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

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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 rhamnoides*, 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, spasmylic, 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 rhamnoides, 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 µl) 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, was determined in relation to a homologues series of n-alkanes ($C_6$-$C_{40}$) 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 chromatograms of mixtures 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 ± standard deviation (n = 3). Values identified by an asterisk 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),6,8-triene, 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: carboxylic 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 from frozen berries of sea buckthorn contain 91.4% - 97.4% of carboxylic 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...
Figure 1. Volatile compounds of different parts of *H. rhamnoides*, GC-MS analysis.
Table 1. Volatile compounds composition of different parts of *H. rhamnoides*.

| Название вещества                        | RT   | RI   | RI лит | seedling | shots | berries |
|-----------------------------------------|------|------|--------|----------|-------|---------|
| 3-methylbutan-1-ol                      | 3208 | 722  | 718    |          |       | 2.65 ± 0.59 |
| Acetaldehyde diethyl acetal             | 3244 | 725  | 730    |          |       | 0.26 ± 0.14* |
| 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 ± 0.08* |
| 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 ± 0.11* |       |
| Camphor                                 | 7410 | 1130 | 1146   | 0.08 ± 0.06* |       |
| 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 ± 0.06* | 0.22 ± 0.15* |
| Benzenecacetic 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 ± 0.05* |       |
| 2,4,5,6,7,7α-Hexahydro-3-(1-methylethyl)-7α-methy l-1H-2-indenone | 8388 | 1284 | 1269   | 0.23 ± 0.07 |       |
| Compound                                                                 | Retention Time (min) | Peaks | Area Ratio (± Standard Deviation) |
|------------------------------------------------------------------------|----------------------|-------|-----------------------------------|
| 2-Acetyl-4-methylphenol                                                 | 8520                 | 1306  | 1285                              |
| (Z)-Theaspirane                                                        | 8520                 | 1306  | 1290                              |
| (E)-Theaspirane                                                        | 8599                 | 1320  | 1303                              |
| Dehydro-ar-ionone                                                      | 8784                 | 1353  | 1355                              |
| Decenoic acid, ethyl ester, (4Z)-                                      | 8837                 | 1363  | 1311                              |
| Pentanoic acid, benzyl ester                                           | 8908                 | 1375  | 1396                              |
| Decanoic acid, ethyl ester                                             | 8916                 | 1377  | 1396                              |
| Methyleugenol                                                          | 8925                 | 1378  | 1366                              |
| 1-tetradecene                                                          | 8987                 | 1389  | 1388                              |
| Copae                                                                 | 8996                 | 1391  | 1380                              |
| β-Elemen                                                               | 9048                 | 1400  | 1391                              |
| Benzoic acid, 3-methylbutyl ester                                      | 9163                 | 1422  | 1439                              |
| Caryophyllene                                                          | 9163                 | 1422  | 1421                              |
| Methyl cis-isoeugenol                                                  | 9198                 | 1429  | 1422                              |
| β-Maaliene                                                             | 9207                 | 1431  | 1405                              |
| Unidentified                                                           | 9322                 | 1453  |                                    |
| 3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one         | 9427                 | 1473  |                                    |
| Benzenacetic acid, 3-methylbutyl ester                                 | 9427                 | 1473  | 1472                              |
| 6-Epishyobunon                                                          | 9533                 | 1493  | 1480                              |
| γ-Selinene                                                             | 9560                 | 1498  | 1493                              |
| Shyobunon                                                              | 9630                 | 1513  | 1502                              |
| Elemicicine                                                            | 9665                 | 1520  | 1522                              |
| Isoshyobunon                                                           | 9718                 | 1531  | 1520                              |
| Unidentified                                                           | 9762                 | 1541  |                                    |
| γ-Asarone                                                              | 9762                 | 1541  | 1533                              |
| α-Calacorene                                                           | 9788                 | 1546  | 1542                              |
| Dodecenoc acid, methyl ester, (5E)-                                    | 9824                 | 1554  |                                    |
| (Z)-lsolemicin                                                         | 9885                 | 1566  | 1560                              |
| Cadina-1(10),6,8-triene                                                | 9894                 | 1568  | 1533                              |
| Germacrene B                                                           | 9930                 | 1575  | 1556                              |
| β-Asarone                                                              | 9973                 | 1584  | 1581                              |
| Cedrenol                                                               | 10,119               | 1615  | 1615                              |
| (E)-lsolemicin                                                         | 10,176               | 1628  | 1628                              |
| Muurola-4,10(14)-dien-1β-ol                                           | 10,194               | 1632  | 1628                              |
| α-Asarone                                                              | 10,255               | 1645  | 1646                              |
| (8Z,11Z,14Z)-Heptadeca-1,8,11,14-tetraene                              | 10,335               | 1662  | 1664                              |
| Isolongifolen-5-one                                                   | 10,493               | 1696  | 1685                              |
| Isolongifolen-9-one                                                   | 10,511               | 1700  | 1728                              |
| Isocalamendiol                                                        | 10,749               | 1755  | 1761                              |
| Myristic acid, ethyl ester                                             | 10,846               | 1777  | 1761                              |
**Continued**

| Substance                                           | Seedlings | Shoots | Fruits |
|-----------------------------------------------------|-----------|--------|--------|
| 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 |
| Dibutylphthalate*                                   | 11,515    | 1931   | 1924   | 0.45 ± 0.03 |
| Hexadecanoic 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)-                                    | 13,352    | 2273   | 2278   | 0.09 ± 0.06* |
| 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   | 0.88 ± 0.14  |
| 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 |
|-----|-----------|--------|--------|
| alkane & monoterpane hydrocarbons.                |          |        |        |
| Socaci *et al.* [11] studied essential oils of berries and juice of both wild and cultivated *H. rhamnoides carpathica*. 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

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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 (C₂-C₆), 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 hydrodistillation 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 (C₂₂-C₃₀), 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-calcacorene. Carboxylic acids and their esters were absent.
In the essential oils isolated from shoots of the sea buckthorn, phenylpropa-noids 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 (C_{22}-C_{30}) 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, isolongifolene, 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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