

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

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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 β -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 family 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.

References

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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 (EI/CI, msⁿ) 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 R_t -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).

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⁾, occasional 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).

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 13 β , 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

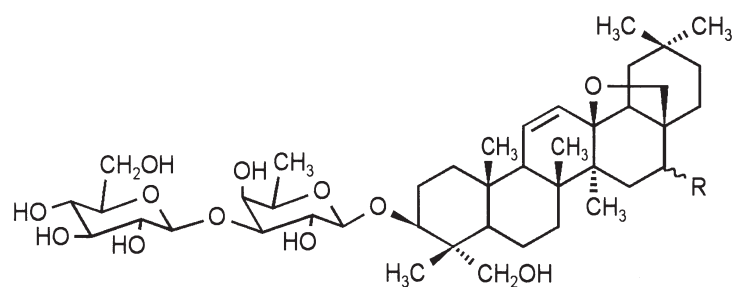
Pharmacology:

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).

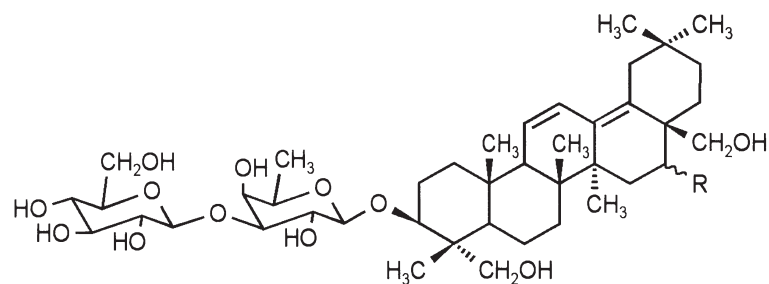
In vivo effects^(3, 4, 15)

- 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



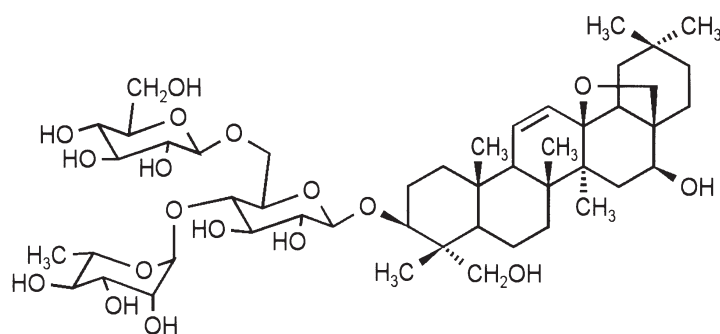
Saikosaponin a: R = β -OH

Saikosaponin d: R = α -OH



Saikosaponin b₁: R = β -OH

Saikosaponin b₂: 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 ultrastrirrer 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 F₂₅₄, 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.

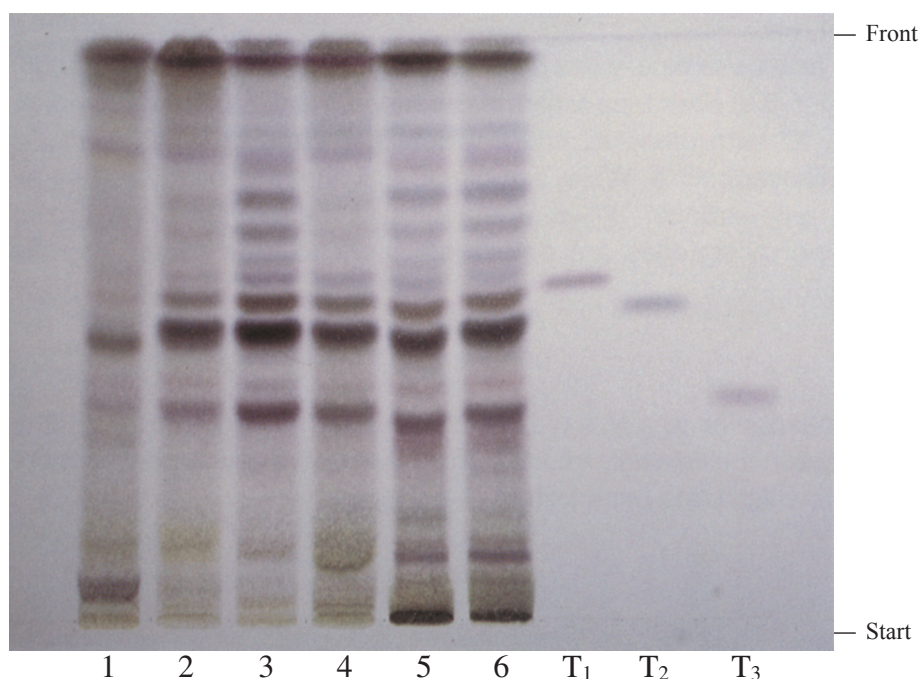


Fig. 2:
Thin layer
chromatogram of
Bupleurum
samples

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. scorzonrifolium* 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 H₂O. 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

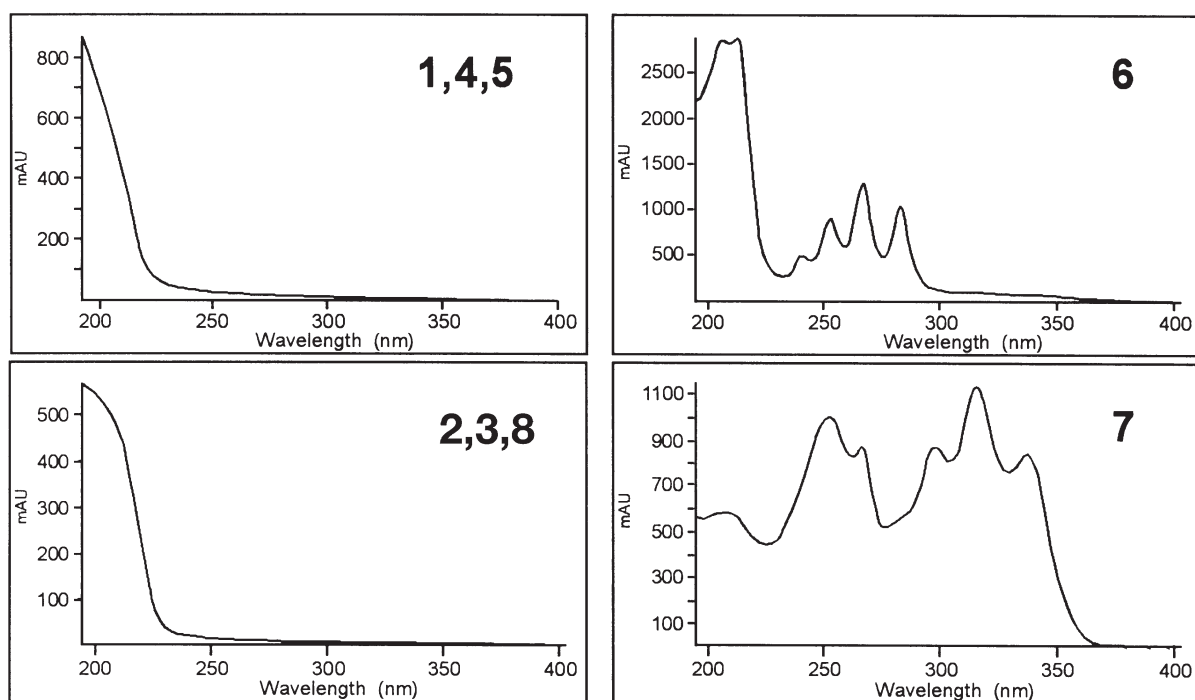


Fig. 3: UV-spectra of the major compounds

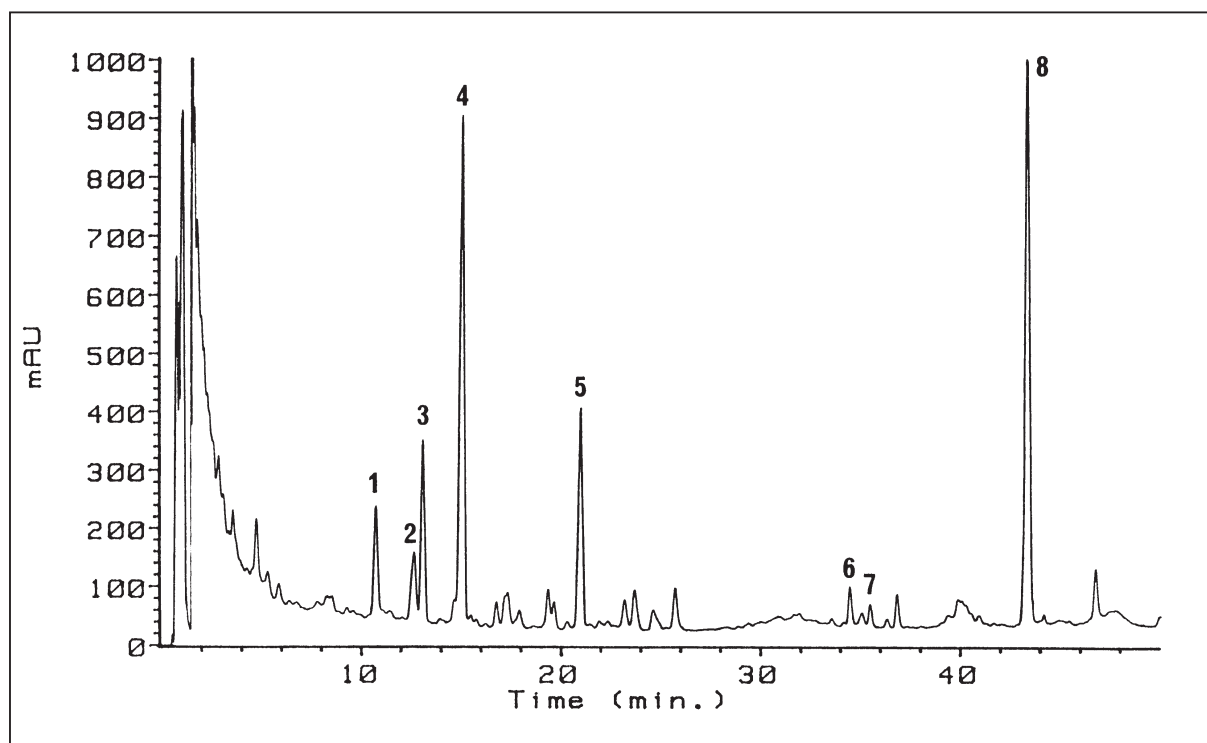


Fig. 4: HPLC fingerprint chromatogram of a drug sample of Chinese origin, Hebei Province (North) (sample 3).

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.

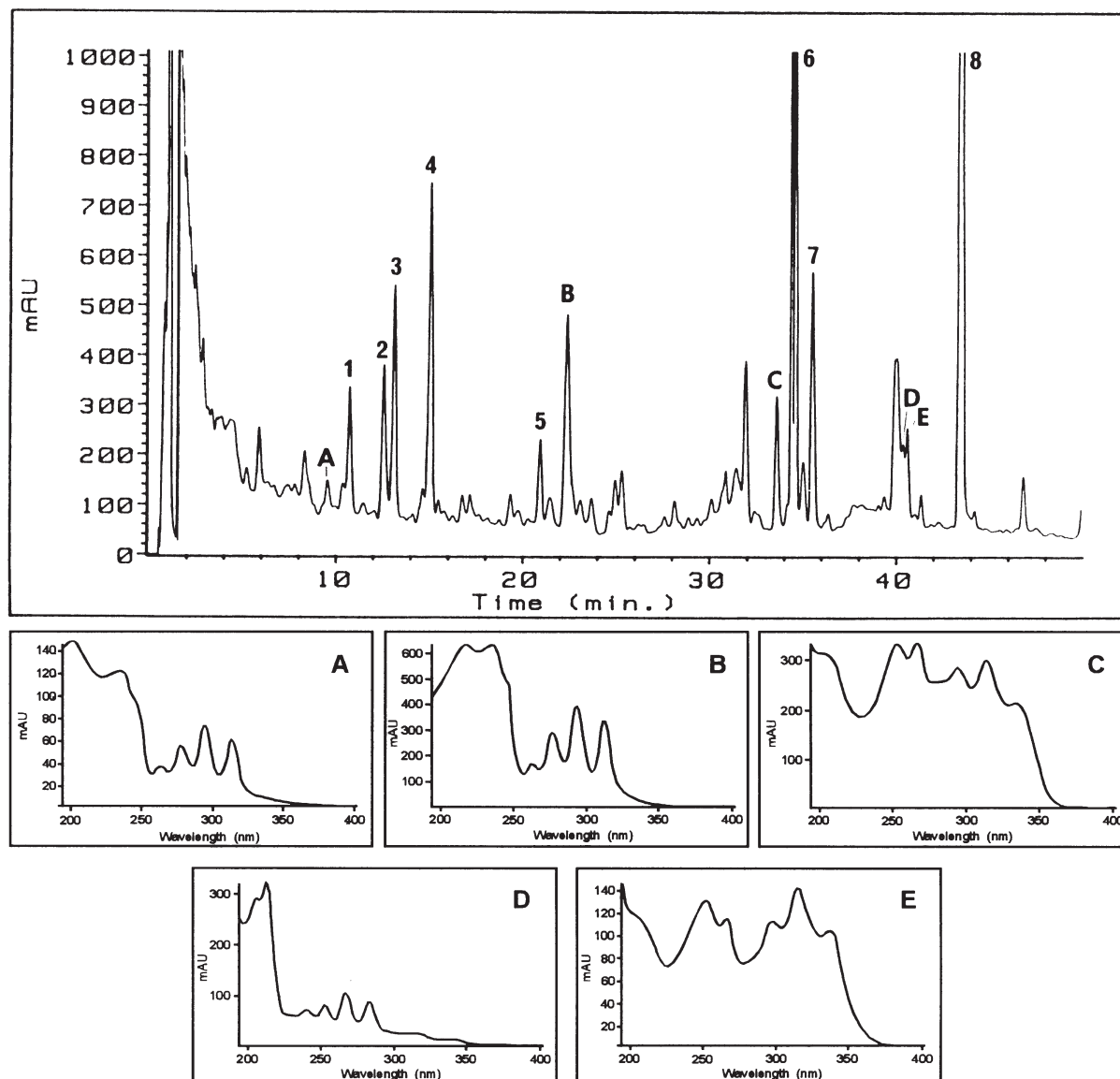


Fig. 5: HPLC fingerprint analysis of a drug sample of Japanese origin (sample 5), with UV-spectra of A–E

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**).

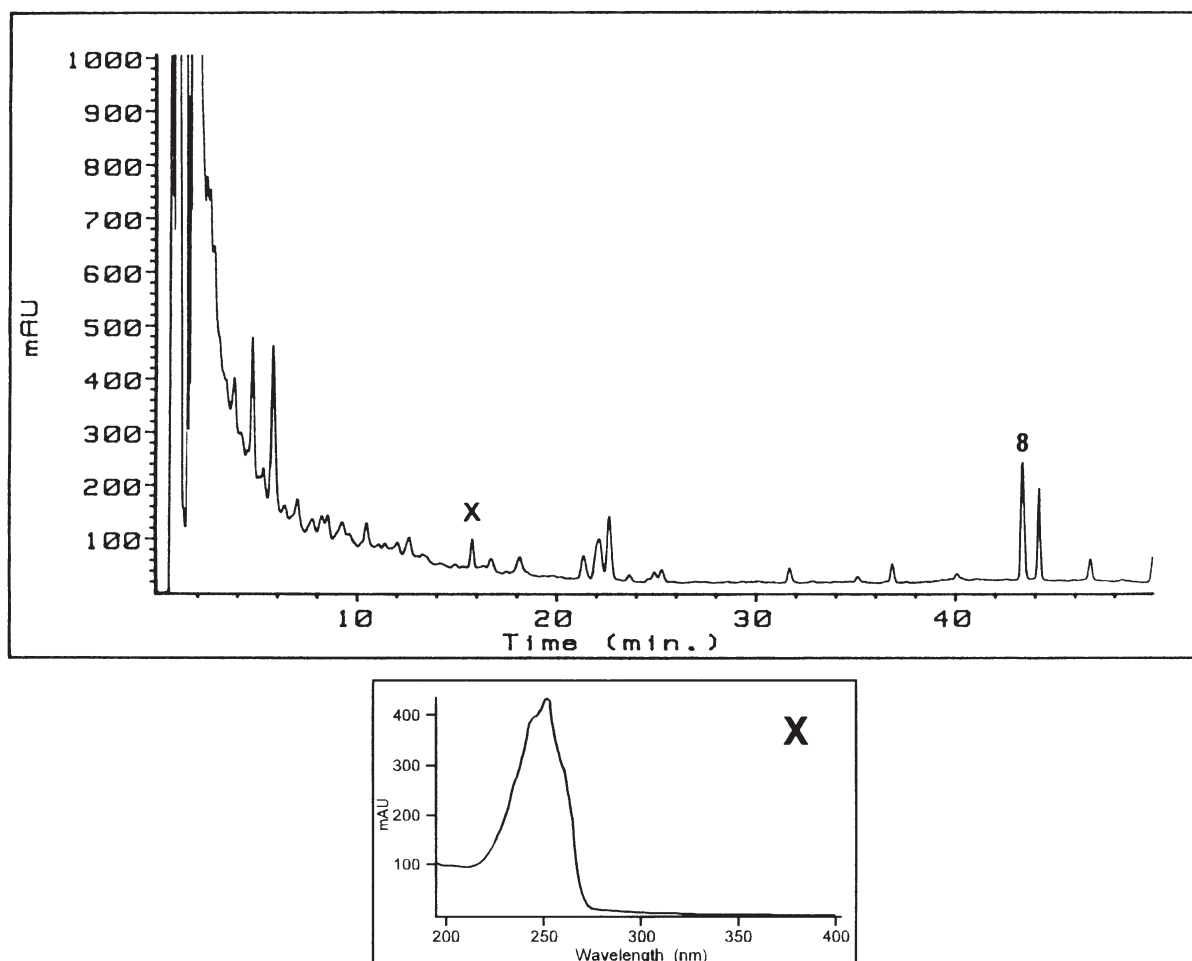


Fig. 6: HPLC fingerprint analysis of a commercial drug imported from China (sample 1), with UV-spectrum of X.

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 saponins 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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Radix *Acanthopanax senticosi*

Ciwujia

Pharmacopoeia:	Radix <i>Acanthopanax senticosi</i> : Pharmacopoeia of the People's Republic of China, English Edition 1997/2005 ⁽¹⁾ Extractum <i>Acanthopanax senticosi</i> : Pharmacopoeia of the People's Republic of China, English Edition 2000/2005 ⁽²⁾
Official drugs^(1,3):	<i>Acanthopanax (Eleutherococcus) senticosus</i> (Rupr. et Maxim.) Harms The drug is commonly known as "Siberian ginseng", – Araliaceae –
Origin⁽³⁻⁵⁾:	<i>Acanthopanax senticosus</i> 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 <i>Acanthopanax senticosus</i> are collected in spring and autumn, washed clean and dried.
Production of the extract⁽²⁾:	To 1000 g of the coarse powder of Radix <i>Acanthopanax senticosi</i> 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 dystonia. 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.

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 <i>qi</i> , 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 E₁ [(-)-syringaresinol-O-β-D-monoglucosid], eleutheroside B₄ [(-)-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 B₁ (isofraxidin-7-O-glucoside)
 - polysaccharide

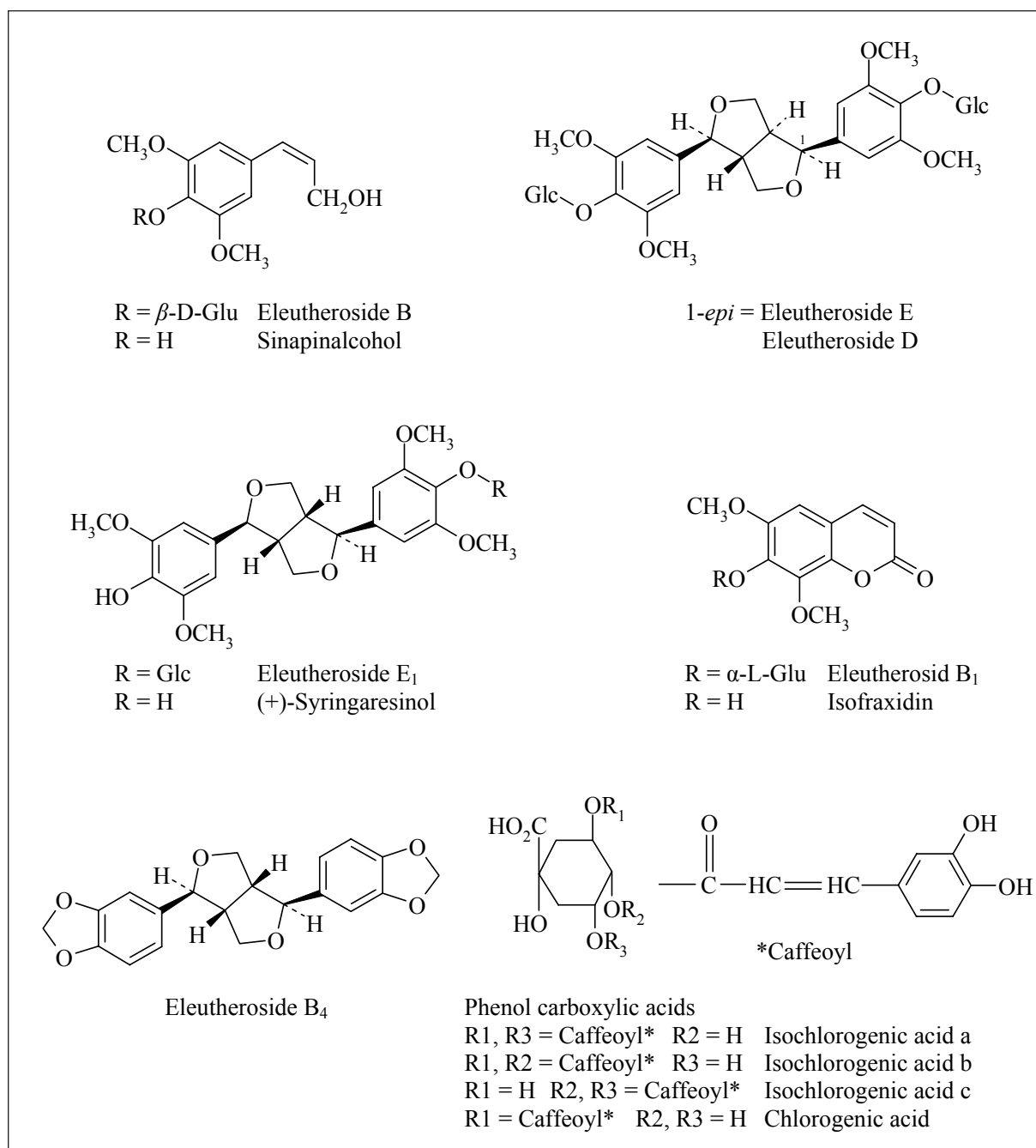


Fig. 1: Formulae of the main constituents of *Acanthopanax senticosi* radix⁽⁵⁻⁷⁾

Contraindication⁽⁵⁾:

Radix Eleutherococci should not be used during pregnancy, lactation or patients with hypertension, *Yin* weakness and depletion 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, E₁, B₄, 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 F₂₅₄; Merck
- Solvent systems: lignans (Fig. 2, 3): chloroform: methanol: water (70:30:4)
coumarin and phenylpropane derivatives (Fig. 4): ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)
- Detection: Spray reagents:
- lignans:
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.
- coumarin and phenylpropane derivatives:
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	<i>Acanthopanax radix</i> / <i>Acanthopanax senticosus</i>	locality Jirin; China
2	<i>Acanthopanax radix</i> / <i>Acanthopanax senticosus</i>	sample of commercial products; China
3	<i>Acanthopanax radix</i> / <i>Acanthopanax senticosus</i>	locality Kirin, Tongfeng; China
4	<i>Acanthopanax radix</i> / <i>Acanthopanax senticosus</i>	locality Kirin, Antun; China
5	<i>Acanthopanax radix</i> / <i>Acanthopanax senticosus</i>	} samples of commercial products; Korea
6	<i>Acanthopanax radix</i> / <i>Acanthopanax senticosus</i>	

Reference compounds		R _f
T1	eleutheroside B	0.63
T2	eleutheroside E	0.54
T3	eleutheroside E ₁	0.82
T4	eleutheroside B ₄	0.98

T5	chlorogenic acid	0.61
T6	caffeic acid	0.93
T7	isofraxidin	0.93
T8	isochlorogenic acids	0.59, 0.80, 0.92

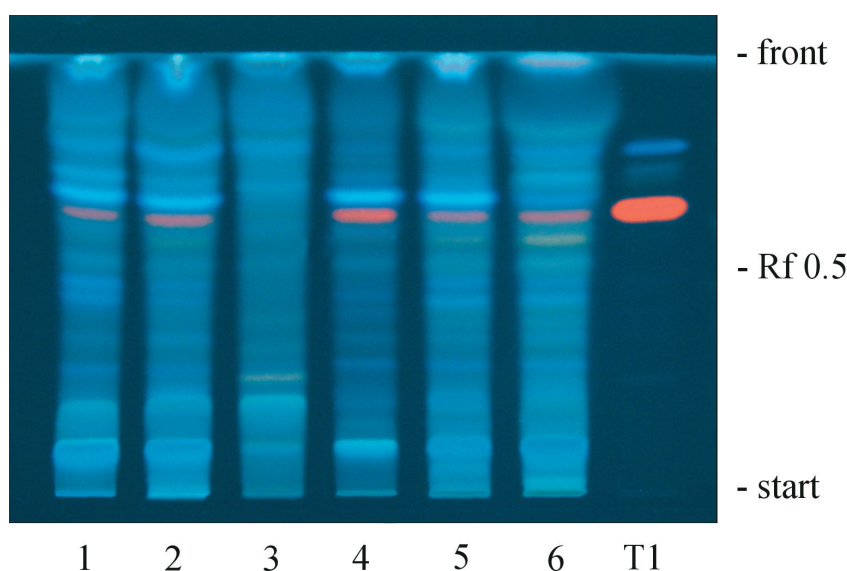


Fig. 2: TLC-fingerprint of lignans of *Acanthopanax senticosi* radix detected with antimony-III-chloride reagent in UV 365 nm

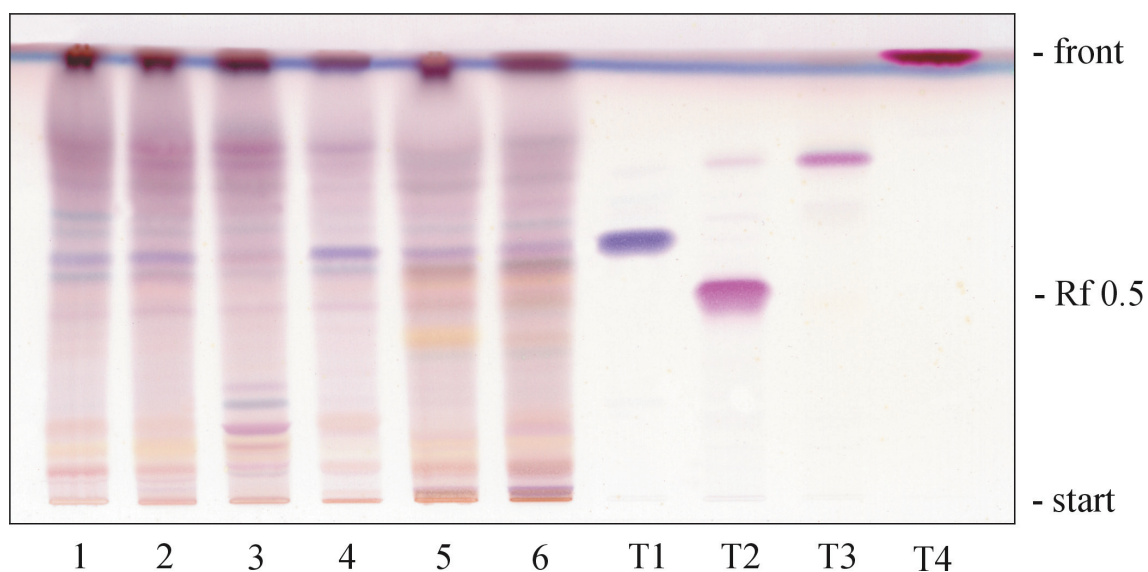


Fig. 3: TLC-fingerprint of lignans of *Acanthopanax senticosi* radix detected with vanillin-phosphoric acid reagent in VIS

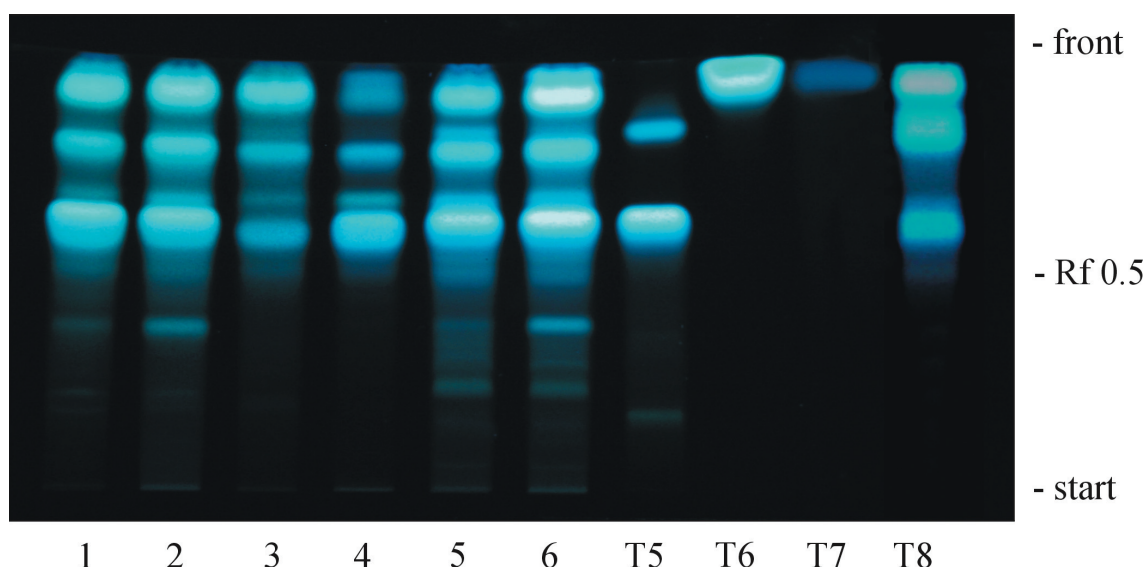


Fig. 4: TLC-fingerprint of coumarin and phenylpropane derivatives of *Acanthopanax senticosi* radix detected with natural products-polyethylene glycol reagent in UV 365 nm

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 Rf 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, E₁, B₄ (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 E₁ (T3) at R_f 0.82 and eleutheroside B₄ (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 ₃ PO 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

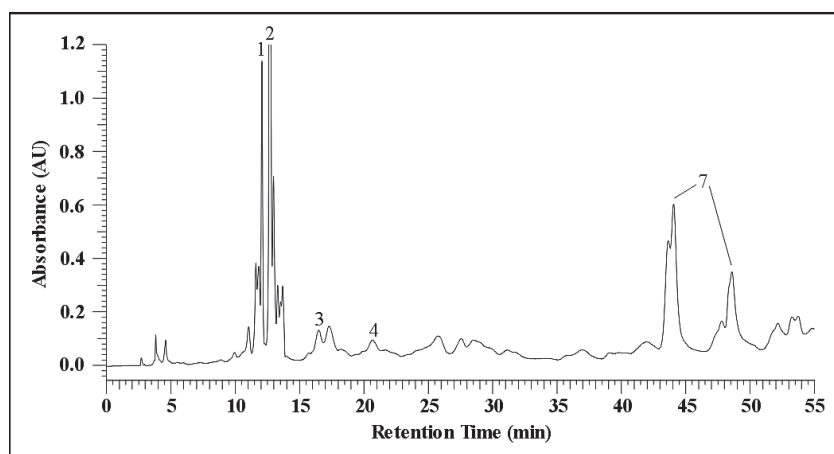


Fig. 5a: HPLC fingerprint of *Acanthopanax senticosi* radix sample 2

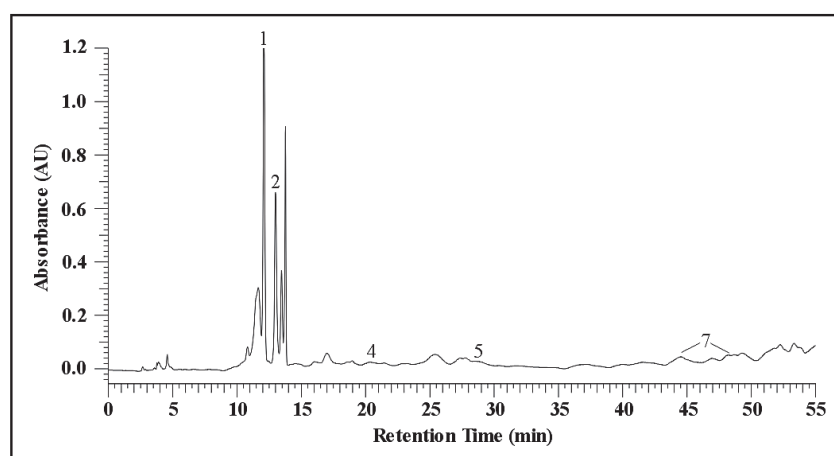


Fig. 5b: HPLC fingerprint of *Acanthopanax senticosi* radix sample 4

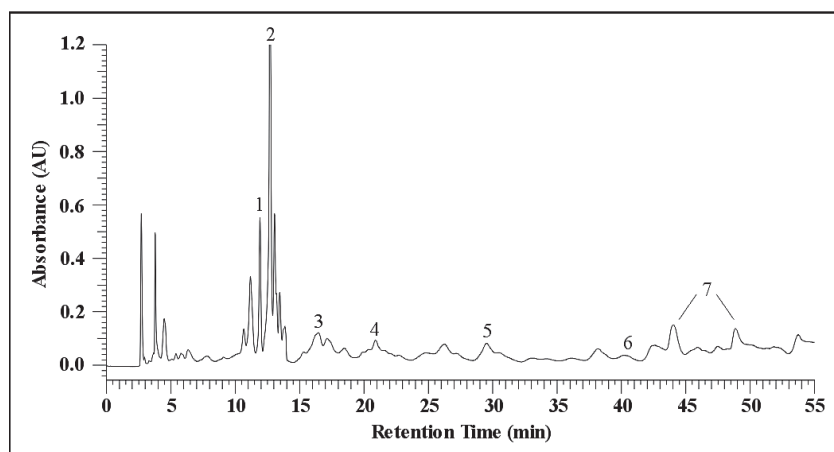


Fig. 5c: HPLC fingerprint of *Acanthopanax senticosi* radix sample 6

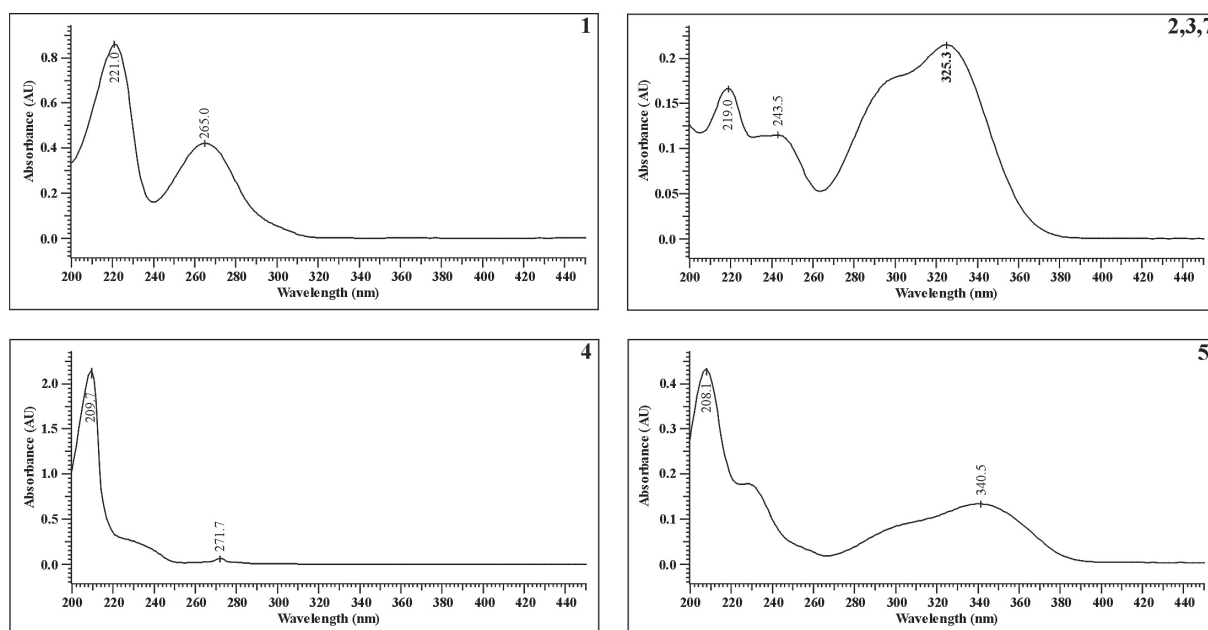


Fig 6: UV-spectra of the main characteristic peaks of the HPLC-fingerprint of *Acanthopanax senticosi* radix:

4) Description of the HPLC-chromatogram, **Fig. 5a**, **Fig. 5b** and **Fig. 5c**:

The HPLC-fingerprint of all *Acanthopanax* 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 E₁ (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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Chromatographic Fingerprint Analysis of Herbal
Medicines

Thin-layer and High Performance Liquid

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