Application of Gas and High Performance Liquid Chromatography Techniques for the Identification of Natural Biologically Active Phenolic Compounds

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Abstract

Using the techniques of Gas and High Performance Liquid Chromatography under standardised conditions, chromatographic profiles ("fingerprints") and spectral characteristics were obtained for some compositions of the major biologically active phytogenous substances: hydoxycinnamic acids, flavonoids, anthocyanins, lignanes, flavolignanes. The data obtained can be used for the analysis of group and individual compositions, for the identification and standardisation of herbal raw material as well as preparations on this base.

INTRODUCTION

Plants produce a large number of biologically active compounds [1, 2] used by humans in various spheres of life. In connection with the urgency of the problem of human health preservation and strengthening, ageing prophylaxis and the treatment of diseases connected with it, the enhancement of human organism resistibility to the influence of combinations of negative natural and anthropogenic factors, the increasing attention is focused on the creation and use of natural biologically active compositions. The necessity of qualitative and quantitative research of natural biologically active compositions is caused as well by that fact that the effect of the components of these mixtures on a human organism is often comparable to the action of pharmaceutical substances.

The complexes of natural biologically active substances are the subject of phytochemical research; due to various kinds of biological activity (antioxidant, immunomodulatory, antiviral, antiallergic, antibacterial, *etc.* [2–4]), they are used in medicine, cosmetology, food-processing industry as well as in agriculture [4–6]. In this connection obtaining the new data on qualitative and quantitative formulation of natural compositions and the development of techniques to analyse them is currently important both in the field of basic and applied research.

Almost all the natural biologically active complexes are multicomponent mixtures. The primary task of phytochemical research consists in the identification as many components as possible in the mixtures of extractive substances. The isolation and identification of individual components from the mixtures of natural compounds similar with respect to the structure and properties is an intricate problem; for solving the latter there are different approaches. The traditional pathway is directed toward the isolation of individual compounds and their identification using physical and chemical methods. The use of the Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) methods on the modern tool base allows one to obtain in some cases the information concerning group composition of multicomponent natural mixtures without isolation of individual compounds as well as without using any large set of reference substances. Within the framework of this concept, an approach is known for the analysis of a wide range of flavorous basing on reversed phase HPLC technique where identification parameters are the relative optical absorbance and the chromatographic retention indices calculated using reference compounds [7–11].

The application of GC method with mass selective detector allows ascertaining the struc-

ture of various groups of volatile compounds. Due to the use of modern mass spectrometric databases the method opens up comprehensive possibilities for compound identification. The use of derivation techniques for heavy and non-volatile compounds essentially extends the possibilities of the gas chromatography method for the identification of natural compounds.

In recent years the methods of capillary electrophoresis [12, 13] and HPLC with mass selective detector [14, 15] are widely applied to the analysis of the mixtures of natural compounds.

Within the framework of the present work using methods GC and HPLC methods in standardized conditions are received chromatographic profiles ("fingerprints") are obtained for a series of the major groups of biologically active phytogenous phenolic compounds as well as spectral and chromatographic characteristics of their basic components. The phenolic compounds those represent one of the most widespread and numerous types of secondary phytometabolites are of various biological activities. A numerous group of natural phenolic compounds is presented by phenylpropanoids such as hydroxycinnamic acids, flavonoids, lignanes, flavolignanes, antocyanins *etc.* containing one or several C_6–C_3 fragments in the structure. Recently these compounds became the subject of researchers' rapt attention in the view of searching prospective biologically active compounds and developing effective pharmaceuticals on this base. Besides, separate groups of phenylpropanoids and their relationships are important chemotaxonomic markers used for the identification and standardization of herbal raw material and the preparations made of it.

The HPLC technique with UV spectrophotometric detection is known to be extensively used for the analysis of natural phenolic compounds [16–18]. For the UV absorption spectra of phenolic compounds, as a rule, the presence of several absorption bands is observed, therefore the detection under HPLC analysis is carried out at the wavelengths those are the most typical for these compounds (for example: 280 nm – catechines, flavanonoles, lignanes, flavolignanes, 320 nm – hydroxicynnamic acids, coumarines, 360 nm – flavononoles, 520 nm – antocyanins) [19]. The separation in the reversed phase HPLC conditions is usually carried out basing on binary systems consisting of methanol or acetonitrile and a dilute acid (formic, acetic, trifluoroacetic, phosphoric).

The creation of electronic collections of standardised chromatographic profiles of the samples of natural raw material and spectroanalytical characteristics of the major groups of biologically active compounds obtained using modern methods of gas and liquid chromatography is the basis for the formation of the approaches to the development of the techniques for the identification and analysis of biologically active compounds and preparations from plant material as well as for the revealing of taxonomic markers.

EXPERIMENTAL

The HPLC assay procedure was carried out using an Agilent 1100 liquid chromatograph (Agilent Technologies, the USA) with an Agilent 1100 G1315B diode array detector, G1313A autosampler, G1311A four-channel pump, G1322A degasser and ChemStation software for chromatography data processing. The column (4.6 × 150 mm) was packed with ZORBAX Eclipse XDB-C8 (5 μm) reversed-phase sorbent. The elution conditions A were as it follows: a linear gradient in the methanol–0.1 % CF_3COOH system (from 0 up to 100 % methanol within 20 min) at the eluent flow rate 0.8 mL/min, the detection at analytical wavelengths of 280, 320 and 360 nm. The elution conditions B were the following: acetonitrile–0.2 % CF_3COOH system (12 : 88) at the eluent flow rate 0.8 mL/min, the detection at analytical wavelength of 520 nm.

The HPLC/MS analysis was performed using an Agilent 1100 Series LC/MSD liquid chromatograph with diode array and mass selective detectors. For the mass selective detector an atmospheric pressure chemical ionisation (APCI) technique was used. The m/z 100–700 positive and negative ion scanning was performed with m/z increment of 0.1. The operation parameters for APCI were as it follows: the flow rate of the drier gas (nitrogen) amounted to 4 L/min, the temperature was 340 °C. The temperature of the vaporizing injector was at 400 °C. The separation was car-

ried out using the 4.6 ×150 mm column packed with Zorbax Rx-C18 (5 μm) reversed-phase sorbent.

The GC/MS analysis procedure was carried out using a HP-6890 gas chromatograph with a HP 5972 MSD. The separation was carried out using a capillary quartz column (30 m × 0.25 mm) with HP-5 stationary phase (0.25 μm); the temperature program mode was 40 °C for 2 min, then the temperature rising rate at 10 °C/min up to 280 °C (during 25 min). The scanning range was 50–500 amu; the injector temperature was 280 °C, the MSD interface temperature 280 °C, the carrier gas was helium (1.0 mL/min), splitless injection. For the identification a NIST 2002 mass spectra library was used.

RESULTS AND DISCUSSION

The use of standardised chromatographic conditions for the group analysis of multicomponent natural compositions allows obtaining such identification parameters as the characteristic chromatographic profile of a mixture of biologically active compounds ("fingerprints") and spectral characteristics of the basic components.

The identification cromatographic parameters for the compounds of various structural types of biologically active natural phenolic compounds, the representatives of those are the derivatives of hydroxycinnamic acids, flavonoids, anthocyanins, lignanes, flavolignanes, are obtained using the HPLC and GC assays. For some groups of phenolic compounds these characteristics were obtained for the first time.

The simplest group of $C_6–C_3$ phenylpropanoids such as hydroxycinnamic acids are contained in different combinations in the free state or as glycosides* almost in each higher plant and exhibit bactericidal properties. Chromatographic profile (Fig. 1) and characteristic spectral quotients (Table 1) for hydroxycinnamic acids are obtained in our work analysing the acidic fraction isolated according the standard pattern [20] from the gum of larch *(Larix Sibirica)*.

The peak assignment was performed using the reference sample of caffeic acid and the data from the literature [16, 21]. For the acids analysed the retention time interval on the chromatogram obtained in standardised conditions, was about 4 min (6–10 min). The data obtained (see Table 1) allow performing the estimation of characteristic spectral peak ratio values A(320/280), A(320/220) and A(220/280) for hydroxycinnamic acids amounting to 1.2±0.4, 1.4±0.6 and 1.0±0.3, respectively.

In order to determine hydroxycinnamic acids using the GC/MS method their preliminary derivation was carried out using diazomethane solution in diethyl ether under standard conditions [22]. The chromatographic profile of the methyl esters of hydroxycinnamic acids of the larch gum is shown in Fig. 2. The peak assignment was performed basing on the comparison of full experimental and library mass spectra. In the mass spectra at the basic peaks the fragmentation with the registration of (M-31) ions characteristic for methyl esters is observed, corresponding to OCH_3 group abstraction. It should be noted that the use of the GC/MS method allows to distinguish *cys*- and *trans*-isomers of hydroxycinnamic acids.

Flavonoids are an integral part of a plenty of higher plants. Besides they form the extracts of some lichens, mosses and algae. The variety of natural flavonoids can be expressed by a general formula $C_6–C_3–C_6$ where several structural types are distinguished depending on the structure of the central fragment and substituent. In the present work we have obtained chromatographic and spectral characteristics for some of them such as flavonoles (I), dihydroflavonoles (II), flavans (III), anthocyanins (IV) (Scheme 1).

To the share of flavonoids such as I, isolated from plant raw material, falls about 40 % of overall natural floavonoids those exhibit antioxidant and capillary protective activity. The list of the plants containing this group of biologically active compounds includes St. John's wort (*Hypericum*), bear's whortleberry (*Arctostaphylos uva-ursi* (L.) Spreng.), borovaya uetrus (*Orthilia secunda*), motherwort (*Leonurus*), hawthorn (*Crataegus*), the leaves of *Ginkgo biloba*, *etc*. [1, 2]. The main flavonoids such as I, isolated from these plants, are such well-known compounds as quercetin, kaempferol, isorhamnetin, myricetin, apigenin and others.

*It should be noted that under the conditions of reversed-phase HPLC the glycosides of phenolic compounds always have shorter retention times than corresponding aglycons.

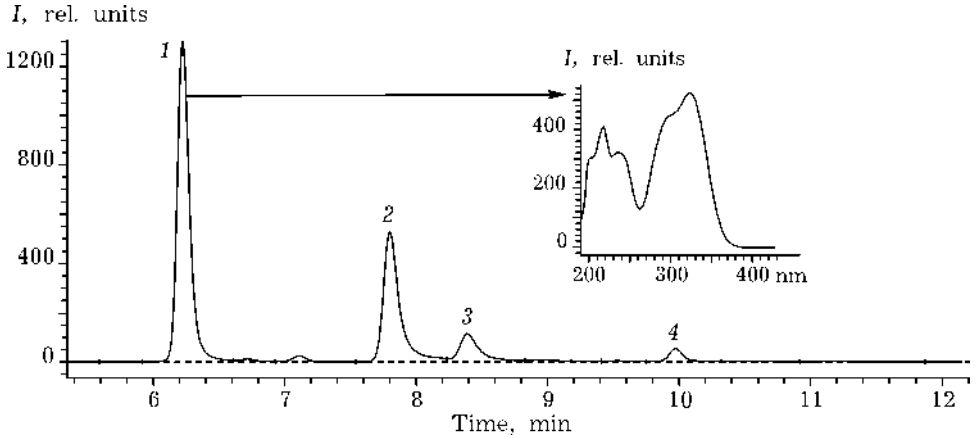


Fig. 1. HPLC profile of hydroxylcinnamic acids of larch gum (λ = 320 nm): *1* – caffeic, *2* – *p*-coumaric, *3* – ferulic, *4* – 3,4-dimethoxycinnamic.

TABLE 1

UV absorption spectral data for hydroxylcinnamic acids of larch gum (see Fig. 1)

Acids	λ_{max}, nm	Spectral peaks ratio values		
		A(320/280)	A(320/220)	A(220/280)
Caffeic	218, 238, 296, 322	1.56	1.25	1.25
p-Coumaric	212, 228, 298, 310	1.21	1.63	0.80
Ferulic	220, 240, 298, 324	1.53	2.38	0.64
3,4- Dimethoxycinnamic	202, 232, 280, 312	0.51	0.44	1.13

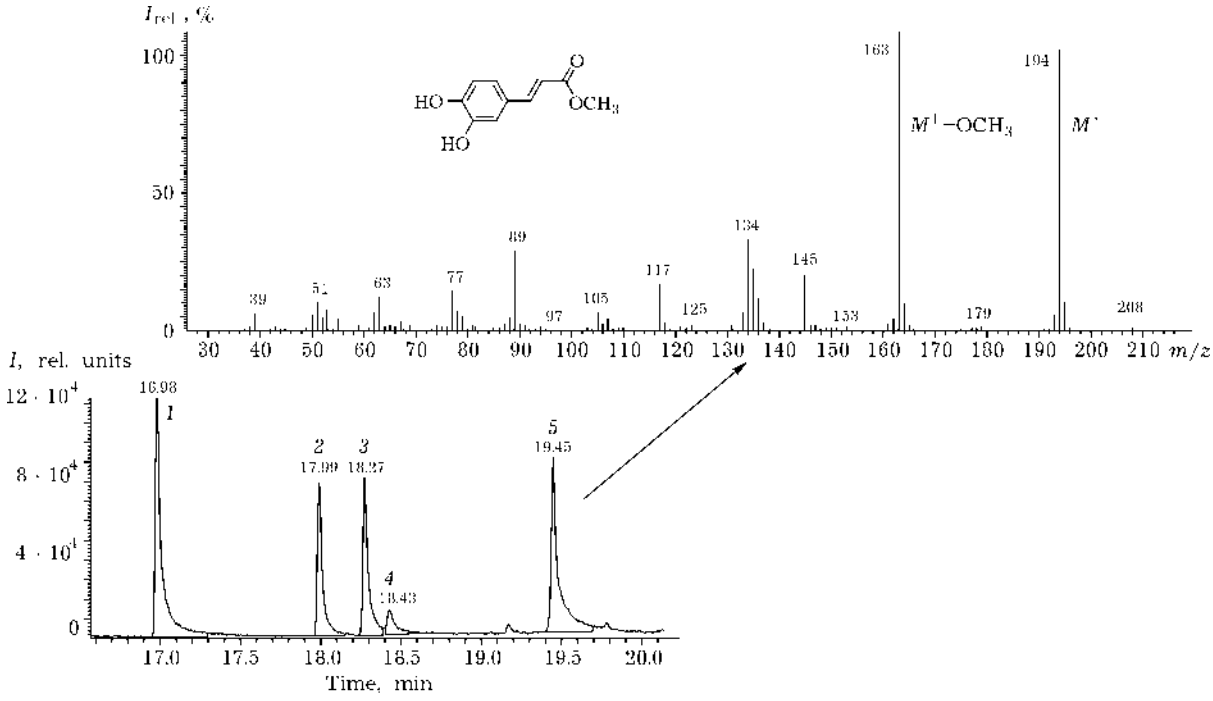
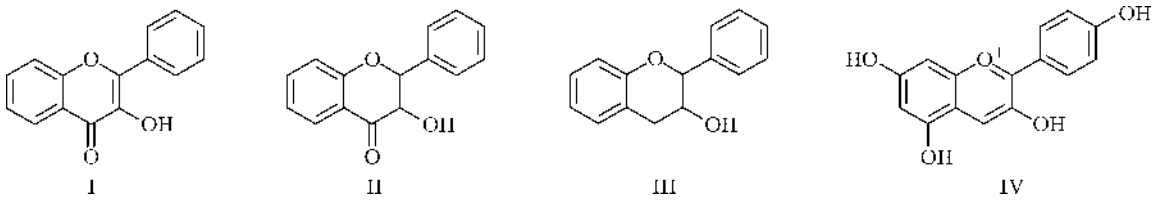


Fig. 2. Chromatogram of methyl esters of hydroxycinnamic acids lof arch gum, obtained using GC/MS technique: *1* – *p*-coumaric, *2* – *cis*-ferulic, *3* – *trans*-ferulic, *4* – dimethoxycinnamic, *5* – caffeic.



Scheme 1.

Figure 3 demonstrates the chromatographic profile of flavonoids such as I from *Orthilia secunda* L. herb obtained using HPLC in standardised conditions. For the sample analysed the retention time interval of the main peaks (quercetin and kaempferol) is ~2 min (14–16 min), amounting to about 10–20 min for wider range of flavonoids (compare to [16]). The UV spectral data for the main components and characteristic spectral peak ratio values (Table 2) are corresponding to the data from the literature (A(254/220) 0.8±0.2; A(360/254) 0.9±0.2, A(360/220) 0.7±0.2 [10]).

The other widespread group of bioflavonoids consists of the compounds such as II. The most known and utilized in the medicine and food-processing industry representative of this group of flavonoids is dihydroquercetin isolated from the timber of larch and exhibiting antioxidant, capillary protective, anti-inflammatory and antihistamine action [23].

In order to obtain the chromatographic profile and UV spectral characteristics of flavonoids such as II we used an aqueous-alcoholic extract of the timber of larch *(Larix Sibirica)* after its CO_2 processing then analysed using HPLC

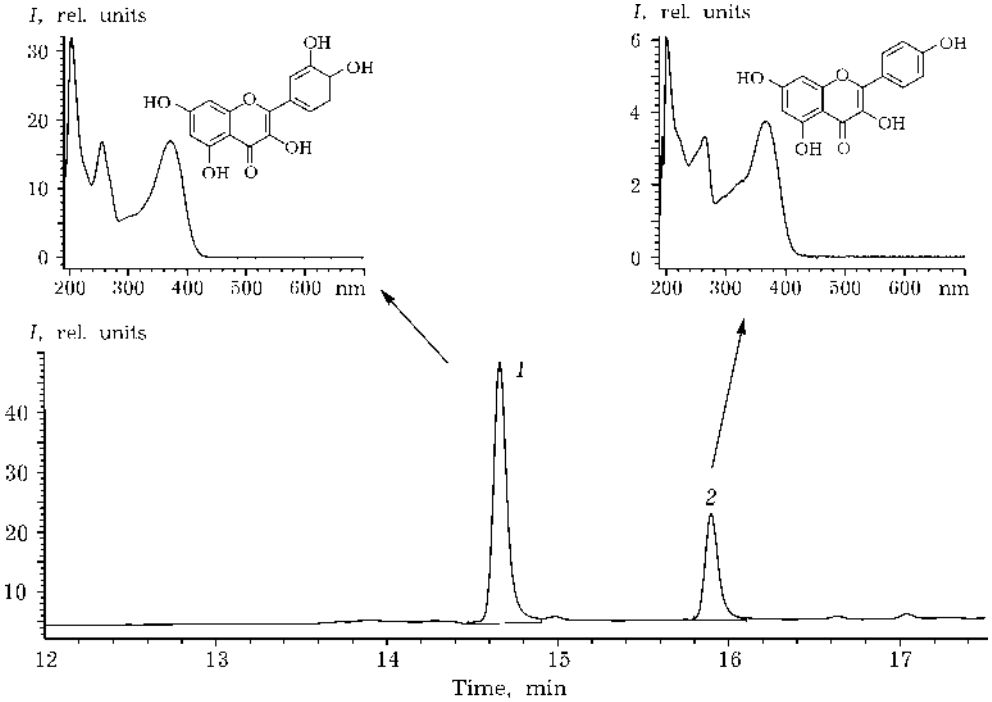


Fig. 3. HPLC profile of the main flavonoids of *Orthilia secunda* L. (λ = 360 nm): *1* – quercetin, *2* – kaempferol.

TABLE 2

UV absorption spectral data for flavonoids of *Orthilia secunda* L. (see Fig. 3)

Compounds	λ_{max}, nm	Spectral peaks ratio values		
		A(254/220)	A(360/254)	A(360/220)
Quercetin	204, 256, 372	0.78	0.92	0.72
Kaempferol	202, 264, 366	0.84	1.12	0.91

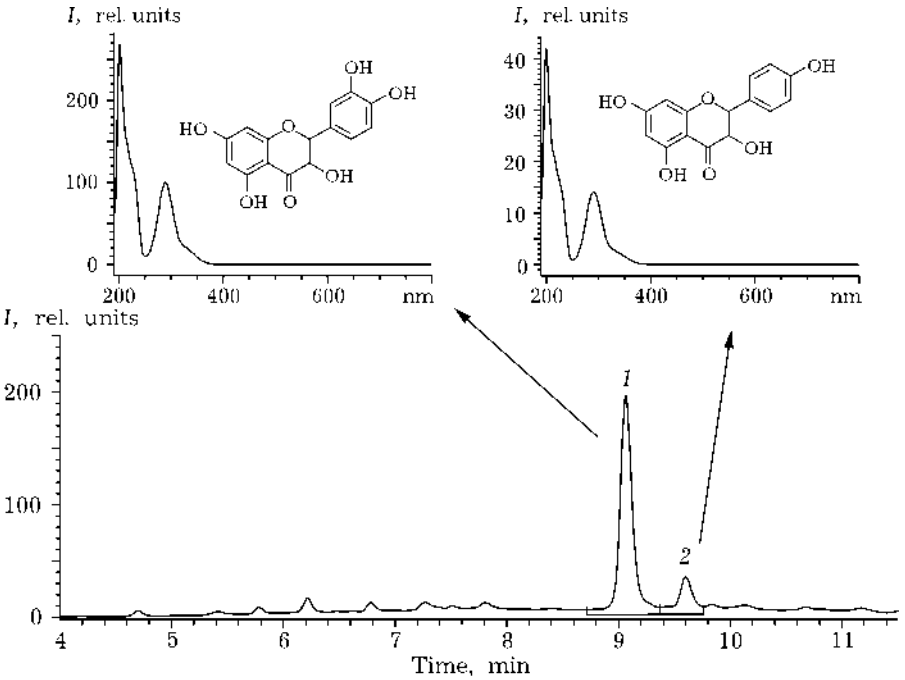


Fig. 4. HPLC profile of larch timber flavonoids (λ = 280 nm): *1* – dihydroquercetin, *2* – dihydrokaempferol.

technique in the standardized conditions of gradient elution. The main peaks of the chromatogram are corresponding to dihydroquercetin and dihydrokaempferol (Fig. 4). The dihydroquercetin peak was identified using a reference sample. In the UV absorption spectra of dihydroquercetin and dihydrokaempferol the absorption bands characteristic for flavonols at the wavelength λ_{max} = (290±2) nm are observed. The retention time interval for the main flavonoids of larch timber is about ~2 min (8–10 min). The characteristic spectral peak ratio values for dihydroquercetin and dihydrokaempferol (Table 3) calculated using the results of the HPLC determination, are corresponding to the data from the literature (A(254/220) 0.19±0.11, A(360/254) 0.18±0.20, A(360/220) 0.03±0.03 [10]).

The chromatographic profile of catechines those belong to the flavonoid group such as III, was obtained using the sample of green tea extract (Fig. 5). Its main components are catechine, epigallocatechine, epigallocatechine gallate, epicatechine and epicatechine gallate. The assignment of the peaks was made basing on the data from the literature [24]. The retention time interval of green tea catechines is ~4 min (6–10 min). The main peaks on the chromatogram *(*peaks Nos. *1–5)* are corresponding to the UV absorption spectra characteristic for catechines with the absorption maximum at λ_{max} = (276±4) nm. The spectral peak ratio values A(254/220) calculated for epigallocatechine, catechine and epicatechine are corresponding to the data from the literature (A(254/220) 0.05±0.02 [10]). For the esters of catechine and gallic acid such as epigallocatechine gallate and epicatechine gallate they slightly differ, which is apparently caused by some changes in the chromophore system (Table 4). For the green tea catechines an estimation was performed for the interval of spectral peak ratio values A(280/220) 0.2±0.1 and A(280/254) 1.0±0.5 those can be

TABLE 3

UV absorption spectral data for flavonoids of larch timber (see Fig. 4)

Compounds	λ_{max}, nm	Spectral peaks ratio values		
		A(254/220)	A(360/254)	A(360/220)
Dihydroquercetin	202, 230 shoulder, 290, 342 shoulder	0.18	0.19	0.04
Dihydrokaempferol	200, 230 shoulder, 292, 344 shoulder	0.24	0.23	0.06

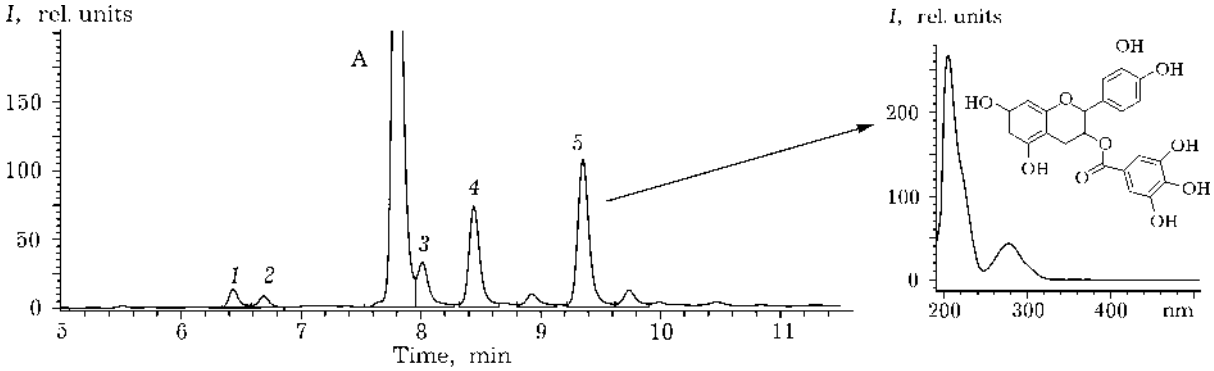


Fig. 5. HPLC profile of green tea catechines (λ = 280 nm): *1* – epigallocatechine, *2* – catechine, *3* – epicatechine, *4* – epigallocatechine gallate, *5* – epicatechine gallate; A – caffeine.

used as the identification characteristics of catechines produced from plant raw material.

An important group of natural phenolic compounds is formed by lignanes, the compounds consisting of two phenylpropane fragments with a general formula $(C_6–C_3)_2$, those in most cases are bound between themselves through β,β-carbon atoms of side chains. The structure of phenylpropane fragments in the molecules of natural lignanes has a great variety of forms depending on the substituents in aromatic rings, the saturation degree of a side chain, the oxidation degree of a propane fragment as well as the features of the bond between aromatic rings. As the subsituents in the aromatic rings, substituents containing oxygen atom are considered such as hydroxy group, methoxy group, methylenedioxy group.

There is also a group of neolignanes for those a linking of the propane fragment of one part of the molecule with the aryl fragment of another part is characteristic, however the variety of forms of aryl and propane fragments in this case is the same as for the lignanes with normal linking.

Lignanes exhibit a wide spectrum of biological action and are the components of extractive substances not only of timber, but also of the seeds of some plants, for example of flax (*Linum*) [25].

Lignanes are widely distributed in the plant world and exist both in the free state and as glycosides. They are inherent to certain groups of plants and can be used as a chemotaxonomic marker.

In order to obtain the identification characteristics of $(C_6–C_3)_2$ lignanes with a guaiacyl type of the aryl fragment substitution, the well-known representatives of those are larch lignanes (pinoresinol, conidendrine, lariciresonol, secoisolariciresinol, *etc*. [26]), the acidic part of aqueous fraction of the CO_2 extract of larch *(Larix Sibirica)* timber was analysed using HPLC, HPLC/MC and GC/MS assays.

The chromatographic profile of the larch lignanes obtained using HPLC technique, as well as their UV spectral characteristics are shown in Fig. 6 and in Table 5, respectively. The retention time interval for larch lignanes is about 4 min (11–15 min). The UV absorption spectra at the main chromatographic peaks demonstrate

TABLE 4

UV absorption spectral data for catechines of green tea (see Fig. 5)

Peak number	λ_{max}, nm	Spectral peaks ratio values		
		A(254/220)	A(280/220)	A(280/254)
1	208, 232 shoulder, 272	0.05	0.04	0.39
2	204, 230 shoulder, 278	0.05	0.12	1.28
3	204, 230 shoulder, 280	0.05	0.15	1.58
4	208, 274	0.11	0.21	1.38
5	206, 224 shoulder, 278	0.11	0.28	1.82

the absorption band with λ_{max} = (282±2) nm characteristic for lignanes corresponding to the data from the literature (see Table 5, compare with [27]). The calculated interval of characteristic spectral ratio values A(254/220) and A(280/254) is 0.2±0.1 and 1.7±0.8, respectively.

Using the HPLC/MS technique under the conditions of positive ionisation it is established that the basic compounds in the sample are characterized by the molecular mass amounting to 356, 358 and 374, that is corresponding to the literature data on the composition of larch lignanes [26].

The data obtained are confirmed with GC/MS technique. The analysis of the chromatography–mass spectrometry data for larch lignanes shows the main registered molecular mass to be 356, 358, 360, 362, 372 and 374. The analysis of full mass spectra at the chromatographic peaks (Fig. 7) allowed to reveal the main fragmentary ions with m/z 151 and 137 that is typical fort the fragmentation of $(C_6–C_3)_2$ lignanes with guaiacyl aromatic ring substitution [28]. Such the fragmentation of molecules is observed for secoisolariciresinol, pinoresinol, lariciresinol, conidendrin and the other lignanes identified in larch timber [26].

Flavolignanes are the flavonoids with an additional phenylpropanoid fragment in the

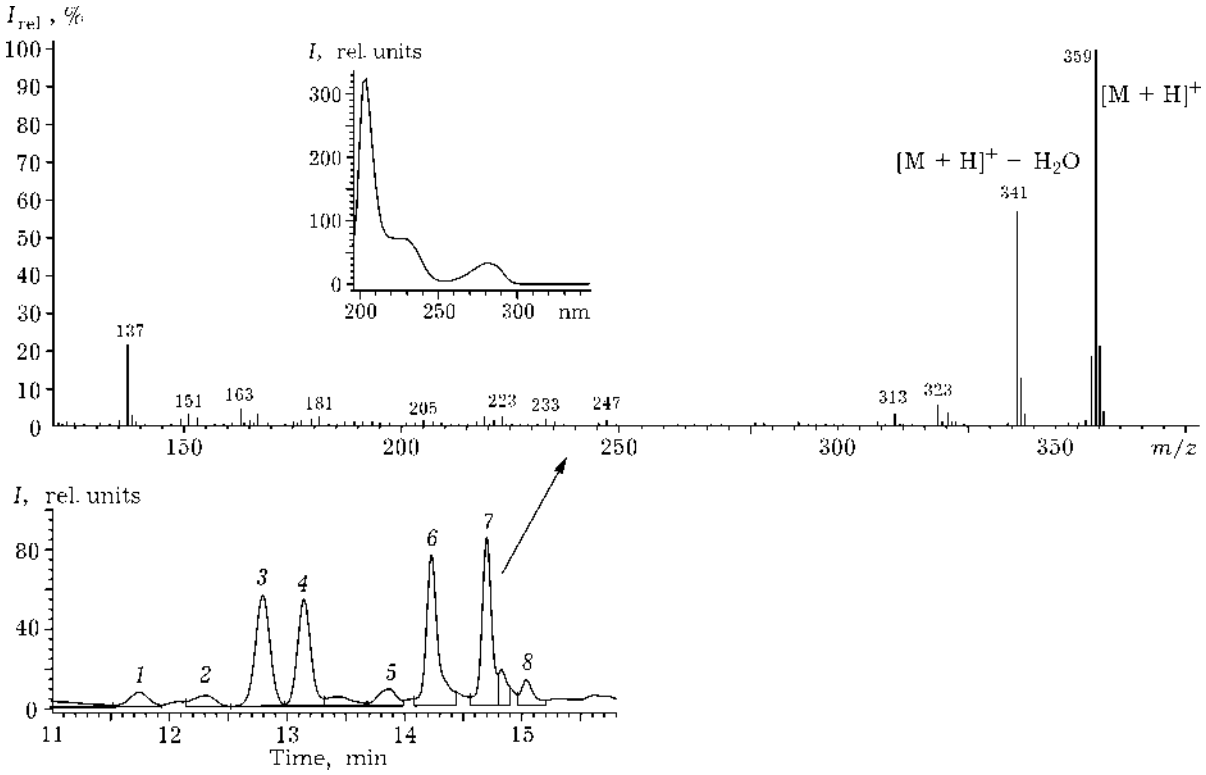


Fig. 6. HPLC profile of larch timber lignanes (λ = 280 nm).

TABLE 5

UV absorption spectral data for lignanes of larch (see Fig. 6)

Peak number	λ_{max}, nm	Spectral peaks ratio values	
		A(254/220)	A(280/254)
1	202, 232, 280, 310 shoulder	0.26	2.51
2	204, 230 shoulder, 284	0.21	1.03
3	202, 228 shoulder, 282	0.14	2.40
4	202, 230 shoulder, 282	0.16	2.21
5	206, 228 shoulder, 284	0.24	1.21
6	206, 232 shoulder, 280	0.12	0.92
7	204, 232 shoulder, 282	0.16	2.32
8	202, 232, 282	0.13	2.52

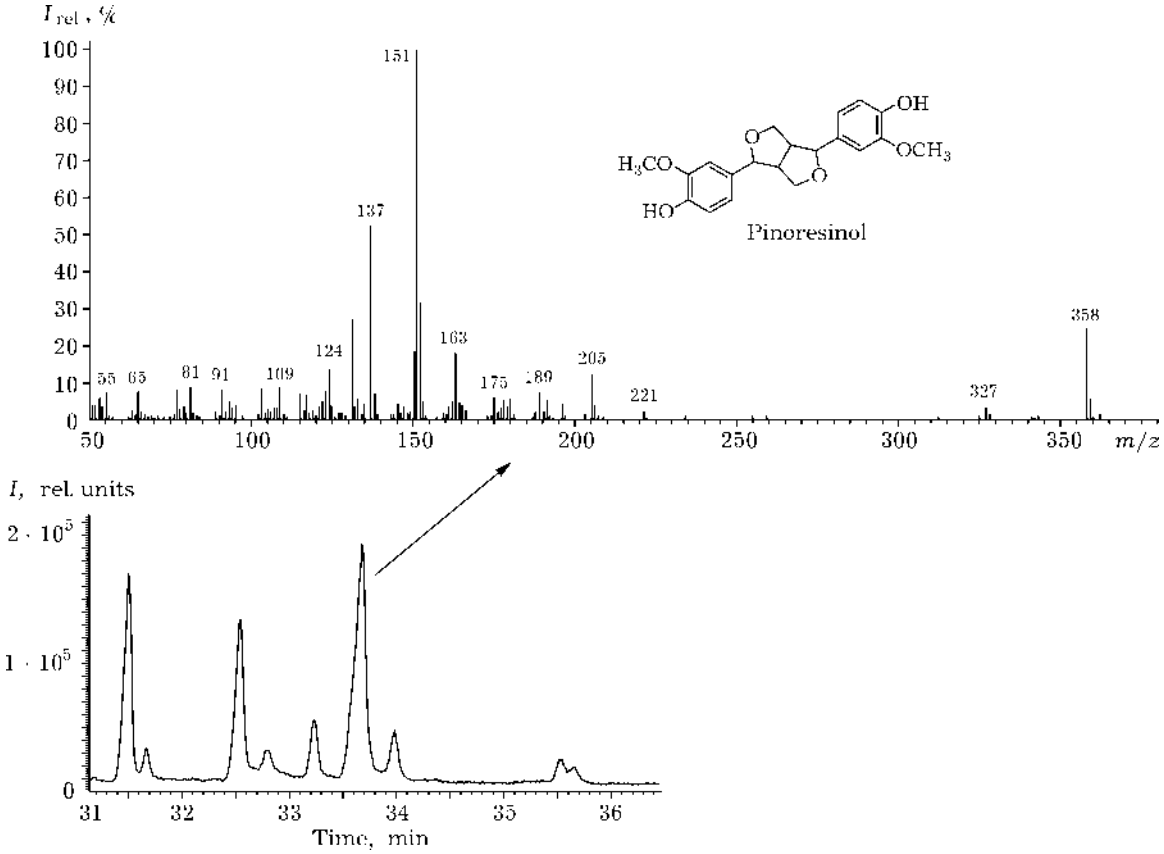


Fig. 7. Chromatogram of the larch lignanes obtained using GC/MS technique.

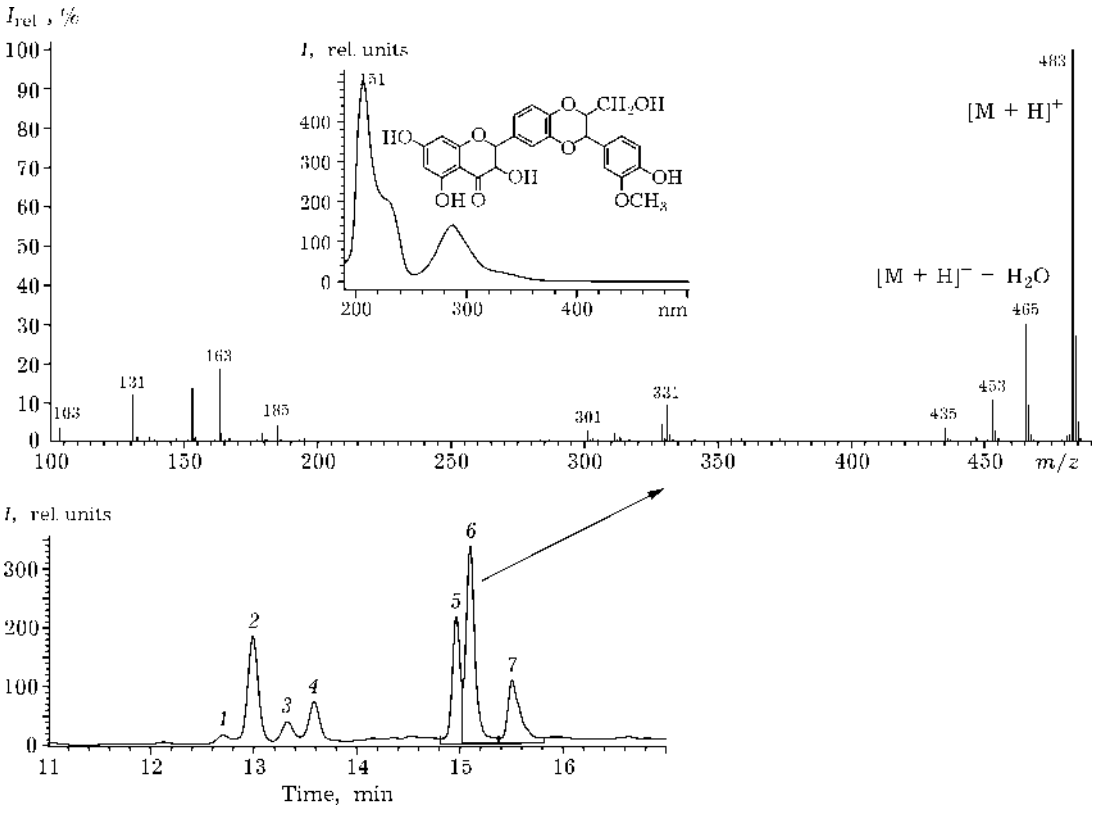


Fig. 8. HPLC profile of flavolignanes of *Silybum marianum* (λ = 280 nm): *1, 3, 5, 7* – non-identified species, *2* – silychristin, *4* – silydianin, *6* – silybin (peak assignment was made basing on the data from [25]).

TABLE 6

UV absorption spectral data for flavolignanes of *Silybum marianum* (see Fig. 8)

Peak number	λ_{max}, nm	Spectral peaks ratio values		
		A(254/220)	A(280/254)	A(280/220)
1	206, 228 shoulder, 290	0.18	1.88	0.36
2	206, 230 shoulder, 288, 338 shoulder	0.20	1.76	0.35
3	206, 232 shoulder, 288, 336 shoulder	0.21	1.67	0.34
4	202, 228 shoulder, 288, 336 shoulder	0.16	2.17	0.36
5	206, 232 shoulder, 288, 336 shoulder	0.17	2.27	0.39
6	206, 230 shoulder, 286, 338 shoulder	0.17	2.29	0.40
7	206, 230 shoulder, 288, 336 shoulder	0.20	1.89	0.37

structure. As an example we considered flavolignanes of the seed extract of *Silybum marianum* Gaertn., the main components of those are silybin, silydianin and silychristin representing the effective agent of a well-known pharmaceutical preparation "Carsil" that exhibits hepatoprotective properties.

In the studies of the holy thistle extract using HPLC technique with diode array and mass selective detectors, chromatographic and spectral characteristics of flavolignanes were obtained (Fig. 8, Table 6). The retention time interval of holy thistle flavolignanes is 4 min (12–16 min). The calculated interval of characteristic spectral ratio values A(254/220), A(280/254) and A(280/220) is 0.20±0.02, 2.00±0.20 and 0.40±0.03, respectively.

The analysis of spectral ratio values for lignanes and flavolignanes (see Tables 5 and 6) shows that they appeared to be closely allied, which it seems to be caused by the similarity of their chromophor systems.

Using the method of HPLC/MS under the conditions of positive ionisation it was established that all the *Silybum marianum* flavolignanes are of molecular mass amounting to 482. According to the literature data [29] the three of them (see Fig. 8, peaks *2*, *4*, *6*) were identified as silychristin, silybin and silydianin, respectively.

The chromatograms of the holy thistle extract and of the effective agent of the preparation "Carsil" are virtually coinciding (compare Figs. 8 and 9).

The group of natural phenolic compounds those exhibit high antioxidant activity is formed by anthocyanins, the interest for the studying and application of those is significant today. Since the characteristic optical absorption for anthocyanins is observed at the wavelength $\lambda = (520\pm10)$ nm, and the majority of natural compounds exhibits no absorption within this range, this wavelength value is used as analytical one to study anthocyanins using HPLC technique [30–32]. As the mobile phase, binary mixtures consisting of methanol or acetonitrile and dilute acids such as formic, acetic, trifluoroacetic, phosphoric under the conditions of both gradient and isocratic elution are generally used. Recently, results with a good resolution are obtained for the HPLC analysis of the sum of anthocyanins using isocratic systems [33].

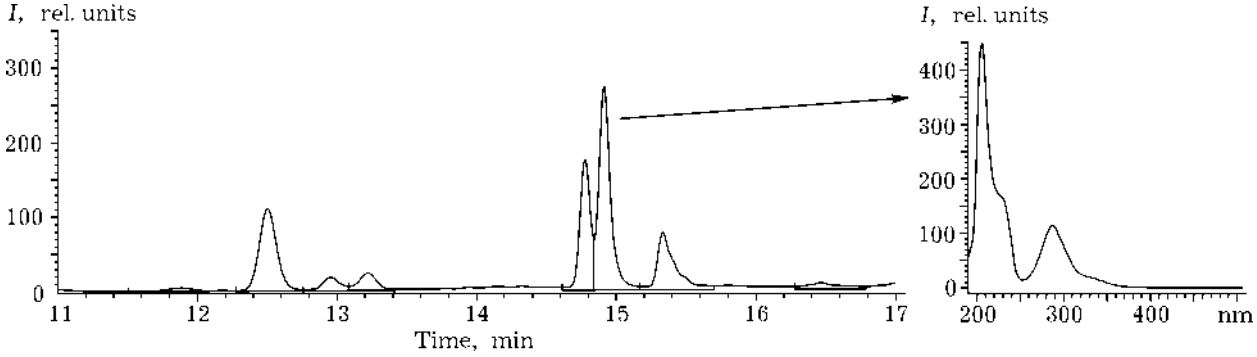


Fig. 9. HPLC profile of the Carsil preparation (λ = 280 nm).

We have obtained chromatographic profiles and UV spectral characteristics of anthocyanins of bilberry (*Vaccinium myrtillus*), aronia (*Aronia melanocarpa* Michx.) (compare [34]) and kalina (*Viburnum opulus* L.) under the conditions of isocratic elution (Figs. 10–12). The UV absorption spectra (Table 7) at the main chromatographic peaks are shown to be almost identical and corresponding to anthocyanins (the characteristic absorption peak at ~520 nm); the

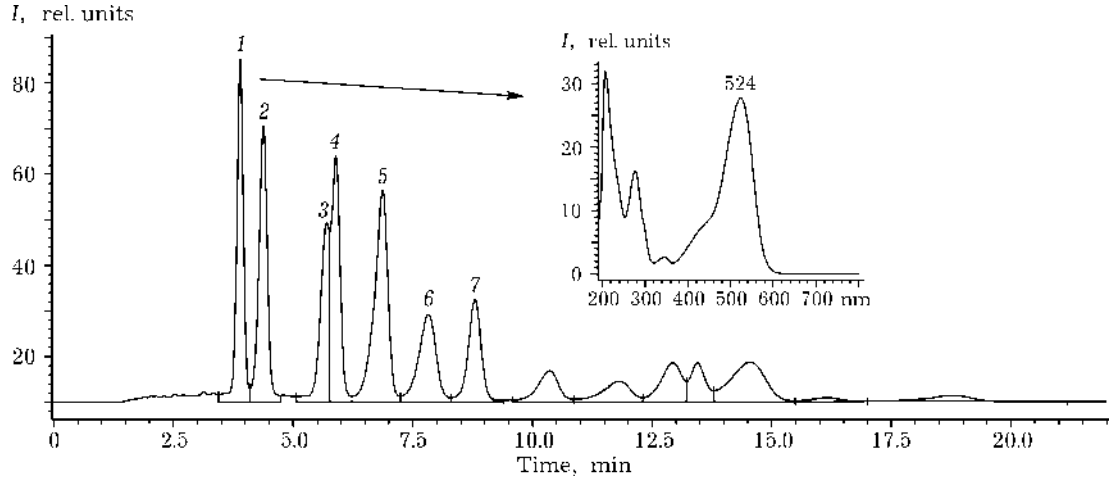


Fig. 10. HPLC profile of the extract of bilberry (*Vaccinium myrtillus* L.) (λ = 520 nm): *1* – delphinidin-3-galactoside, *2* – delphinidin-3-glucoside, *3* – cyanidin-3-galactoside, *4* – delphinidin-3-arabinoside, *5* – cyanidin-3-glucoside, *6* – petunedin-3-galactoside, *7* – cyanidin-3-arabinoside.

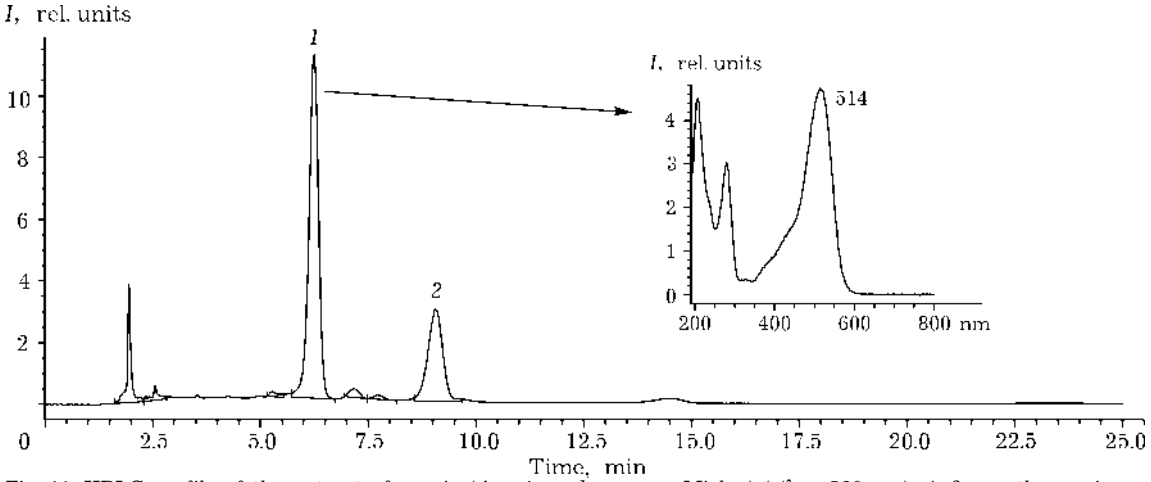


Fig. 11. HPLC profile of the extract of aronia (*Aronia melanocarpa* Michx.) ((λ = 520 nm): *1*, *2* – anthocyanins.

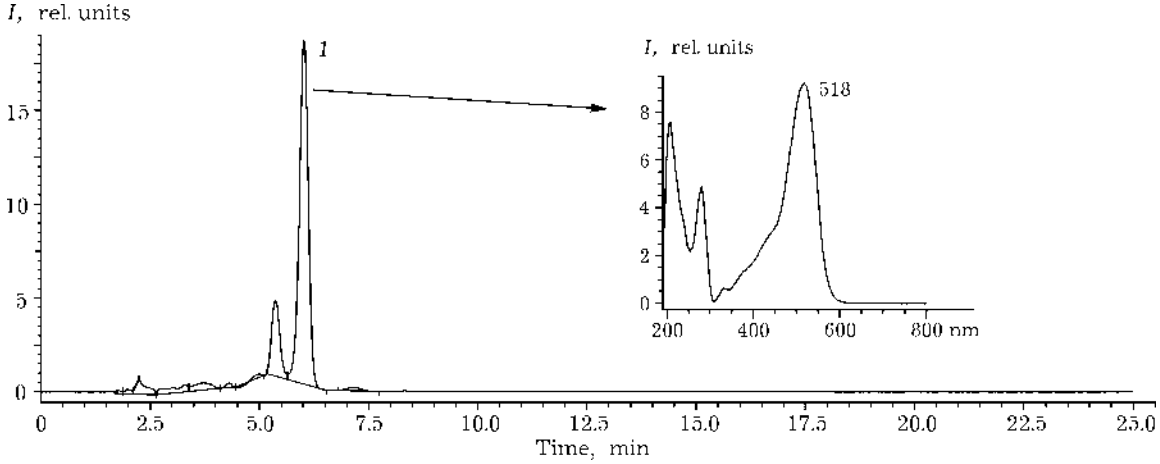


Fig. 12. HPLC profile of the extract of kalina (*Viburnum opulus* L.) (λ = 520 nm): *1* – anthocyanin.

TABLE 7

UV absorption spectral data for the main anthocyanins of bilberry,aronia and kalina(see Fig. 10)

Peak number	λ_{max}, nm	Spectral peaks ratio values	
		A(520/280)	A(280/254)
	Bilberry		
1	210, 278, 350, 430 shoulder, 524	1.82	1.21
2	208, 282, 328, 438 shoulder, 526	1.50	1.22
3	206, 272, 300 shoulder, 344, 426 shoulder, 532	1.85	1.15
4	206, 280, 328, 380 shoulder, 430 shoulder, 514	1.42	1.47
5	206, 280, 328, 378 shoulder, 438 shoulder, 514	1.41	1.54
6	206, 276, 346, 436 shoulder, 526	1.51	1.57
7	208, 280, 334, 380 shoulder, 436 shoulder, 518	1.32	1.88
	Aronia		
1	206, 282, 430 shoulder, 514	1.32	1.35
2	204, 282, 512	1.43	1.33
	Kalina		
1	206, 282, 334, 440 shoulder, 518	1.54	1.49

chromatographic profiles substantially differ from each other and being inherent to each plant. The assignment of the peaks for the main anthocyanins of bilberry (see Fig. 10) was made basing on the data from [33]. The calculated intervals of characteristic spectral ratio values A(520/280) and A(280/254) for the anthocyanins of bilberry are 1.6±0.3 and 1.5±0.3, respectively.

The validity of this approach with respect to the analysis of the group compositions of plant extracts is shown by the example of the investigation of the composition of aqueous-ethanol and ethanol extracts of Siberian fir (*Abies Sibirica*) arboreous greenery, preliminary processed with methyl-*tert*-butyl ether in order to remove low-polar components.

Under the conditions of acid catalysis for aqueous-ethanol extract of Siberian fir arboreous greenery we carried out hydrolysis and the subsequent hydrolysate separation on polyamide using aqueous-methanol mixtures as an eluent with increasing methanol proportion. The isolated fractions were analysed under standardised conditions using HPLC method.

Within one of the fractions a group of lignanes was identified by the matching its chromatographic profile (Fig. 13) and UV spectral characteristics (Table 8) with the identification characteristics of lignanes isolated from larch timber (see Fig. 6, Table 5). In the chromatogram of the fraction, the peaks Nos. *1*–7 with the retention time value lying within the interval of 12–16 min, according to UV absorption spectra and spectral peak ratio values, are corresponding to the lignanes of $(C_6–C_3)_2$ structural type. The calculated intervals of characteristic spectral ratio values A(254/220) and A(280/254) are 0.2±0.1 and 1.7±0.8, respectively, being in a good agreement with the data obtained for the lignanes of $(C_6–C_3)_2$ structural type, isolated from larch timber. The analysis of UV absorption spectra at the peaks A and B allows assuming them to belong to the group of flavones such as II.

Analysing the fraction using HPLC/MS technique under the conditions of positive ionization and GC/MS technique, mass spectrometric information has been obtained that the main compounds are characterised by the molecular mass amounting to 344, 358, 374 and 386. The results are in a good agreement with the data from the literature [35] concerning the content in Siberian fir timber such the lignanes as 3,4-divanilliltetrahydrofuran (M344), pinoresinol (M358), matairesinol (M358), oxymatairesinol (M374), *etc.*

In the other fraction the flavonoids of Siberian fir (*Abies Sibirica*) were identified (Fig. 14). Comparing the chromatographic profile of this fraction with the profile *Orthilia*

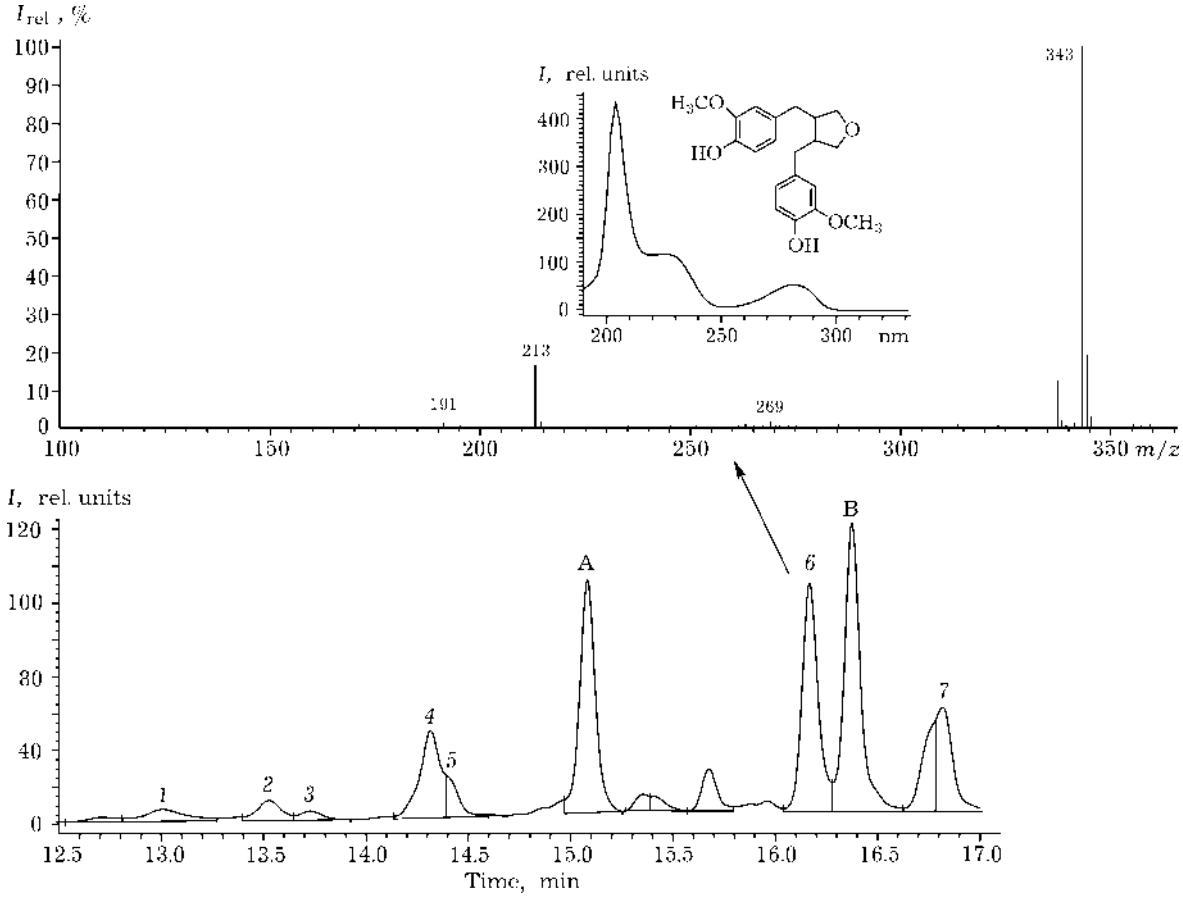


Fig. 13. HPLC profile of the fraction containing lignanes of Siberian fir (*Abies Sibirica* Ledeb.) arboreous greenery (λ = 280 nm): *1*–7 – lignanes of Siberian fir; A, B – non-identified species.

TABLE 8

UV absorption spectral data for lignanes of Siberian fir (see Fig. 13)

Peak number	λ_{max}, nm	Spectral peaks ratio values	
		A(254/220)	A(280/254)
1	204, 230, 284	0.26	0.53
2	204, 232 shoulder, 284	0.20	1.20
3	204, 232 shoulder, 284	0.37	1.21
4	206, 230, 284	0.15	1.91
5	204, 230, 282	0.20	1.50
6	204, 230, 282	0.23	1.69
7	206, 232, 284	0.14	2.01

secunda L. flavonoids (see Fig. 3) and analysing UV spectral characteristics of main peaks quercetin and kaempferol were identified, which is corresponding to the literature data [26].

The group of hydroxycinnamic acids was identified in the ethanol extract of Siberian fir *(Abies Sibirica)* arboreous greenery. After the hydrolysis and chromatographing the extract on polyamide a fraction was isolated containing hydroxycinnamic acids (Fig. 15). The peaks Nos. *1–4* according to the UV absorption spectra and retention time values were assigned to the group of hydroxycinnamic acids.

Thus, by the example of the extracts of the arboreous greenery of Siberian fir (*Abies Sibirica*) the possibility is demonstrated for the group identification of biologically active compounds using the method of comparison of the identification chromatographic characteristics.

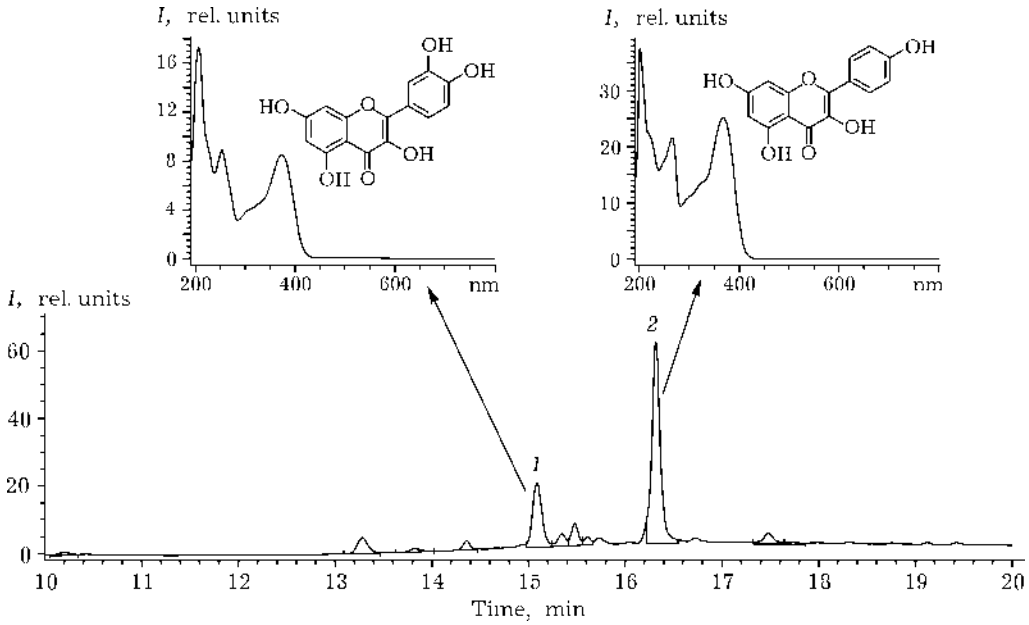


Fig. 14. HPLC profile of flavonoids of Siberian fir (*Abies Sibirica* Ledeb.) arboreous greenery (λ = 360 nm): *1* – quercetin, *2* – kaempferol.

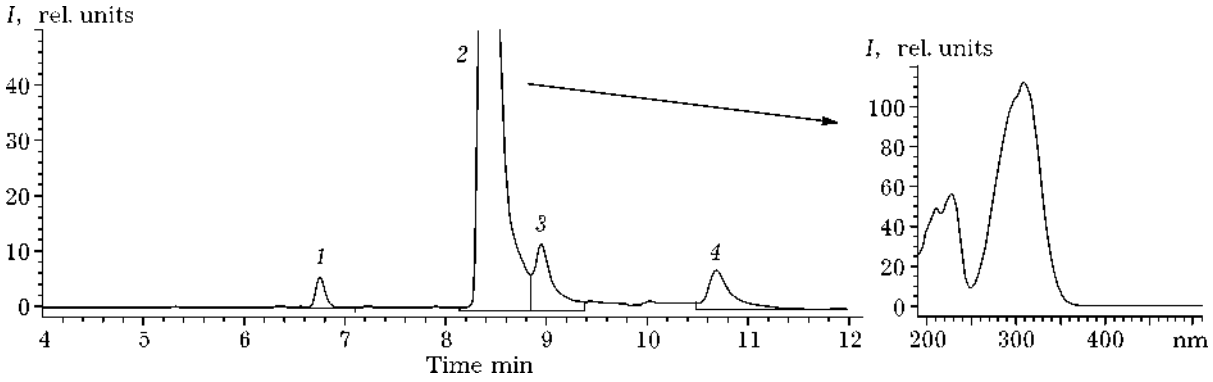


Fig. 15. HPLC profile of hydroxylcinnamic acids of Siberian fir (*Abies Sibirica* Ledeb.) arboreous greenery (λ = 320 nm): *1* – caffeic, *2* – *p*-coumaric, *3* – ferulic, *4* – 3,4-dimethoxycinnamic.

CONCLUSION

1. Using the methods of high performance liquid chromatography and gas chromatography under standardised conditions, chromatographic profiles ("fingerprints") and spectral characteristics are obtained for the major groups of biologically active natural phenolic compounds: hydroxycinnamic acids, flavonoids, anthocyanins, lignanes, flavolignanes.

2. An approach is suggested for the analysis of group and individual compositions of biologically active substances basing on chromatographic profiles and spectral characteristics of the main components. This approach can be used for the development of the methods of identification and standardisation of plant raw material and the preparations on its base.

3. The application of the approach is demonstrated by the example of biologically active substances of Siberian fir (*Abies Sibirica*) arboreous greenery.

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