

THE SIBERIAN FLORA AS A SOURCE OF POLYISOPRENOIDS

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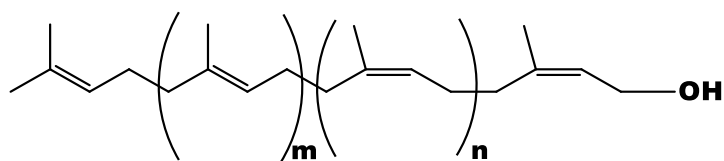
Abstract

Polyprenols were found in a number of plants, animals and microorganisms. Dolichols are partially hydrogenated polyprenols. The physiological activity of dolichols is several times higher than that of polyprenols. We analyzed polyisoprenoid alcohols (dolichols and polyprenols) in the lipophilic extracts from over 40 plant species of the Pinaceae, Onagraceae, Eleagnaceae, Asteraceae, Solanaceae, Vitaceae, Malvaceae, and Rosaceae families by high-performance liquid chromatography method. The constituents of some mushrooms of Tricholomataceae, Boletaceae, and Lycoperdaceae families were tested for the first time. Special attention was paid to the occurrence of dolichols in fruit bodies of mushrooms. Dolichols were determined for the first time in this raw material. These results may be used for chemotaxonomic and medical purposes.

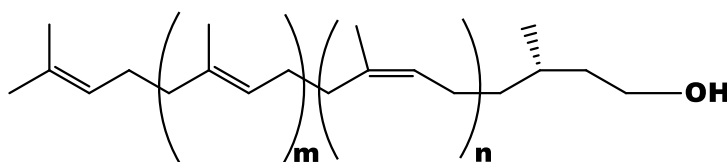
Key words: dolichols, polyprenols, plants, mushrooms, high-performance liquid chromatography (HPLC)

1. INTRODUCTION

Polymers of the five carbon isoprene unit comprise a very large family of natural products with diverse biochemical functions. Isoprene-derived compounds participate in various processes, e.g. blood clotting (vitamin K), respiration (Coenzyme Q), and vision (retinol). Long-chain polyisoprenoids are also essential intermediates in the formation of cell wall peptidoglycans (polyprenols, PPs) and asparagines-linked glycoproteins (dolichols, Ds). Polyisoprenoid alcohols are linear polymers of isoprene with a terminal primary alcohol group. Polyprenols usually consist of 6–22 isoprene units. The mixture of dolichols has been isolated for the first time from pig liver. The term *dolicholis* derived from a Greek word meaning “long”. These compounds common in animal tissues, but they are also found in plants. Their lengths are generally within 14–24 isoprenoid units. The dolichol molecule differs from PP in that their α -isoprene unit is saturated (Fig 1). The number of *trans*-units (*m*) is usually 2 or 3.



(1)



(2)

Fig. 1. Structure of polyprenol (1), and dolichol (2) molecules.

The contents of PPs in plants reach up to 2%. The highest amounts are isolated from gymnosperms, such as the Pinaceae and Cupressaceae families and *Ginkgo biloba* (Rip et al, 1985, Ravi et al, 1984, Khidyrova et al, 2002, Zhang et al, 2015). PPs exert no toxic, mutagenic, teratogenic, or carcinogenic effects on humans. In addition, they demonstrate significant anti-tumour, anti-hepatitis C virus, and anti-HIV effects, and they serve as adjuvants in leukaemia chemotherapy and radiotherapy (Khidyrova et al, 2002, Zhang et al, 2015, Boldyrev et al, 2000, Rizvi et al, 2003). PPs can also remedy hypertension, hypercholesterolemia, diabetes, gout, lupus, and other immune disorders (Simons et al, 2002, Sinensky et al, 2000, Jozwiak et al, 2013, Scalais et al, 2013). PPs have a broad spectrum of biological activities, such as immunostimulating, antiulcer, hypotensive, antistress, adaptogenic, wound-healing, hepatoprotective, antiviral, and cell-repairing (Khidyrova et al, 2002, Zhang et al, 2015). A mixture of PPs exhibits complex and antiviral effects and stimulates natural mechanisms of protection against infections. Therefore, ever-increasing attention is focused on the PPs research and application. Polyprenyl compounds in vivo are involved in membrane stability and sugar chain formation. Another interesting feature of PPs is their important role as lipophilic sugar transporters in the biosynthesis of bacterial polysaccharides and glycoproteins. Ds have an E, E-farnesyl residue at the ω -residue of their prenyl chains, but they are different from other polyprenols because their α -residue is saturated. Ds are important physiological compounds in the human body as they regulate the stability and permeability of membranes. Dolichol phosphates are a kind of glycosyl carrier and essential component for human glycoprotein biosynthesis (Zhang et al, 2013, Subramaniam et al, 2005, Ishiguroa et al, 2014). Ds are mainly isolated from the liver, kidney, pancreas, spleen, thyroid, and other mammals organs. However, their content is generally as low as three to ten thousandths of the wet weight of the organs (Rip et al, 1985, Rupar et al, 1978). Their extraction, isolation, and purification are labour-consuming. For these reasons, they are not easily available and the application of dolichol derivatives and drugs is limited. In the early 1980s, Suzuki found that the structure of S-dolichols in mammals was similar to that of PPs. Hence, PPs became the best semisynthetic precursors of S-dolichols and their derivatives (Steen et al, 1985, Skorupinska-Tudek et al, 2008, Houte et al, 1994). The physiological activity of Ds is several times higher than that of PPs.

The aim of synthetic chemists of the whole world is the chemical conversion of fully unsaturated plant polyprenols into dolichol-like molecules in which the OH-terminal isoprene residue is hydrogenated. The soft spot of the majority of synthetic methods is the formation of a mixture of 3S- and 3R-isomers. It is a matter of common knowledge that the 3R-isomers demonstrate the adverse activities in some tests. Natural Ds, as opposed to synthetic ones, contain only 3S-isomers. It is the reason of reference of natural compounds in medicinal use (Khidyrova et al, 2002, Zhang et al, 2015). Sea buckthorn is a rich source of polyprenols. The contents of these compounds in samples of raw materials investigated in 1984–1990 were about 0.3% of dry weight. Our previous work led to the discovery of Ds in sea buckthorn leaves (Kukina et al, 1991). Later, Ds were found in a variety of plants, but sea buckthorn raw material remained one of the richest sources of Ds. Ds constituted 0.005% of dry weight of sea buckthorn leaves. The yield from pig liver as traditional source is about 0.01% (Mankowski et al, 1976). Twelve species of *Artemisia* demonstrate high level of Ds (up to 0.014% of raw material dry weight) although the PPs level in these plants is poor – 0.017–0.064% of air part dry weight (Kukina, 2016). The content of polyisoprenoid alcohols of alder is lower than that of conifers and *Artemisia*; it varies from 0.001 to 0.009% in leaves and from 0.005 to 0.030% in branches. This amount is gradually decreasing both in leaves and branches from the maximum value to the minimum one from May to August. Ds content found from 5 to 10% of polyisoprenoid fraction. Fractions of polyisoprenoid alcohols isolated from leaves and branches at different collection times were analyzed by the generally accepted for these compounds HPLC method using a reversed phase columns. Identification of components of the group of PPs was done by adding an authentic sample of pentadecaprenol (C_{75}); Ds were identified by comparison of obtained chromatograms with those for leaves of sea buckthorn (Kukina et al, 1991) and needles of siberian cedar *Pinus sibirica* (Roshchin, 1999, Grishko et al, 1994). The last raw material contents about 0.01% Ds.

Various qualitative and quantitative methods for assaying PPs and Ds in plant material are known. However, each of them has certain shortcomings. For instance, comparison of HPLC analyzes of an extract and a chromatographic standard concentrate of PPs does not take into account seasonal cycles

of the component ratio in plant material. Moreover, PPs and Ds are present in certain species as esters of aliphatic acids which are difficult to analyze by HPLC (Kukina et al, 1991, Norin et al, 1974). HPLC with refractive-index detection also has disadvantages. Normalization to the total peak area in the chromatogram does not consider differences in extinction or refraction. The analysis of polyisoprenoid concentrates by PMR is not flawless either. A PMR spectrum of a PP sample is a superposition of resonances of similar structural fragments. The regular structure of the PPs produces total resonances that are stronger than those of impurities. This leads to a substantial overestimation of the quantitative characteristics of the studied PP fraction, as exemplified by solanesol (Rowland et al, 1956, Swiezewska et al, 1994, Roshchin, 1995, Roshchin et al, 1986).

Use of densitometry of thin-layer chromatograms compared against a standard (Swiezewska, 1994) often causes a substantial overestimation of quantitative results because accompanying mono-, sesqui-, and diterpene alcohols overlap the spot of the PP fraction. In particular, borneol, *cis*-abienol, isoabienol, dehydroabietinol, and other alcohols that enhancing the intensity of the PP spot were present as impurities in the total fraction in a study of PPs from conifers (Roshchin, 1995, Roshchin et al, 1986). The chromatographic mobility of PPs in total extracts depends on their chain length and their form (alcohols or esters). Therefore, it is practically impossible to choose an adequate standard for an unstudied raw material. HPLC with a reversed-stationary phase that uses an external standard, and tocopheryl palmitate as an external standard with spectral and chromatographic properties best suited for sample under study is practically devoid of these shortcomings in the analysis of PPs in extracts and fractions (Khidyrova et al, 2012). Furthermore, the standard is typically stable against external factors, as its chemical and spectral properties remain unaffected both in the pure state and in solution for years. The optical density and retention time are important parameters for the method. It should be mentioned that the external standard can be used to study any PP-containing mixtures or mixtures of PP derivatives regardless of their composition and source and the chemotaxonomic and seasonal features of the raw material. An advantage of the proposed method over semi-quantitative estimation by TLC (Swiezewska et al, 1994) is the better differentiation of PPs from accompanying impurities and the ability to distinguish these compounds, which are present in the plant material as esters of fatty aliphatic acids in addition to free and acetylated PPs. We used just this method in our experiments. Extracts from leaves of *Alcea nudiflora* and fractions were analyzed for PP content in a Milichrom chromatograph equipped with a 6.3×0.2 cm column packed with Lichrosorb RP-18 sorbent (5 μm) with methanol:acetone (1:3, v/v) as the mobile phase. Ultraviolet detection was done at 210 nm. Previously, this method was applied to PPs of basidial fungi and extracts of the aerial part of *Hypericum perforatum*, some Malvaceae, and *Chamaenerion angustifolia* (Kukina et al, 2007a, Kukina et al, 2007b, Khidyrova et al, 2012, Kiyamova et al, 2012, Kukina et al, 2014).

2. MATERIAL AND METHODS

Analytical samples were obtained from needles of some coniferous and Eleagnaceae foliage and tree green. The raw material of Pinaceae tree needles, bush leaves and tree green was extracted with methyl-tert-buthyl ether (MTBE) as a convenient universal solvent for plant material treatment. A weighed portion of raw material was placed into a flow-through percolator or Soxlet-apparatus. Samples of extracts collected during the extraction process were analyzed for PPs content without preliminary sample preparation and were compared with an average sample saponified by aqueous alcoholic base. The PPs contained in the raw material in the free form or as the acetates could be analyzed without preliminary processing directly in the extract. The PPs esterified by aliphatic acids required sample preparation, which included hydrolysis of the esters, separation of acidic components, and removal of the hydrolysing reagent. Analysis of Ds needs the oxidation procedure and chromatographic purification.

Raw Material Characteristics. Raw material was collected in the Central Siberian Botanical Garden (CSBG) (Novosibirsk, Russia). Some samples of raw material were collected in Novosibirsk region, Altay region and Kemerovo region. Some samples were supplied by colleagues from CSBG and NIOCH SB RAS.

Raw Material Extraction. Air-dried raw material was ground in an electric grinder and passed through a 2-mm sieve. A weighed portion of raw material was loaded into a percolator, drawn with MTBE, heated to 50°C, and left to stand for 1–1.5 h. The extract was drained through the lower tap. The raw material was treated with solvent so that the solvent above the matter was 1.5–2.0 cm and the solvent:raw-material ratio was 3:1. The process was repeated 3–4 times. The solvent was removed in vacuo. The yield of extracted substances was determined.

Isolation of Free Acids. A weighed portion of the extract was dissolved in MTBE, diluted to 1–5%, and extracted with three 50-mL portions of 2% aqueous NaOH containing 10% EtOH. The pH of the combined aqueous phases, containing sodium salts of the acidic constituents was adjusted to 2 with 10% aqueous HCl. Free acids were extracted by MTBE (3×50 mL). The combined ether extracts were washed in a separatory funnel with distilled H₂O until the water phases were neutral (pH 7) according to universal indicator, dried over anhydrous Na₂SO₄, and evaporated to dryness in vacuo.

Isolation of Unsaponified Substances (USs). Extracts purified of free acids were washed until neutral with distilled H₂O in a separatory funnel to remove traces of the basic aqueous extractant. The ether solutions were dried over anhydrous Na₂SO₄. The solvent was removed. The residues were saponified with a mixture of KOH (15% w/w), distilled H₂O (10%), and EtOH (75%), calculated to be a 10-fold excess of the saponifying mixture relative to the sample weight. The mixture was refluxed in a flask equipped with a water-cooled condenser with vigorous stirring on a magnetic stirrer/heater for 1.5 h. When the reaction was finished (TLC monitoring of the disappearance of the ester fraction), the mixture was diluted with four volumes of H₂O. Unsaponified compounds were extracted with four portion of MTBE. The combined ether extracts were washed with distilled H₂O until neutral, dried over anhydrous Na₂SO₄, and evaporated in vacuo.

Chromatographic Fractionation of Extracts purified of free acids (EPFA). A weighed portion of EPFA (0.5 g) from extract was loaded on to a silica-gel (8.0g) column as a hexane solution. The column was eluted with a linear gradient from 0 to 8% diethyl ether in hexane. The resulting fractions were evaporated to dryness in vacuo and scanned on a Milichrom chromatograph for acetylated polyprenol content. The fractions with acetylated polyprenols were analyzed by HPLC. The fractions of polyprenol esterified by fatty acids were treated as described above for USs obtaining.

Chromatographic Fractionation of USs. A weighed portion of USs (0.5 g) from leaf (needle) extract was loaded on to a silica-gel (8.0g) column as a hexane solution. The column was eluted with a linear gradient from 0 to 50% diethyl ether in hexane. The resulting fractions were evaporated to dryness in a vacuo and scanned on a Milichrom chromatograph for polyprenol content. The fraction with PPs was analyzed by HPLC.

Oxidation of polyprenols. Manganese dioxide was prepared as described earlier (Rip et al, 1985). A weighed portion (50 mg) of polyprenoid alcohols purified from USs was dissolved in CHCl₃ (5 mL) and treated with 0.5 g of MnO₂ at 4°C for 16 hours. Reaction mixture was chromatographed as described above. The fraction with dolichols was analyzed by HPLC. The NMR spectra of dolichols fraction were the similar to literature data (Grishko et al, 1994).

Quantitative Determination of PPs in the Extract. The method was based on HPLC with a reversed stationary phase and tocopheryl palmitate as an external standard with spectral and chromatographic properties fitting best to those of the studied sample. The analytical instrument was a Milichrom LC with UV detection at 210 nm, a column (6.3 × 0.2 cm) packed with Lichrosorb RP-18 sorbent (5 μm), and MeOH:(CH₃)₂CO (1:3, v/v) as a mobile phase. The analysis was carried out under the same conditions used for PPs of sea buckthorn, fungi, Malvaceae plants, and *H. perforatum* (Kukina et al, 2007a, Kukina et al, 2007b, Khidyrova et al, 2012, Kiyamova et al, 2012, Kukina et al, 2014).

The quantitative content of PPs in the studied fraction was calculated using the formula:

$$C = \frac{S_{sumPP} \times M_{est} \times 100 \times K}{S_{est} \times M_s},$$

where *C* is the percent PPs in the analyzed sample;

S_{sumPP} is the sum of PPs peak areas;

Sest is the peak area of the external standard (peak areas were determined as the product of the peak height and width at half-maximum);

Mest is the weight of the external standard (μg);

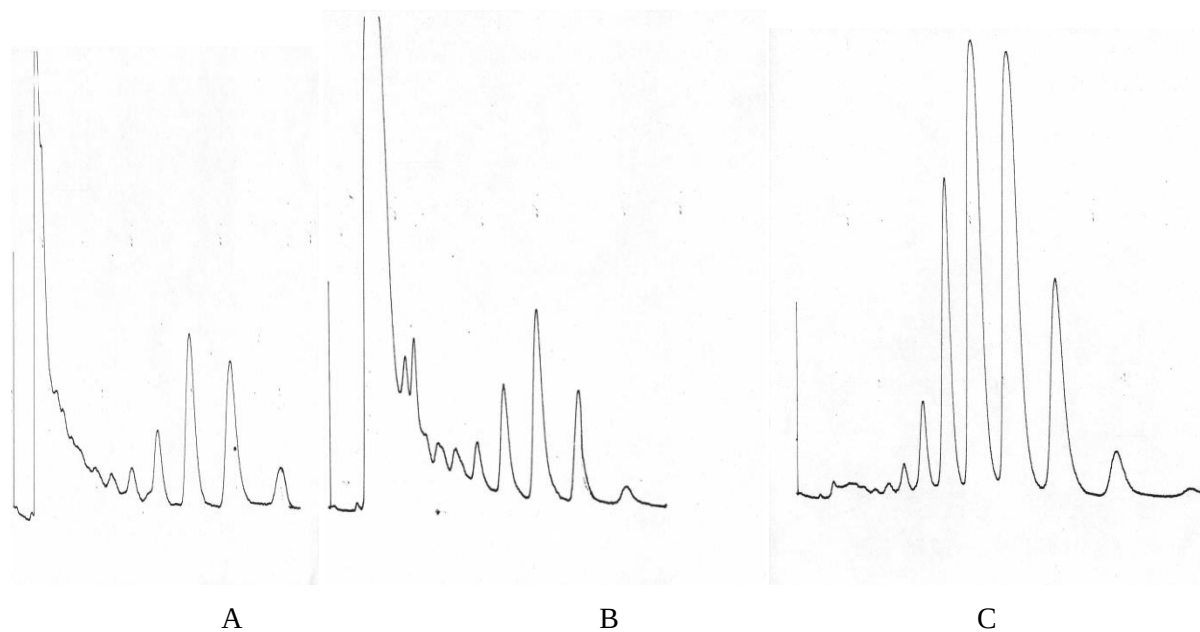
Ms is the weight of sample (μg). Sample and standard weights were determined as the product of the concentration of the corresponding solution and the sample volume injected into the chromatograph.

K is a coefficient reflecting the nature of the external standard. In case of tocopheryl palmitate it is 0.667 as calculated from a chromatogram of a trial mixture of pure PPs and tocopheryl palmitate. Trial mixtures were proposed from decaprenol and undecaprenol from leaves of sea buckthorn those were isolated as before (Kukina, 1991) and from pentadecaprenol and hexadecaprenol generously provided by A. M. Moiseenkov.

The studied sample (4–5 mg, ± 0.0001 g, weighed on an analytical balance) was dissolved in a penicillin vial in the working eluent to the concentration 5 mg/mL immediately prior to the analysis. The external standard solution was prepared at the concentration 0.5 mg/mL. The chromatograph pump was primed with the working eluent degassed by purging with a gentle stream of helium from a cylinder for 20–30 s. The working eluent (4 μL) and/or external standard solution (4 μL) were injected into the sample port through a needle. Samples were taken at 50 $\mu\text{L}/\text{min}$. The elution rate was 100 $\mu\text{L}/\text{min}$. Detection was performed at 210 nm with measurement time 0.3 s and sensitivity 0.8–3.2. The recorder speed was 12 mm/min. The analysis time of a single sample was 12–15 min. Proximate analyses of PPs were performed without additional purification. The composition was determined on enriched PP fractions isolated by column chromatography on silica gel and by preparative thin layer chromatography (TLC).

3. RESULTS AND DISCUSSION

The identification of the compounds from the needles of Pinaceae trees as well as leaves and tree-greens of other plants was based mainly on HPLC data with the aid of references and standards. The table 1 summarizes contents of free, acetylated and total PPs in coniferous raw materials. These results were obtained without additional treatment of extracts and fractions because our method allows analysis of concentrates with low content of polyisoprenoids (Fig 1.).



A-Polyprenyl acetate concentrate (7%); B-Polyprenol concentrate (17%); C-Polyprenol pure (95%)

Fig 2. HPLC-chromatograms of polyprenol concentrates

Genus	Species	Acetylated polyprenols (% of dry weight)	Free polyprenols (% of dry weight)	Total polyprenols (% of dry weight)
<i>Pinus</i>	<i>Densiflora</i>	0.52	0.080	0.600
	<i>Rigida</i>	0.053	0.008	0.061
	<i>Sylvestris</i>	0.420	0.060	0.480
	<i>Thunbergii</i>	0.450	0.070	0.520
	<i>Sibirica</i>	0.210	0.090	0.300
	<i>Koraensis</i>	0.070	0.020	0.090
	<i>Pumila</i>	0.024	0.006	0.030
<i>Picea</i>	<i>Obovata</i>	0.74	0.80	1.54
	<i>Abies</i>	0.66	0.72	1.38
<i>Abies</i>	<i>Sibirica</i>	0.17–0.42		0.22–0.45
	<i>Nephrolepis</i>			0.42
	<i>Balsamea</i>			0.27
	<i>Fraseri</i>			0.20

Table 1. Content of polyprenols in *Pinus*, *Abies* and *Picea* species needles (% of dry weight).

Qualitative and quantitative compositions of the isoprenologues of pine polyprenols in free and acetylated forms are there in tables 2 and 3.

<i>Pinus species</i>	PP-13	PP-14	PP-15	PP-16	PP-17	PP-18	PP-19	PP-20	Sum D*
<i>Densiflora</i>	0.8	3.4	13.2	33.6	33.9	13.7	2.5	1.3	3.6
<i>Sylvestris</i>	1.8	6.6	36.2	49.2	6.7	2.3	0.8		4.4
<i>Thunbergii</i>	0.5	1.8	16.8	47.3	29.9	6.3	0.9		4.5
<i>Sibirica</i>	0.4	2.5	12.7	27.3	34.1	17.9	5.7	2.6	4.8

Table 2. Content of isoprenologues in sum of acetylated PPs of *Pinus* needles, (%).

* – column of Sum D demonstrates the content of total dolichols after oxidation of PPs fraction and chromatographic purification.

<i>Pinus species</i>	PP-13	PP-14	PP-15	PP-16	PP-17	PP-18	PP-19	PP-20	Sum D*
<i>Densiflora</i>	1.1	4.7	11.9	32.3	33.6	12.5	2.4	1.2	ND**
<i>Sylvestris</i>	2.5	9.6	34.5	44.6	5.1	2.2	0.6		ND
<i>Thunbergii</i>	0.9	4.3	24.2	40.2	23.8	4.3	1.5		ND
<i>Sibirica</i>	0.4	2.6	12.0	27.0	32.4	17.6	5.2	2.3	ND

Table 3. Content of isoprenologues in sum of free PPs (%) of *Pinus* needles.

** – not detected

The comparison of data from tables 2 and 3 indicates the absence of Ds in free polyisoprenoid fractions as well as in previous investigation (Grishko et al, 1994).

The tables 4 and 5 represent qualitative and quantitative compositions of the isoprenologues in total PPs from USs of some pines and firs.

<i>Pinus species</i>	PP-13	PP-14	PP-15	PP-16	PP-17	PP-18	PP-19	PP-20	Sum D*
<i>Rigida</i>	1.8	5.1	20.0	43.6	18.2	9.3	3.7		2.3
<i>Koraensis</i>	1.8	2.2	14.1	42.5	27.1	8.1	4.1	1.0	3.1
<i>Pumila</i>	3.1	11.6	20.2	33.4	18.3	10.1	5.0		3.3

Table 4. Content of isoprenologues in sum of polyprenols (%) of *Pinus* needles.

<i>Abies species</i>	PP-14	PP-15	PP-16	PP-17	PP-18	PP-19	Sum D*
<i>Sibirica</i>	2.6	11.3	35.6	32.9	10.2	2.5	2.9
<i>Nephrolepis</i>	5.4	13.5	36.3	32.1	8.6	1.3	2.8
<i>Balsamea</i>	5.3	17.1	38.1	26.5	9.5	0.9	2.6
<i>Fraseri</i>	3.2	8.9	33.0	36.7	12.4	3.4	2.4

Table 5. Content of isoprenologues in sum of polyprenols (%) of *Abies* needles.

The table 6 shows qualitative and quantitative compositions of the isoprenologues in PPs from needles of two spruce species.

<i>Picea species</i>	PP-12	PP-13	PP-14	PP-15	PP-16	PP-17	PP-18
<i>Obovata acetylated</i>	0.7	6.1	19.7	36.1	25.9	9.5	2.0
<i>Obovata free</i>	1.3	4.4	22.5	38.8	24.4	7.5	1.3
<i>Abies total</i>	0.8	9.2	26.8	35.7	19.8	6.4	0.6

Table 6. Content of isoprenologues in polyprenols fractions (%) in *Picea* needles.

The comparison of data from tables 2-6 indicates the difference of quantitative compositions of various coniferous plants. Spruce needles main constituents is PP-15 whereas PP-16 and PP-17 prevail in fir and pine needles.

The table 7 shows qualitative and quantitative compositions of the Ds in pine and fir needles. Spruce needles did not investigate in this aspect.

Species	D-14	D-15	D-16	D-17	D-18	D -19	D -20
<i>Pinus densiflora</i>	2.5	7.8	32.9	28.8	18.7	7.2	2.1
<i>Pinus rigida</i>	2.8	7.2	30.8	32.8	16.5	6.3	3.6
<i>Pinus sylvestris</i>	3.1	8.2	33.0	31.3	15.3	7.4	1.7
<i>Pinus thunbergii</i>	2.6	7.6	30.9	32.6	16.7	6.2	3.4
<i>Pinus ssibirica</i>	1.9	6.9	33.7	32.3	15.9	6.8	2.5
<i>Pinus koraensis</i>	1.7	5.8	33.8	35.8	14.4	6.1	2.4
<i>Pinus pumila</i>	1.5	7.1	32.6	33.6	17.1	6.9	1.2
<i>Abies sibirica</i>	1.8	8.0	34.9	31.1	15.7	6.4	2.1
<i>Abies nephrolepis</i>	0.9	8.8	34.3	31.8	17.4	6.2	0.6
<i>Abies balsamea</i>	0.6	6.8	31.2	33.8	16.9	6.5	3.6
<i>Abies fraseri</i>	2.1	8.1	31.9	32.2	16.3	6.3	3.1

Table 7. Qualitative and quantitative compositions of Ds fractions from some pines and firs

The data from tables 1-7 show the presence of Ds in coniferous needles. The amount of these constituents is the same as in animal tissues and measures up 0.02% of raw material dry weight (*Pinus densiflora*).

The other plant family that we are interesting in is Eleagnaceae as sea buckthorn belongs to this family. We investigated three species of *Eleagnus* in comparison to *Hippophae rhamnoides*. The table 8 shows qualitative and quantitative compositions of the PPs in *Eleagnus angustifolia* leaves, *Eleagnus commutata* leaves, *Eleagnus pungens* leaves and *Eleagnus pungens* tree-green.

Species	% PP*	PP-9	PP-10	PP-11	PP-12	PP-13	Other PP	Sum D
<i>Hippophae rhamnoides</i> leaves	0.62	1.8	29.1	51.2	12.2	0.2	3.5	1.9
<i>Eleagnus angustifolia</i> leaves	0.48	2.0	12.2	44.6	31.2	6.1	3.2	0.7
<i>Eleagnus commutata</i> leaves	0.42	2.8	27.2	48.1	13.4	3.8	3.9	0.8
<i>Eleagnus pungens</i> leaves	0.65	2.4	11.9	56.2	26.1	2.3	0.7	0.5
<i>Eleagnus pungens</i> tree-green	0.42	7.9	13.3	50.9	23.2	1.8	1.7	1.1

Table 8. Qualitative and quantitative compositions of PPs fractions from US of some Eleagnaceae

*– of raw material dry weight

The table data demonstrate the difference in quantitative compositions of PPs and low level of Ds content in *Eleagnus* species in comparison to sea buckthorn. Nevertheless, this amount is discovered up to 0.0046% whereas in investigated sample of sea buckthorn is equal 0.012%.

Nine samples of sea buckthorn leaves were investigated by high-pressure liquid chromatography of ether extract with palmitate of α -tocopherol as internal standard. HPLC was performed as described previously (Kukina, 1991). The average results of investigation of male and female samples were summarized in the table 9.

Month	June	July	August	September	October
Male	0.15	0.11	0.28	0.31	0.50
Female	0.21	0.22	0.55	0.74	0.70

Table 9. Polyprenols and dolichols accumulation in sea buckthorn leaves

Dolichols were investigated in US of ester fraction since these compounds were present only in bonded form. These data show female plants are a richer source of PPs and Ds in comparison with male specimens.

The composition of free and esterified PPs and Ds differs principally. The season distribution of free PPs is presented in table 10. Analogous data for esterified PPs and Ds are in table 11.

	June		July		August		September		October	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
PP-10	33.7	–	–	31.6	28.2	26.7	33.2	–	28.1	25.5
PP-11	51.0	–	–	50.8	57.0	55.7	53.5	–	55.7	54.9
PP-12	15.3	–	–	17.6	14.8	17.7	13.3	–	16.2	19.6

Table 10. Distribution of free polyprenols in sea buckthorn leaves

	June		July		August		September		October	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
PP-10	24.3	–	–	26.6	28.6	18.7	19.8	–	21.6	23.6
PP-11	32.9	–	–	43.7	45.7	45.0	48.7	–	47.7	44.5
PP-12	5.2	–	–	5.3	8.6	8.0	6.7	–	7.8	7.3
PP-14	–	–	–	traces	traces	traces	1.6	–	1.4	1.0
D-14	4.4	–	–	traces	traces	traces	0.4	–	0.4	0.2
PP-15	2.0	–	–	1.2	1.2	7.1	6.7	–	5.2	5.6
D-15	1.7	–	–	0.5	0.2	2.4	1.9	–	4.2	1.7
PP-16	7.6	–	–	4.9	4.5	5.4	8.1	–	7.6	7.9
D-16	5.2	–	–	3.1	2.2	2.5	1.9	–	2.2	2.3
PP-17	7.4	–	–	6.8	5.5	5.2	2.8	–	3.4	3.7
D-17	5.5	–	–	4.0	1.5	2.4	0.5	–	0.7	0.6
PP-18	2.3	–	–	2.4	2.1	2.7	0.8	–	1.2	1.2
D-18	1.2	–	–	1.1	0.4	0.2	traces	–	0.2	0.1
PP-19	0.2	–	–	0.4	0.5	0.4	traces	–	0.4	0.3
%e*	10.4	–	–	29.5	12.0	17.9	11.5	–	23.2	21.4
%d**	14.6	–	–	8.7	4.3	7.5	4.7	–	7.7	4.9
%dr***	0.004	–	–	0.003	0.002	0.004	0.004	–	0.007	0.005

Table 11. Distribution of esterified isoprenoids in sea buckthorn leaves

*– % of esterified isoprenoids in whole polyprenol fraction

**– % of dolichols in fraction of esterified polyprenols

***– % of dolichols in raw material

The table data show the contents of Ds in raw material grow in comparison with initial period although the percentage in fraction of esterified PPs decreases.

The obtained data may serve as a recommendation for the optimal time of raw material collection.

4. CONCLUSIONS

The obtained research data enable us to conclude that

- 1) the HPLC-method with standart tocopheryl palmitate allows analysis of crude concentrates with low content of polyisoprenoids in the case free and acetylated form;
- 2) investigated plants may be source of dolichols as well as animal tissues;
- 3) the content of polyisoprenoids depends on place of growth and phase of vegetation.

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