Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 812 (2004) 291–307
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Hippophae rhamnoides L.: chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects

Vahid Bilaloglu Guliyev[a,*], Mustafa Gul[b], Ali Yildirim[a]

[a] *Department of Chemistry, Faculty of Education, Ataturk University, 25240 Erzurum, Turkey*
[b] *Deparment of Physiology, Faculty of Medicine, Ataturk University, 25240 Erzurum, Turkey*

Received 15 July 2004; accepted 31 August 2004
Available online 18 October 2004

Abstract

There is an increasing interest in the usage of chromatographic methods on the analysis of chemical compounds present in *Hippophae rhamnoides* L. In this paper, the chromatographic techniques applied for the determination, separation and identification of chemical compounds of *H. rhamnoides* L. are reviewed. We examined the existing chromatographic methods based on separations by paper and thin-layer chromatography, gas chromatography, high-performance liquid chromatography and capillary electrophoresis and also methods of detection by ultraviolet absorption, fluorescence, refractive index, electrochemical and mass spectrometry. Biological properties of the plant and its pharmacological effects and use in traditional medicine have also been reviewed.

Keywords: Reviews; *Hippophae rhamnoides* L.; Pharmacological effects; Chromatography

Contents

Abbreviations: APCI, atmospheric pressure chemical ionization; BAW, *n*-butanol–acetic acid–water; CC, column chromatography; CCC, counter current chromatography; CE, capillary electrophoresis; CZE, capillary zone electrophoresis; DAD, diode array detection; ESI, electrospray ionization; ECD, electrochemical detection; FAMEs, fatty acid methyl esters; FID, flame ionization detector; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; HPTLC, high-performance TLC; HSCCC, high-speed counter-current chromatography; IR, infrared; HPLC–MS, HPLC–mass spectrometry; MS, mass spectrometry; PC, paper chromatography; RI, refractive index; SPE, solid phase extraction; TAG, triacylglycerol; TLC, thin-layer chromatography; TMS, trimethylsylil ether; UV, ultraviolet, FD, fluorescence detector

* Corresponding author. Tel.: +90 442 2314012; fax: +90 442 2360955.
E-mail addresses: gbvahid@atauni.edu.tr, vahitbilaloglu@yahoo.com (V.B. Guliyev).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.08.047

1. Introduction

Hippophae rhamnoides L. (sea buckthorn) is a hardy bush, which is belonged to the *Elaeagnaceae* family and naturally distributed over Asia and Europe [1]. Various organs of *H. rhamnoides* L., especially berries, were used in traditional medicines, mainly in Tibetian, Mongolian, Chinese and Middle Asian [2,3]. The berries have been used as a raw material for foods and medicines for decades in China and Russia [2–4]. Recently, the nutritional importance of *H. rhamnoides* L. berries has been increased in North America and Europe [5–9]. Berry products of *H. rhamnoides* L. are among popular foods in the United States, Canada, Finland, Germany, and some other European countries [5,7–10]. Industrial utilizations and having different biological activities of the berries and other parts of *H. rhamnoides* L. caused to increase the necessity of compositional information for selecting the best raw materials for food and drug industries [2].

Hippophae rhamnoides L. contains a series of chemical compounds including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tanins, etc. These compounds are of interest not only from the chemical point of view, but also because many of them possess biological and therapeutic activity including antioxidant, antitumoral, hepato-protective and immunumodulatory properties [3]. Because of these effects, *H. rhamnoides* L. containing bioactive compounds is often used in traditional medicine. This in turn has necessitated the development of new methods for analysis and quantitative measurement of bioactive components of *H. rhamnoides* L.

The methods for determination of chemical compounds of *H. rhamnoides* L. include preliminary chromatographic separation, gas chromatography (GC), high-performance liquid chromatography (HPLC), hyphenated techniques such as GC–mass spectrometry (GC–MS), HPLC–mass spectrometry (HPLC–MS) and capillary electrophoresis (CE). These first three methods were used for quantitation of chemical compounds in plant material. The advantages of recent hyphenated techniques are rapid initial screening of crude plant extract, and providing preliminary information on the content and the nature of constituents in the matrix.

In the present paper, the analytical methods currently available to investigate the analysis of chemical compounds present in *H. rhamnoides* L. are reviewed. In addition, properties of the plant, areas where it grows, and its use in traditional medicine, and its pharmacological effects have been briefly reviewed.

2. Biological properties of *Hippophae rhamnoides* L.

Hippophae rhamnoides, also known as sea buckthorn, is a member of the *Elaeagnaceae* family. *Hippophae rhamnoides* L. is a medium sized deciduous tree or large shrub with 2.5–6 m in height. The main trunk has a thick and rough bark. The young branches are smooth, grey and light ash coloured with needle shaped thorns. The leaves grow either in alternate or in clusters. Each leaf is elongate-oblanceolate or elongate-spathulate with green upper surface and silvery tinged ashy green lower surface. The flowers bloom when the leaf is still in bud condition [11]. The flowers of a plant of *H. rhamnoides* L. are either male or female, but only one sex is to be found on any one plant so both male and female plants must be grown if seed is required, and seeds are pollinated by the wind. The plant is not self-fertile [12]. The fruit is a narrow-elliptic or oval, yellowish orange berry with its external surface covered with silvery dust particles and it is sour in taste [11]. It flowers in April, and the fruits are collected through August to October. The pith of the stem can also be used in medicine.

Hippophae rhamnoides is a perennial plant native to European countries such as Britain, Italy and Spain, and countries in Asia such as Russia, India, Tibet [1] and Turkey [13], and Canadian states like Saskatchewan and British Columbia [14]. *Hippophae rhamnoides* L. is found growing more on riversides of mountains and foothills, sandy and gravel grounds at an elevation of 3300–4500 m above sea level [11].

3. Use in traditional medicine and pharmacological effects

3.1. Use in traditional medicine

Different parts of *H. rhamnoides* L. have been used for the treatment of diseases in traditional medicine in various countries in the world. *Hippophae rhamnoides* L. grown widely in northern and southwestern China, is a traditional herbal medicine, which has long been used for relieving cough, aiding digestion, invigorating blood circulation and alleviating pain since ancient time [15].

The extracts of *H. rhamnoides* L. branches and leaves are used to treat colitis and enterocolitis in humans and animals in Mongolia [16]. Branches and leaves are also used in the treatment of diarrhea [17]. Leaves are used in gastrointestinal and dermatologic disorders [16] and have been applied as compress form in rheumatoid arthritis in the Middle Asia [18].

Flowers of *H. rhamnoides* L. are used as skin softener in Tajikistan [19].

For its hemostatic and antiinflammatory effects, fruits of the plant are added to prescriptions in pulmonary [20–22], gastrointestinal, cardiac, blood and metabolic disorders [21,23] in Indian and Tibetian medicine.

After reviewing the ancient literature Li and Guo [24] point out that sea buckthorn is a mild drug with the characteristic effects such as lowering fever, diminishing inflammation, counteracting toxicity and abscesses, treating cough and colds, keeping warm, easing respiration, clearing sputum, having mildly laxative effect, treating tumors, especially of the stomach and the esophagus, and treating different kinds of gynecological diseases in Tibetian medicine.

Oil extracts obtained from fruits are used in liver diseases, inflammatory processes, absorption disorders in the gastrointestinal system, and are applied externally in hemorrhage [25]. Juice, syrup, and oil of the fruits have been used as pain killer, to heal wounds, in ulcer and other diseases of the stomach, disantheria, cancer, and as a metabolism regulator in traditional medicine [18,26]. The freshly pressed juice is used in the treatment of colds, febrile conditions, and exhaustion [2].

Oil from fruits and seeds is used in the treatment of eczema, lupus erythematosus, chronic wounds that are difficult to heal, inflammatory diseases, erosion of the cervix uteri, in the treatment of burns and frozen parts of the body [27]. Also, its berry oil is reported to treat skin disease [28] and thrombosis [29].

Oil extracts are used externally in dermatologic diseases such as eczema, psoriasis, lupus erythematosus, and chronic dermatoses [30,31]. In ophthalmology, they are used in the treatment of keratitis, trachoma, injuries or burns of eye lid, conjunctivitis [32].

3.2. Pharmacological effects

3.2.1. Antimicrobial and antitumoral effects

A few studies show that *H. rhamnoides* L. has antimicrobial and antitumoral effects. Phenolic compounds of *H. rhamnoides* L. berry inhibit the growth of Gram-negative bacteria [33]. Antitumor effects of fruit juice and seed oil [34], and prevention of aminopyrine plus nitrite induced tumor production in rats by juice of *H. rhamnoides* L. [35] have been reported.

3.2.2. Antiulcerogenic effect

Curative and preventive effects of *H. rhamnoides* L. against experimental gastric ulcers in rats have been reported by various studies. Suleyman et al. have demonstrated the antiulcerogenic effects of a hexane extract from *H. rhamnoides* fruits on indomethacin- and stress-induced ulcer models [36] and on ethanol-induced gastric lesion [37]. Reduction in water-immersion and reserpine-induced ulcer models and the index of pylorus ligation-induced gastric ulcer, and also acceleration of the healing process of acetic acid-induced gastric ulcer by oils of CO_2-extract from the seeds and pulp of *Hippophae rhamnoides* L. have also been reported [38].

3.2.3. Dermatological effects

Beneficial effect of *H. rhamnoides* L. in dermatological disorders is known. Of the 4 months oral supplementation of pulp and seed oils in patients with atopic dermatitis, improvement in dermatitis was followed only in pulp oil supplemented group [39].

3.2.4. Effects on platelet aggregation

Total flavones of *H. rhamnoides* L. fruit have prolonged thrombotic occlusion time in a mouse femoral artery thrombosis model by the photochemical reaction between intravenously injected rose bengal and green light irradiation [40]. In the same study, total flavones have inhibited in vitro platelet aggregation induced by collagen in a concentration dependent manner, in contrast, they did not affect aggregation induced by arachidonic acid and adenosine diphosphate [40].

A small-scale preliminary cross-over study has also been conducted to investigate the effects of supercritical CO_2-extracted *H. rhamnoides* L. berry oil on some risk factors of cardiovascular disease [41]. A clear decrease in the rate of adenosine-5′-diphosphate-induced platelet aggregation and maximum aggregation by *H. rhamnoides* L. berry oil was

found [41]. These findings point out the possible beneficial effects of *H. rhamnoides* L. berry oil in cardiovascular diseases by inhibiting the blood clotting.

3.2.5. Effects on blood lipids

The supplementation of *H. rhamnoides* L. juice increased plasma high density lipoprotein-cholesterol and triacylglycerol concentrations by 20% and 17%, respectively, in healthy male volunteers [42]. In addition, *H. rhamnoides* L. juice supplementation resulted in a moderate decrease in the susceptibility of low density lipoprotein to oxidation in these subjects [42].

3.2.6. Electrophysiological effects

The effects of *H. rhamnoides* L. on action potential of myocardial cells have been tested in culture by Wu et al. [43]. Total flavones (100 mg/L) decreased the duration of repolarization period in both cultured rat myocardial cells and guinea pig papillary muscles. The slope of phase 4 of depolarization in cultured rat myocardial cells was also decreased, and the contractile force in guinea pig papillary muscles was weakened. On the other hand, arrhythmias evoked by strophantin G in guinea pig papillary muscles were suppressed by total flavones [43].

3.2.7. Antioxidant effects

Antioxidant effect of various extracts of *H. rhamnoides* L. has been studied in vitro. Gao et al. [44] have shown that the crude extract of *H. rhamnoides* L. fruits containing both hydrophilic and lipophilic antioxidants has higher inhibitory effect in both 2,2-azobis(2,4-dimethylvaleronitrile) (AMVN) and ascorbate-iron induced lipid peroxidation. The aqueous and ascorbate-free extracts have demonstrated higher inhibition in the AMVN assay, but lower inhibition in ascorbate-iron induced peroxidation, than the lipophilic extract. Interestingly, capacity to scavenge radicals of the crude extract, like the phenolic and ascorbate extracts, has decreased significantly with increased maturation of the fruit [44].

The production of free radicals by chromium and the ability of alcoholic leaf and fruit extracts of *H. rhamnoides* L. to inhibit the oxidative damage induced by chromium (VI) have been investigated in vitro. Alcoholic extracts of leaves and fruits of this plant were found to inhibit chromium (VI)-induced free radical production, apoptosis, DNA fragmentation and restored the antioxidant status of cells. In addition, these extracts were also able to arrest the chromium-induced inhibition of lymphocyte proliferation [45]. Antioxidant effect of *H. rhamnoides* L. on chromium induced oxidative stress was also studied in vivo by Geetha et al. [46]. They have demonstrated that the leaf extract of this plant protects the male albino rats from the chromium induced oxidative injury in serum significantly [46]. Hexane extract of *H. rhamnoides* L. fruits has shown to inhibit nicotine-induced oxidative stress in erythrocytes in rat [47]. However, it could not prevent nicotine-induced oxidative stress in the brain of these rats, probably because it could not have been diffused to the rat brain to exert its antioxidant effect [48]. Hexane extract of *H. rhamnoides* L. has also prevented the depletion of the most abundant thiol, glutathione [49], in gastric tissue of ethanol administered rats [37]. Various in vitro and in vivo studies clearly demonstrate that *H. rhamnoides* L. has antioxidant activity.

3.2.8. Effects on liver injury

The effects of *H. rhamnoides* L. oils have also been tested on experimental injury and clinical diseases of the liver. Protection by seed oil against hepatic injury induced by CCl_4, ethyl alcohol and acetaminophen has been studied in mice [50]. This oil has markedly inhibited malondialdehyde formation of liver induced by CCl_4, acetaminophen and ethyl alcohol. It decreased serum glutamic pyruvic transaminase levels induced by CCl_4 and acetaminophen. In addition, the oil prevented the depletion of glutathione in damaged liver induced by acetaminophen [50]. Clinical effects of oil on liver fibrosis have also been tested recently by Gao et al. [51]. The oil treatment has also notably shortened the duration for normalization of aminotransferases [51]. The results of these studies suggest that seed oil of *H. rhamnoides* L. may also be a useful for prevention and treatment of liver diseases.

3.2.9. Radioprotective effects

Protection against whole body lethal irradiation by whole berries of *H. rhamnoides* L., has been reported in mice. Goel et al. [52] has demonstrated that alcoholic extract have rendered 82% survival as compared to no survival in irradiated control. Furthermore, it has inhibited Fenton reaction and radiation mediated generation of hydroxyl radicals in vitro, and superoxide anion mediated nitroblue tetrazolium reduction and $FeSO_4$ mediated lipid peroxidation in liver [52].

Administration of alcoholic extract 30 min before irradiation has increased the number of surviving crypts in the jejunum and villi cellularity in comparison to the irradiated control in mice [53]. It has also reduced the incidence of apoptotic bodies in the crypts in a time dependent manner and increased cellularity in the crypts and villi (84 h post irradiation) as compared to control. Caspase-3 activity has also been found significantly lower in the mice administered alcoholic extract before irradiation as compared to irradiated control. These results in gastrointestinal mucosa suggest that reduction in the radiation induced loss of cellularity of crypts and villi and also decrease in frequency of apoptosis could have contributed to the protection of mice treated with alcoholic extract before irradiation [53]. To understand the mechanism of radioprotection, the effects of alcoholic extract chromatin organization have been studied. This extract has induced a strong compaction of chromatin as was evident from lack of tail and appearance of intensely stained circular bodies in Comet assay, single cell gel electrophoresis. This could have made the nuclei resistant even to a radiation dose of 1000 Gy [54]. The alcoholic extract has also inhibited radiation and tertiary butyl hydroperoxide induced DNA strand breaks in a dose dependent manner in that study. The results

of these studies suggest that the ability of alcoholic extract of *H. rhamnoides* L. to protect DNA could mainly be attributed to direct modulation of chromatin organization, and the role of its free radical scavenging activity may be limited in this radioprotective effect.

4. Chromatographic methods

Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds, even from complicated matrix. Analytical methods used for the determination, separation and identification of chemical compounds from *H. rhamnoides* L. include paper chromatography (PC), thin-layer chromatography (TLC), GC, HPLC, HPLC–MS, GC–MS, and CE. The first four methods were especially used for quantitation of chemical compounds present in plant material. However, recent hyphenated techniques allow rapid initial screening of crude plant extracts providing preliminary information on the content and the nature of constituents in the matrix. These recent techniques provide a good method for identification of new compounds and assure avoidance of unnecessary isolation of common compounds of minor interest [55].

In this present review, the analytical methods currently available for the analysis of chemical compounds in *H. rhamnoides* L. are summarized.

4.1. Paper chromatography

The paper chromatography is one of the first chromatographic methods used for the analysis of natural products. This technique can be used for isolating known and unknown substances; identifying pure substances and mixtures; purity tests of single substances and mixtures; checking of isolation and determination procedures, and quantitative determination of numerous substances.

PC was commonly used in 1970s for the separation and determination of compounds isolated from plants. This technique was also applied to analysis of flavonoids present in *H. rhamnoides* L. PC can provide valuable information about the type of flavonoids by using special spray reagents [56,57]. However, this method provides less information compared with HPLC– or GC–MS.

Quercetin, kaempferol, isorhamnetin, myricetin, and gallic acid were isolated from ether fraction of methanol extract of *H. rhamnoides* L. leaves by using polyamide and silica gel column chromatography (CC) and PC [(solvent systems: (I) 15% acetic acid and (II) *n*-butanol–acetic acid–water (BAW 4:1:5)] [58].

Rasputina et al. [59] have obtained two polyphenolic compounds from ethyl acetate extract of *H. rhomnaides* L. leaves. R_f values determined by PC were 0.84 and 0.64 for isorhamnetin and kaempferol 3-*O*-β-D-glucoside, respectively (solvent system, BAW 4:1:2).

In another study, quercetin 3-β-D-glucopyranoside, isorhamnetin 3-β-D-glucofuranoside-6-β-D-glucopyranoside, and quercetin 3-galactoglucosides were isolated from methanol extract by using CC and preparative PC [60].

Purve et al. [61] have also studied phenolic compounds of the fruit of the *H. rhamnoides* L. Methanol extract after removing lipophilic compounds was applied to a LH-20 sephadex column and eight flavonoids were obtained subsequently by using preparative paper chromatography (solvent systems 15%-acetic acid, *n*-butanol–acetic acid–water 4:1:5 and 3:1:1) The R_f values of these compounds in BAW systems (4:1:5) were: quercetin 3-β-D-rutinoside (0.76), quercetin (0.64), quercetin 7-*O*-rhamnoside (0.55), isorhamnetin 3-*O*-rutinoside (0.45), and isorhamnetin 3-β-D-glucoside (0.60). However, R_f values of quercetin 3-*O*-methyl ether, isorhamnetin and kaempferol were not provided [61].

Eight flavonoids from the ethanol extracts of *H. rhamnoides* L. fruits were determined by two-dimensional paper chromatography in which the same solvent systems [58] used [62]. R_f values in systems I and II: isorhamnetin, 0.05, 0.79; isorhamnetin 3-*O*-β-D-glucopyranosil-(6-1)-*O*-α-L-rhamnopyranoside (narcissin), 0.33, 0.53; isorhamnetin 3-*O*-β-D-glucopyranoside, 0.47, 0.46; rutin, 0.57, 0.35; and isorhamnetin 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnoside, 0.28, 0.62. These compounds were identified by comparing UV and IR spectrums, NMR spectrums of TMS derivatives, and acidic hydrolysis products with authentic samples [62].

4.2. Thin-layer chromatography

Thin-layer chromatography has frequently been used for the separation and the quantitative or semi-quantitative analysis of natural compounds. It can also be used to control isolation in column chromatography and identity of the compounds. TLC has some advantages such as rapidity, easiness, and cheapness. This method does not require complex instrumental equipment and it is more sensitive than PC. However, this method provides less information compared with HPLC– or GC–MS.

High-performance TLC (HPTLC) is an instrumentalized, quantitative method allowing greater separation efficiency, faster separations, and improved detection limits. It is carried out on layers composed of particles with a smaller particle diameter, compared to conventional TLC. Quantitative HPTLC using a densitometric scanner can produce results that are comparable with GC and column liquid chromatography (HPLC) when optimally performed [63]. Although TLC, GC, and HPLC are highly complementary, TLC has advantages in many respects including simplicity of operation, the availability of many sensitive and selective reagents for detection and confirmation without interference of the mobile phase, and the ability to repeat detection and quantification at any time with changed parameters [63].

TLC on alumina plates was used for separation of total carotenoids from the hexane extracts of *H. rham-*

noides L. fruits [64]. Developing systems were consisting of petroleum ether–acetone (96:4) and petroleum ether–benzene–methanol (60:10:1) mixtures. Nine carotenoids were determined and five of these (α-, β-carotenes, lycopene, polycislicopine and zeaxanthine) were identified. β-Sitosterin was also isolated from unsaponified fractions of the hexane extract by TLC on silica gel plates (hexane–ethyl acetate, 9:1 and 6:4) in the same study [64].

Total lipid extract isolated from *H. rhamnoides* L. fruits was separated by TLC on silica gel plates [65]. Development was performed with solvent system hexane–diethyl ether–acetic acid (90:15:4). Neutral lipids (acylglycerols and sterols) and carotenoids were separated, and polar lipids (phospholipids and galactolipids) remained at the baseline. The polar lipid extract was separated further by HPTLC on silica gel plates. Mobile phase was *n*-propanol:chloroform:methanol:methyl acetate:0.25% aqueous potassium chloride (8.3:8.3:2.7:10:3, by volume). Lipid bands were visualized by spraying the layers with an aqueous solution containing 3% copper acetate and 8% phosphoric acid and scanned at 366 nm using a scanning photodensitometer. The identification of a phospholipid band was made by comparing with the R_f values of standards. The ratio of the integration area of each phospholipid band compared with that of the external standard (SM), together with a calibration curve derived from the appropriate standard, was used for quantitative evaluation. The concentrations of individual polar lipids were estimated as μg/g carotenolipoprotein complexes (CLPs), which were: phosphatidylcholine (338.5), phosphatidylglyserol (88.4), phosphatidylethanolamine (657.2), digaloctosylacylglycerol (554.1) and monogalactosyldiacylglycerol (822.1) [65].

4.3. High-speed counter current chromatography

The conventional methods, such as TLC, column chromatography and HPLC, are tedious and usually require multiple steps [66,67]. Counter-current chromatography (CCC) is describing modern liquid–liquid chromatography, without solid support, requiring two immiscible phases [68]. In most of the reported variants of CCC, one phase remain stationary while the second phase is passed through the stationary solvent phase. The principle of separation involves the partition of a solute between the two phases [69].

High-speed counter-current chromatography (HSCCC) is a useful method for rapid chromatographic purification employing highly efficient fractionation by a hybrid technique of liquid–liquid counter-current distribution and liquid chromatography, in conjunction with the use of centrifugal force. There is no solid support matrix at the HSCCC. Therefore, it eliminates irreversible adsorptive loss of samples onto the solid support matrix used in the conventional column chromatography and HPLC. Consequently, the method is ideal for the preparative separation of several natural products [70–74] such as flavonoids.

The preparative separation of isorhamnetin, kaempferol and quercetin from crude flavone aglycones of *H. rhamnoides* L. has been achieved by a multidimensional counter-current chromatographic system [75]. The system was set for the first time with two sets of high-speed counter-current chromatography instruments. Two-phase solvent system composed of chloroform–methanol–water (4:3:2, v/v/v) was employed. The sample solution was prepared by dissolving the crude flavone aglycons in above solvent system used for the separation. The efficiency of the preparative separation was controlled by RP-HPLC using a C_{18} column and isocratic mobile phase [methanol–0.04% H_3 PO_4 (50:50, v/v)]. The effluent was monitored at 254 nm.

4.4. Gas chromatography

Gas chromatography is one of the most efficient chromatographic techniques for separating volatile mixtures. A variety of columns with different properties are available. However, capillary columns with dimethyl polysiloxane (methyl silicone), non-polar and Carbowax 20 M polar phases are commonly used [76]. Nevertheless, the fused-silica capillary GC columns are mostly preferred. The use of these columns with improved surface provide inertness, thermal stability and a good resolution [77,78]. In capillary GC, the peak resolution is expressed in terms of column efficiency, separation and retention factors [79], those are primarily affected by the polarity of the stationary phase, column length, internal diameter and film thickness [80–82]. With the advent of high-resolution capillary GC using fused-silica columns, separation of complex mixtures of plant extracts was become possible. Moreover, fused-silica columns are highly applicable in practical work due to their flexibility and simplicity in handling and easy connection to GC and mass spectrometers [83].

Identification based on direct comparison of retention times with standards or precise knowledge of retention indices, e.g. Kovats' retention indices [76,84–86]. However, identification based only GC retention data and Kovats' retention indices are not sufficient. Therefore, nowadays the combination of gas chromatography and mass spectrometry in the electron impact mode (GC–EI-MS) is a well-established technique for the routine analysis of plant extracts. This technique offers the possibility to gain additional information by mass spectra.

For flavonoids, GC–MS is a very useful tool for the analysis of complex mixtures. However, sometimes identification is limited when a single chromatographic peak formed by several compounds, which makes difficult to interpret the recorded mass spectra. There are several possibilities to solve this problem. One of them is MS–MS (tandem mass spectrometry), which, when coupled with GC, allows to differentiate each component of such complex peaks. Moreover, the presence of minor constituents can also be confirmed [87,88].

Gas chromatography coupled with mass spectrometric detection (GC–MS) is one of the most widely utilized ana-

lytical techniques. The explosion of applications stems from the excellent qualitative information obtained by high sensitivity inherent with mass spectrometric detection. The great majority of GC–MS applications utilize capillary GC with quadropole MS detection and electron ionization (EI) [89].

In recent years, capillary GC with mass spectrometry (MS) detection has become a primary analytical tool for qualitative and quantitative analysis of complex mixtures. Because of the wide range of applications, the analytical requirements have motivated a variety of chromatographic and detection developments. GC has been the method of choice in fatty acid analysis for about half of a century. Within a few years after the first separations of individual volatile fatty acids performed by James and Martin [90], GC had become widely adopted as a highly applicable tool in micro-scale analytical work in a number of research areas of fatty acids.

The general principle of sample preparation have been comprehensively covered in the previous reviews, including the selection of solvents, their properties and modes of extraction of lipids [91,92], and the purification and separation of lipid classes by solid phase extraction (SPE) and thin-layer chromatography [93,94]. On the analysis of lipids (fatty acids and sterols) and volatile compounds isolated from different parts of *H. rhamnoides* L. GC and GC–MS have been commonly used.

The fatty acid compositions of triglycerols in *H. rhamnoides* L. oil obtained by extraction from dried fruit with petroleum ether were analyzed by Muraveva et al. [95]. Fatty acids methyl ethers (FAMEs) for GC analysis were obtained by MeOH/acetylchloride. FAMEs were analyzed by GC-FID with glass column. Identification of fatty acids was carried out by comparing retention time (R_t) with the standards. Quantification of fatty acids was performed using miristinic acid as an internal standard and it has been reported that palmitic, oleic and palmitoleic acids were predominantly present.

Pintea et al. [65] determined the fatty acid compositions of total and individual polar lipids separated from carotenolipoproten complexes of *H. rhamnoides* L. fruits. The polar lipid fractions were separated by HPTLC. Identity of lipids was confirmed by co-chromatography with a standard mixture of polar lipids. The individual polar lipids were saponified, transesterified and methyl esters of the fatty acids were analyzed by GC-FID. The 17:0 acid was used as internal standard.

The identification of the fatty acid methyl esters were determined from the retention times (R_t) compared to standards, whilst quantification was made by area integration. A composite sample, containing all fatty acids, was submitted to GC–MS in order to confirm the identifications made by R_t comparisons. The main fatty acids in polar lipids were: oleic 18:1 (33.9%), palmitic 16:0 (31.9), palmitoleic 16:1 (9c) (23.0%) and linoleic 18:2 (9c, 12c) (5.2%). The minor acids: miristic 14:0 (0.56%), stearic18:0 (1.45%), linolenic 18:3 (9c, 12c, 15c) (2.86%), arachidonic 20.0 (0.27%), 20:1 (9c) (0.23%), behenic 22:0 (0.31%) and 22:1 acids (13c) (0.27%).

Yang and Kallio [96] investigated fatty acid composition of lipids of seeds and whole berries of *H. rhamnoides* L. Sample preparation included solvent extraction using a mixture of chloroform–methanol, washing with a salt solution of lipids [97], the purification and separation of lipid groups by solid phase extraction.

Total oil, TAG and glyserophospholipid (GPL) fractions transesterified by sodium methoxide and fatty acid methyl esters were analyzed by GC-FID. FAMEs were identified by comparison with a standard mixture of known composition and the fatty acid composition was expressed as a weight percentage of the total fatty acids.

Yang and Kallio [98] studied fatty acid composition TAG and GPL from seed and berries in different origin. Lipids were isolated as described by Christie [97]. TAG and GPL were separated and transesterified by sodium methoxide for GC analysis of FAMEs. Quantification of each fatty acid was carried out using methyl nonadecanoate as the internal standard without the application of any correctional factors for different fatty acids.

The combination of linoleic and α-linolenic acids constituted about 70% of the total fatty acids of seed TAG and 60% of seed GPL. Oleic acid (18:1n-9) (typically 15–20%) and palmitic acid (16:0) (8–13%) were the other major fatty acids in seed TAG and GPL. In whole berries, linoleic and α-linolenic acids comprised around 20–25% in TAG, and 35–40% in GPL. Oleic acid was commonly present 10–20% in both TAG and GPL of whole berries. A striking feature of the whole berry oil was the high proportion of palmitic (typically 25–30% in TAG, 15–20% in GPL) and palmitoleic acids (around 25% in TAG, 15–20% in GPL), the latter being almost absent in seed oil. FAME was identified by comparison with a standard mixture of known composition [97].

Sterols in seeds, pulp/peel fractions, and whole berries of *H. rhamnoides* L. were analyzed as TMS derivatives by GC–MS [99]. Lipids were saponified before the addition of cholesterol palmitate as an internal standard. In order to isolate the sterols from unsaponifiables, they were applied to a Silica Sep-Pak Cartridge and were derivatized by incubation in a mixture of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS). The TMS derivatives of sterols were analyzed by GC–MS with positive ion electronimpact mode. Sterols were identified by comparing the mass spectra and retention times with those of reference compounds: campesterol (campest-5-en-3β-ol), sitosterol (stigmast-5-en-3β-ol), stigmastanol (5α-stigmastan-3β-ol), α-amyrin (5α-urs-12-en-3β-ol), and β-amyrin (5α-olean-12-en-3β-ol) or the mass spectra published in the literature. The list of sterols isolated and identified from *H. rhamnoides* L. is given in Table 1.

The sterol TMS derivatives were analyzed with gas chromatograph equipped with a flame ionization detector (FID). The same column and GC parameters were used as in the GC–MS analysis (Table 2). The quantification of sterol compounds was carried out with a cholesterol internal standard and calculated by applying the detector response of sitosterol.

Table 1
Sterols and triterpenes identified in seeds and pulp/peel of *Hippophae rhamnoides* L. [99]

Identified compound	Occurrence	Identified compound	Occurrence
Campest-5-en-3β-ol (campesterol)	Seeds, pulp/peel	9,19-cylco-5α,9β-lanost-24 en-3βol (cycloartenol)	Seeds, pulp/peel
Stigmastadienol	Seeds, pulp/peel	4α,14α-dimethyl-9β,19-cycloergost-24(241)-en-3β-ol	Seeds, pulp/peel
Stigmast-5-en-3β-ol (sitosterol)	Seeds, pulp/peel	5α-urs-12-en-3β-ol seeds, (α-amyrin)	Pulp/peel
5α-Stigmastan-3β-ol (stigmastanol)	Seeds, pulp/peel	stigmasta-7,24(24^1)-dien-3β-ol	Seeds, pulp/peel
24(*Z*)-stigmasta-5,24(24^1)-dien-3β-ol (isofucosterol)	Seeds, pulp/peel	4α,14α,24^1-trimethylergosta-8,24(24^1)-dien-3β-ol	Seeds, pulp/peel
Stigmast-8-en-3β-ol	Pulp/peel	24-Methyl-5α-cycloart-24(24^1) en-3β-ol (24 methylenecycloar tanol)	Seeds, pulp/peel
4a,14a-Dimethyl-5a-ergosta-8,24(24^1)-dien-3β-ol (obtusifoliol)	Seeds, pulp/peel	24(*Z*)-4α-methyl-5α-stigmasta-7,24(24^1)-dien-3β-ol (citrostadienol)	Seeds, pulp/peel
Stigmasta-5,24(25)-dien-3β-ol	Seeds	Friedelan-3-ol*c*	Pulp/peel
Stigmasta-(8,24)-dien-3β-ol	Pulp/peel	Friedelan-3-ol*c*	Pulp/peel
Stigmast-7-en-3β-ol	Seeds, pulp/peel		

Tian et al. [100] analyzed volatile composition of leaves from 14 populations of *H. rhamnoides* ssp. *yunnanensis* and *H. rhamnoides* ssp. by GC–MS. Sample preparation was carried out by the filtration of dichloromethane extract of dried leaves. The compounds were identified by matching their mass spectral fragmentation patterns with those stored in the spectrometer database. 44 compounds were detected, 40 of them were identified by their mass spectra fragmentation patterns. The main compounds include tetracosane (10–40%), hexadecanoic acid (<0.1–11%), octadecatrienol (5–27%), tetracosene (3–11%), eicosanol (<0.1–13%) and others (Table 2).

Cakir [101] has reported the essential oil and fatty acid composition of the fruits of *H. rhamnoides* L. The volatiles from fruit were extracted by $CHCI_3$. Lipids from mesocarps and seeds were extracted with *n*-hexane. Fatty acids, before saponification, were isolated by TLC and *trans*-esterified. FAMEs were analyzed by GC fused silica capillary column. Quantification of volatiles and FAMEs were obtained from GC peak area integrations using an electronic integrator. Analyses of volatile components were carried out with GC–MS. The identification of the volatile compounds was based on GC retention indices and FAMRs were determined from the retention times compared to standards. Volatile constituents of fruit *H. rhamnoides* L. were determined as aliphatic esters, aliphatic alcohols, aliphatic hydrocarbons and acids.

Table 2 lists GC systems used for detection of fatty acids sterols and volatiles in *H. rhamnoides* L.

4.5. High-performance liquid chromatography

High-performance liquid chromatography is the most powerful and the most frequently used technique for natural compounds (izoflavones, saponins, carotenoids, sesquiterpene lactons) [102–105] and also flavonoids analysis [106].

Table 2
The methods used in the GC determination of fatty acids and volatile compounds in *Hippophae rhamnoides* L.

Samples	Derivatization	Column	Identification/quantitation	Reference
Oil of fruit	Acetylchloride/CH_3OH	10% polyethylene glycol succinat on chromatone N-AW–HMDS, 2.4 m, glass column	FAMEs standards, retention time, internal standard (miristinic acid)	[95]
Total and polar lipids	BF_3/MeOH	BP × 70 (30 m × 25 mm i.d., 0.25 μm), capillary column	Retention time, internal standard, GC–MS	[65]
Oil, TAG and GPL	NaMeO	NB 351 (25 m × 0.32 mm i.d., 0.2 μm) silica capillary column	FAMEs standards	[96]
TAG and GPL	NaOMe	NB 351 (25 m × 0.32 mm i.d., 0.2 μm) silica capillary column	Internal standard (methyl nonadecanoate)	[98]
TAC and GPL	NaOMe	NB 351 (25 m × 0.32 mm i.d., 0.2 μm), silica capillary column	FAMEs standards, internal standard (methyl nonadecanoate)	[143]
Sterols	*N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS)	DB-1710 (30 m × 0.25 mm i.d., 0.25 μm), capillary column	Retention time, internal standard, GC–MS of TMS derivatives	[99]
CH_2CI_2 extract		HP-INNOWax (30 m × 0.25 mm i.d., 0.25 μm) fused-silica capillary column	GC–MS	[100]
$CHCI_3$ extract		SE-52 (25 m × 0.32 mm i.d., 0.15 μm), fused-silica capillary column	Retention time, retention indices, GC–MS	[101]
Lipids	0.5 M MeOH/NaOH BF_3/MeOH	DB-1 (30 m × 0.53 mm i.d., 0.25 μm), fused-silica capillary column		

It has also been used extensively for determination of both aglycones and glycosides.

HPLC offers significant advantages in terms of simplicity, speed, cost (depending on detection method), sensitivity, specificity, precision and sample preservation.

HPLC was first used for the determination of flavonoids in 1976 [107]. This technique has the advantages of simultaneous separation and quantification of the flavonoid compounds without preliminary derivatization [108,109]. However, due to the lack of standard compounds for many flavonoid glycosides and their great numbers, it has become an accepted practice to hydrolyze the glycosides into aglycones before HPLC analysis. For flavonoids, high-performance liquid chromatography with ultra-violet detection (HPLC–UV) is a very convenient approach and has been used for the determination of the total flavonoids in the dried plant extracts [110]. The weakness of the detection method, HPLC–UV, is its non-specificity leading to the possibility of sample matrix interference [106].

The use of diode array detection (DAD) (which enables the collection of on-line spectra and simultaneous quantification by several wavelengths) has become especially common [111–115]. Recently, coulometric electrode array detection has been shown to be a promising technique for flavonoid analysis. Coulometric array detection enables increased selectivity and sensitivity for the HPLC analysis of phenolic and flavonoid compounds based on differences of their voltammetric properties [116–118].

More recently, hyphenated techniques coupling HPLC with different spectroscopic detection methods have been developed. The use of on-line detection and identification systems allowing chemical screening of plant extracts for a number of phytochemicals is a promising breakthrough in the determination and structural analyses of natural products.

HPLC–MS and capillary electrophoresis methods have also been employed to determine phenolic compounds present in foods [119–122]. Most of the different classes of flavonoids and their metabolites are separated by reversed-phase HPLC.

Elution systems are usually binary: (system A) with an aqueous acidified polar solvent such as aqueous acetic acid, phosphoric acid, or formic acid and (system B) a less polar organic solvent such as methanol or acetonitrile, possibly acidified. Less frequently, runs are isocratic or tertiary, and even quaternary systems have been reported [123,124]. Details about the solvent systems and HPLC column have been reviewed by Merken and Beecher [106].

4.5.1. UV detection

HPLC–UV detection was employed to determine various chemical compounds (flavonoids, vitamin C, tocopherols) in *H. rhamnoides* L. Flavonoids are of interest because of their apparent health-promoting effects as antioxidants [45,125] and anticarcinogen [126–128]. Therefore, there is an increasing interest in analyzing flavonols of this plant.

Flavonoids have two characteristic absorption bands in the UV–vis region, Band I (arise from B-ring) and Band II (from A-ring) with a maximum in the 300–550 nm and 240–285 nm range, respectively. UV spectra of flavones and flavonols have a Band II peak at around 240–280 nm and a Band I peak around 300–380 nm [56]. Detection of flavonoids in food analysis is performed usually by UV–vis with diode array detection. Flavones, flavonols, and flavonol glycosides were usually detected at wavelengths such as 270 nm [129], 365 nm [130] and 370 nm [131], although detection at 280 and 350 nm was also used [132]. Problems caused by differences in the wavelengths for maximum UV absorption by individual flavonoids can be solved by using DAD.

Flavonoids and phenolic acids of *H. rhamnoides* L. berries were studied by HPLC–DAD methods [133]. In that study, the pretreatment cleanup procedure for HPLC analysis was adapted from Häkkinen et al. [134]. The solvent gradient elution program used is given in Table 3. The flow rate was 0,5 ml min^{-1} and injection volume 20 μL. Retention times and UV–vis spectra of the peaks were compared with those of the standards. Quercetin and *p*-coumaric acid were used as secondary standards for flavonoids (kaempferol and myricetin) and phenolic acids (ferulic, *p*-hydroxybenzoic, gallic and ellagic acids), respectively. Quercetin (87.3%), *p*-coumaric acid (2.8%), ferulic acid (3.1%), *p*-hydroxybenzoic acid (2.4%), and ellagic acid (4.4%) were detected in berry of *H. rhamnoides*. Phenolic compounds in berries were expressed as the percentages of the total content of all compounds.

Rosch et al. [135] have investigated the phenolic composition of juice of *H. rhamnoides* L. fruits by HPLC–DAD and electrochemical detection (ECD). In this approach, acid hydrolysis [136] was carried out during extraction of foods to convert the various flavonoid glycosides into their respective aglycones.

A chromatographic separation of hydrolyzed and filtered juices was made using reversed phase HPLC. This allows the determination of phenolic acids and flavonoids within one analytical run. The detection was performed by a DAD at 280 and 350 nm. Injection volume was 20 μL of standard solution or filtered juice [135].

Flavonols of *H. rhamnoides* L. juice eluting at times between 35 and 65 min were measured at a wavelength of 350 nm and the structures of the flavonols were identified by comparing with authenthic reference substances (Fig. 1). They have been shown to be the predominating polyphenols in *H. rhamnoides* L. berries [133]. Major components of juice were 3-*O*-rutinoside, 3-*O*-glucoside and 3-*O*-glucoside-7-*O*-rhamnoside of isorhamnetin. Isorhamnetin and isorhamnetin 7-*O*-rhamnoside were minor flavonoids. Furthermore, far smaller concentrations were of quercetin 3-*O*-rutinoside and 3-*O*-glycoside. Quercetin and kaempferol were determined following hydrolysis of *H. rhamnoides* L. juices.

Vitamin C was determined with HPLC–DAD in berries of *H. rhamnoides* L. of different origins [137]. Sample preparation includes diluting of juice with purified water, addition

Table 3
HPLC of chemical compounds of *Hippophae rhamnoides* L.

Food	Analyte	Sample preparation	Stationary phase	Guard	Mobile phase	Detector	Reference
Berries	Flavonoids phenolic acids	Filtration, hydrolyzed with 1.2 M HCI/MeOH by refluxing, filtered	ODS-Hypersil (100 × 4 mm, 3.5 μm)	a	(A) 50 mM ammonium dihydrogen phosphate, pH 2.6; (B) 0.2 mM *ortho*-phosphoric acid, pH 1.5; (C) 20% solvent A in 80% acetonitrile; gradient: 100% A, 0–5 min; 4% C/96% A 5–15 min; 8% C/92% A,15–25 min; 8% C/92% B, 25–25.01 min; 20% C/80% B, 25.01–45 min; 30% C/70% B, 45–50 min; 40% C/60% B, 50–55 min; 80% C/20% B, 55–60 min; 80% C/20% B, 50–65 min; 100 A, 65–70 min; 100 A, 70–75 min	DAD	[133]
	Flavonol aglycones	Extracted and hydrolyzed, filtered	LiChroCART RP C_{18} (125 × 3 mm, 5 μm)	b	(A) 1% formic acid; (B) acetonitrile; gradient: 10–13% B in A, 0–10 min; 13–70% B in A, 10–25 min; 70% B in A, 25–29 min; 70–10% B in A, 29–30 min; 10% B in A, 30–35 min	DAD, 220–450 nm, HPLC-MS	[136]
	Zeaxanthin esters	Extracted with methanol/ethyl acetate/light petroleum (1:1:1), filtered	YMC analytical C_{30} (250 × 4.6 mm, 5 μm)	c	(A) CH_3OH/*tert*-butyl methyl ether/H_2O (81:15:4); (B) CH_3OH/*tert*-butyl methyl ether/H_2O (6:90:4); isocratic: 100% A 10 min; gradient: 50% B 40 min; 100% 50 min; 100 A 55 min; isocratic: 100% A 55–60 min	DAD, 450 nm, HPLC-(APCI) MS	[155]
Juices	Flavonols	Filtration, hydrolyzed with 1.2 M HCI/MeOH by refluxing, filtered	Fluofix 120E (250 × 4.6 mm, 5 μm)	d	(A) Water/phosphoric acid (99.5:0.5); (B) acetonitrile/water/phosphoric acid (50:49.5:0.5); gradient: 0% B 5 min, 0–25% B 40 min, 25–80% B 20 min, 80–100% B 5 min, 0% B 5 min	DAD, 280 and 350 nm ECD	[135]
	Phenolic acids	Filtered, extracted with hexane, extracted HCI/ethylacetate pH 2				ECD	
	Flavan-3-ols	Filtered over a Sephadex LH-20 column				ECD	
	Ascorbic acid	Diluted, filtered and dissolved in 5% meta-phosphoric acid			(A) Water/phosphoric acid (99.5:0.5); isocratic: 100% A	ECD	
	Ascorbic acid	Diluted with purified water, added reducing agent and filtered	LiChrospher 100 RP-18		0.5% KH_2PO_4 water solutions (containing 0.1% dithiothreitol)	DAD, 254 nm	[137]
	Organic acids	Juice separated, aqueous layer utilized, organic acids isolated from sugars	Bio-Rad HPX-87H (300 × 7.8 mm column	e	(A) 0.02N sulfuric acid; isocratic: 100% A	RID	[142]
	Monomeric sugars		Water's Carbohydrate analysis column (300 × 3.9 mm)	f	(A) 85% acetonitrile/water; isocratic: 100% A	RID	
Seeds and pulp/peel	Tocopherols, tocotrienols	Extracted, filtered, purified	LiChroCART 250-4 Superspher Si 60	g	Gradient: 92% hexane/8% diisopropyl ether, 0–5 min; 92% hexane/8% diisopropyl ether to 83% hexane/17 diisopropyl ether, 5–30 min; 83% hexane/17 diisopropyl ether, 30–35 min	FD	[143]

(a) RP-18 (5 μm, 10 mm × 4 mm) guard column; (b) LiChroCART (5 μm, 4 mm × 4 mm, Purospher RP-18e) guard column; (c) C30-reversed phase material including a (10 mm × 4 mm i.d.); (d) Fluofix 120E (250 mm × 4.6 mm, 5 μm) guard column; (e) Brownlee Polypore H 10 μm guard column (30 mm × 4.6 mm); (f) Water's μBondapak NH_2 (20 mm × 3.9 mm) analysis column; (g) Merck LiChroCART 4-4, Lichrospher Si 60 guard column.

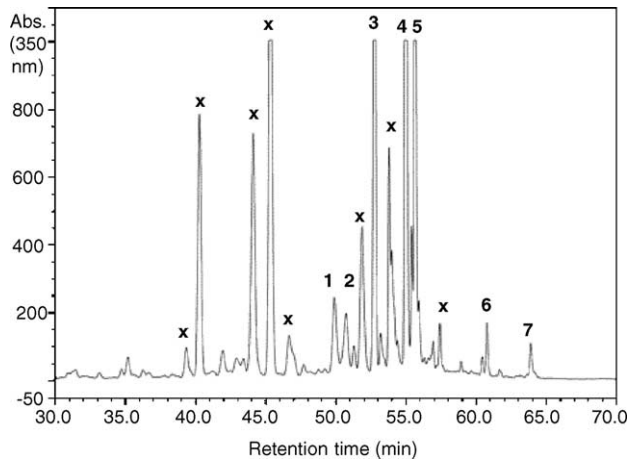

Fig. 1. Typical HPLC–DAD (absorbance, 350 nm) plot of filtered sea buckthorn juice. Peaks: (1) quercetin 3-*O*-rutinoside, (2) quercetin 3-*O*-glucoside, (3) isorhamnetin 3-*O*-glucoside-7-*O*-rhamnoside, (4) isorhamnetin 3-*O*-rutinoside, (5) isorhamnetin 3-*O*-glucoside, (6) isorhamnetin 7-*O*-rhamnoside, (7) isorhamnetin, and (x) unidentified flavonol glycosides. Reprinted with permission from [135]. Copyright 2003 American Chemical Society.

of dithiothreitol (DTT) as a reducing agent and filtering of sample solution. The vitamin C concentration in the sample solution, and, thus, in the juice was determined according to the absorption peak area at 254 nm, using an external standard method.

4.5.2. Electrochemical detection

Electrochemical detection is a particularly useful method for determination of electroactive compounds, such as phenols, with better sensitivity than UV detection for HPLC applications [138–140]. The phytochemicals, which contain phenolic groups (phenolic acids, flavonoids), are generally electroactive and can be detected by ECD.

Rosch et al. [135] have used HPLC–ECD method (Table 3) for the determination of very low amounts of phenolic compounds with a catechol or pyrogallol structure in juice of *H. rhamnoides* L. Gallic acid (3,4,5-trihydroxybenzoic acid) and protocatechuic acid (3,4-dihydroxybenzoic acid) were identified by comparing the retention times of standards. The identity of gallic and protocatechuic acids was confirmed by HPLC–DAD investigation, after removing lipophilic substances and flavonoids from juice, by comparing with the retention times of standards. Detection was performed at 280 and 350 nm. Gallic acid was previously found only in the leaves of *H. rhamnoides* L. [141].

Rosch et al. indicated also the occurrence of (+)-catechin and (−)-epicatechin by HPLC–ECD chromatogram [135]. The identity of these flavan-3-ols was confirmed by comparison of the UV spectra after the juice was cleaned up over a small column filled with Sephadex LH-20 [142]. Detection was performed at 500 mV. All other conditions are given in Table 3.

Ascorbic acid in juice *H. rhamnoides* L. was determined also by HPLC–ECD [135]. Sample preparation was made by the dilution of filtrated juice with metaphosphoric acid (5% in deionized water). The elution was performed isocratically (100% water–phosphoric acid 99.5:0.5, v/v). All other conditions are given in Table 3.

Table 3 lists several modern HPLC methods for detection of phenolic compounds (flavonols, phenolic acids, flavonol-3-ols, tocopherols and ascorbic acid) in *H. rhamnoides* L.

4.5.3. Fluorescence detection

Detection based on fluorescence is generally more sensitive than UV absorption. Fluorescence is measured against a nearly zero background, whereas UV absorption is determined from decreases in the incident light source. It should be noted that the number of compounds that are naturally fluorescent is quite limited. However, fluorescence detection can provide better selectivity in addition to better sensitivity compared to UV detection.

Kallio et al. [143] reported a HPLC method for analysis of tocopherols and tocotrienols in *H. rhamnoides* L. berries. Lipids were extracted using a methanol–chloroform extraction procedure from seeds and the soft parts (pulp and peel) isolated from freeze-dried berries. Lipids were washed up with salt solution for the purification. Purified lipid fraction was filtered and solvent was removed. Tocopherols and tocotrienols were analyzed with a normal phase HPLC-fluorescence detector. The excitation wavelength was 295 nm, and the emission wavelength was 330 nm. The identification of individual peaks was carried out by co-injection with standard compounds. The quantification was carried out with internal standard tocol and corrected with specific correction factors determined by analysis of standard compounds. All the four tocopherol isomers (α-, β-, γ-, δ-tocopherols) and α-tocotrienol were found in seeds. α- and β-tocopherols were the two major isomers, representing typically 40–50% and 20–40%, respectively, of the total tocopherols and tocotrienols. The proportions of each of the other isomers were typically 5–10%.

4.5.4. Refractive index detection

HPLC with refractive index (RI) detection is a powerful technique for fast and reliable HPLC results when analyzing non-UV absorbing substances, such as carbohydrates and lipids. Beveridge et al. [144] have studied neutral sugars and organic acids in juice of berries from *H. rhamnoides* L. by HPLC–RI method. In that paper, aqueous layer separated from berry juices was analysed. Aqueous layer sample was passed through ion-exchange resin to isolate the organic acids from the sugars. Organic acids were separated isocratically and detected by a Water's 410 refractive index detector. Chromatographic control and quantification was achieved by a Waters Millinium System. Five components (oxalic, citric, tartaric, malic and quinic acids) were detected in the organic acid fraction obtained from the anion-exchange resin. Neutral, monosaccarides were also separated isocratically and detected as mentioned above. Juice of berries con-

tains glucose and fructose as the major sugars detected by HPLC.

4.5.5. Mass spectrometry

HPLC coupled to mass spectrometry (MS) has been used in several studies to identify natural compounds from biological samples [145–149]. Much effort has been devoted to developing HPLC–MS methods with mainly atmospheric pressure ionization interfaces (APCI) or electrospray ionization interfaces (ESI) [150–152].

HPLC–electrospray ionization (ESI)-MS offers advantages in terms of sensitivity and capacity for the analysis of large, thermally labile and highly polar compounds [153].

Häkkinen and Auriola [136] applied high-performance liquid chromatography–electrospray ionization-mass spectrometry (HPLC–ESI-MS) for separation and identification of flavonol aglycones and glycosides in *Hippophae rhamnoides* L. berries. All mass spectrometry data were acquired in the positive ionization mode. The sample preparation procedures for aglicon include hydrolysis, filtration followed by solvent evaporation and sample dilution. On the other hand, sample preparation of flavonoid glycosides include thawing and homogenizing the berries, diluting, centrifuging, and evaporating to dryness.

In Häkkinen and Auriola's [136] research, quercetin was identified with both ESI-MS and DAD in *H. rhamnoides* L. berries. Quercetin eluted at 19.9 min in the HPLC–DAD system and at 18.3 min in the HPLC–MS system. A MS spectrum of *m*/*z* 303.3 ion resulted in a fragmentation spectrum MS–MS in which the main ions matched with the fragmentation spectrum of quercetin. Using HPLC–ESI-MS techniques, kaempferol was also identified (retention time, 19.9 min; *m*/*z* 287).

Total ion chromatograms (TICs) and full scan source induced dissociation (SID) chromatogram, as well as MS, MS–MS and MS^3 spectra, were used to identify glycoside in berries.

Two glycosides deoxyhexose–hexoses and hexoses–deoxyhexose (*m*/*z* 611) of quercetin were identified by using HPLC–ESI-MS (retention times, 12.3 and 14.3 min). To confirm the data obtained using the HPLC–ESI-MS procedure, fractions of the glycosides from berries were separated, hydrolyzed, silylated, and the sugars present in glycosides were analyzed using gas chromatography–mass spectrometry. In the GC–MS analysis of both fractions of *m*/*z* 611, glucose and rhamnose were identified.

Electrospray ionization technique provides information on the structure of the aglycones and glycosides without time-consuming, pre-purification or derivatization steps. The HPLC–ESI-MS technique is highly valuable in the identification of flavonol aglycones and glycosides from *H. rhamnoides* L. berry.

HPLC–MS technique was applied also for analysis of carotenoids in *H. rhamnoides* L. berry. HPLC–MS systems have a number of advantages over UV detection: sample quantity required for analysis is very small, with limits of detection around 500 fmol for individual carotenoids [150]. HPLC–MS provides information on carotenoid molecular mass, and fragmentation patterns allow determination of the carotenoid structure.

Dachtler et al. [154] have reported that, using HPLC–MS with atmospheric pressure chemical ionization, the lutein stereoisomers can be distinguished from zeaxanthin stereoisomers in the upper picogram range within one chromatographic run.

Weeler and Breithaupt [155] have reported that liquid chromatography–atmospheric pressure chemical ionization mass spectrometry [LC–(APCI)MS] method allows to identify zeaxanthin esters of a standard mixture and of *H. rhamnoides* L. extracts. Sample preparation includes extraction with slightly polar plus non-polar solvent, filtering, drying with anhydrous sodium sulfate and evaporation. Saponification of zeaxanthin esters was carried out with methanolic KOH. These esters were quantified on the basis of their respective molecular masses using zeaxanthin for calibration. However, total zeaxanthin was determined after saponification of aliquots of the extracts. The zeaxanthin esters are separated using HPLC method with a diode array detector (450 nm) with C_{30} column. Detection limit was 0.4 μg/ml.

For identification, the standard mixture of zeaxanthin esters and *H. rhamnoides* L. extracts were analyzed using LC–(APCI)MS in the positive mode. The quasimolecular ion ($[M + H]^+$) was clearly detectable. The fragmentation pathway was dominated by the loss of one ($[M + H - FA]^+$) or two ($[M + H - 2FA]^+$) fatty acids. In the case of zeaxanthin monoesters, this led to the formation of *m*/*z* 551.4, whereas diesters formed *m*/*z* 533.4, the "backbone" of zeaxanthin. Zeaxanthin, zeaxanthin dipalmitat, and β-cryptoxanthin palmitat were identified in the fruit extract of *H. rhamnoides* L. In that study, zeaxanthin ester patterns as well as the concentration of free (native) and total (after saponification) zeaxanthin of *H. rhamnoides* L. were reported [155].

4.6. Capillary electrophoresis

Capillary electrophoresis is a relatively new separation technique compared to other chromatographic methods such as GC and HPLC. The theory of CE has been discussed in detail in several studies [156–158].

Since first described in its modern format by Jorgenson and Lukcas in 1981 [159,160], CE has been developed into several modes, such as capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC). CZE is the basic mode of CE techniques.

CE has gained widespread interest as a favourable technique for the determination of natural compounds in biological matrices such as plants [161]. Basically, separation by CE is a result of differences in electrophoretic mobilities of charged species in an electric field in small diameter capillaries.

The most attractive advantages of CE are rapidity and high-resolution of separation with sample volumes in the

nanoliter range. Borate buffers with pH 8–11 and a concentration range of 25–200 mM are commonly used. Borate can form complexes with ortho-dihydroxyl groups on the flavonoid nucleus and with vicinal *cis*-dihydroxyl groups of sugar, and therefore makes the separation easy [162]. The influence of structure and buffer composition on electrophoretic behavior of flavonoids has been discussed in several studies [163–166].

Charged species are separated from each other in the capillary, and all neutral species migrate at the same speed. Since most of the flavonoids are weak acids, alkaline buffers are used to ensure that the phenolic moiety is charged for electrophoretic separation.

The instrumentation format of CE is similar to HPLC. Therefore, most detection methods used in HPLC can be adapted to monitor CE separations. The various modes of detection in CE are present and include fluorescence, UV absorbance, and electrochemical detection. The detection limits (ranging from single molecule to 10^{-5} M), detection scheme complexity and the particular applications dictate the selection of detection methodology in CE [167].

4.6.1. UV detection

As mentioned above, the instrumentation format of CE is similar to HPLC-therefore most detection methods used in HPLC can be adapted to monitor CE separations.

Yue et al. [168] have developed a fast capillary zone electrophoresis method, for the determination of three flavonoids (quercetin, kaempferol and isorhamnetin) in the methanol extract from *H. rhamnoides* L. fruits with UV detection at 270 nm. In this method, dimethyl-β-cyclodextrin (DM-β-CD) was used as modifier. Separation capillary was an untreated fused silica capillary with a total length of 35 cm and an effective length of 30 cm (50 μm i.d., 365 μm o.d.).

The effects of several CE parameters on the resolution of flavonols were studied. The effect of borate buffers (20 mM) with different pH on migration behavior was studied. The pH 10.0 was then selected as the reference pH for further optimization. Flavonoids as phenol types are weak acids with pK_a value of 9.0–10.0, therefore, these compounds will be constantly ionized in higher pH, and migration times and resolution will increase with increasing pH values. Influence of organic solvents (methanol, acetone and isopropanol) to enhance the separation was also studied. However, the analytical times were still longer because the electroosmotic flow (EOF) decreased with the addition of organic modifiers. The effect of DM-β-CD concentration on the migration time was determined. The contents of three flavonoids in *H. rhamnoides* L. berry were successfully determined in 4.5 min (Fig. 2), with satisfactory repeatability and recovery. The detection limits were 0.83 μg ml^{-1} for quercetin, 0.83 μg ml^{-1} for kaempferol and 1.65 μg ml^{-1} for isorhamnetin [168]. The results demonstrated that the proposed CE method is very suitable for the fast determination of flavonoid compounds in the extract of the fruits of *H. rhamnoides* L.

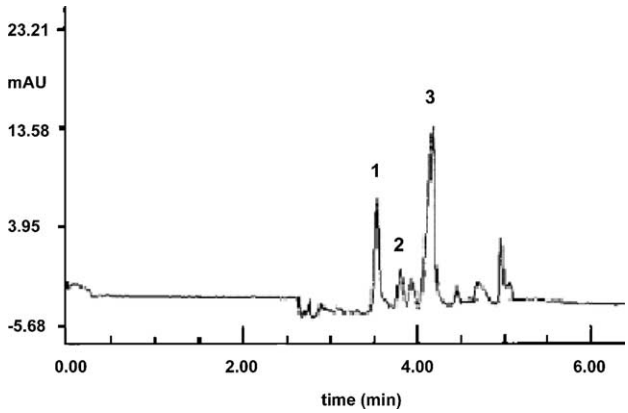

Fig. 2. Chromatogram of mixture of methanol extract of the whole plant of Hippophae rhamnoides: (1) kampferol; (2) isorhamnetin; (3) quercetin. Analytical conditions: borate, 20 mM; pH, 10.0; DM-β-CD, 5 mg ml^{-1}; voltage 15 kV; temperature 25 °C; and UV detection wavelength, 270 nm. From [168].

Vaher and Koel [169] described the CZE method for separation of poliphenolic compounds extracted from berries of *H. rhamnoides* by supercritical fluid extraction with carbon dioxide modified with different alcohols. CE separations were performed using an electropherograph with an UV detector. The separation was monitored at 240 nm and applied voltage was 18 kV. An uncoated capillary with dimensions of 75 cm × 50 μm was used. Before use, the capillary was rinsed with 1 M sodium hydroxide solution, methanol, and separation medium. Effects of electrolytic solutions (disodium hydrogen phosphate, sodium dihydrogen phosphate, ammonium acetate and borate) and of concentrations on separation efficiency were studied. The optimum separation of poliphenolic compounds (resveratrol, catechin and quercetin) carried out in the pH 9.4 with 25 mM disodium tetraborate buffer. Identification of analytes is made by comparison with standard [169].

4.6.2. Electrochemical detection

A high-performance capillary electrophoresis with electrochemical detection (CE–ECD) was developed for the determination of the bioactive flavonoids in *H. rhamnoides* L. [170]. Dried *H. rhamnoides* L. fruit and seed residues were extracted with anhydrous ethanol and H_3BO_3–$Na_2B_4O_7$ buffer (running buffer) (1:1), filtered and injected directly to the CE–ECD. The fused-silica capillary (75 cm × 25 μm i.d.) was used for the separation. Samples injected electrokinetically, applying 14 kV for 8 s. The effect of several factors such as the activity and concentration of running buffer, the separation voltage, the applied potential and the injection time were investigated. The method showed good selectivity, sensitivity and reproducibility. Thus, this quantitative method is useful for the analysis of ethanolic extracts from *H. rhamnoides* L. The detection limits of epicatechin, catechin, rutin, kaempferol and quercetin were 2.5×10^{-7}, 1.3×10^{-7}, 3.1×10^{-7}, 4.3×10^{-7}, and 5.9×10^{-7} mg L^{-1}, respectively.

Table 4
Natural compounds isolated and identified from *Hippophae rhamnoides* L. by chromatographic methods

Substance	Sample	References
Flavonols		
Quercetin	Leaves	[58]
	Fruit	[61,75,133]
Quercetin 3-galactoside	Leaves	[60]
Quercetin 3-*O*-β-D-glucopyranoside (isoquercetin)	Leaves	[60]
Quercetin 3-methyl ether	Leaves	[61]
Quercetin 3-*O*-rutinoside	Fruit	[61,62]
	Juice	[135]
Quercetin 3-*O*-glucoside	Juice	[135]
Quercetin-7-*O*-rhamnoside	Fruit	[61]
Kaempferol	Leaves	[58,75]
	Fruit	[61]
Kaempferol 3-*O*-β-D-glucopiranoside (astragalin)	Leaves	[59,60]
Isorhamnetin (3,5,7,4-tetrahydroxy-3-methoxyflavon)	Leaves	[58,59]
	Fruit	[62,75]
	Juice	[135]
Isorhamnetin 3-*O*-β-D-glucopyranosil-(6-1)-*O*-α-L-rhamnopyranoside (narcissin)	Fruit	[62]
Isorhamnetin 3-*O*-β-D-glucopyranoside	Fruit	[61,62]
Isorhamnetin 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnoside	Fruit	[62]
	Leaves	[59]
	Juice	[135]
Isorhamnetin 3-*O*-glucoside-7-*O*-rhamnoside	Juice	[62,135]
Isorhamnetin 3-*O*-rutinoside	Fruit	[61]
	Juice	[135]
Isorhamnetin 3-*O*-glucoside	Juice	[135]
	Fruit	[61]
Isorhamnetin 7-*O*-rhamnoside	Fruit	[61]
	Juice	[135]
Isorhamnetin 3-*O*-galactoside	Fruit	[61]
Myricetin	Leaves	[58]
	Fruit	[133]
Flavon-3-ols		
(+)-Catechin	Juice	[135]
(−)-Epicatechin	Juice	[135]
Phenolic acids		
Gallic acid (3,4,5-trihydroxybenzoic acid)	Leaves	[58]
	Juice	[135]
Protecatechuic acid (3,4-dihydroxybenzoic acid)	Juice	[135]
p-Coumaric acid	Berries	[133]
Ferulic acid	Berries	[133]
p-Hydroxybenzoic acid	Berries	[133]
Ellagic acid	Berries	[133]
Tocopherols		
α-Tocopherol	Seeds	[143]
β-Tocopherol	Seeds	[143]
γ-Tocopherol	Seeds	[143]
δ-Tocopherol	Seeds	[143]
Carotenoids		
α-Carotene	Fruit	[64]
β-Carotene	Fruit	[64]
γ-Carotene	Fruit	[64]
Lycopene	Fruit	[64]
Zeaxanthin	Fruit	[64,155]
Zeaxantin dipalmitat	Fruit	[155]
β-Cryptoxanthin palmitat	Fruit	[155]
Organic acids		
Oxalic acid	Juice	[144]
Citric acid	Juice	[144]
Tartaric acid	Juice	[144]
Malic acid	Juice	[144]
Quinic acid	Juice	[144]
Ascorbic acid	Fruit	[137,144]
Lipids		
Phosphatidylcholine	Fruit	[65]
Phosphatidylglycerol	Fruit	[65]
Phosphatidylethanolamine	Fruit	[65]
Digalactosyldiacylglycerol	Fruit	[65]
Monogalactosylacylglycerol	Fruit	[65]
Fatty acids	Oil	[95,100]
	Fruit	[65,98,101]
	Seeds	[96,98]
Sterols	Fruit	[99]
Volatile compounds		
Aliphatic esters	Fruit	[101]
Aliphatic alcohols	Fruit	[101]
	Leaves	[100]
Aliphatic hydrocarbons	Fruit	[101]
	Leaves	[100]
Aliphatic aldehyde	Leaves	[100]
Monosaccarides		
Glucose	Juice	[144]
Fructose	Juice	[144]

Natural compounds isolated and identified from *H. rhamnoides* L. by chromatographic methods are listed in Table 4.

5. Conclusion

Hippophae rhamnoides L. is a widely used plant in traditional medicine for various clinical conditions. Antiulcerogenic effect, in vitro and in vivo antioxidant effects, radioprotective effects, beneficial effects on experimental injury and clinical diseases of the liver, inhibition of platelet aggregation are among the pharmacological affects *H. rhamnoides* L. reported.

Hippophae rhamnoides L. has some biologically important compounds. Thus, there is an increasing interest for the analysis of chemical compounds present in this plant. However, there is no a single method that can be recommended as routine procedure for the analysis of complex mixtures.

Therefore, a variety of analytical methods have been used for the analysis of chemical compounds of *H. rhamnoides* L. All methods presented have various advantages and limitations.

Simple chromatographic methods (PC, TLC, TLC–densitometry) can be used to control isolation in open column chromatography and/or separation of compounds present in the extracts. However, compared with HPLC– or GC–MS these chromatographic methods provide less information.

HPLC with DAD–UV detection is the method of choice, since it is equally suited to the quantitative measurement of flavonoids (aglycones and glycosides) and requires minimal sample preparation. Detection using fluorescence may help in certain cases. For well-described matrices on where there are many known flavonoids present, HPLC–ECD is suitable. For studies where the flavonoids in the sample are not known a priori methods based on mass spectrometry are recommended.

The HPLC–ESI-MS technique is highly valuable in the identification of flavonol aglycones and glycosides from berry extracts. This ionization technique requires very little sample work up and provides information on the structure of the aglycones and glycosides without time-consuming prepurification or derivatization steps. HPLC–electrospray ionization (ESI)-MS offers advantages in terms of sensitivity and capacity for the analysis of large, thermally labile and highly polar compounds.

Capillary electrophoresis has been proven to be a useful technique for analyzing natural compounds, such as flavonoids. The amount of the sample required for the analysis is very small, and the other advantage of the CE is its low running cost. CE is also a powerful separation method for phenolic compounds particularly when it is combined with electrochemical and UV detection.

Modern GC separations are converging towards the combination of high speed and high resolution. This technique is proved to be applicable on complex lipidic samples and can certainly be extended to other matrices.

A multidimensional counter-current chromatographic system is a successful method for the preparative separation of flavonol aglycones of *H. rhamnoides*.

References

[1] A. Rousi, Ann. Bot. Fennici 8 (1971) 177.
[2] B. Yang, K.O. Kalimo, R.L. Tahvonen, L.M. Matilla, J.K. Katajisto, H.P. Kallio, J. Nutr. Biochem. 11 (2000) 338.
[3] M.Y. Xu, X.X. Sun, W.X. Tong, Hippophae 7 (1994) 32.
[4] J.Y. Cheng, K. Kondo, Y. Suzuki, Y. Ikeda, X. Meng, K. Umemuraet, Life Sci. 72 (2003) 2263.
[5] G.J. Vetters, Gartenbau 37 (1990) 206.
[6] K.W. Quirin, D. Gerard, Parfum. Kosmet 10 (1993) 618.
[7] T.S.C. Li, W.R. Schroeder, Hort. Technol. 6 (1996) 370.
[8] T.S.C. Li, L.C.H. Wang, in: G. Mazza (Ed.), Functional Foods: Biochemical and Processing Aspects, Technomic Publishing, Lancaster, PA, 1998, pp. 329–356.
[9] P.S. Chikox (Ed.), Areas and Resources of Medicinal Plants in USSR, Moskva, 1983, p. 340 (in Russian).
[10] H. Kallio, High Technology Finland, The Finnish Academies of Technology, Helsinki, Finland, 2001.
[11] Traditional Tibetian Healing Inc., http://store.yahoo.com/tibetansupplements/hiprhaml.html, access date March 29, 2004.
[12] Tibetian medical encyclopedia, http://www.doctorgarma.com/rst/hippophae_rhamnoides/, access date March 29, 2004.
[13] P.H. Davis, Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh, 1972.
[14] T. Beveridge, T.S.C. Li, B.D. Oomah, A. Smith, J. Agric. Food Chem. 47 (1999) 3480.
[15] Z.H. Zheng, Z.H. Dong, J. Yu, Modern Study of Traditional Chinese Medicine, Beijing University of Traditional Chinese Medicine, Xue Yuan Press, Beijing, China, 1997.
[16] D.Ts. Tsybikova, D.B. Rasputina-Sultumova, N.F. Komissarenko, M.N. Bolotova, Utilization problems of medicinal resources in Siberia and Far East, Novosibirsk, 1983 (in Russian).
[17] B.A. Vereshchagin, K.A. Sobolevskaya, A.N. Yakubov, Useful Plants of Western Siberia, 1959 (in Russian).
[18] Kh.Kh. Khalmatov, I.A. Kharlamov, P.K. Alimbaeva, M.O. Karriev, I.Kh. Khaitov, Important Medicinal Plants of Central Asia Tashkent, 1984 (in Russian).
[19] O.D. Barnaulov, L.M. Belenovskaya, in: L.I. Medvedyeva (Ed.), Medicinal Plants of USSR, Nauka, Leningrad, 1988, p. 356 (in Russian).
[20] T.A. Aseeva, B.B. Batuev, I.S. Khapkin, N.N. Fedotovskikh, D.B. Dashiev, Rastit. Res. 21 (1985) 15 (in Russian).
[21] E.G. Bazaron, T.A. Aseeva, Thesis of Indo-Tibetian medicine-"Vaydurya-onbo", Novosibirsk, 1984, p. 118 (in Russian).
[22] A.F. Gammerman, Materials to Learn the Resources of Traditional System of Indo-Tibetian Medicine, Novosibirsk, 1982, p. 53 (in Russian).
[23] B.B. Badareva, Monument of Tibetian medicine-"Dzeytsxar migchjan", Novosibirsk, 1985 (in Russian).
[24] F. Li, T. Guo, Proceedings of the International Symposium on Seabuckthorn, Xi'an China, 1989.
[25] E.G. Bazaron, D.Ch. Chibikova, Rastit. Res. 14 (1978) 67 (in Russian).
[26] J.L. Harwell, Llodia 32 (1969) 153;
J.L. Harwell, Llodia 33 (1970) 288;
J.L. Harwell, Llodia 34 (1971) 386.
[27] V.I. Chopik, L.G. Dudchenko, A.N. Krasnova, Wild Medicinal Plants of Ukrain Kiev, 1983 (in Russian).
[28] B. Yang, K.O. Kalimo, R.L. Tahvonen, et al., J. Nutr. Biochem. 11 (2000) 338.
[29] J.Y. Cheng, K. Kondo, Y. Suzuki, et al., Life Sci. 72 (2003) 2263.
[30] I.A. Damirov, L.I. Prilipko, D.Z. Shukurov, Y.B. Kerimov, Medicinal Plants of Azerbaijan, AARIF, Baku, 1982 (in Russian).
[31] L.N. Yudkina, Vestn. Venerologii i Dermatologii 2 (1954) 20 (in Russian).
[32] A.T. Vedmedenko, Oftalmologicheskiy Jurn. 1 (1959) 48 (in Russian).
[33] R. Puupponen-Pimia, L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia, K.M. Oksman-Caldentey, J. Appl. Microbiol. 90 (2001) 494.
[34] Z. Peizhen, D. Xiaofan, M. Lina, L. Dexing, L. Lanping, Proceedings of the International Symposium on Sea Buckthorn (*H. rhamnoides* L), The Secretariat of International Symposium on Sea Buckthorn, Xi'an, China, 1989, p. 373.
[35] Y. Li, H. Liu, in: J. O'Neill, H. Chen, Barrish (Eds.), Relevance to Human Cancer of N-Nitroso Compounds, Tobacco Smoke and Mycotoxins, International Agency for Research on Cancer, Lyon, France, 1991, p. 568.
[36] H. Suleyman, L.O. Demirezer, M.E. Buyukokuroglu, M.F. Akcay, A. Gepdiremen, Z.N. Banoglu, F. Gocer, Phytother. Res. 15 (2001) 625.
[37] H. Suleyman, M.E. Buyukokuroglu, M. Koruk, F. Akcay, A. Kiziltunc, A. Geptiremen, Indian J. Pharm. 33 (2001) 77.

[38] J.F. Xing, B.R. Yang, Y.L. Dong, B.W. Wang, J.X. Wang, H.P. Kallio, Fitoterapia 73 (2002) 644.
[39] B.R. Yang, K.O. Kalimo, L.M. Mattila, S.E. Kallio, J.K. Katajisto, O.J. Peltota, H.P. Kallio, J. Nutr. Biochem. 10 (1999) 622.
[40] J.Y. Cheng, K. Kondo, Y. Suzuki, Y. Ikeda, X.S. Meng, K. Umemura, Life Sci. 72 (2003) 2263.
[41] A.K. Johansson, H. Korte, B.R. Yang, J.C. Stanley, H.P. Kallio, J. Nutr. Biochem. 11 (2000) 491.
[42] C. Eccleston, Y. Baoru, R. Tahvonen, H. Kallio, G.H. Rimbach, A.M. Minihane, J Nutr. Biochem. 13 (2002) 346.
[43] J. Wu, X.J. Yu, X. Ma, X.G. Li, D. Liu, Acta Pharmacol. Sin. 15 (1994) 341.
[44] X.Q. Gao, M. Ohlander, N. Jeppsson, L. Bjork, V. Trajkovski, J. Agric. Food Chem. 48 (2000) 1485.
[45] S. Geetha, M.S. Ram, V. Singh, G. Ilavazhagan, R.C. Sawhney, J. Ethnopharmacol. 79 (2002) 373.
[46] S. Geetha, M.S. Ram, S.S. Mongia, V. Singh, G. Ilavazhagan, R.C. Sawhney, J. Ethnopharmacol. 87 (2003) 247.
[47] H. Suleyman, K. Gumustekin, S. Taysi, S. Keles, N. Oztasan, O. Aktas, K. Altinkaynak, H. Timur, F. Akcay, S. Akar, S. Dane, M. Gul, Biol. Pharmacol. Bull. 25 (2002) 1133.
[48] K. Gumustekin, K. Altinkaynak, H. Timur, S. Taysi, N. Oztasan, M.F. Polat, F. Akcay, H. Suleyman, S. Dane, M. Gul, Hum. Exp. Toxicol. 22 (2003) 425.
[49] M. Gul, F.Z. Kutay, S. Temocin, O. Hanninen, Indian J. Exp. Biol. 38 (2000) 625.
[50] T.J. Cheng, Zhonghua Yu Fang Yi Xue Za Zhi 26 (1992) 227.
[51] Z.L. Gao, X.H. Gu, F.T. Cheng, F.H. Jiang, World J. Gastroenterol. 9 (2003) 1615.
[52] H.C. Goel, J. Prasad, S. Singh, R.K. Sagar, I.P. Kumar, A.K. Sinha, Phytomedicine 9 (2002) 15.
[53] H.C. Goel, C.A. Salin, H. Prakash, Phytother. Res. 17 (2003) 222.
[54] I.P. Kumar, S. Namita, H.C. Goel, Mol. Cell. Biochem. 238 (2002) 1.
[55] K. Hostettmann, J.L. Wolfender, in: C.R. Yang, O. Tanaka (Eds.), Advances in Plant Glycosides, Chemistry and Biology, Elsevier, Amsterdam, 1999, p. 233.
[56] T.J. Mabry, K.R. Markham, M.B. Thomas, The Systematic Identification of Flavonoids, Springer, Berlin, 1970.
[57] L.K. Klishev, B.A. Bondyukova, L.C. Alyukina, Flavonoids of plants, Alma-Ata, 1978 (in Russian).
[58] T.K. Cumbalov, M.M. Muxamedyarova, V.V. Polyakov, Khim. Prirod. Soed. 2 (1976) 281 (in Russian).
[59] D.P. Rasputina, N.F. Komissarenko, D.Ts. Tsibikova, Khim. Prirod. Soed. 1 (1976) 97 (in Russian).
[60] M.M. Muxamedyarova, T.K. Cumbalov, Khim. Prirod. Soed. 2 (1977) 281 (in Russian).
[61] O. Purve, Ya. Jamyansan, B.M. Malikov, T. Baldan, Khim. Prirod. Soed. 3 (1978) 403 (in Russian).
[62] I.M. Potapova, N.D. Gachechiladze, E.M. Glazunova, X.Y. Yusufbekov, M.D. Isobaev, Khim. Prirod. Soed. 6 (1980) 837 (in Russian).
[63] J. Sherma, J. Chromatogr. A 880 (2000) 129.
[64] E.N. Novruzov, Khim. Prir. Soedin. 1 (1981) 98 (in Russian).
[65] A. Pintea, A. Marpeau, M. Faye, C. Socaciu, M. Gleizes, Phytochem. Anal. 12 (2001) 293.
[66] C.L. Ky, M. Noirot, S. Hamon, J. Agric. Food Chem. 45 (1997) 786.
[67] J.J. Liu, G.L. Zhao, H. Wang, X.H. Zhang, J. Cent. South Univ. T. 9 (2002) 246.
[68] Y. Ito, Biochem. Biophys. Methods 5 (1981) 105.
[69] A. Marston, K. Hostettmann, J. Chromatogr. A 658 (1994) 315.
[70] H.B. Li, F. Chen, T.Y. Zhang, F.Q. Yang, G.Q. Xu, J. Chromatogr. A 905 (2001) 151.
[71] L. Chen, Y.S. Han, F.Q. Yang, T.Y. Zhang, J. Chromatogr. A 907 (2001) 343.
[72] L. Lei, F.Q. Yang, T.Y. Zhang, P.F. Tu, L.J. Wu, Y. Ito, J. Chromatogr. A 912 (2001) 181.
[73] F.Q. Yang, T.Y. Zhang, Q.H. Liu, G.Q. Xu, Y.B. Zhang, S. Zhang, Y. Ito, J. Chromatogr. A 883 (2000) 67.
[74] X.L. Cao, Y. Tian, T.Y. Zhang, X. Li, Y. Ito, J. Chromatogr. A 855 (1999) 709.
[75] F. Yang, J. Quan, T.Y. Zhang, Y. Ho, J. Chromatogr. A 803 (1998) 298.
[76] N.W. Davies, J. Chromatogr. 503 (1990) 1.
[77] S.R. Lipsky, W.J. McMurray, M. Hernandez, J.E. Purcell, K.A. Billeb, J. Chromatogr. Sci. 18 (1980) 1.
[78] W. Jennings, J. High Resol. Chromatogr. Chromatogr. Commun. 3 (1980) 601.
[79] P. Sandra, J. High Resol. Chromatogr. Chromatogr. Commun. 12 (1989) 82.
[80] N.C. Shantha, G.E. Napolitano, J. Chromatogr. 624 (1992) 37.
[81] K. Eder, J. Chromatogr. B 671 (1995) 113.
[82] G. Gutnikov, J. Chromatogr. B 671 (1995) 71.
[83] M.A. Kaiser, M.S. Klee, J. Chromatogr. Sci. 24 (1986) 369.
[84] H. Rotzsche, in: E. Leibnitz, G. Struppe (Eds.), Handbuch der Gaschromatographie, Akadem. Verlagsgesellschaft, Leipzig, 1984, p. 528.
[85] E.E. Stashenko, M.A. Puertas, M.Y. Combariza, J. Chromatogr. A 752 (1996) 223.
[86] E.E. Stashenko, J.R. Martinez, C. Macku, T. Shibamoto, J. High Resolut. Chromatogr. 16 (1993) 441.
[87] A. Cazaussus, R. Pes, N. Sellier, J.-C. Tabet, Chromatographia 25 (1988) 865.
[88] N. Sellier, A. Cazaussus, H. Budzinski, M. Lebon, J. Chromatogr. 557 (1991) 451.
[89] N. Ragunathan, K.A. Krock, C. Klawun, T. Sasaki, C.L. Wilkins, Chromatographia 856 (1999) 349.
[90] A.T. James, A.J.P. Martin, Biochem. J. 50 (1952) 679.
[91] F. Shahidi, J.P.D. Wanasundara, in: C.C. Akoh, D.B. Min (Eds.), Food Lipids: Chemistry, Nutrition, and Biotechnology, second ed., Marcel Dekker, New York, 1998, p. 115.
[92] J.J. Myher, A. Kuksis, J. Chromatogr. B 671 (1995) 3.
[93] J.C. Touchstone, J. Chromatogr. B 671 (1995) 169.
[94] J. Folch, M. Lees, G.H.S. Stanley, Biol. Chem. 226 (1957) 497.
[95] I.A. Muravyeva, D.S. Lagidze, V.S. Bosmoganashvili, Khim. Farm. Zh. 18 (1984) 461.
[96] B. Yang, H.P. Kallio, J. Agric. Food Chem. 49 (2001) 1939.
[97] W.W. Christie, Gas Chromatography and Lipids, The Oily Press, Glasgow, Scotland, 1989.
[98] B. Yang, H. Kallio, J. Food Comp. Anal. 15 (2002) 143.
[99] B. Yang, R.M. Karlsson, P.H. Oksman, H.P. Kallio, J. Agric. Food Chem. 49 (2001) 5620.
[100] C. Tian, P. Nan, J. Chen, Y. Zhong, Biochem. Syst. Ecol. 32 (2004) 431.
[101] A. Cakir, Biochem. Syst. Ecol. 32 (2004) 809.
[102] C.-C. Wang, J.K. Prasain, S. Barnes, J. Chromatogr. B 777 (2002) 3.
[103] W.A. Oleszek, J. Chromatogr. A 967 (2002) 147.
[104] Q. Su, K.G. Rowley, N.D.H. Balazs, J. Chromatogr. B 781 (2002) 393.
[105] I. Merfort, J. Chromatogr. A 967 (2002) 115.
[106] H.M. Merken, G.R. Beecher, J. Agric. Food Chem. 48 (2000) 577.
[107] S. Hasegawa, M.A. Berhow, C.H. Fong, Modern Methods of Plant Analysis, Springer, New York, 1996.
[108] M.G.L. Hertog, Flavonols and flavones in foods and their relation with cancer and coronary heart disease risk, DIP-data Koninklijke Bibliotheek, The Hague, Ph.D. Thesis, Agricultural University Wageningen, 1994.
[109] K. Robards, M. Antolovich, Analyst 122 (1997) 11R.
[110] H. Hasler, O. Sticher, B. Meier, J. Chromatogr. 605 (1992) 41.
[111] C. Chilla, D.A. Guillen, C.G. Barroso, Perez-Bustamante J. Chromatogr. A 750 (1996) 209.

[112] M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, J. Agric. Food Chem. 40 (1992) 2379.
[113] M.G.L. Hertog, P.C.H. Hollman, D.P. Venema, J. Agric. Food Chem. 40 (1992) 1591.
[114] U. Justesen, P. Knuthsen, T. Leth, J. Chromatogr. A 799 (1998) 101.
[115] Y. Nogata, H. Ohta, K.-I. Yoza, M. Berhow, S. Hasegawa, J. Chromatogr. A 667 (1994) 59.
[116] G. Achilli, G.P. Cellerino, P.H. Gamache, J. Chromatogr. 632 (1993) 111.
[117] P. Gamache, E. Ryan, I.N. Acworth, J. Chromatogr. 635 (1993) 143.
[118] C. Guo, G. Cao, E. Sofic, R.L. Prior, J. Agric. Food Chem. 45 (1997) 1787.
[119] B. Fernandez de Simon, I. Estrella, T. Hernandez, Chromatographia 41 (1995) 389.
[120] X.-G. He, L.-Z. Lian, L.-Z. Lin, M. Bernart, J. Chromatogr. A 791 (1997) 127.
[121] G. Poon, J. Chromatogr. A 794 (1998) 63.
[122] F.A. Tomas-Barberan, C. Garcia-Viguera, Analysis 25 (1997) M23.
[123] S. de Pascual-Teresa, D. Treutter, J.C. Rivas-Gonzalo, C. Santos-Buelga, J. Agric. Food Chem. 46 (1998) 4209.
[124] H. Tamura, Y. Hayashi, H. Sugisawa, T. Kondo, Phytochem. Anal. 5 (1994) 190.
[125] C.A. Rice-Evans, N.J. Miller, G. Paganga, Free Radic. Biol. Med. 20 (1996) 933.
[126] B.N. Ames, Science 221 (1983) 1256.
[127] S. Ogava, T. Hirayama, M. Nohara, M. Tokuda, K. Hirai, S. Fukui, Mutat. Res. 142 (1985) 103.
[128] V.B. Guliyev, M. Harmandar, Flavonoids: Molecular Structures, Chemical Characteristics, Determination Techniques, and Biological Activities, Aktif Yayinevi, Erzurum, 1999 (in Turkish).
[129] M. Brolis, B. Gabetta, N. Fuzzati, R. Pace, F. Panzeri, F. Peterlongo, J. Chromatogr. A 825 (1998) 9.
[130] A. Crozier, M.E.J. Lean, M.S. McDonald, C. Black, J. Agric. Food Chem. 45 (1997) 590.
[131] C. Ewald, S. Fjelkner-Modig, K. Johansson, I. Sjoholm, B. Akesson, Food Chem. 64 (1999) 231.
[132] L. De Cooman, E. Everaert, D. De Keukeleire, Phytochem. Anal. 9 (1998) 145.
[133] S. Häkkinen, M. Heinonen, S. Kärenlampi, H. Mykkänen, J. Ruuskanen, R. Törrönen, Food Res. Intern. 32 (1999) 345.
[134] S. Häkkinen, S. Kärenlampi, M. Heinonen, H. Mykkänen, R. Törrönen, J. Sci. Food Agric. 77 (1998) 543.
[135] D. Rosch, M. Bergmann, D. Knorr, L.W. Kroh, J. Agric. Food Chem. 51 (2003) 4233.
[136] S. Hakkinen, S. Auriola, J. Chromatogr. A 829 (1998) 91.
[137] H. Kallio, B. Yang, P. Peippo, J. Agric. Food Chem. 50 (2002) 6136.
[138] D.A. Roston, P.T. Kissinger, Anal. Chem. 55 (1981) 1695.
[139] S.M. Lunte, J. Chromatogr. 2 (1987) 371.
[140] D.J. Jones, C.K. Lim, D.R. Ferry, A. Gescher, Biomed. Chromatogr. 12 (1998) 232.
[141] T.K. Chumbalov, M.M. Mukhamedyarova, V.V. Polyakov, Chem. Nat. Compd. 12 (1976) 597.
[142] G.A. Spanos, R.E. Wrolstad, J. Agric. Food Chem. 38 (1990) 1565.
[143] H. Kallio, B. Yang, P. Petppo, R. Tahvonnen, R. Pan, J. Agric. Food. Chem. 50 (2002) 3004.
[144] T. Beveridge, J.E. Harrison, J. Drover, J. Agric. Food Chem. 50 (2002) 113.
[145] A.Z. Mercadante, A. Steck, H. Pfander, J. Agric. Food Chem. 47 (1999) 145.
[146] C.W. Huck, M. Popp, H. Scherz, G.K. Bonn, J. Chromatogr. Sci. 38 (2000) 441.
[147] F. Khachik, C.J. Spangler, J.C. Smith Jr., Anal. Chem. 69 (1997) 1973.
[148] D. Talwar, T.K.K. Ha, J. Cooney, Clin. Chim. Acta 270 (1998) 85.
[149] T. Hagiwara, T. Yatsuko, K. Funayama, S. Suzuki, J. Chromatogr. B 708 (1998) 67.
[150] C. Rentel, S. Stroschein, K. Albert, E. Bayer, Anal. Chem. 70 (1998) 4394.
[151] R.B. van Breemen, Anal. Chem. News Feat. 1 (1996) 299.
[152] R.B. van Breemen, C.R. Huang, Y. Tan, L.C. Sander, A.B. Schilling, J. Mass Spectrom. 31 (1996) 975.
[153] M. Careri, A. Mangia, M. Musci, J. Chromatogr. A 794 (1998) 263.
[154] M. Dachtler, T. Glaser, K. Kohler, K. Albert, Anal. Chem. 73 (2001) 667.
[155] P. Weller, D.E. Breithaupt, J. Agric. Food Chem. 51 (2003) 7044.
[156] P. Camilleri (Ed.), Capillary Electrophoresis—Theory and Practice, CRC Press, Boca Raton, FL, 1993.
[157] N.A. Guzman (Ed.), Capillary Electrophoresis Technology, Marcel Dekker, New York, 1993.
[158] K.D. Altria, J. Chromatogr. A 856 (1999) 443.
[159] J.W. Jorgenson, K.D. Lukcas, Anal. Chem. 53 (1981) 1298.
[160] J.W. Jorgenson, K.D. Lukcas, J. Chromatogr. 218 (1981) 209.
[161] A. Kulomaa, H. Siren, M.-L. Riekkola, J. Chromatogr. A 781 (1997) 523.
[162] K.R. Markham, S.J. Bloor, in: C.A. Rice-Evans, L. Packer (Eds.), Flavonoids in Heath and Disease, Marcel Dekker, New York, 1999.
[163] P. Pietta, P. Mauri, A. Bruno, C. Gardana, Electrophoresis 15 (1994) 1326.
[164] T.K. McGhie, K.R. Markham, Phytochem. Anal. 5 (1994) 121.
[165] K.R. Markham, T.K. McGhie, Phytochem. Anal. 7 (1996) 300.
[166] G.S. McLeod, M.J. Shepherd, Phytochem. Anal. 11 (2000) 322.
[167] K. Swinney, D.J. Bornhop, Electrophoresis 21 (2000) 1239.
[168] M.-E. Yue, T.-F. Jiang, Y.-P. Shi, Talanta 62 (2004) 695.
[169] M. Vaher, M.K.J. Koel, J. Chromatogr. A 990 (2003) 225.
[170] Q.C. Chy, W.Q. Qu, Y.Y. Peng, Q.H. Cao, J.N. Ye, Chromatographia 58 (2003) 67.