

Essential Oils from Different Parts of the Sea Buckthorn *Hippophae rhamnoides* L.

Nikolay M. Slynko¹, Leonid V. Kuibida², Ludmila E. Tatarova¹, George U. Galitsyn¹, Tatiana N. Goryachkovskaya^{1*}, Sergey E. Peltek^{1*}

¹The Federal Research Center, Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia ²Institute of Chemical Kinetics and Combustion SB RAS, Novosibirsk, Russia Email: nslynko@mail.ru, kuibida@kinetics.nsc.ru, ljudatat@mail.ru, goga@bionet.nsc.ru, *tanago@bionet.nsc.ru, *peltek@bionet.nsc.ru

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Abstract

The study investigated essential oils of berries and green biomass of the sea buckthorn at various growth stages. Plant material of *H. rhamnoides* ssp. *mongolica* was collected in the experimental fields of the Institute of Cytology and Genetics SB RAS (Siberia, Russia). Volatile compounds and essential oils were detected. Green spring shoots without leaves, first-year seedlings without leaves and berries were analyzed. In all studied exemplars, 103 volatile compounds were defined among which considerable proportion wasn't found in a sea-buckthorn earlier. Phenylpropanoids prevailed in essential oils composition from seedlings. Alkanes and phenylpropanoids prevailed in shoots. Berries contained mostly carboxylic acids and their esters. These data can be applied to a fingerprint of various cultivars of a sea-buckthorn with relevance to food science and technology.

Keywords

Hippophae rhamnoídes, Essential Oils, Volatile Compounds, Gas Chromatography-Mass Spectrometry (GC-MS) Analysis, Chemotaxonomy

1. Introduction

Essential oils are complex mixtures of volatile secondary metabolites of plants. They can be extracted from various organs: leaves, fruits, roots, etc. Essential oils often define plant taste, as well as its therapeutic potential. Numerous studies demonstrated their antimicrobial, antioxidant, spasmolytic, anti-inflammatory, and relaxing effects [1] [2] [3].

Individual compositions of volatile compounds may be used to identify species or geographic origin of biomaterial. Moreover, rapid and reliable analytical methods may be developed for high-scale quality control for phytopharmacy. Many volatile compounds also act as signal substances (semiochemicals). Plant signals are often cross-specific: they are used to attract insect pollinators and to scare off pests [4] [5].

The common sea buckthorn (*Hippophae rhamnoídes*, Hippophae, Elaeagnaceae) is a dioecious shrub cultivated for its fruits that are used to produce juice, grist, and oil. Leaves can also be used for medical purposes [6] [7] [8].

Hirvi and Honkanen [9] demonstrated that essential oils of *H. rhamnoides* fruit consist mainly of esters of carboxylic acids. All previous works are constructed on the research of essential oils of berries of a sea-buckthorn. In this study, we investigated essential oils of berries and green biomass of the sea buckthorn at various growth stages.

2. Material and Methods

2.1. Plant Material

Plant material of *H. rhamnoides* ssp. *mongolica* was collected in 2016 in the experimental fields of the Institute of Cytology and Genetics SB RAS. Green spring shoots collected in April contained 37.5 mg of volatile compounds per kg of air-dry material; first-year seedlings without leaves, in June. Berries were harvested in the autumn and stored at -30° C until used.

2.2. Extraction of Volatile Compounds

Dried seedlings or shoots (35.0 g) were suspended in 300 ml of deionized water, boiled in a flask with a reflux condenser for 30 min, and distilled with water vapor to a total volume of 200 ml. A total of 60.0 g of sodium chloride was added to the distillate, and the solution was extracted with 25.0 ml of ethyl acetate. The extract was dried with anhydrous sodium sulfate, filtered and analyzed by GC-MS. The same procedure was performed for 200 g of berries. For quantitation of volatile compounds, 1,2,3,4-tetrafluoronaphthalene was added to water distillates as a reference. All experiments are made in three replicas.

2.3. Chromatographic Analysis

Separation of compounds was performed with an Agilent Technologies 6890 chromatograph with a 5973 mass spectrometry detector. A 30 m capillary chromatographic column with the inner diameter of 0.25 mm was used. The speed of the carrier gas (helium) was 1.0 ml/min; the temperature of the sample heater was set at 250°C; the thermostat was heated from 50°C to 250°C by 25°C per min; sample input rate was 1.0 ml/min. The sample (1 μ l) was applied to the column without flow separation.

Compounds were identified by comparing their mass-spectra with those obtained with NIST14 from the US National Institute of Technology and Standards (NIST) mass spectra libraries, the NIST Chemistry WebBook

(https://webbook.nist.gov/), and the literary references from the latter database.

Linear RI_s was determined in relation to a homologues series of n-alkanes (C₆-C₄₀) run under the same operating conditions. Relative percentage of the compounds was calculated based on the peak areas from the FID data without correction for response factors.

3. Results and Discussion

Shoots, in April, contained 37.5 mg of volatile compounds per kg of air-dry material; seedlings collected in June, 196.7 mg/kg. Berries contained 265 mg of volatile compounds per 1 kg of fresh biomass.

Figure 1 shows chromate grams of mixtries of volatile compounds isolated from different parts of *H. rhamnoides*.

All detected compounds are listed in Table 1.

The results are expressed as the mean \pm standard deviation (n = 3). Values identified by an asterix indicate differences (p < 0.05).

Berries contained mostly carboxylic acids and their esters (**Table 2**), predominantly 3-methylbutyl ester of benzoic acid (11.63%), ethyl ester of hexanoic acid (9.07%), ethyl ester of (9Z)-hexadecenoic acid (8.85%), and 3-methylbutyl ester of 3-methylbutanoic acid (7.77%). The ratio of esters of aromatic to aliphatic acids was about 2:9.

Aromatic acids were represented by benzoic and phenylacetic acids. The alcoholic part of the esters was mostly derived from ethanol and 3-methylbutanol. Alkanes were scarce (0.29%), represented only by n-tricosane; the same was observed for alkenes (0.65%): (8Z, 11Z, 14Z)-heptadeca-1,8,11,14-tetraene and isomeric tricosenes. Phenylpropanoids were abundant in seedlings and shoots, but they accounted only for 1.5% of essential oils (β -asarone and (E)-isoelemicin) in berries. No monoterpene compounds were detected, but the following sesquiterpene hydrocarbons were identified: β -maaliene, γ -selinene, cadina-1(10),6,8triene, and germacrene B. The content of oxidized terpene compounds was 5.12%, which included isocalamendiol, isolongifolanones, shyobunon and its isomers.

Our results were generally similar to those obtained by Hirvi and Honkanen [9] and Tiitinen *et al.* [10], but certain differences were detected. The results of [9] were similar to those of our study: caroboxylic acids and their esters accounted for 94.2% of the sample and were mainly represented by 3-methylbutyl ester of benzoic acid, ethyl ester of hexanoic acid, and 3-methylbutyl ester of 3-methylbutanoic acid (7.77%), as well as by 3-methylbutanoic acid. According to Tiitinen *et al.* [10], headspace volatiles form frozen berries of sea buckthorn contain 91.4% - 97.4% of caroboxylic acids and their esters, and the highly volatile ethanol and 3-methylbutanol accounted for the rest. They found less than 2.9% of esters of aromatic acids, while in [9] and our study they made up 17% of the sample. Both studies failed to detect alkanes, phenylpropanoids, and sesquiterpenes; however, in contrast to our data, they revealed approximately 1% of





Table 1. Volatile compounds composition of different parts of *H. rhamnoides*.

Название вещества	RT	RI	RI _{лит}	seedling	shoots	berries
3-methylbutan-1-ol	3208	722	718			2.65 ± 0.59
Acetaldehyde diethyl acetal	3244	725	730			$0.26\pm0.14^{\star}$
Toluene	3675	759	755			6.57 ± 0.22
Diethyl carbonate	3790	768	765			0.71 ± 0.38
Mesityl oxide	3983	782	798			0.31 ± 0.06
Acetic acid, butyl ester	4080	789	795			0.29 ± 0.08
Furfural	4309	806	833			0.24 ± 0.12
3-methylbutanoic acid	4706	841	848			1.27 ± 0.19
2-methylbutanoic acid, ethyl ester	4723	842	849			0.52 ± 0.16
3-methylbutanoic acid, ethyl ester	4759	845	854			1.38 ± 0.21
3-methyl-3-hydroxybutanoic acid, methyl ester	4935	860	882			0.26 ± 0.06
Acetic acid, 3-methylbutyl ester	5014	866	867			0.18 ± 0.09
Styrene	5182	880	893			$0.12\pm0.08^{\star}$
o-Xylene	5234	884	887			1.45 ± 0.24
2-methyl-2-hydroxybutanoic acid, methyl ester	5490	904	923			0.84 ± 0.14
3-methyl-3-hydroxybutanoic acid, ethyl ester	5781	935	926			2.80 ± 0.27
2-hydroxy-3-methylbutanoic acid, ethyl ester	5957	953	975			0.79 ± 0.19
6-Methyl-5-hepten-2-on	6098	967	986			0.42 ± 0.11
Hexanoic acid	6115	968	990			0.61 ± 0.09
Hexanoic acid, ethyl ester	6256	982	1000			9.07 ± 1.01
Heptanoic acid, ethyl ester	7058	1081	1097			0.21 ± 0.05
Nonanal	7093	1085	1080		2.56 ± 0.44	0.65 ± 0.15
Linaool	7102	1086	1097	0.36 ± 0.09		
2,6-Dimethylcyclohexan-1-ol	7137	1091	1110		0.74 ± 0.21	
3-methylbutanoic acid, 3-methylbutyl ester	7137	1091	1104			7.77 ± 0.64
2-methylbutanoic acid, 3-methylbutyl ester	7146	1092	1107			1.19 ± 0.21
Octanoic acid, methyl ester	7252	1106	1126			$0.14\pm0.11^{\star}$
Camphor	7410	1130	1146	$0.08\pm0.06^{*}$		
Benzoic acid, ethyl ester	7560	1153	1171			0.99 ± 0.18
Octenoic acid, ethyl ester, (4Z)-	7683	1171	1187			2.12 ± 0.20
Octanoic acid, ethyl ester	7745	1179	1196			4.32 ± 0.36
Safranal	7771	1183	1173		0.38 ± 0.13	
1-Dodecene	7807	1188	1187		0.86 ± 0.17	
β -Cyclocitral	7921	1205	1216	$0.09\pm0.06^{\ast}$	$0.22\pm0.15^{*}$	
Benzeneacetic acid, ethyl ester	8000	1219	1246			1.14 ± 0.22
Heptanoic acid, 2-methylbutyl ester	8088	1234	1247			2.34 ± 0.29
Heptanoic acid, 3-methylbutyl ester	8106	1237	1252			0.45 ± 0.16
Hepta-2,4-dienoic acid, ethyl ester	8203	1253	1296			$0.06 \pm 0.05^{*}$
2,4,5,6,7,7 <i>a</i> -Hexahydro-3-(1-methylethyl)-7 <i>a</i> -methy l-1H-2-indenone	8388	1284	1269		0.23 ± 0.07	

Continued

2-Acetyl-4-methylphenol	8520	1306	1285		0.30 ± 0.07	
(Z)-Theaspirane	8520	1306	1290		1.07 ± 0.15	
(E)-Theaspirane	8599	1320	1303		0.36 ± 0.12	
Dehydro-ar-ionene	8784	1353	1355		0.14 ± 0.09*	
Decenoic acid, ethyl ester, (4Z)-	8837	1363	1311			0.60 ± 0.17
Pentanoic acid, benzyl ester	8908	1375	1396			1.43 ± 0.33
Decanoic acid, ethyl ester	8916	1377	1396			0.80 ± 0.17
Methyleugenol	8925	1378	1366	0.12 ± 0.05		
1-tetradecene	8987	1389	1388		1.28 ± 0.28	
Copaene	8996	1391	1380	$0.05 \pm 0.05^{*}$		
β-Elemen	9048	1400	1391	0.16 ± 0.09	0.38 ± 0.15	
Benzoic acid, 3-methylbutyl ester	9163	1422	1439			11.63 ± 1.66
Caryophyllene	9163	1422	1421		0.20 ± 0.09	
Methyl cis-isoeugenol	9198	1429	1422		0.32 ± 0.08	
β-Maaliene	9207	1431	1405			0.93 ± 0.31
Unidentified	9322	1453			0.63 ± 0.12	0.30 ± 0.12
3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0] heptan-2-one	9427	1473			1.07 ± 0.16	
Benzeneacetic acid, 3-methylbutyl ester	9427	1473	1472			2.23 ± 0.32
6-Epishyobunon	9533	1493	1480	0.51 ± 0.12	2.74 ± 0.41	0.11 ± 0.07
γ-Selinene	9560	1498	1493			0.51 ± 0.11
Shyobunon	9630	1513	1502	2.07 ± 0.63	4.36 ± 0.37	0.43 ± 0.11
Elemicine	9665	1520	1522	0.17 ± 0.08		
Isoshyobunon	9718	1531	1520	2.23 ± 0.42	0.76 ± 0.24	0.62 ± 0.14
Unidentified	9762	1541				2.40 ± 0.46
γ-Asarone	9762	1541	1533	2.59 ± 0.94	0.45 ± 0.15	
<i>a</i> -Calacorene	9788	1546	1542	$0.22\pm0.16^{*}$		
Dodecenoic acid, methyl ester, (5E)-	9824	1554				2.17 ± 0.31
(Z)-Isoelemicin	9885	1566	1560	0.46 ± 0.12		
Cadina-1(10),6,8-triene	9894	1568	1533			0.21 ± 0.12
Germacrene B	9930	1575	1556			2.42 ± 0.28
β-Asarone	9973	1584	1581	64.60 ± 7.11	29.20 ± 5.82	0.63 ± 0.13
Cedrenol	10,119	1615	1615		0.69 ± 0.12	
(E)-Isoelemicin	10,176	1628	1628	0.97 ± 0.22		0.97 ± 0.24
Muurola-4,10(14)-dien-1 β -ol	10,194	1632	1628	$0.19\pm0.11^{*}$		
<i>a</i> -Asarone	10,255	1645	1646	3.89 ± 1.04		
(8Z,11Z,14Z)-Heptadeca-1,8,11,14-tetraene	10,335	1662	1664	0.99 ± 0.34	1.23 ± 0.15	0.35 ± 0.09
Isolongifolen-5-one	10,493	1696	1685			0.67 ± 0.08
Isolongifolen-9-one	10,511	1700	1728	1.58 ± 0.26	6.88 ± 0.23	3.00 ± 0.28
Isocalamendiol	10,749	1755	1761	1.09 ± 0.14	1.04 ± 0.19	0.29 ± 0.13
Myristic acid, ethyl ester	10,846	1777	1761			0.75 ± 0.16

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1-Octadecene	10,899	1789	1786		1.45 ± 0.09	
Unidentified	10,969	1805				0.72 ± 0.11
Dodecanoic acid, 3-methylbutyl ester	11,075	1830	1845			0.47 ± 0.10
Hexahydrofarnesylacetone	11,101	1836	1838	0.50 ± 0.11	2.47 ± 0.31	
Hexadecenoic acid, methyl ester, (11Z)-	11,330	1891	1846			0.34 ± 0.05
Hexadecanoic acid, methyl ester	11,418	1910	1886			0.19 ± 0.06
Dibutylphtalate*	11,515	1931	1924		0.45 ± 0.03	
Hexadecenoic acid, ethyl ester, (9Z)-	11,638	1958	1977			8.85 ± 0.73
Hexadecanoic acid, ethyl ester	11,735	1978	1993			2.68 ± 0.39
5-Eicosene	11,797	1991	1990	1.28 ± 0.19		
Octadeca-9,12-dienoic acid, (9Z, 12Z)-	12,651	2146	2169			0.40 ± 0.16
Octadecenoic acid, ethyl ester, (9Z)-	12,731	2159	2173			0.28 ± 0.13
1-Docosene	12,933	2191	2190		0.80 ± 0.21	
Docosane	12,986	2199	2200	0.43 ± 0.05	0.33 ± 0.06	
Retene	13,198	2228	2176		0.47 ± 0.11	
Tricosene, (9Z)-	13,532	2273	2278			$0.09\pm0.06^*$
analogue of (9Z)-tricosene	13,576	2279				0.21 ± 0.08
Tricosane	13,726	2298	2300	1.70 ± 0.08	1.53 ± 0.08	0.29 ± 0.07
Abietic acid, methyl ester	13,999	2329	2323		1.18 ± 0.19	
1-Tetracosene	14,572	2391	2391		0.46 ± 0.12	
Tetracosane	14,651	2399	2400	1.14 ± 0.11	2.69 ± 0.06	
Pentacosane	15,814	2500	2500	2.36 ± 0.14	4.21 ± 0.11	
Hexacosane	17,294	2600	2600	2.24 ± 0.09	6.65 ± 0.14	
Heptacosane	19,179	2702	2700	3.51 ± 0.10	5.47 ± 0.12	
Octacosane	21,610	2799	2800		4.60 ± 0.15	
Squalene	21,927	2810	2805		3.49 ± 0.17	
Nonacosane	24,729	2900	2900	5.63 ± 0.13	4.38 ± 0.14	

Table 2. Major components of essential oils of different parts of *H. rhamnoides*.

	Seedlings	Shoots	Fruits
alkanes	15.84	29.86	0.29
alkenes	2.27	10.31	3.42
phenylpropanoids	72.90	29.97	1.50
sesquiterpenes	0.43	0.58	4.07
oxygenated terpenes	8.10	17.07	5.12
carboxylic acids and their esters	-	1.63	71.45

C₁₀H₁₆ monoterpene hydrocarbons.

Socaci *et al.* [11] studied essential oils of berries and juice of both wild and cultivated *H. rhamnoides carpatica*. The main components were again 3-methylbutyl ester of 3-methylbutanoic acid and ethyl ester of hexanoic acid, as well as ethyl esters of 2-methyl and 3-methylbutyric acids. The share of terpene

Continued

compounds, limonene, and cis-ozymene was below 1%.

The differences of our results from those obtained by Cakir [12] were more pronounced. The last found that volatile compounds except octyl acetate consist mostly of ethyl esters of various aliphatic acids, while phenylpropanoids, terpenes and esters of isomeric valeric acids, were absent. The content of aliphatic hydrocarbons was 13.5%, and of aliphatic alcohols, 15.7%.

In the study of Yue *et al.* [13], palmitic acid accounted for the major part of the acid-ester fraction. However, we consider these results highly questionable based on the reported retention times do not match the corresponding Kovacs indices.

Vítová *et al.* [14] found the majority of the volatile compounds of the sea buckthorn berries to be represented by aliphatic alcohols with low boiling point (C_2-C_6) , oct-2-en-3-ol, octanal, esters (butyl acetate, ethyl acetate and ethyl hexanoate), and ketones (pentane-2-one, and 3-hydroxybutan-2-one). Alkanes, phenylpropanoids, and terpene compounds were not detected.

It is hard to explain the discrepancies among these studies. Hirvi & Honkanen [9] analyzed essential oils from berries of two varieties of the sea buckthorn from Finland, of *H. rhamnoides* ssp. *mongolica* from Russia and the local *H. rhamnoides* ssp. *rhamnoides* in two consecutive seasons. Both varieties are cultivated in southern and southwestern Finland. The authors used the Principal Component Analysis (PCA) and found that the differences were mainly observed among seasons, not among the varieties. However, a similar study of Vítova *et al.* [14] yielded opposite results. PCA analysis of Socaci *et al.* [11] indicated that essential oils of seeds and juice from various sea buckthorn varieties consist of the same substances but in highly varying ratios. On the whole, these studies yielded strongly differing composition of essential oils, which might be attributed to regional (climatic) differences. All aforementioned studies used different methods: solid-phase microextraction (SPME) in [14], vapor SPME in [11], vapor distillation in [12], and hydroextraction distillation in [9]. However, we failed to detect any interconnections caused by differences in extraction procedures.

We used hydrodistillation to extract essential oils from seedlings and shoots of the sea buckthorn. Similar to alkanes, phenylpropanoids prevailed, although their total content was lower in shoots than in seedlings (25.9% vs. 72.9%). Among phenylpropanoids, β -asarone was the most abundant (64.4%), followed by its isomers (α - and γ -asarons), elemicin and cis- and trans-isoelemicins (a total of 8.08%), as well as low amounts of O-methyleugenol. The observed high content of phenylpropanoids is remarkable, as these substances are increasingly used due to their antiplatelet and anticoagulant activity [15].

Compared to berries, essential oils from seedlings had more long chain alkanes (C_{22} - C_{29}), small amounts of oxidized monoterpenes of linalool, β -cyclocitral and camphor (a total of 0.53%), and significantly more oxidized sesquiterpenes (7.5%), almost similar to those found in berries. A small portion of sesquiterpene hydrocarbons was represented by copaene, β -elemen, and α -calacorene. Carboxylic acids and their esters were absent. In the essential oils isolated from shoots of the sea buckthorn, phenylpropanoids were represented only by β - and γ -asarons. Isomers of elemicin were absent, while small amounts of methyl-cis-isoeugenol were detected. Content of n-alkanes (C₂₂-C₃₀) was found to change during vegetative development, from 15.84% in seedlings to 29.86% in shoots. It is well known that cuticular wax that protects shoots from the elements, pathogenic microorganism and phytophagous insects contains long-chain alkanes [16]. Piasentier *et al.* [17] found that n-alkane profiles change during vegetative development, and these changes vary in different tree species. The maximum alkane length in the green mass of the sea buckthorn was n = 29 [18] (Kukina *et al.*, 2016).

We also found the following alkenes in essential oils from the shoots of the sea buckthorn: 1-dodecene, 1-tetradecene, 1-octadecene, and 1-docosene (total content of 4.4%), which were probably formed from the corresponding terminal alkanols; retene (0.47%), the marker substance of forest fires; squalene (4.39%), which is often found in plants; and (8Z, 11Z, 14Z)-Heptadeca-1,8,11,14-tetraene (1.23%), which is characteristic for all essential oils. We failed to found carboxylic acids and their esters that are abundant in berries; however, we detected methylabietate (1.18%) and dibutyl phthalate (0.45%). We believe the latter to be a human-introduced admixture. We also detected sesquiterpenes: β -elemen (0.38%) and caryophyllene (0.20%). We identified several compounds also found in berries, but in higher relative amounts (a total of 17.07%): isocalamendiol, isolongifolenes, shyobunon, and its isomers.

It is well known that shyobunon and its analogues may act as repellents and insecticides [19]. Moreover, Yue *et al.* demonstrated that essential oils from various parts of the sea buckthorn are effective against pathogenic bacteria: *Staphylococcus aureus* was equally suppressed by oils from all studied material, oil from berries had the highest impact on *Bacillus subtilis* and *B. coagulans*, and oils from seedlings, on *E. coli* [13].

Therefore, in this study we extracted essential oils from different parts of the sea buckthorn *Hippophae rhamnoides,* L. by hydrodistillation, identified them using GC-MS, and performed comparative analysis.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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