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Bioactive Components of Sea Buckthorn *Hippophae rhamnoides* L. Foliage

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Abstract—The composition of lipophilic components of sea buckthorn leafy shoots, a large tonnage waste in the production of sea buckthorn oil and during renewing the cultural plantings of sea buckthorn, was studied. Hexane was used as an extraction solvent for raw materials; it provides a high degree of lipophilic component extraction and is an analogue of extraction gasoline used in the food and perfume industries. The chemical composition of the hexane extract of sea buckthorn leafy shoots was studied by gas chromatography–mass spectrometry and high-performance liquid chromatography. Sixty-seven neutral and twenty-nine acidic components, including polyprenols, dolichols, triterpene alcohols and acids, sterols, were identified. β-Sitosterol was the main component of the sterol fraction. Its content was 6.9% of the extract mass, which is much higher than in the essential extracts of leaves and pulp of sea buckthorn fruit. It is mostly found in the free form in the extract. The acidic fraction contains highly active triterpene acids (up to 5% of the extract mass) along with the major aliphatic acids. Components with the chain length of 11 and 17 isoprene units predominate in the fraction of polyprenols and dolichols (up to 4.2%). The results allow us to consider sea buckthorn leafy shoots as a promising source of biologically active compounds.

Keywords: *Hippophae rhamnoides* L., polyprenols, dolichols, sterols, triterpenic alcohols, triterpenic acids, leafy shoots

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INTRODUCTION

The issue of intensive nature management is becoming more acute in the modern world of exhausted natural resources. The rational use of plant raw materials is one such area.

Sea buckthorn *Hippophae rhamnoides* has long been used in traditional medicine as a drug plant. There are associations with fruits and oil of sea buckthorn when mentioning plants of the *Hippophae* genus. But in fact, vegetative parts of the plant such as leaves, roots, shoots, also possess useful properties. The leaves contain up to 10% of tannin, more than 300 mg% of vitamin C, up to 1% of flavonoids. Serotonin can be also found in the leaves, and the bark of branches is especially rich in it [1]. The chemical composition of fruits and leaves of sea buckthorn is widely studied [2–7]. More than 200 biologically active compounds were found in these parts of the plant; these are flavonoids, glycosides, aliphatic acids, hydrocarbons, aldehydes, alcohols, carbohydrates, carotenoids, vitamins, polyprenols, dolichols, sterols, neutral and acid triterpenoids. The literature data [5, 8] indicate that the greatest contribution to the activity of lipophilic preparations (such as sea buckthorn oil) is made by sterols. The β-sitosterol content is high enough in the essential extracts of fruit pulp and leaves of sea buckthorn; it is 1 and 3%, respectively [2, 5]. At the same time it is rarely accompanied by other sterols. Sea buckthorn is also rich in triterpene alcohols, which possess the activity typical for sterols [2, 3, 5].

In turn, the composition of leafy shoots of this plant has not been studied enough, although they are a large tonnage waste in the production of sea buckthorn oil and during renewing the cultural plantings of sea buckthorn. Therefore, the aim of this work was to study the chemical composition of the hexane extract of sea buckthorn leafy shoots.

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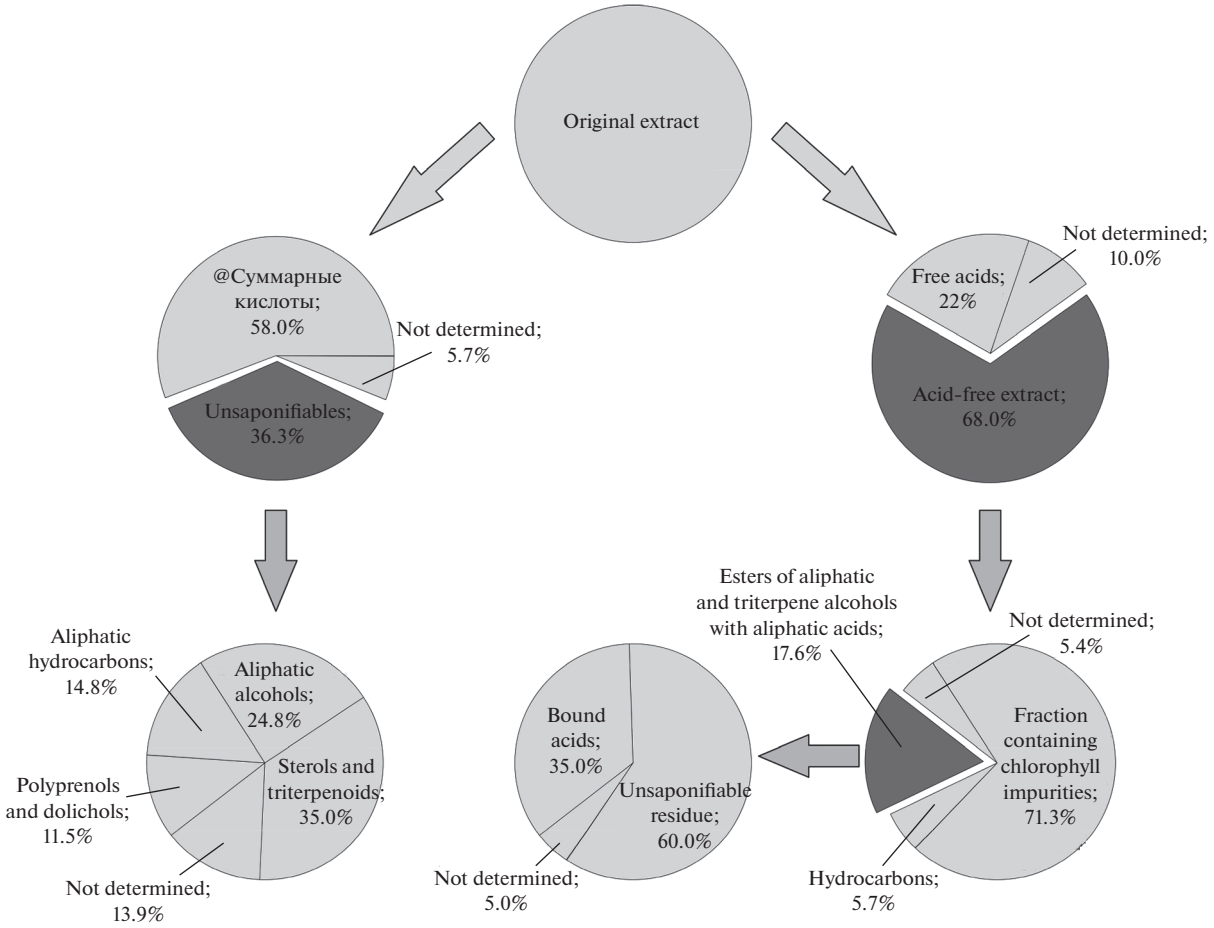

Fig. 1. Scheme for analysis of sea buckthorn leafy shoot extract.

EXPERIMENTAL

The grinding of raw materials was carried out on an electric mill. A rotary evaporator was used (Büchi, Switzerland). Thin layer chromatography was performed on Sorbfil and Armsorb plates in the hexane – methyl *tert*-butyl ether system (6 : 1 and 1 : 1 for the analysis of the fraction of low polar compounds and the remaining fractions, respectively). The chromatogram was developed by spraying the plates with a mixture of vanillin – sulfuric acid – ethanol in the ratio of 1 : 10 : 90 with subsequent heating of the plate. Purification of the target compounds was carried out by column chromatography on a silica gel from Sigma-Aldrich (Merck Grade 7734) 70-230 mesh. Hexane with increasing content of diethyl ether from 1 to 50% was used as an eluent. Mass spectra were recorded on a Hewlett Packard G 1800 A device consisting of a HP 5890 series II gas chromatograph and a HP 5971 mass selective detector. A column 30 m × 0.25 mm × 0.25 μm with a HP-5MS sorbent (5% diphenyl, 95% dimethylsiloxane). Helium was used as a carrier gas (1 mL/min). Column temperature: 2 min at 50°C, then increase the temperature to 300°C at a rate of 4°C/min, and 30 min at 300°C. The evaporator temperature was 280°C, the temperature of the ion source was 170°C. A Milichrom-2 liquid chromatograph with UV detection at the wavelength of 210 nm was used as an analytical device for HPLC; column 7.0 × 0.2 cm filled with a ProntoSil 120-5-C18 sorbent; a mixture of methanol and acetone (1 : 3 by volume) was used as an eluent.

We chose hexane, a low-toxic solvent, as an extraction solvent, which provides a high extraction degree of lipophilic components of plant raw materials, such as esters, including fats, hydrocarbons, free aliphatic acids, polyprenols, dolichols, neutral triterpenoids and sterols in free and bound form. The use of hexane is promising, since it allows us to extend the technological chain by using more polar solvents for exhaustive extraction of biologically active components and complex utilization of large-tonnage raw materials.

Extraction was carried out in a Soxhlet apparatus for 7 h. The extract yield was 4%.

The hexane extract of sea buckthorn was first divided into acidic and neutral components to facilitate the analysis (Figure) [3, 5, 9, 10]. The free acids were isolated from the total extract by alkaline

extraction with a 2% aqueous solution of sodium hydroxide. The neutral part was thus the sum of aliphatic hydrocarbons and aliphatic and triterpene alcohols in free form and in the form of esters with aliphatic acids. Also, an additional step of fractioning, saponification, was required: fractions of total acids and unsaponifiable substances were obtained by alkaline hydrolysis (figure).

RESULTS AND DISCUSSION

Unsaponifiable substances are the sum of aliphatic hydrocarbons, aliphatic and triterpene alcohols, polyisoprenoids (including polyprenols and dolichols), as well as sterols and triterpenoids (Table 1).

Fractions of the unsaponifiable residue of the extract were studied by gas chromatography–mass spectrometry and high-performance liquid chromatography.

Twenty six compounds were identified in the fraction of aliphatic hydrocarbons (Table 2). Paraffins with an odd number of atoms are predominant. The content of even hydrocarbons is much lower. Nonacosane, hentriacontan and heptacosane are the main components of this fraction; they constitute more than 85% of this group of compounds.

Phytol, octadecanol and tetracosanol are the main components of the fraction of aliphatic alcohols; they constitute more than 75% of this group of compounds. It is known [5] that phytol is present in the sea buckthorn leaves not only as a chlorophyll constituent, but also in the form of esters with aliphatic acids. A total of 8 compounds were identified (Table 3).

Seventeen compounds were identified in the polyisoprenoid fraction. The components with the chain lengths of 11 and 17 isoprene units predominate here (Table 4). A high percentage of dolichols in the polyisoprenoid fraction increases the prospects of the lipophilic concentrate of sea buckthorn leafy shoots as a bioactive substance.

β-Sitosterol, as well as a hard-to-separate mixture of α-amirin, lupeol and cycloartenol, are the main component of the terpene fraction (Table 5). Moreover fucosterol and components previously found in the sea buckthorn leaves were found [2–5].

The ratio of aliphatic and triterpene acids in the fractions of the hexane extract of the sea buckthorn leafy shoots is summarized in Table 6. A total of 29 acid components were identified.

Palmitic, linoleic, linolenic, behenic acids typical for the sea buckthorn leaves predominate in all three fractions. Oleanolic and ursolic acids were found in the fractions of free and total acids. Only aliphatic

Table 1. Proportion of groups of chemical compounds in unsaponifiable residue (UR)

Group of compounds	Percentage in UR	Percentage in extract
Aliphatic hydrocarbons	14.8	5.4
Aliphatic alcohols	24.8	9.0
Polyprenols and dolichols	11.5	4.2
Sterols and triterpenoids	35.0	12.7

Table 2. Distribution of components in fraction of aliphatic hydrocarbons (percentage of fraction mass)

Component	Percentage	Component	Percentage
Tridecane	0.03	Hexacosane	0.82
Tetradecane	0.10	Heptacosane	5.16
Pentadecane	0.10	Octacosane	3.18
Hexadecane	0.15	Squalene	1.27
Heptadecane	0.12	Nonacosane	59.66
Octadecane	0.14	Triacontane	4.42
Nonadecane	0.13	Hentriacontan	20.00
Icosane	0.14	Dotriacontane	0.81
Heneicosane	0.14	Tritriacontane	0.70
Docosane	0.15	Tetratriacontane	0.22
Tricosane	0.39	Pentatriacontane	0.17
Tetracosane	0.31	Hexatriacontane	0.14
Pentacosane	1.33	Heptatriacontane	0.10

Table 3. Distribution of components in fraction of aliphatic alcohols (percentage of fraction mass)

Component	Percentage	Component	Percentage
Hexadecanol	0.1	Docosanol	11.6
Phytol	27.1	Tricosanol	0.5
Octadecanol	23.1	Tetracosanol	25.0
Icosanol	9.9	Hexacosanol	2.9

Table 4. Distribution of components in polyprenoid fraction (percentage of fraction mass)

Component	Percentage	Component	Percentage
PP-8*	2.97	PP-15	5.10
PP-9	3.42	D-15	1.00
PP-10	6.32	PP-16	9.56
PP-11	30.18	D-16	1.21
PP-12	10.75	PP-17	16.00
PP-13	4.09	D-17	3.49
D-13**	0.73	PP-18	6.82
PP-14	1.64	D-18	1.62
D-14	0.27		

* Polyprenol containing 8 isoprene units; ** dolichol containing 13 isoprene units

Table 5. Distribution of components in fraction of sterols and triterpenoids (percentage of fraction mass)

Component	Percentage	Component	Percentage
Stigmastanol	0.80	Oleanolic aldehyde	0.82
β-Sitosterol	54.00	Ursolic aldehyde	1.16
Fucosterol	1.12	Uvaol	0.27
β-Amyrin	4.25	Erythrodiol	0.50
α-Amyrin + lupeol + cycloartenol	34.37	28-nor-olean-12,18(17)-dien-3-ol	0.49
24-methylenecycloartanol	1.30	28-nor-urs-12,18(17)-dien-3β-ol	0.65
Citrostadienol	0.18	Obtusifoliol	0.10

Table 6. Distribution of components in acid fractions (percentage of fraction mass)

Acid/Sample	Total acids	Free acids	Acids of ether fraction
Benzoic	0.76	–*	0.11
Lauric C_{12}	0.96	Traces	1.18
Myristic C_{14}	4.24	1.71	5.64
Pentadecanoic C_{15}	1.20	1.39	2.13
Pentadecanoic $C_{15:1}$	0.37	–	0.33
Palmitic C_{16}	16.71	19.16	24.19
Hexadecanedioic C_{16}	0.83	–	–
Palmitoleic $C_{16:1}$	1.02	1.15	0.93
Palmitolinoleic $C_{16:2}$	Traces	–	0.25
Palmitolinolenic $C_{16:3}$	Traces	0.73	1.18
Margaric C_{17}	0.51	0.48	0.55
Heptadecatrienoic	–	–	0.49
Stearic C_{18}	2.14	2.41	2.45
Oleic $C_{18:1}$	1.12	2.36	0.71
Linoleic $C_{18:2}$	16.86	9.50	8.64
Linolenic $C_{18:3}$	10.22	16.66	15.83
Arachidic C_{20}	5.28	3.08	9.01
Heneicosanoic C_{21}	0.12	0.57	1.25
Behenic C_{22}	16.23	7.54	19.51
Tricosanoic C_{23}	1.73	1.14	1.12
Tetracosanoic C_{24}	3.19	1.99	3.11
Pentacosanoic C_{25}	0.43	–	0.18
Hexacosanoic C_{26}	0.32	0.39	0.22
Octacosanoic C_{28}	0.49	0.52	Traces
Nonacosanoic C_{29}	0.18	–	–
Triacontanoic C_{30}	0.31	1.42	–
Dotriacontanoic C_{32}	0.17	–	–
Oleanolic	4.84	9.01	–
Ursolic	6.72	11.95	–

* Not found.

acids are present in the fraction of acids isolated from esters.

CONCLUSIONS

1. The chemical composition of the hexane extract of sea buckthorn leafy shoots was studied by gas chromatography–mass spectrometry and high-performance liquid chromatography.

2. Sixty seven neutral and twenty nine acidic components, including polyprenols, dolichols, triterpene alcohols and acids, sterols, were identified.

3. β-Sitosterol is the main component of the sterol fraction. Its content is 6.9% of the extract mass, which is much higher than in the essential extracts of leaves and pulp of sea buckthorn fruit. It is mostly found in the free form in the extract (free : bound ratio is 30 : 1).

4. The acidic fraction contains highly active triterpene acids (up to 5% of the extract mass) along with the major aliphatic acids.

5. Components with the chain length of 11 and 17 isoprene units predominate in the fraction of polyprenols and dolichols (up to 4.2%). A high percentage of dolichols in the polyisoprenoid fraction increases the prospects of the lipophilic concentrate of sea buckthorn leafy shoots as a bioactive substance.

6. The results obtained allow us to consider sea buckthorn leafy shoots as a promising plant raw material.

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