Determination of Hydrophilic Bioactive Substances in Liquid and Dry Extracts of Sea Buckthorn

A. Chernova[1], A. Davydkina[1,2*], A. Gurenkova[1], A. Bondarenko[1,2], and M. Alekseeva[1]

***Abstract*—**HPLC-UV was used to quantify the content of hydrophilic bioactive substances (serotonin, flavonoids) in liquid and dry extracts of non-fruiting parts of sea buckthorn (leaves, twigs and bark). It has been established that the studied aqueous extracts of sea buckthorn contain up to 14% (wt.) of serotonin in terms of dry matter, and dry extracts - up to 20% (wt.). It was found that flavonoids were found in all the studied extracts of sea buckthorn leaves and branches. The content of dihydroquercetin was found only in the extract of the branches, its content was 0.43% (wt.), Rutin and quercetin were determined in the extract of the leaves, they are contained up to 0.39% (wt.) and 1.13% (wt.), subsequently.

***Index Terms*—**Extraction, HPLC, hydrophilic bioactive substances, sea buckthorn.

I. Introduction

Sea buckthorn or *Hippophae* (lat.) is a perennial shrub belonging to the family *Elaeagnaceae* (suckers). Sea buckthorn got its Latin name (*Hippophae* - "*Hippo*" - horse, "*Phaos*" - radiance) in ancient Greece, where sick or injured horses were fed with its fruits [1]. The plant is widespread and grows mainly near sea or river coasts and in the mountainous regions of Asia (Central Asia, India, China, Tibet, Mongolia, the Caucasus, Turkey, and Russia) and Northwestern Europe [2]. Three main species of sea buckthorn are described in the literature [3]: *H. rhamnoides* — about. buckthorn-shaped, *N. salicifolia* - about. willow, *N. thibetana* - about. Tibetan. In Russia, sea buckthorn (*H. rhamnoides*) mainly grows, which is represented by such regions as the Altai Territory, the Republic of Tuva and the Republic of Buryatia. The Altai Territory ranks first among the regions of Russia in terms of cultivation - sea buckthorn plantations occupy an area of more than 3,000 hectares. In Altai, the North Caucasus and Buryatia, there are about 30 thousand hectares of wild sea buckthorn, which is grown and harvested. It takes root well on poor soils and is able to tolerate extreme temperature regimes from -40°C to +40°C [4], which subsequently affects the content of biologically active substances in it [5].

The beneficial properties of sea buckthorn were discovered in Tibet and China as early as the 9th century BC, where the berries themselves and the leaves, branches, bark and roots of the plant were used for medicinal purposes [6]. In northern countries [7], branches and bushes of sea buckthorn were collected as fuel in the 1940s. Sea buckthorn wood was used to make rake teeth in Trøndelag in Norway. In northern Sweden the bushes were used to produce yellows and browns. In addition, branches from wild shrubs were used for decorative purposes in shop windows and homes, and as hedges to keep cats out. In Germany, shrubs have long been grown for the ecological rehabilitation of degraded lands. To date, it is known [8]–[10] that each of the parts of sea buckthorn is a source of a wide range of biologically active substances and is widely used in various fields (Fig. 1), where the food and pharmaceutical industries are most significant.

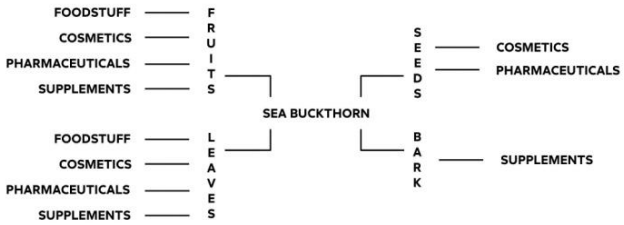

Fig. 1. Scheme of areas of application of sea buckthorn

More and more attention is paid to the study of branches and bark as a new source of biologically active substances of sea buckthorn, especially for use in medicine and the food industry [9], [11]–[12]. At the moment, it has been established that almost all parts of sea buckthorn (berries, roots, leaves, bark) contain phenolic compounds - flavonoids (for example, rutin, quercetin, kaempferol or myricetin [13]), and phenolic acids; tannins, vitamins (tocopherols, carotenoids, ascorbic acid, folic acid, vitamins B1, B2, etc.), protein, amino acids, minerals (Fe, Ca, P and K) [14]–[17], and others [18]–[19]. The fruits of sea buckthorn have found the greatest use, from which cosmetic, pharmaceutical and food products are produced, as well as sports and functional nutrition products. Thus, the study of the influence of technological modes of production of dry and liquid extracts of potentially significant biologically active substances for dietary supplements, functional nutrition is

Manuscript received March 28, 2023. This work was supported in part by the Foundation for Assistance to Small Innovative Enterprises in Science and Technology under Grant 377ГССС15-L/78594.

[1]National Research Tomsk Polytechnic University (TPU), 634050 Tomsk, Russia

[2] Institute of Monitoring of Climatic and Ecological Systems of the Siberian Branch of the Russian Academy of Sciences (IMCES), 634055 Tomsk, Russia.

relevant. The purpose of the work is to study the influence of the extraction and drying regime on the yield of hydrophilic biologically active substances.

II. Methods and Materials

A. Object

The object of the study was vegetable non-fruit raw materials from the Altai Territory harvested in autumn (October) 2021 year: samples of crushed branches and bark, and leaves of sea buckthorn no more than 1.4 mm in size, as well as a dry extract of sea buckthorn bark after CO_2 extraction.

B. Sample Preparation

The samples are represented by 1-times, 2-times, 3-times and 4-times extraction by maceration under dry matter conditions to an extractant of 1:100 for 40 minutes at 25°C. Purified water was used as an extraction agent. The scheme for obtaining a dry extract from the non-fruiting part of sea buckthorn is shown in Fig. 2. To obtain a dry extract, liquid extracts were used after 3-times extraction, which were dried using vacuum freeze dryer Christ Epsilon 2-6D LSCplus lyophilizer (Germany). For testing, dry extracts of sea buckthorn are diluted with purified water in a ratio of 1:10.

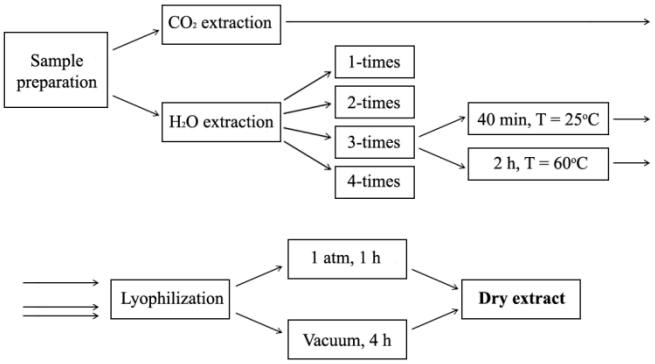

Fig. 2. Scheme for obtaining dry extracts from the non-fruiting part of sea buckthorn

C. Determination of Serotonin in Extracts

The quantitative content of serotonin in objects was determined by HPLC on the microcolumn chromatography "Milichrome A-02" (Russia) with UV detection on a column C18 with a reagent phase in the water (H_2O) : acetonitrile (ACN) system in a ratio of 8:2 in isocratic mode [20]. As standard serotonin chloride (98%, Sigma-Aldrich) was used. For analysis, dry extracts were dissolved in purified water at a concentration of 1:1000. Serotonin detection was carried out at wavelengths of 260-300 nm. For quantitative analysis of serotonin, a series of standard solutions of serotonin of various concentrations mg/cm^3 was preliminarily prepared: 1.0; 0.9; 0.8; 0.7; 0.6; 0.5; 0.4; 0.3; 0.2. Serotonin was identified by retention time Tr = 1.20÷1.23 min. The peak area was processed in the AlphaSpectrum 1.3 program.

D. Determination of Flavonoids in Extracts

The quantitative content of flavonoids in plant materials was carried out by HPLC on the microcolumn chromatography "Milichrome A-02" (Russia) with an C18 column was used for analysis. The mobile phase was the mixture with eluate A, was H_2O and 0.1% (wt.) aqueous solution of H_3PO_4, and eluate B, ACN with an eluates in the A:B system in a ratio of 75%:25% in isocratic mode. UV detection was performed at three wavelengths: rutin at λ = 360 nm, quercetin at 290 nm, and dihydroquercetin at 254 nm [21]. As standard rutin, quercetin and dihydroquercetin (>95%, Sigma-Aldrich) were used. For quantitative analysis of flavonoids, a series of standard solutions of rutin, quercetin, and dihydroquercetin of various concentrations mg/cm^3 was preliminarily prepared: 0.1; 0.05; 0.025; 0.013; 0.003. Chromatography of a series of solutions was carried out under the conditions described above. Flavonoids were identified by retention time Tr (rutin) = 2.67 min, Tr (quercetin) = 2.98 min, Tr (dihydroquercetin) = 3.48 min. The peak area was processed in the AlphaSpectrum 1.3 program.

E. Determination of Tannins in Extracts

Determination of tannins in the studied extracts was carried out by the titrimetric method [22]. A solution of indigo sulfonic acid was added to a liquid or diluted dry extract in bidistilled water, and then the resulting mixture was titrated with constant stirring with a solution of potassium permanganate until a golden yellow color was obtained.

III. Results and Discussion

It is known that the yield of biologically active substances (BAS) is affected by the anatomy and timing of plant harvesting, extraction modes, and the nature of the solvent. We used unprocessed sea buckthorn raw materials (branches with leaves) after separating berries with preliminary freezing to -30°C, collected in the fall of 2021. According to the literature, sea buckthorn bark, which is harvested in autumn, should contain serotonin [23]. On the other hand, the leaves have been shown to be a source of flavonoids, tannins with antioxidant and anti-inflammatory effects. Since the studied biologically active substances are hydrophilic, the paper considers 2 types of extraction using water and liquefied carbonic acid. Thus, in the work, the influence of the extraction and drying regime on the content of valuable hydrophilic biologically active substances was investigated. When studying the complex of hydrophilic substances of the bark and shoots of sea buckthorn, special attention was paid to those substances that can make a certain contribution to the implementation of the gastroprotective action: flavonoids and tannins. Besides, due to the high physiological activity of substances of alkaloid nature, namely, serotonin was conducted. In the work [24] was found that there are flavonoids of flavone and flavonol groups and catechin in a heap of sea buckthorn by thin-layer chromatography. Using spectrophotometry, it was found that the total amount of flavonoids in aqueous extracts in terms of quercetin was 2.7% (leaves) and 3.07% (branches and bark) [25]. In this study, the individual flavonoids (quercetin, dihydroquercetin and rutin)

were separated, and their quantitative content was determined by HPLC methods. The examples of the obtained chromatograms of dihydroquercetin (Fig. 3) and serotonin (Fig. 4) for aqueous extract branches and bark of sea buckthorn are shown.

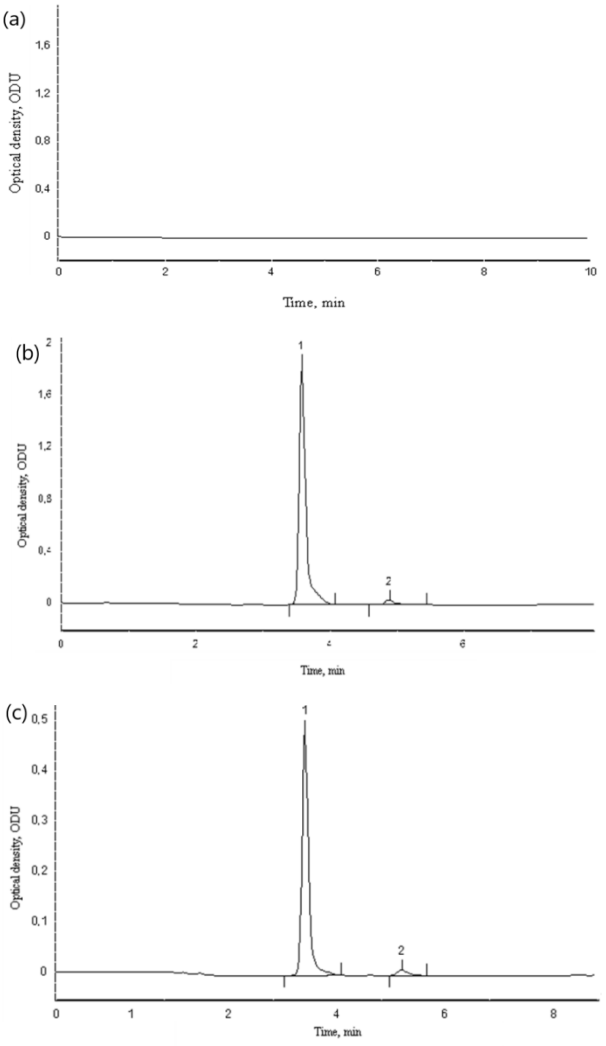

Fig. 3. Chromatograms of dihydroquercetin by 254 nm, where 1 - Tr (dihydroquercetin) = 3.48 min at 25ºC: (a) solvent (H_2O); (b) standard of dihydroquercetin; (c) dried aqueous extract of sea buckthorn branches and bark (2 h extraction).

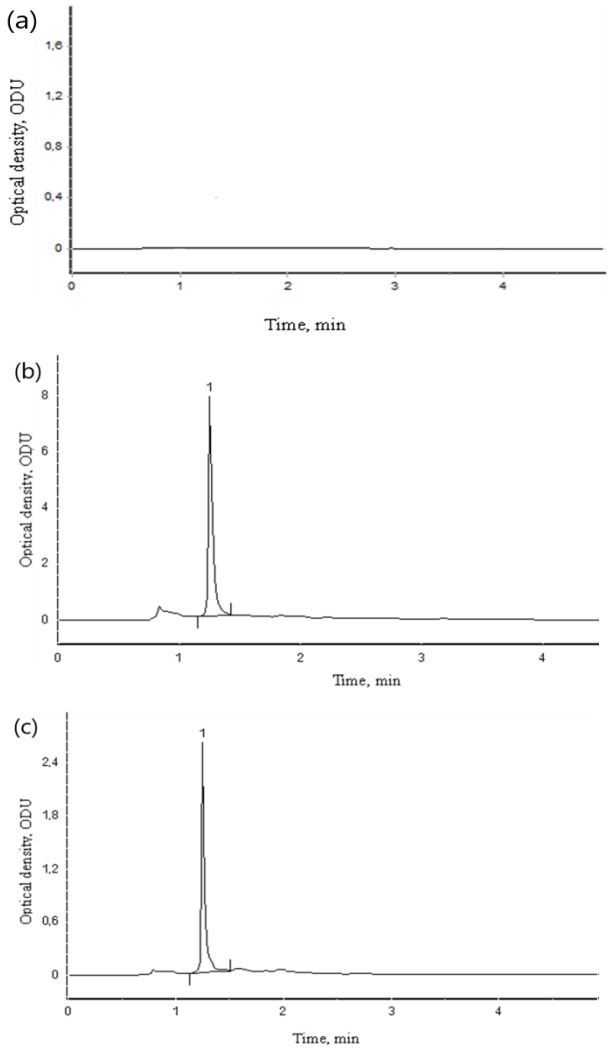

Fig. 4. Chromatograms of serotonin by 280 nm, where 1 - Tr (serotonin) = 1.23 min at 25ºC: (a) solvent (H_2O); (b) standard of serotonin; (c) aqueous 3-times extract of branches and bark.

To obtain a dry extract, liquid extracts were dried using a lyophilizer in two modes: liquid CO_2 extract was dried at 1 atm for an hour and aqueous extracts were dried at vacuum for 4 hours. For the analysis of biologically active substances in dry extracts, they were diluted with purified water. The modes for obtaining liquid and dry extracts and the content of yield hydrophilic substances from non-fruits parts of sea buckthorn are presented in Tables I-III.

TABLE I: THE QUANTITATIVE CONTENT OF SEVERAL FLAVONOIDS IN EXTRACTS OF SEA BUCKTHORN HEAP, %

Extract	Raw material	Extraction time	Rutin	Dihydro-quercetin	Quercetin
Liquid	Leaves	40 min	0.17±0.02	-	0.40±0.01
		2 h	0.37±0.04	-	1.13±0.03
	Branches and bark	40 min	0.13±0.01	0.43±0.06	-
		2 h	0.15±0.02	-	0.6±0.01
Dry	Leaves	40 min	0.33±0.03	-	0.51±0.03
		2 h	0.46±0.02	0.47±0.03	1.20±0.01
	Branches and bark	40 min	0.23±0.01	0.31±0.02	-
		2 h	0.25±0.01	0.38±0.03	0.64±0.02

TABLE II: THE QUANTITATIVE CONTENT OF TANNINS IN EXTRACTS OF SEA BUCKTHORN HEAP, %

Extract	Raw material	Extraction time	Tannins
Liquid	Leaves	40 min	1.8±0.6
		2 h	4.0±0.6
	Branches	40 min	4.5±0.6
	and bark	2 h	7.9±1.2
Dry	Leaves	40 min	5.8±1.1
		2 h	10.4±1.0
	Branches	40 min	16.8±1.2
	and bark	2 h	25.6±1.0

TABLE III: THE QUANTITATIVE CONTENT OF SEROTONIN IN EXTRACTS OF SEA BUCKTHORN HEAP, %

Extract	Raw material	The multiplicity of extraction	Serotonin
Liquid	Leaves	1-times	3.0±0.2
		2-times	3.1±0.2
		3-times	3.2±0.2
		4-times	3.8±0.3
	Branches	1-times	4.6±0.4
	and bark	2-times	10.6±0.8
		3-times	13.9±0.8
		4-times	10.9±0.7
Dry	Leaves	3-times	5.4±0.3
	Branches and bark	3-times	20.1±0.9
	Bark extract after CO_2 extraction	-	10.3±0.8

According to these results, flavonoids (Table I) were found in all the studied extracts of sea buckthorn leaves and branches. However, dihydroquercetin was found only in the extract of branches (extraction for 40 min at room temperature), its content was 0.43% (wt. dry raw material). This may be due to the morphology of the feedstock and the extraction temperature. Leaf extract (extraction for 2 hours at 60°C) contains the largest amount of flavonoids, % (wt.) to dry raw materials: rutin - 0.37 and quercetin - 1.13. Also, serotonin was found in all analyzed samples (Table II). It is shown that the highest extraction of serotonin is observed in a 3-times liquid extract of a mixture of bark and branches, which is approximately 14% (wt.). For liquid extracts from the bark and branches of sea buckthorn, a more significant increase in the content of serotonin with an increase in the multiplicity of extraction is observed, while in the case of using leaves, it is practically absent. The content of serotonin in water extracts of leaves is 1.5-4 times less than in branches and bark. Among dry extracts, the content of serotonin in the CO_2 extract of the bark and branches was 10.3% (wt.), which is comparable to the content of serotonin in liquid extracts of 2-times and 4-times extraction of a mixture of branches and leaves. When drying aqueous extracts, an increase in the content of serotonin by about 1.5 times was observed, it amounted to approximately 20% (wt.). The highest content of tannins is observed in dry extracts during extraction for 2 hours at 60°C with a predominance of tannins in the extract of the branches (Table III). The lowest content of tannins was obtained for the leaf extract (extraction for 40 min at room temperature).

IV. CONCLUSION

It has been established that non-fruit sea buckthorn is a raw material for obtaining valuable substances: alkaloids - serotonin, flavonoids - rutin, quercetin, dihydroquercetin, tannins. Differences in the content of biologically active substances in sea buckthorn are shown depending on the method of extraction. It was revealed that the raw material of sea buckthorn (branches, bark, leaves) after the separation of the berries is a potentially suitable raw material for the isolation of biologically active substances. Also, this raw material can be used for the production of food additives and functional nutrition.

ACKNOWLEDGMENT

The authors are grateful to the center of TPU's "Physical and chemical methods of analysis".

REFERENCES

[1] T. Michel, E. Destandau, G. Le Floch, M. E. Lucchesi, and C. Elfakir, "Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (*Hippophaë rhamnoides L.*) leaf, stem, root and seed," *Food chemistry*, vol. 131, no. 3, pp. 754-760, Oct. 2012. https://doi.org/10.1016/j.foodchem.2011.09.029

[2] T. S. C. Li and T. H. J. Beveridge, *Sea buckthorn (Hippophae rhamnoides L.): production and utilization*, Ottawa, Canada: National Research Council, 2003, 133 p.

[3] A. Yu. Kulenkamp. (May 2022). Sea buckthorn, *Big Russian Encyclopedia*. [Online]. Available: https://bigenc.ru/c/oblepikha-3a220e.

[4] Z. Ciesarová, M. Murkovic, K. Cejpek, F. Kreps, B. Tobolková, *et. al*, "Why is sea buckthorn (*Hippophae rhamnoides L.*) so exceptional? A review," *Food Research International*, vol. 133, pp. 109-170, Mar. 2020. https://doi.org/10.1016/j.foodres.2020.109170

[5] Y. Kanayama, K. Sato, H. Ikeda, T. Tamura, M. Nishiyama, and K. Kanahama, "Seasonal changes in abiotic stress tolerance and concentrations of tocopherol, sugar, and ascorbic acid in sea buckthorn leaves and stems," *Scientia Horticulturae*, vol. 164, pp. 232-237, Oct. 2013. https://doi.org/10.1016/j.scienta.2013.09.039

[6] G. Suryakumar and A. Gupta, "Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides L.*)," *Journal of ethnopharmacology*, vol. 138, no. 2, pp. 268-278, Nov. 2011. https://doi.org/10.1016/j.jep.2011.09.024

[7] S. Ståhlberg and I. Svanberg, "Historical and Contemporary Uses of Sea Buckthorn, *Hippophae rhamnoides L.*, in the Nordic Countries," *Journal of Northern Studies*, vol. 15, no. 2, pp. 34-46, Mar. 2021.

[8] Yu. A. Lupashko, "Features of the composition of *hippophae rhamnoides* in the nutritional aspect," *proceedings of Natural sciences and medicine: theory and practice*, pp. 11-22, Novosibirsk, Russia: Siberian academic book, 2020.

[9] I. Gradt, S. Kuhn, J. Morsel, and G. Zvaigzne, "Chemical composition of sea buckthorn leaves, branches and bark," *Proceedings of the Latvian Academy of Sciences*, vol. 3, pp. 211-216, Jan. 2017. https://doi.org/10.1515/prolas-2017-0035

[10] A. Jastrząb and E. Skrzydlewska, "Composition and biomedical relevance of sea buckthorn," *Acta Poloniae Pharmaceutica-Drug Research*, vol. 76, no. 6, pp. 959-969, Dec. 2019. https://doi.org/10.32383/appdr/111181

[11] K. Tkacz, A. Wojdyło, I. P. Turkiewicz, and P. Nowicka, "Triterpenoids, phenolic compounds, macro-and microelements in anatomical parts of sea buckthorn (*Hippophaë rhamnoides L.*) berries, branches and leaves," *Journal of Food Composition and Analy*sis, vol. 103, pp. 104-107, Oct. 2021. https://doi.org/10.1016/j.jfca.2021.104107

[12] Y. Tian, A. Puganen, H. L. Alakomi, A. Uusitupa, M. Saarela, and B. Yang, "Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants," *Food research international*, vol. 106, pp. 291-303, Apr. 2018. https://doi.org/10.1016/j.foodres.2017.12.071

[13] E. Christaki, "*Hippophae rhamnoides L.* (Sea Buckthorn): a potential source of nutraceuticals," *Food Public Health*, vol. 2, no. 3, pp. 69-72, May 2012

https://doi.org/10.5923/j.fph.20120203.02
[14] T. Beveridge, T. S. Li, B. D. Oomah, and A. Smith “Sea buckthorn products: manufacture and composition,” *Journal of agricultural and food chemistry*, vol. 47, no. 9, pp. 3480-3488, Sep. 1999. https://doi.org/10.1021/jf981331m
[15] N. P. Bekker and A. I. Glushenkova, “Components of certain species of the Elaeagnaceae family,” C*hemistry of natural compounds*, vol. 37, no. 2, pp. 97-116, Mar. 2001. https://doi.org/10.1023/A:1012395332284
[16] A. Zeb, “Chemical and nutritional constituents of sea buckthorn juice,” *Pakistan Journal of Nutrition*, vol. 3, no. 2, pp. 99-106, Feb. 2004. https://doi.org/10.3923/pjn.2004.99.106
[17] P. Malinowska and B. Olas, “Sea buckthorn – valuable plant for health,” *Kosmos*, vol. 2, pp. 285-292, Mar. 2016.
[18] R. Arimboor and C. Arumughan, “Effect of polymerization on antioxidant and xanthine oxidase inhibitory potential of sea buckthorn (*H. rhamnoides*) proanthocyanidins,” *Journal of food science*, vol. 77, no. 10, pp. C1036-C1041, Oct. 2012. https://doi.org/10.1111/j.1750-3841.2012.02884.x
[19] T. Fatima, A. Nazir, B. Naseer, and S. Z. Hussain, “Seabuckthorn (*Hippophae rhamnoides*): A repository of phytochemicals,” *International Journal of Pharmaceutical Science and Research*, vol. 3, pp. 9-12, Sep. 2018.
[20] D. A. Rychkov and E. V. Boldyreva, “Method of obtaining powder preparations containing serotonin from non-fruit parts of sea buckthorn,” Russia Patent 2533818, November 20, 2014.
[21] N. A. Vernikovskaya, “Chromatographic determination of phenolic compounds of flavonoids in medicinal plants,” Ph.D. dissertation, Dept. Anal. Chem., Kuban State Univ., Krasnodar, Russia, 2011.
[22] *State Pharmacopoeia of the Russian Federation*, XIV ed., vol. 2, Ministry of Health of the Russian Federation, Moscow, Russia, 2018.
[23] G. U. Galitsyn, V. K. Kreymer, O. I. Lomovsky, I.O. Lomovsky, “Seabuckthorn as a source of Serotonin,” *Cortex*, vol. 1, pp. 3-6, May 2014.
[24] A. A. Gurenkova, “Determination of the content of hydrophilic biologically active compounds in the bark and shoots of buckthorn buckthorn,” *proceedings of Chemistry and Chemical Technology in the XXI century*, p. 426, vol. 1, Tomsk, Russia: TPU Press, 2022.
[25] A. A. Gurenkova, “Determination of the content of hydrophilic biologically active compounds in the leaves and branches of buckthorn buckthorn,” B.S. thesis, Dept. Nat. Resources, Tomsk Polytech. Univ., Tomsk, Russia, 2022.

A. Chernova was born in Taiga, Kemerovo region, Russia, February 4, 1984. A. Chernova has education in the specialties: Chemistry-ecology, Tomsk State University, Faculty of Chemistry, Department of Analytical Chemistry, Russia, 2001; Translator in the field of professional communications (English), Tomsk State University, Faculty of Foreign Languages and Additional Professional Education, Russia, 2006; PhD Chemistry, analytical chemistry, Tomsk Polytechnic University, Department of the National Research Engineering, Russia, 2011; Master’s degree in “Chemical technology”, Tomsk Polytechnic University, Department of the National Research Engineering, Russia, 2014; specialty: Microbiology, St. Petersburg Institute of Management and Food Technologies, Faculty of professional retraining, Russia, 2022. SCOPUS AuthorID: 35806801900, Web of Science Researcher ID: AAN-5987-2020; Elibrary AuthorID: 607523; Elibrary SPIN-8646-5484, ORCID: https://orcid.org/0000-0001-7002-492X.

She has the work experiments in the next fields: specialist of the center for drug development based on the MAB CJSC "R-Farm", Russia, Yaroslavl, 2014; associate Professor, Department of the National Research Engineering, Tomsk Polytechnic University, Russia, Tomsk, 2011- until now. She delivers the following disciplines: "Biotechnological methods for the synthesis of chemical and pharmaceutical preparations", "Biotechnological methods for creating producers of biologically active compounds", "Technology of finished dosage forms", "Advanced training in English", "Molecular bases of the action of drugs", "Surface phenomena of dispersed systems. In total, more than 84 scientific and educational works have been published, (number of works) including over the past 5 years – 72. Total publications in Scopus – 12 (h = 2), WoS – 2 (h = 2). The scientific interest is biotechnology, ecology, toxicology, analytical chemistry. The samples the significant articles at the last years are presented: Molecular Plasmonic Silver Forests for the Photocatalytic-Driven Sensing Platforms Fatkullin, M., Rodriguez, R.D., Petrov, I., Jia, X., Sheremet, . // Nanomaterials, 2023, 13(5), 923, Photoinduced flexible graphene/polymer nanocomposites: Design, formation mechanism, and properties engineering Lipovka, A., Petrov I., Fatkullin, M., ... Rodriguez, R.D., Sheremet, E. // Carbonthis link is disabled, 2022, 194, pp. 154–161, Influence of Lithium Salt on Escherichia coli Growth and Viability Chernova A., Pukhniarskaia D., Biryukov, M., Plotnikov E. Industrial Biotechnologythis link is disabled, 2022, 18(1), pp. 32–37, Propofol: use, toxicology and assay features Chernova, A.P., Shormanov, V.K., Davydkina, A.E. // Sudebno-Meditsinskaya Ekspertizathis link is disabled, 2022, 65(5), pp. 46–51.
Dr. Chernova.

A. Davydkina was born in Tomsk, Russia, May 8, 1998. A. Davydkina received a bachelor's degree “Chemical Technology”, Tomsk State Polytechnic University, Department of Natural Resources, Russia, 2020; master's degree in "Chemical Technology", Tomsk State Polytechnic University, Department of Natural Resources, Russia, 2022. The master's work was devoted to the determination of extractable substances (serotonin and tryptophan) in water extracts of sea buckthorn.
ORCID: https://orcid.org/0000-0003-3905-6370.
She works at the Institute of Monitoring of Climatic and Ecological Systems of the Siberian Branch of the Russian Academy of Sciences (IMCES SB RAS).
Ms. Davydkina.

A. Gurenkova was born in Tomsk, Russia, October 21, 2000. A. Gurenkova received a bachelor's degree “Chemical Technology”, Tomsk State Polytechnic University, Department of Natural Resources, Russia, 2022. The bachelor's work was devoted to the determination of hydrophilic biologically active compounds such as flavonoids, tannins and alkaloids in a pile of sea buckthorn.
ORCID: https://orcid.org/0009-0004-8631-7945.
She has experience in developing a business plan and participating in a startup group project. Proficiency in such methods as UV spectrometry, HPLC, thin-layer chromatography.
Ms. Gurenkova.

A. Bondarenko was born in Tomsk, Russia, January 26, 2000. A. Bondarenko received a bachelor's degree “Chemical Technology”, Tomsk State Polytechnic University, Department of Natural Resources, Russia, 2022. The bachelor's work was devoted to the determination of antioxidant activity of plant raw materials.
ORCID: https://orcid.org/0009-0001-6659-0520.
She works at the Institute of Monitoring of Climatic and Ecological Systems of the Siberian Branch of the Russian Academy of Sciences (IMCES SB RAS).
Ms. Bondarenko.

M. Alekseeva was born in Kolpashevo, Tomsk region, Russia, July 3, 1992. M. Alekseeva received a master's degree “Biotechnology”, Institute of High-Tech Physics, Tomsk State Polytechnic University, Russia, 2016.
ORCID: https://orcid.org/0009-0004-4694-9729.
She has experience working in biopharmaceutical companies. She was a researcher in the analytical support group at Biocad, developing analytical techniques for anticancer and autoimmune drugs, 2017-2020. She gained experience in the department of biotechnological control of vaccines at the company Microgen, 2020. She has experience with digital technologies in analytical chemistry (Laboratory Information System Chemist Analytical Software) 2021. She is an engineer in the Department of Chemical Engineering at Tomsk Polytechnic University. She is a PhD student/engineer in the Department of Chemical Engineering, National Research Tomsk Polytechnic University, Russia.
Ms. Alekseeva.