Prospects for comprehensive use of sea buckthorn of Novosibirsk selection

Conference Paper · January 2022

DOI: 10.1063/5.0070159

6 authors, including:

Dmitry Shcherbakov
State Research Center of Virology and Biotechnology VECTOR
96 PUBLICATIONS **189** CITATIONS
SEE PROFILE

Tatyana Kukina
Novosibirsk Institute of Organic Chemistry
52 PUBLICATIONS **199** CITATIONS
SEE PROFILE

Petr Kolosov
Altai State University
9 PUBLICATIONS **18** CITATIONS
SEE PROFILE

Prospects for comprehensive use of sea buckthorn of Novosibirsk selection

Cite as: AIP Conference Proceedings **2390**, 030085 (2022); https://doi.org/10.1063/5.0070159
Published Online: 04 February 2022

D. N. Shcherbakov, G. Y. Galitsyn, T. P. Kukina, et al.

Prospects for Comprehensive Use of Sea Buckthorn of Novosibirsk Selection

D. N. Shcherbakov [1, 2, a)], G. Y. Galitsyn [3, b)], T. P. Kukina [4, c)], N. V. Panteleeva [2, d)], O. I. Salnikova [4, e)] and P. V. Kolosov [1, f)]

[1]*Altai State University, Barnaul 656049, Russia*
[2]*State Research Center of Virology and Biotechnology Vector, 630559 Koltsovo, Novosibirsk oblast, Russia*
[3] *Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk 630090, Russia*
[4]*Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk 630090, Russia*

[a)]Corresponding author: dnshcherbakov@gmail.com
[b)]goga@bionet.ncs.ru
[c)]kukina@nioch.nsc.ru
[d)]nino4ka_panteleeva@mail.ru
[e)]olga@nioch.nsc.ru
[f)]petro.kolosov@gmail.com

Abstract. The composition of sea buckthorn lipophilic acids was studied. Acidic and neutral components were determined by gas-chromatography-mass-spectrometry. Using hexane and methyl *tert*-butyl ether (MTBE) as extractants lipophilic extracts with the similar composition were obtained. As a result, comparison with databases some triterpenes and aliphatic acids with a chain lengths 12 to 32 carbon atoms, including saturated, unsaturated and dibasic acids. Some components were discovered in this raw material at first time.

INTRODUCTION

Sea buckthorn (SB) (*Hippophae rhamnoides* L) is an economically important medicinal plant belonging to Elaeagnaceae family. It grows in cold regions of Asia, Europe, and North America. The distribution ranges from Himalayan regions including India, Nepal, Bhutan, Pakistan and Afghanistan, to China, Mongolia, Russia, Kazakstan, Hungary, Romania, Switzerland, Germany, France and Britain, and northwards to Finland, Sweden and Norway. The wide distribution of SB is reflected in its habit-related variation not only in morphology, yield, growth rhythms and cold hardiness, but also in berry related characters such as fresh weight, chemical and sensory attributes [1, 2]. Sea buckthorn berries have high levels of vitamin C, vitamin E, carotenoids, carbohydrates, proteins, organic acids, dietary minerals, β-sitosterol and polyphenolic acids [3]. Extracts of sea buckthorn berries have antioxidative [4], antitumoric [5], anticarcinogenic [6], chemoprotective [7], and nutritional effects [8]. Many studies have shown that this medicinal plant possesses antioxidant and α-glucosidase inhibitory activities and protects the human body against both cellular oxidative reaction and diabetes [9]. Leaves of sea buckthorn (*Hippophae rhamnoides* L., *Elaeagnaceae*) are a source of valuable biologically active substances. They are used in folk medicine for diseases of the skin, gastrointestinal tract. Lipids from leaves have been recommended as antiburn and wound healing agents. Lipids from sea buckthorn leaves have been investigated previously [10–17]. However, the data of detailed pharmacological screening of sea buckthorn leaves extract, fractions, and isolated bioactive compounds are very poor [18–24]. Some authors connect the activity of sea buckthorn leaves with high level of triterpenoic acids [24], but they turn sum of such acids exclusively into ursolic acid. The composition of lipophilic components of sea

Actual Problems of Organic Chemistry and Biotechnology (OCBT2020)
AIP Conf. Proc. 2390, 030085-1–030085-5; https://doi.org/10.1063/5.0070159
Published by AIP Publishing. 978-0-7354-4171-2/$30.00

buckthorn leafy shoots was studied [25, 26]. In this literature data the chemical composition of the hexane extract of sea buckthorn leafy shoots was studied. Sixty-seven neutral and twenty-nine acidic components, including polyprenols, dolichols, triterpene alcohols and acids, sterols, were identified. The acidic fraction contains highly active triterpene acids (up to 5% of the extract mass) along with the major aliphatic acids. The aim of the present study was to isolate and identify of sea buckthorn lipophilic compounds including triterpenoic acids.

The Institute of Cytology and Genetics SB RAS and the Novosibirsk Michurin Zonal Fruit-Berry Experimental Station Siberian Branch of the RAAS have developed sea-buckthorn cultivars (Cvs), suitable for mechanical harvesting. The Cvs differ in ripening terms, color, chemical composition, and taste, which determine the purpose of their use: technical, gastronomic or universal. Sibirsky Rumyanets is an ultra-early cultivar. Fruits ripen in mid-August, have shining bright-red color, contain up to 36 mg% of carotenoids, and have high commodity ratio. Cultivars Podruga, Zolotoi Kaskad, and Kapriz are mid-early, ripen in the first half of September, sugar content is 7-10%. Krasny Fakel, Ognistaya, and Ivushka are late cultivars ripening in the second half of September, have red fruits with high content of carotenoids, resistant to squashing pressure up to 600 g, remain undamaged on branches till the first frosts and may be harvested both in autumn and winter by kicking. Druzhina is mid-early cultivar, most suitable for industrial harvesting in autumn, has reserved growth intensity, trees have compact top and hard shoots with no branches in summer, uniform-sized large rare fruits with hard pericarp, good for light dry pick-up. Required pick-up pressure is 70–90 g, as opposed to 140–210 g for other existing cultivars [1, 2].

EXPERIMENTAL

Sea buckthorn berries, cvs. Zyrianka (Zy), Sibirskiy rumianetz (SR), Krasny fakel (KF), Druzhina (Dr), Podruga (Pod) and Zolotoy kaskad (ZK) were collected from a 15-year old orchard of the Institute of Cytology and Genetics SB RAS. Berries were manually harvested in August-September 2011. Berries were hand cleaned to remove dry leaves, branches, and damaged berries by harvesting and then dried. The first series of experiments deals with whole berries dried by natural ventilation after fermentation at temperature about 35 °C (1). The second series of samples was dried at 120 °C without fermentation (2). These ways simulated two methods of sea buckthorn industry processing. Cleaned berries were separated of seeds. Exhaustive extraction of sea buckthorn samples was performed in Soxhlet apparatus. Plant material (100–120 g) was placed in the thimble holder of the apparatus and extracted with hexane for 15 h. We extracted seedless dry berries to obtain the sum of lipids without polar substances. Lipophilic extracts of seedless sea buckthorn berries were obtained by usual procedure and separated towards neutral and acid constituents by saponification. Sea buckthorn leaves from male (MP) and female (KF and SR) plants were collected from 8-10-year old orchard of the Institute of Cytology and Genetics SB RAS. Leaves were manually harvested in July 2011. Leaves were hand cleaned to remove branches and berries damaged by harvesting and then winded. Exhaustive extraction of sea buckthorn samples was performed in Soxhlet apparatus. The samples KF and MP were extracted by MTBE, SR leaves were extracted by two-step pathway: first step with hexane (SRH) and second step with MTBE (SRM) as extractant.

Free (F), bonded (B), and total (T) acids of SR leaves were isolated from the total sample by alkaline extraction with a 2% aqueous solution of sodium hydroxide and hydrolysis by 15% aqua-ethanol solution of potassium hydroxide [25, 26]. The investigation of the acid fractions was carried out after full methylation by diazomethane using a Hewlett-Packard G 1800 A system with mass-selective detector HP 5971 and HP-5 MS column (30 m × 0.25 mm × 0.25 μm). Helium (1 mL/min) was used as carrier gas. The GC oven temperature was kept at 50 ° C for 2 min and programmed to 300 ºC as a rate 4º/min and then kept constant at 300 ºC for 30 min. The injecting port temperature was at 280 ºC and ion source temperature was at 170 ºC. MS were recorded at 70 eV. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram and given in mg% for the convenience of comparing raw material samples.

RESULTS AND DISCUSSION

Six cultivars of sea buckthorn of Siberian selection were investigated as a raw material for oil production. We used hexane as an extractant. This solvent ensured the quantitative extraction of fatty acids, tocopherols and tocotrienols, total carotenoids, sterols. The extracted quantities were compared on two series.

The first series of experiments deals with whole berries dried by natural ventilation after fermentation at temperature about 35 °C (1). The second series of samples was dried at 120 °C without fermentation (2). These

ways simulated two methods of sea buckthorn industry processing. We extracted seedless dry berries to obtain the sum of lipids without polar substances. Oil percentage of the dried berries is presented in table 1.

TABLE 1. Oil yield in the different ways of processing.

Cultivar	Oil yield (% of dry seedless berries) (1)	Oil yield (% of dry seedless berries) (2)
Zyrianka (Z)	46	21
Sibirskiy rumianetz (SR)	19	17
Krasny fakel (KF)	35	23
Druzhina (D)	25	22
Podruga (P)	26	not tested
Zolotoy kaskad (ZK)	not tested	20

The table data show the distinctions of yield depending on the processing way. The yield increases in first way with application of fermentation technology.

Some samples of oil were investigated by GC/MS-analysis. The quality and quantity composition is presented in table 2.

TABLE 2. Distribution of components in fractions of aliphatic total acids in hexane extracts of sea buckthorn seedless berries in mg/100g of sample weight.

Acid	Content in raw material (mg%)				
	KF	SR	SR-2	Zy	Pod
Lauric	56.1	19.0	traces	traces	78
Dodecenoic	traces	traces	not detected	not detected	52.2
Tetradecenoic	not detected	36.1	not detected	46.1	46.8
Miristic	658	245.1	268.6	299.1	348.4
Pentadecanoic	73.5	traces	traces	traces	62.4
Pentadecenoic	35.2	not detected	not detected	not detected	not detected
Palmitic	9419.5	5080.6	4323.1	17296.2	7880.6
Palmitoleic	13510.1	8337.5	6303.6	19228.4	7391.8
Palmitolinoleic	not detected	39.4	not detected	not detected	not detected
Stearic	455	182.3	136.2	460.1	304.2
Oleic	1057.5	431.3	411.4	1651.4	559.8
Vaccenic	2506.2	1913.4	1436.5	3243.1	1588.6
Linoleic	3878.8	2069.1	1589.5	2382.8	2256.8
Linolenic	2026.5	729.6	1292.7	1660.6	663.3
Arachidic	150.5	53.2	40.8	138.4	83.2
Gadoleinic	112.4	34.2	19.4	82.8	44.2
Behenic	140.0	36.1	23.8	69.4	130.5
13-Docosenoic	133.1	32.3	16.9	59.8	72.8
Tricosanoic	56.2	18.0	13.3	19.8	28.6
Lignoceric	227.6	47.5	45.9	78.2	262.6
Nervonic	161.3	30.5	27.3	59.8	182.9
Pentacosanoic	35.1	15.2	8.4	6.7	31.2
Hexacosenoic	traces	traces	traces	traces	57.2
Cerotic	66.5	45.6	54.4	115.2	360.2
Heptacosanoic	traces	traces	traces	traces	28.6
Octacosenoic	traces	traces	traces	traces	78.2
Montanic	91.2	77.9	47.6	289.8	665.2
Triacontenoic	traces	traces	traces	traces	93.6
Melissic	77.3	41.8	27.2	179.4	298.9
Laceric	traces	traces	traces	traces	91.2
Dotriacontenoic	traces	not detected	not detected	not detected	44.2
Oleanolic	343.6	89.3	132.6	243.8	291.2
Ursolic	479.5	108.3	215.9	317.4	174.2

The table data show the presence of 31 fatty acids and 2 triterpenoic acids. Bioactive acids such as palmitoleic, linoleic, linolenic and vaccenic form a significant part of the composition. Extracts of meal after hexane extraction (yield 6-8% of sample) contained up to 87% of triterpenic acids with a predominance of ursolic (up to 65% of the total triterpenoids) according to HMS and HPLC. The GC-MS-analysis also made it possible to identify acetylursolic (up to 1.5%), oleanolic (up to 30%), ursonic (up to 1%), olive (up to 3%), corosol (up to 4%), grinding (up to 5%). Ursonic, acetylursolic and corosolic acid were found for the first time in sea buckthorn fruits. Studied Cvs may be used also as a source of triterpenoic acids. The qualitative and quantitative compositions of aliphatic and triterpenoic neutral constituents are similar to one referred in literature data [25].

TABLE 3. Distribution of components in fractions of aliphatic total acids in hexane and total, free, and bonded acids MTBE extracts of sea buckthorn leaves in mg/100g of sample weight.

Acid	**Content in raw material (mg%)**				
	KFT	**MPT**	**SRHT**	**SRMF**	**SRMB**
Lauric	8.6	33.2	traces	traces	traces
Nonanedioic	51.8	not detected	not detected	not detected	not detected
Miristic	24.3	34.8	18.0	2.0	12.3
Pentadecanoic	8.1	2.7	4.8	not detected	2.7
Palmitolinolenic	35.5	not detected	not detected	not detected	not detected
Palmitic	389.3	477.1	287.7	12.2	109.7
Palmitoleic	32.9	62.9	132.2	5.6	65.0
Hexadecanedioic	15.1	35.6	traces	traces	traces
Palmitolinoleic	not detected	not detected	2.7	not detected	2.0
Margaric	10.0	3.0	3.5	not detected	1.8
Stearic	38.9	73.4	23.3	2.2	10.4
Oleic	12.4	34.3	76.8	1.0	20.4
Vaccenic	traces	traces	39.4	traces	10.1
Linoleic	191.7	113.1	124.7	4.8	57.8
Linolenic	521.6	309.4	234.5	12.1	88.7
Octadecanedioic	19.7	72.1	not detected	not detected	traces
Arachidic	50.2	34.6	56.4	1.8	14.4
Heneicosanoic	18.1	3.0	5.6	3.4	1.8
Behenic	27.0	62.9	167.7	6.8	43.0
13-Docosenoic	traces	traces	not detected	not detected	not detected
Tricosanoic	14.0	traces	6.1	1.8	1.8
Lignoceric	19.2	57.5	25.3	4.6	6.3
Nervonic	traces	not detected	not detected	not detected	traces
Pentacosanoic	2.7	2.2	not detected	not detected	not detected
Cerotic	78.1	3.2	3.8	1.4	17.8
Heptacosanoic	traces	not detected	traces	traces	traces
Montanic	20.5	15.4	traces	8.4	0.7
Nonacosanoic	traces	traces	traces	1.6	traces
Melissic	43.7	39.2	traces	13.6	1.7
Laceric	2.7	2.2	traces	traces	traces
Oleanolic	262.7	334.8	traces	151.8	14.7
Ursolic	551.2	767.9	0.9	388.0	42.5
Oleanonic	2.3	3.1	not detected	2.0	not detected
Ursonic	2.5	3.2	not detected	1.9	not detected
Betulinic	3.6	4.1	not detected	4.8	not detected
Pomolic	2.3	1.9	not detected	2.0	not detected
Acetylursolic	9.9	7.9	not detected	17.9	not detected
Acetyloleanolic	5.8	4.8	not detected	11.4	not detected
Maslinic	7.9	9.6	not detected	15.9	not detected
Corosolic	18.3	20.3	not detected	20.9	not detected

Hexane extract of sea buckthorn leaves and bonded acids does not content triterpenoic components.

CONCLUSION

The composition of sea buckthorn seedless berries and leaves lipophilic acids was studied. Acidic and neutral components were determined by gas-chromatography-mass-spectrometry.

Two pathways of industry processing were modeling in experiment. The first series of experiments deals with whole berries dried by natural ventilation after fermentation at temperature about 35 °C. The second series of samples was dried at 120 °C without fermentation. The first pathway was more prospective.

Studied Cvs may be used as a source of bioactive triterpenoic and aliphatic acids. Seedless berries after hexane extraction is the most prospective for triterpenoic acids isolation.

REFERENCES

1. D. Dhyani, R. K. Maikhuri, K. S. Rao K. S., L. Kumar, V. K. Purohit, M. Sundriyal, and K. G. Saxena, Curr. Sci. **92**, 1148–1152 (2007).
2. Zeb A. J., Biol. Sci. **4**, 687–693 (2004).
3. H. Kallio, B. Yang B, and P. J. Peippo, Agric. Food Chem. **50**, 6136–6142 (2002).
4. D. Rösch, A. Krumbein, and L. Kroh, W. Eur. Food Res. Technol. **219**, 605–613 (2004).
5. K. Yasukawa, S. Kitanaka, K. Kawata, and K. Goto, Fitoterapia. **80**, 164–167 (2009).
6. A. Zeb, Asian Pac. J. Cancer Prev. **7**, 32–35 (2006).
7. B. Padmavathi, M. Upreti, V. Singh, A.R. Rao, R.P. Singh, and P. S. Rath, Nutr. Cancer **51**, 59–67 (2005).
8. S. M. Sabir, H. Maqsood, I. Hayat, M. Q. Khan, and A. Khaliq, J. Med. Food **8**, 518–522 (2005).
9. J. S. Kim, Y. S. Kwon, Y. J. Sa, and M. J. Kim, J. Agric. Food Chem. **5** (9), 138–144 (2011).
10. Y. A. Koshelev and L.D. Ageeva, *Seabuckthorn*. (NIC BPGU, Biysk, 2004) 320 P. (in Russian).
11. T. Kukina, Materials, Methods & Technologies **11**, 63–75 (2017)
12. T. P. Kukina and V. A. Raldugin, Sib. Khim. Zh. (2), P. 92–93 (1992) (In Russian).
13. T. P. Kukina and V. A. Raldugin, "Neutral and acid lipids of extracts from sea buckthorn leaves", in *New trends in biology, chemistry and pharmacology of the sea buckthorn*, edited by V. A. Pentegova (Nauka, Sib. Div., Novosibirsk, 1991), pp. 94-98.
14. O. M. Melnikov, A. L. Vereshchagin, and Y. A. Koshelev, Himija rastitelnogo syr'ja (Chemistry of plant raw material) (2), 113–116 (2010).
15. V. L. Salenko, T. P. Kukina, V. N. Karamyshev, V. N. Sidelnikov, and V. A. Pentegova, Chemistry of Nat. Comps. **21**, 481–485 (1985).
16. N. D. Gachechiladze, "Chemical research of sea buckthorn growing on West Pamir," Ph.D. thesis, Dushanbe, 1984.
17. N. P. Goncharova and A. I. Glushenkova, Chemistry of natural Compounds **32**, 585–586 (1996).
18. M. Bao and Y. J. Lou, Cardiovasc. Pharmacol. **48**, (1), 834–841 (2006).
19. A. Gupta, The International Journal of Lower Extremity Wounds **4** 88–92 (2005).
20. J. Tang, "Effects of sea buckthorn leaf on the hepatocarcinogenesis of aflatoxin B" In *Proceedings of the International Symposium on sea buckthorn*, (Xian, China, 1989), pp. 384–386.
21. C. L. Liu, H. Q. Liu, Y. H. Deng and M. L. Liu, and Zhong Yao Cai. **29**, 151–154 (2006).
22. S. Saggu, H. M. Divekar, V. Gupta, R. C. Sawhney, P. K. Banerjee, and R. Kumar, Food and Chemical Toxicology **45**, 609–617 (2007).
23. Arun Prabhu R. et al. Review on Curative assets of Sea buckthorn. Journal of Pharmacy Research **4** (1), 164–166 (2011).
24. C. Grey, C. Widen, P. Adlercreutz, K. Rumpune, and R.-D. Duan, Food Chemistry **20** (1), 1004–1010 (2010).
25. T. P. Kukina, "Isoprenoids of sea buckthorn leaves", Ph.D. thesis, Novosibirsk, 1992.
26. T. P. Kukina, D. N. Shcherbakov, K. V. Gensh, E. A. Tulysheva, O. I. Salnikova, A. E. Grazhdannikov, E. A. Kolosova, Russian Journal of Bioorganic Chemistry **43** (7), 747–751 (2017).