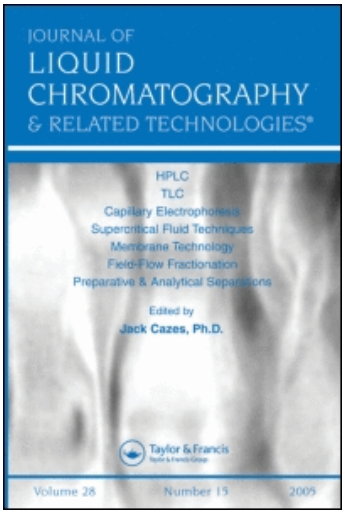

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 28 August 2002

To cite this Article Cimpan, Gabriela and Gocan, Simion(2002) 'ANALYSIS OF MEDICINAL PLANTS BY HPLC: RECENT APPROACHES', Journal of Liquid Chromatography & Related Technologies, 25: 13, 2225 — 2292
To link to this Article: DOI: 10.1081/JLC-120014003
URL: http://dx.doi.org/10.1081/JLC-120014003

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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES
Vol. 25, Nos. 13–15, pp. 2225–2292, 2002

ANALYSIS OF MEDICINAL PLANTS BY HPLC: RECENT APPROACHES

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ABSTRACT

Medicinal plants are widely used, nowadays, for the preparation of various pharmaceutical forms, or as food additives; therefore, the research focuses on validated methods of analysis by single or hyphenated HPLC. This review includes references to recent work for the analysis of flavonoids, terpenes, alkaloids, coumarins, alkamides, polyacetylene and some other compounds of interest which can be found in medicinal plants. Details of experimental conditions (sample preparation, chromatography) are included.

Key Words: Medicinal plants; HPLC; Review; Flavonoids; Phenolic acids; Terpenes; Terpenoids; Carotenoids; Alkaloids; Coumarins; Alkamides; Polyacetylenes

INTRODUCTION

Medicinal plants are widely used, nowadays, for the preparation of different pharmaceutical forms, or as food additives. Their routine analysis was mainly

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DOI: 10.1081/JLC-120014003
1082-6076 (Print); 1520-572X (Online)

based on TLC, but single or hyphenated HPLC methods are being validated.[1–6] It is well known that the content and composition of active compounds in herbal medicines are strongly influenced by many factors, such as genotype, climate, harvest, and preparation processes. Therefore, the analytical investigation should take into consideration these conditions, and should also be accurate, reproducible, with low detection limits. The main problem in separating complex mixtures, such as plant extracts, by chromatography, is in finding systems which have specific selectivities. Coupling normal phase with reversed phase liquid chromatography can be useful for the separation in oa single run for many compounds, from non-polar to very polar. Often, thin-layer chromatography (TLC) can give important information about a plant extract; it is used for the preliminary screening of the separated compounds prior to HPLC. Complex mobile phase gradients are often used for the separation of compounds with different polarities.

The traditional methods of preparing plant extracts include steam distillation and organic solvent extractions using percolation, maceration, or Soxhlet techniques. These methods are sometimes very complex, including a number of steps, and significant quantities of organic solvents. Care should be taken when selecting the temperature and when solvent removal is important (extract concentration, evaporation to dryness), because these steps can lead to the degradation of the compounds or to loss of material. Supercritical fluid extraction (SFE) shows several advantages in the extraction of different compounds from natural matrices, such as suitability of the extraction controlled by diffusion in plant tissues, and easy modification of solvent strength by changing the temperature or pressure of the experimental conditions. Carbon dioxide is the most commonly used supercritical fluid because of its low toxicity, low cost, low critical temperature, non-flammability, and easy removal from the system.[7] Microwave assisted extraction of compounds of interest from a medicinal plant have also been used but, in this case, the extraction should be performed in alternative steps, power on-heating, power off-cooling, as the solvent should not boil.

Hyphenated techniques, such as HPLC–NMR or HPLC–MS play an increasing role in the analysis of natural products, since they permit the fast screening of the crude biological extracts or pharmaceutical preparations. The HPLC–NMR technique can give valuable information about the structures of investigated compounds by the observation of exchangeable protons (e.g., N−H, O−H) usually in deuterated aprotic solvents. The investigations can also be performed in protic solvents, such as $^{1}H_2O$, with a solvent suppression scheme, and the results can be compared with the HPLC–MS data obtained by analyzing the exchange of $^{1}H_2O$ against $^{2}H_2O$. Two-dimensional NMR (2D-NMR) experiments such as TOCSY (total correlation spectroscopy) and NOESY (nuclear Overhausser effect spectroscopy) have been used for the investigation of *Triphyophyllum peltatum* (Dioncophyllaceae).[8]

There are situations where both NMR and MS data are needed for structure determination (e.g., positional isomers of substituents on an aromatic ring). Newer HPLC compatible interfaces allow the application of multiple hyphenation techniques like HPLC–NMR–MS, and more ambitious combinations with UV-diode array spectrophotometry or IR are under investigation. These techniques have an enormous potential for the rapid investigation of plant extracts.[9]

Ecdysteroids from crude extracts of *Silene otites, Silene nutans*, and *Silene frivaldiskiana* were investigated by a combination of spectrometers coupled with HPLC, which enables the on-flow collection of UV, ^{1}H NMR, IR, and MS spectra. The multiple hyphenation of several spectroscopies in a single system has the potential of better identification of compounds from plant extracts. The dried plant material was extracted with 96% ethanol, the extract evaporated to dryness, and the residue was dissolved in a small volume of deuterated methanol (CD_3OD) before HPLC. The extracts were subjected to a qualitative screening for the identification of ecdysteroids by normal-phase HPLC (on silica gel) with UV detection using a solvent system of dichloromethane–2-propanol–water (125 : 40 : 3 or 125 : 20 : 1.5, v/v). The pump of the chromatographic system delivered D_2O at 0.8 mL/min to a C8 XTerra column (150 × 4.6 mm, 5 μm) or to a C_{18} XTerra column (150 × 4.6 mm, 5 μm). Columns were placed in an oven for chromatography at elevated temperatures (160°C). The eluent from the oven was cooled immediately in a water bath kept at 0–4°C using ice. The D_2O was maintained in a liquid state at temperatures greater than 100°C by the back pressure generated by the PEEK tubing connecting the column to the various detectors. A small part of the effluent was subject to MS, and the rest of the flow (ca. 95%) was directed to an FTIR. The effluent from the FTIR was directed to a UV-diode array detector where the UV spectra were collected over the wavelength range 188–1000 nm. From the UV detector, the effluent was sent to the NMR flow probe. The method enables full spectral characterization of ecdysteroid-containing plants although, in some cases, lower sensitivities can be obtained.[10]

The review is focused on some recent development of plant material analysis by HPLC during recent years. However, the published material related to medicinal plants and liquid chromatography is numerous and an extensive approach of the plant analysis by HPLC is beyond the aim of the present paper.

FLAVONOIDS

Flavonoids are benzopyrane derivatives with a phenyl group in the second position; they are natural polyphenols, widely spread among different plant species. Flavonoids can be O-glycosides, usually in the positions three or seven. According to the degree of oxidation, the flavonoids can be classified as: calcones, flavanones, flavones, flavonols, catechins, terpenylflavonols, isoflavonols, etc.

Many flavonoids show activity related to the sanguine capillary, have an antioxidant activity, and are involved in the oxidation processes which take place in the cell. Their biological activity is very important and many allopathic or homeopathic drugs which are flavonoid-rich are on the market. The consumer demands are for naturally processed, additive-free, safer products. Organic foods are in great demand, and the necessity for new and more reliable analytical methods is of concern. Modern pathological prevention has been linked to a number of natural phenolic biomolecules from fruits and vegetables. From this point of view, the qualitative and quantitative analysis of a plant extract becomes very important.[11]

Flavonoids can be extracted from plants by various methods, most of them involving an extraction in ethanol, followed by a precipitation, at room temperature, or by the help of lead acetate, and purification. Flavonoids are generally stable compounds and may be extracted from the dried, ground medicinal plant material and pharmaceutical preparation with cold or hot solvents (aqueous mixtures with ethanol, methanol, acetone, and dimethylformamide). Modern separation methods involve chromatography on magnezol, cellulose, or polyamide packings. Other methods involve successive extractions with different solvents and chromatographic separation on a specific fraction. Usually, the aglycons can be extracted in low-polarity solvents, but the extractions are not very selective, so a further step must involve defatting with petroleum ether or hexane. The flavonoid recovery will not be very high because of the large number of steps involved. The use of a large number of methods, individually or hyphenated, has been published (TLC, HPLC, GC, electrophoresis, gravimetry, spectrophotometry, coulometry, IR, NMR, MS) for the quantification of flavonoids from plants. Table 1 includes a selection of the most recent work in this field.

Flavonoids have a diphenylpropane skeleton. The monomeric flavonoids commonly found in food can be divided into five subclasses: anthocyanidins, catechins (flavan-3-ols), flavanones, flavones, and flavonols. Glycosylation is often on C3 and, less often, on C5, C7, and C4′. The most common sugar is glucose, but other sugars are found, including rhamnose, galactose, xylose, rutinose, and neohesperidose.[39,40]

Thirty two biophenols and polyphenols were analysed in *Olea europaea* L. (olives).[41] The plant material (fruits), was extracted by following four different procedures, so that different biophenols can be analysed. Procedure A involves the extraction of olives with 6 N HCl, then with ethyl acetate until negative $FeCl_3$ and H_2SO_4 tests. The organic phase was treated with acetonitrile–hexane 1 : 1 (v/v), resulting in two immiscible organic phases and an insoluble precipitate. The biophenols were contained in the acetonitrile phase. In procedure B, the isolation of soluble and alkalyhydrolysable phenolic fractions was performed as follows: olives were left at room temperature for 24 h in

Table 1. Flavonoids Analysed by HPLC (F = Flow Rate; DAD = Diode-Array Detection; All Proportions Are v/v Unless Otherwise Stated)

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Anthemisnobilis L.	Apigenin-7-O-glycoside Apiegin-7-O-apioglucoside Luteolin-7-O-glucoside	Dried flowers were extracted with 60% methanol for 15 min at 60°C; extract evaporated to dryness under vacuum; residue dissolved in methanol and percolated through a Sep-Pak C_{18} cartridge.	Column: C_8 Aquapore RP 300 (250 × 4 mm, 7 μm). Isocratic elution with 2-propanol/water (15 : 85) F = 2 mL/min; Detection: UV spectra 230–430 nm.	12
Arachis hypogaea, Hemizonia increscens, Eriodictyon glutinosum, Thymus vulgaris	Enantiomeric flavanones: eriodictyol, homoeriodictyol and naringenin.	Dried sample was extracted with methanol with the exception of *T. vulgaris* which was extracted in *t*-butylmethylether. Extracts were dried in vacuum at 40°C. Residues were dissolved in methanol and percolated by SPE on C18 cartridge. The extract was racemisated at elevated temperatures in aqueous alcoholic solution.	Column packed with cellulose triacetate supported on silica gel diol (125 × 4.6 mm). Gradient 1: A = *n*-hexane/2-propanol (9 : 1); B = methanol/2-propanol (2 : 1) Starting: 80% A and 20% B; after 1 min linear gradient 20% B to 60% B in 20 min. Gradient 2: A = water/methanol/acetic acid (90 : 10 : 1) and B = methanol. Initial 90% A and 10% B for	13

(continued)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
			5 min.Then 10% B to 80% B in 30 min F = 1 mL/min. Detection: UV 295 nm	
Astragalus mongolicus Bge. and *A. membranaceus* Bge. Var *Mongolicus*	Eighth flavonoids were identified. This is the first report of flavonoid glycoside malonates in the two species, and malonate calycosin-7-O-β-D-glucoside-6″-O-malonate is a new compound, completely identified structurally.	The sample of ground dried roots was extracted with ethanol/water (9 : 1) at room temperature using sonication for 60 min. After filtration the sample is ready for analysis.	Column: Symetry C_{18} (150 × 2.1 mm, 5 μm); guard column: Symetry C_{18}, (20 × 3.9 mm, 5 μm); temp. = 45°C Linear gradient: A = water containing 0.25% acetic acid; B = acetonitrile containing 0.25% acetic acid. 18–42% B in 40 min. Detection: UV–Vis spectra 200–500 nm; LC-electro-spray interface (ESI)-MS in positive ion mode	14
Coffea arahica, Coffea canephora	7 chlorogenic acids (5-CQA, 5-FQA, 3,4-diCQA, 3,5 = diCQA, 4,5-diCQA, 3,4-CFQA, 4,4-CFQA, where CQA = caffeoylquinic acid, FQA = feruloylquinic acid).	Ground coffee extracted 4 times with aqueous ethanol 80%. Alcohol fractions were combined and concentrated under reduced pressure. Followed extractions with petroleum ether,	Column: Lichrosorb RP-18 precolumn and a Hibar Lichrospher RP-18 column (250 × 4.6 mm, 5 μm) Mobile phase: (A) 2 mM H_3PO_4 and (B) MeOH, the elution programe at	15

		anhydrysation and extraction with chloroform; another extraction was made with ethyl acetate to extract the chlorogenic acids. The extraction of total chlorogenic acid was also performed.	room temperature (25°C) was: 0–10 min 60% B (isocratic); 10–25 min 100% B (linear gradient) F = 1 mL/min Detection: HPLC–DAD (diode array detection) UV spectra 220–340 nm HPLC–MS and comparison with the reference spectra.	
Crataegus	Catechins ((−)-epicatechin Procyani dimmers: (−)-epicatechin-(4$\beta \rightarrow$ 8)- (−)-epicatechin; (−)-epicatechin-4$\beta \rightarrow$ 6)-(−)-epicatechin; (+)-catechin-(4$\alpha \rightarrow$ 8)-(−)-epicatechin; and trimer (−)-epicatechin-(4$\beta \rightarrow$ 8)- (−)-epicatechin-(4$\beta \rightarrow$ 8)-(−)-epicatechin.	Powder of dried leaves was macerated with aqueous acetone 70% for 15 min. The suspension was transferred to a column and percolated. The extract solution was evaporated under vacuum at 30°C, then diluted with methanol to produce a 50% aqueous solution and filtered. The methanolic solution was evaporated under vacuum, immediately frozen and lyophilized. Final purification by SPE on Sep-Pak C_{18} cartridges.	Column: LiChrosorb RP-18 (250 × 4 mm, 5 μm) and a guard column (10 × 4 mm) of the same material; temp. 24°C. Gradient elution: A = methanol; B = 0.5% o-phosphoric acid in water; (18% A–82% B to (24% A–76% B) in 10 min, then constant from 10–20 min, (24% A–76% B) to (40% A–60% B) in 25 min, then 100% A from 45–50 min.	16

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
			F = 1.0 mL/min Detection at 280 and 220 nm. Recorded spectra in the range 190–600 nm.	
Crataegus monogya Jacq	Vitexin after hydrolysis Quercetin after hydrolysis	Dried leaves, flowers and fruits were refluxed with 80% methanol 60 min, evapored under vacuum. Then SPE on Bond Elut C_{18}. Hydrolysation with 25% HCl refluxed for 90 min.	Column: Hypersil ODS (100 × 4 mm, 5 μm); temp. = 25°C. For vitexin: Mobile phase: A = THF/2-propanol/acetonitrile (18 : 8 : 3) and B = 0.5% *o*-phosphoric acid. Isocratic elution (0–13 min 12% A in B); F = 1 mL/min. UV detection at 336 and 260 nm. For quercetin: Gradient elution 0–15 min 30% A = methanol in B = 0.5% *o*-phosphoric acid to 55% A in B UV detection at 370 and 260 nm.	17

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Dissotis rotundifolia T.	Four C-glycosilflavone: isoorientin, orientin, vitexin and isovitexin.	The whole plant was air-dried and grounded, then successively extracted at room temp. with dichlormethane, then with methanol. Methanolic extracts were dissolved in methanol/water (50 : 50). A small quantity of the plant material was extracted with ethanol/water (50 : 50) at room temp. for 24 h. This extract was dissolved in methanol/water (50 : 50). "Sirop de Dissotis" was extracted for 24 h with 2-butanol. The solvent was evaporated and the residue dissolved in methanol/water (50 : 50).	HPLC-UV-TSP-MS-MS Column: Nova-Pak RP-18 (300 × 3.9 mm, 4 µm) and Nova-Pak guard precolumn. Isocratic elution: acetonitrile/water (14 : 86) and trifluoracetic acid (0.05%) to reduce tailing. F = 0.9 mL/min Spectral window 200–400 nm. HPLC-UV-TSP-MS System A: Nucleosil RP-18 (250 × 4 mm; 5 µm); Gradient elution: acetonitrile/water 15 : 85 to 25 : 75 in 25 min and trifluoracetic acid (0.05%); F = 1 mL/min. Spectral window UV 200–400 nm and MS 120–800 amu. System B: Nova-Pak RP-18 4 µm (300 × 3.9 mm) and Nova-Pak guard	18

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
			precolumn. Isocratic elution: acetonitrile/water (14 : 86) trifluoracetic acid (0.05%); F = 0.8 mL/min. Spectral window UV: 200–400 nm The MS system consisted of a triple stage, quadrupole MS equipped with a TSP-2 interface; spectral window 100–600 amu.	
Equisetum arvense L.	Isoquercitrin Quercetin-3-O-soforoside Astragalin	Dried leaves were extracted with 60% methanol for 15 min at 60°C; the extract dried under vacuum, and the residue dissolved in methanol and percolated through a Sep-Pak C_{18}.	Column: C_8 Aquapore RP 300 (250 × 4 mm, 7 μm). Isocratic elution with 2-propanol/water (15 : 85); F = 2 mL/min; Detection UV spectra 230–430 nm.	12
Eucalyptus camaldulensis	Flavonoids Flavone: luteolin, apigenin, chrysin, flavone Flavanones: eriodictyol, naringenin, hesperetin, sakuranetin, pinocem-	Wood, bark and leaves samples were grounded and extracted with methanol/water (80 : 20) at room temp. for 24 h.	Column: Hypersil ODS (200 × 4 mm); temp. 30°C Gradient elution: A = methanol/*o*-phosphoric acid (999 : 1); B = water/	19

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	brin, isosakuranetin, flavone Dihydroflavonolols and flavonols: taxifolin, myricetin, fisetin, quercetin, kaempferol Isoflavones: prunetin tectochrysin Flavone Glycosides: vitexin, luteolin-7-glucoside Flavanone Glycosides: hesperidin, naringin Flavonol Glycosides: hyperoside, rutin, isoquercitrin, kaempferol-7-neohesperidoside, quercitrin.	The extract was filtered, and methanol removed under vacuum distillation. The aqueous solution was extracted with diethyl ether, the etheric extract was dried and the residue redisolved in methanol.	*o*-phosphoric acid (999 : 1) Starting: 80% B+20% A; in 0–40 min to 100% A and kept at 100% a from 40–45 min. F = 1 mL/min Detection at 325 nm.	
Gentiana ottonis	Flavone C-glycoside; swertisin	The whole plant was extracted with methanol.	Column: Nova Pak C_{18} (150 × 3.9 mm), and Nova Pak C_{18} precolumn. Gradient elution: acetonitrile/water 5 : 95% to 65 : 35% in 50 min. Detection at 254 nm. LC-UV spectrum of swertisin LC/TSP-MS spectrum of swertisin LC/TSP-MS/MS of $[M\text{-}120]^+$ spectrum LC/^{1}NMR with the stop-flow; spectrum of swertisin.	20

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Ginkgo biloba L.	Quercetin-3-O-glicoside 5 compounds Kaempferol-3-O-glicoside 5 compounds Isorhamnetin-3-O-glycoside	Dried leaves were extracted 15 min under reflux with 60% aqueous acetone.	Column: C_8 Aquapore RP 300 (250×4 mm, 7 μm) Linear gradient: (A) water/2-propanol (95 : 5); (B) 2-propanol/THF/water (40 : 10 : 50) from 20 to 60% B in 40 min F = 2 mL/min UV spectra 230–430 nm.	12
G. biloba L.	Biflavones: bilobetin, ginkgetin, isoginkgetin and sciadopitysin	*G. biloba* L. extract was dissolved in ethanol, centrifugated at 5,000 g for 3 min and supernatant was filtered through a 0.45 μm membrane.	HPLC: column, Novapak C_{18}; Mobile phase: THF/propanol/water (21 : 10 : 69); F = 1 mL/min Detection UV at 270 nm.	21
G. biloba L.	Five new flavonol glycosides: K 3-[2″-glu]rha; K 3-[2″-6‴-{*p*-(7⁗-glu) coumaroyl}-glu]rha; Q 3-[2″-glu]rha; Q 3-[2″-6‴-{*p*-(7⁗-glu) coumaroyl}-glu]rha; Q 3-[2″-6‴-*p*-coumaroy)-glu]rha-7-glu. K = kaempferol Q = quercetin	Powder of dried leaves was extracted with petrol followed by acetone and finally methanol. Each extract was concentrated by rotary evaporation at 30°C. Methanolic extract was partitioned between water/*n*-butanol. *n*-Butanol extract was	CC: Sephadex LH-20 (800×49 mm) and Separalyte RP-18, 40 μm (713×18.5 mm); Mobile phase: methanol/water gradient. HPLC: Hypersil ODS (100×4 mm, 3 μm); Linear gradient: A = methanol and	22

		fractionated by Craig distribution. The purification by CC (column chromatography) lead to five pure flavonol glycosides.	B = 0.5% *o*-phosphoric acid in water. Gradient from 38 to 48.2% A in 12 min. Structural characterization by: ^{1}H and ^{13}C NMR, UV and FAB-MS spectra FAB = Fast Atom Bombardment.	
G. biloba L. *Calendula officinalis*	Kampferol-3-O-[6‴-O-(*p*-coumaroyl)-α-D-glucopyranosyl-(1 → 4)-α-L-rhamnoside] (I) and Isorhamnetin-3-O-2^{G}-rhamnosyl-rutinoside (II)	Compounds (I) and (II) were isolated from a purified extract of *G. biloba* and *C. officinalis* The methanolic solutions of compounds (I) and (II) were used for chromatography	Column: Aquapore RP-300 (220 × 4.6 mm) Eluents: 2-propanol/THF/water (10 : 5 : 85), and 2-propanol/THF/ ammonium acetate pH 4.5 (10 : 5 : 85). F = 1.8 and 1.2 mL/min, respectively. LC-TSP-MS, full-scan spectra *m/z* 260–800	23
G. biloba L. and Terapeutical extract	33 flavonoids	Method A: refluxed with methanol/25% HCl (70 : 10) for 60 min; the extract was dried under vacuum; further purification on Bond Elut C_{18} Method B: extraction by a high-speed mixer	Column: Nucleosil 100-C_{18}, 3 μm; temp. 30°C Ternary gradient: (a) A = methanol, B = THF, C = 0.5% *o*-phosphoric acid; F = 1 mL/min Detection at 360 nm	24

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		(20,000 rpm) with 80% ethanol 2 min; evaporated under vacuum; Solid phase extraction (SPE) Bond Elut C_{18}	(b) A = 2-propanol/THF (25 : 65), B = acetonitril, C = 0.5% *o*-phosphoric acid F = 1 mL/min Detection at 360 nm.	
Humulus lupulus, H. japonicus, (Cannabinaceae)	22 flavonoids (chalcones, flavanones), xanthohumol	The plant material was extracted with MeOH–HCOOH (99 : 1) for 2 h. A portion of clear extract was directly analysed by HPLC–MS.	HPLC-MS-MS Column: reversed-phase C18 (250 × 4 mm, 5 µm) Linear solvent gradient from 40 to 100% B (MeCN) in A (1% aq. HCOOH) over 15 min, followed by 100% B for 7 min. F = 0.8 mL/min MS with atmospheric pressure chemical ionization source operated in posigtive ion mode. Argon–nitrogen (9 : 1) was used as target gas in the collision cell.	25
Hypericum perforatum L. Var. *angustifolium* DC	Flavonoids: quercetin quercitrin, hyperoside, isoquercitrin and biapigenin	Dried flower were extracted in methanol, filtered and immediately injected in the sample injector.	Column: SGX C_{18}, 7 µm (150 × 3 mm) Gradient elution: A = acetonitrile/water/	26

			o-phosphoric acid (19 : 80 : 1) B = acetonitril/ methanol/*o*-phosphoric acid (59 : 40 : 1) 0 min 25% B, 5 min 30% B, 10 min 55% B, 15 min 100% B, 25 min 100%, 30 min 25% B. Detection: UV–Vis spectra	
Hypericum perforatum L (St. John's wort)	Chlorogenic acid, hyperforins, hypericins, lipids, quercetin derivatives, shikimic acid, sucrose.	A commercial sample of the extract of *H. perforatum*, lyophilized extract. The dried extract 2.5 mg/mL was dissolved in a mixture of HPLC grade solvents (methanol/ acetonitrile/water, acidified to pH 2 with formic acid, 3 : 1 : 1), sonicated and before HPLC.	Column: Protein C4 (250 × 0.5 mm, 5 μm) UV monitorization at 230, 254, 270, 350, 590 nm and detection at 270 nm. Parallel NMR experiments as an alternative for the routine HPLC analysis, with very good identification results.	27
Hypericum perforatum L.	Flavonoids, naphthodianthrones, and other constituents.	100 mg crude plant extract was solved in 1 mL of a mixture of 80% of d-methanol (CD_3OD) in D_2O, and was used for analysis. For flavonoid	Column: Knauer packed with Apex-1 ODS (120 × 4 mm, 5 μm) Mobile phase: (A) acetonitrile–0.1% acetic acid (5 : 95); (B) acetonitrile–	28

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		concentration, 50 mg of crude extract was extracted with 75% methanol and retained on a SPE cartrige, and eluted with methanol. The methanolic fraction containing flavonoids was prepared as above for LC-NMR-MS analysis.	20 mM ammonium acetate (95 : 5); all buffers were solutions in D_2O. Linear gradient: time 0, 10% B; at time 10 min, 20% B; at 20 min, 100% B; at 30 min, 100% B; at 32 min, 10% B (run time 40 min). F = 1 mL/min Coupling with NMR, and MS/MS (neg. electrospray ionization).	
Matricaria recutita L. (or *Chamomilla recutita* L.)	24 flavonoids, polyphenolic compounds.	100 mg dried head flowes were extracted with 80 mL methanol for 1 h and filtered.	Column: Inertsil ODS-3 (3.0 × 150 mm, 3 μm) with a precolumn (2.0 × 10 mm, 5 μm) packed with the same material; temp. = 30°C. Mobile phase: (A) water adjusted to pH 2.5 by CF_3COOH, (B) acetonitrile; a concave solvent gradient from 92% A in B to 30% A in B within 50 min.	29

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			F = 0.4 mL/min Detection: 335 nm, UV-Vis spectra 200–450 nm.	
Origanum vulgare	Flavones (5,7,3,4-tetra-OH-flavones, 5,7,4′-tri-OH-flavones, 5,7,3′-tri-OH-4′Ome-Flavone)	The aerial parts were air dried, and kept in a dark, cool place until the analysis. The sample was extracted with acetone containing 5% of 25% HCl. The extract was extracted again with ethyl acetate. The ethyl acetate fractions were collected, dried with anhydrous sodium sulphate, filtered and evaporated to dryness under low pressure. The residue was dissolved in 2 mL of mixture of methanol and chloroform (1 : 1), and this solution was used for analysis of flavone aglycons.	Column: RP-C18 (250 × 4.6 mm, 5 µm), temp 30°C Mobile phase: (A) 5% CH_3COOH and (B) CH_3OH, and the elution programe for screening the extracts was: 0–5 min 70% A; 10–15 min 60% A; 20–25 min 50% A; 30–35 min, 40% A; 40–45 min 20% A. F = 1.3 mL/min Diode array detection, UV 254, 348 nm.	30
Paliurus spina-christi Mill. (Rhamnaceae) extracts	Flavonoids	Powdered plant material was extracted with methanol under reflux for 15 min then filtered, solvent evaporated and the residue	Column: LiChroCart RP-8 supersphere (250 × 4 mm, 10 µm) Mobile phase: Solvent A: H_2O–H_3PO_4 (99 : 1),	31

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		dissolved in a small amount of methanol.	solvent B: acetonitrile; gradient 0–30 min 86–83% A, 14–17% B. F = 1 mL/min Detection: UV 254 nm.	
Phaseolus vulgaris v. *vulgaris*	Polyphenolic compounds, flavonoids, and flavonols.	Fresh samples (pod and seeds) and commercial canned beans were extracted at room temperature, in the absence of light, with aqueous methanol (20% v/v) containing 1% 2,6-di-*tert*-butyl-4-*p*-cresol using an ultrasonic bath. The extracts were stored at −20°C prior to HPLC.	Column: Nucleosil 120 C18 (25 × 0.46 cm i.d., 5 µm) Mobile phase: A: aqueous 0.01 M phosphoric acid, and B: 100% methanol. Gradient: 5% B as initial conditions; 50% for 10 min; 70% B for 5 min; 80% b for 5 min; and finally 100% B for 5 min. F = 1 mL/min. Detection: 280 nm. Absorption spectra recorded in the range 210–350 nm.	32
Pisum sativum and *Vigna radiata*	Isoflavonoids	Seeds were desintegrated in a mixer and extracted with 80% ethanol for 72 h at 4°C. The extract was analysed directly by	Column: Nucleosil 100–5 C18 (250 × 4 mm, 5 µm); temp. 40°C Mobile phase: (A) 40% methanol in water; (B)	33

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		radioimmunoassays or fractionated by chromatography. Before chromatography, the extract was evaporated to dryness and redissolved in water.	100% methanol. Gradient (all steps linearly): 0 min, A = 100%, B = 0%; 10 min, B = 20%; 25 min, B = 50%; 30 min, B = 100%, next 10 min B = 100%, then step to A = 100% for 10 min. F = 1 mL/min Detection: UV 254 nm.	
Plants available as food in supermarkets	17 monomeric flavonoid aglycones representative for all five common subclasses.	Standard solutions and extracts of flavonoids from the plants.	HPLC-DAD Column: Zorbax Eclipse XDB-C18 (25034.6 mm, 5 μm) with guard column (12.5 × 4.6 mm) of the same stationary phase; temp. 30°C. Different gradient mixtures of water, methanol, and acetonitrile, each containing 0.05% (w/w) trifluoroacetic acid (TFA). F = 1.0 mL/min. Detection: 210, 260, 278, 370, 520 nm.	34

(*continued*)

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Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Radix puerariae	Isoflavone (puerarin)	Powdered plant material is covered with 30–70% ethanol or water and a microwave-assisted extraction was performed for 30 min. The extract was filtered and analysed by HPLC.	Column: HP ECLIPSE-XDB-C18 Column (150 mm × 4.6 mm); temp. 40°C Mobile phase: methanol/ water (22 : 78) F = 0.4 mL/ min Detection: UV 250 nm.	35
Tilia cordata	Q-3-glu-7-rha K-3-glu-7-rha Q-3,7-di-rha K-3,7-di-rha	Powdered leaves were suspended in 50% methanol and left overnight at room temperature. The solution was evapored to dryness in vacuum and dissolved in methanol.	Column: Aquapore RP-300 (220 × 4.6 mm) Eluents: 2-propanol/THF/ water (10 : 5 : 85), and 2-propanol/THF/ ammonium acetate pH 4.5 (10 : 5 : 85). F = 1.8 and 1.2 mL/min, respectively. Detection: UV 360 nm LC-TSP-MS, full-scan spectra *m/z* 260–800 in PI, except for (II) in NI.	23
Trifolium pratense L. (leguminosae)	14 isoflavone glycoside malonates and 6 acetyl glycosides.	Dried powdered plant material was extracted by sonication with methanol/ water (7 : 3), diluted with water and loaded to	HPLC-MS I. Column: Zorbax Exlipse XDB C8 (150 × 4.6 mm, 5 μm); temp. 40°C Mobile	36

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		a SPE (reversed phase) cartridge. Isoflavones were eluted with 1 mL of 60 and 80% methanol containing 2% ammonium hydroxyde. The samples were evaporated to dryness and dissolved in 0.5 mL of mobile phase, then analysed by HPLC.	phase: (A) 0.2% acetic acid, (B) acetonitrile; a linear gradient from 15–50% B in 20 min, up to 55% B in the next 25 min and followed by a negative gradient up to 15% B in 30 min. $F = 0.8$ mL/min II. Column: MetaChem Polaris C18A (150×2.0 mm; 3 μm); temp. 40°C Mobile phase: a linear gradient from 15 up to 25% B in 36 min, up to 55% B in 90 min and followed by a negative gradient up to 15% B in 100 min. $F = 0.3$ mL/min Detection: 280 nm; DAD 190–400 nm for spectra recording MS- positive mode; gas temp. 300°C, scan 100–800 *m/z*.	
Vaccinium species	Flavonols (myricetin, quercetin, kaempferol) in blueberries.	Berries frozen in liquid nitrogen and kept in dry ice until stored at −80°C prior to analysis (2–3 weeks). The frozen berries were extracted with	Column: LiChroCART (125×3 mm, Purospher RP-18e, 5 μm), guard column (4×4 mm, 5 μm) Gradient elution using 1% formic acid (A) and	37

(*continued*)

Table 1. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		50% aqueous methanol, containing 0.3 mg of morin as an internal standard and 20 mg of tert-butylhydroquinone as antioxidant at 35°C in a water bath for 2 h.The acid hydrolysis of the flavonoid glycosides with 6 M HCl, and the flavonol aglycons were extracted in ethyl acetate. Ethyl acetate was evaporated to dryness under vacuum, the residue dissolved in 0.5 mL methanol and filtered prior to HPLC analysis.	acetonitrile (B): 0–10 min, 5–40% of B in A, 10–20 min, 40–70% of B in A, 20–22 min, 70–90% of B in A (F = 0.5 mL/min); 22–25 min, 90–5% of B in A F = 0.4 mL/min with the exception above Detection: 360 nm UV.	
Vitis vinifera L.	Flavan fingerprints were determined for different plant extract.	Total extract was concentrated under vacuum on a rotary evaporator at 30°C. pH was corrected to 7.0. The neutral and acidic phenolic constituents were separated using SPE on C_{18} Sep Pak cartridges.	Column: Lichrosorb RP C-18 (250 × 4 mm, 10 μm) Gradient elution: acetic acid (0% 10 min; 0–5% in 10–60 min) with continuous in flow of methanol (10%). The flow rate was 1.2–2.0 mL in 60 min. Detection at 280 nm.	38

argon saturated 2 N NaOH, under argon. The aqueous phases were acidified at pH 2.0 with conc. HCl at a temp below 4°C, treated with anhydrous sodium sulphate, and evaporated to dryness under vacuum at room temp. The residue was treated as described in procedure A and the acetonitrile fraction was subjected to HPLC. Procedure C (isolation of cytoplasmatic biophenols): the olives were left at room temp. for 24 h in CH_2Cl_2. Aqueous and organic phase were separated. The aqueous phase contained water-soluble components. Charcoal was added to the CH_2Cl_2 water-free solution. The resulting suspension, stratified on a Gooch funnel, was salt and simple sugar removed by 5% water and 10% EtOH elution, whereas discontinuous gradient elution, with EtOH from 20 to 90% (300 mL fractions each, were increased each time 10% EtOH content), separated glucosidic biophenols. Collected ethanolic fractions and volatile material evaporation resulted in crude biophenols, which were chromatographed on Si gel in n-BuOH saturated with H_2O, performing the separation according to polarity. Successive separations were obtained on Si gel with $CHCl_3$–MeOH in a 9 : 1–7 : 3 (v/v) ratio, depending on component polarity. Isolated glucosidic biophenols were obtained by medium pressure chromatography columns with a discontinuous gradient of H_2O–MeOH as eluent (25 mL fractions, increasing 5% MeOH content). Cytoplasmatic biophenols were identified by comparison with authentic samples, according to their HPLC, ^{1}H NMR, and ^{13}C-NMR spectra. Olives were frozen under liquid nitrogen and freeze dried (procedure D), then extracted with methanol–acetone (1 : 1) and centrifuged under 4°C. The supernatant containing soluble-esterified biophenols was extracted with hexane for defatting, then with ether/ethyl acetate. The acetate extract was evaporated to dryness, redissolved in a small quantity of methanol, and analysed by HPLC. The residue was analysed for insoluble-bond biophenols, by direct hydrolysis with 1 N NaOH under similar conditions as for the soluble esters, then analysed by HPLC. Chromatography was performed on a Bakerbond reversed phase C_{18} column (250×4.6 mm, 5 μm), with a precolumn (50×4 mm) containing the same stationary phase at 25°C. Mobile phase was water/acetic acid, pH 3.1 (98 : 2) (solvent A), and methanol (solvent B). The gradient started with 95% A-5% B for 3 min, 80% A-20% B in 15 min, 80% A-20% B for 2 min, 60% A-40% B in 10 min, 50% A-50% B in 10 min, 100% B in 10 min, then kept constant for 10 min. The flow rate was 1 mL/min, with diode-array detection (DAD) at 230, 278 nm. For NMR, the solvents were: D_2O, internal standard HDO at 4.70 ppm from TMS; CD_3OD, internal standard TMS; $CDCl_3$, internal standard TMS.[41]

Isoflavonoids are typically stored as 7-O glycosides, whilst secretion of aglycones is elicited by different physiologic or pathologic situations.[42] Isoflavonoids can be found in legumes and other plant species such as *Iridaceae, Rosaceae, Podocarpaceae, Moraceae, Armanthaceae*[43,44] and influence the endocrine system in animals.[45] More than 3000 flavones and more than 700

known isoflavones exist in plants. Their structures are based on a 3-phenylbenzpyrone (3-phenylchromone) group. The structure differs in the degree of methylation, hydroxylation, and glycosylation.[46]

Antioxidants (flavonoids) from *Thymus vulgaris* L. were analysed by HPLC–CL (chemiluminescence) on an Alltima C_{18} analytical column (250 × 4.6 mm, 5 μm), with photodiode array detector in the range 210–450 nm. The mobile phase was (A) water–acetonitrile (3 : 1) acidified with 0.25% glacial acetic acid; (B) acetonitrile acidified with 0.25% glacial acetic acid; linear gradient: A decreased over 40 min from 95 to 10%, was held for the following 15 min, and then returned from 10 to 95% in 5 min. The flow rate was 0.85 mL/min with fluorescence quenching detection obtained by on-line post-column addition of CL reagents. Several chemiluminescence reagents were investigated: hydrogen peroxide-1 M NaOH mixture; horseradish microperoxidase sodium salt MP-11 in disodium tetraborate buffer, and brought to pH 10 by addition of 0.1 M of NaOH; and Luminol 1.6 mM in methanol. The plant material was dried under forced ventilation at room temperature for two days, then ground and extracted with methanol for 72 h. The extract was filtrated and vacuum dried at 45°C, and the obtained extract was dissolved in MeOH–H_2O (1 : 1) for the HPLC–DPPH (2,2′-diphenyl-1-picrylhydrazyl) analysis and in acetonitrile–H_2O (1 : 1) for the HPLC–CL (chemiluminescence) analysis. The mobile phases for the HPLC–DPPH analysis were mixtures methanol–water or acetonitrile-water acidified with acetic acid. The reagent was added to the organic solvent and the detection wavelength was 517 nm.[47]

The flavonoids' UV-Vis spectra show strong absorption at the following wavelengths: flavones, biflavones (310–350 nm) and (250–280 nm); isoflavones (310–330 nm) and (245–275); flavonols (350–385 nm) and (250–280 nm); flavanones (310–330 nm) and (275–295 nm); chalcones (365–390 nm) and (240–260); and anthocyanins (465–560) and (265–275).[48]

Phenolic acids and polyphenols are compounds which can be found together with the flavonoids in plants. Some of phenolic acids show potential immunomodulating activity (e.g., rosmarinic, gentisic, chlorogenic, and caffeic acids). Polyphenols are polyphenylcarboxylic acids, and can be found in plants as esters, glycosides, or acylglycosides, especially in the species *Asteraceae* and *Labiatae*. Some examples are: caffeic acid, cichoric acid, rosmarinic acid, frulic acid, *p*-cumaric acid, and chlorogenic acids. Polyphenols show antioxidant and hepatoprotective activity and are assumed to contribute to the health of fruits and vegetables. Polyphenols can be extracted with a binary mixture of ethanol–water and analysed by a variety of analytical methods. Tables 2 and 3 show a selection of recent methods applied for the analysis of phenolic acids and polyphenols in plants.

Polyphenols (procyanidins) from *Vitis vinifera* were extracted from the fresh plant material with acetone for 24 h, filtered, then evaporated under reduced

Table 2. Phenolic Acids in Plants Analysed by HPLC (F = Flow Rate, DAD = Diode-Array Detector; All Proportions Are v/v Unless Otherwise Stated)

Medicinal Plant	Analyte	Sample Preparation	Chromatographic Conditions	Ref.
Betula pubescens *B. pendula*	Phenolics with non-flavonoid structure: 1-O-galloyl-β-d-(2-O-acetyl)-glucopyranose, 1-(4″-hydroxyphenyl)-3′-oxopropyl-β-d-glucopyranose, gallic, chlorogenic, neo-chlorogenic, *cis*- and *trans*-forms of 3- and 5-*p*-coumaroylquinic acids.	Fresh leaves were transferred in methanol, ground with an Ultra-turrax for 3 min then let to stand for 60 min with continuous stirring. The mixture was centrifuged and pellet was re-extracted twice with 15 mL of 80% methanol. The combined extract was evaporated in a rotary evaporator. The dry residue was dissolved in water and centrifuged.	Analytical and preparative HPLC Column: Spherisorb ODS-2 (250 × 4 mm, 5 μm). Mobile phase: A = 5% formic acid; B = acetonitrile. Gradient: 100% A, 0–5 min; 0–30% B in A for 5–60 min; 30–60% B in A for 60–70 min and 60% B in A for 70–80 min. F = 1 mL/min Detection with DAD, 280 nm, and the acquisition of UV spectra (210–370 nm) Preparative column: μBondapak C_{18} (30 × 19 mm) Gradient elution: A = 2.5% acetic acid; B = 95% ethanol Spectroscopic UV, ^{1}H and ^{13}C NMR, MS techniques were applied for the identification of these phenolics.	49

(continued)

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Table 2. Continued

Medicinal Plant	Analyte	Sample Preparation	Chromatographic Conditions	Ref.
Echinacea purpurea	*p*-Cumaric acid	A sample of press juice was taken and filtered.	Column: RP-18 LiChroCart (250 × 4 mm, 5 μm) Gradient elution: A = acetonitrile and B = 0.1 M *o*-phosphoric acid. 0–30 min (15% A–85% B) to (30% A–70% B) F = 0.7 mL/min UV detection at 310 nm.	50
	Cicoric acid	Dried sample (root, flower, stem and leaf) were extracted with 80% methanolic solution and the mixture subsequently sonicated for 10 min.	Column: RP-C_{18} (150 × 4.6 mm, 5 μm) and C_{18} guard column, at 40°C. Gradient elution: A = acidified (1% of 0.1 M *o*-phosphoric acid) methanol, B = water; 0–20 min (10% A–90% B) to (50% A–50% B) F = 1 mL/min Detection 330 nm.	51
E. purpurea *E. palida* *E. angustifolia*	Caffeic acid Caftaric acid Cicoric acid Chlorogenic acid Echinacosid	Two consecutive Soxhlet extractions of the herb with methanol, each of 1 h, the extract evaporated under vacuum at 40°C.	Column: LiChrospher 100 CH-18(2) Hibar (125 × 4 mm, 5 μm); guard column LiChroCart (4 × 4 mm, 5 μm) with LiChrospher 100 CH-18.	52

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			Gradient elution: A = water/ 0.1 N *o*-phosphoric acid (99 : 1), B = acetonitrile/ 0.1 N *o*-phosphoric acid (99 : 1). (95% A–5% B) to (75% A–25% B) in 20 min. F = 1 mL/min Detection at 330 nm.	52
Echinacea species: *E. purpurea, E. angustifolia, E. montana, E. multiflora, E. commutata, E. umbellate*	Phenolic acids: Protocatechuic Chlorogenic, *p*-Hydroxibenzoic Vanillic, Caffeic, Syryngic, *p*-Coumaric, Ferulic	Milled aerial parts of plant were extracted with methanol in a Soxhlet for 6 h. Methanolic extract were evaporated in a rotary vacuum evaporator at 50°C. Residues were dissolved in 30% methanol. The plant extract was purified on ODS and quaternary amine SPE columns.	Column: ODS Hypersil (200 × 4.6 mm, 5 μm). Elution under isocratic conditions: methanol/ water/ acetic acid (25 : 75 : 1). F = 1 mL/min, Detection at 254 nm.	53
Eleutherococcus senticosus (Rupr. et Maxim.) Maxim. = *Acanthopanax senticosus* (Rupr. et Maxim.) Harms	Free phenolic acids	Dried, pulverised samples of plant roots were extracted twice with methanol, for 1 h, under reflux. SPE extraction. The combined methanolic extracts were concentrated, and retained on a SPE C18 cartrige. Eluates to pH 7.0–7.2 with	HPLC Column I: ODS Hypersil (200 × 4.6 mm, 5 μm); room temp. Mobile phase: methanol–water–acetic acid (23 : 77 : 1) Column II: Symmetry™ C18 (250 × 4.6 mm,	54

(continued)

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Table 2. Continued

Medicinal Plant	Analyte	Sample Preparation	Chromatographic Conditions	Ref.
		5% sodium bicarbonate aqueous solution and passed through a quaternary amine SPE-cartridge. The analytes were eluted with 0.2 M phosphoric acid and methanol (1 : 1 v : v). The eluates were adjusted to pH 3 with 1 M sodium hydroxide, and analysed by RP-HPLC. LL extraction. The methanolic extracts were concentrated in vacuum, diluted with hot water, filtered, then washed with petroleum ether and the organic layer rejected. The aqueous solution was extracted with diethyl ether. The etheric layer was treated with 5% sodium bicarbonate, acidified with 36% HCl until pH 3.0, then extracted again with diethyl ether. Each of the	5 μm); temp. 30°C. Mobile phase: methanol–0.001 M phosphoric acid (23 : 77) F = 1 mL/min Photodiode array detector, 254 and 280 nm Fluorescence detector: excitation at 230 and 265 nm, emission at 350 nm.	

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		ether extracts was evaporated to dryness, and the residues were dissolved in methanol (10 mL), giving fractions of free phenolic acids.		
Krameria triandra	Nine rhataniaphenol constituents were determined in petroleum extract, in chloroform and in a commercial extract.	The roots were dried, finely powdered and exhaustively extracted with acetone/water (8 : 2). The acetone was removed in vacuum. The aqueous residue was extracted three times with petrol, then with chloroform and butylacetate, and concentrated in vacuum. The crude extracts was dissolved in methanol.	LC/MS experiments were carried out on a mass spectrometer equipped with a particle beam interface [60°C; ionization mode, 70 eV electron inpact (EI)]. Column: Ultrasphere ODS (250 × 4 mm, 5 μm) Linear gradient: A = 0.045 M acetic acid/ methanol (1 : 1), B = acetonitrile; (50% A–50% B) to (10% A–90% B) in 45 min. F = 1 mL/min. FAB mass spectra (NI) FAB-MS/MS (NI mode detection; daughter and parent ion) spectra were obtained.	55
Lamiaceae plants (*Satureja hortensis,*	Caffeic and rosmarimic acid	The plant material was air-dried at room temperature in the dark. Two types of	HPLC-DAD: Column: Nucleosil 188–5 C18 (125 × 4 mm, 5 μm)	56

(*continued*)

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Table 2. Continued

Medicinal Plant	Analyte	Sample Preparation	Chromatographic Conditions	Ref.
Origanum vulgare, Origanum onites, Satureja thymbra, Salvia triloba)		Extracts were used: crude ethanolic extracts obtained in a Soxhlet apparatus (6 h) and methanolic extracts obtained by using a sequence of nonpolar and polar solvents.	Mobile phase: solvent A was 1% formic acid, and solvent B was acetonitrile. The elution system was as follows: 0–10 min, 10–13% B in A; 10–25 min, 13–70% of B in A; 25–29 min, 70% B in A; 29–30 min, 70–10% of B in A; 30–40 min, 10% of B in A F = 1 mL/min NMR: 1H-13C and the result compared with the HPLC analysis.	
Lamiaceae family *Salvia officinalis* L., *Melissa officinalis* L., *Mentha piperita* (L.) Hudson, *Thymus vulgaris* L., *Lavandula officinalis* Chaix, Rosmarinus officinalis L., *Origanum majorana* L.,	Free phenolic acids	Dry, pulverized samples were refluxed with methanol for 1 h, the extract concentrated under reduced pressure. The dry residue was diluted with 30% methanol and passed through an C18 cartridge. The eluate containing phenolics was adjusted to pH 7.0–7.2 with 5% sodium bicarbonate aqueous solution	Column: ODS Hypersil (200 × 4.6 mm, 5 μm) Mobile phase: isocratic acetonitrile/water/acetic acid (20 : 80 : 1) or methanol/water/acetic acid (25 : 75 : 1). F = 1 mL/min Detection: 280 nm for rosmarinic acid, and 254 for other phenolic acids.	57

Hyssopus officinalis L., Ocimum basilicum L. and Satureja hortensis L.		and passed through quaternary amine microcolumns. The phenolic acids were desorbed with 0.2 M phosphoric acid–methanol (1 : 1), adjusted to pH 3 with 1 M NaOH and analysed by HPLC.		
Malus sylvestris (apple) and *Pyrus communis* (pear)	Phenolic acid and flavonoids.	Fruits were extracted with aqueous acetone (70%) or ethyl acetate. Optional, the extracts were evaporated to dryness, redissolved in a small amount of water, retained on a silica gel C18 SPE cartridge, and the compounds of interest eluted with methanol.	HPLC-MS Column: Aqua C18 (240 × 4.6 mm, 5 μm) with aguard column C18 ODS (4 × 3.0 mm); temp. 25°C. Mobile phase: (A) 2% acetic acid in water, (B) 0.5% acetic acid in water and acetonitrile (50 : 50). The gradient program was: 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10% B (5 min). F = 1 mL/min Detection: DAD with simultaneous monitoring at 280 nm (catechins, proanthocyanidins and benzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols). MS: negative mode, m/z range 100–800.	58

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Table 3. Terpenoids Analysed by HPLC (F = Flow Rate; DAD = Diode-Array Detection; All Proportions Are v/v Unless Otherwise Stated)

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Achillea asiatica	Sesquiterpenoids, guaianolides	Dried plant material was extracted with dichloromethane by ultrasonification for 10 min at room temp. Extract evaporated to dryness at 40°C, redissolved in dichloromethane–aqueous methanol (1 : 1), and evaporated at 40°C till only the methanol fraction remain. This containes the polar sesquiterpenes.	Column: Li-Chrospher 100-RP8 (250 × 4.0 mm, 5 μm) Zorbax SB-C8 (75 × 4.6, 3.5 μm) Guard column: LiChrospher 100-RP8 (4 × 4 mm, 5 μm) Mobile phase: various methanol–water mixtures F = 1 mL/min DAD, monitoring wavelength 220 nm.	65
Aesculus species, *A. parviflora, A. rubicunda*	Carotenoids: violaxanthin, neoxanthin, aesculaxanthin, lutein, β-carotene, β-cryptoxanthin β-citraurin and their *cis*-isomers.	Fresh plant material was extracted three times with methanol, and finally twice with diethylether. The extract was saponified in ether with 30% KOH–MeOH at room temp. The saponified pigments were stored in benzene at −20°C under nitrogen, away from light.	Column: Chromsyl C18 (250 × 4.6 mm, 6 μm) endcapped. Mobile phase: (A) 12% water in methanol, (B) methanol, (C) 30% dichloromethane in methanol in the following linear gradient: 100 A 2 min, to 50 A-50 B, 16 min, to 100 B 8 min, to 100 C, 7 min, 100 C 8 min. F = 1.2 mL/min Diode-Array Detection: 300–500 nm.	66

Alomia myriadenia Schultz-Bip. ex Baker (Asteraceae)	Diterpene	The dried plant material was macerated in ethanol, then extracted with hexane followed by CH_2Cl_2. The fractions were further purified by preparative HPLC.	Preparative and analytical HPLC Column: Shimpak ODS RP-18 (250 × 4.6 mm—analytical, 250 × 20 mm—preparative, 5 μm) Eluent: acetonitrile–water (7 : 3) F = 1, respective 15 mL/min Detection: UV 254 nm.	67
Astrogalus species: *A. melanophrurius, A. oleifolius, A. cephalotes, A. microcephalus, A. trojanus*	Triterpene glycosides (saponins)	1 g of fine powdered root was extracted three times with 10 mL methanol, sonicated for 10 min and centrifugated. The combined extracts were evaporated to dryness under a stream of nitrogen, and the residue redissolved in 2 mL of methanol.	Column: Phenomenex Luna C18 column (150 × 4.6 mm, 5 μm). Mobile phase: water (A) and acetonitrile (B), in the following gradient elution: 72 A/28 B to 70 A/30 B in 30 min, in 12 min to 62 A/38 B, then in 10 min to 30 A/70 B, and held at this composition for the another 5 min. F = 1 mL/min Detection: UV at 200 nm and evaporative light scattering detector, operated at 40°C and the nitrogen pressure at 2.4 bar.	68

(*continued*)

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Table 3. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Cimicifuga racemosa	Triterpene glycosides: Cimiracemoside, H26-deoxyactein, and actein.	1 g *C. racemosa* root powder was extracted three times with 3 mL methanol by sonication for 10 min. After centrifugation, the extracts were combined on a 10 mL volumetric flask and adjusted to the final volume with methanol.	HPLC-ELS (Evaporative Light Scattering Detection Column: Discovery C-18 (150 × 4.6 mm, 5 µm) Mobile phase: water (A), acetonitrile (B) and reagent alcohol (C), with gradient elution: from 58A/21B/21C within 35 min to 52A/14B/34C, using a slightly concave gradient profile (Waters curve type 7). F = 1 mL/min. Detection: UV 200 nm. The ELS detector was set up to 40°C.	69
Euphorbia leuconeura, Euphorbiaceae	Diterpene esters of the tigliane and ingenane types.	Latex drained from the plant was extracted with methanol, centrifuged and the supernatant diluted with 200 mM NH_4OAc, and used for HPLC.	HPLC-MS-MS Column: Supersphere RP-18 (C-18) (250 × 4 mm, 4 µm) Mobile phase: linear water–acetonitrile gradient from 0 to 88% acetonitrile over 25 to 60 min. F = 0.8 mL/min Detection at 220 nm MS:	70

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			the flow from the column was split 50%; N_2 was used as nebulizer, curtain and collision gas in the MS-MS mode.	
G. biloba L.	Diterpenes: Ginkgolide A Ginkgolide B Ginkgolide C Sesquiterpene: Bilobalide	Dried, powdered, purified *G. biloba* L. leaf extract was treated with boiling ethyl acetate. Then concentrated and extracted with chloroform, cooled, decanted and filtred. The solvent removed and the residue extracted with diethyl ether and dried over anhydrous sodium sulphate, then distilled under reduced pressure.	Column: LiChrosorb RP-18 Hibar (250 × 4 mm, 10 μm); Isocratic systems: A: water/methanol (70 : 30), 1 mL/min B: water/acetonitrile (80/20), 1 mL/min C: water/ tetrahydrofuran 80 : 20), 1 mL/min D: water/methanol/ tetrahydrofuran (75 : 5 : 15), 1.5 mL/min. The best resolution was obtained with system D. Detection: 220 nm.	71
G. biloba L.	Ginkgolide A, Ginkgolide B, Ginkgolide C, Bilobalide B	Extracts were purchased from different commercial sources. The extract were dissolved in 50% acetone.	Column: Microsorb Spherical C_{18} (100 × 4.6 mm, 3 μm) Semipreparative column C_{18} (250 × 10 mm, 5 μm); Mobile phase: 2-propanol/water (10 : 90) F = 1 mL/min Detection at 220 nm.	72

(*continued*)

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Table 3. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
G. biloba L.	Ginkgolide A, Ginkolide B, Ginkgolide C, and Ginkgolide J Bilobalide	Leaves were dried as soon as possible after collection for 24 h at 70°C in an oven with forced ventilation. The dried leaves were pulverised and refluxed in 10% aqueous methanol for 15 min. The hot solution is filtred. The leaves were washed with 2% aqueous methanol, then extracted with 15% aq. methanol. After filtration the solution is transferted to the polyamide column and drawn through the two columns at a rate of 1 drop/s and a SPE on C_{18}.	Column: Spherisorb C_{18}, column (250 × 10 mm, 5 µm). Mobile phase: A: water/methanol/THF (7 : 2 : 1); B: water/acetonitrile/THF (7 : 2 : 1); C: water/methanol (67 : 33). F = 1.0 mL/min (analytical), 4.0 mL/min (preparative) Detection: refractive index (RI) detector 3.10^{-5} full-scale, at 20°C.	73
G. biloba L.	Ginkgolide A, Ginkolide B, Ginkgolide C, and Ginkgolide J Bilobalide	Leaves of *G. biloba* L. were extracted with methanol/water (1 : 1). Methanolic extract was then partitioned between ethyl acetate and water. The organic phase was analysed.	LC-TSP-MS Column: Nucleosil C_{18} (125 × 4 mm, 5 µm) Mobile phase: methanol/water (40 : 60) F = 1 mL/min Detection: UV 220 nm TSP: vaporizer 62°C; source	74

			225°C; filament on, 600 V and 200 mA; buffer, 0.5 M ammonium acetate at 0.2 mL/min; 1.2 s/scan.	
Helicteres angustifolia	Lupane type triterpenoids	The dried plant material was extracted with methanol, and the extract concentrated under reduced pressure. The residue was suspended in water and partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH.	Column: Inertsil ODS-2 (250 × 4.6 mm, 5 μm) Linear solvent gradient of A-B (A, acetonitrile; B, water) as follows: 0 min, 85 : 15 and 25 min, 100 : 0. F = 1.5 mL/min DAD set at 230 nm.	75
Lisianthius seemanii (Gentinaceae)	A dimeric secoiridoid, lisianthoside	Metanolic extract	Column: RP-18 Novapak, 1(50 × 3.9 mm, 4 μm) Gradient elution: Acetonitrile/water (0.05% THF) 5 : 95 to 70 : 30 in 50 min F = 1 mL/min. Termospray: vaporizer, 100°C; source, 250°C. (a) Ammonium acetate buffer (0.5 M, 0.2 mL/min) or (b) diaminoethane buffer (0.5 M, 0.2 mL/min); PI mode.	76

(*continued*)

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Table 3. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Panax ginseng, Panax quinquefolius L.	Ginsenoside (triterpene saponins)	Pulverised samples of ginseng roots were extracted with chloroform followed by methanol. Methanolic extracts were evaporated under reduced pressure at 35°C, the residue redissolved in a small volume of methanol–water (1 : 1) and filtered immediately before LC-MS-MS. Pure 24(R)-pseudoginsenoside F1 was obtained after successive column chromatographic purification by RP-LC on silica gel C18, with acetone–water (4 : 6) as mobile phase. Solutions of the reference ginsenosides were prepared in methanol–water (1 : 1, v/v), in the concentration range 12–9,600 ng/mL.	HPLC-MS-MS Waters Spherisorb C18 narrow-bore analytical column (2.0 × 150 mm, 5 μm). Mobile phase: (A) water containing 0.1% acetic acid and 5 μM sodium acetate, and (B) acetonitrile containing 0.1% acetic acid; Gradient from 25 to 40% (B) in (A) over 30 min. F = 200 μL/min. or Waters Spherisorb ODS 2 C18 analytical column (250 × 4.6 mm). Isocratic water–acetonitrile (80 : 20) for 20 min, then gradient from 20 to 40% (B) over the next 40 min. F = 1.6 mL/min. The entire effluent was directed to the electrospray interface (140°C in positive ion mode, N_2 as nebulizing and drying gas).	77

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P. ginseng, *P. quinquefolius*	Ginsenosides, ginsenoside Rf, 24(R)-pseudoginsenoside	Powedered plant material (300 mg) was extracted with 10 mL chloroform, 3 h reflux, then filtrated and the chloroformic extract discarded. The solid residue was extracted again with 10 mL methanol, 3 h reflux; the methanolic extract brought to dryness under vacuum. The residue containing ginsenosides was redissolved in 3 mL water. The aqueous solution was passed through a SEP-PAK C18 cartrige, washed with water, and eluted with 10 mL acetonitrile 50%. The solution was filtered prior to HPLC-MS.	Column: 46 × 150 mm packed with 3 µm hydrophobic C18. Mobile phase: (A) 50% acetonitrile; (B) 10 mM ammonium acetate. Gradient elution: linear change of solvent B from 70 to 50% in A in the first 30 min, and to 10% in the next 30 min, maintained 10 min before returning to 70% B in A in 2 min. F = 1 mL/min MS: nitrogen as nebulising gas; full-scan spectra in the range m/z 300–2300.	78
Pentemon serrulatus Menz.	Non-esterified glucoiridoids: Harpagide, Aucuboside, Loganine, Plantarenaloside Valerian-type	Dried and powdered callus tissues of *P. serrulatus* were extracted twice with boiling ethanol. Ethanol was removed, residue was washed with hot water. Aqueous solution was	Column: LiChrosorb RP-18 (250 × 4 mm, 10 µm); temp. 21°C. Isocratic elution: methanol/water (30 : 70) F = 2 mL/min. Detection at 220 nm.	79

(*continued*)

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Table 3. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
	glicoiridoids: 8-epi-Valerosidate, Penstemide, Serrulatoloside, Serrulatoside	evaporated under reduced pressure. Subsequent purification on alumina column. Glucoiridoids were eluted with methanol. SPE clean-up of crude extracts was performed on C_{18}-bonded silica cartridges.		
Salvia miltiorrhiza	Diterpenes (tanshinones)	Roots were extracted with methanol at room temp. The solvent removed under reduced pressure and the residue redissolved in water. The aqueous solution was extracted six times with light petroleum, and the combined extracts evaporated to dryness. Solutions of 5 mg/mL were prepared in the mobile phases.	Column: Intersil ODS-3 (150 × 4.6 mm); temp. 30°C. Mobile phase: methanol–water (80 : 20) F = 1.0 mL/min Photodiode array detector. The results were compared with those obtained by high-speed counter-current chromatography.	80
Salvia miltiorrhiza bunge	Diterpenes, tanshinones	Dried roots were extracted with ethanol, methanol, acetone, *n*-butanol, ethyl acetate and tetrahydrofuran, in a microwave oven	Column: Zorbax-ODS (150 × 4.6 mm, 5 μm); Zorbax-SB guard column Mobile phase: methanol–tetrahydrofuran–glacial	81

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		at 80°C, with intermitent power on and off to avoid the boiling. All solvents extracted the same amount of tanshinones.	acetic acid–water (16 : 37.5 : 1 : 45.5) F = 1 mL/min Detection: UV 254 nm.	
Sesamum angolense (Pedaliaceae)	Iridoid and secoiridoid glycoside represent a large group of cyclopentane pyran monoterpenoids. Phlomiol and seasamoside.	Methanolic extract	Column: RP-8 Nucleosil (125 × 4 mm, 5 μm) Gradient elution: Acetonitrile/water (0.05% THF) 2 : 98 to 7 : 93 in 30 min and 7 : 93 to 37 : 63 in 30 min. F = 1 mL/min. Termospray: vaporizer, 110°C, source, 220°C; ammonium acetate buffer (0.5 M, 0.2 mL/min); positive ionization (PI) mode.	76
Spinacia oleracea (spinach)	Carotenoids: lutein, zeaxanthin	Pure all-E carotenoid standards were prepared as solutions in chloroform, 4 mg/mL. The isomerization was carried out by adding one drop of a solution of one iodine crystal in 2 mL hexane to the pure all-E standards, followed by exposure to	Column: ProntoSIL C30 reversed-phase (250 × 4.6 mm, 3 μm), temp. 22°C Mobile phase: for carotenoid stereoisomers: binary mixtures of acetone and water for spinach extracts: isocratic mixture of acetone–water (84 : 14) for	82

(*continued*)

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Table 3. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		sunlight for a few hours, then kept in the dark at −40°C. The extraction of carotenoids from spinach was done according to the literature.	21 min, then 4 min linear gradient to acetone–water (97 : 3) maintained until the end of the chromatographic run at 40 min. F = 1 mL/min Detection: absorbance at 450 nm MS: positive ion mode in a mass range of m/z 200–800; nitrogen as used gas.	82
Tetrapleura tetraptera	Saponins: four aridanin derivatives	The dried powdered pulp of fruits was extracted with methanol.	LC-TSP-MS Column: μBondepak (300 × 3.9 mm, 10 μm) Gradient elution: water/acetonitrile 30 : 70 to 80 : 20 in 30 min;	74

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			F = 1 mL/min Detection: 206 nm. TSP: vaporizer 100°C source 270°C, filament off; buffer, 0.5 M ammonium acetate, at 0.2 mL/min, 1.5 s/scan.	
Valeriana species (*V. officinalis, V. wallichii, V. edulis*)	Sesquiterpenoids (valerianic acid, valerian, valepotriates)	The roots samples were dried at 30°C for 72 h. The finely milled powder was extracted with methanol, filtered and analysed by HPLC.	Column: ApexPrep. ODS (250 × 4.6 mm, 8 μm), Mobile phase: MeOH–H_2O 0.5% H_3PO_4, pH = 2 (80 : 20) F = 1.5 mL/min Detection: 220 nm (valerenic acid related compounds), 255 nm (valepotriates).	83

pressure to remove acetone. The solutions were diluted with water, the procyanidins precipitated with 0.1 M caffeine solution, centrifuged and the supernatant was applied to a C_{18} SPE cartridge. The adsorbed phenolics were eluted with methanol and, together with the precipitate, was dissolved in methanol. The procyanidines can be analysed directly by NP-HPLC or they can be thyolized with a 0.08 M HCl solution in MeOH, containing 12% v/v benzyl mercaptan, and analysed by RP-HPLC. The chromatographic conditions were: LiChrospher RP-18 column (250×4 mm, 5 μm) with a guard column (10×4 mm) containing the same packing material. Mobile phase: binary gradient (A) 1 mL 85% H_3PO_4 in 1 L H_2O, and (B) methanol; linear gradient from 20 to 70% B for 35 min, 90% B for 5 min and to 20% B for 5 min. The flow rate was 1 mL/min and the detection wavelength set at 280 nm.[59]

Catechins were analysed from *Malus pumila* by liquid chromatography. The fruits were homogenized in potassium pyrophosphate 0.1% (w/w) at 4°C for 24 h. The juice was centrifugated and filtered. The filtrate (juice) was applied to a Sepabeads SP-850 preparative column (285×25 mm) and the column was washed with water to remove soluble components. The crude apple polyphenol fraction was obtained after elution with 80% ethanolic eluent. Ethanol was removed to obtain a concentrated crude apple polyphenol fraction, and a portion of this fraction was loaded onto a TSG gel Toyopearl HW-40EC column (285×25 mm). This column was washed with water, then the phenolic compounds were eluted with 40% aqueous ethanol and 60% aqueos acetone. The fraction eluted with 40% ethanol contained, mainly, monomeric catechins, dimeric catechins, and phloretin glycosides, and was further purified using Sep-pak C_{18} ENV to eliminate phloretin glycosides. The resulting solution and the latter 60% aqueous acetone eluate containing, mainly, oligomeric catechin were mixed and the solution was lyophilized. The characteristic profile of catechin in the UV spectrum is at 280 nm. The identification of catechin oligomers was performed by matrix-assisted laser desorption/ionization time-of-flight MS and FAB/MS.[60]

Various catechins and epicatechins were analyzed from *Crataegus* by RP-HPLC. The dried leaves were extracted with aqueous acetone (70%) for 15 min. The suspension was percolated through a column and the extract was evaporated under vacuum at 30°C. Methanol was added to the residue to produce a 50% aqueous solution, further purified by SPE on Sep-Pak tC_{18} cartridges. RP-HPLC was performed on a LiChrosorb RP-18 column (250×4 mm, 5μm) protected by a guard column (10×4 mm) of the same material, at 24°C. The gradient elution involved solvents: (A) methanol, and (B) 0.5% *o*-phosphoric acid in water, following the profile (18% A–82% B) to (24% A–76% B) in 10 min, then constant from 10–20 min, (24% A–76% B) to (40% A–60% B) in 25 min, then 100% A from 45–50 min and then reconditioning with the starting concentration. The flow rate was 1 mL/min and

the detection was set at 280 and 220 nm. Spectra were recorded in the range 190 to 600 nm.[61]

TERPENOIDS

The large class of terpenoids includes the compounds with a triterpenic and steroidal structure (saponins, sterols, cardiotonic glycosides), but also the compounds with an isoprenic structure, such as volatile oils, terpenes (bitter principles) and carotenoids.

The volatile oils are aliphatic or aromatic hydrocarbons, aldehydes, alcohols, acids, esters, etc., and are widely distributed in nature. Structurally, the volatile oils are monoterpenes, sesquiterpenes, and azulenes. Volatile oils are obtained by distillation with water, or with non-polar solvents. The most used method for their analysis is GC, but some HPLC methods are reported in the literature on a silica stationary phase and a binary mixture, *n*-pentane/water, as mobile phase.[62] LC-MS methods have also been applied.[63] Standardized extracts of *Ginkgo biloba* leaves contain large quantities of terpene-like compounds and are mainly used in the treatment of peripheral and cerebral circulation disorders, or as a remedy against asthma, coughs, bladder inflammation, blenorrhagia, and alcohol abuse. The leaf extracts contain biflavones, flavonol glycosides, and terpene lactones. A method based on liquid chromatography, coupled with electrospray mass spectrometry, has been reported for the analysis of terpenoids in *G. biloba* extracts. This method allows the rapid isocratic separation of underivatized ginkgolides (GA, GB, GC, and GJ) and bilobalide at very low levels (10 pg on the column) and their quantitative detection by external standardization with relative standard deviations of 3 and 5% for intra- and inter-day analyses, respectively.[63]

The terpenes (bitter principles) can be found in different species such as: *Gentianaceae, Asteraceae, Labiatae, Fabaceae, Rutaceae, Asclepiadaceae, Papaveraceae, Menispermiaceae, Solanaceae*, etc. This group of compounds doesn't have a uniform composition; it includes monoterpenes, sesquiterpenes, diterpenes, and triterpenes. The bitter principles are barely soluble in water, but are soluble in organic solvents, and can be extracted into ethanol, water, or chloroform. The biological activity includes chronic gastritis, anorexia, antibiotic activity, etc.

Carotenoids are compounds with a terpenoid structure with 30–50 carbon atoms in the molecule. Carotenoids are widely spread in nature, being the yellow/red pigments which can be found together with chlorophylls. Carotenoids have a provitamin A structure and have an important biological activity in the visual process and regarding the epithelial tissue. These compounds can be

extracted in non-polar solvents and can be analysed by liquid chromatography (TLC, HPLC, etc.).

A mixture of terpene reference substances was analysed by coupling a commercial capillary HPLC system with a diode array spectrophotometric detector and a custom-built nuclear magnetic resonance (NMR) flow microprobe. The flow from the HPLC was stopped when a peak of interest had reached the NMR flow cell. The system featured a light-guided flow cell for UV–Vis absorbance detection via a photodiode array. The column was a Symmetry 300 C_{18} (150×0.32 mm, 5 μm) and the pressure was monitored by the HPLC instrument. The mobile phase was a mixture of acetonitrile–D_2O (70 : 30, v/v).[64] Table 3 contains some examples of terpenoid analyses by HPLC.

ALKALOIDS

Alkaloids can be found in many plants, have various structures, and have been used for a long time for their biological activity. Many pharmacological activities have been reported (antiarrhythmic, hypotensive, platelet-aggregation-inhibiting, histamine-antagonizing, anti-flagellated protozoa, etc.).

An interesting review describes the analysis of isosteroidal alkaloids from *Fritillaria* species by different analytical methods, including HPLC. The described alkaloids do not contain strong chromophores for a direct UV detection, and a pre-column derivatization must be performed. The UV-absorbing groups were introduced via esterification of hydroxyl groups in the alkaloids using 1-naphthoyl chloride with thionyl chloride as a catalyst. The resulting mixture was analysed by HPLC on a Nova-Pak C_{18} reversed-phase column (150×3.9 mm, 4 μm) using a simple isocratic mobile phase of methanol containing 0.2% diethylamine, and detected at 224 nm.[84] Table 4 includes some examples of alkaloid from plants analysed by HPLC.

COUMARINS

Coumarins (α-benzopyrones) are natural derivatives of benzopyrane with a lactonic structure. The name comes from "coumara" the name of Tonka seeds which contain large quantities of coumarins. Coumarins can be found in numerous species: algae, mushrooms, and lichens, but also in superior plants (Umbelliferae, Rutaceae, Labiatae, Orchidaceae, etc.). Biochemically, coumarins are formed by photosynthesis from phenylalanine and cinnamic acid.

As crystals, coumarins have blue, green, or violet fluorescence, show a good absorbance of the UV light, being an effective UV-screen. Many of them are thermally labile. Coumarin derivatives are biologically active on the nervous

Table 4. Alkaloids Analysed by HPLC (F = Flow Rate; DAD = Diode-Array Detection; All Proportions Are v/v Unless Otherwise Stated)

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Adhatoda vasica	Quinazoline alkaloids, vasicine and vasicinone	Powdered leaves were soaked with ethanol for 24 h, the extract filtered and concentrated. The concentrated solution was treated with 5% acetic acid, warmed for 15 min and filtered. The filtrated was defatted with hexane, basified with ammonia (pH 9.0), then extracted with chloroform. The chloroformic extract contains pure vasicine and vasicinone, which were isolated by column chromatography over silica gel with chloroform/ methanol/ethyl acetate mixtures as mobile phases with incresing polarity.	HPLC-DAD Column: Merck Hibar C18 (250 × 4 mm, 10 µm), temp. 26°C Mobile phase: acetonitrile–0.1 M phosphate buffer–glacial acetic acid (15 : 85 : 1), pH 3.9 F = 0.7 mL/min Detection: 300 nm.	85
Catharantus roseus L.	Vinca alkaloids (vincristine, vinblastine)	Powdered leaves were extracted with 90% methanol (3 × 30 mL, 12 h each) at room temp. The	Column: Waters µ Bondapak C18 RP-column (300 × 3.9 mm, 10 µm), temp 26°C	86

(*continued*)

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Table 4. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		alcohol extract was filtered, concentrated in vacuum to 10 mL, diluted with water 10 mL, acidified with 3% HCl (10 mL) and washed with hexane (3 × 30 mL) The aqueous portion was basified with ammonia at pH 8.5, extracted with chloroform (3 × 30 mL). Chloroform extract was washed with water, dried over sodium sulphate, and concentrated under vacuum. The residue was redissolved in 10 mL methanol.	F = 0.6 mL/min Mobile phase: acetonitrile-0.1 M phosphate buffer-glacial acetic acid, 38 : 62; 0.3, pH 4.14 Detection: multidimesional UV–Vis detector, 254 nm.	
Dehaasia triandra (Berberidaceae, Menispermaceae, Monimiaceae, Ranunculaceae, Lauraceae)	Bisbenzylisoquinoline alkaloids	The plant dried powder was extracted with 95% ethanol. The extract was concentrated and the obtained residue containing the alkaloids was extracted with 0.1 M HCl. The aqu-	HPLC-DAD Column: Luna C18 (250 × 4.6 mm, 5 μm) Mobile phase: linear gradient from (A) [acetonitrile–0.2% phosphoric acid (85%), 50 : 50, adjusted	87

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		eous layer was extracted with chloroform before (fraction A) and after (fraction B) the alkalinisation with ammonia water to pH 9.0. Fraction A contained the non-phenolic alkaloids and fraction B contained both phenolic and non-phenolic alkaloids. The fractions were concentrated and redissolved in a small amount of methanol.	with ammonia water (5%) to pH 8.0] to (B) [acetonitrile–0.5%–phosphoric acid (85%), 80 : 20, adjusted with ammonia water (5%) to pH 8.0]. Gradient profile of 100% A–100% B/ 0–15 min followed by 100% B for 5 min. F = 1 mL/min Detection: 215 nm; spectra in the range 200–400 nm. HPLC-ESI-MS Mobile phase: ammonium acetate buffer–acetonitrile (42 : 58); the buffer prepared as above. F = 0.1 mL/min Positive ion mode.	
Ephedrae herba	2,3,5,6-tetramethylpyrazine	Crude plant material was extracted with acidified water (pH 4 with 1 M HCl) by refluxing on a water bath for 1 h then on ultrasonic bath for 10 min. The filtered solution was used for HPLC.	Column: Nova-Pak C18 column (150 × 3.9 mm); temp. 30°C Mobile phase: methanol–water–35% acetic acid (35 : 65 : 0.5) F = 0.8 mL/min Detection: UV 290 nm.	88

(*continued*)

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Table 4. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
Fritillaria (Bulbus Fritillariae, *F. cirrhosa, F. thunbergii, F. hupehensis*)	Non-chromophoric alkaloids: verticine, verticinone, isoverticine, ebeiedinone, hupehenine, ebeiedine, peimissine, imperialine.	Dried sample powder was extracted with diethyl ether pre-alkalized with ammonium hydroxide and internal standard solution (1 mg/ mL) was added. The mixture was mechanicaly shaken for 2 h, centrifugated, and the supernatants evaporated to dryness. The residue was redissolved in a small quantity of methanol and was subjected to HPLC-ELSD analysis.	HPLC-ELSD (Evaporative Light Scattering Detection) Column: Supelco reversed-phase C8 (150 × 4.6 mm, 3 μm), temp. 28°C. Mobile phase: acetonitrile–methanol–water (6.5 : 3.5 : 3.0) containing 0.006% triethylamine F = 1 mL/min Temp. of detector drift tube and LTA were set at 65°C. Nitrogen flow = 2.64 standard L/min, five bar nebulising gas.	89
Papaver sominiferum L.	Morphine, codeine, oripavine, codeinone, reticuline, thebaine, papaverine, narcotine	Samples of air dried and powdered capsules (1 g) was extarcted with methanol three times (10 mL each for 3 h); the combined extracts were filtered, concentrated under vacuum, and made up to 1 mL in volume in methanol, which was filtered again before HPLC.	HPLC-DAD: Column: Merck Durasil C18 (250 × 4.6 mm, 10 μm); temp 26°C Mobile phase: acetonitrile-0.1 M phosphate buffer-glacial acetic acid, 20 : 80 : 0.4) pH 3.8 F = 1 mL/min Detection 240 nm.	90

Symphytum sp. (comfrey), *Petasites hybridus* and *Petasites albus* (butterbur), *Tussilago farfara* (coltsfoot), *Emilia coccinea* (tassel flower) and *Doronicum columnae* (leopard's bane).	Pyrrolizidine alkaloids	Powdered plant material was extracted with 1% solution of tartaric acid in methanol under reflux. The cooled, filtered extract was evaporated to dryness at 44°C. The residues were dissolved in a small quantity of 0.05 M hydrochloric acid for the cation-exchange SPE. The alkaloids were eluted from the sorbent with different mixtures of methanol-ammonia. The fraction were evaporated to dryness and the residue dissolved in methanol, ready for chromatography.	HP-IPLC (high-performance ion-pair liquid chromatography) Column: Hypersil BDS C8 (250 × 4.6, 5 µm); temp 40°C. Mobile phase: (A) hexane-1-sulfonic acid in 1% aqueous phosphoric (pH 3.2); (B) 100% acetonitrile. Gradient program: 0–7 min, isocratic at 20% B; 7–25 min, jump to 40% B; 25–28 min, jump to 60% B; 28–33 min, jump to 80%; 33–35 min, isocratic at 80% B; 35–40 min, jump to 20% B. F = 0.8 mL/min Detection: 220 nm; spectra 200–350 nm.	91
Triphyophyllum peltatum (Dioncophyllaceae)	Dioncophylline A, *N*-methyl-dioncophylline A, korupensamine A, ajmalicine hydrochloride, and (−)-nicotine.	5 g of dried and ground twigs were extracted with a mixture of water (pH 2 with TFA-trifluoracetic acid)–MeCN (8 : 2) (ultrasonic bath, room temp., 2 h). The extract solution was filtered and lyophilized. A 4.5-mg amount of	HPLC Column: Symmetry C18 column (250 × 4.6 mm, 5 µm) Mobile phase: (A) 0.01% TFA in $^{2}H_2O$, kept constant at 5% of the overall solvent mixture; (B) 0.01% TFA in $^{1}H_2O$; (C) MeCN, was added to A	92

(*continued*)

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Table 4. Continued

Medicinal Plant	Analyte	Sample Preparation	Method	Ref.
		this extract was redissolved in 300 μL ^2H_2O–MeCN (8 : 2) and filtered prior to HPLC-NMR.	and B to make up the full percentage of 100%. The HPLC gradient: 0 min 90% B, 12 min 75% B, 19 min 65% B, 25 min 55% B, 30 min 35% B. F = 1 mL/min Detection UV 254 nm NMR spectra of the standards alkaloids. HPLC-ESI-MS-MS Nitrogen-sheath and auxiliary gas, argon-collision gas. Positive ions were detected by scanning from 200 to 500 u.	92

system level, and some of them have an anticoagulant activity. Coumarin glycosides are soluble in water and polar organic solvents, while terpenylcoumarins and furanocoumarins are soluble in non-polar organic solvents. The coumarins have been analysed by TLC or HPLC, and easily detected due to their natural fluorescence. HPLC has also been extensively used, mainly with reversed phase stationary phases.

Coumarins from *Citrus aurantifolia* (Bergamottin, 5-Geranyloxy-7–methoxycumarin, 8-Geranyloxypsoralen, Isoimperatorin, Xanthyletin, Citropten, Phellopterin, Bergapten, Imperatorin, Isopimpinellin) were analysed by HPLC on a μPorasil normal phase column (300 × 3.9 mm, 10 μm) and ethyl acetate/hexane (1 : 4, v/v) as mobile phase or on a Nova-Pak C_{18} column (5 μm) and water/methanol (8 : 2, v/v), water/acetonitrile (1 : 1, v/v) as mobile phases. Detection was performed at 335 and 310 nm.[93]

Coumarin glycosides (Daphnin, Daphnetin-8-β-glucoside) were extracted from dry, powdered *Daphne acuminata* (Royle) by percolating four times with cold methanol. Extracts were evaporated to a semi-solid residue that was suspended in water and extracted four times with chloroform, then evaporated and partitioned between light petroleum and 95% methanol (1 : 1, v/v). The methanolic phase was used for chromatography after a treatment with charcoal followed by evaporation of the solvent. The residue was chromatographed on silica gel by step gradient elution: light petroleum, benzene, and chloroform/methanol. The chloroform/methanol (1 : 9, v/v) fraction contained daphnetin-8-β-glucoside and daphnin. ^{13}C-NMR was used for compound identification.[94]

Coumarins from *Archangelica officinalis* were analysed by liquid chromatography, HPLC and TLC, on reversed and normal phase, respectively. Twelve coumarins (0.1% solutions in methanol) and 1% solution of *Archangelica* extract were injected in a Alltech RP-18 column (250 × 4.6 mm, 5 μm) and separated with a mixture of methanol-water as mobile phase (8 : 2; 7 : 3; 6 : 4, v/v). The flow rate was 1 mL/min and the detection wavelength 254 nm. The fractions of coumarins, partially separated by HPLC, were collected, evaporated at 30°C, redissolved in a small amount of methanol, and separated by HPTLC on silica gel layers and dichloromethane, *n*-heptane, and ethyl acetate mixtures as mobile phases.[95]

ALKAMIDES

Akamides are a distinct class of natural products, containing an aliphatic acid (mostly unsaturated) residue linked with various amine moieties. Approximately 200 alkamides have been isolated from nature.[96] It is well known that alkamides from *Echinacea* species have immunostimulating properties[97] and 15 new isobutyl- and 2-methyl-butylamides have been identified in *Echinacea angustifolia* and *purpurea* roots.[98]

Soxhlet extraction with chloroform is widely used for the dried, powdered plant material. The extract is usually evaporated to dryness and the residue redissolved in a small volume of ethanol.[98,99] Methanol is also used for extraction by different methods: Soxhlet, high speed agitation, and sonication.[100]

Alkamides have been separated from *E. purpurea, E. pallida* and *E. angustifolia* by high performance liquid chromatography with a photodiode array detection system. The experimental conditions were as follows: Hibar column (125 × 4 mm, 5 μm), with LiChrospher 100 CH-18(2) packing material; LiChroCART precolumn (4 × 4 mm, 5 μm) with LiChrospher 100-CH; linear gradient elution: A = water and B = acetonitrile. The solvent gradient was from 40 to 80% B within 30 min, and the flow rate was 1 mL/min. Detection in the UV at 210 nm and 254 nm. More than 20 compounds have been separated from each sample (roots and aerial parts). HPLC determination of the main amide constituents dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutyl-amide (8/9) in root and aerial parts showed that *E. purpurea* roots contained 0.004–0.0039% and *E. angustiflia* roots, 0.009–0.151%, respectively. The total amount of alkamides was lower in the aerial parts as compared with the roots. The concentration of 8/9 in the herb was found to be 0.001–0.003%. The various structural types of alkamides could be distinguished by their UV spectra. Alkamides with a 2,4-diene moiety showed an absorption maximum at 259 nm, while 2-monoene compounds had an absorption maximum at 210 nm. Additional olefinic groups, as in compounds 8 or 9, resulted in a shoulder at 230 nm.[98,101]

Alkamides can be separated on a RP-C_{18} column (150 × 4.6 mm, 5 μm) with a C_{18} guard column, at 40°C, but using slightly modified conditions. The eluted peaks were detected at 254 nm.[100] Mobile phase was a gradient mixture of water and acetonitrile, starting with 40% acetonitrile for 10 min followed by a linear gradient to 53% acetonitrile at 35 min. *E. purpurea* plants collected from two sites were analyzed for alkylamides content at four growth stages: pre-flower, flowering, mature, and senescent. Total alkylamide concentration in the root, stem, and leaf decreased throughout the first growing season, while the concentration in flowers increased. In mature plants, the root contained about 70% of the total alkylamides and approximately 20% in flower, 10% in stem, and 1% in leaf tissue. The relative proportion of individual alkylamides in the root did not change during plant growth. Quantification was performed by the external standard method. The reference compound was trans, *trans*-2,4-dodecadienal, initially calibrated against an isomeric mixture of compound (8/9),[98] leading to a conversion factor of 0.978. The reference compound was used for all quantification calculations of alkylamides, and the same response factor and similar extinction coefficients were assumed for all alkylamides.

Alkamides in roots and achenes of *E. purpurea* (L) Moench were analysed by HPLC–electrospray mass spectrometry (ES-MS) with emphasis on the difference between the chromatographic "fingerprints" for the two samples.[99]

Nine alkamides were identified in the root of *E. purpurea*, similar to other works.[98] The technique enables the identification of small peaks which were unidentified in previously published studies[98,102] due to the fact that no standards were available for the comparison of retention times. The isomeric pair, tetraene (8/9), was purified as a standard for the quantification of alkamide content in *E. purpurea* roots and achenes, as well as for the achenes of *E. angustifolia* DC and *E. pallida* Nutt.

Alkamides in *E. Pallida* (Asteraceae) were analysed by HPLC, using isocratic conditions, on a silica gel C_{18} column (7.5 cm, 3 μm), mobile phase: acetonitrile–water (6 : 4, v/v), flow rate 1 mL/min, and UV detection at 210, 260 nm. The plant material was extracted with 95% aqueous ethanol for 24 h under mechanical agitation. Filtered extracts were concentrated in vacuum.[103]

POLYACETYLENES

Eleven polyacetylenes were isolated from the aerial and subterranean parts of water hemlock, *Cicuta virosa* L. (Apiaceae, Apioideae), including isocicutoxin, isocicutol, and two incompletely characterised isomers with two *cis*-double bonds (falcarindiol, (1,8*E*,10*E*)-hetptadecatriene4,6-diyn-3-ol, already known from other Apiaceae, and the novel polyacetylene (1,8*E*/*Z*, 10*E*, 12*E*) heptadecatetraene-4,6-diyn-3-ol).[104] Keto compounds, postulated to occur in water hemlock, could not be detected. Fresh plant material was extracted with methanol at room temperature for several days after mincing in the solvent. The methanol extract was evaporated at 40°C, and extracted with a petroleum ether/diethyl ether mixture (1 : 1, v/v). The concentrated extract of the subterranean parts of the plants was used for analytical and preparative separation of polyacetylenes. HPLC was performed on a Spherisorb S5 ODS2 column (290 × 4 mm) with gradient elution, methanol 60 to 100% in 20 min in aqueous buffer (*o*-phosphoric acid 0.015 M, tetrabutylammonium hydroxide 0.0015 M, pH = 3), flow rate 1 mL/min. Detection was carried out with a diode array spectrophotometer at 230 nm. The compounds with two hydroxyl groups show retention times in the range 10–15 min, and those with only one hydroxyl group in the range 17–20 min. The elution order of the compounds was according to decreasing polarity. The structures of the separated substances were identified by UV, IR, ^{1}H-, and ^{13}C-NMR spectra.

MISCELLANEOUS

The screening of active compounds in *Gastrodia elata Blume* (Orchidaceae), a plant used in Chinese folk medicine, was performed by extraction from

dried roots with 75% ethanol by refluxing on a water bath at 80°C for 1 h, then placed in an ultrasonic bath for 20 min. The ethanolic extracts were filtered and the organic solvent removed with a rotary evaporator at 40°C under vacuum. The residue was diluted to a proper concentration with methanol, and the sample was filtered through a 0.45 μm membrane before HPLC. The ethanolic extracts, dissolved in water, were extracted with petroleum ether to remove esters. Then, the aqueous solution was successively extracted with ethyl ether and butanol, three times, respectively. The organic solvents were removed with a rotary evaporator at 40°C under vacuum to obtain ethyl ether and butanol extracts. Each of them was redissolved in methanol and filtered before HPLC. HPLC was performed on an ODS Zorbax SB-C_{18} column (250 × 4.5 mm, 5 μm) temp 30°C, mobile phase: methanol–water–isopropyl alcohol (35 : 55 : 10, v/v), flow rate 0.4 mL/min, and detection at 270 nm.[105]

The chromatographic fingerprints of soybean seeds and roots obtained from the phytoalexins (glyceollins, daidzein, genistein) separation were investigated for reproducibility. Dried plant material powder was successively extracted with hexane, under reflux, for 2 h, then with methanol for 2 h. The hexane extract was evaporated to dryness and the residue redissolved in 1 mL hexane. The methanolic extract was evaporated under reduced pressure at 40°C to provide a clear oil. The clear oil was diluted with 10 mL methanol, and 1 mL of the resulting solution was evaporated and the rest was partitioned between 50 mL water and 50 mL dichloromethane. Slightly modified procedures were applied too. RP-HPLC was performed with a Nova-Pak C_{18} column (150 × 3.9 mm, 4 μm) with a two-component gradient mobile phase: (A) methanol; (B) aqueous buffer made from 0.01 M KH_2PO_4 (pH 2.4 with HCl) and 0.1% Et_3N (final pH about 2.46). (A) increased from 5% to 55% over 50 min; (A) maintained at 55% for 10 min; then (A) returned to 5% over 5 min. The flow rate was 1 mL/min and the detection was at 220 nm. NP-HPLC was performed on a Hypersil Si column (150 × 4.6 mm, 5 μm) with hexane as mobile phase. The flow rate was 0.8 mL/min and the detection was set at 254 nm.[106]

The separation of five rhubarb anthraquinones (standard substances, not extracted from plant) is described, and the migration times, peak heights, area, and reproducibility have been investigated. Due to the increased interest in capillary electrochromatography (CEC), a hybrid of HPLC and CE, many compounds of interest from medicinal plants are being analysed by this method. Rhubarb (*Rheum palmatum* L.) is one of the oldest and best known herbal medicines and is officially listed in pharmacopoeias of many countries. The separation of anthraquinones from the roots of this plant was performed on a packed ODS electochromatography column. The instrumentation was a laboratory made CEC system comprising an Isco CV4 capillary electrophoresis absorbance detector (Lincoln, NE, USA), a 9323 HVPS high-voltage power supply, and a TL-9800 chemstation for data acquisition and handling. Fused silica

capillary tubing (100 μm i.d., 365 μm o.d.), L = 45 cm, and 25 cm packed with HPLC stationary phase. Mobile phase was a mixture of different concentrations of acetonitrile–sodium dihydrogenphosphate (100 mM) with the pH adjusted with 2-[*N*-morpholine] ethanesulphonic acid.[107]

Phytoestrogens (8-prenylnaringenin) from *H. lupulus* L. were extracted from dried flowers by supercritical fluid CO_2, or by three times extraction with methanol–water (3 : 1), refluxing under N_2 atmosphere. The combined methanolic extracts were filtered and concentrated at 30°C. HPLC was coupled with MS. Liquid chromatography was performed on Alltima RP C_{18} column (250 × 4.6 mm, 5 μm), with linear gradient elution with 500 ppm formic acid in water (A) and acetonitrile (B), following the pattern: 0–2 min, 40% B in A; 2–20 min, 40% to 60% B in A; 20–30 min, 60% to 95% B in A; 30–35 min, 95% B in A. The flow rate was 0.9 mL/min, and the detection was at 280 nm. Mass spectrometry was in positive and negative ionization mode, N_2 the employed gas, and quantification by selecting ion monitoring at m/z 341.[108]

Twenty ecdysteroids from *Lychnis flos-coculi* (Caryopyllaceae) were analysed by multiple hyphenated methods, HPLC–UV–FTIR–MS and HPLC–UV–FTIR–NMR. The powder from air dried plant material was extracted with 96% ethanol (1 L for every 100 g) with continuous stirring for 2–3 days. The sample was filtered, evaporated to dryness, redissolved in a small volume of methanol, and centrifuged. Immediately prior to HPLC the sample was evaporated to dryness and taken up in a small volume of deuterium oxide. Chromatography was performed on Hypersil HIRPB C_{18} column (100 × 4.6 mm, 5 μm) or a Hypersil H5BDS-C_{18} column (250 × 4.6 mm, 5 μm) with acetonitrile and D_2O 99.8% isotopic purity as mobile phase. The flow rate was 1 mL/min, and diode-array detection (DAD), UV spectra in the range 190–360 nm. FTIR spectra were obtained with 20 scans per spectrum (5 s acquisition time) with a sensitive MCT (mercury cadmium telluride) liquid nitrogen cooled detector; 8 cm^{-1} spectral resolution. MS with electrospray ionisation (ESI) and a Z Spray source, operated in positive ion mode, was obtained for the mass range 100–900 Daltons.[109]

Antioxidants from *Sideritis* species (Labiatae, *S. scardica*) were extracted, from the air-dried, ground plant material, with methanol, the solvent evaporated to dryness, followed by successive partitioning between water and *tert*-butyl methyl ether, ethyl acetate, and 1-butanol. The obtained subextracts were evaporated to dryness under vacuum, at 50°C, and the remaining aqueous layer was freeze-dried. Chromatography was performed on an Alltima C_{18} column (250 × 4.6 mm, 5 μm); mobile phase was 90, 70, 50, 30, 10% methanol or acetonitrile in water or acetate buffer, mixed with 10^{-5} M 2,2′-diphenyl-1-picrylhydrazyl (DPPH) stable free radical; the flow rate 0.7 mL/min. The detection of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) bleaching (as a negative peak) was set at 517 nm.[110]

Paclitaxels (taxanes and 12 related taxanes) were analysed from *Taxus cuspidata* Siebold & Zucc. cv *Densiformis* (Taxaceae). Callus of *T. cuspidata* cv *Densiformis* was grown on Gamborg's B5 medium supplemented with sucrose, casein hydrolysate, agar, picloram, and α-naphthaleneacetic acid. Eight milli liter of medium were dispensed into each culture tube and autoclaved at 121.1°C for 20 min. Samples were taken over a period of 28 days. Samples were extracted four times with 1 mL of methylene chloride. The combined methylene chloride extract was evaporated to dryness at room temperature and then redissolved in 100 mL of methanol for HPLC analysis. The column was Microsorb-MV C8 (50 × 4.6 mm, 5 μm); mobile phase: acetonitrile–water (25 : 75, v/v) to acetonitrile–water (50 : 50, v/v) in 10 min; flow rate 2.50 mL/min, and detection at 227 nm.[111]

Taxol is a promising anti-cancer drug that can be found naturally in many Taxus species (*T. brevilofolia, T. baccata, T. canadensis, T. cuspidata, T.X media, T. X media Nigra, T. X media Hicksii, T. X media Densiformis, T. cuspidata capitata, T. chinensis, T. floridana, T. yunnanensis*). Taxol and related taxanes were isolated and analysed from *Taxus wallichiana* extract by HPLC on different columns: Nova-Pak C_{18} (150 × 3.9 mm, 4 μm), Symmetry C_{18} (150 × 3.9 mm, 5 μm), Nova-Pak Phenyl (150 × 3.9 mm, 4 μm) and Curosil-B (250 × 4.6 mm, 3 μm); detection was at 228 nm. The plant green needles were extracted with methanol, the extract concentrated under reduced pressure, diluted with water, defatted with hexane, and extracted again with chloroform. The chloroform extract contained taxoids, and was further prepared for HPLC by concentration and dilution in methanol–chloroform. The mobile phase was a mixture of methanol–acetonitrile–water, investigated for different proportions and various gradients.[112]

Naphthodianthrones (hypericin, pseudohypericin) were extracted with ethanol from capsules and tablets, so that the concentration will be comparable to *H. perforatum* plant material. Alcoholic tinctures were centrifuged and analysed directly. The HPLC column was Ultrasphere ODS RP-C_{18} (250 × 4.6 mm, 5 μm); the mobile phase: (A) methanol–acetonitrile (5 : 4), (B) triethylammonium acetate buffer. Initial conditions A-B (70 : 30) for 2 min, then A incresed linearly to 90% over 8 min, held constant for 4 min, then A to 100% over 2 min and held constant for 5 min, returning to initial conditions over 1 min and held for 5 min. The flow rate was 1 mL/min, and the diode-array detection provided the UV-Vis spectra in the range 200–600 nm, max at 236 and 592 nm. For the fluorescence detection, the excitation wavelength was 236 nm, and the emission was at 592 nm. Mass spectrometry scanned the m/z ratio 350–700.[113]

Anthocyanins are widely distributed in various plant species, mainly in fruits and flowers. They have high potential as food colorants because of their low toxicity. The anthocyanins were extracted from *Daucus carota* (carrot) using acidified methanol (1% HCl) overnight at 4°C. The extract was concentrated at

reduced pressure, washed with chloroform and diethyl ether to remove chlorophyll and other lipid materials. The concentrate was loaded onto a Dowex 50 W-4X, H^+ type resin column. Anthocyanins were eluted from the column with acidified methanol. The eluate was concentrated and separated on a Sephadex LH-20 column, with methanol : acetic acid : water (10 : 1 : 9). The anthocyanin fractions were hydrolysed with 2 N HCl for 1 h, the acyl groups were extracted with diethyl ether, the aglycons with amyl alcohol, and the aqueous solution was used for carbohydrate analysis. HPLC of anthocyanidins (aglycones) was performed on a μBondapack C_{18} column (250 × 4.6 mm, 10 μm). Mobile phase was methanol : acetic acid : water (7 : 1 : 2, v/v), the flow rate 1 mL/min, and the detection was at 530 nm. For MS, xenon was used as FAB gas (6 kV, 10 mA), and thioglycerol as the matrix. For NMR measurements, CD3 OD + 10% TFA-d was used as solvent and the chemical shifts were recorded for both proton and ^{13}C NMR as ppm.[114]

Polyprenols present in the leaves of *G. biloba* were analysed by HPLC and SFC (supercritical fluid chromatography).[115] The method allows the quantification of C_{85}, C_{90}, C_{95}, and C_{120} polyprenols. The last one cannot be identified by HPLC. Pulverized leaves were extracted in *n*-hexane, then the *n*-hexane extract was washed three times with 90% aqueous methanol and vigorously stirred with potassium carbonate and methanol. After a further washing, once with water and twice with saturated sodium chloride solution, the resulting *n*-hexane solution was passed through sodium sulfate. The eluted *n*-hexane solution was evaporated under nitrogen, and the residue was dissolved in *n*-hexane prior to separation of the polyprenols by SFC.

A chemically bonded SB-Phenyl-50 capillary column (10 m × 50 mm i.d., film thickness 0.25 mm) was used for the separation of polyprenols. Separation of polyprenols was achieved by pressure gradient in which the initial pressure of 200 atm was increased at a rate of 20 atm/min to a final pressure of 400 atm. The oven temperature was maintained at 100°C. The SFC system was equipped with a flame ionization detector. The concentration of each polyprenol (C_{85}, C_{90}, and C_{95}, respectively) was calculated from the standard curves. SFC is an interesting an alternative method for HPLC.

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Received April 1, 2002
Accepted May 1, 2002
Manuscript 5820