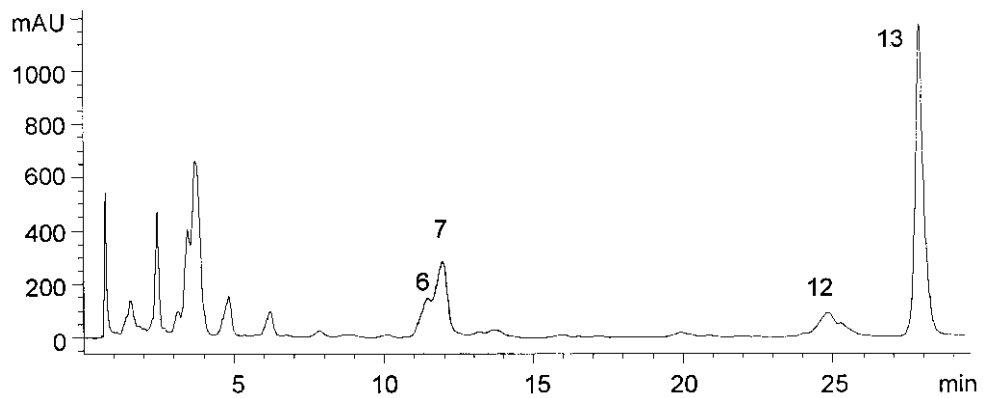

Fig. 3c: HPLC-fingerprint chromatogram of *A. acutiloba* *n*-hexane extract (detection at 210 nm)

Description and Discussion:

The HPLC-analysis of *A. sinensis* drug samples (1–5) from different sources resulted in very similar fingerprints.

The main difference observed was the content of the main compound *Z*-ligustilide. Generally the amount of *Z*-ligustilide was much higher in the *n*-hexane extracts of root stocks which were purchased as whole drug compared to the *n*-hexane extracts of the drugs which had been already sliced (root stock, main root or branching roots).

For the identification of *A. sinensis*, HPLC-analysis is a very suitable method. It allows the differentiation between this species and its Japanese substitute *Angelica acutiloba* on the basis of quantitative and qualitative differences. The HPLC-fingerprint of *A. acutiloba* shows prominent peaks between Rt 0 min and Rt 7 min which are derived from furanocoumarins according to the UV-spectra and are not found in the chromatogram of *A. sinensis*.

Recently RAPD analysis has been applied for the discrimination of *A. sinensis, A. acutiloba, A. gigas* and *A. pubescens*[31]. High performance capillary electrophoresis has been used for the quantitative analysis of ferulic acid in Danggui[32].

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Radix Angelicae dahuricae
Baizhi

Pharmacopoeias:

Chinese Pharmacopoeia X

Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[1]

Official drugs:[1]

Angelica dahurica (Fisch. Ex Hoffm.) Benth. et Hook f.

Angelica dahurica (Fisch. Ex Hoffm.) Benth. et Hook f. var. *formosana* (Boiss.) Shan et Yuan

– Apiaceae –

The drug is known as *Xiang Bai Zhi*, *Byakushi* (Japanese), *Paegchi* (Korean) and *Chinese Angelica Root* (English).

Description of the drug:[1,4]

Long-conical, 10–25 cm long, 1.5–2.5 cm in diameter. Externally greyish-brown or yellowish-brown, the root stock obtusely quadrangular or subrounded, with longitudinal wrinkles, rootlet scars and lenticel-like transverse protrudings, some of them arranged in four longitudinal rows. Apex with dented stem scars. Texture compact, fracture white or greyish-white and starchy, cambium ring brown, subsquare or subrounded, scattered with many brown oil dots in bark. Odour aromatic; taste pungent and bitter.

Pretreatment of the raw drug[1]

Eliminate foreign matter, grade according to size, soak briefly, cut into thick slices and dry.

Medicinal use[1,2,3,4]

In Traditional Chinese Medicine the decoction is used as an antipyretic and analgesic for patterns of externally contracted windcold, especially those with headache; it is also used for supraorbital pain, nasal congestion, and toothache.

Externally it reduces swelling and expels pus in early stages of surface sores and carbuncles.

The drug expels dampness and alleviates discharge, in particular vaginal discharge.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,2,3,4]

Taste:	acrid
Temperature:	warm
Channels entered:	*lung, stomach*
Effects:	dispels wind, removes damp, clears blocked noses, relieves pain and promotes subsidence of swelling and drainage of pus.
Symptoms and indications:	headache, particularly pain in the forehead, and blocked nose due to colds; sinusitis; toothache; excessive leukorrhea; swelling, painfull sores and wounds.

Main constituents (see Fig. 1)[5–20]

- **furanocoumarins:** e.g. imperatorin, isoimperatorin, phellopterin, bergapten, cnidilin[6–10]
- **coumarins:** e.g. scopoletin, 7-demethylsuberosin, cedrelopsin[6–8]
- **coumarin glycosides:** e.g. nodakenin, 3′-hydroxymarmesinin[11]
- **polyacetylenes:** falcarindiol[12]
- **steroles:** stigmasterol, sitosterol,
- **lactones:** β-angelica lactone, 2-hydroxy-3,4-dimethyl-2-butene-4-olide, γ-nonalactone and γ-decalactone[13–15]
- unsaturated fatty acids[12]

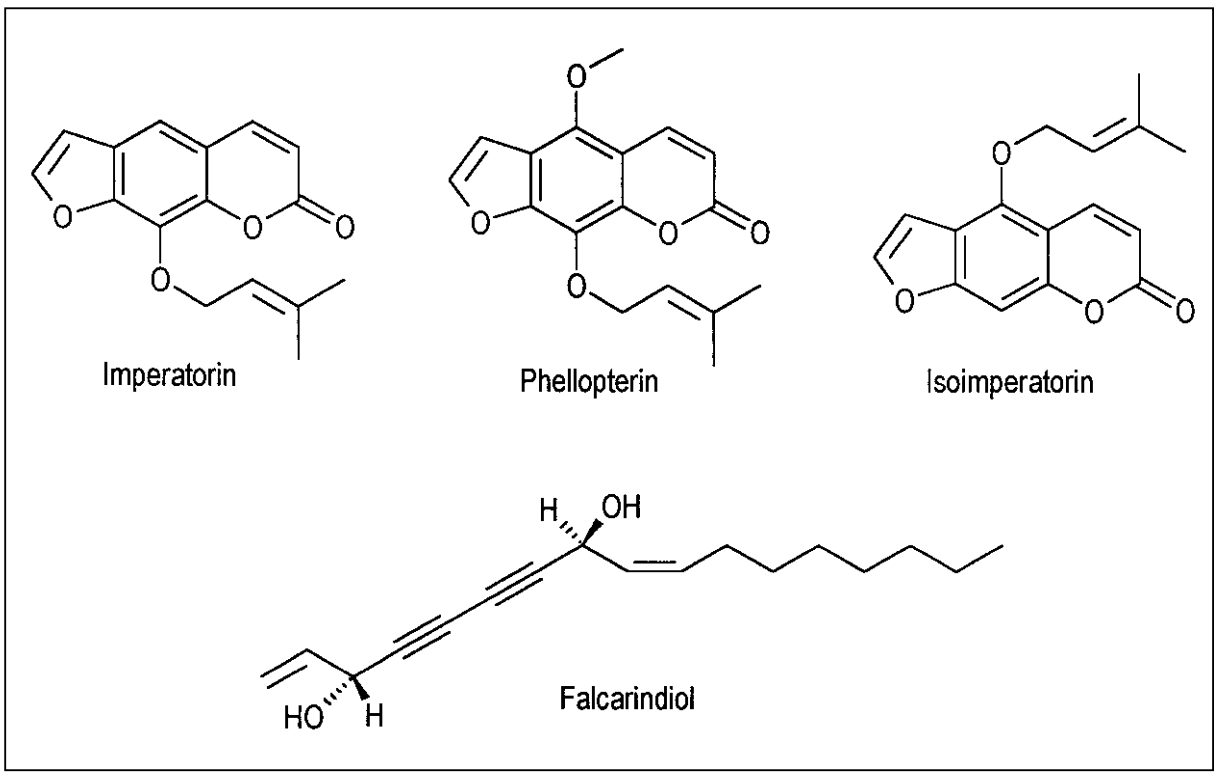

Fig. 1: Formulae of the main constituents

Pharmacology:[12, 21–37]

- Antimicrobial activity: *in vitro* inhibitory activity against *Shigella* and *Salmonella* species, *Bacillus subtilis, Escherichia coli, Cladosporium herbarum* and *Aspergillus candidus* (phellopterin, byak angelicin, scopoletin, heraclenol).
- Effective in promotion of healing and avoiding sequelae of corneal ulcers secondary flash burns.
- A powder composed of Angelicae dahuricae radix and borneol has been effective in treating headache and toothache when inhaled through nostrils. It also appears to be usefull in treating trigeminal neuralgia.
- Some coumarins activated adrenaline-induced lipolysis (oxypeucedanin, bergapten, xanthotoxin, imperatorin, phellopterin), ACTH-induced lipolysis (oxypeucedanin hydrate, imperatorin, phellopterin) or insulin-stimulated lipogenesis (byakangelicin, neobyakangelicin, isopimpinellin).
- Oral administration of psoralene derivatives in combination with photochemotherapy using long wave UV radiation is used to treat psoriasis.
- Mutagenic and carcinogenic activities of psoralene derivatives after photoactivation.
- Inhibition of histamin release *in vivo* (mice) (bergapten, byak angelicin, oxypeucedanin hydrate).
- Inhibition of diazepam binding *in vitro* (phellopterin).
- Inhibition of aldose-reductase *in vivo* (rats) (byak angelicin).
- Inhibition of cyclooxygenase-1 and 5-lipoxygenase *in vitro* (*n*-hexane extract, falcarindiol).

TLC-fingerprint analysis:

1) Extraction: 5 g coarsely ground drug are soxhlet-extracted with 50 ml *n*-hexane p.a. for one hour. The extract is evaporated to dryness and redissolved in 2.5 ml ethanol and filtered over Millipore® 0.45 µm filtres.

2) Reference compounds: isoimperatorin (R*f* 0.52), imperatorin (R*f* 0.40), linoleic acid (R*f* 0.38), falcarindiol (R*f* 0.19), dissolved in ethanol (1 mg/ml)

3) Separation parameters:

Applied amount:	20 µl extract, 5 µl standard solution
Plates:	Silica gel 60 F_{254} (Merck)
Solvent system:	Toluene – ethyl acetate – acetic acid (90+10+1), tank saturation, 15 cm

4) Detection:

Direct evaluation:	$UV_{254\ nm}$ and $UV_{365\ nm}$
Spray reagent:	Anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde, 10 ml glacial acetic acid, 85 ml methanol and 5 ml conc. sulphuric acid, mixed in this order). The TLC plate is intensively sprayed with 10 ml of the reagent and heated for 5–10 min. at 100 °C under observation. The evaluation is carried out in VIS.

Drug samples	Origin	Species
1	China 96	*A. dahurica*
2	Hongkong 25.10.94	*A. dahurica*
3	Sichuan 94	*A. dahurica*
4	Pharmacy Hangzhou 09.94	*A. dahurica*
5	Beijing Bungantang	*A. dahurica*
R	Reference compounds	
6	Koetzting 08.07.93	*Angelica sinensis*
7	China, authentic	*Angelica pubescens*

Description of the chromatograms:

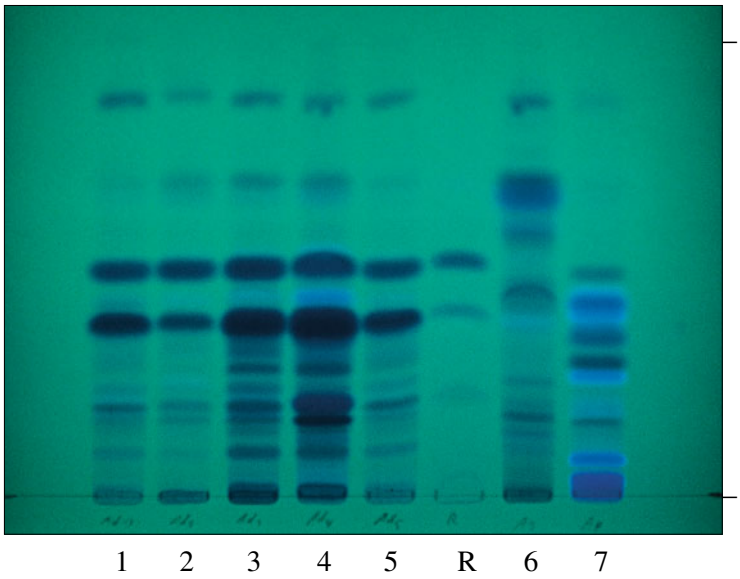

Fig. 2a:
TLC separation of *Angelica dahurica* *n*-hexane extracts (UV_{254} nm)

Fig. 2a: $UV_{254\ nm}$:

The TLC-chromatograms of the five *A. dahurica* samples are dominated by two strongly absorbing zones: isoimperatorin (R*f* 0.52) and imperatorin, which coelutes with phellopterin at R*f* 0.4. Drug samples 1, 2 and 5 hardly show any differences. In sample 2 the furanocoumarins are less concentrated, therefore this drug sample seems to be of minor quality. On the other hand, samples 3 and 4 show the highest concentration of compounds. In the chromatogram of these drug samples additional absorbing zones become visible between R*f* 0.10 and R*f* 0.36, as well as at R*f* 0.45.

In comparison to *A. dahurica* the TLC-chromatograms of *Angelica sinensis* and *Angelica pubescens*, which are also used in Traditional Chinese Medicine, show completely different characteristics. Although the absorbing spots of isoimperatorin, phellopterin and imperatorin are also found in the TLC-chromatogram of *A. pubescens* (trace 7), this species can easily be distinguished by the blueish zones of simple coumarins between R*f* 0 and R*f* 0.58 (see[39]). The UV absorbing zones of isoimperatorin, imperatorin and phellopterin are missing in the TLC-chromatogram of *A. sinensis* (trace 6). The chromatogram of this *Angelica* species is

characterized by a blueish-dark zone at R*f* 0.68 and a strong absorbing zone at R*f* 0.72 which are derived from the phthalides in this plant (see [38,40]).

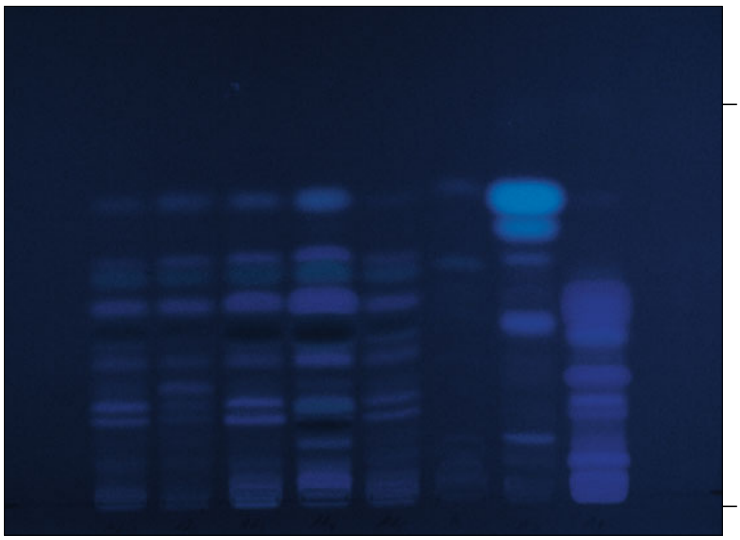

Fig. 2b:
TLC separation of *Angelica dahurica* *n*-hexane extracts (UV_{365} nm)

Fig. 2b: UV_{365nm}:
The furanocoumarins isoimperatorin (R*f* 0.52), imperatorin (R*f* 0.40) and phellopterin (R*f* 0.40) appear as whitish fluorescent zones and are detected in all five *A. dahurica* samples examined. In addition, blue fluorescent bands of further coumarins are visible between R*f* 0 and R*f* 0.38 and at R*f* 0.45. The highest concentration of coumarins is found in drug sample 4.

Again the TLC-chromatograms of the two other *Angelica* species show distinct differences: the chromatogram of *A. sinensis* (trace 6) is dominated by a strong blue fluorescent zone at R*f* 0.75. The chromatogram of *A. pubescens* (trace 7) is characterized by many striking blue fluorescent zones between the start and R*f* 0.53.

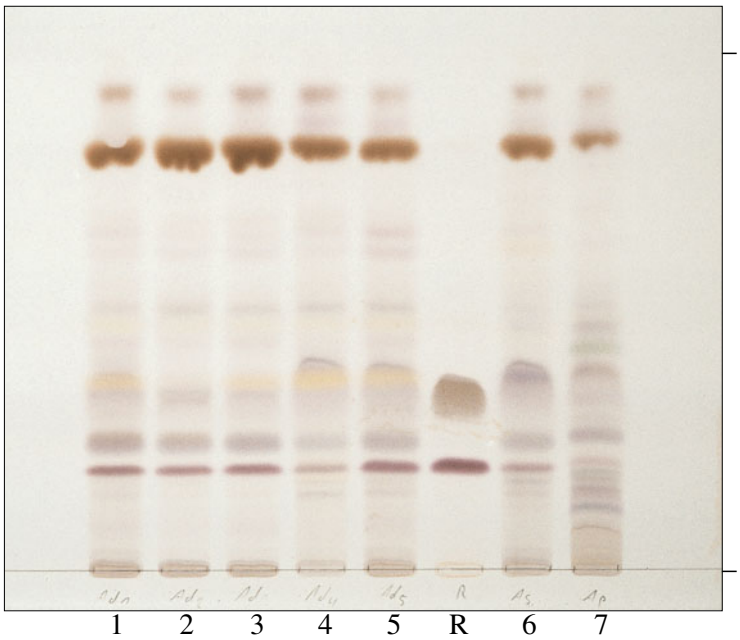

Fig. 2c:

TLC separation of *Angelica dahurica* *n*-hexane extracts after spraying with anisaldehyde sulphuric acid reagent (VIS)

Fig. 2c: AS-reagent, VIS:

After detection with AS-reagent, falcarindiol appears as greenish-brown zone (R*f* 0.19), and linoleic acid as a striking violet spot (R*f* 0.3 – 0.4), indicating that the roots of *A. dahurica* are rich in this unsaturated fatty acid.

After detection with AS-reagent the TLC-fingerprints of *Angelica sinensis* and *Angelica pubescens* look very similar and cannot be distinguished from *A. dahurica*.

Discussion:

All *Angelica dahurica* drug samples examined showed a very constant pattern of constituents when analyzed by TLC. The TLC-chromatograms of the two other *Angelica* species which can be found in the Chinese Pharmacopoeia, *Angelica sinensis* and *Angelica pubescens*, are completely different from the chromatogram of *Angelica dahurica* under UV_{254nm} and UV_{365nm}.

TLC-analysis is therefore a suitable method for the identification of *A. dahurica* and the safe differentiation between the three official Chinese *Angelica* species.

HPLC fingerprint analysis:[12]

1)	Sample preparation:	The same extracts are used as for TLC
2)	Injection volume:	10 µl ethanolic solution
3)	HPLC parameters:	
	Apparatus:	Liquid chromatograph HP 1050 with photodiode array detector HP 1050
	Column:	LiChroCART® 125–4 with LiChrospher® 100 RP 18 (5 µm), Merck
	Pre-column:	LiChroCART® 4–4 with LiChrospher® 100 RP 18 (5 µm), Merck
	Solvent system:	A: water; B: acetonitrile
	Gradient:	40–55 % B in 15 min. (linear), 55–95 % B in 18 min. (linear), 95 % B for 2 min. (isocratic)
	Flow rate:	1.0 ml/min.
	Detection:	210 nm

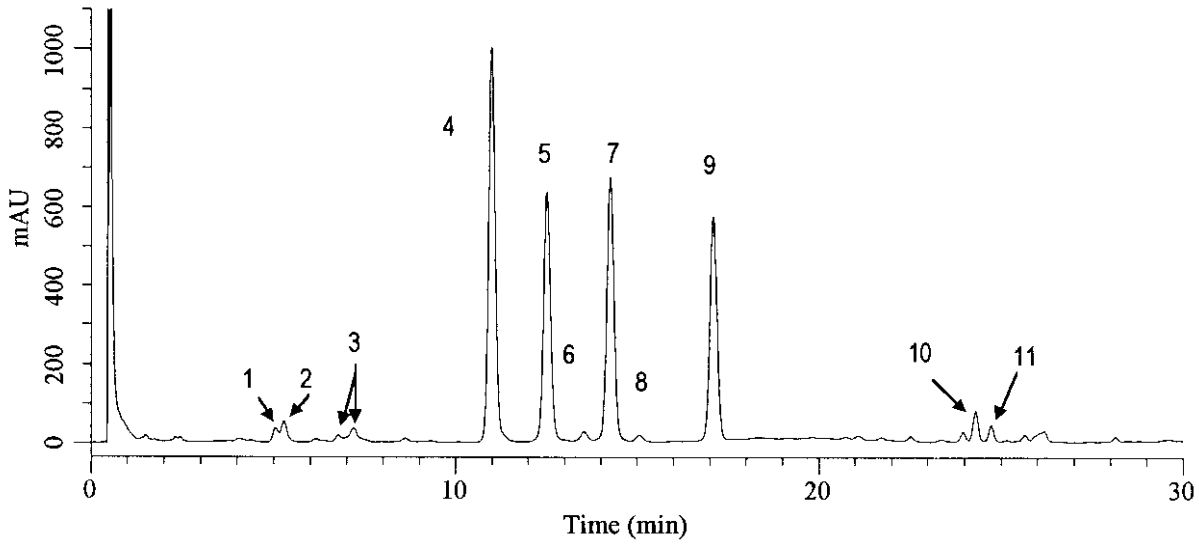

Fig. 3a: HPLC-fingerprint chromatogram of *A. dahurica n*-hexane extract (detection at 210 nm)

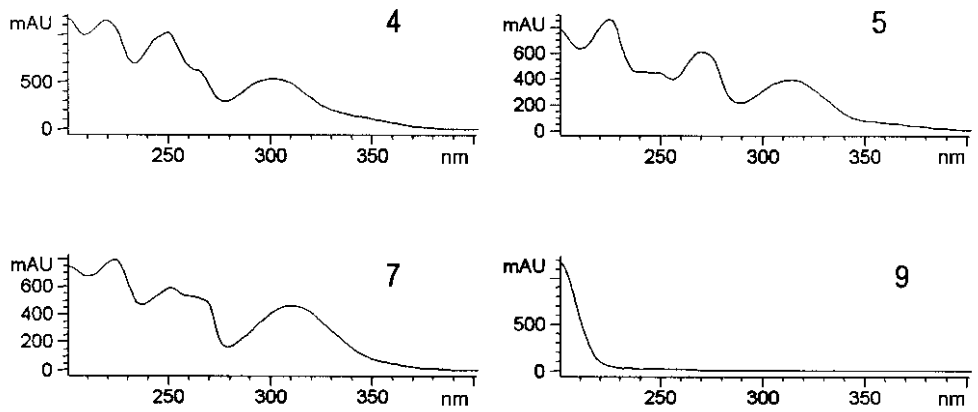

Fig. 3b: Online recorded UV-spectra of the main peaks in the HPLC-separation of *A. dahurica*.

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	5,1	furanocoumarin
2	5,3	bergaptene
3	6,8–7,2	furanocoumarin
4	11,0	imperatorin
5	12,5	phellopterin
6	13,5	cnidilin
7	14,3	isoimperatorin
8	15,0	simple coumarin
9	17,1	falcarindiol
10	24,3	coumarin dimer
11	24,7	coumarin dimer

Description:

The HPLC-fingerprint of *Angelica dahurica* is very characteristic with 4 sharp main peaks. These main peaks represent the furanocoumarins imperatorin (peak **4**), phellopterin (peak **5**) and isoimperatorin (peak **7**) as well as the polyacetylene falcarindiol (peak **9**). The HPLC-chromatograms of all *A. dahurica* drug samples examined appear very similar. Mainly quantitative differences were observed. Therefore *A. dahurica* can easily be distinguished from the other official *Angelica* species (see [38,39,40]).

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Radix Ligustici chuanxiong
Chuanxiong

Pharmacopoeias:

Chinese Pharmacopoeia X

Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[1]

Official drugs:

Ligusticum chuanxiong HORT. (syn. *Ligusticum wallichii* FRANCH.)

– Apiaceae –

The drug is known as Senkyu (Japanese), Ch'onkung (Korean), and Szechuan lovage root (English).

The Japanese substitute *Cnidium officinale* MAKINO (syn. *Ligusticum officinale* KITAGAWA) is not official in China.

Description of the drug:[1–3]

Shaped in irregular knotty and fist-like masses, 2–7 cm in diameter. Externally yellowish-brown, rough and shrunken, with many parallel and raised annulations, showing dents and subrounded breathing scars on the summit and numerous tuberculous rootlet scars beneath the summit and at the annulations. Texture compact; not easily broken; fractures yellowish-white or greyish-yellow, scattered with yellowish-brown oil cavities. Cambium in an undulate ring.

Odour: strongly aromatic; taste: bitter, pungent, leaving a slight numbness, but sweetish afterwards.

Pretreatment of the raw drug:[1]

Eliminate foreign matter, grade according to size, soak briefly, cut into thin slices and dry.

Medicinal use:[1–3]

The drug is used for any blood stasis pattern. It is important in gynecology for problems as dysmenorrhea, amenorrhea, difficult labour or lochioschesis.

Chuanxiong is a leading herb for headaches, dizziness or painful obstruction.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1–3]

Taste:	acrid
Temperature:	warm
Channels entered:	*liver, gallbladder, pericardium*
Effects:	dispels wind, removes damp, clears blocked noses, relieves pain and promotes subsidence of swelling and drainage of pus.
Symptoms and indications:	dysmenorrhea, amenorrhea, difficult labour and lochioschesis; headache, dizziness and painful obstruction.

Main constituents (see Fig. 1)[4–19]

- **Alkylphthalides:** e.g.: *Z*-ligustilide, senkyunolide A, butylphthalide, *Z*-butylidenephthalide, neocnidilide, cnidilide[5–11,19]
- **Hydroxy alkylphthalides:** e.g.: senkyunolide B - M[10–16]
- **Phthalide dimers:** e.g.: wallichilide, levistolide A, tokinolide B[10,12,17]
- **Phenolic constituents:** ferulic acid, coniferyl ferulate[12]
- **Nitrogen containing substances:** tetramethylpyrazine, perlolyrine[15,18]
- **Polyacetylenes:** falcarindiol[12]
- Quinones: sekyunone[13]
- Steroles: pregnenolone[12]
- Unsaturated fatty acids[11]

Pharmacology: [12, 20–37]

- Effect on central nervous system: sedative effect in rats and mice. It prolongs the hypnotic effect of barbiturates, but does not counteract the stimulant effect of caffeine.
- Cardiovascular effect: orally: weak hypertensive effect in animal experiments; intravenous or intramuscular: significant hypotensive effect in animal experiments; the phthalides have also been shown to exhibit antiarrhythmic effects and dilating activity on coronary arteries. Ligustrazine increases the coronary flow and decreases myocardial contractile force. It lowers the arterial blood pressure and increases beating rate, coronary blood flow and myocardial oxygen consumption.
- Effect on smooth muscles: small amounts stimulate the uteri of pregnant rabbits; large amounts stop contractile effects completely; continued injections result in the death of the fetus; small amounts inhibit the contraction of small intestines; large amounts stop all contraction; also inhibitory effects on the contraction of nonpregnant rat uteri *in vitro* have been observed.
- Antimicrobial effect: *in vitro* inhibitory effect against *Shigella sonnei, Pseudomonas aeruginosa, Shigella typhi* and *Vibrio cholerae*. It also has *in vitro* inhibitory effects against many dermatomycoses.
- Prevention of thrombus formation: ligustrazine inhibits platelet aggregation and might have the ability to displace Ca^{2+} from platelet membranes. Increases phagocytosis activity.
- Anti asthmatic and spasmolytic effects.
- Inhibition of cyclooxygenase-1 and 5-lipoxygenase *in vitro.*

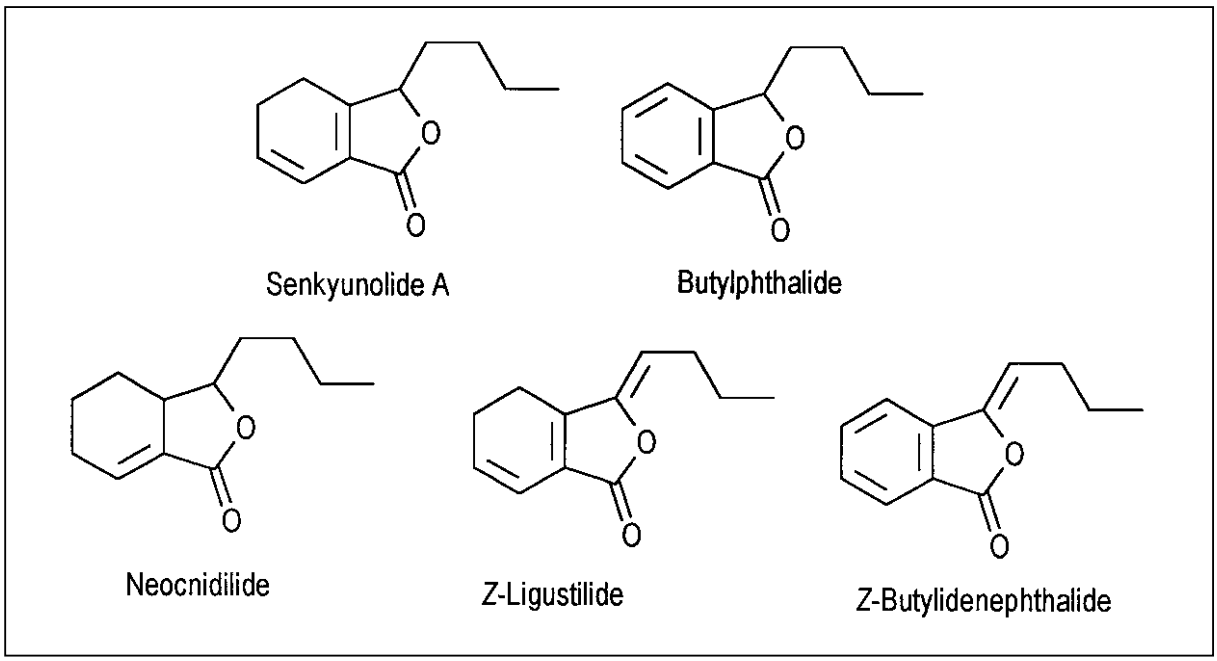

Fig. 1: Formulae of the main constituents

TLC-fingerprint analysis

1) Extraction: 5 g coarsely ground drug are soxhlet-extracted with 50 ml *n*-hexane p.a. for 1 hour. The extract is evaporated to dryness and redissolved in 2.5 ml ethanol and filtered over Millipore® 0.45 µm filtres.

2) Reference compounds: *Z*-Ligustilide (R*f* 0.7), linoleic acid (R*f* 0.35), neocnidilide (R*f* 0.48), dissolved in ethanol (1mg/ml).

3) Separation parameters:

   Applied amounts: 20 µl extract, 5 µl standard solution

   Plates: Silica gel 60 F_{254} (Merck)

   Solvent system: Toluene - ethyl acetate - glacial acetic acid (90+10+1), chamber saturation, 15 cm

4) Detection:

   Direct evaluation: $UV_{254\ nm}$ and $UV_{365\ nm}$

   Spray reagent: Anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde, 10 ml glacial acetic acid, 85 ml methanol and 5 ml conc. sulphuric acid, mixed in this order).

   The TLC plate is intensively sprayed with 10 ml of the reagent and heated for 5–10 min at 100 °C under observation. The evaluation is carried out in VIS.

Drug samples	Origin	Species
1	Koetzting	*Ligusticum chuanxiong*
2	Koetzting 17.07.96	*Ligusticum chuanxiong*
3	Koetzting 26.01.95	*Ligusticum chuanxiong*
4	Singapore 07.07.96	*Ligusticum chuanxiong*
5	China	*Ligusticum chuanxiong*
R	Reference compounds	
6	Koetzting 08.07.93	*Angelica sinensis*
7	Japan, authentic	*Cnidium officinale*

5) Description of the chromatograms:

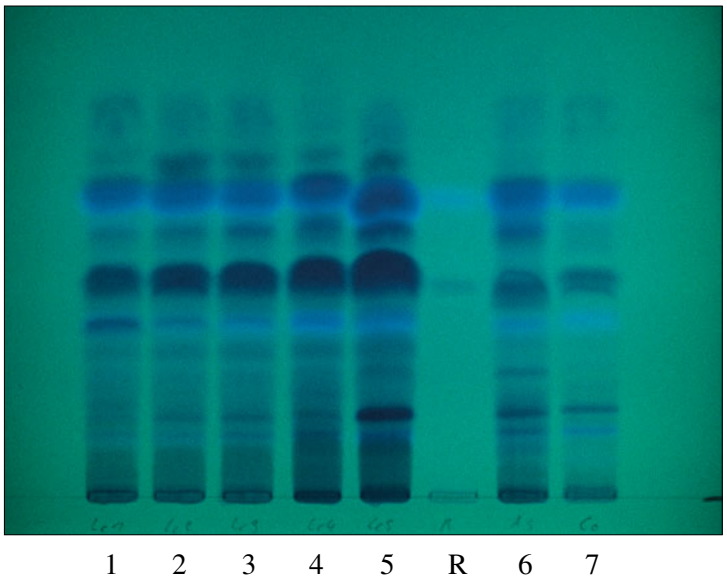

Fig. 2a:
TLC separation of *Ligusticum chuanxiong* *n*-hexane extracts (UV_{254} nm)

Fig. 2a: UV_{254nm}:

The five *L. chuanxiong* samples examined give very similar TLC-patterns. The chromatograms are characterized by the fluorescence quenching zones of *Z*-butylidenephthalide at R*f* 0.73, tokinolide B at R*f* 0.58, neocnidilide (overlapped by another strong UV-absorbing compound) at R*f* 0.48 and coniferylferulate at R*f* 0.18, the blueish fluorescing spot of *Z*-ligustilide which appears dark blue in the middle at R*f* 0.7 and another blue fluorescing zone at R*f* 0.43. Sample 5 showed the highest concentration of constituents.

The TLC chromatograms of *Angelica sinensis* (trace 6) and *Cnidium officinale* (trace 7) look very similar and cannot be distinguished from *L. chuanxiong*.

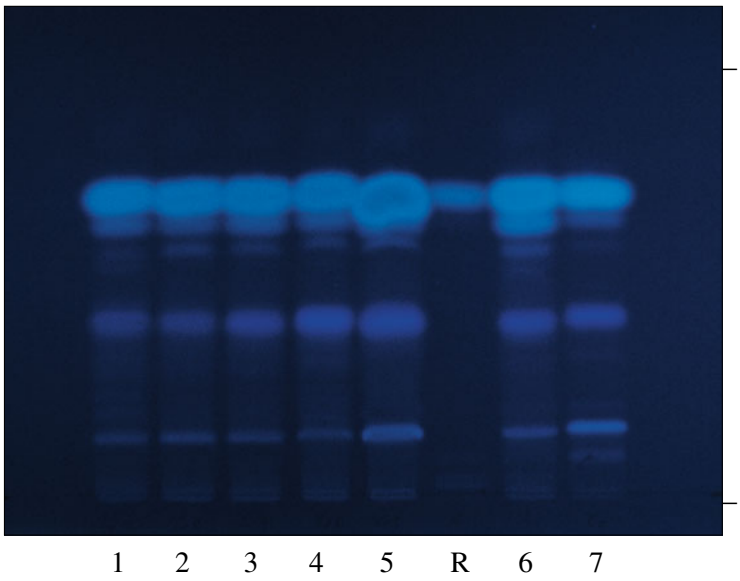

Fig. 2b:
TLC separation of *Ligusticum chuanxiong n*-hexane extracts (UV_{365} nm)

Fig. 2b: UV_{365nm}:
Under UV_{365nm} the TLC-chromatogram of *L. chuanxiong* is dominated by the bright blue fluorescent zone of *Z*-ligustilide at R*f* 0.7. Further blue fluorescent zones are detected at R*f* 0.62 (*E*-ligustilide), R*f* 0.39 and R*f* 0.15. The thin layer chromatograms of *Angelica sinensis* look very similar. The only difference can be seen in a higher content of *Z*-ligustilide in *A. sinensis* (trace 6) which we often observed in the course of our investigations. *C. officinale* (trace 7) looks practically identical to *L. chuanxiong*.

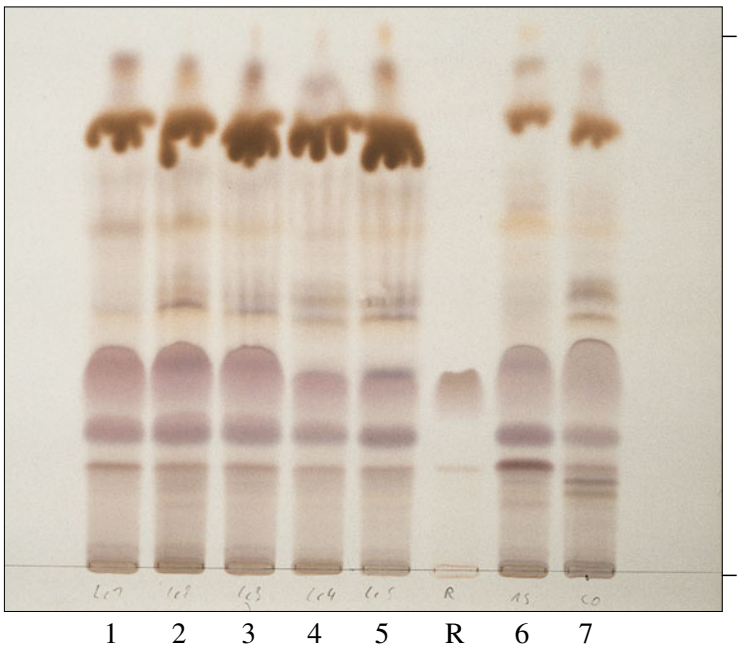

Fig. 2c:
TLC separation of *Ligusticum chuanxiong n*-hexane extracts after spraying with anisaldehyde sulphuric acid reagent (VIS)

Fig. 2c: VS-reagent, VIS:
After detection with AS-spray reagent linoleic acid becomes visible as prominent violet spot at R*f* 0.3-0.4, indicating that the roots of the species examined are rich in this unsaturated fatty acid. In addition another violet band at R*f* 0.24 is detected in all drug samples investigated.

Furthermore *A. sinensis* (trace 6) contains falcarindiol in detectable amounts. Falcarindiol can be seen as greenish-brown band at R*f* 0.2 which only occurs as very weak spot in the chromatograms of *L. chuanxiong* and *C. officinale*. Nevertheless, there is no striking difference between the TLC-chromatograms of *L. chuanxiong*, *A. sinensis* and *C. officinale*.

HPLC fingerprint analysis[11,38,40]

1) Sample preparation: The same extracts are used as for TLC

2) Injection volume: 10 µl ethanolic solution

3) HPLC parameters

Apparatus:	Liquid chromatograph HP 1050 with photodiode array detector HP 1050
Column:	LiChroCART® 125–4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART® 4–4 with LiChrospher® 100 RP 18 (5 µm), Merck
Solvent system:	A: water; B: acetonitrile
Gradient:	40–55 % B in 15 min. (linear), 55–95 % B in 18 min. (linear), 95 % B for 2 min. (isocratic)
Flow rate:	1.0 ml/min.
Detection:	210 nm

Retention times of the main peaks of *L. chuanxiong* HPLC-fingerprint:

Peak	Rt (min.)	Compounds
1	8,6	senkyunolide A
2	9,2	butylphthalide
3	11,8	neocnidilide
4	12,2	*Z*-ligustilide
5	12,6	*Z*-butylidenephthalide
6	17,1	falcarindiol
7	21,0	tokinolide B
8	21,9	levistolide A

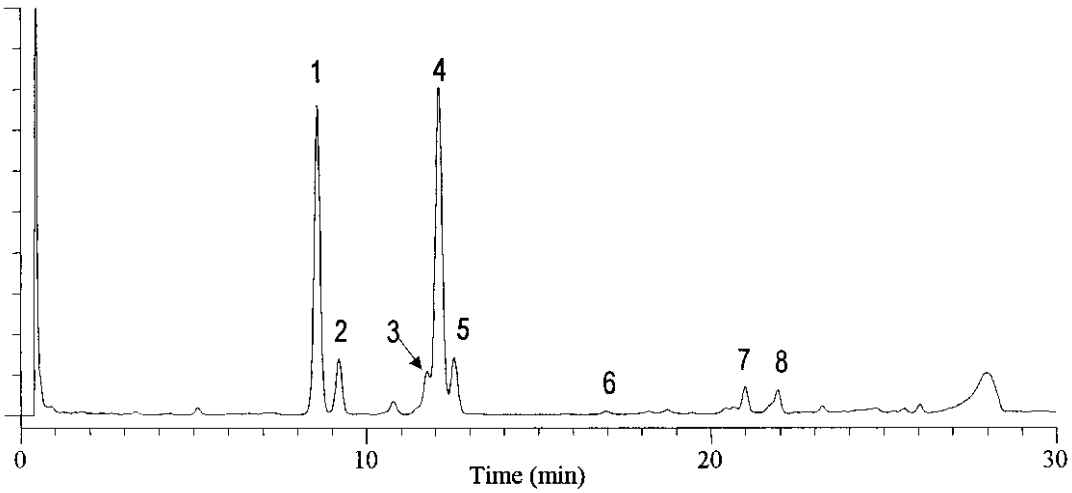

Fig. 3a: HPLC-fingerprint chromatogram of *L. chuanxiong n*-hexane extract (detection at 210 nm)

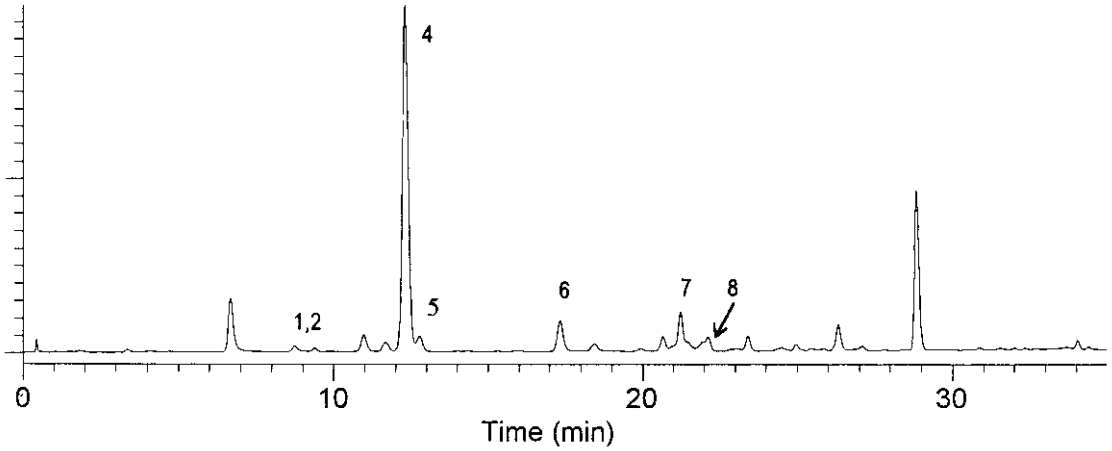

Fig. 3b: HPLC-fingerprint chromatogram of *A. sinensis n*-hexane extract (detection at 210 nm)

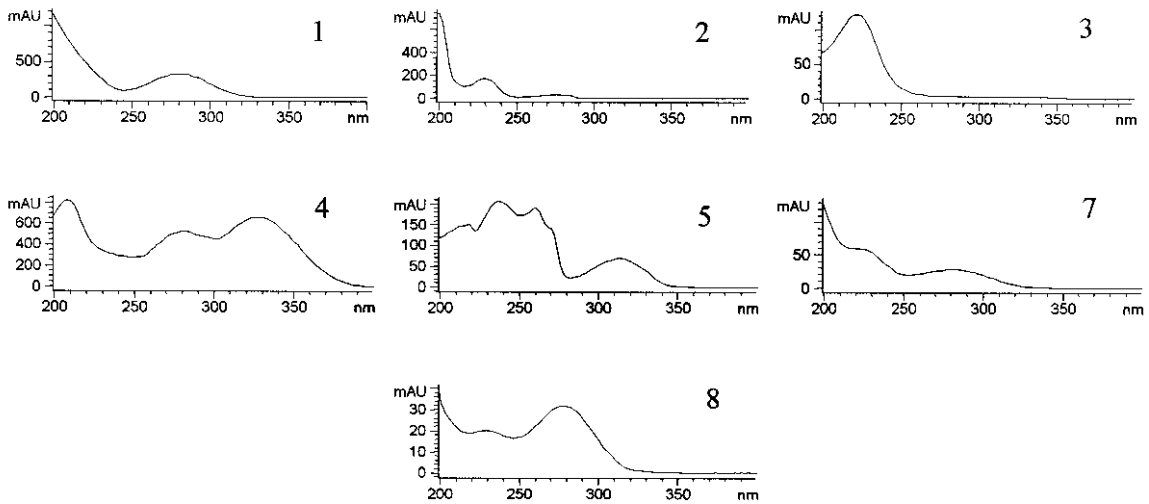

Fig. 3c: Online recorded UV-spectra of the main peaks in the HPLC-separation of *L. chuanxiong*.

Discussion:

Fig. 3a shows the typical HPLC-fingerprint of an *n*-hexane extract of *Ligusticum chuanxiong*. The monomeric phthalides senkyunolide A, butylphthalide, neocnidilide, *Z*-ligustilide and *Z*-butylidenephthalide represent the main peaks in the chromatogram and give very characteristic UV-spectra (see Fig. 3c).

HPLC-analysis of five *Ligusticum chuanxiong* drug samples gave very similar chromatograms, differing mainly in the quantities of the main constituents.

Although the differentiation between *L. chuanxiong* and *Angelica sinensis* was almost impossible by TLC-analysis, these species are easy to distinguish by HPLC-analysis. Fig 3b gives the HPLC-fingerprint of the *n*-hexane extract of the roots of *A. sinensis*. Here *Z*-ligustilide is the dominating main compound whereas the other phthalides are only present in small quantities. Neocnidilide is missing in *A. sinensis*. Furthermore coniferyl ferulate (Rt 6.7 min) and falcarindiol (Rt 17.3 min) are more prominent peaks in the *A. sinensis* chromatogram although these compounds were also described for *L. chuanxiong* (see also[38]).

On the other hand, the HPLC-chromatograms of *L. chuanxiong* and *C. officinale* are so similar that they cannot be distinguished clearly by HPLC-analysis. For detailed information about the quantitative differences between these two closely related species see[39].

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Pericarpium Zanthoxyli
Huajiao

Pharmacopoeias:

Chin. Ph. X

Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[1]

Official drugs:

Zanthoxylum schinifolium Sieb. & Zucc

Zanthoxylum bungeanum Maxim.

– Rutaceae –

The drug is known as Prickly ash Peel and Szechuan-Pepper (English); Kasho and Shokusho (Japanese).

Not official *Zanthoxylum* spec. : *Z. armatum/Z. piperitum*

Description of the drug[1,3–7]

Z. schinifolium:

Mostly 2–3 small follicles, the upper part apocarpusate, grouped on a fruit stalk; follicles spherical, spliting along the ventral suture, 3–4 mm diameter. Outer surface greyish-green or dark green, scatterd with numerous oil dots and fine reticulated, raised wrinkles; inner surface allmost white, smooth. Endocarp commonly separated from exocarp at the base. Remains of seed ovoid, 3–4 mm long, 2–3 mm diameter, externally black, lustrous. Odour aromatic; taste slightly sweet and pungent.

Z. bungeanum:

Most follicles singly, 4–5 mm diameter, outer surface purplish-red or brownish-red, scattered with numerous warty oil dots, translucent when observed against the light; inner surface yellowish. Odour strongly aromatic; taste lastingly pungent and numbing.

Pretreatment of the raw drug[1]

The drug is collected in autumn when ripe, dried in the sun, removed of seeds and foreign matter.

Sometimes stir-fried (*Pericarpium Zanthoxyli stir fried*)

Medicinal use[1,3,5–10]

In Traditional Medicine in China as a decoct mainly for the treatment of stomach-ache accompanied by feelings of coldness and wetness, vomiting, intestinal disorders, diarrhoea, infections of ascarids as well as rheumatic inflammations of joints. The drug is applied externally to bruises, excema and snake-bites.

In Indian and Nepalese folk medicine the decoct is used as an aromatic and tonic in cases of fever, as carminative and stomachic against dyspepsia, cholera and tooth-ache.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1–5]

Taste:	pungent
Temperature:	hot and slightly toxic
Channels entered:	*spleen, stomach, kidney*
Effects:	warms spleen and stomach, stops pain and kills parasites
Symptoms and indications:	stomach-ache, intestinal disorders, diarrhoea, infections with ascarids, bruises, excema, snake-bite, fever, dyspepsia, cholera, rheumatic inflammations, tooth-ache

Main constituents (see Fig. 1)[11–14]

- **Essential oil**, in particular limonene (27 %), β-myrcene (16.6 %), β-oximene X (9.7 %), β-phellandrene (6.0 %), α-pinene (4.9 %), 3-thujanol (5.4 %), piperitone (3.6 %), linalool (3.25 %)[15,24]
- **Alkamides:** hydroxy-α-sanshooil (0.3 %), hydroxy-β-sanshooil (0.15%), hydroxy-γ-sanshooil (0.13 %)[25–29]
- **Flavonoids**, including the aglycons tambulin, tambuletin and nevadensin, quercetin-glycosides (e.g. hyperoside); isoquercitrin, luteolin- and kempferol-glycosides[26,30–32]
- **Fatty acids** (cis-9-hexadecenoic acid, eicosaenoic acid, palmitic acid, hydroxyalk-4Z-enoic acids) in the fatty oil of the seeds[33,34]
- **Lignans**, e.g. kobusin, planinin, fargesin, eudesmin, epieudesmin, sesamin and asarinin[26,35–37]
- **Cinnamic acid derivatives**, the **coumarin** umbelliferone, ubiquitous components like β-sitosterol and its β-D-glucoside.

Pharmacology:

- Antimicrobial effects of the essential oil: effective against *E. coli*, *S. typhi*, *S.aureus* and *V. cholerae*. The anti-bacterial effectiveness against *K. pneumoniae* and *P. aeruginosa* corresponds to that of penicillin G (filter-paper diffusion method).[38]
- Antihelminthic effects of the essential oil: in the concentration range 0.1 to 0.4% *in vitro* effective against rainworms and ascarids which is comparable with those of piperazin phosphate, while effectiveness against taeniae is half of that of piperazin phosphate.[38]
- Antifungal effects of the essential oil: a dilution of 1:10 had relatively good antifungal effects in the agar-diffusion test against *C. tropicalis, C. albicans, T. mentagrophytes* and *M. canis*; not effective against *A. niger* and *A. terreus*. Only mild fungistatic active against *Trychophyton rubrum* and hardly effective against *Microsporum gypseum* and *T. equinum*.[39,40]
- Anti-inflammatory effects: the hexane extract inhibits 5-lipoxygenase from porcine leukocytes. This activity is probably due to the furofuranolignans kobusin, asarin and sesamin. The extract also inhibited prostaglandin synthetase-1 *in vitro*. This effect is due to β-sitosterol and fatty acids.[26,41]
- Immunological effects: the water extract stimulated phagocytosis *in vitro*.[26]
- Insect repellent effects: the extracts of Zanthoxylum species are highly repellent to insects. The repellent activity is mainly caused by piperitone, 4-terpineol and linalool.[42–46]
- Liver protective and antioxidative effects.[47,48]
- Anti HBV DNA replication activity.[12]

– Inhibition of the mutagenicity of Trp-P-1.[49]
– Inhibition of platelet aggregation.[13,50]
– Positive chronotropic effect: extracts of Huajiao increase the beating rate of embryonic mouse myocardial cell sheets. Hydroxy-β-sanshooil, xanthoxylin, hyperosid and quercitrin have been identified as active compounds.[51]

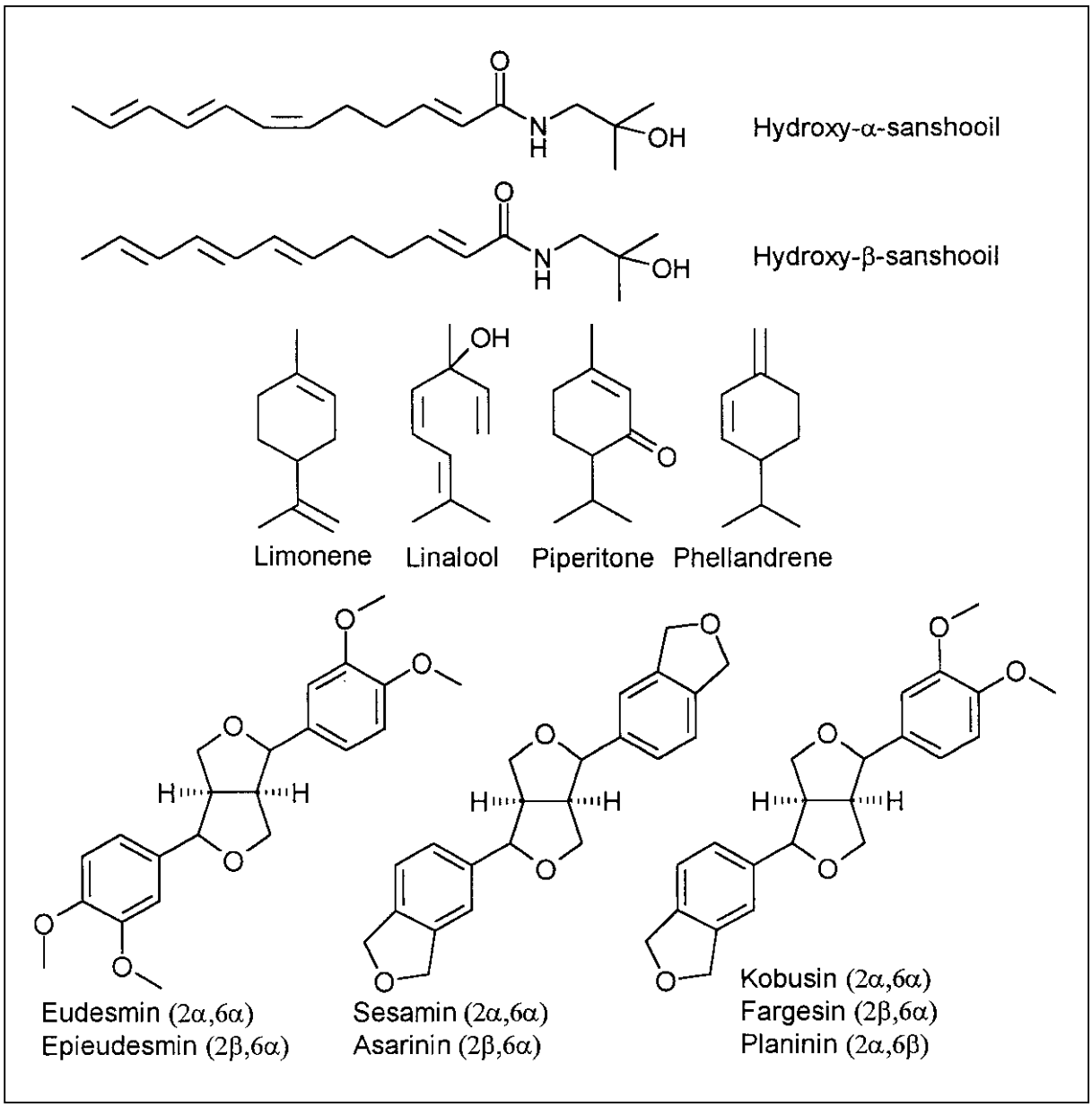

Fig. 1: Formulae of the main constituents

TLC-fingerprint analysis[22,46]

1) Extraction: 2 g of powdered drug are soxhlet-extracted for 2 hrs with 100 ml *n*-hexane. The extract is evaporated to dryness and redissolved in 6 ml methanol and filtered. In order to obtain contrast-rich TLC and to avoid the tailing of fatty acids and terpenoids these compounds can be removed by filtrating the methanolic solution via RP18 Sep-Pak®cartridges (Millipore).

2) Reference compounds: R1: cinnamic acid methylester (R*f* 0.74), tambulin (0.64) and cinnamic acid (0.59), 1 mg each dissolved in 1 ml methanol.

R2: asarinin (R*f* 0.69), sesamin (0.63), fargesin and planinin (0.52), kobusin (0.47), epieudesmin (0.37) and eudesmin (0.32), 1 mg each dissolved in 1 ml methanol.

3) Separation parameters:

Applied amount: 40 µl extract, 5 µl standard solution

Plates: Silica gel 60 F_{254} (Merck)

Solvent system: toluene-ethyl acetate-formic acid (80+15+10), chamber saturation, 15 cm

4) Detection:

Direct evaluation: UV 254 nm and UV 365 nm

Spray reagent: Vanillin/sulphuric acid reagent (solution 1: 1 % ethanolic solution of vanillin; solution 2: 5 % ethanolic sulfuric acid).

The TLC plate is intensively sprayed with 10 ml of solution 1 and then with 5–10 ml of solution 2; then heated for 5–10 min at 110 °C under observation. The evaluation is carried out in VIS.

Drug samples	Origin	Species
1	Huajiao 31.12.97 Shenyang	*Z. schinifolium*
2	Huajiao 01.01.97 Shenyang	*Z. schinifolium*
3	Huajiao 24.12.96 Shenyang	*Z. bungeanum*
4	Huajiao 31.12.96 Shenyang	*Z. bungeanum*
5	Huajiao Shanghai	*Z. bungeanum*
6	*Zanthoxylum armatum* 13.05.91, Nepal	*Z. armatum*
7	University of TCM, Beijing	unknown
8	*Zanthoxylum piperitum,* Japan	*Z. piperitum*

5) Description of the chromatograms:

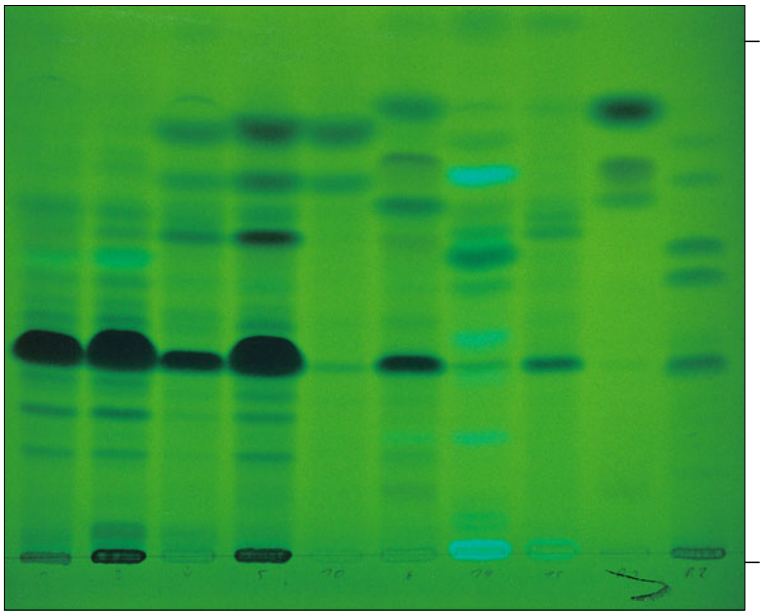

Fig. 2a:
TLC separation of *n*-hexane extracts of the fruits of *Zanthoxylum* species ($UV_{254\ nm}$)

Fig. 2a: $UV_{254\ nm}$:
The samples of *Z. schinifolium* and *Z. bungeanum* all show major quenching zones R*f* 0.57, R*f* 0.39, R*f* 0.33, R*f* 0.25 and R*f* 0.18 and a green fluorescent zone at R*f* 0.53.

The samples 1 and 2 originating from *Z. schinifolium* exhibit a blue fluorescent zone at R*f* 0.50.

Samples 3 and 4 originating from *Z. bungeanum* show additional quenching zones at R*f* 0.71, R*f* 0.63, and R*f* 0.53, but no blue zone.

Sample 6 (*Z. armatum*) exhibits quenching zones at R*f* 0.75 (cinnamic acid methylester), R*f* 0.58 (cinnamic acid) and R*f* 0.33; green zones at R*f* 0.67 (tambulin), R*f* 0.53 and R*f* 0.11 and a blue zone at R*f* 0.21.

Sample 7 originating from an unknown *Zanthoxylum* species shows quenching zones at R*f* 0.49 and R*f* 0.33; no green zone but five blue zones between R*f* 0.62 and 0.20.

Sample 8 (*Z. piperitum*) shows only two major quenching zones at R*f* 0.53 and R*f* 0.33.

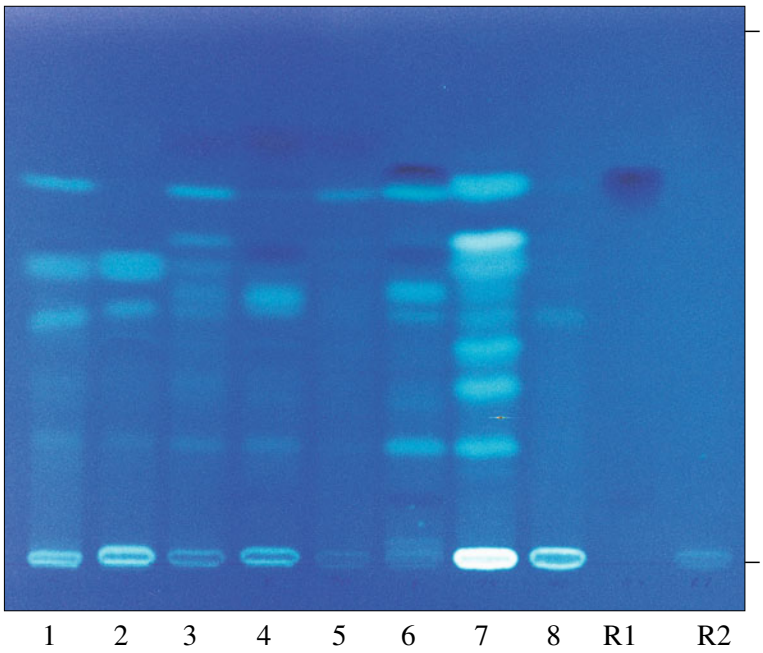

Fig. 2b:
TLC separation of *n*-hexane extracts of the fruits of *Zanthoxylum* species ($UV_{365\ nm}$)

Fig. 2b: UV_{365nm}:
The samples 1 to 6 are characterized by two or three different blue fluorescent compounds between R*f* 0.43 and 0.63.

Z. armatum shows an additional compound at R*f* 0.21, and cinnamic acid methylester at R*f* at 0.74.

Sample 7 is characterized by nine fluorescent compounds between R*f* 0.21 and 0.63. *Z. piperitum* is almost lacking fluorescent spots.

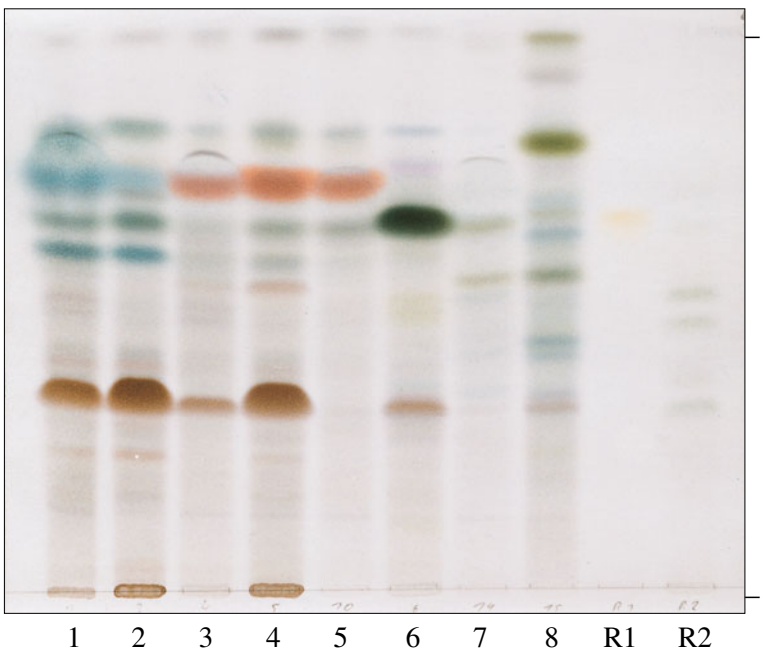

Fig. 2c:
TLC separation of *n*-hexane extracts of the fruits of *Zanthoxylum* species after spraying with vanillin-sulphuric acid reagent (VIS)

Fig. 2c: VS-reagent, VIS:
The different drugs can be distinguished from each other by TLC fingerprints best after spraying with vanillin-sulphuric acid reagent.

The official drugs (samples 1 to 4) show a big brown zone at R*f* 0.4, the samples of *Z. schinifolium* four blue or green zones between R*f* 0.57 and 0.85, the samples of *Z. bungeanum* an orange red zone at R*f* 0.72.

Z. armatum shows an intensive green zone at R*f* 0.66 and a weak brown zone at R*f* 0.4.

Sample 7 (unknown *Zanthoxylum* species) does not show any characteristic zones.

Z. piperitum is characterized by many green or blue zones but not the specific zones of samples 1 to 4.

HPLC fingerprint analysis[22]

Sample preparation:	The same extracts as for TLC are used.
Injection volume:	5 µl methanolic extract
Apparatus:	Liquid chromatograph HP 1050 and photodiode array detector HP 1050
Column:	LiChroCART® 125–4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART® 4–4 with LiChrospher® 100 RP 18, Merck
Solvent system:	A: Water with 1% (V/V) 0.1 N phosphoric acid B: Acetonitrile with 1% (V/V) 0.1 N phosphoric acid
Gradient:	40% B for 12 min. (isocratic); 40–95% B in 23 min. (linear)
Flow rate:	1.0 ml/min.
Detection:	210 nm

Retention times and identity of the main peaks:

Peak	Rt (min.)	Compounds
1	2.77	Cinnamic acid
2	5.15	Not identified
3	6.73	Dodeca-(2E,6Z,8Z,10E)-tetraenoicacid-2′-hydroxybutylamide
4	7.21	Hydroxy-α-sanshooil
5	7.95	Hydroxy-β-sanshooil
6	8.27	Cinnamic acid methylester
7	9.52	Linalool
8	16.61	Lignan
9	17.37	Tambulin
10	19.80	Sanshooil
11	22.56	Not identified
12	24.92	Not identified
13	26.33	Not identified
14	26.38	Not identified
15	28.69	Not identified
16	29.94	Not identified
17	30.88	Not identified

Description of the chromatograms:

The different *Zanthoxylum* species can be distinguished unambiguously from each other by the HPLC fingerprint chromatograms.

The sanshooils (peaks **3**,**4**,**5**) appear as major constituents in the Huajiao-drugs *Z. schinifolium* and *Z. bungeanum*. But they are also present in *Z. armatum* and in lower concentration in *Z. piperitum*.

The two Huajiao drugs can be distinguished by the peaks **10** (*Z. bungeanum*) and **14** (*Z. schinifolium*).

Cinnamic acid, cinnamic acid methylester, linalool and tambulin (peaks **1**,**6**,**7**,**9**) appear not in the offical drugs. They are typical for *Z. armatum.*

Compound **11** seems to be characteristic for *Z. piperitum* and the compounds **2**, **8**, **12** and **13** seem to be typical for the unknown *Zanthoxylum* species.

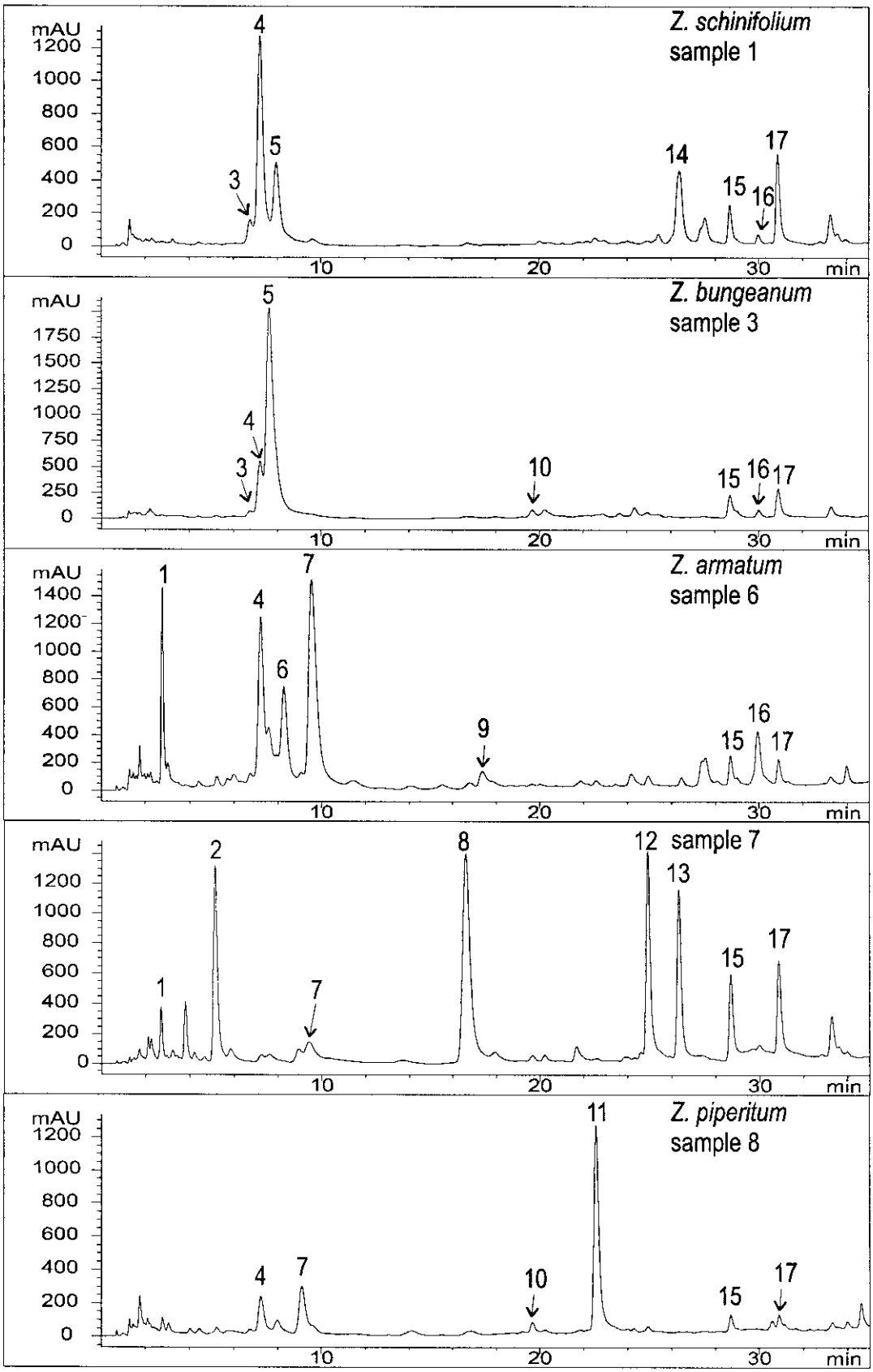

Fig. 3a: HPLC-fingerprint chromatograms of *Zanthoxylum* species (detection at 210 nm)

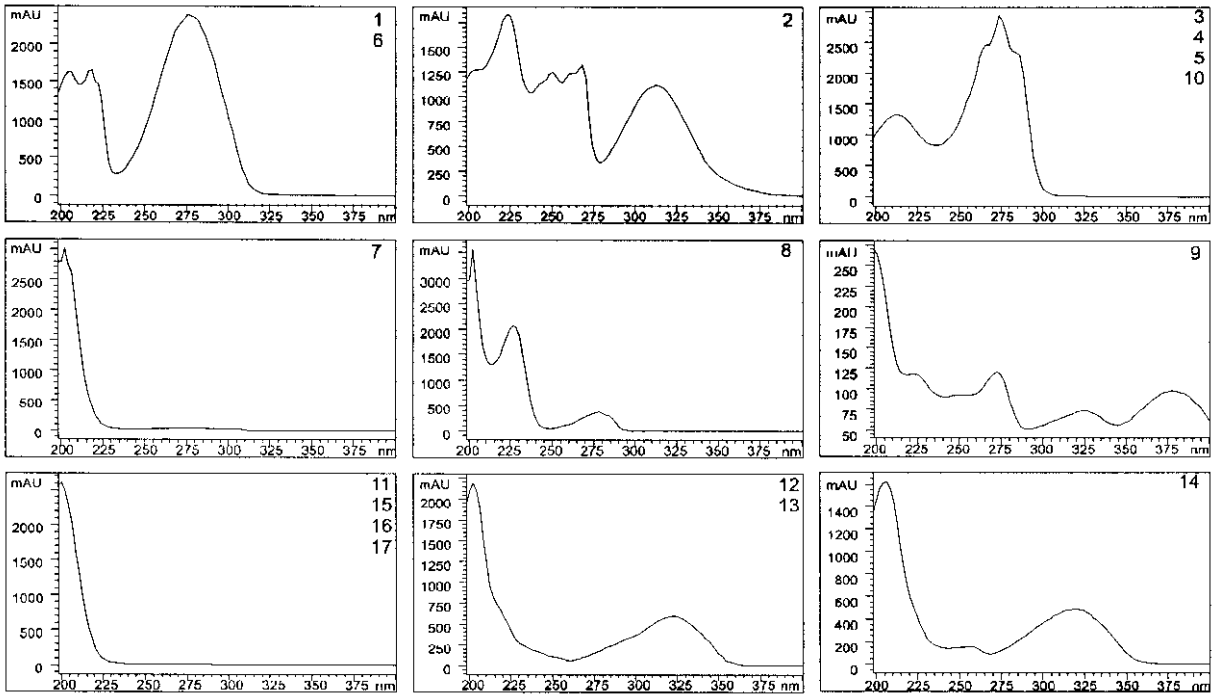

Fig. 3b: Online recorded UV-spectra of the main peaks in the HPLC-separation of *Zanthoxylum* species

Discussion:

The different pericarps can be easily distinguished by TLC or HPLC.

In TLC the official drugs originating from *Z. schinifolium* are characterized by compounds which can be detected by vanillin-sulphuric acid as brown (R*f* 0.4), bluish-green (R*f* 0.57–0.85, *Z. schinifolium*) or orange-red (R*f* 0.72, *Z. bungeanum*) spots. *Z. armatum* and *Z. piperitum* show different patterns of constituents.

In HPLC Huajiao is characterized by the sanshooils and missing of cinnamic acid, cinnamic acid methylester and linalool.

However there are also other qualities of Huajiao on the market (samples 5 and 7) which differ strikingly in their chemical composition. Therefore the different qualities have to be identified and only the official drugs should be used. A morphologic description of different *Zanthoxylum* species is given in[3].

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Cortex Magnoliae officinalis
Houpo

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[(1)]

Official drug: Magnoliae officinalis cortex

Magnolia officinalis REHD. et WILS. or
Magnolia officinalis REHD. et WILS. var. *biloba* REHD et WILS.

– Magnoliaceae –

Origin[2)]: China (provinces of Si Chuan, Hu Bei, Zhe Jiang, Gui Zhou and Hu Nan) and Japan.

The drug is collected in April, May or June. The root bark and the branch bark are dried in the shade. The stem bark is slightly decocted in boiling water and piled up in a wet place until its inner surface turns purplish-brown or brown, steamed to rolled, and dried.

Description of the drug[1)]:

Stem bark: Quilled singly or double quilled. 30–35 cm long, 2–7 mm thick, commonly known as "Tongpo"; one end near the root spread out like a bell, 13–15 cm long, 3–8 mm thick, commonly known as "Xuetongpo". Outer surface greyish-brown, rough, sometimes scaled, easily exfoliated, with distinct elliptical lenticels and longitudinal wrinkels, appearing yellowish-brown when the coarse outer layer peeled; inner surface purplish-brown or dark purplish-brown, relatively smooth, with fine longitudinal striations and exhibiting oily traces on scratching. Texture hard, uneasily broken, fracture granular, greyish-brown in outer layer and purplish-brown or brown in inner layer, oily, sometimes numerous small bright spots visible.
Odour: aromatic; taste: pungent and slightly bitter.

Root bark (Genpo): Quilled singly or pieced irregularly, some curved like chicken intestines, commonly known as "Jichangpo". Texture hard, easily broken, fracture fibrous.

Branch bark (Zhipo): Quilled singly, 10–20 cm long, 0,1–0,2 cm thick. Texture fragile, easily broken, fracture fibrous.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Pretreatment of the raw drug[1]:

Magnoliae officinalis cortex:	Scrape off the coarse outer layer, wash clean, soften thoroughly, cut into slivers, and dry in the sun. The slivers curved, fracture fibrous, outer surface yellowish-brown, inner surface dark purplish-brown.
Magnoliae officinalis cortex (processed with ginger):	Stirfry the slivers of *Magnoliae officinalis* cortex as described under the method for stir-frying (Appendix II D, Ch AB) with ginger-juice to dryness. The slivers are curved, fracture fibrous, externally purplish-brown.
Medicinal use:	stroke[3], headache[3], gastrointestinal disorders (diarrhea, dysenteria)[4,5,6], lack of appetite, cough, fever, bronchitis[7], neuroses[6], anxiety[8]

Effects and indications according to Traditional Chinese Medicine[2]

Taste:	bitter and pungent in flavor
Temperature:	warm
Channels entered:	acts on the spleen, stomach, lung and large intestine channels
Symptomes and indications:	promotes the flow of Qi, dries dampness, removes food stagnation, alleviates cough and asthma

Main constituents:

- **biphenols** (with a propenyl side chain)[9,6]: magnolol (2–11 %), honokiol (0,3–4,6 %)
- **hydroxybiphenyls**[10]: 8,9-dihydroxy-dihydro-honokiol, 8,9- dihydroxy-7-methoxy-dihydro-honokiol, 8,9-dihydroxymagnolol, bornylmagnolol
- **essential oil**[11]: cadinol, α-, β-, γ-eudesmol, guajol
- **alkaloids**[12]: magnocurarine, magnosprengerine, salicifoline

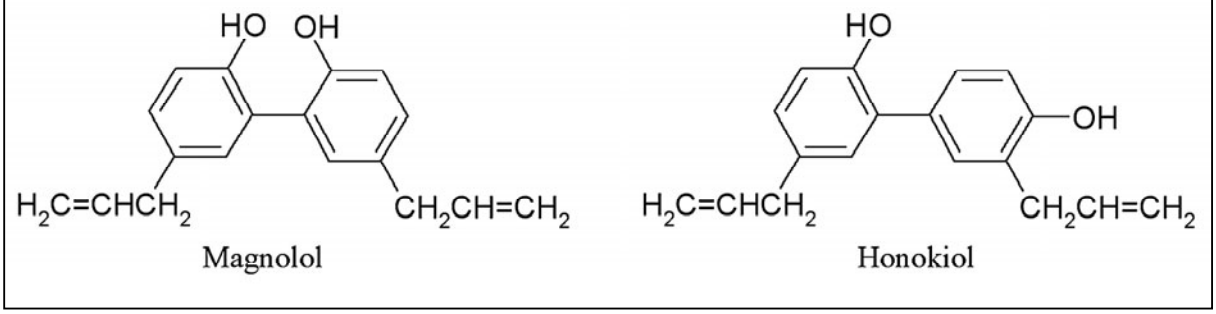


Fig. 1: Formulae of the main constituents

Pharmacology:

- antiallergic[13]
- analgesic-antiphlogistic[8]
- antiemetic[14]
- muscle relaxing[6]
- anticonvulsive[16]
- lowering the blood pressure[16]
- antiviral[17]
- inhibits the lipoxygenase[18]
- antitumoral

Toxicology: No toxic adverse effects in animals and humans were observed. The kidney toxicity reported in women is probably due to a falsification of a Magnoliae cortex and Stephaniae tetr. radix prescription containing Aristolochiae fangchi radix.

TLC-fingerprint-analysis: methanol extract

1) Extraction: 0,5 g powdered drug is shaken with 5 ml methanol for 30 minutes and the extract filtered.

2) Reference compound: magnolol, honokiol are dissolved in methanol (1 mg/ml)

3) Separation parameters:

Applied amount: 20 µl extract, 20 µl standard solution

Plate: Silicagel 60 $F_{254;}$ Merck

Solvent system: benzol-methanol (27:1)

Detection: Spray reagent: vanillin sulphuric acid reagent: the plate is intensively sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid. The plate heated for 10 minutes at 110 °C and evaluated in VIS.

	Drug samples	**Origin**
1	Magnoliae officinalis cortex	sample of commercial drug, Uchida, Japan
2	Magnoliae officinalis cortex	sample of commercial drug, China
3	Magnoliae officinalis cortex	sample of commercial drug, China

	Reference substances	**R*f***
T1	Magnolol	~0,24
T2	Honokiol	~0,16

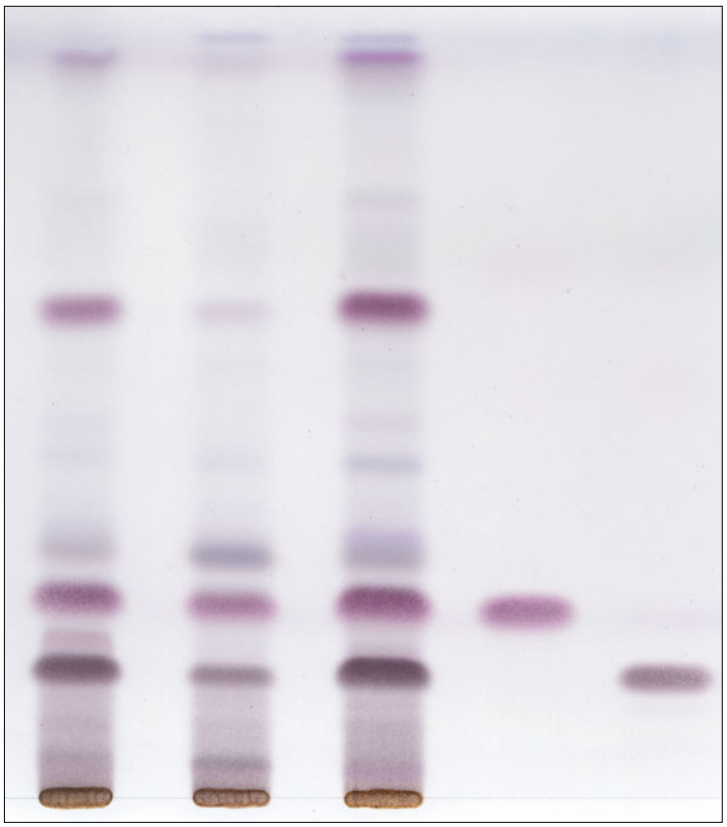


Fig. 2: Thin layer chromatogram of the methanol extract of Magnoliae officinalis cortex

4) Description of the chromatogram:

VIS: The *Magnolia officinalis* bark extract is characterized by a darkviolet spot of Honokiol at R*f* ~ 0,16 and a pinkviolett spot of Magnolol at R*f* ~ 0,24. Further main violet zones are visible at R*f* ~ 0,31, 0,63 and on the solvent front.

TLC-fingerprint-analysis: essential oil

1) Extraction:	The powdered drug is subjected to a water steam distillation in a Neo Clevenger apparat.
2) Separation parameters:	
Applied amount:	10 µl essential oil
Plate:	Silicagel 60 F_{254}; Merck
Solvent system:	toluol-ethyl acetate (93: 7)
Detection: Spray reagent:	vanillin sulphuric acid reagent: the plate is intensively sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid. The plate heated for 10 minutes at 110 °C and evaluated in VIS.

	Drug sample	Origin
2	Magnoliae officinalis cortex	sample of commercial drug, China

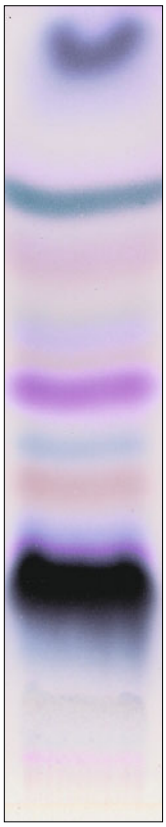


Fig. 3: Thin layer chromatogram of the essential oil of Magnoliae officinalis cortex

3) Description of the chromatogram:

VIS: The chromatogram is characterized by a strong blue zone at R*f*~ 0,26 (probably cadinol), a violet zone at R*f*~ 0,49, a greyblue zone at R*f*~ 0,70 and a violet zone at R*f*~ 0,88 (Terpene Hydrocarbon). The pattern of terpenoids differs distinctly from that of the essential oil of Magnoliae flos.

HPLC-fingerprint-analysis:

1) Sample preparation: filtration of the extract used for TLC over Millipore® (Type HV 0,45 µm)

2) Injection volume: 15 µl methanol extract
5 µl standard solution

3) HPLC data:

Apparatus: L- 6200A Intelligent Pump, AS- 2000 Autosampler, L- 4500A Diode Array Detector, D- 6000A Interface; Merck, Hitachi

Column: LiChroCART® 125 x 4 mm with LiChrospher® 100 RP 18 (5 µm); Merck

Solvent System: A: water, for HPLC; Acros Organics
B: acetonitrile, for HPLC; Acros Organics

Gradient: 10–90 % B in 30 min.
90–95 % B in 1 min.
95 % B for 9 min.
95–10 % B in 7 min.
10 % B for 8 min.

Flowrate:	1,0 ml/min.
Detection:	210 nm

4) Description of the HPLC-chromatogram:

Retention times and identity of the main peaks:

Peak	Rt (min.)	Compounds
1	22.27	Honokiol
2	23.72	Magnolol

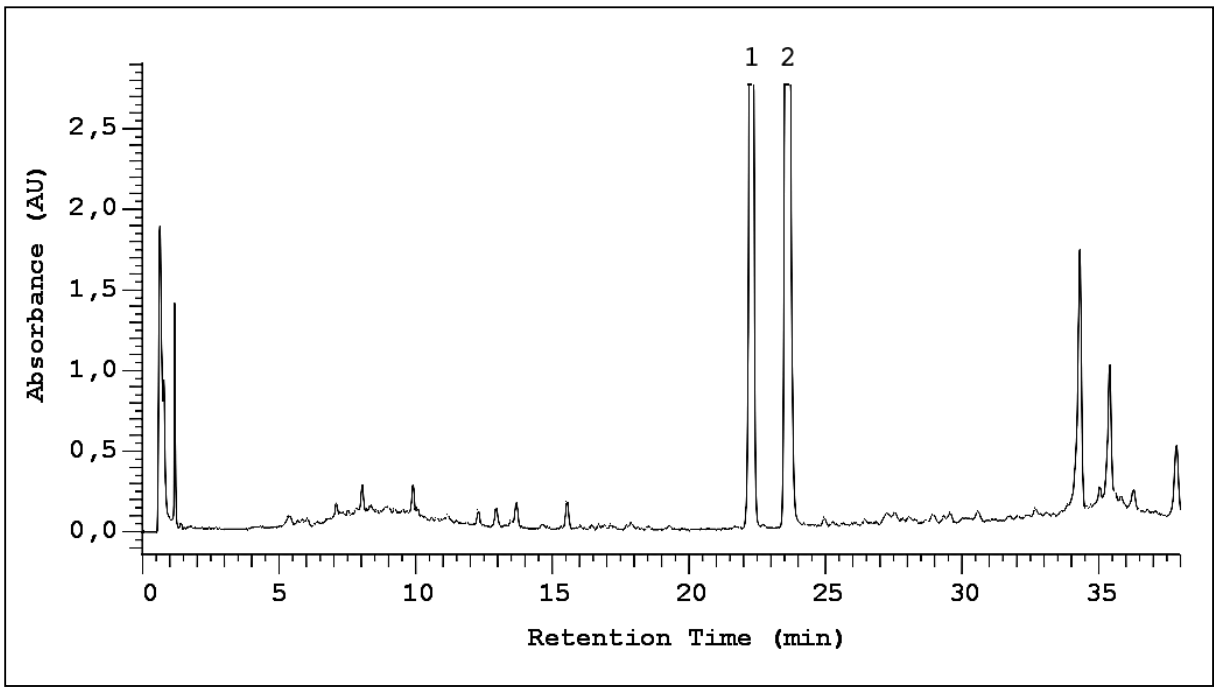
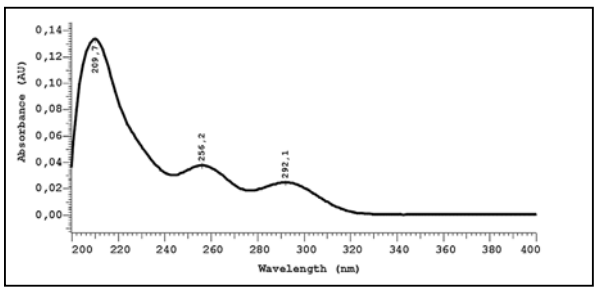
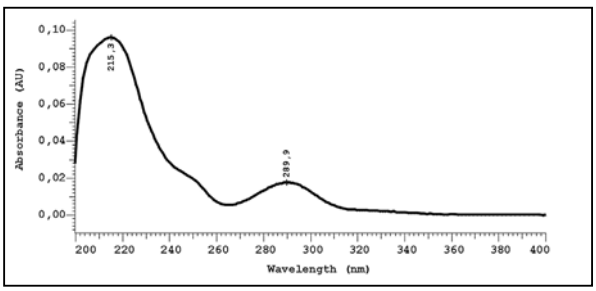


Fig. 4: HPLC-fingerprint of Magnoliae officinalis cortex



Honokiol



Magnolol

Fig 5: on line UV- spectra of the main constituents

Description:

The HPLC-fingerprint of Magnoliae officinalis cortex is characterized by the dominating biphenols honokiol (Rt: 22,27) and magnolol (Rt: 23,72). Further peaks with higher Rt-values at Rt 35 are probably due to terpenylbiphenols.

Note: According to the Chinese Pharmacopoeia 2005 the Cortex drug should contain not less than 2.0 % of the total amount of magnolol and honokiol, calculated with reference to the dried drug.

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Wu YT, Lin LC, Tsai TH, Simultaneous determination of honokiol and magnolol in Magnolia officinalis by liquid chromatography with tandem mass spectrometric detection, Biomed Chromatogr. 20(10):1076-81 (2006)

Rhizoma Drynariae
Gusuibu

Pharmacopoeia:[1]	Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[1]
Official drug:[1]	Drynariae rhizoma *Drynaria fortunei* (KUNZE) J. SM. *Drynaria baronii* (CHRIST) DIELS – Polypodiaceae –
Origin:[2]	China (provinces of Hubei, Zhejang, Guangdong, Guangxi, Sichuan), Taiwan, Japan, Korea
Description of the drug:[1]	Flattened long slat-shaped, mostly curved, branched, 5–15 cm long, 1– 1,5 cm wide, 2– 5 mm thick. The surface closely covered with deep brown to dark brown hair-like ramenta, and brown or dark brown when burnt, upper surface and both sides marked by raised or depressed circular frond, scars, rarely by frond-bases and remains of fibrous roots. Texture light, fragile, easily broken, fracture reddish-brown, vascular bundles yellow dotted and arranged in a ring. Odourless; taste: weak and slightly adstringent.
Pretreatment of the raw drug:[1]	Foreign matters and hairy parts are eliminated, washed, cut into thick slices and dried.
Medicinal use:[1, 3]	In Traditional Chinese Medicine the decoction, pills or powder are used for injuries and bone fractures and osteoarthritis, for pain in loins, weakness in the feet, tinnitus and deafness, toothache, chronic diarrhoea due to kidney deficiency and externally macerated into wine for Alopecia areata and Vitiligo. The drug tonifies the kidney, promotes blood circulation, stops bleeding and treats injuries. It is contraindicated in patients with heat due to yin deficiency and for patients without blood stasis.

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Effects and indications according to Traditional Chinese Medicine[1, 2, 3]

Taste:	bitter in flavor
Temperature:	warm
Channels entered:	kidney, liver
Effects:	strenghtens the kidney, heals ligaments and bone fractures, stimulates hair growth
Symptomes and indications:	traumatic injuries, fractures, contusions, distorsions, weakness in the knee, diarrhoea, tinnitus, loss of tooth, bleeding from the gums

Main constituents: (see Fig. 1):

- **flavanones:** naringin[4], neoeriocitrin[5] and their aglycons
- **anthraguinone:** physcion
- **phenols:** protocatechuic acid[5], p-hydroxybenzoic acid[5], 3,4-dihydroxybenzaldehyde[5], catechin[5], gallic acid[5], paeonol[5]
- **tetracyclic triterpenes:** cycloeucalenol, cyclotrichosantol, 4,6,8 (14), 22-ergostatetraene-3-on[5], (24R)-cyclolaudenyl-acetate, (24R)-cyclomargenyl-acetate, diplopten, diplopterol, fern-9(11)-en, hop-21-en cyclolaudenol, cyclolaudenone, cyclomargenol, cyclotrichosantol[5], cycloartenol, cycloartenone, 25-en-cycloartanol, 5-stigmasten-3-on, β-sitosterin[6]
- **fatty acids:** n-dotriacontanoic acid[7], palmitic acid[5]

Pharmacology:

in vitro effects:

- antibacterial
- antiviral
- stimulation of osteoblast proliferation[5, 10, 11]

in vivo effects:

- antisclerotic[12]
- antihypercholesteremic[12]
- antihyperlipemic[12]

TLC-fingerprint-analysis:[13]

1) Extraction:

hexane extract:	10,0 g coarsely ground drug are soxhlet-extracted with 150 ml n-hexane p.a. for 4–5 hours. The extract is evaporated to 5–6 ml and hexane added to 10 ml.
methanol/ethyl acetate extract:	1,0 g powdered drug is extracted with 10 ml methanol for 5 minutes on the water bath at about 60 °C and then filtered and evaporated. The residue is dissolved in water, 10 ml ethyl acetate are added and shaken several times. The ethyl acetate phase is separated, reduced to a volume of 1 ml and used for TLC.
2) Reference compounds:	naringin, neoeriocitrin, protocatechuic acid, β-sitosterin, are dissolved in methanol or dichlormethane respectively (1 mg/ml)

3) Separation Parameters:

Applied amount:	50 µl extract, 25 µl standard solution
Plates:	Silicagel 60 F_{254} (Merck)
Solvent system:	**hexane extract:** n-hexane-ethyl acetate (80 : 20)
	methanol/ethyl acetate extract: ethyl acetate-glacial acetic acid-formic acid-water (100 : 11: 11: 26)

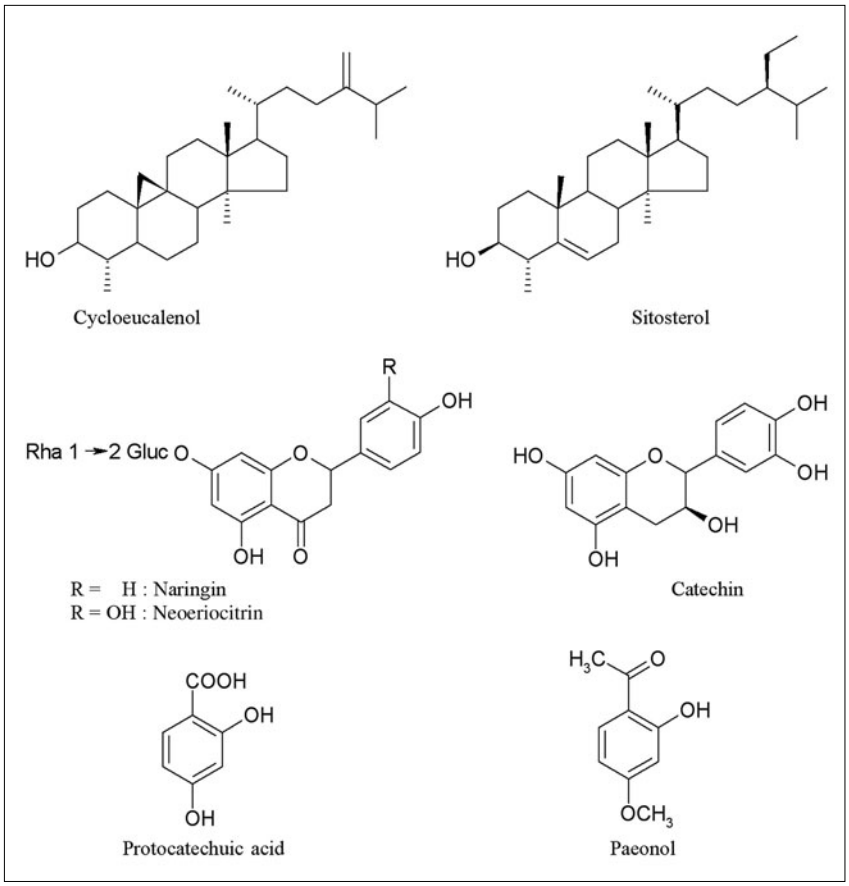

Fig. 1: Formulae of the main constituents

Detection:	Direct evaluation:	visible light, UV_{254nm} and UV_{365nm}
	Spray reagents:	**hexane extract:** vanillin-sulphuric acid-reagent: the plate is intensively sprayed successively with 1 % ethanolic vanillin solution and 10 % ethanolic sulphuric acid followed by ca. 10 minutes heating at 110 °C. The evaluation is carried out in VIS.
		methanol/ethyl acetate extract: natural product-polyethylenglycol reagent (NP/PEG): the plate is sprayed successively with a 1 % methanolic solution of diphenylboric acid-β-ethyl-aminoester (NP) and a 5 % ethanolic polyethylenglycol-4000 solution (PEG). The evaluation is carried out in VIS and UV_{365nm}.

Drug samples	**Origin**	**Species**
1	Kwangsi, China	*Drynaria fortunei*
2	Quinghai, China	*Drynaria baronii*
3	Yunnan, China	*Drynaria fortunei*
4	China	*Drynaria fortunei*
Reference compounds		**R*f*-value**
T1	β-Sitosterin	R*f* = 0,27
T2	Naringin/Neoeriocitrin	R*f* = 0,45/0,40
T3	Protocatechuic acid	R*f* = 0,95

4) Description of the TLC-chromatograms:

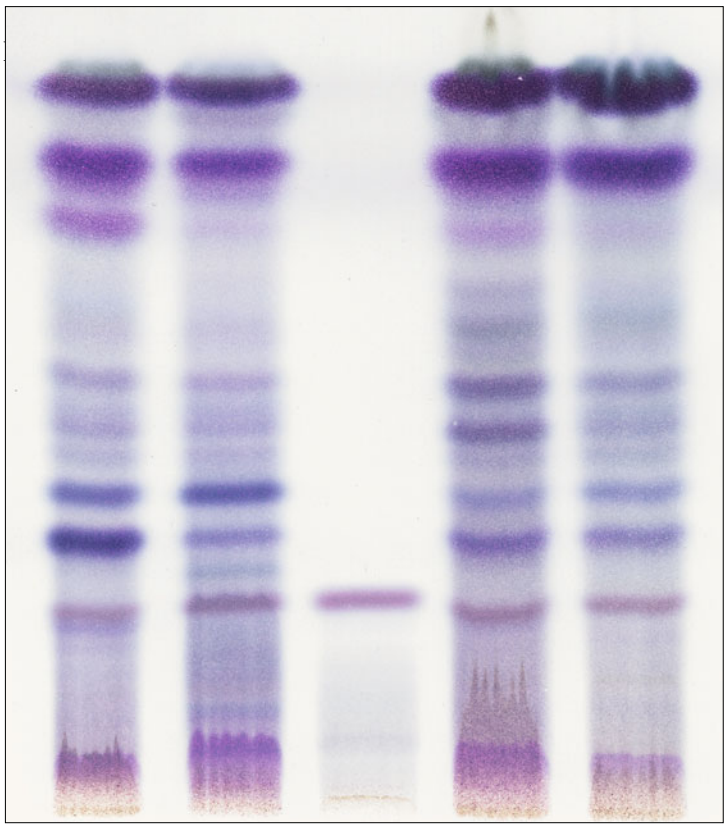

Fig. 2: Thin layer chromatogram of the hexan extract of *Drynariae rhizoma* (VIS)

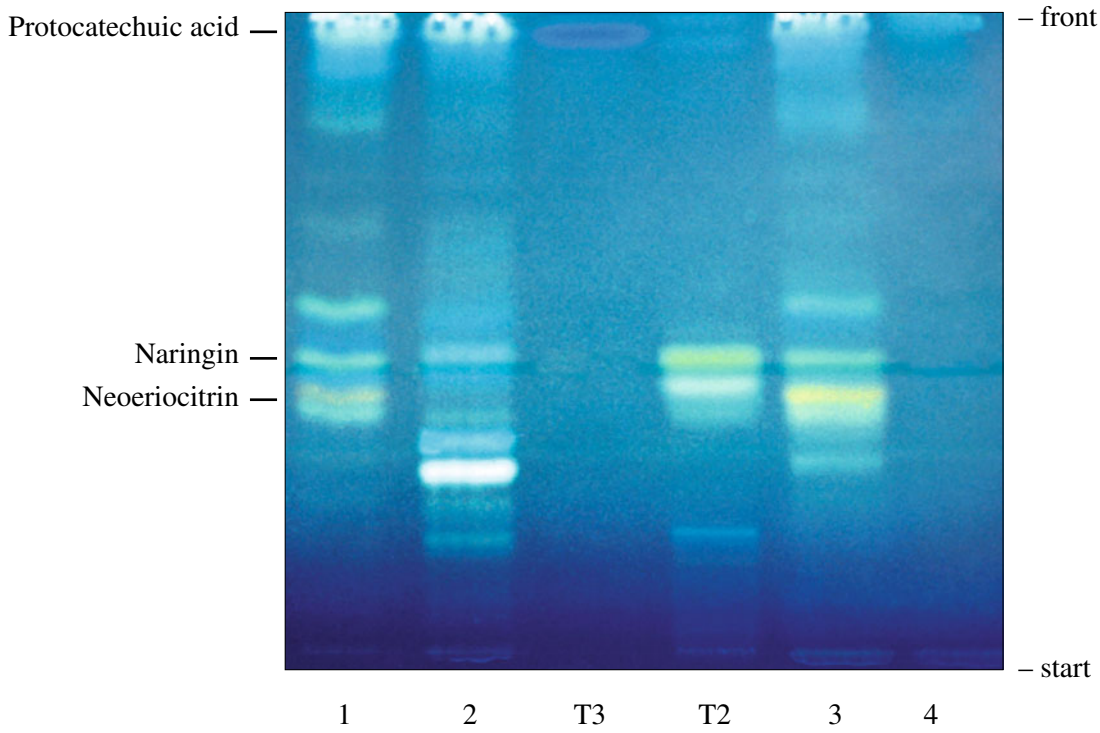

Fig. 3: Thin layer chromatogram of the methanol/ethyl acetate extract of *Drynariae rhizoma* (UV_{365nm})

VIS: All samples of *Drynaria fortunei* and *baronii* show in the R*f*- range of 0,25 to 0,95 a nearly equal pattern of 8–10 grey to violettblue spots. The spot at R*f*~ 0,27 can be assigned to β-sitosterin, whereas the spot above with R*f*~ 0,34 is identical probably with cycloeucalenol.

Fig 3: methanol/ethyl acetate extracts:

UV_{365nm}/VIS: The *Drynaria fortunei* extracts (**1**,**3**,**4**) are characterized in UV_{365nm} by a yellowgreen fluorescent spot of naringin at R*f*~ 0,45, a further spot at R*f*~ 0,52 and a greenorange fluorescent spot of neoeriocitrin at R*f*~ 0,4. Both spots appear in VIS with yellow and pink colour respectively. Additionally in all samples of *Drynaria fortunei* a deep blue fluorescent spot at R*f*~ 0,95 (protocatechuic acid) is detectable in UV_{365nm}.

In *Drynaria baronii* (**2**) only traces of naringin and neoeriocitrin can be detected in UV_{365nm}. In the R*f*-range of 0,27 to 0,32 two light blue zones are visible.

HPLC-fingerprint-analysis:

1)	Sample preparation:	Filtration of the methanol and ethyl acetate fraction used for TLC over Millipore® (Type HV 0,45 µm)
2)	Injection volume:	10 µl extract
3)	HPLC data:	
	Apparatus:	Liquid chromatograph HP 1090 and photodiode array detector HP 1040 and HP Chemstation; Hewlett-Packard
	Column:	LiChroCART® 125 × 4 mm with LiChrospher® 100 RP 18 (5 µm), Merck
	Solvent system:	A: dist. water + 0,01 % 10N H_3PO_4 B: acetonitrile Chrom AR; Pirochem + 0,01 % 10N H_3PO_4
	Gradient:	5- 35 % B linear in 35 min.
	Flow rate:	1,0 ml/min.
	Detection:	210 nm

4) Description of the HPLC of Fig 4a , 4b and 5:

Retention times and identity of the main peaks of Fig. 4a and 4b:

Peak	Rt (min.)	Compound
1	5,82	protocatechuic acid
2	7,18	catechin
3	7,55	caffeoyl derivative
4	12,72	catechin derivative
5	18,29	neoeriocitrin
6	21,19	naringin

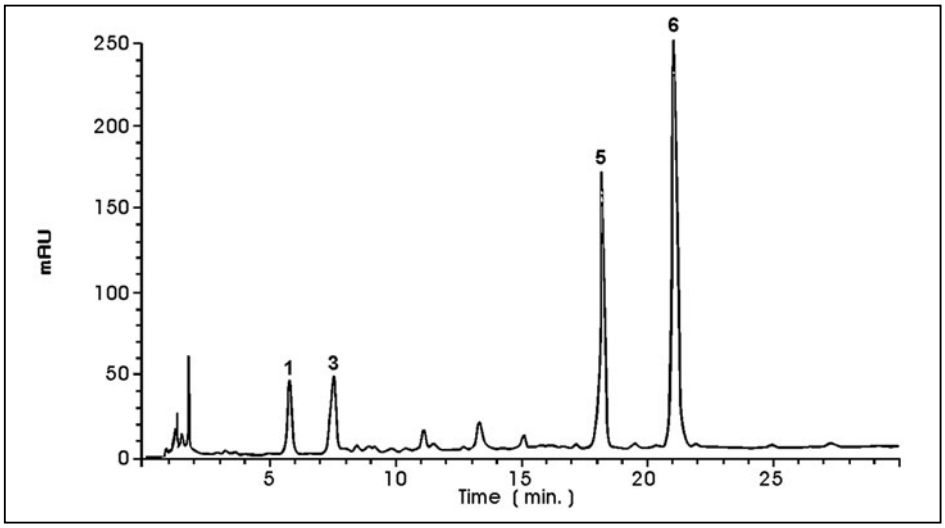

Fig. 4a: HPLC-fingerprint of *Drynaria fortunei* methanol extract

The HPLC-fingerprint of *Drynaria fortunei* (Fig. 4a) is characterized by the dominating flavanone glycosides neoeriocitrin (**5**) and naringin (**6**), protocatechuic acid (**1**) and a caffeoyl derivative (**3**) of minor concentration.

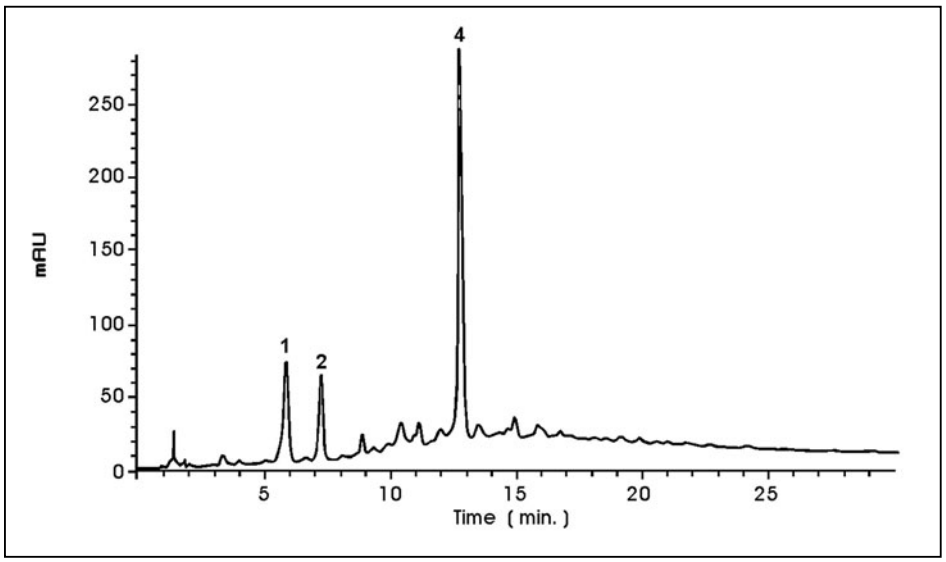

Fig. 4b: HPLC-fingerprint of *Drynaria baronii* methanol fraction

In the HPLC-fingerprint of *Drynaria baronii* (Fig. 4b) naringin and neoeriocitrin are lacking.
The chromtogram is characterized by the presence of a catechinderivat (**4**) (probably meta-digallic acid), protocatechuic acid (**1**) and catechin (**2**).

Therefore a discrimination of both Drynaria spezies can be easily achieved by HPLC of the methanol extracts.

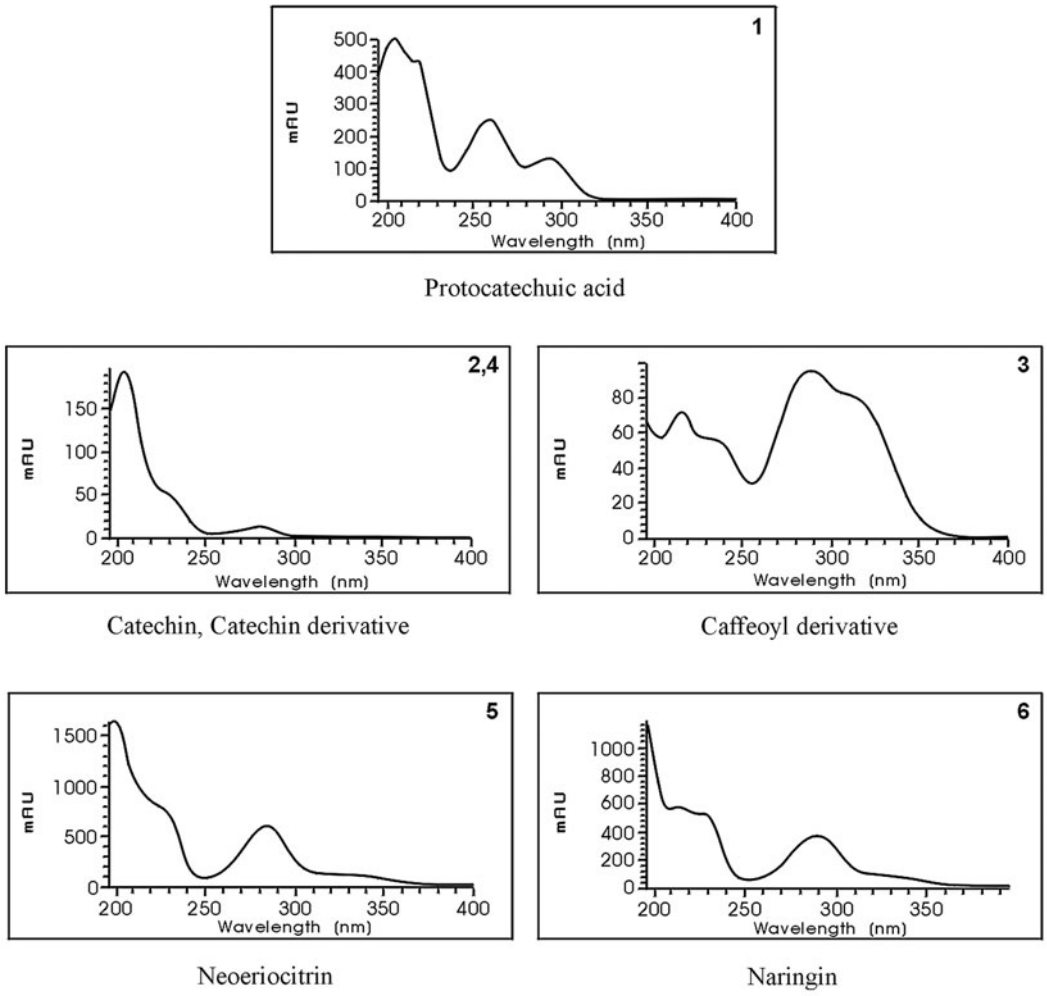

Fig. 5: On line UV-spectra of the main constituents detected in HPLC Fig. 4a and 4b

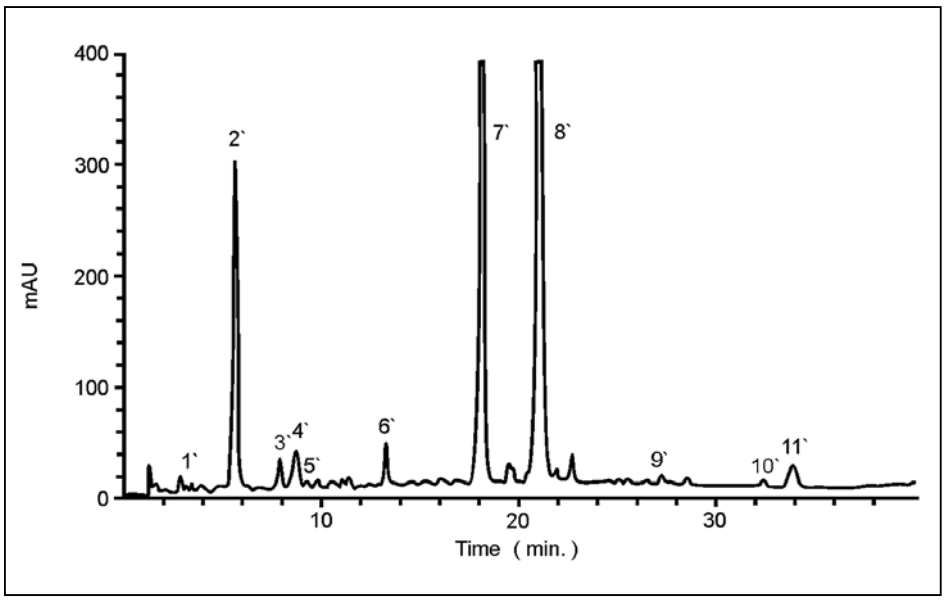

Fig. 6: HPLC-fingerprint of *Drynaria fortunei* ethyl acetate fraction

5) Description of the HPLC of Fig. 6:

Retention times and identity of the main peaks of Fig. 6:

Peak	Rt (min.)	Compound
1′	3,10	gallic acid
2′	5,69	protocatechuic acid
3′	7,83	3,4-dihydroxybenzaldehyde
4′	8,75	p-hydroxybenzoic acid
5′	9,77	catechin
6′	13,31	catechin derivative
7′	18,14	neoeriocitrin
8′	21,05	naringin
9′	27,10	eriodictyol
10′	32,19	naringenin
11′	33,81	paeonol

In the HPLC of the ethyl acetate fraction (Fig. 6), obtained from the methanol extract of *Drynaria fortunei,* besides neoeriocitrin (**7′**), naringin (**8′**) and protocatechuic acid (**2′**) further compounds, present in minor quantities, can be detected in the Rt-range of 2.0 – 16.0 and 22.0 – 35.0.

Note: According to the Chinese Pharmacopoeia 2005 the drug should contain not less than 0.50 % naringin, calculated with reference to the dried drug.

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Radix Puerariae
Ge Gen

Pharmacopoeia:
Pharmacopoeia of the People's Republic of China, English Edition, 1997/2005[1]
Japanese pharmacopoeia, English Edition, 1986 [2]

Official drug:
Pueraria lobata (Willd.) Ohwi[1,2]
Pueraria thomsonii Benth[1]. (Fam. Fabaceae)
The drug is collected in autumn and winter.
P. lobata is often cut into thick slices or pieces when fresh and dried.
P. thomsonii, known as „Starchy Radix Puerariae", is removed from the outer bark, fumigated with sulfur, dried for a while, then cut into sections or cut again longitudinally into two parts and dried.[1]

Description of the drugs:

Radix Puerariae lobatae:
Longitudinally cut rectangular, thick slices or small square pieces. 5 – 35 cm long, 0.5 – 1 cm thick. The outer bark pale brown, with longitudinal wrinkles, rough, cut surface yellowish-white, striations indistinct. Texture pliable and strongly fibrous.
Odourless, taste: slightly sweet.[1]

Radix Puerariae thomsonii:
Cylindrical, almost fusiform or semi-cylindrical, 12 – 15 cm long, 4 – 8 cm in diameter; some longitudinally or obliquely cut thick slices, varying in size. Externally yellowish-white or pale brown, or greyish-brown when unpeeled. Transverse section showing pale brown concentric ring formed by fibres. Heavy, texture hard and starchy.[1]

Substitute drugs:
Pueraria edulis Pamp.[3]
Pueraria omeineisis Wang et Tang[3]
Pueraria phaseoloides (Roxb.) Benth.[3]
Pueraria peduncularis Grah. ex Benth. - poisonous!

Pretreatment of the raw drug:
Eliminate foreign matter, wash clean, soften thoroughly, cut into thick pieces and dry in the sun.[1]

Medicinal use:
In Chinese Traditional Medicine internally as spasmolytic, antipyretic, secretory, antidiarrheal[4] and for the treatment of alcohol addiction[8], angina pectoris and hypertension[5].
It is thought to induce the eruption of measles at the early stage[4].

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Effects and indications according to Traditional Chinese Medicine[1,3,4,6,7]

Taste:	sweet, pungent
Temperature:	cool, neutral
Channels entered:	spleen, stomach
Effects:	relieves fever, promotes the production of body fluid, faciliates eruption, invigorates the spleen Yang to arrest diarrhea
Symptoms and indications:	fever, headache, stiff and painful nape in hypertension, measles, diarrhea

Main constituents (see ref. 8) (see Fig. 1)**:**

Isoflavonoids and derivatives[5,8,9,10,11]

- isoflavone-C-glycoside (puerarin and its 7-xyloside) and other isoflavone-derivatives (e.g. genistin, daidzein, daidzin, daidzein-4',7-diglucoside, 8-*C*-glucosyl-7,3', 4'-trihydroxyisoflavone = PG-1, 2'-hydroxy-4'-*O*-β-D-glucosylpuerarin, 3'-methoxydaidzin, malonylesters of daidzin, genistin and puerarin and various methoxyisoflavonoids)
- aromatic glycosides (pueroside A, pueroside B)

Sapogenins and saponins[5,8,12,13,14]

- sapogenins (kudzusapogenol A, kudzusapogenol B, kudzusapogenol C, sophoradiol, cantoniensistriol, soyasapogenol A, soyasapogenol B, various kudzu-saponins, soyasaponins and other oleanene-typ triterpene glycosides)

Miscellaneous compounds:

- β-sitosterin[3] and its glucoside, 6,7-dimethoxycoumarin, 5-methylhydantoin[5], arachinic acid.

Pharmacology (see ref. 8)**:**

<u>*In vitro*-effects:</u>

- inhibition of platelet-aggregation: puerarin[4].
- smooth muscle effects[15]
- inhibition of thrombocyte aggregation[16]
- estrogenic activity: isoflavone aglycone-fraction[17]
- antioxidant activity[8]
- hepatoprotective activity[8]

In vivo effects:

- effect on coronary blood vessel[18,19]
- effect on myocardial ischemia and arrhythmia[20,21]
- effect on myocardial metabolism[22]
- effect on blood pressure[23,24]
- effect on blood vessel microcirculation[25,26]
- effect on blood vessel of retina[27]
- antidipsotropic activity (in Syrian hamster): daidzin and daidzein[11]

Toxicology:

Oral administration of 10 and 20g/kg of dried ethanol extract daily for 3 days to mice did not result in any toxic effects. The LD_{50} of dried ethanol extract injected *i.v.* into mice was determined as 2.1± 0.12 g/kg.

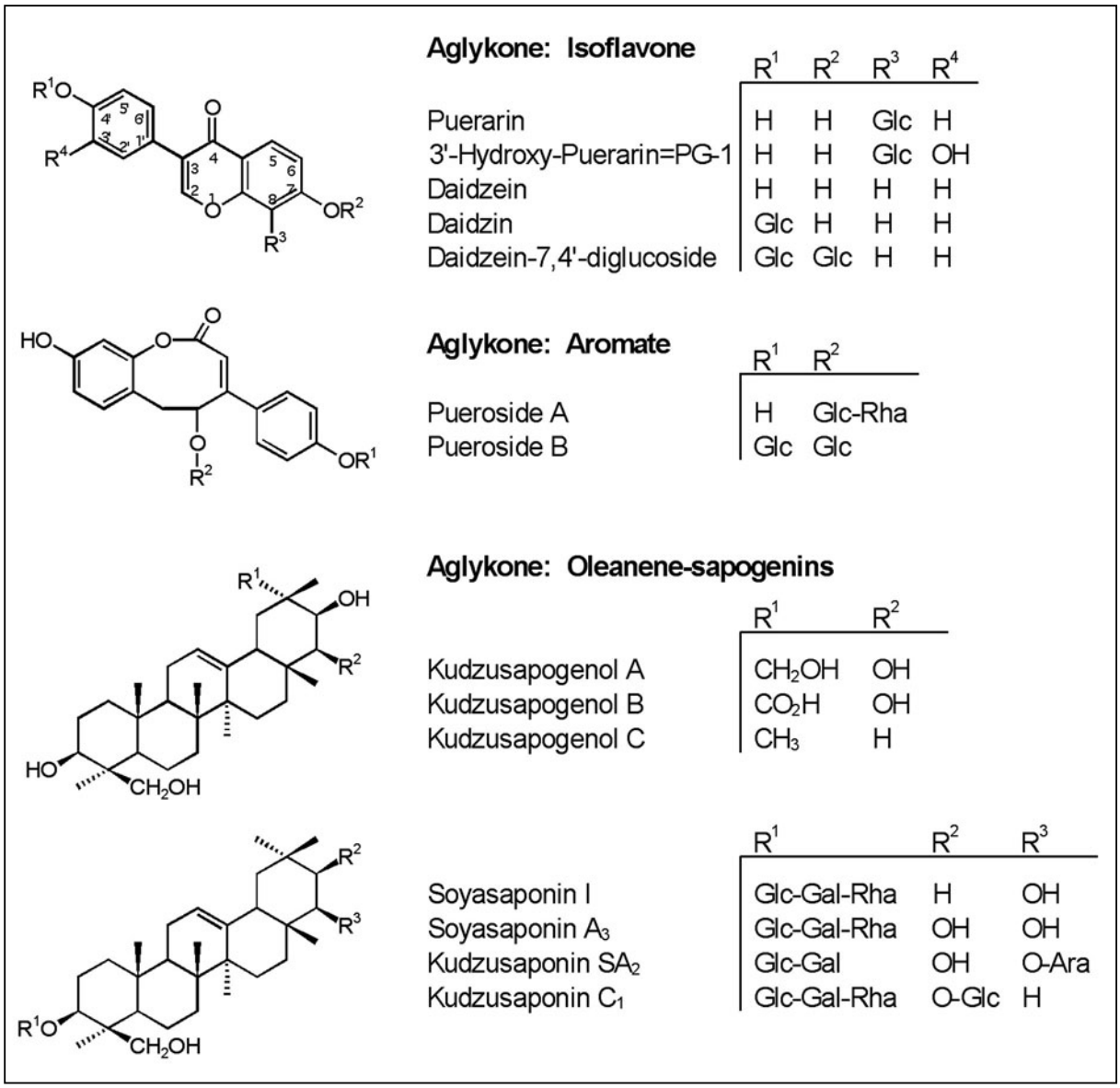

Fig. 1: Formulae of the main constituents

TLC-fingerprint analysis

Drug sample	Species	Origin
1	Puerariae lobatae radix	Japan
2	Puerariae lobatae radix	Japan
3	Puerariae lobatae radix	China
4	Puerariae thomsonii radix	China
5	Puerariae thomsonii radix	China
6	Puerariae thomsonii radix	China

1. Isoflavones: (see Fig. 2a and 2b)

1) Extraction: 1.6 g pulverized drug are extracted light protected for 2 hours with 20 ml methanol at room temperature. The extract is then filtered and the filtrate concentrated to dryness. The residue is dissolved up to 1.0 ml with methanol.

2) Reference compounds:

	Reference compound	R*f*
T 1	formononetin	0.88
T 2	genistin	0.41
T 3	daidzin	0.35
T 4	puerarin	0.28
T 5	PG-1	0.17
T 6	puerosid A	0.13

(T1: 1 mg dissolved in 1 ml n-butanol; T2 – T5: 1 mg dissolved in 1 ml methanol; T6: 2 mg dissolved in 1 ml methanol; applied amounts: 10 µl each)

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: *P. lobata*-methanol-extract: 5 µl
P. thomsonii-methanol-extract: 20 µl
T1 – T6: each 10 µl

Solvent system: chloroform – methanol – formic acid – water (70 + 27 + 1.5 + 1.5)

The plates are run in a glas chamber strongly saturated with the solvent mixture.

Detection: on two *separate* plates

Spray reagents: a) aluminium(III)chloride-reagent
(5 % $AlCl_3$ x 6 H_2O dissolved in ethanol 80 %)

The plate is intensively sprayed with 10 ml solution and then exposed to UV 366 nm for 30 minutes.

b) fast blue salt-reagent [28]
I: 0.5 % fast blue salt B = 3,3'-dimethoxybiphenyl–4,4' bis (diazonium)-dichloride is dissolved in methanol 80 %

II: 5 % potassiumhydroxid in methanol 80 %

The plate is intensively sprayed with 10 ml solution I and then immediately sprayed with 10 ml solution II.
The evaluation is carried out in VIS.

Saponines: (see Fig. 3)

1) Extraction: 4.0 g pulverized drug are soxhlet-extracted for 2 hours with 80 ml methanol. The extract is evaporated to dryness. To the residue 4 ml distilled water are added and heated in a waterbath at 40 °C. The suspension is transformed into a funnel and extracted two times each with 2.5 ml of water saturated n-butanol. n-butanol-phases are combined, shaken with 5 ml distilled water and centrifuged. The organic phase is filled up to 5.0 ml with water-saturated n-butanol.

2) Reference compounds:

	Reference compound	R*f*
T 7	kudzusaponines mixed with soyasaponine I	0.18–0.41
T 8	kudzusaponines	
T 9	soyasaponine I	0.41

(T7, T8, T9: 2.5 mg dissolved in 1 ml methanol; applied amounts: 10 µl each)

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: *P. lobata*-butanol-extract: 10 µl
P. thomsonii-butanol-extract: 40 µl
T7, T8, T9: each 10 µl

Solvent system: chloroform – glacial acetic acid – distilled methanol – water (64 + 32 + 12 + 8)

The plates are run in a strongly with the solvent mixture saturated glas chamber.

4) Detection:

Spray reagent: vanillin-sulphuric acid-reagent[27]:
1 % vanillin dissolved in sulphuric acid 50 %

The plate is intensively sprayed with 10 ml solution and then heated for 5 minutes at 110 °C. The evaluation is carried out in VIS.

Description of the TLC-chromatograms:

Fig. 2a: Isoflavone-TLC detected with aluminium(III)chloride-reagent, UV-365 nm

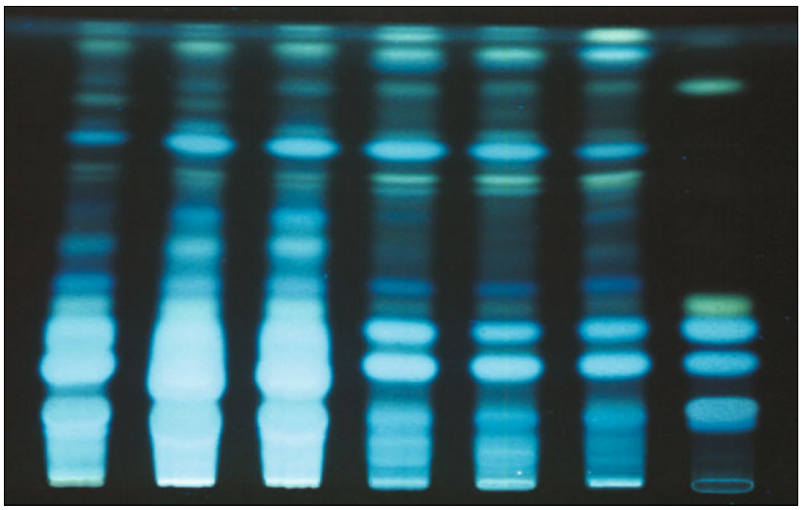

Fig. 2a:
Thin layer chromatogram of methanolic extract of *P. lobata* and *P. thomsonii* after spraying with aluminium(III)chloride-reagent in UV 365 nm

A dark blue fluorescent spot of puerosid A (R*f* = 0.13) is visible only in samples 4 – 6. *P. lobata* samples 1 – 3 show three very intensive blue fluorescent spots of isoflavon-glycosides: PG-1 (R*f* = 0.17), puerarin (R*f* = 0.28) and daidzin (R*f* = 0.35). They are also detectable in samples 4 – 6 of *P. thomsonii* in lower concentration. A more green fluorescent spot of genistin (R*f* = 0.41 = **T2**) can be detected in samples 1 – 6. The blue fluorescent spots of *P. lobata* in the R*f*-range 0.45 – 0.55 are missing in *P. thomsonii*. In the upper range a second green fluorescent spot of the isoflavone-aglycone formononetin (R*f* = 0.88) is present in all samples.

Fig. 2b: Fast blue salt-reagent, VIS

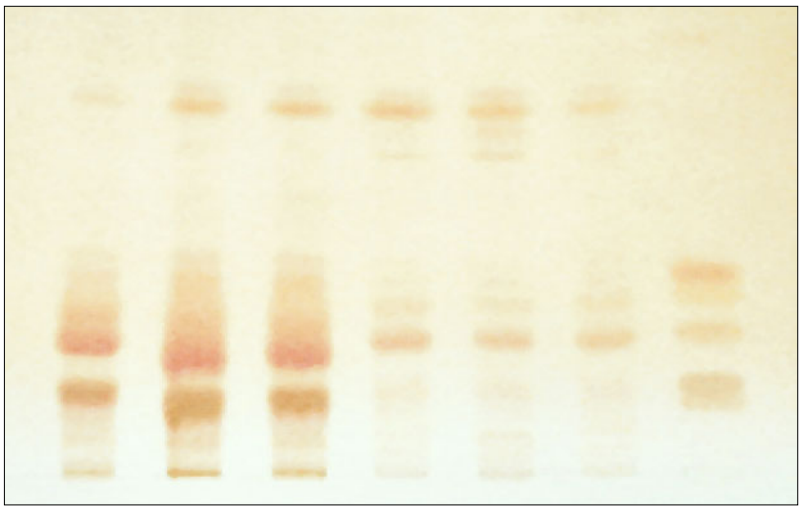

Fig. 2b:
Thin layer chromatogram of methanolic extracts of *P. lobata* and *P. thomsonii* after spraying with fast blue salt-reagent (VIS)

In the range of R*f* 0.10 to R*f* 0.38 isoflavone-glycosides puerosid A (orange-brown), PG-1, puerarin (red) and daidzin (weak orange) are visible. One further red spot of genistin (R*f* = 0.41) could be also detected in sample 1 – 3 and in lower concentration in sample 4 – 6. For identifying the isoflavonaglycone formononetin (R*f* = 0.88) in the samples the detection limit of fast blue salt-reagent is not sufficient.

Fig. 3: Saponines and sapogenins detected with vanillin-sulphuric acid-reagent, VIS

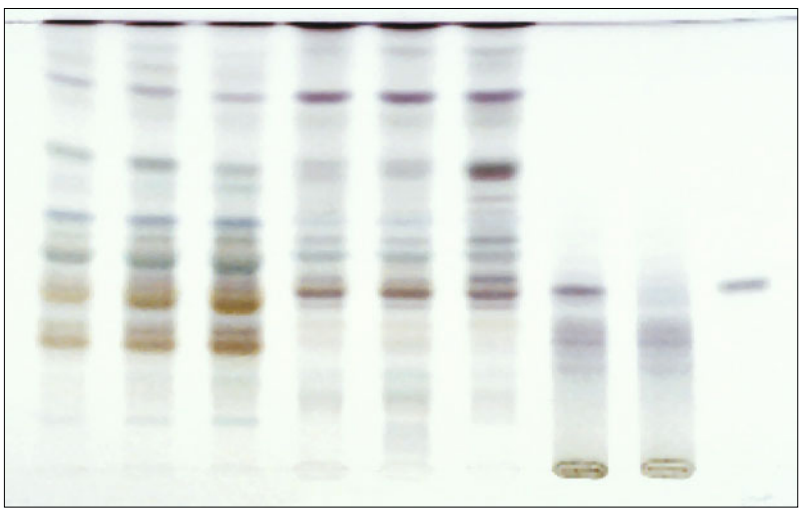

Fig 3:
Thin layer chromatogram of n-butanolic extracts of *P. lobata* and *P. thomsonii* after spraying with vanillin-sulphuric acid-reagent (VIS)

Soyasaponine I (R*f* = 0.42) could be identified clearly in *P. thomsonii* (sample 4 – 6) and as a weak spot only in sample 1 – 3 because overlapped by a prominent brown spot. Below this spot in *P. lobata* three strong brown violet kudzusaponine zones (R*f* = 0.18 – 0.41) appear, in *P. thomsonii* in a very low concentration only. In the upper R*f*-range in all 6 samples strong sapogenine zones appear at R*f* = 0.72 and R*f* = 8.8.

HPLC fingerprint analysis:

1) Sample preparation: 0.5 ml *P. lobata*-methanolic extract (see TCL: isoflavones) is filled up to 5.0 ml with distilled methanol and filtered over Millipore® filtration unit, type 0.45 µm.

   The *P. thomsonii* -methanolic extract (see TLC: isoflavones) is also filtered over Millipore® filtration unit, type 0.45 µm.

2) Injection volume: 10 µl *P. thomsonii*-sample
   2 µl *P. lobata*-sample

3) HPLC parameter:

Apparatus:	Liquid chromatograph HP 1090 Photodiode array detector HP 1040 A, Hewlett Packard
Separation column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
Solvent:	A: distilled water + 1 % 0.1 N H_3PO_4 B: acetonitrile + 1 % 0.1 N H_3PO_4
Gradient:	0 – 25 % B in 30 min., 25 – 45 % B in 5 min., 45 – 95 % B in 5 min., linearly, 95 % B isocratic 5 min., total runtime: 45 min.
Flow:	1.0 ml/min.
Detection:	200 nm

Description of the chromatograms:

Retention times of the main peaks (in Fig 4a and 4b).

Peak	Rt (min.)	Compound
1	10.5	8-*C*-glucosyl-7,3',4'-trihydroxyisoflavone = PG-1
2	13.4	puerarin
3	14.9	puerarin-apioside
4	16.9	daidzin
5	19.4	pueroside A
6	22.2	genistin
7	30.7	daidzein
8	36.8	soyasaponine I

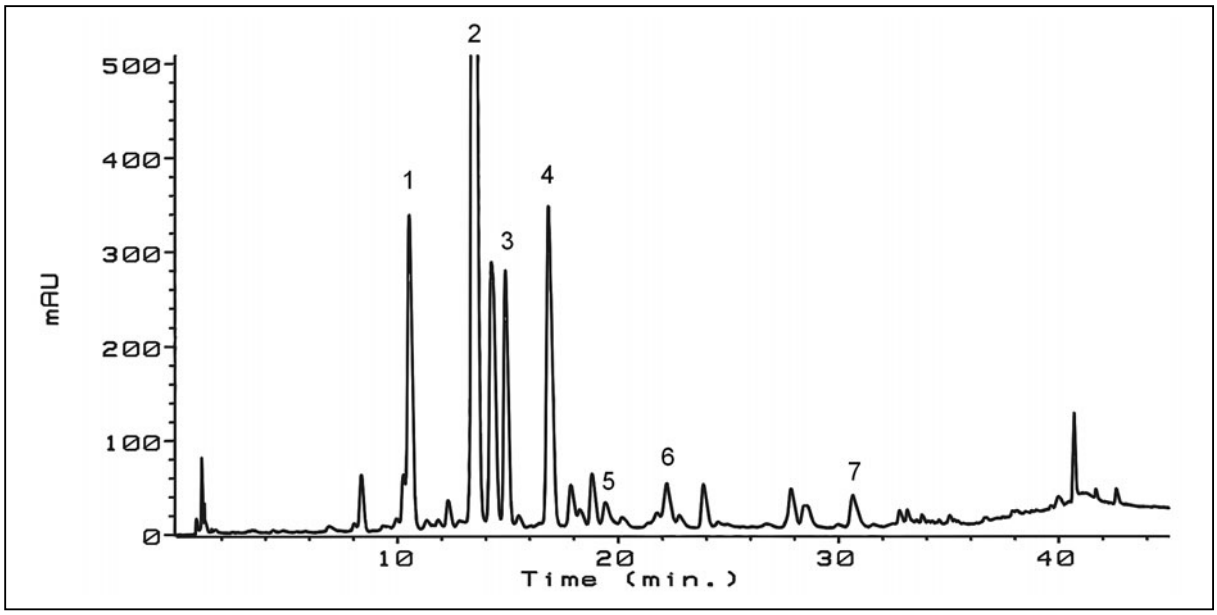

Fig. 4a: HPLC-fingerprint chromatogram of *P. lobata* (sample 4) methanolic extract.
The HPLC-fingerprint of *Pueraria lobata* methanolic extract is characterized by the strong peaks of PG-1 (**1**), puerarin (**2**), puerarin-apioside (**3**), daidzin (**4**) and one further not identified peak at Rt = 14.3 min. Daidzein (**7**) appears in small concentration in comparison to the main peak in *Pueraria thomsonii* chromatogram. Pueroside A (**5**) and genistin (**6**) are also detectable. Soyasaponin I (**8**) and other kudzusaponines are not recordable because of their low concentrations in the sample.

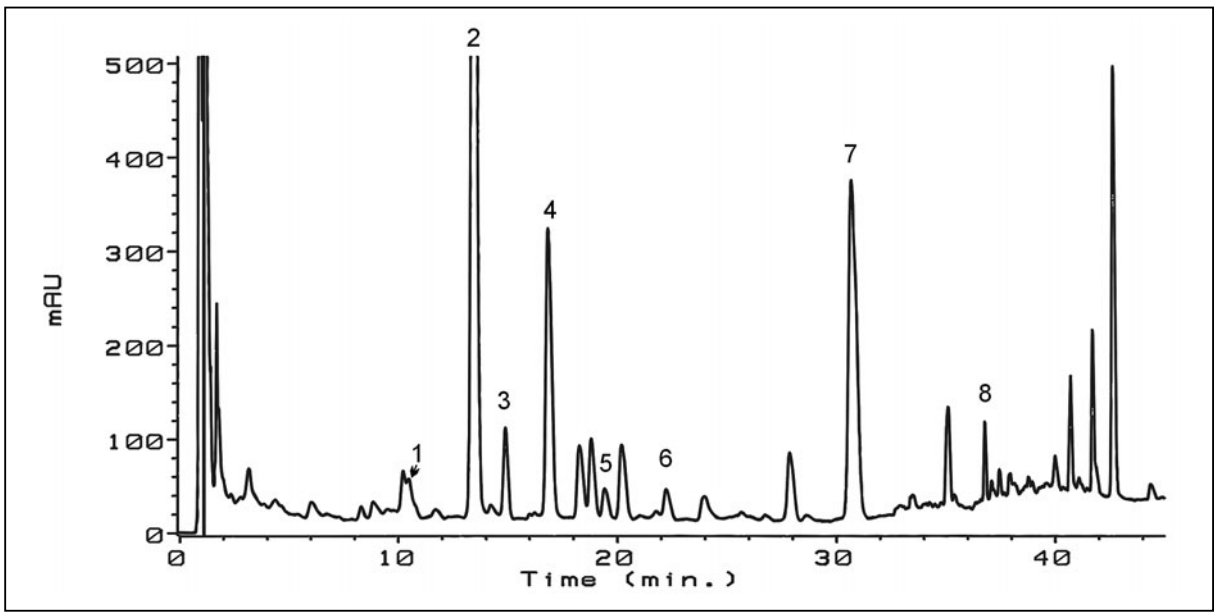

Fig. 4b: HPLC-fingerprint chromatogram of *P. thomsonii* (sample 3) methanolic extract.
The main compounds of *Pueraria thomsonii* methanolic extract are puerarin (**2**), daidzin (**4**) and daidzein (**7**). Furthermore PG-1 (**1**), puerarin-apioside (**3**), pueroside A (**5**) and genistin (**6**) appear in minor concentration. Soyasaponin I (**8**), eluted with Rt = 36.5 min., can be detected in small amount. In the range of Rt = 40 – 43 min. appear sapogenins.

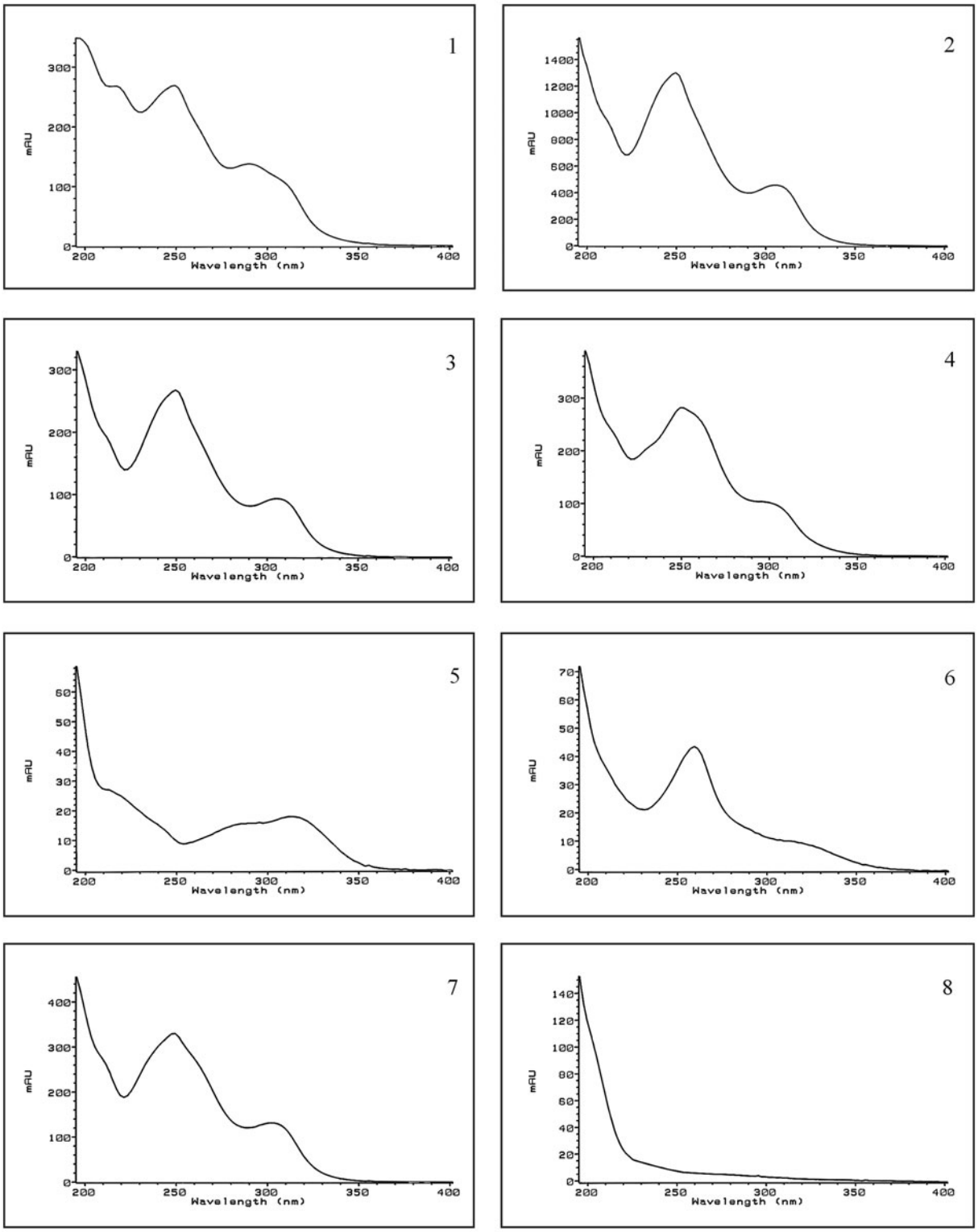

Fig. 5: UV-spectra of the main compounds (Peak 1 – 8) of *P. lobata* and *P. thomsonii* extracts.

Note: According to the Chinese Pharmacopoeia 2005 *P. lobata (P. thomsonii)* not less than 2.4 (0.30)% of Puerarin calculated with reference to the dried drug are demanded.

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Radix Codonopsis pilosulae
Dangshen

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 1997/2005[1]

Official drug: Dangshen is the dried root of *Codonopsis pilosula* (FRANCH.) NANNF, *Codonopsis pilosula* NANNF. var. *modesta* (NANNF.) L.T. SHEN or *Codonopsis tangshen* OLIV. (Fam. Campanulaceae).
English name: pilose asiabell root.

Dangshen is mainly produced in the China provinces *Shan Xi*, *Shanan Xi*, *Gan Su* and *Si Chuan*, collected in autumn, washed clean, and dried.

Description of the drug: Root of *Codonopsis pilosula*[1]:

Long cylindrical, slightly curved, 10 – 34 cm long, 0.4 – 2 cm in diameter. Externally yellowish-brown to greyish-brown, with numerous warty prominent stem scars and buds on the root stock, and the apex of each stem scar sunkenly dotted; dense transverse annulations occuring below the root stock, gradually sparse downwards, some up to half length of the root while the transverse annulations rare or absent in same cultivars; whole root showing longitudinal wrinkles and scattered transverse lenticels, frequently with blackish-brown gelatinous substances at the fractured area of the rootlets. Texture slightly hard or tenacious, fracture somewhat even, cleft or striated radially, bark pale yellowish-white to pale brown, wood pale yellow. Odour: characteristic and aromatic; taste: sweetish.

Root of *Codonopsis pilosula* var. *modesta*[1]:

10 – 35 cm long, 0.5 – 2.5 cm in diameter. Externally yellowish-white to pale yellow, dense transverse annulations occurring below the root stock, frequently up to over half length of the root. Fracture more cleft, bark greyish white to pale yellow, wood light yellow.

Root of *Codonopsis tangshen*[1]:

10 – 45 cm long, 0.5 – 2 cm in diameter. Externally greyish yellow to yellowish brown, with distinctly longitudinal wrinkles. Texture slightly soft and compact, fracture less cleft, bark yellowish white, wood pale yellow.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Pretreatment of the raw drug[1]: Foreign matters are eliminated, washed clean, softened thoroughly, cut into thick slices and dried.

Medicinal use: In Traditional Chinese Medicine internally as a tonic, for accelerating the recovery from diseases, to increase the capacity for physical work and intellectual performance, to increase the resistance of the organism in stress situations (adaptogenic activity), for poor appetite, lassitude, weakness, shortness of breath and loose stool[3, 4, 5] – in former times used as a Ginseng substitute.

Effects and indications according to Traditional Chinese Medicine[1–5]

Taste:	sweet
Temperature:	warm
Channels entered:	spleen, lung
Effects:	Invigorates the spleen and replenishes Qi, promotes the production of body fluid and nourishes the blood
Symptoms and indications:	Weakness of the spleen and the lung manifested by shortness of breath, cough, palpitation, anorexia, loose stool; diabetes caused by internal heat

Contraindication: not to be used for heat syndromes and shock patients as in the case of Ginseng medication

Main constituents (see also Tang and Eisenbrand[23] (see Fig. 1):

- **phenylpropanoids**[14,15]:
  tangshenoside I, tangshenoside II, syringin, syringaaldehyde, coniferylalcohol, dihydroconiferylalcohol pinoresinol
- **furan derivatives**[14,16]:
  furan-2-carboxylic acid, 5-(hydroxymethyl-2-furaldehyde, 5-(methoxymethyl)-2-furfuraldehyde
- **(Polyacetylenes alkanyl- and alkenyl-glycosides; alcins**[14,17,18]**)**
  ethyl-α-D-fructofuranoside, n-hexyl-β-D-glucopyranoside, (Z)-3-hexenyl-β-D-glucopyranoside, (E)-hexenyl-β-D-glucopyranoside; tetradeca-4,12-dien-8,10-diin-1,6,7-triol(-6-O-β-D-glucoside)
- **steroids and triterpenoids**[6,7,8,9,10,11,12]:
  α-spinasterol, (β-D-glucopyranoside), δ^7-stigmasterol, (β-D-glucopyranoside), δ-stigmasterol, taraxerol, taraxeryl acetat and friedelin
- **alkaloids**[12,13]:
  codonopsine, perlolyrine
- sugars and polysaccharides[19,20]:
  glucose, fructose, galactose, arabinose, mannose, rhamnose, xylose, ribose, fructose; inulin, heteroglycans
- organic acids[14]:
  protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid

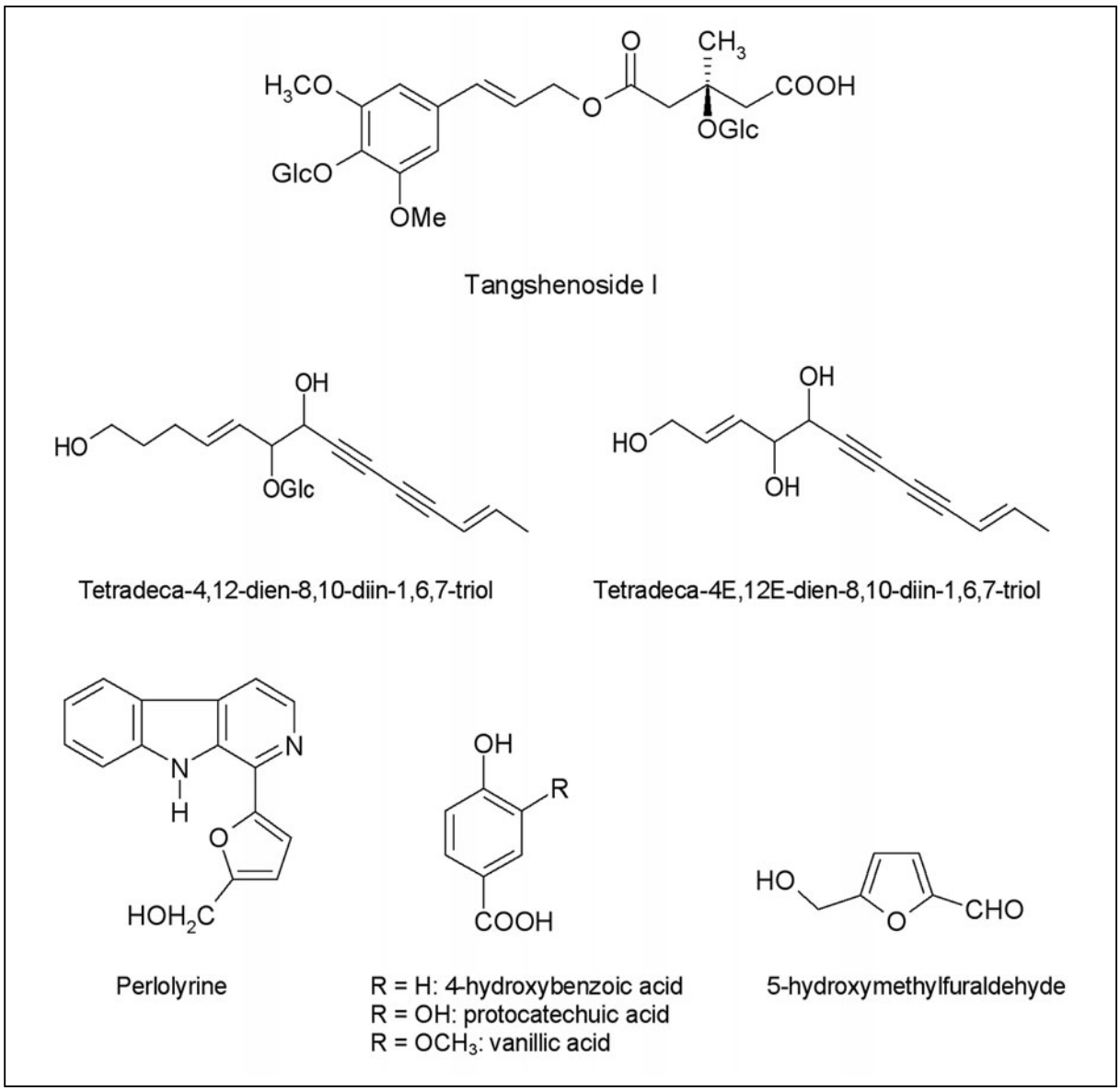


Fig. 1: Formulae of the main constituents

Pharmacology:

– Antistress effect[21]:
Enhancing of swimming capacity of weight loaded mice, increasing tolerance of mice to high temperatures (45 - 47 °C), prolongation of life span of mice under hypoxic environment by reduced oxygen consumption, reduction of stress induced (acetic acid, aspirin, indometacin, pylorus ligation) stomac ulcers.

– Effect on CNS (nootropic effect)[21,22]:
Compensation of Dexamethason induced decrease of corticosteron level = adaptogenic effect, increasing of learning and memory activity in animal models, reduction of impairment of cognitive function in different phases of learning and memory caused by scopolamine, sodium nitrite and alcohol in mice, increasing of m-cholinergic receptors in mice brain.

– Effect on the cardiovascular and gastrointestinal system[21]:
  Reduction of heart rate and cardiac contraction, production of transient hypotension, inhibition of the spontaneous intestinal movement, induced by neostigmine.
– Immunostimulatory effect[21]:
  Increasing phagocytosis and antibody production in cyclophosphamide treated mice.
– Antidiabetic effect[21]

TLC-fingerprint analysis

Drug sample	Species	Supplier	Origin
1	Codonopsis pilosulae radix	Chinamed Teisendorf	unknown
2	Codonopsis pilosulae radix	Herbasin Hilsdorf GmbH Schwabach	China
3	Codonopsis pilosulae radix	Nibelungen Apotheke Munich	Japan
4	Codonopsis pilosulae radix	TCM Klinik Kötzting	China
5	Codonopsis pilosulae radix	TCM Klinik Kötzting	China

1) Extraction:
4.0 g pulverised drug are soxhlet-extracted with 160 ml distilled methanol for 3 hours. Afterwards the extract is filtered through the filterpaper, wetted with distilled methanol. The extract was evaporated to dryness and the residue redissolved in 5.0 ml distilled methanol.

3.0 g methanol extract are evaporated to dryness. The residue is suspended in 5 ml distilled water and transferred into a 100 ml separatory funnel. Then it is extracted with 3 x 15 ml water saturated n-butanol.
The n-butanol phases are pooled and washed with 10 ml distilled water. The n-butanol phases are evaporated to dryness and the residue redissolved in 1.0 ml distilled methanol.

2) Reference compound: β-sitosterin (R)
1 mg dissolved in 1.0 ml methanol

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: *Codonopsis pilosula*-methanol extract: 12 µl
Reference compound: 5 µl

Solvent system: chloroform - methanol - water
(35 + 25 + 10)
The lower phase is used for TLC-fingerprint analysis.

4) Spray reagent: Vanillin-sulphuric acid reagent:
Solution I: 1 % vanillin dissolved in ethanol 100 %
Solution II: 15 % sulphuric acid 98 % dissolved in ethanol 100 %

The plate is intensively sprayed with 10 ml solution I and then immediately sprayed with 10 ml solution II. Afterwards the plate is heated for 5 minutes at 110 °C. The evaluation is carried out in VIS.

Description of the TLC-chromatogram:

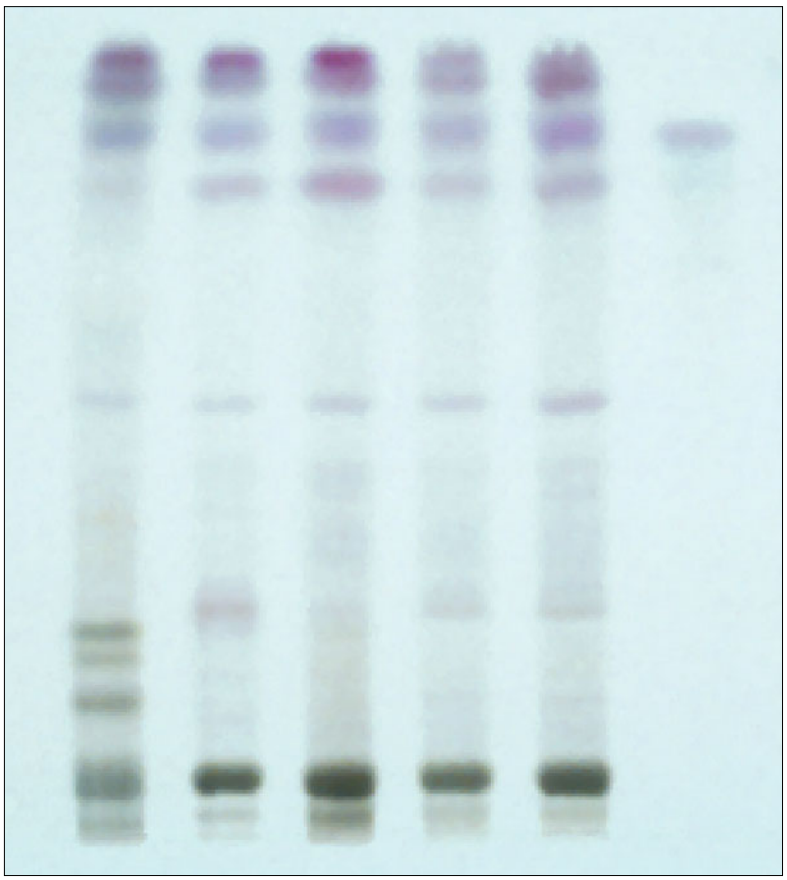


Fig. 2: Thin layer chromatogram of methanolic extract of Codonopsis pilosulae radix after spraying with vanillin-sulphuric acid-reagent (VIS)

All samples show a very homogeneous pattern of grey or violet spots centered in the R*f*-range of 0 – 0.3 (3 – 4 spots) and 0.5 – 1.0 (5 – 7 zones).

In the R*f* ~ 0.1 – 0.15 appear tangshenoside I and dehydrodiconiferylalcohol. At R*f* ~ 0.25 tetradeca-4,12-dien-8,10-diin-1,6,7-triol-6-O-β-D-glucoside is visible. In the higher R*f*-area (0.8 – 1.0) the tetradeca-4,12-dien-8,10-diin-1,6,7-triol, 5-hydroxymethylfurfural and several triterpenoids can be detected.

HPLC-fingerprint analysis:

Retention times of the main peaks:

Peak	Rt (min.)	Compound
C1	1 – 2	5-hydroxymethylfurfural
C5	~8	Tangshenoside I
C8	14 – 15	Tetradeca-4E,12E-dien-8,10-diin-1,6,7-triol-6-O-β-D-glucoside
C11	18 – 19	Tetradeca-4E,12E-dien-8,10-diin-1,6,7-triol

---

1. HPLC of the water phase and n-butanol phase of Codonopsis pilosulae radix:

Sample preparation:

1.0 g pulverised drug is soxhlet-extracted with distilled methanol for 3 hours and the extract evaporated to dryness. After that the residue is dissolved in 10 ml water and extracted with n-butanol.

1.1 Water phase:

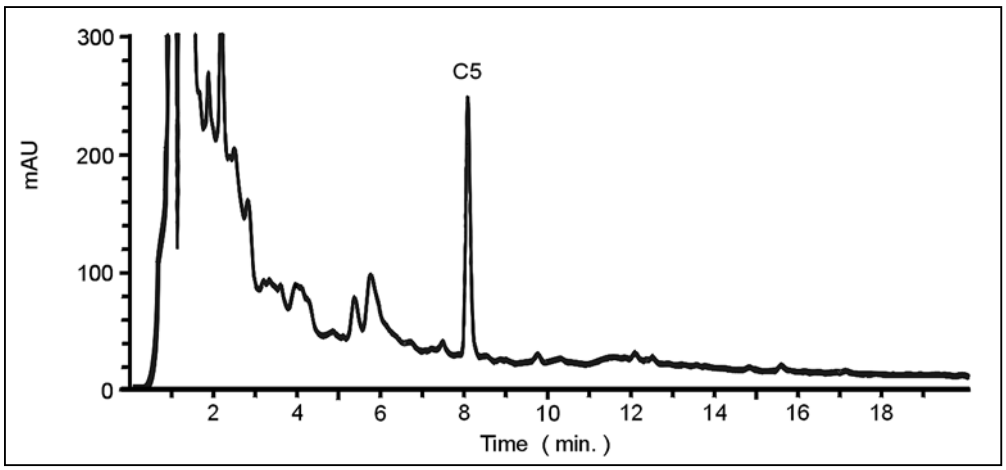
Sample preparation of the water-phase:

The above-mentioned water-phase is separated, evaporated and dissolved in a 1:1 methanol-water mixture.

After filtration of the extract over Millipore® filtration unit, type HV 0.45 µm direct injection of 25 µl

HPLC parameter:

Apparatus:	Liquid chromatograph HP 1090 Hewlett Packard
Separation column:	Hibar® commercial-column 125-4 mm LiChrospher 100 CH-18/2 (+ RP18 – pre-column), Merck Darmstadt
Solvent system:	A: distilled water + 1 % (V/V) 0.1 N H_3PO_4 B: acetonitrile + 1 % (V/V) 0.1 N H_3PO_4
Gradient:	10 – 50 % B in 40 minutes (linear)
Flow:	1.0 ml/min.
Detection:	λ= 210 nm



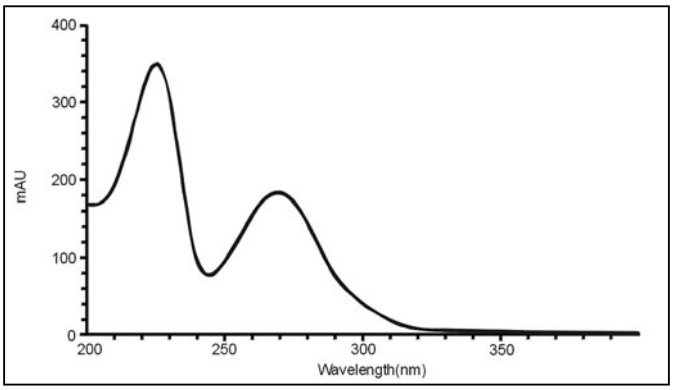


Fig. 3a: HPLC-fingerprint of *Codonopsis pilosula*-water phase (210 nm) with UV-Spectrum (on line) of substance C5 (Tangshenoside I)

Fig. 3a Besides the peak sequence between Rt ~ 0.5 and 3.0, which comprises the bulk of sugars, at Rt ~ 8.0 tangshenoside I (**C5**) appears as sharp peak. It can be identified by its on line UV-spectrum with maxima at 220 nm and 265 nm.

1.2 n-butanol phase:

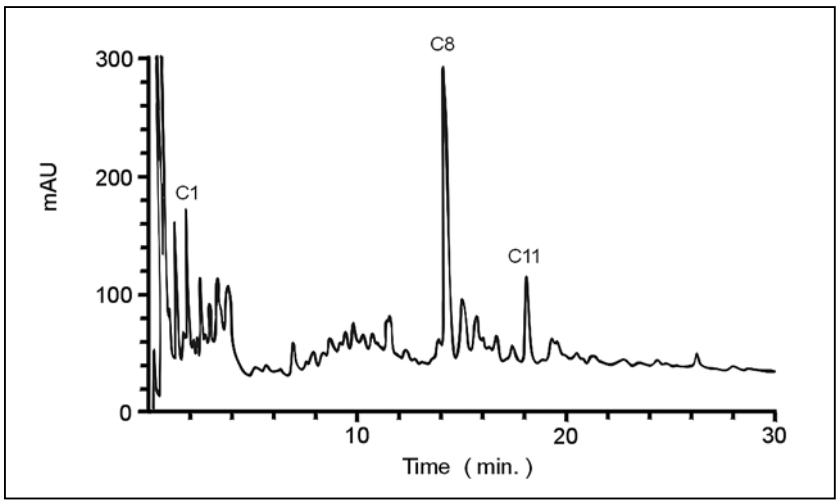
Sample preparation of n-butanol-phase:

The above-mentioned n-butanol-phase is shaken with water to remove free sugars. The n-butanol phase is evaporated to dryness and the residue dissolved in methanol.

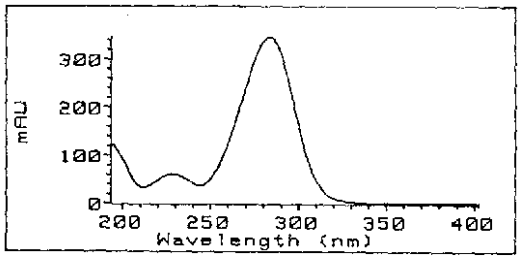
After filtration of the extract over Millipore® filtration unit, type HV 0.45 µm, direct injection of 25 µl.

HPLC parameter:

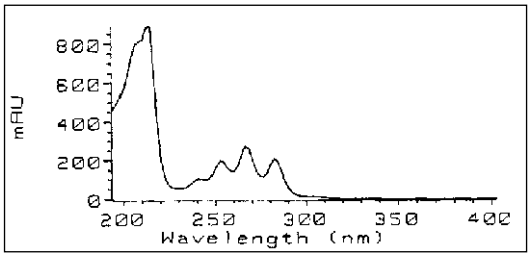
Apparatus:	Liquid chromatograph HP 1090 Hewlett Packard
Separation column:	Hibar® commercial-column 125-4 mm LiChrospher 100 CH-18/2 (+ RP18 – pre-column), Merck Darmstadt
Solvent system:	A: distilled water + 1 % (V/V) 0.1 N H_3PO_4 B: acetonitrile + 1 % (V/V) 0.1 N H_3PO_4
Gradient:	10 – 50 % B in 40 minutes (linear)
Flow:	1.0 ml/min
Detection:	λ = 210 nm



UV-spectrum C1



UV-spectrum C8



UV-spectrum C11

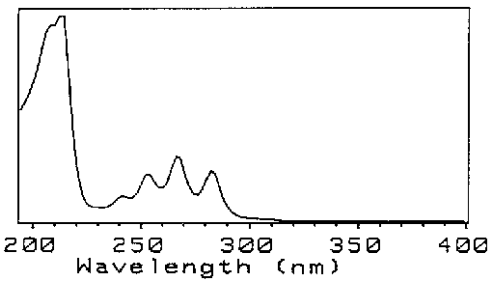


Fig. 3b: HPLC-fingerprint of *Codonopsis pilosula*-n-butanol phase (210 nm) with UV-Spectra (on line) of substances C1, C8 and C11

Fig 3b n-butanol phase:

The HPLC-fingerprint shows 5-hydroxymethylfurfural (**C1**) at Rt ~ 2.5, tetradeca-4,12-dien-1,6,7-triol-6-O-glucoside (**C8**) at Rt ~ 14.7 and tetra-4,12-dien-8,10-diin-1,6,7-triol (**C11**) at Rt ~ 18.5.

1. Qualitative HPLC-comparison of different *Codonopsis pilosula* root samples:

Drug sample	Species	Supplier	Origin
A	Codonopsis pilosulae radix	Public Pharmacy	unknown
B	Codonopsis pilosulae radix	TCM Klinik Kötzting	China
C	Codonopsis pilosulae radix	TCM Klinik Kötzting	China

Peak	Rt (min.)	Compound
C5	18 – 19	Tangshenoside I
C8	22 – 23	Tetradeca-4E,12E-dien-8,10-diin-1,6,7-triol-6-O-β-D-glucoside
C11	~ 29	Tetradeca-4E,12E-dien-8,10-diin-1,6,7-triol

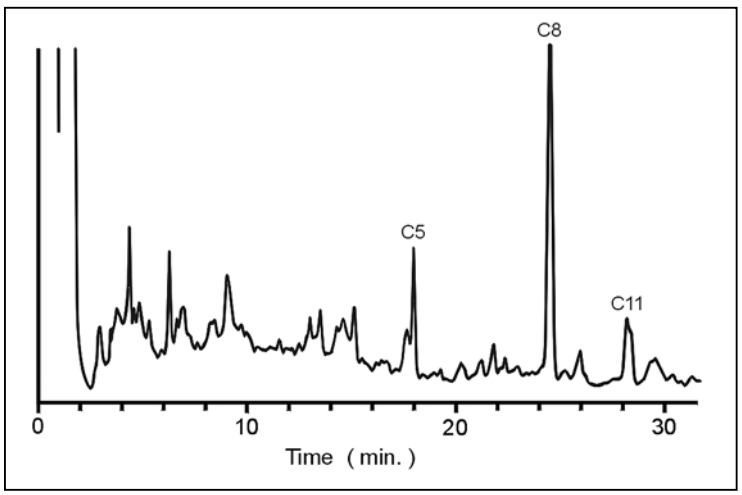
Sample preparation:

Each 1.0 g of the drug-samples A, B and C is pulverised, defatted with dichlormethan and for 3 hours soxhlet-extracted with distilled methanol. The extracts are evaporated to 10.0 ml, filtered over Millipore® filtration unit, type HV 0.45 µm and 25 µl methanol extract directly injected.

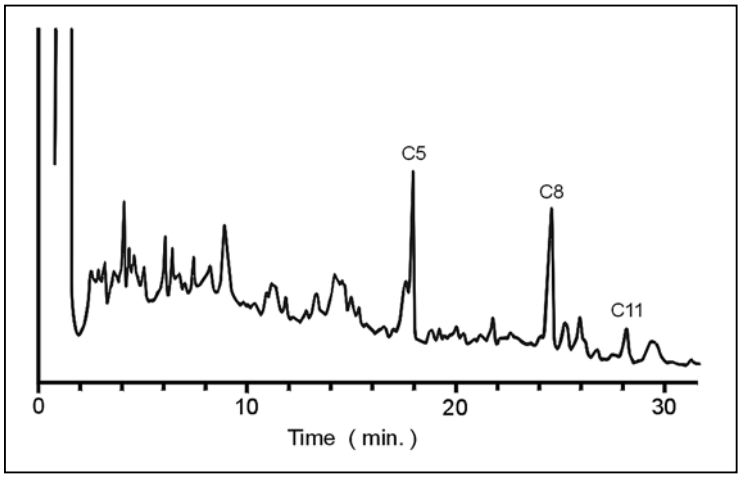
HPLC parameter:

Apparatus:	Liquid chromatograph HP 1090 Hewlett Packard
Separation column:	Hibar® commercial-column 125-4 mm LiChrospher 100 CH-18/2 (+ RP18 – pre-column), Merck Darmstadt
Solvent system:	A: distilled water + 1 % (V/V) 0.1 N H_3PO_4 B: acetonitrile + 1 % (V/V) 0.1 N H_3PO_4
Gradient:	00 – 40 % B in 40 minutes (linear)
Flow:	1.0 ml/min.
Detection:	λ = 210 nm

Drug sample A:



Drug sample B:



Drug sample C:

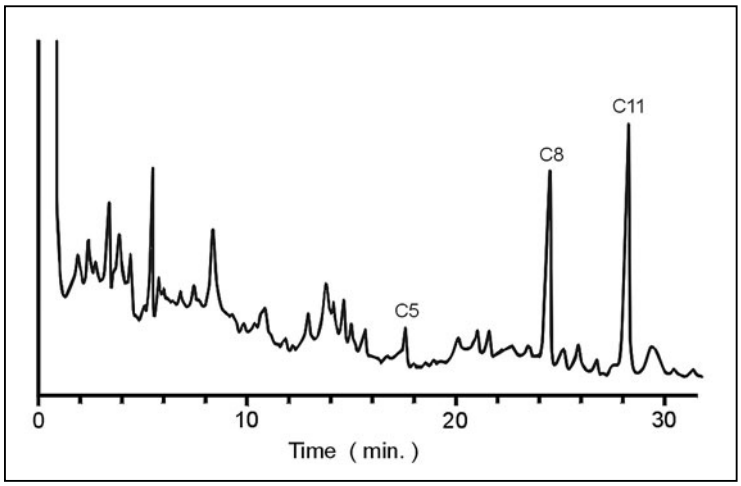


Fig. 4: HPLC-comparison of the *Codonopsis pilosula* drug samples A, B and C (210 nm)

Description of the Fig. 4

The HPLC-fingerprint of the three *Codonopsis* methanol root extracts shows in the Rt-range 16.6 to 30.0 the three peaks of tangshenoside I (**C5**), tetradeca-4,12-dien-1,6,7-triol-6-O-glucoside (**C8**) and tetradeca-4,12-dien-8,10-diin-1,6,7-triol (**C11**). In most of the Codonopsis root extracts analysed tangshenoside is the most prominent compound, whereas the two other can vary in their concentrations.

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Fructus Gardeniae
Zhizi

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drug[1]: *Gardenia jasminoides* Ellis.

The drug is also known under the English name Cape Jasmine Fruit.

– Rubiaceae –

Origin[2,3]: China (provinces Jiangsi, Zhejiang, Anhui, Jiangxi, Guangdong, Guangxi, Guizhou, Sichuan, Hubei, Fujian), Japan, Taiwan

Description of the drug[1]: Prolate-ovoid or ellipsoid, 1.5 – 3.5 cm long, 1– 1.5 cm in diameter. The outer surface reddish-yellow or brownish-red, with 6 longitudinal winged ribs and a conspicuous longitudinal winged and branched vein between two ribs. Summit bearing remains of sepals, base somewhat tapering and having a remain of fruit stalk. Pericarp thin and brittle, somewhat lustrous; the inner surface relatively pale in colour, lustrous, with 2–3 raised false septa.
Seeds numerous, flattened-ovoid, aggregated in a mass, deep red or reddish-yellow, with fine and dense warts on the surface.
Odour slight; taste slightly sour and bitter.

Pretreatment of the raw drug[1]: The drug is collected from September to November when it turns reddish-yellow, removed from the fruit stalk and foreign matters steamed thoroughly or treated with boiling water for a moment. Then the drug is dried and grounded to pieces.

Gardeniae fructus (stir-baked): The clean crude drug is placed in a pot, stir-baked with gentle heat until its outer part turns to yellowish-brown, then taken out and cooled. Then the drug is baked to dryness or dried in the sun.

Gardeniae fructus (charred): The clean drug is placed in a pot, stir-baked with relatively high temperature until its outer part is charred, the drug turns brown and the fractures become darkened. Then it is taken out and cooled, baked to dryness or dried in the sun.

Medicinal use[4,5]: Acute hepatitis, uterine gastric and Oesophagus bleeding hematoms, painful dysfunctions and inflammations of the bile bladder, keratoconjunctivitis

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Effects and indications according to Traditional Chinese Medicine[1–4]

Taste:	slightly sour and bitter
Temperature:	cold
Channels entered:	heart, liver, lungs, stomach, the Sanjiao channel
Effects:	reduces pathogenic fire, eases the mind, eliminates damp heat, relieves irritability, dispels excessive heat, promotes diuresis, removes heat from blood and counteracts toxicity
Symptoms and indications:	febrile diseases with restlessness jaundice with dark urine, hematuria with difficult painful urination, hemoptysis and epistaxis caused by heat in the blood, inflammation of the eye, boils and sores, external use for sprains, bruises and for wound healing

Contraindication: Contraindicated in patients with diarrhea due to spleen deficiency.

Main constituents[6] (see Fig. 1):

- **iridoids**: gardenoside and its aglycone gardenogenin A, geniposide, shanzhisside, genipin-gentiobioside, gardoside, scandoside, geniposidic acid, scandoside methyl ester, deacetylasperulosidic acid methyl ester
- **pigments**: crocetin, crocin, picrocrocinic acid
- 3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl)quinic acid and 3-caffeoyl-4-sinapolyquinic acid
- **aglycone of gardenoside:** gardenogenin A
- **terpenoids:** oleanolic acid acetate, stigmasterol (stem and root)
- **essential oil:** benzylacetate, hydroxycitronellal, eugenol (flower)
- D-mannitol

Pharmacology:

- antiphlogistic[7]
- hypoglycemic[8]
- antithrombotic[9]
- hypotensive[10]
- choleretic[11]
- antihepatotoxic[12]

} iridoids and extract

- antioxidant[13,14]
- chemopreventive[15]

} crocetin/crocin

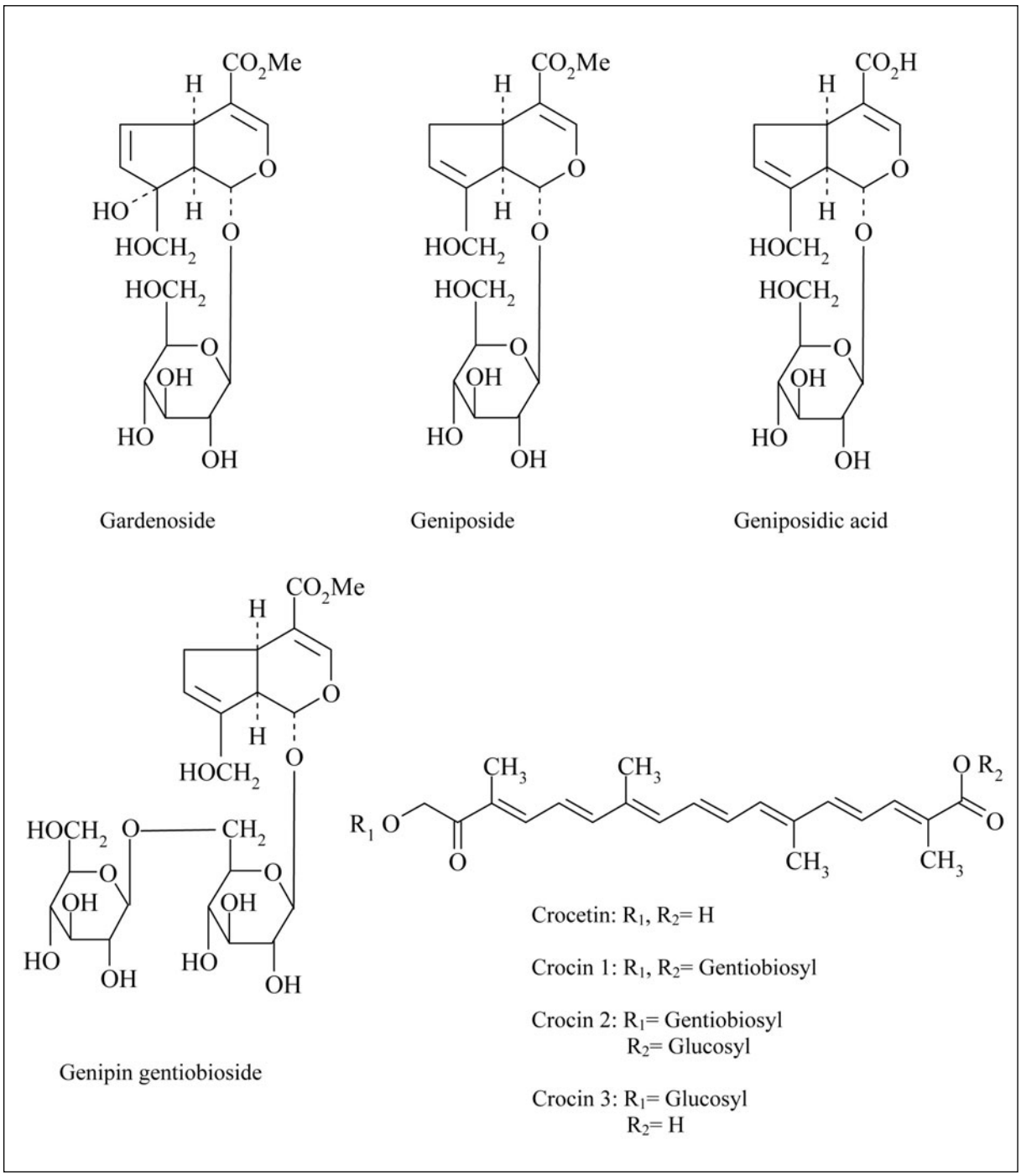

Fig. 1: Formulae of the main constituent[6]

TLC-fingerprint-analysis:

1) Extraction: 1.0 g of the powdered drug is macerated with 10 ml of 75 % ethanol on a warm water bath for 15 min. and filtered.

2) Reference compounds: gardenoside, oleanolic acid, β-sitosterol and mannitol are dissolved in methanol (1 mg/ml)

3) Separation parameters:

Applied amount: 30 µl extract, 20 µl standard solution

Plate: Silicagel 60 F_{254}; Merck

Solvent system: chloroform-methanol-water (70 : 30 : 4)

Detection: Spray reagents:

Vanillin-sulphuric acid-reagent:

The plate is sprayed intensively with 1 % ethanolic vanillin solution and 10 % ethanolic sulphuric acid followed by ca. 10 minutes heating at 110 °C. The evaluation is carried out in VIS.

Natural product-polyethylenglycol reagent:

The plate is sprayed successively with 1 % methanolic solution of diphenylboric acid-β-ethyl-aminoester (NP) and a 5 % ethanolic polyethylenglycol – 4000 solution (PEG). The evaluation is carried out in UV365 nm.

Drug samples		**Origin**
1	Gardeniae fructus / *Gardenia jasminoides*	province Jiangxi, China
2	Gardeniae fructus / *Gardenia jasminoides*	province Sichuan, China
3	Gardeniae fructus / *Gardenia jasminoides*	commercial product of Uchida Company, Japan
4	Gardeniae fructus / *Gardenia jasminoides*	sample of commercial drug, China

Reference compounds		**R*f***
T1	gardenoside and ß-sitosterol	0.40 and 0.95
T2	oleanolic acid	0.92

4) Description of the TLC-chromatogram Fig. 2 in VIS:

Fig. 2: TLC-fingerprint of Gardeniae fructus extract detected with vanillin- sulphuric acid-reagent in VIS

Before spraying the plate with vanillin-sulphuric acid reagent, in the lower R*f*-range the yellow pigments of crocin and crocetin (R*f* 0.13 and R*f* 0.16) can be seen.

After spraying the plate with vanillin sulphuric acid gardenoside appears as a blue-grey zone at R*f* 0.40 in all *Gardenia jasminoides* samples. Another intense zone of violet colour at R*f* 0.59 can be assigned to geniposide. In the higher R*f*-range β-sitosterol (R*f* 0.95) and oleanolic acid (R*f* 0.92) can be detected.

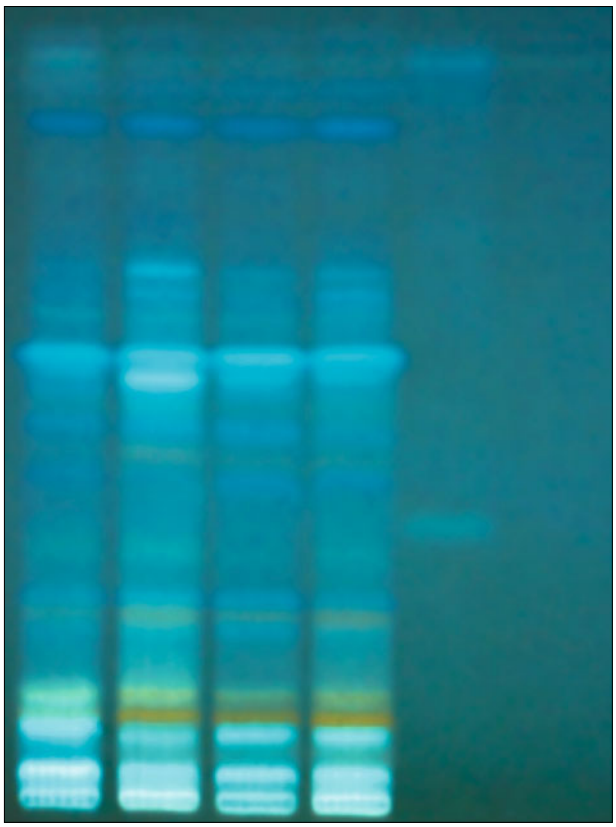

Fig. 3: TLC-fingerprint of Gardeniae fructus extract detected with natural product-polyethylenglycol reagent in UV 365 nm

Description of the TLC-chromatogram Fig. 3 in UV 365 nm:
The pigments (picrocrocinic acid, crocin, crocetin) can be detected in UV365 nm as yellow-orange zones at R*f* 0.13, R*f* 0.16 and R*f* 0.26. 3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl) quinic acid and 3-caffeoyl-4-sinapoylquinic acid show flashy blue fluorescence bands at R*f* 0.05 and 0.09. The iridoid glycosides are visible in the higher R*f*-range as light blue zones between R*f* 0.25 and 0.85 with geniposide as the main compound.

HPLC-fingerprint analysis[16]**:**

1)	Sample preparation:	The ethanol extract, used for TLC, is filtered over Millipore® (Type HV 0.45 µm).
2)	Injection volume:	10 µl extract
3)	HPLC-data:	
	Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck, Hitachi
	Column:	LiChroCART® 125 – 4 mm with LiChrospher® 100 RP 18 (5 µm), LiChroCART® 4 – 4 mm with LiChrospher® 100 RP 18 (5 µm); Merck

Solvent system:	A: 0,1 % aqueous phosphoric acid; Acros Organics
	B: methanol for HPLC; Acros Organics
Gradient:	10 % B to 90 % B in 25 min. (linear)
Flow rate:	1.0 ml/min.
Detection:	240 nm, respectively 315 nm and 438 nm

Retention times and identity of the main peaks of Fig. 4:

Peak	Rt (min.)	Compound
1	7.0	geniposidic acid
2	7.8	gardenoside
3	10.7	genipin gentiobioside
4	12.0	geniposide
5 and 5´	18.0; 18.9	3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl)quinic acid, 3-caffeoyl-4-sinapoylquinic acid
6	20.1; 22.0; 25.3; 26.1; 27.1	picrocrocinic acid, crocin 1, 2, 3, crocetin

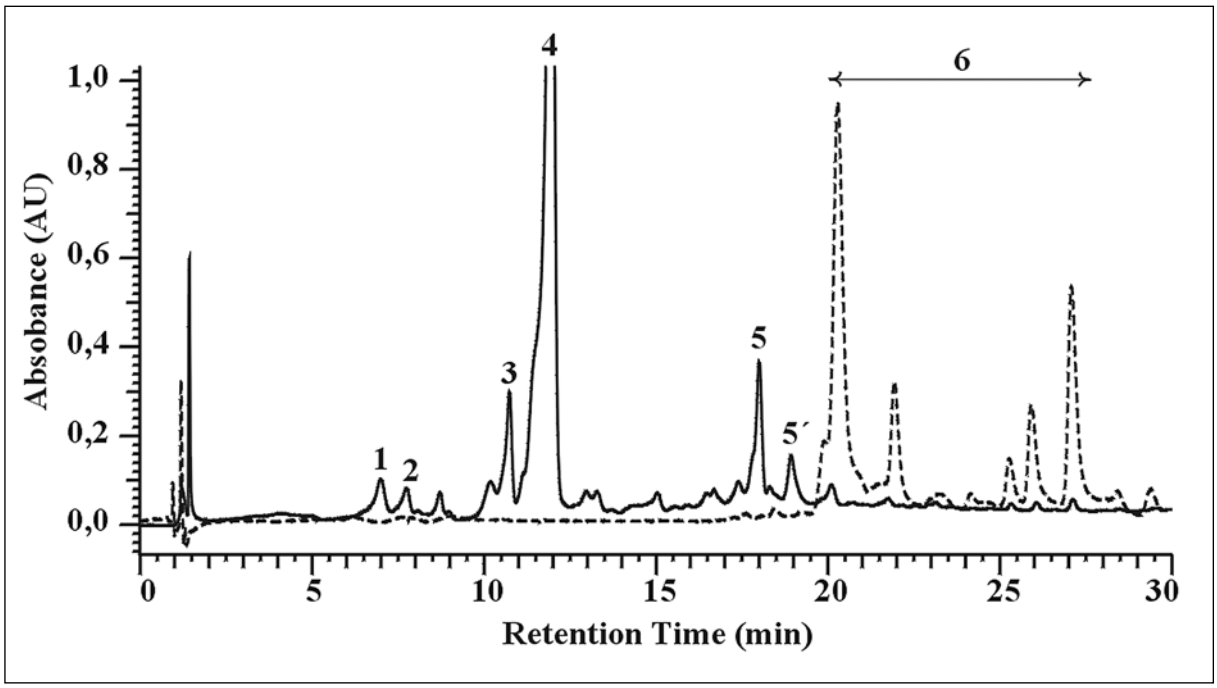

Fig. 4: HPLC-fingerprint of Gardeniae fructus detected at 240 nm (———) and 438 nm (– – – –)

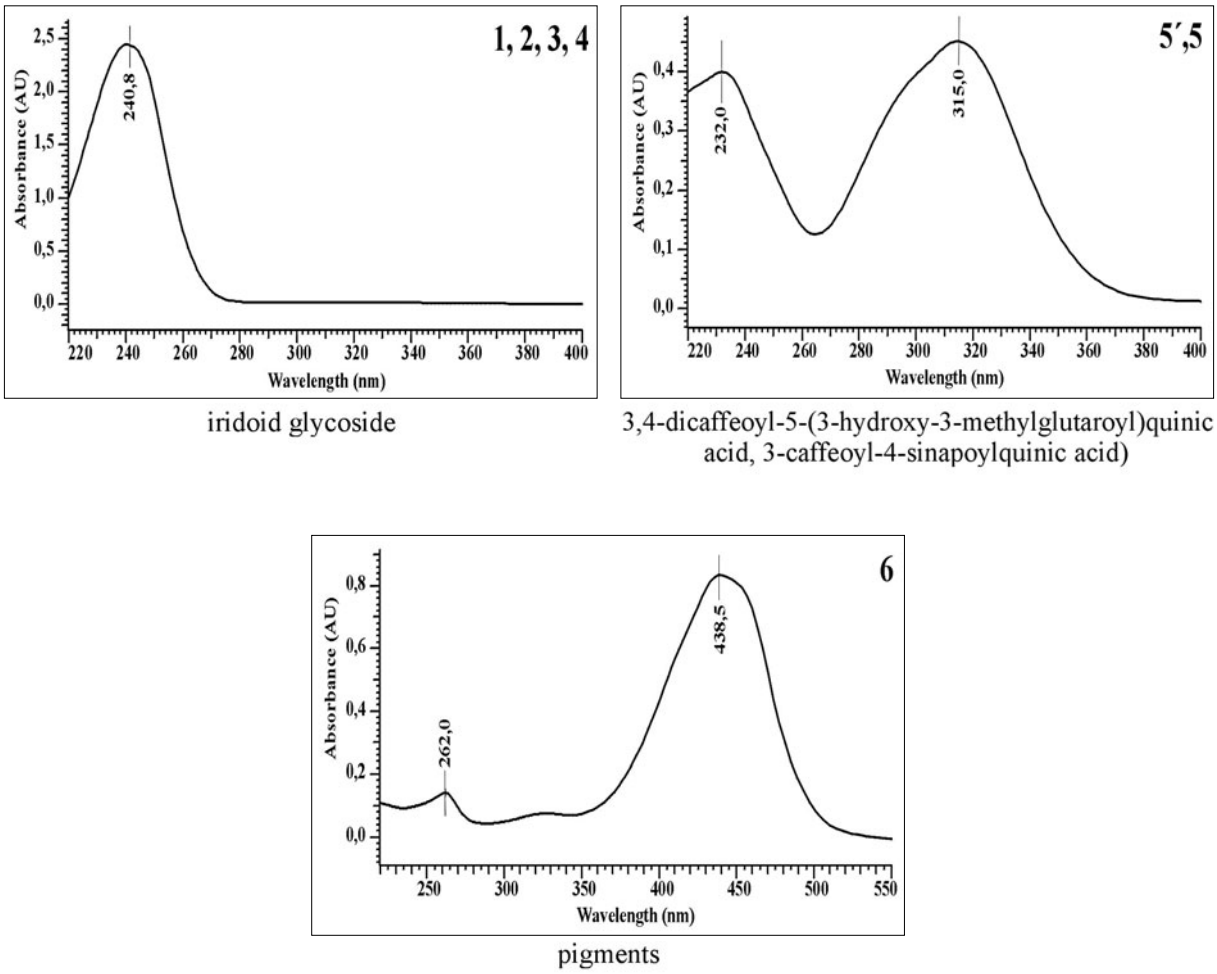

Fig. 5: online UV-spectra of the main constituents

4) Description of the HPLC-chromatogram:

The HPLC-chromatograms at 240 nm are characterized by the iridoid glycosides pattern. Four major peaks can be assigned to geniposidic acid (**1**) at Rt 7.0, gardenoside (**2**) at Rt 7.8, genipin gentiobioside (**3**) at Rt 10.7 and geniposide (**4**) at 12.0. All iridoid glycosides show UV-spectra which are superimposable to geniposide (**4**) with its characteristic UV-maximum at 240 nm.

At Rt 18.0 and 18.9 3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl)quinic acid and 3-caffeoyl-4-sinapolyquinic acid (**5**,**5**´) are detectable. They show their characteric UV-spectrum (**5**) for cinnameic acid derivatives with maxima at 232 nm and 315 nm.

The pigments picrocrocinic acid, crocin 1, 2, 3 and crocetin appear at 240 nm as small peaks and at 438 nm as main peaks with retention times from 20.1 to 27.1. Their nearly superimposible UV-maxima at 438.5 nm are characteristic for carotinoids (**6**).

(A quantitative determination of geniposide, gardenoside, geniposidic acid and genipin-1-β-

gentiobioside in *Gardenia jasminoides* fruit extract by HPLC using a ODS silica gel column and as mobile phase water-methanol-phosphoric acid (870:130:1) has been described by Japanese authors[16]).

Note: According to the Chinese Pharmacopoeia 2000 and 2005 Fructus Gardeniae should contain not less than 1.8 % of gardenoside, calculated with reference to the dried drug.

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Rhizoma Gastrodiae
Tianma

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drug[1]: *Gastrodia elata* BLUME
– Orchidaceae –

Origin[2,3]: Western China (provinces Sichuan, Yunnan, Shaanxi, Gansu), Tibet, Korea, Japan

Description of the drug[1]: Ellipsoid or slat-shaped, slightly compressed, shrunken and somewhat curved, 3-15 cm long, 1.5-6 cm wide, 0.5-2 cm thick. Externally yellowish-white to pale yellowish-brown, with longitudinal wrinkles and many transverse annulations arranged by latent buds, sometimes brown funiculi visible. Apex with reddish-brown to deep brown parrot-beak like buds or remains of stem, the lower end with a rounded scar. Texture hard and uneasily broken, fracture fairly even, yellowish-white to brownish, horny. Odour, slight; taste, sweetish.

Pretreatment of the raw drug[1]: The tuber of *Gastrodia elata* is collected from winter to next spring, washed clean immediately, softened thoroughly or steamed to soften, cut into thin slices, spread out and dried at a low temperature.

Medicinal use[1-3]: The drug is used for the treatment of headache, dizziness and numbness of the limbs, infantile convulsion, vertigo, rheumatism, epilepsy and tetanus.

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Effects and indications according to Traditional Chinese Medicine[1-4]

Taste:	sweet
Temperature:	warm
Channels entered:	liver
Effects:	stops wind to relieve convulsion, soothes the liver and supresses hyperactive liver Yang
Symptoms and indications:	for infantile acute convulsion, for dizziness and headache, for numbness of the limbs or windstroke due to liver and kidney Yin deficiency, quadriplegia and rheumatic arthralgia

Main constituents[5] (see Fig. 1):

– **phenolic glucosides**: gastrodin and gastrodioside [bis(4-hydroxybenzyl)ether-mono-β-D-glycopyranoside]
– aglycones: 4-hydroxybenzyl alcohol (gastrodigenin), 4-hydroxybenzaldehyde and 4-hydroxy-3-methoxybenzaldehyd (vanillin)
– **organic acids:** succinic acid, citric acid with its monomethyl ester and palmitic acid
– **sterols:** β-sitosterol, daucosterol
– sugar: sucrose

Pharmacology:

in vitro:
– promotion of energy metabolism of heart cell under hypoxia condition[7]
– inhibition of GABAergic neurotransmission[8]
– antioxidant and free radical scavenging activities[9]

in vivo:
– sedative (animals and humans)[10]
– anticonvulsant (mice)[10,11]
– antiepileptic (rabbits, guinea pig)[11,12]
– antimotion sickness (mice)[13]
– neuroprotective (mice)[14]
– improving learning (rats)[15]
– memory enhancing[16]
– antiischemic[17]

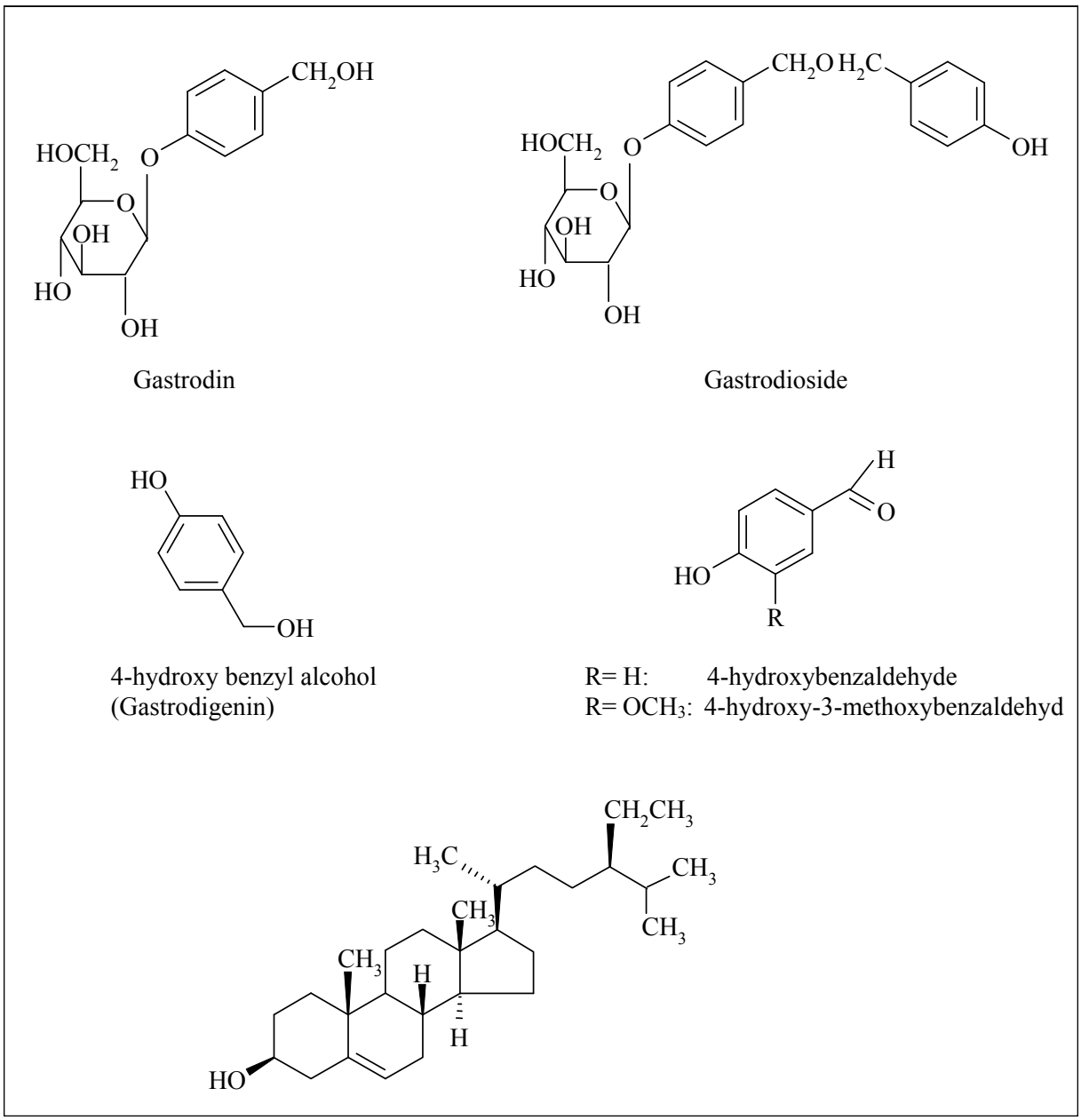

Fig. 1: Formulae of the main constituents of Gastrodiae rhizoma[5,6]

TLC-fingerprint-analysis[6]:

1) Extraction:	3.0 g powdered drug are extracted by heating under reflux with 30 ml methanol for 30 min, allowed to cool down and then filtered. The filtrate is evaporated to dryness and redissolved in 1 ml methanol.
2) Reference compounds:	gastrodin, β-sitosterin, 4-hydroxybenzylalcohol and 4-hydroxybenzaldehyde are dissolved in methanol (2 mg/ml)

3) Separation parameters:

Applied amount:	50 µl extract and 25 µl standard solution
Plate:	Silicagel 60 F_{254}; Fa. Merck
Solvent system:	ethyl acetate-methanol-water-formic acid (77:13:10:2)
Detection:	Spray reagent: Vanillin-sulphuric acid reagent (VIS): The plate is sprayed intensively with 1 % ethanolic vanillin solution, followed immediately with 10 % ethanolic sulphuric acid. After heating at 110 °C for 5 – 10 min. under observation, the plate is evaluated in VIS.

Drug samples		**Origin**
1	Gastrodiae rhizoma / *Gastrodia elata*	province Shaanxi, China
2	Gastrodiae rhizoma / *Gastrodia elata*	province Si-chuan,China
3	Gastrodiae rhizoma / *Gastrodia elata*	province Gui-zhou, China
4	Gastrodiae rhizoma / *Gastrodia elata*	province Gui-zhou, China

Reference compounds		**R*f***
T1	gastrodin	0.34
T2	β-sitosterin	0.94
T3	4-hydroxybenzylalcohol	0.88
T4	4-hydroxybenzaldehyde	0.92

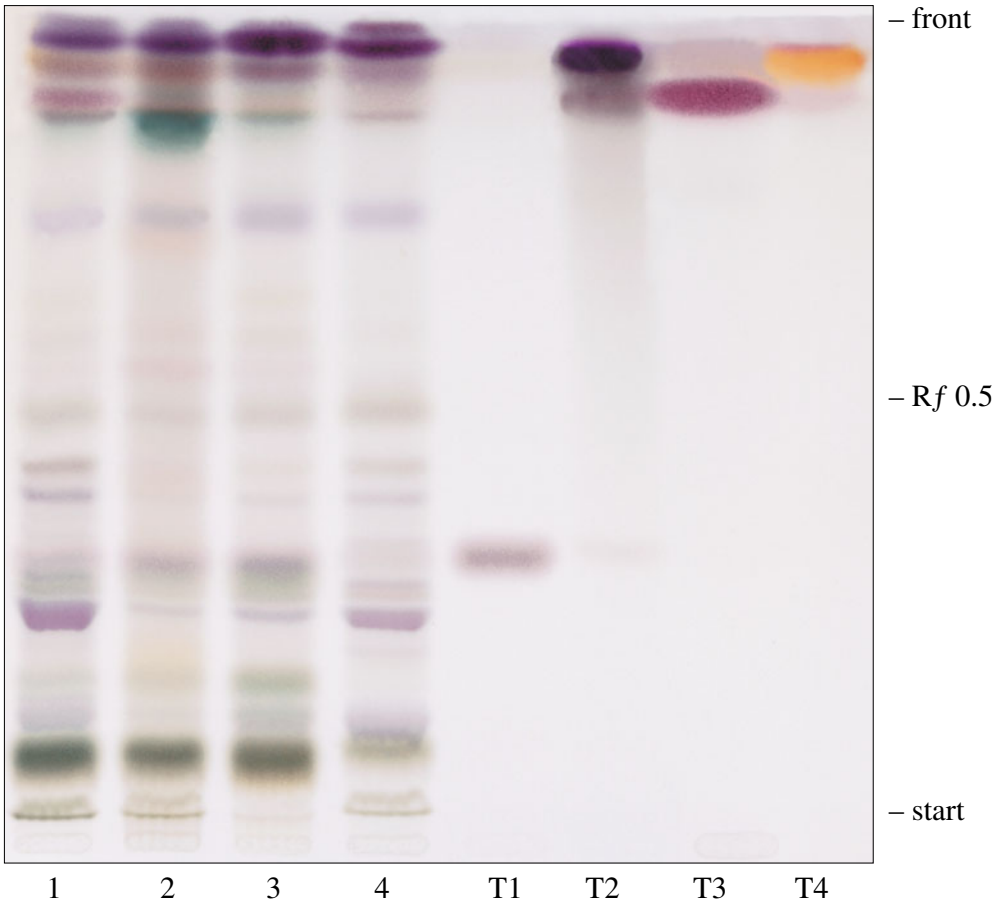

Fig. 2: TLC-fingerprint of Gastrodiae elatae rhizoma detected with vanillin-sulphuric acid-reagent in VIS

4) Description of the TLC-chromatogram:

All four samples of *Gastrodia elata* show at R*f* 0.10 the green-brownish zones of sucrose. The methanol extract of Gastrodiae rhizoma is characterized by gastrodin, a pink-violet zone at R*f* 0.34 (**T1**) and a second one at R*f* 0.27. The gastrodin contents in *Gastrodia elata* samples vary, depending on the seasons of collection and the regional provenance.
A light pink zone of 4-hydroxybenzylalcohol at R*f* 0.88 and an orange zone of 4-hydroxybenzaldehyd can be detected at R*f* 0.92. The darkpink zone at R*f* 0.94 can be assigned to β-sitosterin.

HPLC-fingerprint analysis:

1) Sample preparation: The extracts used for TLC are filtered over Millipore® (Type HV 0.45 µm).

2) Injection volume: 10 µl extract

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck, Hitachi
Column:	LiChroCART® 125–4 mm with LiChrospher® 100 RP 18 (5 µm), LiChroCART® 4–4 mm with LiChrospher® 100 RP 18 (5 µm); Merck
Solvent system:	A: 0,1 % phosphoric acid ; Acros Organics B: methanol for HPLC; Acros Organics
Gradient:	10 % – 90 % B in 16 min.
Flow rate:	1.0 ml/min.
Detection:	222 nm

Retention times and identity of the main peaks of Fig. 3a and 3b:

Peak	Rt (min.)	Compound
1	1.6	gastrodin
2	2.7	4-hydroxybenzylalcohol
3	9.2	4-hydroxybenzaldehyde
4	10.4	β-sitosterin

4) Description of the HPLC-chromatogram:

The HPLC-fingerprint of *Gastrodia elata* samples is characterized by the major peak of gastrodin at Rt 1.6 (**1**). The peak of 4-hydroxybenzylalcohol appears at Rt 2.7 (**2**) and the peak of 4-hydroxybenzaldehyde at Rt 9.6 (**3**). The peak of β-sitosterin is detected at Rt 10.4 (**4**).

The contents of the compounds vary depending on the season and provenance of collection.

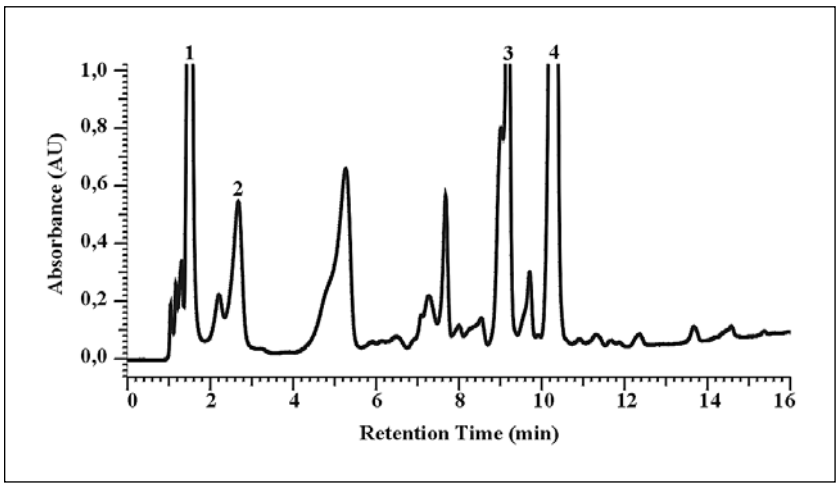

Fig. 3a: HPLC-fingerprint chromatogram of the methanol extract of *Gastrodia elata*, Shaanxi (drug sample 1):

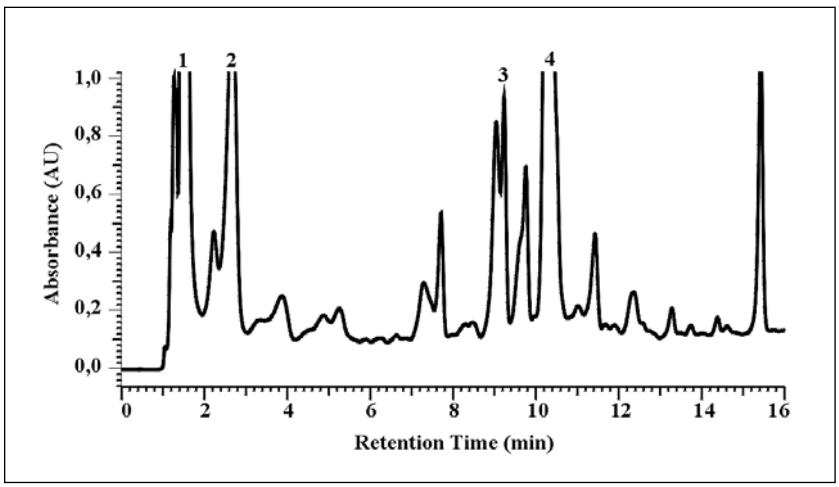

Fig. 3b: HPLC-fingerprint chromatogram of the methanol extract of *Gastrodia elata*, Si-chuan (drug sample 2):

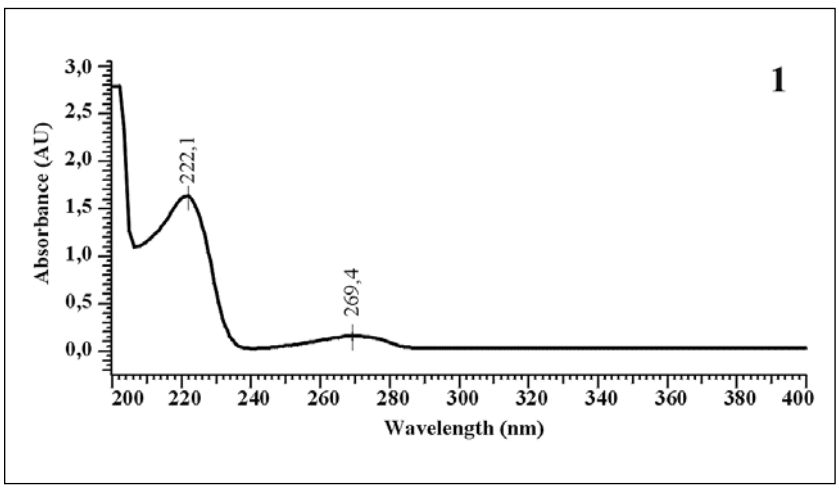

Fig. 4: online UV-spectrum of gastrodins

Note: According to the Chinese Pharmacopoeia 2005 Rhizoma Gastrodiae should contain not less than 0.20 % of gastrodin, calculated with reference to the dried drug.

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Herba Ecliptae
Mohanlian

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drug[2]: *Eclipta prostrata L.*
Synonyms: *Eclipta alba* Hassk., *Eclipta erecta L., Eclipta thermalis* Bunge, *Eclipta marginata* Boiss.
The drug is also known under the English name Yerbadetajo herb.

Origin[2]: China (provinces of Jiang Su, Jinag Xi, Zhe Jiang), Taiwan, Korea, India, Philippines and Japan

– Asteraceale –

Description of the drug[1]: White pubescent wholly.
Stems cylindrical, with longitudinal ridges, 2-5 mm in diameter; externally greenish-brown or dark green.
Leaves opposite, almost sessile, lamina crumpled and rolled or broken, when whole, long-lanceolate, margin entire or shallowly dentate, dark green.
Capitulum 2-6 mm in diameter.
Achenia elliptical and flattened, 2-3 mm long, brown or pale brown.
Odour, slight; taste, slightly salty.

Pretreatment of the raw drug[1]: The drug is collected at flowering from July to September, eliminated from foreign matters, washed briefly, cut into sections and dried in sunlight.

Medicinal use[1-5]: Infectious hepatitis, jaundice, liver cirrhosis, aching and weakness of the knees and loins, spitting of blood, epistaxis, hematuria and diarrhea with bloody stools, abnormal uterine bleeding.

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Effects and indications according to Traditional Chinese Medicine[1-3]

Taste:	sweet-sour
Temperature:	cold
Channels entered:	liver, kidney
Effects:	replenishes the liver, tonifies the kidney and stops bleeding by removing heat from the blood
Symptoms and indications:	dizziness, tinnitus, hematuria and diarrhoea with bloody stool due to heat in the blood, and impaired vision

Main constituents (see Fig. 1):

- **coumestanes:** wedelolactone, desmethylwedelolactone, desmethylwedelolactone-7-glucoside[6-8]
- **flavonoids:** apigenin, luteolin and apigenin-7-glucoside, luteolin-7-glucoside[9]
- **alkaloide:** nicotine ("ecliptine")[10]
- triterpenoid saponins (ecliptasaponins)[9]
- **sterine:** β-amyrin, stigmasterol, β-sitosterol[6]
- **long-chain alcohols:** hentriacontan-16-ol, heptacosan-14-ol[11]
- **thiophenacetylene:** α-terthienylmethanol (= terthienylcarbinol)[6,11]
- polyacetylenes[12]
- peptides[13]
- protocatechuic acid, 4-hydroxy-benzoic acid, isochlorogenic acids

Pharmacology:

- antihepatotoxic[7,14,15]
- antiphlogistic[7]
- immunosuppression antagonizing[15]
- antisnake bite (antimyotoxic, antihemorragic)[16]

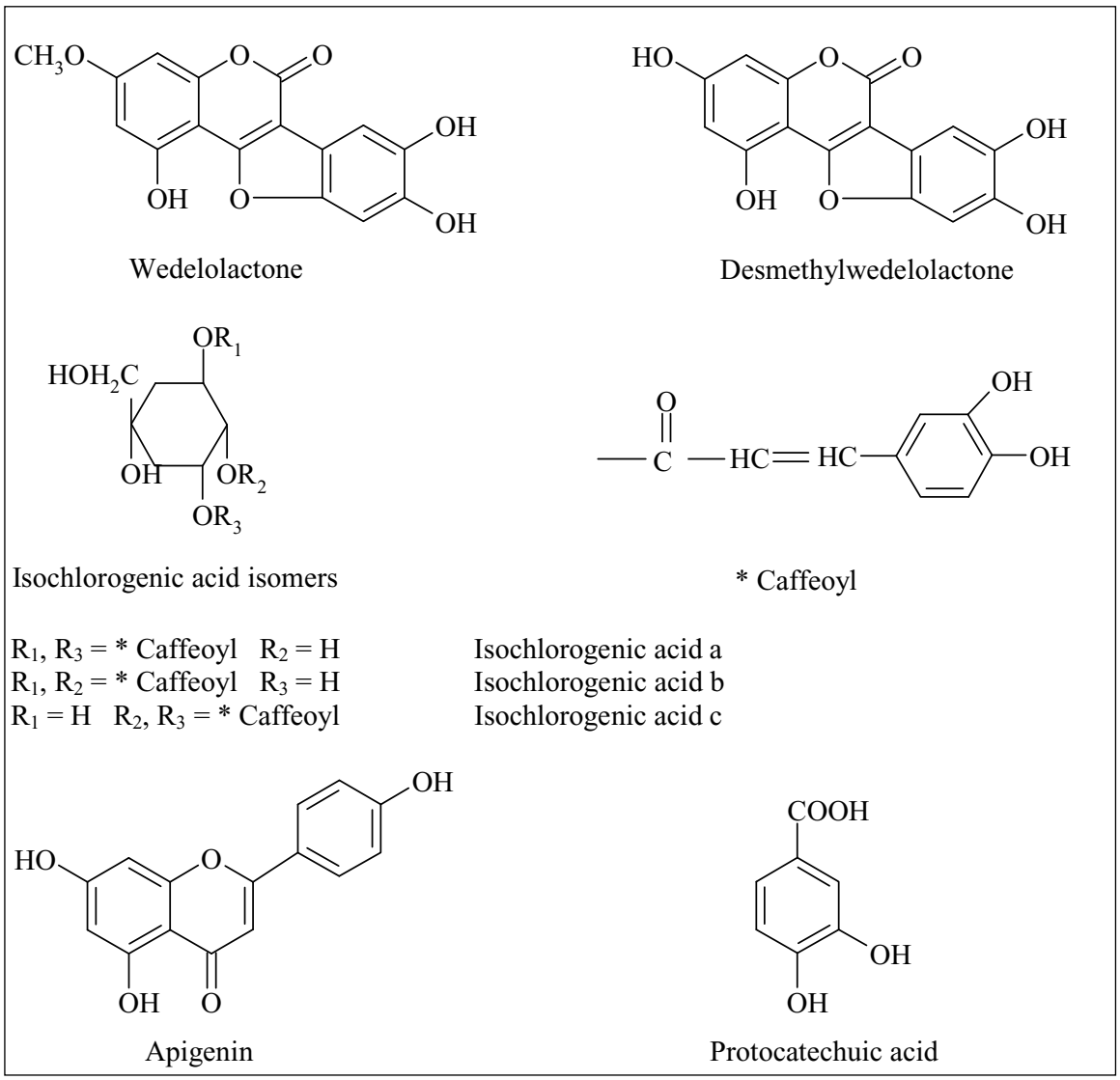

Fig. 1: Formulae of the main constituents[4]

TLC-fingerprint-analysis[5]:

1) Extraction: 5.0 g powdered drug are soxleth-extracted with 100 ml methanol for 1 hour and filtered. The extract is evaporated to dryness and the residue redissolved in 50 ml distilled water. The aqueous suspension is transfered to a separation funnel, 25 ml ethyl acetate are added and shaken. The ethyl acetate phase is evaporated to dryness and redissolved in 2 ml methanol.

2) Reference compounds: wedelolactone, desmethylwedelolactone, isochlorogenic acid, protocatechuic acid, ß-sitosterin and apigenin, each dissolved in methanol (1 mg/ml)

3) Separation parameters:

Applied amount:	30 µl extract, 10 µl standard solution
Plates:	Silicagel 60 F_{254}; Merck
Solvent system:	toluol-acetone-formic acid (11: 6: 1)
Detection:	Spray reagents: Natural product-polyethylenglycol reagent (NP/PEG): The plate is sprayed successively with a 1 % methanolic solution of diphenylboric acid-β ethyl-aminoester (NP) and a 5 % ethanolic polyethylenglycol-4000 solution (PEG). The evaluation is carried out in UV 365 nm. Iron-III-chloride solution: The plate is sprayed with a 10 % aqueous solution of iron-III-chloride. The evaluation is carried out in VIS.

Drug samples		**Origin**
1	Ecliptae herba/*Eclipta alba*	sample of commercial drug, China
2	Ecliptae herba/*Eclipta alba*	sample of commercial drug, China
3	Ecliptae herba/*Eclipta alba*	province Anhui, China
4	Ecliptae herba/*Eclipta alba*	province Jangtsui, China

Reference compounds		**R*f***
T1	wedelolactone and desmethylwedelolactone	0.57 and 0.47
T2	isochlorogenic acid mixture and protocatechuic acid	0.05/0.16 and 0.52
T3	apigenin	0.60

4) Description of the TLC-chromatogram Fig. 2 in UV 365 nm:

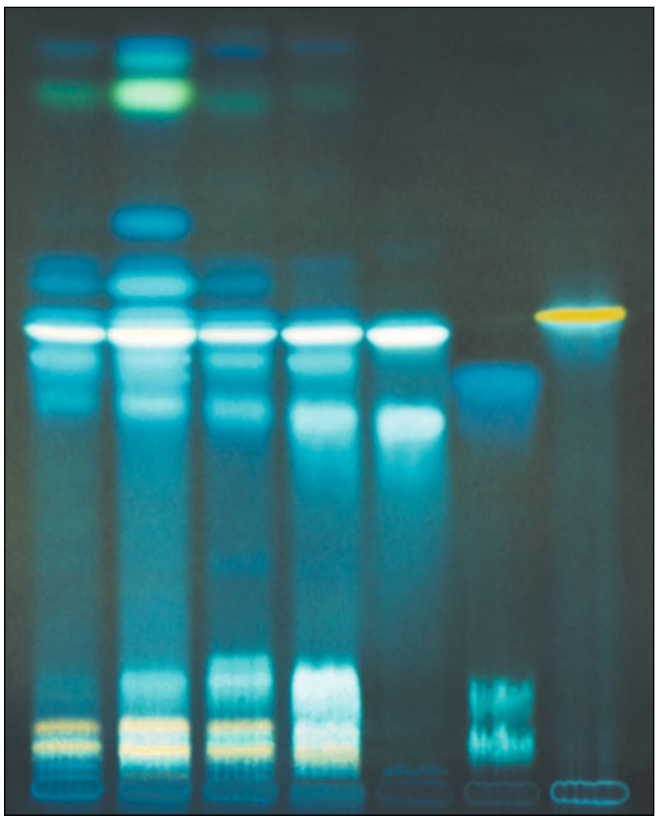

Fig. 2: TLC-fingerprint of Ecliptae herba extract detected with natural product-polyethylenglycol-reagent in UV 365 nm

With natural product-polyethylenglycol reagent the two main constituents wedelolactone (R*f* 0.57) and desmethylwedelolactone (R*f* 0.47), appear in UV 365 nm as white-blue zones. The isochlorogenic acid and its isomers appear as blue zones in the lower R*f*-range from 0.05 to 0.16. A dark blue zone of protocatechuic acid in very low concentration is visible at R*f* 0.52. In some samples of *Eclipta prostata* apigenin can be detected right above wedelolactone (R*f* 0.60).

Description of the TLC-chromatogram Fig. 3 in VIS:

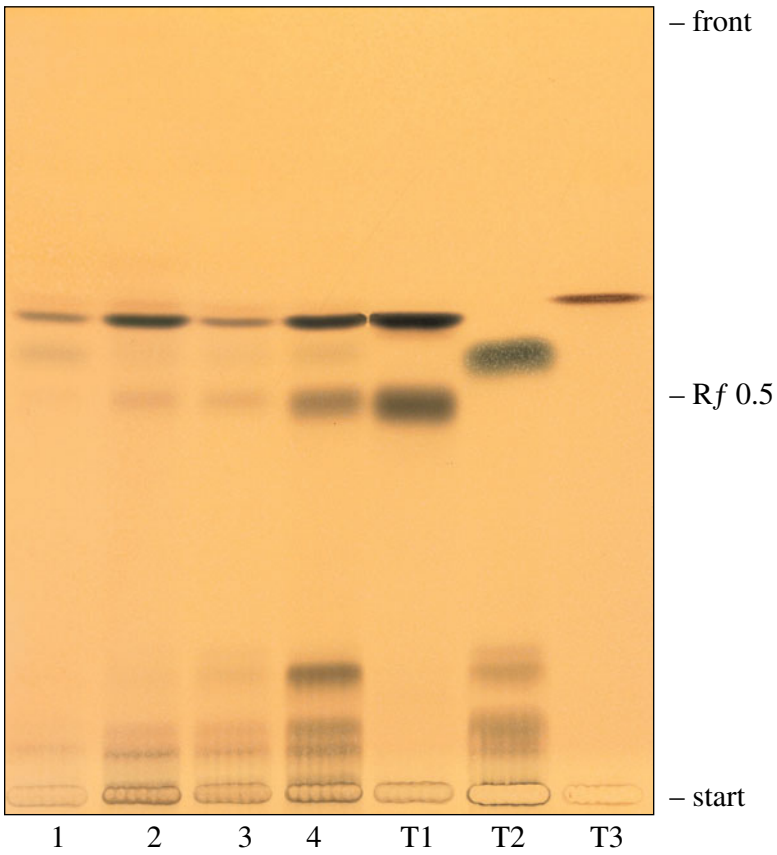

Fig. 3: TLC-fingerprint of Ecliptae herba extract detected with iron-III-chloride in VIS

With iron-III-chloride as reagent all phenolic componds appear with violet-brown colour and green-brown colour (coumestans) respectively.

HPLC-fingerprint analysis:

1) Sample preparation: The extracts used for TLC is filtered over Millipore® (Type HV 0.45 µm).

2) Injection volume: 20 µl extract

3) HPLC-data:

Apparatus: L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface, Merck Hitachi

Column: LiChroCART® 125 – 4 mm with LiChrospher® 100 RP 18 (5 µm), LiChroCART® 4 – 4 mm with LiChrospher® 100 RP 18 (5 µm), Merck

Solvent system:	A: water for HPLC, Acros Organics + 10 ml 0.1N H_3PO_4/l B: acetonitrile for HPLC, Acros Organics + 10 ml 0.1N H_3PO_4/l
Gradient:	15 % B to 40 % B in 25 min. (linear)
Flow rate:	1.0 ml/min.
Detection:	254 nm, respectively 350 nm

Retention times and identity of the main peaks of Fig. 4:

Peak	Rt (min.)	Compound
1	2.21	protocatechuic acid
2	8.73	isochlorogenic acid isomer
2´	9.94	isochlorogenic acid isomer
2´´	11.00	isochlorogenic acid isomer
3	12.07	desmethylwedelolactone
4	18.45	wedelolactone

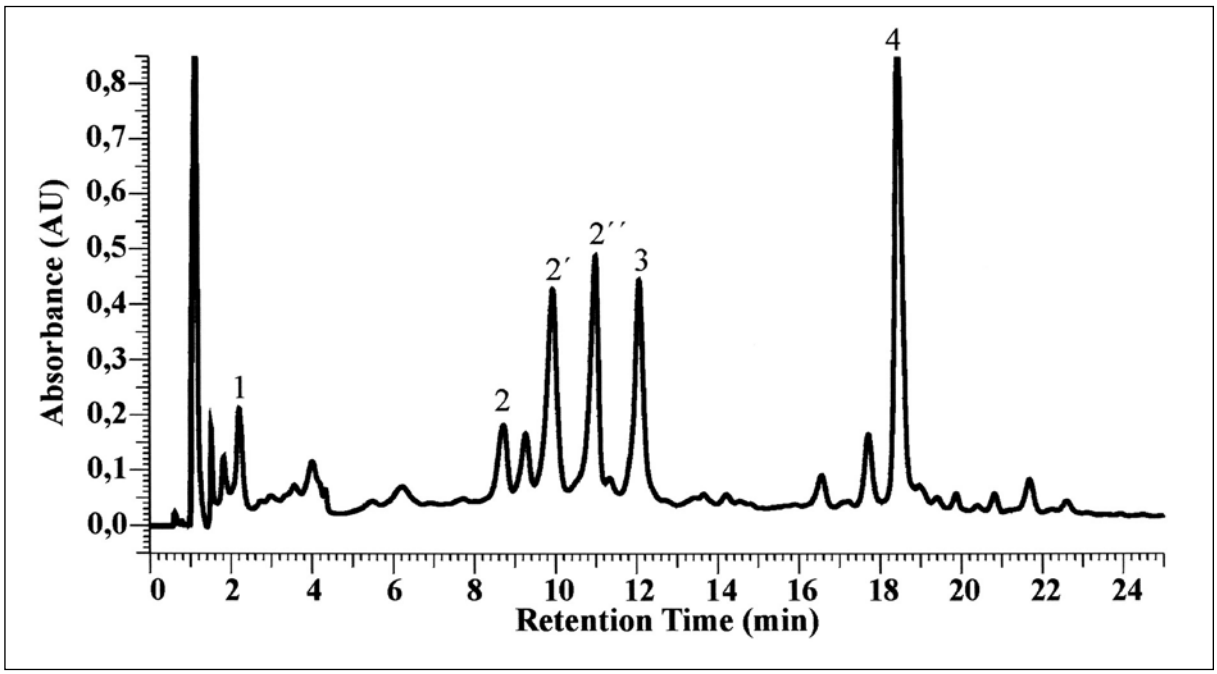

Fig. 4: HPLC-fingerprint of Ecliptae herba

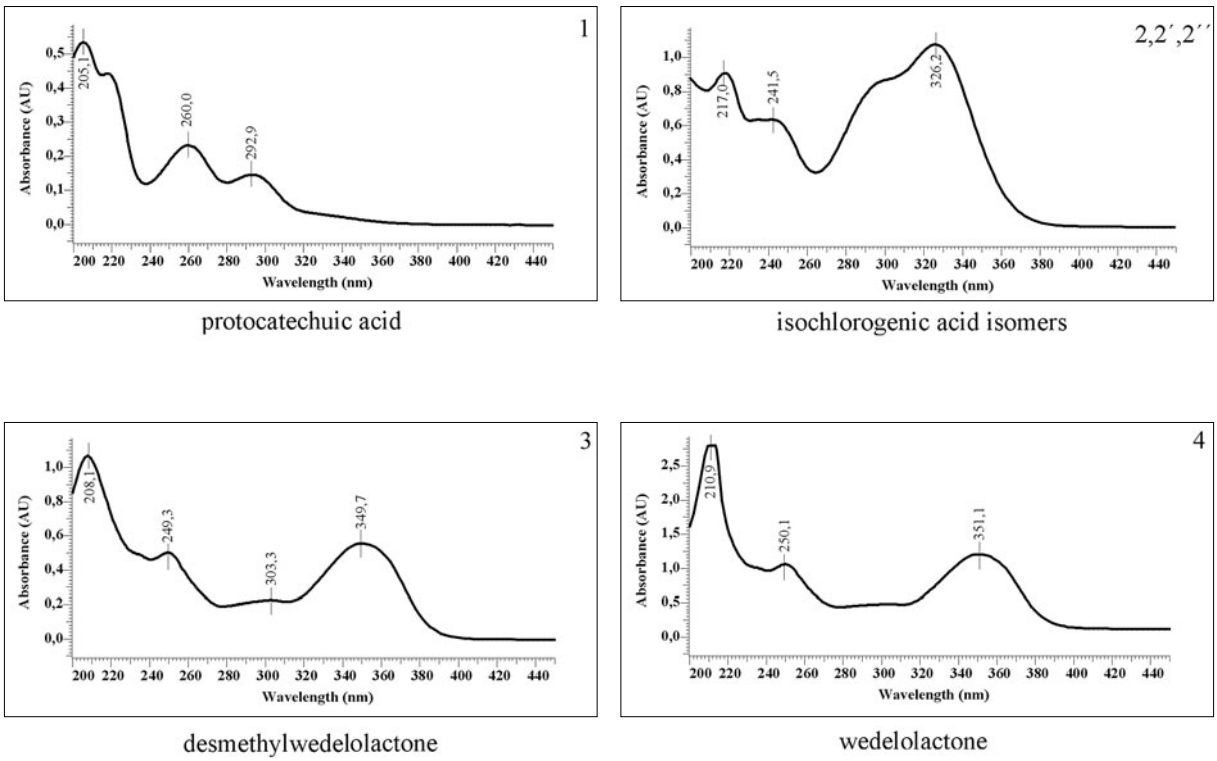

Fig. 5: online UV-spectra of the main constituents

4) Description of the HPLC-chromatogram:

The HPLC-fingerprints of all investigated samples are characterized by the prominent peak 4 of wedelolactone at Rt 18.45 and a sequence of four peaks between Rt 8.2 and 12.5 with desmethylwedelolactone peak (**3**) at Rt 12.07 and the isochlorogenic acid peaks (**2**, **2´**, **2´´**) at Rt 8.73, 9.94 and 11.00. Wedelolactone and desmethylwedelolactone are present in the Eclipta extracts in a concentration ratio of about 6 (8) : 1. A low concentrated peak (**1**) of protocatechuic acid appears at Rt 2.21.

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Herba Andrographis
Chuanxinlian

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005

Official drug[1]: Common Andrographis Herb is the dried aerial part of *Andrographis paniculata* (Burm. f.) Nees (Fam. Acanthaceae). The drug is collected in early autumn when foliage branch growing luxuriantly, sliced and dried in the sun.

Descripition of the drug[1]: Stems square and frequently branched, 50 – 70 cm long, nodes slightly swollen; texture fragile, easily broken. Leaves simple, opposite, short petioled or nearly sessile; lamina crumpled and easily broken, when whole, lanceolate or ovate-lanceolate, 3 – 12 cm long, 2 – 5 cm wide, with acuminate apex and cuneate-decurrent base, margin entire of undulate; the upper surface green, the lower surface greyish-green, glabrous on both surfaces. Odour, slight; taste, extremely bitter.

Pretreatment of the raw drug[1]: Foreign matters and legumes are eliminated, washed clean, cut into sections and dried.

Medicinial use[1,2]: Inflammations, hepatitis, febrile diseases, common cold, laryngitis, cough, diarrhoe, mastitis, externally carbuncles, sores and nodules

Effects and indications according to Traditional Chinese Medicine[1]

Taste:	extremely bitter
Temperature:	cold
Channels entered:	acts on the lung, stomach, large intestine and small intestine channels
Effects:	clears pathogenic heat, relieves depressed liver, removes dampness, alleviates pain and promotes diuresis
Symptoms and indications:	jaundice with hypochondriac distress, epigastric distensions and pain, acute and chronic hepatitis, mastitis

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Main constituents[2]**:**

- **diterpene lactones**:
  andrographolide, neoandrographolide, deoxy-didehydroandrographolide, deoxy-oxoandrographolide, deoxyandrographolide, dideoxyandrographolide (andrograpanin), andrographiside, deoxyandrographoside (andropanoside), deoxy-methoxyandrographolide
- **flavone derivatives:**
  oroxylin, wogonin, andrographidine A, B, C, D, E, F
- sesquiterpen lactones:
  paniculide A, B, C
- acidic polysaccharides PA, PB

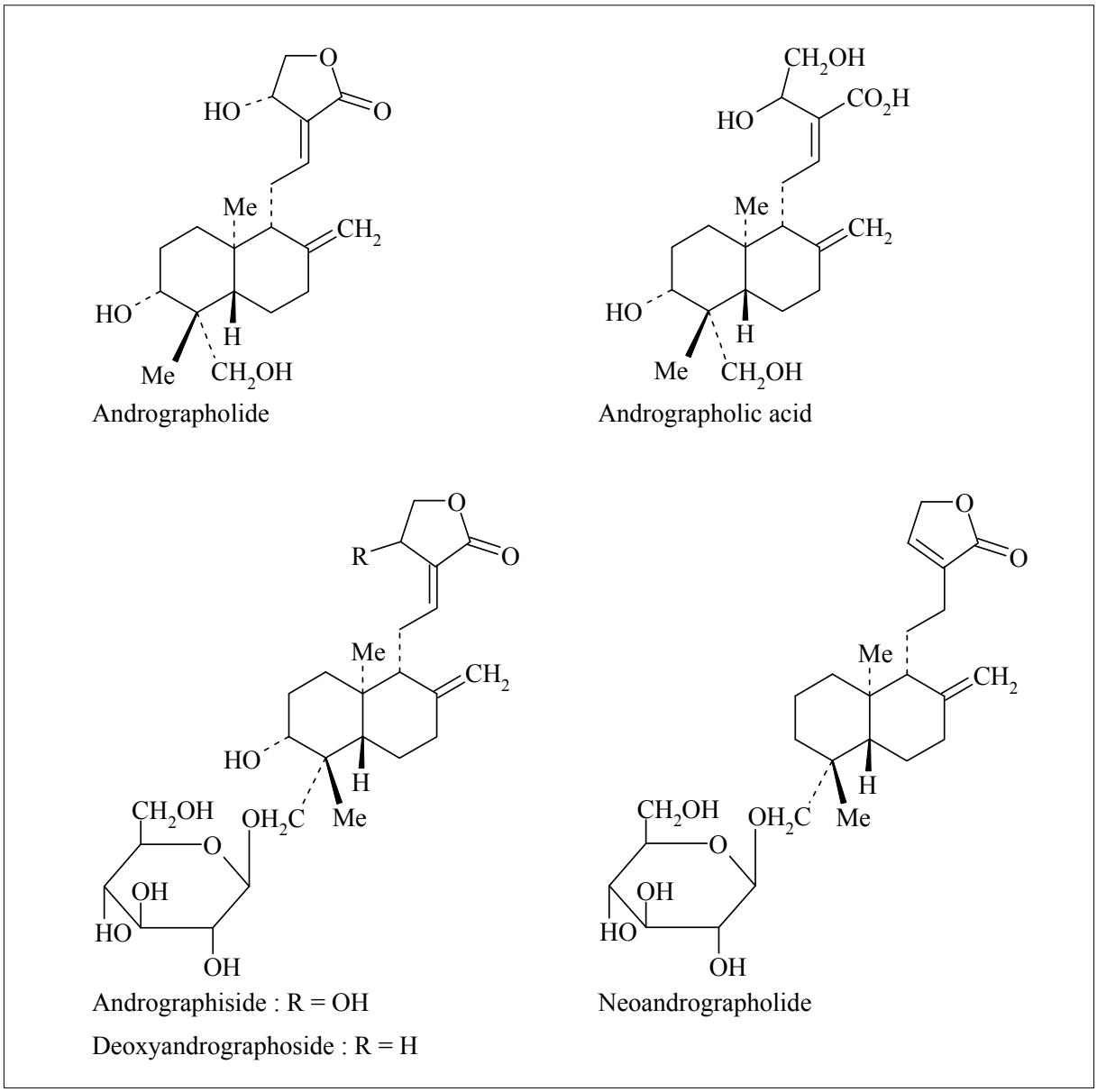
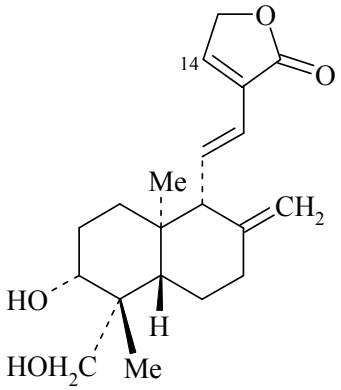
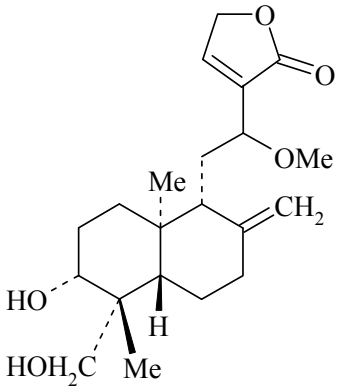


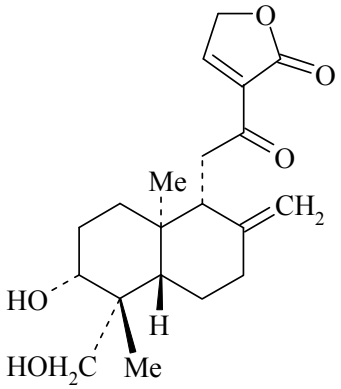
Fig. 1: Formulae of the main constituents



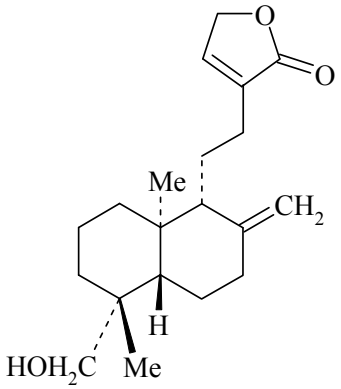
14-deoxy-11,12-didehydroandrographolide



Deoxy-methoxy-andrographolide



Deoxy-oxo-andrographolide



Dideoxy-andrographolide

Pharmacology: (in vitro/in vivo)

extracts, andrographolide and derivatives:

– antiinflammatory [3]
– antihepatoxic and liverprotective [4,5]
– NO inhibitory in macrophages (neoandrographolide) [6]
– superoxide scavenging effect [7,8]
– antihyperglycemic [9,10]
– antithrombotic [11,12]
– hypotensive [13]
– immunstimulatory [14]

Clinical trial: Common cold [15]

TLC fingerprint analysis

1) Extraction: 0.5 g of the powdered drug is macerated for 30 minutes with 30 ml 96 % ethanol. Afterwards the macerate is ultrasonicated for 30 minutes, filtered and the residue washed thrice with 10 ml of ethanol 96 %. The washings are combined to the filtrate and the total solution evaporated to dryness. The residue is dissolved in a small amount of ethanol 96 %, transferred to a 5 ml volumetric flask and filled up to the 5 ml mark with ethanol 96 %.

2) Reference compound: andrographolide (T 1): 1 mg is dissolved in 1 ml 96 % ethanol

3) Separation parameters:

Plates:	Silica gel F_{254} Merck
Applied amounts:	Andrographis herba-ethanol-extract: each 25 µl reference compound: 20 µl
Solvent system:	chloroform : ethyl acetate : methanol 4 3 0.4
Detection:	Vanillin-sulphuric acid reagent: Solution I: 1 % ethanolic vanillin solution Solution II: 50 % ethanolic sulphuric acid The plate is intensively sprayed with 10 ml solution I followed immediately by 10 ml solution II. Afterwards the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

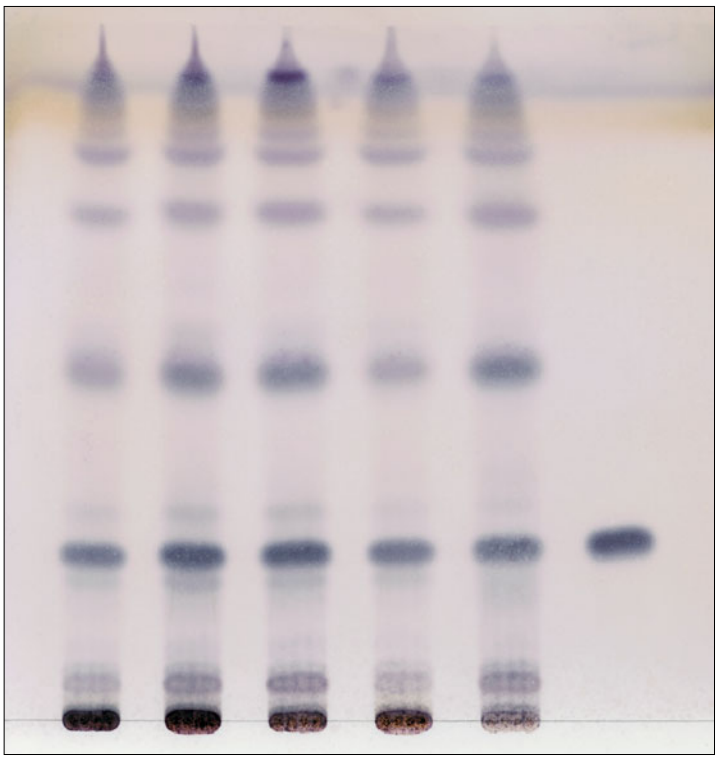


Fig. 2: Thin layer chromatogram of ethanolic extracts of Andrographis herba after spraying with vanillin-sulphuric acid reagent in VIS

Drug samples		Origin
1	Andrographis herba	province Fujian, China
2	Andrographis herba	sample of commercial drug, China
3	Andrographis herba	sample of commercial drug, China
4	Andrographis herba	sample of commercial drug, China
5	Andrographis herba	sample of commercial drug, China

Reference compound		R*f*
T 1	andrographolide	0.25

4) Description of the TLC-chromatogram:

The chromatograms of all investigated samples of *Andrographis* extracts show a very homogeneous pattern of six violett grey zones at R*f* = 0.06, 0.25, 0.50, 0.73, 0.81 and R*f* = 0.92. The most prominent are andrographolide (R*f* = 0.25) and 14-deoxy-11,12-didehydroandrographolide (R*f* = 0.50). The zone with the R*f* = 0.06 is one of the diterpenglucosides (andrographiside, neoandrographolide or andrographolic acid).

HPLC-fingerprint analysis:

1) Sample preparation: The same extract, used for the TLC, is filtered over Millipore® filtration unit type 0.45 µm and injected into the HPLC.

2) Injection volume: 20.0 µl extract

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
Solvent:	A: dist. water (Acros Organics) B: methanol (Acros Organics)
Gradient:	40 – 60 % B in 5 minutes 60 % B in 10 minutes 60 – 100 % B in 5 minutes total runtime: 20 minutes
Flow rate:	0.7 ml/min
Detection:	229 nm

Retention times of the main peaks:

peak	Rt (min.)	compound
1/2	1.2 – 1.6	andrographiside, neoandrographolide or andrographolic acid
3	6.9	14-deoxy-andrographolide
4	9.9	andrographolide
5	15.1	14-deoxy-11,12-didehydroandrographolide

4) Description of the HPLC chromatogram:

The chromatograms are characterized by the dominant andrographolide peak **4** at Rt = 9.9. Peak **1** and **2** at Rt = 1.2 and 1.5 can be assigned to andrographiside, neoandrographolide or andrographolic acid, whereas peak **5** (Rt = 15.1) must be identical with 14-deoxy-11,12-didehydroandrographolide. Peak **3** (Rt = 6.9), which shows an UV-spectrum superimpossible to andrographoside, could be 14-deoxy-andrographolide.

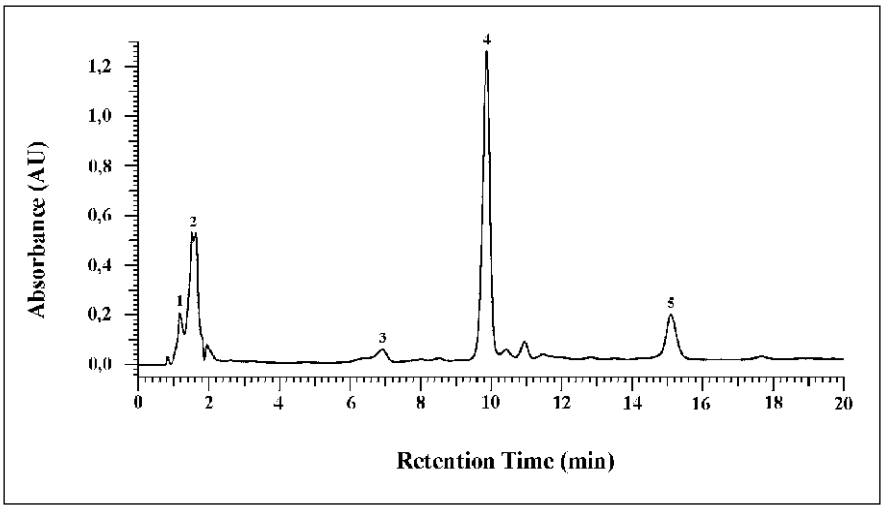


Fig. 3: HPLC-fingerprint chromatogram of Andrographis herba

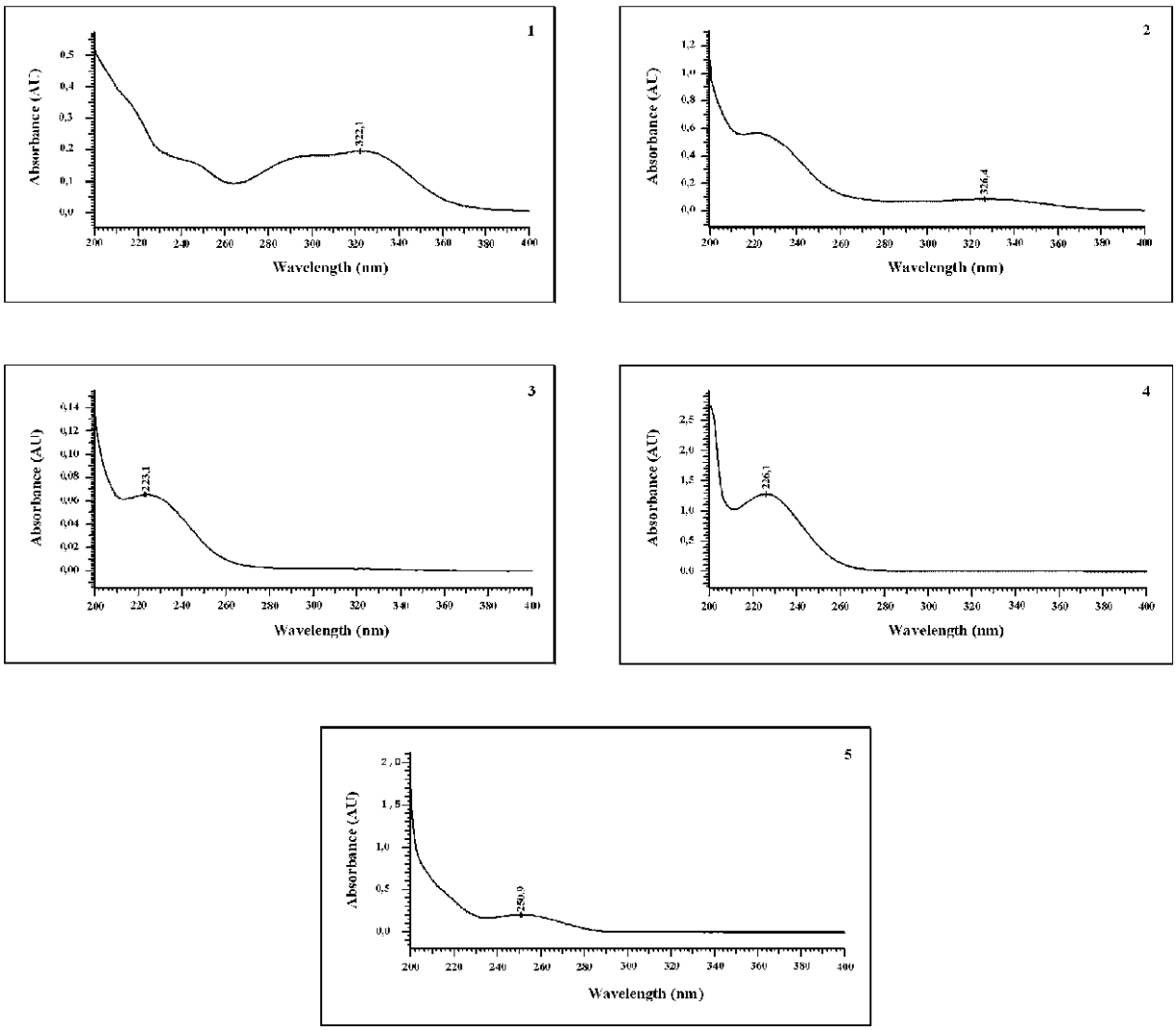


Fig. 4: UV-spectra of the main compounds (peak) of Andrographis herba

Note: According to the Pharmacopoeia of the People's Republic of China, English Edition, 2000 and 2005 Andrographis herba should contain not less than 0.80 % of total amount of andrographolide and dehydroandrogrpholide, calculated on the dried raw drug.

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Radix Paeoniae albae/rubrae
Baishao/Chishao

Pharmacopoeias: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[(1)]
Japanese Pharmacopoeia, English Edition, 1986 [(2)]

Official drug[(1)]:

Paeoniae albae radix (White Peony Root):
Paeonia lactiflora Pall. (Fam. Ranunculaceae / Paeoniaceae)
The drug is collected in summer and autumn, washed clean, removed from root stock, the lower part and rootlet, either peeled after boiling in water or boiled after peeling, and dried in the sun.

Paeoniae rubrae radix (Red Peony Root):
Paeonia lactiflora Pall. or *Paeonia veitchii* Lynch
The drug is collected in spring and autumn, removed from rhizome, rootlet and dried in the sun.

Description of the drug[(1)]:

Paeoniae albae radix:
Cylindrical, straight or slightly curved, two ends truncate, 5 – 18 cm long, 1 – 2.5 cm in diameter. Externally whitish or pale reddish-brown, glossy or with longitudinal wrinkles, rootlet scars and occasional remains of brown cork. Texture compact, uneasily broken, fracture relatively even, whitish or pale brownish-red, cambium ring distinct and rays radial. Odour, slightly; taste, slightly bitter and sour.

Paeoniae rubrae radix:
Cylindrical, somewhat curved, 5 – 40 cm long, 0.5 – 3 cm in diameter. Externally brown, rough, longitudinally furrowed and wrinkled, and showing rootlet scars and transversely prominent lenticels, sometimes the outer bark easily exfoliated. Texture hard and fragile, uneasily broken, fracture chalk-white or pink, bark narrow, wood with distinct radial striations, sometimes with clefts. Odour, slightly; taste, somewhat bitter, sour and adstringent.

Pretreatment of the raw drug[(1)]:

Paeoniae albae radix:
Washed, softened thoroughly, cut into thin slices, and dried.
When frying, the drug is stirred constantly on a homogeneous fire under control of the frying temperature, duration and extent of individual drugs.

Simple stir-frying:
The cleaned crude drugs are placed in a pot, stirred-fry with gentle heat until it becomes yellowish, taken out and cooled. For the crude drugs which should be fried to charring, the drug is fried at a relatively high temperature until the surfaces of the crude drugs are turning brown and the fractures become darkened, then they are taken out and cooled.

Stir-frying with wine:
The cleaned crude drugs are mixed with wine thoroughly in a closed vessel until it is infused completely. Then the drugs are placed in a pot and roasted with gentle heat until they become yellowish, then they are taken out and cooled. Unless otherwise specified, 10 kg of yellow rice wine are used for each 100 kg of clean crude drugs.

Paeoniae rubrae radix:
The foreign matters are eliminated, graded according to size, washed clean, softened thoroughly, cut into slices, and dried.
They occur in cylindrical slices, 0.5 – 3 cm in diameter, 0.3 – 0.5 cm thick, cut surface yellowish-white or pink.

Medicinal use[1,3,4,5,6]: Paeoniae albae radix:
Internally as antispasmodic drug, for the treatment of menstrual disorder, dysentery, stomach-, liver and intestine disorders, spontaneous perspiration, perspiration in the night, vertigo, hyperactivity of the liver, dizziness.

Effects and indications according to Traditional Chinese Medicine[1]

Taste:	sour, bitter
Temperature:	neutral with cold tendency
Channels entered:	spleen, liver (orbis hepaticus)
Effects:	subdues hyperactivity of the liver and relieves pain, nourishes blood, regulates menstruation, checks excessive perspiration
Symptoms and indications:	headache and dizziness, costal and abdominal pain, spasmodic pain of the limbs, anemia, menstrual disorders, spontaneous sweating and night sweating

Paeoniae rubrae radix:

Internally for the treatment of stomach-, intestine and liver diseases, ingestion, intestine infection and gynecological disorders, inflammation of the eye, pain in the chest and costal regions, boil, sore, traumatic injuries.

Effects and indications according to Traditional Chinese Medicine[1]

Taste:	bitter
Temperature:	neutral with cold tendency
Channels entered:	spleen, stomach
Effects:	removes heat from blood, eliminates blood stasis, relieves pain
Symptoms and indications:	maculation in epidermic diseases, spitting of blood, epistaxis, inflammation of the eye, pain in the chest and costal regions, amenorrhea, dysmenorrhea, mass formation in the abdomen, traumatic injuries, boils and sores

Main constituents[7,8]: Paeoniae albae radix, Paeoniae rubrae radix:

- **monoterpene glucosides:**
  paeoniflorin, albiflorin, oxypaeoniflorin, benzoylpaeoniflorin, benzoyloxypaeoniflorin, β-pinen-10-yl-β-vicianoside and lactiflorin
- **monoterpenes:**
  paeoniflorigenone, paeonilactones A, B and C
- gallotannins
- acidic polysaccharide peonan

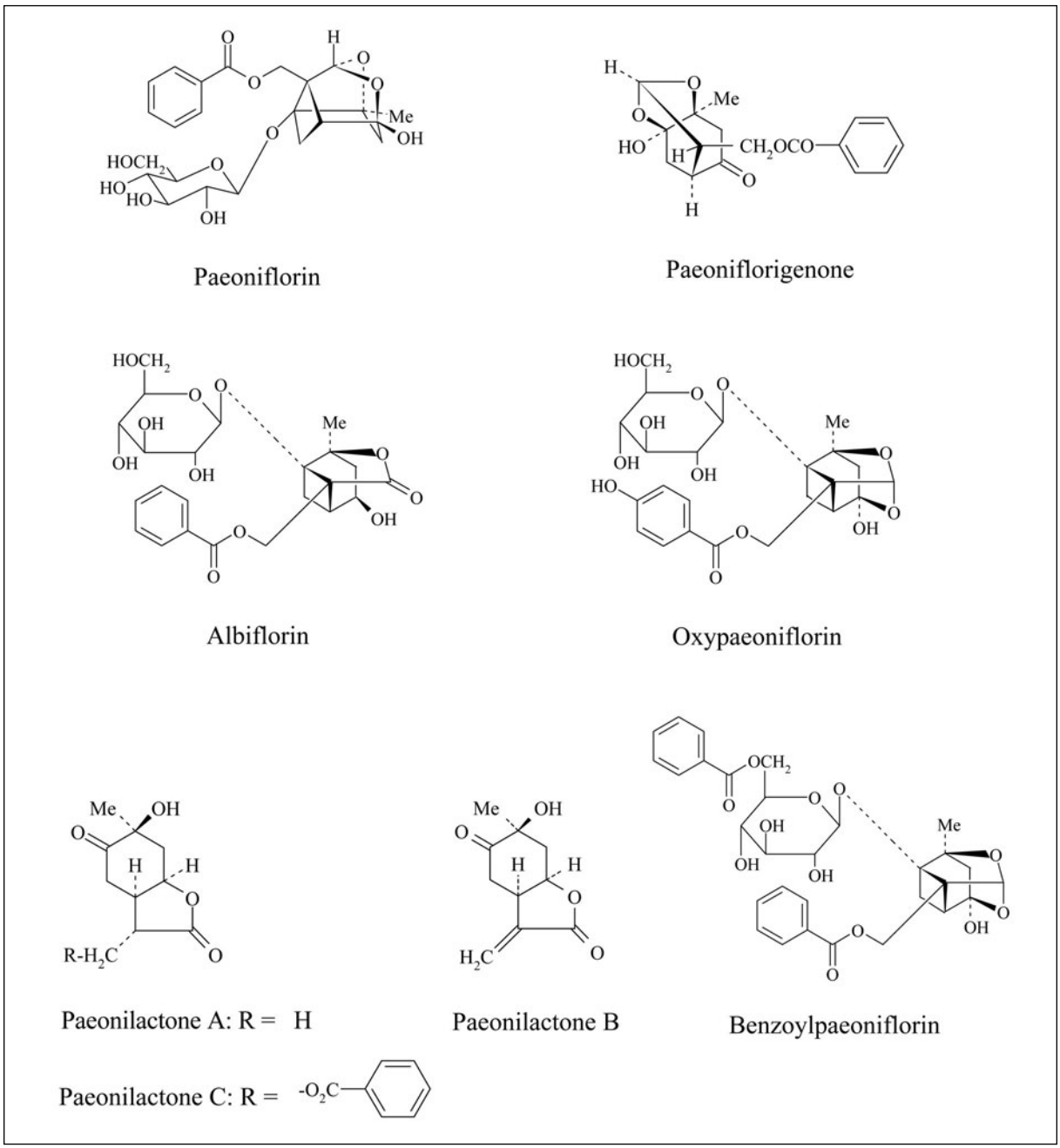

Fig. 1: Formulae of the main constituents

Pharmacology[9]:

Paeonia root extract:

– antioxidant
– imunostimulating activity (polysaccharide)[10]
– analgesic effect[11]
– apoptosis inducing effect[12]
– protective effect on endothelial cells[13]
– antithrombotic effect[14]
– anticoagulant[15]
– antihyperglycemic effect[16]
– hepatoprotective[17]
– antifibrinolytic[18]

Paeoniflorin:

– antispasmodic[4]
– antiinflammatory
– preventive effect on stress ulcer
– hypotensive
– inhibitory effect on plasminogen and plasmin

TLC fingerprint analysis

1) Extraction: 1.0 g pulverised drug is soxhlet-extracted with 50 ml ethanol 95 % for 1 hour. The extract is evaporated to dryness and the residue redissolved in 10 ml ethanol 95 %.

2) Reference compounds: paeoniflorin (T1): 1 mg is dissolved in 1 ml ethanol 96% oxypaeoniflorin (T2) and albiflorin (T3): 2 mg are dissolved in 1 ml ethanol

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: Paeonia alba-ethanol-extract: each 50 µl
Paeonia rubra-ethanol-extract: each 50 µl
reference compound: each 50 µl

Solvent system: chloroform – ethyl acetate - methanol – formic acid (40 + 5 + 15 + 0.2)

Detection: a) Vanillin-sulphuric acid reagent:
Solution I: 1 % ethanolic vanillin solution
Solution II: 50 % ethanolic sulphuric acid

The plate is intensively sprayed with 10 ml solution I followed immediately with 10 ml solution II. Afterwards the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

b) UV 254 nm

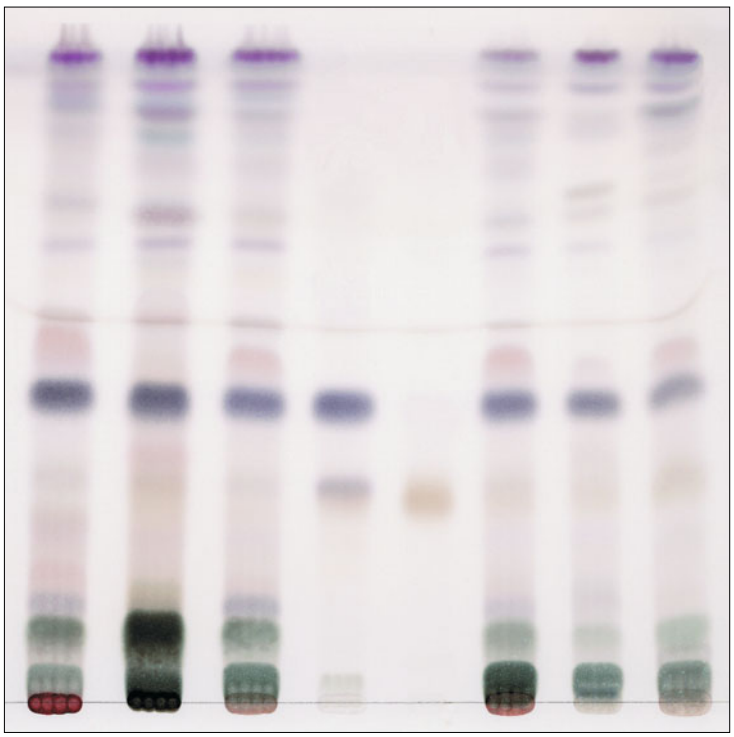

Fig. 2a: Thin layer chromatogram of ethanolic extract of Paeoniae rubrae radix and Paeoniae albae radix after spraying with vanillin-sulphuric acid reagent (VIS)

Drug sample		**origin**
1	Paeoniae rubrae radix	sample of commercial drug, China
2	Paeoniae rubrae radix	sample of commercial drug, China
3	Paeoniae rubrae radix	sample of commercial drug, Inner Mongolia
4	Paeoniae albae radix	sample of commercial drug, Japan
5	Paeoniae albae radix	sample of commercial drug, China
6	Paeoniae albae radix	province Anhui, China

Reference compounds		**R*f***
T1	paeoniflorin	0.44
T2	oxypaeoniflorin	0.32
T3	albiflorin	0.30

4) Description of the TLC-chromatogram:

Fig. 2a: Paeonia rubra root samples 1 – 3 show the dominant blue grey zone of paeoniflorin at R*f* = 0.44, oxypaeoniflorin and albiflorin as weak grey zones at R*f* = 0.32 and R*f* = 0.30 respectively. In the upper part from R*f* 0.65 to R*f* 0.95 6 – 8 violet zones can be detected.

In the lower R*f*-range between R*f* = 0.03 and R*f* = 0.1 appear 3 – 4 grey and green coloured zones. Paeonol, which has a R*f*-value of 0.9, could not be detected.

Paeonia alba root (4 - 6) shows about the same chromatographic pattern of zones as Paeonia rubra root. According to the lower content of paeoniflorin of the official drug (not less than 0.8 %) the zone of paeoniflorin is of lower intensity compared with that of Paeonia rubra root (not less than 3.8 %).

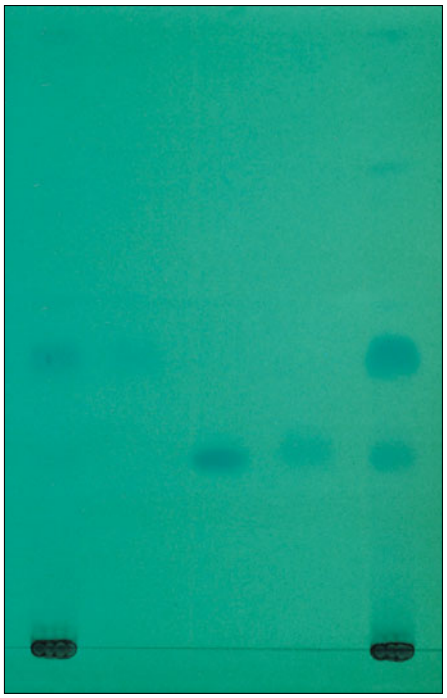

Fig. 2b: Thin layer chromatogram of ethanolic extract of Paeoniae rubrae radix and Paeoniae albae radix (UV 254 nm)

Drug samples		**origin**
3	Paeoniae rubrae radix	sample of commercial drug, Inner Mongolia
4	Paeoniae albae radix	sample of commercial drug, Japan
Reference compounds		**R*f***
T 1	paeoniflorin	0.44
T 2	oxypaeoniflorin	0.32
T 3	albiflorin	0.30

Fig. 2b: Both Paeonia root-extracts show the bluegreen zone of paeoniflorin at R*f* 0.44 and oxypaoeniflorin (albiflorin) at R*f* 0.31. In the upper part 3 – 4 zones appear more pronounced in Paeoniae rubrae radix in comparison to those of Paeoniae albae radix.

HPLC-fingerprint analysis:

1)	Sample preparation:	The ethanol extract, used for TLC, is filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC.
2)	Injection volume:	Paeoniae albae radix/Paeoniae rubrae radix extract: each 10 µl
3)	HPLC parameter:	
	Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
	Separation column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
	Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
	Solvent:	A: 33 µl H_3PO_4 / l dist. water (Acros Organics) B: 33 µl H_3PO_4 / l acetonitrile (Acros Organics)
	Gradient:	12 – 15 % B in 8 minutes 15 – 35 % B in 5 minutes 35 – 40 % B in 7 minutes 40 – 95 % B in 5 minutes total runtime: 25 minutes
	Flow rate:	1.0 ml/min.
	Detection:	235 nm

Retention times of the main peaks:

peak	Rt (min.)	compound
1	0.8	not identified
2	1.2	not identified
3	6.4 / 6.6	oxypaeoniflorin / albiflorin
4	8.0 / 8.2	paeoniflorin
5 / 6 / 7	14.0 – 17.0	benzoyl- and other (oxy)paeoniflorin derivatives
8	18.0 – 19.2	

4) Description of the HPLC chromatograms (Fig. 3a + 3b):

The HPLC-pattern of *Paeonia alba* and *Paeonia rubra* root extracts are characterized by the paeoniflorin peak at Rt = 8.0 / 8.2 (**4**), the oxypaeoniflorin / albiflorin peak at Rt = 6.4 / 6.6 (**3**), a strong peak at Rt = 1.2 (**2**) and a sequence of several peaks of benzoyl- and other (oxy)paeoniflorin derivatives between Rt = 14.0 and 20.0.

Paeonia rubra extract can be discriminated from *Paeonia alba* extract by a much higher concentration of paeoniflorin (**4**) and the compounds in the 14.0 – 17.0 Rt-range (**5,6,7**).
In contrast to the HPLC of *Paeonia rubra, Paeonia alba* shows a higher concentration of oxypaeoniflorin (albiflorin) (**3**).

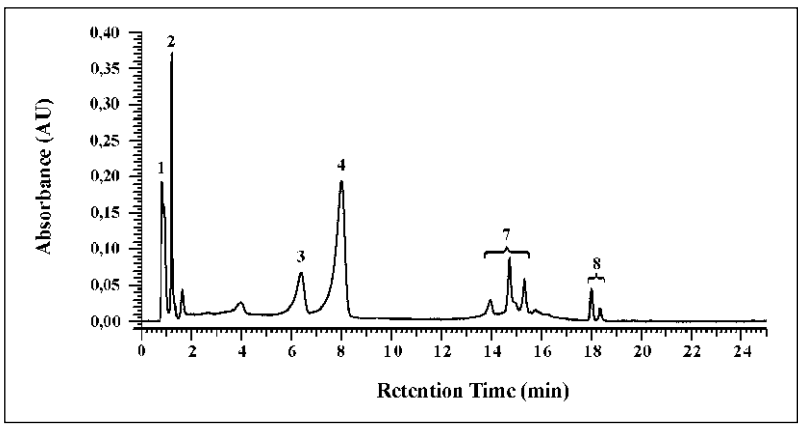

Fig. 3a: HPLC-fingerprint chromatogram of Paeoniae albae radix

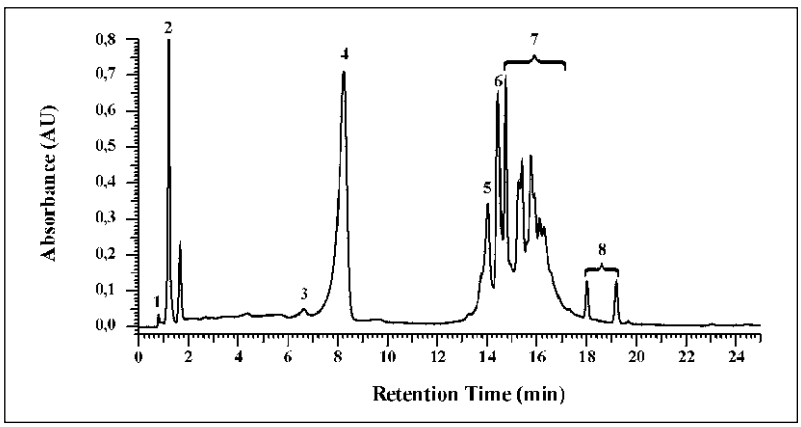

Fig. 3b: HPLC-fingerprint chromatogram of Paeoniae rubrae radix

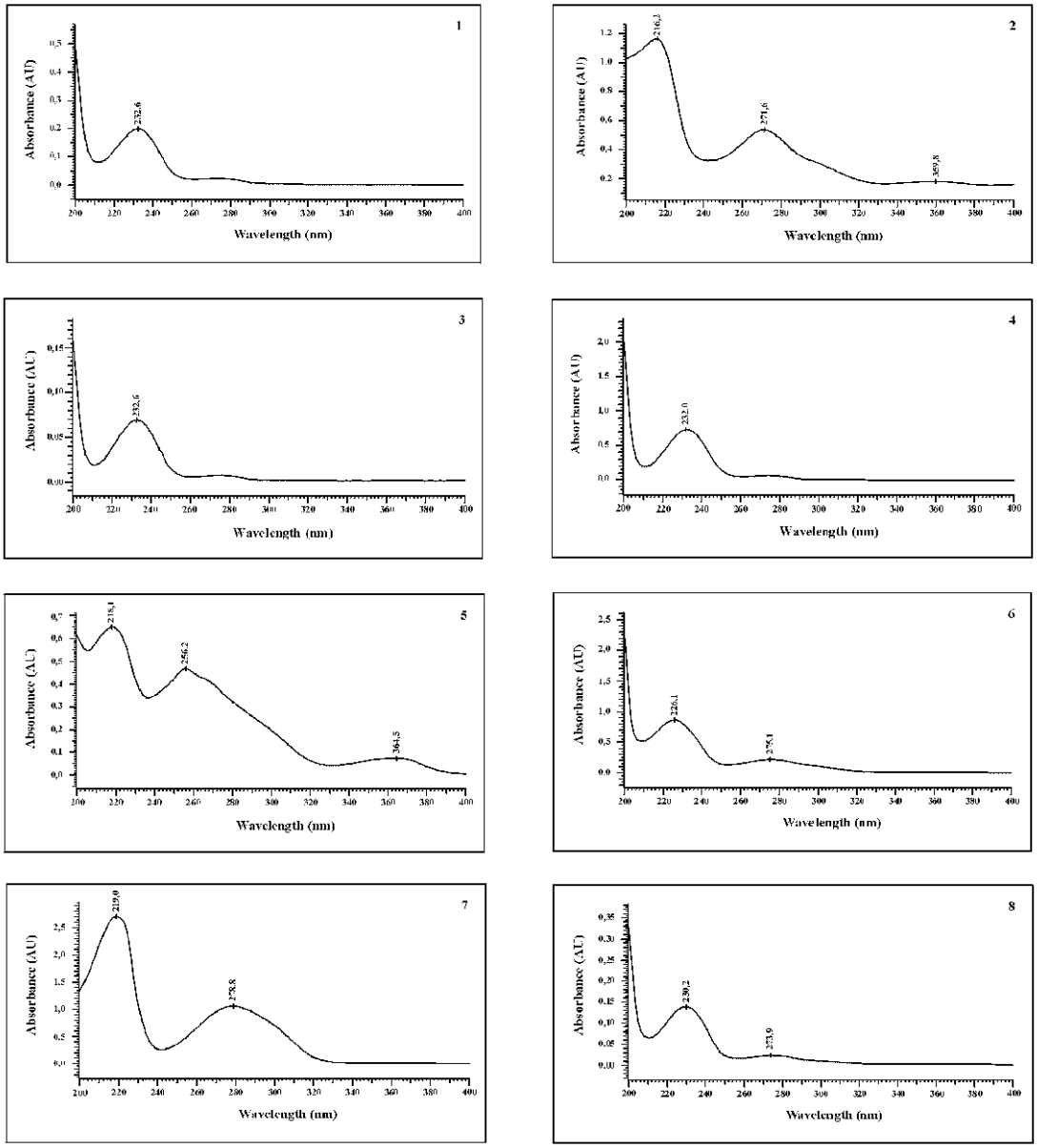

Fig. 4: UV-spectra of the main compounds (peaks) of Paeoniae albae radix and Paeoniae rubrae radix extracts

Note: The TLC and HPLC-chromatograms confirm that *Paeonia alba* and *Paeonia rubra* extracts differ from each other only in the quantitative chemical composition of the major compounds.

For further chromatographic methods to characterize the chemical composition of *Paeonia albiflora* (*Paeonia veitchii*) root see also references 19 and 20.

Note: According to the Chinese Pharmacopoeia 2005 Radix Paeoniae albae should contain not less than 1.6 % paeoniflorin and Radix Paeoniae rubrae not less than 1.8 % paeoniflorin, calculated with reference to the dried drug.

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Flos Sophorae
Huaimi / Huaihua

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005

Official drug[1]: Sophorae flos immaturus and Sophorae flos from *Sophora japonica* L. (Fabaceae)
The drug is collected in summer when flower buds are forming or at flowering, dried in time and removed from branch, pedicel and foreign matters. The former is known as „Huaimi" and the later as „Huaihua".

Origin[1]: China (primarily in the provinces Guangdong, Guangxi, Shandong), Japan, North Vietnam

Description of the drug[1]:

Huaimi (flower buds):
Ovoid and ellipsoidal, 2 ~ 6 mm long, about 2 mm in diameter. Calyx with several longitudinal striations at the lower part. Petals yellowish-white, unflowering, occurring above the calyx. Pedicels slender. Texture light, broken after rubbing. Odourless; taste, slightly bitter and adstringent.

Huaihua (flowers):
Crumpled and rolled, petals mostly fallen off. When whole, calyx campanulate, yellowish-green, 5-lobed at the apex. Petals 5, yellow or yellowish-white, 1 larger, subrounded, the apex retuse, the other 4 oblong. Stamens 10, 9 accreted at the base. Filaments slender. Pistil cylindrical, curved. Texture light. Odourless, taste slightly bitter.

Pretreatment of the raw drug [1]:

Flos Sophorae (Huaimi):
Foreign matters are eliminated.

Flos Sophorae (Chaohuaimi: qingchao-method; stir-baked):
Clean crude drug is placed in a pot, stir-baked with gentle heat until a dark yellow colour is produced externally, then taken out and cooled. The crude drugs which should be baked to charring are baked at a relatively high temperature until the surfaces of the crude drugs turn brown and the fractures become darkened, then taken out and cooled. For the crude drugs inflammable on baking to char, a small amount of

water may be sprayed on the crude drugs, then the crude drugs are baked to dryness or dried in the sun.

Flos Sophorae (Huaimitan: chaotan-method; carbonized):
The nature of the crude drugs should be preserved and prevented from ashing. The clean crude drug is placed in a hot pot and stir-baked at a high temperature until a charred brown colour is produced externally. A small quantity of water is sprayed, taken out and dried in the air.

Medicinal use[2,3]: various external and internal hemorrhagic diseases (e.g. retinopathia), ulcerative colitis, cerebral thrombosis, hypertension

Effects and indications according to Traditional Chinese Medicine[1,2]

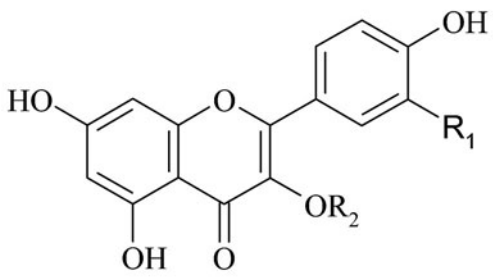
Taste:	bitter
Temperature:	neutral, with cold tendency
Channels entered:	liver, colon
Effects:	arrest bleeding by reducing heat in blood, quench excess fire in the liver
Symptoms and indications:	hematochezia, hemorrhoidal bleeding, dysentery with bloody stools, abnormal uterine bleeding, spitting of blood, epistaxis, redness of the eyes, headache and dizziness due to excess fire in the liver

Main constituents[4]:

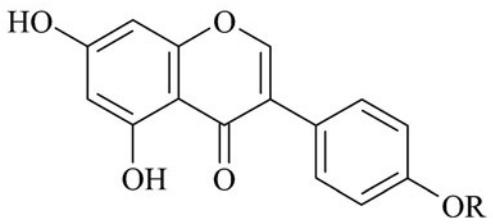
– **flavonoids:**
Rutin (6 % Huaimi, 15 % Huaihua) quercetin-, kaempferol-, genistein- and other isoflavone-glycosides and their aglycones

– **triterpenoids:**
sophoradiol (=olean-12-ene-3.22-diol); soyasapogenol- and betulin-glycosides

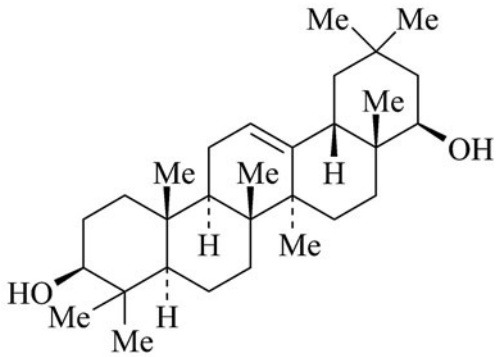
– **disaccharide:**
sophorose (2-O-β-D-glucopyranosyl-D-glucose)



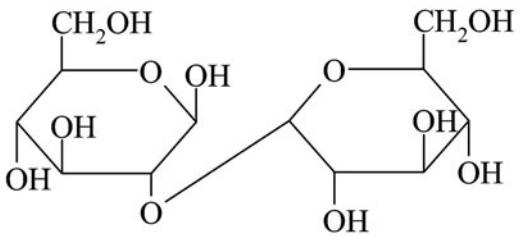
Quercetin: R_1 = OH; R_2 = H
Kaempferol: $R_1 + R_2$ = H
Rutin: R_1 = OH; R_2 = rutinosyl (1 → 6 rhamnoglucosyl)
Kaempferol-3-O-sophoroside: R_1 = H; R_2 = sophorosyl (1 → 2 glucoglucosyl)



Genistein: R = H
Sophoricoside: R = 4'-O-glucosyl
Sophorabioside: R = 4'-O-(1 → 2) rhamnoglucosyl



Sophoradiol



Sophorose

Fig. 1: Formulae of the main constituents

Pharmacology: Most of the pharmacological activities reported for Sophorae flos refer to the main flavonoids rutin, quercetin and isoflavonolglycosides.
– antihemorrhagic[5]
– antioxidant[6]
– antianaphylactic[7]
– antiinflammatory[8]
– antithrombotic[9]
– cardiotonic[10]

TLC-fingerprint analysis

1) Extraction: 8.0 g pulverised drug are soxhlet-extracted with 80 ml methanol until the extract turns colorless, followed by filtration of the cooled extract and evaporation to 5 ml. The extract is filtered and filled up in a volumetric flask with methanol up to 10 ml. 1.0 ml of this extract is given into a 5 ml volumetric flask and filled up to the mark with methanol.

2) Reference compounds: rutin (T 1), hyperoside (T 2), quercetin (T 3), kaempferol-3-gentiobioside (T 4), kaempferol-3-sophoroside (T 5):
1 mg is dissolved in 2 ml methanol

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: Sophorae flos immaturus-extract - *Huaimi*: each 10 µl
Sophorae flos-extract - *Huaihua*: each 10 µl
reference compounds: each 20 µl

Solvent system:

ethyl acetate	:	formic acid	:	glacial acetic acid	:	water
100		11		11		26

The plate is developed in a glas chamber, strongly saturated (half an hour) with the solvent mixture before chromatography.

Spray reagent: Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester (=diphenylboryloxyethylamine, NP) in methanol
II: 5 % polyethylene glycol-4000 (PEG) in ethanol
The plate is sprayed first with solution I and then with solution II.

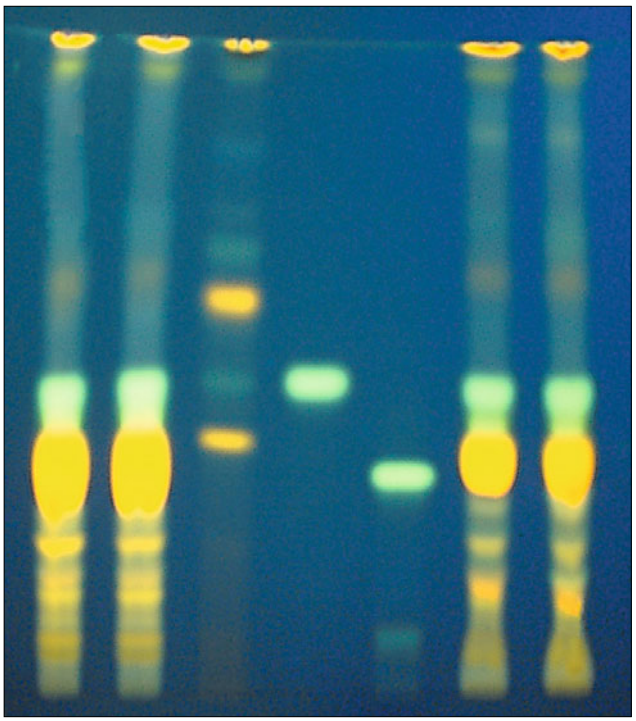


Fig. 2: Thin layer chromatogram of methanolic extract of Sophora flower buds – *Huaimi* and Sophora flowers – *Huaihua*, after spraying with natural products-polyethylene glycol reagent (NP/PEG) (UV 365 nm)

Drug samples		**Origin**
1	Sophora flower buds - *Huaimi*	sample of commercial drug, China
2	Sophora flower buds - *Huaimi*	sample of commercial drug, China
3	Sophora flowers - *Huaihua*	province Anhwei, China
4	Sophora flowers - *Huaihua*	province Shandong, China

Reference compounds		**R*f***
T 1	rutin	0.35
T 2	hyperoside	0.62
T 3	quercetin	0.99
T 4	kaempferol-3-gentiobioside	0.46
T 5	kaempferol-3-sophoroside	0.33

4) Description of the TLC-chromatogram:

Sophorae flos immaturus and Sophorae flos are characterized by the dominating yellow zone of rutin at R*f* = 0.35. The extract of the Sophorae flos immaturus contains a higher rutin content (> 20 %) than the extract of Sophorae flos (> 8 %). Above the rutin zone appear two green-

yellow fluorescent zones of kaempferol-diglycosides (R*f* = 0.42 and 0.46). The upper zone is identical with kaempferol-3-gentiobioside. Overlapped by the rutin zone (R*f* = 0.28) a third kaempferol-diglycoside (probably kaempferol-3-sophoroside) can be seen. Five further green-yellow fluorescent zones in the low R*f* – range (between R*f* ~ 0.06 to R*f* ~ 0.2) can be assigned to kaempferol-tri-glycosides. All extracts contain small amounts of hyperoside (R*f* = 0.62) and the aglycones quercetin (R*f* = 0.99). The isoflavonglycosides cannot be detected without special enrichment.

HPLC-fingerprint analysis:

1) Sample preparation:	The same extract, used for the TLC, is filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC.
2) Injection volume:	Sophorae flos immaturus / Sophorae flos methanol extract: each 1 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 mm with LiChrospher® 60 RP Select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 mm with LiChrospher® 60 RP Select B (5µm), Merck
Solvent:	A: acetonitrile – dist. water – 0.1 M H_3PO_4 (110 – 890 – 20) (Acros Organics) B: acetonitrile – dist. water – 0.1 M H_3PO_4 (500 – 500 – 20) (Acros Organics) 0.1 M H_3PO_4 85%: 11.4 g H_3PO_4 are dissolved in water and filled up to 1000 ml with water
Gradient:	0 – 25 % B in 20 minutes 25 – 70 % B in 10 minutes 70 % B in 10 minutes total runtime: 40 minutes
Flow rate:	1.0 ml / min.
Detection:	210 nm

Retention times of the main peaks:

peak	Rt (min.)	compound
1	17.1	rutin
2	18.6	hyperoside
3	20.6	kaempferol-3-sophoroside
4	21.0	kaempferol-3-gentiobioside
5	29.9	quercetin

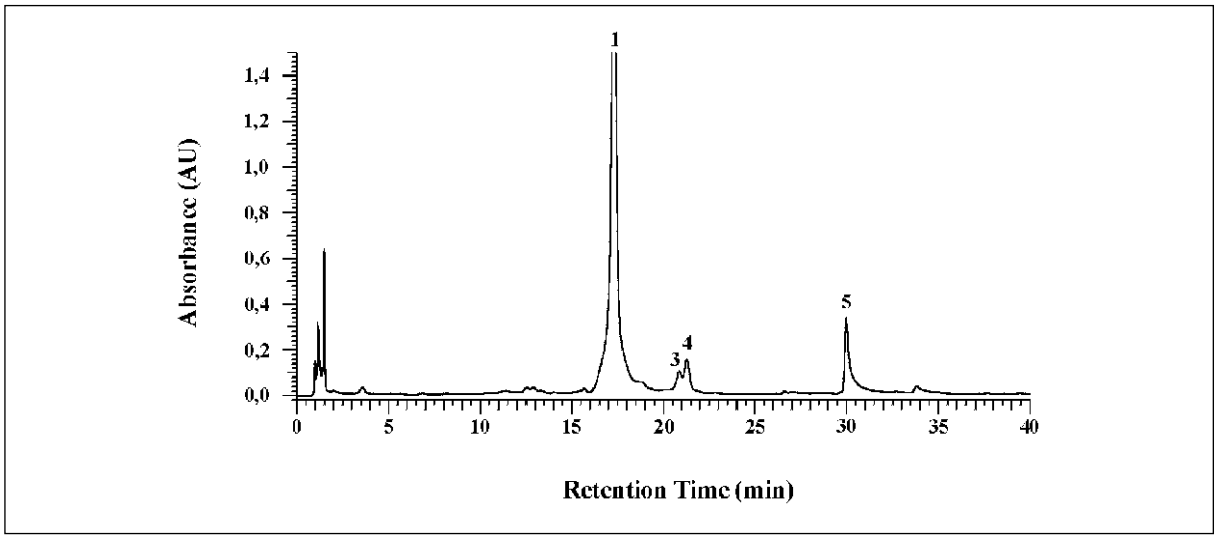


Fig. 3a: HPLC-fingerprint chromatogram of Sophorae flos – *Huaihua*

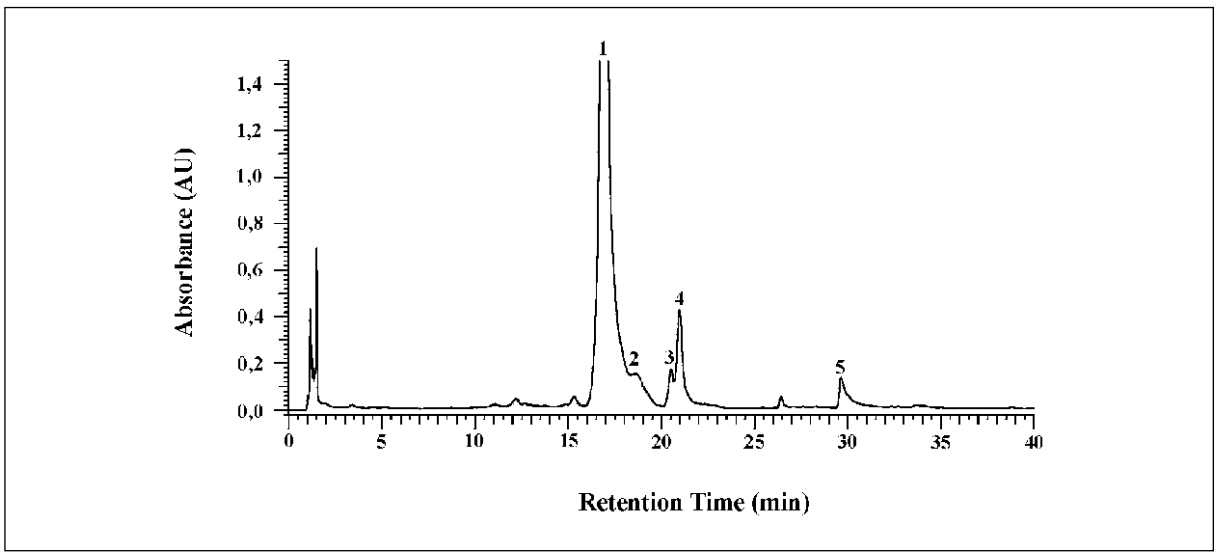


Fig. 3b: HPLC-fingerprint chromatogram of Sophorae flos immaturus – *Huaimi*

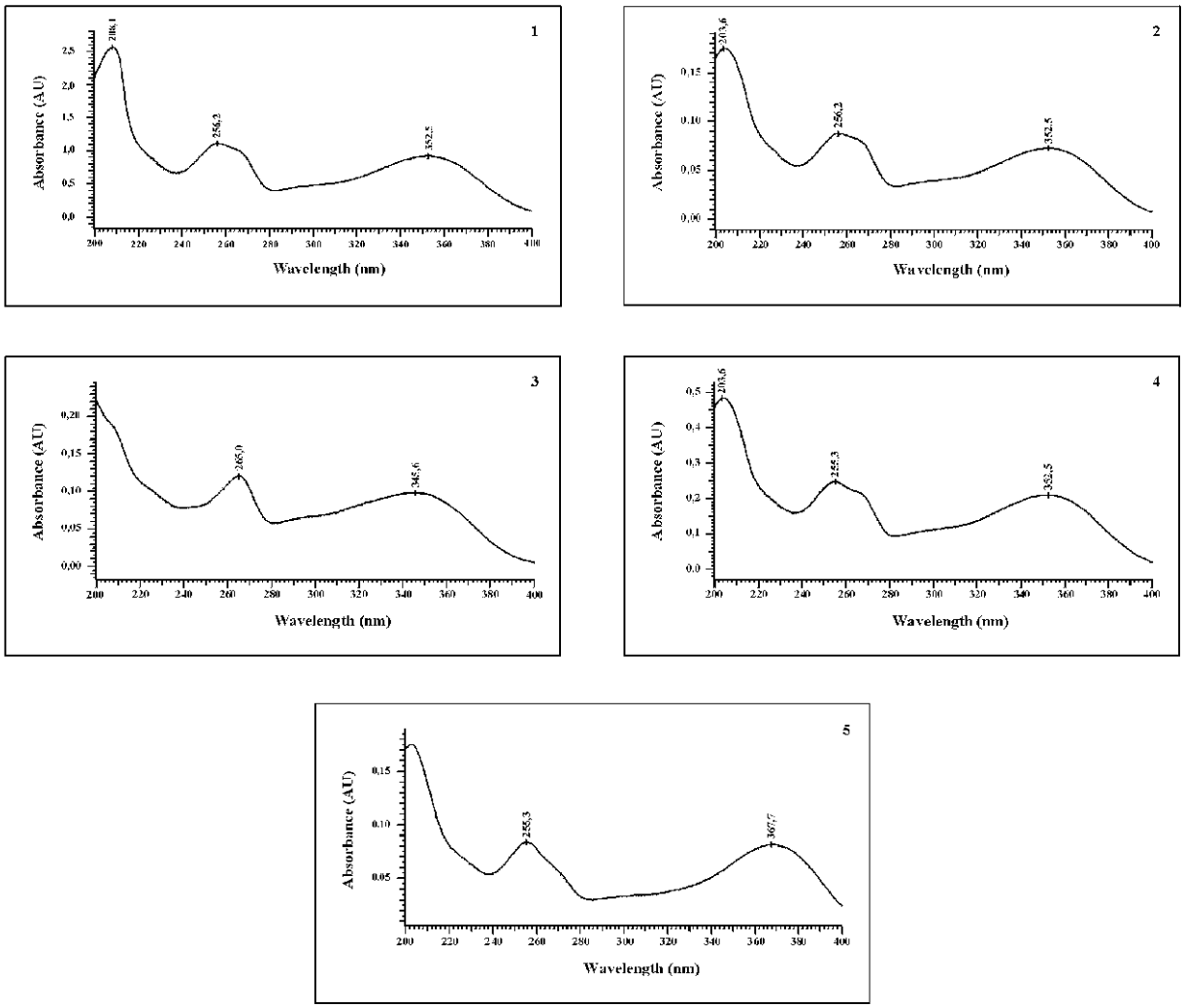


Fig. 4: UV-spectra of the main compounds (peaks) of Sophorae flos immaturus – *Huaimi* and Sophorae flos – *Huaihua*

4) Description of the HPLC chromatograms (Fig. 3a and 3b):

The chromatograms of both *Sophora* extracts show rutin as major peak at Rt = 17.5, the kaempferolglycosides at Rt = 20.6 and Rt = 21.0 and quercetin at Rt = 30.05. The square ratio of peak 1 in the HPLC of Flos Sophorae immaturus extract compared to that of Sophorae flos is in accordance with the mean content of rutin noted in the Chinese Pharmacopoeia (Sophorae flos immaturus > 20 %, Sophorae flos > 8 %).

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Rhizoma Coptidis – *Huanglian*

Pharmacopoeia:	Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]
Official drugs[1]:	*Coptis chinensis* Franch. (Weilian) *Coptis deltoidea* C. Y. Cheng et Hsiao (Yalian) *Coptis teeta* Wall. (Yunlian) The drug is also known as Oren (Japan), Hwangnyon (Korea) and as Golden Thread (English). – Ranunculaceae –
Origin:	Middle and southern regions of China, northern India and Japan.
Description of the drug[1]:	
Rhizome of Coptis chinensis:	Mostly gathered to a cluster, curved, like 'chicken's feet', single rhizome 3-6 cm long, 0.3-0.8 cm in diameter. Externally greyish-yellow or yellowish-brown, rough, bearing irregular nodular protrudings, rootlets and remains of rootlets, some internodes smooth as stem. The upper part mostly remained with brown scale leaves, apex often bearing remains of stems or petioles. Texture hard, fracture uneven, bark orange-red or dark brown , wood brightly yellow or orange-yellow, radially arranged, pith sometimes hollowed. Odour, slight; taste, very bitter.
Rhizome of *Coptis deltoidea:*	Mostly single, somewhat cylindrical, slightly curved, 4-8 cm long, 0.5-1 cm in diameter. Internodes smooth and relatively long. Apex with some remains of stems.
Rhizome of *Coptis teeta:*	Curved hook-like, mostly single, relatively small.
Pretreatment of the raw drug[1]:	The drug is collected in autumn, removed from rootlet and soil, and dried.
Coptidis rhizoma:	From the harvested rhizoma foreign matters are removed, softened thoroughly, cut into thin slices, dried in air, or broken to pieces before use.
Coptidis rhizoma (processed with wine):	Sometimes the rhizoma slices are stir-fried with wine in a closed vessel until it is infused completely. 12.5 kg of yellow rice wine for 100 kg of Rhizoma Coptidis are roasted in a pot and heated gentle to dryness.
Coptidis rhizoma (processed with ginger):	To the rhizoma slices ginger juice is added and mixed well. For 100 kg Rhizoma Coptidis 12.5 kg of ginger are used The whole is stir-baked in a pot with gentle heat until the ginger juice is absorbed completely to dryness.
Coptidis rhizoma (processed with Fructus Evodiae):	Fructus Evodiae is cooked with water, the decoction continued with clean Rhizoma Coptidis and then stirbaked to dryness. To

each 100 kg of Rhizoma Coptidis 10 kg of Fructus Evodiae are added.

Medicinal use[1-5]**:** For the treatment of gastroenteritis, diarrhea, vomiting, icterus, fever, insomnia, hematemesis, nose bleeding, conjunctivitis, toothache, carbuncle and abscess, as a bitter digestive for the treatment of indigestion diabetes and eczema by external application.

Effects and indications according to Traditional Chinese Medicine[1-9]

Taste:	bitter
Temperature:	cold
Channels entered:	large intestine, liver, stomach, heart
Effects:	clears heat and dry dampness, reduces fire and dispels toxins, stops bleeding, drains stomach and abdomen, acts on liver and heart
Symptoms and indications:	for damp heat with suffiness and fullness of the abdomen, high fever accompanied by impairment of consciousness; restlessness and insomnia due to exuberant fire; nosebleeding, blood in the urine; spitting of blood and epistaxis caused by heat in the blood; irritability; delirium, disorientation; topical for red eyes; sore throat carbuncles and abscesses, ulceration of the tongue and the mouth; diarrhoea, vomiting; diabetes; digestive dysfunction; dysmenorrhoea; arthritis; gout; malaria; renal disease

Main constituents[2,7,9-11]**:** (see Fig. 1)
- **quaternary protoberberine-type alkaloids:** berberine, coptisine, palmatine, epiberberine besides columbamine, jatrorrhizine, worenine, groenlandicine, berberastine etc.
- **quaternary aporphine alkaloid:** magnoflorine
- **flavonoids:** baicalin, wogonoside, baicalein, wogonin
- **tetracyclic triterpenes:** limonin
- **organic acids:** ferulic acid, gentisic acid, quinic acid

Pharmacology: *in vitro* effects:
- antibacterial (*Shigella-, Brucella-, Staphyllococcus* and *Streptococcus* sp.)[7]
- antifungal (e.g. *Candida albicans* or *Penicillium* sp.)[7,12]
- antiprotozoic (*Entamoeba histolytica*)[7]

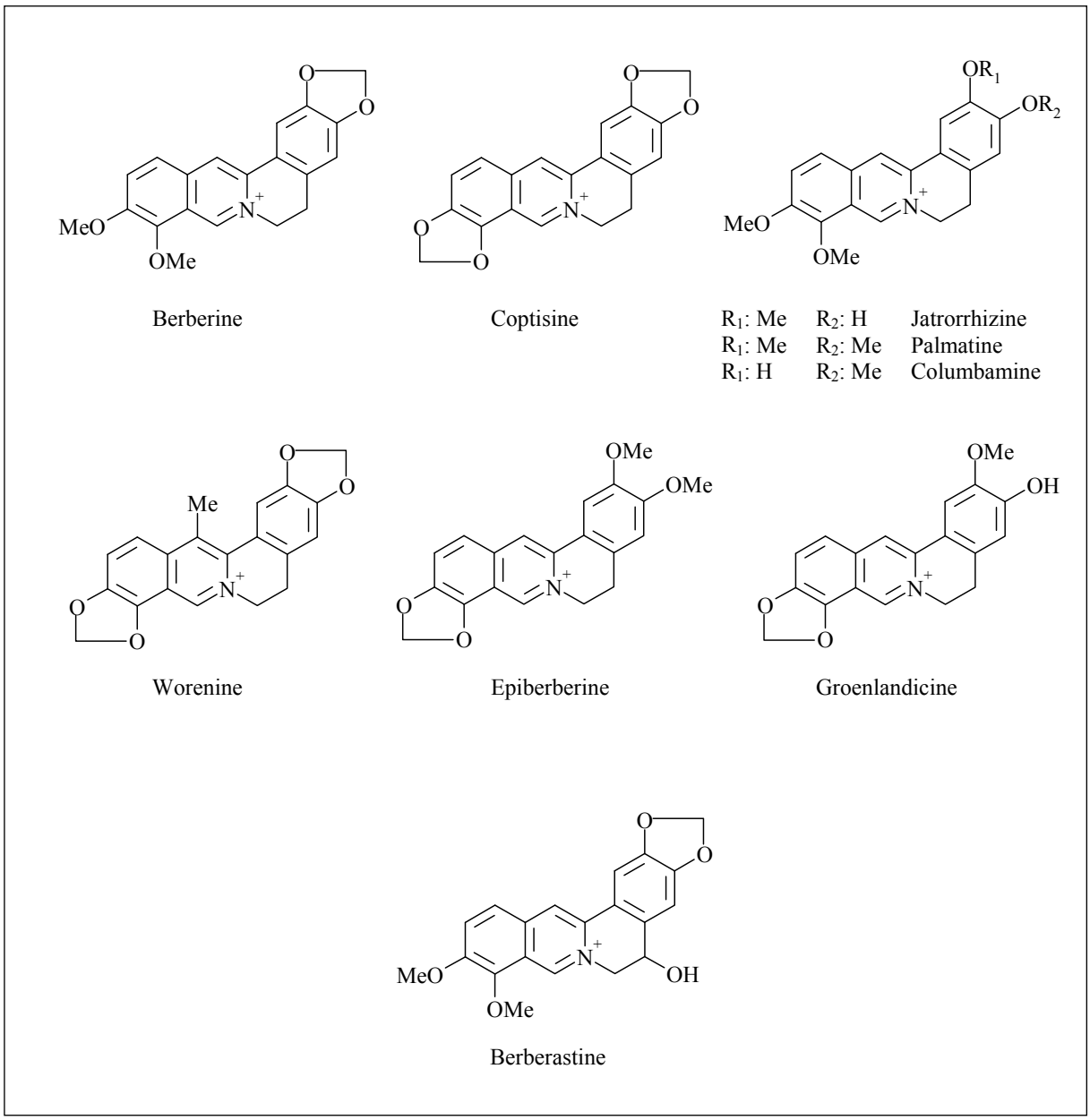

Fig. 1: Formulae of the main constituents[7]:

– antitrypanosomal[13]
– berberine type alkaloids inhibit elastase (berberine, coptisine)[14]
– competitive inhibition of the alcohol dehydrogenase (berberine)[7]
– specific inhibition of cholinesterase (palmatine, berberine)[7]
– inhibition of IL-1-induced IL-6 mRNA expression in rabbit spleen[16]
– NO radical scavenging effect (alkaloids)[17]
– inhibition of cytokine induced neutrophil chemoattractant[18]
– calcium channel blocking and α-adrenoreceptor blocking action in rat aorta (extract, alkaloids)[19]

in vivo effects:

– effect against virus influenza in chicken embryos[2]

– gastric-mucous membrane protection (coptisine)[10,20]

– protoberberine like alkaloids increase the concentration of polar drugs in the skin and enhance the skin permeation similarly to surfactants[15]

– hypoglycemic effects in mice (aqueous extract, berberine)[9]

– lowering of blood pressure (cats, dogs, rabbits)[2]

– vasodilatory effects, stimulation of smooth muscle concentration in uterus, bladder, bronchioles and other organs (animals)[2]

– cognitive enhancing[21]

– antitumoral[7,22-25]

– monoaminoxidase-inhibiting[26]

Toxicology: Toxic adverse effects are reported when overdosages of herbal preparations are administered. The adverse effects (general depression, vomiting, dyspnoea, salivation, defecation, micturition) are caused by overdoses of berberine alkaloids.[6]

TLC-fingerprint-analysis[27,28]:

1) Extraction: 50 mg powdered drug are extracted with 5 ml methanol on a water bath for 15 min and filtered.

2) Reference compounds/drug: berberine, coptisine, jatrorrhizine, palmatine chloride, columbamine, each dissolved in methanol (1 mg/ml)
Berberidis radicis cortex powdered drug are extracted according to the method above.

3) Separation parameters:

Applied amount: 10 µl extract and standard solution

Plates: Silicagel 60 F_{254}; Merck

Solvent system: In one trough of a twin trough glass chamber a mixture of toluol-ethyl acetate-isopropanol-methanol-water (6: 3: 1,5: 1,5: 0,3) and in the other trough ammonia 25 % solution are poured in.
Equilibration of the chamber for 15 min.

Detection: Direct evaluation:
UV 365 nm (without spray reagent)

Iodine reagent:
0.05 g iodine is dissolved in 10 ml ethanol 96 %.
The plate is evenly sprayed until background appears yellow.
Examination in VIS when background has been turned to white again.

Drug samples		Origin
1	Coptidis rhizoma/*Coptis chinensis*	Wan-Xian (province), Chong-giing (City), China
2	Coptidis rhizoma/*Coptis chinensis*	commercial product from Hebei An-guo Drug Market, China
3	Coptidis rhizoma/*Coptis teeta*	province Yunnan, China
4	Coptidis rhizoma	commercial product of Uchida company, Japan

Reference compounds/drugs		R*f*	
T1	coptisine and palmatine	0.65/0.13	Institute of Pharmac. Biology, LMU Germany and Sigma, Germany
T2	berberine and jatrorrhizine	0.25/0.08	Institute of Pharmac. Biology, LMU Germany
T3	columbamine	0.04	Institute of Pharmac. Biology, LMU Germany
T4	Berberidis radicis cortex		sample of commercial drug, Germany

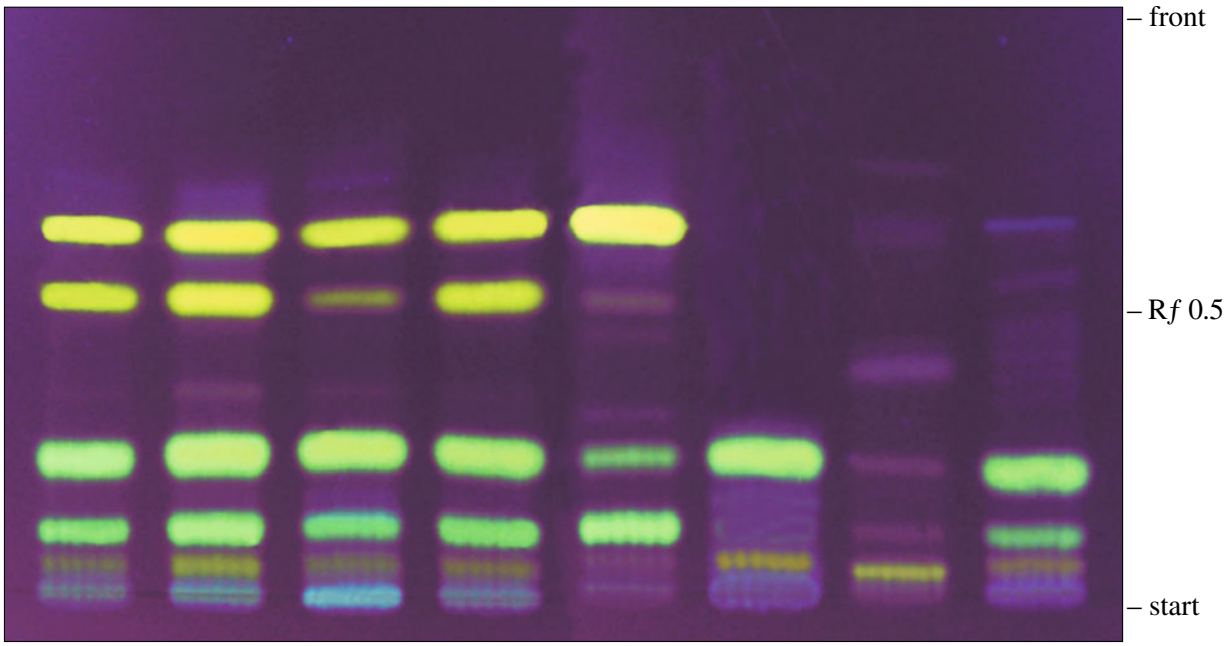

Fig. 2: TLC-fingerprint of Coptidis rhizoma in UV 365 nm

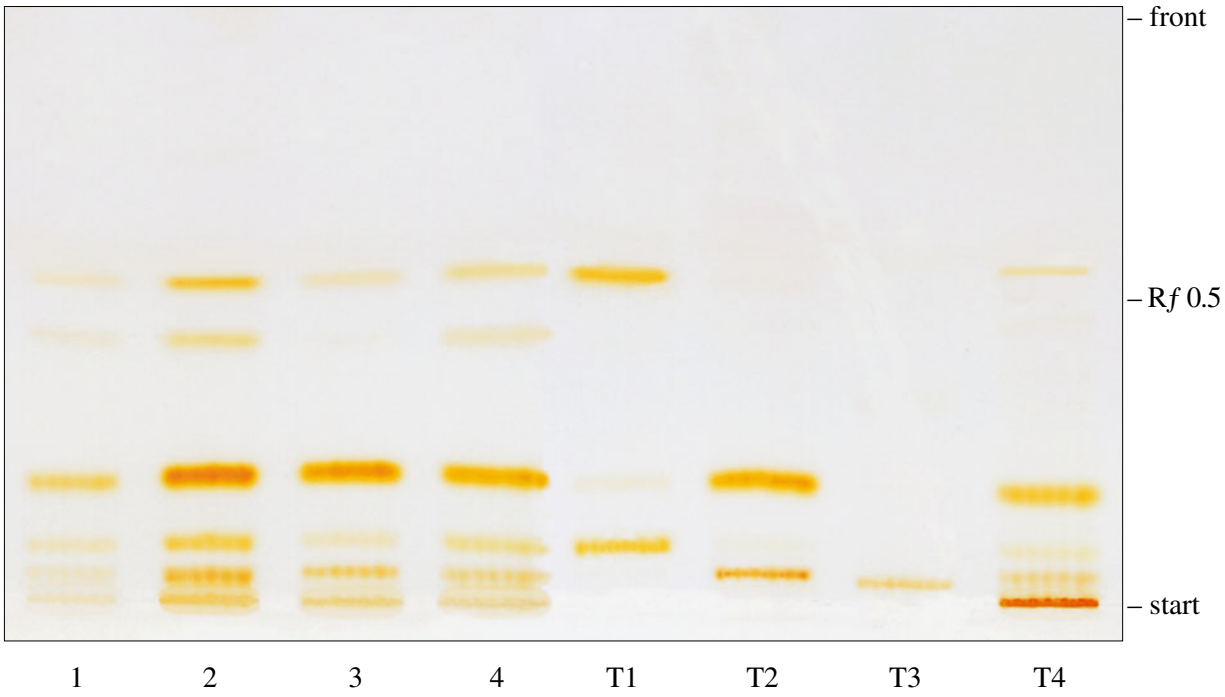

Fig. 3: TLC-fingerprint of Coptidis rhizoma detected with iodine reagent in VIS

4) Description of the TLC-chromatogram, without spray reagent, **Fig. 2** in UV 365 nm:
All four Coptidis rhizoma samples are characterized by four major yellow to green fluorescent zones: Coptisine (R*f* 0.65), epiberberine (R*f* 0.52), berberine (R*f* 0.25) and palmatine (R*f* 0.13). Columbamine (R*f* 0.04) and jatrorrhizine (R*f* 0.08) appear in lower concentrations as yellow-orange zones. The extract of *Coptis teeta* differs from that of *Coptis chinensis* by a low content of epiberberine.
Berberidis radicis cortex (T4) differs from Coptidis rhizoma by lacking the alkaloids coptisine and epiberberine.

Description of the TLC-chromatogram, sprayed with iodine reagent, **Fig. 3** in VIS:
The Coptidis rhizoma samples show the same qualitative alkaloid pattern as Fig. 2 with yellow-orange coloured zones.

HPLC-fingerprint-analysis[29]:

1) Sample preparation: The same extracts as used for TLC are filtered over Millipore® (Type HV 0.45 µm).
2) Injection volume: 10 µl extract

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D- 6000A Interface; Merck, Hitachi
Column:	LiChroCART® 125-4 LiChrospher® 60 RP-select B with LiChroCART® 4-4 LiChrospher® 60 RP-select B (5 µm); Merck
Solvent system:	A: acetonitrile for HPLC; Acros Organics B: puffer pH 4.2 (0.87 mol acetic acid, 0.12 mol sodium acetate, 11.56 mol sodium dodecyl sulfate and 16.12 mmol diethylamine are dissolved in 1 litre water for HPLC; Acros Organics)
Gradient:	60 % B isocratic in 18 min.
Flow rate:	0.8 ml/min.
Detection:	270 nm

Retention times and identity of the main peaks of Fig. 4 and Fig. 5:

Peak	Rt (min.)	Compound
1	6.2	columbamine
2	6.4	jatrorrhizine
3	7.0	epiberberine
4	7.9	coptisine
5	9.5	palmatine
6	10.6	berberine

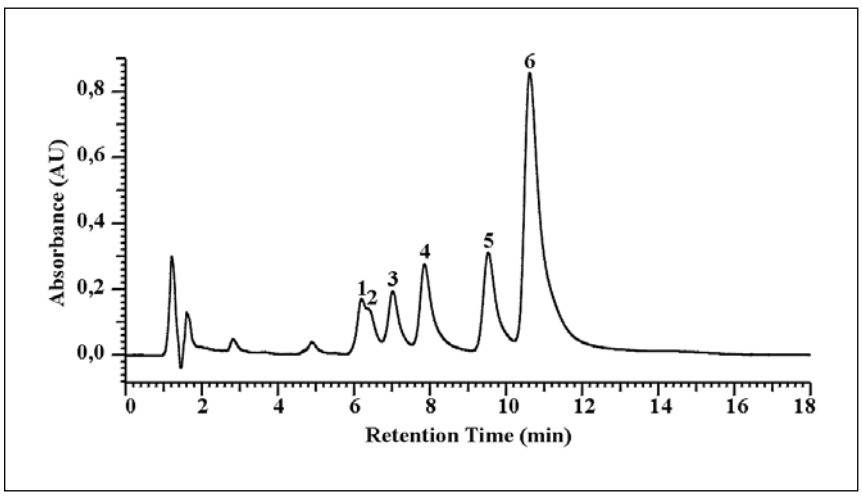

Fig. 4: HPLC-fingerprint chromatogram of Coptidis rhizoma extract

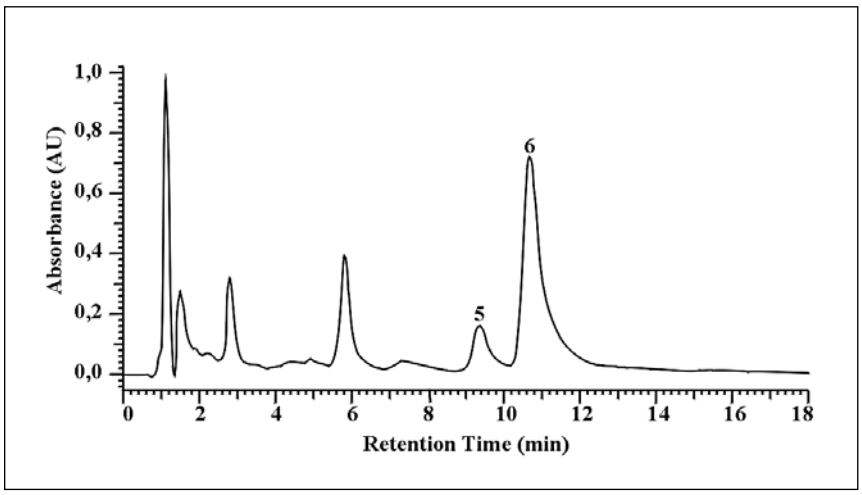

Fig. 5: HPLC-fingerprint chromatogram of Berberidis radicis cortex extract

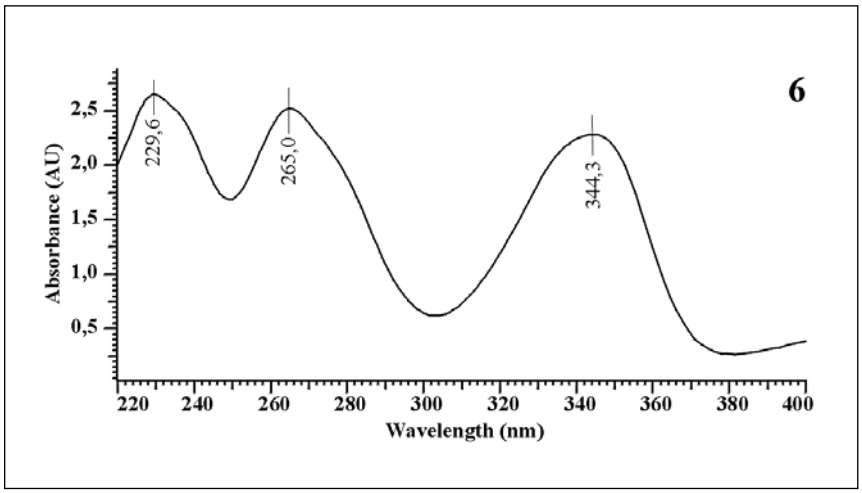

Fig. 6: online UV-spectrum of berberine

4) Description of the HPLC-chromatograms:

Coptidis rhizoma (Fig. 4) and Berberidis radicis cortex (Fig. 5) for comparison show the major alkaloid peaks in the Rt-range 6.0 to 12.0 min both with berberine (**6**) as the dominant peak at 10.6 min. The other alkaloids, of Coptidis rhizoma, columbamine (**1**; Rt 6.2), jatrorrhizine (**2**; Rt 6.4), epiberberine (**3**; Rt 7.0), coptisine (**4**; Rt 7.9) and palmatine (**5**; Rt 9.5) possess nearly equal UV-spectra, as shown for berberine (Fig. 6). The percent ratio of coptisine to berberine in official Coptidis rhizome of about 1:3 corresponds with that of the HPLC-peak pattern.

The peak pattern of Berberidis radicis cortex extract for comparison shows also the main peaks of berberine (**6**) and palmatine (**5**) but differs from Coptidis rhizoma in two additional peaks at Rt 2.8 and 5.8.

According to the Chinese Pharmacopoeia 2005 the berberine content of Coptidis rhizoma should be not less than 3.6 %, whereas for Berberidis radicis cortex often contents up to 4.5 % are reported.

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Radix Stephaniae tetrandrae
Hanfangji

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[1]

Official drug[1]: Fourstamen Stephaniae tetrandrae radix is the dried root of *Stephania tetrandra* S. Moore (Fam. Menispermaceae).

Origin[2]: The provinces Shaanxi, Hunan, Anhui, Guangdong and Guangxi

Description of the drug[1]: Irregularly cylindrical, semi-cylindrical or lump-shaped, mostly tortuous, 5 – 10 cm long, 1 – 5 cm in diameter. Externally greyish-yellow, usually exhibiting deeply depresessed transverse grooves and appearing knotty-knobby at the curved part. Texture heavy and compact, fracture even, greyish-white, starchy, sparsely. Odour, slight; taste, bitter.

Pretreatment of the raw drug[1]: The drug is collected in autumn, washed clean, removed from the outer coarse bark, half-dried in the sun, cut into section; the large one is cut longitudinally and dried.

Processing[1]: Foreign matters are removed, soaked briefly, washed clean, softened thoroughly, cut into thick slices, and dried. Occuring in subrounded or broken thick slices, edges rather dark in colour, cut surface greyish-white, starchy, with sparse radial striations. Odour slight; taste bitter.

Medicinal use: Cardiovascular diseases (hypertension arrythmia, angina, pulmonary and portal hypertension), for the protection of ischemia and induced myocardial injury and myocardium infarction.

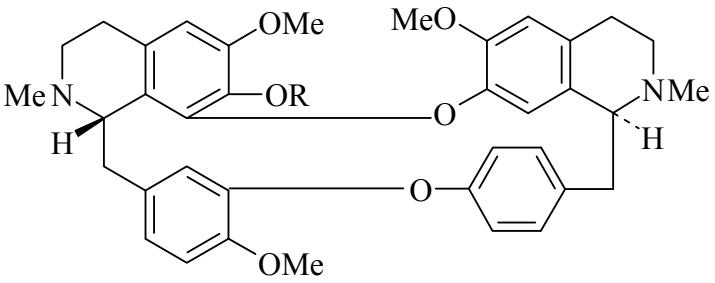
Effects and indications according to Traditional Chinese Medicine[1,2]

Taste:	bitter
Temperature:	cold
Channels entered:	kidney, spleen, urinary bladder
Effects:	removes fluid and eliminates 'wind and damp' from the body
Symptoms and indications:	edema with oliguria, eczema, rheumatic arthritis, hypertension

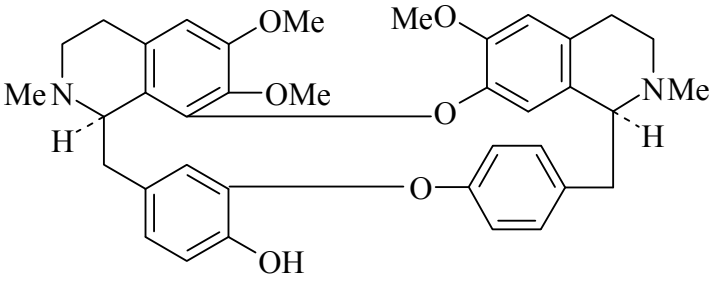
Main constituents[3]:

– **alkaloids of the bisbenzylisoquinoline type:** tetrandrine, fangchinoline, berbamine, oxofanchirine
– **quaternary alkaloid of the protoberberine type:** cyclanoline
– **alkaloid with a phenanthrene skeleton and a tertiary amine side chain:** stephenanthrine

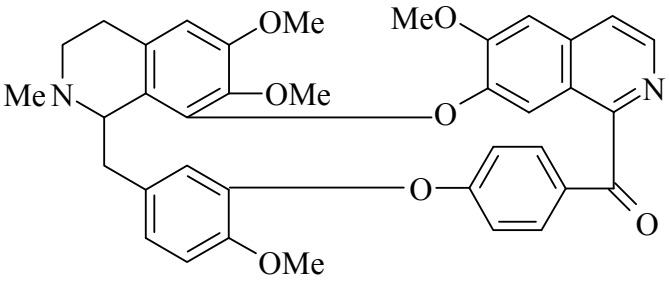
H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*



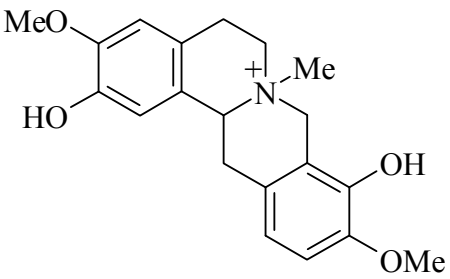
Tetrandrine: R = CH_3
Fangchinoline: R = H



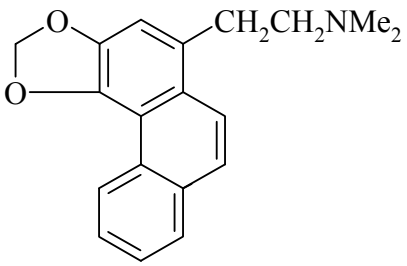
Berbamine



Oxofanchirine



Cyclanoline



Stephenanthrine

Fig 1: Formulae of the main constituents

Pharmacology:

in vitro:

- Ca-antagonistic (verapamil like) activity (pig coronary artery strips and rat uterus)[4,5]
- inhibiton of platelet aggregation[6,7]
- antagonizes the transient inward current of Ca^{2+} in neuroblastoma cells[8]
- inhibition of the vascularisation of retinal capillary in diabetic rats[9]
- induction of apoptosis (human long carcinoma cell)[10]
- antiinflammatory[11]

in vivo:

- antiarrythmic action[6,8]
- antimyocardial infarction (dog)[12], slight decrease of blood pressure and heart rate[13]
- antiallergic[14]
- antihyperglycemic[15]
- antifibrotic[16]
- antiinflammatory[17]
- antiangiogenetic[18]
- preventive effect on myocardial ischemia-reperfusion injury[19]

Adulterations: Adulterations with Aristolochiae radix have been reported. Therefore Stephaniae radix has to be examined microscopically and chromatographically on the absence of *Aristolochia* adulterations or impurities.

TLC-fingerprint analysis

1) Extraction: To 4.5 g pulverised root 45 ml methanol are added and the suspension treated in an ultrasonic bath for 1 hour. After that the extract is cooled and filtered. The filtrate is evaporated to dryness and the residue dissolved in 10.0 ml methanol.

2) Reference compounds: fangchinoline (T 1), tetrandrine (T 2): 1 mg each are dissolved in 1 ml chloroform

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: Stephaniae tetrandrae radix extracts: each 20 µl
fangchinoline (T1), tetrandrine (T2): each 25 µl

Solvent system:

chloroform	:	methanol	:	water
60		30		6.5

Detection: Dragendorff reagent:

Solution I: 0.85 g basic bismuth nitrate are dissolved under heating in a mixture of 10 ml glacial acetic acid and 40 ml water.

Solution II: 8 g potassium iodide are dissolved in 30 ml water.

5 ml of spray reagent I and II are mixed with 20 ml glacial acetic acid in a volumetric flask and water added up to 100 ml.
The plate is sprayed with this mixture.

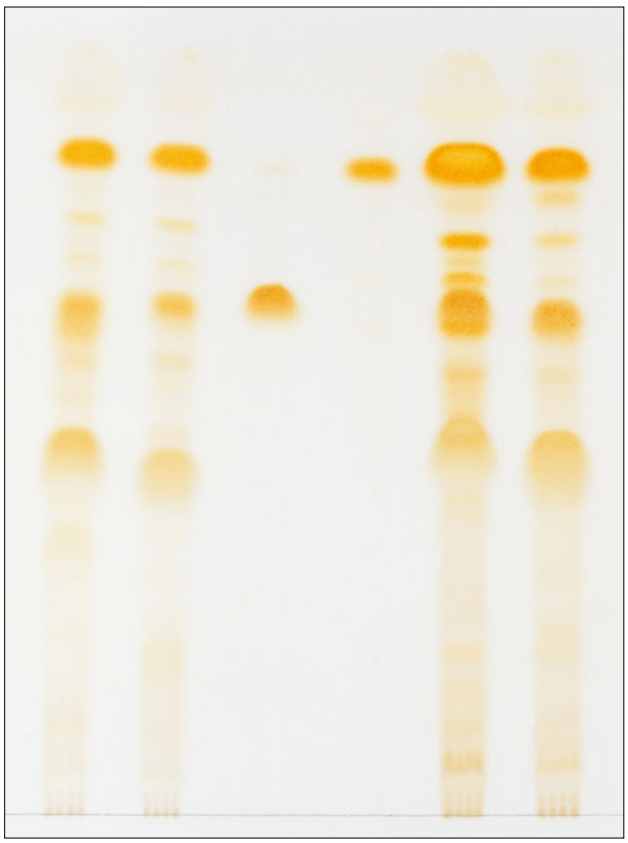


Fig. 2: Thin layer chromatogram of methanolic extract of Stephaniae tetrandrae radix after spraying with Dragendorff reagent (VIS)

Drug samples		**Origin**
1	Stephaniae tetrandrae radix	province An-hui, China
2	Stephaniae tetrandrae radix	province Beijing, China
3	Stephaniae tetrandrae radix	province Jiang-su, China
4	Stephaniae tetrandrae radix	province Zhejiang, China

Reference compounds		**R*f***
T 1	fangchinoline	0.65
T 2	tetrandrine	0.85

4) Description of the thin layer chromatogram (Fig. 2):

Stephania tetr. root samples 1, 2, 3 and 4 show a very homogenous alkaloid pattern with tetrandrine (T 2; R*f* = 0.85), fangchinoline (T 1; R*f* = 0.65) and a third alkaloid (not identified) at R*f* = 0.48 as the main alkaloids. Further 3 – 4 alkaloids appear in minor concentrations between tetrandrine and fangchinoline and between fangchinoline and the non identified alkaloid at R*f* = 0.48. 4 – 5 very low concentrated alkaloids can be seen in the lower R*f*–range between the start and R*f* = 0.48.

HPLC-fingerprint analysis:

1) Sample preparation: 1.0 g of pulverised *Stephania* root is extracted under reflux with 15 ml of ethanol for 1 hour. The extract is cooled, filtered, evaporated to dryness and the residue redissolved in 5.0 ml ethanol. 2.0 ml of this extract are evaporated to dryness and the residue treated with 500 µl of a solution of 2 g hexanesulfonic acid / 1 l dist. water + H_3PO_4 85 % (pH = 3.0) in an ultrasonic bath for 1 hour. To the extract 1.0 ml ethanol is added, filtered over Millipore® filtration unit type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Stephaniae tetrandrae radix extract: 3 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing) (pH = 3.0) B: acetonitrile (Acros Organics)
Gradient:	10 – 50 % B in 25 min., 50 % B in 5 min., total runtime: 30 min.
Flow rate:	1.0 ml / min.
Detection:	210 nm

Retention times of the main peaks:

peak	Rt (min.)	compound
1	15.7	not identified
2	19.1	fangchinoline
3	20.0	tetrandrine
4	21.1	not identified

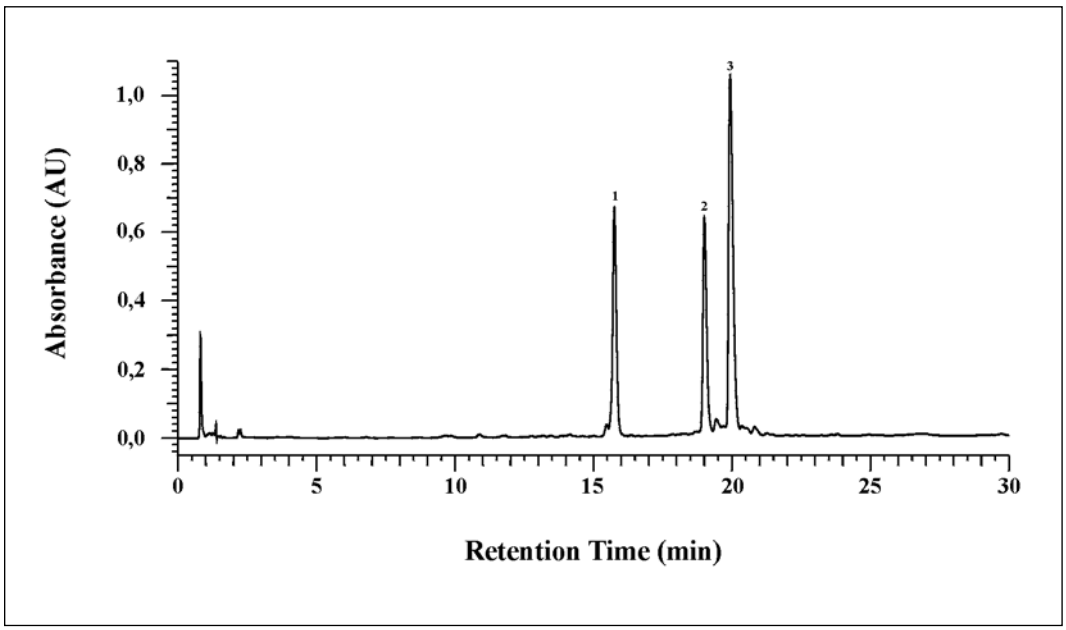


Fig. 3a: HPLC-fingerprint chromatogram of Stephaniae tetrandrae radix extract (sample 1, province An-hui, China)

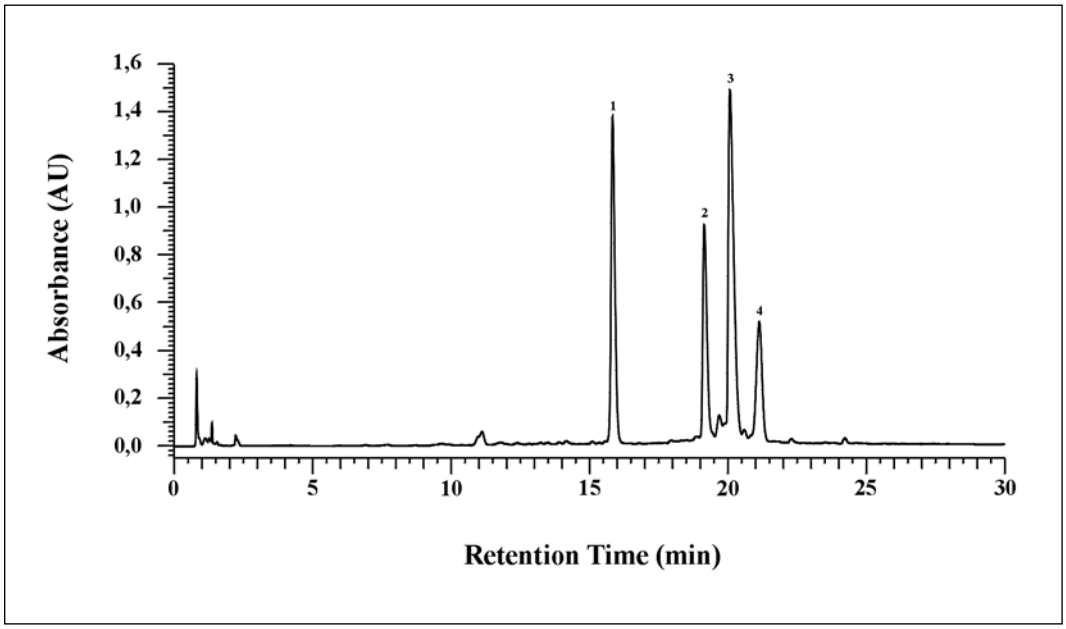


Fig. 3b: HPLC-fingerprint chromatogram of Stephaniae tetrandrae radix extract (sample 4, province Zhejiang, China)

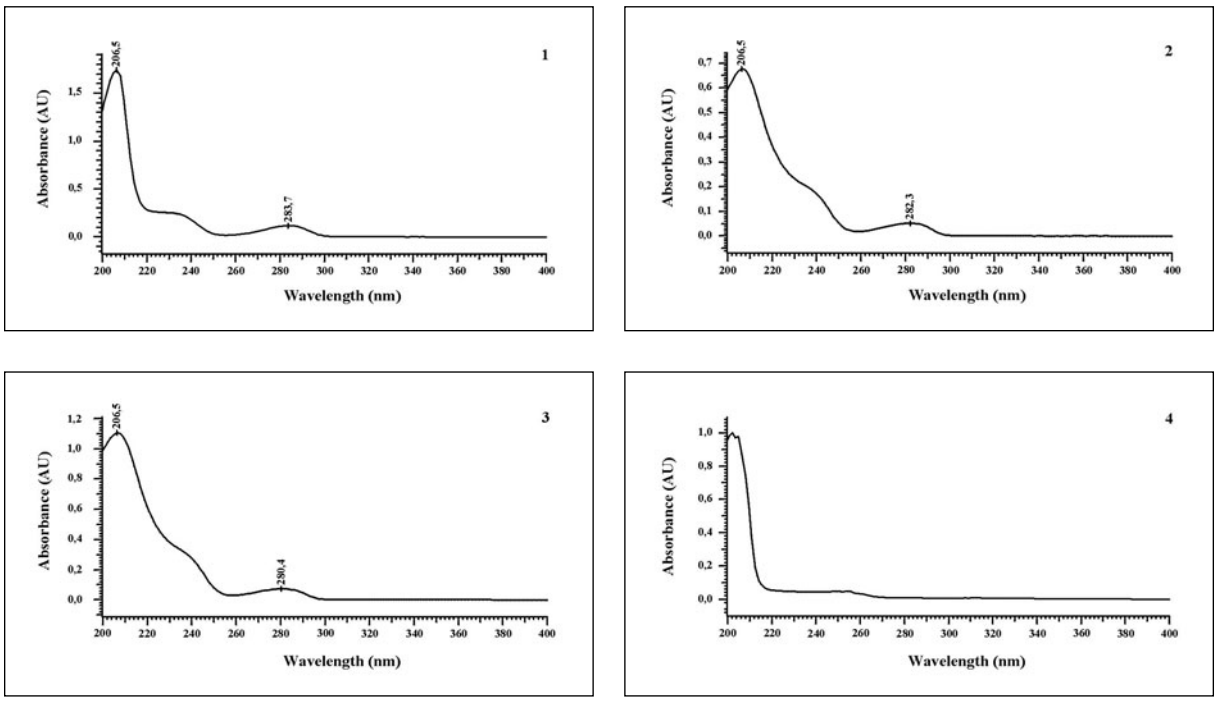


Fig. 4: UV-spectra of the main compounds (peaks) of Stephaniae tetrandrae radix

4) Description of the HPLC chromatograms (Fig. 3a and 3b):

The HPLC-fingerprint is characterized by a three or four peak pattern with tetrandrine (**3**) at Rt = 20.0 as the major alkaloid and fangchinoline (**2**) at Rt = 19.1. Another non-identified alkaloid (**1**) with a typical tetrahydroisochinolin UV-spectrum appears at Rt = 15.7. A fourth peak (**4**) with an Rt-value of 21.1 was found in the root of *Stephania tetr.* from the province Zhejiang only. Its UV-spectrum shows an endabsorption at 205 nm.

Quantitation of Tetrandrine

Deviating from the method of the Pharmacopoeia of the People's Republic of China (Engl. Edition 2000) which describes a half quantitative TLC-methode by comparing the zone squares of the extracts with those of several reference zones of known tetrandrine concentrations, a new HPLC-method has been developed:

1) Extraction: 1.0 g root powder (through No. 3 sieve) four hours dried at 80 °C and accurately weighed, is soaked with 6 drops of 25 % ammonia solution and kept standing for 1 hour at room temperatur. After that 60 ml chloroform are added and the solution heated under reflux for 6 hours. After cooling, the solution is evaporated on a water bath, the residue dissolved in ethanol, the solution transferred to a 2 ml volumetric flask and ethanol added up to the 2 ml mark. 1.0 ml of this solution is given into a second 2 ml volumetric flask, 250 µl of a solution of 2 g hexanesulfonic acid / 1 l dist. water + phosphoric acid 85 % (pH = 3.0) added and the solution again filled up to the 2 ml mark. The solution is filtered over Millipore® filtration unit type 0.45 µm and injected into the HPLC apparatus.

2) Reference compound: 1.0 mg accurately weight tetrandrine is dissolved in 600 µl DMSO (dimethyl sulfoxide) and 400 µl of a solution containing 2 g hexanesulfonic acid / 1 l dist. water + phosphoric acid 85 % (pH = 3.0) added. For establishing a calibration straight line 10, 15, 20, 25, 30, 35 and 40 µl are injected into the HPLC apparatus.

3) Injection volume: Stephaniae tetrandrae radix extract: 10 µl

4) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing GmbH) (pH = 3.0) B: acetonitrile (Acros Organics)
Gradient:	10 – 50 % B in 25 min., 50 % B in 5 min., total runtime: 30 min.
Flow rate:	1.0 ml / min.
Detection:	280 nm

4) Calculation of the tetrandrine % content of *Stephania* root:
A calibration straight line is established using the HPLC-peak areas and their corresponding tetrandrine reference concentrations and injected. The % content of tetrandrine in *Stephania* root is calculated with the following equation:

$$\% \text{ tetrandrine} = \frac{\left(\dfrac{\mu g \text{ tetrandrine / injection volume} \times \mu l \text{ tetrandrine solution}}{\mu l \text{ injection volume tetrandrine solution}} \right) \times 100}{\mu g \text{ Stephania root / injection volume}}$$

Proof of Stephaniae tetr. radix on the absence of Aristolochiae radix:

To prove Stephaniae tetr. radix samples on a possible falsification or blending with root of *Aristolochia* spec. the following TLC- and HPLC-methods have been worked out:

TLC-fingerprint analysis:

1) Extraction:	4.5 g pulverised drug are added with 45 ml methanol and treated in an ultrasonic bath for 1 hour. After that the extract is cooled and filtered. The filtrate is evaporated to dryness, the residue dissolved in methanol and filled up in a volumetric flask to 10.0 ml with methanol.
2) Reference compounds:	fangchinoline (T1), tetrandrine (T2), aristolochic acid I + II (T3): 1 mg each is dissolved in 1 ml chloroform
3) Separation parameters:	
Plates:	Silica gel 60 F_{254}, Merck
Applied amounts:	drug samples 1 – 7: 20 µl each reference compounds T 1, T 2, T 3: 25 µl each
Solvent system:	toluol : ethyl acetate : dist. water : formic acid (upper phase) 20 10 1 1
Spray reagent:	Tin-(II)-chloride reagent: 1.5 ml hydrochloric acid (36 %) are diluted with 8 ml water. 1 g of tin-(II)-chloride x 2 H_2O is dissolved in this mixture. This reagent has to be prepared always freshly. The plate is sprayed until slightly wet and then heated at 100 ° C for 5 minute.

The tin-(II)-chloride reagent for the identification of aristolochic acids in herbal drugs has been first applied in DAC [20]. A HPTLC method for prooving Stephaniae radix on impurities or falsification with Aristolochiae radix has been described by Blatter and Reich (2004)[21].

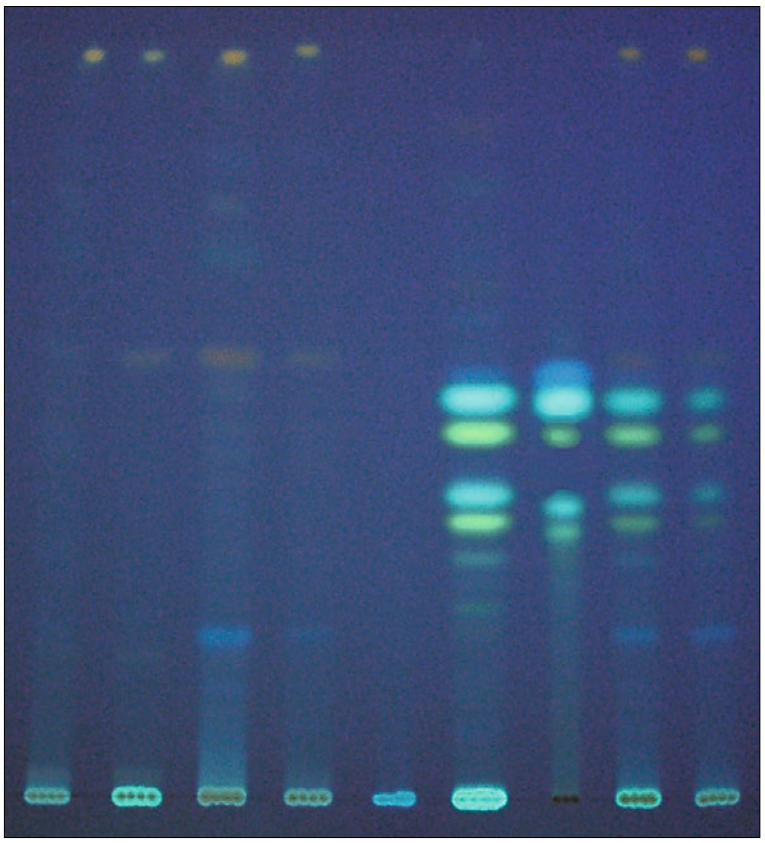


Fig. 5: Thin layer chromatogram of methanolic extracts of Stephaniae tetrandrae radix after spraying with tin-(II)-chloride reagent (UV 365 nm)

Drug samples		Origin
1	Stephaniae tetrandrae radix	province An-hui, China
2	Stephaniae tetrandrae radix	province Beijing, China
3	Stephaniae tetrandrae radix	province Jiang-su, China
4	Stephaniae tetrandrae radix	province Zhejiang, China
5	Aristolochiae radix	sample of commercial drug, China
6	artificial mixture of drug samples 3 and 5 (80 : 20)	
7	artificial mixture of drug samples 3 and 5 (95 : 5)	

Reference compounds		R*f*
T 1	tetrandrine	–
T 2	fangchinoline	–
T 3	Aristolochic acid I + II (Acros organics)	0.48 + 0.53

4) Description of the TLC proof on absent of aristolochic acid (Fig. 5):

All *Stephania* root samples 1 – 4 show in the UV 365 nm on the start the light-blue fluorescent zones of their alkaloids. The 80 : 20 and 95 : 5 mixtures of sample 6 and 7 show the typical aristolochic acids I + II zones (T 3) at R*f* = 0.48 and R*f* = 0.53 respectively and two further aristolochic acid compounds at R*f* = 0.35 and 0.39. The same alkaloid pattern shows sample 5 (*Aristolochia* root). In none of the *Stephania tetr.* root samples examined aristolochic acids could be detected.

With this method still 400 pg Aristolochic acid in herbal drug can be detected [21]

HPLC-fingerprint analysis:

1) Sample preparation: 1.0 g of pulverised root of *Stephania* and *Aristolochia* are extracted separately for 1 hour with ethanol. The extracts are filtered, evaporated to dryness and the residues redissolved in 5.0 ml ethanol. 2.0 ml of these extracts are evaporated to dryness and the residues are treated with 500 µl of a solution of 2 g hexanesulfonic acid / 1 l dist. water + H_3PO_4 (pH = 3.0) in an ultrasonic bath for 1 hour. To the extracts 1.0 ml ethanol are added and filtered over Millipore® filtration unit type 0.45 µm. *Stephania* root extract and *Aristolochia* root extract are mixed in a ratio of 50 : 50 and 80 : 20 and injected into the HPLC apparatus.

2) Injection volume: mixture 50 : 50 and 80 : 20: each 3 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing) (pH = 3.0) B: acetonitrile (Acros Organics)
Gradient:	10 – 50 % B in 25 min., 50 B in 5 min., total runtime: 30 min.
Flow rate:	1.0 ml/min.
Detection:	254 nm

Two other HPLC-methods to examine Stephaniae radix and other herbal drugs on the absence of Aristolochiae radix and aristolochic acid I + II respectively have been described in DAC [20] and published by Sun et al (2003)[22].

Retention times of the main peaks:

peak	Rt (min.)	compound
1	12.0 / 12.3	flavonoid derivatives
2	22.4	caffeic acid derivative
3	27.9 / 28.0	aristolochic acid I + II

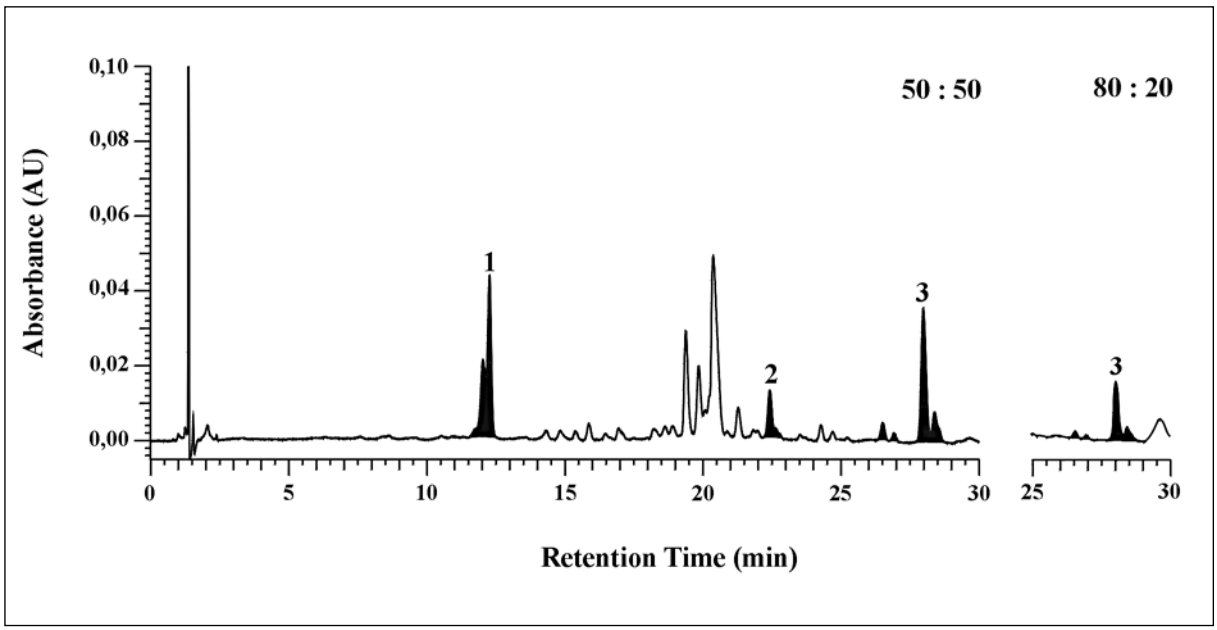


Fig. 6: HPLC-fingerprint chromatogram of artificial mixtures of drug samples 3 and 5 (50 : 50 and 80 : 20)

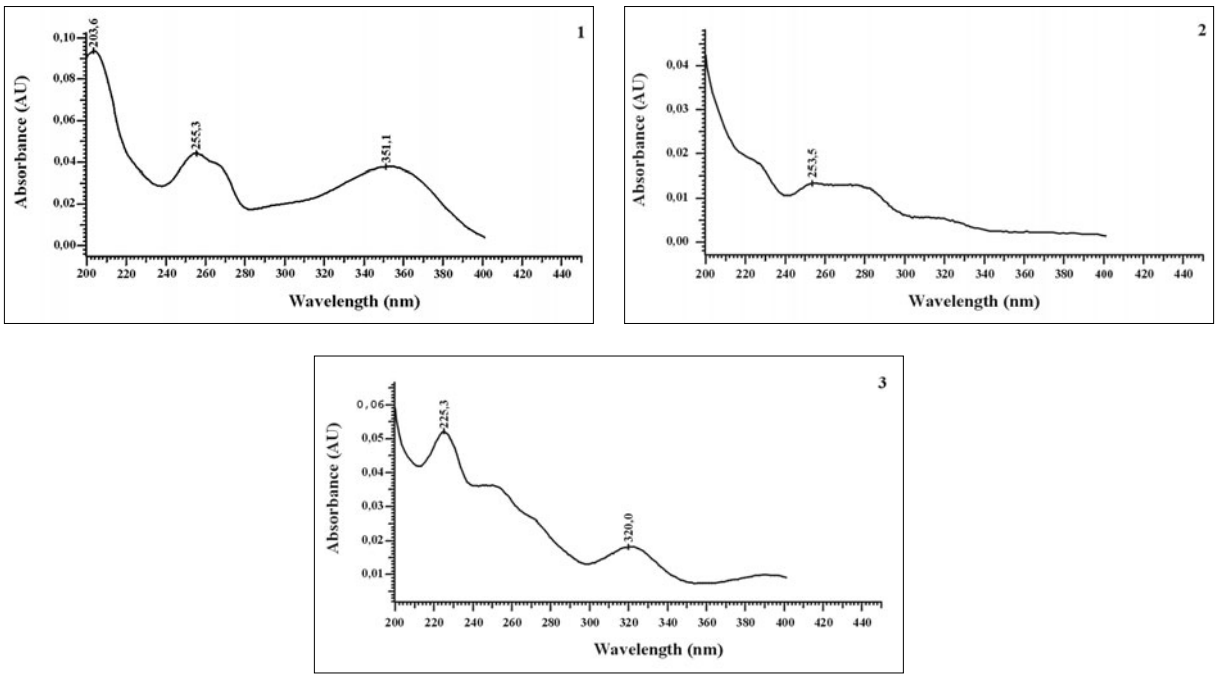


Fig. 7: UV-spectra of the main compounds (peaks) of Aristolochiae radix

4) Description of the HPLC proof on the absence of aristolochic acid in artificial 50 : 50 and 80 : 20 mixtures of Stephaniae- and Aristolochiae radix:

   Aristolochiae radix shows a characteristic peak pattern with major peaks at Rt = 12.0, 12.3 (**1**), 22.4 (**2**), and 27.9 (**3**) respectively 28.0 (black marked). The peaks at Rt = 27.9 and 28.0 respectively can be assigned to aristolochic acid I + II. The peaks at 12.0, 12.3 show characteristic UV-spectra for flavonoids and a caffeic acid derivative (Rt = 22.4) respectively. In the artificial *Stephania* / *Aristolochia* root mixtures the corresponding black marked peaks, characteristic for *Aristolochia* root at Rt = 12.0, 12.3, 22.4, 27.9 and 28.0 can be easily detected. All other peaks (white) originate from *Stephania* root.

   With this method still 6 µg aristolochic acid / g herbal drug can be detected[20].

   (See also Monographs Radix Clematidis and Caulis Sinomenii)

Note: According to the Chinese Pharmacopoeia 2005 Radix Stephaniae tetrandrae should contain not less than 1.4 % of the total amount of tetrandrine and fanchinoline, calculated with reference to the dried drug.

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Semen Ziziphi spinosae
Suanzaoren

Pharmacopoeia:	Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]
Official drugs[1]:	*Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H. F. Chou – Rhamnaceae –
Origin[2,3]:	China (mainly Hebei, Shandong, Liaoning, Henan, Shaanxi), Japan, India, Afghanistan, Malaysia, Australia, tropical Africa.
Description of the drug[1]:	Oblate or flattened ellipsoidal, 5-9 mm long, 5-7 mm wide, about 3 mm thick seeds. Externally purplish-red or purplish-brown, smooth, lustrous, some fissured. One surface even, with 1 raised longitudinal line in the centre; the other surface slightly raised. One end dented, showing a linear hilum; the other end having a finely raised chalaza. Testa fragile; endosperm white; cotyledons 2, pale yellow, oily. Odour, slight; taste, weak.
Pretreatment of the raw drug[1]:	The ripe fruits are collected in later autumn and early winter. The seeds are collected, removed from the pulp and shell (endocarp) and dried in the sun.
Semen Ziziphus spinosae:	Remove the remained shells, break to pieces before use.
Semen Ziziphus spinosae: (stir-baked)	The clean drug is placed in a pot, stir-baked with gentle heat until the drug is inflated and the colour slightly darkened. Then it is taken out and cooled, baked to dryness or dried in the sun. The drug is broken into pieces before use.
Medicinal use[1,4]:	Insomnia, anxiety, restlessness, allergies, anorexia, prophylaxis of liver diseases and stress ulcers

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

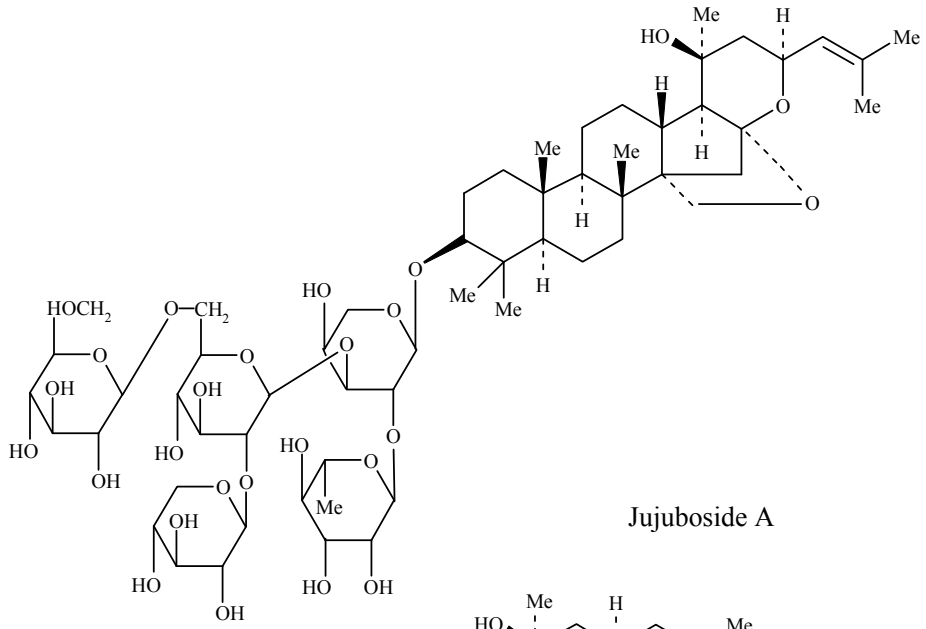
Effects and indications according to Traditional Chinese Medicine [1,3,5,6]	
Taste:	weak sweet and sour
Temperature:	neutral
Channels entered:	heart, spleen, liver, stomach, gall bladder
Effects:	supporting and additional *qi,* harmonizing and supporting *orbes*, moisturizing *ariditas*, promote the production of body fluid, arrest excessive perspiration, and cause tranquillization and balance, replenish the liver
Symptoms and indications:	dream-disturbed sleep, exhaustibility, nervousness, excessive sweating due to debility, thirst due to consumption of body fluid, palpitation, insomnia

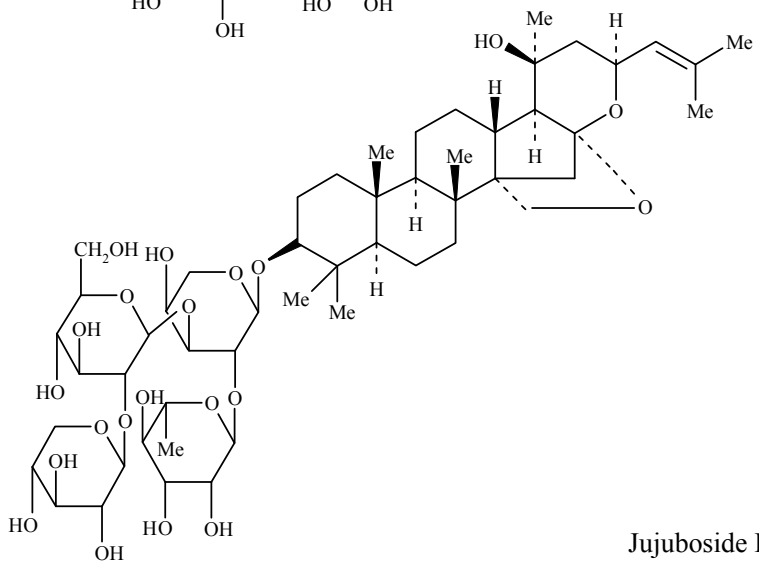
Contraindication[6]**:** The effect fails when combined with Radix Stephaniae.

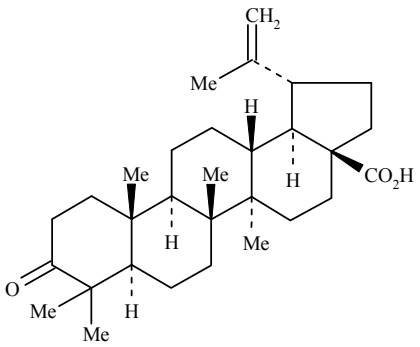
Main constituents[7]**:** (see Fig. 1)

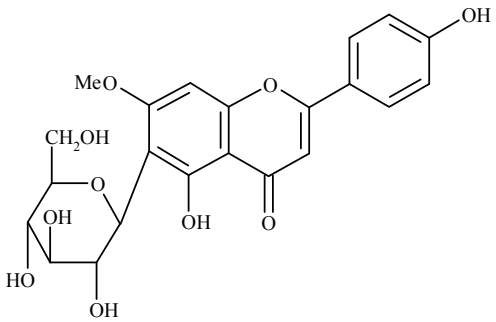
- **steroid-saponins:** jujuboside A, jujuboside B, ziziphin
- **triterpenoic acids:** betulinic acid, alphitolic acid, betulonic acid, oleanolic acid, maslinic acid and ursolic acid
- **flavone C-glycosides:** swertisin, spinosin, sinapoylspinosin, feruloylspinosin, coumaroylspinosin
- **alkaloids:** zizyphusine
- **cyclopeptide:** daechucyclopeptide-1
- **cyclic nucleotides:** adenosine-3´,5´-monophosphate, guanosine-3´,5´-monophosphate
- ascorbic acid
- fatty oil
- polysaccharides
- ferulic acid

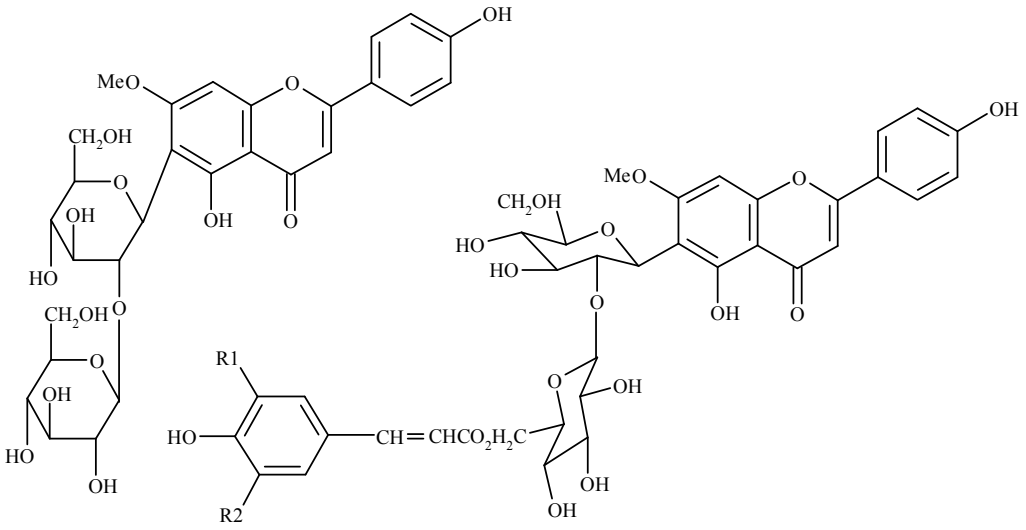
Fig. 1: Formulae of the main constituents[23]











Spinosin

Sinapoylspinosin: R1=R2=OCH_3
Feruloylspinosin: R1=H R2=OCH_3
p-Coumaroylspinosin: R1=R2=H

Pharmacology: *in vitro* and *in vivo*:
- antiallergic[8,9]
- antiinflammatory[10-14]
- analgesic[13,15,16]
- CNS depressant (jujuboside A)[17,18] and toxicology [12,13,19-23]
- hypoglycemic[24]
- immunostimulatory[11,25]
- platelet aggregation inhibiting[26]
- cytotoxic (triterpenoic acids)[27]
- sedative (flavonoids)[28]

TLC-fingerprint-analysis[29]:

1) Extraction: 2.5 g powdered drug are first defatted by heating under reflux for 2 h with 25 ml petroleum ether high boiling.
The petroleum extract is discarded and the drug residue heated under reflux for 2 h with 25 ml methanol.
The extract is filtered and the filtrate is evaporated to about 1 ml.
The residue is dissolved in 10 ml water and shaken several times with 10 ml water-saturated *n*-butanol. The *n*-butanol phase is separated and concentrated to 0.5 ml. The residue is diluted with 1.5 ml methanol and filtered over Millipore® (Type HV 0.45 µm).

2) Reference compounds: jujuboside A and B, jujubogenin 3-O-{(α-L-Rha1→2)-[β-D-Glc-(1→3)]α-L-ara}, spinosin, swertisin, ferulic acid, betulinic acid, ascorbic acid (1 mg/ml MeOH)

3) Separation parameters:

Applied amount: 15 µl extract and standard solution

Plate: HPTLC Silicagel 60 F_{254}; Merck

Solvent system: steroid-saponins and flavone C-glycosides: chloroform-glacial acetic acid-methanol- water (64:32:12:8). Equilibration of the chamber for 5 min (Fig. 2+3)

triterpenoic acids: ethyl acetate-ethanol-water-ammonia solution (25 %) (70:25:9:1) (Fig. 4).

Detection: Spray reagent:

steroid-saponins and triterpenoic acids: anisaldehyde-sulphuric acid reagent:
0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid. The TLC plate is sprayed with 10 ml, heated at 100 °C for 5-10 min and then evaluated in VIS.

flavone C-glycosides: Natural product-polyethylenglycol reagent:
The plate is sprayed successively with 1 % methanolic solution of diphenyl-boric acid-ß-ethyl-aminoester (NP) and a 5 % ethanolic polyethylenglycol-4000 solution (PEG). The evaluation is carried out in UV 365 nm.

Drug samples	Drug name/Chinese identification	Origin
1	Semen Ziziphi spin./ *Ziziphus jujuba* Mill. var. *spinosa*	sample of commercial drug, China
2	Semen Ziziphi spin./ *Ziziphus jujuba* Mill. var. *spinosa*	sample of commercial drug, China
3	Semen Ziziphi spin./ *Ziziphus jujuba* Mill. var. *spinosa*	sample of commercial drug, China
4	Semen Ziziphi spin./ *Ziziphus jujuba* Mill. var. *spinosa*	sample of commercial drug of Uchida company, Japan
5	Semen Ziziphi spin./ *Ziziphus jujuba* Mill. var. *spinosa*	sample of commercial drug of Uchida company, Japan

Reference compounds		R*f*-values
T1	jujuboside A	0.34
T2	jujuboside B	0.62
T3	jujubogenin 3-O-{(α-L-Rha1→2)-[β-D-Glc-(1→3)]α-L-ara}	0.90
T4	spinosin	0.45-0.50
T5	swertisin	0.73
T6	ferulic acid	0.97
T7	betulinic acid	0.70
T8	ascorbic acid	0.04

4) Description of the TLC-fingerprints:

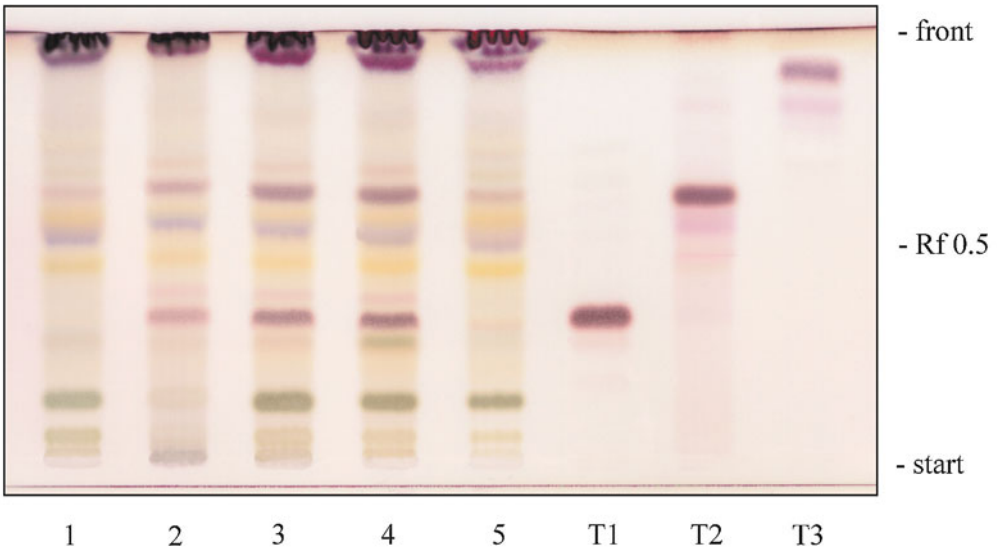


Fig. 2: Saponin TCL-fingerprint of Ziziphi semen extracts detected with anisaldehyde-sulphuric acid reagent in VIS

The TLC is characterized by a very homogenous pattern of the major saponins showing jujuboside A at R*f* 0.34 (T1), jujuboside B at R*f* 0.62 (T2) and a third Ziziphus-saponin, a jujubogenin-triglycoside at R*f* 0.90 (T3).

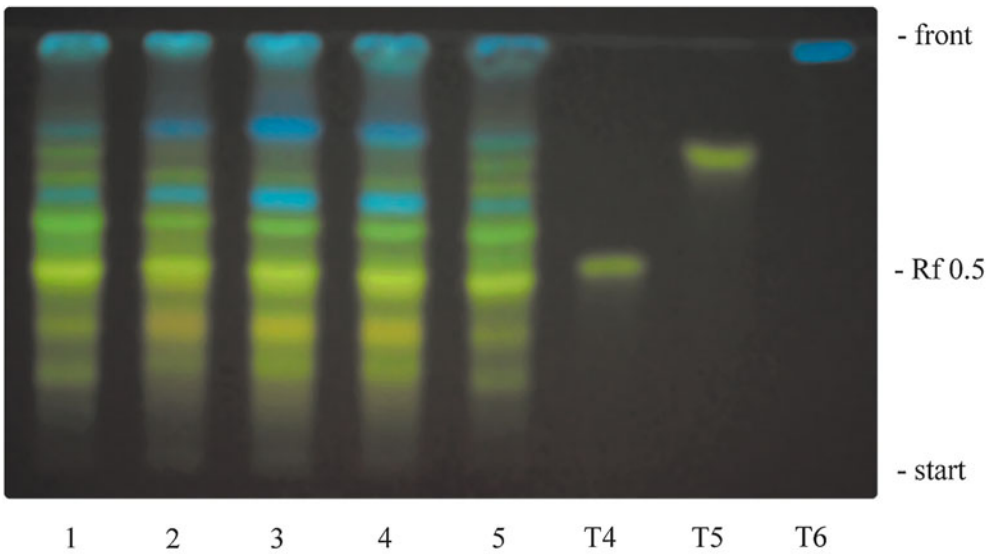


Fig. 3: Flavone C-glycosides TCL-fingerprint of Ziziphi semen extracts detected with Natural product-polyethylenglycol reagent in UV 365 nm

The TLC shows about 8 yellow and green fluorescent zones and 3 major blue fluorescent zone in the upper R*f*-range. The yellow and green fluorescent zones represent the flavone C-glycosides and their corresponding acylglycosides respectively with spinosin

at R*f* 0.46 (T4) and swertisin at R*f* 0.73 (T5). The blue fluorescent zones derive from phenolcarboxyl acids like ferulic acid (R*f* 0.97) (T6).

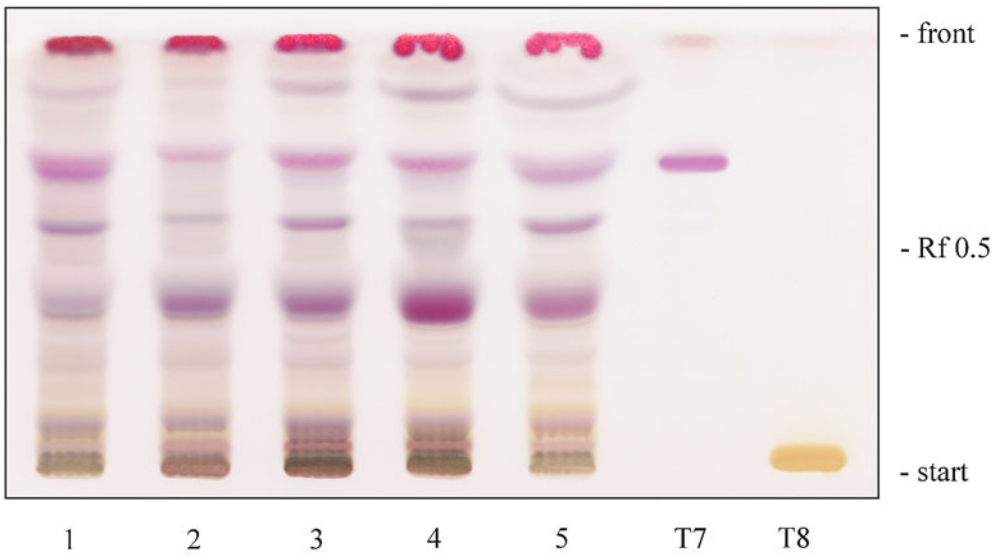


Fig. 4: Triterpenoic acid TCL-fingerprint of Ziziphi semen extracts detected with anisaldehyde-sulphuric acid reagent in VIS

The TLC of all *Ziziphus* samples shows the characteristic mixture of triterpenoic acids with the violet coloured zone of betulinic acid (R*f* 0.70) (T7) and the betulonic-, alphitolic-, maslinic- and oleanolic acids above and below the betulinic acid zone. Ascorbic acid can be found near the start at R*f* 0.04 (T8).

HPLC-fingerprint analysis:

1) Sample preparation: The extract used for TLC is injected in the HPLC-chromatograph.
2) Injection volume: 15 µl extract
3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck, Hitachi
Column:	LiChrospher® 60 RP-18 (5 µm) 250 × 4 mm with LiChrocart® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: water + 10 ml 0.1 % H_3PO_4 / l, HPLC quality, Acros Organics B: acetonitrile + 10 ml 0.1 % H_3PO_4 / l, HPLC quality Acros Organics
Gradient:	10 % B for 5 min. (isocratic) 10 % B to 20 % B in 5 min. (linear) 20 % B to 30 % B in 20 min. (linear) 30 % B to 80 % B in 10 min. (linear) 80 % B for 25 min. (isocratic)
Flow rate:	1.0 ml/min.
Detection:	210 nm

Retention times and identity of the main peaks of Fig. 5:

Peak	Rt (min.)	Compound
1	2.2	ascorbic acid
2	17.9	spinosin
3	19.2 and 19.4	swertisin and ferulic acid
4	37.4	jujuboside A
5	38.3	jujuboside B
6	57.2	betulinic acid

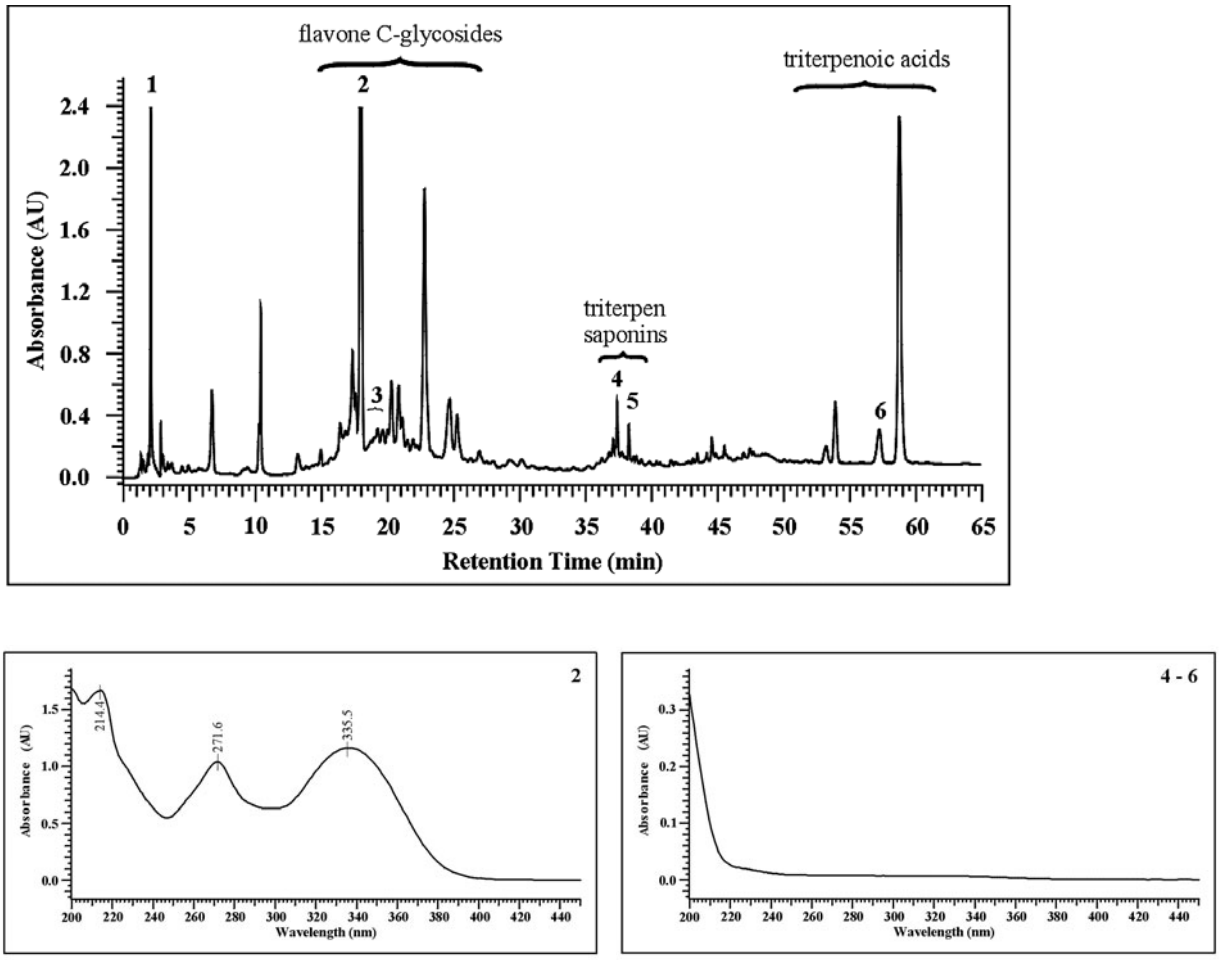


Fig. 5: HPLC-fingerprint of Ziziphi semen extract

4) Description of the HPLC-fingerprint:

The gradient solvent system used is suitable to detect all major constituents shown in the three TLC (Figs. 2, 3 and 4).

The jujuboside A and B can be detected at Rt 37.4 (**4**) and 38.3 (**5**), respectively with an endabsorption of the UV-spectra. The flavone C-glycosides show their peaks at Rt 17.9 (**2**, spinosin) and Rt 19.2 (**3**, swertisin). The online UV-spectra show the typical flavone – UV-maxima at 214.4, 271.6 and 335.5 nm.

Ascorbic acid shows a very prominent peak (**1**) at Rt 2.2, betulinic acid (**6**) at Rt 5.7.

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Fructus Amomi – *Sharen* / Fructus Amomi rotundi – *Doukou*

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drugs[1]:

Fructus Amomi
Amomum villosum Lour.
Amomum villosum Lour. var. *xanthioides* T. L. Wu et Senjen
Amomum longiligulare T. L. Wu

Fructus Amomi rotundi:
Amomum kravanh Pirrw ex Gagnep.
Amomum compactum Soland ex Maton
The drug is also classified as *Amomum cardamomum* L., "Protogenic Round Cardamon Fruit" and "Indonesian Round Cardamon Fruit" according to the different localities of production.

– Zingiberaceae –

Origin[2,3]: China (Guangdong, Guangxi, Yunnan), South-east Asia (Thailand, Vietnam, India, Indonesia)

Description of the drug[1]:

Fructus Amomi: Fruit of *Amomum villosum* and *Amomum villosum* var. x*anthioides*: Ellipsoidal or ovoid, indistinctly 3-ridged, 1.5-2 cm long, 1-1.5 cm in diameter. Externally brown, densely covered with spiny protrudings, apex with remains of perianth, and base often bearing a fruit stalk. Pericarp thin and soft. Seeds agglutinated into a mass, 3-ridged obtusely, divided into 3 groups by white septa, and each group containing 5-26 seeds. Seeds irregularly polyhedral, 2-3 mm in diameter; externally brownish-red or dark brown, finely wrinkled, covered with pale brown membranous aril; texture hard, endosperm greyish-white.
Odour, strongly aromatic; taste, pungent, cool and slightly bitter.

Fructus Amomi rotundi: Fruit of *Amomum longiligulare*: Long ellipsoidal or ovoid, distinctly 3-ridged, 1.5-2 cm long, 0.8-1.2 cm in diameter. Externally with flaky and branched soft spines, base showing a scar of fruit stalk.
Pericarp thickened and hard. Masses of seeds relatively small, each group containing 3-24 seeds, 1.5-2 mm in diameter.
Odour and taste slightly weak.

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Fructus Amomi rotundi: Fruit of *Protogenic Round Cardamon*: Subspherical, 1.2-1.8 cm in diameter. Externally yellowish-white to pale yellowish-brown with 3 relatively deep longitudinal furrows, apex possessing a prominent stylopodium, base with dented scar of fruit stalk, both ends bearing pale brown pubescence. Texture of pericarp light and brittle, easily broken longitudinally, 3 loculi, each containing about 10 seeds irregularly polyhedral, dorsal surface slightly raised, 3-4 mm in diameter, externally dark brown, with wrinkles and remains of aril. Odour, aromatic; taste pungent and cool, slightly camphor-like.

Fruit of *Indonesian Round Cardamon*: Relatively small, externally yellowish-white, sometimes slightly purplish-brown, testa relatively thin and blighted.
Odour, relatively weak.

Pretreatment of the raw drug[1]: The drugs are collected between summer and autumn when ripe, and dried in the sun or at low temperature. Foreign matters are eliminated and the drugs broken to pieces before use.

Medicinal use: The drugs are used for the treatment of gastrointestinal dyspepsia, diarrhea, flatulence, stomach pains and lack of appetite, pregnance vomiting, heart burn (eructation).

Effects and indications of Fructus Amomi according to Traditional Chinese Medicine [1,3,4]	
Taste:	pungent
Temperature:	warm
Channels entered:	spleen, stomach, kidney
Effects:	eliminates damp, promotes the flow of *qi*, improves appetite, warms the spleen, checks diarrhoea, and prevents abortion
Symptoms and indications:	accumulation of damp in the spleen and the stomach marked by epigastric stuffiness and anorexia, vomiting and diarrhoea due to deficiency-cold of the spleen and the stomach; pernicious vomiting at pregnancy, threatened abortion

Effects and indications of Fructus Amomi rotundi according to Traditional Chinese Medicine [1,3,4]	
Taste:	pungent
Temperature:	warm
Channels entered:	lung, spleen, stomach
Effects:	resolves dampness, promotes the flow of *qi*, removes stagnancy of food, warms the spleen and stomach, promotes digestion, stops vomiting
Symptoms and indications:	loss of appetite due to accumulation of turbid dampness in the spleen and stomach, feeling of suffocation in the chest with anorexia at the early stage of damp-warm syndromes, nausea, vomiting, distension and pain in the chest and abdomen caused by cold-dampness, indigestion with retention of food

Main constituents[5-7]**:** (see Fig. 1)

Fructus Amomi: bornyl acetate, camphor, borneol, nerolidol, linalool, camphene, limonene

Fructus Amomi rotundi: 1,8-cineole, d-borneol, camphor, α and β-pinene, comphene, limonene, ρ-cymene, α-terpinene, α-humulene

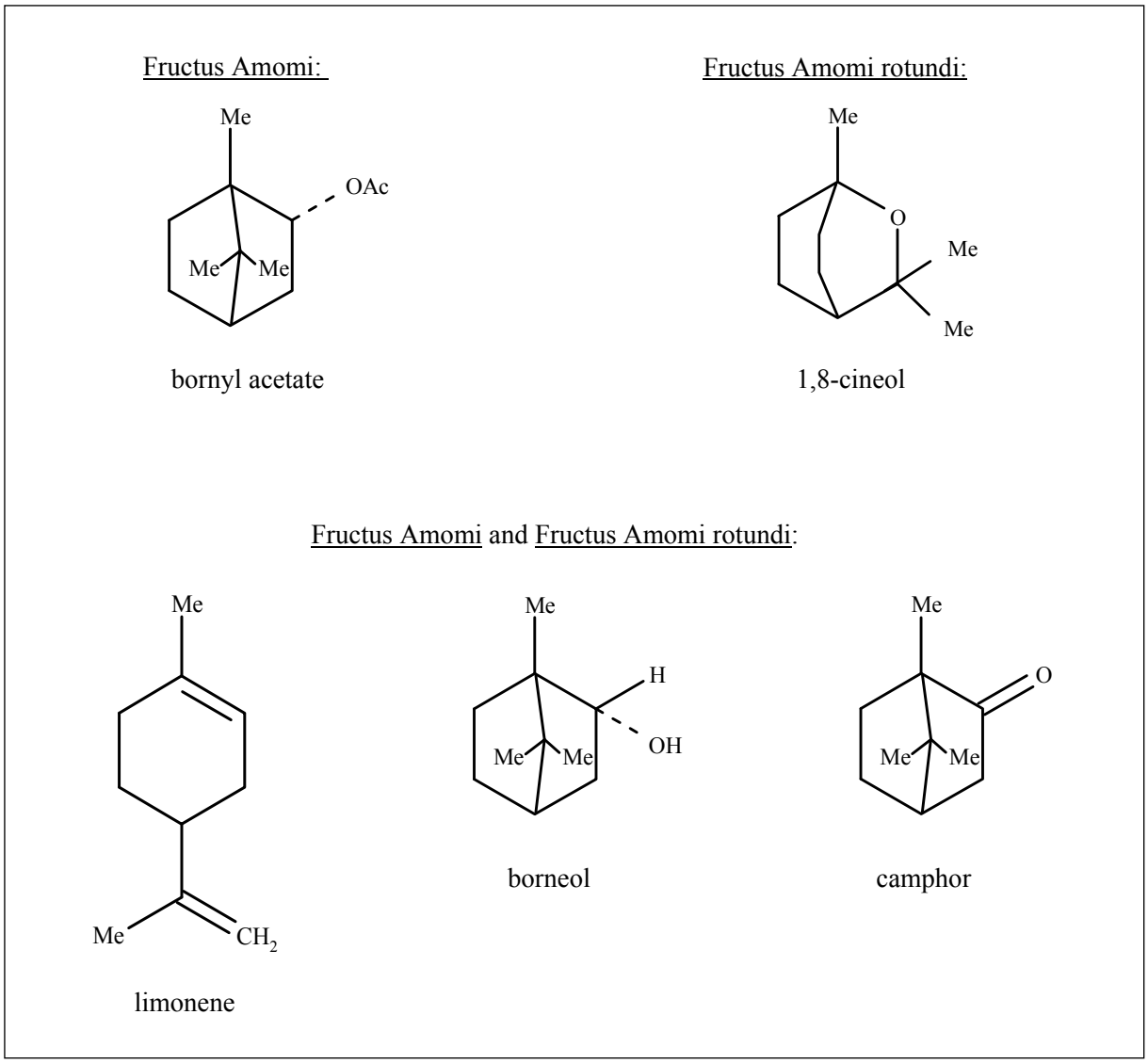

Fig. 1: Formulae of the main characteristic constituents[5]:

Pharmacology:
- inhibitory effect on gastric acid secretion (alcoholic and water extract)[8]
- protective effect against alloxan-induced diabetes (extract)[9]
- antibacterial effect (isolated alcenones)[10]

Toxicology[4]**:** Toxic adverse effects are reported when overdoses of herbal preparations are administered. The toxic symptoms (general irritations of the stomach and the urinary tract collection system) are caused by overdoses of the pungent compounds.

TLC-fingerprint-analysis[11]**:**

1) Extraction: The powdered drug is subjected to a water steam distillation in a Neo Clevenger apparat. The essential oil is diluted with xylene (Fructus Amomi 1:3 and Fructus Amomi rotundi 1:6).

2) Reference compounds/ drugs: bornyl acetate diluted with xylene (1:1), camphor dissolved in xylene (5 mg/ml), cineol diluted with xylene (1:6)

   The essential oil of Fructus Cardamomi is obtained by water distillation.

3) Separation parameters:

   Applied amount: 5 µl extract and standard solution

   Plate: TLC-Plate Silicagel 60 F_{254}; Merck

   Solvent system: toluene-ethyl acetate (93:7)

   Detection: Spray reagent:
   Vanillin-sulphuric-acid-reagent:
   The plate is intensively sprayed with 1 % ethanolic vanillin-solution, subsequently with 10 % ethanolic sulphuric acid followed by heating under supervision for 10 minutes at 110 °C.

Drug samples	Drug name/Species	Origin
1	Fructus Amomi/*Amomum villosum*	province Guangdong, China
2	Fructus Amomi/*Amomum villosum* var. *xanthioides*	province Guangdong, China
3	Fructus Amomi/*Amomum longiligulare*	province Hainan, China
4	Fructus Amomi rotundi	sample of commercial product, China
5	Fructus Amomi rotundi	sample of commercial product, China
Reference drug and compounds		
T1	Fructus Cardamomi/*Elettaria cardamomum*	Galke GmbH, Germany
T2	bornyl acetate (R*f* = 0.65)	Merck, Germany
T3	camphor (R*f* = 0.30)	Merck, Germany
T4	cineol (R*f* = 0.46)	Merck, Germany

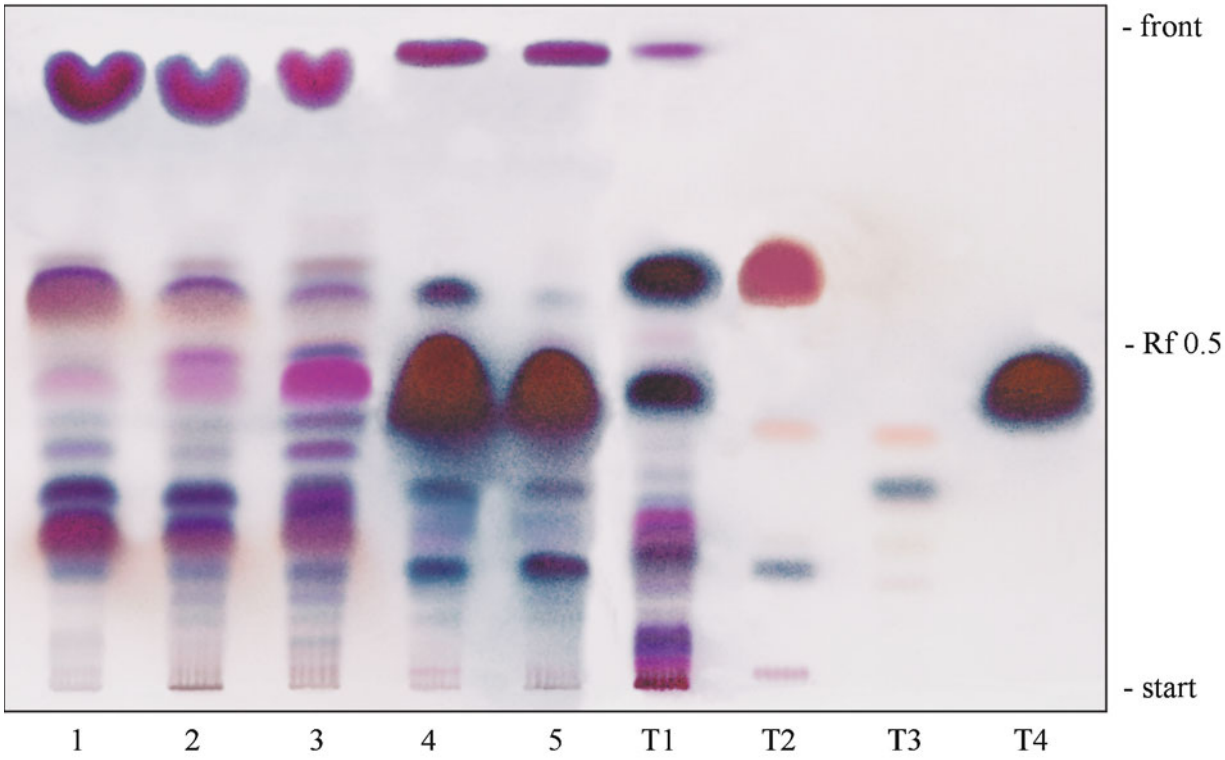

Fig. 2: Essential oil TLC-fingerprint of Fructus Amomi and Fructus Amomi rotundi detected with vanillin-sulphuric reagent

4) Description of the TLC-fingerprint sprayed with vanillin-sulphuric acid spray reagent:

The TLC-fingerprint of Fructus Amomi (sample 1-3) is characterized by a strong pink violett limonene zone at R*f* 0.93 and by 9-10 pink to bluegrey coloured zones from R*f* 0.70 down to R*f* 0.18 with the dominating pink bornyl acetate zone (T2) at R*f* 0.65 and the blue zones of camphor (T3) at R*f* 0.30 partly overlapped by linalool at R*f* 0.32. Borneol gives a weak blue-violett zone at R*f* 0.26.

The TLC-fingerprint of Fructus Amomi rotundi (sample 4,5) is dominated by a blue-brown zone of cineol (T4) at R*f* 0.46. A weak pink zone of limonene at R*f* 0.97, a blue zone of camphor (T3) at R*f* 0.30 and one of borneol at R*f* 0.26 characterize the fingerprints.

Fructus Cardamomi (T1) shows for comparison a major blue zone of α-terpinyl acetate at R*f* 0.63 and another one, cineol, at R*f* 0.46. In very low concentration appear limonene at R*f* 0.97 as pink coloured zone and α-terpineol at R*f* 0.18 as a blue coloured zone. Linalool appears at R*f* 0.32.

GC-fingerprint-analysis:

1) Sample preparation: The essential oils and standard solutions are diluted with n-hexane (1:2).

2) Injection volume: 5 µl diluted essential oil and standard solution, ratio 10:1

3) GC-data:

Apparatus:	AutoSystem Gas Chromatography, Perkin Elmer Injector system: Splitinjector Detector: flame ionization detector
Column:	SPB™-1701, Fused Silica Capillary Column 30 m × 0.25 mm × 0.25 µm film thickness; Supelco
Carrier gas:	Helium, flow rate 0.9 ml/min.
Temperature program:	30 °C to 180 °C, 3 °C/min., total run time 50 min. Injector Temperature: 210 °C Detector Temperature: 230 °C

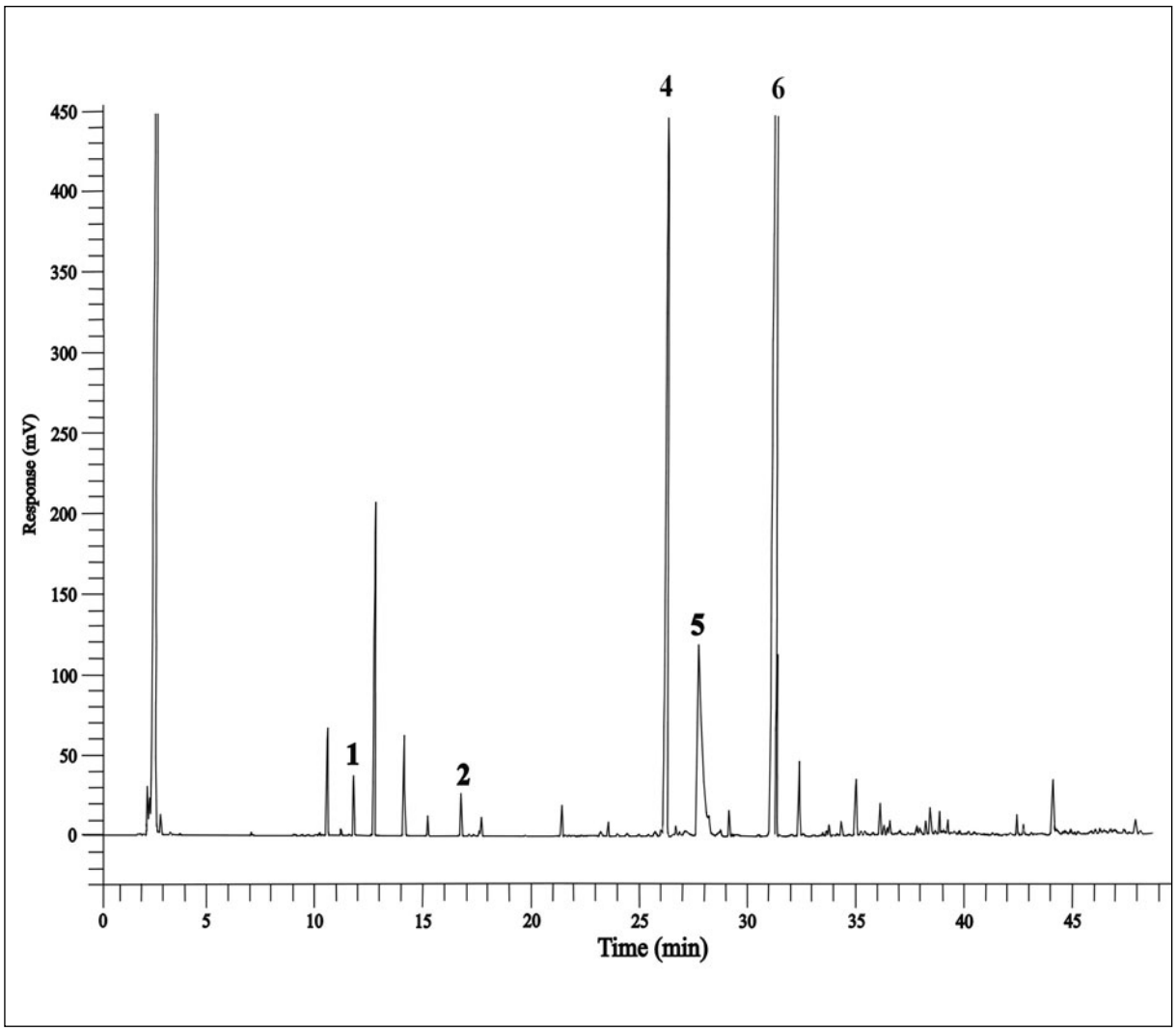

Fig. 3a: GC-fingerprint of Fructus Amomi sample 1 essential oil

Retention times and identity of the main peaks of Fig. 3a and 3b:

Peak	Rt (min.)	Compound
1	12.4	α-pinene
2	17.5	limonene
3	18.5	cineol
4	27.2	camphor
5	28.3	borneol
6	31.8	bornyl acetate

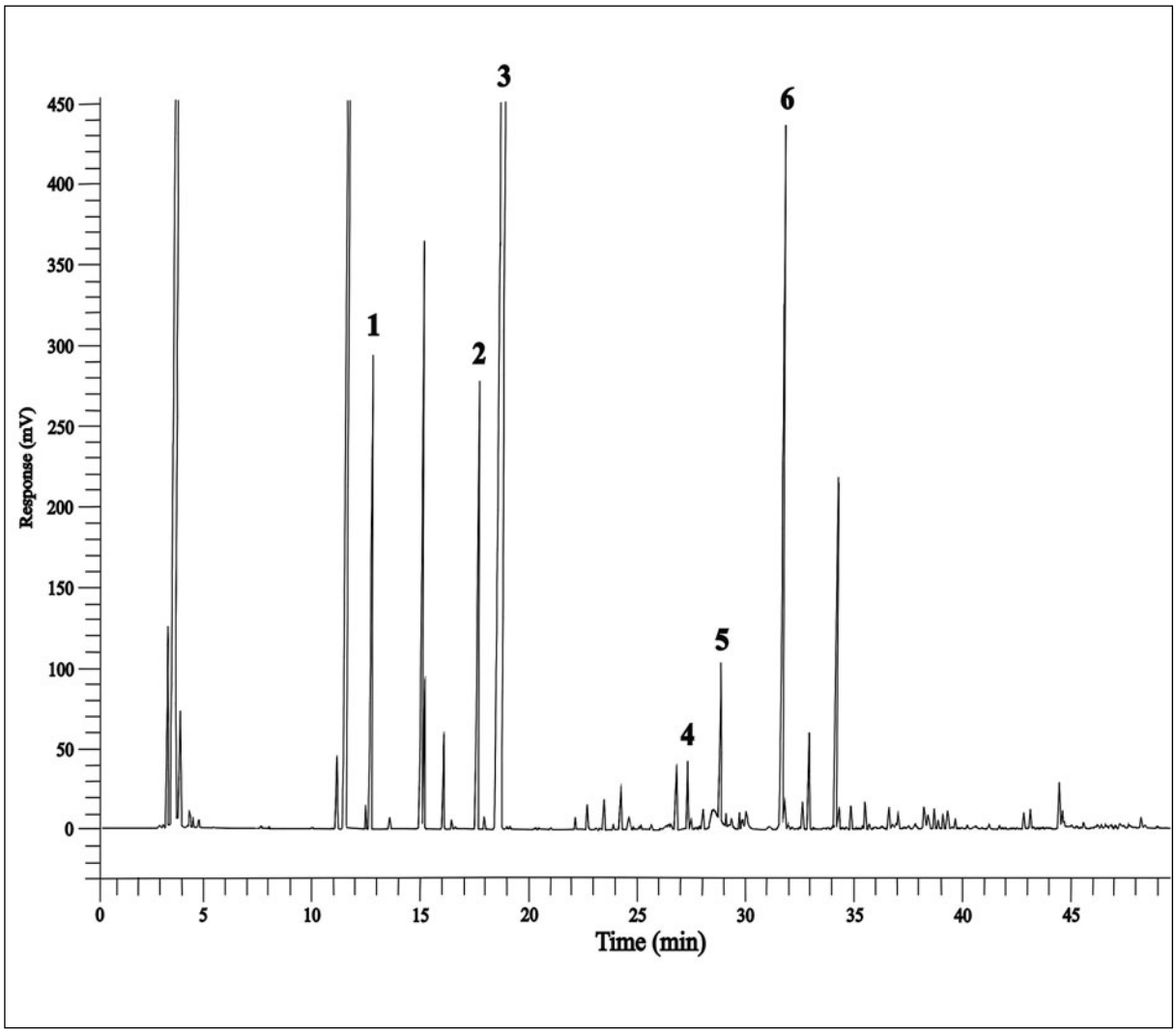

Fig. 3b: GC-fingerprint of Fructus Amomi rotundi sample 4 essential oil

4) Description of the GC-chromatogram of Fructus Amomi, Fig. 3a:

The GC-fingerprint of Fructus Amomi is charaterized by one major peak of camphor (**4**) at Rt 27.2 and bornyl acetate at Rt 31.8 (**6**). Other compounds like α-pinene (**1**, Rt 12.4), limonene (**2**, Rt 17.5) and borneol (**5**, Rt 28.3) can be identified.

Description of the GC-chromatogram of Fructus Amomi rotundi, Fig. 3b:

The GC-fingerprint of Fructus Amomi rotundi is characterized by one major peak of cineol (**3**) at Rt 18.5. The content of α-pinene (**1**, Rt 12.4), limonene (**2**, Rt 17.5), camphor (**4**, Rt 27.2), borneol (**5**, Rt 28.3) and bornyl acetate (**6**, Rt 31.8) vary in dependence of the province and season of collection.

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Ramulus Uncariae cum Uncis
Gouteng

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drugs[1,2]: *Uncaria rhynchophylla* (Miq.) Jacks.,
Uncaria macrophylla Wall.,
Uncaria hirsuta Havil.,
Uncaria sinensis (Oliv.),
Uncaria sessilifructus Roxb.

– Rubiaceae –

Origin[2]: China (Kweichow, Kwangsi, Kwangtung, Hunan, Kiangsi, Fukien), Japan and South-east Asia, Africa and Madagascar.

Description of the drug[1]: Cylindrical or subsquare, 2-3 cm long, 2-5 mm in diameter. Externally reddish-brown to purplish-red, with fine longitudinal striations and glabrous; or yellowish-green to greyish-brown, sometimes with white dotted lenticels, covered with yellowish-brown pubescences. Most nodes bearing with two opposite downward curved hooks (sterile peduncles), someones with a hook at one side and a raised scar at another side; hooks slightly flattened or rounded, apex acute, base relatively broad; dotted scars of falling petiole and ring-shaped scars of stipule visible on the branch connected with hook base. Texture hard and tenacious, fracture yellowish-brown, bark fibrous, pith yellowish-white or hollowed.
Odourless; taste, weak sweet.

Pretreatment of the raw drug[1]: The drug is collected in autumn and winter, removed from leaves, cut into section and dried in the sun.

Medicinal use[3]: For the treatment of hypertension, arrythmia, headache, dizziness, nausea, hepatitis, stroke, epileptic cramps, fever, facialis paresis, nose bleeding.

Effects and indications according to Traditional Chinese Medicine[4]	
Taste:	sweet
Temperature:	tendency cold
Channels entered:	liver meridian, pericardium meridian
Effects:	Yang diminishing, heat cooling, wind expelling, intern wind calming
Symptoms and indications:	cramps, dizziness**,** headache, fever, dampness, irritability, topical for red eyes

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Main constituents[5-7]**:** (see Fig. 1)

oxindole alkaloids:
- **tetracyclic alkaloids:** rhynchophylline, isorhynchophylline, mitraphylline, isomitraphylline, corynoxeine, corynoxine B, isocorynoxeine, cadambine, dihydrocadambine, uncarine F, geissochizine
- **pentacyclic oxindol-alkaloids:** pteropodine, isopteropodine (*Uncaria tomentosa*)[26].

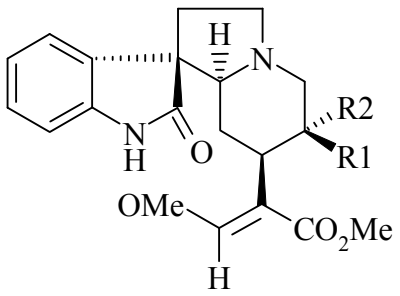
heteroyohimbin alkaloids:
- pentacyclic alkaloids: akuammigine
- **tetracyclic alkaloids:** hirsuteine, hirsutine, corynantheine, dihydro-corynantheine

pyridino-indolo-chinolizidon alkaloids: angustine, angustoline

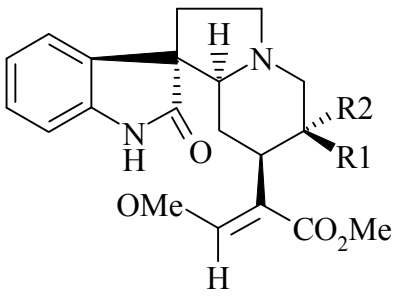
triterpenes: oleanolic acid, ursolic acid, quinovic acid derivatives

tanninoids; phenolics: catechin, epicatechin, cinchonain I + II (proanthocyanidins)

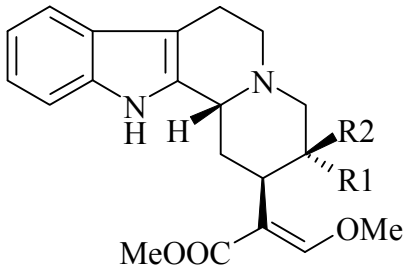
(not all alkaloids and other constituents listed are contained in each *Uncaria* species)



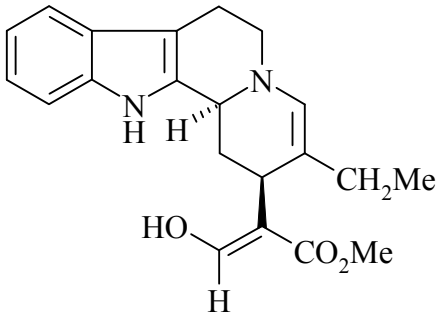
R1: H R2: $CH=CH_2$ Corynoxeine
R1: CH_2CH_3 R2: H Corynoxine B
R1: H R2: C_2H_5 Rhynchophylline



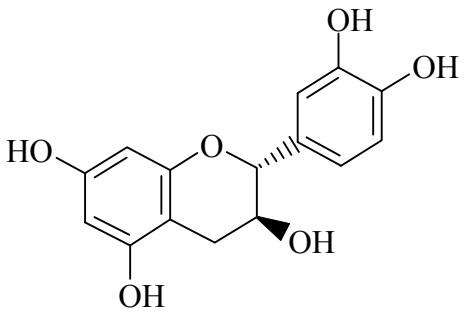
R1: H R2: $CH=CH_2$ Isocorynoxeine
R1: H R2: C_2H_5 Isorhynchophylline



R1: CH_2CH_3 R2: H Hirsutine
R1: $CH=CH_2$ R2: H Hirsuteine



Geissoschizine



Catechin

Fig. 1: Formulae of the main constituents[3,5]

Pharmacology: *in vitro* and *in vivo*
- blood pressure lowering effect (hypotensive) (alkloid mixture)[5,7,8]
- antiarrythmic (rhynchophylline/isorhynchophylline)[9]
- antiischemic (rhynchophylline/isorhynchophylline)[9]
- vasorelaxant (geissoschizine)[10]
- neuroprotective (extract)[11]

- anticonvulsive[12]
- protective effect on glutamate-induced neuronal death in cerebellar granula cells of rats (extract and indol-and oxyindol-alkaloids)[13]
- antilocomotoric effect (mice)[6,14]
- antioxidant (extract)[15]
- antiinflammatory (extract)[16]
- Ca-antagonistic effect (hirsutine)[17,18]
- 5-HT-receptor agonistic effect (various alkaloids)[19]
- serotonin antagonistic/agonistic effects (alkaloid fractions)[19]
- ganglia blocking effect (isorhynchophylline)[20]
- antidementia effect (extract)[21]
- respiratory frequency increasing effect (rhynchophylline)[22]

TLC-fingerprint-analysis:

1) Extraction:

alkaloids: ca. 2.0 g powdered drug are macerated with 5 ml ammonia solution 10 % for 10 min. The wet drug is soxleth extracted with 150 ml methanol for 4 hours. This extract is filtered and evaporated to dryness, the residue dissolved in 2 ml methanol and transferred to an separation funnel. 20 ml distilled water are added and the solution extracted two times with 20 ml ethyl acetate. The ethyl acetate phase is evaporated to dryness and dissolved in 2 ml methanol.

triterpenes and tanninoids: ca. 2.0 g powdered drug are extracted under reflux with 30 ml methanol on a 60 °C warm water bath for 15 min. The extract is filtered and the filtrate is evaporated to 2 ml.

2) Reference compounds: rhynchophylline, isorhynchophylline, corynoxine B, isocorynoxeine, corynoxeine, hirsutine, hirsuteine, isopteropodine, catechin, oleanolic acid and ursolic acid are dissolved in methanol (1 mg/ml).

3) Separation parameters:

Applied amount: 15 µl extract and standard solution

Plate: HPTLC-Silicagel 60 F254; Merck

Solvent system: alkaloids: chloroform-methanol (90: 10)

triterpenes and tanninoids: chloroform- aceton-conc. formic acid (75: 16.5: 8.5)

Detection: Direct evaluation:

alkaloids: UV 254 nm (without chemical treatment)

Spray reagents:

alkaloids: iodine reagent: 0.05 g iodine is dissolved in 10 ml ethanol 96 %. The plate is evenly sprayed with this solution until plate background appears yellow. Examination in VIS when background turned to white again.

triterpenes and tanninoids: anisaldehyde-sulphuric acid reagent: 0.5 ml anisaldehyd is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid. The TLC plate is sprayed with 10 ml, heated at 100 °C for 5-10 min. and then evaluated in VIS.

Drug samples	Drug name/Chinese identification	Origin
1	Ramulus Uncariae cum Uncis/ *Uncaria rhynchophylla*	province Jiangxi, China
2	Ramulus Uncariae cum Uncis/ *Uncaria rhynchophylla*	province Guangxi, China
3	Ramulus Uncariae cum Uncis/ *Uncaria rhynchophylla*	province Guangxi, China
4	Ramulus Uncariae cum Uncis/ *Uncaria macrophylla*	province Yunnan, China
5	Ramulus Uncariae cum Uncis/ *Uncaria sinensis*	province GuieChon, China
6	Ramulus Uncariae cum Uncis/ *Uncaria sinensis*	province Guizhou, China
7	Ramulus Uncariae cum Uncis/ *Uncaria sinensis*	sample of commercial drug, China
8	Ramulus Uncariae cum Uncis/ *Uncaria tomentosa*	sample of commercial drug, Peru

Reference compounds		R*f*-values
T1	rhynchophylline and isorhynchophylline	0.48 and 0.75
T2	corynoxine B and isocorynoxine	0.55 and 0.78
T3	corynoxeine	0.55
T4	hirsutine and hirsuteine	0.34 and 0.46
T5	pteropodine	0.81
T6	isopteropodine	0.80
T7	catechin	0.18
T8	oleanolic acid	0.95
T9	ursolic acid	0.95

4) Description of the TLC-fingerprints:
A definite identification of the various *Uncaria* spec. listed in the Chinese Pharmacopoeia requires TLC-detection of the alkaloids, triterpenoic acids and tanninoids. This can be achieved with three chromatograms only.

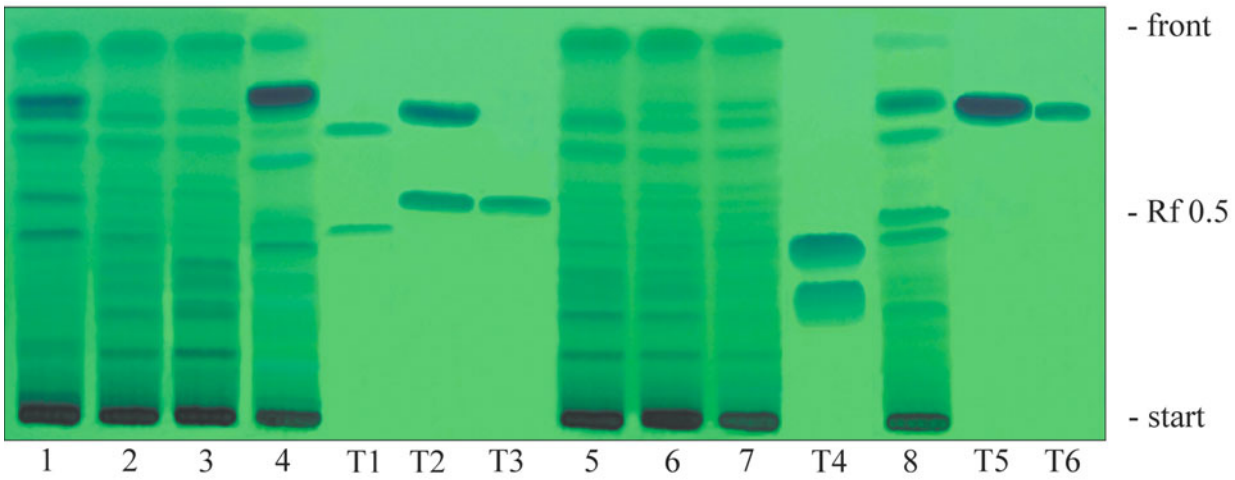


Fig. 2a: TLC-alkaloid fingerprint of the various extracts of *Uncaria* spec. in UV 254 nm

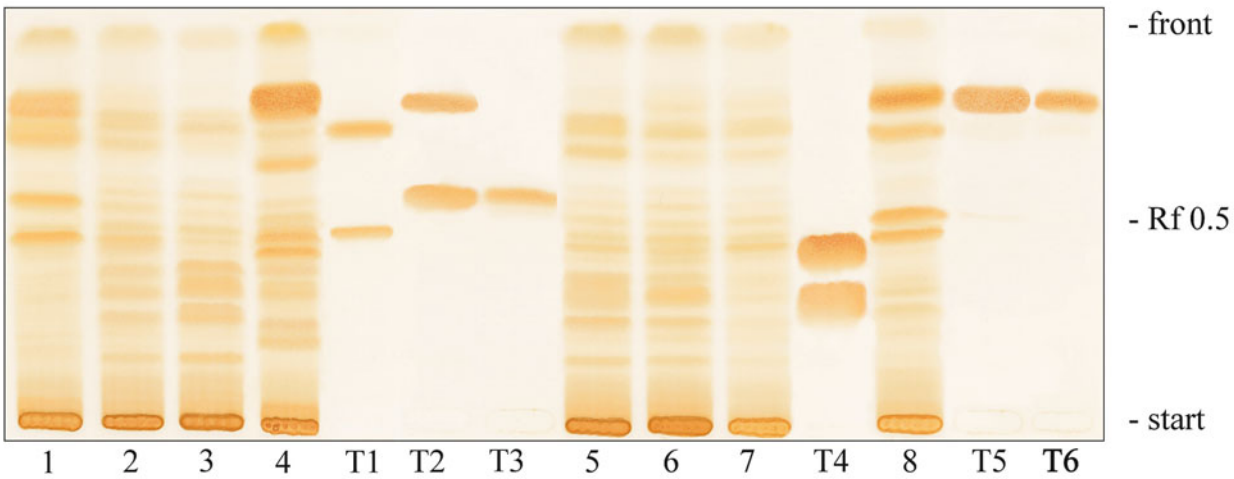


Fig. 2b: TLC-alkaloid-fingerprint of the various extracts of *Uncaria* spec., detected with iodine reagent, in VIS

The extracts of *Uncaria rhynchophylla* (sample 1-3) show (with the exception of sample 1 from Jiangxi province) a relatively homogenous alkaloid pattern with more than 10 alkaloid zones spread over the entire R*f*-range. All samples are characterized by one alkaloid zone at R*f* 0.98. Two (three) zones between R*f* 0.78 and R*f* 0.75, probably isocorynoxine and isorhynchophylline, and a sequence of very pronounced zones between R*f* 0.6 and R*f* 0.05 are detected. Corynoxine B and corynoxeine at R*f* 0.55, rhynchophylline at R*f* 0.48, hirsuteine at R*f* 0.46 and hirsutine at R*f* 0.34 can be identified within this range. Additional alkaloids which appear in the 0.15 R*f*-area might belong to the class of alkaloid glycosides, e.g. cadambine or dihydrocadambine.

The sample 1 of Jiangxi origin differs from the two others in two conspicuous strong zones at R*f* 0.78, which can be assigned to the alkaloids isocorynoxeine and isorhynchophylline. Corynoxine B and corynoxeine are also present in much higher concentrations than in sample 2 and 3.

Uncaria macrophylla (sample 4) differs from *Uncaria rhynchophylla* sample 2 and 3 and *Uncaria sinensis* by high concentrations of isocorynoxine and isorhynchophylline.

Uncaria sinensis (sample 5-7) shows an alkaloid pattern very similar to those of *Uncaria rhynchophylla* samples 2 and 3.

The "Peruvian" *Uncaria tomentosa* species, commonly known in English under "Cat's claw" and not common in TCM, but very prominent in the western traditional medicine, can be distinguished from the TCM-species by the presence of the alkaloids pteropodine (R*f* 0.81) and isopteropodine (R*f* 0.80), and the minor alkaloids speciophyllin, mitraphylline and isomitraphylline which appear in the lower R*f*-range.

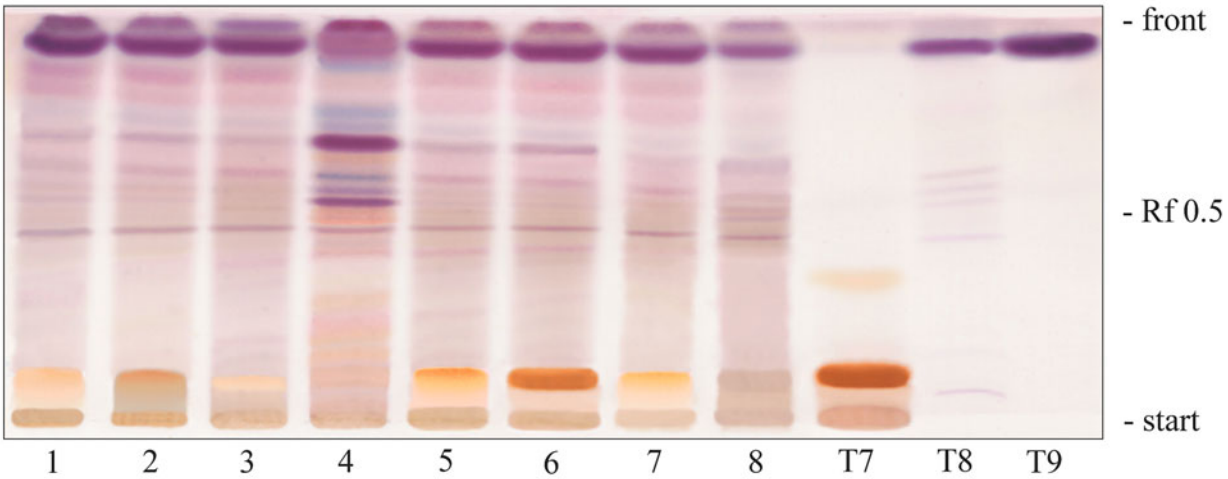


Fig. 3: TLC-fingerprint of the various methanolic extracts of *Uncaria* spec., detected with anisaldehyde-sulphuric acid reagent, in VIS

The extracts of *Uncaria rhynchophylla* and *Uncara sinensis* show a very homogenous triterpenoid-pattern with strong zones of oleanolic and ursolic acid in the R*f* range of 0.90 to 0.95 and additional weak violet zones in the R*f*-range between 0.40 and 0.88. *Uncaria macrophylla* differs from the two other *Uncaria* species by strong additional terpenoid zones between R*f* 0.5 and R*f* 0.8.

The brown-coloured zones of catechin appear in all *Uncaria* extracts at R*f* 0.18.

A comprehensive TLC-screening of sixty alkaloids of *Uncaria* species was also described by Phillipson and Hemingway[23].

HPLC-fingerprint analysis:

1) Sample preparation: The alkaloid extract used for TLC is filtered over Millipore® (Type HV 0.45 µm).

2) Injection volume: 10 µl extract

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck, Hitachi
Column:	LiChrospher® 60 RP-select B (5 µm) 125x4 with LiChrocart® 4-4 LiChrospher® 60 RP-select B (5 µm); Merck
Column temperature:	15 °C
Solvent system:	A: 10 mM phosphat puffer: pH 6.6 (Solution I: 10 mM potassium dihydrogen phosphate solution, Solution II: 10 mM di-sodium hydrogen phosphate solution. 65.3 ml solution I and 34.7 ml solution II are mixed and the pH-value is controlled with the pH-meter)
	B: acetonitrile-methanol (1: 1) for HPLC; Acros Organics
Gradient:	20 % B to 75 % B in 25 min. (linear) 75 % B for 13 min. (isocratic)
Flow rate:	1.0 ml/min.
Detection:	245 nm

Retention times and identity of the main peaks of Fig. 4a, 4b, 5, 6 and 7:

Peak	Rt (min.)	Compound
1	20.1	uncarine F
2	20.4	speciophylline
3	21.0	mitraphylline
4	21.6	isomitraphylline
5	21.7	isocorynoxeine
6	22.1	pteropodine
7	22.7	rhynchophylline
8	23.3	corynoxeine
9	24.4	isorhynchophylline
10	24.7	corynoxine B
11	24.9	isopteropodine
12	26.3	hirsuteine
13	27.8	hirsutine

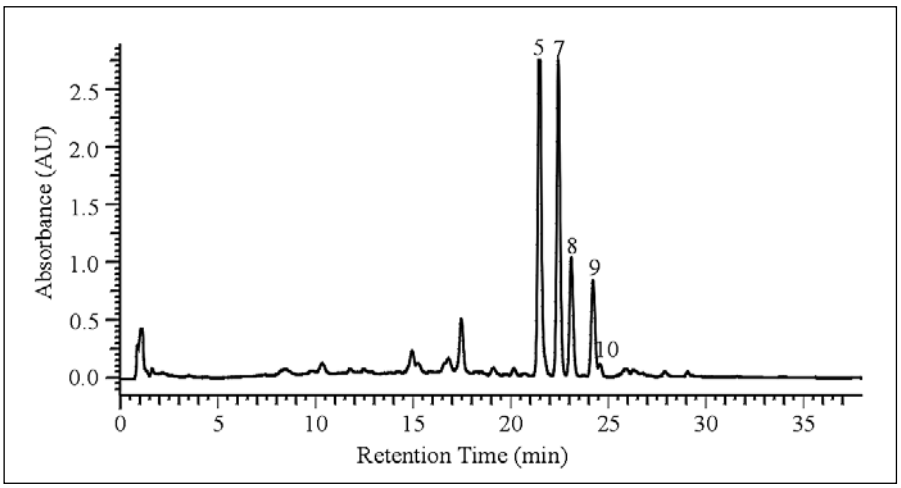


Fig. 4a: HPLC-fingerprint chromatogram of *Uncaria rhynchophylla,* sample 1

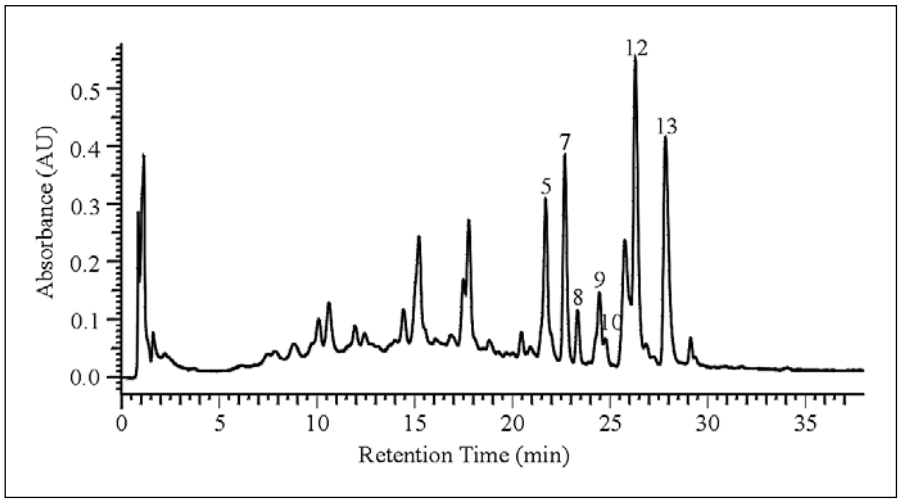


Fig. 4b: HPLC-fingerprint chromatogram of *Uncaria rhynchophylla,* sample 2

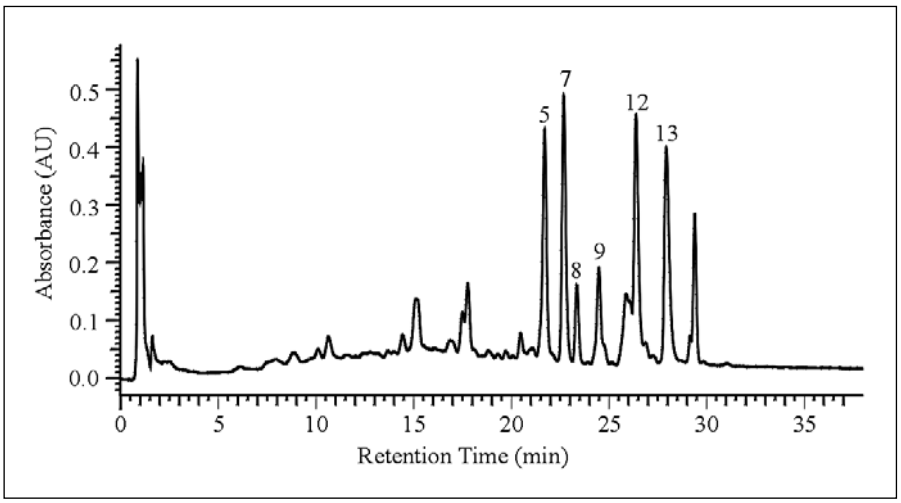


Fig. 5: HPLC-fingerprint chromatogram of *Uncaria sinensis,* sample 7

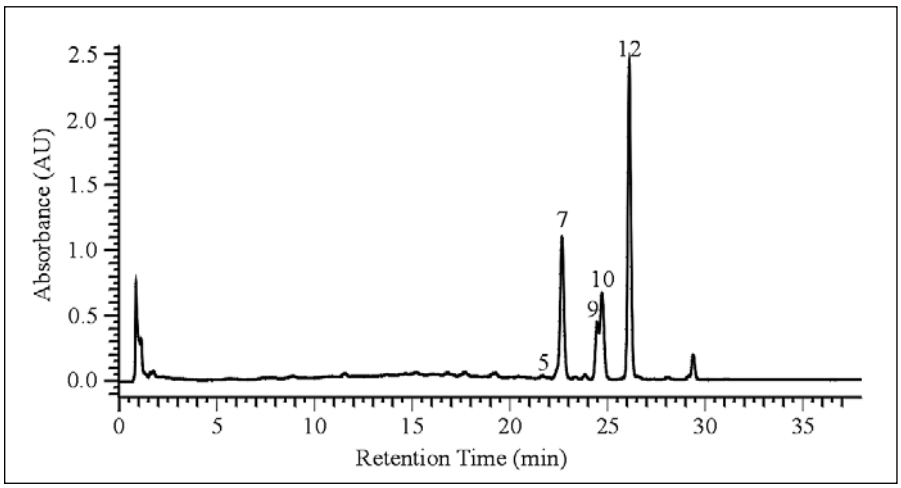


Fig. 6: HPLC-fingerprint chromatogram of *Uncaria macrophylla,* sample 4

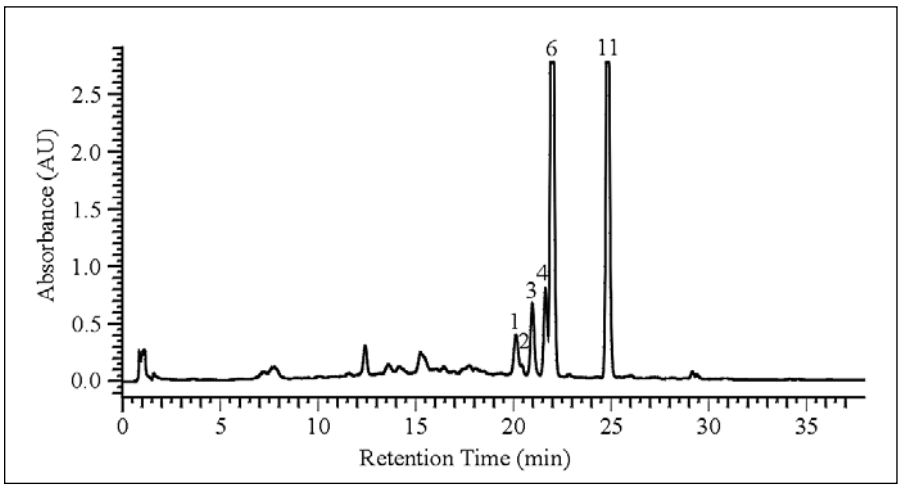


Fig. 7: HPLC-fingerprint chromatogram of *Uncaria tomentosa*, sample 8

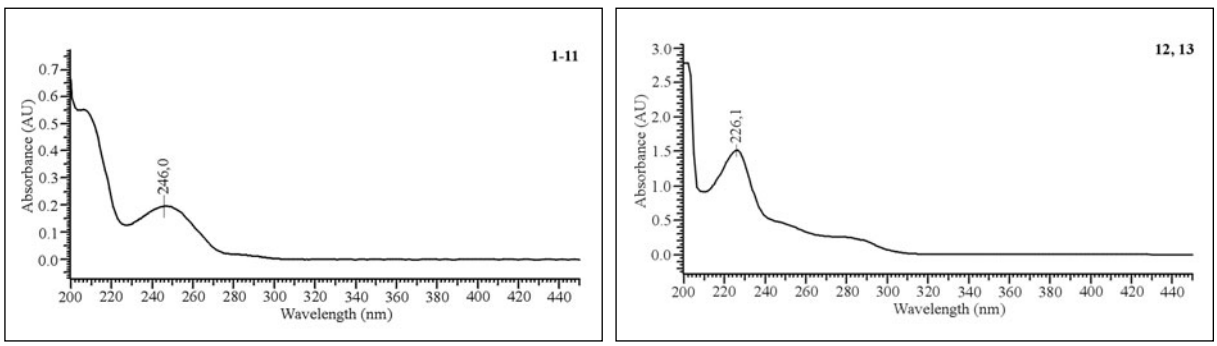


Fig. 8: The characteristic nearly superimposabel on line UV-spectra of the HPLC-peaks 1-11 (oxindole alkaloids) and the HPLC-peaks 12 and 13 (heteroyohimbin alkaloids)

4) Description of the HPLC fingerprints:

Fig. 4a, 4b and **5:** The peak patterns of *Uncaria rhynchophylla* and *Uncaria sinensis* extract samples show a nearly superimposable qualitative peak pattern. The peaks in the Rf-range 21.7 to 27.8 can be assigned to isocorynoxeine (**5**), rhynchophylline (**7**), corynoxeine (**8**), isorhynchophylline (**9**), corynoxine B (**10**), hirsuteine (**12**) and hirsutine (**13**).

Uncaria rhynchophylla sample 1 differs from samples 2 and 3 by the absence of hirsuteine (**12**) and hirsutine (**13**) (see also TLC-pattern).

Fig. 6: The HPLC profile of *Uncaria macrophylla* shows a more simple peak pattern, characterized by a very prominent peak at Rf 26.2, which can be probably assigned to hirsuteine.

Fig. 7: The alkaloid pattern of *Uncaria tomentosa*, differs from all other species in the main alkaloids isopteropodin (**11**), pteropodin (**6**), uncarine F (**1**), speciophylline (**2**), mitraphylline (**3**) and isomitraphylline (**4**).

Other HPLC-methods for the determination of oxindol alkaloids of *Uncaria tomentosa* are described by Ganzera et al.[24], Stuppner et al.[25] and Sakikabara et al.[6].

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Radix Clematidis
Weilingxian

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[1]

Official drug[1]: Chinese Clematis Root is the dried root and rhizome of *Clematis chinensis* Osbeck, *Clematis hexapetala* Pall. or *Clematis manshurica* Rupr. (Fam. Ranunculaceae). The drugs are collected in autumn, removed from soil and dried in the sun.

Origin[2]: Cultivated in the provinces of Jiang Su, An Hui and Zhe Jiang

Description of the drug[1]:

Root of *Clematis chinensis*:
Rhizomes cylindrical, 1.5 – 10 cm long, 0.3 – 1.5 cm in diameter; externally pale brownish-yellow, crowned by remains of stems, the lower part bearing numerous rootlets. Texture relatively tough, fracture fibrous. Roots slender cylindrical, somewhat curved, 7 – 15 cm long, 1 – 3 cm in diameter; externally blackish-brown, longitudinally fine-wrinkled, sometimes showing yellowish-white wood when bark falling off. Texture hard and fragile, easily broken, fracture showing bark relatively broad, wood yellowish and slightly square, often with cleft between bark and wood. Odour slight, taste weak.

Root of *Clematis hexapetala*:
Rhizomes short cylindrical, 1 – 4 cm long, 0.5 – 1 cm in diameter. Roots 4 – 20 cm long, 1 – 2 mm in diameter, externally brown to brownish-black, wood in fracture subrounded. Taste salty.

Root of *Clematis manshurica*:
Rhizomes cylindrical, 1 – 11 cm long, 0.5 – 2.5 cm in diameter. Roots relatively dense, 5 – 23 cm long, 1 – 4 cm in diameter. Externally brownish-black, wood in fracture subrounded. Taste pungent.

Pretreatment of the raw drug[1]: Foreign matters are eliminated, washed clean, softened thoroughly, cut into sections and dried.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Medicinial use[1,3,4]**:** Edema, arthralgia, rheumatic and joint pain, migraine, headache, thrombophlebitis

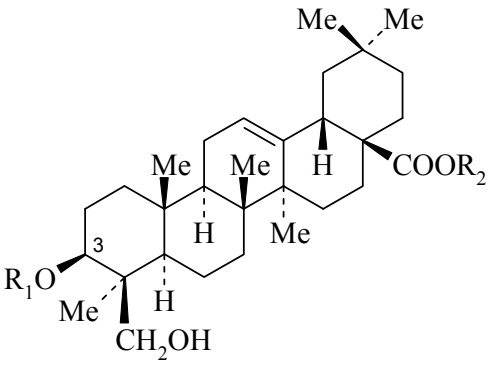
Effects and indications according to Traditional Chinese Medicine [1,2]	
Taste:	*Clematis chinensis*: weak *Clematis hexapetala*: salty *Clematis manshurica*: pungent
Temperature:	warm
Channels entered:	bladder channel
Effects (functions):	expels wind dampness to relieve pain, activates the channels, softens and dissolves fish bones when lodged in the throat
Symptoms and indications:	rheumatic or rheumatoid arthralgia caused by wind dampness, numbness of the extremities, stiffness of the joints, muscle contracture and limitation of motion, fish bone stuck in the throat

Main constituents[5]**:** *Clematis chinensis, Cl. hexapetala, Cl. manshurica:*

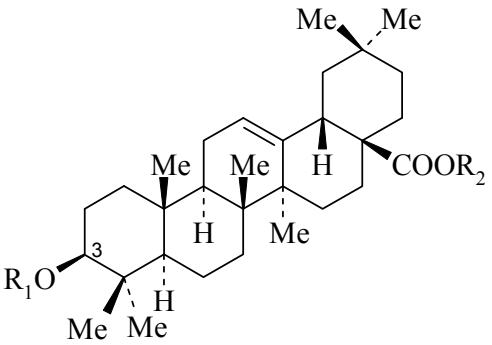
Triterpenoids:

- hederagenin and hederagenin-mono-, di-, tri-, tetra- and pentaglycosides containing glucose, rhamnose, xylose and arabinose as sugar moieties
- oleanolic acid and oleanolic acid-di-, tri-, tetra-, and pentaglycosides containing glucose, rhamnose, xylose and arabinose as sugar moieties (clematoside A, A', B, B' and C as constituents of *Clematis manshurica)*
- 4-epihederagenin as aglycone

Clematis chinensis, *Clematis hexapetala*, *Clematis manshurica*:

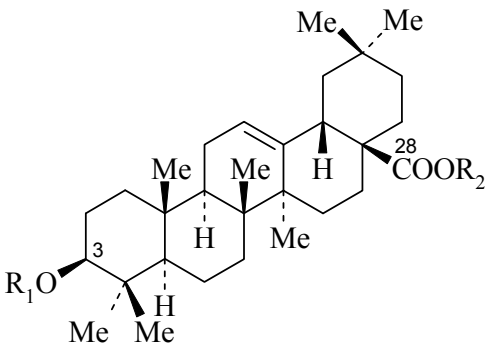


Hederagenin and Hed.-glycosides:
R_1 = oligoglycosyl (1 - 5 sugars)
R_2 = H



Oleanolic acid and Ol.-glycosides:
R_1 = oligoglycosyl (2 - 5 sugars)
R_2 = H

Clematis manshurica:



Clematoside A:
R_1 = diglucosyl-diarabinosyl-xylosyl
R_2 = triglucosyl-rhamnosyl

Fig. 1: Formulae of the main triterpenoids

Pharmacology:
- antiinflammatory[4]
- antiedemic[4]
- antitumoral[6]
- analgesic[7]
- diuretic[7]
- insecticidal[7]

TLC fingerprint analysis:

1) Extraction: 2 g of the powdered drug are extracted for 1 hour with 10 ml of ethanol, the extract filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol.

2) Reference compounds: 1 mg of oleanolic acid and the hedera-compounds are dissolved in 1 ml methanol

3) Separation parameters:

Plates: Silica gel 60 F_{254}, Merck

Applied amounts: Clematidis radix drug samples 1/2/4/6/7: each 25 µl
Clematidis radix drug samples 3/5: each 50 µl
reference compounds: each 25 µl

Solvent system: chloroform : ethyl acetate : methanol
4 3 0.4

Detection: Spray reagents:
a) Vanillin-sulphuric acid reagent:
Solution I: 1 % ethanolic vanillin solution
Solution II: 5 % ethanolic sulphuric acid

The plate is intensively sprayed with solution I and then with solution II. Subsequently the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

b) Blood-reagent:
Solution I: 3.6 % sodium citrate solution
Solution II: Phosphate buffer:
20.0 ml potassium dihydrogen phosphate solution (27.281 g potassium dihydrogen phosphate dissolved in water up to a volume of 10.0 ml) are mixed with 39.3 ml 0.1 M sodium hydroxide and filled up to 100 ml with water.

10 ml of solution I are added to 90 ml fresh bovine blood. 2 ml of this blood solution are then mixed with 30 ml solution II. The plate is sprayed in a horizontal position.

Detection of saponins: white zones are formed against the reddish background of the plate. Hemolysis may occur immediately or after the plate has been dried.

Drug samples		Origin
1	Clematidis radix / *Clematis chinensis*	sample of commercial drug, China
2	Clematidis radix / *Clematis chinensis*	sample of commercial drug, China
3	Clematidis radix / *Clematis chinensis*	province Jiangxi, China
4	Clematidis radix / *Clematis chinensis*	sample of commercial drug, China
5	Clematidis radix / *Clematis chinensis*	sample of commercial drug, China
6	Clematidis radix / *Clematis hexapetala*	province Hebei, China
7	Clematidis radix / *Clematis manshurica*	province Liaoning, China

Reference compounds		R*f*
T1	oleanolic acid	0.95
T1	hederagenin-3-O-arabinoside (isol. from Hederae helicis herba)	~ 0.85
T1	hederagenin-3-O-arabinosyl-rhamnoside (isol. from Hederae helicis herba)	~ 0.80
T2	hederagenin (isol. from Hederae helicis herba)	0.93
T2	hederasaponin-mixture (isol. from Hederae helicis herba)	~ 0.5 / 0.6

4) Description of the TLC-chromatograms:

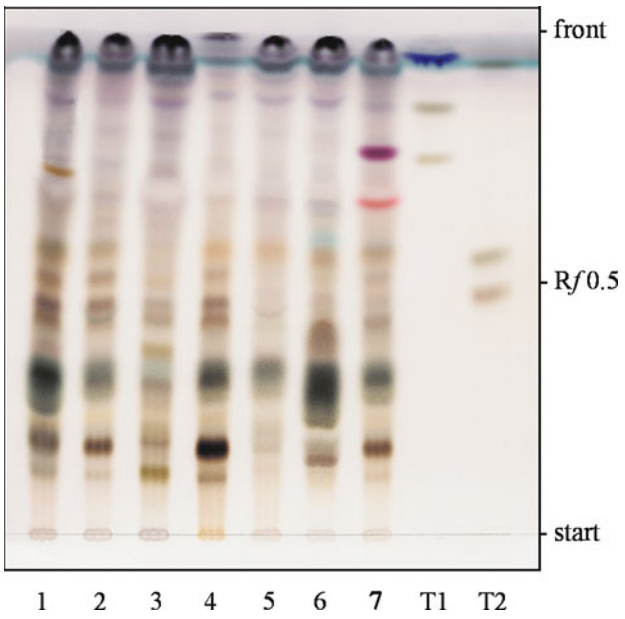


Fig. 2a:
Thin layer chromatogram of ethanolic extracts of Clematidis radix after spraying with Vanillin-sulphuric acid reagent (VIS)

All samples of Clematidis radix show a similar pattern of more than 10 grey or violet-grey zones distributed over the entire R*f*-distance, representing the various hederagenin- and oleanolic acid-glycosides described in the literature.

The triterpen-mono-, di- and triglycosides appear in lower concentration in the R*f*-range 0.45 to 0.95, whereas the higher glycosylated saponins are located in the low R*f*-range from 0.15 to 0.45. A discrimination of the root of *Clematis chinensis*, *Clematis hexapetala* and *Clematis manshurica* by TLC alone is not possible.

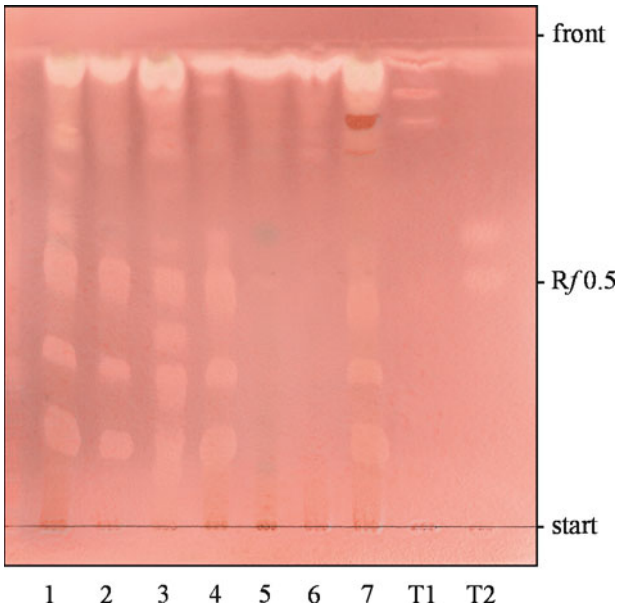


Fig. 2b:
Thin layer chromatogram of ethanolic extracts of Clematidis radix after spraying with Blood reagent (VIS)

All major zones which show a positiv reaction with the vanillin-sulfuric acid reagent also react with blood reagent, forming characteristic white hemolytic zones against a reddish background and thereby confirm their triterpen-saponin nature.

HPLC-fingerprint analysis:

1) Sample preparation: 2 g of the powdered drug are extracted 1 hour with 10 ml ethanol, then the extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol, filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: *Clematis chinensis*, *Clematis hexapetala* and *Clematis manshurica*: each 10.0 µl

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 125-4 with LiChrospher® 60 RP select B (5 µm), Merck

Precolumn: LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck

Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing) (pH = 3) B: acetonitrile (Acros Organics)
Gradient:	5 % B in 5 minutes 5 - 20 % B in 5 minutes 20 % B in 20 minutes 20- 90 % B in 25 minutes 90 % B in 5 minutes total runtime: 60 minutes
Flow:	1.0 ml/min.
Detection:	210 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1, 3 – 6, 8, 9	12.0 – 54.0	hederagenin- or oleanolic acid-glycosides
2, 7	18.3, 39.5	flavonoids?
10	54.7 – 55.0	oleanolic acid

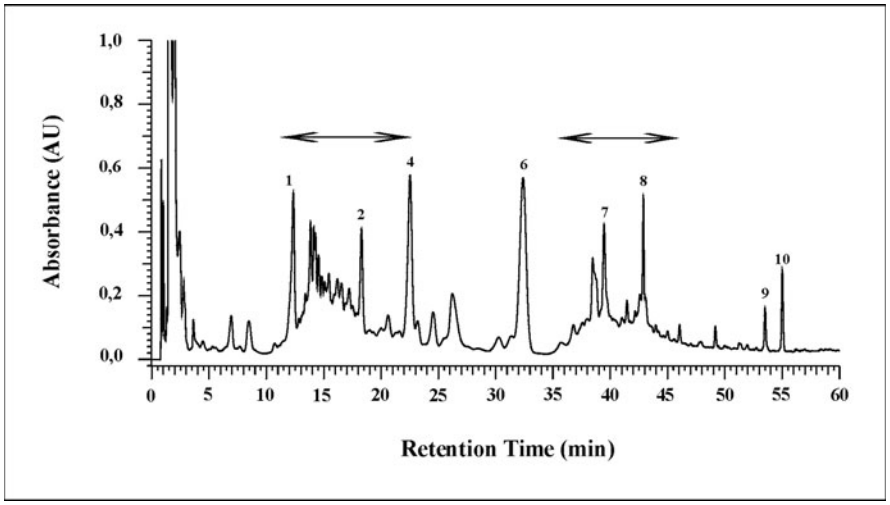


Fig. 3a: HPLC-fingerprint chromatogram of *Clematis chinensis* root extract

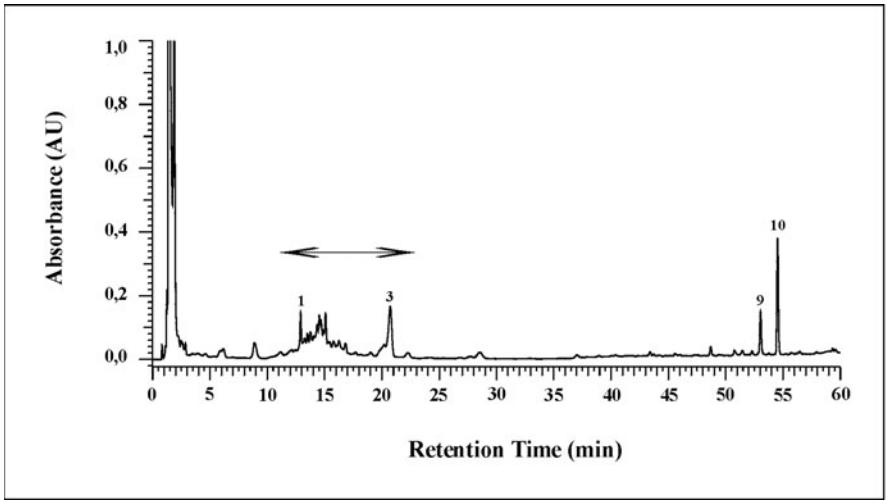


Fig. 3b: HPLC-fingerprint chromatogram of *Clematis hexapetala* root extract

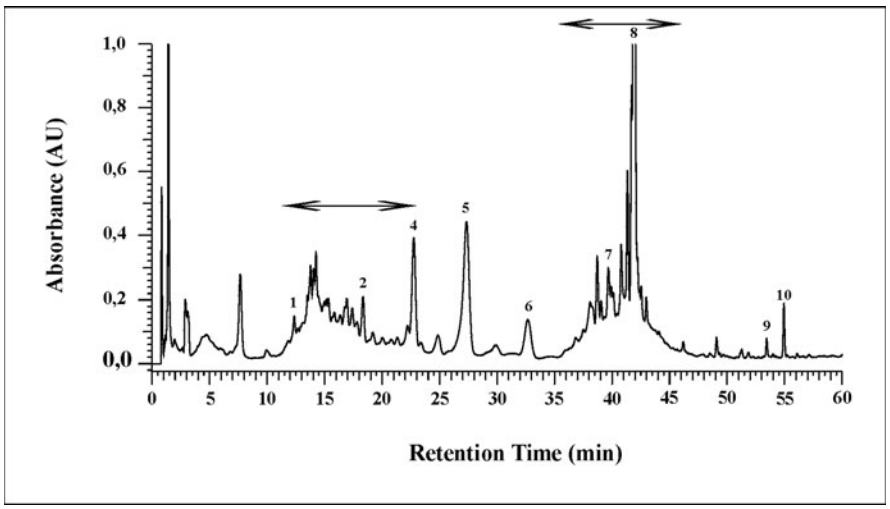


Fig. 3c: HPLC-fingerprint chromatogram of *Clematis manshurica* root extract

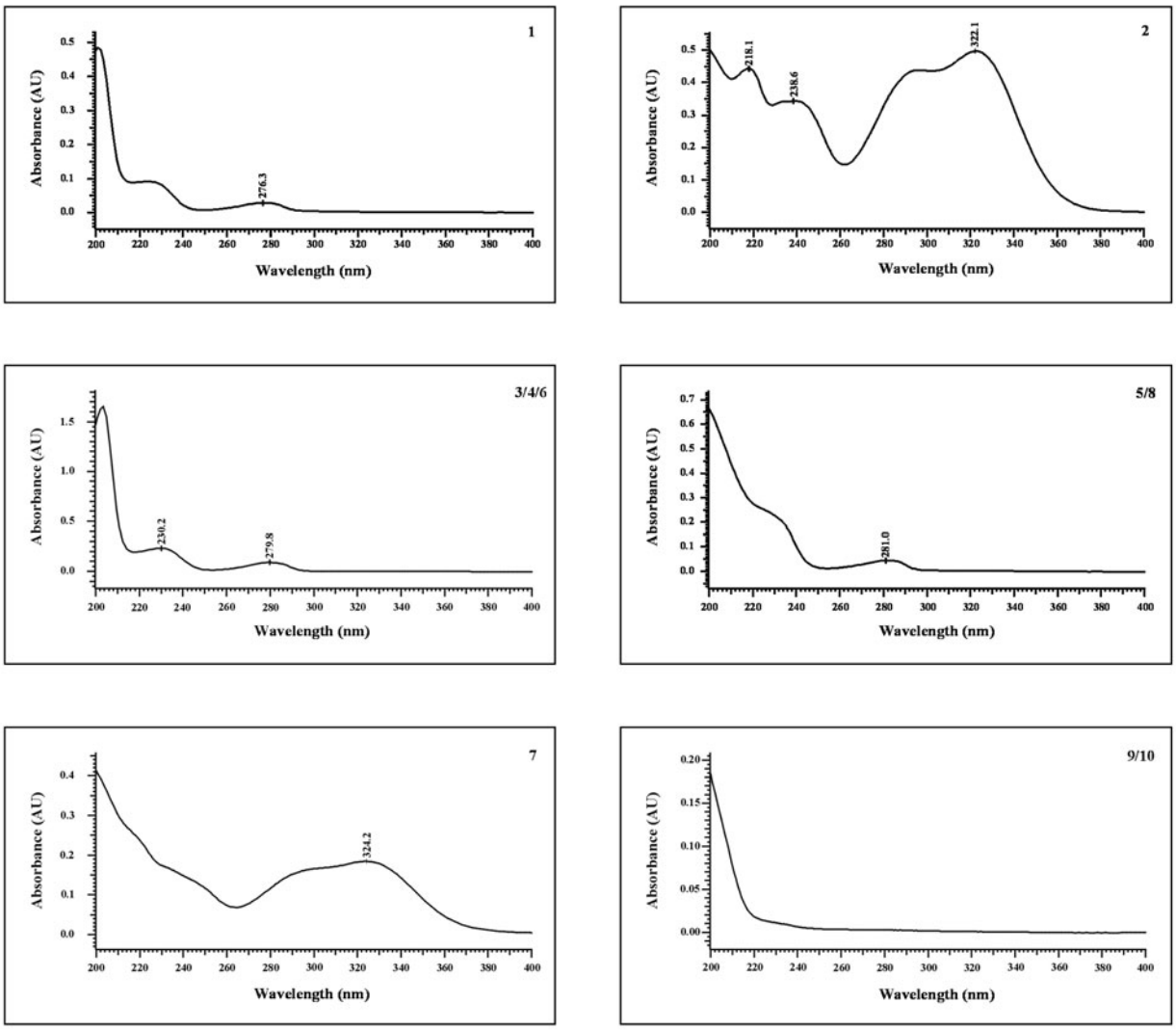


Fig. 4: UV-spectra of the main compounds (peak) of the root extract of *Clematis chinensis*, *hexapetala* and *manshurica*

4) Description of the HPLC chromatogram:

Figure 3a:
The HPLC-fingerprint of the *Clematis chinensis* root extract is characterized by two major peak complexes in the Rt-range 11.0 – 23.0 (↔) and Rt-range 35.0 – 46.0 (↔), which represent (with the exception of peak **2** and **7**) the highly glycosylated and the low glycosylated triterpen saponins, respectively. The UV-spectra of peak **2** and **7** indicate the presence of flavonoids.

Figure 3b:
The HPLC-fingerprint of *Clematis hexapetala* root is characterized by the peak complex in the Rt-range 11.0 – 23.0 (↔) only. The peaks **9** and **10** correlate with those of *Clematis chinensis* and *Clematis manshurica.*

Figure 3c:
The HPLC-fingerprint of *Clematis manshurica* root extract shows a peak pattern very similar to that of *Clematis chinensis* with the exception of a higher concentration of the low glycosylated saponins.

Proof of Clematidis radix on the absence of Aristolochiae radix

Since Clematidis radix can be changed by mistake or adulterated with the Caulis Clematidis armandii (Chuanmutong) which itself can be falsified by Aristolochiae manshuriensis caulis (Guanmutong), it is imperative to proof Clematis root on the absence of aristolochic acid[4,8].

TLC-fingerprint analysis:

1) Extraction: 2 g of the powdered drug are extracted 1 hour with 10 ml of ethanol. Afterwards the extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol.

2) Reference compound: aristolochic acid: 1 mg is dissolved in 1 ml chloroform

3) Separation parameters:

Plates: Silica gel 60 F_{254} Merck

Applied amounts: Clematidis radix drug samples 1/2/4/6/7: each 25 µl
Clematidis radix drug samples 3/5: each 50 µl
artificial mixtures 8/9: each 25 µl
Aristolochiae radix extract 10: 25 µl
reference compound: 25 µl

Solvent system: toluol : ethyl acetate : dist. water : formic acid (upper phase)
20 10 1 1

Detection: Tin-(II)-chloride reagent:
1.5 ml hydrochloric acid (36 %) is diluted with 8 ml water. 1 g of tin-(II)-chloride x 2 H_2O is dissolved in this mixture. This reagent has to be prepared always freshly.
The plate is sprayed until slightly wet and then for 5 minute heated at 100 °C.

The tin-(II)-chloride reagent for the identification of aristolochic acids in herbal drugs has been first applied in DAC [9]. A HPTLC method for prooving Clematidis radix on impurities or a falsification by Aristolochiae radix has been described by Blatter and Reich (2004)[8].

Drug samples		Origin
1	Clematidis radix chinensis / *Clematis chinensis*	sample of commercial drug, China
2	Clematidis radix chinensis / *Clematis chinensis*	sample of commercial drug, China
3	Clematidis radix chinensis / *Clematis chinensis*	province Jiangxi, China
4	Clematidis radix chinensis / *Clematis chinensis*	sample of commercial drug, China
5	Clematidis radix chinensis / *Clematis chinensis*	sample of commercial drug, China
6	Clematidis radix hexapetala / *Clematis hexapetala*	province Hebei, China
7	Clematidis radix manshurica / *Clematis manshurica*	province Liaoning, China
8	artificial mixture of drug samples 1 and 10 (95 : 5)	
9	artificial mixture of drug samples 1 and 10 (80 : 20)	
10	Aristolochiae radix / *Aristolochia fangchi* (Guangfangji)	sample of commercial drug, China

Reference compound		R*f*
T 1	Aristolochic acid I, II, III and IV	0.52 - 0.34

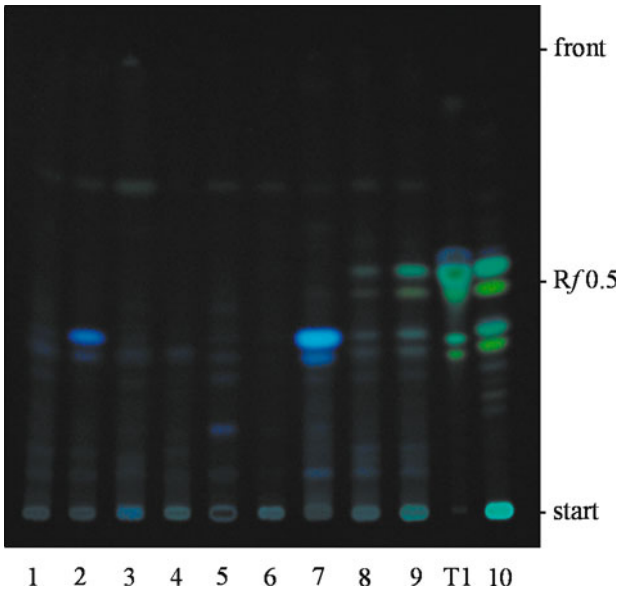


Fig. 5:
Thin layer chromatogram of ethanolic extracts of Clematidis radix, Aristolochiae radix and artificial Clematis-Aristolochia-mixtures after spraying with tin-(II)-chloride reagent (UV 365 nm)

4) Description of the TLC proof on the absence of aristolochic acid (Figure 5):

Green fluorescent zones could not be detected in any of the Clematidis radix extract samples. Aristolochic acid I, II, III and IV (T1) appear in the R*f*-range of 0.34 – 0.52, as shown in samples 8, 9 and 10. The blue fluorescent zones, seen especially in samples 2 and 7 at R*f* = 0.38, originate from coumarins or phenolcarboxylic acids characteristic of Clematis roots.

HPLC-fingerprint analysis:

1) Sample preparation: 2.0 g of pulverised roots of *Clematis* and *Aristolochia* are extracted separately 1 hour with 10 ml of ethanol. The extracts are filtered and the filtrates evaporated to dryness. The residues are dissolved in 20 ml ethyl acetate, each solution is given into a separation funnel and shaken with about 20 ml water. The ethyl acetate phases are separated and evaporated to dryness. Each residue is dissolved in 1 ml ethanol and filtered over Millipore® filtration unit, type 0.45 µm. Clematis root and Aristolochia root extract are mixed in a ratio of 50 : 50 and 80 : 20 and injected into the HPLC apparatus.

2) Injection volume: mixture 50 : 50 and 80 : 20: each 10 µl

3) HPLC parameter:

Apparatus:
MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 125-4 with LiChrospher® 60 RP select B (5 µm), Merck

Precolumn: LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck

Solvent: A: 2.0 g hexanesulfonic acid (Aldrich)/1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing) (pH = 3)
B: acetonitrile (Acros Organics)

Gradient: 10 – 50 % B in 25 min.,
50 B in 5 min.,
total runtime: 30 min.

Flow rate: 1.0 ml/min.

Detection: 254 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	26.0	Aristolochic acid II
2	27.4	Aristolochic acid I

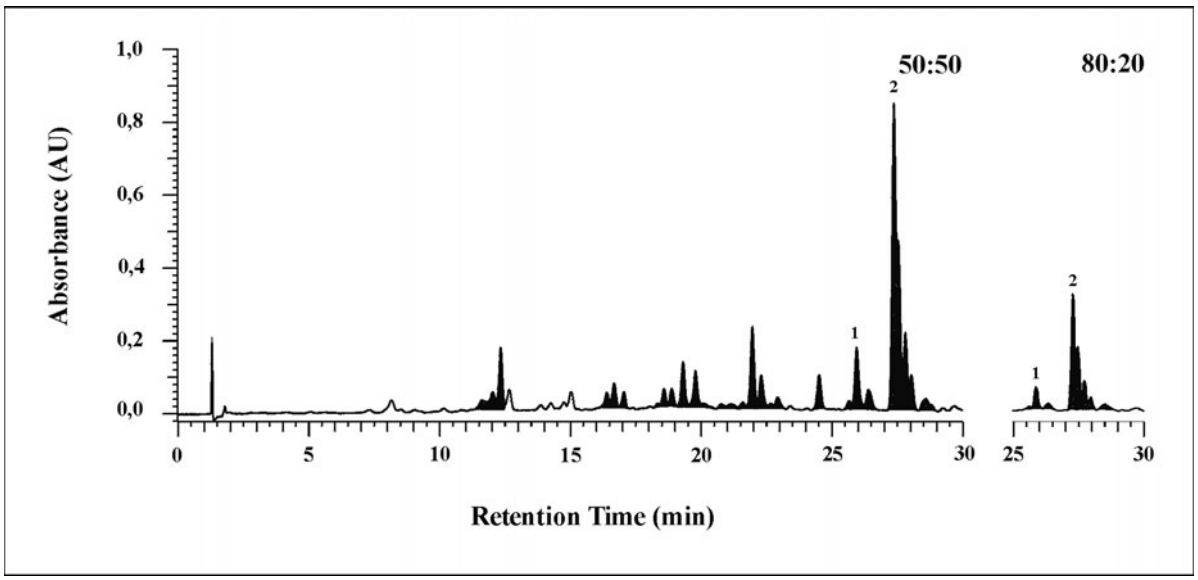


Fig. 6: HPLC-fingerprint of the artificial mixtures of ethyl acetate extract samples 1 and 10 (50 : 50 and 80 : 20)

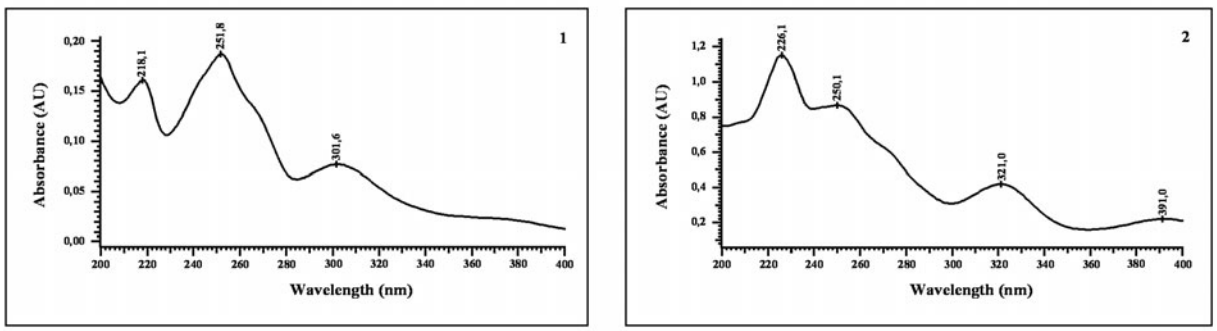


Fig. 7: On line UV-spectra of the main HPLC-peaks of Aristolochiae radix fangchi (aristolochic acid 1 and 2)

4) Description of the HPLC-fingerprint of Figure 6:

Aristolochic acids I and II appear in the HPLC-fingerprint of the two artificial extract mixtures of Clematidis radix chinensis and Aristolochiae radix (50 : 50 and 80 : 20) in the prominent black marked peaks at Rt = 26.0 (**1**) and Rt = 27.4 (**2**). The peaks of aristolochic acids III and IV appear in the Rt-range of 20.0 to 22.0. The other black marked peaks between Rt = 11.5 and 24.5 derive from other Aristolochiae radix constituents. The minor peaks at Rt = 8.1, 12.7 and 15.0 (white) originate from Clematidis radix.

Through this method, still 6 µg aristolochic acid/g herbal drug can be detected[9].

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Caulis Sinomenii
Qingfengteng

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[1]

Official drug[1]: Orientvine Stem is the dried lianoid stem of *Sinomenium acutum* (Thunb.) Rehd. et Wils. or *Sinomenium acutum* (Thunb.) Rehd. et Wils. var. *cinereum* Rehd. et Wils. (Fam. Menispermaceae).

The drug is collected in late autumn and early winter, tied up in bundle or cut into long section, and dried in the sun.

Description of the drug[1]: Long cylindrical, usually somewhat curved, 20 – 70 cm long or more, 0.5 – 2 cm in diameter. Externally greenish-brown to brown, some greyish-brown, with fine longitudinal striations and lenticels. Nodes slightly swollen and branched. Texture light, hard and fragile, easily broken, fracture uneven, greyish-yellow or pale greyish-brown, bark narrow, wood rays arranged radially, pith pale yellowish-white or yellowish-brown. Odour, slight; taste, bitter.

Pretreatment of the raw drug[1]: Foreign matters are eliminated, soaked briefly, softened thoroughly, cut into thick slices and dried.

Medicinial use[1]: rheumatic diseases (arthritis)

Effects and indications according to Traditional Chinese Medicine[1]	
Taste:	bitter, acrid, mild
Temperature:	neutral
Channels entered:	liver meridian and spleen meridian
Effects (functions):	relieves rheumatic condition, removes obstruction of the channels and collaterals, causes diuresis
Symptoms and indications:	rheumatic joint pain and swelling, paralysis, itching

Main constituents:

- **Aporphinalkaloids:**
  magnoflorine[2,4], liriodenine[3,4], N-demethyl-N-formyldehydronuciferine[5], Tuduranine[5]
- **Phenanthrenalkaloids:**
  Sinomenine[5], Sinoacutine[5], Isosinomenine[6]

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

- **Protoberberine alkaloids:**
  Sinactine[5]
- Other alkaloids:
  Acutumidine[5], Acutumine[5], Acutuminine[6]
- Cumarin:
  Scopoletine[4,7]

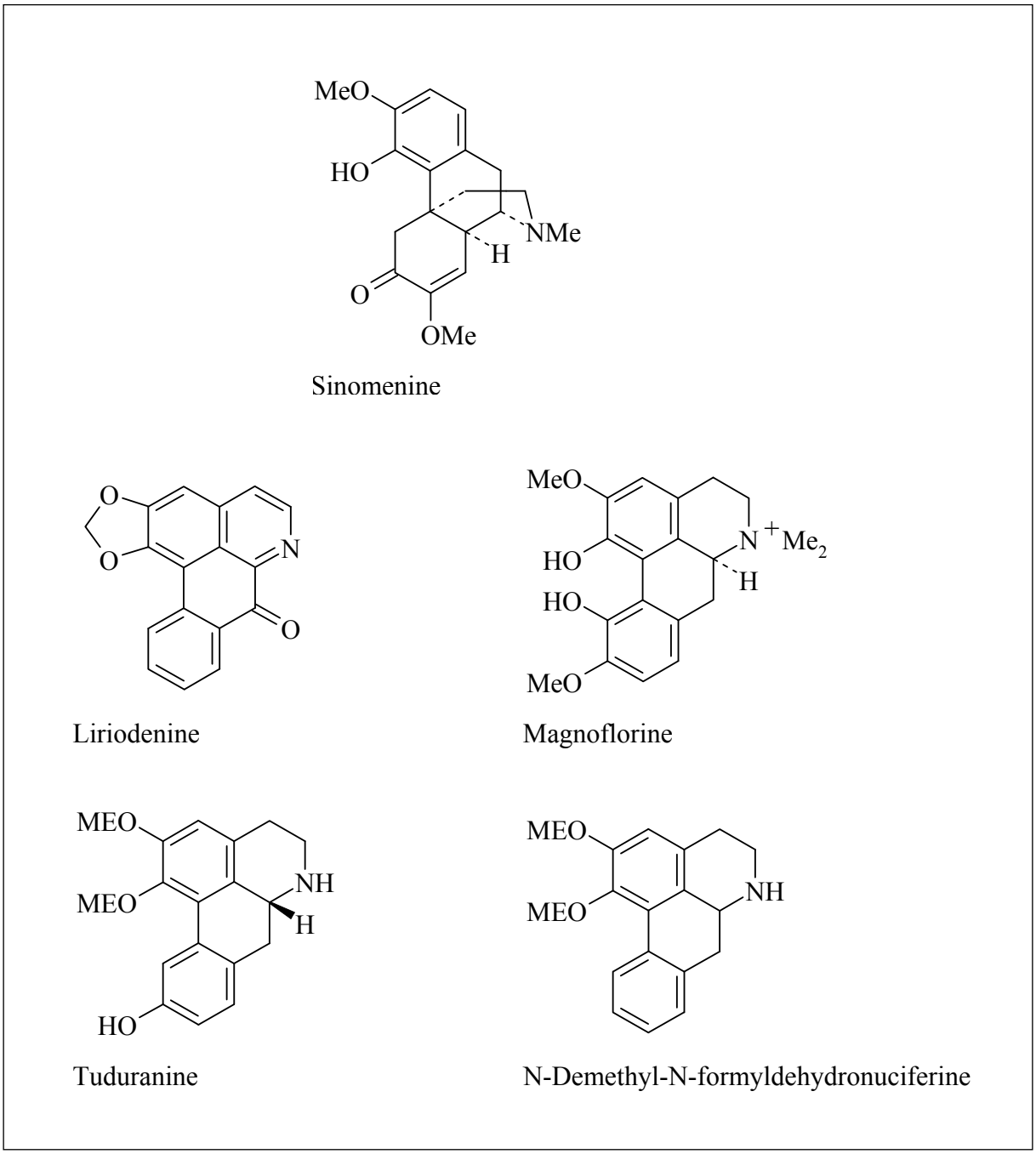

Fig. 1: Formulae of the main compounds

Pharmacology:

- antiarthritic (sinomenine)[8]
- antirheumatic (sinomenine)[9]
- antiinflammatory (COX-1, COX-2, Phospholipase A inhibition)[10]
- antioxidant activity (scopoletin)[7]
- antianaphylactic[11]
- immunomodulatory (sinomenine)[12]
- hypotensive (other alkaloids)[13]
- x-irradiation protective effect[14]

Toxicity:

- mutagenicity for liriodenine is described[3]
- a decoction developed eruption (fever and edematous erythema) in a patient which was judged to be caused by sinomenine and magnoflorine[2]

TLC fingerprint analysis:

1) Extraction: To 1 g of the powdered drug 1 ml ammonia solution 10 % is added. 10 ml methanol are added and extracted under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml methanol.

2) Reference compounds: 1 mg of sinomenine, liriodenine, magnoflorine are dissolved in 1 ml methanol

3) Separation parameters:

Plates: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Sinomenii caulis extract: each 10 µl
reference compounds: each 10 µl

Solvent system:

chloroform	methanol	water
60	30	6.5

Detection: Iodine reagent:
0.05 g iodine is dissolved in 10 ml ethanol 96 %.
The plate is evenly sprayed until background appears yellow.

Drug samples		Origin
1	Sinomenii caulis / *Sinomenium acutum*	sample of commercial drug, China
2	Sinomenii caulis / *Sinomenium acutum*	province Anhui, China
3	Sinomenii caulis / *Sinomenium acutum*	province Hebei, China
4	Sinomenii caulis / *Sinomenium acutum*	province Hubei, China
5	Sinomenii caulis / *Sinomenium acutum*	province Hunan, China

Reference compounds		R*f*
T1	liriodenine	0.97
T2	sinomenine	0.75
T3	magnoflorine	0.29
T4	scopoletin	0.96

4) Description of the TLC-chromatogram:

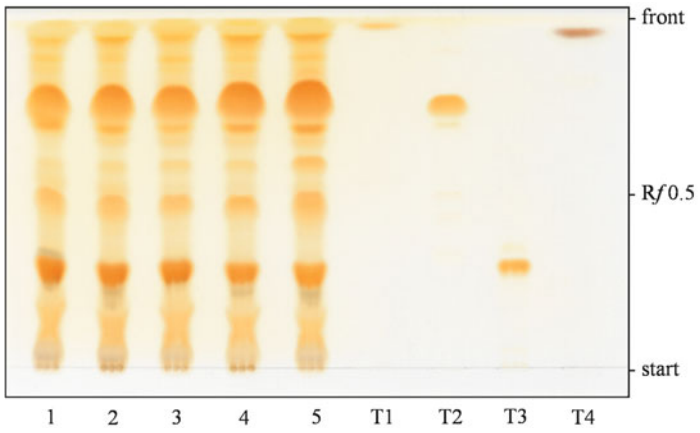

Fig. 2a: Thin layer chromatogram of methanolic extracts of Sinomenii caulis sprayed with iodine reagent (VIS)

The chromatogram of the 5 used extracts of commercial samples from different provinces of China show a very homogeneous pattern of 5 – 6 orange brownish spots which are spread over the entire TLC. The major alkaloid sinomenine has the R*f* value 0.75 wheras magnoflorine and liriodenine appear in minor concentration at R*f* = 0.29 and R*f* = 0.97. Scopoletin, described in the literature, could not be found in any of the samples investigated.

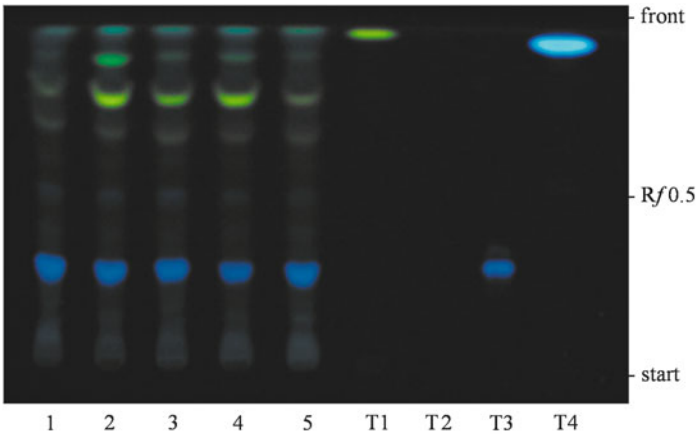

Fig. 2b: Thin layer chromatogram of methanolic extracts of Sinomenii caulis (UV 365 nm)

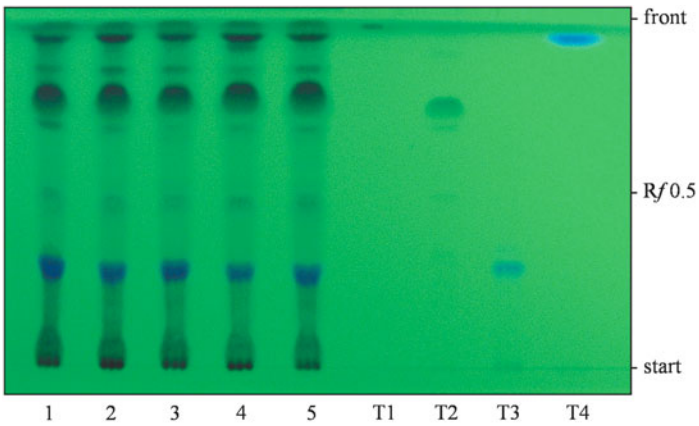

Fig. 2c: Thin layer chromatogram of methonolic extracts of Sinomenii caulis (UV 254 nm)

The chromatogram Fig. 2b shows in UV 365 nm the blue violet fluorescent spot of magnoflorine (T3) at R*f* = 0.29 and four yellowgreen fluorescent spots from R*f* = 0.6 up to R*f* = 0.97 which might belong to the same class of alkaloids as liriodenine. The fluorescent spot at R*f* = 0.97 is liriodenine. In UV 254 nm Sinomenine does not show any fluorescence but can be identified as black quenching zone in Figure 2c at R*f* = 0.75. Scopoletin was not detectable at all.

HPLC-fingerprint analysis:

1) Sample preparation: To 1 g of the powdered drug 1 ml ammonia solution 25 % is added. 10 ml methanol are added and extracted under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml methanol, filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: Sinomenii caulis extract: 10.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing) (pH = 3) B: acetonitrile (Acros Organics)
Gradient:	10 – 25 % B in 10 minutes 25 – 35 % B in 20 minutes 35 – 90 % B in 5 minutes 90 % B in 10 minutes total runtime: 45 minutes
Flow:	1.0 ml/min.
Detection:	262 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	2.5	non identified
2 / 3	13.6 / 14.2	non identified alkaloids
4	15.1	sinomenine
5	15.6	non identified alkaloid
6	17.8	non identified alkaloid
7	21.1	magnoflorine
8	39.9	non identified alkaloid

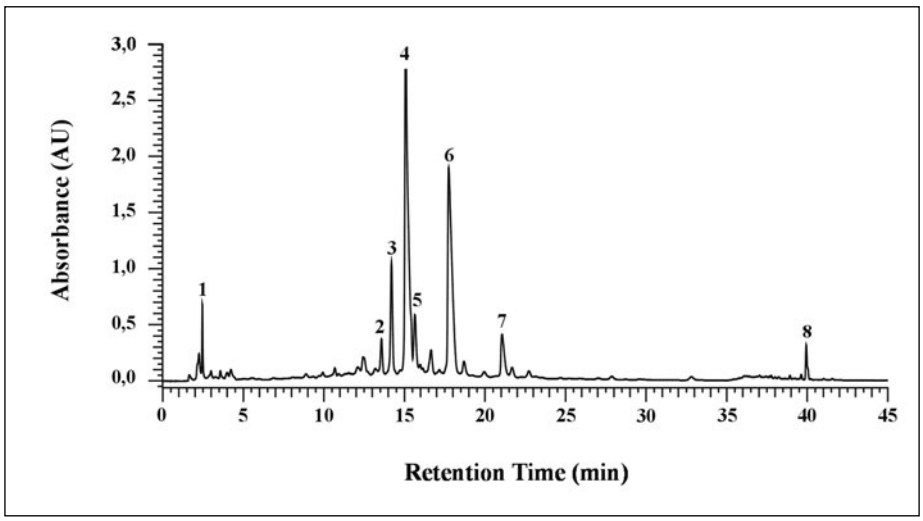

Fig. 3: HPLC-fingerprint chromatogram of the extract of Sinomenii caulis

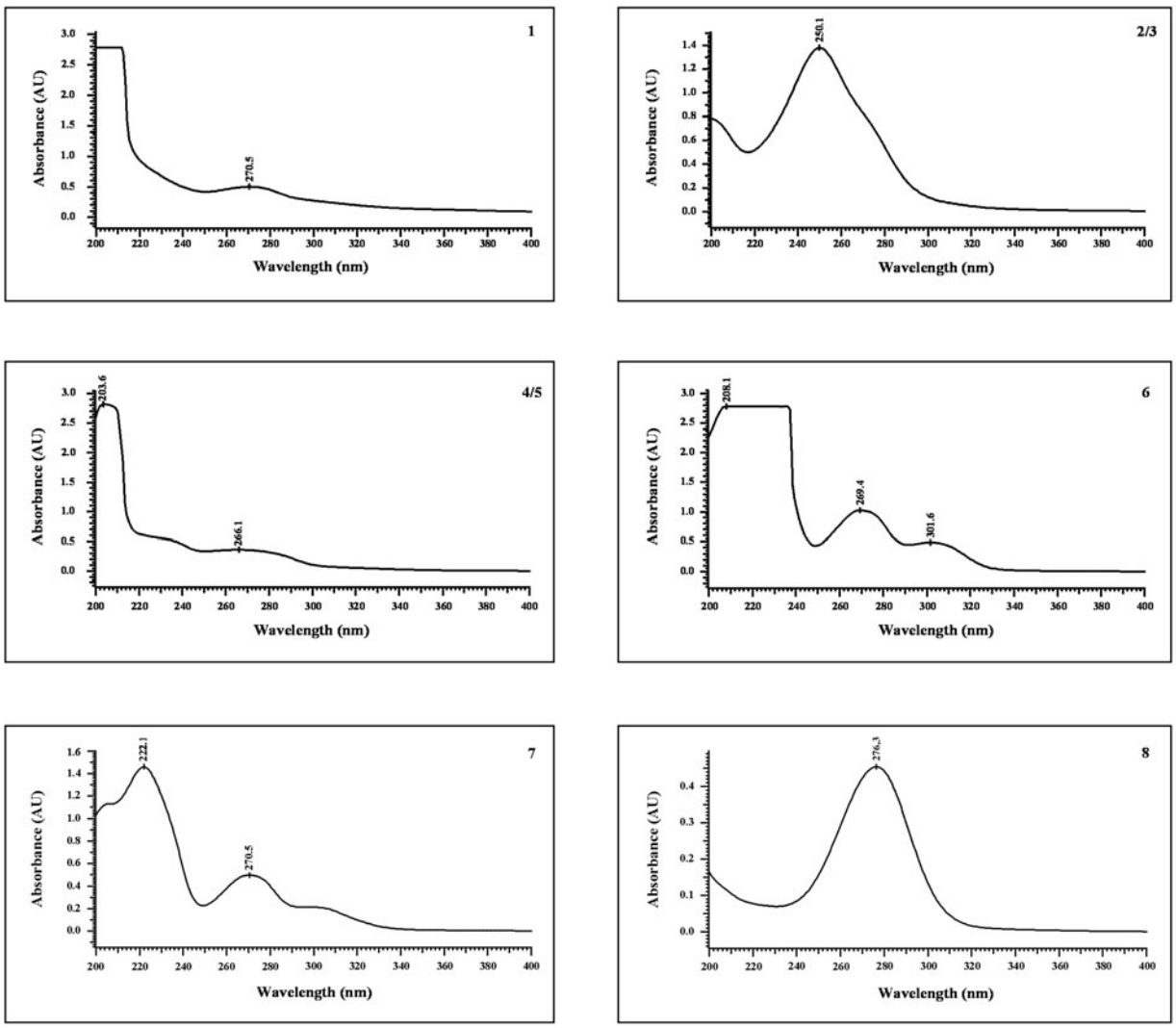

Fig. 4: UV-spectra of the main compounds (peak) of the extract of Sinomenii caulis

4) Description of the HPLC chromatogram:

The HPLC is characterized by two major alkaloids (**4**,**6**) with Rt of 15.1 (sinomenine) and 17.8 (nonidentified alkaloid). Magnoflorine has the Rt of 21.1 (**7**). The other peaks (**1**, **2**, **3** and **5**) most likely identical with the yellowgreen fluorescent alkaloids shown in Fig. 2 b, cannot be structurally assigned. Peak **8** might be a common phenol or phenol carboxylic acid.

Proof of Sinomenii caulis on the absence of Aristolochiae radix

To prove Sinomenii caulis samples on a possible falsification or blending with root of *Aristolochia* spec. the following TLC- and HPLC-methods have been worked out:

TLC-fingerprint analysis:

1) Extraction: To 1 g of the powdered drug 1 ml ammonia solution 10 % is added. 10 ml methanol are added and extracted under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml methanol

2) Reference compound: aristolochic acid: 1 mg is dissolved in 1 ml chloroform

3) Separation parameters:

Plates: Silica gel 60 F_{254} Merck

Applied amounts: Sinomenii caulis: each 25 µl
artificial mixtures 6/7: each 25 µl
Aristolochiae radix extract: 25 µl
reference compound: 25 µl

Solvent system:

toluol	:	ethyl acetate	:	dist. water	:	formic acid (upper phase)
20		10		1		1

Detection: Tin-(II)-chloride reagent:

1.5 ml hydrochloric acid (36 %) is diluted with 8 ml water. 1 g of tin-(II)-chloride x 2 H_2O is dissolved in this mixture. This reagent has to be prepared always freshly.

The plate is sprayed until slightly wet and then for 5 minute heated at 100 °C.

The tin-(II)-chloride reagent for the identification of aristolochic acids in herbal drugs has been first applied in DAC [15]. A HPTLC method for prooving Sinomenii caulis on impurities or a falsification by Aristolochiae radix has been described by Blatter and Reich (2004)[16].

Drug samples		Origin
1	Sinomenii caulis / *Sinomenium acutum*	sample of commercial drug, China
2	Sinomenii caulis / *Sinomenium acutum*	province Anhui, China
3	Sinomenii caulis / *Sinomenium acutum*	province Hebei, China
4	Sinomenii caulis / *Sinomenium acutum*	province Hubei, China
5	Sinomenii caulis / *Sinomenium acutum*	province Hunan, China
6	artificial mixture of drug samples 1 and 8 (80 : 20)	
7	artificial mixture of drug samples 1 and 8 (95 : 5)	
8	Aristolochiae radix / *Aristolochia fangchi* (Guangfangji)	sample of commercial drug, China

Reference compound		R*f*
T 5	Aristolochic acid I, II, III and IV	0.55 – 0.32

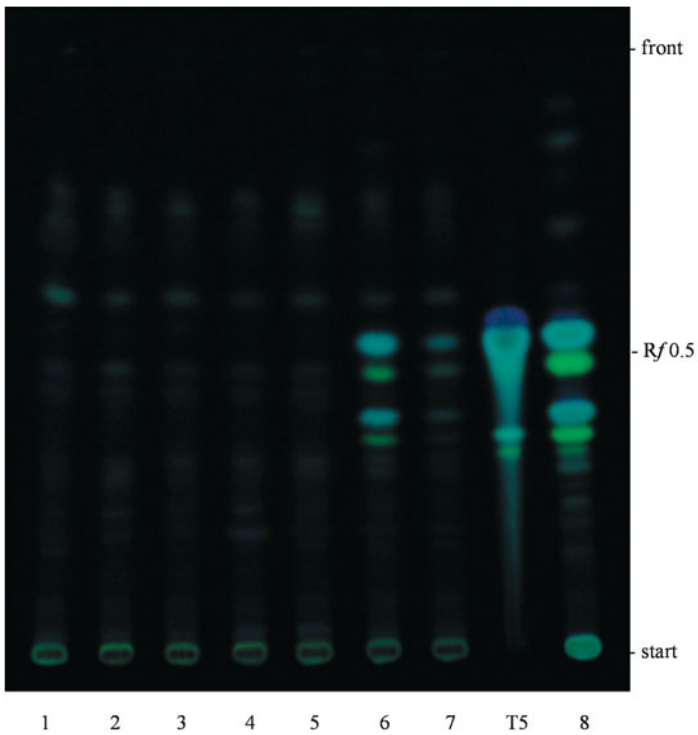

Fig. 5: Thin layer chromatogram of methanolic extracts of Sinomenii caulis, Aristolochiae radix and artificial Sinomenium-Aristolochia-mixtures after spraying with tin-(II)-chloride reagent (UV 365 nm)

4) Description of the TLC proof on the absence of aristolochic acid (Fig. 5):

Green fluorescent zones could not be detected in any of the Sinomenii caulis extract samples. Aristolochic acid I, II, III and IV appear in the R*f*-range of 0.34 – 0.52, as shown in samples 6, 7 and 8.

HPLC-fingerprint analysis:

1) Sample preparation: To 1 g of the powdered drug 1 ml ammonia solution 10 % is added. 10 ml methanol are added and extracted under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm. Sinomenii caulis and Aristolochiae radix extract are mixed in a ratio of 50 : 50 and 80 : 20 and injected into the HPLC apparatus.

2) Injection volume: mixture 50 : 50 and 80 : 20: each 10 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 60 RP-select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 2.0 g hexansulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Grüssing) (pH = 3) B: acetonitrile (Acros Organics)
Gradient:	10 – 50 % B in 25 min., 50 B in 5 min., total runtime: 30 min.
Flow rate:	1.0 ml/min.
Detection:	254 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	25.0	Aristolochic acid II
2	26.5	Aristolochic acid I

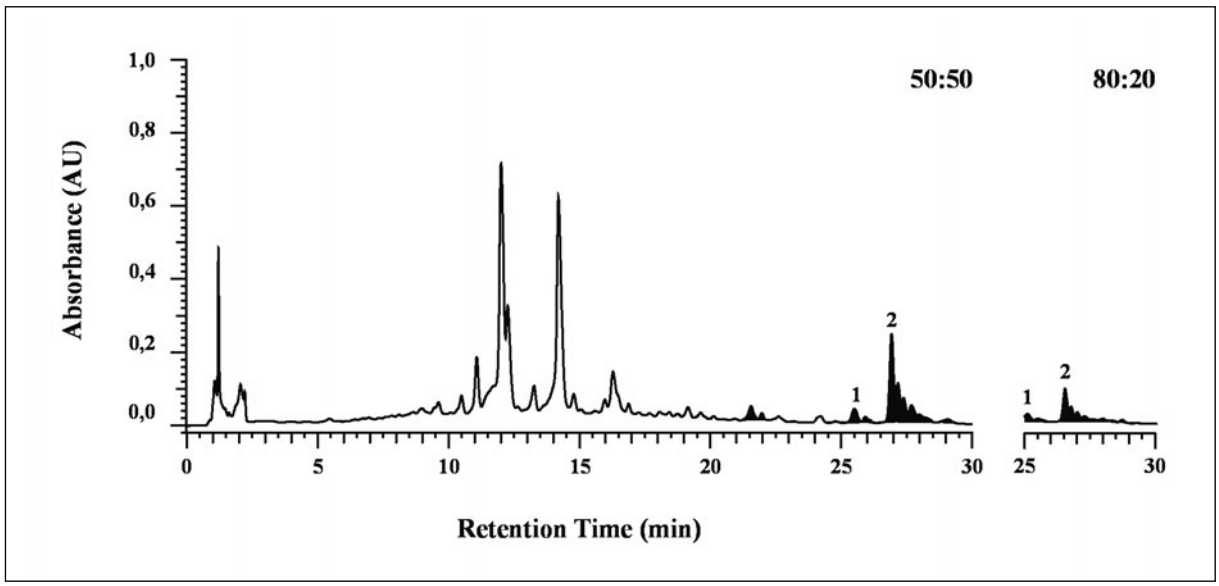

Fig. 6: HPLC-fingerprint of the artificial mixtures of samples 1 and 8 (50 : 50 and 80 : 20)

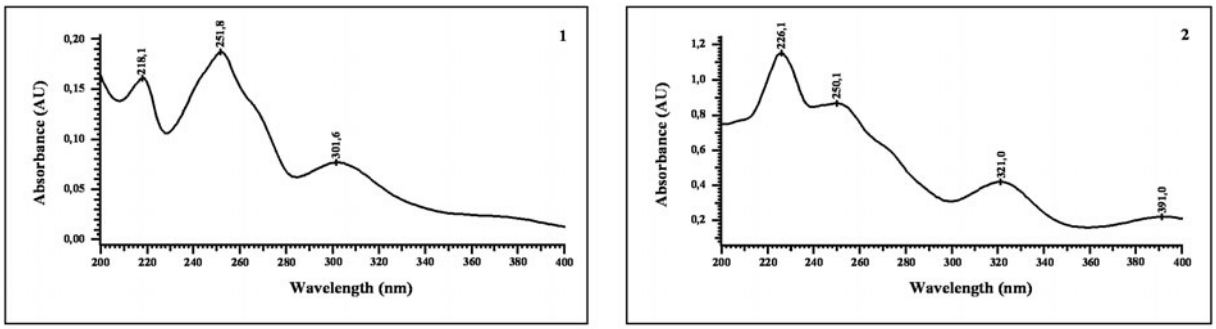

Fig. 7: On line UV-spectra of the main HPLC-peaks of Aristolochiae radix fangchi extract (aristolochic acid 1 and 2)

4) Description of the HPLC-fingerprint of Fig. 6:

In the HPLC-fingerprint of the two artifical extract mixtures of Sinomenii caulis with Aristolochiae radix 50 : 50 and 80 : 20 aristolochic acids I and II give significant black marked peaks at Rt = 26.5 (**2**) and Rt = 25.0 (**1**). The peaks of aristolochic acid III and IV appear with lower Rt in the range of 20.0 and 22.0. The other black marked peak at Rt = 21.0 derives also from Aristolochiae radix constituents, the other peaks (white) originate from Sinomenii caulis. With this method still 6 µg aristolochic acid / g herbal drug can be detected[13].

Note: According to the Chinese Pharmacopoeia 2005 Caulis Sinomenii should contain not less than 0.50 % of sinomenine calculated with reference to the dried drug.

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Additional reference (HPLC-analysis)

Zhao ZZ, Liang ZT, Zhou H, Jiang ZH, Liu ZQ, Wong YF, Xu HX, Liu L, Quantification of sinomenine in caulis sinomenii collected from different growing regions and wholesale herbal markets by a modified HPLC method, Biol. Pharm. Bull., 28(1), 105 – 109 (2005)

Fructus Forsythiae
Lianqiao

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[1]

Official drug[1]: Weeping Forsythia Capsule is the dried fruit of *Forsythia suspensa* (Thunb.) Vahl (Fam. Oleaceae).
The drug is collected in autumn when nearly ripe and still greenish, removed from foreign matter, steamed thoroughly and dried in the sun (known as "Qingqiao" = green Forsythia); or the drug is collected when fully ripe, dried, and removed from foreign matter (known as "Laoqiao" = yellow or brown Forsythia).

Description of the drug[1]: Long ovoid to ovoid, slightly compressed, 1.5 – 2.5 cm long, 0.5 – 1.3 cm in diameter. Externally with irregular longitudinal wrinkles, numerous raised small maculaters, and a longitudinal furrow on each of the two surfaces. Apex acute, bearing a small fruit stalk or its scar at the base. "Qingqiao" mostly indehiscent, externally greenish-brown, with less small greyish-white maculates, texture hard; seeds numerous, yellowish-green, slender, winged at one side. "Laoqiao" dehiscent from apex or to segments. Outer surface yellowish-brown or reddish-brown, inner surface mostly pale yellowish-brown, smooth, with a longitudinal septum. Texture brittle; seeds brown, mostly fallen off. Odour, slightly aromatic; taste bitter.

Medicinial use[1,2]: fever, headache, exanthema, carbuncles, mastitis, swellings and inflammation of the upper respiratory tract, pharyngitis

Effects and indications according to Traditional Chinese Medicine[1,2]	
Taste:	bitter, pungent, neutral
Temperature:	neutral with cold tendency
Channels entered:	orbis cardialis, liver and bile
Effects (functions):	clears pathogenic heat, acts on the lung, heart and gallblader, reduces swelling and dissovels lumps
Symptoms and indications:	carbuncles, boils, lymphadenitis, mastitis, erysipelas, upper respiratory infection, febrile diseases at the early stage and at the stage with high fever, dire thirst, delirium and maculation, acute urinary infection with oliguria

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents[3]:

- **phenolic glycosides:**
  forsythoside A, C, D and E
- **lignans:**
  phillygenin, phillyrin (forsythin), pinoresinol and pinoresinol-glucoside;
  matairesinol, arctigenin and their glycosides matairesinoside and arctiin; O-methylarctigenin
- plant alcohols:
  rengyol and its glucoside rengyoside A, rengyoxide, rengyolone cornoside and salidroside; suspenol
- triterpenes:
  betulinic acid; oleanolic acid; ursolic acid; β-amyrin acetate
- flavon glycoside:
  rutin

Pharmacology:

- antibacterial[4]
- antiviral[5]
- antioxidant[6]
- protective effect against acute hepatic injury[7]
- protective effect against irradiation[8]
- antiinflammatory[9,10,11]
- antiallergic[9]
- PAF antagonistic effect[12]
- inhibition of low density lipoprotein oxidation[13]

TLC fingerprint analysis

Drug sample		Origin
1	Forsythiae fructus / *Forsythia suspensa*	sample of commercial drug, China
2	Forsythiae fructus / *Forsythia suspensa*	sample of commercial drug, China
3	Forsythiae fructus / *Forsythia suspensa*	sample of commercial drug, China
4	Forsythiae fructus / *Forsythia suspensa*	sample of commercial drug, China
5	Forsythiae fructus / *Forsythia suspensa*	sample of commercial drug, China
6	Forsythiae fructus / *Forsythia suspensa*	sample of commercial drug, Japan

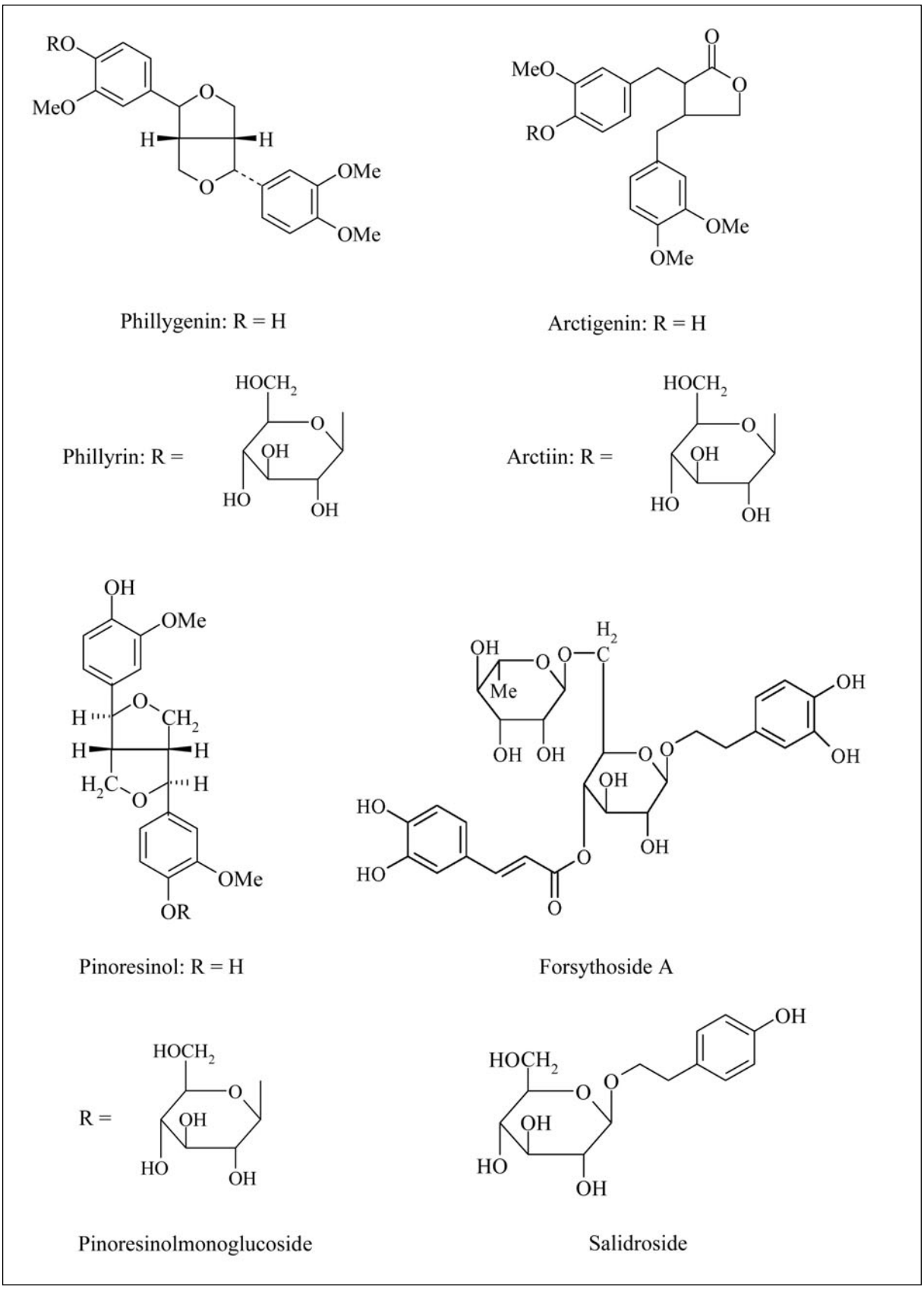

Fig. 1: Formulae of the main constituents

1) Extraction: 1.0 g of the powdered drug is extracted under reflux with 10 ml of methanol. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 2 ml methanol.

2) Reference compounds: 1 mg are dissolved in 1 ml methanol

3) Separation parameters:

Figure 2a:

Plate:	HPTLC-plate Silica gel F_{254} Merck
Applied amounts:	Forsythiae fructus-methanol-extracts: each 10 µl reference compounds: each 10 µl
Solvent system:	Chloroform : Methanol 90 10
Detection:	Vanillin-sulphuric acid reagent: Solution I: 1 % ethanolic vanillin solution Solution II: 10 % ethanolic sulphuric acid The plate is intensively sprayed with 10 ml solution I followed immediately by 10 ml solution II. Afterwards the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

Figure 2b:

Plate:	TLC-plate Silica gel F_{254} Merck
Applied amounts:	Forsythiae fructus-methanol-extracts: each 15 µl reference compounds: each10 µl
Solvent system:	Toluene : Ethylacetate 70 30
Detection:	Vanillin-sulphuric acid reagent: Solution I: 1 % ethanolic vanillin solution Solution II: 10 % ethanolic sulphuric acid The plate is intensively sprayed with 10 ml solution I followed immediately by 10 ml solution II. After then the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

Figure 2c:

Plate:	HPTLC-plate Silica gel F_{254} Merck
Applied amounts:	Forsythiae fructus-methanol-extracts 1, 2, 3, 5, 6: each 10 µl Forsythiae fructus-methanol-extract 4: 3 µl reference compound: 10 µl

Solvent system:	ethyl acetate	: formic acid	: glacial acetic acid	: water
	100	11	11	26

Detection: Natural products-polyethylene glycol reagent (NP/PEG):
Solution I: 1 % diphenylboric acid-β-ethylaminoester
Solution II: 5 % polyethylene glycol-4000 (PEG) in ethanol
The plate is sprayed first with solution I and then with solution II.

Reference compounds of Fig. 2a		**R*f***
T 1	Phillyrin	0.25
T 2	Arctiin	0.28
T 3	Salidroside	0.07
T 4	Pinoresinol monoglucoside	0.13
T 5	Pinoresinol	0.87

Reference compounds of Fig. 2b		**R*f***
T 5	Pinoresinol	0.18
T 6	Ursolic acid	0.40
T 7	Oleanolic acid	0.41
T 8	Betulinic acid	0.49

Reference compound of Fig. 2c		**R*f***
T 9	Rutin	0.45

4) Description of the TLC-chromatograms:

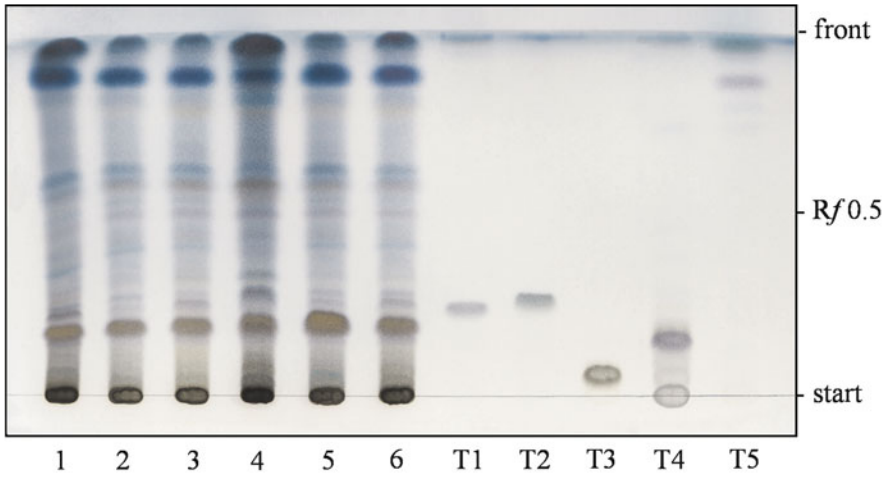

Fig. 2a: Thin layer chromatogram of methanolic extracts of Forsythiae fructus after spraying with vanillin-sulphuric acid reagent in VIS

Samples 1 – 6 of Forsythiae fructus show a very homogenous pattern of violett spots over the whole plate. Phillyrin (T 1) and arctiin (T 2) can be found at R*f* = 0.25 and R*f* = 0.28 respectively. Whereas pinoresinolmonoglucoside (T 4) appears in all samples as a prominet violett-brown spot at 0.13, its aglycone (T 5) gives a weak spot at 0.87. Salidroside has the lowest R*f*-value (T 3; R*f* = 0.07), present in traces only.

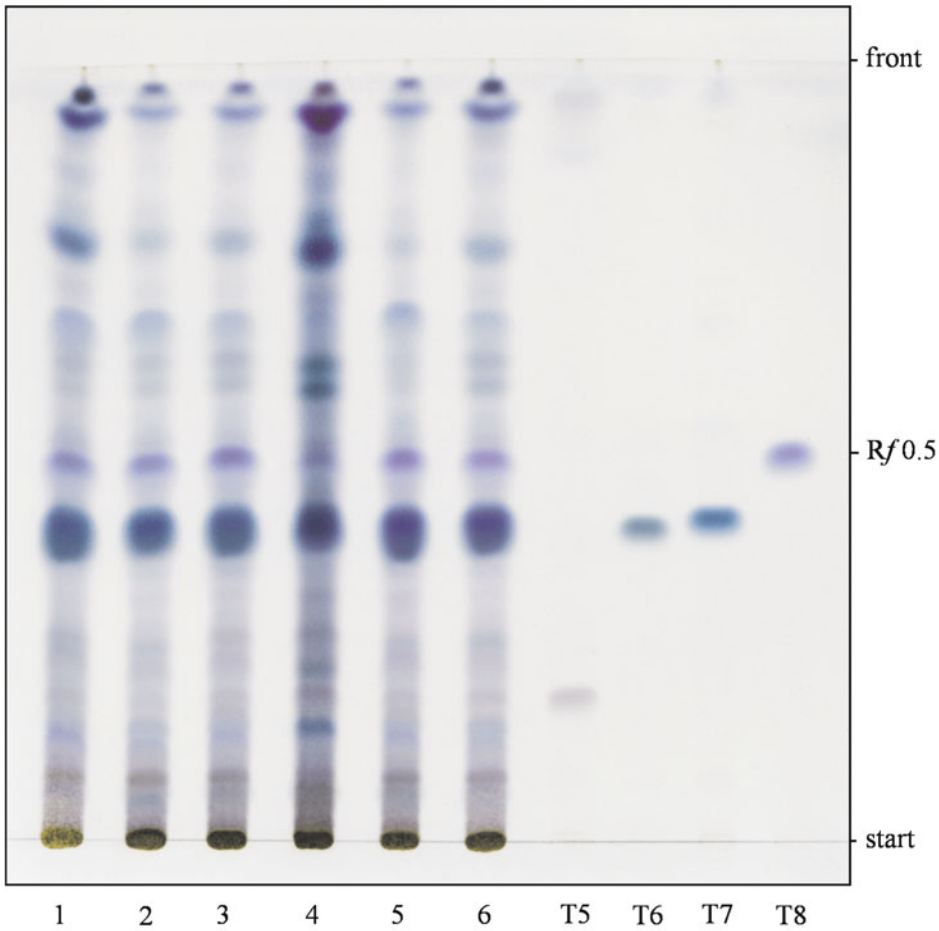

Fig. 2b: Thin layer chromatogram of methanolic extracts of Forsythiae fructus after spraying with vanillin-sulphuric acid reagent in VIS

The TLC shows also a very homogenous pattern of about eight violet spots with ursolic- and oleanolic acid (T 6 / T 7) overlapped in one major spot and betulinic acid above it (T 8). Pinoresinol (T 5) appears at R*f* = 0.18.

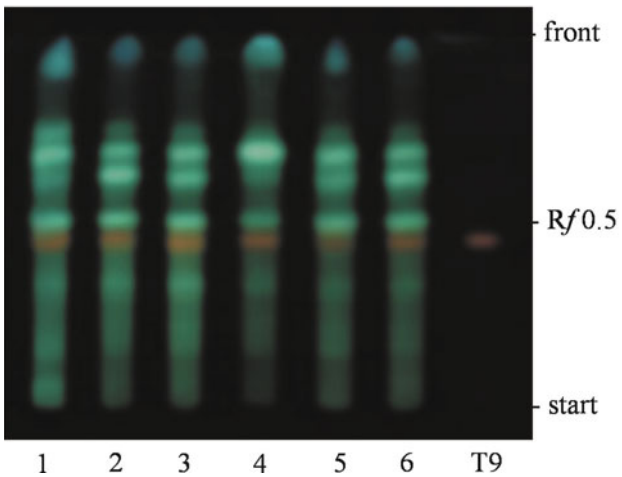

Fig. 2c: Thin layer chromatogram of methanolic extracts of Forsythiae fructus after spraying with Natural products-polyethylene glycol reagent (NP/PEG) (UV 365 nmm)

This TLC is characterized by a pattern of about five green fluorescent zones in the R*f*-range of 0.20 up to 0.75 and a orange fluorescent zone at R*f* = 0.45. The orange one is rutin, whereas the green fluorescent zone can be assigned to the caffeic acid containing glycosides forsythoside A, C, D and E.

HPLC-fingerprint analysis:

1) Sample preparation: 1.0 g of the powdered drug is extracted under reflux with 10 ml of methanol. The extract is cooled, filtered and evaporated to dryness. To the residue 10 ml water and 10 ml butanol are added and the mixture given into a separation funnel. The butanol phase is separated and evaporated to dryness. The residue is dissolved in 2 ml methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC.

2) Injection volume: Forsythiae fructus extract: 2 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: dist. water + 10 ml H_3PO_4 0,1 % (Merck) B: acetonitrile + 10 ml H_3PO_4 0,1 % (Merck)
Gradient:	5 – 30 % B in 5 minutes 30 % B in 20 minutes 30 – 95 % B in 10 minutes 95 % B in 20 minutes total runtime: 55 minutes
Flow:	0.6 ml / min.
Detection:	210 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	14.6	Forsythoside, not exactly identified
2	14.8	Rutin
3	16.0	Pinoresinolmonoglucoside
4	20.6	Phillyrin

5	21.0	Arctiin
6	27.6	Pinoresinol
7	37.2	not identified
8	48.1	Betulinic acid, Oleanolic acid, Ursolic acid

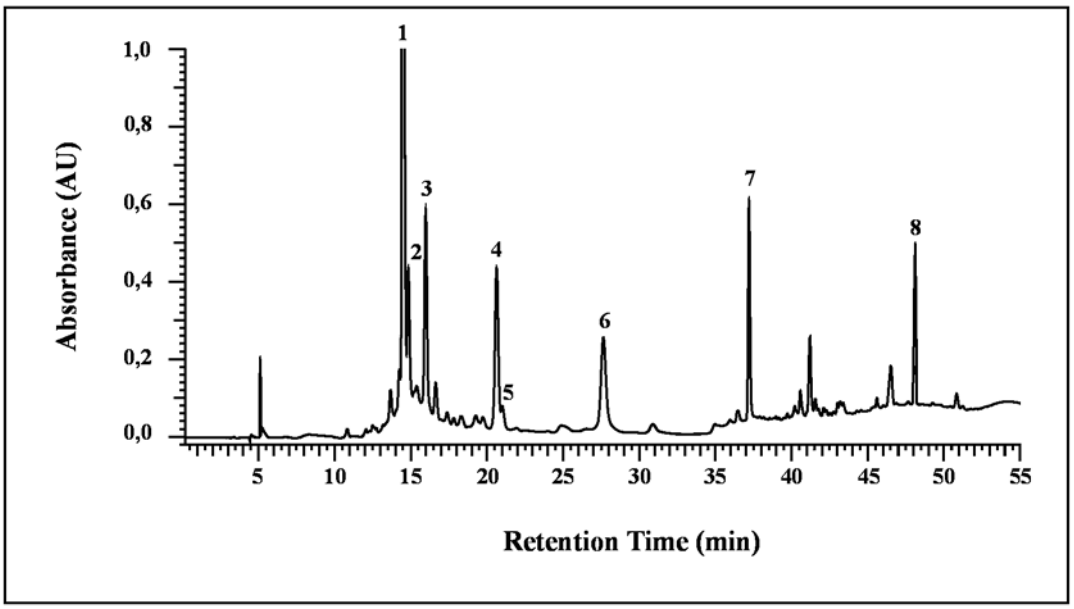

Fig. 3: HPLC-fingerprint chromatogram of Forsythiae fructus

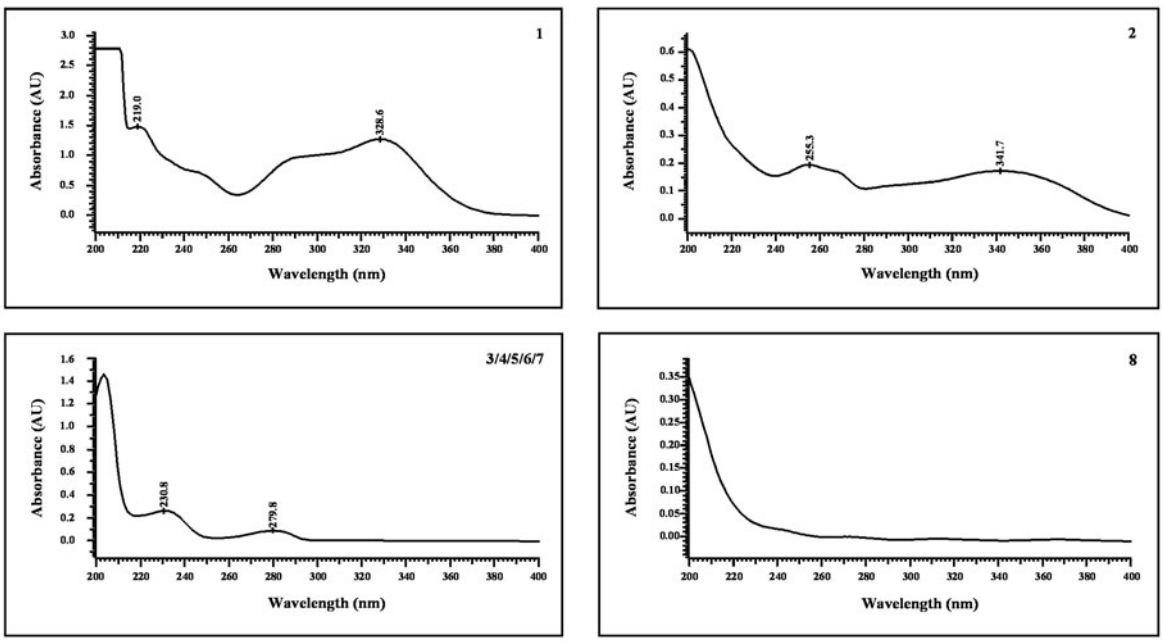

Fig. 4: UV-spectra of the main compounds (peak) of Forsythiae fructus

4) Description of the HPLC chromatogram:

The HPLC-fingerprint is characterized by a dominant peak at Rt = 14.6, identified as one Forsythoside (**1**) and Rutin (**2**) at Rt = 14.8 next to it. The lignans Pinoresinolmonoglucoside (**3**), Phillyrin (**4**), Arctiin (**5**) and Pinoresinol (**6**) appear at Rt = 16.0, 20.6, 21.0 and 27.6. Another substance (**7**) with an UV-spectrum typical for lignans appears at Rt = 37.2. Betulinic acid, oleanolic acid or ursolic acid (**8**) appear together non separated in one peak at Rt = 48.1.

Note: The Chinese Pharmacopoeia 2005 demands for Fructus Forsythiae not less than 0.15 % forsythin (phillyrin) with reference to the dried drug.

References:

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Additional references (HPLC-analysis)

Guo H, Liu AH, Li L, Guo DA, Simultaneous determination of 12 major constituents in Forsythia suspense by high performance liquid chromatography—DAD method, J. Pharm. Biomed. Anal. 43(3), 1000-1006 (2007)

Guo H, Liu AH, Ye M, Yang M, Guo DA, Characterization of phenolic compounds in the fruits of Forsythia suspense by high-performance liquid-chromatography coupled with electrospray ionization tandem mass spectrometry, Rapid. Commun. Mass Spectrm. 21(5), 401-405 (2007)

Fructus Evodiae
Wuzhuyu

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drugs[1]: *Evodia rutaecarpa* (Juss.) Benth.
Evodia rutaecarpa (Juss.) Benth. var. *officinalis* (Dode) Huang
Evodia rutaecarpa (Juss.) Benth. var. *bodinieri* (Dode) Huang

– Rutaceae –

Origin[2]: South-east China (provinces Gui Zhou, Guang Xi, Hu Nan Yun Nan, Shan Xi, Zhe Jiang, Si Chuan), Japan and India.

Description of the drug[1]: Spheroidal or slightly flattened-pentageon spheroidal, 2-5 mm in diameter. Externally brown, rough, with numerous spotted protrudings or depressed oil dots. A pentagon-stellate cleft present at the apex and a yellow-tomentose fruit stalk at the base. Texture hard and fragile. Transverse section showing 5-locular ovary, each locule possesing 1-2 yellowish seeds.
Odour, strong aromatic; taste, pungent and bitter.

Pretreatment of the raw drug[1]:

Fructus Evodiae: The fruit spur is cut off from August to November before it bursts. The drug is dried in the sun or at a low temperature, removed from shoots, leaves, fruit stalks and foreign matters eliminated.

Fructus Evodiae (prepared): Radix Glycyrrhizae is pounded to pieces and decocted in a proper amount of water. The residue is removed, clean Fructus Evodiae is added in a covered container to absorb the decoction entirely. Stir-baked with either salt water or a decoction of licorice root (Gan cao) until partially dry and then dried in the sun. To each 100 kg of Fructus Evodiae 6 kg of Radix Glycyrrhizae in the processing are added.

Medicinal use: The drug is used as antihypertonic, cardiotonic, antiemetic, analgesic, anti-inflammatory and antigastric medicine.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,2]	
Taste:	pungent and bitter
Temperature:	hot
Channels entered:	acts on the liver, spleen and stomach channels
Effects:	dispels cold, relives pain, soothes the liver, alleviates rebellious q*i*, dries dampness, antiemetic
Symptoms and indications:	effective for cankers scores and hypertension

Main constituents[3,4]**:** (see Fig. 1)

- **indolopyridoquinazoline alkaloids:**
  evodiamine and rutaecarpine, dihydrorutaecarpine, 14-formyl dihydrorutaecarpine, 7-carboxyevodiamine and rhetisinine

- **quinolone alkaloids and other nitrogen-containing compounds:**
  evocarpine, dihydroevocarpine, 1-methyl-2-pentadecyl-4(1H)-quinolone, 1-methyl-2-undecyl-4(1H) quinolone, synephrine

- **limonoids:**
  evodin, evodol (limonin diosphenol), evodinon (rutaevin), obacunone, jangomolide, rutaevin acetate, graucin A, 12α-hydroxylimonin, 12α-hydroxyevodol, 6α-acetoxy-5-epilimonin and 6β-acetoxy-5-epilimonin

- **flavonoid:**
  4´,5,7-trihydroxy-6(or 8)-(3-methylbut-2enyl)flavanone 7,4′-di-O-β-D-glucopyranoside

- monoterpens/phenylpropens:
  myrcene, β-phellandrene, limonene, α-terpineol, linalool, β-elemene, trans-caryophyllene; methyl eugenol

- tryptamine derivatives

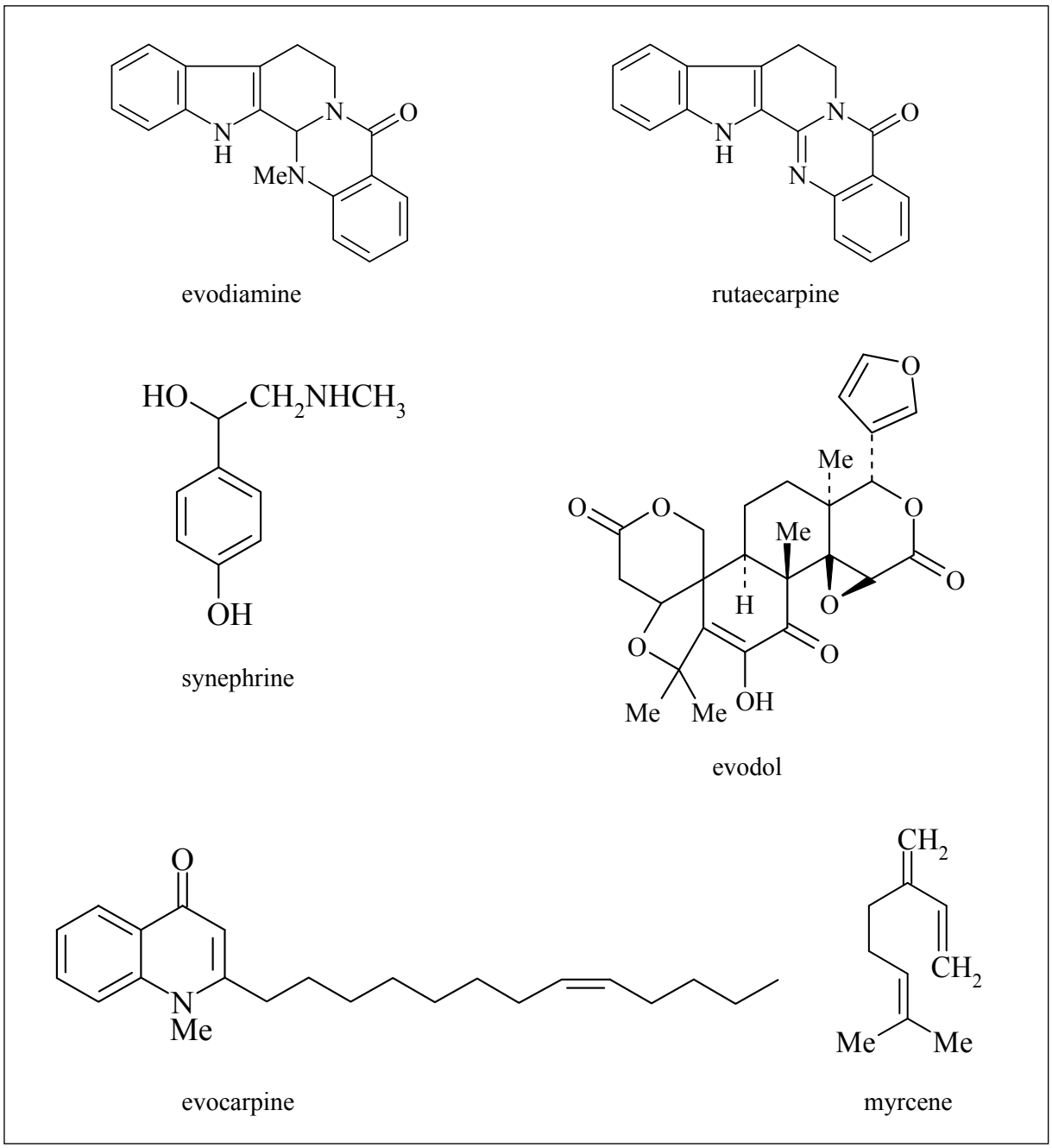

Fig. 1: Formulae of the main constituents[3]:

Pharmacology:

- uterotonic (rutaecarpine and dehydroevodiamine)[2]
- positive inotropic and chronotropic effects[5]
- cardiotonic activity (evodiamine)[6]
- antiplatelet activity (rutaecarpine)[7]
- vasodilatory effects (evodiamine, dehydroevodiamine, rutataecarpine)[8-10]
- cardioprotective effects (rutaecarpine)[11]
- COX-inhibitory effect (rutaecarpine)[12]

- analgesic effect (evodiamine)[13]
- antibacterial effect (*Helicobacter pylori*) (quinolone alkaloids)[14]
- NO-inbibiting effect in murine macrophages[15]
- inhibitory effect on cytochrome P450 1A (mouse and human liver microsomes)[16]
- anti-inflammatory (antiedemic) effect (limonin)[17]
- anti-diarrheal effect (water extrat of *Evodia rutaecarpa*)[18]
- carminativ and stomachic effect[2]
- leukotrienes inhibiting (quinolone alkaloids)[19]

Toxicology: Contraindicated for use in patients with Yin deficiency, heat syndromes and at pregnancy.

TLC-fingerprint analysis[20]:

Chloroform extract:

1) Extraction: 3.0 g powdered drug are defatted by extraction with 30 ml *n*-hexane. The *n*-hexane fraction is discarded and the drug dried at room temperature.
The dried defatted powdered drug is grounded in a mortar for about 1 min with 2 ml 10 % ammonia solution and then thoroughly mixed with 7 g basic aluminium oxide (activity grade I). This mixture is then packed loosely into a glass column (diameter, 1.5 cm length, 20 cm) and 10 ml chloroform are added. The alkaloid bases are eluted with about 5 ml chloroform, the eluate collected and in vacuum evaporated to 2 ml methanol.

2) Reference compounds: evodiamine, rutaecarpine, synephrine and evocarpine are dissolved in methanol (1 mg/ml MeOH)

3) Separation parameters:

Applied amount: 7 µl extract and standard solution

Plate: HPTLC Silicagel 60 F_{254}; Merck

Solvent system: toluene-ethyl acetate-isopropanol-methanol (43: 15: 7: 12) in one trough of a twin trough glass chamber, ammonia 25 % in the other trough.
Equilibration of the chamber for 15 min.

Detection: Direct evaluation:
UV 245 nm (Fig. 2a)

Spray reagent:
Iodine reagent: 0.05 g iodine is dissolved in 10 ml ethanol 96 %.
The plate is evenly sprayed until background appears yellow.
Examination in VIS when background has been turned to white again (Fig. 2b).

Essential oil:

1) Extraction: About 50 g powdered drug are subjected to a water steam distillation in a Neo Clevenger apparat. The obtained essential oil is diluted 1:1 with xylene.

2) Separation parameters:

Applied amount:	5 µl diluted essential oil and standard solution
Plate:	TLC Silicagel 60 F_{254}; Merck
Solvent system:	toluene-ethyl acetate (97: 3)
Detection:	Spray reagent: Vanillin-sulphuric acid reagent: The plate is intensively sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid, followed by heating under supervision for 10 minutes at 110 °C (Fig. 3).

Drug samples		**Origin**
1	Evodiae fructus/ *Evodia rutaecarpa*	commercial product of Uchida company; Japan
2	Evodiae fructus/ *Evodia rutaecarpa*	samples of commercial products; China
3	Evodiae fructus/ *Evodia rutaecarpa*	
4	Evodiae fructus/ *Evodia rutaecarpa*	
5	Evodiae fructus/ *Evodia rutaecarpa*	

Reference compounds		**R*f***
T1	evodiamine and rutaecarpine	0.64 and 0.75
T2	synephrine and evocarpine	0.23 and 0.57

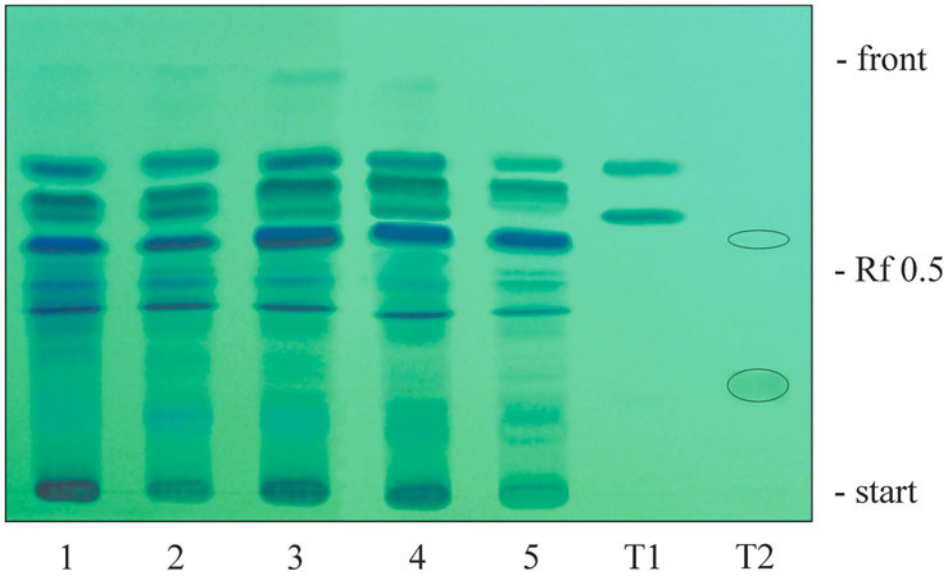

Fig. 2a:
TLC-fingerprint of Evodiae fructus chloroform extracts in UV 245 nm

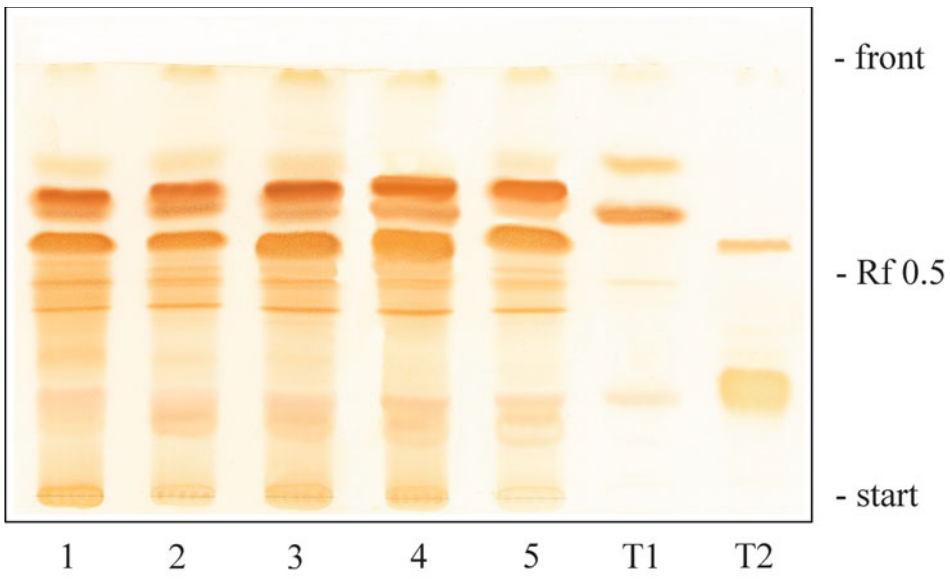

Fig. 2b:
TLC-fingerprint of Evodiae fructus chloroform extracts detected with iodine spray reagent in VIS

4) Description of the TLC-chromatograms **Fig. 2a** and **Fig. 2b**:

- All samples of Evodiae fructus chloroform extracts show in UV 245 nm (**Fig. 2a**) in the R*f*-range of 0.55-0.85 four main blue-green fluorescent zones of rutaecarpine (R*f* 0.75) on the top, followed by a nonidentified alkaloid (R*f* 0.70), evodiamine (R*f* 0.64) and the mixture of quinolone alkaloids evocarpine, 1-methyl-2-undecyl-4-(1H)-quinolone, 1-methyl-2-(6Z)-6-undecyl-4-(1H)-quinolone, 1-methyl-2-(6Z,9Z)-6,9-pentadecadienyl-4-(1H)-quinolone (R*f* 0.57). Synephrine appears at R*f* 0.23.
- Sprayed with iodine reagent the same chloroform extracts show in VIS (**Fig. 2b**) the two major yellow-orange zones of evodiamine and the mixture of quinolone alkaloids. With this reagent rutaecarpine and synephrine give weak yellow zones only.

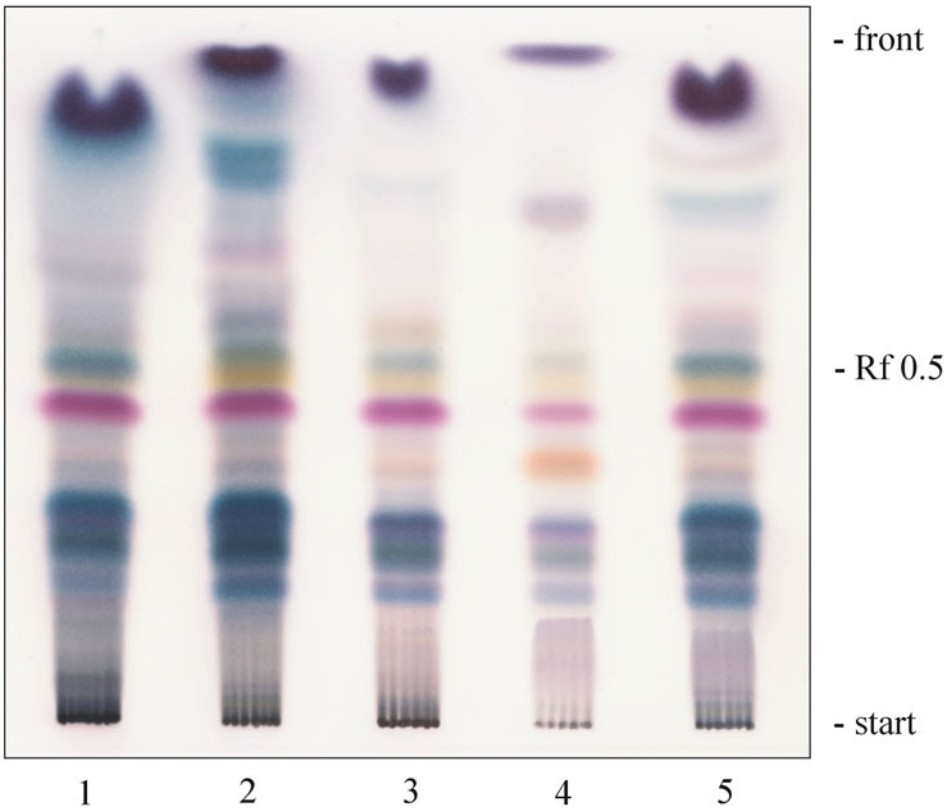

Fig. 3:
TLC-fingerprint of essential oil of Evodiae fructus detected with vanillin-sulphuric reagent

Description of the TLC-chromatogram, sprayed with vanillin-sulphuric acid spray reagent, **Fig. 3**:[21]

The essential oil of the Evodiae fructus samples shows a very homogeneous terpenoid pattern with the dark violet zones of myrcene, β-phellandrene, limonene and caryophyllene in the R*f*-range of 0.92-0.96. Between R*f* 0.5 and 0.8 appear several blue and grey zones, which can be assigned to terpene alcohol esters. The phenylpropene methyl eugenol is detectable as yellow-brown zone at R*f* 0.48, followed by the characteristic pink violet zone at R*f* 0.45 of caryophyllene oxide. The monoterpene alcohols (e.g. linalool and terpineol) appear in the R*f*-range of 0.18-0.34 as blue grey zones.

HPLC-fingerprint analysis[22]:

1) Sample preparation: The same chloroform extracts as used for TLC are filtered over Millipore® (Type HV 0.45 µm).

2) Injection volume: 3 µl extract

3) HPLC-data:

Apparatus: L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi

Column: LiChroCART® 125-4 LiChrospher® 60 RP-select B with LiChroCART® 4-4 LiChrospher® 60 RP-select B (5 µm); Merck

Solvent system: A: solution of ionic pair reagent in 0.1 N H_3PO_4 and water for HPLC; Acros Organics (5.0 g sodium dodecyl sulfate dissolved among careful heating in 20 ml 0.1 N H_3PO_4 and mixed with 1000 ml water)

B: acetonitrile for HPLC; Acros Organics

Gradient: 35% B to 85% B in 30 min.

Flow rate: 1.0 ml/min.

Detection: 225 nm

Retention times and identity of the main peaks of Fig. 4a and Fig. 4b:

Peak	Rt (min.)	Compound
1	2.6	synephrine
2	9.2	evodiamine
3	10.3	rutaecarpine
4	25.5–30.0	quinolone alkaloids

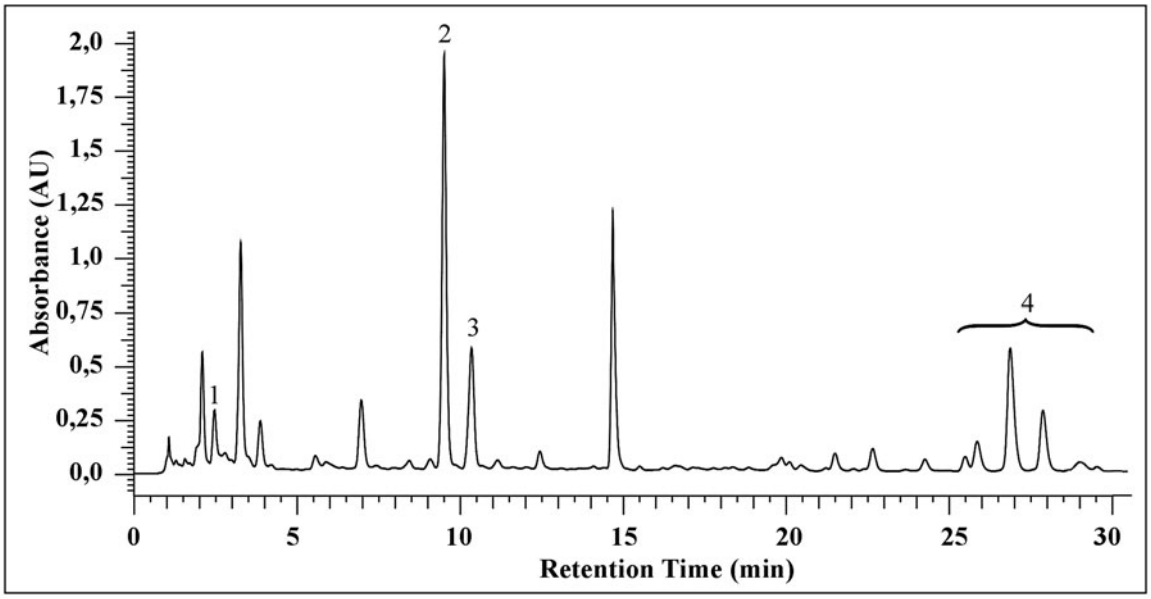

Fig. 4a: HPLC chromatograms *of Fructus Evodiae* methanol extract sample 2

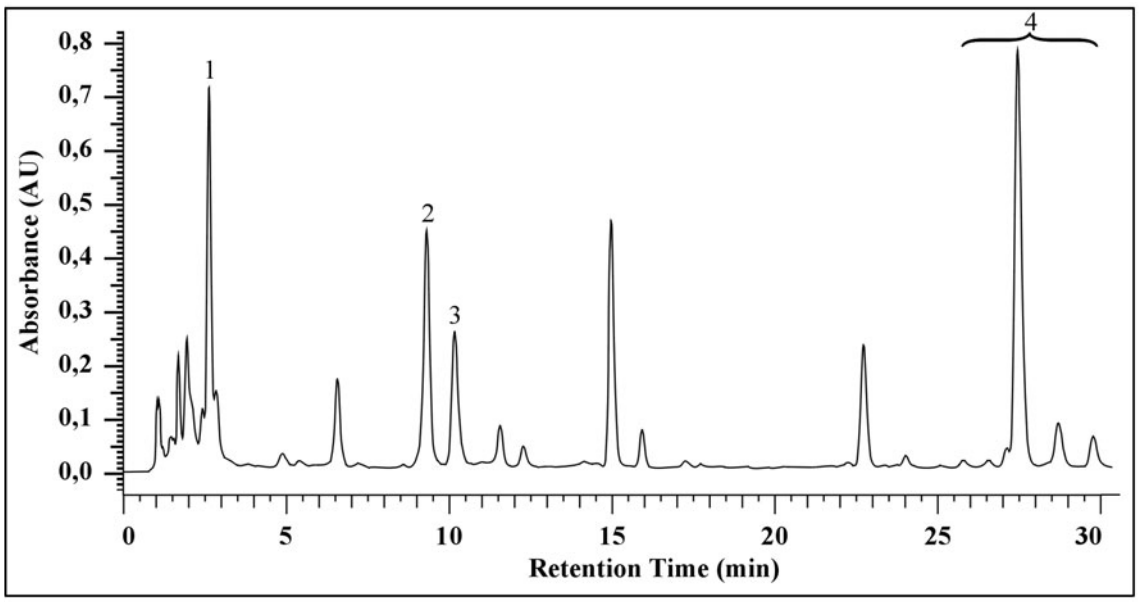

Fig. 4b: HPLC chromatograms *of Fructus Evodiae* methanol extract sample 4

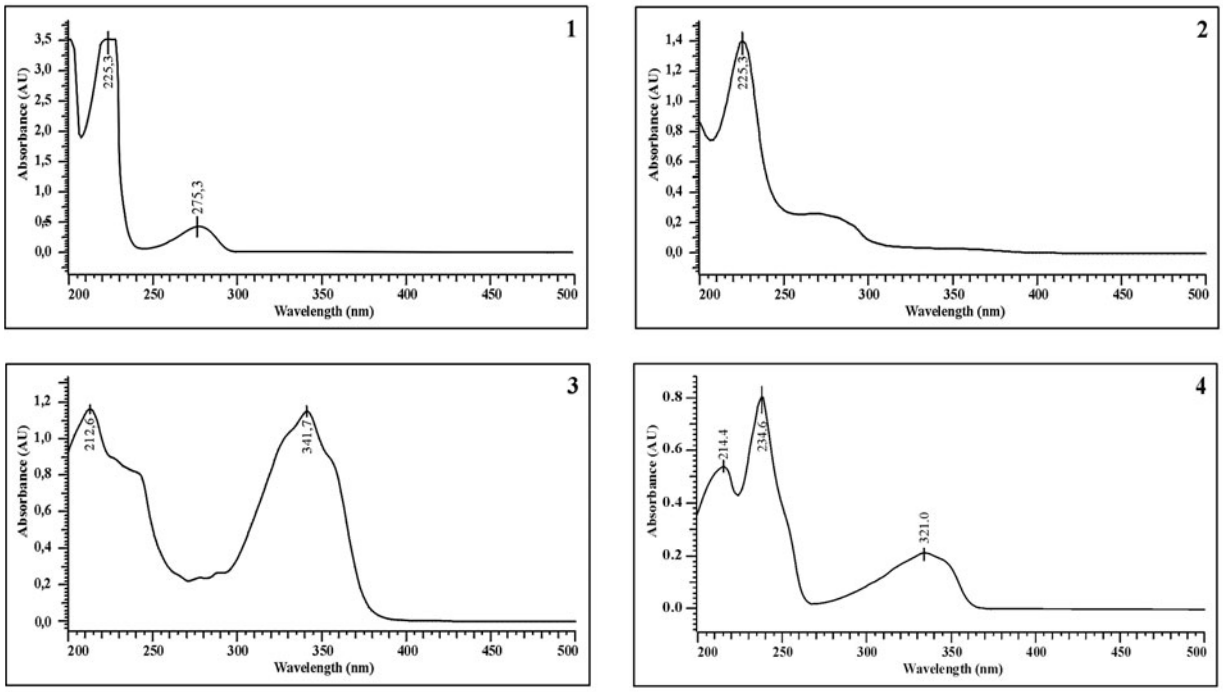

Fig 5: UV-spectra of synephrine (**1**), evodiamine (**2**) and rutaecarpine (**3**) and quinolone alkaloids (**4**)

4) Description of the HPLC-chromatogram, **Fig. 4a** and **4b**:

The HPLC-chromatograms of all *Evodia* extracts (sample 1-5) show at 225 nm in the Rt-range of 2.0 to 11.0 the three alkaloids synephrine (**1**) at Rt 2.6, evodiamine (**2**) at Rt 9.2 and rutaecarpine (**3**) at Rt 10.3 in different concentrations. Additionally in all extracts at Rt 14.8 and Rt 22.2 appear two not identified peaks. The lipophilic quinolone alkaloids (**4**) can be detected in the Rt-range from 25.5 to 30.0 min.

A HPLC separation of Evodiae rutaecarpae fructus has also been published by Chuang et al.[23]

Note: The Chinese Pharmacopoeia 2005 demands for Fructus Evodiae not less than 0.15 % of evodiamine and rutaecarpine, calculated with reference to the dried drug.

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Rhizoma Anemarrhenae
Zhimu

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005

Official drug[1]: Common Anemarrhena Rhizome is the dried rhizome of *Anemarrhena asphodeloides* Bge. (Fam. Liliaceae).

The drug is collected in spring and autumn, removed from fibrous root and soil and dried in the sun.

Description of the drug[1]: Slat-shaped, slightly curved, somewhat compressed, branched occasionally, 3 – 15 cm long, 0.8 – 1.5 cm in diameter. One end exhibiting pale yellowish stem and leaf scars. Externally yellowish-brown to brown, the upper surface exhibiting a concave groove and closely arranged annular nodes with dense yellowish-brown remains to leaf bases growing upward bilaterally; the lower surface raised and somewhat shrivelled, exhibiting depressions or protruding dotted root scars. Texture hard, easily broken, fracture yellowish-white, Odour slight; taste, slightly sweetish, bitterish and viscous on chewing

Provinces[2]: Hebei, Henan, Shan Xi, Hubei, Guang Dong, Jiangsu, Inner Mongolia, Ningxia

Pretreatment of the raw drug[1]: *Rhizoma Anemarrhenae:*
Foreign matters are eliminated, washed, softened thoroughly, cut into thick slices, dried and removed from hairs and scraps.

Rhizoma Anemarrhenae (processed with salt):
Salt-water is added to clean crude drugs, mixed well in a closed vessel until they are infused thoroughly. They are placed in a pot, stir-baked with gentle heat until they are dry, taken out and cooled.

Medicinial use[3]: infectious and febrile diseases, chronic bronchitis, dry cough

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,2]	
Taste:	Slightly sweetish, bitterish and viscous on chewing
Temperature:	cool
Channels entered:	Stomach, lung, kidney
Effects (functions):	Removes heat and quenches fires, replenishes Yin, promotes the production of body fluid and relieves dryness syndrome
Symptoms and indications:	Febrile diseases with high fever and dire thirst; heat in the lung with dry cough; consumptive fever; diabetes due to internal heat; constipation

Toxicity:

Contraindicated for patients with diarrhea due to a spleen deficiency

Main constituents:

– **steroid sapogenins and saponins:**
sarsasapogenin[4], markogenin[4], timosaponin A-I[4], A-II[4], A-III[4], A-IV[4], B[5], B-I[4], B-II[4], B-III[6], E1[7], E2[7], F[5], pseudoprototimosaponin A III[8], anemarrhenasaponin I[5,6,7], anemarrhenasaponin Ia[5,6], anemarsaponin A1[9], A2[9], B[9], F[10], G[10], smilageninoside[11]

– **norlignans:**
hinokiresinol[4], cis-hinokiresinol[12], oxy-hinokiresinol[4]

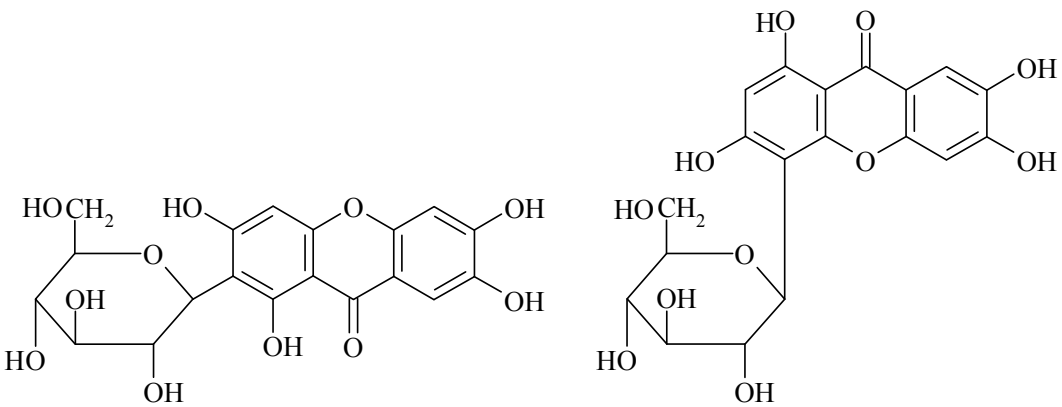
– **xanthone C-glucosides:**
mangiferin[4], isomangiferin[4], neomangiferin[13]

– glycans:
anemarans A, B, C, D[4]

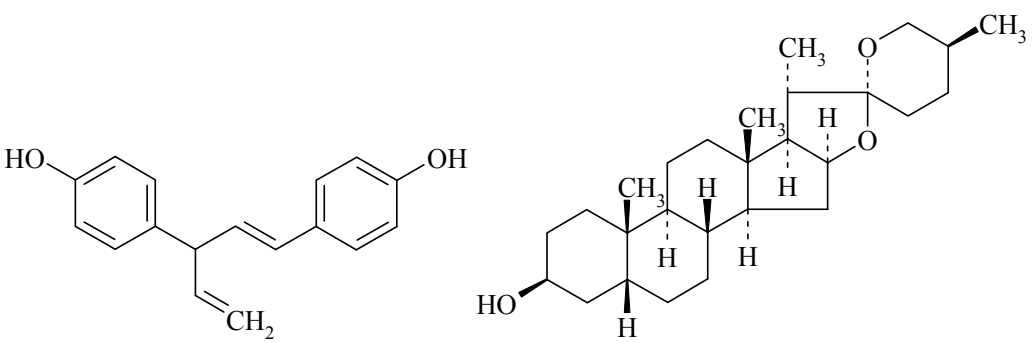
– other constituents:
2,6,4'-trihydroxy-4-methoxy-benzophenone[4], p-hydroxy-phenyl-crotonic acid[4], nyasol = (z)-1,3-bis(4-hydroxyphenyl)-1,4-pentadiene[14]

Pharmacology:

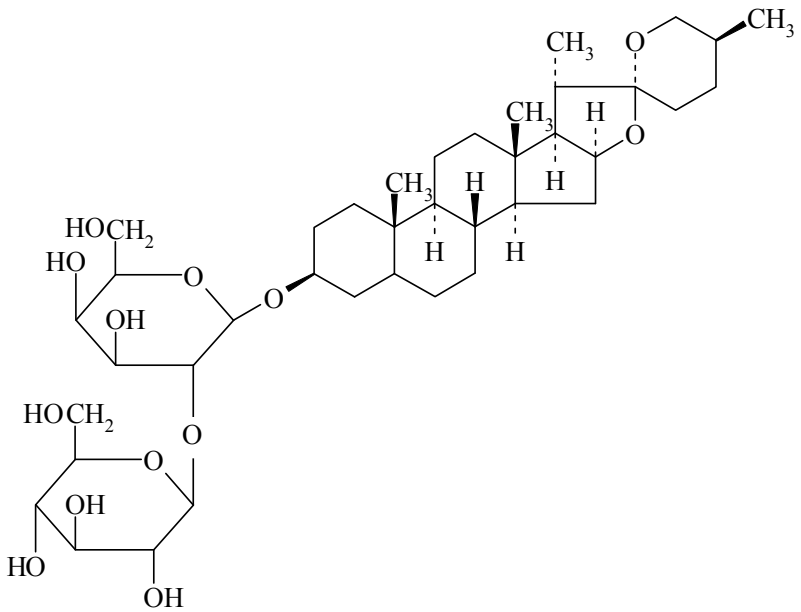
- antifungal[14]
- antioxidative[15]
- antiviral[15]
- anticancer[15]
- inhibits platelet aggregation[7]
- antipyretic[13]
- anti-inflammatory[13]
- diuretic[13]
- antidiabetic (mangiferin)[13,16]
- antihypertonic[17]



Mangiferin Isomangiferin



Hinokiresinol Sarsasapogenin



Timosaponin A-III

Fig. 1: Formulae of the main compounds

TLC fingerprint analysis

Drug samples		Origin
1	Anemarrhenae rhizoma / *Anemarrhena asphodeloides*	province Anhui, China
2	Anemarrhenae rhizoma / *Anemarrhena asphodeloides*	province Hebei, China
3	Anemarrhenae rhizoma / *Anemarrhena asphodeloides*	province Inner Mongolia, China
4	Anemarrhenae rhizoma / *Anemarrhena asphodeloides*	province Ningxia, China
5	Anemarrhenae rhizoma / *Anemarrhena asphodeloides*	sample of commercial drug, China

Reference compounds		R*f*
T 1	mangiferin	0.77
T 2	sarsasapogenin	0.41

1. Thin layer chromatogram of xanthones and saponins:

1) Extraction: 1 g of the powdered drug is extracted under reflux with 10 ml ethanol 99 % for 40 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol 99 %.

2) Reference compound: 1 mg of mangiferin is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Anemarrhenae rhizoma extract: each 3 µl
reference compound: 7 µl

Solvent system: ethyl acetate : acetic acid : formic acid : water
100 11 11 26

The plate is developed in a glass chamber, strongly saturated (at least 1 hour) with the solvent mixture before chromatography.

Detection: a) Detection of xanthones:
Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol
II: 5 % polyethylene glycol-4000 (PEG) in ethanol
The plate is sprayed first with solution I and then with solution II.
The evaluation is carried out in UV 365 nm.

b) Detection of saponins:
Anisaldehyde-sulphuric acid reagent (AS):
0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The plate is sprayed with this mixture, heated at 100 °C for 5 – 10 minutes and then evaluated in VIS.

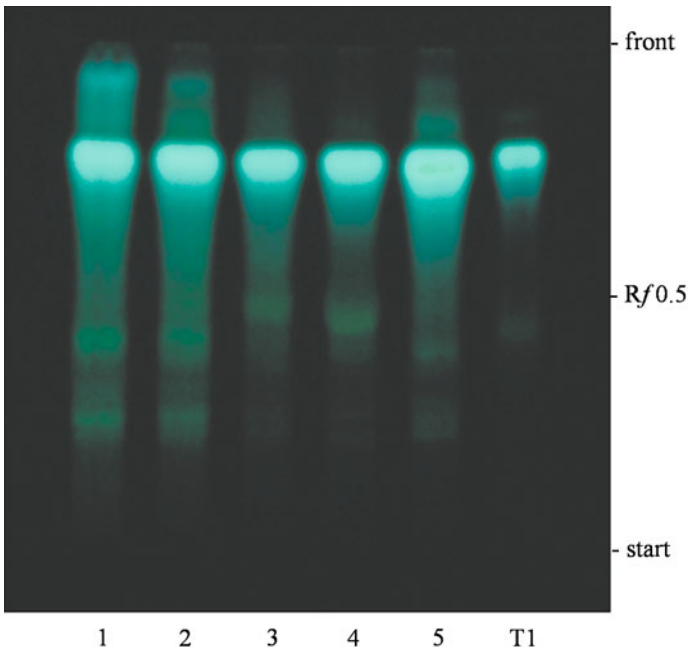


Fig. 2a:
Thin layer chromatogram of the ethanolic extracts of Anemarrhenae rhizoma after spraying with natural products-polyethylene glycol reagent (UV 365 nm)

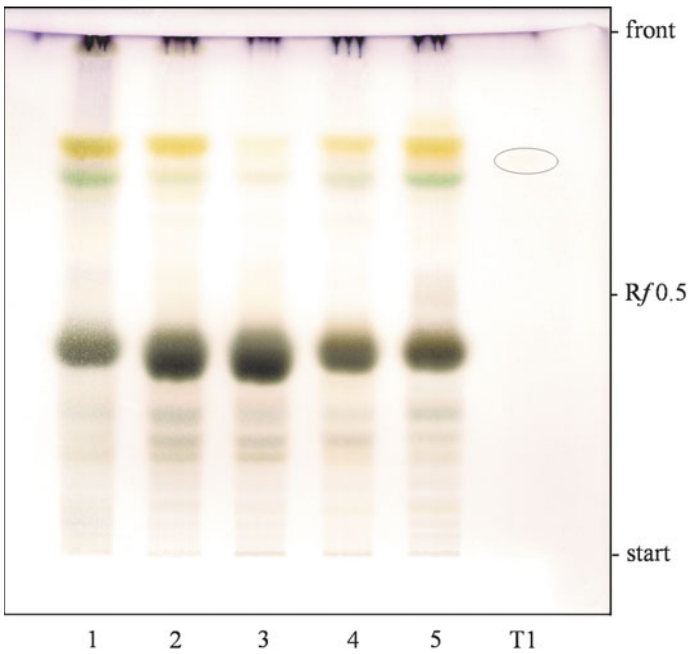


Fig. 2b:
Thin layer chromatogram of the ethanolic extracts of Anemarrhenae rhizoma after spraying with anisaldehyde-sulfuric reagent (VIS)

4) Description of Figure 2a and 2b

Figure 2a:

The chromatogram with the five commercial samples from different provinces of China show mangiferin as prominent turquoise fluorescent spot at R*f* = 0.77. In the lower R*f*-range appear some light green fluorescent spots which might derive from other xanthonglycosides.

Figure 2b:

In the R*f*-range of 0.18 – 0.45 grey green spots of the various saponins with a dominant dark green spot at R*f* = 0.38 (Timosaponin?) can be seen. In the R*f*-range 0.75 – 0.85 a yellow (Mangiferin) and green zone can be detected.

2. Thin layer chromatogram of sapogenins:

1) Extraction: 1 g of the powdered drug is extracted under reflux with 10 ml ethanol 99 % for 40 minutes. The extract is cooled, filtered and 1 ml hydrochloric acid 37% is added. The mixture is heated under reflux for 1 hour, 10 ml of water are added, extracted by shaking with 20 ml of toluene and the toluene extract evaporated to dryness. The residue is dissolved in 2 ml toluene.

2) Reference compound: 5 mg of sarsasapogenin are dissolved in 1 ml toluene

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Anemarrhenae rhizoma extract: each 5 µl
reference compound: 5 µl

Solvent system: toluene : acetone
9 1

Detection: Vanillin sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I immediately followed by solution II. Afterwards the plate is heated for 5 – 10 minutes at 105 °C. The evaluation is carried out in VIS.

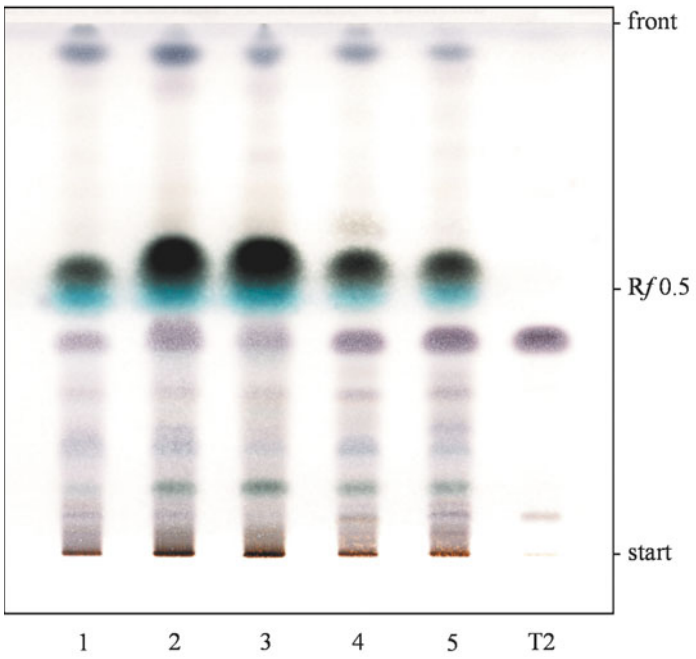


Fig. 2c:
Thin layer chromatogram of the toluene extracts of Anemarrhenae rhizoma after spraying with vanillin-sulfuric acid reagent (VIS)

Figure 2c:

Chromatogram 2c shows the violet spot of sarsasapogenin at R*f* = 0.41. Beneath several violet blue zones of other sapogenins or phytosterols are visible. Above the sarsasapogenin zone a characteristic blue and prominent black zone of unknown structures appears.

HPLC-fingerprint analysis:

1) Sample preparation:	To 1 g of the powdered drug 10 ml ethanol 99 % are added and extracted under reflux for 1 hour. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol 99 %, filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.
2) Injection volume:	Anemarrhenae rhizoma extract: 2.0 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck

Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Acros Organics) + H_3PO_4 85 % (Merck) (pH = 3)
	B: acetonitrile (Acros Organics)
Gradient:	5 – 30 % B in 5 minutes 30 % B in 15 minutes 30 – 95 % B in 10 minutes 95 % B in 15 minutes total runtime: 45 minutes
Flow:	0.6 ml/min.
Detection:	210 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	12.3	xanthone C-glucoside
2	13.5	mangiferin
3	13.7	xanthone C-glucoside
4	15.3	unknown flavonoid
5	26.6	non identified
6 / 7	34.6 / 36.9	non identified
8	38.9 – 43.6	steroid sapogenins

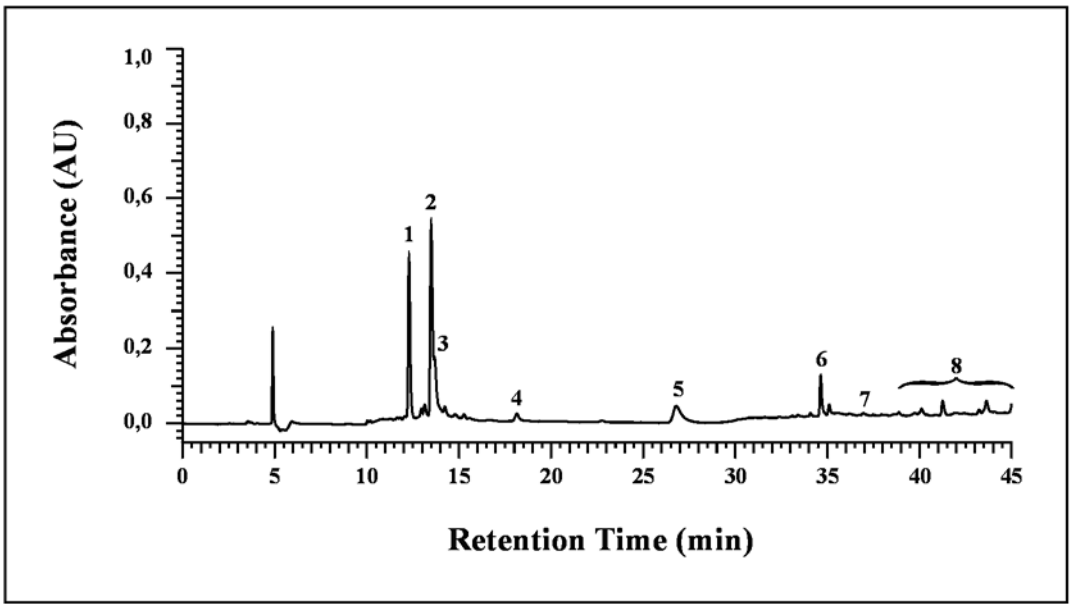


Fig. 3a: HPLC-fingerprint chromatogram of the ethanol extract of Anemarrhenae rhizoma (Province Hebei)

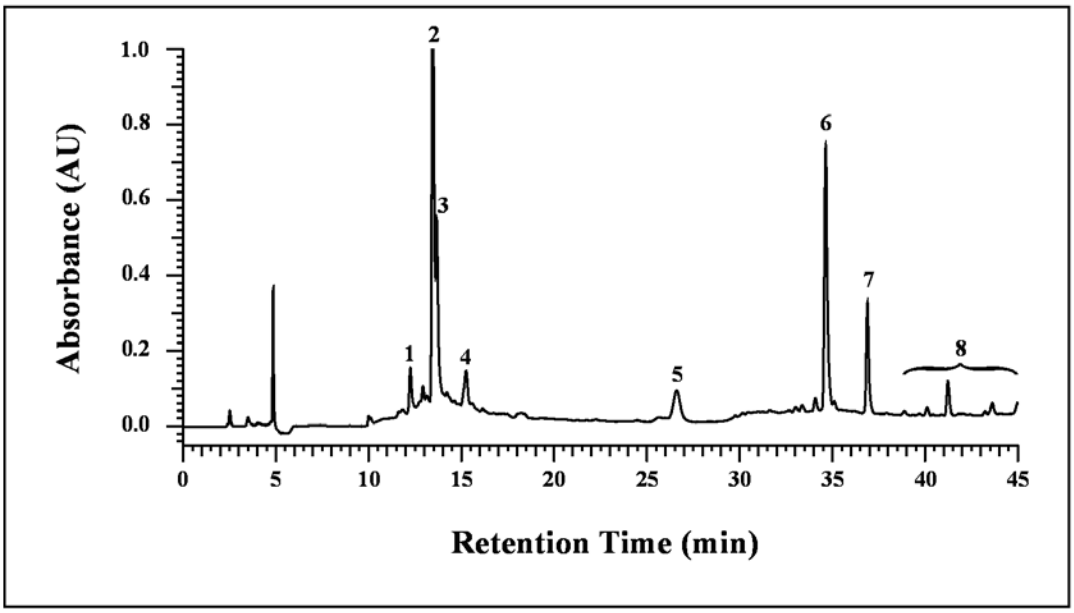


Fig. 3b: HPLC-fingerprint chromatogram of the ethanol extract of Anemarrhenae rhizoma (Province Ningxia)

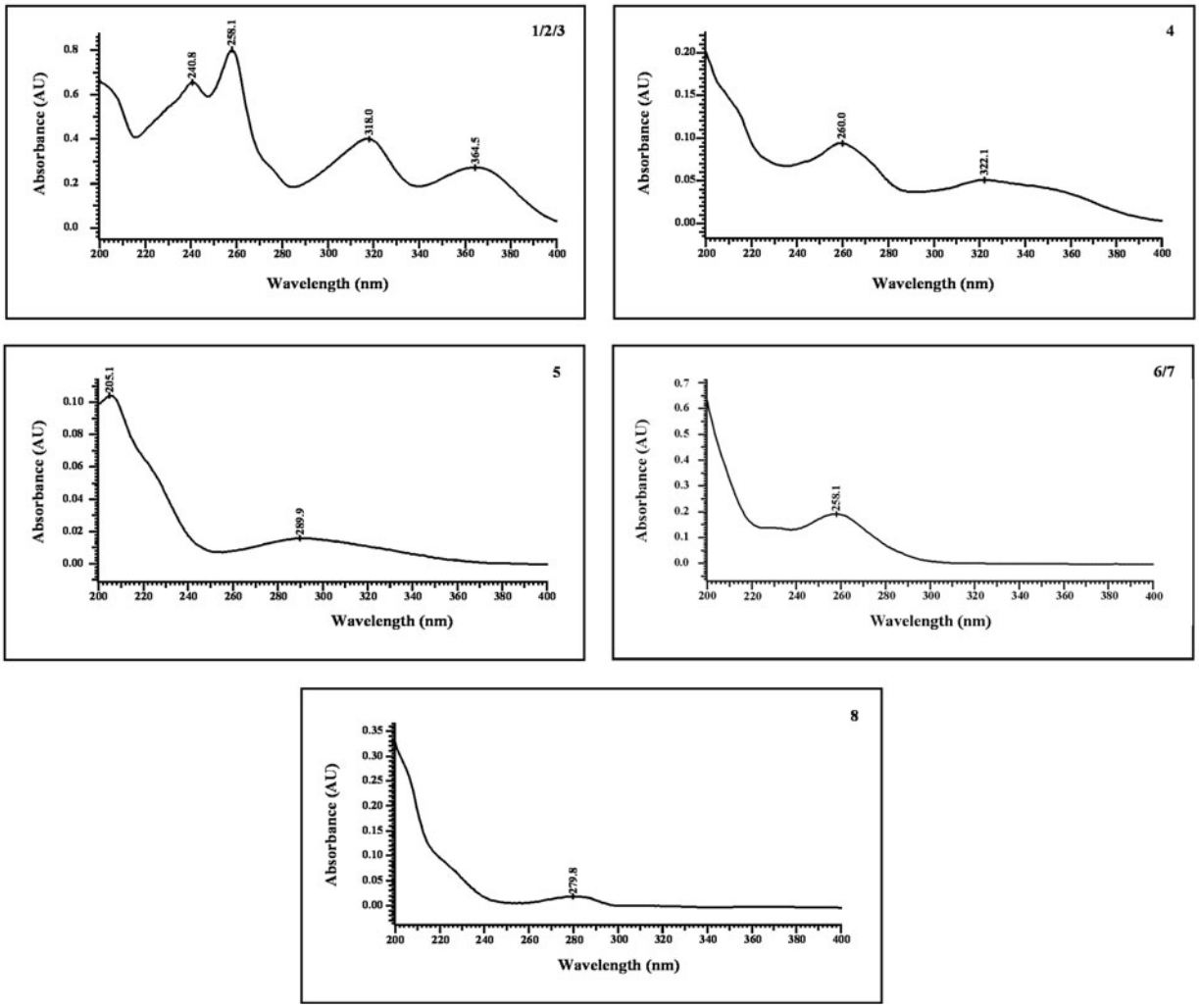


Fig. 4: UV-spectra of the main compounds (peaks) of the ethanol extracts of Anemarrhenae rhizoma

4) Description of the HPLC of Figure 3a and 3b:

The HPLC features of Anemarrhenae rhizoma from the Provinces Hebei and Ningxia are qualitatively very similar. Both HPLC are characterized by one major peak of mangiferin (**2**) at Rt = 13.5. The spectra of peak **1** – **3** show a similar UV-spectrum as mangiferin and can be assigned to other hydroxylated xanthon-C-glucosides. Peak **4**, **6** and **7** (Rt = 15.3, 34.6 and 36.9) might be flavonoids. The peaks from Rt = 38.9 to 43.6 derive probably from steroid sapogenins. Both extract samples differ in the quantities of the individual compounds only.

Note: The Chinese Pharmacopoeia 2005 demands for Rhizoma Anemarrhenae not less than 1.0 % of sarsasapogenin, calculated with reference to the dried drug.

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Radix Acanthopanacis senticosi
Ciwujia

Pharmacopoeia:

Radix Acanthopanacis senticosi: Pharmacopoeia of the People's Republic of China, English Edition 1997/2005[1]

Extractum Acanthopanacis senticosi: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[2]

Official drugs[1,3]:

Acanthopanax (Eleutherococcus) senticosus (Rupr. et Maxim.) Harms
The drug is commonly known as "Siberian ginseng",

– Araliaceae –

Origin[3-5]:

Acanthopanax senticosus grows in Northern China (province Shansi, Hopei), Siberia (Khabarovsk, Primorsk), Korea and Japan (island Sachalin) above 500 m altitude.

Description of the drug[1]:

Rhizomes irregular, nodular cylindrical, 1.4 – 4.2 cm in diameter. Root cylindrical, mostly tortuous , 3.5 – 12 cm long, 0.3 – 1.5 cm in diameter; external greyish-brown or blackish-brown, rough, with fine longitudinal furrows and wrinkles, bark relatively thin, sometimes exfoliated, the exposed surface appearing greyish-yellow. Texture hard, fracture yellowish-white, fibrous.
Odour, characteristic and aromatic; taste, slightly pungent somewhat bitter and adstringent.

Pretreatment of the raw drug[1]:

The dried root and rhizome of *Acanthopanax senticosus* are collected in spring and autumn, washed clean and dried.

Production of the extract[2]:

To 1000 g of the coarse powder of Radix Acanthopanacis senticosi 7 volumes of 75 % ethanol are added, heated under reflux for 12 hours, filtered, ethanol recovered from the filtrate and the solution concentrated to 50 g of extract.

Medicinal use[1,3,5]:

For the treatment of general weakness, lassitude, anorexia, insomnia, dream-disturbed sleep and vegetative dystony. As a prophylactic and restorative tonic for enhancement of mental and physical capacities in cases of weakness, exhaustion and tiredness, and during convalescence.

In Western medicine used as an immunostimulant, adaptogenic and antistress drug.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1,5]	
Taste:	bitter and adstringent, acrid and persistent
Temperature:	warm
Channels entered:	kidney and liver
Effects:	stimulation of the immune system, promotion of an overall improvement in physical and mental performance, reinforces the *qi*, invigorates the function of the spleen and kidney
Symptoms and indications:	varities of adverse conditions (stress, immobilization or chemical challenge), hypofunction of the spleen and kidney, aching of the loins and knees

Main constituents[5-7]**:** (see Fig. 1)

- **lignans:** eleutheroside E [(-)-syringaresinol-4,4´-O-β-D-diglucosid), eleutheroside E_1 [(-)-syringaresinol-O-β-D-monoglucosid], eleutheroside B_4 [(-)sesamin)], eleutheroside D, (-)syringaresinol
- **phenylpropane derivatives:** eleutheroside B (syringin), caffeic acid derivatives, caffeic acid ethyl ester, sinapinalcohol, chlorogenic acid, isochlorogenic acids a, b, c
- triterpene saponins, sterols: daucosterol
- coumarins: isofraxidin, eleutheroside B_1 (isofraxidin-7-O-glucoside)
- polysaccharide

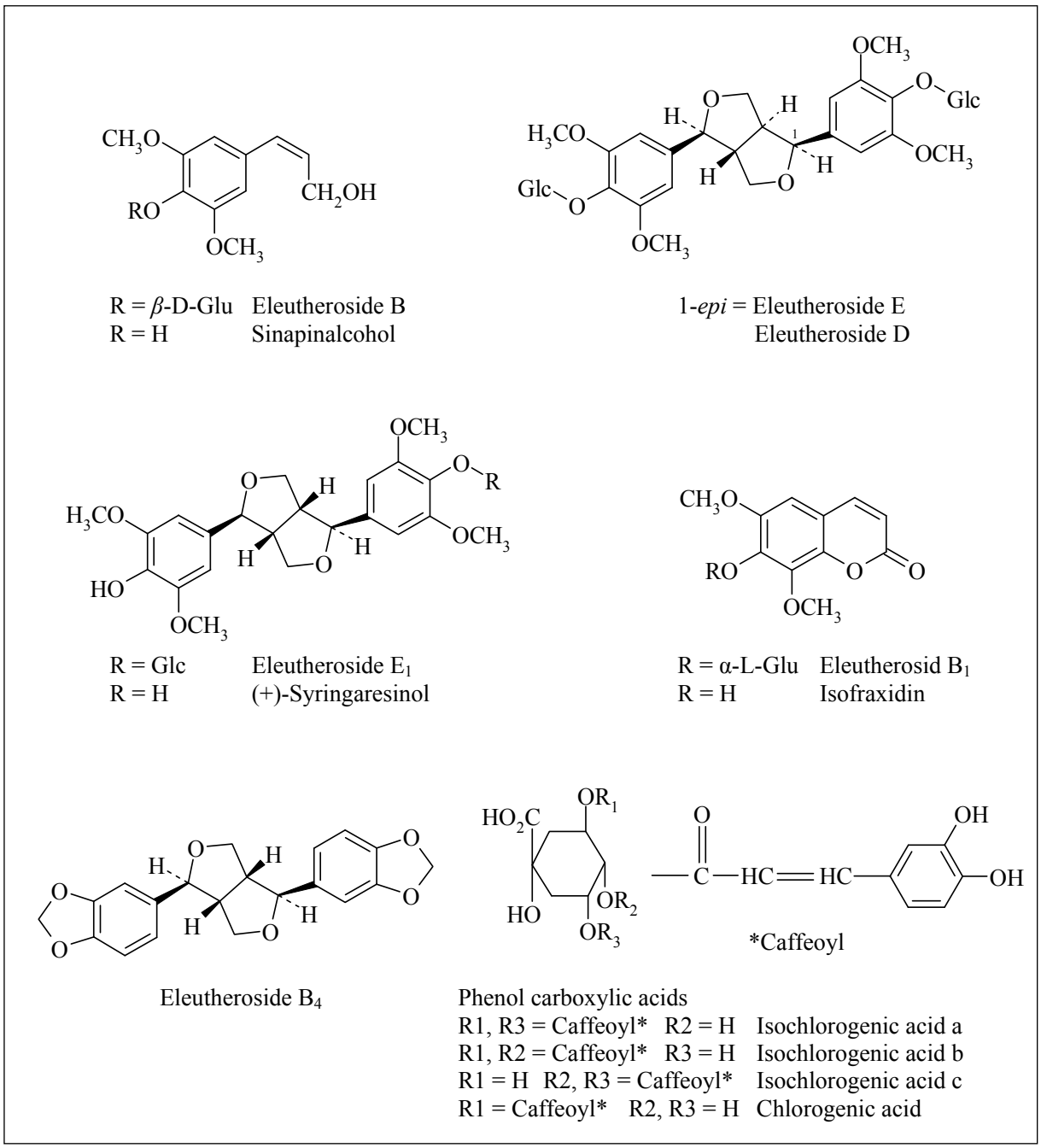

Fig. 1: Formulae of the main constituents of Acanthopanacis senticosi radix[5-7]

Contraindication[5]: Radix Eleutherococci should not be used during pregnancy, lactation or patients with hypertension, *Yin* weakness and depletio of *Yin* with calor.

Pharmacology/clinic: *in vitro*/*in vivo*:
- adaptogenic activity (antistress effect)[8-14]
- immunomodulating activity[15-18]
- apoptose inducing effect[19]
- antitumoral effect[20]

- antiaging effect[21]
- antiallergic effect[22,23]
- antiischemic effect in patients[24]
- inhibitory effect on platelet aggregation effect[25]
- effect on acute cerebral infarction[26]
- cardioprotective effect[27]
- CAMP-phosphodiesterase inhibiting activity[28]
- hypoglycemic activity[29]
- effect on the pituitary-adrenal system[30]

TLC-fingerprint analysis[7,31]:

1) Extraction: 1.2 g powdered drug are heated under reflux for 15 min with 15 ml 50 % methanol. After cooling down the extract is filtered and evaporated to dryness. The residue is dissolved in 10 ml water and shaken with 10 ml water-saturated *n*-butanol. The *n*-butanol layer is separated, evaporated to dryness and the residue dissolved in 1 ml methanol 50 %.

2) Reference compounds: eleutheroside B, E, E_1, B_4, chlorogenic acid, caffeic acid, isofraxidin, isochlorogenic acid (1mg/ml MeOH)

3) Separation parameters:

Applied amount: 30 µl extract and 10 µl standard solution

Plates: HPTLC Silicagel 60 F_{254}; Merck

Solvent systems: <u>lignans (Fig. 2, 3):</u> chloroform: methanol: water (70:30:4)
<u>coumarin and phenylpropane derivatives (Fig. 4):</u> ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)

Detection: Spray reagents:

<u>lignans:</u>
Antimony-III-chloride reagent (Fig. 2): The TLC-plate must be sprayed with 20 % solution of antimony-III-chloride in chloroform and then heated for 5–6 min. by 110 °C. Evaluation in VIS or UV 365 nm.
Vanillin-phosphoric acid reagent (Fig. 3): 1 g vanillin are dissolved in 100 ml 50 % phosphoric acid. After spraying the plate is heated for 10 min. at 100 °C. Evaluation in VIS or in UV 365 nm.

<u>coumarin and phenylpropane derivatives:</u>
Natural products-polyethylene glycol reagent (Fig. 4): The plate is sprayed with 1% methanolic diphenylboric acid-β-ethylamino ester (NP), followed by 5 % ethanolic polyethylene glycol-4000 (PEG). Evaluation in UV 365 nm.

Drug samples		Origin
1	Acanthopanacis radix / *Acanthopanax senticosus*	locality Jirin; China
2	Acanthopanacis radix / *Acanthopanax senticosus*	sample of commercial products; China
3	Acanthopanacis radix / *Acanthopanax senticosus*	locality Kirin, Tongfeng; China
4	Acanthopanacis radix / *Acanthopanax senticosus*	locality Kirin, Antun; China
5	Acanthopanacis radix / *Acanthopanax senticosus*	samples of commercial products; Korea
6	Acanthopanacis radix / *Acanthopanax senticosus*	

Reference compounds			Rf	
T1	eleutheroside B	Fig. 2	0.63	phenylpropane derivatives
T2	eleutheroside E	Fig. 3	0.54	lignans
T3	eleutheroside E_1		0.82	
T4	eleutheroside B_4		0.98	
T5	chlorogenic acid	Fig. 4	0.61	coumarin and phenylpropane derivatives
T6	caffeic acid		0.93	
T7	isofraxidin		0.93	
T8	isochlorogenic acids		0.59, 0.80, 0.92	

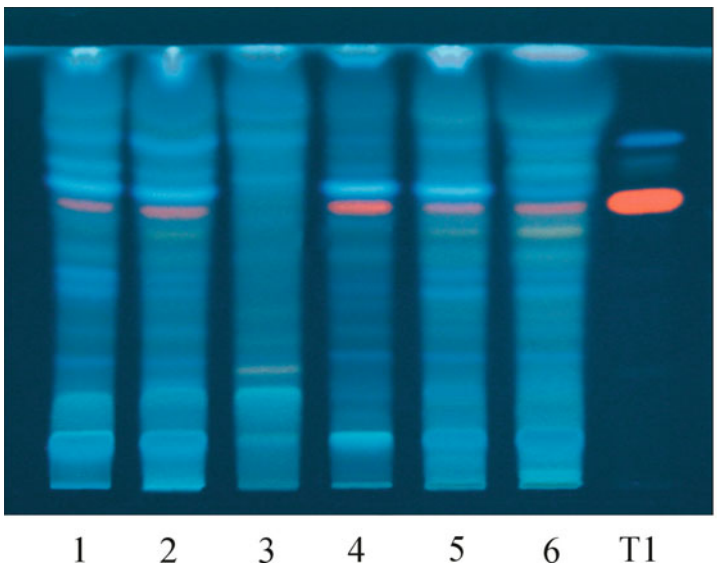

Fig. 2: TLC-fingerprint of lignans of Acanthopanacis senticosi radix detected with antimony-III-chloride reagent in UV 365 nm

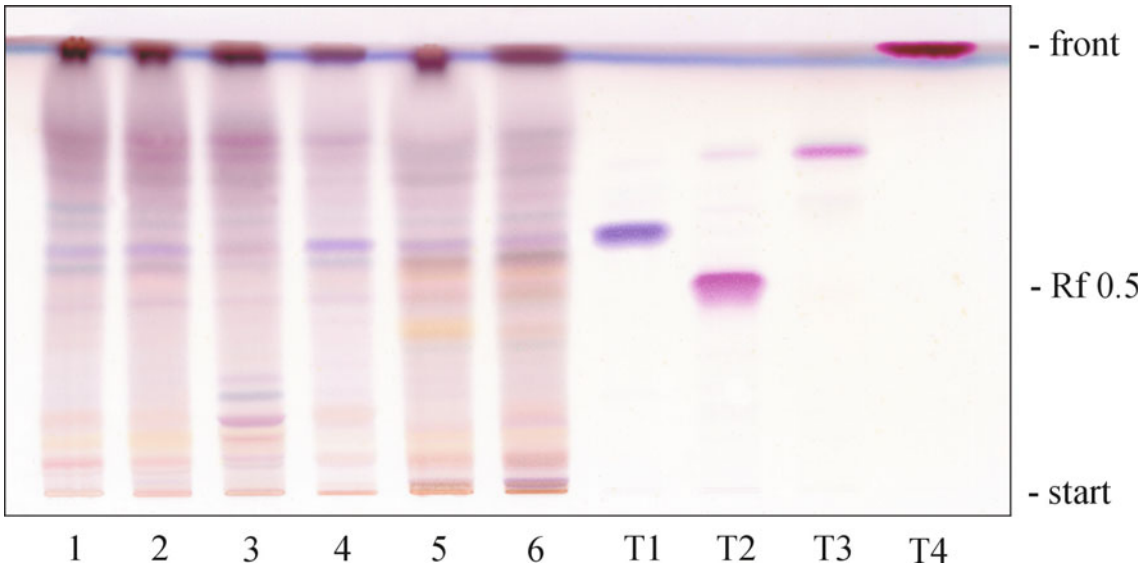

Fig. 3: TLC-fingerprint of lignans of Acanthopanacis senticosi radix detected with vanillin-phosphoric acid reagent in VIS

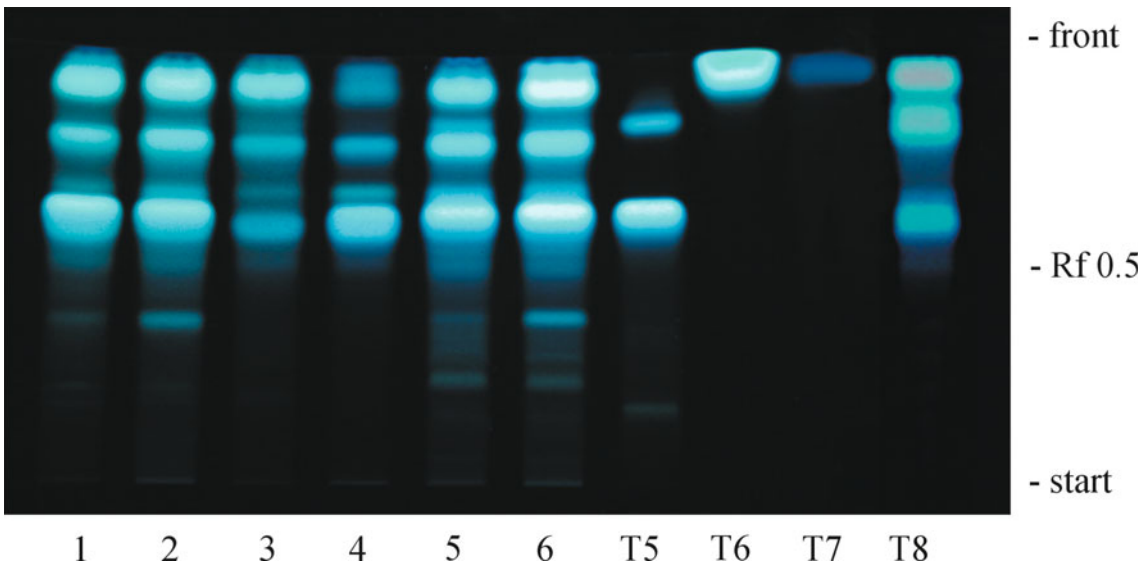

Fig. 4: TLC-fingerprint of coumarin and phenylpropane derivatives of Acanthopanacis senticosi radix detected with natural products-polyethylene glycol reagent in UV 365 nm

Description of the TLC-fingerprint of **Fig. 2**, sprayed with antimony-III-chloride reagent in UV 365 nm:

In samples 1, 2, 4, 5 and 6 appears at R*f* 0.63 the characteristic orange-red fluorescence zone of eleutheroside B (T1). Eleutheroside B can be absent or found in extremely low concentration as shown in sample 3. Eleutheroside E, E_1, B_4 (T2 - T3) are better detectable with vanillin-phosphoric acid reagent in VIS (see **Fig. 3**).

Description of the TLC-fingerprint of **Fig. 3**, sprayed with vanillin-phosphoric acid reagent in VIS:

Eleutherococci radix samples are characterized by a violet zone of eleutheroside B (T1) at R*f* 0.63. Pink zones of eleutheroside E (T2) at R*f* 0.54, its monoglucoside eleutheroside E_1 (T3) at R*f* 0.82 and eleutheroside B_4 (T4) near the solvent front at R*f* 0.98 are detectable in all samples. The brown zones in the R*f*-range 0.05-0.15 may partly derive from free sugars.

Description of the TLC-fingerprint of **Fig. 4**, sprayed with natural product-polyethylenglycol reagent in UV 365 nm:

Eleutherococci radix samples 1–6 show the phenol carboxylic acids and coumarins as blue-azure fluorescent zones: Chlorogenic acid (T5) at R*f* 0.61 and the mixture of isochlorogenic acids (T8) are detectable as 3 zones at R*f* 0.59, 0.80 and 0.92. Caffeic acid (T6) and the coumarin isofraxidin (T7) are detectable overlapped at R*f* 0.93.

As shown in Figs. 2–4, the amount and presence of the constituents of Eleutherococci radix samples 1–6 vary depending on the origin of the plant and season of collection.

HPLC-fingerprint analysis[7]:

1) Sample preparation: The same extracts as used for TLC are filtered over Millipore® (Type HV 0.45 µm).

2) Injection volume: 25 µl extract, 10 µl reference solution

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi
Column:	LiChroCART® 250-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: water + 10 ml 0.1% H_3PO_4 / l, HPLC quality, Acros Organics B: acetonitrile, HPLC quality Acros Organics
Gradient:	10 % B to 17 % B in 4 min. (linear) 17 % B for 21 min. (isocratic) 17 % B to 30 % B in 30 min. (linear)
Flow rate:	0.6 ml/min.
Detection:	220 nm

Retention times and identity of the main peaks of **Fig. 5a**, **Fig. 5b** and **Fig. 5c** detected at 220 nm:

Peak	Rt (min.)	Compound
1	12.0	eleutheroside B
2	12.8	chlorogenic acid
3	16.4	caffeic acid
4	20.7	eleutheroside E
5	28.9	isofraxidin
6	40.9	eleutheroside E_1
7	44.1, 48.6	isochlorogenic acids

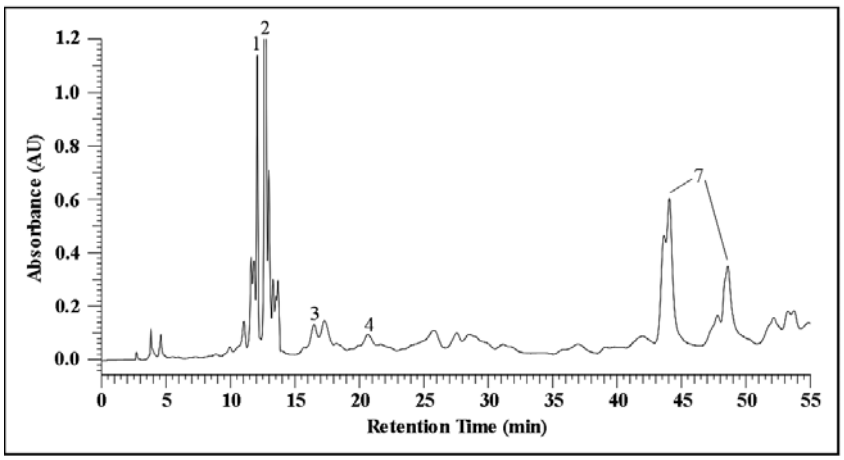

Fig. 5a: HPLC fingerprint of Acanthopanacis senticosi radix sample 2

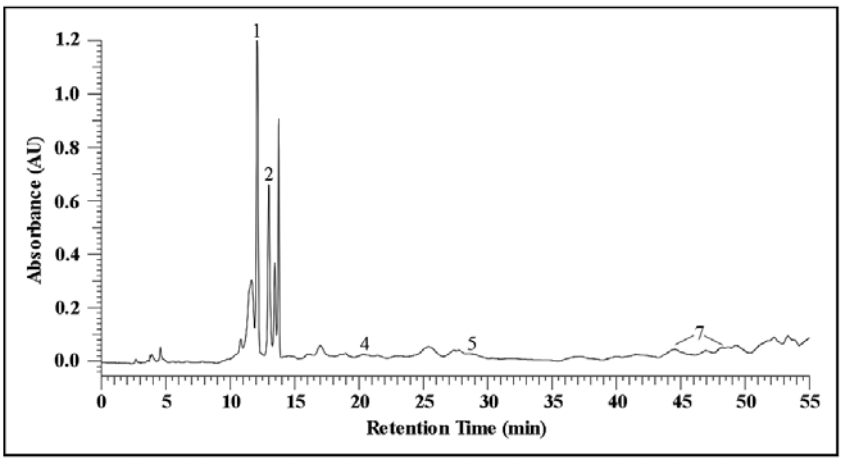

Fig. 5b: HPLC fingerprint of Acanthopanacis senticosi radix sample 4

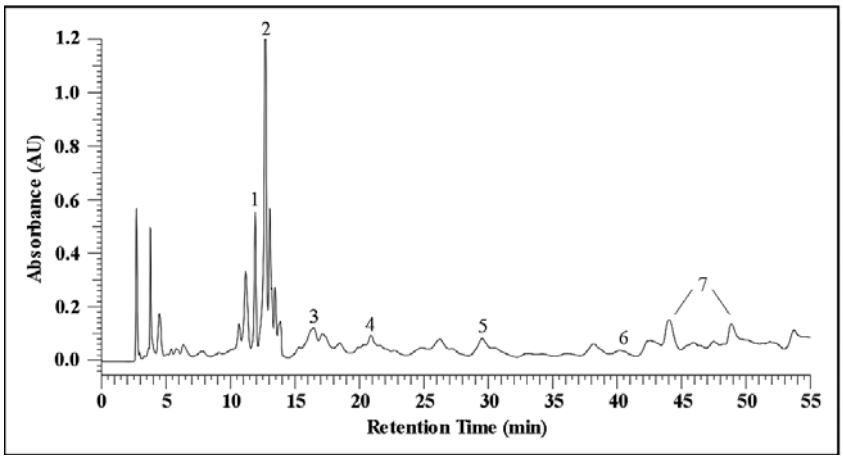

Fig. 5c: HPLC fingerprint of Acanthopanacis senticosi radix sample 6

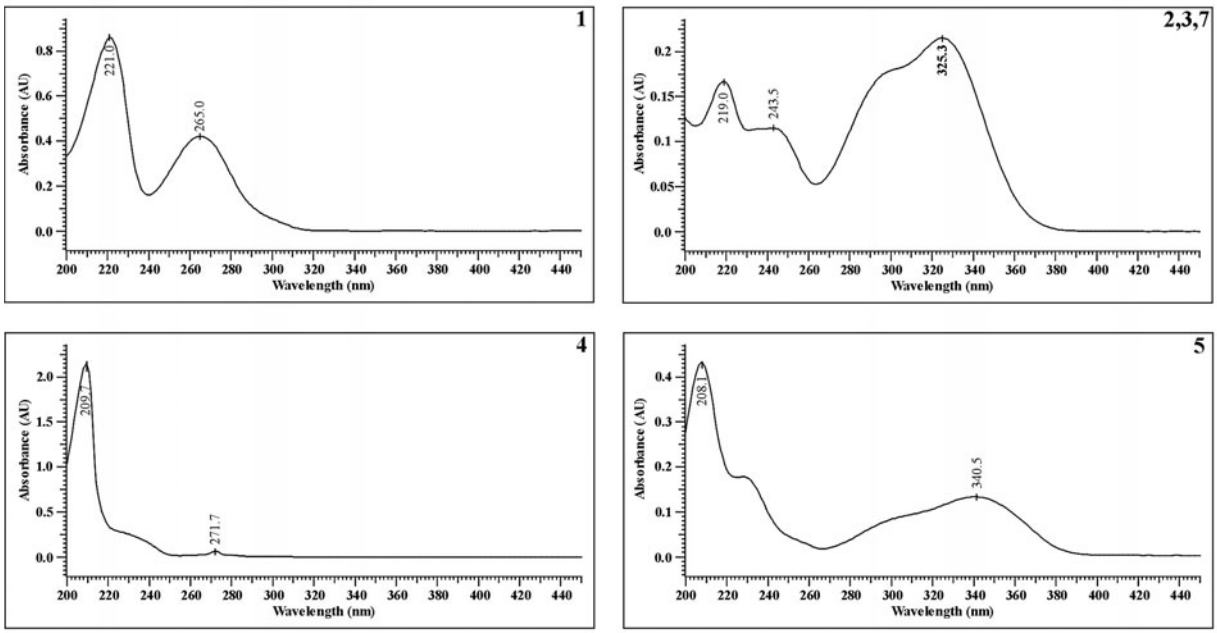

Fig 6: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of Acanthopanacis senticosi radix:

4) Description of the HPLC-chromatogram, **Fig. 5a, Fig. 5b** and **Fig. 5c**:

The HPLC-fingerprint of all Acanthopanacis radix extracts (sample1-6) show at 220 nm a major peak of the lignan eleutheroside B (**1**) at Rt 12.0. Characteristic peaks of chlorogenic acid (**2**) appear at Rt 12.8 min., caffeic acid (**3**) at Rt 16.4 and isochlorogenic acid derivatives (**7**) at Rt 44.1 and 48.6. Eleutheroside E appears only as small peak at Rt 20.7 (**4**).

The coumarin derivative isofraxidin (**5**) and the lignan eleutheroside E_1 (**6**) are hardly detectable at Rt 28.9 and 40.9 respectively.

Caffeic acid, chlorogenic acid and isochlorogenic acids are better detectable at 332 nm.

The concentrations of the compounds in the extracts vary again depending on the season and province of collection.

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Radix Scrophulariae
Xuanshen

Pharmacopoeia:

Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drugs[1,2]:

Figwort Root is the dried root of *Scrophularia ningpoensis* (Hemsl.).

The following substitute drugs are not official in the Pharmacopoeia of the People's Republic of China:
Radix Scrophulariae buergerianae Miq.,
Radix Scrophulariae grossheimi and
Radix Scrophulariae nodosae L.

– Scrophulariaceae –

Origin[2,3]:

Scrophularia ningpoensis grows in the coastal provinces Anhui, Szuchuan and Shensi of Middle China and along the Yangzi river.

Description of the drug[1]:

Subcylindrical, slightly thick at the middle part or thick at the upper part and slender at the lower part, sometimes slightly curved, 6–20 cm long, 1–3 cm in diameter. Externally greyish-yellow or greyish-brown, with irregular longitudinal grooves, transverse lenticels, sparese transverse fissures and numerous rootlet scars. Texture compact, uneasily broken, fracture black, somewhat lustrous.
Odour, characteristic resembling caramel; taste, sweetish and slightly bitter.

Pretreatment of the raw drug[1]:

The drug is collected in winter when stem and leaves wither, removed from rhizome, bud, rootlet and soil, dried in the sun or baked to be half-dried, piled up for 3–6 days, processed until dried completely.

Medicinal use[2-4]:

- bronchitis with dry cough and slight mucus
- pharyngitis, laryngitis with bulge and pain
- infectious disease exanthema (furuncle and carbuncle)
- lymph gland inflammation, struma, lump formation
- ear and eye infection (conjunctivitis)
- obstipation
- blain of the abdomen

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1-4]	
Taste:	sweet, bitter, salty
Temperature:	cold
Channels entered:	acts on the stomach, lung and kidney channels
Effects:	• reduces heat from blood • supplements, nourishes and moistens *Yin*, quenches heat and fire from inside • eliminates toxins • disintegrates agglomerate
Symptoms and indications:	febrile diseases with impairment of *Yin* manifested by deep red tongue and dire thirst or with eruptions, constipation due to impairment of body fluid; phtisis with cough, conjunctivitis, sore throat, scrofula, diphteria, boils and sores, internal bleeding

Main constituents:
(see Fig. 1):

iridoid glycosides[5-7]:
- harpagoside, harpagide, 8-O-feruloyl harpagide, 8-O-(2-hydroxycinnamoyl) harpagide, 6-O-α-D-galacto-pyranosyl harpagoside
- catalpol derivatives: aucubin, loganin, verbenalin

phenylpropanoid glycosides:
- ningposides A, B and C[8]
- sibirioside A[8]
- cistanoside D and F[8]
- angoroside C[8,9]
- acteoside,

decaffeoylacteoside[8,9]
phenol carboxylic acids[10]:
- cinnamic acid and its derivatives, caffeic acid

Other compounds:
fatty acids[2]:
- linoleic acid, oleic acid, stearic acid

alkaloids in traces[2]

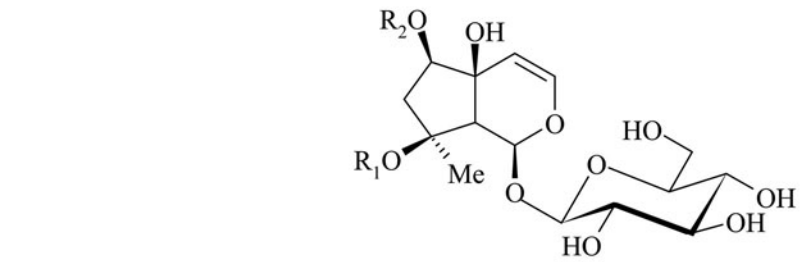
essential oil compounds[2]

carotene[2]

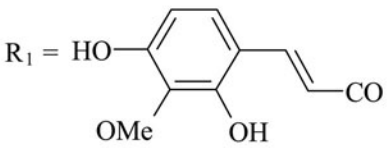
saponins[11]:
- aescin

amino acid[2]:
- 1-asparagin

steroide[2]:
- sterol

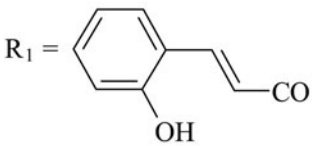


8-O-feruloyl harpagide:



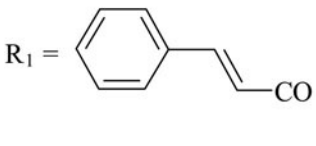
R2 = H

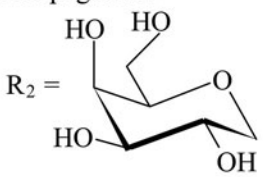
8-O-(2-hydroxy-cinnamoyl)harpagide:



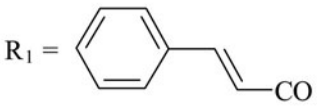
R2 = H

6-O-α-D-galacto pyranosyl harpagoside:





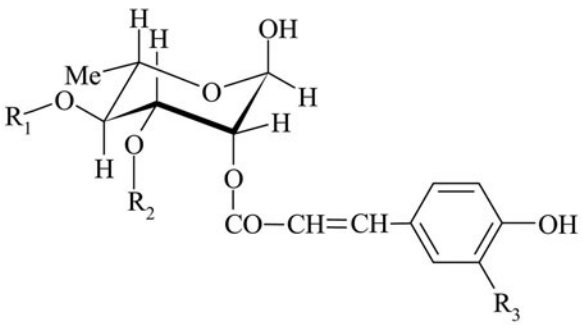
harpagoside:



R2 = H

harpagide:

R1 = H R2 = H



ningposide A:	$R_1 = H$	$R_2 = CO\text{-}CH_3$	$R_3 = OCH_3$
ningposide B:	$R_1 = CH_3CO$	$R_2 = H$	$R_3 = OCH_3$
ningposide C:	$R_1 = H$	$R_2 = CO\text{-}CH_3$	$R_3 = H$

Fig. 1: Formulae of the main constituents[5,8,12,13]

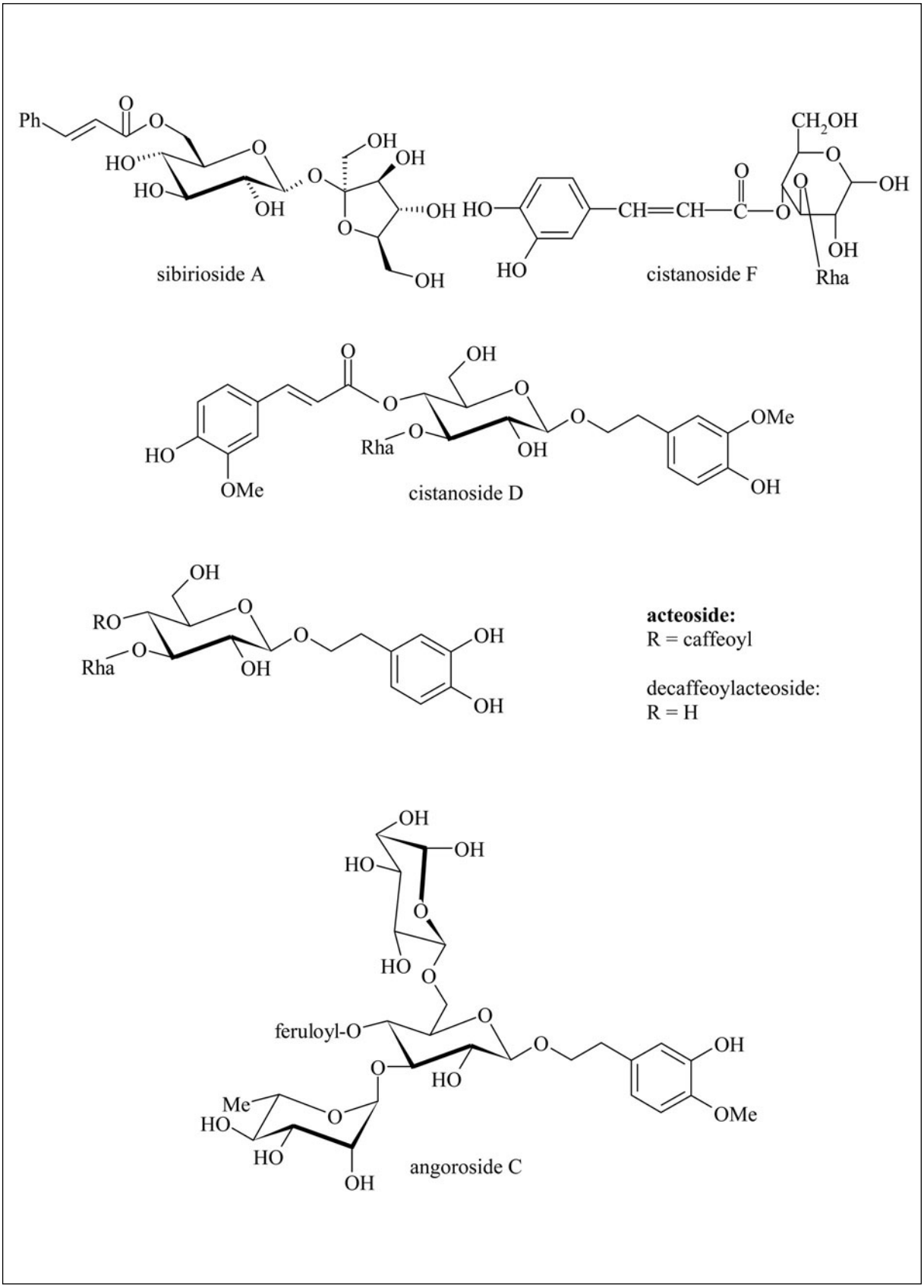


Fig. 1: Formulae of the main constituents[5,8,12,13]

Pharmacology:

- anti-inflammatory activity[6]
- antioxidant effect[14]
- antidepressant effect[15]
- inhibitory activity against substance P-induced itching[16]
- antagonizing adverse effects of high doses of chemotherapeutics (MTX, vincristine)[17]

TLC-fingerprint-analysis[1,7,10]:

1) Extraction: 1.0 g powdered drug is ultrasonicated with 10 ml *n*-butanol for 30 min. After cooling down the extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2) Reference compounds: harpagoside, aucubin, aescin, acteoside, angoroside C, catalpol (1 mg/ml MeOH)

3) Separation parameters:

   Applied amount: 10 µl extract and standard solution

   Plate: HPTLC Silicagel 60 F_{254}; Merck

   Solvent system: ethyl acetate : methanol : water (77 : 15 : 8)

   Detection: Spray reagent:

   Vanillin-sulphuric-acid-reagent:
   The plate is intensively sprayed with 1 % ethanolic vanillin-solution, and with 10 % ethanolic sulphuric acid followed by heating for 10 minutes under supervision at 110 °C. The evaluation is carried out in VIS.

Drug samples		Origin
1	Radix Scrophulariae/ *Scrophularia ningpoensis*	locality Zhejiang; China
2	Radix Scrophulariae/ *Scrophularia ningpoensis*	sample of Anguo market; China
3	Radix Scrophulariae/ *Scrophularia ningpoensis*	locality Guizhen; China
4	Radix Scrophulariae/ *Scrophularia ningpoensis*	sample from Peking market; China
5	Radix Scrophulariae	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
6	Radix Scrophulariae	sample of commercial product, Uchida company; Japan
7	Radix Harpagophyti/ *Harpagophytum procumbens*	sample of commercial product, Caelo; Germany
8	Herba Scrophulariae/ *Scrophularia nodosa*	sample of commercial product, Caelo; Germany

Reference compounds		R*f*
T1	mixture of harpagid, procumbid and harpagoside	0.18 0.54
T2	aucubin aescin	0.25 0.06
T3	acteoside angoroside C	0.62 0.46/0.43
T4	catalpol	0.23

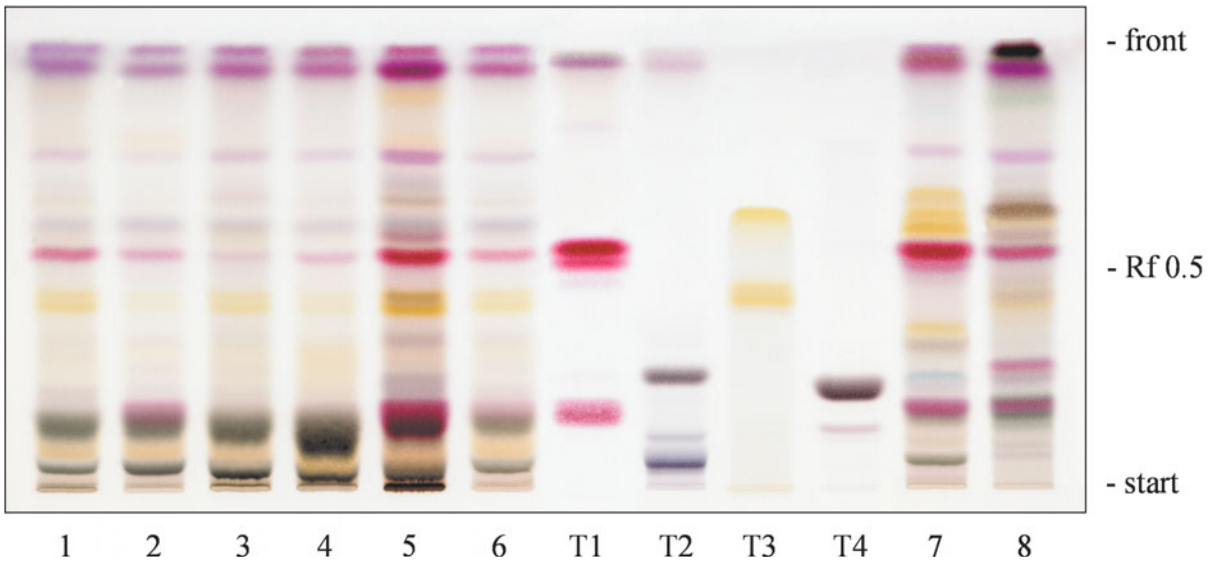


Fig. 2: TLC-fingerprint of Radix Scrophulariae *n*-butanol extracts, detected with vanillin-sulphuric-acid-reagent in VIS

4) Description:

All Radix Scrophularia samples (1–6) show a nearly homogenous pattern of pink-violet zones of harpagid and procumbid (T1, R*f*=0.18), harpagosid (T1, R*f*=0.54) and two further iridoid derivatives on the solvent front overlapped by carotenes. The double zone of harpagosid is due to the cis-, trans-isomerism in the cinnamoyl moiety. Cinnamic acid appears as grey-green zone at R*f* = 0.15.

A conspicuous yellow zone of angoroside C (split into two zones) is detected at R*f* 0.46/0.43 (T3) and a hardly visible yellow zone of acteoside is detected at R*f* 0.62 (T3).

In the lower R*f*-range (0–0.25) appear some grey-brown zones which originate from the saponin aescin (T2) and catalpol (T4).

The *Scrophularia nodosa* sample (8) shows a similar pattern but lacking most of the grey-brown zones in the lower R*f*-range. The zone pattern of *Harpagophytum procumbens* (7), a drug originated from South Africa and used in Europe as antirheumatic drug, can serve as reference drug: It contains harpagoside and harpagid as the major components, but differs from *Scrophularia* spec. by several strong yellow zones in the R*f*-range 0.57–0.68 (probably acteoside), and can so be discriminated from *Scrophularia ningpoensis.*

HPLC-fingerprint-analysis[10,18]**:**

1) Sample preparation: 1.0 g powdered drug is extracted with 10 ml methanol on the 70°C warm water bath under reflux for 30 min. The cooled extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol, filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-apparat.

2) Injection volume: 20 µl extract and 10 µl reference solution

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi
Column:	LiChroCART® 250-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: water, Millipore Ultra Clear UV plus® filtered, containing 10 ml 0,1 % H_3PO_4 /l B: acetonitrile, HPLC quality Acros Organics, containing 10 ml 0,1 % H_3PO_4 /l
Gradient:	0 % B for 5 min. (isocratic) 0 % B to 15 % B in 5 min. (linear) 15 % B for 5 min. (isocratic) 15 % B to 35 % B in 10 min. (linear) 35 % B for 10 min. (isocratic)
Flow rate:	1.0 ml/min.
Detection:	278 nm

Retention times and identity of the main peaks of **Fig. 3a** and **Fig. 3b**:

Peak	Rt (min.)	Compound
1	24.5	acteoside
2	26.0	angoroside C
3	28.5	harpagoside
4	30.4	cinnamic acid

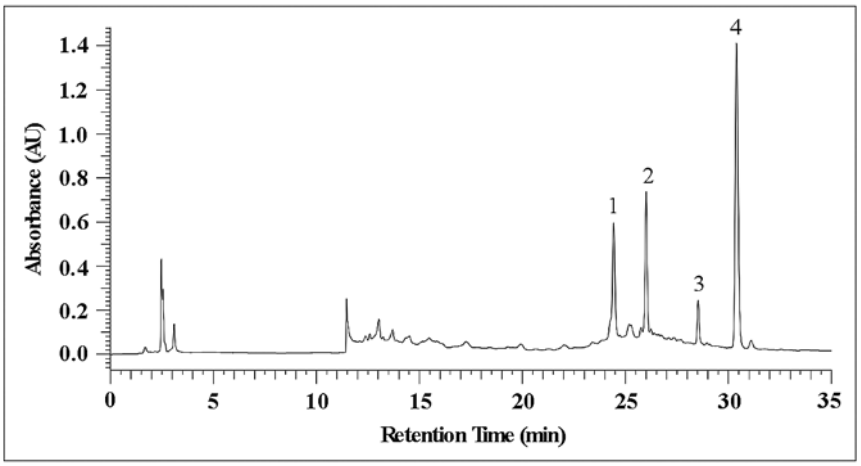


Fig. 3a: HPLC fingerprint of Radix Scrophulariae methanol extract, sample 3

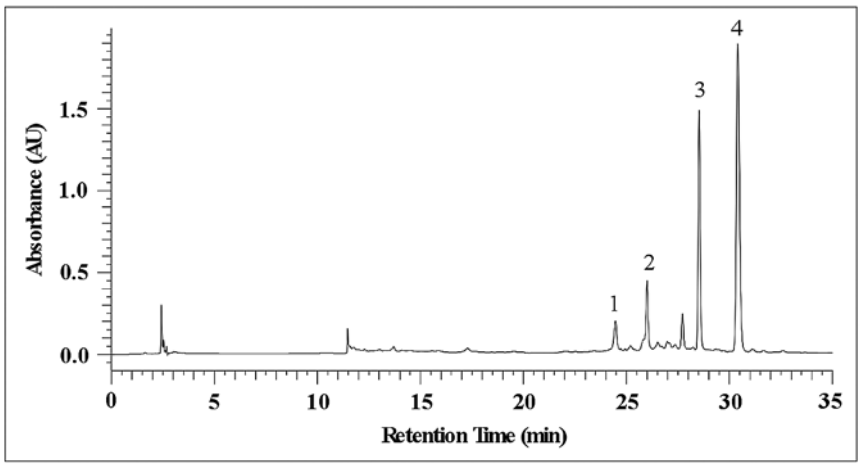


Fig. 3b: HPLC fingerprint of Radix Scrophulariae methanol extract, sample 5

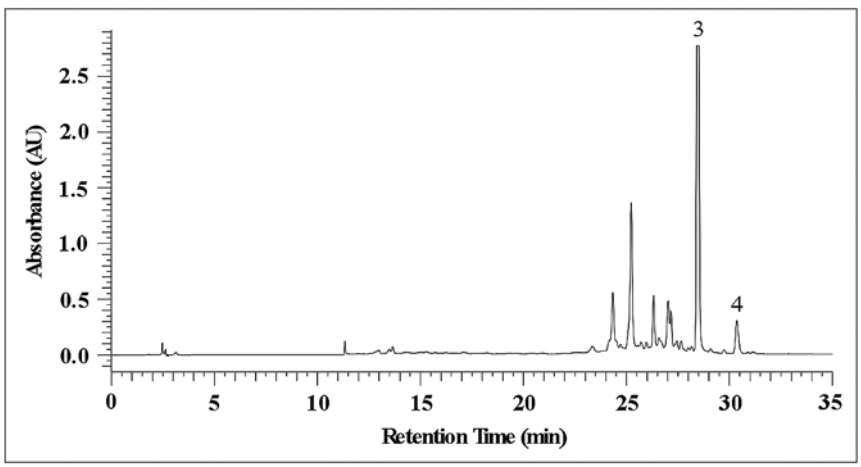


Fig. 4: HPLC fingerprint of Radix Harpagophyti methanol extract, sample 7

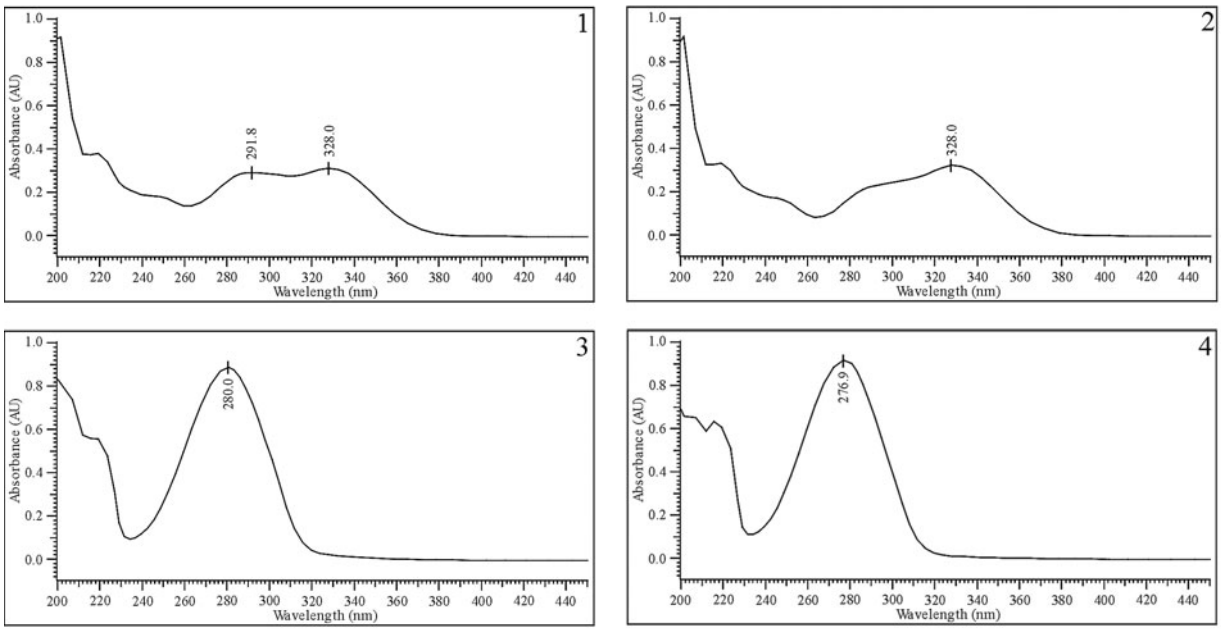


Fig. 5: Online UV-spectra of the main constituents of *Scrophularia ningpoensis*

4) Description:

The HPLC-fingerprint of all Radix Scrophulariae samples show at 278 nm four major peaks of acteoside at Rt 24.5 (**1**), angoroside C at Rt 26.0 (**2**), harpagoside Rt 28.5 (**3**) and cinnamic acid at Rt 30.4 (**4**).

The amount of the main constituents (e.g. harpagoside and cinnamic acid) of Radix Scrophulariae varies depending on the origin, harvest time and storage period of the drug and plant.

Radix Scrophulariae (Fig. 3a, 3b) can be discriminated from the reference drug Radix Harpagophyti (Fig. 4) by a lower harpagoside concentration (**3**) and a higher concentration of cinnamic acid (**4**). This difference can be also seen on TLC.

Note: The Chinese Pharmacopoeia 2005 demands for Radix Scropholariae not less than 0.050 % of harpagoside, calculated with reference to the dried drug.

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Additional references (HPLC-chromatography)

Li J, Huang X, Du X, Sun W, Zhang Y, Study of chemical composition and antimicrobial activity of leaves and roots of *Scrophularia ningpoensis*, Nat Prod Res. 23(8):775-80 (2009)

Lee MK, Choi OG, Park JH, Cho HJ, Ahn MJ, Kim SH, Kim YC, Sung SH, Simultaneous determination of four active constituents in the roots of *Scrophularia buergeriana* by HPLC-DAD and LC-ESI-MS, J Sep Sci. 30(15):2345-50 (2007)

Radix Polygoni multiflori
Heshouwu

Pharmacopoeia:	Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]
Official drugs[1,2]**:**	Fleeceflower root is the dried root tuber of *Polygonum multiflorum* Thunb. – Polygonaceae –
Origin[3,4]**:**	Cultivated especially in the Yangtse regions (Jiangsu, Zhejiang, Anhui, Guangdong, Hunan, Henan, Shandong and Sichuan).
Description of the drug[1]**:**	Mass of irregular fusiform, 6–15 cm long, 4–12 cm in diameter. Externally reddish-brown, shrunken and uneven, shallowly grooved, with transverse elongated lenticels and fine rootlet scars. Texture heavy, compact, uneasily broken, fracture pale yellowish-brown or reddish-brown, bark exhibiting 4–11 subrounded rings of abnormal vascular bundles, forming brocaded patterns, wood in central part relatively large, some having a woody core. Odourless; taste, bitterish, sweetish and astringent.
Pretreatment of the raw drug[1]**:**	The drug is collected in autumn and winter when leaves wither, removed from two ends, washed clean, the large one cut into pieces and then dried.
Processing[1]**:**	Foreign matters are eliminated from the drug, washed, macerated, softened thoroughly, cut into thick slices or pieces and dried.
Medicinal use[1,2]**:**	For treatment of hypercholesterinemia and malaria infections, externally also for the treatment of carbuncles.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine	
Taste[1]**:**	bitterish, sweetish and astringent
Temperature[5,6]**:**	neutral, tendency warm
Channels entered[4,5]**:**	liver and kidney
Effects[3-6]**:**	supplying energy *orbis hepaticus et renalis*, nourishing the blood and adding the essence *Jing*, *Xue* replenishing, moisturising the bowel, detoxicating and disinfecting
Symptoms and indications[1,4-6]**:**	· liver- and kidney-blood absence: dizziness, impaired vision, premature greying of the hair, debility of the haunches and the knees, spermatorrhoe · obstipation and constipation · wind invasion underlying blood deficiency with skin rash: urticaria with itching, dry skin, carbuncles, furuncles and abscesses · lymphadentitis · hyperlipidemia

Main constituents[2,7-10]: (see **Fig. 1**)

- **anthraquinones:** emodin and physcion, chrysophanol
- **hydroxylated stilbene glycosides and derivatives:** 2,3,5,4´-tetrahydroxystilbene 2-*O*-*β*-D-glucopyranoside and its 2- and 3-*O*-monogalloyl esters
- **acetophenone glycoside:** polygoacetophenoside (2,3,4,6-tetrahydroxyacetophenone 3-*O*-*β*-D-glucopyranoside)
- scoparone

Pharmacology:

in vitro / *in vivo*:

- hypocholesterolemic[5,6,11-13]
- myocardial protective effect[14,15]
- reduces cerebral ischemia-induced infarct volume[16]
- anti-inflammatory[17]
- antioxidant[18-20]
- inhibitory potency on six active major cytochrome P450 enzymes[21]
- improving the learning and memory deficit[22,23]
- beneficial effects on hippocampus morphology[24]
- protective effect on acetyl-choline esterase projecting neurofibers[25]
- protecting rat heart mitochondria against lipid peroxidation[26]
- $Ca^{(2+)}$-ATPase inhibiting[27]
- antibacterial and antibiotic[5,6]
- antimutagenic[28,29]
- estrogenic activity[30]

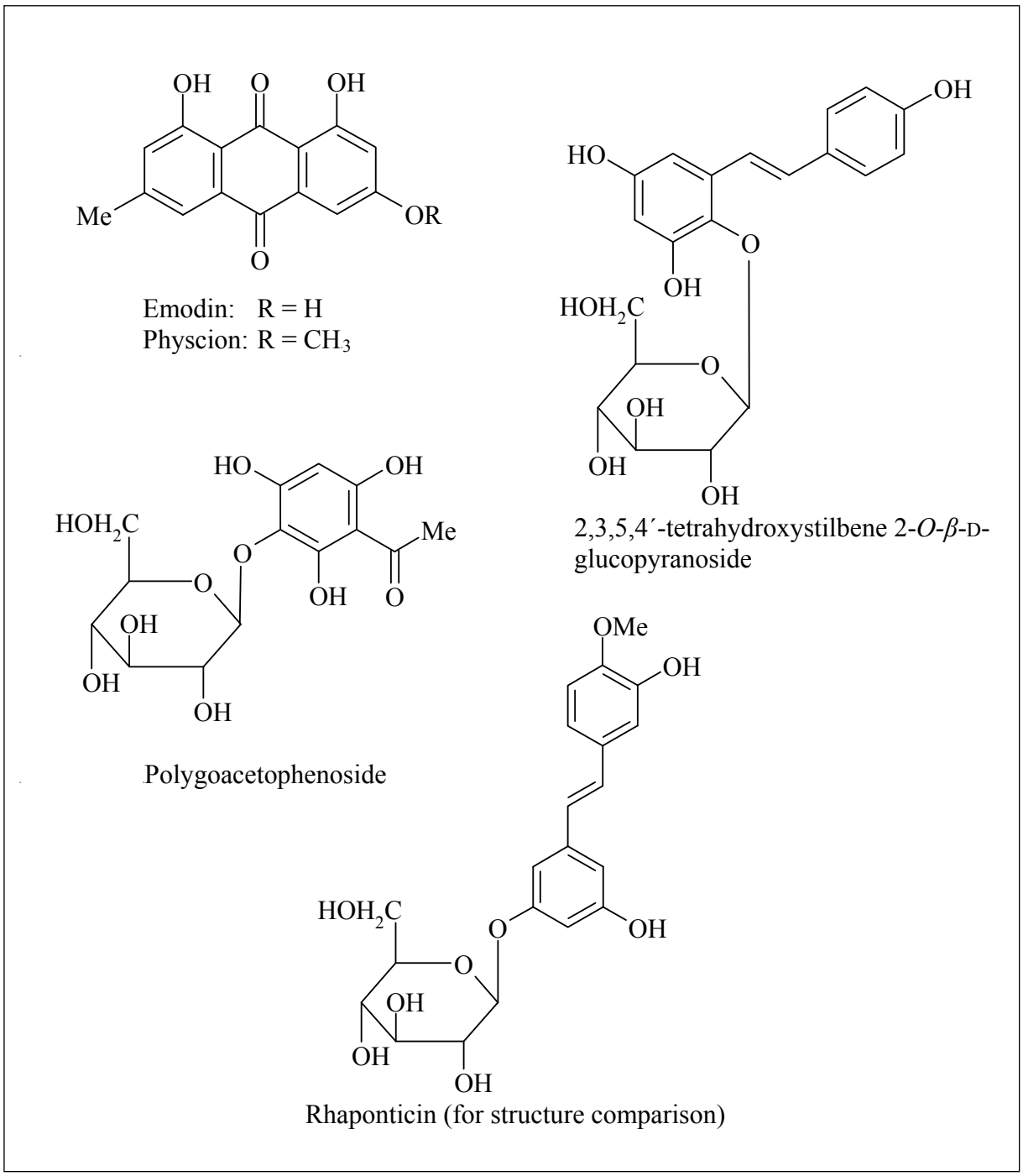

Fig. 1: Formulae of the main constituents of *Polygonum multiflorum*[2]

TLC-fingerprint-analysis[1,31]**:**

1) Extraction: 1.0 g powdered drug of Radix Polygoni multiflori is extracted with 25 ml ethanol on the water bath under reflux for 1 hour. The cooled extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml methanol.

Extracts of other *Polygonum* species and Rhizoma Rhei rhapontici are used as references for comparative chromatography. These drugs were extracted with the same method described above for Radix Polygoni multiflori.

2) Reference compounds: physcion, emodin (1 mg/ml methanol)

3) Separation parameters:

Applied amount: 10 µl extract and standard solution

Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: toluene - ethanol (3 : 1)

Detection: Spray reagents:

Fig. 2a/2b: Natural products-polyethylenglycol reagent:
The plate is sprayed successively with 1 % methanolic solution of diphenylboric acid-ß-ethyl-aminoester (NP) and a 5 % ethanolic polyethylenglycol-4000 solution (PEG).
The evaluation is carried out in UV 365 nm (Fig. 2a) and VIS (Fig. 2b).

Fig. 3: Phosphomolybdic acid reagent with sulphuric acid:
4 g phosphomolybdic acid dissolved in 40 ml hot water;
60 ml concentrated sulphuric acid is carefully added to the cooled solution. The plate is sprayed with 10 ml and then heated at 100°C for 5 min. under observation and evaluated in VIS.

Drug samples		Origin
1	Radix Polygoni multiflori/ *Polygonum multiflorum*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
2	Radix Polygoni multiflori/ *Polygonum multiflorum*	locality SiChuan Ya-an; China
3	Radix Polygoni multiflori/ *Polygonum multiflorum*	locality SiChuan; China
4	Radix Polygoni multiflori/ *Polygonum multiflorum*	locality Quizhou; China
5	Radix Polygoni multiflori/ *Polygonum multiflorum*	locality Guangdong; China
6	Rhizoma Rhei rhapontici/ *Rheum rhaponticum* (for comparison)	sample of commercial product obtained from Alfred Galke GmbH; Germany
7	Radix Polygoni multiflori praeparata/ *Polygonum multiflorum*	sample of commercial product obtained from Herbasin Company; Germany
8	Caulis Polygoni multiflori/ *Polygonum multiflorum*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
9	Rhizoma Polygoni cuspidati/ *Polygonum cuspidatum*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany

Reference compounds		R*f*
T1	physcion	0.95
T2	emodin (impurified with physcion)	0.76

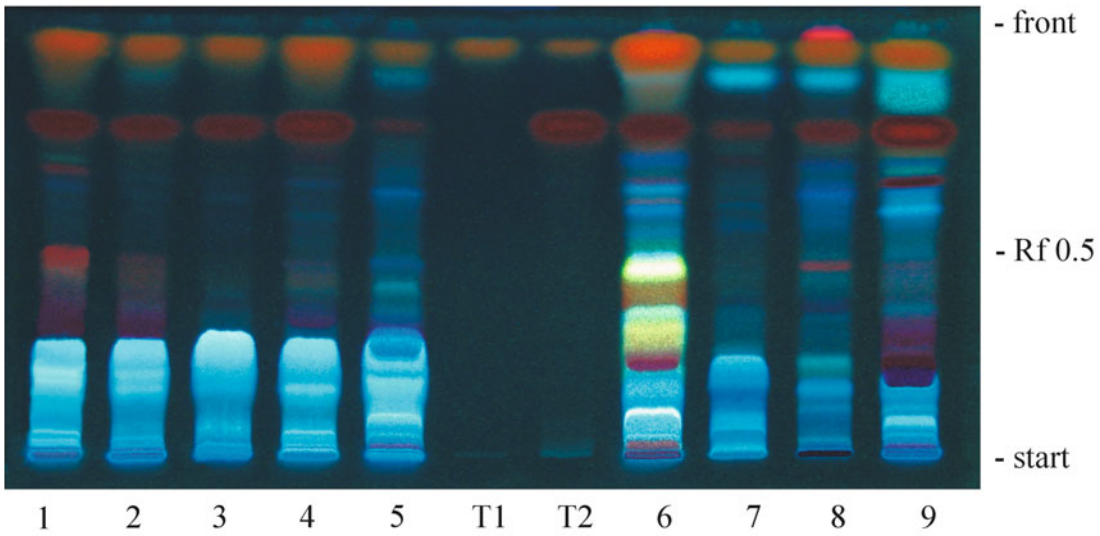

Fig. 2a: HPTLC-fingerprint of Radix Polygoni multiflori extract, detected with natural products-polyethylenglycol reagent in UV 365 nm

4) Description of the HPTLC-fingerprint of **Fig. 2a**:

All *Polygonum multiflorum* root samples (1-5) show an orange fluorescent zone of physcion (T1, R*f* 0.95) and a red-purple fluorescent zone of emodin (T2, R*f* 0.76).

In the R*f*-range of 0.25–0.45 red-violet zones of anthraquinone-mono- and diglucoside can be detected. From start up to R*f* 0.28 blue fluorescent zones appear with 2,3,5,4´-tetrahydroxystilbene 2-*O*-*β*-D-glucoside as the major characteristic constituent.

Rhizoma Rhei (sample 6) used as reference drug shows besides physcion and emodin the characteristic stilbene glucoside rhaponticin (R*f* 0.49). Radix Polygoni multiflori praeparata (sample 7) differs from the non processed *Polygonum* samples by the weak blue fluorescence zone at R*f* 0.28. Caulis Polygoni multiflori (sample 8) and Radix Polygoni multiflori (sample 1–5) show a similar pattern of anthraquinones and stilbenglycosides. Rhizoma Polygoni cuspidati (sample 9) contains besides the usual anthraquinones (emodin and physcion) a high amount of anthraquinone diglycosides in the R*f*-range of 0.20–0.30.

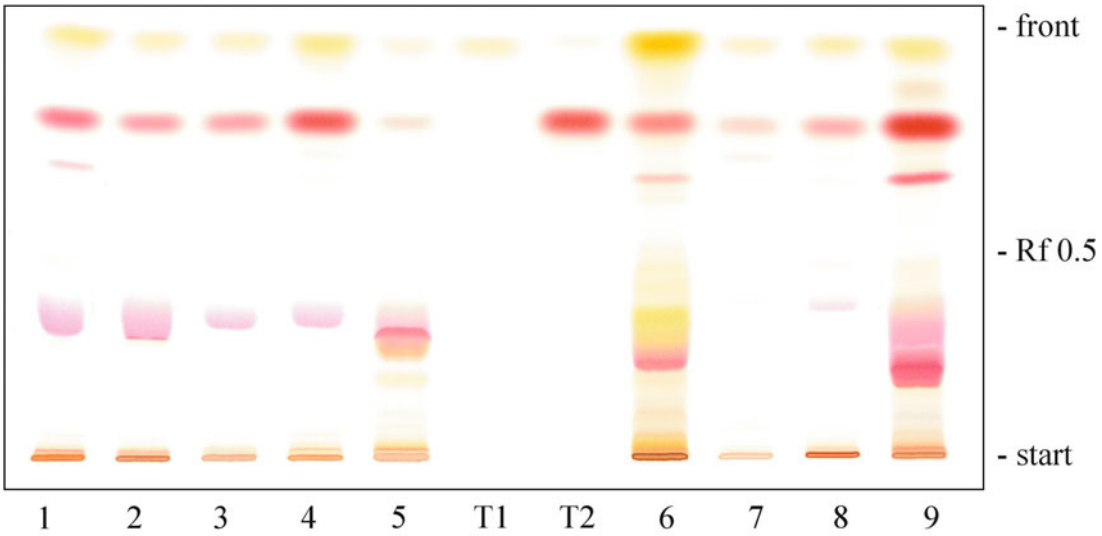

Fig. 2b: HPTLC-fingerprint of Radix Polygoni multiflori extract, detected with natural products-polyethylenglycol reagent in VIS

Description of the HPTLC-fingerprint of **Fig. 2b**:

All anthraquinone aglycones and glycosides show in VIS with the exception of physcion a red colour. Physcion is characterized by a yellow colour.

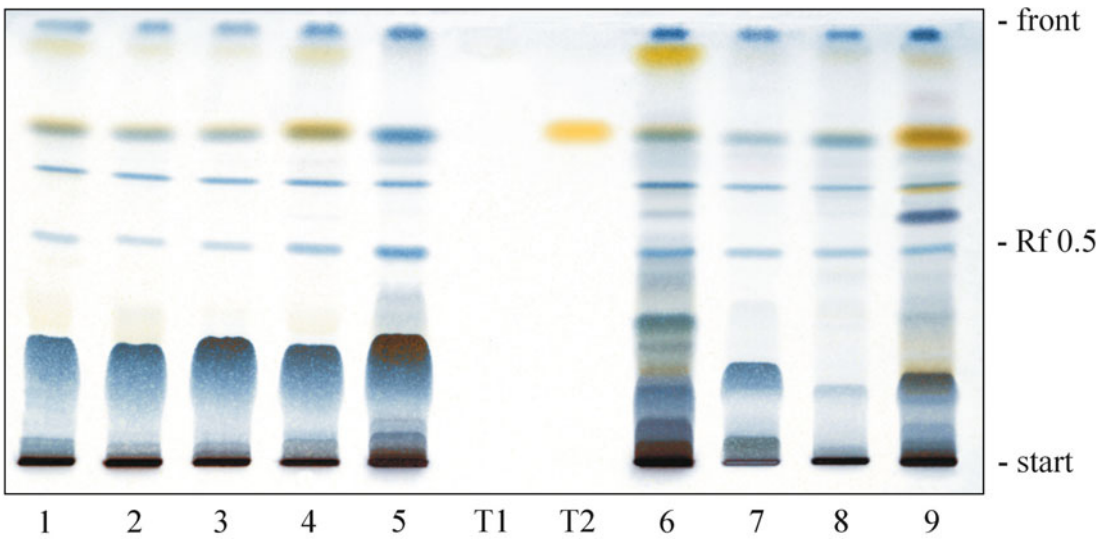

Fig. 3: HPTLC-fingerprint of Radix Polygoni multiflori extract, detected with phosphomolybdic acid reagent with sulphuric acid in VIS

Description of the HPTLC-fingerprint of **Fig. 3**:

All Radix Polygoni multiflori (1–5) samples show a predominating blue zone of 2,3,5,4´-tetrahydroxystilbene 2-*O*-β-D-glucoside in the Rf-range 0.0–0.28. Physcion (T1) can be detected only as weak yellow zone at Rf 0.95. Emodin (T2) can be detected at Rf 0.76 as yellow zone overlapped by a blue zone.

A TLC-method to identify Radix Polygoni multiflori and its main constituent 2,3,5,4′-tetrahydroxystilbene 2-*O*-*β*-D-glucoside is described in the Pharmacopoeia of the People's Republic of China.[1]

HPLC-fingerprint-analysis[1,8]:

1) Sample preparation: The ethanol extract, used for HPTLC, filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-apparat.

2) Injection volume: 10 µl extract and reference solution

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi
Column:	LiChroCART® 125-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: water, Millipore Ultra Clear UV plus® filtered B: acetonitrile, HPLC quality Acros Organics
Gradient:	0 % B to 95 % B in 40 min. (linear)
Flow rate:	1.0 ml/min.
Detection:	254 nm

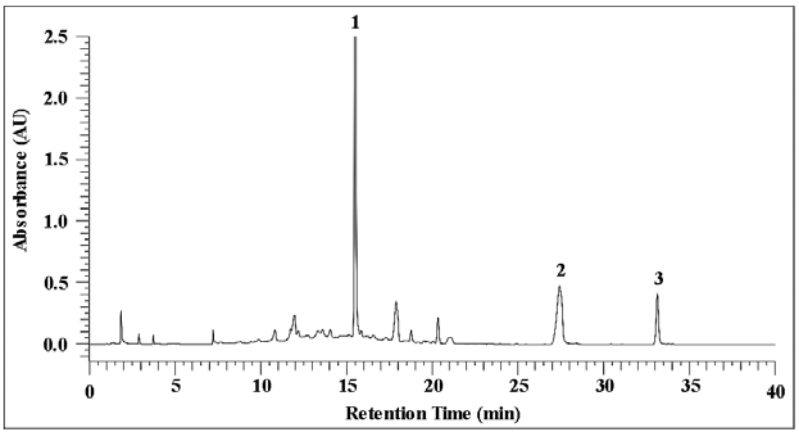

Fig. 4a: HPLC fingerprint of Radix Polygoni multiflori extract, sample 1

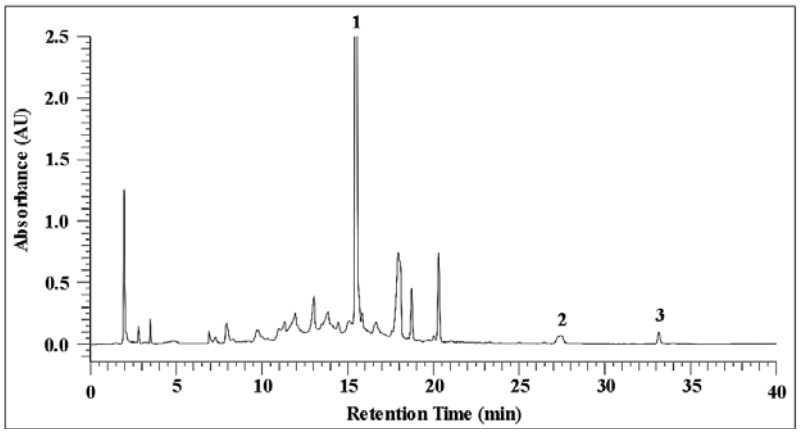

Fig. 4b: HPLC fingerprint of Radix Polygoni multiflori extract, sample 5

Retention times and identity of the main peaks of **Fig. 4a** and **Fig. 4b**:

Peak	Rt (min.)	Compound
1	15.5	2,3,5,4′-tetrahydroxystilbene 2-*O*-β-D-glucoside
2	27.4	emodin
3	31.1	physcion

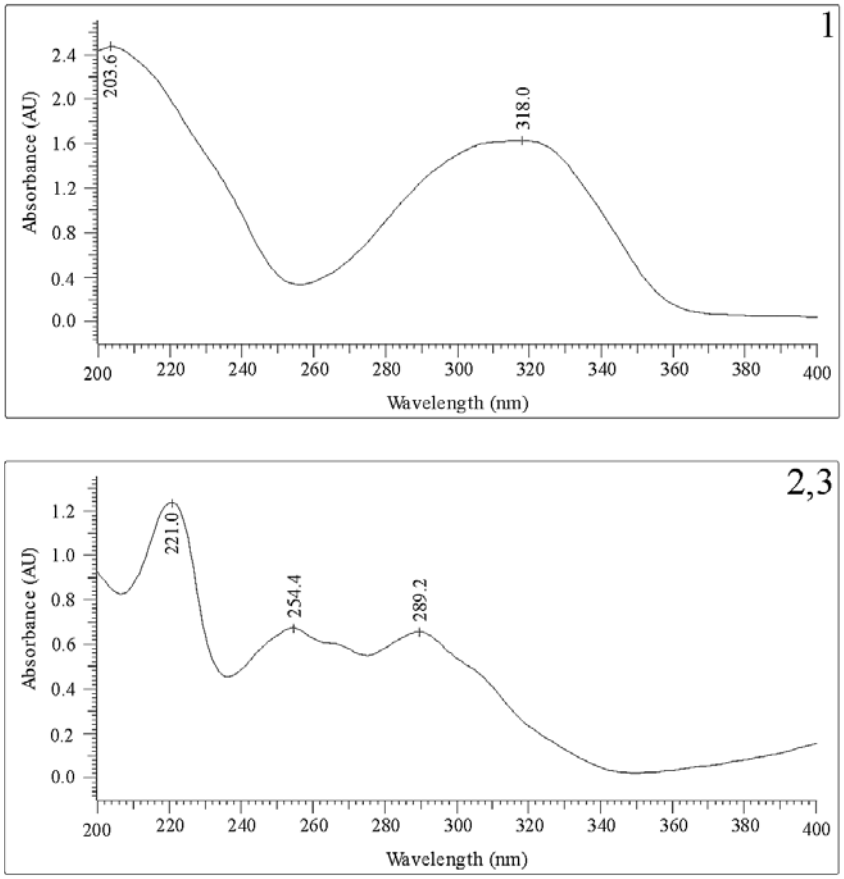

Fig. 5: Online UV-spectra of the main constituents of Radix Polygoni multiflori radix extracts

4) Description of the HPLC-fingerprints of **Fig. 4a** and **Fig. 4b** and the online UV-spectra of **Fig. 5**:

   The HPLC-fingerprint of all Radix Polygoni multiflori samples shows one major peak of 2,3,5,4′-tetrahydroxystilbene 2-*O*-*β*-D-glucoside (**1**) at 15.5 min. with two UV-maxima at ~204 and ~320 nm. The two anthraquinones emodin (**2**, Rt 27.4 min.) and physcion (**3**, Rt 31.1 min.) can be detected in different concentrations giving a typical online UV-spectrum with maxima at 221.0, 254.4 and 289.2 nm.

Note: The Chinese Pharmacopoeia 2005 demands for Radix Polygoni multiflori not less than 1.0 % of 2,3,5,4′-tetrahydroxystilbene-2-O-*β*-D-glucoside, calculated with reference to the dried drug.

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Rhizoma Alismatis
Zexie

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drugs[1]: The official drug is the rhizoma of *Alisma orientalis* (Sam.) Juzep. The drug is known under the English name "Oriental Waterplantain Rhizome".

– Alismataceae –

Origin[2,3]: *Alisma orientalis* grows in Middle and Southern China (Fujian, Sichuan, Hunan and Jianxi), Sinkiang and Taiwan.

Description of the drug[1-4]: Subspherical, elliptical or ovate, 2–7 cm long, 2–6 cm in diameter. Externally yellowish-white or yellowish-brown, with irregular transverse-annular shallow furrows and numerous small raised fibrous root scars, occasionally tuberculate bud scars attached to the base.
Texture compact, fracture yellowish-white, starchy, with numerous small pores.
Odour, slight; taste, slightly bitter or sweet.

Pretreatment of the raw drug[1]: The drug is collected in winter when the stem withers, washed clean, dried, and removed from the fibrous root and coarse outer tissue.

Rhizoma Alismatis: Foreign matters are eliminated from the drug, soaked briefly, softened thoroughly, cut into thick slices and dried.

Rhizoma Alismatis (processed with salt): Salt water is added to clean slices of Rhizoma Alismatis and mixed well in a closed vessel until the drug is infused thoroughly. The drug is placed in a pot, stir-baked with gentle heat to a specified condition, taken out and cooled.

Medicinal use[1,5]: The drug is used as diuretic and sudatory agent, for lowering the blood sugar and cholesterol level. Additional indications are: tinnitus, vertigo, edemas, chronic nephritis, diarrhea and oliguria.

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Effects and indications according to Traditional Chinese Medicine[1-5]	
Taste:	slightly bitter or sweet
Temperature:	cold
Channels entered:	kidney, bladder
Effects:	diuretic, purges dampness, cools up heat, purges damp and ardor, disposes heat because of effeteness
Symptoms and indications:	edema with oliguria, diarrhoea with diminished discharge of urine, vertigo due to retention of fluid, acute urinary infection with painful urination; hyperlipidemia; deficiency heat because of kidney-*Yin*-deficiency

Contraindication[4]**:** Rhizoma Alismatis should not be used during kidney dysfunction, kidney-*Yang*-deficiency and moisture coldness.

Main constituents:
(see **Fig. 1**)

- **protostane and dammarane triterpenoids:**
  alisol A, alisol A monoacetate, alisol B, alisol B and alisol C monoacetate, *epi*-alisol[6]
- sesquiterpenoids:
  alismol, alismoxide[6]
- sulfosesquiterpenes:
  sulfoorientalols a, b, c[7,8]

- polysaccharides:
  glucans[9]

Pharmacology:

- anticholesterinemic (alisol A, B, C, -monoacetate)[10]
- diuretic[11]
- liver protective activity[12]
- anticomplementary activity (alisol B 23-acetate, alisol A 24-acetate, alisol B)[13]
- inhibitory effect on renal stone formation[14]
- nitrooxide inhibitory activity (alismaketones-B 23-acetate, alismaketones-C 23-acetate)[15]
- antiallergic effects (alisol A, B, -monoacetate, alismol, alismoxide)[16,17]
- immunostimulatory activity (glucans)[18]
- cytotoxic effects (alisol B 23-acetate, alisol C 23-acetate, alisol B, alisol A 23-acetate)[19]

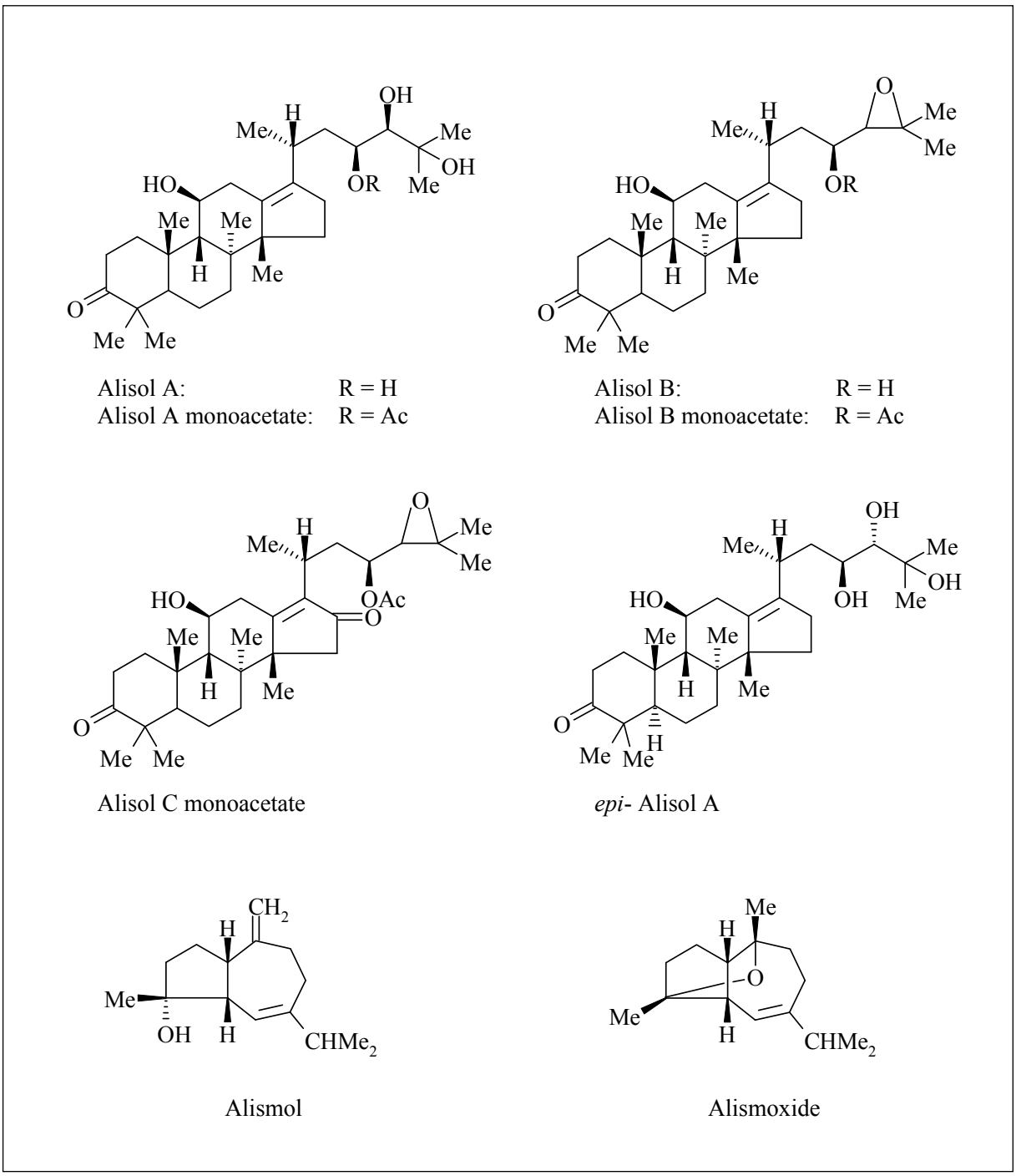

Fig. 1: Formulae of the main constituents of *Alisma orientalis*[6]

TLC-fingerprint-analysis[20]:

1) Extraction: 1.0 g powdered drug is ultrasonicated with 30 ml methanol for 30 min. After cooling down the extract is filtered and evaporated to dryness. The residue is dissolved in 2 ml methanol and afterwards filtered through a Sep-pak® Cartridge C18, Waters Millipore. The cartridge is eluted with 50 ml methanol 60 %. The elute is evaporated to dryness and the residue redissolved in 1 ml methanol.

2) Reference compounds: alisol B, alisol B monoacetate (1 mg/ml)

3) Separation parameters:

Applied amount: 10 µl extract and standard solution

Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: petroleum ether – ethyl acetate (8 : 9)

Detection: Spray reagent:

Anisaldehyde-sulphuric acid reagent:
0.5 ml anisaldehyd is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid. The TLC plate is sprayed with 10 ml, heated at 100 °C for 5–10 min. and then evaluated in VIS.

Drug samples		**Origin**
1	Alismatis rhizoma/*Alisma orientalis*	commercial product obtained from the Chinese University of Hong Kong; School of Chinese Medicine, China
2	Alismatis rhizoma/*Alisma orientalis*	sample of commercial product; China
3	Alismatis rhizoma/*Alisma orientalis*	province Sichuan; China
4	Alismatis rhizoma/*Alisma orientalis*	province Fujian; China
5	Alismatis rhizoma/*Alisma orientalis*	province Fujian; China

Reference compounds		**R*f***
T1	alisol B	0.25
T2	alisol B monoacetate	0.72

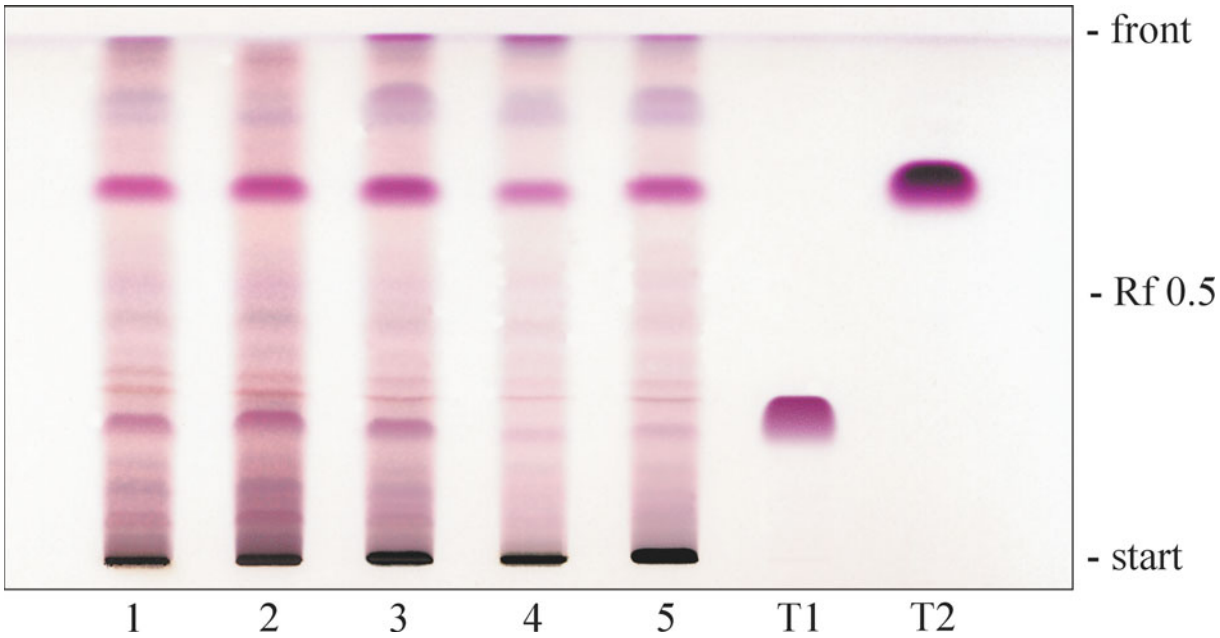

Fig. 2: TLC-fingerprint of Alismatis rhizoma methanol extract, detected with anisaldehyde-sulphuric acid reagent in VIS

4) Description of the HPTLC-fingerprint of **Fig. 2**, sprayed with anisaldehyde-sulphuric acid reagent in VIS:

   All *Alisma orientalis* samples (1–5) are characterized by a very homogenous fingerprint with two main pink-violet zones of alisol B (T1) at Rf 0.25 and alisol B monoacetate (T2) at Rf 0.72. Further weak pink violet zones appear in the Rf-region of 0.75 up to 1.0.

HPLC-fingerprint-analysis:

1) Sample preparation: 1.0 g powdered drug is macerated with 25 ml petroleum ether (35–60 °C) for 30 min. The extract is heated under reflux for 1 hour. After cooling down the extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol, filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-apparat.

2) Injection volume: 10 µl extract and reference solution

3) HPLC-data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi
Column:	LiChroCART® 125-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: water, Millipore Ultra Clear UV plus® filtered B: acetonitrile, HPLC quality Acros Organics

Gradient:	15 % B for 3 min. (isocratic) 15 % B to 95 % B in 5 min. (linear) 95 % B for 22 min. (isocratic)
Flow rate:	0.8 ml/min.
Detection:	210 nm

Retention times and identity of the main peaks of **Fig. 3a** and **Fig. 3b**:

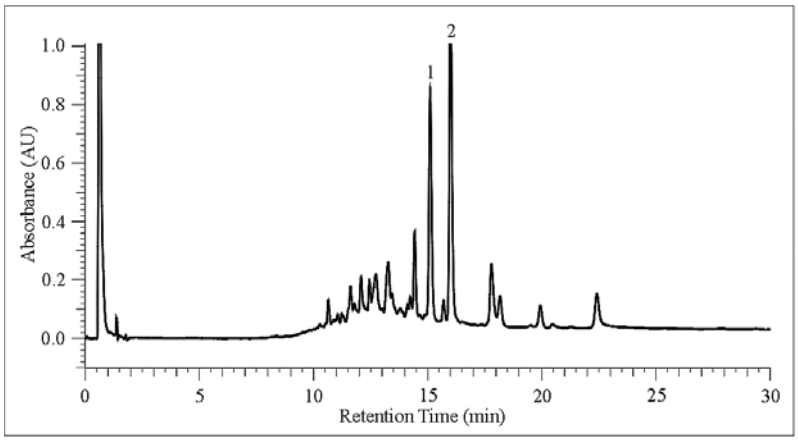

Fig. 3a: HPLC fingerprint of Alismatis rhizoma petroleum ether extract, sample 1

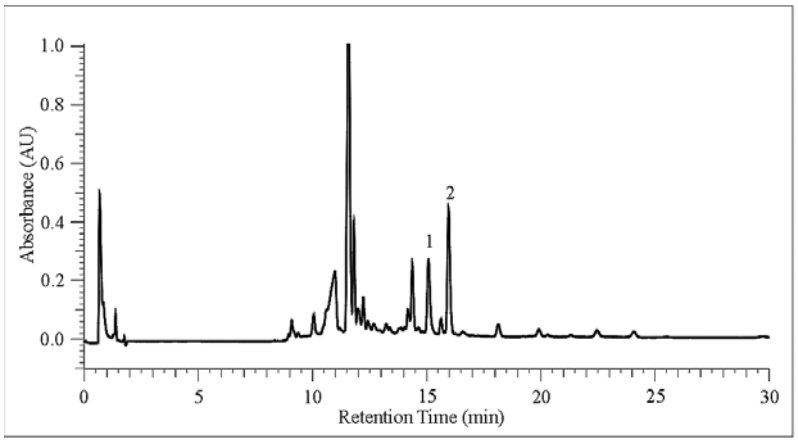

Fig. 3b: HPLC fingerprint of Alismatis rhizoma petroleum ether extract, sample 3

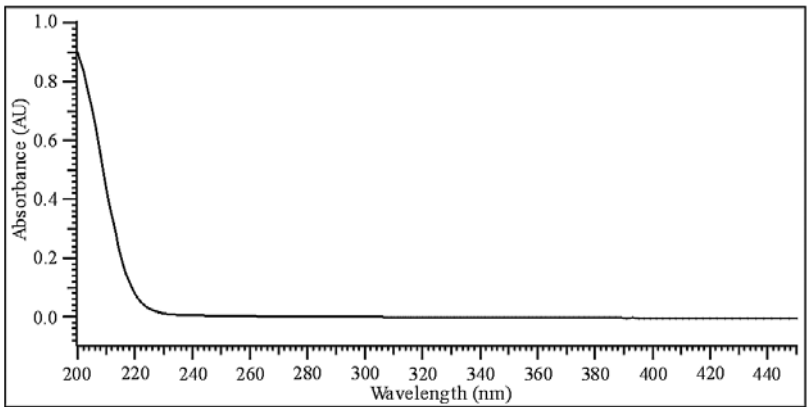

Fig. 4: Online UV-spectrum of alisol B and alisol B monoacetate

Peak	Rt (min.)	Compound
1	15.1	alisol B
2	16.0	alisol B monoacetate

Note: Qualitative and quantitative HPLC-investigations of triterpene constituents of Alismatis rhizoma were described also from some other laboratories.[20-22]

4) Description of the HPLC-chromatogram, **Fig. 3a** and **Fig. 3b**:

The HPLC-fingerprint of all Alismatis rhizoma petroleum ether extracts (sample1-5) show at UV 210 nm two major peaks of the triterpenes alisol B at Rt 15.1 (**1**) and alisol B monoacetate at Rt 16.0 (**2**) with very similar online UV-spectra (endabsorption) (**Fig. 4**).

Due to the online UV-spectra the peak assembly in the Rt-range of 10–14.5 min. may derive from other triterpenoids.

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Flos Carthami
Honghua

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[1]

Official drug[1]: Safflower is the dried flower of *Carthamus tinctorius* L. (Fam. Asteraceae).

The drug is collected in summer when its colour turns from yellow to red, and dried in shade or in the sun.

Description of the drug[1]: The drug consisting of tubular flowers without ovaries, 1 – 2 cm long. Externally reddish-yellow or red. Corolla tubes slender, 5-lobed at the apex, the lobes narrowly belt-shaped, 5 – 8 mm long. Stamens 5, anthers aggregated to a tube, yellowish-white. Stigma long cylindrical, slightly 2-cleft. Texture pliable. Odour slightly aromatic, taste slightly bitter.

Medicinal use[2]: for the treatment of coronary heart disease, hematomas, swelling and edemas

Effects and indications according to Traditional Chinese Medicine[1,2]	
Taste:	acrid
Temperature:	warm
Channels entered:	orbis cardialis, orbis hepaticus
Effects (functions):	activates blood circulation and stimulates menstrual discharge, removes blood stasis and relieves pain
Symptoms and indications:	amenorrhea, dysmenorrhea, retention of lochia, formation of mass in the abdomen, traumatic injuries, sores and ulcers with swelling and pain

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents:

- **red and yellow pigments (chalcones, quino-chalcones):**
  carthamin[3], isocarthamin[4], carthamidine[3], isocarthamidine[3], tinctormine[4], safflor yellow A[3], safflor yellow B[3], safflomin A[3], safflomin C[5], hydroxysafflor yellow A[6,7], anhydrosafflor yellow B[8], precarthamin[9]

- **flavones:**
  luteolin[3], 7-O-β-D-glucopyranoside[3], neocarthamin[7], kaempferol[7], quercetin[10], 6-hydroxykaempferol-3-O-glucoside[4], 6-hydroxykaempferol-7-O-glucoside[4], kaempferol-3-O-rutinoside[4], rutin[10], quercetin-3-O-glucoside[4],

- fatty acids:
  lauric acid[3], myristic acid[3], arachidic acid[3], palmitic acid[3], linoleic acid[3], oleic acid[3]

- polyacetylene derivatives:
  l-tridecene-3,5,7,9,11-pentayne[3], (11Z)-trideca-1,11-diene-3,5,7,0-tetraene-7,9-diyne[3], (3Z,5E,11E)-trideca-1,3,5,11,-tetraene-7,9-diyne[3]

- other constituents:
  ubiquinone[3], β-sitosterol[3], and its 3-O-β-D-glucopyranoside[3], adenosine[10], erythro-alkane-6,8-diols[11]

- 5-hydroxy-1H-indol-3-yl-ethyl-cinnamoylamide[12]

- polysaccharides[13]

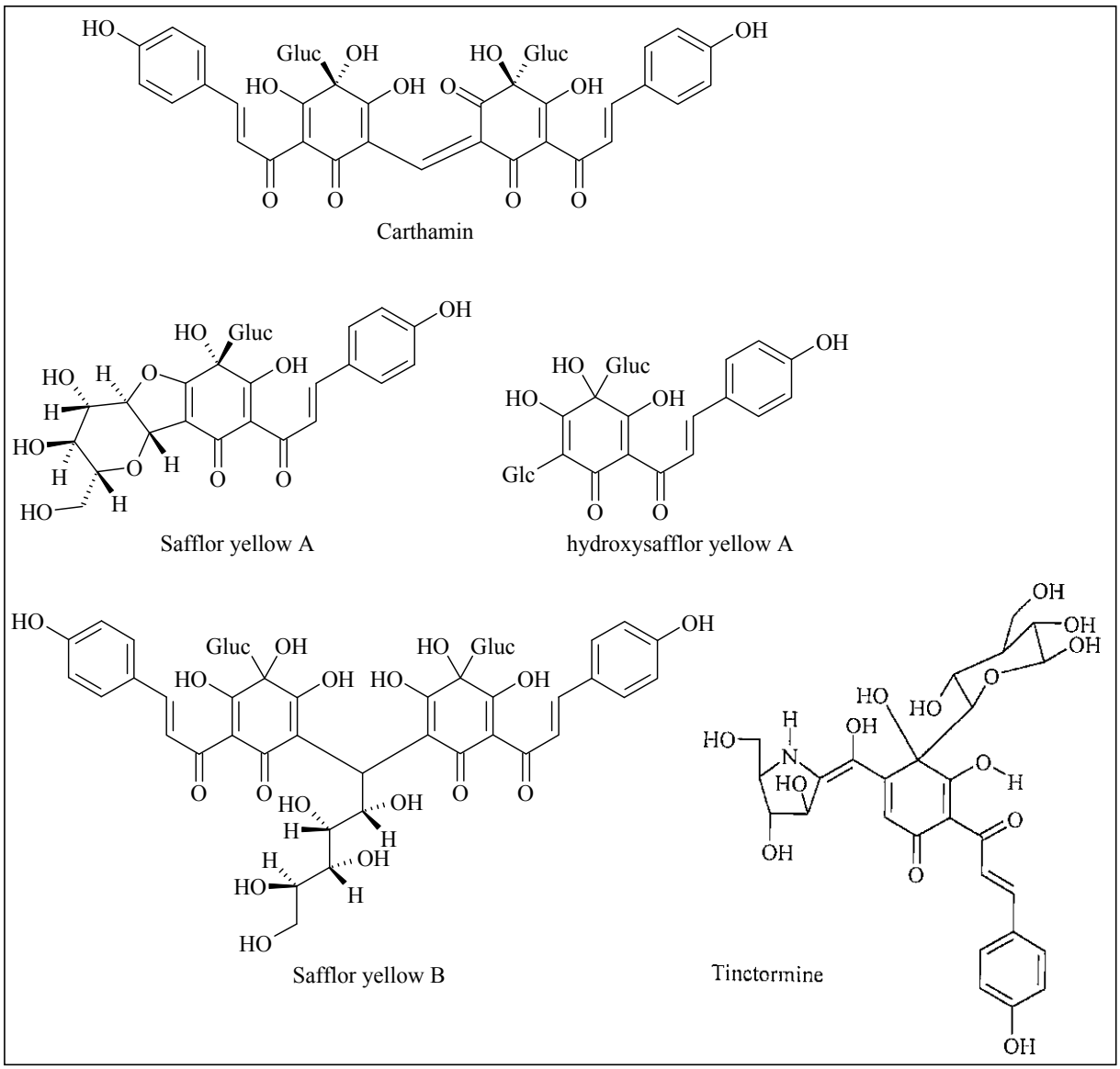

Fig. 1: Formulae of the main compounds[5,7]

Pharmacology:

- neuroprotective[6]
- analgesic[3,7]
- antiinflammatory[4,7]
- antipyretic[7]
- antihepatotoxic[7]
- antimicrobial[7]
- cardiovascular effects[7]
  - antithrombotic
  - Ca-channel blocking effect (tinctormine)[5]
  - antiischemic effect
  - hypotensive[14]
- activating NF-kappa B (polysaccharides)[13]
- antioxidative[15]
- antimycotic[16]
- cytotoxic[17]

Caution[1,7]:

Used with caution in pregnancy.
The median lethal dose (LD_{50}) of a decoction of the crude drug after intraperitoneal administration to mice was 1.2 g/kg body weight (Chang and But 1986).

TLC-fingerprint analysis

1) Extraction:

Extraction of Carthami flos:
To 0.5 g of the powdered drug 5 ml of 80 % acetone solution is added, stoppered tightly, shaken constantly for 15 minutes and filtered. The filtrate is used for TLC.

Extraction of Croci stigma:
To 0.25 g of the powdered drug 5 ml of 80 % acetone solution is added, stoppered tightly, shaken constantly for 15 minutes and filtered. The filtrate is used for TLC.

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Carthami flos extracts: each 5 µl
Croci stigma extract: 5 µl

Solvent system:

n-butanol	glacial acetic acid	water
20	5	10

Detection:
Detection:
Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol
II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II.
The evaluation is carried out in UV 365 nm.

Drug samples		Origin
1	Carthami flos/*Carthamus tinctorius*	province Guizhou, China
2	Carthami flos/*Carthamus tinctorius*	province Henan, China
3	Carthami flos/*Carthamus tinctorius*	sample of commercial drug, China
4	Carthami flos/*Carthamus tinctorius*	sample of commercial drug, China
5	Carthami flos/*Carthamus tinctorius*	sample of commercial drug, China
6	Carthami flos/*Carthamus tinctorius*	sample of commercial drug, Japan
7	Croci stigma/*Crocus sativus*	sample of commercial drug, Germany

4) Description of the TLC-chromatograms:

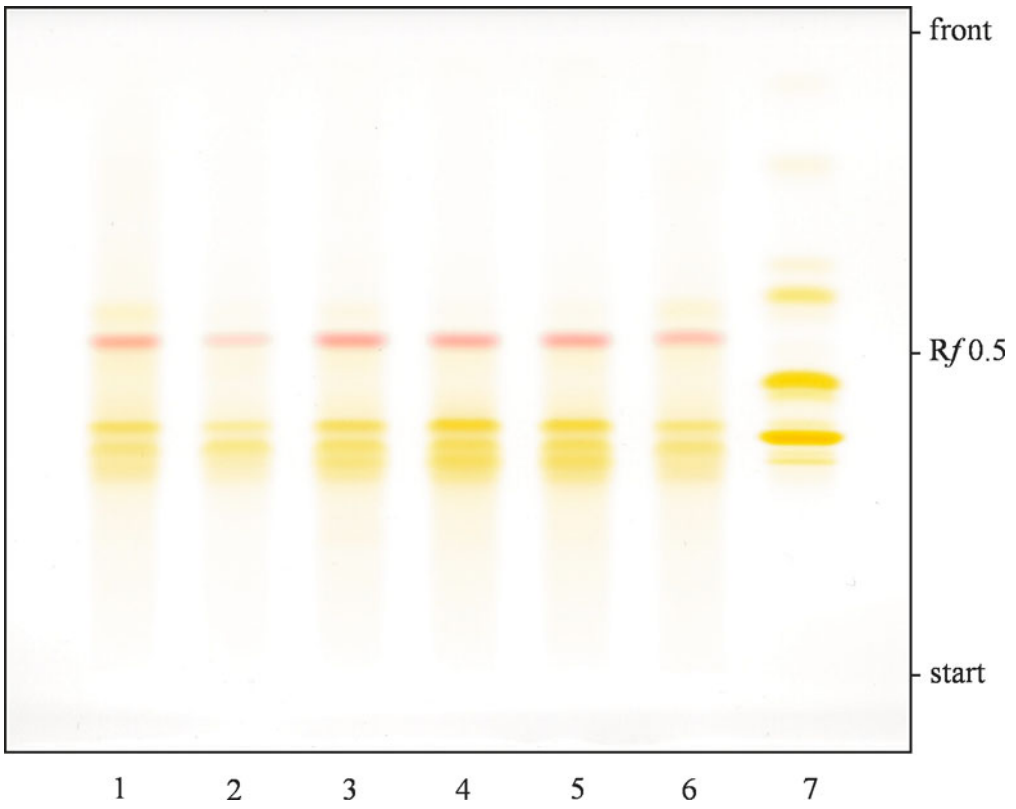

Fig. 2a: Thin layer chromatogram of the acetone extracts of Carthami flos and Croci stigma (VIS)

Samples 1 – 6 of Carthami flos show a very homogeneous pattern of yellow and red pigments with carthamin as red pigment at R*f* = 0.52 and several yellow pigments (e.g. hydroxysafflor yellow A) between R*f* = 0.32 and 0.49.

The sample of Croci stigma (7) which is very often adulterated with or substituted by safflower shows a yellow zone of crocin at R*f* = 0.49. Picrocrocin seen in UV 254 nm as a violet zone, appears at R*f* = 0.67.

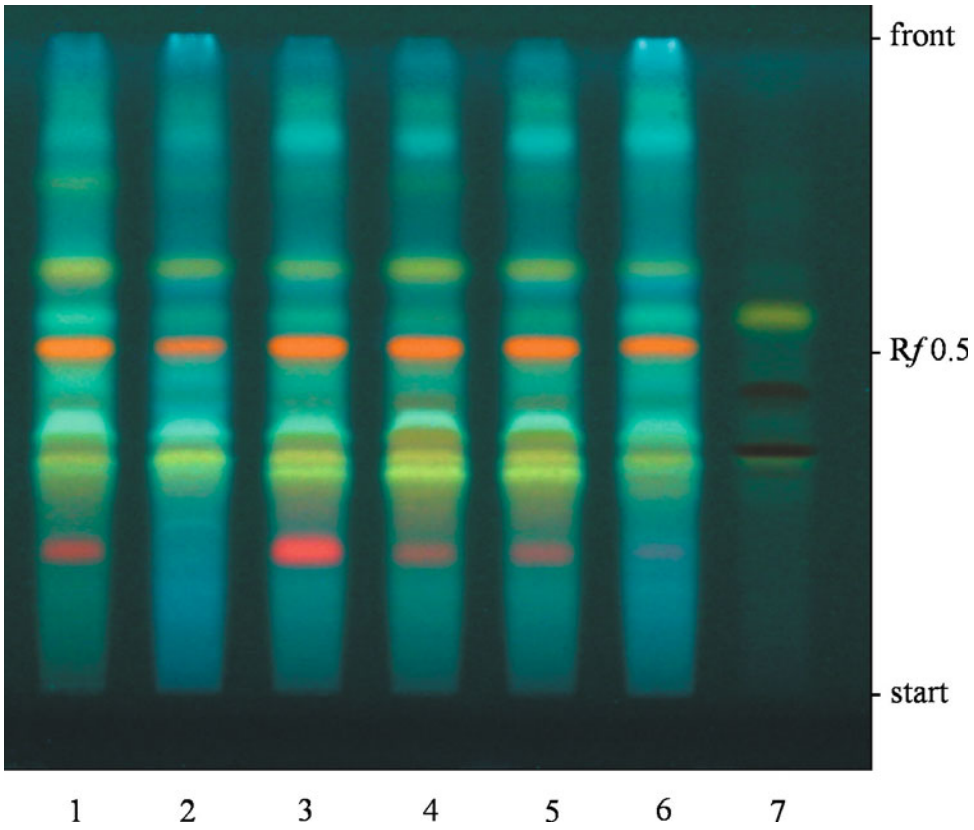

Fig. 2b: Thin layer chromatogram of the acetone extracts of Carthami flos and Croci stigma sprayed with natural products polyethylene glycol reagent (UV 365 nm)

Chromatogramm 2b shows also a homogeneous pattern of Carthami flos samples. The red pigment carthamin can be found as an orange fluoreszent zone at R*f* = 0.52. At R*f* = 0.20 a red fluoreszent zone appears in almost each Carthami flos samples with the exception of the sample from the province Henan. The yellow pigments can be found between R*f* = 0.31 and 0.37 as yellow zones. In addition a lot of yellow spots appear between R*f* = 0.55 and 0.87 which might derive from flavones. The sample of Croci stigma shows two yellow fluoreszent zones at R*f* = 0.36 and R*f* = 0.57 and two red brown spots at R*f* = 0.37 and R*f* = 0.45.

HPLC-fingerprint analysis:

1) Sample preparation: To 0.5 g of the powdered drug 5 ml of 80 % acetone solution is added, stoppered tightly, shaken constantly for 15 minutes and filtered. The filtrate is filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: Carthami flos extract: 20.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 /l water B: acetonitrile
Gradient:	0 % B in 8 minutes 0 – 50 % B in 42 minutes 50 – 100 % B in 2 minutes 100 % B in 3 minutes total runtime: 55 minutes
Flow:	0.6 ml/min.
Detection:	400 and 510 nm

Retention times of the main peaks:

Peak (area)	Rt (min.)	Compound
1	26.6 – 38.7	yellow pigments (e.g. hydroxysafflor yellow A)
2	43.0	red pigment carthamin

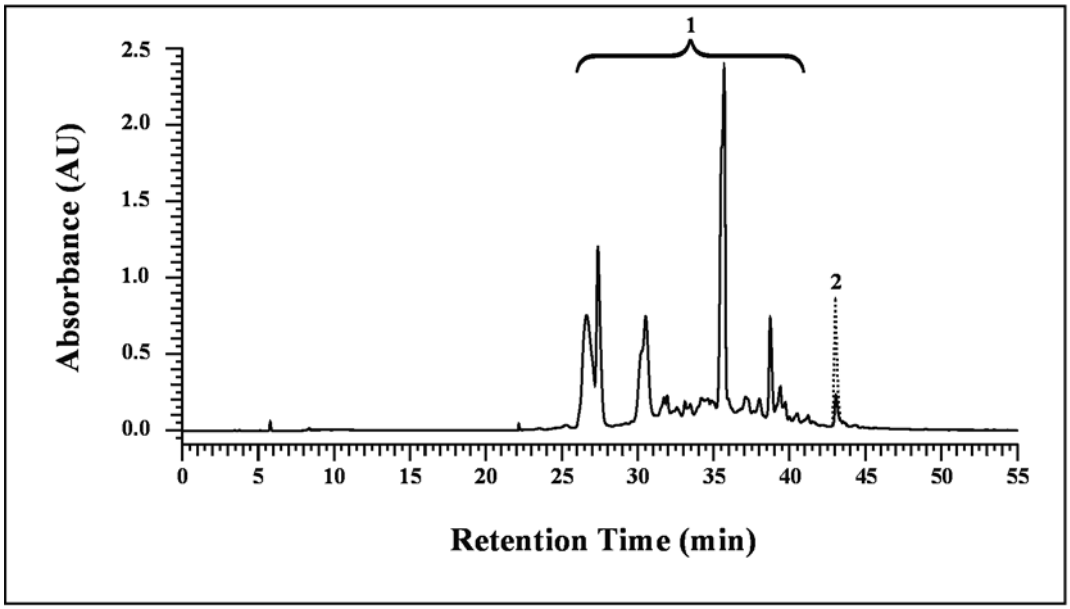

Fig. 3: HPLC-fingerprint chromatogram of Carthami flos (sample of commercial drug, China) UV 400 nm (dotted peak of carthamin at 510 nm)

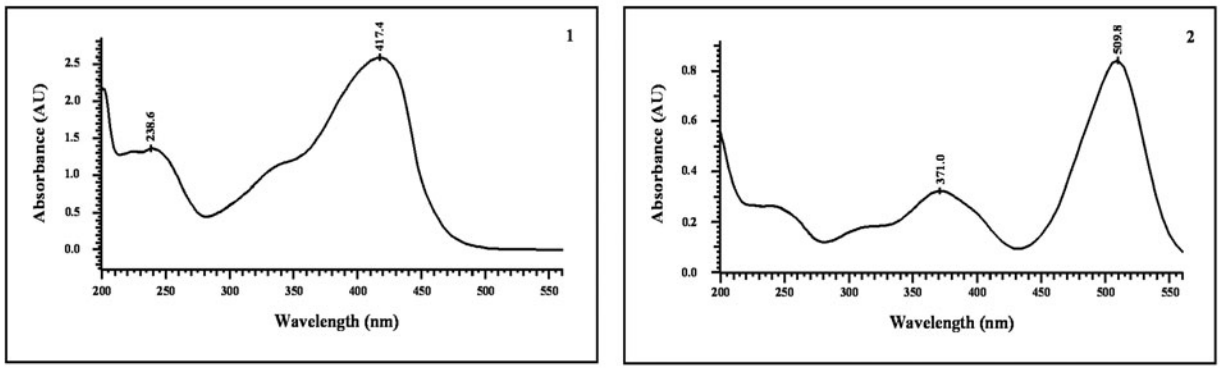

Fig. 4: UV-spectra of the main peaks of Carthami flos

4) Description of the HPLC of Figure 3:

The HPLC of Carthami flos shows at UV 400 nm a series of yellow pigments between Rt = 26.6 and 38.7 (**1**) with an UV spectrum with maxima at 238.6 and 417.4 nm. At Rt = 43.0 (**2**) the red pigment carthamin appears as a small peak which can be better detected at UV 510 nm (dotted peak). The red pigment shows an UV-spectrum with major maxima at 371.0 and 509.8 nm.

Other HPLC-fingerprint analyses are described by Nakano K et al (1988)[18] and Zhu M et al (2000)[19]

Note:

- According to the Chinese Pharmacopoeia Flos Carthami should contain not less than 1.0 % hydroxysafflor yellow A, calculated with reference to the dried drug.
- According to the Chinese Pharmacopoeia Flos Carthami should contain not less than 0.050 % kaempferol CRS, calculated with reference to the dried drug.

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Herba Epimedii
Yinyanghuo

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005[1]

Official drug[1]: Epimedium herb in the Chinese Pharmacopoeia includes the dried aerial part of *Epimedium brevicornum* Maxim., *Epimedium sagittatum* (Sieb. Et Zucc.) Maxim., *Epimedium pubescens* Maxim., *Epimedium wushanense* T. S. Ying or *Epimedium koreanum* Nakai (Fam. Berberidaceae).

The drug is collected in summer and autumn when foliage branch growing luxuriantly, removed from the thick stalks and foreign matter and dried in the sun or in the shade.

Origin[2]: *Epimedium* species are cultivated in the provinces of Si Chuan, Henan, Hu Bei, Sha'anxi, Shanxi and Guang Xi.

Description of the drug[1]: Herb of *Epimedium brevicornum*:
Stem slenderly cylindrical, about 20 cm long, externally yellowish-green or pale yellow, lustrous. Cauline leaves opposite, double ternately compound; leaflets ovate, 3 – 8 cm long, 2 – 6 cm wide; apex slightly acute, terminal leaflets cordate at the base, bilateral leaflets relatively small, oblique-cordate, the outer sider relatively large, auriculate, margin with yellow and thorny serrulations; the upper surface yellowish-green, the lower surface greyish-green, main veins 7 – 9, occurring sparsely slender hairs at the base, thin veins prominent on both surfaces, reticulated veins distinct; petiolules 1 – 5 cm long, Lamina subleathery. Odourless; taste, slightly bitter.

Herb of *Epimedium sagittatum*:
Leaves terrately compound, leaflets long-ovoid to ovoid-lanceolate, 4 – 12 cm long, 2.5 – 5 cm wide; acuminate at the apex; bilateral leaflets distinctly oblique at the base, the outer side arrow-shaped. The lower surface sparsely covered with thick, short and pronated hairs or nearly glabrous. Lamina leathery.

Herb of *Epimedium pubescens*:
The lower surface of lamina and petioles densely covered with flossy pubescences.

Herb of *Epimedium wushanense*:
Leaflets lanceolate to narrow-lanceolate, 9 – 23 cm long, 1.8 – 4.5 cm wide; acuminate or long-acuminate at the apex, margin thornydentate, basal lobes of the bilateral leaflets oblique, the inside lobes small and rounded, the outside lobes large, triangular, acuminate. The lower surface tomentose or bare.

Herb of *Epimedium koreanum*:
Leaflets relatively large, 4 – 10 cm long, 3.5 – 7 cm wide, long-acuminate at the apex. Lamina relatively thin.

Pretreatment of the raw drug[1]:

Herba Epimedii:
Foreign matters are eliminated, the leaves are picked, sprayed with water, softened slightly, cut into slivers and dried.

Herba Epimedii (stir-baked):
The slivers of Herba Epimedii are stir-baked with refined suet by gentle heating until an evenly lustre is produced, removed and cooled. 20 kg of refined suet is used per 100 kg of Herba Epimedii.

Medicinal use[3]:

rheumatic pain, arthralgic and paralytic diseases, climacteric hypertension, neurasthenia, chronic bronchitis, viral myocarditis, leucopenia, used also as tonic

Effects and indications according to Traditional Chinese Medicine[1,4]	
Taste:	Pungent and sweet in flavor
Temperature:	warm
Channels entered:	acts on the liver and kidney channels
Effects (functions):	reinforces the kidney yang, expels the wind and *dampness*, strengthens the tendons and bones and relieves rheumatic conditions
Symptoms and indications:	Impotence, seminal emission, weakness of the limbs, rheumatic or rheumatoid arthralgia with numbness and muscle contracture; climacteric hypertension

Contraindication[2]:

in patients with excess fire due to *Yin* deficiency

Main constituents:

Epimedium brevicornum:
- **prenylflavonol-glycosides:**
  icariin[3], icariside I[3], baohuoside I[5], II (= icariside II)[5], baohuoside VI[6], sagittatoside B[5], ikarisosides A[7], C[5], F[5], 2'-O-rhamnosylicariside II[5], III[7], wushanicariin[6], hexandraside E[6], epimedoside A[6], epimedins B[8], C[8], breviflavone B[9]

- flavonols:
  kaempferol-3,7-O-α-L-dirhamnoside[6], hyperoside[8]

- sugar:
  inositol[8]

Epimedium sagittatum:
- **prenylflavonol-glycosides:**
  icariin[3], anhydroicaritin-3-O-rhamnoside[3], anhydroicaritin 3-O-α-L-rhamnopyranoside[3], icaritin-3-O-rhamnoside[3], sagittatosides A[3], B[3], C[3], sagittatins A[3], B[3], epimedins A[3], B[3,10], C[3], icariside I[3], II[10], ikarisoside A[10], epimedoside A[10], epimedokoreanoside-I[10], yinyanghuo A – E[11]

- lignans:
  Icarisides E6[12], E7[12], icariols A1[12], A2[12]

- flavon(ol)s:
  chrysoeriol[11], quercetin[11], apigenin[11], apigenin-7,4'-dimethylether[11], kaempferol[11], luteolin[11]

- polysaccharides[3,13]

- other constituents:
  icariside B9[12], D3[12], H1[12]

Epimedium pubescens:
- **prenylflavonol-glycosides:**
  icariin[3], baohuoside VI[14]

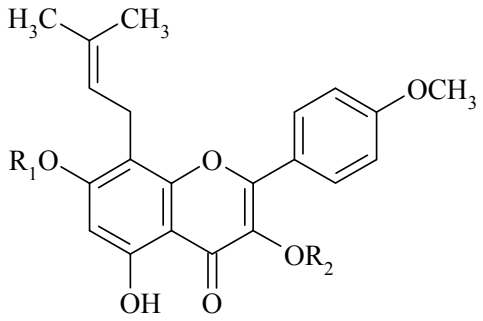
Epimedium wushanense:
- **prenylflavonol-glycosides:**
  icariin[3], wushanicariin[3], anhydroicaritin-3-O-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnpyranoside[15], desmethylanhydroicaritin-3-O-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside[15], sagittatoside A[3,15], B[3,15,16], ikarisoside A[16], B[15,16], wanepimedoside A[16], epimedin B[16], C[16], anhydroicaritin[16], desmethylanhydroicaritin[16], icarisid I[16], II[16], 2"-O-rhamnosylicarisid II[16], 2"-O-rhamnosylikarisoside A[16], diphylloside A[16], B[16]

- flavonol:
  quercetin[16]

Epimedium koreanum:
- **prenylflavonol-glycosides:**
  icariin[3], epimedoside A[3], epimedins A[3], B[3], C[3,14], sagittatosides A[3], B[3], anhydroicaritin 3-O-rhamnoside[3], 2"-O-rhamnosylicarisoside A (desmethylanhydroicaritin-3-O-α-L-rhamnosyl-(1→2)-α-L-rhamnoside)[14], 2"-O-rhamnosylicariside II (anhydroicaritin-3-O-α-L-rhamnosyl-(1→2)-α-L-rhamnoside)[14], epimedokoreanoside I[17,18], icariside II[17], caohuoside-B[18],

3-O-β-glucopyranosyl-(1→3)-α-L-(4-actyl)-rhamnopyranoside-anhydroicaritin-7-O-β-D-glucopyranoside[19], korepimedosides A[20], B[20]

- other constituents:
  2-(p-hydroxyphenoxy)-5,7-dihydroxy-6-prenylchromene[19], 7-hydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene-2-O-β-D-glucopyranoside[19], magnoflorine[21], chlorogenic acid[22], hyperosid[23], syringaresinol[24]
- polysaccharides[3]



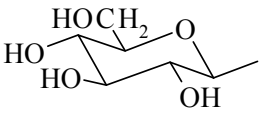
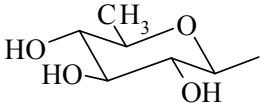
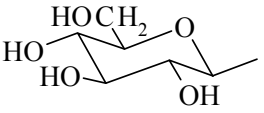
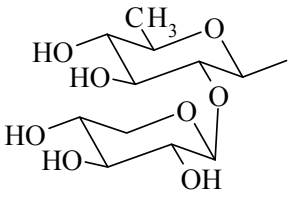
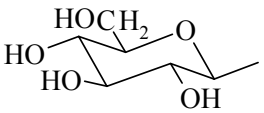
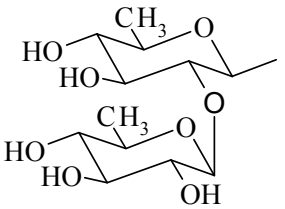
Compound	R_1	R_2
icariin		
icariside I		H
sagittatoside B	H	
epimedin C		

Fig. 1: Formulae of the main compounds[3]

Pharmacology:

Epimedium species:
- immunomodulatory[3]
- antihypertonic[3]
- antihyperlipidemic[3]
- cardiotonic[25]

Epimedium brevicornum:
- antitumoral[9]
- anti-osteoporotic[25]
- estrogenic[26]
- neuroendocrino-immunogenic[27]

Epimedium sagittatum:
- angiogenetic[28]
- cytotoxic effects[29]

Epimedium wushanense:
- invigorates kidney[16]
- strengthens 'Yang'[16]

Epimedium koreanum:
- tonic[18]
- antirheumatic[18]
- aphrodisiac[18]
- neuritogenetic[24]
- antihepatotoxic[30]

TLC-fingerprint analysis

1) Extraction: To 0.5 g of the powdered drug 10 ml of ethanol are added, heated under reflux for 30 minutes, cooled and filtered. The filtrate is evaporated to dryness, the residue is dissolved in 1 ml of ethanol and used for TLC.

2) Reference compounds: 1 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Epimedii herba extracts: each 5 µl
reference compounds: each 10 µl

Solvent system:

ethyl acetate	formic acid	glacial acetic acid	water
50	5.5	5.5	5

Detection:

Detection of flavonoids:
Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol
II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. After 30 minutes the evaluation is carried out in UV 365 nm.

2. Vanillin-sulphuric acid reagent

Solution I: 1 % ethanolic vanillin solution
Solution II: 10 % ethanolic sulphuric acid

The plate is sprayed with 10 ml solution I, followed immediately by 10 ml solution II. After heating at 110°C for 5-10 minutes under observation, the plate is evaluated in VIS.

Drug samples		**Origin**
1	Epimedii herba/*Epimedium brevicornum*	province Shaanxi, China
2	Epimedii herba/*Epimedium sagittatum*	province Sichuan, China
3	Epimedii herba/*Epimedium pubescens*	province Sichuan, China
4	Epimedii herba/*Epimedium koreanum*	province Jilin, China
5	Epimedii herba (botanical species unknown)	sample of commercial drug, China
6	Epimedii herba/*Epimedium acuminatum*	province Shaanxi, China

Reference compounds		**R*f***
T1	epimedin C	0.28
	icariin	0.65
	sagittatoside B	0.72
T2	epimedokoreanoside	0.28
	epimedoside A	0.59
	anhydroicaritin	0.93
T3	ikarisoside C	0.15
	ikarisoside F	0.66
	baohuoside I (icariside II)	0.93
T4	magnoflorin	0.09
	hyperoside	0.63
	syringaresinol	0.99
T5	chlorogenic acid	0.52

4) Description of the TLC-chromatogram:

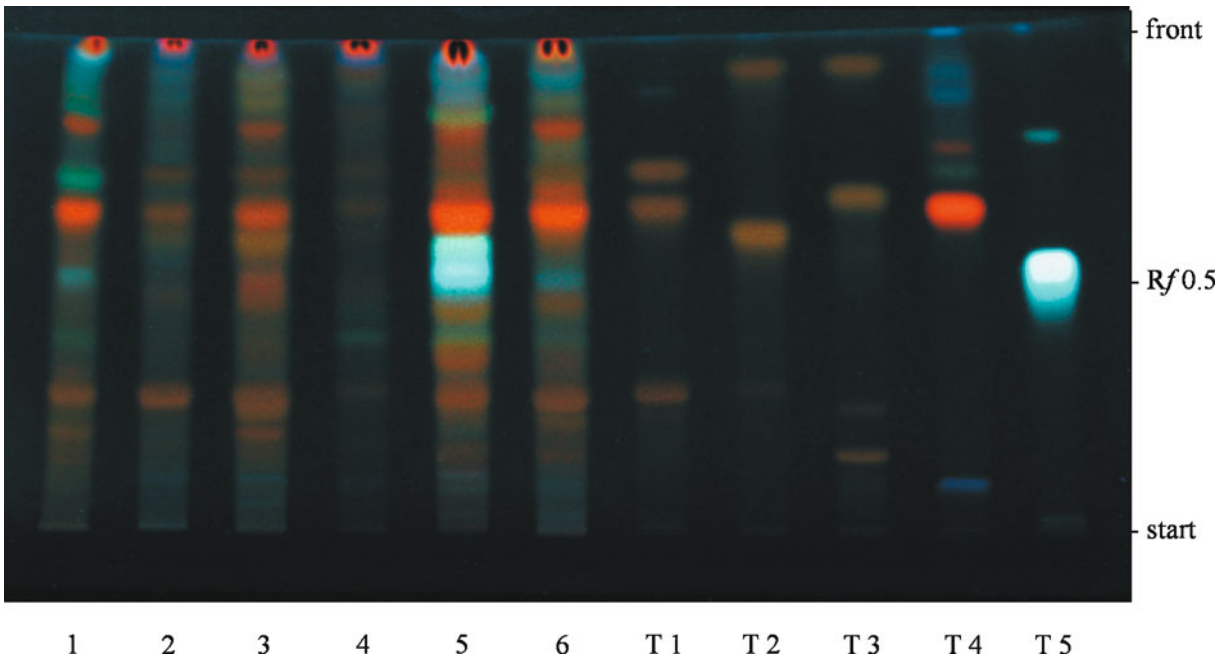


Fig. 2a: Thin layer chromatogram of ethanol extracts of Epimedii herba sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

All *Epimedium* samples with the exception of sample 4 show a relatively homogeneous chromatographic fingerprint pattern with the red to red orange fluorescent zone of hyperoside at R*f* = 0.63. This flavonolglycoside is overlapped by the orange fluorescent icariin with nearly the same R*f*-value (R*f* = 0.65). The other prominent flavonoids with orange brown fluorescent zones are epimedin C, two non identified flavonoids with R*f*-values at 0.36, 0.47 and sagittatoside B (R*f* = 0.72). Ikarisoside F (R*f* = 0.66) has the same R*f*-value as icariin (R*f* = 0.65) and the flavonoid baohuoside I (R*f* = 0.93) is overlapped by blue fluorescent constituents. Magnoflorin (R*f* = 0.09) and syringaresinol (R*f* = 0.98) reported for *Epimedium koreanum* could be hardly detected (blue fluorescence). A turquoise fluorescent spot of chlorogenic acid could be found at R*f* = 0.52 in sample 5 only.

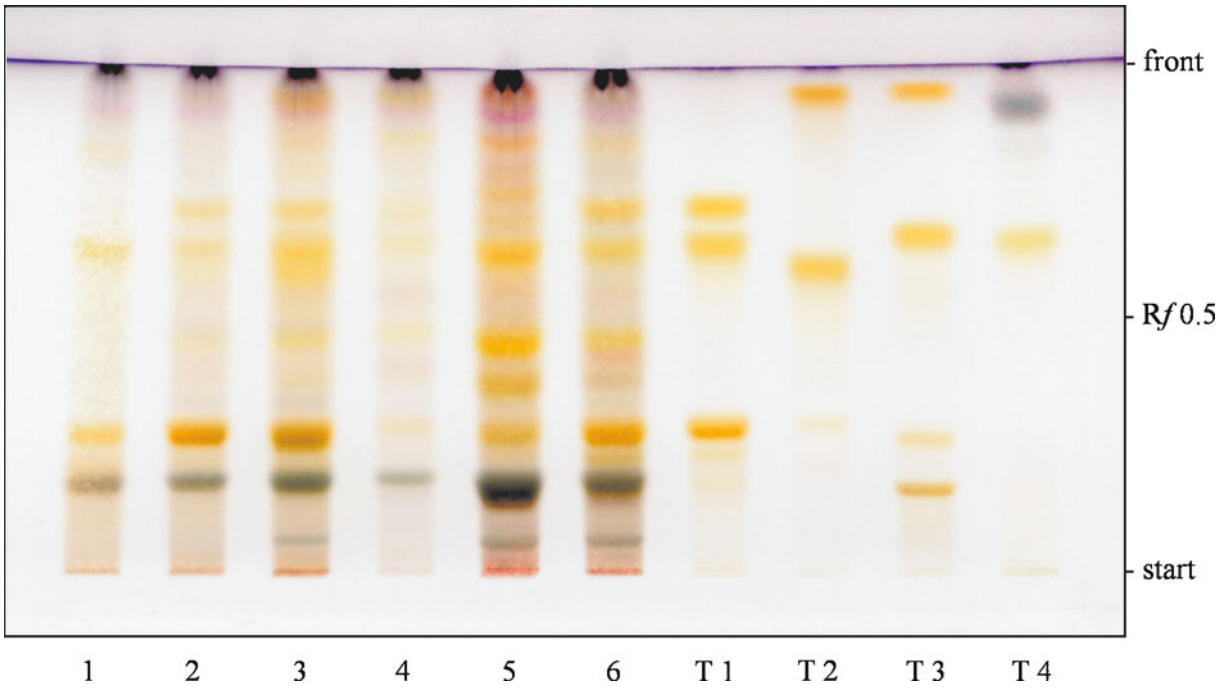


Fig. 2b: Thin layer chromatogram of ethanol extracts of Epimedii herba sprayed with vanillin-sulfuric acid reagent (VIS)

The different *Epimedium* samples detected with vanillin-sulphuric acid spray reagent show again with the exception of sample 4 a homogenous chromatographic pattern of mainly yellow to yellow orange spots of prenylflavon glycosides with the same R*f*-value as in Figure 2a. The grey brown spots in all drug samples at R*f* = 0.18 derive probably from sugars.

HPLC-fingerprint analysis:

1) Sample preparation:	To 0.5 g of the powdered drug 10 ml of ethanol are added, heated under reflux for 30 minutes, cooled and filtered. The filtrate is evaporated to dryness, the residue is dissolved in 1 ml of ethanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.
2) Injection volume:	Epimedii herba extract: 10.0 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck

Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 /litre water B: acetonitrile
Gradient:	20 – 48 % B in 55 minutes 48 – 70 % B in 5 minutes 70 % B in 12 minutes total runtime: 72 minutes
Flow:	0.6 ml/min.
Detection:	270 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	10.7	hyperoside
2	13.3	ikarisoside C
3	16.9	epimedoside A
4	23.3	epimedin C
5	24.3	epimedokoreanoside
6	25.3	icariin
7	34.4	ikarisoside F
8	42.7	sagittatoside B

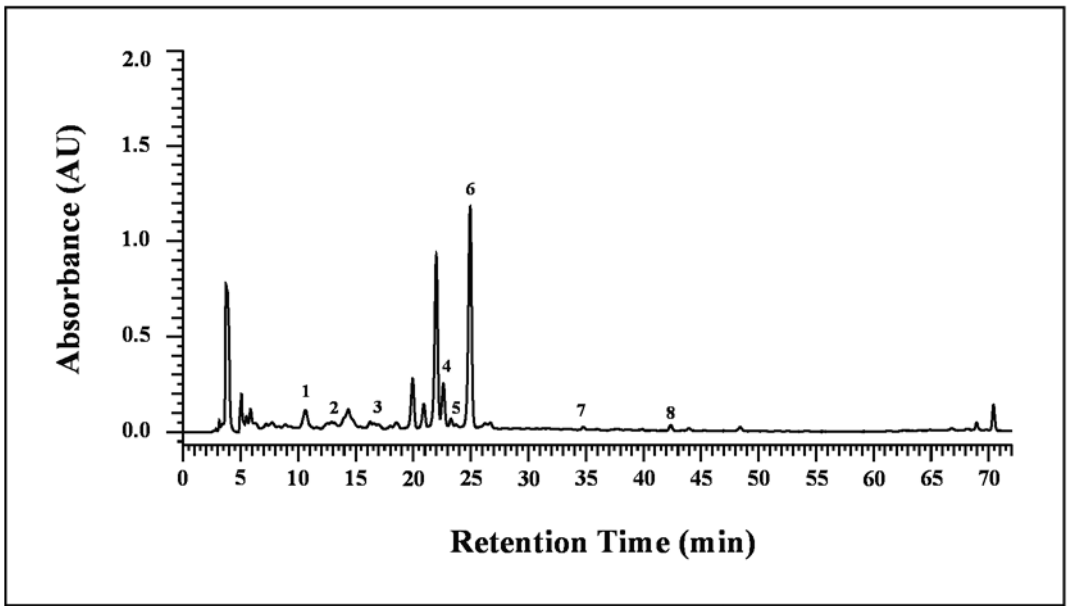


Fig. 3a: HPLC-fingerprint chromatogram of the ethanol extract of *Epimedium brevicornum* (Province Shaanxi) (sample 1)

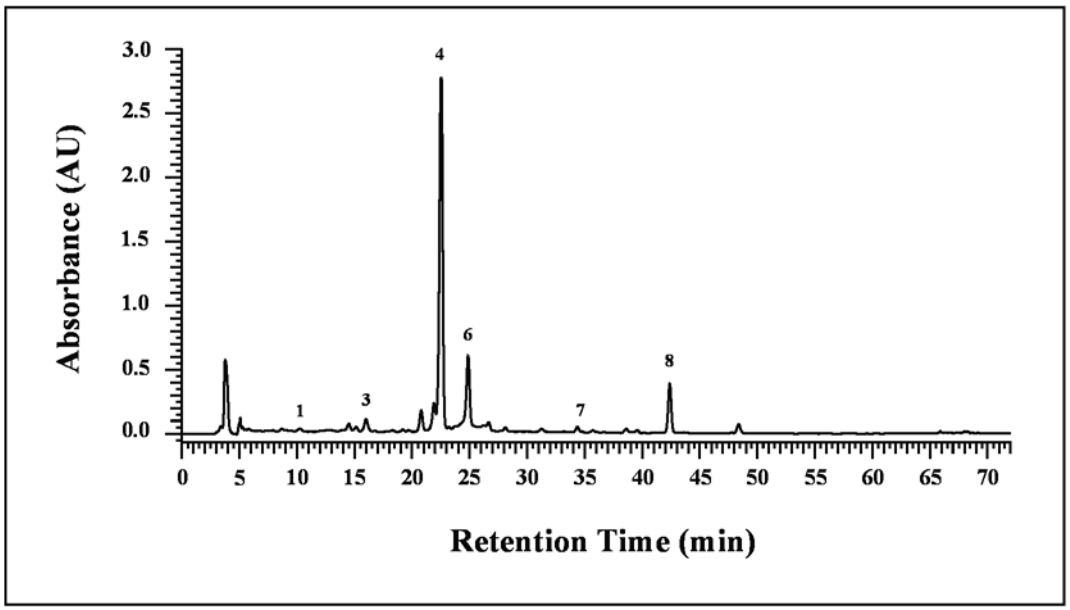


Fig. 3b: HPLC-fingerprint chromatogram of the ethanol extract of *Epimedium sagittatum* (Province Sichuan) (sample 2)

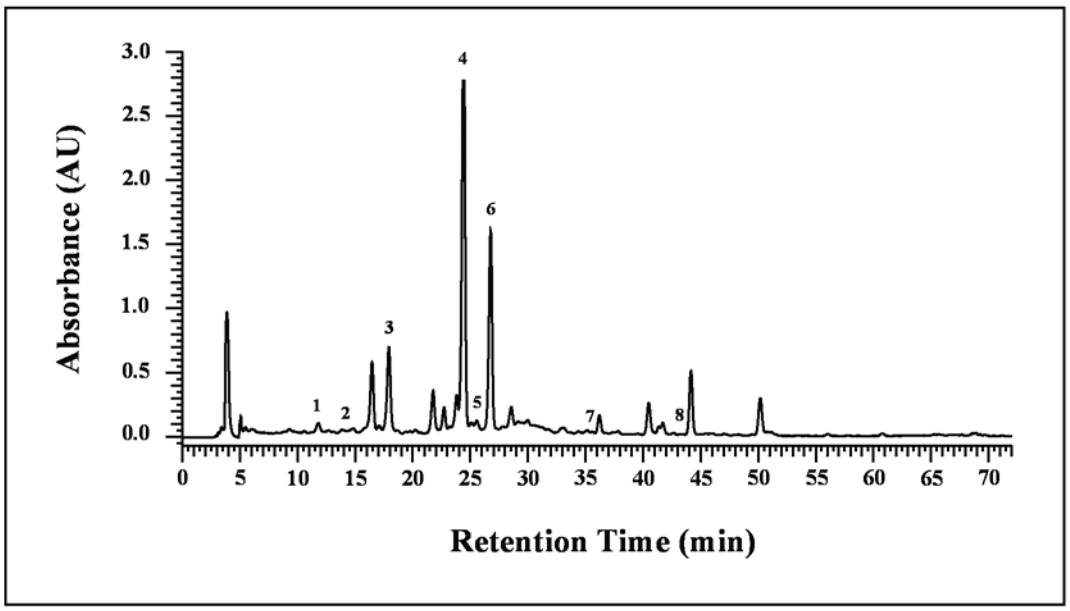


Fig. 3c: HPLC-fingerprint chromatogram of the ethanol extract of *Epimedium pubescens* (Province Sichuan) (sample 3)

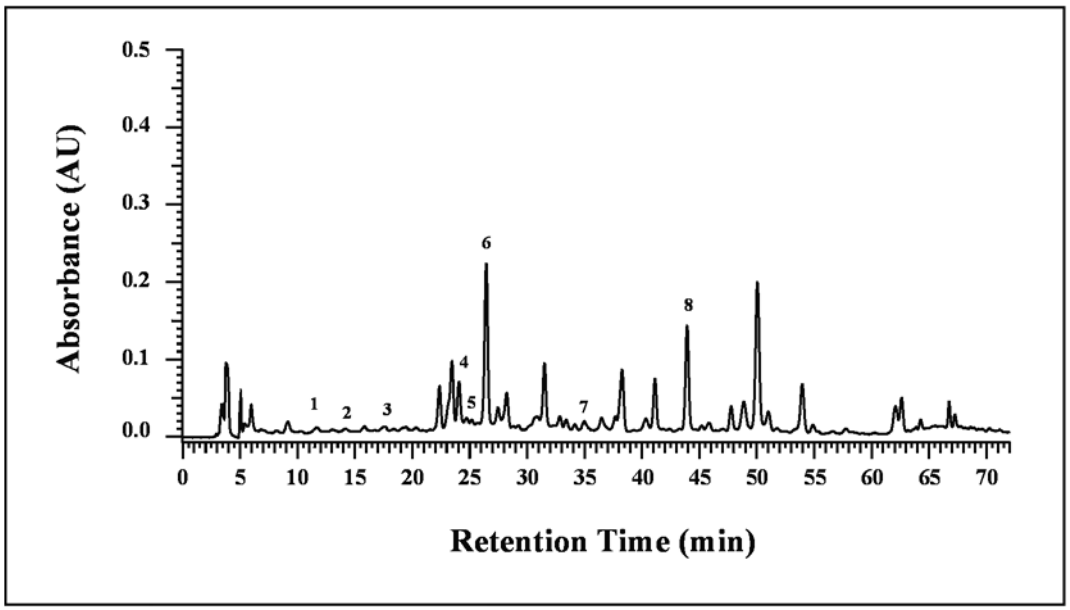


Fig. 3d: HPLC-fingerprint chromatogram of the ethanol extract of *Epimedium koreanum* (Province Jilin) (sample 4)

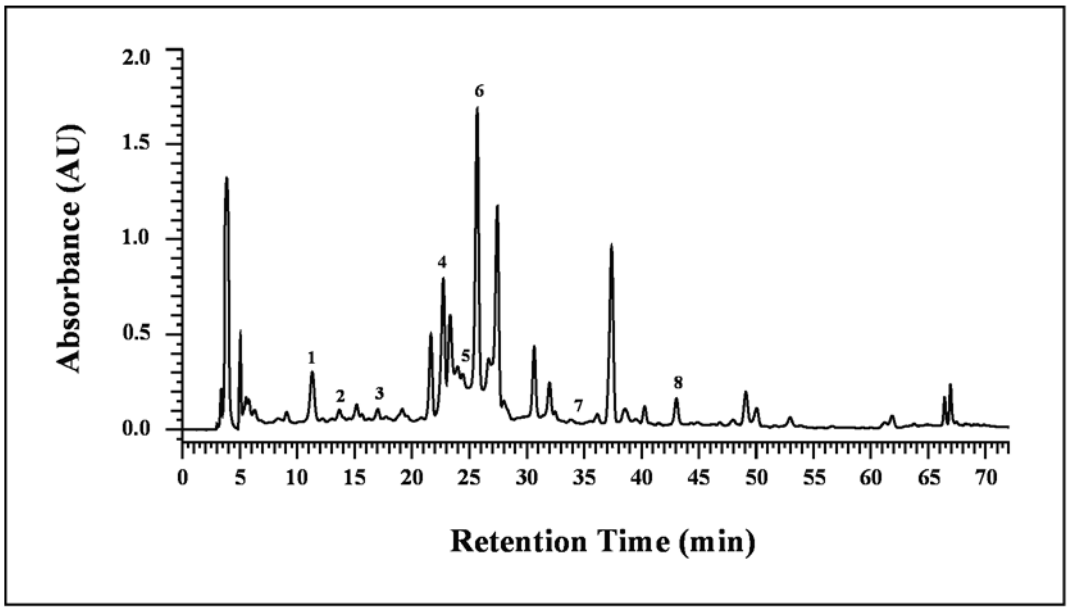


Fig. 3e: HPLC-fingerprint chromatogram of the ethanol extract of Epimedii herba (sample of commercial drug, China) (sample 5)

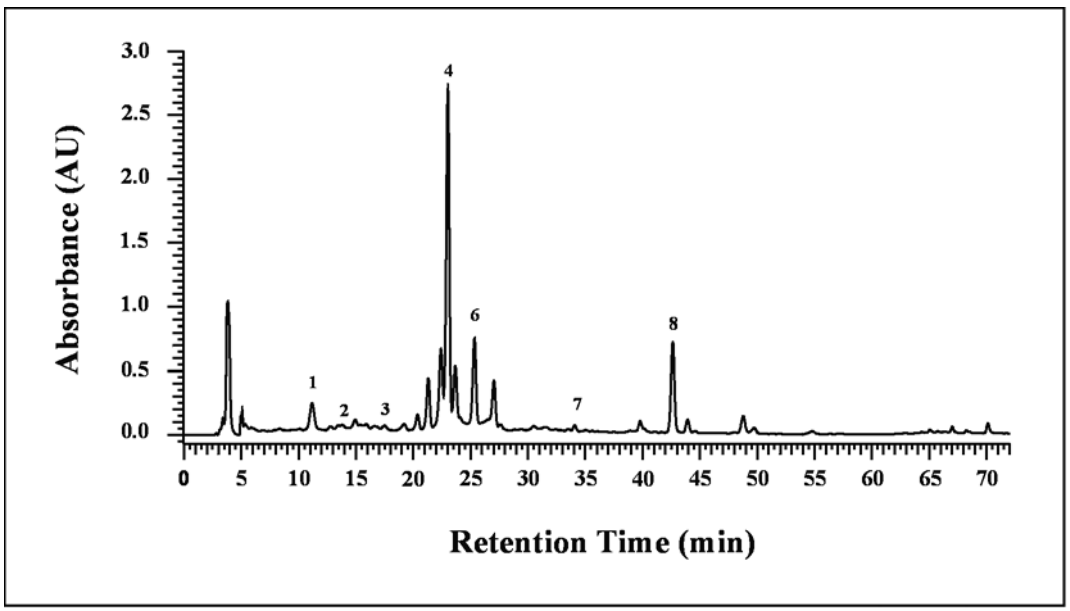


Fig. 3f: HPLC-fingerprint chromatogram of the ethanol extract of *Epimedium acuminatum* (Province Shaanxi) (sample 6)

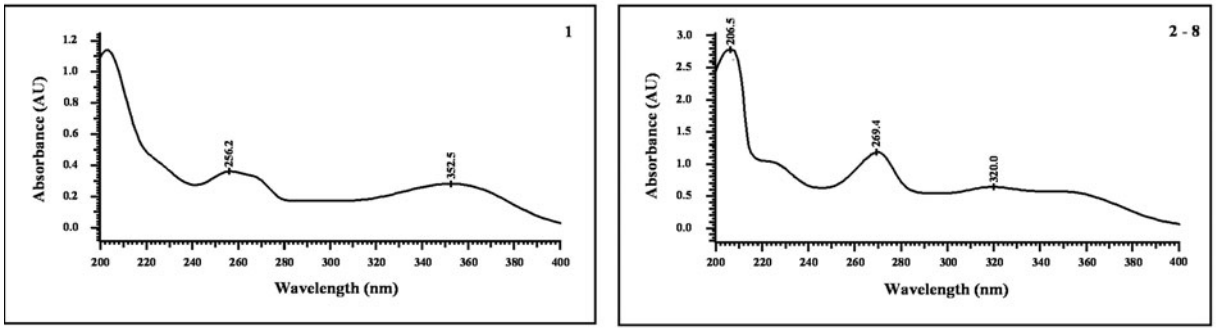


Fig. 4: UV-spectra of the main peaks of the ethanol extracts of Epimedii herba

4) Description of the HPLC of Figure 3a-f:

The various *Epimedium* species show in an overall view a deviating HPLC-peak pattern but are all characterized by a dominant peak composition with icariin* and epimedin C at Rt = 25.3 (**6**) and Rt = 23.3 (**4**). In the Rt-range 10 – 17 appear ikarisoside C (Rt = 13.3) (**2**), epimedoside A (Rt = 16.9) (**3**) and epimedokoreanoside (Rt = 24.3) (**5**), whereas ikarisoside F and sagittatoside B can be detected at Rt = 34.4 (**7**) and Rt =42.7 (**8**) respectively. All prenylflavon glycosides show about the same UV-spectrum with maxima at 206.5, 269.4 and 320.0 nm. Hyperosid can be determined at Rt = 10.7 (**1**) with UV-maxima at 256.2 and 352.5 nm.

***Note:** According to the Chinese Pharmacopoeia 2005 Herba Epimedii contains not less than 5.0 % of total flavones calculated as icariine, with reference to the dried drug.

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Fructus Cnidii
Shechuangzi

Pharmacopoeia:

Pharmacopoeia of the People´s Republic of China, English Edition 2005[1]

Official drug[1]:

Common Cnidium fruit is the dried ripe fruit of *Cnidium monnieri* (L.) Cuss. The drug is known under the English name "snake's bed seeds".

– Apiaceae –

Origin[2]:

Cnidium monnieri grows in the whole Republic of China, especially in the northern part.

Description of the drug[1]:

Cremocarp, ellipsoidal, 2-4 mm long, about 2 mm in diameter. Externally greyish-yellow or greyish-brown; with 2 outcurved stylopods at the summit, and sometimes with a fine fruit stalk at the base. Dorsal surface of mericarps with five thin and longitudinal ridges, commissural surface flattened, with two brown and slightly raised longitudinal ribs. Pericarp lax and fragile, easily rubbed off, seed small, greyish-brown and oily. Odour: aromatic. Taste: pungent,bitter, cool and numb.

Pretreatment of the raw drug[1]:

The drug is collected in summer or autumn when ripe, removed from foreign matter, and dried in the sun.

Medicinal use[2-4]:

internal use:
impotence, infertility either genera, leukorrhea, lumbago, renal disorders, rheumatism

external use:
psoriasis, eczema of external genitalia, vulval itching, dermatomycosis, trichomonas vaginitis, hemorrhoids

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[1-4]	
Taste:	bitter, pungent
Temperature:	warm
Channels entered:	acts on the kidney and spleen, site of action are the lower *calorium* and the lung
Effects:	*Yang* supporting and warming, warms the kidney and promotes virility, removes *damp*, dispels *wind* and kills parasites
Symptoms and indications:	• kidney-*Yang*-deficiency: impotence, infertility caused by kidney-debility or coldness in the uterus • *cold-damp* especially of the lower *calorium*: excessive leukorrhea, lumbago • external affection of the skin: psoriasis, parasites, itching skin lesion

Contraindication[3,4]**:** Contraindicated in *calor humidus* in the lower calorium and *Yin*-deficiency with *calor*- and *ardor*-signs

Main constituents (see Fig. 1)**:**

furanocoumarins[5,6]:
- osthol
- bergapten
- imperatorin
- xanthotoxin
- isopimpinellin

biscoumarins and coumarin derivatives[7]:
- cnidimonal
- cnidimarin
- 5-formylxanthotoxol
- 2'-deoxymeranzin hydrate

sesquiterpenes[8]:
- torilin
- torilolone
- 1-hydroxytorilin

glucides[9]:
- glycerol 2-O-alpha-L-fucopyranoside and D-quinovitol (6-deoxy-D-glucitol)

constituents of the essential oil[10,11]:
- β-ocimene
- limonene
- camphene
- β-myrcene
- β-pinene
- borneol
- terpineol

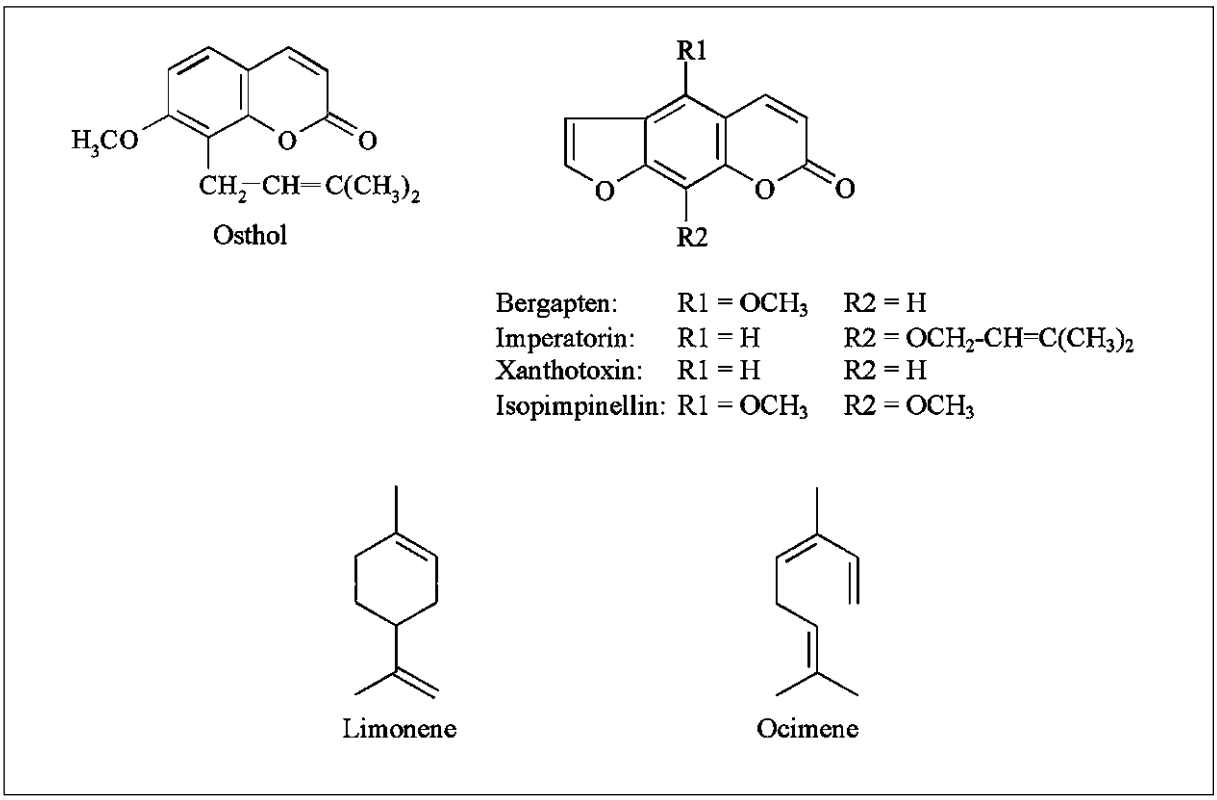

Fig. 1: Formulae of the main constituents[6,12,13]:

Pharmacology:
- anti-allergic activity (osthol)[14,15]
- antiosteoporotic effect (osthol, bergapten and imperatorin)[16-18]
- antimutagenic effect[19,20]
- anti-inflammatory effect[21]
- antipruritic effect (isopimpinellin and osthol)[20], inhibition of itch-scratch[23]
- oxygen radical scavaging effect and brain neurons protecting effect (osthol)[24], cognition-enhancing activities and anti-amnestic effects[25]
- anti-asthmatic effect[26]
- local anesthetic effect[27]
- cytotoxic activity on tumor cell lines[28]
- androgen-like effect and gonadotropin-like effect (osthol)[29]
- vasorelaxing effect[5]
- hepaprotective activity(sesquiterpene)[8]

TLC-fingerprint-analysis[1,13]**:**

Coumarins:

1) Extraction: 1.5 g powdered drug are ultrasonicated with 25 ml ethanol for 30 min. After cooling down the extract is filtered and evaporated to dryness. The residue is dissolved in 1.5 ml methanol.

2) Reference compounds: Osthol, imperatorin, xanthotoxin, bergapten, isopimpinellin (1 mg/ml)

3) Separation parameters:

Applied amount: 10 µl extract and standard solution

Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: toluene : ethyl acetate (8 : 2)

Detection: Direct evaluation in UV 365 nm and UV 254 nm.

Drug samples		Origin
1	Fructus Cnidii / *Cnidium monnieri*	locality Shanxi; China
2	Fructus Cnidii / *Cnidium monnieri*	sample of commercial product from Beijing market; China
3	Fructus Cnidii / *Cnidium monnieri*	locality Hebei; China
4	Fructus Cnidii / *Cnidium monnieri*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
5	Fructus Cnidii / *Cnidium monnieri*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany

Reference compounds		R*f*
T1	Osthol	0.59
T2	Imperatorin	0.54
T3	Xanthotoxin	0.47
T4	Bergapten	0.51
T5	Isopimpinellin	0.46

4) Description of the HPTLC-fingerprint of **Fig. 2** in UV 365 nm:

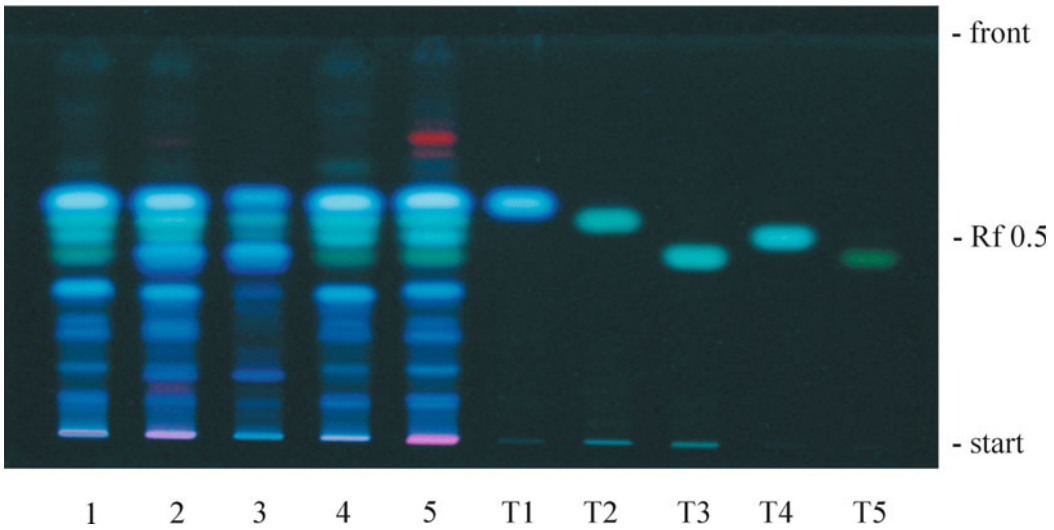

Fig. 2: HPTLC-fingerprint of Fructus Cnidii ethanol extract in UV 365 nm

All Fructus Cnidii samples (1-5) show a dominating blue fluorescent zone of the main coumarin osthol (T1) at R*f* 0.59. Three turquoise zones can be identified as imperatorin (T2, R*f* 0.54), xanthotoxin (T3, R*f* 0.47) and bergapten (T4, R*f* 0.51). Isopimpinellin (T5) can be detected as a blue-green zone at R*f* 0.46, overlapped by the turquoise zone of xanthotoxin (T3). In the lower R*f*-range (R*f* 0.4) down to the start appear 6-7 further blue zones of other coumarin derivatives (biscoumarins and coumarin glycosides).

Description of the HPTLC-fingerprint of **Fig. 3** in UV 254 nm:

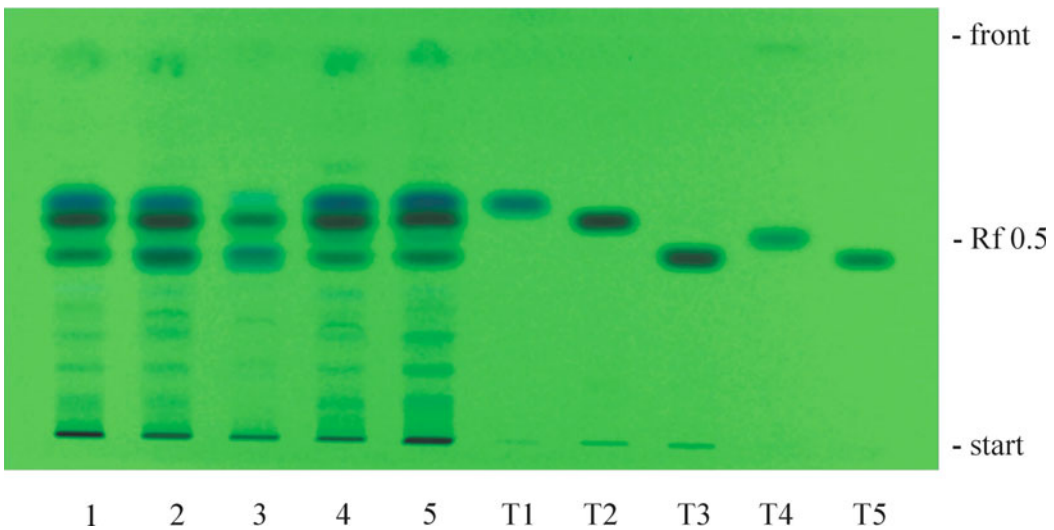

Fig. 3: HPTLC-fingerprint of Fructus Cnidii ethanol extract in UV 254 nm

In Fig. 3 the main coumarine osthol (T1) is detected as a green zone at R*f* 0.59. All other coumarins (T2-T5) show dark green fluorescent zones.

In the Pharmacopoeia of the People's Republic of China 2005[1] a TLC-method is described for the identification of osthol as main constituent. Fructus Cnidii should contain not less than 1.0 % of osthol, as estimated quantitatively by the TLC-scanning method.

Essential oil:

According to the literature[11] (Qiu et al. 2002) the precentages of the major terpenoids are reported as 37.96 % β-ocimene, 35.44 % limonene, camphene (6.28 %), β-myrcene (2.79 %) and β-pinene (1.16 %).

1) Extraction: The powdered drug is subjected to a water steam distillation in a Neo Clevenger apparat. The essential oil is diluted with hexane (1:5).

2) Separation parameters:

Applied amount:	5 µl extract and standard solution
Plate:	TLC-Plate Silicagel 60 F_{254}; Merck
Solvent system:	toluene-ethyl acetate (93 : 7)
Detection:	Spray reagent: Vanillin-sulphuric-acid-reagent: The plate is intensively sprayed with 1 % ethanolic vanillin-solution, subsequently with 10 % ethanolic sulphuric acid followed by heating for 10 minutes at 110 °C under supervision.

3) Description of the HPTLC-fingerprint of **Fig. 4** in VIS[11,13]:

Fig. 4: TLC-fingerprint of Fructus Cnidii essential oil in VIS

The constituents of the essential oil of Fructus Cnidii are distributed over the whole R*f*-range. In the R*f*-range from R*f* 0.7 up to the solvent front appear the spots of β-ocimene, limonene, camphene, β-myrcene and β-pinene. In the lower R*f*-range the monoterpenoids borneol, terpineol and other terpene alcohols can be localized.

HPLC-fingerprint-analysis:

Coumarins:

1) Sample preparation: The ethanol extract, used for HPTLC is filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-apparatus.

2) Injection volume: 2 µl extract and 5 µl reference solution

3) HPLC-data:

Apparatus: L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi

Column: LiChroCART® 250-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck

Solvent system: A: water, Millipore Ultra Clear UV plus® filtered; containing 10 ml 0,1 % H_3PO_4 /l

B: acetonitrile, HPLC quality Acros Organics

Gradient: 0 % B to 95 % B in 30 min. (linear)

Flow rate: 1.0 ml/min.

Detection: 320 nm

Retention times and identity of the main peaks of **Fig. 5a** and **Fig. 5b**:

Peak	Rt (min.)	Compound
1	19.1	Xanthotoxin
2	20.3	Isopimpinellin
3	20.6	Bergapten
4	24.5	Imperatorin
5	25.7	Osthol

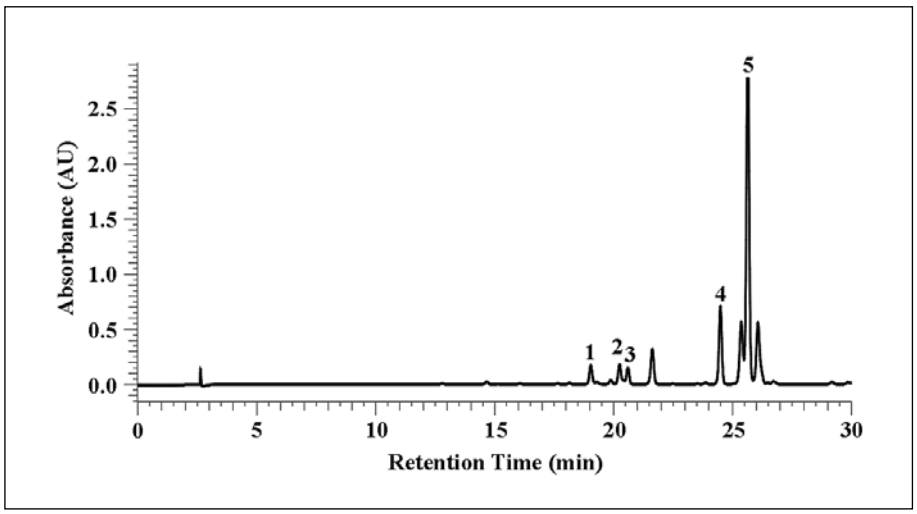

Fig. 5a: HPLC fingerprint of Fructus Cnidii extract, sample 2

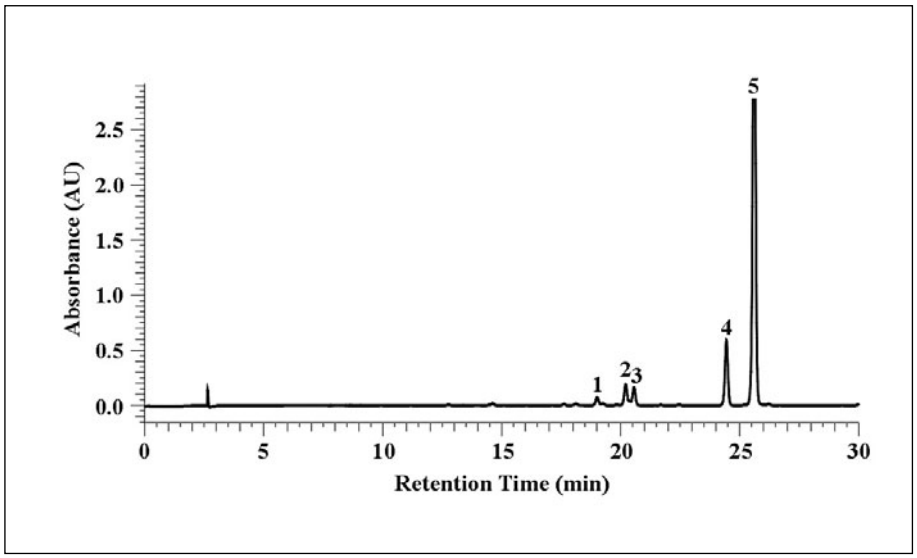

Fig. 5b: HPLC fingerprint of Fructus Cnidii extract, sample 5

4) Description of the HPLC-fingerprints of **Fig. 5a** and **Fig. 5b** and the online UV-spectra of Fig. 6:

The HPLC-fingerprint of all Fructus Cnidii samples shows a very similar qualitative and quantitative peak-pattern with xanthotoxin (**1**, Rt 19.1 min.), isopimpinellin (**2**, Rt 20.3 min.), bergapten (**3**, Rt 20.6 min.), imperatorin (**4**, Rt 24.5 min.) and osthol (**5**, 25.7 min.) as the dominant coumarins. Osthol differs in its UV-spectrum distinctly from those of the other coumarins (1-4).

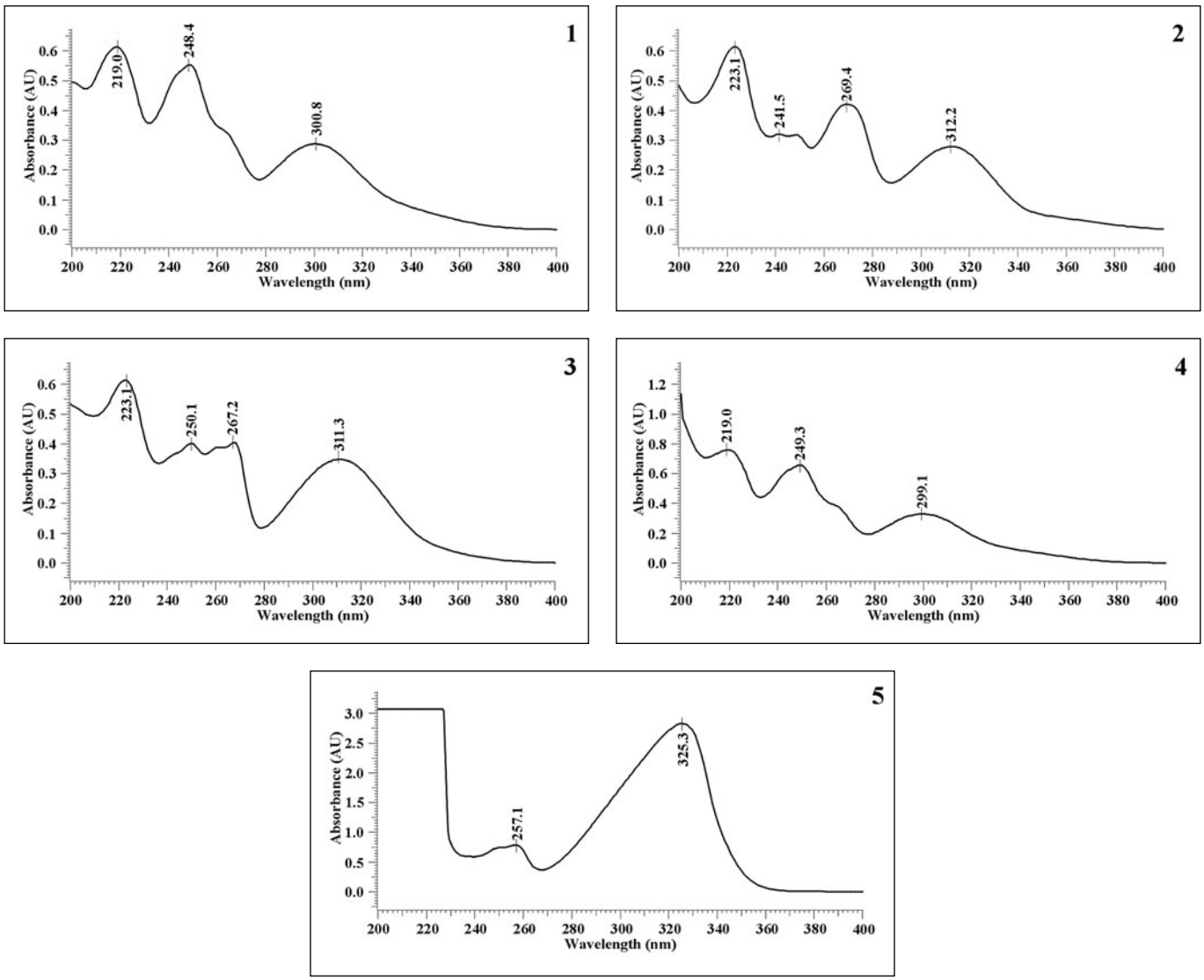

Fig. 6: Online UV-spectra of the main constituents of *Cnidium monnieri* detected in HPLC

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Cortex Lycii radicis
Digupi

Pharmacopoeia:

Pharmacopoeia of the People's Republic of China, English Edition 2005[1]

Official drug[1-3]:

Lycium chinense Mill. or *Lycium barbarum* L.
The drug is known under the English names "Chinese Wolfberry root-bark", "Bark of Wolfberry root" and "Earth bone bark".

– Solanaceae –

Origin[4-6]:

China (Ningxia, Gansu, Xinjiang, Hebei, Shaanxi, inner Mongolia), Japan, Korea and Taiwan.

Description of the drug[1]:

Quilled or channelled, 3-10 cm long, 0.5-1.5 cm wide, 1-3 mm thick. Outer surface greyish-yellow to brownish-yellow, rough, with irregular longitudinal fissures, easily exfoliated. Inner surface yellowish-white to greyish-yellow, relatively even, with fine longitudinal wrinkles. Texture light and fragile, easily broken, fracture uneven, outer layers yellowish-brown and inner layers greyish-white.

Odour , slight; taste, sweetish and then bitter.

Pretreatment of the raw drugs[1]:

The Chinese Wolfberry root-bark is the dried root bark of *Lycium chinese* Mill. or *Lycium barbarum* L. The root is collected in early spring or late autumn and washed clean. Then the root bark is stripped and dried in the sun.

Medicinal use[1-3]:

Cortex Lycii radicis is used for the treatment of hypertension, cough, hemoptysis, epistaxis, afternoon fever and night sweating in consumptive diseases and as adjuvant of diabetes.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of Cortex Lycii radicis according to Traditional Chinese Medicine[1-4,6]	
Taste:	sweetish and then bitter
Temperature:	cold
Channels entered:	acts on the lung, liver and kidney channels
Effects:	*Xue* and *Calor* refrigerant, repels and reduces heat in the blood and lung
Symptoms and indications:	epistaxis caused of *Xue* and *Calor*, subfebril temperature: night fever, much sudor, irritability, cough, aggravating respiration, bloody expectoration or urine or emesis

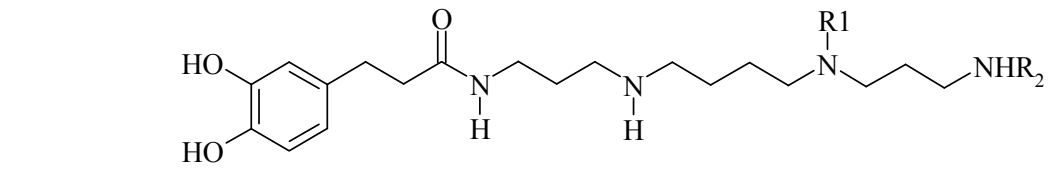
Contraindications and interactions[4,6]**:**

Contraindicated in cases of external common cold and spleen deficiency with diarrhea.

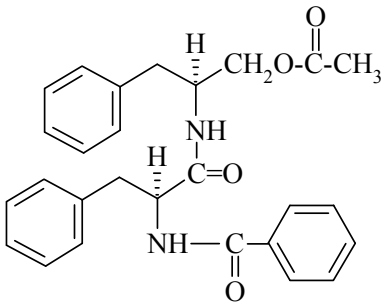
Main constituents[3,4,7-17]: (see Fig. 1)**:**

- **alkaloids*)**: kukoamine A, B (spermine alkaloid)
- *other nitrogenous compounds:* dihydro-N-caffeoyltyramine, trans-N-feruloylactopamine, trans-N-caffeoyltyramine, cis-N-caffeoyltyramine, betaine
- *peptides*, *dipeptide***:** lyciumamide, *octapeptide*: lyciumin A and B
- *fatty acids*: linoleic acid, linolenic acid,
- **coumarin:** scopoletin
- *various minor compounds*: cinnamic acid, vanillic acid, melissic acid, E-ferulic acid octacosyl ester, sugiol
- *sterols, triterpenoic acid*: β-sitosterol, ursolic acid, 5α-stigmastan-3,6-dione
- *sesquiterpenes*: dehydro-α-cyperone and solvavetione
- *acyclic diterpene glycosides:* lyciumoside
- *flavonoids*
- **lignan:** (+)-lyoniresinol-3α-O-β-D-glucopyranoside

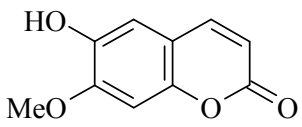
*)Harsh[18] reported 1989 the presence of tropane alkaloids, atropine and hyoscyamine, in plant parts of *Lycium barbarum* originated from Indian arid zone and tissue culture. This could be not confirmed by Han et al.[19] and also recently by Adams et al.[20] using the LC-MS-method as new high selective analytical method.



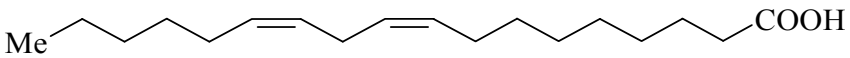
Kukoamine A: R_1=H, R_2=dihydrocaffeoyl
Kukoamine B: R_1=dihydrocaffeoyl, R_2=H



Lyciumamide



Scopoletin



Linoleic acid

Fig. 1: Formulae of the main constituents

Pharmacology:

- hypotensive activity (kukoamine A)[3,9]
- antimicrobial activity [(+)-lyoniresinol-3α-O-β-D-glucopyranoside][17]
- angiotensin converting enzyme inhibitory (lyciumin A)[3,21]
- antioxidant activity[22]
- antiinflammatory activity[22]
- hepatoprotective activity[22]
- antifungal effects (phenolic amides)[10,21]

TLC-fingerprint-analysis:

Coumarines of Cortex Lycii radicis (Fig. 2)[1]:

1) Extraction: 2.0 g powdered drug are mixed with 35 ml water in a flask. The extract is heated on the water bath (100 °C) under reflux for 15 min., cooled down, filtered and the filtrate shaken with 15 ml ethyl acetate. The ethyl acetate layer is separated and evaporated to dryness. The residue is redissolved in 1 ml ethyl acetate.

2) Reference compound: scopoletin (1 mg/ml)

3) Separation parameters:

   Applied amount: 15 µl extract and standard solution

   Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

   Solvent system: ethyl acetate – chloroform – formic acid (60 : 40 : 20)

   Detection: Direct evaluation in UV 365 nm.

   Spray reagent:

   Natural product-polyethylenglycol reagent:
   The plate is sprayed successively with 1 % methanolic solution of diphenylboric acid-ß-ethyl-aminoester (NP) following a 5 % ethanolic polyethylenglycol-4000 solution (PEG).
   The evaluation is carried out in UV 365 nm.

4) Description of Fig. 2[23]:

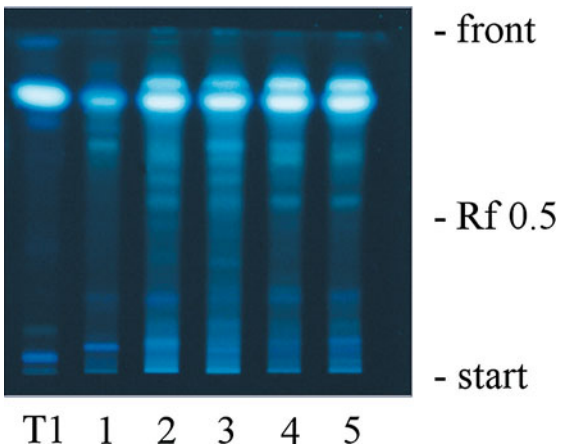


Fig. 2: HPTLC-fingerprint of Cortex Lycii radicis extracts: coumarin (scopoletin) detected with natural product-polyethylenglycol reagent in UV 365 nm

Similar to the fruit extract a predominate blue fluorescent zone of scopoletin in UV 365 nm at R*f* 0.81 is detectable in all bark extract samples.

Fatty acids of Cortex Lycii radicis (Fig. 3)[24]:

1) Extracts/Extraction: 1.0 g powdered drug is heated on the water bath (60 °C) under reflux for 30 min. with 25 ml methanol. After cooling down the extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2) Reference compound: linoleic acid (1 µl/ml)

3) Separation parameters:

   Applied amount: 15 µl extract and 10 µl standard solution

   Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

   Solvent system: toluene - ethyl acetate - acetone (50 : 20 : 20)

   Detection: Spray reagent:

   Vanillin-sulphuric-acid-reagent:
   The plate is intensively sprayed with 1 % ethanolic vanillin-solution and with with 10 % ethanolic sulphuric acid. Following by heating for 10 minutes at 110 °C under supervision. The evaluation is carried out in VIS.

4) Description of Fig. 3[24]:

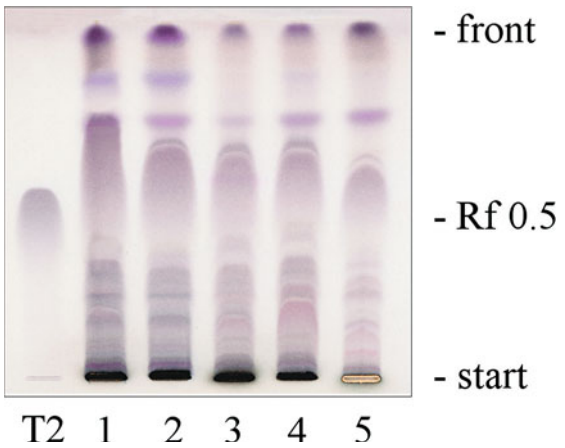


Fig. 3: HPTLC-fingerprint of Cortex Lycii radicis methanol extract (linoleic acid) detected with vanillin-sulphuric reagent in VIS

Linoleic acid (T2) and linolenic acid appear overlapped in all extracts in about the same R*f* range (0.52-0.67) as one characteristic violet zone.

Nitrogenous and peptide compounds of Cortex Lycii radicis (Fig. 4a and 4b)[23]:

1) Extracts: methanol extract used for TLC – Fig. 3

2) Reference compounds: lyciumin A and B, atropine (1 mg/ml)

3) Separation parameters:

Applied amount: 15 µl extract and standard solution

Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: ethyl acetate - formic acid - glacial acetic acid - water (90 : 11 : 11 : 26)

Detection: Spray reagents:

Fig. 4a: Ninhydrin reagent:
30 mg ninhydrin are dissolved in 10 ml *n*-butanol and 0.3 ml glacial acetic acid added. After spraying, the plate is heated for 5-10 min. at 110 °C. The evaluation is carried out in VIS.

Fig. 4b: Dragendorff reagent:
Solution I: 0.85 g basic bismuth nitrate are dissolved in 10 ml glacial acetic acid and 40 ml water under heating. If necessary filter.

Solution II: 8 g potassium iodide are dissolved in 30 ml water.

Stock solution: solution I and II are mixed 1:1.

Spray reagent: The plate is intensively sprayed with 1 ml stock solution mixed with 2 ml glacial acetic acid and 10 ml water. The evaluation is carried out in VIS.

4) Description of Fig. 4a,b[8,9]:

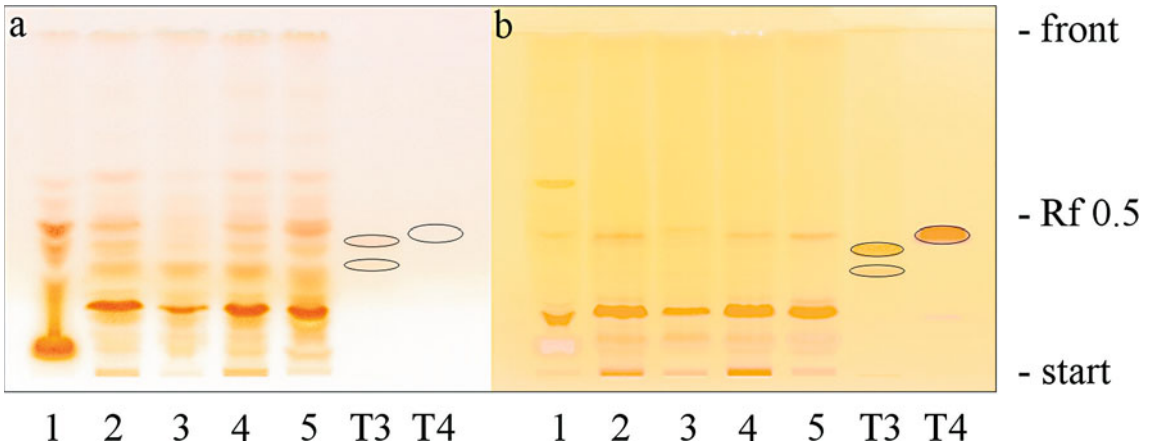


Fig. 4: HPTLC-fingerprint of Cortex Lycii radicis methanol extract (nitrogenous and peptide compounds) detected with ninhydrin (Fig. 4a) reagent and Dragendorff reagent (Fig. 4b) in VIS

Fig. 4 shows the pattern of alkaloids and other nitrogen compounds detected with Dragendorff reagent and the amino acids with ninhydrin reagent. The methanol extract of Cortex Lycii radicis shows a major orange zone at R*f* 0.20, propably the alkaloid kukoamine A. The dipeptide lyciumamide is not detectable with Dragendorff reagent. The two octapeptides lyciumin A and B (T5) are hardly detectable at R*f* 0.32 and 0.39.

With ninhydrin and Dragendorff reagent about the same alkaloid (acid amide) pattern can be obtained (Fig. 4a and 4b). It consists at least of 4-6 orange zones in the R*f*-range from the start to ~R*f* 0.65. A major orange zone at R*f* 0.20 can be assigned probably to kukoamine A. The two octapeptides lyciumin A and B (T3) can be detected at R*f* 0.32 and R*f* 0.39 respectively.
The dipeptide lyciumamide could not be assigned to one of the zones.

The alkaloid atropine (T4) gives with Dragendorff reagent (Fig. 4b) an orange zone at R*f* 0.43. Since atropine is not dectectable with ninhydrine reagent (Fig. 4a), this zone at the same R*f*-value in sample 2-5 cannot be derived from atropine. This is in agreement with the non-detectability of atropine in any part of *Lycium chinense* or *Lycium barbarum*[19,20].

Drug samples		Origin
1	Cortex Lycii radicis/*Lycium chinense*	province Hebei, China
2	Cortex Lycii radicis/*Lycium chinense*	province Shangxi, China
3	Cortex Lycii radicis/*Lycium chinense*	province Shangxi, China
4	Cortex Lycii radicis	Uchida Company, Japan
5	Radix Lycii	sample of commercial product, Korea

Reference compounds		R*f*
T1	scopoletin	0.81
T2	linoleic acid	0.52-0.67
T3	lyciumin A	0.32
	lyciumin B	0.39
T4	atropine	0.43

HPLC-fingerprint-analysis:

Nitrogenous and peptide compounds of Cortex Lycii radicis (Fig. 5a and 5b)[23,25]:

1) Sample preparation: 1.0 g powdered drug is grounded in a mortar for about 1 min with 2 ml 10 % ammonia solution and then thoroughly mixed with 7 g basic aluminium oxide activity grade I. This mixture is then packed loosely into a glass column (1.5 cm diameter, 20 cm length) and 10 ml chloroform are added. The column is eluted with about 5 ml chloroform and the eluate is collected and evaporated to dryness. The residue is redissolved in 1 ml methanol.

2) Injection volume: 15 µl extract

3) HPLC-data:

Apparatus: L-6200A Intelligent Pump,
AS-2000 Autosampler,
L-4500A Diode Array,
D-6000A Interface; Merck Hitachi

Column: LiChroCART® 250-4 LiChrospher® 60 RP-18 with
LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm);
Merck

Solvent system: A: 1 mM aqueous trifluoracetic acid
B: acetonitrile

Gradient: 90 % A to 30 % A in 10 min. (linear)
30 % A for 5 min. (isocratic)
30 % A to 5 % A in 10 min. (linear)
5 % A for 15 min. (isocratic)

Flow rate: 0.7 ml/min.

Detection: 210 nm

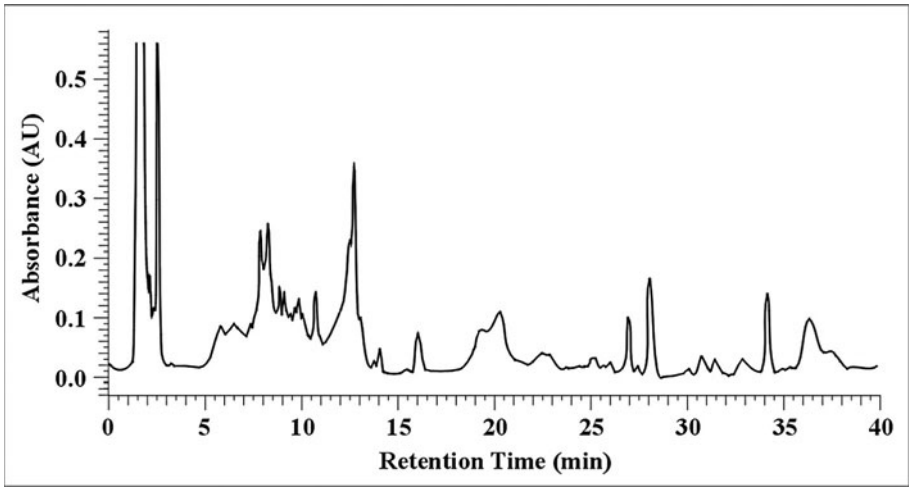


Fig. 5a: HPLC fingerprint of Cortex radicis of *Lycium chinensis*, province Shangxi China (sample 2)

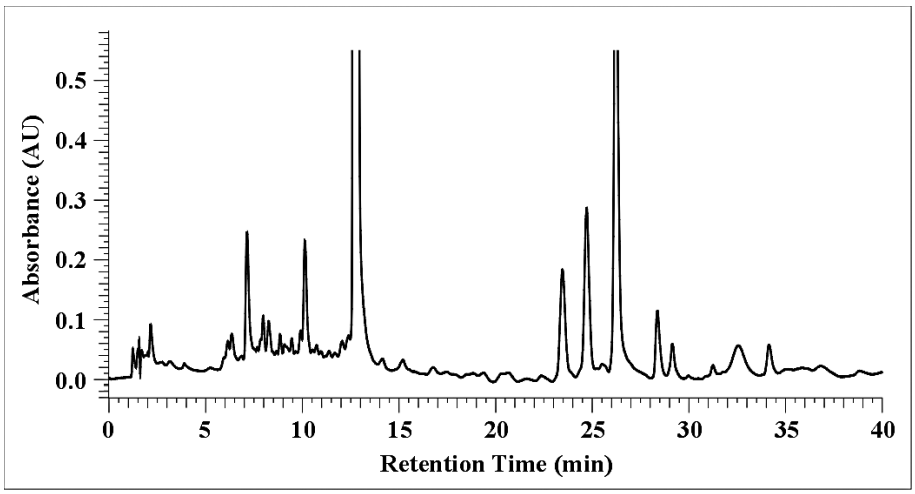


Fig. 5b: HPLC fingerprint of Cortex Lycii radicis, Uchida Company, Japan (sample 4)

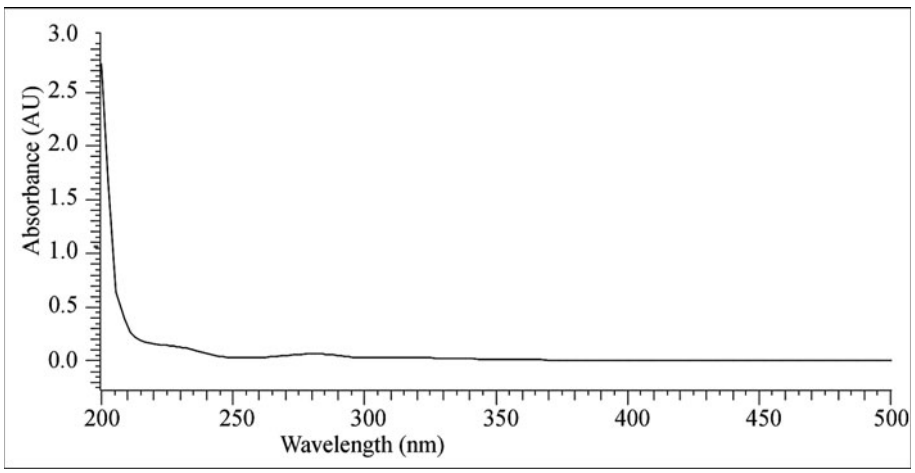


Fig. 6: Characteristical UV-endabsorption of nitrogenous and peptide compounds detected in HPLC

4) Description of the HPLC-chromatogram of Cortex Lycii radicis (Fig. 5a, b):

The main nitrogenous constituents of Cortex Lycii radicis extracts (e.g. lycium A and B) are detectable in the Rt-range of 5 to 15 min with one major compound at Rt 13.0 and some others between Rt 24.0 and 28.0 min. An exact assignment was not possible.

All nitrogenous and peptide compounds show UV-spectra (Fig. 6) with endabsorption at 210 nm.

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Fructus Lycii
Gouqizi

Pharmacopoeia:

Pharmacopoeia of the People's Republic of China, English Edition 2000/2005[1]

Official drug[1-3]:

Lycium barbarum L.

The *Lycium chinense* Mill. species is not listed as official drug in the Chinese Pharmacopeia.

The drug is known under the English names "Chinese Wolfberry fruit", "Matrimony vine fruit" and "Chinese boxthorn".

– Solanaceae –

Origin[4-6]:

China (Ningxia, Gansu, Xinjiang, Hebei, Shaanxi, inner Mongolia), Japan, Korea and Taiwan.

Description of the drug[1]:

Subfusiform or ellipsoid, 6-20 cm long, 3-10 cm in diameter. Externally scarlet or dark red, marked with a protrudent style scar at the apex, and a white fruit stalk scare at the base. Pericarp pliable and shrunken, sarcocarp fleshly, soft and viscous. Seeds 20-50, subreniform, flat and bent upwards, 1.5-1.9 mm long, 1-1.7 mm wide, pale yellow or brownish-yellow on surface.

Odourless; taste, sweet.

Pretreatment of the raw drugs[1]:

Barbary Wolfberry fruit is the dried ripe fruit of *Lycium barbarum* L. The drug is collected in summer and autumn when the fruit turns orange-red. After drying in the shade to make the pericarp shrunken, the drug is exposed to strong sun light until the exocarp is dried and hard, and the pulp soft, and removed from the fruit stalk.

Medicinal application (a) and dietetic use (b)[1,4-7]:

a) For stimulating the immune system and as adjuvant for the treatment of dizziness, tinnitus, impaired vision (macula degeneration) and general debility.

b) As nutrient and antiaging dietary supplement for all kinds of deficiency.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of Fructus Lycii according to Traditional Chinese Medicine[1-7]	
Taste:	sweet
Temperature:	neutral
Channels entered:	acts on the liver and kidney channels
Effects:	on a progressive fill-up basis of kidney- and liver-*Yin*; *Jing* supplementing, blood nourishing, improves eyesight, moistening the lungs
Symptoms and indications:	kidney- and liver-*Yin*-deficiency: impotence, debility or pain in the back and legs, fits of dizziness, common amyasthenia; *Jing*-absence: infertility either genera, amblyopia, amblyacousia, diminished visual acuity, ambiguous sight, treatment of bone marrow deficiency

Main constituents[4,6-13] (see Fig. 1)**:**

- **carotenoids:** β-carotene, β-cryptoxanthin, zeaxanthin, β-cryptoxanthin palmitic acid ester, zeaxanthin monopalmitic acid ester and zeaxanthin dipalmitic acid ester (= physalien)
- **fatty oil:** linoleic acid, γ-linolenic acid, oleic acid
- **phosphatides**
- **volatile compounds:** sesquiterpenes dehydro-α-cyperone, solavetivone and various neutral volatile compounds (hydrocarbons, alcohols, aldehydes, ketones, ketals, esters and lactones)
- **coumarin:** scopoletin
- **polysaccharides:** acidic arabinogalactans
- **glycoproteins**

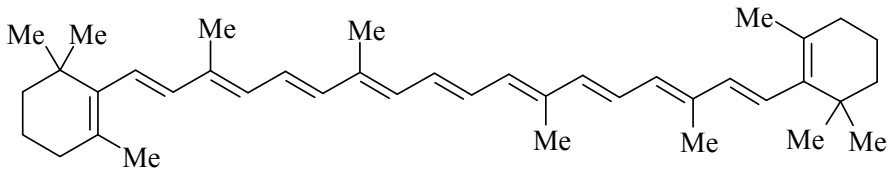
Minor constituents[4,6-13]**:**

- steroid compounds, triterpene 3 β-monoalcohols: cycloartanol, cycloartenol, 24-methyl-cycloartanol and 4,4-dimethylsterols and derivatives (gramisterol), lanosterol derivatives, β-sitosterol
- nitrogenous compounds: perlolyrin and 1-carboxy-methoxycarbolin, betaine, nicotinamine, amino acids
- mineral nutrients
- organic acids: vanillic acid, melissic acid
- cerebroside
- vitamins: A, B_1, B_2, B_6, C, E

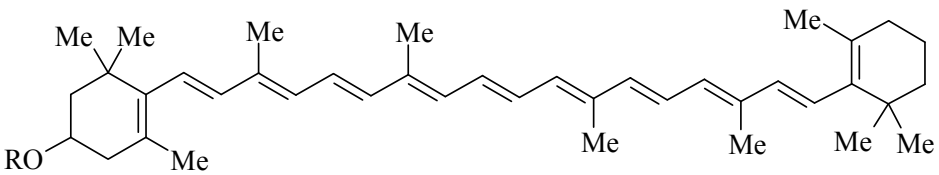
NOTE: Harsh ML[14] reported 1989 the presence of tropane alkaloids, atropine and hyoscyamine, in plant parts of the Indian arid zone and tissue culture of *Lycium barbarum*. This could not be confirmed by Han BH et al.[15] and also recently by Adams M et al.[16] using the LC-MS-method

as new high selective analytical method. The detected amounts were in all samples of *Lycium barbarum* fructus examined far below the toxic level [max. 19 ppb (w/w)].

Fig. 1: Formulas of the main constituents

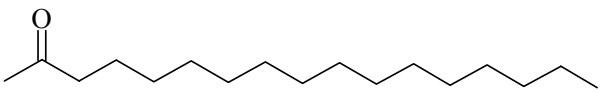


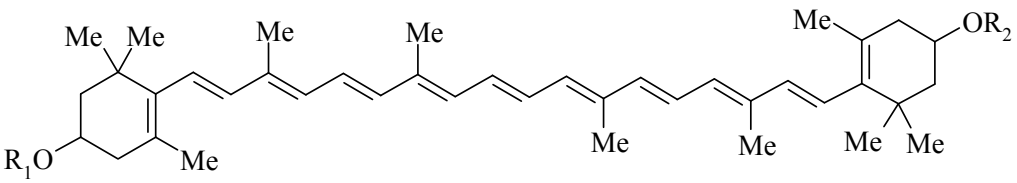
β-Carotene



Cryptoxanthin: R=H

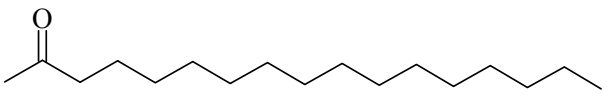
Cryptoxanthin palmitic acid ester: R=



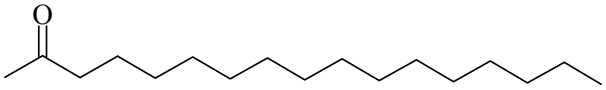


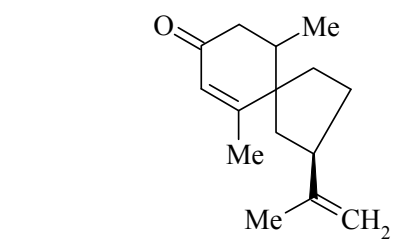
Zeaxanthin: R_1=R_2=H

Zeaxanthin monopalmitic acid ester: R_1=H, R_2=

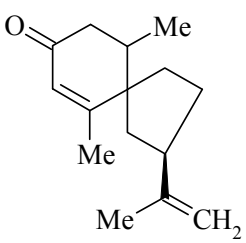


Zeaxanthin dipalmitic acid ester: R_1=R_2=
(=Physalien)

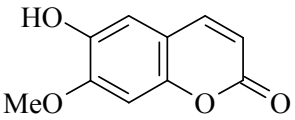




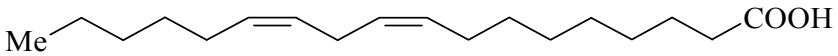
Dehydro-α-cyperone



Solavetivone



Scopoletin



Linoleic acid

Pharmacology:

- antioxidant effects[17,18]
- antiaging effects[19]
- immunomodulating activities (glycopeptides)[20,21]
- antitumoral activities (polysaccharides)[22]
- antihepatotoxic effects[13,23]
- hypoglycemic and hypolipidemic effects[24]
- blood sugar lowering effect[24,25]
- blood pressure lowering effect[26]

Contraindications and interactions[2,4,6,27]:

Contraindicated in cases with externally-contracted excess heat patterns, and cases of spleen deficiency with diarrhea, dampness and loose stools. Possible interaction between warfarin and *Lycium barbarum* fruits has been reported (Lam et al.[27])

TLC-fingerprint-analysis:

Drug samples		Origin
1	Fructus Lycii/*Lycium barbarum*	Yuxi City, province Yunnan, China
2	Fructus Lycii/*Lycium barbarum*	province Ningxia, China
3	Fructus Lycii/*Lycium barbarum*	province Hebei, county Julu, China
4	Fructus Lycii/*Lycium barbarum*	province Ningxia, China
5	Fructus Lycii/*Lycium chinense*	Uchida Company, Japan
6	Fructus Lycii/*Lycium chinense*	province Ningxia, China

Reference compounds		R*f*
T1	β-carotene	0.92
	β-cryptoxanthin	0.09
T2	physalien	0.56
	zeaxanthin	0.01
T3	linoleic acid	0.02 (Fig. 2) respectively 0.52-0.57 (Fig. 5)
T4	scopoletin	0.81

Carotenoids of Fructus Lycii (Fig. 2)[7]:

1) Extraction: 5.0 g drug are cut in a blender to small pieces with 50 ml *n*-hexane. The extract is filtered and the filtrate is evaporated to about 1 ml.

2) Reference compounds: β-carotene, β-cryptoxanthin, physalien, zeaxanthin (1 mg/ml)

3) Separation parameters:

Applied amount: 15 µl extract and standard solution

Plate: TLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: methylene chloride - *n*-hexane - ethyl acetate (30 : 20 : 0.5)
Equilibration of the chamber for 30 min. The R*f*-range can be displaced depending on the equilibration time used.

Detection: Direct evaluation in VIS.

Spray reagent:

Vanillin-sulphuric-acid-reagent:
The plate is intensively sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid, followed by heating for 10 minutes at 110°C under supervision.
The evaluation is carried out in VIS.

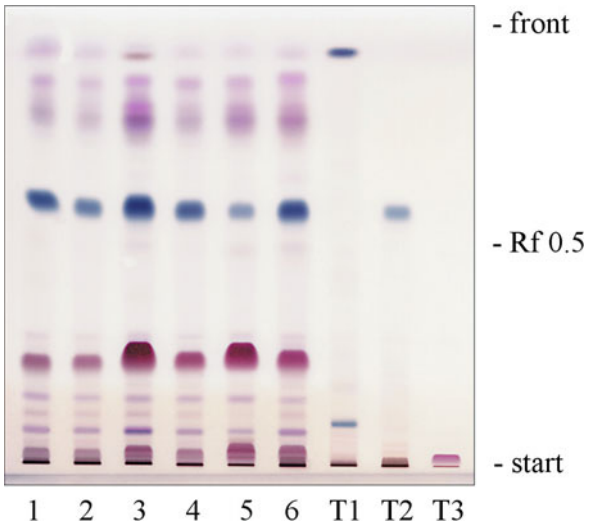


Fig. 2: TLC-fingerprint of Fructus Lycii hexane extracts (carotenoids) detected with vanillin-sulphuric reagent in VIS

4) Description of Fig. 2 [7,28]:

The various hexane extracts of Fructus Lycii samples show a very homogenous pattern of carotenoids and their esters: β-Carotene appears at R*f* 0.92 (**T1**) in a very low concentration. Between R*f* 0.71 and R*f* 0.89 the violet zones of the carotinoid-diesters are visible. At R*f* 0.56 the blue zone of zeaxanthin dipalmitic acid ester (= physalien, **T2**) can be detected. Among the violet zones between the start and ~R*f* 0. 3 zeaxanthin monopalmitic ester appears as prominent zone at R*f* 0.24. β-Cryptoxanthin (R*f* 0.09, **T1**) could not be detected. Linoleic acid (on the start, **T3**) has to be chromatographed in another solvent system (see Fig. 5).

Essential oil of Fructus Lycii (Fig. 3)[11,29]:

1) Extraction: About 200 g powdered drug are subjected to a water steam distillation in a Neo Clevenger apparat. Because of the small amount of volatile oil 1 ml *n*-hexane is given into the reflux condenser.

2) Separation parameters:

Applied amount:	20 µl diluted essential oil and standard solution
Plate:	HPTLC-Plate Silicagel 60 F_{254}; Merck
Solvent system:	toluene - ethyl acetate (93 : 7)
Detection:	Spray reagents:
	Fig. 3a: Vanillin-sulphuric-acid-reagent: The plate is intensively sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid, followed by heating for 10 minutes at 110 °C under supervision. The evaluation is carried out in VIS.

Fig. 3b: Dinitrophenylhydrazine reagent:

The plate is intensively sprayed with 10 ml solution of 0.1 g 2,4-dinitrophenylhydrazine in 100 ml methanol, followed by the addition of 1 ml 36 % hydrochloric acid. The evaluation is carried out in VIS.

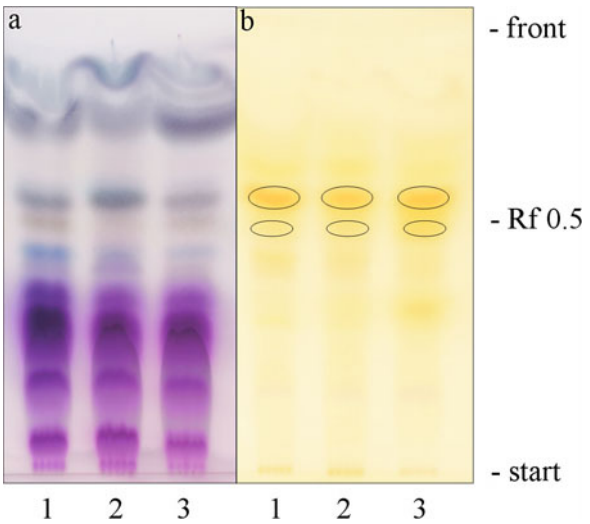


Fig. 3: HPTLC-fingerprint of Fructus Lycii essential oils detected with vanillin-sulphuric reagent (Fig. 3a) and dinitrophenylhydrazine reagent (Fig. 3b) in VIS

4) Description of Fig. 3a, b[8,11]:

In Fig. 3a the essential oil of the various samples of Fructus Lycii show in the R*f* range from start to R*f* 0.42 5-6 strong pink-violet zones which could be not assigned to defined chemical structures. The sesquiterpenes α-cyperone and solavetivone give with vanillin-sulphuric acid reagent weak blue-grew zones. With dinitrophenylhydrazine reagent (Fig. 3b) appear yellow-orange zones which are characteristic for ketones (e.g. dehydro-α-cyperone).

Coumarines of Fructus Lycii (Fig. 4)[1]:

1) Extraction: To 5.0 g Fructus Lycii 35 ml water dest. are added and the fruits cut in a blender to small pieces. The extract is heated on the water bath (100 °C) under reflux for 15 min, cooled down, filtered and the filtrate shaken with 15 ml ethyl acetate. The ethyl acetate layer is separated, evaporated to dryness and the residue redissolved in 1 ml ethyl acetate.

2) Reference compound: scopoletin (1 mg/ml)

3) Separation parameters:

Applied amount: 15 µl extract and standard solution

Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

Solvent system:	ethyl acetate – chloroform - formic acid (60 : 40 : 20)
Detection:	Direct evaluation in UV 365 nm.
	Spray reagent: Natural product-polyethylenglycol reagent: The plate is sprayed successively with 1 % methanolic solution of diphenylboric acid-ß-ethyl-aminoester (NP) and a 5 % ethanolic polyethylenglycol-4000 solution (PEG). The evaluation is carried out in UV 365 nm.

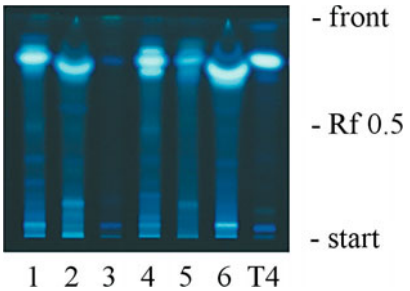


Fig. 4: HPTLC-fingerprint of Fructus Lycii extracts: coumarin (scopoletin) detected with natural product-polyethylenglycol reagent in UV 365 nm

4) Description of Fig. 4[1,29]:

In UV 365 nm a strong blue fluorescent zone of scopoletin (**T4**) is detectable in all Fructus Lycii extracts at R*f* 0.81.

Note: In the Chinese Pharmacopoeia an identification method is described for scopoletin (**T4**).

Fatty acids of Fructus Lycii (Fig. 5)[28]:

1) Extracts:	The same *n*-hexane extract as used for TLC- Fig. 2
2) Reference compound:	linoleic acid (1 µl/ml)
3) Separation parameters:	
Applied amount:	15 µl extract and 10 µl standard solution
Plate:	TLC- Plate Silicagel 60 F_{254}; Merck
Solvent system:	toluene - ethyl acetate - acetone (50 : 20 : 20)
Detection:	Direct evaluation in VIS.
	Spray reagent: Vanillin-sulphuric-acid-reagent: The plate is intensively sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid, followed by heating for 10 minutes at 110°C under supervision. The evaluation is carried out in VIS.

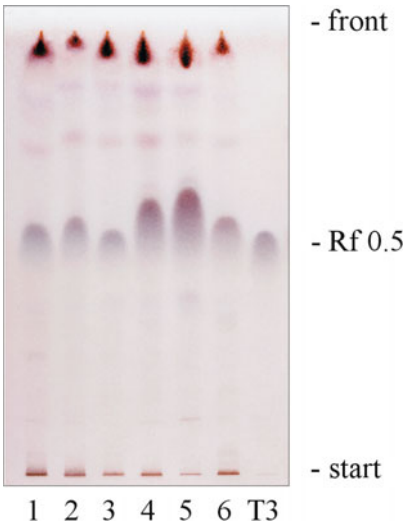


Fig. 5: TLC-fingerprint of Fructus Lycii hexane extracts (linoleic acid) detected with vanillin-sulphuric reagent in VIS

4) Description of Fig. 5[28]:

In Fig. 5 all Fructus Lycii extracts linoleic acid (**T3**) and γ-linolenic acid appear overlapped in the same R*f* range (0.52-0.57) as one characteristic violet zone.

HPLC-fingerprint-analysis:

Carotenoids of Fructus Lycii (Fig. 6)[30,31]

1) Sample preparation: 1.5 g Fructus Lycii are cut in a blender to small pieces in the presence of 10 ml methanol. After 5 min the extract is mixed with 10 ml Tris-HCl (50mM, pH 7.5, containing 1mM NaCl) and kept standing for 10 min. Then 40 ml chloroform are added and the mixture incubated for 10 min followed by centrifugation at 3000 *g* for 5 min to obtain a clear partition into two phases. The upper phase is removed with a pipette and the aqueous extract re-extracted with 40 ml chloroform.

   The chloroform-phases were evaporated to dryness and the residues redissolved in 2 ml ethyl acetate.

2) Injection volume: 10 µl extract and reference solution

3) HPLC-data:

   Apparatus: L-6200A Intelligent Pump,
   AS-2000 Autosampler,
   L-4500A Diode Array Detector
   (monitoring wavelength 200–400 nm),
   D-6000A Interface; Merck Hitachi

Column:	LiChroCART® 250-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: methanol B: methanol - water (80:20) containing 0.2 % ammonium acetate C: *tert*-butyl methyl ether
Gradient:	90 % A, 5 % B and 5 % C for 5 min. (isocratic) 45 % A, 5 % B and 50 % C in 35 min. (linear) 35 % A, 5 % B and 60 % C in 25 min. (linear)
Flow rate:	0.5 ml/min.
Detection:	Monitoring wavelength: 290 or 275 nm Absorbance: 445 nm Wavelength range: 250 to 550 nm

Retention times and identity of the main peaks of **Fig. 6a-d recorded at 445 nm:**

Peak	Rt (min.)	Compound
1	11.1	zeaxanthin
2	23.0	β-cryptoxanthin
3	37.7	β-carotene
4	50.4	physalien

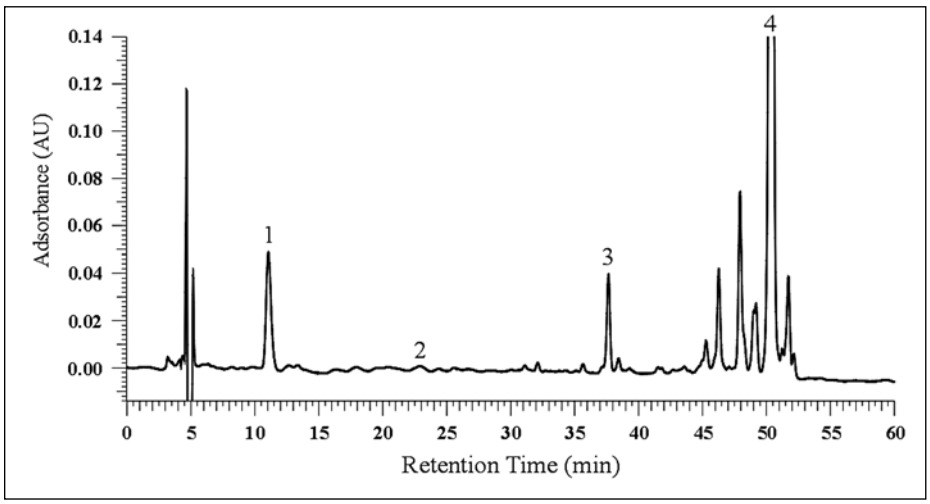


Fig. 6a: HPLC fingerprint of Fructus *Lycium barbarum*, Yuxi City, province Yunnan, China (sample 1)

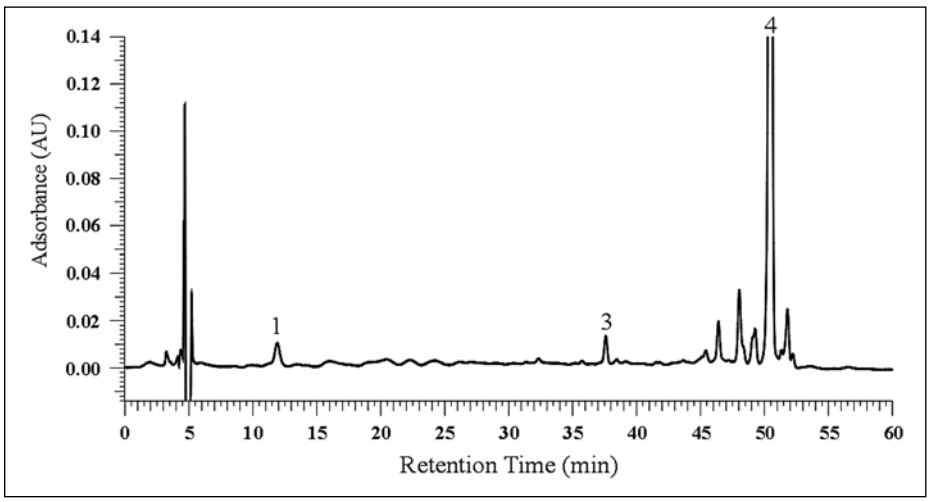


Fig. 6b: HPLC fingerprint of Fructus *Lycium barbarum*, province Ningxia, China (sample 2)

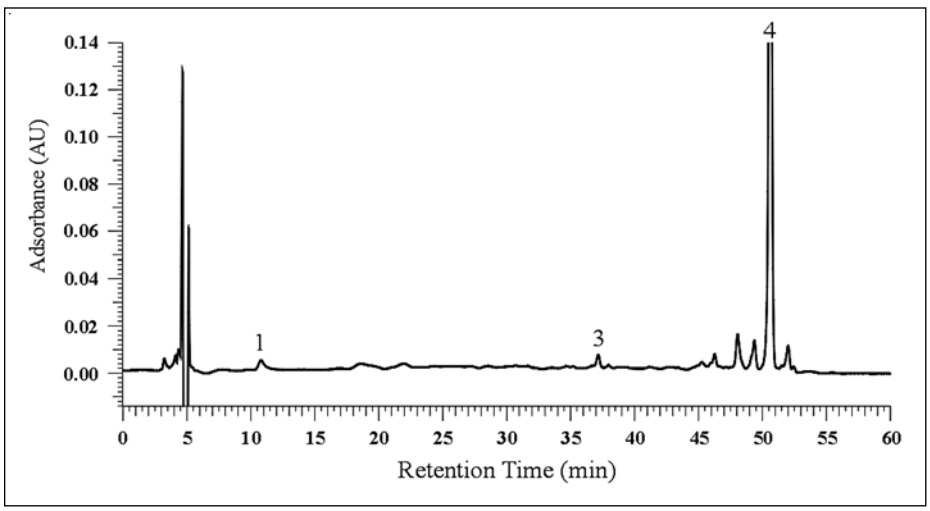


Fig. 6c: HPLC fingerprint of Fructus *Lycium chinense,* Uchida Company, Japan (sample 5)

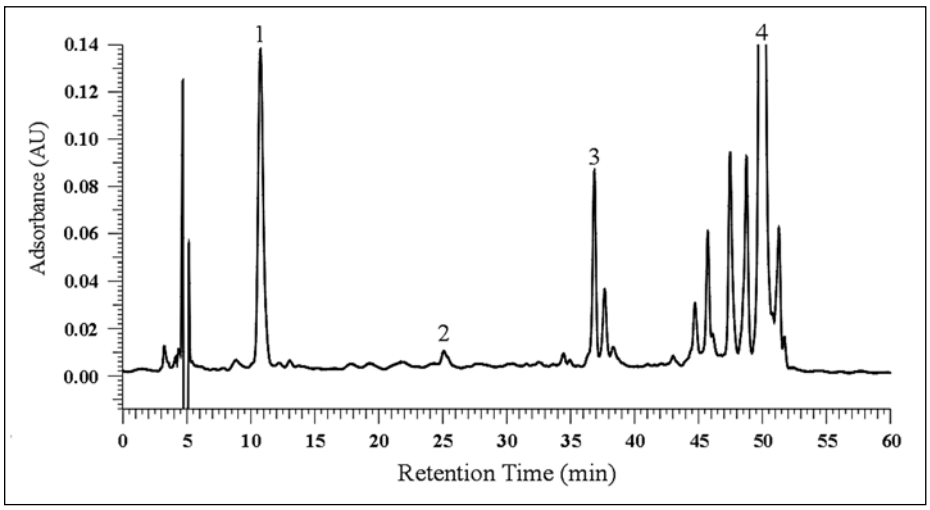


Fig. 6d: HPLC fingerprint of Fructus *Lycium chinense,* province Ningxia, China (sample 6)

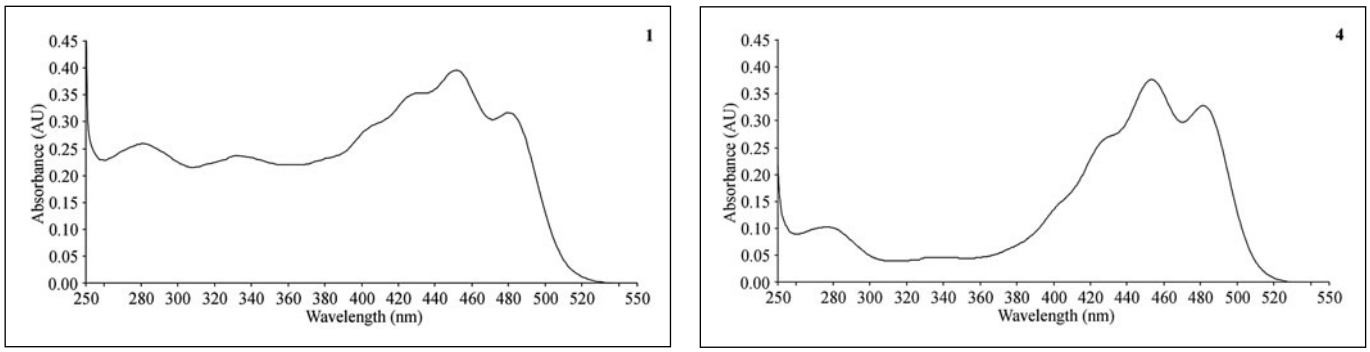


Fig. 7: Characteristic UV-spectra of carotenoids measured with UV/VIS spektrophotometer Lambda Bio 20; Perkin Elmer

4) Description of the HPLC- chromatograms of Fructus Lycii (Fig. 6a-d):

All HPLC-fingerprints of the carotenoids of various Fructus Lycii samples show a characteristic peak pattern.

The main prominent peak at Rt 50.4 can be assigned to physalien (**4**). All other identified carotene peaks as zeaxanthin (**1**) at Rt 11.1, β-cryptoxanthin (**2**) at Rt 23.0 and β-carotene (**3**) at Rt 37.7 are detectable in minor concentration only.

All carotenes show nearly the same charcteristic UV-spectra (Fig. 7) with maxima at 290, 450 and 478 nm.

The carotenoid pattern of both Fructus *Lycium barbarum* samples (Fig. 6a and 6b) differ in the quantitative composition of the single carotenoids. The same differences can be seen also in Fructus *Lycium chinense* samples (Fig. 6c and 6d). The differences are probably due to the climatic influences or cultivating conditions rather than to genetic characteristics.

Notes: A clear differentation of both Lycium species is only possible by using the random amplified polymorphic DNA-analytical method[32].

According to the Chinese Pharmacopoe 2005 the fruits should contain "not less than 1.8 % polysaccharides" (calc. as glucose) and "not less than 0.3 % betaine with reference to the dried drug".

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Cortex Mori radicis
Sangbaipi

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005

Official drug[1]: White Mulberry Root-bark is the dried root bark of *Morus alba* L. – Moraceae –

The root is collected in late autumn while leaf falling off and in early spring before germination, removed from the yellowish-brown coarse bark, cut longitudinally. The root bark is stripped off and dried in the sun.

Origin[2]: China, Indochina, Japan, Philippines

Descriptions of the drug[1]: Quilled, channelled or flat pieced, twisted, varying in length and width, 1 – 4 mm thick. Outer surface white or pale yellowish-white, relatively even, some with orange-yellow or brownish-yellow remains of scaly bark; inner surface yellowish-white or greyish-yellow, with fine longitudinal striations. Texture light and tenacious, strongly fibrous, uneasily broken, but easily stripped longitudinally, dusting on stripping. Odour, slight; taste, slightly sweet.

Pretreatment of the raw drug[1]: The drug is washed clean, softened briefly, cut into slivers and dried.

Cortex Mori (stir-baked with honey) (= Misangbaipi):
The refined honey should be diluted at first with a quantity of boiling water. Then it is added to the clean slivers and mixed well in a closed vessel until they are infused thoroughly. They are roasted in a pot with gentle heat until it is no more sticky to fingers, taken out and cooled. 25 kg of refined honey is used for each 100 kg of clean crude drug.

Medicinal use[2,3]: Hypertension, asthma, cough, inflammations

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of Cortex Mori according to Traditional Chinese Medicine[1,4]	
Taste:	sweet
Temperature:	cold
Channels entered:	orbis pulmonalis, orbis hepaticus, orbis lienalis
Effects (functions):	removes heat from the lung, relieves asthma, decongestant and induces diuresis
Symptoms and indications:	cough and asthma caused by heat in the lung; anasarca with oliguria, fever

Main constituents:

- prenylflavone- and flavanone-compounds:
mulberrin (kuwanone C)[5], cyclomulberrin[5], mulberrochromene (morusin)[5], cyclomulberrochromene (cyclomorusin)[5], mulberranol[5], oxydihydromorusin (morusinol)[5], kuwanon A[5], B[5], D – F[5], G (albanin F, moracenin B)[5], H (albanin G, moracenin A)[5], I – T[5], V – Z[5], kuwanol A[5], B[5], sanggenone A – Q[5], moracenin C[5], D[5], mulberrofuran A – Q[5], albanol B[5], albafuran A – C[5], mulberroside A[6], mulberroside C[7], moralbanone[7], eudraflavone B hydroperoxide[7], leachianone G[7], alpha-acetyl-amyrin[7], cudraflavone B[8], C[8]

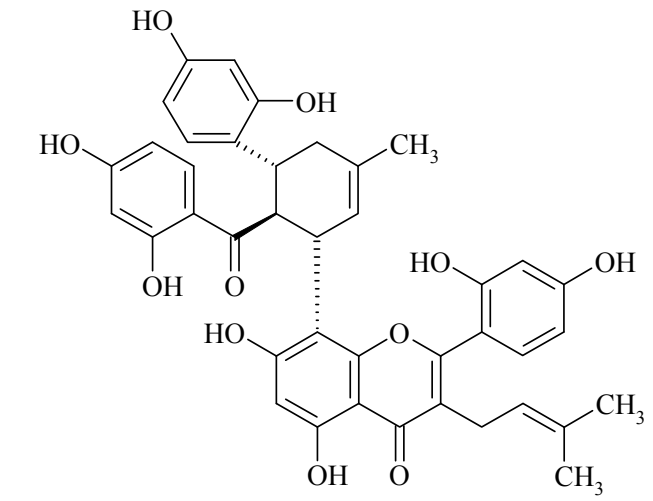
- phenylbenzofurane derivatives:
moracin A – M[5], dimoracin[5]

- other phenolic compounds:
umbelliferone[5], scopoletin[5], ethyl 2,4-dihydroxybenzoate[5], 5,7-dihydroxychromone[5], oxyresveratrol[6], 5,7-dihydroxy-coumarin-7-methylether[8]

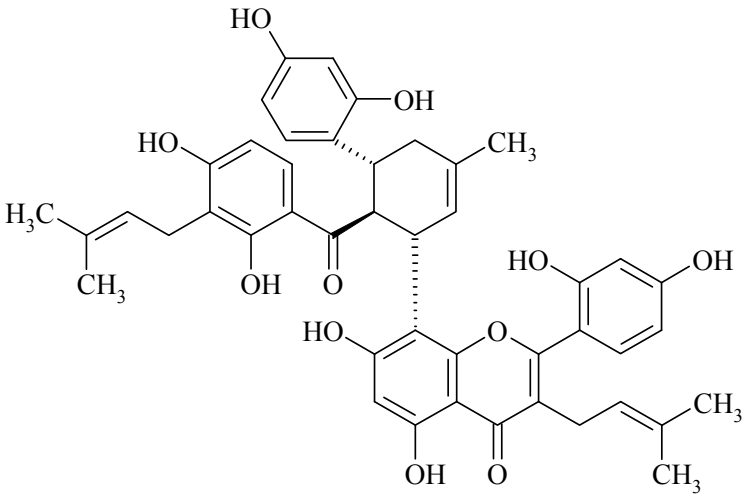
- alkaloids:
moranoline (piperidine alkaloid)[5], 1,4-dideoxy-1,4-imino-D-ribitol (pyrrolidine alkaloid)[9], (2R, 3R, 4R)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-N-propionamide[10]

- other constituents:
β-tocopherol[5], moran 20k[11], moran A[12]

Fig. 1: Formulae of the main compounds of Cortex Mori[5]



Kuwanon G



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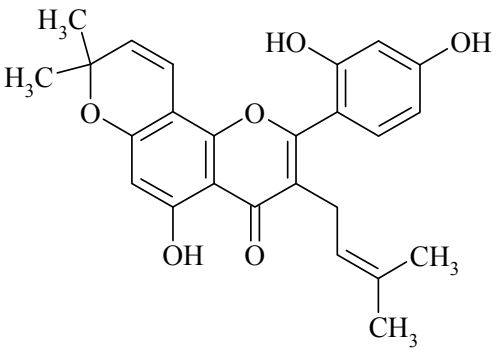
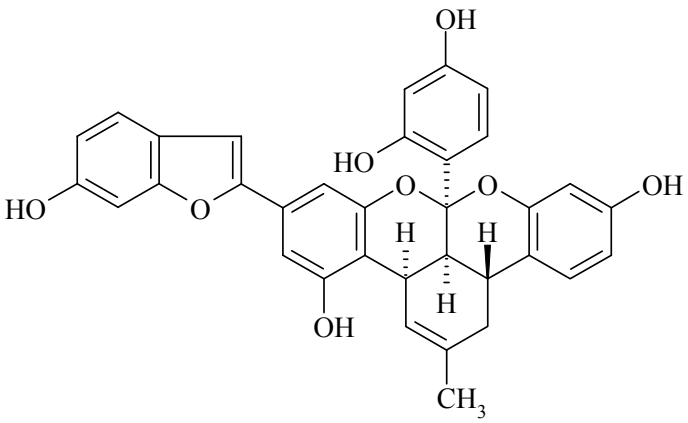


Morusin



Mulberrofuran G

Pharmacology:

- antitussive[5]
- anticonvulsive[5]
- antiviral[7]
- expectorant[13]
- antiinflammatory[6]
- antiphlogistic[13]
- hypotensive[5]
- antioxidative[6]
- antinephritis[14]
- analgesic[5]
- diuretic[5]
- antiedemic[5]
- sedative[5]
- antifungal[5]
- antibacterial[5]
- hepatoprotective[8]
- antidiabetic[11]
- cytotoxicity against tumor cells[13]
- cathartic[15]

TLC fingerprint analysis:

Drug samples		Origin
1	Mori cortex / *Morus alba* L.	province Sichuan, China
2	Mori cortex / *Morus alba* L.	province Guizhou, China
3	Mori cortex / *Morus alba* L.	Beijing market, China
4	Mori cortex / *Morus alba* L.	sample of commercial drug, China
5	Mori cortex / *Morus alba* L.	sample of commercial drug, Japan
6	Mori cortex / *Morus alba* L.	sample of commercial drug, China

Reference compounds of Figure 2a		**R*f***
T 1	kuwanon G	~ 0.25
T 2	kuwanon H	~ 0.45
T 3	Mulberrofuran G	~ 0.4
T 4	morusin	~ 0.73

Reference compounds of Figure 2b		**R*f***
T 3	mulberrofuran G	0.5
T 5	scopoletin	0.89

Reference compounds of Figure 2c		**R*f***
T 6	morin	0.95
T 7	chlorogenic acid	0.5

1. Thin layer chromatogram of an ethyl acetate-extract (see Figure 2a):

1) Extraction: 0.75 g of the powdered drug is extracted under reflux with 15 ml of hexane for 30 minutes. The extract is cooled, filtered and discarded. Afterwards the powdered drug is extracted under reflux with 15 ml ethyl acetate for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of isopropanol and used for TLC

2) Reference compounds: kuwanon G, H, morusin, mulberrofuran G:
each 1 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Mori radicis cortex extract: each 20 µl
reference compounds: each 10 µl

Solvent system: chloroform : methanol
8.5 1.5

Detection: Vanillin-sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I followed immediately with solution II. Then the plate is heated for 5 – 10 minutes at 105 °C and evaluated in VIS.

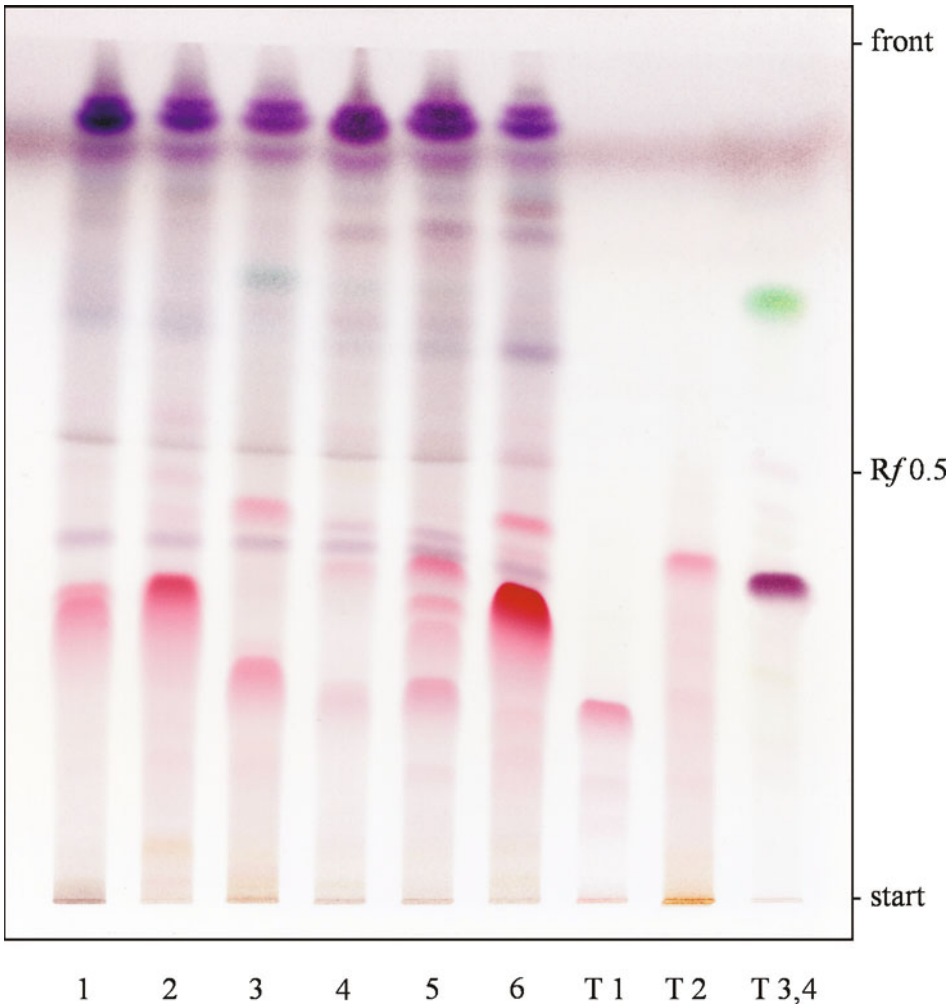


Fig. 2a: Thin layer chromatogram of the ethyl acetate extracts of Mori cortex sprayed with vanillin-sulphuric acid reagent (VIS)

4) Description of Figure 2a:

All 6 *Morus alba* samples show in the upper part of the TLC (R*f* = 0.5 – solvent front) a similar pattern of 7 – 8 violet zones with an additional weak turquoise zone of morusin (**T4**) at R*f* ~ 0.73, only well visible in sample 3. The lower R*f*-range between the start and R*f* ~ 0.5 is characterized by several red zones which can be suggested to derive from prenylflavones. One of them at R*f* = 0.35, especially prominent in sample 1, 2 and 6, could be not assigned. The red zone at R*f* ~ 0.25 and ~ 0.45 in samples 3, 4 and 5 could be identified as kuwanon G (**T1**) and H (**T2**) respectively. A small violet zone at R*f* = 0.4 visible in all samples could be identified as mulberrofuran G (**T3**).

2. Thin layer chromatogram of a MeOH-extract (see Figure 2b):

1) Extraction: 1.5 g of the powdered drug are extracted under reflux with 25 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol and used for TLC.

2) Reference compounds: scopoletin, mulberrofuran G:
each 1 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Mori radicis cortex extracts: each 20 µl
reference compounds: each 10 µl

Solvent system: chloroform : methanol
8.5 1.5

Detection: Natural products-polyethylene glycol reagent (NP/PEG):

I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol

II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. The evaluation is carried out in UV 365 nm.

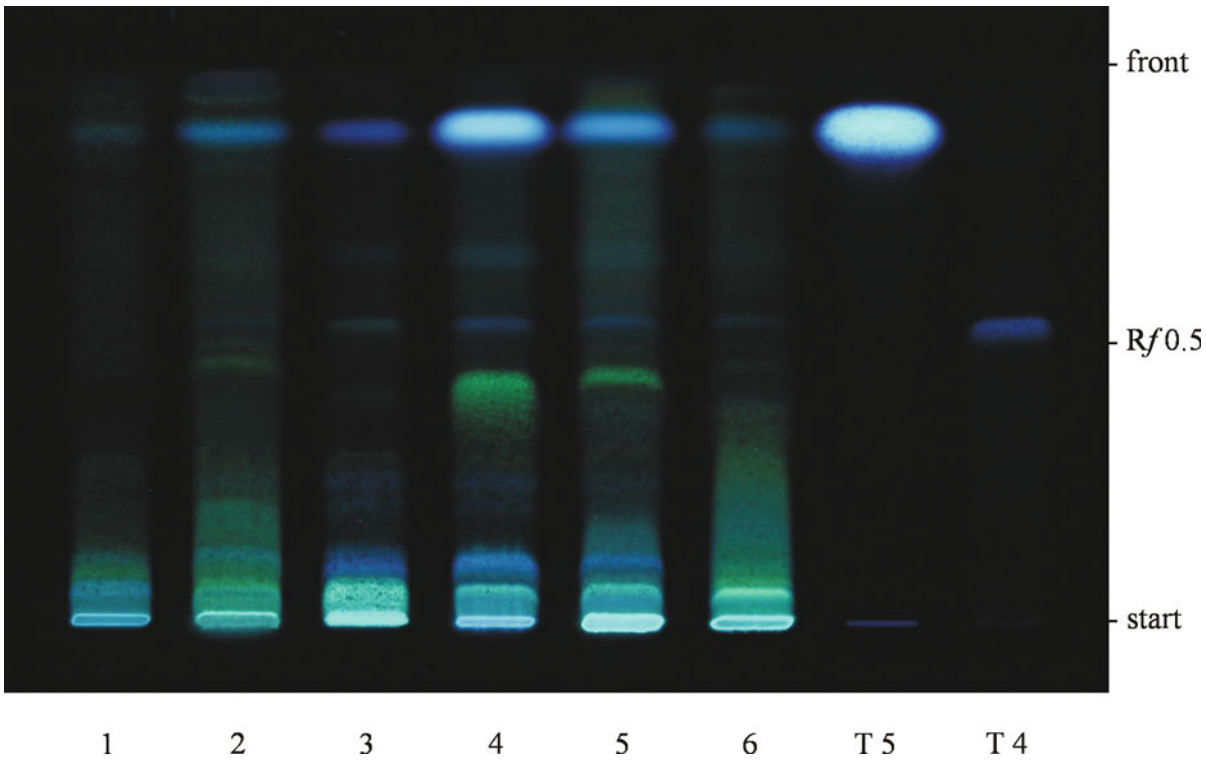


Fig. 2b: Thin layer chromatogram of the methanol extracts of Mori cortex sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

4) Description of Figure 2b:

The different *Morus alba* samples show from the start up to the solvent front 8 – 9 turquoise-blue fluorescent zones with scopoletin at R*f* = 0.89 and mulberrofuran G at 0.5. Most of the detected zones especially from start to R*f* ~ 0.4 derive from prenylated flavones and flavanones described for *Morus alba*.

3. Thin layer chromatogram of flavones (see Figure 2c):

1) Extraction: 1.5 g of the powdered drug are extracted under reflux with 25 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol and used for TLC.

2) Reference compounds: morin, chlorogenic acid:
each 0.5 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Mori radicis cortex extracts: each 10 µl
reference compounds: each 5 µl

Solvent system:

ethyl acetate	:	formic acid	:	glacial acetic acid	:	water
100		11		11		26

Detection: Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol
II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. After 15 minutes the evaluation is carried out in UV 365 nm.

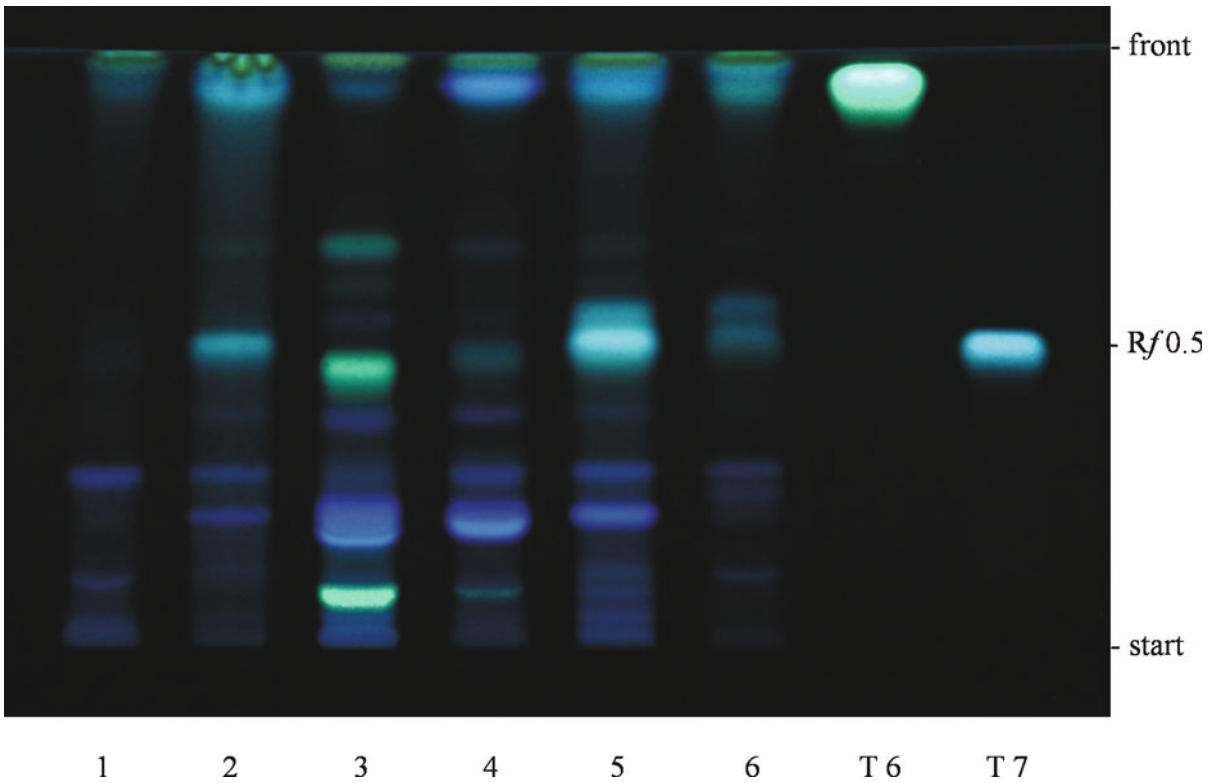


Fig. 2c: Thin layer chromatogram of the methanol extracts of Mori cortex sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

4) Description of Figure 2c:

The TLC sprayed with natural products-polyethylenglycol reagent shows well separated ~10 deep blue or turquoise fluorescent zones with morin (**T6**) and chlorogenic acid (**T7**) at R*f* = 0.95 and 0.5 respectively. The violet zones between chlorogenic acid and the start originate from prenylflavonoids.

HPLC-fingerprint analysis:

1) Sample preparation:	0.75 g of the powdered drug is extracted under reflux with 15 ml of hexane for 30 min. The extract is cooled, filtered and discarded. Afterwards the powdered drug is extracted under reflux with 15 ml ethyl acetate for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of isopropanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.
2) Injection volume:	Mori radicis cortex extract: 20.0 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 /1 l water B: acetonitrile
Gradient:	30 – 70 % B in 60 minutes 70 – 95 % B in 5 minutes 95 % B in 7 minutes total runtime: 72 minutes
Flow:	0.6 ml/min.
Detection:	280 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	30.7	unknown
2	33.9	mulberrofuran G
3	35.6	unknown
4	36.2	unknown
5	36.4	unknown
6	39.6	kuwanon G
7	40.8	unknown
8	43.5	unknown
9	44.5	kuwanon H
10	52.5	morusin
11	56.6	unknown

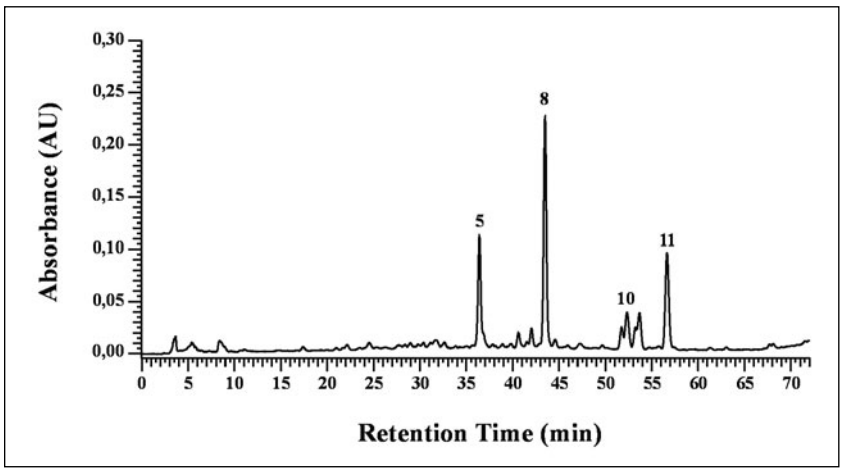


Fig. 3a: HPLC-fingerprint chromatogram of the ethyl acetate extract of Mori radicis cortex (sample 1, province Sichuan)

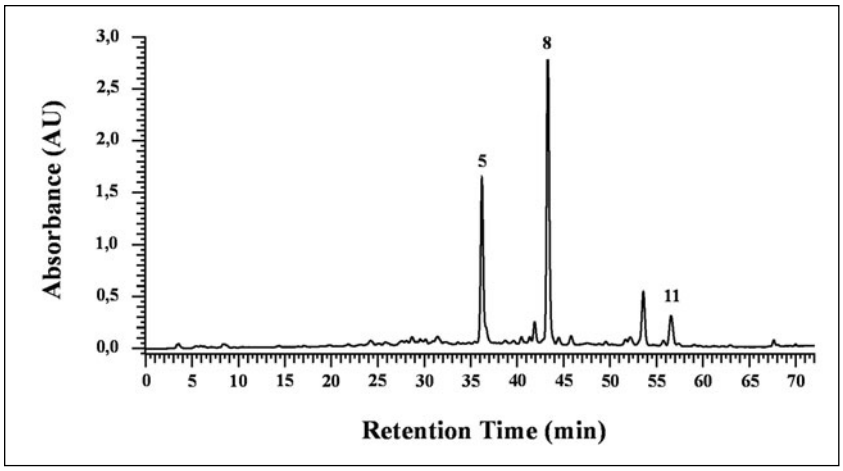


Fig. 3b: HPLC-fingerprint chromatogram of the ethyl acetate extract of Mori radicis cortex (sample 2, province Guizhou)

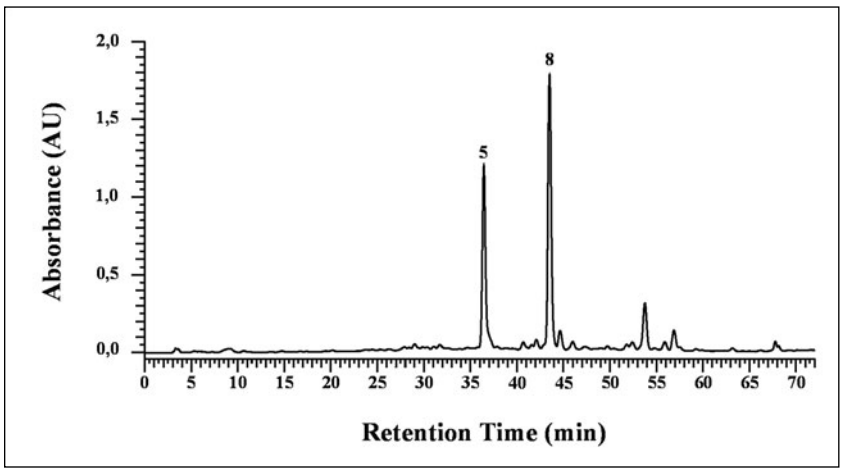


Fig. 3c: HPLC-fingerprint chromatogram of the ethyl acetate extract of Mori radicis cortex (sample 6, commercial drug of unknown origin)

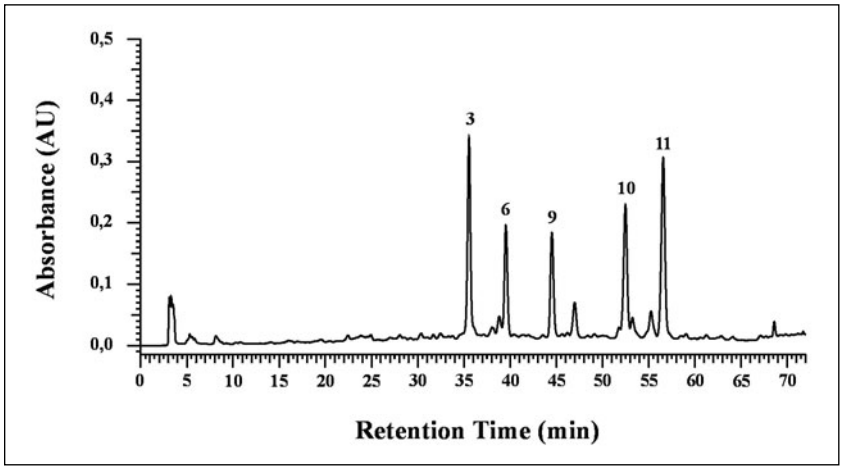


Fig. 3d: HPLC-fingerprint chromatogram of the ethyl acetate extract of Mori radicis cortex (sample 3, Beijing market)

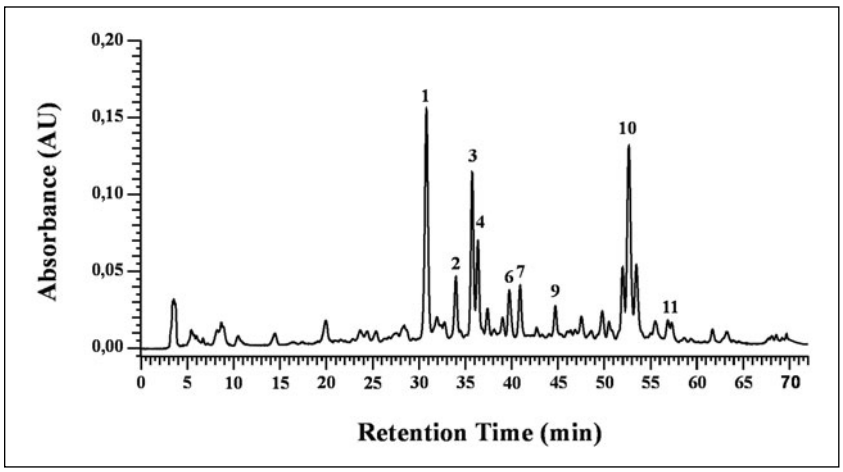


Fig. 3e: HPLC-fingerprint chromatogram of the ethyl acetate extract of Mori radicis cortex (sample 4, commercial drug of unknown origin)

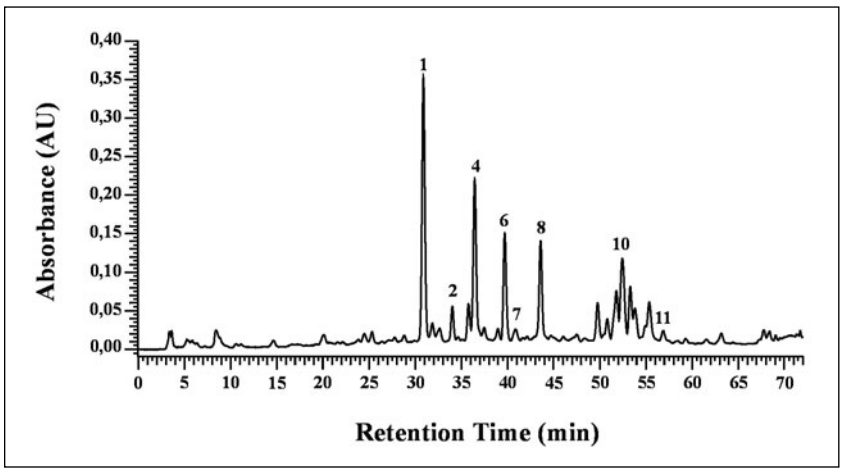


Fig. 3f: HPLC-fingerprint chromatogram of the ethyl acetate extract of Mori radicis cortex (sample 5, commercial drug of unknown origin)

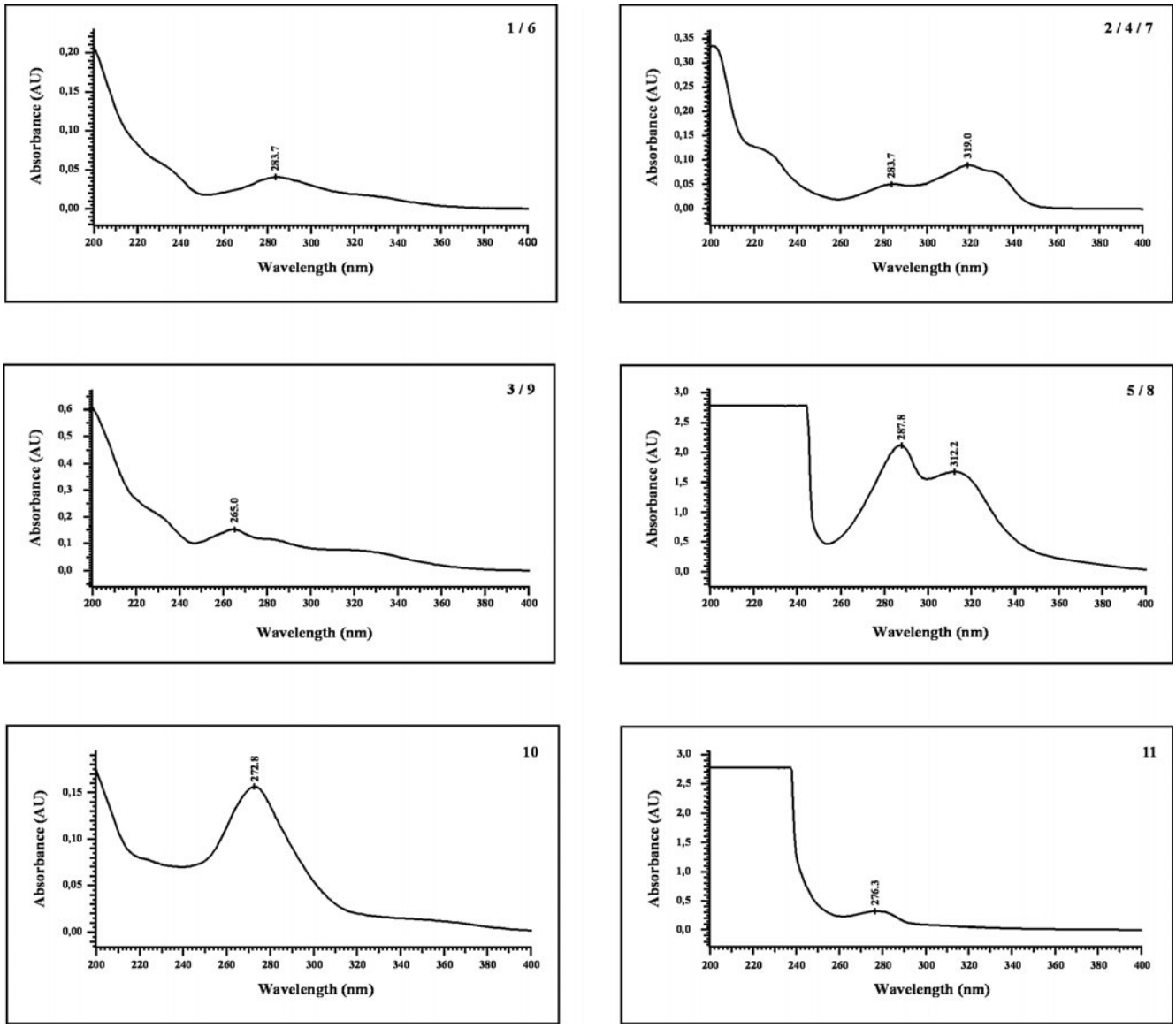


Fig. 4: UV-spectra of the main peaks of the ethyl acetate extracts of Mori radicis cortex

4) Description of the HPLC of Figure 3a–f:
Because of the great number of prenylflavonoids reported in the literature for Morus root bark (> 70) no homogeneous HPLC-peak pattern of the samples collected could be expected. Among the six samples investigated, three types of peak pattern could be evaluated.

HPLC of Figure 3a–c (samples 1, 2 and 6):
The samples of the province Sichuan (sample 1), the province Guizhou (sample 2) and sample 6 of unknown origin show a very similar peak pattern with peaks at Rt = 36.4 (**5**) and 43.5 (**8**). There is a relatively good correlation of this HPLC peak pattern with the TLC-spot pattern of Figure 2a. In comparison of the graphs it could be suggested that the HPLC-peak at Rt = 43.5 of sample 1, 2 and 6 corresponds with the strong red spot on TLC (Fig. 2a) at R*f* = 0.35.

HPLC of Figure 3d (sample 3):
The peak pattern of sample 3 is more complex. It consists of 5 major peaks at Rt = 35.6 (**3**), 39.6 (**6**), 44.5 (**9**), 52.5 (**10**) and 56.6 (**11**). The peak at 39.6 could be identified as kuwanon G, the peak at 44.5 as kuwanon H and the peak with a Rt of 52.5 might be originate from morusin. This peak pattern corresponds with the red zone pattern of TLC of Figure 2a.

HPLC of Figure 3e, f (samples 4, 5):
Both samples show a great peak similarity. In both samples kuwanon G at Rt = 39.6 (**6**), mulberrofuran G at Rt = 33.9 (**2**) and morusin at Rt = 52.5 (**10**) could be identified. Kuwanon H at Rt = 44.5 (**9**) could be found only in sample 4.

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Folium Mori
Sangye

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2000/2005

Official drug[1]: Mulberry leaf is the dried leaf of *Morus alba* L.
– Moraceae –
The drug is collected in early frost season, removed from foreign matter and dried in the sun.

Origin: All parts of China, whole Asia and in the western hemisphere

Descriptions of the drug[1]: Mostly crumpled and broken. When whole, petioled, ovate or broadly ovate, 8 – 15 cm long, 7 – 13 cm wide; apex acuminate, base truncate, round or cordate, margin dentate or obtuse-dentate, some irregularly partite. Upper surface yellowish-green or pale yellowish-brown, some with small warty protrudings; lower surface relatively light in colour veins prominent, lateral veins reticulate, sparsely pubescent on the veins, cluster of hairs occurring at the vein base. Texture fragile. Odour, slight; taste, weak, slightly bitter and astringent.

Pretreatment of the raw drug[1]: Foreign matters are eliminated, rubbed to break, the petioles removed and the dust sifted off.

Medicinal use[2,3]: fever, headache, sore throat in connection with cold, externally also for skin- and eye-diseases

Effects and indications of Folium Mori according to Traditional Chinese Medicine[1,4]	
Taste:	bitter and sweet in flavor
Temperature:	cold in property
Channels entered:	orbis pulmonalis, orbis hepaticus
Effects (functions):	dispels wind-heat and removes heat from the lung, subdues hyperactivity of the liver and improves eyesight
Symptoms and indications:	upper respiratory infection, heat in the lung with dry cough; dizziness, headache, inflammation of the eye, blurred vision

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Main constituents:

- flavones:
quercetin[5], rutin[5], quercetin-3-O-glucoside (= isoquercitrin)[5], quercetin 3-(6-malonyl)glucoside (6), quercetin-3-O-(6''-O-acetyl)-β-D-glucopyranoside[7], quercetin-3,7-di-O-β-D-glucopyranoside[7], quercetin-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside[7], kaempferol-3-O-(6''-O-acetyl)-β-D-glucopyranoside[7], kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside[7], astragalin[8]

- sterols/steroids:
β-sitosterol[5], campesterol[5], β-sitosterolglycoside[5], β-ecdysone[5], inokosterone[5]

- other constituents:
moraprenol-11[5], bombiprenone[5], scopolin[8], skimmin[8], roseoside II[8], benzyl D-glucopyranoside[8], mulberroside F[9], 1-deoxynojirimycin[10]

- essential oil constituents[5]:
o-, m-, p-cresol, guaiacol, eugenol, methyl salicylate, isobutanol, isoamylalcohol, isoamylacetate, acetophenone, acetic-, propionic-, butyric-, isobutyric-, isovaleric-, caproic-, isocaproic- and lactic-acids, benzaldehyde, phenylacetaldehyde

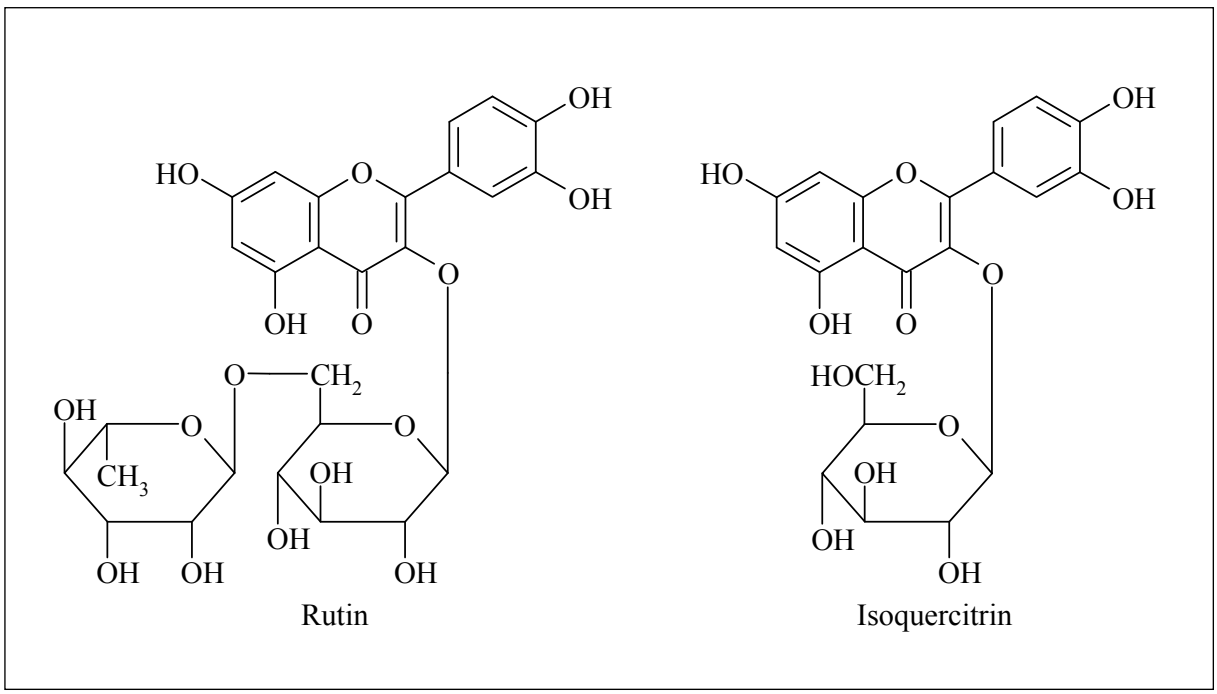

Fig. 1: Formulae of the main compounds of Folium Mori[5]

Pharmacology:
- antiatherogenic[6]
- antioxidative[6]
- antihypertonic[8]
- antiatherosclerotic[8]
- antihyperglycemic[11]
- antibacterial activity[12]

TLC fingerprint analysis

1) Extraction: To 1.0 g of the powdered drug 15 ml of petroleum ether (60 – 90 °C) are added, heated under reflux for 30 minutes and cooled. The petroleum ether solution is discarded. The residue is evaporated to dryness, 15 ml of ethanol are added, heated under reflux for 30 minutes, cooled and filtered. The filtrate is concentrated to about 2 ml and used for TLC.

2) Reference compounds: chlorogenic acid, rutin, isoquercitrin, scopoletin:
1 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Mori folium extracts: each 5 µl
reference compounds: 5 – 10 µl

Solvent system:

ethyl acetate	formic acid	acetic acid	water
50	5.5	5.5	5

Detection: Detection of flavones:
Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol

II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. After 30 minutes the evaluation is carried out in UV 365 nm.

Drug samples		Origin
1	Mori folium / *Morus alba* L.	Beijing market, China
2	Mori folium / *Morus alba* L.	province Guangxi, China
3	Mori folium / *Morus alba* L.	province Shandong, China
4	Mori folium / *Morus alba* L.	sample of commercial drug, China
5	Mori folium / *Morus alba* L.	sample of commercial drug, China
6	Mori folium / *Morus alba* L.	sample of commercial drug, China
7	Mori folium / *Morus alba* L.	sample of commercial drug, China

Reference compounds		R*f*
T 1	rutin	0.35
T 2	quercetin-3-glucoside (= isoquercitrin)	0.67
T 3	scopoletin	0.93
T 4	chlorogenic acid	0.50

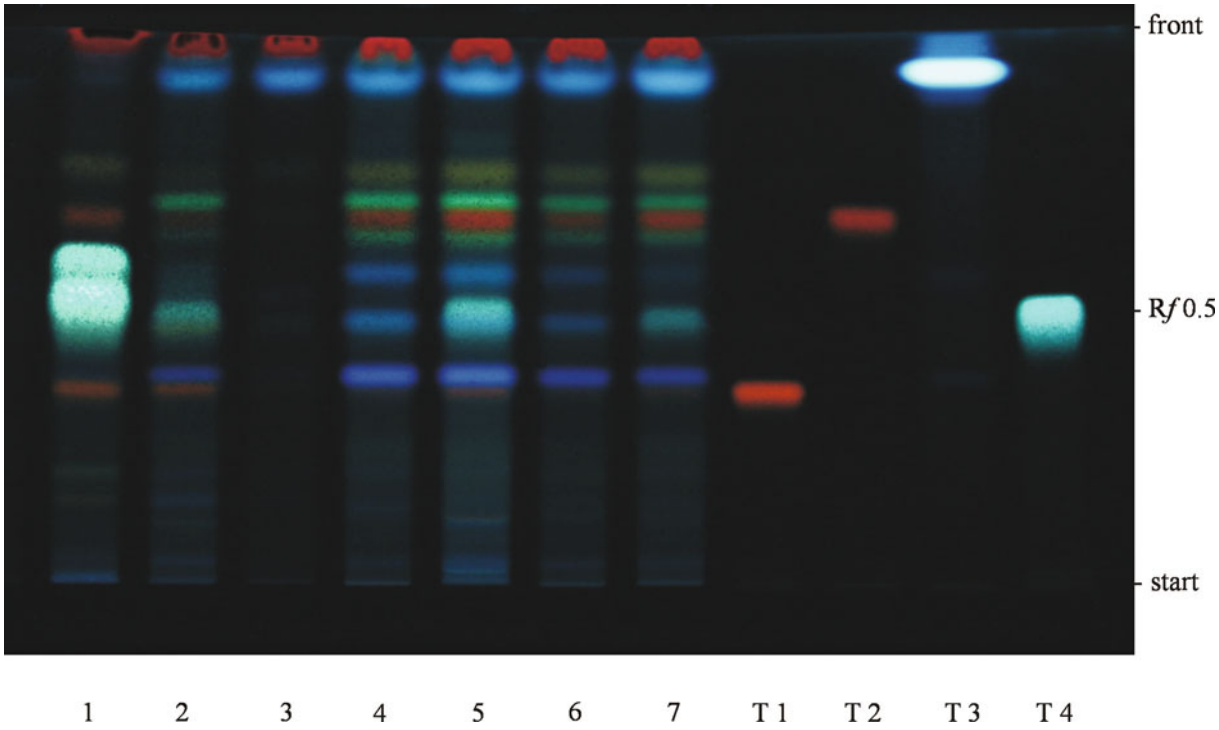

Fig. 2: Thin layer chromatogram of the ethanolic extracts of Mori folium sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

4) Description of Figure 2:

The samples 1 – 7 except those from the Beijing market, the province of Guangxi and Shandong show a relatively homogeneous pattern of about eight mainly blue and green fluoreszent zones.

With the exception of sample 3 all samples show quercetin-3-glucoside as orange red fluorescent zone at R*f* = 0.67. In sample 3 only the blue fluorescent zone of scopoletin at R*f* = 0.93 and chlorophyll as red fluorescent zone at R*f* = 0.99 are visible.

Scopoletin can be found in all samples at R*f* = 0.93. The violet blue fluoreszent zone at R*f* = 0.37 might be scopolin.

Chlorogenic acid can be detected in samples 1, 2 and 5 as turquoise fluorescent zone at R*f* = 0.50. In these samples also the orange red fluorescent zone of rutin at R*f* = 0.35 can be seen.

HPLC-fingerprint analysis:

1) Sample preparation: To 1.0 g of the powdered drug 15 ml of petroleum ether (60 – 90 °C) are added, heated under reflux for 30 minutes and cooled. The petroleum ether solution is discarded. The residue is evaporated to dryness, 15 ml of ethanol are added, heated under reflux for 30 minutes, cooled and filtered. The filtrate is concentrated to about 2 ml, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Mori folium extract: 10.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 /1l water B: acetonitrile
Gradient:	0 – 40 % B in 33 minutes 40 – 60 % B in 12 minutes 60 – 100 % B in 5 minutes 100 % B in 20 minutes total runtime: 70 minutes
Flow:	0.6 ml/min.
Detection:	260 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	21.5	chlorogenic acid
2	27.8	rutin
3	29.5	quercetin-3-glucoside
4	31.2	quercetin-acetylglucoside?
5	43.7	not identified
6	44.3	not identified
7	47.3	not identified
8	57.9	not identified

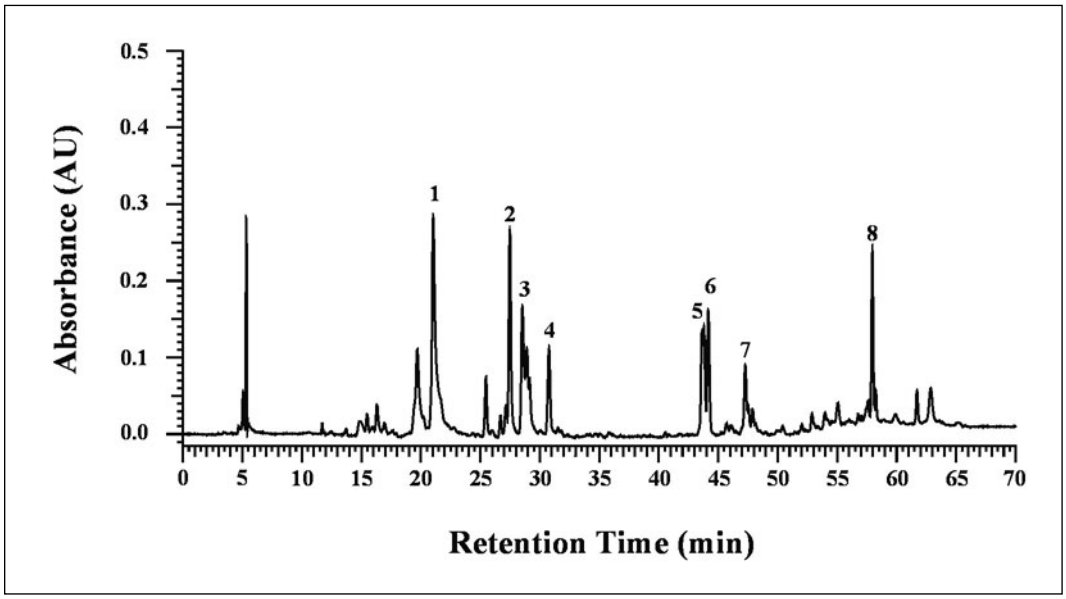

Fig. 3a: HPLC-fingerprint chromatogram of the ethanol extract of Mori folium (sample 1)

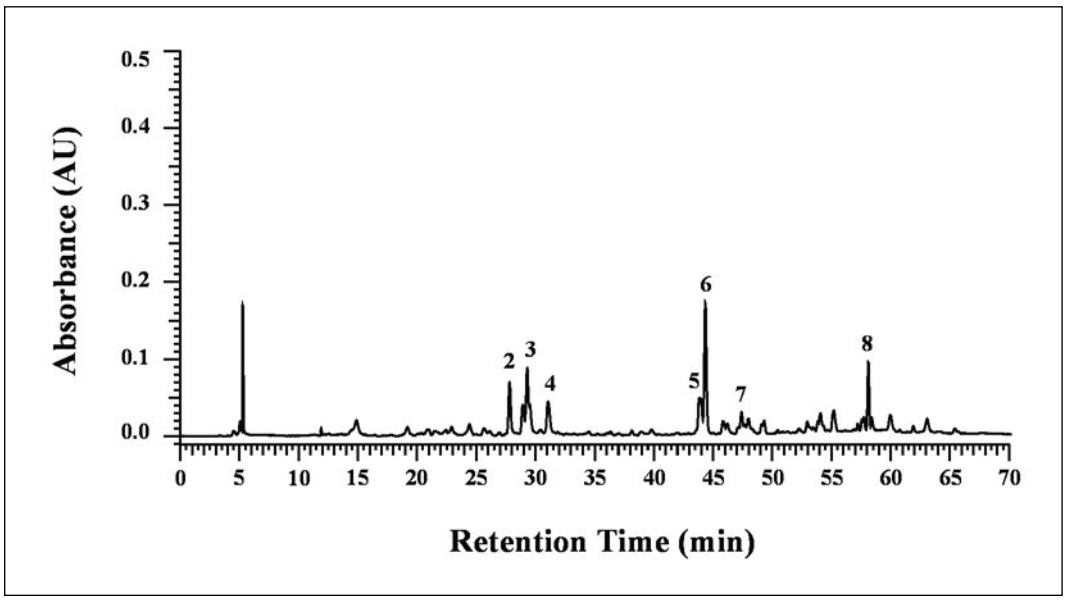

Fig. 3b: HPLC-fingerprint chromatogram of the ethanol extract of Mori folium (sample 3)

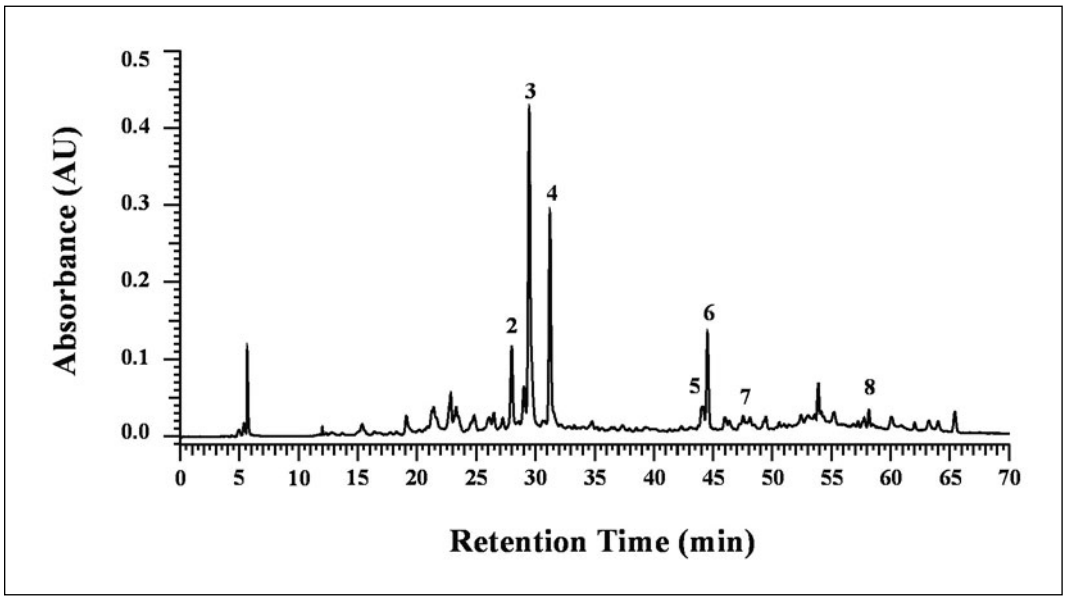

Fig. 3c: HPLC-fingerprint chromatogram of the ethanol extract of Mori folium (sample 7)

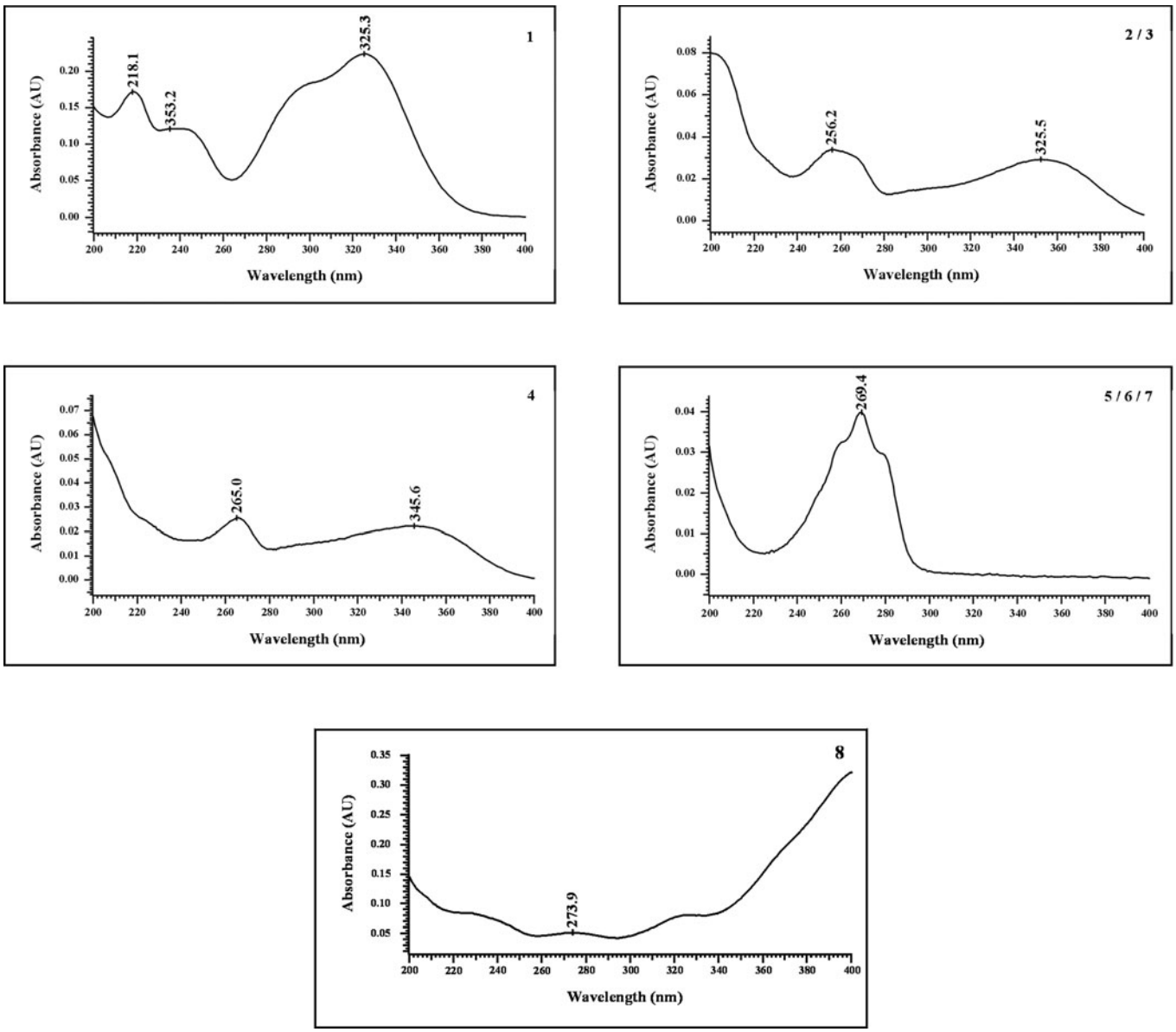

Fig. 4: UV-spectra of the main peaks of the ethanol extracts of Mori folium

4) Description of the HPLC of Figure 3a – c:

The HPLC chromatograms of Figure 3a – c show a qualitatively similar but quantitatively different peak pattern.

The most prominent peaks are rutin and quercetin-3-glucoside at Rt = 27.8 (**2**) and Rt = 29.5 (**3**) respectively. Only in sample 1 chlorogenic acid can be found at Rt = 21.5 (**1**). At Rt = 31.2 (**4**) another major peak occurs which could not be identified, but has an UV-spectrum with maxima at 265.0 and 345.6 nm, characteristic for flavonoids. Between Rt = 43.7 and 47.3 appear various peaks (5 – 7) with the same UV-spectrum having a maximum at 269.4 nm and two inflexions at 260 and 280 nm respectively. The peak 8 shows an UV-spectrum with a major maximum at ~ 400 nm, characteristic for polyenic compounds (carotenoids?).

Note: The Chinese Pharmacopoeia 2005[(1)] demands for Folium Mori not less than 0.10 % of rutin, calculated with reference to the dried drug.

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Rhizoma Cimicifugae
Shengma

Pharmacopoeia[1]:	Pharmacopoeia of the People's Republic of China, English Edition, 2005
Official drug[1]:	Largetrifolious Bugbane Rhizome is the dried rhizome of *Cimicifuga heracleifolia* Kom., *Cimicifuga dahurica* (Turcz.) Maxim. or *Cimcifuga foetida* L. (Fam. Ranunculaceae). The drug is collected in autumn, removed from soil and fibrous root, and then dried in the sun.
Origin[2]:	Provinces Jilin, Liaonling, Heilongjiang, Hunan, Shanxi (China)
Descriptions of the drug[1]:	Irregular long pieces, frequently branched, nodular, 10 – 20 cm long, 2 – 4 cm in diameter. Externally blackish-brown or brown, rough, with remains of many wiry fibrous roots, the upper part showing several round and hollow remains of stems, the inner walls of the hole with reticulate furrows, and the lower part lumpy, with fibrous root scars. Texture light and hard, uneasily broken, fracture uneven, cracked, fibrous, yellowish-green or yellowish-white. Odour, slight; taste, slightly bitter and adstringent.
Pretreatment of the raw drug[1]:	Foreign matters are eliminated, soaked briefly, washed clean, softened thoroughly, cut into thick slices, and dried.
Medicinal use[3]:	Used as antipyretic, analgesic and anti-inflammatory drug for the treatment of febril diseases (e.g. influenza infection), inflammations of the upper respiratory tract and exanthemas. Note: Interestingly the rhizome of the species *Cimicifuga racemosa*, used in Europe and other western countries contrary to the Chinese concept primarily for the treatment of dysmenorrhoic, postmenopausal disorders and to reduce the risk of developing osteoporosis, possesses a very similar composition of chemical constituents.

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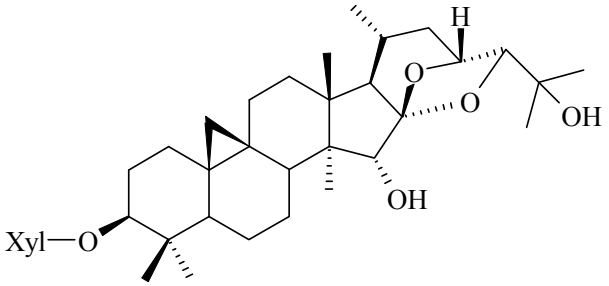
Effects and indications of Rhizoma Cimicifugae according to Traditional Chinese Medicine[1,4]	
Taste:	acrid, weakly sweet
Temperature:	neutral with cold tendency
Channels entered:	orbis pulmonalis, orbis lienalis, orbis stomachi, orbis intestini crassi
Effects (functions):	Induces perspiration and promotes eruption, removes toxic heat and elevates yang
Symptoms and indications:	Headache caused by wind-heat, toothache, ulcers in the mouth, sore throat; measles with inadequate eruptions and other eruptive febrile diseases; prolapse of the rectum or the uterus

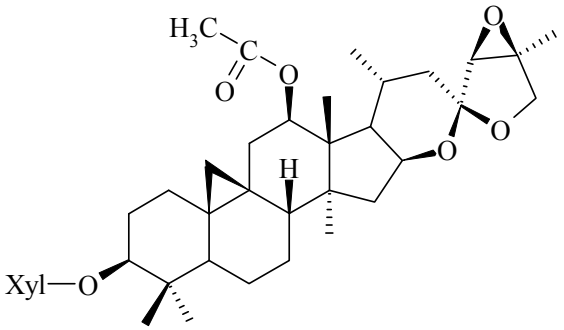
Main constituents:

- **triterpenoids, triterpene glycosides:**
  actein[5], 23-epi-26-deoxyactein[5], 7,8-didehydro-27-deoxyactein[6], cimigenol-3-O-xyloside (= cimicifugoside)[5,6], cimigenol[6], 23-O-acetylshengmanol-3-O-xyloside[5], 24-O-acetylhydroshengmanol-3-O-xyloside[5], cimicifugoside H-1[5,7,8], H-2[7,8], H-3[7], H-4[7], H-5[7,8], H-6[7], 7,8-didehydrocimigenol-3-O-xyloside[5], dahurinol[9,10], dehydroxydahurinol[10], isodahurinol[10], 25-O-methylisodahurinol[10], shengmanol-3-O-D-xylopyranoside[9], cimicifugamide[11], 2'-O-acetylactein[12], 2'-O-acetyl-27-deoxyactein[12], 15-hydroxycimicidol-3-O-xyloside[12], shengmanol xyloside[13], cimicifugenol[14], 25-O-acetylcimigenoside[15], 25-O-methylcimigenoside[15], cycloartenol-3-O-mono- and triglycosides[16,17], cycloartenol-3,15-O-diglycosides[18,19]
- **caffeic acid and tartaric acid derivatives:**
  ferulic acid[5], isoferulic acid[5], caffeic acid[5], caffeic acid methyl ester[20], 4-O-acetyl-caffeic acid[20], sinapic acid[20], cimicifugic acid A[5,21], B[5,21], C[21], D[21], E[21], F[21], fukinolic acid[5], fukiic acid[22], 2-feruloyl piscidic acid[5], 2-isoferuloyl piscidic acid[5], sodium ferulate (= 3-methoxy-4-hydroxy-cinamate)[23]
- furochromones:
  cimifugin[5], cimifugin-glucoside[5], visamminol[9], visnagin[9], norvisnagin[9]
- other constituents:
  3-(3'-methyl-2'-butenylidene)-2-indolinone[9], esculetin[20]

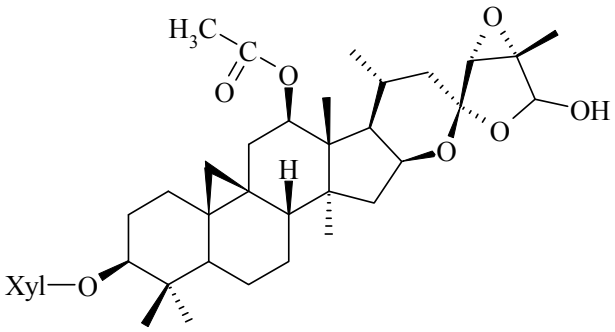
Note: The presence of the isoflavonoid formononetin reported for *C. racemosa* and some other *C.* species, could not be confirmed or detected only in minor quantities[24,25]



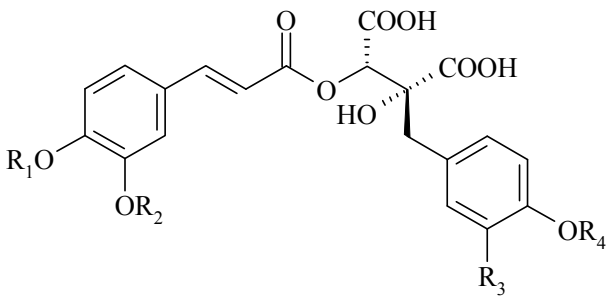
cimigenol-3-O-xyloside

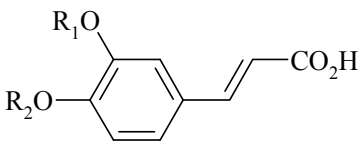


23-epi-26-deoxyactein



actein





cimicifugic acid A: $R_1 = H$, $R_2 = Me$, $R_3 = OH$, $R_4 = H$
cimicifugic acid B: $R_1 = Me$, $R_2 = H$, $R_3 = OH$, $R_4 = H$
fukinolic acid: $R_1 = R_2 = H$, $R_3 = OH$, $R_4 = H$

ferulic acid: $R_1 = CH_3$, $R_2 = H$
isoferulic acid: $R_1 = H$, $R_2 = CH_3$
caffeic acid: $R_1, R_2 = H$

Fig. 1: Formulae of the main compounds of Cimicifugae rhizoma[5]

Pharmacology:

- antibiotic[2]
- antihypertensive[2]
- antipyretic[5]
- analgesic[5]
- anti-inflammatory[5]
- hepatoprotective[9]
- antihyperlipidemic activity[9]
- spasmolytic activity[9]
- immunosuppressive activity[16]
- vasoactive effect (cimicifugic acids C,D and fukinolic acid)[22]
- antithrombotic[23]
- cardiovascular and cerebrovascular activity[23]
- antitumoral[26,27]
- antihyperglycemic activity[28]
- antimycotic[3]
- antiprotozoal activity (malaria)[29]
- sedative[30]

TLC fingerprint analysis:

	Drug sample	Origin
1	Cimicifugae rhizome/*Cimicifuga heracleifolia*	sample of commercial drug, China
2	Cimicifugae rhizome/*Cimicifuga heracleifolia*	sample of commercial drug, China
3	Cimicifugae rhizome/*Cimicifuga heracleifolia*	sample of commercial drug, China
4	Cimicifugae rhizome/*Cimicifuga heracleifolia*	sample of commercial drug, China
5	Cimicifugae rhizome/*Cimicifuga dahurica*	province Jilin, China
6	Cimicifugae rhizome/*Cimicifuga dahurica*	province Jilin, China
7	Cimicifugae rhizome/*Cimicifuga dahurica*	province Liaoning, China
8	Cimicifugae rhizome/*Cimicifuga foetida*	province Hebei, China
9	Cimicifugae rhizome/*Cimicifuga foetida*	province Hebei, China
10	Cimicifugae rhizome/*Cimicifuga racemosa*	sample of commercial drug, Yugoslavia

Reference compounds of Figure 2a		**R*f***
T 1	cimigenol-3-O-xyloside	~ 0.51
T 2	actein	~ 0.48
T 3	23-epi-26-deoxyactein	~ 0.40

Reference compounds of Figure 2b		**R*f***
T 4	ferulic acid	0.67
T 5	isoferulic acid	0.66
T 6	caffeic acid	0.62

1. Thin layer chromatography of triterpenoids (see Figure 2a):

1) Extraction: 0.5 g of the powdered drug is ultrasonicated with 10 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. To the residue 10 ml ethyl acetate and 10 ml water are added and given into a separation funnel. The ethyl acetate phase is separated and evaporated to dryness. The residue is dissolved in 1 ml of methanol and used for TLC.

2) Reference compounds: cimigenol-3-O-xyloside, actein, 23-epi-26-deoxyactein: 0.5 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Cimicifugae rhizoma extract: each 10 µl
reference compounds: each 10 µl

Solvent system: ethyl formate : toluene : formic acid
50 50 15

The plate is developed in a glass chamber, saturated for 30 minutes with the solvent mixture before chromatography.

Detection: Anisaldehyde-sulphuric acid reagent:

0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The plate is sprayed with the mixture, heated at 105 °C for 5 – 10 minutes and evaluated in VIS.

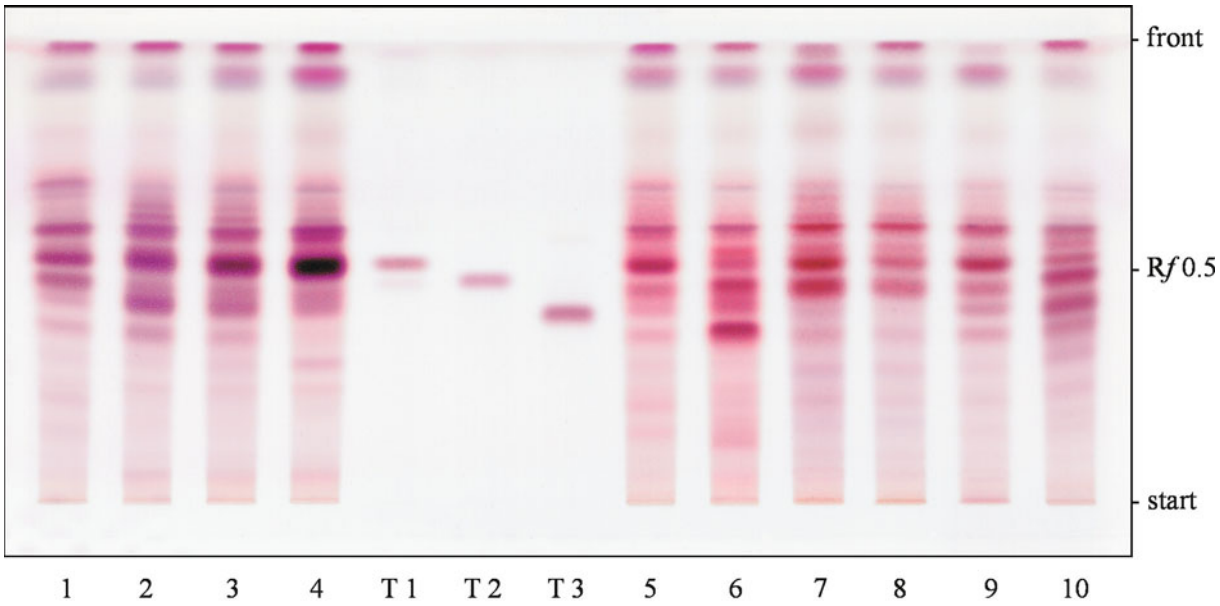


Fig. 2a: Thin layer chromatogram of the methanol extracts of Cimicifugae rhizoma sprayed with Anisaldehyde-suphuric acid reagent (VIS)

4) Description of Figure 2a:

All *Cimicifuga* samples show a very similar pattern of 6 – 7 violet brown bands in the R*f*-range of 0.4 – 0.75 and 2 – 3 additional bands of the same colour directly below the solvent front.

In the centre of the first R*f*-range appear the triterpenoids cimigenol-3-O-xyloside (**T 1**), actein (**T 2**) and 23-epi-26-deoxyactein (**T 3**). Between the samples of *Cimicifuga dahurica* (5,6,7), *C. foetida* (8,9) and those from the Chinese drug market (1–4) only quantitative differences can be noticed. Interestingly *Cimcifuga racemosa* although used in western countries for another indication (see Note "Medicinal use"), shows a quite similar band pattern as the *Cimicifuga* species of the Chinese Pharmacopoeia 2005.

2. Thin layer chromatography of caffeic acid and tartaric acid derivatives (see Figure 2b):

1) Extraction: 0.5 g of the powdered drug is ultrasonicated with 10 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol and used for TLC.

2) Reference compounds: ferulic acid, isoferulic acid, caffeic acid:
1 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Cimicifugae rhizoma extracts: each 10 µl
reference compounds: each 10 µl

Solvent system:	ethyl formate : toluene : formic acid 50 50 15
	The plate is developed in a glass chamber, saturated for 30 minutes with the solvent mixture
Detection:	UV 365 nm

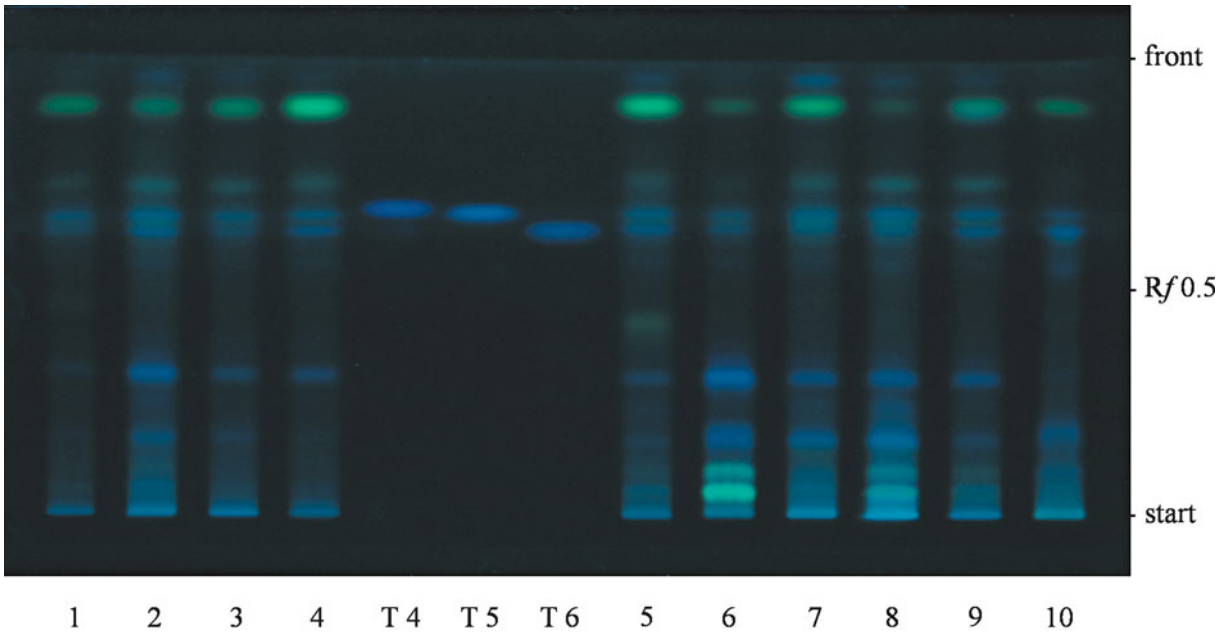


Fig. 2b: Thin layer chromatogram of the methanol extracts of Cimicifugae rhizoma (UV 365 mm)

4) Description of Figure 2b:

The TLC-fingerprint of all *Cimicifuga* samples is characterized by a green fluorescent band at R*f* = 0.9 and 2 – 3 blue violet fluorescent bands in the R*f*-range 0.6 – 0.7, identified as ferulic acid (**T 4**), isoferulic acid (**T 5**) and caffeic acid (**T 6**). In the lower R*f*-range between R*f* = 0.05 and R*f* = 0.35 appear in low concentration further blue violet fluorescent bands which may derive from cimicifugic acids A – E, fukinolic acid or fukiic acid. They are present in all three offical *Cimicifuga* species. *Cimicifuga racemosa* (10) shows a deviating quantitative pattern of acids.

Note: Chromatographic fingerprints of C. racemosae rhizoma and other *Cimicifuga* species are published in the Monograph Black Cohosh (Rhizoma Acteae racemosae synonym to *C. racemosa*) of the American Herbal Pharmacopoeia 2002[31]

HPLC-fingerprint analysis:

1) Sample preparation: 0.5 g of the powdered drug is ultrasonicated with 10 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. To the residue 10 ml ethyl acetate and 10 ml water are added and given into a separation funnel. The ethyl acetate phase is separated and evaporated to dryness. The residue is dissolved in 1 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Cimcifugae rhizoma extract: 50.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4/1 l water (Millipore Ultra Clear UV plus® filtered) + H_3PO_4 85 % (pH = 3.0) (Merck) B: acetonitrile (VWR)
Gradient:	20 – 50 % B in 40 minutes 50 – 100 % B in 25 minutes 100 % B in 7 minutes total runtime: 72 minutes
Flow:	1.0 ml/min.
Detection:	205 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	7.3	unknown
2	10.9	ferulic acid/isoferulic acid
3	58.6	cimigenol-3-O-xyloside
4	59.9	actein/23-epi-26-deoxyactein

4) Description of the HPLC of Figure 3A–C:

The HPLC-peak pattern of sample 7 (*Cimicifuga dahurica*), sample 9 (*Cimicifuga foetida*) and also sample 10 (*Cimicifuga racemosa*) are characterized by the major peak of cimigenol-3-O-xyloside at Rt = 58.61 (**3**). The samples 5, 6 and 8 deviate only in the quantitative peak pattern. Between Rt = 9.0 and 22.0 (**I**) caffeic acid and tartaric acid derivatives with their characteristic UV-spectra can be found. Ferulic acid (**2**) and isoferulic acid (**2**) respectively can be detected at Rt = 10.9. Between Rt = 52.4 and Rt = 59.9 (**III**) appear triterpenes and triterpene glycosides. At Rt = 59.9 actein (**4**) and 23-epi-26-deoxyactein (**4**) are detectable.

Notes: For a detailed HPLC-discrimination and quality control of 10 *Cimicifuga* species and a method to distinguish between *C. racemosa* and other *Cimicifuga* species see ref. 5.

A preliminary RAPD-PCR analytical method was also described to distinguish *C. racemosa*, *C. americana* and *C. rubifolia*[32].

The Chinese Pharmacopoeia 2005 uses isoferulic acid as marker compound and demands its quantitative determination (not less than 0.10 %)

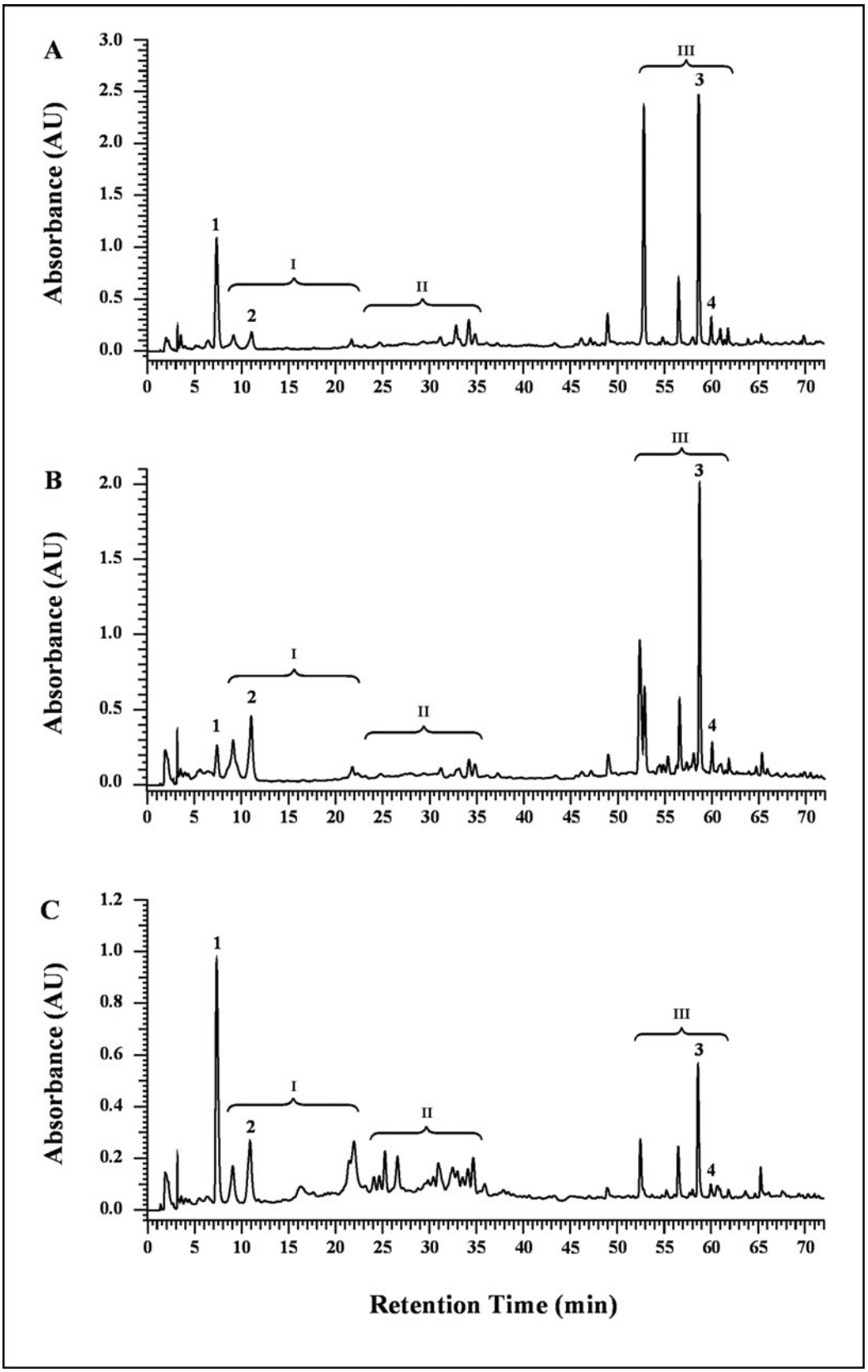


Fig. 3: HPLC-fingerprint chromatogram of *Cimicifuga dahurica* (sample 7; A), *Cimicifuga foetida* (sample 9; B) and *Cimicifuga racemosa* (sample 10; C)

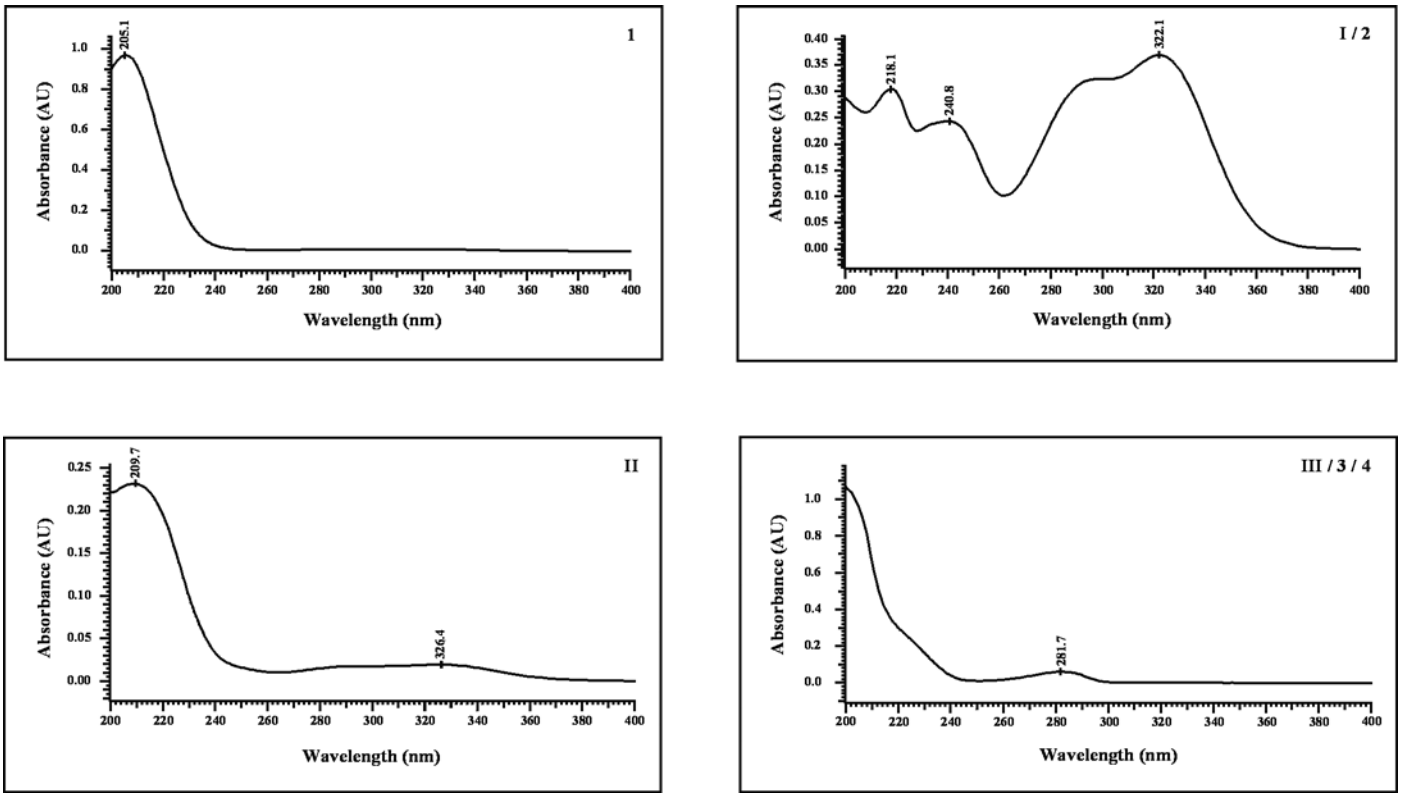


Fig. 4: UV-spectra of the main peaks of Cimicifugae rhizoma

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Cortex Phellodendri amurensis
Guanhuangbo
Cortex Phellodendri chinensis
Huangbo

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2005

Official drugs[1]:

Cortex Phellodendri amurensis:

"Amur cork-tree" is the dried bark of *Phellodendron amurense* Rupr. (Fam. Rutaceae). The drug is collected, removed from coarse bark, and dried in the sun.

Cortex Phellodendri chinensis:

"Chinese Cork-tree" is the dried bark of *Phellodendron chinensis* Schneid. (Fam. Rutaceae). The drug is commonly called "Chuanhuangbo".

The drug is collected, removed from coarse bark, and dried in the sun.

Origin[2,3,4]: Provinces Sichuan, Hebei, Hubei, Guizhou, Yunnan, Jiangxi, Zhejiang, Shaanxi, Hunan, Inner Mongolia (China); Sibiria; Japan

Descriptions of the drugs [1]:

Cortex Phellodendri amurensis:

Tabular of shallowly channelled, varying in length and width, 2 – 4 mm thick. Outer surface yellowish-green or pale brownish-yellow, relatively even with irregular longitudinal fissures, lenticel scars small and infrequently visible, occasionally remaining greyish-white coarse bark. Inner surface yellow or yellowish-brown. Texture light and relatively hard, fracture fibrous, showing lobelike layers, bright yellow or yellowish-green. Odour, slight; taste, very bitter, viscous on chewing.

Cortex Phellodendri chinensis:

Tabular or shallowly channelled, varying in length and width, 1 – 6 mm thick. Outer surface yellowish-brown, even or longitudinally furrowed, some showing scars of lenticels, and remains of greyish-brown coarse bark, inner surface dark yellow

or pale brown, with fine longitudinal ridges. Texture light and hard, fracture fibrous, showing lobelike layers, dark yellow. Odour, slight; taste, very bitter, viscous on chewing.

Pretreatment of the raw drugs[1]:

Cortex Phellodendri amurensis and chinensis:

Foreign matters are eliminated, sprayed with water, softened thoroughly, cut into slivers and dried.

Cortex Phellodendri amurensis and chinensis (processed with salt):

Salt-water is added to clean slivers, mixed well in a closed vessel until they are infused thoroughly, placed in a pot and stir-baked with gently heat to dryness, taken out and cooled.

Cortex Phellodendri amurensis and chinensis (carbonized):

The slivers are placed in a hot pot, stir-baked at a high temperature until the surfaces of the slivers became charred-black, sprayed a small quantity of water, taken them out and cooled in the air.

Medicinal use[4,5]:

Internal:

genito-urinary infection, leucorrhoea, diarrhoea, arthralgia hemorrhoids, ulcera,

External:

aphtons, dermatitis, eczema, pustules, burn and scalds

Effects and indications of Cortex Phellodendri amurensis according to Traditional Chinese Medicine[1,5]	
Taste:	acrid
Temperature:	cold
Channels entered:	orbis renalis, orbis vesicalis, orbis intestini crassi / tenuis
Effects (functions):	clears heat and dampness, purges fire and eliminates steaming of bone, relieves toxicity and cures sores. Stir-baked Cortex Phellodendri with salt nourishes yin and lessens fire
Symptoms and indications:	Diarrhea or dysentery, jaundice, morbid leukorrhea, stranguria, beriberi, joint or muscular disorders with pain due to wind, cold or dampness and flaccidity of limbs, steaming of bone with hectic fever, night sweat, seminal emission, pyocutaneous diseases, eczema with pruritus Stir-baked Cortex Phellodendri with salt used at night sweat and steaming of bone due to flaming of fire from yin deficiency

Effects and indications of Cortex Phellodendri chinensis according to Traditional Chinese Medicine[1,5]	
Taste:	acrid
Temperature:	cold
Channels entered:	orbis renalis, orbis vesicalis, orbis intestini crassi / tenuis
Effects (functions):	Removes damp-heat, quenches fire, counteracts toxicity and relieves consumptive fever Cortex Phellodendri chinensis (processed with salt) nourishes yin and reduces fire
Symptoms and indications:	Dysentery, jaundice and morbid leukorrhea caused by damp-heat; urinary infections; weakness and edema of legs; consumptive fever and night sweating; seminal emission; sores and skin infection with local redness and swelling; eczema with itching Cortex Phellodendri chinensis (processed with salt) used at night sweating and consumptive fever due to exuberant fire secondary to deficiency of yin

Main constituents:

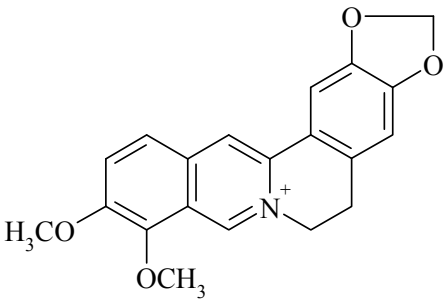
- **alkaloids**:
  berberine[6], palmatine[6], magnoflorine[6], phellodendrine[6], candicine[6], jatrorrhizine[6], 7,8-dihydroxyrutaecarpine[7], 7-hydroxyrutaecarpine[7], menisperin[2]

- steroids[2]:
  campesterin, sitosterin, 7-dehydrostigmasterin

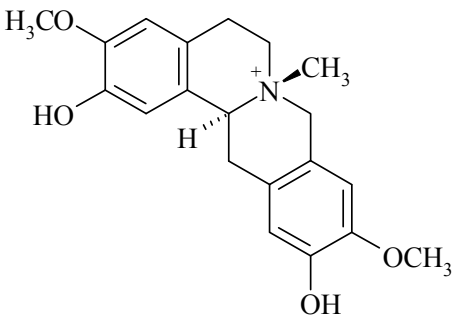
- **limonoidal triterpenoids**:
  limonin (obakulactone)[5,8], obakunone[2,8] and their γ-hydroxybutenolides[8]

- **phenolic constituents** (lignans, flavonoids, phenolcarboxylicacid-esters)[8]:
  syringin, lyoniresinol, coniferin, syringaresinol-di-O-D-glucopyranoside, sinapic aldehyde-4-O-D-glucopyranoside, vanilloloside, methyl-5-O-feruloyl-quinate, 3-O-feruloylquinic acid, 3-O-ferluoylquinic acid methylester

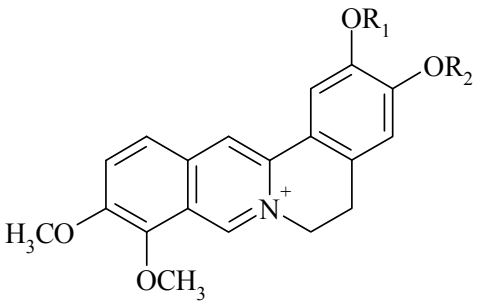
- other constituents:
  nomilin[9], 3-acetyl-3,4-dihydro-5,6-dimethoxy-1H-2-benzopyran-1-one[10]



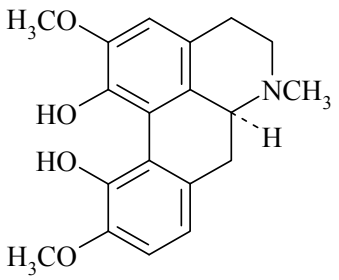
Berberine



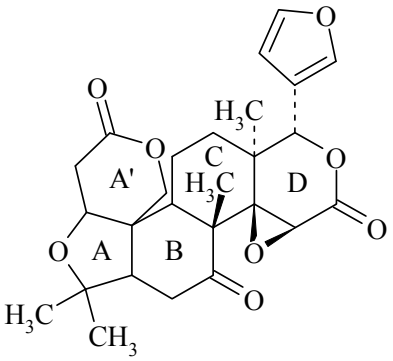
Phellodendrine



Jatrorrhizine: $R_1 = CH_3$; $R_2 = H$
Palmatine: $R_1 = CH_3$; $R_2 = CH_3$



Magnoflorine



Limonin

Fig. 1: Formulae of the main compounds of Cortex Phellodendri[6]

Pharmacology: most of the pharmacological effects reported can be assigned to berberine and the other alkaloids (see also Monograph Rhizoma Coptidis, No. 28)

- antiinflammatory[6]
- antiallergic[8]
- antipyretic[8]
- anti-ulcer activity[11]
- antibacterial[6]
- antimicrobial[12]
- antifungal[6]
- antitumoral[6]
- hypotensive[6]
- choleretic action[6]
- competitive inhibition of the alcohol dehydrogenase[6]
- immune-stimulating activity[13]
- antidiarrhoic[14]
- shortening of sleeping time in mice (limonoids)[15]

Toxicology[6]: Toxic adverse effects (depression, dyspnoea, salivation, defecation, micturation) are reported when overdosages of herbal preparations (berberine overdoses!) are administered.

TLC fingerprint analysis:

Drug samples		Origin
1	Phellodendri cortex/*Phellodendron amurensis*	sample of commercial drug, China
2	Phellodendri cortex/*Phellodendron amurensis*	province Jilin, China
3	Phellodendri cortex/*Phellodendron amurensis*	province Jilin, China
4	Phellodendri cortex/*Phellodendron chinensis*	sample of commercial drug, China
5	Phellodendri cortex/*Phellodendron chinensis*	province Yan Nan, China
6	Phellodendri cortex/*Phellodendron chinensis*	province Sichuan, China

Reference compounds of Figure 2a/b		R*f*
T 1	berberine hydrochloride *	0.60
T 2	palmatine chloride *	0.46
T 3	jatrorrhizine chloride *	0.56
T 4	magnoflorine **	0.15

Reference compounds of Figure 2c		**R*f***
T 5	limonin ***	0.67

* detectable in UV 365 nm
** detectable only with Dragendorff-reagent
*** detectable only with vanillin-sulphuric acid reagent

1. Thin layer chromatography of alkaloids:

1) Extraction: 0.1 g of the powdered drug is extracted under reflux with 5 ml of methanol for 15 minutes. The extract is cooled, filtered and filled up with methanol to 5 ml.

2) Reference compounds: berberine, jatrorrhizine, palmitine, magnoflorine: each 0.5 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Phellodendri cortex extract: each 20 µl
reference compounds: each 10 µl

Solvent system:

n-butanol	ethyl acetate	formic acid	water
30	50	10	10

Detection: Direct evaluation:
UV 365 nm

Dragendorff reagent:

Solution I: 0.85 g basic bismuth nitrate is dissolved under heating in 10 ml glacial acetic acid and 40 ml water

Solution II: 8 g potassium iodide are dissolved in 30 ml water.

Stock solution: I and II are mixed 1 : 1

Spray reagent: 1 ml stock solution is mixed with 2 ml glacial acetic acid and 10 ml water.
The plate is evenly sprayed until background appears yellow.

4) Description of Figure 2a and 2b:

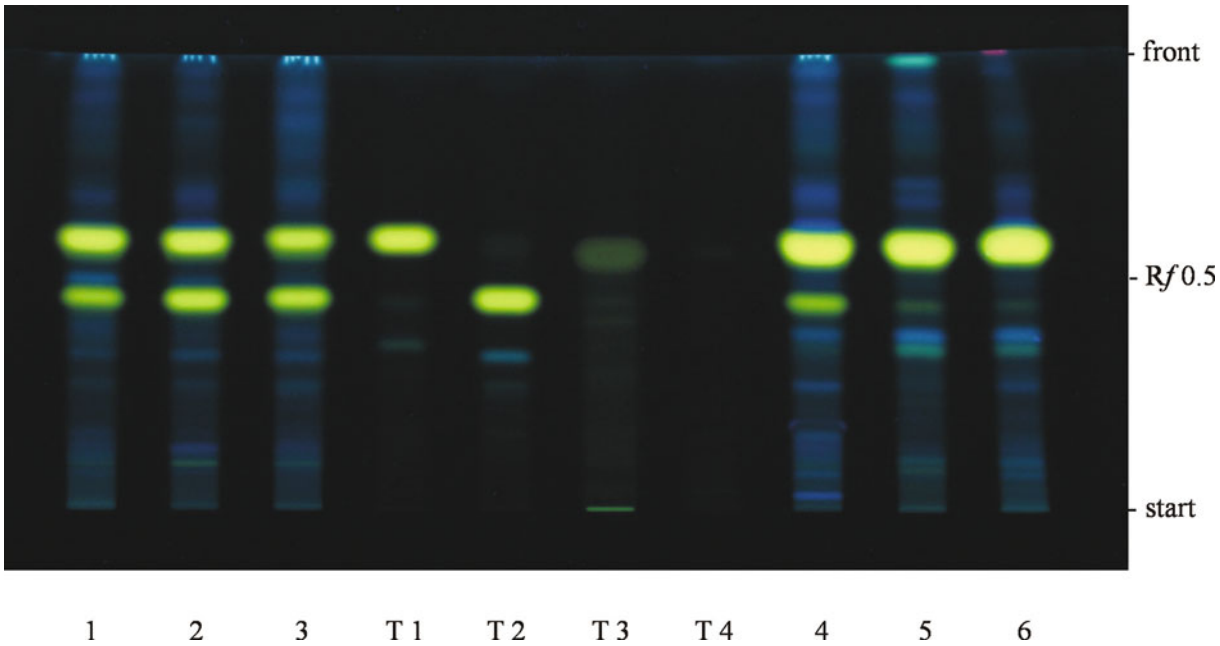


Fig. 2a: Thin layer chromatogram of the methanol extracts of Phellodendri cortex (UV 365 nm)

In UV 365 nm the three samples of *Phellodendron amurensis* (1, 2, 3) are characterized by berberine (**T 1**) and palmatine (**T 2**). In the R*f*-range of 0.15 to 0.4 2 – 3 other not identified alkaloids in very small concentration can be detected.

In samples 4, 5 and 6 of *Phellodendron chinensis* berberine is present in higher concentration, which is in accordance with the berberine content as demanded in the Chinese Pharmacopoe 2005 (not less than 3.0 %). In sample 4 palmatine is also detectable.

The alkaloid jatrorrhizine (**T 3**) as described for both Phellodendron species, is present in very low concentration and in this solvent system overlapped by berberine. Magnoflorine is only detectable with Dragendorff-reagent (see Fig. 2b).

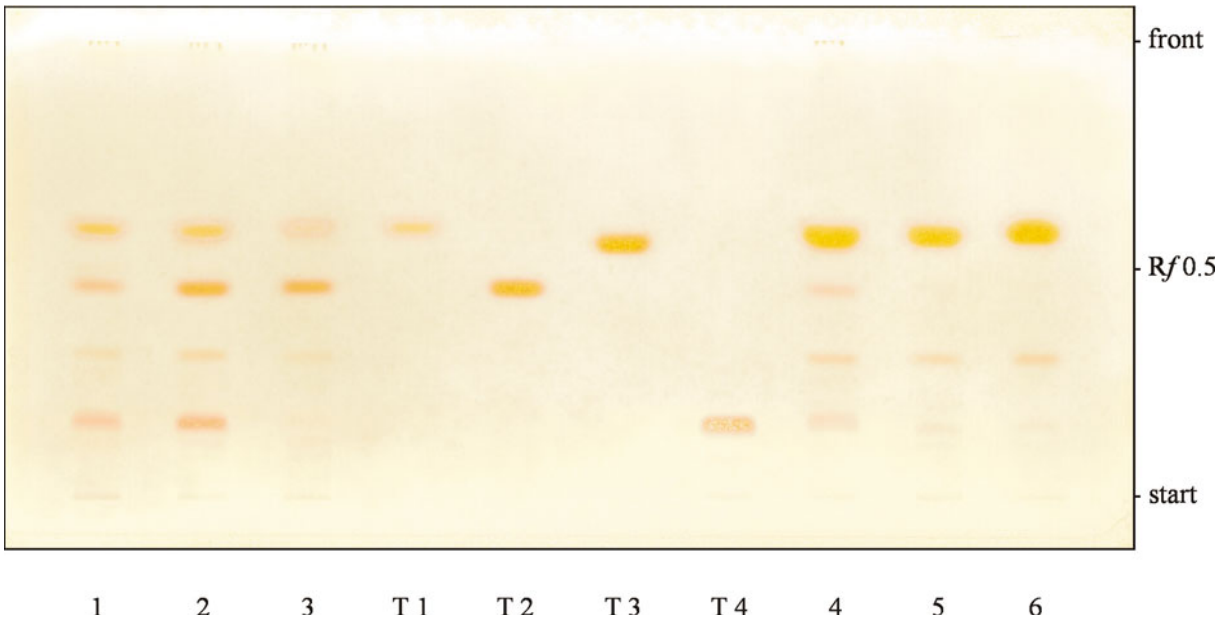


Fig. 2b: Thin layer chromatogram of the methanol extracts of Phellodendri cortex sprayed with Dragendorff Reagent (VIS)

With Dragendorff reagent all alkaloids, berberine, palmatine, jatrorrhizine and magnoflorine (R*f* = 0.15) appear with yellow-brownish colour.

2. Thin layer chromatogram of limonin:

1) Extraction: 0.1 g of the powdered drug is extracted under reflux with 5 ml of methanol for 15 minutes. The extract is cooled, filtered and filled up with methanol to 5 ml.

2) Reference compound: limonin:
1.0 mg is dissolved in 1 ml methanol

3) Separation parameters:

Plate: HPTLC plate, Silica gel 60 F_{254}, Merck

Applied amounts: Phellodendri cortex extract: each 20 µl
reference compound: 10 µl

Solvent system: chloroform : methanol
95 5

Detection: Vanillin-sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I followed immediately with solution II. Then the plate is heated for 5 – 10 minutes at 105 °C and evaluated in VIS.

4) Description of Figure 2 c:

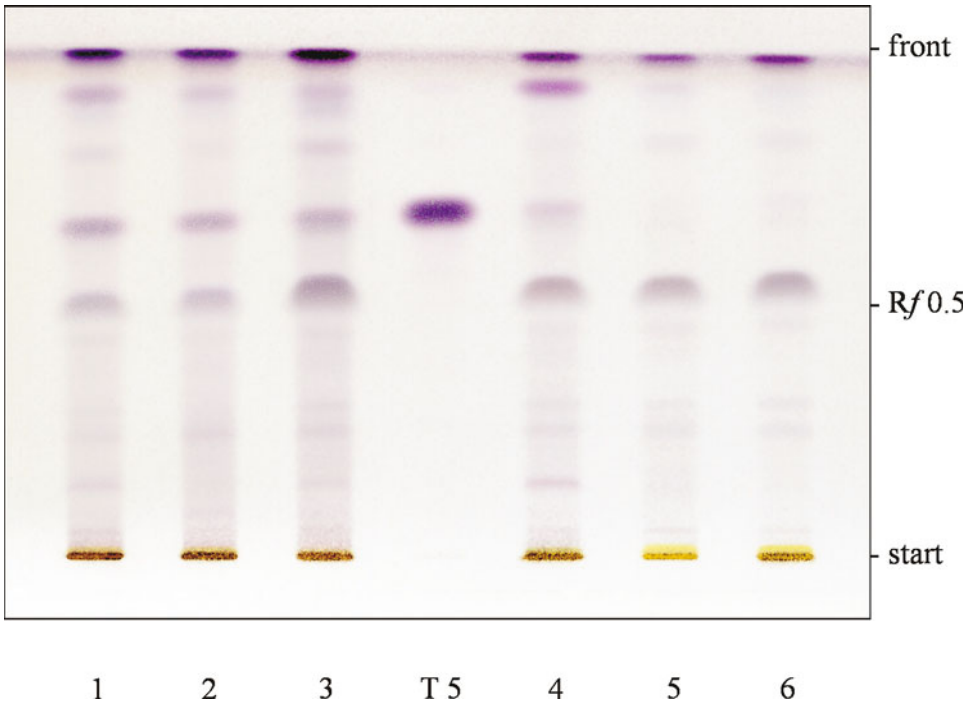


Fig. 2c: Thin layer chromatogram of the methanol extracts of Phellodendri cortex sprayed with vanillin-sulphuric acid reagent (VIS)

The triterpenoid limonin gives with the Vanillin-H_2SO_4-reagent a violet coloured zone at R*f* = 0.67. The other zone at R*f* = 0.53 might be obakunone. In the drug samples 5 and 6 limonin is absent.

HPLC-fingerprint analysis:

1) Sample preparation: 0.1 g of the powdered drug is extracted under reflux with 5 ml of methanol for 15 minutes. The extract is cooled, filtered, filled up with methanol to 5 ml, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Phellodendri cortex extract: 10.0 µl

3) HPLC parameter:

Apparatus:
MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 250-4 with LiChrospher® 60 RP-select B (5 µm), Merck

Precolumn: LiChroCART® 4-4 with LiChrospher® 60 RP-select B, Merck

Solvent:
A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. water (Millipore Ultra Clear UV plus® filtered) + H_3PO_4 85 % (pH = 3.0) (Merck)
B: acetonitrile (VWR)

Gradient:
5 – 60 % B in 30 minutes
60 % B in 10 minutes
total runtime: 40 minutes

Flow: 1.0 ml/min.

Detection: 210 nm

Retention times of the main peaks:

Peak	Rt (min.)	compound
1	16.3	not identified alkaloid
2	17.1	magnoflorine
3	22.4	jatrorrhizine
4	24.3	palmatine
5	25.1	berberine
6	26.8	limonin

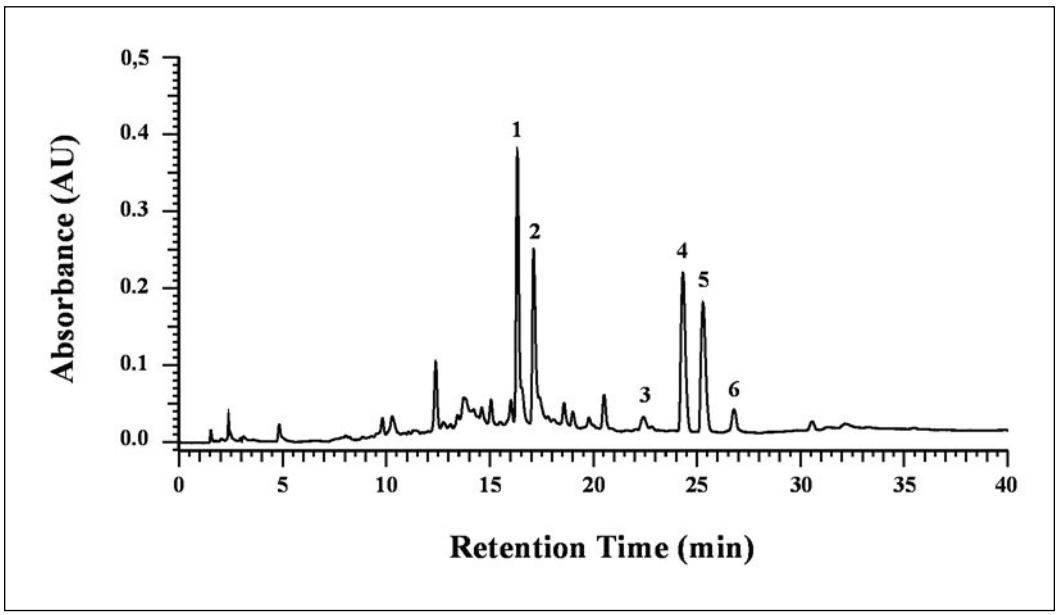


Fig. 3a: HPLC-fingerprint chromatogram of the methanol extract of Cortex Phellodendri amurensis (sample 2, province Jilin)

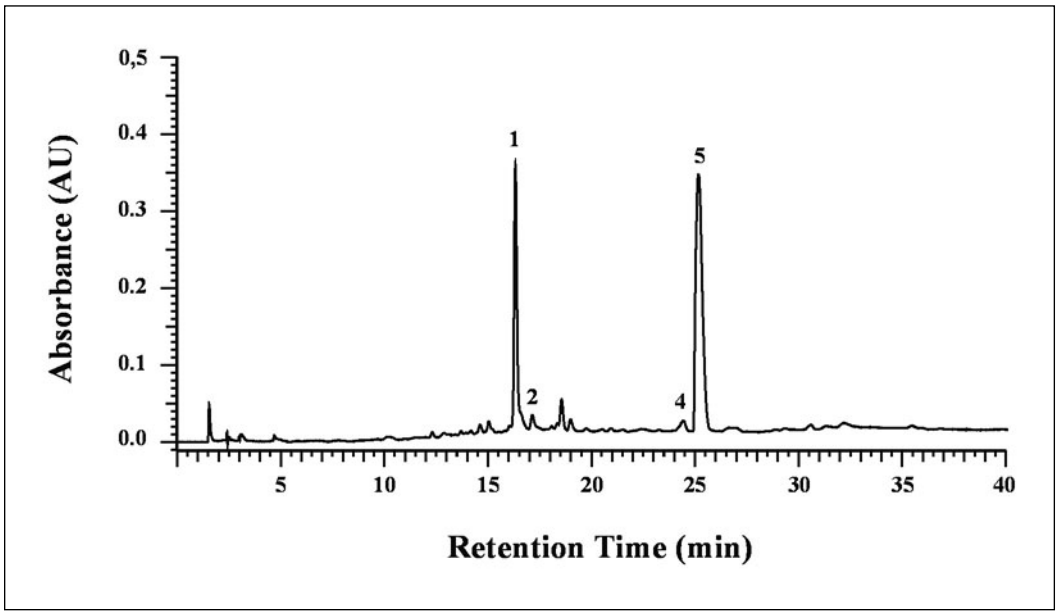


Fig. 3b: HPLC-fingerprint chromatogram of the methanol extract of Cortex Phellodendri chinensis (sample 5, province Yan Nan)

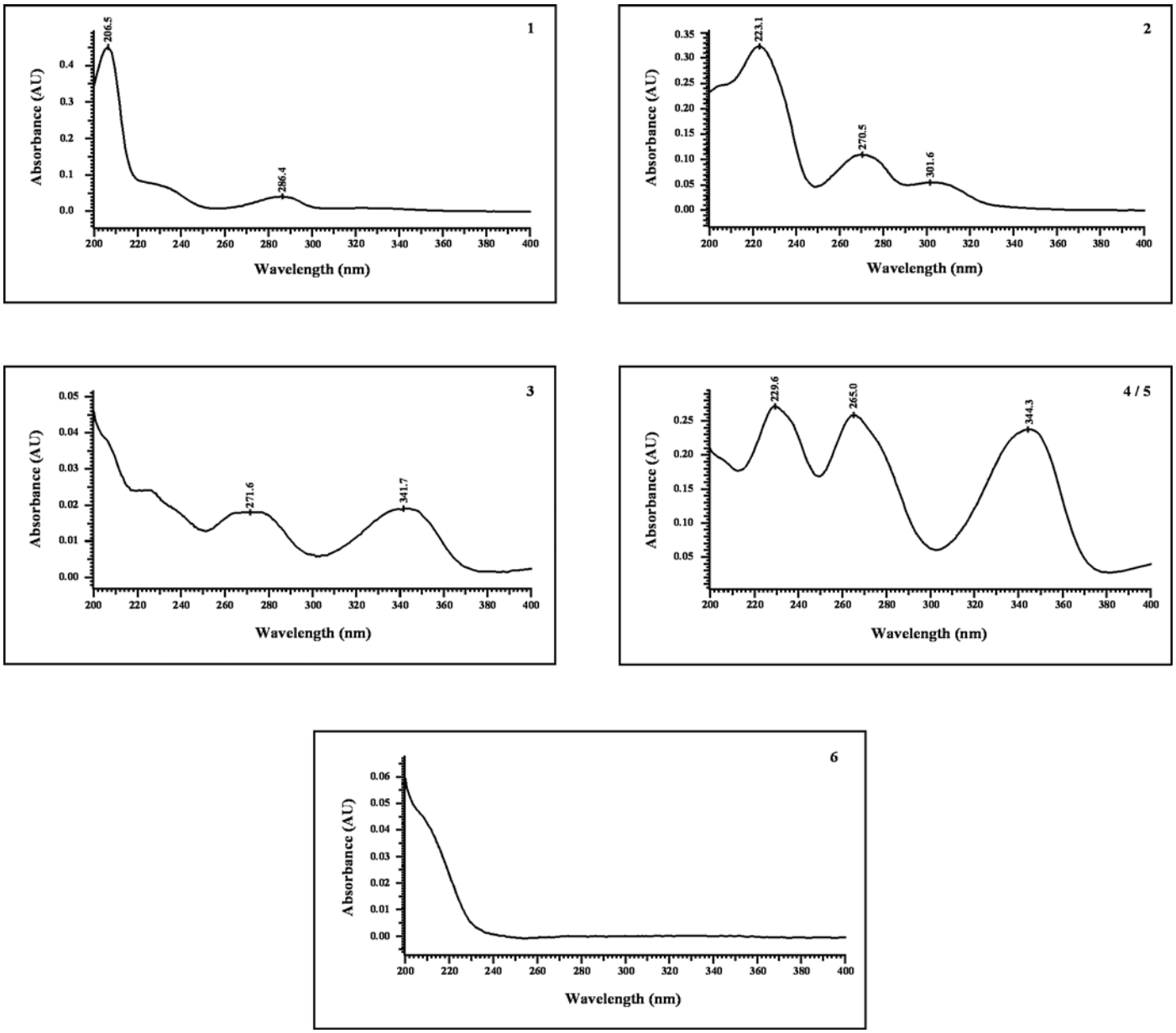


Fig. 4: UV-spectra of the main peaks of the methanol extract of Cortex Phellodendri

4) Description of the HPLC of Figure 3a and b:

The HPLC-peak pattern of *Phellodendron amurensis* samples 1 – 3 (Fig. 3a) are characterized by the four major alkaloids **1**, **2**, **4**, **5** and the small peak of jatrorrhizine (**3**) (Rt = 22.4) and the triterpenoid limonin (**6**) (Rt = 26.8), the latter of them is absent in samples 4 – 6.

In the samples 4 – 6 of *Phellodendron chinensis* (Fig. 3b) the not identified alkaloid of peak 1 and berberine can be detected. The "not identified" alkaloid may correspond with the not identified alkaloid between palmatine and magnoflorine in TLC of Fig. 2b.

Note: The Chinese Pharmacopoeia 2005[(1)] demands not less than 0.60 % (Cortex Phellodendri Amurensis) respectively 3.0 % (Cortex Phellodendri Chinensis) of berberine-HCl, calculated with reference to the dried drug.

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Additional References (Chromatographic analysis/Chemistry of *Ph. amurense* + *Ph. chinensis*)

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Chen ML, Xian YF, Ip SP, Tsai SH, Yang JY, Che CT, Chemical and biological differentiation of Cortex Phellodendri Chinensis and Cortex Phellodendri Amurensis, Planta Med. 76(14):1530-5 (2010)

Flos Lonicerae – *Shanyinhua*
Flos Lonicerae Japonicae – *Jinyinhua*
Caulis Lonicerae Japonicae – *Rendongteng*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition, 2005

Official drugs[1]:

Flos Lonicerae:

Honeysuckle Flower is the dried flower bud or opening flower of *Lonicera hypoglauca* Miq., *Lonicera confusa* DC. or *Lonicera macranthoides* Hand.-Mazz. (Fam. Caprifoliaceae)

The drug is collected before flowering in early summer and dried.

Flos Lonicerae Japonicae:

Honeysuckle Flower is the dried flower bud or opening flower of *Lonicera Japonica* Thunb. (Fam. Caprifoliaceae).

The drug is collected before flowering in early summer and dried.

Caulis Lonicerae Japonicae:

Honeysuckle Stem is the dried stem and branch of *Lonicera Japonica* Thunb. (Fam. Caprifoliaceae).

The drug is collected in autumn and winter and dried in the sun.

Origin[2,3]: Primarily from the provinces Henan, Shandong (China) and Japan, Korea or Taiwan

Descriptions of the drug[1]:

Flos Lonicerae:

Flower of *Lonicera macranthoides*:

Clavate, slightly curved, 3 – 4.5 cm long, about 2 mm in diameter in upper part and 1 mm in diameter in lower part. Externally greenish-brown or yellowish-white. Flowers grouped in clusters, the length of lobes of corolla shorter than the ½ of the whole length of opened flowers. Texture slightly hard and springy. Odour, delicately aromatic; taste, slightly bitter and sweet.

Flower of *Lonicera confusa*:

1.6 – 3.5 cm long, 0.5 – 2 mm in diameter. Calyx tube and corolla densely covered with grayish-white hairs. Ovary hairy.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Flower of *Lonicera hypoglauca*:

2.5 – 4.5 cm long, 0.8 – 2 mm in diameter externally yellowish-white to yellowish-brown, glabrous of sparsely pubescent. Calyx tube glabrous, 5-lobed at the apex, lobes long-triangular, pubescent. The lower lip of corolla recurved when open. Style glabrous.

Flos Lonicerae Japonicae:

Clavate, stout in upper part and tapered downwards, slightly curved, 2 – 3 cm long, about 3 mm in diameter in upper part and 1.5 mm in diameter in lower part. Externally yellowish-white or greenish-white, gradually darken on keeping, densely pubescent. Foliaceous bracts occasionally visible. Calyx green, 5-lobed at the apex, lobes pubescent, about 2 mm long. Corolla tubular when open, apex 2-lipped; stamens 5, epipetalous, yellow; pistil 1, ovary glabrous. Odour, delicately aromatic; taste, weak and slightly bitter.

Caulis Lonicerae Japonicae:

Long cylindrical, frequently branched, usually twisted into a bundle, 1.5 – 6 mm in diameter. Externally brownish-red to dark brown, some greyish-green, glabrous or pubescent; outer bark easily fallen off. Branches muchnodose, internodes 6 – 9 cm long, showing remains of leaves and leaf scars. Texture fragile, easily broken, fracture yellowish-white, hollow. Odour, slight; taste of older branches slightly bitter, and the younger ones weak.

Pretreatment of the raw drug [1]:

Caulis Lonicerae Japonicae:

Foreign matter are eliminated, washed clean, softened thoroughly, cut into sections and dried.

Medicinal use[3,4]:

Flos Lonicerae Japonicae:

Prescribed as diuretic, refrigerant, antiphlogistic in acute infectious diseases, chronic conjunctivitis, keratitis, mastitis, as antidiarrhoeic in dysentery and enteritis.

Effects and indications of Flos Lonicerae according to Traditional Chinese Medicine[1,4]	
Taste:	sweet
Temperature:	cold
Channels entered:	orbis pulmonalis, orbis cardialis, orbis stomachi
Effects (functions):	clears toxic heat, eliminates wind-heat with herbs cool in property
Symptoms and indications:	carbuncles and sores, laryngalgia, erysipelas, bloody dysentery due to toxic-heat, common cold due to wind-heat and warm diseases

Effects and indications of Flos Lonicerae Japonicae according to Traditional Chinese Medicine[1,4]	
Taste:	sweet
Temperature:	cold
Channels entered:	orbis pulmonalis, orbis cardialis, orbis stomachi
Effects (functions):	removes toxic heat and dispels wind-heat
Symptoms and indications:	carbuncles, boils, erysipelas, acute dysentery, pharyngitis, upper respiratory infection, epidemic febrile diseases

Effects and indications of Caulis Lonicerae Japonicae according to Traditional Chinese Medicine[1,4]	
Taste:	sweet
Temperature:	cold
Channels entered:	orbis pulmonalis, orbis stomachi
Effects (functions):	removes toxic heat, dispels wind from the channels and collaterals
Symptoms and indications:	epidemic febrile diseases, acute dysentery, carbuncles, sores, acute arthritis with redness, swelling and pain of the joint

Main constituents: **Flos Lonicerae / Flos Lonicerae Japonicae / Caulis Lonicerae:**

- **phenolic carboxylic acids and esters:**
  chlorogenic acid[5], isochlorogenic acid a[5], b[5], c[5] , 3-caffeoylquinic acid[6], 3-caffeoylquinic acid methyl ester[6], 3,5-dicaffeoylquinic acid[6], 3,5-dicaffeoylquinic acid methyl ester[6], 3,5-dicaffeoylquinic acid butyl ester[6], methyl-3,4-di-O-caffeoylquinate[7], methyl caffeate[7], 3,4-di-O-caffeoylquinic acid[7], 3,4-di-O-caffeoylquinic acid[8], 3,5-di-O-caffeoylquinic acid[8], 4,5-di-O-caffeoylquinic acid[8], methyl-3,4-di-O-caffeoylquinic acid[8], methyl-3,5-di-O-caffeoylquinic acid[8]

- **iridoid glycosides:**
  loganin[5], secoxyloganin[5], secologanin dimethylacetat[5], vogeloside[5], epivogeloside[5], 7-epi-loganin[9], sweroside[9], loniceracetalides A[10], B[10]), L-phenylalaninosecologanin[11], 7-O-(4-β-D-glucopyranosyloxy-3-methoxybenzoyl)-secologanolic acid[11], 6'-O-(7α-hydroxyswerosyloxy)-loganin[11], (Z)-aldosecologanin[11], (E)-aldosecologanin[11],

- β-sitosterol[12]

- **flavones:**
  lonicerin (luteolin-7-O-rhamnoglucoside)[5], loniceraflavone[5], rutin[12], quercetin[12], luteolin-7-O-β-D-galactoside[12], tetratriacontane[12], ochnaflavone[13], luteolin (3',4',5,7-tetrahydroxyflavone)[14]

- **triterpenoid saponins**[5,15,16,17,18]:
  hederagenin-mono-, di-, tri-, tetraglycosides containing glucose, rhamnose and arabinose as sugar moieties, oleanolic acid-mono-, di-, tri-, tetraglycosides containing glucose, rhamnose and arabinose as sugar moieties (e.g. macranthoidin A, B, dipsacoside B, macranthoside A, B, loniceroside A, B, C)

- essential oil:
  linalool[5], 2,6,6-trimethyl-2-vinyl-5-hydroxytetrahydropyran[5], pinene[5], hex-1-ene[5], hex-3-en-1-ol[5], cis- and trans-2-methyl-2-vinyl-5-(α-hydroxyisopropyl)-tetrahydrofuran[5], geraniol[5], α-terpineol[5], benzyl alcohol[5], β-phenylethyl alcohol[5], carvacrol[5], eugenol[5], aromadendrene[19], ethylpalmitate[20], palmatic acid[21], linoleic acid[21]

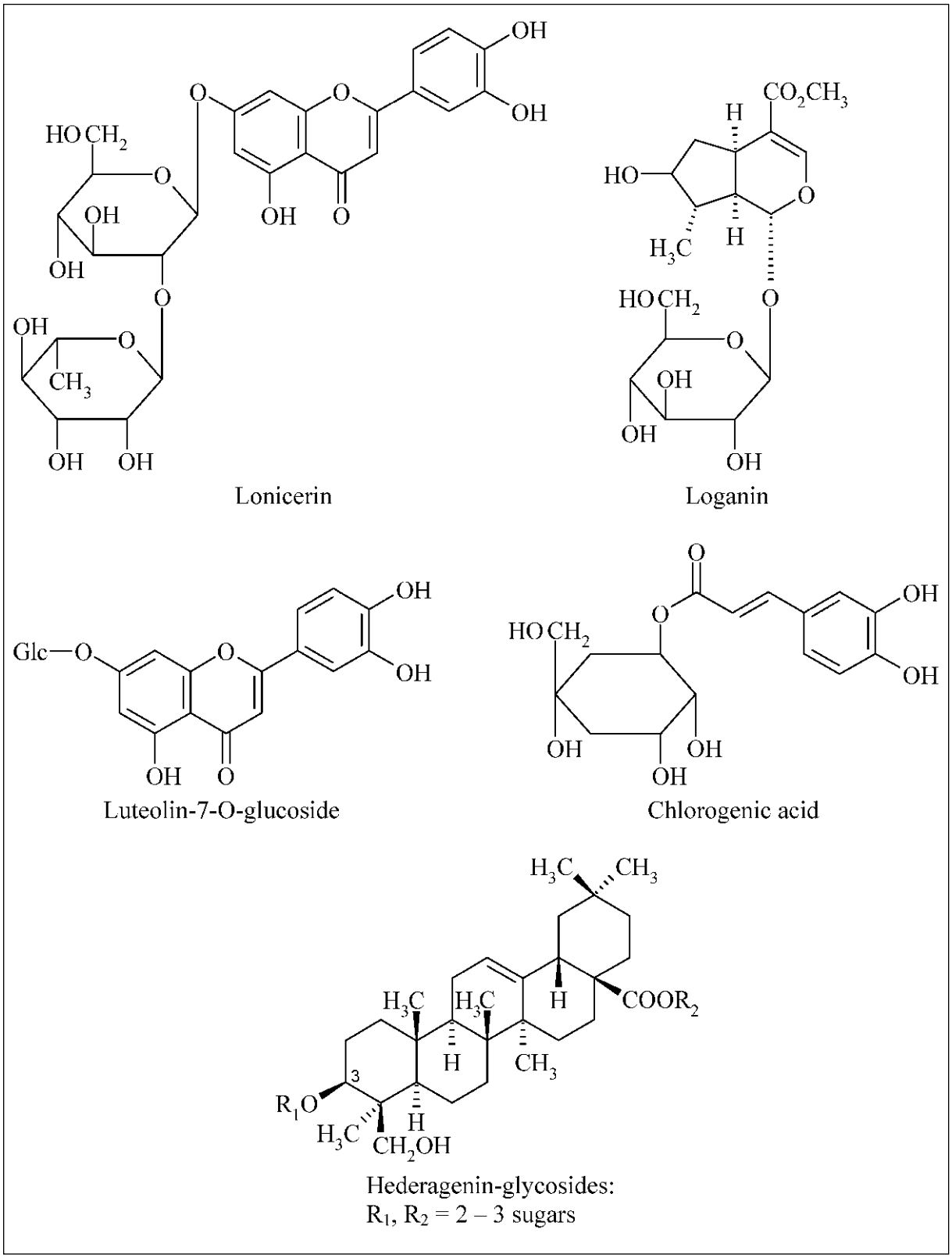

Fig. 1: Formulae of the main compounds of Flos Lonicerae, Flos Lonicerae Japonicae and Caulis Lonicerae Japonicae[5]

Pharmacology:

Flos Lonicerae:
- antipyretic[9,10]
- anti-inflammatory[5,9,10]
- antimicrobial[5,22]
- diuretic[9]
- hepatoprotective[9]
- anti-tumoral activity[14]

Caulis Lonicerae:
- antipyretic[11]
- anti-inflammatory[5,11]
- bacteriostatic[5]
- diuretic[11]
- anti-tumoral activity[14]

TLC fingerprint analysis:

Drug samples		Origin
1	Lonicerae flos / *Lonicera hypoglauca*	sample of commercial drug, China
2	Lonicerae flos / *Lonicera hypoglauca*	sample of commercial drug, China
3	Lonicerae flos / *Lonicera confusa*	province Guangdong, China
4	Lonicerae flos / *Lonicera Japonica*	province Hebei, China
5	Lonicerae flos / *Lonicera Japonica*	province Henan, China
6	Lonicerae flos / *Lonicera Japonica*	province Shangdong, China
7	Lonicerae flos / *Lonicera macranthoides*	province Guangxi, China
8	Lonicerae caulis	sample of commercial drug, China
9	Lonicerae caulis	sample of commercial drug, China
10	Lonicerae caulis	sample of commercial drug, China
11	Lonicerae caulis	sample of commercial drug, China
12	Lonicerae caulis	sample of commercial drug, China

Reference compounds of Figure 2 a		R*f*
T 1	chlorogenic acid	0.54
T 2	isochlorogenic acids	0.80 / 0.95
T 3	luteolin-7-O-glucoside	0.68
T 4	lonicerin (luteolin-7-O-rhamno-glucoside)	0.41
T 5	rutin	0.41

Reference compound of Figure 2b		**R*f***
T 6	loganin	0.43

1) Extraction: To 0.5 g of the powdered drug 10 ml of methanol are added and ultrasonicated for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol and used for TLC.

2) Reference compounds: chlorogenic acid, isochlorogenic acid, luteolin-7-glucoside, lonicerin, rutin, loganin:
0.5 mg of each are dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Lonicerae flos extract: each 3 µl
Lonicerae caulis extract: each 8 µl
reference compounds: each 10 µl

Solvent system:

ethyl acetate	:	formic acid	:	glacial acetic acid	:	water
100		11		11		26

Detection: Natural products-polyethylene glycol reagent (NP/PEG):

I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol

II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. The evaluation is carried out in UV 365 nm.

Vanillin-sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I followed immediately with solution II. The plate is heated for 5 – 10 minutes at 105°C and evaluated in VIS.

4) Description:

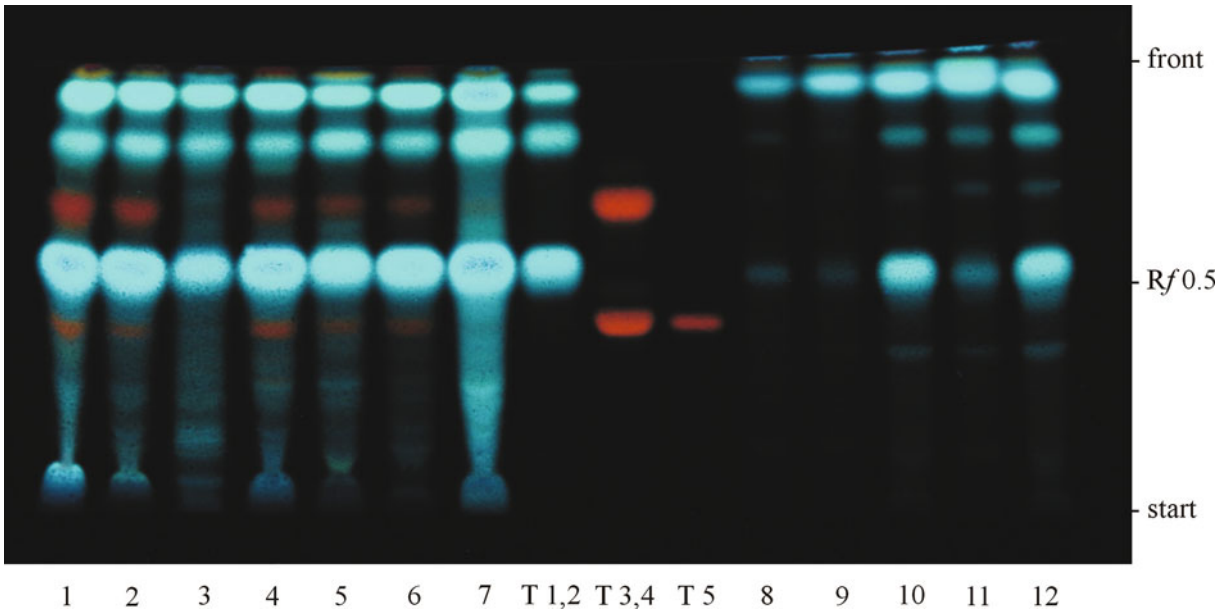

Fig. 2a: Thin layer chromatogram of the methanol extracts of Lonicerae flos and Lonicerae caulis sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

The Flos Lonicerae samples (samples 1 – 7) show a very similar pattern of acids with dominant zones of chlorogenic acid (T 1) at R*f* = 0.54 and isochlorogenic acids (T 2) at R*f* = 0.80 and 0.95.

In the samples of Caulis Lonicerae (8 – 12) only the samples 10 – 12 contained the acids in about the same distribution and concentration as in Flos Lonicerae. In Caulis Lonicerae samples 8 and 9 chlorogenic acid and one isochlorogenic acid (R*f* = 0.80) could be detected in traces only.

With the exception of *Lonicera confusa* (sample 3) and *Lonicera macranthoides* (sample 7) in all other Lonicerae flos samples orange-red bands of lonicerin (T 4) at R*f* = 0.41 and luteolin-7-glycoside (T 3) at R*f* = 0.68 can be detected.

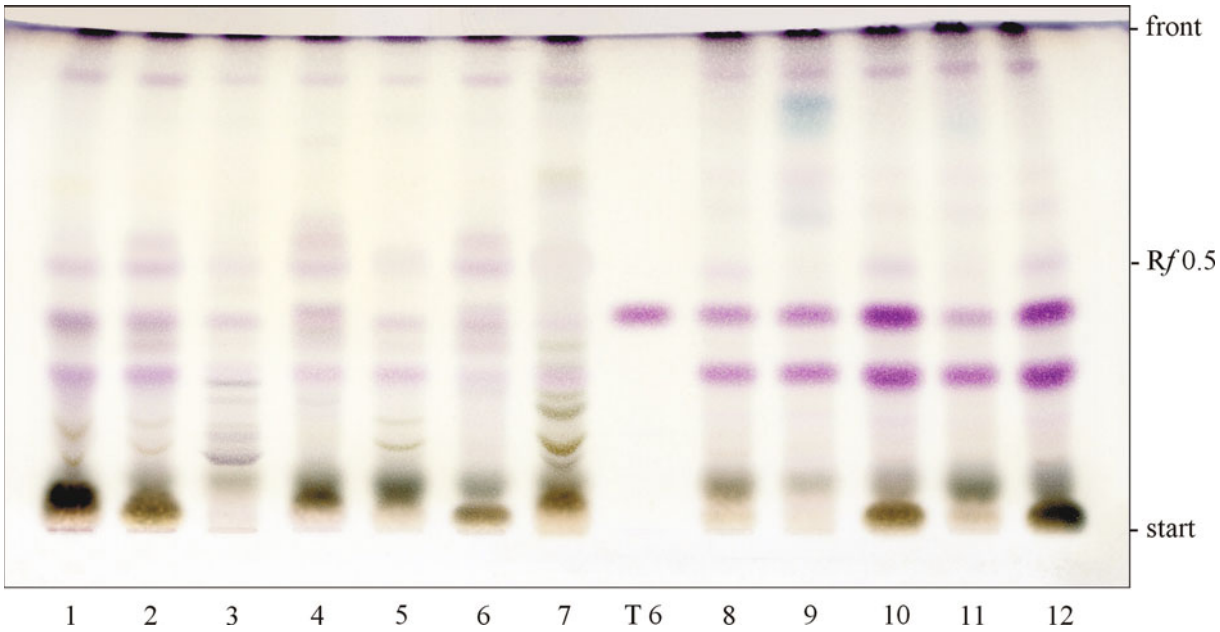

Fig. 2b: Thin layer chromatogram of the methanol extracts of Lonicerae flos and Lonicerae caulis sprayed with vanillin-suphuric acid reagent (VIS)

Between R*f* ~ 0.25 and R*f* ~ 0.6 the iridoid glycosides appear as violet zones. Loganin (T 6) and a second iridoid glycoside can be seen in Caulis Lonicerae (8 – 12) (R*f* = 0.43 and R*f* = 0.27) in high concentration, in Flos Lonicerae (1 – 7) only in lower concentration. The hederagenin glycosides (R*f* = 0.05 – 0.15) can be separated and detected in the solvent system chloroform: ethyl acetate: methanol (4:3:0.4) with brown/black colour.

HPLC-fingerprint analysis:

1) Sample preparation: To 0.5 g of the powdered drug 10 ml of methanol are added and ultrasonicated for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Flos Lonicerae, Flos Lonicerae Japonicae, Caulis Lonicerae Japonicae extract: each 10.0 µl

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 250-4 with LiChrospher® 100 RP 18 (5 µm), Merck

Precolumn: LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck

Solvent:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	2 – 10 % B in 10 minutes 10 – 20 % B in 40 minutes 20 – 30 % B in 5 minutes 30 % B in 10 minutes total runtime: 65 minutes
Flow:	0.8 ml/min.
Detection:	215 nm/327 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	15.0	chlorogenic acid isomer?
2	18.3	iridoidglycoside?
3	18.7	chlorogenic acid
4	27.0	loganin
5	47.5	isochlorogenic acid
6	~ 49.5	lonicerin / luteolin-7-O-glucoside

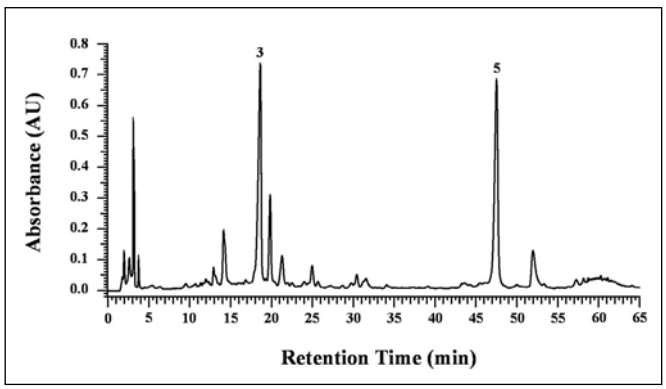

Fig. 3a: HPLC-fingerprint chromatogram of the methanol extract of Flos Lonicerae hypoglauca (sample 1) and L. confusa (sample 3, province Guangdong) at wavelength 215 nm

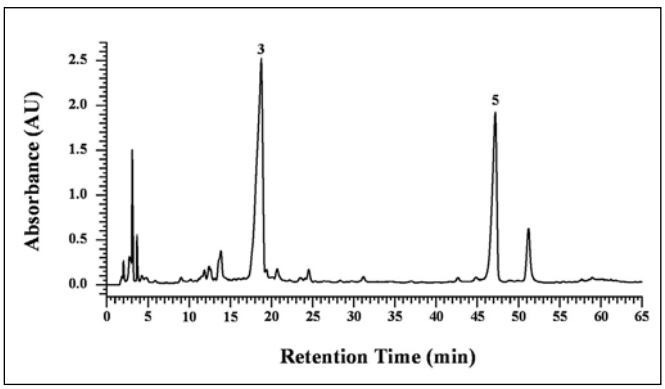

Fig. 3b: HPLC-fingerprint chromatogram of the methanol extract of Flos Lonicerae macranthoides (sample 7, province Guangxi) at wavelength 215 nm

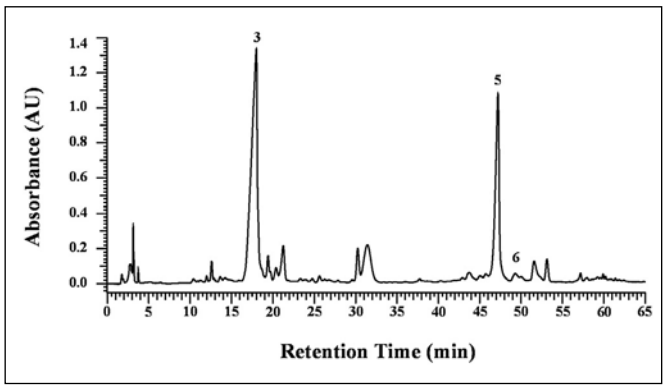

Fig. 3c: HPLC-fingerprint chromatogram of the methanol extract of Flos Lonicerae Japonicae (sample 6, province Shangdong) at wavelength 215 nm

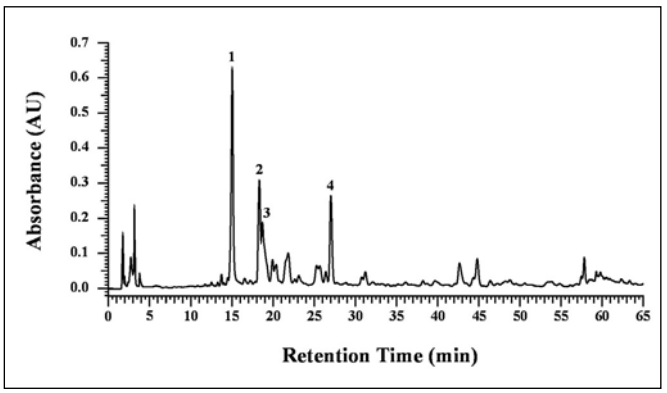

Fig. 3d: HPLC-fingerprint chromatogram of the methanol extract of Caulis Lonicerae (sample 10, sample of commercial drug, China) at wavelength 215 nm

4) Description of the HPLC:

Figure 3a–c:

The HPLC-peak pattern of the various Flos Lonicerae samples (1 – 7) (Fig. 3 a - c) are characterized by the major peaks of chlorogenic acid (**3**) at Rt = 18.7 and isochlorogenic acid (**5**) at Rt = 47.5. In contrast to *Lonicera confusa* and *Lonicera macranthoides*, only in *Lonicera Japonica* (samples 4 – 6) luteolin-7-glucoside (**6**) and lonicerin (**6**), can be detected unseparated in one small peak at Rt ~ 49.5. Loganin was not detectable in the chosen column and solvent system.

Figure 3d (Caulis Lonicerae sample 10, 215 nm):

The samples of Caulis Lonicerae Japonicae (8 – 12) shown for sample 6 are characterized at 215 nm by a non identified chlorogenic acid isomer at Rt = 15.0 (**1**), a non identified iridoid glycoside (**2**) at Rt = 18.5, the chlorogenic acid (**3**) (Rt = 18.7) and another iridoid glycoside (**4**) at Rt = 27.0. Between Rt = 43.0 and 45.0 appear two further phenolcarboxylic acid esters.

Figure 3e (Caulis Lonicerae sample 10, 327 nm):

In this HPLC-graph appear the non identified chlorogenic isomer 1 and 5 and the chlorogenic acid (**3**).

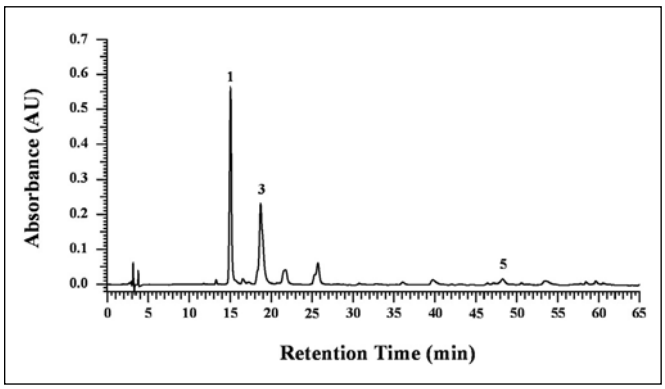

Fig. 3e: HPLC-fingerprint chromatogram of the methanol extract of Caulis Lonicerae (sample 10, sample of commercial drug, China) at wavelength 327 nm

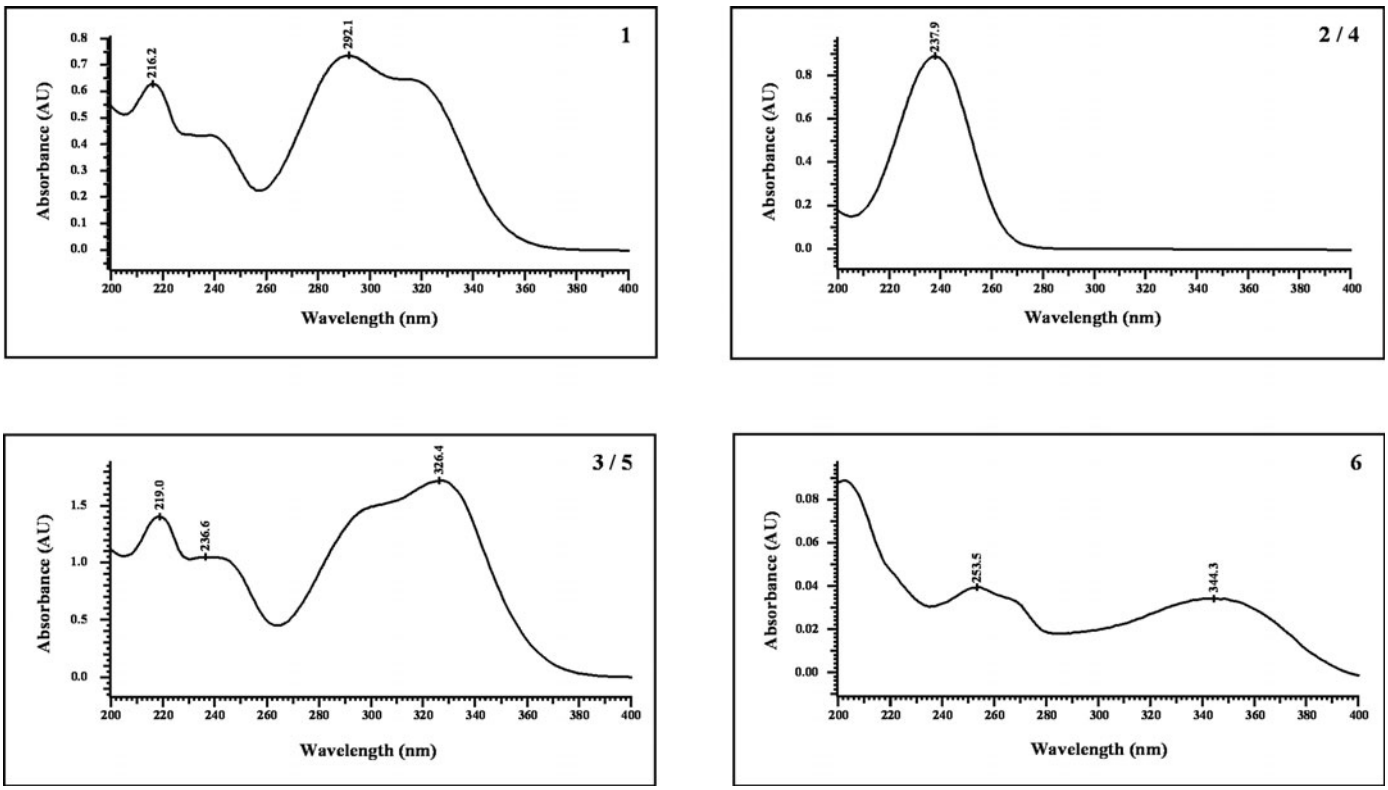

Fig. 4: UV-spectra of the main peaks of the methanol extracts of Flos Lonicerae, Flos Lonicerae Japonicae and Caulis Lonicerae Japonicae

Note: In the Chinese Pharmacopoeia 2005 for Flos Lonicerae is demanded a content of chlorogenic acid of "not less than 0.08 %", for Flos Lonicerae Japonica "not less than 1.5 % chlorogenic acid" and "not less than 0.1 % of luteolin-7-O-glucoside". Caulis Lonicerae Japonicae should contain "not less than 0.1 % chlorogenic acid".

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Rhizoma Curcumae longae – *Jianghuang*
Rhizoma Curcumae – *Ezhu*
Radix Curcumae – *Yujin*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition 2005

Official drugs[1]: The Chinese Pharmacopeia contains three official *Curcuma* (engl. = turmeric) monographs which descend from six different *Curcuma* species. The Pharmacopoeia discriminates also between rhizomes and radix tuber drugs of *Curcuma*.

Rhizoma Curcumae longae *(Jianghuang)*:

Turmeric is the dried rhizome of *Curcuma longa* L. (syn. = *Curcuma domestica* Valeton)

Rhizoma Curcumae *(Ezhu)*:

Zedoary Rhizome is the dried rhizome of *Curcuma phaeocaulis* Valeton, *Curcuma kwangsiensis* S.G. Lee et C.F. Liang or *Curcuma wenyujin* Y.H. Chen et C. Ling

Radix Curcumae *(Yujin)*:

Turmeric Root tuber is the dried root tuber of *Curcuma wenyujin* Y.H. Chen et C. Ling, *Curcuma longa* L., *Curcuma kwangsiensis* S.G. Lee et C.F. Liang or *Curcuma phaeocaulis* Valeton.

– Zingiberaceae –

Note:

Rhizoma Curcumae xanthorrhizae Roxb., the Javanese turmeric rhizome, is the official drug of some European Pharmacopoeias. In this monograph it is used for comparative TLC- and HPLC-analysis.

Origin[2,3]: Cultivated in Asia and Africa and imported from China (Sichuan, Zhejiang, Guangdong, Guangxi, Yunnan), India and Indonesia.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Description of the drugs[1]:

Rhizoma Curcumae longae:

Irregulary ovoid, cylindrical or fusiform, frequently curved, some short branched in Y-shape, 2-5 cm long, 1-3 cm in diameter. Externally dark yellow, rough, with wrinkled striations and distinct rings, and exhibiting some rounded scars of branched rhizome and fibrous root scars. Texture hard, uneasily broken, fracture brownish-yellow to golden-yellow, horny with wax lustre, endodermis ring distinct, scattered with dotted vascular bundles.

Odour: characteristic and aromatic. Taste: bitter and pungent.

Rhizoma Curcumae phaeocaulis:

Ovoid elongate ovoid, concical or elongate fusiform, frequently apex obtuse and rounded, 2-8 cm long, 1.5-4 cm in diameter. Externally greyish-yellow to greyish-brown, the upper part conspicuously raised-annulated and with rounded and slightly dented rootlet scars, or remaining rootlets, some exhibiting a row of concave bud scars and subrounded lateral rhizome scars on two sides, respectively, and showing knife cut traces. Heavy, texture hard, fracture greyish-brown, waxy, usually attached with greyish-brown powder, bark and stele easily detachable, endodermal ring deep brown.

Odour: slight aromatic. Taste: slight bitter and pungent.

Radix (root tuber) Curcumae longae:

Fusiform, sometimes slender at one end, 2.5-4.5 cm long, 1-1.5 cm in diameter. Externally brownish-grey or greyish-yellow, with fine wrinkles. Fracture orange, but edges brownish-yellow to brownish-red.

Odour: aromatic. Taste: pungent.

Radix (root tuber) Curcumae wenyujin:

Oblong or ovoid, slightly compressed or curved, the two ends tapering, 3.5-7 cm long, 1.2-2.5 cm in diameter. Externally pale brown or greyish-brown, with irregular longitudinal wrinkles, the raised longitudinal wrinkles pale in colour. Texture compact, fracture greyish-brown and horny. Endodermis ring distinct.

Odour: slightly aromatic. taste: slightly bitter.

The description of Rhizoma Curcumae kwangsiensis, Rhizoma Curcumae wenyujing, Radix (root tuber) Curcumae kwangsiensis and Radix Curcumae phaeocaulis which is very similar to that of the above listed drugs, see Pharmacopoeia of the People's Republic of China, English Edition 2005[1].

Pretreatment of the raw drugs[1]:

Rhizoma Curcumae longae *(Jianghuang)*:

The drug is collected in winter when the aerial part withers, washed clean, boiled or steamed thoroughly, cut into thick slices, dried in the sun and removed from fibrous root.

Rhizoma Curcumae *(Ezhu)*:

The drug is collected in winter when stem and leaves wither, washed clean, steamed or boiled thoroughly, cut into slices, dried in the sun or dried at low temperature, removed from fibrous root and foreign matter.

Processed with vinegar: The clean drug is boiled with vinegar until vinegar is absorbed entirely, cut into slices and dried.

Radix Curcumae *(Yujin)*:

The drug is collected in winter when stem and leaves wither, removed from soil and rootlet, washed clean, steamed or boiled thoroughly, cut into thin slices and dried or washed clean, dried and broken to pieces.

Medicinal use [4]:

Rhizoma Curcumae longae *(Jianghuang)*:

The drug is chiefly used internely for the treatment of nonulcer dyspepsia, hepatobiliary disorders and rheumatic complaints, rheumatoid arthritis; externally of traumatic diseases (sprains and swellings caused by injury).

Rhizoma Curcumae *(Ezhu)*:

The drug is used for the treatments of nonulcer dyspepsia associated with impaired bile secretion and flow, and of cervical treatments at early stage.

Radix Curcumae *(Yujin)*:

The drug is used as a choleretic, analgesic, sedative and similar as the rhizome for the treatment of hepatobiliary disorders.

Effects and indications of Rhizoma Curcumae longae (*Jianghuang*) according to Traditional Chinese Medicine	
Taste:	bitter and pungent[1,5]
Temperature:	warm[5]
Channels entered:	acts on *orbis lienalis, orbis stomachi, orbis hepaticus*[5]
Effects:	to eliminate *blood stasis*, to promote the flow of *qi* and *xue*, to remove *humor venti*, to stimulate menstrual discharge and relieve pain[1,5]
Symptoms and indications:	pricking pain in the chest and hypochondriac regions; amenorrhea; mass formation in the abdomen; rheumatic pain of the shoulders and arms; traumatic swelling pain[1]

Effects and indications of Rhizoma Curcumae (*Ezhu*) according to Traditional Chinese Medicine	
Taste:	bitter and pungent[1,5]
Temperature:	warm[5]
Channels entered:	acts on *orbis hepaticus* and *orbis lienalis*[5]
Effects:	to promote the flow of *qi* and *xue*, to eliminate *blood stasis* with strong effect, and to relieve pain by removing the stagnation of undigested food[1,5]
Symptoms and indications:	mass in the abdomen, amenorrhea due to *blood stasis*, distension and pain due to stagnation of undigested food; carcinoma of cervix at early stage[1]

Effects and indications of Radix Curcumae (*Yujin*) according to Traditional Chinese Medicine	
Taste:	bitter and pungent[1,5]
Temperature:	cold[5]
Channels entered:	acts on *orbis cardialis, orbis pulmonalis, orbis hepaticus*[5]
Effects:	to promote the flow of *qi*, to eliminate *blood stasis*, to calm the nerves and ease the mind, and to increase the flow of bile, remove *calor humidus*, to cool *calor* and *xue*[1,5]
Symptoms and indications:	amenorrhea, dysmenorrhea, distending or pricking pain in the chest and abdomen; impairment of consciousness in febrile diseases, epilepsy, mania; jaundice with dark urine[1]

Contraindication:

Rhizoma Curcumae longae *(Jianghuang)*:

Contraindicated in every *depletio*, specially in stagnation of *Xue* and *Qi* [5]

Rhizoma Curcumae *(Ezhu)*:

Contraindicated in pregnancy [1], in *depletio* of *xue* and *qi* and strong menstrual disorders [5]

Radix Curcumae *(Yujin)*:

Contraindicated in pregnancy [2]

Main constituents[4] (see Fig. 1):

Rhizoma Curcumae longae:

• **diarylheptane derivatives (curcuminoids)** (~3.5 %):

- bisferuloyl-methane = curcumin I
- 4-hydroxycinnamoyl feruloyl methane (demethoxy-curcumin) = curcumin II
- bis(4hydroxy-cinnamoyl)-methane (bisdemethoxy-curcumin) = curcumin III

• **essential oil** (-4 %):

- sesquiterpene ketones: *ar*-turmerone, turmerone α, β-turmerone, curlone
- mono-terpenoids: α-, β-pinene, camphene, limonene, terpinene, curcumene, caryophyllene, linalool, borneol, isoborneol, camphor, eugenol, cineol, curdione, curzerenone

Rhizoma Curcumae kwangsiensis:

• low concentrations of curcumin I, II and III (see Fig. 2a, sample 12)

• essential oil composed mainly of monoterpenoids and sesquiterpenoids (e.g. β-pinene, 1,8-cineol, linalool, and some ketones such as germacrene)

Rhizoma Curcumae wenyujinae:

• no curcumin I, II and III (see Fig. 2a, samples 7–9)

• essential oil (e.g. sesquiterpenoids such as curcumol, germacrene D,β-elemene, wenjine and germacrone-diepoxide)

Rhizoma Curcumae xanthorrhizae (for TLC-comparison):

• absence of bisdemethoxycurcumin

• essential oil: 1-cyclo-isoprenmyrcene, xanthorrhizol (hydroxy-*ar*-curcumene) and 4-tolylcarbinol as the major constituents

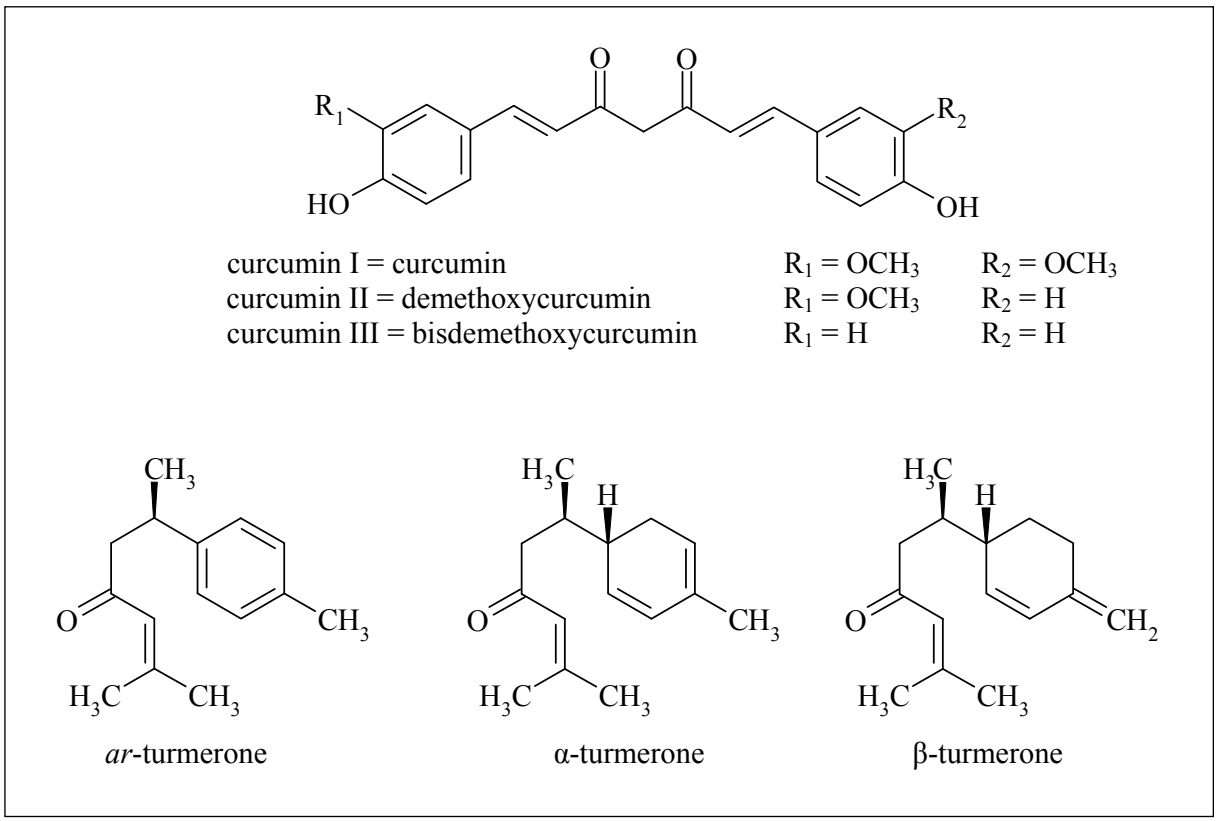

Fig. 1: Formulae of the main constituents of Rhizoma Curcumae longae[4,6]:

Pharmacology:

Curcuminoids:

- anti-inflammatory and antioxidant activity[7,8]
- wound healing[7,8]
- antipeptic ulcer and liver protecting activity; choleretic effects[9]
- effect on the cardiovascular system[7]
- anticoagulant activity[7]
- antimicrobial and antibacterial effects[7,8]
- antifungal effects[7]
- antitumoral activity (antiangiogenic)[8,9,10,11]
- anticarcinogenic activity[7,8,12]
- antifibrotic activity[13]
- neuroprotective activity[14]
- cholekinetic [15]
- antihyperlipidemic [15]

Essential oil[15]:

- antiinflammatory
- antioxidant
- antitumoral
- antimicrobial
- antifebril

TLC- fingerprint- analysis of the MeOH-extracts of various Rhizoma and Radix Curcuma species (Fig. 2a and Fig. 2b)[16]:

1) Extraction: Powdered drug (1.0 g) is extracted with 5 ml methanol for 15 min. under reflux on the 80 °C warm water bath. The extract is filtered.

2) Reference compound: Curcumin (1 mg/ml)

3) Separation parameters:

Applied amount:
7 µl Rhizoma Curcumae longae extract
17 µl Radix Curcumae extract
12 µl Rhizoma Curcumae extract
7 µl curcumin standard solution

Plate: HPTLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: chloroform : ethanol : acetic acid (95 : 5 : 1)

Detection: a) Direct evaluation in UV 365 nm (Fig. 2a)

b) Spray reagent:
Vanillin-sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I followed immediately with solution II. Then the plate is heated for 5 – 10 minutes at 105 °C and evaluated in VIS (Fig. 2b)

TLC-fingerprint-analysis of essential oil of various Rhizoma and Radix Curcumae species (Fig. 3)[16]:

1) Extraction: The powdered drug is subjected to a water steam distillation in a Neo Clevenger apparat. The essential oil is diluted with hexane 1 : 5.

2) Separation parameters:

Applied amount: 7 µl diluted essential oil

Plate: TLC-Plate Silicagel 60 F_{254}; Merck

Solvent system: toluene – ethyl acetate (93 : 7)

Detection: Spray reagent:
Vanillin-sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I followed immediately with solution II. Then the plate is heated for 5 – 10 minutes at 105 °C and evaluated in VIS

Drug samples		Origin
1	Rhizoma Curcumae longae / *Curcuma longa*	locality Sichuan; China
2	Rhizoma Curcumae longae / *Curcuma longa*	locality Sichuan; China
3	Rhizoma Curcumae longae / *Curcuma longa*	locality Sichuan; China
4	Rhizoma Curcumae longae / *Curcuma longa*	sample of commercial product obtained from Hellmuth Carroux GmbH & Co. KG Hamburg; Germany
5	Rhizoma Curcumae longae / *Curcuma longa*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
6	Rhizoma Curcumae longae / *Curcuma longa*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
7	Radix Curcumae / *Curcuma wenyujin*	locality Zhejiang, Wenzhou; China
8	Radix Curcumae / *Curcuma wenyujin*	locality Zhejiang; China
9	Radix Curcumae / *Curcuma wenyujin*	locality Zhejiang, Jin Hua; China
10	Radix Curcumae (from unknown species)	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
11	Radix Curcumae (from unknown species)	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
12	Rhizoma Curcumae / *Curcuma kwangsiensis*	sample of commercial product obtained from Herbasin Hilsdorf GmbH Rednitzhembach; Germany
13 (for comparison)	Rhizoma Curcumae xanthorrhizae / *Curcuma xanthorrhiza*	sample of commercial product obtained from Alfred Galke GmbH Gittelde/Harz; Germany
14 (for comparison)	Rhizoma Curcumae xanthorrhizae / *Curcuma xanthorrhiza*	sample of commercial product obtained from Heinrich Klenk GmbH & Co. KG Schwebheim; Germany

Reference compound		R*f*
T	Curcumin	0.67

4) Description of the HPTLC-fingerprints of the MeOH-extracts of various Rhizoma and Radix Curcuma spezies in UV 365 nm (Fig. 2a) and in VIS after sprayed with vanillin-sulphuric-acid-reagent (Fig. 2b)[16]:

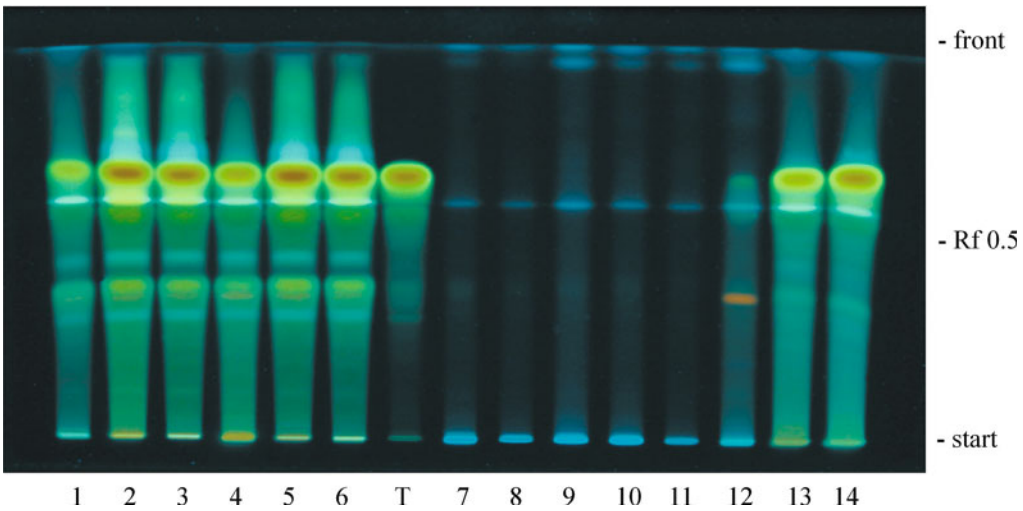

Fig. 2a: HPTLC-fingerprint of the MeOH-extracts of various Rhizoma and Radix Curcuma species in UV 365 nm

All extract samples of Rhizoma Curcumae longae (1-6) show in UV 365 nm prominent yellow-green fluorescent zones of curcumin (T) at R*f* 0.67, directly below demethoxycurcumin at R*f* 0.58 and bisdemethoxycurcumin at R*f* 0.39.

In all Radix Curcumae wenyujin extract samples (7-9) only a weak blue fluorescent zone (dihydrocurcumin?) at R*f* 0.6 can be detected. Obviously they are devoid of any of the curcuminoids.

In the extract sample of Rhizoma Curcumae kwangsiensis (12) curcuminoids are also absent or present only in traces.

The Rhizoma Curcumae xanthorrhizae extract samples (13,14) contain high amount of curcumin (T, R*f* 0.67) and a small amount of demethoxycurcumin. Bisdemethoxycurcumin (R*f* 0.39) as the characteristic curcuminoid of all other Rhizoma Curcumae longae samples (1 – 6) is not detectable.

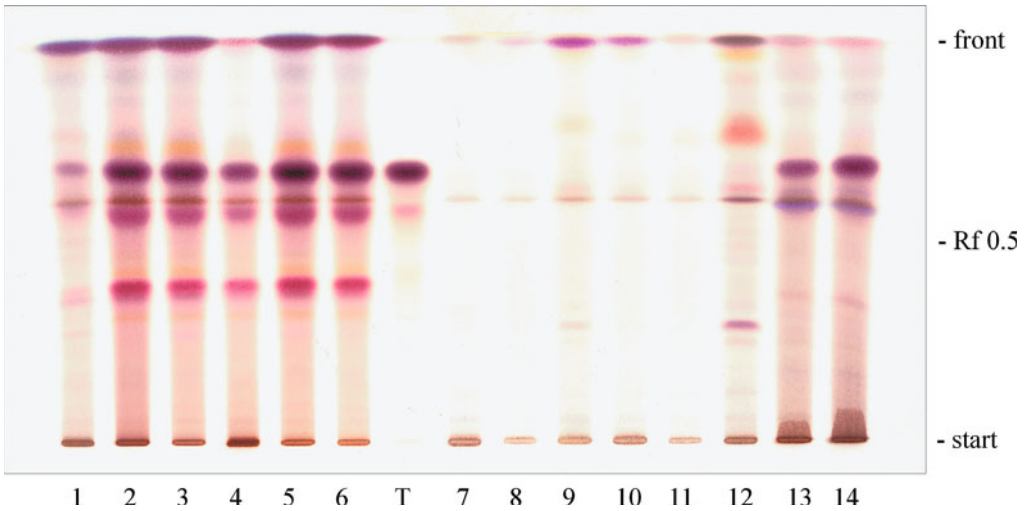

Fig. 2b: HPTLC-fingerprint of the MeOH-extracts of various Rhizoma and Radix Curcuma species detected with vanillin-sulphuric-acid-reagent in VIS

All curcuminoids sprayed with vanillin-sulphuric-acid-reagent appear in VIS as pink-violet zones.

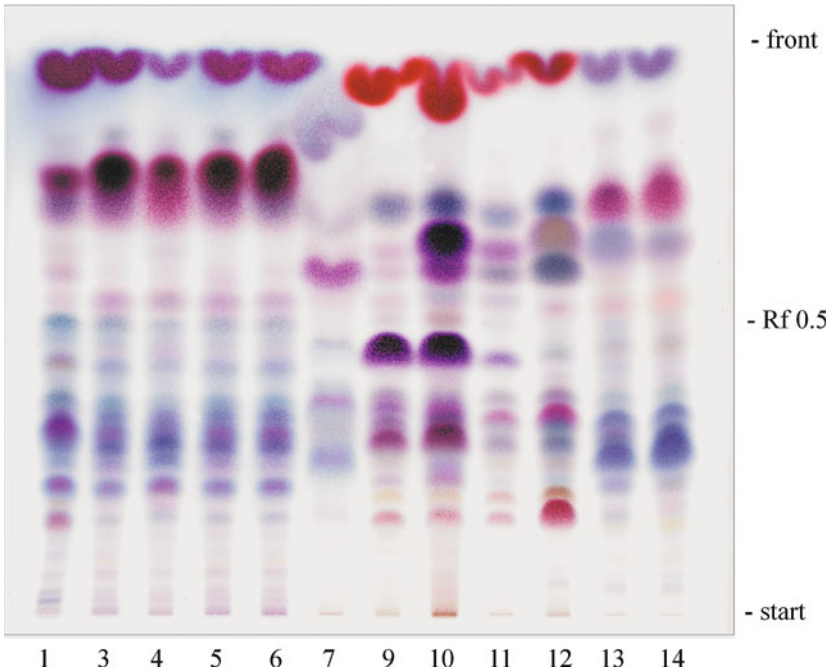

Fig. 3: TLC-fingerprint of the essential oil of various Rhizoma and Radix Curcuma species detected with vanillin-sulphuric-acid-reagent in VIS

In the fingerprint pattern of the essential oil we can discriminate three types:

Samples 1 – 6; 7, 9 – 12 and 13/14.

The essential oil samples 1 – 6 are characterized by one violet zone of mono- and sesquiterpen-hydrocarbons on the solvent front and a strong violet zone at R*f* 0.75.

In sample 7, 9 – 12 the zone on the solvent front has a characteristic carminred colour.

In the R*f*-range of 0.6 – 0.75 the ketons and phenolic terpenoids can be localized. In the R*f*-range 0.2 – 0.4 the monoterpene alcohols are detectable. The terpenoid pattern of samples 13 and 14 (*C. xanthorrhiza*) is very similar to that of the samples 1 – 6.

Note: In the Pharmacopoeia of the People's Republic of China 2005 for Rhizoma Curcumae longae a TLC-method for the identification of the main constituent curcumin is described.

For Rhizoma Curcumae longae and Rhizoma Curcumae only the quantitative determination of volatile oil content is demanded (not less than 7.0 % in Rhizoma Curcumae longae and 1.5 % in Rhizoma Curcumae respectively)[1].

HPLC- fingerprint- analysis:[1,17]:

1) Sample preparation: The methanol extract, used for HPTLC is filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-apparatus.

2) Injection volume: 5 µl extract and reference solution

3) HPLC- data:

Apparatus:	L-6200A Intelligent Pump, AS-2000 Autosampler, L-4500A Diode Array Detector, D-6000A Interface; Merck Hitachi
Column:	LiChroCART® 250-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck
Solvent system:	A: 0.2 % acetic acid (made with water, Millipore Ultra Clear UV plus® filtered and Suprapur® acetic acid (glacial) 100 % Merck KGaA) B: acetonitrile, HPLC quality Acros Organics
Gradient:	50 % B to 60 % B in 3.5 min. (linear) 60 % B for 6.5 min. (isocratic) 60 % B to 70 % B in 5 min. (linear)
Flow rate:	1.0 ml/min.
Detection:	422 nm

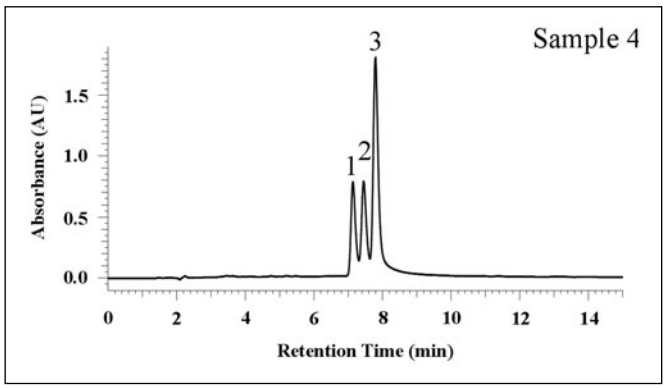

Fig. 4a: Characteristic HPLC fingerprint of all Rhizoma Curcumae longae extracts (1 – 6), shown for sample 4

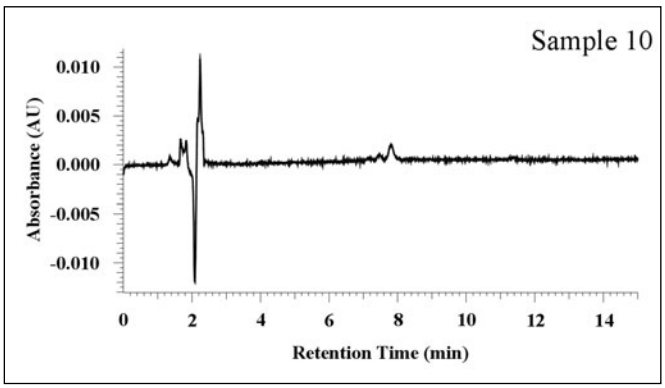

Fig. 4b: Characteristic HPLC fingerprint of all Radix Curcumae wenyujin extracts (7 – 9), shown for sample 7

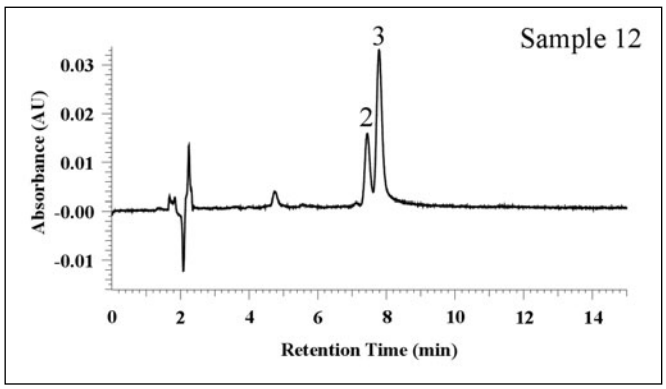

Fig. 4c: HPLC fingerprint of Rhizoma Curcumae kwangsiensis extract, sample 12

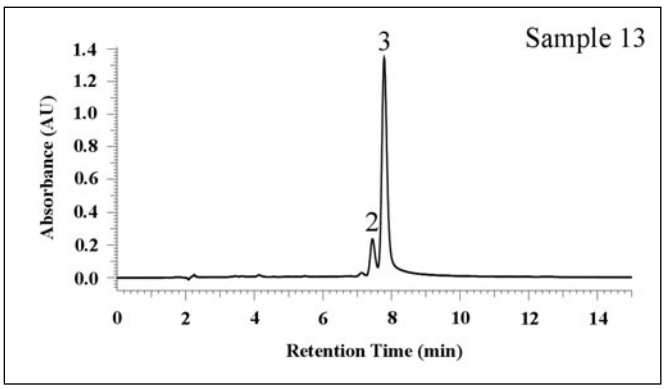

Fig. 4d: Characteristic HPLC fingerprint of all Rhizoma Curcumae xanthorrhizae extracts (13, 14), shown for sample 13

Retention times and identity of the main peaks of Fig. 4a – Fig. 4d:

Peak	Rt (min.)	Compound
1	7.1	Bisdemethoxycurcumin
2	7.5	Demethoxycurcumin
3	7.8	Curcumin

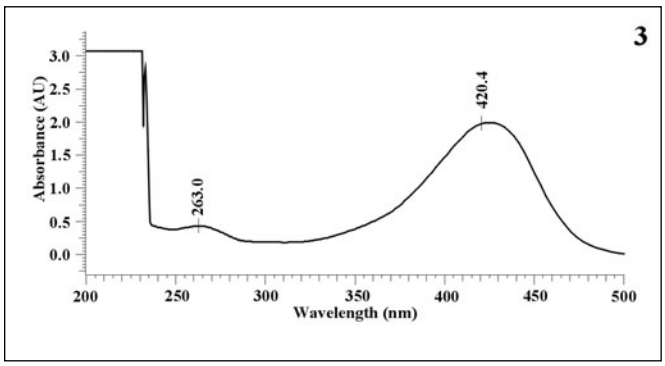

Fig. 5: Online UV-spectrum of the main constituent curcumin of *Curcuma* spp.

4) Description of the HPLC-fingerprints of Fig. 4a – Fig. 4d and the online UV-spectra:

The HPLC-fingerprints of all Rhizoma Curcumae longae (1 – 6) samples show a very similar qualitative and quantitative peak-pattern with the predominant peak of curcumin (**3**) at Rt 7.8 min. and the smaller peaks of demethoxycurcumin (**2**) at Rt 7.5 and bisdemethoxycurcumin (**1**) at Rt 7.1.

The HPLC-fingerprints of all Radix Curcumae wenyujin samples (7 – 11) samples show only traces of curcuminoids.

The HPLC-fingerprint of Rhizoma Curcumae kwangsiensis sample (12) shows only weak peaks of Demethoxycurcumin and Curcumin at Rt 7.8 and 7.5.

The HPLC-fingerprints of all Rhizoma Curcumae xanthorrizae samples (13, 14) show one predominant peak of curcumin at Rt 7.8 and a small peak of demethoxycurcumin at R*f* 7.5. The peak of bisdemethoxycurcumi at Rt 7.1 is missing.

All curcuminoids of *Curcuma* spp. are characterized by an UV-spectrum with a maximum at 422 nm.

Note: In the Pharmacopoeia of the People's Republic of China 2005 for Rhizoma Curcumae longae a HPLC-assay is described for the quantitative determination of curcumin (not less than 1.0 %) [(1)].

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Rhizoma Dioscoreae oppositae – *Shanyao*
Rhizoma Dioscoreae hypoglaucae – *Fenbixie*
Rhizoma Dioscoreae nipponicae – *Chuanshanlong*
Rhizoma Dioscoreae septemlobae – *Mianbixie*

Pharmacopoeia[1]**:** Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1-4]**:** Common Yam Rhizome is the dried rhizome of *Dioscorea opposita* Thunb. ("Shanyao"). The drug is also known under the name *Dioscorea batatas* Decaisne.

Hypoglaucous Collett yam Rhizome is the dried rhizome of *Dioscorea hypoglauca* Palibin ("Fenbixie").

Nippon Yam Rhizome is the dried rhizome of *Dioscorea nipponica* Makino ("Chuanshanlong").

Sevenlobed Yam Rhizome is the dried rhizome of *Dioscorea septemloba* Thunb. or *Dioscorea futschauensis* Uline ex R. Kunth ("Mianbixie").

– Dioscoreaceae –

Origin[4,5]**:** Different provinces of southern China (Henan, Zhejiang, Jiangsu, Hunan, Yunnan and Fujian)

Description of the drugs: *Dioscorea opposita*[1,3]: Subcylindrical, curved or somewhat flattened, 15-30 cm long, 1.5-6 cm in diameter. Externally yellowish-white or pale yellow, longitudinally furrowed and wrinkled, and bearing fibrous root scars, with occasional patches of brownish cork. Texture heavy, compact and tough; uneasily broken, fracture white and starchy.

Odourless. Taste: weak, sweet, acidulous and viscous when chewed.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Dioscorea hypoglauca[1]: Occurring in irregular thin slices, border uneven, varying in size, about 0.5 mm thick, some with brownish-black or greyish-brown outer bark. Cut surface yellowish-white or pale greyish-brown, vascular bundles scattered. Texture loose, slightly elastic.

Odour: weak. Taste: pungent and slightly bitter.

Dioscorea nipponica[1]: Subcylindrical, slightly curved, 15-20 cm long, 1.0-15 cm in diameter. Externally yellowish-white or brownish-yellow, irregularly and longitudinally furrowed, bearing spinous remains of roots and protuberant stem scars on one side. Texture hard, fracture even, white or yellowish-white, scattered pale brown dotted vascular bundles.

Odour: slight. Taste: bitter and astringent.

Dioscorea septemloba and *Dioscorea futschauensis*[1]: Occuring in irregular oblique slices, border uneven, varying in size, about 2-5 mm thick. Externally yellowish-brown or brownish-black, sparsely bearing remains of fibrous roots, protruding conically. Cut surface greyish-white to pale greyish-brown, yellowish-brown spots of vascular bundle scattered. Texture loose, somewhat spongy. Odour: slight. Taste: slightly bitter.

Pretreatment of the raw drugs[1]:

Dioscorea opposita: The drug is collected in winter when the stem and leaves are withered, deprived of root stock, washed clean and deprived of outer bark and fibrous root, fumigated with sulfur, and then dried("Shanyao"). Otherwise the thick large straight and dried rhizome is sorted, soaked in clean water until the central portion of the drug gets wet and softened thoroughly, then fumigated with sulfur, cut two ends to smooth plane and rubbed on a board to become cylindrical in shape, dried in the sun and finally polished ("Guang Shanyao").

Processing:
Rhizoma Dioscoreae: Eliminate foreign matter, grade according to size, soak and soften thoroughly, cut into thicker slices, and dry.

Rhizoma Dioscoreae (stir-baked with bran): The bran is put in a hot pot and heated until it smokes, then the clean crude drug is added and stirred quickly until the surface of crude drugs turns yellow or dark, then taken out, sifted out and cooled. 10 kg bran are used for each 100 kg of the clean crude drug.

Dioscorea hypoglauca: The drug is collected in autumn and winter, removed from the fibrous root, washed clean, cut into slices and dried in the sun.

Dioscorea nipponica: The drug is collected in autumn or spring, washed clean, removed from fibrous roots and outer bark, cut into slices and dried in the sun.

Dioscorea septemloba and *Dioscorea futschauensis*: The drug is collected in autumn and winter, removed from fibrous root, washed clean, cut into slices, and dried in the sun.

Medicinal use:

Dioscorea opposita:
Used mainly for the treatment of diarrhea, asthma, polyuria, and diabetes[2].

Dioscorea hypoglauca:
Used against rheumatoid arthritis[2,6].

Dioscorea nipponica:
Used in folk medicine mainly for rheumatic diseases, pain and numbness of the lower back and legs, chronic bronchitis, cough and asthma[1]. The major sapogenin diosgenin is an important starting material for the synthesis of steroid hormones[2].

Dioscorea septemloba and *Dioscorea futschauensis*:
Used against rheumatic diseases[2].

Effects and indications of *Dioscorea opposita* according to Traditional Chinese Medicine	
Taste:	sweet[3-5,7,8] or pungent and slightly bitter[1]
Temperature:	neutral[3,7,8]
Channels entered:	*Orbis lienalis, orbis pulmonalis, orbis renalis*[8]
Effects:	To replenish the spleen and stomach, to promote fluid secretion and benefit the lung, and to strengthen the kidney and restrain seminal discharge[1].
Symptoms and indications:	*Qi* and *Yin* supporting and supplementing, *Orbes* harmonising and supporting[7]: Anorexia and chronic diarrhea due to diminished function of the spleen, cough and dyspnea due to diminished function of the lung, seminal emission, excessive leukorrhea, frequency of urination or diabetes due to deficiency of the kidney[1].

Effects and indications of *Dioscorea hypoglauca* according to Traditional Chinese Medicine	
Taste:	pungent and bitter[1,7,8]
Temperature:	neutral[7,8]
Channels entered:	*Orbis renalis, orbis stomachi, orbis hepaticus, orbis vesicalis*[7,8]
Effects:	To remove turbid *damp*, and to relieve rheumatic condition[1].
Symptoms and indications:	*Humor, Humor venti* and *calor humidus* purging[7]: Chyluria, turbid urine mixed with whitish substances or whitish discharge from urethra, excessive leukorrhea, rheumatic arthralgia with limitation of motion and pain in the loins and knees[1].

Effects and indications of *Dioscorea nipponica* according to Traditional Chinese Medicine [1]	
Taste:	bitter and astringent
Temperature:	warm
Channels entered:	*Orbis pulmonalis*
Effects:	To expel *wind-dampness*, relieve pain, relax muscles and tendons, promote blood circulation, to relieve cough and asthma and resolve phlegm[1].
Symptoms and indications:	Rheumatic arthritis, pain and numbness of the lower back and legs, Kaschin-Bek disease, injury from falling, lumbar sprain, chronic bronchitis, cough and asthma[1].

Effects and indications of *Dioscorea septemloba* and *Dioscorea futschauensis* according to Traditional Chinese Medicine[1]	
Taste:	slightly bitter
Temperature:	neutral
Channels entered:	*Orbis vesicalis, orbis stomachi*
Effects:	To remove turbid *damp*, and to relieve rheumatic conditions.
Symptoms and indications:	Gonorrhea with whitish discharge from the urethra, excessive leukorrhea; ulcers caused by *damp-heat*, arthralgia of the lumber spine and knees

Contraindication/ Precaution:

<u>*Dioscorea opposita:*</u> Contraindicated in every kind of repletio[7], obstipation[5] and retention of food[3].

<u>*Dioscorea hypoglauca*:</u> Contraindicated in *Yin*-deficiency and debility in *orbis depletio* or *renalis*[7].

<u>*Dioscorea nipponica*:</u> Defend from anaphylaxis while crushing or processing[1].

Constituents (see Fig. 1):

Steroid saponins and sapogenins are the main characteristic constituents of all *Dioscorea* species.

Dioscorea opposita:
Main constituents:
- **steroid saponins and sapogenins** (diosgenin)[2,9]
- batatasins I (phenanthrenderivatives), II, III, IV and V (dihydrostilben derivatives)[2,9]
- 3,4,6-trihydroxyphenanthrene-3-O-β-D-glucopyranoside[9]
- mucilages[9]

Minor constituents:
- soyacerebroside[9], adenosine[9], β-sitosterol[9], palmitic acid[9], palmitoyloleoylphosphatidcholine[9], giberellins[9], allantoin[9,10], phytoalexin[9], dioscorin[9]

Dioscorea hypoglauca:
- dioscin, prosapogenin A of dioscin[18]
- methyl protodioscin[11,12], protoneodioscin[13] and methylprotoneosdioscin[14]
- gracillin, methyl protoneogracillin[16,17,18]
- hypoglaucine A and protohypoglaucine A[2], hypoglaucin G (pregnane glycoside)[15]
- diosgenin, diosgenin acetate and diosgenin palmitate[2]
- $\Delta^{3,5}$-deoxytigogenin and - $\Delta^{3,5}$-deoxyneotigogenin[2]
- yamogenin, yamogenin acetate and yamogenin palmitate[2]
- β-sitosterol[2]

Dioscorea nipponica:
Main constituents:
- dioscin[2]
- diosgenin[2]

Minor constituents:
- piscidic acid (= p-hydroxy benzyl tartaric acid)[2]

Dioscorea septemloba and *Dioscorea futschauensis*:

- dioscin[2,19] and prosapogenin A[19] and B of dioscin[20]
- gracillin[2,19] and protogracillin[21]
- dioscorone A[22]
- trillin[2]
- yamogenin[2]
- diosgenin and diosgenin palmitate[2]
- $\Delta^{3,5}$-deoxytigogenin[2]
- β-sitosterol[2]

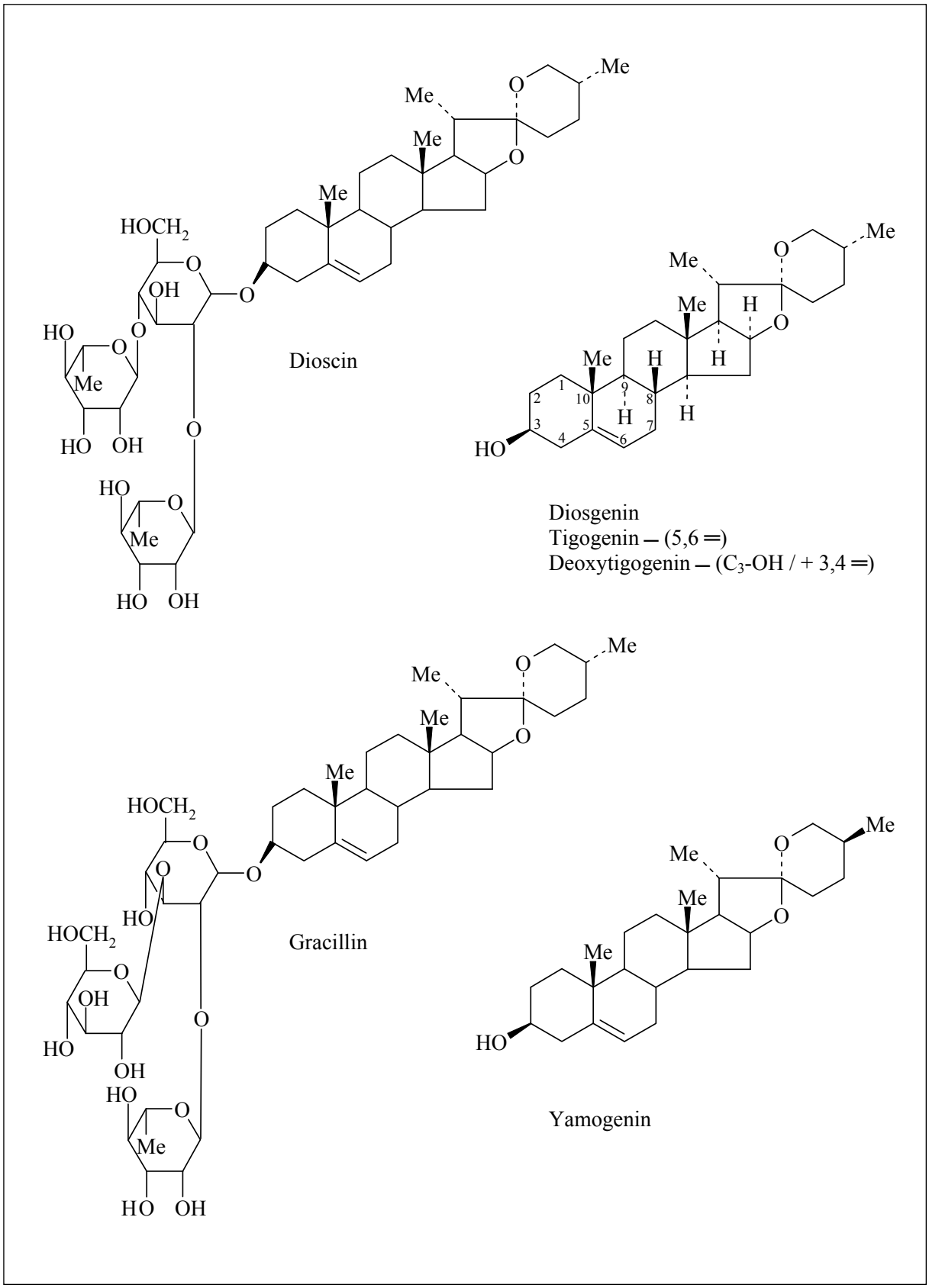

Fig. 1: Formulae of the main constituents[2]

Pharmacology:

Dioscorea opposita:
- immunostimulating activity (phagocytosis enhancing effect)[23]

Dioscorea hypoglauca:
- antitumoral activity (leukemia, CNS- and prostate cancer) (methylprotodioscin, gracillin)[11-14,16-18]

Dioscorea nipponica:
- anti-obesity effect[24]
- anti-brucellosis activity[25]

Dioscorea futschauensis:
- anticancer activity[19-22,26]
- antifungal activity[19,21,22,27]
- osteoplastic proliferation stimulatory activity[27]

TLC fingerprint analysis of steroid saponins and steroid sapogenins[28,29]:

1) Extraction: 1.0 g powdered drug is extracted with 20 ml ethanol 70 % under reflux for 15 min. The extract is filtered and the filtrate evaporated to about 1 ml. The residue is dissolved in 10 ml water and shaken with 10 ml water-saturated *n*-butanol. The *n*-butanol phase is separated and concentrated to dryness. The hydrophile phase is shaken again with 5 ml water-saturated *n*-butanol. The *n*-butanol phases are combined and evaporated to dryness. The residue is dissolved in 1.0 ml ethanol.

2) Reference compounds: Diosgenin, dioscin, progenin II, parrisaponin = diosgenin 3-O-α-L-rhamnopyranosyl-(1→4)- α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, methylprotodioscin, asperoside, sarsapogenin, prosapogenin A of dioscin, β-sitosterol (1 mg/ml MeOH)

3) Separation parameters:

Applied amount: 10 µl extract and standard solution

Plate: HPTLC- Plate Silicagel 60 F_{254}; Merck

Solvent system: chloroform – methanol – water (13 : 7 : 2), lower phase

Detection: Spray reagents:

Anisaldehyde-sulphuric acid reagent:
0.5 ml anisaldehyde are mixed with 10 ml glacial acetic acid and then 85 ml methanol and 5 ml concentrated sulphuric acid are added. The TLC plate is sprayed with 10 ml, heated for 5-10 minutes at 100 °C and then evaluated in VIS and in UV 365 nm.

TLC – fingerprint – analysis of steroid sapogenins after hydrochloric acid hydrolysis of *Dioscorea* spp. extracts[1]:

1) Extraction:	1.0 g powdered drug is ultrasonicated with 50 ml methanol for 30 min. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 20 ml 3 mol/l hydrochloric acid solution and heated on a water bath for 30 min. to hydrolyze all steroid glycosides of the extract. After cooling, 20 ml chloroform are added and heated under reflux for 15 min. After cooling the chloroform-layer is separated, evaporated to dryness and the residue dissolved in 2.5 ml methanol.
2) Reference compound:	Diosgenin (1 mg/ml MeOH)
3) Separation parameters:	
Applied amount:	10 µl extract and standard solution
Plate:	HPTLC-Plate Silicagel 60 F_{254}; Merck
Solvent system:	toluene – acetone (9 : 1)
Detection:	Spray reagents:
	Anisaldehyde-sulphuric acid reagent:
	0.5 ml anisaldehyde are mixed with 10 ml glacial acetic acid and then 85 ml methanol and 5 ml concentrated sulphuric acid are added. The TLC plate is sprayed with 10 ml, heated for 5-10 minutes at 100 °C and then evaluated in VIS and in UV 365 nm.

Drug samples		Origin
1	Rhizoma Dioscoreae	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
2	Rhizoma Dioscoreae / *Dioscorea opposita*	locality Hebei; China
3	Rhizoma Dioscoreae / *Dioscorea opposita*	locality Henan; China
4	Rhizoma Dioscoreae hypoglaucae / *Dioscorea hypoglauca*	locality Zhejiang; China
5	Rhizoma Dioscoreae hypoglaucae / *Dioscorea hypoglauca*	sample of commercial product obtained from sinoMed GmbH Kötzting; Germany
6	Rhizoma Dioscoreae septemlobae / *Dioscorea septemloba*	locality Fujian; China
7	Rhizoma Dioscoreae septemlobae / *Dioscorea septemloba*	locality Guangdong; China
8	Rhizoma Dioscoreae septemlobae / *Dioscorea septemloba*	locality Zhejiang; China
9	Rhizoma Dioscoreae septemlobae / *Dioscorea futschauensis*	locality Fujian; China
10	Rhizoma Dioscoreae septemlobae / *Dioscorea futschauensis*	locality Hebei; China
11	Rhizoma Dioscoreae nipponicae / *Dioscorea nipponica*	locality Hebei; China

Reference compounds		R*f*
T1	Diosgenin Dioscin	0.97 (Fig. 2a/2b), 0.37 (Fig. 3a/3b) 0.59
T2	Progenin II Dioscin Parrisaponin	0.76 0.59 0.50
T3	Methylprotodioscin Dioscin derivative Asperoside	0.33 0.28 0.19
T4	Sarsapogenin Prosapogenin A of dioscin	0.97 0.67
T5	β-Sitosterol	0.97

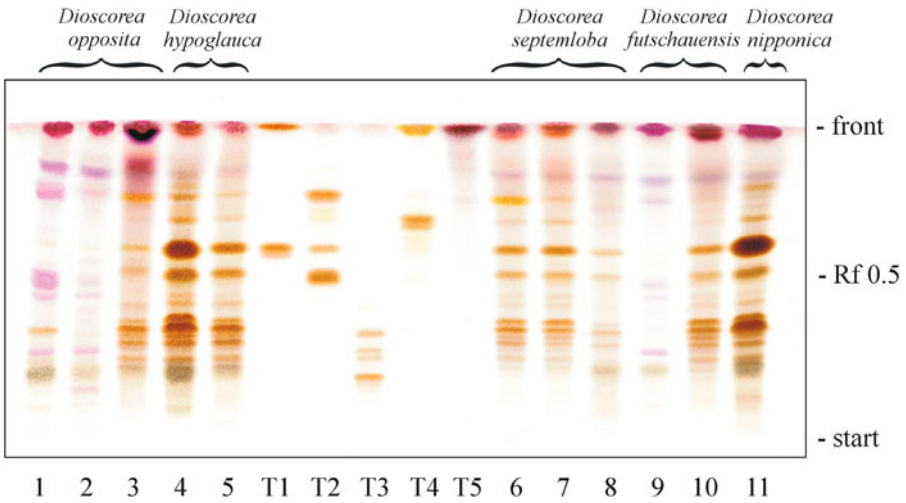

Fig. 2a: HPTLC-fingerprint analysis of the *n*-butanol phases of the ethanol extracts of *Dioscorea* spp. in VIS

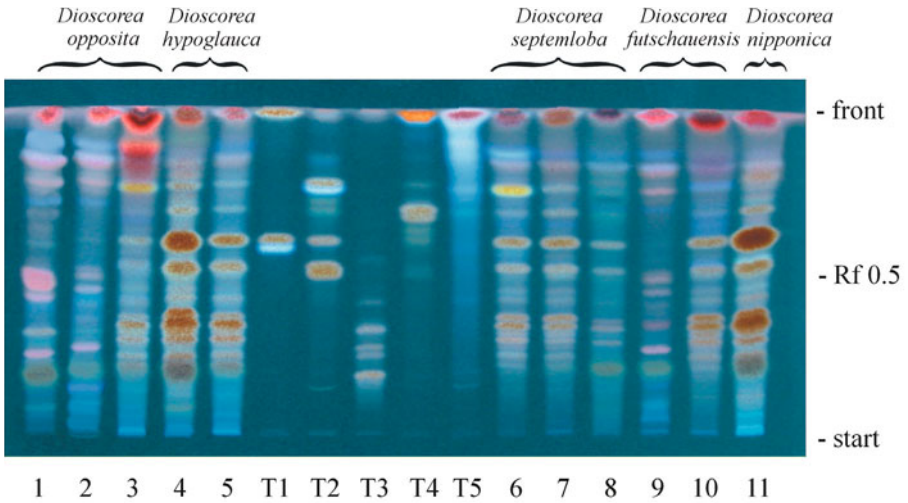

Fig. 2b: HPTLC-fingerprint analysis of the *n*-butanol phases of the ethanol extracts of *Dioscorea* spp. in UV 365 nm

4) Description of the HPTLC-fingerprint of the *n*-butanol phases of the ethanol extracts of *Dioscorea* spp. in VIS (Fig. 2a) and UV 365 nm (Fig. 2b):

The HPTLC-fingerprint of all extract samples (1-11) sprayed with anisaldehyde-sulphuric acid reagent show with some exceptions (sample 1, 2 and 8, 9 of *Dioscorea opposita* and *Dioscorea futschauensis*) a qualitatively homogenous but quantitatively different pattern of brownish zones in two R*f*-ranges:

- The R*f*-range from ~0.7 to the solvent front with the zones of the steroid sapogenins diosgenin (T1) and β-sitosterol (T5) at R*f* 0.97.
- The R*f*-range from ~0.25 to ~0.6 with the various zones representing the bulk of steroid saponins.

Two dubletts in the R*f*-range of dioscin and another one in the R*f*-range 0.3 maybe the characteristic chromatographic fingerprints for *Dioscorea* species.

A distinct chromatographic discrimination between the various *Dioscorea* species is not possible with the *n*-butanol phases alone.

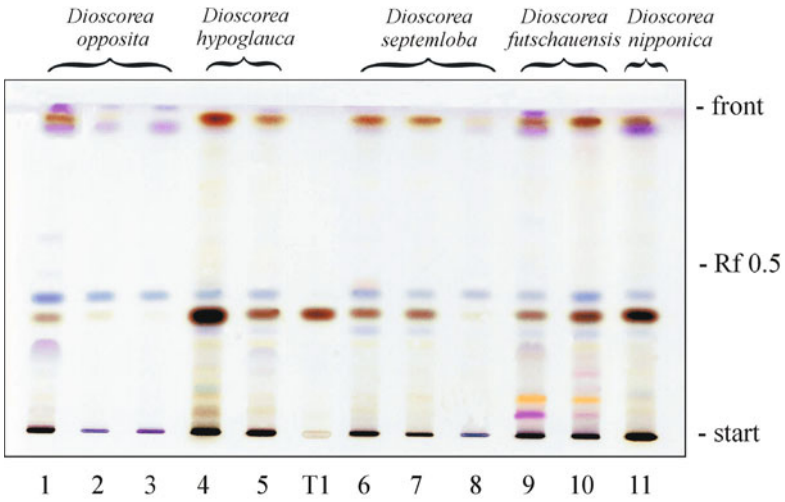

Fig. 3a: HPTLC-fingerprint analysis of the steroid sapogenins of *Dioscorea* spp. after acidic hydrolysis in the chloroform phases in VIS

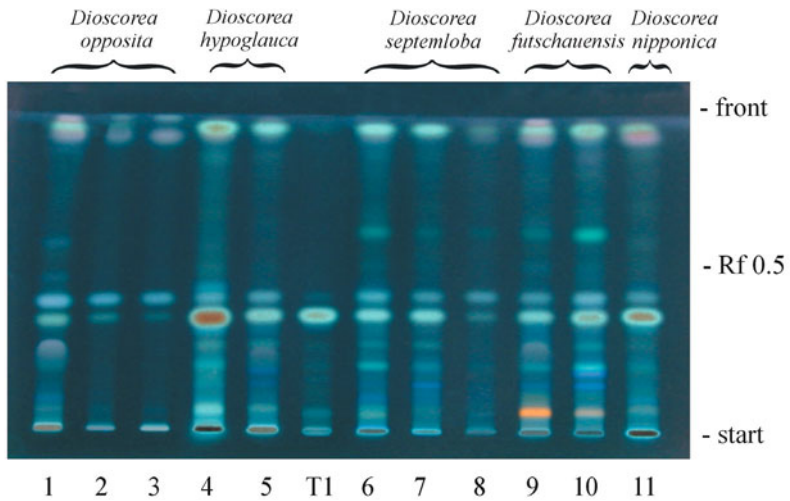

Fig. 3b: HPTLC-fingerprint analysis of the steroid sapogenins of *Dioscorea* spp. after acidic hydrolysis in the chloroform phases in UV 365 nm

Description of the HPTLC-fingerprint of the steroid sapogenins of *Dioscorea* spp. after acidic hydrolysis in the chloroform phases in VIS (Fig. 3a) and UV 365 nm (Fig. 3b):

Both chromatograms show a very simplified pattern of brownish and pink or blue fluorescent zones with diosgenin at R*f* 0.37 and a second just above which might be tigogenin, yamogenin or any other steroid sapogenin. The hydrolized extracts of samples 2, 3 and sample 8, *Dioscorea opposita* and *Dioscorea septemlobae* respectively, show only traces of diosgenin.

This chromatogram confirms that the major characteristic steroid saponins of *Dioscorea* species derive from diosgenin.

HPLC-fingerprint – analysis[1]**:**

1) Sample preparation: The extracts (steroid saponins and sapogenins) used for HPTLC are filtered through Millipore® Type HV 0.45 µm and injected into the HPLC-apparatus.

2) Injection volume: 25 µl extract and reference solution

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Column: LiChroCART® 125-4 LiChrospher® 60 RP-18 with LiChroCART® 4-4 LiChrospher® 60 RP-18 (5 µm); Merck

Solvent system: A: water, Millipore Ultra Clear UV plus® filtered
B: acetonitrile, HPLC quality Acros Organics

Gradient: 5 % B for 3 min. (isocratic)
5 % B to 95 % B in 27 min. (linear)
95 % B for 13 min. (isocratic)

Flow rate: 1.0 ml/min.

Detection: 210 nm

Retention times and identity of the reference compounds of *Dioscorea* spp.:

Peak	Rt (min.)	Compound
1	14.5	Dioscin
2	21.5 – 22.3	Parrisaponin
3	32.5	Progenin

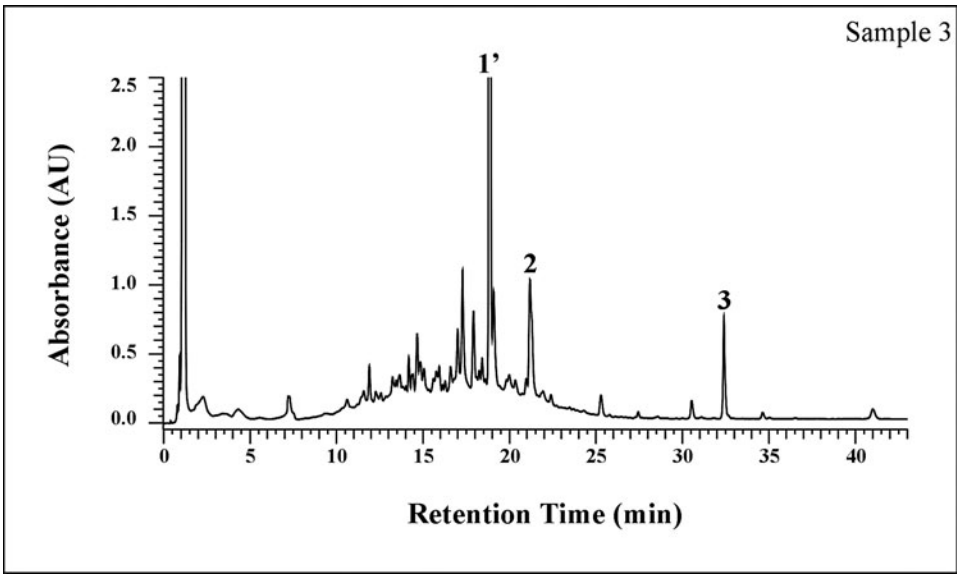

Fig. 4a: HPLC-fingerprint analysis of the *n*-butanol phases of the ethanol extracts of *Dioscorea opposita,* shown for sample 3

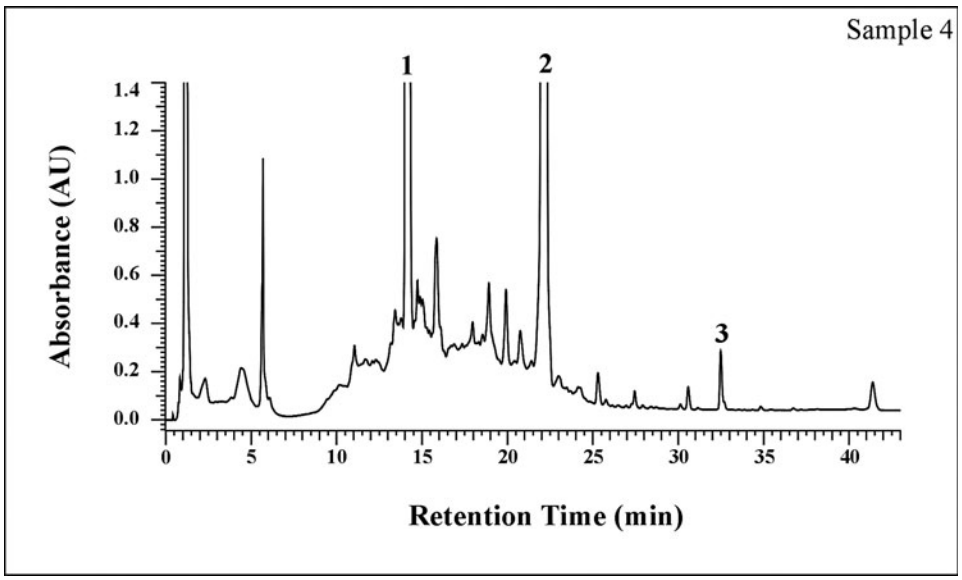

Fig. 4b$_1$: HPLC-fingerprint analysis of the *n*-butanol phases of the ethanol extracts of *Dioscorea hypoglauca,* shown for sample 4 and 5

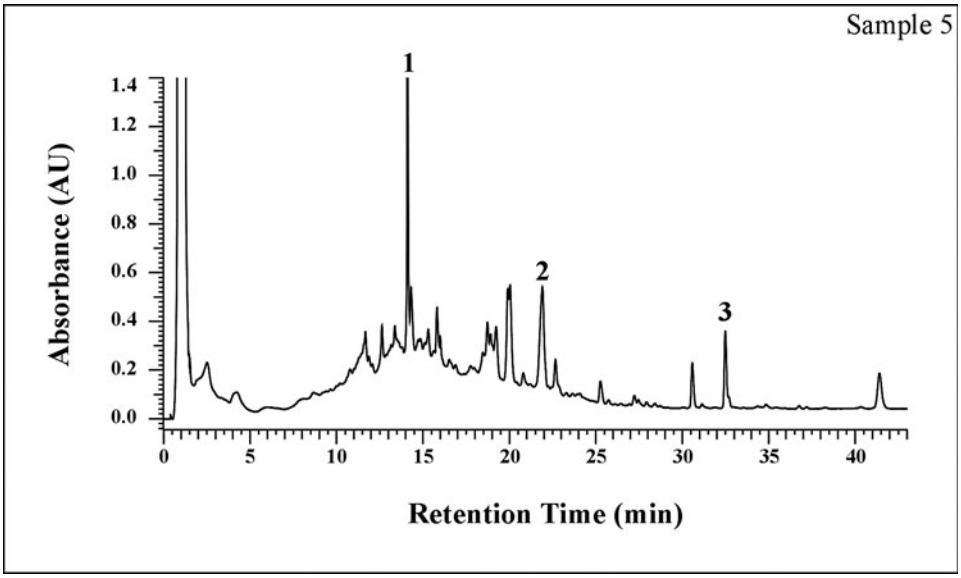

Fig. 4b$_2$: HPLC-fingerprint analysis of the *n*-butanol phases of the ethanol extracts of *Dioscorea hypoglauca,* shown for samples 4 and 5

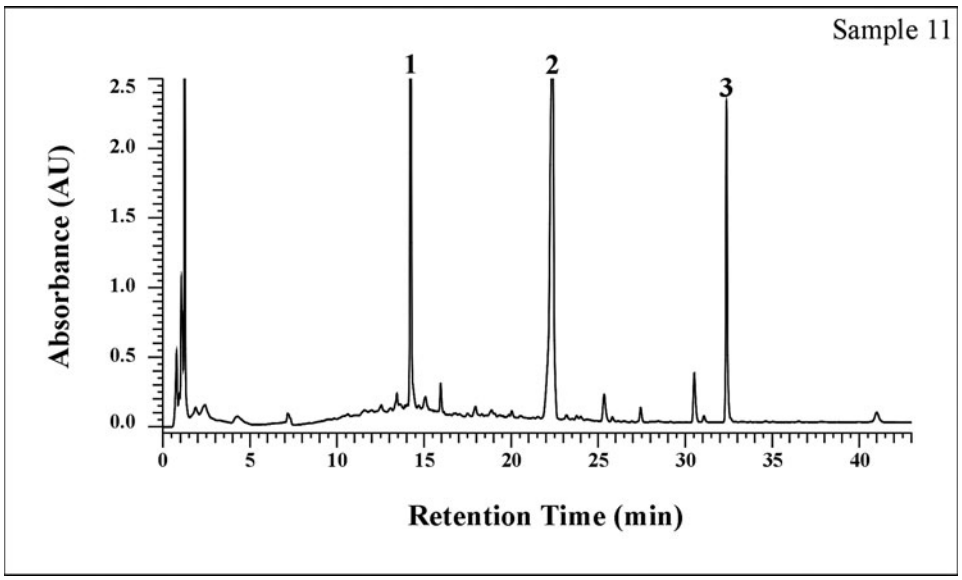

Fig. 4c: HPLC-fingerprint analysis of the *n*-butanol phases of the ethanol extracts of *Dioscorea nipponica,* shown for sample 11

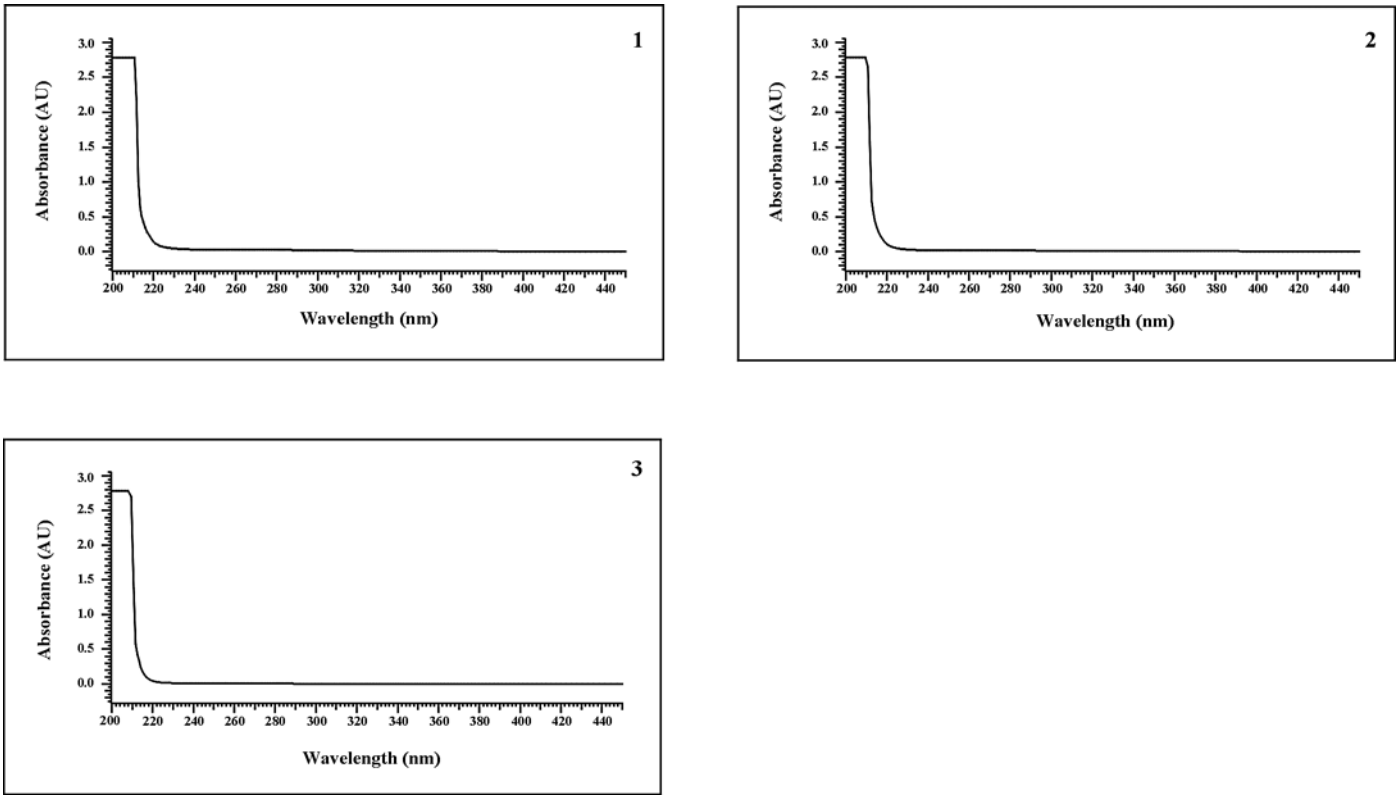

Fig. 5: Online UV-spectra of the typical saponins of *Dioscorea* spp. detected in HPLC

4) Description of the HPLC-fingerprints of the *n*-butanol phases of the ethanol extracts

Figure 4a

The HPLC-fingerprint analysis of *Dioscorea opposita* (sample 3) shows in the Rt-range of 10.0 to 25.0 an assembly of at least 8-10 peaks with a dominant but not identified saponin at Rt = 19.0 (**1'**) and four minor peaks at Rt = 14.8, 17.5, 17.8 and 21.5 (**2**) and a further peak of lower intensity at Rt = 32.5 (**3**). It is likely that in the assembly of the peaks between Rt ~ 10.0 and ~ 25.0 most of them derive from (Methyl)Dioscin derivatives. Since, however, *Dioscorea opposita* in contract to all other *Dioscorea* spezies contains additional phenanthrene- and stilben compounds, the deviating fingerprint of *D. opposita* from those of the other *Dioscorea* species might be the reason.

Figure $4b_{1,2}$

The sample 4 and 5 of *Dioscorea hypoglauca* show in their HPLC-fingerprints three distinct peaks at Rt = 14.5 (**1**), 22.3 (**2**) and 32.5 (**3**) beside minor peaks between the peaks 1 and 2. According to the TLC-chromatogram of Figure 2a the peaks **1**, **2** and **3** can be assigned to Dioscin, Parrisaponin and Progenin respectively.

Figure 4c

The HPLC-fingerprint of *Dioscorea nipponica* samples is characterized by three prominant peaks at Rt = 14.5 (**1**), 22.3 (**2**) and 32.5 (**3**). The latter peak is also present in the HPLC-fingerprints of *D. opposita* and *D. hypoglauca* but in much lower concentration. The distinct peak at Rt = 32.5 could be correspond with Progenin shown in TLC-Figure 2a as Progenin II with its R*f* = 0.76.

Note: *Dioscorea septemloba* and especially *D. futschauensis* differ in their TLC- and HPLC-fingerprints in many respects from those of the others *Dioscorea* species. Therefore we have renounced of a reproduction of the HPLC-fingerprints of both *Dioscorea* species, which do not allow a clear authenticity.

Conclusion

The samples of *Dioscorea* species investigated show especially in their TLC-fingerprints a characteristic zone feature, however, as with many saponins containing TCM-herbs, it is difficult to identify them through definable marker compounds. The HPLC-fingerprints analyses showed only for *D. opposita*, *D. hypoglauca* and *D. nipponica* species characteristic and fairly reproducible peak patterns.

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Ganoderma – *Lingzhi*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Ganoderma is the dried sporophore fruiting body of *Ganoderma lucidum* (Leyss. Ex Fr.) *Karst.* or *Ganoderma sinense (Ganoderma japonicum)* Zhao, Xu et Zhang; reishi (jap.); reishi mushroom (engl.) (Fam. Polyporaceae).

The drug is collected all the year, removed from foreign matter, attached rotten wood, sand or the lower stipe of the culture matrix, dried in the shade or stove at 40-50 °C.

Origin[2]: China, Japan, Korea, North America, Europe

Descriptions of the drug[1]:

Ganoderma lucidum:
Firmbriate, pileus reniform, semi-rounded or subrounded, 10-18 cm in diameter, 1-2 cm thick. Shell hard, yellowish-brown to redish-brown, lustrous, with circular arrised stripe and radiate wrinkle, edge thin and even, frequently incurved slightly. The inner part white to brownish. Stip cylinder, laterally grown, few leaning grown, 7-15 cm long, 1-3.5 cm in diameter, reddish-brown to purplish brown, luminous. Spore small and fine, yellowish-brown. Odour, slightly aromatic, taste, bitter and puckery.

Ganoderma sinense:
Shell purplish-black, with lacquer-like lustre. Sporophore rusty-brown. Stip 17-23 cm long.

Medicinal use[2]: Treatment of various cardiovascular diseases (hypertension, hyperlipidemia, atherosclerosis), hepatopathia, neurasthenia, asthma, cancer prevention.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of *Ganoderma lucidum/Ganoderma sinense* according to Traditional Chinese Medicine[1,2,3]	
Taste:	sweet
Temperature:	neutral
Channels entered:	*orbis cardialis, orbis hepaticus, orbis pulmonalis*
Effects (functions):	To replenish *qi cardiale* et *pulmonale* and ease the mind, relieve cough and asthma
Symptoms and indications:	Dizziness, insomnia, palpitation, shortness of breath, asthenic cough and asthma

Main constituents[4,5]:

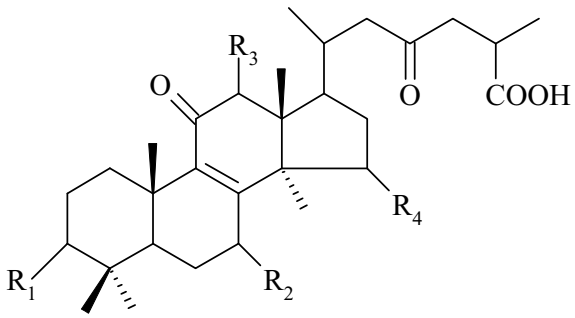
- **triterpenoids:**
  ganoderic acid A, B, AM_1, C_2, D, G, H, J, K
  ganoderenic acid B
  ganolucidic acids A-F
  3β-hydroxy-4, 4, 14-trimethyl-7, 11, 15-trioxochol-8-en-24-oic acid
  lucidenic acids A-M, lucidone A-C
  ganodermanontriol, ganodermatriol
  lucidadiol, lucidal
  epoxyganoderiol A-C

- ergosterol and steryl esters

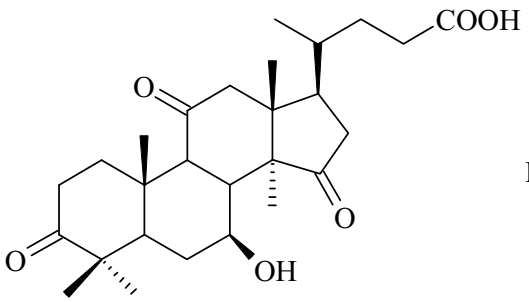
- **amino acids:**
  serine, alanine, glycine, threonine, aspartic acid, glutamic acid, proline, valine.

- **polysaccharides:**
  homo- and heteroglucans, arabinoxyglucan, peptido-heteroglucans, ganoderans B and C

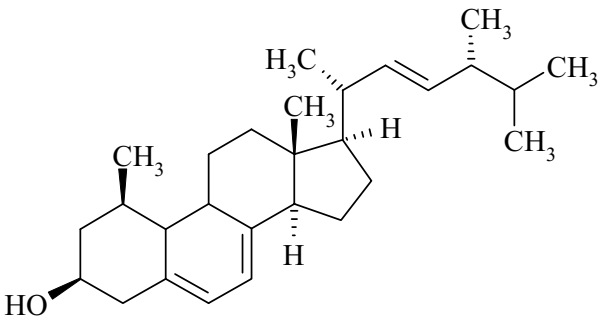
Other constituents[4]: adenosine, fungal lysozymes, fatty acids



Ganoderic acid A:	R_1: =O R_2: OH R_3: OH R_4: H		
Ganoderic acid C_2	R_1, R_2: β-OH R_3: α-OH R_4: H	Ganoderic acid B	R_1, R_2: β-OH R_3: =O R_4: H
Ganoderic acid AM_1	R_1: β-OH R_2, R_4: =O R_3: H	Ganoderic acid K	R_1, R_2: β-OH R_3: =O R_4: β-OAc
Ganoderic acid H	R_1: β-OH R_2, R_4: =O R_3: β-OAc	Ganoderic acid D	R_1, R_3: =O R_2: β-OH R_3: H R_4: H



Lucidenic acid A



Ergosterol

Fig. 1: Formulae of the main compounds of *Ganoderma lucidum/Ganoderma sinense*

Pharmacology:

- angiotensin converting enzyme – inhibitory activity[6]
- anticholesterolemic[7]
- antihepatitis[8]
- hepatoprotective effects[9]
- analgesic[10]
- anti-inflammatory[10]
- antihistaminic[11]
- immunomodulating effects[12]
- anti-HIV-1[13]
- anticomplement[14]
- antiandrogenic[16]
- antioxidant[17]

TLC fingerprint analysis:

Drug samples		Origin
1	Ganoderma / *Ganoderma lucidum*	Province Hebei, China
2	Ganoderma / *Ganoderma lucidum*	Province Anhui, China
3	Ganoderma / *Ganoderma lucidum*	Province Beijing, China
4	Ganoderma / *Ganoderma lucidum*	Province Hebei, Ankuo, China
5	Ganoderma / *Ganoderma lucidum*	sample of commercial drug, China
6	Ganoderma / *Ganoderma sinense*	Province Yunnan, China

Reference compounds of Figure 2		R*f*
T 1	ganoderic acid A	0.46
T 2	ganoderic acid C_2	0.30
T 3	ganoderic acid H	0.60
T 4	ergosterol	0.93

TLC-fingerprint analysis:

1) Extraction: 2.0 g of the powdered drug are extracted under reflux with 30 ml of methanol for 1 hour. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 2.0 ml of methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Ganoderma extracts: each 15 µl
reference compounds: each 10 µl

Solvent system: dichloromethane : methanol
9 1

Detection: Vanillin-sulphuric acid reagent:
I: 1 % ethanolic vanillin solution
II: 10 % ethanolic sulphuric acid

The plate is sprayed with solution I followed immediately with solution II. The plate is heated for 5 – 10 minutes at 105 °C and evaluated in VIS.

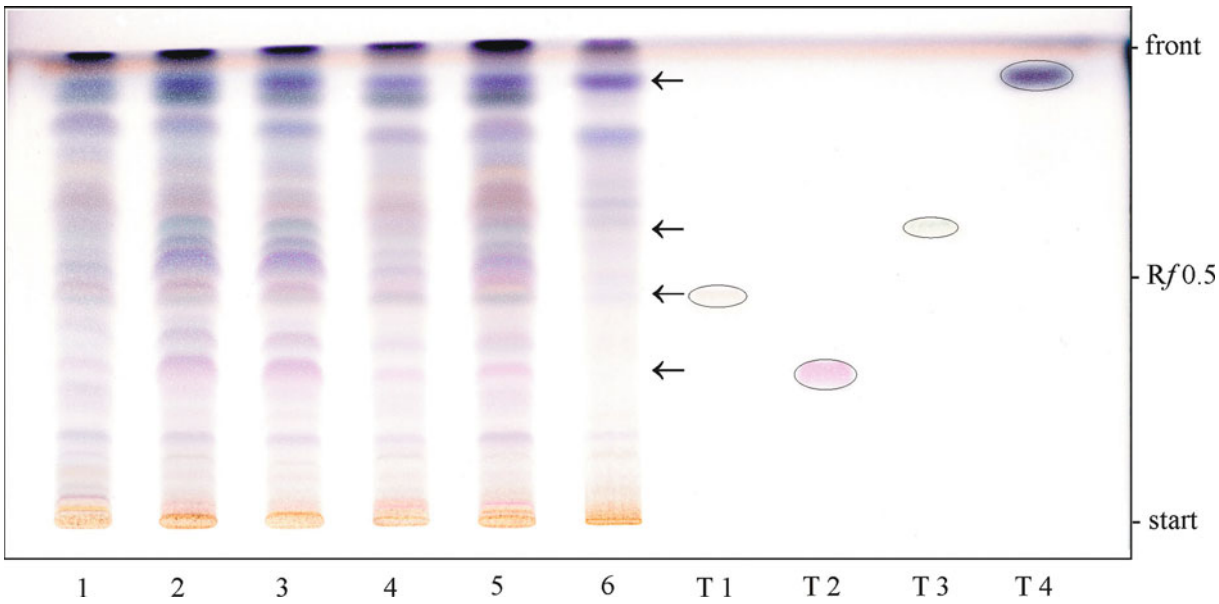


Fig. 2: Thin layer chromatogram of the methanol extracts of Ganoderma sprayed with vanillin-sulphuric acid (VIS)

4) Description of Figure 2:

All methanol extract samples of *Ganoderma lucidum* (1-5) show from the start to the solvent front a very homogeneous pattern of about 15 violett-pink zones of tripenoid compounds.

The sample of *Ganoderma sinense* (sample 6) contains the triterpenoids in the region between R*f* ~ 0,1 and R*f* ~ 0,85 in relatively low concentration in comparison to those of *Ganoderma lucidum:* Ganoderic acid C_2 (**T2**) with its three hydroxyl-groups in the molecule has the lowest R*f* value (~ 0,30), Ganoderic acid H (**T3**) with only one hydroxyl group, two carbonyl groups and one OAc group the highest R*f* value (~ 0,60). Ergosterol (**T4**), a characteristic steroid of mushrooms and fungi, appears at R*f* ~ 0,93.

Note: A nearly identical terpenoid zone pattern on HPTLC developed with the same solvent system and sulphuric acid as spray reagent, is described for Reishi mushroom also in the American Herbal Pharmacopoeia[4].

HPLC-fingerprint analysis:

I. HPLC-fingerprint analysis of triterpenes (Fig3a–c):

1) Sample preparation: 2.0 g of the powdered drug are extracted under reflux with 30 ml of methanol for 1 hour. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 2.0 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Ganoderma extracts: each 30.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	0-20 % B in 7 min., 20-95 % B in 48 min., 95 % B in 10 min., total runtime: 65 minutes
Flow:	0.8 ml/min.
Detection:	205 nm

II. HPLC-fingerprint analysis of ergosterol[24] (Fig 3d):

1) Sample preparation: 2.0 g of the powdered drug are extracted under reflux with 30 ml of methanol for 1 hour. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 2.0 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Ganoderma extracts: each 20.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 125-4 with LiChrospher® 60 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: 0,05 % acetic acid (Merck) (dist. water (Millipore Ultra Clear UV plus® filtered)) / methanol (VWR), 90 : 10 v/v
	B: methanol (VWR)
Gradient:	10 % B in 10 minutes 10 - 90 % B in 10 minutes 100 % B in 30 minutes total runtime: 50 minutes
Flow:	1.0 ml/min.
Detection:	280 nm

Retention times of the main peaks recorded at 205 nm in **Fig. 3a, b** and **c** and 280 nm in **Fig. 3d**:

Peak	Rt (min.)	Compound
1	24.11	ganoderic acid C_2
2	26.91	ganoderic acid A
3	27.77	ganoderic acid H
4	50.61	triterpenoid *)
5	53.51	triterpenoid *)
6	55.25	triterpenoid *)
7	56.66	triterpenoid *)
8	48.87 **)	**Ergosterol (Fig. 3d)**

*) not identified
**) recorded at 280 nm (see Fig. 3d)

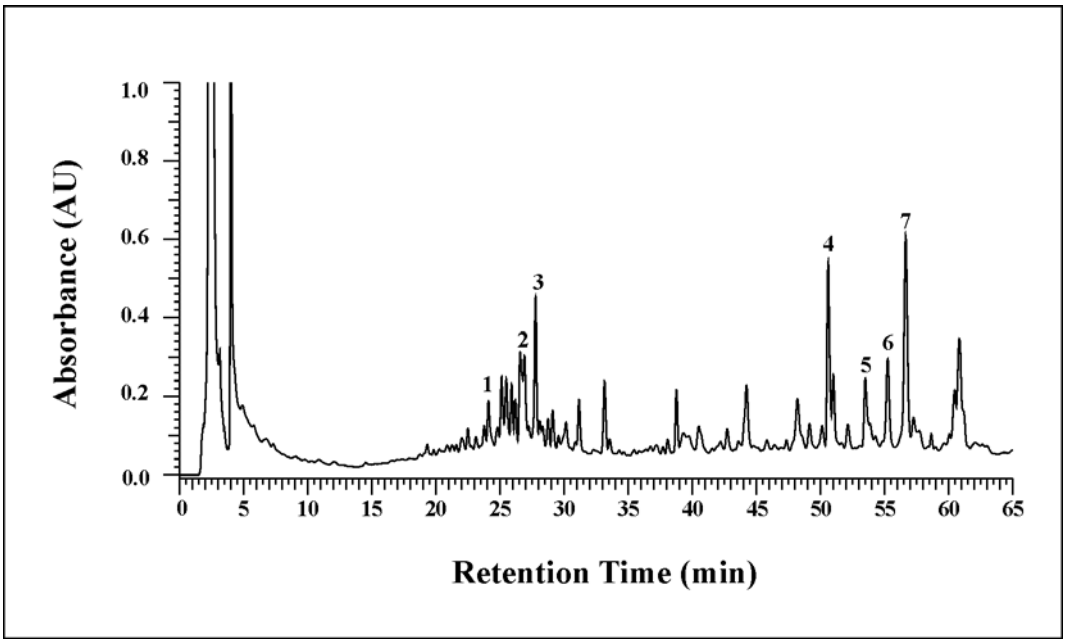


Fig. 3a: HPLC-fingerprint analysis of the MeOH-extract of *Ganoderma lucidum* sample 1 recorded at 205 nm

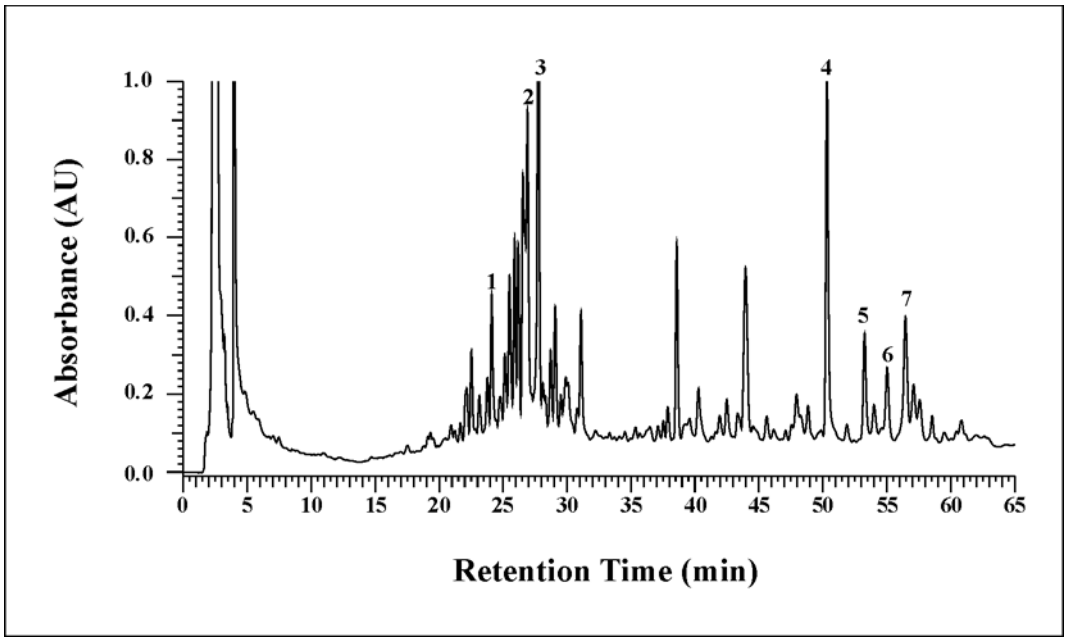


Fig. 3b: HPLC-fingerprint analysis of the MeOH-extract of *Ganoderma lucidum* sample 2 recorded at 205 nm

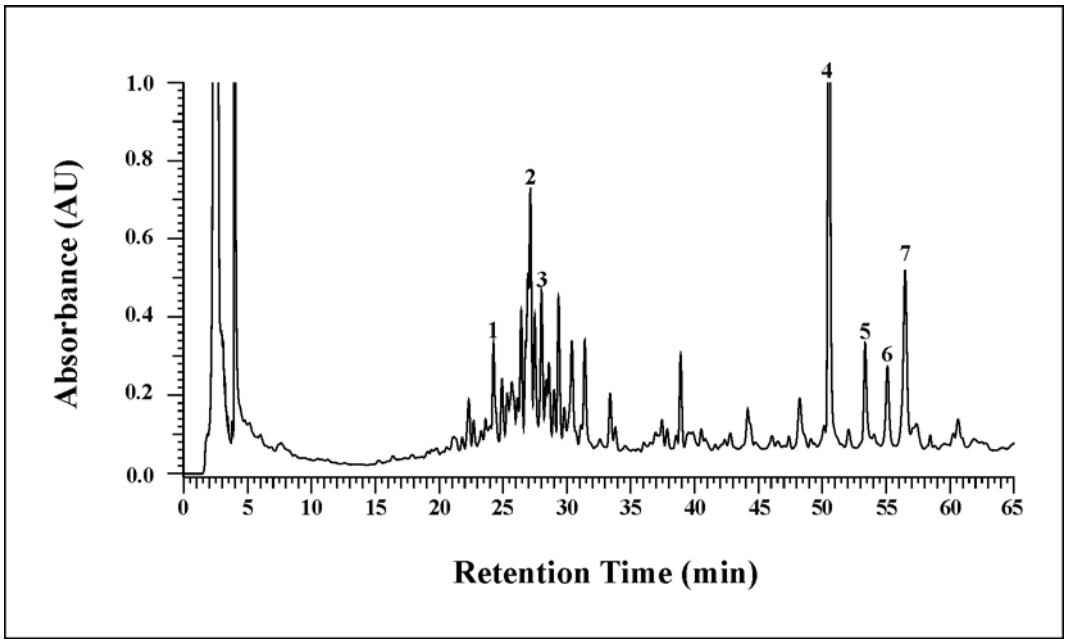


Fig. 3c: HPLC-fingerprint analysis of the MeOH-extract of *Ganoderma lucidum* sample 3 recorded at 205 nm

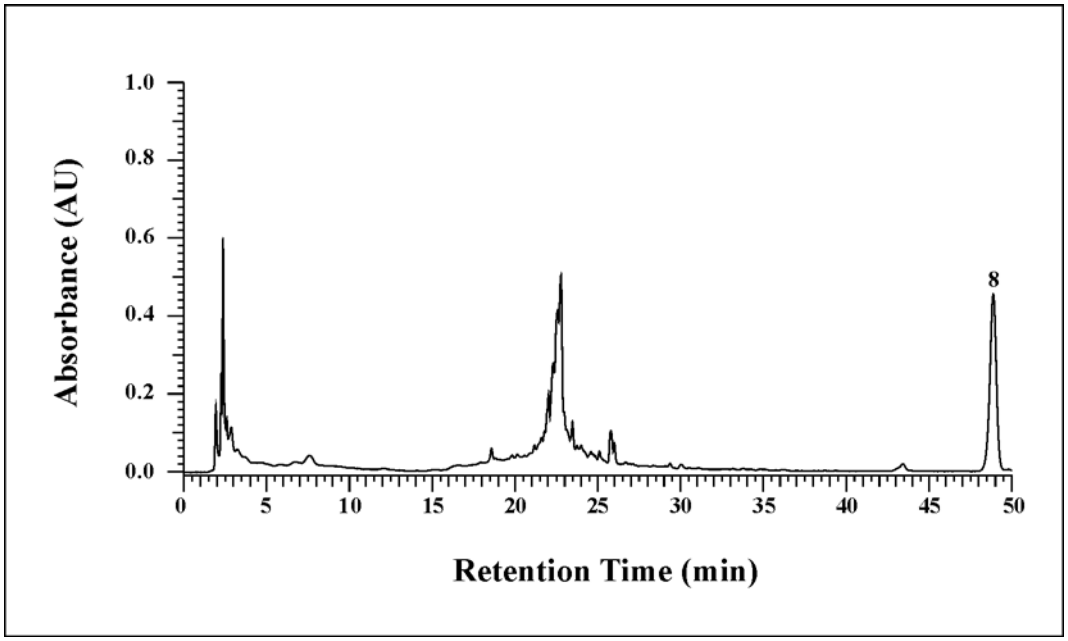


Fig. 3d: HPLC-fingerprint analysis of the MeOH-extract of *Ganoderma lucidum* sample 1 recorded at 280 nm

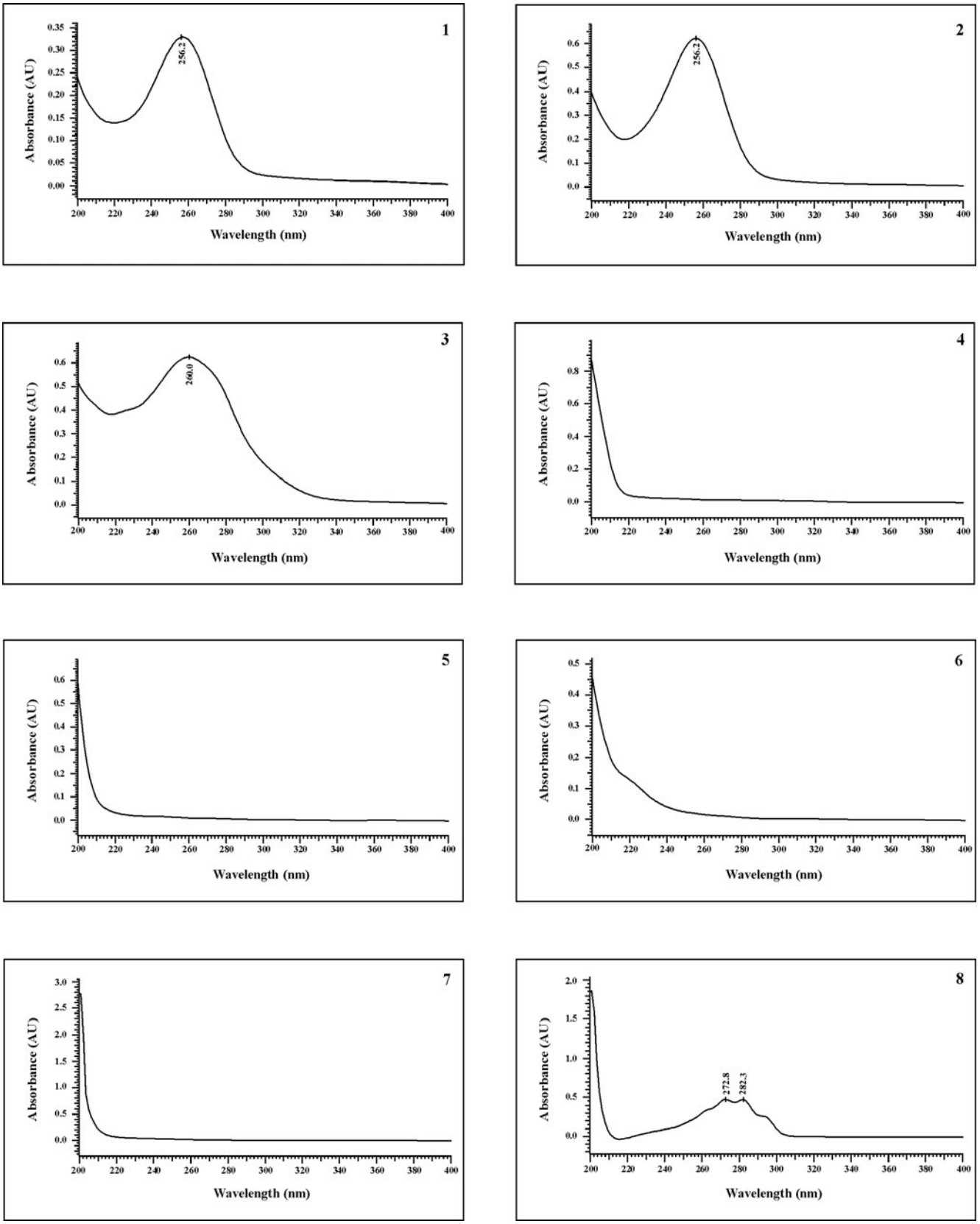


Fig. 4: On line UV-spectra of *Ganoderma lucidum* sample 1

4) Description of the HPLC-Figure **3a**, **3b**, **3c** and **3d**:

- The HPLC-fingerprint of all *Ganoderma lucidum* samples 1 – 5 is characterized by a complex peak accumulation in the Rt-ranges of Rt ~20 to 34 and Rt ~50 min. to 62 min. The first peak accumulation comprises at least 8 – 10 triterpenoic acid with ganoderic acid C_2 at Rt = 24.11 (**1**), ganoderic acid A at Rt = 26.91 (**2**) and ganoderic acid H at Rt = 27.77 (**3**). In the second peak accumulation in all samples appear four peaks at Rt = 50.61, Rt = 53.51, Rt = 55.25 and Rt = 56.66 with two dominant peaks (**4**, **7**). They can be probably assigned to ganoderic alcohols.
- The peak at Rt = 48.87 (**8**) in Fig. 3d, identified as ergosterol, was recorded at 280 nm after fingerprint analysis in an acetic acid – water – methanol solvent system.
- The HPLC-fingerprint of *Ganoderma sinense* shows nearly the same peak pattern but with much lower concentration of the triterpenoids.
- All ganoderic acids A – D with an α-unsaturated keto group in ring C give a characteristic UV-spectrum with a main maximum at 250 – 260 nm.

Note: Qualitative HPLC-fingerprint analyses of Ganoderma extracts and quantitative determination of the major ganoderic acids inclusive ganoderic alcohols and ergosterol are also described in a series of preceding publications[18,19,20,21].

Conclusion

Ganoderma lucidum and *Ganoderma sinense* can be easily identified by TLC and HPLC based on the characteristic features of triterpenoid and the presence of ergosterol which is a characteristic constituent of many mushrooms. A discrimination of *G. lucidum* and *G. sinense* is only possible by a macro- and microscopic analysis.

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Pericarpium Citri Reticulatae – *Chenpi*
Pericarpium Citri Reticulatae Viride – *Qingpi*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]:

Pericarpium Citri reticulatae (mature)
Dried Tangerine Peel is the dried pericarp of the ripe fruit of *Citrus reticulata* Blanco or its cultivars (Fam. Rutaceae). The drug is subdivided into two classes, known respectively as *"Chenpi"*, and *"Guang Chenpi"*.

The pericarp is peeled off when the fruit is ripe and dried in the sun or at a low temperature.

Pericarpium Citri reticulatae Viride (immature)
Green Tangerine Peel is the dried pericarp of the young or immature fruits of *Citrus reticulata* Blanco and its cultivars (Fam. Rutaceae).

The fallen young fruit is collected in May and June, dried in the sun and known commonly as *"Geqingpi"*. The immature fruit is collected in July and August, cut longitudinally into four-valved but connected at the base, removed from the emergences completely and dried in the sun, and known commonly as *"Sihuaqingpi"*.

Origin[2]: Provinces of Guang Dong, Fu Jian, Si Chuan, Jiang Su and Zhe Jiang (China)

Descriptions of the drug[1]:

Pericarpium Citri reticulatae (mature):

Chenpi:
Often peeled in several lobes connecting at the base, or in irregular slices, 1-4 mm thick. Outer surface orange-red to reddish-brown, with fine wrinkles and sunken oil cavity spots; inner surface pale yellowish-white, rough, bearing yellowish-white or yellowish-brown vein-like vascular bundles. Texture slightly hard and fragile. Odour, aromatic; taste, pungent and bitter.

Guang Chenpi:
Often in three lobes connected at the base, regular in shape and even in thickness, about 1 mm thick. The sunken oil cavity spots relatively large, transparent when observed against light. Texture slightly soft.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Pericarpium Citri reticulatae Viride (immature):

Sihuaqinpi:
Pericarp cut into four prolate elliptic lobes, long-elliptical, 4-6 cm long, 0.1-0.2 cm thick. The outer surface greyish-green or blackish-green, with numerous oil cavities; the inner surface almost white or yellowish-white, rough, with yellowish-white or yellowish-brown veins. Texture, slightly hard, easily broken, fracture showing 1-2 layers of oil cavities at the outer part. Odour, aromatic; taste, bitter and pungent.

Geqingpi:
Subspheroidal, 0.5-2 cm in diameter. Externally greyish-green or blackish-green, slightly rough, with numerous fine and sunken oil cavities. Remains of style, slightly projecting at the apex, and a rounded scar of fruit stalk at the base. Texture, hard, fractured pericarp yellowish-white or pale yellowish-brown, 1-2 mm thick, with 1-2 layers of oil cavities at the outer part. Pulp vescles 8-10, pale brown. Odour, delicately aromatic; taste, sour, bitter and pungent.

Medicinal use[3, 4, 5]: Distension of epigastrium and abdomen with anorexia, vomiting, and diarrhoea; venous insufficiency and allergic diseases such as allergic rhinitis, atopic dermatitis and food allergy; chronic bronchitis and cough.

Effects and indications of Pericarpium Citri reticulatae according to Traditional Chinese Medicine[1,2,13]	
Taste:	bitter, acrid
Temperature:	warm
Channels entered:	*orbis lienalis, orbis pulmonalis, orbis stomachi*
Effects (functions):	to regulate the flow of *qi*, invigorate the spleen function, eliminate *damp*, and resolve phlegm
Symptoms and indications:	Distension and fullness in the chest and epigastrium with anorexia, vomiting and diarrhea, cough with copius phlegm

Effects and indications of Pericarpium Citri reticulatae viride according to Traditional Chinese Medicine[1,2,14]	
Taste:	bitter, acrid
Temperature:	warm
Channels entered:	*orbis hepaticus, orbis felleus, orbis stomachi*
Effects (functions):	to soothe the liver, disintegrate stagnated *qi*, and remove retained food
Symptoms and indications:	distending pain in the chest and costal regions hernia; mass formation in the breast, mastitis; abdominal pain due to retention of undigested food

Main constituents:

- **flavanonglycosides**[6]:
  hesperidin, naringin, neoeriocitrin

- **polymethoxylated flavones (flavanones)**[7]:
  sinensetin (3′,4′,5,6,7-pentamethoxyflavone), isosinensetin
  tetramethoxy-isoscutellarein, tetramethoxy-scutellarein
  nobiletin (3′,4′,5,6,7,8-hexamethoxyflavone)
  5-demethylnobiletin
  tangeretin (4′,5,6,7,8-pentamethoxyflavone)
  5-demethyltangeretin
  citromitin (3′,4′,5,6,7,8-hexamethoxyflavanone)
  4′,5, 7, 8-tetramethoxyflavone
  5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone
  4′-heptamethoxyflavone
  auranetin, 5-hydroxyauranetin

- **alkaloids**[7]:
  synephrine

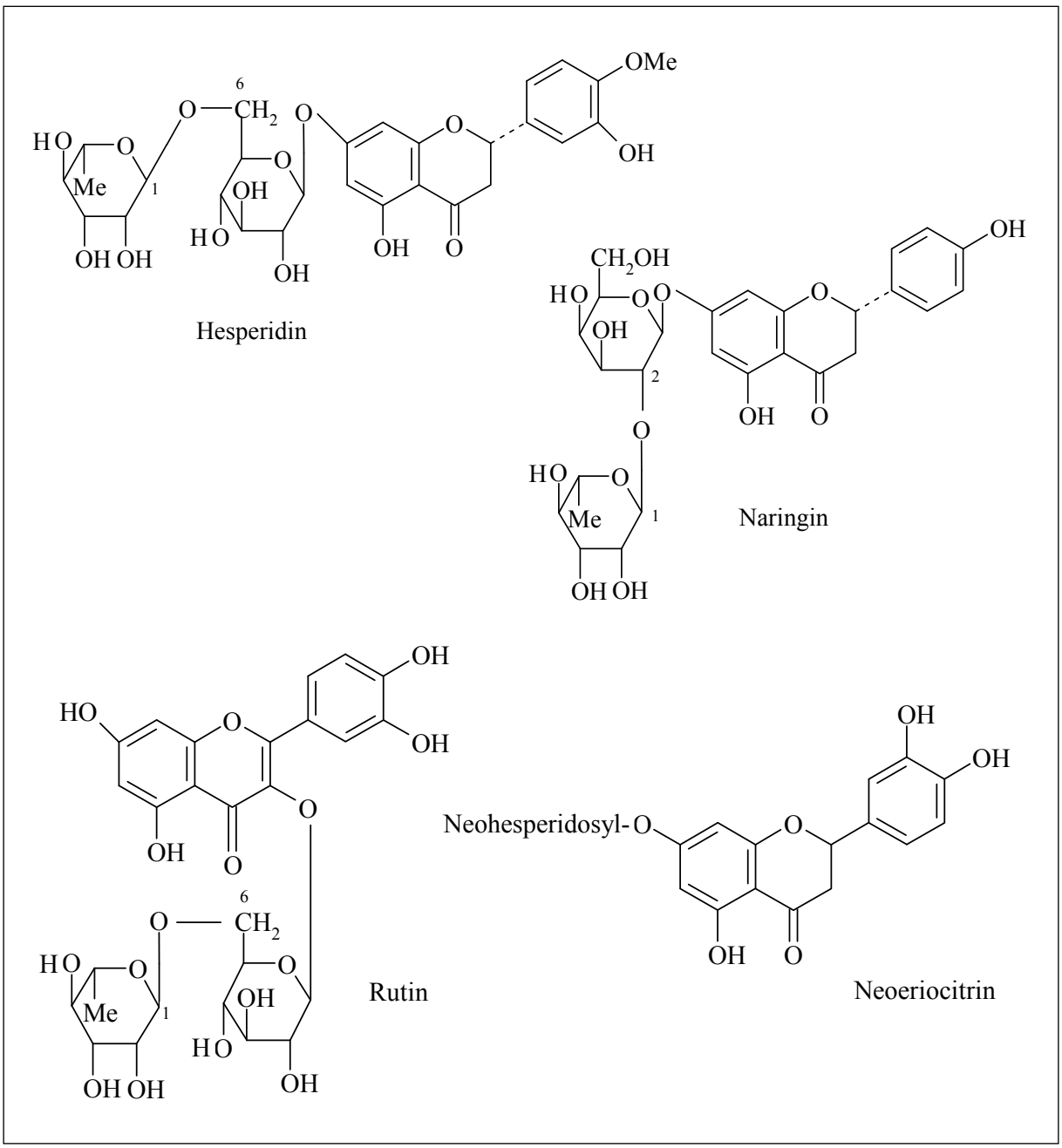
- **carotene**[8]:
  cryptoxanthin, 5, 5′, 6, 6′-tetrahydro-β, β-carotene, luteoxanthin, mutatochrome, auroxanthin, zeaxanthin, phytoene, phytofluene, sintaxanthin, β-apo-10′-carotenal

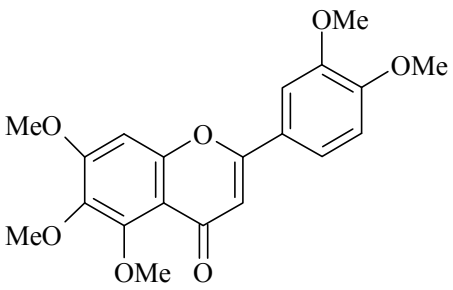
- **essential oil**[8]:
  β-caryophyllene, α-copaene, β-copaene, β-cubebene, p-cymene, β-elemene, farnesene, heptane, hexane, α- und β-humulene, limonene, myrcene, nootkatene, α-pinene, sabinene, epi-α-selinene, valencene, cis-carveol, trans-carveol, citronellol

- **triterpenes**[8]:
  limonin, deoxylimonin, nomilin, obacunone, deacetylnomilin

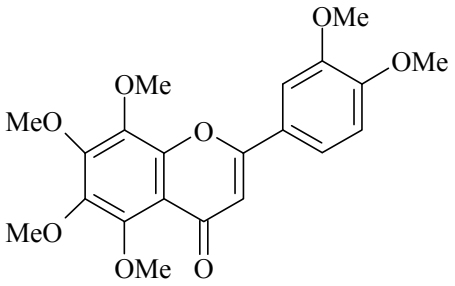
- **phenyl propanoids**[8]:
  citrusin A, citrusin B, citrusin C,
  syringin, coniferin, dehydrodiconiferyl alcohol

Fig. 1: Formulae of the main compounds of Pericarpium Citri reticulatae/ Pericarpium Citri reticulatae viride

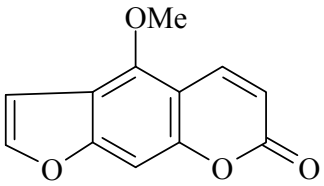




Sinensetin



Nobiletin



Bergapten

Pharmacology:

- antiallergic[9] (Hesperidin)
- antileukemic activity[10]
- antioxidant[11]
- immunomodulating effects[12]
- expectorant[13]
- antitussive[13]
- antiemetic[13]
- stomachic[13]
- antiulcer[14]
- aorta-dilatory effects[15]
- anti-inflammatory[9]

TLC-fingerprint analysis

Drug samples		Origin
1	Pericarpium Citri reticulatae viride / *Citrus reticulata* Blanco	province Fujian, China
2	Pericarpium Citri reticulatae viride / *Citrus reticulata* Blanco	province Jiangxi, China
3	Pericarpium Citri reticulatae viride / *Citrus reticulata* Blanco	province Jiangxi, China
4	Pericarpium Citri reticulatae / *Citrus reticulata* Blanco	province Sichuan, China
5	Pericarpium Citri reticulatae / *Citrus reticulata* Blanco	province Guangdong, China
6	Pericarpium Citri reticulatae / *Citrus reticulata* Blanco	sample of commercial product obtained from HerbaSinica, Germany
7	Pericarpium Citri / *Citrus limon*	sample of commercial drug 2007
8	Pericarpium Citri / *Citrus limon*	sample of commercial drug 2008
9	Pericarpium Citri viride / *Citrus aurantium*	province Guangxi, China
10	Pericarpium Citri / *Citrus aurantium*	sample of commercial drug 2007
11	Pericarpium Citri / *Citrus aurantium*	sample of commercial drug 2008

Reference compounds of Figure 2a		R*f*
T 1	rutin	0.37
T 2	neoeriocitrin	0.42
T 3	hesperidin	0.45
T 4	naringin	0.48
T 5	caffeic acid	0.93

Reference compounds of Figure 2c		R*f*
T 6	sinensetin	0.28
T 7	nobiletin	0.32
T 8	bergapten	0.60
T 9	hesperetin	0.49

TLC-fingerprint analysis

1. Thin layer chromatograms of Citrus-flavanon-glycosides (see Figure 2a and 2b):

1) Extraction: 0.3 g of the powdered drug is extracted under reflux with 10 ml of methanol for 20 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Pericarpium Citri extract: each 10 µl
reference compounds: each 10 µl

Solvent system: ethyl acetate : formic acid : glacial acetic acid : water
100 11 11 27

Detection: Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol

II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. The evaluation is carried out in UV 365 nm

2. Thin layer chromatogram of lipophilic methoxylated flavones (see Figure 2c):

1) Extraction: 0.3 g of the powder drug is extracted under reflux with 10 ml of methanol for 20 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Pericarpium Citri extract: each 10µl
reference compounds: each 10 µl

Solvent system: I ethyl acetate : methanol : water
100 17 13

After developing about 3 cm and removal of the plate, dried in air, and then used solvent system II.

II toluene : ethyl acetate : formic acid : water
20 10 1 1
(upper phase)

After developing 8 cm and removal of the plate, dried in air.

Detection: Aluminium chloride TS reagent:
Dissolve 1 g of aluminium chloride in 100 ml ethanol.

The plate is sprayed with the solution and the evaluation is carried out in UV 365 nm.

Description of the TLC – fingerprint analysis:

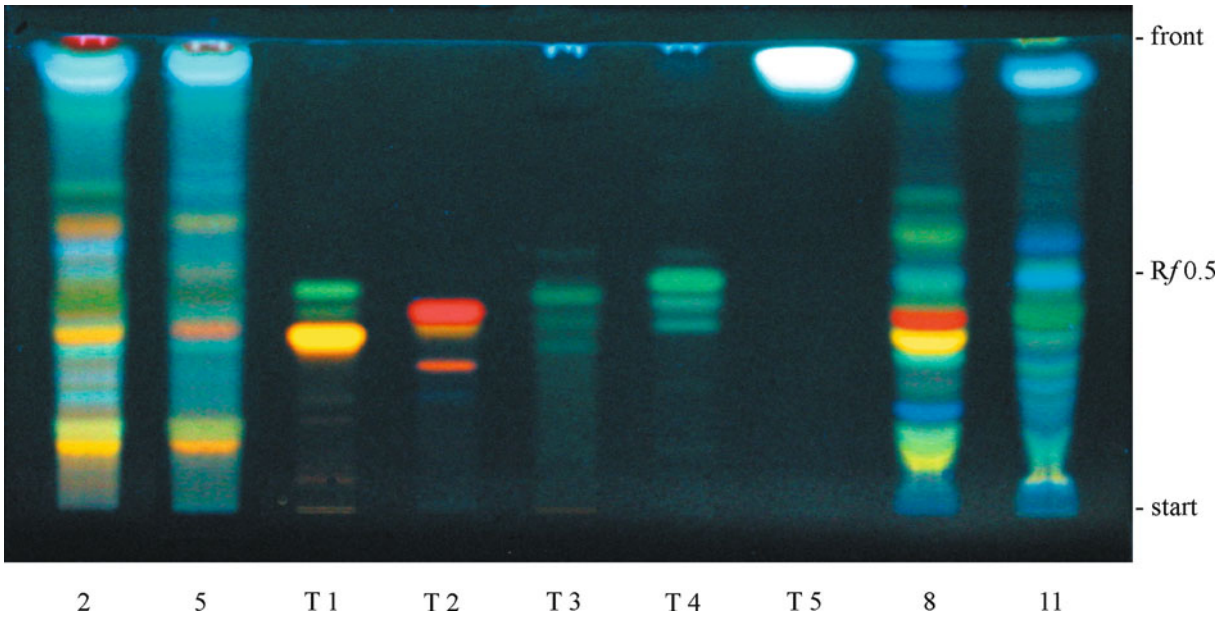


Fig. 2a: Thin layer chromatogram of the methanol extracts of Pericarpium Citri reticulatae, Citri limonis and Citri aurantii sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

The TLC of Fig. 2a shows at 365 nm the two methanol-extracts of Pericarpium Citri reticulatae (samples 2 and 5) and for comparison the extracts of Pericarpium Citri limonis (sample 8) and Pericarpium Citri aurantii (sample 11).

Between them the partly impurified reference compounds Rutin (T1), Neoeriocitrin (T2), Hesperidin (T3), Naringin (T4) and Caffeic acid (T5) are applied.

Pericarpium Citri reticulatae extracts are characterized by a weak orange zone at R*f* = 0.60 (Eriodictyol?), two green fluorescent zones between R*f* = 0.48 and 0.45 as Naringin and Hesperidin, directly below the orange-yellow zone of Rutin and in the R*f*-range of 0.1 and 0.25 additionally some light green and yellow zones of flavonol- and flavanone-triglycosides.

The Pericarpium Citri limonis (sample 8) shows the conspicuous carmine red zone of Neoeriocitrin, directly above Hesperidin, Naringin directly below Rutin and in the lower R*f*-range a dark blue and light green zone. In the R*f*-range 0.6 up to the front appear some weak blue zones inclusive caffeic acid (R*f* = 0.98).

Pericarpium Citri aurantii (sample 11) contains no Neoeriocitrin but mainly Naringin and Hesperidin.

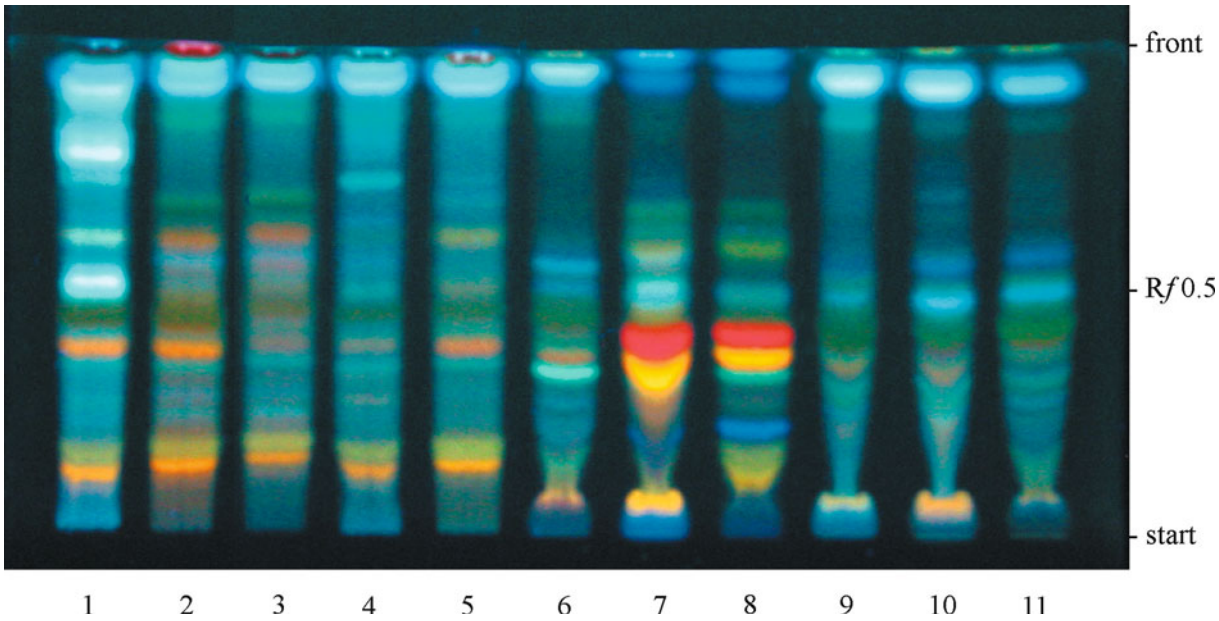


Fig. 2b: Thin layer chromatogram of the methanol extracts of Pericarpium Citri sprayed with natural products-polyethylene glycol reagent (UV 365 nm)

Fig. 2b gives once more an overview of the methanol-extracts of the samples 1-11, among them 1-6 those of Pericarpium Citri reticulatae, 7 and 8 of Pericarpium Citri limonis and 9-11 those of Pericarpium Citri aurantii.

The Pericarpium Citri reticulatae extracts show a very homogeneous pattern of orange, green and blue fluorescent zone pattern as described for Fig. 2a. The extract of Pericarpium Citri limonis shows the characteristic strong carmine red and yellow zones of Neoeriocitrin and Rutin, and the zones of Hesperidin and Naringin, whereas on the tracks of Pericarpium Citri aurantii (9-11) appear only the green zones of Hesperidin and Naringin and on the TLC-front the light blue zone of caffeic acid.

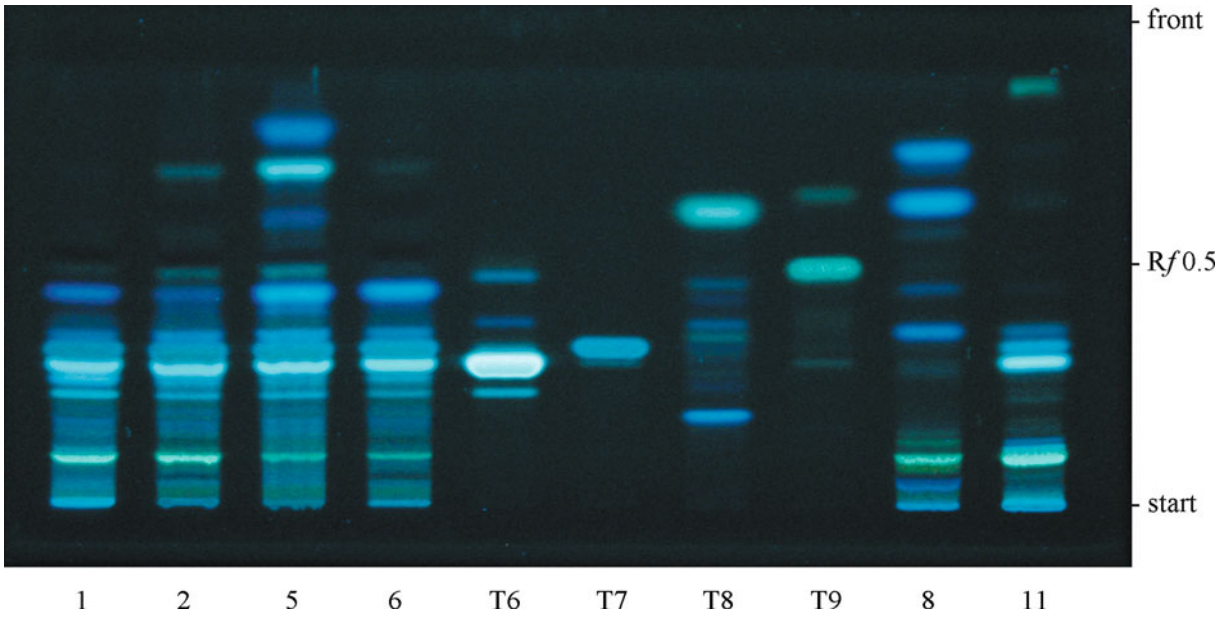


Fig. 2c: Thin layer chromatogram of the methanol extracts of Citri pericarpium sprayed with aluminium chloride reagent (UV 365 nm)

The extracts of Pericarpium Citri reticulatae 1, 2, 5, 6 show with the exception of sample 5 a very homogeneous pattern of blue/violet zones of the polymethoxylated flavonoids, Sinensetin at R*f* = 0.28 and Nobiletin at R*f* = 0.32 with a further light blue zone at R*f* = 0.15 and a violet zone at R*f* ~ 0.4. The chromatograms of extract sample 2 and 5 differ from the others by additional blue/violet zones in the upper R*f*-range. Pericarpium Citri limonis (sample 8) does not contain Sinensetin but contains a light blue zone at R*f* = 0.15 and two distinct violet zones at R*f* = 0.63 and 0.75, one of them probably the furanocumarin Bergapten. The zones pattern of Pericarpium Citri aurantii shows a zone similarly to that of Pericarpium Citri reticulatae but is devoid of all blue fluorescent zones above the R*f* – range 0.5 up to the front.

HPLC-fingerprint analysis:

1) Sample preparation: 0.3 g of the powder drug is extracted under reflux with 10 ml of methanol for 20 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol. The extract is filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: Pericarpium Citri extract: 5.0 µl

3) HPLC parameter:

Apparatus:
MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column:	LiChroCART® 125-4 with LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP–18 (5 µm), Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered)
	B: acetonitrile (VWR)
Gradient:	5-30 % B in 25 min., 30-95 % B in 15 min., 95 % B in 5 min., total runtime: 45 minutes
Flow:	1.0 ml/min.
Detection:	255 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	~ 13.2	Flavonoid – glycoside?
2	~ 13.8	Flavonoid – glycoside?
3	~ 15.7	Caffeic acid
4	~ 17.4	Neoeriocitrin?
5	~ 18.0	Rutin
6	~ 18.5	Naringin
7	~ 19.7	Hesperidin
8	~ 31.4	Bergapten
9	~ 32.1	Sinensetin
10	~ 33.1	methoxylated Flavone
11	~ 34.5	methoxylated Flavone
12	~ 35.2	Nobiletin

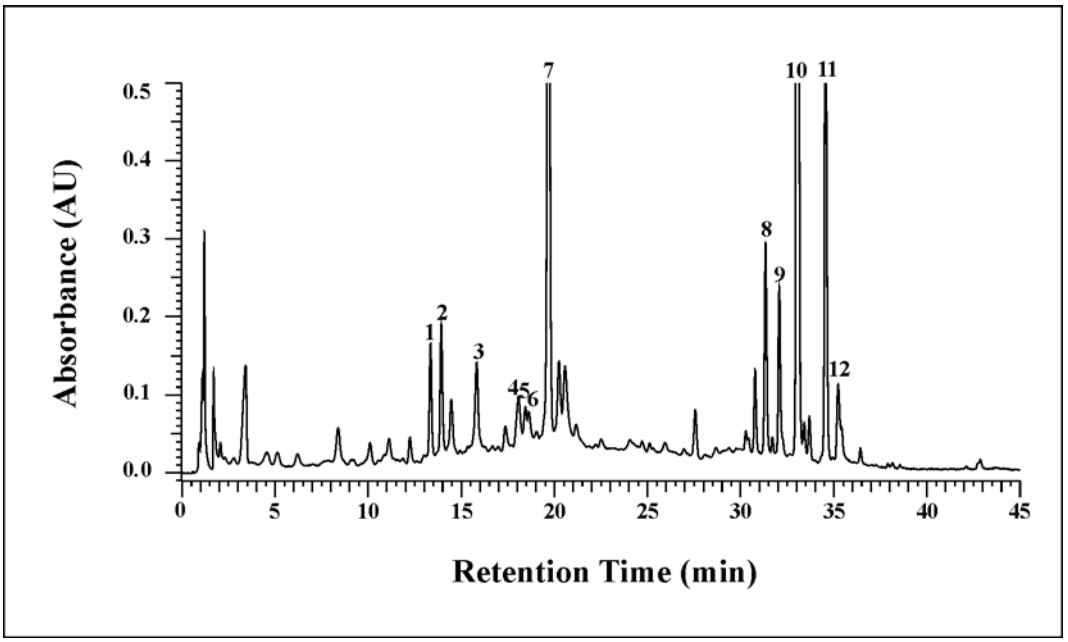


Fig. 3a: HPLC fingerprint of Pericarpium Citri reticulatae viride (*Citrus reticulata*) sample 3

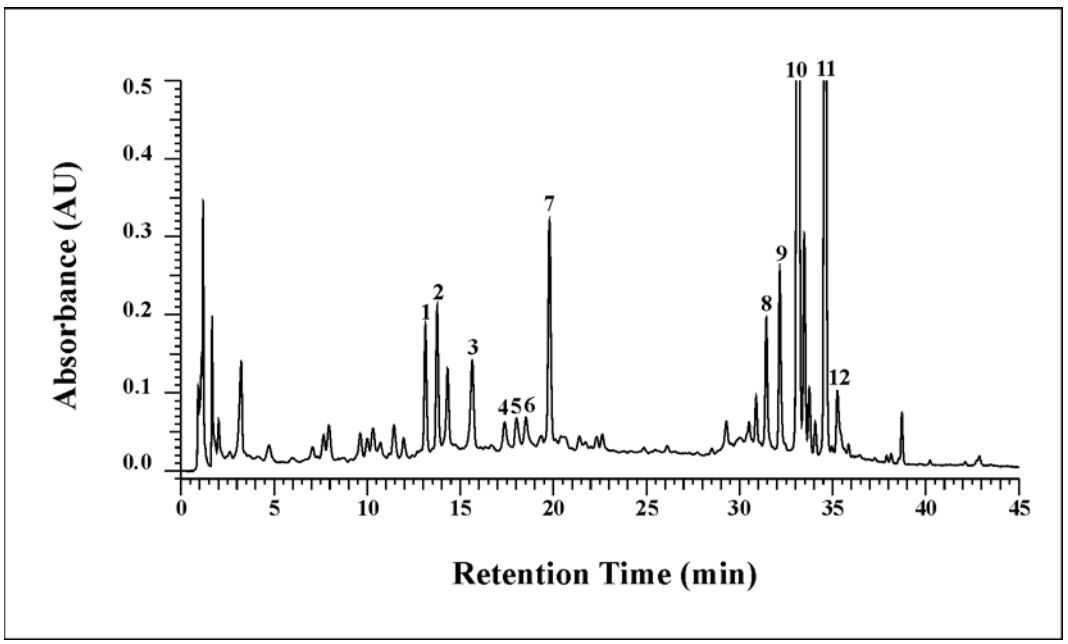


Fig. 3b: HPLC fingerprint of Pericarpium Citri reticulatae viride (*Citrus reticulata* sample) 5

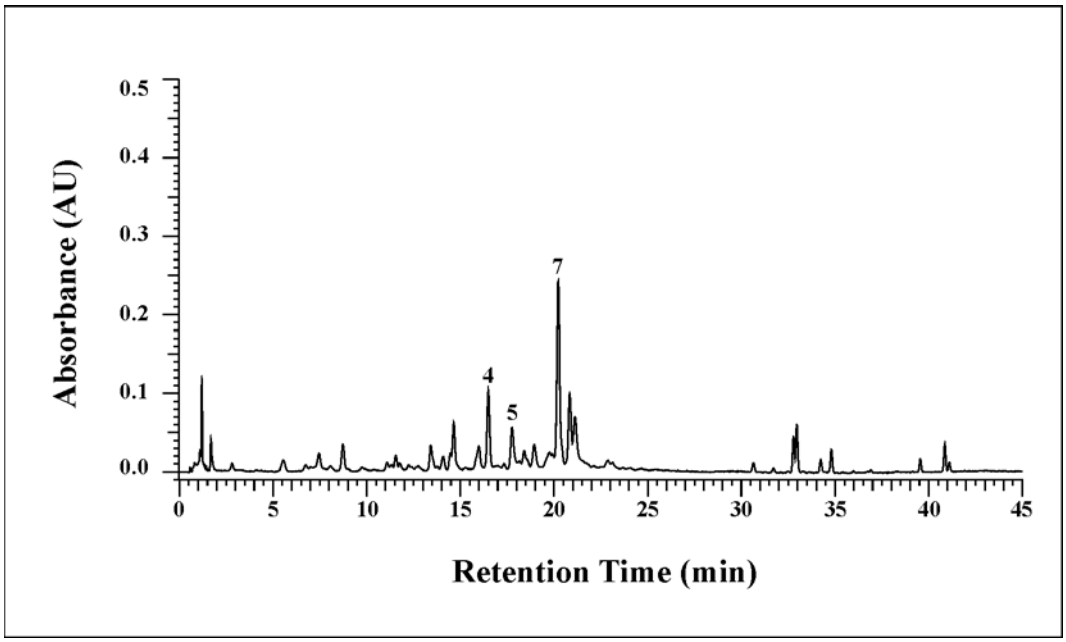


Fig. 3c: HPLC fingerprint of *Citrus x limon* sample 8

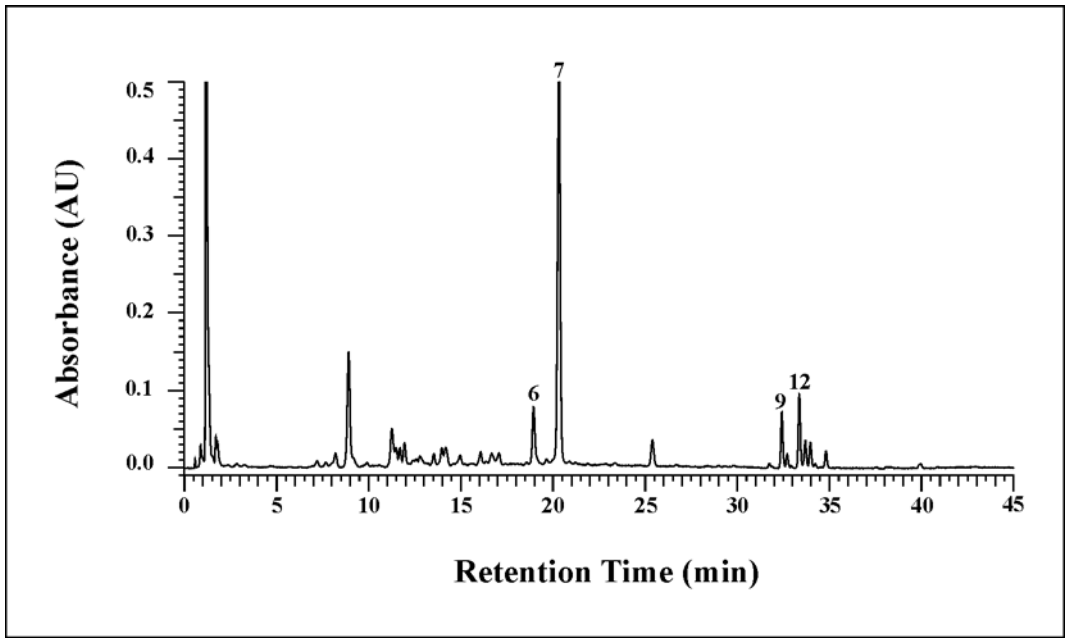
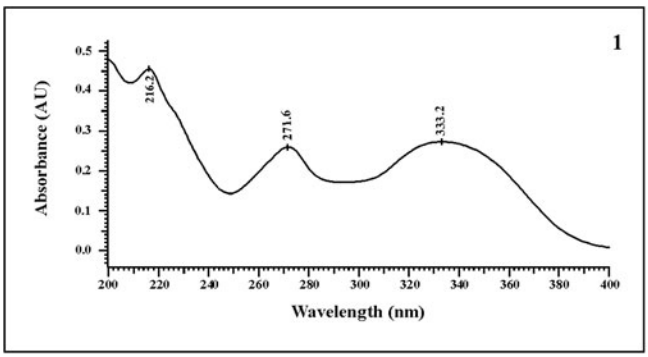
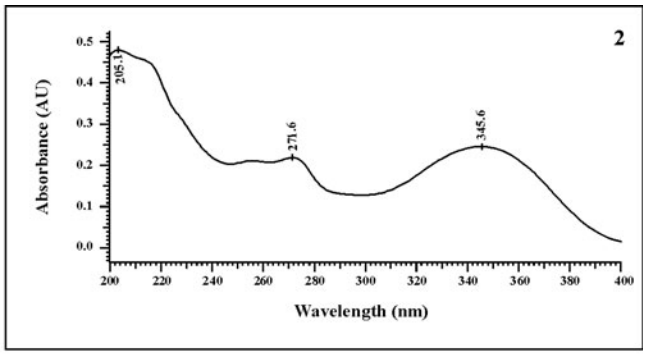
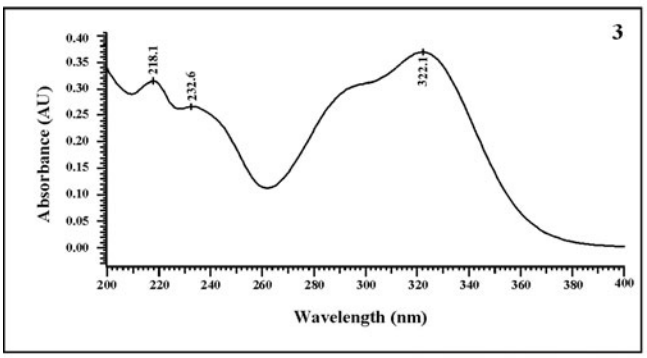
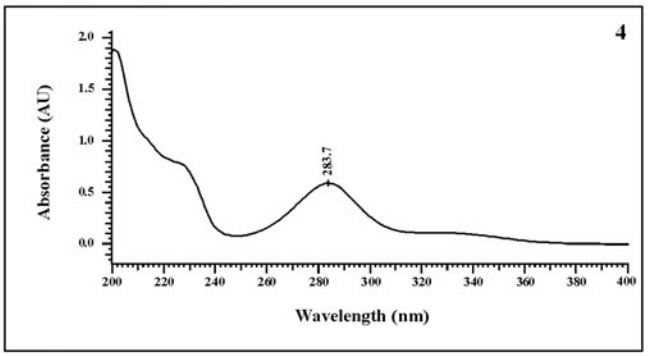


Fig. 3d: HPLC fingerprint of *Citrus aurantium* sample 11


1
Absorbance (AU)
Wavelength (nm)
216.2
271.6
333.2


2
Absorbance (AU)
Wavelength (nm)
205.1
271.6
345.6


3
Absorbance (AU)
Wavelength (nm)
218.1
232.6
322.1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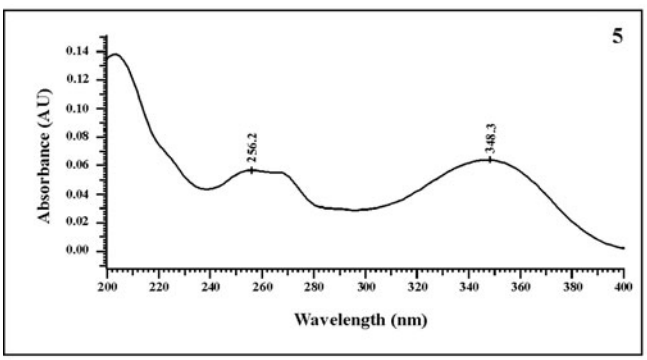
Absorbance (AU)
Wavelength (nm)
283.7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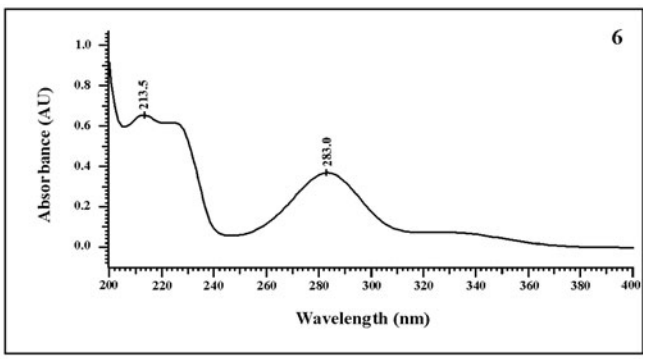
Absorbance (AU)
Wavelength (nm)
256.2
348.3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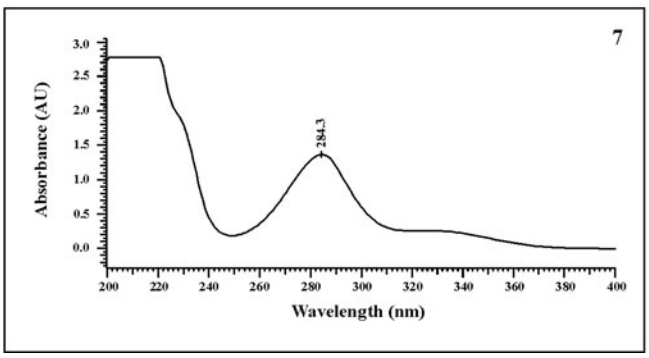
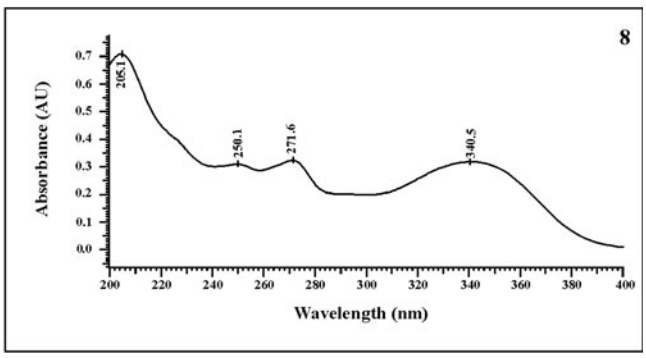
Absorbance (AU)
Wavelength (nm)
213.5
283.0


7
Absorbance (AU)
Wavelength (nm)
284.3


8
Absorbance (AU)
Wavelength (nm)
205.1
250.1
271.6
340.5

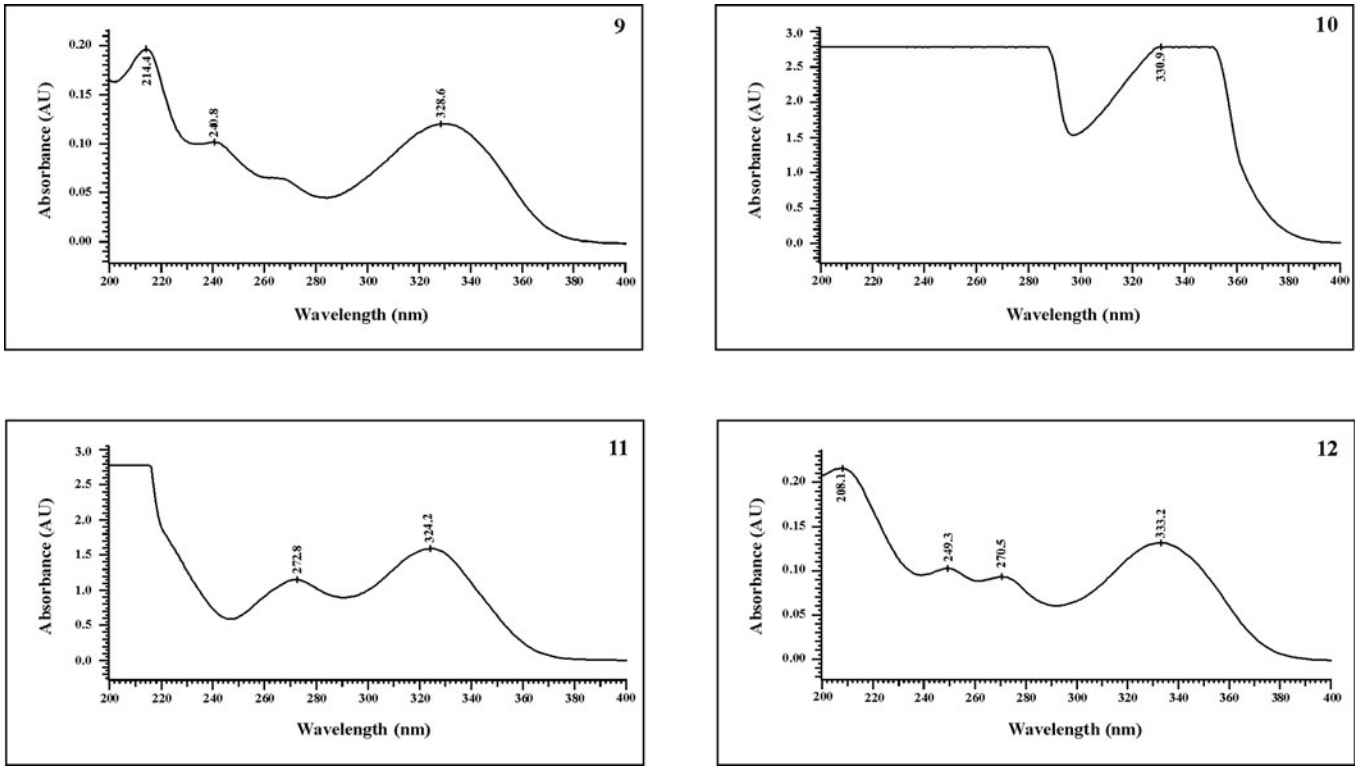


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of *Citrus reticulata*

4) Description of the HPLC:

Fig. 3a and 3b:

The HPLC – peak fingerprints of Pericarpium Citri reticulatae of sample 3 and 5 are nearly superimposed. The peaks assemblies in the Rt–range ~10.0 to 23.0 correspond with the polar R*f*-range in the TLC – Fig. 2a and 2b whereas the peaks at Rt = 30 to 36 with the TLC – pictures of Fig. 2c. According to the reference compounds and their on line UV – spectra the peaks **1** – **7** can be identified as follows:

- Peak **1** and **2** with Rt ~ 13.2 and 13.8 are not identified flavanon- or flavonol-(tri)glycosides.
- Peak **3** (Rt ~ 15.7) is caffeic acid.
- The peaks **4**, **5**, **6** with Rt ~ 17.4, 18.0 and 18.5 could be identified as Neoeriocitrin, Rutin and Naringin respectively.
- The dominant peak **7** at Rf~19.7 of this first peak assembly is identical with Hesperidin.
- The peak sequence **8** – **12** from Rt ~ 30 to 36 correspond with the nonglycosidic lipophilic flavonoids which appear in TLC – Fig. 2c with blue or violet fluorescence.
- The peak **8** with Rt ~31.4 is Bergapten, present also in most essential oils of orange species.
- The peak **9** at Rt ~32.1 shows Sinensetin.
- The peaks **10** and **11** at Rt ~33.1 and 34.5 are further methoxylated flavones.
- The peak **12** at Rt ~35.2 is identical with Nobiletin.

Fig. 3c:

The peak pattern of Pericarpium Citri limonis sample 8 corresponds with that of the HPLC of Figure 3a. The samples 3 and 5 contain besides the dominant Hesperidin **7**, the distinct peaks of **4** (Neoeriocitrin) and **5** (Rutin) (see TLC – Figure 2a and b).

A small concentration of nonglycosidic flavonoids (Sinensitin and Nobiletin) appear in the Rt–range of 30 to 36 (**9** and **12**).

Fig. 3d:

The peak pattern of Pericarpium Citri aurantii is dominated by Naringin (**6**) and Hesperidin (**7**). In the second peak assembly only the peaks **9** (Sinensetin) and **12** (Nobiletin) are prevalent.

Note:

- Further chromatographic methods for fingerprint analyses of Pericarpium Citri reticulatae and quantitation of single main flavonoids (e.g. Hesperidin) are described in the publications[16-23].
- The Chinese Pharmacopoeia 2005 describes for the Pericarpium Citri reticulatae viride (Qingpi) a Hesperidin content not less than 5 % with reference to the dried drug.

  For Pericarpium Citri reticulatae (Chenpi) not less than 3.5 % with reference to the dried drug are demanded.

Conclusion

The fingerprints of Pericarpium Citri reticulatae can be very well authenticated by TLC and especially by HPLC. The “European” fruits of Pericarpium Citri limonis and Pericarpium Citri aurantii as falsifications or adulterations can be easily distinguished from each other in TLC as well as in HPLC.

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Rhizoma Corydalis – *Yanhusuo*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Rhizoma Corydalis is the dried tuber of *Corydalis yanhusuo* W.T. Wang (Fam. Papaveraceae).

The drug is collected in early summer when the plant is withered, removed from fibrous root, washed clean, boiled in water until no dry core visible, and dried in the sun.

Origin[2]: China (provinces Zhejiang, Jiangsu), Siberia, Manchuria, Japan

Description of the drug[1]: Irregularly oblate, 0.5-1.5 cm in diameter. Externally yellow or yellowish-brown, irregularly reticulate-wrinkled. Apex with slight dented stem scar, base usually tuberculate. Texture hard and fragile, fracture yellow, horny, waxy-sheeny. Odour, slight; taste, bitter.

Medicinal use[1,3,7]: To promote circulation of blood and relieve pain. Reinforce vital energy and alleviate pain such as headache and chest pain. For the treatment of inflammation, skin diseases and headache

Effects and indications of *Corydalis yanhusuo* according to Traditional Chinese Medicine[1,2,3,4]	
Taste:	acrid, sharp
Temperature:	warm
Channels entered:	*Orbis hepaticus, orbis lienalis, orbis stomachi, orbis pulmonalis*
Effects (functions):	Inhibitory activity on platelet aggregation, neuroprotective effects, inhibits calcium anion entry into cells to prevent neuronal death in ischemia-reperfusion rats, reduces cerebral infarct lesions in focal ischemia-reperfusion injured rats
Symptoms and indications:	Chest pain, epigastric pain, amenorrhea, dysmenorrhea, blood stasis after childbirth, traumatic swelling and pain

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents:

- **isoquinoline alkaloids**[5,6,7,8,9,10,11,12,13,14] **:**
  tetrahydroberberine
  tetrahydro-5-methyl bis-(1-3)benzdioxide-(4,5-C:5′,6)-azecine-13(5H)-one (protopine)
  6,7-methylenedioxy-2-(6-acetyl-2,3-methylenedioxybenzyl)-1 (2 H)-isoquinolinone
  dehydrocavidine
  tetradehydroscoulerine
  caseanidine
  clarkeanidine
  glaucine (dibenzoquinoline)

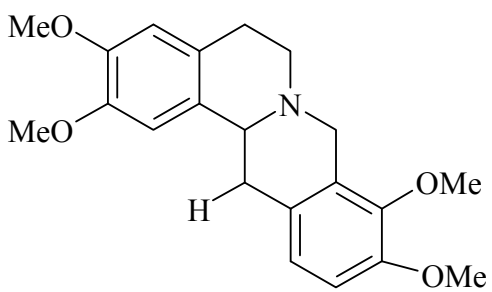
- **benzophenanthridine alkaloids**[15]**:**
  sanguidimerine
  sanguinarine
  spallidamine

- **tetrahydroprotoberberine N-oxide alkaloids**[16]**:**
  (-)-cis-corydalmine N-oxide
  (-)-trans-isocorypalmine N-oxide
  (-)-trans-corydalmine N-oxide

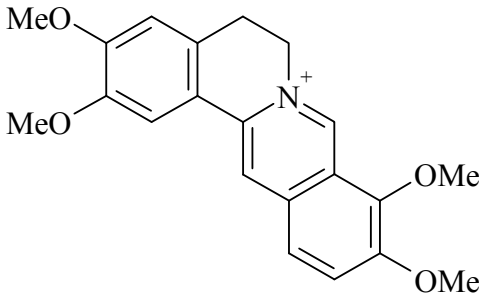
- **protoberberine type 1 alkaloids**[17]**:**
  palmatine
  coptisine
  dehydroapocavidine
  dehydrocorydaline

- **protoberberine type 2 alkaloids**[18]**:**
  isoapocavidine
  corydaline
  tetrahydropalmatine
  scoulerine
  isocorypalmine

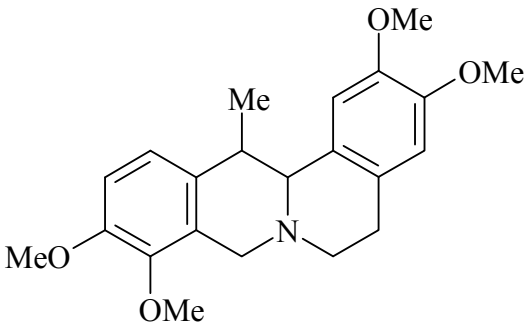
- **flavonol-O-glycosides**[19]**:**
  3-O-α-arabinopyranosyl-β-glucopyranoside, 7-O-glucopyranosides of kaempferol and quercetin
  kaempferol 3,7-di-O-glusoside, quercetin 3-O-glucoside, quercetin 7-O-glucoside, quercetin 3,7-di-O-glucoside, quercetin 3-O-rutinoside, quercetin 3-O-rutinosyl 7-O-glucoside



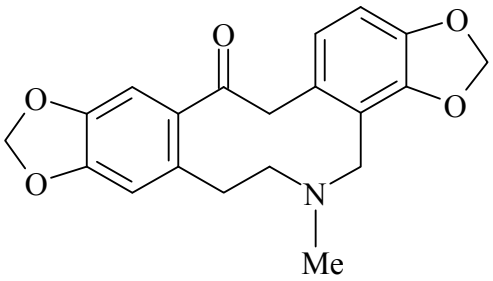
DL-Tetrahydropalmatine



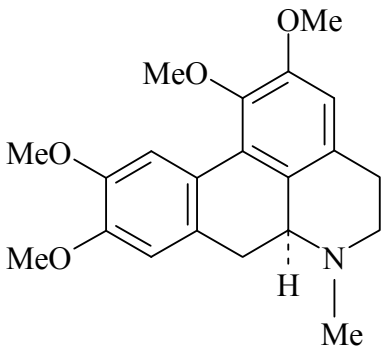
Palmatine



Corydaline



Protopine



Glaucine

Fig. 1: Formulae of the main compounds of *Corydalis yanhusuo*

Pharmacology:

- analgesic[20]
- neuroleptic[20]
- antihypertensive[21,25]
- antiinflammatory[22]
- anticholinergic[22]
- antihistaminic[22]
- anthelmintic[22]
- antibacterial[23]
- antiviral[23]
- anticancer[23]
- anti-ulcer effect[24]
- gastroprokinetic[24]
- hepaprotective[26]
- antimicrobial[22]

TLC-fingerprint analysis

Drug samples		Origin
1	Rhizoma Corydalis / *Corydalis yanhusuo*	province Zhejiang, China
2	Rhizoma Corydalis / *Corydalis yanhusuo*	province Jiangsu, China
3	Rhizoma Corydalis / *Corydalis yanhusuo*	province Jiangsu, China
4	Rhizoma Corydalis / *Corydalis yanhusuo*	sample of commercial drug, China
5	Rhizoma Corydalis / *Corydalis yanhusuo*	sample of commercial drug, China
6	Rhizoma Corydalis / *Corydalis yanhusuo*	sample of commercial drug, China

Reference compounds of Figure 2a + b		R*f*
T 1	Corydaline	0.70
T 2	Tetrahydropalmatine	0.68
T 3	Protopine	0.64
T 4	Palmatine	0.56

TLC-fingerprint analysis

1) Extraction: To 2 g of the powdered drug 1 ml ammonia solution 10 % and 10 ml methanol are added, followed by extraction under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml of methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: HPTLC Silica gel 60_{F254}, Merck

Applied amounts: Rhizoma Corydalis extract: each 10 µl
reference compounds: each 10 µl

Solvent system:

ethyl acetate	methyl ethyl ketone	formic acid	water
50	30	10	10

Direct evaluation: UV 365 nm

Detection: Dragendorff reagent:

Solution I: 0.85 g basic bismuth nitrate are dissolved under heating in a mixture of 10 ml glacial acetic acid and 40 ml water.

Solution II: 8 g potassium iodide are dissolved in 30 ml water.
5 ml of solution I and II are mixed with 20 ml glacial acetic acid in a volumetric flask and water added up to 100 ml. The plate is sprayed with ca. 10 ml of this mixture and evaluated in VIS.

4) Description:

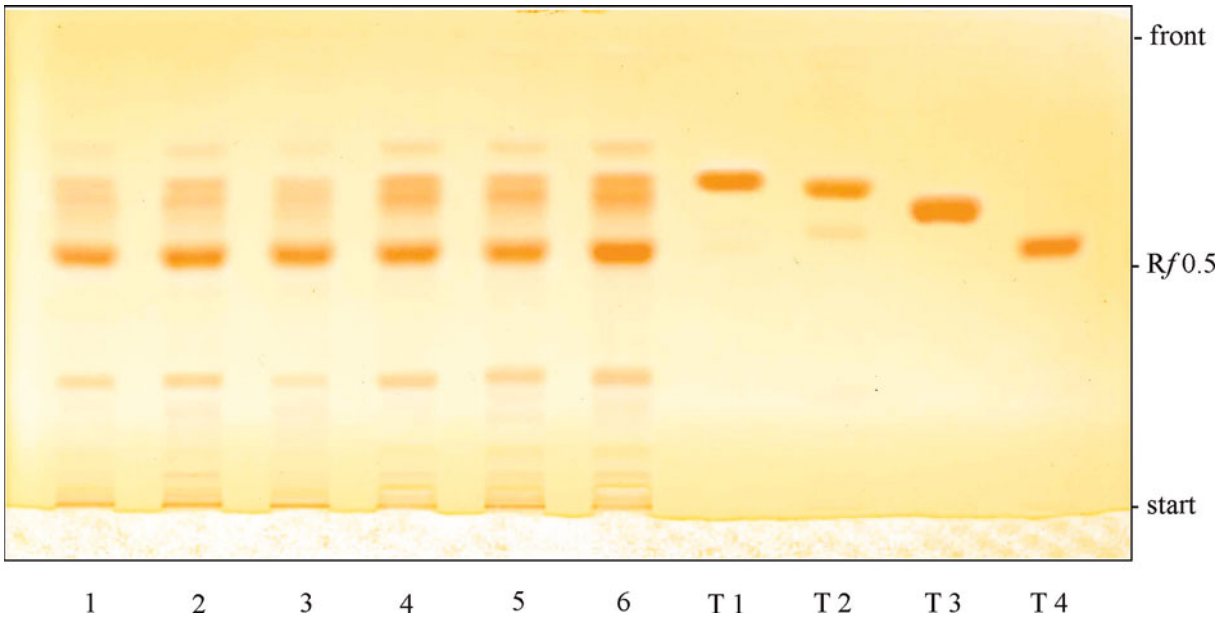


Fig. 2a: TLC of the methanol extracts of Rhizoma Corydalis sprayed with Dragendorff reagent (VIS)

All extracts show a very homogeneous pattern of five distinct orange-brown bands in the R*f*-range of 0.20 to 0.80. Palmatine is the dominant and characteristic zone at R*f* = 0.58. Above Palmatine in the R*f*-range of 0.65 – 0.80 appear partly overlapped the weaker zones of Protopine (R*f* = 0.64), Tetrahydropalmatine (R*f* = 0.68) and Corydaline (R*f* = 0.70). The small alkaloid zones at R*f* = 0.77 and 0.29 could not be assigned.

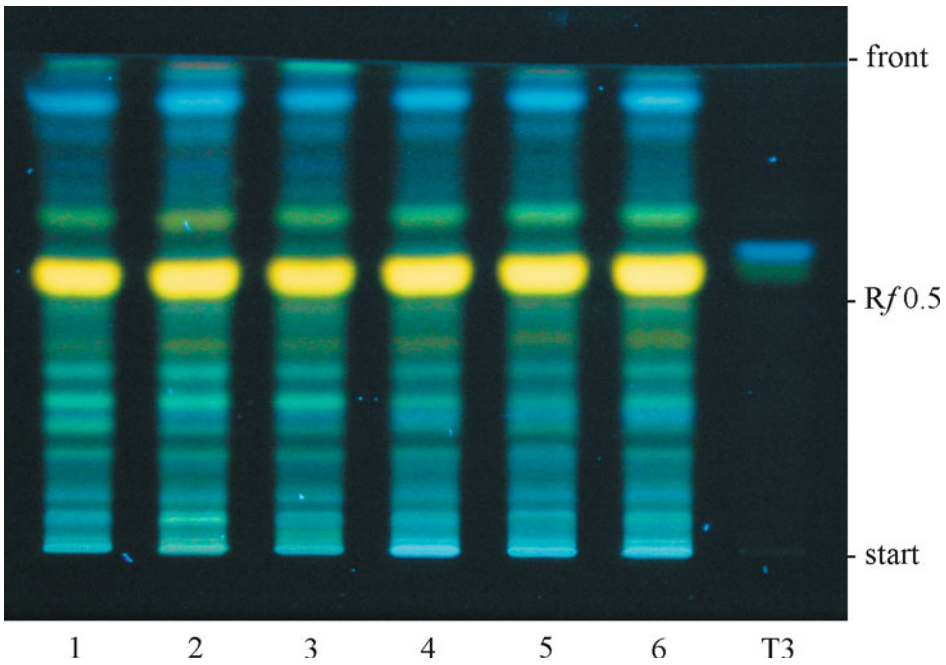


Fig. 2b: Thin layer chromatogram of the methanol extracts of Rhizoma Corydalis (UV 365)

All six *Corydalis* extracts show the same homogeneous pattern of ~ 15 fluorescent zones distributed over the whole R*f*-range with the conspicuous bright yellow Palmatine alkaloid at R*f* = 0.58. The other alkaloids fluorescence light green or deep blue because of their nonconjugated ring system.

HPLC-fingerprint analysis:

1) Sample preparation:	To 1 g of the powdered drug 1 ml ammonia solution 25 % and 10 ml methanol are added, followed by extraction under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.
2) Injection volume:	Rhizoma Corydalis extract: each 10.0 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: 2.0 g hexanesulfonic acid (Aldrich) / 1 l dist. Water (Millipore Ultra Clear UV plus® filtered) + H_3PO_4 85 % (Merck) (pH = 3) B: acetonitrile (VWR)
Gradient:	10-25 % B in 10 minutes 25-35 % B in 20 minutes 35-90 % B in 5 minutes 90 % B in 10 minutes total runtime: 45 minutes
Flow:	1.0 ml/min.
Detection:	262 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	25.11	Protopine
2	27.01	(not identified)
3	29.56	Corydaline
4	30.17	Tetrahydropalmatine
5	33.10	(not identified)
6	34.93	Palmatine

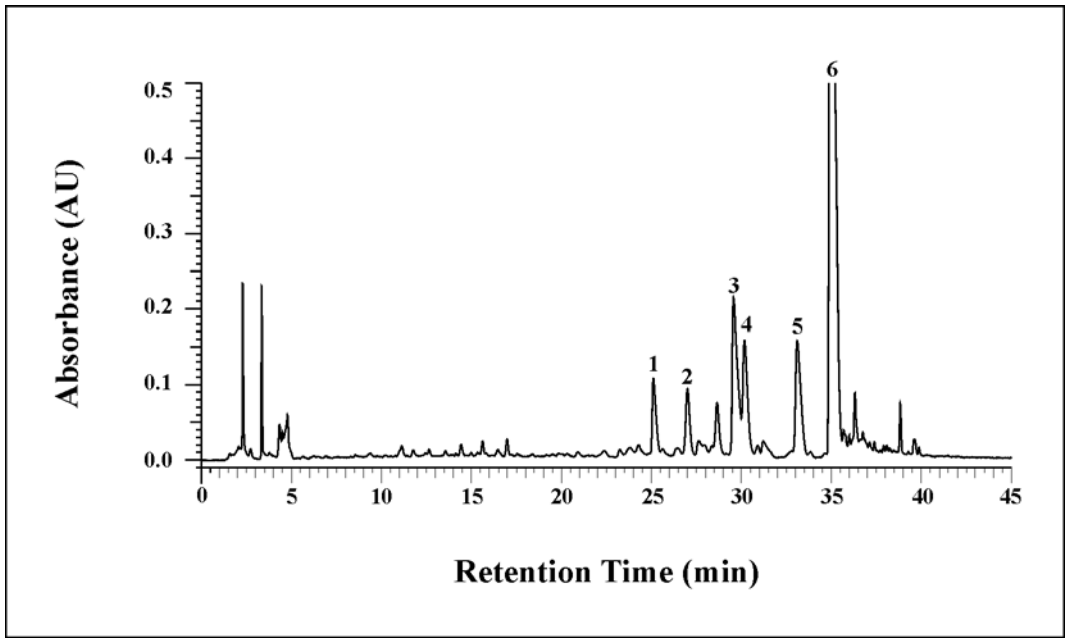


Fig. 3a: HPLC fingerprint of Rhizoma Corydalis sample 1

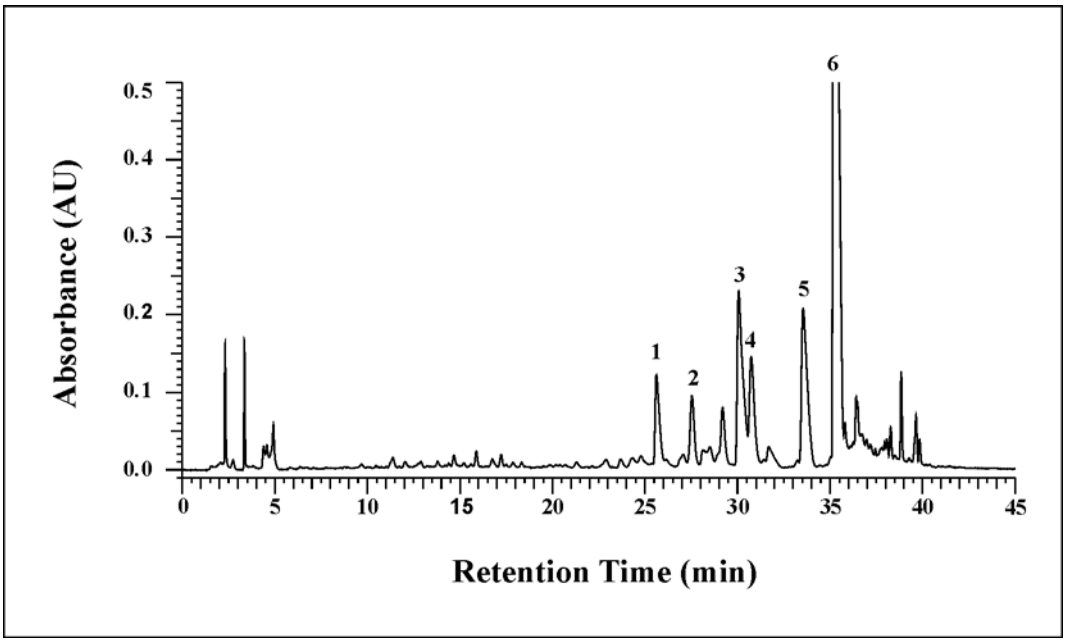


Fig. 3b: HPLC fingerprint of Rhizoma Corydalis sample 2

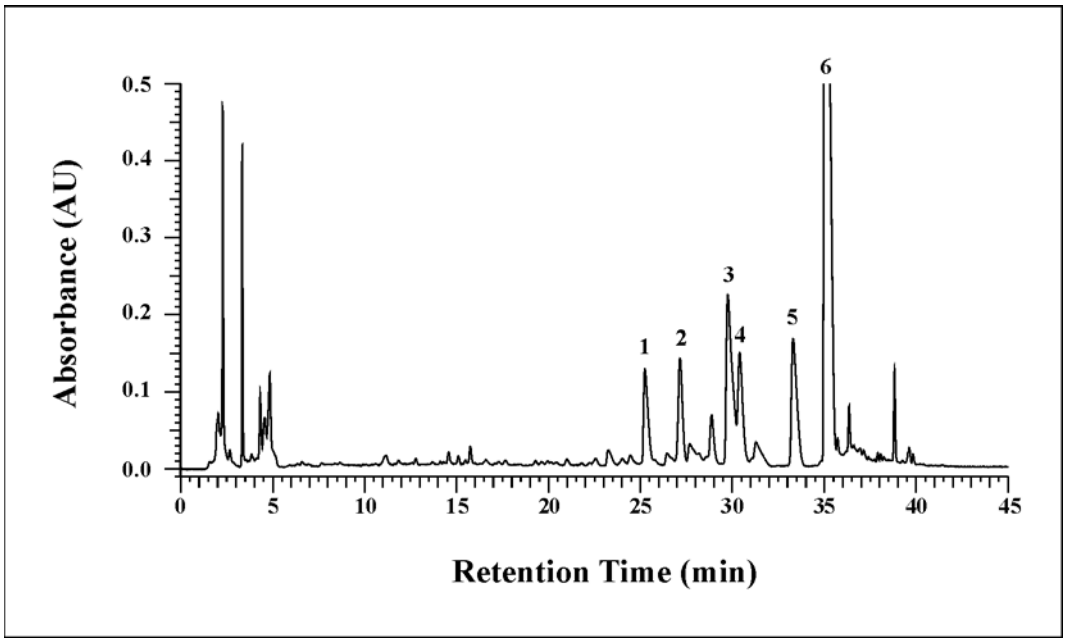


Fig. 3c: HPLC fingerprint of Rhizoma Corydalis sample 4

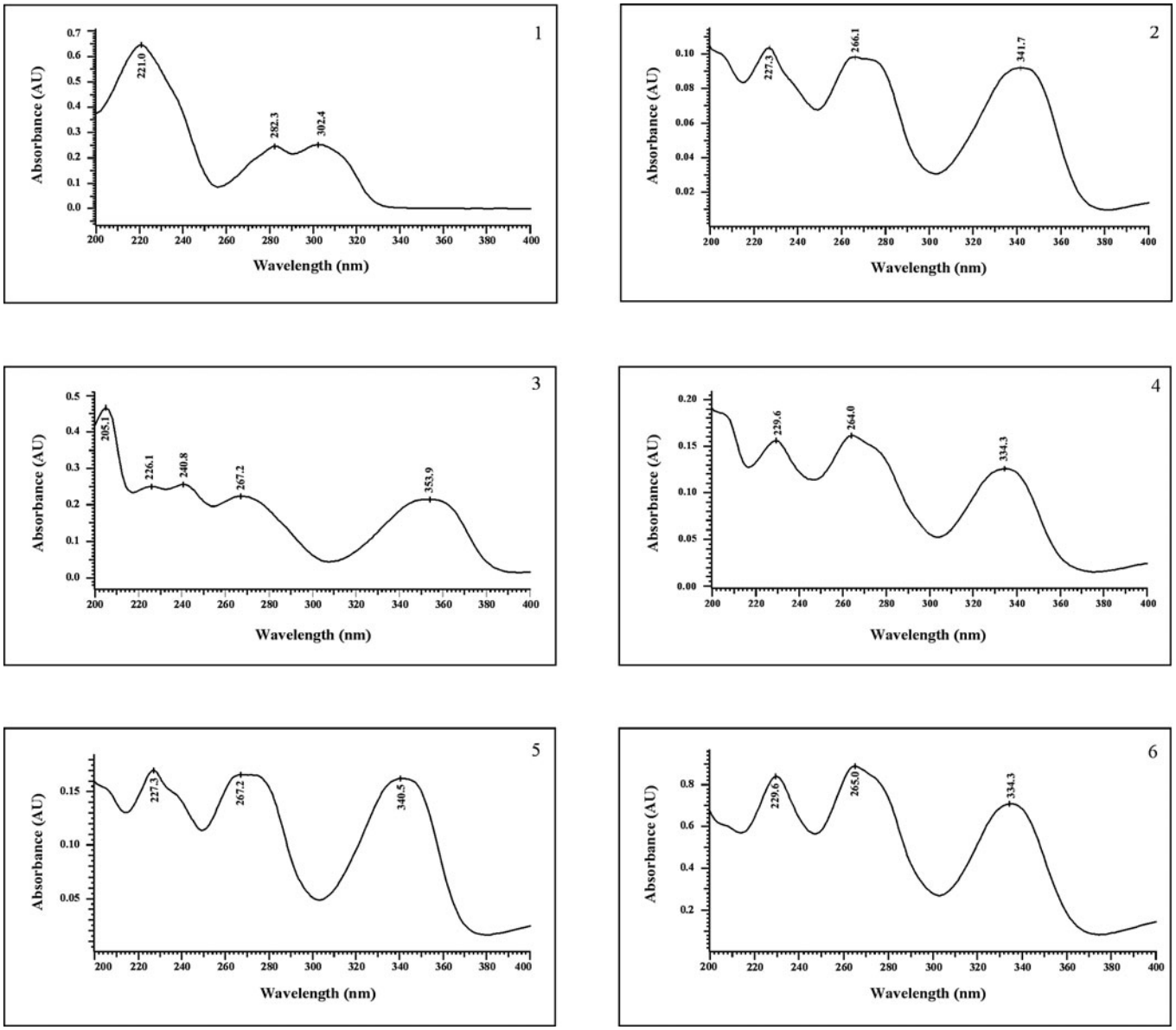


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of Rhizoma Corydalis

4) Description of Fig 3a, b, c:

All six Rhizoma Corydalis MeOH-extracts – shown are only the extracts of drug sample 1, 2 and 4 – possess a very uniform pattern of six major alkaloid peaks in the Rt-range of 25.0 till 40.0. Palmatine (Rt = 34.93, **6**) and the alkaloids **2**, **3**, **4**, **5** are characterized by three UV-maxima at 220-229 nm, 260-265 nm and 340-353 nm respectively.

According to reference alkaloids the peaks **1**, **3** and **4** could be assigned to Protopine (Rt ~25.1-25.5, **1**), Corydaline (Rt = 29.56, **3**) and Tetrahydropalmatine (Rt ~30.2-30.7, **4**). The UV-spectrum of Protopine (**1**) differs from that of the other alkaloids because of its additional carbonyl-group. The alkaloid peaks **2** and **5** could not be identified.

Conclusion

The TLC and HPLC of the extracts of all 6 samples of Rhizoma Corydalis show a very great homogeneity in their characteristic TLC-zones and HPLC-peak pattern. Palmatine is the dominant alkaloid in all samples. The various alkaloid types possess very similar UV-spectra with 3 strong conspicuous maxima between 220 and 350 nm.

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Radix Dipsaci – *Xuduan*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]: Radix Dipsaci is the dried root of *Dipsacus asperoides* C.Y. Cheng et T.M.Ai (Fam. Dipsacaceae).

The drug is collected in autumn, removed from the root stock and rootlet, baked to half-dryness, piled up until a green colour is developed inside, and then baked to dryness.

Origin[2,3]: Produced mainly in the provinces of Si Chuan and Hu Bei (China).

Description of the drug[1]: Cylindrical, somewhat flattened, some slightly curved, 5-15 cm long, 0.5-2 cm in diameter. Externally greyish-brown or yellowish-brown, with slightly twisted or obviously twisted longitudinal wrinkles and furrows, showing transversal lenticel-like cicatrices and sparse rootlet scars. Texture soft and hardened after long storage, easily broken, fracture uneven, bark dark green or brown, the outer part brown or pale brown; wood yellowish-brown, vessel bundles arranged radially. Odour, slightly aromatic; taste, bitter, slightly sweet, then astringent

Pretreatment of the raw drug[1]: Washed clean, softened thoroughly, cut into slices, and dried.

Medicinal use[3]: for internal and external treatment of low back and knee pains, inflammation and traumatic injuries of the bones joints and legs; gynecological bleedings (e.g. during pregnancy), general weakness and lack of strength

Effects and indications of *Dipsacus asperoides* according to Traditional Chinese Medicine [1,3,4,5,6]	
Taste:	Bitter, acrid
Temperature:	Neutral with warm tendency
Channels entered:	*orbis hepaticus, orbis renalis*
Effects (functions):	Replenishes the liver and the kidney, strengthens tendons and bones, heals bone fractures, arrests abnormal uterine bleeding
Symptoms and indications:	Aching and weakness of loins and knees; rheumatic arthralgia, abnormal uterine bleeding or menorrhalgia, due to liver and kidney deficiency, uterine bleeding during pregnancy, traumatic injuries

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents:

- phenolic carboxylic acids[7]**:**
methyl-3,4-di-O-caffeoylic acid ; methyl-3,4-di-O-caffeoylquinat; 3,5-di-O-caffeoylquinic acid, methyl-3,5-di-O-caffeoyl quinat; 4,5-di-O-caffeoylquinic acid; methyl-4,5-di-O-caffeoyl quinat

- iridoid terpenoids[8,9,10,11]**:**
loganin, loganic acid, loganic acid-6`-O-β-D-glucoside, sweroside, cantleyoside, dipsanoside A and B

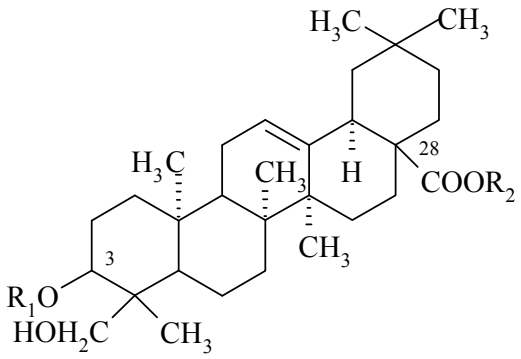
- triterpenoid saponins[11-14]**:**
hederagenin-mono-, di-, tri-, tetraoctaglycosides containing glucose, rhamnose, arabinose and xylose as sugar moieties: (e.g. asperosaponin VI = akeboside D), oleanolic acid-mono-, di-, tri-, tetraglycosides containing glucose, rhamnose, arabinose, and xylose as sugar moieties, besides the aglycones hederagenin and oleanolic acid

- sterols[15]**:**
sitosterol, daucosterol

- essential oil[16]

- alkaloids[17,18]**:**
venoterpine, cantleyine

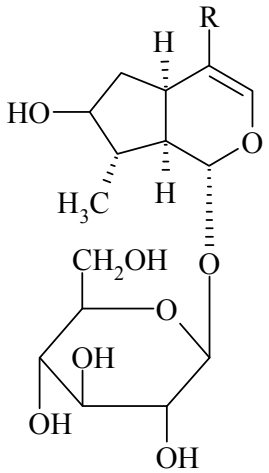
- polysaccharides[19]



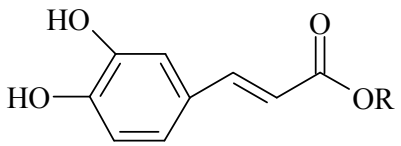
Hederagenin, - mono-,di-,tri-,tetra→octaglycosides
(3-O-or 3,28-O-glycosides)
Marker compound: Asperosaponin VI
= Akebeoside D
R_1 = Ara R_2 = Glu (1-6) Glu

Reference compounds:

Hederagenin	R_1 = H R_2 = H
Hederin	R_1 = Rha-Ara R_2 = H
Hederacosid C	R_1 = Rha-Ara R_2 = Glu-Glu-Rha



Loganin: R = $COOCH_3$
Loganic acid: R = COOH



Chlorogenic acid: R = quinic acid

Isochlorogenic acid: R = caffeoylquinic acid

Caffeic acid: R = H

Fig. 1: Formulae of the main compounds of *Dipsacus asperoides*

Pharmacology: (in vitro/in vivo)

triterpenoids and phenolcarboxylic acids:

- analgesic[7, 20]
- antioxidant[7]
- antiinflammatory[21]
- antitumoral (cytotoxic)[22]
- neuroprotective[23]
- immunomodulating[19]:
  anticomplementary ➞ triterpensaponins[19]
  mitogenic activity on lymphocytes, phagocytosis stimulating ➞ polysaccharides[19]

TLC fingerprint analysis

Drug samples		Origin
1	Radix Dipsaci / *Dipsacus asperoides*	sample of commercial drug, China
2	Radix Dipsaci / *Dipsacus asperoides*	province Anhei, China
3	Radix Dipsaci / *Dipsacus asperoides*	sample of commercial drug, China
4	Radix Dipsaci / *Dipsacus asperoides*	sample of commercial drug, China
5	Radix Dipsaci / *Dipsacus asperoides*	sample of commercial drug, China
6	Radix Dipsaci / *Dipsacus asperoides*	province Sichuan, China
7	Radix Dipsaci / *Dipsacus asperiodes*	sample of commercial drug, China

Reference compounds of Figure 2a		R*f*
T1	hederacosid C	0.60
T2	α-hederin	0.90
T3	loganin	0.81
T4	loganic acid	0.44
T5	oleanolic acid	0.91

Reference compounds of Figure 2b		R*f*
T6	chlorogenic acid	~ 0.50
T7	isochlorogenic acids mixture	0.79 / 0.92
T8	caffeic acid	0.95

1. Thin layer chromatogram of the triterpenoid saponins:

1) Extraction: 1.0 g of the powdered drug is extracted under reflux with 20 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 2 ml of methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Radix Dipsaci extract: each 5 µl
reference compounds: each 10 µl

Solvent system:	chloroform : methanol : water 64 50 10
Detection:	Vanillin-sulphuric acid reagent: I: 1 % ethanolic vanillin solution II: 10 % ethanolic sulphuric acid The plate is sprayed with solution I followed immediately by solution II. The plate is heated for 5-10 minutes at 105 °C and evaluated in VIS.

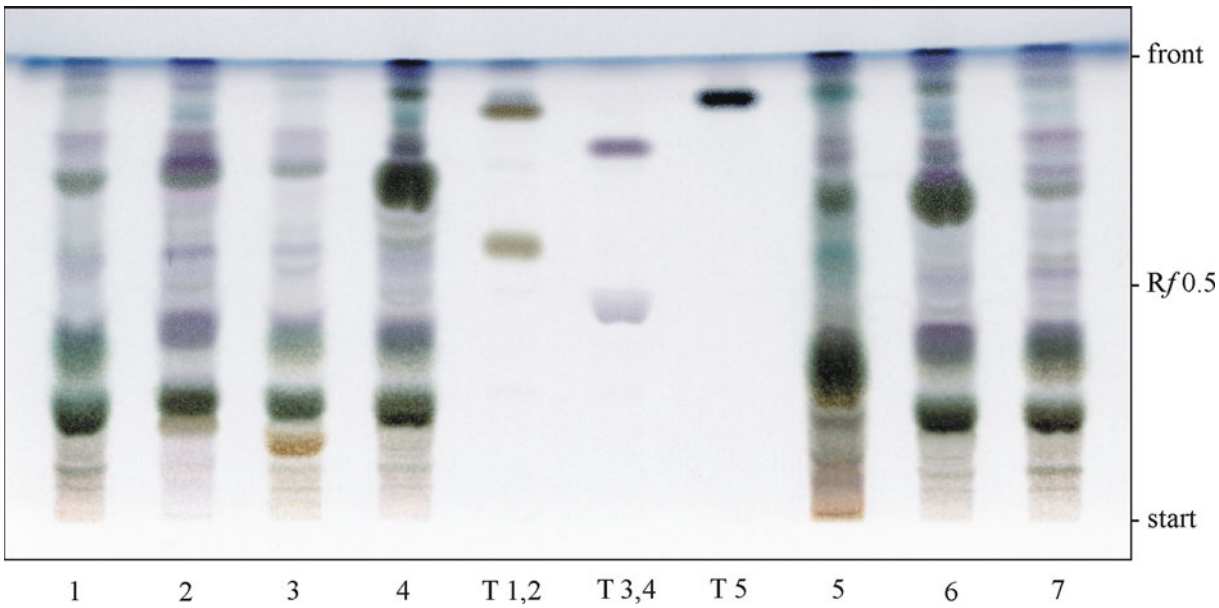


Fig. 2a: Thin layer chromatogram of methanolic extracts of Radix Dipsaci sprayed with vanillin-sulphuric acid reagent (VIS)

4) Description:

All samples of Radix Dipsaci (Tab. 1-7) show in the TLC fingerprint analysis a very similar qualitative pattern of about 12-15 violet-brown zones distributed over the entire R*f*-distance. They represent the hederagenin/oleanolic acid saponins inclusive their aglycones and the diterpenoids.

Just below the solvent front lie the zones of the aglycones oleanolic acid and hederagenin, followed at R*f* = 0.70-0.75 by the marker substance asperosaponin VI, a hederagenin-triglycoside*. In sample 2, 4 and 6 this saponin was detected in high concentration, in sample 1, 3 and 7 only in relatively low concentration. In the R*f*-range of R*f* = 0.4-0.65 appear saponins with four sugars, in the R*f*-range 0.2-0.4 the penta-, hexa-, hepta- and octa-glycosides of hederagenin. With the exception of the genuine terpenoids loganin, loganic acid (**T3**, **T4**) and oleanolic acid (**T5**), hederin and hederacosid C (**T1**, **T2**) are no genuine terpenoids of Radix Dipsaci.

* This saponin is used as marker compound also in the Chinese Pharmacopoeia of 2005 (not less than 2 %)[(1)]

Note: - the alkaloids described in the literature and in the Chinese Pharmacopoeia 2005 could not be detected even after solvent enrichment.

- the reference compounds α-hederin **T2** (hederagenin-diglycoside) and hederacosid C **T1**(hederagenin-pentaglycoside) are not present in Radix Dipsaci and serve to assign the zones of the various Dipsacus-saponins

2. Thin layer chromatogram of the phenolcarboxylic acids:

1) Extraction: 1.0 g of the powdered drug is extracted under reflux with 20 ml of methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 5 ml of water and shaken twice with 5 ml ethyl acetate. The ethyl acetate layer is separated, evaporated to dryness, the residue dissolved in 2 ml methanol and used for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{250}, Merck

Applied amounts: Radix Dipsaci extract: each 10 µl
reference compounds: each 10 µl

Solvent system:

ethyl acetate	formic acid	glacial acetic acid	water
100	11	11	26

Detection: Natural products-polyethylene glycol reagent (NP/PEG):
I: 1 % diphenylboric acid-β-ethylamino ester
(= diphenylboryloxyethylamine, NP) in methanol
II: 5 % polyethylene glycol-4000 (PEG) in ethanol

The plate is sprayed first with solution I and then with solution II. The evaluation is carried out in UV 365 nm.

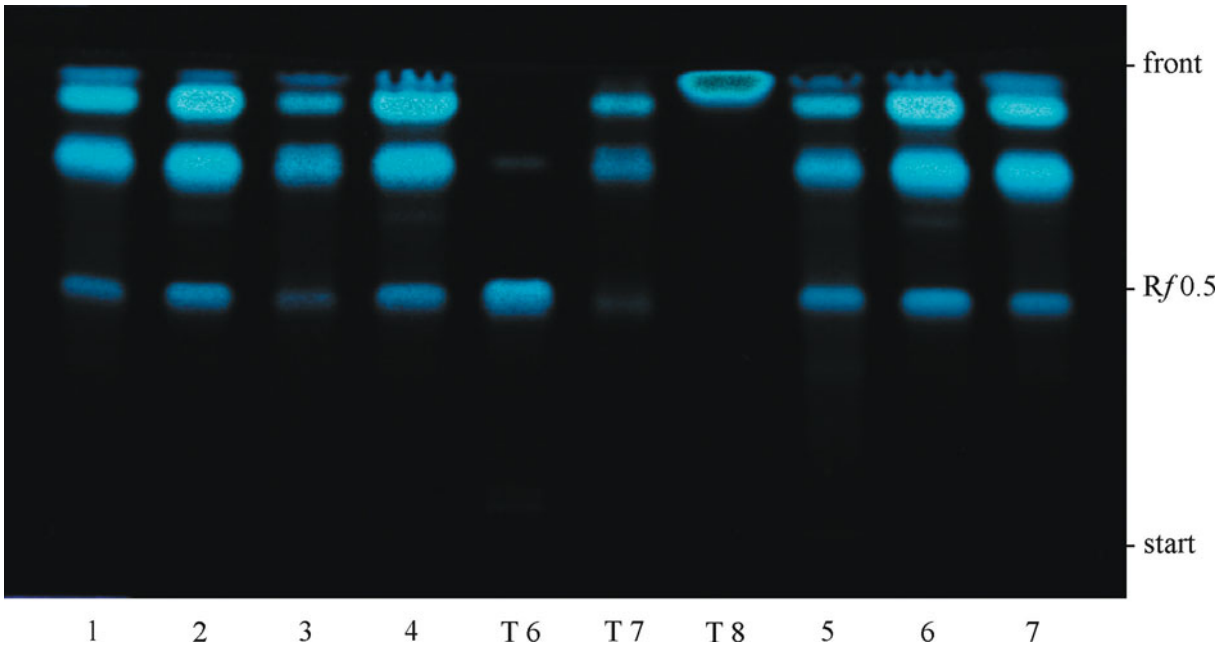


Figure 2b: Thin layer chromatogram of ethyl acetate phases of Radix Dipsaci methanol extracts sprayed with natural products-polyethylene glycol reagent (UV 356 nm).

4) Description:

The samples 1-4 and 5-7 give in the R*f*-range 0.5-1.0, four prominent light blue fluorescent zones at R*f* = 0.50 (chlorogenic acid), R*f* = 0.79 / 0.92 (isochlorogenic acids) and at R*f* = 0.95 (caffeic acid), as evidenced by the three reference compounds T6, T7 and T8.

HPLC-fingerprint analysis:

1) Sample preparation: 1.0 g of the powdered drug is extracted for 30 minutes under reflux with 20 ml of methanol. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 2 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Radix Dipsaci extract: each 5.0 µl

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 125-4 with LiChrospher® 60 RP select B (5 µm), Merck

Precolumn: LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck

Solvent: A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered)
B: acetonitrile (VWR)

Gradient: 5-35 % B in 45 min.,
35-95 % B in 10 min.,
95 % B in 10 min.
total runtime: 65 minutes

Flow: 0.8 ml/min.

Detection: 205 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	~14.0	loganin
2	25.5-26.0	isochlorogenic acids
3	41.0-41.5	asperosaponin VI
4	61.5-62.0	non identified terpenoid
5	10.5-11.0	chlorogenic acid

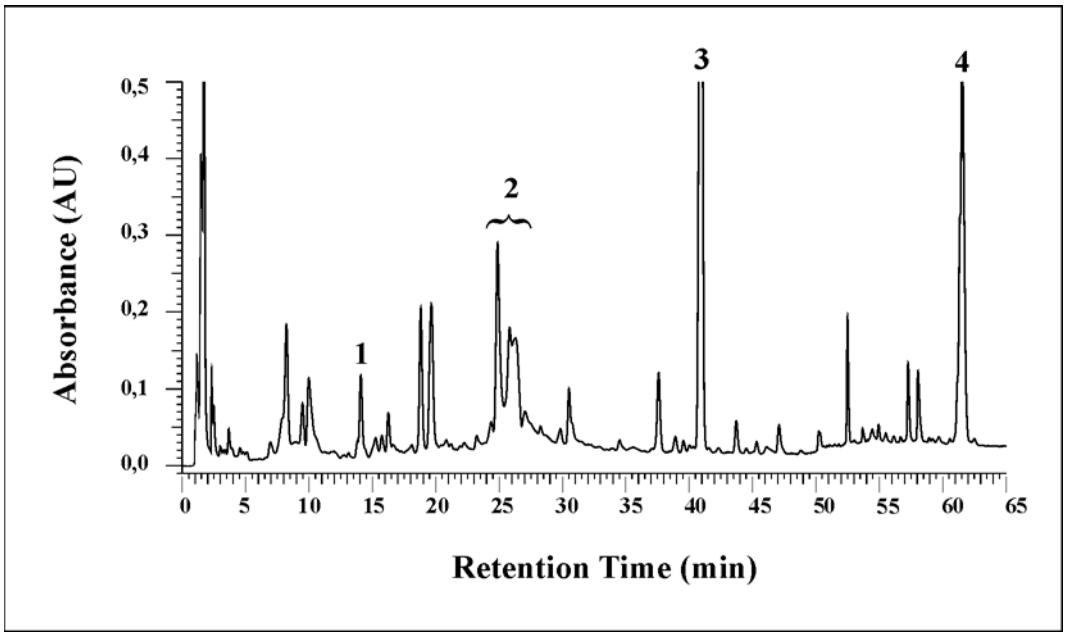


Figure 3a: HPLC-fingerprint chromatogram of the methanol root extract of Radix Dipsaci extract sample 4

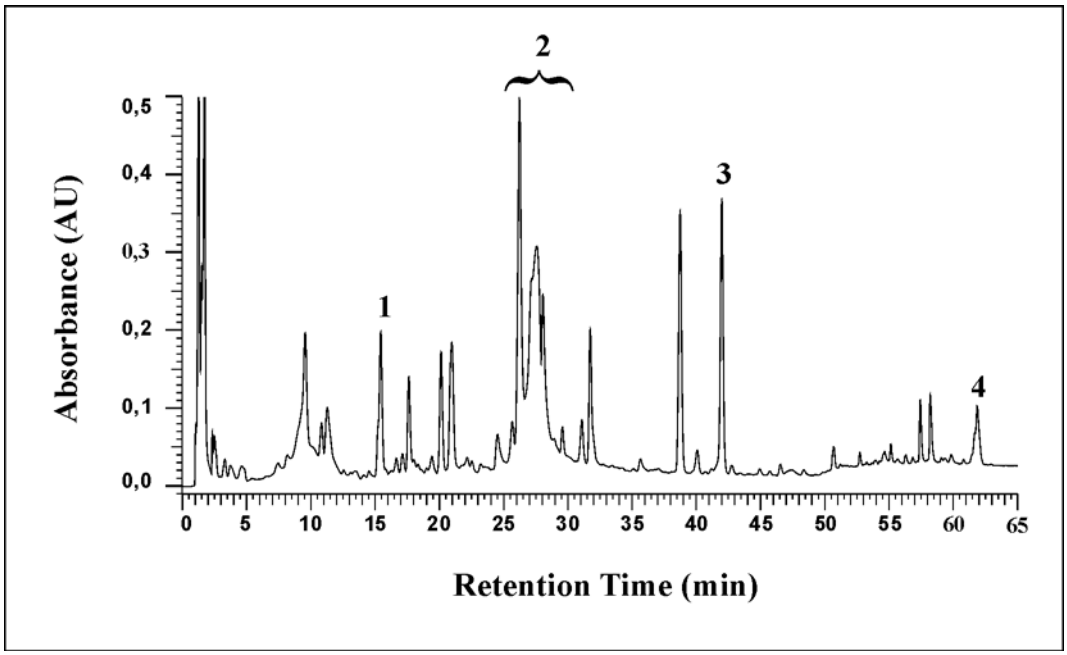


Figure 3b: HPLC-fingerprint chromatogram of the methanol root extract of Radix Dipsaci sample 2

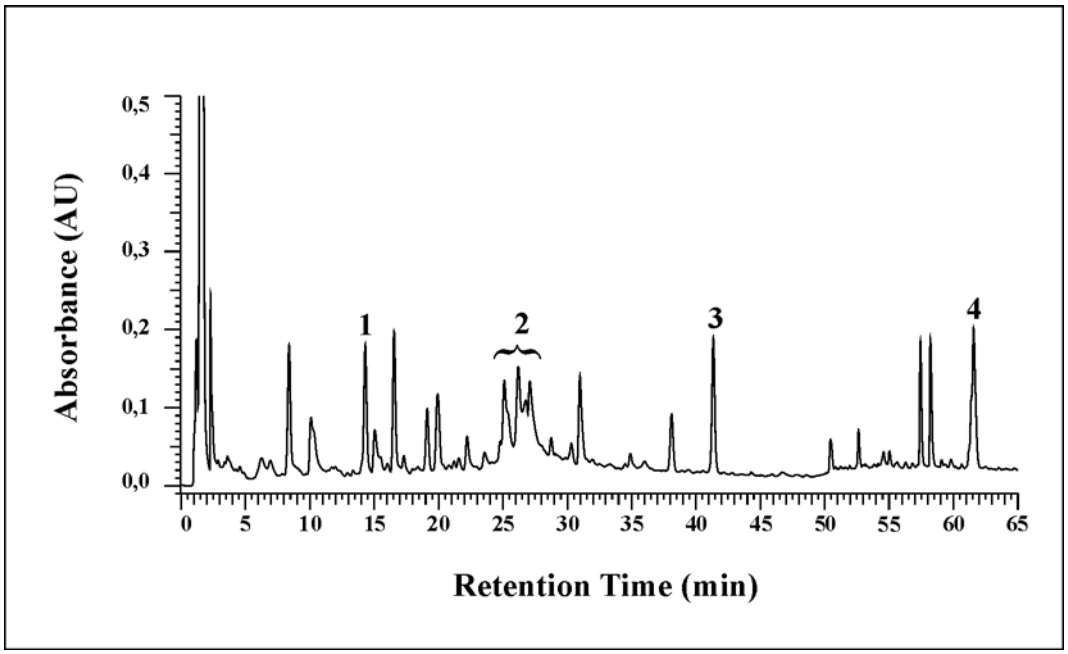


Figure 3c: HPLC-fingerprint chromatogram of the methanol extract of Radix Dipsaci sample 7

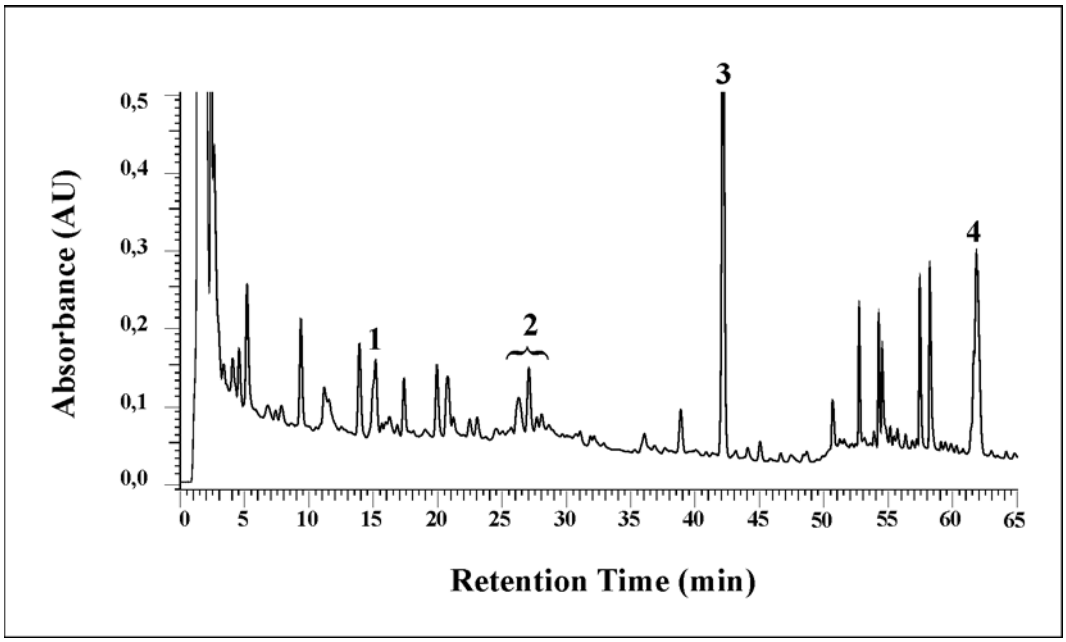


Figure 3d: HPLC-fingerprint chromatogram of the methanol root extract of Radix Dipsaci sample 5

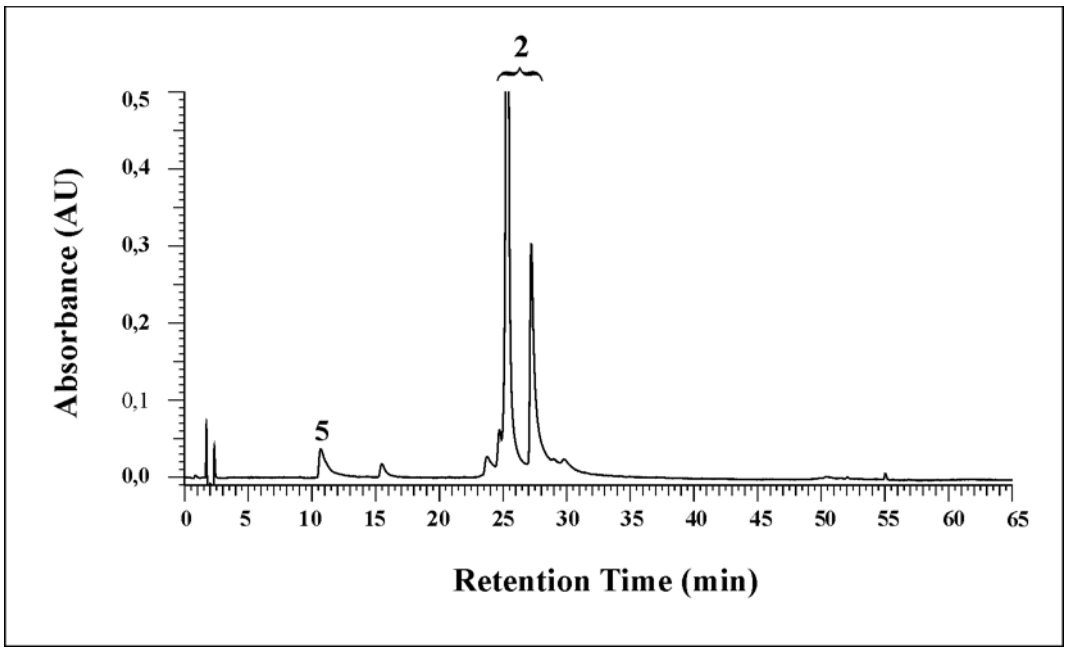


Figure 3e: HPLC-fingerprint chromatogram of the ethyl acetate phase of Radix Dipsaci sample 2

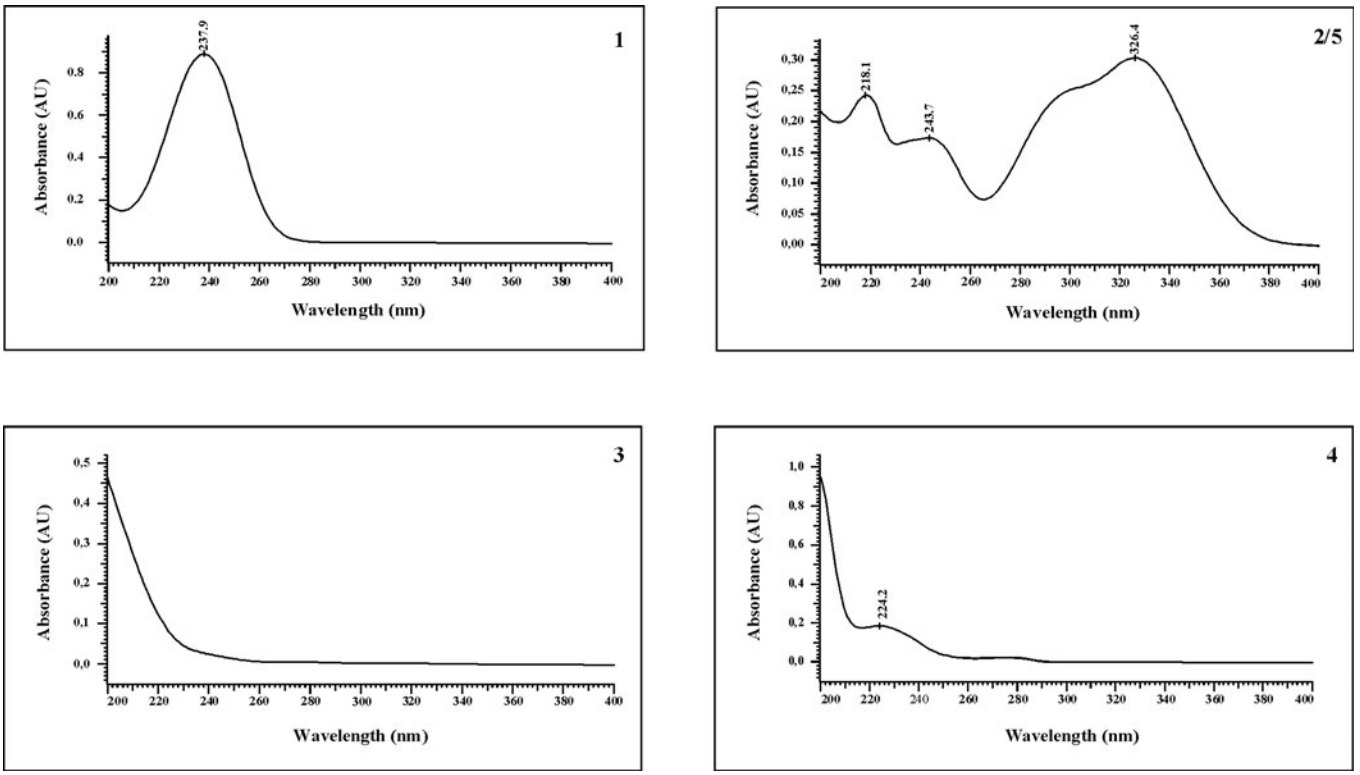


Figure 4: UV-spectra of the main compounds (peak) of the root extract of Radix Dipsaci

4) Description of the HPLC

The characteristic types of compounds of the methanol extracts of Radix Dipsaci, the triterpene saponins and the caffeic acid derivatives, can be detected in one HPLC-fingerprint at 205 nm in Fig. 3 a, b, c and d. For identifying exclusively the chlorogenic- and isochlorogenic acids we propose to use the ethyl acetate phase and record them at 325 nm (Fig. 3e)

Note: In the HPLC-fingerprints of all samples investigated (Fig. 3a, b, c and d) appear in the Rt-range 18.5-21.5 and 56.0-58.0 two characteristic peak doublets of non identified compounds (terpenoids?).

Description of Figure 3a:

Figure 3a shows the HPLC-fingerprint of the methanol extracts of sample 4. It is characterized by a nearly superimposed peak pattern with the marker compound loganin (**1**) (Rt ~ 14.0), isochlorogenic acids (**2**) (Rt = 25.5-26.0), asperosaponin VI (**3**) at Rt = 41.0-41.5 and a non identified terpenoid (**4**) at Rt = 61.5-62.0.

Description of Figure 3b and 3c:

Figure 3b and 3c of samples 2 and 7 respectively show a peak pattern which deviates from each other and from sample 4 mainly in the concentration of the peaks 2, 3 and 4.

Figure 3d :

The fingerprint of sample 5 in Figure 3d is again characterized by a high concentration of the marker saponin, asperosaponin VI (**3**), at Rt = 42.0 and compound **4** at Rt = 62.5, whereas all peaks in the Rt-range of 12.0-40.0 appear in relative low concentrations.

Figure 3e:

Figure 3e shows a representative fingerprint of the ethyl acetate phase prepared from the methanol extract of sample 2, with chlorogenic acid at Rt = 10.7 and isochlorogenic acids at Rt = 25.0 and 27.0.

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Rhizoma Atractylodis lanceae
Cangzhu

Pharmacopoeias:

Pharmacopoeia of the People's Republic of China, English Edition Vol. 1, 2005[1]

Japanese Pharmacopoeia, English Edition 1996 (Jap. XIII)

Official drugs:

The rhizomes of *Atractylodes lancea* (Thunb.) DC. and *Atractylodes chinensis* (Bunge) Koidz. – Asteraceae – .

The title of the official Chinese *Cangzhu* monograph is "Rhizoma Atractylodis".

The rhizome of *Atractylodes macrocephala* Koidz., *Baizhu* is a second official drug in the Chinese Pharmacopoeia edition 2005 and the TLC- and HPLC-analysis described in a separate monograph in this book.

In the Japanese Pharmacopoeia, the rhizomes of the two *Cangzhu* species are described in the Chinese monograph "Atractylodis lanceae Rhizoma (Sojutsu)", whereas "Atractylodis Rhizoma (Byakujutsu)" means the rhizomes of *Atractylodes ovata* DC. *(= Atractylodes macrocephala* Koidz., *Baizhu)* or of *Atractylodes japonica* Koidz. ex Kitam.

Origin:

China (provinces Jiangsu, Anhui, Henan, Hubei, Hebei), Korea, Japan

Synonyms:

***Atractylodes lancea* (Thunb.) DC.** = *A. lyrata* Sieb. et Zucc., *A. ovata* Thunb., *Atractylodes lancea* Thunb., *Atractylodes ovata* Thunb., *Atractylodes lyrata* (Sieb. et Zucc.) Nakai, *Atractylodes separata* Bailey, *Arcana lancea* (Thunb.) Willd.

English: Swordlike Atractylodes, Japanese: *Sojutsu*[4,5]

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

***Atractylodes chinensis* (Bunge) Koidz.** = *A. lancea* var. *chinensis* (Bunge) Kitam., *Atractylis chinensis* (Bunge) DC., often not correctly: "*Atractylodes chinensis* (DC.) Koidz"[5]

Description of the drug:[1,6,7]

Rhizoma Atractylodis lanceae: Irregularly moniliform or nodular-cylindrical, somewhat curved, occasionally branched. 3 - 10 cm long, 1 - 2 cm in diameter. Externally greyish-brown, wrinkled, transversly twisted-lined, with remains of rootlets, and stem scars or remains of stems attached at apex. Texture compact, fracture yellowish-white or greyish-white, scattered with many orange-yellow or brownish-red oil cavities that - as a sign of good quality - crystallize out as white fine needle crystals on the surface of the drug in storage. Odour: characteristic, taste: sweetish, pungent and bitter.

Rhizoma Atractylodis chinensis: Knotty-lumpy or nodular-cylindrical, 4 - 9 cm long, 1 - 4 cm in diameter. Externally blackish-brown, yellowish-brown when peeled. Texture lax, fracture scattered with yellow oil cavities. Odour: weakly aromatic, taste: pungent and bitter.

Pretreatment of raw drug:[1,6]

After elimination of the foreign matter the drug is washed, softened thoroughly, cut into thick slices and dried (*Cangzhu*).
The slices are stir-fried with bran until its outer surface becomes deep yellow (*Fuchaocangzhu*).

Medicinal use:[1,3,6]

In Tradional Chinese Medicine *Cangzhu* is used as a remedy against rheumatic diseases, edema, particularly edema of the legs with lameness, digestive disorders (mild diarrhea, nausea, vomiting, loss of appetite), night blindness and common cold.

Effects and indications according to Traditional Chinese Medicine:[1,2,3,6]	
Taste:	pungent, bitter
Temperature:	warm
Channels entered:	*spleen, stomach*
Effects:	dries dampness and strengthens the *spleen*, dispels *wind-cold*, promotes sweating and improves eyesight.

Symptoms and indications:	– dampness blocking the *spleen* and *stomach* - symptoms include epigastric distension and fullness, reduced appetite, nausea or vomiting, lassitude and sticky tongue coating; – *wind-cold-damp* obstruction syndrome with swollen and painful knee joints and weakness of the lower limbs; – exterior syndrome due to invasion by exogenous pathogenic *wind, cold* and *dampness* manifested as soreness and heaviness of the limbs, chills, fever, headache; – downward flow of *damp-heat* (as in leg *qi*), vaginal discharge, and swollen, sore joints; – night blindness and diminished vision with a rough sensation in the eyes.

Main constituents (see Fig. 1):

- **essential oil:** monoterpenes (e.g. borneol, 3-carene, *p*-cymene, α-/β-phellandrene, α-terpinene), sesquiterpenes (atractylon, elemol, α-bisabolol, muurolene, valencene, δ-cadinene, and "atractylol", a mixture of β-eudesmol and hinesol), phenolic constituents (thymol, carvacrol, *o*-cresol, *p*-cresol), atractylodin (a polyacetylene), *n*-dodecanol[8,9,10]
- further sesquiterpenes (3β-acetoxyatractylon, 3β-hydroxyatractylon, atractylenolide I, II and III)[11,12,13]
- sesquiterpene glycosides (guaiane- and eudesmane-type)[14]
- **triterpenes and sterols**[12]
- further acetylenic compounds (e.g. atractylodin, atractylodinol, acetylatractylodinol and its 1*Z*-derivatives, (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate, (4*E*,6*E*,12*E*)-tetradecatriene-8,10-diyne-1,3-diyl diacetate, *threo*-1-(2-furyl)-(7*E*)-nonene-3,5-diyne-1,2-diyl diacetate, (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diynyl-1-*O*-(*E*)-ferulate)[12,15,16, 17,18]
- 2-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-6-methyl-2,5-cyclohexadiene-1,4-dione (atractyloquinone)[12,13]
- atractylochromene[12,13]
- atractylohydroquinone[12]
- monosaccharides (arabinose, galactose, glucose)[19]
- **furanocoumarin** (osthol)[12,13,17]
- **polysaccharides** (actractans A,B,C)[20]

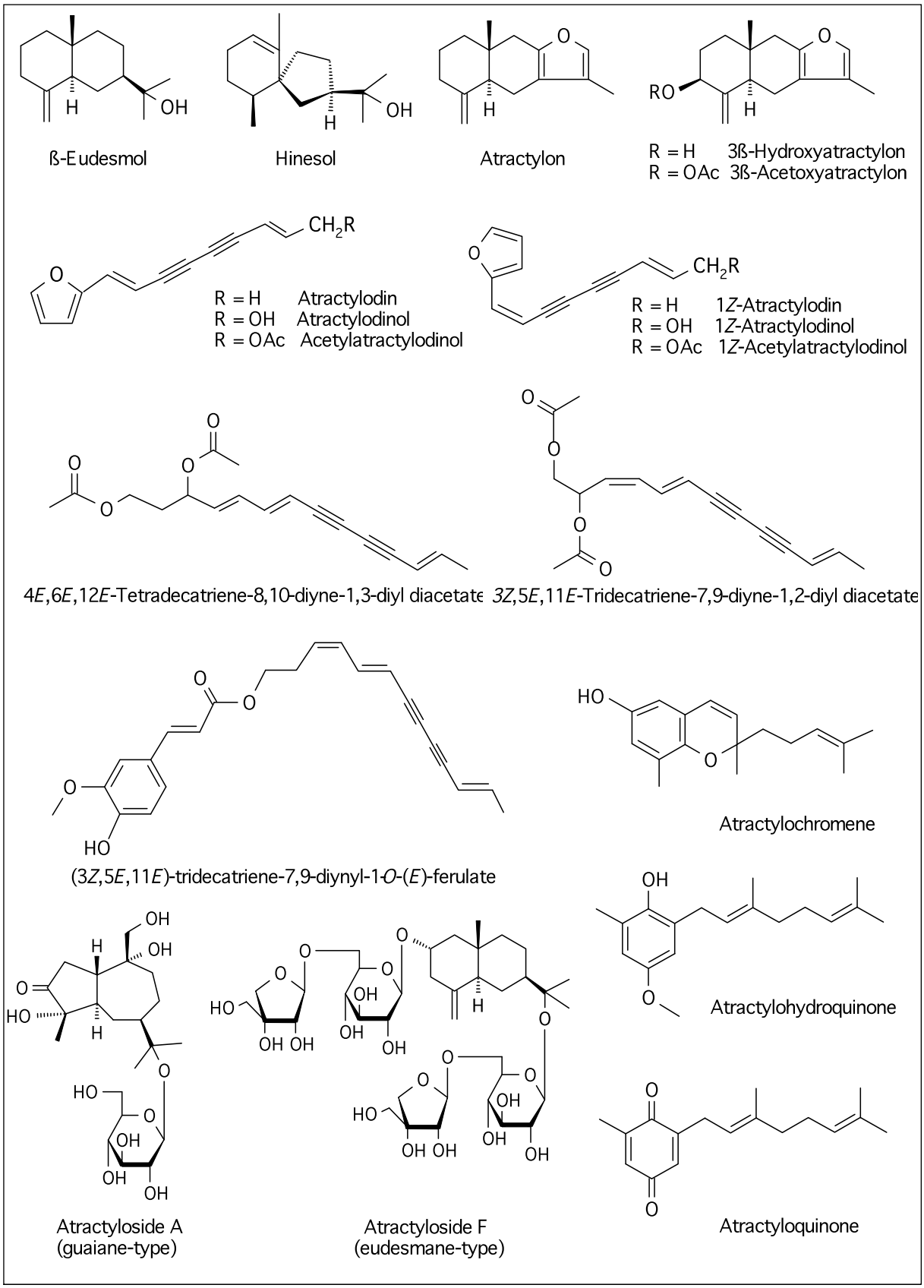


Fig. 1: Formulae of the main compounds

Pharmacology (see also [12,21]):

In vitro effects

- anti-inflammatory activity: inhibitory effect on 5-lipoxygenase (atractylochromene, atractylohydroquinone, atractyloquinone, (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diynyl-1-*O*-(*E*)-ferulate), cyclooxygenase-1 and -2[12,13,18,22]
- antihepatotoxicity and cytoprotection (atractylon, β-eudesmol, hinesol)[23]
- inhibitory actions on esophageal carcinoma cells (hinesol, eudesmol)[21]
- inhibiton of Na^+/K^+-ATPase activity(β-eudesmol)[24]
- alleviates muscular pain: block of the neuromuscular junction in mouse skeletal muscles (β-eudesmol)[25]
- modulation of the intestinal immune system (polysaccharides)[26]
- adenylate cyclase stimulation[27]
- phototoxic, antibiotic activity against *Escherichia coli, Saccharomyces cerevisiae, Candida albicans*[28]

In vivo effects:

- antiulcerative and antihistamine effects in rats (β-eudesmol, hinesol)[29,30]
- antiepileptic activities in mice (β-eudesmol)[31]
- intestinal motility enhancing activities in mice (β-eudesmol, hinesol)[32]
- antiemetic effects in chicken (β-eudesmol, hinesol)[33]
- cholagogue effect in rats (atractylodin)[34]
- antianoxic action in mice (β-eudesmol)[35]
- influence on the fertility of rats: luteolytic effect[36]
- central nervous system depressant action in mice (β-eudesmol, hinesol)[37]

TLC fingerprint analysis

1) Extraction: 5 g coarsely ground drug are soxhlet-extracted with 120 ml *n*-hexane p.a. for 2 hours. The extract is evaporated to dryness, redissolved in 5.0 ml ethanol p.a. and filtered over Millipore® 0.45 µm filters.

2) Reference compounds: Atractylodin, atractylodinol, acetylatractylodinol, (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate, atractylon, β-eudesmol, mixtures of triterpenes and sterols, dissolved in ethanol p.a. (1 mg/ml).

3) Separation parameters:

Applied amount: 15 µl extract, 10 µl standard solution

Plates: Silica gel 60 F_{254} (Merck)

Solvent system: *n*-hexane - ethyl acetate (93 + 7), no saturation in the TLC chamber, 15 cm

Direct evaluation: UV_{254} nm

Spray reagents: Anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde + 10 ml glacial acetic acid + 85 ml methanol + 5 ml conc. sulphuric acid are mixed in this order).

The plate is intensively sprayed with ca. 10 ml of the reagent and heated for 5-10 min. at 100 °C under observation. The evaluation is carried out in VIS.

4) Thin layer chromatograms and descriptions:

Drug samples:

1 *Atractylodes lancea* rhizomes, TCM hospital Bad Kötzting, Germany

2 *Atractylodes lancea* rhizomes, Institute of Chinese Materia Medica, Academy of Traditional Medicine, Peking, China

3 *Atractylodes lancea* rhizomes, Yu Qing Yu Tang pharmacy store, Hangzhou, China

4 *Atractylodes lancea* rhizomes, Hubei drug market, Sichuan, China

5 *Atractylodes lancea* rhizomes, pharmacy store, Shaanxi, China

6 *Atractylodes lancea* rhizomes, pharmacy store, Guangzhou, China

7 *Atractylodes lancea* rhizomes, Botanical Garden of the University of Düsseldorf, Germany

8 *Atractylodes lancea* rhizomes, pharmacy store, Nagoya, Japan

9 *Atractylodes macrocephala* rhizomes (*Baizhu*), TCM hospital Bad Kötzting, Germany

10 Atractylodes chinensis rhizomes, Dong Zhimen hospital, Beijing, China

11 Atractylodes koreana rhizomes, Yang-Gu, Kang Weon Province, South Korea

Reference compounds:

T1 atractylodin (R*f* 0.97)

T2 atractylon (R*f* > 0.98)

T3 acetylatractylodinol (R*f* 0.61)

T4 (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate (R*f* 0.41)

T5 mixture of triterpenes (α-, β-amyrine, taraxerol; R*f* 0.31)

T6 β-eudesmol (R*f* 0.26)

T7 atractylodinol (R*f* 0.20)

T8 mixture of sterols (β-sitosterol, stigmasterol, campesterol; R*f* 0.19)

5) Description of the TLC-chromatograms

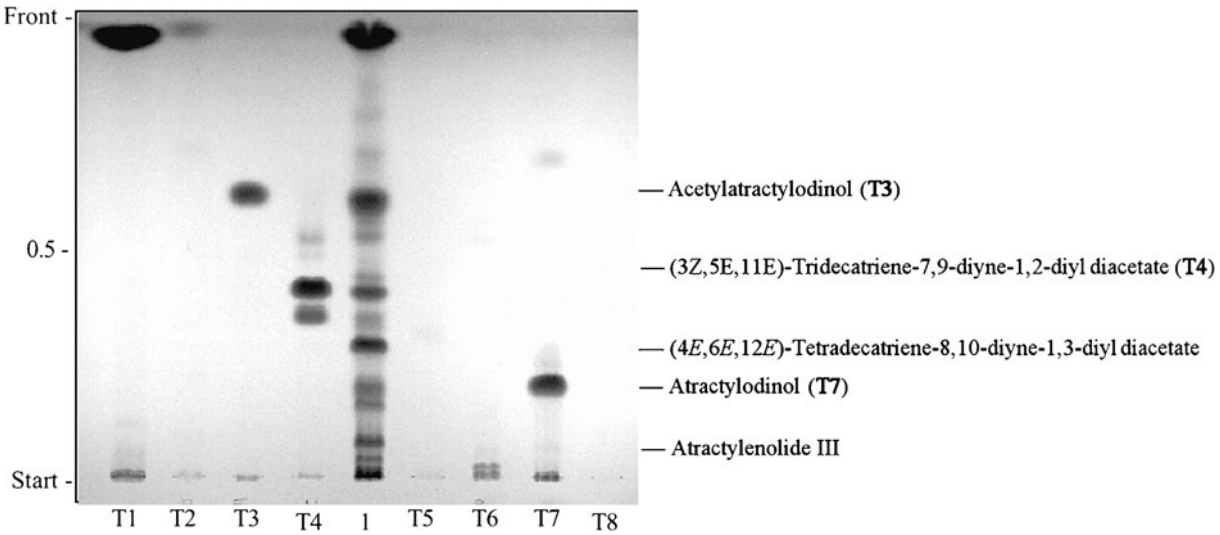


Fig. 2a: Reference compounds with Sample 1, evaluated in UV_{254} nm:

Direct evaluation in UV_{254} nm shows a major absorbing spot below the front at R*f* 0.97 (atractylodin, **T1**). Three more intensively quenching zones are detected at R*f* 0.61 (acetylatractylodinol, **T3**), R*f* 0.41 ((3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate, **T4**) and at R*f* 0.29 (4*E*,6*E*,12*E*)-tetradecatriene-8,10-diyne-1,3-diyl diacetate). Atractylodinol (R*f* 0.20, **T7**) and atractylenolide III (R*f* 0.14) as well as unidentified compounds at R*f* 0.52, 0.35 and 0.16 give zones with less intensity.

Under UV_{365} nm, no fluorescent zones appear in *Atractylodes lancea* rhizomes.

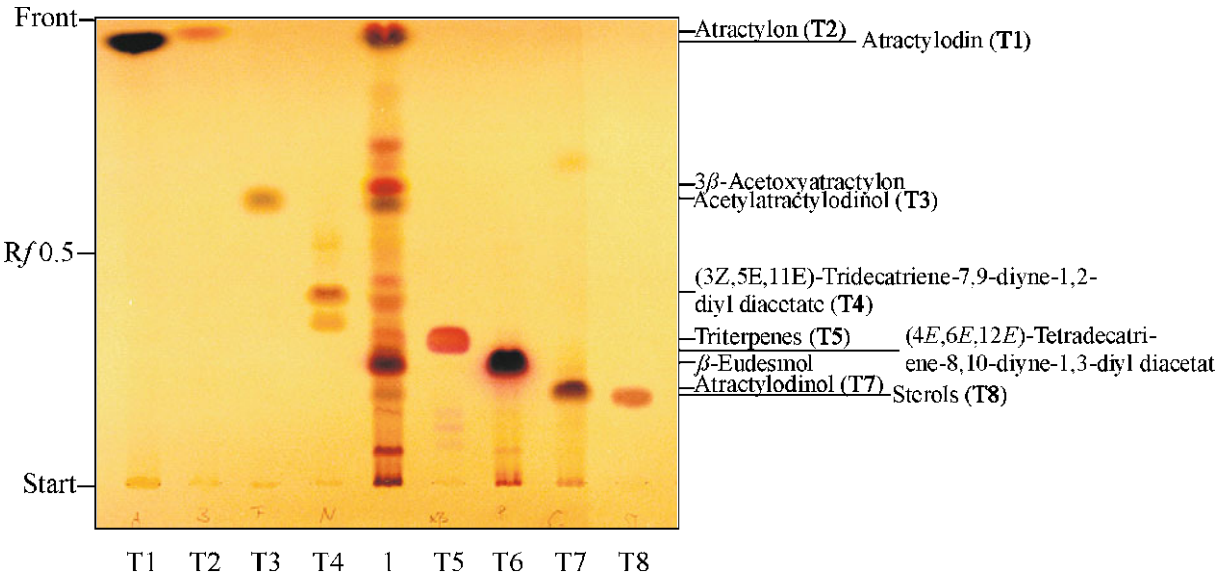


Fig. 2b: Reference compounds with Sample 1 after spraying with anisaldehyde sulphuric acid reagent in VIS:

Atractylon (**T2**, R*f* > 0.98) appears as a yellow-orange zone directly above the intensive black-green spot of atractylodin (R*f* 0.97, **T1**). Heated more intensively, its colour changes typically to orange-red, finally to brown-purple. As the atractylon zone under these conditions is mostly covered by violet constituents of the essential oil running with the front, this effect is even better visible at the zone of its derivative 3β-acetoxyatractylon (R*f* 0.65). Below the zone of 3β-acetoxyatractylon, acetylatractylodinol (**T3**) appears as a dark-green zone at R*f* 0.61. At lower R*f* values, the plate is dominated by the zones of (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate (**T4**, R*f* 0.41, grey or green-brown), the mixture of α-, β-amyrine and taraxerol (**T5**, R*f* 0.31, pink), β-eudesmol (**T6**, R*f* 0.26, violet), atractylodinol (**T7**, R*f* 0.20, grey) and the different sterols (**T8**, 0.19, greyish-pink). As the drug contains lots of the triterpenes and β-eudesmol, (4*E*,6*E*,12*E*)-tetradecatriene-8,10-diyne-1,3-diyl diacetate at R*f* 0.29 is almost invisible. It would be detected as a brown(-green) zone with anisaldehyde sulphuric acid reagent. Atractylenolide III is not visualized by this spray reagent. Nevertheless, at R*f* 0.14, a grey coloured zone becomes visible.

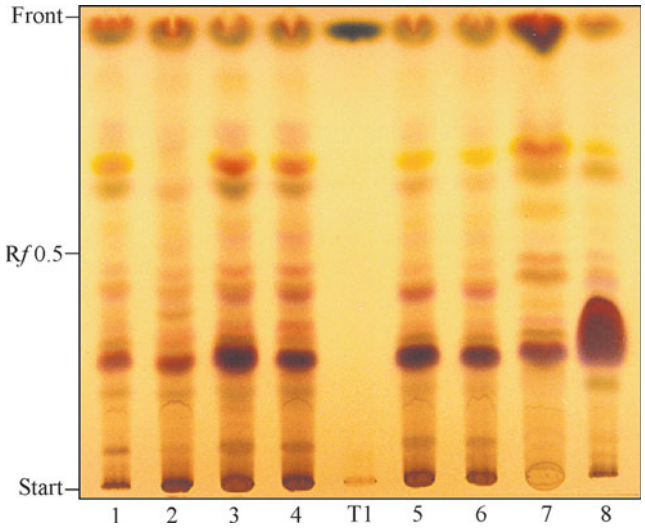


Fig. 3a: Samples 1-8 with reference compound **T1** after spraying with anisaldehyde-sulphuric acid reagent in VIS:

Different samples of *Atractylodes lancea* rhizomes gave a very similar zone pattern. All of them contain atractylodin as a major constituent (**T1**, R*f* 0.97). In most of the samples, the other known compounds (see Fig. 2a/b) can also be detected. Due to the extreme instability of 3β-acetoxyatractylon (at R*f* 0.33), its content decreases under storage (see extract **2** which was some months later analysed). Therefore, fresh prepared Atractylodes extracts should be used for quality investigations.

Sample **8** of Japanese origin, differs macroscopically from the rhizomes of the other proveniences by crystals called "atractylol" on the outer surface of the drug (an effect that traditionally was regarded as a sign of good drug quality, see Monograph Rhizoma Atractylodes macrocephalae No. 10. "Atractylol" was found to be a mixture of β-eudesmol and hinesol[9]. The extremely high content of these two sesquiterpenes in sample **8** forms a broad, dark red-violet zone at R*f* 0.26.

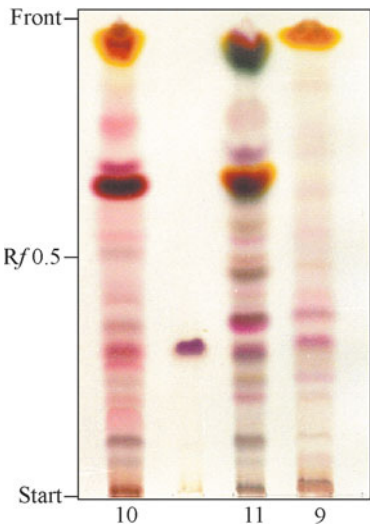


Fig. 3b: Anisaldehyde-sulphuric acid reagent, VIS:

The rhizomes of *Atractylodes chinensis* (**10**) show the same pattern of constituents as those from *Atractylodes lancea*. From the rhizomes of other *Atractylodes* species (e.g. *Atractylodes macrocephala,* **9**, and *Atractylodes koreana*, **11**), the two *Cangzhu* species can be easily distinguished by the presence of the major constituents atractylodin (R*f* 0.97) and acetyl-atractylodinol (R*f* 0.61). In general, under these TLC conditions especially the rhizomes of *Atractylodes macrocephala* (*Baizhu*) show much lesser compounds compared to *Cangzhu* drugs.

HPLC fingerprint analysis:

1) Sample preparation: Filtration of the extract used for TLC over Millipore® filtration unit, type HV 0.45 µm.

2) Injection volume: 2 µl ethanolic solution of the extracts (conc. = 5 g drug/5ml)

3) HPLC parameters:

Apparatus:	Liquid Chromatograph HP 1050 with photodiode array detector HP 1040 M (Hewlett Packard)
Column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5µm), Merck
Pre-column:	LiChroCART® 4-4 with LiChrospher® 100 RP 18 (5µm), Merck
Solvent system:	A: water; B: acetonitrile
Gradient:	62 - 70 % B in 10 min. (linear), 70 - 95 % B in 2 min. (linear), 95 % B for 8 min. (isocratic)
Flow:	1.0 ml/min.
Detection:	215 nm, 365 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compounds
1	2.6	atractylodinol
2	4.3	(1*Z*)-acetylatractylodinol
3	4.9	(3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate
4	5.2	acetylatractylodinol
5	5.7	(4*E*,6*E*,12*E*)-tetradecatriene-8,10-diyne-1,3-diyl diacetate
6	6.7	(1Z)-atractylodin
7	7.3	3β-acetoxyatractylon
8	7.6	"atractylol"
9	8.2	atractylodin
10	8.9	atractylohydroquinone
11	13.6	atractylon
12	15.4	mixture of sesquiterpenes?

Description of HPLC fingerprints

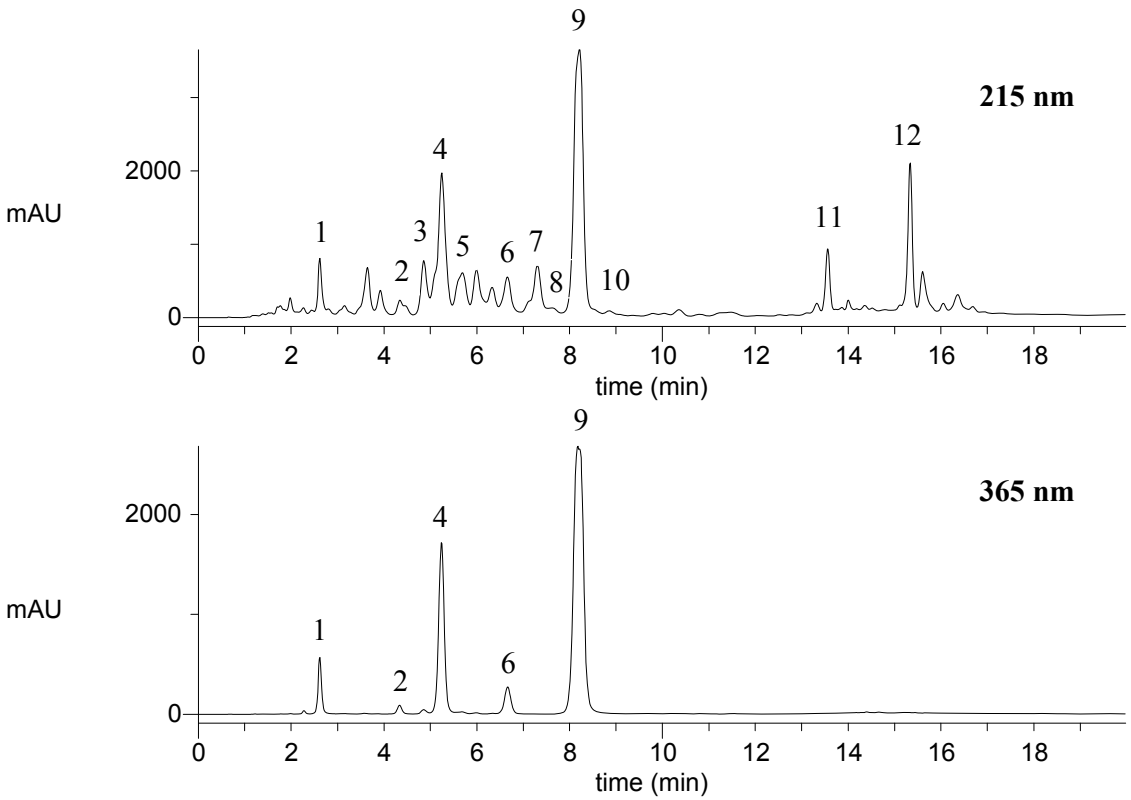


Fig. 4: HPLC fingerprint chromatograms of the *n*-hexane extract of *Atractylodes lancea* rhizomes (drug sample **1**, TCM-hospital Bad Kötzting, Germany)

Eight out of nine examined *Atractylodes lancea* rhizomes from different proveniences gave fingerprints similar to drug sample **1**: The acetylene atractylodin (peak **9**) is the major compound in this chromatogram. Its derivatives atractylodinol, 1*Z*-acetyl-atractylodinol, acetylatractylodinol and 1*Z*-atractylodin (peaks **1**,**2**,**4**,**6**) were also detectable in relatively large amounts. Several other acetylenes with the typical UV spectrum of an ene-diyne-diene-chromophore appear at Rt-s similar to acetyl-atractylodinol, for example (3*Z*,5*E*,11*E*)-tridecatriene-7,9-diyne-1,2-diyl diacetate (peak **4**) and (4*E*,6*E*,12*E*)-tetradecatriene-8,10-diyne-1,3-diyl diacetate (peak **6**). The sesquiterpene atractylon – characteristic for all *Atractylodes* species - and 3β-acetoxyatractylon can be found in various amounts, depending on the storage conditions of the drugs and extracts, as these compounds are not stable. Another often intensive peak (**12**) - possibly a degradation product of several sesquiterpenes - is present at Rt = 15.4 min. Interestingly, atractylohydroquinone, which is a strong 5-LOX-/COX-1-inhibitor *in vitro* [12,18]) is also visible in some *Cangzhu*-chromatograms at Rt = 8.9 min. It might be useful for the quality proof of *Atractylodes* drugs.

Due to their extended chromophore, atractylodin and its four derivatives show intensive peaks at detection wavelengths 365 nm, which indicate the presence of the *Cangzhu* drug.

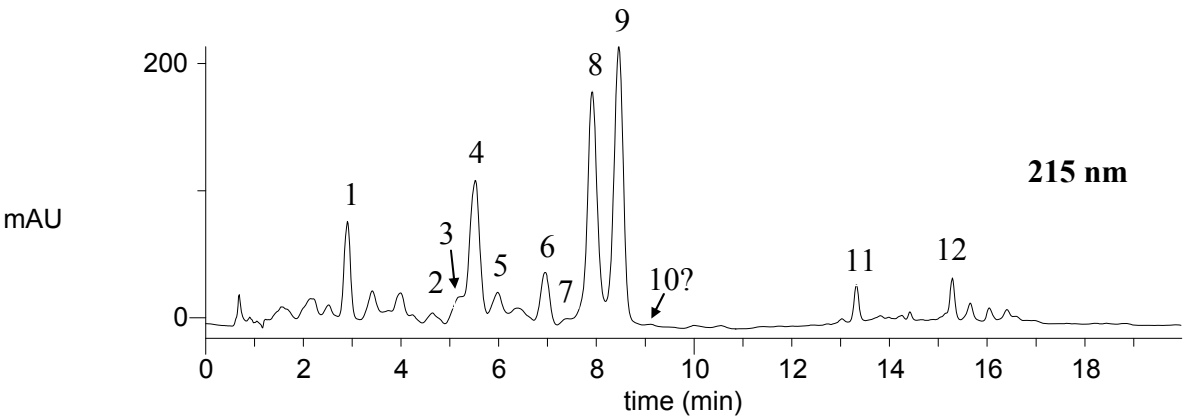


Fig. 5: HPLC fingerprint chromatogram of the *n*-hexane extract of *Atractylodes lancea* rhizomes (drug sample **8** from Nagoya, Japan)

The HPLC chromatogram reveals that also drug sample **8** of Japanese origin, contains the main constituents of *Atractylodes lancea* rhizomes (see Fig. 4). The great difference to other drugs of this species, however, is of quantitative nature: "Atractylol" that in general exhibits just a minor peak in the chromatogram – in accordance with its UV spectrum (Fig. 7) - shows a peak as intensive as the same of atractylodin. This observation can be explained with the very high concentration of "atractylol" in this drug sample (for comparison, see the TLC fingerprint in Fig. 3a **T6**).

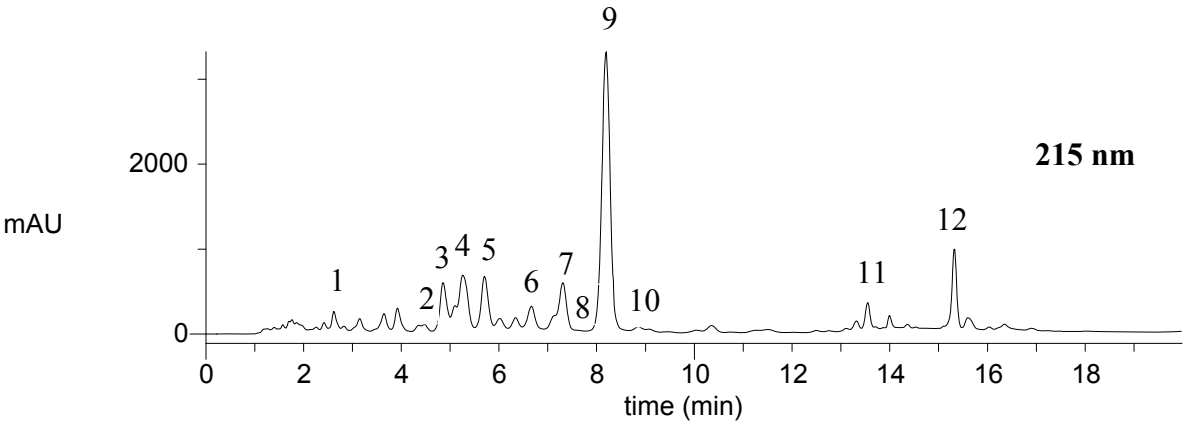
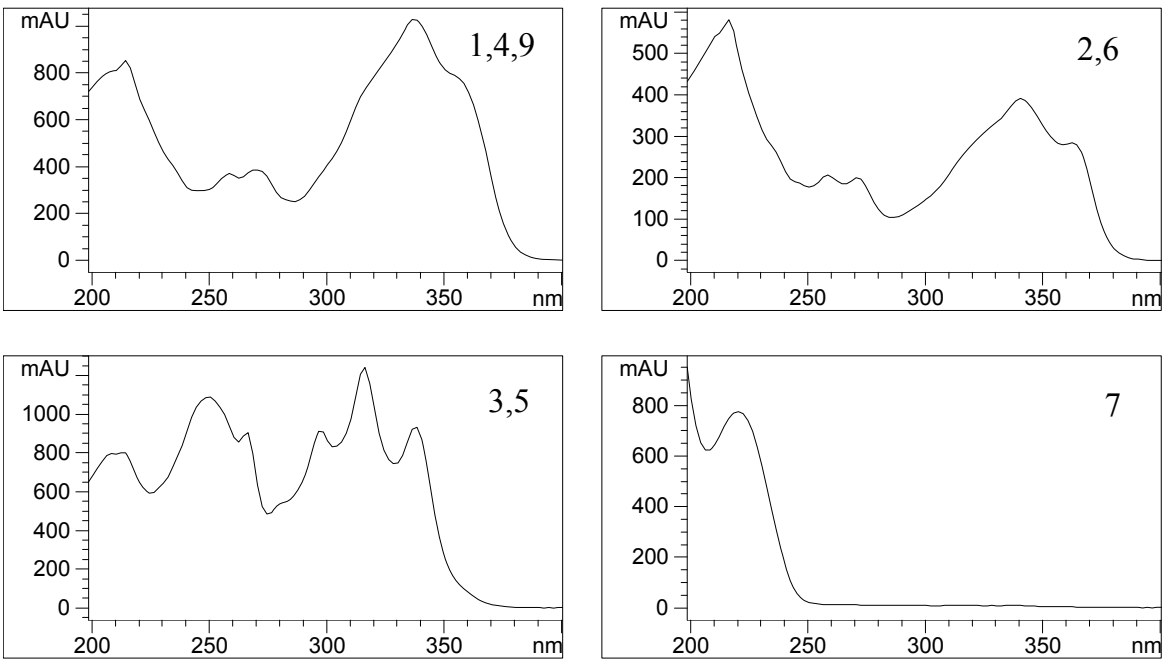


Fig. 6: HPLC fingerprint chromatogram of the *n*-hexane extract of *Atractylodes chinensis* rhizomes (Drug sample **12** from Kunming, China)

In the HPLC chromatogram of the *Atractylodes chinensis* rhizomes from Kunming, all characteristic peaks of *Atractylodes lancea* rhizomes (Fig. 4) are present in similar amounts. Therefore a discrimination between this drug and the majority of the *Atractylodes lancea* samples is not possible.

For the differentiation of the HPLC fingerprint of *Atractylodes lancea* rhizomes from *Atractylodes macrocephala* rhizomes (Baizhu), see the special monograph No 10.



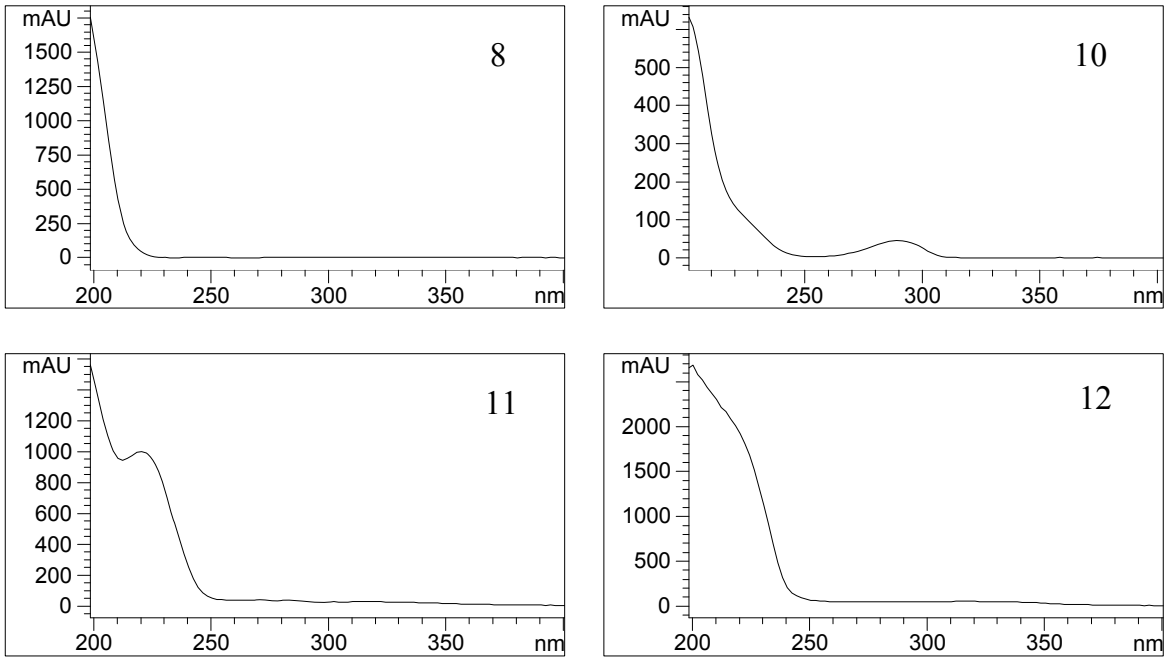


Fig. 7: Online recorded UV spectra of the main peaks in the HPLC fingerprint of *Cangzhu* extracts

Discussion

When analyzed by TLC and HPLC, the drug samples of *Atractylodes lancea* showed - with one exception - a very constant pattern of constituents with the acetylene atractylodin and its derivatives as characteristic compounds. Rhizomes of Japanese origin, represented the exceptional group: This drug differed at first sight from the other *Atractylodes lancea* rhizomes by the presence of white, cotton-like crystals ("atractylol") on its surface, what is traditionally considered as a sign of good quality[38]. In fact, "atractylol" consists of β-eudesmol and hinesol, two sesquiterpenes that possess numerous pharmacological activities (see "pharmacology"). Therefore the contents of β-eudesmol and hinesol can indeed be an indication for the quality of *Atractylodes lancea* drugs. In this context, the occurrence of a small atractylohydroquinone peak in the HPLC chromatogram could also be of interest, as this substance showed strong *in vitro* antiinflammatory activity in two bioassays[12,18].

The analyzed sample of *Atractylodes chinensis* rhizome (**10**) showed an identical HPLC fingerprint as the majority of the *Atractylodes lancea* drugs. As both species are accepted to supply *Cangzhu* drugs, it is not necessary to distinguish between them. In the whole *Atractylodes* literature there is a consent that *Atractylodes lancea* and *Atractylodes chinensis* really have equal chemical composition[12,39,40].

On the other hand, the differentiation of the two *Cangzu* drugs from rhizomes of other *Atractylodes* species is quite simple: By applying the methods described in this monograph, the major acetylenic constituent atractylodin and its derivatives are missing in the TLC and HPLC fingerprints of the rhizomes from *Atractylodes macrocephala*, *Atractylodes koreana*, and *Atractylodes japonica* (see Figure 3b and literature[12,37]).

Acknowledgements - The authors are very grateful to Prof. Dr. Zhong-liang Chen, Institute for Materia Medica, Shanghai, for providing the reference compounds 3β-acetoxyatractylon and atractylenolide III, and to Dr. T.A. van Beek, Vakgroep Organic Chemistry, University of Wageningen, The Netherlands, for a sample of β-eudesmol.

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Herba Leonuri – *Yimucao*

Pharmacopoeia:[1] Chinese Pharmacopoeia of the People's Republic of China, English Edition, Vol. 1, 2005

Official drug:[1] *Leonurus japonicus* Houtt.

Synonyms: *Leonurus heterophyllus* Sweet ChinPix.(= *L. artemisia* (Lour.) S.Y.HU)

Leonurus heterophyllus Sweet f. *leucanthus* C.Y.Wu et H.W.Li

Leonurus sibiricus L.

– Lamiaceae –

The drug is known as *Chinese motherwort* (English), *yakumoso* (Japanese), *ikmocho* (Korean) and *Chinesisches Mutterkraut* (German)

The herb *Leonurus cardiaca* is used in Europe.

Description of the drug:[1] Fresh herb of *Leonurus japonicus:*

Perennial Herb, with rounded-cordate basal leaves. Stem square, frequently branched at the upper part, furrowed longitudinally on 4 sides, 30-60 cm long, 0.2-0.5 cm in diameter, externally dark green, containing pith. Leaves opposite, petioled, lamina dark green, juicy and soft. The lower stem leaves palmately ternate, the upper leaves pinnatilobately ternate, lobes entire or slightly serrate. Odor, slight; taste, slightly bitter.

Dried herb of *Leonurus japonicus:*

Stems externally greyish-green or yellowish-green; texture light and pliable, fracture medullated in the centre. Lamina pieces greyish-green, mostly wrinkly, often deteched from stem. Verticillaster axillary, florets pale purple, calyx tubular, corolla bilabiate. Cutting sections about 2 cm long.

Pretreatment of raw drug:[1,6,11] The fresh herb is collected in spring to early summer at the time of its best growth before flowering season and dried in sun or shade after cutting into sections.

Medicinal use:[1,6] To regulate menstruation by activating blood circulation and to induce diuresis.

Used against dysmenorrhea, amenorrhea, incessant drippling of lochia and for uterus contraction post partum; edema and oliguria such as edema in acute nephritis.

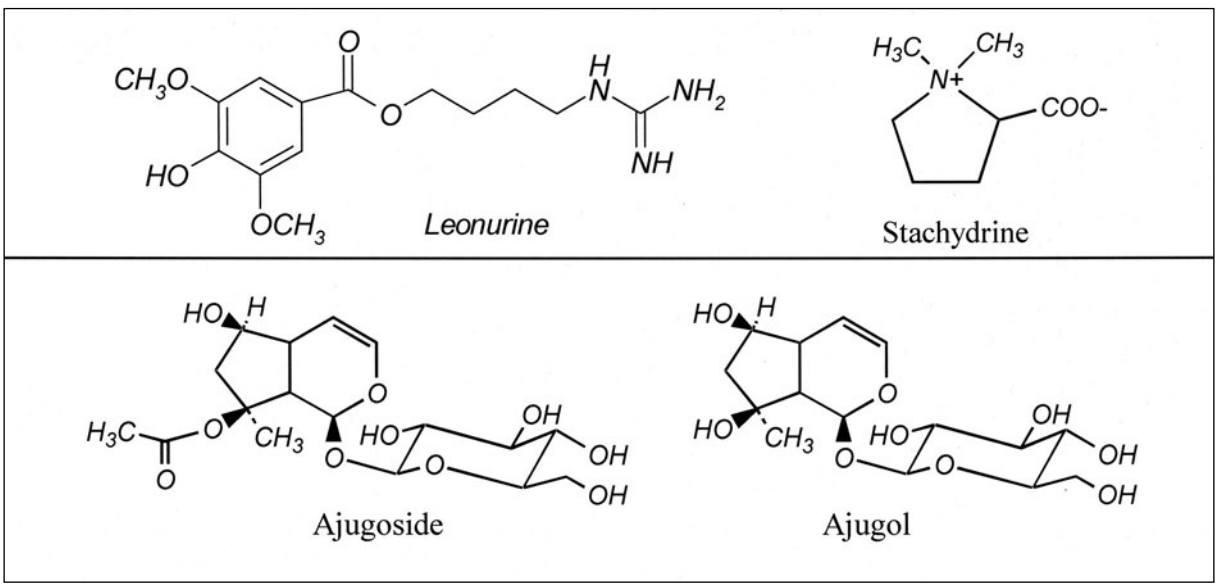
H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

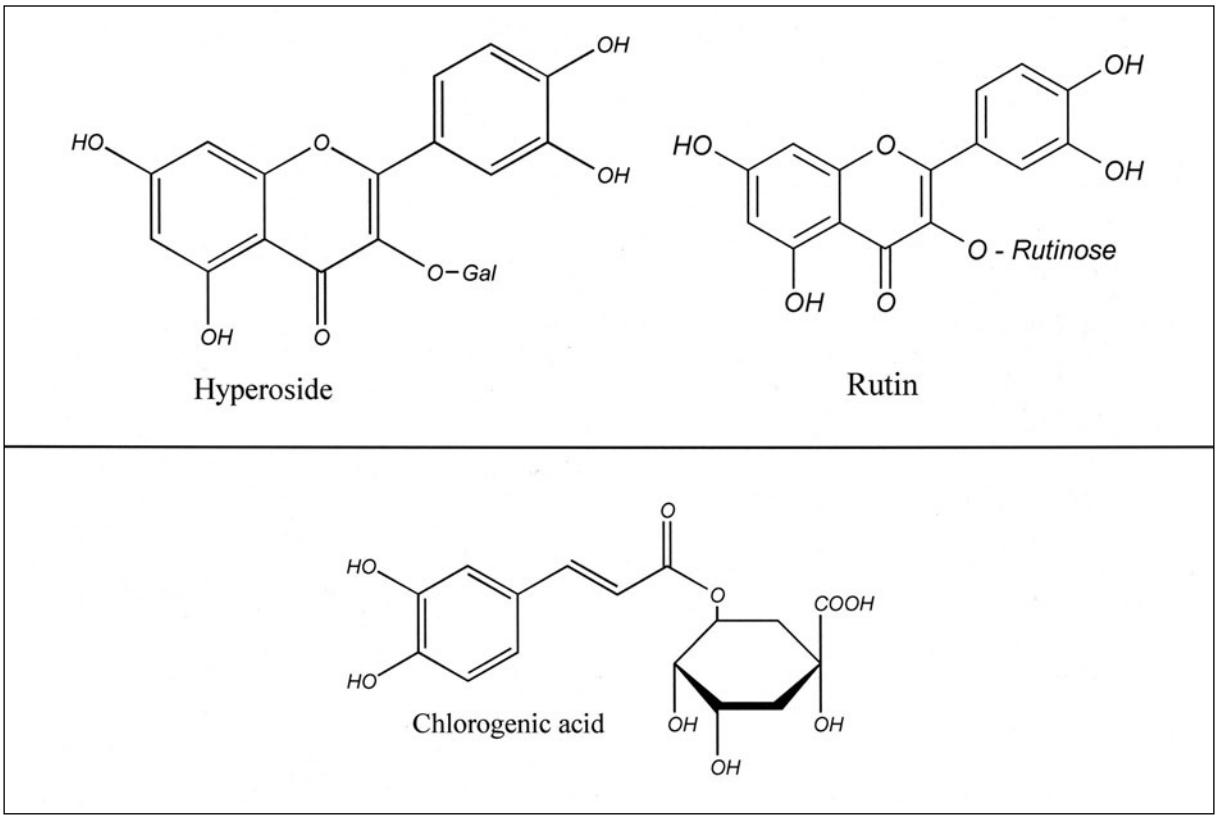
Effects and indications according to Traditional Chinese Medicine[1,2]	
Taste:	acrid, bitter
Temperature:	slightly cold
Channels entered:	heart, liver, bladder
Symptoms and indications:	Used for menstrual disorders, dysmenorrhea, amenorrhea, incessant drippling of lochia; edema and oliguria such as edema in acute nephritis

Main Constituents (see Fig. 1)[2,7,8,9]:

- **Alkaloids:** leonurine, stachydrine, leonuridine, leonurinine
- **Iridoid glycosides:** ajugol, ajugoside, galiridoside
- **Flavonoids:** rutin, kaempferol, quercetin, apigenin, genkwanin
- **Diterpenes:** prehispanolone, hispanolone, galeopsin, preleoheterin, leoheterin, leocardin
- **Essential oil:** 1-octen-3-ol, 3-octanol, β-ocimene, linalool, nonanol, copaene, caryophyllene, oxide, humulene, γ-elemene, cadinene, hexahydrofarnesylacetone, methyl palmitate, dibutylphthalate, nonadecane
- **Organic acids:** palmitic acid, fumaric acid, lauric acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, stearic acid
- Other constituents: phytol, leonuramide, bufenolide and tannins

Fig. 1: Formulae of the main constituents





Pharmacology:[3,4,10]

In vitro effects:

- Inhibition of platelet aggregation[4]
- Cytotoxic effect of ursolic acid on lymphocytic leukemia, human lung carcinoma, KB, human colon and mammary tumour cells.[4]
- Stimulant effect of a decoction, ethanolic extract of Hb. Leonuri and of leonurine on the uteri of animals including rabbit, cat, dog, and guinea pig.[3]
- Dosage-dependent effect of leonurine on contractility and contraction frequency.[3]
- Enhancement of contraction of the isolated frog heart by leonurine.[3]

In vivo effects:

- Slow onset of uterine contracting effect of Hb. Leonuri-fluidextract or decoction.[3,10]
- Effect of Hb. Leonuri (decoction) on edema caused by acute or chronic nephritis: rapid removal of edema, increased urinary and fecal output.[3]
- Blood-flow stimulatory effect and elimination of stasis.[3]
- Hypertensive effect of decoction, tincture, or aqueous extract of Hb. Leonuri.[3]

Toxicology:[2,3]

Due to its alkaloid content, overdosage of this herb has slightly toxic effects, appearing 4-6 hours after ingestion. Symptoms include sudden general weakness, stiffness and paralysis, general body pains, an oppressive sensation in the chest, excessive sweating, low blood pressure, cold extremities, and, in severe cases, shock, cyanosis, and respiratory paralysis. Overdosage can also cause miscarriage.[2]

Multiple and long-term oral doses, however, produced no adverse reaction. The intramuscular dose did not cause toxic side effects other then xerostomia and shortened sleep. No side effects were known from the clinical use of the sterilized injection of the total alkaloids 15mg/ml.[3]

TLC fingerprint analysis: flavonoids and phenolcarboxylic acids

1) Extraction: 0.5 g of powdered drug in 5 ml methanol is heated on a water-bath under reflux for 10 minutes, the extract is filtered and filled up with methanol in a 5 ml volumetric flask.

2) Reference compounds: chlorogenic acid, hyperoside, isoquercitrin and rutoside are dissolved in methanol (1 mg/ml)

3) Separation parameters:

Applied amount: 20 µl extract, 10 µl standard solution

Plates: Silica gel 60 F_{254} (Merck) 105554.

Solvent system: Ethyl acetate – formic acid – glacial acetic acid – water (100 : 11 : 11 : 27) development distance 8 cm

4) Detection: Natural product – polyethyleneglycol reagent:
The plate is sprayed successively with 1 % methanolic solution of diphenylboric acid-β-ethyl-aminoester (NP) and a 5 % ethanolic polyethyleneglycol – 4000 solution (PEG). The evaluation is carried out in UV 365 nm and VIS.

Drug samples	Origin	Species
1	Weihenstephan, Germany	*Leonurus japonicus* 2000
2	Weihenstephan, Germany	*Leonurus japonicus* 2001
3	Weihenstephan, Germany	*Leonurus japonicus* 2002
4	Weihenstephan, Germany	*Leonurus heterophyllus* 2001
5	Complemedis, Switzerland	Leonuri herba (spec. unknown)
6	Kottas-Heldenberg+Sohn, Vienna, Austria	*Leonurus cardiaca* 2000
T1	Reference compound	Hyperoside, R*f* = 0.56
T2	Reference compound	Rutin, R*f* = 0.39
T3	Reference compound	Chlorogenic acid, R*f* = 0.50
T4	Reference compound	Caffeic acid, R*f* = 0.96

5) Description of the chromatograms (Fig. 2 and 3):

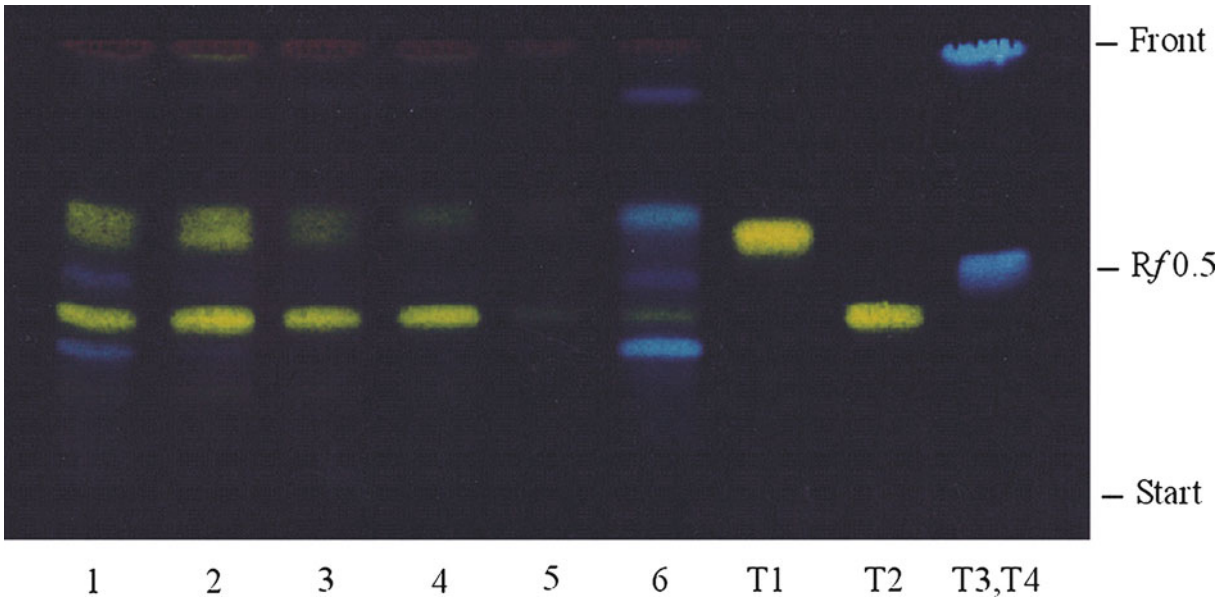


Fig. 2: TLC fingerprint analysis: flavonoids and phenolcarboxylic acids detected with Natural product – polyethyleneglycol reagent in UV 365

Leonurus japonicus / *Leonurus heterophyllus* samples 1,2,3 and 4 are characterized by the flavonoid pattern of two yellow spots: R*f* 0.39 rutin (**T2**) and R*f* 0.56 hyperoside (**T1**), in sample 3 and 4 with diminished concentration of hyperoside. In sample 5 (Leonuri herba) of non defined species, flavonoids are present only in traces. Most *Leonurus* spec. contain additionally in small amount or traces blue fluorescent spots of chlorogenic acid (R*f* = 0.50) and caffeic acid (R*f* = 0.96). The latter are absent in *L. heterophyllus*. In contrast *Leonurus cardiaca* shows both acids in high concentration.

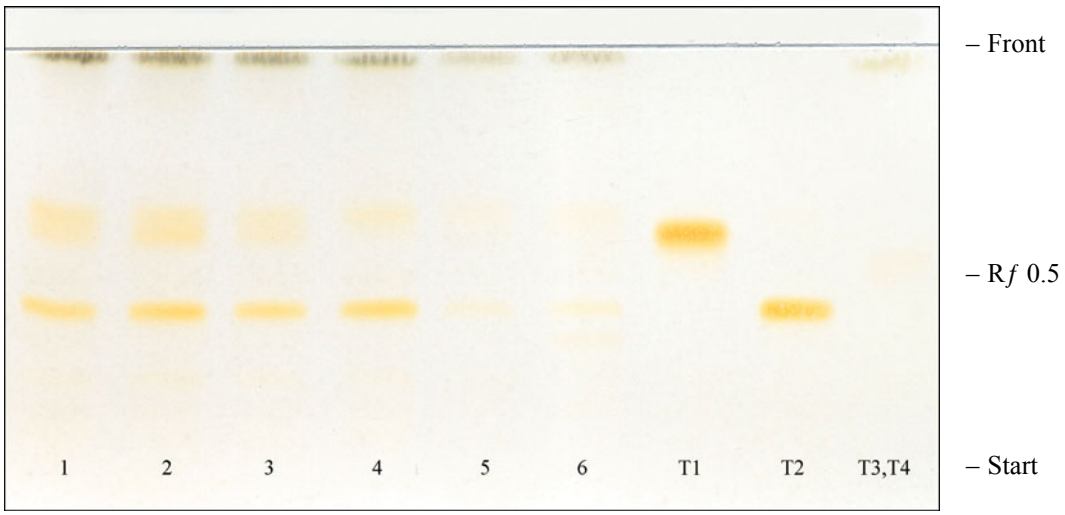


Fig. 3: TLC-fingerprint analysis: flavonoids and phenolcarboxylic acids detected with Natural product – polyethyleneglycol reagent in VIS

In VIS *Leonurus japonicus* and *Leonurus cardiaca* show a very similar pattern of only yellow flavonoid spots but in different concentrations.

Note: In the monograph of the Chinese Pharmacopoeia 2005 for Herba Leonuri the alkaloid stachydrine is quantified by TLC using iodobismuthante TS and 1 % ferric chloride TS as spray reagent followed by a TLC scanning quantitation at 510 nm. The dried drug should contain not less than 0.5 % of stachydrine hydrochloride.

TLC fingerprint analysis: iridoids

1) Extraction: 0.5 g of powdered drug in 5 ml methanol is heated on water-bath under reflux for 10 minutes, the extract is filtered and filled up with methanol in a 5 ml volumetric flask.

2) Separation parameters:

Applied amount: 20 µl extract, 10 µl standard solution

Plates: Silica gel 60 F_{254} (Merck) 105554.

Solvent system: Toluol – ethyl acetate – glacial acetic acid (70:25:5), development distance 8 cm

3) Detection: Plate is sprayed with 1 % ethanolic vanillin-solution and with 10 % ethanolic sulphuric acid. After heating at 110 °C, the evaluation is carried out in VIS

4) Description of the chromatogram (Fig. 4):

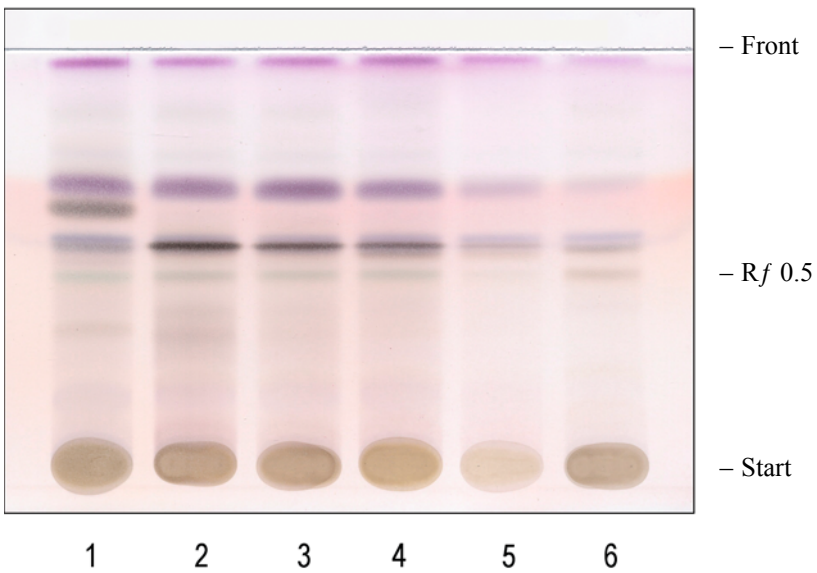


Fig. 4: TLC-fingerprint analysis: iridoids detected with vanillin-sulphuric acid reagend in VIS

Leonurus japonicus (1-5) shows three major violet spots which can be assigned to iridoid compounds (e.g. Ajugoside or Ajugol). These spots can be seen at R*f* 0.53, R*f* 0.68, and R*f* 0.98. The difference between *L. japonicus* and *L. cardiaca* is obvious: Extracts of *L. japonicus* show a green spot at R*f* 0.50 whereas *L. cardiaca* shows a violet-brown one. Differences, however, between these two species are better visible in the flavonoid-TLC.

HPLC fingerprint analysis

1) Sample preparation: 0.5 g of powdered drug in 5 ml methanol is heated on water-bath under reflux for 10 minutes and the extract is filtered into a 5 ml volumetric flask. Methanol is filled up to volume and the solution is filtered over 3 ml solid phase extraction columns [200mg per column packed with reversed phase octadecylsilane (C18) bonded to silicagel] under vacuum.

2) Injection volume: 10 µl of the methanol extracts

3) HPLC parameters:

Apparatus: G1379A Degasser, G1311A QuatPump, G1313A ALS, G1316A Colcom, G1315B DAD (Agilent Series)

Column: LiChroCART®125 × 4 with LiChrospher 100 RP-18 (5 µm), Merck

Pre-column: LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck

Solvent system: A: water + 10 ml 0.1 % H_3PO_4/liter; B: acetonitrile

Gradient:

Time (min.)	% A
0	90
4	70
6	5
10	5
11	90
12	90

Flow rate: 1.0 ml/min.

Detection: 205 nm

Peak	Rt (min.)	Compounds
1	0.89	Ajugoside
4	4.91	Rutin
5	5.21	Hyperoside

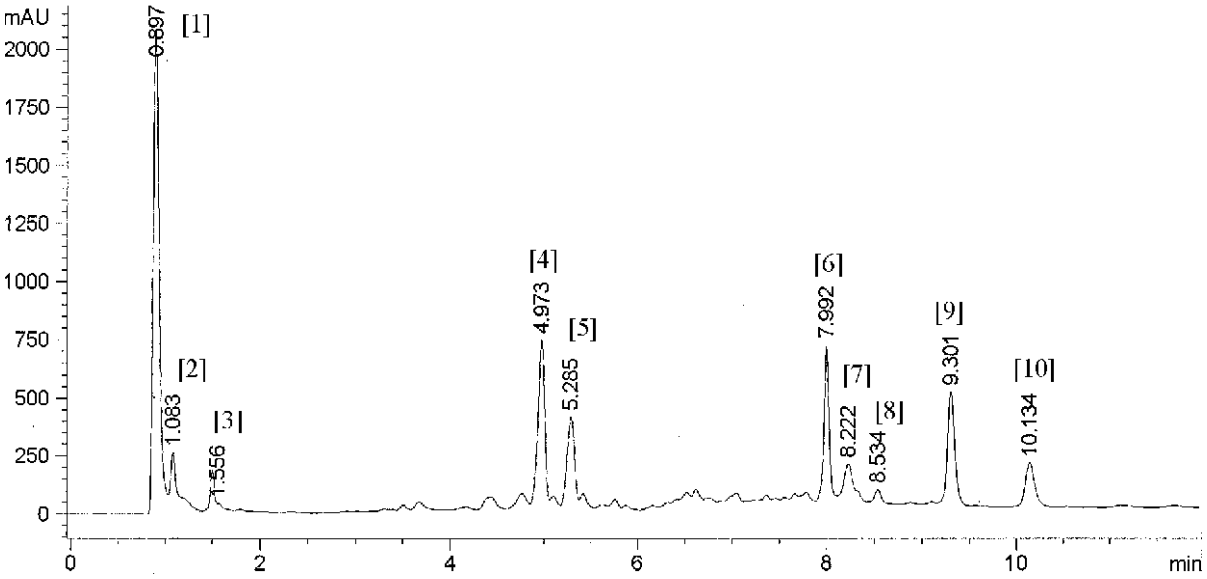


Fig. 5: HPLC of the methanol extract of *Leonurus japonicus*

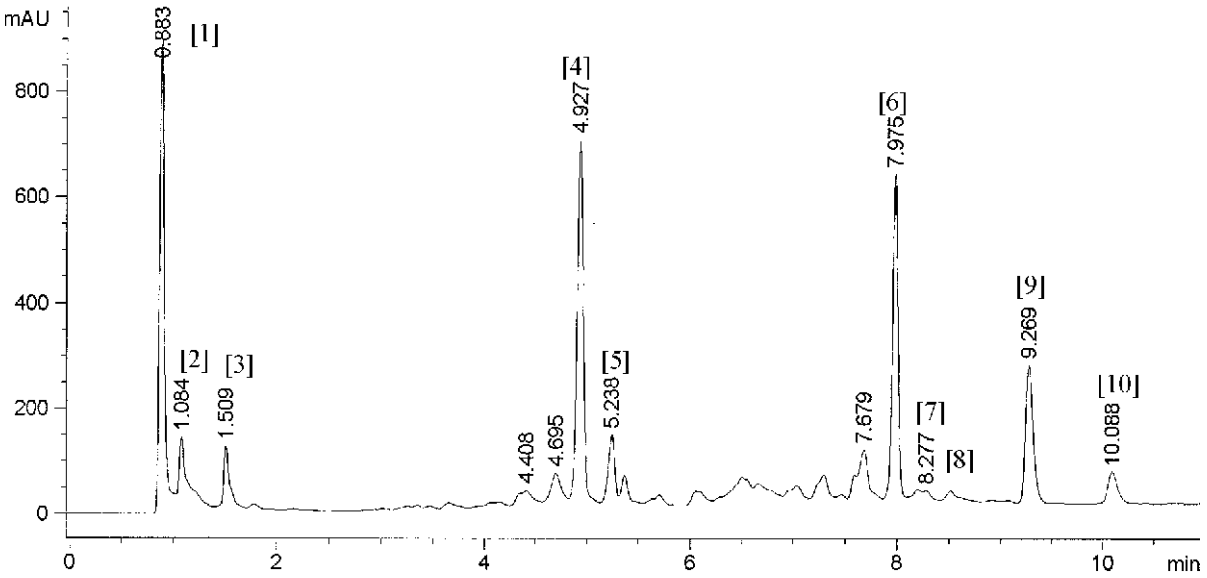


Fig.6: HPLC of the methanol extract of *Leonurus heterophyllus*

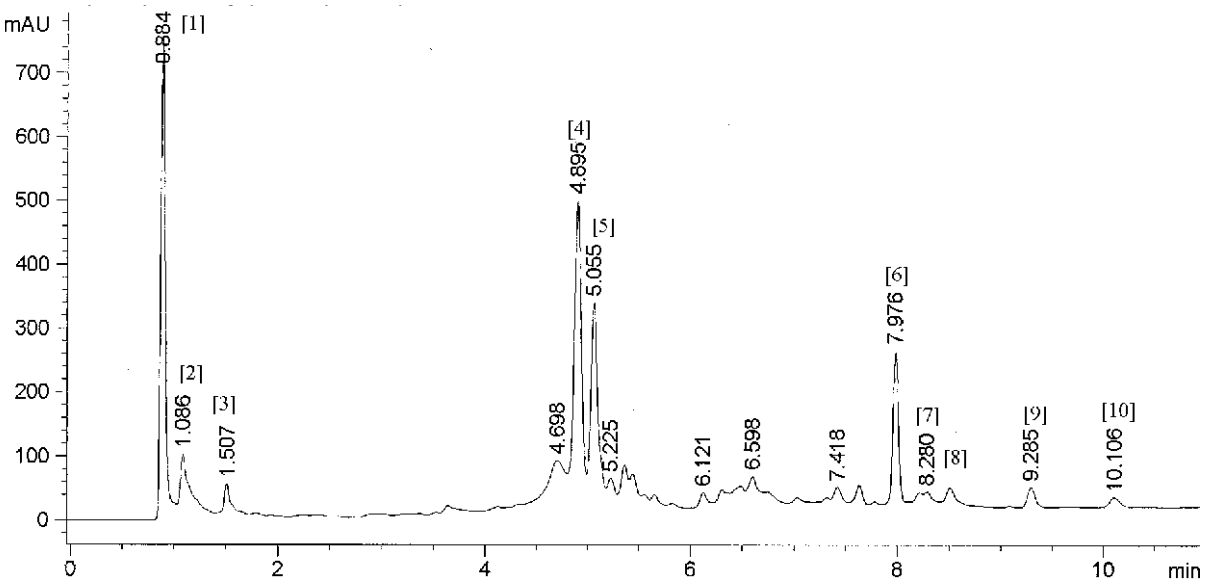


Fig. 7: HPLC of the methanol extract of *Leonurus cardiaca*

Description of the HPLC-chromatograms (Fig. 5–7):

Methanol extracts of *Leonurus japonicus* and *L. heterophyllus* show a nearly identical HPLC-fingerprint with major peaks at Rt = 0.89 (Ajugoside [**1**]), Rt = 4.91 (Rutin [**4**]), Rt = 5.21 (Hyperosid [**5**]) and the peaks **6**-**7** in the Rt-range of 7.9-10.2. The iridoidglycosides (e.g. peak [**1**]) show in the on line UV-spectrum endabsorption at 220 nm, whereas the flavonoids Peak **3** and **4** can be identified by their typical flavonol UV-maxima at 255 nm and 350 nm.

Leonurus cardiaca differs from *L. japonicus* and *L. heterophyllus* only in a relatively low concentration of the peaks **7**-**10**.

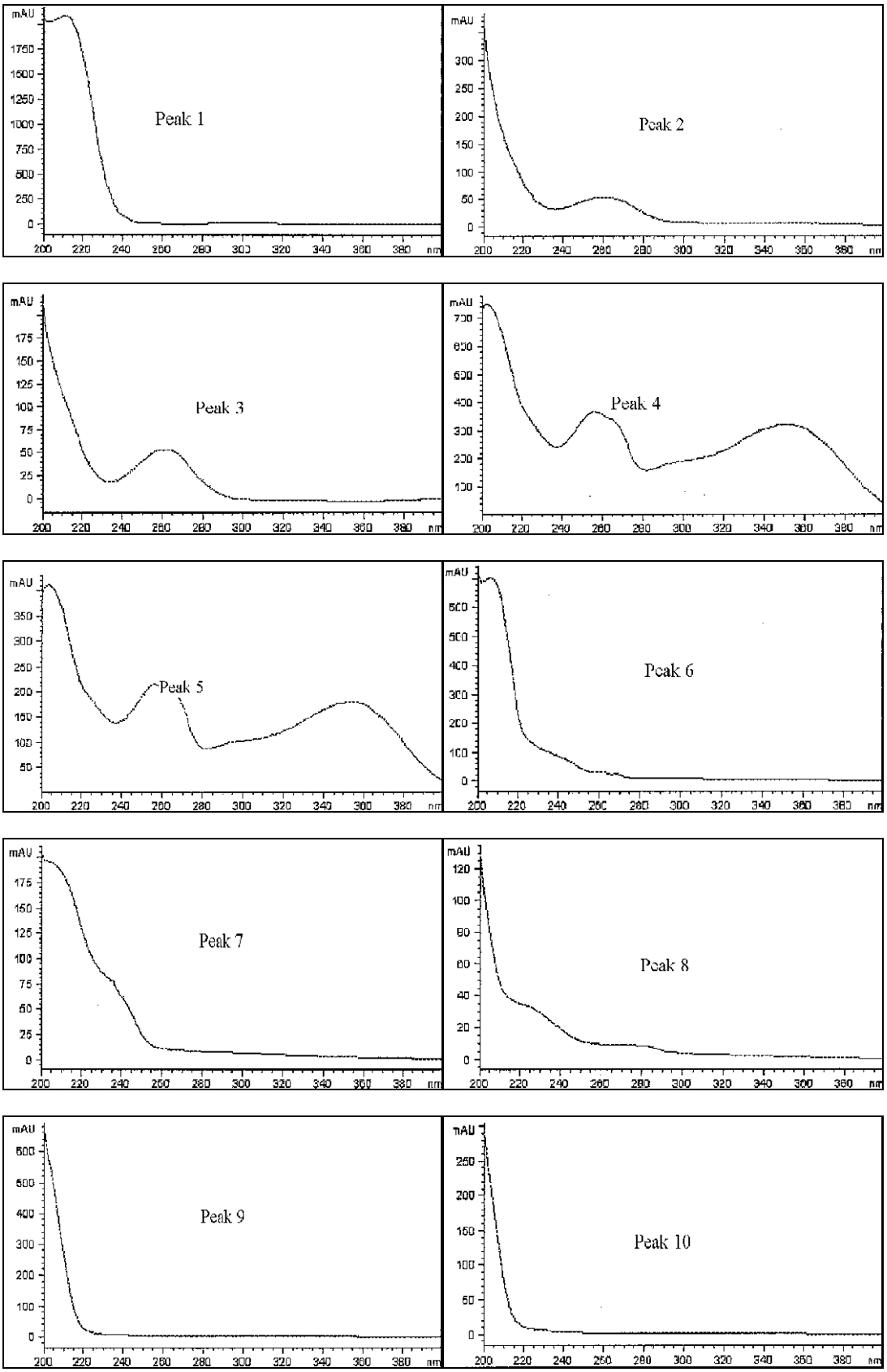


Fig. 8: UV-spectra of the main peaks

Discussion:

Leonurus japonicus can be best identified by TLC-fingerprint analysis of the methanol extract using the Natural product polyethyleneglycol reagent and detection under UV 365 and by HPLC-registered at 205 nm.

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Flos Magnoliae – *Xinyi*

Pharmacopoeia:[1] Chinese Pharmacopoeia of the People's Republic of China, English Edition, Vol. I, 2005

Official drugs:[1] *Magnolia biondii* Pamp.
Magnolia denudata Desr.
Magnolia sprengeri Pamp.
– Magnoliaceae –
The drug is known as Shini (Japanese), Sinihwa (Korean) and Magnolia flower (English).[3]

The following substitute drugs are not official:[4,5,6]
-*Magnolia liliiflora* Desr.
-*Magnolia fargesii* Cheng
-*Magnolia campbellii* Hook. f. et Thoms.

Origin: China (especially in provinces of He Nan, An Hui and Sichuan)

Description of the drug:[1] Flower bud of *M. biondii:*

Long ovoid, like the tip of a writing brush, 1.2-2.5 cm long, 0.8-1.5 cm in diameter. Mostly the base with a pedicel, about 5 mm long, exhibiting whitish dotted lenticels. Bracts 2-3 layers, each layer 2 segments, bearing small scaly buds between 2 layers of bracts, outer surface of bract densely covered with greyish-white or greyish-green tomenta, inner surface brownish, glabrous. Perianth-segments 9, brownish, outer ones 3, stripe-shaped, about ¼ in length of the inner ones, sepaloid, inner ones 6, arranged in 2 whorls of 3. Stamens and pistils numerous, spirally arranged. Texture light and fragile. Odour: aromatic; taste: pungent, cool and slightly bitter.

Flower bud of *M. denudata*:

1.5-3 cm long, 1-1.5 cm in diameter. Base with a stouter pedicel, lenticels brownish. Outer surface of bract densely covered with greyish-white or greyish-green hairs. Perianth-segments 9, outer whorls and inner whorls homogeneous.

Flower bud of *M. sprengeri*:

2-4 cm long, 1-2 cm in diameter. Pedicels stout, lenticels red-brown. Outer surface of bract densely covered with yellowish or yellowish-green tomenta, sometimes the outermost bracts appearing blackish-brown after the hairs fallen off. Perianth-segments 10-12-15, less differentiated between the outer and inner whorls.

Pretreatment of raw drug:[1] The drug is collected in late winter and early spring before flowering, removed from branchlet, and dried in the shade.

Medicinal use:[4,5,7] For the treatment of allergic and acute rhinitis, nasal discharge, loss of sense of smell, sinus problems also headache and toothache. It is also recommended as analgesic and decongestant.

Effects and indications according to Traditional Chinese Medicine[1,2,3]	
Taste:	pungent
Temperature:	warm
Channels entered:	lung and stomach
Symptoms and indications:	dispels *wind-cold* and to unblock the nasal passages: for nasal obstruction and headache in colds

Constituents (see Fig. 1)[3,5,8]:

- **tetrahydrofurofuran-type lignans:** magnolin, eudesmin, yangambin, fargesin, kobusin, aschantin, epimagnolin A
- tetrahydrofuran-type lignans: biondinin B/E
- neolignans: biondinin A
- **monoterpenes:** 1,8 cineol, α-terpineol, linalool, sabinen, α,β-pinen, camphor, biondinin C/D
- **sesquiterpenes:** parthenolide, oplodiol[12]
- **alkaloids:** magnocurarine, magnoflorine, salicifoline, magnosprengerine

Pharmacology:

In vitro effects:

- antibacterial (*Shigella-, Bacillus-, Staphylococcus-, Corynebacterium-* and *Streptococcus* sp.)[5]
- antifungal activity (e.g. *Candida albicans* or *Trichophyton interdigitalis*)[5]
- antiviral (*influenza*)[5]
- stimulates smooth muscle contraction in uterus and intestine (rabbits, dogs, rats)[5,3]
- antagonistic activity against platelet-activating factor (tetrahydrofurofuran-lignans)[9]
- anti-inflammatory (magnolin)[10]
- TNF-α-inhibition (magnolin, eudesmin, yangambin)[11]

In vivo effects:

- lowering blood pressure (cats, dogs, rabbits)[5]
- skeletal muscle relaxing (frogs)[5]
- local astringent[5]
- anesthetic (guinea pigs)[5]

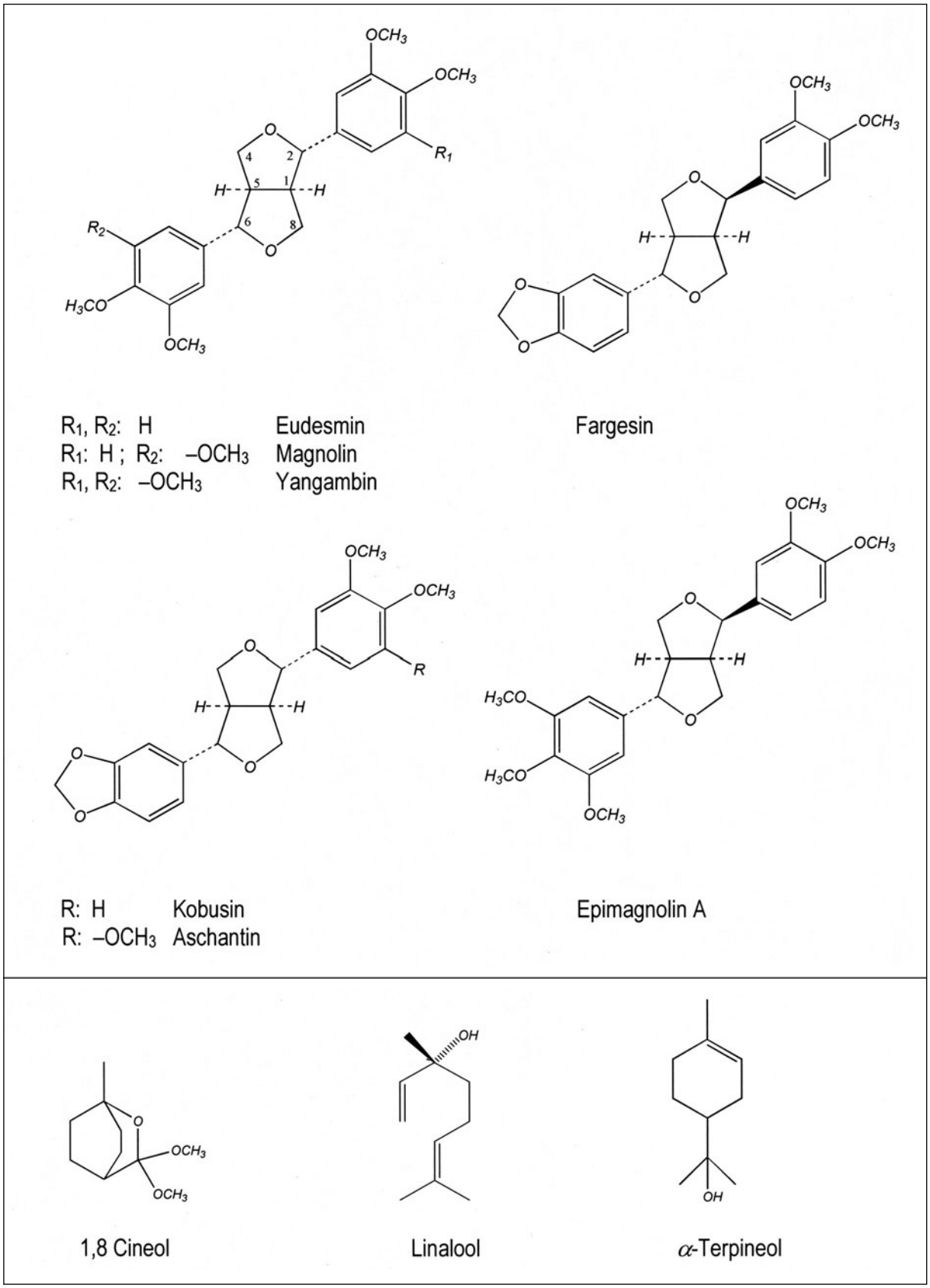

Fig. 1: Formulae of the main constituents

Toxicology:

The flower buds are not known to be toxic. Since the alkaloid content is very low and their intestinal absorption hindered, there is good evidence that the drug is non-toxic. The usual clinical doses used for oral or external application do not cause any changes in respiration, blood pressure, and muscular tone.[5] Overdosage may cause dizziness or redness of the eyes.[3]

TLC fingerprint analysis: tetrahydrofurofuran-type lignans

1) Extraction: 0.5 g of powdered drug in 5 ml methanol is shaken for 30 minutes and the extract is filtered

2) Reference compounds: Magnolin, eudesmin, fargesin, kobusin are dissolved in methanol (1 mg/ml)

3) Separation parameters:

Applied amount: 20 µl extract, 10 µl standard solution

Plates: Silica gel 60 F_{254} (Merck)

Solvent system: Ethyl acetate - toluene - formic acid (15+80+10), development distance 8 cm

4) Detection: The TLC plate is sprayed with 1 % ethanolic vanillin-solution 10 % and with ethanolic sulphuric acid. After heating at 110 °C, the evaluation is carried out in VIS

Drug samples	Origin	Species
1	Complemedis, Switzerland	*Magnolia biondii*
2	Complemedis, Switzerland	*Magnolia biondii*
3	Pharmacy Casa medica, Graz, Austria	*Magnolia biondii*
4	Kunming Institute of Botany, authentic	*Magnolia biondii*
5	Kunming Institute of Botany, authentic	*Magnolia denudata*
6	Kunming Institute of Botany, authentic	*Magnolia liliiflora*
T1	Reference compound	Magnolin, R*f* = 0.23
T2	Reference compound	Eudesmin, R*f* = 0.25
T3	Reference compound	Fargesin, R*f* = 0.44
T4	Reference compound	Kobusin, R*f* = 0.40

5) Description of the chromatogram:

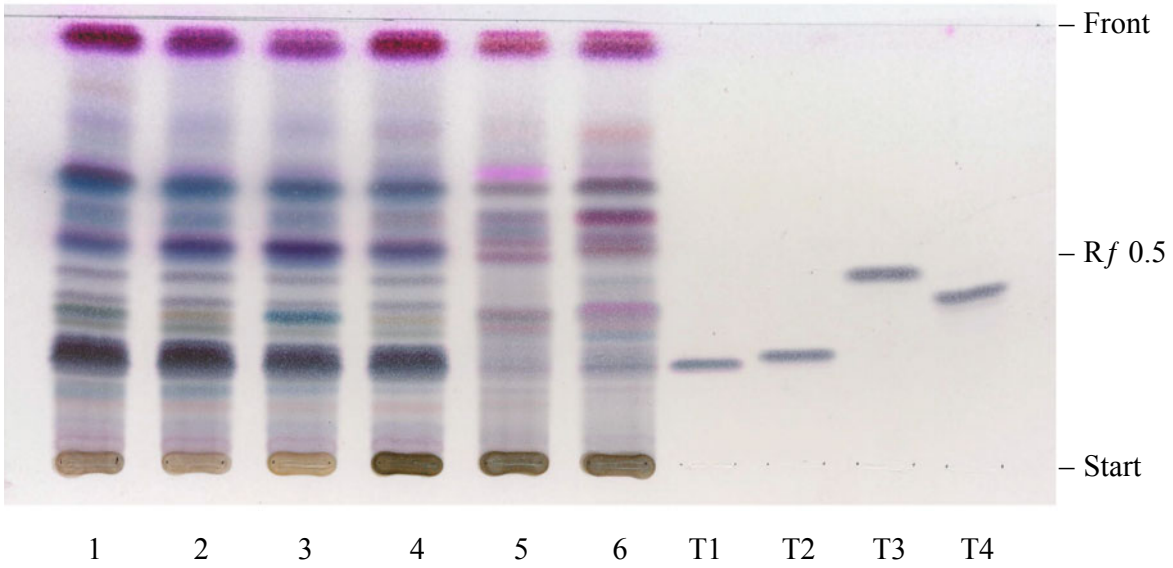

Fig. 2: TLC-fingerprint analysis: tetrahydrofurofuran-type lignans, vanillin-sulphuric acid reagent, VIS

The *Magnolia biondii* samples 1,2,3 and 4 are characterized by the lignan pattern of four prominent grey-violet zones: R*f* 0.23 magnolin (=**T1**), R*f* 0.25 eudesmin (=**T2**), R*f* 0.44 fargesin (=**T3**) and R*f* 0.40 kobusin (=**T4**). Yangambin is located directly under the magnolin zone and aschantin directly under the kobusin spot.

Magnolia liliiflora (sample 6) shows magnolin and fargesin only in low concentration, whereas in *Magnolia denudata* (sample 5) the magnolin zone is hardly visible and fargesin seems to be absent. On the solvent front appear the violet-pink spots of sterol-type compounds.

TLC fingerprint analysis: essential oils

1)	Extraction:	0.5 g of powdered drug in 5 ml *n*-hexane is shaken for 30 minutes and the extract is filtered
2)	Reference compounds:	1,8 cineol, α-terpineol and linalool are dissolved in *n*-hexane to give a solution of 0.1 %
3)	Separation parameters:	
	Applied amount:	30 µl extract, 10 µl standard solution
	Plates:	Silica gel 60 F_{254} (Merck)
	Solvent system:	Toluene - ethyl acetate (93+7), development distance 8 cm
4)	Detection:	same as above described

Drug samples	Origin	Species
1	Complemedis, Switzerland	*Magnolia biondii*
2	Complemedis, Switzerland	*Magnolia biondii*
3	Pharmacy Casa medica, Graz, Austria	*Magnolia biondii*
4	Kunming Institute of Botany, authentic	*Magnolia biondii*
5	Kunming Institute of Botany, authentic	*Magnolia denudata*
6	Kunming Institute of Botany, authentic	*Magnolia liliiflora*
T1	Reference compounds	1,8 cineol, R*f* = 0.34
T2	Reference compounds	linalool, R*f* = 0.25
T3	Reference compounds	α-terpineol, R*f* = 0.15

5) Description of the chromatogram:

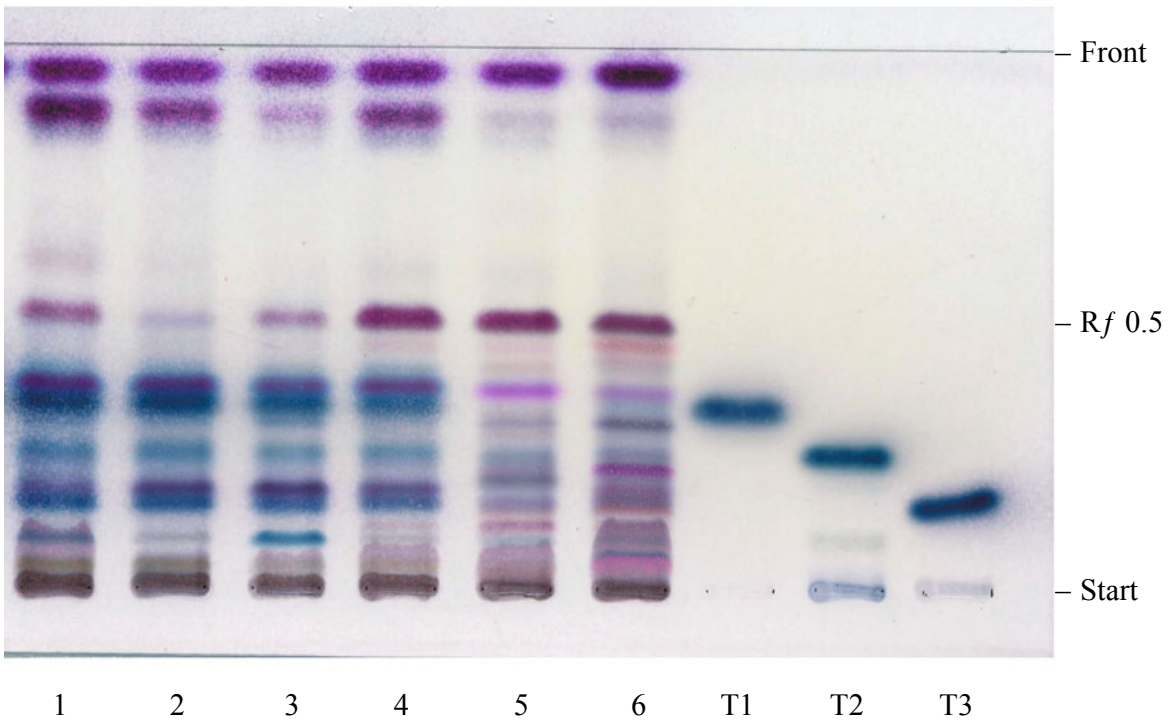

Fig. 3: TLC-fingerprint analysis: essential oils, vanillin sulphuric acid reagent, VIS

The reference compounds appear as dark-blue spots at R*f* 0.34 (**T1**=1,8 cineol), R*f* 0.25 (**T2**=linalool) and R*f* 0.15 (**T3**=α-terpineol). These zones are visible in all four *Magnolia biondii* samples (1-4) with 1,8 cineol as major component.

Magnolia liliiflora (6) shows none of these monoterpene alcohols, neither does *Magnolia denudata* (5).

All samples contain on the solvent front sterol-type compounds (violet zones at R*f* = 0.9-1.0).

Discussion:

On TLC, the drug samples of *Magnolia biondii* show a very homogenous pattern of the tetrahydrofurofuran-type lignans and essential oils.

The TLC fingerprints of the official *Magnolia denudata* and its substitute *Magnolia liliiflora* differ from *Magnolia biondii* mainly by absence of magnolin and the zones at R*f* = 0.48 and R*f* = 0.63.

HPLC fingerprint analysis[28]

1) Sample preparation: 0.5 g of powdered drug in 5 ml methanol is shaken for 30 minutes and the extract is filtered in a 10 ml volumetric flask. Methanol is added to a volume of 10 ml and filtered over Millipore® filters (Type HV 0.45 µm)

2) Injection volume: 5 µl of the methanol extracts

3) HPLC parameters:

Apparatus: L-7100 Pump, L-7455 Diode Array Detector, D-7000 Interface, L-7300 Column Oven (Merck Hitachi)

Column: LiChroCART® 125 × 4 mit LiChrospher® 100 RP-18 (5 µm), Merck

Pre-column: LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck

Solvent system: A: acetonitrile; B: water (nanopure)

Gradient:

Time (min.)	% A
0	30
2	40
7	40
15	50
16	100
20	100
21	30
30	30

Flow rate: 0.5 ml/min.

Detection: 220 nm

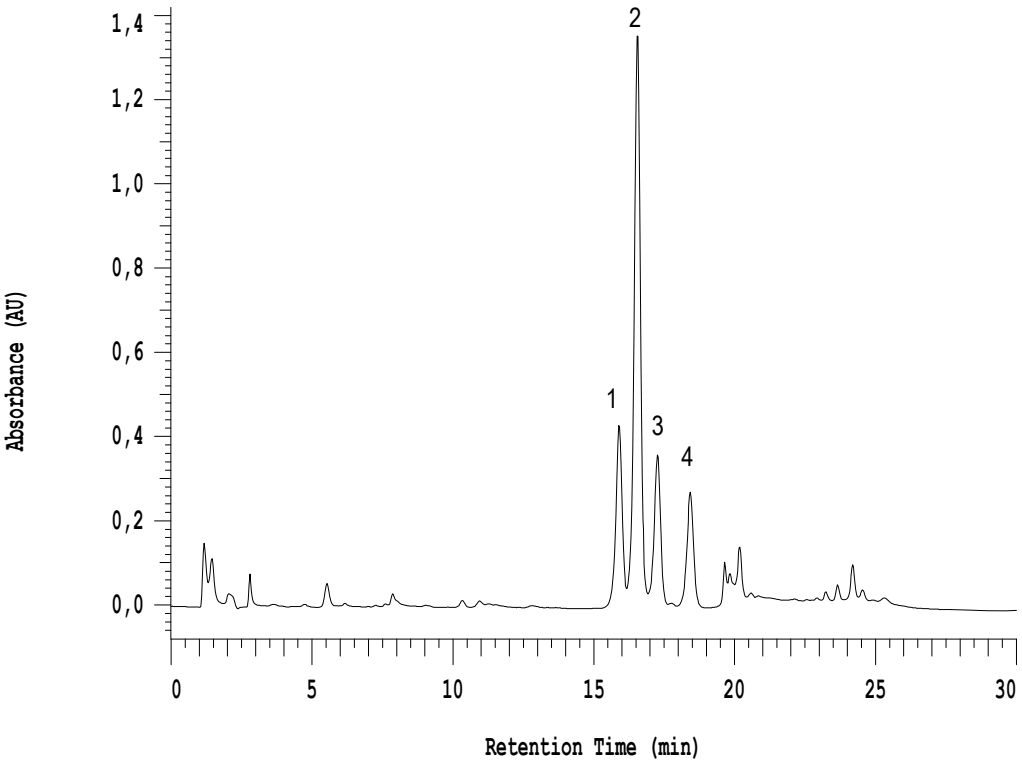

Fig. 4: HPLC of *Magnolia biondii* extract

Retention times of the main peaks:

Peak	Rt (min.)	Compounds
1	15.9	eudesmin
2	16.6	magnolin
3	17.3	yangambin
4	18.4	epimagnolin A

Description of the HPLC-chromatogram:

The chromatograms of all *Magnolia biondii* samples investigated are characterized by the major lignan peaks of eudesmin (Rt 15.9 =**1**), magnolin (Rt 16.6 =**2**), yangambin (Rt 17.3 =**3**) and epimagnolin A (Rt 18.4 =**4**). Kobusin, aschantin and fargesin are present only at lower concentrations in the Rt-range 19.7 to 20.2.

Note: The Chinese Pharmacopoeia 2005 demands a magnolin content not less than 0.4% as measured by a HPLC-method.

Discussion:

The HPLC-analysis of the various *Magnolia biondii* drug samples from different sources resulted in very similar fingerprints.

Acknowledgement:

We thank Dr. Owi Nandi for his cooperation in 2004.

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Fructus Piperis longi – *Bibo*

Pharmacopoeia: Pharmacopoeia of the People's Republic of China, English Edition, Vol. I, 2005[1]

Official drugs:[1] *Piper longum* L., Fam. Piperaceae

The drug is known as *Bibo* or *bì bá* (Chinese); *[Small] Peepal*, *Pipal*, *Pipli* (Indian); *hihatsu* (Japanese); *p'ilhal* (Korean) and *[Indian] Long Pepper* (English);.

Substitutes: The following substitute drugs are not official in Chin. Pharm. 2005
- *Piper retrofractum* Vahl syn. *Piper officinarum (Miq.)* C. DC. (Bari or Large Peepal, India)[3,4]
- *Piper sarmentosum* Roxb. (Jia Ju, China)
- *Piper peepuloides* Roxb. (Choti or Savali Peepal, India)[3,4]

Synonym:[6] *Chavica roxburghii* Miq.

Origin:[6] China, India, Malaysia

Description of the drug:[1,3] The drug consists of the dried fruiting spikes of *Piper longum* L. They are cylindrical to irregulary cylindrical, aggregated by numerous small berries, 1-2.5 cm long (rarely longer than 2.5 cm), 3-5 mm in diameter, blackish-brown to almost black. The spikes are quite compact, tough, composed of small fruits firmly fixed on the receptacle in regular to oblique rows. The small berries are spherical and about 1 mm in diameter. The bracts are black, small, dot-like, confined to depressions between adjacent berries. Sometimes a remnant of the peduncle is still present at the base of the cylinder. Fruiting spikes can be easily broken, the fracture is irregular and granular. The drug exhibits a characteristically aromatic odour, its taste is pungent, slightly similar to black pepper, and followed by salivation and numbness of the tongue/mouth.

Pretreatment of the raw drug[1]: Eliminate foreign matter. Break to pieces before use.

Medicinal use[1,2,5,6]: In Traditional Chinese Medicine the drug is used as an analgesic in epigastric and abdominal pain, for belching, acid regurgitation, nausea, vomiting, diarrhea and flatulence, especially when caused by cold; it is also used for headache, migraine as well as deep source nasal congestion.

Externally applied it relieves toothache and therefore is used for dental caries.

Effects and indications according to Traditional Chinese Medicine[1,2,5,7,8]	
Taste:	acrid, hot, pungent
Temperature:	warm
Channels entered:	spleen, stomach, large intestine, kidney
Effects:	dispels cold from the spleen and the stomach, relieves pain, stomachic, dispels wind and *qi.*
Symptoms and indications:	for stomach cold with such symptoms as nausea, vomiting, belching, diarrhea, and gastralgia; headache and migraine; deep source nasal congestion; topically for toothache.

Main constituents (see Fig. 1)[9-20]:

- **amides** with different amine portions (e.g. isobutylamine, piperidinamine), often with a methylenedioxybenzene moiety, e.g. piperine, piperanine, N-[7-(3',4'-methylendioxyphenyl)-2*E*,6*E*-heptadienoyl]piperidine, dehydropipernonaline, piper-longuminine, dihydropiperlonguminine, futoamide, guineensine, pellitorine, and N-isobutyl-2*E*,4*E*-octadecadienamide[9-16]
- **lignans**, e.g. asarinin, sesamin, diaeudesmin and fargesin[9,13,14,17,18]
- **terpenes**, e.g. *p*-cymene, dihydrocarveol, terpinolene, α-thujene, and zingiberene[19]
- **steroles:** stigmasterol, sitosterol[13]
- **paraffines**, e.g. *n*-triacontane, *n*-octadecane, *n*-hexadecane, *n*-eicosane[19,20]
- **organic acid**: 3-(3',4',5'-trimethoxyphenyl)-propionic acid[9]

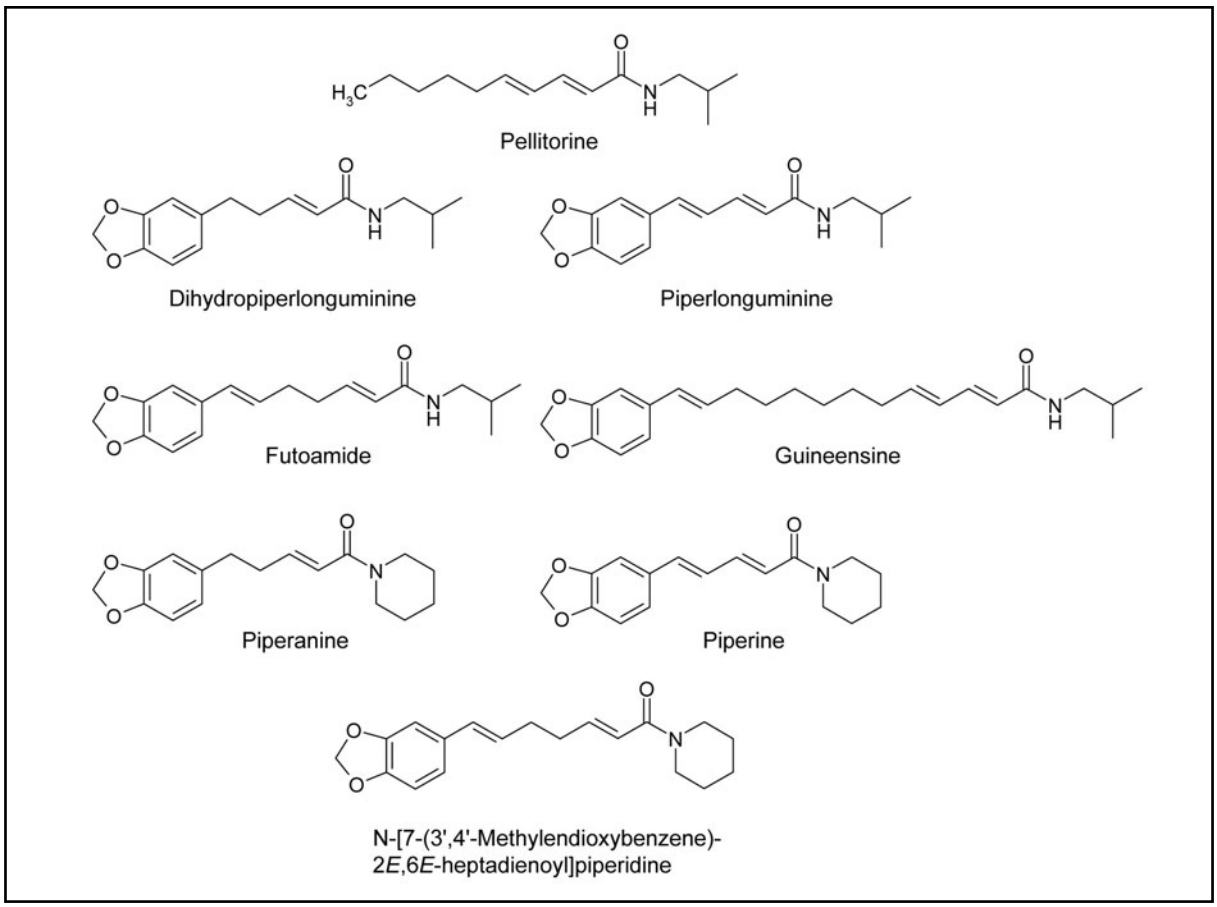

Fig. 1: Formulae of the main constituents

Pharmacology:[2,7,21-34]

- Liver protective potential: significant protection against *tert*-butyl hydroperoxide and carbon tetrachloride induced hepatotoxicity in male swiss mice (piperine)[21]
- Coronary vasodilating activity in rabbits (dehydropipernonaline)[22]
- Bioavailability enhancing activity; inhibition of drug metabolism: this property has been patented with a special formulation in the United States; studies with healthy volunteers (piperine)[23-26]
- Antiamoebic activity against *Entamoeba histolytica in vitro* and *in vivo* in rats (several lipophilic extracts)[27]
- Antigiardial effects on giardiasis due to *Giardia lamblia* infection in mice and *in vitro* (aqueous and ethanolic extract)[28,29]
- Contraceptive activity: antifertility effects in rats (piperine)[30,31]
- Antiallergic and antiasthmatic activity shown in a study with children suffering from asthma[32,33]
- Anti-microbial effect: the oil of Fructus Piperi longi has shown an inhibitory effect against *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus in vitro*[2,7]
- anti-inflammatory activity: inhibitory effect on cyclooxygenase-1 and TNF-α-induced expression of cell adhesion molecules by inhibiting NF-*k*B activation and microsomal lipid peroxidation[41]

TLC-Fingerprint analysis:[35,36]

1) Extraction: 5 g coarsely ground drug are soxhlet-extracted with 50 ml *n*-hexane p.a. for one hour. The extract is evaporated to dryness and redissolved in 2.5 ml ethanol and filtered over Millipore® 0.45 µm filters.

2) Reference compound: Piperine (R*f* 0.26), dissolved in ethanol (1mg/ml)

3) Separation parameters:

   Applied amount: 20 µl extract, 5 µl standard solution

   Plates: Silica gel 60 F_{254} (Merck)

   Solvent system: *n*-hexane - ethyl acetate (5+3), champer saturation, 15 cm

4) Detection:

   Direct evaluation: UV_{254} nm and UV_{365} nm

   Spray reagent: Anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde, 10 ml glacial acetic acid, 85 ml methanol and 5 ml conc. sulphuric acid, mixed in this order).

   The TLC plate is intensively sprayed with 10 ml of the reagent and heated for 5-10 min at 100°C under observation. The evaluation is carried out in VIS.

Drug sample	Species	Origin	Description
1	*P. retrofractum*	China, Beihang Pharmacy, Shenyang	Sold as "Bibo = *P. longum*"
2	*P. retrofractum*	China, Dongteng Pharmacy, Shenyang	Sold as "Bibo = *P. longum*"
3	*P. longum*	China, Institute for Medicinal Plant Development, Beijing	Bibo
4	*P. longum*	India, Zandu Pharmaceutical Works Ltd., Bombay	Peepal
5	*P. longum*	Thailand, Chiang-Mai University, Chiang-Mai	Navsari Peepal
6	*P. longum var.?*	Thailand, Chiang-Mai University, Chiang-Mai	Round Peepal
7	*P. retrofractum*	Thailand, Chiang-Mai University, Chiang-Mai	Large Peepal
8	*P. retrofractum*	Thailand, Chiang-Mai University, Chiang-Mai	Sold as "*P. chaba*"
R	Reference compound piperine (R*f* = 0.26)		

5) Description of the chromatograms:

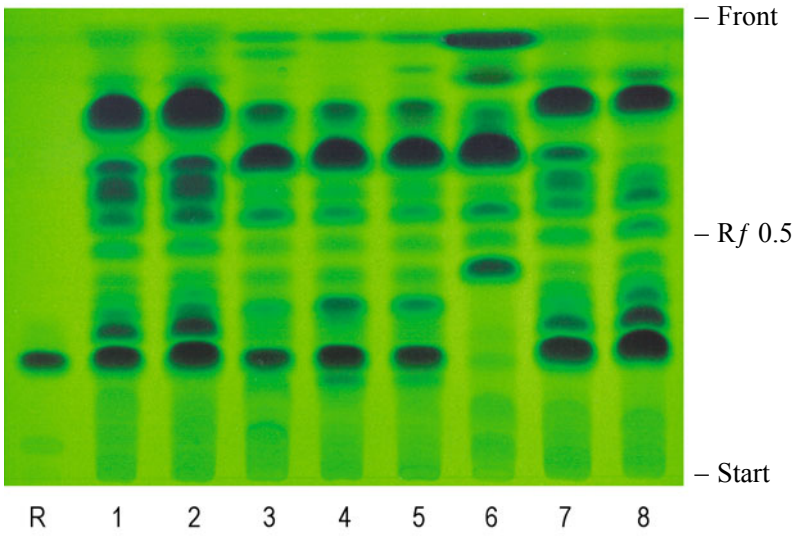

Fig. 2a: UV_{254} nm:

The TLC-chromatograms of the three *P. longum* samples (traces 3, 4, 5) are dominated by two strongly absorbing zones: piperine (R*f* 0.26) and pellitorine (R*f* 0.72). Further three less strong absorbing zones can be found at R*f* 0.37, R*f* 0.58 and R*f* 0.80 (N-isobutyl-2*E*,4*E*-octadecadienamide). At R*f* 0.20, R*f* 0.43, R*f* 0.51, R*f* 0.62 and R*f* 0.96 (pipataline) additional weak absorbing zones are visible. Drug samples 3, 4 and 5 only show slight differences. In sample 3 and 5 one respectively two additional absorbing zones can be observed between R*f* 0.88 and R*f* 0.95 whereas the zone at R*f* 0.20 seems to be missing in sample 3.

In comparison to *P. longum* the TLC-chromatograms of *P. retrofractum* (Large Peepal, traces 1, 2, 7, 8) show distinct different characteristics. Although the strongly absorbing zone of piperine (R*f* 0.26) can also be found, the second dominating zone is at R*f* 0.81 and not at R*f* 0.72. Pellitorine is completely missing. Additional zones, which are absent or less visible in the TLC-chromatograms of *P. longum* appear at R*f* 0.33 and between R*f* 0.61and R*f* 0.66. In contrast to *P. longum* only one weak spot can be observed above R*f* 0.81 (R*f* 0.88). The remaining pattern between R*f* 0.26 and R*f* 0.72 is quite similar to that of *P. longum*.

The TLC-chromatogram of *P. longum var.* (Round Peepal, trace 6) can also be clearly distinguished from that of *P. longum.* Whereas the strongly absorbing zone of pellitorine (R*f* 0.72) is found, the piperine-zone at R*f* 0.26 is missing. Besides the zone of pellitorine, two additional dominating zones are present at R*f* 0.46 and R*f* 0.98. Another more or less prominent zone is visible at R*f* 0.88. The pattern between R*f* 0.46 and R*f* *733*0.72 is quite similar to that of *P. longum*.

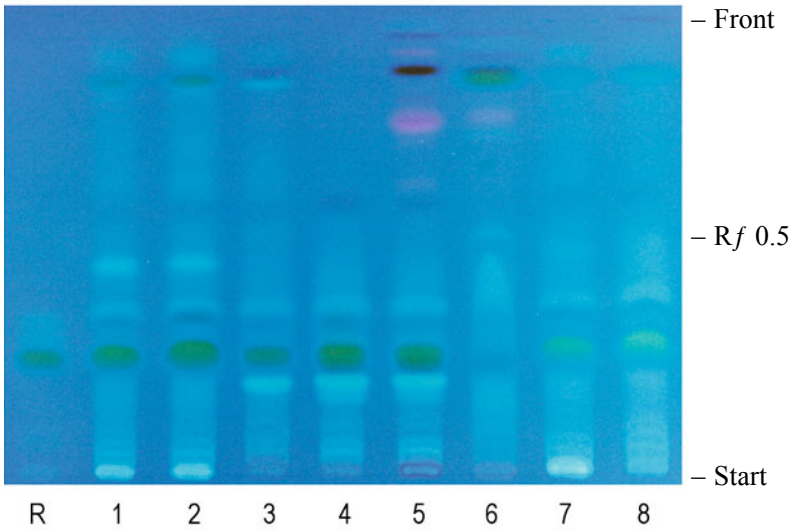

Fig. 2b: UV_{365} nm:

Piperine (R*f* 0.26) appears as green fluorescent zone in all three *P. longum* samples examined as well as in all chromatograms of *P. retrofractum*. In addition, the chromatograms of *P. longum* show one strong light blue fluorescent zone at R*f* 0.20 and a weak one at R*f* 0.35. The magenta fluorescence at R*f* 0.77 and R*f* 0.88 in sample 5 (and partly in sample 3) is probably caused by residual chlorophyll.

Again the TLC-chromatograms of the four *P. retrofractum* samples show distinct differences: Whereas an additional light blue band is visible at R*f* 0.45, no distinct blue fluorescent zone can be found at R*f* 0.20. Furthermore a weak yellowish fluorescence is present at R*f* 0.86.

In contrast to the above mentioned two species the TLC-chromatogram of *P. longum var.* (Round Peepal) lacks the distinct fluorescent zones between R*f* 0.20 and R*f* 0.45, however a weak yellowish fluorescence at R*f* 0.86 is observable in *P. retrofractum*.

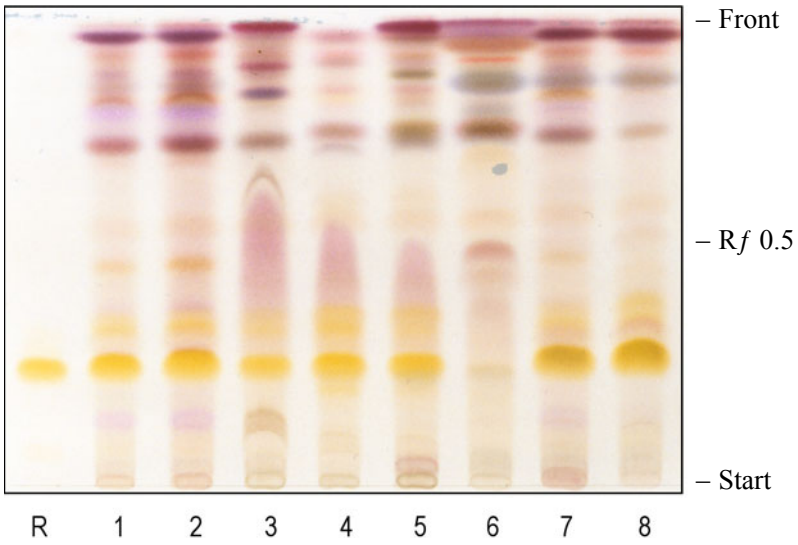

Fig. 2c: AS-reagent, VIS:

After detection with AS-reagent, piperine appears as yellow spot (R*f* 0.26) together with another yellowish zone directly above (between R*f* 0.31 and R*f* 0.37), which can also be seen in the chromatograms of *P. retrofractum*. The TLC-chromatograms of *P. longum* exclusively show a broad tailing zone between R*f* 0.35 and R*f* 0.63 in violet colour, indicating that the extracts are rich in unsaturated fatty acids (e.g. linoleic acid).

Between R*f* 0.65 and R*f* 0.99 the TLC-fingerprints of Large and Round Peepal (*P. retrofractum* and *P. longum var.*) show a lot of mainly violet to brown coloured zones. They look very similar to and can hardly be distinguished from *P. longum*.

Discussion:

All *Piper longum* drug samples examined showed a very constant pattern of constituents when analyzed by TLC. The TLC-chromatograms of the two other pepper species, *Piper retrofractum* (Large Peepal) and *Piper longum var.* (Round peepal), differ from the chromatogram of *Piper longum* and can be easily distinguished under UV_{254} nm and UV_{365} nm.

Therefore TLC-analysis is a suitable method for the identification of *P. longum* and one possibility to differentiate between the two closely related species mentioned above.

HPLC fingerprint analysis:[35-39]

1) Sample preparation: The same extracts are used as for TLC.

2) Injection volume: 10 µl ethanolic solution

3) HPLC parameters:

Apparatus:	Liquid chromatograph HP 1050 with DAD (photodiode array detector) HP 1050
Column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART® 4-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Solvent system:	A: water; B: acetonitrile
Gradient:	40-50 % B in 15 min. (linear), 50-95 % B in 15 min. (linear), 95-100 % B in 15 min. (linear)
Flow rate:	1.0 ml/min.
Detection:	254 nm

Retention times of the assigned peaks in *P. longum*, *P. retrofractum* and *P. longum var.*:[35-39]

Rt (min.)	Peak	Compound
7.3	1	Dihydropiperlonguminine
7.6	2	Piperlonguminine
8.7	3	Piperanine (= dihydropiperine)
9.7	4	Piperine
11.8	5	Futoamide
13.9	6	N-[7-(3',4'-Methylendioxyphenyl)-2E,6E-heptadienoyl]piperidine
17.5	12	Retrofractamide A
17.9	7	Pellitorine
21.5	13	Retrofractamide D
22.3	14	Retrofractamide B
25.7	8	Guineensine
28.4	9	Brachystamide B
30.3	10	N-Isobutyl-2E,4E-hexadecadienamide
32.3	15	N-Isobutyl-2E,4E-octadecadienamide
33.2	11	Pipataline

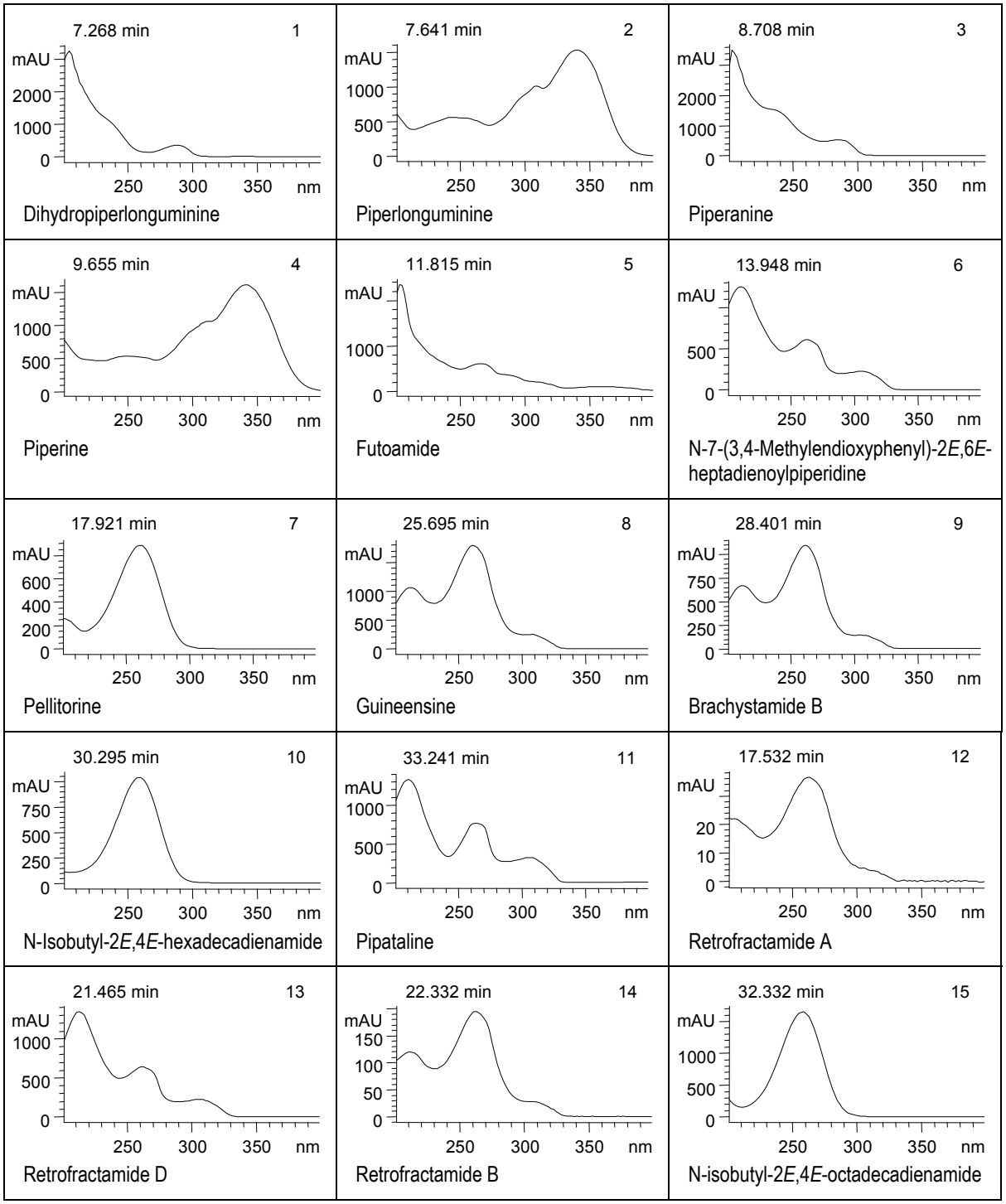

Fig. 3: Online recorded UV-spectra of the assigned peaks in the HPLC-separations of the *n*-hexane extracts of *P. longum*, *P. retrofractum* and *P. longum var.*

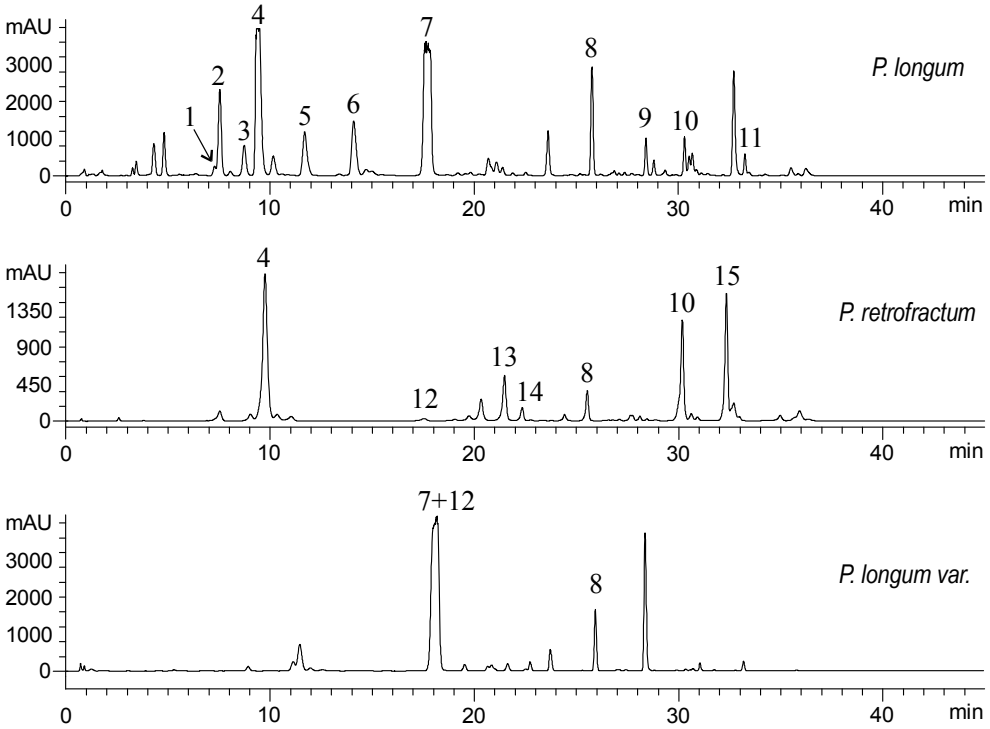

Fig. 4: Comparison of the HPLC-fingerprint chromatograms of *P. longum*, *P. retrofractum* and *P. longum var.* (UV 254 nm)

Description:

Piper longum:

The HPLC-chromatogram of *Piper longum* shows a homogeneous distribution of numerous peaks, indicating that the separation conditions are suitable for this specific pattern of constituents. The assignment of most of the corresponding compounds was done recently by means of LC-MS and LC-NMR[10,35,36]. Except pipataline[35,36,39], a methylendioxybenzene with a long aliphatic side chain (peak **11**) all compounds assigned are amides consisting of an unsaturated aliphatic acid and isobutylamine (peaks **1**, **2**, **5**, **7**, **8**, **9**, **10**) or piperidinamine (peaks **3**, **4**, **6**) as amine portion. Except pellitorine and N-isobutyl-2*E*,4*E*-hexadecadienamide all of them show an additional methylenedioxybenzene moiety.

As in the TLC-chromatogram, piperine (peak **4**) and pellitorine (peak **7**) are also the predominant peaks in the HPLC-fingerprint. Moderate intensity can be found for piperlonguminine (peak **2**), futoamide (peak **5**), N-7-(3,4-Methylene-dioxyphenyl)-2*E*,6*E*-heptadienoylpiperidine (peak **6**), guineensine (peak **8**) and a peak at Rt 32.7, which shows a very similar UV-spectrum with that of pellitorine and therefore might be a homologous compound. The remaining peaks are smaller (**1**, **3**, **9**, **10**, **11**) and less characteristic.

Note: The Chinese Pharmacopoeia 2005 demands for Fructus Piperis longi not less than 2.5 % of piperine ($C_{17}H_{19}O_3N$) calc. with reference to the dried drug as measured by HPLC.

Piper retrofractum, Piper longum var.:

In comparison to *P. longum* the HPLC-chromatograms of *P. retrofractum* (Large Peepal) and *P. longum var.* (Round Peepal) are less complex.

From Rt 0 to 18 min. the HPLC-fingerprint of *P. retrofractum* only shows one predominant peak at 9.7 min. (piperine) and a very small one at Rt 17.5 which does not correspond to pellitorine but to a different compound, retrofractamide A (peak **12**). In contrast to the chromatogram of *P. longum* the retrofractamides D and B (peaks **13** and **14**) can be detected between Rt 21 and 23 min., which are also not present in the HPLC-fingerprints of *P. longum var.*. At almost 100 % acetonitrile (Rt 32.3 min.) N-isobutyl-2*E*,4*E*-octadecadienamide elutes with a peak of high intensity (**15**).

The HPLC-chromatogram of *P. longum var.* is dominated by one single peak between Rt = 17.8 and 18.2 min., where pellitorine and retrofractamide A coelute. The remaining HPLC-fingerprint only shows two more major peaks, guineensine (peak **8**) and a peak at 28.4 min., with a UV-spectrum very similar to that of retrofractamide D.

In summary, the HPLC-analysis is a suitable tool to characterize the *n*-hexane extract of *Piper longum* and allows the certain distinction between this species and the closely related *P. retrofractum* and *P. longum* var. The HPLC-fingerprints of these three species are widely different.

Discussion: *Piper longum* and related pepper species

In the course of our investigation we analysed ten different drug samples from the People's Republic of China sold as "*Piper longum*" or "Bibo". The respective samples where obtained from five different pharmacies (including sample 1 and 2 of this publication) as well as one commercial medical and pharmaceutical company in Shenyang and one drug store as well as one drug market in Beijing. Two "authentified samples" came to us via Shenyang University and the Institute for Medicinal Plant Development in Beijing respectively (sample 3).

Except of one sample (3) all drugs turned out to be derived from *Piper retrofractum* and not from *Piper longum*. Two additional "*Piper longum*" samples from India and Thailand (4 and 5) could be confirmed in their identity. Further two samples from a drug store in Shanghai, also identified as *Piper retrofractum* had been correctly labeled as "Big Bibo".

With this background the following remarks seem to be helpful. According to the monograph "Fructus Piperis Longi, Bibo" in the Chinese Pharmacopoeia 2005[1] only the fruits of *Piper longum* L. are official. The macroscopic description of the drug in the Pharmacopoeia is not sufficient to achieve an unambiguous distinction of *Piper longum* L. and the related species *Piper retrofractum* Vahl. This species is neither mentioned as substitute nor as adulteration of the official drug. As our investigation suggests, a not insignificant portion of the drugs marketed in China labelled "Bibo" or "Long Pepper" is derived from *Piper retrofractum* Vahl. Several books dealing with the Chinese Materia Medica clearly describe properties of *Piper retrofractum* Vahl under the chapter "*Piper longum L.*" and even illustrate them with pictures of this related species[5,6].

In India "Long Pepper (Peepal)" is also traditionally used. Here this term is associated with several different pepper species, which all have a long fruiting spike in common. A distinction is made between Small Peepal (*Piper longum* L.), Savali or Choti Peepal (*Piper peepuloides* Roxb.) and Large or Bari Peepal (*Piper retrofractum* Vahl). Several publications describe how

these species can easily be distinguished[3,4]. According to Govindarajan [40] the fruits of *Piper retrofractum* Vahl are sold at the Indian market at relatively low prices compared to the product of *Piper longum* L. Additionally Mehra and Puri[4] state, that fruiting spikes of *Piper longum* L. are rarely available in India and instead often substituted by the above mentioned related pepper species.

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Radix Sophorae flavescentis – *Kushen*

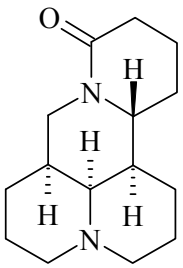
Pharmacopoeias:	Pharmacopoeia of the People's Republic of China, English Edition, Vol. 1, 2005[1] Japanese Pharmacopoeia, English Edition 1996 (Jap. XIII)
Official drugs:	According to Chinese Pharmacopoeia: the root of *Sophora flavescens* Ait. (Fam. Fabaceae). "Radix Sophorae flavescentis, lightyellow sophora root", *Kushen*. The monograph "Radix Sophorae tonkinensis, Gagnep., Vietnamese sophora root", *Shandougen,* is not official in the Chinese Pharmacopoeia 2005. The Japanese Pharmacopoeia includes two monographs of *Sophora flavescens* roots, "*Sophora* root" and "Powdered *Sophora* root".
Origin:	China (Provinces Shanxi, Hubei, Henan, Hebei)
Synonyms [2-5]:	*Sophora angustifolia* Sieb. et Zucc., S*ophora galegoides* Pall., *Sophora kronei* Hance, *Sophora soroia* Hance. The drug is also named *kujin* (Japanese), *kosam* (Korean), *bitter ginseng* (literal English translation).
Adulteration[4]:	*Glycyrrhiza pallidiflora* Maxim.
Description of the drug [1,6,7,8]:	Long cylindrical, usually branched in lower part, 10-30 cm long, 1-6.5 cm in diameter. Externally greyish-brown or brownish-yellow, exhibiting longitudinal wrinkles and transverse elongated lenticel like protrudings. Outer bark thin, mostly broken and recurved, easily exfoliated, the exposed surface appearing yellow and smooth. Texture hard uneasily broken, fracture fibrous. Slices 3-6 mm thick, transversely cut, surface yellowish-white with radial lines and cracks, some exhibiting abnormal vascular bundles arranged in concentric rings or scattered irregularly. Odour slight; taste bitter.
Pretreatment of raw drug[3]:	After removing the root stock and rootlets, the drug is washed, softened thoroughly, cut into thick slices and dried (*Kushen).*
Medicinal use[9,10]:	In Traditional Chinese Medicine the herb is used for the treatment of arrhythmia, diarrhea, gastrointestinal haemorrhage, skin disorders, eczema, jaundice, vaginitis and asthma. Furthermore people recommend it as *trichomoniasis*-, adstringent- and stomachic drug for the treatment of dysentery and enterorrhagia.

Effects and indications according to Traditional Chinese Medicine[3-7]:	
Taste:	bitter
Temperature:	cold
Channels entered:	heart, liver, kidney, large intestine and small intestine, urinary bladder
Effects:	1. removes heat and *damp,* used *in jaundice with oliguria*, acute dysentery with bloody stool 2. dispels pathogenic wind and kills parasites, used in eczema, sores with exudation; itching of the skin, scabies and leprosy 3. promotes diuresis
Symptoms, Indications:	Acute dysentery with bloody stools; jaundice with oliguria; bloody and purulent leukorrhea; pudendal swelling and itching; eczema, scores with exudation, itching of the skin, scabies and leprosy, external use for *Trichomonas vaginitis*. Painful urination caused by damp-heat is a further indication of Kushen.
Dosage	3-10 g
Cautions, Contraindications	The herb should never be used with the herb Black false hellebore (Lilu) and Veratri nigri rhizoma and radix. It is contraindicated in cases with weakness and cold in the spleen and stomach.

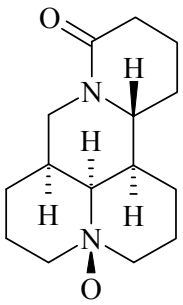
Main constituents (see Fig. 1):

- **quinolizidine alkaloids** (matrine, oxymatrine, sophoranol, sophocarpine, 5-episophocarpine, isomatrine, sophocarpine-N-oxide)[11,12]
- **flavanone derivatives** (kushenols A-X, sophoraflavanone B, sophoraflavanone G, kurarinone, kosamol A, kuraridinol, kurarinol, neokurarinol, norkurarinone, isokurarinone) [13,14]
- **pterocarpans** (maackiain, medicarpin, kushecarpines A-C)[14,15]
- **chalcones** (kuraridin, kuraridinol, kushenol D)[13,16]
- **triterpene saponines** (soyasaponine I, sophorasaposides I- IV)[17]
- the quinone kushequinone A[18]
- Other compounds: ß-sitosterol, sucrose, umbelliferon, sinapic acid hexadecyl ester and lignoceric acid[19]

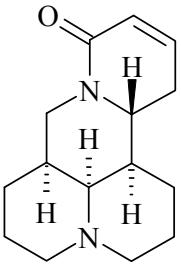
Alkaloids:



Matrine

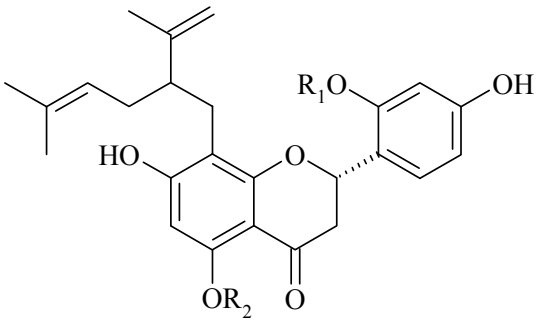


Oxymatrine



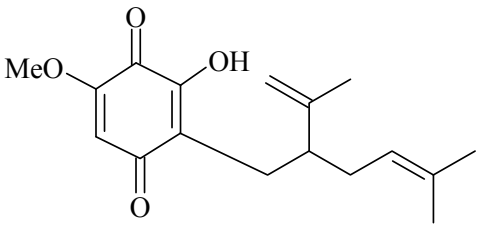
Sophocarpine

Flavanones:



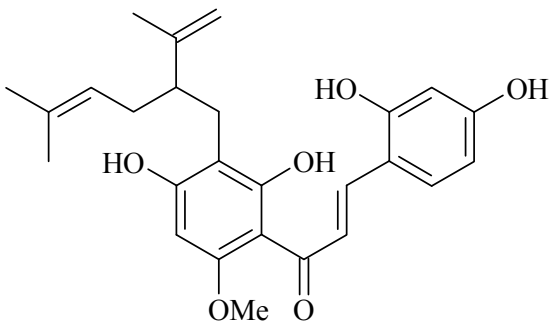
R_1:H R_2:H Sophoraflavanone G
R_1: CH_3 R_2:H Isokurarinone
R_1: H R_2: CH_3 Kurarinone

Quinone:



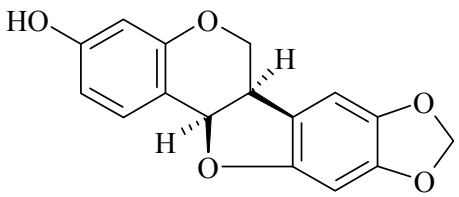
Kushequinone A

Chalcones:



Kuraridine

Pterocarpans:



Maackiain

Fig. 1: Formulae of the main compounds

Pharmacology:

In vitro effects:

- inhibitory activities for melanogenesis[18]
- inhibition of phospholipase $C\gamma_1$[20]
- antiproliferative effects[21]
- induction of apoptosis[21]
- inhibition of 5-lipoxygenase[22]
- antitumor activity[23]
- cytotoxic activity[25]
- effects against chronic hepatitis B (kurarinone)[26]
- antibacterial and antiandrogen action[27]

In vivo effects:

- antiinfective effect on hepatitis B[18]
- vasodilatory effect[18]
- antiulcer action[28-32]
- antiasthmatic and antitussive activity[33,34]
- antineoplastic[2]
- antiarrhythmic activity with dose dependant negative chronotropic, negative automatotropic and negative dromotropic effect (rats)[35]

Toxicology[3]:

Mild dizziness, nausea, vomiting and constipation may occasionally occur with individual patients.

TLC fingerprint analysis:

1) Extractions:
   a) detection of the flavonoids

      5 g coarsely ground drug are powdered and soxhlet-extracted with 120 ml dichloromethane p.a. for 2 hours. The extract is evaporated to dryness and the residue redissolved in 5.0 ml ethanol p.a.

   b) detection of the alkaloids

      5 g coarsely ground drug are powdered and extracted with 50 ml water for 30 min at room temperature by stiring, then shortly boiled. After cooling down it is filtered over a blue band filter. To concentrate the lipophilic compounds, 25 ml of the filtrate is given on an Extrelut® 20 column (Merck) and extracted with 60 ml of chloroform 30 min later.
      The eluate is evaporated to dryness and the residue redissolved in 1 ml ethanol p.a.

2) Standards:

   each 1 mg is dissolved in 1 ml ethanol p.a.

3) Separation parameters:

Applied amount:	10 µl extract solution, 10 µl standard solution
Plates:	Silica gel 60 F_{254}, Merck
Solvent system:	Lower phase of the mixture chloroform-methanol-ammonia solution (50+10+3)
Spray reagents:	a) Vanillin-sulphuric acid reagent (0.5 ml vanillin + 85 ml methanol + 10 ml glacial acetic acid + 5 ml conc. sulphuric acid are mixed in this order). The plate is sprayed with ca. 20 ml of the reagent and heated for 1-2 min. at 100°C. The evaluation is carried out in VIS.
	b) Dragendorff reagent (0.85 g basic $Bi(NO_3)_3$ + 40 ml water + 10 ml glacial acetic acid + 20 ml potassiumiodide *R*) The plate is sprayed with approx. 10 ml of the reagent and evaluated in VIS after drying.

Drug samples:

1 *Sophora flavescens* roots, drugstore, Beijing
2 *Sophora flavescens* roots, TCM hospital, Beijing
3 *Sophora flavescens* roots, drugstore, Chinatown, Los Angeles (USA)
4 *Sophora flavescens* roots, TCM-Hospital Bad Kötzting, Germany
5 *Sophora flavescens* roots, TCM-Hospital Bad Kötzting, Germany
6 *Sophora flavescens* roots, Beijing
7 *Sophora tonkinensis* roots, Beijing
8 *Glycyrrhiza pallidiflora* roots, Bot. Garden, Beijing

Reference compounds:

T1	Maackiain	R*f* = 0.76
T2	Isokurarinone	R*f* = 0.64
T3	Sophoraflavanone G	R*f* = 0.33
T4	Kurarinone	R*f* = 0.22
T5	Matrine	R*f* = 0.71
T6	Oxymatrine	R*f* = 0.15
T7	Sophoridine	R*f* = 0.45

4) Descriptions of thin layer chromatograms:

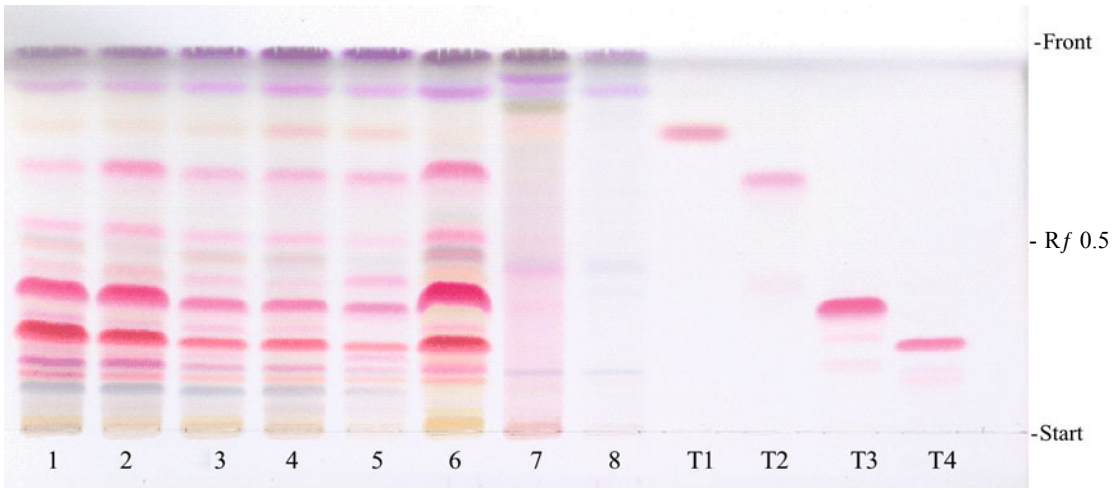


Fig. 2: Thin layer chromatogram of different *Sophora flavescens* roots (**1-6**) from various origins, *Sophora tonkinensis* (**7**) and *Glycyrrhiza pallidiflora* (**8**) with reference compounds (**T1-T4**) (detection: vanillin-sulphuric acid reagent)

All samples of the *Sophora flavescens* samples show a very similar pattern of constituents. Violet zones appearing at R*f* 0.87 and 0.97 (mainly fatty acids) are followed by the red zones of maackiain at R*f* 0.76 (**T1**) and isokurarinone at R*f* 0.64 (**T2**). Additional intensive red zones of flavonoids appear in the R*f*-range between R*f* 0.14 and R*f* 0.50. The most intensive zones are seen at R*f* 0.33 and 0.22 with sophoraflavanone G (**T3**) and kurarinone (**T4**), respectively. The intensity of the zones is variable. Sample 6 showed the highest content, sample 3-5 the lowest.

The fingerprints of *Sophora tonkinensis* and *Glycyrrhiza pallidiflora* (7, 8) are quite different from those of *Sophora flavescens* (1-6) and therefore easily discriminable. Besides the violet zones at R*f* 0.87 and 0.98, in both samples no red spots in the chromatograms of *Sophora tonkinensis* (7) and *Glycyrrhiza pallidiflora* (8) can be detected.

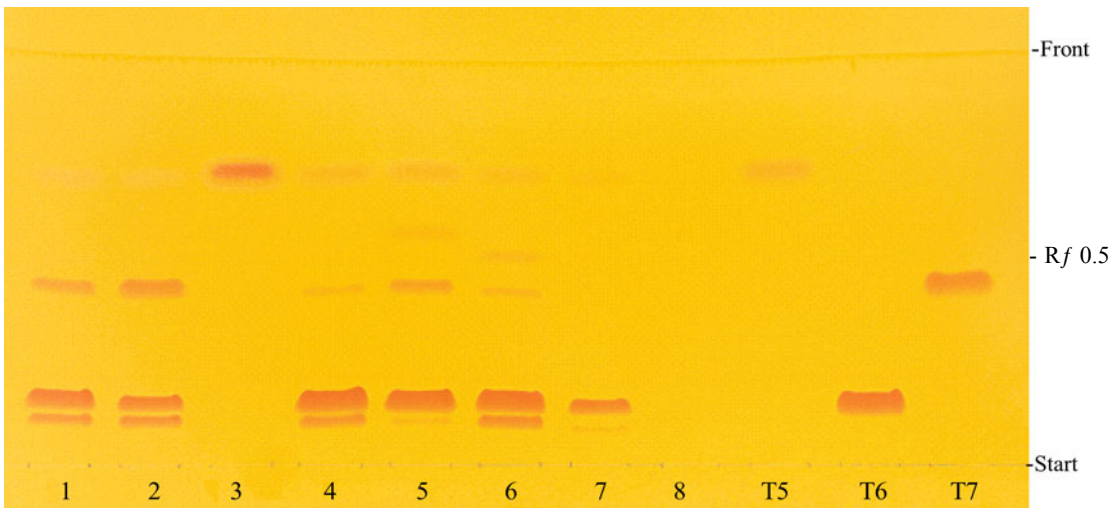


Fig. 3: Thin layer chromatogram of different samples of *Sophora flavescens* roots (**1-6**) *Sophora tonkinensis* (**7**) *Glycyrrhiza pallidiflora* (**8**) and reference compounds (**T5-T7**) (detection: Dragendorff reagent, VIS)

In all *Sophora flavescens* samples 1-6 with exception of sample 3, the dominant Oxymatrine (R*f* 0.15, **T6**) accompanied by oxysophocarpine (R*f* 0.10) just below oxymatrine can be detected as orange zones. Sophorodine at R*f* 0.45 (**T7**) is also present in sample 1,2,4,5 and 6. Further alcaloids in very low concentrations appear at R*f* 0.72 and 0.15. In sample 3 (*Sophora flavescens* of USA origin) only matrine (**T5**) at R*f* 0.71 can be seen but with a higher yield in comparison to the other samples. Oxymatrine (strong) and matrine (weak) are also present in *Sophora tonkinensis* (sample 7), whereas sophoridine is missing. *Glycyrrhiza pallidiflora* (sample 8) does not contain any of these alkaloids.

Discussion:

The TLC enables a distinct identification of *Sophora flavescens* roots and discrimination from *Sophora tonkinensis* and the possible adulteration with *Glycyrrhiza pallidiflora* by detecting the non characteristic compounds and the Quinolizidine alkaloids of *Sophora flavescens*.

HPLC fingerprint analysis:

1) Sample preparation: The extracts used for TLC (Extraction method 1a) are filtered over Millipore® filtration unit, type HV 0.45 µm and directly injected.

2) Injection volume: 2 µl ethanolic solution (conc. = 5 g drug/5ml)

3) HPLC parameters:

Apparatus:	Liquid Chromatograph HP 1050 (Hewlett Packard)
Column:	LiChroCART® 125-4 with LiChroSpher® 100 RP18 (5µm), Merck
Pre column:	LiChroCART® 4-4 LiChrospher® 100 RP18 (5µm), Merck
Solvent system:	A: water, B: acetonitrile
Gradient:	isocratic: 40 % B (15 min.) linear: 40-80 % B in 15 min.
Flow:	1.0 ml/min.
Detection:	210 nm and 365 nm

4) Description of the HPLC chromatograms:

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	4.8	Norkurarinol
2	6.1	Maackiain
3	9.7	Kurarinone
4	19.1	2`-Methoxykurarinone
5	19.7	Sophoraflavanone G
6	20.2	Not identified
7	23.6	Isokurarinone
8	24.2	Kuraridine

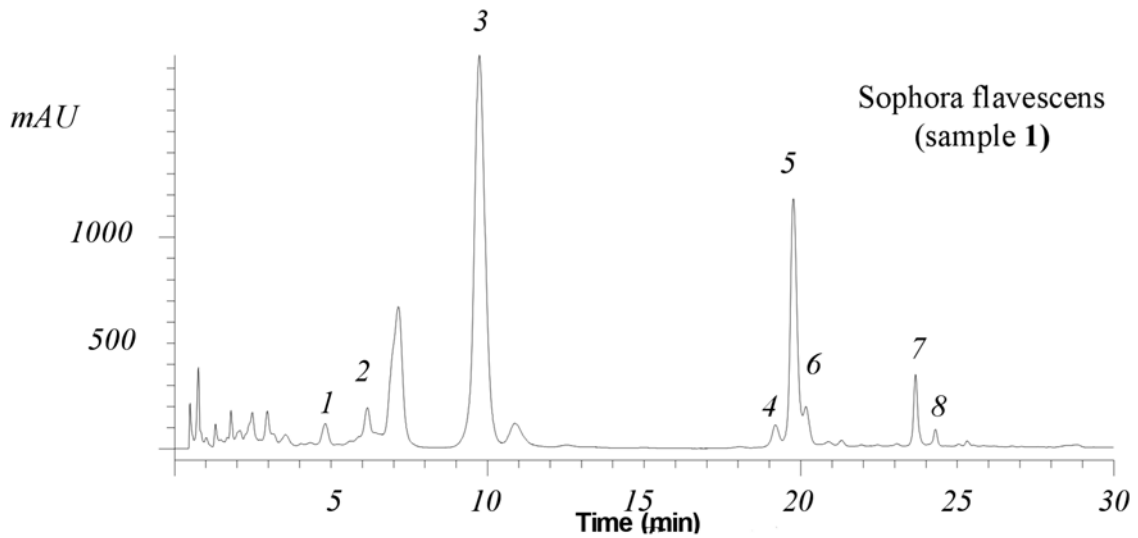


Fig. 4: HPLC fingerprint analysis of the dichloromethane extract of *Sophora flavescens* sample 1 detected at UV 210 nm

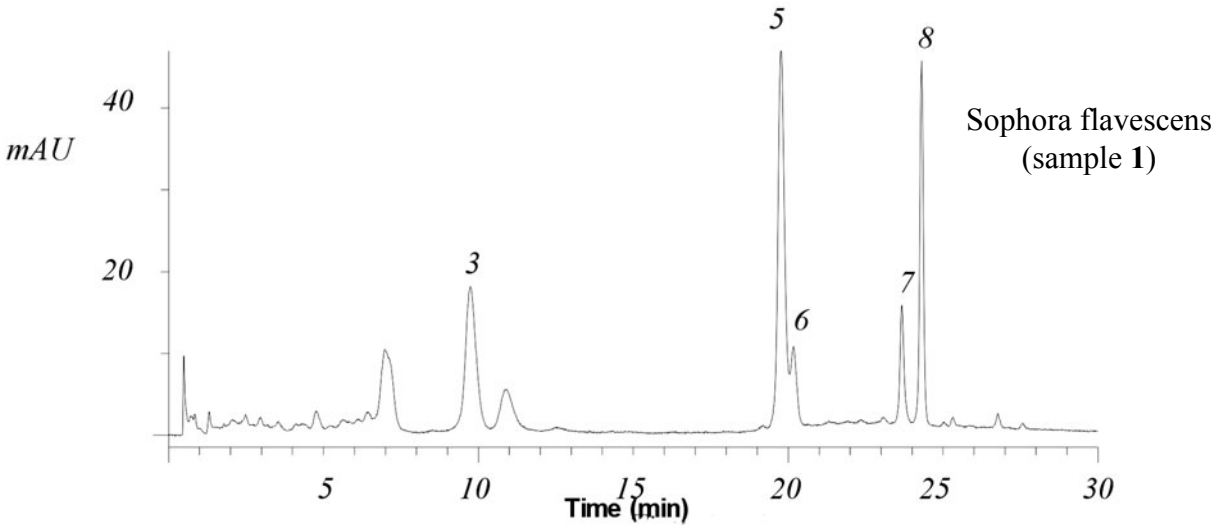
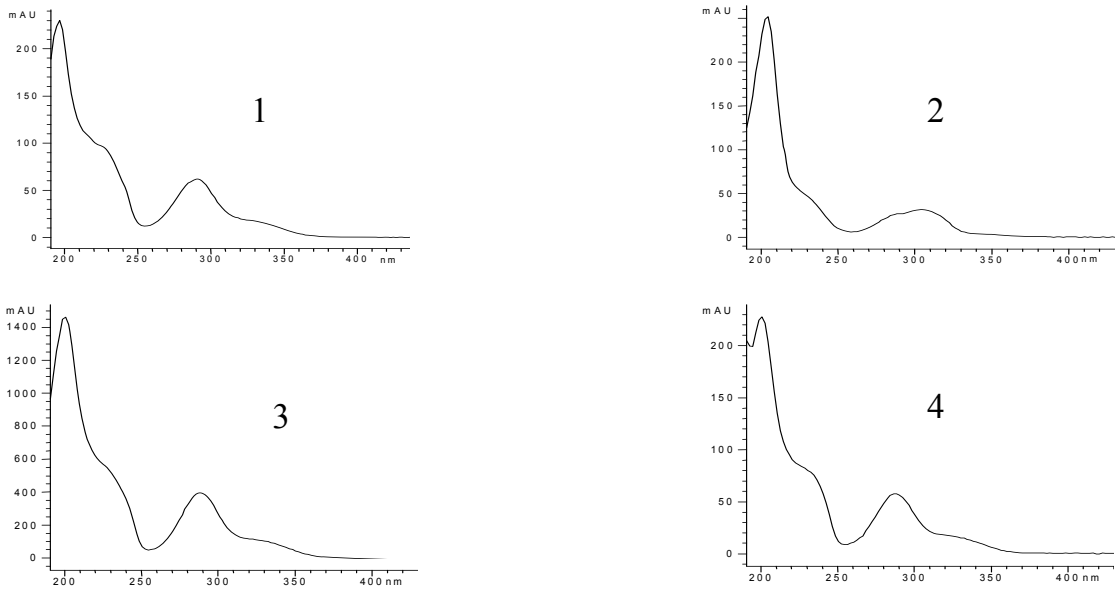


Fig. 5: HPLC separations of the dichloromethane extract of *Sophora flavescens* sample 1 detected at UV 365 nm



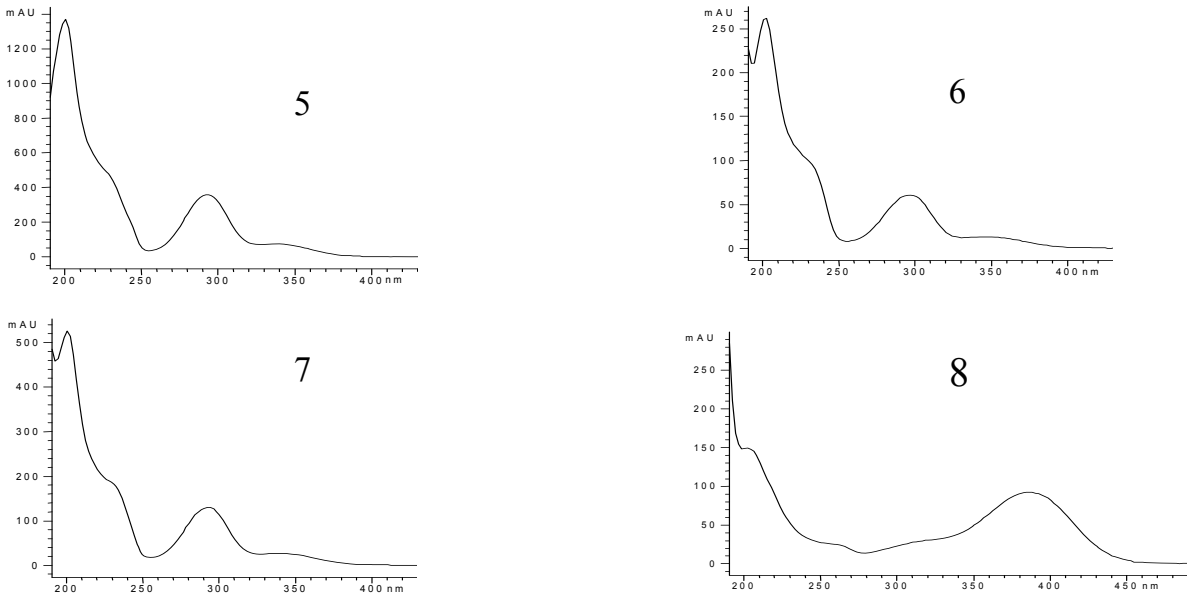


Fig. 6: on line UV spectra of the main compounds

Fig. 4:

HPLC-fingerprint of the dichloromethane extract of *Sophora flavescens* sample 1 detected at UV 210 nm.

- This fingerprint of *Sophora flavescens*, sample 1 is except of quantitative deviations characteristic for all other *Sophora flavescens* samples 2-6. All peaks detected in **Fig. 4** (**1**-**8**) derive from Flavanones, Chalcones and the pterocarpan derivative Maackiain.

  The major peaks are Kurarinone (peak **3**) and Sophoraflavanone G (peak **5**). All other peaks (peak **1**,**2**,**4**,**6**,**7** and **8**) appear with minor concentration. The peak **6**, with the typical flavanone UV-spectrum, could not be assigned.

- The HPLC-fingerprints of *Sophora tonkinensis* and *Glycyrrhiza pallidiflora*-extracts detected also at 210 nm, did not show any relevant peak at all.

Fig. 5:

HPLC-fingerprint of the dichloromethane extract of *Sophora flavescens* sample 1 detected at UV 365 nm.

This fingerprint differs in its peak area from that of **Fig. 4**. The peak of Kurarinone (**3**) at 365 nm appears smaller in comparison to the greater peaks **5**,**7** and **8**.

Note: According to the Chinese Pharmacopoeia 2005[1] Radix Sophorae flavescentis should contain not less than 1.2 % of the total amount of matrine and oxymatrine, calculated with reference to the dried drug.

Conclusion

The TLC-chromatograms of *Sophora flavescens* root extract samples except of some quantitative deviation show a rather uniform zone pattern when detected with vanillin-sulphuric acid reagent. The *Sophora tonkinensis* sample did not contain the characteristic flavanones, chalcones and pterocarpan but showed only a small amount of the alkaloid Oxymatrine. *Glycyrrhiza pallidiflora* was devoid of both classes of compounds. In the HPLC-fingerprints of all *Sophora flavescens* extract samples 1-6 the characteristic flavanones, chalcones and pterocarpan were detectable at 210 and 365 nm. Neither *Sophora tonkinensis* nor *Glycyrrhiza pallidiflora* extract showed any relevant peaks which could be used for the authentity proof. For the HPLC-analysis of the alkaloids of *Sophora flavescens* another extraction procedure and HPLC-system has to be used.

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Radix Scutellariae – *Huangqin*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]: Baical Skullcap Root is the dried root of *Scutellaria baicalensis* Georgi (Fam. Lamiaceae). The drug is collected in spring or autumn, removed from rootlet and soil, dashed to peel the rugged outer bark after being sun-dried, and then dried thoroughly.

Origin[6,7]: Originated from the provinces Hebei and Shanxi; Inner Mongolia, Japan, Korea and Russia.

Description of the drug[1]: Conical, twisted, 8–15 cm long, 1–3 cm in diameter. Externally brownish-yellow or dark yellow, bearing sparse warty traces of rootlets, the upper part rough with twisted longitudinal wrinkles or irregular reticula, the lower part with longitudinal striations and fine wrinkles. Texture hard and fragile, easily broken, fracture yellow, reddish-brown in the centre; the central part of an old root dark brown or brownish-black, withered or hollowed. Odour, slight; taste, bitter.

Cultivar roots slender, mostly branched. Externally yellowish-brown, outer bark closely adhering to wood, with relatively thin longitudinal wrinkles. Fracture yellow or yellowish, slightly horny. Taste bitter.

Substitute drugs: Several other species of *Scutellaria* from other regions of China have been described as substitutes for *Scutellaria baicalensis*. These include *S. amoena, S. hypericifolia, S. likiangensis, S. rehderiana, S. tenax* and *S. viscidula.*

In Western Herbal Medicine, especially in USA and Canada the aerial parts (herb, leaves) of *Scutellaria lateriflora* are commonly used.

Pre-treatment of the raw drug[1]: The drug is removed from rootlet and soil, dashed to peel the rugged outer bark after being sun-dried, and then dried thoroughly.

a) Processed with water:

The dried root is boiled in water for 10 min. (or steamed for 30 min.) to soften thoroughly, then cut into thin slices and dried, protecting from strong sunlight. Occurring in sub rounded or irregular thin slices; externally yellowish-brown, cut surface yellowish-brown or yellowish-green, striated radially.

b) Processed with wine:

Radix Scutellariae is described under the method for stir-frying with wine to dryness. Occurring in sub rounded or irregular thin slices; externally brown; cut surface yellowish-brown, striated radially, showing less burnt patches, sometimes appearing brown in the centre.

Medicinal use[7]: In TCM to treat fever, nausea and vomiting, acute dysentery, jaundice, coughs, carbuncles and sores, and threatened abortion. In folk medicine to treat allergies, atherosclerosis, diarrhea, dermatitis and hypertension.

Effects and indications of Radix Scutellariae according to Traditional Chinese Medicine[1, 2, 3, 4]	
Taste:	bitter
Temperature:	cold
Channels entered:	*Orbis cardialis et intestini tenuis, Orbis pulmonalis et intestini crassi, Orbis felleus, Orbis stomachi.*
Effects (functions):	To remove *damp-heat,* quench fire and counteract *toxicity,* arrest bleeding, and prevent abortion.
Symptoms and indications:	Discomfort in the chest, nausea and vomiting in epidemic febrile diseases caused by *damp-heat* or *summer-heat;* feeling of stuffiness in the abdomen, acute dysentery or jaundice caused by *damp-heat;* cough due to heat in the lung; high fever with dire thirst; spitting of blood and epistaxis due to *heat* in blood; carbuncles and sores; threatened abortion.

Main constituents[5, 6, 7, 8, 9,10]:

Main flavonoids:

- Baicalin (Baicalein-7-O-glucuronide)
- Baicalein (5,6,7-trihydroxyflavone)
- Wogonoside (Wogonin-7-O-glucuronide)
- Wogonin (5,7-dihydroxy-8-methoxyflavone)
- Scutellarin (Scutellarein-7-O-glucuronide)
- Scutellarein (5,6,7-4’-tetrahydroxyflavone)
- Oroxyloside (Oroxylin-A-7-O-glucuronide)
- Oroxylin A (5,7-dihydroxy-6-methoxyflavone)

Minor flavonoids and other constituents:

- Viscidulin III-2'-O-glucoside
- Viscidulin III
- Chrysin-6-C-arabinosyl-8-C-glucoside
- Chrysin
- Skullcapflavone I and II
  (I = 5,2'-OH, 7,8-OCH_3-flavone;
  II = 5,2'-OH, 6,7, 8,5'-OCH_3-flavone)
- trans-Verbascoside
- trans-Martynoside
- 5,7,2',6'-Tetrahydroxyflavone
- Dihydro-Oroxylin A

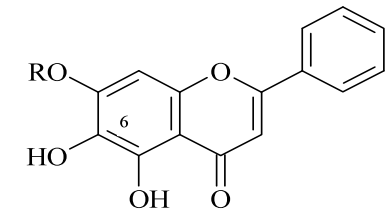
Note:

In the literature[8, 9] at least more than 20 other flavonoids and phenylpropan-derivates (*trans*-Martynoside, *trans*-Verbascoside) are described for the various *Scutellaria* species. Most of them described under "Minor flavonoids" occur in *Scutellaria* species which are listed as "substitute drugs" of *Scutellaria baicalensis.*

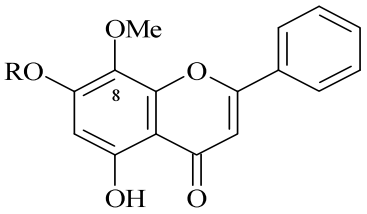
Trans-Martynoside, *trans*-Verbascoside, Viscidulin III-2'-O-glucoside, and Viscidulin III, primarily described as constituents of the herb of *Scutellaria lateriflora*, could be detected also in small amounts in the HPLC fingerprint of *Scutellaria baicalensis* root.

Pharmacology:

– ***in vitro* and *in vivo* effects:**
- anti-inflammatory activity[7,12,15,20]
- antihepatotoxic activity[7]
- antioxidant activity[7,12,15]
- antimicrobial activity[7,8,11]
- antitumor activity[7,8,19]
- antiviral activity[7,17,21,22]
- sedative and anxiolytic activity[7,14,16]
- inhibitory effect of neurotoxicity[13]
- platelet aggregation inhibition[7,8]
- anticonvulsant activity[7,9]
- inhibition of mast cell histamine release[18]



R = H: Baicalein
R = GlcA: Baicalein-7-O-glucuronide (Baicalin)



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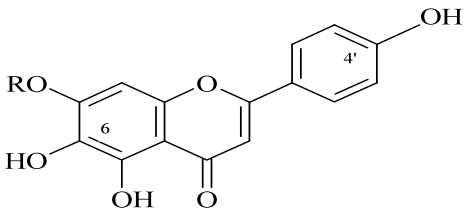
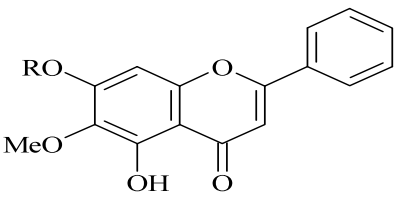
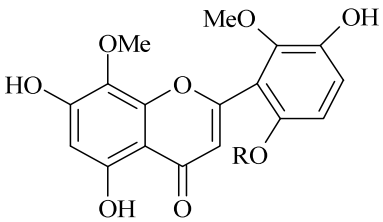


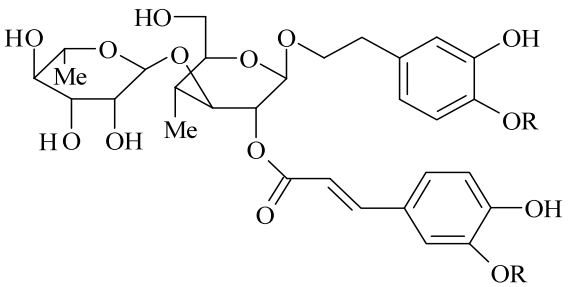
R = H: Scutellarein
R = GlcA: Scutellarin



R = H: Oroxylin A
R = GlcA: Oroxylin A-7-O-glucuronide (Oroxyloside)



R = Glc: Viscidulin III-2'-O-glc



R = H: trans-Verbascoside
R = Me: trans-Martynoside

Fig. 1: Formulae of the main compounds as detected in TLC or HPLC of Radix Scutellariae[8, 9]

TLC-fingerprint analysis:

1) Extraction[1]: 1 g powdered drug is extracted under reflux for 30 minutes with a mixture of 20 ml ethyl acetate and 10 ml methanol. After cooling and filtration the extract is evaporated to dryness. The residue is dissolved in 5 ml methanol and once more filtrated to use the solution for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Radix Scutellariae extracts: each 25 µl
reference compounds: each 25 µl

Solvent system: toluene : ethyl acetate : methanol : formic acid
10 3 1 2

Detection: Iron-III-chloride (1g in 100 ml EtOH)
After spraying the plate is heated at 100°C for 10 minutes.

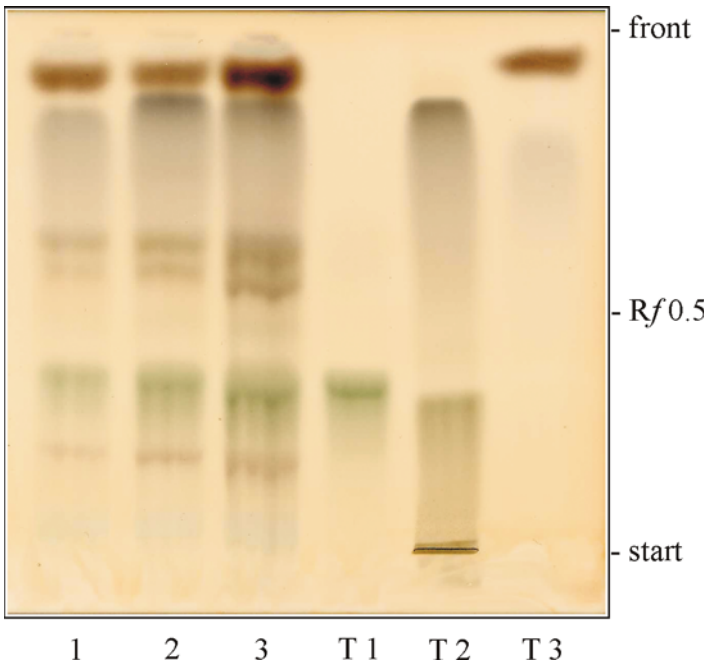


Fig. 2: Thin layer chromatogram of the methanol root extracts of *Scutellaria baicalensis* sprayed with Iron-III-chloride reagent (VIS)

4) Description:

All five extract samples show in VIS a very homogenous fingerprint pattern of green and violet brown zones respectively. In Fig. 2 only the samples 1, 2 and 3 are shown. Baicalin (**T1**), one main flavonolglycoside, shows its green zones at R*f* = 0.31. Scutellarin (**T2**) at R*f* = 0.28 is present only in a very low concentration and partly overlapped by Baicalin. The aglycone Baicalein together with Scutellarein appear at R*f* = 0.85. Directly above Baicalein lies Wogonin at R*f* = 0.94. In the middle R*f*-range between R*f* = 0.5 and 0.65, two main brownish zones, can be assigned to Oroxylin-A-7-glucuronide (Oroxyloside) and Wogonin-7-O-glucuronide (Wogonoside). In the deep R*f*-range at 0.2 to 0.25 a further red brown band might be identical with Chrysin-di-C-glycoside or Viscidulin III-2'-O-glucoside analogous to the peaks between Rt 4.0 and 10.3 in the HPLC-fingerprint (see Table 1).

Drug samples		**Origin**
1	Radix Scutellariae / *Scutellaria baicalensis*	Sample of commercial drug obtained from public pharmacy, Munich, Germany
2	Radix Scutellariae / *Scutellaria baicalensis*	Sample of commercial drug obtained from HerbaSinica, Germany (origin: Neimenggu, China)
3	Radix Scutellariae / *Scutellaria baicalensis*	Sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany
4	Radix Scutellariae / *Scutellaria baicalensis*	Sample of commercial drug obtained from China Medica, Germany
5	Radix Scutellariae / *Scutellaria baicalensis*	Sample from German cultivation obtained from China Medica, Germany
6	Radix Scutellariae / *Scutellaria baicalensis*	Sample of commercial extract obtained from China Medica, Germany
7	Radix Scutellariae / *Scutellaria baicalensis*	Province Anhui, China
8	Radix Scutellariae / *Scutellaria baicalensis*	Province Hebei, China

Reference compounds of Figure 2		**R*f***
T 1	Baicalin	0.31
T 2	Scutellarin / Baicalein (Scutellarein)	0.28 / 0.85
T 3	Wogonin	0.94

HPLC-fingerprint analysis[22,23,24,25]:

1) Sample preparation[1]: 1 g powdered drug is extracted under reflux for 30 minutes with a mixture of 20 ml ethyl acetate and 10 ml methanol. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 5.0 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Radix Scutellariae extracts: each 10.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 0.1 % H_3PO_4 (phosphoric acid, Merck / water, Millipore Ultra Clear UV plus® filtered) B: Methanol (VWR)
Gradient:	40 % B in 25 minutes 40–60 % B in 20 minutes 60 % B in 15 minutes total runtime: 60 minutes
Flow:	1.0 ml/min.
Detection:	276 nm

Table 1: Retention times of the main peaks

Peak	Rt (min.)	Compound
1	~ 4.1	Viscidulin III-2'-O-glc*
2	~ 6.5	Chrysin-6-C-ara-8-C-glc*
3	~ 8.6 / ~ 9.6 ?	*trans*-Verbascoside*
4	~ 10.3	Viscidulin III*
5	~ 20.6	Baicalin
6	~ 28.5 /~ 31.5?	*trans*-Martynoside*
7	~ 33.6	Oroxylin A-7-O-glucuronide*
8	~ 36.2	Wogonin-7-O-glucuronide (Wogonoside)*
9	~ 46.4	Baicalein*
10	~ 54.4	Wogonin
11	~ 59.3	Oroxylin A*

* identified analogous to the HPLC-analysis of American Skullcap[9], the herb of *Scutellaria lateriflora*, which contains Baicalin as dominant flavonoid and small amounts of Oroxylin- and Wogonin-7-glucuronide.

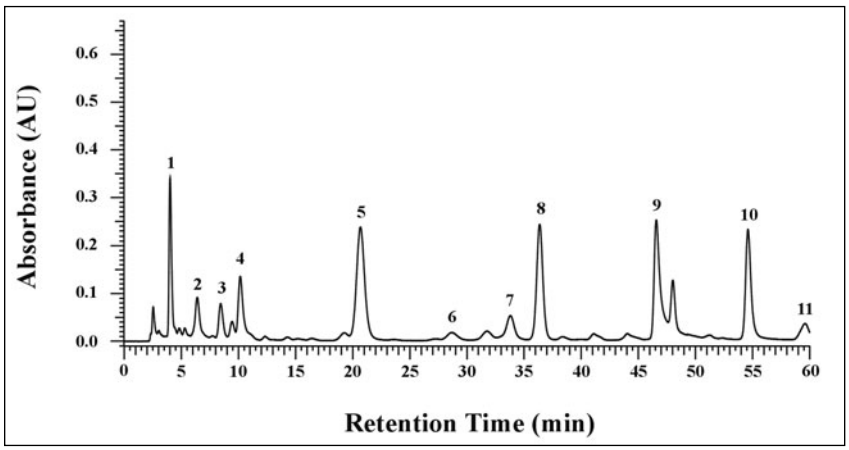


Fig. 3a: HPLC fingerprint of *Scutellaria baicalensis* root extract sample 1

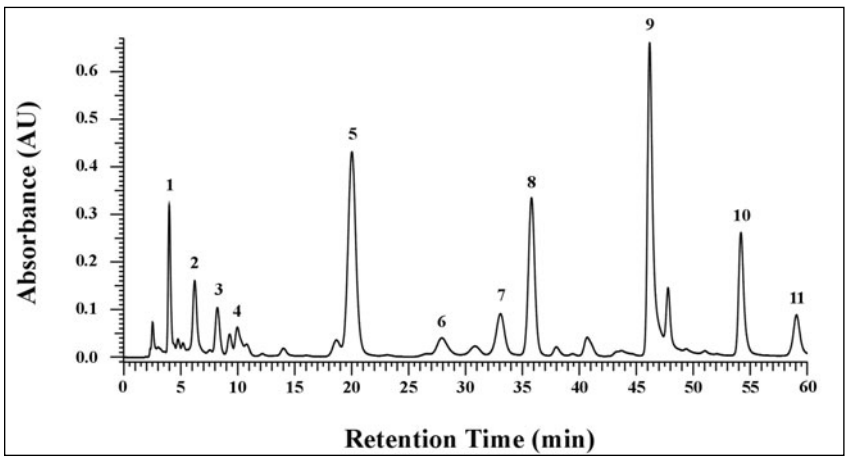


Fig. 3b: HPLC fingerprint of *Scutellaria baicalensis* root extract sample 2

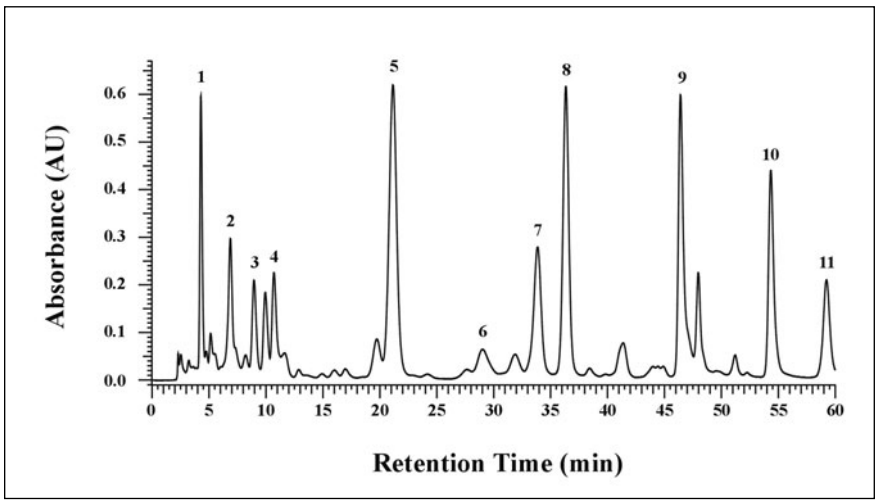
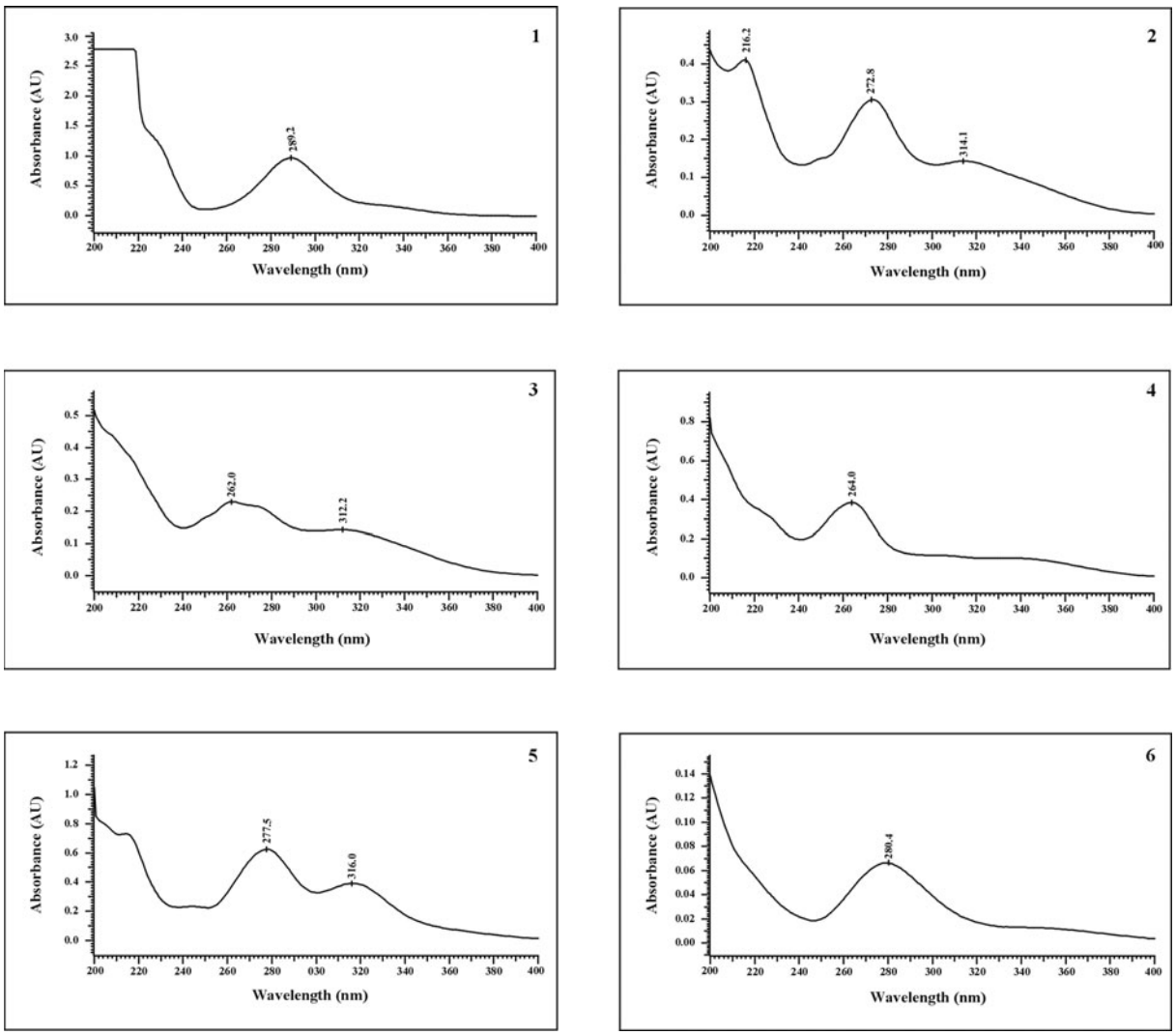


Fig. 3c: HPLC fingerprint of *Scutellaria baicalensis* root extract sample 3



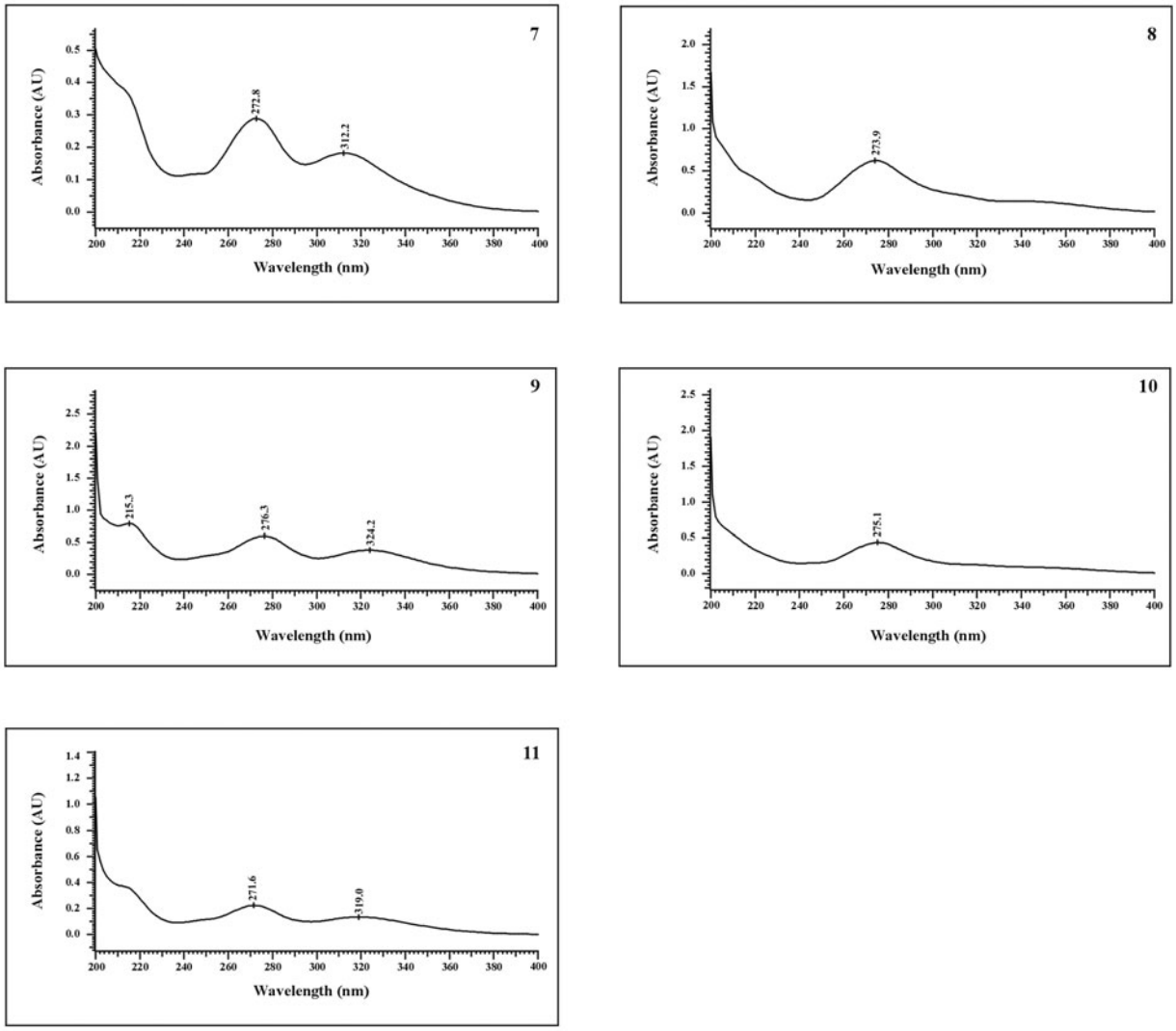


Fig. 4: On line UV-spectra of the main characteristic peaks of the HPLC-fingerprint of the root extracts of *Scutellaria baicalensis*

4) Description of the HPLC:

All three *Scutellaria* root extract samples recorded at 276 nm show a very identical pattern of 11 main peaks: The detected flavones are Baicalin (peak **5**), Wogonin-7-O-glucuronide (peak **8**), Baicalein (peak **9**) and Wogonin (peak **10**). The other peaks **1** – **4**, **6**, **7** and **11** could be identified according to the publication of Zhang et al.[9] who used a very similar HPLC-system for the HPLC-fingerprint-analysis of the American Skullcap, the herb of *Scutellaria lateriflora*. The on line UV-spectra are in agreement with those of Zhang's publication.

Note: The Chinese Pharmacopoeia 2005 describes a content not less than 8 % of baicalin calculated with reference to the dried drug as determined by HPLC.

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Fructus Chaenomelis – *Mugua*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I (2005)

Official drug[1]: The fruits of *Chaenomeles speciosa* (Sweet) Nakai

– Rosaceae –

Origin: China (provinces of An Hui, Zhe Jiang, Hu Bei and Sichuan)

Synonyms[3]: *Chaenomeles speciosa* Bartl, *Cydonia japonica var. lagenaria* Loisel.

Description of the drug[1,2]: The dried fruits of *Chaenomeles speciosa* are cut to longitudinal oblong strips, 4–9 cm long, 2–5 cm wide and 1–2.5 cm thick, purplish-red to reddish-brown. The strips possess irregular, deep wrinkles. The edges of cut are rolled inwards. The pulp is reddish-brown, the central part is dented, brownish-yellow. Seed long-triangular, mostly falling off. The texture is hard. It has a slightly aromatic odour and sour taste.

Pretreatment of raw drug[1,2]: The collected greenish-yellow fruits are blanched and boiled in water until the exocarp becomes greyish-white in colour, halved longitudinally and dried in the sun.

Medicinal use[4,5]: In Traditional Chinese Medicine it is used for the treatment of rheumatism, arthritis, chronic cough and diarrhea.

It is also used for arthritis with ankylosis, aching and heaviness sensation of the loins and knees as well as for systremma due to vomiting and diarrhea, edema and weakness of the legs.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications according to Traditional Chinese Medicine[4,5]	
Taste:	sour
Temperature:	slightly warm
Channels entered:	liver and spleen
Functions:	- promotes blood circulation in the channels and collaterals - relaxes muscles and tedons - dispels dampness and regulates stomach function
Indications:	- convulsions and spasms - painful and swollen legs with irritability - wind-damp obstruction syndrome manifested as rheumatic pain, numbness of limbs and joint pain

Main constituents[6,7,8,9] (see Fig. 1):

- **pentacyclic triterpenoic acids** (oleanolic acid, maslinic acid, 3*β*-O-Acetylursolic acid, 3*β*-O-Acetylpomolic acid, pomolic acid, tormentic acid, euscaphic acid)
- *β*-sitosterol, campesterol
- saponins and flavones without known structures
- longchain saturated alcohols
- longchain saturated paraffines
- linoleic acid
- ascorbic acid, fumaric acid, citric acid, malic acid, tartaric acid, sorbitol

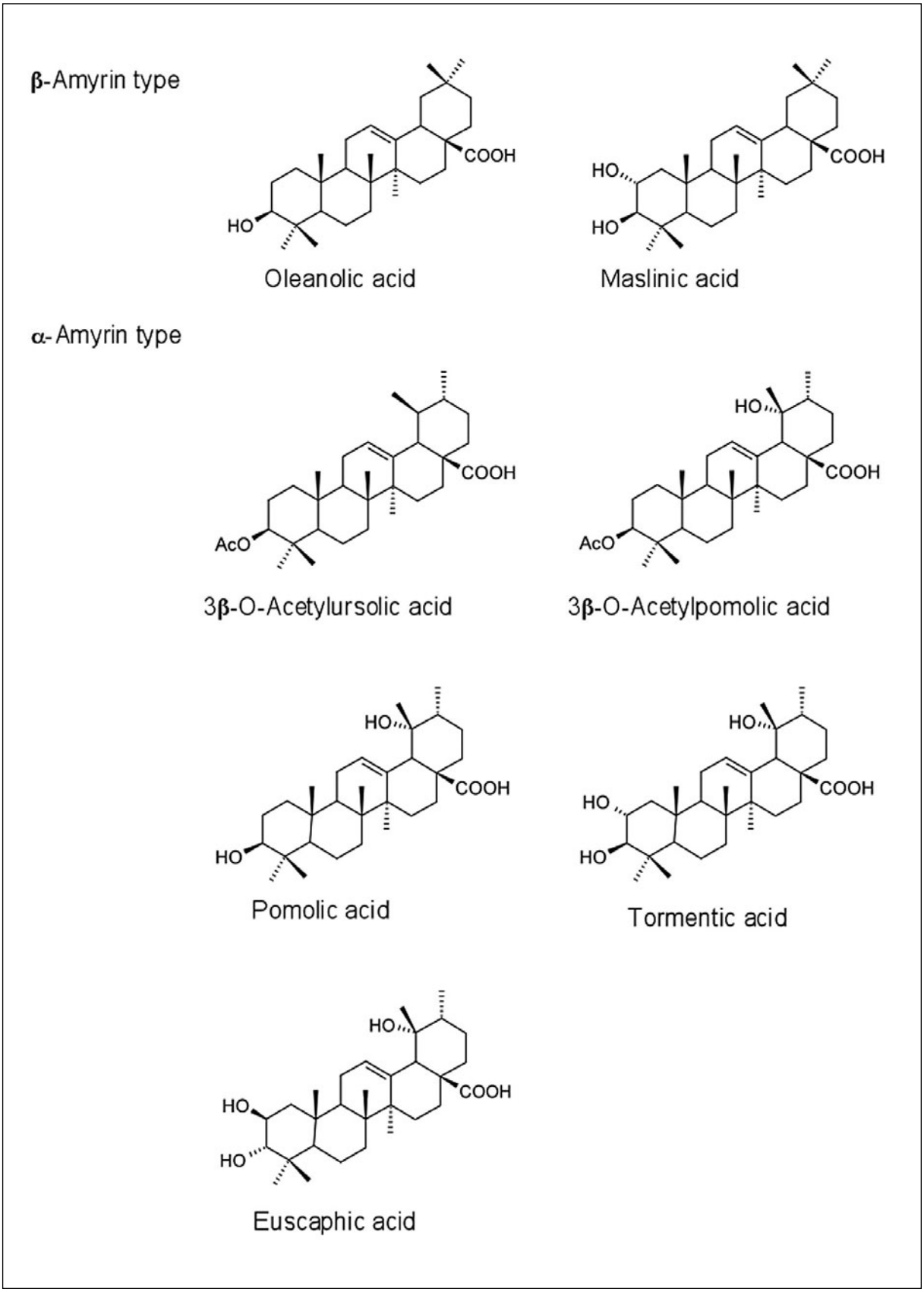

Fig. 1: Formulae of the main triterpenoid acids of Fructus Chaenomelis

Pharmacology[4,6,10]:

In vitro effects:	COX-1, -2 inhibition, antibacterial effect
In vivo effects:	immunomodulatory effects, lowering spleen-index, inhibition phagocytosis activity of macrophages
	antiinflammatory effects: inhibition of protein induced mice paw edemas

TLC fingerprint analysis:

1) Extraction:	2 g powdered drug are extracted with 10 ml 90 % ethanol under reflux for 10 minutes. The filtrate is evaporated to about 5 ml.
2) Reference compounds:	oleanolic acid, pomolic acid, euscaphic and tormentic acid, 3β-O-acetylursolic acid, 3β-O-acetylpomolic acid, β-sitosterol and campesterol, nonacosan-10-ol (each 1 mg dissolved in 1 ml ethanol p.a.)
3) Separation parameters:	
Applied amount:	15 µl extract, 10 µl standard solutions
Plates:	Silicagel 60 F_{254}, Merck
Solvent system:	Toluene / ethyl acetate / glacial acetic acid (80 : 15 : 5)
Direct evaluation:	UV 254 nm and UV 365 nm
Spray reagent:	Vanillin-sulphuric acid reagent
	(1.0 g vanillin dissolved in a mixture of 85 ml methanol, 10 ml glacial acetic acid and 5 ml conc. sulphuric acid in this order).
	The plate is sprayed (approx. 20 ml) and heated for 5–10 min. at 100–105 °C. The evaluation is carried out in VIS.

4) Description of the TLC-chromatograms (Fig. 2 and Fig. 3):

Tab. 1: Origin and date of supply of different commercial drug samples of Chaenomelis fructus

Drug sample	Origin	Date
1	TCM-Hospital Bad Kötzting	August 1997
2	TCM-Hospital Bad Kötzting	November 1998
3	TCM-Hospital Bad Kötzting	December 1998
4	Pharmacy Beijing	April 1999

Tab. 2: R*f*-values and colour after detection of different reference substances with vanillin sulphuric acid (see Fig. 2)

Compound		R*f*	Vanillin-sulphuric acid-reagent (VIS)
Oleanolic acid	T1	0.50	blue/violet
Pomolic acid	T2	0.47	light blue
Euscaphic acid / Tormentic acid	T3	0.24/0.27	light blue
3β-O-Acetylursolic acid	T4	0.63	reddish-violet
3β-O-Acetylpomolic acid	T5	0.71	bluish-violet
Nonacosan-10-ol	T6	0.99	light violet
β-Sitosterol / Campesterol	T7	0.49	purple
Linoleic acid	T8	0.60	light red violet with slightly tailing

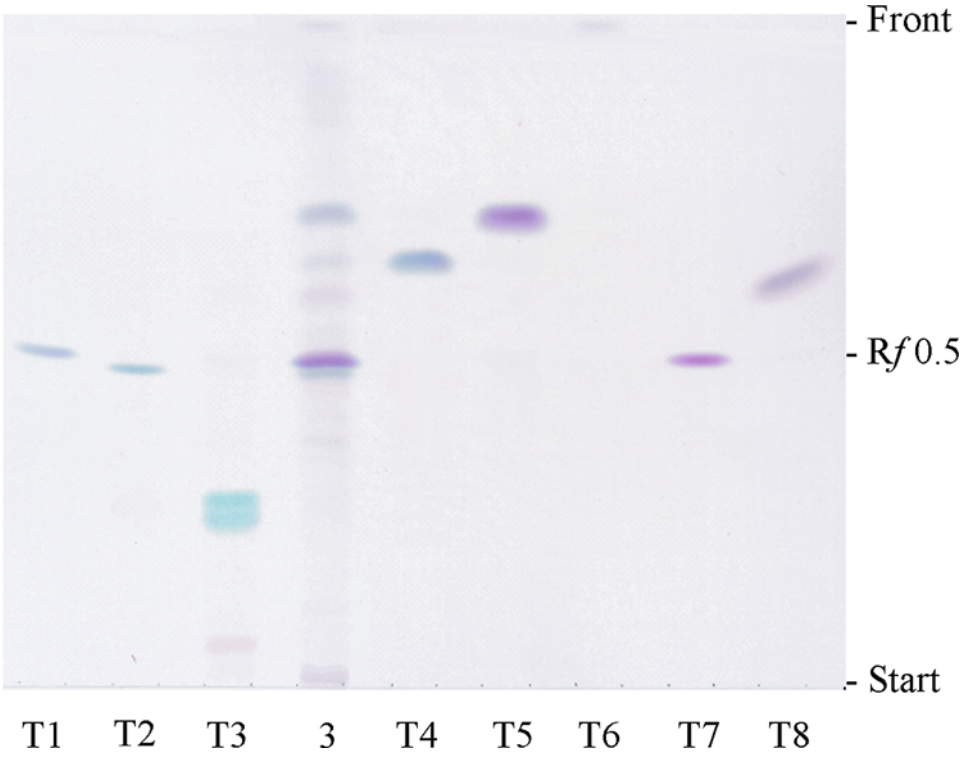

Fig. 2: Thin layer chromatogram of the reference compounds of Fructus Chaenomelis

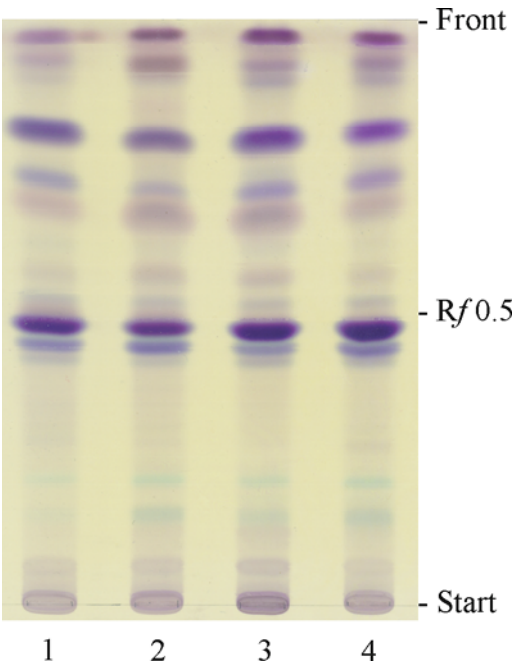

Fig. 3: Thin layer chromatogram of different Fructus Chaenomelis samples (origin see Tab. 1)

The TLC-fingerprint of the Fructus Chaenomelis samples 1–4 is very homogeneous and differs only in the concentrations of the single compounds. In all drug samples oleanolic acid (R*f* 0.50 = **T1**) appears as the major zone. In this range also the phytosterols (R*f* 0.49 = **T7**) and the small zone of pomolic acid (R*f* 0.47 = **T2**) can be detected. 3β-O-acetylursolic acid is the second

significant zone (R*f* 0.63 = **T4**). The zones of the paraffines and longchain alcohols (nonacosan-10-ol) appear at the front (R*f* 0.99 = **T6**). 3β-Acetylpomolic acid (R*f* 0.71 = **T5**) is apparent as a violet zone. Linoleic acid (= **T8**) is detected at R*f* 0.60 as a slightly tailing spot with red-violet colour. The colours of the spots can differ dependent on the concentrations of the compounds and the heating temperature. The hydroxylated triterpenoic acids (euscaphic and tormentic acid) exhibit zones with light blue colour (R*f* 0.24/0.27 = **T3**).

HPLC fingerprint analysis:

1) Sample preparation: see TLC fingerprint analysis

2) Injection volume: 15 µl (conc. 1 mg/ml in ethanol p.a.)

3) HPLC data:

Apparatus:	Liquid chromatograph HP 1050, Photodiode array detector HP 1040 M, Hewlett Packard
Column:	LiChroCART® 125-4 with LiChrospher® 100 RP 18 (5 µm), Merck
Pre-column:	LiChroCART® 4-4 with LiChrospher® 100 RP 18, Merck
Solvent system:	A: water
	B: acetonitrile
Gradient:	20–30 % B in 8 min., 30–60 % B in 2 min., 60–95 % B in 30 min., 95 % B for 5 min.
	total runtime: 45 minutes
Flow:	1.0 ml/min.
Detection:	200 nm

Retention times of the main peaks:

Peak	Rt [min.]	Compound
1	13.3	Euscaphic acid
2	15.7	Pomolic acid
3	17.1	Maslinic acid
4	23.1	3β-O-Acetylpomolic acid
5	25.7	Oleanolic acid
6	32.1	3β-O-Acetylursolic acid

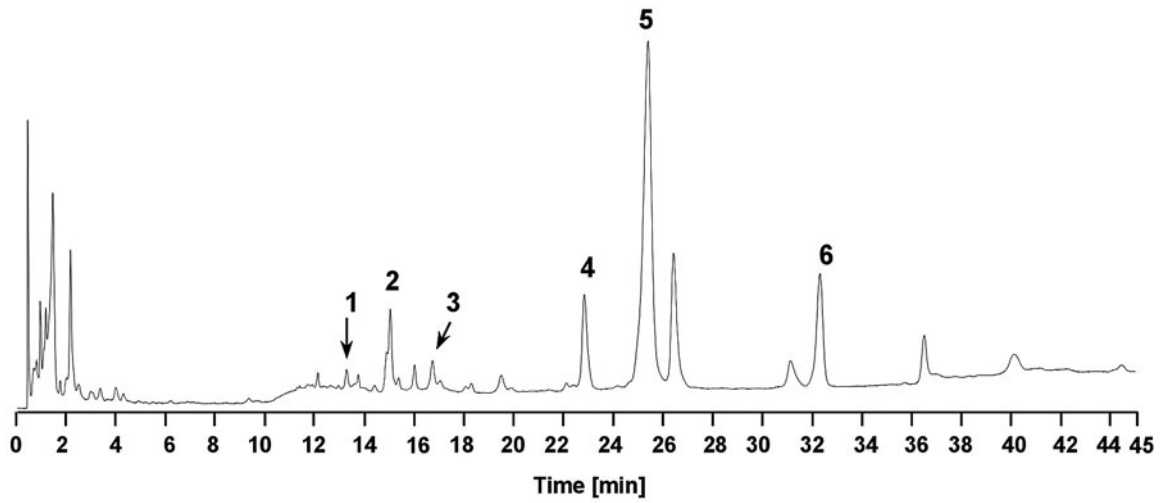

Fig. 4: HPLC fingerprint analysis of commercial drug sample 3 (*Chaenomeles speciosa*)

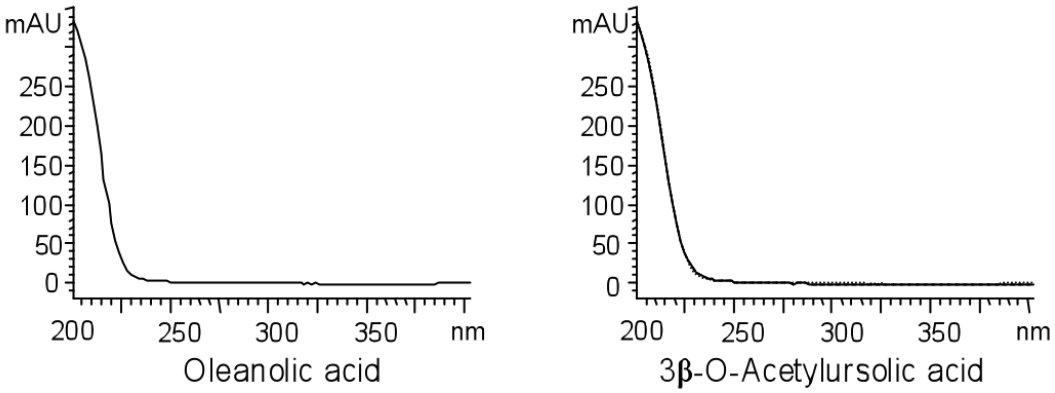

Fig. 5: UV spectra of oleanolic acid and 3β-O-acetylursolic acid

Description of the HPLC-Chromatogram:

The HPLC-chromatogram is dominated by the peak of oleanolic acid (peak **5**). 3β-O-acetyl-ursolic acid is the second conspicuous peak (peak **6**). All other compounds are present only in minor concentrations. All triterpenoid acids show the same UV-spectrum with end absorption. Between Rt 12 min. and 19 min. other compounds are eluted along with the triterpenoid acids **1**,**2** and **3**.

The HPLC chromatograms of different drug batches showed the same peak pattern, and differed only in the concentrations of compounds. A simultaneous and direct determination of the different acids inclusive vitamin C by HPLC was described in J. of Chromatography[11].

Discussion:

Ethanol extracts of Fructus Chaenomelis showed a very homogeneous and significant pattern of constituents. Since all major constituents of the ethanolic extract of Fructus Chaenomelis did not possess a chromophor, a detection with vanillin-sulphuric acid reagent is necessary for TLC and

a low detection wavelength for HPLC (200 nm). The main compound of the extracts is oleanolic acid. Its concentration in the extracts usually is so high that it precipitates in the cold.

Because oleanolic acid is very common in plants, it is necessary to use the whole spectrum of hydrophilic triterpenoic acids for identification. In TLC-analysis euscaphic and tormentic acid, in HPLC-analysis especially pomolic and 3β-O-acetylpomolic acid can be used for the authentication of Fructus Chaenomelis.

RAPD and isoenzyme analysis have been successfully applied for the discrimination of *Chaenomeles speciosa*, *C. japonica*, *C. thibetica* and *C. cathayensis*[12].

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Rhizoma Acori calami – *Zangchangpu*
Rhizoma Acori tatarinowii – *Sichangpu*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]:

Rhizoma Acori calami * (engl. = seeflag)

Rhizoma Acori calami is the dried rhizome of *Acorus calamus* L. (Fam. Araceae). The drug is collected in autumn and winter, removed from fibrous roots and soil and dried in the sun.

Rhizoma Acori tatarinowii *

Rhizoma Acori tatarinowii is the dried rhizome of *Acorus tatarinowii* Schott (syn. *Acorus gramineus*) (Fam. Araceae). The drug is collected in autumn and winter, removed from fibrous roots and soil, and dried in the sun.

* The existence of polyploidy races and sub varieties are described under "Constituents".

Origin[2]: Southern China, Japan, India (Himalaya area)

Description of the drugs[1]:

Rhizoma Acori calami

Compressed-cylindrical, slightly curved, 4–20 cm long, 0.8–2 cm in diameter. Externally grayish-brown to brown, nodes distinct, internodes 0.5–1.5 cm long, with longitudinal wrinkles, one surface with dense and rounded remains of fibrous roots; leaf scars obliquely triangular, arranged alternately, stem-base remains at the lateral surface surrounded by remains of scaly leaf bases and hairy fibrous roots. Texture hard, fracture pale brown, an endodermis ring distinct, numerous brown dotted oil cells visible. Odor, strong and characteristic; taste, pungent.

Rhizoma Acori tatarinowii

Compressed-cylindrical, frequently tortuous, often branched, 3–20 cm long, 0.3–1 cm in diameter. Externally brown or grayish-brown, rough, with uneven annulations, internodes 2–8 mm long, with fine longitudinal wrinkles, one surface with remains of fibrous roots or rounded root scars; leaf scars triangular, arranged alternately, some with hairy and scaly remains of leaf bases. Texture hard, fracture fibrous, whitish or reddish, an endodermis ring distinct, numerous dotted vascular bundles and brown oil cells visible. Odour, aromatic; taste, bitter and slightly pungent.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Pretreatment of the raw drug[1]:

Rhizoma Acori calami
Foreign matters are eliminated, cut into slices, and dried.

Rhizoma Acori tatarinowii
Foreign matters are eliminated, washed clean, softened, cut into thick slices and dried.

Medicinal use[4,5,6]: Dyspeptic disorders, anorexia, diarrhea, externally ointment for rheumatic pains, as gargling for angina.

Effects and indications of Rhizoma Acori calami according to Traditional Chinese Medicine[1,2,3,5]	
Taste:	sharp
Temperature:	warm
Channels entered:	*orbis cardialis, orbis hepaticus*
Effects (functions):	to warm the stomach, counteract inflammation and alleviate pain
Symptoms and indications:	declined *yang* of stomach, indigestion, stagnated food, diphtheria and anthracnose, etc.

Effects and indications of Rhizoma Acori tatarinowii according to Traditional Chinese Medicine[1,2,3,5]	
Taste:	acrid, sharp
Temperature:	warm
Channels entered:	*orbis cardialis, orbis stomachi*
Effects (functions):	to eliminate dampness and phlegm, whet appetite, and restore consciousness and improve intelligence
Symptoms and indications:	Stuffiness sensation in the epigastrium with anorexia; severe dysentery with total loss of appetite; impairment of consciousness in epilepsy; forgetfulness and impaired hearing

Main constituents[6-9]: - **essential oil with phenylpropan derivatives*)**
β-asarone (*cis*-Isoasarone)
α-asarone (*trans*-Isoasarone)
1,2-dimethoxy-4-(2-propenyl)-benzene
1,2-dimethoxy-4-(1-propenyl)-benzene
cis-methyl-isoeugenol, *trans*-methyl-isoeugenol, eugenol, methyl chavicol, safrole, iso-shyobunone, acorone, acorenone, gramenone, asarylaldehyde
(see the detailed composition in Table 1 and 2 of p. 8 + 9).

- **terpenoids**
camphene, e-*β*-ocimene, *β*-farnesene, *β*-sesquiphellandrene, calarene, α-selinene, elemicine, caryophyllene, acoradiene, cedrene, α-patchoulene, eucalyptol, ɾ-cadinol, terpinen-4-ol, linalool
(see the detailed composition in Table 1 and 2 of p. 8 + 9).

- **other constituents**
polysaccharides (starch, mucilages)

- **tannins**

***)** Note: The chemical composition of the essential oil of *Acorus* species, inclusive *β*-Asarone varies depending on the grade of polyploidy of the various *Acorus* cytotypes, sub varieties and/or species[9,10].

The Calamus roots of plants belonging to the diploid race (var. *americanus*) (2x = 24) are reported to contain ~ 2 – 6 % essential oil with no or only traces (0.2 %) of *β*-Asarone[11]. The oil of the European *Acorus* var. *calamus,* determined as triploid race (3x = 36), possesses 3 – 13 % *β*-Asarone content[9]. The *Acorus* species of East Asian origin is defined as a tetraploid race and reported with *β*-Asarone contents up to 82 %[12].

In the Chinese Pharmacopoeia 2005 the roots of two Calamus species, the roots of *Acorus calamus* and *Acorus tatarinowii,* are described. For Rhizoma Acori calami not less than 2.0 % (ml/g) volatile oil is demanded, but the *β*-Asarone content is not specified. For Rhizoma Acori tatarinowii, not less than 1.0 % (ml/g) essential oil also without specification of the *β*-Asarone content is described.

***Acorus calamus* L.**	**Origin**	**n**	**essential oil (%)**	***β*-Asarone[13] content (%)**
var. *americanus* WULFF	USA	diploid	2 – 6	0 – 0.5
var. *calamus* L.	Europe	triploid	2 – 6	3 – 13
var. *angustata* ENGLER	East Asia	tetraploid	up to 7	up to 80

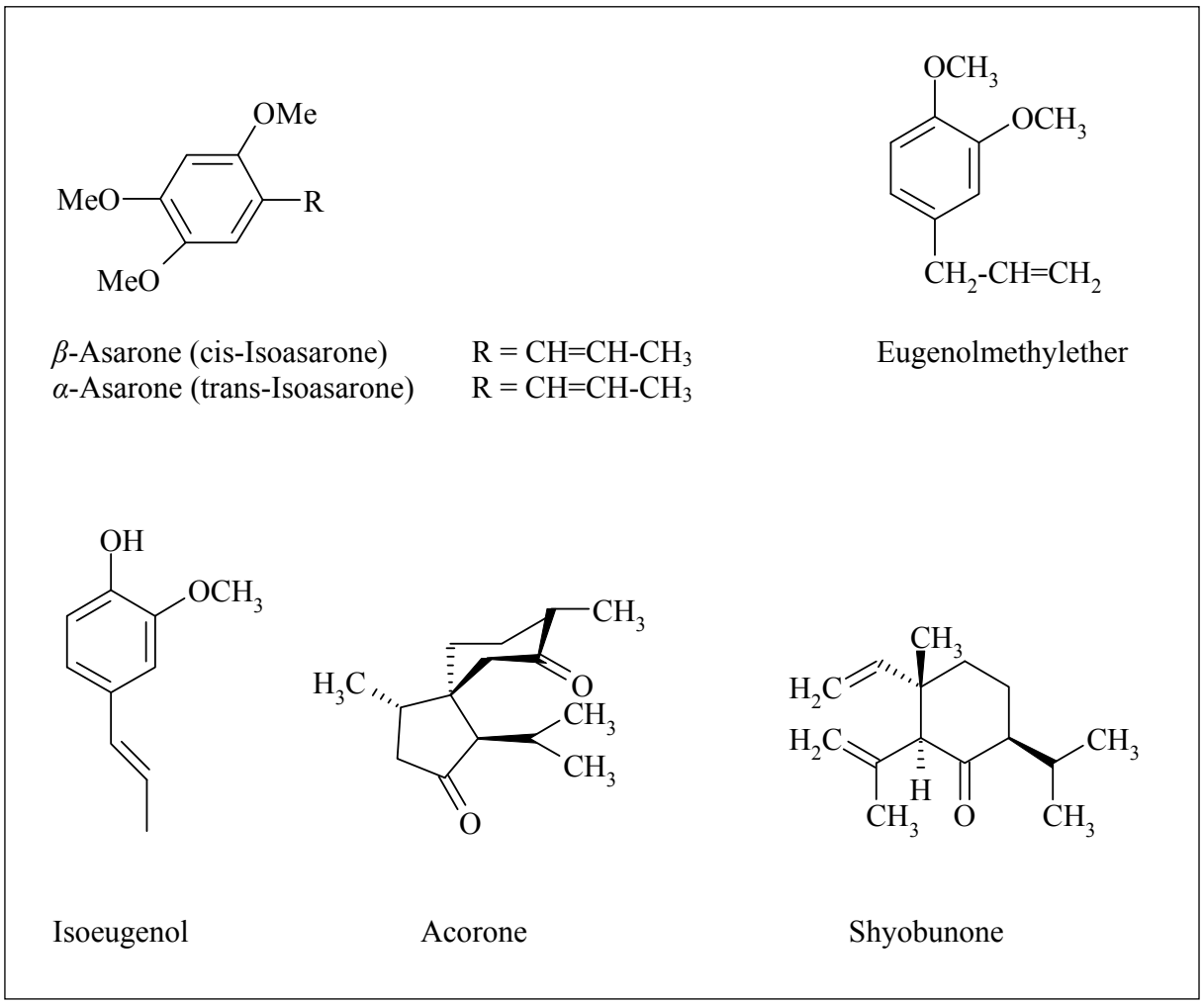

Fig. 1: Formulae of the main compounds of Rhizoma Acori calami/tatarinowii

Pharmacology:

- CNS-activity:
  sedative, antidepressant → *β*-Asarone[16,18,19,20];
  antiepileptic → *α*-Asarone[21]
- antiasthmatic[14]
- spasmolytic[15,16]
- cardiovascular[16,17]
- antimicrobial[22,23]
- hypolipidemic[24]

Toxicology:

Among the constituents of the essential oil of *Acorus* species *β*-Asarone is the most characteristic component and considered as potential carcinogenic[25,26,27], mutagenic[28] and immunosuppressive[29]. (see Note, p. 3)

TLC- fingerprint-analysis:

	Drug samples	Origin
1	Rhizoma Acori calami /*Acorus calamus* L. → toluene extract	sample of commercial drug obtained from Galke, Germany
2	Rhizoma Acori calami /*Acorus calamus* L. → toluene extract	province Sichuan, China
3	Rhizoma Acori tatarinowii /*Acorus tatarinowii Schott* → toluene extract	sample of commercial drug obtained from HerbaSinica, Germany
4	Rhizoma Acori tatarinowii /*Acorus tatarinowii Schott* → toluene extract	province Jiangsu, China
5	Rhizoma Acori calami /*Acorus calamus* L. → essential oil	sample of commercial drug obtained from Caelo, Germany
6	Rhizoma Acori tatarinowii /*Acorus tatarinowii Schott* → essential oil	sample of commercial drug obtained from Caelo, Germany

Reference compounds of Figure 2a		R*f*
T 1	*β*-Asarone	0.36
T 2	Isoeugenol	0.41

1) Extraction:

I. Toluene extracts:

1 g powdered drug is extracted by shaking 15 minutes with 10 ml dichloromethane. The suspension is filtered and the clear filtrate evaporated to dryness. The residue is dissolved in 1 ml toluene.

II. Water steam distillation:

100 g powdered drug are distilled for 3 hours. For TLC the essential oil is diluted 1 : 10 with Xylene.

III. Water extracts (decoction):

1 g powdered *Acorus calamus* and *Acorus tatarinowii* are extracted by boiling with 20 ml water for 20 minutes. Both extracts are cooled, filtered and shaken twice with 20 ml ethyl acetate. The water phases are discarded, the ethyl acetate phases combined and evaporated to dryness on a Büchi rotary evaporator.

The residues are dissolved in 1 ml ethanol.

2) Reference compounds: 0.5 mg each is dissolved in 0.5 ml of methanol

3) Separation parameters:

Applied amount: *Acorus calamus/tatarinowii*:
- toluene extracts (see Extraction I.): 15 µl each
- essential oils (see Extraction II.): 5 µl each
- water extracts (see Extraction III.): 15 µl each

reference compounds (*β*-Asarone/Isoeugenol):
10 µl each of a 1:500 diluted ethanolic solution

Plate: HPTLC-plate Silicagel 60 F_{254}; Merck

Solvent system: Toluene : Ethyl acetate
93 : 7

Detection: Anisaldehyde-sulphuric acid reagent

0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The TLC plate is sprayed with about 10 ml reagents, heated at 105 °C for 10 minutes and evaluated in VIS.

The reagent has only limited stability and is no longer useable when the color has turned to red-violet.

4) Description

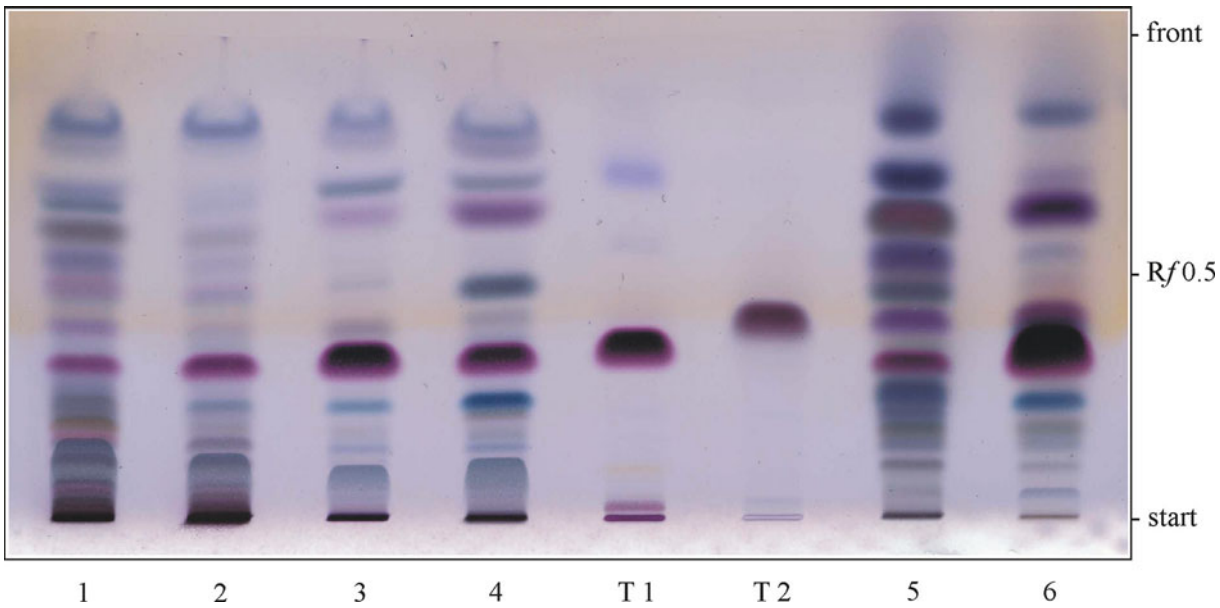

Fig. 2a: Thin layer chromatogram of the toluene extracts samples 1 - 4 and essential oils (5 + 6) of *Acorus calamus* and *A. Tatarinowii* sprayed with anisaldehyde-sulphuric acid reagent (VIS)

The toluene extracts of the roots of *Acorus calamus* (samples 1 + 2) and *Acorus tatarinowii* (samples 3 + 4) show the dominant violet-red bands of *β*-Asarone at R*f* = 0.36 (**T1**). It is visible that the *β*-Asarone content of *Acorus tatarinowii* is much higher than that of *Acorus calamus.* In all four samples Isoeugenol (R*f* = 0.41 (**T2**)) is present in relatively low concentration. In the R*f* – range above *β*-Asarone up to the solvent front most of the phenylpropan-derivatives appear together with the main monoterpenes (e.g. Camphene or *β*-Pinene). In the R*f* – range below *β*-Asarone the terpene alcohols (e.g. linalool or terpineol) can be found. In the essential oils of both *Acorus*-species (samples 5 + 6) obtained by water steamed distillation all major compounds, especially those in the upper R*f* – range, are present in much higher concentration than in the toluene extracts. In *Acorus tatarinowii* oil *β*-Asarone, Methylisoeugenol and at R*f* = 0.63 a highly concentrated phenylpropan derivative, probably Methyleugenol appear as dominant compounds. A great difference between *Acorus calamus* and *Acorus tatarinowii* is the qualitative and quantitative composition of the phenylpropan derivatives and terpenoids in the R*f* – range above *β*-Asarone up to the solvent front (see also the corresponding Rt – ranges in the GC/MS-fingerprints).

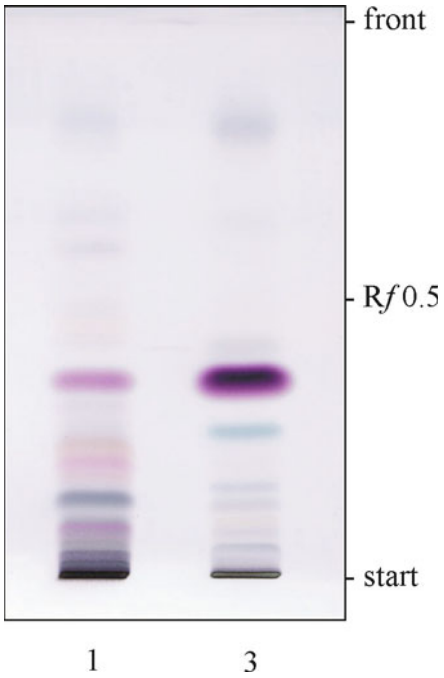

Fig. 2b: Thin layer chromatogram of the water phases of *Acorus calamus* (1) and *A. Tatarinowii* (3) obtained by boiling for 20 min. with water, followed by ethyl acetate extraction and after TLC spraying with anisaldehyde-sulphuric acid reagent (VIS)

In both water extracts of *Acorus calamus* (sample 1) and *Acorus tatarinowii* (sample 3) *β*-Asarone could be still detected.

GC-MS analysis[8,35–38]:

1) Sample preparation: 100 g powdered drug are distilled in a neo-Clevenger apparatus for 3 hours and the essential oil obtained diluted 1 : 100 with *tert* butylmethylether.

2) Injection volume: *Acori calami/Acori tatarinowii* oil : 1.0 µl each

3) GC-MS data:

Apparatus:	Varian GC 3800 Varian Saturn 2200 (El/Cl, ms^n) ion trap-mass spectrometer Autosampler: CTC CombiPal
Separation column:	Varian VF-5ms with 10 m precolumn (deactivated methyl-polysiloxan)
Carrier gas:	Helium (99.9990 %), split ratio 1 : 50, flow rate 1.2 ml/min.

Temperature program:	60 °C for 2 min. 10°C/min. to 160 °C 12 °C/min. to 310 °C Total runtime: 24.5 min. Injector Temperature: 270 °C
MS-parameter:	Temperature of the ion trap: 200 °C Manifold-Temperature: 50 °C Transfer Line-Temperature: 270 °C Total Ion Count: between m/z = 70 and m/z = 400 Ionization El AGC with 20 µA filament current, 70 eV ionization energy

4) Results:

Table 1: Chemical Composition of both essential oils:

Essential oil	Compounds	Areas	Relative content of the determinable compounds in the Phenyl ether fraction
Acorus calamus (sample 5)	Methyleugenol	2337	0.3 %
	Methylisoeugenol	159579	22.2 %
	Elemicin	n.d.*	–
	Isoelemicin	21079	2.9 %
	α-Asarone	n.d.*	–
	β-Asarone	536266	74.6 %
Acorus tatarinowii (sample 6)	Methyleugenol	5234	0.0 %
	Methylisoeugenol	481325	4.6 %
	Elemicin	7649	0.1 %
	Isoelemicin	113158	1.1 %
	α-Asarone	16575	0.2 %
	β-Asarone	9868000	94.1 %

* below the detection limit

Table 2: Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	5.41	+Camphene
2	5.83	*β*-Pinene
3	6.51	+Cymene
4	7.03	Terpinene
5	7.62	Linalool
6	8.47	Campher
7	8.93	Terpinen-4-ol
8	9.13	+*α*-Terpineol
9	10.40	Isobornylacetat
10	11.84	+Azulen
11	11.92	Methyleugenol
12	12.30	Cedren
13	12.43	+Gurjunene
14	12.60	Methylisoeugenol
15	13.10	Shyobunone
16	13.35	Shyobunone-isomer
17	13.54	+Copaene
18	13.73	Calacorene
19	14.09	8,14-Cedranoxid
20	14.16	Spathulenol
21	14.40	*β*-Asarone
22	14.48	+Isolongifolenepoxid
23	14.76	Dehydroxy-Isocalamendiol
24	15.03	+Isoelemicin
25	15.19	1,8-Dimethyl-4-(1-methylethyl)-spiro[4.5]
26	15.33	Azulenone
27	17.29	Palmitic acid C16:0
28	18.67	+Linoleic acid C18:2(9,12)

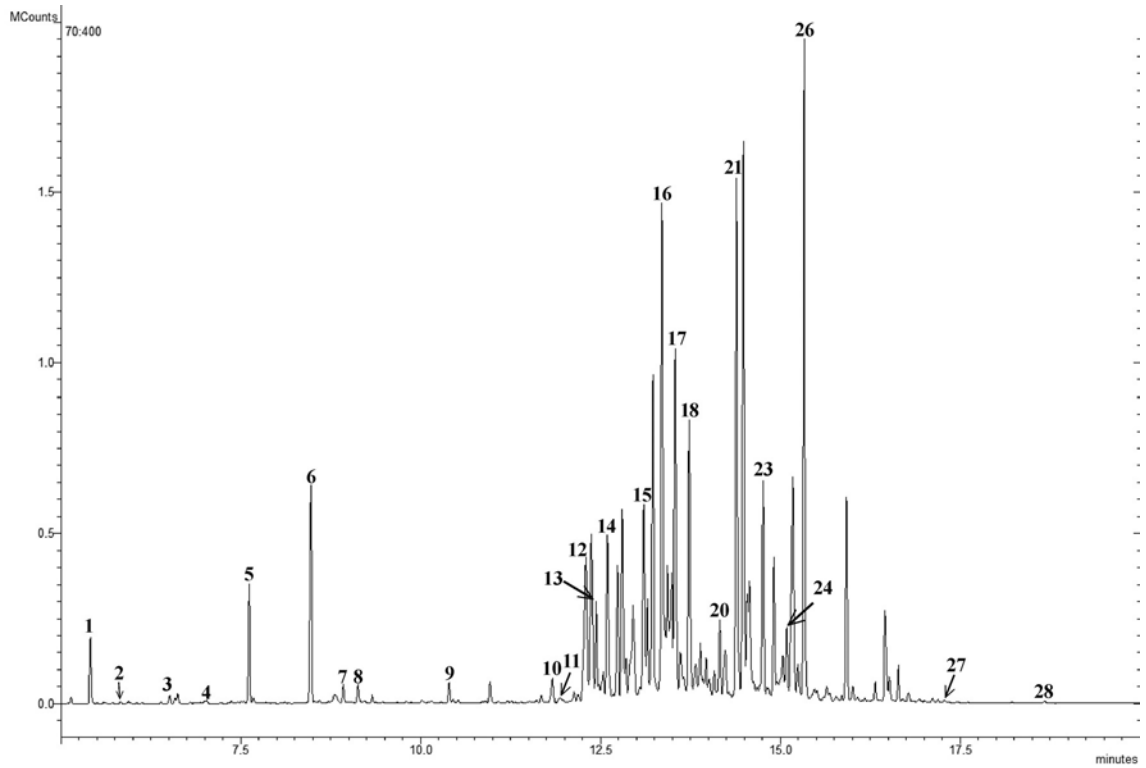

Fig. 3a: GC-fingerprint of the essential oil of *Acorus calamus* sample 5

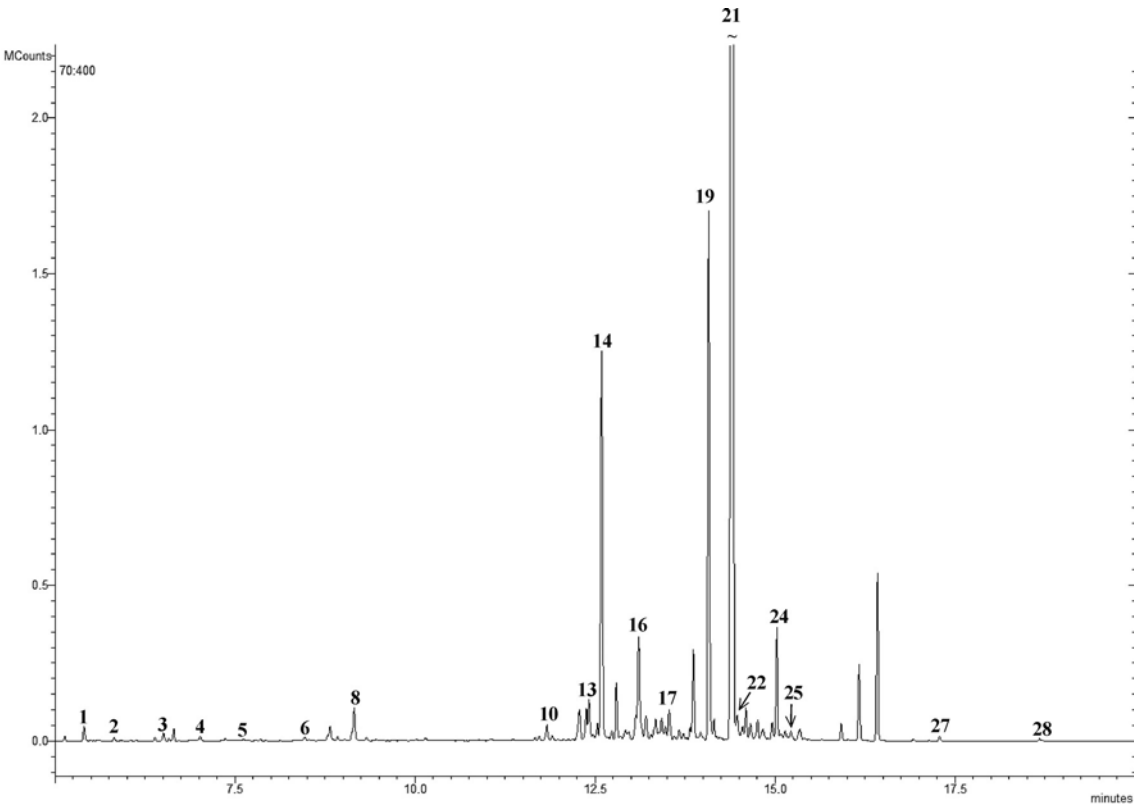

Fig. 3b: GC-fingerprint of the essential oil of *Acorus tatarinowii* sample 6

5) Description of Fig. 3a) and 3b)

As shown in the GS-MS-analysis of the distilled essential oil of *Acorus calamus* root (sample 5) and *Acorus tatarinowii* root (sample 6) 28 phenylpropan-derivatives and terpenoids inclusive traces of fatty acids could be detected.

The chemical composition of the oil of *Acorus calamus* was more complex than that of *Acorus tatarinowii,* however the *β*-Asarone (peak **21**) content of *Acorus tatarinowii* with a content of 475 mg/ml essential oil was much higher than that of *Acorus calamus* with a content of ~ 60 mg/ml. The other differences between both oils refer to the different content of Shyobunone, +Copaene, Calacorene, 8,14-Cedranoxid (peaks **15**–**19**) and Azulenone (peak **26**). Obvious differences could be seen also in the Rt – range of Rt = 5.0–8.0 corresponding with the major terpenehydrocarbons and monoterpenalcohols. In *Acorus calamus* oil (sample 5) *α*-Asarone was not detectable and in *Acorus tatarinowii* (sample 6) only in very small amounts (~ 0.2 %).

Quantification of *β*-Asarone and *α*-Asarone:

Several methods are described in the literature to quantify the *β*-Asarone content in the rhizomes and the essential oil of *Acorus calamus* and *Acorus tatarinowii* species and their cytotypes.[7,11,30-34]

In a publication of Deng et al. (2004), the carcinogenic *β*-Asarone and the non-carcinogenic but antiepileptic *α*-Asarone of the oils were reported as 44.76 % and 29.65 %, respectively. These high concentrations could be not confirmed through investigations of our samples.

Conclusion

- The identification and quality proof of all Rhizoma Acori extracts with their essential oils can be easily carried out using TLC and GC/MS with the methods described.
- Despite of the very lipophilic nature of *β*-Asarone it could be determined also in the water decoction. This means that a potential risk in administering *Acorus calamus* or *Acorus tatarinowii* as decoction cannot be excluded.
- The contradictory reports about the content of *β*-Asarone and *α*-Asarone in *Acorus calamus* and *Acorus tatarinowii* rhizomes can be cleared only through detailed cytogenetic (chromosomal) analyses[8].

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Radix Isatidis – *Banlangen*

Pharmacopoeias[1]: Pharmacopoeia of the People's Republic of China, English Edition 2005, Volume I
Japanese Pharmacopoeia, English Edition 1996 (Jap. XIII)

Official Drugs[1,2]: According to the Chinese Pharmacopoeia:
The dried root of *Isatis indigotica* Fort. – Brassicaceae – .
The dried roots of *Isatis tinctoria* L., Brassicacae, and *Baphicacanthus cusia* (Nees) Brem., Acanthaceae, are sometimes also used as Banlangen.

Synonyms: English: woad root, isatis root
Japanese: banrankon

Description of the drug[1,3]: Cylindrical, slightly tortuous, 10–20 cm long, 0.5–1 cm in diameter. Externally grayish-yellow or brownish-yellow, wrinkled longitudinally and lenticellate horizontally, with rootlets or rootlet scars. Root stock slightly expanded exhibiting dark green or dark brown petiole-bases arranged in whorls and dense tubercles. Texture compact and soft, fracture yellowish-white in bark and yellow wood. Odor: slight; taste: sweetish, then bitter and astringent.

Pretreatment of raw drug[1]: After elimination of the foreign matter the drug has to be washed, softened thoroughly, cut into thick slices and dried.

Medicinal use[2,4-7]: In modern Chinese medicine the herb is used for treating a wide variety of viral infections, and in combination with other herbs for febrile and inflammatory diseases, mumps, pain and swelling in the throat, herpes, acute tonsillitis, laryngitis, diphtheria, measles, influenza, pneumonia, trachoma, arthritis and also for infectious acute hepatitis and encephalitis B.

Effects and indications according to Traditional Chinese Medicine[1,2,8-10]	
Taste:	Sweetish, then bitter and astringent
Temperature:	cold
Channels entered:	heart, lung and stomach
Effects:	- It drains heat. - It reduces heat in blood. - It relieves fire toxicity. - It soothes and benefits the sore throat.
Symptoms, Indications:	Eruptive epidemic diseases with dark red or purplish tongue; mumps; pharyngitis; laryngitis; scarlet fever; erysipelas; carbuncles.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents (see Fig. 1):

– **indolyliden-compounds:**

– indigo, indigotin, indirubin, indican, isatan A and B[11,12]

– isaindigotidione and isaindigotone[13,14]

– sulphur-containing epigoitrin and 2-hydroxy-3-butenyl thiocyanate[11,15,16]

Minor constituents:

– tryptanthrin (indolo-[2,1-*b*]-quinazoline-6,12-dione), 2,5-dihydroxyindole, 2,3-dihydro-4-hydroxy-2-oxoindole-3-acetonitrile and indole-3-acetonitrile 6-*O*-D-glucopyranoside[17-19]

– β- and γ-sitosterol and several amino acids[11,12]

– (E)-3-(3',5'-dimethoxy-4'-hydroxy-benzylidene)-2-indolinone[5,20], quinazoline-2,4-dione[21]

– 2,3-dihydro-1*H*-pyrrolo[2,1-*c*][1,4]benzo-diazepine-5,11(10*H*,11a*H*)-dione[5,20]

– 4-(4-hydroxy-3,5-dimethoxyphenyl)-3-buten-2-one[5,20]

– 3-(2'-Carboxyphenyl)-4(3*H*)-quinazolinone 5,7 and 3-(2'-Hydroxyphenyl)-4(3*H*)-quinazolinone[14]

– isolariciresinol and lariciresinol derivatives, 3-formylindole, 1-methoxy-3-indolecarb-aldehyde, 1-methoxy-3-indoleacetonitrile[22]

– 2-aminobenzoic acid, syringic acid, benzoic acid, salicylic acid and qingdainone[5,11,20]

– sphingolipids[23]

– neohesperidin, liquiritigenin and isoliquiritigenin[24]

Pharmacology[4,5]:

In vitro effects:

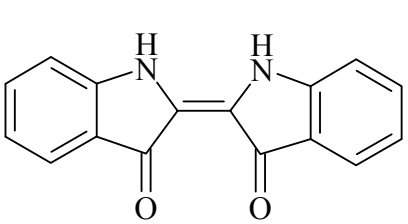
– anti-nociceptive, anti-inflammatory and antipyretic effects[27]

– antibacterial activity: *Staphylococcus aureus, Diplococcus pneumoniae, alpha Streptococcus, Hemophilus influenzae, Escherichia coli, Salmonella thyphi, Salmonella enteritidis, Shigella dysenteriae* and *Shigella flexneri*[2,5,28,29]

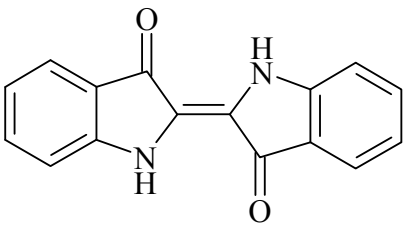
– antiviral activity: influenza virus strains PR_8 and JK_{68-1} both *in vitro* and in chicken embryo[5,28]; human cytomegalovirus (HCMV AD169)[30]

– antiparasitic effect: Leptospirosis[2]

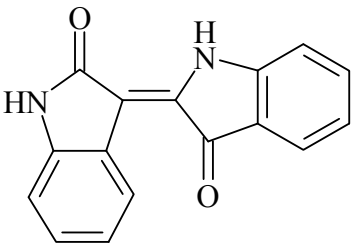
– anti-endotoxic activity: In the limulus amoebocyte lysate (LAL) test, 3-(2'-carboxyphenyl)-4(3*H*)-quinazolinone, 2-aminobenzoic acid, syringic acid, benzoic acid and salicylic acid exhibited significant anti-endotoxic activities[5,20], anti-inflammatory and antihypertensive properties (quinazoline-2,4-dione)[21]



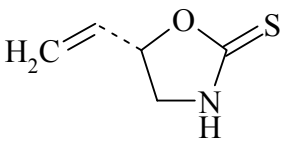
Indigo



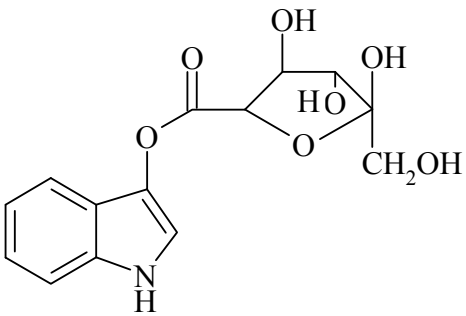
Indigotin



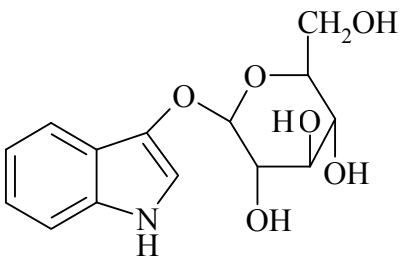
Indirubin



Epigoitrin



Isatan B



Indican

Fig. 1: Formulae of the main constituents[25,26]

In vivo effects:

– Indirubin exhibits a moderate inhibitory activity against transplanted tumors in animals[11]

– Indirubin inhibits Lewis lung carcinoma in mice and Walker carcinosarcoma 256 in rats, but not leukemia L 7212 or P 388 in mice[11]

– Polysaccharides extracted from the root of *Isatis indigotica* increase the weight of spleen and number of white blood cells and lymphocytes in peripheral blood of normal ICR *mice* and antagonize the immunosuppressive actions of hydrocortisone[31]

– The drug increases blood flow, improves microcirculation and lowers blood pressure, reduces capillary permeability and reduces oxygen consumption of the heart muscle[4]

TLC and HPLC fingerprint analysis:

Drug samples		**Origin**
1	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2003 I)
2	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1999)
3	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1996)
4	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1995)
5	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1994)
6	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1990)
7	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from HerbaSinica, Germany (origin: Anhui, China)
8	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2007)
9	Radix Isatidis / *Isatis indigotica* Fort.	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2003 II)

Reference compounds of Figure 2a		**R*f***
T1	Indigo	0.91
T2	Indirubin	0.87

TLC fingerprint analysis:

1) Extraction: 5.0 g powdered drug are extracted under reflux with 50 ml ethanol on a water bath for 60 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 1ml ethanol.

2) Reference compounds: Indirubin: 0.1 mg is dissolved in 1.0 ml acetonitrile[32];
Indigo: 0.1 mg is dissolved in 1.0 ml chloroform.

3) Separation parameters:

Applied amount: Radix Isatidis extracts: 10 µl each
reference compounds: 30 µl each

Plates: HPTLC Silica gel 60 F_{254}, Merck

Solvent system: chloroform : ethyl acetate : methanol
4 : 3 : 2

Spray reagents: a) Vanillin-sulphuric acid reagent: 0.5 ml vanillin, 85 ml methanol, 10 ml glacial acetic acid and 5 ml conc. sulphuric acid are mixed in this order.

b) Van Urk reagent: 0.2 g of 4-dimethylaminobenzaldehyde is dissolved in 100 ml 25 % hydrochloric acid with the addition of one drop of 10 % iron-III-chloride solution.

The plates are sprayed with approx. 10 ml of reagent a) and heated at 105°C, or with reagent b) without heating. The evaluation is carried out in a) VIS or b) UV366 nm.

4) Thin layer chromatograms and descriptions:

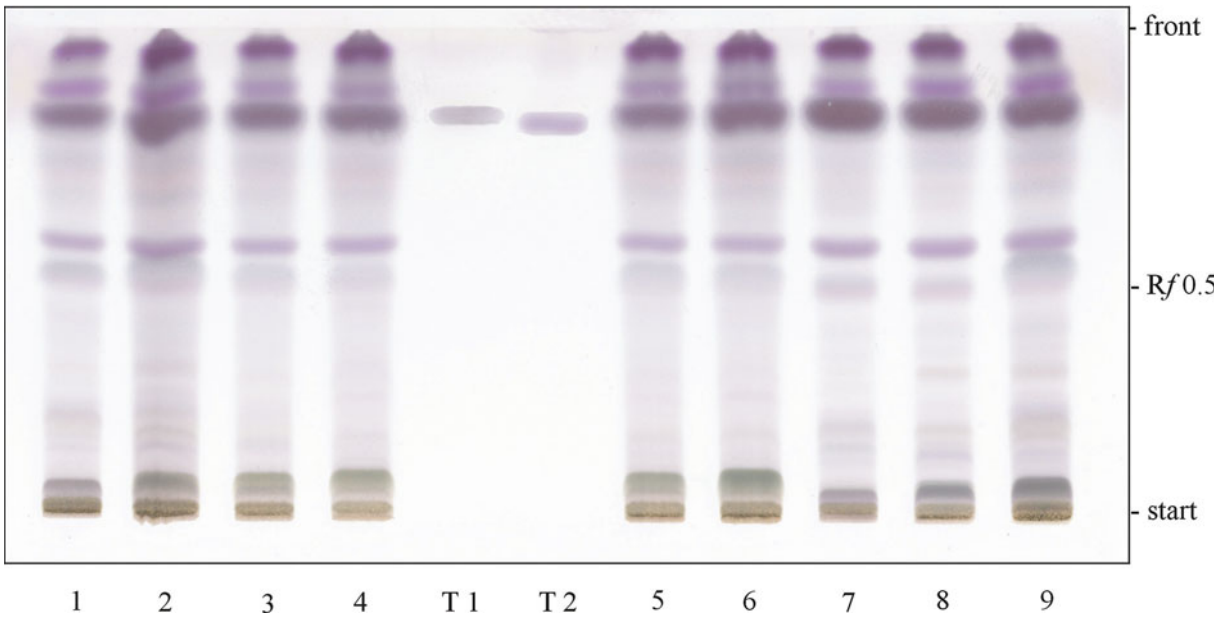


Fig. 2a: TLC sprayed with vanillin-sulphuric acid reagent in VIS

All Radix Isatidis samples (1 – 9) show a very homogeneous pattern of violet zones mainly in the R*f*-range of 0.8 up to the R*f*-front and in the R*f*-range of 0.45 – 0.65. Above the start appear brown-violet bands of polymeric substances. The characteristic constituents of Radix Isatidis, Indirubin and Indigo appear overlapped in the violet zone at R*f* = 0.89 (**T1**/**T2**).

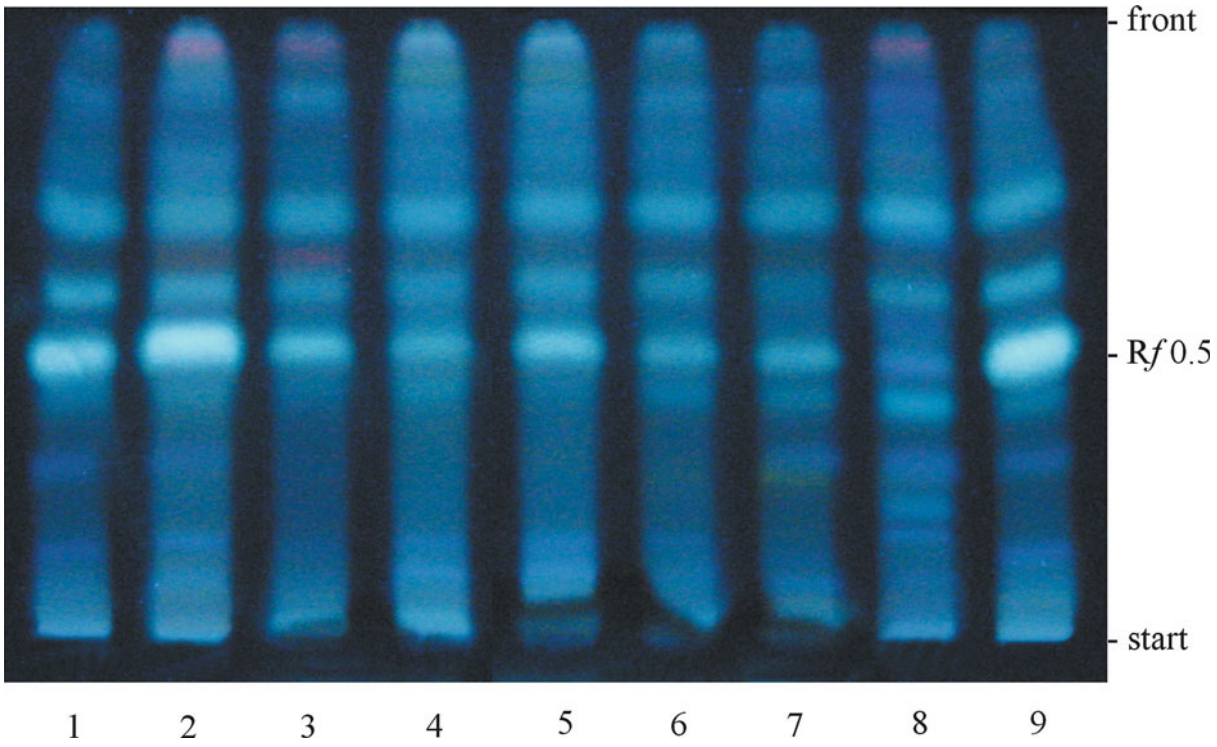


Fig. 2b: TLC sprayed with van Urk reagent in UV 366 nm

The Radix Isatidis samples 1 – 9 show with this reagent again a very homogeneous pattern of four characteristic light blue fluorescent zones at R*f* = 0.95, 0.75, 0.65 and 0.50. For the assignments of these bands no reference compounds were available. Indirubin and Indigo give no visible quenching zones in the same R*f*-range as shown in Fig. 2a.

HPLC fingerprint analysis[33]:

1) Sample preparation: The extract used for TLC is filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

   Reference compounds:
   Indirubin: 0.1 mg is dissolved in 1.0 ml acetonitrile[32];
   Indigo: 0.1 mg is dissolved in 1.0 ml DMSO.

2) Injection volume: Radix Isatidis extracts and reference compounds: 60 µl each

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent:	A: water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	5–45 % B in 45 minutes 45–100 % B in 20 minutes total runtime: 65 minutes
Flow:	1.0 ml/min.
Detection:	280 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	12.9	not identified
2	36.8	not identified
3	38.4	not identified
4	39.7	not identified
5	43.2	not identified
6	51.0	Indigo
7	54.0	Indirubin
8	58.9	not identified
9	59.5	not identified
10	61.1	not identified

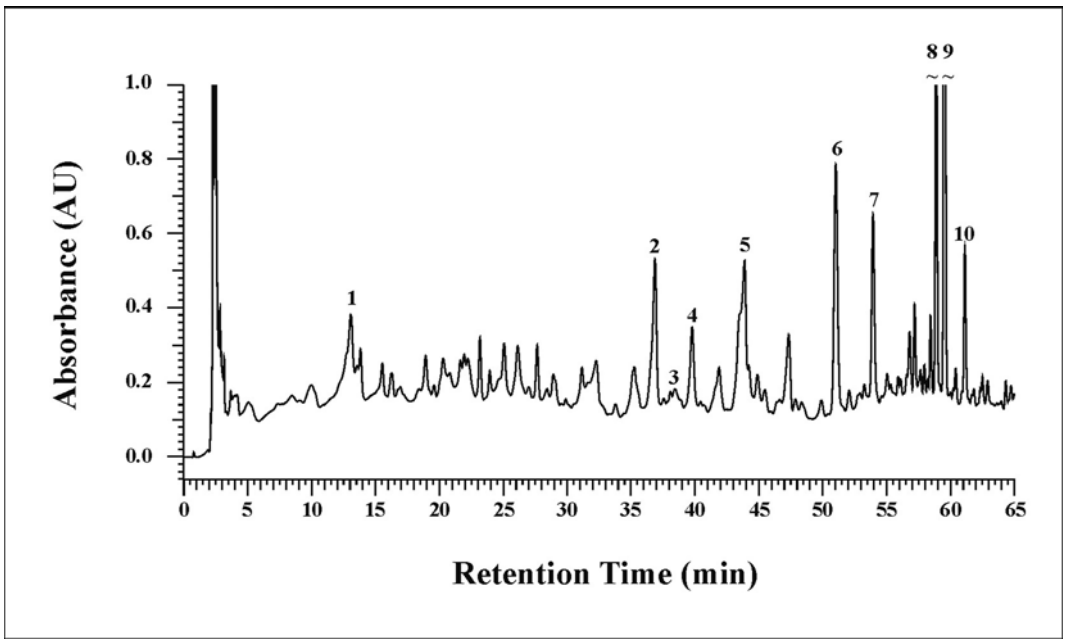


Fig. 3a: HPLC fingerprint of sample 2, Radix Isatidis

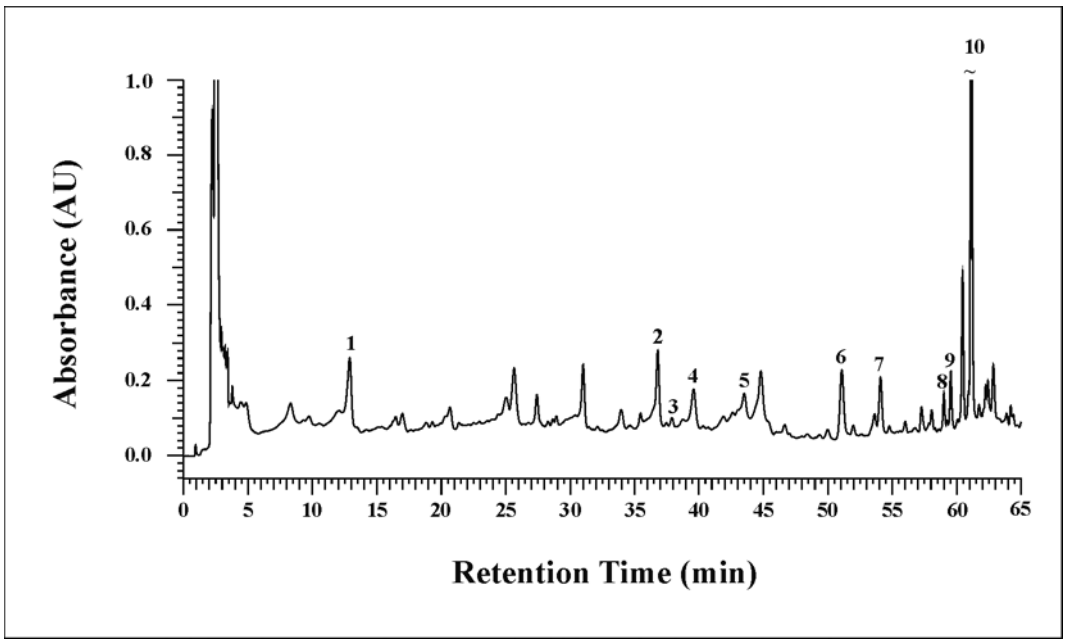


Fig. 3b: HPLC fingerprint of sample 8, Radix Isatidis

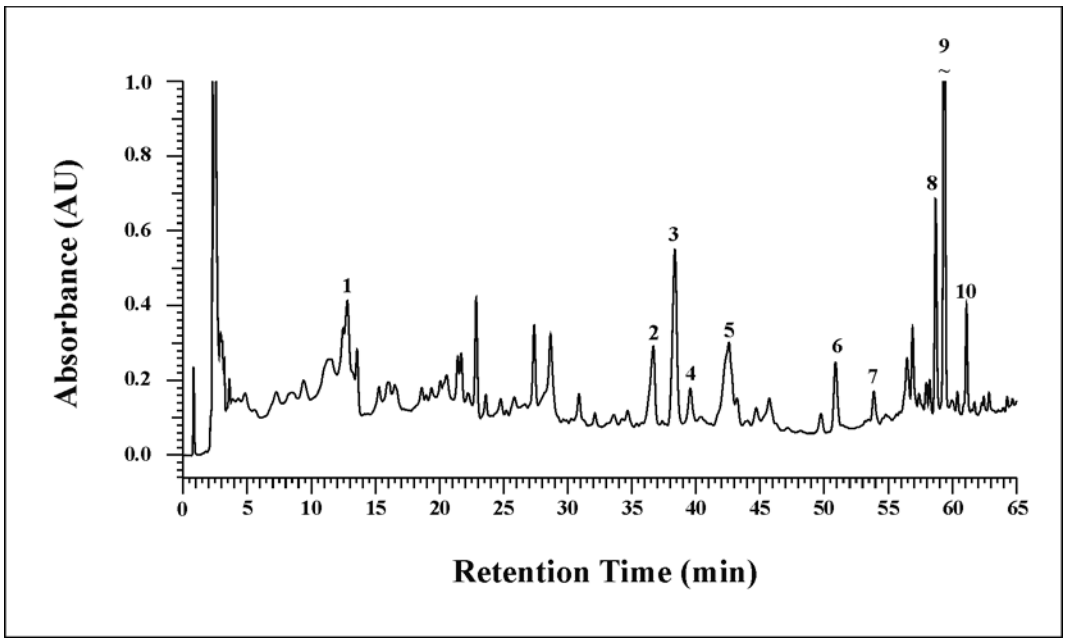


Fig. 3c: HPLC fingerprint of sample 9, Radix Isatidis

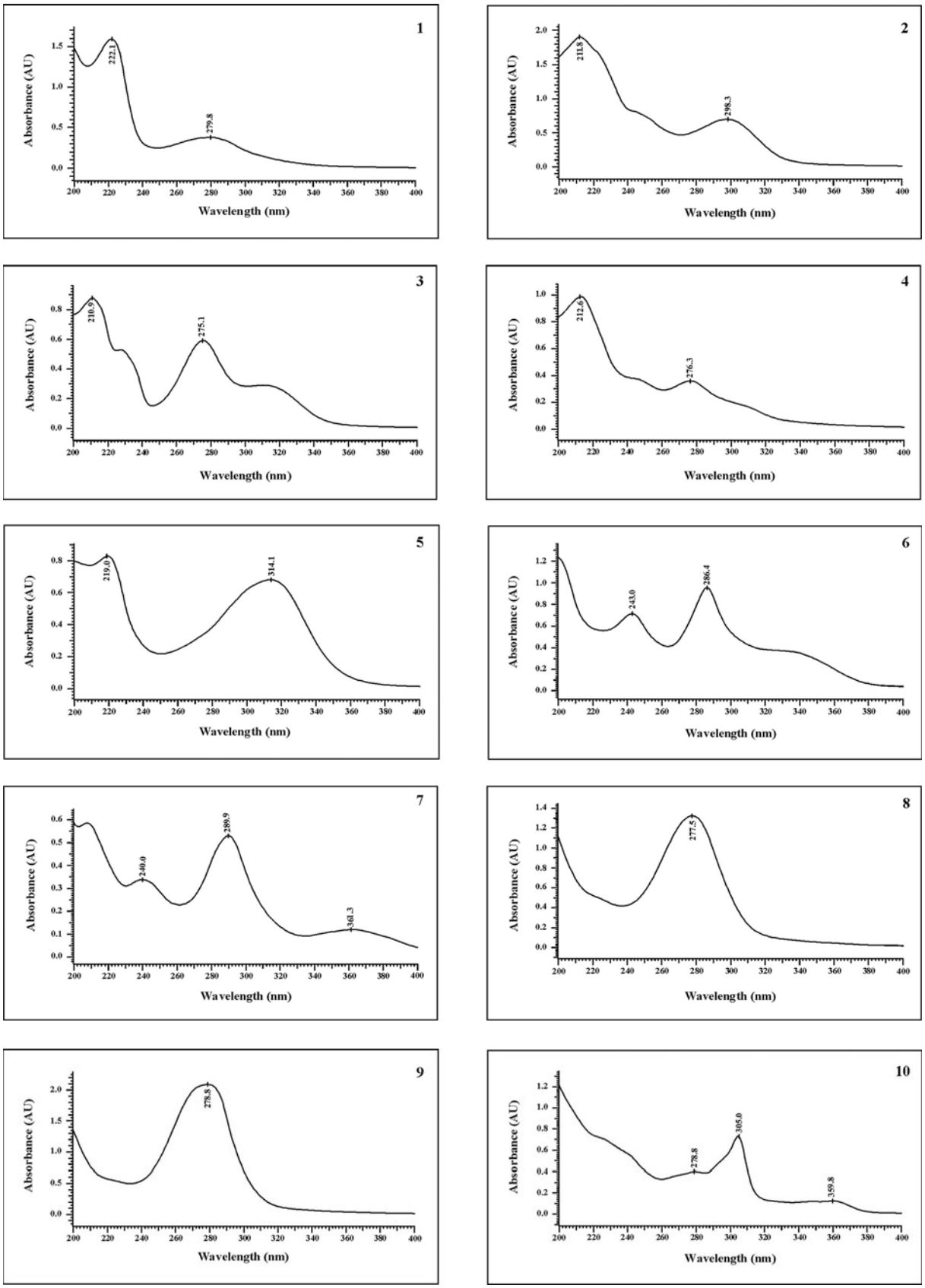


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprints of Radix Isatidis

4) Description of the HPLC-chromatograms:

The HPLC-fingerprint analysis of all *Isatis* samples shows 10 distinct peaks mainly in the Rt-range Rt = 36.0 to ~ 63.0. Indigo and Indirubin appear in varying concentrations as peak **6** and **7**, easily identifiable by their characteristic UV-spectra with end absorption at 210 nm and maxima in the UV-ranges 240 nm – 243 nm and 286 nm – 289 nm with an additional inflexion at 340 and 360 nm.

Since the peaks **3** and **4** at Rt = 38.4 and 39.7 show similar UV-spectra they may be structurally closely related to Indirubin or Indigo respectively.

Note:

- A quantitative TLC scanning method for the determination of Isatin and Indigotin in Banlangen has been described by Liang et al.[34].
- A LC-APCI-MS method for detection and analysis of Tryptanthrin, Indigo and Indirubin in Daqingye and Banlangen was described by Liau et al.[25]. For the determination of Indican, Isatin, Indirubin and Indigotin also a liquid chromatography / electro spray ionization tandem mass spectrometry method was proposed[35].
- The qualitative analysis of Indigo precursors from woad by HPLC and HPLC-MS was performed by Gilbert et al.[36].
- Indigowoad root and leaf extracts were qualitatively evaluated by chemical pattern recognition by Sun et al.[37]. The authors have divided them into five classes by the fuzzy clustering technique ISODATA.
- Chemical fingerprinting of *Isatis indigotica* root by RP-HPLC and hierarchical clustering analysis was reported by Zou et al.[33]. Comparison of the chromatograms showed that the samples can be divided into three groups.
- For the quality control of extracts of Radix Isatidis an additional method for the determination of total organic acids and salicylic acid was developed[38].
- A chromatographic discrimination of the root of *Isatis indigotica* and *Isatis tinctoria* does not exist.

Conclusion

Although most of the constituents after spraying with vanillin-sulphuric acid reagent show on TLC characteristic violet purple colored spots, the other spots, with the exception of Indigo and Indirubin, could be not assigned because of nonexistent reference compounds. In the HPLC-fingerprint the presence of Indirubin and Indigo and of a third constituent structurally closely related to the two others (peak **3** in samples 1, 3 and 9) can be easily identified by their characteristic on line UV-spectra.

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Fructus Tribuli – *Jili (Baijili)*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]: Punctuverine Caltrop Fruit is the dried ripe fruit of *Tribulus terrestris* L. (Fam. Zygophyllaceae).

Origin[2]: In many provinces of China, especially in Hebei, Shandong and Sichuan.

Description of the drug[1]: Fruit consisting of 5 mericarps, radially arranged, 7–12 mm in diameter. Often splitting into single mericarp, hatched-shaped, 3–6 mm long; dorsal surface yellowish-green, prominent, with longitudinal ribs and numerous spin lets; two lateral surface rough, with reticular striations, greyish-white. Texture hard. Odour, slight; taste, bitter and pungent.

Pretreatment of the raw drug[1]: The drug is tapped out and removed from foreign matters.

Medicinal use[4,5]: In TCM for the treatment of cardiovascular diseases, sexual impotence and abdominal distension.

Effects and indications of Fructus Tribuli according to Traditional Chinese Medicine[1, 2, 3, 4]	
Taste:	acrid, sharp
Temperature:	neutral with a warm tendency
Channels entered:	*Orbis hepaticus*, *orbis pulmonalis*.
Effects (functions):	To subdue hyperactivity of the liver, promote blood circulation, dispel *wind* to nebula, and to arrest itching.
Symptoms and indications:	Headache and dizziness, distending pain in the hypochondrium; cessation of lactation, mastitis; bloodshot eyes of nebula; urticaria with itching.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents[5,6,7,8,9,10,11,12]:

- **furostanol saponins:**
  dioscin
  protodioscin
  pseudoprotodioscin
  tribestin
  prototribestin
  tribufurosides B and C
  tribulosin
  terrestrosin A-E

- **steroidal saponins/sapogenins:**
  tribulosaponins A and B
  terrestrosin A-K
  diosgenin
  tigogenin
  hecogenin
  desgalactotigonin
  f-gitogenin
  gitonin
  desglucolanatigonin
  β-sitosterol

- **organic acid:**
  caffeic acid

- **flavonoid glycosides:**
  25 flavonoid glycosides derived from kaempferol, quercetin, isorhamnetin or tricin, inter alia:
  kaempferol-3-glucoside
  kaempferol-3-gentiobioside
  quercetin-3-glucoside
  quercetin-3-gentiobioside
  tiliroside (kaempferol-7-*p*-coumaroyl-3-*β*-glucoside)

- **alkaloids/amides, amines:**
  terrestribisamide
  tribulusterine
  N-*p*-coumaroyltyramine
  terrestriamide
  aurantiamide acetate
  tribulusamide A and B
  N-trans-feruloyltyramine
  terrestriamide
  N-trans-coumaroyltyramine
  xanthosine

Pharmacology:

- hypoglycemic activity[14,15]
- hypolipidemic activity[14,16]
- cytotoxic activity[17]
- antiulcerogenic activity[18]
- antihypertensive (vasodilatory) activity[21,22]
- antifungal activity[23]
- apoptosis-inducing[24]
- aphrodisiac activity[19,20]
- ACE inhibitory effects[9]
- anti-inflammatory activity[9]
- diuretic activity[9]
- hepatoprotective activity[9,11]
- melanocyte proliferation stimulation[9]
- reproductive effects[9]
- anthelmintic effect[25]

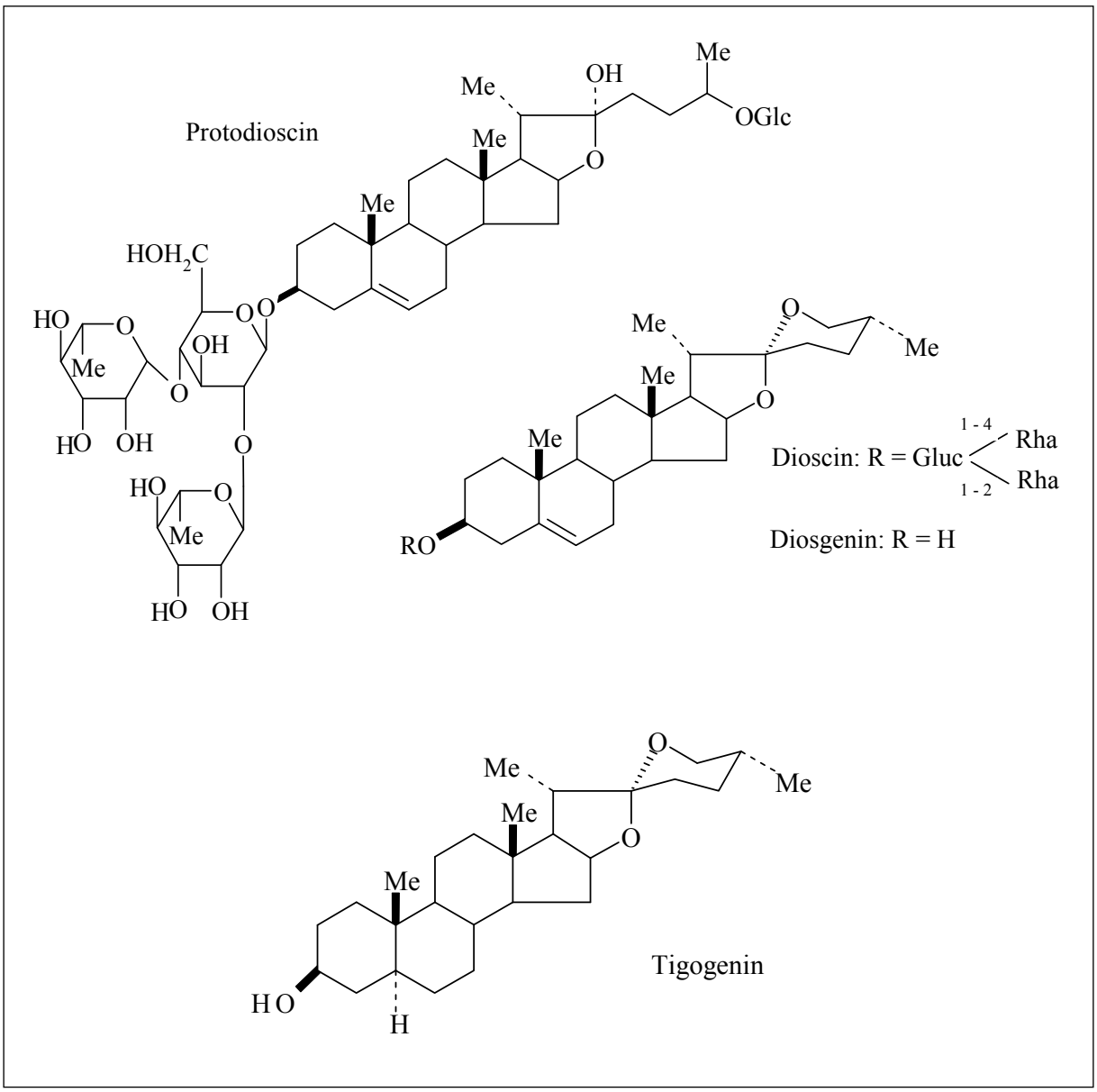
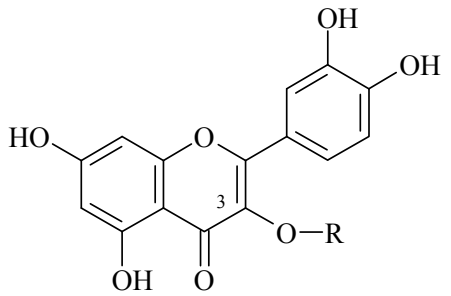
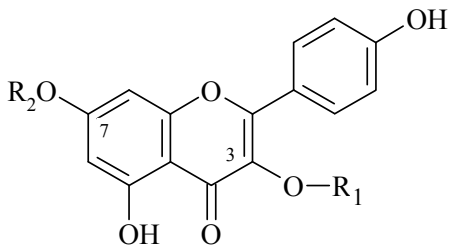


Fig. 1: Formulae of the main compounds of Fructus Tribuli[13]



R = H: Quercetin
R = Glc-Glc-Glc: Quercetin-triglucoside
R = Glc(1-6)Glc: Quercetin-3-O-gentiobioside
R = Glc(1-6)Rha: Rutin
R = Gal: Hyperoside



R_1/R_2 = H:
Kaempferol

R_1= Glc-Glc-Glc, R_2 = H:
Kaempferol-triglycoside

R_1= Glc(1-6)Glc, R_2 = H:
Kaempferol-3-O-gentiobioside

R_1= glucosyl, R_2= *p*-cumaroyl:
Tiliroside (Kaempferol-7-*p*-cumaroyl-3-*β*-glucoside)

TLC fingerprint analysis:

	Drug samples	Origin
1	Fructus Tribuli / *Tribulus terrestris*	Province Sichuan, China
2	Fructus Tribuli / *Tribulus terrestris*	Province Shandong, China
3	Fructus Tribuli / *Tribulus terrestris*	Province Hebei, China
4	Fructus Tribuli / *Tribulus terrestris*	sample of commercial drug obtained from Fraunhofer Apotheke, Munich, Germany
5	Fructus Tribuli / *Tribulus terrestris*	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2009)
6	Fructus Tribuli / *Tribulus terrestris*	Province Henan, China
7	Fructus Tribuli / *Tribulus terrestris*	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2004)
8	Fructus Tribuli / *Tribulus terrestris*	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2003)
9	Fructus Tribuli / *Tribulus terrestris*	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2000)
10	Fructus Tribuli / *Tribulus terrestris*	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1997)

Reference compounds of Figure 2a		R*f*
T1	Protodioscin	0.29
T2	Dioscin	0.50
T3	Diosgenin	0.95
T4	*β*-Sitosterin	0.96

Reference compounds of Figure 2c		R*f*
T5	Quercetin-triglycoside	0.25
T6	Quercetin-3-gentiobioside	0.27
T7	Kaempferol-3-gentiobioside	0.34
T8	Rutin	0.42
T9	Hyperoside	0.63
T10	Kaempferol-monoglycoside, Tiliroside, Kaempferol	0.73, 0.92, 0.98

TLC-fingerprint analysis:

1. Thin layer chromatograms of saponins (see Figure 2a and 2b):

1) Extraction: 1.5 g powdered drug are extracted under reflux with 20 ml petroleum ether on a water bath for 30 minutes. The extract is cooled, filtered and the filtrate discarded. The extract residue is dried and re-extracted under reflux with 20 ml 70 % ethanol for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml of ethanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Fructus Tribuli extracts: each 15 µl
reference compounds: each 15 µl

Solvent system: chloroform : methanol : water (lower layer)
13 : 7 : 2

Detection: Anisaldehyde-sulphuric acid reagent:

0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order. The TLC plate is sprayed with about 10 ml reagent, heated at 100 °C for 15 minutes and then evaluated in VIS.

The reagent has only limited stability and is no longer useable when the colour has turned to red-violet.

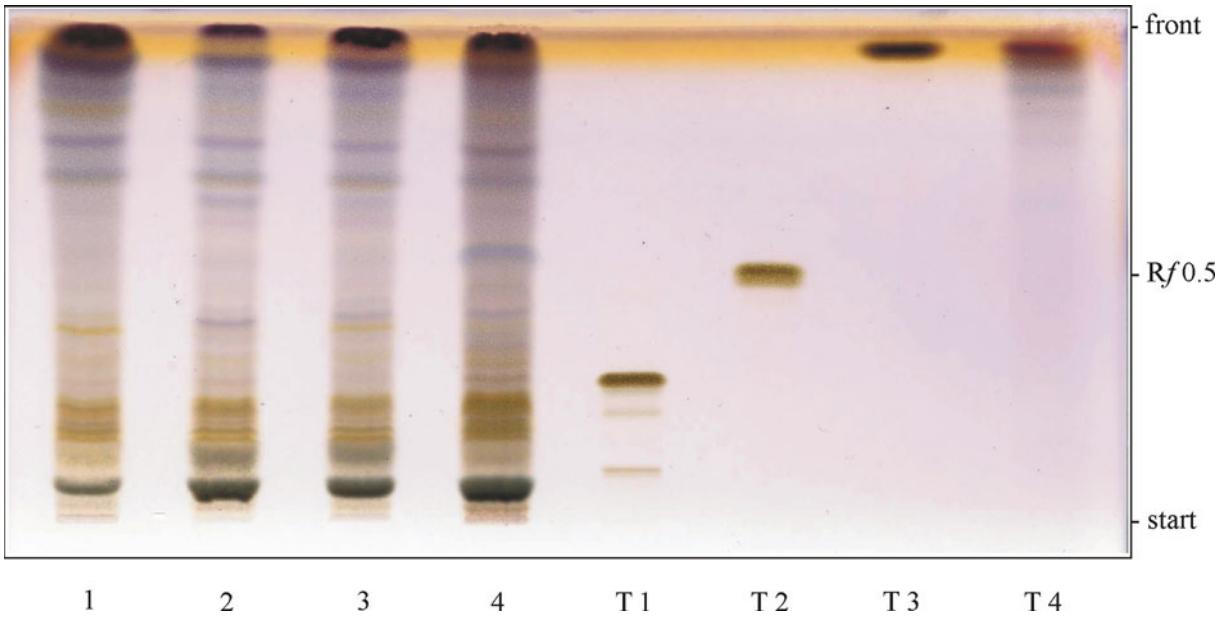


Fig. 2a: Thin layer chromatogram of the ethanol extracts of Fructus Tribuli with reference compounds T1-T4 sprayed with anisaldehyde-sulphuric acid reagent (VIS)

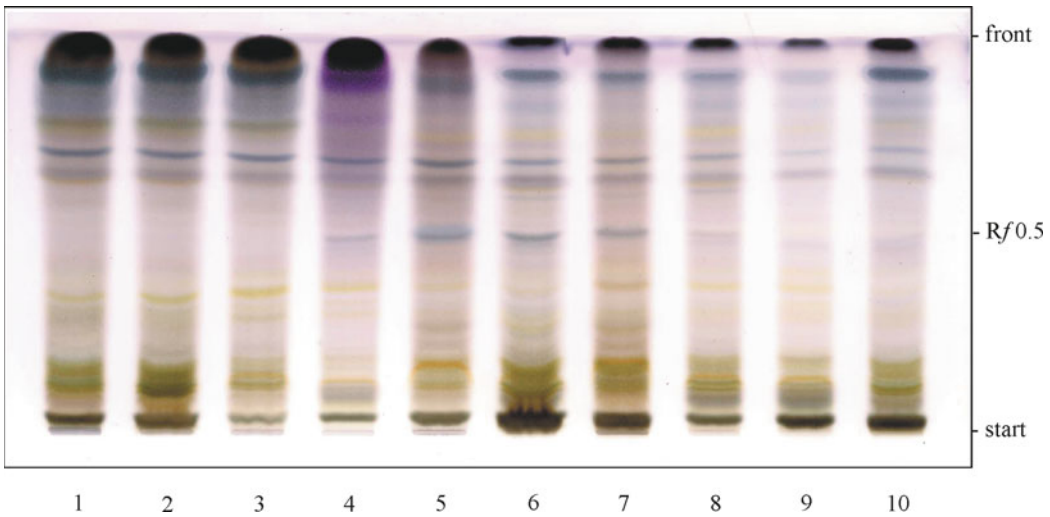


Fig. 2b: Thin layer chromatogram of the ethanol extracts of Fructus Tribuli sprayed with anisaldehyde-sulphuric acid reagent (VIS)

4) Description:

Description of TLC-chromatograms of Fig. 2a and 2b:

All chromatograms of the 10 extract samples show a very homogeneous qualitative pattern of ~ 12–14 violet or violet-green bands which in the upper R*f*-range (R*f* = 0.8 to solvent front) can be assigned to steroid aglycones (e.g. Diosgenin, Tigogenin or *β*-Sitosterol), whereas in the middle and deeper R*f*-range (R*f* = 0.5 – 0.75 and R*f* = 0.1 – 0.3) the detected bands can be assigned to steroidsaponins with 2–3 and 4–5 sugars respectively.

The reference compounds Dioscin and Protodioscin serve as marker compounds which inform about the R*f*-range in which the tri-, tetra- and pentaglycosidated steroidsaponins can be found. The strong bands above or directly on the start are saponins or free sugars (e.g. saccharose) which additionally are overlapped by the yellow/green coloured Quercetin-/Kaempferol-glucosides **T5–T7** shown in Fig. 2c.

2. Thin layer chromatogram of flavones (see Figure 2c):

1) Extraction: 1 g powdered drug is extracted under reflux with 20 ml 70 % ethanol on a water bath for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 1ml of ethanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Fructus Tribuli extracts: each 20 µl
reference compounds: each 10 µl

Solvent system:

ethyl acetate		formic acid		glacial acetic acid		water
100	:	11	:	11	:	26

Detection: Natural products-polyethylene glycol reagent (NP/PEG) (=NEU-reagent):

The plate is sprayed with 1 % methanolic diphenylboric acid-*β*-ethyl amino ester (=diphenylboryloxyethylamine, NP), followed by 5 % ethanolic polyethylene glycol-4000 (PEG) (10 ml and 8 ml, respectively).

The plate is evaluated in UV 365 nm.

4) Description of the TLC-chromatogram of Fig. 2c:

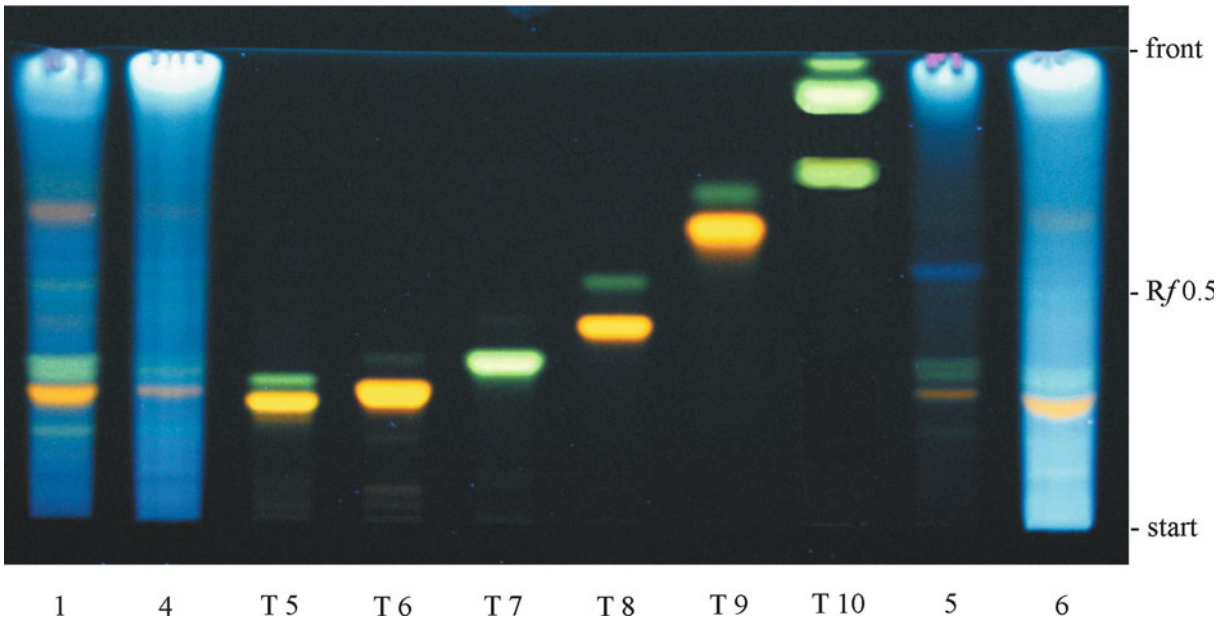


Fig. 2c: Thin layer chromatogram of the ethanol extracts of Fructus Tribuli sprayed with NP/PEG (UV 366)

All extract samples of Fructus Tribuli contain flavonolglycosides, which especially in the extract samples 1, 4 and 6 could be assigned with the help of the reference flavonolglycosides **T5–T10** to Quercetin-triglycoside (**T5**), Quercetin-3-gentiobioside (**T6**), Kaempferol-3-gentiobioside (**T7**), Rutin (**T8**) and Hyperoside (**T9**). Tiliroside and Kaempferol-monoglucoside (**T10**) are detectable only in small amounts and are partly overlapped by caffeic acid in R*f* –range 0.9 – 1.0.

HPLC-fingerprint analysis:

1) Sample preparation: 1 g powdered drug is extracted under reflux with 20 ml 70 % ethanol on a water bath for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 1ml of ethanol, filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: Fructus Tribuli extracts: each 15.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck

Solvent:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	5–40 % B in 32 minutes 40–95 % B in 10 minutes 95 % B in 18 minutes total runtime: 60 minutes
Flow:	1.0 ml/min.
Detection:	205 nm

Retention times of the main peaks:

Peak	Rt (min.)	Compound
1	~18.9	Quercetin-3-gentiobioside
2	~20.8	Kaempferol-3-gentiobioside
3	~27.4	Caffeic acid
range 4	~27.7 - 40.2	Tiliroside and Protodioscin
5	~40.9	Dioscin
6	~43.5	not identified
7	~44.8	Diosgenin
8	~46.9	*ß*-Sitosterin
9	~48.4	not identified

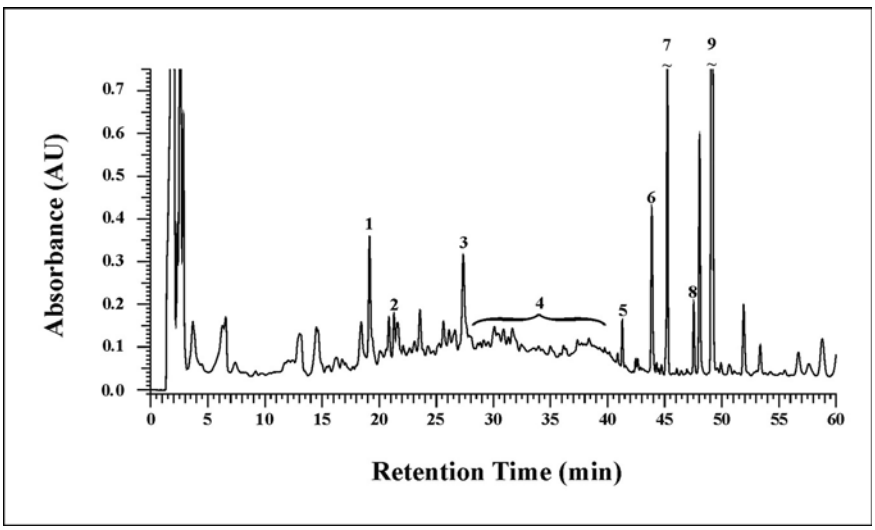


Fig. 3a: HPLC fingerprint of *Tribulus terrestris* extract sample 1

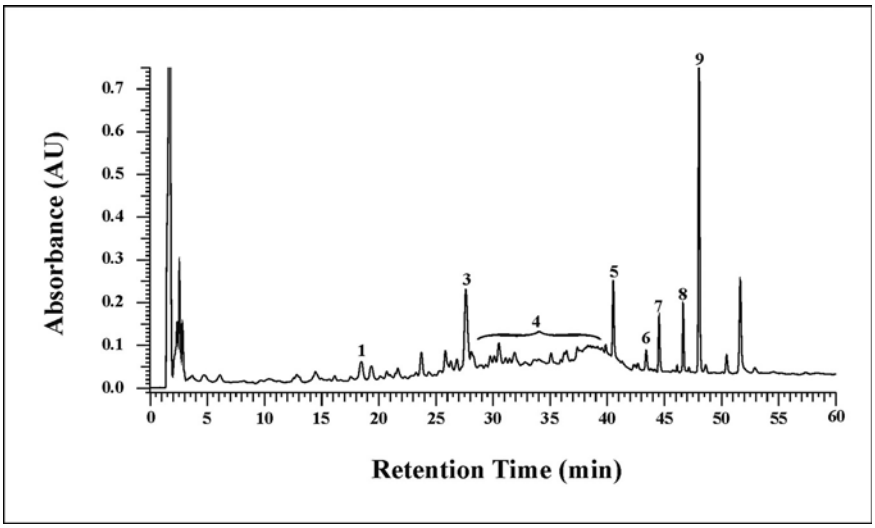


Fig. 3b: HPLC fingerprint of *Tribulus terrestris* extract sample 6

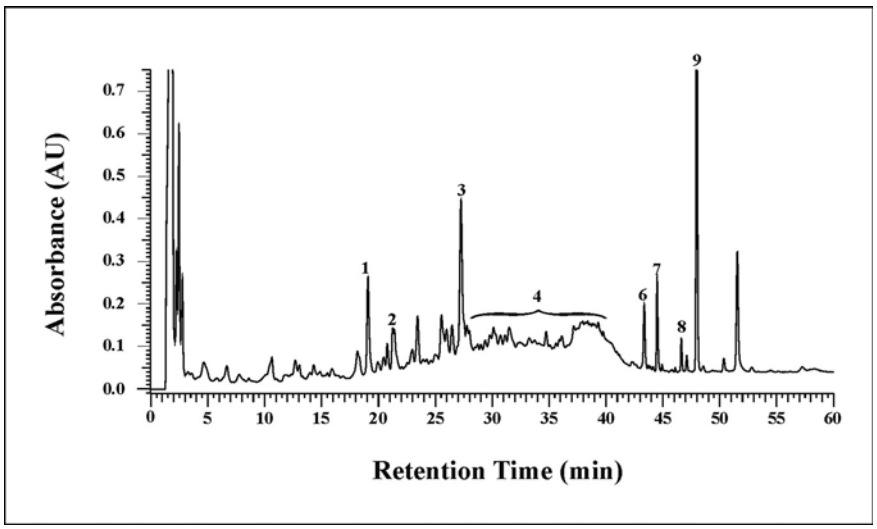


Fig. 3c: HPLC fingerprint of *Tribulus terrestris* extract sample 7

4) Description of the HPLC:

The HPLC-graphs mirror approximately the TLC-pictures of Fig. 2a, 2b and 2c. In the Rt-range from 15.6 to 33.0 we find the peaks of the flavonolglycosides Quercetin- and Kaempferol-3-gentiobioside (**1** and **2**), followed by caffeic acid **3** (Rt = 27.4), and Tiliroside with Kaempferol-3-glucoside and Protodioscin in the Rt-range 28 – 40. Dioscin appears at Rt = 40.9. In the Rt-range 43 to 50.0 the strong peaks can be assigned to the aglycones Tigogenin, Diosgenin **7** (Rt = 44.8) and *ß*-Sitosterin **8** (Rt = ~46,9). The strong peak at Rt = 48.4 could be any of the sapogenins (e.g. hecogenin or gitogenin) as reported in the literature.

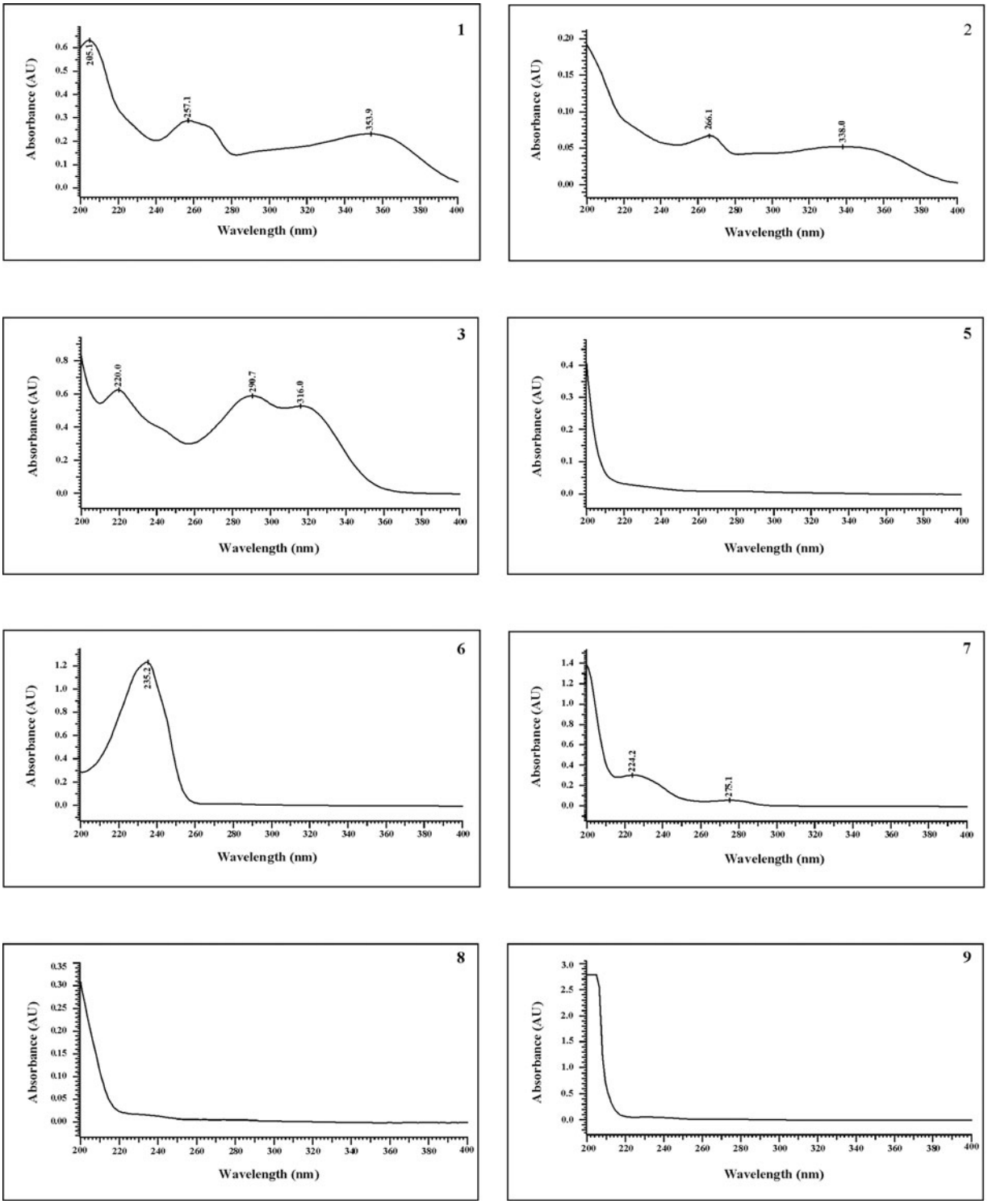


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of *Tribulus terrestris* extracts

Conclusion

The authenticity of Fructus Tribuli can be very well characterized in TLC- and in HPLC-fingerprint by the presence of the characteristic flavonolglycosides and saponins.

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Radix Ophiopogonis – *Maidong*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]: Dwarf Lilyturf Tuber is the dried root tuber of *Ophiopogon japonicus* (Thunb.) Ker-Gawl. (Fam. Liliaceae).

The drug is collected in summer, washed clean, sun-dried and piled up repeatedly until nearly dry, removed from rootlet, and dried.

Origin[2]: Provinces of Zhejiang and Sichuan, China.

Description of the drug[1]: Fusiform, with two ends slightly tapering, 1.5–3 cm long, 3–6 mm in diameter. Externally yellowish-white, finely wrinkled longitudinally. Texture tough, fracture yellowish-white, translucent, stele small. Odour, slightly aromatic; taste, sweetish and bitterish.

Pretreatment of the raw drug[1]: Foreign matters are eliminated, washed clean, softened thoroughly, pressed to be flattened, and dried.

Medicinal use[3]: Diseases of the lung and the bronchial tubes, cough, xerostomia, constipation, hypotonia, insomnia and obliviousness.

Effects and indications of Radix Ophiopogonis according to Traditional Chinese Medicine[1, 2, 4, 5]	
Taste:	Sweet, bitterish
Temperature:	Neutral, slightly cold
Channels entered:	*Orbis cardialis, orbis pulmonalis, orbis stomachi, orbis intestini crassi.*
Effects (functions):	To nourish *yin* and promote the production of body fluids, moisten the lung, and anchor the mind.
Symptoms and indications:	Dry cough, phthisical cough; thirst due to impairment of body fluids; fidgets and insomnia; wasting-thirst caused by internal *heat*; constipation; diphtheria.

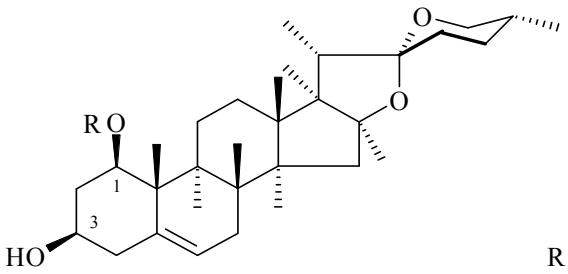
H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

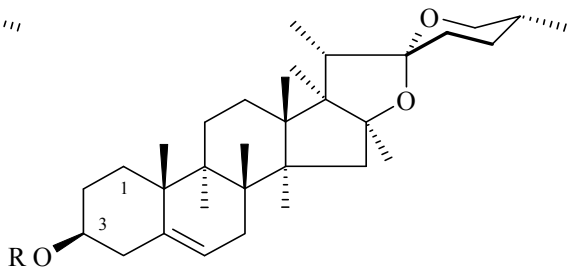
Main constituents:

– **steroidal saponins/sapogenins:**

ophiofurospiside A[8]	(26-O-β-D-glucopyranosyl-(22S,25R)-furospirost-5-ene-3β, 17α, 26-triol-3-O-[α-1-rhamnopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→4)]-glucopyranoside)
ophiopogonin A[16]	(ruscogenin 1-O-[(3-O-acetyl)-α-L-rhamnopyranosyl(1→2)]-β-D-fucopyranoside)
ophiopogonin B[16]	(ruscogenin 1-O-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside)
ophiopogonin B'[16]	(diosgenin 3-O-[(4-O-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside)
ophiopogonin C[16]	(mono-O-acetylophiopogonin D)
ophiopogonin C'[16]	(diosgenin 3-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside) (= prosapogenin A of dioscin)
ophiopogonin D[16]	(ruscogenin 1-O-[α-L-rhamnopyranosyl(1→2)]-[β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside)
ophiopogonin D'[16]	(diosgenin 3-O-[α-L-rhamnopyranosyl-(1→2)] [β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside)
ophiopogonin E[11]	(pennogenin 3-O-β-D-xylopyranosyl(1→4)]-β-D-glucopyranoside)
ruscogenin[10]	

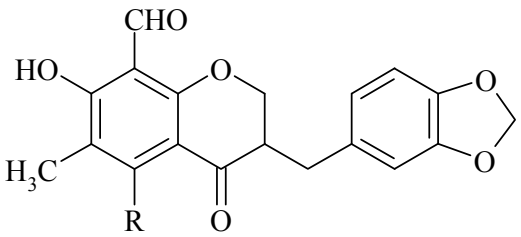
- **homoisoflavonoids:**
  ophioponanone C, D, E, F[15]
  ophiopogonone C[15]
  6-aldehydoisoophiopogonone[15]
  5,7,2'-trihydroxy-6-methyl-3-(3',4'-methylene- dioxybenzyl)chromone[15]
  2'-hydroxymethylophiopogonone A[15]
- **monoterpene glycosides**[17]
- **steroidal glycosides**[17]
- phenolic glycosides:
  ophiopojaponin[12]
- sesquiterpene glycosides:
  liriopeoside A[21]
  ophiopogoside A[21]
- polysaccharides :
  Opaw-2 (fructan)[9]
  MDG-1 (β-D-fructosan)[13]
  Md-1, Md-2 (D-Glucose units joined by α-(1→4))[14]
- lectin[25]



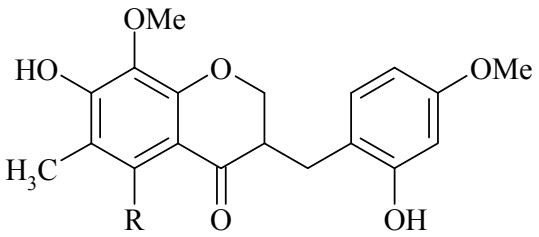


R = H: (R, S)-Ruscogenin
R = 2 - 3 sugars: Ophiopogonin A/B/C/D

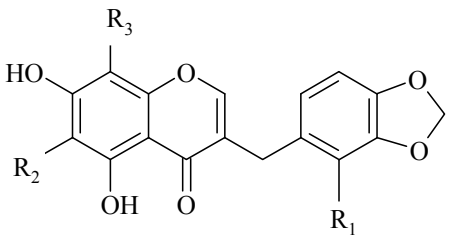
R = H: Diosgenin
R = 2 - 3 sugars: Ophiopogonin B′/C′/D′



1. R=OH Ophiopogonanone C
2. R=OCH_3 Ophiopogonanone D



4. R=OH Ophiopogonanone E
5. R=OCH_3 Ophiopogonanone F



3. R_1=H R_2=CH_3 R_3=CHO Ophiopogonone C
6. R_1=H R_2=CHO R_3=CH_3 6-aldehydoisoophiopogonone A
7. R_1=OH R_2=CH_3 R_3=H 5,7,2′-trihydroxy-6-methyl-3-(3′,4′-methylenedioxybenzyl)chromone
8. R_1=OH R_2=CH_3 R_3=CH_3 2′-hydroxymethylophiopogonone A

Fig. 1: Formulae of the main compounds of Radix Ophiopogonis [15,18]

Pharmacology:

- hypolipidemic activity[24]
- anti-inflammatory activity[19,23]
- antithrombotic activity[20,23,26]
- anti-ischemic activity[20]
- anti-arrhythmic activity[20,22]
- inhibiting platelets aggregation[20]
- protecting endothelium from apoptosis[20]
- improving microcirculation[20]
- apoptosis-inducing activity[25]
- antiproliferative activity[25]

TLC fingerprint analysis:

Drug samples		Origin
1	Radix Ophiopogonis / *Ophiopogon japonicus*	Province Hebei, China
2	Radix Ophiopogonis / *Ophiopogon japonicus*	Province Hunan, China
3	Radix Ophiopogonis / *Ophiopogon japonicus*	Province Sichuan, China
4	Radix Ophiopogonis / *Ophiopogon japonicus*	Sample of commercial drug obtained from HerbaSinica, Germany (origin: Sichuan, China)
5	Radix Ophiopogonis / *Ophiopogon japonicus*	Sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (2000)
6	Radix Ophiopogonis / *Ophiopogon japonicus*	Sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1999)
7	Radix Ophiopogonis / *Ophiopogon japonicus*	Sample of commercial drug (origin: Sichuan, China)
8	Radix Ophiopogonis / *Ophiopogon japonicus*	Sample of commercial drug (origin: unknown)
9	Radix Ophiopogonis / *Ophiopogon japonicus*	Sample of commercial drug obtained from Fraunhofer Apotheke, Munich, Germany

Reference compounds of Figure 2b		R*f*
T 1	Dioscin	0.34
T 2	Diosgenin	0.90

1) Extraction: 1.5 g powdered drug are extracted with 20 ml 70 % ethanol under reflux for 15 min. The extract is filtered and the filtrate evaporated to about 1 ml. The residue is dissolved in 10 ml water and shaken with 10 ml water-saturated *n*-butanol. The phases are separated. The hydrophile phase is shaken again with 5 ml water-saturated *n*-butanol. The *n*-butanol phases are combined and evaporated to dryness. The residue is dissolved in 1.0 ml ethanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Radix Ophiopogonis extracts: each 10 µl
reference compounds: each 10 µl

Solvent system: Chloroform : methanol : water (lower layer)
13 7 2

Detection: Anisaldehyd-sulphuric acid reagent:

0.5 ml anisaldehyd is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The TLC plate is sprayed with about 10 ml, heated at 100° C for 15 minutes, and then evaluated in VIS.

The reagent has only limited stability and is no longer useable when the colour has turned to red-violet.

4) Description:

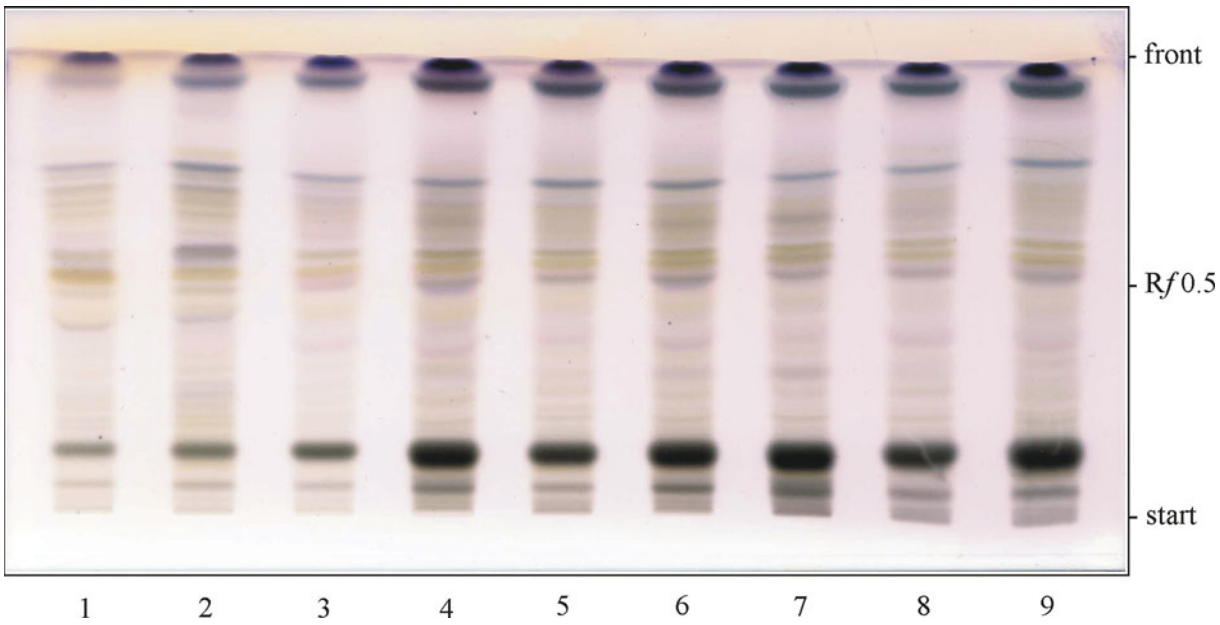


Fig. 2a: Thin layer chromatogram of the ethanol extracts of Radix Ophiopogonis sprayed with anisaldehyde-sulphuric acid reagent (VIS)

The TLC-fingerprint of Radix Ophiopogonis samples 1 – 9 is characterized by a very homogeneous pattern of 2 – 3 dark violet-brown bands directly below the solvent front and further 10 – 12 violet-brown or violet and green-yellow bands in the R*f*-range from start to R*f* ~ 0.85. The bands below the solvent front can be assigned to the steroid- and triterpene sapogenins Diosgenin and Ruscogenin respectively, whereas the bands above the start till R*f* = 0.4 are in a R*f*-range where in this TLC solvent system the steroid-glycosides with 2 – 4 sugars (e.g. ophiopogonin D) appear. In the R*f*-range between R*f* = 0.5 – 0.8 are located the homoisoflava(o) nones (e.g. Ophiopogonin C and A) partly overlapped by steroid glycosides such as Ophiopogonin D. The strong dark brown-violet band at R*f* = 0.13 could be identified as sucrose.

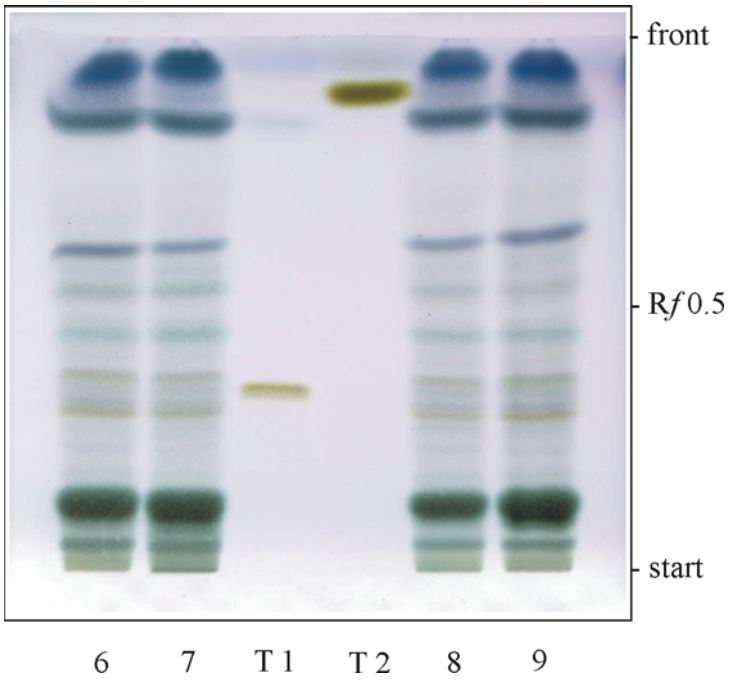


Fig. 2b: Thin layer chromatogram of the ethanol extracts of Radix Ophiopogonis sprayed with anisaldehyde-sulphuric acid reagent (VIS)

In this TLC the samples 6, 7, 8, and 9 mirror more distinctly the TLC-pattern of Fig. 2a showing the steroid glycones in the R*f*-range of 0.85 – 0.95. The steroid- tri- and tetra-glycosides lie in the R*f*-range 0.05 – 0.12 of sucrose (saccharose). The reference compounds **T1** and **T2** mark the position of Dioscin and Diosgenin respectively, but it is questionable whether they are present in the various root samples at all. The relatively strong violet band at R*f* = 0.61 could be assigned to Ophiopogonin D.

HPLC-fingerprint analysis:

1) Sample preparation: To 1 g of the powdered drug 1 ml ammonia solution 25 % is added. 10 ml methanol are added and extracted under reflux for 30 minutes. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm, and the solution injected into the HPLC apparatus.

2) Injection volume: Radix Ophiopogonis extracts: each 15.0 µl

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck

Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent:	A: dist. Water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	5 – 90 % B in 55 minutes 90 % B in 5 minutes total runtime: 60 minutes
Flow:	1.0 ml/min.
Detection:	205 nm

Retention times of the main peaks*:

Peak	Rt (min.)	Peak	Rt (min.)
1	~ 9.8	5	~ 40.9
2	~ 33.3	6	~ 45.5
3	~ 37.1	7	~ 51.7
4	~ 40.1 Ophiopogonin D	8	~ 55.3

***** Because of lacking reference compounds a correct assignment to the various steroid glycosides was not possible.

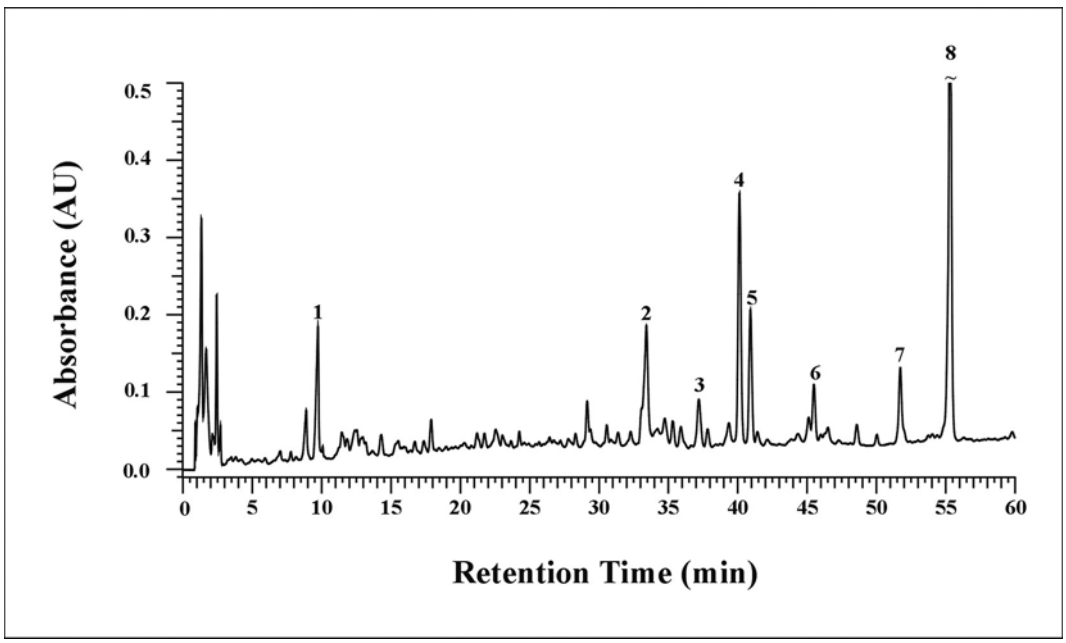


Fig. 3a: HPLC fingerprint of Radix Ophiopogonis sample 6

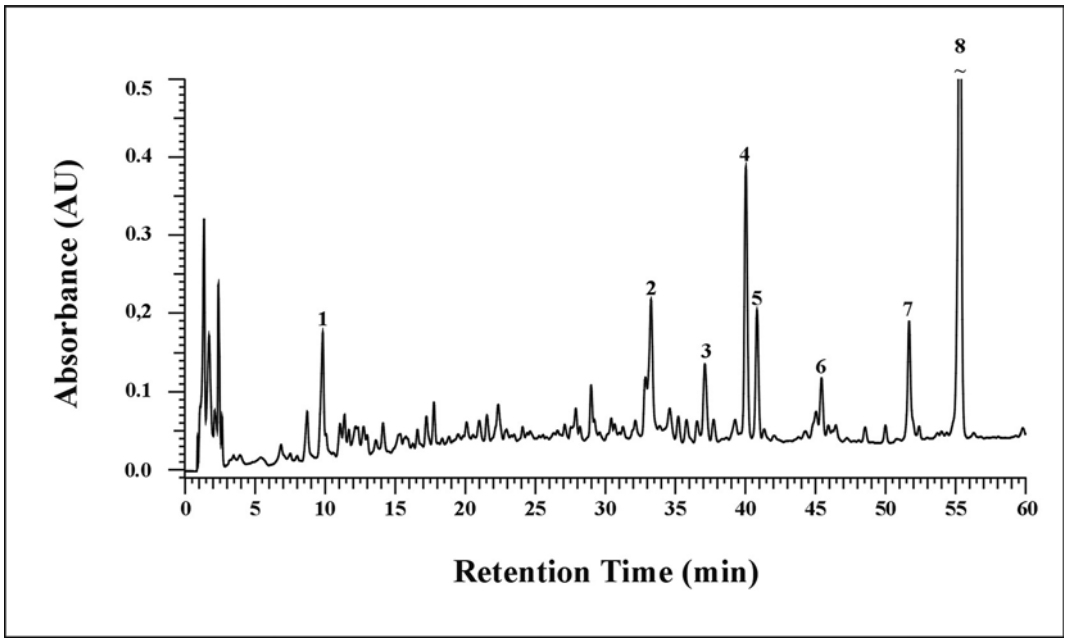


Fig. 3b: HPLC fingerprint of Radix Ophiopogonis sample 9

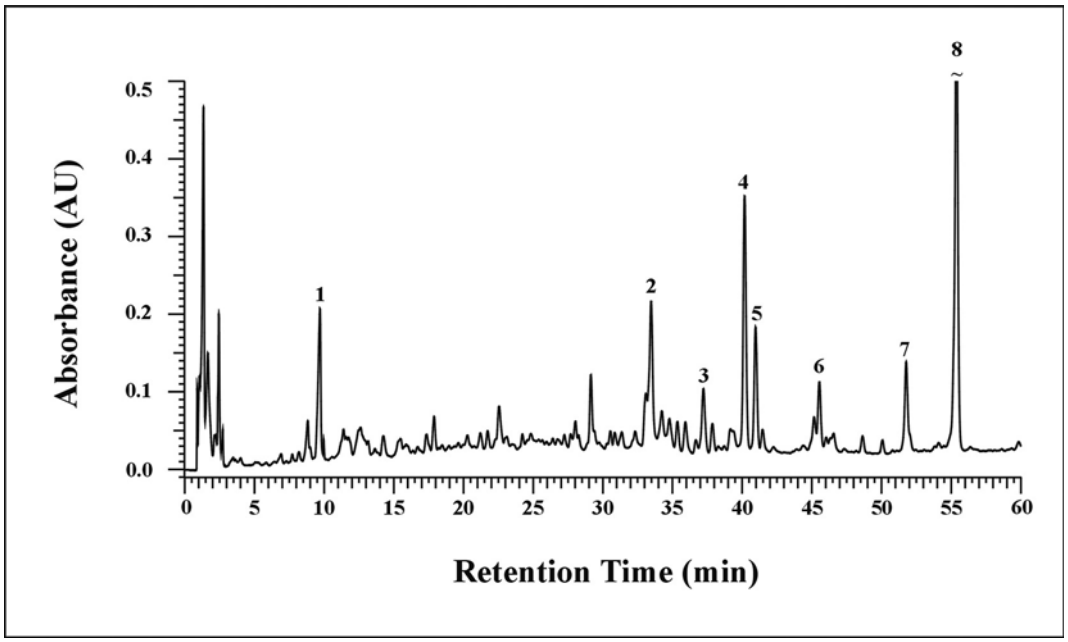


Fig. 3c: HPLC fingerprint of Radix Ophiopogonis sample 4

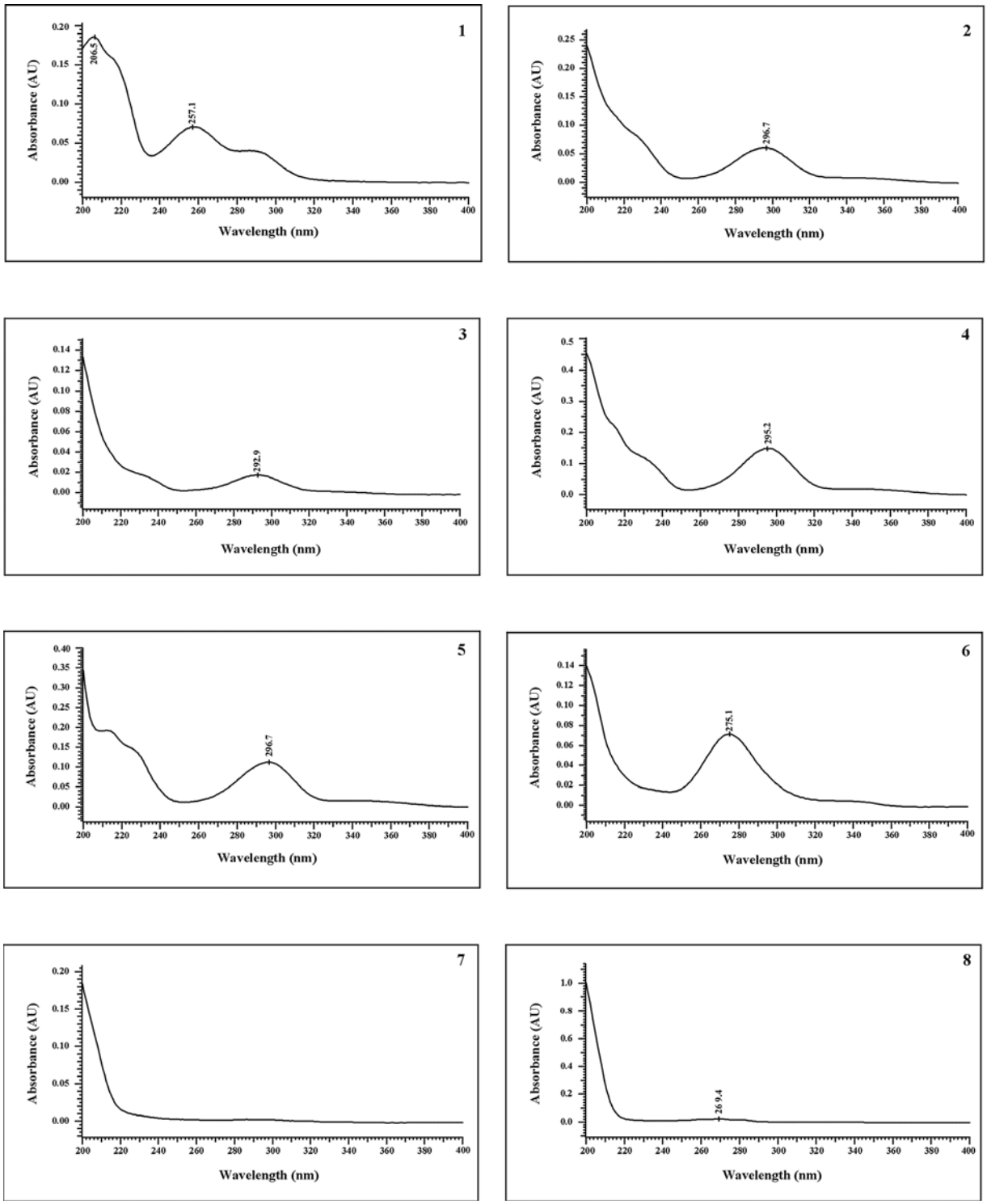


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of Radix Ophiopogonis

4) Description of the HPLC:

On the HPLC-graphs only the fingerprints of the samples 4, 6, and 9 are shown. All supply a peak-pattern of 8 peaks with the dominant peaks **1**, **2**, **4**, and **8**. Peak **8** might be Ruscogenin or Diosgenin. According to the on-line UV-spectra the peaks **1** – **6** could be assigned to homoisoflavanones, whereas the peaks **7** and **8** represent the steroidaglycones Ruscogenin and/or Diosgenin respectively. The steroid saponins containing 2 – 4 sugars are spread over the whole Rt-range from Rt = 10 to ~35. The marker compound Ophiopogonin D could be identified as peak **4**.

Conclusion

Without references the exact assignment of the various TLC- and HPLC-zones and peaks respectively is difficult. The TLC and HPLC together yield very characteristic fingerprints which differ only quantitatively in their zones- or peak-pattern.

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Cortex Eucommiae – *Duzhong*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]: "Eucommia Bark" is the dried stem bark of *Eucommia ulmoides* Oliv. (Fam. Eucommiaceae)

The drug is collected from April to June, removed from the coarse bark, piled up until the inner surface becomes purplish-brown and dried in the sun.

Origin[2]: mainly produced in the provinces of Si Chuan, Yun Nan, Gui Zhou and Hu Bei

Description of the drug[1]: Flat pieces, or two edges somewhat curved inwards, varying in size, 3–7 mm thick. Outer surface pale brown or greyish-brown, markedly wrinkled or fissured and channelled longitudinally; some barks relatively thin, showing distinct lenticels when the coarse bark unscraped; inner surface dark purple, smooth. Texture fragile, easily broken, fracture linked up by fine, dense, silvery and elastic rubber threads. Odour, slight; taste, slightly bitter.

Pretreatment of the raw drug [1]: The remains of coarse bark are scrapped off, washed clean, cut into pieces and dried.

Medicinal use[1,8]: Treatment of hypertension, depression and inflammatory diseases, used also as immunostimulant.

Effects and indications of Cortex Eucommiae according to Traditional Chinese Medicine[1, 2, 3, 4]	
Taste:	acrid, sharp
Temperature:	neutral with a warm tendency
Channels entered:	*Orbis hepaticus* et *renalis*
Effects (functions):	tonifies the liver and kidney, strengthens the tendons and bones, and prevents miscarriage
Symptoms and indications:	Deficiency condition of the kidney marked by lumbago and lack of strength, for sareness and pain in the loins and knees, weakness of the muscles due to liver deficiency; threatened abortion; hypertension
Contraindications:	patients with *Yin* – deficiency with excessive ardor

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Main constituents:

- **lignan derivatives and glucosides**[4]
  hydroxy-pinoresinol di *O*-*β*-D-glucopyranoside, pinoresinol di-*O*-*β*-D-glucopyranoside, syringaresinol *O*-*β*-D-glucopyranoside, olivil di-*O*-*β*-D-glucopyranoside, medioresinol di-*O*-*β*-D-glucopyranoside, eucommin A, liriodendrin, guaiacylglycerol-*β*-medioresinol ether-di-*O*-*β*-D-glucopyranoside, syringlycerol-*β*-syringaresinol ether 4′′, 4′′′-di-*O*-*β*-D-glucopyranoside, cycloolivil

  erythro-dihydroxy-dehydrodiconiferyl alcohol, dehydroconiferyl alcohol - di-*O*-*β*-D-glucopyranoside

- **iridoid glycosides**[4]
  aucubin, harpagide acetate, ajugoside, reptoside, eucommiol, ulmoside, geniposidic acid, geniposide

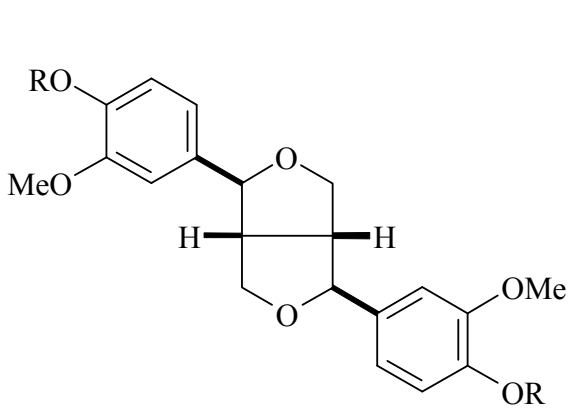
- **polysaccharides**[5]
- **free sugars**

- **trans-1,4-polyisoprene (guttapercha)**[6]

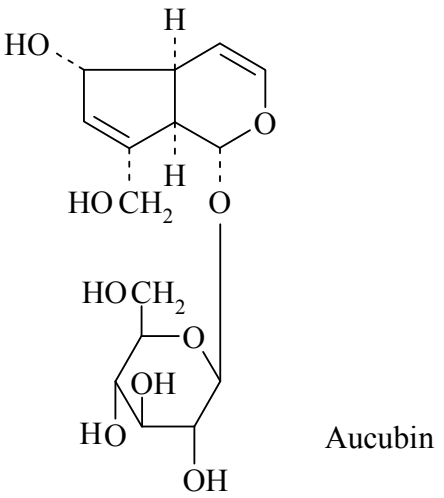
Pharmacology:

- antihypertensive[4,5,6,7]
- antioxidant[8]
- antiosteoporotic[9]
- immunomodulating (anticomplement) activity[10]

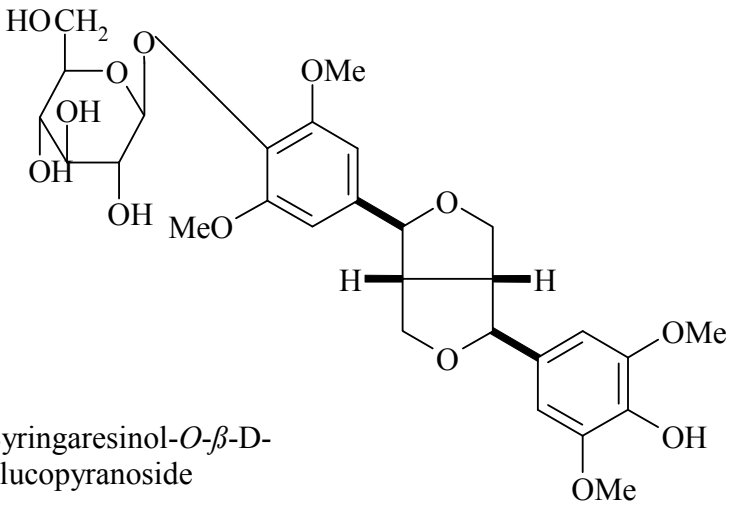
Fig. 1: Formulae of the main compounds of Cortex Eucommiae



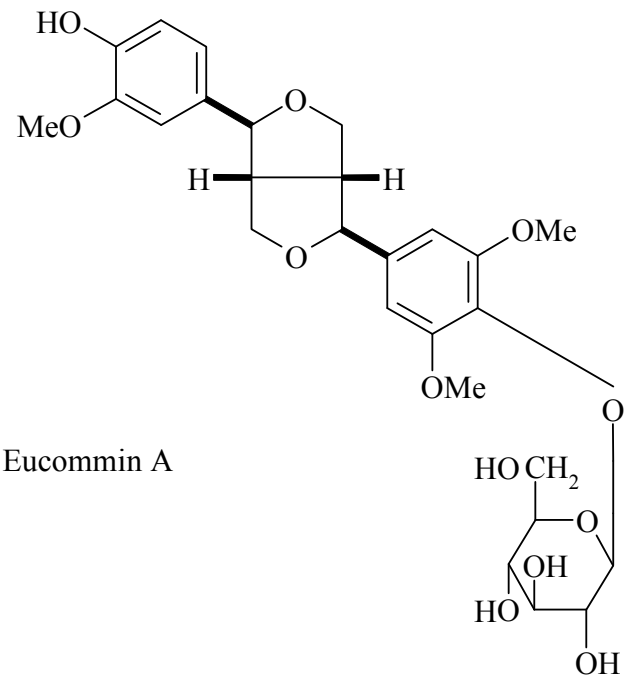
Pinoresinol R: H
Pinoresinoldiglucoside R: Gluc



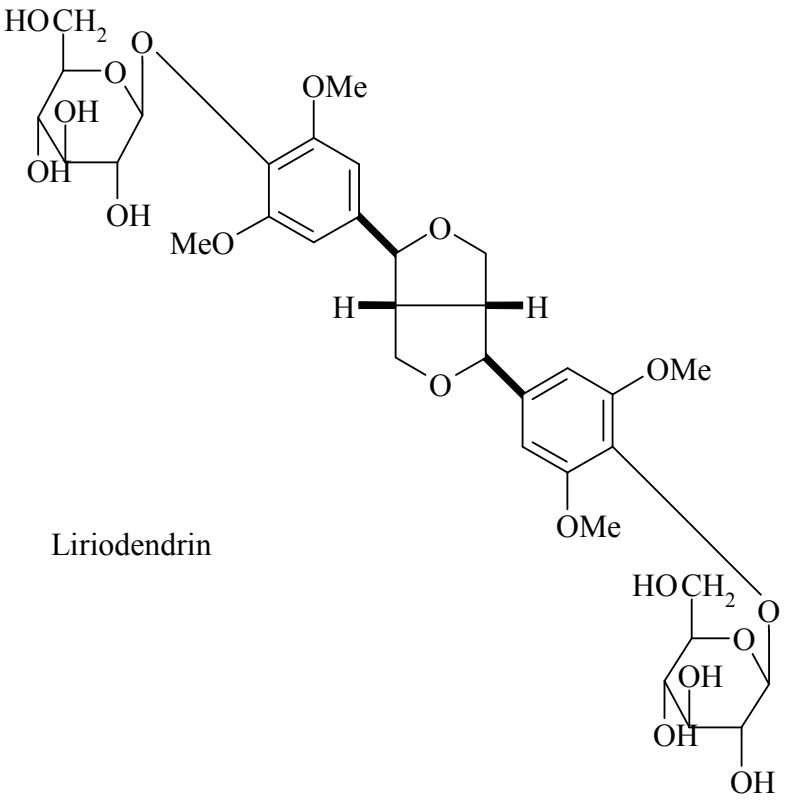
Aucubin



Syringaresinol-*O*-*β*-D-glucopyranoside



Eucommin A



Liriodendrin

TLC fingerprint analysis:

Drug samples		Origin
1	Cortex Eucommiae / *Eucommia ulmoides*	province Guangdong, China
2	Cortex Eucommiae / *Eucommia ulmoides*	province Sichuan, China
3	Cortex Eucommiae / *Eucommia ulmoides*	commercial drug obtained from TCM-Hospital Bad Kötzting, Germany
4	Cortex Eucommiae / *Eucommia ulmoides*	province Hebei, China

Reference compounds of Figure 2		R*f*
T 1	Pinoresinol-diglucopyranoside	0.35
T 2	Pinoresinol	0.97
T 3	Aucubin	0.40
T 4	Syringaresinol	0.97

TLC-fingerprint analysis:

1) Extraction: 2.5 g powdered drug are extracted with 30 ml methanol for 30 minutes on ultrasonic bath, then filtered and the filtrate evaporated to dryness. The residue is dissolved in 20 ml water and extracted with 50 ml dichloromethane under reflux for 1 hour. The aqueous layer is extracted again with 50 ml *n*-butanol under reflux for 1 hour. The *n*-butanol layer is separated and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Cortex Eucommiae extract: each 10 µl
reference compounds: each 20 µl

Solvent system: dichloromethane : methanol : formic acid
3 : 1 : 0.1

Detection: Sulphuric acid reagent:

Add slowly 20 ml sulphuric acid to 80 ml water.

The TLC plate is sprayed with about 10 ml of the solution, heated at 120 °C for 15 minutes and then evaluated in VIS.

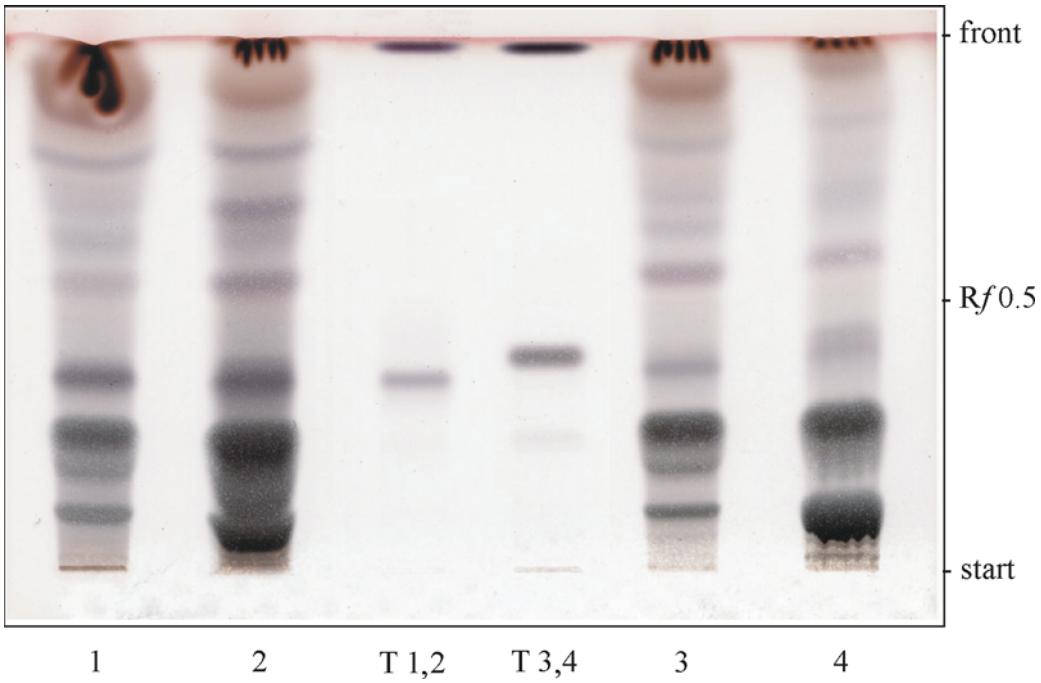


Fig. 2: Thin layer chromatogram of the methanol extracts of *Eucommia ulmoides* sprayed with sulphuric acid reagent (VIS)

4) Description:

All four extract samples show a very homogeneous pattern of 9–10 violet brown bands, which from the R*f*-range 0.25 up to the solvent front can be assigned to the various lignans described for Cortex Eucommiae. Pinoresinol-diglucosid (**T1**) has the R*f*-value 0.35 with its aglycone Pinoresinol (**T2**) at R*f* = 0.97. The Pinoresinol-monoglucoside (see also HPLC-fingerprint) is one of the violet brown bands between R*f* = 0.6 and 0.8. The lignan-aglycone Pinoresinol lies directly under the solvent front, Aucubin is present in a very low concentration (see also peak **6** in the HPLC-fingerprint) and can be localized at R*f* = 0.40. The strong dark brown bands in the R*f*-range between R*f* = 0.12 and 0.26 can be assigned to sugars such as saccharose or glycuronic acid.

HPLC-fingerprint analysis:

I. HPLC-fingerprint analysis of Cortex Eucommiae samples 1-4 (Figure 3a and b):

1) Sample preparation: 2 g powdered drug are extracted with 75 ml dichloromethane under reflux for 1 hour. The dichloromethane phase is discarded and the residue extracted with 75 ml methanol under reflux for 6 hours. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 4 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Cortex Eucommiae extracts: each 20.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 100 RP-18 (5 µm), Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	0–30 % B in 45 min. 30–95 % B in 20 min. 95 % B in 5 min. total runtime: 70 minutes
Flow:	0.6 ml/min.
Detection:	210 nm

II. HPLC-fingerprint analysis of Cortex Eucommiae sample 2 for the identification of aucubin[13]

1) Sample preparation: 2 g powdered drug are extracted with 75 ml dichloromethane under reflux for 1 hour. The dichloromethane phase is discarded and the residue extracted with 75 ml methanol under reflux for 6 hours. The extract is cooled, filtered and the filtrate evaporated to dryness. The residue is dissolved in 4 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm and 20.0 µl injected into the HPLC apparatus.

2) Injection volume: Cortex Eucommiae extracts: each 20.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 with LiChrospher® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 with LiChrospher® 60 RP select B, Merck
Solvent:	A: water (Millipore Ultra Clear UV plus® filtered) + H_3PO_4 85 % (VWR) (pH = 4) B: acetonitrile (VWR)

Gradient:	0 % B in 1 min. 0–10 % B in 15 min. 10–100 % B in 1 min. 100–0 % B in 1 min. total runtime: 18 minutes
Flow:	1.2 ml/min.
Detection:	210 nm

Retention times of the main peaks recorded at 210 nm:

Peak	Rt (min.)	Compound
1	~ 21.0	pinoresinol-di-O-glucoside
2	~ 23.3	not identified
3	~ 24.7	not identified
4	~ 33.0	pinoresinol-mono-O-glucoside
5	~ 48.9	pinoresinol
6	~ 12.4	aucubin (Fig. 3c)

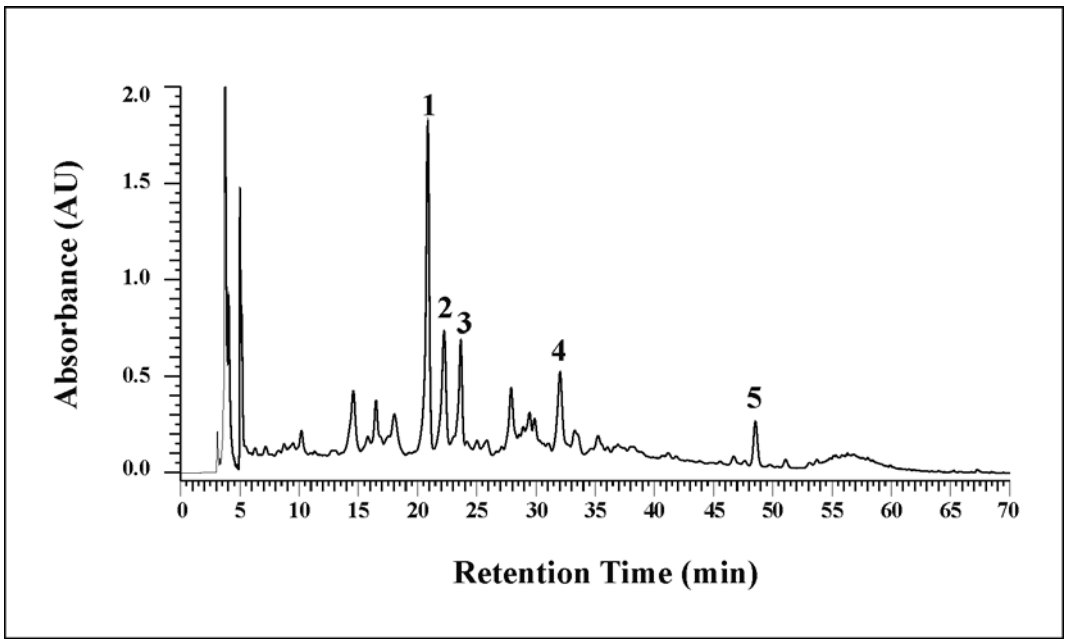


Fig. 3a: HPLC-fingerprint chromatogram of the methanol extract of *Eucommia ulmoides*, shown for sample 1

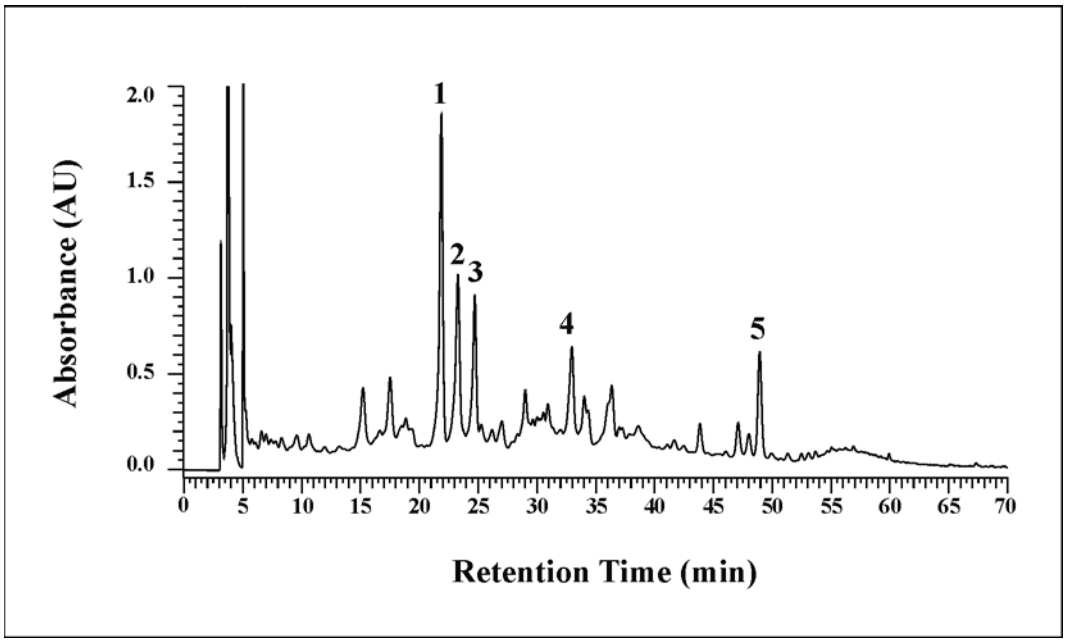


Fig. 3b: HPLC-fingerprint chromatogram of the methanol extract of *Eucommia ulmoides*, shown for sample 2

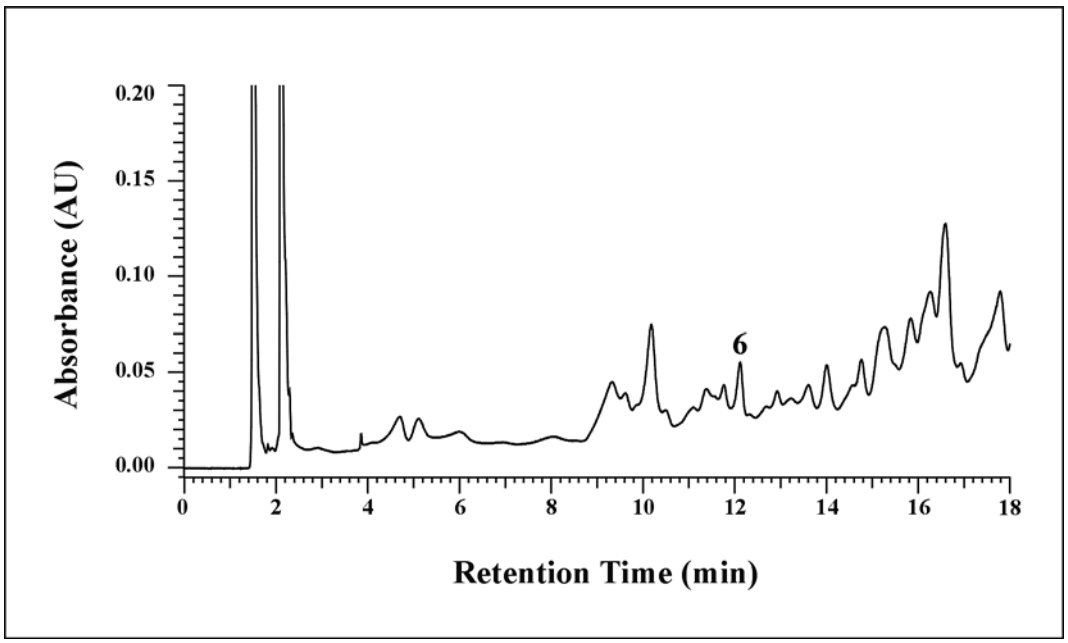


Fig. 3c: HPLC-fingerprint chromatogram of the methanol extract of *Eucommia ulmoides*, shown for sample 2 (see HPLC-analysis II)

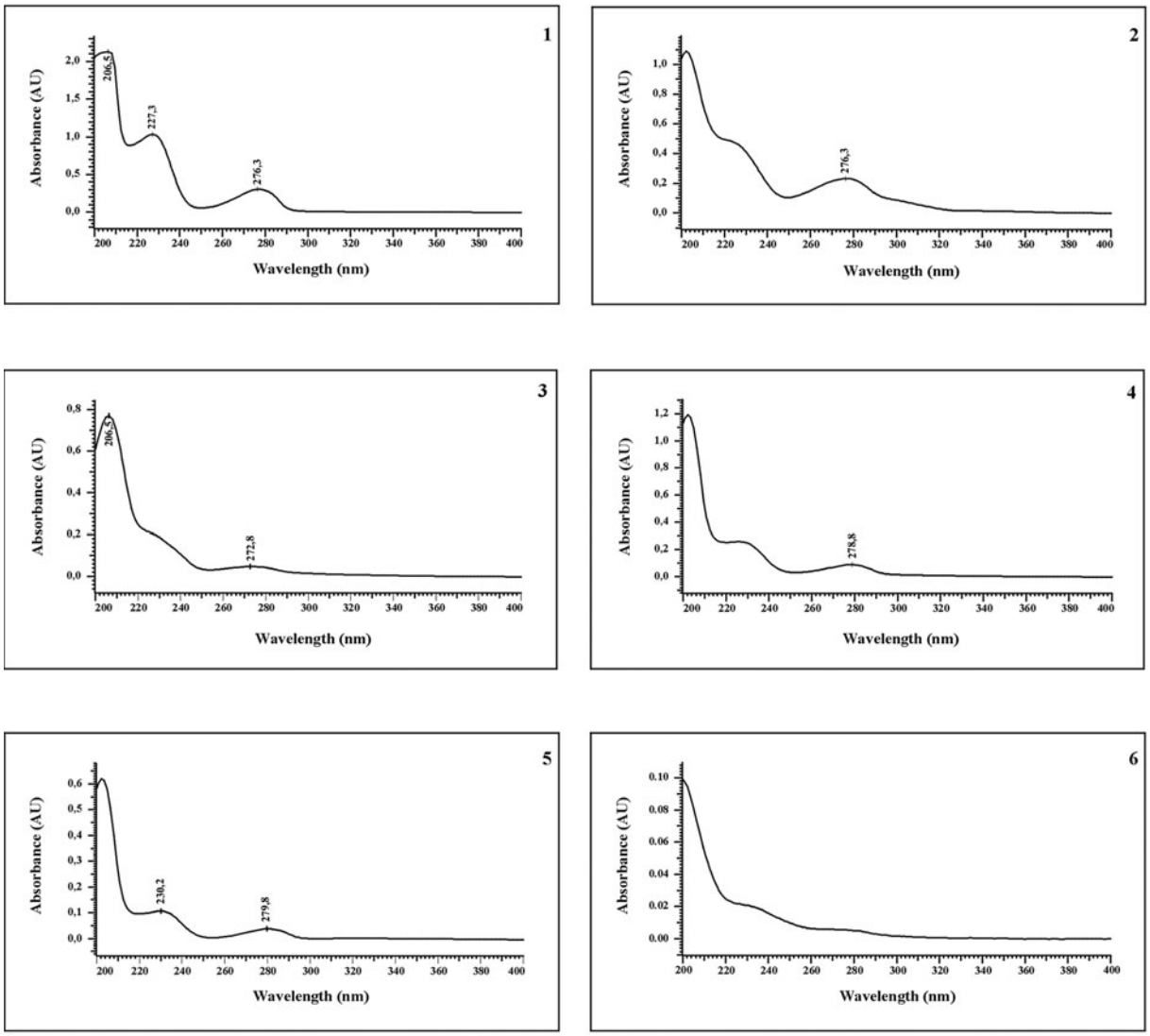


Fig. 4: UV-spectra of the main compounds (peaks) of the methanol extracts of *Eucommia ulmoides*

4) Description :

Figure 3a and b:

All HPLC – graphs of samples 1 – 4 are characterized by a very homogenous peak pattern with 5 pronounced peaks at Rt = 21.0, 23.3, 24.7, 33.0 and 48.9. Peak **1** is identical with the marker compound pinoresinol-di-*O*-glucoside. Peak **2** and **3** show nearly identical UV – spectra as pinoresinol-diglucoside with Maxima at 205, 227 and 267 nm and can be assigned to analogeous lignandiglucosides, such as Liriodendrin or Medioresinol-diglucoside. Peak **4** at Rt = 33.0 should be pinoresinol-mono-*O*-glucoside and peak **5** at Rt = 48.54 can be identified as pinoresinol. According to their on line UV – spectra the minor peaks in the Rt – range 14.0-18.0 can to be assigned to caffeic acid (Rt = 14.58) and two further lignanglucosides.

Figure 3c:

For the detection of Aucubin another HPLC-fingerprint analysis has to be performed (see HPLC-method No. II). Aucubin appears as distinct peak **6** at Rt = 12.4.

Note: The Pharmacopoeia of the People's Republic of China demands for Cortex Eucommiae a concentration not less than 0.10 per cent of pinoresinol-diglucoside.

Conclusion

Cortex Eucommiae shows in the TLC and in HPLC a very homogeneous composition of the lignanglycosides which can be unequivocally identified.

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Radix et Rhizoma Notoginseng
Sanqi

Pharmacopoeia[1]:	Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005
Official drug[1]:	Sanchi is the dried root of *Panax notoginseng* (Burk.) F.H. Chen (Fam. Araliaceae). The drug is collected before flowering in autumn, washed clean, graded into main root, branch root and rhizome, and dried. The branch root is known as "Jintiao" and rhizome is known as "Jiankou".
Origin[4]:	China (mainly districts Yunnan, Sichuan, Guangxi), cultivated mainly in prefectura Wenshan.
Description of the drug[1]:	Main roots subconical or cylindrical, 1–6 cm long, 1–4 cm in diameter. Externally greyish-brown or greyish-yellow with interrupted longitudinal wrinkles and branch root scars. Stem scars at the apex surrounded by warty protrudings. Texture heavy and compact, fracture greyish-green, yellowish-green or greyish-white, wood slightly radially arranged. Odour, slight; taste, bitter but afterwards sweetish. *Jintiao:* Cylindrical or conical, 2–6 cm long, the upper end 0.8 cm in diameter, the lower end 0.3 cm in diameter. *Jiankou:* Irregularly shrunken lump-shaped or slat-shaped, externally with several conspicuous stem scars and annulations; fracture greyish-green or greyish-white in the centre and deep green or grey at the margin.
Pretreatment of the raw drug[1]:	Washed clean, dried, and pulverized to fine powder.
Medicinal use[2,3]:	Used in the treatment of diabetes and gastrointestinal disorders such as gastritis and ulcers. It is also used as a hemostatic drug in the treatment of different types of bleeding.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of *Panax notoginseng* according to Traditional Chinese Medicine[1,5,6]	
Taste:	sweet
Temperature:	warm
Channels entered:	*Orbis hepaticus*, *orbis stomachi*
Effects (functions):	To eliminate blood stasis, arrest bleeding, cause subsidence of swelling and alleviate pain
Symptoms and indications:	Hemoptysis, hematemesis, epistaxis, hematochezia, abnormal uterine bleeding, traumatic bleeding; pricking pain in the chest and abdomen, traumatic swelling and pain

Characteristic main constituents *:

- **saponines**[3]
  ginsenosides R*x* (*x* = a, **b1**, b2, c, **d**, e, f, **g1**, **h1**)
  glucoginsenoside (R*f*)
  notoginsenosides R*x* (*x* = **1**, 2, 3, 4, 6), Fa, Fc, Fe, K

- **sapogenins**[3]
  panaxadiol (dammar-20(22)-ene-3*β*,12*β*-diol), panaxatriol (dammar-20(22)-ene-3*β*,12*β*,26-triol), 20(R)-dammarane-3*β*,12*β*,20,25-tetrol, 20(R)-protopanaxatriol

- **essential oil**[7]
  α-guaiene, *β*-guaiene, octadecane

Minor constituents **:

- polyacetylenes[8]
  Falcarinol (panaxynol), Falcarindiol (panaxydol), 1,8-Heptadecadiene-4,6-diyne-3,10-diol

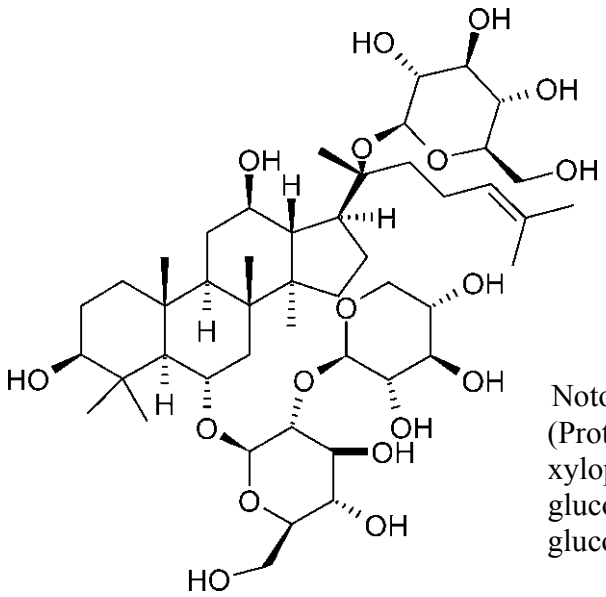
- nucleosides[9]
  uracil, cytidine, uridine, guanosine, adenosine

- amino acids[6]
- flavonoids[6]

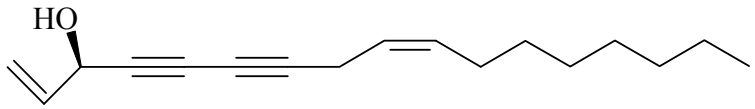
* **Note:** The ginsenosides Rb1, Rd, Rg1 and the Notoginsenoside R1 are regarded as the quantitatively main, characteristic Dammarane-triterpenoid glycosides of *Panax notoginseng* but the genetic diversity and variation affect the contents of the main saponins.[6]

** **Note:** The polyacetylenes present in *P. notoginseng* root may derive originally from the fungus *Paecilomyces species*. Whether they are also biosynthetsized from *Panax notoginseng* root is not yet investigated.[10]

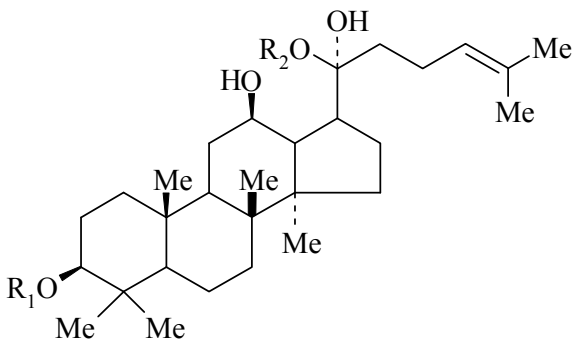
Fig. 1: Formulae of the main constituents of *Panax notoginseng*



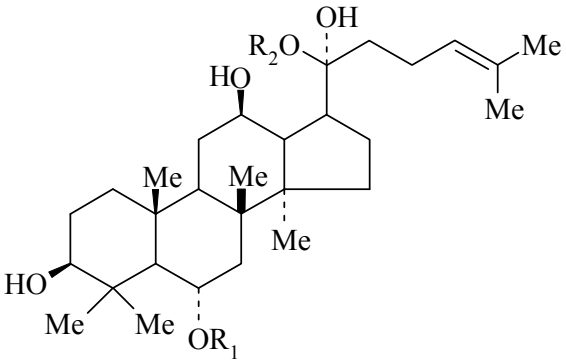
Notoginsenoside R1
(Protopanaxatriol 20-*O*-*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranosyl 6-*O*-*β*-D-glucopyranoside)



Falcarinol (panaxynol)



Protopanaxadiol-Type



Protopanaxatriol-Type

Ginsenosides

	R_1	R_2
Protopanaxadiol-Type		
Rb1	*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-glucopyranosyl	*O*-*β*-D-Glucopyranosyl-(1→6)-*β*-D-glucopyranosyl

	R_1	R_2
Protopanaxatriol-Type		
Re	*O*-*α*-L-Rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl	*O*-*β*-D-Glucopyranosyl
Rg1	*O*-*β*-D-Glucopyranosyl	*O*-*β*-D-Glucopyranosyl

Pharmacology:

a) Dammarane-triterpenoids:

Immunomodulatory activity	***Cardiovascular and metabolic effects***	***Enhancement of CNS activities***
adaptogenic[15]	platelet aggregation inhibitory activity[3,14,16,9]	protective effects on neurodegeneration[16] and injured brain[17]
anti-fatigue effects[17,22]	hemostatic activity[3]	neuroprotective[14]
anti-cancer[13,18]	anti-arrhythmic[9]	anti-oxidant[11]
anti-stress[15,16]	anti-hyperlipidemic[16,9]	anti-aging[11,16]
	anti-inflammatory[3,14]	
	hepatoprotective activity[12]	
	anti-anoxia[11]	
	anti-diabetic[20]	

b) Polyacytelenes:

antifungal, antimutagenic and antitumor properties[10,13,20,23]

TLC fingerprint analysis:

Drug samples		Origin
1	Radix et Rhizoma Notoginseng / *Panax notoginseng*	sample of commercial drug obtained from HerbaSinica, Germany
2	Radix / Rhizoma Notoginseng / *Panax notoginseng*	Province Yunnan (Wenshan), China
3	Radix / Rhizoma Notoginseng / *Panax notoginseng*	Province Yunnan (Maguan), China
4	Radix / Rhizoma Notoginseng / *Panax notoginseng*	Province Guangxi, China
For comparison		
5	Radix / Rhizoma Ginseng / *Panax ginseng*	sample of commercial drug obtained from HerbaSinica, Germany
6	Radix / Rhizoma Ginseng / *Panax ginseng*	sample of commercial drug obtained from China Medica, Germany
7	Radix / Rhizoma Panacis Quinquefolii / *Panax quinquefolium*	Washington, USA
8	Radix / Rhizoma Panacis Quinquefolii / *Panax quinquefolium*	Wisconsin, USA

Reference compounds of Figure 2a + b		R*f*
T 1	Ginsenoside Rg1	0.57
T 2	Ginsenoside Re	0.34
T 3	Ginsenoside Rb1	0.12

TLC-fingerprint analysis:

1) Extraction: 2 g powdered drug are extracted with 10 ml 90% ethanol under reflux for 10 minutes. The filtrate is evaporated to about 5 ml.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: Silica gel 60 F_{254}, Merck

Applied amounts: Notoginseng extracts: each 10 µl
reference compounds: each 10 µl

Solvent system:	Chloroform	: methanol	: water	
	70	30	4	

Detection: Vanillin-phosphoric acid reagent:
1 g vanillin is dissolved in 100 ml of 50 % phosphoric acid.
The plate is sprayed with this solution, heated for 5 minutes at 105°C and evaluated in VIS and 365 nm.

4) Description of TLC-fingerprint analysis:

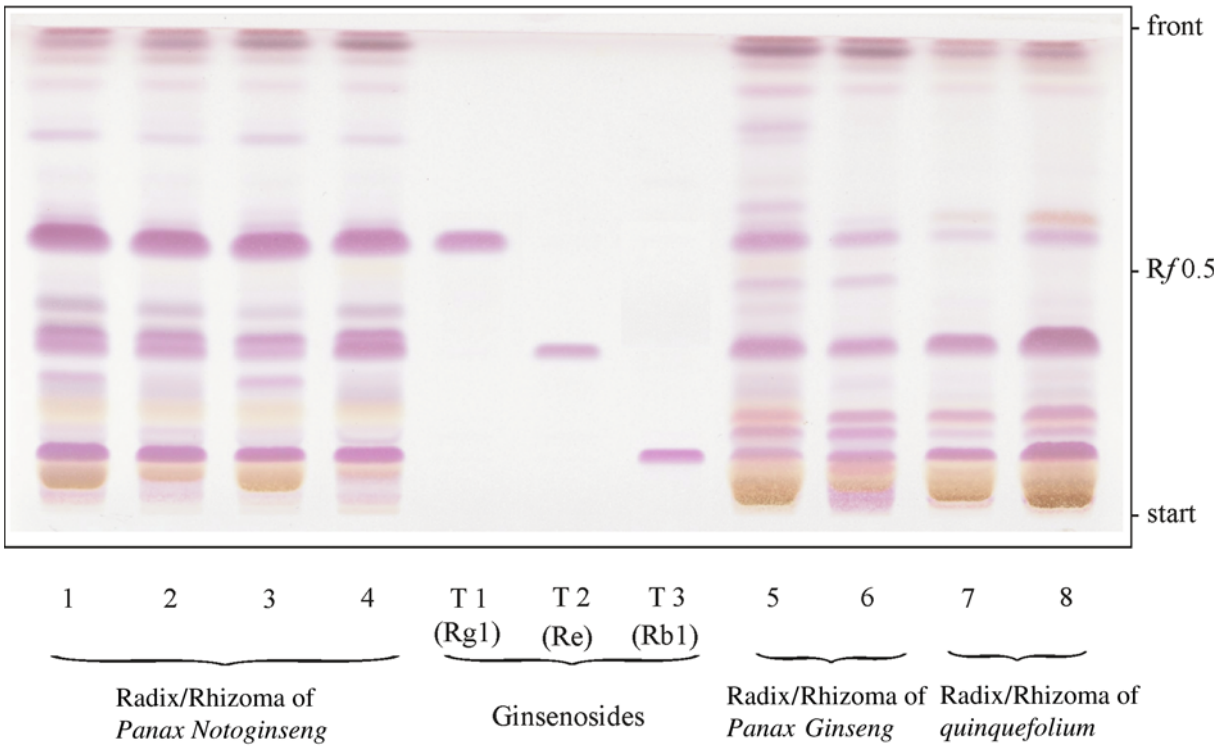


Fig. 2a: Thin layer chromatogram of the ethanol extracts of *Panax* ssp. sprayed with vanillin-phosphoric acid reagent (VIS)

On Fig. 2a are seen the TLC-fingerprints of the root/rhizome extracts of *Panax Notoginseng* (samples 1–4) and for comparison those of *Panax Ginseng* (samples 5 + 6) and of *Panax quinquefolium* (samples 7 + 8).

All Notoginseng root extracts show a very homogeneous pattern of 10–12 pink bands. Directly below the solvent front can be localized the sapogenins of the Ginsenosides (Protopanaxadiol and Protopanaxatriol). Notoginseng root is characterized by the dominating Ginsenoside Rg1 (Diglucoside) at R*f* = 0.57 (=**T1**) and Ginsenoside Re (Triglucoside) at R*f* = 0.34 (=**T2**) accompanied by Rd (R*f* = 0.36) and as fourth Ginsenoside Rb1 (Tetraglucoside) at R*f* = 0.12 (=**T3**).

The TLC of the root extract of *Panax Ginseng* (samples 5+6) differs in three points: a lower concentration of Ginsenoside Rg1, Ginsenoside Re as the dominant Ginsenoside and besides Ginsenoside Rb1 two further ginsenosides (probably Ginsenoside Rb2 and Rc*) above Rb1

forming together a triplet with Rb1. The root extracts of *Panax quinquefolium* (sample 7+8) differ from *P. Notoginseng* by a very low concentrated Ginsenoside Rg1 but very dominant Ginsenosides Re and Rb1.

	R_1	R_2
* Rb2 = Protopanaxadiol	*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-glucopyranosyl	*O*-α-L-Arabinopyranosyl-(1→6)-*β*-D-glucopyranosyl
Rc = Protopanaxadiol	*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-glucopyranosyl	*O*-α-L-Arabinofuranosyl-(1→6)-*β*-D-glucopyranosyl

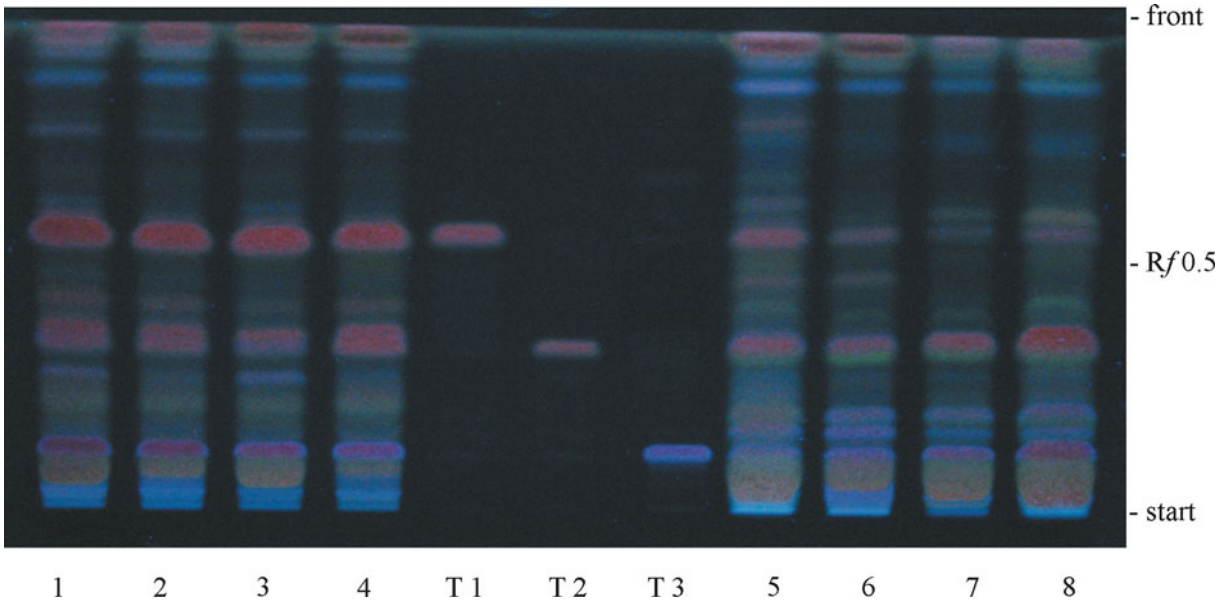


Fig. 2b: Thin layer chromatogram of the ethanol extracts of *Panax* ssp. sprayed with vanillin-phosphoric acid reagent (UV 366 nm)

In UV 365 nm of Fig. 2b Notoginseng root extracts can be discriminated from the two other Ginseng spec. by the two strong concentrated deep red fluorescent Ginsenosides Rg1 and Ginsenoside Re.

HPLC-fingerprint analysis[14]:

1) Sample preparation: 4 g powdered drug are extracted twice with 15 ml methanol overnight at room temperature, followed by extraction once with 15 ml 80 % methanol. The methanol extracts were combined and adjusted to a final volume of 50 ml methanol. The solution is filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Notoginseng extracts: each 20.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l dist. Water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	0 –20 % B in 20 min. 20–40 % B in 20 min. 40–100 % B in 32 min. total runtime: 72 minutes
Flow:	1 ml/min.
Detection:	205 nm

Retention times of the main peaks recorded at 205 nm

Peak	Rt (min.)	Compound
1	~ 23.7	ginsenoside Rg1
2	~ 26.3	ginsenoside Re
3	40.6	ginsenoside Rb1
4	57.0–59.9	Polyacetylenes (Falcarinol, Falcarindiol)
5	60.5–62.0	Stigmasterol
6	66.3	Panaxadiol
7	69.0	Panaxatriol

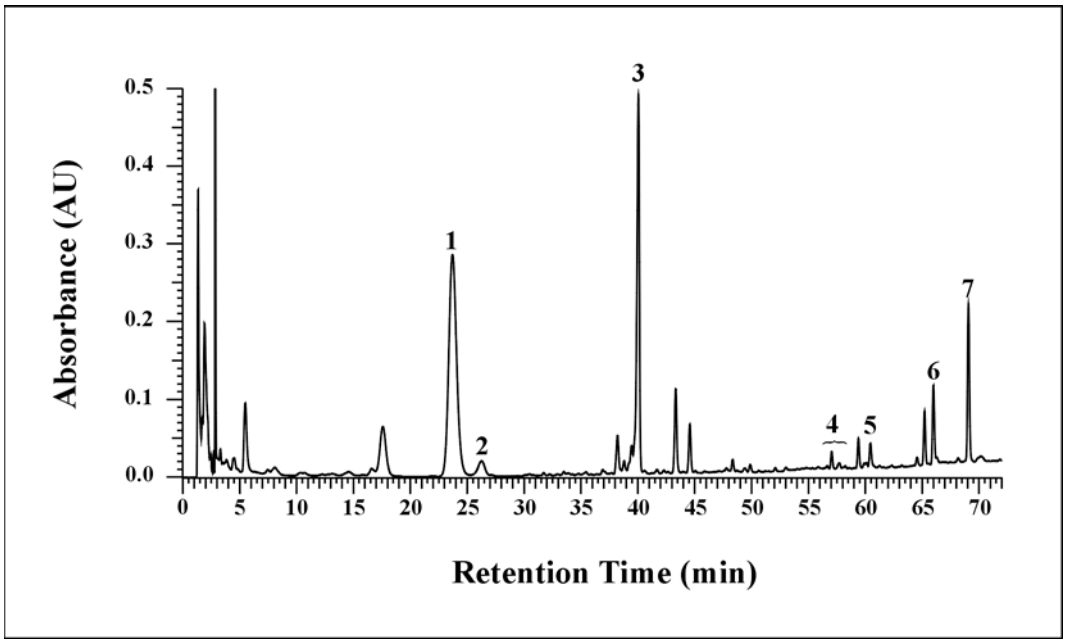


Fig. 3a: HPLC-fingerprint analysis of the MeOH-extract of *Panax notoginseng* sample 3

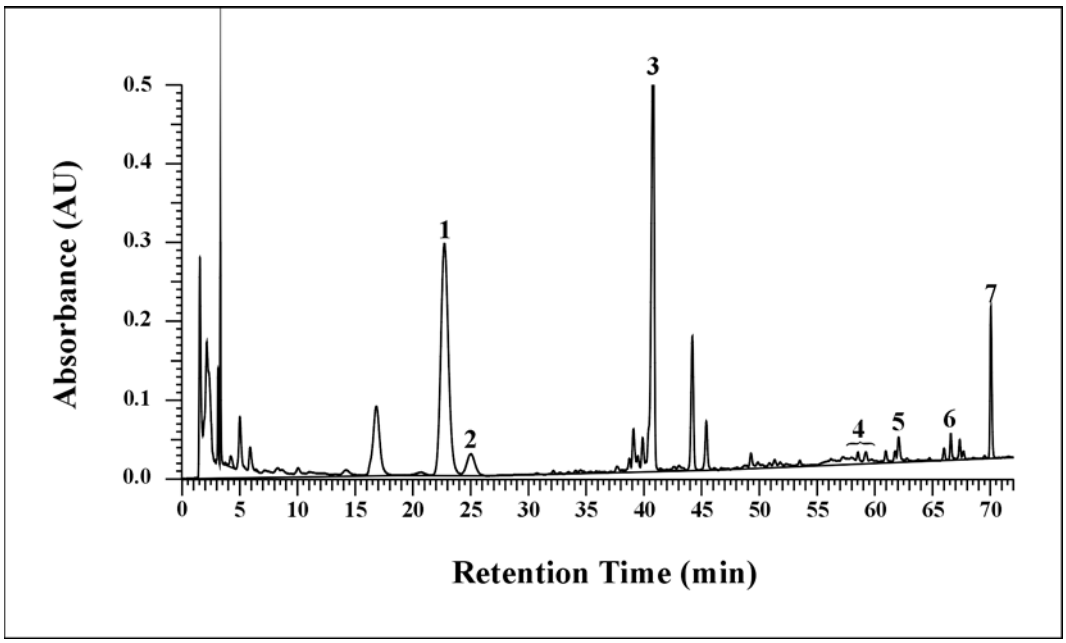


Fig. 3b: HPLC-fingerprint analysis of the MeOH-extract of *Panax notoginseng* sample 4

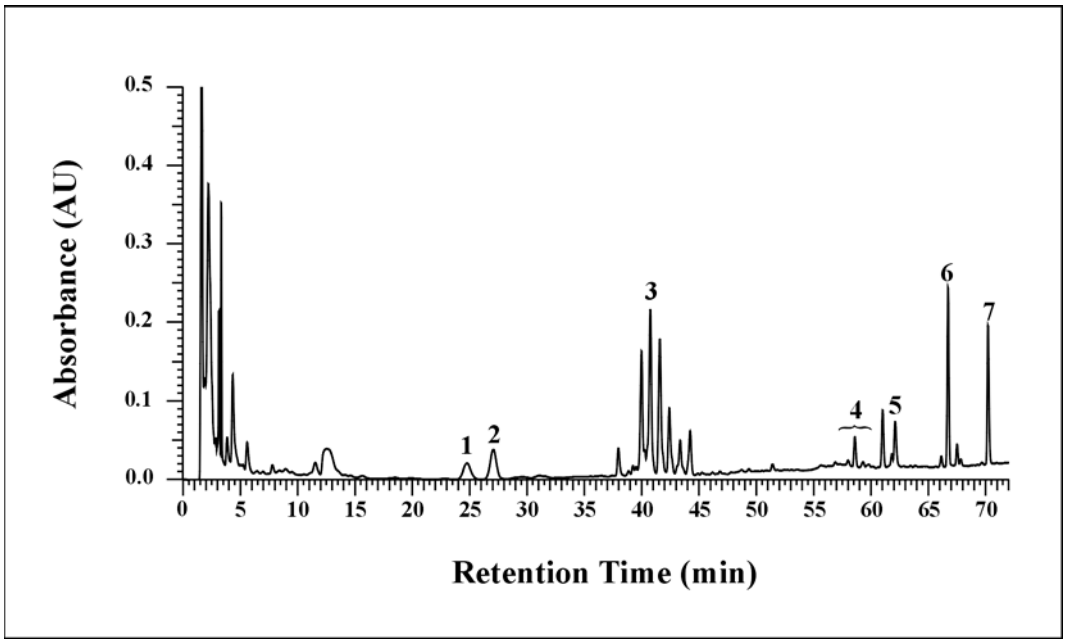


Fig. 3c: HPLC-fingerprint analysis of the MeOH-extract of *Panax ginseng* sample 5

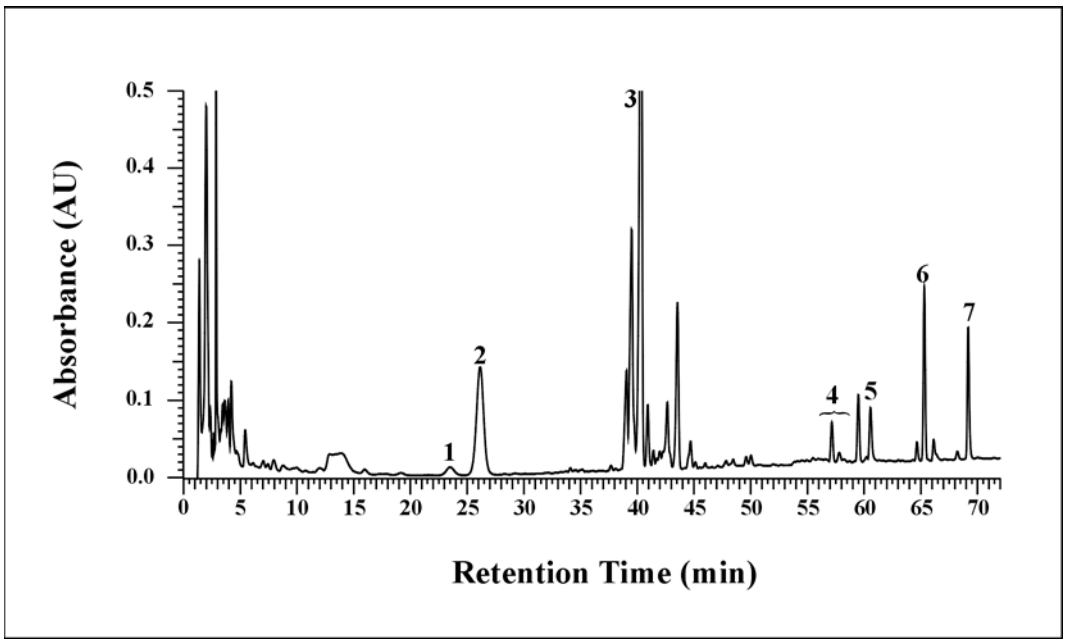


Fig. 3d: HPLC-fingerprint analysis of the MeOH-extract of *Panax quinquefolium* sample 7

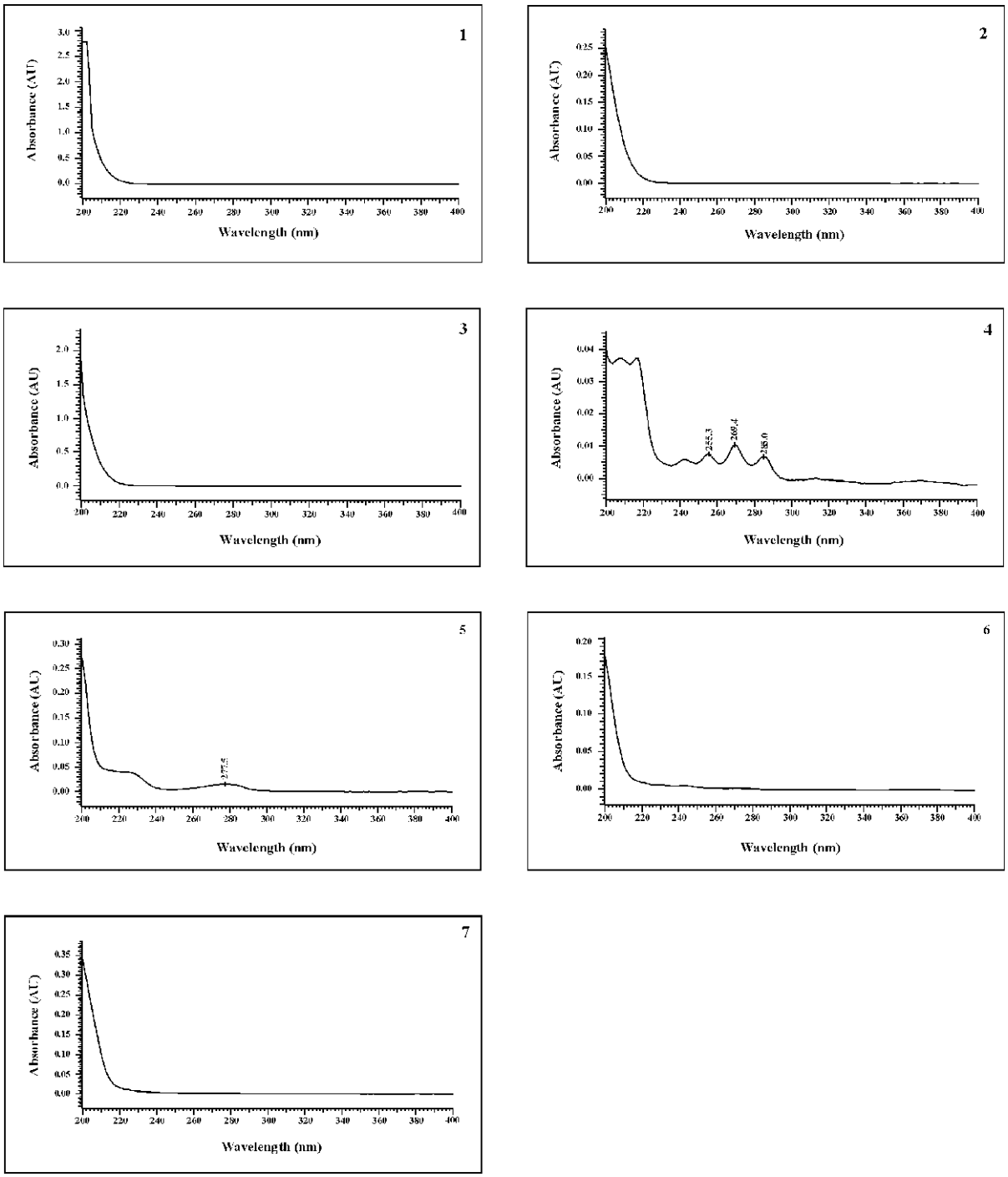


Fig. 4: On line UV-spectra of the main characteristic peaks of the HPLC-fingerprint of *Panax notoginseng*

4) Description of the HPLC-Figures

Fig. 3a, 3c, 3d:

All root extracts of *Panax Notoginseng* (only shown samples 3 and 4) are characterized by two dominating peaks of ginsenoside Rg1 (peak **1**) and ginsenoside Rb1 (peak **3**), a very low

concentrated ginsenoside Re (peak **2**) and peak accumulation **4**, the latter according to their typical polyacetylene UV-spectra identifiable as falcarinol and the falcarindiol stigmasterol appears at 60.5-62.0. The other peaks at Rt = 66.0 and 68.0 can be assigned to panaxadiol and –triol.

The *Panax Notoginseng* fingerprints can be discriminated from those of *Panax Ginseng* (Fig. 3c) (shown for sample 5) by a very low peak doublet at Rt = 25.0/25.3 (peak **1**+**2**) and an accumulation of 5-6 peaks between 40 and 46 Rt with the dominating ginsenoside Rb1(**3**). Discrimination of *Panax Notoginseng* from *Panax quinquefolium* (Fig. 3d) is possible on the basis of a peak accumulation at Rt 40 with a dominating peak **3** (Ginsenoside Rb1).

Note:

In several publications special methods for the quantitation of the main triterpen-saponins and polyacetylenes of *Panax Notoginseng* and other *Panax* species are described.[8, 14, 15, 17, 21, 22]

Conclusion

The Chinese Pharmacopoeia 2005 demands for dried Notoginseng root a total content of Ginsenosides Rg1 and Rb1 not less than 5.0 % as determined by HPLC.

The discrimination of the root of *Panax Notoginseng* from the other *Ginseng* spec. can be easily achieved by TLC and HPLC as well.

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Radix et Rhizoma Rhei – *Dahuang*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Rhubarb is the dried root and rhizome of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. or *Rheum officinale* Baill. (Fam. Polygonaceae).

The drugs are collected in late autumn when stem and leaves are withered or in next spring just before budding, removed from rootlet and the outer bark, cut into segment or section, either stringed together to be dried, or dried directly.

Origin[2]: High Mountains of Western China, especially in the provinces Sichuan, Gansu and Shaanxi

Description of the drug[1,24]: In sub cylindrical, conical, ovoid or irregular pieces, 3–17 cm long, 3–10 cm in diameter. Externally yellowish-brown to reddish-brown when peeled, sometimes whitish reticulations and scattered star spots (abnormal vascular bundles) visible, occasionally with brownish-black patches of cork, mostly with a hole through which the string passed, and coarse wrinkles. Texture compact, sometimes rather loose and lost in the centre, fracture reddish-brown or yellowish-brown, granular. Pith of the rhizome broad, with star spots arranged in a ring or irregularly scattered. Wood of the root well developed, lined radially, cambium ring distinct, without star spots. Odour, delicately aromatic; taste bitter and slightly astringent, sticky and gritty on chewing.

Pretreatment of the raw drug[1]: Foreign matters are eliminated, washed clean, softened thoroughly, cut into thick slices or pieces, and dried in the air.

I. Radix et Rhizoma Rhei (processed with wine):

The slices of Radix et Rhizoma Rhei are stir-baked as described under the method for stir-baking with wine (appendix II D of [1]) to dryness.

II. Radix et Rhizoma Rhei (prepared):

The pieces of Radix et Rhizoma Rhei are stewed or steamed as described under the method for stewing or steaming with wine (appendix II D of[1]) until the drug darkens thoroughly.

III. Radix et Rhizoma Rhei (carbonized):

The slices of Radix et Rhizoma Rhei are stir-baked as described under the method for carbonizing by stir-baking (appendix II D of[1]) until the outer surface is charred and the inner turns to be dark brown.

Medicinal use[3]: Depending on the content of anthraquinone derivatives and tannins and dosage Radix et Rhizoma Rhei is used as laxative or astringent and stomachicum. Inter alia in case of hemorrhoids, anal fissures, liver and bile diseases or gastroenteritis.

Table 1:

Effects and indications of Radix et Rhizoma Rhei according to Traditional Chinese Medicine[1, 2, 4]	
Taste:	bitter
Temperature:	cold
Channels entered:	*Orbis linealis et stomachi*, *Orbis intestini crassi*, *Orbis pericardialis*, *Orbis hepaticus*.
Effects (functions):	To purge *fire* and *dredge* intestines, reduce *heat* in blood and counteract *toxicity*, and eliminate blood stasis and stimulate menstruation. Preparation I: To remove *toxic heat* from the blood in the upper portion of the body. Preparation II: To purge *heat* and remove toxic substances, but with less effect. Preparation III: To reduce *heat* in blood, remove blood stasis, and arrest bleeding.
Symptoms and indications:	Fever with constipation, retention of feces and abdominal pain; dysentery; jaundice caused by *damp-heat*; hematemesis, epistaxis, inflammation of eyes and sore throat due to *heat* in blood; appendicitis with abdominal pain; boils, sores and abscess; amenorrhea due to blood stasis; traumatic injuries; hemorrhage from the upper gastrointestinal tract; external use for scalds and burns. Preparation I: Inflammation of the eye, swelling of the throat and painful swelling of the gums. Preparation II: Boils, sores and abscess. Preparation III: Hemorrhage due to stagnation and *heat* in the blood.

Main constituents[8-10]:

- **Anthraquinone glycosides and their aglycones**

  Physcion 1-*O*-*β*-D-glucopyranoside
  Aloe emodin 1-*O*-*β*-D-glucopyranoside
  Rheum emodin 1-, 3- and 8-*O*-*β*-D-glucopyranoside
  Chrysophanol 1- and 8-*O*-*β*-D-glucopyranoside
  Rhein 1-*O*-*β*-D-glucopyranoside
  Physcion 8-*O*-*β*-D-gentiobioside

Minor constituents:

- **Bianthrones and their glycosides**

  Chrysophanol bianthrone
  Aloe emodin bianthrone
  Sennidin A/B/C
  Palmidin A/B/C
  Rheidin A/B/C and Rheinosides A/B/C/D
  Sennoside A/B/C/D/E/F

- **Chromones and chromone derivatives**

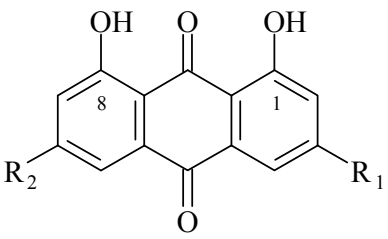
- **Tannins**

  > 20 various types of galloyl-, cinnamoyl- and coumaroyl-glucose derivatives e. g.
  Piceatannol and *O*-galloylglucosides and galloyl-procyanidins

- **Stilbenes**

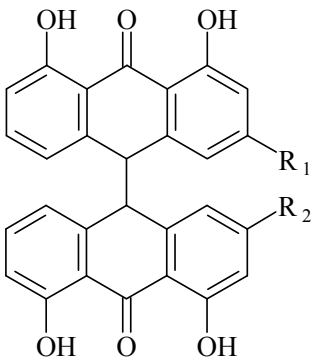
  Resveratrol dimers
  Resveratrol-*O*-galloylglucosides
  In *Rheum rhaponticum* and *Rheum undulatum:*
  Rhaponticin/Isorhaponticin
  Desoxyrhaponticin
  (Des)oxyrhapontigenin *O*-actoylglucosides

Fig. 1: Formulae of the main compounds of Radix et Rhizoma Rhei[8]

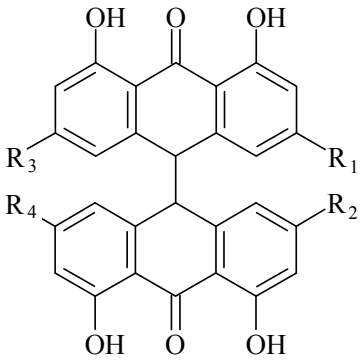


R_1 = Me, R_2 = H: Chrysophanol
R_1 = CH_2OH, R_2 = H: Aloe emodin
R_1 = COOH, R_2 = H: Rhein
R_1 = OH, R_2 = Me: Emodin
R_1 = OMe, R_2 = Me: Physcion

and their 1- or 8-*O*-glycosides

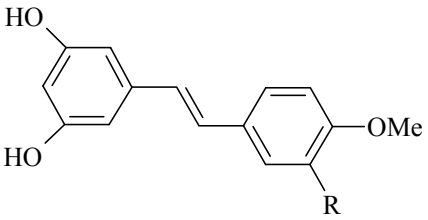


R_1/R_2 = Me: Chrysophanol bianthrone
R_1/R_2 = CH_2OH: Aloe emodin bianthrone
R_1/R_2 = COOH: Sennidin A
R_1 = Me, R_2 = CH_2OH: Palmidin B
R_1 = Me, R_2 = COOH: Rheidin B
R_1 = CH_2OH, R_2 = COOH: Sennidin C



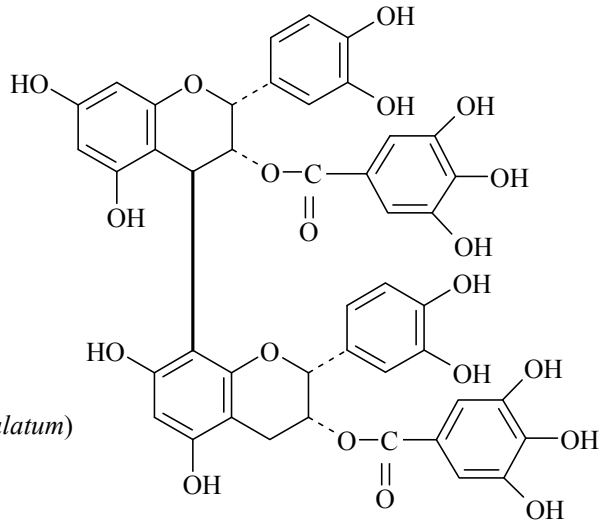
R_1 = CH_2OH, R_2 = OH, R_3 = H, R_4 = Me: Palmidin A
R_1 = Me, R_2 = OH, R_3 = H, R_4 = Me: Palmidin C
R_1 = Me, R_2 = COOH, R_3 = OH, R_4= H: Rheidin A
R_1 = Me, R_2 = COOH, R_3 = MeO, R_4 = H: Rheidin C

Stilbenes



R = H: Desoxyrhaponticin
R = OH: Rhaponticin
R = OMe: Isorhaponticin
(adulteration: *Rheum rhaponticum* or *Rheum undulatum*)

Galloylprocyanidines



3,3´-Di-*O*-galloyl-procyanidin B-2

Pharmacology[11-18]:

- purgative effect
- antibiotic activity (→anthraquinone derivatives)
- antifungal activity
- antiviral activity (→chrysophanol 8-*O*-*β*-D-glucoside)
- antitumor activity (→emodin, rhein)
- anti-inflammatory activity (→lindleyin)
- inhibition of hyaluronidase (→catechin, epicatechin, *O*-galloylglucose)
- decrease of cholesterol and urea-nitrogen (→rhatannin, oligostilbenes)
- hemostatic activity (→anthraquinone derivatives, catechin, gallic acid)
- diuretic effect (→emodin)
- vasorelaxant effects (→emodin)
- anti-diabetic effects (→chrysophanol and its glycosides)
- anti-atherogenic effects
- anti-nephrotoxic

TLC fingerprint analysis:

Table 2: (see Fig. 2a – c)

Drug samples		Origin
1	Radix et Rhizoma Rhei / *Rheum palmatum*	Province Kansu, China
2	Radix et Rhizoma Rhei / *Rheum palmatum*	Province Qinghai, China
3	Radix et Rhizoma Rhei / *Rheum palmatum*	Province Xizang, China
4	Radix et Rhizoma Rhei / *Rheum tanguticum* I	Province Qinghai, China
5	Radix et Rhizoma Rhei / *Rheum tanguticum* II	Province Qinghai, China
6	Radix et Rhizoma Rhei / labeled as *Rheum tanguticum* III, but identified as *Rheum rhaponticum* or *Rheum undulatum*	Province Qinghai, China
7	Radix et Rhizoma Rhei / *Rheum officinale*	Province Sichuan, China
8 - 12	Radix et Rhizoma of non identified *Rheum* species	samples of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany (1995, 2000, 2003, 2005)
13	Radix et Rhizoma Rhei / *Rheum officinale*	reference sample from Phytochem®, Ulm
14	Radix et Rhizoma Rhei / *Rheum rhaponticum* (Fig. 2b+c only)	reference sample from Pharm. Comp. Müller-Göppingen®

Table 3:

Reference compounds of Figure 2b		R*f*
T1	Rhein-glucoside	0.09
T2	Rhein	0.42
T3	Aloe-emodin-monoglucoside	0.41
T4	Rhaponticin (only in adulterations)	0.45
T5	Chrysophanol-monoglucoside	0.51
T6	Physcion-glucoside	0.52
T7	Aloe-emodin	0.89
T8	Chrysophanol	0.93

Table 4:

Reference compounds of Figure 2c		R*f*
T9	Aloe-emodin	0.17
T10	Rhein	0.21
T11	Emodin (= Rheum–emodin)	0.31
T12	Physcion	0.52
T13	Chrysophanol (impured with Physcion)	0.60

TLC-fingerprint analysis[17]:

1. Thin layer chromatograms of anthraquinones (Fig. 2a + b):

1) Extraction: 0.5 g powdered drug is extracted in a water bath with 5 ml methanol for 5 minutes. The filtrate is used for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Radix et Rhizoma Rhei extracts: each 10 µl
reference compounds: each 10 µl

Solvent system: ethyl acetate : methanol : water
100 : 13.5 : 10

Detection: without chemical treatment → UV-366 nm

Drug samples	labeled as
1 – 3	*Rheum palmatum* species
4 – 6	*Rheum tanguticum* species (sample 6 identified as *Rheum rhaponticum* / *Rheum undulatum*)
7 + 13	*Rheum officinale* species
8 – 12	Not identified *Rheum* species

4) Description (Figure 2a/b):

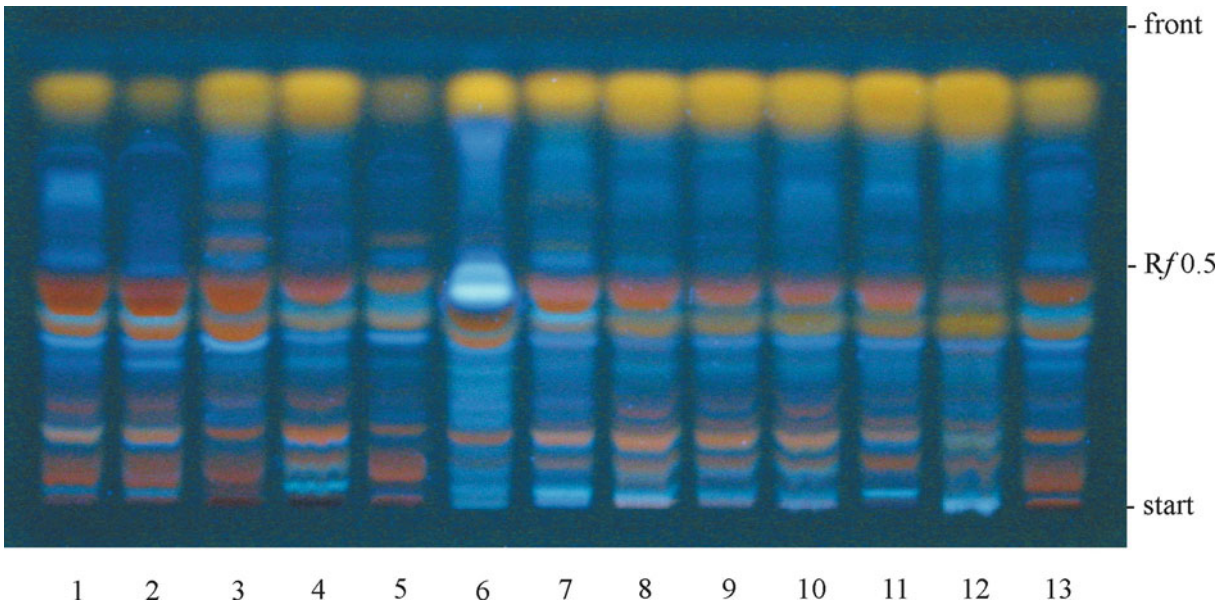


Fig. 2a: Thin layer chromatogram (antraquinone glycosides) of the methanol extracts of Radix et Rhizoma Rhei detected without chemical treatment at UV 366 nm

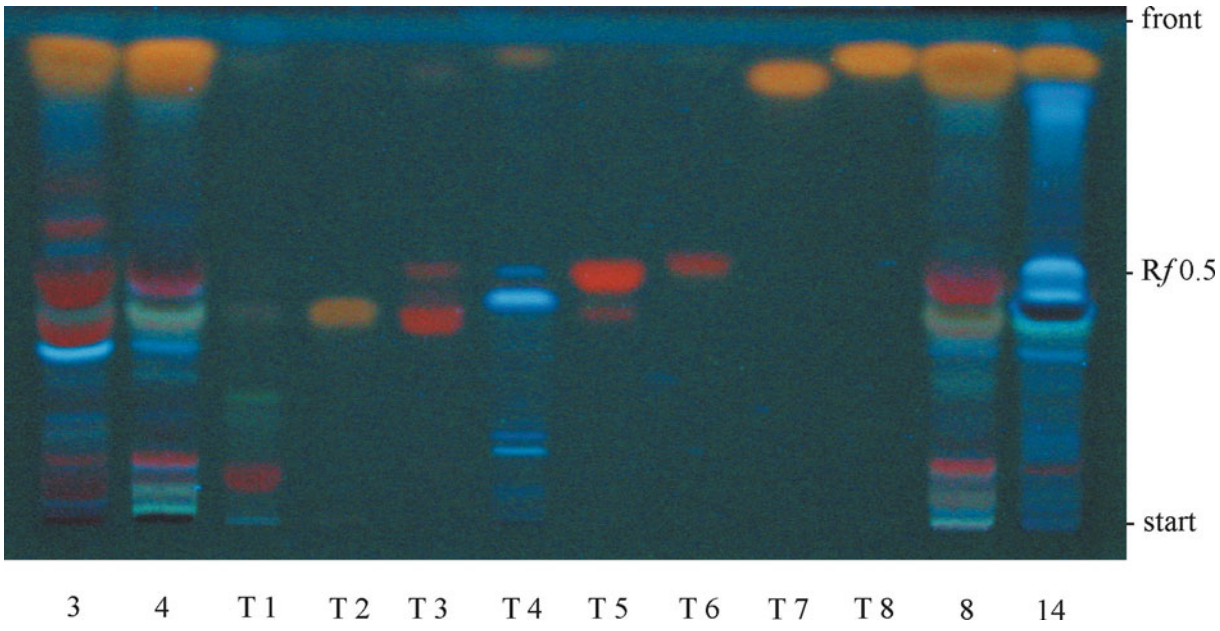


Fig. 2b: Thin layer chromatogram (antraquinone glycosides) of the methanol extracts of Radix et Rhizoma Rhei detected without chemical treatment at UV 366 nm

All 13 samples with the exception of sample 6 show a very homogeneous pattern of 7 – 9 light brown, red or orange colored bands.

- Just below the TLC-front at R*f* = 0.85 – 9.0 appears a strong light brown zone which consists of the Rheum-aglycone. Chrysophanol overlapped by Aloe-emodin.
- In the middle R*f* – range one bright red zone at R*f* = 0.51 and directly below at R*f* = 0.41 a light brown band can be identified as Chrysophanol-monoglucoside and Aloe-emodin-monoglucoside respectively. The latter are overlapped in samples 4, 5, 7 – 12 by the aglycone Rhein.
- In the R*f* – range from R*f* = 0.09 – 0.25 three zones can be seen, one of them identified as Rhein-monoglycoside at R*f* = 0.09. The other bands might be anthraquinone-diglycosides and Bianthronoids.
- Sample 6 differs from all the other samples in the middle and deep R*f* – range by a relatively low concentration of anthraquinones, and in the R*f* – range of 0.40 – 0.50 by strong blue fluorescent zones of Rhaponticin and Desoxyrhaponticin.

2. Thin layer chromatogram of aglycones (Fig. 2c):

1) Extraction: (the same extraction procedure as at 1.1))

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Radix et Rhizoma Rhei extracts: each 10 µl reference compounds: each 10 µl
Solvent system:	light petroleum : ethyl acetate : formic acid 75 25 1
Detection:	without chemical treatment → UV-366 nm

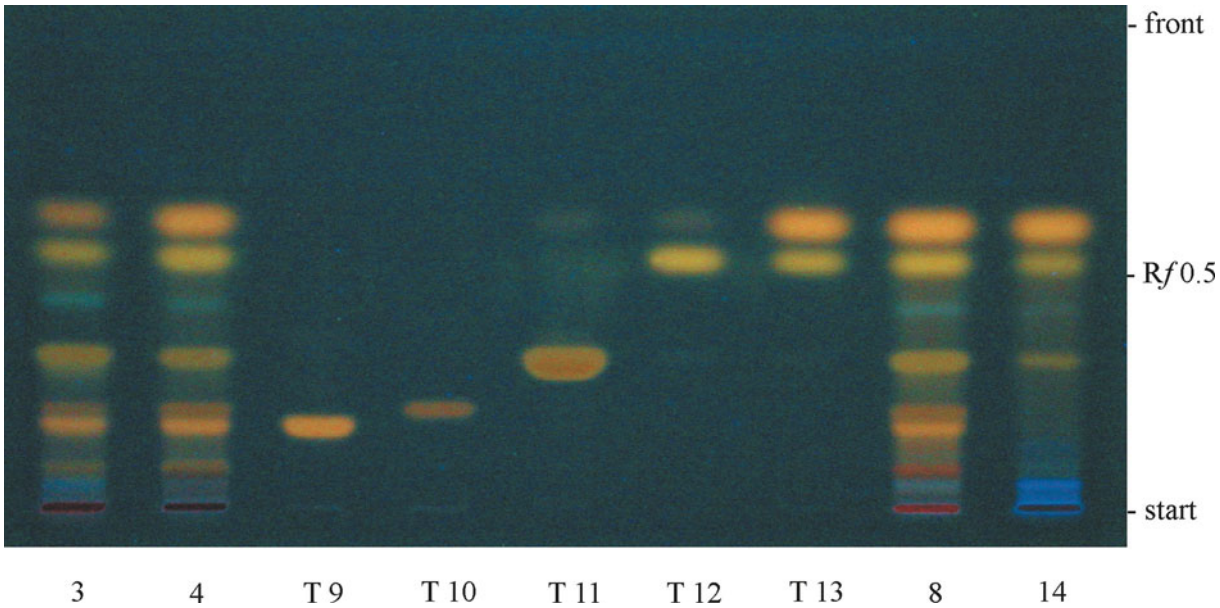


Fig. 2c: Thin layer chromatogram (antraquinone aglycones) of the methanol extracts of Radix et Rhizoma Rhei detected without chemical treatment at UV 366 nm

4) Description (Figure 2c):

In contrast to the method of the Chinese Pharmacopoeia 2005[1] which describes the detection of the anthraquinone aglycones after hydrochloric acid hydrolysis of the glycosides, we propose a very simple method of detecting the free aglycones present in the MeOH-extracts by using the solvent system light petroleum/ethyl acetate/formic acid (75 : 25 : 1). In this chromatogram (Fig. 2c) the antraquinone aglycones without pretreatment with any reagent appear in UV-366 nm in ascendent sequence:

Aloe-emodin (**T9**) at R*f* = 0.17, Rhein (**T10**) at R*f* = 0.21, Rheum-emodin (**T11**) at R*f* = 0.31, Physcion (**T12**) at R*f* = 0.52 and Chrysophanol (**T13**) at R*f* = 0.60.

The MeOH-root/rhizome-extracts of *Rheum palmatum* (sample 3), *Rheum tanguticum* (sample 4) and *Rheum officinale* (sample 8) contain all free aglycones with a dominance of Chrysophanol and Emodin. The red/orange zones in the R*f* – range of 0.05 – 0.15 are monoglycosides of Aloe-emodin and Rhein.

HPLC-fingerprint analysis[10,19-24] :

1) Sample preparation: 0.5 g powdered drug is extracted for 5 minutes on a water bath with 5 ml methanol. The extract is filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: Radix et Rhizoma Rhei extracts: each 5.0 µl

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector

	MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent:	A: 0.05 % H_3PO_4 (phosphoric acid, Merck / water, Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	6 – 20 % B in 8 minutes 20 – 32 % B in 16 minutes 32 – 48 % B in 14 minutes 48 – 97 % B in 5 minutes 97 % B for 12 minutes total runtime: 55 minutes
Flow:	1.0 ml/min.
Detection:	280 nm

Retention times of the main peaks:

Table 5:

Peak	Rt (min.)	Compound
1	4.8	Gallic acid
2	10.2	Catechin
3	14.4	Rhaponticin or Isorhaponticin (only in adulterations)
4	14.7	Aloe-emodin-monoglucoside
5	15.7	Rhein-glucoside
6	16.8	Not identified gallotannin
7	17.3	Isorhaponticin or Rhaponticin (only in adulterations)
8	19.1	Not identified gallotannin
9	24.3	Chrysophanol-glucoside
10	24.8	Not identified anthraquinonglucoside
11	25.1	Desoxyrhaponticin (only in adulterations)
12	28.9	Physcion-glucosid
13	35.8	Not identified gallotannin
14	37.1	Aloe-emodin
15	39.1	Rhein
16	44.8	Emodin (= Rheum-emodin)
17	47.3	Chrysophanol
18	48.3	Physcion

4) Description of the HPLC-fingerprints of the root/rhizome *Rheum palmatum, Rheum tanguticum, Rheum officinale* and *Rheum rhaponticum* (see Fig. 3a–3e, recorded at UV-280 nm):

- In Table 5 are listed the detected HPLC-peaks with their Rt-values and the corresponding assigned *Rheum* constituents. All three *Rheum* species contain the same anthraquinone glycosides and aglycones but in different concentrations analogue to the TLC-pattern in Figure 2a. *Rheum palmatum* seems to have the highest content of anthraquinone glycosides, followed by *Rheum officinale*/*R. tanguticum*. All anthraquinone aglycones (Rhein, Aloe-emodin, Chrysophanol and Physcion) appear in contrast to the anthraquinone-monoglycosides in lower concentration than the glycosides.
- The *Rheum rhaponticum* extract shows a distinctly different peak pattern, characterized by the strong peaks **3**, **7** and **11**, which according to their UV-spectra can be assigned to the estrogenic Isorhaponticin, Rhaponticin and Desoxyrhaponticin respectively.
- The gallic acid can be identified in peak **1**, the various gallotannins (glucosides) in the peaks **6**, **8** and **13** and Catechin in peak **2**.

Note:

There are further publications [1–9, 19–23] in which further detailed results of HPLC-investigations of *Rheum palmatum, Rheum tanguticum, Rheum officinale, Rheum rhaponticum* and other *Rheum* species are described. In the Chinese Pharmacopoeia 2005 and in Vol. II of the Hong Kong Chinese Materia Medica Standard[24] the various *Rheum* species are characterized through the HPLC-fingerprints of their anthraquinone aglycones after HCl-hydrolysis of all glycosides of the *Rheum* spec. extracts.

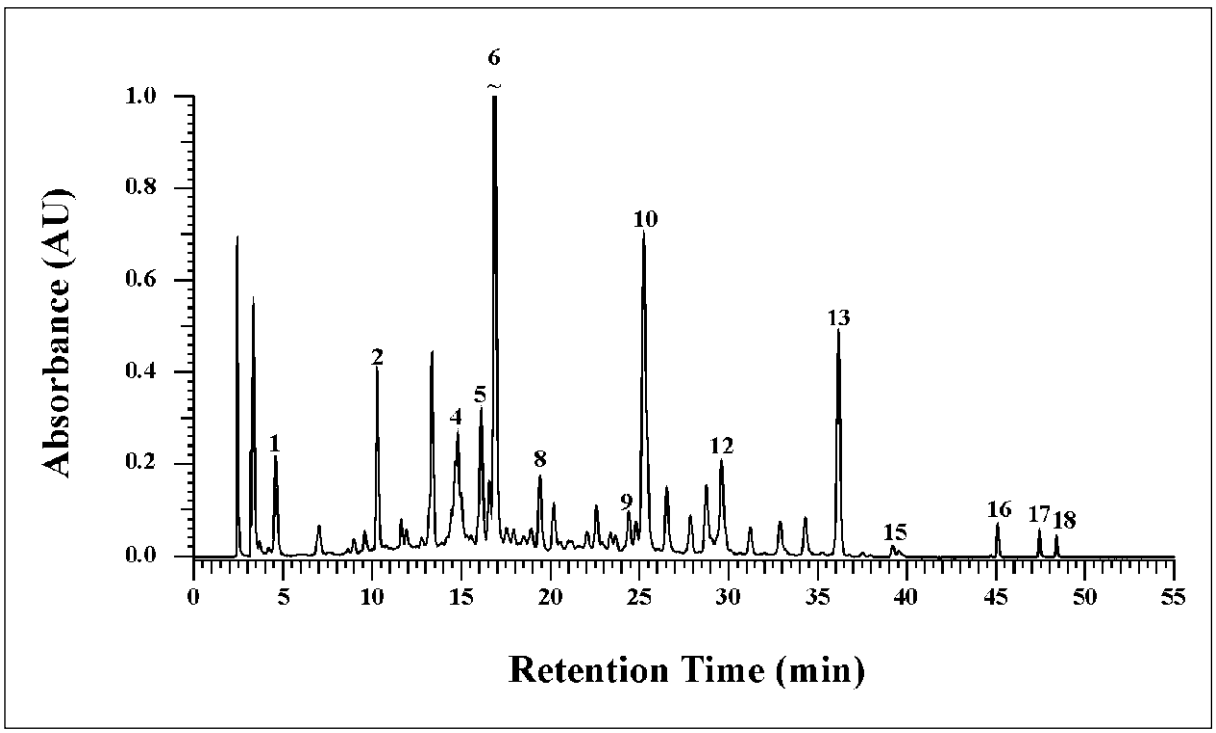


Fig. 3a: HPLC fingerprint of sample 1, root of *Rheum palmatum*

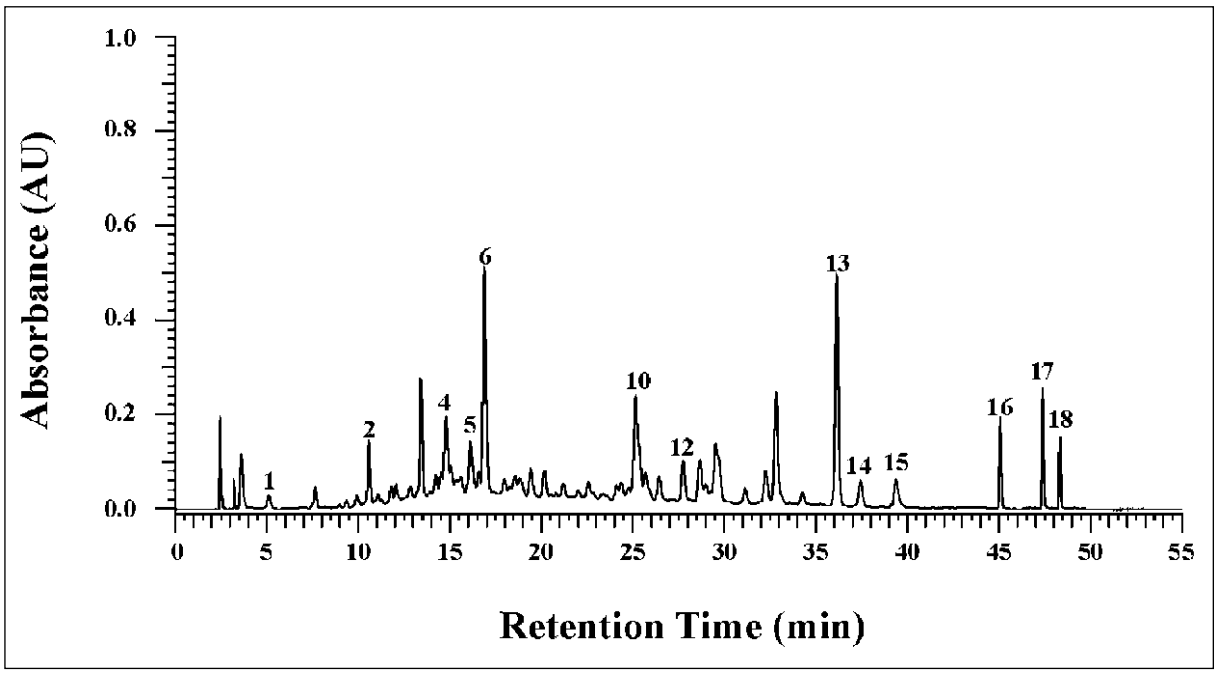


Fig. 3b: HPLC fingerprint of sample 4, root of *Rheum tanguticum*

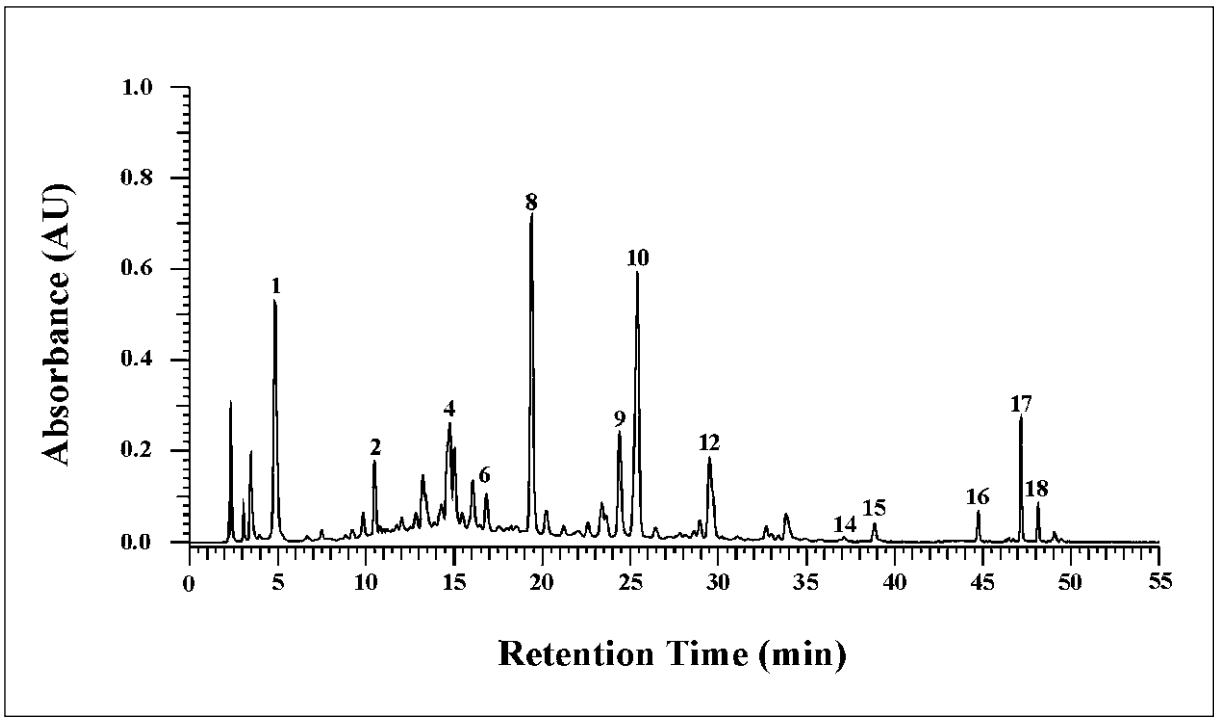


Fig. 3c: HPLC fingerprint of sample 7, root of *Rheum officinale*

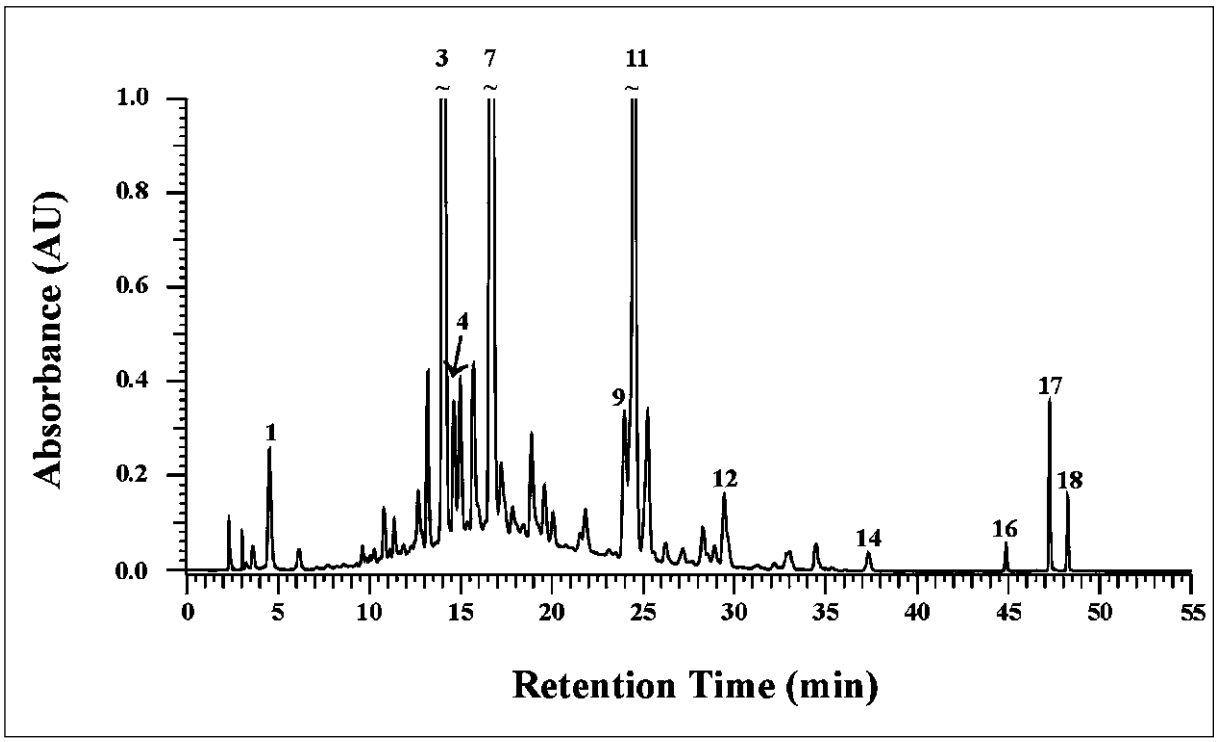


Fig. 3d: HPLC fingerprint of sample 6, labeled as root of *Rheum tanguticum* but identified as root of *Rh. raphonticum* or *Rh. undulatum*

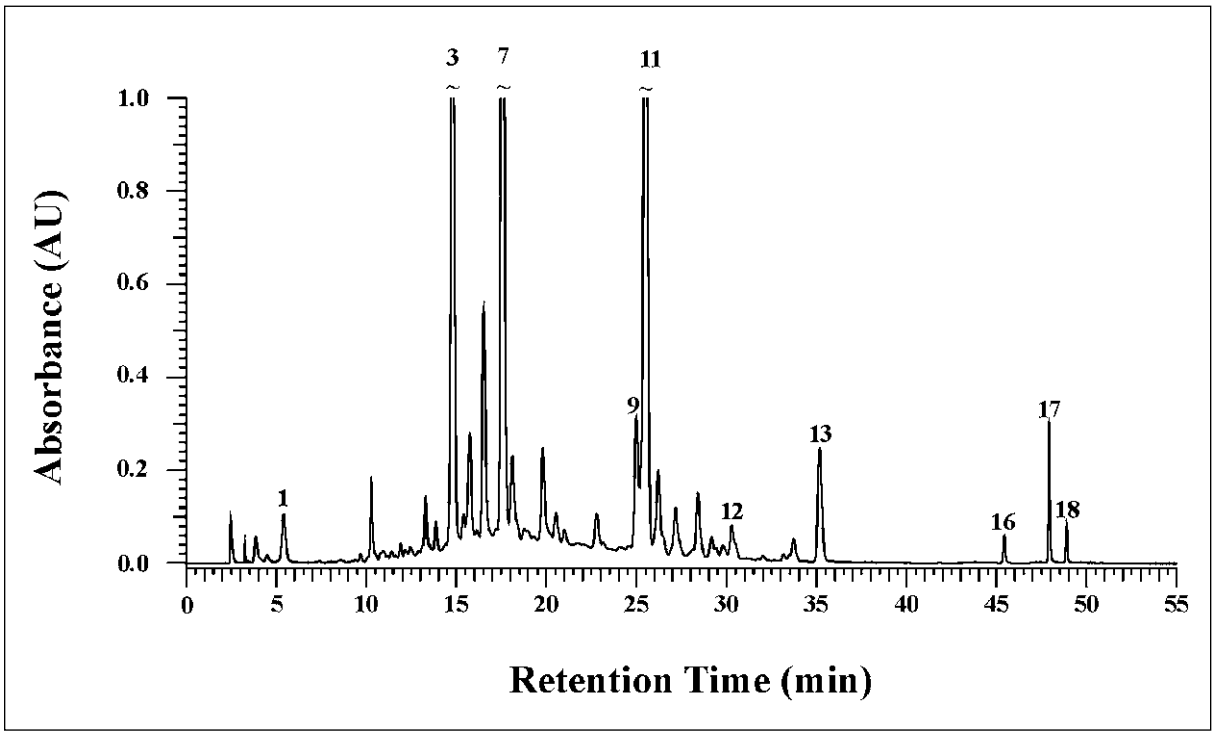
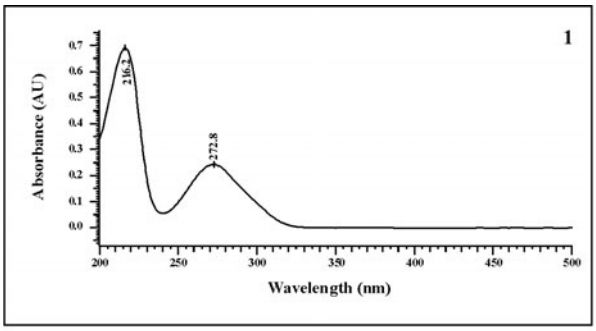
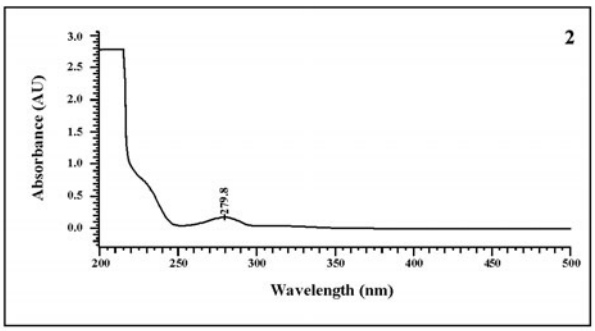
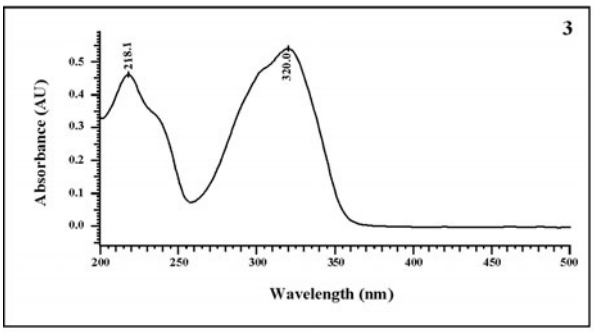


Fig. 3e: HPLC fingerprint of sample 14, authentic root of *Rheum rhaponticum*


1
Absorbance (AU)
Wavelength (nm)
216.2
272.8


2
Absorbance (AU)
Wavelength (nm)
279.8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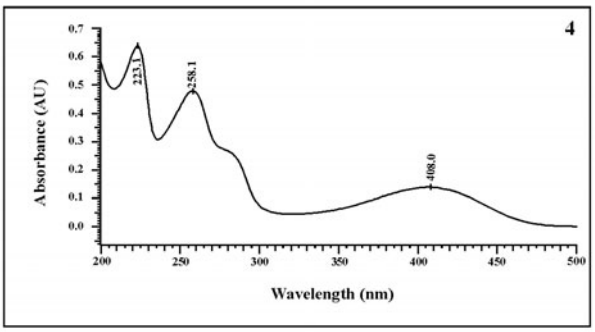
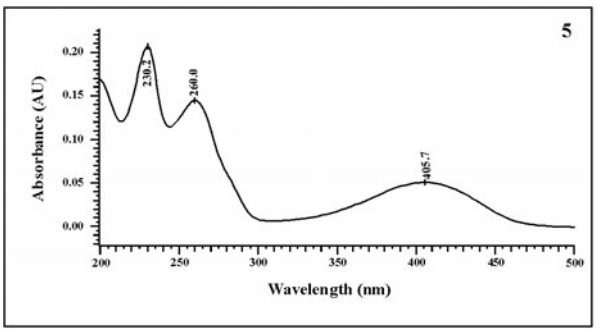
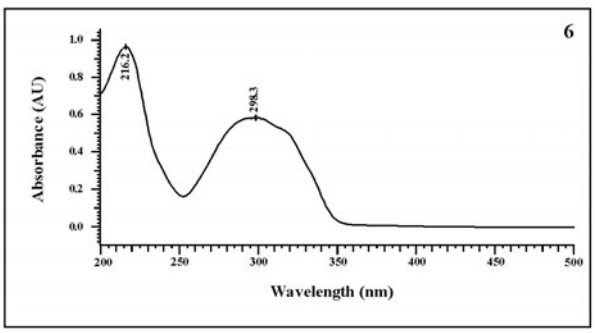
Absorbance (AU)
Wavelength (nm)
218.1
320.0


4
Absorbance (AU)
Wavelength (nm)
223.1
258.1
408.0


5
Absorbance (AU)
Wavelength (nm)
230.2
260.0
405.7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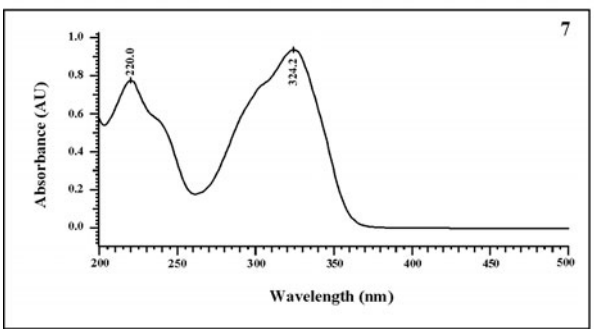
Absorbance (AU)
Wavelength (nm)
216.2
298.3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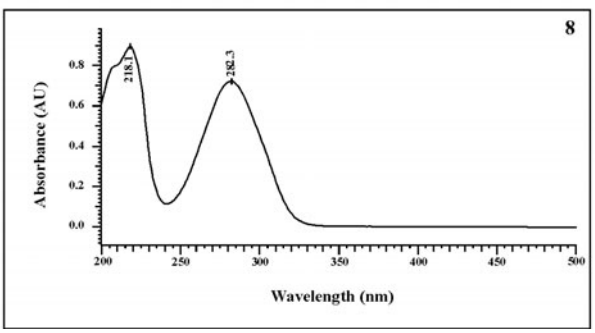
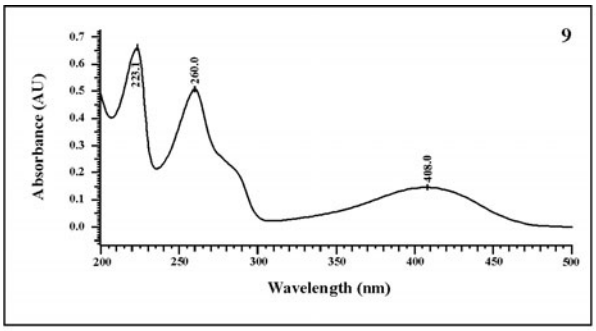
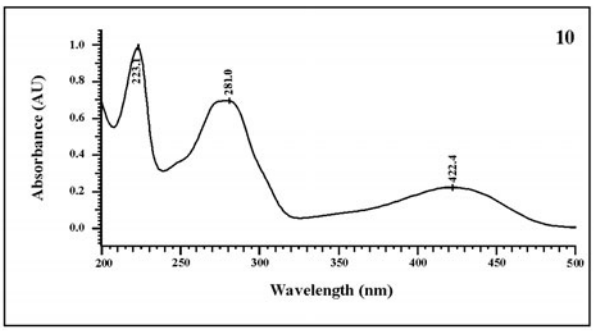
Absorbance (AU)
Wavelength (nm)
220.0
324.2


8
Absorbance (AU)
Wavelength (nm)
218.1
282.3


9
Absorbance (AU)
Wavelength (nm)
223.1
260.0
408.0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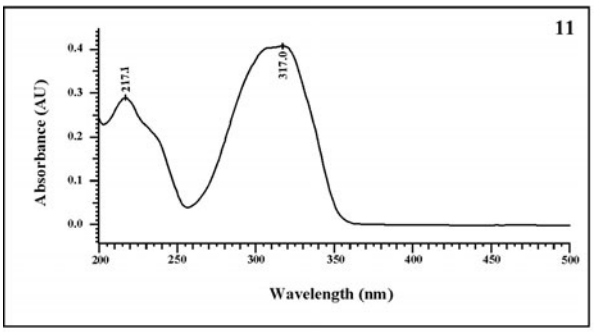
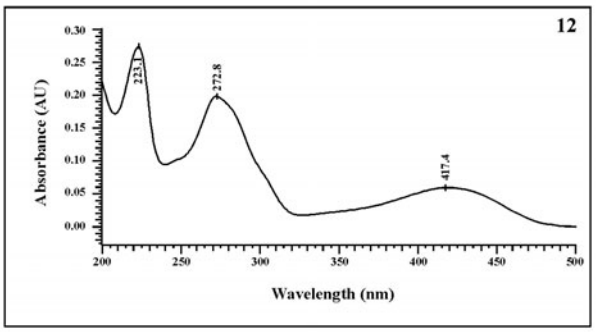
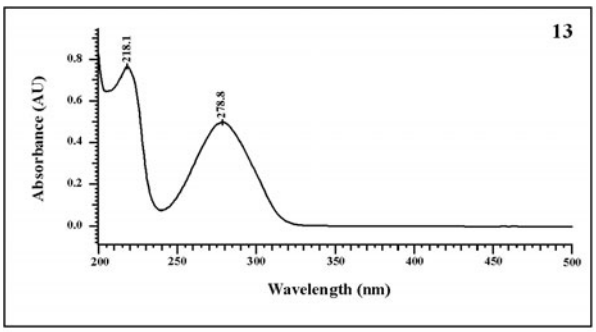
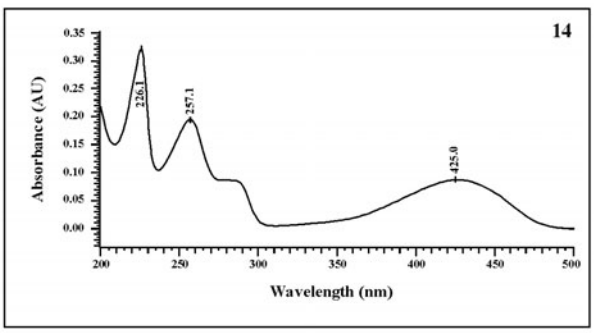
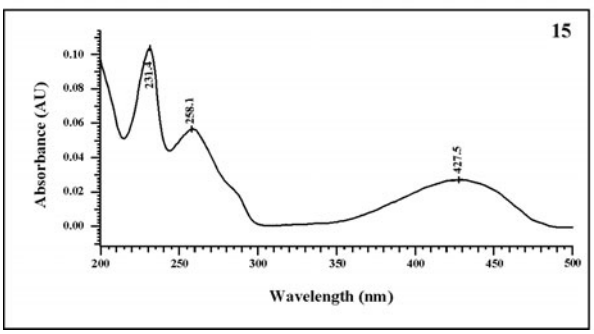
Absorbance (AU)
Wavelength (nm)
225.1
281.0
422.4

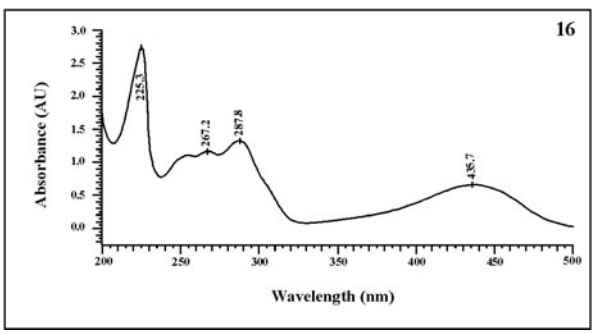

11
Absorbance (AU)
Wavelength (nm)
217.1
317.0


12
Absorbance (AU)
Wavelength (nm)
223.1
272.8
417.4


13
Absorbance (AU)
Wavelength (nm)
218.1
278.8


14
Absorbance (AU)
Wavelength (nm)
226.1
257.1
425.0


15
Absorbance (AU)
Wavelength (nm)
231.2
258.1
427.5


16
Absorbance (AU)
Wavelength (nm)
225.3
267.2
287.8
435.7

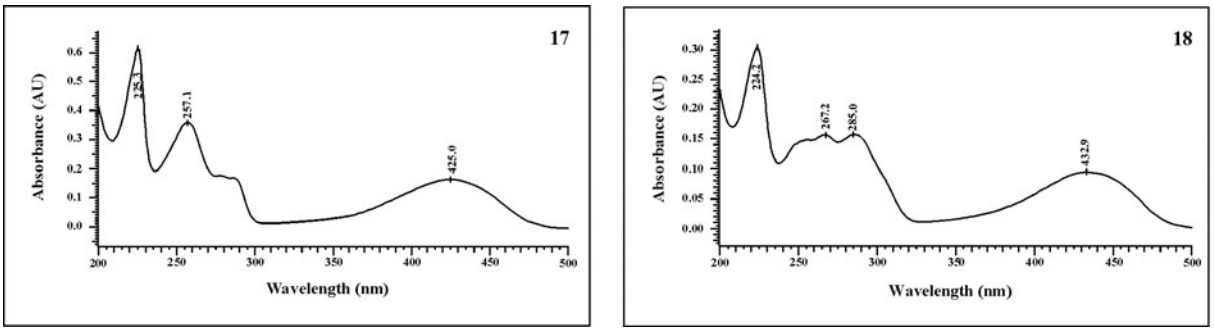


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of *Rheum* spec.

Conclusion

The extracts of the three official *Rheum* spec., *Rheum palmatum, Rheum tanguticum* and *Rheum officinale* possess a very similar qualitative composition of anthraquinone glycosides and their free aglycones. They can be hardly distinguished from each other by TLC alone. The anthraquinone aglycones show quantitatively also a very similar fingerprint pattern. The adulterations, *Rheum rhaponticum* or *Rheum undulatum,* characterized by a high content of the phytoestrogenic stilbenes (rhaponticin, isorhaponticin and desoxyrhaponticin) can be easily detected by TLC due to two distinct blue fluorescent bands at R*f*-value around 0.5. The corresponding graphs of the HPLC-fingerprints of the three official *Rheum* spec. show also a very similar qualitative peak pattern, without the possibility of a clear discrimination, whereas the adulterations of *Rheum rhaponticum* or *Rheum undulatum* can be again easily detected due to three significant peaks, not present in other *Rheum* species.

Note: According to the Chinese Pharmacopoeia 2005[1] Radix et Rhizoma Rhei should contain not less than 1.5 % of the total amount of aloe-emodin, rhein, emodin, chrysophanol and physcion, calculated with reference to the dried drug.

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Radix et Rhizoma Ginseng – *Renshen*
Radix Panacis Quinquefolii – *Xiyangshen*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]:

Radix et Rhizoma Ginseng

Ginseng is the dried root of *Panax ginseng* C.A. Mey. (Fam. Araliaceae).

The drug obtained from the cultivated form is known as "Yuanshen" (garden ginseng) and the drug obtained from the wild origin is known as "Shanshen" (wild ginseng).

The drug is collected in autumn and washed clean. Sun - dried or bake - dried Yuanshen is known as "Shengshaishen" (sun - dried ginseng). Sun - dried Shanshen is known as "Shenshaishanshen" (sun - dried wild ginseng).

Radix Panacis Quinquefolii

American Ginseng is the dried root of *Panax quinquefolium* L. (Fam. Araliaceae).

All the commercial supplies are obtained from cultivated forms.

The drug is collected in autumn, washed clean, and dried in the sun or at a lower temperature.

Origin:

Radix et Rhizoma Ginseng: [2, 5]

north-eastern China (Jilin, Liaoning), South-Korea, Japan, Russia

Radix Panacis Quinquefolii:[7]

cultivated in United States, Canada, France and northern China

Description of the drugs[1]:

Radix et Rhizoma Ginseng:

Main roots fusiform or cylindrical, 3 – 15 cm long, 1 – 2 cm in diameter; externally greyish-yellow, the upper part or entire root exhibiting sparse, shallow, interrupted and coarse transverse - striations and distinct longitudinal wrinkles; the lower part bearing 2 – 3 branch roots and numerous slender rootlets with inconspicuous minute tubercles. Rhizomes (Lutou) 1 – 4 cm long, 0.3 – 0.5 cm in diameter, mostly constricted and curves, bearing adventitious roots (Ding) and showing sparse depressed-circular stem scars (Luwan). Texture relatively hard, fracture yellowish-white, starchy, cambium ring brownish-yellow, bark exhibiting

yellow - brown dotted resin canals and radial clefts. Odour characteristic; taste, slightly bitter and sweet.

Alternatively, main roots as long as or shorter than rhizome, cylindrical, rhomboid or V - shaped, 1–6 cm long; externally greyish-yellow, longitudinally wrinkled, the upper or middle - lower part with annulations, branch roots mostly 2, rootlets less and slender, orderly arranged and showing some distinct warts. Rhizomes slender, a kew stout, the upper part exhibiting sparse or dense deep depressed stem scars adventitious roots relatively thin, mostly reclinate.

Radix Panacis Quinquefolii:

Fusiform, cylindrical or conical, 3 – 12 cm in length, 0.8 – 2 cm in diameter. Externally pale yellowish-brown or yellowish-white, exhibiting transverse - striations and linear lenticel - like protrudings, and showing fine and dense longitudinal wrinkles, and rootlet scars. The middle and lower part of the main root with 1 to several lateral roots, mostly broken off. Sometimes, the upper part with rhizome (Lutou) showing distinct annulations, rounded or semi-rounded stem scars (Luwan), and bearing adventitious roots (Ding) or broken off. Texture heavy and hard, uneasily broken, fracture even, yellowish-white, slightly starchy, bark exhibiting yellowish-brown dotted resin canals, cambium ring brownish-yellow, wood exhibiting less distinct radiate striations. Odour, slight and characteristic; taste, slightly bitter and sweet.

Pretreatment of the raw drugs[1]:

Radix et Rhizoma Ginseng:
The drug is softened thoroughly, cut into thin slices and dried, pulverized or broken to pieces before use.

Radix Panacis Quinquefolii:

After removal of the Rhizome (Lutou), the remaining Radix is softened thoroughly, cut into thin slices, dried, or pounded to pieces before use.

Medicinal use:

Radix et Rhizoma Ginseng:[2]
Prophylactic and restorative agent for enhancement of mental and physical capacities, in cases of weakness, exhaustion, tiredness and loss of concentration, during convalescence. It is also used in the treatment of diabetes, impotence, prevention of hepatotoxicity and gastrointestinal disorders such as gastritis and ulcers.

Radix Panacis Quinquefolii:[3]

Internally used as a diuretic, digestive, tonic and a stimulant. It is also used to enhance stress resistance, to treat atherosclerosis, bleeding disorders, cough, loss of appetite, colic, vomiting, dysentery, insomnia, neuralgia, rheumatism and headaches.

Effects and indications of *Panax ginseng* according to Traditional Chinese Medicine[1,5,7]	
Taste:	Sweet
Temperature:	Warm
Channels entered:	*Orbis lienalis*, *orbis pulmonalis*
Effects (functions):	To reinforce *qi*, rescue collapse and restore the normal pulse, to benefit the spleen and lung, promote the production of body fluids, and anchor the mind.
Symptoms and indications:	Fainting in debilitated patients marked by cold limbs and faint pulse, hypofunction of the spleen with loss of appetite, cough and dyspnea due to hypofunction of the lung, thirst due to impairment of body fluids, wasting – thirst caused by internal *heat*, general weakness from chronic diseases, palpitation, insomnia, impotence or cold in the uterus; heart failure, cardiogenic shock.

Effects and indications of *Panax quinquefolium* according to Traditional Chinese Medicine[1,5,7]	
Taste:	Bitter and sweet
Temperature:	Cold
Channels entered:	*Orbis cardialis*, *orbis renalis*, *orbis pulmonalis*, *orbis stomachi*
Effects (functions):	To tonify *qi* and nourish *yin*, remove *heat* and promote the production of body fluids.
Symptoms and indications:	Used for deficiency of *qi* and *yin*, internal – *heat*, cough and asthma, bloody phlegm, *fire* in the deficiency syndrome, dysphoria and tiredness, diabetes, dry and thirsty mouth and throat. Treatment of cardiovascular disorders as well as hypoimmunity.

Characteristic main constituents of *Panax ginseng*:

- triterpene saponines *[4]
ginsenoside R*x* (*x* = a1, a2, a3, **b1**, b2, b3, c, d, **e**, f, **g1**,)
notoginsenosides R*x* (*x* = 1, 4)

- sapogenins[4]
panaxadiol (dammar-20(22)-ene-3*β*,12*β*-diol), panaxatriol (dammar-20(22)-ene-3*β*,12*β*,26-triol), 20(R)-dammarane-3*β*,12*β*,20,25-tetrol, 20(S)-protopanaxatriol, 20(R)-protopanaxatriol

- sterols[9]
β-sitosterin
stigmasterin

- volatile components[4]
eremophilene, *β*-gurjunene, *trans*- and *cis*-caryophyllene, ε-muurolene, γ-patchoulene, *β*-eudesmol, *β*-farnesene,

β-bisabolene, aromadendrene, alloaromadendrene, *β*-guaiene, γ-elemene, mayurone, pentadecane, 2,5-dimethyltridecane

- polysaccharides[9,18]

Minor constituents:

- polyacetylenes **[4,15,19]
  Falcarinol (Panaxynol), Falcarindiol (Panaxydol), 1,8-Heptadecadiene-4,6-diyne-3,10-diol
- nucleosides[9]
  uracil, cytidine, uridine, guanosine, adenosine
- amino acids[6]
- flavonoids[6]

Characteristic main constituents of *Panax quinquefolium*:

- **saponines ***[13, 16]
  ginsenoside R*x* (*x* = **b1**, b2, c, d, **e**, **g1**,)
- **sapogenins**[13]
  20(S)-protopanaxatriol
- **polysaccharides**[9,18]

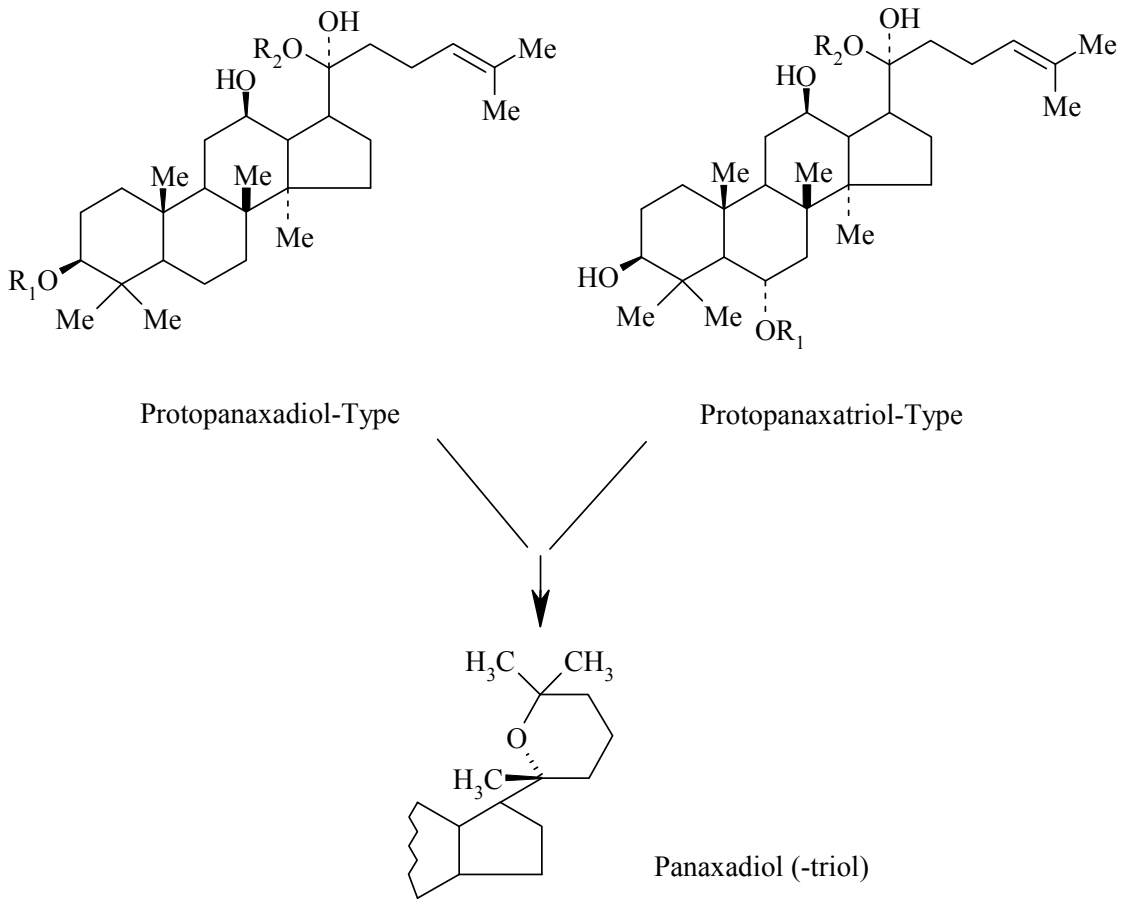
Minor constituents:

- polyacetylenes **[4,15,19]
  Falcarinol (Panaxynol), Falcarindiol (Panaxydol), 1,8-Heptadecadiene-4,6-diyne-3,10-diol
- pseudoginsenoside F11[11,17,18]

* Note: After acidic or enzymatic hydrolysis of the saponins the aglycones formed are protopanaxadiol or protopanaxatriol respectively followed by a spontaneous cyclization to panaxadiol or panaxatriol respectively (see Fig. 1).

** Note: The polyacetylenes present in the roots of *Panax ginseng* and *Panax quinquefolium* may derive originally from the fungus *Paecilomyce* species which is obviously a concomitant fungus of the living plants. Whether the polyacetylenes are also biosynthesised from *Panax* spec. is not yet investigated.[9]

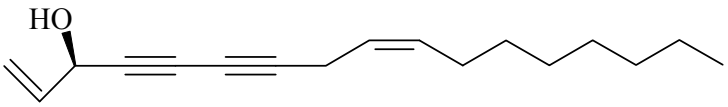
Fig. 1: Formulae of the main constituents



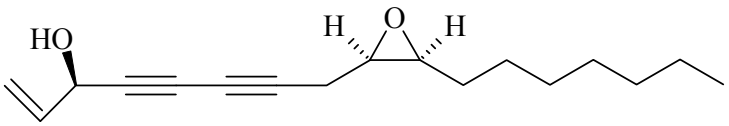
Ginsenosides

	R_1	R_2
Protopanaxadiol – Type		
Rb1	*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-gluco-pyranosyl	*O*-*β*-D-Glucopyranosyl-(1→6)-*β*-D-gluco-pyranosyl
Rb2	*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-gluco-pyranosyl	*O*-α-L-Arabinopyranosyl-(1→6)-*β*-D-gluco-pyranosyl
Rc	*O*-*β*-D-Glucopyranosyl-(1→2)-*β*-D-gluco-pyranosyl	*O*-α-L-Arabinofuranosyl-(1→6)-*β*-D-Gluco-pyranosyl

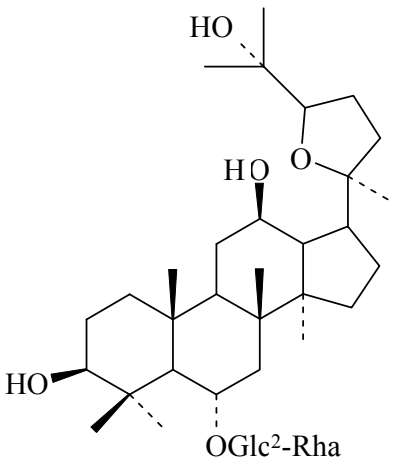
	R_1	R_2
	Protopanaxatriol – Type	
Re	*O*-*α*-L-Rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl	*O*-*β*-D-Glucopyranosyl
Rg1	*O*-*β*-D-Glucopyranosyl	*O*-*β*-D-Glucopyranosyl



Falcarinol (panaxynol)



Falcarindiol (panaxydol)



Pseudo-ginsenoside F11

Pharmacology of *Panax ginseng* and *Panax quinquefolium*

Panax ginseng:

Immunomodulatory activity	***Cardiovascular and metabolic effects***	***Enhancement of CNS activities and hormone like activities***
adaptogenic[2, 9, 17, 21]	inhibition of platelet aggregation and 15 – hydroxyprostaglandin dehydrogenase[17, 19, 20, 23]	protective effects on neurodegeneration[8] Rg3 exhibits neuroprotective effects, scavenging free radicals and improving energy metabolism[8]
anti-stress[17, 18, 21]	anti-diabetic[18]	anti-aging[9]
anti-tumor[4, 22]	hepatoprotective[9, 21]	aphrodisiac properties[21]
cytotoxic activity against leukemia cells[19]	anti-inflammatory activity [15, 19, 20, 23] anti-atherosclerotic effects[18] inhibits leukotriene release[18] increase of ischemia-induced cell proliferation[8]	Rg3 exhibits neuroprotective effects, scavenging free radicals and improving energy metabolism[8]
anti-viral[21]	anti-obesity[10]	
	antinociceptive[21]	

Panax quinquefolium:

Immunomodulatory activity	***Cardiovascular and metabolic effects***	***Enhancement of CNS and hormone like activities***
anti-fatigue [11, 14, 16]	anti-hyperglycemic[3, 18] anti-lipid peroxidation[10, 11]	anti-aging[9, 18]
anti-stress [11, 14, 15, 17, 18, 21]	Re and Rg1 enhance angiogenesis[18] Rb1, Rg3 and Rh2 inhibit angiogenesis[18]	protective effects on neurodegeneration[18]
anti-carcinogenic[15]	hepatoprotective[21]	anti-oxidant[3, 11, 12]
anti-viral[21]	anti-obesity[10]	anxiolytic[11]
anti-HIV-activity[12]	anti-inflammatory activity [3, 13, 15, 18, 19, 20 ,23] antinociceptive[21]	aphrodisiac properties[21]
cancer prevention[18]	anti-arrhythmic[11]	
antifungal[12, 22]	anti-thrombotic[3]	

immunosuppressive effects[15]	platelet aggregation inhibitory activity[15,17,19,20,23]
	Rb1, Re and Rg1 enhance recovery of the brain, heart and other ischemia injury to organs[11,18]
	Rg1 and Rg3 relax vascular smooth muscle and inhibit endothelin production[18]

TLC fingerprint analysis

Drug samples		Origin
1	Radix et Rhizoma Ginseng / *Panax ginseng*	Commercial drug sample from HerbaSinica ®, Germany (origin: Jillin, China)
2	Radix et Rhizoma Ginseng / *Panax ginseng*	Commercial drug sample from China Medica ®, Germany
	Radix et Rhizoma Ginseng / *Panax ginseng*	Commercial drug sample from China Medica ®, Germany
3	Radix Panacis Quinquefolii / *Panax quinquefolium*	Washington, USA
4	Radix Panacis Quinquefolii / *Panax quinquefolium*	Wisconsin, USA
	Radix Panacis Quinquefolii / *Panax quinquefolium*	Ontario, Canada
	Radix Panacis Quinquefolii / *Panax quinquefolium*	British Columbia, Canada
	Radix Panacis Quinquefolii / *Panax quinquefolium*	Wisconsin, USA
	Radix Panacis Quinquefolii / *Panax quinquefolium*	unknown
5	Radix Notoginseng / *Panax notoginseng*	Commercial drug sample from HerbaSinica ®, Germany (origin: Yunnan, China)
6	Radix Notoginseng / *Panax notoginseng*	Province Yunnan (Wen shan), China
	Radix Notoginseng / *Panax notoginseng*	Province Yunnan (Ma Guan), China
	Radix Notoginseng / *Panax notoginseng*	Province Yunnan, China
	Radix Notoginseng / *Panax notoginseng*	Province Guang Xi, China

Reference compounds of Figure 2 a+b		R*f*
T 1	Ginsenoside Rg1	0.57
T 2	Ginsenoside Re	0.36
T 3	Ginsenoside Rb1	0.13

TLC – fingerprint analysis

1) Extraction: 2 g powdered drug are extracted with 10 ml 90 % ethanol under reflux for 10 minutes and the filtrate evaporated to about 5 ml.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: *Panax ginseng* extract: each 10 µl
Panax quinquefolium extract: each 10 µl
reference compounds: each 10 µl

Solvent system: chloroform : methanol : water
70 30 4

Detection: Vanillin – phosphoric acid reagent:
1 g Vanillin is dissolved in 50 % phosphoric acid.

The plate is sprayed with this solution, heated for 5 minutes at 105 °C and evaluated in VIS and at 365 nm.

4) **Description of the TLC – fingerprints of *Panax ginseng* (1 + 2) and *Panax quinquefolium* (3 + 4)**

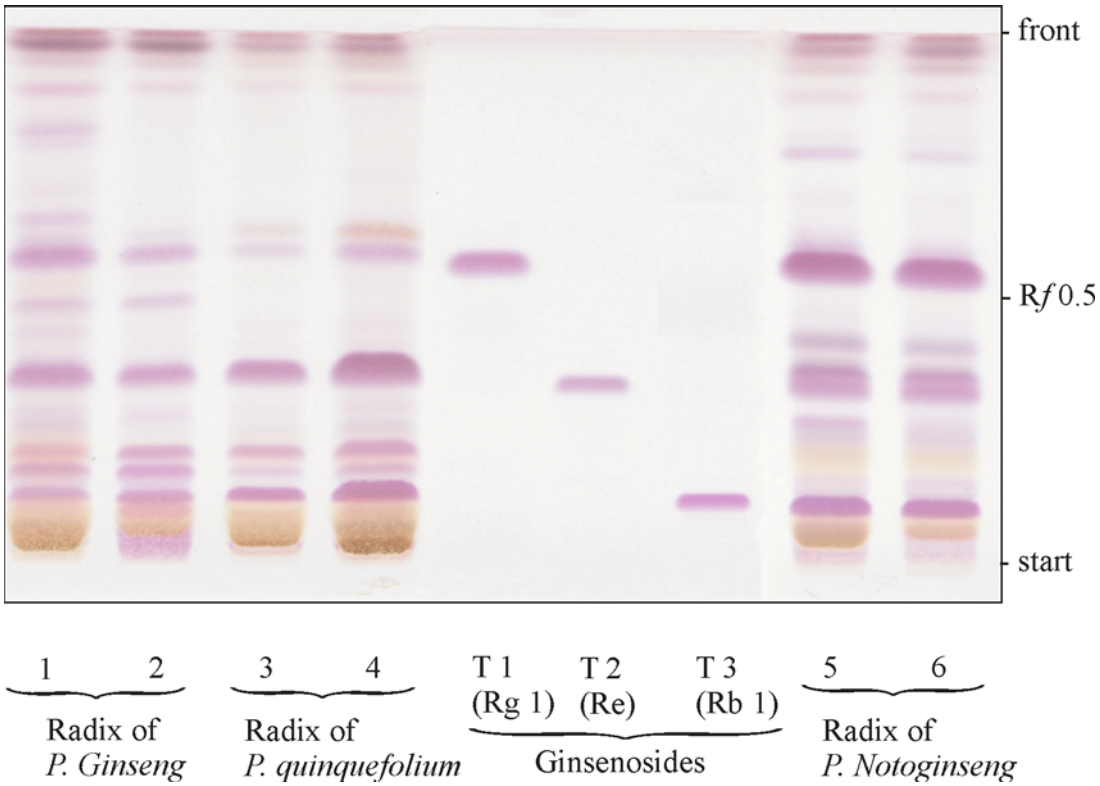


Fig. 2a: Thin layer chromatogram of the ethanol extracts of *Panax* ssp. sprayed with vanillin-phosphoric acid reagent (VIS)

All *Panax ginseng* ethanol root extracts (shown for sample 1 and 2) are characterized by two red violet strong zones of sapogenins directly on the solvent front, two further strong zones at R*f* = 0.57 (Ginsenoside Rg1 = **T1**) and R*f* = 0.36 (Ginsenoside Re = **T2**) and a triple zone pattern between R*f* = 0.1 and 0.2 with the dominant Ginsenoside Rb1 (**T3)** at R*f* = 0.13. The two other zones can be assigned to Ginsenoside Rb2 (R*f* = 0.18) and Rc (R*f* = 0.21). Further red violet zones in very low concentration appear in the upper R*f* – range between R*f* = 0.65 and 0.90 and one at R*f* ~ 0.48.

The red violet zone pattern of *Panax quinquefolium* (sample 3 and 4) differs from that of *Panax ginseng* (sample 1 and 2) by a lower concentration of Ginsenoside Rg1 (**T1**) but with stronger zones of Ginsenoside Re (**T2**) and Rb1 (**T3**).

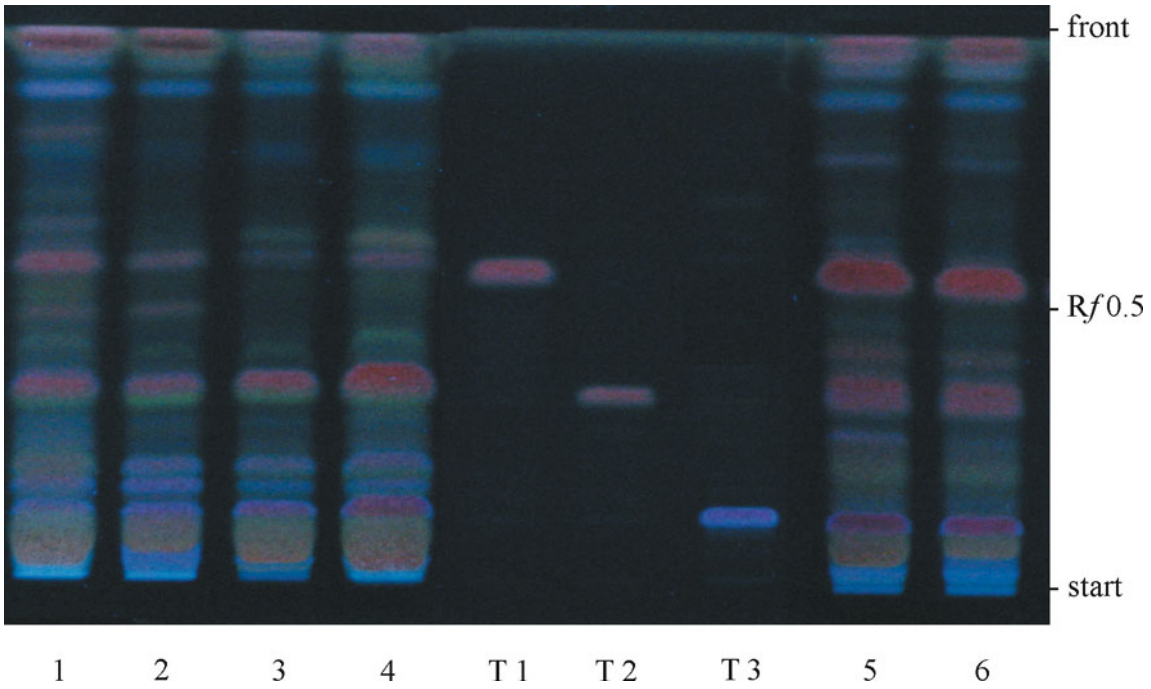


Fig. 2b: Thin layer chromatogram of the ethanol extracts of *Panax* ssp. sprayed with vanillin-phosphoric acid reagent (UV 366 nm)

The red-violet fluorescent zones on dark black background mirror the zone pattern of the TLC-fingerprint in VIS.

Comparison with *Panax notoginseng* root TLC – fingerprint (sample 5 + 6) (Fig. 2a and b)

Panax notoginseng roots show in comparison to the fingerprints of the roots of *Panax ginseng* and *Panax quinquefolium*, three dominant red violet zones at R*f* = 0.57, 0.34/0.36 and 0.13. Ginsenoside Re (**T2**) is here concomitant with a second very strong zone directly above Ginsenoside Re. The triple band pattern in the R*f* – range between 0.18 to 0.21 characteristic for *P. ginseng* and *P. quinquefolium* is not present in *Panax notoginseng* fingerprint.

HPLC – fingerprint analysis:[15]

1) Sample preparation: 4 g powdered drug are extracted twice with 15 ml methanol overnight at room temperature, followed by extraction once with 15 ml 80 % methanol. The methanol extracts were combined and adjusted to a final volume of 50 ml methanol. The solution is filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: *Panax ginseng* extract: each 20.0 µl
Panax quinquefolium extract: each 20.0 µl

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector

	MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l dist. water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (Merck)
Gradient:	0–20 % B in 20 min., 20–40 % B in 20 min., 40–100 % B in 32 min. total runtime: 72 minutes
Flow:	1 ml/min.
Detection:	205 nm

Retention times of the main peaks recorded at 205 nm

Peak	Rt (min.)	Compound
1	24.6	Ginsenoside Rg1
2	26.9 – 27.5	Ginsenoside Re
3	40.6	Ginsenoside Rb1
4	57.0 – 58.5	Polyacetylenes (Falcarinol, Falcarindiol)
5	~ 61.0	Stigmasterol
6	66.3	Panaxadiol
7	69.0	Panaxatriol

4) **Description of the HPLC – fingerprints of the root extracts of *Panax ginseng* and *Panax quinquefolium***

Fig. 3a: The HPLC – pattern of all *Panax ginseng* methanol extract samples, shown for sample 2, is characterized by the presence of Ginsenoside Rg1 (peak **1**, Rt = 24.7) and Ginsenoside Re (peak **2**, Rt = 27.0), an accumulation of 7 peaks with the dominant Ginsenoside Rb1 as peak **3** (Rt = 40.6) (described in the TLC - fingerprint Fig. 2a and 2b as a characteristic triple zone pattern). The polyacetylenes appear as a triple peak between Rt = 57.0 – 58.5. The UV spectra of the peaks **4** (Fig. 4/4) are characterized by two diin maxima of Falcarinol and Falcarindiol at 255, 269 and 285 nm. Stigmasterol appears at Rt ~ 61.0 and the aglyones Panaxadiol at peak **6** (Rt = 66.3) and Panaxatriol at peak **7** (Rt = 69.0).

Fig. 3b: The HPLC – fingerprint of the root methanol extracts of *Panax quinquefolium* shows Ginsenoside Re (peak **2**), the accumulations of ginsenosides with the dominant Ginsenoside Rb1 (peak **3**), the polyacetylenes (peak accumulations **4**), Stigmasterol (peak **5**) and the Panaxadiol and Panaxatriol as the peaks **6** and **7**, all in much higher concentration than in *Panax ginseng*.

Fig. 3c: *Panax notoginseng* HPLC-fingerprint for comparison can be discriminated from that of *Panax ginseng*, by two dominating peaks of Ginsenoside Rg1 (peak **1**), Ginsenoside Rb1 (peak **3**) and relatively low concentrated Ginsenoside Re (peak **2**) and peak accumulation **4**. The characteristic 5 – 6 peaks in the Rt - range of around 40 to 45 Rt is lacking.

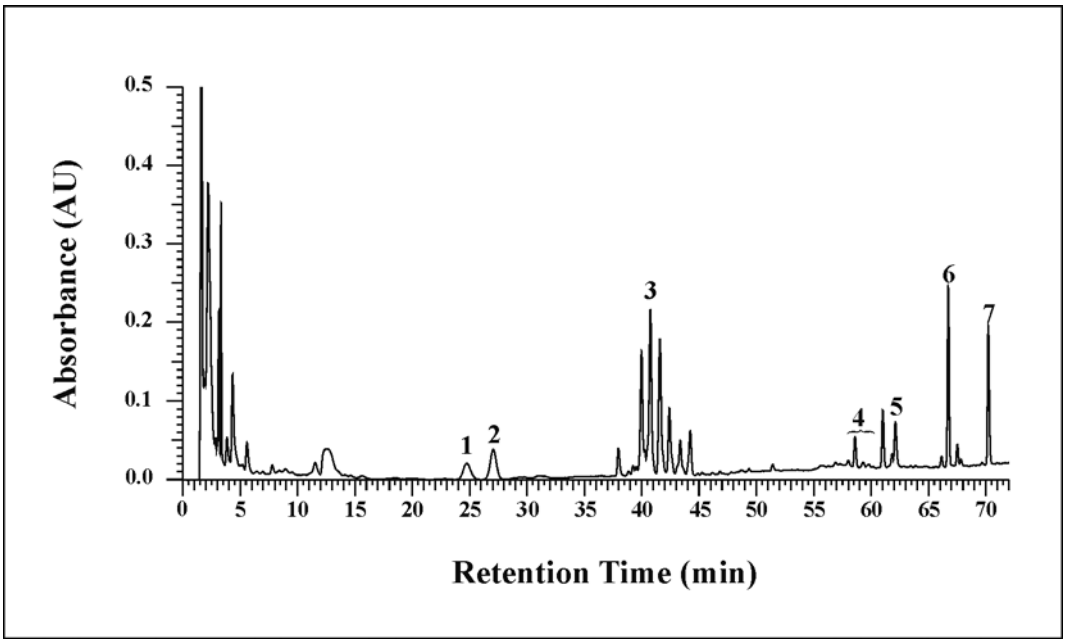


Fig. 3a: HPLC-fingerprint analysis of the MeOH-extract of *Panax ginseng* sample 2

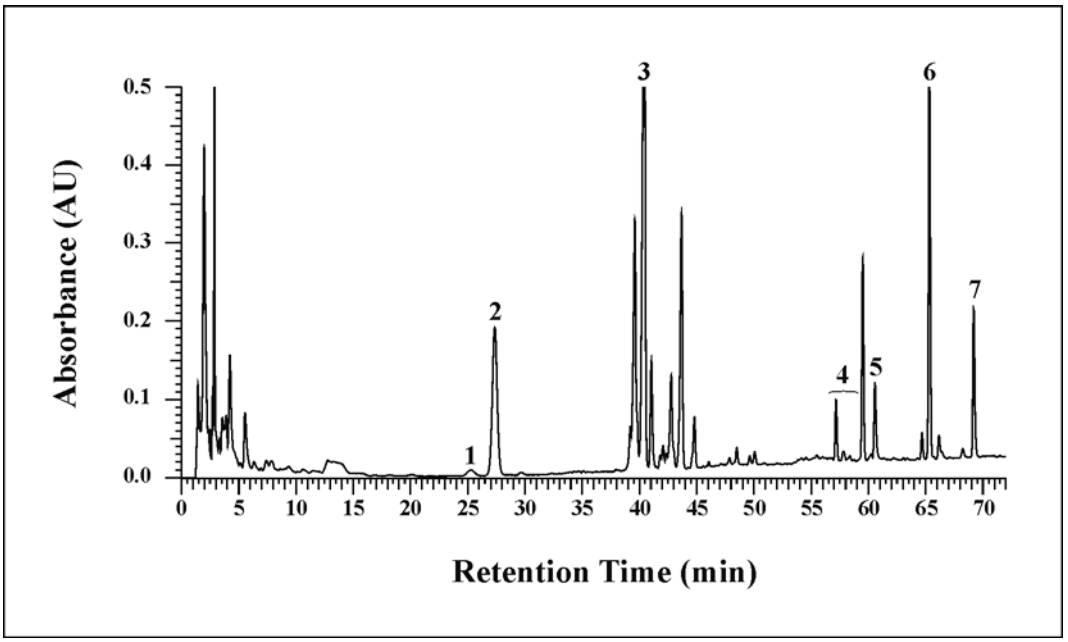


Fig. 3b: HPLC-fingerprint analysis of the MeOH-extract of *Panax quinquefolium* sample 4

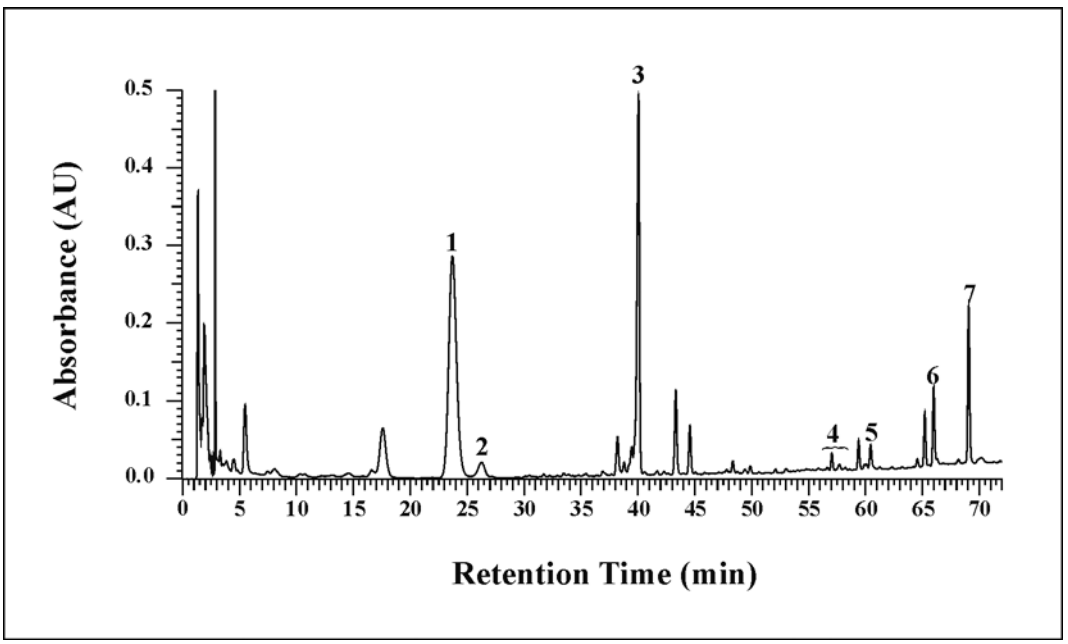


Fig. 3c: HPLC-fingerprint analysis of the MeOH-extract of *Panax notoginseng* sample 6

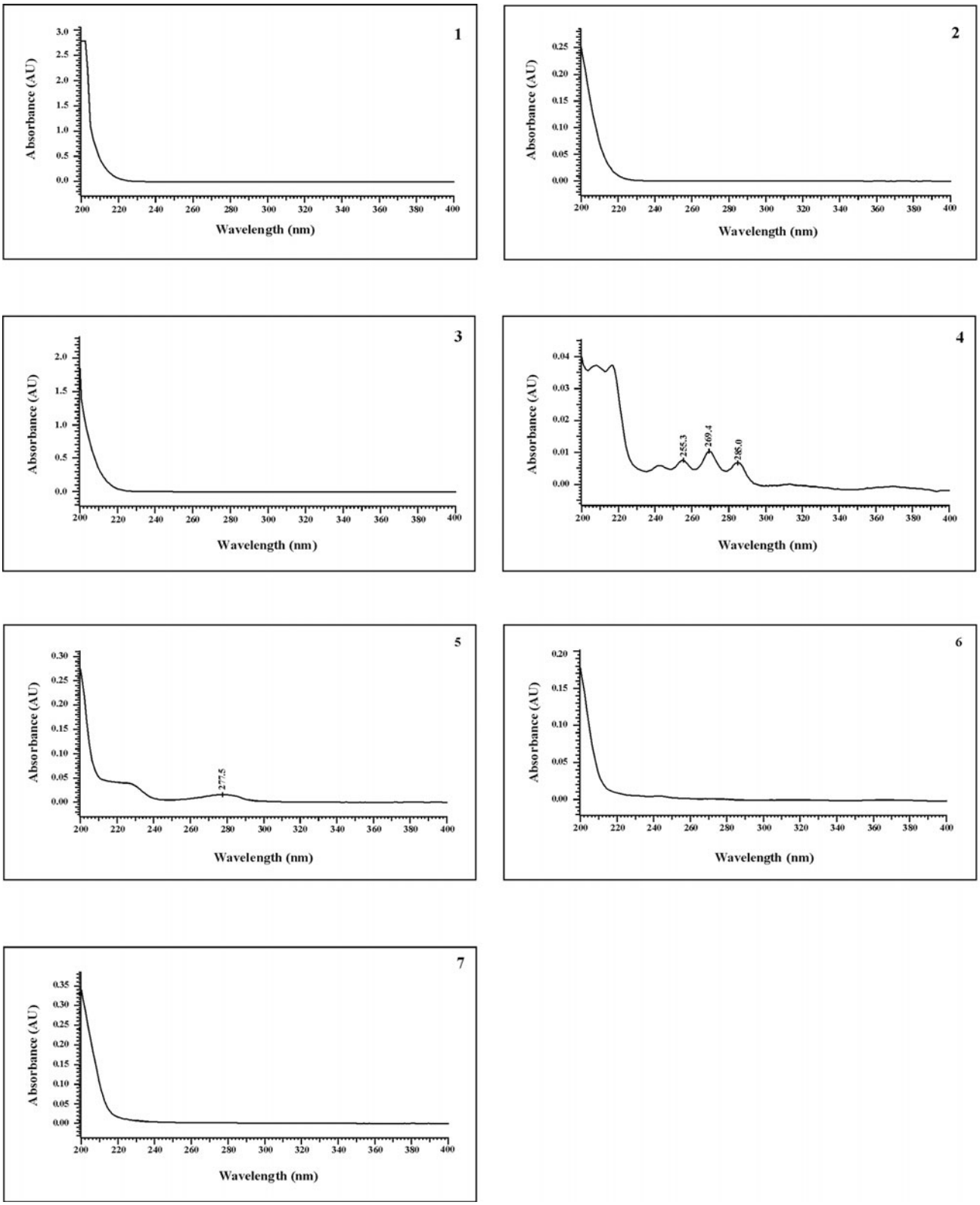


Fig. 4: On line UV-spectra of the main characteristic peaks of the HPLC-fingerprint of *Panax ginseng* and *Panax quinquefolium* extracts

Notes:

In several publications special methods for the quantitation of the main triterpen-saponins [8,14,17,21] and polyacetylenes [15, 22] of *Panax ginseng* and other *Panax* species are described.

The Chinese Pharmacopoeia 2005 demands for Radix et Rhizoma Ginseng not less than 0.26% of Ginsenoside Re, and not less than 0.20% of Ginsenoside Rb1, as determined by HPLC and calculated with reference to the dried drug.

For Radix Panacis Quinquefolii are demanded not less than 2.0% of the total amount of Ginsenoside Rg1, Ginsenoside Re and Ginsenoside Rb1 as determined by HPLC and calculated with reference to dried drug.

Conclusion

Panax ginseng and *Panax quinquefolium* roots show very similar qualitative TLC- and HPLC-fingerprints but can be discriminated from each other by a higher concentration of Ginsenoside Re in *P. quinquefolium* than in *P. ginseng* and vice versa a higher concentration of Ginsenoside Rg1 in *P. ginseng* than in *P. quinquefolium*.

Panax notoginseng roots can be discriminated from the two other *Panax* species by the lacking zone/peak pattern in the ranges of R*f* = 0.1 – 0.2 and Rt = 40.0 – 45.0 respectively (see monograph Radix et Rhizoma notoginseng).

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Herba Siegesbeckiae
Xixiancao

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Siegesbeckia Herb is the dried aerial part of *Siegesbeckia orientalis* L., *Siegesbeckia pubescens* Makino or *Siegesbeckia glabrescens* Makino (Fam. Asteraceae).

The drug is collected in summer and autumn before or at flowering, removed from foreign matter, and dried in the sun.

Origin[2,3]: China (provinces of Zheijiang, Jiangsu, Sichuan), Indochina, Philippines, Java, India

Descriptions of the drug[1]: Stems subsquare, frequently branched, 30–110 cm long, 0.3–1 cm in diameter; externally greyish-green, yellowish-brown or purplish-brown, with longitudinal furrows and fine striations, covered with grey pubescences; nodes distinct, slightly swollen; texture fragile, easily broken, fracture yellowish-white or greenish; pith broad, almost white, hollowed. Leaves opposite, lamina frequently crumpled and rolled, when whole, ovate, greyish-green, margin obtusely serrate; both surfaces with white pubescences, trinervious. Some bearing yellow capitulum; involucre spatulate. Odour, slight; taste, slightly bitter.

Pretreatment of the raw drug[1]:

Herba Siegesbeckiae:
Foreign matters are eliminated, washed clean, softened slightly, cut into sections, and dried.

Herba Siegesbeckiae (processed with wine):
The sections of Herba Siegesbeckiae are thoroughly steamed as described under the method for steaming with wine (Appendix II D), using 20 kg of yellow rice wine per 100 kg of Herba Siegesbeckiae.

Medicinal use[3,10]: Treatment of cardiovascular diseases such as hypertension and angina pectoris and externally for ulcers, abscesses and boils.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of Herba Siegesbeckiae according to Traditional Chinese Medicine [1,2,4,5]	
Taste:	Bitter, pungent
Temperature:	Cold
Channels entered:	*Orbis hepaticus*, *orbis renalis*, *orbis lienalis*
Effects (functions):	To relieve rheumatic conditions, improve the motility of joints, and counteract *toxicity*.
Symptoms and indications:	Rheumatic arthralgia with aching and weakness of the lower back and knees, numbness of limbs; hemiplegia; rubella, sores with exudation. Treatment of asthma, paralysis and allergic disorders.

Main constituents:

- ***ent*-pimarane diterpenoids and diterpenoid glycosides:**
  kirenol[6], 16-acetylkirenol, isopropylkirenol[9]
- hythiemoside B, darutigenol, darutoside, *ent*-16-acetoxypimar-8(14)-ene-3*β*,(15*R*)-diol 3-*O*-*β*-D-glucopyranoside (hythiemoside A); *ent*-(15*R*),16,19-trihydroxypimar-8(14)-ene 19-*O*-*β*-D-glucopyranoside[7]
- *ent*-12α,16-epoxy-2*β*,15α,19-trihydroxypimar-8-ene;
  ent-12α,16-epoxy-2*β*,15α,19-trihydroxypimar-8(14)-ene;
  ent-2-oxo-15,16,19-trihydroxypimar-8(14)ene;
  ent-2α,15,16,19-tetrahydroxypimar-8(14)-ene;
  ent-15-oxo-2*β*,16,19-trihydroxypimar-8(14)-ene;
  ent-2-oxo-15,16-dihydroxypimar-8(14)-en-16-*O*-*β*-glucopyranoside;
  ent-2-oxo-3*β*,15,16-trihydroxypimar-8(14)-en-3-*O*-*β*-glucopyranoside;
  ent-2*β*,15,16,19-tetrahydroxypimar-8(14)-en-19-*O*-*β*-glucopyranoside[9]
- pubeside A, B, C, D[8, 9], E[8], siegesbeckioside, siegesbeckiol, siegesbeckic acid, orientalin A, B[8],
- **Sterins**[7]:
  β-Sitosterol, stigmasterol
- **Flavonoids:**
  rutin[7], orientin[12]
- **Phenolcarboxylic acid**[7]:
  caffeic acid

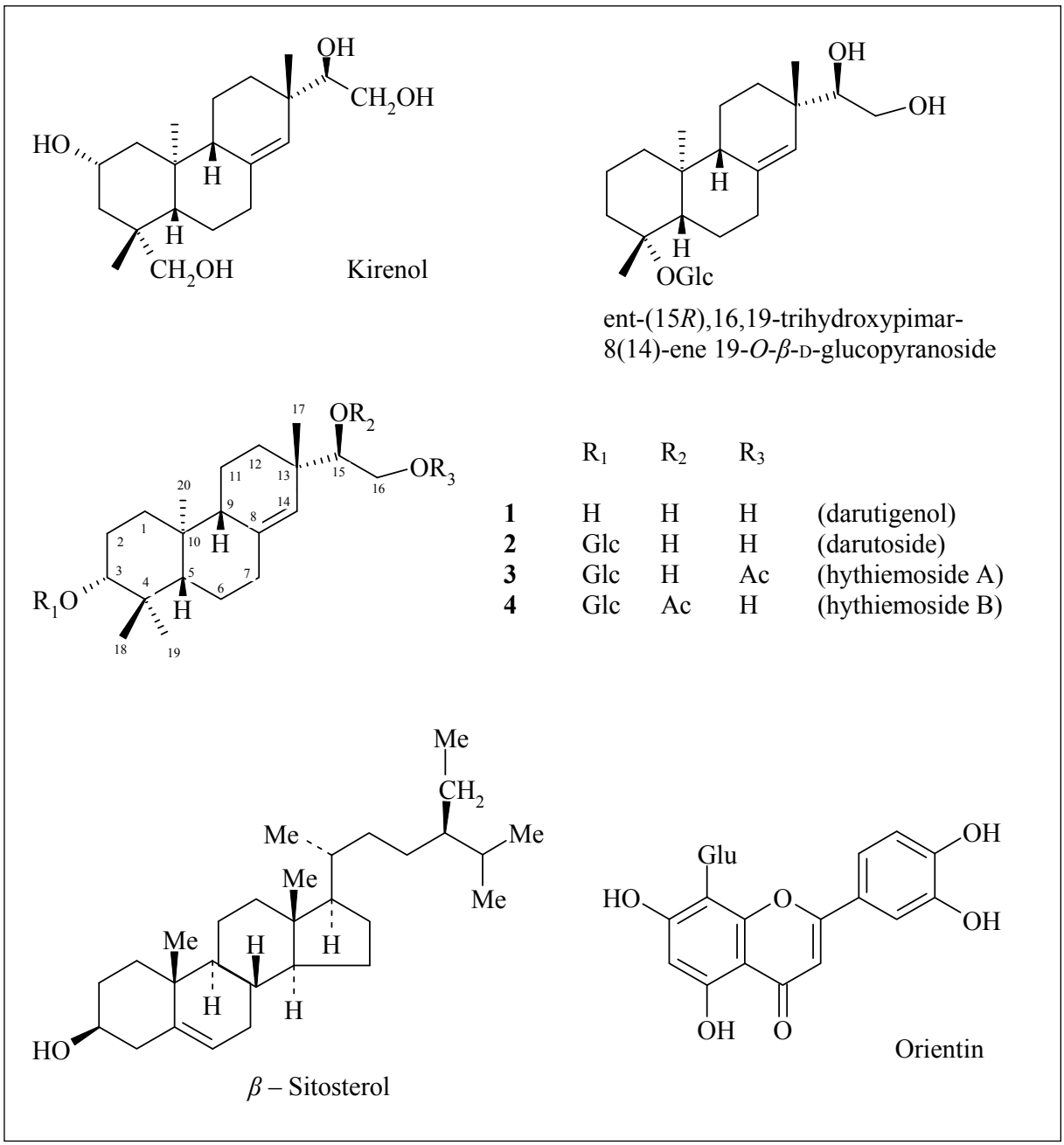

Fig. 1: Formulae of the main constituents of Herba Siegesbeckiae

Pharmacology:

- analgesic[3]
- anti-rheumatic[3,6]
- anti-oxidative[5,6,9,11]
- anti-allergic[5,9,11]
- anti-hypertensive[5]
- anti-tumor[5]
- anti-inflammatory activities[5,6]
- immunosuppressive activity[6]
- exhibits infertile activity[9]

TLC fingerprint analysis:

Drug samples		Origin
1	Herba Siegesbeckiae / *Siegesbeckia pubescens*	commercial drug sample from TCM Hospital, Bad Kötzting, Germany
2	Herba Siegesbeckiae / *Siegesbeckia pubescens*	commercial drug sample from TCM Hospital, Bad Kötzting, Germany
3	Herba Siegesbeckiae / *Siegesbeckia orientalis*	province Shaanxi, China
4	Herba Siegesbeckiae / *Siegesbeckia glabrescens*	province Hunan, China

Reference compounds of Figures 2a and 2b		R*f*
T 1	*β*-Sitosterol	0.91
T 2	Kirenol	0.26

TLC-fingerprint analysis:

1) Extraction: 2 g powdered drug are extracted under reflux with 20 ml of methanol for 1 hour. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, VWR

Applied amounts: Herba Siegesbeckiae extracts: each 10 µl
reference compounds: each 10 µl

Solvent system: chloroform : methanol : water (lower layer)
25 5 1

Detection: conc. sulphuric acid : ethanol (50%)
1 9
The plate is sprayed with this solution, heated for 10 minutes at 105 °C and evaluated at 366 nm and VIS.

4) Description of TLC-fingerprints of Herba Siegesbeckiae

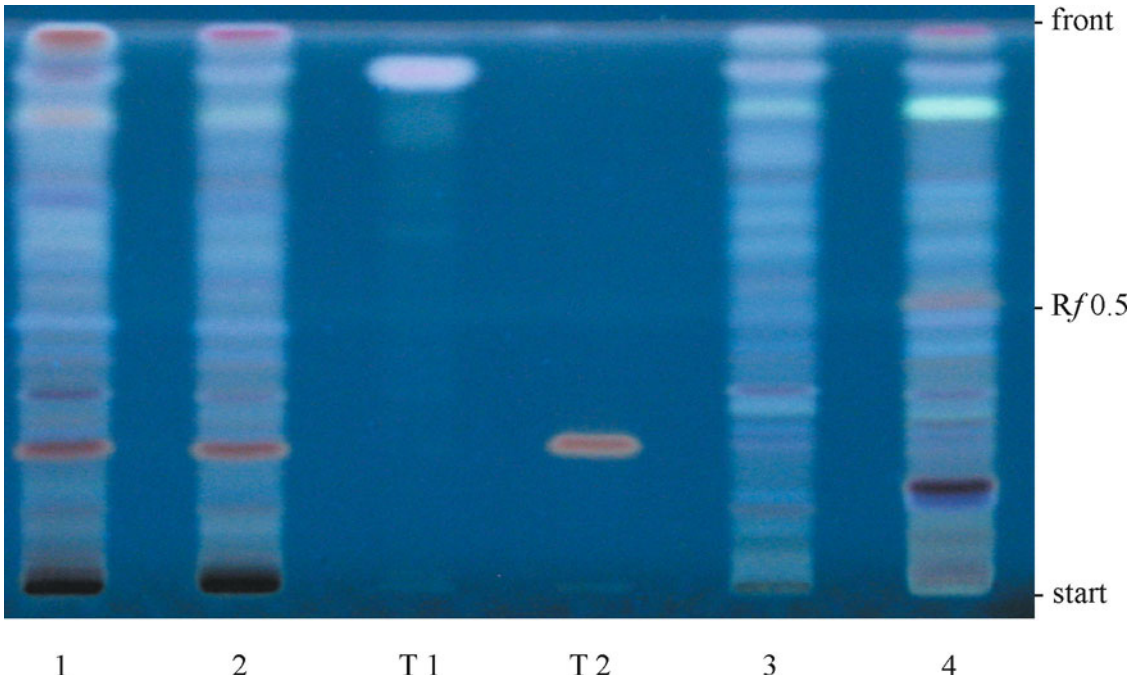

Fig. 2a: Thin layer chromatogram of the methanol extracts of Herba Siegesbeckiae sprayed with conc. sulphuric acid: 50 % ethanol (1 : 9) (UV 366 nm)

All extract samples of *Siegesbeckia pubescens* **1** and **2** are characterized by the light violet fluorescent zone of *β*-Sitosterol (**T1** = 0.91) and the red zone of Kirenol (**T2** at R*f* = 0.26). Above Kirenol appear four distinct also light violet fluorescent compounds which can be assigned to the more lipophilic 16-acetylkirenol, isokirenol and other kirenol derivatives. The red fluorescent band on the solvent front derives from another sterol.

The weak fluorescent bands in the middle and low R*f*-range cannot be assigned to the other hydroxylated diterpenoids of Siegesbeckia but might be glycosides of them.

The extract samples of *Siegesbeckia orientalis* **3** and *S. glabrescens* **4** differ from the samples 1, 2 by their very low concentrations of Kirenol but contain instead of it high concentrations of diterpenoids above Kirenol. The only extract sample of *S. glabrescens* which was available possesses a distinct deep violet zone at R*f* = 0.17, not present in the other spezies and violet zones in the middle and low R*f*-range.

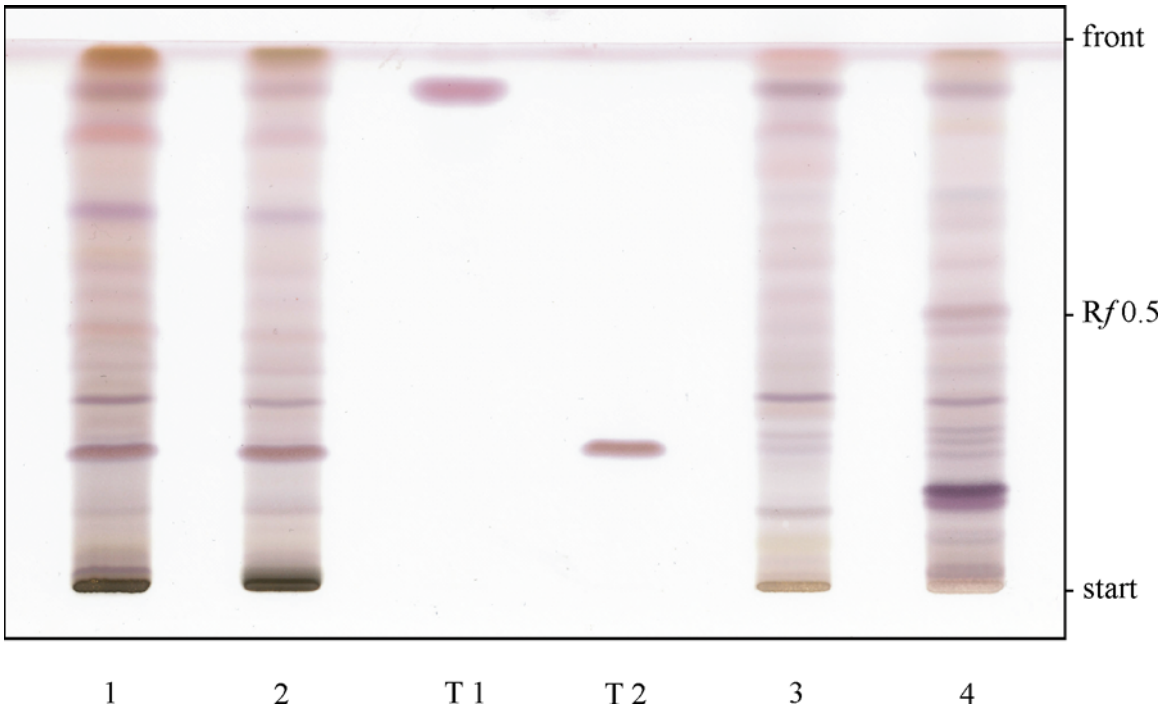

Fig. 2b: Thin layer chromatogram of the methanol extracts of Herba Siegesbeckiae sprayed with conc. sulphuric acid : 50 % ethanol (1 : 9) (VIS)

The MeOH extracts of samples 1 and 2 show in the same solvent system in VIS six distinct red violet zones at R*f* = 0.91 (**T1** = Sitosterol), R*f* = 0.84, R*f* = 0.69, R*f* = 0.35 and R*f* = 0.26 (**T2** = Kirenol). The extract sample of *Siegesbeckia orientalis* (sample 3) shows only the zone of Sitosterol and a further small zone at R*f* = 0.35. The extract sample of *Siegesbeckia glabrescens* (sample 4) shows besides Sitosterol further zones at R*f* = 0.52 and R*f* = 0.35, followed by Kirenol with two small other zones above of Kirenol and a strong overlapped zone at R*f* = 0.17.

HPLC-fingerprint analysis:

1) Sample preparation: 2 g powdered drug are extracted under reflux with 20 ml of methanol for 1 hour. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol and filtered over Millipore® filtration unit, type 0.45 µm and injected into the HPLC apparatus.

2) Injection volume: Herba Siegesbeckiae extracts: each 30.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), VWR
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), VWR

Solvent System:	A: water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	10 % B in 5 min., 10 – 40 % B in 35 min., 40 – 90 % B in 25 min. total runtime: 65 minutes
Flow:	1 ml/min.
Detection:	210 nm

Retention times of the main peak recorded at 210 nm

Peak	Rt (min.)	Compound
1	29.84	Kirenol

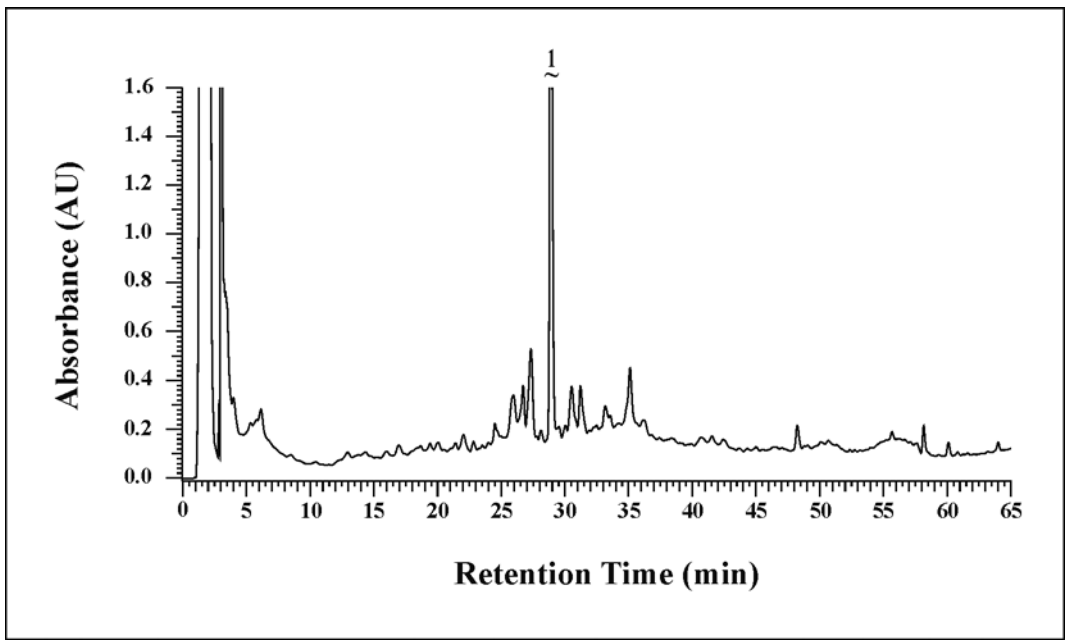

Fig. 3a: HPLC-fingerprint analysis of the methanol extract of *Siegesbeckia pubescens*, extract sample 2

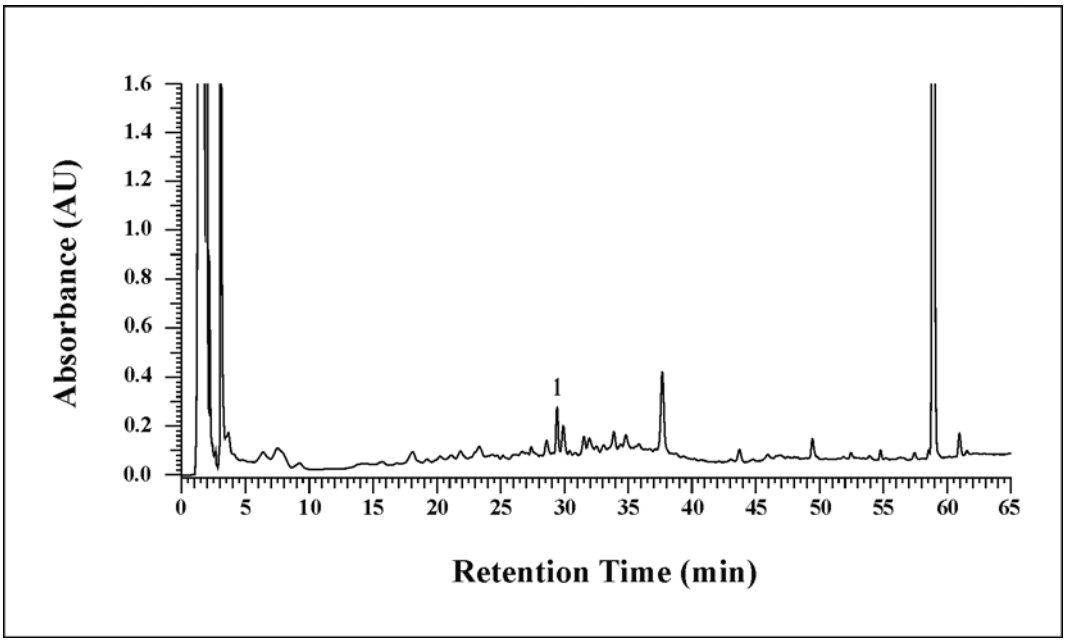

Fig. 3b: HPLC-fingerprint analysis of the methanol extract of *Siegesbeckia orientalis*, extract sample 3

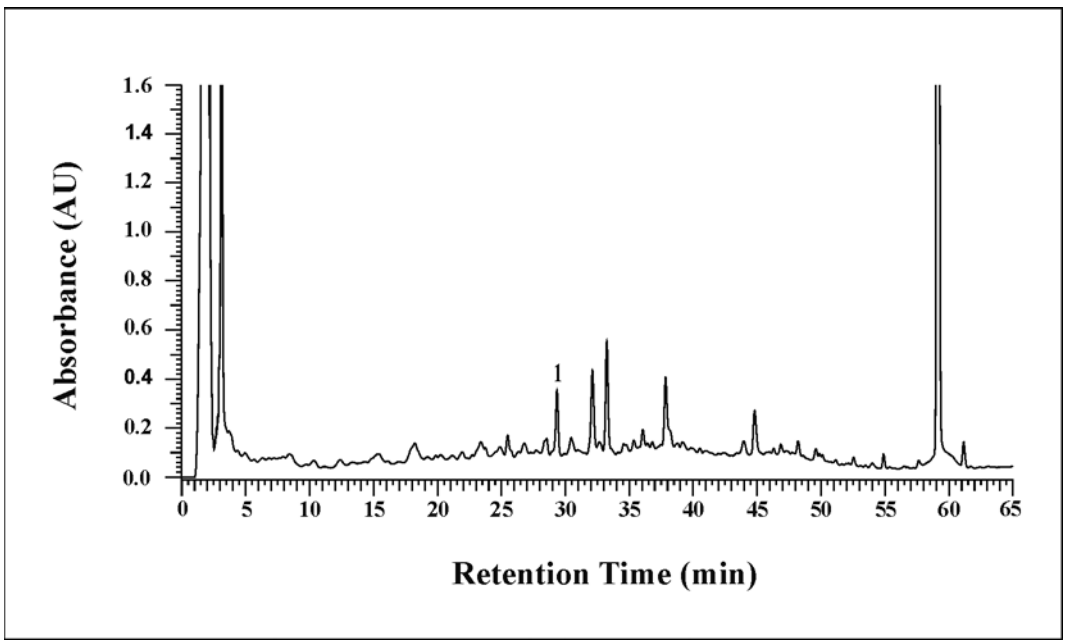

Fig. 3c: HPLC-fingerprint analysis of the methanol extract of *Siegesbeckia glabrescens*, extract sample 4

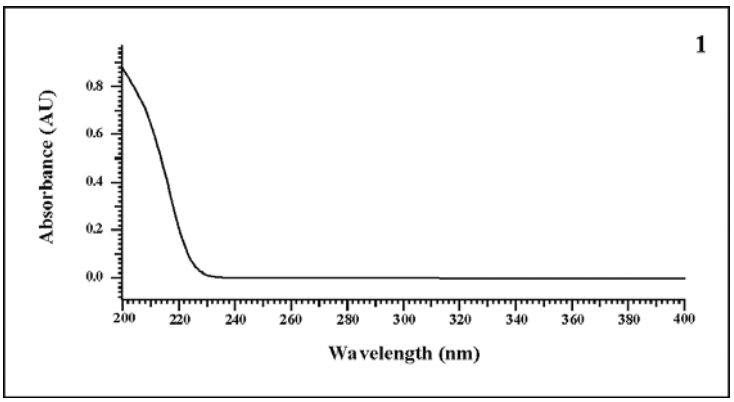

Fig. 4: On line UV-spectrum of Kirenol

4) Description of the HPLC-Figures

All *Siegesbeckiae pubescens* extract samples are characterized by a very strong peak at Rt = 29.8 (**1**) shown here for sample 2. They are accompanied by an accumulation of 7–8 peaks with lower and higher Rt-values between Rt = ~ 25.0 and 35. The extract samples 3 and 4 of *S. orientalis* and *S. glabrescens* are characterized by a strong peak at Rt = 59.5, which has no direct correlation with one conspicuous zone in the corresponding TLC-range R*f* 0.75 – 0.95. Kirenol is present in both species only in a very small concentration as shown also in the TLC.

Note: The Pharmacopoeia of the People's Republic of China describes for Herba Siegesbeckiae a concentration not less than 0.050 per cent of Kirenol.

Conclusion

Siegesbeckia pubescens can be well characterized by the high concentration of Kirenol as shown in TLC and HPLC. A clear discrimination between *S. pubescens* and *S. orientalis* or *S. glabrescens* respectively is not necessary because they show the same composition of constituents.

References:

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Radix et Rhizoma Salviae miltiorrhizae
Danshen

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1] Danshen Root is the dried root and rhizome of *Salvia miltiorrhiza* Bge. (Fam. Lamiaceae).

Origin[2,3]: Provinces Hebei, Anhui, Jiangsu and Sichuan, Northwestern China, Manchuria, Japan.

Description of the drug[1]: Rhizomes short and stout, sometimes with remains of a stem at the apex. Several roots, long cylindrical, slightly curved, some branched and with rootlets, 10 – 20 cm long, 0.3 – 1 cm in diameter. Externally brownish-red or dark brownish-red, rough, longitudinally wrinkled. The bark of old roots loose, mostly purplish-brown, usually scaling off. Texture hard and fragile, fracture loose, cleft or slightly even and dense, with brownish-red bark and grayish-yellow or purplish-brown wood, showing bundles of vessels, yellowish-white, arranged radially. Odor, slight; taste slightly bitter and astringent.

Cultivars relatively stout, 0.5 – 1.5 cm in diameter. Externally reddish-brown, longitudinally wrinkled, the bark closely adhering to wood and uneasy to be scaled off. Texture compact, fracture relatively even, slightly horny.

Pretreatment of the raw drug[1]: The drug is collected in spring or autumn, removed from soil, and dried.

Medicinal use (Clinical application)[5,9]: *Salvia miltiorrhiza* is used for:

(1) - Hypertension
- Arrhythmias
- Hyperhomocysteinemia
- Stroke prevention and recovery
- Peripheral and pulmonary vascular diseases

(2) - Coronary artery diseases
- Angina pectoris
- Myocardial infarction
- Hyperlipidemia Hypercholesterolemia, Hypertriglyceridemia

(3) - Renal diseases and Diabetes

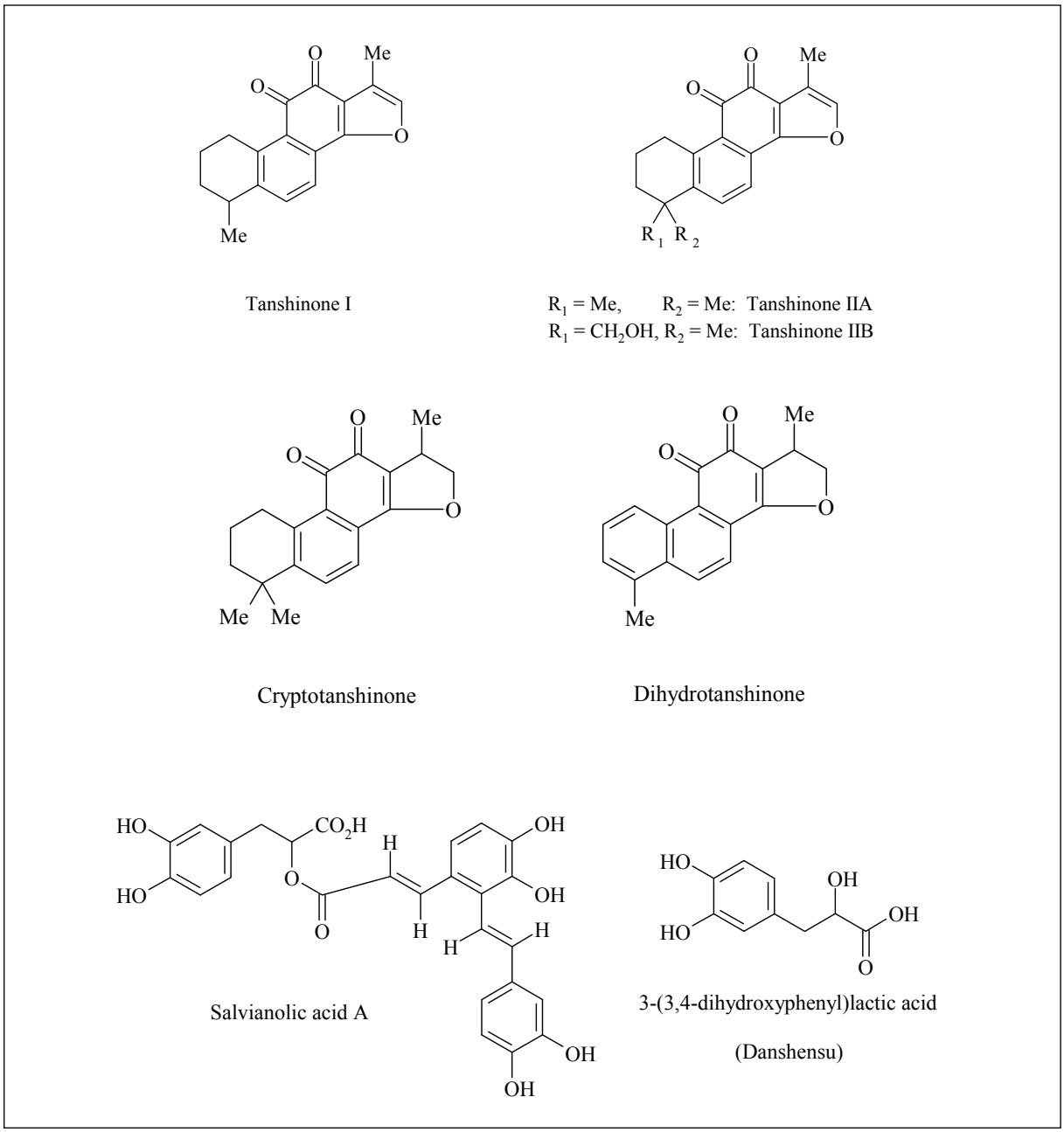
Effects and indications of Radix et Rhizoma Salviae miltiorrhizae according to Traditional Chinese Medicine[1,6,7,8]	
Taste:	bitter
Temperature:	neutral, slightly cold
Channels entered:	*Orbis cardialis, Orbis pericardialis, Orbis hepaticus*
Effects (functions):	To remove blood stasis and relieve pain, promote the flow of blood and stimulate menstruation and clear *heart-fire* and remove restlessness.
Symptoms and indications:	Menstrual disorders, amenorrhea, dysmenorrhea; mass formation in the abdomen; pricking pain in the chest and abdomen, pain in acute arthritis and subcutaneous infection; fidgets and insomnia; hepatosplenomegaly; angina pectoris.

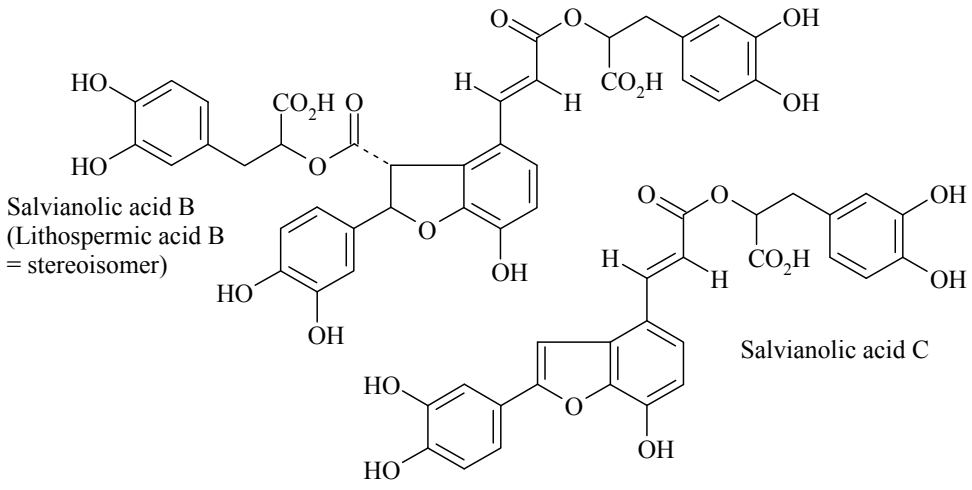
Constituents [4,9-12]

Main constituents	Minor constituents
I. Lipophilic diterpenoid compounds:	
1. phenanthrofurane quinone derivatives:	
- phenathro[1,2-*b*]furan-10,11-diones:	
tanshinones I, IIA and IIB	tanshindiols
cryptotanshinone	methyltanshinonate
dihydrotanshinone	methylene tanshinquinone
	tanshindiols A, B and C
	nortanshinone
	7α-hydroxytanshinone II
	dihydrotanshinquinone
- phenanthro[3,2-*b*]furan-7,11-diones:	isotanshinones I, II and IIB
	isocryptotanshinone
	dihydroisotanshinone I
2. phenanthrenes:	danshenxinkuns A, B and C
	miltirone, salviol, ferruginol, dehydromiltirone;
	miltiodol, miltionone I
3. spiro ketal lactones:	danshenspiroketal lactone
	epi-danshenspiroketal lactone
4. phenalenofuran diterpene derivative	salvilenone
5. phenanthropyrandione derivative	danshenxinkun D
6. furonaphthopyrane derivative	tanshinlactone
7. phenathro[1,2-*b*]furan-10,11-dione	miltionone II
II. Water-soluble phenol carboxylic acids:	
3-(3′,4′-dihydroxyphenyl)lactic acid (danshensu)	protocatechuic acid and -aldehyde

salvianolic acid B (stereoisomer: lithospermic acid B)	salvianolic acid A, salvianolic acids C - G
lithospermic acid	
rosmarinic acid	
III. Other components:	
1. sterols:	*β*-sitosterol, daucosterol, ursolic acid
2. flavonoids:	5,3´-dihydroxy-7,4´-dimethoxy flavanone, baicalin

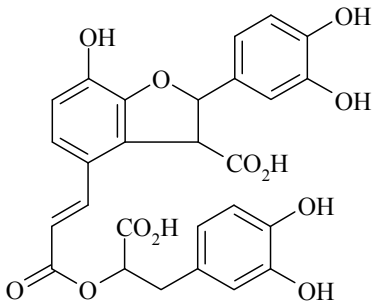
Fig. 1: Formulae of the main compounds of Radix et Rhimoma Salviae miltiorrhizae [4,9-12]



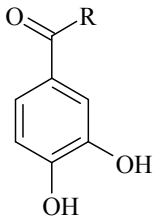


Salvianolic acid B
(Lithospermic acid B
= stereoisomer)

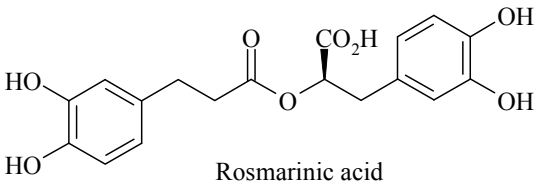
Salvianolic acid C



Lithospermic acid



R = OH: Protocatechuic acid
R = H: Protocatechualdehyde



Rosmarinic acid

Pharmacology reported in the literature

***Salvia miltiorrhiza* extract**	- alleviates Angina pectoris comparable with Isosorbiddinitrate: improves microcirculation, causes coronary vasodilatation, inhibits platelet adhesion and aggregation, protects against myocardial ischemia, suppresses the formation of thromboxane[5,9] - cardio protective effects comparable with ramipril[5] - improves cardiac function comparable with captopril[5] - reduces the myocardial reperfusion injury in patients with acute myocardial infarction[5] - reduces the incidence of arrhythmias[5] - improves peripheral circulation, vasorelaxant effects[5] - increases right ventricular myocardial contractility[5] - improves the rheology of erythrocytes[5] - reduces total cholesterol, triglyceride, and LDL cholesterol levels[5] - renoprotective effects[5] - anti-HIV-1 activity[10] - causes apoptotic cell death[10]
Tanshinone IIA	- reduces myocardial infarct size[9] - suppresses ischemic arrhythmias[15] - antihypertensive activity[16] - inhibits LDL oxidation[9] - insulin-sensitizing activity[20] - attenuates cardiac cell hypertrophy[9] - inhibits *in vivo* metastasis of colon carcinoma cells[13] - inhibits leukemia cell growth by induction of apoptosis[19] - effects against postmenopausal syndrome[17]
Danshensu (3-(3′,4′-dihydroxy-phenyl)lactic acid)	- dilates coronary arteries[9] - inhibits platelet aggregation[9] - improves microcirculation[9] - scavenges oxygen-free radicals[9] - inhibits myocardial cell apoptosis[9] - beneficial effects on homocysteine metabolism[18]
Salvianolic acid B (=Lithospermic acid B)	- protects brain and heart from ischemia-reperfusion injury[9] - inhibits platelet aggregation[9] - inhibits oxidative modification of LDL[9] - antihypertensive effect[5] - inhibitory activity against hepatic fibrosis[10] - antioxidant activity[10] - anti-secretory and anti-ulcer activities by inhibiting the gastric H+, K+-ATPase[10]
Rosmarinic acid	- protects HepG2 cells against cell death[23] - neuroprotective effect[21] - anxiolytic-like effect[22] - antiviral, antibacterial, antiinflammatory and antioxidant[22]

TLC-fingerprint analysis:

Drug samples		Origin
1	Radix et Rhizoma Salviae miltiorrhizae	sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany
2	Radix et Rhizoma Salviae miltiorrhizae	sample of commercial drug obtained from China Medica, Germany
3	Radix et Rhizoma Salviae miltiorrhizae	Province Hebei, China
4	Radix et Rhizoma Salviae miltiorrhizae	Province Henan, Lushi, China
5	Radix et Rhizoma Salviae miltiorrhizae	sample of commercial drug obtained from Public pharmacy, Munich

Reference compounds of Figure 2a and 2b		R*f*
T1	Tanshinone IIA	0.81
T2	Cryptotanshinone*	0.49

*according to the reference[14]

Reference compounds of Figure 2c and 2d		R*f*
T3	Protocatechuic acid	0.64
T4	Rosmarinic acid	0.50
T5	Lithospermic acid	0.38
T6	Salvianolic acid B	0.27

TLC-fingerprint analysis of tanshinones and phenol/caffeoyl carboxylic acids of *Salvia miltiorrhiza* root/rhizome extract:

1. Thin layer chromatograms of the lipophilic tanshinones (see Figure 2a and 2b):[14]

1) Extraction: 1 g powdered drug is extracted with 10 ml **diethyl ether** for 1 hour at room temperature with occasional shaking. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 0.5 ml of methanol and then used for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: TLC Silica gel 60 F_{254} (aluminium sheets), Merck

Applied amounts: Radix et Rhizoma Salviae miltiorrhizae extracts: 10 µl each, reference compounds: 10 µl each

Solvent system:

petroleum ether	:	ethyl acetate	:	cyclohexane
5		3		2

Detection: a) Without any reagent treatment: The plate is evaluated in VIS.

b) Anisaldehyde-sulphuric acid reagent:
0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, 85 ml methanol and 5 ml concentrated sulphuric acid (96 %), in that order.
The TLC is sprayed with about 10 ml, heated at 110 °C for 5 min, and then evaluated in VIS

2. Thin layer chromatograms of water-soluble caffeoyl carboxylic acids (see Figure 2c and 2d):[14]

1) Extraction: 0.5 g powdered drug is extracted under reflux with 25 ml **water** on a water bath for 30 minutes. The extract is cooled, filtered and **acidified** with 30 µl concentrated hydrochloric acid (37 %). The extract is filtered again and shaken three times in a separating funnel with 5 ml ethyl acetate. The combined organic layer is concentrated to dryness under vacuum. The residue is dissolved in 1 ml ethyl acetate and then used for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: TLC Silica gel 60 F_{254} (aluminium sheets), Merck

Applied amounts: Radix et Rhizoma Salviae miltiorrhizae extracts: 15 µl each, reference compounds: 10 µl each

Solvent system:

chloroform		ethyl acetate		toluene		formic acid		methanol
15	:	20	:	10	:	10	:	1

Detection: c) Without any reagent treatment:
The plate is evaluated in UV 254 nm.

d) Iron-III-chloride reagent ($FeCl_3$):
The plate is sprayed with 2 % $FeCl_3$/EtOH, heated at 110 °C for 5 minutes and is immediately evaluated in VIS.

Note:

a) In Chinese Pharmacopoeia 2005[1] the lipophilic tanshinones are chromatographed on silica gel G plates with a mixture of benzene and ethylacetate (19:1) and evaluated in VIS.

b) The caffeoyl carboxylic acids are chromatographed on silica gel G F_{254} plates with a mixture of toluene-chloroform- ethyl acetate-methanol-formic acid (2:3:4:0.5:2) and evaluated under UV 254 nm[1].

c) Other TLC-methods for *Salvia miltiorrhiza* extracts are reported in references[6,14,23].

4) **Descriptions of TLC-fingerprints of *Salvia miltiorrhiza:***

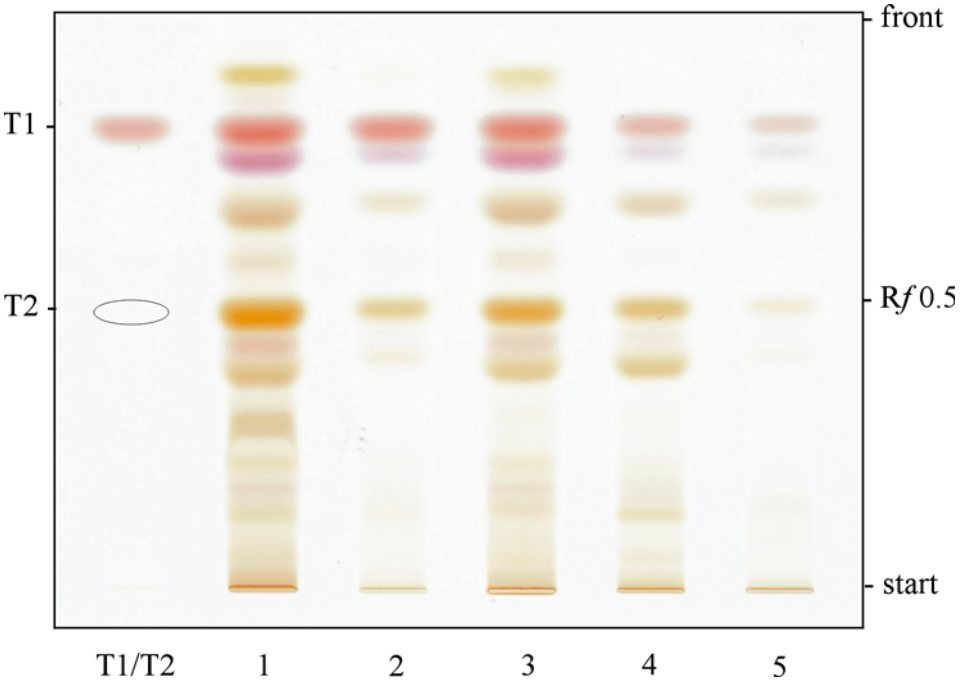


Fig. 2a: Thin layer chromatogram of the diethyl ether extracts of *Salvia miltiorrhiza* (VIS)

The TLC fingerprint of all five Salvia diethyl ether extract samples are characterized by 11 – 13 red orange or light orange brown bands distributed over the whole plate. The prominent red orange zones are the Tanshinone IIA at R*f* = 0.81 (**T1**) with a second one directly below (R*f* = 0.75) and Cryptotanshinone at R*f* = 0.49 (**T2**) accompanied by a further diterpenquinone at R*f* = 0.45. In the R*f* - range from Cryptotanshinone up to the solvent front and in the low R*f* - range further non identified diterpenquinones of lower concentration, can be detected.

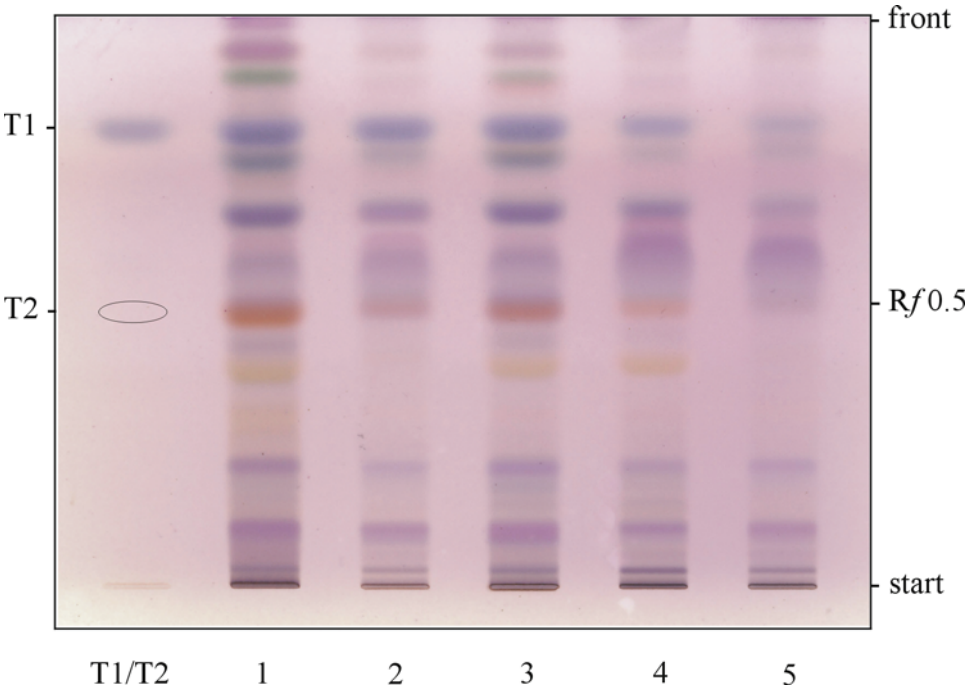


Fig. 2b: Thin layer chromatogram of the diethyl ether extracts of *Salvia miltiorrhiza* sprayed with anisaldehyde-sulphuric acid reagent (VIS)

The TLC was developed in the same solvent system but sprayed with the anisaldehyde-sulphuric acid agent. It shows for all diethyl ether extracted samples the same constituents but in more distinct red violet color. The extract samples 1 and 3 are characterized by a very homogeneous pattern of all known constituents, whereas in the samples 2, 4, and 5 most of the constituents are present only in lower concentration.

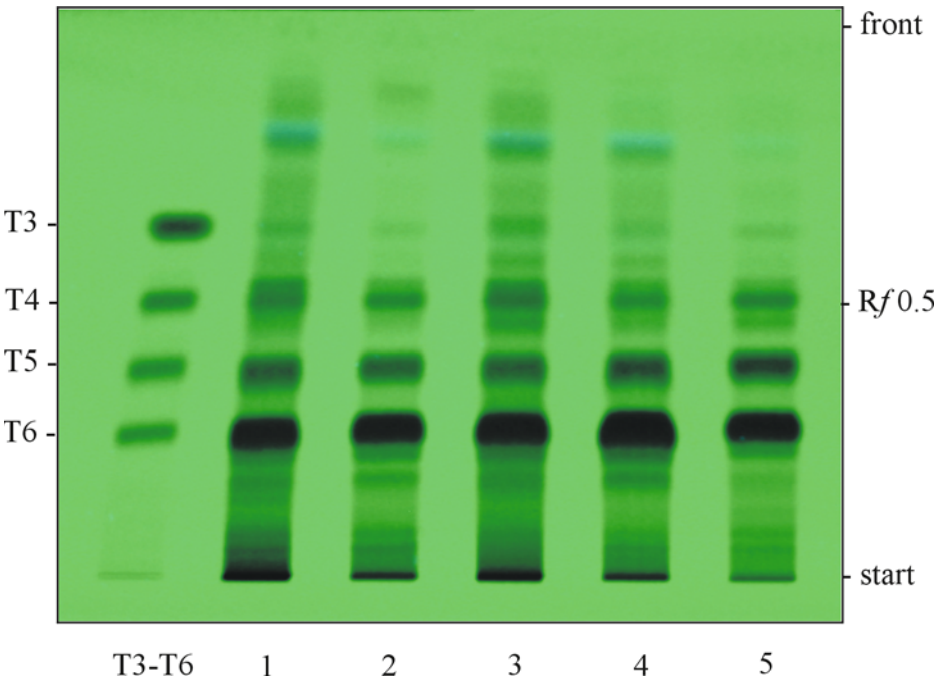


Fig. 2c: Thin layer chromatogram of the water extracts of *Salvia miltiorrhiza* after enrichment of the main polar constituents in the ethyl acetate phase (UV 255 nm)

The water extracts of samples 1 – 5 provide under UV 255 nm a very homogeneous pattern of four to five green black colored constituents on green background: Salvianolic acid B (R*f* = 0.27/ **T6**), Lithospermic acid (R*f* = 0.38/**T5**), Rosmarinic acid (R*f* = 0.50/**T4**), and Protocatechuic acid (R*f* = 0.64/**T3**). Salvianolic acid B is the dominant acid present in the highest concentration. Further light green zones between the main compounds are present in very low concentration and can be assigned to other phenol carboxylic acids.

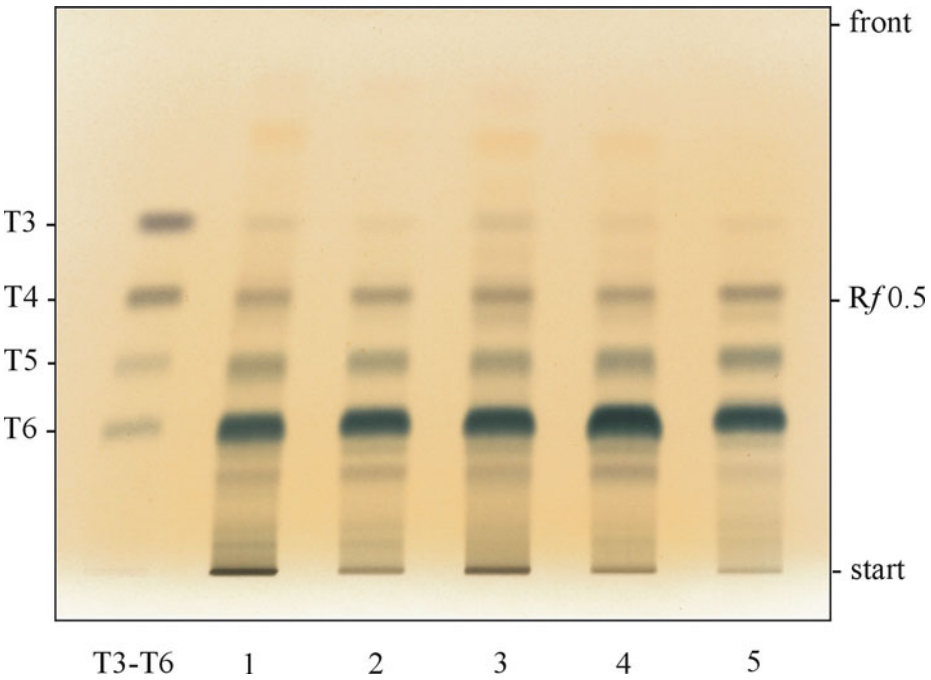


Fig. 2d: Thin layer chromatogram of the water extracts of *Salvia miltiorrhiza* after enrichment of the main polar constituents in the ethyl acetate phase and treatment with Iron-III-chloride reagent (VIS)

The same phenol carboxylic acids sprayed with Iron-III-chloride reagent, appear with black-grey color on light yellow background.

HPLC-fingerprint analysis of tanshinones and phenol/caffeoyl carboxylic acids of *Salvia miltiorrhiza* root/rhizome extracts:

1) Sample preparation:
   a) 1 g powdered drug is extracted under reflux with 10 ml **methanol** on a water bath for 30 minutes. After cooling the extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm, and the solution is injected into the HPLC apparatus.
   b) 1 g powdered drug is extracted under reflux with 10 ml **methanol** on a water bath for 30 minutes. After cooling the extract is filtered, **acidified** with 10 µl concentrated hydrochloric acid (37 %) and evaporated to dryness. The residue is dissolved in 1 ml of methanol, filtered over Millipore® filtration unit, type 0.45 µm, and the solution is injected after 16 hours into the HPLC apparatus.

c) 1 g powdered drug is extracted under reflux with 10 ml **water** on a water bath for 30 minutes. After cooling the extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml of water (Millipore Ultra Clear UV plus® filtered), filtered over Millipore® filtration unit, type 0.45 µm, and the solution injected into the HPLC apparatus.

2) Injection volume: Radix et Rhizoma Salviae miltiorrhizae extracts: 5.0 µl each

3) HPLC parameter:

Apparatus:
MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck

Precolumn: LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck

Solvent:
A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l water (Millipore Ultra Clear UV plus® filtered)

B: acetonitrile (VWR)

Gradient:

Gradient Fig. 3a – 3c:	Gradient Fig. 3d: (1)
0 – 23 % B in 18 minutes 23 – 25 % B in 11 minutes 25 – 60 % B in 11 minutes 60 – 85 % B in 20 minutes.	55 % B in 50 minutes.
Total runtime: 60 minutes	Total runtime: 50 minutes

Flow: 1.0 ml/min.

Detection:

Detection Fig. 3a – 3c:	Detection Fig. 3d: (1)
281 nm	270 nm

Retention times of the main peaks

Peak	Rt (min.) Fig. 3a	Rt (min.) Fig. 3b	Rt (min.) Fig. 3c	Rt (min.) Fig. 3d	Compounds
1	n. d.	10.0	n. d.	n. d.	Danshensu*
2	21.8	20.3	19.2	n. d.	Rosmarinic acid
3	n. d.	27.0 + 28.0	n. d.	n. d.	Lithospermic acid
4	22.5	37.9	20.1	n. d.	Salvianolic acid B
5	48.0	47.5	n. d.	16.4	Dihydrotanshinone*
6	50.1	49.3	n. d.	21.8	Cryptotanshinone*
7	52.3	50.4	n. d.	26.2	Tanshinone I*
8	55.0	53.1	n. d.	41.9	Tanshinone IIA

n. d.: not detectable * according to the reference[14]

4) **Description of the HPLC-fingerprints of various Salvia-extracts:**

Figure 3a: The **methanol** extract of sample 1 shows at Rt 21.8 Rosmarinic acid (peak **2**) in a very low concentration and Salvianolic acid (peak **4**) as the dominant caffeoyl carboxylic acid. The tanshinones appear in the range of Rt = 48.0 to Rt = 55.0 (peak **5** = Dihydrotanshinone, peak **6** = Cryptotanshinone, peak **7** = Tanshinone I and peak **8** = Tanshinone IIA).

Figure 3b: In the HPLC-fingerprint of the **acidified methanol** extract the phenol (caffeoyl) carboxylic acid fraction shows five peaks: **1** = Danshensu (3-(3′,4′-dihydroxy-phenyl)lactic acid) at Rt 10.0; **2** = Rosmarinic acid at Rt 20.3; **3** = Lithospermic acid with its stereoisomer at Rt 27.0 and 28.0 and **4** = Salvianolic acid B at Rt 37.9. The tanshinones appear again in the Rt - range between 47.5 to 53.1.

Figure 3c: In the **water** extract appear only the phenol (caffeoyl) carboxylic acids Rosmarinic acid in peak **2** and Salvianolic acid B in peak **4** with further acids in very low concentration in the Rt – range of 15.0 to 22.0.

Figure 3d: In this HPLC-fingerprint obtained with a different solvent gradient the single tanshinone (**5, 6, 7, 8**) of the **methanol** extract appear in a Rt - distribution, which is best suitable for the quantitative determination.

Note:

a) The Tanshinones and caffeoyl carboxylic acids are chromatographed in Chinese Pharmacopoeia 2005 monographs[1] on octadecylsilane bonded silica gel with a mixture of methanol and water (75:25) and detected at 270 nm. The caffeoyl carboxylic acids are chromatographed on the same column with methanol, acetonitrile, formic acid and water (30:10:1:59) and detected at 286 nm.

b) Alternative HPLC-methods of *Salvia miltiorrhiza* extracts are reported in references[6,11,14,24-31].

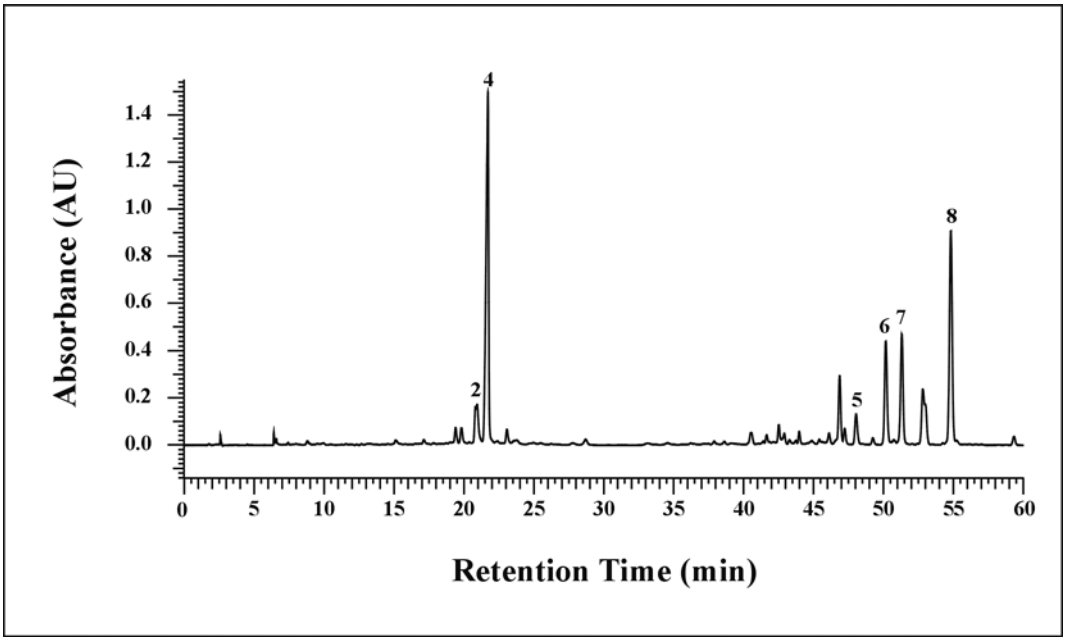


Fig. 3a: HPLC fingerprint of tanshinones and phenol/caffeoyl carboxylic acids of *Salvia miltiorrhiza*, sample 1 (methanol extraction, see sample preparation 1a)

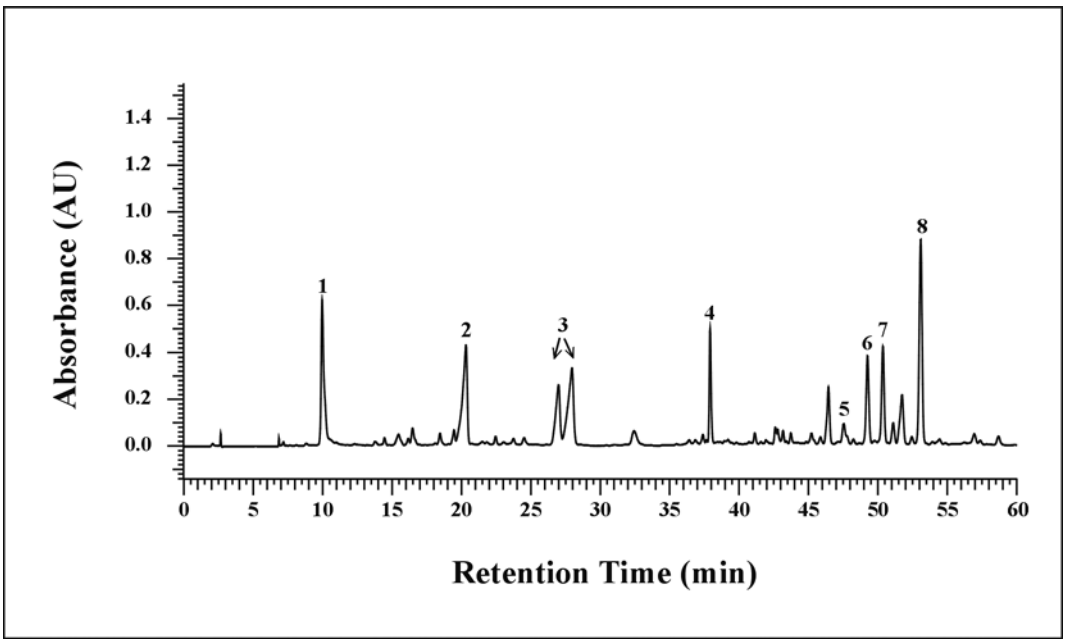


Fig. 3b: HPLC fingerprint of tanshinones and phenol/caffeoyl carboxylic acids of *Salvia miltiorrhiza*, sample 1 (acidified methanol extraction, see sample preparation 1b)

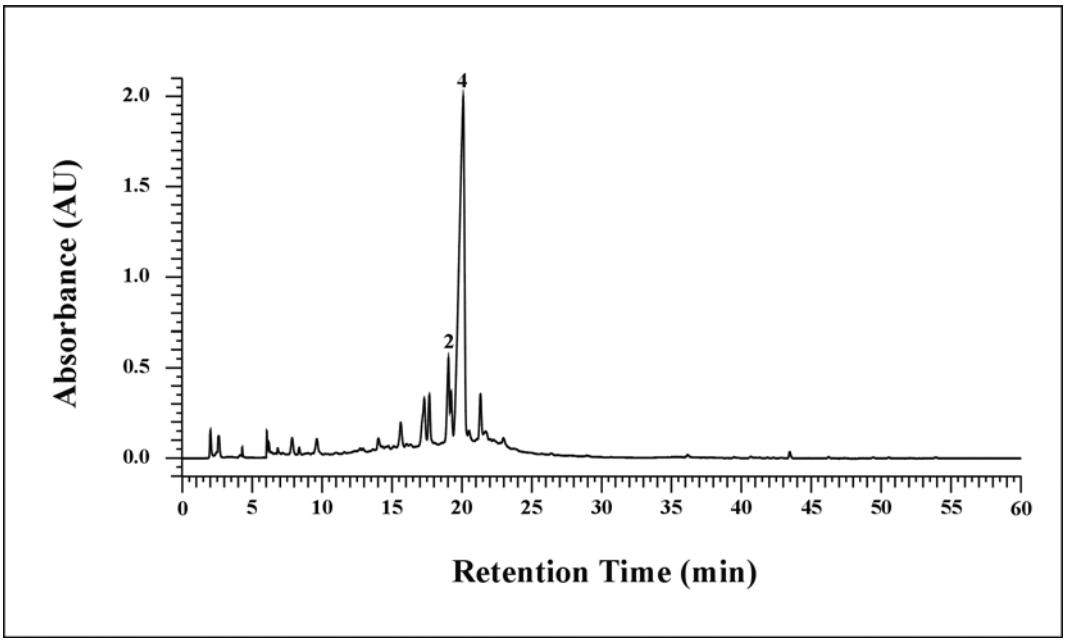


Fig. 3c: HPLC fingerprint of phenol/caffeoyl carboxylic acids of *Salvia miltiorrhiza*, sample 1 (water extraction, see sample preparation 1c)

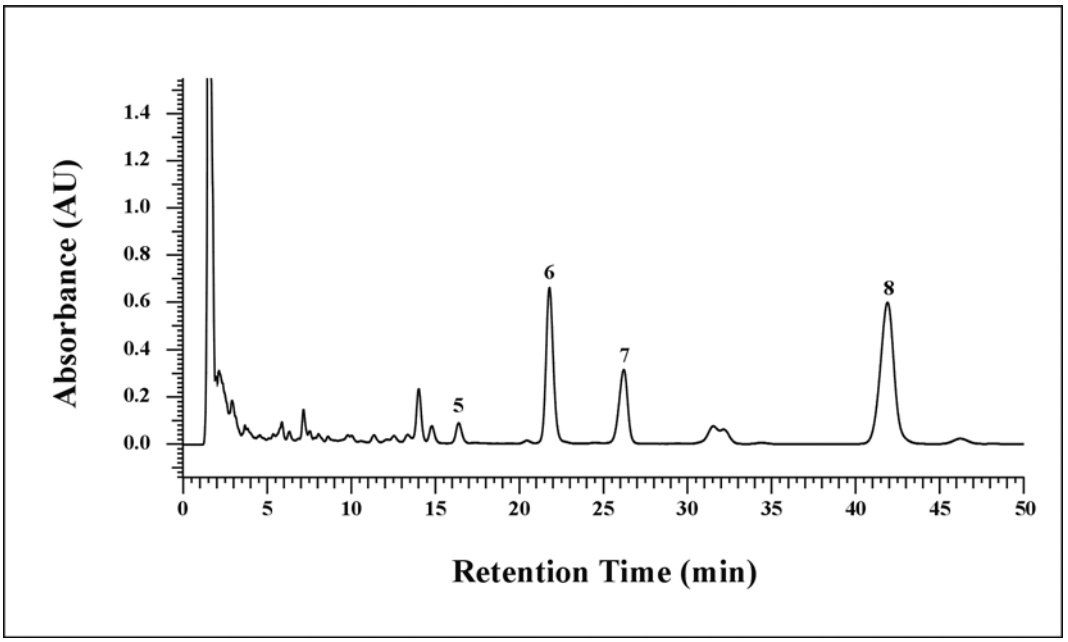


Fig. 3d: HPLC fingerprint of tanshinones of *Salvia miltiorrhiza*, sample 1 (methanol extraction with a different gradient, see sample preparation 1a and gradient)

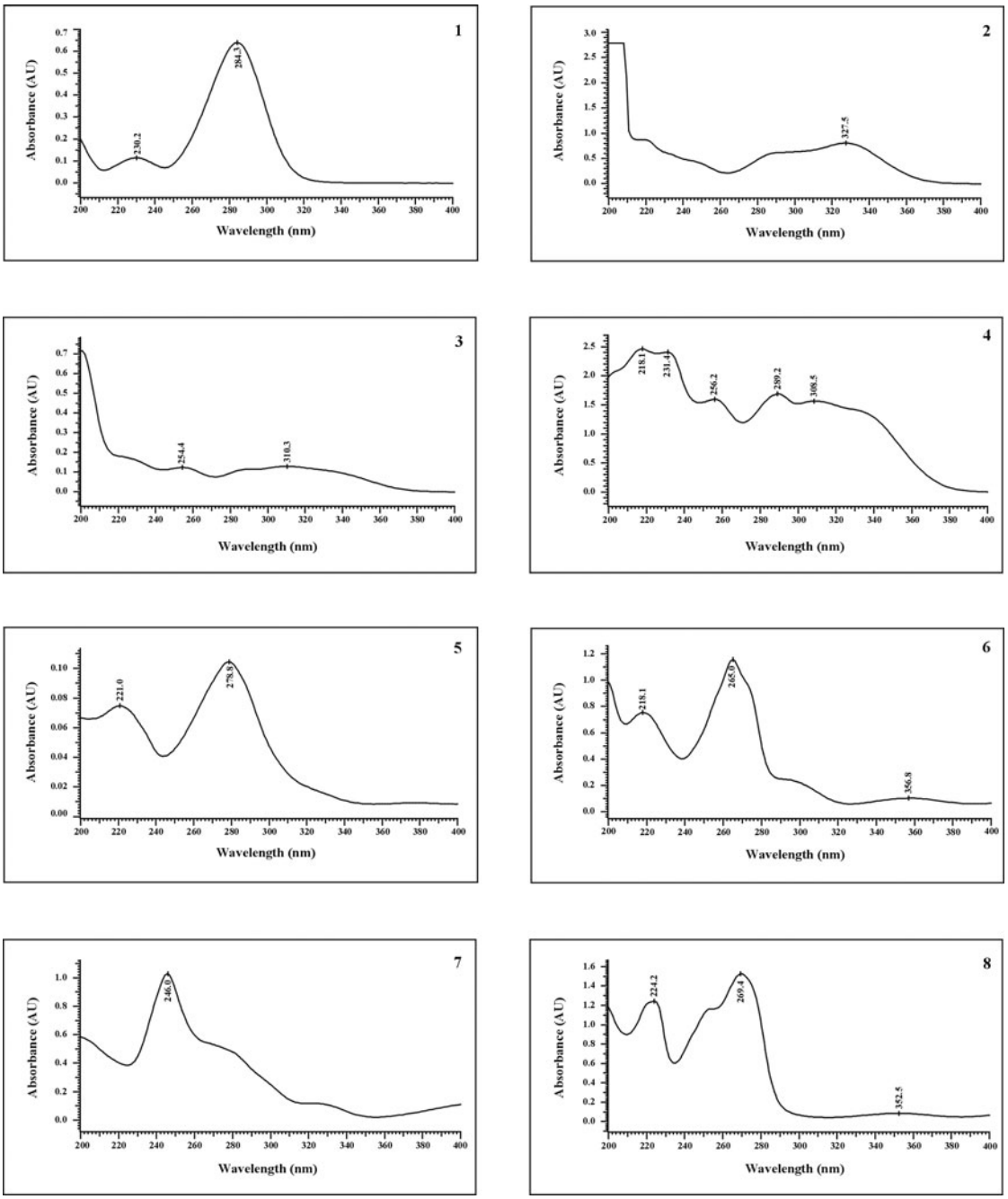


Fig. 4: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of Radix et Rhizoma Salviae miltiorrhizae

- Quantitative HPLC-Analysis of tanshinones in root/rhizome of *Salvia miltiorrhiza*[1]

1) Sample preparation:	1.0 g powdered drug is extracted under reflux with 10 ml methanol on a water bath for 30 minutes. After cooling the extract is filtered and evaporated to dryness. The residue is dissolved in 2 ml of methanol. The solution is transferred to a 5 ml volumetric flask, and filled up to the measuring mark. Before injection a sample is filtered over Millipore® filtration unit, type 0.45 µm.
2) Injection volume:	Radix et Rhizoma Salviae miltiorrhizae extracts: 5.0 µl each
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent:	A: 10 ml 0.1 % H_3PO_4 (Merck) / 1 l Water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	isocratic 55 % B in 50 minutes. total runtime: 50 minutes
Low:	1.0 ml/min.
Detection:	270 nm

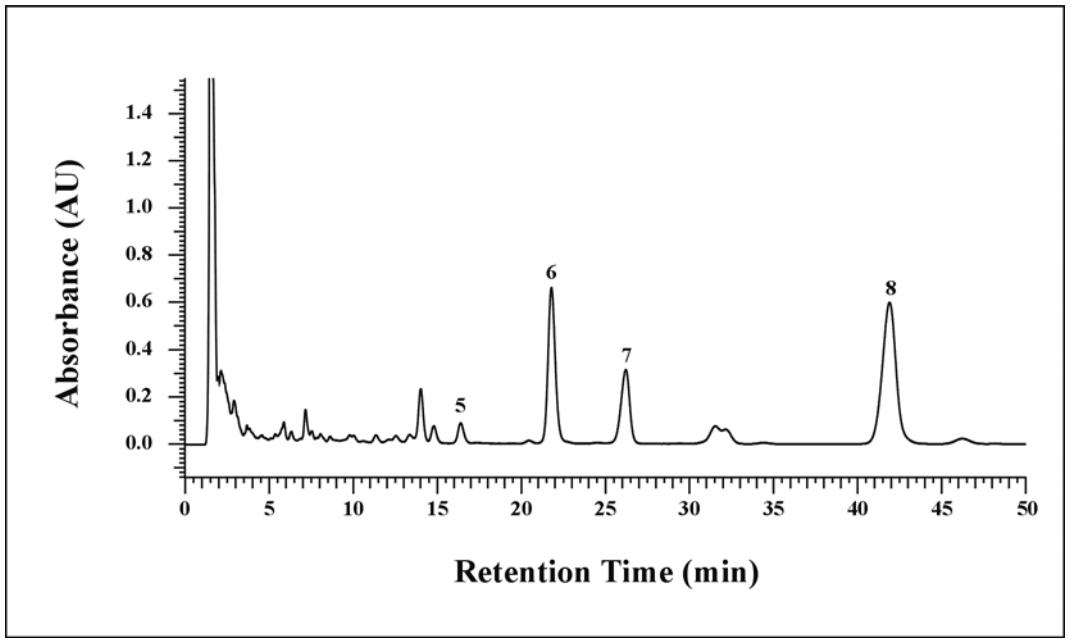


Fig. 5: HPLC fingerprint of the methanol-extract of Radix et Rhizoma Salviae miltiorrhizae, sample 1

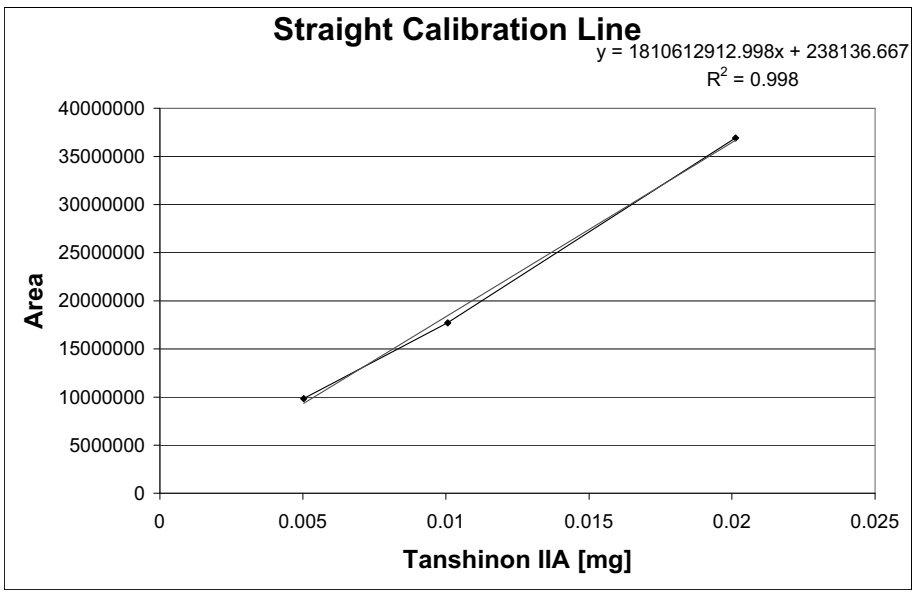


Fig. 6: Straight Calibration Line

The concentrations of reference standard Tanshinone IIA used for calibration were 0.25, 0.50, and 1.0 mg/ml in methanol. Each of the three solutions was injected three times.

(Relative) Content of tanshinones in methanol-extract of Radix et Rhizoma Salviae miltiorrhizae sample 1 as calculated as Tanshinone IIA with reference to the dried drug of the methanol-extract:

Peak	Rt (min.)	Compound	(relative) content in extract sample 1
5	16.39	Dihydrotanshinone	(0.01 %)
6	21.79	Cryptotanshinone	(0.11 %)
7	26.21	Tanshinone I	(0.06 %)
8	41.90	Tanshinone IIA	0.19 %
		total concentration of tanshinones	(0.37 %)

Note:

The Chinese Pharmacopoeia 2005[1] demands for Tanshinone IIA a content not less than 0.2 %. Additionally also a quantitative determination of Salvianolic acid B is described and should be not less than 3.0 % calculated to the dried root/rhizome.

Conclusion

The lipophilic ether soluble tanshinones and the water soluble phenol/caffeoyl carboxylic acids of *Salvia miltiorrhiza* which both contribute to the cardiovascular, antiischemic, antihyperlipidemic and antidiabetic activity of *Salvia miltiorrhiza* root can be best separated and evaluated using two different TLC- and HPLC-fingerprint analytical methods for the tanshinones and the phenol/caffeoyl carboxylic acids. A fast detection of both fractions can be achieved with the same MeOH-extract (Fig. 3a). The characteristic marker compounds for *Salvia miltiorrhiza* are Tanshinone IIA and Salvianolic acid B.

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Poria – *Fuling*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Indian Bread is the dried sclerotium of the fungus, *Poria cocos* (Schw.) Wolf (Fam. Polyporaceae).

The drug is collected mostly in July to September, removed from soil, piled up, spread, and air-dried on the surface. This operation has to be repeated for several times until wrinkles appear and the inside water is evaporated, then dried in the shade. This is known as "Fulingge". The fresh sclerotium can be also cut and dried in the air. Accordingly the cut portions are known as "Fulingpi" and "Fulingkuai" respectively.

Synonyms[2,3]: *Wolfiporia cocos* (Wolf) Ryvarden *et* Gilbertson, "Hoelen" in Japanese

Origin[4]: Chinese provinces Yunnan, Anhui, Hubei, Zhejiang.

Description of the drug[1]: *Fulingge*: Subglobose, ellipsoid, oblate or irregular-shaped, variable in size. The outer skin thin and rough, brown to blackish-brown, conspicuously shriveled and striated. Texture hard and compact, fracture granular, some cracked, the outer layer pale brown, inner part white, rarely reddish, some showing the penetrating roots of pine in the center. Odor, slight; taste, weak and sticky when chewed.

Fulingpi (pared skin of Poria): Variable in form and size. Externally brown to blackish-brown, internally white or pale brown. Relatively loose and soft, slightly elastic.

Fulingkuai (peeled and sliced Poria): Occurring in pieces or slices, variable in size. White, pale red or pale brown.

Pretreatment of the raw drug[1]: Poria is soaked in water, washed clean and steamed briefly after softened. This product is cut into skin pieces and thick slices separately in time, and then dried.

Medicinal use[4]: Poria is used to treat diarrhea, urethritis, dropsy, insomnia and digestive disorders.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Table 1:

Effects and indications of Poria according to Traditional Chinese Medicine[1,4-7]	
Taste:	Sweet
Temperature:	Neutral
Channels entered:	*Orbis cardialis, Orbis pulmonalis, Orbis lienalis, Orbis stomachi, Orbis renalis.*
Effects (functions):	Poria causes urination, invigorates the spleen function, and calms the mind.
Symptoms and indications:	Edema with oliguria; dizziness and palpitation caused by retained phlegm and morbid fluid; diminished function of the spleen marked by anorexia, loose stools or diarrhea; restlessness and insomnia.

Constituents

Main constituents	Minor constituents
1. Lanostane-type triterpenoids:	
ebricoic acid[8]	3-acetyloxy-16α-hydroxytrametenolic acid[9]
pinicolic acid[8]	dehydrotumulosic acid[9,11,14]
pachymic acid[8,9,10]	3-*epi*-dehydrotumulosic acid[9,11]
tumulosic acid[8,9,10,11]	15α-hydroxydehydrotumulosic acid[13]
dehydrotrametenolic acid[11,12,13]	5α,8α-peroxydehydrotumulosic acid[13]
polyporenic acid C[9,10,15]	29-hydroxypolyporenic acid C[9]
poricoic acids[11,13]	dehydropachymic acid[9,10]
dehydroebriconic acid[11]	25-hydroxypachymic acid[16]
	3-*epi*-dehydropachemic acid[9]
	16α,25-dihydroxydehydroeburicoic acid[13]
	25-hydroxyporicoic acid H[13]
	16-deoxyporicoic acid B[13]
	poriacosone A and B[17]
2. Polysaccharides:	
β-pachyman[8]	beta-glucan PCM3-II[18]
	(1→3)-alpha-D-glucan Pi-PCM3-I[19]
	ac-PCM0[20]
	(1→3)-alpha-D-glucan ab-PCM3-I and ac-CM3-I[21]
	PCS1, PCS2, PCS3-I, PCS3-II, PCS4-I, PCS4-II[22]
	wc-PCM0, wc-PCM1, wc-PCM2[23]
	wb-PCM0, wb-PCM1, wb-PCM2[23]
	ac-PCM0, ac-PCM1, ac-PCM2[23]
	ab-PCM0, ab-PCM1, ab-PCM2[23]

3. Other components:

proteins, fats, lecithin, sterols, gum [8]

2,4,6-triacetylenic octane diacid [8]

2,4,5,6-tetrahydroxyhexanoic acid [8]

3,4-dihydroxy-2-keto-*n*-butyl 2,4,5,6-tetrahydroxy-hexanate [8]

(S)-(+)-turmerone [10]

ergosterol peroxide [10]

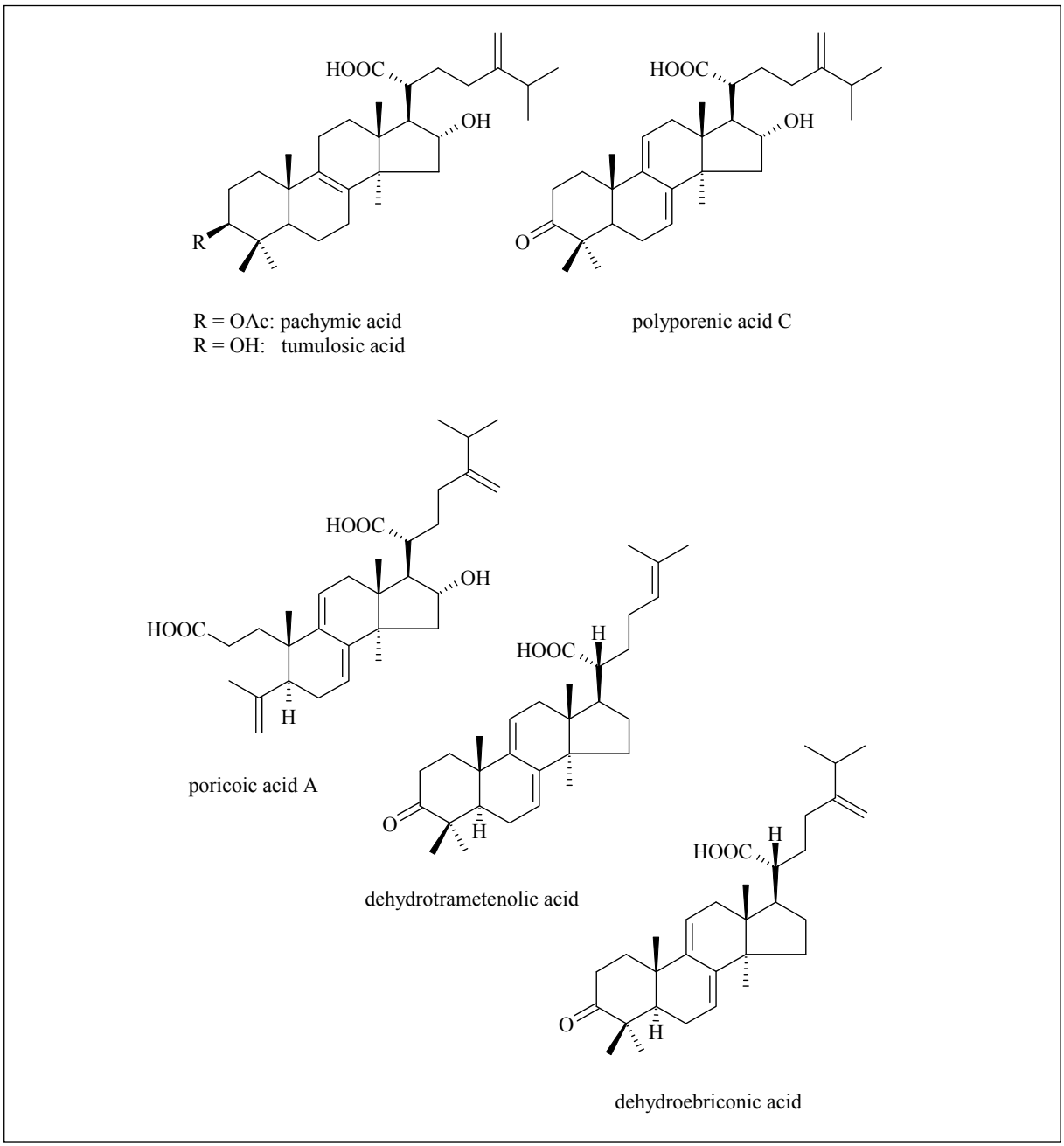


Fig. 1: Formulae of the main compounds of Poria [9,11]

Pharmacology:

- cytotoxic[9,10,13,15,18,21,23]
- anti-oxidant[9,24]
- anti-inflammatory activity[25,26]
- immunomodulatory effect[27]
- angiogenesis-inhibitory effect[28]
- insulin-sensitizing[12]
- antinephritic effect[29]
- anti-emetic effect[30]
- nematicidal activity[8]

TLC- and HPLC-fingerprint analysis:

Table 2: (see Fig. 2)

Drug samples		Origin
1	Fuling/*Poria cocos* (Schw.) Wolf	sample of commercial drug (HerbaSinica, Germany (origin: Province Anhui, China))
2	Fuling/*Poria cocos* (Schw.) Wolf	Province Hunan, China
3	Poria/*Poria cocos* (Schw.) Wolf	sample of commercial drug (Fraunhofer Apotheke, Munich)
4	Poria/*Poria cocos* (Schw.) Wolf	sample of commercial drug (China Medica, Germany)
5	Fulingkuai/*Poria cocos* (Schw.) Wolf	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany)
6	Fulingkuai/*Poria cocos* (Schw.) Wolf	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany)
7	Fulingpi/*Poria cocos* (Schw.) Wolf	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany)

Table 3:

Reference compound of Figure 2		R*f*
T1	Ursolic acid	0.72

TLC-fingerprint analysis[2]:

1) Extraction: 2 g powdered drug are extracted under reflux in a water bath with 20 ml diethyl ether for 30 minutes. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and used for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: TLC Silica gel 60 F_{254}, Merck

Applied amounts: Poria extracts: each 10 µl
Fulingpi (sclerotium of Poria) extract: 2.5 µl
reference compound: 10 µl

Solvent system: chloroform : methanol
9 1

Detection: Anisaldehyde-sulphuric acid reagent:
0.5 ml anisaldehyde is mixed with 10 ml glacial acetic acid, followed by addition of 85 ml methanol and 5 ml conc. sulphuric acid (96 %), in that order.
The TLC is sprayed with about 10 ml, heated at 110 °C for 5 min., then evaluated in VIS.

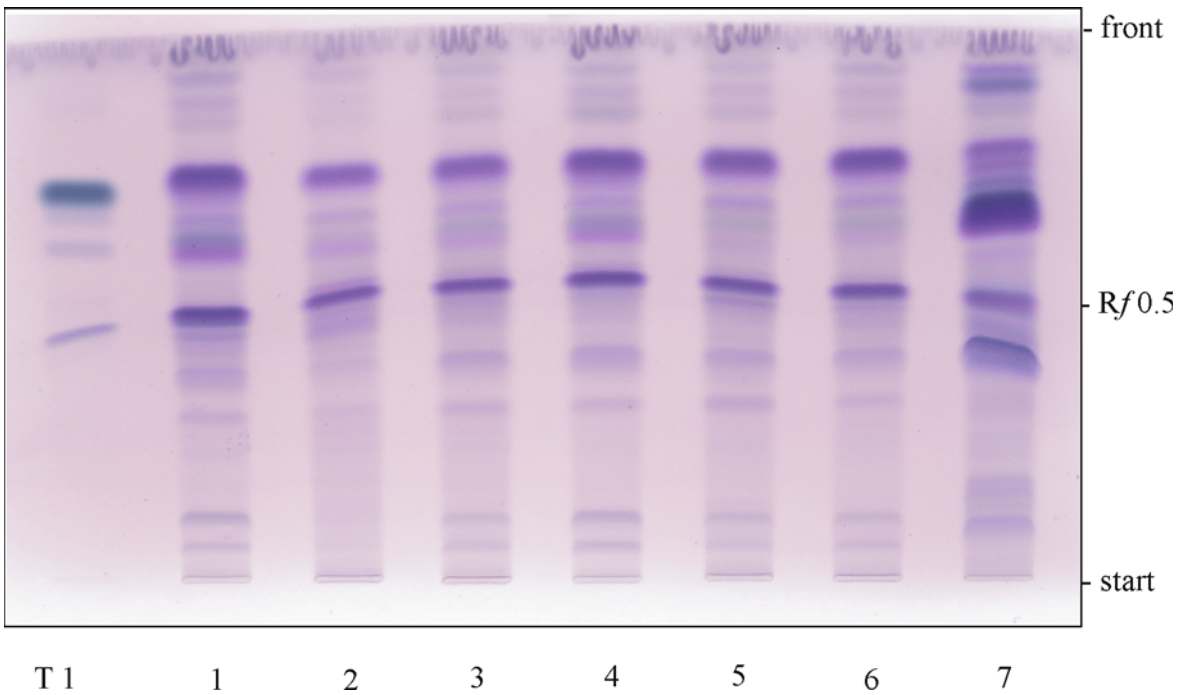


Fig. 2: Thin layer chromatogram of the diethyl ether extracts of *Poria cocos* sprayed with anisaldehyde-sulphuric acid reagent (VIS)

4) Description of Fig. 2:

All seven extract samples show in VIS a very homogeneous pattern of 3 – 5 distinct violet bands in the R*f*-range of 0.45 to 0.85. Sample 7 deviates from the others by two strong violet zones at R*f* = 0.42, 0.67 and 0.91. Additional weak zones appear in all samples in the low R*f*-range from start to 0.4 and between R*f* 0.85 to solvent front. The reference compound ursolic

acid **T1** (blue-violet), not present in *Poria cocos*, lies in the R*f*-range close to a triterpenoic acid at R*f* = 0.85. Because of lacking reference compounds the main zones could not be assigned to the compounds listed on the formula chart, but obviously the main triterpenoic acids with one carboxyl group like pachymic acid or polyporenic acid are located in higher and middle R*f*-ranges whereas e.g. poricoic acid with two carboxyl groups may be located in the middle or low R*f*-range.

HPLC-fingerprint analysis:

1) Sample preparation: 2 g powdered drug are extracted under reflux in a water bath with 20 ml diethyl ether for 30 minutes. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol, filtered over Millipore® filtration unit, type 0.45 µm, and injected into the HPLC apparatus.

2) Injection volume: Poria extracts: each 30.0 µl
Fulingpi (sclerotium of Poria) extract: 7.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent:	A: 0.001 % H_3PO_4 (Merck) / Water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	50 – 100 % B in 20 minutes 100 % B for 10 minutes total runtime: 30 minutes
Flow:	1.0 ml/min.
Detection:	240 nm

Retention times of the main peaks:*

Table 4:

Peak	Rt (min.)	Peak	Rt (min.)
1	6.1 – 6.5	7	14.2 – 15.4
2	7.6 – 8.1	8	16.4 – 17.8
3	10.7 – 11.7	9	17.5 – 19.0
4	11.9 – 13.1	10	19.9 – 21.2
5	12.8 – 13.9	11	21.8 – 23.3
6	13.7 – 15.0	12	23.2 – 24.6

***** A correct assignment to the various triterpenoids was not possible.

4) Description of the HPLC-fingerprints (Fig. 3a-c, Fig. 4):

The HPLC-fingerprint of all samples shows a very homogeneous qualitative and quantitative pattern of 12 peaks distributed over the whole Rt-range between 5 to 25 minutes. The peaks **3** – **10** possess in all samples about the same quantitative profile. Analogous to the TLC-zone pattern sample 7 (Fulingpi) differs from the other samples in two distinct strong peaks **4** and **11** which may correspond with the violet bands in TLC at R*f* = 0.42 and 0.67. It can also be suggested, that the online UV-spectrum of sample 8 with its weak additional maximum at 324 nm derives from the C = O – functions and conjugated double bounds of dehydrotrametenolic and dehydroebriconic acid.

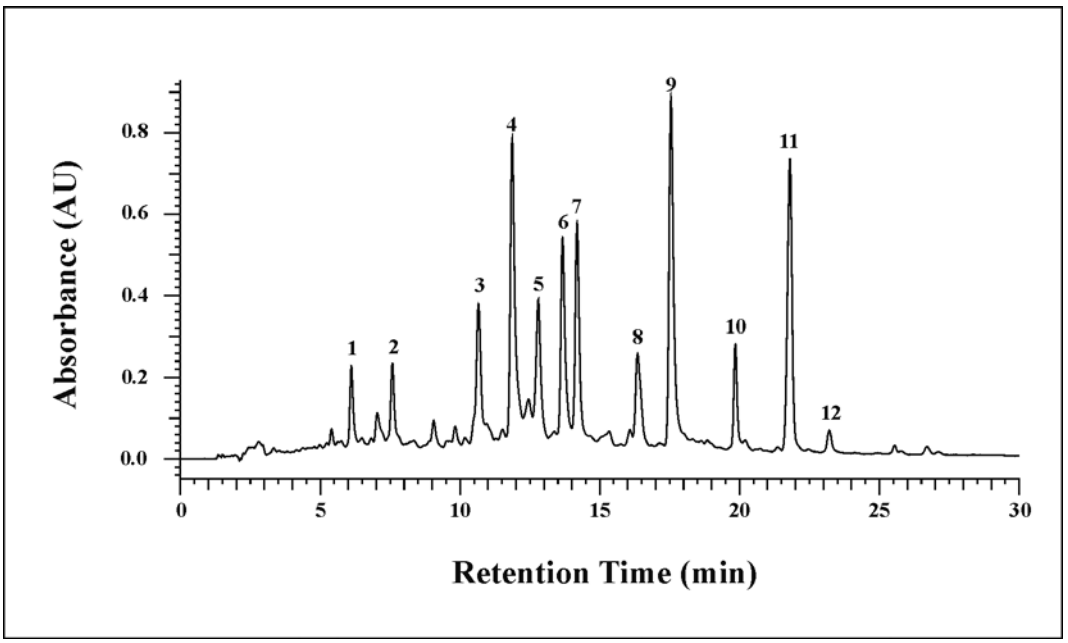


Fig. 3a: HPLC fingerprint of Poria (Fuling), sample 1

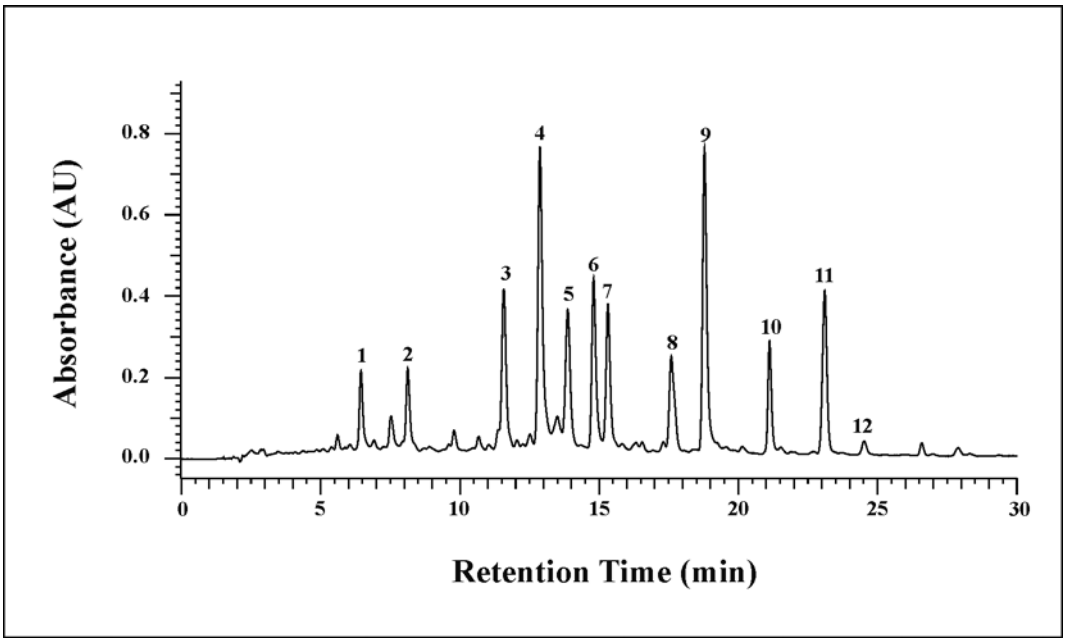


Fig. 3b: HPLC fingerprint of Poria, sample 4

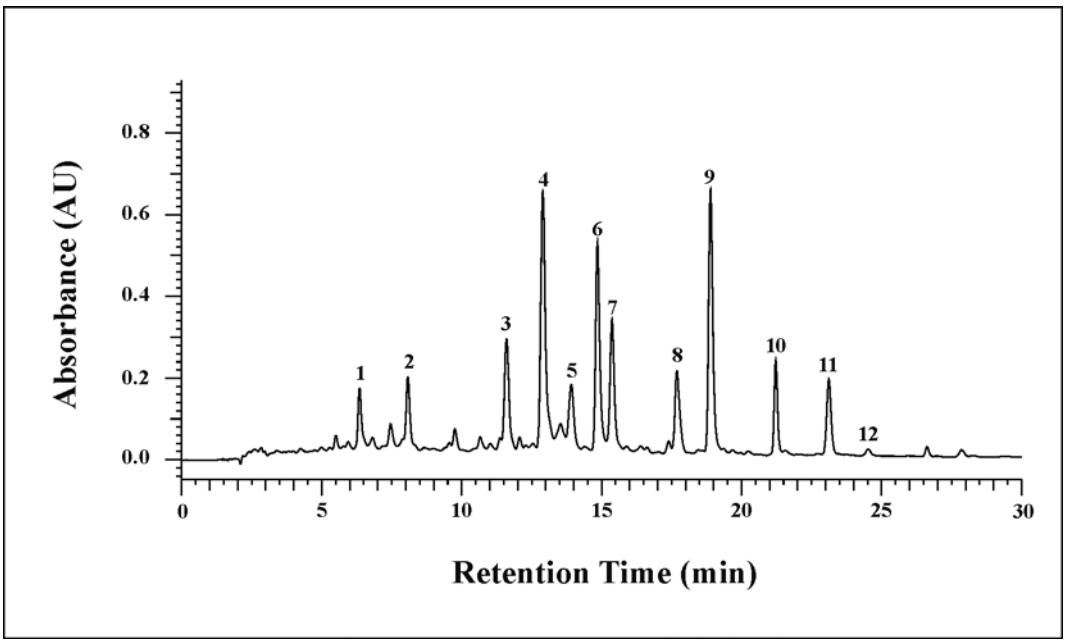


Fig. 3c: HPLC fingerprint of Poria (Fulingkuai), sample 6

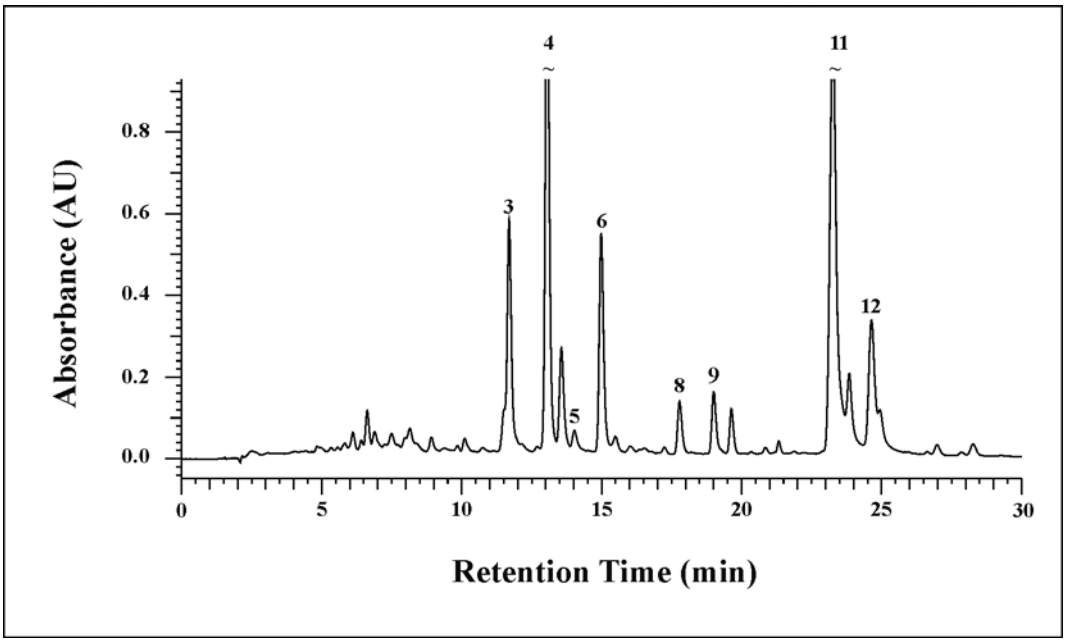
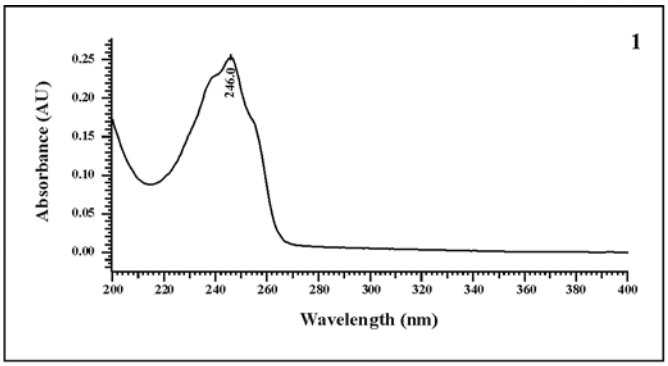
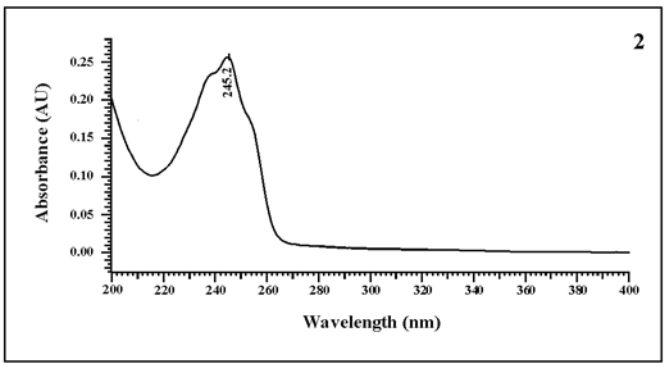
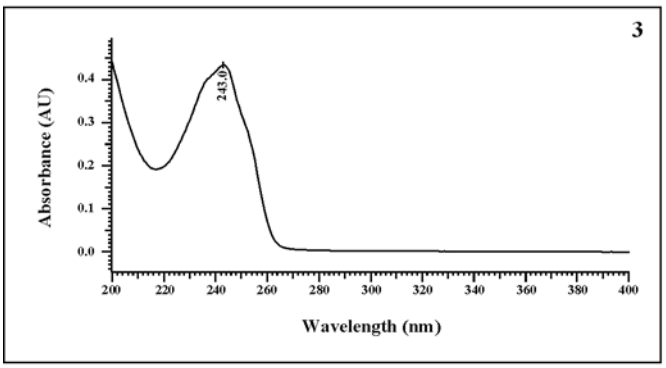
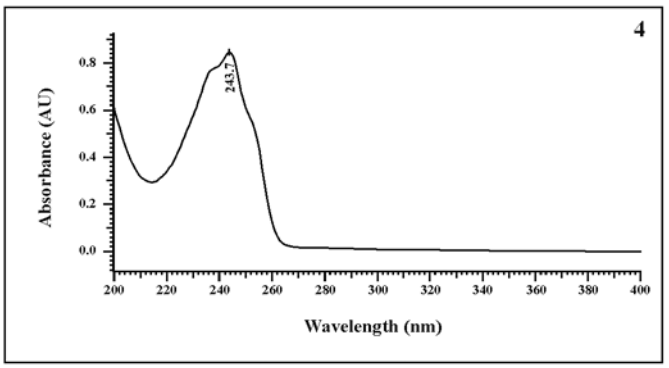


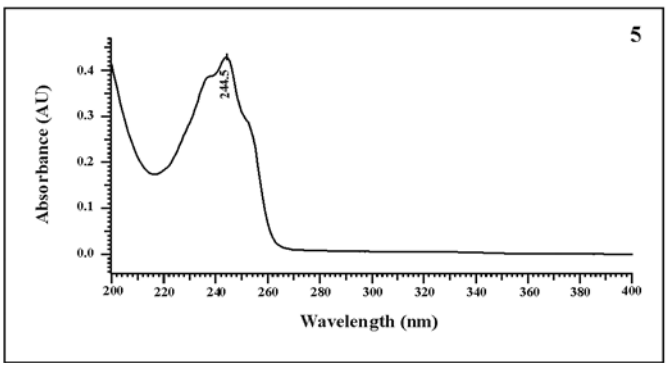
Fig. 3d: HPLC fingerprint of Poria (Fulingpi), sample 7

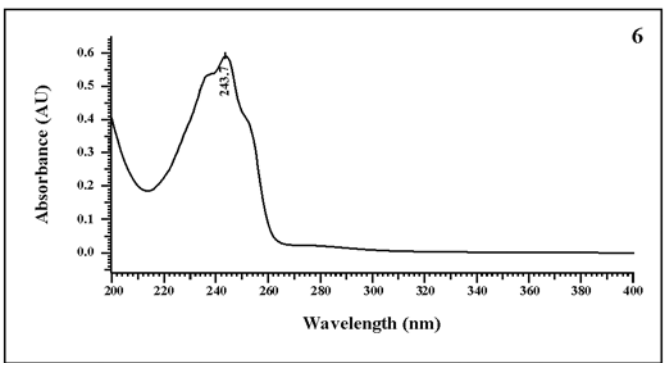

1
Absorbance (AU)
246.0
Wavelength (nm)

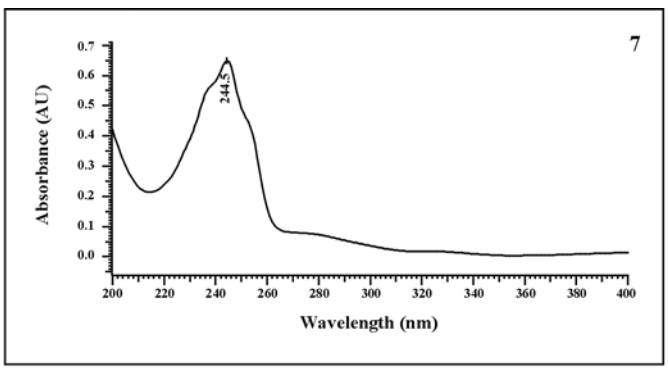

2
Absorbance (AU)
245.2
Wavelength (nm)

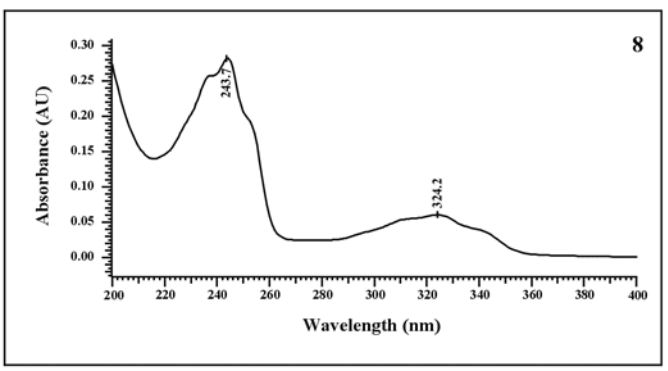

3
Absorbance (AU)
243.0
Wavelength (nm)


4
Absorbance (AU)
243.7
Wavelength (nm)


5
Absorbance (AU)
244.5
Wavelength (nm)


6
Absorbance (AU)
243.7
Wavelength (nm)


7
Absorbance (AU)
244.5
Wavelength (nm)


8
Absorbance (AU)
243.7
324.2
Wavelength (nm)

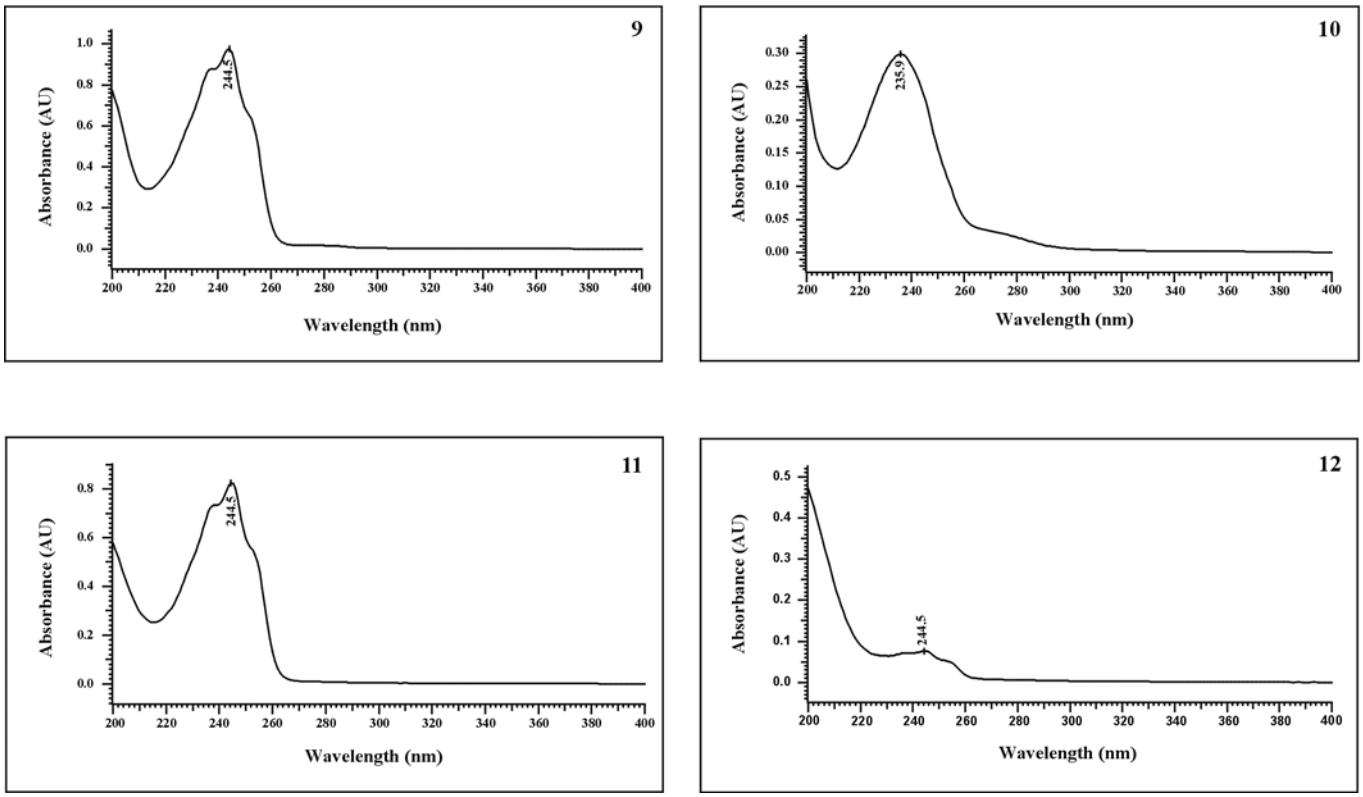


Fig. 4: Online UV-spectra of the main characteristic peaks of the HPLC-fingerprint of Poria

Conclusion

The *Poria cocos* samples can be easily authenticated in TLC and HPLC by their characteristic very homogeneous zone- and peak patterns. The Fulingpi sample 7 (Poria skin) can be discriminated from Fulingge and Fulingkuai samples 1, 4 and 6 due to very strong zones and peaks **4** and **11**, which are weak in Fulingge and Fulingkuai correspondingly.

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Semen Cassiae – *Juemingzi*

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Cassia Seed is the dried ripe seed of *Cassia obtusifolia* L. ("sicklepod") or *Cassia tora* L. (Fam. Caesalpinaceae).

The ripe legume is collected in autumn and dried in the sun, then the seed is tapped out and removed from foreign matter.

Origin[2]: Southern China, Laos, Cambodia, Vietnam, India, Japan, Philippines, Java.

Description of the drugs[1]: Semen Cassiae obtusifoliae: Slightly rhomboidal-cuboid or shortly cylindrical, both ends parallel and oblique, 3-7 mm long, 2-4 mm wide. Externally greenish-brown or dark brown, smooth and lustrous. One end relatively even, the other end oblique and acuminate, dorsal and ventral surfaces exhibiting a raised rib respectively, with an obliquely symmetrical and paler-colored dented line on each side of a rib. Texture hard and uneasily broken. Testa thin, cotyledons 2, yellow, S-shaped. Odor, slight; taste, slightly bitter.

Semen Cassiae torae: Shortly cylindrical, relatively small, 3-5 mm long, 2-3 mm wide, with broad yellowish-brown bands on both sides of the rib. (The other anatomical characteristics are like those of Semen Cassiae obtusifoliae.)

Pretreatment of the raw drug[1]: Semen Cassiae: Foreign matter is eliminated, washed clean and dried. Before use the drug is broken into pieces.

Semen Cassiae (stir-baked): The clean Semen Cassiae is stir-baked as described under the method for simple stir-baking (Appendix II D of[1]) until slightly scented. Before use the drug is broken into pieces.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Medicinal use[3]: *Cassia obtusifolia* and *Cassia tora* are widely distributed in tropical Asian countries and their seeds have been used as a traditional medicine for constipation, asthenia, eye disease, hepatitis, hemoglobin disorders and as an antidysenteric and diuretic. Hypotensive activity of the seed extract has also been reported. In India the plant is used for the treatment of snakebites and scorpion stings.

Effects and indications of Semen Cassiae according to Traditional Chinese Medicine[1,4]

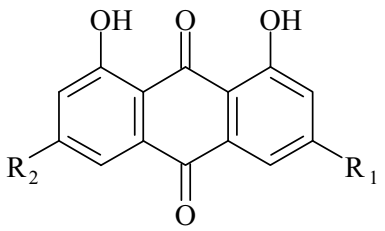
Taste:	Sweet, bitter, briny
Temperature:	Cold tendency
Channels entered:	*Orbis hepaticus, orbis intestini crassi*
Effects:	To remove *heat*, improve eyesight, and relax bowels.
Symptoms and indications:	Inflammation of the eyes with pain, photophobia and lacrimination; headache, dizziness, blurred vision and constipation.

Main constituents:

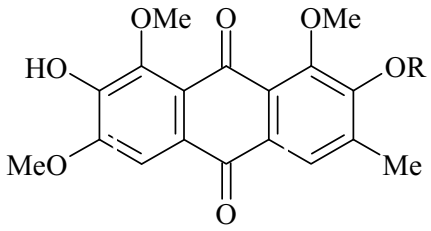
	Cassia obtusifolia	***Cassia tora***
Anthraquinones:	(Aloe-)Emodin[6,12,13,16]	(Aloe-)Emodin[20-22,27]
	Chrysophanol[6,12,13,16]	Chrysophanol[3,20-22,28]
	Physcion[6,12,13,16]	Physcion[3,20-22]
	Rhein[13]	Rhein[27]
	Obtusin[6]	Obtusin[22]
	1-Hydroxy-7-methoxy-3-methylanthraquinone[12,16]	Obtusifolin (1-Methoxy-2,8-dihydroxy-3-methylanthrachinone)[21,22]
	1,2-Dihydroxyanthraquinone[14]	Aurantio-obtusin[3,20-22,28]
	1,2,8-Trihydroxy-6,7-dimethoxyanthraquinone[16]	Alaternin[20]
	1-*O*-Methylchrysophanol[12,16]	Chryso-obtusin[20-22,28]
	8-*O*-Methylchrysophanol[12,6,16]	
Anthraquinone glycosides:	Obtusifolin-2-*O*-*β*-D-(6′-*O*-acetyl) glucopyranoside[6]	Chrysophanol triglucoside[3,20]

	Gluco-obtusifolin[18]	Obtusifolin-2-*O*-*β*-D-glucoside[22]
	Gluco-aurantio-obtusin[18]	Aurantio-obtusin 6-*O*-*β*-D-glucoside[3]
	Gluco-chryso-obtusin[18]	Chryso-obtusin-2-*O*-*β*-D-glucoside[21,22]
Naphthopyrone (glucosides):	Rubrofusarin 6-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside (Cassiasides B2)[17]	Cassiaside[20,24,28]
	Toralactone 9-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (Cassiaside C2)[15]	
		Rubrofusarin[3]
		nor-Rubrofusarin[3,21]
	Rubrofusarin gentiobioside[9]	Rubrofusarin-6-*O*-*β*-D-gentiobioside[20,24,28]
		Rubrofusarin triglucoside[3,27]
		nor-Rubrofusarin gentiobioside[24]
		Toralactone[27]
		nor-Toralactone[3]
		Toralactone 9-*O*-*β*-D-gentiobioside[3,24]
		Toralactone 9-*O*-[*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside][3]
		9-Methoxychrysophanoltoralactone[3]
		Demethylflavasperone gentiobioside[27]
Naphthalene (glycosides):		Cassitoroside[20]
		Torachrysone[3,27]
		Torachrysone 8-*O*-*β*-D-gentiobioside[3,27]
		Torachrysone tetraglucoside[3,27]
		Torachrysone 8-*O*-[*β*-D-glucopyranosyl(1→3)-*O*-*β*-D-glucopyranosyl(1→6)-*O*-*β*-D-glucopyranoside][3]

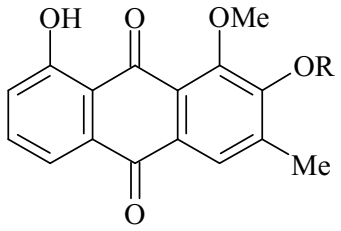
		6-Hydroxymusizin[3] 6-Hydroxymusizin 8-*O*-*β*-D-glucoside[3]
Phytosterols:	*β*-Sitosterol[6,16] Stigmasterol[12,16]	*β*-Sitosterol[20]
Coumarin:	Obtusin[6]	Obtusin[21,22]
quaternized galactomannan:	quaternized as hydroxypropyltrimonium chloride[10]	quaternized as hydroxypropyltrimonium chloride[10]
monosaccharide:		Ononitol[19]
triterpene:	Betulinic acid[12,16]	
soluble fiber (gum)[23]		soluble fiber (gum)[23]



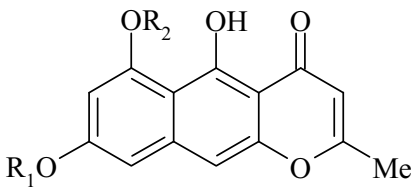
R_1 = Me, R_2 = H: Chrysophanol
R_1 = CH_2OH, R_2 = H: Aloe emodin
R_1 = COOH, R_2 = H: Rhein
R_1 = OH, R_2 = Me: Emodin
R_1 = OMe, R_2 = Me: Physcion



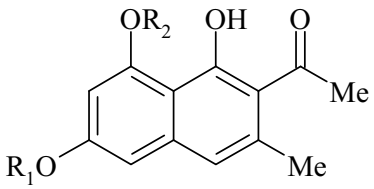
R = H: Obtusin
R = glc: Chryso-obtusin-2-*O*-*β*-D-glucoside



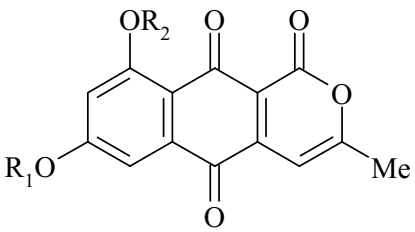
R = H: Obtusifolin
R = glc: Obtusifolin-2-*O*-*β*-D-glucoside



R_1 = Me, R_2 = H: Rubrofusarin
R_1 = H, R_2 = H: nor-Rubrofusarin
R_1 = Me, R_2 = glc(6→1)glc(3→1)glc: Rubrofusarin triglucoside



R_1 = Me, R_2 = H: Torachrysone
R_1 = Me, R_2 = glc(6→1)glc(3→1)glc(6→1)glc: Torachrysone tetraglucoside
R_1 = Me, R_2 = gentiobioside: Torachrysone-8-*O*-*β*-D-gentiobioside
R_1 = H, R_2 = H: 6-Hydroxymusizin
R_1 = H, R_2 = glc: 6-Hydroxymusizin-8-*O*-*β*-D-glucoside



R_1 = Me, R_2 = H: Toralactone
R_1 = H, R_2 = H: nor-Toralactone
R_1 = Me, R_2 = gentiobioside: Toralactone-9-*O*-*β*-D-gentiobioside

Fig. 1: Formulae of the main compounds of Semen Cassiae[3,22]

Pharmacology:

***Cassia obtusifolia*:**	▪ Laxative effect[7] ▪ ACE-inhibitory effect[5,11] ▪ Inhibition of platelet aggregation[11,18] ▪ Antiallergic activity[15,17] by inhibition of histamine release from mast cells[5,11] ▪ Estrogenic activity[5,11] ▪ Neuroprotective effect[5,8] ▪ Larvicidal activity against *Anopheles stephensi*[7], *Aedes aegypti* and *Culex pipiens*[25] ▪ Growth inhibition of *Clostridium perfringens*, *Escherichia coli*[11,14] and *Helicobacter pylori*[11] ▪ Growth-promoting activity to *Bifidobacterium bifidum*[14] ▪ Hair conditioning effect[10]
***Cassia tora*:**	▪ Antioxidant property[20,21,22] ▪ Laxative[19,21,22] and diuretic effect[22] ▪ Hepatoprotective effect[3,19,20,22] ▪ Hypotensive effect[20,21] ▪ ACE-inhibitory effect[20] ▪ Hypolipidemic and cholesterol-lowering effect[3,20,21,23] ▪ Hypoglycemic effect[21] by inhibition of protein glycation and aldose reductase[22,24] ▪ Antiallergic activity[3,22] ▪ Estrogenic activity[3] ▪ Antinociceptive activity[26] ▪ Spasmogenic effects[26] ▪ Antimutagenic activity[3,21,22,28] ▪ Inhibition of cadmium-accumulating[21] ▪ Anthelmintic activity[19] ▪ Larvicidal on larvae of *Aedes aegypti* and *Culex pipiens*[25] ▪ Antimicrobial[3,22], particularly in *Staphylococcus aureus*[27] ▪ Antifungal activity[3,20,22] ▪ Hair conditioning effect[10]

TLC fingerprint analysis

Drug samples		Origin
1	Semen Cassiae/*Cassia obtusifolia*	sample of commercial drug (HerbaSinica, Germany, origin: Shanxi)
2	Semen Cassiae/*Cassia obtusifolia/tora* (botanically not determined)	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany, 2003II)
3	Semen Cassiae/*Cassia obtusifolia/tora* (botanically not determined)	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany, 2003I)
4	Semen Cassiae/*Cassia obtusifolia/tora* (botanically not determined)	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany, 2001)
5	Semen Cassiae/*Cassia obtusifolia/tora* (botanically not determined)	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany, 1996)
6	Semen Cassiae/*Cassia obtusifolia/tora* (botanically not determined)	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany, 1995)
7	Semen Cassiae/*Cassia tora*	sample of commercial drug (CHINA MEDICA, Germany)

Reference compounds		R*f* in Fig. 2a	R*f* in Fig. 2b
T 1	Chrysophanol	0.64	0.97
T 2	Physcion	0.57	0.97
T 3	Emodin	0.40	0.96

TLC-fingerprintanalysis[1,4]

1. Thin layer chromatogram of aglycones after acidic hydrolysis of the extract (Figure 2a):

1) Extraction: 1 g powdered drug is extracted with 30 ml methanol under reflux for 1 hour. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 10 ml hydrochloric acid (10%) and heated on a water bath for 30 minutes. The extract is cooled immediately and extracted twice with 20 ml ethyl acetate. The ethyl acetate phases are combined and evaporated to dryness. The residue is dissolved in 1 ml ethyl acetate and filtered over Millipore® filtration unit, type 0.45 µm.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Machery-Nagel
Applied amounts:	Semen Cassiae extracts: 5 µl each reference compounds: 10 µl each
Solvent system:	petroleum ether (40-60 °C) : ethyl formate : formic acid 15 5 1
Detection:	without chemical treatment → UV-366 nm

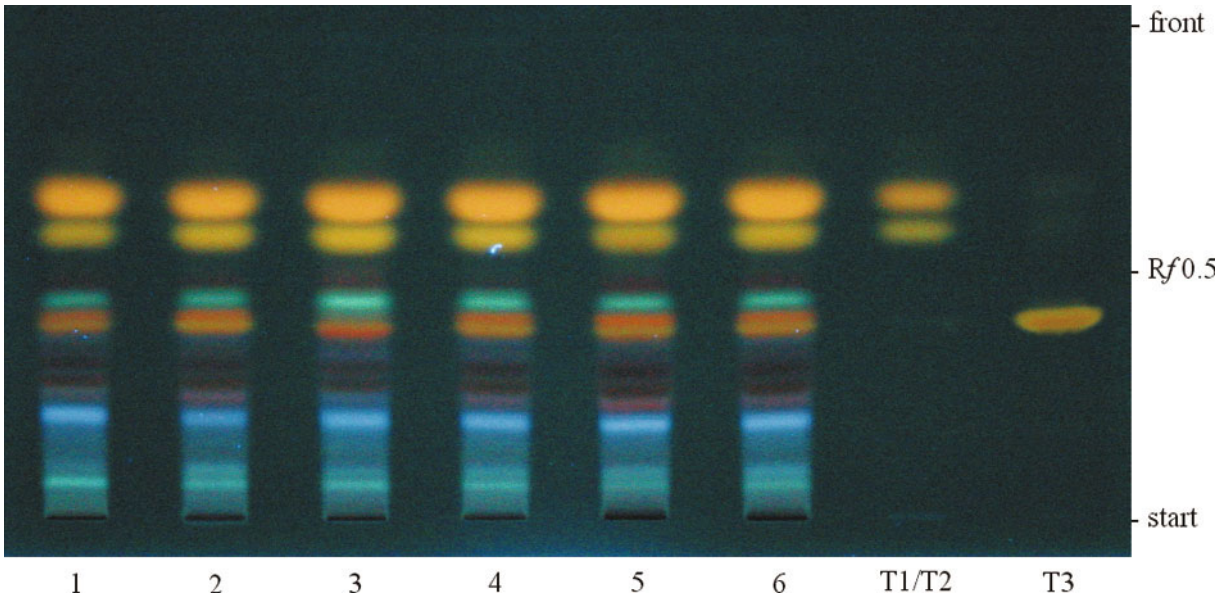


Fig. 2a: Thin layer chromatogram of the hydrolyzed methanol extracts of Semen Cassiae (UV 366 nm)

4) Description:

All hydrolyzed *Cassia obtusifolia*/*Cassia tora* MeOH-extract samples show under UV-366 nm a very homogeneous pattern of orange/yellow and blue/green spots. The yellow bands at R*f* = 0.64, 0.57 and 0.40 are identified as Chrysophanol, Physcion and Emodin respectively. The red spot directly above Emodin may be another non identified anthraquinone and the second red/violet band at R*f* = 0.23 should be the anthraquinone Rhein. The blue and green fluorescent zones in the deep R*f*-range derive from naphthopyrones.

2. Thin layer chromatogram of the total genuine extracts (Figure 2b):

1) Extraction:	1 g powdered drug is extracted with 30 ml methanol under reflux for 1 hour. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm.
2) Reference compounds:	each 0.5 mg is dissolved in 0.5 ml methanol
3) Separation parameters:	
Plate:	HPTLC Silica gel 60 F_{254}, Machery-Nagel
Applied amounts:	Semen Cassiae extracts: 5 µl each reference compounds: 10 µl each
Solvent system:	ethyl acetate : methanol : water 80 20 10
Detection:	without chemical treatment → UV-366 nm

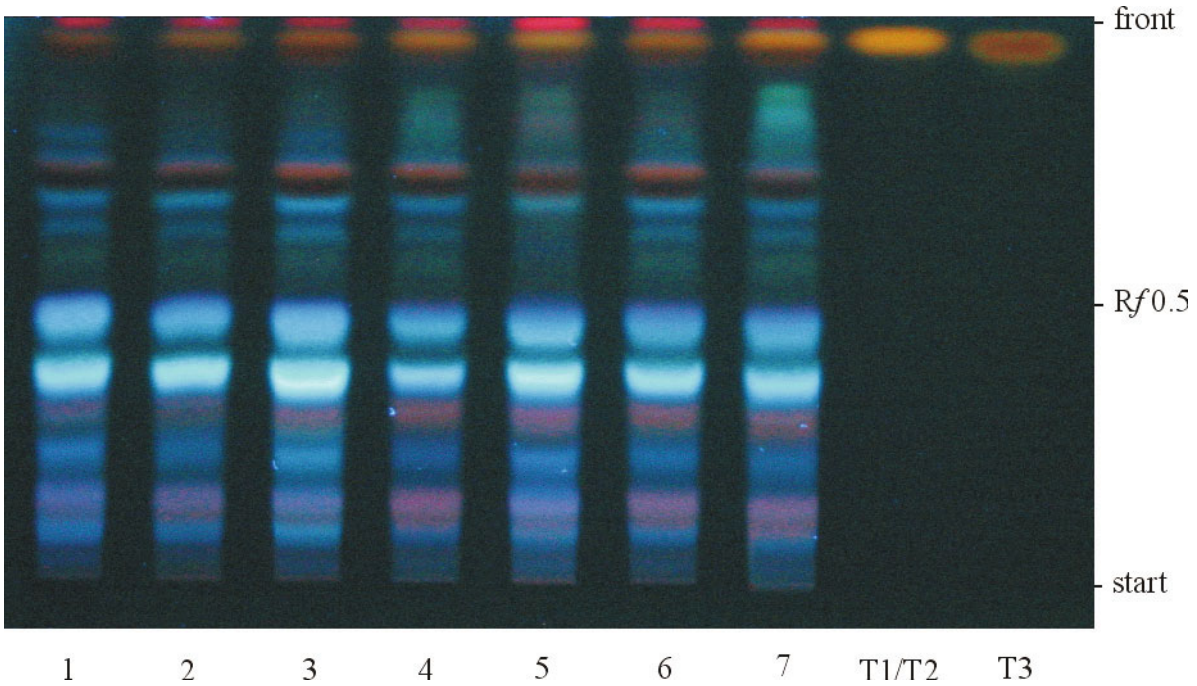


Fig. 2b: Thin layer chromatogram of the methanol extracts of Semen Cassiae (UV 366 nm)

4) Description:

The genuine *Cassia obtusifolia* / *Cassia tora* extracts show under UV 366 nm also a very homogeneous pattern of orange, red violet, and blue fluorescent bands which are distributed over the whole R*f*-plate: the anthraquinone aglycones on the TLC-front, two conspicuous blue fluorescent in the R*f*-range of 0.35-0.45 and three violet zones at R*f* = 0.10/0.15 and 0.30

respectively. Their structural assignment was not possible but it is likely that they derive from aceto-napthalene- or naphthopyrone-glycosides.

HPLC-fingerprint analysis:[1]

1) Sample preparation:

Fig. 3a: Hydrolized methanol extract

1 g powdered drug is extracted with 30 ml methanol under reflux for 1 hour. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 10 ml hydrochloric acid (10%) and heated on a water bath for 30 minutes. The extract is cooled immediately and extracted twice with 20 ml ethyl acetate. The ethyl acetate phases are combined and evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm.

Fig. 3b and 3c: Methanol extract

1 g powdered drug is extracted with 30 ml methanol under reflux for 1 hour. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm.

2) Injection volume: Semen Cassiae extracts: 5 µl each

3) HPLC parameter:

Apparatus:
MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck

Precolumn: LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck

Solvent:
A: 0.001 % Phosphoric acid/Water (Millipore Ultra Clear UV plus® filtered)
B: Acetonitrile (VWR)

Gradient:

Figure 3a:	Figure 3b and 3c:
60 % B for 40 min, total runtime: 40 minutes	20-21 % B in 20 min 21-40 % B in 2 min 40-100 % B in 18 min total runtime: 40 minutes

Flow: 1 ml/min.

Detection: 280 nm

Retention times of the main peaks recorded at 280 nm

peak	Rt (min.) Fig. 3a	Rt (min.) Fig. 3b/c	compound
1	-	3.9	(not identified)
2	-	9.0	(not identified)
3	-	10.9	(not identified)
4	-	11.5	(not identified)
5	-	14.4	(not identified)
6	-	18.0	(not identified)
7	-	26.2	(not identified)
8	-	29.8	(not identified)
9	3.8	30.2	(not identified)
10	5.1	32.2	(not identified)
11	8.0	35.3	Emodin
12	17.0	37.6	Chrysophanol
13	23.8	-	Physcion

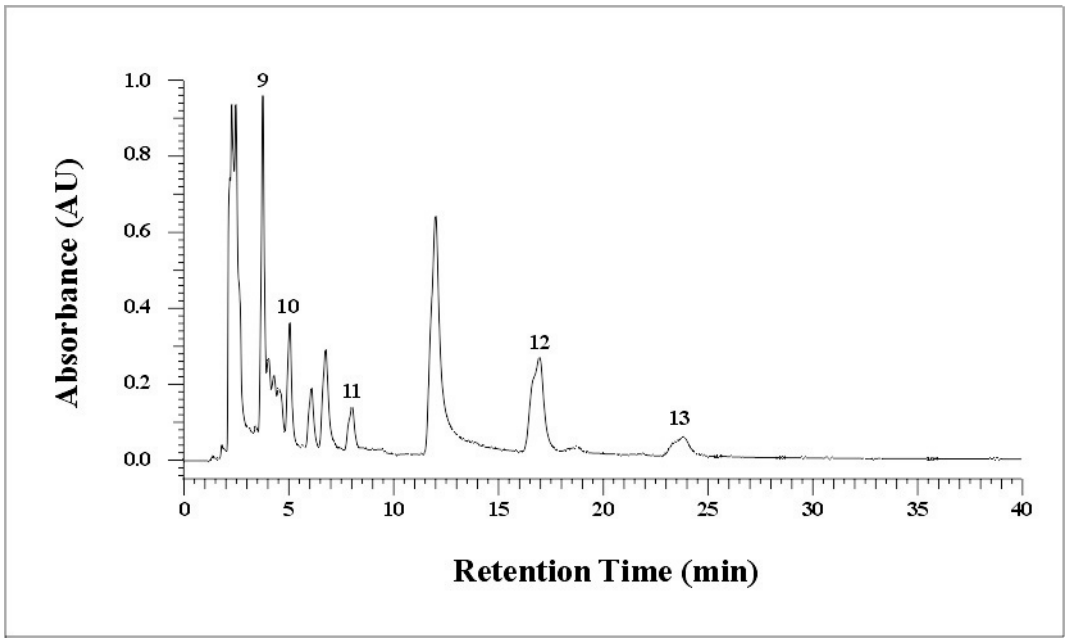


Fig. 3a: HPLC-fingerprint analysis of the hydrolyzed methanol extract of *Cassia tora* (sample 7)

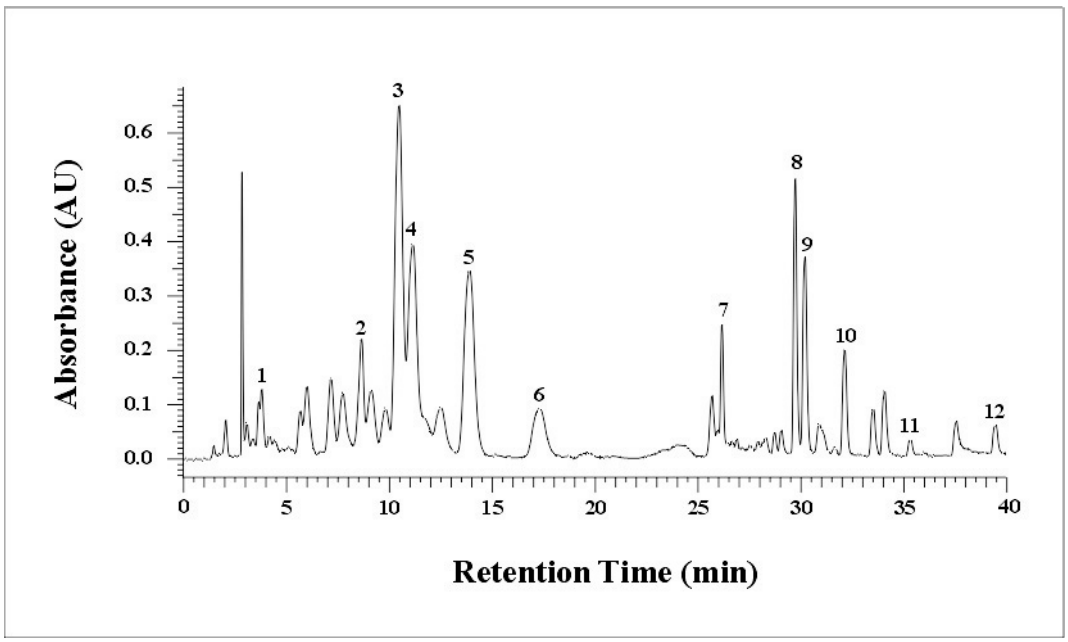


Fig. 3b: HPLC-fingerprint analysis of the methanol extract of *Cassia tora* (sample 7)

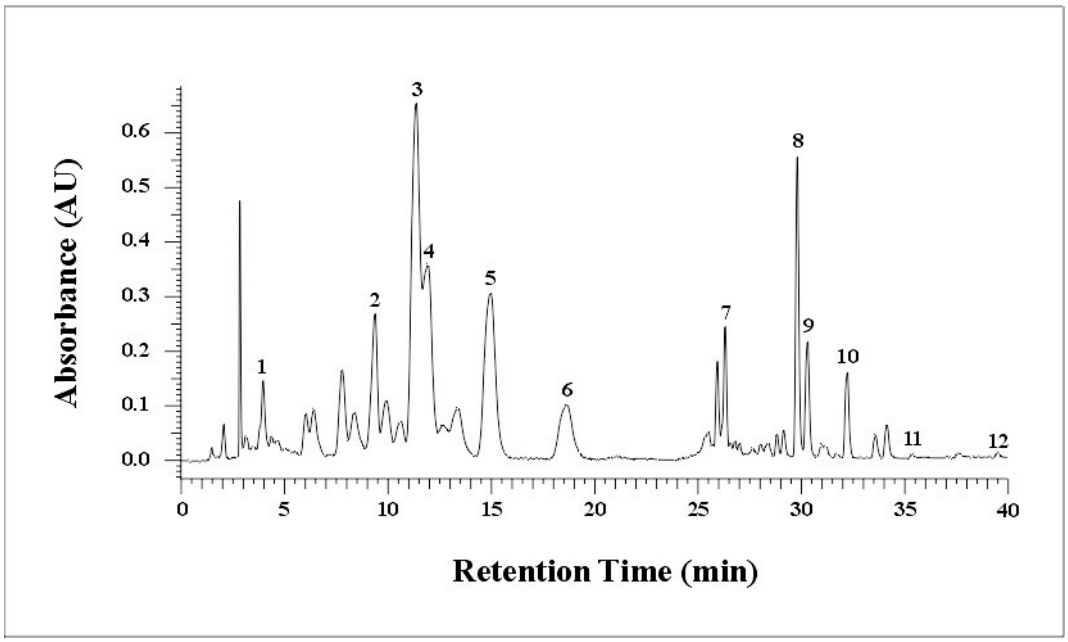
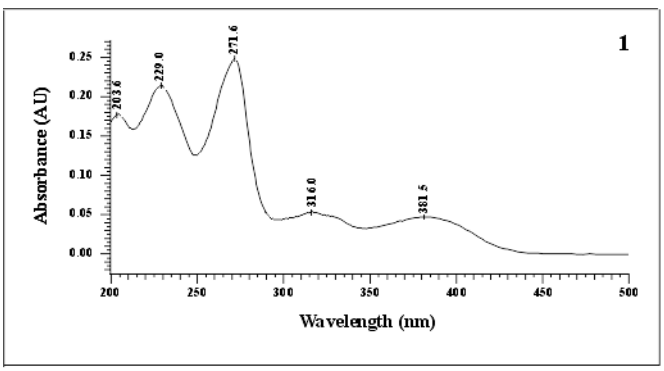
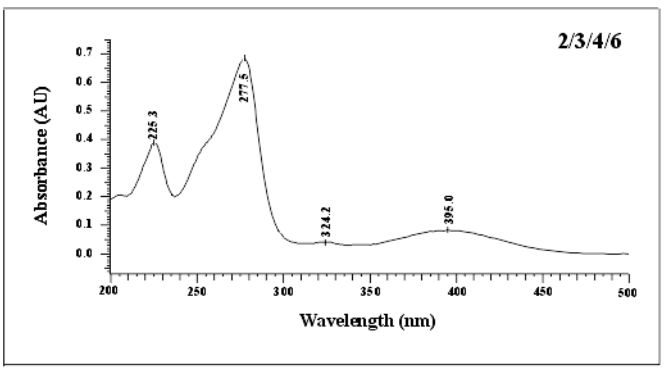
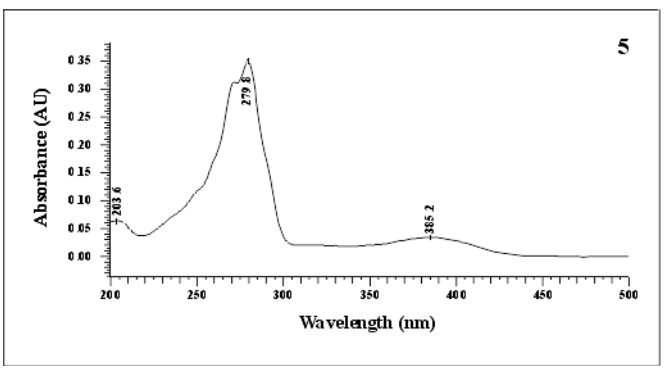
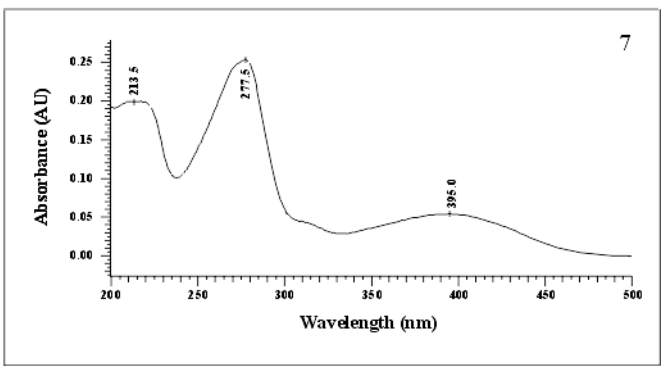


Fig. 3c: HPLC-fingerprint analysis of the methanol extract of *Cassia obtusifolia* (sample 1)









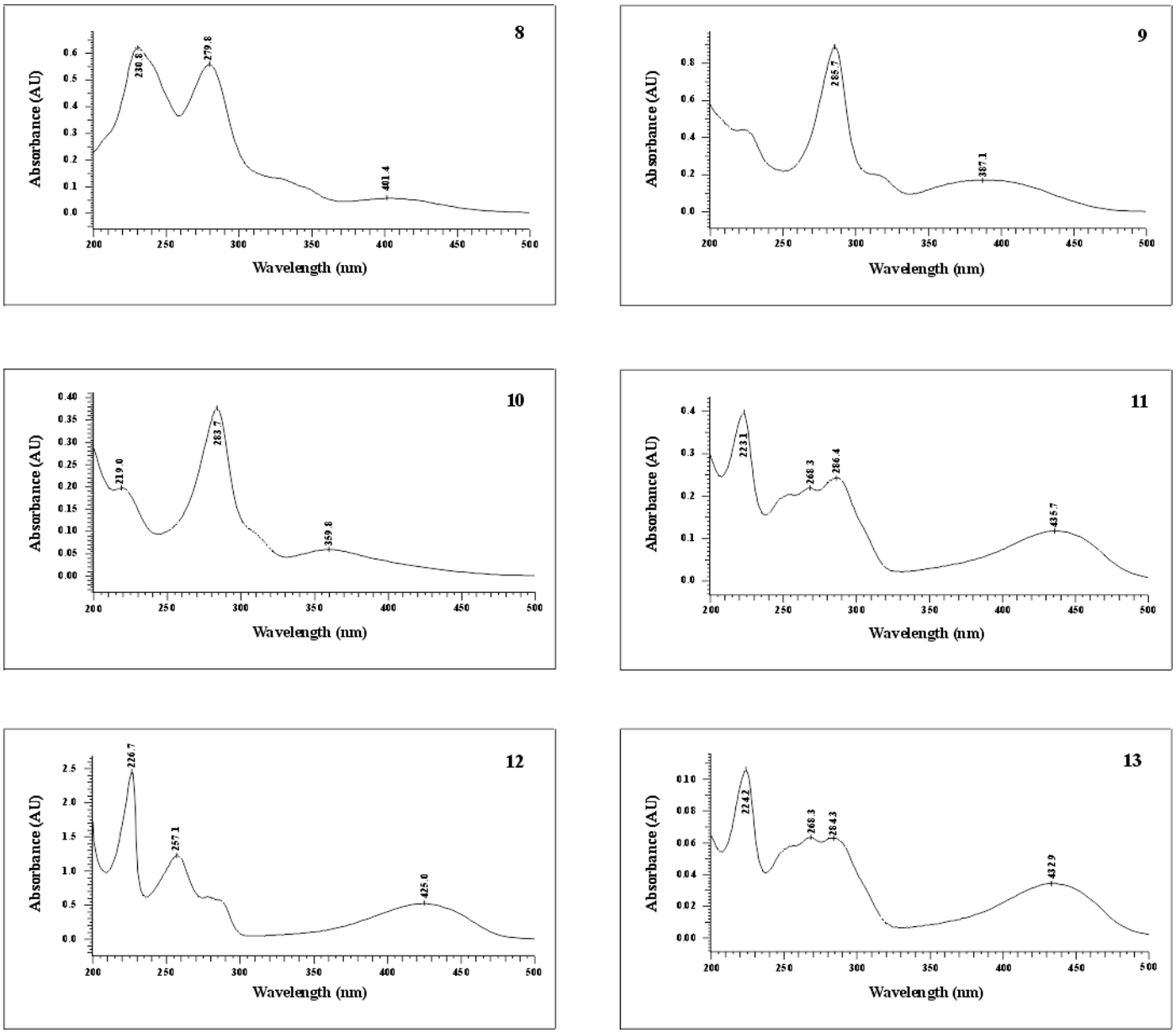


Fig. 4: On line UV-spectra of Semen Cassiae

4) Description of the HPLC-Figures

Figure 3a:

The HPLC-fingerprint analyses of all hydrolyzed *Cassia obtusifolia*/*Cassia tora* extract samples (shown for *Cassia tora* sample 7) registered at 280 nm show only in the aglycone range from Rt = 3.0 till 25.0 five distinct peaks **9**, **10**, **11**, **12**, and **13**. The peak **11** and **13** can be identified due to their characteristic UV-spectra as Emodin and Physcion and peak **12** as Chrysophanol. Peak **9** and **10** possess deviating UV-spectra and be assigned to naphthopyrone derivatives.

Figure 3b and 3c:

The HPLC-fingerprint analyses of all *Cassia obtusifolia*/*Cassia tora* extract samples (shown for extract sample 1 and 7) supplied a nearly identical peak pattern which is characterized by two peak assemblies in the Rt-range from Rt 0.0 till 20.0 and Rt = 25.0 till 45.0. According to their UV-spectra the peaks **1**-**6** derive from di- and monoglycosides of anthraquinones, naphthopyrones and aceto-naphthalenes. The peaks **7** till **12** are naphthopyrone- and anthraquinone aglycones which are present also in both genuine Cassia-alcohol extracts.

Note: The Chinese Pharmacopoeia 2005 describes only the TLC-chromatography of the *Cassia* MeOH raw extract after acidic hydrolysis (10 % HCl) with Emodin and Chrysophanol as reference compounds. Accordingly also a HPLC-analysis is performed with quantitation of the Chrysophanol content is described. *Cassia* drug should contain not less than 0.080 % Chrysophanol calculated with reference to the dried drug.

Further HPLC-fingerprint analytical methods can be found in the following references[4,29,30].

Conclusion

Since the TLC- and HPLC-fingerprint analyses of both *Cassia* species show a completely identical band- and peak-pattern they are therapeutically equivalent and can be mutually exchanged.

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Folium Camelliae – *Cha-yeh*

Pharmacopoeia:	Not contained in the Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005
Official drug[1]:	Non fermented and fermented dried leaves of *Camellia sinensis* G. Kuntze (Fam. Theaceae). The non fermented leaves can be purchased on the market as "green tea".
Origin[2,8]:	China, Indochina, India, Burma (Myanmar), Thailand (Siam), Laos and Vietnam
Descriptions of the drug[2]:	A shrub or small tree 10 m tall in its natural habitat, but under 2 m in cultivation. Leaves alternate, briefly petiolate, oval-oblong, acuminate, finely dentate, 2-15 cm long by 1-6 cm wide, slightly coriaceous. Flowers fragrant, nodding, axillary, solitary or agglomerate 3-4; calyx short, sepals 5; corolla white, petals 5; stamens numerous; ovary globular, 3-celled. Fruit a trigonal, ligneous capsule. The leaves are used as beverages and medicinally. Odour, aromatic; taste, astringent and slightly bitter.
Pretreatment of the raw drug[1]:	After collection of the leaves they are heated in a pan over a flame, rolled and after roasting dried at 40-60 °C → Green tea Partial fermented leaves → Oolong tea After short heating and rolling the leaves are fermented at 100 % atmospheric humidity and dried at 23-25 °C → Black or red tea
Medicinal use[5]:	Reduction of serum cholesterol, inhibition of low density lipoprotein (LDL), treatment of obesity and as chemoprevention of cardiovascular diseases, diabetes and cancer.

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Effects and indications of *Camellia sinensis* according to Traditional Chinese Medicine[2,3,4]	
Taste:	Bitter, sweet
Temperature:	Neutral, tendency cold
Channels entered:	*Orbis cardialis*, *O. hepaticus*, *O. renalis*, *O. stomachi*, *O. lienalis*
Effects (functions):	Prescribed as cardiotonic, central nerve stimulant, diuretic and intestinal astringent
Symptoms and indications:	Increased mentality and relief from muscular and mental fatigue and from their attendant unpleasant sensations. Exhibits dyspepsia, restlessness, nervous excitability, tremor, insomnia, anorexia, headache, vertigo, confusion, palpitation and dyspnea.

Main constituents

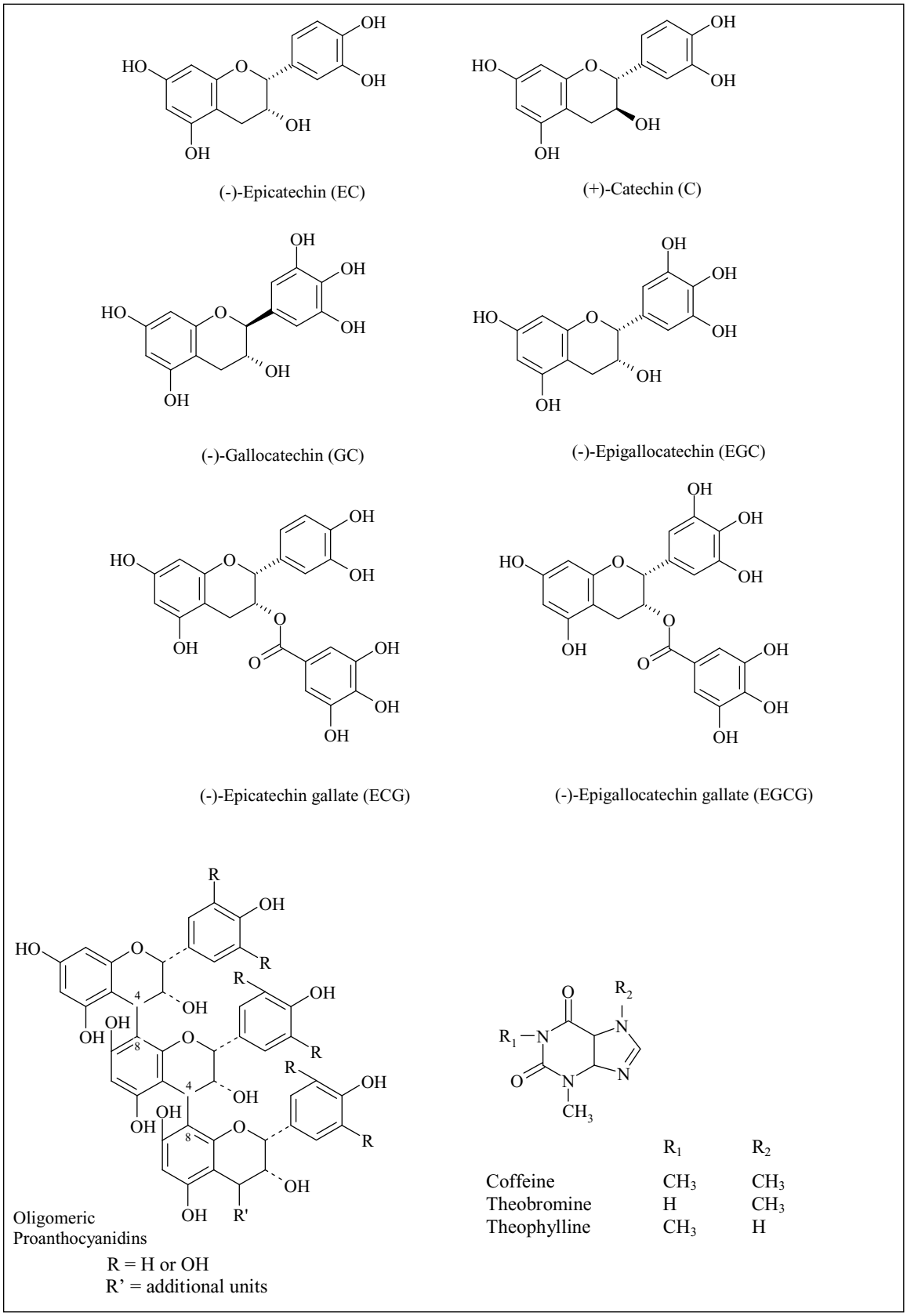
Green Tea
• 2.5 – 4.5 % Coffeine, ~ 0.15 % Theobromine, 0.01 – 0.04 % Theophylline • **Polyphenols:** 10 – 25 % (+)-Catechin, (-)-Epicatechin, (-)-Gallocatechin, (-)-Epigallocatechin, (-)-Epicatechin gallate (0.3 – 0.6 %), Benzotropolon derivatives Theaflavin, Theaflavingallate, Thearubigins • Flavonols: (glycosides of quercetin, kaempferol and myricetin) • Tannins: 1 – 30 % • Esters of phenolcarboxylic acids: 3 – 5 % chlorogenic acid, 5-caffeoylquinic acid, 5-p-coumaroylquinic acid, 5-galloylquinic acid

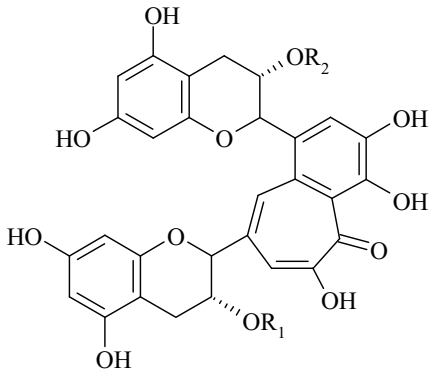
Black Tea
• lower concentrations of alkaloids (1.4 – 3.5 % Coffeine), higher concentrations of oligomeric and polymeric polyphenols such as theaflavins and thearubigins due to the fermentation process • Tannins: 5 – 30 %

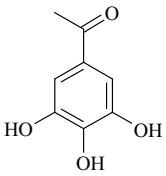
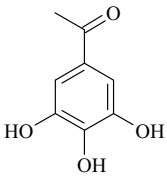
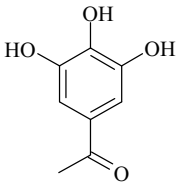
Minor constituents

triterpensaponins E_1 and E_2, Amino acids, Theanin (Monoethylamide of glutamic acid), Lignin, Proteins, Vitamin K

Fig. 1: Formulae of the main constituents of *Camellia sinensis*





	R_1	R_2
Theaflavin	H	H
Theaflavin-3-gallate		H
Theaflavin-3,3'-digallate		

Pharmacology/Molecular biology:

Polyphenolics:	
- anti-diarrheic[1] - anti-oxidative[1,5,6,7,9,10] - anti-thrombotic[6] - blood pressure reducing effect[20] - anti-inflammatory[6] - NO-dependent vasodilatory effects[6,20] - insulin sensitizing effect[19] - anti-proliferative[6] - anti-mutagenic[5,7,9] - anti-carcinogenic[5,9,10] - anti-bacterial (*Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhi*, *S. typhimurium*, *S. enteritidis*, *Shigella flexneri*, *S. dysenteriae* and *Vibrio cholerae*)[8,9]	- anti-viral (against HIV reverse transcriptase and against RNA polymerase)[8,9] - inhibition of DNA synthesis in HTC rat hepatoma cells and DS 19 mouse erythroleukemia cells in culture[8] - inhibition of protein kinase C activation (epigallocatechin gallate)[8] - inhibition of growth of lung and mammary cancer cell lines in culture[8] - prevention of cancer, heart-, diabetes and neurodegenerative diseases[11] - anti-cancer[16] - inhibition of cholesterol and low density lipoprotein oxidation[29] - inhibition of lipid accumulation in 3T3-L1 adipocytes[30]

Alkaloids:	Saponins:
- diuretic activity[1] - stimulation of wakefulness, facilitation of ideas association and decrease of the sensation of fatigue[13]	(e.g. theasaponines E_1 and E_2) have anti-sweet, gastroprotective, gastricemptying inhibitory and gastrointestinal transit accelerating activities[12]

Toxicology[17,18]:

Increased consumption of coffeine in animals has been shown to be teratogenic. However, studies have been inconclusive about the effect of consumption of a moderate portion of the herb on the fetus.

Pharmacokinetic[21-24] and pre/clinical studies[16, 25,26]:

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b) B.A. Warden, L.S. Smith, G.R. Beecher, D.A. Balentine, B.A. Clevidence, Catechins are bioavailable in men and women drinking black tea throughout the day, J. of Nutr. 131, 1731-1737 (2001)[22]

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d) M. Zhu, Y. Chen, R.C. Li, Oral Absorption and Bioavailability of Tea Catechins, Planta Medica 66(5), 444-447 (2000)[24]

e) C.L. Nance, W.T. Shearer, Preclinical and clinical development of the green tea catechin, epigallocatechin gallate, in treating HIV-1 infection (Chapter 5), Botanical Medicine: From Bench to Bedside, 92-108 (Edited by R. Cooper and F. Kronenberg) © Mary Ann Liebert, Inc. (2009)[25]

f) M. Rondanelli, A. Opizzi, S.B. Solerte, R. Trotti, C. Klersy, R. Cazzola, Administration of a dietary supplement (*N*-oleyl-phosphatidylethanolamine and epigallocatechin-3-gallate formula) enhances compliance with diet in healthy overweight subjects: a randomized controlled trial, Brit. J. of Nutr. 101, 457-464 (2009)[26]

g) J.J. Johnson, H.H. Bailey, H. Mukhtar, Green tea polyphenols for prostate cancer chemoprevention: A translational perspective, Phytomedicine 17(1), 3-13 (2010)[16]

TLC fingerprint analysis

Drug samples		Origin
1	Folium Camelliae (Green Tea, non fermented)/*Camellia sinensis*	Province Zheijiang, China (green tea named "Long-jing")
2	Folium Camelliae (Green Tea, non fermented)/*Camellia sinensis*	sample of commercial drug, Special Gunpowder, Heuschen & Schrouff OFT B.V., Netherlands
3	Black Tea (fermented)/*Camellia sinensis*	sample of commercial drug, Teefix®

Reference polyphenols of Figure 2a		R*f*
T 1	(±)-Catechin	0.83
T 2	(-)-Epicatechin	0.83
T 3	(-)-Epigallocatechin-3-gallate	0.71

Reference alkaloids of Figure 2b		R*f*
T 4	Coffeine	0.52
T 5	Theophylline	0.58
T 6	Theobromine	0.45

TLC-fingerprint analysis

1. Thin layer chromatogram of the polyphenols of the tea extracts (Fig. 2a):

1) Extraction: 1 g powdered green tea and black tea are extracted with 10 ml methanol under reflux for 30 minutes. After cooling, the extracts are filtered and evaporated to dryness. The residues are dissolved in 1 ml methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:
Plate: HPTLC-Silica gel 60 F_{254}, Merck

Applied amounts: green and black tea extracts: each 10 µl
reference compounds: each 10 µl

Solvent system:	ethyl acetate	: water	: formic acid	: glacial acetic acid	
	70	30	3	2	(upper layer)

Detection: Vanillin-phosphoric acid reagent:

To 1 g vanillin 100 ml of 50% ethanolic phosphoric acid are added and the plate sprayed with this solution, heated for 10 minutes at 110 °C and then evaluated in VIS.

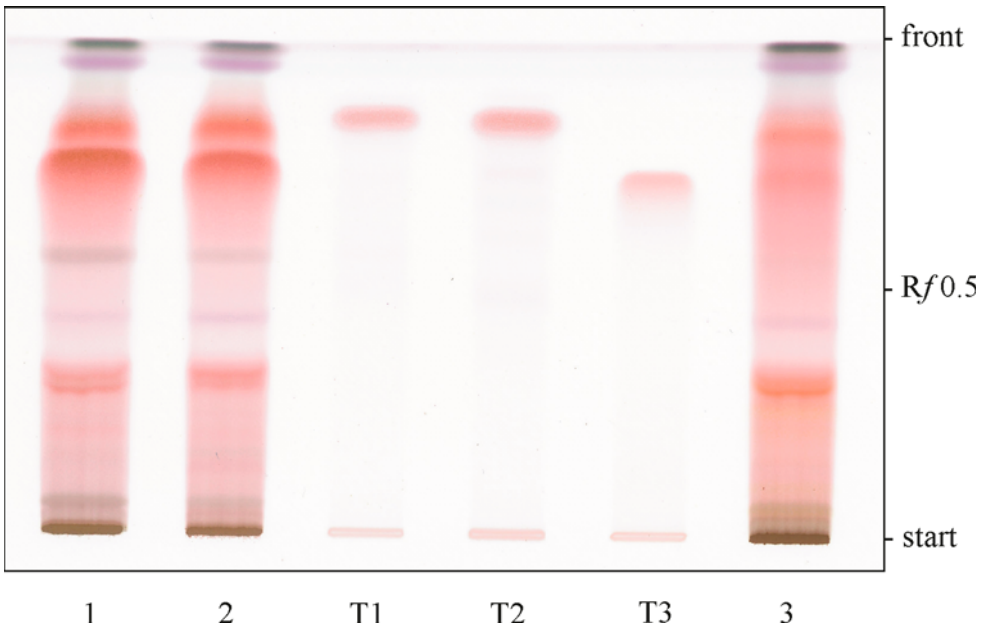


Fig. 2a: Thin layer chromatogram of the methanol extracts of *Camellia sinensis* sprayed with vanillin-phosphoric acid reagent (VIS)

4) Description:

In the MeOH-extract the polyphenols catechin (**T1**) and epicatechin (**T2**) appear overlapped in VIS with red colour at R*f* = 0.83, the epigallocatechin gallate (**T3**) in higher concentration as the dominant catechin derivate at R*f* = 0.71. At R*f* = 0.30 an oligomeric procyanidin and on the start the polymeric tannins can be identified. In green tea the monomeric catechins and the epigallocatechingallate are present in much higher concentration than in black tea (sample 3).

2. Thin layer chromatogram of the alkaloids of the tea extracts (Fig. 2b):

1) Extraction: 1 g powdered green and black tea are extracted with 10 ml methanol under reflux for 30 minutes. After cooling, the extracts are filtered and evaporated to dryness. The residues are dissolved in 1 ml methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate:	HPTLC-Silica gel 60 F_{254}, Merck
Applied amounts:	green and black tea extracts: each 10 µl reference compounds: each 15 µl
Solvent system:	ethyl acetate : methanol : water 100 13.5 10
Detection:	UV 254 nm (without chemical treatment)

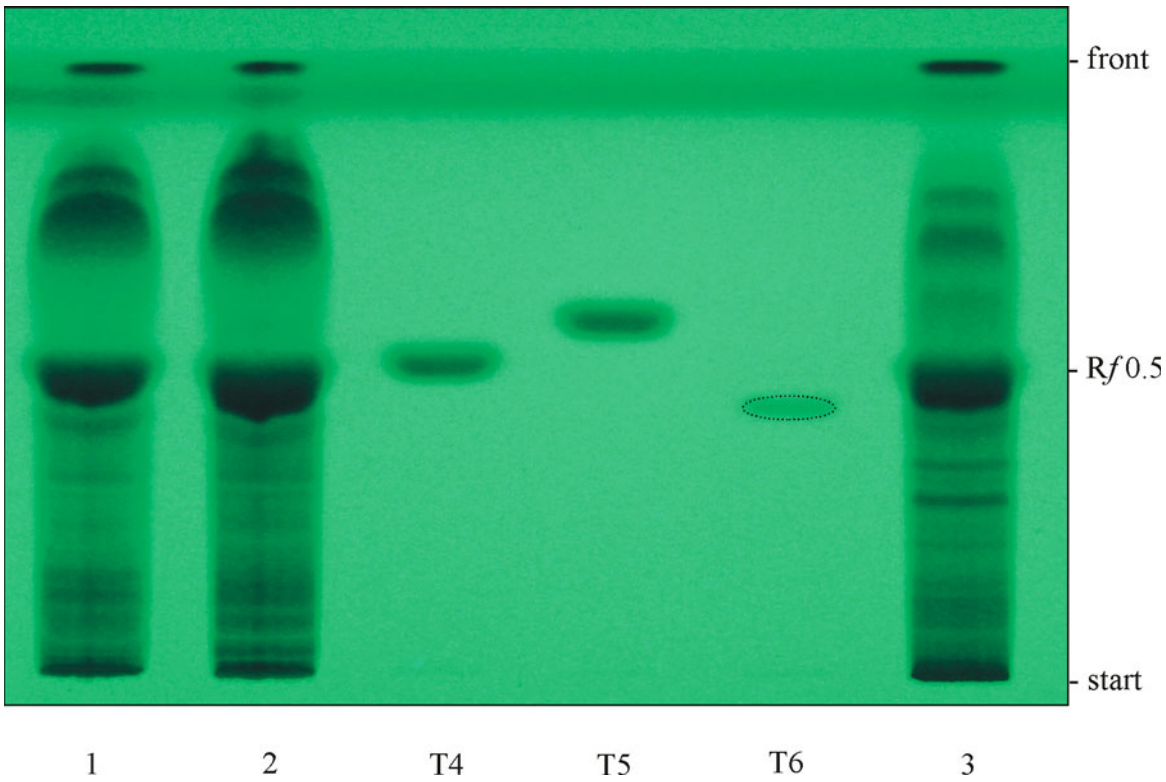


Fig. 2b: Thin layer chromatogram of the methanol extracts of *Camellia sinensis* without chemical treatment (UV 254 nm)

4) Description:

The alkaloids (**T4**, **T5**, **T6**) and catechins appear in the MeOH-extract in UV 254 nm as green black zones on light green background. The differences of the concentration of alkaloids and catechins between non fermented green tea (1, 2) and fermented black tea (3) are evident.

HPLC-fingerprint analysis of green and black tea:

1) Sample preparation:

a) water extract[14]

To 1 g powdered drug 18 ml of boiling water are added. After 3 minutes the brew is filtered over a folded filter (3 hw, 110 mm, 65 g/m²) and the solution further filtered over Millipore® filtration unit, type 0.45 µm. The last solution is injected into the HPLC apparatus.

b) methanol extract[15]

2 g powdered drug are extracted for 3 hours under reflux with 20 ml 80% methanol on a water bath. After cooling the extract is filtered over a folded filter (3 hw, 110 mm, 65 g/m²), followed by a further filtration over Millipore ® filtration unit, type 0.45 µm. The last solution is injected into the HPLC apparatus.

2) Injection volume:

Green tea extracts (water and methanol): each 2.5 µl
Black tea extracts (water and methanol): each 2.5 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 10 ml 0.1 % H_3PO_4 (Merck)/1 l dist. water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (Merck)
Gradient:	5-15 % B in 20 min. 15-20 % B in 15 min. isocratic for 5 min. total runtime: 40 minutes
Flow:	1 ml/min.
Detection:	210 nm

Retention times of the main peaks recorded at 210 nm

peak	Rt (min.)	compound
1	4.2	Gallic acid
2	8.0	Theobromine
3	13.1	Catechin
4	17.4	Coffeine
5	19.3	(-)-Epicatechin
6	20.5	Epigallocatechin-3-gallate
7	28.1	Epicatechin gallate

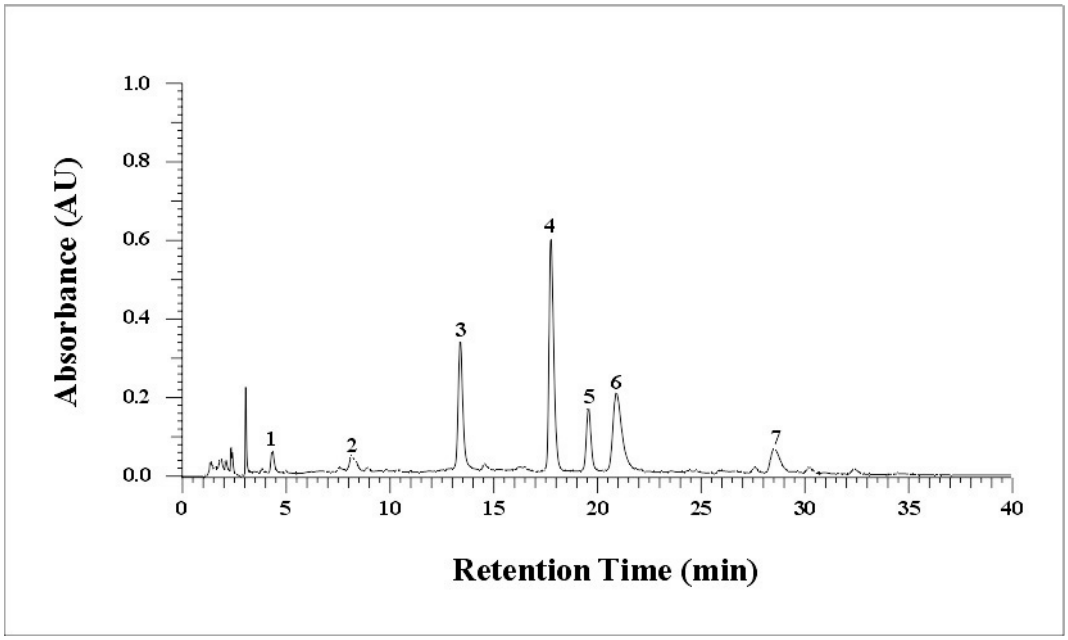


Fig. 3a: HPLC-fingerprint analysis of the **water** extract of **Green Tea** (*Camellia sinensis*) sample 2

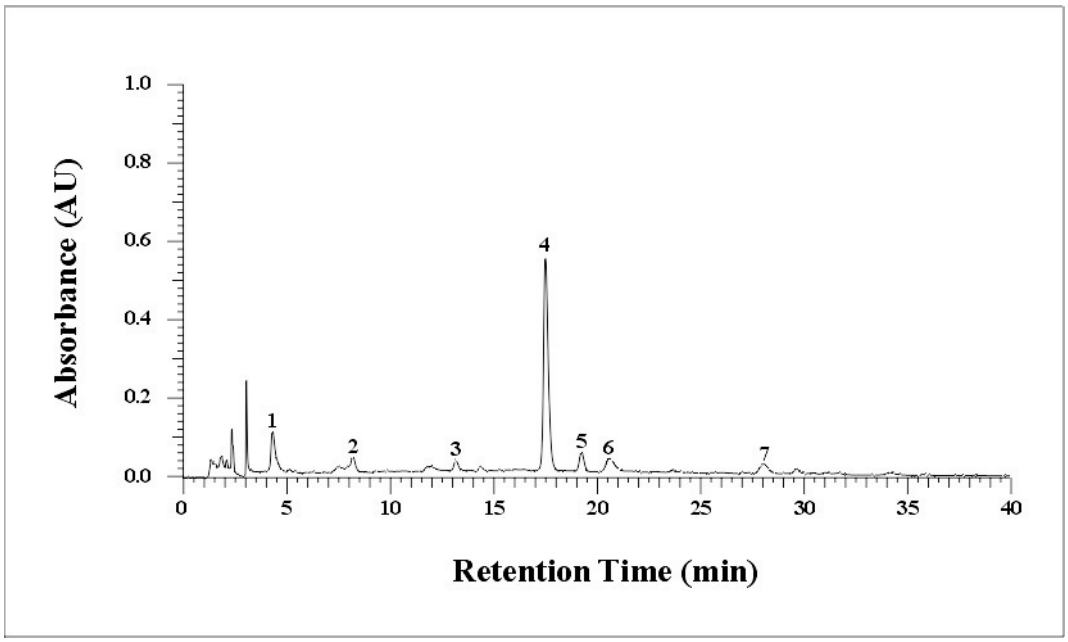


Fig. 3b: HPLC-fingerprint analysis of the **water** extract of **Black Tea** sample 3

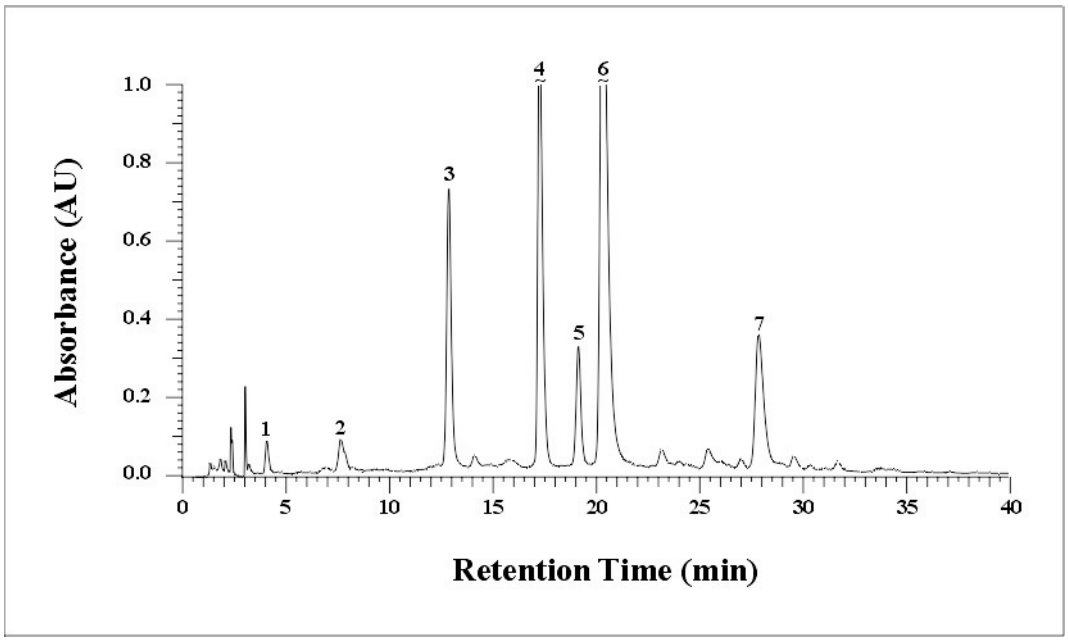


Fig. 3c: HPLC-fingerprint analysis of the **methanol** extract of **Green Tea** (*Camellia sinensis*) sample 2

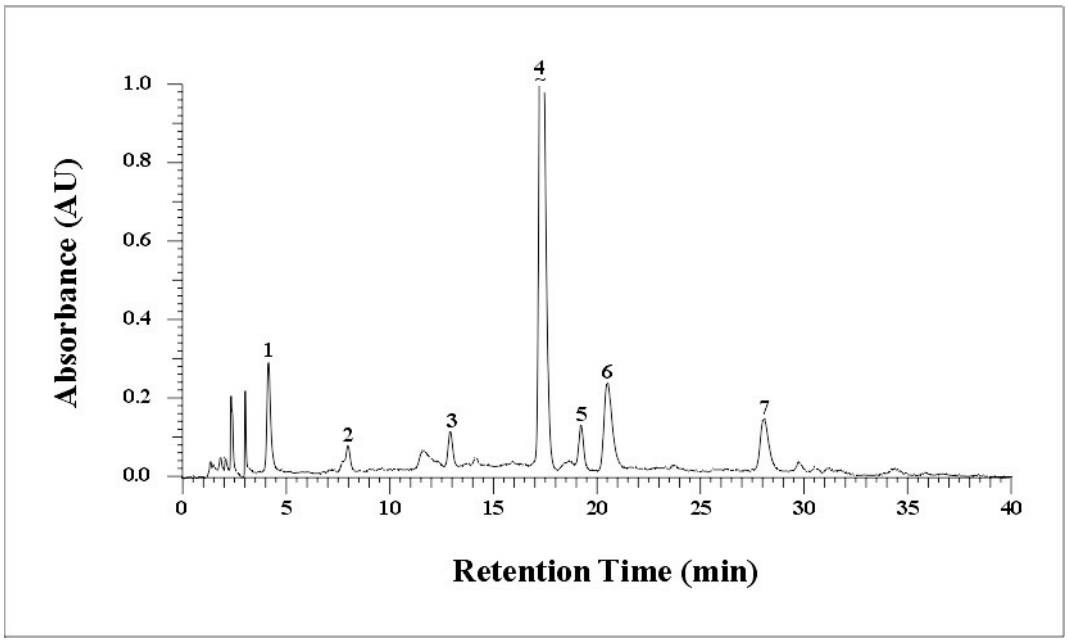
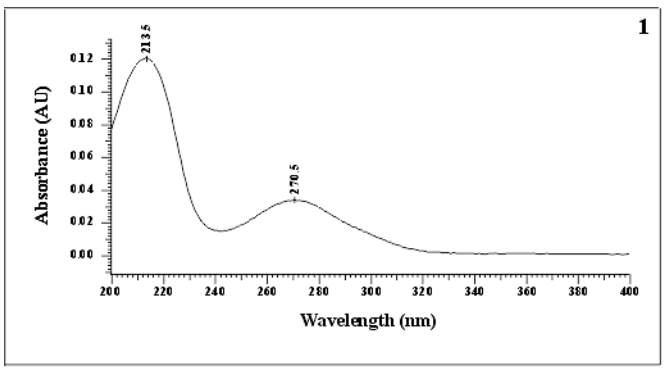
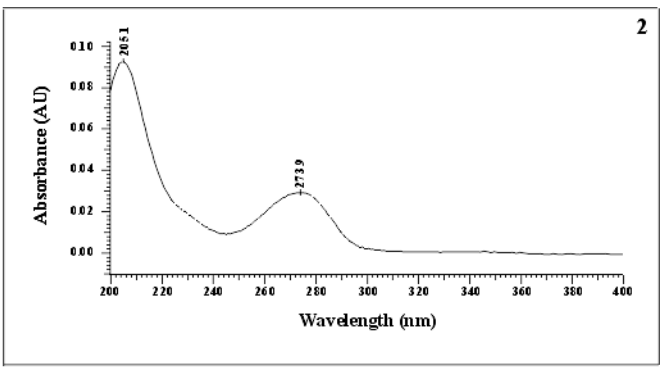
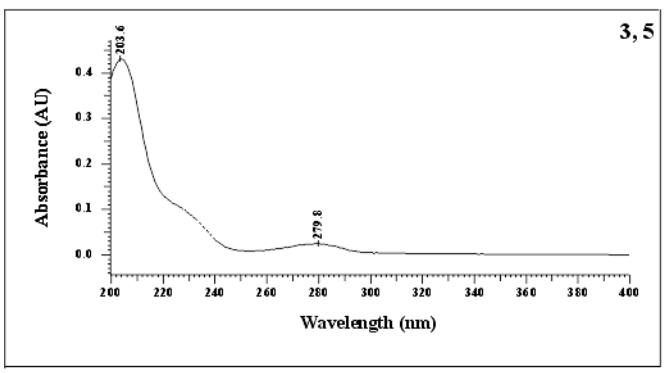
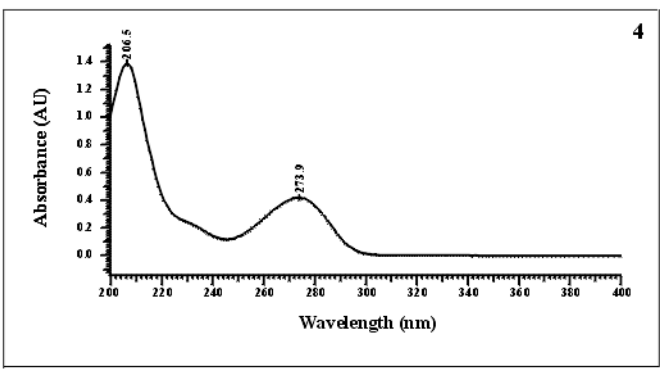


Fig. 3d: HPLC-fingerprint analysis of the **methanol** extract of **Black Tea** sample 3









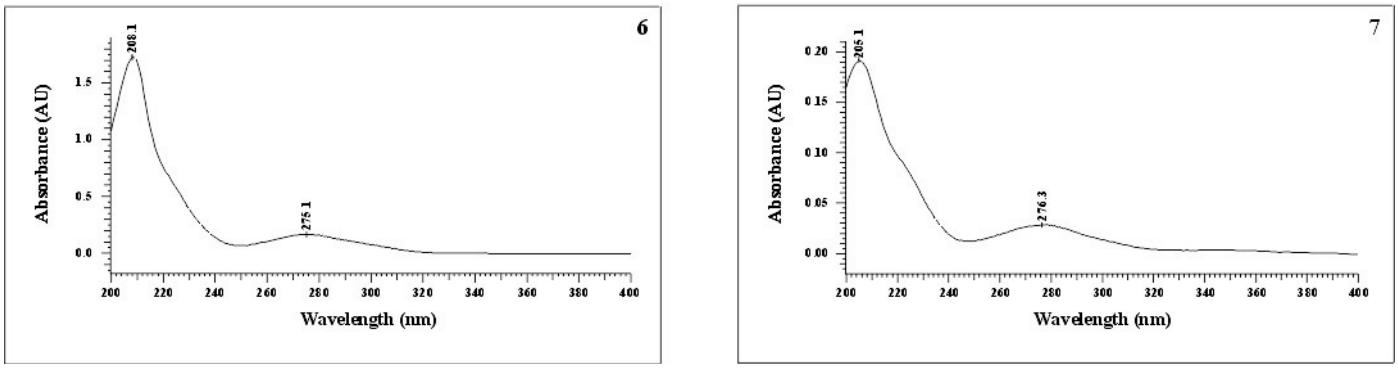


Fig. 4: On line UV-spectra of *Camellia sinensis* extracts

4) Description of the HPLC-Figures

Fig. 3a and 3b:

The HPLC-fingerprint of the **water** extracts of green (sample 2) and black tea (sample 3) show coffeine (peak **4**) as dominant peak followed by all main catechin derivatives (peaks **3**, **5**, **6**, **7**) in much lower concentration.

Fig. 3c and 3d:

The HPLC-graphs of the MeOH-extracts of green tea (sample 2) and black tea (sample 3) shown coffeine also as main peak. The epigallocatechingallate in particular and the other chatechin derivatives, however, dominate in green tea in contrast to black tea.

Conclusion

For the isolation of the pharmacologically most interesting epigallocatechingallate the MeOH-extract of green tea is the best appropriate starting plant material.

Quantitative composition of the Green tea catechins compounds and alkaloids

- Meanwhile publications have appeared describing the HPLC- or HPLC/MS-fingerprint analysis with quantitation of the main catechin derivatives and Theaflavin, inclusive their various gallate derivatives, quinic esters and flavonolglycosides [D. Del Rio et al., J. Agric. Food Chem., 52(10), 2807-2815 (2004)[14] and Zuo et al., Talanta 57(2), 307-316 (2002)[15]].

- Since 1991 29 publications appeared studying over several weeks the daily consumption of green tea water decoction on plasma flavanol concentrations with quantitation of the single catechin derivatives (Eichenberger et al., Int. J. Vitam. Nutr. Res. 79(1), 24-33 (2009)[27]). The mean values of the flavanol intake/day of the 29 studies were calculated with 1.67% for C, 11.36% for EC, 9.93% for ECG, 26.50% for EGC and 50.53% for EGCG (see Fig. 5).

- In more than 30 publications the quantitative chemical composition of alkaloids and catechin derivatives in Green tea extracts (decoction) was described using the HPLC / HPLC/MS-analytical methods. The following graph shows the mean values of the catechin derivatives in take/day of 29 studies[28].

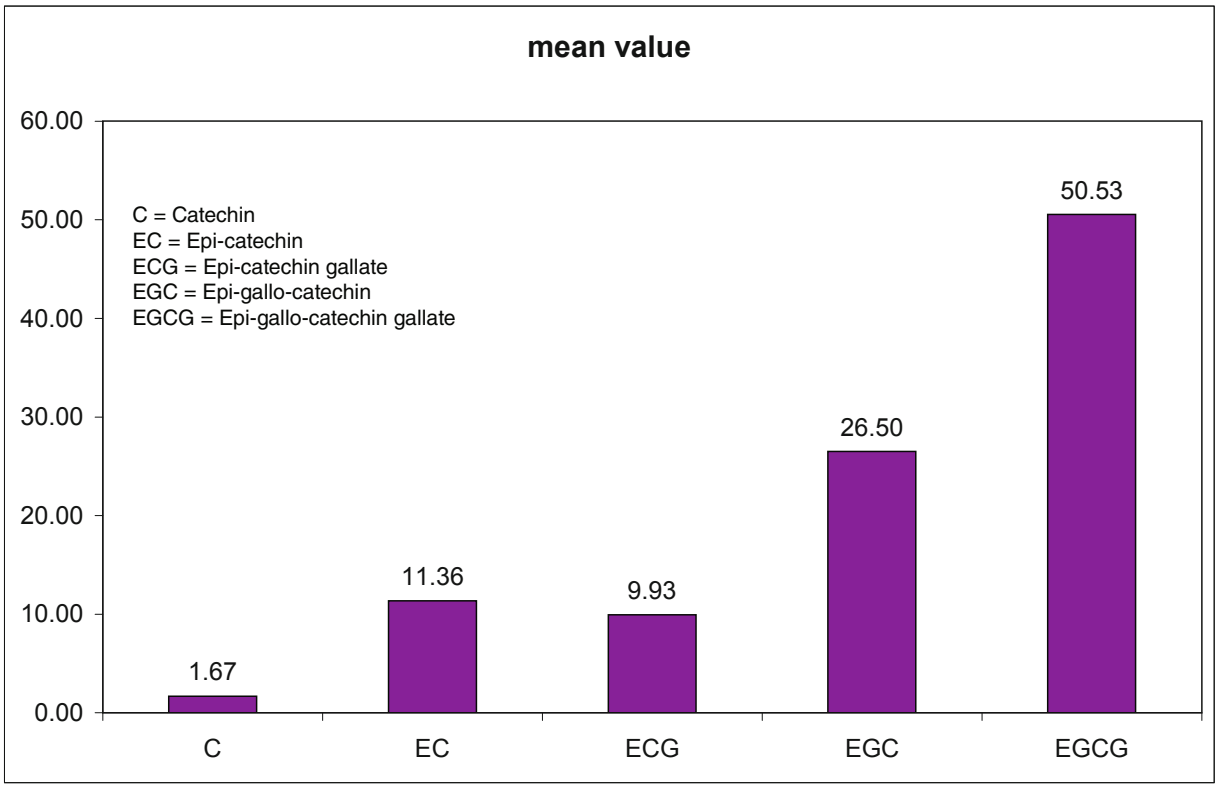


Fig. 5: Mean values of the catechin derivatives

These studies confirm that epigallocatechingallate is by far the catechin derivate of the highest amount in green tea used in the studies[28].

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Herba Artemisiae Scopariae
Yinchen

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drugs[1]: Virgate Wormwood Herb is the dried aerial part of *Artemisia scoparia* Waldst. et Kit. or *Artemisia capillaris* Thunb. (Fam. Asteraceae).

The drug is collected in spring when the seedling is 6-10 cm high, or in autumn when the bud is forming, removed from foreign matter and older stem, and dried in the sun. The drug collected in spring is known as "Mianyinchen" and collected in autumn is known as "Yinchenhao".

Origin[4, 5]: China (province Shaanxi, Shanxi and Anhui), Japan, Taiwan

Description of the drugs[1]:

Mianyinchen

Mostly rolled into masses. Greyish-white, or greyish-green, densely covered with white pubescences throughout, soft like a nap. Stems thin and small, 1.5-2.5 cm long, 1-2 mm in diameter, longitudinal striations distinct after removing the white pubescences on the surface; texture fragile, easily broken. Leaves petioled, when whole, 1-3 pinnatipartisect, lamina 1-3 cm long, about 1 cm wide; segment ovoid or slightly oblanceolate, stripe-shaped, apex acute. Odour, delicately aromatic; taste, slightly bitter.

Yinchenhao

Stems cylindrical, frequently branched, 30-100 cm long, 2-8 mm in diameter; externally pale purple or purple, striated longitudinally, pubescent; texture light, fragile, fracture almost white. Leaves densely gathered, or mostly fallen off. Basal leaves 2-3 pinnatipartite, segments stripe-shaped or finely stripe-shaped, densely covered with white pubescences on both surfaces; cauline leaves 1-2 pinnatipartisect, amplexicaul at the base, segments filamentous; capitulum ovoid, mostly gathered in conical, 1.2-1.5 mm long, 1-1.2 mm in diameter; short petioled; involucres 3-4 layers, ovoid, bracts 3-lobed; the outer female flowers 6-10, some times up to 15. the inner bisexual flowers 2-10; achenes oblong, yellowish-brown. Odour. Aromatic; taste, slightly bitter.

Pretreatment of the raw drug[1]: Remains of roots and foreign matters are eliminated, rubbed or cut into pieces. For "Mianyinchen" (see page 1, Description of the drugs), sifted to removed dust.

Medicinal use[3]: Used for the treatment of hypertension, respiratory diseases, chronic cervicitis and also for the treatment of epidemic hepatitis.

Effects and indications of Herba Artemisiae according to Traditional Chinese Medicine[1,2,4]	
Taste:	Bitter
Temperature:	Cold
Channels entered:	*Orbis lienalis et stomachi*, *orbis hepaticus et felleus*
Effects (functions):	To remove *damp-heat* and relieve jaundice.
Symptoms and indications:	Infectious icteric hepatitis; sores with exudation and itching; for the remedy of liver diseases such as hepatitis, jaundice and fatty liver.

Main constituents[3]:

- **Flavones**
  cirsilineol (5,4′-dihydroxy-6,7,3′-trimethoxyflavone), cirsimaritin (5,4′-dihydroxy-6,7-dimethoxyflavone), genkwanin (5,4′-dihydroxy-7-methoxyflavone), rhamnocitrin (3,5,4´-trihydroxy-7-methoxy-flavone), arcapillin (5,2′,4′-trihydroxy-6,7,5′-trimethoxyflavone), eupatolitin (3,5,3′,4′-tetrahydroxy-6,7-dimethoxyflavone), arcapillin, capillarisin

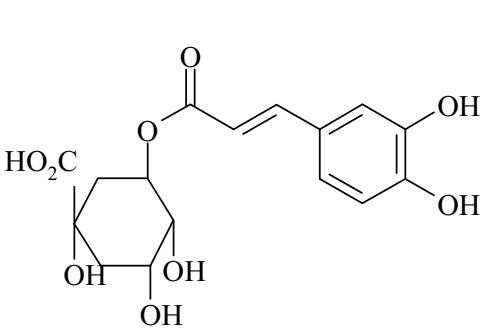
- **Phenylcarboxylic acid**
  chlorogenic acid, isochlorogenic acids

- **Cumarins/p-Cumaric acid derivatives**
  scoparone, scopoletin, capillartemisin A, B, umbelliferone

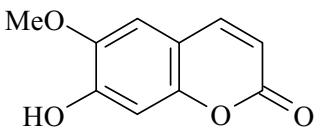
- **Terpenoids/Essential oil (*Artemisia scoparia*)**
  p-cymene, Δ^3-carene, α-terpinol, bornyl acetate, methyleugenol, *β*-elemene, *β*-caryophyllene α-pinene, *β*-pinene, myrcene, cineol, *p*-cymol, carvone, thujone, apiole, isoeugenol, cadinene, caryophyllene epoxide, vanillin, capillin, 1-phenyl- 2,4-hexadiyne-1-ol

- **Acetylenes derivatives**
  capillene, capillone, capillin, capillarin, dehydrofalcarinone, dehydrofalcarinol, norcapillene, capillanol, methoxycapillene, neocapillene

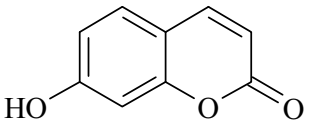
Fig. 1: Formulae of the main constituents of *Artemisia capillaris*



Chlorogenic acid

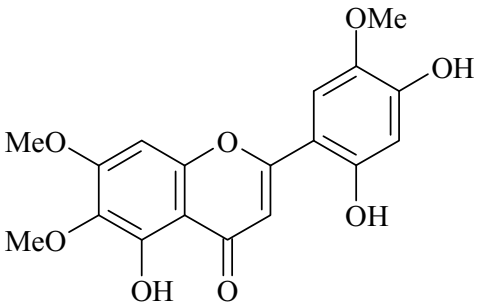


Scopoletin

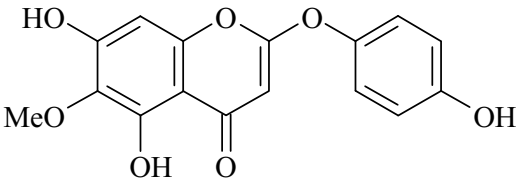


Umbelliferone

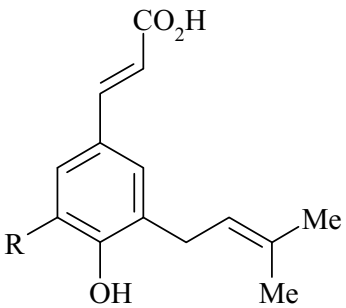
Flavones:

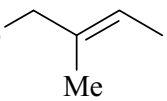


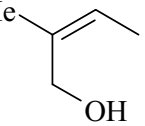
Arcapillin



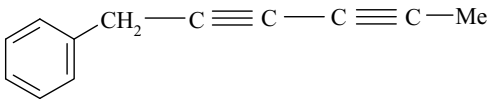
Capillarisin



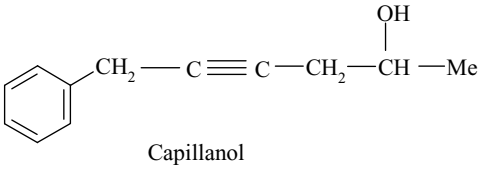
Capillartemisin A R = 

Capillartemisin B R = 

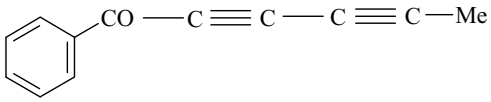
Acetylenes derivatives:



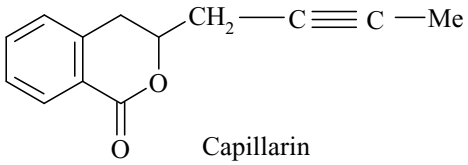
Capillene



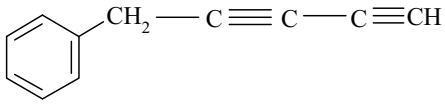
Capillanol



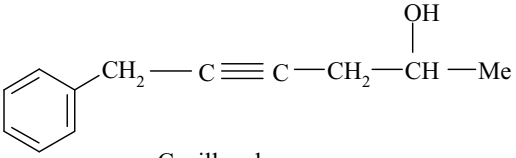
Capillin



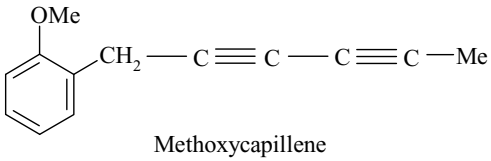
Capillarin



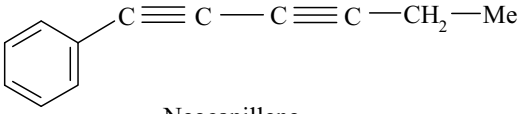
Norcapillene



Capillanol



Methoxycapillene



Neocapillene

Pharmacology:

- cholagogic[6, 8, 12], choloretic[3, 10, 11]
- anti-pyretic[6, 7, 8]
- hepatoprotective[9, 12]
- anti-hypertensive[9]
- anti-platelet aggregation activity[10]
- anti-HIV activity[10]
- antioxidant activity[12]
- anti-microbial (e.g. *Streptococcus ratti*, *S. sanguinis*, *S. gordonii*, obligate anaerobic bacteria)[9], antiviral[12]
- anti-inflammatory[6, 7, 8, 10, 11] (inhibits expression of inflammatory proteins including iNOS, COX-2 and TNF-alpha[12]
- inhibits the EtOH-, IL-1alpha-, TNF-alpha-induced cytotoxicity and the EtOH-induced apoptosis of Hep G2 cells[12]
- neuroprotective, neurotrophic effect[12]

Toxicology[13]**:**

Overdose of capillarisin is characterized by lethargy and salivation. The LD_{50} for capillarisin in mice is 262.5 mg/kg after intraperitoneal injection.[14, 15]

TLC fingerprint analysis

Drug samples		origin
1	Herba Artemisiae scopariae/*Artemisia capillaris*	Province Ningxia (Gu-yuan), China
2	Herba Artemisiae scopariae/*Artemisia capillaris*	Province Quizhou, China
3	Herba Artemisiae scopariae/*Artemisia capillaris*	Province Hubei, China
4	Herba Artemisiae scopariae/*Artemisia capillaris*	Sample of commercial drug obtained from TCM-Hospital, Bad Kötzting, Germany
5	Herba Artemisiae scopariae/*Artemisia scoparia* or *Artemisia capillaris*	Sample of commercial drug obtained from company China Medica, Germany

Reference compounds of Fig. 2		R*f*
T 1	Umbelliferone	0.91
T 2	Scopoletin	0.88
T 3	Mixture of Chlorogenic acid and Isochlorogenic acid	0.35 **0.62 / 0.82**
T 4	Chlorogenic acid	0.35

1. Thin layer chromatogram of the chlorogenic acids and coumarins:

1) Extraction: 1 g powdered drug is extracted under reflux with 20 ml methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Herba Artemisiae extracts: each 10 µl reference compounds: each 10 µl
Solvent system:	toluene : ethyl acetate : formic acid : water 5 100 10 10
Detection:	Natural products-polyethylene glycol reagent (NP/PEG): I: 1% diphenylboric acid-β-ethylamino ester (= diphenylboryloxyethylamine, NP) in methanol II: 5% polyethylene glycol-4000 (PEG) in ethanol The plate is sprayed first with solution I and then with solution II. The evaluation is carried out in UV 365 nm.

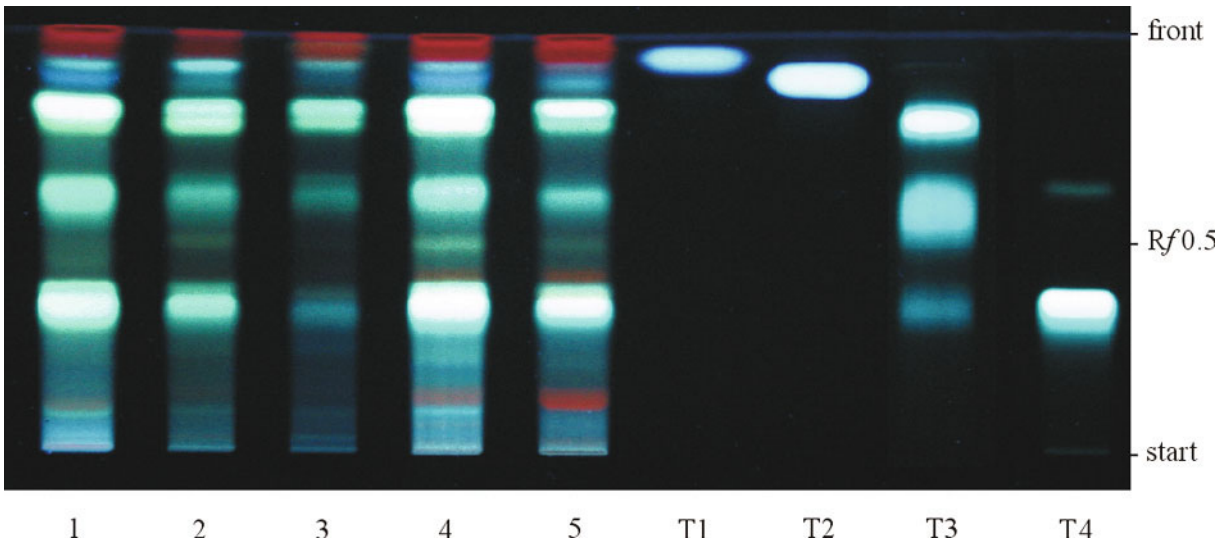


Fig. 2: Thin layer chromatogram of the methanol extracts of Herba Artemisiae scopariae sprayed with Natural products-polyethylene glycol reagent (NP/PEG) (UV 366 nm)

4) Description:
The TLC-fingerprint of all 5 *Artemisia scoparia* extract samples is characterized by the dominant presence of chlorogenic acid and isochlorogenic acids besides smaller amounts of the coumarins scopoletin and umbelliferone. The red zones on the solvent front derive from chlorophyll.

HPLC-fingerprint analysis:

1) Sample preparation: 1 g powdered drug is extracted under reflux with 20 ml methanol for 30 minutes. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2) Injection volume: Herba Artemisiae extracts: each 15.0 µl

3) HPLC parameter:

Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 10 ml 0.1% H_3PO_4 (Merck) / 1 l dist. Water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (Merck)
Gradient:	0-20 % B in 20 min. 20-40 % B in 35 min. 40-100 % B in 17 min total runtime: 72 minutes
Flow:	1 ml/min.
Detection:	205 nm

Retention times of the main peaks recorded at 205 nm

Peak	Rt (min.)	Compound
1	15.75	Chlorogenic acid
2	23.50	Umbelliferone
3	26.35	Scopoletine
4	66.71	Acetylenes derivatives (e.g. capillene)

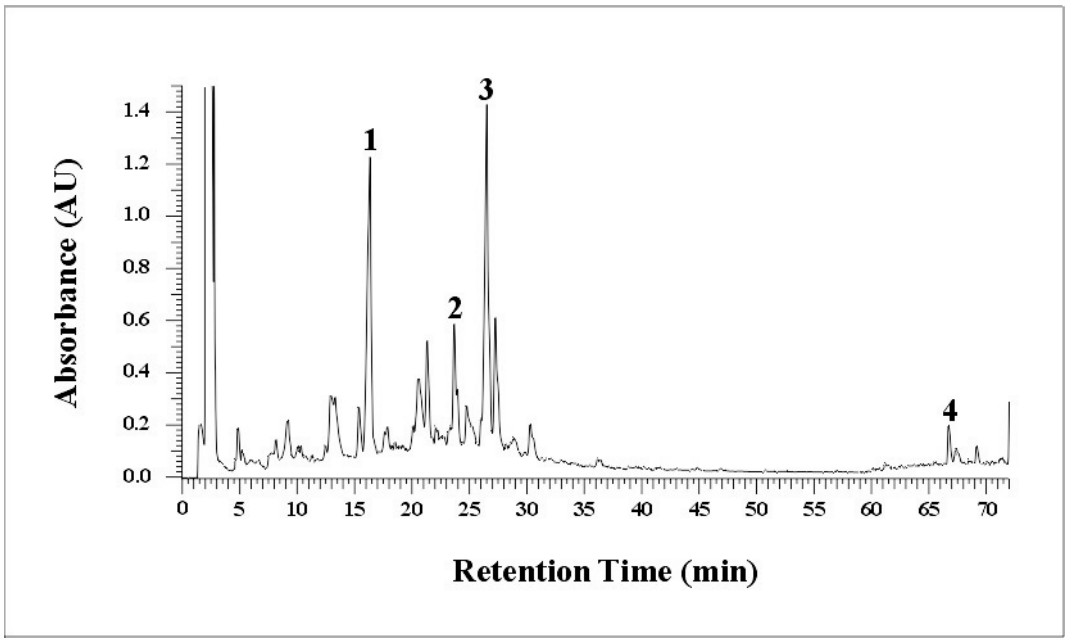


Fig. 3a: HPLC-fingerprint analysis of the extract of *Artemisia capillaris*, sample 1

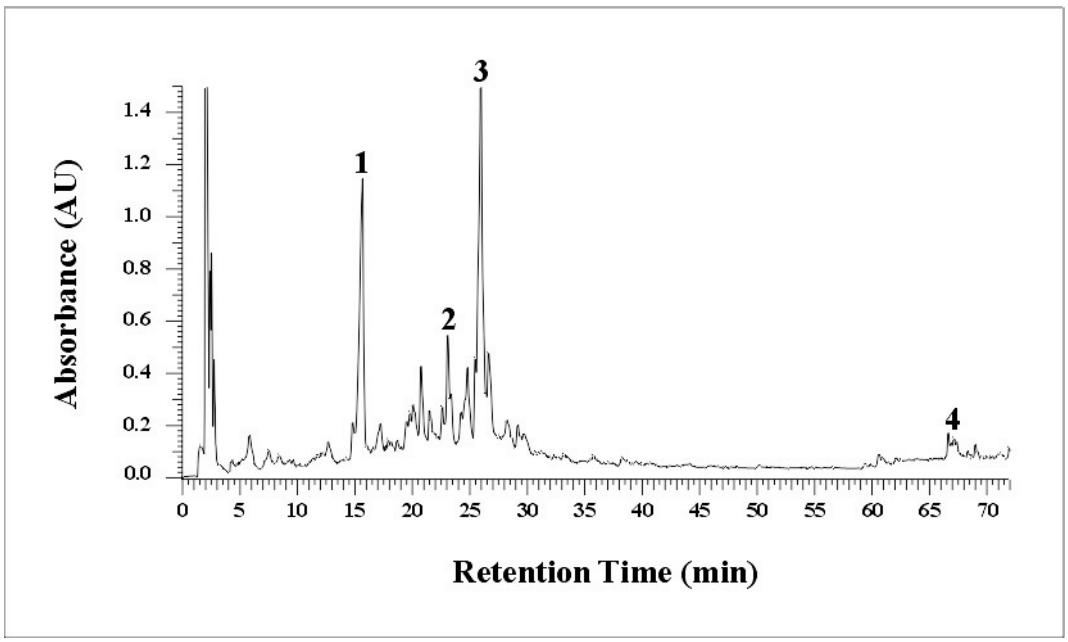


Fig. 3b: HPLC-fingerprint analysis of the methanol extract of *Artemisia capillaris*, sample 4

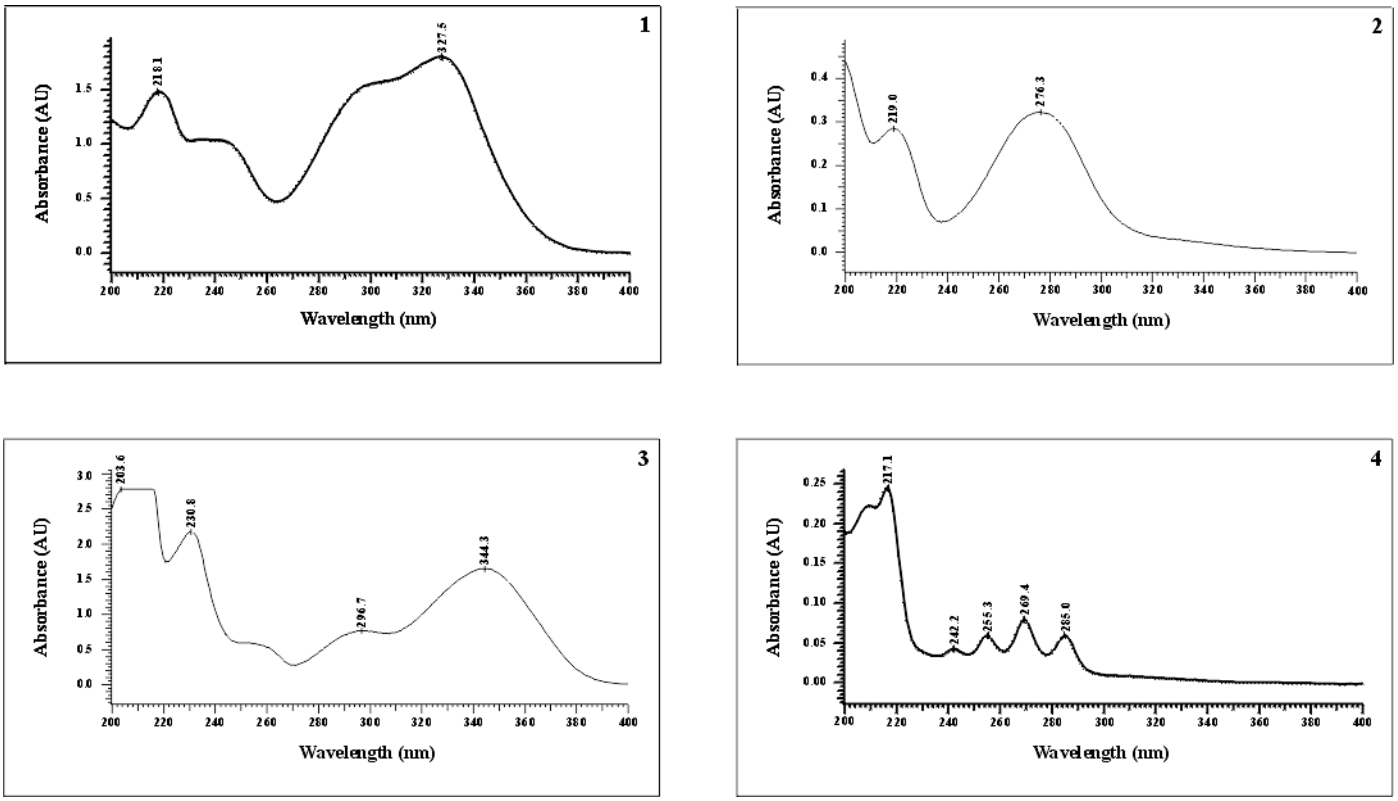


Fig. 4: On line UV-spectra of *Artemisia capillaris*

4) Description of the HPLC-Figures

The HPLC-fingerprint is characterized by an assembly of peaks in the Rt – range Rt = 15-30 and low concentrated peaks between Rt = 65-70. The four characteristic peaks can be assigned to chlorogenic acid (peak **1**, Rt = 15.75), umbelliferone (**2**, Rt = 23.50), scopoletin (**3**, Rt = 26.35) and the different polyacetylenes (**4**, Rt = 66.5 – 67.8).

Conclusion

The TLC- and HPLC-fingerprints of Herba Artemisiae supply the perfect identity of the herb. A good indicator is the on line UV-spectrum of the acetylenic compounds.

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Radix Aconiti Lateralis praeparata
Fuzi
Radix Aconiti Kusnezoffii praeparata
Zhicaowu

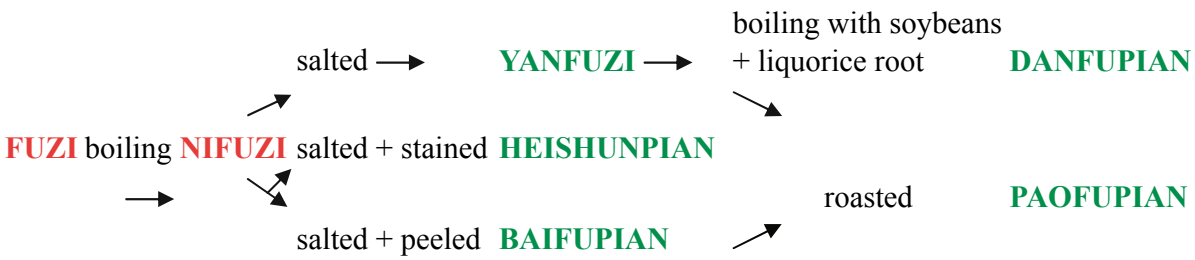
Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005
There exist 5 monographs of *Aconitum*:
- Radix Aconiti (*A. carmichaeli*) – *Chuanwu*
- Radix Aconiti Lateralis praeparata (*A. carmichaeli*) – *Fuzi*
- Radix Aconiti praeparata (*A. carmichaeli*) – *Zhichuanwu*
- Radix Aconiti Kusnezoffii (*A. kusnezoffii*) – *Caowu*
- Radix Aconiti Kusnezoffii praeparata (*A. kusnezoffii*) – *Zhicaowu*

Radix Aconiti Lateralis praeparata:

Official drug[1]: Prepared Common Monkshood Daughter Root is the processed daughter root (→ "lateralis") of *Aconitum carmichaeli* Debx. (Fam. Ranunculaceae).

The drug is collected in late June to early August. After removal of the parent root, rootlet and soil, the daughter roots are dried and usually used only after processing.

Processed forms[1, 3]:



Origin[2]: From Yunnan and Sichuan in the west to Jiangsu and Zhejiang in the east and from Shaanxi and Shandong in the north to the northern parts of Guangxi and Guangdong in the south

Descriptions of the drugs[1]: 1. Yanfuzi *(salted aconite daughter root)*
Conical, 4-7 cm long, 3-5 cm in diameter. Externally grayish-black, covered with fine powder of salt, topped with depressed bud scars and encircled with tubercled short rootlets or rootlet scars. Texture heavy. Transversely cut surface grayish-brown, showing small clefts filled with fine powder of salt and a polyangular cambium ring and vascular bundles arranged irregularly inside the ring. Odor, slight; taste, salty, numb und pungent.

2. Heishunpian *(black slices)*
Longitudinal slices, the upper portion wide and the lower portion narrow, 1.7-5 cm long, 0.9-3 cm wide, 0.2-0.5 cm thick. The outer bark blackish-brown, cut surface dark yellow, oily and lustrous, translucent and showing longitudinal vascular bundles. Texture hard and fragile. Fracture horny. Odor, slight; taste, weak.

3. Baifupian *(white slices)*
Without outer bark, yellowish-white, translucent, about 3 mm thick.

Radix Aconiti Kusnezoffii praeparata:

Official drug[1]: Prepared Kusnezoff Monkshood Root is the processed root tuber of *Aconitum kusnezoffi* Reichb. (Fam. Ranunculaceae).

The drug is collected in autumn when the aerial part is withered. After removal of rootlet and soil, the roots are dried and usually used only after processing.

Processing-Method[1]: Boiling in water
For further information on processing see:[1, 3]

Origin[2]: Shaanxi, Hebei, Inner Mongolia, and throughout the North-East Provinces

Description of the drug[1]: Irregular rounded or nearly triangular slices. Externally dark brown, with a grayish-white polyangular cambium ring, dotted vascular bundles and clefts; the edges wrinkled or curved. Texture fragile. Odor, slight; taste, slightly pungent and numb.

Medicinal use[3,4]: The tubers and roots of *Aconitum* spec. in mainly processed form are commonly applied as painkillers for collapse, syncope, rheumatic fever, painful joints, gastroenteritis, diarrhea, edema, bronchial asthma, various tumors, and some endocrinal disorders like irregular menstruation.

The cardio- and neurotoxicity of the unprocessed drug is potentially lethal, and the improper internal use of *Aconitum* in China, India, Japan and some other countries results in a high risk of severe intoxications. Therefore the unprocessed roots are only used for external application as anesthetics.

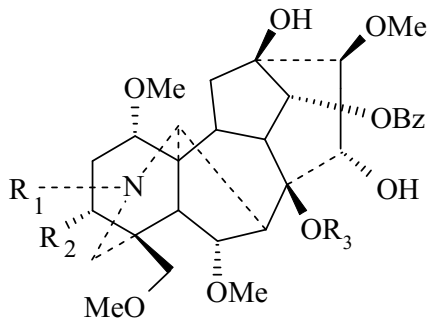
For further information on toxicity see:[2,3,5,6]

Effects and indications of Radix Aconiti Lateralis Praeparata according to Traditional Chinese Medicine[1,7]	
Taste:	Acrid
Temperature:	Hot
Channels entered:	*Orbis cardialis, Orbis renalis, Orbis lienalis*
Effects (functions):	To cause restoration from collapse, reinforce *fire* and *yang*, and dispel *wind, cold* and *damp*.
Symptoms and indications:	Collapse with cold limbs and faint pulse due to prostration of *yang*; impotence, *cold* in uterus; cold pain in the heart and abdomen, vomiting and diarrhea due to deficiency and *cold*; edema from *yin-cold*; afflictions from external pathogenic factors due to *yang* insufficiency; arthralgia.

Effects and indications of Radix Aconiti Kusnezoffii Praeparata according to Traditional Chinese Medicine[1,7]	
Taste:	Pungent and numb
Temperature:	Hot
Channels entered:	*Orbis cardialis, Orbis renalis, Orbis lienalis*
Effects (functions):	To relieve rheumatic conditions, warm the *meridians* and alleviate pain.
Symptoms and indications:	Rheumatic and rheumatoid arthralgia; precordial and abdominal pain with cold sensation; abdominal colic caused by cold; anesthesia also used as an analgesic.

Main and minor **constituents** of *Aconitum carmichaeli*/*kusnezoffii* and pharmacology[3]:

Constituent	Pharmacology
Monoester diterpene alkaloids Lappaconitine, Benzoylnapelline, 6-Benzoylheteratisine, Benzoylheteratisine	Antinociceptive, anti-arrhythmic, and anti-epileptic activity
Diester diterpene alkaloids **Aconitine**	Antinociceptive, anti-inflammatory, and local anesthetic activity
Mesaconitine	Antinociceptive and anti-inflammatory activity
Hypaconitine	Antinociceptive and anti-inflammatory activity
Benzoylmesaconine	Antinociceptive and anti-arrhythmic activity
Benzoylhypaconitine, Jesaconitine, Lipodeoxyaconitine, Beiwutine, Deoxyaconitine, Yunaconitine, Guan-fubase A	Antinociceptive, anti-arrhythmic, anti-inflammatory, antihypotensive, and local anesthetic activity
Triester diterpene alkaloids Benzoylaconitine, 3-Acetylaconitine, Acetylaconitine	Antinociceptive, anti-arrhythmic, and anti-inflammatory activity
Non-esterfied diterpene alkaloids Songorine, Napelline, Heteratisine, Talastisamine	Antinociceptive, anti-arrhythmic, and hypotensive activity
Lipo-alkaloids[4] 14-benzoylaconine-8-palmitate, 14-benzoylaconine-8-linoleate	COX-2 inhibitory activity
Other **Polysaccharides**[8], Steroid: *β*-Sitosterol	Antitumoral and immunostimulating activity[8]



Aconitine	R_1: C_2H_5, R_2: OH, R_3: acetyl
Mesaconitine	R_1: Me, R_2: OH, R_3: acetyl
Hypaconitine	R_1: Me, R_2: H, R_3: acetyl
Benzoylmesaconine	R_1: Me, R_2: OH, R_3: H
Deoxyaconitine	R_1: C_2H_5, R_2: H, R_3: acetyl

Fig. 1: Formulae of the main compounds of *Aconitum carmichaeli* and *Aconitum kusnezoffii*[9]

A: Content (μg/g) of Aconitine, Mesaconitine, and Hypaconitine in raw and processed Radix Aconiti (data are taken from references[9,10]):

	A. lateralis, raw		A. lateralis, processed		A. kusnez., raw		A. kusnez., processed	
	min.	max.	min.	max.	min.	max.	min.	max.
Aconitine	64.61	281.13	3.41	21.47	19.78	1580.75	8.81	34.54
Mesaconitine	219.64	2707.40	3.39	52.51	19.23	1216.57	10.24	144.18
Hypaconitine	533.67	1498.60	12.30	237.05	122.71	640.41	7.87	142.16

B: Content of Aconitine and acute toxicity of Fuzi (Radix Aconiti lateralis praeparata) and its processed products:[11]

Group		Aconitine (μg/g)	LD_{50} value (g/kg)
Fuzi	(60.0 mg/kg, p.o.)	25.2 ± 0.2	1.4 ± 3.2
Yanfuzi	(60.0 mg/kg, p.o.)	3.4 ± 0.2*	9.9 ± 4.3*
Heishunpian	(60.0 mg/kg, p.o.)	1.4 ± 0.2*	10.7 ± 5.2*
Baifupian	(60.0 mg/kg, p.o.)	1.1 ± 0.1*	20.3 ± 5.2*

Values are reported as mean ± S.E.M. Each LD_{50} value was obtained from a group of eight animals (in BALB/c mice).
* $P < 0.01$ vs. value from Fuzi-treated group.

C: Toxicity (LD_{50} (mg/kg)) of the pure alkaloids in mice, p.o.[3]

Aconitine	Mesaconitine	Hypaconitine	Benzoylmesaconine
1.00 – 1.80	1.90	5.80	810

D: Detoxification processes

a) Reduction of the total alkaloid content[2]
b) Hydrolysis of diester alkaloids in monoesters or unesterified compounds e.g. Aconitine → Benzoylaconine → Aconine[3]
c) Increase of the Lipo-alkaloid content (mice, i.v.: LD_{50} >10 mg/kg for lipo-alkaloids versus 0.3 mg/kg for aconitine)[4]

TLC-fingerprint analysis

Drug samples		Origin
1	Radix Aconiti "Chuanwu" *Aconitum carmichaeli*	Jiang you County, Sichuan, China
2	Radix Aconiti lateralis **praep**.* *Aconitum carmichaeli*	Jiang you County, Sichuan, China
3	Radix Aconiti lateralis **praep**.* "Nifuzi" *Aconitum carmichaeli*	Han zhong County, Shaanxi, China
4	Radix Aconiti kusnezoffii "Caowu" *Aconitum kusnezoffii*	Beijing
5	Radix Aconiti kusnezoffii **praep**.* "Zhicaowu" *Aconitum kusnezoffii*	sample of commercial drug (TCM-Hospital, Bad Kötzting, Germany, 1999)

* informations concerning the exact processing method are lacking

Reference compounds of Figure 2		**R*f***
T1	Aconitine	0.64
T2	Mesaconitine	0.55
T3	Hypaconitine	0.58
T4	Deoxyaconitine	0.67

1) Extraction:[1] 5.0 g powdered drug are extracted by stirring at room temperature with 37.5 ml diethyl ether for 10 minutes. After addition of 2.5 ml ammonia solution 25 % it is further extracted for 30 minutes and allowed to stand for 2 hours. The extract is filtered and the filtrate evaporated in vacuum to dryness. The residue is dissolved in 0.5 ml ethanol and used for TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol.

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Raw Radix Aconiti extracts (sample 1 + 4): 15 µl each
Processed Radix Aconiti extracts (sample 2, 3 + 5): 25 µl each
reference compounds: 20 µl each

Solvent system: Ethyl acetate : 2-Butanone : Formic acid 98 % : Water
5 + 3 + 1 + 1

Detection: Dragendorff reagent (DRG; MUNIER and MACHEBOEUF):
Solution (a): 0.85 g basic bismuth nitrate is dissolved in 10 ml glacial acetic acid and 40 ml water.
Solution (b): 8 g potassium iodide are dissolved in 30 ml water.
Spray reagent: 0.5 ml (a) + 0.5 ml (b) is mixed with 2 ml glacial acetic acid and 10 ml water.

The plate is sprayed first with 10 ml of Dragendorff reagent. After drying at room temperature the plate is additionally sprayed with 5 ml of a 10 % sodium nitrite aqueous solution. The evaluation is carried out in VIS.

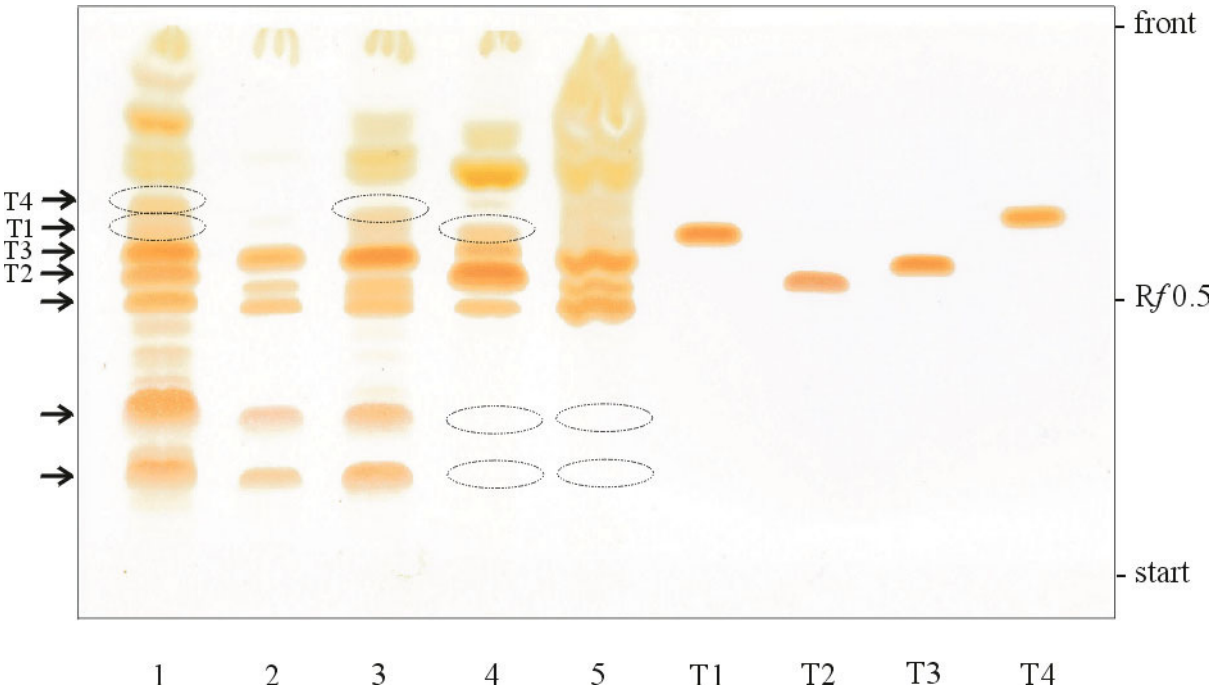


Fig. 2: Thin layer chromatogram of the ether extraxts of Radix Aconiti sprayed with Dragendorff and sodium nitrite reagent (VIS)

4) Description:

Sample **1**:
The TLC-view of sample **1** shows the alkaloid composition of the raw (unprocessed) root extract of *Aconitum carmichaeli*. The alkaloid zones in the R*f*-range of 0.45 up to 0.65: R*f* = 0.50 (not identified), R*f* = 0.55 (**T2** = Mesaconitine), R*f* = 0.58 (**T3** = Hypaconitine), R*f* = 0.64 (**T1** = Aconitine). In the R*f*-range above Aconitine up to the front 3 – 4 Dragendorff positive zones are visible: R*f* = 0.67 (**T4** = Deoxyaconitine), R*f* = 0.75 and R*f* = 0.85 (not identified).

Samples **2** + **3**:
- The TLC-view of samples **2** and **3** shows the alkaloid composition of two processed root samples of the same *Aconitum* species as in **1** but of different origin (Sichuan and Shaanxi)
- In sample **2** Aconitine (**T1**) is absent (degraded), in sample **3** it is detectable but in very low concentration. Both samples show in the deep R*f*-range at 0.21 and 0.31 two further alkaloids which can be assigned to Benzoylmesaconine and Benzoylaconine respectively.

Sample **4**:

The alkaloid composition of the raw/unprocessed root of *Aconitum kusnezoffii* in the middle R*f*-range is very similar to that of sample **1**, but differs in a high alkaloid concentration of **T2** (Mesaconitine) and contains only traces of alkaloids in the deep R*f*-range compared with those of sample **1**.

Sample **5**:

The alkaloid composition of the processed root of *Aconitum kusnezoffii* approximately corresponds with the processed root of *Aconitum carmichaeli* (sample **2** and **3**).

HPLC-fingerprint analysis:

1) Sample preparation:[1] The extract used for TLC is filtered over Millipore® filtration unit, type 0.45 µm.

2) Injection volume: Radix Aconiti extracts: 25 µl each

3) HPLC parameter:

Apparatus: MERCK HITACHI D-6000 A Interface
MERCK HITACHI L-4500 A Diode Array Detector
MERCK HITACHI AS-2000 Autosampler
MERCK HITACHI L-6200 A Intelligent Pump

Separation column: LiChroCART® 250-4 LiChrospher® 60 RP select B (5 µm), Merck

Precolumn: LiChroCART® 4-4 LiChrospher® 60 RP select B (5 µm), Merck

Solvent: A: 2.0 g hexanesulfonic acid (SigmaAldrich)/1 l dist. Water (Millipore Ultra Clear UV plus® filtered) + H_3PO_4 85 % (VWR) (pH 3)
B: Acetonitrile (SigmaAldrich)

Gradient: 5-90 % B in 60 min.
total runtime: 60 minutes

Flow: 1 ml/min.

Detection: 230 nm

Retention times of the main peaks recorded at 230 nm

peak	Rt (min.)	compound
1	7.1	Not identified (no alkaloid)
2	22.8	Not identified alkaloid
3	23.9	Benzoylmesaconine
4	26.5	Not identified diester diterpene alkaloid
5	27.4	Not identified diester diterpene alkaloid
6	29.2	Mesaconitine
7	30.8	Aconitine
8	32.0	Hypaconitine overlapped by Deoxyaconitine
9	55.5	Not identified alkaloid (Lipo-alkaloid?)

identified by reference compounds and references [9,10,12]

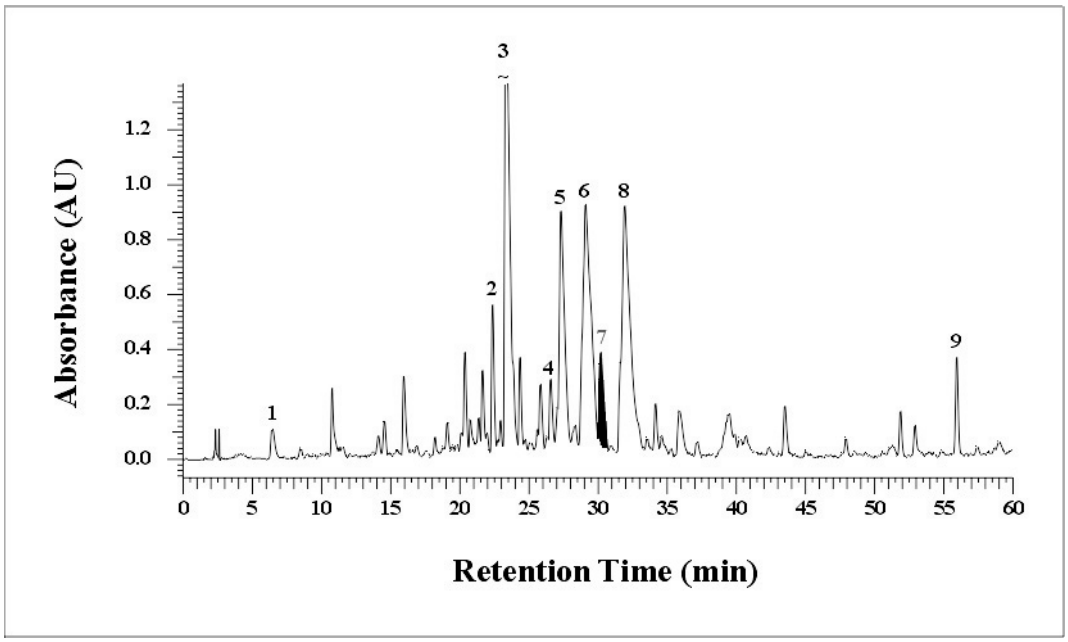


Fig. 3a: HPLC-fingerprint analysis of the ether extract of unprocessed Radix Aconiti (*Aconitum carmichaeli*; sample **1**)

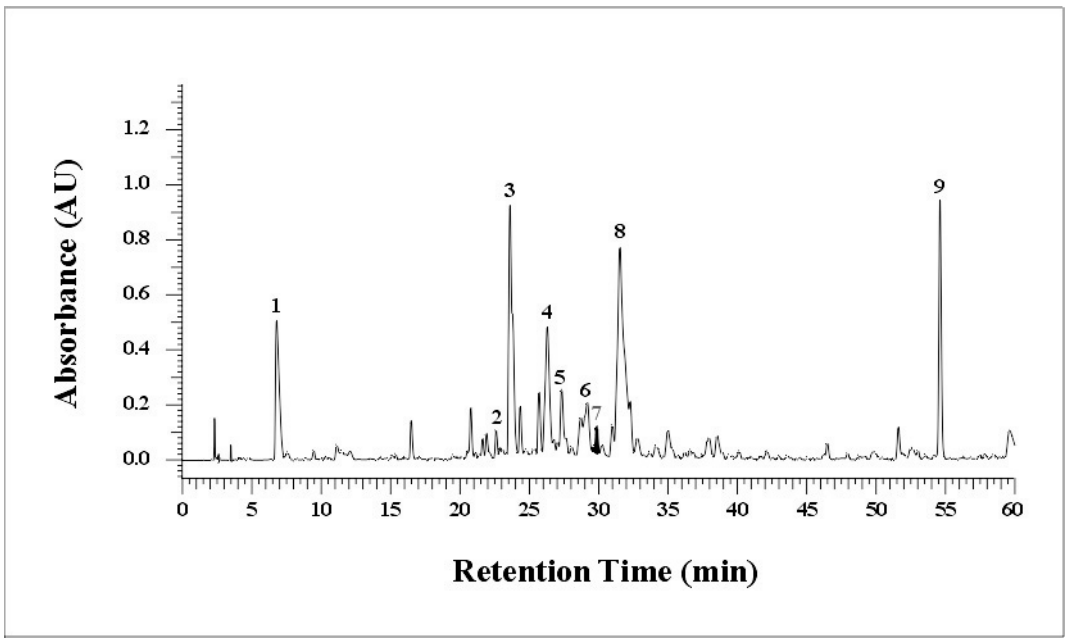


Fig. 3b: HPLC-fingerprint analysis of the ether extract of Radix Aconiti lateralis praeparata (*Aconitum carmichaeli*; sample **3**)

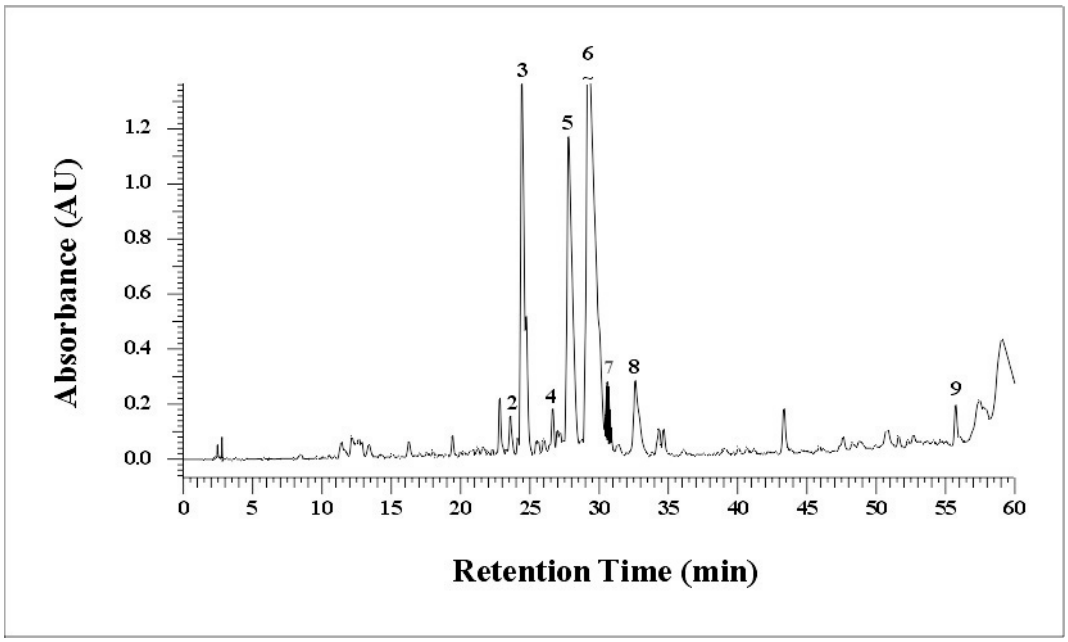


Fig. 3c: HPLC-fingerprint analysis of the ether extract of unprocessed Radix Aconiti kusnezoffii (*Aconitum kusnezoffii*; sample **4**)

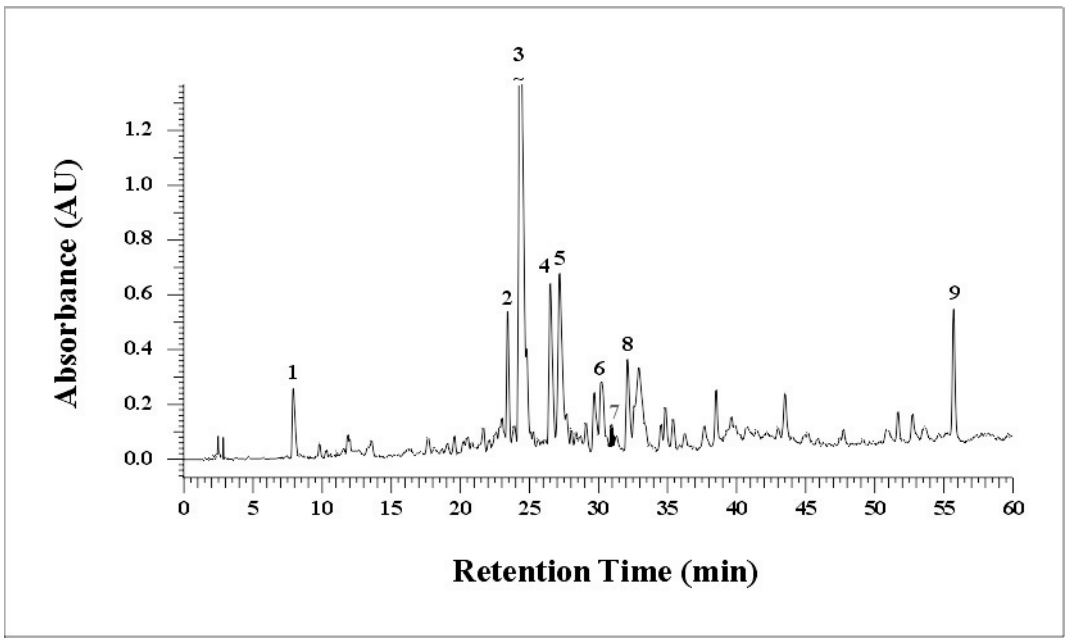


Fig. 3d: HPLC-fingerprint analysis of the ether extract of Radix Aconiti kusnezoffii praeparata (*Aconitum kusnezoffii*; sample **5**)

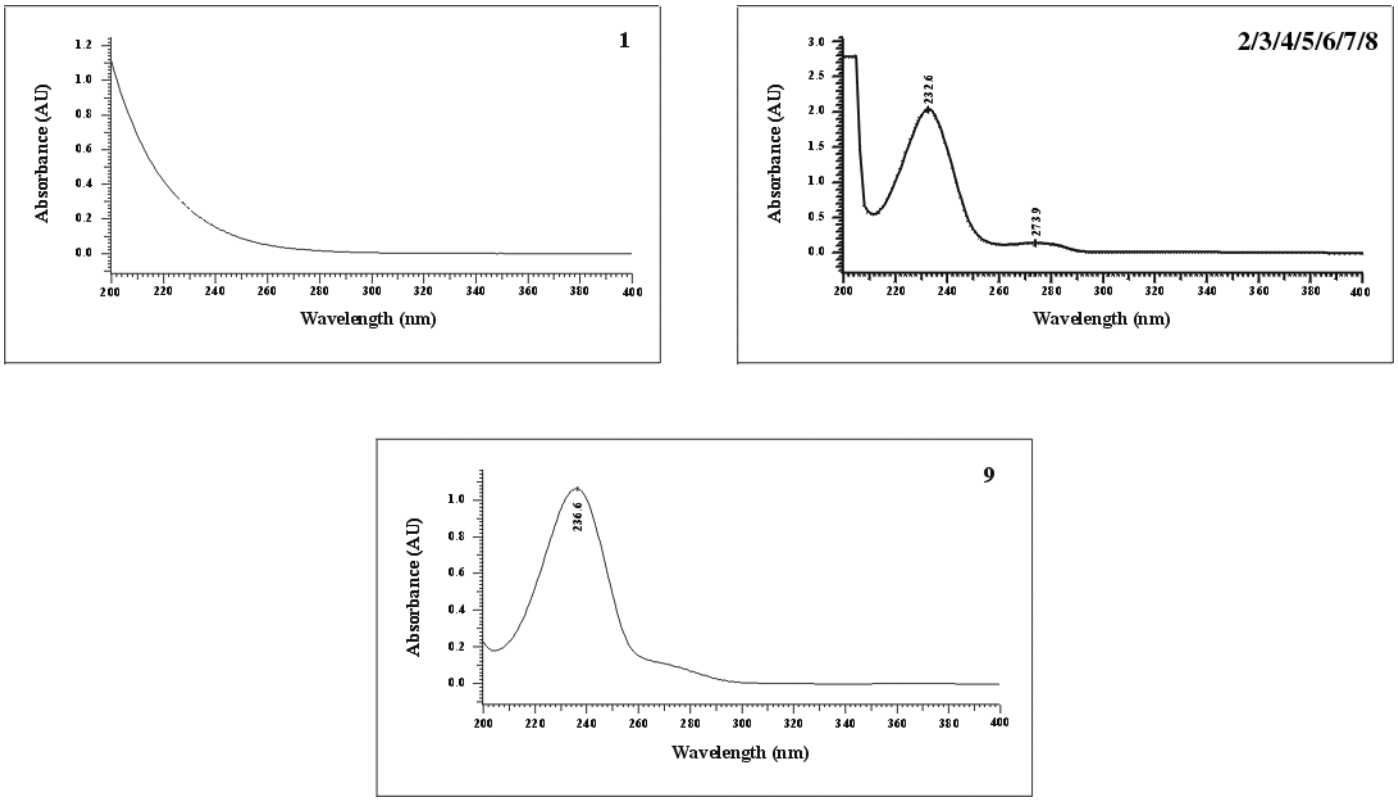


Fig. 4: On line UV-spectra of Radix Aconiti

4) Description of the HPLC-Figures

Sample 1:

The root extract of raw (unprocessed) *Aconitum carmichaeli* shows in the Rt-range 22.0 – 32.0 an assembly of 5 – 6 main peaks identified as Benzoylmesaconine (**3**; Rt = 23.3), a not identified diester diterpene alkaloid (**5**; Rt = 27.3), Mesaconitine (**6**; Rt = 29.1), Aconitine (**7**; Rt = 30.2) and Hypaconitine overlapped by Deoxyaconitine (**8**; Rt = 32.0). The peaks in the Rt-range Rt = 6.0 – 22.0 can be assigned to Benzoylaconine or Benzoylhypaconine. The peaks in the Rt-range of 45 – 60 could be not identified but may derive from the long chain acyl alkaloids: the lipo-alkaloids.

Sample 3:

The processed root sample **3** of *Aconitum carmichaeli* differs from the non processed sample of the same species **1** by a strong reduction of the peaks **3**, **5** and **6** inclusive **7** (Aconitine). Therefore the processing has degraded most of the alkaloids in this Rt-range, namely Benzoylmesaconine, Aconitine and Mesaconitine. Instead of them the concentrations of two further alkaloids at Rt = 6.8 and 54.6 are increased.

Sample 4:

The raw (unprocessed) root of *Aconitum kusnezoffii* shows in the Rt-range 22.8 – 33.0 a very similar peak pattern except the peak **8** (Hypaconitine/Deoxyaconitine) the concentration of which was strongly reduced.

Sample 5:

The processed root of *Aconitum kusnezoffii* again shows a similar peak profile as compared with sample **2** or **3**. The Aconitine peak has nearly disappeared.

Note:

- For the dried drug Radix Aconiti praeparata (*Aconitum carmichaeli*) the Chinese Pharmacopoeia 2005[1] prescribes a content of not more than 0.15 % of diestheralkaloids (calculated as aconitine), and less than 0.20 % of alkaloids (calculated as aconitine).
- For quantification of the alkaloids see also the publications[9,10,12]

Conclusion

The TLC- and HPLC-fingerprint analysis has confirmed that in both processed *Aconitum* roots the extremely toxic alkaloid Aconitine was strongly degraded. Therefore the demand of the Chinese Pharmacopoeia seems to be fulfilled. However, since neither the processing methods used are standardized nor safe limits were stipulated for the toxic Aconitine, Mesaconitine and the other main alkaloids, it is compulsory to work out reliable, quantitative HPLC-methods to guarantee the safety of processed *Aconitum* species for prescription and the preparations of decoctions. It is also compulsory to perform toxicity studies to asses how safety limits[3] can be achieved without simultaneous loss of the medicinal potential. If this aim cannot be guaranteed all unprocessed and processed *Aconitum* drugs should be banned from the Pharmacopoeias and excluded from the market.

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Cortex Cinnamomi
Rougui

Pharmacopoeia[1]: Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005

Official drug[1]: Cassia Bark is the dried stem bark of *Cinnamomum cassia* Presl. (Fam. Lauraceae).

The drug is frequently collected in autumn and dried in the shade.

In other Chinese districts also the species *Cinnamomum tamada* and *Cinnamomum burmanii* are used[6].

Origin[4,5]: Southern China (Guangdong, Guangxi, Fujian), Laos, Vietnam, Sumatra

Description of the drugs[1]: Channelled or quilled, 30-40 cm long, 3-10 cm wide or in diameter, 2-8 mm thick. Outer surface greyish-brown, slightly rough, with irregular fine wrinkles and transverse protrudering lenticels, some showing greyish-white streaks; inner surface reddish-brown, somewhat even, with fine longitudinal striations and exhibiting oily trace on scratching. Texture hard and fragile, easily broken, fracture uneven, outer layer brown and relatively rough, inner layer reddish-brown and oily, showing a yellowish-brown line between two layers. Odour, strongly aromatic; taste, sweet and pungent.

Pretreatment of the raw drug[1]: Foreign matters are eliminated and bark roughed. Pounded to pieces before use.

Medicinal use[5]: Mainly used in the adjuvant treatment of diabetes type II, vascular disorders, prescribed as aromatic stomachic, astringent, tonic, analgesic and stimulant

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*

Effects and indications of *Cinnamomum casssia* according to Traditional Chinese Medicine[1,2,4]	
Taste:	Sweet, pungent
Temperature:	Hot
Channels entered:	*O. hepaticus, O.renalis*, *O.lienalis*
Effects (functions):	To reinforce *yang*, and lead the *fire* back to the kidney, dispel *cold* and relieve pain, and to activate blood circulation and stimulate menstrual discharge
Symptoms and indications:	Impotence, frigidity, feeling of cold and pain in the loins and knees, dyspnoea in deficiency of the kidney; dizziness, inflammation of the eyes and sore throat due to *yang-deficiency*; precordial and abdominal pain with cold syndrome; neurosis with a feeling of gas rushing up through the chest to the throat from the lower abdomen; amenorrhea, dysmenorrhea

Main constituents[6]:

- trans-cinnamaldehyde, methoxy-cinnamaldehyde
- cinnamic acid
- coumarin (*C. cassia* 7 %)
- Procyanidins B1, B2, B5, B7, A2
- choline
- protocatechuic acid
- vanillic acid
- syringic acid
- *β*-sitosterol
- salicylaldehyde
- diterpenoids (cinncassiols)
- essential oil in *C. cassia* (eugenol/methyleugenol, safrol, cineol, piperitone, linalool) (~4 %)

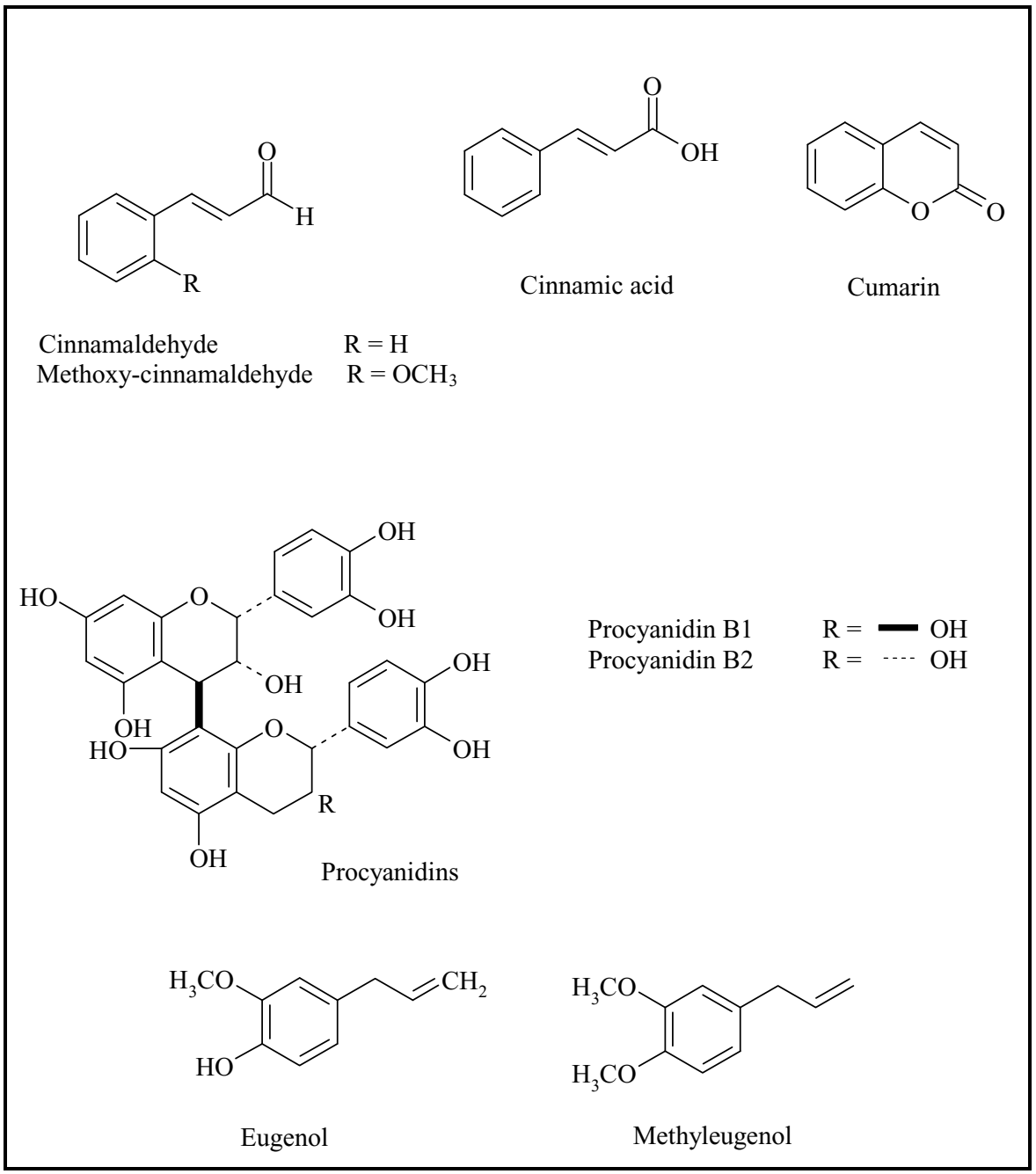

Fig. 1: Formulae of the main constituents of Cortex Cinnamomi[6]

Pharmacology:

- antidiabetic[8,10,11,13,14,16-19] → procyanidins B1/B2-rich extract
- antifungal[3,6]
- antibacterial[3,6,9,12]
- antiulcerogenic[3,6]
- promotes motility[3]
- stimulates gastric juices[3,7,13]
- anti-inflammatory[7,8]
- lack of appetite, dyspepsia, intestinal winds[3,7]
- anti-viral[9]
- antiparasitic[9]
- lowering blood glucose[8]
- antioxidant[12,13]
- anticomplementary (Procyanidin)[13]
- antitumoral[13]
- inhibition of protein kinase[13]
- antipyretic[6]
- antihypertensive[6,13]
- antihelmintic[9]
- attenuated cell swelling and mitochondrial dysfunction[8]
- inhibits cancer cell proliferation[8]

Therapeutic perspectives:

For clinical studies and the application of Chinese herbal drugs in China see publications of Jia et al.[17], Evans JL[18], Li et al.[21], Mang et al.[22], Vanschoonbeek et al.[23], Blevins et al.[24] and Baker et al.[25]

Toxicology[3]:
Incorrect prescribing: flushing, red eyes, dry mouth and bleeding.
Overdoses: nausea, vomiting, abdominal pain, dysuria, anuria, dizziness, red face, visual disturbances, numbness of the tongue, respiratory distress, convulsions.
Not recommended to use during pregnancy.

Safety:

The Federal Institute of Germany for Risk Assessment published an alternative limit of 0.1 mg Coumarin per kg body mass and day which is considered harmless as average intake[27]. See also the publications of Cao et al.[8] and Woehrlin et al.[26].

TLC fingerprint analysis:

Drug samples		origin
1	Cortex Cinnamomi/*Cinnamomum cassia*	Sample of commercial drug, HerbaSinica, Charge: 070401H011)
2	Cortex Cinnamomi/*Cinnamomum cassia*	Sample of official pharmacy, Munich, Charge: A 26.09.2002)
3	Cortex Cinnamomi/*Cinnamomum ceylanicum*	Sample of commercial drug, Gewürzmühle Brecht GmbH, Eggenstein
4	Cortex Cinnamomi/*Cinnamomum ceylanicum*	Sample of commercial drug, Sonnentor GmbH, Sprögnitz, Austria (origin: Madagascar)
5	Cortex Cinnamomi/*Cinnamomum cassia* → **essential oil**	Sample of commercial drug, HerbaSinica, Charge: 070401H011)
6	Cortex Cinnamomi/*Cinnamomum ceylanicum* → **essential oil**	Sample of commercial drug, Gewürzmühle Brecht GmbH, Eggenstein

Reference compound Fig. 2a		**R*f***
T 1	Coumarin	0.25

Reference compounds Fig. 2b		**R*f***
T 2	trans-Cinnamaldehyde	0.41
T 3	Methyleugenol	0.43
T 4	Eugenol	0.43

Reference compound Fig. 2c		**R*f***
T 5	Procyanidin B2	0.57

TLC-fingerprint analysis

1. Analysis of the main compounds of Cortex Cinnamomi[20]

1) Extraction:

1. dichloromethane extract
1 g powdered drug is shaken with 10 ml dichloromethane for 15 minutes. The extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml toluene.

2. essential oil
30 g powdered drug are distilled for 3 hours on a Neo-Clevenger apparatus with 400 ml water (acidified with 1.5 ml hydrochloric acid 37 %). The collected essential oil was dissolved with 0.5 ml Xylene and used for the TLC.

2) Reference compounds: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Cortex Cinnamomi extracts: each 10 µl
reference compounds: each 10 µl
essential oil extracts: each 3 µl

Solvent system: Toluene : ethyl acetate
93 7

Detection: Spray reagents

1. 5% Potassium hydroxide solution **(Fig. 2a)**:

5g potassium hydroxide are dissolved in 100 ml ethanol. The plate is sprayed with 10 ml of the solution and after 10 minutes evaluated under UV 366 nm.

2. Anisaldehyde – Sulphuric acid – Reagent **(Fig. 2b)**:

0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The plate is sprayed with 10 ml reagent, heated at 110°C for 5 minutes and evaluated in VIS.

Note: The reagent has only limited stability and is no longer useable when colour has turned to red-violet.

3. Dinitrophenylhydrazine reagent (DNPH) (**Fig. 2b – T 2**)

0.1 g 2,4-Dinitrophenylhydrazine is dissolved in 100 ml methanol, followed by the addition of 1 ml of 36 % hydrochloric acid.

After spraying with 10 ml the plate is evaluated immediately in VIS.

Drug samples of Fig. 2a

1+2	Dichloromethane extracts of the bark of *Cinnamomum cassia*
3+4	Dichloromethane extracts of the bark of *Cinnamomum ceylanicum*
T 1	Coumarin

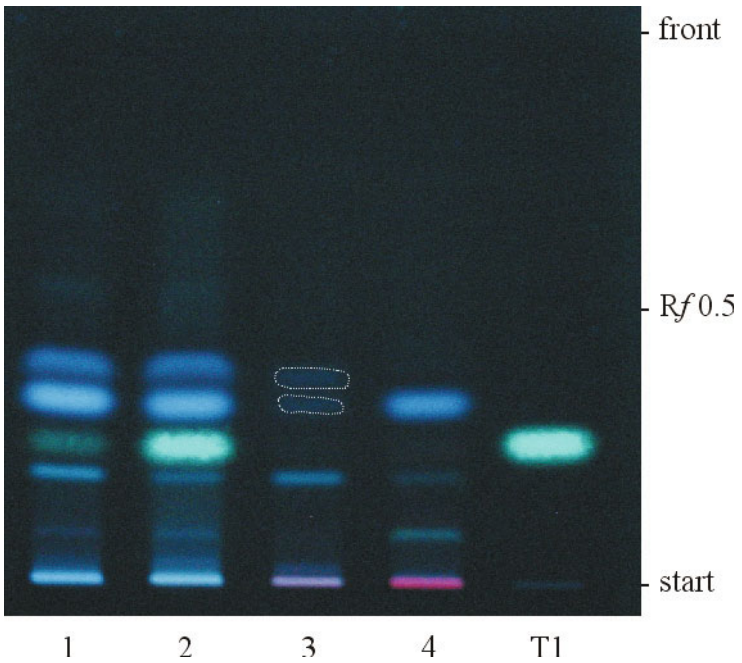

Fig. 2a: Thin layer chromatogram of the dichloromethane extracts of Cortex Cinnamomi sprayed with 5% potassium hydroxid solution, UV 366 nm

4) Description of Fig. 2a:
The dichloromethane extracts of *Cinnamomum cassia* bark **1** and **2** show in the Rf-range 0.19-0.40 three distinct blue-green fluorescent zones of Coumarin (R*f* = 0.25), o-Methoxy-cinnamaldehyde (R*f* = 0.33) and Cinnamaldehyde (R*f* = 0.40). The corresponding extract of *Cinnamomum ceylanicum* bark **3** contains the same compounds only in very small concentrations. In the bark extract **4** only o-Methoxy-cinnamaldehyde could be detected. The blue-green zones on the start are degradation products of cinnamaldehyde.

Drug samples of Fig. 2b

1	Dichloromethane extract of the bark of *Cinnamomum cassia*
3	Dichloromethane extract of the bark of *Cinnamomum ceylanicum*
5	**Essential oil** of the bark of *Cinnamomum cassia*
6	**Essential oil** of the bark of *Cinnamomum ceylanicum*
T 2	trans-cinnamaldehyde
T 3	Methyleugenol
T 4	Eugenol

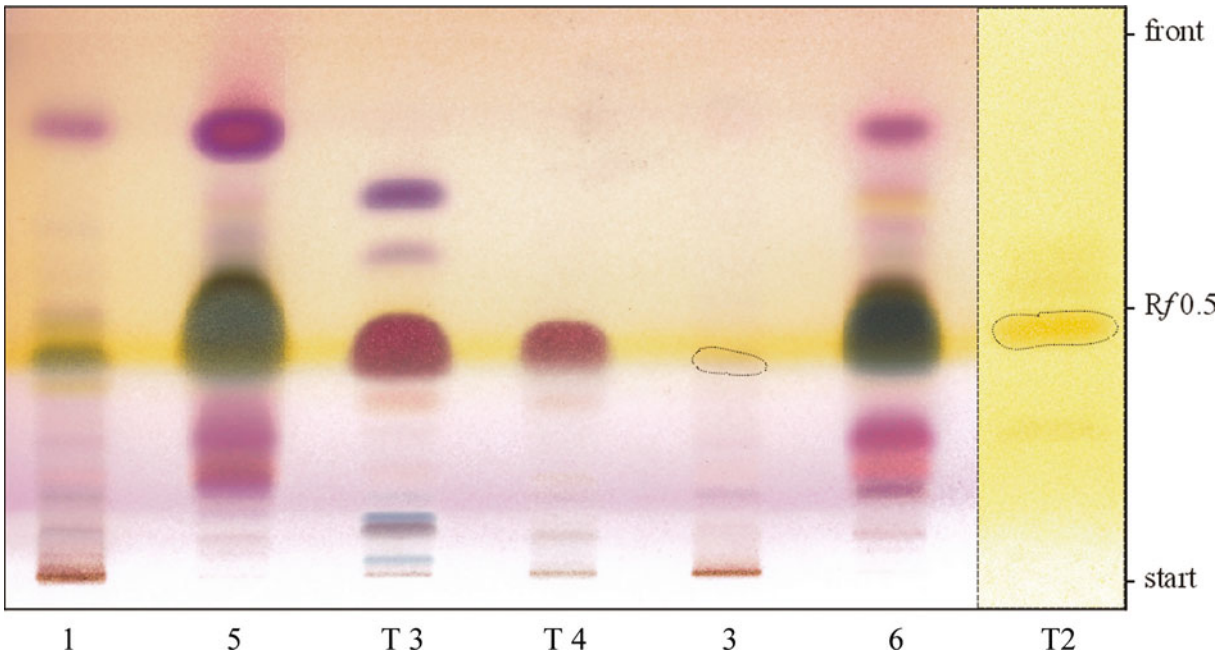

Fig. 2b: Dichloromethane and essential oil extracts of Cortex Cinnamomi sprayed with Anisaldehyde - Sulphuric acid - Reagent, VIS + Cinnamaldehyde (T2, DNPH)

4) Description of Fig. 2b:

- The dichloromethane extracts of *Cinnamomum cassia* **1** and *C. ceylanicum* **3** show only cinnamaldehyde as grey zone.
- The essential oils of *Cinnamomum cassia* and *C. ceylanicum* are characterized by the main grey zone of cinnamaldehyde at R*f* = 0.41 which has the same R*f*-value as eugenol, a red-violet zone of safrol at R*f* = 0.83 and two red zones in the R*f*-range of 0.17-0.30, with piperitone at R*f* = 0.26.
  The cinnamaldehyde also detectable with DNPH-reagent gives a yellow zone at R*f* = 0.48.

2. Analysis of the procyanidines of Cortex Cinnamomi[15]

1) Extraction: 2 g powdered drug are extracted under shaking for 2 hours with 10 ml 50 % acetone-water at room temperature. The solution was filtered and the residue extracted again with the same solvent under the same conditions. The two extracts were mixed in a separation funnel and shaken with 10 ml diethyl ether. The water layer was collected and used for the TLC.

2) Reference compound: each 0.5 mg is dissolved in 0.5 ml methanol

3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Cortex Cinnamomi extracts: each 10 µl
reference compound: each 10 µl

Solvent system: Ethyl acetate : methanol : water
100 20 10

Detection: Anisaldehyde – Sulphuric acid – Reagents **(Fig. 2c)**:

0.5 ml Anisaldehyde is mixed with 10 ml glacial acetic acid, followed by 85 ml methanol and 5 ml concentrated sulphuric acid, in that order.

The plate is sprayed with 10 ml reagent, heated at 110°C for 5 minutes and evaluated in VIS.

Note: The reagent has only limited stability and is no longer useable when colour has turned to red-violet.

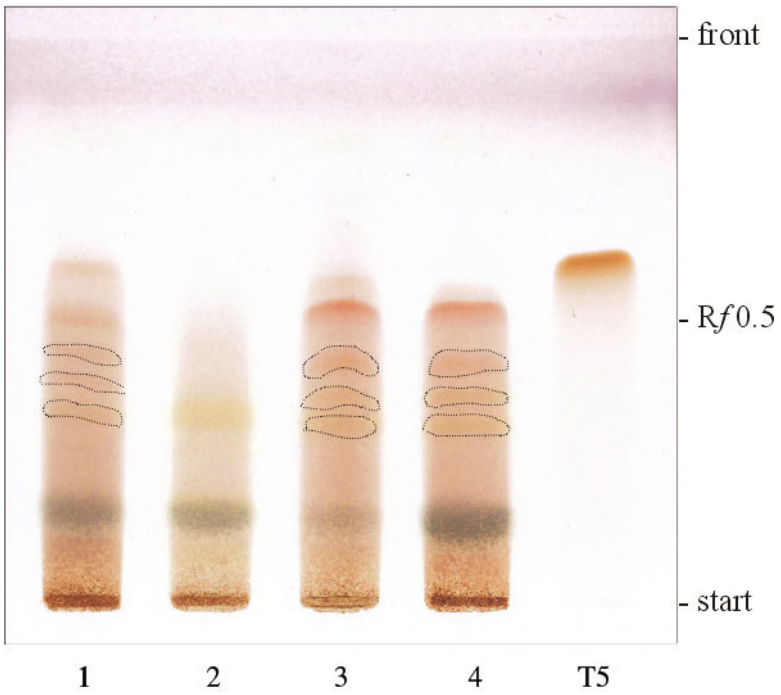

Fig. 2c: Thin layer chromatogram of the methanol extracts of Cortex Cinnamomi sprayed with Anisaldehyde - Sulphuric acid - Reagent, VIS

4) Description of Fig. 2c:
The oligomeric procyanidins extracted with acetone-water mixture appear as brown red zones in the R*f*-range of 0.32 to 0.54. Procyanidin B2* (**T5**) is visible at R*f* = 0.52. On the start the higher polymeric procyanidins (Cinnamtannins?) are visible.
* The higher R*f*-value of pure Procyanidin B2 in comparison to the same in the extracts is due to the TLC borderline effect.

HPLC-fingerprint analysis:

1. Analysis of the main compounds of Cortex Cinnamomi[7]

1) Sample preparation:	0.5 g powdered drug is filled into a 50 ml flask and 25 ml methanol are added. The flask is weight and ultrasonicated for 30 minutes. The lost volume of methanol is added and the solution filtered over a folded filter (3 hw, 110 mm, 65 g/m²) followed by a further filtration over Millipore® filtration unit, type 0.45 µm and the solution injected into the HPLC apparatus.
2) Injection volume:	Cortex Cinnamomi extracts: each 15.0 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump

Separation column:	LiChroCART® 125-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 0.4 ml glacial acetic acid/1 l dist. water (Millipore Ultra Clear UV plus® filtered)
	B: acetonitrile (VWR)
Gradient:	10-50 % B in 60 min.
	total runtime: 60 minutes
Flow:	1 ml/min.
Detection:	280 nm

Retention times of the main peaks recorded at 280 nm

peak	Rt (min.)	compound
1	17.4	Coumarin
2	23.4	Cinnamic acid
3	26.2	trans-Cinnamaldehyde
4	32.4	Eugenol

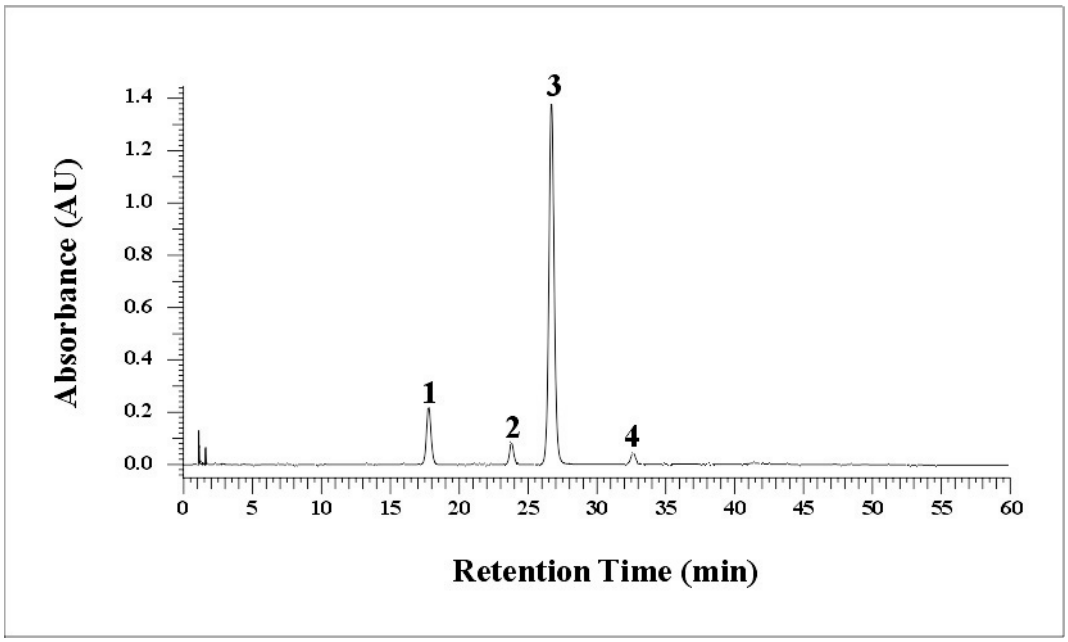

Fig. 3a: HPLC-fingerprint analysis of the methanol extract of *C. cassia*, sample 2

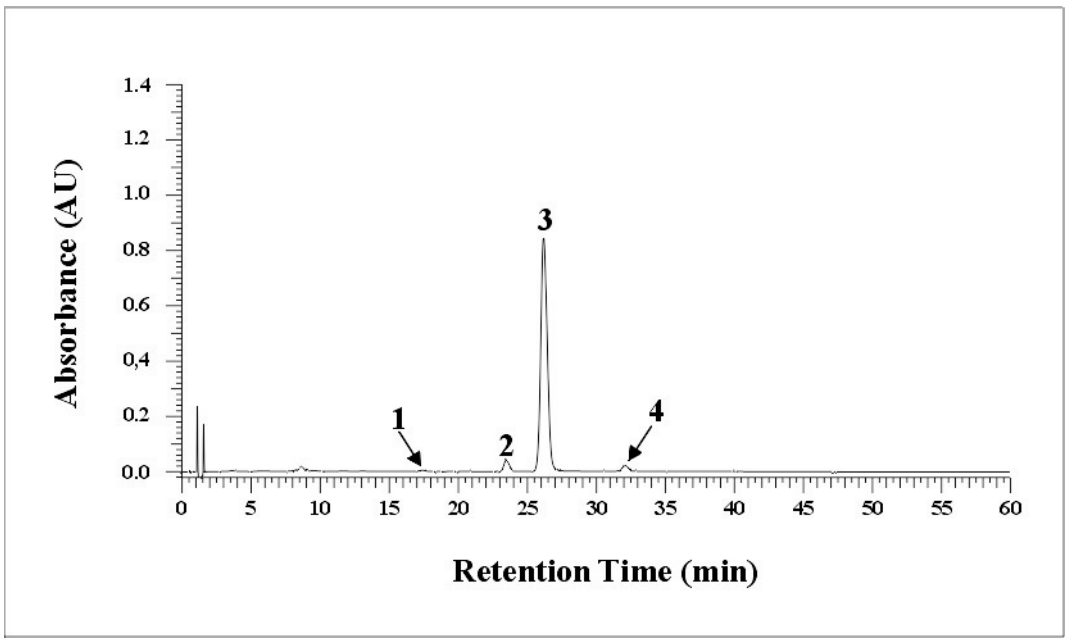

Fig. 3b: HPLC-fingerprint analysis of the methanol extract of *C. ceylanicum,* sample 3

4) Description of Fig. 3a and 3b:

Both *Cinnamomum* spec. provide in the MeOH-extracts the same peak pattern with cinnamaldehyde as dominant compound at Rt = 26.3 (**3**) and Coumarin at Rt = 17.4 (**1**),

Cinnamic acid at Rt = 23.4 (**2**) and Eugenol at Rt = 32.4 (**4**). *Cinnamomum cassia* extract differs in its peak profile from that of *C. ceylanicum* by higher contents of Cinnamaldehyde and Coumarin.

2. Analysis of the procyanidines of Cortex Cinnamomi[10]

1) Sample preparation:	2 g powdered drug are extracted under shaking for 2 hours with 10 ml 50 % acetone-water at room temperature. The solution is filtered and the residue extracted again with the same solvent under the same conditions. The two extracts are mixed in a separation funnel and shaken with 10 ml diethyl ether. The water layer is collected and filtered over Millipore ® filtration unit, type 0.45 µm.
2) Injection volume:	Cortex Cinnamomi extracts: each 30.0 µl
3) HPLC parameter:	
Apparatus:	MERCK HITACHI D-6000 A Interface MERCK HITACHI L-4500 A Diode Array Detector MERCK HITACHI AS-2000 Autosampler MERCK HITACHI L-6200 A Intelligent Pump
Separation column:	LiChroCART® 250-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 2 ml formic acid / 1 l dist. water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	5-12 % B in 5 min. 12-30 % in 20 min. 30 % B for 1 min. Total runtime: 26 minutes
Flow:	1.0 ml/min.
Detection:	280 nm

Retention times of the main peaks recorded at 280 nm

peak	Rt (min.)	compound
X	4.4	Gallic acid
5	11.1	Procyanidin B_2
6	12.8	Oligomeric procyanidin

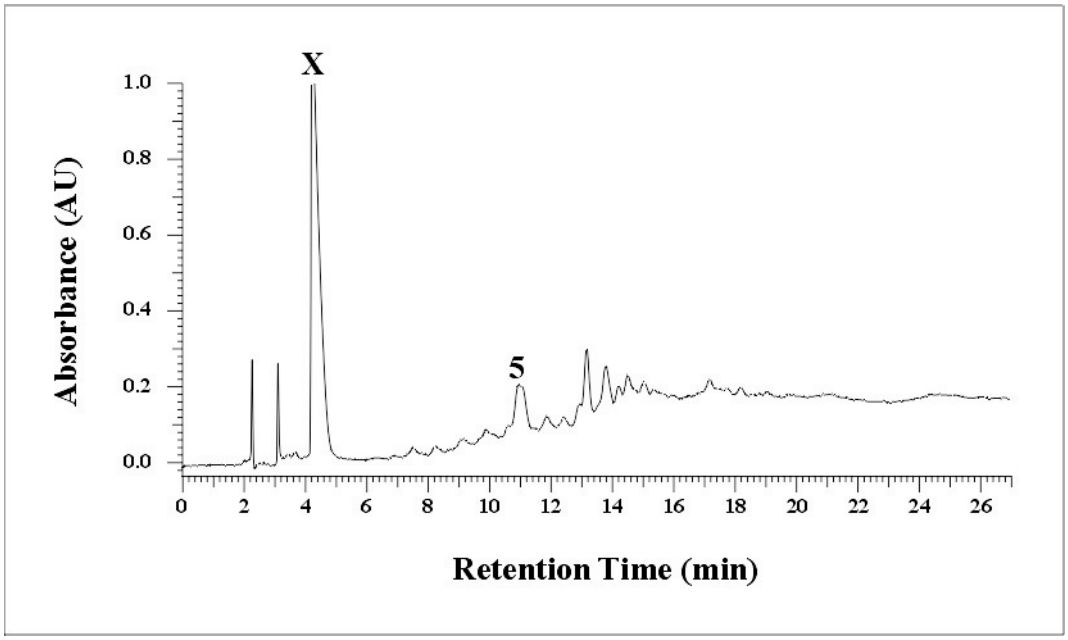

Fig. 4a: HPLC-fingerprint analysis of the methanol extract of *C. cassia*, sample 2

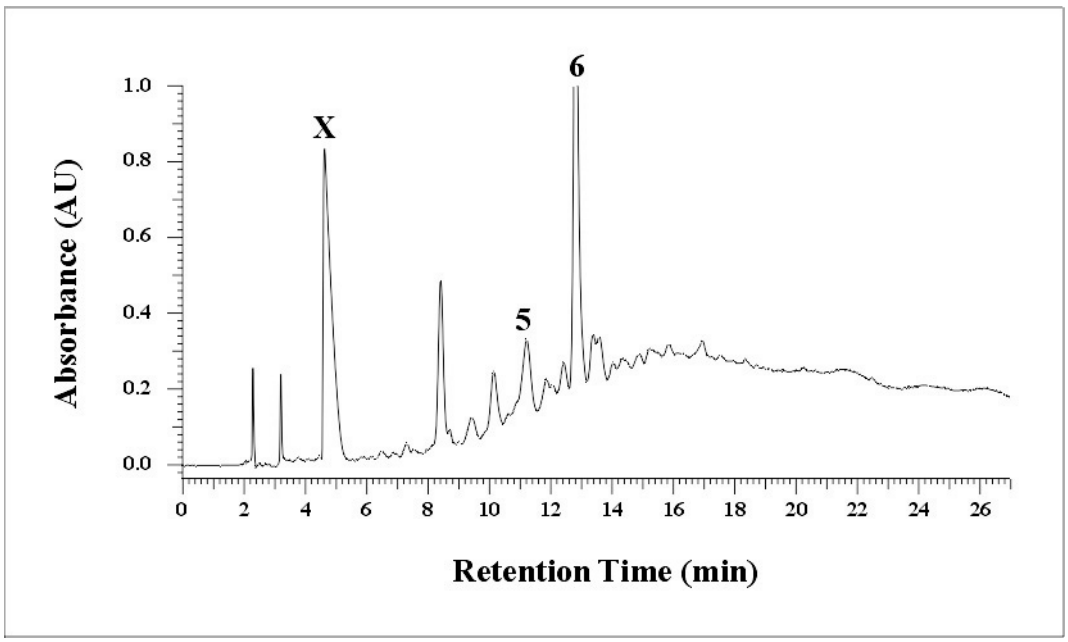

Fig. 4b: HPLC-fingerprint analysis of the methanol extract of *C. ceylanicum*, sample 3

4) Description of Fig. 4a and 4b:

The acetone-water extracts of *Cinnamomum cassia* and *C. ceylanicum* show the bulk of oligomeric procyanidins in the Rt-range from Rt = 8.0 till Rt = 18.0 with Procyanidin B2 at Rt = 11.2. The peak **X** at Rt = 4.4 could be identified as gallic acid, whereas the compound **6** at Rt = 12.8 in *C. ceylanicum* might be assigned to another oligomeric procyanidin.

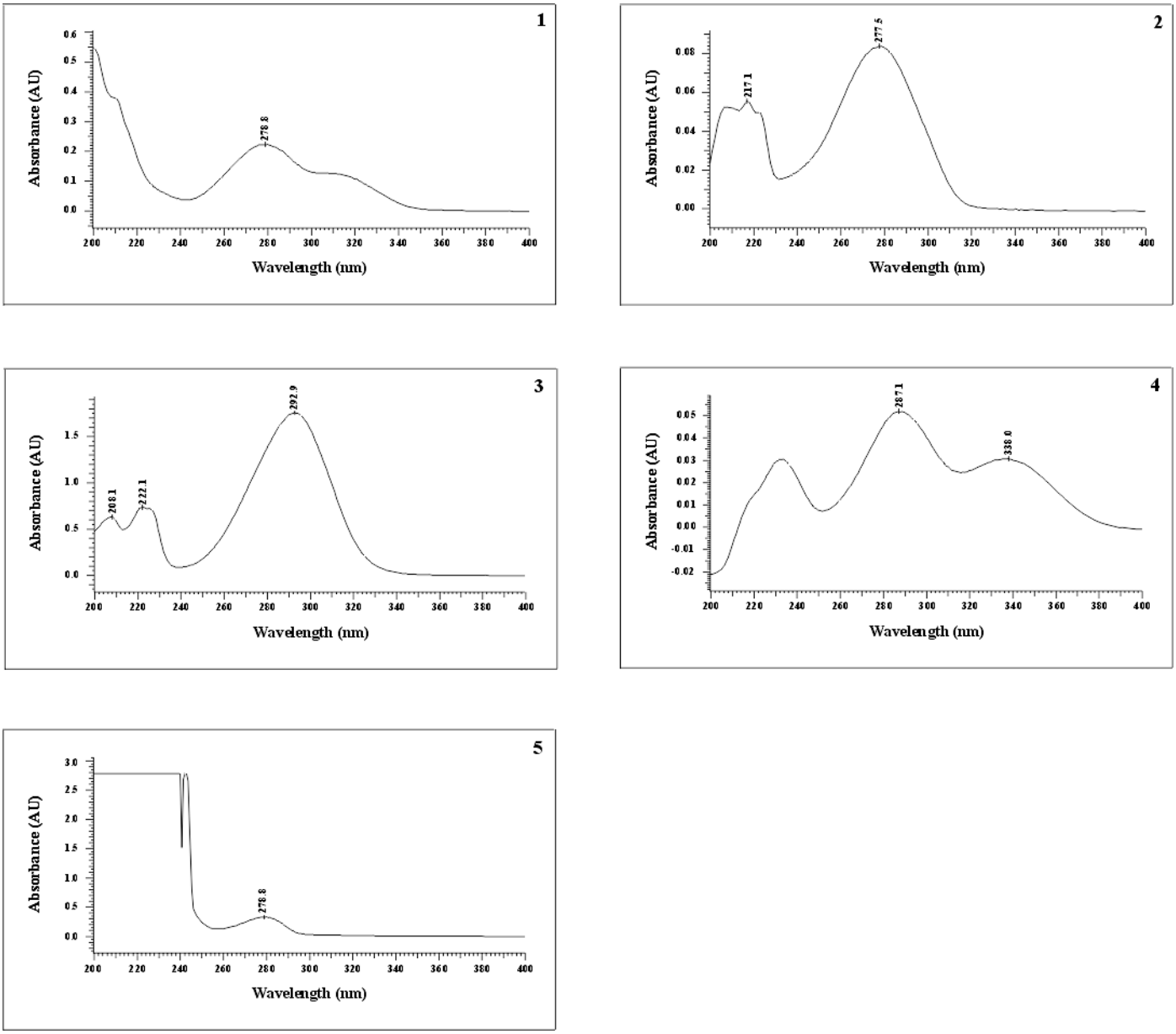

Fig. 5: On line UV-spectra of *Cinnamomum cassia* and *C. ceylanicum*

Note: Further HPLC-fingerprint analyses of Cinnamon extracts were published by Yang et al.[7] and Cao et al.[8].

Quantification of Coumarin in *Cinnamon cassia* bark of different origin were published by Woehrlin et al.[26].

Note: The Chinese Pharmacopoeia 2005 demands not less than 1.5 % of Cinnamaldehyde calc. with reference to the dried drug.

Conclusion

Cortex Cinnamomi cassiae, the official Chinese drug cannot be easily discriminated (distinguished) from Cortex Cinnamomi ceylanici alone by TLC- and HPLC-fingerprint analysis, because the chemical composition of their extracts and essential oils vary in dependence of the origin. For the therapeutic use further pharmacological investigations are needed.

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Appendix

Basic Solvent Systems, reagents and columns for the TLC-, GC- and HPLC-fingerprint Analysis of main structure types of natural products

1. Alkaloids
2. Amides
3. Phenolics
    a) Flavones
    b) Procyanidines
    c) Cumarins
    d) Anthraquinones
4. Essential oils
5. Lignans
6. Triterpene-/Steroidsaponins
7. Other Terpenoids (Diterpenoids)
8. Phenolcarboxylic acids

1. Alkaloids

(see Monographs No. 2, 28, 29, 32, 34, 36, 45, 50, 56, 62, 79)

TLC-Analysis	**HPLC-Analysis**
Ammonia solution 10%; MeOH-Extraction	Ammonia solution 10%; MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-select B column (C-8)
Chloroform-Methanol-Water (6 + 3 + 0.65)	A: 2 g Hexanesulfonic acid (pH 3) B: Acetonitrile
Iodine reagent, VIS	210 nm
TLC-Analysis	**HPLC-Analysis**
MeOH-Extraction	MeOH-Extraction
HPTLC Silica gel	RP-select B column (C-8)
Ethyl acetate-Methyl ethyl ketone-Formic acid-Water (5 + 3 + 1 + 1)	A: 0.68 g KH_2PO_4/1 l, pH = 9 (KOH) B: Acetonitrile
UV 366 nm, Dragendorff reagent, VIS	270 nm

2. Amides
(see Monograph No. 61)

TLC-Analysis	HPLC-Analysis
n-hexane-Extraction	n-hexane-Extraction
Silica gel	RP-18 column
n-Hexane-Ethyl acetate (5+3)	A: Water B: Acetonitrile
UV 254 nm Anisaldehyde sulphuric acid reagent, VIS	254 nm

3a. Flavones
(see Monographs No. 20, 27, 43, 48, 51, 55, 59, 62, 63, 67)

TLC-Analysis	HPLC-Analysis
MeOH-Extraction	MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Ethyl acetate-Formic acid-Glacial acetic acid-Water (10+1.1+1.1+2.6)	A: 0.001% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm	210/270 nm

3b. Procyanidines
(see Monographs No. 77, 80)

TLC-Analysis	HPLC-Analysis
Acetone/Water (1:1)-Extraction; Diethylether	Acetone/Water (1:1)-Extraction; Diethylether
Silica gel/HPTLC Silica gel	RP-18 column
Ethyl acetate-Methanol-Water (10+2+1)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	210/270 nm

3c. Cumarines
(see Monographs No. 13, 38, 45, 46, 78)

TLC-Analysis	HPLC-Analysis
50% MeOH/Butanol-Extraction	50% MeOH/Butanol-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Toluene-Ethyl acetate-Acetic acid (90+10+1)	A: 0.001% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm	210/280 nm

3d. Anthraquinones
(see Monographs No. 40, 71,76)

TLC-Analysis	HPLC-Analysis
MeOH-Extraction	MeOH-Extraction
HPTLC Silica gel	RP-18 column
Light petroleum-Ethyl acetate-Formic acid (75+25+1) for aglycones and glycosides Ethyl acetate-MeOH-Water (100+13.5+10)	A: 0.05% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm Phosphomolybdic acid, VIS	254/280 nm

4. Essential oils
(see Monographs No. 31, 36, 46, 52, 60, 65, 80)

TLC-Analysis	GC-Analysis
Distillation or n-Hexane-Extraction	Distillation (dil. 1:100 oil/*tert*Butylmethylether)
Silica gel	Varian VF-5 ms with 10 m precolumn (deactivated methyl-polysiloxan)
Toluene-Ethyl acetate (93 + 7)	Helium
Vanillin sulphuric acid reagent, VIS	270 °C

5. Lignans
(see Monographs No. 4, 7, 17, 35, 38, 60, 69)

TLC-Analysis	HPLC-Analysis
MeOH-Extraction	MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Toluene-Ethyl acetate-Formic acid (80 + 15 + 10)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	210 nm

TLC-Analysis
50% MeOH/Butanol-Extraction
Silica gel/HPTLC Silica gel
Chloroform-MeOH (9+1)
Vanillin phosphoric/sulphuric acid reagent, VIS

6. Triterpene-/Steroidsaponins
(see Monographs No. 1, 20, 30, 33, 37, 51, 53, 57, 67, 68, 70)

TLC-Analysis	HPLC-Analysis
MeOH/Water/Butanol-Extraction	MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Chloroform-MeOH-Water, lower layer	A: Water B: Acetonitrile
Vanillin phosphoric/sulphuric acid reagent or Anisaldehyde sulphuric acid reagent, VIS	210 nm

7. Other Terpenoids (Diterpenoids)
(see Monographs No. 25, 41, 49, 54, 59, 64, 73, 74, 75)

TLC-Analysis	HPLC-Analysis
MeOH/Ethyl acetate/Water-Extraction	MeOH/Ethyl acetate/Water-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Ethyl formate-Toluene-Formic acid (50 + 50 + 15)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	205 nm
TLC-Analysis	**HPLC-Analysis**
Diethyl ether-Extraction	Diethyl ether-Extraction
Silica gel	RP-18 column
Petroleum ether-Ethyl acetate-Cyclohexane (5 + 3 + 2)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	270 nm

8. Phenolcarboxylic acids
(see Monographs No. 49, 51, 57, 59, 74, 78)

TLC-Analysis	**HPLC-Analysis**
MeOH-Extraction	MeOH-Extraction
Silica gel	RP-18 column
Ethyl acetate-Formic acid-Glacial acetic acid-Water (10 + 1.1 + 1.1 + 2.6)	A: 0.001% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm	220 nm
TLC-Analysis	**HPLC-Analysis**
acidified MeOH-Extraction	acidified MeOH-Extraction
Silica gel	RP-18 column
Chloroform-Ethyl acetate-Toluene-Formic acid-MeOH (15 + 20 + 10 + 10 + 1)	A: 0.001% Phosphoric acid B: Acetonitrile
UV 254 nm	281 nm

Index

Drug monograph, Marker compounds, Chemical classification, Processing

	Drug monograph	Marker compounds	Chemical classification	Processing
1	Bupleuri radix	Saikosaponin		
			Polyacetylenes	
			Phyto-Fungi	
2	Fritillariae bulbus	Verticine		
3	Rehmanniae radix	Aucubin		
		Acteoside		
4	Schisandrae fructus	Schisandrin		
5	Asari radix et rhizoma	Asarinin		
		Sesamin		
			Alkamides	
			Lignans	
6	Houttuyniae cordatae herba	Sesamin		
			Lignans	
7	Pinelliae rhizoma	Pinoresinol		
			Lignans	
				processing
8	Astragali radix	Astragaloside		
			Isoflavones	
9	Angelicae pubescentis radix		Furanocoumarins	
			Phyto-Fungi	
			Polyacetylenes	
10	Atractylodis macrocephalae rhizoma		Polyacetylenes	
			Phyto-Fungi	
11	Belamcandae sinensis rhizoma	Iridin		
		Tectoridin		
		Resveratrol		
			Isoflavonoids	
			Stilbenes	

12	Lycopi lucidi herba	Luteolin--glucoside		
		Betulinic acid		
		Rosmarinic acid		
13	Notopterygii rhizoma seu radix		Furanocoumarins	
			Phyto-Fungi	
			Polyacetylenes	
14	Angelicae sinensis radix	Ligustilide		
			Phyto-Fungi	
			Polyacetylenes	
15	Angelicae dahuricae radix	Scopoletin		
			Furonacoumarins	
			Phyto-Fungi	
			Polyacetylenes	
16	Ligustici chuanxiong radix	Ligustilide		
			Phyto-Fungi	
			Polyacetylenes	
17	Zanthoxyli pericarpium	Sesamin		
			Alkamides	
			Lignans	
18	Magnoliae officinalis cortex	Magnolol		
		Honokiol		
19	Drynariae rhizoma	Naringin		
			Anthraquinones	
20	Puerariae radix	Puerarin		
			Isoflavonoids	
21	Codonopsis pilosulae radix	Tangshenoside		
22	Gardeniae fructus	Gardenoside		
		Crocetin		
23	Gastrodiae rhizoma	Gastrodin		
24	Ecliptae herba	Wedelolactone		
			Polyacetylenes	
			Phyto-Fungi	
25	Andrographis herba	Andrographolide		
26	Paeoniae albae/rubrae radix	Paeoniflorin		

27	Sophorae flos		Isoflavone	
28	Coptidis rhizoma	Berberine		
		Coptisine		
29	Stephaniae tetrandrae radix	Tetrandrine		
		Aristolochia acid		
30	Ziziphi spinosae semen	Jujuboside		
31	Amomi rotundus fructus			
32	Uncariae cum Uncis ramulus	Rhychnophylline		
		Pteropodine		
		Catechins		
33	Clematidis radix	Hederagenin-glycosides		
		Aristolochia acid		
34	Sinomenii caulis	Magnoflorine		
		Aristolochia acid		
		Sinomenine		
35	Forsythiae fructus	Forsythoside		
		Phillyrin (Forsythin)		
		Pinoresinol		
			Lignans	
36	Evodiae fructus	Evodiamine		
		Rutaecarpine		
		Evocarpine		
37	Anemarrhenae rhizoma	Sarsasapogenin		
		Magniferin		
			Lignans	
38	Acanthopanacis radix	Eleutheroside		
		Syringaresinol		
			Lignans	
39	Scrophulariae radix	Harpagoside		
		Aucubin		
		Loganin		
40	Polygoni multiflori radix	Emodin		
		Physcion		

			Anthraquinones	
			Stilbenes	
41	Alismatis rhizoma	Alisol		
42	Carthami flos	Carthamin		
		Safflor yellow		
43	Epimedii herba	Icariin		
		Epimedin		
44	Cnidii fructus	Osthol		
			Furanocoumarins	
45	Lycii radicis cortex	Scopoletin		
			Lignans	
46	Fructus Lycii	Scopoletin		
		Physalien		
			Carotenoids	
47	Mori radicis cortex	Kuwanone		
48	Mori folium			
49	Cimicifugae rhizoma	Actein		
		Fukinolic acid		
50	Phellodendri cortex	Berberine		
		Palmatine		
		Limonin		
51	Lonicerae flos, caulis	Loganin		
		Hederagenin-glycosides		
		Luteolin-glucoside		
52	Curcumae longae rhizoma/radix	Curcumin		
53	Dioscoreae ssp. rhizoma	Dioscin		
		Diosgenin		
54	Ganoderma	Ganoderic acids		
		Ergosterol		
55	Citri reticulatae pericarpium	Hesperidin		
		Limonin		
		Naringin		
56	Corydalis rhizoma	Palmatine		

		Coptisine		
		Corydaline		
57	Dipsaci radix	Loganin		
		Hederagenin-glycosides		
		Asperosaponin		
58	Atractylodis lanceae rhizoma	Eudesmol		
		Atractylon		
			Phyto-Fungi	
			Polyacetylenes	
			Furanocoumarins	
59	Leonuri herba	Leonurine		
60	Magnoliae flos	Magnolin		
			Lignans	
61	Piperis longi fructus	Piperine		
		Sesamin		
			Lignans	
62	Sophorae flavescentis radix	Matrine		
		Sophoraflavanone		
63	Scutellariae radix	Baicalin		
		Scutellarin		
64	Chaenomelis fructus	Pomolic acid		
		Maslinic acid		
65	Acori rhizoma	β-Asarone		
66	Isatidis radix	Indigo		
		Indigotin		
67	Tribuli fructus	Diosgenin		
		Dioscin		
68	Ophiopogonis radix	Ophiopogonines		
69	Eucommiae cortex	Pinoresinol		
		Syringaresinol		
		Aucubin		
			Lignans	
70	Notoginseng radix et rhizoma	Ginsenoside		
			Polyacetylenes	

			Phyto-Fungi	
71	Rhei radix et rhizoma	Emodin		
		Physcion		
		Resveratrol		
			Stilbenes	
			Anthraquinones	
72	Ginseng/Quinquefolii radix/rhizoma	Ginsenosides		
			Polyacetylenes	
			Phyto-Fungi	
73	Siegesbeckiae herba	Kirenol		
74	Salviae miltiorrhizae radix/rhizoma	Tanshinone		
		Rosmarinic acid		
		Salvianolic acid		
75	Poria	Pachymic acid		
		Tumulosic acid		
76	Cassiae semen	Emodin		
		Chrysophanol		
		Physcion		
			Anthraquinones	
77	Camelliae folium	Catechins		
78	Artemisiae scopariae herba	Scopoletin		
79	Aconiti radix praeparata	Aconitine		
		Mesaconitine		
				processing
80	Cinnamomi cortex	Cinnamaldehyde		
		Procyanidins		

Appendix

Basic Solvent Systems, reagents and columns for the TLC-, GC- and HPLC-fingerprint Analysis of main structure types of natural products

1. Alkaloids
2. Amides
3. Phenolics
    a) Flavones
    b) Procyanidines
    c) Cumarins
    d) Anthraquinones
4. Essential oils
5. Lignans
6. Triterpene-/Steroidsaponins
7. Other Terpenoids (Diterpenoids)
8. Phenolcarboxylic acids

1. Alkaloids
(see Monographs No. 2, 28, 29, 32, 34, 36, 45, 50, 56, 62, 79)

TLC-Analysis	**HPLC-Analysis**
Ammonia solution 10%; MeOH-Extraction	Ammonia solution 10%; MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-select B column (C-8)
Chloroform-Methanol-Water (6 + 3 + 0.65)	A: 2 g Hexanesulfonic acid (pH 3) B: Acetonitrile
Iodine reagent, VIS	210 nm
TLC-Analysis	**HPLC-Analysis**
MeOH-Extraction	MeOH-Extraction
HPTLC Silica gel	RP-select B column (C-8)
Ethyl acetate-Methyl ethyl ketone-Formic acid-Water (5 + 3 + 1 + 1)	A: 0.68 g KH_2PO_4/1 l, pH = 9 (KOH) B: Acetonitrile
UV 366 nm, Dragendorff reagent, VIS	270 nm

2. Amides
(see Monograph No. 61)

TLC-Analysis	HPLC-Analysis
n-hexane-Extraction	n-hexane-Extraction
Silica gel	RP-18 column
n-Hexane-Ethyl acetate (5 + 3)	A: Water B: Acetonitrile
UV 254 nm Anisaldehyde sulphuric acid reagent, VIS	254 nm

3a. Flavones
(see Monographs No. 20, 27, 43, 48, 51, 55, 59, 62, 63, 67)

TLC-Analysis	HPLC-Analysis
MeOH-Extraction	MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Ethyl acetate-Formic acid-Glacial acetic acid-Water (10 + 1.1 + 1.1 + 2.6)	A: 0.001% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm	210/270 nm

3b. Procyanidines
(see Monographs No. 77, 80)

TLC-Analysis	HPLC-Analysis
Acetone/Water (1:1)-Extraction; Diethylether	Acetone/Water (1:1)-Extraction; Diethylether
Silica gel/HPTLC Silica gel	RP-18 column
Ethyl acetate-Methanol-Water (10 + 2 + 1)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	210/270 nm

3c. Cumarines
(see Monographs No. 13, 38, 45, 46, 78)

TLC-Analysis	HPLC-Analysis
50% MeOH/Butanol-Extraction	50% MeOH/Butanol-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Toluene-Ethyl acetate-Acetic acid (90 + 10 + 1)	A: 0.001% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm	210/280 nm

3d. Anthraquinones
(see Monographs No. 40, 71,76)

TLC-Analysis	HPLC-Analysis
MeOH-Extraction	MeOH-Extraction
HPTLC Silica gel	RP-18 column
Light petroleum-Ethyl acetate-Formic acid (75+25+1) for aglycones and glycosides Ethyl acetate-MeOH-Water (100+13.5+10)	A: 0.05% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm Phosphomolybdic acid, VIS	254/280 nm

4. Essential oils
(see Monographs No. 31, 36, 46, 52, 60, 65, 80)

TLC-Analysis	GC-Analysis
Distillation or n-Hexane-Extraction	Distillation (dil. 1:100 oil/*tert*Butylmethylether)
Silica gel	Varian VF-5 ms with 10 m precolumn (deactivated methyl-polysiloxan)
Toluene-Ethyl acetate (93 + 7)	Helium
Vanillin sulphuric acid reagent, VIS	270 °C

5. Lignans
(see Monographs No. 4, 7, 17, 35, 38, 60, 69)

TLC-Analysis	HPLC-Analysis
MeOH-Extraction	MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Toluene-Ethyl acetate-Formic acid (80 + 15 + 10)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	210 nm

TLC-Analysis
50% MeOH/Butanol-Extraction
Silica gel/HPTLC Silica gel
Chloroform-MeOH (9+1)
Vanillin phosphoric/sulphuric acid reagent, VIS

6. Triterpene-/Steroidsaponins
(see Monographs No. 1, 20, 30, 33, 37, 51, 53, 57, 67, 68, 70)

TLC-Analysis	HPLC-Analysis
MeOH/Water/Butanol-Extraction	MeOH-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Chloroform-MeOH-Water, lower layer	A: Water B: Acetonitrile
Vanillin phosphoric/sulphuric acid reagent or Anisaldehyde sulphuric acid reagent, VIS	210 nm

7. Other Terpenoids (Diterpenoids)
(see Monographs No. 25, 41, 49, 54, 59, 64, 73, 74, 75)

TLC-Analysis	HPLC-Analysis
MeOH/Ethyl acetate/Water-Extraction	MeOH/Ethyl acetate/Water-Extraction
Silica gel/HPTLC Silica gel	RP-18 column
Ethyl formate-Toluene-Formic acid (50+50+15)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	205 nm
TLC-Analysis	**HPLC-Analysis**
Diethyl ether-Extraction	Diethyl ether-Extraction
Silica gel	RP-18 column
Petroleum ether-Ethyl acetate-Cyclohexane (5+3+2)	A: 0.001% Phosphoric acid B: Acetonitrile
Anisaldehyde sulphuric acid reagent, VIS	270 nm

8. Phenolcarboxylic acids
(see Monographs No. 49, 51, 57, 59, 74, 78)

TLC-Analysis	**HPLC-Analysis**
MeOH-Extraction	MeOH-Extraction
Silica gel	RP-18 column
Ethyl acetate-Formic acid-Glacial acetic acid-Water (10 + 1.1 + 1.1 + 2.6)	A: 0.001% Phosphoric acid B: Acetonitrile
NP/PEG, UV 366 nm	220 nm
TLC-Analysis	**HPLC-Analysis**
acidified MeOH-Extraction	acidified MeOH-Extraction
Silica gel	RP-18 column
Chloroform-Ethyl acetate-Toluene-Formic acid-MeOH (15 + 20 + 10 + 10 + 1)	A: 0.001% Phosphoric acid B: Acetonitrile
UV 254 nm	281 nm

Index

Drug monograph, Marker compounds, Chemical classification, Processing

	Drug monograph	Marker compounds	Chemical classification	Processing
1	Bupleuri radix	Saikosaponin		
			Polyacetylenes	
			Phyto-Fungi	
2	Fritillariae bulbus	Verticine		
3	Rehmanniae radix	Aucubin		
		Acteoside		
4	Schisandrae fructus	Schisandrin		
5	Asari radix et rhizoma	Asarinin		
		Sesamin		
			Alkamides	
			Lignans	
6	Houttuyniae cordatae herba	Sesamin		
			Lignans	
7	Pinelliae rhizoma	Pinoresinol		
			Lignans	
				processing
8	Astragali radix	Astragaloside		
			Isoflavones	
9	Angelicae pubescentis radix		Furanocoumarins	
			Phyto-Fungi	
			Polyacetylenes	
10	Atractylodis macrocephalae rhizoma		Polyacetylenes	
			Phyto-Fungi	
11	Belamcandae sinensis rhizoma	Iridin		
		Tectoridin		
		Resveratrol		
			Isoflavonoids	
			Stilbenes	

12	Lycopi lucidi herba	Luteolin--glucoside		
		Betulinic acid		
		Rosmarinic acid		
13	Notopterygii rhizoma seu radix		Furanocoumarins	
			Phyto-Fungi	
			Polyacetylenes	
14	Angelicae sinensis radix	Ligustilide		
			Phyto-Fungi	
			Polyacetylenes	
15	Angelicae dahuricae radix	Scopoletin		
			Furonacoumarins	
			Phyto-Fungi	
			Polyacetylenes	
16	Ligustici chuanxiong radix	Ligustilide		
			Phyto-Fungi	
			Polyacetylenes	
17	Zanthoxyli pericarpium	Sesamin		
			Alkamides	
			Lignans	
18	Magnoliae officinalis cortex	Magnolol		
		Honokiol		
19	Drynariae rhizoma	Naringin		
			Anthraquinones	
20	Puerariae radix	Puerarin		
			Isoflavonoids	
21	Codonopsis pilosulae radix	Tangshenoside		
22	Gardeniae fructus	Gardenoside		
		Crocetin		
23	Gastrodiae rhizoma	Gastrodin		
24	Ecliptae herba	Wedelolactone		
			Polyacetylenes	
			Phyto-Fungi	
25	Andrographis herba	Andrographolide		
26	Paeoniae albae/rubrae radix	Paeoniflorin		

27	Sophorae flos		Isoflavone	
28	Coptidis rhizoma	Berberine		
		Coptisine		
29	Stephaniae tetrandrae radix	Tetrandrine		
		Aristolochia acid		
30	Ziziphi spinosae semen	Jujuboside		
31	Amomi rotundus fructus			
32	Uncariae cum Uncis ramulus	Rhychnophylline		
		Pteropodine		
		Catechins		
33	Clematidis radix	Hederagenin-glycosides		
		Aristolochia acid		
34	Sinomenii caulis	Magnoflorine		
		Aristolochia acid		
		Sinomenine		
35	Forsythiae fructus	Forsythoside		
		Phillyrin (Forsythin)		
		Pinoresinol		
			Lignans	
36	Evodiae fructus	Evodiamine		
		Rutaecarpine		
		Evocarpine		
37	Anemarrhenae rhizoma	Sarsasapogenin		
		Magniferin		
			Lignans	
38	Acanthopanacis radix	Eleutheroside		
		Syringaresinol		
			Lignans	
39	Scrophulariae radix	Harpagoside		
		Aucubin		
		Loganin		
40	Polygoni multiflori radix	Emodin		
		Physcion		

			Anthraquinones	
			Stilbenes	
41	Alismatis rhizoma	Alisol		
42	Carthami flos	Carthamin		
		Safflor yellow		
43	Epimedii herba	Icariin		
		Epimedin		
44	Cnidii fructus	Osthol		
			Furanocoumarins	
45	Lycii radicis cortex	Scopoletin		
			Lignans	
46	Fructus Lycii	Scopoletin		
		Physalien		
			Carotenoids	
47	Mori radicis cortex	Kuwanone		
48	Mori folium			
49	Cimicifugae rhizoma	Actein		
		Fukinolic acid		
50	Phellodendri cortex	Berberine		
		Palmatine		
		Limonin		
51	Lonicerae flos, caulis	Loganin		
		Hederagenin-glycosides		
		Luteolin-glucoside		
52	Curcumae longae rhizoma/radix	Curcumin		
53	Dioscoreae ssp. rhizoma	Dioscin		
		Diosgenin		
54	Ganoderma	Ganoderic acids		
		Ergosterol		
55	Citri reticulatae pericarpium	Hesperidin		
		Limonin		
		Naringin		
56	Corydalis rhizoma	Palmatine		

		Coptisine		
		Corydaline		
57	Dipsaci radix	Loganin		
		Hederagenin-glycosides		
		Asperosaponin		
58	Atractylodis lanceae rhizoma	Eudesmol		
		Atractylon		
			Phyto-Fungi	
			Polyacetylenes	
			Furanocoumarins	
59	Leonuri herba	Leonurine		
60	Magnoliae flos	Magnolin		
			Lignans	
61	Piperis longi fructus	Piperine		
		Sesamin		
			Lignans	
62	Sophorae flavescentis radix	Matrine		
		Sophoraflavanone		
63	Scutellariae radix	Baicalin		
		Scutellarin		
64	Chaenomelis fructus	Pomolic acid		
		Maslinic acid		
65	Acori rhizoma	β-Asarone		
66	Isatidis radix	Indigo		
		Indigotin		
67	Tribuli fructus	Diosgenin		
		Dioscin		
68	Ophiopogonis radix	Ophiopogonines		
69	Eucommiae cortex	Pinoresinol		
		Syringaresinol		
		Aucubin		
			Lignans	
70	Notoginseng radix et rhizoma	Ginsenoside		
			Polyacetylenes	

			Phyto-Fungi	
71	Rhei radix et rhizoma	Emodin		
		Physcion		
		Resveratrol		
			Stilbenes	
			Anthraquinones	
72	Ginseng/Quinquefolii radix/rhizoma	Ginsenosides		
			Polyacetylenes	
			Phyto-Fungi	
73	Siegesbeckiae herba	Kirenol		
74	Salviae miltiorrhizae radix/rhizoma	Tanshinone		
		Rosmarinic acid		
		Salvianolic acid		
75	Poria	Pachymic acid		
		Tumulosic acid		
76	Cassiae semen	Emodin		
		Chrysophanol		
		Physcion		
			Anthraquinones	
77	Camelliae folium	Catechins		
78	Artemisiae scopariae herba	Scopoletin		
79	Aconiti radix praeparata	Aconitine		
		Mesaconitine		
				processing
80	Cinnamomi cortex	Cinnamaldehyde		
		Procyanidins		