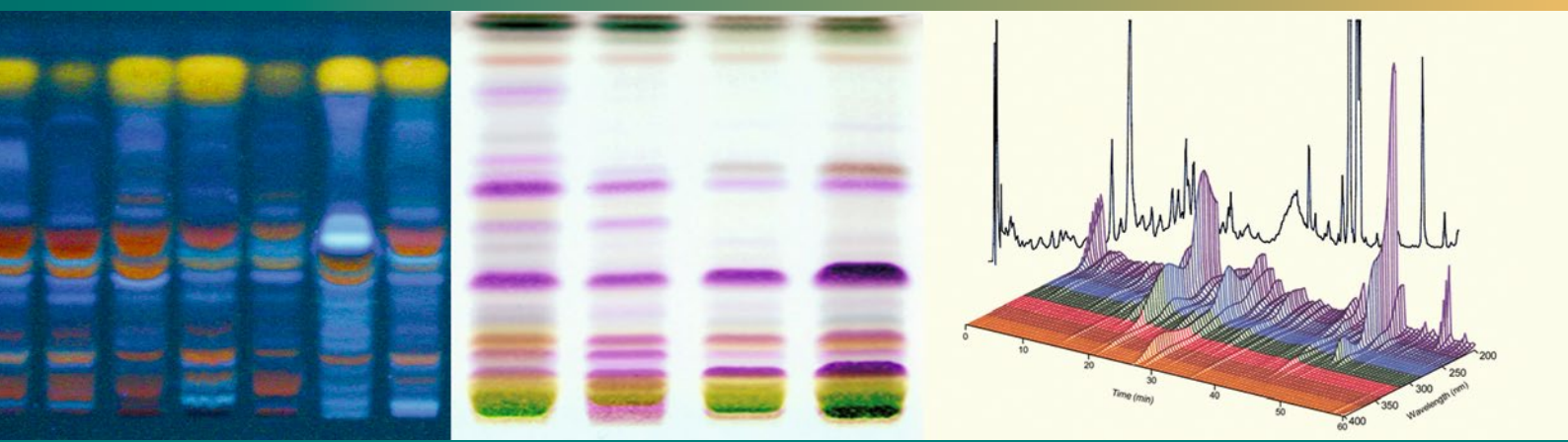
Hildebert Wagner · Rudolf Bauer · Dieter Melchart
Pei-Gen Xiao · Anton Staudinger *Editors*

Chromatographic Fingerprint Analysis of Herbal Medicines

Thin-Layer and High Performance Liquid Chromatography of Chinese Drugs



Volume 3

Springer

TCM-KLINIK BAD KÖTZTING
Erste deutsche Klinik für Traditionelle Chinesische Medizin
Fachklinik für Psychosomatik und Psychotherapie

University Hospital at Beijing University of Chinese Medicine

Chromatographic Fingerprint Analysis of Herbal Medicines

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

Vol. 3

TCM-KLINIK BAD KÖTZTING
Erste deutsche Klinik für Traditionelle Chinesische Medizin
Fachklinik für Psychosomatik und Psychotherapie

Springer

University Hospital at Beijing University of Chinese Medicine

Editors
Hildebert Wagner
Department of Pharmacy
Ludwig-Maximillians-University Center of Pharma Research
Munich
Germany

Rudolf Bauer
Institute of Pharmaceutical Sciences
Department of Pharmacognosy
University of Graz
Graz
Austria

Dieter Melchart
Technische Universität München
Kompetenzzentrum f. Komplementärmedizin
Klinikum rechts der Isar
Munich
Germany

Pei-Gen Xiao
Beijing Institute of Medicinal Plant Development
Beijing
China

Anton Staudinger
TCM-Klinik Bad Kötzting
Bad Kötzting
Germany

ISBN 978-3-319-06046-0 ISBN 978-3-319-06047-7 (eBook)
DOI 10.1007/978-3-319-06047-7
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014945949

© Springer International Publishing Switzerland 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Contents

Vol. I: Monographs No. 1 – 40
Vol. II: Monographs No. 41 – 80

Table of Contents Vol. I

TCM-Analytical Monographs Vol. I

Table of Contents Vol. II

Vol. I: Monographs No. 1 – 40
Vol. II: Monographs No. 41 – 80

TCM-Analytical Monographs Vol. II

Appendix:

Table of Contents Vol. III

Vol. I: Monographs No. 1 – 40
Vol. II: Monographs No. 41 – 80

TCM-Analytical Monographs Vol. III

Contents Alphabetically

Acknowledgements

- The editors wish to express their deep gratitude to the TCM-Clinic Bad Kötzting Mr. A. Staudinger for financial support and Prof. A. Vollmar, LMU Munich, Department of Pharmacy, for the supply of laboratory space and various facilities for the chemical and technical investigations of the TCM-Drugs.
- We are deeply indebted to our technical assistants Bächer Silvia, Barghouti Talee, Botond Carolin and Koch Stefanie.

Introduction

Facts and Perspectives on Chinese Herbal Drugs

When we began our work on the new analytical monographs 20 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[1] 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* (Guangfangji) and administered to women 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.

[1]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

- **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 2010 (People's Republic of China, English Edition Vol I Appendix II A – 25–27) 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 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 longknown 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

Chinese Pharmacopoeia of the People's Republic of China, English Edition 2005, vol. 1. Appendix II A – 24

Heubl, G.: New aspects of DNA-based authentication of Chinese medicinal plants by molecular biological techniques. Planta. Med. **76**, 1963–1974 (2010)

Liang, Y-Z., Xie, P., Chan, R.: Perspective of chemical fingerprinting of Chinese herbs. Planta. Med. **76**, 1997–2003 (2010)

Li, W.C., Zhou, J, Guo, S.Y., Guo, L.D.: Endophytic fungi associated with lichens in Baihua mountain of Beijing, China. Fungal Divers. **25**, 95–106 (2007)

Singhuber, I., Zhu, M., Painz, S., Kopp, B.: *Aconitum* in traditional Chinese medicine: a valuable drug or an unpredictable risk? J. Ethnopharmacol. **126**, 18–30 (2009)

Stierle, A., Strobel, G., Stierle, D.: Taxol and taxane production by *Taxomyces andreanae* an endophytic fungus of Pacific yew. Science. **5105**, 214–216 (1993)

Strobel, G., Daisy, B.: Bioprospecting for microbial Endophytes and their natural products. Microbiol. Mol. Biol. Rev. **5**, 535–544 (2009)

Wagner, H., Bladt, S.: Plant drug analysis, 2nd ed. Springer, Berlin/Heidelberg/New York (2001)

Wagner, H., Bauer, R., Melchart, D., Pei-Gen, X., Staudinger, A. (eds.): Chromatographic fingerprint analysis of herbal medicines. Thin-layer and high performance liquid chromatography of Chinese drugs, vol. I+II, Springer, Wien, New York (2011)

Zhao, Z., Liang, Z., Chan, K., Lu, G., Lee, GLM., Chen, H., Li, L.: A unique issue in the standardization of Chinese materia medica: processing. Planta Med. **76**, 1975–1986 (2010)

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 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, 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 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).

Folium Crataegi – *Shanzhaye*
Fructus Crataegi – *Shanzha*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005/2010

Official drugs:[1]

Folium Crataegi:
Hawthorn Leaf is the dried leaf of *Crataegus pinnatifida* Bge. var. *major* N.E.Br. or *Crataegus pinnatifida* Bge.

The drug is collected in summer and autumn, and dried in the air.

Fructus Crataegi:
Hawthorn fruit is the dried ripe fruit of *Crataegus pinnatifida* Bge. var. *major* N.E.Br. or *Crataegus pinnatifida* Bge.

The drug is collected in autumn when ripe, cut into slices, and dried.

Rosaceae

Synonyms:[2] *Crataegus pentagyna* Waldst. et Kit

Origin:[3] Eastern areas of North America, parts of South America, east Asia and Europe

Description of the drugs:[1]

Folium Crataegi:

Mostly broken, when whole, broadly ovate, 6–12 cm long, 5–8 cm wide. Green to brownish-yellow, apex acuminate, base broadly cuneate, 2–6 pinnate-lobed margin acutely biserrate; petiole 2–6 cm long, stipule ovate to ovate-lanceolate. Odour, slight; taste, astringent, slightly bitter.

Fructus Crataegi:

Rounded slices shrunken an uneven, 1–2.5 cm in diameter, 2–4 mm thick. Externally red, wrinkled, with small greyish-white spots. Pulp dark yellow to pale brown. Transverse slices of the middle part showing 5 pale yellow kerns, mostly fallen off, and loculi hollowed. Some slices exhibiting a slender fruit stalk or remains of calyx. Odour, slightly aromatic; taste, sour and slightly sweet.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_1, © Springer International Publishing Switzerland 2015

Pretreatment of the raw drugs:[1]

Folium Crataegi:

_

Fructus Crataegi:

Foreign matters and fallen kernels are eliminated.

Processing:[1]

Folium Crataegi:

_

Fructus Crataegi:

Stir baked: The clean Fructus Crataegi is stir-baked as described under the method for simple stir-baking (Appendix II D) until darken in colour.

Charred: The clean Fructus Crataegi is stir-baked as described under the method for simple stir-baking (Appendix II D) until it becomes burnt-brown externally and yellowish-brown internally.

Medicinal use:[3] Treatment of chronic congestive heart failure stage II, hypertonia, atherosclerosis, hypercholesteremia

Effects and indications of Folium Crataegi according to Traditional Chinese Medicine[1]

Taste:	Astringent, slightly bitter
Temperature:	Neutral with warm tendency
Channels entered:	*Orbis hepaticus*
Effects (functions):	To activate blood circulation to remove blood stagnation and regulate *qi* flow to activate meridians (2005). To activate blood to resolve stasis (2010).
Symptoms and indications:	Constriction in the chest, palpitation, amnesia, vertigo and tinnitus due to stagnation of *qi* and blood (2005). Chest impediment and heart pain, oppression in the chest and labored breathing, palpitation and forgetfulness, dizziness and tinnitus, hyperlipidemia (2010).

Effects and indications of Fructus Crataegi according to Traditional Chinese Medicine[1, 4–6]

Taste:	Sour, slightly sweet
Temperature:	Neutral with warm tendency
Channels entered:	*Orbis stomachi*, *o. hepaticus*, *o. lienalis*
Effects (functions):	To stimulate digestion and promote the functional activity of the stomach, improve the normal flow of *qi* and dissipate *blood stasis* (2005). To promote digestion and invigorate the stomach, move *qi* and dissipate stasis, resolve turbidity and lower lipid (2010).

Symptoms and indications:	Stagnation of undigested meat with epigastric distension, diarrhea and abdominal pain; amenorrhea due to *blood stasis*, epigastric pain or abdominal colic after childbirth, hernial pain, hyperlipemia (2005). Meet food accumulation and stagnation, distention and fullness in the stomach duct, abdominal pain caused by diarrhea and dysentery, blood-stasis amenorrhea, postpartum stasis and obstruction, stabbing pain in heart and abdomen, chest impediment and heart pain, pain caused by genital disease, and hyperlipidemia. (2010).

Main Constituents [3, 7–9]

Folium Crataegi	**Fructus Crataegi**
Flavonoids	Flavonoids
Rutin, Hyperoside, Orientin, Vitexin, Vitexin-2″-O-rhamnoside	Hyperoside, Isoquercitrin
Polyphenols	Polyphenols
Catechin, Epicatechin	Epicatechin
Proanthocyanidins	Proanthocyanidins
Procyanidin B2, B4, B5, C1	Procyanidin B2, B5, C1
Phenolcarboxylic acids	Phenolcarboxylic acids
Chlorogenic acid, Caffeic acid	Chlorogenic acid, Caffeic acid
Pentacyclic triterpenoic acids	Pentacyclic triterpenoic acids
Ursolic acid, Oleanolic acid, Crataegolic acid	Ursolic acid
	Organic acids
	Tartaric acid, Citric acid, Malic acid, Ascorbic acid (Vitamin C)

Pharmacology

Folium Crataegi	**Fructus Crataegi**
	Anti-arteriosclerotic[4]
Antispasmodic[3]	Positive inotropic effect[4]
Sedative[3, 12–14]	Dilates blood vessels[4]
Antihypertensive[3, 13, 15, 16]	Antihypertensive[4, 15, 16]
Anti-ischemic[13, 17]	Antibiotic[4]
Anti-arrhythmic[12, 13, 17, 18]	Antibacterial[4]
Hypolipidemic[13, 16–18]	Hypolipidemic[13, 16–18]

Folium Crataegi	**Fructus Crataegi**
Chronotropic effects[3]	Anti-ischemia[17]
Antinflammatory[3, 16, 18]	Anti-arrhythmic[17, 18]
Diuretic effects[3]	Hypocholesterolemic effects[17]
Hypocholesterolemic effects[12, 17]	Antioxidant[13, 16–18]
Antioxidant[13, 16–18]	Antiinflammatory[13, 16]
Digestive[16]	Digestive[16]
Somnolent[16]	Somnolent[16]

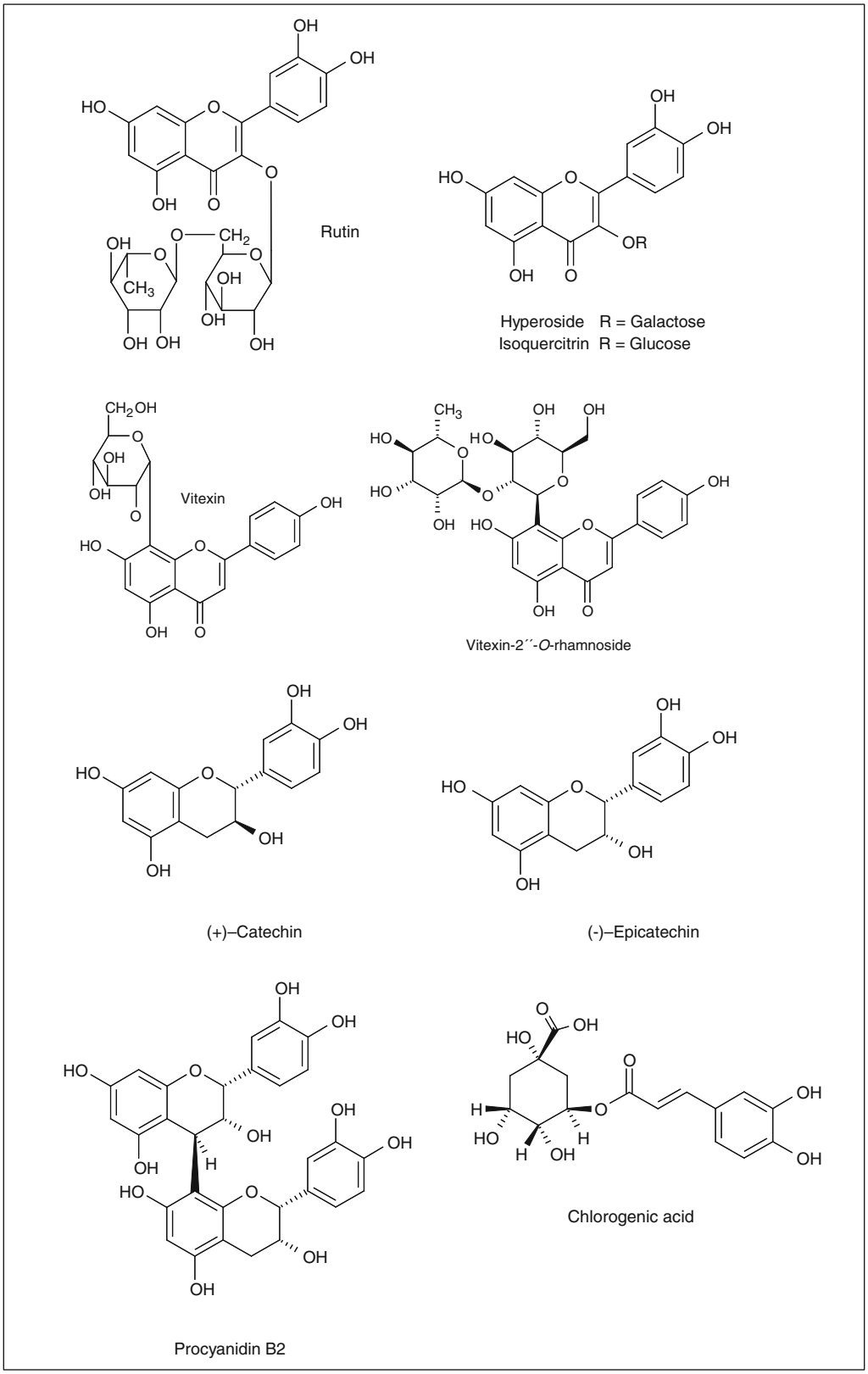


Fig. 1: Formulae of the main constituents of Crataegus sp. [8, 10–12]

TLC-Fingerprint Analysis

	Drug samples	Origin
1	Crataegi fructus/*Crataegus monogyna* or *C. laevigata*	Sample of commercial drug obtained from Caelo, Germany
2	Crataegi fructus/unknown species	Sample of commercial drug obtained from China Medica, Germany (loc.: Hebei, China)
3	Crataegi fructus/*Crataegus pinnatifida*	Sample of commercial drug obtained from HerbaSinica, Germany (loc.: Shandong, China)
4	Crataegi fructus/*Crataegus monogyna* or *C. laevigata*	Sample of commercial drug obtained from Klenk, Germany
5	Crataegi fructus/*Crataegus pinnatifida* var. *major*	Beijing, China (Authentic sample)
6	Crataegi fructus/*Crataegus pinnatifida* var. *major*	Province Hebei, China
7	Crataegi fructus/*Crataegus pinnatifida* var. *major*	Province Hebei, China
8	Crataegi fructus/*Crataegus pinnatifida* var. *major*	Beijing, China
9	Crataegi folium/unknown species	Collected in May, Munich, Germany
10	Crataegi folium/unknown species	Collected in May, Munich, Germany
11	Crataegi folium/*Crataegus pinnatifida* var. *major*	Beijing, China (Authentic sample)
12	Crataegi folium/*Crataegus pinnatifida* var. *major*	Beijing, China (Authentic sample)
13	Crataegi folium/*Crataegus pinnatifida*	Province Hebei, China
14	Crataegi folium/*Crataegus pinnatifida*	Beijing, China
15	Crataegi folium cum flore/unknown species	Sample of commercial drug obtained from E. Reck, Baiersdorf, Germany
16	Crataegi folium cum flore/*Crataegus monogyna* or *C. laevigata*	Sample of commercial drug obtained from Klenk, Germany
17	Crataegi folium cum flore/*Crataegus monogyna* or *C. laevigata*	Sample of commercial drug obtained from Caelo, Germany

	Reference compounds Fig. 2a	R*f*
T1	Hyperoside	0.70
T2	Rutin	0.46
T3	Isoquercitrin	0.73
T4	Orientin	0.70
T5	Caffeic acid	0.94
T6	Chlorogenic acid	0.59
T7	Vitexin	0.78
T8	Vitexin-2″-O-rhamnoside	0.48

1. TLC-fingerprint analysis of flavonoids[10]

(1) Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 5 min. The extract is filtered 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:	Fructus Crataegi extracts: each 10 µl Folium Crataegi extracts: each 10 µl Folium cum Flore Crataegi extracts: each 10 µl Reference compounds: each 10 µl
Solvent system:	Ethyl acetate + formic acid + glacial acetic acid + water (100 + 11 + 11 + 2)
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 366 nm. **Note**: The fluorescence behaviour is dependent on the day of evaluation.

(4) Description:

Figure 2a gives a TLC-chromatographic overview of Crataegi fructus (**1**–**5**), Crataegi folium (**10**–**14**) and Crataegi folium cum flore (**16**). Between the Crataegus extracts samples the reference substances **T1**–**T8** are applied.

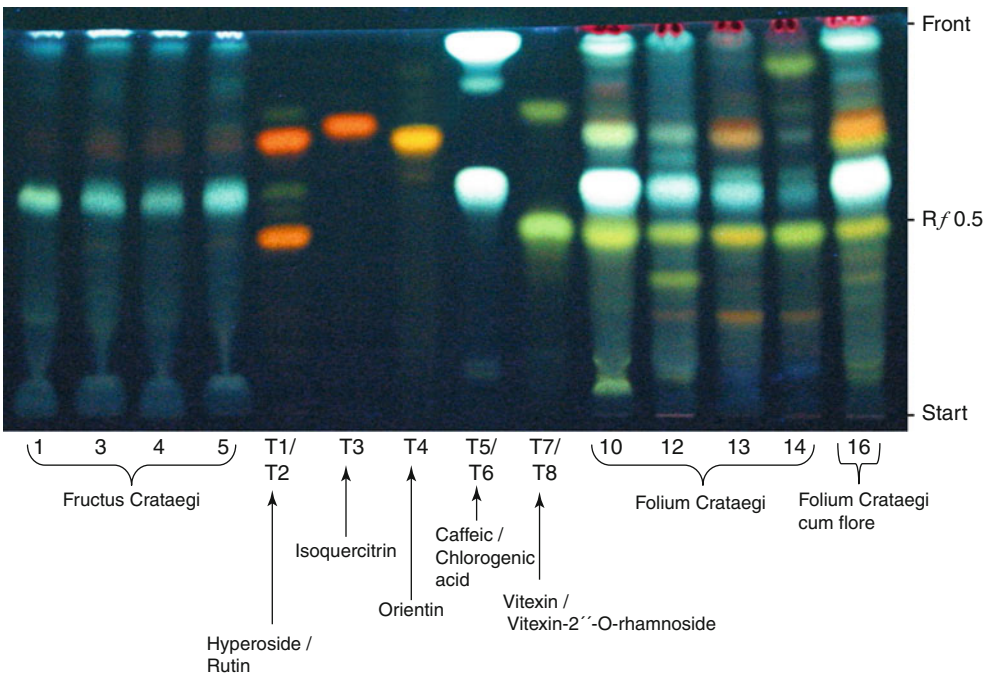


Fig. 2a: Thin layer chromatogram of Crataegi fructus, C. folium and C. folia cum flore, sprayed with natural product reagent (UV 366 nm)

All samples show the white spots of caffeic acid (**T5**) and chlorogenic acid (**T6**). Hyperosid (**T1**) and rutin (**T2**) appear only in traces in Crataegi fructus and stronger concentrated but overlapped by orientin (**T4**) in Crataegi folium sample **13** and in Crataegi folium cum flore sample **16**. The marker flavonoid vitexin-2″-O-rhamnoside (**T8**) appears high concentrated as yellow green zone only in C. folium and C. folium cum flore.

Hyperoside (**T1**), isoquercitrin (**T3**) and orientin (**T4**) appear overlapped in sample **1**3 and **16**. (see also Fig. 2b)

1.1 Comparison of Crataegi folium cum flore and C. folium [10]

(1) Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 5 min. The extract is filtered and used for the TLC.

(2) Reference compounds: No reference compounds are applied.

(3) Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Folium Crataegi extracts: each 10 µl
	Folium cum Flore Crataegi extracts: 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 366 nm.
	Note: The fluorescence behaviour is dependent on the day of evaluation.

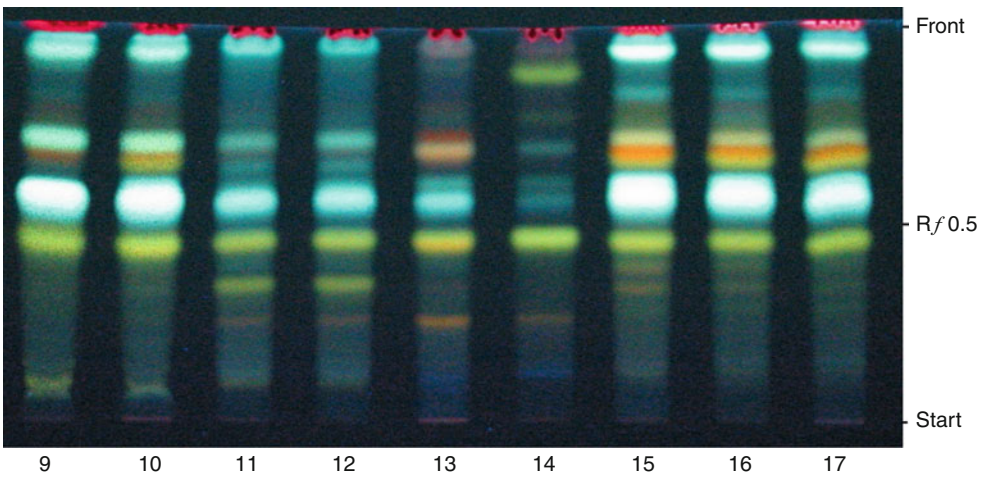


Fig. 2b: Thin layer chromatogram of Crataegi folium and Crataegi folia cum flore, sprayed with NP/PEG (UV 366 nm)

(4) Description of Fig. 2b:

The most homogeneous flavonoid and phenolcarboxylic acid pattern show the Crataegi folium cum flore extract samples **15**–**17**. They derive probably from *Crataegus monogyna* or *Crataegus leavigata* which where purchased from German herbal drug firms.

The same may be true for the Crataegi folium extracts **9**, **10** and **13**, labelled as folium but possibly mixed with small amounts of Flos Crataegi.

2. TLC-fingerprint analysis of Pentacyclic triterpenoic acids [1]

	Reference compounds Fig. 3	R*f*
T1	Ursolic acid	0.32
T2	Oleanolic acid	0.32

(1) Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 5 min. The extract is filtered 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: Fructus Crataegi extracts: each 10 µl
Folium Crataegi extracts: each 10 µl
Folium cum Flore Crataegi extracts: each 10 µl
Reference compounds: each 10 µl

Solvent system: Toluene + ethyl acetate + formic acid (20 + 4 + 0.5)

Detection: 10 % ethanolic Sulphuric acid
The plate is sprayed with 10 ml reagent, heated at 105 °C for 5 min and evaluated in VIS.

(4) Description of Fig. 3:

In all extract samples **1**–**5** and **10**–**16** the yellow-pink zones of ursolic acid (**T1**) and oleanolic acid (**T2**) are detectable as overlapped zones. All Folium Crataegi samples 10–16 differ from the Fructus samples (1–5) by two distinct red Chlorophyll zones at R*f* = 0.42 and 0.53.

3. TLC-fingerprint analysis of Catechins and Proanthocyanidines [19]

	Reference compounds Fig. 4	R*f*
T1	Catechin	0.88
T2	Epicatechin	0.87
T3	Procyanidin B2	0.65

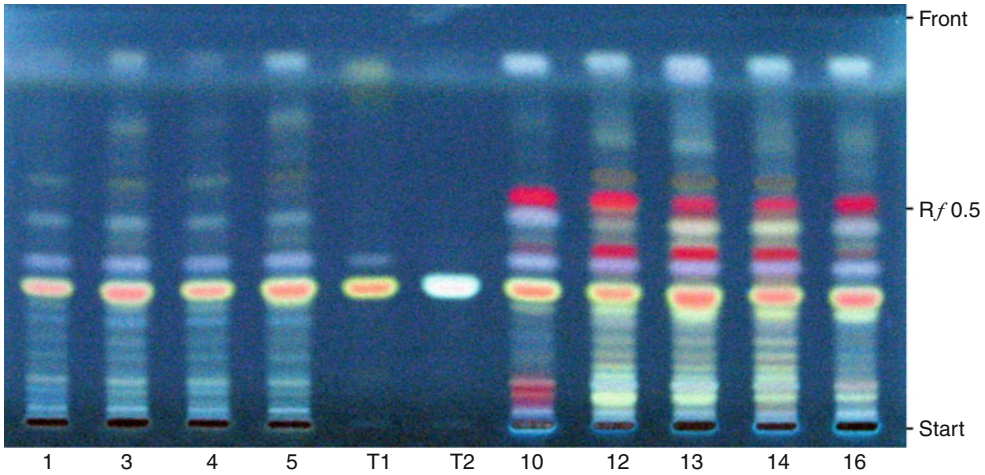


Fig. 3: Thin layer chromatogram of Crataegi fructus, C. folium and C. folium cum flore, sprayed with 10 % ethanolic sulphuric acid (UV 366 nm)

(1) Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 5 min. The extract is filtered 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: Fructus Crataegi extracts: each 10 µl
Folium Crataegi extracts: each 10 µl
Folium cum Flore Crataegi extracts: each 10 µl
Reference compounds: each 10 µl

Solvent system: Ethyl acetate + formic acid + water (upper phase) (100 + 10 + 40)

Detection: Vanillin – Phosphoric acid reagent:

1 g vanillin is dissolved in little ethanol and filled up to 100 ml with 50 % aqueous phosphoric acid. After spraying, the plate is heated for 10 min at 105 °C and evaluated in VIS.

(4) Description of Fig. 4:

Crataegi folium extract samples **10**–**14** and the Crataegi folium cum flore sample **16** show the distinct brown zones of Catechin (**T1**) and epicatechin (**T2**) at R*f* = 0.88/0.87 and procyanidin B2 (**T3**) at R*f* = 0.65. In the R*f*-range from 0.55 down to R*f* = 0.15 appear further 5–6 brown zones of oligomeric procyanidines containing 3-6 catechin/epicatechin molecules with decreasing R*f*-values.

In Crataegi fructus samples **1-5** catechin/epicatechin and the procyanidins are only detectable in very small amounts.

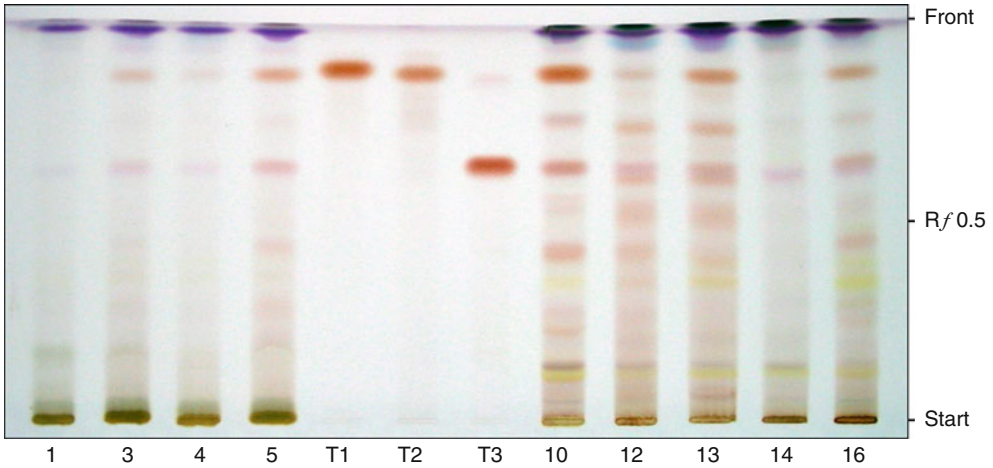


Fig. 4: Thin layer chromatogram of Crataegi fructus, C. folium and C. folia cum flore, sprayed with vanillin – phosphoric acid (VIS)

HPLC-Fingerprint Analysis

(1) Sample preparation: 1 g powdered drug is extracted with 10 ml methanol under reflux for 5 min. The extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol, filtered over Chromafil®, Typ 0.20 µm and injected into the HPLC apparatus.

(2) Injection volume:
Fructus Crataegi extract: each 10 µl
Folium Crataegi extract: each 10 µl
Folium cum Flore Crataegi 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® 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_4/1 l. dist. water (Millipore Ultra Clear UV plus® filtered)
B: acetonitrile (VWR)

Gradient:
0–5 % B in 5 min,
5–30 % B in 35 min,
30 % B for 5 min
Total runtime: 45 min

Flow: 1.0 ml/min

Detection: 210 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	9.77	Chlorogenic acid
2	13.8–15.01	Procyanidins (e.g. Catechin)
3	19.12	Vitexin
4	19.74	Rutin
5	20.71	Proanthocyanidins (e.g. Procyanidin B2)
6	21.33	Hyperosid
7	21.42	Isoquercitrin

(4) Description of Fig. 5a, 5b, and 5c:

- The MeOH-extract of Crataegi fructus (sample 5) shows in the Rt-range of 13.8–15.0 2–3 main peaks inclusive several minor peaks which can be assigned to several procyanidins (catechins). They are characterized through UV-spectra with endabsorption and a small inflexion at 276 nm. Peak **6** might be the flavonol-galactoside hyperoside.
- The extract of Crataegi folium (sample 13) is characterized by chlorogenic acid (**1**), the bulk of procyanidins (**2**), the main flavonol-/and flavon O- and C-glycoside vitexin (**3**), rutin (**4**), hyperosid (**6**) and traces of isoquercitrin (**7**), and procyanidin B2 (**5**).
- The extract of Crataegi folium cum flore (sample 16) shows some slight differences to the folium extract: high concentration of chlorogenic acid (**1**), procyanidins (**2**), vitexin (**3**), rutin (**4**), hyperosid (**6**) and isoquercitrin (**7**).

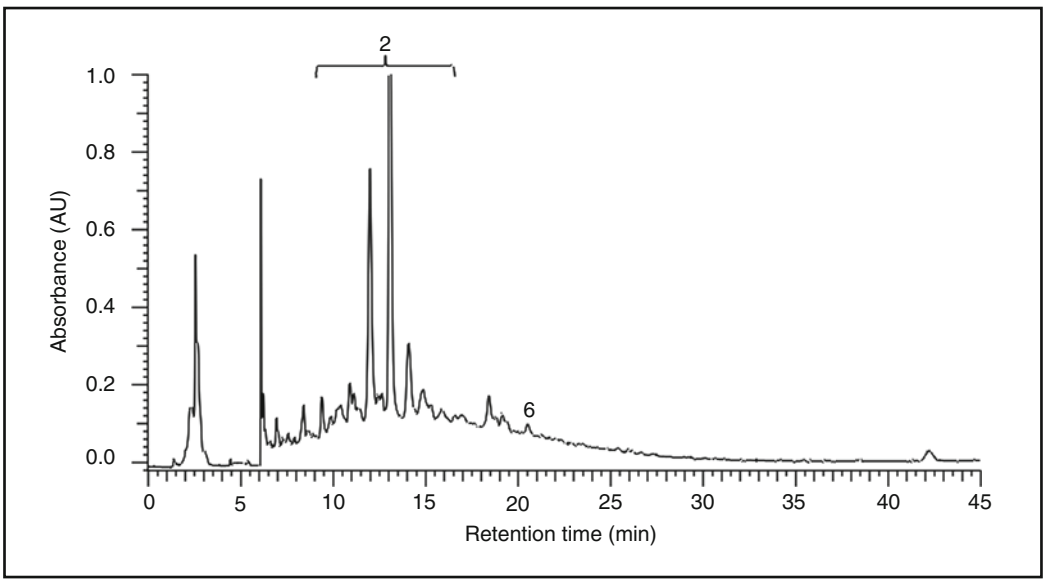


Fig. 5a: HPLC-fingerprint analysis of the methanol extract of Fructus Crataegi, sample 5

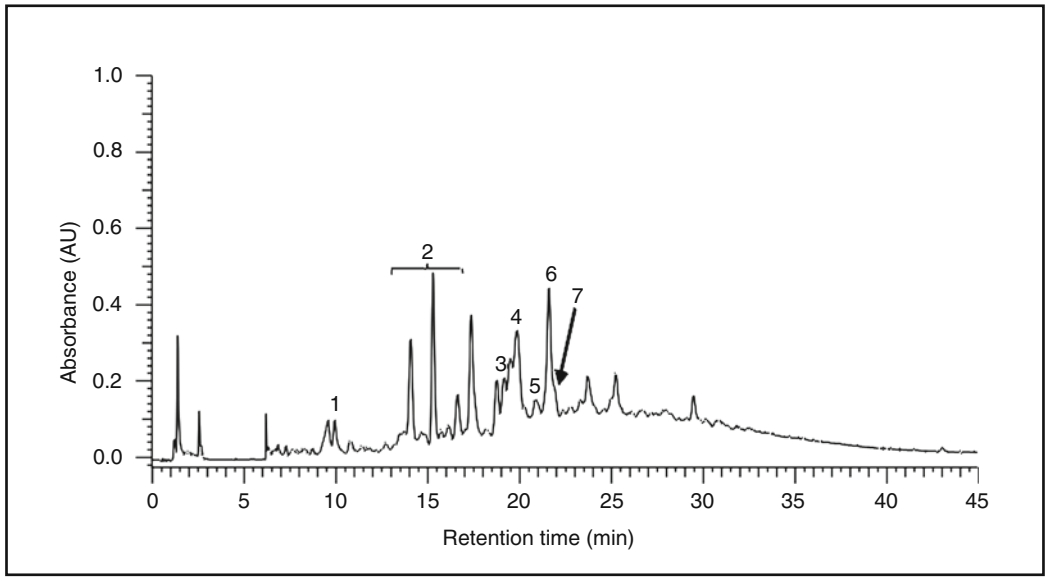


Fig. 5b: HPLC-fingerprint analysis of the methanol extract of Folium Crataegi, sample 13

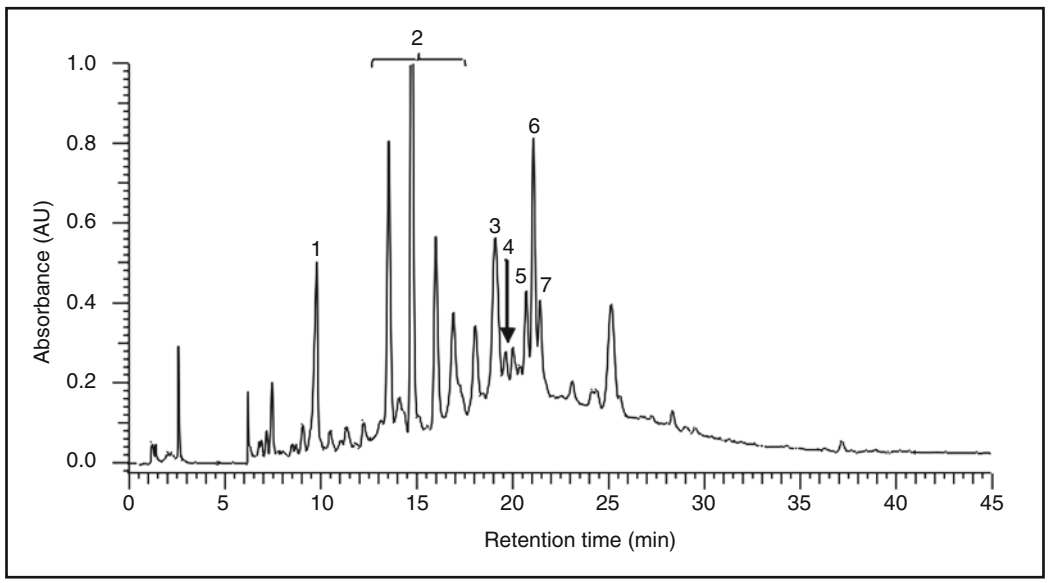


Fig. 5c: HPLC-fingerprint analysis of the methanol extract of Folia cum Flore Crataegi, sample 16

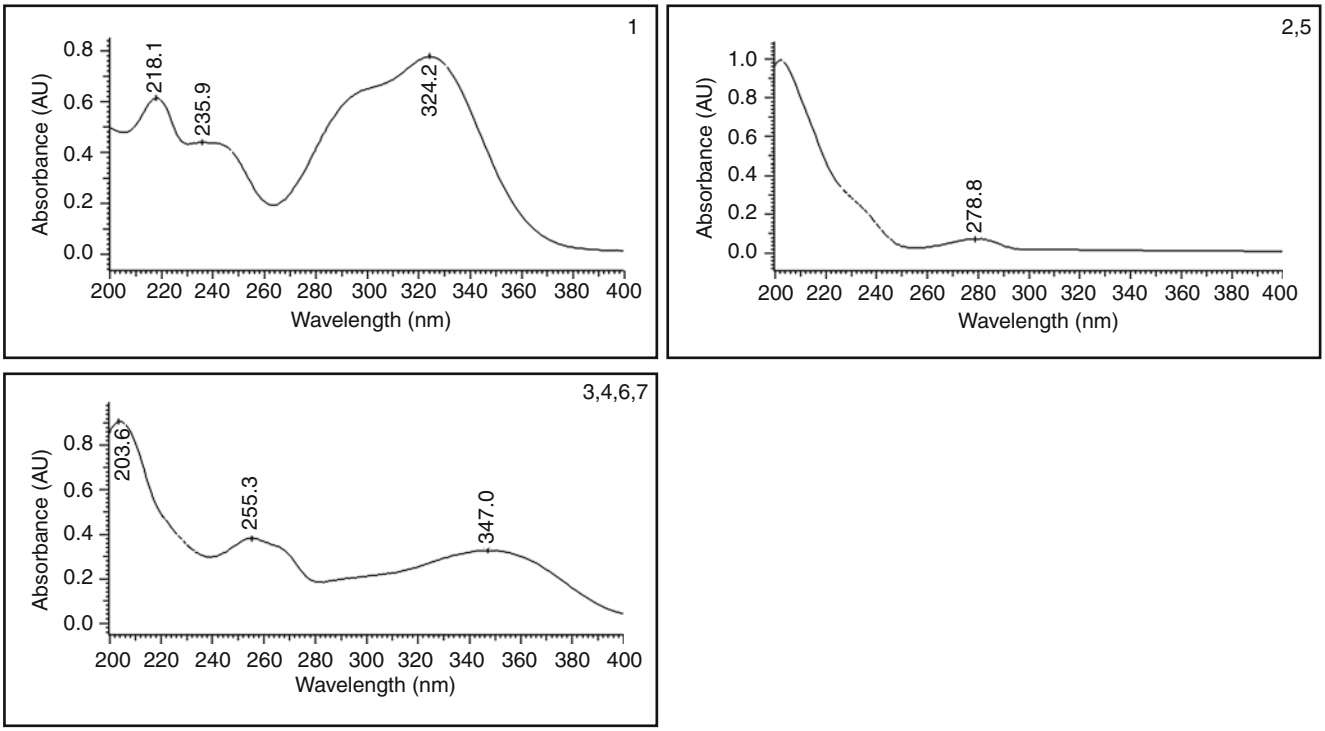


Fig. 6: On line UV-spectra of the main compounds of Crataegus sp

Note: The Chinese Pharmacopeia 2010 demands for Crataegi folium not less than 7.0 % of total flavonoids calc. as anhydrous Rutin with reference to the dried drug and not less than 0.050 % hyperoside calc. with reference to the dried drug. For Crataegi fructus it demands not less than 5.0 % of organic acids calc. as citric acid with reference to the dried drug.

Conclusion

The authentication of *Crataegus pinnatifida* leaves and fruits can be easily achieved by the described TLC- and HPLC method in this monograph.

Deviations in the TLC/HPLC-fingerprints may be caused by changing amounts of added folium and flos drugs or substitutes by other Crataegus species.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. People's Medical Publishing House, Beijing (2005/2010)
2. Keys, J.D.: Chinese herbs – their botany, chemistry, and pharmacodynamics. Charles E. Tuttle Company, Rutland/Tokyo (1976)
3. WHO Monographs on selected medicinal plants, vol. 2: Folium cum Flore Crataegi, World Health Organization, Geneva (2002)
4. Hempen, C.H., Fischer, T.: Leitfaden Chinesische Phytotherapie, 2nd edn. Urban & Fischer, Munich (2007)
5. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
6. Paulus, E., Ding, Y.H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)

7. Wang, S.Y., Chai, J.Y., Zhang, W.-J., Liu, X., Du, Y., Cheng, Z.Z., Ying, X.X., Kang, T.G.: HPLC determination of five polyphenols in rat plasma after intravenous administrating hawthorn leaves extract and its application to pharmacokinetic study. Yakugaku Zasshi **130**(11), 1603–1613 (2010)
8. Cui, T., Li, J.Z., Kayahara, H., Ma, L., Wu, L.X., Nakamura, K.: Quantification of the polyphenols and triterpene acids in Chinese hwathorn fruit by hogh-performance liquid chromatography. J. Agric. Food Chem. **54**(13), 4574–4581 (2006)
9. Rehwald, A., Meier, B., Sticher, O.: Qualitative and quantitative reversed-phase high-performance liquid chromatography of flavonoids in Crataegus leaves and flowers. J. Chromatogr. A **677**(1), 25–33 (1994)
10. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin (1996)
11. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin. Springer, Berlin/Heidelberg (1992)
12. Cheng, S., Qiu, F., Huang, J., He, J.: Simultaneous determination of vitexin-2″-*O*-glucoside, vitexin-2″-*O*-rhamnoside, rutin, and hyperoside in the extract of hawthorn (*Crataegus pinnatifida* Bge.) leaves by RP-HPLC with ultraviolet photodiode array detection. J. Sep. Sci. **30**(5), 717–721 (2007)
13. Cui, T., Nakamura, K., Tian, S., Kayahara, H., Tian, Y.L.: Polyphenolic content and physiological activities of Chinese hawthorn extracts. Biosci. Biotechnol. Biochem. **70**(12), 2948–2956 (2006)
14. Martino, E., Collina, S., Rossi, D., Bazzoni, D., Gaggeri, R., Bracco, F., Azzolina, O.: Influence of the extraction mode on the yield of hyperoside, vitexin and vitexin-2″-*O*-rhamnoside from *Crataegus monogyna* Jacq. (Hawthorn). Phytochem. Anal. **19**(6), 534–540 (2008)
15. Svedström, U., Vuorela, H., Kostiainen, R., Huovinen, K., Laakso, I., Hiltunen, R.: High-performance liquid chromatographic determination of oligomeric procynidins from dimers up to the hexamer in hawthorn. J. Chromatogr. A **968**(1–2), 53–60 (2002)
16. Long, S.R., Carey, R.A., Crofoot, K.M., Proteau, P.J., Filtz, T.M.: Effect of hawthorn (*Crataegus oxycantha*) crude extract and chromatographic fractions on multiple activities in a cultured cardiomyocyte assay. Phytomedicine **13**(9–10), 643–650 (2006)
17. Dalli, E., Colomer, E., Tormos, M.C., Cosín-Sales, J., Milara, J., Esteban, E., Sáez, G.: *Crataegus laevigata* decreases neutrophil elastase and has hypolipidemic effect: a randomized, double-blind, placebo-controlled trial. Phytomedicine **18**(8–9), 769–775 (2011)
18. Fintelmann, V.: Crataegus-Spezialextrakte bei Patienten mit chronischer Herzinsuffizienz. Z. Phytother. **25**, S27–S34 (2004)
19. Kaul, R.: Der Weißdorn. Wiss. Verl.-Ges, Stuttgart (1998)

Rhizoma Cyperi – *Xiangfu*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005/2010

Official drug:[1]

Nut grass Galingale Rhizome is the dried rhizome of *Cyperus rotundus* L. (Fam. Cyperaceae).

The drug is collected in autumn, burnt off the fibrous roots, boiled briefly or steamed thoroughly and dried in the sun, or dried in the sun directly after burning off the fibrous roots.

Origin:[2, 3, 19]

Chinese Provinces Guangdong, Sichuan, Henan, Zhejiang, Anhui, Shandong and Hunan.

Descriptions of the drug:[1]

Frequently fusiform, some slightly curved, 2–3.5 cm long, 0.5–1 cm in diameter. Externally dark brown or blackish-brown, longitudinally wrinkled and with 6–10 slightly prominent annular nodes with brown fibrous roots and broken roots; or slightly smooth and exhibiting indistinct annular nodes on the ones of fibrous roots completely removed. Texture hard, fracture of steamed rhizomes appearing yellowish-brown or reddish-brown, horny: fracture of the unsteamed ones white and starchy, an endodermis ring obvious, stele darkened in colour, with scattered dotted vascular bundles. Odour, aromatic; taste, slightly bitter.

Pretreatment of the raw drug:[1]

Remove fibrous roots and foreign matter, pound to pieces or cut into thin slices.

Processing:[1]

Cyperi Rhizoma (processed with vinegar)

Stir-bake the pieces or slices of Cyperi Rhizoma as described under the method for stir-baking with vinegar (Appendix II D) to dryness.

Medicinal use:[2]

For the treatment of digestive disorders, vomitus, menstrual disorders, internal bleeding, acute hearing loss, otitis media, migraine, and depression.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_2, © Springer International Publishing Switzerland 2015

Effects and indications of Rhizoma Cyperi according to Traditional Chinese Medicine[1, 4, 5]

Taste:	Acrid, sweet, bitter
Temperature: Channels entered:	Neutral, with tendency to cold *Orbis hepaticus*, *orbis lienalis*, *orbis tricolorii*
Effects (functions):	To remove stagnation of *qi*, regulate menstruation and relieve pain (2005). To soothe the liver to resolve depression, regulate *qi* and soothe the middle, regulate menstruation and relieve pain (2010).
Symptoms and indications:	Stagnation of the *liver-qi* characterized by distending pain in the chest, hypochondria and epigastrium, indigestion, feeling of stuffiness in the chest and epigastrium, abdominal colic, distending pain in the breast, menstrual disorders, amenorrhea or dysmenorrhoea (2005). Liver depression and *qi* stagnation, distending pain in the chest and the hypochondrium, pain caused by genital disease, distending pain in the breasts. Spleen-stomach *qi* stagnation, stuffiness and oppression in the epigastrium and abdomen, pain, distention and fullness, menstrual irregularities, amenorrhea and dysmenorrhoea (2010).

Main constituents:

- Sesquiterpeneoids[6, 7, 10, 12, 17, 20]

  Epi-guaidiol A, sugebiol, guaidiol A, sugetriol triacetate, cyperenoic acid, cyperotundone, rotundines A-C

- Norsesquiterpenes[7]

  norcyperone

- Essential oil[9–13, 17, 20]

  α-**cyperone**, *β*-**cyperone**, cyperol, isocyperol, **cyperene**, cyprotene, cyperotundone, cypera 2,4-diene, caryophyllene, rotundine, α-copaene, α-selinene, epi-α-selinene, *β*-selinene, rotundene, valercene, ylanga-2,4-diene, γ-gurjune, trans calamenene, δ-cadinene, γ-calacorene, α-muurolene, γ muurolene, cadalene, nootkatene, mustakone, α-copaene, isolongifolen-5-one + γ-gurjunenepoxide, (*E*)-pinocarveol, myrtenal, dihydrocarvone, verbenone, (*E*)-carveol, valencene

- Flavonoids[8, 12–14, 17]

  Vitexin, isovitexin, orientin, epiorientin

- Cardiac glycosides[12, 13, 17]

- Alkaloids[15]

- Saponins[15]

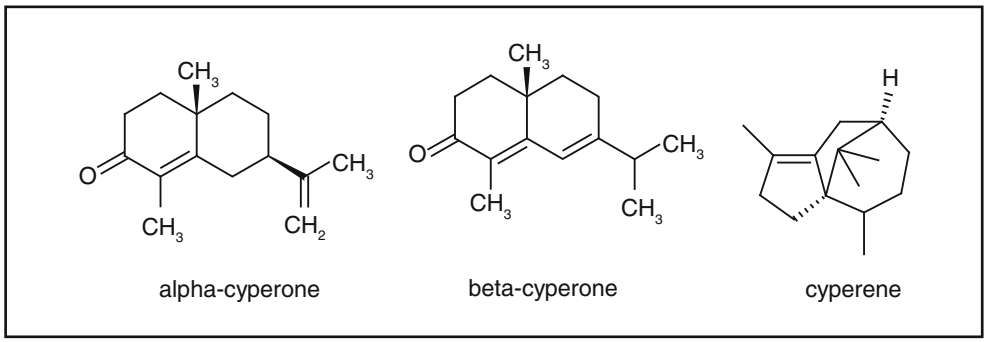


Fig. 1: Formulae of the main compounds of Rhizoma Cyperi [10]

Reported Pharmacological Activities

Anti-inflammatory[6, 7, 12, 13, 15, 17, 20]

Anti-estrogenic activity[3, 7, 14]

Antimicrobial[14, 16]

Anthelmintic[7, 14]

Anti-histaminic[14]

Anti-emetic[7, 14]

Antipyretic[7, 12–15, 17, 20]

Antidiabetic[6, 7, 14, 20]

Anti-diarrhoeal activity[3, 7, 20]

Antimalarial[7, 15, 16, 20]

Antispasmodic [17]

Hepatoprotective [7]

Acetylcholinesterase inhibitory activity [6]

Protein glycation inhibitory activity [6]

Antidepressant [20]

Inhibition of nitric oxide and superoxide production[6, 20]

Hypotensive[7, 12, 13, 17]

Aphrodisiac[7]

Diuretic[7]

Sedative[7, 17]

Carminative[7]

Anticolic[7]

Stimulant[7]

Stomachic[7]

Removes renal calculi[7]

Emmenagogue activity [16]

TLC-Fingerprint Analysis

	Drug samples	Origin
1	Rhizoma Cyperi/*Cyperus rotundus*	Sample of commercial drug obtained from HerbaSinica (origin: Zhejiang)
2	Rhizoma Cyperi/*Cyperus rotundus*	Sample of commercial drug obtained from Herbasin (origin: unknown)
3	Rhizoma Cyperi/*Cyperus rotundus*	Sample of commercial drug obtained from TCM-Clinic Bad Kötzting (origin: unknown)
4	Rhizoma Cyperi/*Cyperus rotundus*	Province Shandong (China)
5	Rhizoma Cyperi/*Cyperus rotundus*	Province Hebei (China)
6	Rhizoma Cyperi/*Cyperus rotundus*	Province Anhui (China)

Reference compound Fig. 2a and 2b		**R*f***
T	α-Cyperone	0.41

Reference compound Fig. 2c and 2d		**R*f***
T	α-Cyperone	0.34

1. Extraction: 2 g powdered drug are extracted with 20 ml methanol for 1 h under reflux, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.
2. Reference compound: 1 mg is dissolved in 1 ml ethyl acetate
3. Separation parameters:
   - Plate: HPTLC Silica gel 60 F_{254}, Merck
   - Applied amounts: Rhizoma Cyperi extracts: each 10 µl
     Reference compound: 10 µl
   - Solvent system: Toluene + ethyl acetate + glacial acetic acid (92 + 5 + 5)

Detection:

1. Without chemical treatment (**Fig. 2a**)

254 nm

2. Dinitrophenylhydrazine reagent (**Fig. 2b**)

1.5 g 2,4-dinitrophenylhydrazin are dissolved in 20 ml sulphuric acid (25 %), filled up with water to 100 ml and filtered.

After spraying with 10 ml, the plate is evaluated after 10 min in VIS.

3. Anisaldehyde – Sulphuric acid reagent (**Fig. 2c** and **2d**)

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, heated at 100 °C for 5 min, then evaluated in VIS and under 366 nm.

Note: The reagent has only limited stability and is no longer useable when the colour has turned to red-violet.

4. Description:

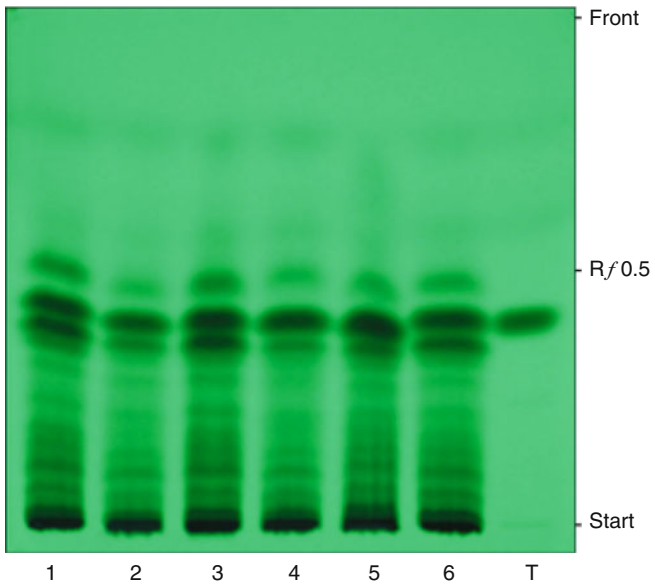


Fig. 2a: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi without chemical treatment (UV 254 nm)

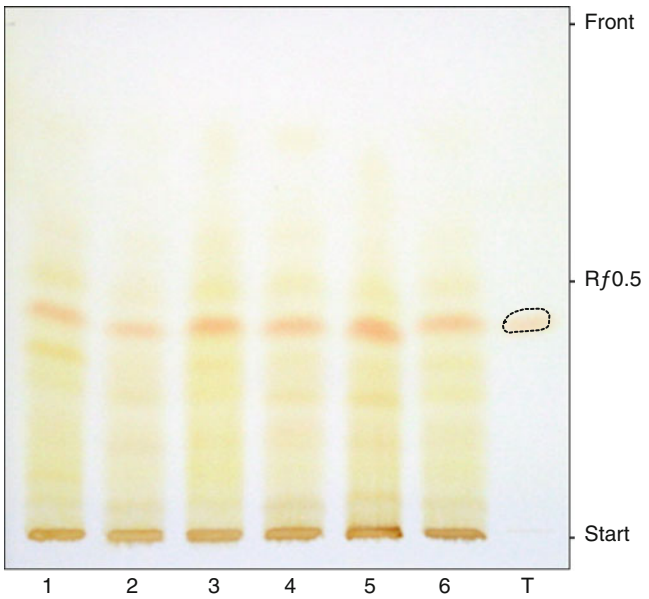


Fig. 2b: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with 2,4-dinitrophenylhydrazine (VIS)

Figure 2a shows the six samples of Rhizoma Cyperi under UV 254 nm without chemical treatment. In all samples several black zones are detectable in the R*f* – range from the start up to 0.5. The main zone at R*f* = 0.41 (**T**) could be identified as α-cyperone. The second zone at R*f* = 0.39 might be *β*-cyperone.

After spraying with 2,4-dinitrophenylhydrazin (**Fig. 2b**) the zones appeared in yellow/orange colours. In all samples the orange spot of α-cyperone at R*f* = 0.41 is clearly detectable.

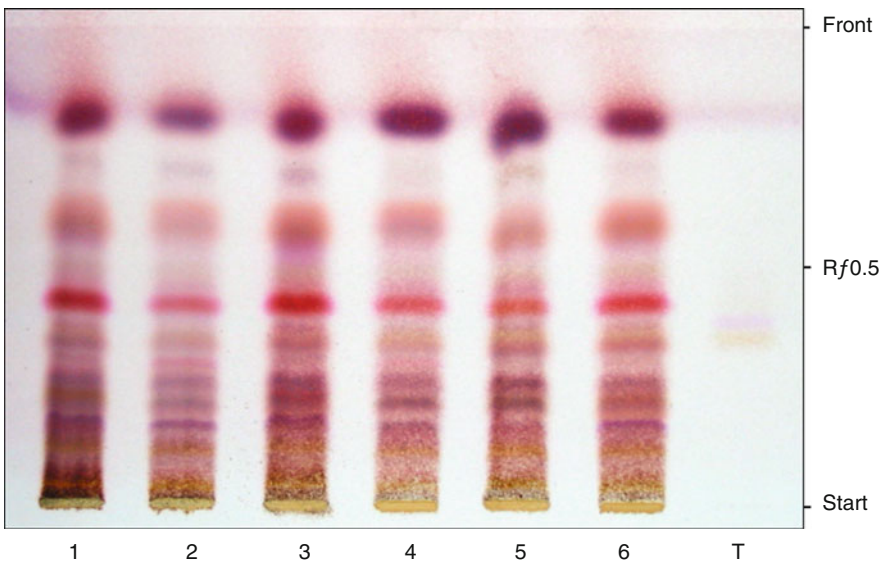


Fig. 2c: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with Anisaldehyde – Sulphuric acid (VIS)

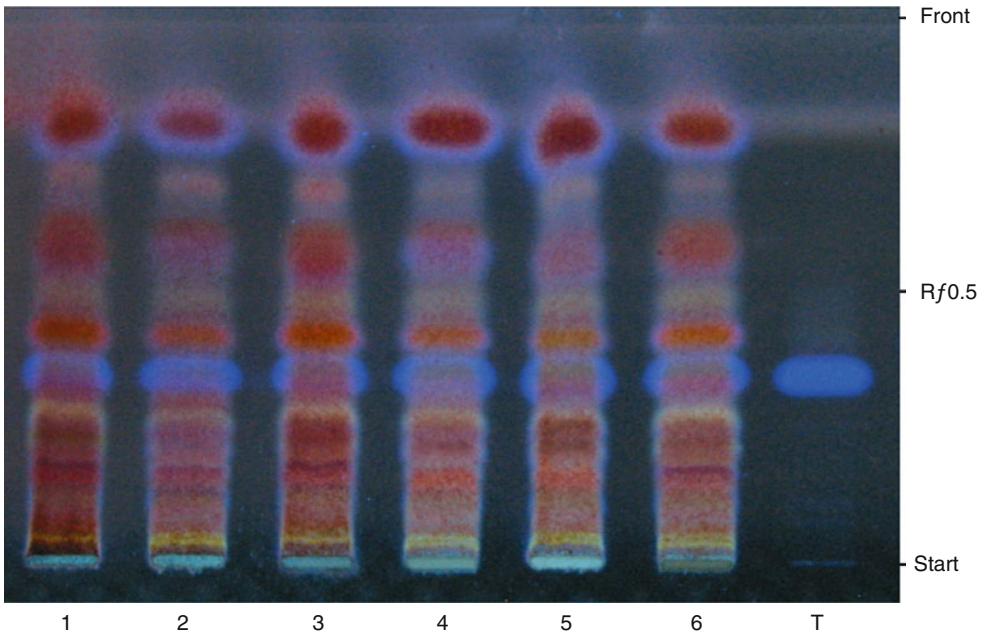


Fig. 2d: Thin layer chromatogram of the methanol extracts of Rhizoma Cyperi sprayed with Anisaldehyde – Sulphuric acid (UV 366 nm)

Fig. 2c and **d**: With the solvent system generally used for essential oils several pink and violet zones from the start up to R*f* = 0.85 are detectable. In VIS (**Fig. 2c**) α-cyperone is not exactly distinguishable, but under UV 366 nm (**Fig. 2d**) the compound can be detected by a light blue coloured spot at R*f* = 0.34.

HPLC-Fingerprint Analysis [18]

1. Sample preparation: 2 g powdered drug are extracted with 20 ml methanol for 1 h under reflux, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, Type 0.45 µm.
2. Injection volume: Rhizoma Cyperi 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 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18, Merck
Solvent:	A: water (Millipore Ultra Clear UV plus® filtered) B: methanol (VWR)
Gradient:	10–100 % B in 45 min, total runtime: 45 min
Flow:	1 ml/min
Detection:	254 nm

Retention times of the main peaks recorded at 254 nm

Peak	Rt (min)	Compound
1	40.8	*β*-Cyperone ?
2	43.2	α-Cyperone

4. Description of the HPLC-Figures

   In the Rt – range 27.0–39.0 there a several minor peaks in both samples. The two main peaks at Rt 40.8 and 43.2 can be assigned to *β*- and α-cyperone, respectively.

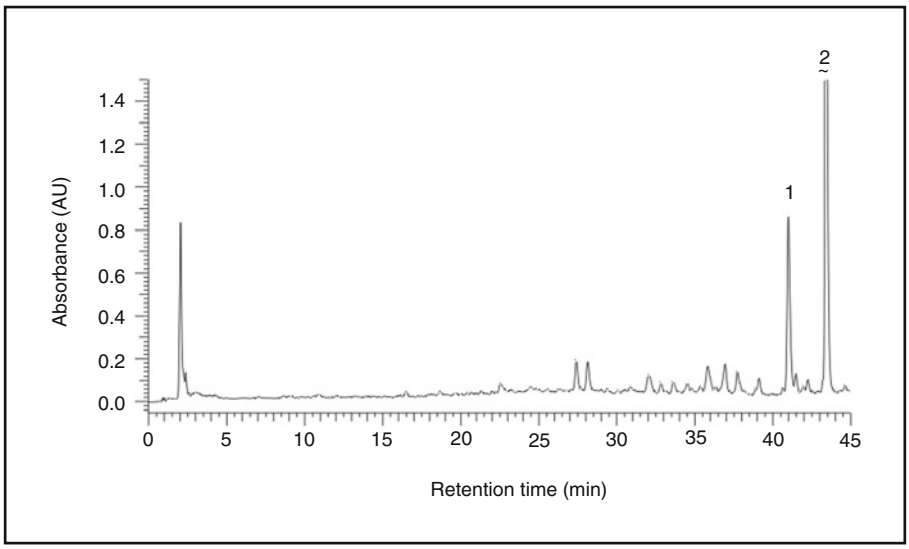


Fig. 3a: HPLC-fingerprint analysis of the methanol extract of Rhizoma Cyperi, sample 2

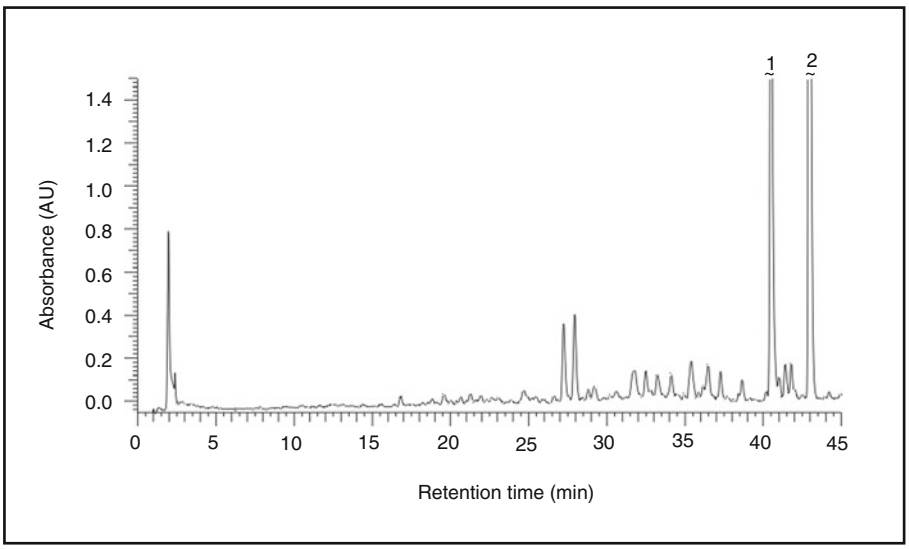


Fig. 3b: HPLC-fingerprint analysis of the methanol extract of Rhizoma Cyperi, sample 6

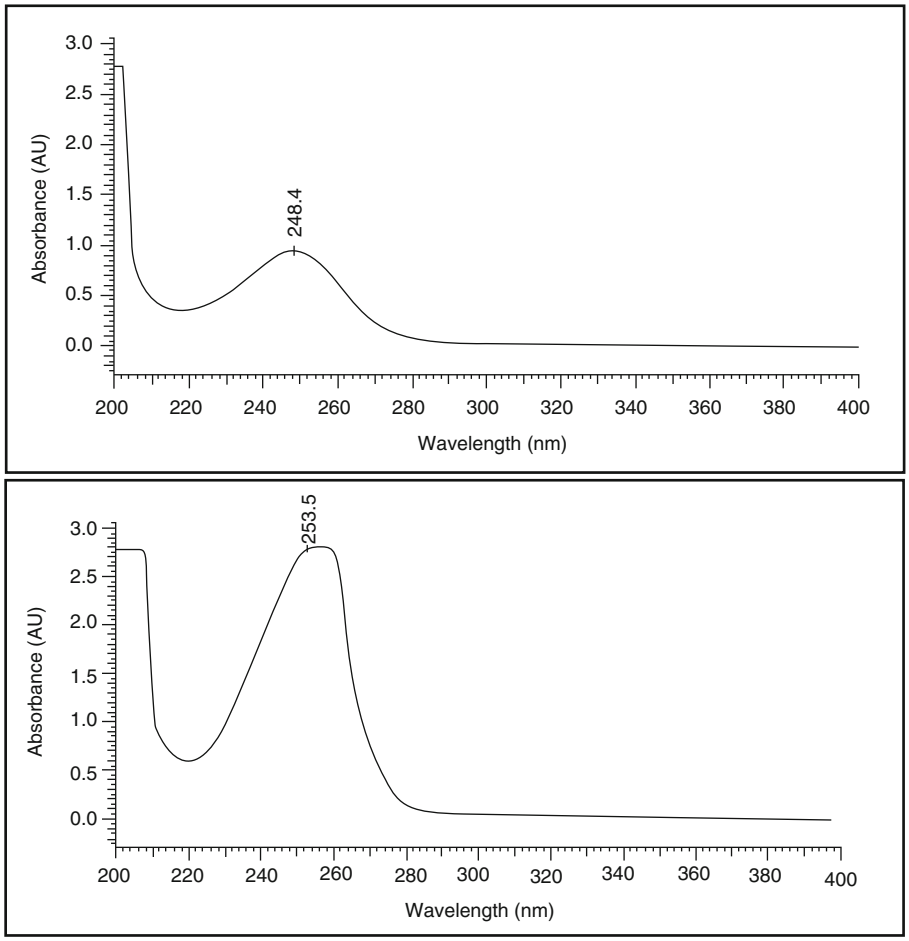


Fig. 4: On line UV-spectra of main peaks of Rhizoma Cyperi

Note: Rhizoma Cyperi should contain not less than 1.0 % of volatile oil, according to the Chinese Pharmacopoeia [1].

Conclusion

The identity of Rhizoma Cyperi can be easily determined by TLC- and HPLC-analysis using MeOH-extract or essential oil by means of the characteristic α-β-cyperone dublett in HPLC.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. People's Medical Publishing House, Beijing (2005/2010)
2. Paulus, E., Ding, Y.H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)
3. Keys, J.D.: Chinese herbs – their botany, chemistry, and pharmacodynamics. Charles E. Tuttle Company, Ruttland/Tokyo (1976)
4. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
5. Hempen, CH., Fischer, T.: A materia medica for Chinese medicine (plants, minerals and animal products), 2 edn. Churchill Livingstone, Elsevier, New York (2009)

6. Xu, Y., Zhang, H.W., Wan, X.C., Zou, Z.M.: Complete assignments of ^{1}H and ^{13}C NMR data for two new sesquiterpenes from *Cyperus rotundus* L. Magn. Reson. Chem. **47**(6), 527–531 (2009)
7. Xu, Y., Zhang, H.W., Yu, C.Y., Lu, Y., Chang, Y., Zou, Z.M.: Norcyperone, a novel skeleton norsesquiterpene from *Cyperus rotundus* L. Molecules **13**(10), 2474–2481 (2008)
8. Saved, H.M., Mohamed, M.H., Farag, S.F., Mohamed, G.A., Omobuwajo, O.R., Proksch, P.: Fructose-amino acid conjugate and other constituents from *Cyperus rotundus* L. Nat. Prod. Res. **22**(17), 1487–1497 (2008)
9. Sonwa, M.M., König, W.A.: Chemical study of the essential oil of *Cyperus rotundus*. Phytochemistry **58**(5), 799–810 (2001)
10. Tam, C.U., Yang, F.Q., Zhang, Q.W., Guan, J., Li, S.P.: Optimization and comparison of three methods for extraction of volatile compounds from *Cyperus rotundus* evaluated by gas chromatography-mass spectrometry. J. Pharm. Biomed. Anal. **44**(2), 444–449 (2007)
11. Kilani, S., Ledauphin, J., Bouhlel, I., Sghaier, M.B., Boubaker, J., Skandrani, I., Mosrati, R., Ghedira, K., Barillier, D., Chekir-Ghedira, L.: Comparative study of *Cyperus rotundus* essential oil by a modified GC/MS analysis method. Evaluation of its antioxidant, cytotoxic, and apoptotic effects. Chem. Biodivers **5**(5), 729–742 (2008)
12. Nima, Z.A.M., Jabier, M.S., Wagi, R.I., Hussain, H.A.A.K.: Extraction, identification and antibacterial activity of cyperus oil from Iraqi C. rotundus. Eng. Technol. **26**(10), 1156–1163 (2008)
13. Zhu, M., Luk, H.H., Fung, H.S., Luk, C.T.: Cytoprotective effects of *Cyperus rotundus* against ethanol induced gastric ulceration in rats. Phytother. Res. **11**(5), 392–394 (1997)
14. Shivakumar, S.I., Suresh, H.M., Hallikeri, C.S., Hatapakki, B.C., Handiganur, J.S., Sankh, K., Shivakumar, B.: Anticonvulsant effect of *Cyperus rotundus* Linn rhizomes in rats. J. Nat. Rem. **9**(2), 192–196 (2009)
15. Uddin, S.J., Mondal, K., Shilpi, J.A., Rahman, M.T.: Antidiarrhoeal activity of *Cyperus rotundus*. Fitoterapia **77**(2), 134–136 (2006)
16. Shi, X., Wang, X., Wang, D., Geng, Y., Liu, J.: Separation and purification of α-cyperone from *Cyperus rotundus* with supercritical fluid extraction and high-speed counter-current chromatography. Sep. Sci. Technol. **44**(3), 712–721 (2009)
17. Kilani, S., Abdelwahed, A., Ammar, R.B., Hayder, N., Ghedira, K., Chraief, I., Hammami, M., Chekir-Ghedira, L.: Chemical composition, antibacterial and antimutagenic activities of essential oil from (Tunisian) *Cyperus rotundus*. J. Essent. Oil. Res. **17**(6), 695–700 (2005)
18. Venkatasubramanian, P., Kumar, S.K., Nair, V.S.N.: *Cyperus rotundus*, a substitute for *Aconitum heterophyllum*: studies on the ayurvedic concept of Abhava Pratinidhi Dravya (drug substitution). J. Ayurveda. Intergr. Med. **1**(1), 33–39 (2010)
19. Zhao, Z.Z.: An illustrated Chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
20. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)

Herba Lycopodii – *Shenjincao*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Common Clubmoss Herb is the dried herb of *Lycopodium japonicum* Thunb. (Fam. Lycopodiaceae).

The drug is collected in summer and autumn when foliage branch growing luxuriantly, removed from foreign matter, and dried in the sun.

Origin:[2] Provinces Guangdong, Guangxi, Yunnan and Guizhou, China.

Description of the drug:[1] Stolons slender cylindrical, slightly curved, up to 2 m long, 1–3 mm in diameter, with yellow-white rootlets underneath. Erect stems bifurcated. Leaves densely growing on the stems, spirally arranged, crumpled and curved, linear or needle-shaped, 3–5 mm long, yellowish-green to pale yellowish-brown, glabrous, aristate at the apex, margin entire, easily broken. Texture soft, fracture pale yellow in bark and whitish in wood. Odour, slight; taste, weak.

Pretreatment of the raw drug:[1] Foreign matters are eliminated, washed clean, cut into sections, and dried.

Medicinal use:[11] For treatments of arthritic pain, quadriplegia, dysmenorrhea and contusion.

Effects and indications of Herba Lycopodii according to Traditional Chinese Medicine[1]

Taste:	Weak
Temperature:	Warm
Channels entered:	*Orbis renalis*, *orbis lienalis, orbis hepaticus*
Effects (functions):	To relieve rheumatic condition and muscular contracture
Symptoms and indications:	Arthralgia with immobilized joints.

Main constituents:[2, 3, 7, 8, 10] **Diterpenoids** (8α,9α-Epoxy-7-oxoroyleanon)

Triterpenoids [lycoclavanol; (3β,8β,14α,21α)-26,27-dinoronocerane-3,8,14,21-tetrol, (3β,8β,14α,21β)-26,27-dinoronocerane-3,8,14,21-tetrol, α-onocerin, lycopodiin A, serratenes (Japonicumins A-D)]

Minor constituents:[2, 3, 9] Flavones (lycopodone; tricin; tricetin 3′,4′,5′-OMe; 5,7,4′-trihydroxy-3′-methoxy flavone)

Alkaloids (miyoshianine A + C, α-obscurine, lycodoline, lucidioline)

Anthraquinones, organic acids

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_3, © Springer International Publishing Switzerland 2015

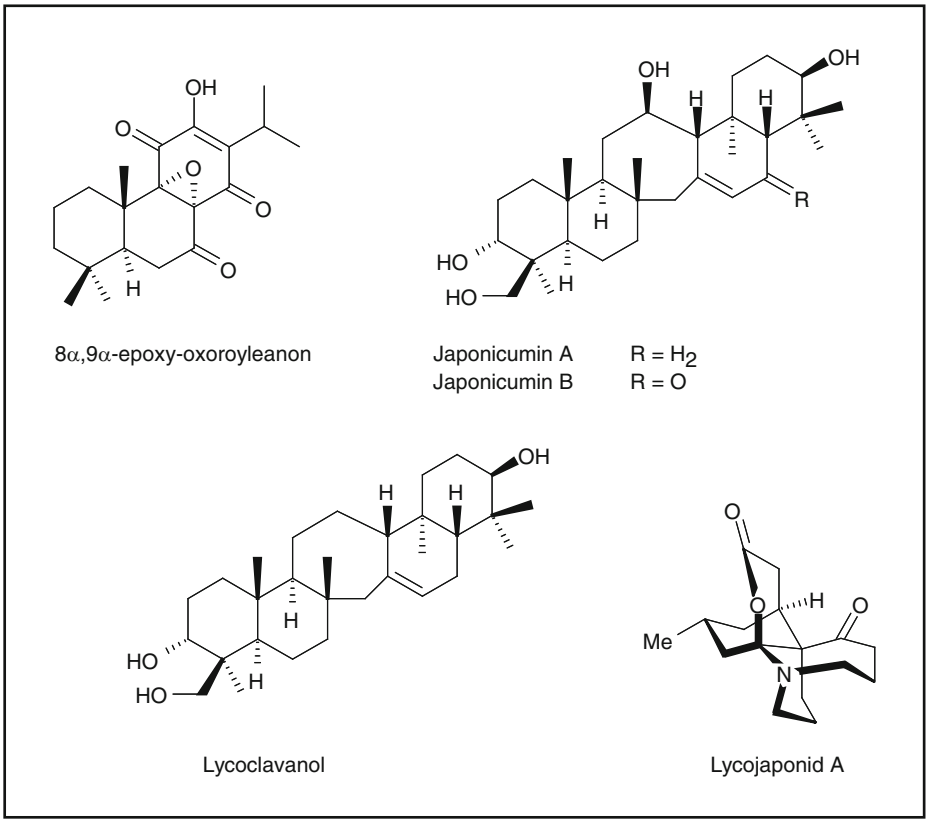


Fig. 1: Formulae of the main terpenoids (diterpenoids) of Herba Lycopodii[2, 8, 10]

Pharmacology Triterpenoids + Lycojaponide

- inhibition of acetylcholinesterase[4–7]
- memory enhancing[4, 5, 7]
- anti-oxidative[7]
- anti-proliferative effects on liver[7]
- anti-HIV activity[14]
- antitumor activities (A549/K562 cells)[13, 14]

TLC-Fingerprint Analysis[1]

Drug samples		Origin
1	Herba Lycopodii/*Lycopodium clavatum*	Sample of commercial drug, obtained from an official pharmacy (unknown origin)
2	Herba Lycopodii/*Lycopodium japonicum*	Sample of commercial drug, obtained from TCM-Clinic Bad Kötzting
3	Herba Lycopodii/*Lycopodium japonicum*	Province Hubei, China
4	Herba Lycopodii/*Lycopodium japonicum*	Province Jiangsu, China

(1) Extraction: 1 g powdered drug is extracted with 20 ml dichloromethane under reflux for one hour. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol.

(2) Reference compounds: Not applied

(3) Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Herba Lycopodii extract: each 10 µl

Solvent system: Chloroform + methanol (40 + 10)

Detection: 10 % ethanolic sulphuric acid

The plate is sprayed with 10 ml reagent and heated at 105 °C for 10 min.
The plate is evaluated in VIS and under 366 nm.

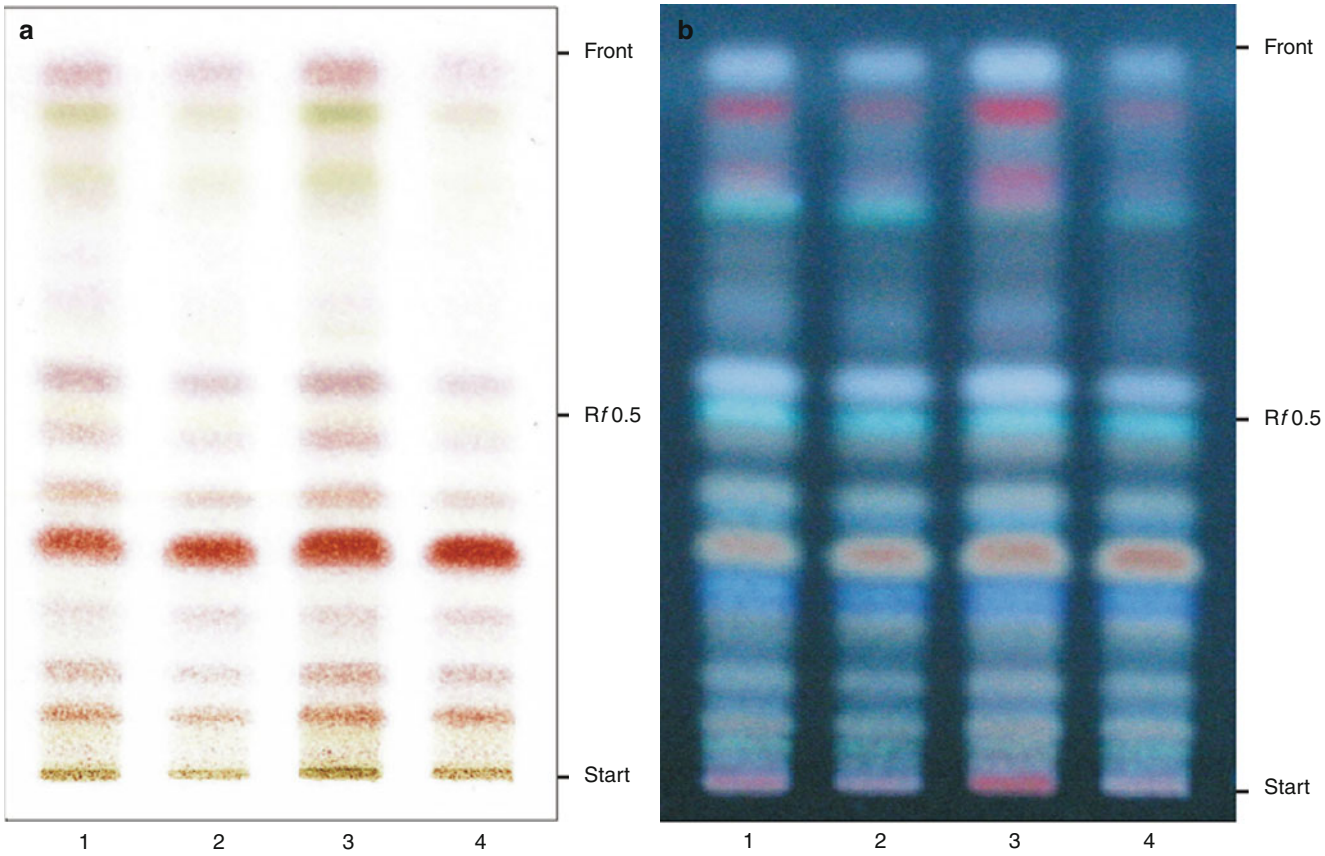


Fig. 2: (**a**, **b**) Thin layer chromatograms of the dichloromethane extracts of Herba Lycopodii sprayed with 10 % ethanolic sulphuric acid in VIS (**a**) and 366 nm (**b**)

(4) Description:

Figure 2a: All four extract samples of the herbal drug showed in VIS 8 red-brown zones distributed over the whole TLC-plate with a dominant zone at R*f* = 0.31. Since Lycojaponide A is designated in the literature as the main constituent, it is likely that the red-brown zone at R*f* = 0.31 might be identical with the alkaloid Lycojaponid A.

Figure 2b: In the 366 nm UV the characteristic zone at R*f* = 0.31 shows a weak violet-lilac fluorescence whereas all other zones appear with light blue fluorescence.

HPLC-Fingerprint Analysis:[15]

1. Sample preparation: 1 g powdered drug is extracted with 10 ml methanol under reflux for 30 min. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol, filtered over Millipore®, Type 0.45 µm and injected into the HPLC-apparatus.
2. Injection volume: Herba Lycopodii extract: each 25 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® 60 RP select B (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 60 RP select B (5 µm), Merck
Solvent System:	A: 2.0 g hexanesulfonic acid/1 l water (Millipore Ultra Clear UV plus® filtered) + H_3PO_4 85 % (pH = 3.0)
	B: acetonitrile (VWR)
Gradient:	10–80 % B in 40 min,
	80–90 % B in 10 min,
	total run time: 50 min
Flow:	1 ml/min
Detection:	210, 262 nm

Retention times of the main peaks recorded at 262 nm (——) and 210 nm (·····)

Peak	Rt (min)
1	11.83
2	17.95
3	21.96
4	25.19
5	26.22
6	27.67
7	29.10
8	36.77
9	47.29

None of the peaks could be assigned to any specific di- or triterpenoid structure of the Serratene structure type.

4. Description of Fig. 3a and 3b:

In both Herba Lycopodii extract samples 9 peaks distributed over the whole Rt-range with a major peak at Rt = 21.9 could be recorded. All peaks can be assigned to the class of serratanes triterpenoids. According to the UV-spectra all substances, except peak 8/9, possess OH–, C = O-groups and α-β-unsaturated or chinoid moities.

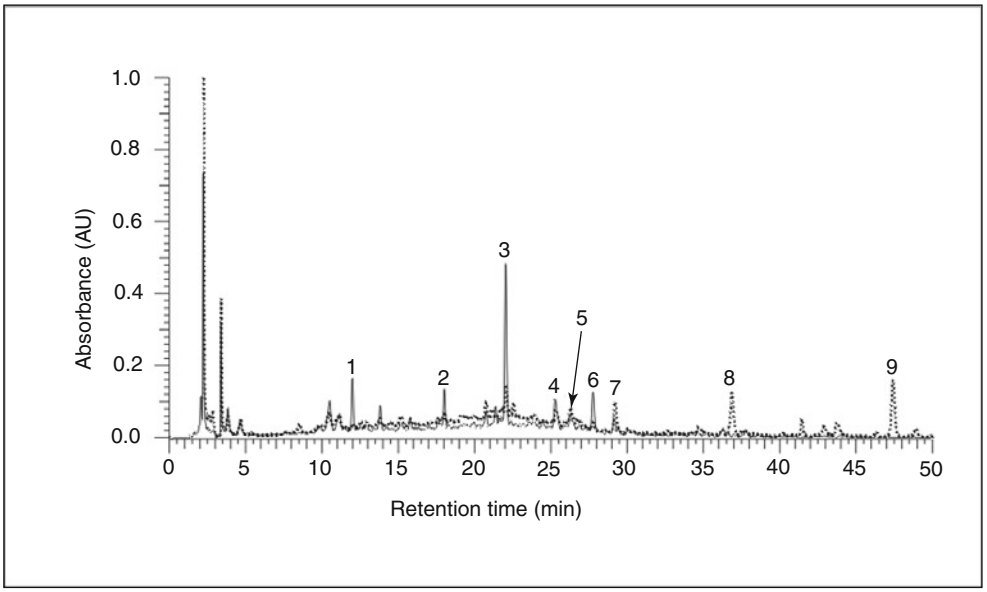


Fig. 3a: HPLC-fingerprint analysis of the methanol extract of Herba Lycopodii japonici, sample 1

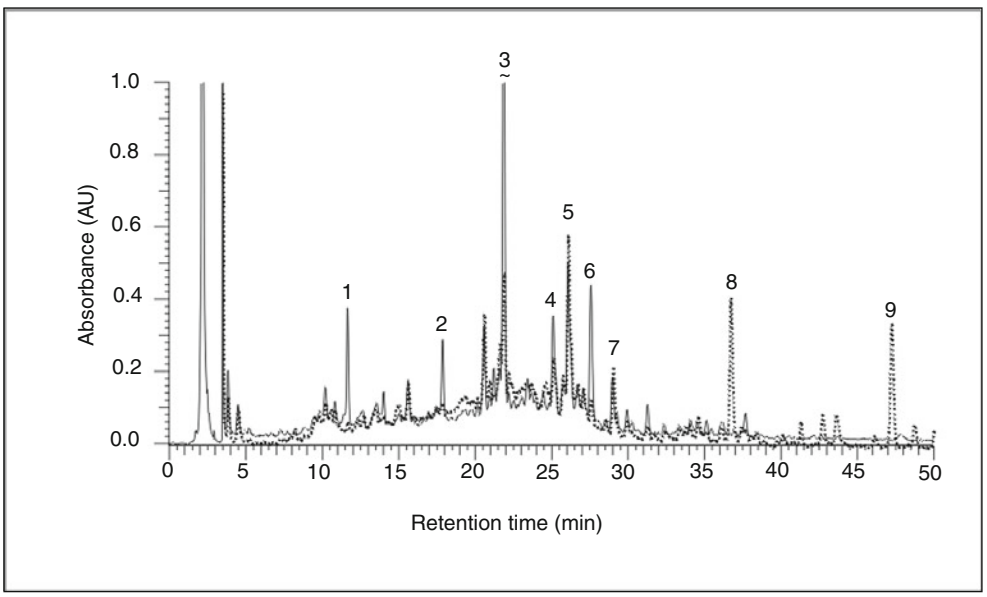


Fig. 3b: HPLC-fingerprint analysis of the methanol extract of Herba Lycopodii japonici, sample 2

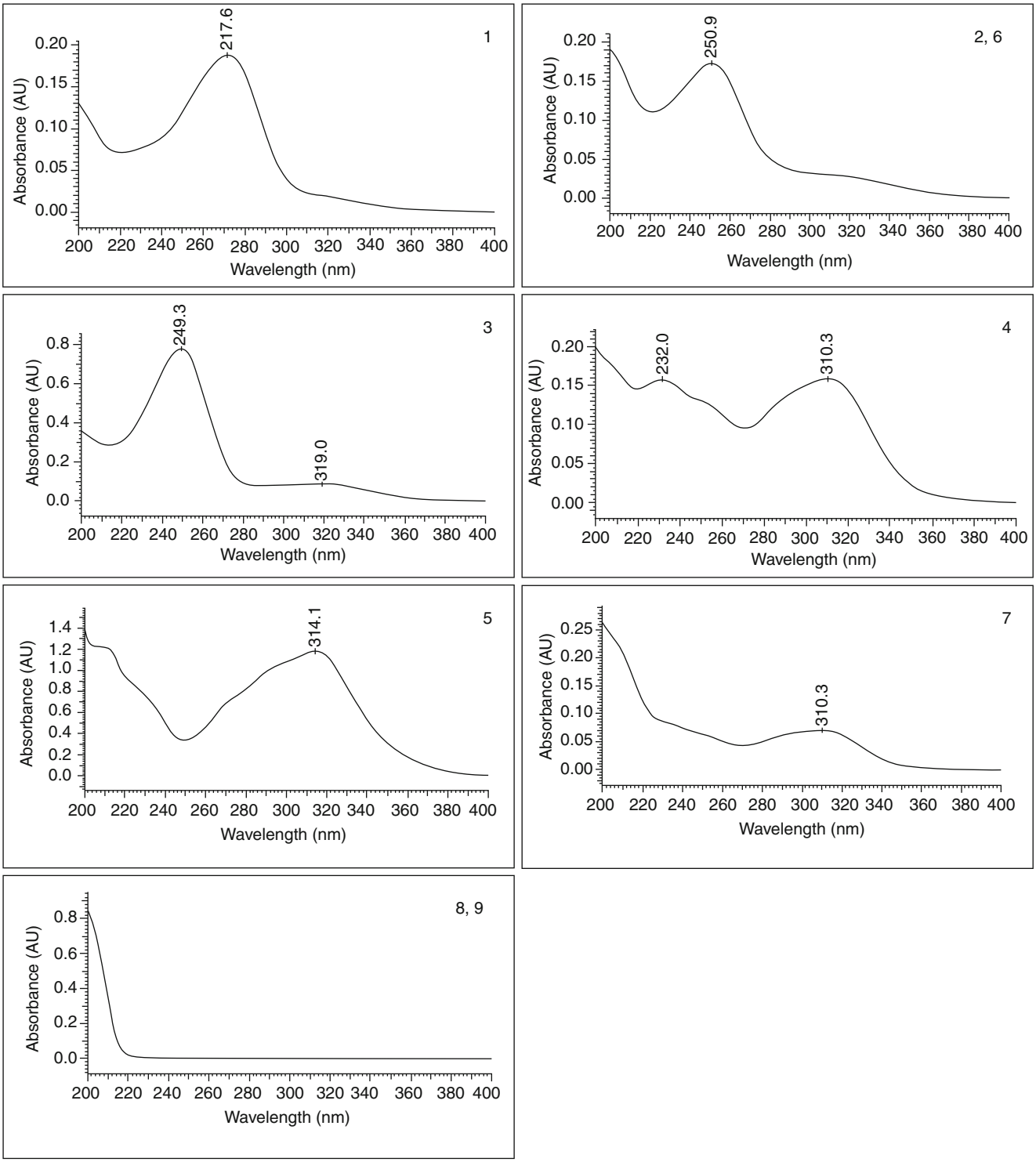


Fig. 4: On line UV-spectra of the main peaks of Herba Lycopodii japonici extracts

Conclusion

The extracts of Herba Lycopodii provide very homogeneous TLC- and HPLC-fingerprints which indicate the identity of these herbs. It is likely that most of the TLC-zones and HPLC-profiles can be assigned to the unusual pentacyclic triterpenoids of the serratane type which are characteristic constituents of Lycopodium plants[10]. The alkaloids which according to some publications[2, 3], e.g. Lycojapodine, were also isolated from *Lycopodium japonicum* could not be detected because of lacking reference substances and the very low concentration of substances obtained by the isolation methods used[2]. For the isolation of the novel alkaloid Lycojapodine 50 kg powdered herb material were used to obtain 11 mg Lycojapodine. Huperzin A + B, also characteristic alkaloids for the Lycopodiaceae family, were never found in *Lycopodium japonicum* except in *Huperzia serrata* (0.007 % yield). These latter alkaloids are suggested to derive from some phytofungi of the *Shiraia* sp. Slf14 type[12] and seem to be not genuine constituents of the plants.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol 1. People's Medical Publishing House, Beijing (2010)
2. He, J., Chen, X.-Q., Li, M.-M., Zhao, Y., Xu, G., Cheng, X., Peng, L.-Y., Xie, M.-J., Zheng, Y.-T., Wang, Y.-P., Zhao, Q.-S.: Lycojapodine A, a novel alkaloid from *Lycopodium japonicum*. Org. Lett. **11**(6), 1397–1400 (2009)
3. Sun, Y., Yan, J., Meng, H., He, C.-L., Yi, P., Qiao, Y., Qiu, M.-H.: A new alkaloid from *Lycopodium japonicum* THUNB. Helv. Chim. Acta. **91**(11), 2107–2109 (2008)
4. Yang, Q.P., Kou, X.L., Fugal, K.B., McLaughlin, J.L.: Determination of huperzine A in formulated products by reversed-phase-liquid chromatography using diode array and electrospray ionization mass spectrometric detection. Phytomedicine **10**(2–3), 200–205 (2003)
5. Ma, X.Q., Tan, C.G., Zhu, D.Y., Gang, D.R.: Is there a better source of huperzine a than *Huperzia serrata*? huperzine a content of huperziaceae species in China. J. Agric. Food Chem. **53**(5), 1393–1398 (2005)
6. Wang, Y., Zeng, Q.G., Zhang, Z.B., Yan, R.M., Wang, L.Y., Zhu, D.: Isolation and characterization of endophytic huperzine A-producing fungi from *Huperzia serrata*. J. Ind. Microbiol. Biotechnol. **38**(9), 1267–1278 (2011)
7. Mandal, S.K., Biswas, R., Bhattacharyya, S.S., Paul, S., Dutta, S., Pathak, S., Khuda-Bukhsh, A.R.: Lycopodine from *Lycopodium clavatum* extract inhibits proliferation of HeLa cells through induction of apoptosis via caspase-3 activation. Eur. J. Pharmacol. **626**(2–3), 115–122 (2010)
8. Wu, L., Lu, Y., Zheng, Q.-T., Li, X.-L., Zhao, Q.-S.: 8α,9α-Epoxy-7-oxoroyleanon. Acta. Crystallogr. (Structure Reports) **E62**, o3269–o3270 (2006)
9. Yan, J., Sun, L., Zhang, X., Qiu, M.: A new Flavone from *Lycopodium japonicum*. Heterocycles **65**(3), 661–666 (2005)
10. Li, X.-L., Zhao, Y., Cheng, X., Tu, L., Peng, L.-Y., Xu, G., Zhao, Q.-S., Japonicumins, A.-D.: Four new compounds from *Lycopodium japonicum*. Helv. Chim. Acta. **89**(7), 1467–1473 (2006)
11. Yan, J., Zhang, X.-M., Li, Z.-R., Zhou, L., Chen, J.-C., Sun, L.-R., Qiu, M.-H.: Three new triterpenoids from *Lycopodium japonicum* THUNB. Helv. Chim. Acta **88**(2), 240–244 (2005)
12. Zhu, D., Wang, J., Zeng, Q., Zhang, Z., Yan, R.: A novel endophytic Huperzine A-producing fungus, Shiraia sp. Slf14, isolated from *Huperzia serrata*. J. Appl. Microbiol. **109**(5), 1469–1478 (2010)
13. Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., Boyd, M.R.: Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer. Res. **48**(3), 589–601 (1988)
14. Niu, X.M., Li, S.H., Li, M.L., Zhao, Q.S., Mei, S.X., Na, Z., Wang, S.J., Lin, Z.W., Sun, H.D.: Cytotoxic *ent*-Kaurane Diterpenoids from *Isodon eriocalyx* var. *laxiflora*. Planta Med. **68**(6), 528–533 (2001)
15. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines: thin layer and high performance liquid chromatography of Chinese drugs, vol. 1 and 2. Springer, Wien (2011)

Radix Saposhnikoviae – *Fangfeng*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2005/2010

Official drug:[1]

Divaricate Saposhnikovia Root is the dried root of *Saposhnikovia divaricata* (Turcz.) Schischk. (Fam. Apiaceae)

The drug is collected in spring or autumn before growing of the flowering stem, removed from the rootlet and soil, and dried in the sun.

Synonyms:[2, 3]

Ledebouriella divaricata and *Ledebouriella seseloides* (Hoffm.) Wolff

Origin:[2, 4, 5]

North-east China (provinces Hebei, Henan, Shandong, Shaanxi, Shanxi, Hunan, Gansu and Sichuan), Inner Mongolia (Neimeng)

Description of the drug:[1]

Long conical or long cylindrical, gradually tapering towards the lower part, some slightly tortuous, 15–30 cm long, 0.5–2 cm in diameter. Externally greyish-brown, rugged, with longitudinal wrinkles, numerous transverse-elongated lenticel-like protrudings and dotted raised rootlet scars. Root stock with obvious dense annulations, some annulations marked by brown hair-like remains of leaf bases. Texture light, easily broken, fracture uneven, bark brownish and cracked, wood yellowish. Odour, characteristic; taste, sweetish.

Pretreatment of the raw drug:[1]

Foreign matters are eliminated washed clean, soften thoroughly, cut into thick slices and dried.

Medicinal use:[2, 5]

Used for the treatment of common cold, headache and dizziness, additionally for migraine, rheumatic disorders and diarrhoea.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_4, © Springer International Publishing Switzerland 2015

Effects and indications of Radix Saposhnikoviae according to Traditional Chinese Medicine [1–4, 6, 7]	
Taste:	Pungent, sweet
Temperature:	Warm
Channels entered:	*Orbis hepaticus*, *oo. lienalis et stomachi*, *o. vesicalis*, *o. pulmonalis*
Effects (functions):	To induce diaphoresis, to dispel *wind*, to alleviate rheumatic condition and to relieve spasm (2005). To dispel *wind* to release the exterior pattern, dispel dampness and relieve pain, arrest convulsions (2010).
Symptoms and indications:	Headache in colds; urticaria, rheumatic arthralgia, tetanus (2005). Common cold, headache, painful impediment caused by wind-dampness, itching caused by rubella, tetanus (2010).

Main constituents:[4, 5, 8–14]

Chromones (hamaudol, cimifugin, *prim-O*-glucosylcimifugin 5-*O*-methylvisamminol, khellin, divaricatol)

Furanocoumarins (imperatorin, isoimperatorin,psoralen, xanthotoxin, bergapten)

Polyacetylenes (falcarinol, falcarindiol, falcarinone, anaxynol, panaxydol, panaxytriol)

Essential oils (caryophylene oxide, sabinene, β-pinene, myrtenal, myrtenol, α-terpineol, p-cymene, α-pinene, nonanoic acid)

Minor constituents:[8, 14]

Alkapolyalkynes
Polysaccharide

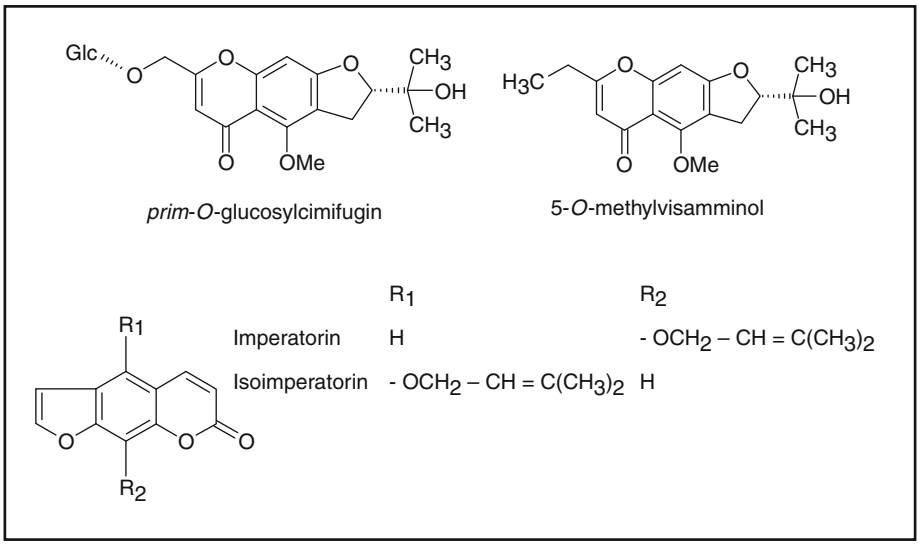


Fig. 1: Formulae of the main constituents of Radix Saposhnikoviae [8]

Pharmacology: Anti-inflammatory activities [9, 11, 14]
Adjuvant arthritis [9]
Inhibitory effects on CNS and peptic ulcers [9]
Anti-pyretic activities [10–12, 14]
Inhibition of nitrite production by iNOS [14]
Antioxidant activities [11, 14]
Anti-convulsant activities [9, 14]
Inhibition of platelet aggregation [4]
Antibiotic activities [10, 12]
Antivirus activities [10]
Antiproliferative [14]

TLC-Fingerprint Analysis [1]

Drug samples		**Origin**
1	Radix Saposhnikoviae/*Saposhnikovia divaricata*	Sample of commercial drug obtained from Herbasin, Germany
2	Radix Saposhnikoviae/*Saposhnikovia divaricata*	Sample of commercial drug obtained from HerbaSinica, Germany (Origin: Neimenggu)
3	Radix Saposhnikoviae/*Saposhnikovia divaricata*	Sample of commercial drug obtained from China Medica, Germany
4	Radix Saposhnikoviae/*Saposhnikovia divaricata*	Sample of commercial drug obtained from TCM-Clinic Bad Kötzting, Germany

Reference compounds		**R*f***
T1	*prim-O*-glucosylcimifugin	0.20
T2	Imperatorin	0.92

(1) Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 30 min. 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:	Radix Saposhnikoviae extract: each 10 µl Reference compounds: each 10 µl
Solvent system:	Chloroform + methanol (4 + 1)
Detection:	Without chemical treatment → 254 nm (**Fig. 2a**)
	Anisaldehyde – Sulphuric acid (**Fig. 2b**)
	0.5 ml anisaldehyde are mixed with 10 ml glacial acetic acid, 85 ml methanol and 5 ml conc. sulphuric acid, in this order.
	The plate is sprayed with 8 ml reagent and heated at 105 °C for 5 min. The plate is evaluated in VIS.
	Note: The reagent has only limited stability and is no longer useable when the colour has turned to red-violet.

4. Description of Fig. 2a and 2b:

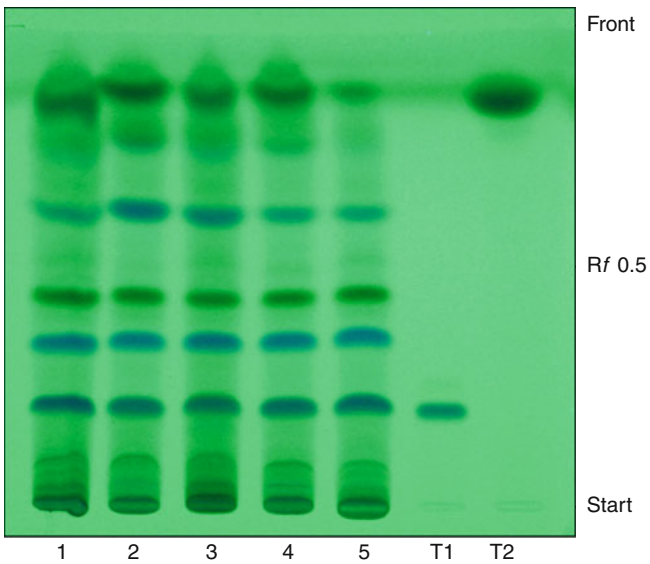


Fig. 2a: Thin layer chromatogram of the methanol extracts of Radix Saposhnikoviae, without chemical treatment (UV 254 nm)

All *Saposhnikovia divaricata* root samples show four green-blue zones. The lowest zone can be assigned to *prim*-*O*-glucosylcimifugin (**T1**). The second above (R*f* = 0.36) might be 5-*O*-methylvisamminol. A dark green zone at the solvent front is imperatorin (**T2**).

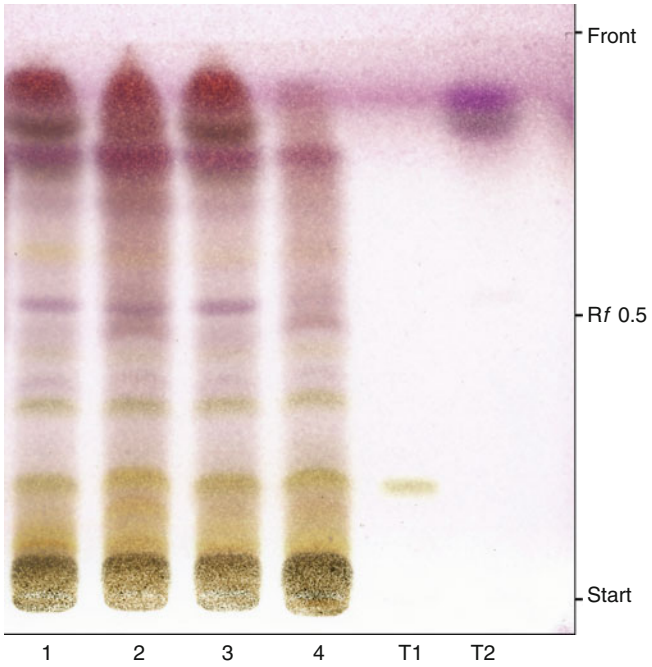


Fig. 2b: Thin layer chromatogram of the methanol extracts of Radix Saposhnikoviae sprayed with Anisaldehyde – Sulphuric acid (VIS)

In analogy to Fig. 2a the former dark green-blue zones turned brown/violet with the same chemical assignments. The strong brown-violet zones from R*f* = 0.85 up to the front can be assigned to imperatorin, isoimperatorin, bergapten and other compounds of the essential oils

HPLC-Fingerprint Analysis

1. Sample preparation: 1 g powdered drug is extracted with 10 ml methanol under reflux for 30 min. The extract is cooled, filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, Type 0.45 µm.
2. Injection volume: Radix Saposhnikoviae 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® 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_4/1 l water (Millipore Ultra Clear UV plus® filtriert) B: acetonitrile (VWR)
Gradient:	0–100 % B in 60 min total run time: 60 min
Flow:	1.0 ml/min
Detection:	254 nm

Retention times of the main peaks:

Peak	**Rt (min)**	**Compound**
1	13.8	*prim*-*O*-glucosylcimifugin
2	16.5	5-O-methylvisamminol
3	37.9	Imperatorin

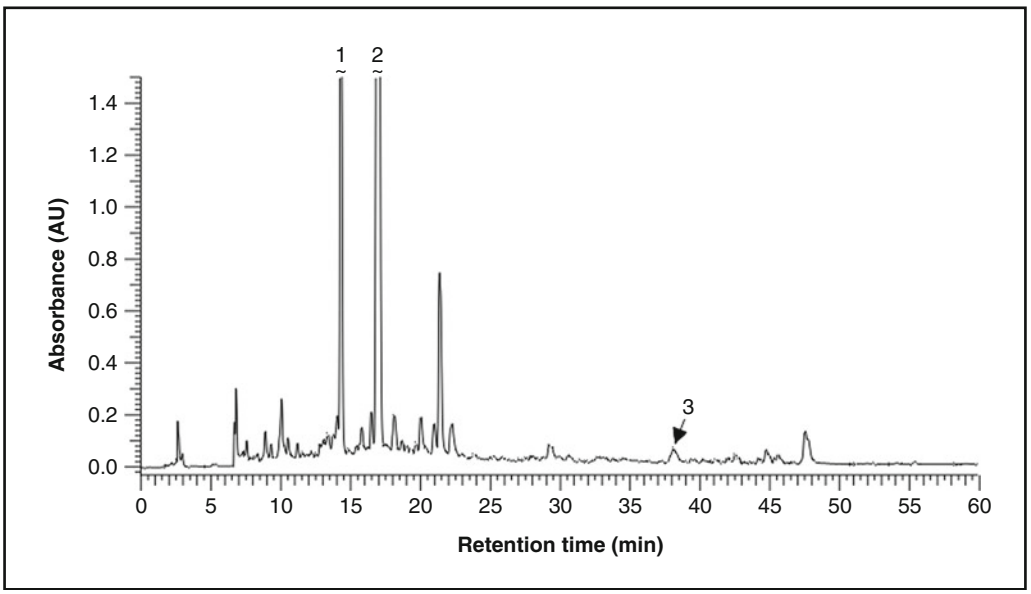


Fig. 3a: HPLC-fingerprint analysis of the methanol extract of Radix Saposhnikoviae, sample 1

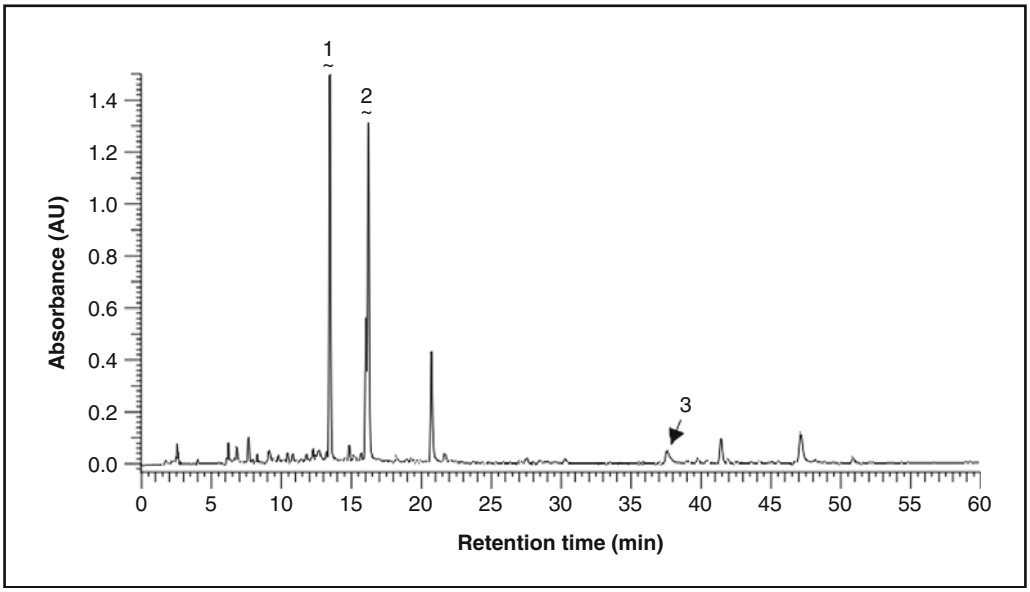


Fig. 3b: HPLC-fingerprint analysis of the methanol extract of Radix Saposhnikoviae, sample 3

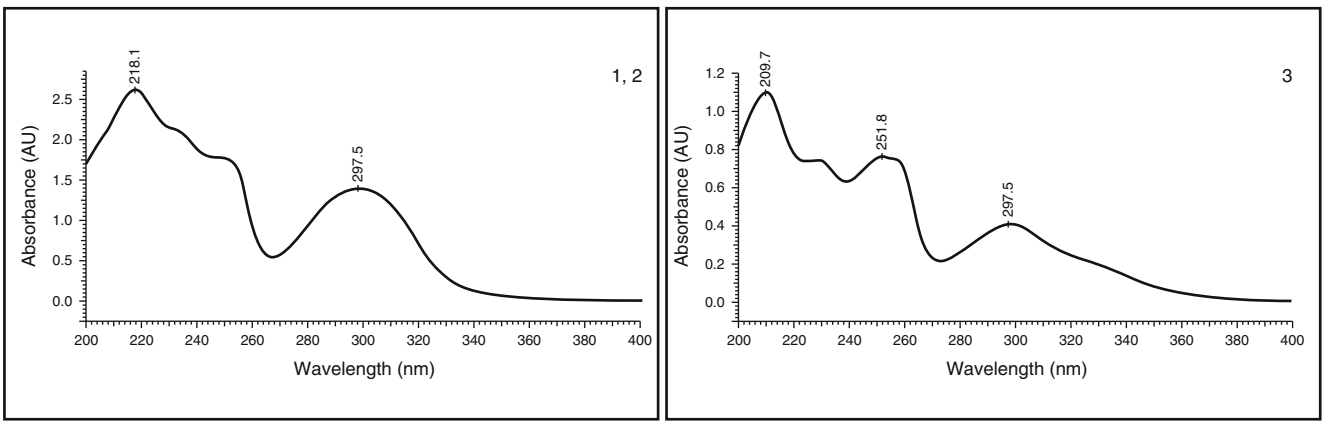


Fig. 4: On line UV-spectra of the reference compounds of Radix Saposhnikoviae

4. Description of Fig. 3a and 3b:

All samples of Radix Saposhnikoviae show a similar peak profile. In the range of Rt 12–22 three main peaks are seen. The first peak **1** is *prim*-O-glucosylcimifugin. According to the HPTLC – chromatogram peak **2** (Rt = 16.5) can be assigned to 5-*O*-methylvisamminol. Peak **3** is imperatorin.

Note: According to the Chinese Pharmacopoeia Radix Saposhnikoviae contains not less than 0.24 % of the total amount of prim-O-glucosylcimicifugin and 5-O-methylvisamminoside, calculated with reference to the dried drug. [1]

Conclusion

The TLC- and HPLC-fingerprints are best suitable for authentication of the *Saposhnikovia* (*Lederbouriella*) *divaricata* herbal drug samples.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. People's Medical Publishing House, Beijing (2005/2010)
2. Paulus, E., Ding, Y.-H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F Haug Verlag, Heidelberg (1987)
3. Hempen, C.H., Fischer, T.: A materia medica for Chinese medicine (plants, minerals and animal products), 2nd edn, Churchill Livingstone Elsevier, New York (2009)
4. Deng, C., Yang, X., Zhang, X.: Rapid determination of panaxynol in a traditional Chinese medicine of *Saposhnikovia divaricata* by pressurized hot water extraction followed by liquid-phase microextraction and gas chromatography-mass spectrometry. Talanta **68**(1), 6–11 (2005)
5. Zhao, Z.Z.: An illustrated Chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2007)
6. Hempen, C.H., Fischer, T.: Leitfaden Chinesische Phytotherapie, 2nd edn. Urban & Fischer, Munich (2007)
7. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer GmbH, Heidelberg (1978)
8. Kang, J., Sun, J.H., Zhou, L., Ye, M., Han, J., Wang, B.R., Guo, D.A.: Characterization of compounds from the roots of *Saposhnikovia divaricata* by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Rapid Commun. Mass Spectrom. **22**(12), 1899–1911 (2008)
9. Okuyama, E., Hasegawa, T., Matsushita, T., Fujimoto, H., Ishibashi, M., Yamazaki, M.: Analgesic components of saposhnikovia root (*Saposhnikovia divaricata*). Chem. Pharm. Bull. **49**(2), 154–160 (2001)
10. Wang, C.N., Shiao, Y.J., Kuo, Y.H., Chen, C.C., Lin, Y.L.: Inducible nitric oxide synthase inhibitors from *Saposhnikovia divaricata* and *Panax quinquefolium*. Planta Med. **66**(7), 644–647 (2000)
11. Li, W., Wang, Z., Chen, L., Znag, J., Han, L., Hou, J., Zheng, Y.: Pressurized liquid extraction followed by LC-ESI/MS for analysis of four chromones in Radix Saposhnikoviae. J. Sep. Sci. **33**(17–18), 2881–2887 (2010)
12. Tai, J., Cheung, S.: Anti-proliferative and antioxidant activities of *Saposhnikovia divaricata*. Oncol. Rep. **18**(1), 227–234 (2007)
13. Gui, Y., Tsao, R., Li, L., Liu, C.M., Wang, J., Zong, X.: Preparative separation of chromones in plant extract of *Saposhnikovia divaricata* by high-performance counter-current chromatography. J. Sep. Sci. **34**(5), 520–526 (2011)
14. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)

Radix et Rhizoma Glycyrrhizae – *Gancao*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1]

Liquorice (= Licorice) Root is the dried root and rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat. or *Glycyrrhiza glabra* L. (Fam. Fabaceae).

The drug is collected in spring or autumn, removed from rootlets and dried in the sun.

Origin:[2–4]

Northern/Northeastern China (Gansu, Xinjiang), Inner Mongolia, Siberia, South Russia.

Description of the drugs:[1]

Root of *Glycyrrhiza uralensis*: Roots cylindrical, 25–100 cm long, 0.6–3.5 cm in diameter. The outer bark loose or tight. Externally reddish-brown or grayish-brown, obviously longitudinally wrinkled, furrowed, lenticel-like protruded and with sparse rootlet scars. Texture compact, structure slightly fibrous, yellowish-white, starchy, cambium ring distinct, rays radiate, some with clefts. Rhizomes cylindrical, externally with bud scars, pith present in the center of fracture. Odour, slight; taste, sweet and characteristic.

Root of *Glycyrrhiza inflata*: Roots and rhizomes woody and stout, some branched, the outer bark rough, mostly grayish-brown. Texture compact, lignified fibers abundant and less starchy. Rhizomes with more and large adventitious buds.

Root of *Glycyrrhiza glabra*: Texture of root and rhizome relatively compact, some branched, the outer bark not rough, mostly grayish-brown, lenticels small and indistinct.

Pretreatment of the raw drug:[1]

Foreign matters are eliminated, washed clean, softened thoroughly, cut into thick slices and dried.

Note: In addition the Chinese Pharmacopeia 2010 lists the monograph Radix et Rhizoma Glycyrrhizae Praeparata cum Melle (*Zhigancao*):

The slices of Glycyrrhizae Radix are stir-baked as described under the method for stir-baking with honey (Appendix II D) until it becomes yellow to deep yellow and not sticky to the fingers, taken out and cooled in the air.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_5, © Springer International Publishing Switzerland 2015

Medicinal use:[5, 6]

Licorice has a long history of medicinal use in Europe and Asia. It is reported to be effective in the treatment of diabetes, peptic ulcer disease, cystitis, kidney stones, Addison's disease, constipation, lung ailments, cough, gastrointestinal system diseases (e.g. in the stomach, liver), problems of the arteries, scabies of the bladder, skin and eye diseases. It is also used as an anabolic, contraceptive and aphrodisiac. It has the property of quenching thirst, reducing fevers, tumors of the limbs and indurations.

Effects and indications of Radix et Rhizoma Glycyrrhizae according to Traditional Chinese Medicine [1, 7–9]

Taste:	Sweet, characteristic, slightly aromatic
Temperature:	Neutral
Channels entered:	*Orbis cardialis*, *o. pulmonalis*, *o. lienalis*, *o. stomachi*, *o. hepaticus*
Effects (functions):	To reinforce the function of the spleen and replenish *qi*, remove *heat* and counteract *toxicity*, dispel phlegm and relieve cough, alleviate spasmodic pain and moderate drug actions.
Symptoms and indications:	Hypofunction of the spleen and the stomach marked by lassitude and weakness; cardiac palpitation and shortness of breath; cough with much phlegm; spasmodic pain in the epigastrium, abdomen and limbs; carbuncles and sores, also used for reducing the toxic or drastic action of other drugs.

Precaution:[5]

The consumption of large amounts of Licorice may result in hypertension, hypocalcemia and edema.

Published Constituents[4, 5, 8, 10–13]

Triterpene saponins:	(Total 2–15 %), **Glycyrrhizin** (Synonyms: Glycyrrhizic or Glycyrrhizinic acid (4–5 %), as Ca- and K-salts of Glycyrrhizic acid)
Other triterpenes:	**Glycyrrhetinic acid** (Synonym: Glycyrrhetic acid; two isomers: 18α- and 18β-glycyrrhetic acid)
	Liquiritic acid (similar formula as Glycyrrhetic acid)
	Glycyrretol
	Glabrolide
	Isoglabrolide
	Licorice acid

Flavonoids, (Retro-) Chalcones:	(Total 1–1.5 %)
	Liquiritin
	Liquiritigenin
	Licoricidin
	Rhamnoliquiritin
	Neoliquiritin
	Isoliquiritin
	Isoliquiritigenin
	Neoisoliquiritin
	Licuraside
	Licoflavonol
	5,8-dihydroxy-flavone-7–O-β-D-glucuronide
	5-hydroxy-8-methoxyl-flavone-7–O-β-D-glucuronide
	Glychionide A and B
	Licochalcone A (0.8 %), B, C and D
	Echinatin
	Isotrifoliol
	Glisoflavanone
Isoflavones:	Glabridin
	Galbrene
	Glabrone
	Shinpterocarpin
	Licoisoflavone A and B
	Formononetin
	Glyzarin
	Kumatakenin
	Hispaglabridin A and B
	4′-O-methylglabridin
	3′-hydroxy-4′-O-methylglabridin
	Glabroisoflavanone A and B
Coumarins:	Liqcoumarin
	Glabrocoumarone A and B
	Herniarin
	Umbelliferone
	Glycyrin
	Glycocoumarin
	Licofuranocoumarin
	Licopyranocoumarin
	Glabrocoumarin

Stilbenoids:

Dihydro-3,5-dihydroxy-4′-acetoxy-5′-isopentenylstilbene
Dihydro-3,3′,4′-trihydroxy-5-O-isopentenyl-6-isopentenylstilbene
Dihydro-3,5,3′-trihydroxy-4′-methoxystilbene
Dihydro-3,3′-dihydroxy-5-β-D-O-glucopyranosyloxy-4′-methoxystilbene

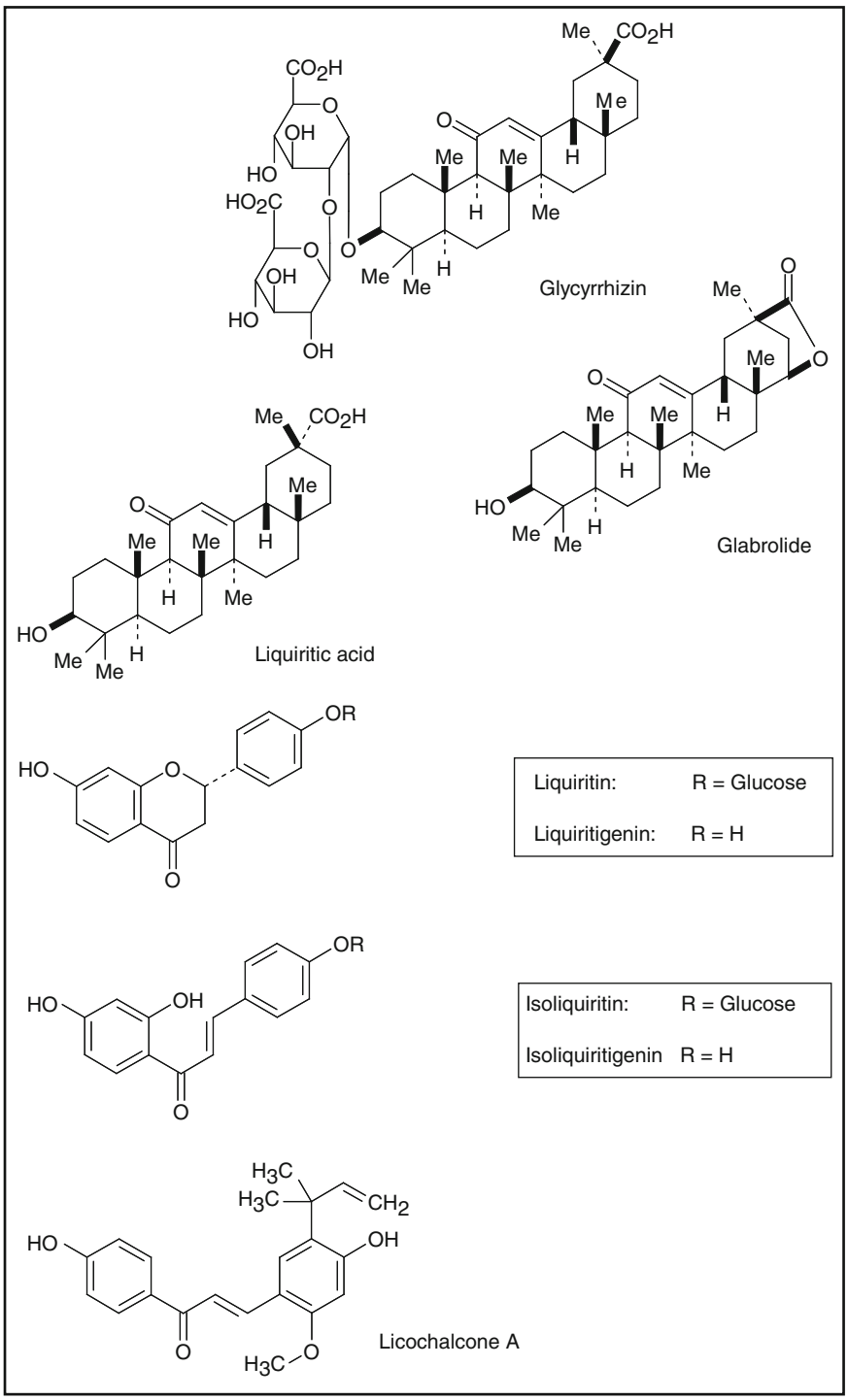


Fig. 1: Formulae of the main compounds of Radix et Rhizoma Glycyrrhizae [2, 6, 10–18]

Reported Pharmacological Effects[5, 6, 9, 11]

In vitro and *in vivo*:

- Antiinflammatory (β-Glycyhrritinic acid, Glycyrrhizin, Glycyrrhetinic acid, Glabridin, Glyderinine)
- Antimicrobial and antiviral (Glabridin, Glabrene, Licochalcone A, Glycyrrhizol A, Glycyrrhizin, Glycocoumarin, Licopyranocoumarin)
- Antiprotozoal (Licochalcone A)
- Antioxidative (Licochalcone A, B, C, D, Echinatin, Glabridin, Hispaglabridin A, B, Isoprenylchalcone derivatives, Isoliquiritigenin)
- Hepatoprotective (Glycyrrhizin, Glycyrrheitinic acid)
- Antitumor (Glycyrrhetinic acid, Licochalcone A, E, Isoliquiritigenin)
- CNS-activities: antidepressant, protective in ischemic-reperfusion injuries, memory enhancing, anticonvulsant, sedative, muscle relaxant (Glabridin, Isoliquiritigenin, Carbenoxolone)
- Cardiovascular effects: antiplatelet aggregation, antithrombotic, vasorelaxant (Glycyrrhizin, Isoliquiritigenin, Glabridin)
- Estrogen-like activities (Glabridin)
- Immunomodulatory (Glycyrrhizin, Glycyrrhetinic acid, Licochalcone A, polysaccharides, saponins)
- Renoprotective (Glabridin, Glycyrrhizin)
- Cytotoxic (Isoliquiritigenin)
- Antitussive (Liquiritin apioside, Liquiritigenin, Liquiritin)

Note: Recently in the edible roots of *Glycyrrhiza foetida* and *Glycyrrhiza glabra* some isoprenyl polyphenols (amorfrutins) were detected which exhibit antidiabetic activity. The compounds bind to and activate PPARγ (peroxisome proliferator-activated receptor gamma) which regulates the glucose- and lipid metabolism and may play a role in the prevention of diabetes 2 and obesity.

TLC-Fingerprint Analysis[1, 7]

	Drug samples	Origin
1	Radix et Rhizoma Glycyrrhizae/*Glycyrrhiza* spec.	Sample of commercial drug obtained from HerbaSinica (Charge: 070701H020)
2	Radix Glycyrrhizae/*Glycyrrhiza uralensis*	Sample of commercial drug obtained from China Medica (Charge: 140015)
3	Radix Glycyrrhizae/*Glycyrrhiza glabra*	Provinve Xingjian, China
4	Radix Glycyrrhizae/*Glycyrrhiza inflata*	Province Xingjian, China
5	Radix Glycyrrhizae/*Glycyrrhiza uralensis*	Province Neimenggu, China
6	Radix Glycyrrhizae/*Glycyrrhiza uralensis*	Province Neimenggu, China
7	Radix Glycyrrhizae/*Glycyrrhiza uralensis*	Province Neimenggu, China
8	Radix et Rhizoma Glycyrrhizae praeparata cum melle/*Glycyrrhiza uralensis*	Sample of commercial drug obtained from HerbaSinica (Charge: 070601H411, origin: Neimenggu)

Drug samples		Origin
9	Radix Glycyrrhizae tosta (roasted)/*Glycyrrhiza uralensis*	Sample of commercial drug obtained from China Medica (Charge: 040017)
10	Radix et Rhizoma Glycyrrhizae/*Glycyrrhiza uralensis*	Sample of commercial drug obtained from Caelo (Origin: inner Mongolia)
11	Radix Liquiritiae/*Glycyrrhiza glabra*	Sample of commercial drug obtained from Caelo
12	Radix Liquiritiae (peeled)/*Glycyrrhiza glabra*	Sample of commercial drug obtained from Klenk
13	Radix Liquiritiae/*Glycyrrhiza glabra*	Sample of commercial drug obtained from Bombastus

Reference compounds		R*f*
T1	Glycyrrhetinic acid (18β-glycyrrhetic acid)	0.97
T2	Glycyrrhizin	0.13
T3	Licochalcone A	0.95
T4	Liquiritin	0.55

1. Extraction: 0.5 g powdered drug is extracted under reflux with 40 ml ethyl acetate for 30 min and filtered. The dried residue is extracted under reflux with 30 ml methanol for 1 h and filtered. The filtrate is evaporated to dryness and the residue dissolved in 40 ml water. The aqueous solution is extracted three times with 20 ml quantities of water saturated *n*-butanol. The combined *n*-butanol solutions are washed three times with 10 ml *n*-butanol saturated water and evaporated to dryness under vacuum. The residue is dissolved in 1 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

Plate:	0.5 % NaOH impregnated HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Radix et Rhizoma Glycyrrhizae extracts: 5 µl each
	Reference compounds: 10 µl each
Solvent system:	Ethyl acetate + formic acid + glacial acetic acid + water (15 + 1 + 1 + 2)
Detection:	10 % ethanolic Sulphuric acid
	The plate is sprayed with 10 ml solution, heated at 105 °C until the bands become visible and evaluated under UV 366 nm.

Description of Fig. 2:
The samples 2, 5, 6 and 7 of *Glycyrrhiza uralensis* show identical fingerprints with blue or green fluorescent zones between R*f* = 0.1–0.2 and R*f* = 0.4–0.98. Glycyrrhizin (**T2**) appears at R*f* = 0.13 with olive-green colour and glycyrrhetinic acid (**T1**) in weak concentration at R*f* = 0.97. The zones between R*f* = 0.4–0.98 can be assigned to flavanones and coumarins. Licochalcone A (**T3**) can be identified at R*f* = 0.95 as a reddish-yellow zone, best represented in sample 4, *Glycyrrhiza inflata*. The grass-green spot at R*f* = 0.55 is Liquiritin (**T4**). The *Glycyrrhiza glabra* samples 3 and 11 differ from *Glycyrrhiza uralensis* by the weak or lacking zones in the R*f*-range of 0.4–0.7. The samples 8 and 9 of the roasted or otherwise treated *Glycyrrhiza* roots show the same TLC-fingerprints as sample 5–7.

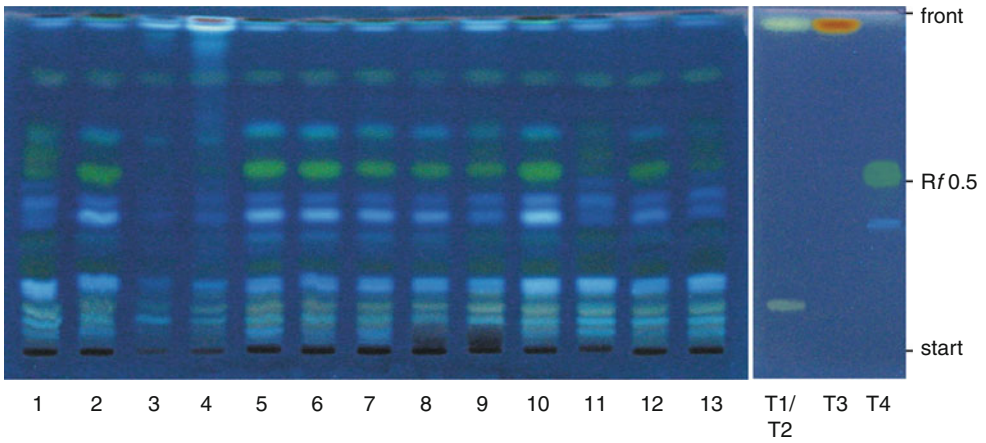


Fig. 2: Thin layer chromatogram of Radix et Rhizoma Glycyrrhizae extracts, sprayed with 10 % ethanolic sulphuric acid (UV 366 nm)

HPLC-Fingerprint Analysis

1. Sample preparation: 1.0 g powdered drug is extracted under reflux with 40 ml methanol 80 % for 30 min and filtered. The filtrate is evaporated to dryness, dissolved in 2 ml methanol 80 % and filtered over Millipore® filtration unit, type 0.45 µm.

2. Injection volume: Radix et Rhizoma Glycyrrhizae 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: 10 ml 0.1 H_3PO_4/1 l water (Millipore Ultra Clear UV plus® filtered)
	B: acetonitrile (VWR)
Gradient:	10–28 % B in 35 min,
	28 % B for 10 min,
	28–95 % B in 15 min,
	Total runtime: 60 min
Flow:	1.5 ml/min
Detection:	254 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	6.2–8.5	Flavonoid
2	7.9	Liquiritin
3	14.1	Flavonoid//Stilbene?
4	17.3	Coumarin/Flavonoid?
5	18.8	Flavonoid//Stilbene?
6	23.8	Flavonoid
7	32.3	Glycyrrhizin
8	52.5	Licochalcon A

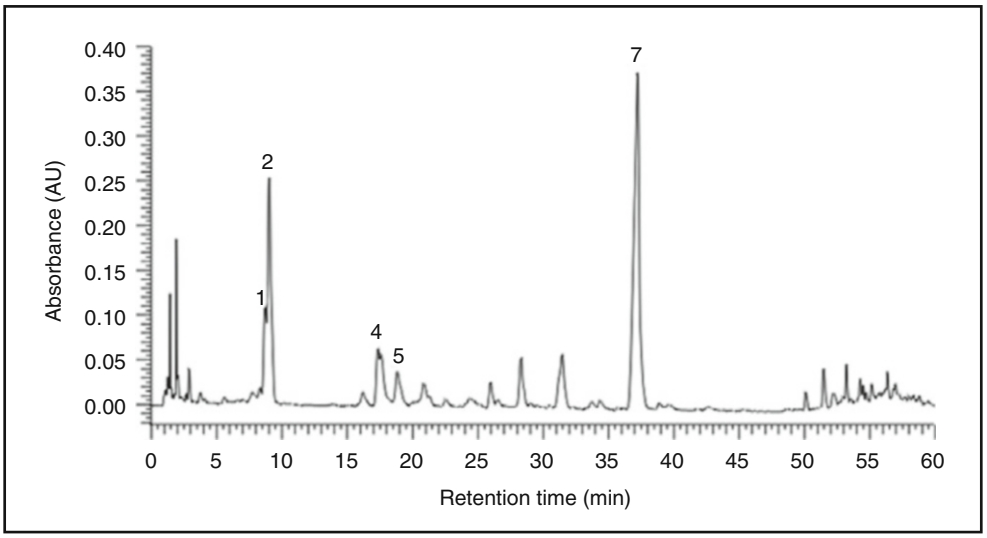


Fig. 3a: HPLC-fingerprint analysis of the methanol extract of Radix et Rhizoma Glycyrrhizae (sample 10, *Glycyrrhiza uralensis*)

4. Description of the HPLC-Figures

Figure 3a:
The fingerprint of the root of *Glyzyrrhiza uralensis* (samples 2, 5, 6 and 7) are characterized by the distinct peaks of liquiritin (Peak **2** at Rt = 7.9) and glyzyrrhizin (Peak **7** at Rt = 32.3). The peaks **1**, **4** and **5** can be assigned to a flavonoid, coumarin and isoliquiritin.

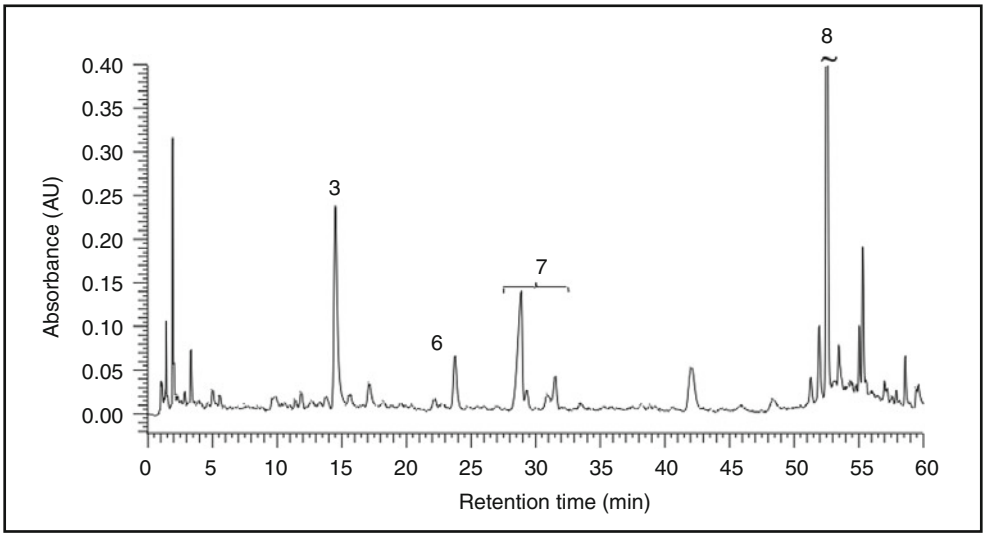


Fig. 3b: HPLC-fingerprint analysis of the methanol extract of Radix et Rhizoma Glycyrrhizae (sample 4, *Glycyrrhiza inflata*)

Figure 3b:
The fingerprint of the root of *Glyzyrrhiza inflata* (sample 4) showed a strong peak of a flavanoid or stilbene (peak **3**, Rt = 14.5), a flavonoid at Rt = 23.8 (peak **6)** and glyzzyrhizin at Rt = 31.3 (peak **7**) and in contrast to all other *Glyzyrrhiza* species at Rt = 52.5 (peak **8**) Licochalcone A.

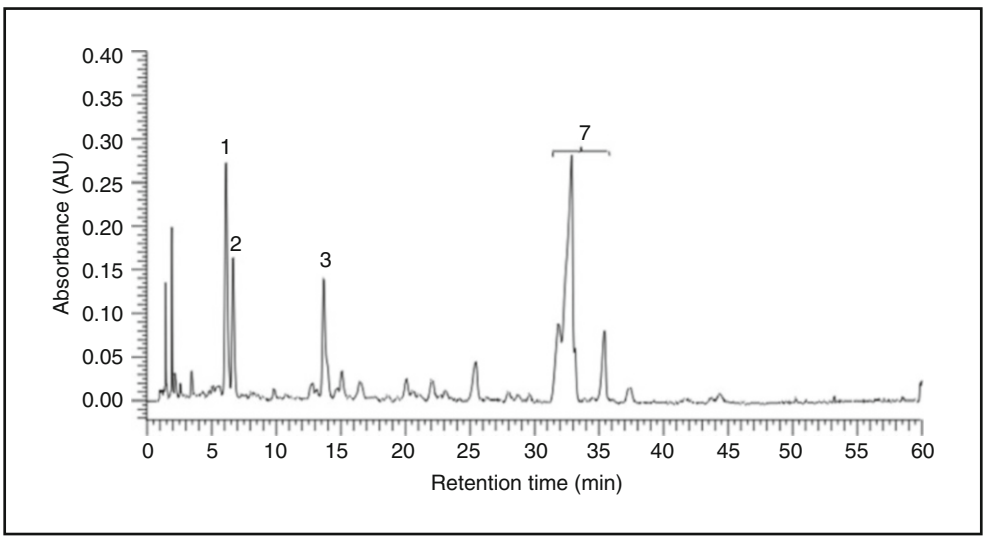


Fig. 3c: HPLC-fingerprint analysis of the methanol extract of Radix et Rhizoma Glycyrrhizae (sample 12, *Glycyrrhiza glabra*)

Figure 3c:
The HPLC-fingerprint of the root of *Glyzyrrhiza glabra*, (official in the European Pharmacopoeia) shows similarity with the fingerprint of *Glyzyrrhiza uralensis* and *Glyzyrrhiza inflata* with the exception of the lacking peaks of **4** and **5**, but with flavonoid/stilbene (peak **3**) and a strong concentration of glycyrrhizinic acid (peak **7**). The Licochalcone A (peak **8**) is lacking.

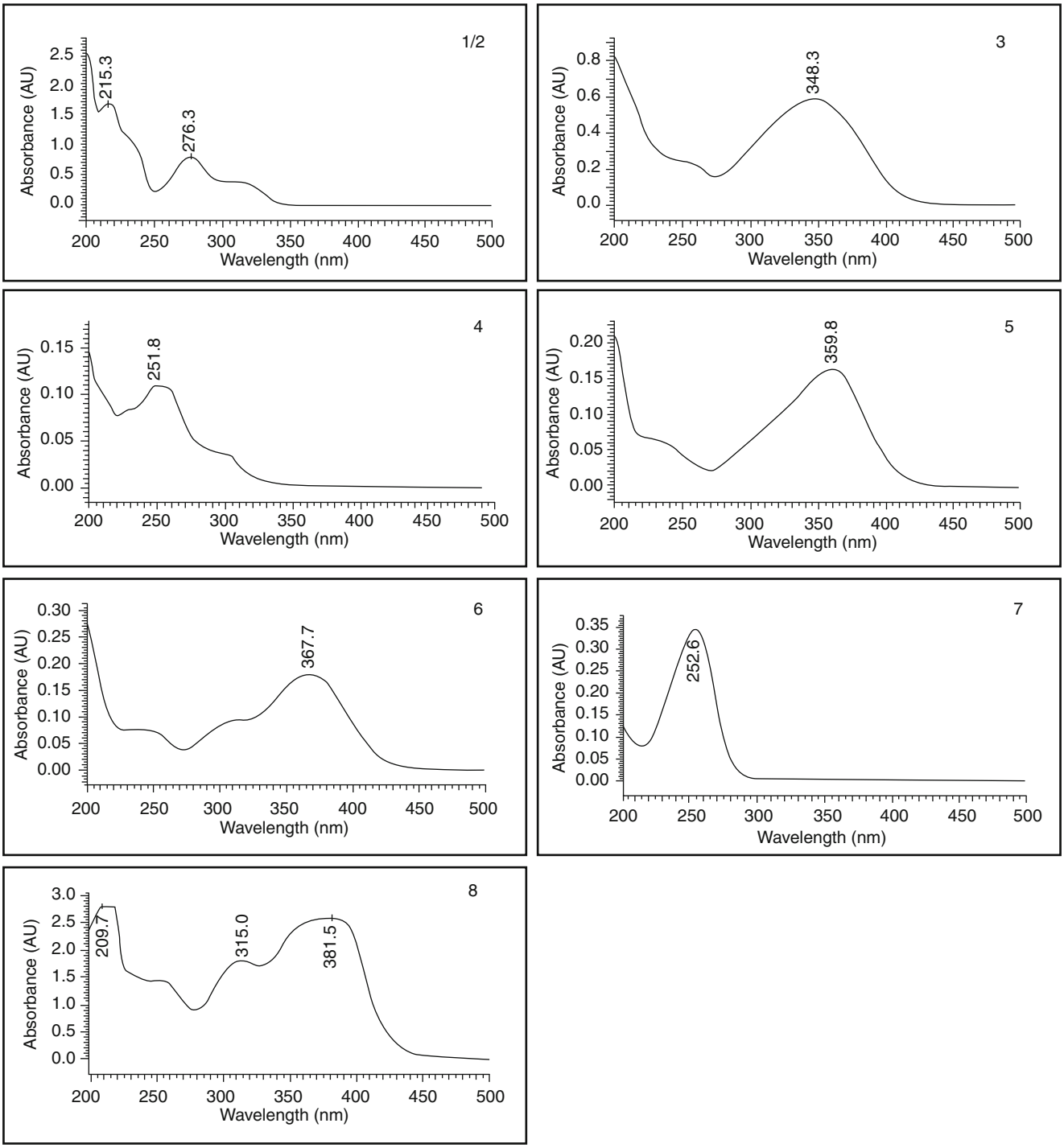


Fig. 4: On line UV-spectra of the main peaks of Radix et Rhizoma Glycyrrhizae

Note: The Chinese Pharmacopoeia 2010 demands for Radix et Rhizoma Glycyrrhizae a content not less than 2.0 % of Glycyrrhizin and 0.5 % of Liquiritin calculated with reference to the dried drug.

Further HPLC-fingerprint analytical methods can be found in the following references:[1, 7, 13–16, 19]

Conclusion

The HPLC-fingerprints of the root of *Glyzyrrhiza uralensis* and *Glyzyrrhiza glabra* are nearly identical. They differ from *Glyzyrrhiza inflata* which can be characterized by the presence of Licochalcone A.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. People's Medical Publishing House, Beijing (2010)
2. WHO monographs on selected medicinal plants, vol. 2. World Health Organization, Geneva (2002)
3. Keys, J.D.: Chinese herbs – their botany, chemistry and pharmacodynamics. Charles E. Tuttle Company, Rutland/Tokyo (1987)
4. Zhao, Z.Z.: An illustrated Chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
5. Asl, M.N., Hosseinzadeh, H.: Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. Phytother. Res. **22**(6), 709–724 (2008)
6. Fiore, C., Eisenhut, M., Ragazzi, E., Zanchin, G., Armanini, D.: A history of the therapeutic use of liquorice in Europe. J. Ethnopharmacol. **99**(3), 317–324 (2005)
7. Stöger, E.A.: Arzneibuch der chinesischen Medizin. Deutscher Apotheker Verlag, Stuttgart (2009)
8. Wichtl, M.: Teedrogen – Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage, 2nd edn. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart (1989)
9. Hempen, C.H., Fischer, T.: A materia medica for Chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone Elsevier, New York (2009)
10. Shibata, S.: A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. Yakugaku Zasshi. **120**(10), 849–862 (2000)
11. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 1. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)
12. Sabbione, C., Mandrioli, R., Ferranti, A., Bugamelli, F., Saracino, M.A., Forti, G.C., Fanali, S., Raggi, M.A.: Separation and analysis of glycyrrhizin, 18β-glycyrrhetic acid and 18α-glycyrrhetic acid in liquorice roots by means of capillary zone electrophoresis. J. Chromatogr. A **1081**(1), 65–71 (2005)
13. Tan, G., Zhu, Z., Zhang, H., Zhao, L., Liu, Y., Dong, X., Lou, Z., Zhang, G., Chai, Y.: Analysis of phenolic and triterpenoid compounds in licorice and rat plasma by high-performance liquid chromatography diode-array detection, time-of-flight mass spectrometry and quadrupole ion trap mass spectrometry. Rapid. Commun. Mass. Spectrom. **24**(2), 209–218 (2010)
14. Hong Kong Chinese materia medica standards, vol. 2, Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region, Hong Kong (2008)
15. Zhang, Q., Ye, M.: Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). J. Chromatogr. A. **1216**(11), 1954–1969 (2009)
16. Hayashi, H., Hosono, N., Kondo, M., Hiraoka, N., Ikeshiro, Y., Shibano, M., Kusano, G., Yamamoto, H., Tanaka, T., Inoue, K.: Phylogenetic relationship of six Glycyrrhiza species based on rbcL sequences and chemical constituents. Biol. Pharm. Bull. **23**(5), 602–606 (2000)
17. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin/Heidelberg/New York (2001)
18. Kimura, Y., Okuda, T., Okuda, H.: Effects of flavonoids from licorice roots (*Glycyrrhiza inflata* Bat.) on arachidonic acid metabolism and aggregation in human platelets. Phytother. Res. **7**(5), 341–347 (1993)
19. Sticher, O., Soldati, F.: Glycyrrhizinsäure-Bestimmung in Radix Liquiritiae mit Hochleistungs-flüssigkeitschromatographie (HPLC). Pharm. Acta Helv. **53**(2), 46–52 (1978)

Herba Gynostemmatis – *Jiaogulan*

Pharmacopoeia:	Not official in the Pharmacopoeias of the People's Republic of China, English Edition Vol. I, 2005 and 2010
Official drug:[1, 2]	Jiaogulan is a plant of the genus *Gynostemma* (Fam. Cucurbitaceae). The species *Gynostemma pentaphyllum* (Thunb.) Makino is the most widespread herb which is used as Jiaogulan. There exist over 21 species of *Gynostemma*, whose analytical discrimination is uncertain. The best harvest time in a greenhouse is June and outdoor in August to achieve the highest concentration of gypenosides.
Synonyma:[1]	"Southern Gingseng", Xiancao Falsifications are reported with *Cayratia japonica*. There are also polyploidy subspecies of *G. pentaphyllum*, as inferred from multiple gene sequences [3].
Origin:[2]	In China *Gynostemma pentaphyllum* is growing especially in the southern provinces of Shaan Xi and areas south of the Yangtze River. Additionally also spread over India, Nepal, Bangladesh, Sri Lanka, Laos, Myanmar, Korea and Japan.
Description of the fresh drug:[2]	The plant *G. pentaphyllum* consists of slender stems of thin, soft leaves arranged like fingers on a hand, bearing 3–9 (mainly 5–7) leaves. The leaflets are long and pliable, broadest below the middle and tapering to a point like a lance.
Pretreatment of the raw drug:[1]	Teas are made mainly from dried leaves with the highest concentration of saponins. If available also the other plant parts can be chopped and used.
Cultivation:[1]	Jiaogulan can be found growing wild, but hardly in cold climates. It can be successfully cultivated also in a greenhouse but requires warm, not too dry temperatures.
Medicinal use:[2]	Clinical studies have shown that *G. pentaphyllum* is effective in the treatment of diabetes, migraine, chronic bronchitis, hepatitis-B, dysrhythmia, chronic gastritis, gastric ulcer and leucopenia.

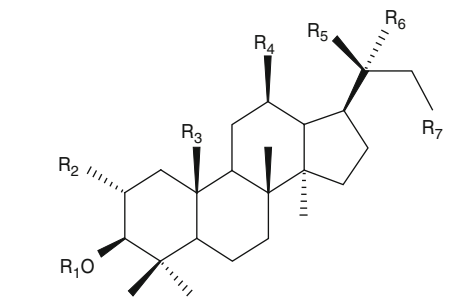
H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_6, © Springer International Publishing Switzerland 2015

Effects and indications of Herba Gynostemmatis according to Traditional Chinese Medicine [2, 4]

Taste:	Slightly bitter and sweet
Temperature:	Neutral to warm
Channels entered:	*Orbis pulmonaris*, *Orbis cardialis*
Effects (functions):	Enhancing *Yin* and supporting *Yang*
Symptoms and indications:	Increases the resistance to infection and for antiinflammation. Heat clearing, detoxification, antitussive, heart palpitation, fatigue syndrome, chronic bronchitis and relieving cough. Treatment of hematuria, edema, pain of the pharynx, heat and edema of the neck, tumours and trauma. Indications include hyperlipidemia, palpitation, shortness of breath, chest congestion, tingling sensation in the limbs, dizziness, headache, forgetfulness, tinnitus, spontaneous perspiration, general weakness, swelling of abdomen, Qi deficiency of heart and spleen and stagnation of phlegm and blood.

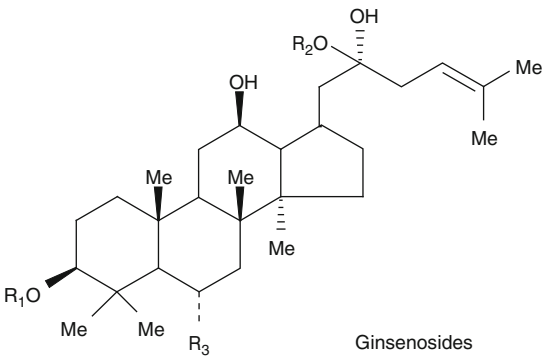
Main Constituents of *G. pentaphyllum* [2, 4–9]

Dammarane saponins	Gypenosides I, II, III, IV ,V, VI, VII, VIII, IX, X, XI, XIII, XIV, XV, XVI, XVII, XIX, XXII, XXIII, XXV, XXVIII, XXXVIII, XLIX, LV, LVIII, LXII, LXIII, LXXV (= gynosides A, B, C, D, E, F, O, Q, R, T, U, TN-1, TN-2, TR-1)
	Ginsenosides Rb_1, Rb_2, F_2, Rc, R_f, Rg_3, malonyl-Rb_1, malonyl-Rd
Sterols	Sitosterol, stigmasterol, chondrillasterol, spinasterol, ergostanol
	Steroids: 3β-hydroxy-14α-dimethyl-5α-ergosta-9(11),
	24(28)-diene, 3β-hydroxy-4α-methyl-5α-ergosta-7,9(11), 24(28)-triene, 3β-hydroxy-(24R)-14α-methyl-5α-ergosta-9(11)-ene, 3β-hydroxy- 24(S)-14α-dimethyl-5α-ergosta-9(11)-ene, 3β-hydroxy-24, 24-dimethyl-5α-cholestan
Flavonoids/phenol-carboxylic acids	Quercetin-rhamno-glucoside (rutin), kaempferol-3-*O*-rhamno-glucoside (ombuoside), vitexin, yixingensin, caffeic acid
Other compounds	Amino acids, proteins and vitamins, carotenoids, allantoin
Monosaccharides	β-D-glucose, β-D-xylose, α-L-arabinose, α-L-rhamnose



Gypenosides

R_1 = Glucose (Arabinose, Rhamnose)
R_2 = H /OH
R_3 = CH_3 / CH_2OH/ CHO
R_4 = H /OH
R_5 = Glucose (Rhamnose, Xylose)
R_6 = CH_3 / CH_2OH/ OH/ CH_2O-Glucose
R_7 =various C_5-residues (a-i)[4]



Ginsenosides

Ginsenosides	R_1	R_2	R_3
Rb_1	O-β-D-Glucopyranosyl-(1→2)-β-D-gluco-pyranosyl	O-β-D-Glucopyranosyl-(1→6)-β-D-gluco-pyranosyl	-
Rb_3	O-β-D-Glucopyranosyl-(1→2)-β-D-gluco-pyranosyl	O-β-D-Xylopyranosyl-(1→6)-β-D-gluco-pyranosyl	-
Rc	O-β-D-Glucopyranosyl-(1→2)-β-D-gluco-pyranosyl	O-β-D-Arabinofuranosyl-(1→6)-β-D-gluco-pyranosyl	-
Rd	O-β-D-Glucopyranosyl-(1→2)-β-D-gluco-pyranosyl	O-β-D-Glucopyranosyl	-
Malonyl-GinsenosideRd	6-O-Malonyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl	O-β-D-Glucopyranosyl	-
Rg_1	H	O-β-D-Glucopyranosyl	O-β-D-Gluco-pyranosyl

Fig. 1: Formulae of the main compounds of Herba Gynostemmatis [2, 8, 10, 11]

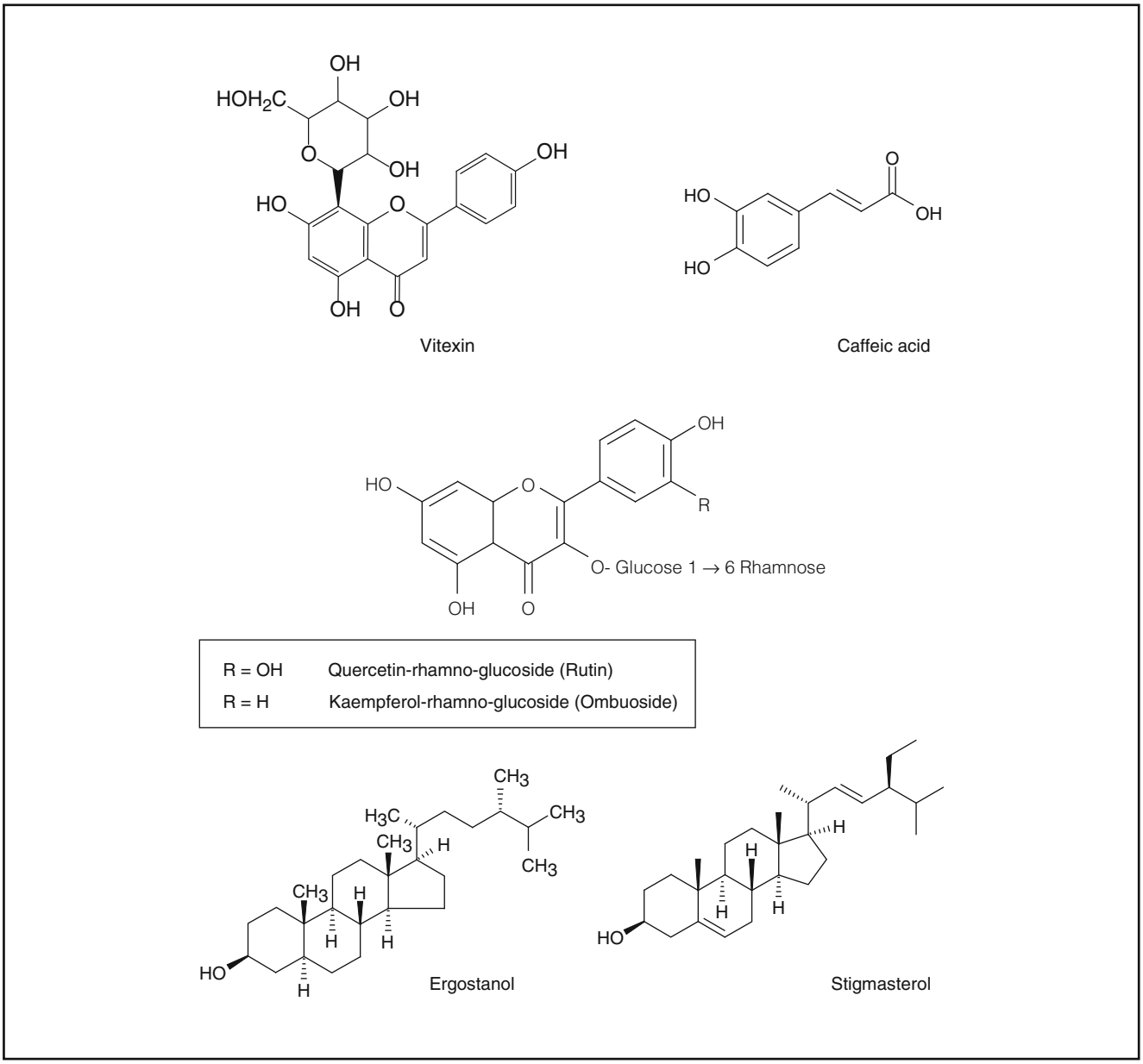


Fig. 1: (continued)

Reported Pharmacological Activities

In vitro, *in vivo*, Clinic research

Antihyperglycemic Activity [2]

- hypoglycemic [2, 7, 12–16]
- stimulating insulin release [2, 12–15]
- improving glucose tolerance [2, 14]

Effects on the Lipid Metabolism [2]

- hypolipidemic (TG, LDL, VLDL) [6, 12, 14–17]
- hypocholesterolemic (TC) [4, 6, 9, 12, 14, 16]
- raising high-density lipoprotein (HDL) [14]
- reduction of post-prandial hypertriglyceridemia [14]

Cardiovascular Activities [2, 10, 14, 15, 17, 18]

- hypotonic [2, 6, 12]
- antiarrhythmic [2, 6]
- protecting myocardial cells during infarction [2]

Effects on Central Nervous System [2, 10]

- antischemic [2]
- improving post-ischemic damages [2]
- alleviating dysmnesia [2]

Effects on Immune Functions [2, 10]

- immunomodulatory / -stimulating [2, 7, 12, 16]
- anticarcinogenic [2, 4, 9, 12, 14, 17, 19]
- anti-inflammatory [2, 6, 9, 12, 14–17]
- antioxidative / antioxidant [12, 16–18]
- anti-allergic [12]

Effects on Platelet Aggregation and Arachidonic Acid Metabolism [2, 9]

- antithrombotic [2, 17, 18]

Various Cell (Organ) Effects [2, 9, 10, 12, 17, 19, 20]

- reduction of free radical injuries [2]
- hepatic regeneration / liver cancer [2, 6, 20]
- inhibition of mutagenicity [6]
- antidiabetic effects [10, 12, 14, 16, 21]
- antitumoral activities [2, 6, 10, 11, 16]
- antileukemic activities [17, 22]
- metabolic syndrome (see also: improving glucose tolerance, hypolipidemic, antiarrhythmic, hypotonic, hypoglycemic) [21]
- radiation protective [5]
- anti-atherosclerotic [21]

TLC-Fingerprint Analysis [23, 24]

	Drug samples	Origin
1	Herba Gynostemmatis/*Gynostemma pentaphyllum*	Sample of commercial drug (China Medica, Germany)
2	Herba Gynostemmatis/*Gynostemma pentaphyllum*	Province Fujian, China
3	Herba Gynostemmatis/*Gynostemma pentaphyllum*	Province Anhui, China
4	Herba Gynostemmatis/*Gynostemma pentaphyllum*	sample of commercial drug (Beijing, China)
5	Herba Gynostemmatis/*Gynostemma pentaphyllum*	sample of commercial drug (Dein-Teeladen, Germany)
6	Herba Gynostemmatis/*Gynostemma sp.*	S001 (PLANTASIA GmbH, Mag. E. Stöger, Oberndorf, 5110 Österreich)
7	Herba Gynostemmatis/*Gynostemma sp.*	S002 (PLANTASIA GmbH, Mag. E. Stöger, Oberndorf, 5110 Österreich)
8	Herba Gynostemmatis/*Gynostemma sp.*	S003 (PLANTASIA GmbH, Mag. E. Stöger, Oberndorf, 5110 Österreich)
9	Herba Gynostemmatis/*Gynostemma sp.*	S005 (PLANTASIA GmbH, Mag. E. Stöger, Oberndorf, 5110 Österreich)
10	Herba Gynostemmatis/*Gynostemma sp.*	S007 (PLANTASIA GmbH, Mag. E. Stöger, Oberndorf, 5110 Österreich)

1. TLC fingerprint analysis of flavonoids and phenylcarboxylic acids:

	Reference compounds of Fig. 2	**R*f***
T1	Rutin	0.41
T2	Caffeic acid	0.95
T3	Quercitrin	0.82
n.a.	Kaempferol-triglycoside	~ 0.20
n.a.	Kaempferol-diglycoside	0.38
n.a.	Kaempferol-3-*O*-rhamno-glucoside (Ombuoside)	0.44
n.a.	Chlorogenic acid	0.48
n.a.	Vitexin	0.65
n.a.	Isoquercitrin	0.65
n.a.	Kaempferol/ Quercetin	0.97–0.99

n.a not applied

1. Extraction: 1 g powdered drug is extracted with 10 ml ethanol (90 %) under reflux for 10 min. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol (90 %) and filtered over Chromafil® filtration unit, type 0–20 µm /25 mm.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Herba Gynostemmatis extracts: 8 µl each

Reference compounds: 10 µl each

Solvent system and detection for flavonoids and organic acids (**Fig. 2**)

Solvent system: Ethyl acetate + formic acid + glacial acetic acid + water (10 + 1.1 + 1.1 + 2.6)

Detection: Natural products – Polyethylene glycol reagent (NP/PEG):

I: 1 % diphenylboric acid-β-ethylamino ester (= diphenylboryloxyethyl-amine, 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 under UV 366 nm

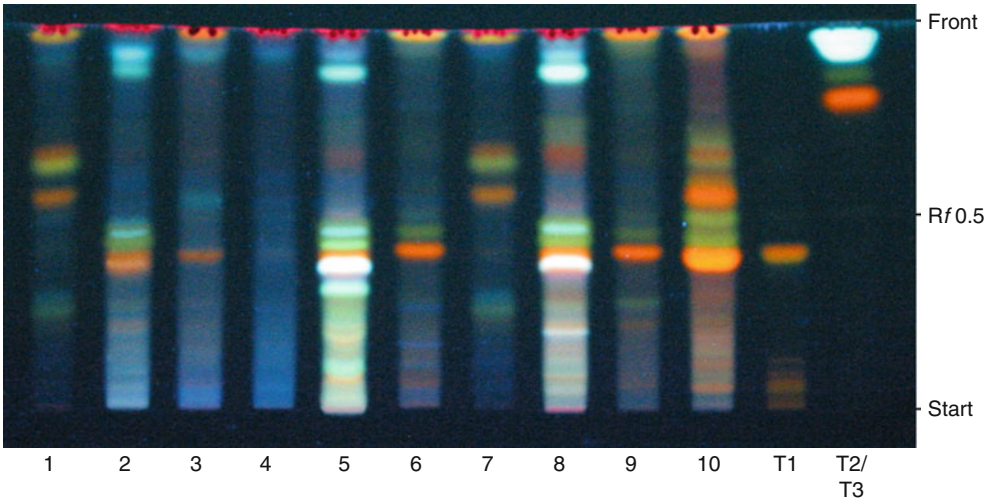


Fig. 2: Thin layer chromatogram of the ethanol extracts of Herba Gynostemmatis (organic acids and flavonoids) sprayed with NP/PEG (UV 366 nm)

Description of Fig. 2:

The ten extract samples of *Gynostemma pentaphyllum* show a very inhomogeneous flavonoid and phenolcarboxylic acid pattern of orange, green, blue and yellow fluorescent zones distributed over the whole plate distance. This inhomogeneity might be due to a different composition of the stems, leaves and flowers in the commercial overground parts of the herbs. Another reason could be the unknown origin of some samples or a possible falsification with *Cayratia japonica*. With respect to the known composition of the main constituents reported in the literature for the herbs of *Gynostemma pentaphyllum* the samples No. 5, 8 and 10 represent best the characteristic chemical composition of official *Gynostemma* species with kaempferol-triglycoside at R*f* = ~ 0.20, kaempferol-diglycoside at R*f* = 0.38, rutin (**T1**) at R*f* = 0.41, kaempferol-3-*O*-rhamno-hexoside (ombuoside) at R*f* = 0.44, chlorogenic acid at R*f* = 0.48, vitexin at R*f* = 0.64 (green zone), isoquercitrin at R*f* = 0.65 (orange zone), quercitrin (**T3**) at R*f* = 0.82, caffeic acid (**T2**) at R*f* = 0.95, kaempferol/ quercetin at R*f* = 0.97–0.99.

2. TLC fingerprint analysis of gypenosides and ginsenosides:

	Reference compounds of Fig. 3	**R*f***
T4	Ginsenoside Re	0.34
T5	Ginsenoside Rb1	0.11
T6	Ginsenoside Rg1	0.55
T7	Ginsenoside Rd	0.33
n.a	Stigmasterol/ Ergostanol/Steroids	0.85–0.98

n.a. not applied

Solvent system and detection for gypenosides and ginsenosides (**Fig. 3**)

Solvent system: Chloroform + methanol + water (7 + 3 + 0.4)

Detection: Vanillin – Phosphoric acid reagent

1 g vanillin is dissolved in small quantity of ethanol and filled up to 100 ml with 50 % aqueous phosphoric acid.

The plate is sprayed with this solution, heated for 5 min at 105 °C and evaluated in VIS.

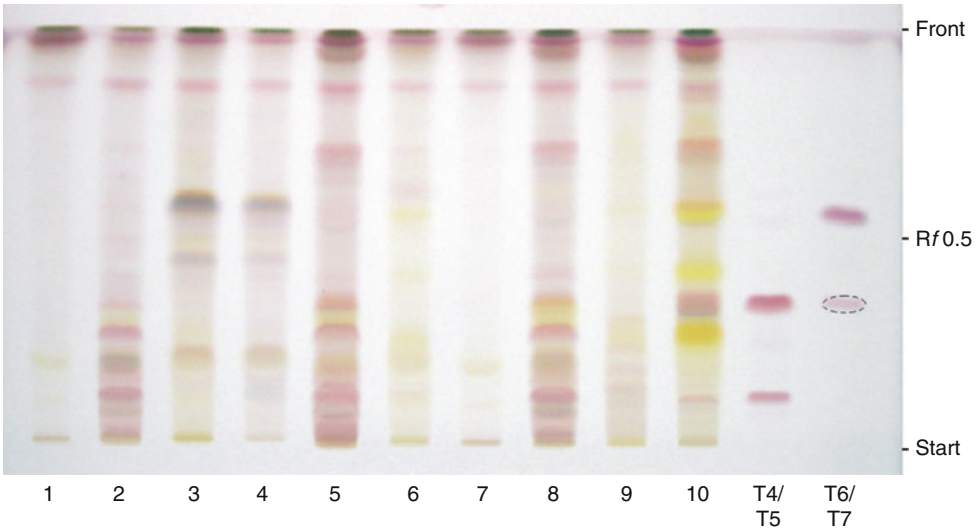


Fig. 3: TLC of the ethanol extracts of Herba Gynostemmatis (ginsenosides and gypenosides) sprayed with Vanillin – Phosphoric acid reagent (VIS)

Description of Fig. 3:

In this TLC-Fingerprint profile the *Gynostemma* samples 2, 5 and 8 show best the characteristic dammarane triterpene-, tri- and tetraglycosides pattern: Ginsenoside Rb1 (**T5**) at R*f* = 0.11, Ginsenoside Rd (**T7**) at R*f* = 0.33, Ginsenoside Re (**T4**) at R*f* = 0.34, Ginsenoside Rg1 (**T6**) at R*f* = 0.55. The mono-, diglycosides and gypenosides appear in the upper R*f* -range: ~ 0.5–0.9.
Note: Further TLC-fingerprint analytical methods can be found in the following references: [17, 25]

HPLC-Fingerprint Analysis

1. Sample preparation: 1 g powdered drug is extracted with 50 ml methanol under reflux for 3 h at 60 °C. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 5 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm.
2. Injection volume: Herba Gynostemmatis extracts: 10 µl each
3. HPLC parameters:

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_4/1 l water (Millipore Ultra Clear UV plus® filtered)
	B: acetonitrile (VWR)
Gradient:	5–45 % B in 60 min
Flow:	1 ml/min
Detection:	205 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	19.4	Rutin
2	19.9	Ombuoside?
3	39.7	Ginsenoside Rb1
4	41.7	Ginsenoside Rd?

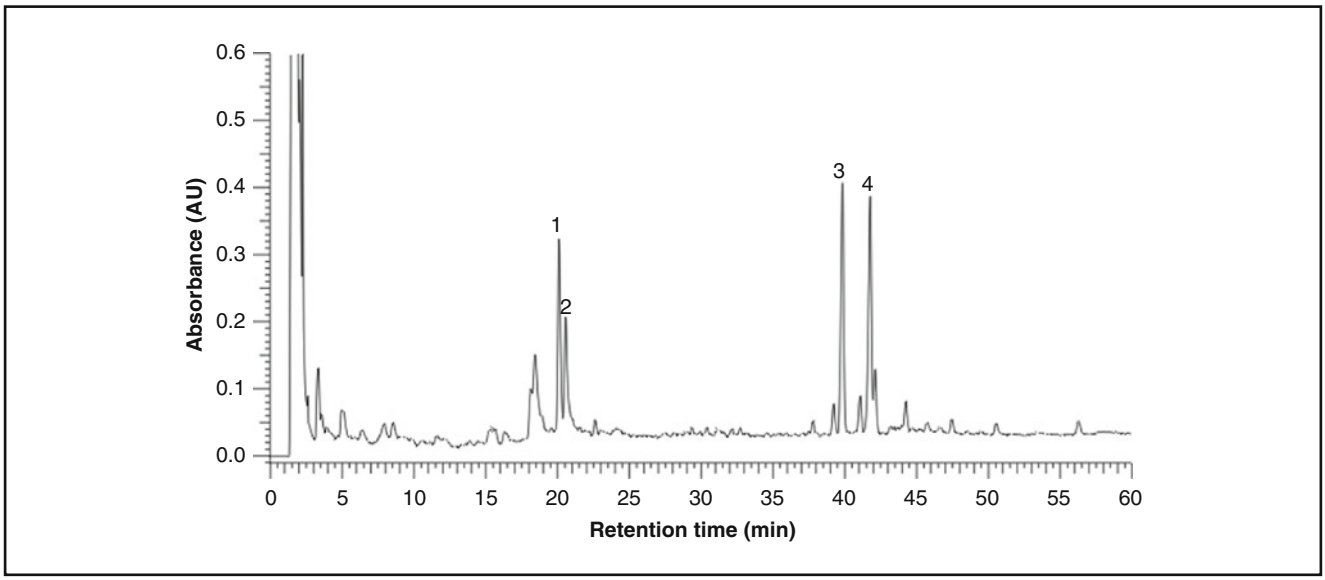


Fig. 4a: HPLC-fingerprint analysis of the methanol extract of Herba Gynostemmatis, sample 2

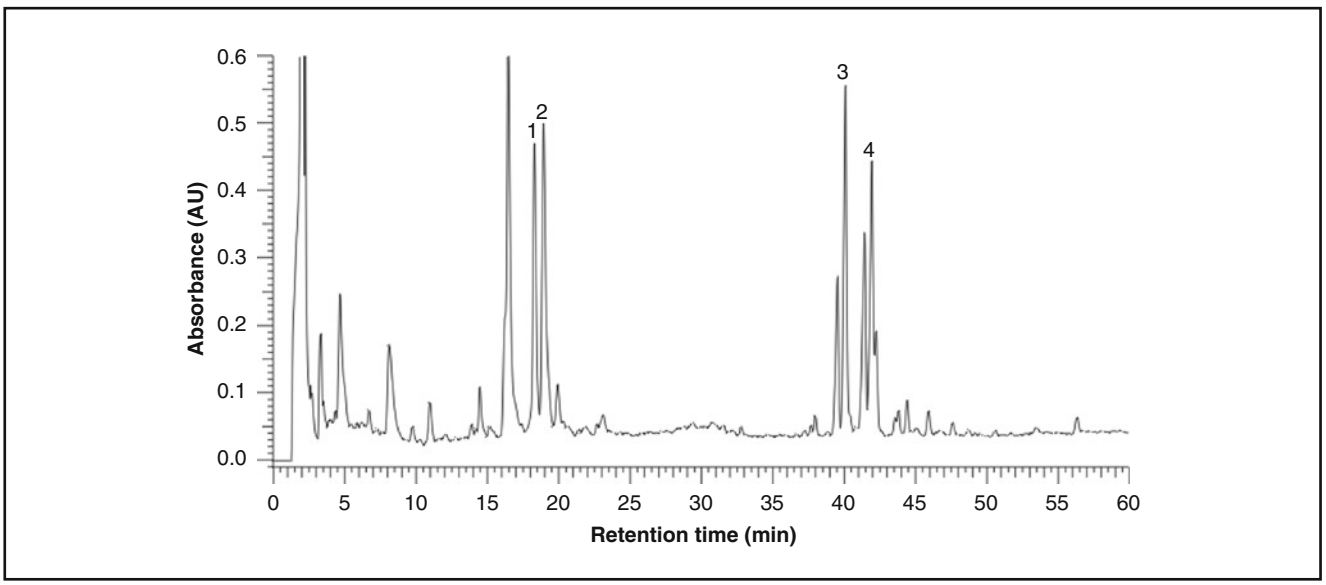


Fig. 4b: HPLC-fingerprint analysis of the methanol extract of Herba Gynostemmatis, sample 5

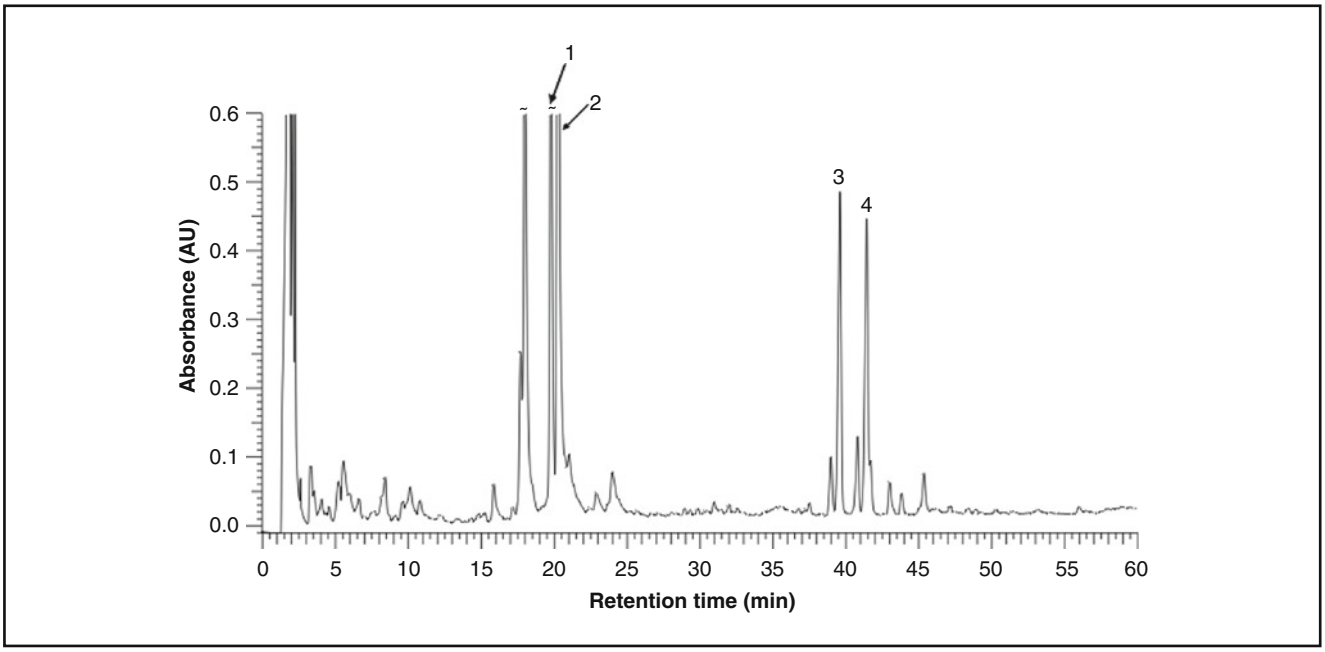


Fig. 4c: HPLC-fingerprint analysis of the methanol extract of Herba Gynostemmatis, sample 8

4. Description of the HPLC-Figures 4a, b and c:

The three most characteristic peak pattern of *Gynostemma pentaphyllum* extract samples 2, 5 and 8 consist of a peak block between Rt 15.0–22.0 and a second one between Rt 39.0–43.0. The first consists of flavonoids e.g. rutin at Rt = 19.4 (**1**), with two or three peaks nearby, probably kaempferol-3-*O*-rhamno-glucoside (ombuoside) at Rt = 19.9 (**2**). In the Rt-range of 39.0–43.0 we find at Rt = 39.7 ginsenoside Rb1 (**3**) associated with several further peaks. Peak **4** might be identical with ginsenosid Rd.

Note: Further HPLC-fingerprint analytical methods for identification of the characteristic triterpene glycosides can be found in the following references: [6, 8, 9, 11, 13, 16, 19–21, 25]

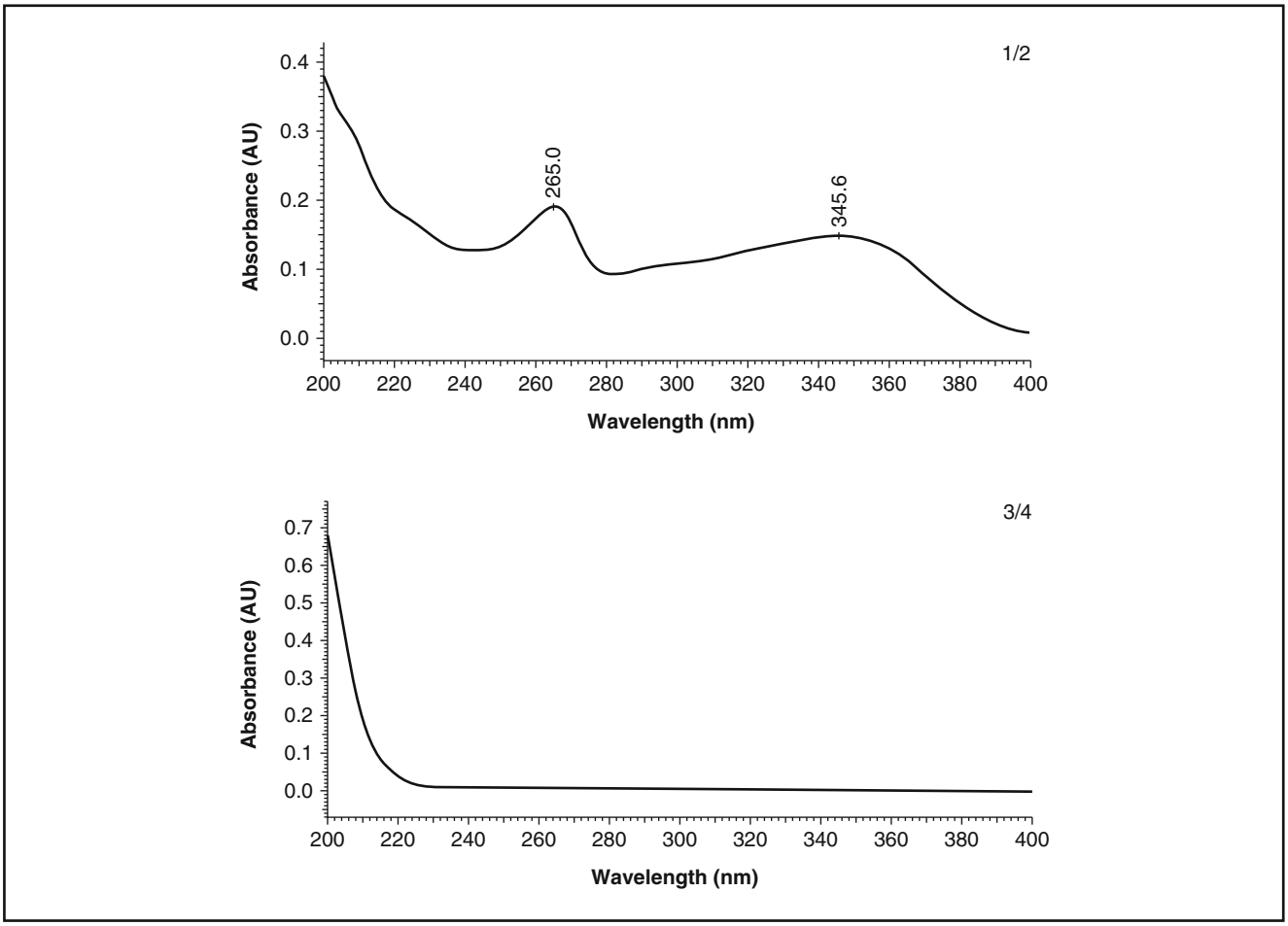


Fig. 5: On line UV-spectra of the detected peaks of Herba Gynostemmatis

Conclusion

The official *Gynostemma* species are best represented by the TLC- and HPLC-fingerprints of samples 5, 8, 10 (TLC, **Figs.** 2 and 3) and samples 2, 5 and 8 (HPLC, **Fig.** 4a, 4b and 4c). The authentication of the herb can be achieved by the characteristic polyphenol flavonoid- and triterpene glycoside fingerprints.

References

1. Blumert, M., Liu, J.: Jiaogulan – China's "immortality" herb, 3rd edn. Torchlight Publishing, Inc, Badger CA (2003)
2. Razmovski-Naumovski, V., Huang, T., Tran, V., Li, G., Duke, C., Roufogalis, B.: Chemistry and pharmacology of *Gynostemma pentaphyllum*. Phytochem. Rev. **4**(2–3), 197–219 (2005)
3. Jiang, L.Y., Qian, Z.Q., Guo, Z.G., Wang, C., Zhao, G.F.: Polyploid origins in *Gynostemma pentaphyllum* (Cucurbitaceae) inferred from multiple gene sequences. Mol. Phylogenet. Evol. **52**(1), 183–191 (2009)
4. Chen, J.: Gynostemma: an undiscovered treasure. Acupunct Today **3**(9), 1–4 (2002)

5. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants: chemistry, pharmacology, toxicology. WILEY-VCH Verlag, GmbH & Co. KGaA, Weinheim/Berlin (2011)
6. Cheng, T.C., Lu, J.F., Wang, J.S., Lin, L.J., Kuo, H.I., Chen, B.H.: Antiproliferation effect and apoptosis mechanism of prostate cancer cell PC-3 by flavonoids and saponins prepared from Gynostemma pentaphyllum. J. Agric. Food Chem. **59**(20), 11319–11329 (2011)
7. Schmitz-Hübsch, M., Koch, A., Hanssen, H.-P.: Jiaogulan – Das "Kraut der Unsterblichkeit", Deutsche Apotheker Zeitung. **149**. Jahrgang (10), 1024 (2009)
8. Schild, L., Chen, B.H., Makarov, P., Kattengell, K., Heinitz, K., Keilhoff, G.: Selective induction of apoptosis in gliom tumour cells by a *Gynostemma pentaphyllum* extract. Phytomedicine **17**(8–9), 589–597 (2010)
9. Kao, T.H., Huang, S.C., Stephen Inbaraj, B., Chen, B.H.: Determination of flavonoids and saponins in *Gynostemma pentaphyllum* (Thunb.) Makino by liquid chromatography-mass spectrometry. Anal. Chim. Acta **626**(2), 200–211 (2008)
10. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin: chemistry, pharmacology and medicinal use in taditional and modern medicine. Springer, Berlin/Heidelberg (1992)
11. Liu, F., Ren, D., Guo, D.A., Pan, Y., Zhang, H., Hu, P.: Method development for gypenosides fingerprint by high performance liquid chromatography with diode-array detection and the addition of internal standard. Chem. Pharm. Bull. **56**(3), 389–393 (2008)
12. Norberg, A., Hoa, N.K., Liepinsh, E., Van Phan, D., Thuan, N.D., Jörnvall, H., Sillard, R., Östenson, C.G.: A novel insulin-releasing substance, phanoside, from the plant *Gynostemma pentaphyllum*. J. Biol. Chem. **279**(40), 41361–41367 (2004)
13. Hoa, N.K., Norberg, A., Sillard, R., Van Phan, D., Thuan, N.D., Dzung, D.T., Jörnvall, H., Östenson, C.G.: The possible mechanisms by which phanoside stimulates insulin secretion from rat islets. J. Endocrinol. **192**(2), 389–394 (2007)
14. Megalli, S., Davies, N.M., Roufogalis, B.D.: Anti-hyperlipidemic and hypoglycemic effects of *Gynostemma pentaphyllum* in the Zucker fatty rat. J. Pharm. Pharm. Sci. **9**(3), 281–291 (2006)
15. Zhang, H.J., Ji, B.P., Chen, G., Zhou, F., Luo, Y.C., Yu, H.Q., Gao, F.Y., Zhang, Z.P., Li, H.Y.: A combination of grape seed-derived procyanidins and gypenosides alleviates insulin resistance in mice and HepG2 cells. J. Food Sci. **74**(1), H1–H7 (2009)
16. Hung, T.M., Hoang, D.M., Kim, J.C., Jang, H.S., Ahn, J.S., Min, B.S.: Protein tyrosine phosphatase 1B inhibitory by dammaranes from Vietnamese Giao-Co-Lam tea. J. Ethnopharmacol. **124**(2), 240–245 (2009)
17. Hsu, H.Y., Yang, J.S., Lu, K.W., Yu, C.S., Chou, S.T., Lin, J.J., Chen, Y.Y., Lin, M.L., Chueh, F.S., Chen, S.S., Chung, J.G.: An experimental study on the antileukemia effects of gypenosides *in vitro* and *in vivo*. Integr. Cancer Ther. **10**(1), 101–112 (2011)
18. Circosta, C., De Pasquale, R., Occhiuto, F.: Cardiovascular effects of the aqueous extract of *Gynostemma pentaphyllum* Makino. Phytomedicine **12**(9), 638–643 (2005)
19. Chen, J.C., Lu, K.W., Tsai, M.L., Hsu, S.C., Kuo, C.L., Yang, J.S., Hsia, T.C., Yu, C.S., Chou, S.T., Kao, M.C., Chung, J.G., Wood, W.G.: Gypenosides induced G0/G1 arrest via CHk2 and apoptosis through endoplasmic reticulum stress and mitochondria-dependent pathways in human tongue cancer SCC-4 cells. Oral Oncol. **45**(3), 273–283 (2009)
20. Tsai, Y.C., Lin, C.L., Chen, B.H.: Preparative chromatography of flavonoids and saponins in *Gynostemma pentaphyllum* and their antiproliferation effect on hepatoma cell. Phytomedicine **18**(1), 2–10 (2010)
21. Tan, Y., Kamal, M.A., Wang, Z.Z., Xiao, W., Seale, J.P., Qu, X.: Chinese herbal extracts (SK0506) as potential candidate for therapy of the metabolic syndrome. Clin. Sci. **120**(7), 297–305 (2011)
22. Lin, J.J., Hsu, H.Y., Yang, J.S., Lu, K.W., Wu, R.S., Wu, K.C., Lai, T.Y., Chen, P.Y., Ma, C.Y., Wood, W.G., Chung, J.G.: Molecular evidence of anti-leukemia activity of gypenosides on human myeloid leukemia HL-60 cells *in vitro* and *in vivo* using a HL-60 cells murine xenograft model. Phytomedicine **18**(12), 1075–1085 (2011)
23. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer Verlag, Berlin/Heidelberg/New York (2001)
24. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines: thin layer and high performance liquid chromatography of Chinese drugs, vol. 1 and 2. Springer, Wien (2011)
25. Schild, L., Roth, A., Keilhoff, G., Gardemann, A., Brödemann, R.: Protection of hippocampal slices against hypoxia/hypoglycaemia injury by a *Gynostemma pentaphyllum* extract. Phytomedicine **16**(8), 734–743 (2009)

Herba Sarcandrae – *Zhongjiefeng*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Glabrous Sarcandra Herb is the dried herb of *Sarcandra glabra* (Thunb.) Nakai (Fam. Chloranthaceae).

The drug is collected in summer and autumn, removed from foreign matters and dried in the sun.

Synonym:[2] *Chloranthus glaber* (Thunb.) Makino.

Origin:[3–5] Southern China: provinces Jiangxi, Fujian, Zhejiang, Sichuan and Guangxi.

Description of the drug:[1] 50–120 cm long. Rhizomes relatively large, with numerous rootlets. Stems cylindrical, frequently branched, 0.3–1.3 cm in diameter; externally dark green to dark brown, with distinct fine longitudinal striations, longitudinal lenticels scattered, nodes swollen; texture fragile, easily broken, fracture medullated or hollowed. Leaves opposite, lamina ovate-lanceolate to ovate-elliptical, 5–15 cm long, 3–6 cm wide; externally green, greenish-brown to dark brown or brownish-red, glabrous, margin roughly serrate, the tips of serrations with blackish-brown glandular bodies, petiole about 1 cm long; texture nearly leathery. Spikes terminal, frequently branched. Odour, slightly aromatic; taste, slightly pungent.

Medicinal use:[3, 6] Traditionally used in China as herbal tea or food supplement to enhance mental efficiency and for recoverment from stress and fatigue, effective in the treatment of cancer, pneumonia, appendicitis, gastritis, enteritis, diarrhea, rheumatism, and injuries from falls and fracture.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_7, © Springer International Publishing Switzerland 2015

Effects and indications of Herba Sarcandrae according to Traditional Chinese Medicine:[1]

Taste:	Bitter and pungent
Temperature:	Neutral
Channels entered:	*Orbis hepaticus, Orbis cardialis*
Effects (functions):	To reduce *heat* in the blood, activate blood circulation and remove ecchymoses, expel *wind* and remove obstruction from meridians.
Symptoms and indications:	Purpura due to *heat* in the blood, impediment disease with pain due to *wind-dampness*, traumatic injuries.

Main Constituents [2, 3, 5, 7–11]

Coumarins	Isofraxidin and other coumarins (e.g. sarcandracoumarin, biisofraxidin, esculetin, scopoletin)
Phenolic carboxylic acids	Caffeic acids and derivatives (e.g. isochlorogenic acids, rosmarinic acid, 4′-O-β-D-glycopyranosyl rosmarinic acid and other carboxylic acids)
Butendicarbonic acid	Fumaric acid
Sesquiterpenes (glycosides)	E.g. atractylenolide II and III, eudesmanolide, elemanolide, lindenana, germacranolide (aglycone)
Flavonoids	(E.g. quercetin, kaempferol-glycosides, dihydrochalcones, dihydroxy-flavanones)
Perhydronaphtofuran derivatives	Istanbulin A
Triterpene saponines	Sarcandrosides A+B

Pharmacology

In vitro and *in vivo*

- antioxidative effects [3]
- immunomodulatory effects [3]
- anti-tumor, cytotoxic effects [8, 9, 13]
- antimicrobial: anti-bacterial, antifungal effects [8, 9, 13]
- anti-inflammatory effects [8, 9]
- hepatoprotective effects [5, 14]

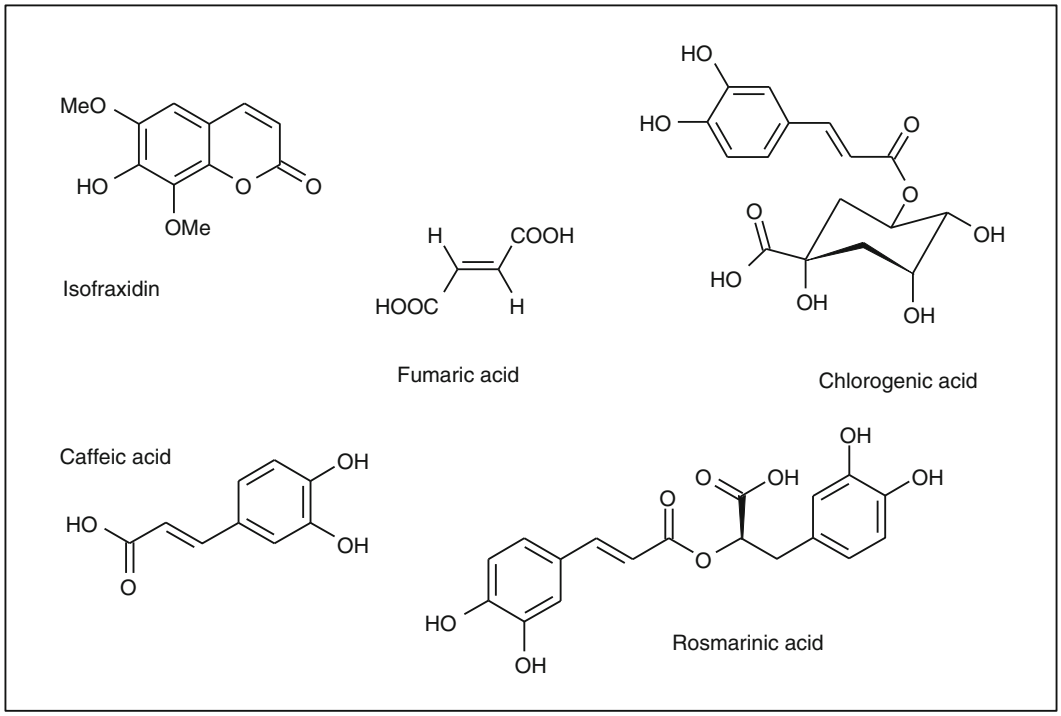


Fig. 1: Formulae of the main compounds of Herba Sarcandrae [3, 6, 12]

TLC-Fingerprint Analysis[1, 15]

	Drug samples	Origin
1	Herba Sarcandrae/*Sarcandra glabra*	Sample of commercial drug (Sinomed, TCM-Clinic Bad Kötzting)
2	Herba Sarcandrae/*Sarcandra glabra*	Sample of commercial drug (Marketing in Beijing, origin: Jiangxi)
3	Herba Sarcandrae/*Sarcandra glabra*	Sample of commercial drug (Marketing in Beijing, origin: Jiangxi)
4	Herba Sarcandrae/*Sarcandra glabra*	Sample of commercial drug (Marketing in Beijing, origin: Jiangxi)
5	Herba Sarcandrae/*Sarcandra glabra*	Sample of commercial drug (Marketing in Beijing)

	Reference compounds	R*f* (Fig. 2)	R*f* (Fig. 3)
T1	Isofraxidin	0.39	0.37
T2	Fumaric acid	n.d.	0.15
T3	Chlorogenic acid and Isochlorogenic acid	0.10 + 0.17	0.06 + 0.08
T4	Caffeic acid (impure)	0.46	0.30
T5	Rosmarinic acid	0.28	0.12

1. Extraction: 2 g of the powdered drug are extracted with 50 ml methanol in an ultrasonic bath for 30 min. 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(a) Separation parameters **Fig. 2**:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Herba Sarcandrae extracts: 5 µl each reference compounds: 10 µl each
Solvent system:	Toluene + ethyl acetate + formic acid (9 + 4 + 1)
Detection:	Natural products – Polyethylene glycol reagent (NP/PEG) I: 1 % diphenylboric acid-*β*-ethylamino ester (= diphenylboryloxyethylamin, 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 under UV 366 nm.

Description of Fig. 2:

All five Herba Sarcandrae extracts show a very homogeneous pattern of three main and 3–4 minor blue-green fluorescent zones from start up to R*f* = 0.5 with caffeic acid (**T4**) at R*f* = 0.46, isofraxidin (**T1**) at R*f* = 0.39, rosmarinic acid (**T5**) at R*f* = 0.28 and chlorogenic//isochlorogenic acid (**T3**) at R*f* = 0.10//0.17. Fumaric acid (**T2**) is not detectable with this reagent (see **Fig. 3**). In the upper R*f*-range from R*f* = 0.55 up to R*f* = 0.90 appear 4 and 5 red zones which can be assigned to chlorophyll-derivatives.

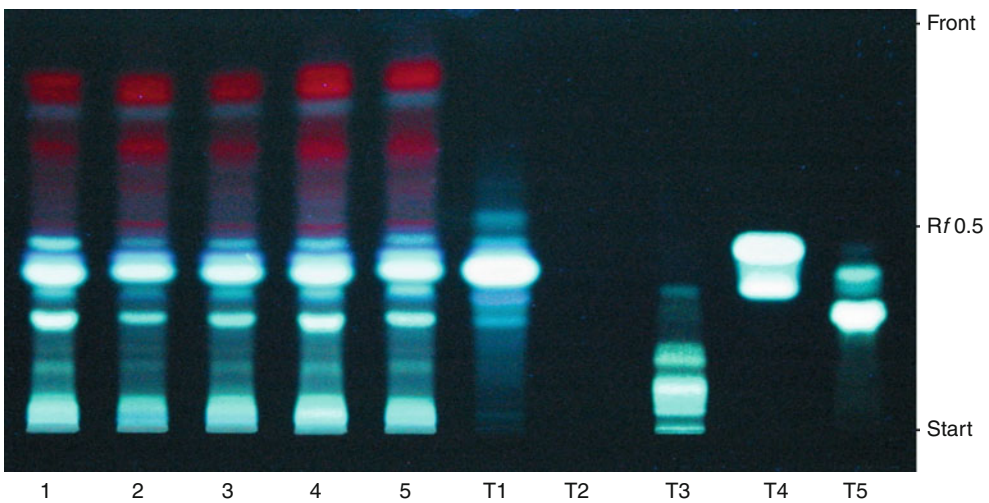


Fig. 2: Thin layer chromatogram of the methanol extracts of Herba Sarcandrae, sprayed with NP/PEG reagent (UV 366 nm)

3(b) Separation parameters **Fig. 3**:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Herba Sarcandrae extracts: 10 µl each reference compounds: 10 µl each
Solvent system:	I ethyl acetate + methanol + water (100 + 17 + 13) After developing about 3 cm and removal of the plate, dry in air, and then use solvent system II. II toluene + ethyl acetate + formic acid + water (upper phase) (20 + 10 + 1 + 1) After developing up to 8 cm and removal of the plate, dry in air.
Detection:	Bromocresol Green reagent 0.02 g bromocresol is dissolved in 15 ml ethanol. Ammonia solution is added drop wise until the orange color turns to blue. The plate is sprayed with this solution and heated at 105 °C for 20 min.

Description of Fig. 3:

This reagent serves only to detect the marker compound fumaric acid, which appears immediately after heating as yellow zone at R*f* = 0.30. After one day also the other organic acids inclusive the coumarin isofraxidin and the chlorophyll derivatives can be detected as small dark green zones.

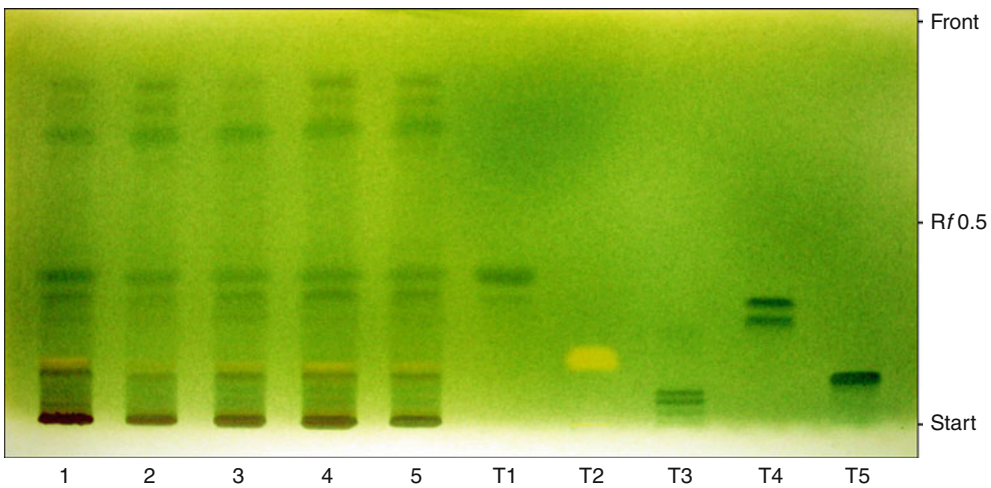


Fig. 3: Thin layer chromatogram of the methanol extracts of Herba Sarcandrae, sprayed Bromocresol Green reagent (VIS), evaluated after one day

HPLC Fingerprint Analysis [1, 3]

1. Sample preparation: 2 g of the powdered drug are extracted with 50 ml water in an ultrasonic bath for 30 min. The extracts are filtered and the filtrates extracted twice with 25 ml ethyl acetate each. The ethyl acetate phases are combined and evaporated to dryness. The residues are dissolved in 1 ml methanol and filtered over Millipore® filtration unit, type 0.45 µm.
2. Injection volume: Herba Sarcandrae extracts: 10 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:	5–50 % B in 35 min
Flow:	1.0 ml/min
Detection:	330 nm

Retention times of the main peaks

Peak	Rt (min)	Compounds
1	13.5	Caffeic acid
2	17.8	4′-O-β-D-glycopyranosyl rosmarinic acid[a]
3	18.6	Isofraxidin

[a]Based on Ref. [3]

4. Description of the HPLC-Figures

The methanol extracts of Herba Sarcandrae sample 1 and 3 provide the three peaks: **1** (= caffeic acid), **2** (= 4′-O-β-D-glycopyranosyl rosmarinic acid) and **3** (= isofraxidin). Fumaric acid (UV-max. at 210 nm) contained in the methanol extract cannot be clearly detected by HPLC, because it appears on the start at Rt = 2.0 together with the solvent peaks.

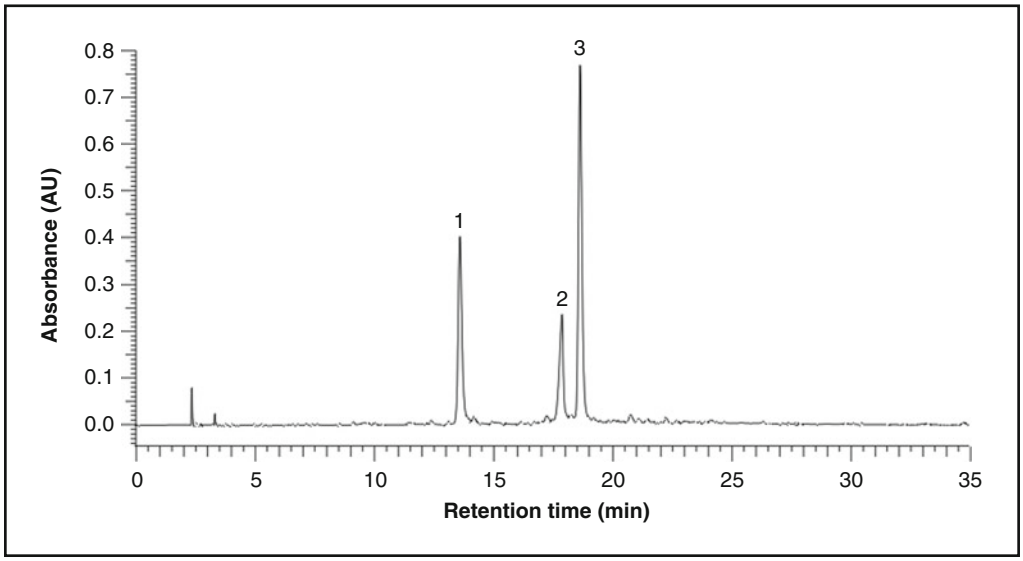


Fig. 4a: HPLC fingerprint analysis of the water/ethyl acetate extract of Herba Sarcandrae (sample 1)

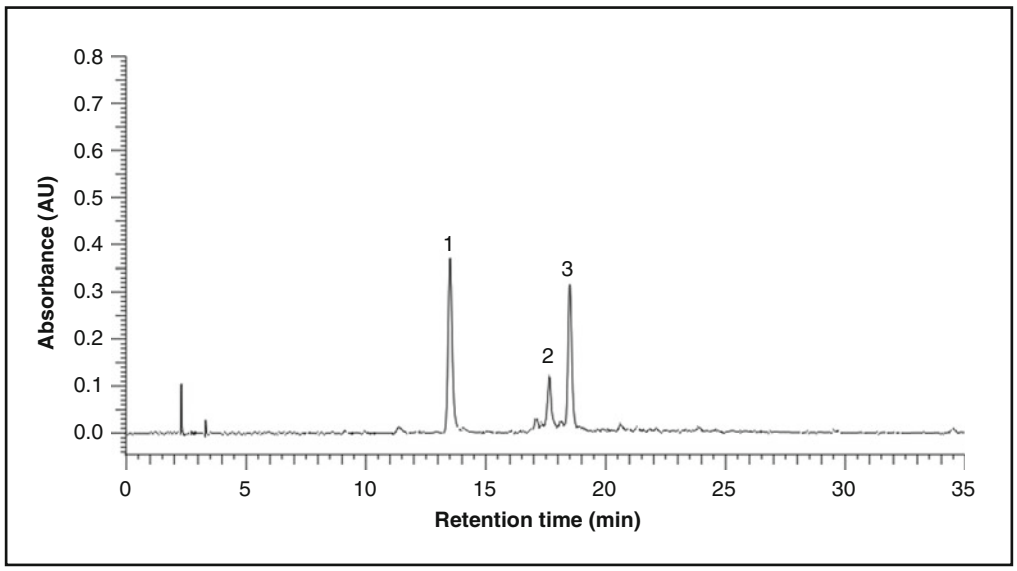


Fig. 4b: HPLC fingerprint analysis of the water/ethyl acetate extract of Herba Sarcandrae (sample 3)

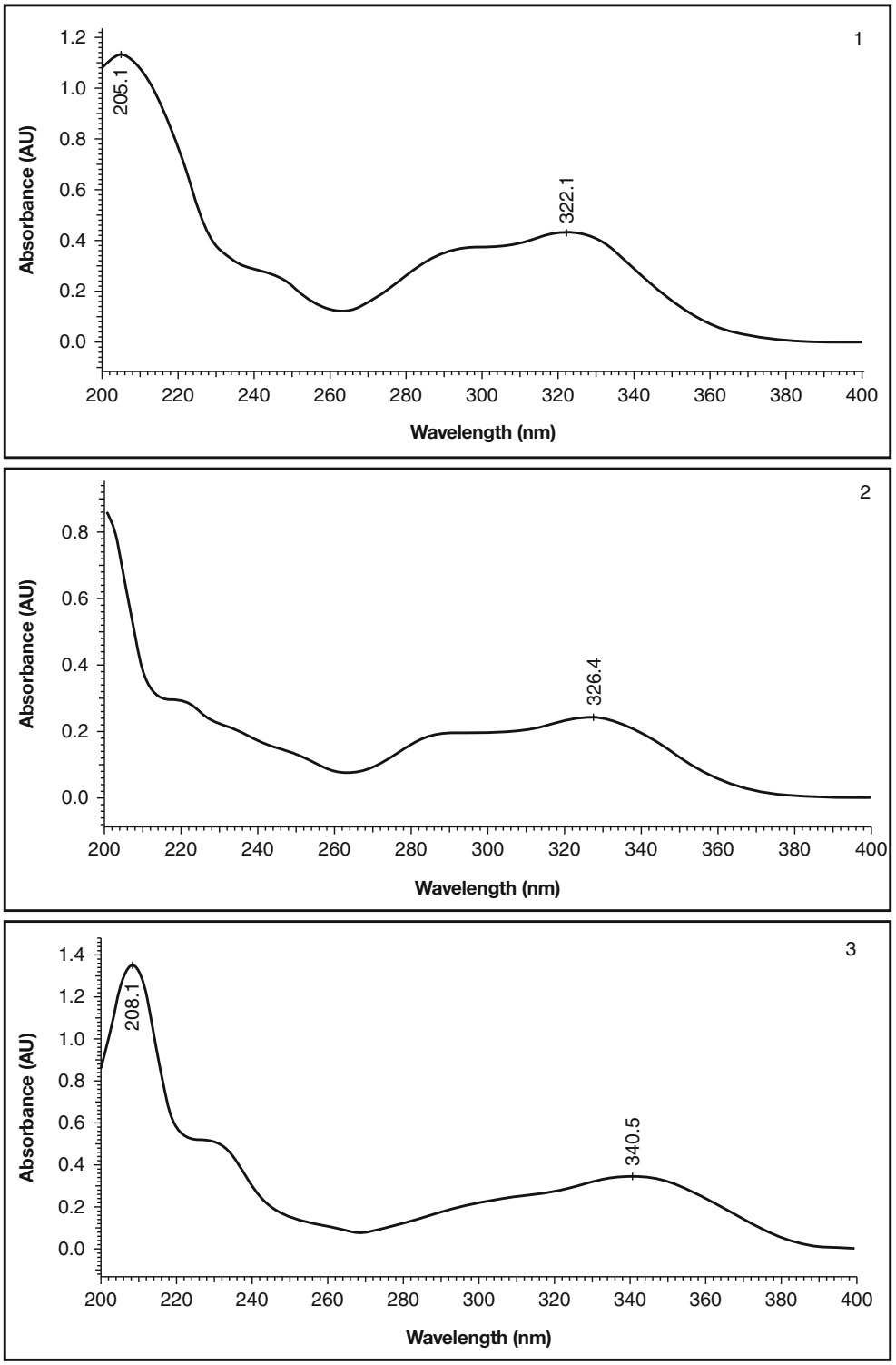


Fig. 5: On line UV-spectra of detected peaks of Herba Sarcandrae

Note: The Chinese Pharmacopoeia 2010 demands for Herba Sacandrae a content of not less than 0.02 % isofraxidin and 0.02 % rosmarinic acid, calculated with reference to the dried drug.

Conclusion

The authentication of Herba Sarcandrae is possible without difficulties using the TLC- and HPLC-techniques.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. People's Medical Publishing House, Beijing (2010)
2. Hu, X.R., Yang, J.S., Xu, X.D.: Three novel sesquiterpene glycosides of *Sarcandra glabra*. Chem. Pharm. Bull. **57**(4), 418–420 (2009)
3. He, R.R., Yao, X.S., Li, H.Y., Dai, Y., Duan, Y.H., Li, Y.F., Kurihara, H.: The anti-stress effects of *Sarcandra glabra* extract on restraint-evoked immunocompromise. Biol. Pharm. Bull. **32**(2), 247–252 (2009)
4. Min, F., Si, J.P., Huang, W.H., Huang, H.H., Lou, S.Q., Zhu, G.Q.: Studies on furmaric acid and isofraxidin content in *Sarcandra glabra* of different provenances. Zhongguo Zhong Yao Za Zhi **33**(15), 1849–1853 (2008)
5. Zhao, Z.Z.: An illustrated Chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
6. Zheng, W., Wang, S., Chen, X., Hu, Z.: Analysis of *Sarcandra glabra* and its medicinal preparations by capillary electrophoresis. Talanta **60**(5), 955–960 (2003)
7. Oanh do, T., Ky, P.T., Hang, N.T., Yen, P.H., Hanh, T.H., Cuong, N.X., Luong, D.V., Minh, C.V., Kiem, P.V.: Two new sesquiterpenes from *Sarcandra glabra*. Nat. Prod. Commun. **5**(11), 1717–1720 (2010)
8. Feng, S., Xu, L., Wu, M., Hao, J., Qiu, S.X., Wei, X.: A new coumarin from *Sarcandra glabra*. Fitoterapia **81**(6), 472–474 (2010)
9. Zhou, B., Liu, K., Chang, J., Cheng, C.: Advances on chemical constituents and pharmacological activities of *Sarcandra glabra*. Chin JMAP **26**(12), 982–986 (2009)
10. Li, X., Zhang, Y., Zeng, X., Yang, L., Deng, Y.: Chemical profiling of bioactive constituents in *Sarcandra glabra* and its preparations using ultra-high-pressure liquid chromatography coupled with LTQ Orbitrap mass spectrometry. Rapid Commun. Mass Spectrom. **25**(17), 2439–2447 (2011)
11. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)
12. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin. Springer, Berlin (1992)
13. He, X.F., Yin, S., Ji, Y.C., Su, Z.S., Geng, M.Y., Yue, J.M.: Sesquiterpenes and dimeric sesquiterpenoids from *Sarcandra glabra*. J. Nat. Prod. **73**(1), 45–50 (2010)
14. Li, Y., Zhang, D.M., Li, J.B., Yu, S.S., Li, Y., Luo, Y.M.: Hepatoprotective sesquiterpene glycosides from *Sarcandra glabra*. J. Nat. Prod. **69**(4), 616–620 (2006)
15. Hilp, M.: Identification of fumaric, maleic and malic acid; analytical methods of pharmacopoeias with DBH in respect to environmental and economical concern part 5. Pharmeuropa **13**(4), 697–703 (2001)

Fructus Ligustri lucidi – *Nüzhenzi*

Pharmacopoeia:[1]	Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010
Official drug:[1]	Glossy Privet Fruit is the dried ripe fruit of *Ligustrum lucidum* Ait. (Fam. Oleaceae). The drug is collected when ripe in winter, removed from branch and leaf, steamed or treated with boiling water for a moment, and dried, or dried directly.
Origin:[2]	Different origins of Eurasia; China (especially provinces in the middle)
Description of the drug:[1]	Ovoid, elliptical or reniform, 6–8.5 mm long, 3.5–5.5 mm in diameter. Externally blackish-purple or greyish-black, shrunken and uneven, with a fruit stalk scar or persistent calyx and a short fruit stalk at the base. Texture light. Epicarp thin, mesocarp relatively lax and soft, easily stripped off, endocarp woody, yellowish-brown, with longitudinal ribs. Seed 1 reniform, purplish-black, oily. Odour, slight; taste, sweet but slightly bitter and astringent.
Pretreatment of the raw drug:[1]	Foreign matters are eliminated, washed and dried.
Processing:[1]	Fructus Ligustri lucidi (processed with wine) The clean Fructus Ligustri lucidi is stewed or steamed as described under the method for stewing or steaming with wine (Appendix II D) until the wine is entirely absorbed or steamed thoroughly.
Medicinal use:[3]	Hyperlipidemia, diabetes and as anti-hepatotoxic and anti-inflammatory drug.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_8, © Springer International Publishing Switzerland 2015

Effects and indications of Fructus Ligustri lucidi according to Traditional Chinese Medicine [1–3]	
Taste:	Sweet and bitter
Temperature:	Cold
Channels entered:	*Orbis hepaticus, o. renalis*
Effects (functions):	To nourish the liver and kidney, improve vision and blacken hairs.
Symptoms and indications:	Liver-kidney yin deficiency, dizziness and tinnitus, soreness and weakness in the low back and knees, premature greying, dim and blurred vision, interior heat wasting-thirst, bone-steaming and tidal fever.

Main constituents:

- **Triterpenoids**[4–11]

  (Acetyl)-oleanolic acid, ursolic acid, crataegolic acid, lupeol, betulin

- **Dammarane triterpenes**[10, 12]

  Dammar-24-ene-3-beta-acetyl-20S-ol; dammarenediol II 3-*O*-palmitate; dammarenediol-II; (E)-25-hydroperoxydammar-23 ene-3-beta,20-diol; 20S,24R-dammarane-25-ene-24-hydro-peroxy-3-beta,20-diol; 25-epoxydammarane-3-beta,24-alpha-diol; 3-beta-acetyl-20,25-epoxydammarane-24-alpha-ol; 5α-dammar-25-ene-3*β*,20,24-triol (fouquierol); oliganthas A; 3-beta-acetyl-20S,24R-dammarane-25-ene-24-hydroperoxy-20 ol; 3-beta-acetyl-20S,25-epoxydammarane-24-alpha-ol; 20S,24R-dammarane-25-ene-24-hydroperoxy-3-beta,20-diol; 20S,25-epoxydammarane-3-beta,24-alpha-diol; 20S-dammarane-23-ene-3-beta,20,25-triol

- **Iridoid and secoiridoid glycosides**[5, 7–9, 11, 13–15]

  (Iso)ligustrosidic acid, 6′–*O*-*cis*-cinnamoyl-8-epikingisidic acid, 6′–*O*-*trans*-cinnamoyl-8-epikingisidic acid, nuzhenals A + B, oleopolynuzhenide A, lucidumosides A – D, oleoside dimethyl ester, nuezhenide, isonuezhenide, neonuezhenide, nuezhenidic acid, specnuezhenide, oleuropein, ligustroside, ligustaloside A + B

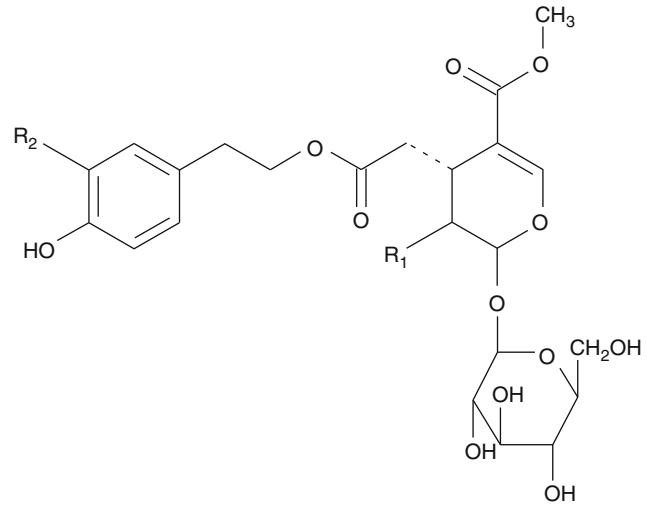
- Phenylethane glycosides[5, 9, 11]

  Salidroside, verbascoside (acteoside), 2-(4-hydroxy phenyl)ethyl-*β*-D-apiosyl-(1→6)-*β*-*D*-*glucopy* ranoside (osmanthuside H), 2-(3,4-dihydroxyphenyl) ethanol, 2-(3,4-dihydroxyphenyl)-ethyl-*O*-*β*-D-glucopyranoside

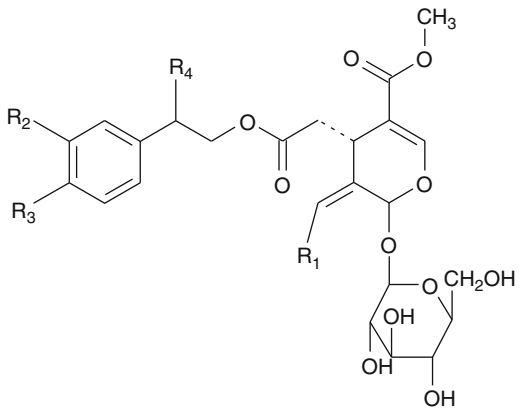
- Essential oils (α/β-pinene, limonene, 4-terpineol, 2-phenyl-1-ethanol, eugenol) and phenolic compounds[7–9, 11]

- **Flavonoids**[7, 8, 16]

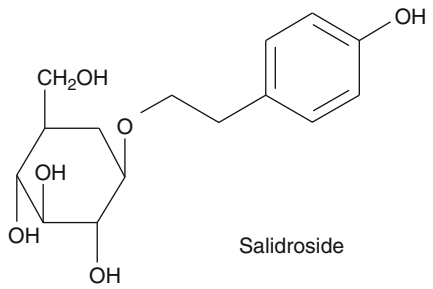
  Apigenin, cosmosiin, apigenin-7-*O*-acetyl-*β*-D-glucoside, apigenin-7-*O*-*β*-D-lutinoside, luteolin, luteolin-7-*O*-*β*-D-glucopyranoside, quercetin



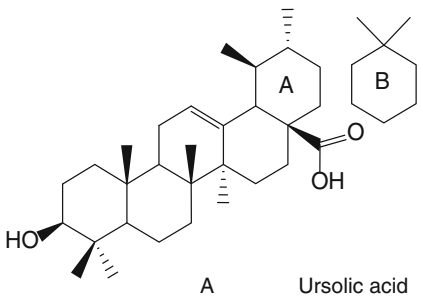
	R_1	R_2
Ligustaloside A	CH_2CHO	OH
Ligustaloside B	CH_2CHO	H
Lucidumoside A	CH_2CH_3	H
Lucidumoside B	CH_2CH_3	OH



	R_1	R_2	R_3	R_4
Oleuropein	CH_3	OH	OH	H
Ligustrosidic acid	COOH	H	H	OH
Lucidumoside C	CH_3	OH	OH	CH_2CH_3
Lucidumoside D	CH_3	OCH_3	OCH_3	H



Salidroside



A Ursolic acid
B Oleanolic acid

Fig. 1: Formulae of the main constituents of Fructus Ligustri lucidi [4, 5, 11]

Reported pharmacology:

Immune stimulating (leucocytes)[3, 11]
Lowers serum glucose levels[3, 14]
Lowers serum cholesterol and lipid levels [3, 6, 7]
Promotes hematopoiesis[3]
Anti-inflammatory[3–5, 7, 11, 14]
Hepatoprotective[3, 5, 7, 11]
Antiobiotic[3]
Anti-oxidative[4, 7, 11, 14, 17]
Anti-protozoal[4]
Anti-mutagenic[4, 11]
Anti-cancer[4, 11]
Immunomodulatory[5, 7, 9]
Anti-tumor[5, 7, 11]
Anti-aging[5, 7, 9]
Increased coronary blood flow[14]
Anti-arrhythmic[14]
Spasmolytic effects[14]
Anti-bacterial[14]
Anti-viral[14, 17]
Inhibition of platelet aggregation[14]
Increases vitamin D-dependent CaBPs expression[17]
Promotes osteogenesis of mesenchymal stem cells[17]
Improves Ca^{2+} balance in aged female rats by increasing serum 1,25 $(OH)_2D_3$ levels[17, 18]

TLC-Fingerprint Analysis[1]

Drug samples		Origin
1	Fructus Ligustri lucidi/ *Ligustrum lucidum*	Sample of commercial drug obtained from China Medica (origin: Anyue, Sichuan, charge: 91 0301)
2	Fructus Ligustri lucidi/ *Ligustrum lucidum*	Sample of commercial drug obtained from TCM-Clinic Bad Kötzting, Germany, (charge: K 07.06.1999)
3	Fructus Ligustri lucidi/ *Ligustrum lucidum*	Sample of commercial drug obtained from TCM-Clinic, Bad Kötzting, Germany, (charge: 13801022012)

Drug samples		Origin
4	Fructus Ligustri lucidi/ *Ligustrum lucidum*	Province Sichuan, Pengzhou, Xin Cha (China)
5	Fructus Ligustri lucidi/ *Ligustrum lucidum*	Province Hubei, Lou Ping, Ping Hu (China)
6	Fructus Ligustri lucidi/ *Ligustrum lucidum*	Province Sichuan, Pengzhou, Xin Xing (China)

Reference compounds		R*f* in Figs. 2a and 2b	R*f* in Figs. 3a and 3b
T 1 T 4	Oleanolic acid/ Ursolic acid	0.93	0.57
T 2	Oleuropein	0.69	–
T 3	Verbascoside	0.60	0.70

1. Extraction: 0.5 g powdered drug are extracted with 20 ml methanol under reflux for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml ethanol.

2. Reference compounds: Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Fructus Ligustri lucidi extracts: 10 µl each
Reference compounds: 10 µl each

Solvent system: 1. Ethyl acetate + methanol + water (77 + 15 + 8) (**Figs. 2a and 2b**)
2. Chloroform + methanol + formic acid (40 + 1 + 1) (**Figs. 3a and 3b**)

Detection: 1. Vanillin – Sulphuric acid (**Figs. 2a and 2b**)

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 min at 105 °C and evaluated in VIS and under UV 366 nm.

2. 10 % ethanolic sulphuric acid (**Figs. 3a and 3b**)

The plate is sprayed with the reagent, heated at 105 °C for 5–10 min and evaluated in VIS and under UV 366 nm.

Note: The several zones changed their colour depending on the time of heating.

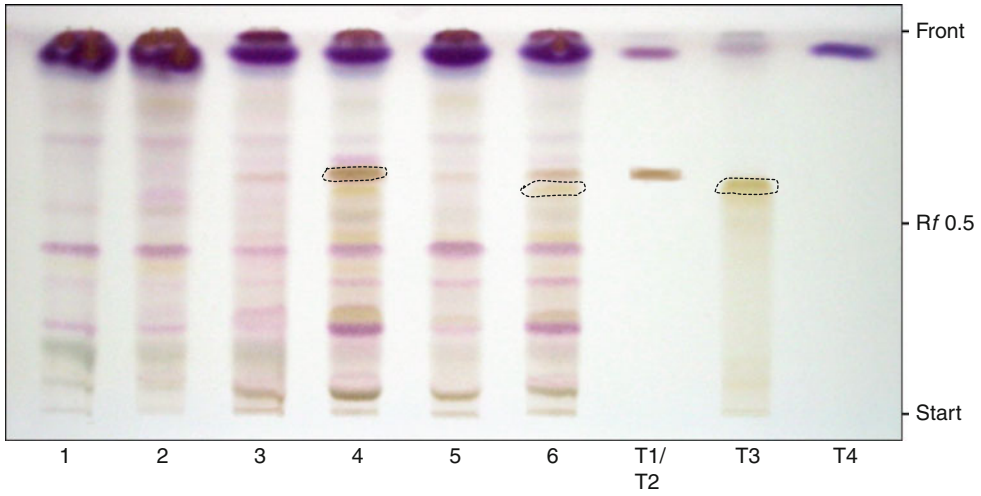


Fig. 2a: Thin layer chromatogram of the methanol extracts of Fructus Ligustri lucidi sprayed with Vanillin – Sulphuric acid reagent (VIS)

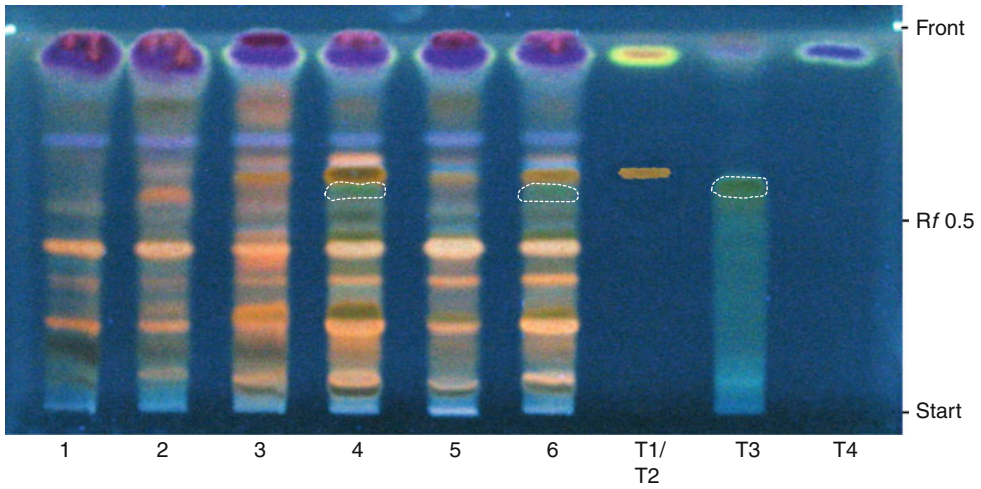


Fig. 2b: Thin layer chromatogram of the methanol extracts of Fructus Ligustri lucidi sprayed with Vanillin – Sulphuric acid reagent (UV 366 nm)

4.1. Description of Figs. 2a and 2b:

- In VIS the chromatogram is characterized by 7–8 weak violet/brown zones in R*f*-range from the start up to R*f*=0.7, with oleuropein (**T2**) at R*f*=0.69 and verbascoside (**T3**) at R*f*=0.60. Oleanolic and ursolic acid (**T1, T4**) appear together with the various dammarane triterpenoids with deep brown/blue colour from R*f*=0.95 up to the front.
- Under UV 366 nm oleuropein appears as light yellow-brown zone. The other 5–6 light orange zones down to the start can be assigned to the various ligustalosides, ligustrosidic acids, lucidumosides inclusive salidroside.

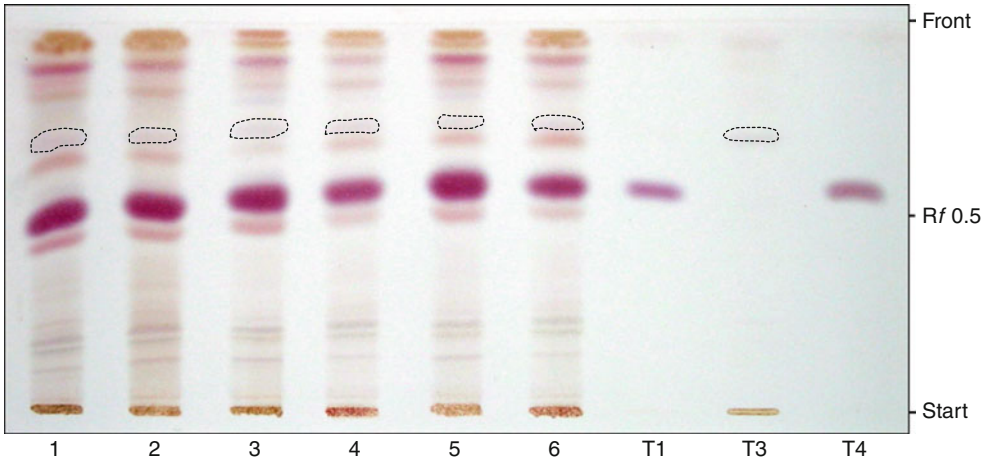


Fig. 3a: Thin layer chromatogram of the methanol extracts of Fructus Ligustri lucidi sprayed with 10 % ethanolic sulphuric acid (VIS)

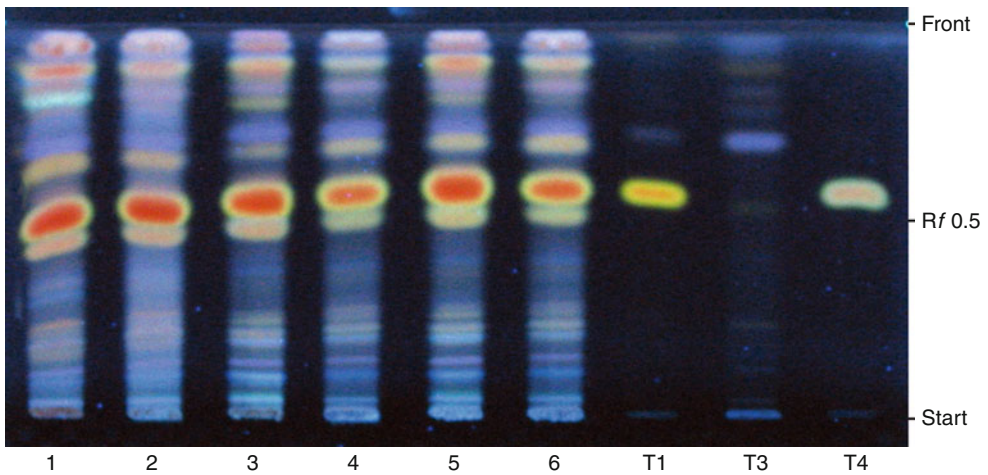


Fig. 3b: Thin layer chromatogram of the methanol extracts of Fructus Ligustri lucidi sprayed with 10 % ethanolic sulphuric acid (UV 366 nm)

4.2. Description of Figs. 3a and 3b:

In these chromatograms oleanolic (**T1**) and ursolic acid (**T4**) appear as distinct pink marker compounds at R*f* = 0.57 separated from the dammarane triterpenoids which can be identified beneath the front in two light pink zones. Verbascoside (**T3**) could be identified under UV 366 nm as weak blue zone at R*f* = 0.70.

HPLC-Fingerprint Analysis

1. Sample preparation: 0.5 powdered drug are extracted with 20 ml methanol under reflux for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml ethanol and filtered over Chromafil®, type 0.20 µm.

2. Injection volume: Fructus Ligustri lucidi: 10 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® 125–4 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®) B: acetonitril (VWR)
Gradient:	0–15 % B in 5 min, 15–50 % B in 20 min, 50–95 % B in 5 min, 95 % B for 10 min, Total runtime: 40 min
Flow:	1 ml/min
Detection:	210 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	11.7	Verbascoside
2	14.5	Oleuropein
3	34.6	Oleanolic acid
3′	34.7	Ursolic acid

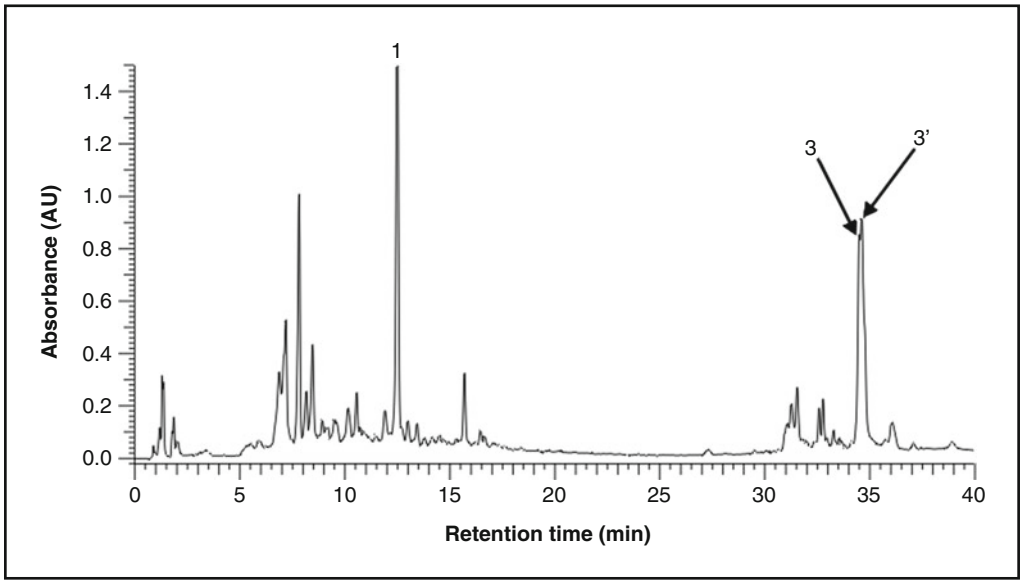


Fig. 4a: HPLC-fingerprint analysis of the methanol extract of Fructus Ligustri lucidi, sample 2

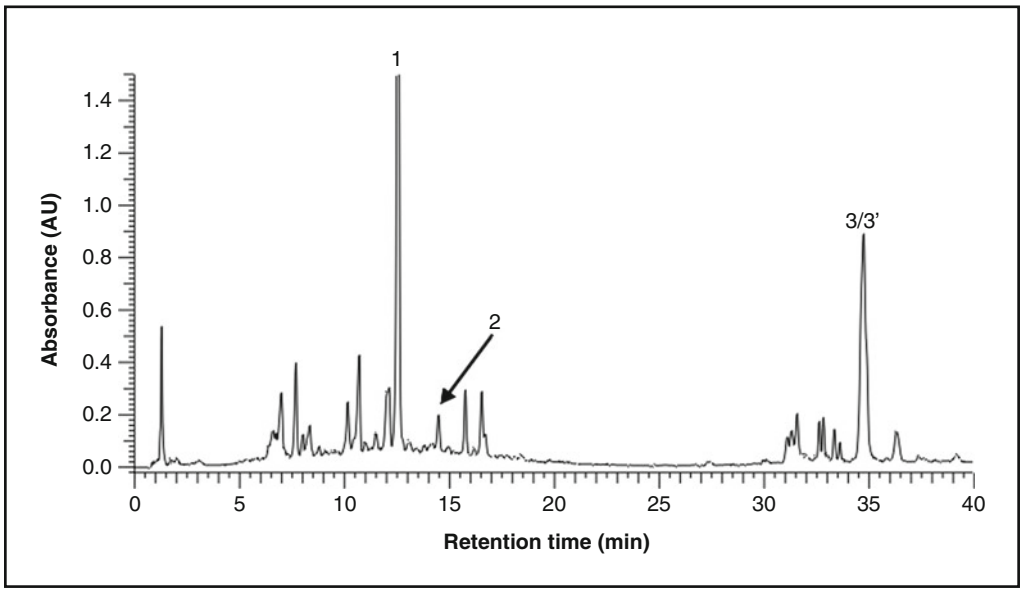


Fig. 4b: HPLC-fingerprint analysis of the methanol extract of Fructus Ligustri lucidi, sample 5

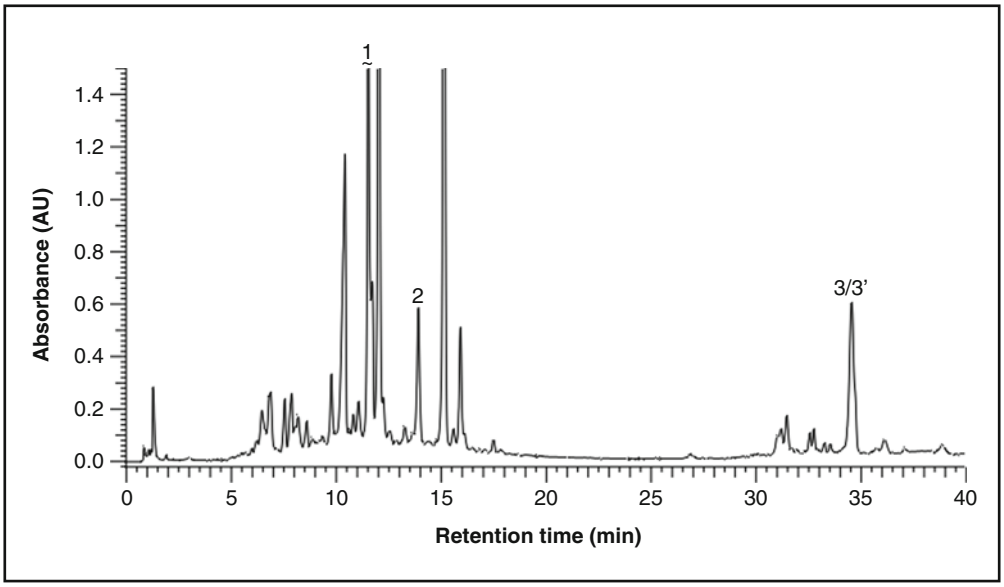


Fig. 4c: HPLC-fingerprint analysis of the methanol extract of Fructus Ligustri lucidi, sample 6

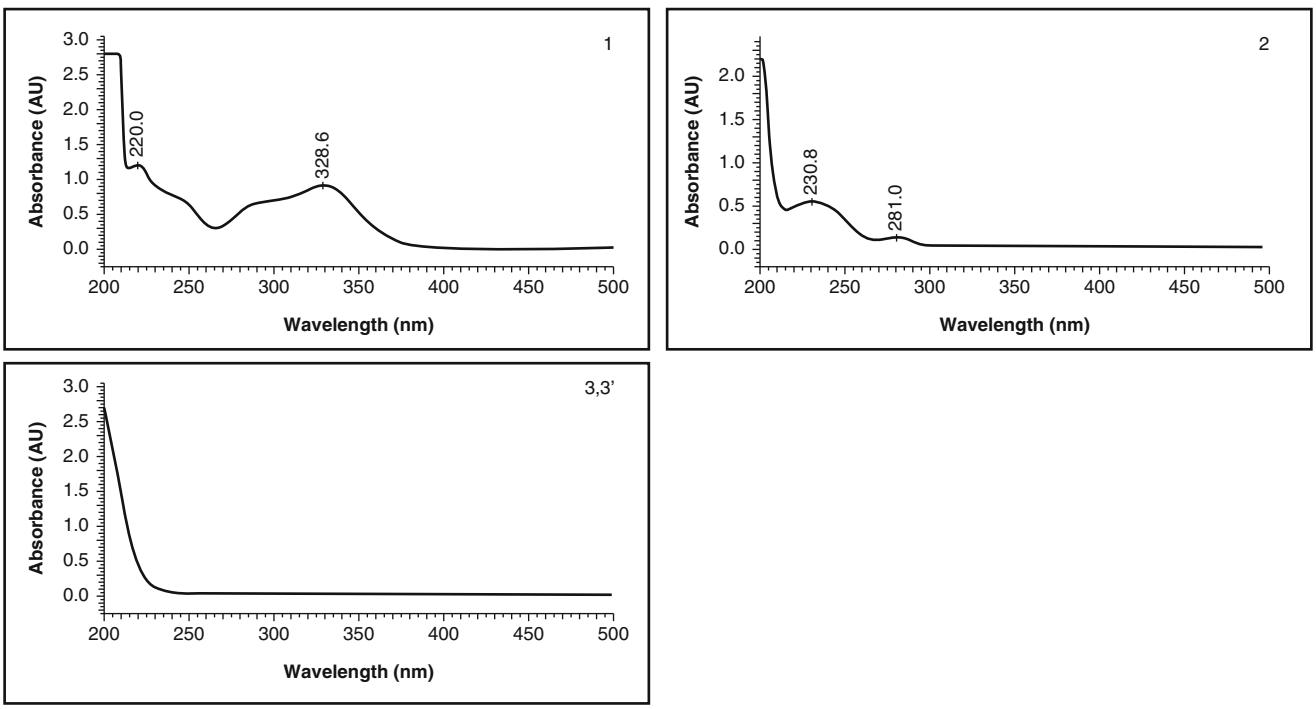


Fig. 5: On line UV-spectra of the main peaks of Fructus Ligustri lucidi

4. Description of the HPLC-Figures:

   The extract samples 2, 5 and 6 are characterized by two peak profiles in the Rt-range of 5.0 to 17.0 and 30.0 to 36.0. The first range contains as marker compounds verbascoside (**1**) at Rt = 11.7 and oleuropein (**2**) at Rt = 14.3. In the second peak block the peaks **3** and **3′** can be assigned to oleanolic and ursolic acid as marker compounds.

   In very low concentration appear in the Rt-range of 6.8 to 11.0 the ligustalosides and lucidumosides, whereas in Rt-range 31.0 to 34.0 the dammarane triterpenoids can be assigned.

Note: The Chinese Pharmacopeia 2010 describes for Ligustri Lucidi Fructus a Nuezhenoside content not less than 0.70 % with reference to the dried drug.[1]

Conclusion

The TLC- and HPLC-fingerprints provide characteristic marker profiles which facilitate the authentication of the herbal TCM drug.

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
3. Hempen, C.H., Fischer, T.: Leitfaden Chinesische Phytotherapie 2. Auflage. Urban & Fischer, Munich (2007)
4. Xia, E.Q., Yu, Y.Y., Xu, X.R., Deng, G.F., Guo, Y.J., Bi, H.B.: Ultrasound-assisted extraction of oleanolic acid and ursolic acid from *Ligustrum lucidum* Ait. Ultrason. Sonochem. **19**(4), 772–776 (2012)
5. Guo, N., Yu, Y., Ablajan, K., Li, L., Fan, B., Peng, J., Yan, H., Ma, F., Nie, Y.: Seasonal variations in metabolite profiling of the fruits of *Ligustrum lucidum* Ait. Rapid Commun. Mass Spectrom. **25**(12), 1701–1714 (2011)
6. Yim, T.K., Wu, W.K., Pak, W.F., Ko, K.M.: Hepatoprotective action of an oleanolic acid-enriched extract of *Ligustrum lucidum* fruits is mediated through an enhancement on hepatic glutathione regeneration capacity in mice. Phytother. Res. **15**(7), 589–592 (2001)
7. He, Z.D., But, P.P.H., Chan, T.W.D., Dong, H., Xu, H.X., Lau, C.P., Sun, H.D.: Antioxidative glucosides from the fruits of *Ligustrum lucidum*. Chem. Pharm. Bull. **49**(6), 780–784 (2001)
8. He, Z.D., Dong, H., Xu, H.X., Ye, W.C., Sun, H.D., But, P.P.H.: Secoiridoid constituents from the fruits of *Ligustrum lucidum*. Phytochemistry **56**(4), 327–330 (2001)
9. Shi, L., Ma, Y., Cai, Z.: Quantitative determination of salidroside and specnuezhenide in the fruits of *Ligustrum lucidum* ait by high performance liquid chromatography. Biomed. Chromatogr. **12**(1), 27–30 (1998)
10. Huang, X., Yin, Z., Ye, W., Shen, W.: Chemical constituents from fruits of *Ligustrum lucidum*. Zhongguo Zhong Yao Za Zhi **35**(7), 861–864 (2010)
11. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)
12. Xu, X.H., Yang, N.Y., Qian, S.H., Xie, N., Duan, J.A.: Dammarane triterpenes from *Ligustrum lucidum*. J. Asian Nat. Prod. Res. **10**(1–2), 33–37 (2008)
13. Aoki, S., Honda, Y., Kikuchi, T., Miura, T., Sugawara, R., Yaoita, Y., Kikuchi, M., Machida, K.: Six new secoiridoids from the dried fruits of *Ligustrum lucidum*. Chem. Pharm. Bull. **60**(2), 251–256 (2012)

14. Ma, S.C., He, Z.D., Deng, X.L., But, P.P.H., Ooi, V.E.C., Xu, H.X., Lee, S.H.S., Lee, S.F.: *In vitro* evaluation of secoiridoid glucosides from the fruits of *Ligustrum lucidum* as antiviral agents. Chem. Pharm. Bull. **49**(11), 1471–1473 (2001)

15. Willems, M.: Quantitative Bestimmung von Secoiridoidglucosiden aus den Früchten von *Ligustrum vulgare* mit HPLC. Planta Med. **54**(1), 66–68 (1988)

16. Xu, X.H., Yang, N.Y., Qian, S.H., Xie, N., Yu, M.Y., Duan, J.A.: Study on flavonoids in *Ligustrum lucidum*. Zhong Yao Cai **30**(5), 538–540 (2007)

17. Jeong, J.C., Kim, J.W., Kwon, C.H., Kim, T.H., Kim, Y.K.: *Fructus ligustri lucidi* extracts induce human glioma cell death through regulation of Akt/mTOR pathway *In Vitro* and reduce glioma tumor growth in U87MG xenograft mouse model. Phytother. Res. **25**(3), 429–434 (2011)

18. Li, G., Zhang, X.A., Zhang, J.F., Chan, C.Y., Yew, D.T.W., He, M.L., Lin, M.C.M., Leung, P.C., Kung, H.F.: Ethanol extract of *Fructus Ligustri lucidi* promotes osteogenesis of mesenchymalm stem cells. Phytother. Res. **24**(4), 571–576 (2010)

Cortex Moutan – *Mudanpi*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1, 27]

Tree Peony Bark is the dried root bark of *Paeonia suffruticosa* Andr. (Fam. Ranunculaceae/Paeoniaceae).

The root is collected in autumn, removed from rootlets and soil, the root bark is stripped off, and dried in the sun; or scraped coarse bark off, removed from woody part, and dried in the sun. The former is known as Liandanpi and the latter as Guadanpi.

Origin:[2]

Provinces Hebei, Henan, Shandong, Sichuan, Shaanxi and Gansu

Description of the drugs:[1]

Liandanpi
Quilled or semiquilled, with longitudinal cut fissures, somewhat involute or opened. 5–20 cm long, 5–12 mm in diameter, 1–4 mm thick. Outer surface greyish-brown or yellowish-brown, showing numerous transverse lenticel-like prominences and rootlet scras, the exposed surface where cork fallen off appearing pink; inner surface pale greyish-yellow or pale bron, with obvious fine longitudinal striations, usually showing bright crystals. Texture hard and fragile, easily broken, fracture relatively even, mealy, pale pink. Odour, aromatic; taste, slightly bitter and astringent.

Guadanpi
Outer surface exhibiting the scraping traces, reddish-brown or pale greyish-yellow, sometimes greyish-brown spotted remains of outer bark visible.

Pretreatment of the raw drug:[1]

Washed clean rapidly, softened, cut into thin slices, and dried in the sun.

Medicinal use:[15]

Used as a therapeutic medicine for the treatment of hypertonia, myalgia, rheumatic pain and neuralgia.

Effects and indications of Cortex Moutan according to Traditional Chinese Medicine[1–4]

Taste:	Bitter and pungent
Temperature:	Mild cold
Channels entered:	*Orbis cardialis*, *o. hepaticus*, *o. renalis*, *o. pericardialis*
Effects (functions):	To clear heat and cool the blood, activate blood toresolve stasis.
Symptoms and indications:	Heat entering nutrient-blood aspects, macula and papule caused by warm toxin, hematemesis and epistaxis, fever at night and cool in the morning, steaming bone without sweating, amenorrhoea and dysmenorrhoea, pain caused by injuries from falls, swelling abscess, sore and toxin.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_9, © Springer International Publishing Switzerland 2015

Main constituents:
[5, 6, 8–12, 15, 17, 21–23, 26]

- **Phenolic compounds and glycosides**

Paeonol, paeonoside (= paeonol-*β*-D-glucopyranoside), apiopaeonoside, paeonolide (= paeonol-*α*-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside), paeoniflorigenone, suffruticosides A-E

- **Monoterpenoids**

Paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin, benzoyloxypaeoniflorin, galloylpaeoniflorin, galloyloxypaeoniflorin, paeonisuffrone, paeonisuffral, α- and β-benzoylpaeoniflorin, mudanpiosides A-F, paeonisothujone, deoxypaeonisuffrone, isopaeonisuffral

- Other compounds

Benzoic acid, gallic acid, catechin, quercetin, kaempferol, resacetophenone, paeoniflorigenone, β-sitosterol, betulinic acid, oleanolic acid, quercetin, caffeic acid stearyl ester, mudanpinoic acid A, mudanoside B, 1,2,3,4,6-penta-*O*–galloyl-β-D-glucose

Note 1: Paeonol was found **only** in the root of ***P. suffruticosa***, whereas **Paeoniflorin** occurs in **all *Paeonia* species**. This difference may be employed as a chemical criterion to distinguish *P. suffruticosa* from *P. lactiflora* [7, 26]. See TLC and HPLC-fingerprint analyses of Radix Paeoniae albae/rubrae (Vol. I, p. 281–290) [24].

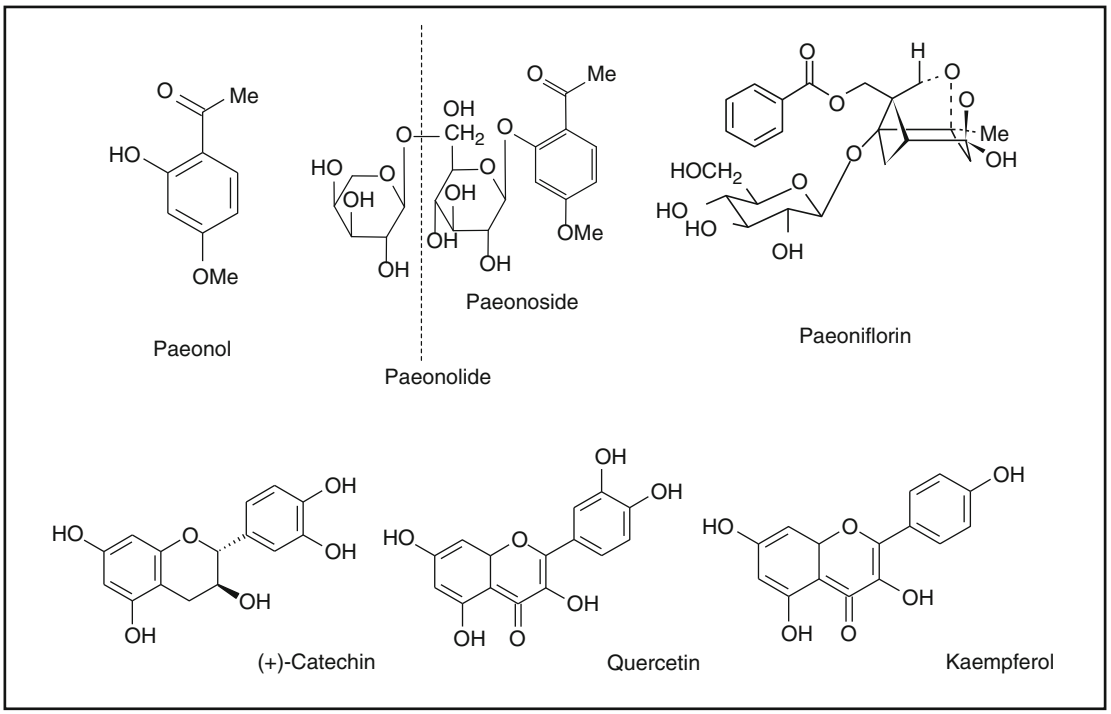


Fig. 1: Formulae of the main constituents of Cortex Moutan[5]

Reported pharmacology:

- Antithrombotic[5]
- Inhibition of blood platelet coagulation[5, 15, 19]
- Antihypertensive[5, 6, 14]
- Vasodilation effects[18]
- Depressant effects[5]
- Calming/sedative[5, 6, 12]
- Sleep-promoting[6]
- Neuroprotective[13]
- Anxiolytic-like effects[18]
- Antispasmodic[5, 16, 17]
- Antimutagenic[5]
- Inhibition of growth of *Escherichia coli*, *Bacillus subtilus*, *Staphylococcus auereus* and *Streptococcus faecalis*[5, 14, 15]
- Anti-inflammatory[5, 6, 9, 10, 12–19, 21]
- Anti-pyretic[6, 14, 19]
- Immune-stimulating[6, 10, 15]
- Analgesic[5, 6, 12, 14, 16, 17, 21, 26]
- Antibiotic[6, 13]
- Antioxidant[9, 10, 18, 19, 21]
- Anti-allergic[13, 15, 18]
- Antiproliferative[18]
- Osteoclastogenesis effects[18]
- Anti-diabetic[20]
- Anaphylactic[21]
- Hemostyptic[26]
- Bacteriostatic agent[26]

TLC-Fingerprint Analysis

Drug samples		Origin
1	Cortex Moutan/*Paeonia suffruticosa*	Sample of commercial drug, obtained from HerbaSinica (origin: Shandong)
2	Cortex Moutan/*Paeonia suffruticosa*	Sample of commercial drug, obtained from China Medica (Charge: Bozhou, Anhui)
3	Cortex Moutan/*Paeonia suffruticosa*	Sample of commercial drug, obtained from TCM-Clinic Bad Kötzting (Charge: K07.01.2000)

Drug samples		Origin
4	Cortex Moutan/*Paeonia suffruticosa*	Province Shaanxi (China)
5	Cortex Moutan/*Paeonia suffruticosa*	Province Anhui (China)
6	Cortex Moutan/*Paeonia suffruticosa*	Province Beijing (China)
7[a]	**Radix Paeoniae albae**/*Paeonia lactiflora*	Province Anhui (China)
8[a]	**Radix Paeoniae rubrae**/*Paeonia lactiflora*	Sample of commerical drug obtained from SinoMed, TCM-Clinic Bad Kötzting, Charge: 9203062005

[a]For comparison

1. **TLC-fingerprint analysis of Paeonol, Paeoniflorin and Oxypaeoniflorin:**[24]

Reference compounds of Fig. 2		R*f*
T1	Paeonol	0.93
T2	Paeoniflorin	0.52
T3	Oxypaeoniflorin	0.40
T4	Catechin	0.65

1. Extraction: 1 g powdered drug is extracted with 10 ml ethanol (95 %) under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml ethanol (95 %).

2. Reference compounds: Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Cortex Moutan extracts: 5 µl each
Radix Paeoniae extracts: 5 µl each
Reference compounds: 10 µl each

Solvent system: Chloroform + ethyl acetate + methanol + formic acid (40 + 5 + 15 + 0.2)

Detection: Vanillin – Sulphuric acid

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 min at 105 °C and evaluated in VIS.

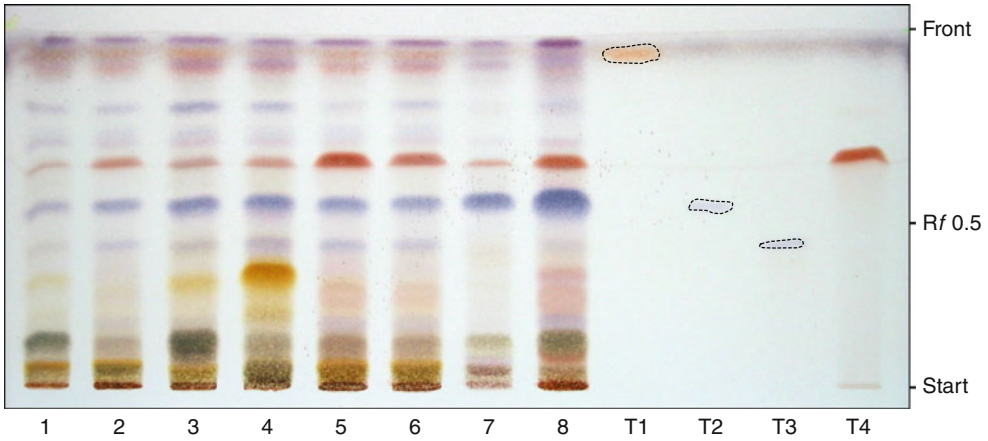


Fig. 2: Thin layer chromatogram of the ethanol extracts of Cortex Moutan (1 − 6) and Radix Paeoniae (7 + 8), sprayed with Vanillin – Sulphuric acid reagent (VIS)

4. Description of Fig. 2:

The Cortex Moutan extract samples 1–6 provide a very homogeneous TLC-fingerprint profile with paeonol (**T1**, orange) at R*f* = 0.93, Catechin (**T4**, red-brown) at R*f* = 0.65, paeoniflorin (**T2**, blue-grey) at R*f* = 0.52, oxy-paeoniflorin (**T3**, blue-grey) at R*f* = 0.40 and several red-brown and blue-grey zones in the low Rf-range (from start up to R*f* = 0.35).

The extract samples of Radix Paeoniae albae/rubrae (7 + 8) differ from Cortex Moutan by absence of paeonol.

2. **TLC-fingerprint analysis of Paeonol, Quercetin and Kaempferol:**[25]

Reference compounds of Fig. 3a, b		**R*f***
T5	Paeonol	0.77
T6	Quercetin	0.48
T7	Kaempferol	0.55

1. Extraction: 1 g powdered drug is extracted with 10 ml ethanol (95 %) under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml ethanol (95 %).

2. Reference compounds: Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Cortex Moutan extracts: 5 µl each Radix Paeoniae extracts: 5 µl each Reference compounds: 10 µl each
Solvent system:	Toluene + ethyl formate + formic acid (50+40+10)
Direct evaluation:	UV 254 nm
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 under UV 365 nm after 4 h. **Note:** The fluorescence behavior is dependent on the day of evaluation.

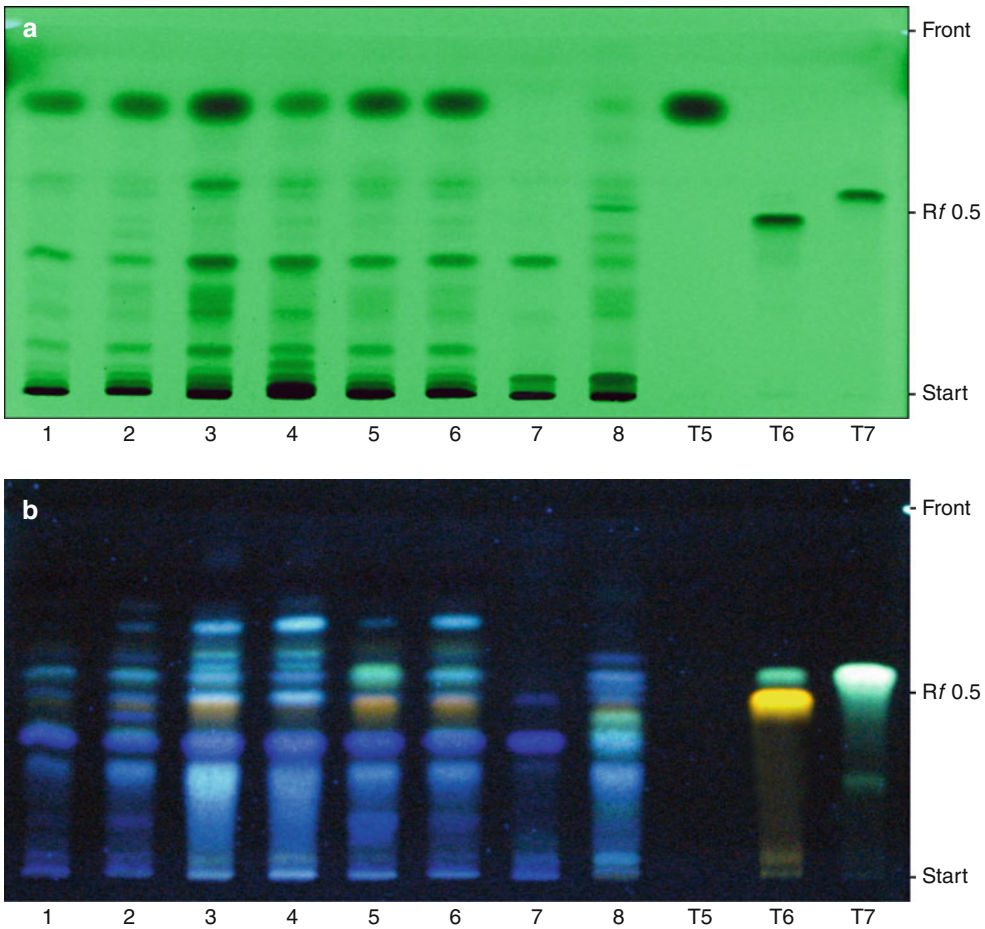


Fig. 3: (**a**, **b**) Thin layer chromatogram of the ethanol extracts of Cortex Moutan and Radix. Paeoniae ((**a**) UV 254 nm; (**b**) UV 366 nm, sprayed with NP/PEG)

4. Description of Fig. 3a, b:
   (a) The TLC-picture under UV 254 nm confirms the presence of paeonol (**T5**) in the Cortex Moutan extract samples 1–6 and the absence in Radix Paeoniae (7 + 8).
   (b) The TLC-plate sprayed with NP/PEG reagent shows under UV 366 nm at R*f* = 0.55 the flavonoid kaempferol (**T7**) and at R*f* = 0.48 quercetin (**T6**). The various monoterpene glycosides appear with blue fluorescence in the R*f*-range from start up to R*f* = 0.65.

HPLC-Fingerprint Analysis: [12]

1. Sample preparation: 1 g powdered drug is extracted with 10 ml ethanol (95 %) under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml ethanol (95 %).

2. Injection volume: Cortex Moutan extracts: 10 µl each
   Radix Paeoniae extracts: 10 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 system:	A: 0.1 % aqueous formic acid (Millipore Ultra Clear UV plus® filtrated) B: acetonitril (VWR)
Gradient:	5–15 % B in 15 min, 15 % B for 10 min, 15–25 % B in 25 min, 25–45 % B in 10 min, 45–70 % B in 5 min, 70 % B for 7 min, Total runtime: 72 min
Flow:	0.5 ml/min
Detection:	230/254 nm

Retention times of the main peaks recorded at 230 nm (▬) and 254 nm (▬)

Peak	Rt (min)	Compound
1	6.5	Gallic acid
2/3	10.7–14.7	Oxypaeoniflorin/Catechin
4	19.2	Paeoniflorin
5	22.9	Monoterpen glycosides
6	25.5	
7	27.2	
8	31.1	
9	34.8	
10	41.2	
11	63.6	Quercetin
12	69.0	Paeonol

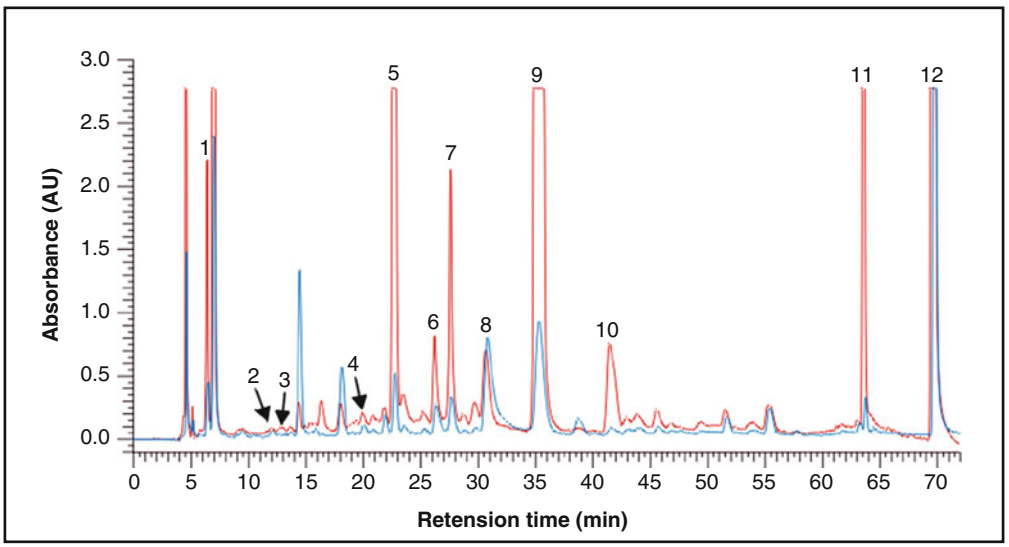


Fig. 4a: HPLC-fingerprint analysis of the ethanol extract of Cortex Moutan, sample 3

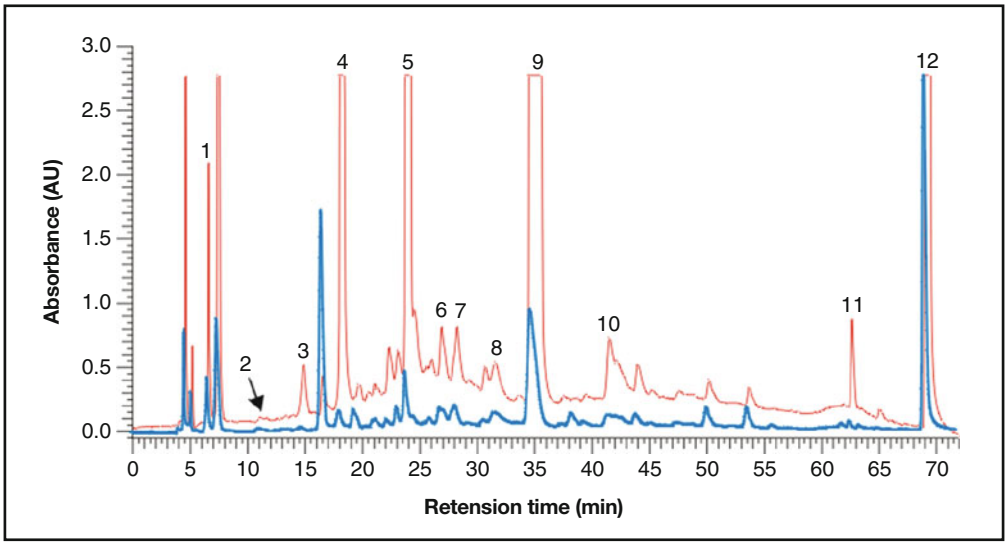


Fig. 4b: HPLC-fingerprint analysis of the ethanol extract of Cortex Moutan, sample 5

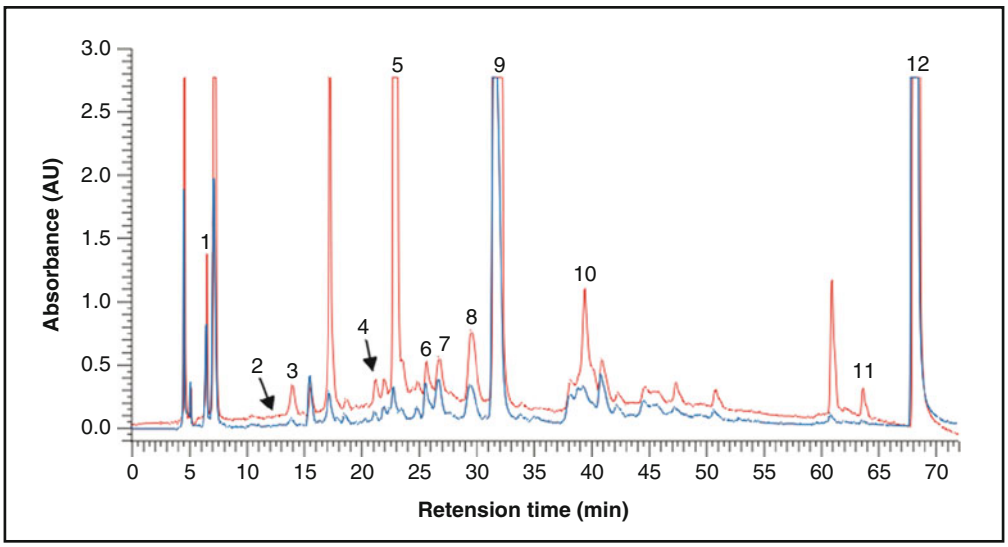


Fig. 4c: HPLC-fingerprint analysis of the ethanol extract of Cortex Moutan, sample 6

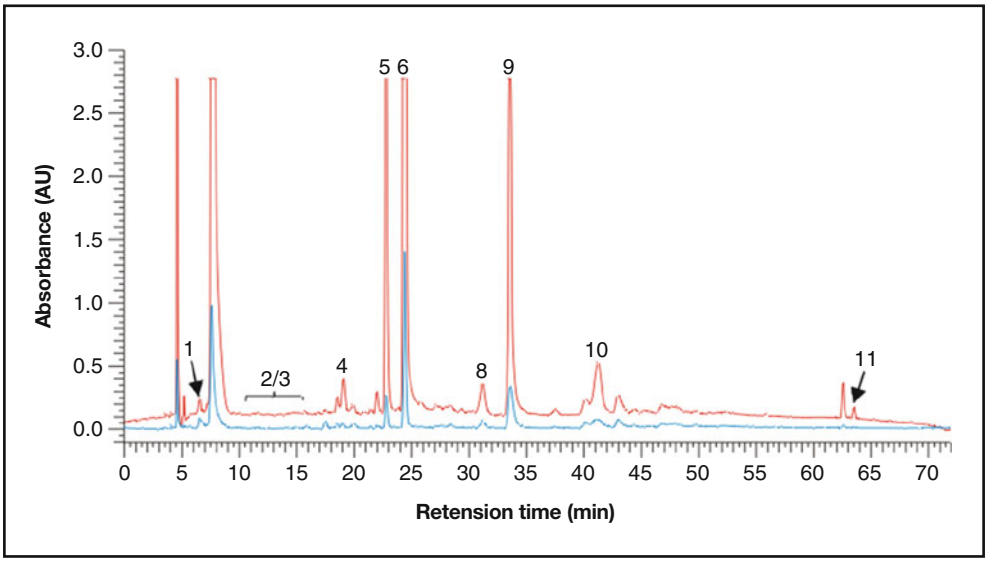


Fig. 4d: HPLC-fingerprint analysis of the ethanol extract of Radix Paeoniae albae, sample 7

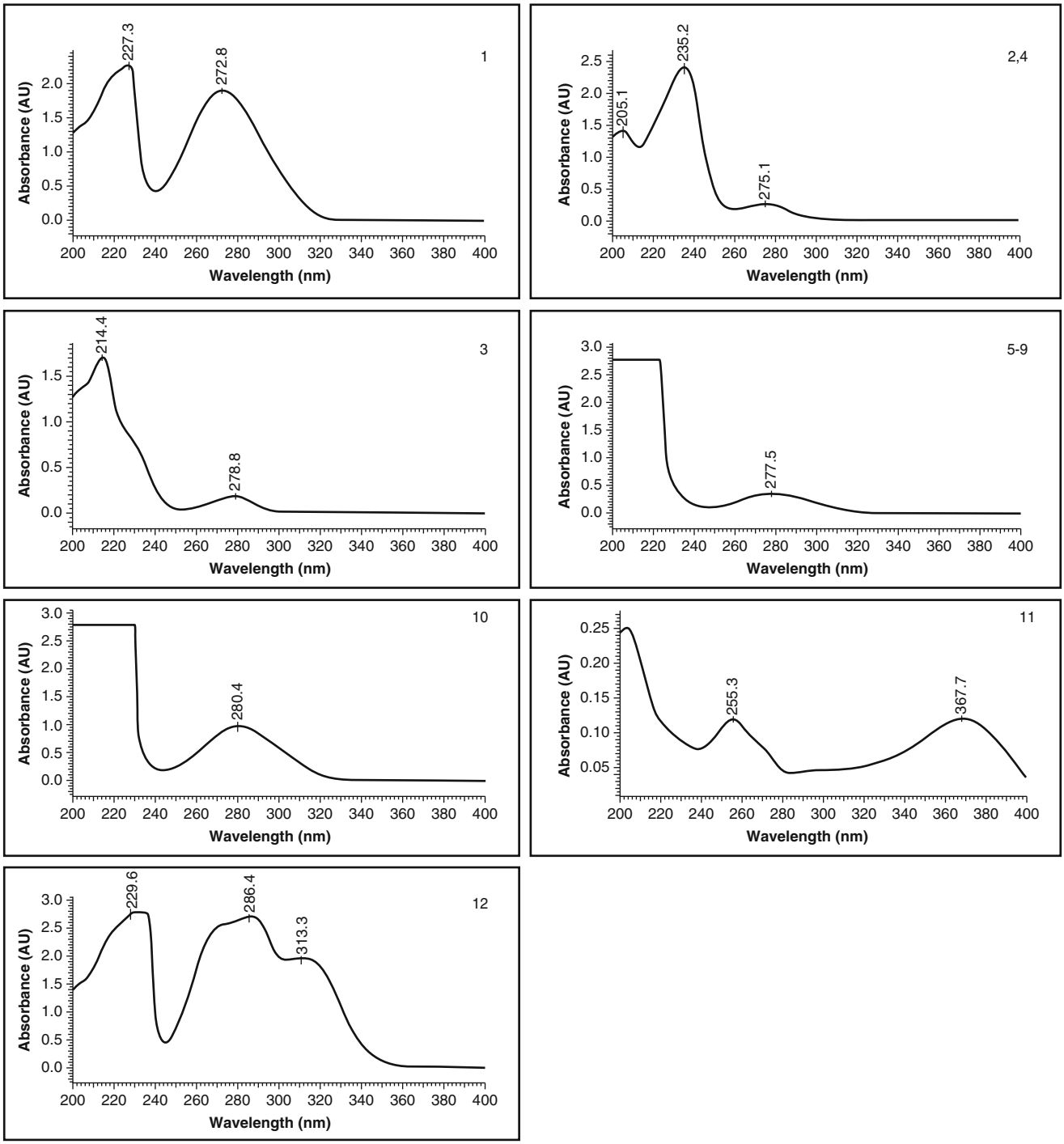


Fig. 5: On line UV-spectra of the main compounds of Cortex Moutan

4. Description of the HPLC-Figures

The HPLC-graphs of Cortex Moutan extract samples 3 + 5 show a superimposable peak profile with gallic acid (**1**) at Rt = 6.5, oxypaeoniflorin (**2**) and catechin (**3**) at Rt 10.7–14.7, a sequence of seven peaks (**4**–**10**) which can be assigned to the characteristic monoterpene glycosides of the paeoniflorin-type. Peak **11** was identified as quercetin and peak **12** as paeonol.

Sample 6 differs from the other samples by a lower concentration of peak **6**, but shows again a significant peak of paeonol confirmed by the characteristic UV-spectrum.

In sample 7 for comparison the peak profile of Radix Paeoniae albae is shown. The absence of peak **12** at Rt = 69.0 shows clearly that both Paeonia species differ from each other in the presence and absence of paeonol, respectively.

Note: The Chinese Pharmacopeia 2010 describes for Cortex Moutan a paeonol content not less than 1.2 % with reference to the dried drug[1, 26].

Conclusion

A definitive chromatographic authentication of Cortex Moutan and discrimination from Radix Paeoniae spec. can be best achieved by the chromatographic confirmation of the presence of paeonol in Cortex Moutan.

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Paulus, E., Ding, Y.H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)
3. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
4. Hempen, C.H., Fischer, T.: Leitfaden Chinesische Phytotherapie, 2nd edn. Urban & Fischer, Munich (2007)
5. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin. Springer, Berlin/Heidelberg (1992)
6. Keys, J.D.: Chinese herbs – their botany, chemistry, and pharmacodynamics. Charles E. Tuttle Company, Rutland/Vermont/Tokyo (1976)
7. Yu, J., Lang, H.Y., Xiao, P.G.: The occurrence of paeoniflorins and paeonols in Paeoniaceae. Acta. Pharm. Sin. **20**(3), 229–234 (1985)
8. He, Q., Hu, X.J., Cheng, Y.Y.: Analysis of 'SHUANGDAN' granules by high-performance liquid chromatography-diode array detection-electrospray ionization tandem mass spectrometry. J. Pharm. Biomed. Anal. **41**(2), 485–492 (2006)
9. Zhang, C., Hu, S., Cao, M., Xiao, G., Li, Y.: Antiproliferative and apoptotic effects of Paeonol on human hepatocellular carcinoma cells. Anticancer Drugs **19**(4), 401–409 (2008)
10. Xing, G., Zhang, Z., Liu, J., Hu, H., Sugiura, N.: Antitumor effect of extracts from moutan cortex on DLD-1 human colon cancer cells *in vitro*. Mol. Med. Rep. **3**(1), 57–61 (2010)
11. Matsuda, H., Ohta, T., Kawaguchi, A., Yoshikawa, M.: Bioactive constituents of chinese natural medicines. VI. Moutan cortex. (2): structures and radical scavenging effects of suffruticosides A, B, C, D, and E and galloyl-oxypaeoniflorin. Chem. Pharm. Bull. **49**(1), 69–72 (2001)
12. Xu, S.J., Yang, L., Zeng, M., Wang, Z.T.: Characterization of compounds in the Chinese herbal drug Mu-Dan-Pi by liquid chromatography coupled to electrospray ionization mass spectrometry. Rapid Commun. Mass Spectrom. **20**(22), 3275–3288 (2006)
13. Wu, H., Zhu, Z., Zhang, G., Zhao, L., Zhang, H., Zhu, D., Chai, Y.: Comparative pharmacokinetic study of Paeoniflorin after oral administration of pure Paeoniflorin, extract of Cortex Moutan and Shuang-Dan prescription to rats. J. Ethnopharmacol. **125**(3), 444–449 (2009)
14. Wu, X., Chen, H., Chen, X., Hu, Z.: Determination of Paeonol in rat plasma by high-performance liquid chromatography and its application to pharmacokinetic studies following oral administration of Moutan cortex decoction. Biomed. Chromatogr. **17**(8), 504–508 (2003)

15. Chen, G., Zhang, L., Yang, P.: Determination of three bioactive constituents in moutan cortex by capillary electrophoresis with electrochemical detection. Anal. Sci. **21**(10), 1161–1165 (2005)

16. Kim, J., Lee, H., Lee, Y., Oh, B.G., Cho, C., Kim, Y., Shin, M., Hong, M., Jung, S.K., Bae, H.: Inihibition effects of moutan cortex radicis on secretion of eotaxin in A549 human epithelial cells and eosinophil migration. J. Ethnopharmacol. **114**(2), 186–193 (2007)

17. Rho, S., Chung, H.S., Kang, M., Lee, E., Cho, C., Kim, H., Park, S., Kim, H.Y., Hong, M., Shin, M., Bae, H.: Inhibition of production of reactive oxygen species and gene expression profile by treatment of ethanol extract of moutan cortex radicis in oxidative stressed PC12 cells. Biol. Pharm. Bull. **28**(4), 661–666 (2005)

18. Tseng, Y.T., Hsu, Y.Y., Shih, Y.T., Lo, Y.C.: Paeonol attenuates microglia-mediated inflammation and oxidative stress-induced neurotoxicity in rat primary microglia ad cortical neurons. Shock **37**(3), 312–318 (2012)

19. Hsieh, C.L., Cheng, C.Y., Tsai, T.H., Lin, I.H., Liu, C.H., Chiang, S.Y., Lin, J.G., Lao, C.J., Tang, N.Y.: Paeonol reduced cerebral infarction involving the superoxide anion and microglia activation in ischemia-reperfusion injured rats. J. Ethnopharmacol. **106**(2), 208–215 (2006)

20. Lau, C.H., Chan, C.M., Chan, Y.W., Lau, K.M., Lau, T.W., Lam, F.C., Law, W.T., Che, C.T., Leung, P.C., Fung, K.P., Ho, Y.Y., Lau, C.B.S.: Pharmacological investigations of the anti-diabetic effect of Cortex Moutan and its active component paeonol. Phytomedicine **14**(11), 778–784 (2007)

21. He, Q., Ge, Z.W., Song, Y., Cheng, Y.Y.: Quality evaluation of cortex moutan by high performance liquid chromatography coupled with diode array detector and electrospray ionization tandem mass spectrometry. Chem. Pharm. Bull. **45**(9), 1271–1275 (2006)

22. Wu, M., Gu, Z.: Screening of bioactive compounds from moutan cortex and their anti-inflammatory activities in rat synoviocytes. Evid. Based. Complement. Alternat. Med. **6**(1), 57–63 (2009)

23. Ha, D.T., Trung, T.N., Hien, T.T., Dao, T.T., Yim, N., Ngoc, T.M., Oh, W.K., Bae, K.H.: Selected compounds derived from Moutan Cortex stimulated glucose uptake and glycogen synthesis *via* AMPK activation in human HepG2 cells. J. Ethnopharmacol. **131**(2), 417–424 (2010)

24. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines: thin-layer and high performance liquid chromatography of chinese drugs, vol. I+II. Springer, Wien/New York (2011)

25. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin/Heidelberg/New York (2001)

26. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)

27. Hempen, C.H., Fischer, T.: A materia medica for chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone, Elsevier, New York (2009)

Radix Peucedani – *Qianhu*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Hogfennel Root is the dried root of *Peucedanum praeruptorum* Dunn (Fam. Apiaceae).

The drug is collected in winter to next spring when stem and leaves wither or before floral stem grows, removed from rootlet, washed clean and dried in the sun or at lower temperature.

Other source plant:[2–4] *Peucedanum decursivum* (*Angelica decursiva*) → not contained in the Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Origin:[3] Provinces Zheijiang, Hunan, Anhui, Jiangxi, Shandong, Sichuan (China)

Description of the drug:[1] Irregular cylindrical, conical or fusiform, slightly twisted, the lower part frequently branched, 3–15 cm long, 1–2 cm in diameter. Externally blackish-brown or greyish-yellow, frequently with stem scars and fibrous remains of pericladia at the root stock, with numerous fine annular striations at the upper end, and longitudinal furrows or wrinkles and transverse lenticel-like cicatrices at the lower part. Texture relatively flexible, hard when dried, easily broken, fracture uneven, pale yellowish-white, numerous brownish-yellow oily spots scattered in bark, cambium ring brown, rays radiated. Odour, aromatic; taste, slightly bitter and pungent.

Pretreatment of the raw drug:[1] Foreign matters are eliminated, washed clean, softened thoroughly, cut into thin slices and dried in the sun.

Processing:[1] *Peucedani Radix (processed with honey)*

The slices of Peucedani Radix are stir-baked as described under the method for stir-baking with honey (Appendix II D) until not sticky to fingers.

Medicinal use:[4–9] In China used for the treatment of certain respiratory diseases such as asthma, chronic bronchitis and pulmonary hypertension. It is also used to treat gastric ulcers.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_10, © Springer International Publishing Switzerland 2015

Effects and indications of Radix Peucedani according to Traditional Chinese Medicine[1, 3, 5, 6, 10]	
Taste:	Pungent, bitter
Temperature:	Cold tendency
Channels entered:	*Orbis pulmonalis, o. lienalis*
Effects (functions):	To direct *qi* downward and resolve phlegm, disperse *wind* and clear heart.
Symptoms and indications:	Phlegm-heat panting and fullness, yellow thick phlegm, wind-heat cough and profuse sputum.

Main constituents:

- *Peucedanum praeruptorum*[2–4, 6, 11–15]
  - **Pyranocoumarins and -glycosides**
    Praeruptorin A-F; praeroside II-V; pteryxin; qianhucoumarins A-D, F, H and I
  - **Furanocoumarin glycosides**
    Praeroside I + II, isorutarin, rutarin, marmesinin (= nodakenin), psoralen, bergapten, xanthotoxin, byakangelicin, qianhucoumarin G, apterin
  - Coumarin glycosides
    Scopoline, skimming, apiolskimmin

- *Peucedanum decursivum*[2, 3, 15]
  - **Furo-, Pyranocoumarins and -glycosides**
    Bergapten, nodakenetin, nodakenin, decurosides I-VI, xanthyletin, decursin, decursidin, decursidate, 7-angeloyloxy-6-isovaleroyloxy-6,7-dihydroxanthyletin, 7-angeloyloxy-6-senescioyloxy-6,7-dihydroxanthyletin, 7-senecioyloxy-6-hydroxy-6,7-dihydroxanthyletin, 7-hydroxy-6-senecioyloxy-6,7-dihydroxanthyletin, 7-acetoxy-6-isovaleroyloxy-6,7-dihydroxanthyletin, 7-acetoxy-6-angeloyloxy-6,7-dihydroxanthyletin,

Minor constituents: Spongesterin, mannit, estragol (chavicol methylether), limonen

Note: Coumarin content in the root of *P. praeruptorum* and *P.decursivum* are 0.6 and 1.1 %, respectively[2, 15].

Note: Furocoumarins may provoke photosensitivity[5].

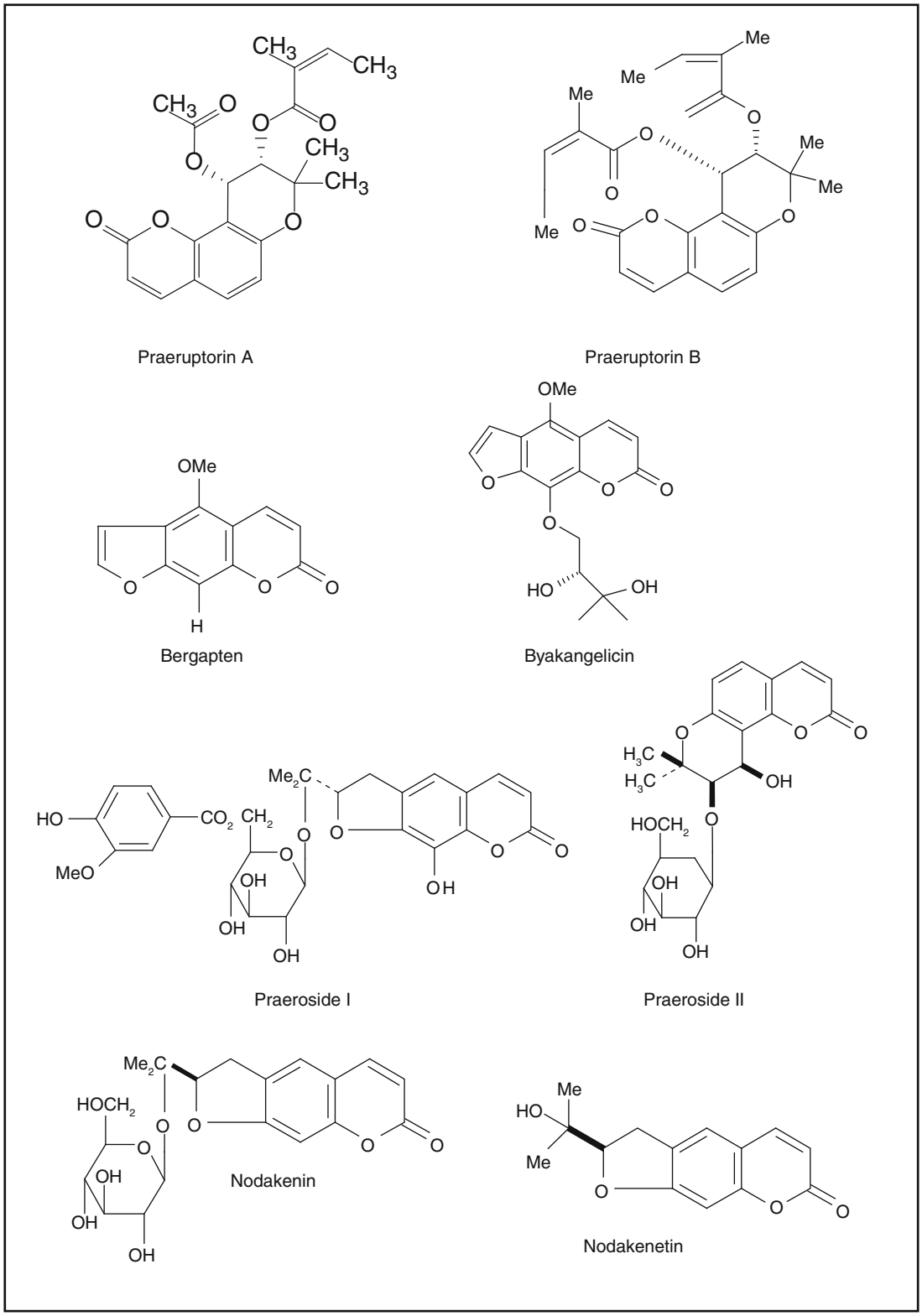


Fig. 1: Formulae of the main constituents of Radix Peucedani[2]

Reported Pharmacological Activities

Lungs/bronchia	Heart/circulation
• Anti-spasmodic activity[2, 15]	• Inhibition of human platelet aggregation[2, 11, 15–19]
• Inhibition of the contraction of human pulmonary artery induced by norepinephrine[4]	• Coronary dilatory[11, 15]
• Relaxation of tracheal and vascular smooth muscles of rabbits in vitro[6]	• Myocardial protection[11, 15, 17]
• Inhibition of the release of anaphylactic mediator from rat mast cells induced by concanavalin A[6]	• Calcium antagonistic action[11, 13, 15, 19, 20]
• Anti-asthma[13]	• Lowers levels of superoxide dismutase and malondialdehyde after local myocardial ischemia-reperfusion injury in rats[6]
• Relaxation of gut musculature, trachea, vessels and uterus[7, 13, 21]	• Increases intermediate filament desmin and vimentin contents in ischemia/reperfusion myocardiocytes[16, 20, 22]
• Inhibition of inflammatory response in LPS-stimulated murine macrophages[14]	• Anti-hypertension[16, 19]
• Anti-inflammatory[16, 21]	• Anti-heart failure properties[16]
• Reduction the level of proinflammatory factors[20, 22]	• Vasodilatation[17, 19]

Miscellaneous effects:	Praeruptorin B shows remarkable relaxant effect on the smooth muscles of ileum and taenia coli[4] Antitumor[11, 18] Anti-cancer[11, 14, 16, 17] Anti-leukemia[11, 17] Anti-oxidant[14, 21] Inhibits the expression of apoptosis related proteins[22] Antimutagenic[15] Ameliorates hypoxia-induced pulmonary hypertension in dogs[4]

TLC-Fingerprint Analysis

Drug samples	Origin
1 Radix Peucedani/*Peucedanum praeruptorum*	Sample of commercial drug, obtained from SinoMed, TCM-Clinic Bad Kötzting (Charge: 15114022004)
2 Radix Peucedani/*Peucedanum praeruptorum*	Sample of commercial drug, obtained from PharmaChin GmbH (Charge: 405051)
3 Radix Peucedani/*Peucedanum praeruptorum*	Sample of commercial drug, obtained from SinoMed, TCM-Clinic Bad Kötzting, (Charge: 15119042010)
4 Radix Peucedani/*Peucedanum praeruptorum*	Sample of commercial drug, obtained from China Medica (Charge: 12 0092; origin: Bozhou, Anhui, China)

Drug samples		Origin
5	Radix Peucedani/*Peucedanum praeruptorum*	Province Ningguo, Anhui, China
6	Radix Peucedani/*Peucedanum praeruptorum*	Province Guangde, Anhui, China
7	Radix Peucedani/***Angelica decursiva***	Province Guangde, Anhui, China
8	Radix Peucedani/***Angelica decursiva***	Province Fengqiao, Guangde, Anhui, China

1. **TLC-fingerprint analysis of Radix Peucedani and praeruptorin B:**[23]

Reference compound of Fig. 2		*Rf*
T 1	Praeruptorin B	0.63

1. Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml methanol.
2. Reference compound: 0.5 mg is dissolved in 0.5 ml ethanol
3. Separation parameters:

   Plate: HPTLC Silica gel 60 F_{254}, Merck

   Applied amounts: Radix Peucedani extracts: 10 µl each
   Reference compound: 10 µl

   Solvent system: Toluene 1 + Diethyl ether (saturated with 10 % glacial acetic acid) 1
   Toluene (50 ml) and diethyl ether (50 ml) are shaken with 50 ml 10 % glacial acetic acid in a separating funnel for 5 min. The lower phase is discarded and the saturated toluene-diethyl ether mixture used for the TLC.

   Detection: 10 % ethanolic KOH

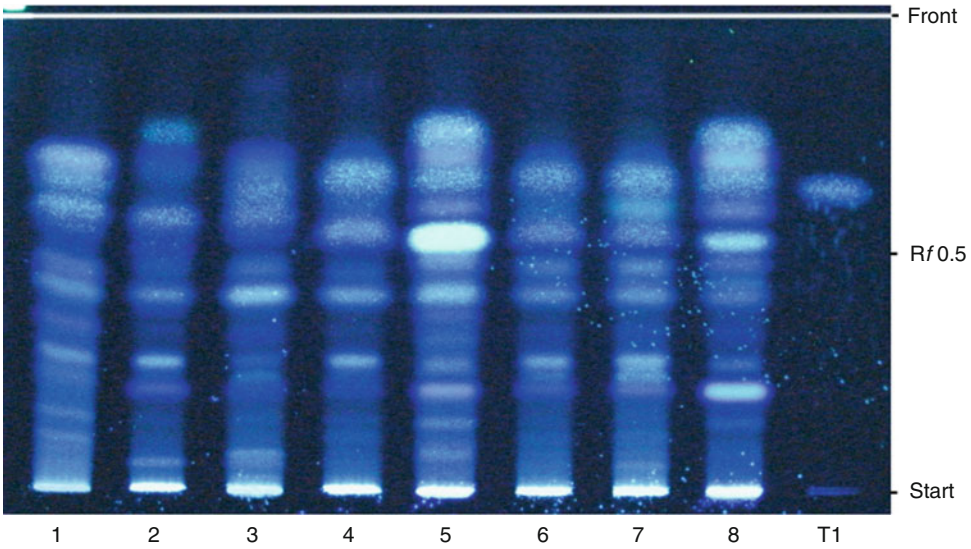


Fig. 2: Thin layer chromatogram of the methanol extracts of Radix Peucedani sprayed with 10 % ethanolic potassium hydroxide solution (UV 366 nm)

4. Description of Fig. 2:

   The various extract samples show a very homogenous zone profile of 7–9 bluish fluorescent zones from the start up to R*f* = 0.7 with praeruptorin B (**T1**) at R*f* = 0.63 and in sample 5 with a chlorogenic acid isomer at R*f* = 0.53. The start is marked by the various white fluorescent coumarin-glycosides.

2. **TLC-fingerprint analysis of Radix Peucedani, praeruptorin B, with the phenolcarboxylic acids and coumarin-glycosides:** [24]

Reference compounds of Fig. 3		**R*f***
T 1	Praeruptorin B	0.95
T 2	Caffeic acid	0.86
T 3	Mixture of Chlorogenic acids isomers	0.34/0.62
T 4	Scopoletin	0.86

1. Extraction: 1 g powdered drug is extracted with 10 ml methanol under reflux for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2. Reference compounds: 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Radix Peucedani extracts: 10 µl each Reference compounds: 10 µl each
Solvent system:	Toluene + ethyl acetate + formic acid + water (5 + 100 + 10 + 10)
Detection:	Without chemical treatment

4. Description of Fig. 3:

In this solvent system the caffeic acid (**T2**, R*f* = 0.86) and the coumarin scopoletin (**T4**, R*f* = 0.86) appear separated from praeruptorin B (**T1**, R*f* = 0.95). The main chlorogenic acid isomers (**T3**) at R*f* = 0.34 and at R*f* = ~0.62. could be not detected in all Peucedanum samples.

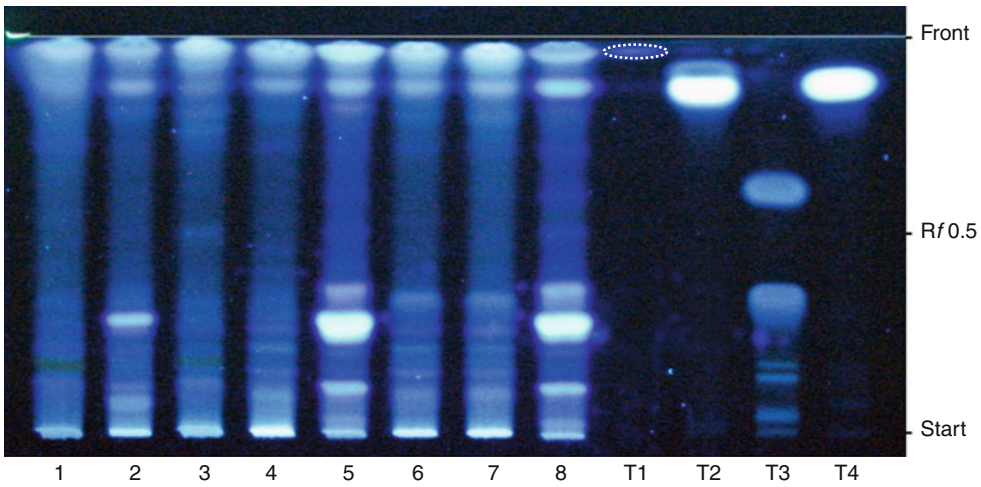


Fig. 3: Thin layer chromatogram of the methanol extracts of Radix Peucedani without chemical treatment (UV 366 nm)

HPLC-Fingerprint Analysis:[25, 26]

1. Sample preparation: 1 g powdered drug is extracted with 10 ml methanol under reflux for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml methanol.

2. Injection volume: Radix Peucedani extract: 10 µl each

3.1. 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: 10 ml 0.1 H_3PO_4/1 l water (Millipore Ultra Clear UV plus® filtered) B: acetonitril (VWR)
Gradient:	0–30 % B in 6 min, 30–60 % B in 6 min, 60 % B for 2 min, 60–100 % B in 11 min, 100 % B for 10 min, Total runtime: 35 min
Flow:	1 ml/min
Detection:	320 nm

Retention times of the main peaks recorded at 320 nm

Peak	Rt (min)	Compound	
1	5.6	Not identified	A
2	6.5	Not identified	
3	7.5	Scopoletin	
4	14.0	Not identified Pyranocoumarin	B
5	15.9	Not identified Pyranocoumarin	
6	16.1	Not identified Pyranocoumarin	
7	17.7	Not identified Pyranocoumarin	
8	18.6	Praeruptorin B	
9	19.1	Not identified Pyranocoumarin	
10	19.7	Not identified Pyranocoumarin	

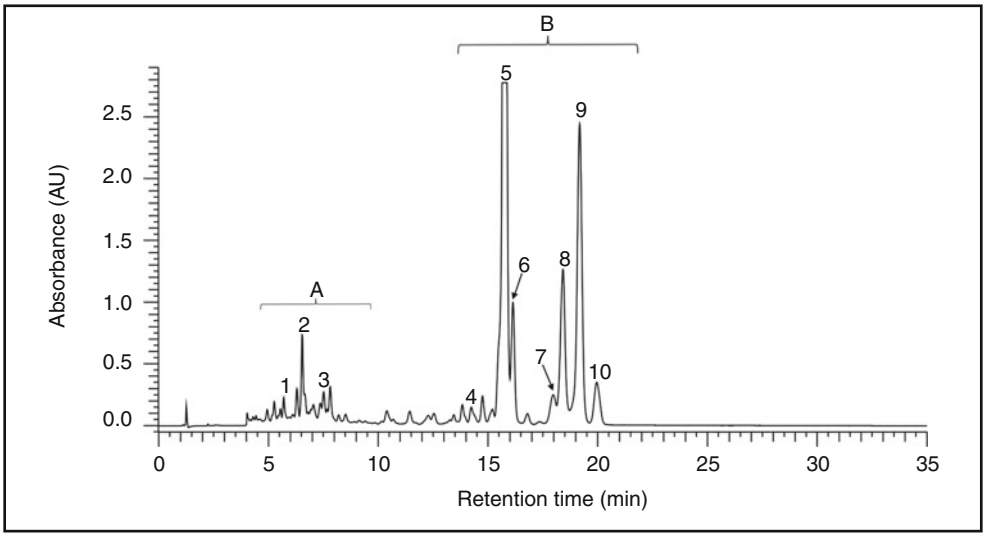


Fig. 4a: HPLC-fingerprint analysis of the methanol extract of Radix Peucedani, sample 4

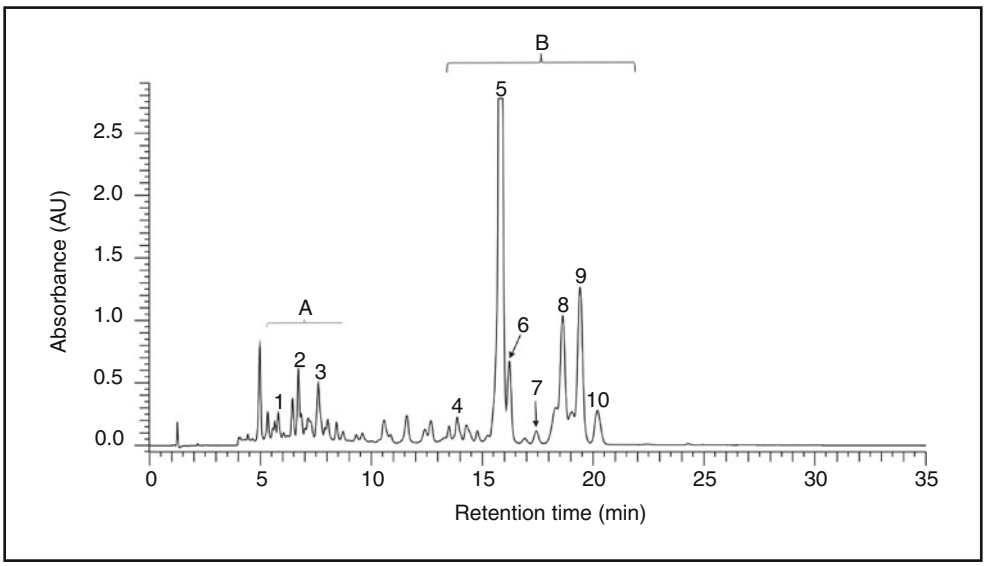


Fig. 4b: HPLC-fingerprint analysis of the methanol extract of Radix Peucedani, sample 12

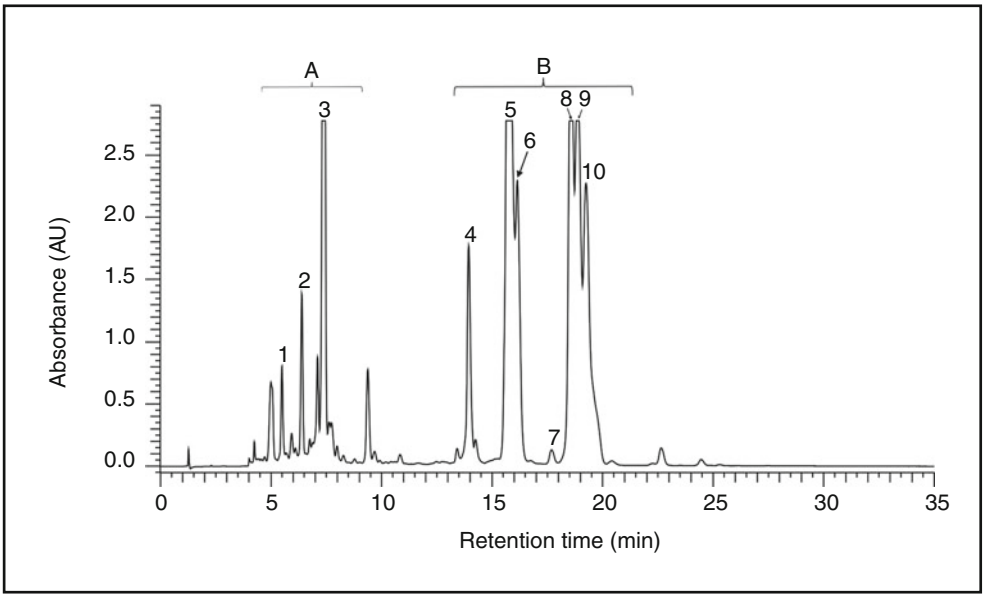


Fig. 4c: HPLC-fingerprint analysis of the methanol extract of Radix Peucedani, sample 14

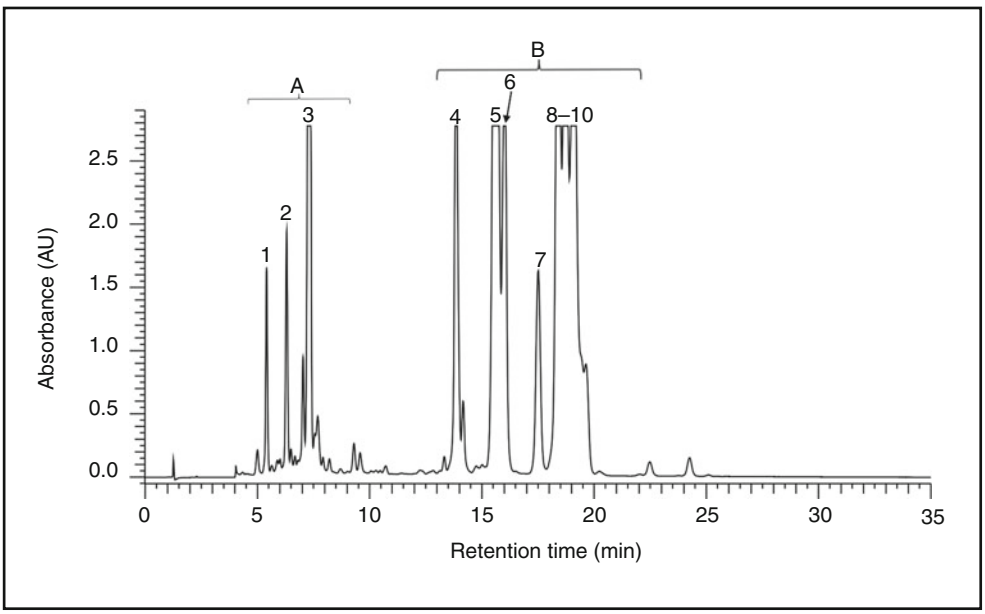


Fig. 4d: HPLC-fingerprint analysis of the methanol extract of Radix Peucedani (*Angelica decursivum*), sample 7

4.1. Description of Figs. 4a, 4b, 4c, and 4d:

All three *Peucedanum praeruptorum* extract samples 4,12 and 14 and sample 7, labelled as *Angelica decursivum*, showed the two peak block **A** (between Rt = 5.0 – 10.0) and **B** (between Rt = 13.0 – 21.0).
In peak block **A** scopoletin (**3**) could be recorded at Rt = 7.5 with phenylcarboxylic acids (e.g. caffeic acid and chlorogenic acid).
In peak block **B** only praeruptorin B (**8**) was identified at Rt = 18.6. All other peaks can be assigned to pyrano- or furanocoumarins.

3.2. 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: water (Millipore Ultra Clear UV plus®) B: methanol (VWR)
Gradient:	5–40 % B in 12 min, 40–50 % B in 15 min, 50–65 % B in 5 min, 65–90 % B for 28 min, Total runtime: 60 min
Flow:	1 ml/min
Detection:	320 nm

Retention times of the main peaks recorded at 320 nm

Peak	Rt (min)	Compound	
1	10.3	Not identified	A
2	14.2	Not identified	
3	18.2	Scopoletin	
4	36.7	Not identified	B
5	39.8	Not identified	
6	40.7	Not identified	
7	44.3	Not identified	
8	44.9	Praeruptorin B	
9	46.1	Not identified	
10	47.5	Not identified	

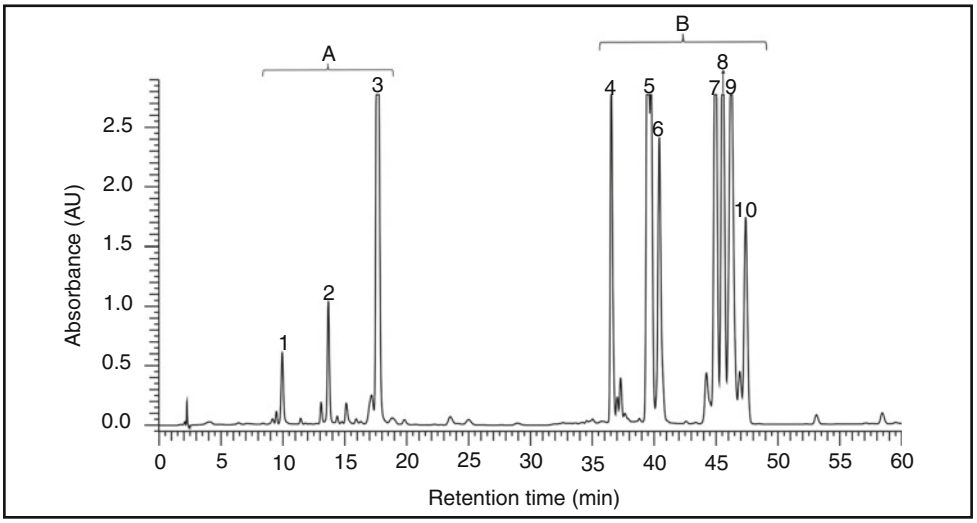


Fig. 5: HPLC-fingerprint analysis of the methanol extract of Radix Peucedani (*Angelica decursivum*), sample 7

4.2. Description of Fig. 5:

Sample 7 (*Angelica decursivum*) was HPLC-fingerprinted in a different solvent system and with another gradient which provided a better separation of all main peaks in block **B**.

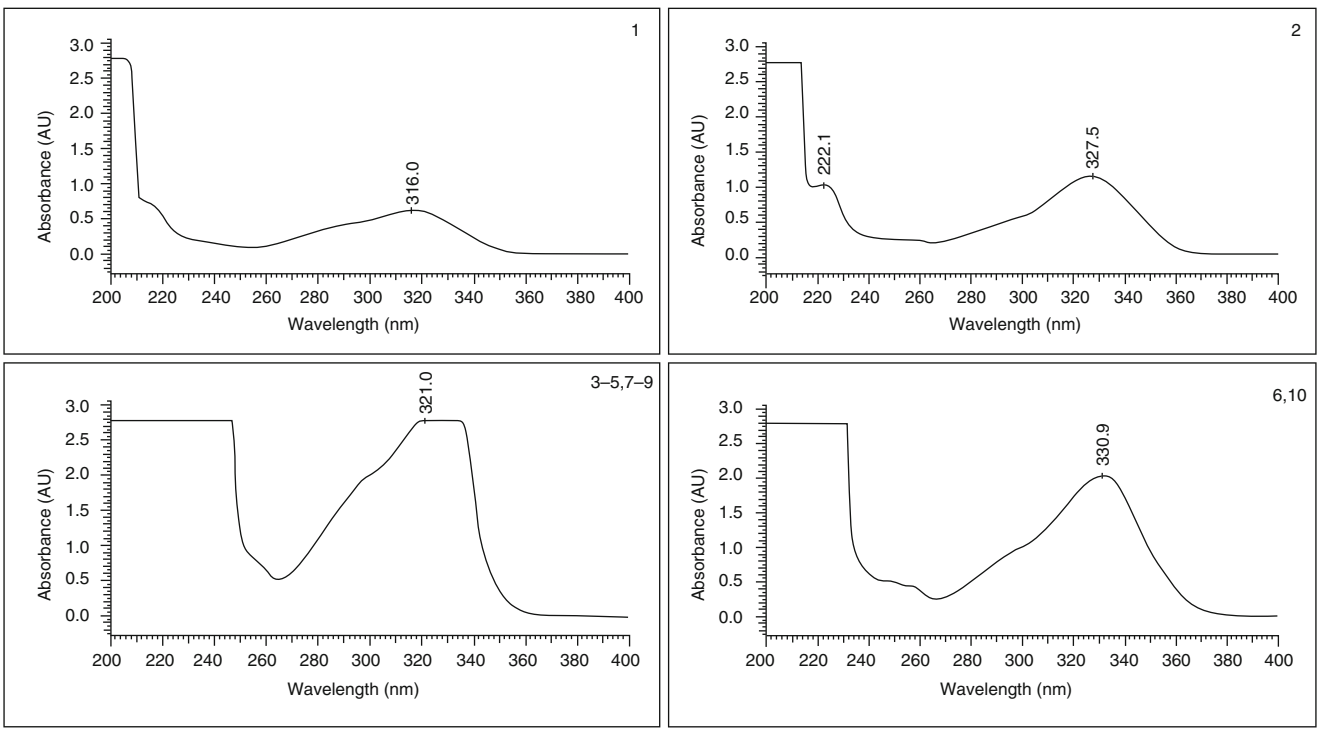


Fig. 6: On line UV-spectra of the main peaks of Radix Peucedani

Note: According to the Chinese Pharmacopoeia, Radix Peucedani contains not less than 0.90 % of praeruptorin A and 0.24 % of praeruptorin B calculated with reference to the dried drug.

Conclusion

The best identity proof of Radix Peucedani can be achieved with the HPLC-method. Since according to the Chinese Pharmacopeia Radix Peucedani praeruptori can be substituted by *Peucedanum decursivum* (= *Angelica decursiva*) it might be of interest to compare also the HPLC-fingerprint analysis technique described for Radix Angelica pubescentis (pp. 99–111[24]).

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin. Springer, Berlin/Heidelberg (1992)
3. Paulus, E., Ding, Y.-H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)
4. Zhao, N.C., Jin, W.B., Zhang, X.H., Guan, F.L., Sun, Y.B., Adachi, H., Okuyama, T.: Relaxant effects of pyranocoumarin compounds isolated from a chinese medical plant, Bai-Hua Qian-Hu, on isolated rabbit tracheas and pulmonary arteries. Biol. Pharm. Bull. **22**(9), 984–987 (1999)
5. Hempen, C.-H., Fischer, T.: Leitfaden Chinesische Phytotherapie 2. Auflage. Urban & Fischer, Munich (2007)
6. Xiong, Y., Wang, J., Wu, F., Li, J., Zhou, L., Kong, L.: Effects of (±)-praeruptorin A on airway inflammation, airway hyperresponsiveness and NF-κB signaling pathway in a mouse model of alergic airway disease. Eur. J. Pharmacol. **683**(1–3), 316–324 (2012)
7. Xu, Z., Wang, X., Dai, Y., Kong, L., Wang, F., Xu, H., Lu, D., Song, J.: Hou Z, (±)-Praeruptorin A enantiomers exert distinct relaxant effects on isolated rat aorta rings dependent on endothelium and nitric oxide synthesis. Chem. Biol. Interact. **186**(2), 239–246 (2010)
8. Zhang, J.X., Fong, W.F., Wu, J.Y., Yang, M., Cheung, H.Y.: Pyranocoumarins isolated from *Peucedanum praeruptorum* as differentiation inducers in human leukemic HL-60 cells. Planta Med. **69**(3), 223–229 (2003)
9. Ishii, H., Okada, Y., Baba, M., Okuyama, T.: Studies of coumarins from the chinese drug Qianhu, XXVII[1]: structure of a new simple coumarin glycoside from Bai-Hua Qianhu, *Peucedanum praeruptorum*. Chem. Pharm. Bull. **56**(9), 1349–1351 (2008)
10. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
11. Liang, T., Yue, W., Li, Q.: Chemopreventive effects of *Peucedanum praeruptorum* DUNN and its major constituents on SGC7901 gastric cancer cells. Molecules **15**(11), 8060–8071 (2010)
12. Rao, M.R., Shen, X.H., Zou, X.: Effects of praeruptorin C and E isolated from 'Qian-Hu' on swine coronary artery and guinea-pig atria. Eur. J. Pharmacol. **155**(3), 293–296 (1988)
13. Lu, M., Nicoletti, M., Battinelli, L., Mazzanti, G.: Isolation of praeruptorins A and B from *Peucedanum praeruptorum* Dunn. and their general pharmacological evaluation in comparison with extracts of the drug. Farmaco **56**(5–7), 417–420 (2011)
14. Yu, P.J., Jin, H., Zhang, J.Y., Wang, G.F., Li, J.R., Zhu, Z.G., Tian, Y.X., Wu, S.Y., Xu, W., Zhang, J.J., Wu, S.G.: Pyranocoumarins isolated from *Peucedanum praeruptorum Dunn* suppress lipopolysaccharide-induced inflammatory response in murine macrophages through inhibition of NF-κB and STAT3 activation. Inflammation **35**(3), 967–977 (2011)
15. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)
16. Yu, P.J., Ci, W., Wang, G.F., Zhang, J.Y., Wu, S.Y., Xu, W., Jin, H., Zhu, Z.G., Zhang, J.J., Pang, J.X., Wu, S.G.: Praeruptorin A inhibits lipopolysaccharide-induced inflammatory response in murine macrophages through inhibition of NF-κB pathway activation. Phytother. Res. **25**(4), 550–556 (2011)
17. Liu, R., Feng, L., Sun, A., Kong, L.: Preparative isolation and purification of coumarins from *Peucedanum praeruptorum* Dunn by high-spedd counter-current chromatography. J. Chromatogr. A. **1057**(1–2), 89–94 (2004)
18. Chang, H., Okada, Y., Okuyama, T., Tu, P.: Spectral assignments and reference data, ^{1}H and ^{13}C NMR assignments for two new angular furanocoumarin glycosides from *Peucedanum praeruptorum*. Magn. Reson. Chem. **45**(7), 611–614 (2007)
19. Aida, Y., Kasama, T., Takeuchi, N., Tobinaga, S.: The antagonistic effects of khellalactones on platelet-activating factor, histamine, and leukotrien D_4. Chem. Pharm. Bull. **43**(5), 859–867 (1995)

20. Zhang, Z., Liu, Y.Y., Su, M.Q., Liang, X.F., Wang, W.F., Zhu, X.: Pharmacokinetics, tissue distribution and excretion study of *dl*-*praeruptorin* A of *Peucedanum praeruptorum* in rats by liquid chromatography tandem mass spectrometry. Phytomedicine **18**(6), 527–532 (2011)
21. Xiong, Y.Y., Wu, F.H., Wang, J.S., Li, J., Kong, L.Y.: Attenuation of airway hyperreactivity and T helper cell type 2 response by coumarins from *Peucedanum praeruptorum* Dunn in a murine model of allergic airway inflammation. J. Ethnopharmacol. **141**(1), 314–321 (2012)
22. Zhang, Z., Liang, X.F., Su, M.Q., Yang, Q., Li, L.P., Zhang, X.H., Wang, X.M., Zhu, X.: Pharmacokinetics of *dl*-praeruptorin A after single-dose intravenous administration to rats with liver cirrhosis. Daru **19**(3), 210–215 (2011)
23. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin/Heidelberg/New York (2001)
24. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines: thin layer and high performance liquid chromatography of chinese drugs, vol. 1 and 2. Springer, Wien (2011)
25. Eeva, M., Rauha, J.-P., Vuorela, P., Vuorela, H.: Computer-assisted, high-performance liquid chromatography with mass spectrometric detection for the analysis of coumarins in *Peucedanum palustre* and *Angelica archangelica*. Phytochem. Anal. **15**(3), 167–174 (2004)
26. Hong Kong chinese materia medica standards, vol 4. Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region, The People's Republic of China (2011)

Radix Achyranthis bidentatae – *Niuxi*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Twotoothed Achyranthes Root is the dried root of *Achyranthes bidentata* Bl. (Fam. Amaranthaceae).

The drug is collected in winter when the aerial part is withered, removed from rootlet and soil, tied up in a small bundle, sun-dried to be wrinkled externally, cut evenly at the summit, and then dried thoroughly.

Origin:[2, 3] Chinese Districts: Henan, Sichuan, Hebei, Shandong, Liaoning. Tropical areas of Asia and Africa

Description of the drug:[1] Slender cylindrical, straight or slightly curved, 15–70 cm, 0.4–1 cm in diameter. Externally grayish-yellow or pale brown, with slightly twisted and fine longitudinal wrinkles, transverse lenticel-like protrudings and sparse rootlet scars. Texture hard and fragile, easily broken, softened when moistened, fracture even, pale brown, slightly horny and oily. Xylem of vascular bundles arranged in 2–4 whorls. Odour, slight; taste, slightly sweet, somewhat bitter and astringent.

Pretreatment of the raw drug:[1] Foreign matters are eliminated, the drug is washed clean, softened thoroughly, remains of rhizomes removed, cut into sections and dried.

Processing:[1] Achyranthis bidentatae Radix (processed with wine)

The sections of Achyranthis bidentatae Radix are stirbaked as described under the method for stir-baking with wine (Appendix II D) to dryness.

Medicinal use:[4, 5] Used as antihypertonic, antispasmotic, antiasthmatic and diuretic drug, at menopausal syndrome, cerebrovascular insult and arthritis.

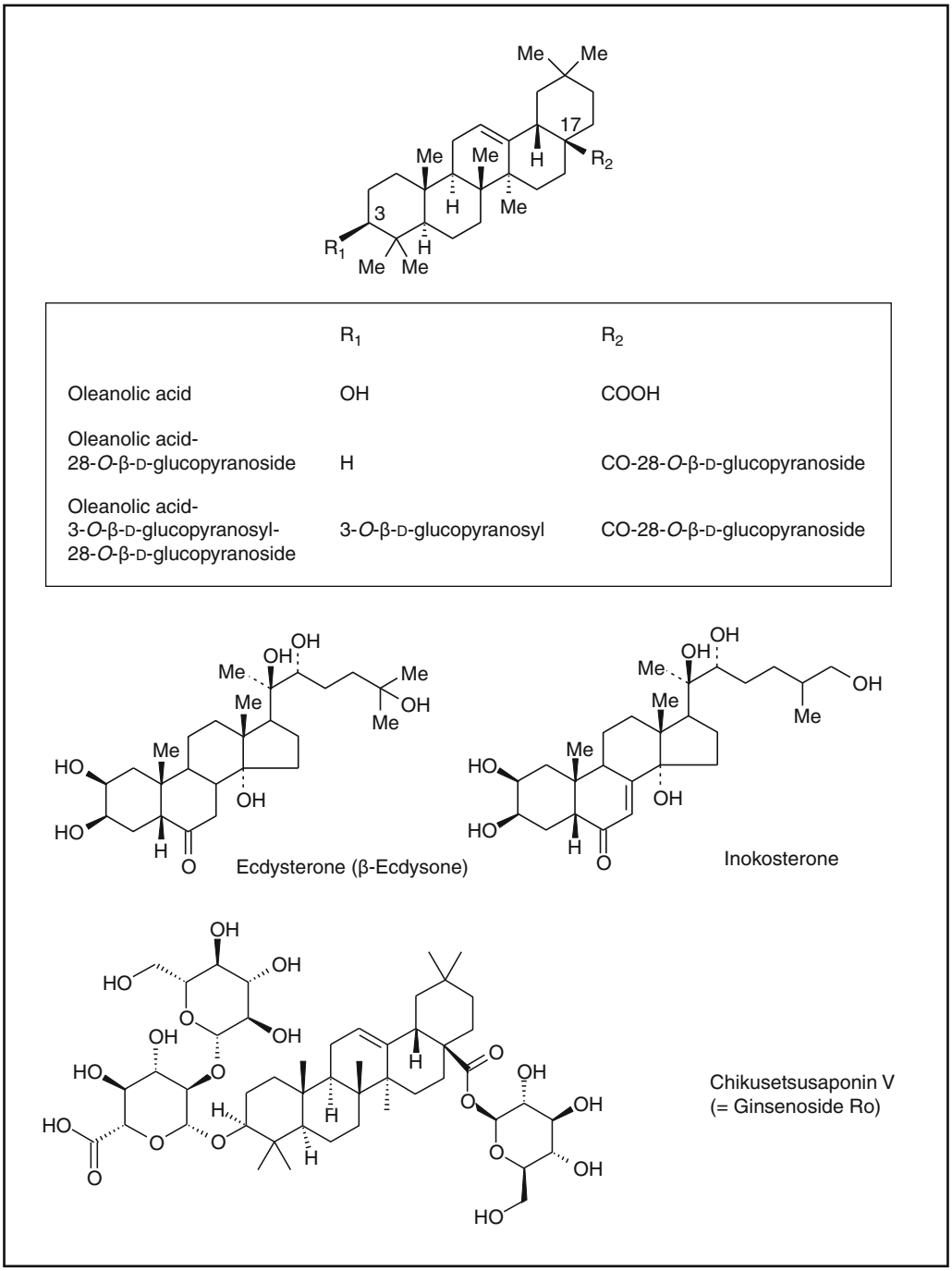
H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_11, © Springer International Publishing Switzerland 2015

Effects and indications of Radix Achyranthis bidentatae according to Traditional Chinese Medicine[1–4, 6–13]

Taste:	Bitter, sour
Temperature:	Neutral
Channels entered:	*Orbis hepaticus, o. renalis*
Effects (functions):	To expel stasis to unblock the merdian, tonify liver kidney, strengthen sinew and bone, disinhibit urine and relieve stranguria, and conduct blood downward.
Symptoms and indications:	Amenorrhea, dysmenorrheaa, excessively lochia, menopausal complaints, frigidity, limp aching in the lower back and knees, lack of strength of sinew and bone, stranguria, edema, swellings following blunt trauma, blurred vision, headache, dizziness, toothache, mouth sore, hematemesis, epistaxis. It is also applied in fields like rheumatic diseases, hypertension, apoplexy, diabetes, gastroenteritis. In traditional medicine it is used as a tonic, diuretic, antifertility and immunostimulatory agent, against osteodynia of the lumbar region and knees, spasm and flaccidity of limbs, abdominal pain and trauma, soreness, flaccidity of extremeties, dredging the channels, nourishing the liver and kidney, as an emmemanogue, antiarthritic, agent and to invigorate circulation.

Main Constituents of Radix Achyranthis bidentatae[1, 2, 5, 8, 10–17]

Oleanolic acid-glycosides (Saponins):	Oleanolic acid, oleanolic acid-28–*O*–β-D-glucopyranoside, oleanolic acid-3-*O*–β-D-glucopyranosyl-28-O–β-D-glucopyranoside, bidentatoside I, PJS-1, bidentatoside II (= 3-*O*–β-[2′-(2”-O-glycolyl)-glyoxylyl]-oleanolic acid-28-O-β-D-glucopyranoside), momordin IIa, momordin Ib, zingibroside R_1
Dammarane saponins:	Chikusetsusaponin IVa, chikusetsusaponin IVa ethyl ester, chikusetsusaponin IVa methyl ester, 28-deglucosyl-chikusetsusaponin IVa, 28-deglucosyl-chikusetsusaponin IVa butyl ester, chikusetsusaponin V (= ginsenoside Ro) chikusetsusaponin V methyl ester achyranthoside A trimethyl ester, achyranthoside C dimethyl ester, achyranthoside C butyl dimethyl ester, achyranthoside D trimethyl ester, achyranthoside E butyl methyl ester, achyranthoside E dimethyl ester
Steroids:	β-ecdysone (= β-ecdysterone, 20-hydroxyecdysone), rubrosterone, polypodine B, inokosterone, (25*S*)-20,22-*O*-(*R*-ethylidene)inokosterone, 20,22-*O*-(*R*-ethylidene)-20-hydroxyecdysone, 20,22-*O*-(*R*-3-methoxycarbonyl)propylidene-20-hydroxyecdysone, 20-hydroxyecdysone-20,22-monoacetonide, (25*S*)-inokosterone, (25*R*)-inokosterone, (25*S*)-inokosterone-20,22-acetonide, niuxixinsterone A-C, serfurosterone A,
Anthraquinones:	Emodin, physcione (parietin)
Flavonoids and phenolcarboxylic acids:	Quercetin, rutin, caffeic acid
Others:	18-(β-D-glucopyranosyloxy)-28-oxoolean-12-en-3β-yl 3-*O*-(β-D-glucopyranosyl β-D-glucopyranosiduronic acid methyl ester, allantoin



	R_1	R_2
Oleanolic acid	OH	COOH
Oleanolic acid-28-*O*-β-D-glucopyranoside	H	CO-28-*O*-β-D-glucopyranoside
Oleanolic acid-3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside	3-*O*-β-D-glucopyranosyl	CO-28-*O*-β-D-glucopyranoside

Ecdysterone (β-Ecdysone)

Inokosterone

Chikusetsusaponin V
(= Ginsenoside Ro)

Fig.1: Formulae of the main compounds of Radix Achyranthis bidentatae[14]

Pharmacology

Effects on cardiovascular system:
Aantihypertensive[2, 14]
Reduce blood pressure[14]
Increase blood flow[3]

Effects on immune functions:
Anti-inflammatory[2, 3]
Immunomodulatory[3, 8, 10, 13, 18]

Gynecological effects:
Stimulates uterine contractions[2, 18]
Decreases fertility[2, 13, 18]

Effects on hyperglycemia:
Suppression of hyperglycemia[14]
Effect on carbohydrate metabolism in blood[8, 10]

Effects on lipid metabolism:
Improvement of lipid metabolism[8, 10]
Insulin-independent lowering of glucose levels[9]

Effects on central nervous system:[8]
Cognition-enhancing[18]
Anti-senility[3]
Improvement of the learning and memory[9]

Cell protective effects:
Protection of PC12 cell cytotoxicity[9]

Other protective effects:
Analgesic[2, 3, 13, 18]
Vasodilatory effect[3, 13, 14]
Prevention of experimental liver damage[14]
Epstein-Barr-Virus inhibiting effect[14]
Anti-osteoporosis[3, 18]
Antitumor[3, 18]
Stimulation of RNA and protein synthesis[8, 10]
Promotion of wound healing[9]
Antibacterial[18]

TLC-Fingerprint Analysis[1]

Drug samples	Origin
1 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Sample of commercial drug (HerbaSinica, origin: Henan, China)
2 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Sample of commercial drug (Pharmacy of Munich, Germany)
3 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Sample of commercial drug, SinoMed, TCM-Clinic Bad Kötzting
4 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Sample of commercial drug (China Medica, origin: Henan, Jiaouzuo, China)
5 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Sample of commercial drug (Caesar & Loretz, origin: Henan, China)
6 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Province Hebei, Angon, Zhengzhang (China)

Drug samples	Origin
7 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Province Henan, Wuzhi, Dapeng (China)
8 Radix Achyranthis bidentatae/*Achyranthes bidentata*	Province Hebei (China)

Reference compounds		R*f*
T 1	Ecdyson	0.65
T 2	Ginsenoside Re	0.34
T 3	Ginsenoside Rb1	0.16
T 4	Ginsenoside Rg1	0.52
T 5	Ginsenoside Rb2	0.23
n.a.	Oleanolic acid	0.99
n.a.	Glucose	0.17
n.a.	Saccharose	0.08

n.a. not applied

1. Sample preparation: 2 g powdered drug are extracted with 30 ml *n*-hexane under reflux for 30 min. The solvent is discarded and the residue re-extracted with 30 ml methanol under reflux for 2 h, filtered and the filtrate evaporated to dryness.
   15 ml water and 30 ml *n*-butanol are added, shaken and separated in a separating funnel. The *n*-butanol phase is evaporated to dryness, the residue dissolved in 2.5 ml methanol and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.
2. Reference compounds: 1 mg is dissolved in 1 ml methanol
3. Separation parameters:

   Plate: HPTLC Silica gel 60 F_{254}, Merck

   Applied amounts: Radix Achyranthis bidentatae extracts: 10 µl each
   reference compounds: 10 µl each

   Solvent system: Chloroform + methanol + formic acid + water (14 + 6 + 0.1 + 1)

   Detection: Komarowsky reagent (KOM)

   1 ml 50 % ethanolic sulphuric acid and 10 ml 2 % methanolic 4-hydroxybenzaldehyd are mixed shortly before use.

   The sprayed plate is heated at 105 °C for 5 min and evaluated in VIS.

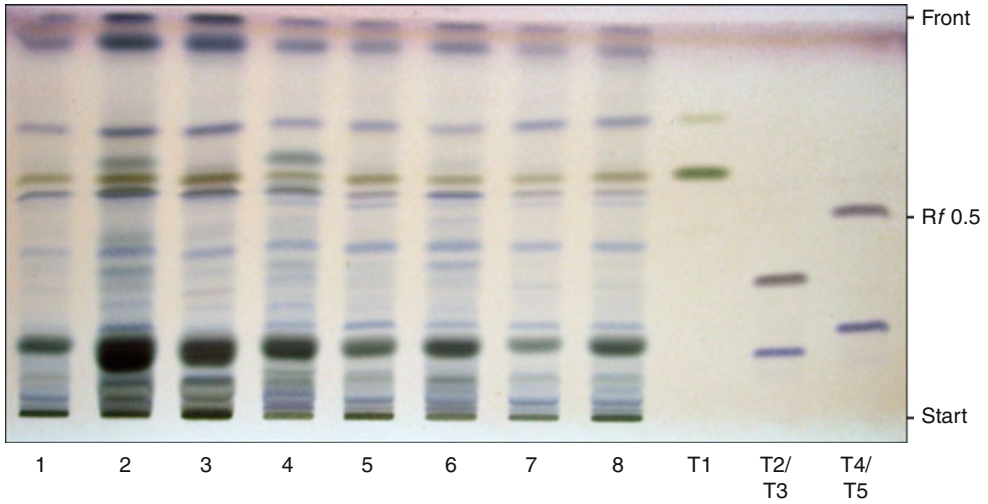


Fig. 2: Thin layer chromatogram of the butanol phase of the Radix Achyranthis bidentatae extracts detected with Komarowsky reagent (VIS)

4. Description of Fig. 2:

   All 8 methanol extract samples of Radix Achyranthis bidentatae show a very homogenous pattern of >15 grey-blue zones over the whole range of the TLC-plate.
   In the R*f*-range 0.6–0.85 appear the ecdyson derivatives (esters) with ecdyson at R*f* = 0.65 (**T1**). In the R*f*-range 0.10 and 0.45 are detectable the ginsenosides Rb1 (**T2**), Re (**T3**), Rb2 (**T4**) and Rg1 (**T5**). One of the triterpenoid glycosides at R*f* = 0.16, is overlapped by glucose. Saccharose lies direct above the start (R*f* = 0.08). Two zones at R*f* = 0.99 and R*f* = 0.95 may derive from oleanolic acid and sitosterin.

Note: Further TLC-fingerprint analytical methods are reported in the following references:[10, 14]

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts are used as for TLC.
2. Injection volume: Radix Achyranthis bidentatae extracts: 30 µl each
3. HPLC parameters:

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: 0.001 % phosphoric acid/water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)

Gradient:	0–20 % B in 7 min, 20–95 % B in 41 min, 95 % B for 10 min, Total runtime: 58 min
Flow:	0.8 ml/min
Detection:	205 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	7.7	Steroid (Ecdyson derivative)
2	10.0	Steroid (Ecdyson derivative)
3	10.6	Steroid (Ecdyson derivative)
4	10.8	Steroid (Ecdyson derivative)
5	14.9	Steroid (Ecdyson derivative)
6	15.2	Ecdyson
7	20.4	Steroid (Ecdyson derivative)
8	21.6	Phenol carboxylic acid
9	45.7	Oleanolic acid
10	52.8	β-sitosterol

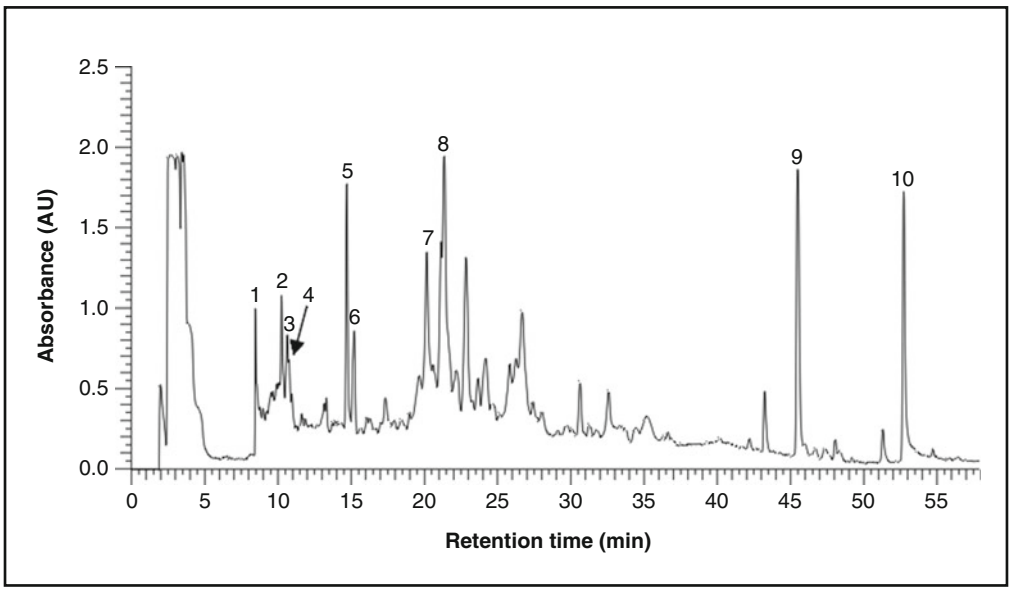


Fig. 3a: HPLC-fingerprint analysis of Radix Achyranthis bidentatae extract, sample 2

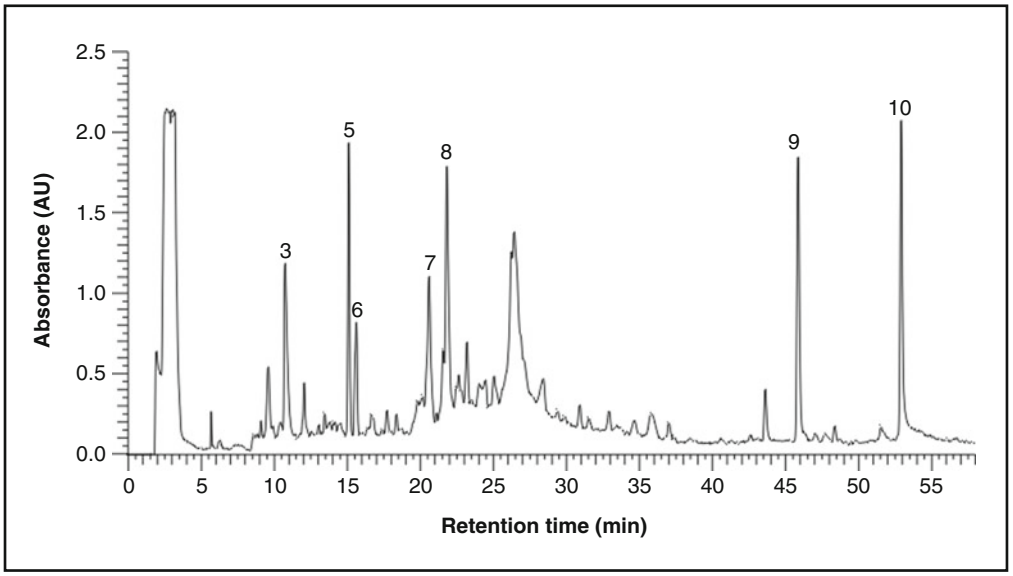


Fig. 3b: HPLC-fingerprint analysis of Radix Achyranthis bidentatae extract, sample 3

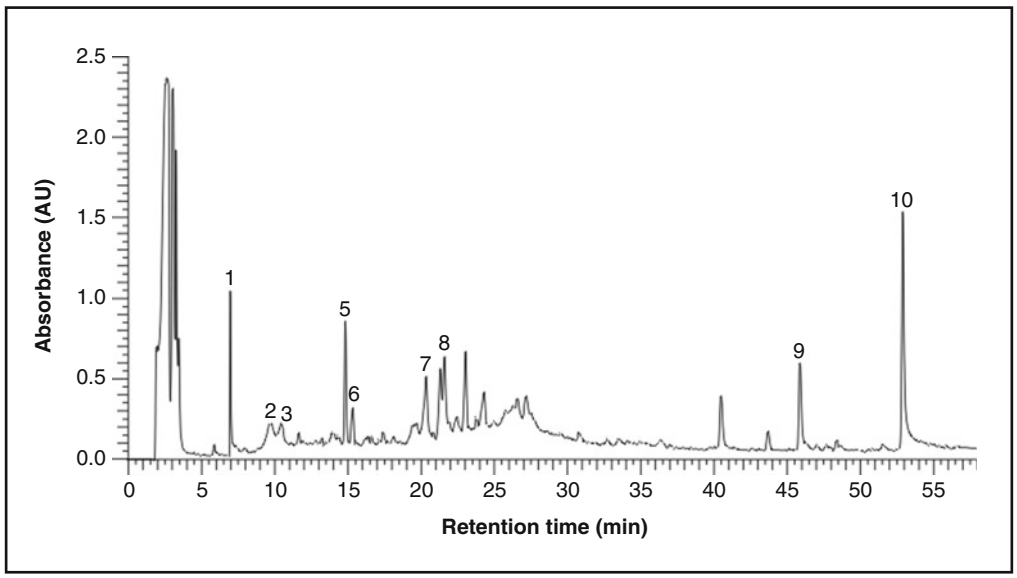


Fig. 3c: HPLC-fingerprint analysis of Radix Achyranthis bidentatae extract, sample 5

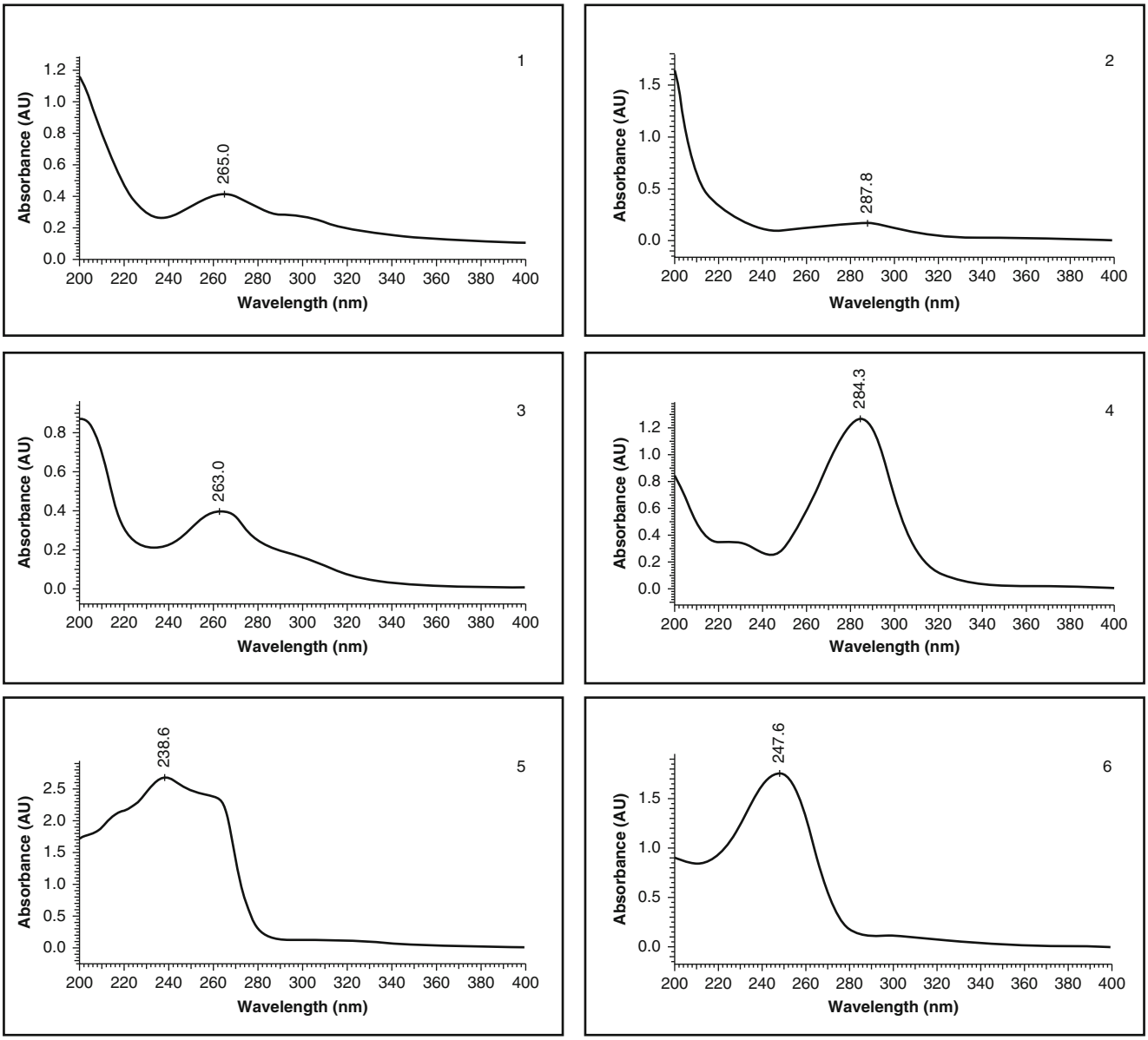


Fig. 4: On line UV-spectra of the detected peaks of Radix Achyranthis bidentatae

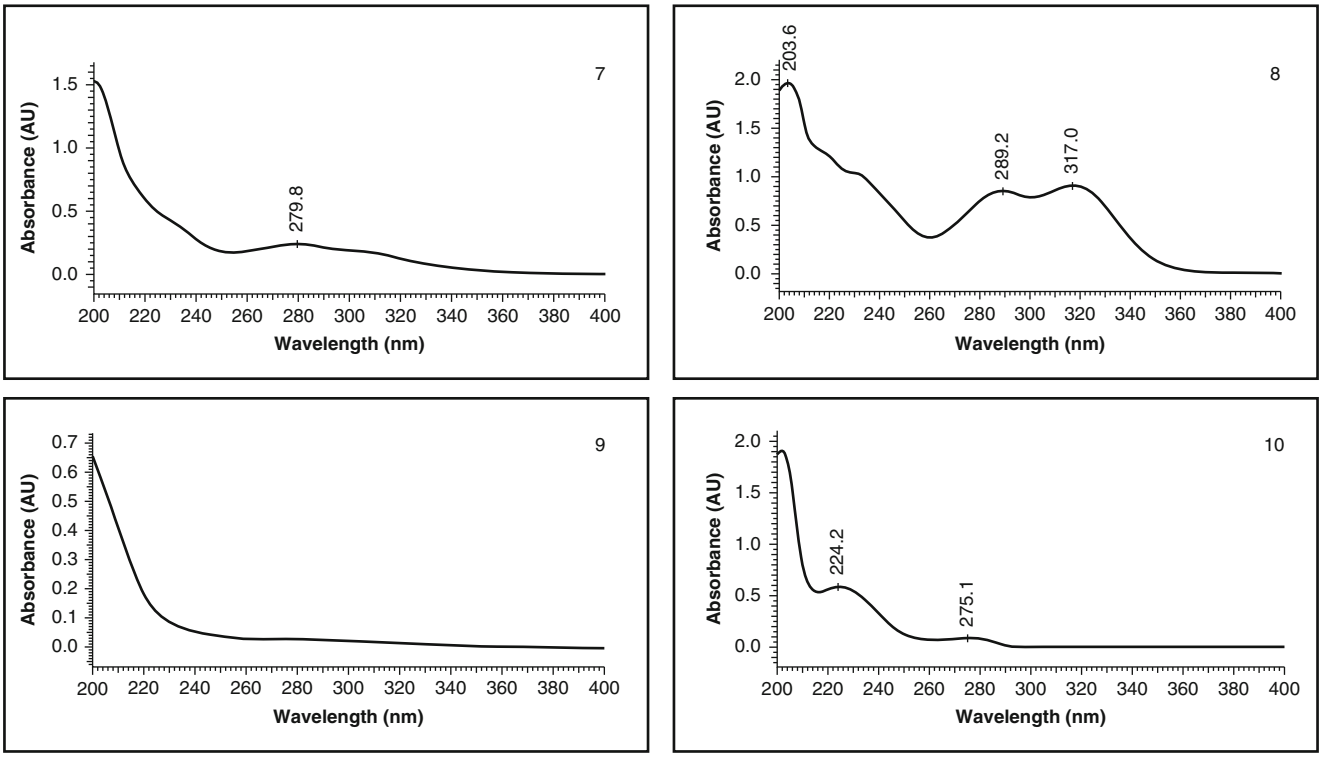


Fig. 4: (continued)

4. Description of the HPLC-Figures:

   The HPLC-graphs of the various sample extracts show two characteristic peak accumulations in the Rt-range of 5.0–16.0 and 19.0–30.0. The peaks of the first peak-accumulation can be assigned to the various ecdyson derivatives (esters) with ecdyson at Rt = 15.1 (peak **6**). In the second peak accumulation according to their UV-spectra appear the various oleanolic acid- and chikusetsuglycosides with the exception of peak **8** which might be assigned to a phenol-carboxylic acid. The peaks **9** (Rt = 45.7) and **10** (Rt = 53.0) were identified as oleanolic acid and β-sitosterol.

Further HPLC-fingerprint analytical methods for identification of the characteristic marker compounds can be found also in the following references:[8–10]

Note: The Chinese Pharmacopoeia 2010 demands for Radix Achyranthis bidentatae, a content not less than 0.03 % of β-ecdysone calculated with reference to the dried drug [1]. The Hong Kong Chinese Materia Medica Standards Vol. 2, demands for Radix Achyranthis bidentatae, a content not less than 1.1 % of oleanolic acid calculated with reference to the dried substance[19].

Conclusion

The Radix Achyranthis bidentatae samples are characterized in the TLC by a very homogenous pattern of triterpene glycosides and steroids (ester) and a high concentration of sugar (glucose) content which is overlapping one or two triterpene glycosides. In the HPLC-fingerprint two peak accumulations in the Rt-range of 5.0–16.0 and 19.0–35.0 characterize the HPLC peak profile.

References

1. Pharmacopoeia of the people's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Paulus, E., Ding, Y.H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)
3. Mitaine-Offer, A.C., Marouf, A., Pizza, C., Khanh, T.C., Chauffert, B., Lacaille-Dubois, M.A.: Bidentatoside I, a new triterpene saponin from *Achyranthes bidentata*. J. Nat. Prod. **64**(2), 243–245 (2001)
4. Hempen, C.H., Fischer, T.: A materia medica for chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone, Elsevier (2007)
5. Vetrichelvan, M., Jegadeesan, M.: Effect of alcoholic extract of *Achyranthes bidentata* Blume on acute and subacute inflammation. Indian J. Pharmacol. **34**(2), 115–118 (2002)
6. Porkert, M.: Klinische Chemische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
7. Stöger, E.A.: Arzneibuch der chinesischen Medizin. Deutscher Apotheker Verlag, Stuttgart (2009)
8. Li, J., Li, H.J., Li, P., Qi, H.: Simultaneous qualitation and quantification of four phytoecdysones in Radix Achyranthis Bidentatae by high-performance liquid chromatography with diode array detection. Biomed. Chromatogr. **21**(8), 823–828 (2007)
9. Zheng, Y., Liu, B., Chen, M., Chen, T.: Supercritical fluid extraction of ecdysterone from the roots of Achyranthes bidentata Bl. J. Sep. Sci. **31**(8), 1393–1398 (2008)
10. Li, J., Qi, H., Qi, L.W., Yi, L., Li, P.: Simultaneous determination of main phytoecdysones and triterpenoids in Radix Achyranthis Bidentatae by high-performance liquid chromatography with diode array-evaporative light scattering detectors and mass spectrometry. Anal. Chim. Acta **596**(2), 264–272 (2007)
11. Mitaine-Offer, A.C., Marouf, A., Hanquet, B., Birlirakis, N., Lacaille-Dubois, M.A.: Two triterpene saponins from *Achyranthes bidentata*. Chem. Pharm. Bull. **49**(11), 1492–1494 (2001)
12. Li, J.X., Hareyama, T., Tezuka, Y., Zhang, Y., Miyahara, T., Kadota, S.: Five new oleanolic acid glycosides from *Achyranthes bidentata* with inhibitory activity on osteoclast formation. Planta Med. **71**(7), 673–679 (2005)
13. Marouf, A., Desbene, S., Khanh, T.C., Wagner, H., Correia, M., Chauffert, B., Lacaille-Dubois, M.A.: Triterpene saponins from the roots of *Achyranthes bidentata*. Pharmac. Bio. **39**(4), 263–267 (2001)
14. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin: chemistry, pharmacology and medicinal use in taditional and modern medicine. Springer, Berlin/Heidelberg (1992)
15. Zhang, M., Zhou, Z.Y., Wang, J., Cao, Y., Chen, X.X., Zhang, W.M., Lin, L.D., Tan, J.W.: Phytoecdysteroids from the roots of *Achyranthes bidentata* blume. Molecules **17**(3), 3324–3332 (2012)
16. Hänsel, R., Keller, K., Rimpler, H., Schneider, G.: Hagers Handbuch der pharmazeutischen Praxis – 4 Drogen A-D. Springer, Berlin (1999)
17. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 1. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)
18. Wang, Q.H., Yang, L., Jiang, H., Wang, Z.B., Yang, B.Y., Kuang, H.X.: Three new phytoecdysteroids containing a furan ring from the roots of *Achyranthes bidentata* Bl. Molecules **16**(7), 5989–5997 (2011)
19. Hong Kong chinese materia medica standards, vol 2. Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region – the People's Republic of China, Hong Kong (2008)

Caulis Bambusae in Taenia – *Zhuru*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drugs:[1] Bamboo Shavings are the dried middle shavings of stem of *Phyllostachus nigra* (Lodd.) Munro var. *henonis* (Mitf.) Stapf ex Rendle, *Bambusa tuldoides* Munro or *Sinocalamus beecheyanus* (Munro) McClure var. *pubescens* P. F. Li (Fam. Poaceae).

Synonym:[8] *Bambusa breviflora* Munro (Syn. of *Bambusa tuldoides* Munro)

Origin:[2, 6] Coast provinces of China, e. g. Huzhou, Zhejiang Province

Description of the drug:[1] Occuring in masses formed by numerous rolled irregular slivers, or in long slat-shaped shavings, varying in width and thickness, greenish or yellowish-green. Texture light, loose, flexible and elastic. Odour, slight; taste, weak.

Pretreatment of the drug:[1] The drug is collected all the year round. After peeling, the greenish middle layer of fresh stem is cut into sliver or shaving, bundled, and dried in the shade. The former is called "Sanzhuru" (Scattered Bamboo Shavings) and the latter "Qizhuru" (Uniform Bamboo Shavings).

Foreign matters are eliminated, cut into sections or crumpled up into small masses.

Processing:[1] *Caulis bambusae in Taenia (processed with ginger)*
The clean drug is stir-baked as described under the method for stir-baking with ginger juice (Appendix II D) until it becomes yellow.

Medicinal use:[12] Diabetes mellitus

Effects and indications of Caulis Bambusae in Taenia according to Traditional Chinese Medicine[1–3, 7–9, 14]

Taste:	Sweet
Temperature:	Neutral, with cold tendency
Channels entered:	*Orbis pulmonalis, Orbis stomachii, Orbis vesica fellis*
Effects (functions):	Removes *heat*, resolves *phlegm*, relieves restlessness and arrests vomiting.
Symptoms and indications:	Cough due to heat and phlegm; restlessness, nausea, vomiting, morning sickness, palpitation and insomnia caused by excessive fire in the gallbladder, stroke with impairment of consciousness; stiff tongue and vomiting due to heat in the stomach; hyperemesis gravidarum, threatened abortion. Descends stomach and lung *qi*, dries heaves and cough. It can also open depression and eliminate vexation and is particularly suitable for oppression and vexation due to depression binding of phlegm and heat. Treatment of skin diseases such as scabies, eczema and atopic dermatitis, hypertension and cardiovascular disease. It is also used against constraint, bloody sputum, nosebleeds, hematemesis, diarrhea, chest diaphragm inflammation, stomach-ache and excessive thirst.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_12, © Springer International Publishing Switzerland 2015

Identified Constituents[4, 6–9, 11]

(Tri)terpenoids	Olean-12-ene, friedelan-3-one (friedelin), friedelan-3-ol, α-amyrin, lup-20(29)-en-3-on, lup-20(29)-en-3-ol, squalene, oleanene, triterpenoid saponins
Flavonoid (glycosides)	Vitexin, rutin
Other compounds	2,5-dimethoxy-*p*-benzoquinone, *p*-hydroxy-benzaldehyde, syringaldehyde, tannins, waxes, lignin, resins

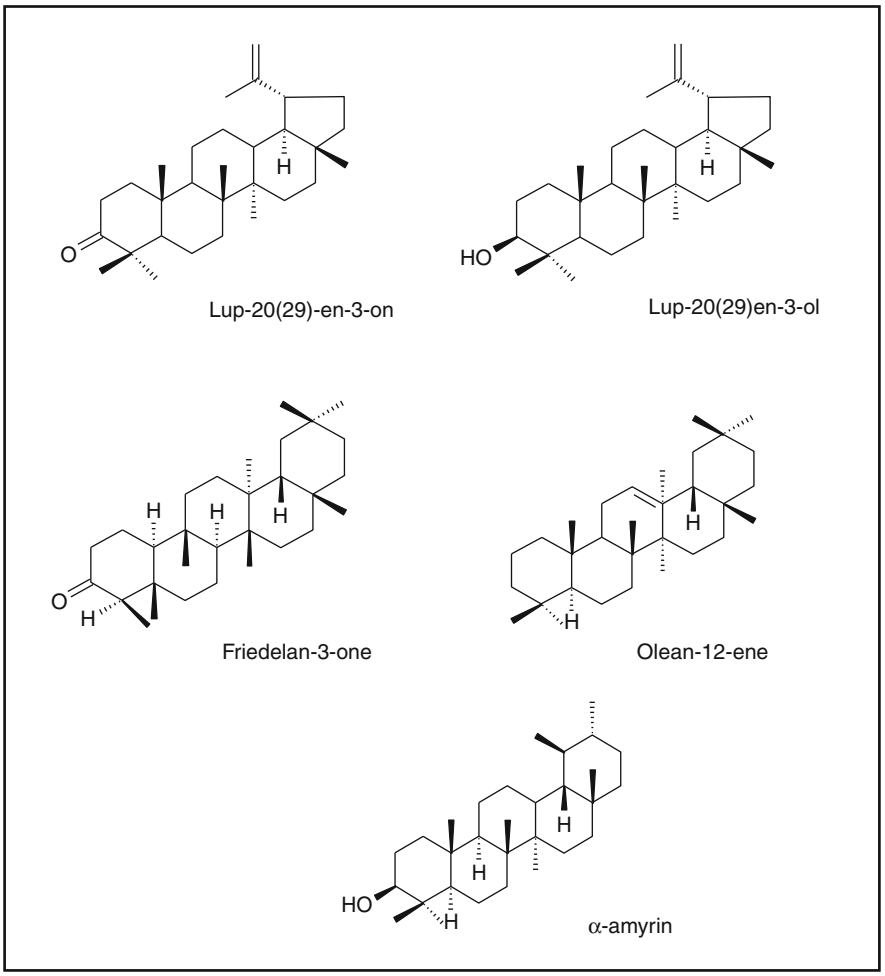


Fig. 1: Formulae of the main compounds of Caulis bambusae in Taenia[7]

Reported Pharmacological Effects

In vitro, *in vivo*, clinical research

- hypolipidemic[4, 6]
- anti-allergic[5]
- antioxidative[5, 11]
- antidiabetic[12]
- antihypertensive[6, 11]
- anti-inflammatory[5, 13, 15]
- antibiotic[3, 12]
- modulates neuroprotective and anti-neuroinflammatory effects in hippocampal and microglial cells[14]
- antifatigue effect[7]
- vasoconstrictor effects on phenylephrine-induced vasoconstriction in the thoracic aortas[6]
- inhibits Staphylococcus albus, Escherichia coli and Salmonella typhi[8]
- raises blood sugar level[8]
- increases discharge of chloride in the urine[8]

TLC-Fingerprint Analysis[10]

	Drug samples	Origin
1	Caulis Bambusae in Taenia / (source plant not listed)	Sample of commercial drug obtained from China Medica
2	Caulis Bambusae in Taenia / *Phyllostachus nigra* (Lodd.) Munro var. *henonis* (Mitf.) Stapf ex Rendle	Sample of commercial drug obtained from HerbaSinica (Origin: Sichuan, China)
3	Caulis Bambusae in Taenia / *Phyllostachys nigra* var. *henonis*	Province Shandong, China
4	Caulis Bambusae in Taenia / *Phyllostachys nigra* var. *henonis*	Province Jiangsu, China

1. **TLC fingerprint analysis of triterpenoids:**
   1. Extraction: 1.5 g powdered drug are extracted with 10 ml chloroform under reflux for 2 h. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 0.5 ml methanol.
   2. Reference compounds: 1 mg is dissolved in 1 ml methanol

      Friedelin = 1 mg is dissolved in 1 ml chloroform

3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Caulis Bambusae extracts: 15 µl each Reference compounds: 10 µl each
Solvent system:	*n*-hexane + ethyl acetate + glacial acetic acid (7+3+0.1)
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 10 ml reagent, heated at 110 °C for 5 min and evaluated under VIS and UV 366 nm.

Note: The reagent has only limited stability and is no longer useable when colour has turned to red-violet.

Reference compounds of Fig. 2a, b		**R*f***
T 1	Friedelin	0.77
T 2	β-sitosterol	0.41

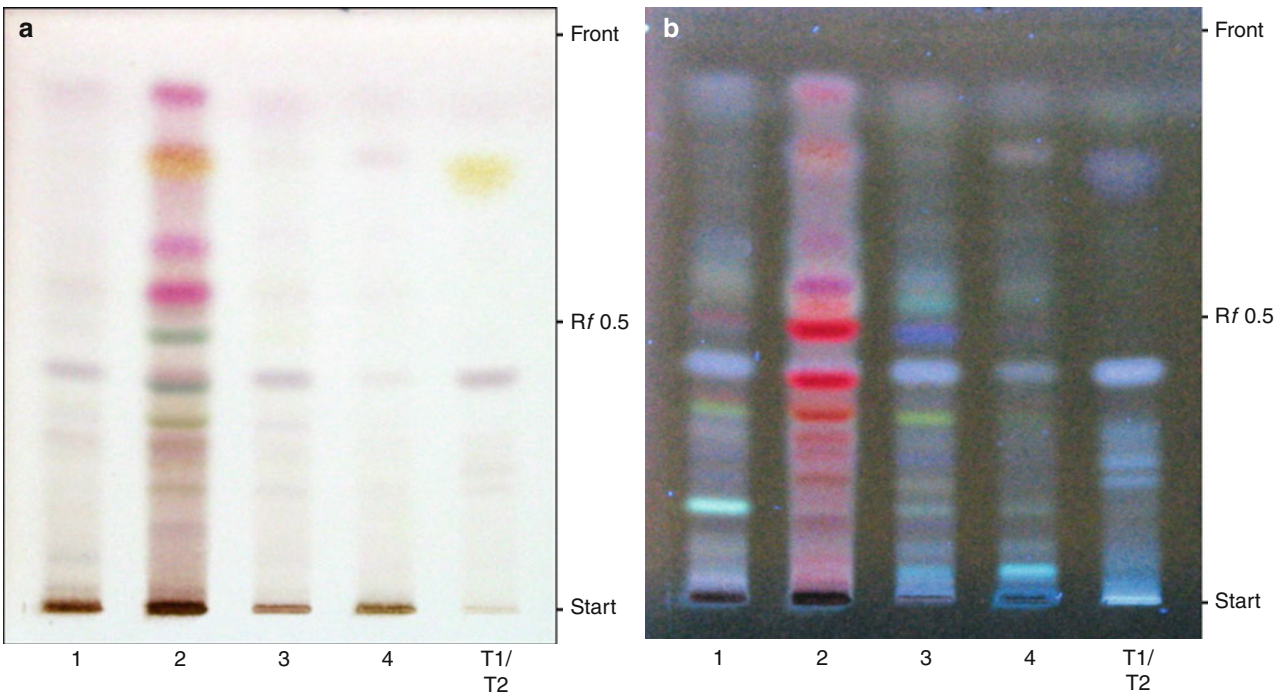


Fig. 2a/b: Thin layer chromatogram of the chloroform extracts of Caulis Bambusae in Taenia sprayed with Anisaldehyde – Sulphuric acid reagent in VIS (**a**) and under UV 366 nm (**b**)

Description of Fig. 2a, b:
The TLC-fingerprint of the Caulis Bambusae in Taenia chloroform extract samples 1, 3 and 4 show in VIS (Fig. 2a) weak grey zones from start up to R*f* = 0.9 with a dominant sitosterol (**T2**) zone at R*f* = 0.41. Sample 2 differs from the others by strong grey-brown zones from start up to R*f* = 0.5 and by four further carmine-red zones from R*f* = 0.5 up to R*f* = 0.9. Whereas the grey zones derive from triterpenoids, the pink-red zones can be assigned to chlorophyll compounds. These originate from Caulis Bambusae in Taenia which have still chlorophyll containing surfaces or from crushed leaves added to the stem parts. Friedelin (**T1**, R*f* = 0.77) shows a weak yellow zone which is hardly visible in samples 1, 3 and 4.

Under UV 366 nm (Fig. 2b) the samples 1, 3 and 4 provide over the whole plate range 9–10 bluish fluorescent zones with sitosterol (**T1**) as dominant zone at R*f* = 041. Sample 2 differs from the others by only carmine-red or brown zones. Here sitosterol is overlapped by a strong carmine-red chlorophyll compound.

2. **TLC fingerprint analysis of flavonoids:**

1. Extraction: 1.5 g powdered drug are extracted with 10 ml methanol under reflux for 2 h. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 0.5 ml methanol.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Caulis Bambusae extracts: 15 µl each
Reference compounds: 10 µl each

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 (= Diphenylboryloxyethylamin, 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 under UV 366 nm.

Reference compounds of Fig. 3		**R*f***
T 3	Isochlorogenic acids	0.51/0.72/0.9
T 4	Apigenin	0.97
T 5	Rutin	0.42

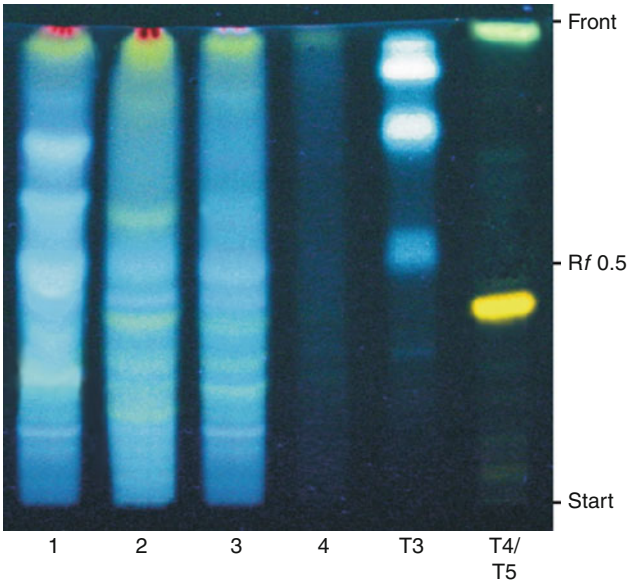


Fig. 3: Thin layer chromatogram of the methanol extracts of Caulis Bambusae in Taenia sprayed with NP/PEG (UV 366 nm)

Description of Fig. 3

All samples, except sample 4, show light bluish and yellow-green zones over the whole R*f*-range. Rutin (**T5**) can be identified in these samples at R*f*=0.42. The mixture of isochlorogenic acids (**T3**) at R*f*=0.51/0.72/0.9 can be seen particularly in sample 1. The green zone at R*f*=0.97 can be assigned to apigenin (**T4**). In sample 4 flavonoids can be not distinctly detected.

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts (methanol and chloroform) which are used for the TLC.
2. Injection volume: Caulis Bambusae in Taenia extracts: 20 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:	5–50 % B in 45 min
Flow:	1.0 ml/min
Detection:	330 nm → methanol extracts
	210 nm → chloroform extracts

Methanol extracts of Caulis Bambusae in Taenia:
Retention times of the main peaks recorded at 330 nm

Figures 4a and 4b			**Figure 4c**		
Peak	**Rt**	**Compound**	**Peak**	**Rt (min)**	**Compound**
1	3.1	Not identified	1	3.1	Not identified
2	4.4	Not identified	a	10.9	Phenolic compound?
3	10.8	Flavonoid?	b	13.7	Flavonoid, Sterol?
4	13.7	Phenolic compound (iso/chlorogenic acid?)	c	14.2	Flavonoid, Sterol?
5	15.6	Flavonoid?	d	19.3	Flavonoid?
6	26.5	Flavonoid, Sterol?	e	22.3	Not identified
			f	22.8	Not identified

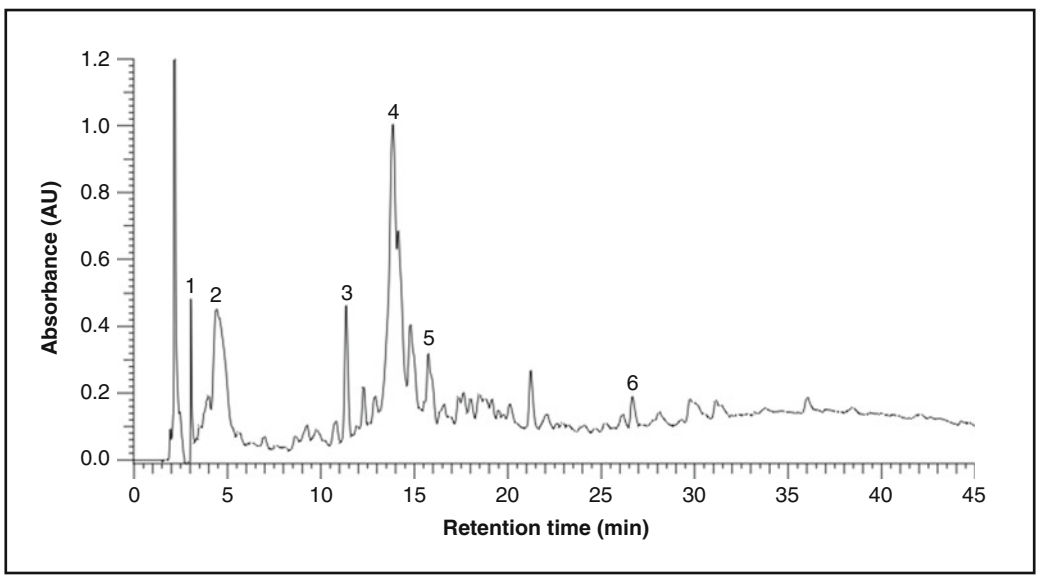


Fig. 4a: HPLC-fingerprint analysis of the methanol extract of Caulis Bambusae in Taenia, sample 1

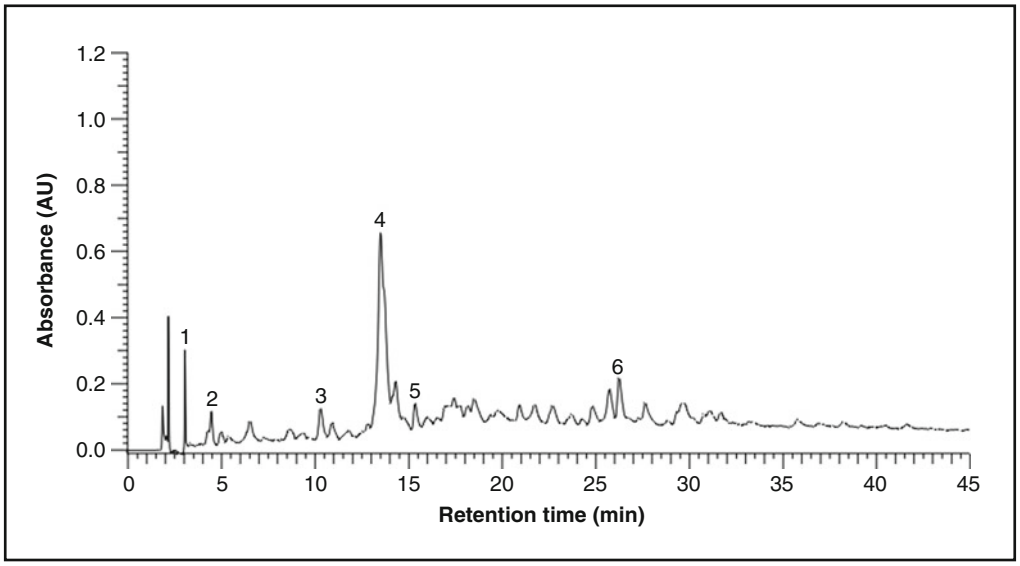


Fig. 4b: HPLC-fingerprint analysis of the methanol extract of Caulis Bambusae in Taenia, sample 3

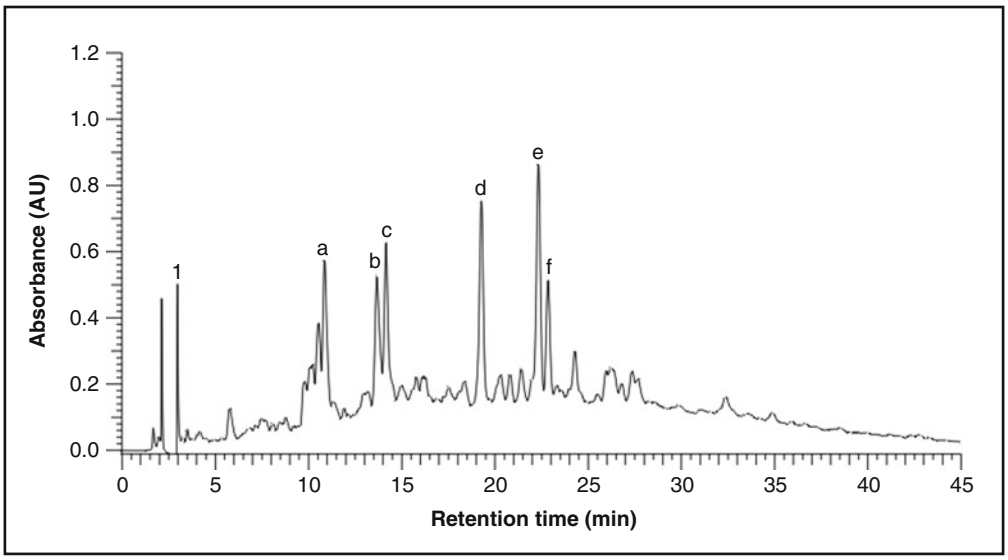


Fig. 4c: HPLC-fingerprint analysis of the methanol extract of Caulis Bambusae in Taenia, sample 2

4.1. Description of the HPLC-Figures (methanol extracts):

Figures 4a and 4b: Both HPLC-fingerprints are characterized by 6 peaks which can be assigned to phenolic carboxylic acids and flavonoids. The dominant peak **4** (Rt = 13.7) could be identified as chlorogenic acid.

Figure 4c: the HPLC-fingerprint of sample 2 differs from these of Figs. 4a and 4b by a peak profile containing 7 distinct peaks, numerated with a – f, which according to the online UV-spectra primarily can be assigned to flavonoids. A coincidence exists with the TLC-profile of Fig. 2a, b which also differs from those of sample 1 and 3 by a great number of chlorophyll spots.

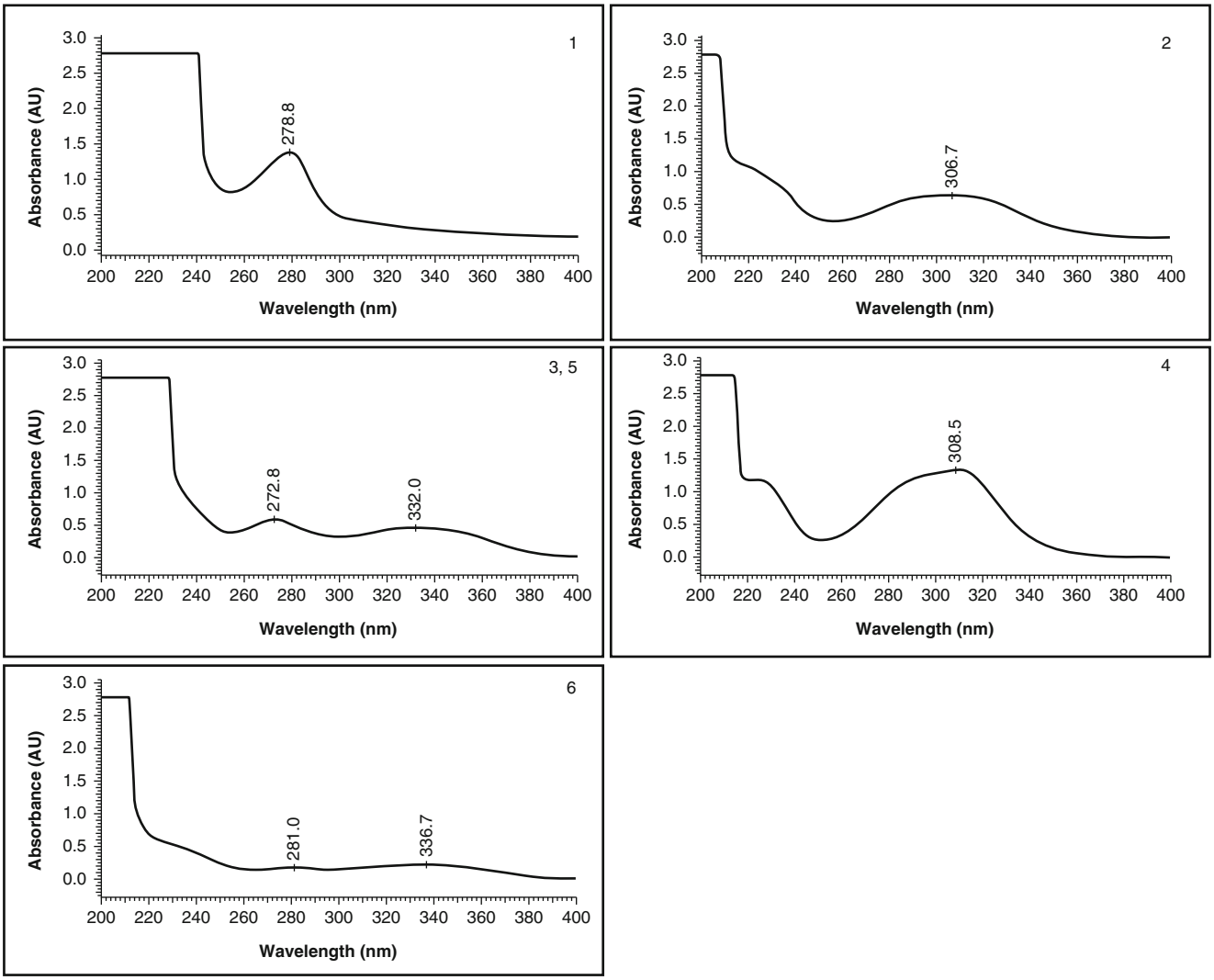


Fig. 5a: On line UV-spectra of the detected peaks of the methanol extracts of Caulis Bambusae sample 1 + 3 (Figs. 4a and 4b)

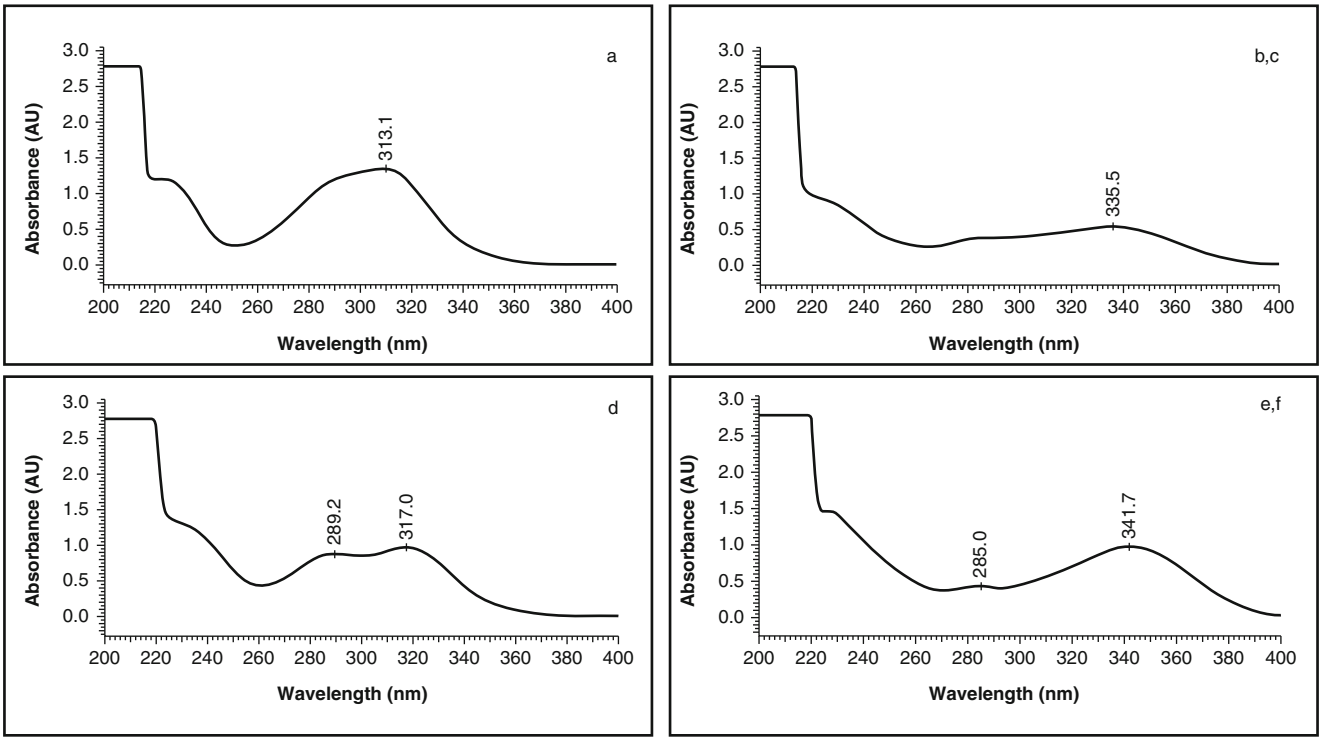


Fig. 5b: On line UV-spectra of the detected peaks of the methanol extracts of Caulis Bambusae sample 2 (Fig. 4c)

Chloroform extracts of Caulis Bambusae in Taenia:

Retention times of the main peaks recorded at 210 nm

Peak	Rt (min)	Compound
1	8.1	Not identified
2	29.1	Friedelin
3	44.7	Not identified

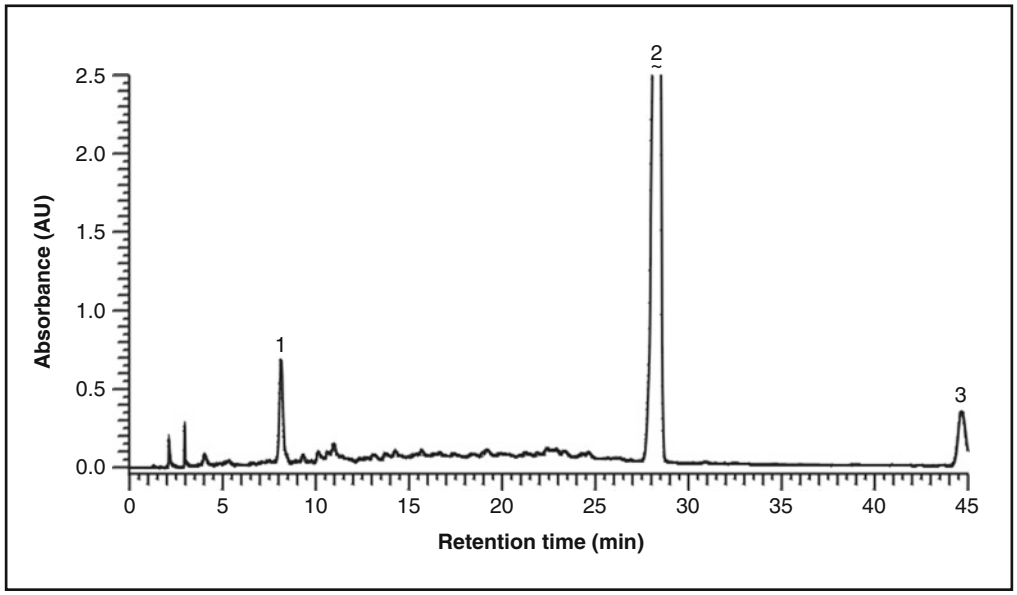


Fig. 6a: HPLC-fingerprint analysis of the chloroform extract of Caulis Bambusae in Taenia, sample 2

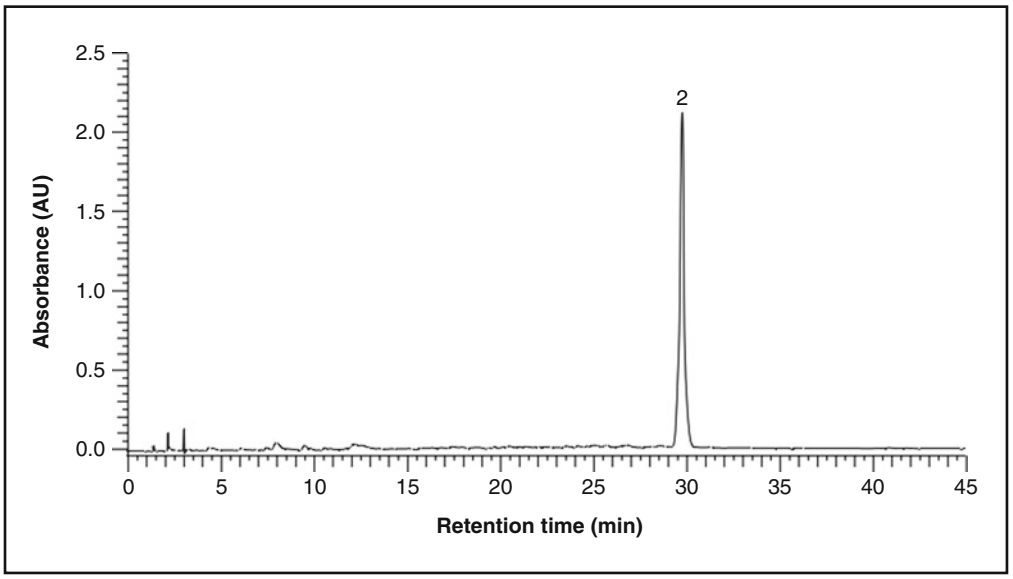


Fig. 6b: HPLC-fingerprint analysis of the chloroform extract of Caulis Bambusae in Taenia, sample 3

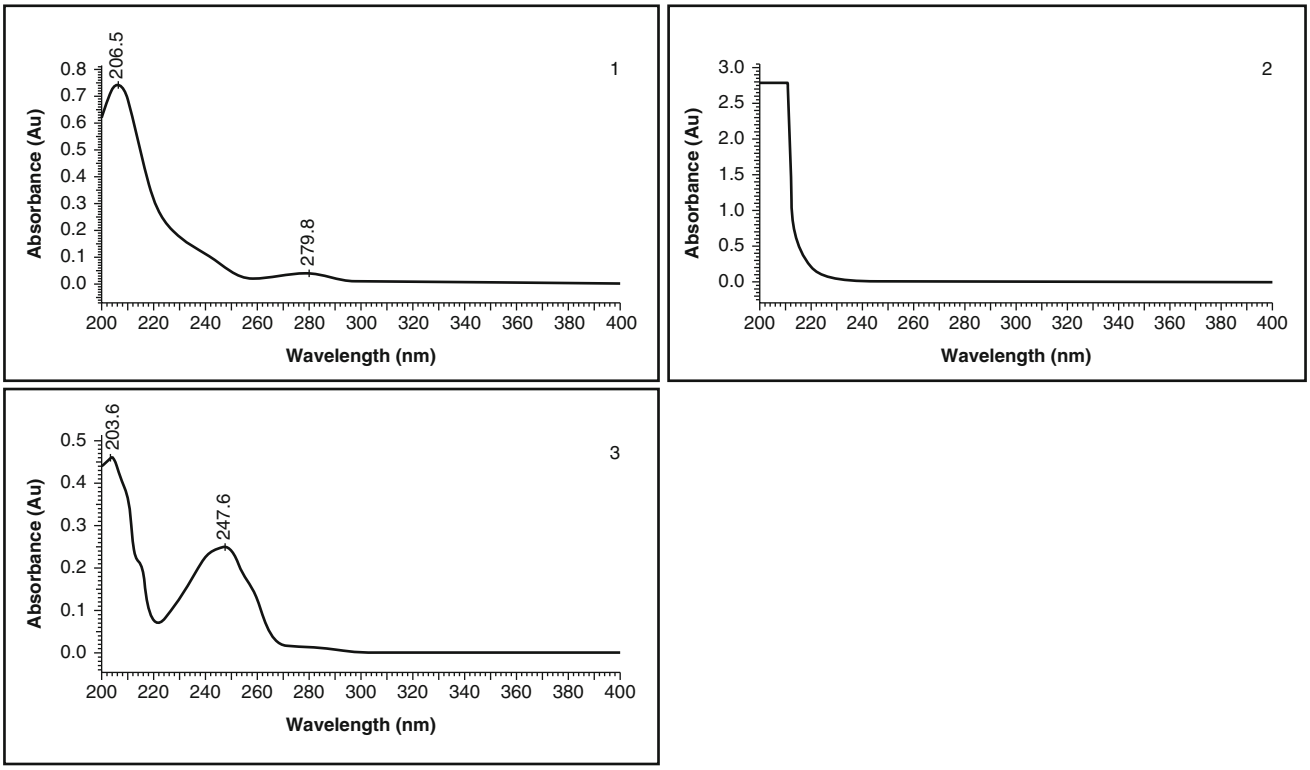


Fig. 7: On line UV-spectra of the detected peaks of the chloroform extracts of Caulis Bambusae in Taenia

4.2. Description of the HPLC-Figures (chloroform extracts):

Here the peak profile of the chloroform extracts of sample 2 and 3 shows the dominating friedelin peak (**2**) which is a characteristic marker compound of Caulis Bambusae in Taenia.

Note: Further HPLC-fingerprint analytical methods for identification of the characteristic marker compounds can be found in the following references:[13]

Conclusion

The described TLC- and HPLC-methods give sufficient indications to authenticate the herbal drug and estimate its quality.

References

1. Pharmacopoeia of the people's Republic of China, english edition, vol. I, People's Medical Publishing House, Beijing (2010)
2. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
3. Hempen, C.H., Fischer, T.: A materia medica for chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone/Elsevier, New York (2009)
4. Ham, I., Yang, G., Lee, J., Lee, K.J., Choi, H.Y.: Hypolipidemic effect of MeOH extract of Bambusae Caulis in Taeniam in hyperlipidemia induced by Triton WR-1339 and high cholesterol diet in rats. Immunopharmacol. Immunotoxicol. **31**(3), 439–445 (2009)

5. Qi, X.F., Kim, D.H., Yoon, Y.S., Li, J.H., Jin, D., Deung, Y.K., Lee, K.J.: Effects of *Bambusae caulis* in Liquamen on the development of atopic dermatitis-like skin lesions in hairless mice. J. Ethnopharmacol. **123**(2), 195–200 (2009)
6. Jiao, J., Zhang, Y., Lou, D., Wu, X., Zhang, Y.: Antihyperlipidemic and antihypertensive effect of triterpenoid-rich extract from bamboo shavings and vasodilator effect of friedelin on phenylephrine-induced vasoconstriction in thoracic aortas of rats. Phytother. Res. **21**(12), 1135–1141 (2007)
7. Zhang, Y., Yao, X., Bao, B., Zhang, Y.: Anti-fatigue activity of triterpenoid-rich extract from chinese bamboo shavings (*Caulis Bambusae* in taeniam). Phytother. Res. **20**(10), 872–876 (2006)
8. Li, X., Wei, W.: Chinese materia medica: combinations and applications. Donica Publishing, St. Albans (2002)
9. Zhang, Y., Wu, X., Ren, Y., Fu, J., Zhang, Y.: Safety evaluation of a triterpenoid-rich extract from bamboo shavings. Food Chem. Toxicol. **42**(11), 1867–1875 (2004)
10. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin (2001)
11. Zhang, Y., Wu, X., Yu, Z., Zhu, YL., Chen, L., Lou, S.: Composition containing total triterpenoid saponins extracted from bamboo, and the preparation method and use thereof, United States patent application 20060148733. A1 (2006)
12. Greten, H.J.: Checkliste – Chinesische Phytotherapie. Hippokrates, Stuttgart (2009)
13. Ra, J., Lee, S., Kim, H.J., Jang, Y.P., Ahn, H., Kim, J.: Bambusae Caulis in Taeniam extract reduces ovalbumin-induced airway inflammation and T helper 2 responses in mice. J. Ethnopharmacol. **128**(1), 241–247 (2010)
14. Eom, H.W., Park, S.Y., Kim, Y.H., Seong, S.J., Jin, M.L., Rye, E.Y., Kim, M.J., Lee, S.J.: Bambusae Caulis in Taeniam modulates neuroprotective and anti-neuroinflammatory effects in hippocampal and microglial cells via HO-1- and Nrf-2-mediated pathways. Int. J. Mol. Med. **30**(6), 1512–1520 (2012)
15. Jin, G.H., Park, S.Y., Kim, E., Ryu, E.Y., Kim, Y.H., Park, G., Lee, S.J.: Anti-inflammatory activity of Bambusae Caulis in Taeniam through heme oxygenase-1 expression via Nrf-2 and p38 MAPK signaling in macrophages. Environ. Toxicol. Pharmacol. **34**(2), 315–323 (2012)

Herba Lysimachiae christiniae – *Jinqiancao*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China,
English Edition Vol. I, 2010

Official drug:[1]

Christina Loosestrife is the dried herb of *Lysimachia christina* Hance (Fam. Primulaceae).

The drug is collected in summer and autumn, removed from foreign matters and dried in the sun.

The drug 'Herba' consists of leave and stem parts in different amounts. Since most stems of plants contain a lower concentration of constituents than leaves, it has to be suggested that the TLC- and HPLC- fingerprints of the herbal extracts may show different profiles of constituents according to the different ratios of stems and leaves in the test drug.

Other source plants:[3, 4]

Desmodium styracifolium (Osbeck) Merr. (see Monograph of Herba Desmodii styracifolii)

Adulterations were reported with the plants of other species as e.g. *Lysimachia congestiflora* Hemsl. or *Lysimachia hemsleyana* Maxim. [19]

Note:
In the official Chinese Pharmacopoeia (2010) Herba Lysimachiae and Herba Desmodii are listed as two different monographs, although according to the literature both plants are used for the same medicinal indication.

Origin:[14]

South, Southwest and Central China.

Description of the drug:[1]

Frequently twisted into masses, glabrous or sparsely pubescent. Stems twisted, externally brown or dark brownish-red, striated longitudinally, stem nodes of the lower part sometimes with rootlets, fracture solid. Leaves opposite, mostly crumpled, when whole, broadly ovate or cordate, 1–4 cm long, 1–5 cm wide, base slightly concave, margin entire; the upper surface grayish-green or dark brown, the lower surface pale in colour, midrib distinctly prominent, after soaking in water, the black or brown stripes visible under the light; petioles 1–4 cm long. Some with flowers, yellow, solitary and axillary, longpetioled. Capsules globose. Odour, slight; taste, weak.

Pretreatment of the raw drug:[1]

Foreign matters are eliminated, the drugs are washed briefly, cut into sections and dried in the sun.

Medicinal use:[4, 5, 13]

For the treatment of strangury, pain and stones in the urinary tract, abdominal colics, renale and bilary stones and mastitis.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_13, © Springer International Publishing Switzerland 2015

Effects and indications of Herba Lysimachiae christinae according to Traditional Chinese Medicine [1, 3, 4, 21]

Taste:	Neutral, with a tendency to saltiness, bitter and slightly sweet
Temperature:	Neutral, cold
Channels entered:	*Orbis hepaticus* et *felleus, Orbis renalis* et *vesicalis*
Effects (functions):	To drain dampness to abate jaundice, disinhibit urine and relieve stranguria, remove toxin and disperse swelling.
Symptoms and indications:	Dampness-heat jaundice, gallbladder distention and hypochondric pain, stone strangury, heat strangury, slow and painful urination, swelling abscess, deep-rooted boil and sore, bite wound of insect, worm or snake or other infected wounds, lack of appetite, tiredness, heaviness of the body, cools blood.

Main constituents of *Lysimachia christina* Hance:[5, 6, 8, 11, 12, 19, 20]

Flavonoids and flavonoid glycosides:	Kaempferol, quercetin, vitexin, isovitexin, quercetin-3-*O*–β-D-glucopyranoside (isoquercitrin), kaempferol-3-*O*– galactoside, kaempferol-3-*O*-β-D-glucopyranoside (astragalin), kaempferol-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-gluco-pyranoside, eriodictyol kaempferol-3-*O*- lysimachiatrioside, 3,2′,4′,6′-Tetrahydroxy,4,3′di-methoxychalcone
Sterols:	β-sitosterol, daucosterol (=β-sitosterol-glucoside, eleutheroside A)
Others:	Phenols, phenolic acids and triterpene saponins, alkaloids, tannins, pectic-like substances, lipids

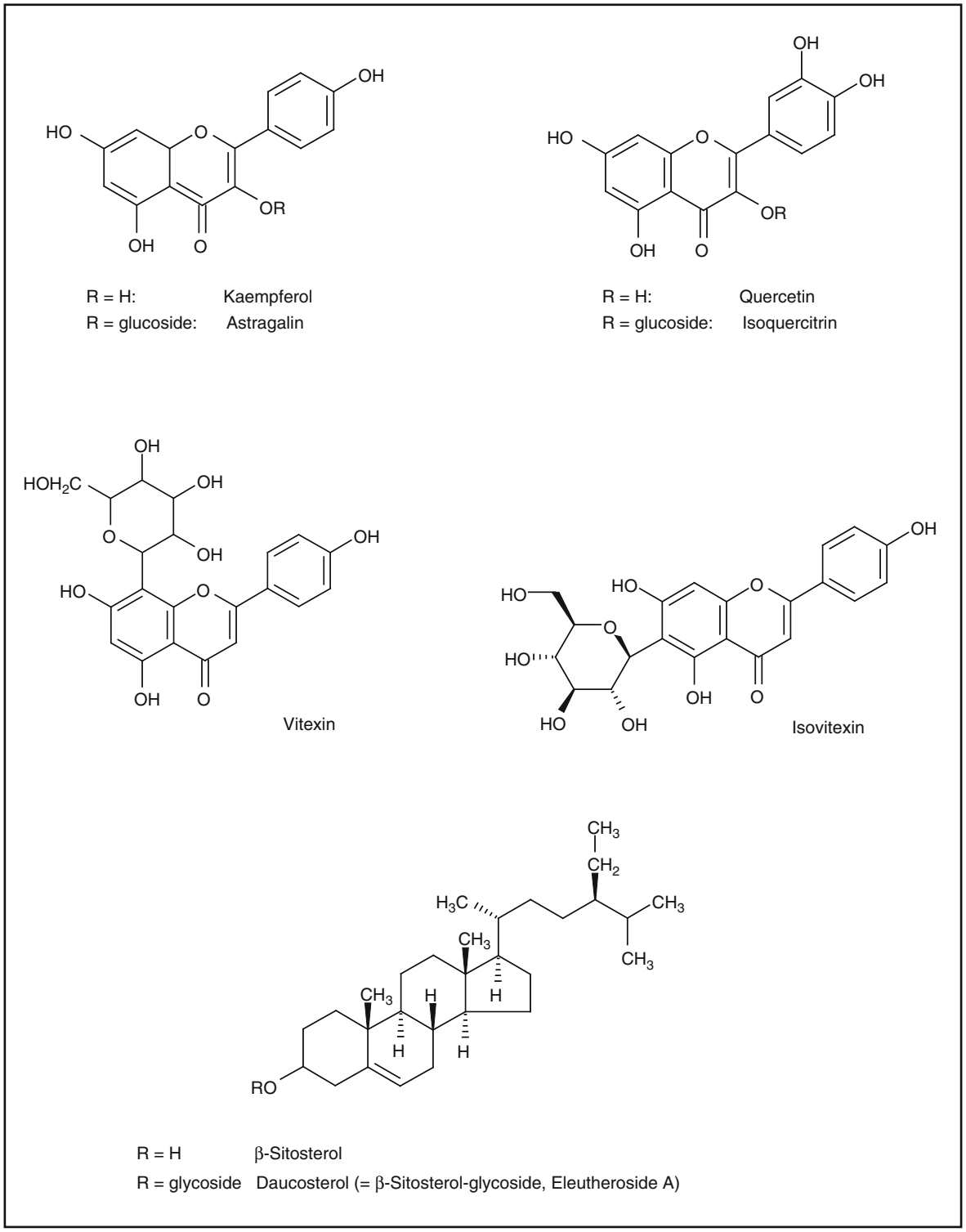


Fig. 1: Formulae of the main compounds of Herba Lysimachiae [6–18]

Reported Pharmacological Activities

In vitro, *in vivo*, clinical research
***Lysimachia christina* Hance:**

Effects on immune functions:

- immune modulatory [6]
- anti-inflammatory [12]
- antioxidative [6, 9, 10]

Enzymatic effects:

- decrease of lipid peroxidation levels (LPO) [5]
- increase of superoxide dismutase (SOD), catalase (CAT), glutathione-s transferase (GST), glutathione peroxidase (GPx) [5]

Protective effects:

- enhancement of the phagocytic activities of macrophages and neutrophile granulocytes [7]
- inhibition of lipid peroxidation damage of erythrocyte membranes [10]
- diuretic [4]
- antibiotic [4]
- stimulation of bile juice secretion [4, 13]
- anticholecystitic [4, 13]
- protective against alcohol-induced liver injury [5]

TLC-Fingerprint Analysis: [15, 17]

Drug samples		Origin
1	Herba Lysimachiae/unknown species	Sample of commercial drug, Sinomed, (TCM-Clinic Bad Kötzting)
2	Herba Lysimachiae/*Lysimachia christina* Hance	Sample of commercial drug (China Medica, origin: province Sichuan, Bazhong, China)
3	Herba Lysimachiae/unknown species	Sample of commercial drug, Sinomed, (TCM-Clinic Bad Kötzting)
4	Herba Lysimachiae/*Lysimachia christina* Hance	Chinese Province Anhui, Jixi, Yangxi, Chao or Kengkou
5	Herba Lysimachiae/*Lysimachia christina* Hance	Chinese Province Anhui, Jixi, Fuling or Libian Kang
6	Herba Lysimachiae/*Lysimachia christina* Hance	Chinese Province Anhui, Jixi, Yangxi, or Dingjiadian

1. Sample Preparation: 2 g of the powdered drug are extracted with 50 ml ethanol (80 %) in an ultrasonic bath for 30 min. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 2 ml ethanol (p. a.) and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

   Plate: HPTLC Silica gel 60 F_{254}, Merck

1. **TLC fingerprint analysis of the flavonoids kaempferol and quercetin** (Fig. 2)**:**

   Applied amounts: Herba Lysimachiae extract: 15 µl each
   Reference compounds: 10 µl each

   Solvent system: *n*-hexane + ethyl acetate + formic acid (10 + 6 + 1)

   Detection: Aluminium chloride TS reagent:
   0.2 g aluminium chloride are dissolved in 10 ml ethanol.
   The plate is sprayed with the solution and evaluated under UV 366 nm.

Reference compounds of Fig. 2		**R*f***
T1	Kaempferol	0.56
T2	Quercetin	0.46

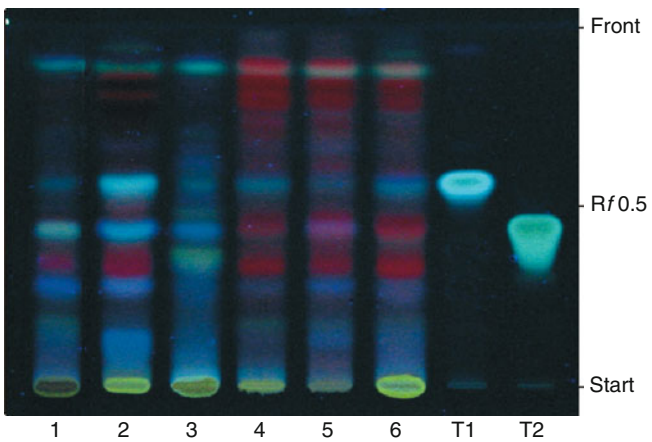


Fig. 2: Thin layer chromatogram of the ethanol extracts of Herba Lysimachiae detected with Aluminium chloride TS reagent (UV 366 nm)

Description of Fig. 2:

All *Lysimachia christina* extract samples (1–6) provide a characteristic fingerprint with the green fluorescent zones of the flavonol aglycones, kaempferol (**T1**) at R*f* = 0.56 and quercetin (**T2**) at R*f* = 0.46. Sample 1 and 3 show a weaker concentration of kaempferol than quercetin, whereas in samples 4–6 quercetin is overlapped by carmine-red zones of chlorophyll. On the start appear various not chromatographically separated flavonol-glycosides with light-green colour. From R*f* = 0.30 upwards to R*f* = 0.90 appear 5–7 carmine red fluorescent chlorophyll zones.

2. **TLC fingerprint analysis of the flavonoids and phenol carboxylic acids** (Figs. 3a and 3b):

Applied amounts:	Herba Lysimachiae extract: 10 µl each Reference compounds: 10 µl each
Solvent system:	Ethyl acetate + formic acid + glacial acetic acid + water (10 + 1.1 + 1.1 + 2.6)
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 under UV 366 nm.

Description of Fig. 3a:

The chromatogram of one leaf and stem extract shows clearly that the leaves of *Lysimachia christina* contain a much higher concentration of flavonoids and phenol carboxylic acids than the stems.

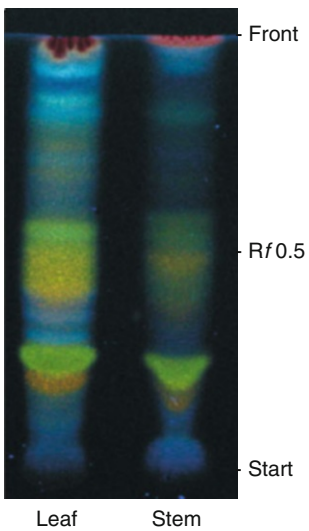


Fig. 3a: Thin layer chromatogram of an ethanol leaf and stem extract of Herba Lysimachiae sprayed with NP/PEG (UV 366 nm)

Reference compounds of Fig. 3b		Rf
T3	Vitexin	0.76
T4	Isovitexin	0.67
T5	Isorhamnetin-3-O-neohesperidin	0.52
T6	Mixture of Isochlorogenic and	0.81–0.98
	Chlorogenic acids	0.57
T7	Rutin	0.48
T8	Eriodictyol	0.99
T9	Isoquercitrin	0.71
n.a.	Schaftoside	0.34

n.a. not applied

Description of Fig. 3b:

In the more polar solvent system the TLC-fingerprints of *Lysimachia christina* extracts 1, 2, 3 and 6 (except the samples 4 and 5) show a similar qualitative profile of green, blue and orange fluorescent zones distributed over the whole TLC-plate (sample 3 shows at once yellow zones). The obviously very low concentration of flavonoids and phenolcarboxylic acids in the samples 4 and 5 may be due to the higher percentage of stems in these samples (see also Fig. 3a).

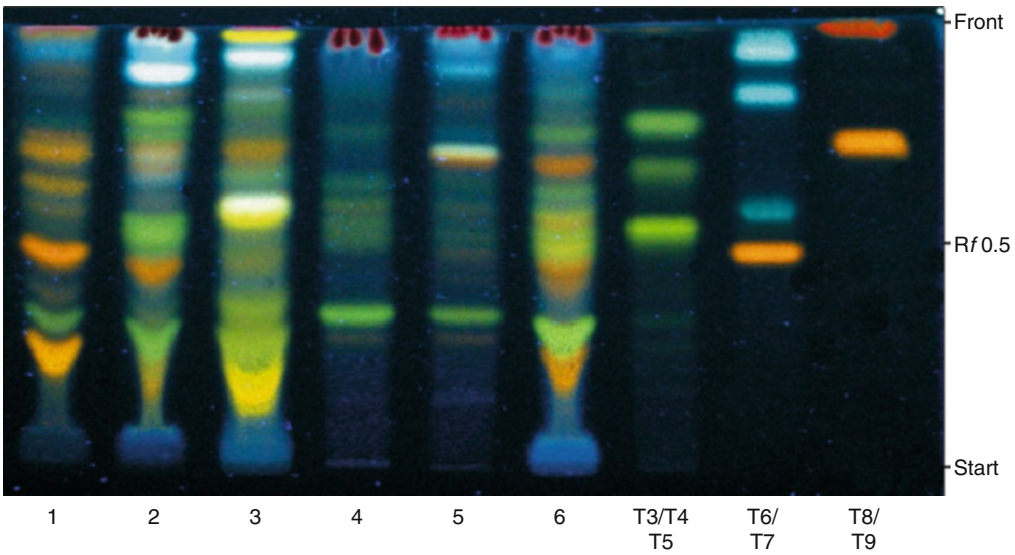


Fig. 3b: Thin layer chromatogram of the ethanol extracts of Herba Lysimachiae sprayed with NP/PEG (UV 366 nm)

3. **TLC fingerprint analysis of triterpenoids** (Fig. 4):

Applied amounts: Herba Lysimachiae extracts: 10 µl each
Reference compounds: 10 µl each

Solvent system: Chloroform + methanol + water (13 + 7 + 2) (lower layer)

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 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.

Reference compounds of Fig. 4		**R*f***
T10	β-Sitosterol	0.97
T11	Oleanolic acid	0.95
n.a.	Daucosterol	0.80

Description of Fig. 4:

All samples show the characteristic zones of β-Sitosterol at R*f* = 0.97, oleanolic acid at R*f* = 0.95 and daucosterol at R*f* = 0.80, and an unidentified pink zone in sample 4 at R*f* = 0.74. In the lower range appear dark and bright green zones which could be assigned to sterol-glycosides.

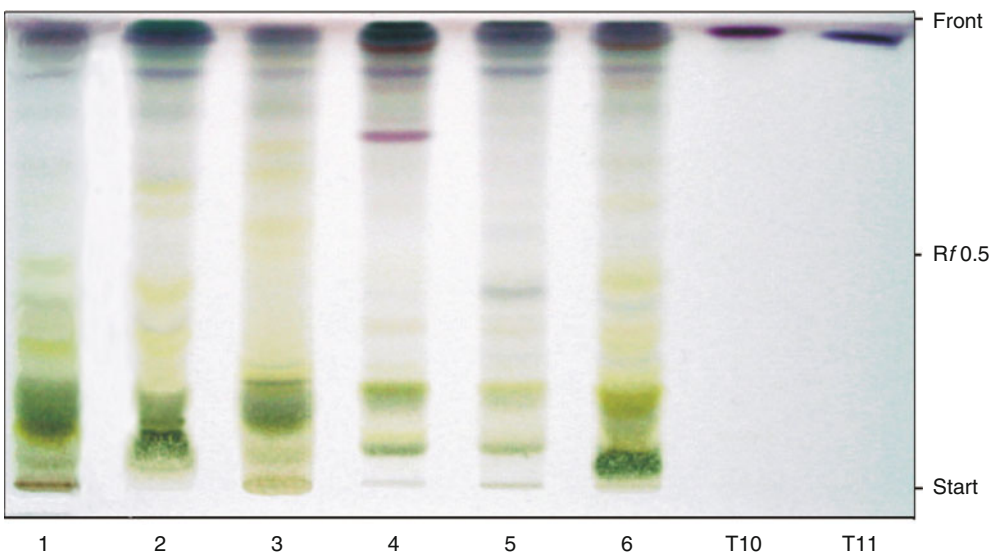


Fig. 4: Thin layer chromatogram of the ethanol extracts of Herba Lysimachiae sprayed with Anisaldehyde – Sulphuric acid reagent (VIS)

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts are used as for the first HPTLC (see above).
2. Injection volume: Herba Lysimachiae extracts: 20 µl each
3. HPLC parameters:

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: 0.001 % Phosphoric acid/Water (Millipore Ultra Clear UV plus® filtered) B: Acetonitrile (VWR)
Gradient:	5–40 % B in 32 min, 40–95 % B in 10 min, 95 % B for 18 min, Total runtime: 60 min
Flow:	1.0 ml/min
Detection:	205 nm

Retention times of the main peaks

Peak		Rt (min)	Compound
1		5.3	Flavonoid or Phenolic compound
2		6.7	Flavonoids or Phenolic carboxylic acids
A	3	14.6	Quercetin and Kaempferol-glycosides
	4	16.0–21.1	

Peak		Rt (min)	Compound
B	5	43.1	Sterol or Triterpenoic acid
	6	44.9	
	7	45.2	
	8	45.9	
	9	47.3	(Peak No. 9 or 10 = Oleanolic acid)
	10	48.8	Sterols or Triterpenoic acids
	11	52.0	

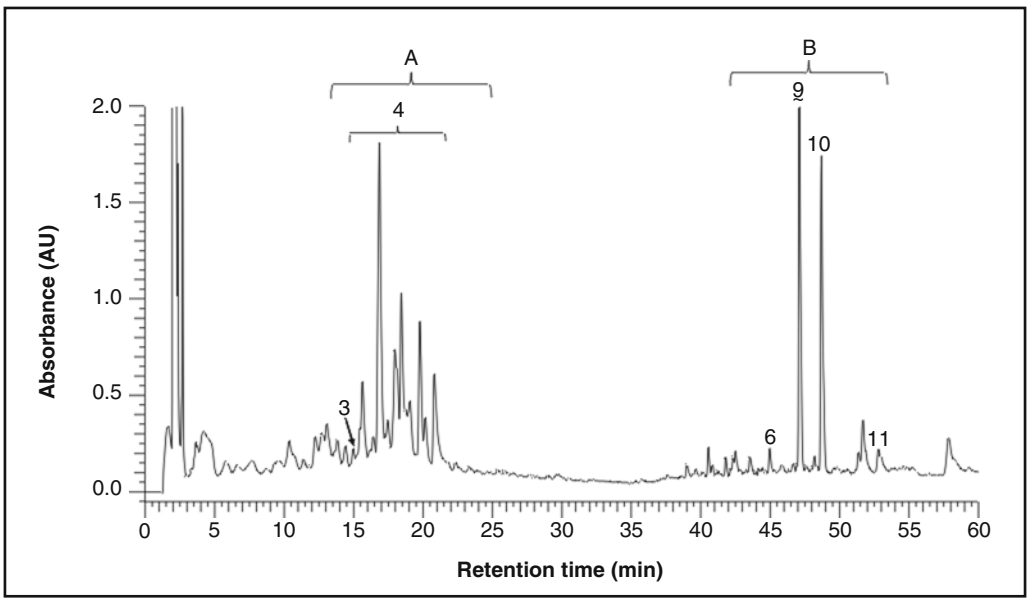


Fig. 5a: HPLC-fingerprint analysis of the ethanol extract of *Lysimachia christina* (sample 3)

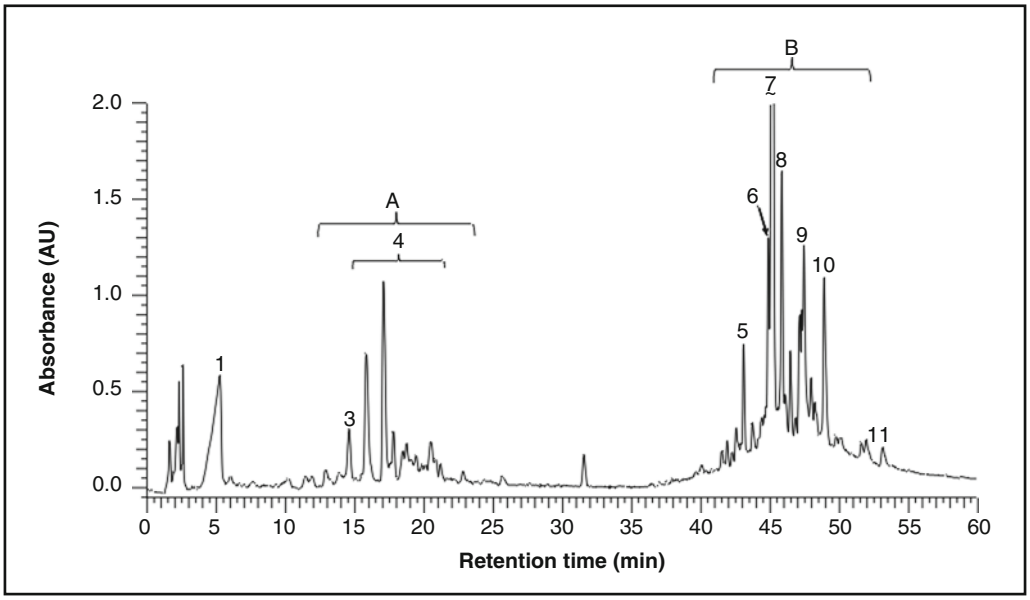


Fig. 5b: HPLC-fingerprint analysis of the ethanol extract of *Lysimachia christina* (sample 5)

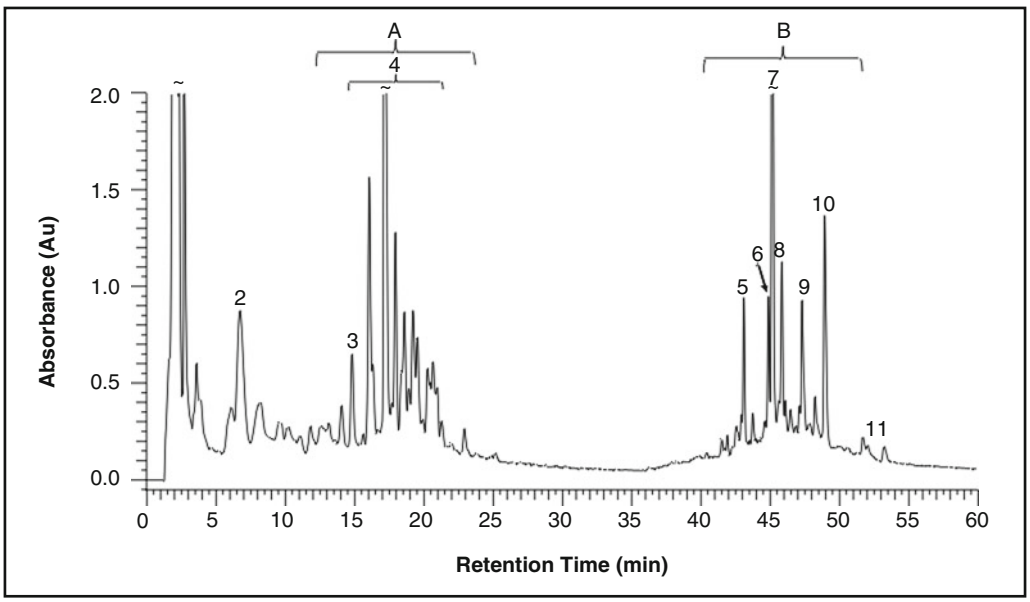


Fig. 5c: HPLC-fingerprint analysis of the ethanol extract of *Lysimachia christina* (sample 6)

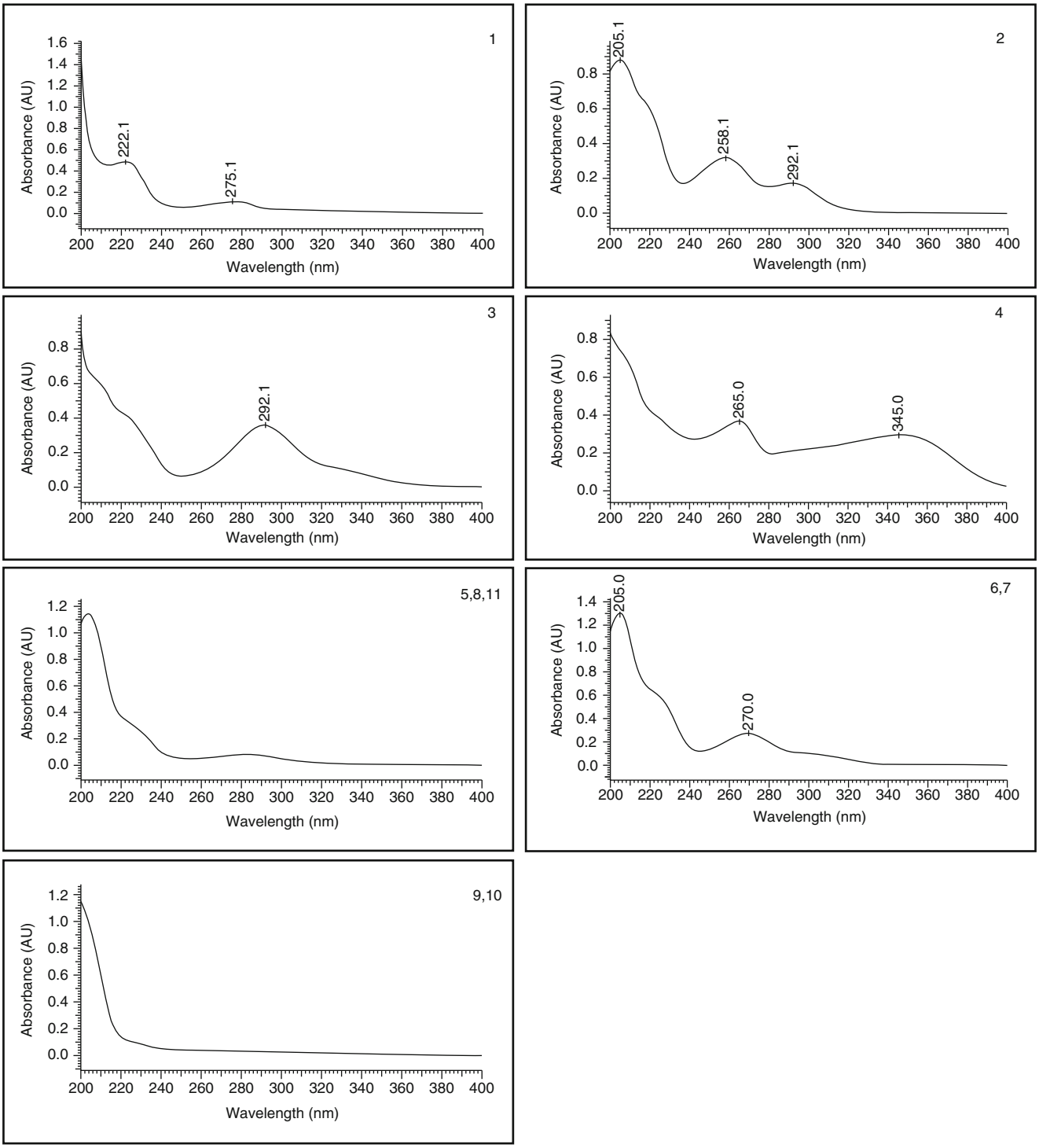


Fig. 6: On line UV-spectra of the detected peaks of Herba Lysimachiae

4. Description of the HPLC-Figures 5a, 5b, and 5c:

   All *Lysimachia christina* samples (sample 3, 5 and 6) are characterized by two characteristic HPLC-peak accumulations in the Rt-range of ~13.0–25.0 (**A**) and Rt-range ~ 40.0–54.0 (**B**). The first peak accumulation represents the main amount of flavonoids and phenol-carboxylic acids whereas the second one shows the accumulation of all sterol- and triterpenoic aglycones, inclusive β-sitosterol and oleanolic acid. A higher leaf content can be supposed for the *Lysimachia christina* sample 3 whereas in sample 5 a lower content of leaves than stems can be suggested. In sample 6 the leave and stem percentages seem to be present in about equal amounts.

Note: The Chinese Pharmacopoeia 2010 demands for Herba Lysimachiae a content not less than 0.1 % of the total amount of quercetin and kaempferol calculated with reference to the dried drug.

Further HPLC-fingerprint analytical methods for identification of the characteristic marker compounds can be found also in the references: [5, 15]

Conclusion

The obvious often different stem and leave content of flavonol glycosides and phenolcarboxylic acids in the various *Lysimachia christina* samples available on the herbal drug market, determine the HPLC-profiles which provide a better authenticity proof than alone with TLC. For the authentication and differentiation between *Lysimachia*- and *Desmodium* species see the Monograph of Folium Desmodii styracifolii (Vol. 3, pp. 159–169).

References

1. Pharmacopoeia of the people's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
3. Geng, J., Huang, W., Ren, T., Ma, X.: Materia Medica der chinesischen Arzneimitteltherapie (Praxis der chinesischen Arzneimitteltherapie; Bd. 2), Verlag für Ganzheitliche Medizin Dr. Erich Wühr GmbH, Bad Kötzting (1993)
4. Hempen, C.H., Fischer, T.: A materia medica for chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone, Elsevier (2007)
5. Wang, J., Zhang, Y., Zhang, Y., Cui, Y., Liu, J., Zhang, B.: Protective effect of *Lysimachia christinae* against acute alcohol-induced liver injury in mice. Biosci. Trends **6**(2), 89–97 (2012)
6. Huang, H., Xu, B., Duan, C.: Antioxidative activity and components of *Lysimachia christinae* Hance extract. China Oils. Fats. 12 (2006)
7. Yao, C., Zhang, Z., Liu, Y., Cheng, J., Liu, Y., Zhao, S.: Influence of chinese herb *Lysimachia hemsleyana* Maxim on immune responses in mice. II. Depletion of lymphoid tissue. Zhongguo Yi Xue Ke Xue Yuan Xue Bao **4**(5), 286–289 (1982)
8. Shen, L.D., Yao, F.R.: Studies on the chemical constituents of the herb *Lysimachia christinae* Hance. Zhong Yao Tong Bao **13**(11), 31–34 (1988)
9. Wang, B., et al.: Antioxidative activity of *Lysimachia christinae* Hance in edible oils and fats. China Oils. Fats. 05 (1991)
10. Lei, J., Liao, Z., Yu, J., Liu, H.: Protective effect of extract of Herba *Lysimachia christinae* against lipid peroxidation damage of erythrocyte membrane. J. Yunnan College Trad. Chin. Med. 01 (2007)
11. Wang, Y., Sun, Q.: Chemical constituents of *Lysimachia christinae* Hance. Chin. J. Medic. Chem. 06 (2005)
12. Gu, L., Zhang, B., Nan, J., Wang, R.: Studies on the anti-inflammatory effects of two species of *Lysimachia christinae* Hance and *Desmodium styracifolium* (Osbeck) Merr. China J Chinese Materia Medica. 07 (1988)
13. Yang, X., Wang, B.C., Zhang, X., Liu, W.Q., Qian, J.Z., Li, W., Deng, J., Singh, G.K., Su, H.: Evaluation of Lysimachia christinae Hance extracts as anticholecystitis and cholagogic agents in animals. J. Ethnopharmacol. **137**(1), 57–63 (2011)
14. Zheng, W., Xu, X., Zhao, K.G., Chen, L.: Lysimachia christinae 'Zixin': a new groundcover plant. Hort. Sci. **44**(2), 474–475 (2009)

15. Hong Kong Chinese materia medica standards, vol 5. Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region – the People's Republic of China (2012)
16. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin: chemistry, pharmacology and medicinal use in taditional and modern medicine. Springer, Berlin/Heidelberg (1992)
17. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin/Heidelberg/New York (2001)
18. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines: thin layer and high performance liquid chromatography of chinese drugs, vol. 1 and 2. Springer, Wien (2011)
19. Zhang, L.J.: Comparative anatomy and histochemistry of sectretory structures in *Lysimachia christinae, Lysimachia congestiflora* and *Lysimachia hemsleyana*, Master Theses, posted by Agricultural Science Paper (2012)
20. Marr, K.L., Bohm, B.A., Cooke, C., Gunning, P.: Flavonoids of hawaiian endemic *Lysimachia* in honour of Professor G: H. Neil Towers 75th birthday. Phytochemistry **49**(2), 553–557 (1998)
21. Suter, R., Lian Chinaherb, A.G.: Newsletter "Extrakt" 2, 12–13 (2006)

Herba Desmodii styracifolii – *Guangjinqiancao*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1]

Snowbellleaf Ticklover Herb is the dried aerial part of herb of *Desmodium styracifolium* (Osbeck) Merr. (Fam. Fabaceae).

The drug is collected in summer and autumn, removed from foreign matter, and dried in the sun.

Other source plant:[2, 13]

Herb of *Desmodium capitatum* DC., *Desmodium retroflexum* (L.) DC, *Hedysarum capitatum* Burm. f., *Meibomia capitata* (Burm. f.) O. Kuntze and *Nicolsonia styracifolia* (Osb.) Desv.

Note:
In the official Chinese Pharmacopoeia (2010) Herba Lysimachiae and Herba Desmodii are listed as two different monographs. Various literatures, however, name both source plants as synonyms and use them both for the same indication.

Origin:[3, 7, 13, 15]

Distributed in tropical and subtropical regions like China, India (Assam, Karnataka, Kerala, Meghalaya and Sikkim), Bangladesh, Burma, Malaysia, Sri Lanka, Thailand and Vietnam.

Description of the drug:[1]

Stems cylindrical, up to 1 m long, densely covered with yellow spreading pubescens; texture slightly fragile, fracture medullated in the centre. Leaves alternate, leaflets 1–3, rounded or oblong, 2–4 cm in diameter; retuse at the apex, cordate or obtusely rounded at the base, margin entire; the upper surface yellowish-green or grayish-green, glabrous, the lower surface densely covered with grayish-white tomenta, lateral veins pinnate; petiole 1–2 cm long; stipules 2, lanceolate, about 8 mm long. Odour, slightly aromatic; taste, slightly sweet.

Pretreatment of the raw drug:[1]

Foreign matters are eliminated, cut into sections and dried in the sun.

Medicinal use:[2]

Especially for the treatment of bladder problems, renal stones and strangury.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_14, © Springer International Publishing Switzerland 2015

Effects and indications of Herba Desmodium styracifolii according to Traditional Chinese Medicine[1, 3, 4, 7, 14, 15]

Taste:	Slightly sweet
Temperature:	Cold
Channels entered:	*Orbis hepaticus, Orbis renalis* et *vesicalis*
Effects (functions):	To drain dampness to abate jaundice, disinhibit urine and relieve stranguria.
Symptoms and indications:	Heat clearing, urinary diseases (like cholelithiasis, jaundice and red urine, heat strangury, stone strangury, slow and painful urination, edema and small quantity of urination, bladder and kidney stones). Rheumatism, pyrexia, dysentery, wounds, cough, malaria, hepatitis, hemoptysis, choloplania, stomatitis, laryngitis, urticaria, hepatitis.

All constituents of *Desmodium styracifolium* listed in the literature:[1, 3, 4, 6, 7, 9, 10, 15, 17]

Flavonoids: Chrysoeriol, kaempferol, orientin, ambonin, astragalin, quercetin, quercetin 3–O-β-D-glucopyranoside (isoquercitrin), vicenin 1, vicenin 2, vicenin 3, hydnocarpin-D, apigenin, 6-C-glycopyranosyl-8-C-arabinosyl apigenin, 6-C-glycopyranosy l-8-C-glycopyranosyl apigenin luteolin, 6-C-glycopyranosyl luteolin, katuranin, 2,3-trans-3,5,7,2′,4′-pentahydroxy-flavanone, homoadonivernite, schaftoside, isoschaftoside, vitexin, isovitexin, isoorientin, isoorientin 3′-O-methyl ether

Isoflavonoids, cumaranochromones: 5,7-dihydroxy-2′,3′,trimethoxy-isoflavanone, 5,7-dihydroxy-2′-methoxy-3′, 4′-methylenedioxy-isoflavanone; 5,7-dihydroxy-2′,3′,4′-trimethoxy-isoflavanone 7-O-β- glucopyranoside; 5,7-dihydroxy-2-methoxy-3′,4′-methylenedioxy-isoflavanone 7-O β-glucopyranoside; 5,7, 4′-trihydroxy-2′,3′-dimethoxy - isoflavanone 7-O-β glucopyranoside; genistin, 2′-hydroxygenistein,7,4′-dihydroxy-3′-methoxy-isoflavone, formononetin, orobol, homoferreirin, isoferreirin, secundiflorol H, dalbergiodin, 3,9-dihydroxypterocarpan, desmoxyphyllin A, 3,5,7,4′-tetrahydroxy-coumaronochromone, aromadendrin, 5,7,4′-trihydroxy-coumaronochromone, panchovillin

Phenolic acids: Chlorogenic acid, ferulic acid, salicylic acid, vanillic acid, cimicifugic acid

Alkaloids: Desmodimine, desmodilactone, (3α,4β,5γ)-4,5-dihydro-4,5-dimethyl-3(1-pyrrol)-furan-2(3H)-one

Terpenoids: Lupeol, lupenone, sophoradiol, soyasaponin I, soyasapogenol B, soyasapogenol E, 19-cycloart-23-ene-3β,25-diol

Steroides: β-sitosterol, τ-sitosterol, daucosterol (=β-sitosterol-glycoside, eleutheroside A), stigmasterol, stigmasterol-3-O-β-D-glucopyranoside,

Volatile oils: Tritriacontane, eiconsanoic acid, eiconsanoic acid ethyl ester, eicosyl ester, oxalic acid, tetradecanoic acid, 3,7,11,15-tetramethyl-2-hexadecon-1-ol, 6,10,14-trimethyl-2-pentadecanone, pentadecanoic acid, hexadecanoic acid methylester, isophytol, n-hexadecanoic acid, hexadecanoic acid ethylester, heptadecanoic acid, phytol, 9,12-octadecadienoic acid, octadecanoic acid, octadecan oic acid methylester, octadecanoic acid ethylester, 4,8,12,16-tetramethylheptadecan-4-olide

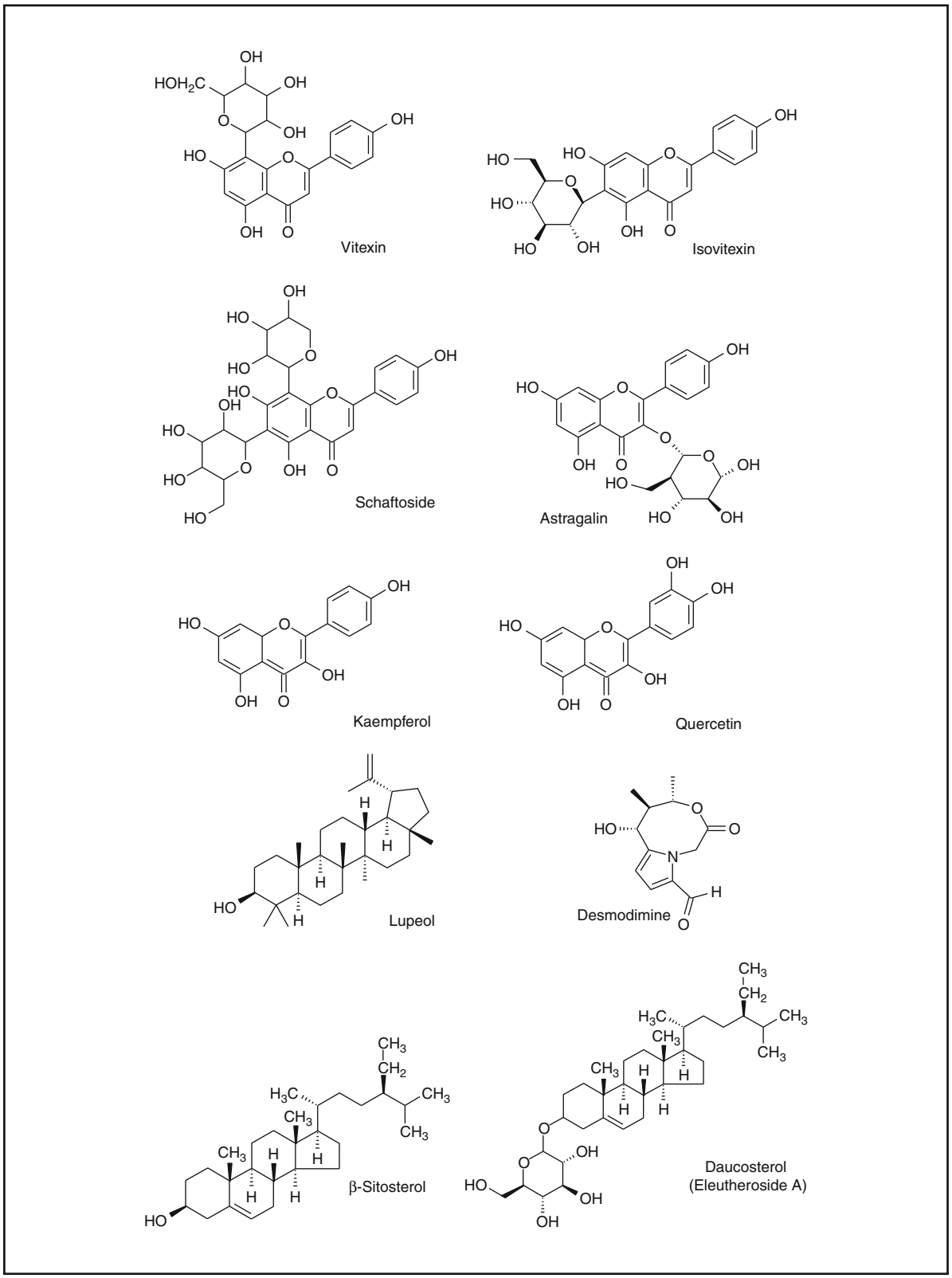


Fig. 1: Formulae of the main compounds of Herba Desmodii styracifolii [3, 4, 6, 7, 9, 10, 15]

Pharmacology

In vitro, *in vivo*, clinical research

Antinephrolithic Activity

- effective against urolithiasis (prophylaxis of calcium oxalate renal stones) [2, 3, 5, 8, 17]
- diuretic (increased urine volume) [3, 4]

Effects on Immune Functions

- immunopotentiating [3]
- anti-inflammatory [12, 17]
- anti-oxidative [3]
- lymphocyte transformation [3]
- induction of lymphokine-activated killer (LAK) cell activity [3]

Cardio-Cerebrovascular Effects [4, 7, 11]

Hypotensive Activity

- cholinergic receptor stimulation [3, 12]
- blockades autonomic ganglion and α-adrenoceptor [3, 12, 17]

TLC-Fingerprint Analysis [16]

Drug samples	Origin
1 Herba Desmodii/*Desmodium stryracifolium*	Province Guangdong, China
2 Herba Desmodii/*Desmodium stryracifolium*	Province Guangdong, China
3 Herba Desmodii/*Desmodium stryracifolium*	Province Guangxi, China
4[a] Herba Lysimachiae/*Lysimachia christina* Hance	Province Anhui, Jixi, Fuling, Libian Kang, China

[a]For comparison

1. Sample Preparation: 2 g of the powdered drug are extracted with 50 ml ethanol (80 %) in an ultrasonic bath for 30 min. The extracts are filtered and the filtrates evaporated to dryness. The residue is dissolved in 2 ml ethanol (p. a.) and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Herba Desmodii styracifolii: 10 µl each
Reference compounds: 10 µl each

1. **TLC-fingerprint analysis of flavonoids and organic acids** (Fig. 2)

Solvent system: Ethyl acetate + formic acid + glacial acetic acid + water (10+1.1+1.1+2.6)

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 under UV 366 nm.

Reference compounds of Fig. 2		**R*f***
T1	Vitexin	0.80
T2	Isovitexin	0.70
T3	Isoquercitrin	0.75
T4	Schaftoside	0.31/0.40

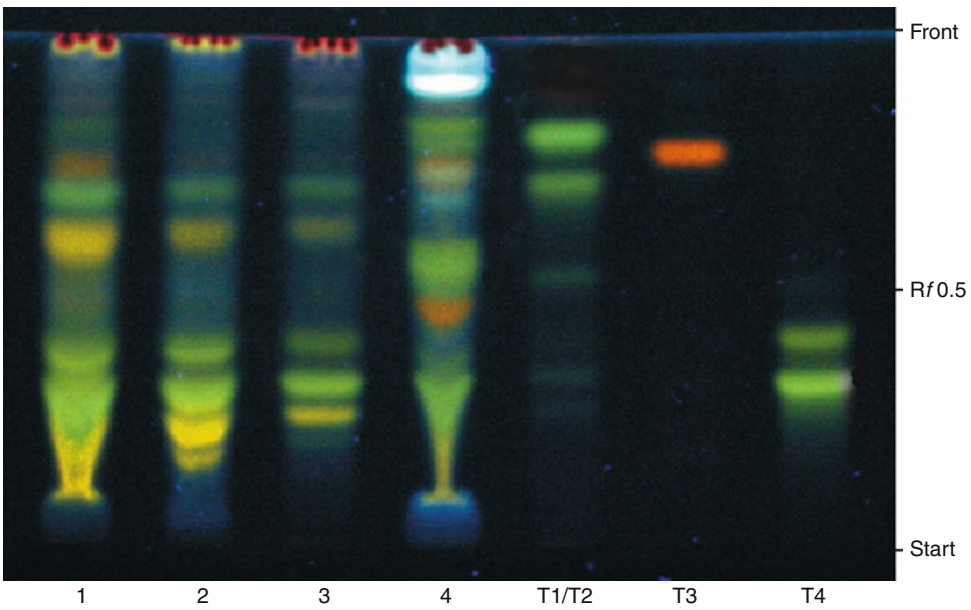


Fig. 2: Thin layer chromatogram of the ethanol extracts of Herba Desmodii styracifolii (flavonoids and organic acids) sprayed with NP/PEG (UV 366 nm)

Description of Fig. 2:

The three *Desmodium styracifolium* samples show the typical Isovitexin (**T2**) zone at R*f* = 0.70 but no or only weak traces of Vitexin (**T1**) at R*f* = 0.80. Isoquercitrin is most visible in sample 1.
All *Desmodium styracifolium* and the *Lysimachia christina* sample 4 show two strong green zones at R*f* = 0.31 and R*f* = 0.40 which can be assigned to Schaftoside (**T4**) and a second not identified flavon-C-glycoside.

2. **TLC-fingerprint analysis of triterpenoids** (Fig. 3)

Solvent system: Chloroform + methanol + water (13 + 7 + 2) (lower layer)

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 10 ml reagent, heated at 110 °C for 5 min and evaluated in VIS.

Note: The reagent has only limited stability and is no longer useable when colour has turned to red-violet.

Reference compounds of Fig. 3		**R*f***
T5	β-Sitosterol	0.97
T6	Oleanolic acid	0.96
T7	Daucosterol	0.88

Description of Fig. 3:

All samples show the characteristic zones of β-Sitosterol at R*f* = 0.97, oleanolic acid at R*f* = 0.96. and at R*f* = 0.74 a further brown zone which might be assigned to daucosterol or lupeol. In the lower range further dark and bright green zones are visible which derive from triterpen-glycosides.

Note: In the samples investigated no alkaloids could be detected.

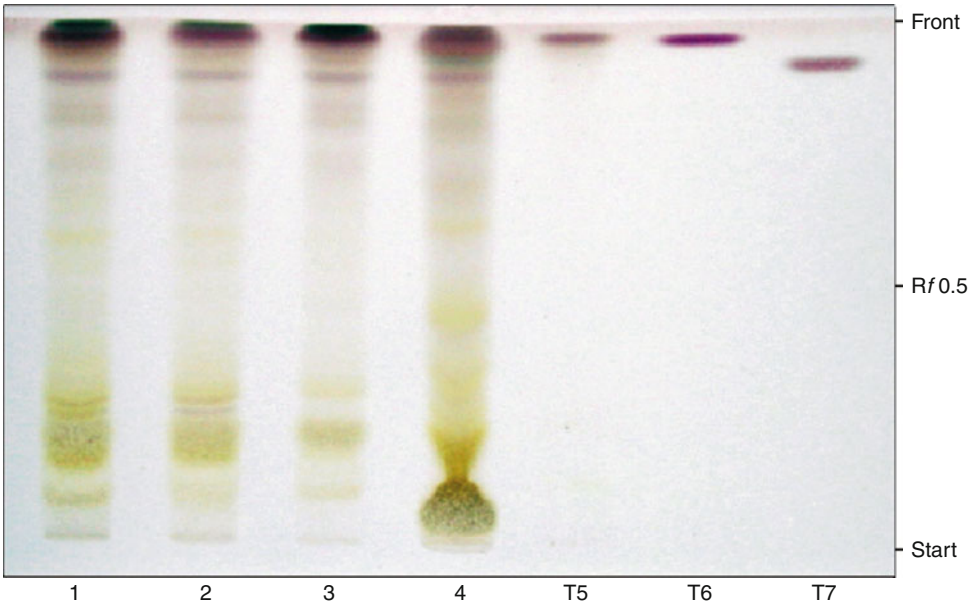


Fig. 3: Thin layer chromatogram of the ethanol extracts of Herba Desmodii styracifolii (triterpenoids) sprayed with Anisaldehyde – Sulphuric acid reagent (VIS)

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts are used as for the first HPTLC (see above).
2. Injection volume: Herba Desmodii styracifolii extracts: 20 µl each

3. HPLC parameters:

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, Merck
Solvent:	A: 0.001 % aq. H_3PO_4 (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	5–40 % B in 32 min, 40–95 % B in 10 min, 95 % B for 18 min, Total runtime: 60 min
Flow:	1.0 ml/min
Detection:	205 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	12.6	
2	13.0	
3	13.5	Flavonoids or phenolcarboxylic acids.
4	13.8	Assignments of the peaks see Fig. 4a–c
5	14.0	and the analogue HPLC peak profile of
6	14.5	Herba Lysimachiae
7	15.8	
8	17.0	
9	47.1	Daucosterol/Lupeol
10	48.8	

4. Description of the HPLC-Figures:

All *Desmodium styracifolium* extract samples show analogue to those of *Lysimachia christina* the accumulation of two distinct peak accumulation between Rt = 10.0 to 25.0 and 45.0 to 50.0. The first contains primarily the flavonolglycosides, the second the various sterols as evidenced by the UV-spectra designed with No. 1–6 and 9 + 10.

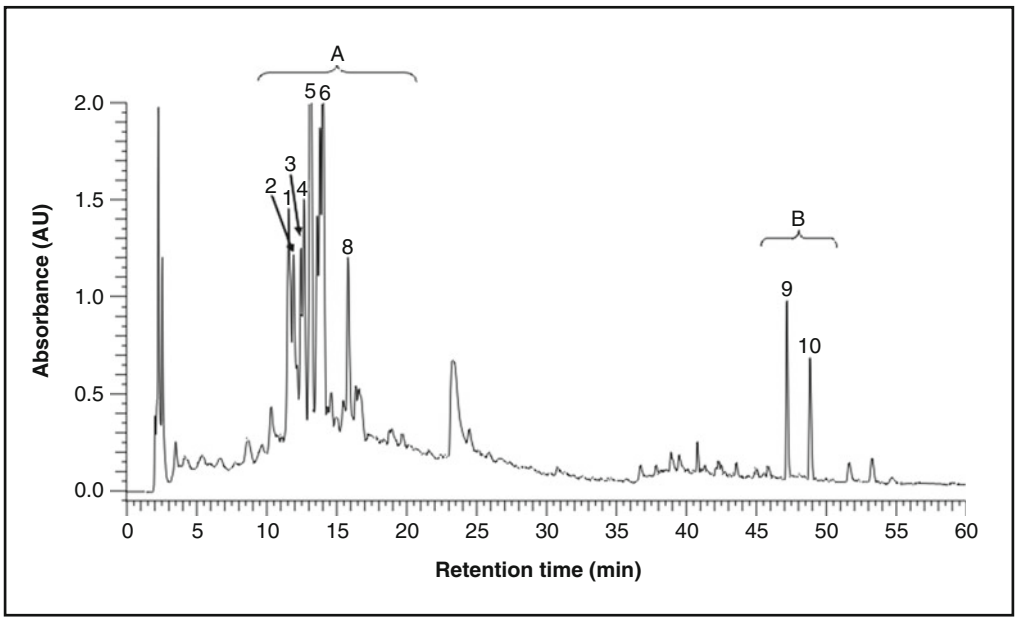


Fig. 4a: HPLC-fingerprint analysis of the ethanol extract of Herba Desmodii styracifolii, sample 1

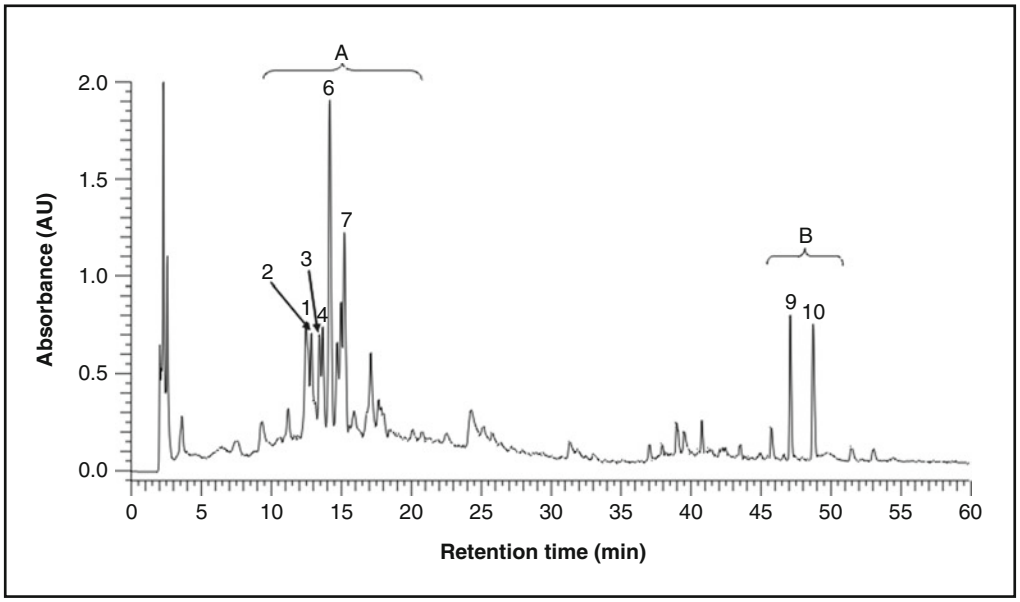


Fig. 4b: HPLC-fingerprint analysis of the ethanol extract of Herba Desmodii styracifolii, sample 2

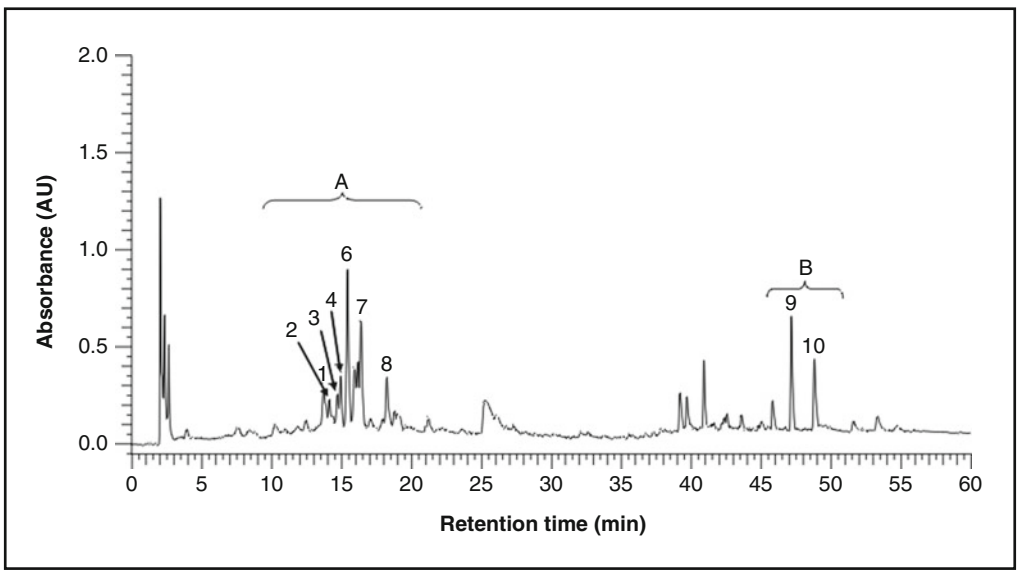


Fig. 4c: HPLC-fingerprint analysis of the ethanol extract of Herba Desmodii styracifolii, sample 3

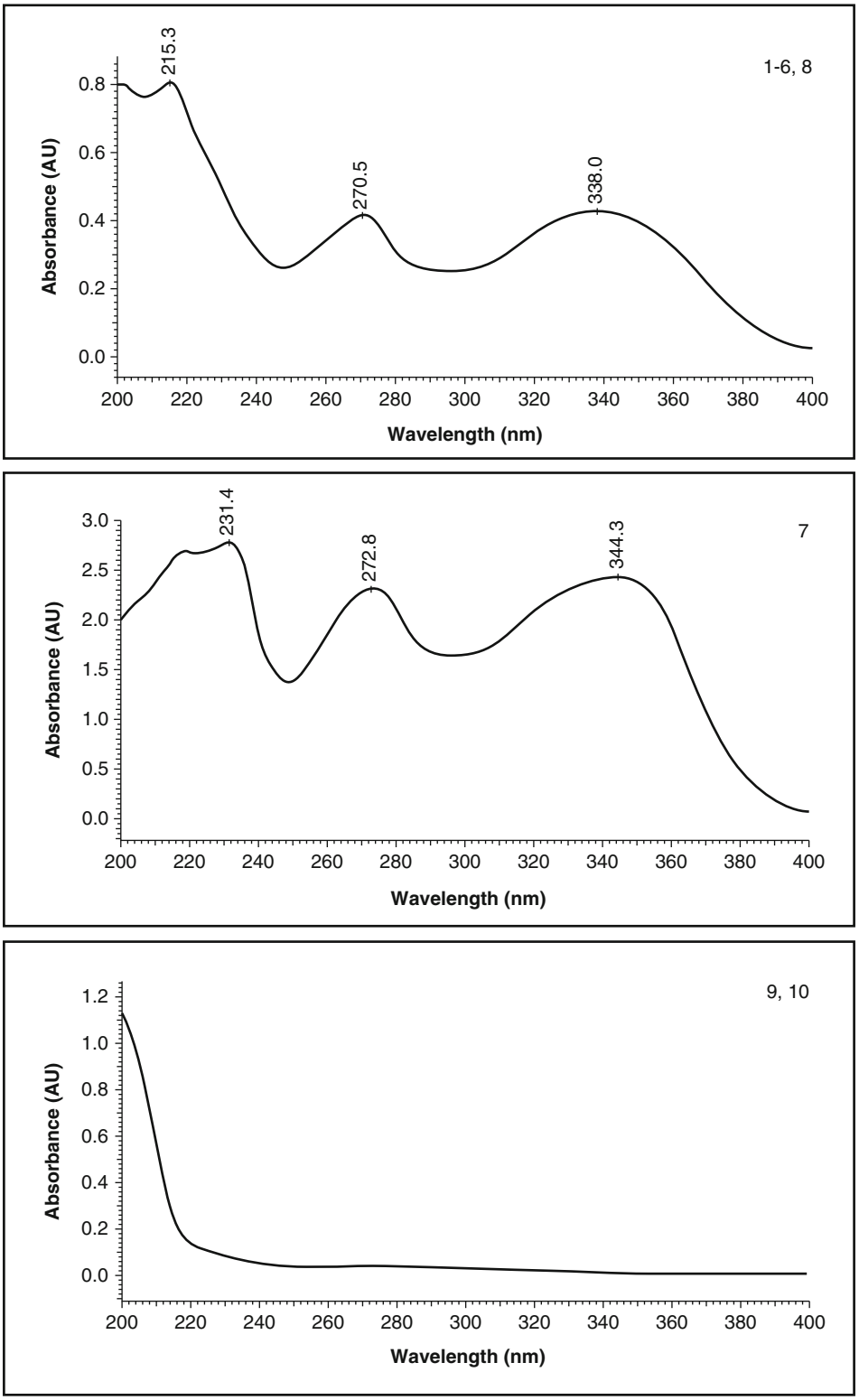


Fig. 5: On line UV-spectra of the detected peaks of Herba Desmodii styracifolii

Note: The Chinese Pharmacopoeia 2010 demands for Herba Desmodii styracifolii a content not less than 0.13 % of the total amount of schaftoside calculated with reference to the dried drug.

Further HPLC-fingerprint analytical methods for identification of the characteristic markers can be found in the following references: [4, 15]

Conclusion

If all herbal drug samples obtained from China were botanically correctly authenticated, the performed TLC- and HPLC-fingerprint analyses of Lysimachia and Desmodium show a nearly equal composition of the constituents. The slight differences in the content of polyphenols and triterpenoids may be due to geographical differences, other times of collection or other storage conditions.

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. China Medical Science Press, Beijing (2010)
2. Hempen, C.-H., Fischer, T.: A materia medica for chinese medicine: plants, minerals and animal products, 1st edn. Churchill Livingstone, Elsevier (2009)
3. Ma, X., Zheng, C., Hu, C., Rahman, K., Qin, L.: The genus *Desmodium* (Fabaceae)-traditional uses in chinese medicine, phytochemistry and pharmacology. J. Ethnopharmacol. **138**(2), 314–332 (2011)
4. Zhou, C., Luo, J.G., Kong, L.Y.: Quality evaluation of *Desmodium styracifolium* using high-performance liquid chromatography with photodiode array detection and electrospray ionisation tandem mass spectrometry. Phytochem. Anal. **23**(3), 240–247 (2012)
5. Mi, J., Duan, J., Zhang, J., Lu, J., Wang, H., Wang, Z.: Evaluation of antiurolithic effect and the possible mechanism of *Desmodium styracifolium* and *Pyrrosiae petiolosa* in rats. Urol. Res. **40**(2), 151–161 (2012)
6. Li, X.L., Wang, H., Liu, G., Zhang, X.Q., Ye, W.C., Zhao, S.X.: Study on chemical constituents from *Desmodium styracifolium*. Zhong Yao Cai **30**(7), 802–805 (2007)
7. Zhao, M., Duan, J.A., Che, C.T.: Isoflavanones and their *O*-glycosides from *Desmodium styracifolium*. Phytochemistry **68**(10), 1471–1479 (2007)
8. Hirayama, H., Wang, Z., Nishi, K., Ogawa, A., Ishimatu, T., Ueda, S., Kubo, T., Nohara, T.: Effect of Desmodium styracifolium-triterpenoid on calcium oxalate renal stones. Br. J. Urol. **71**(2), 143–147 (1993)
9. Yang, J.S., Su, Y.L., Wang, Y.L.: Studies on the chemical constituents of *Desmodium styracifolium*, (Osbeck) Merr. Yao Xue Xue Bao **28**(3), 197–201 (1993)
10. Kubo, T., Hamada, S., Nohara, T., Wang, Z.R., Hirayama, H., Ikegami, K., Yasukawa, K., Takido, M.: Study on the constituents of *Desmodium styracifolium*. Chem. Pharm. Bull. **37**(8), 2229–2231 (1989)
11. Ho, C.S., Wong, Y.H., Chiu, K.W.: The hypotensive action of *Desmodium styracifolium* and *Clematis chinensis*. Am. J. Chin. Med. **17**(3–4), 189–202 (1989)
12. Gu, L.Z., Zhang, B.S., Nan, J.H., Wang, R.Q.: Studies on the anti-inflammatory effects of two species of *Lysimachia christinae* Hance and *Desmodium styracifolium* (Osbeck) Merr. Zhong Yao Tong Bao **7**, 40–42 (1988), 63
13. Trout, K., Friends – Mydriatic Productions: Trout's notes on *The Genus Desmodium* (chemistry, ethnomedicine, pharmacology, synonyms & miscellany). URL: http://www.troutsnotes.com/sc/D2_2004_Trout.pdf, (Status: 09/05/2012)
14. Hong Kong chinese materia medica standards, vol 2. Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region – the People's Republic of China (2008)
15. Phan, M.G., Phan, T.S., Matsunami, K., Otsuka, H.: Flavonoid compounds from Desmodium styracifolium of Vietnamese origin. Chem. Nat. Comp. **46**(5), 797–798 (2010)
16. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin/Heidelberg/New York (2001)
17. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)

Fructus Retinervus Luffae – *Sigualuo*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Luffa Vegetable Sponge is the dried vascular bundles of ripe fruit of *Luffa cylindrica* (L.) Roem. (Fam. Cucurbitaceae).

Synonyms:[3, 5, 7] *Luffa aegyptica* Mill, *Luffa foetida* Sieb. et Zucc., *Luffa petola* Ser., *Momordica cylindrica* L.

Origin:[5, 7] Tropical countries of Asia and Africa. Indo-Burma. The main commercial production countries are China, Korea, India, Japan and Central America. Vietnam, Thailand, Laos, Philippines.

Description of the drug:[1] Interweaved silvery vascular bundles, mostly prolate cylindrical, somewhat curved, 30–70 cm long, 7–10 cm in diameter. Externally pale yellowish-white. Texture light, tenacious and springy, uneasily broken. 3 loculi visible, transverse section hollowed. Odour, slight; taste, weak.

Pretreatment of the raw drug:[1] The drug is collected in summer and autumn when the fruit is ripe, the pericarp turns to yellow and the inner part withered, removed from exocarp and sarcocarp, washed clean, dried in the sun and removed from the seed.

Remained seeds and exocarp are removed and cut into sections.

Medicinal use:[16] For the treatment of cough, fever, allergies, asthma, bronchitis and inflammations (rheumatic diseases). In western medicine primarily as homoeopathic tinctures.

Effects and indications of Fructus Retinervus Luffae according to Traditional Chinese Medicine [1, 3, 4]

Taste:	Sweet
Temperature:	Neutral
Channels entered:	*Orbis hepaticus*, *Orbis pulmonalis* et *stomachi*
Effects (functions):	To dispel wind, unblock the collaterals, activate blood, promote lactation.
Symptoms and indications:	Impediment pain, spasm, cramping, distending pain in the chest and the hypochondrium, agalactia, acute mastitis with swelling and pain. Hemostatic and analgesic in enterorrhagia, dysentery, metrorhagia, orchitis, hemorrhoids. Also to treat variola, boils. Toxic swelling, sores, abscesses (especially breast abscesses). Cough with sputum and pulmonary inflammation, very high fever, *Bi* syndrome, aching pain in the lower extremities. Stiff joints, pain and numbness in the muscles and sinews, stops bleeding, such as blood in the stools, heavy menstruation.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_15, © Springer International Publishing Switzerland 2015

Main constituents of *Luffa cylindrica*:[2–5, 7, 8, 10–15]

Pentacyclic and tetracyclic triterpensaponins:	Lucyoside A-P hederagenin, hederagenin-3-*O*–β-D-glucopyranosyl, oleanolic acid, oleanolic acid-3-*O*-β-D-glucopyranosyl, ginsenoside Re, ginsenoside Rg1 (protopanaxadiol- and triolglycosides), oleanolic acid saponins
Tetracyclics triterpenoids:	Cucurbitacine B, D, E, I, L
Flavonoids:	Diosmetin-7-*O*-β-D-glucuronide methyl ester, apigenin-7-*O*-β-D-glucuronide methyl ester, luteolin-7-*O*-β-D-glucuronide methyl ester
Phenolic acids/glucosides:	*p*-coumaric acid, 1-*O*-feruloyl-β-D-glucose, 1-*O*-*p*-coumaroyl-β-D-glucose, 1-*O*-caffeoyl-β-D-glucose, 1-*O*-(4-hydroxybenzoyl)glucose
Sterols:	22,23-dihydroxy spinasterol
Naphthalenes:	3-hydroxy-1-methylene-2,3,4,4 tetrahydroxynaphthalene-2-carbaldehyde
Others:	Terpenoids, xylose, mannosan, galactan, lignin, vitamin A, B and C, 4-*O*-methyl-D-glucurono-D-xylan

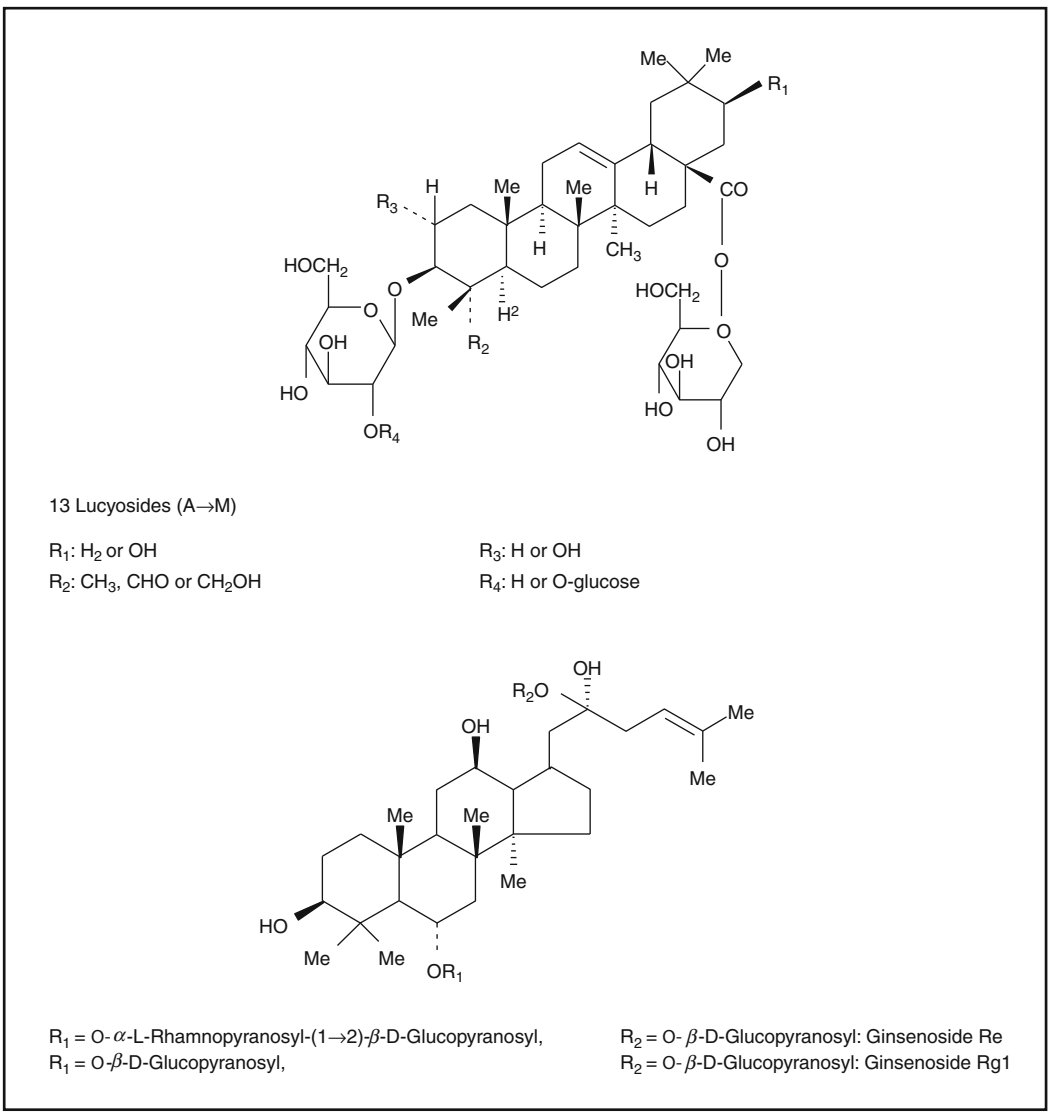


Fig. 1: Formulae of the main compounds of Fructus Retinervus Luffae [2, 7]

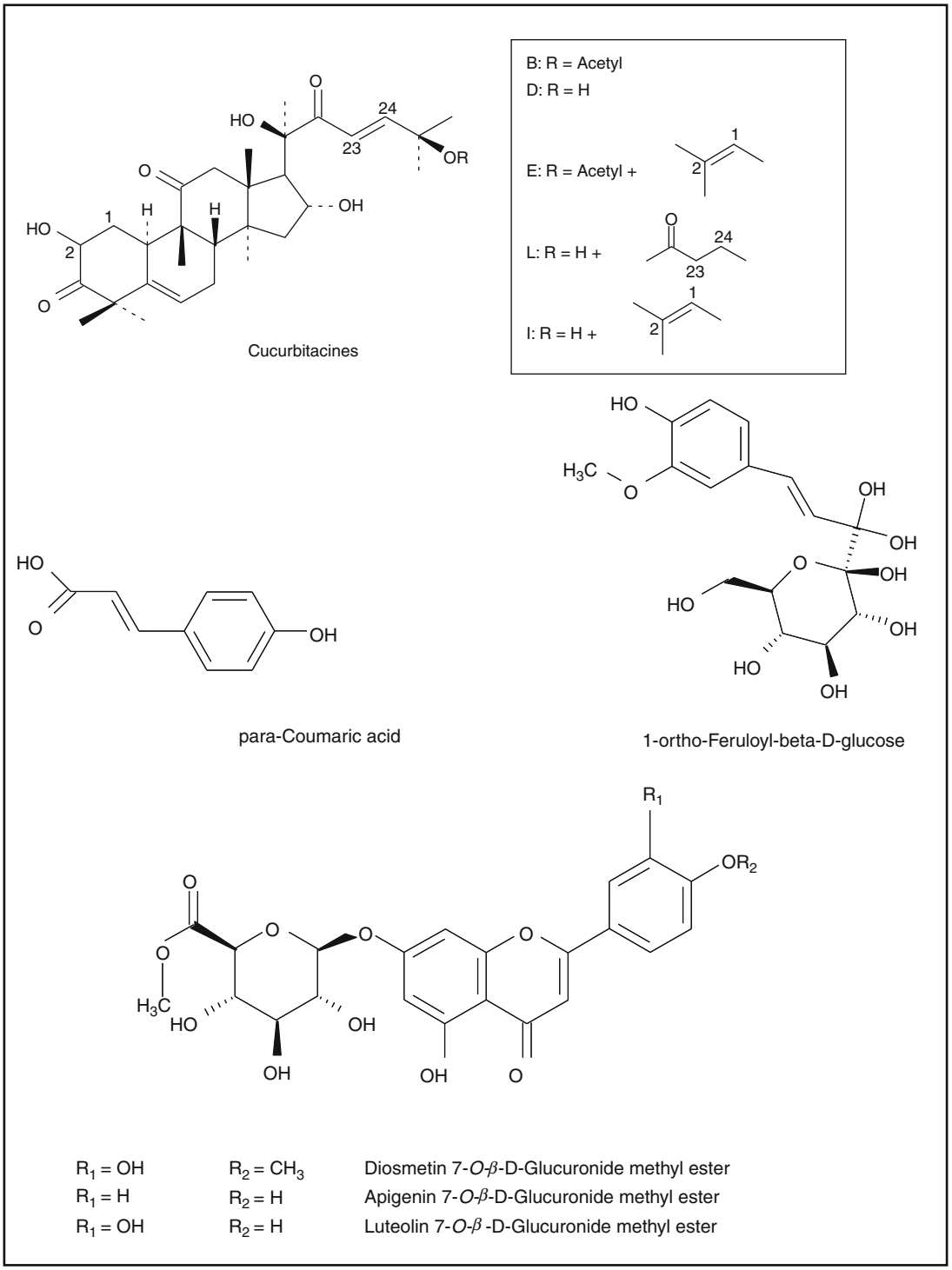


Fig. 1: (continued)

Pharmacology

In vitro, *in vivo*, clinical research of the fruit of *Luffa cylindrica* (L.)

Effects on Immune Functions

- antioxidative [5]
- immunopotentiating [6]
- anti-inflammatory [6]
- antiallergical [5]

Cardio-Vascular/Anti-Ischemic Effects [4, 7]

- cardiotonic
- antimyocardial ischemia
- lowers Twave increase in electrodiogram
- inhibits the decrease of heart rate
- inhibits the raise in serum lactate dehydrogenase and myocardial malondialdehyde level
- enhances the activity of myocardial superoxide dismutase

Analgesia and Sedation [5, 7]

- inhibits acetic acid-induced writhing
- raises the pain threshold in hot plate and electric shock tests
- reduces spontaneous activities
- synergizes the effects of pentobarbital sodium
- relieves pain

Anti-Hypertriglyceride [5, 7]

- decreases serum cholesterol and triglyceride levels
- increases high density lipoprotein-cholesterol
- reduce body weight

Anti-Asthma, Anti-Tussive and Expectorant Effects [5, 7]

- suppresses SO2- and ammonium aerosol-induced cough
- increases the respiratory tract phenol red excretion
- inhibits histamine induced asthma

Miscellaneous Actions [4–7]

- anti-acute hepatic injury
- cardiac stimulation
- S180 sarcoma inhibitory
- antihuman immunodeficiency virus
- antibacterial
- antifungal

Other Effects

- carrier for immobilization of microorganisms and plants and animal cells [12]

TLC-Fingerprint Analysis

	Drug samples	Origin
1	Fructus Retinervus Luffae/*Luffa cylindrica*	Sample of commercial drug obtained from China Medica (origin: Sichuan, Bazhong, China)
2	Fructus Retinervus Luffae/*Luffa cylindrica*	Province Henan (China)
3	Fructus Retinervus Luffae/*Luffa cylindrica*	Province Beijing (China)
4	Fructus Retinervus Luffae/*Luffa cylindrica*	Province Hebei (China)
5	Fructus Retinervus Luffae/*Luffa cylindrica*	Province Sigualo (China)
6	Fructus Retinervus Luffae/*Luffa cylindrica*	Sample of commercial drug obtained from TCM-clinic Bad Kötzting
7	Fructus Retinervus Luffae/*Luffa cylindrica*	Unknown Chinese Province
8	'Buchinha' (brazilian folk medicine) ***Luffa operculata***	Sample of commercial drug obtained from Cfm Oskar Tropitzsch (Brazilian province Bahia)

1. Sample Preparation: 2.5 g of the crushed drug are washed fat-free with 25 ml petroleum ether under reflux for 20 min, the filtrate is discarded and the residue is dried over night. Then the residue is extracted with 25 ml chloroform under reflux for 1 h. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml ethanol (p. a.) and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.

2. Reference compound: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Fructus Retinervus Luffae extracts: 10 µl each
Reference compound: 10 µl each

Solvent system: Chloroform + ethyl acetate + methanol (8+6+2)

Detection: Vanillin – Phosphoric acid reagent

1 g vanillin is dissolved in a small amount of ethanol and filled up with phosphoric acid (50 % aqu.) to 100 ml.

The plate is sprayed with 10 ml reagent, heated at 110 °C for 5 min and evaluated under VIS and UV 366 nm.

Reference compounds of Fig. 2a		**R*f***
T 1	Cucurbitacin D	0.54
T 2	Cucurbitacin E	0.75
T 3	Cucurbitacin B	0.71
T 4	Cucurbitacin L	0.59
T 5	Cucurbitacin I	0.63

4.1. Description of Fig. 2a:

The Fructus Luffae extract sample 1 shows the sterols at R*f*=0.90//0.93, curcubitacin E (**T2**) at R*f*=0.75, curcubitacin D (**T1**) at R*f*=0.54 and another zone at R*f*=0.20 which could be assigned to ginsenoside Re or Rg1 (see also Fig. 2b).
Extract sample 8 may contain both ginsenosides.

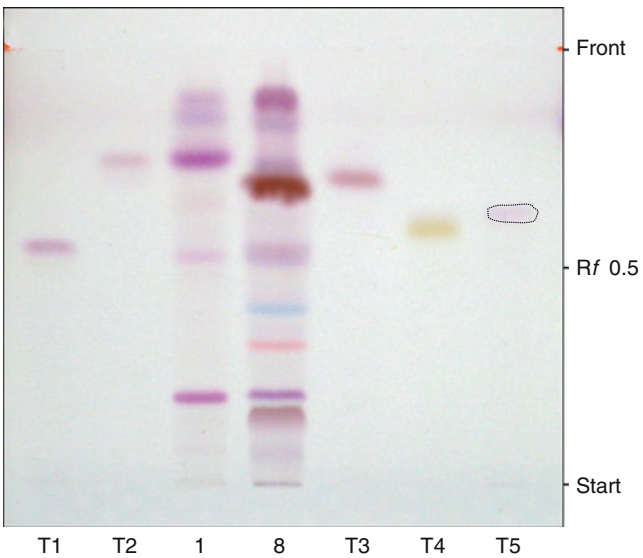


Fig. 2a: TLC of chloroform extracts of Fructus Retinervus Luffae (sample **1**) and Fructus Luffae operculatae (sample **8**), sprayed with Vanillin – Phosphoric acid reagent (VIS)

Reference compound of Figs. 2b and 2c		Rf
T 1	Cucurbitacin B	0.77

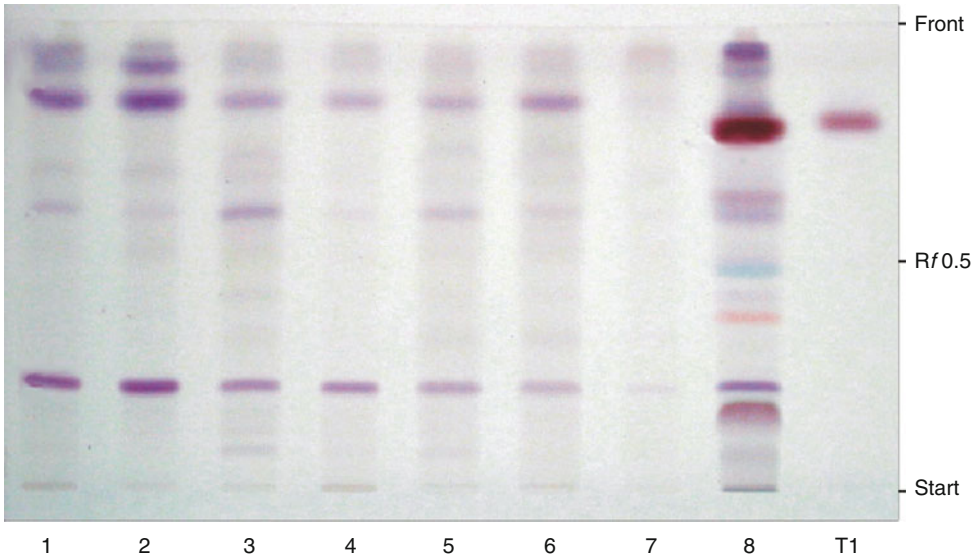


Fig. 2b: Thin layer chromatogram of the chloroform extracts of Fructus Retinervus Luffae sprayed with Vanillin – Phosphoric acid reagent (VIS)

4.2. Description of Fig. 2b:

The *Luffa cylindrica* samples 1–6 show a homogeneous pattern of violet-brown bands in the upper, middle and lower range of the plate. The bands in the upper R*f*-range at R*f*=0.90/0.93 and 0.83 can be assigned to sterols and cucurbitacin E (see also Fig. 2a). In the middle R*f*-range appear 3–4 weak bands with one distinct band at R*f*=0.60 which can be assigned to cucurbitacin D. In the low R*f*-range appear distinct bands which can be assigned to ginsenosides. Luffa sample 7 possesses very low concentrations of cucurbitacins and other compounds. Lucyoside could be not identified in any Luffa extract sample, probably because of too low solubility in the extraction solvent.

The extract sample of Fructus Luffae operculatae (**8**) possesses a cucurbitacin pattern with the dominating red brown cucurbitacin band at R*f*=0.77 which is identical with cucurbitacin B. A second cucurbitacin right above curcubitacin B has to be assigned to cucurbitacin E. From the other violet, green and orange bands in the middle R*f*-range one of the two violet bands at R*f*=0.63 may be identical with cucurbitacin D. The two bands at R*f*=0.20 and 0.15 might be assignably to ginsenosides Re and Rg1 which are described for Luffa spec.

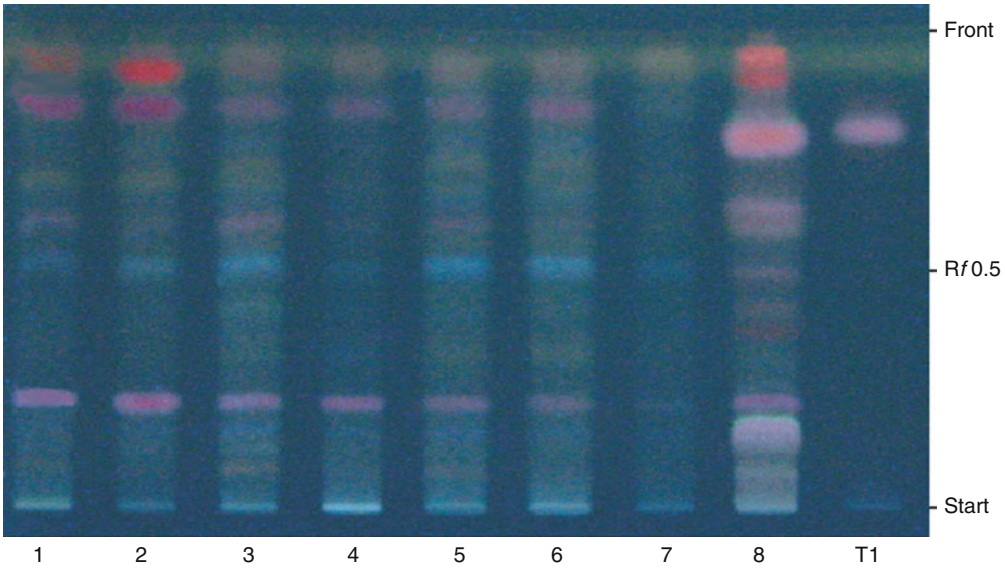


Fig. 2c: Thin layer chromatogram of the chloroform extracts of Fructus Retinervus Luffae sprayed with Vanillin – Phosphoric acid reagent (UV 366 nm)

4.3. Description of Fig. 2c:

The Fig. 2c (UV 366 nm) shows the same band pattern of Luffa sample as in Fig. 2b with light brown, red, green and blue fluorescence colours. Exact assignments except for Fructus Luffae operculatae (sample 8) cannot be given.

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts are used as for the HPTLC (see above).
2. Injection volume: Fructus Luffae extracts: 20 µl each

3. HPLC parameters:

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 % aq. H_3PO_4 (Millipore Ultra Clear UV plus® filtered) B: Acetonitrile (VWR)
Gradient:	0–40 % B in 40 min, 40–100 % B in 20 min, 100 % B for 12 min, Total runtime: 72 min
Flow:	1.0 ml/min
Detection:	205 nm

Retention times of the main peaks

Peak	**Rt (min)**	**Compound**
1	30.6	Cucurbitacin D
2	41.5	Cucurbitacin B
3	45.9	Cucurbitacin E
4	48.3	Unknown
5	51.0–63.0	Not assignable
6	64.8	β-sitosterol

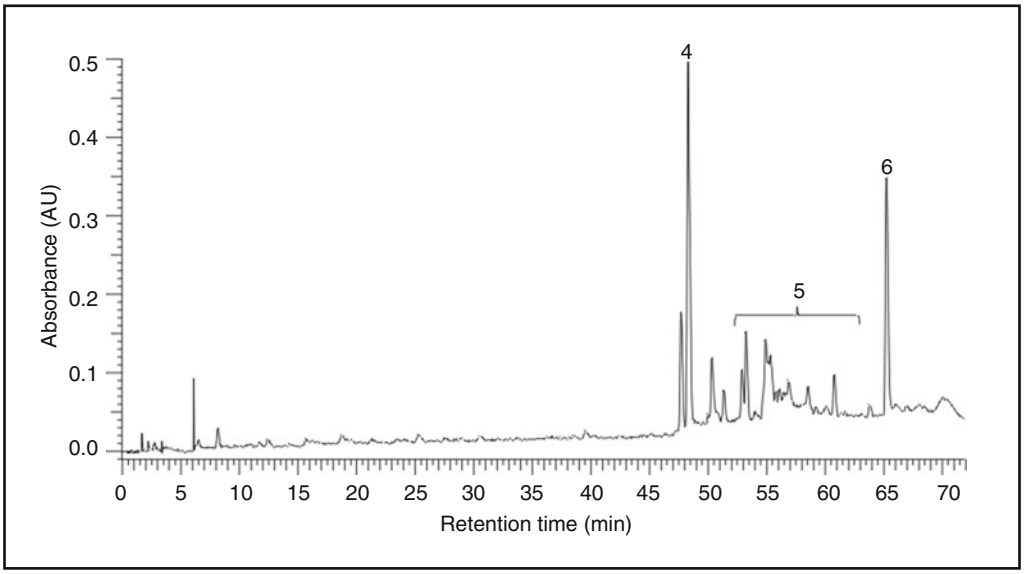


Fig. 3a: HPLC-fingerprint analysis of the chloroform extract of *Luffa cylindrica* (sample 1)

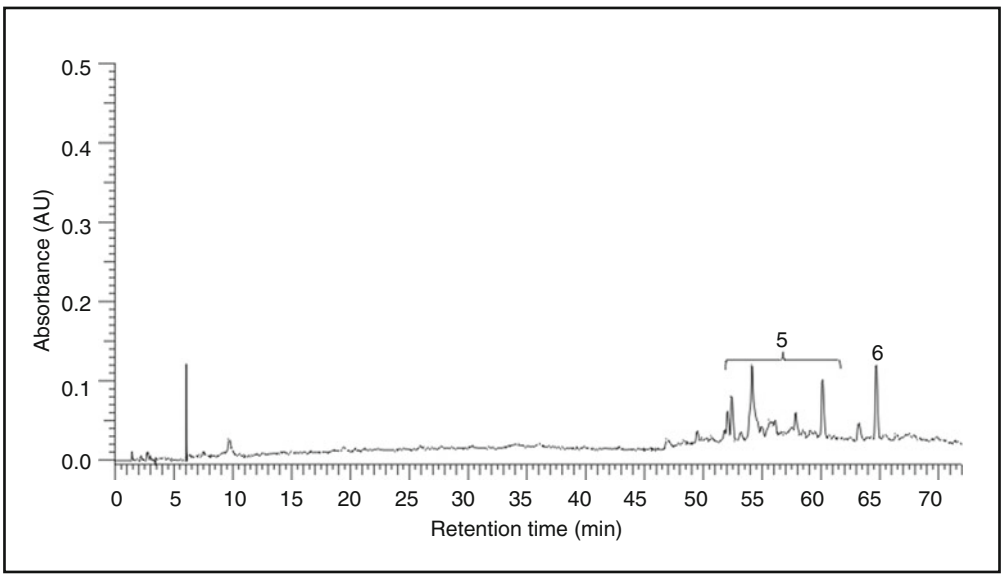


Fig. 3b: HPLC-fingerprint analysis of the chloroform extract of *Luffa cylindrica* (sample 2)

4. Description of Figs. 3a and 3b:

Sample 1 shows in the Rt-range of Rt 45.0–70.0 two distinct peaks at 48.5 and 65.5. The first accompanied by a smaller peak at 47.7 can be assigned to cucurbitacin E. The on line UV-spectrum with maximum at 230 nm is characteristic for most of the available cucurbitacin reference compounds (see also TLC-Figs. 2a and 2b). The little 7–8 peaks of peak-accumulation **5** between the Rt-range 50.0–62.0 could be not assigned but maybe also triterpenoids. The peak at 65.5 possesses a UV spectrum with end absorption which is characteristic for triterpenoids/sterins. The small peak at Rt = 6.1 could be not identified.

Sample 2 shows a similar peak assignment in lower concentration but without the two dominant peaks at Rt 48.5 and 65.5.

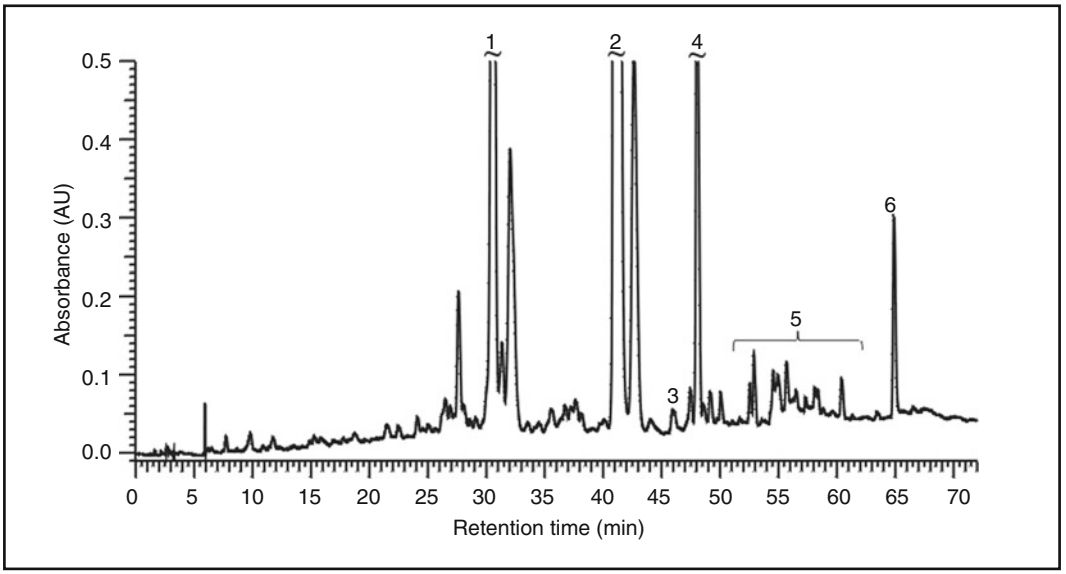


Fig. 3c: HPLC-fingerprint analysis of the ethanol chloroform of *Luffa operculata* (sample 8)

Description of Fig. 3c:

The HPLC-profile of Luffa operculata (sample 8) shows two dominant double peaks in the Rt-range of 30.0–33.5 and 41.0–44.0. All four single peaks possess the characteristic cucurbitacin UV-spectra with maxima at 230 nm and thereby assignable to cucurbitacin D (1) and B (2). The peak (4) at 48.0 could be not assigned. The peak accumulation between 45.0 and 65. corresponds with the peak profile of extract sample 1 and 2.

Note: Further HPLC-fingerprint analytical methods for identification of the characteristic marker compounds can be found in the following references: [9]

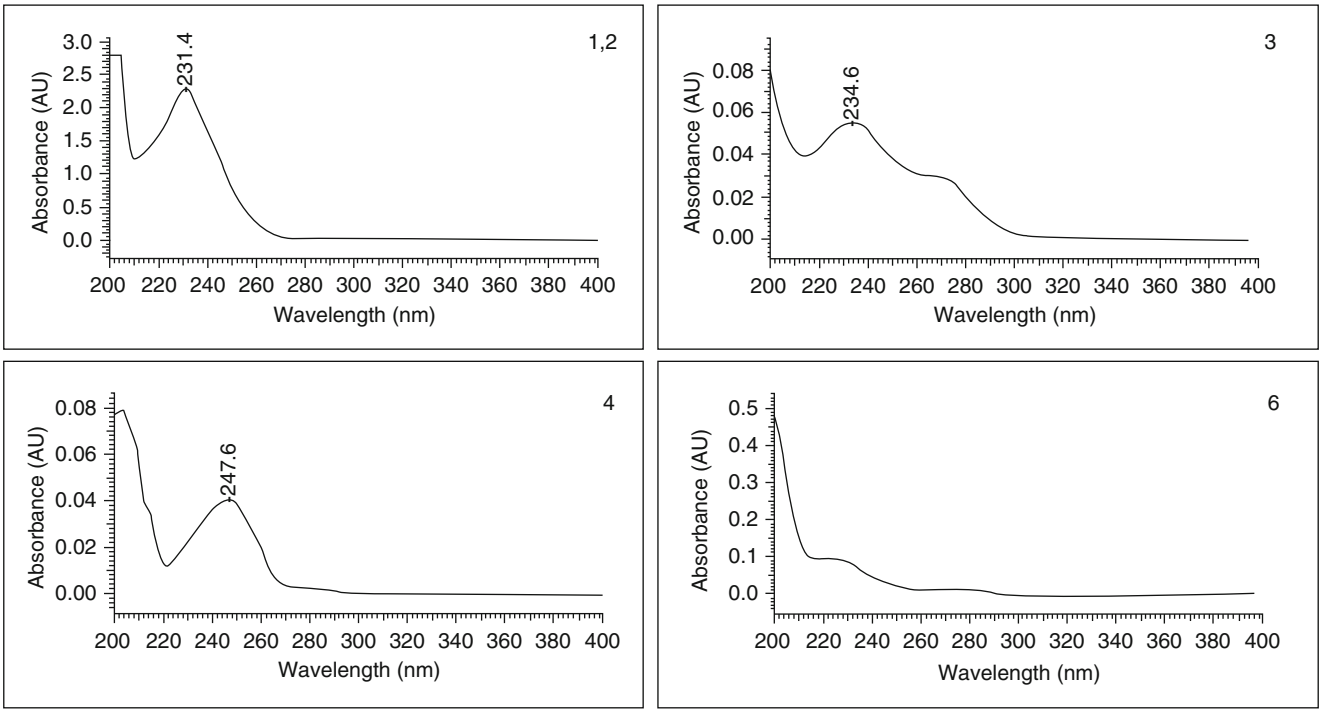


Fig. 4: On line UV-spectra of the detected peaks of Fructus Luffae

Conclusion

The dried Luffa sponges extracts show TLC- and HPLC-fingerprints which permit only the detection of the cucurbitacins which thereby have to be considered as the most characteristic pharmaceutically important constituents of *Luffa cylindrica*. Apart of the Brazilian *Luffa operculata* other Luffa species were not available and seem to be of no interest. The triterpenglycosides Lucyosides reported in the literature could be not detected. Flavonoids could be also not identified.

The TLC seems to be the best analytical method to use it for the quality proof. The presence of cucurbitacins could be also confirmed, because of their characteristic UV – Maxima at ~230 nm with or without an inflexion at 270 nm in the online UV – spectra.

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Tang, W., Eisenbrand, G.: Chinese drugs of plant origin: chemistry, pharmacology and medicinal use in traditional and modern medicine. Springer, Berlin/Heidelberg (1992)
3. Keys, J.D.: Chinese herbs – their botany, chemistry, and pharmacodynamics. Charles E. Tuttle Company, Ruttland/Vermont/Tokyo (1976)
4. Hempen, C.H., Fischer, T.: A materia medica for chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone/Elsevier, New York (2009)
5. Lim, T.K.: Edible medicinal and non medicinal plants. In: Fruits, vol. 2. Springer Science/Business Media B.V, Dordrecht/Heidelberg/London/New York (2012)
6. Saxena, P., Dey, S., Arora, A., Nagarajan, K., Singh, P.K., Sing, S.P.: *Luffa cylindrica*: biological actions and medicinal applications. Int. J. Adv. Pharm. Biol. Sci. **1**(1), 36–41 (2011)

7. Partap, S., Kumar, A., Sharma, N.K., Jha, K.K.: *Luffa cylindrica*: an important medicinal plant. J. Nat. Prod. Plant Resour. **2**(1), 127–134 (2012)
8. Du, Q., Xu, Y., Li, L., Zhao, Y., Jerz, G., Winterhalter, P.: Antioxidant constituents in the fruits of *Luffa cylindrica* (L.) roem. J. Agric. Food Chem. **54**(12), 4186–4190 (2006)
9. Bauer, R., Wagner, H.: Cucurbitacinhaltige Drogen – Analyse und Standardisierung von Arzneidrogen und Phytopräparaten durch Hochleistungsflüssigchromatographie (HPLC) und andere chromatographische Verfahren (II). Deutsche Apotheker Zeitung **123**(27), 1313–1321 (1983)
10. Fai, Y.M., Tao, C.C.: A review of presence of oleanolic acid in natural products. Sample review for Natura Proda Medica (2009)
11. Du, Q., Cui, H.: A new flavone glycoside from the fruits of *Luffa cylindrica*. Fitoterapia **78**(7–8), 609–610 (2007)
12. Hideno, A., Ogbonna, J.C., Aoyagi, H., Tanaka, H.: Acetylation of loofa (*Luffa cylindrica*) sponge as immobilization carrier for bio-processes involving cellulase. J. Biosci. Bioeng. **103**(4), 311–317 (2007)
13. Joshi, B.K., Hari, B.K.C., Tiwari, R.K., Ghale, M., Sthapit, B.R., Upadhyay, M.P., NARC, LIBIRD, IPGRI: Descriptors for sponge gourd [*Luffa cylindrica* (L.) roem]. NARC, Kathmandu (2004)
14. Ismail, M., Hussain, M.M., Dastagir, M.G., Billah, M., Quader, A.: Phytochemical and antimicrobial investigation of *Luffa cylindrica*. Bol. Latinoam. Caribe Plant Med. Aromat. **9**(5), 327–332 (2010)
15. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)
16. Chen, J.K., Chen, T.T.: Chinesische Pharmakologie I: 523 Arzneimonogrpahien. Verlag Systemische Medizin AG, Bad Kötzting (2012)

Herba Oldenlandiae – *Bai hua she she cao*

Pharmacopoeia: Not listed in the Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2000, 2005, 2010

Official drug:[3] Herba Oldenlandiae is the dried whole herb of *Oldenlandia diffusa* (Willd.) Roxb. (Fam. Rubiaceae).

Oldenlandia tenelliflora Bl. and *Oldenlandia corymbosa* (L.) Lam are often used indiscriminately as Herba Oldenlandiae in the markets.

Synonym:[1, 5] Herba Hedyotidis, *Hedyotidis diffusa*

Origin:[3, 11] Southern regions of China, Chinese provinces like Guangxi, Jiangsu, Guangdong, Sichuan, Jiangxi, Fujian, Anhui and Hubei. Taiwan, Japan, Korea.

Description of the drug:[2] Interwined into a loose mass; grayish-green or grayish brown; 1 axial root; rootlets fine. Stem fine and curled; marked with longitudinal ridges. Leaves opposite (borne in pairs at each node), mostly in fragments, extremely shriveled, fall off easily; when intact, blades linear (long and narrow); with stupiles, 1–2 mm long, membranous, lower part accrete (grown or joined together), top end serrulate (finely notched). Flowers usually solitary (occurring singly) and axillary (grown at axil); mostly pedicellate (with a pedicel). Capsules flattened and spherical; top end with 4-toothed persistent calyx. Odour: faint; taste: mild.

Pretreatment of the raw drug:[2] The whole herbs are harvested in summer and autumn, dried under the sun and used while fresh.

Medicinal use:[12] It has been used as anti-cancer drug and for the treatment of liver diseases (hepatitis) and appendicitis.

Effects and indications of Herba Oldenlandiae according to Traditional Chinese Medicine[1, 5, 8, 9, 11, 12, 14–16, 18]

Taste:	Sweet, bland and lightly bitter
Temperature:	Cool
Channels entered:	*Orbis intestinorum* et *stomachi*
Effects (functions):	Clears heat, resolves toxin, quickens blood
Symptoms and indications:	Disperses swelling (e.g. skin and intestinal abscesses, boils and snakebites), disinhibits urine. Treatment of tonsillitis, sore throat, appendicitis and urethral infection. Useful for some tumours (e.g. liver, lung and stomach, esophagus, leukemia). Treatment of sphagitis, bronchitis, dysentery, mastitis, pneumonia, appendicitis, pelvitis. Hepatitis, rheumatism, arthritis, autoimmune disease, furunculosis, enteritis and bleeding. Removes toxic damp heat, clears abscesses, infections with fever.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_16, © Springer International Publishing Switzerland 2015

Described constituents of *Oldenlandia diffusa*:[3–6, 9–13, 16–18, 20]

Iridoids, iridoid glycosides and esters:	Asperuloside, deacetylasperuloside, asperulosidic acid, asperulosidic acid methyl ester, iridoid V_3 *E*-6–O–*p*-coumaroylscandoside methyl ester (Oldenlandoside I), *E*-6-O–*p-coumaroylscandoside* methyl ester-10-methyl ether, 10-*O*–benzoyl-6′-*O*–α-L-arabino(1→6)-*β*-D-gluco-pyranosylgeniposidic acid, deacetyl-6-ethoxyasperulosidic acid methyl ester, galioside, gardenoside, 4-epiborreriagenin, 6-*O*–methyldeacetylasperulosidic acid methyl ester, *E*-6-*O*–feruloylscandoside methyl ester, *Z*-6-*O*–feruloylscandoside methyl ester, scandoside methyl ester, asperulosidic acid methyl ester, oldenlandoside III, geniposidic acid, scandoside, feretoside, diffusoside A and B
Triterpenoids:	Oleanolic acid, ursolic acid
Flavonoid glycosides:	Rutin (quercetin-3-O–(6-O-α-L-rhamnoside)-D-gluco-pyranoside), quercetin (3,5,7,3′,4′-pentahydroxyflavone), quercetin-3-O-(2-O-beta-D-glucopyranosyl)-beta-D-glucopyranoside, quercetin-3-O-[2-O-(6-O-E-sinapoyl)-beta-D-glucopyranosyl]- beta-glucopyranoside, quercetin-3-O-[2-O-(6-O-E-feruloyl)-beta-D-glucopyranosyl]- beta-glucopyranoside, quercetin-3-O-sambubioside, quercetin-3-O-sophoroside, kaempferol-3-O-[2-O-(6-O-E-feruloyl)-beta-D-glucopyranosyl]-beta-galactopyranoside
Phenolic acids:	*p*-coumaric acid, ferulic acid
Sterols:	Stigmasterol, β-sitosterol, β-sitosteryl glucoside
Anthraquinones:	2,6-dihydroxy-1-methoxy-3-methylanthraquinone, 2-hydroxy-1-methoxy-3-methylanthraquinone, 2-hydroxy-3-methylanthraquinone
Others:	Polysaccharides, phenylpropanoids, chlorophyll 10-(*S*)-hydroxypheophytin a, hentriacontane

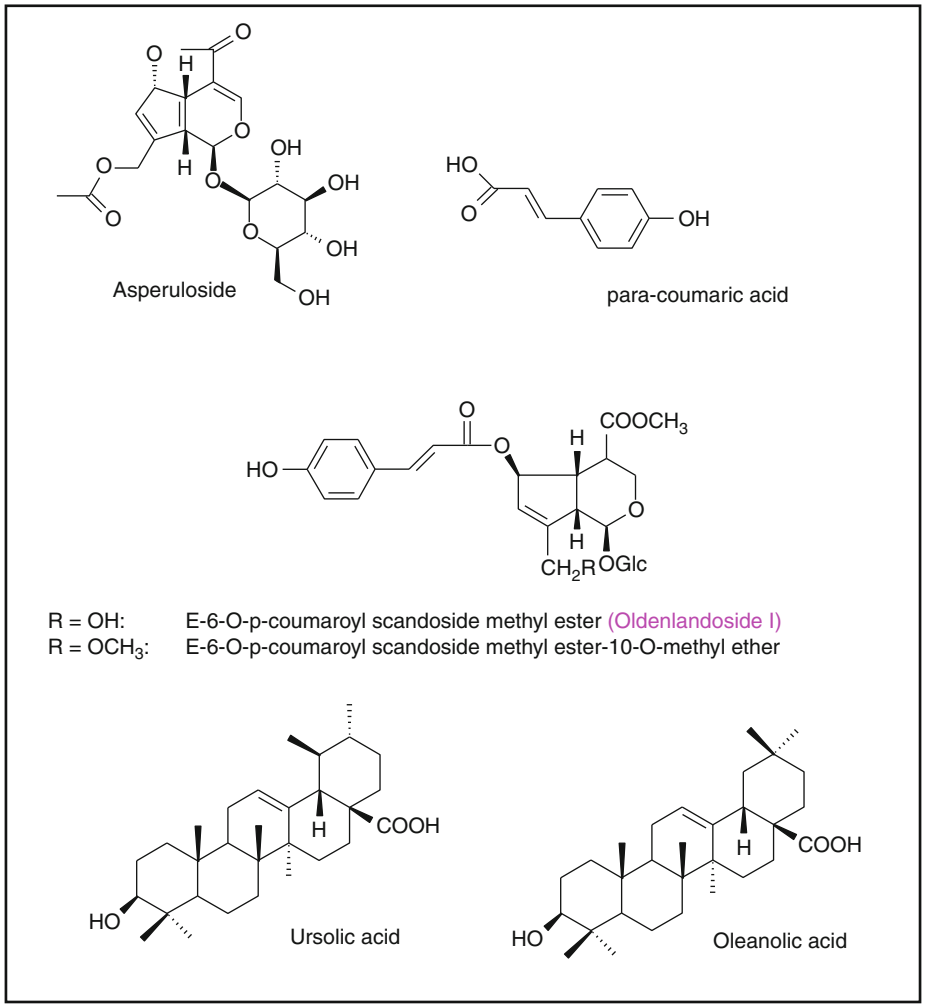


Fig. 1: Formulae of the main compounds of Herba Oldenlandiae [6, 10]

Reported Pharmacological Activities

Effects on Immune Functions

- immunoregulatory [9–11]
- anti-inflammatory [6, 10–12]
- antioxidative [6, 10, 12, 18]
- antimicrobial [11]

Miscellaneous Actions

- inhibits allelochemicals and DNA polymerase [12]
- antitumoural [6, 9–11, 20]
- pro-apoptotic [6, 8]
- anti-angiogenic [6, 9, 12]

- antiprolerative of breast and prostate cancer cells [7–9, 13, 20]
- cytotoxic [9]
- anti-histaminic [9]
- anti-thrombotic [9]
- increases phagocytosis [9]
- lowers fever [9]
- arrests growth of spermatogenesis [9]
- empties convoluted seminiferous tubules [9]
- antimutagenic [10, 20]
- hepatoprotective [10]
- neuroprotective [10]

TLC-Fingerprint Analysis

Drug samples	Origin
1 Herba Oldenlandiae/*Oldenlandia diffusa*	Chinese Province Guangdong
2 Herba Oldenlandiae/*Oldenlandia diffusa*	Chinese Province Guangxi
3 Herba Oldenlandiae/*Oldenlandia diffusa*	Chinese Province Fujian
4 Herba Oldenlandiae/*Oldenlandia sp.*	Sample of commercial drug (China Medica GmbH)
5 Herba Oldenlandiae/*Oldenlandia sp.*	Sample of commercial drug (PharmaChin GmbH, origin: province Zhe Jiang, China)
6 Herba Oldenlandiae/*Oldenlandia diffusa*	Sample of commercial drug, Sinomed, (TCM-Clinic Bad Kötzting)

1. Sample Preparation: 1 g powdered drug is extracted with 25 ml methanol under reflux for 1 h. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

   Plate: HPTLC Silica gel 60 F_{254}, Merck

   Applied amounts: Herba Oldenlandiae extract: 12 µl each

   Reference compounds: 10 µl each

1. **Solvent system and detection for triterpenoids and iridoid glycosides** (Figs. 2a and 2b)

Solvent system: Ethyl acetate + methanol + water + glacial acetic acid (15.4 + 3 + 2.5 + 0.1) (upper layer)

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 10 ml reagent, heated at 110 °C for 5 minutes and evaluated under VIS and UV 366 nm.

Note: The reagent has only limited stability and is no longer useable when colour has turned to red-violet.

Reference compounds of Figs. 2a and 2b		**R*f***
T1	Asperuloside	0.30
T2	Ursolic acid	0.94
T3	Oleanolic acid	0.95

Description of Fig. 2a:

All samples show a very intensive blue zone at R*f* = 0.34 which may be assigned to *E*-6-O-*p*-coumaroylscandoside methyl ester (oldenlandoside I). Asperuloside at R*f* = 0.30 (**T1**) and 2–3 other diterpenoids in the R*f*-range from start to R*f* = 0.25 appear in all extract samples but only in very low concentrations. The characteristic pink zones at ~ R*f* = 0.95 can be attributed to ursolic- (**T2**) and oleanolic acid (**T3**).

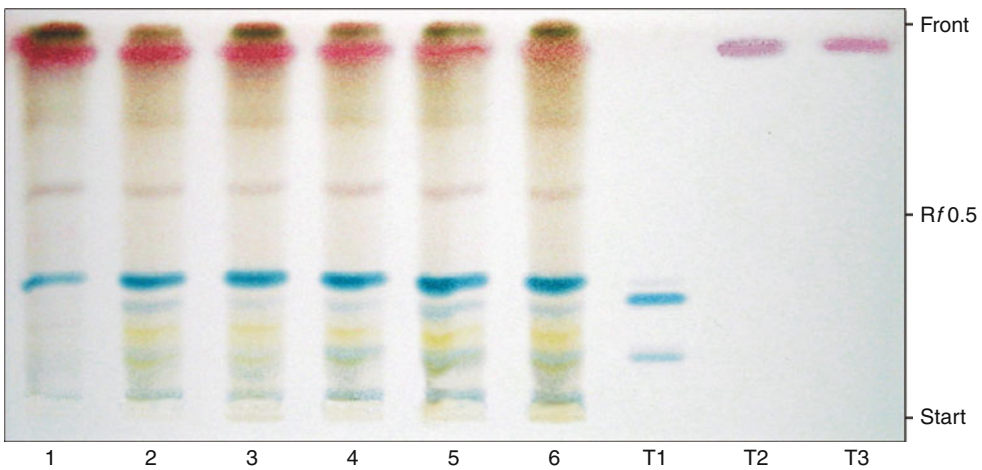


Fig. 2a: Thin layer chromatogram of the methanol extracts of Herba Oldenlandiae (triterpenoids and iridoid glycosides) sprayed with Anisaldehyde – Sulphuric acid reagent (VIS)

Description of Fig. 2b:

All samples show an intensive dark-blue zone at R*f* = 0.34 which can be assigned to E-6-O-p-coumaroylscandoside methyl ester (oldenlandoside I) with asperuloside at R*f* = 0.30. Ursolic and oleanolic acid appear under UV 366 nm yellow-green overlapped by chlorophyll.

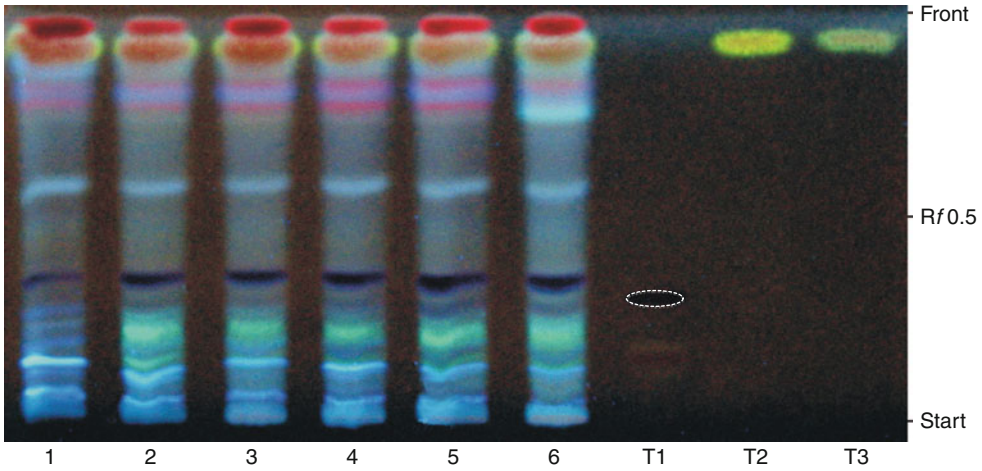


Fig. 2b: Thin layer chromatogram of the methanol extracts of Herba Oldenlandiae (triterpenoids and iridoid glycosides) sprayed with Anisaldehyde – Sulphuric acid reagent (UV 366 nm)

2. **Solvent system and detection for flavonoids and organic acids** (Fig. 3)

Sample preparation: 1 g powdered drug is extracted with 25 ml methanol under reflux for 1 h. The extract is filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.

Solvent system: Ethyl acetate + formic acid + glacial acetic acid + water (10 + 1.1 + 1.1 + 2.6)

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 under UV 366 nm.

Reference compounds of Fig. 3		***Rf***
T1	Rutin	0.45
T2	Chlorogenic acid	0.55

Description of Fig. 3:

The *Oldenlandia diffusa* extract samples 2–6 with the exception of extract sample 1 show a very homogenous TLC-fingerprint analytical profile with yellow-orange and blue fluorescent zones over the whole plate range: rutin (**T1**, R*f* = 0.45), quercetin- and kaempferol glycosides as orange/yellow zones at R*f* = ~ 0.4 and between R*f* = 0.15 and 0.25. The light-blue fluorescent zone above rutin can be assigned to chlorogenic acid (**T2**, R*f* = 0.55).

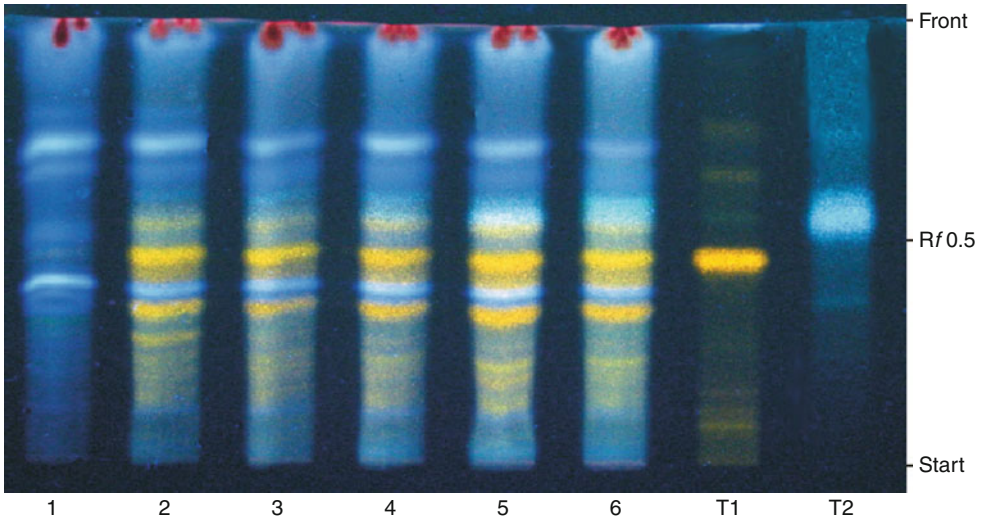


Fig. 3: Thin layer chromatogram of the methanol extracts of Herba Oldenlandiae (flavonoids) sprayed with NP/PEG (UV 366 nm)

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts as used for the HPTLC (see above).
2. Injection volume: Herba Oldenlandiae extracts: 15 µl each
3. HPLC parameters:

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:	0–7 % B in 10 min, 7–20 % B in 20 min, 20–75 % B in 20 min, 75–100 % B in 12 min, 100 % B for 10 min, Total runtime: 72 min
Flow:	1.0 ml/min
Detection:	210 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	6.3	Not identified
2	10.4–14.8	Asperuloside
3	14.8–21.2	Rutin
4[a]	23.3–29.9	*E*-6-O-*p*-coumaroylscandoside methyl ester
5[a]	24.3–30.8	*E*-6-O-p-coumaroylscandoside methyl ester-10-methyl ether
6[a]	24.7–31.2	6-O-feruloylscandoside methyl ester
7	41.6	Not identified
8	46.7	Diterpene derivative?
9	49.3	Diterpene derivative?
10	61.1	Oleanolic acid
11	66.0	Ursolic acid

[a]According to Ref. [3]

4. Description of Fig. 4a, 4b and 4c:

   The HPLC of the extract samples 3, 4 and 6 show at 210 nm the same Rt-profile with distinct peaks at Rt = 6.3 (**1**), Rt = 23.3–29.9 (**4**), Rt = 46.7 (**8**) and Rt = 66.0 (**11**). The assignment of the other minor peaks at Rt = 24.3–30.8 (**5**), Rt = 24.7–31.2 (**6**), Rt = 41.6 (**7**), Rt = 49.3 (**9**), Rt = 61.1 (**10**), could be not exactly assigned.

Note: Further HPLC-fingerprint analytical methods for identification of the characteristic marker compounds can be found in the following references:[3, 18, 19]

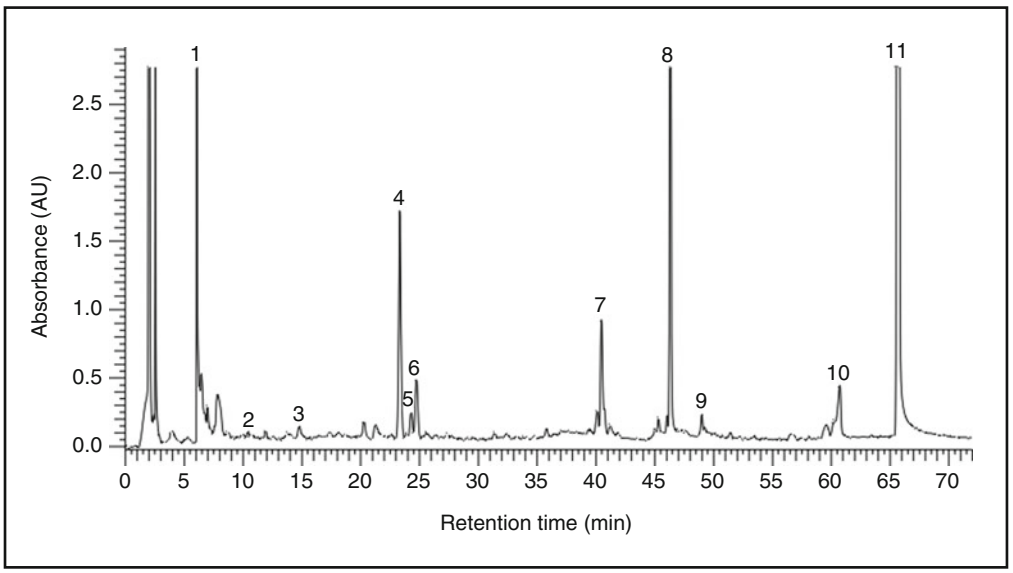


Fig. 4a: HPLC-fingerprint analysis of the methanol extract of Herba Oldenlandiae (sample 3)

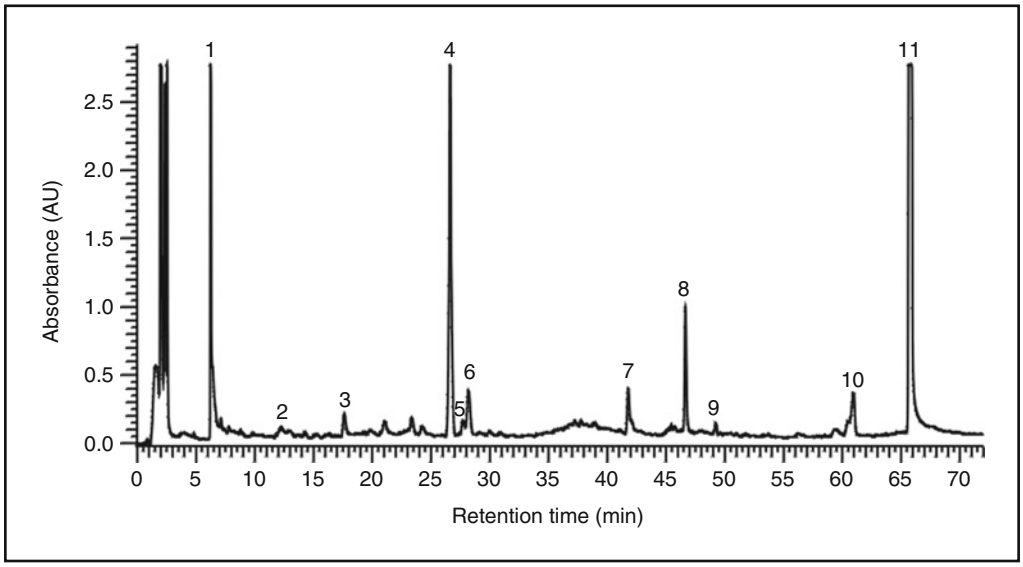


Fig. 4b: HPLC-fingerprint analysis of the methanol extract of Herba Oldenlandiae (sample 4)

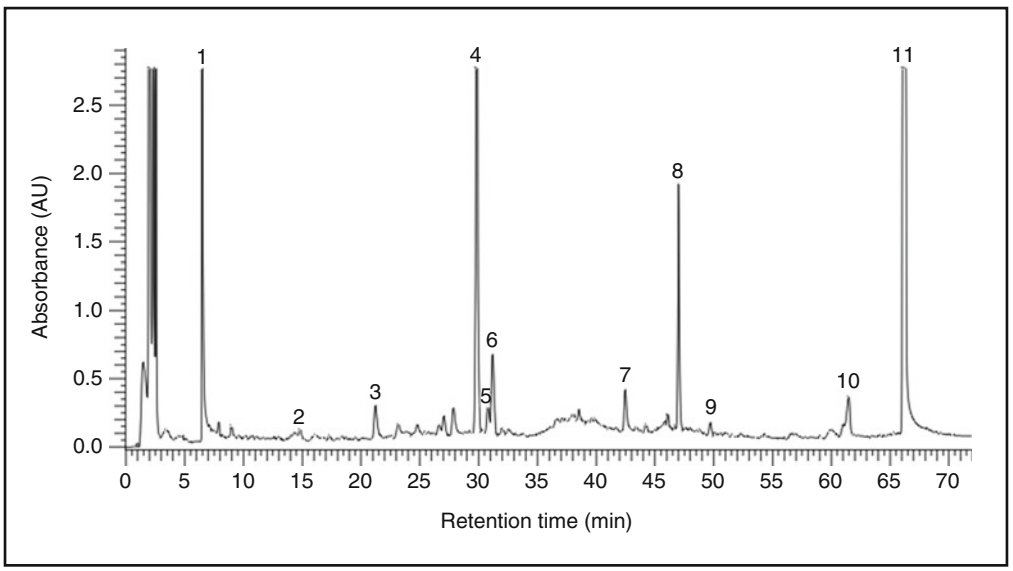


Fig. 4c: HPLC-fingerprint analysis of the methanol extract of Herba Oldenlandiae (sample 6)

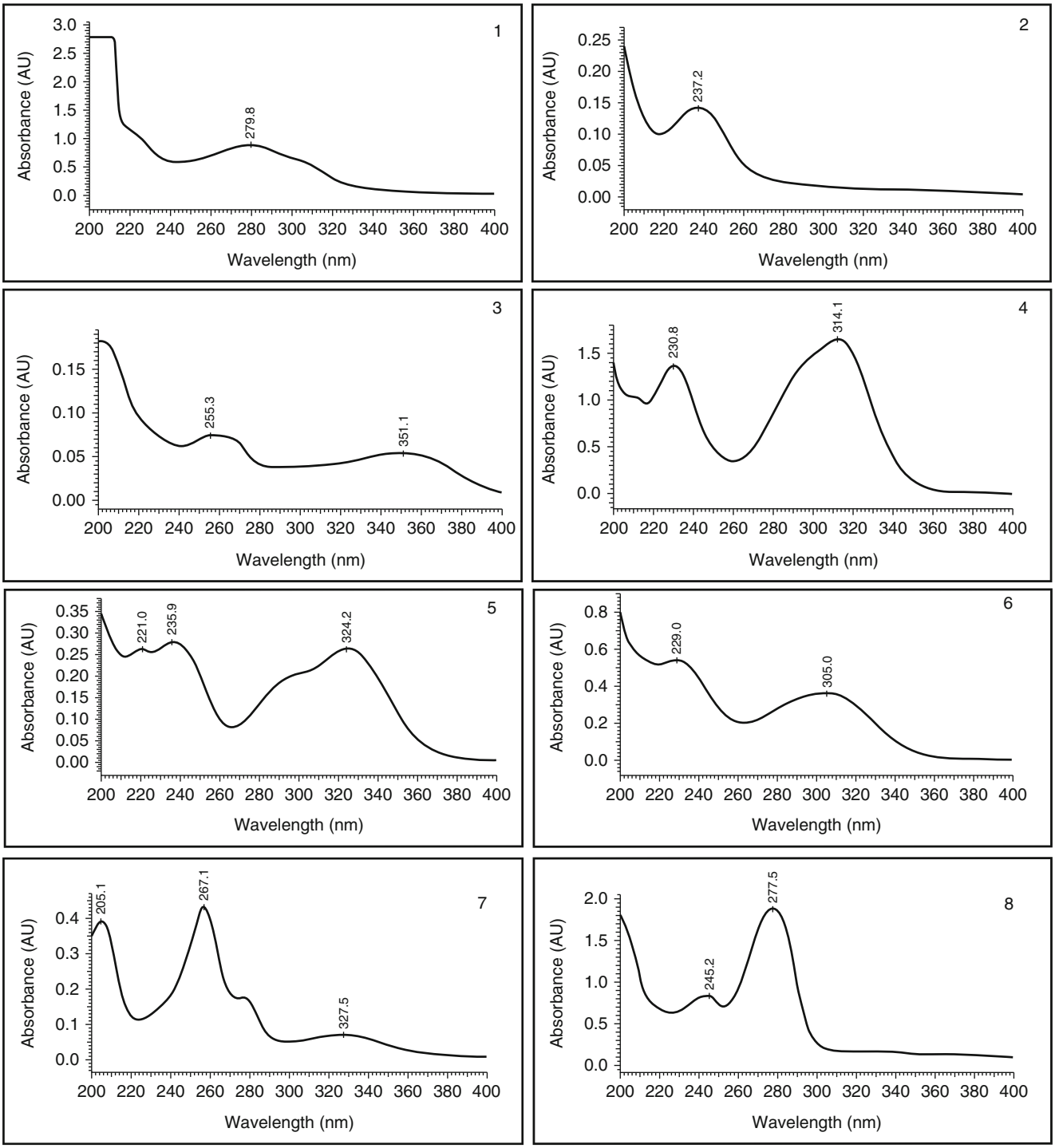


Fig. 5: On line UV-spectra of the detected peaks of Herba Oldenlandiae

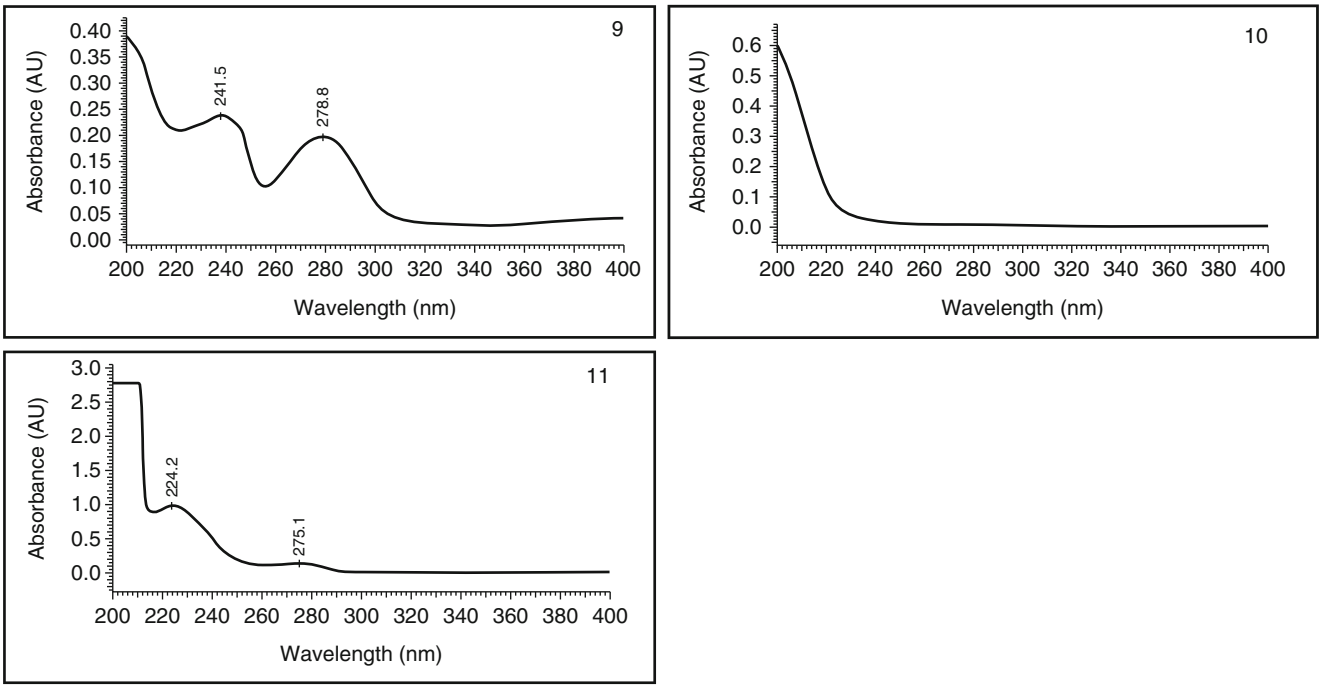


Fig. 5: (continued)

Conclusion

The available herbal drug samples of *Oldenlandia diffusa* showed in TLC and HPLC very homogeneous pattens of flavonoids-, diterpenoid- and triterpenoid markers. Therefore the authentication of official Oldenlandia herbal drug can be easily performed using the described analytical methods.

References

1. Hempen, C.H., Fischer, T.: A materia medica for chinese medicine (plants, minerals and animal products), 2nd edn. Churchill Livingstone/Elsevier, New York (2009)
2. Zhao, Z.Z.: An illustrated chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
3. Liang, Z., Jiang, Z., Ho, H., Zhao, Z.: Comparative Analysis of *Oldenlandia diffusa* and its substitutes by high performance liquid chromatographic fingerprint and mass spectrometric analysis. Planta Med. **73**(14), 1502–1508 (2007)
4. Nishihama, Y., Masuda, K., Yamaki, M., Takagi, S., Sakina, K.: Three new iridoid glucosides from *Hedyotidis diffusa*. Planta Med. **43**(1), 28–33 (1981)
5. Kim, D.H., Lee, H.J., Oh, Y.J., Kim, M.J., Kim, S.H., Jeong, T.S., Baek, N.I.: Iridoid glycosides isolated from *Oldenlandia diffusa* inhibit LDL-oxidation. Arch. Pharm. Res. **28**(10), 1156–1160 (2005)
6. Wu, P.K., Chi Shing Tai, W., Liang, Z.T., Zhao, Z.Z., Hsiao, W.L.: Oleanolic acid isolated from *Oldenlandia diffusa* exhibits a unique growth inhibitory effect against *ras*-transformed fibroblasts. Life Sci. **85**(3–4), 113–121 (2009)
7. Gupta, S., Zhang, D., Yi, J., Shao, J.: Anticancer activities of *Oldenlandia diffusa*. J. Herb. Pharmacother. **4**(1), 21–33 (2004)
8. Gu, G., Barone, I., Gelsomino, L., Giodarno, C., Bonofiglio, D., Statti, G., Menichini, F., Catalano, S., Andó, S.: *Oldenlandia diffusa* extracts exert antiproliferative and apoptotic effects on human breast cancer cells through ERα/Sp1-mediated p53 activation. J. Cell. Physiol. **227**(10), 3363–3372 (2012)
9. Beinfield, H., Korngold, E.: Chinese medicine and cancer care. Altern. Ther. Health Med. **9**(5), 38–52 (2003)

10. Liang, Z., He, M., Fong, W., Jiang, Z., Zhao, Z.: A comparable, chemical and pharmacological analysis of the traditional chinese medicinal herbs *Oldenlandia diffusa* and *O. corymbosa* and a new valuation of their biological potential. Phytomedicine **15**(4), 259–267 (2008)
11. Ding, B., Ma, W.W., Dai, Y., Gao, H., Yu, Y., Tao, Y., Zhong, Y., Yao, X.S.: Biologically active iridoids from *Hedyotis diffusa*. Helv. Chim. Acta **93**(12), 2488–2494 (2010)
12. Liang, Z.T., Jiang, Z.H., Leung, K.S., Zhao, Z.Z.: Determination of iridoid glucosides for quality assessment of herba oldenlandiae by high performance liquid chromatography. Chem. Pharm. Bull. **54**(8), 1131–1137 (2006)
13. Li, M., Jiang, R.W., Hon, P.M., Cheng, L., Li, L.L., Zhou, J.R., Shaw, P.C., But, P.P.H.: Authentication of the anti-tumor herb Baihuasheshecao with bioactive marker compounds and molecular sequences. Food Chem. **119**(3), 1239–1245 (2010)
14. Jiao, S.D.: Yong Yao Xin de Shi Jiang – clinical chinese medicine series – miscellaneous medicinals (trans: Wiseman, N., Mitchell, C.). Paradigm Publications, Brookline, Mass (2003)
15. Liang, Z.T., Jiang, Z.H., Leung, K.S., Peng, Y., Zhao, Z.Z.: Distinguishing the medicinal herb *Oldenlandia diffusa* from similar species of the same genus using fluorescence microscopy. Microsc. Res. Tech. **69**(4), 277–282 (2006)
16. Zhang, Y., Chen, Y., Fan, C., Ye, W., Luo, J.: Two new iridoid glucosides from *Hedyotis diffusa*. Fitoterapia **81**(6), 515–517 (2010)
17. Zhou, Y.J., Wu, K.S., Zeng, G.Y., Tan, J.B., Xu, K.P., Li, F.S., Tan, G.S.: Studies on constituents of *Oldenlandia diffusa*. Zhongguo Zhong Yao Za Zhi **32**(7), 590–593 (2007)
18. Yang, T., Yang, Y.H., Yang, J.Y., Chen, B.M., Duan, J.P., Yu, S.Y., Ouyang, H.T., Cheng, J.P., Chen, Y.X.: Fingerprint of *Hedyotis diffusa* Willd. by HPLC-MS. Phytochem. Anal. **19**(6), 487–492 (2008)
19. Lu, C.M., Yang, J.J., Wang, P.Y., Lin, C.C.: A new acylated flavonol glycoside and antioxidant effects of *Hedyotis diffusa*. Planta Med. **66**(4), 374–377 (2000)
20. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, 2nd edn. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim (2011)

Fructus Siraitiae/Momordicae – *Luohanguo*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] The former "Grosvenor Momordica" fruit is the dried fruit of *Siraitia grosvenorii* (Swingle) C. Jeffrey ex A. M. Lu et Z. Y Zhang (Fam. Cucurbitaceae).

The drug is collected in autumn when the fruit turns from pale green to deep green, dried in the air for several days and dried at a low temperature.

Origin:[2] Mainly in Guangxi provinces in China.

Description of the drug:[1] Ovoid, elliptical or spherical, 4.5–8.5 cm long, 3.5–6 cm in diameter. Externally brown, yellowish-brown or greenish-brown, marked with dark-coloured patches and covered with yellow fine hairs, some exhibiting 6–11 longitudinal lines. Apex with a style scar, and base with a fruit stalk scar. Texture light, fragile, pericarp thin, easily broken. Sarcocarp spongiform, pale brown. Seeds oblate, abundant, about 1.5 cm long and 1.2 cm wide, pale red to brownish-red, slightly dented between two surfaces, surrounded by radial furrows, and channelled at the edge. Odour, slight; taste, sweet.

Medicinal use:[3] Used for treatment of cough, pharyngitis, asthma and also as laxative and sweetening agent.

Effects and indications of Fructus Siraitiae according to Traditional Chinese Medicine[1, 2, 4]

Taste:	Sweet
Temperature:	Cool
Channels entered:	*Orbis pulmonalis, orbis intestini crassi*
Effects (functions):	To clear heat and moisten the lung, soothe the throat to restore the voice, lubricate intestine to relax the bowels.
Symptoms and indications:	Lung heat and dryness cough, sore throat and loss of voice, constipation caused by intestinal dryness.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_17, © Springer International Publishing Switzerland 2015

Main constituents: **<u>Triterpenes</u> (Cucurbitane type)**[3, 5]

Mogrosides V, Mogroside IV A, Mogroside IV E, mogroside III, mogroside III-E, mogroside II-E, Mogroside I A_1, Mogroside I E_1, siamenoside I, 11-oxo-mogroside I A_1, 11-Oxo-mogroside 1 E_1, 11-oxo-mogroside V, 5-dehydrokarounidiol-dibenzoate, karounidiol-dibenzoate, karounidiol-3-benzoate, 5α,6α-epoxymogroside I E_1, isomultiflorenol, β-amyrin, 10α-cucurbitadienol

<u>Flavonol glycosides</u>[3]

Kaempferol-3,7-O-di-α-L-rhamnopyranoside, grosvenorin (kaempferol-3-O-α-L-rhamnopyranosyl-7-O-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside)

Minor constituents: β-Sitosterin, camposterin, volatile oil

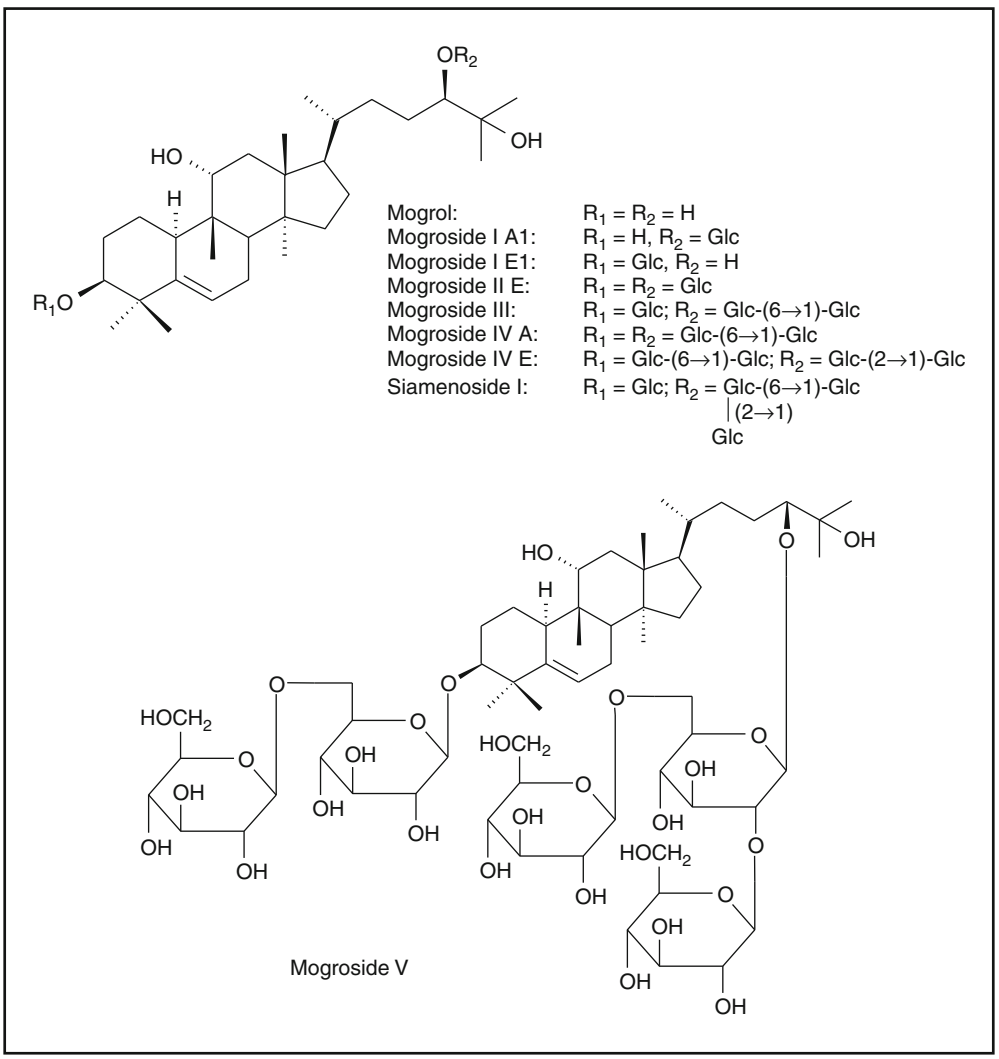


Fig. 1: Formulae of the main constituents of Fructus Siraitiae [3, 5]

Pharmacology: Immunmodulating activity[3]
Antiinflammatory activity[3, 6]
Antioxidant effect[7]
Antistress effect[7]
Anticarcinogenic activity[8]
Antidiabetic/insulin secreting activity[9]

TLC Fingerprint Analysis

Drug samples		Origin
1	Fructus Siraitiae/*Siraitia grosvenorii*	District Lingui, Province Guangxi (China)
2	Fructus Siraitiae/*Siraitia grosvenorii*	District Yongfu, Province Guangxi (China)
3	Fructus Siraitiae/*Siraitia grosvenorii*	Province Guangxi (China)

1. **TLC-fingerprint analysis of Triterpenes and Flavones:**[1, 4]

Reference compounds of Fig. 2		R*f*
T 1	Mogroside V	0.28
T 2	Kaempferol-3,7-dirhamnoside	0.64

1. Extraction: 2 g powdered drug are extracted with 20 ml ethanol 50 % under reflux for 30 min. The extract is cooled, filtrated and evaporated to about 5 ml. The residue is extracted further by shaking with two quantities of *n*-butanol (10 and 5 ml). The *n*-butanol extracts are combined and evaporated to dryness under reduced pressure. The residue is dissolved in 1.5 ml methanol.

2. Reference compounds: 1 mg is dissolved in 1 ml methanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Fructus Siraitiae extracts: 5 µl each
Reference compounds: 5 µl each

Solvent system: *n*-butanol + ethanol + water (40 + 10 + 15)

Detection: Vanillin – Sulphuric acid
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 min at 105 °C and evaluated in VIS.

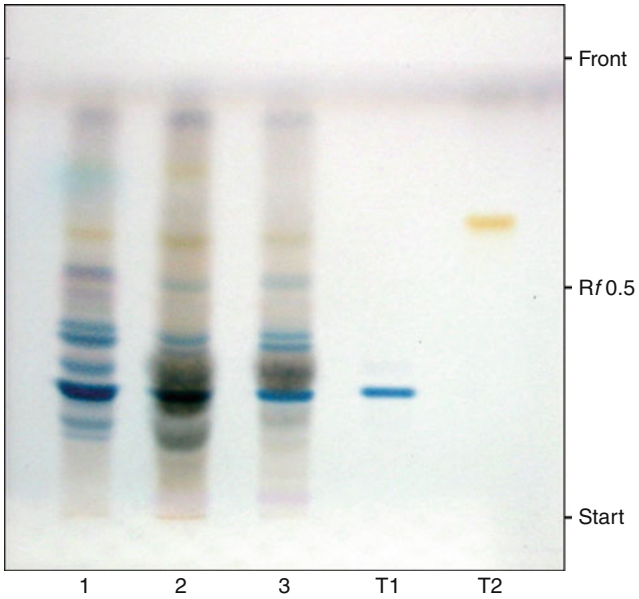


Fig. 2: Thin layer chromatogram of the 50 % ethanol/butanol extracts of Fructus Siraitiae, sprayed with Vanillin – Sulphuric acid reagent (VIS)

4. Description of the Fig. 2:

   The Fructus Siraitiae 50 % ethanol/butanol extract samples 1, 2 and 3 show in R*f*-range of R*f* 0.2 up to R*f* 0.55 6–7 dark green/blue zones with the dominant zone of mogroside V (**T1**) at R*f* = 0.28. The zones above mogroside V with increasing R*f*-values can be assigned to mogroside IV and mogroside I according to the decreasing number of sugar moieties.

HPLC-Fingerprint Analysis

1. Sample preparation: The 50 % ethanol/butanol extract, used for TLC, is diluted 1:10 with methanol, filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-aparatus.

2. Injection volume: Fructus Siraitiae extracts: 15 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® 125–4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4–4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 0.001 % H_3PO_4 in water (Millipore Ultra Clear UV plus®) B: acetonitril (VWR)

Gradient:	2 % B for 5 min, 2–30 % B in 25 min, 30–95 % B in 15 min, 95 % B for 10 min, Total run time: 55 min
Flow rate:	1 ml/min
Detection:	203 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	18.6	Kaempferol-3,7-dirhamnoside
2	23.5	Mogroside V

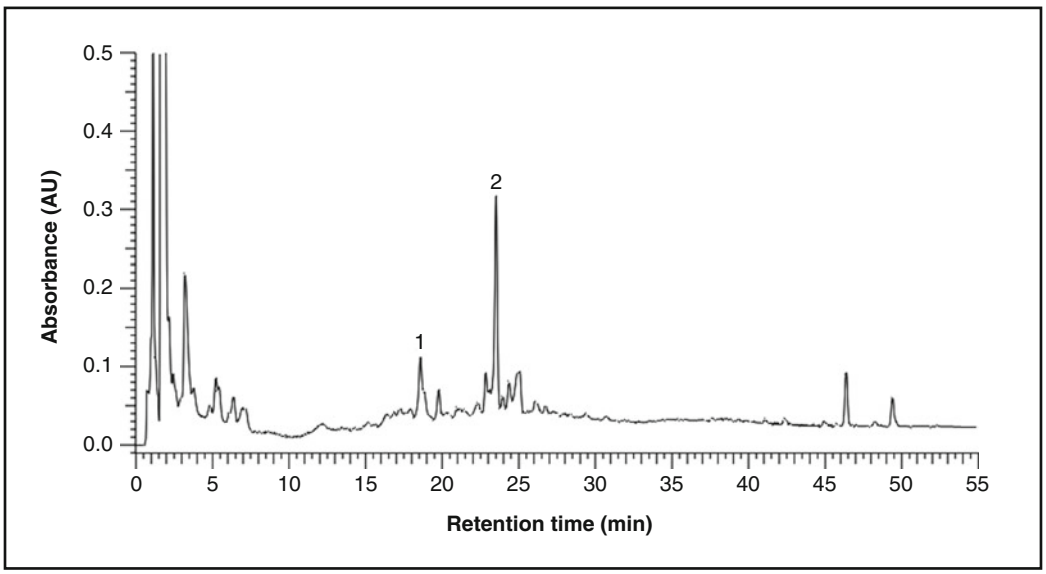


Fig. 3a: HPLC-fingerprint analysis of the 50 % ethanol/butanol extract of Fructus Siraitiae, sample 1

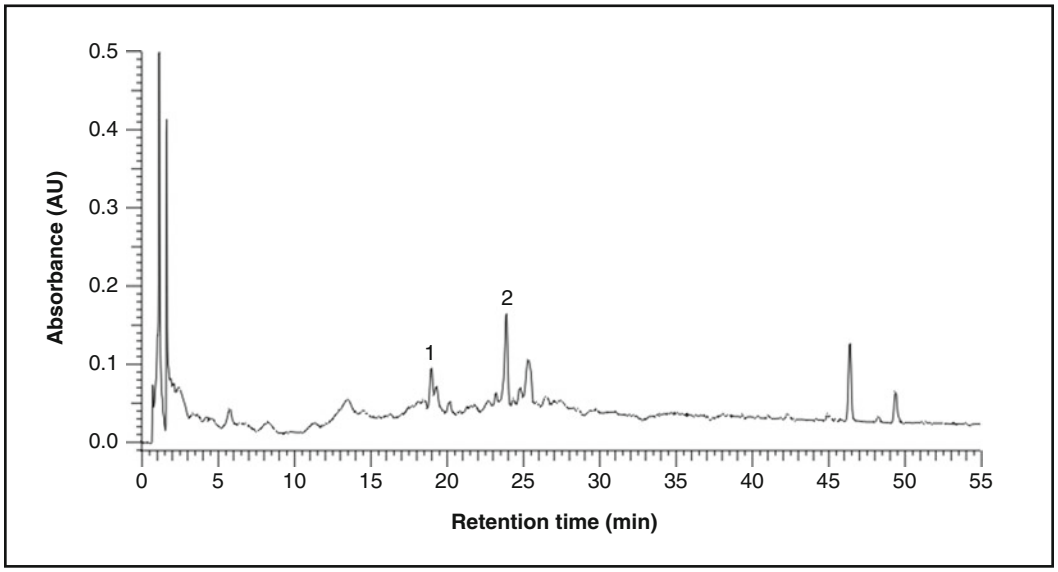


Fig. 3b: HPLC-fingerprint analysis of the 50 % ethanol/butanol extract of Fructus Siraitiae, sample 3

4. Description of Figs. 3a and 3b

The Fructus Siraitiae 50 % ethanol/butanol extacts show a characteristic peak pattern in the range of Rt 15–30 min with the dominant mogroside V peak **2** at Rt = 23.5 and a second prominent peak at Rt = 18.6 assignable to kaempferol-3,7-dirhamnoside (**1**).

The other minor peaks right and left of mogroside V according to the UV-spectra can be assigned to triterpenes or flavone glycosides, respectively.

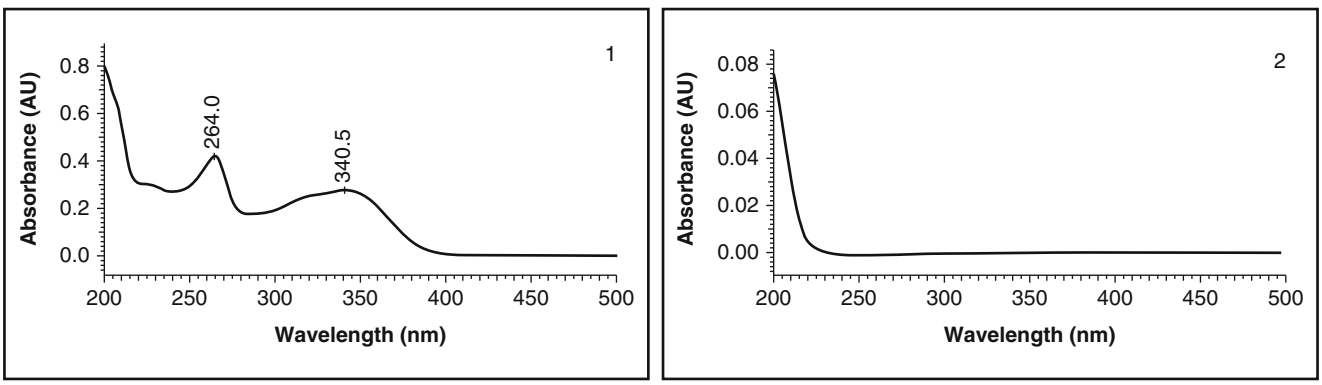


Fig. 4: On line UV-spectra of the main constituents of Fructus Siraitiae

Note: Fructus Siraitiae contains not less than 0.50 % of mogroside V, calculated with reference to the dried drug [1].

Conclusion

The quality proof (botanical authentication) of Siraitiae fructus was concentrated primarily on the HPTLC- and HPLC-detection of the cucurbitacin saponins (mogrosides) with the dominant mogroside V. These glycosides possess comprehensive pharmacological activites (see [5–9]).

References

1. Pharmacopoeia of the people's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Zhao, Z.Z.: An illustrated chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
3. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)
4. Stöger, E.A.: Arzneibuch der chinesischen Medizin. Deutscher Apotheker Verlag, Stuttgart (2009)
5. Ukiya, M., Akihisa, T., Tokuda, H., Toriumi, M., Mukainaka, T., Banno, N., Kimura, Y., Hasegawa, J., Nishino, H.: Inhibitory effects of cucurbitane glycosides and other triterpenoids from the fruit of Momordica grosvenori on Epstein-barr virus early antigen induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate. J. Agric. Food Chem. **50**(23), 6710–6715 (2002)
6. Di, R., Huang, M.T., Ho, C.T.: Antiinflammatory activities of mogrosides from Momordica grosvenori in murine macrophages and a murine ear edema model. J. Agric. Food Chem. **59**(13), 7474–7481 (2011)
7. Wang, X.Y., Liu, J.K., Zhao, Y., Sanada, F.M., Okada, S., Mori, A.: The antioxidant and antistress activities of the extract of Fructus Momordica. In: Packer, L.S., Traber, M.G.S., Xin, W.J.S. (eds.) Proceedings international symposium on natural antioxidants: molecular mechanosms and health effects. AOCS Press, Champaign (1996)
8. Taksaki, M., Konoshima, T., Murata, Y., Sugiura, M., Nishino, H., Tokuda, H., Matsumoto, K., Kasai, R., Yamasaki, K.: Anticarcinogenic activity of natural sweeteners, cucurbitane glycosides from Momordica grosvenori. Cancer Lett. **198**(1), 37–42 (2003)
9. Song, F., Chen, W., Jia, W., Yao, P., Nussler, A.K., Sun, X., Liu, L.: A natural sweetener, Momordica grosvenori, attenuates the imbalance of cellular immune functions in alloxan-induced diabetic mice. Phytother. Res. **20**(7), 552–560 (2006)

Radix Morindae officinalis – *Bajitian*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Morinda Root is the dried root of *Morinda officinalis* How (Fam. Rubiaceae).

The drug is collected throughout the year, removed from rootlet, dried in the sun partially, beaten gently to be compressed, and then dried in the sun.

Other formally used plants:[2] *Polygala reinii* Franchet et Savatier (Fam. Polygalaceae), *Bacopa monniera* Wetttstein (Fam. Scrophulariaceae), *Damnacanthus indicus* Gaertner var. *gigantean* Nakai (Fam. Rubiaceae).

Origin:[3] Mainly in Chinese provinces such as Guangdong, Guangxi and Fujian.

Description of the drug:[1] Compressed-cylindrical, somewhat curved, varying in length, 0.5–2 cm in diameter. Externally greyish-yellow or dark grey, with longitudinal wrinkles and transverse cracks, some bark transversely broken and wood exposed. Texture tough, fracture bark thick, purple or yellowish-brown or yellowish-white, 1–5 mm in diameter. Odour, slight; taste, sweetish and slightly astringent.

Pretreatment of the raw drug:[1] Foreign matters are eliminated and dried in the sun.

Medicinal use:[4] Used for treatment of rheumatoid arthritis, the metabolic syndrome, impotence, infertility and menstrual disorders.

Effects and indications of Radix Morindae officinalis according to Traditional Chinese Medicine [1, 5]

Taste:	Mild warm; sweet and pungent
Temperature:	Neutral with warm tendency
Channels entered:	*Orbis renalis, orbis hepaticus*
Effects (functions):	To tonify the kidney yang, strengthen sinew and bone, dispel wind-dampness.
Symptoms and indications:	Impotence and seminal emission, infertility caused by uterine coldness, menstrual irregularities, cold pain in the lower abdomen, painful impediment caused by wind-dampness, limp wilting sinew and bone.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_18, © Springer International Publishing Switzerland 2015

Reported constituents:

- **Anthraquinones:**[4, 6–10]

Rubiadin, rubiadin-1-methyl ether, 1-hydroxyanthraquinone, 1-hydroxy-2-methylanthraquinone, 1,6-dihydroxy-2,4-dimethoxyanthraquinone, 1,6-dihydroxy-2-methoxyanthraquinone, 1-hydroxy-2-methoxyanthraquinone, physcion, 2-methyl-anthraquinone, 1-hydroxy-2-hydroxymethyl-anthraquinone, 1,3-dihydroxy-2-methoxy-anthraquinone, 1,4-dimethoxy-2-hydroxy-anthraquinone, 1,4-dihydroxy-2-methyl-anthraquinon, 1-methoxy-2-hydroxy-anthraquinone, alizarin-1-methylether, Lucidin-ω-methylether, 1-hydroxy,2,3-dimethyl-anthraquinone, 1-hydroxy-3-hydroxymethyl-anthraquinone, 3-hydroxy-1,2-dimethoxy-anthraquinone, 2-hydroxy-1-methoxy-anthraquinone, 1,2-dihydroxy-3-methyl-anthraquinone, 1,3,8-trihydroxy-2-methoxy-anthraquinone, 2-hydroxymethyl-3-hydroxy-anthraquinone, 2-methoxy-anthraquinone, alizarin-2-methylether, 1,2-dimethoxyanthraquinone

- **Iridoids (Diterpenoids):**[4, 8]

Glucosides:

Asperuloside, monotropein, asperuloside tetraacetate, morofficinaloside, asperulosidic acid, desacetyl-asperulosidic acid

Lactone:

Morindolide

- **Terpenes:**[8]

Monoterpenglucoside:

L-borneol-6-O-ß-D-apiosyl-ß-D-glucoside

Triterpene:

Rotungenic acid

- **Phytosterols:**[4, 8]

β-Sitosterol, Oxositosterol

- **Saccharides:**[4, 11–13]

Oligosaccharides:

Nystose, fructofuranosylnystose, inulin-type hexasaccharide and heptasaccharide, sucrose, inulin-type trisaccharide, inulotriose, inulotetrose, inulopentose,1-kestose

Monosaccharides:

Arabinose, galactose, galacturonic acid

Acidic polysaccharides

- **Others:**[4, 6, 8, 10]

Succinic acid, 24-ethylcholesterol, (4R,5S)-5-hydroxyhexan-4-olide, scopoletin

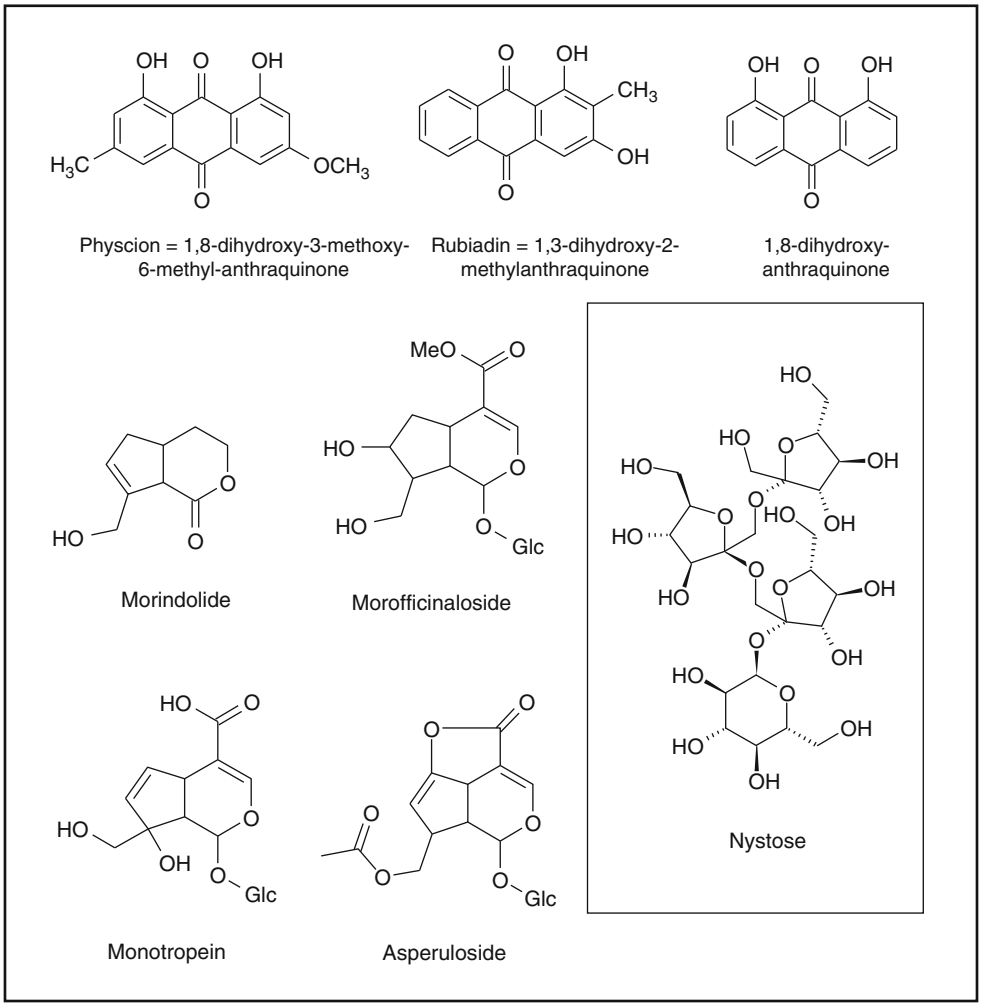


Fig. 1: Formulae of the main constituents of Radix Morindae officinalis

Reported Pharmacological Activities

In vitro and in vivo effects:

- antinociceptive[14, 15]
- anti-inflammatory[14, 15]
- induction of apoptosis[16]
- promotion of angiogenesis[17]

- antioxidant[13, 18]
- antiosteoporotic[19]
- antineurotoxic[20]
- antistress[21]
- enhancement of adipocyte differentiation[22]

TLC-Fingerprint Analysis

Drug samples		Origin
1	Radix Morindae officinalis/*Morinda officinalis*	Gaolian, Deqing District, Province Guangdong (China)
2	Radix Morindae officinalis/*Morinda officinalis*	Gaolian, Deqing District, Province Guangdong (China)
3	Radix Morindae officinalis/*Morinda officinalis*	Locality Guangxi (China)
4	Radix Morindae officinalis/*Morinda officinalis*	Province Guangdong (China)
5	Radix Morindae officinalis/*Morinda officinalis*	Province Guangdong (China)

1. **TLC-fingerprint analysis of anthraquinones and scopoletin:** [1]

Reference compounds of Figs. 2a and 2b		***Rf***
T1	Rubiadin	0.64
T2	1,8-dihydroxy-anthraquinone	0.86
T3	Physcion	0.86
T4	Scopoletin	0.19

---

1. Extraction: 2.5 g of the powdered drug are extracted with 25 ml ethanol under reflux for 1 h. The extract is cooled, filtrated and evaporated to about 1 ml.
2. Reference compounds: Each 1.0 mg is dissolved in 1.0 ml methanol
3. Separation parameters:

   Plate: HPTLC Silica gel 60 F_{254}, Merck

   Applied amounts: Radix Morindae officinalis extracts: 10 µl each
   Reference compounds: 10 µl each

   Solvent system: Toluene + ethyl acetate + formic acid (40 + 10 + 0.5)

   Detection: (a) UV 366 nm (Fig. 2a)

   (b) 5 % sodium hydroxide solution in ethanol (Fig. 2b):

   The plate is sprayed with this solution and the evaluation is carried out in VIS.

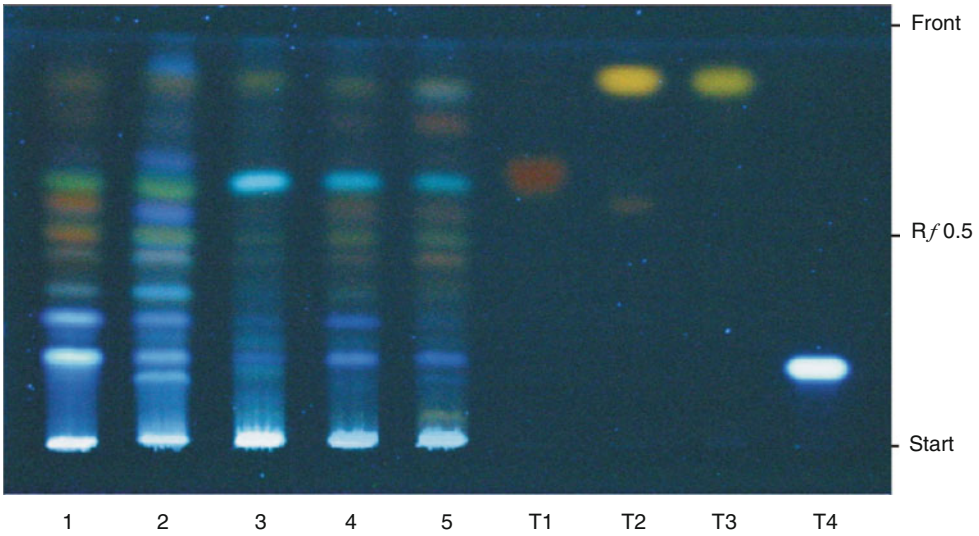


Fig. 2a: Thin layer chromatogram of the ethanol extracts of Radix Morindae officinalis (UV 366 nm)

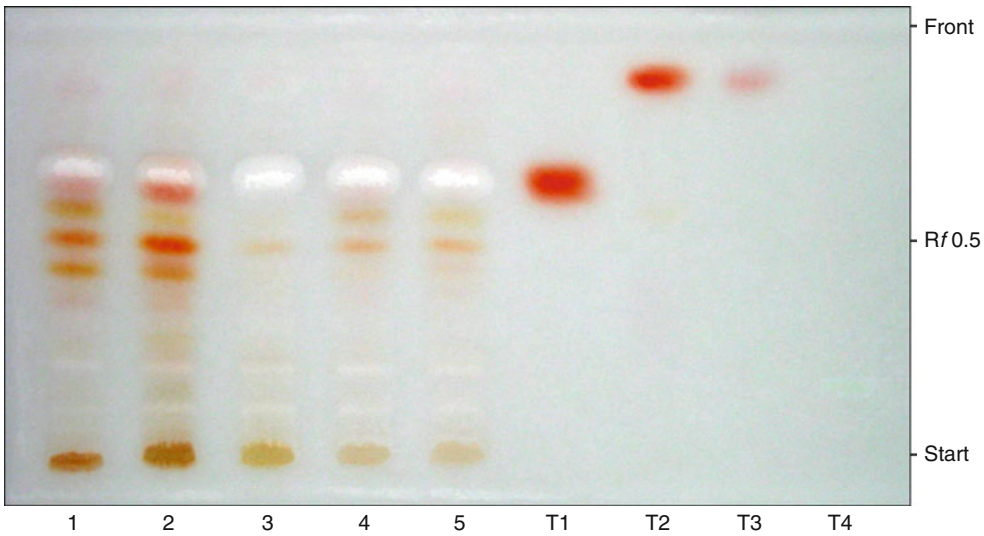


Fig. 2b: Thin layer chromatogram of the ethanol extracts of Radix Morindae officinalis, sprayed with sodium hydroxide solution (VIS)

4. Description of Figs. 2a and 2b:

   Figure 2a (UV 366 nm):

   The Morinda extract samples 1–5 show a relatively homogenous blue, green and orange/brown fluorescent zone pattern in the R*f*-range from start up to the front. The weak orange/brown zones derive from anthraquinones (**T1**, **T2**, **T3**) whereas the blue/green and white appearing zones can be assigned to iridoid-compounds, and cumarins (e.g. scopoletin, **T4**).

   Figure 2b (VIS):

   In this TLC only the orange-red coloured anthraquinones (glycosides) on the start and in the R*f*-range of ~0.45 up to R*f* = 0.65 are detectable.

2. **TLC-fingerprint analysis of oligosaccharides and iridoids:**[23]

Reference compounds of Fig. 3		R*f*
T 5	Nystose	0.40
T 6	Monotropein	0.75
T 7	Asperuloside	0.88

1. Extraction: To 0.5 g powdered drug 20 ml methanol are added and ultrasonicated for 15 min. The extract is filtrated.
2. Reference compounds: Nystose: 0.5 mg is dissolved in 5 ml methanol
   Monotropein, asperuloside: each 1.0 mg is dissolved in 1 ml methanol
3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Radix Morindae officinalis: 10 µl each
Reference compounds: Nystose: 15 µl; Monotropein, Asperuloside: each 10 µl

Solvent system: Ethyl acetate + water + formic acid + glacial acetic acid (30 + 15 + 10 + 10)

Detection: 11 ml of conc. sulphuric acid is added to 89 ml ethanol. 2.75 g naphthol is dissolved and the solution is added with 7 ml water. This reagent has to be prepared freshly.
The plate is sprayed with this solution, heated for 5 min at 105 °C and evaluated in VIS.

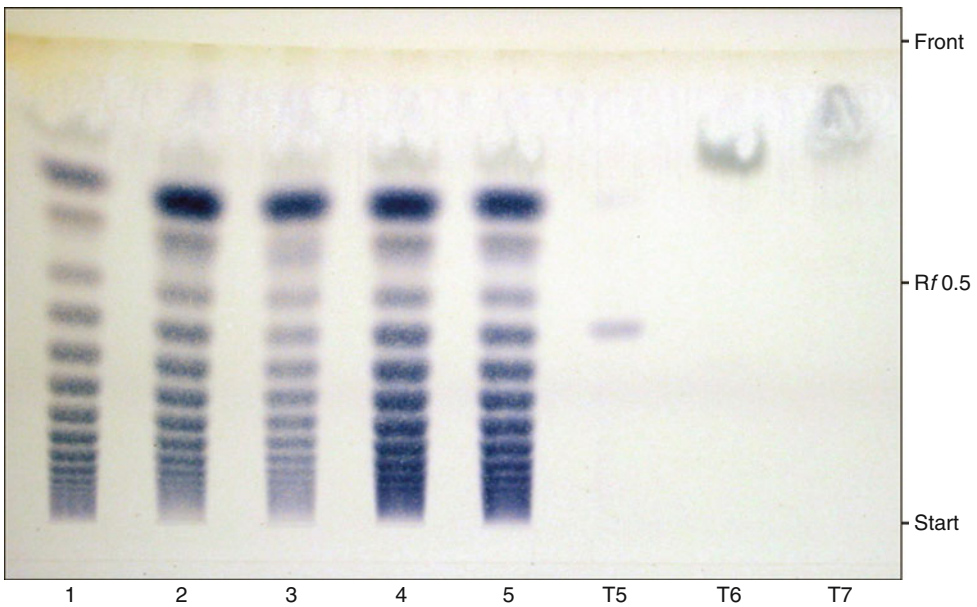


Fig. 3: Thin layer chromatogram of the methanol extract of Radix Morindae officinalis, sprayed with ethanolic sulphuric acid (VIS)

4. Description of Fig. 3:

   This chromatogram, developed with a special solvent system and detected with ethanolic sulphuric acid, shows in the R*f*-range from start up to R*f* = 0.70 the 6–7 oligosaccharides with violet colour. The tetra-saccharide nystose (**T5**) has the R*f*-value ~0.40, monotropein (**T6**) and asperuloside (**T7**) appear at R*f* = 0.75 and 0.88, respectively (hardly to detectable).

HPLC-Fingerprint Analysis

1. Sample preparation: The ethanol extract, used for the HPTLC of anthraquinones and scopoletin, is filtered through Millipore® (Type HV 0.45 µm) and injected into the HPLC-aparatus.
2. Injection volume: Radix Morindae officinalis extracts: 10 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 system:	A: 0.001 % H_3PO_4 in water (Millipore Ultra Clear UV plus®) B: acetonitril (VWR)
Gradient:	0–10 % B in 10 min, 10–40 % B in 15 min, 40 % B for 5 min, 40–95 % B in 15 min, 95 % B for 10 min, Total run time: 55 min
Flow:	1.0 ml/min
Detection:	280 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	6.2	Monotropein
2	13.2	Asperuloside
3	17.1	Scopoletin
4	29.8	Anthraquinone
5	30.5–39.0	Anthraquinones
6	40.0	Rubiadin

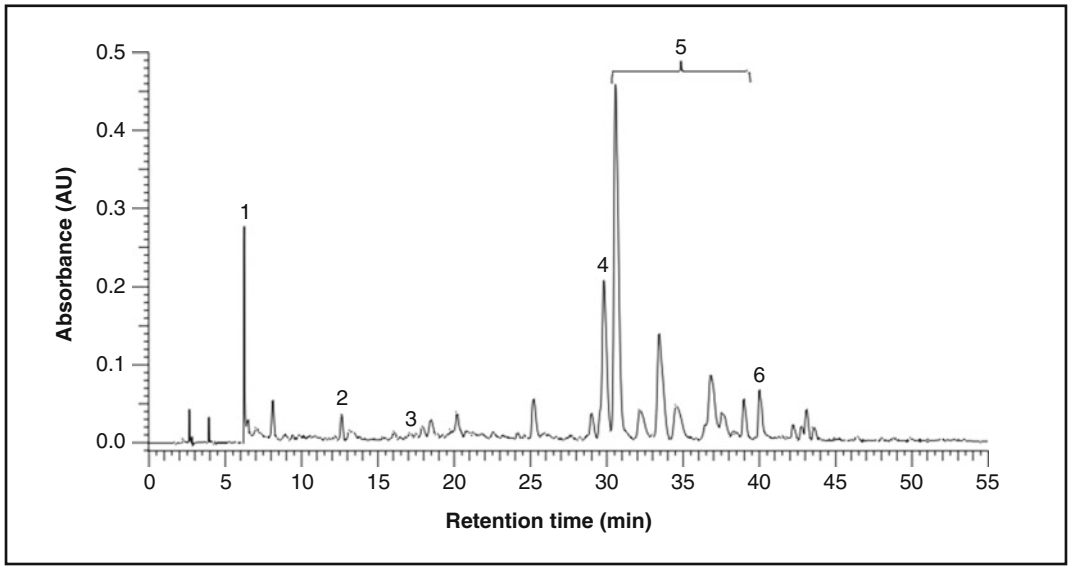


Fig. 4a: HPLC-fingerprint analysis of the ethanol extract of Radix Morindae officinalis sample 1

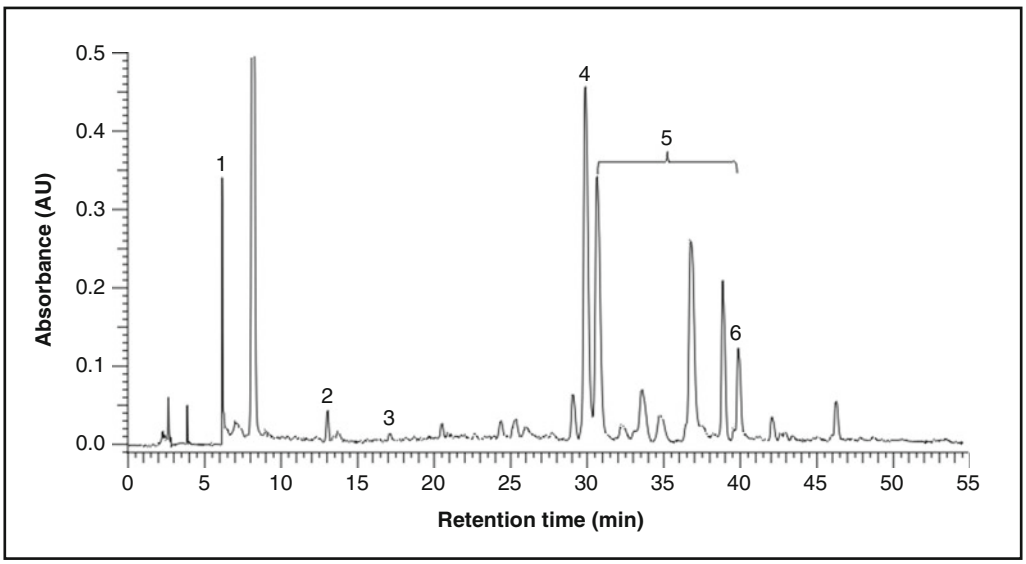


Fig. 4b: HPLC-fingerprint analysis of the ethanol extract of Radix Morindae officinalis sample 2

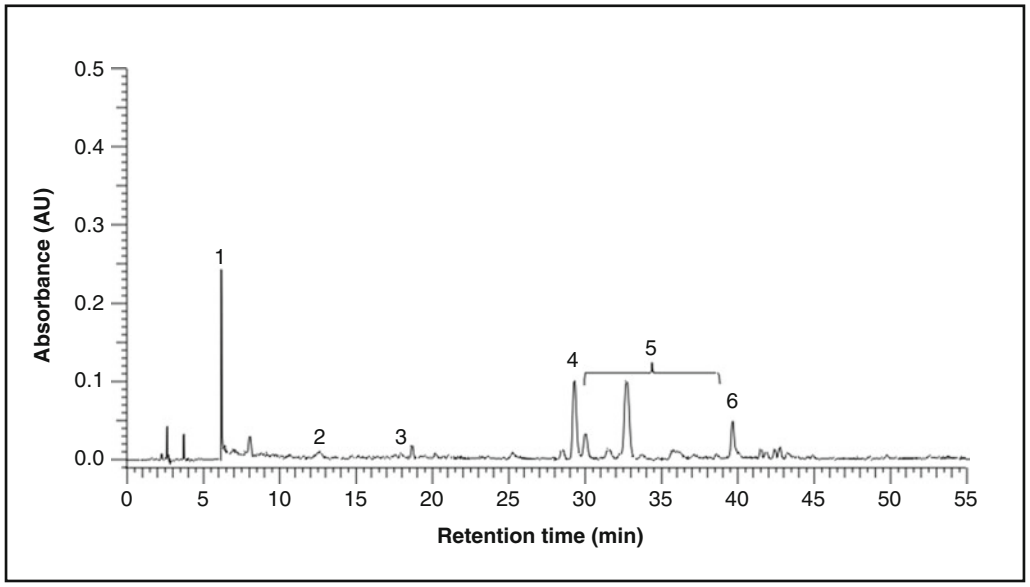


Fig. 4c: HPLC-fingerprint analysis of the ethanol extract of Radix Morindae officinalis sample 5

4. Description of the HPLC-figures:

   The HPLC of extract samples of Fig. 4a (sample 1) and Fig. 4b (sample 2) show a nearly equal peak profile with monotropein (**1**, Rt = 6.2 min), asperuloside (**2**, Rt = 13.2), scopoletin (**3**, Rt = 17.1), various anthraquinones (**4**, Rt = 29.8 and peaks in the Rt-range **5**, Rt = 30.5–39.0) and rubiadin (**6**, Rt = 40.0).

   Extract sample of Fig. 4c (sample 5) shows the same peak profile as in Figs. 4a and 4b, however, in a weaker peak concentration. These different peak profiles correspond with the HPTLC-profile of this extract sample (see Figs. 2a and 2b).

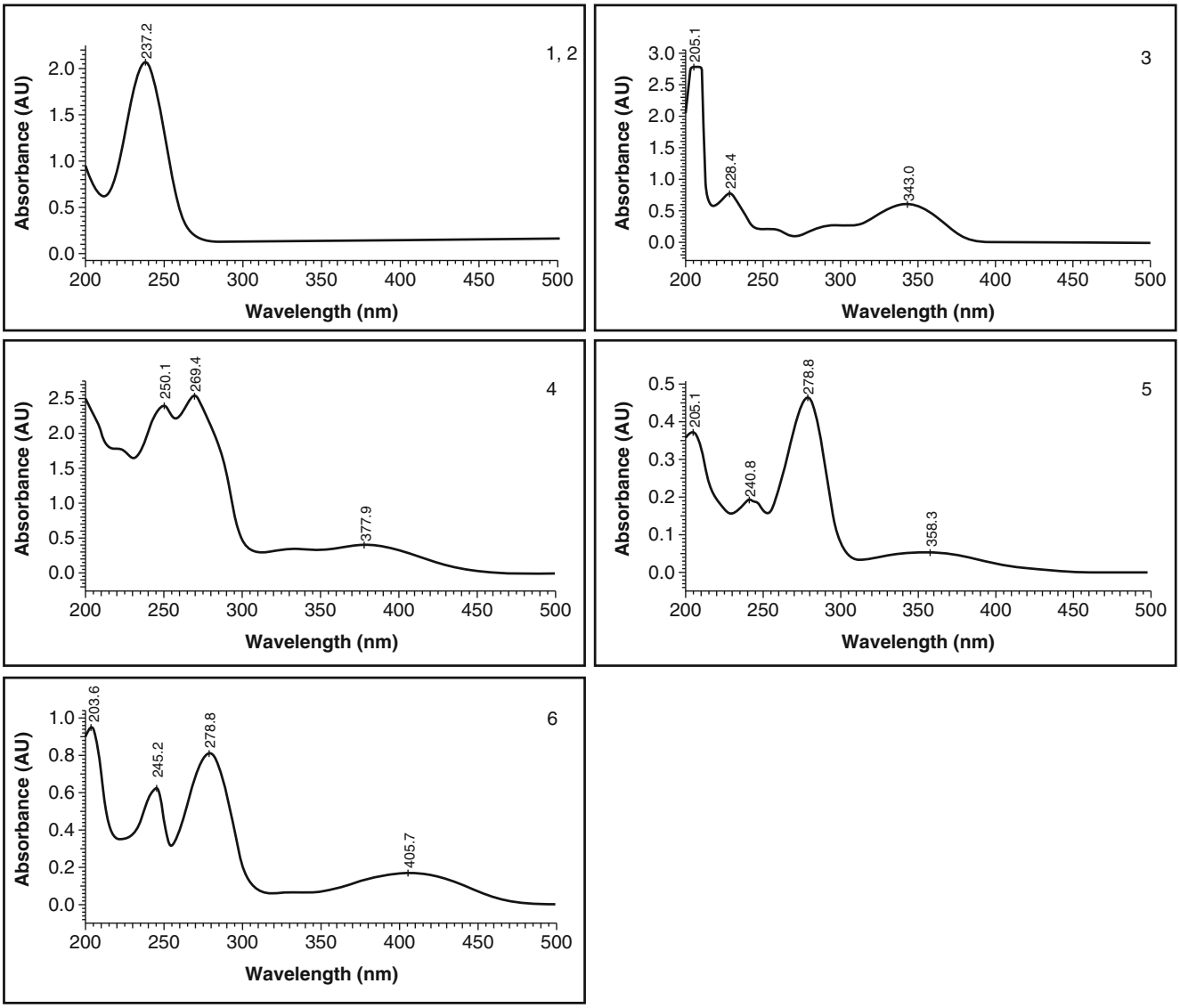


Fig. 5: On line UV-spectra of the main peaks of Radix Morindae officinalis ethanol extracts

Note: According to the Chinese Pharmacopoeia Radix Morindae officinalis contains not less than 2.0 % of nystose, calculated with reference to the dried drug. [1]

Conclusion

The HPTLC and HPLC profiles of the various Radix Morindae officinalis extract samples provide the authentication of the herbal drug of different Chinese origins. The composition of constituents, however, varies in dependence of other not defined reasons such as different cultivation or climatic conditions, time of collection and preservation method.

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
3. Zhao, Z.Z.: An illustrated chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
4. Tang, W., Eisenbrand, G.: Handbook of chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)
5. Stöger, E.A.: Arzneibuch der chinesischen Medizin. Deutscher Apotheker Verlag, Stuttgart (2005)
6. Li, S., Ouyang, Q., Tan, X., Shi, S., Yao, Z.: Chemical constituents of Morinda officinalis How. Zhongguo. Zhong. Yao. Za. Zhi. **16**(11), 675–676 (1991)
7. Yang, Y.J., Shu, H.Y., Min, Z.D.: Anthraquinones isolated from Morinda officinalis and Damnacanthus indicus. Yao Xue Xue Bao **27**(5), 358–364 (1992)
8. Yoshikawa, M., Yamaguchi, S., Nishisaka, H., Yamahara, J., Murakami, N.: Chemical constituents of Chinese natural medicine, Morindae radix, the dried roots of Morinda officinalis How: structures of morindolide and morofficinaloside. Chem. Pharm. Bull. **43**(9), 1462–1465 (1995)
9. Xu, Y.J., Yang, X.X., Zhao, H.B.: 3-Hydroxy-1,2-dimethoxy-anthraquinone. Acta. Crystallogr. Sect. E. Struct. Rep. Online. **65**(Pt 7), o1524 (2009)
10. Wu, Y.B., Zheng, C.J., Qin, L.P., Sun, L.N., Han, T., Jiao, L., Zhang, Q.Y., Wu, J.Z.: Antiosteoprotic activity of anthraquinones from Morinda officinalis on osteoblasts and osteoclasts. Molecules **14**(1), 573–583 (2009)
11. Feng, F., Wang, L.L., Lai, X.P., Li, Y.B., Cao, Z.M., Zhou, Y.J.: Study on oligosaccharides from Morinda officinalis. Zhong Yao Cai **35**(8), 1259–1262 (2012)
12. Deng, S.D., Xiao, F.X., Lin, L., Zhang, P., Lin, J.R., Zhang, S.B.: HILIC-eLSD determination of five oligosaccharides contained in Morinda officinalis. Zhongguo Zhong Yao Za Zhi **37**(22), 3446–3450 (2012)
13. Zhang, H., Li, J., Xia, J., Lin, S.: Antioxidant activity and physicochemical properties of an acidic polysaccharide from Morinda officinalis. Int. J. Biol. Macromol. **58**, 712 (2013)
14. Choi, J., Lee, K.T., Choi, M.Y., Nam, J.H., Jung, H.J., Park, S.K., Park, H.J.: Antinociceptive anti-inflammatory effect of monotropein isolated from the root of Morinda officinalis. Biol. Pharm. Bull. **28**(10), 1915–1918 (2005)
15. Kim, I.T., Park, H.J., Nam, J.H., Park, Y.M., Won, J.H., Choi, J., Choe, B.K., Lee, K.T.: In-vitro and in-vivo anti-inflammatory and antinociceptive effects of the methanol extract of the roots of Morinda officinalis. J. Pharm. Pharmacol. **57**(5), 607–615 (2005)
16. Li, Y.F., Gong, Z.H., Yang, M., Zhao, Y.M., Luo, Z.P.: Inhibition of the oligosaccharides extracted from Morinda officinalis, a Chinese traditional herbal medicine, on the corticosterone induced apoptosis in PC12 cells. Life Sci. **72**(8), 933–942 (2003)
17. Yang, J., Feng, G., Yu, S., Qiao, P.: Angiogenesis promoting effect of Morinda officinalis oligosaccharides on chicken embryo chorioallantoic membrane. Zhongguo Zhong Yao Za Zhi **35**(3), 360–363 (2010)
18. Soon, Y.Y., Tan, B.K.: Evaluation of the hypoglycemic and antioxidant activities of Morinda officinalis in streptozotocin-induced diabetic rats. Singapore Med. J. **43**(2), 77–85 (2002)
19. Li, N., Qin, L.P., Han, T., Wu, Y.B., Zhang, Q.Y., Zhang, H.: Inhibitiory effects of Morinda officinalis extract on bone loss in ovariectomized rats. Molecules **14**(6), 2049–2061 (2009)
20. Chen, D.L., Zhang, P., Lin, L., Shuai, O., Zhang, H.M., Liu, S.H., Wang, J.Y.: Protective effect of Bajijiasu against β–amyloid induced neurotoxicity in PC12 cells. Cell. Mol. Neurobiol. **33**(6), 837–850 (2013)
21. Li, Y.F., Yuan, L., Xu, Y.K., Yang, M., Zhao, Y.M., Luo, Z.P.: Antistress effect of oligosaccharides extracted from Morinda officinalis in mice and rats. Acta Pharmacol. Sin. **22**(12), 1084–1088 (2001)
22. Liu, Q., Kim, S.B., Ahn, J.H., Hwang, B.Y., Kim, S.Y., Lee, M.K.: Anthraquinones from Morinda officinalis roots enhance adipocyte differentiation in 3 T3-L1 cells. Nat. Prod. Res. **26**(18), 1750–1754 (2012)
23. Hong Kong chinese materia medica standards, vol 5. Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region, the People's Republic of China (2012)

Folium Apocyni veneti – *Luobumaya*

Pharmacopoeia:[1]	Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010
Official drug:[1]	Dogbane Leaf is the dried leaf of *Apocynum venetum* L. (Fam. Apocynaceae). The drug is collected in summer, removed from foreign matters and dried.
Synonym:[25]	*Trachomitum venetum* L. Woodson
Substitutes:[10, 31]	*Poacynum pictum* (Schrenk) Baill. and *Poacynum hendersonii* (Hook.f.) Woodson.
Origin:[2, 4, 32]	Gansu, Hebei, Henan, Jiangsu, Liaoning, Nei Mongol, Qinghai, Shaanxi, Shandong, Shanxi, Xinjiang, Xizang (China), north-western, north-eastern and northern China, India, Japan, Mongolia, Pakistan and Russia.
Description of the drug:[1]	Mostly crumpled and rolled, some broken, when whole, elliptical-lanceolate or ovate-lanceolate, 2–5 cm long, 0.5–2 cm wide, pale green or greyish-green, apex obtuse and mucronate, base obtuse or cuneate, margin serrulate, usually recurved, glabrous on both surfaces, veins on lower surface prominent; petioles thin, about 4 mm long. Texture fragile. Odour, slight; taste, weak.
Pre-treatment of the raw drug:[2]	Foreign matters are eliminated and dried in the sun.
Medicinal use:[2]	Treatment of headache, dizziness, irritability, insomnia, hypertension, conjunctivits, chronic cough, dyspnoea, edema and dysuria.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_19, © Springer International Publishing Switzerland 2015

Effects and indications of Folium Apocyni veneti according to Traditional Chinese Medicine[1, 3]

Taste:	Bitter and sweet
Temperature:	Cold
Channels entered:	*Orbis hepaticus*
Effects (functions):	To pacify the liver to tranquilize the mind, clear heat to induce diuresis.
Symptoms and indications:	Liver yang dizziness, palpitation and insomnia, puffiness and small quantity of urination.

Main constituents:

- **Flavonoids**[2, 5–7, 10–15, 17–21, 29, 31, 32]

Quercetin-3-O-galactoside (**hyperoside**), quercetin-3-O-glucoside (**isoquercitrin**), quercetin-3-O-β-D-glucosyl-β-D-glucopyranoside, quercetin-3-O-glucuronide (**miquelianin**), quercetin-3-O-rutinoside (**rutin**), quercetin-3-O-sophoroside (**baimaside**), quercetin-3-O-arabino-furanoside (**avicularin**), kaempferol-3-O-galactoside (**trifolin**), kaempferol-3-O-glucoside (**astragalin**), kaempferol-3,7-dirhamnosid (**lespedin**), chlorogenic acid, kaempferol-6′-O-acetate, isoquercetin-6′-O-acetate, isoquercetin (trifoliin), quercetin, kaempferol, biapigenin

- **Polyphenols (Flavan-3-ols)**[8, 12–16, 31, 32]

Catechin, catechin-[8,7-*e*]-4α-(3,4-dihydroxyphenyl)-dihydro-2(3*H*)-pyranone, epicatechin, epicatechi n-(4β-8-)gallocatechin, epigallocatechin, epigallocatechin-(4β-8)-epicatechin, gallocatechin, procyanidin B2, apocynin A-D, cinchonain Ia

Minor constituents:

- Phloroglycinols (hyperforin, adhyperforin)
- Ionone glucosides (apocynosides I + II)
- Cardiac glycosides (cymarin)
- β-sitosterol, lupeol
- Scopoletin, isofraxidin
- Polysaccharides

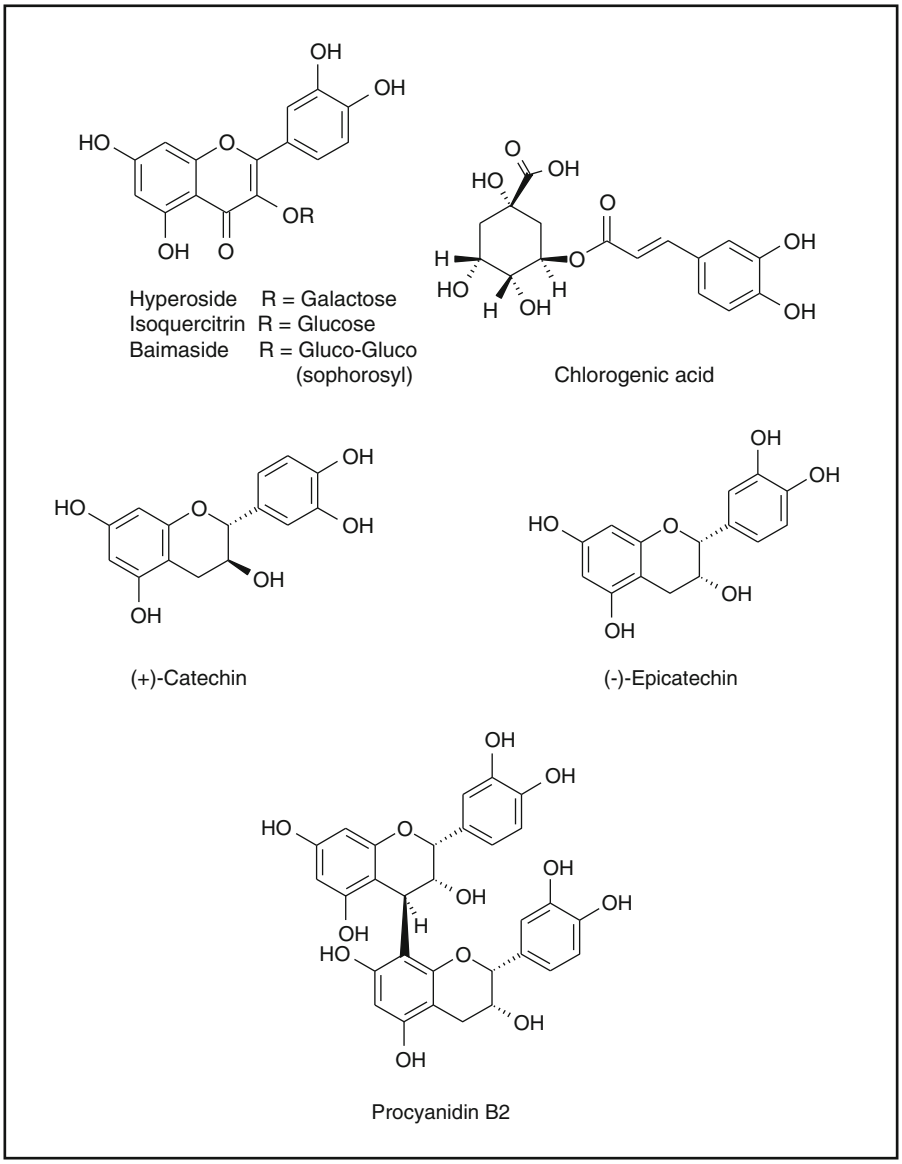


Fig. 1: Formulae of the main constituents of Folium Apocyni veneti[5, 7]

Reported Pharmacological Activities

- diuretic[5, 13, 14, 22, 23, 25–30]
- anti-hyperlipemic[5, 13, 14, 23, 25, 27–29]
- sedative/anxiolytic-like activity[5, 8–10, 12, 13, 16, 24, 25, 30, 32]
- anti-aging/anti-oxidant[5, 7, 9, 10, 12, 14, 15, 18, 23–25, 27–32]
- inibititory effect in lipid-peroxidation assay/cholesterol lowering effects/anti-low-density-lipoprotein oxidation[5, 8, 13, 15, 25–29]

- anti-depressant[6, 7, 9–12, 19, 23–25, 30–32]
- anti-hepatotoxic[7, 12, 29, 30, 32]
- anti-allergic/anti-inflammatory[7]
- anti-osteoprotic[7]
- anti-hypertensive[8–11, 13–15, 18, 22–24, 26–31]
- caspase-inhibitory effect[8]
- protects against oxygen and glucose deprivation (OGD) induced apotosis in rat cortical neurons[8]
- anti-nephritis[11, 19]
- anti-neurasthenia[11, 19]
- suppressing growth of tumor cells[18]
- inhibitory activity against the formation of AGEs[29]
- vasorelaxing activity[29]

TLC-Fingerprint Analysis

Drug samples		Origin
1	Folium Apocyni veneti/*Apocynum venetum*	Province Xinjiang (China)
2	Folium Apocyni veneti/*Apocynum venetum*	Province Liaoning (China)
3	Folium Apocyni veneti/*Apocynum venetum*	Province Tiangjin, Jinhai (China)
4	Folium Apocyni veneti/*Apocynum venetum*	Province Shanxi, Pinglin (China)
5	Folium Apocyni veneti/*Apocynum venetum*	Province Hebei, Cangzhou (China)
6	Folium Apocyni veneti/*Apocynum venetum*	Province Xinjiang, Altay (China)
7	*Poacynum hendersonii* → for comparison	Province Xinjiang (China)

1. **TLC-fingerprint analysis of Flavonoides:**[34]

Reference compounds of Figs. 2a and 2b		***Rf***
T1	Rutin	0.47
T2	Hyperoside	0.68
T3	Chlorogenic acid	0.58
T4	Isoquercitrin	0.71
T5	Quercetin	0.98

1. Extraction: 1 g powdered drug is ultrasonicated with 10 ml ethanol (70 %) for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml 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: Folium Apocyni veneti extracts: 10 µl each
   Reference compounds: 10 µl each

   Solvent system: Ethyl acetate + formic acid + glacial acetic acid + water (10 + 1.1 + 1.1 + 2.6)

   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 with solution I and then with solution II. The evaluation is carried out in VIS and under UV 365 nm after one hour.
   Note: The fluorescence behaviour is dependent on the day of evaluation.

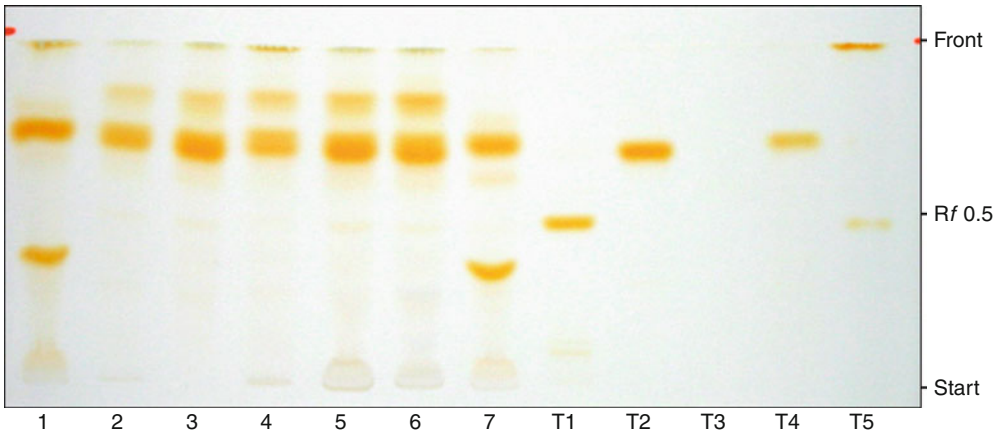


Fig. 2a: Thin layer chromatogram of the ethanol extracts of Folium Apocyni veneti, sprayed with NP/PEG reagent (VIS)

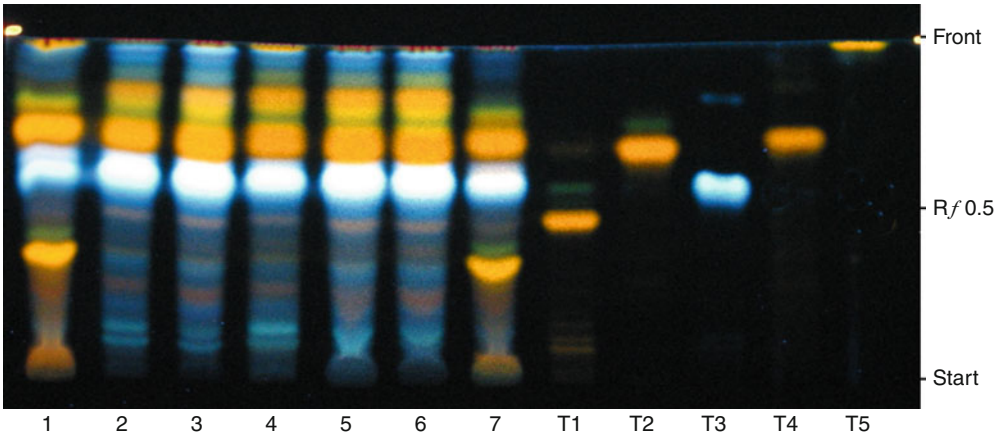


Fig. 2b: Thin layer chromatogram of the ethanol extracts of Folium Apocyni veneti, sprayed with NP/PEG reagent (UV 366 nm)

4. Description:

- Figure 2a: In VIS the extract samples 2-7 show the dominant orange zones of hyperoside (**T2**, R*f* = 0.68), Isoquercitrin (**T4**, R*f* = 0.71) and quercetin (R*f* = 0.82). Whereas rutin (**T1**, R*f* = 0.47) is present in all samples only in very low concentration, another orange zone at R*f* = 0.36 may be assigned to quercetin-3-sophoriside.
- Figure 2b: In this TLC (UV 366 nm) besides hyperoside (**T2**) and isoquercitrin (**T4**) the blue-white fluorescent chlorogenic acid (**T3**) at R*f* = 0.58 dominates in the chromatogram. Above and below quercitrin with more yellow colour kaempferol-glucoside and -galactoside can be detected. Quercetin (**T5**) is best visible in sample 1 and 4.

2. **TLC-fingerprint analysis of Polyphenols:** [33]

Reference compounds of Fig. 3a/b		**R*f***
T6	Catechin	0.81
T7	Procyanidin B2	0.79

1. Extraction: 1 g powdered drug is extracted with 20 ml ethanol under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 2 ml methanol.
2. Reference compounds: Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Folium Apocyni veneti extracts: 10 µl each Reference compounds: 10 µl each
Solvent system:	Ethyl acetate + water + formic acid + glacial acetic acid (70+30+3+2) → **Upper phase**
Detection:	Vanillin – Phosphoric acid reagent: 1 g vanillin is dissolved in a small amount ethanol and filled up to 100 ml with 50 % aqueous phosphoric acid. The plate is sprayed with this solution, heated for 5 min at 105 °C and evaluated in VIS and under UV 366 nm.

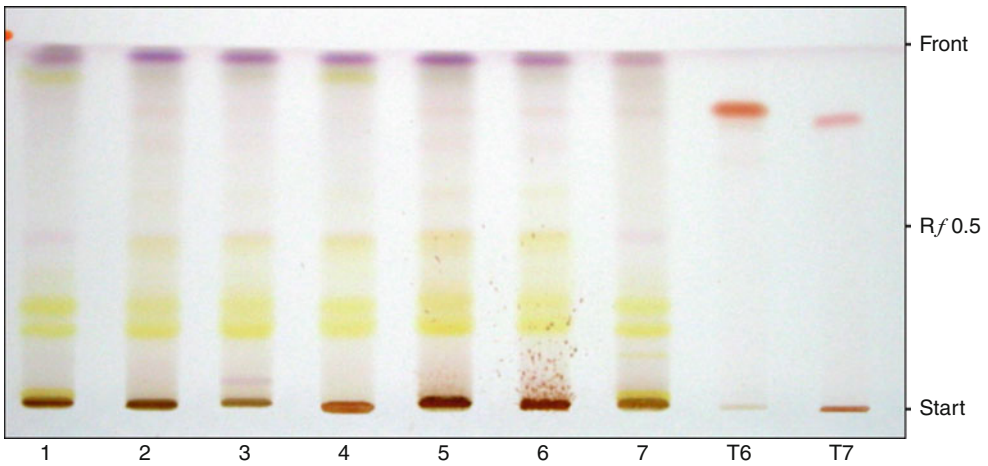


Fig. 3a: Thin layer chromatogram of the ethanol extracts of Folium Apocyni veneti, sprayed with Vanillin – Phosphoric reagent (VIS)

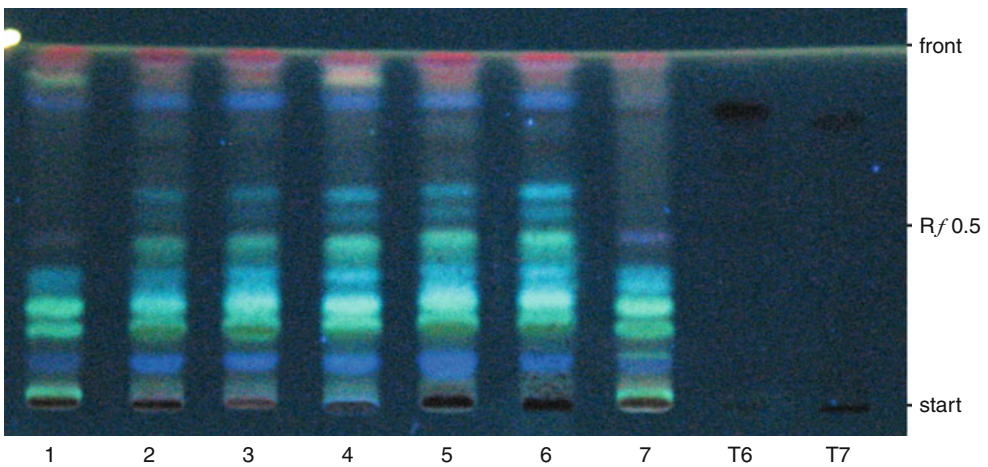


Fig. 3b: Thin layer chromatogram of the ethanol extracts of Folium Apocyni veneti, sprayed with Vanillin – Phosphoric reagent (UV 366 nm)

4. Description:
   - At R*f* = 0.81/0.79 of Fig. 3a the brown zones of catechin/epicatechin are visible. Procyanidin B2 is contained only in very low concentration and in most extract samples hardly detectable. On the start appear the oligomeric procyanidins with brown colour.
   - In Fig. 3b appear in the R*f*-range from 0.2 up to R*f* = 0.5 4–5 blue green fluorescent zones which might be assigned to the apocynins A-D and the ionone glucosides I + II.

HPLC-Fingerprint Analysis

1. Sample preparation: 1 g powdered drug is ultrasonicated with 10 ml ethanol (70 %) for 30 min. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 1 ml ethanol and filtered over Chromafil® filtration unit, type 0–20 µm/25 mm.
2. Injection volume: Folium Apocyni veneti extracts: 10 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 system:	A: 0.1 % Phosphoric acid/Water (Millipore Ultra Clear UV plus® filtered) B: Acetonitril (VWR)
Gradient:	0–5 % B in 5 min, 5–30 % B in 35 min, 30 % B for 5 min Total runtime: 45 min
Flow:	1 ml/min
Detection:	210 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	9.7	Chlorogenic acid
2	16.6	Hyperoside
3	16.9	Isoquercitrin
4	18.4–21.6	Flavonoids
5	28.8	Quercetin
X	13.0	Quercetin-3-sophoroside?

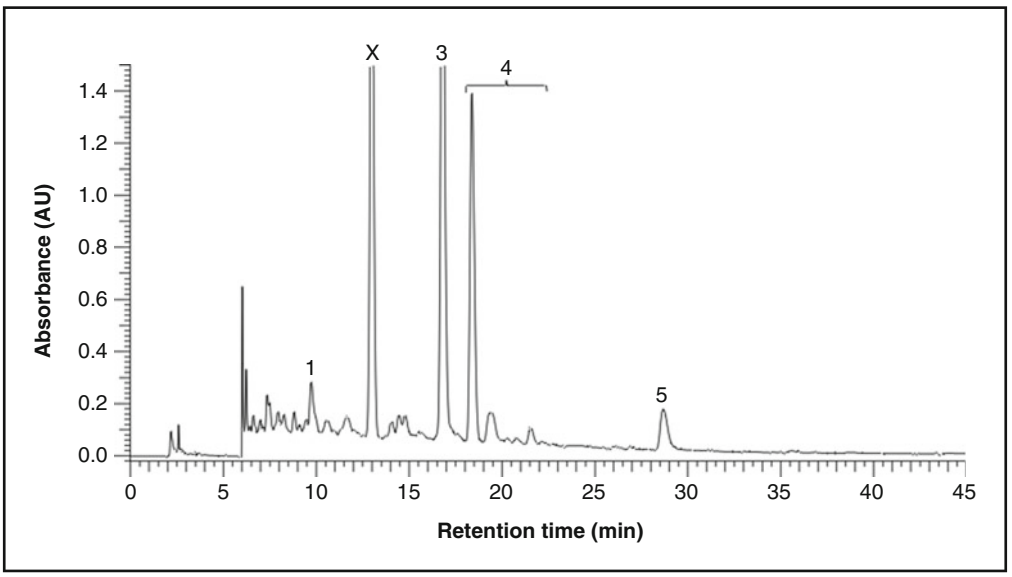


Fig. 4a: HPLC-fingerprint analysis of Folium Apocyni veneti extract, sample 1

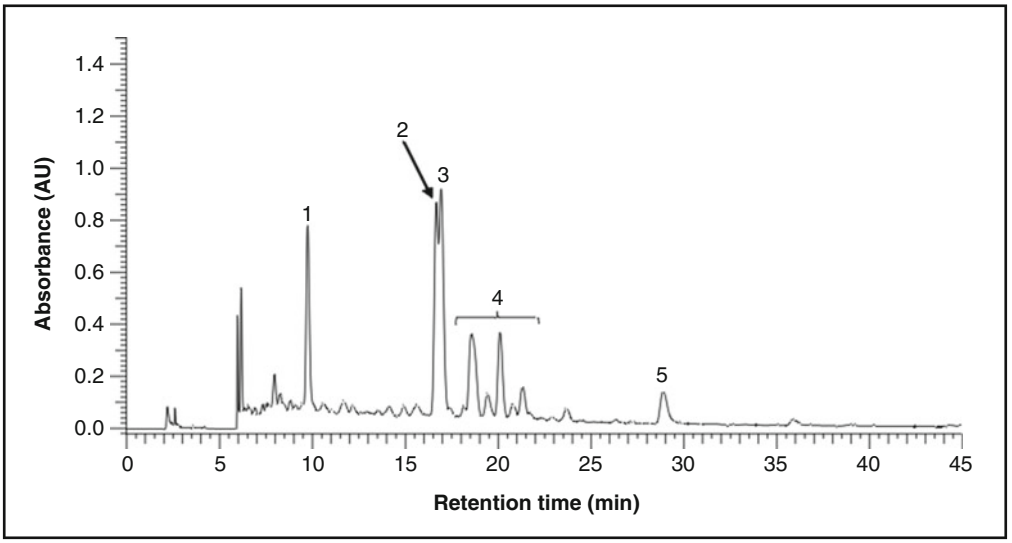


Fig. 4b: HPLC-fingerprint analysis of Folium Apocyni veneti extract, sample 4

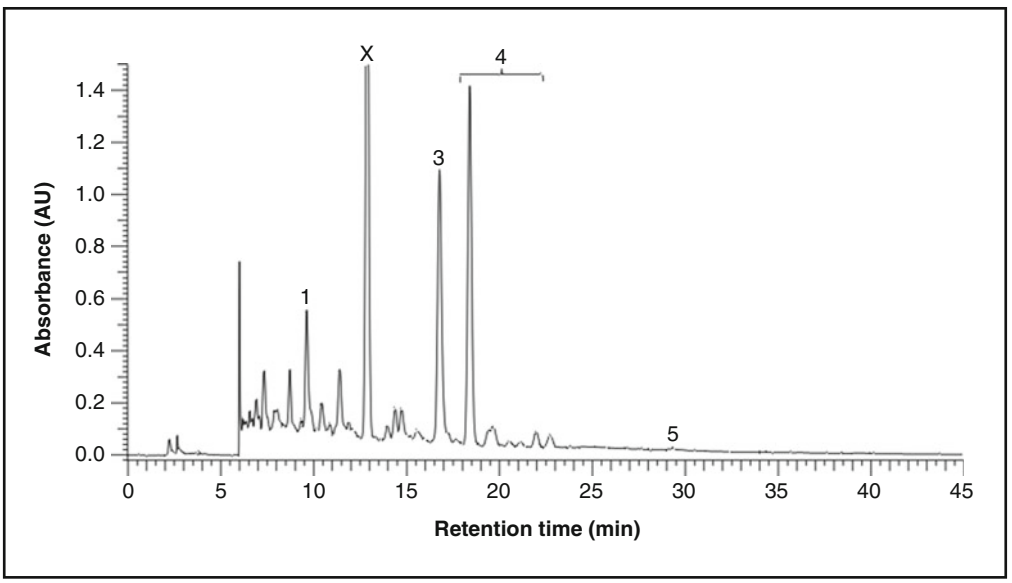


Fig. 4c: HPLC-fingerprint analysis of *Poacynum hendersonii*, sample 7

4. Description of the HPLC-Figures

The HPLC-profile is characterized by a peak accumulation between Rt = 5.0 and 22.0 with chlorogenic acid (**1**), hyperoside (**2**), isoquercitrin (**3**), quercitrin and kaempferol-glycoside (**4**) and quercetin (**5**). The peak X might be quercetin-sophoroside as shown in TLC in the extract samples 1 and 7.

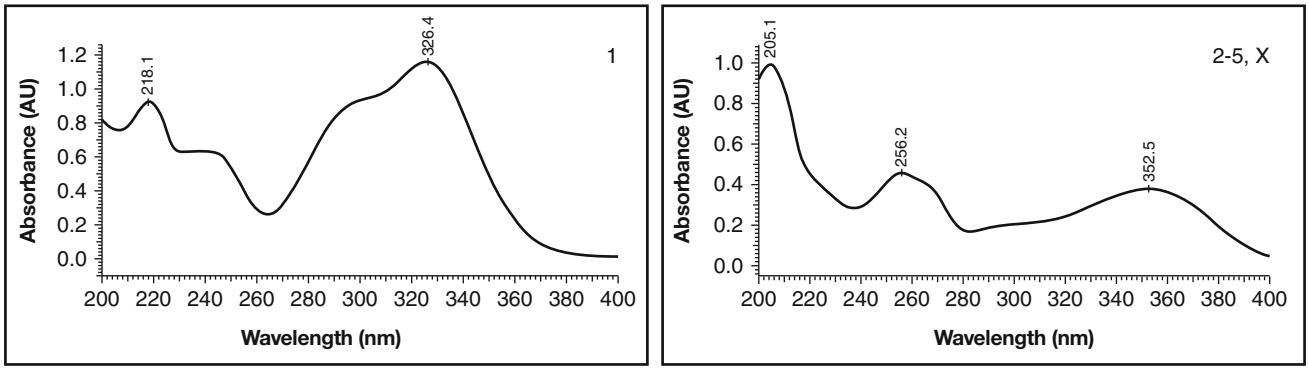


Fig. 5: On line UV-spectra of the detected peaks of Folium Apocyni veneti

Note: According to the Chinese Pharmacopoeia Folium Apocyni veneti contains not less than 0.30 % of hyperoside, calculated with reference to the dried drug.

Conclusion

HPLC and TLC-fingerprint analysis are best suitable for the authentication of Folium Apocyni veneti based on the characteristic flavonoid-glycosides profiles.

References

1. Pharmacopoeia of the people's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Zhang, Z.: An illustrated chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
3. Geng, J., Huang, W., Ren, T., Ma, X.: Materia Medica der chinesischen Arzneimitteltherapie (Praxis der chinesischen Arzneimitteltherapie; Bd. 2). Verlag für Ganzheitliche Medizin Dr. Erich Wühr GmbH, Bad Kötzting (1993)
4. Flora of China, Apocynum venetum, FOC 16, 181 (www.eFloras.org)
5. Butterweck, V., Nishibe, S., Sasaki, T., Uchida, M.: Antidepressant effects of *Apocynum venetum* leaves in a forced swimming test. Biol. Pharm. Bull. **24**(7), 848–851 (2001)
6. Zheng, M., Fan, Y., Shi, D., Liu, C.: Antidepressant-like effect of flavonoids extracted from *Apocynum venetum* leaves on brain monoamine levels and dopaminergic system. J. Ethnopharmacol. **147**(1), 108–113 (2013)
7. Zheng, M., Liu, C., Pan, F., Shi, D., Zhang, Y.: Antidepressant-like effect of hyperoside isolated from *Apocynum venetum* leaves: possible cellular mechanisms. Phytomedicine **19**(2), 145–149 (2012)
8. Xiang, J., Lan, R., Tang, Y.P., Chen, Y.P., Cai, D.F.: *Apocynum venetum* leaf extract attenuated disruption of the blood–brain barrier and upregulation of matrix metalloproteinase-9/-2 in a rat model of cerebral ischemia-reperfusion injury. Neurochem. Res. **37**(8), 1820–1828 (2012)
9. Irie, K., Sato, T., Tanaka, I., Nakajima, J., Kawaguchi, M., Himi, T.: Cardiotonic effect of *Apocynum venetum* L. extracts on isolated guinea pig atrium. J. Nat. Med. **63**(2), 111–116 (2009)
10. Liang, T., Yue, W., Li, Q.: Comparison of the phenolic content and antioxidant activities of *Apocynum venetum* L. (Luo-Bu-Ma) and two of its alternative species. Int. J. Mol. Sci. **11**(11), 4452–4464 (2010)

11. Zhang, Y., Liu, C., Zhang, Z., Wang, J., Wu, G., Li, S.: Comprehensive separation and identification of chemical constituents from *Apocynum venetum* leaves by high-performance counter-current chromatography and high performance liquid chromatography coupled with mass spectrometry. J. Chromatogr. B **878**(30), 3149–3155 (2010)
12. Kamata, K., Seo, S., Nakajima, J.: Constituents from leaves of *Apocynum venetum* L. J. Nat. Med. **62**(2), 160–163 (2008)
13. Xiong, Q., Fan, W., Tezuka, Y., Adnyana, I.K., Stampoulis, P., Hattori, M., Namba, T., Kadota, S.: Hepatoprotective effect of *Apocynum venetum* and its active constituents. Planta Med. **66**(2), 127–133 (2000)
14. Kim, D.W., Yokozawa, T., Hattori, M., Kadota, S., Namba, T.: Inhibitory effects of an aqueous extract of *Apocynum venetum* leaves and its constituents on Cu^{2+}-induced oxidative modification of low density lipoprotein. Phytother. Res. **14**(7), 501–504 (2000)
15. Yokozawa, T., Nakagawa, T.: Inhibitory effects of Luobuma tea and its components against glucose-mediated protein damage. Food Chem. Toxicol. **42**(6), 975–981 (2004)
16. Grundmann, O., Nakajima, J., Kamata, K., Seo, S., Butterweck, V.: Kaempferol from the leaves of *Apocynum venetum* possesses anxiolytic activities in the elevated plus maze test in mice. Phytomedicine **16**(4), 295–302 (2009)
17. Murakami, T., Kishi, A., Matsuda, H., Hattori, M., Yoshikawa, M.: Medicinal foodstuffs. XXIV.1) Chemical constituents of the processed leaves of *Apocynum venetum* L.: absolute stereostrucutres of apocynosides I and II. Chem. Pharm. Bull. **49**(7), 845–848 (2001)
18. Ma, M., Hong, C.L., An, S.Q., Li, B.: Seasonal, spatial, and interspecific variation in quercetin in *Apocynum venetum* and *Poacynum hendersonii*, chinese traditional herbal teas. J. Agric. Food Chem. **51**(8), 2390–2393 (2003)
19. Zhang, Y., Liu, C., Zhang, Z., Qi, Y., Wu, G., Li, S.: Solvent gradient elution for comprohensive separation of constituents with wide range of polarity in *Apocynum venetum* leaves by high-spedd counter-current chromatography. J. Sep. Sci. **33**(17–18), 2743–2748 (2010)
20. Cheng, X.L., Zhang, S.Q., Li, Q.S.: Chemical constituents of flavonoids from *Apocynum venetum*. Zhong. Yao. Cai. **30**(9), 1086–1088 (2007)
21. Chen, M., Liu, F.: Sedative chemical constituents of leaves of *Apocynum venetum* Linn. Zhongguo. Zhong. Yao. Za. Zhi. **16**(10), 609–611 (1991)
22. Kwan, C.Y., Zhang, W.B., Nishibe, S., Seo, S.: A novel in vitro endothelium-dependent vascular relaxant effect of *Apocynum venetum* leaf extract. Clin. Exp. Pharmacol. Physiol. **32**(9), 789–795 (2005)
23. Grundmann, O., Nakajima, J., Seo, S., Butterweck, V.: Anti-anxiety effects of Apocynum venetum L. in the elevated plus maze test. J. Ethnopharmacol. **110**(3), 406–411 (2007)
24. Kuo, C.S., Kwan, C.Y., Gong, C.L., Tsai, M.F., Nishibe, S., Tatsuzaki, J., Leung, Y.M.: *Apocynum venetum* leaf aqueous extract inhibits voltage-gated sodium channels of mouse neuroblastoma N2A cells. J. Ethnopharmacol. **136**(1), 149–155 (2011)
25. Lau, Y.S., Kwan, C.Y., Ku, T.C., Hsieh, W.T., Wang, H.D., Nishibe, S., Dharmani, M., Mustafa, M.R.: *Apocynum venetum* leaf extract, an antihypertensive herb, inhibits rat aortic contraction induced by angiotensin II: a nitric oxide and superoxide connection. J. Ethnopharmacol. **143**(2), 565–571 (2012)
26. Kim, D.W., Yokozawa, T., Hattori, M., Kadota, S., Namba, T.: Effects of aqueous extracts of *Apocynum venetum* leaves on spontaneously hypertensive, renal hypertensive and NaCl-fed-hypertensive rats. J. Ethnopharmacol. **72**(1–2), 53–59 (2000)
27. Butterweck, V., Simbrey, K., Seo, S., Sasaki, T., Nishibe, S.: Long-term effects of an *Apocynum venetum* extract on brain monoamine levels and β-AR density in rats. Pharmacol. Biochem. Behav. **75**(3), 557–564 (2003)
28. Zheng, M., Liu, C., Pan, F., Shi, D., Ma, F., Zhang, Y., Zhang, Y.: Protective effects of flavonoid extract from *Apocynum venetum* leaves against corticosterone-induced neurotoxicity in PC12 cells. Cell. Mol. Neurobiol. **31**(3), 421–428 (2011)
29. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants – chemistry, pharmacology, toxicology, vol. 2. WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim (2011)
30. An, H., Wang, H., Lan, Y., Hashi, Y., Chen, S.: Simultaneous qualitative and quantitative analysis of phenolic acids and flavonoids for the quality control of *Apocynum venetum* L. leaves by HPLC-DAD-ESI-IT-TOF-MS and HPLC-DAD. J. Pharm. Biomed. Anal. **85**, 295–304 (2013)
31. Shi, J., Li, G., Wang, H., Zhang, J., Suo, Y., You, J., Liu, Y.: One-step separation of three flavonoids from *Poacynum hendersonii* by high-speed counter-current chromatography. Phytochem. Anal. **22**(5), 450–454 (2011)
32. Xie, W., Zhang, X., Wang, T., Hu, J.: Botany, traditional uses, phytochemistry and pharmacology of Apocynum venetum L. (Luobuma): a review. J. Ethnopharmacol. **141**(1), 1–8 (2012)
33. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines, vol. I+II. Springer, Wien/New York (2011)
34. Wagner, H., Bladt, S.: Plant drug analysis: a thin layer chromatography atlas, 2nd edn. Springer, Berlin (2001)

Flos Eriocauli – *Gujingcao*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Pipewort Flower is the dried capitulum with peduncle of *Eriocaulon buergerianum* Koern. (Fam. Eriocaulaceae).

The drug is collected in autumn, the capitulum is picked up with peduncle, and dried in the sun.

Origin:[2] Provinces Zhejiang, Guangdong and Fujian (China), Taiwan, Japan

Description of the drug:[1] Capitulum hemispherical, 4–5 mm in diameter. Bracts densely arranged in numerous layers at the base, pale yellowish-green, lustrous, densely pubescent at the upper margin. The top of the capitulum greyish-white. After rubbing, numerous black anthers and fine yellowish-green unripe fruits visible. Peduncles slender, varying in length, less than 1 mm in diameter, pale yellowish-green, bearing numerous twisted ridges. Texture pliable. Odour, slight; taste, weak.

Pretreatment of the raw drug:[1] Foreign matters are eliminated, and cut into sections.

Medicinal use:[3–5] Used mainly as ophthalmic.

Effects and indications of Flos Eriocauli according to Traditional Chinese Medicine[1]

Taste:	Pungent and sweet
Temperature:	Neutral
Channels entered:	*Orbis hepaticus*, *orbis pulmonalis*
Effects (functions):	To disperse wind-heat, improve vision and remove nebula.
Symptoms and indications:	Wind-heat red eyes, photophobia with swelling and pain, nebula, wind-heat headache.

Main constituents:[2–5]

- **Flavonoids**

Patuletin (quercetagetin-6-methyl ether), patuletin-3-*O*– β-D-glucopyranoside, patuletin-3-O-β-D-gentiobioside, patuletin-3-*O*-β-D-rutinoside, hispidulin, hispidulin-7-*O*-β-D-glucopyranoside, quercetin, quercetagetin and -derivatives, 5,7,3′-trihydroxy-6,4′,5′-trimethoxyisoflavone, gerontoisoflavone A

Minor constituents:[2–5]

- Palmitic acid, (*Z*,*Z*)-9,12-octa-cosane-dienoic acid
- Anthraquinones (emodin) and -glycosides
- Naphthopyranones
- γ-Tocopheryl acetate, ferulic acid, vanillic acid, protocatechuic acid

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_20, © Springer International Publishing Switzerland 2015

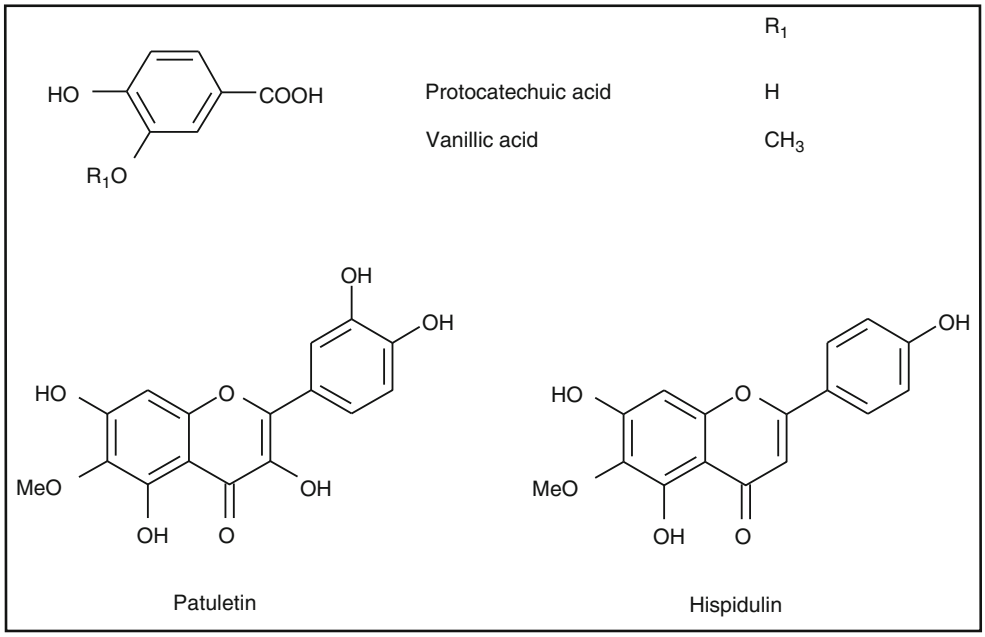


Fig. 1: Formulae of the main constituents of Flos Eriocauli [5]

Pharmacology:

- Anti-inflammatory [2]
- Anti-microbial [2]
- Anti-fungal [6]

TLC-fingerprint Analysis [1]

Drug samples		Origin
1	Flos Eriocauli/*Eriocaulon buergerianum*	Province Anhui (China)
2	Flos Eriocauli/*Eriocaulon buergerianum*	Province Jiangsu (China)
3	Flos Eriocauli/*Eriocaulon buergerianum*	Province Anhui (China)
4	Flos Eriocauli/*Eriocaulon buergerianum*	Sample of commercial drug, obtained from Kronen Apotheke Wuppertal

Reference compounds of Fig. 2a, b		R*f*
T1	Rutin	0.48
T2	Patuletin	0.98
n.a.	Hyperoside	0.70

n.a. not applied

1. Extraction: 1 g powdered drug is extracted with 20 ml ethanol (80 %) for 2 h under reflux. The extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml ethanol

2. Reference compounds: Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Flos Eriocauli extracts: 10 µl each

Reference compounds: 10 µl each

Solvent system: Ethyl acetate + formic acid + glacial acetic acid + water (20+2.2+2.2+5.4)

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 under VIS and UV 366 nm.

Note: The fluorescence behaviour is dependent on the time of evaluation.

4. Description:

Figure 2a: The chromatogram of the extract samples show in the R*f*-range from R*f*=0.1 up to R*f*=0.65 four yellow/orange zones of flavonolglycosides.

Figure 2b: The samples 1–4 show under UV 366 nm from the start up to the front 8–9 yellow/orange zones and further two white/blue fluorescent zones in between. Rutin (**T1**) appears at R*f*=0.48 and patuletin (**T2**)

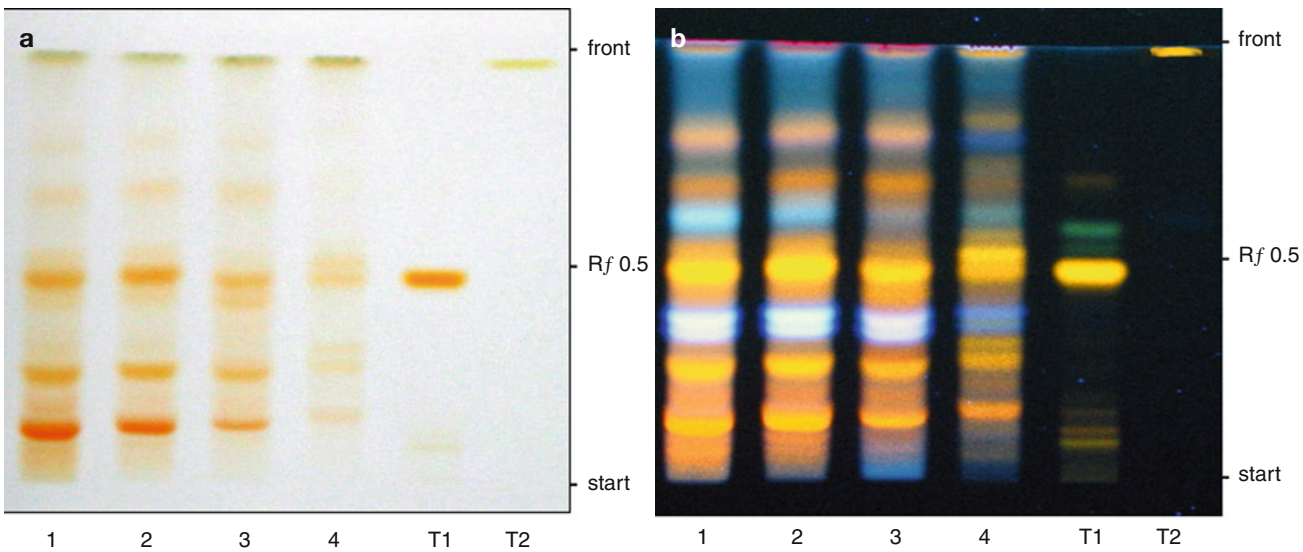


Fig. 2: (**a, b**) Thin layer chromatogram of the ethanol extracts of Flos Eriocauli, sprayed with NP/PEG reagent (a=VIS, b=UV 366 nm)

at R*f* = 0.98. Further flavonol-glycosides in the deep R*f*-range can be assigned to hispidulin or quercetin-glycosides. Hyperoside can be seen at R*f* = 0.70 and one anthraquinone aglycone at R*f* = 0.80. The anthraquinone-glycosides, not identified, are visible with orange colour in the R*f*-range 0.1 to 0.3.

HPLC-fingerprint Analysis

1. Sample preparation: 1 g powdered drug is extracted with 20 ml ethanol (80 %) for 2 h under reflux. The extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml ethanol, filtered over Chromafil®, Type 0.20 µm and injected into the HPLC apparatus.

2. Injection volume: Flos Eriocauli extracts: 10 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), VWR
Precolumn:	LiChroCART® 4–4 LiChrospher® 100 RP-18 (5 µm), VWR
Solvent System:	A: 0.1 % H_3PO_4/water (Millipore Ultra Clear UV plus® filtered)
	B: acetonitrile (VWR)
Gradient:	5–100 % B in 55 min,
	100 % B for 5 min,
	Total runtime: 60 min
Flow:	1 ml/min
Detection:	210 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	2.2	Not identified flavonoid glycoside
2	5.3	Not identified flavonoid glycoside
3	8.3	Rutin
4	9.5	Hyperoside
5	12.3	Anthraquinones

4. Description of the HPLC-Figures

Extract samples 2 and 3 show an uniform peak profile in the Rt-range from 2.0 to 14.0 with the peaks **1** and **2** (flavonol di- or triglycosides), peak **3** (rutin), **4** (patuletin) and the peak **5** (anthraquinone) as derivable from the UV-spectrum of Fig. 4.

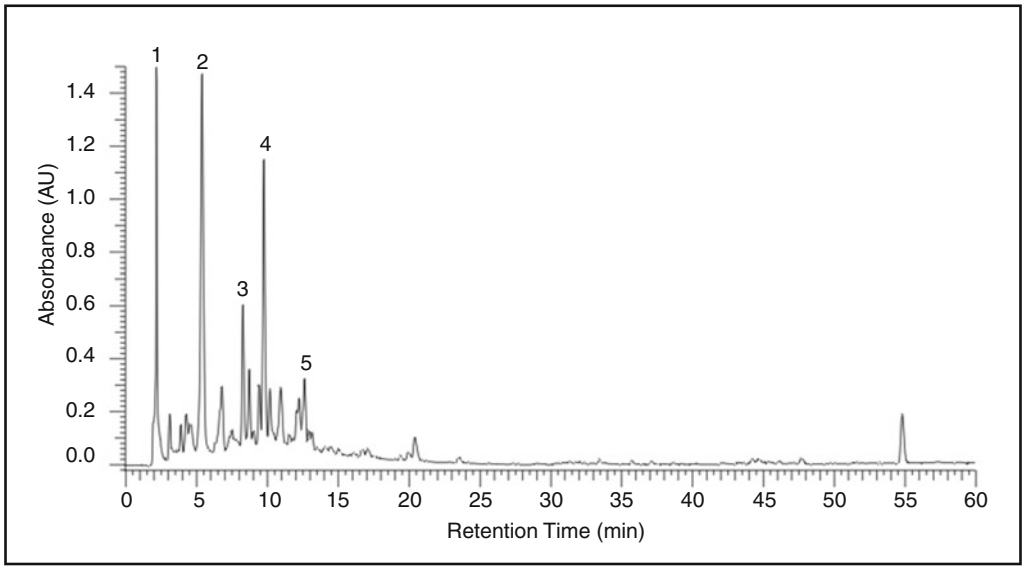


Fig. 3a: HPLC-fingerprint analysis of the ethanol extract of Flos Eriocauli, sample 2

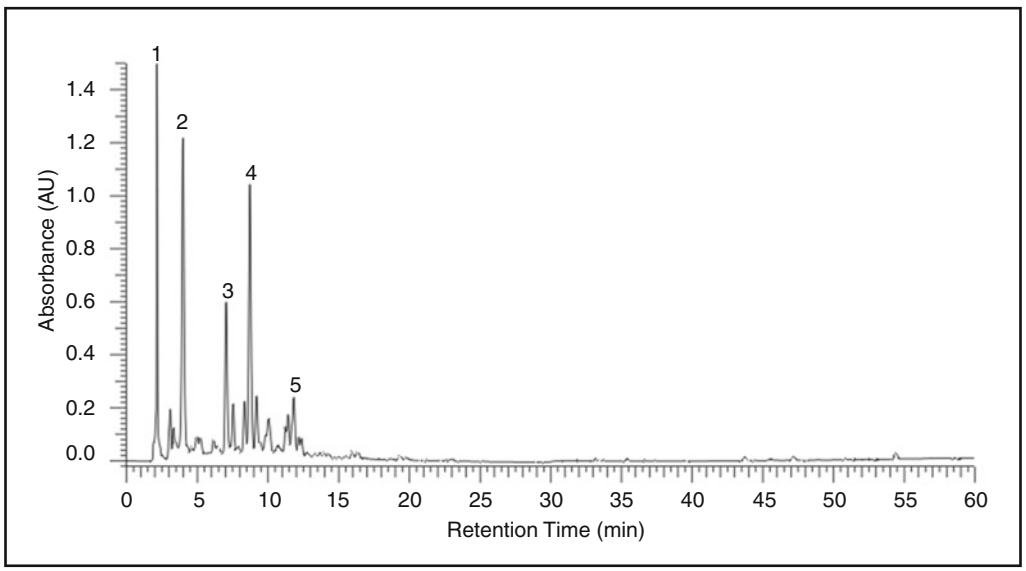


Fig. 3b: HPLC-fingerprint analysis of the ethanol extract of Flos Eriocauli, sample 3

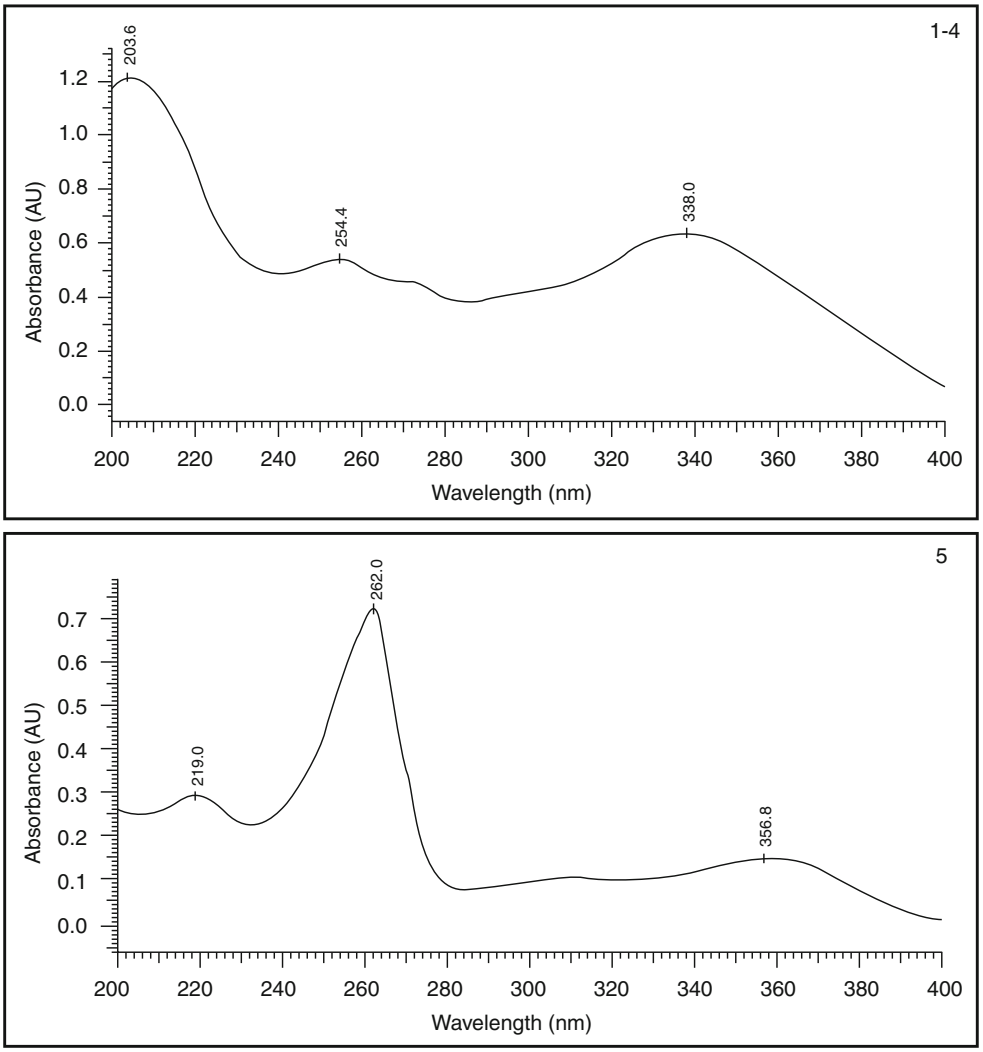


Fig. 4: On line UV-spectra of the detected peaks of Flos Eriocauli

Conclusion

The authentication of Flos Eriocauli extracts can be best performed by the TLC-method as described in the monograph.

References

1. Pharmacopoeia of the People's Republic of China, english edition, vol. I. People's Medical Publishing House, Beijing (2010)
2. Wang, M., Zhang, Z., Cheang, L.C.V., Lin, Z., Lee, S.M.Y.: *Eriocaulon buergerianum* extract protects PC12 cells and neurons in zebrafish against 6-hydroxydopamine-induced damage. Chin. Med. **6**, 16 (2011)
3. Ho, J.C., Chen, C.M.: Flavonoids from the aquatic plant *Eriocaulon buergerianum*. Phytochemistry **61**(4), 405–408 (2002)
4. Fang, J.J., Ye, G., Chen, W.L., Zhao, W.M.: Antibacterial phenolic components from *Eriocaulon buergerianum*. Phytochemistry **69**(5), 1279–1286 (2008)
5. Qiao, X., Ye, G., Liu, C.F., Zhnag, Z.X., Tu, Q., Dong, J., Li, Y.Q., Guo, D.A., Ye, M.: Chemical analysis of *Eriocaulon buergerianum* and adulterating species by high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry. J. Pharm. Biomed. Anal. **57**, 133–142 (2012)
6. Kimura, T., But, P.P.H., Guo, J.X., Sung, C.K.: International collation of traditional and folk medicine – Northeast Asia – part 2, p. 184. World Scientific Publishing, Singapore (1997)

Caulis Spatholobi – *Jixueteng*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Suberect Spatholobus Stem is the dried lianoid stem of *Spatholobus suberectus* Dunn (Fam. Leguminosae).

The drug is collected in autumn and winter, removed from branch and leaf, cut into slices, and dried in the sun.

Origin:[2] Provinces Yunnan, Fujian, Guangdong, Sichuan

Description of the drug:[1] Elliptical, oblong or irregular oblique slices, 0.3–1 cm thick. Cork greyish-brown, sometimes greyish-white patches visible and appearing reddish-brown when the cork exfoliated. Texture compact and hard. In the transversely cut surface, xylem reddish-brown or brown, showing numerous pores of vessels; phloem with resinous secretion reddish-brown to blackish-brown, arranged alternately with xylem, forming several concentric elliptical or eccentric semi-circular ring; pith inclined to one side. Odour, slight; taste, astringent.

Pretreatment of the raw drug:[1] Foreign matters are eliminated, washed clean, softened thoroughly, cut into pieces, and dried in the sun.

Medicinal use:[3] Used for the improvement of blood circulation and treatment of dysmenorrhea, anemia, paralysis and arthralgia.

Effects and indications of Caulis Spatholobi according to Traditional Chinese Medicine [1, 2, 4]

Taste:	Bitter and sweet
Temperature:	Warm
Channels entered:	*Orbis hepaticus*, *o. renalis*, *o. cardialis*, *o. lienalis*
Effects (functions):	To activate blood and nourish blood, regulate menstruation and relive pain, relax sinews and activate collaterals.
Symptoms and indications:	Menstrual irregularities, dysmenorrhoea, amenorrhoea, painful impediment caused by wind-dampnes, numbness, paralysis, blood deficiency and sallow complexion.

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_21, © Springer International Publishing Switzerland 2015

Main constituents:

- **Isoflavones** [3, 5–10]

  Formononetin (biochanin B, glycoside: ononin), genistein, daidzein, calycosin, pseudobatigenin, prunetin

- **Flavanoles** [6–9]

  Catechin, epicatechin, epigallocatechin, gallocatechin,

- **Flavanones** [3, 5, 7, 10]

  Eriodictyol; 6-methoxyeriodictyol; hesperetin; naringenin; liquiritigenin; butin; (2S)-7-hydroxy-6-methoxy-flavanone suberectin (= 7,3′,4′-trihydroxy-6-methoxy flavanone); plathymenin (= 6,7,3′,4′-tetra-hydroxyflavanone)

- Flavanonol [5]

  Dihydroquercetin (Taxifolin), dihydrokaempferol (Aromadedrin)

- Phenolic acids [6, 7, 9]

  Syringic acid, vanillic acid, protocatechuic acid

- Phytosterols [9, 10]

  β-Sitosterol, daucosterol (glycoside of β-sitosterol)

- Other compounds [3, 5, 6, 10, 11]

  Betulinic acid; hexacosanoic acid (cerotic acid); neoisoliquiritigenin; 3′,4′,7-trihydroxyflavone; sativan; pyromucic acid; succinic acid (butanedioic acid); 1,3,5-benzenetriol; 2-methoxy-4-(2′-hydroxyethyl)-phenyl-1-*O*-β-D-glucopyranoside; n-butyl-*O*-β-D-fructopyranoside; glycerol-α-penta-cosanoate; (6a*R*,11a*R*)-maackiain; (6a*R*,11a*R*)-medicarpin

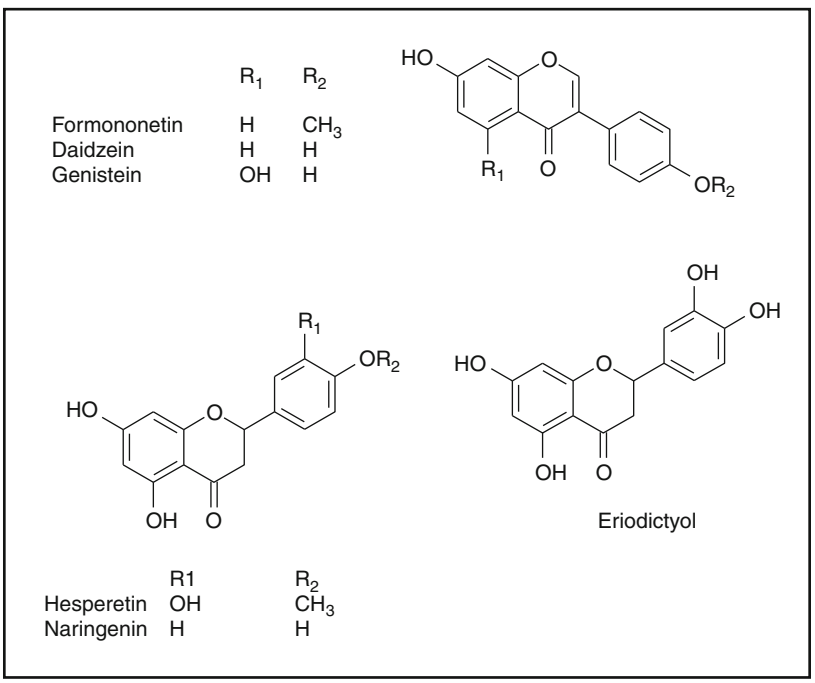


Fig. 1: Formulae of the main constituents of Caulis Spatholobi [5, 7]

Pharmacology:

- Sedative effects [4]
- Antihypertensive effects [4]
- Uterus-stimulating effects [4]
- Hematological effects [5, 6, 12]
- Inhibition of HIV type-1 protease in vivo [5, 7]
- Anti-inflammatory [5, 7]
- Regulation of plasma lipid concentrations [5, 12]
- Enhancement of immunity in cancer patients [6]
- Inhibition of the proliferation of various cancer cell lines in vitro [6]
- Blood circulation improvement [7, 12]
- Tyrosinase inhibition [7]
- Hypocholesterolemic effects [13]
- Radical scavenging effect in vitro and in vivo [13]
- Inhibition of cervical cancer cell proliferation [13]
- Inhibition of tumor cell-induced platelet aggregation [12, 14] and tumor cell invasion [14]

TLC-Fingerprint Analysis

Drug samples		origin
1	Caulis Spatholobi/*Spatholobus suberectus*	Sample of commercial drug, obtained from TCM-Clinic Bad Kötzting (Charge: K07.01.2003)
2	Caulis Spatholobi/*Spatholobus suberectus*	Sample of commercial drug, obtained from TCM-Clinic Bad Kötzting (Charge: K08.10.2004)
3	Caulis Spatholobi/*Spatholobus suberectus*	Province Guangxi (China)
4	Caulis Spatholobi/*Spatholobus suberectus*	Sample of commercial drug, obtained from HerbaSinica
5	Caulis Spatholobi/*Spatholobus suberectus*	Sample of commercial drug, obtained from China Medica
6	Caulis Spatholobi/*Spatholobus suberectus*	Province Guangdong, Pe-ging (China)
7	Caulis Spatholobi/*Spatholobus suberectus*	Province Guangdong (China)
8	Caulis Spatholobi/*Spatholobus suberectus*	Province Guangxi (China)

1. **TLC-fingerprint analysis of Isoflavones**:[15]

Reference compounds of Fig. 2		*Rf*
T1	Formononetin	0.36
T2	Genistein	0.24
T3	Daidzein	0.21

1. Extraction — 1 g powdered drug is extracted with 20 ml ethanol under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 2 ml methanol.

2. Reference compounds — Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate:	TLC Silica gel 60 F_{254} (aluminium sheets), Merck
Applied amounts:	Caulis Spatholobi extracts: 10 µl each Reference compounds: 20 µl each
Solvent system:	Dichloromethane + methanol (30 + 1)
Detection:	Aluminium(III)-chlorid solution 5 g aluminium(III)-chloride hexahydrate are dissolved in 100 ml ethanol (80 %). The plate is sprayed and evaluated under UV 366 nm.

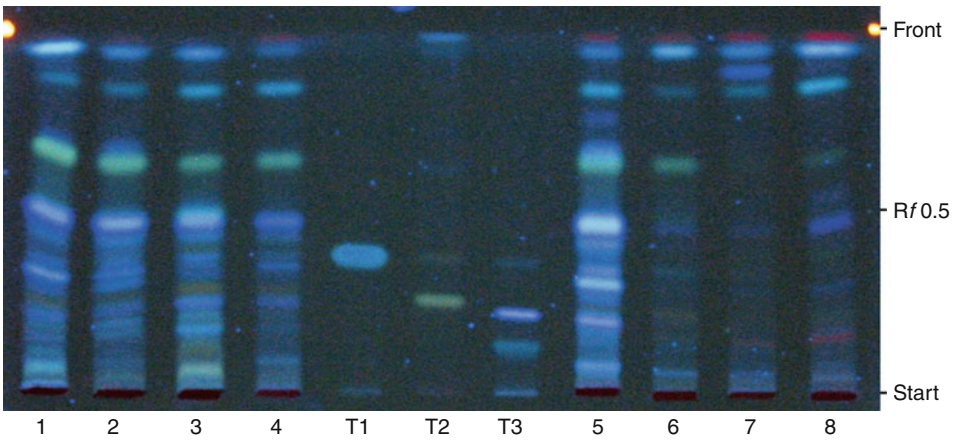


Fig. 2: Thin layer chromatogram of the ethanol extracts of Caulis Spatholobi, sprayed with 5 % ethanolic $AlCl_3$ solution (UV 366 nm)

4. Description:

The extract samples 1–5 show a homogeneous pattern of blue/green fluorescent isoflavonoids in the deep R*f*-range with the reference compounds formononetin (**T1**, R*f* = 0.36), genistein (**T2**, R*f* = 0.24) and daidzein (**T3**, R*f* = 0.21). The green fluorescent zone at R*f* = 0.65 can be assigned to naringenin. The other two blue fluorescent zones above naringenin might be the flavanones hesperetin or eriodictyol.

2. **TLC-fingerprint analysis of Procyanidins**:[16]

Reference compounds of Fig. 3		**R*f***
T4	Catechin	0.81
T5	Epicatechin	0.79

1. Extraction: 1 g powdered drug is extracted with 20 ml ethanol under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 2 ml methanol.

2. Reference compounds: Each 0.5 mg is dissolved in 0.5 ml ethanol

3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Caulis Spatholobi extracts: 10 µl each
	Reference compounds: 10 µl each
Solvent system:	ethyl acetate + water + formic acid + glacial acetic acid (70+30+3+2) → **upper phase**
Detection:	Vanillin – Phosphoric acid reagent:
	1 g vanillin is dissolved in a small amount of ethanol and filled up to 100 ml with 50 % aqueous phosphoric acid.
	The plate is sprayed with this solution, heated for 5 min at 105 °C and evaluated in VIS.

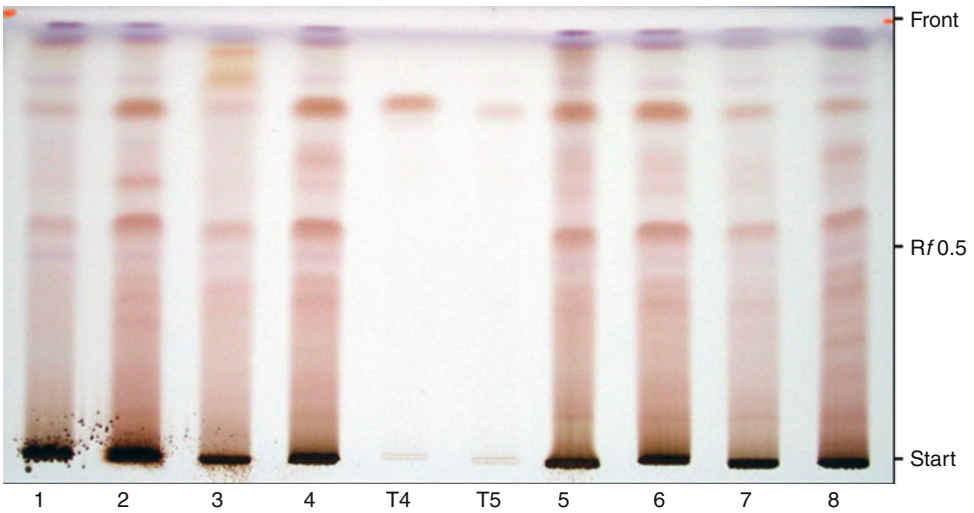


Fig. 3: Thin layer chromatogram of the ethanol extracts of Caulis Spatholobi, sprayed with Vanillin – Phosphoric acid reagent (VIS)

4. Description:

In all extract samples appear in the R*f*-range from R*f* = 0.5 to the front 5–6 reddish brown zones of the catechins (procyanidins). Catechin (**T4**) and epicatechin (**T5**) appear at R*f* = 0.81 and 0.79, respectively, the dimeric and trimeric procyanidins at R*f* = 0.85 and 0.55 and in the deep R*f*-range and on the start the polymeric procyanidins.

HPLC-Fingerprint Analysis

1. Sample preparation: 1 g powdered drug is extracted with 10 ml ethanol under reflux for 1 h. The extract is cooled, filtrated and evaporated to dryness. The residue is dissolved in 5 ml water and shaken with 10 ml ethyl acetate. The ethyl acetate phase is evaporated to dryness, the residue dissolved in 1 ml methanol and filtered over Chromafil®, Type 0.20 µm.

2. Injection volume: Caulis Spatholobi extracts: 10 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® 125-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 0.1 % Phosphoric acid/Water (Millipore Ultra Clear UV plus® filtered) B: Acetonitril (VWR)
Gradient:	0–25% B in 30 min, 25–45% B in 5 min, 45–95 % B in 5 min, 95 % B for 5 min, Total runtime: 45 minutes
Flow:	1 ml/min
Detection:	210 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	7.5	Catechin
2	8.0	Epicatechin

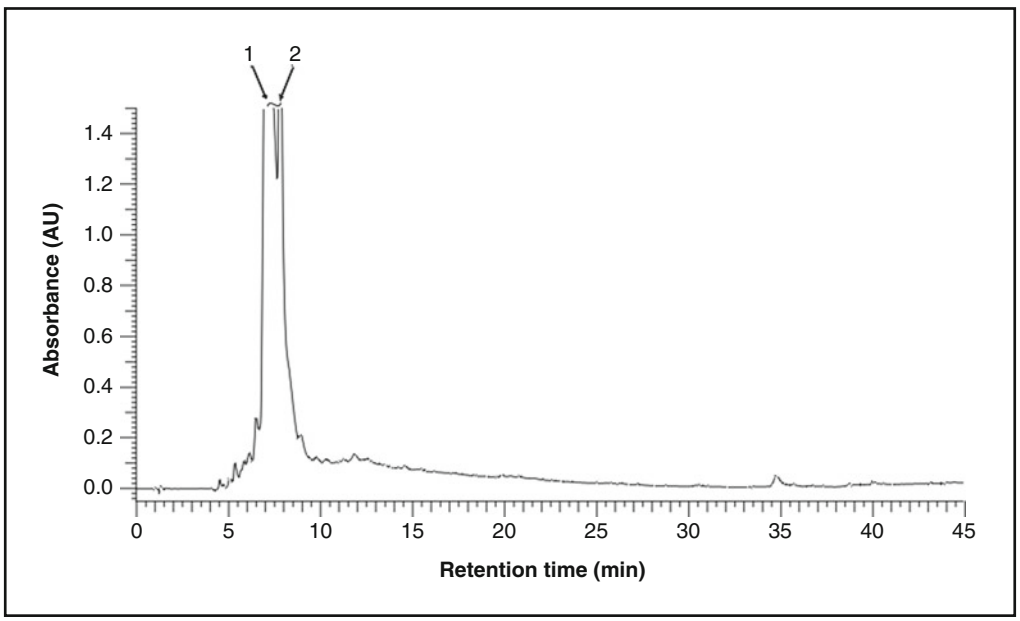


Fig. 4a: HPLC-fingerprint analysis of the ethanol extract of Caulis Spatholobi, sample 2

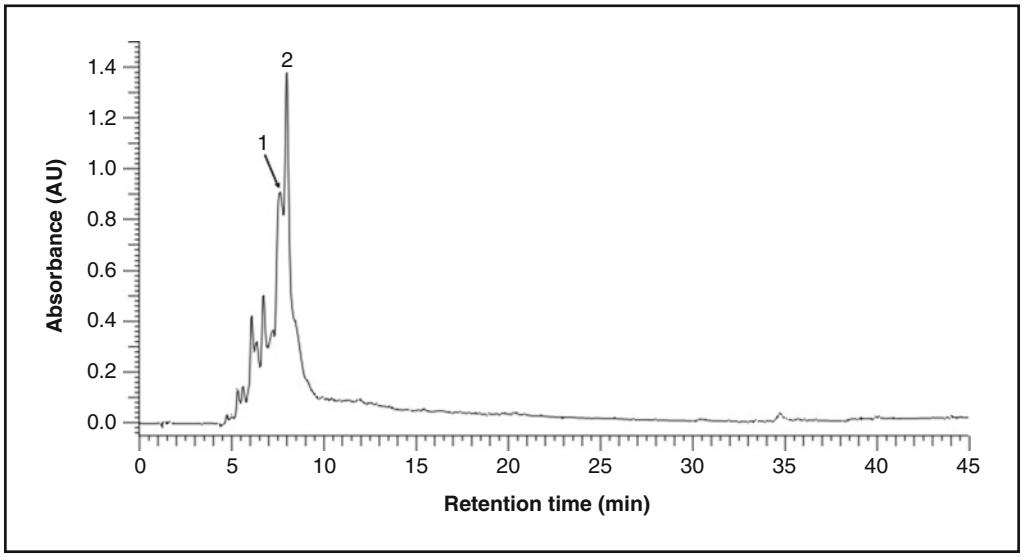


Fig. 4b: HPLC-fingerprint analysis of the ethanol extract of Caulis Spatholobi, sample 4

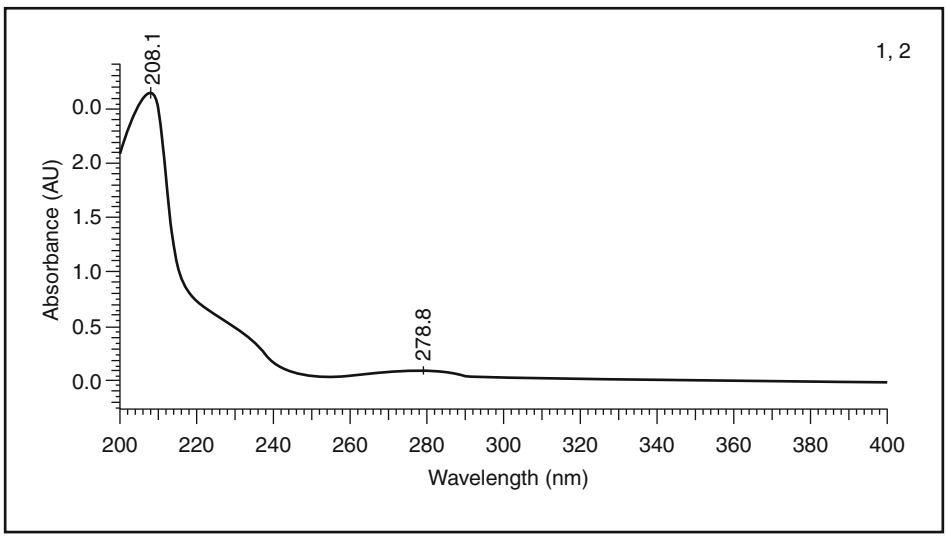


Fig. 5: On line UV-spectra of the detected peaks of Caulis Spatholobi

4. Description of the HPLC-Figures

   Both extract samples show a characteristic peak accumulation in the Rt-range of 4.5–10.0 with the dominant catechin (**1**) and epicatechin (**2**) peaks at Rt = 7.5 and 8.0

Conclusion

Caulis Spatholobi extracts can be easily authenticated based on the characteristic isoflavonoid- and procyanidin pattern in TLC and the distinct catechin/epicatechin peaks in HPLC.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, Vol. 1. People's Medical Publishing House, Beijing (2010)
2. Paulus, E., Ding, Y.-H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)
3. Yoon, J.S., Sung, S.H., Park, J.H., Kim, Y.C.: Flavonoids from *Spatholobus suberectus*. Arch. Pharm. Res. **27**(6), 589–592 (2004)
4. Hempen, C.H., Fischer, T.: Leitfaden Chinesische Phytotherapie, 2nd edn. Urban & Fischer, Munich (2007)
5. Lee, M.H., Lin, Y.P., Hsu, F.L., Zhan, G.R., Yen, K.Y.: Bioactive constituents of Spatholobus suberectus in regulating tyrosinase-related proteins and mRNA in HEMn cells. Phytochemistry **67**(12), 1262–1270 (2006)
6. Wang, Z.Y., Wang, D.M., Loo, T.Y., Cheng, Y., Chen, L.L., Shen, J.G., Yang, D.P., Chow, L.W.C., Guan, X.Y., Chen, J.P.: *Spatholobus suberectus* inhibits cancer cell growth by inducing apoptosis and arresting cell cycle at G2/M checkpoint. J. Ethnopharmacol. **133**(2), 751–758 (2011)
7. Cheng, X.L., Wan, J.Y., Li, P., Qi, L.W.: Ultrasonic/microwave assisted extraction and diagnostic ion filtering strategy by liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid characterization of flavonoids in *Spatholobus suberectus*. J. Chromatogr. A **1218**(34), 5774–5786 (2011)
8. Liu, C., Ma, L., Chen, R.Y., Liu, P.: Determination of catechin and its analogues in *Spatholobus suberectus* by RP-HPLC. Zhongguo Zhong Yao Za Zhi **30**(18), 1433–1435 (2005)
9. Cui, Y.J., Liu, P., Chen, R.Y.: Studies on the active constituents in vine stem of *Spatholobus suberectus*. Zhongguo Zhong Yao Za Zhi **30**(2), 121–123 (2005)
10. Cui, Y.J., Liu, P., Chen, R.Y.: Studies on the chemical constituents of *Spatholobus suberectus* Dunn. Yao Xue Xue Bao **37**(10), 784–787 (2002)
11. Chen, J., Liang, H., Wang, Y., Zhao, Y.Y.: Studies on the constituents from the stems of Spatholobus *suberectus*. Zhongguo Zhong Yao Za Zhi **28**(12), 1153–1155 (2003)
12. Lee, B.J., Jo, I.Y., Bu, Y., Park, J.W., Maeng, S., Kang, H., Jang, W., Hwang, D.S., Lee, W., Min, K., Kim, J.I., Yoo, H.H., Lew, J.H.: Antiplatelet effects of *Spatholobus suberectus* via inhibition of the glycoprotein IIb/IIIa receptor. J. Ethnopharmacol. **134**(2), 460–467 (2011)
13. Shim, S.H.: 20S proteasome inhibitory activity of flavonoids isolated from *Spatholobus suberectus*. Phytother. Res. **25**(4), 615–618 (2011)
14. Ha, E.S., Lee, E.O., Yoon, T.J., Kim, J.H., Park, J.O., Lim, N.C., Jung, S.K., Yoon, B.S., Kim, S.H.: Methylene chloride fraction of Spatholobi Caulis induces apoptosis *via* caspase dependent pathway in U937 cells. Biol. Pharm. Bull. **27**(9), 1348–1352 (2004)
15. Hong Kong Chinese Materia Medica Standards, Vol. 5. Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region, The People's Republic of China (2012)
16. Wagner, H., Bauer, R., Melchart, D., Xiao, P.G., Staudinger, A.: Chromatographic fingerprint analysis of herbal medicines: thin layer and high performance liquid chromatography of Chinese drugs, vol. 1 and 2. Springer, Wien (2011)

Radix Aucklandiae – *Muxiang*

Pharmacopoeia:[1] Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1] Common Aucklandia Root is the dried root of *Aucklandia lappa Decne*. (Fam. Asteraceae).

The drug is collected in winter or spring, removed from soil and rootlet, cut into sections, and the large ones further longitudinally cut into pieces, dried, and removed from the rough outer bark by dashing.

Drug substitutes (Synonyms):[2] 1 – Radix Vladimiriae denticulatae Ling

2 – Radix Vladimiriae souliei (Franch.) Ling (var.)

Adulteration:[22] Radix Aristolochiae (Qing- Muxiang)[a] from *Aristolochia debilis* (Aristolochiaceae)

Origin:[2] China (Yunnan, Guangxi, Sichuan)

Description of the drug:[1] Cylindrical or semicylindrical, 5–10 cm long, 0.5–5 cm in diameter. Externally yellowish-brown to greyish brown, with distinct wrinkles, longitudinal furrows and lateral root scars. Texture hard, uneasily broken, fracture greyish-brown to dark brown, the outer layer greyish-yellow or brownish-yellow, cambium ring brown, having radial lines and scattered brown dotted oil cavities. Odour, characteristic and aromatic; taste, slightly bitter.

Pretreatment of the raw drug:[1] Foreign matters are eliminated, washed clean, soaked briefly, softened thoroughly, cut into thick slices, and dried in the shade.

Medicinal use:[3] General gastrointestinal diseases as chalogog, diuretic and expectorant.

[a]For proof of Radix Aucklandiae on the absence of cancerogenic aristolochic acid see monograph of Radix Stephaniae tetrandrae (Wagner et al).[23]

Effects and indications of Radix Aucklandiae according to Traditional Chinese Medicine[1, 3]

Taste:	Slighty bitter, pungent
Temperature:	Warm
Channels entered:	*Orbis lienales*, *o. stomachi*, *o. intenstini crassi*, *o. polmonalis*
Effects (functions):	Moves and regulates qi, relieves pain, tonifies the qi, dispels cold
Symptoms and indications:	Gastric pain, abdominal pain, distension lack of appetite, Anorexia, nausea, vomiting

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_22, © Springer International Publishing Switzerland 2015

Main constituents:[4–9, 15, 18, 20]

Sesquiterpenes

Costunolide, α-dehydrocostus lactone, santamarin, β-cyclocostunolide, 4α-hydroxy-4-β methyldihydrocostol, 10-α-hydroxyl-artemisinic acid

Lignans

Syringaresinol, ascleposide E, (+)-1-hydroxy-pinoresinol-4″methyl ester-4′-β-D-glucopyranoside, (+)-1-hydroxypinoresinol-4″-O-β-D-glucopyranoside, (+)-1-hydroxypinoresinol-1-O-P-D-glucopyranoside, phenyl-β-D-glucopyranoside-benzyl-β-D-glucopyranoside, n-butyl-β-D-glucopyranoside, ilicic alcohol

Phenol carboxylic acids

(Iso)chlorogenic acid

Other constituents:[2, 6, 7, 14–16, 18–20] Betulinic acid, betulinic acid methyl ester, mokkolactone, betulin

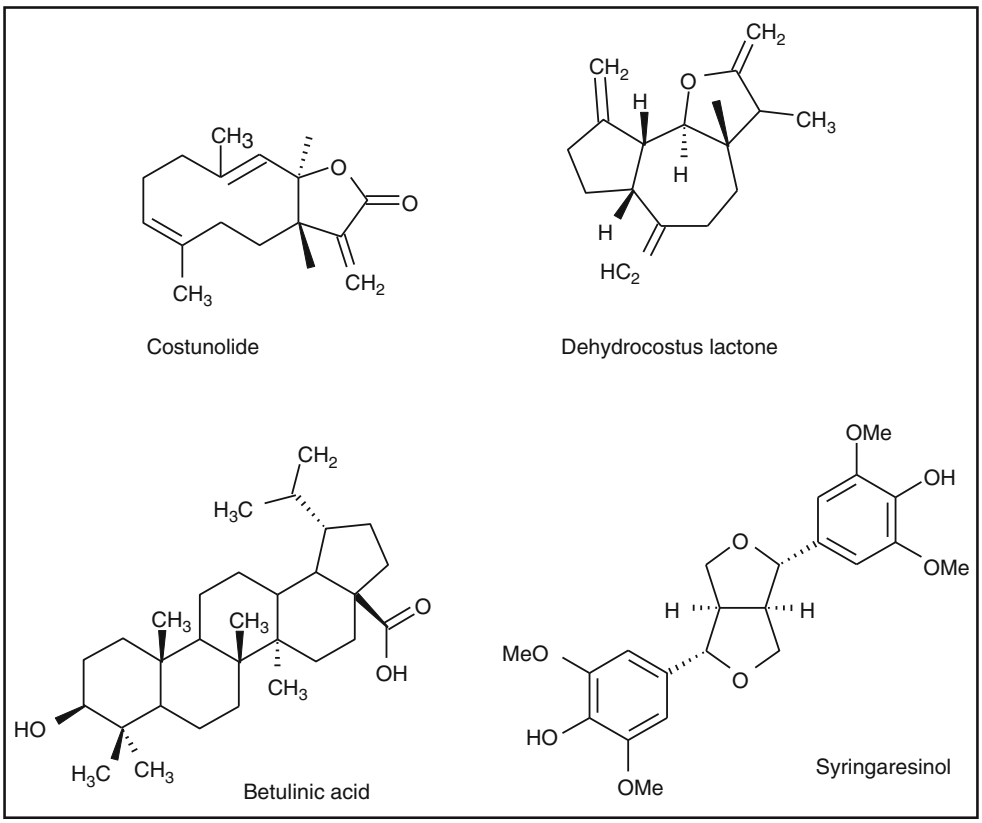


Fig. 1: Formulae of the main constituents of Radix Aucklandiae [6, 7, 9, 17, 20, 21]

Pharmacology: Anticancer effect[6, 7, 9–11, 15]

Anti-inflammatory effect[10, 12, 13]

Anti-ulcer effect[10, 17]

Strong suppressive effect of costunolide and dehydrocostus lactone on the expression of hepatitis B surface antigen (HBsAg) in human hepatoma Hep3B cells [8]

TLC-Fingerprint Analysis

	Drug samples	Origin
1	Radix Aucklandiae/*Aucklandia lappa*	Province Wantang, Wufeng, Hubei (China)
2	Radix Aucklandiae/*Aucklandia lappa*	Sample of commercial drug, obtained from China Medica
3	Radix Aucklandiae/*Aucklandia lappa*	Province Santai, Wufeng, Hubei (China)
4	Radix Aucklandiae/*Aucklandia lappa*	Province Wantang, Wufeng, Hubei (China)
5	Radix Aucklandiae/*Aucklandia lappa*	Province Santai, Wufeng, Hubei (China)
6	Radix Aucklandiae/*Aucklandia lappa*	Province Wantang, Hubei (China)
7	Radix Aucklandiae/*Aucklandia lappa*	Province Sichuan (China)
8	**Radix Vladimiriae/*Vladimiria souliei***	Province Sichuan (China)

1. **TLC-Fingerprint analysis of sesquiterpenes**:

1. Extraction: 1 g powdered drug is ultrasonicated with 20 ml dichloromethane for 30 min, filtrated and evaporated to dryness. The residue is dissolved in 1 ml methanol.
2. Reference compounds: 1.0 mg is dissolved in 1.0 ml methanol
3. Separation parameters:

Plate: HPTLC Silica gel 60 F_{254}, Merck

Applied amounts: Radix Aucklandiae extracts: 5 µl each
Reference compound: 10 µl

Solvent system: Dichloromethane + cyclohexane (5 + 1)

Detection: 1 ml of diluted aq. sulphuric acid (50 % v/v) mixed with 10 ml of *p*-hydroxybenzaldehyde in methanol (2 % w/v).
The plate is sprayed with the solution and evaluated under Vis.

	Reference compound of Fig. 2	R*f*
T1	Costunolide	0.28
n.a	Dehydrocostus lactone	0.37

n.a. not applied

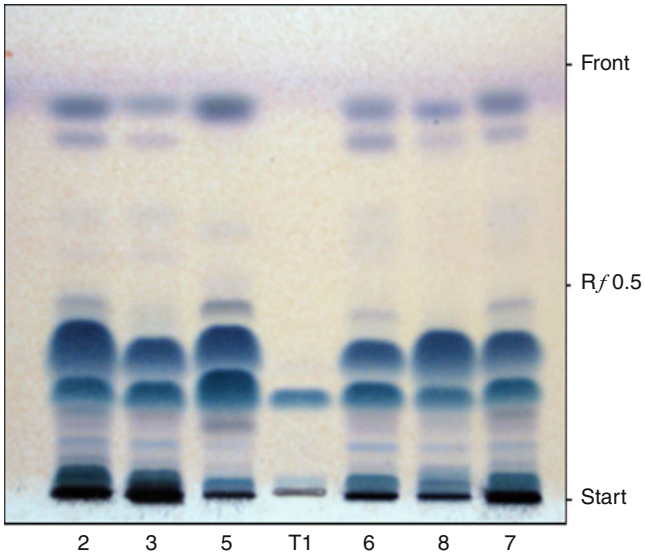


Fig. 2: Thin layer chromatogram of the dichloromethane extracts of Radix Aucklandiae (VIS)

4. Description of Fig. 2:

   The Radix Aucklandiae extracts samples 2, 3, 5, 6 and 7 and the Radix Vladimeriae sample 8 show in the R*f* -range from start till R*f* = 0.45 the dominant dark violet bands of costunolide (**T1**) at R*f* = 0.28 and dehydrocostus lacton at R*f* = 0.37.

   A third weak just above the start at R*f* = 0.05 might be the hydroxypinoresinol glucoside. On the solvent front appear any of the terpenoids (e.g. mokko lactone or betulinic acid methyl- ester).

2. TLC-Fingerprint analysis of costunolide and other constituents:

1. Extraction: 1 g powdered drug is ultrasonicated with 20 ml dichloromethane for 30 min, filtrated and evaporated to dryness. The residue is dissolved in 1 ml methanol
2. Reference compounds: Each 1.0 mg is dissolved in 1.0 ml methanol
3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Reference compounds:	each 10 µl
Solvent system:	Chloroform + methanol (98 + 2)
Detection:	<u>Vanillin – Sulphuric acid</u> 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 min at 105 °C and evaluated in VIS.

Reference compounds of Fig. 3		R*f*
n.a	Dehydrocostus lactone	0.98
T1	Costunolide	0.95
T2	Betulinic acid	0.50
T3	Syringaresinol	0.47
T4	β-sitosterol	0.58

4. Description of Fig. 3:

   The Radix Aucklandiae extract samples show a very homogenous pattern of 7–8 brown violet zones with the dominant zone of costunolide (**T1**) at R*f*=0.93 and dehydrocostus lactone at R*f*=0.98. At R*f*=0.48 appears betulinic acid (**T2**), at R*f*=0.49 syringaresinol (**T3**) and β-sitosterol (**T4**) at R*f*=0.58. Radix Vladimiriae extract sample 8 shows the same zone pattern as the Radix Aucklandiae samples.

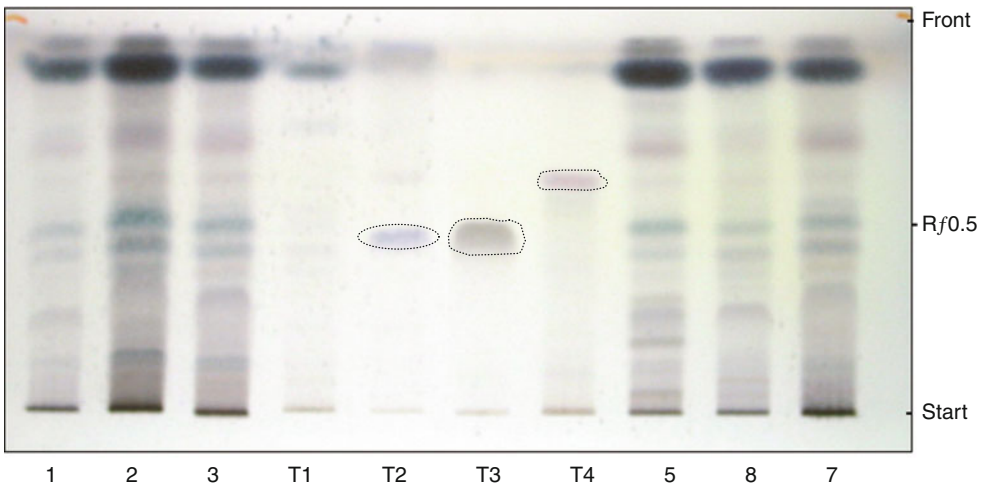


Fig. 3: Thin layer chromatogram of the dichloromethane extracts of Radix Aucklandiae, sprayed with Vanillin – Sulphuric acid reagent (VIS)

HPLC-Fingerprint Analysis

1. Sample preparation: 1.0 g powdered drug is ultrasonicated with 10 ml methanol for 30 min. The extract is filtered and evaporated to dryness. The residue is dissolved in 1 ml methanol, filtered over Chromafil®, Type 0.20 µm and injected into the HPLC apparatus.

2. Injection volume: Radix Aucklandiae extracts: 10 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® 125-4 LiChrospher® 100 RP-18 (5 µm), Merck
Precolumn:	LiChroCART® 4-4 LiChrospher® 100 RP-18 (5 µm), Merck
Solvent System:	A: 0.1 % trifluoroacetic acid (v/v) in water B: acetonitril (VWR)
Gradient:	0–30 % B in 20 min, 30 % B for 8 min, 30–100 % B in 17 min, 100 % B for 15 min, Total runtime: 60 min
Flow:	1 ml/min
Detection:	254 nm

Retention times of the main peaks

Peak	Rt (min)	Compound
1	4–6	Not identified
2	6–12	Syringaresinol
3	6–13	Caffeic acid
4	11–20	Chlorogenic acid
5	12–21	Isochlorogenic acid
6	35–41	Costunolide
7	36–42	Dehydrocostus lactone

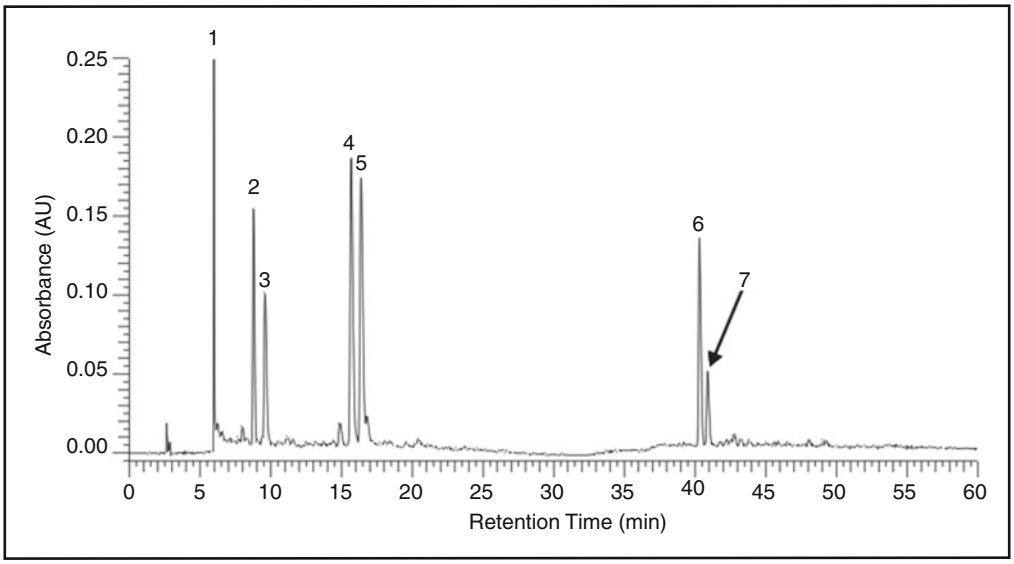


Fig. 4a: HPLC-fingerprint analysis of the methanol extract of Radix Aucklandiae, sample 1

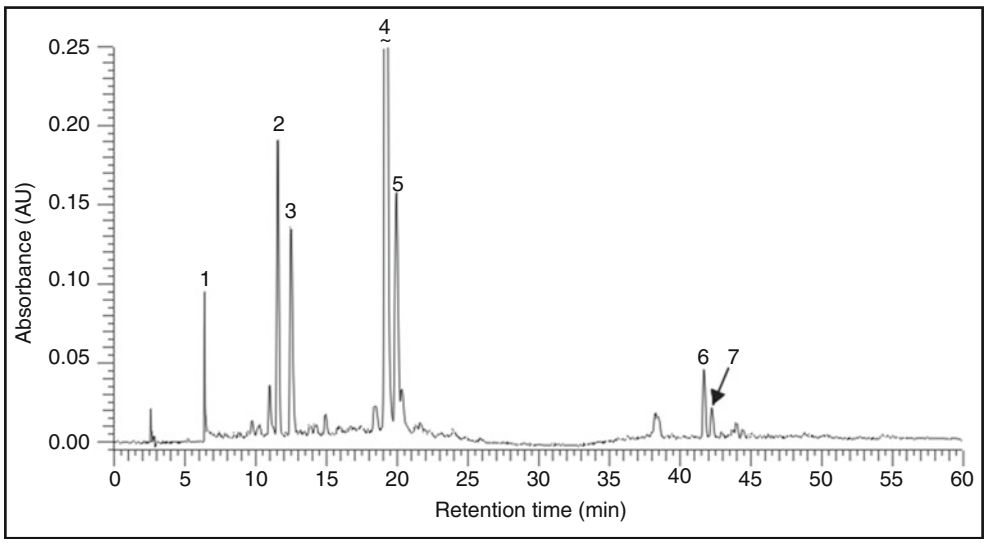


Fig. 4b: HPLC- fingerprint analysis of the methanol extract of Radix Aucklandiae, sample 3

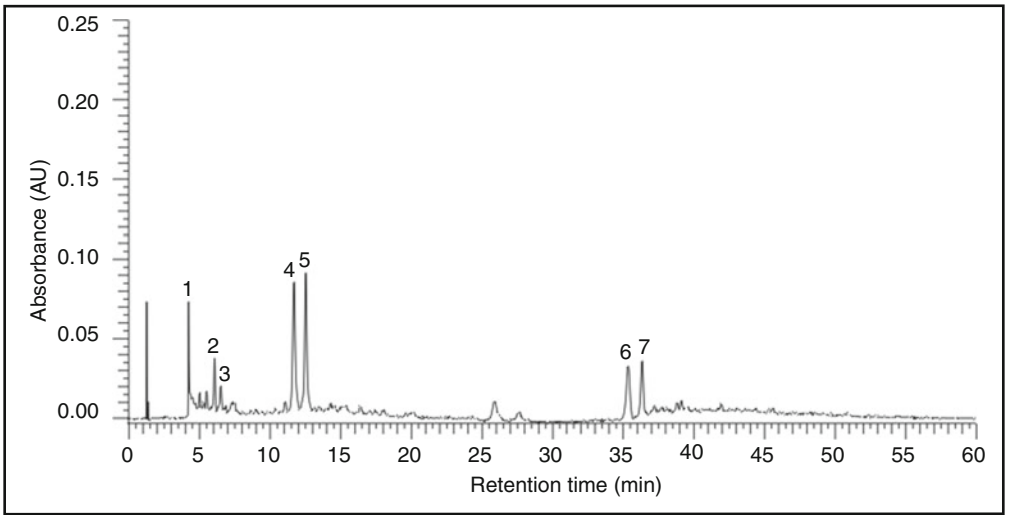


Fig. 4c: HPLC fingerprint analysis of the methanol extract of Radix Aucklandiae, sample 7

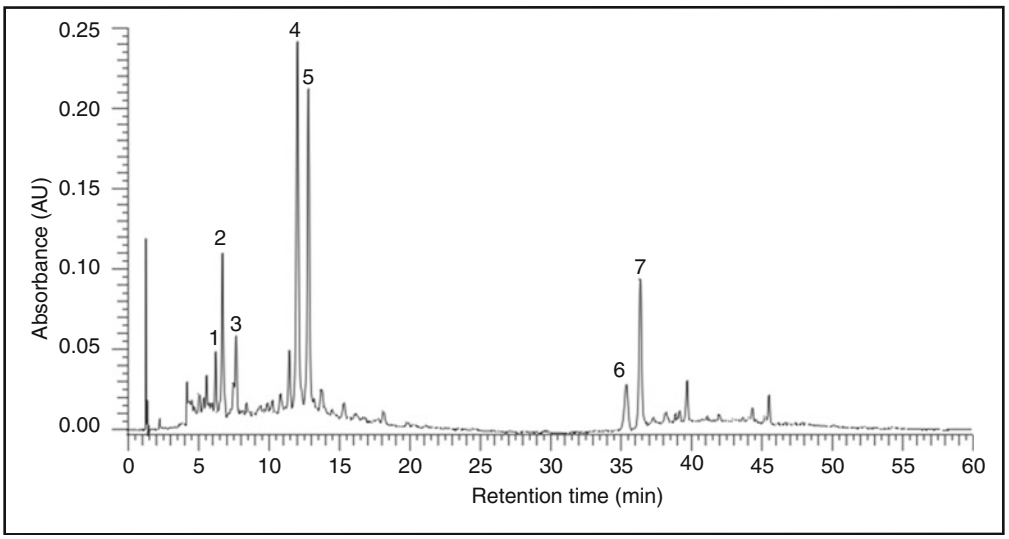


Fig. 4d: HPLC fingerprint analysis of the methanol extract of Radix Vladimiriae, sample 8

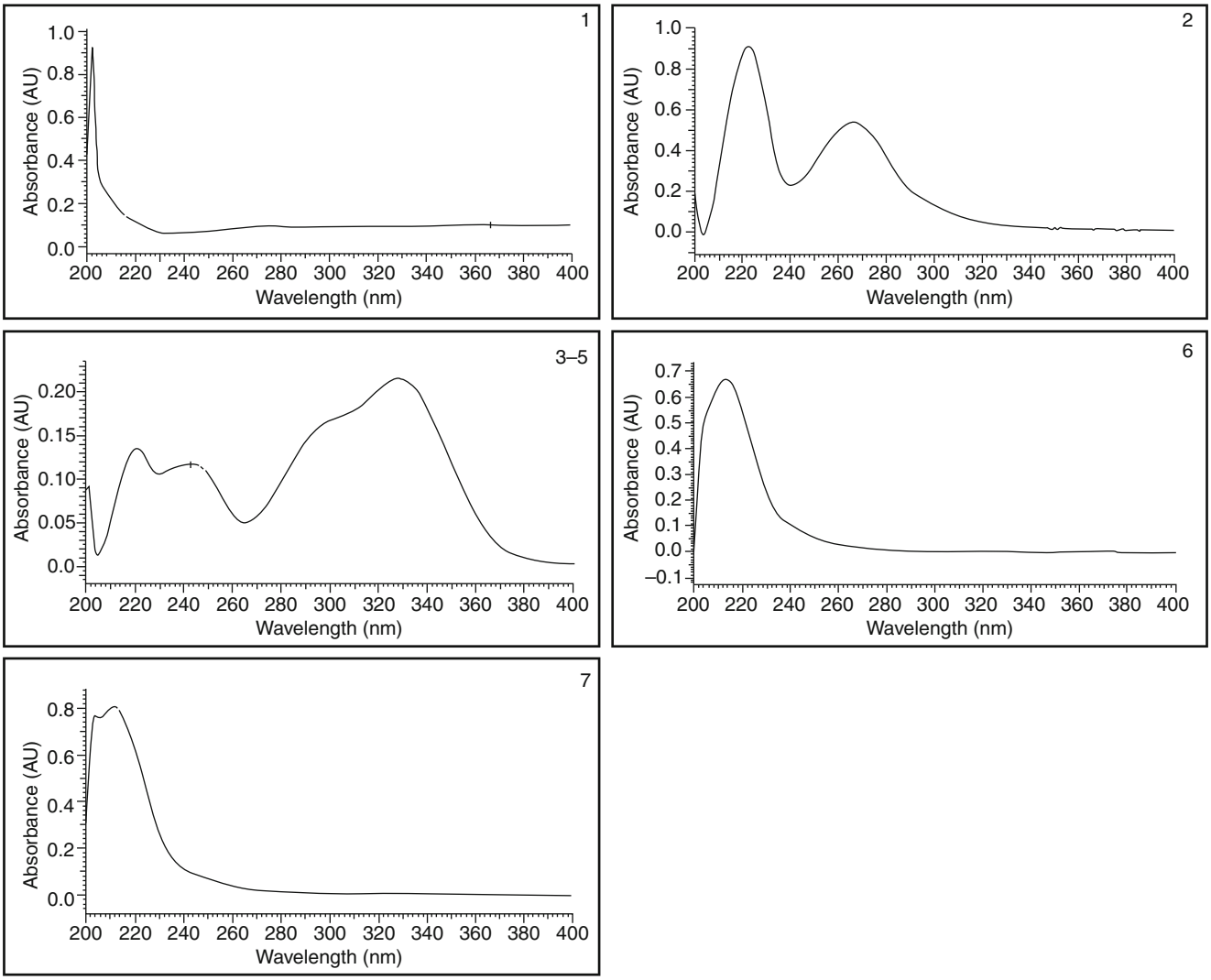


Fig. 5: On line UV-spectra of the main characteristic peaks of Radix Aucklandiae and Radix Vladimiriae

4. Description of the HPLC-Figures

All Radix Aucklandiae extract samples 1, 3 and 7 including Radix Vladimiriae extract sample 8 show the same peak pattern in the Rt-range 5.0 (3.5) to 20.1 (17.0) numbered as **1**, **2**, **3**, **4**, and **5**. According to the UV-spectra the peaks **2**, **3**, **4**, and **5** can be assigned to aromatic compounds [e.g. syringaresinol (**2**), caffeic acid (**3**) and other phenol carboxylic acids (peaks **4** and **5**)]. The peaks **6** and **7** can be assigned to costunolide and dehydrocostus lactone, respectively.

Note: The Chinese Pharmacopeia 2010 demands for Radix Aucklandiae a content not less than 1.8 % of the total amount of costunolide and dehydrocostuslactone, calculated with reference to the dried drug.[1]

Conclusion

According to the TLC und HPLC analyses of the seven extracts of Radix Aucklandiae and one extract obtained from China and labelled as Radix Vladimiriae, both herbal drugs possess the same chemical composition and can be interchanged.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. People's Medical Publishing House, Beijing (2010)
2. Paulus, E., Ding, Y.-H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag, Heidelberg (1987)
3. Hempen, C.H., Fischer, T., A materia medica for Chinese medicine: plants, minerals and animal products, 1st Edition Churchill Livingstone Elsevier, New York (2009)
4. Tang, W., Eisenbrand, G.: Handbook of Chinese medicinal plants, vol. 1. Wiley-VCH Verlag, Weinheim (2011)
5. Stöger, E.A.: Arzneibuch der chinesischen Medizin. Dtsch. Apoth.- Verlag, Stuttgart (2001)
6. Hong Kong Chinese materia medica standards, vol. 2, Chinese Medicine Division – Department of Health – Government of the Hong Kong Special Administrative Region. Hong Kong (2008)
7. Tan, R.X., Jakupovic, J., Jia, Z.J.: Aromatic Constituents from *Vladimiria Souliei*. Planta Med. **56**(5), 475–477 (1990)
8. Chen, H.C., Chou, C.K., Lea, S.D., Yeh, S.F.: Active compounds from *Saussurea lappa* Clarks that suppress hepatitis B virus surface antigen gene expression in human hepatoma cells. Antiviral Res. **27**(1–2), 99–109 (1995)
9. Sun, C.M., Syu, W.J., Don, M.J., Lu, J.J., Lee, G.H.: Cytotoxic sesquiterpene lactones from the root of *Saussurea lappa*. J. Nat. Prod. **66**(9), 1175–1180 (2003)
10. Damre, A.A., Damre, A.S., Saraf, M.N.: Evaluation of sesquiterpene lactone fraction of *Saussurea lappa* on transudative, exudative and proliferative phases of inflammation. Phytother. Res. **17**(7), 722–725 (2003)
11. Lee, M.G., Lee, K.T., Chi, S.G., Park, J.H.: Costunolide induces apoptosis by ROS-mediated mitochondrial permeability transition and cytochrome C release. Biol. Pharm. Bull. **24**(3), 303–306 (2001)
12. Chun, J., Choi, R.J., Khan, S., Lee, D.S., Kim, Y.C., Nam, Y.J., Lee, D.U., Kim, Y.S.: Alantolactone suppresses inducible nitric oxide synthase and cyclooxygenase-2 expression by down-regulating NF-κB, MAPK and AP-1 via the MyD88 signaling pathway in LPS-activated RAW 264.7 cells. Int. Immunopharmacol. **14**(4), 375–383 (2012)
13. Choi, H.G., Lee, D.S., Li, B., Choi, Y.H., Lee, S.H., Kim, Y.C.: Santamarin, a sesquiterpene lactone isolated from *Saussurea lappa*, represses LPS-induced inflammatory responses via expression of heme oxygenase-1 in murine macrophage cells. Int. Immunopharmacol. **13**(3), 271–279 (2012)
14. Yun, Y.G., Oh, H., Oh, G.S., Pae, H.O., Choi, B.M., Kwon, J.W., Kwon, T.O., Jang, S.I., Chung, H.T.: In vitro cytotoxicity of Mokko lactone in human leukemia HL-60 cells: induction of apoptotic cell death by mitochondrial membrane potential collapse. Immunopharmacol. Immunotoxicol. **26**(3), 343–353 (2004)
15. Choi, J.Y., Choi, E.H., Jung, H.W., Oh, J.S., Lee, W.H., Lee, J.G., Son, J.K., Kim, Y., Lee, S.H.: Melanogenesis inhibitory compounds from Saussureae Radix. Arch. Pharm. Res. **31**(3), 294–299 (2008)
16. Zhang, T., Wang, H., Du, G., Chen, R.: Study on chemical constituents from roots of *Saussurea lappa*. Zhongguo. Zhong. Yao. Za. Zhi. **34**(10), 1223–1224 (2009)
17. Robinson, A., Kumar, T.V., Sreedhar, E., Naidu, V.G., Krishna, S.R., Babu, K.S., Srinivas, P.V., Rao, J.M.: A new sesquiterpene lactone from the roots of *Saussurea lappa*: structure-anticancer activity study. Bioorg. Med. Chem. Lett. **18**(14), 4015–4017 (2008)
18. Zhang, T., Ma, L., Wu, F., Chen, R.: Chemical constituents from a portion of ethanolic extract of *Saussurea lappa* roots. Zhongguo. Zhong. Yao. Za. Zhi. **37**(9), 1232–1236 (2012)
19. Duan, J.A., Hou, P., Yang, Y., Liu, P., Su, S., Liu, H.: A new sesquiterpene and other constituents from *Saussurea lappa* root. Nat. Prod. Commun. **5**(10), 1531–1534 (2010)

20. Choi, J.Y., Na, M.K., Hwang, I.H., Lee, S.H., Bae, E.Y., Kim, B.Y., Ahn, J.S.: Isolation of betulinic acid, its methyl ester and guaiane sesquiterpenoids with proteine thyrosine phosphatase 1B inhibitory activity from the roots of *Saussurea lappa* C.B.Clarke. Molecules **14**(1), 266–272 (2009)
21. Yoshikawa, M., Hatakeyama, S., Inoue, Y., Yamahara, J., Saussureamins, A.: B, C, D, and E, new anti-ulcer principles from Chinese Saussureae Radix. Chem. Pharm. Bull. **42**(1), 214–216 (1993)
22. Chen, F., Chan, H.Y., Wong, K.L., Wang, J., Yu, M.T., But, P.P., Shaw, P.C.: Authentication of *Saussurea lappa*, an endangered medicinal material, by ITS DNA and 5S rRNA sequencing. Planta Med. **74**(8), 889–892 (2008)
23. Wagner, H., Bauer, R., Melchart, D., Pei-Gen Xiao, A. (eds.): Chromatographic Fingerprint Analysis of Herbal Medicines, vol. I, pp. 311–324. Staudinger, Springer, Wien/New York (2011)

Radix Platycodonis – *Jiegeng*

Pharmacopoeia:[1]

Pharmacopoeia of the People's Republic of China, English Edition Vol. I, 2010

Official drug:[1, 2]

Platycodon Root is the dried root of *Platycodon grandiflorum* (Jacq.) A. DC. (Fam. Campanulaceae).

The drug is collected in spring and autumn, washed clean, removed from rootlet, peeled when fresh or unpeeled, and dried.

Synonyms:[2, 4, 16]

Platycodon chinensis Lindl., *P. autumnalis* Decne., *P. sinensis* Lem., *Campanula grandiflora* Jacq., *C. glauca* Thunb., *C. gentianoides* Lam.

Origin:[4, 5, 17]

Chinese provinces Anhui, Jiangsu, Sichuan, Shandong, Hebei, Hunan, Hubei, Guangxi. Korea and Japan.

Description of the drug:[1]

Cylindrical or slightly fusiform, gradually becoming tapering downwards, some branched, slightly twisted, 7–20 cm long, 0.7–2 cm in diameter. Externally white or pale yellowish-white, or yellowish brown to greyish-brown when unpeeled; longitudinally twisted-furrowed, with transverse lenticel-like scars and branch root scars, and with transverse striations at the upper part. Sometimes the apex showing a relatively short or inconspicuous rhizome, which is marked by several crescent-shaped stem scars. Texture fragile, fracture uneven, cambium ring brown, bark whitish, with cleft, wood pale yellowish-white. Odour, slight; taste, slightly sweet and then bitter.

Pretreatment of the drug:[1]

Foreign matters are eliminated, washed clean, softened thoroughly, cut into thick slices and dried.

Medicinal use:[9]

Treatment of upper respiratory infections, acute and chronic bronchitis, atopic dermatitis and other skin diseases.

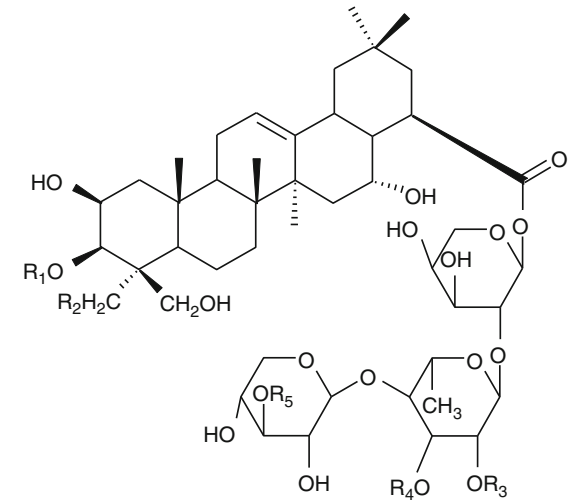
H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7_23, © Springer International Publishing Switzerland 2015

Effects and indications of Radix Platycodonis according to Traditional Chinese Medicine [1–4, 6, 9, 11, 16]

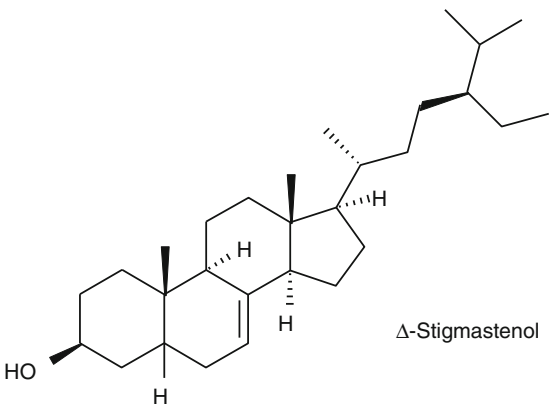
Taste:	Slightly sweet and bitter, pungent, neutral
Temperature:	Warm tendency
Channels entered:	*Orbis pulmonalis*, *Orbis stomachii*, *Orbis intestini crassi*
Effects (functions):	To diffuse the lung, soothe the throat, dispel phlegm, expel pus.
Symptoms and indications:	Cough and profuse sputum, oppression and discomfort in the chest, sore throat and hoarseness, lung abscess with pyemesis. Allows the lung to unfold, cough, breathing difficulties, wind-cold, wind-heat. Bronchitis, asthma, colds, constipation, disturbances of micturition, oedema, water accumulation. Phlegm in the lungs, with cough, abscesses in the lungs. Loss of voice, swelling of the throat, yellow sputum, pulmonary ulcerations, purulent bronchitis, pneumonia, abscesses, purulent sputum, tonsillitis, laryngitis, pharyngitis, dysentery. Unbinds and restrains the intestines. Pulmonary tuberculosis, hyperlipidemia, hypercholesterolemia and inflammatory diseases.

Main Constituents [2, 4, 7–10, 12, 15, 18–20]

Triterpene saponins	Platycodin A, C and D, deapioplatycoside E, deapioplatycodin D3, platycodin D3, platycodin D2, platycodin D, polygalacin D, polygalacin D2, polygalacin D3, platycoside B, C, E, J, F, O, M-3, N platyconic acid B lactone, deapio-platyconic acid B lactone, platyconic acid A, deapio-platycodin D, deapio-platycodin D2, platycodigenin, polygalacinacid A, B and C, 3-O-β-glucosylplatycodigenin
Sterols	Δ-stigmastenol, α-spinasterin, betulin, α-spinasteryl-β-D-glucopyranoside
Other compounds	Polysaccharides [(1 → 2)-β-D-fructan, arabinogalactan (PGAW1), inulin], essential oils, fatty acids



Compounds	R_1	R_2	R_3	R_4	R_5
Platycodin A	Glucosyl	OH	Acetyl	H	Apiosyl
Platycodin C	Glucosyl	OH	H	Acetyl	Apiosyl
Platycodin D	Glucosyl	OH	H	H	Apiosyl
Deapioplatycodin D	Glucosyl	OH	H	H	H



Δ-Stigmastenol

Fig. 1: Formulae of the main compounds of Radix Platycodonis [21]

Pharmacology

In vitro, *in vivo*, clinical research

Antihyperglycemic Activity

- ameliorates obesity and insulin resistance[9]
- improves glucose homeostasis[10]
- improves glucose metabolism[10]
- improves insulin sensivity[10]
- inhibits adipogenesis[9]
- diabetes [9]
- antihyperglycemic[20]

Cardiovascular Activities

- inhibits angiogenesis[16, 17]

Effects on Immune Functions

- anti-inflammatory[9, 10, 20]
- antioxidative/antioxidant[14, 20]
- anti-allergic[3, 13]
- antimycotic[3]
- antipyretic[3]
- antiphlogistic[3]
- antibacterial[3]
- immunological activity[17]
- chemopreventive[20]

Protective and Antiproliferativ Effects

- hepatoprotective[9, 20]
- inducing apoptosis[9]

Other Activities

- calms the respiratory tracts and promotes expectoration[3]
- inhibits gastric secretion[3]

- heals ulcers[3]
- analgesic[3]
- suppresses development of atopic dermatitis-like skin lesions[11]
- inhibitory effect on anaphylactic reaction[13]
- reduces elevation of plasma triglycerides[16]
- neuroprotective[20, 21]

Effects on the Lipid Metabolism

- antihypolipidemic/hyperlipidemic[9, 10]
- anti-inflammation[9, 10, 20]
- causes weight loss in rodents (inhibits lipases)[16]

Note: Secretolytic and hemolytic effects were reported[3, 4]

TLC-Fingerprint Analysis

	Drug samples	**Origin**
1	Radix Platycodonis/*Platycodon grandiflorum*	sample of commercial drug (HerbaSinica, origin: province Hunan, China)
2	Radix Platycodonis/*Platycodon grandiflorum*	sample of commercial drug (Pharmacy of Munich, Germany)
3	Radix Platycodonis/*Platycodon grandiflorum*	sample of commercial drug, Sinomed, TCM-clinic Bad Kötzting
4	Radix Platycodonis/*Platycodon grandiflorum*	Province Sichuan, China
5	Radix Platycodonis/*Platycodon grandiflorum*	Province Anhui, China
6	Radix Platycodonis/*Platycodon grandiflorum*	Province Hebei, China
7	Radix Platycodonis/*Platycodon grandiflorum*	Province Shandong, China

Reference compounds of Fig. 2		**R*f***
T1	Platycodin D	0.58
T2	Saccharose	0.38
T3	Glucose	0.48

1. Extraction: 1.0 g powdered drug is extracted with 20 ml methanol (50 % in water) under reflux for 1 h. The extract is filtered and the filtrate evaporated to about 10 ml. The water extract is shaken two times with 10 ml water – saturated *n*-butanol. The *n*-butanol phase is separated and evaporated to dryness. The residue is diluted with 0.5 ml methanol and filtered over Chromafil® Type 0.20 µm.

2. Reference compounds:
   1 mg platycodin D is dissolved in 1 ml methanol
   1 mg glucose is dissolved in 1 ml methanol
   1 mg saccharose is dissolved in I ml ethanol

3. Separation parameters:

Plate:	HPTLC Silica gel 60 F_{254}, Merck
Applied amounts:	Radix Platycodonis extracts: 5 µl each Reference compounds: 10 µl each
Solvent system:	Chloroform + methanol + water (64 + 50 + 10)
Detection:	Vanillin – Sulphuric acid: 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 min at 105°C and evaluated in VIS.

Description of Fig. 2:

The extracts samples of Radix Platycodonis show in VIS 8–9 grey/blue zones from the start to R*f* = 0.75. With the exception of platycodin D (**T1**, R*f* = 0.58) all other zones can be assigned to the various triterpensaponins listed under the rubric "Main constituents". The saponins above platycodin D contain only 2–5 sugar moieties whereas the other zones in the deep R*f*-range down to the start possess 7–10 sugar moieties. The dominant blue spot centered at R*f* ~ 0.48 and overlapping some triterpensaponins consists of a mixture of saccharose and glucose.

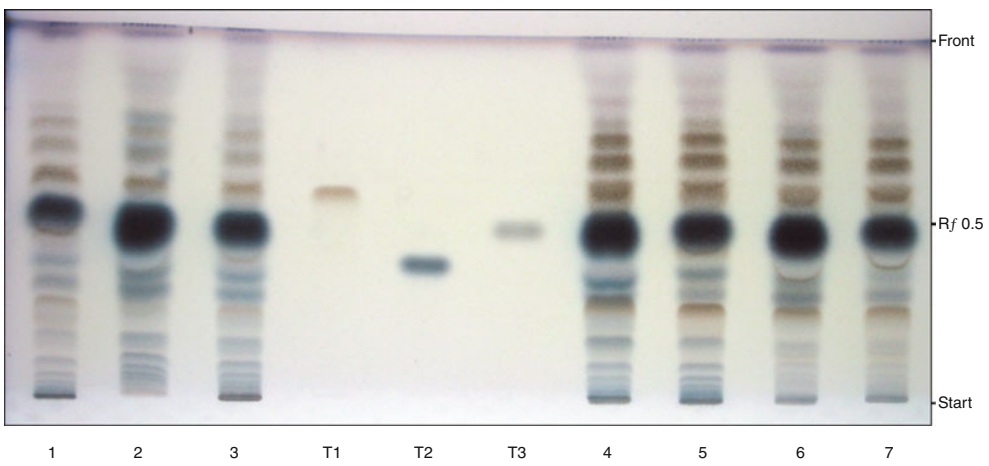


Fig. 2: Thin layer chromatogram of the 50 % methanol extract of Radix Platycodonis, sprayed with Vanillin – Sulphuric acid reagent (VIS)

HPLC-Fingerprint Analysis

1. Sample preparation: The same extracts used for TLC fingerprint analysis

2. Injection volume: Radix Platycodonis extracts: 10 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: 0.1 % phosphoric acid//water (Millipore Ultra Clear UV plus® filtered) B: acetonitrile (VWR)
Gradient:	10 % B for 5 min, 10–20 % B in 5 min, 20–30 % B in 20 min, 30–80 % B in 10 min, 80–95 % B in 25 min Total runtime: 65 min
Flow:	1 ml/min
Detection:	210 nm

Retention times of the main peaks

Peak	**Rt (min)**	**Compound**
1	18.5	Platycodin D

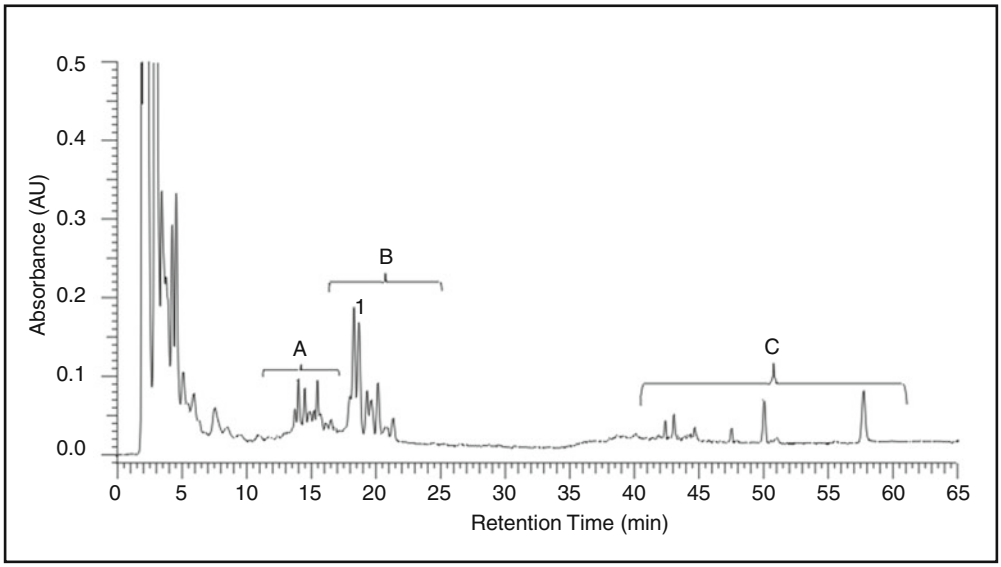


Fig. 3a: HPLC fingerprint analysis of the 50 % methanol extract of Radix Platycodonis (sample 3)

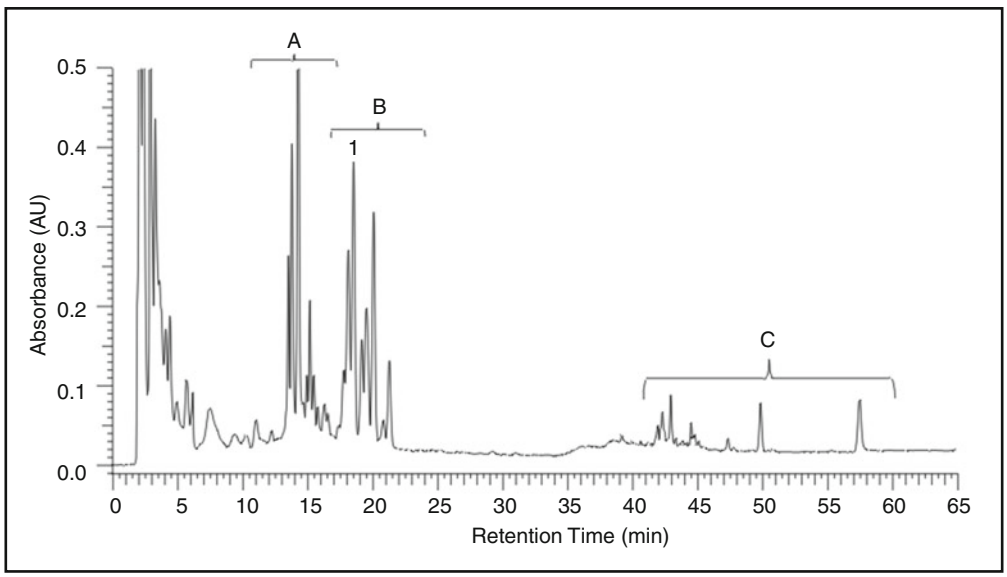


Fig. 3b: HPLC fingerprint analysis of the 50 % methanol extract of Radix Platycodonis (sample 4)

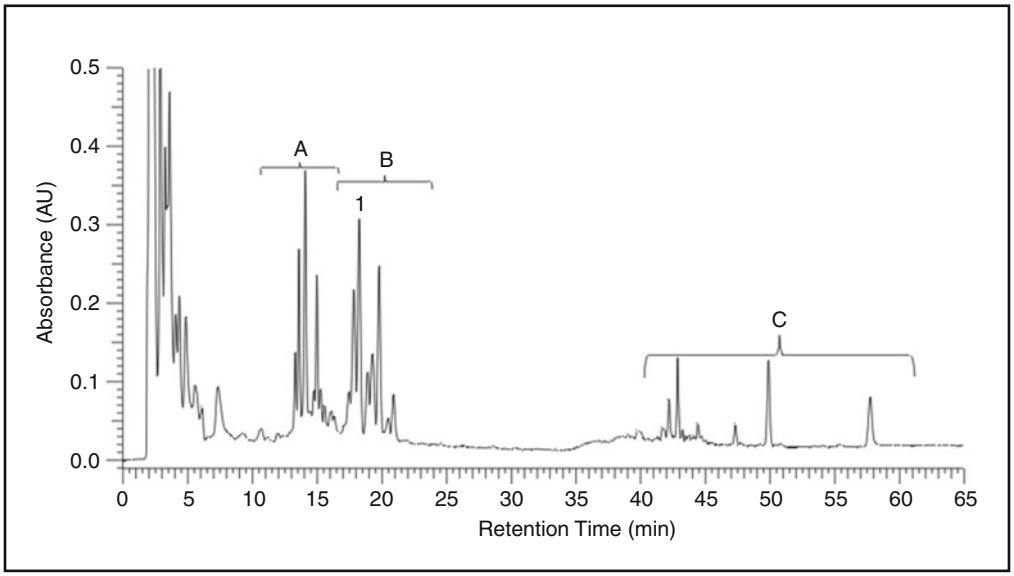


Fig. 3c: HPLC fingerprint analysis of the 50 % methanol extract of Radix Platycodonis (sample 5)

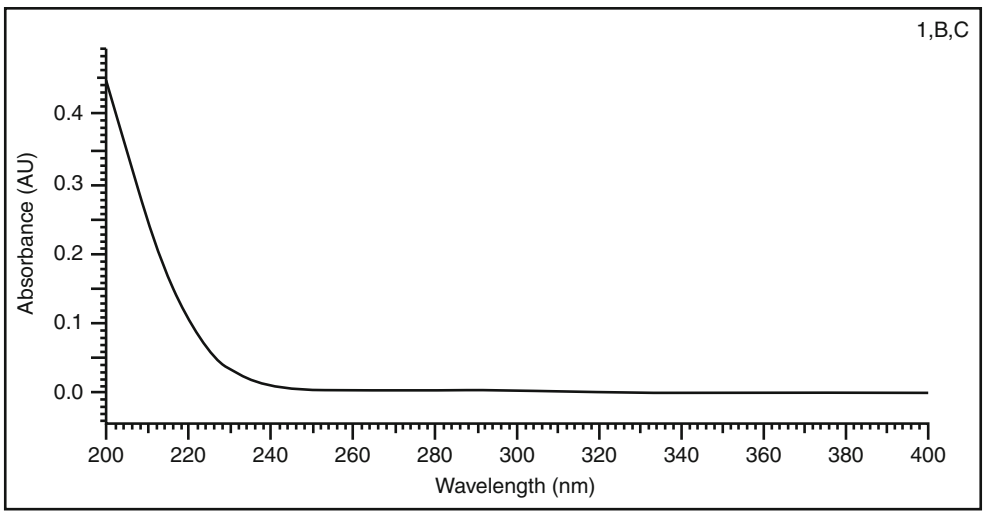


Fig. 4: On line UV-spectra of the main characteristic peak of Radix Platycodonis

4. Description of the HPLC-Figures

   The Peak profiles of the Radix Platycodonis extract samples consists of three characteristic peak ranges. The peak range **A** contains triterpenoid saponins with high sugar content, the second peak range **B** contains the triterpene glycosides with lesser sugar moieties. In this peak accumulation appears platycodin D (**1**) at Rt = 18.5. In the peak range **C** between Rt = 40.0–60.0 the triterpene aglycons and the sterols can be identified as e.g. Δ-stigmastenol or α- spinasterin.

Note: According to the Chinese Pharmacopoeia Radix Platycodonis contains not less than 0.10 % of platycodin D, calculated with reference to the dried drug [1].

Conclusion

The authentication of Radix Platycodonis can be performed very easily using the TLC- and HPLC-methods described in the Monograph.

References

1. Pharmacopoeia of the People's Republic of China, English Edition, vol. 1. China Medical Science Press, Beijing (2010)
2. Keys, J.D.: Chinese herbs – their botany, chemistry and pharmacodynamics. Charles E. Tuttle Company, Rutland/Tokyo (1987)
3. Hempen, C.H., Fischer, T., A materia medica for Chinese medicine: plants, minerals and animal products, 2. edn, Churchill Livingstone Elsevier, New York (2007)
4. Paulus, E., Ding, Y.H.: Handbuch der traditionellen chinesischen Heilpflanzen. Karl F. Haug Verlag GmbH & Co. KG, Heidelberg (1987)
5. Porkert, M.: Klinische Chinesische Pharmakologie. Verlag für Medizin Dr. Ewald Fischer, Heidelberg (1978)
6. Geng, J., Huang, W., Ren, T., Ma, X.: Materia medica der Chinesischen Arzneimitteltherapie. Verlag für Gesundheitliche Medizin Dr. Erich Wühr GmbH, Bad Kötzting (1993)
7. Zhao, Z.Z.: An illustrated Chinese materia medica in Hong Kong. School of Chinese Medicine, Hong Kong Baptist University, Hong Kong (2004)
8. He, M., Li, Y., Yan, J., Cao, D., Liang, Y.: Analysis of essential oils and fatty acids from Platycodi Radix using chemometric methods and retention indices. J. Chromatogr. Sci. **51**(4), 318–330 (2013)
9. Lee, C.E., Hur, H.J., Hwang, J.T., Sung, M.J., Yang, H.J., Kim, H.J., Park, J.H., Kwon, D.Y., Kim, M.S.: Long-term consumption of Platycodi Radix ameliorates obesity and insulin resistance via the activation of AMPK pathways. Evid. Based Complement. Altern. Med. 2012 ID 759143 (2012)
10. Kwon, D.Y., Kim, Y.S., Ryu, S.Y., Choi, Y.H., Cha, M.R., Yang, H.Y., Park, S.: Platyconic acid, a saponin from Platycodi Radix, improves glucose homeostasis by enhancing insulin sensitivity in vitro and in vivo. Eur. J. Nutr. **51**(5), 529–540 (2012)
11. Choi, J.H., Han, E.H., Park, B.H., Kim, H.G., Hwang, Y.P., Chung, Y.C., Lee, Y.C., Jeong, H.G.: Platycodi Radix suppresses development of atopic dermatitis-like skin lesions. Environ. Toxicol. Pharmacol. **33**(3), 446–452 (2012)
12. Zhou, L., Tang, Y., Wu, D., Fan, X., Ding, A.: Comparative analysis of volatile oils of Wuao decoction and its major constituing herbs by GC-MS. Zhongguo Zhong Yao Za Zhi **34**(10), 1245–1250 (2009)
13. Han, E.H., Park, J.H., Kim, J.Y., Chung, Y.C., Jeong, H.G.: Inhibitory mechanism of saponins derived from roots of *Platycodon grandiflorum* on anaphylactic reaction and IgE-mediated allergic response in mast cells. Food Chem. Toxicol. **47**(6), 1069–1075 (2009)
14. Fu, X.J., Liu, H.B., Wang, P., Guan, H.S.: A study on the antioxidant activity and tissues selective inhibition of lipid peroxidation by saponins from the roots of Platycodon grandiflorum. Am. J. Chin. Med. **37**(5), 967–975 (2009)
15. Li, W., Zhao, L.C., Wang, Z., Zheng, Y.N., Liang, J., Wang, H.: Response surface methodology to optimize enzymatic preparation of deapio-platycodin D and platycodin D from Radix Platycodi. Int. J. Mol. Sci. **13**(4), 4089–4100 (2012)
16. Twiner, E.M., Liu, Z., Gimble, J., Yu, Y., Greenway, F.: Pharmacokinetic pilot study of the antiangiogenic activity of standardized Platycodi Radix. Adv. Ther. **28**(10), 857–865 (2011)

17. Xu, Y., Dong, Q., Qiu, H., Cong, R., Ding, K.: Structural characterization of an arabinogalactan from *Platycodon grandiflorum* roots and antiangiogenic activity of its sulfated derivate. Biomacromolecules **11**(10), 2558–2566 (2010)
18. Fu, W.W., Fu, J.N., Zhang, W.M., Sun, L.X., Pei, Y.H., Liu, P.: Platycoside O, a new triterpenoid saponin from the roots of *Platycodon grandiflorum*. Molecules **16**(6), 4371–4378 (2011)
19. Li, W., Zhang, W., Xiang, L., Wang, Z., Zheng, Y.N., Wang, Y.P., Zhang, J., Chen, L.: Platycoside N: a new oleanane-type triterpenoid saponin from the roots of *Platycodon grandiflorum*. Molecules **15**(12), 8702–8708 (2010)
20. Choi, Y.H., Yoo, D.S., Cha, M.R., Choi, C.W., Kim, Y.S., Choi, S.U., Lee, K.R., Ryu, S.Y.: Antiproliferative effects of saponins from the roots of *Platycodon grandiflorum* on cultured human tumor cells. J. Nat. Prod. **73**(11), 1863–1867 (2010)
21. Son, I.H., Park, Y.H., Lee, S.I., Yang, H.D., Moon, H.I.: Neuroprotective activity of triterpenoid saponins from platycodi radix against glutamate-induced toxicity in primary cultured rat cortical cells. Molecules **12**(5), 1147–1152 (2007)

Index

H. Wagner et al. (eds.), *Chromatographic Fingerprint Analysis of Herbal Medicines*, Vol. 3,
DOI 10.1007/978-3-319-06047-7, © Springer International Publishing Switzerland 2015