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Polyphenolic Compounds and Antioxidant Activity of Sea Buckthorn (*Hippophae rhamnoides* L.)

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ABSTRACT

The fruits of the Sea buckthorn (*Hippophae rhamnoides* L.) are a popular plant food and a valuable health product. Cultivating of plants produces a lot of leaves with fine branches as an unnecessary residue, which need valorization. The aim of the study was to estimate (by HPLC-MS/MS) the qualitative and quantitative content of polyphenolic compounds in different parts of *H. rhamnoides* (HR), and to determine the level of antioxidant activity of leaves and fruits (by DPPH, ABTS methods and ferrozine test). Up to 19 compounds were identified in different parts of HR. The fruits are rich in flavonoids, including various glycosides of flavonols isorhamnetin, quercetin, and kaempferol. Two isorhamnetin glycosides were not identified in the leaves, while isorhamnetin-3-rhamnosylglactosides and the ellagitannins hippophaenin B, stachyurin and casuarinin were present only in the leaves of the plant. The bark and roots contained considerably more catechins, but minimal flavonols than the fruits and leaves of HR. The total phenolics and flavonols were most abundant in water infusions from leaves of HR (especially quercetin-3-O-glucoside-7-O-rhamnoside), compared to methanolic extracts. No significant differences in the quantitative and qualitative content of the fresh and dried leaves were detected. The highest antioxidant activity by all three methods was detected in the leaves of HR. In addition to the fruits, the leaves may have a perspective as a source of biologically active substances of HR.

KEYWORDS

Bark; flavonols; fruits; leaves; phenols; roots

1 Introduction

Hippophae rhamnoides L. (family–*Elaeagnaceae*, common name–Sea buckthorn) is a flowering shrub native to cold temperate regions of Eurasia. Berries, seeds, and leaves of the plant are widely used as a folk medicine to treat hypertension, oedema, inflammation, tissue-regeneration, skin grafts, burns/injury, wounds, and ulcers. Berries are the most prominent feature of the plant [1].



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The chemical composition and biological activities of *H. rhamnoides* (*HR*) have been studied by many authors [1–37]. Wide variety of secondary metabolites, including phenolics–quercetin, kaempferol, chlorogenic acid, catechins, isorhamnetin, myricetin; carotenoids–lutein, carotene, zeaxanthin, lycopene, tocopherol; tannins, ursolic acid, hippophae cerebroside, cirsiumaldehyde, oleanolic acid, dolichoic acid, 5-hydroxymethyl-2-furancarboxaldehyde, palmitic acid, 19-alpha-hydroxyursolic acid, 1-o-hexadecanolenin, octacosanoic acid, linolenic acid (omega-3), linoleic acid (omega-6), palmitoleic acid (omega-7), oleic acid (omega-9), palmitic acid and phytosterols, etc., have been isolated from the plant. About 190 bioactive compounds were identified in its raw materials [1–5]. This wide range of active ingredients present in *HR* has been used effectively in the cosmetics industry as well as in medicine. The most studied product of *HR* is its oils [4], dried fruit pulp and berry residues yielding 8%–20%, 20%–25% and 15%–20% of oil, respectively [6]. The valuable substances present in the oil of *HR* are responsible for healthy and beautiful skin as well as for the proper functioning of the human body [1,7].

The whole plant of *HR*–berries, roots, leaves, stems and branches–contain various kinds of phenolics, including flavonoids, hydrolysable tannins and phenolic acids [24,25]. The leaves of the plant contain nutrients and bioactive components including various phenolic components–flavonols such as quercetin, kaempferol and isorhamnetin (mostly in glycosidic forms), flavons-3-ols such as various catechins (epicatechin, gallicocatechin, epicatechin, epigallocatechin), phenolic acids like gallic, ferulic, and ellagic acids, procyanidins, leucoanthocyanidins [8,9], but also amino acids such as 0.13% methionine, 0.73% lysine, as well as 0.13% cysteine, 20.7% proteins [10]; folic acids, isoprenols, esterified sterols, triterpenols [11], vitamin E and minerals like K, Ca and Mg [8,11]. The fresh leaves of *HR* are a rich source of chlorophyll (98.8 mg/100 g), which is an indicator of the excellent trait of green vegetables while carotenoids concentration is 26.3 mg/100 g. Hydrolysable ellagitannins and gallotannins of monomeric type like casuarictin, strictinin, casuarinin, isostrictinin, isorhamnetin, quercetin-3-glucoside, quercetin-3-*O*-galactoside, and kaempferol in leaf extracts of the plant were also identified [9].

The pharmacological studies have demonstrated *HR* to exhibit antibacterial, anti-sebum, antifungal, anti-psoriasis, anti-atopic dermatitis, and wound healing activities. Besides, sea buckthorn has also been included in various cosmeceuticals for its use in skin-eventone, smoothening, rejuvenation, removal of wrinkles, scars, and pigmentation, and in hair related problems [1].

Various compounds found in *HR* raw materials (including vitamins, phenolic compounds, and lipids) play an important role in modifying the progression of diseases associated with oxidative stress, i.e., cancer, neurological and cardiovascular diseases [12]. Moreover, the oxidative stress in blood platelets or plasma may promote the development of cardiovascular diseases, neurological diseases, and cancer. Previous experiments showed that the polyphenolic fraction of sea buckthorn fruits, dominated by flavonoids, acts as an antioxidant and antiplatelet aggregation agent [13–15]. Thus, other parts of sea buckthorn that can act as an antioxidant are also interesting, and it is advisable to study their antioxidant activity for complex using.

The authors of the review [17] concluded that the berries, leaves and bark of *HR* are rich in many bioactive substances valuable for nutritional and health-promoting properties. Leaves of the plant are occasionally used for tea-type infusion because they are rich in nutrients, macro- and microelements. Šne et al. [16] studied total phenolic compounds in *HR* leaves, shoots, berries, and buds, but not in bark and roots. The leaves from female plants contained the highest total phenolics concentration and antioxidant activity, and less valuable were the shoots from male trees. Michel et al. [37] have been performed the antimicrobial, antioxidant and phytochemical investigations (by HPTLC-method) of *HR* leaves, stems, roots and seeds. The content of polyphenols in the fruits and leaves of *HR* has been studied by HPLC-method in several studies [1,6,8,17,18,20,21,23–27,34] but not performed yet in all parts of the plant.

The aim of the study was to estimate the qualitative and quantitative content of main polyphenolic compounds in fruits, leaves, bark and roots of *HR*. The other focus of this research was determination of the antioxidant activity of leaves and fruits (fresh/dried) extracts of *HR* employing DPPH, ABTS methods and Ferrozine test for iron chelating capacity.

To the best of our knowledge, this paper is the first to systematically investigate the content of phenolic compounds in fruits, leaves, bark and roots of *HR* in details using LC-MS/MS-method, and to compare the composition of fresh and dried leaves extracted by different technologies.

2 Materials and Methods

2.1 Plant Material

Sea buckthorn fruits and leaves (with thin stems) (*HR* 'Botanicheskaya') used in the study were obtained from Mr. Lauri Andressel, a board member of the company Hiiu Astelpaju OÜ, from the company's Hiiumaa plantation harvested in October 2018. During harvesting, the branches with bunches of fruit were cut from sea buckthorn bushes and frozen. Then the fruits were removed from the frozen branches and the remaining parts crushed, resulting in a mass of shredded leaves and thin branches with individual fruits separated from the plant material (hereinafter: leaves). Fresh fruits and leaves (about a 100 g of each) were stored in a refrigerator (-18°C) until analysis. The leaves were dried at room temperature for 7 days for comparative analyses (samples 1–8).

For comparance, parts of *HR* (fruits, leaves, bark and roots) were also collected in September 2022 from the forest by the Lilleoru road in Viimsi municipality, Harju county, Estonia. The research material was collected from one bush, from which the branches were cut, the bark was separated with a knife, and the fruits and leaves were removed by hand, while collecting the roots, the finer roots were cut off. The collected plant parts were stored fresh in a freezer at -18°C until the experiments started (samples 9–12).

The Moldavian samples (13–15) were obtained from plants *HR*, cultivated in the collection of the Scientific Center of Medicinal Plants Cultivation of Nicolae Testemitanu State Medical and Pharmaceutical University in October, 2017. Leaves and fruits were harvested during the fruiting stage. Plant materials (leaves and fruits) were divided into 2 parts. One part was dried at room temperature (25°C) inside the laboratory room, in the dark, weighed, and then extracted, the second part was used fresh.

2.2 Sample Preparation

Samples 1–8 were extracted from plant material collected in 2018 and samples 9–12 in 2022. Samples 1 and 2: A pre-weighed amount of fresh (1) and dried (2) leaves were crushed in a coffee grinder for 10 s and sieved on a 3 mm mesh sieve. The mass remaining on the sieve was further ground in a coffee grinder for 10 s and sieved again on a 3 mm sieve. Methanol was added to the ground mass in a ratio of 1:10, and the sample was allowed to macerate for 24 h and filtered through a paper filter. Sample 3: Fresh fruits were weighed approximately 1 g, the fruits were crushed with a glass rod, but the seeds in the fruits were left intact. Methanol was added in a ratio of 1:10, allowed to macerate for 24 h and filtered through a paper filter. Experiments with dried fruits were not performed, because drying sea buckthorn fruits is very complicated.

Samples 4–7: Approximately 2 g of unground fresh (samples 4 and 5) and dried (samples 6 and 7) leaves, corresponding to one tablespoon, were taken to prepare aqueous extracts. One part of the samples (samples 4 and 6) was poured with 200 ml of boiling water and left to infuse for ten minutes, the other part (samples 5 and 7) was placed on a sieve and poured with 200 ml of boiling water, and filtered through a paper filter. Sample 8: Approximately one tablespoon of fruit was weighed to prepare fruit extract. The fruits were crushed with a glass rod, but the seeds inside the fruit were left intact. 200 ml of boiling water was added to the sample and left to infuse for 10 min. The sample was paper filtered and

centrifuged at ~7000 rpm for ten minutes. After filtration, samples 1–8 were centrifuged with a “Centrifuge type MPW-310” at approximately 7000 rpm for 10 min.

Samples 9–12: were extracted as samples 3 (fruits) and 1 (leaves, bark, roots). In addition, before the analyses, the roots, leaves and bark were cut into ~1 cm long pieces with scissors and then ground with a coffee grinder for 10 s. The seeds of the fruits were preserved intact by breaking only the outer skin of the fruits.

The samples 13–15 of fresh or dried leaves and fresh fruits of *HR* (10–15 g) for study of antioxidant activity were extracted with 60% aqueous ethanol at room temperature. After 30 min of constant shaking, the extracts were filtered through Whatman no. 2 filter paper by vacuum suction, using Buchner funnel. The procedure was repeated 6 times. The combined extracts were evaporated to dryness under reduced pressure at 40°C and stored at –4°C until analysis.

2.3 HPLC-DAD-MS/MS Analysis and Quantification

The compounds were identified and quantified by liquid chromatography-mass spectrometry (LC-MS/MS). Samples 1–8 were analysed using an 1100 series LC/MSD Trap-XCT (ion trap) detector (Agilent Technologies, Palo Alto, USA) with electrospray ionisation in negative mode. The MS and MS/MS chromatograms were obtained in the range of 50–1000 atomic mass units (amu); target mass was set to 400 amu; number of fragment ions, 2; maximum ion collection time, 100 ms; compound stability, 100%; drying gas, nitrogen from the generator; collision gas, helium. HPLC 2D ChemStation software was used for process control and initial processing of the results. Samples 9–12 were analysed on a 1290 Infinity system (Agilent Technologies, Waldbronn, Germany), coupled to an Agilent 6450 Q-ToF mass spectrometer equipped with a Jetstream ESI source working in negative ionisation mode. Data acquisition and initial data processing were conducted using the MassHunter software (Agilent Technologies). The constituents were separated on a reversed-phase column (Zorbax 300SB-C18, 2.1 mm × 150 mm; 5 µm; Agilent Technologies) using a stepwise mobile phase gradient (component A-0.1% formic acid and component B-acetonitrile). For samples 1–8 the flow rate was set to 0.3 ml/min, column temperature was 35°C and sample injection volume 5 µl. For samples 9–12, the flow rate was 0.4 ml/min, column temperature was 40°C and injection volume 1 µl. Substances were identified based on MS/MS fragmentation spectra, retention times, standard compounds, and literature data [38–40].

2.4 Determination of Antioxidant Activity by DPPH Method

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of the extracts. DPPH is a free radical which at room temperature produces violet colour in ethanol what is reduced in the presence of an antioxidant molecule, to produce a colourless solution. The use of DPPH provides an easy and rapid way to evaluate antioxidants-radical scavengers. Sample stock solutions (1 mg/ml) were diluted to final concentration of 200, 100, 50, 25, 10, 5 and 1 µg/ml in methanol. Different volumes of each extract were added to 0.75 ml of methanolic solution of DPPH (1.5 ml, 20 mg/l). After 15 min at room temperature, the absorbance was recorded at 517 nm. Methanol was used as the blank. DPPH solution (1.5 ml, 20 mg/l) and methanol (0.75 ml) was used as the negative control. The IC₅₀ values denoting the sample concentration required to scavenge 50% of DPPH free radicals were calculated graphically [41,42].

2.5 Determination of Antioxidant Activity by ABTS Assay

The basis of the ABTS/PP assay is the interaction between an antioxidant and the pre-generated ABTS^{•+} (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation compared with antioxidant standard (Trolox) [43]. The ABTS^{•+} was generated by chemical reaction with potassium persulfate (K₂S₂O₈) Firma. For this purpose, 10 ml of ABTS^{•+} 2 mM was spiked with 0.1 ml of K₂S₂O₈ (70 mM) and allowed to stand in

darkness at room temperature for 12–16 h (the time required for formation of the radical). The working solution was prepared by taking a volume of the previous solution (1 ml) and diluting it in 24 ml of methanol until its absorbance at $\lambda = 734$ nm was 0.70 ± 0.02 . The reaction took place directly in the measuring cuvette. For this purpose, 10 μ l of sample or standard were added at 0.99 ml of ABTS⁺ solution, at which point the antioxidants present in the sample began to inhibit the radical, producing a reduction in absorbance, with a quantitative relationship between the reduction and the concentration of antioxidants present in the sample [44]. At the same time a Trolox calibration curve was prepared for a concentration range of 2.5–30 μ M and the inhibition percentage obtained for the sample was interpolated to calculate the concentration in Trolox equivalents (μ M TEAC).

2.6 Metal Chelating Activity by Ferrozine Test

The chelation of ferrous ions by extracts was estimated by method of Ma et al. [22]. Briefly, 50 μ l of 2 mM FeCl₃ was added to 60 μ l of samples (10 mg/ml). The reaction was initiated by the addition of 200 μ l of 5 mM ferrozine solution (FerroZine®Iron Reagent, Hach). The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine–Fe²⁺ complex formation was calculated as $[(A_0 - A_s) / A_s] \times 100$, where A_0 was the absorbance of the control, and A_s was the absorbance of the extract/standard. EDTA was used as a positive control.

2.7 Statistical Analysis

Data were expressed as mean of three replicates and standard deviation (SD). Statistical significance ($p < 0.05$) was evaluated by the Student's test. All analyses were performed using GraphPad Prism, version 6.01, 2012.

3 Results

3.1 Identification of Phenolic Compounds in HR

A total of 19 compounds were identified in different parts of *HR*, including 16 in fruits, 19 in leaves, 15 in bark and 10 in roots (Table 1). *HR* contains organic acids such as quinic, malic and citric acids in all its parts. In addition, the fruits also contain ascorbic acid. The fruits are particularly rich in flavonoids, including various glycosides of isorhamnetin, quercetin, and kaempferol. The leaves also contain several flavonoids found in the fruits, but two isorhamnetin glycosides were not identified in the leaves. Instead, isorhamnetin-3-rhamnosylgalactoside that was not found in other parts of *HR*, was identified in leaves. The composition of leaves differs from other parts of the plant due to the presence of catechin and ellagic acid derivatives. Ellagitannins such as hippophaenin B, stachyurin and casuarinin are characteristic compounds found in the leaves, but not in other parts of the plant. The bark of *HR* is rich in various derivatives of gallic acid and catechin, like procyanidin dimers and trimers. In contrast, roots contain the fewest phenolic components and have minimal phytochemical similarity to the other parts of the plant.

3.2 Total Polyphenols and Flavonols in Extracts of *H. rhamnoides*

The highest total polyphenols and the lowest total flavonols (1084.5 and 0.8 mg/100 g, respectively) are contained in the methanolic extract of *H. rhamnoides* roots, followed by the bark (859.5 and 0.8 mg/100 g, Fig. 1). The amounts of polyphenols and flavonols in the samples 9 and 10 was equal, but in the sample 1 flavonols were half as much as other polyphenols (77.6 and 148.9 mg/100 g, respectively). The best results regarding the content of total polyphenols and flavonols are obtained from the aqueous extract of fresh and dried leaves with a maceration time of 10 min (418.5–421.4 and 285.0–287.3 mg/100 g). Both total polyphenols and flavonols are extracted better with water than with methanol. The aqueous extract of fresh leaves contains more polyphenols and flavonols than fresh fruits (1.8 and 1.7 times, respectively). Thus, in addition to the fruits, the antioxidant activity of the leaves of *HR* is also promising.

Table 1: Phenolic compounds identified in different parts of *H. rhamnoides*

t_R^*	[M-H] ⁻	Compounds	Fruits	Leaves	Bark	Roots
1.01	191	Quinic acid	+	+	+	+
1.05	439	Syringic acid sulfoglucopyranoside	-	-	+	+
1.06	175	Ascorbic acid	+	-	-	-
1.1	133	Malic acid	+	+	+	+
1.22	191	Citric acid	+	+	+	+
2.4	331	Galloylglucose	-	-	+	-
3.26	783	Catechin glucoside	-	+	-	-
3.31	305	Epigallocatechin	-	-	+	-
3.43	865	Procyanidin C type trimer	-	-	+	-
4.21	329	Vanillic acid hexoside	-	-	-	+
4.98	593	(Epi) gallocatechin-(epi) catechin (procyanidin)	-	-	+	-
8.28	577	Procyanidin B type dimer	-	-	+	+
8.77	289	Catechin	-	-	+	+
9.28	865	Procyanidin C type trimer	-	-	-	+
9.46	325	p-Coumaric acid glucoside	+	-	-	-
9.48	1039	A-type procyanidin trimer glucuronide	-	-	+	-
10.34	451	Catechin glucoside	-	+	-	-
10.46	1067	Ellagitannin (unknown)	-	+	-	-
10.89	633	Galloyl-hexahydroxydiphenoyl (HHDP)-glucose	-	+	-	-
11.04	935	Galloyl-bis-hexahydroxydiphenoyl (HHDP)-hexoside 1	-	+	-	-
11.38	935	Galloyl-bis-hexahydroxydiphenoyl (HHDP)-hexoside 2	-	+	-	-
11.54	447	Ellagic acid rhamnoside	-	+	-	-
11.71	953	Ellagitannin (unknown)	-	+	-	-
11.89	577	Procyanidin B type dimer	-	-	+	+
11.67	865	Procyanidin C type trimer	-	-	+	-
12.43	1103	Hippophaenin B	-	+	-	-
13.62	771	Quercetin-3-sophoroside-7-rhamnoside	+	-	-	-
14.89	785	Isorhamnetin-3-sophoroside-7-rhamnoside	+	-	-	-
15.25	755	Kaempferol-3-sophoroside-7-rhamnoside	+	+	-	-
15.75	433	Ellagic acid arabinoside	-	+	-	-
15.95	301	Ellagic acid	-	+	-	-
16.58	609	Quercetin rutinoside (rutin)	+	+	-	-
16.73	463	Quercetin-O-glucoside	+	+	-	-
16.87	623	Isorhamnetin rutinoside	+	-	-	-

(Continued)

Table 1 (continued)							
t_R^*	[M-H]	Compounds	Fruits	Leaves	Bark	Roots	
17.24	623	Isorhamnetin glucoside-rhamnoside 1	+	+	–	–	
17.65	593	Kaempferol rhamnosyl-hexoside	+	–	–	–	
18.62	623	Isorhamnetin glucoside-rhamnoside 2	+	–	–	–	
18.63	623	Isorhamnetin-3-rhamnosyl-galactoside	–	+	–	–	
18.84	477	Isorhamnetin glucoside	+	–	–	–	

Note: *Retention times obtained on Agilent 6450 Q-ToF mass spectrometer.

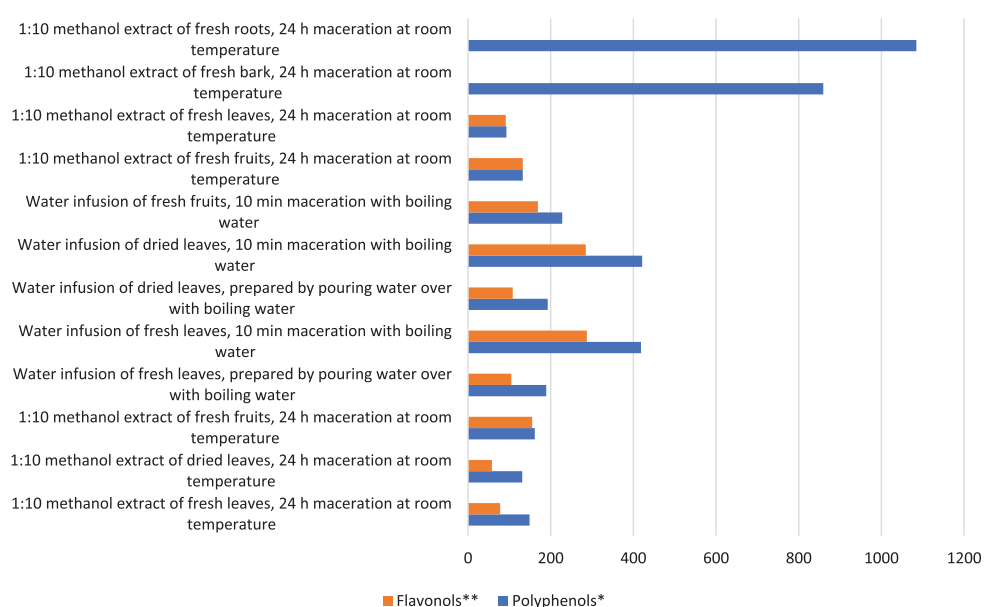


Figure 1: Total polyphenols and flavonols content in samples (mg/100 g DW)

Note: *Calculated as the sum of all compounds in Table 2. **Calculated as the sum of all polyphenols excluding caffeic acid glucoside, dimeric catechin/procyanidin B1 and catechin trimer in Table 2. DW-dry weight.

3.3 Content of Phenolic Compounds Extracted by Different Methods from the Parts of *H. rhamnoides*

The methanolic extract of dried leaves (sample 2) contained the most catechin dimer –35.6 mg/100 g (Table 2), which concentration was higher (55.7 mg/100 g) in fresh leaves (sample 1). There were also significant amounts of quercetin-3-*O*-glucoside-7-*O*-rhamnoside and its isomer (5.7 and 20.3 mg/100 g, respectively), caffeic acid glucoside (26.3 mg/100 g) and catechin trimer (11.6 mg/100 g). In the methanolic extract of fresh leaves (sample 1) catechin dimer was also dominant –55.7 mg/100 g, which exceeds by 1.5 times its content in dried leaves. The dried leaves also contained more quercetin-3-*O*-glucoside-7-*O*-rhamnoside and its isomer (8 and 29.5 mg/100 g) than fresh leaves. The largest amount of isorhamnetin rutinoside was found in the fruits –48.5 mg/100 g, almost 10 times more than in the leaves. Also, more quercetin rutinoside (20.5 mg/100 g), isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside (15.9 mg/100 g) and isorhamnetin-glucoside (21.6 mg/100 g) were found in the fruits.

Table 2: Content of polyphenolic compounds in *H. rhamnoides* samples

t_R^*	[M- H] ⁻	Content (mg/100 g) in samples DW											
		1 Fresh leaves, MeOH	2 Dried leaves, MeOH	3 Fresh fruits, MeOH	4 Fresh leaves, W pou-ring	5 Fresh leaves, W 10 min	6 Dried leaves, W pou-ring	7 Dried leaves, W 10 min	8 Fresh fruits, W 10 min	9 Fresh fruit, MeOH	10 Fresh leaves, MeOH	11 Fresh bark, MeOH	12 Fresh roots, MeOH
0.7	341	nd	26.3	6.4	19.1	44.2	21.5	48.8	8.5	nd	nd	nd	nd
	Caffeic acid +377 glucoside												
12.8	577	55.7	35.6	nd	29.3	46.6	28.7	48.1	20.3	nd	1.3	596.2	717.6
	Dimeric catechin/ procyanidin B1												
13.8	865	15.6	11.6	nd	36.1	40.4	34.6	39.5	30.1	nd	0.3	263.2	366.1
	Catechin trimer												
21.4	609	8.0	5.7	2.8	6.3	37.6	7.7	38.8	3.1	nd	2.2	nd	0.005
	Quercetin-3- <i>O</i> - glucoside-7- <i>O</i> - rhamnoside												
21.9	609	29.5	20.3	14.4	12.8	99.7	15.5	96.2	15.5	4.4	14.1	nd	0.03
	Quercetin-3- <i>O</i> - glucoside-7- <i>O</i> - rhamnoside isomer												
23.4	609	11.0	8.2	20.5	6.3	27.9	6.3	30.3	20.2	4.3	19.2	0.02	0.1
	Quercetin rutinoside (rutin)												
23.7	593	4.3	3.1	8.6	13.2	18.8	13.1	18.3	11.1	0.3	1.0	nd	nd
	Kaempferol- rhamnosyl-hexoside												
23.7	623	3.3	2.6	9.5	13.6	16.2	13.1	15.4	11.9	4.1	5.3	nd	0.05
	Isorhamnetin-3- <i>O</i> - glucoside-7- <i>O</i> - rhamnoside isomer												
23.7	463	3.6	2.8	10.9	10.3	13.0	10.1	12.7	12.2	4.5	3.8	nd	0.04
	Quercetin glucoside												
24.2	623	4.3	3.3	15.9	14.0	20.2	13.8	20.3	16.3	62.2	21.3	0.01	0.3
	Isorhamnetin-3- <i>O</i> - glucoside-7- <i>O</i> - rhamnoside												
25.6	593	1.8	1.4	2.4	2.8	6.7	2.8	5.9	2.7	0.2	0.6	nd	0.02
	Kaempferol-3- <i>O</i> - rutinoside												
25.8	447	2.6	2.2	nd	10.0	11.9	9.7	11.5	8.2	0.03	0.8	nd	nd
	Kaempferol glucoside												
26.2	623	5.1	4.5	48.5	4.8	20.8	5.5	21.3	44.4	40.0	19.8	0.02	0.2
	Isorhamnetin rutinoside												
26.5	477	4.1	3.6	21.6	10.7	14.5	10.5	14.3	23.3	12.5	3.3	nd	0.04
	Isorhamnetin glucoside												

Note: nd-not detected. DW-dry weight. *Retention times obtained on 1100 series LC/MSD Trap-XCT mass spectrometer.

When comparing different water extracts, it becomes clear that the extract obtained by pouring boiling water over the fresh leaves (sample 4) contained the most catechin trimer (36.1 mg/100 g), approximately the same amount was in the water extract standing for 10 min (sample 5 –40.4 mg/100 g, Table 2). It is logical that the sample macerated for 10 min (sample 5) contains 2.3 times more caffeic acid glucoside, 1.5 times more catechin dimer, 7 times more quercetin-3-*O*-glucoside-7-*O*-rhamnoside and its isomer, 4.5 times more rutin and 5 times more isorhamnetin rutinoside than the pour-through sample (sample 4). The content of other compounds was approximately the same both in the sample that was macerated in boiling water for 10 min and in the sample prepared by pouring. The methanol and water samples of the fruits (3 and 8) contained significant amounts of isorhamnetin rutinoside (48.5 and 44.4 mg/100 g, respectively), isorhamnetin glucoside (21.6 and 23.3 mg/100 g) and quercetin rutinoside (both about 20 mg/100 g) (Table 2).

Bark and roots extracts contained less than 1 mg/100 g of identified compounds except dimeric catechin/procyanidin B1 and catechin trimer (596.2–717.6 and 263.2–366.1 mg/100 g, respectively).

3.4 Antioxidant Activity of Fresh/Dried Leaves and Fruits

In all assays, *HR* leaf (fresh/dried) extracts showed significantly ($p < 0.05$) higher antioxidant (radical scavenging) activity than the fruit extracts (Table 3). Leaf extracts also showed strong iron chelating ability, possibly due to presence of malic and citric acids [45] whereas fruit extracts had no activity (Table 3).

Table 3: Antioxidant activity (DW) of *H. rhamnoides* extracts in 60% aqueous ethanol.^{1,2}

<i>H. rhamnoides</i> dried extract		DPPH, IC ₅₀ μg/ml	ABTS, μM TE/g DW	Iron chelating capacity, %
Leaves (samples 13-14)	Fresh	28.5 ± 2.2 ¹	28.3 ± 0.5 ^a	42.1 ± 2.6 ¹
	Dried	25.5 ± 1.8 ¹	28.6 ± 1.4 ^a	53.1 ± 2.2 ²
Fruits (sample 15)	Fresh	355.0 ± 1.8 ²	16.0 ± 0.4 ²	N.A.
	Dried	740.9 ± 1.3 ²	9.2 ± 0.2 ²	N.A.
Standard	Gallic acid	1.5 ± 0.3	–	–
	Trolox	5.3 ± 0.9	–	–
	EDTA	–	–	98.9 ± 0.2

Note: ¹Mean of three replications ± standard error.

²Means followed by the different small letters within a column denote significant differences ($p < 0.05$). DW-dry weight.

4 Discussion

Different ratios of ethanol and water have been used as extractants in solvent extraction process of bioactive substances from *HR* [28]. Our study showed that the method chosen to prepare the water extract affects the amounts of extracted bioactive substances. Comparing the contents of polyphenols and flavonols in samples 4 and 6 prepared by pouring, it turns out that the contents of both fresh and dried samples were practically equal (Fig. 1). The same result also appears when comparing samples 5 and 7 of dried and fresh leaves that have stood for 10 min. Therefore, it does not matter whether the leaves are used fresh or dried.

In the case of *HR* fruits, the use of fresh drug is optimal, as their drying under standard conditions is complicated due the high level of oil. Geng et al. [19] analyzed the effects of hot air drying and infrared drying on flavonoid compounds and antioxidant capacity in *HR* fruits: isorhamnetin and quercetin contents increased in response to both drying methods, while (-)-epigallocatechin and (-)-gallocatechin contents decreased in the fruits. In other similar study [20], they concluded that the pulsed-vacuum–

drying of *HR* fruits had the highest ascorbic acid and total phenolics contents, and is an optimal drying technology for berries.

In this work, the extractability of polyphenolic substances using methanol and water was compared. In the sample 7 macerated with boiling water for 10 min, the concentration of quercetin-3-*O*-glucoside-7-*O*-rhamnoside was 6.5 times higher than in the methanol extract of dried leaves (sample 2, Table 2). The amounts of all other compounds in sample 7 are also significantly higher (1.3–4.8 times) than in the extract prepared with methanol. The same tendency is also visible in the extract of fresh leaves prepared with two different solvents (samples 1 and 5), the only exception is the catechin dimer content. Thus, extracting *HR* leaves with boiling water for 10 min, as is commonly done at home, is optimal. Michel et al. [37] compared the content of hexane, ethyl acetate and water extracts and concluded that the major phenolic compounds were found in water extracts.

In the fruits, the amounts of analysed substances were practically the same in methanol (sample 3) and water extracts (sample 8) (Table 2). However, the water extract also contained 30.1 mg/100 g of catechin trimer, 20.3 mg/100 g of catechin dimer and 8.2 mg/100 g of kaempferol glucoside, which could not be measured in the methanol extract. Therefore, the preparation of water extracts by maceration for 10 min is also justified for fresh fruits.

The technology of reflux extraction of flavonoids from *HR* leaves with water was studied by Wang. The optimal extraction temperature was 90°C, and the extraction time was 2 h [29]. Various authors [30,31] have optimized the ethanol/water extraction of *HR* flavonoids, showing ethanol concentration of 60% or 70% as the best choice. On the other hand, the disadvantage of the reflux extraction method is too long extraction time. The comprehensive use of multiple extraction methods compared to ethanol reflux, microwave-assisted extraction, and ultrasound-assisted extraction, increased the extraction rate of flavonoids from *HR* by 15%, 24%, and 37%, respectively [28]. Guliyev et al. [32] investigated different chromatographic methods to determine the chemical composition of *HR*, including the use of methanol as an extractant, but they did not determine the chemical composition of the bark and roots. The male leaves of *HR* are low in caffeine [33], which dissolves easily in water. This may be the reason why tea made from fresh leaves is recommended as a refreshing drink.

If to compare the extractability of polyphenolic compounds when poured with boiling water (samples 4 and 6) and after 10 min of maceration (samples 5 and 7), it is logical that a longer extraction time leads to a significant increase in the concentration of polyphenols. The differences are particularly large in the content of quercetin-3-*O*-glucoside-7-*O*-rhamnoside isomers, quercetin-3-*O*-glucoside-7-*O*-rhamnoside and isorhamnetin-rutinoside (1.4–7.7: 5.5 times, Table 2). Thus, adequate extraction time (10 min) is necessary for the preparation of aqueous extracts containing the maximum amounts of polyphenolic substances of *HR*.

The concentration of flavonol glycosides in leaves of *HR* is higher than in fruits [23]. Isorhamnetin glycosides, followed by quercetin glycosides are typically the most important flavonols of *HR* fruits and leaves [23]. The fruits are rich in flavonoid glycosides such as isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, isorhamnetin-3-glucoside-7-rhamnoside, isorhamnetin-3-neohesperidoside, quercetin-3-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-sorphanoside-7-*O*-rhamnoside, isorhamnetin-3-*O*-sorphanoside-7-*O*-rhamnoside, rutin and free isorhamnetin [23,26,27]. According to the Pop et al. [23], isorhamnetin-3-rhamnosylglucoside, isorhamnetin-3-neohesperidoside, isorhamnetin-3-glucoside, quercetin-3-pentoside, kaempferol-3-rutinoside, and quercetin-3-glucoside were predominant in leaves of *HR*. By Ma et al. [21], isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside and isorhamnetin-3-*O*-rutinoside were the two major flavonol glycosides in leaves and fruits of *HR*. Our study revealed that quercetin glycosides are more abundant in leaves, but the level of isorhamnetin glycosides depends on the specific compound (Table 2).

Tannins were present in higher concentrations in *HR* seeds, roots, flowers, green berries and stems [37]. The bark and root extracts contained the highest amounts of total polyphenols (859.5 and 1084.5 mg/100 g, respectively), but the least of flavonols (0.05 and 0.8 mg/100 g, Fig. 1). The principal polyphenols in bark and roots are catechin di- and trimers. However, these parts of *HR* contain the identified compounds in such low concentrations that are not of further interest as sources of biologically active substances (Table 2). Ma et al. [21] mentioned that in the leaves of *HR* ellagitannins are the most abundant polyphenols.

Traditionally, the fruits and leaves of *HR*, but not the bark and/or roots, have been used in several countries (China, Turkey, Greece, Mongolia, Tajikistan) [34]. In Central and Southeastern Asia every part of *HR*, including twig, root and bark has been traditionally used as medicine, dietary supplement, fence or firewood [8]. On the other hand, flavonoids are mentioned as the main chemical compounds also in the branches and roots of *HR*. According to our results (Table 2), the bark and roots contain only minimal amounts of flavonoids. The biological activity of *HR* roots and bark has been very little studied. For example, root extract has been shown to have antimicrobial effect (63% of inhibition) against *Enterococcus durans* [1].

Phenolic compounds (flavonoids, tannins and phenolic acids) of *HR* are considered to be the major constituents possessing antioxidant activity [16]. Li et al. [18] showed strong correlations between total phenolics content, gallic acid and antioxidant capacity. Also, Ciesarová et al. [17] concluded that phenolic compounds are dominantly responsible for antioxidative effect of *HR*. They mentioned also carotenoids and tocopherols as lipophilic antioxidants. Geng et al. [19] showed that the antioxidant activity of the dried fruits was considerably lower than that of fresh *HR* berries.

The antioxidant activity of fresh and dried leaves and fruits of *HR* were investigated in our study by three different methods. All these three methods showed that the antioxidant activity of the leaves is stronger than that of the fruits, which can be explained by the higher concentration of polyphenols in the leaves. This study suggests that *HR* leaf extracts have great potential for antioxidant activity and may be beneficial for their nutritional and medicinal functions. This is an important problem in the valorisation of plant residues because *HR* fruits are often collected with the tips of the branches, which are frozen and from which the fruits are later separated.

In our study, the fruits contain more isorhamnetin derivatives than the leaves (Table 2), but the leaves showed stronger activity (Table 3). The antioxidant effect of different parts, particularly leaves of *HR*, is not only due to polyphenols, but also to the content of vitamins, definite organic acids and polysaccharides [35]. We performed the antioxidant test with 60% aqueous ethanol instead of water, because a previous study [36] showed that ethanol extracts possess a significantly higher antioxidant power than water extracts. More generally, due their free-radical scavenging and anti-inflammatory properties, the polyphenols can be used to treat different conditions associated with metabolic disorders: dyslipidemia, atherosclerosis, obesity, hypertension, elevated blood sugar, accelerating aging, liver intoxication, etc. [8].

The study has some limitations. First, the antioxidant activity of roots and bark was not investigated in this work. It has no practical importance, because the mass collection of these plant parts means the destruction of the bushes. In addition, the organoleptic properties of bark and root water extract are unpleasant. On the other hand, the infusion of the *HR* leaves has a pleasant taste, somewhat reminiscent of green tea. Michelet al. [37] concluded that root ethanol extracts of *HR* have higher antioxidant activity (by DPPH and Ferric reducing antioxidant power (FRAP) assays) than extracts from leaves and stems. Thus, the antioxidant activity of roots needs additional study.

Secondly, the phytochemical and antioxidant studies have been performed on plant material collected from different places (Estonia and Moldova, respectively). Therefore, exact correlations between chemical composition and efficacy cannot be calculated. On the other hand, several previous studies [16–18,21]

show that polyphenols are more abundant in *HR* leaves than in fruits and are the most important substances in the plant in terms of antioxidant activity.

Third, the phytochemical studies were performed in plant material collected from two growing sites and antioxidant analyzes only in raw material from one growing place. Probably, the general tendencies in the content and activity of phenolics and flavonols will remain the same, but for more detailed conclusions, the experiments should be repeated with plant parts of *HR* collected from different places.

5 Conclusion

A total of 19 phenolic compounds were identified in different parts of *H. rhamnoides*. Among flavonoids, such compounds as kaempferol glycosides, quercetin glycosides and isorhamnetin glycosides were the most significant ones identified in fruits and leaves. The compositions of bark and roots were very different compared to fruits and leaves as they contain considerably more amounts of polyphenols (mainly catechins but minimal flavonols). The total polyphenols and flavonols contents were the most abundant in water infusions from the leaves of *H. rhamnoides*. The studied phenolic substances were found in the highest concentration in water extracts that stood for 10 min, while the result was practically the same for fresh and dried raw material and did not depend on the method of preparing the water extract. There were no significant differences in the quantitative or qualitative content of phenolic compounds in the fresh and dried raw materials. Quercetin-3-*O*-glucoside-7-*O*-rhamnoside was the most abundant compound in the aqueous extracts. The fresh and dried leaves of *H. rhamnoides* showed the highest values of antioxidant activity and may have a perspective as sources of biologically active compounds in addition to fruits.

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References

1. Pundir, S., Garg, P., Dwiwedi, A., Ali, A., Kapoor, V. K. et al. (2021). Ethnomedicinal uses, phytochemistry and dermatological effects of *Hippophae rhamnoides* L.: A review. *Journal of Ethnopharmacology*, 266, 113434.
2. Leskinen, H. M., Suomela, J. P., Yang, B., Kallio, H. P. (2010). Regioisomer compositions of vaccenic and oleic acid containing triacylglycerols in sea buckthorn (*Hippophae rhamnoides*) pulp oils: Influence of origin and weather conditions. *Journal of Agriculture and Food Chemistry*, 58(1), 537–545.

3. Kallio, H., Yang, B., Peippo, P. (2002). Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophae rhamnoides*) berries. *Journal of Agriculture and Food Chemistry*, 50(21), 6136–6142.
4. Yang, B., Kallio, H. (2002). Composition and physiological effects of sea buckthorn (*Hippophae*) lipids. *Trends in Food Science & Technology*, 13(5), 160–167.
5. Zheng, R. X., Xu, X. D., Tian, Z., Yang, J. S. (2008). Chemical constituents from the fruits of *Hippophae rhamnoides*. *Natural Products Research*, 23, 1451–1456.
6. Kumar, R., Phani Kumar, G., Chaurasia, O. P., Singh, S. B. (2011). Phytochemical and pharmacological profile of sea buckthorn oil: A review. *Research Journal of Medicinal Plants*, 5(5), 491–499.
7. Zielinska, A., Nowak, I. (2017). Abundance of active ingredients in sea-buckthorn oil. *Lipids in Health and Disease*, 16(1), 95–106.
8. Suryakumar, G., Gupta, A. (2011). Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *Journal on Ethnopharmacology*, 138(2), 268–278.
9. Upadhyay, N. K., Yogendra Kumar, M. S., Gupta, A. (2010). Antioxidant, cytoprotective and antibacterial effects of sea buckthorn (*Hippophae rhamnoides* L.) leaves. *Food Chemistry and Toxicology*, 48(12), 3443–3448.
10. Biswas, A., Bharti, V. K., Acharya, S., Pawar, D. D., Singh, S. B. (2010). Sea buckthorn: Newfeed opportunity for poultry in cold arid Ladakh region of India. *World's Poultry Science Journal*, 66(4), 707–714.
11. Guan, T. T. Y., Cenkowski, S., Hydamaka, A. (2006). Effect of drying on the nutraceutical quality of sea buckthorn (*Hippophae rhamnoides* L. ssp. *sinensis*) leaves. *Journal of Food Science*, 70(9), E514–E518.
12. Olas, B., Żuchowski, J., Lis, B., Skalski, B., Kontek, B. et al. (2018). Comparative chemical composition, antioxidant and anticoagulant properties of phenolic fraction (a rich in non-acylated and acylated flavonoids and non-polar compounds) and non-polar fraction from *Elaeagnus rhamnoides* (L.) A. Nelson Fruits. *Food Chemistry*, 247, 39–45.
13. Olas, B. (2016). Sea buckthorn as a source of important bioactive compounds in cardiovascular diseases. *Food Chemistry and Toxicology*, 97, 199–204.
14. Olas, B., Kontek, B., Malinowska, P., Zuchowski, J., Stochmal, A. (2017). *Hippophae rhamnoides* L. fruits reduce the oxidative stress in human blood platelets and plasma. *Oxidative Medicine and Cellular Longevity*, 2016, 4692486.
15. Olas, B., Kontek, B., Szczesna, M., Grabarczyk, L., Stochmal, A. et al. (2017). Inhibition of blood platelet adhesion by phenolics' rich fraction of *Hippophae rhamnoides* L. fruits. *Journal of Physiology and Pharmacology*, 68(2), 23–29.
16. Šne, E., Seglina, D., Galoburda, R., Inta Krasnova, I. (2013). Content of phenolic compounds in various sea buckthorn parts. *Proceedings of the Latvian Academy of Sciences. Section B*, 67(4/5), 411–415.
17. Ciesarová, Z., Murkovic, M., Cejpek, K., Kreps, F., Tobolková, B. et al. (2020). Why is sea buckthorn (*Hippophae rhamnoides* L.) so exceptional? A review. *Food Research International*, 133, 109170.
18. Li, Y., Li, P., Yang, K., He, Q., Wang, Y. et al. (2021). Impact of drying methods on phenolic components and antioxidant activity of sea buckthorn (*Hippophae rhamnoides* L.) berries from different varieties in China. *Molecules*, 26(23), 7189.
19. Geng, Z., Wang, J., Zhu, L., Yu, X., Zhang, Q. et al. (2023). Metabolomics provide a novel interpretation of the changes in flavonoids during sea buckthorn (*Hippophae rhamnoides* L.) drying. *Food Chemistry*, 413, 135598.
20. Geng, Z., Zhu, L., Wang, J., Yu, X., Li, M. et al. (2023). Drying sea buckthorn berries (*Hippophae rhamnoides* L.): Effects of different drying methods on drying kinetics, physicochemical properties, and microstructure. *Frontiers in Nutrition*, 10, 1106009.
21. Ma, X., Yang, W., Kallio, H., Yang, B. (2021). Health promoting properties and sensory characteristics of phytochemicals in berries and leaves of sea buckthorn (*Hippophae rhamnoides*). *Critical Reviews in Food Science and Nutrition*, 62(14), 3798–3816.
22. Ma, X., Moilanen, J., Laaksonen, O., Yang, W., Tenhu, E. et al. (2019). Phenolic compounds and antioxidant activities of tea-type infusions processed from sea buckthorn (*Hippophae rhamnoides*) leaves. *Food Chemistry*, 272, 1–11.

23. Pop, R. M., Socaciu, C., Pinte, A., Buzoianu, A. D., Sanders, M. G. et al. (2013). UHPLC/PDA-ESI/MS analysis of the main berry and leaf flavonol glycosides from different Carpathian *Hippophaë rhamnoides* L. varieties. *Phytochemical Analysis*, 24(5), 484–492.
24. Arimboor, R., Arumughan, C. (2012). HPLC-DAD-MS/MS profiling of antioxidant flavonoid glycosides in sea buckthorn (*Hippophaë rhamnoides* L.) seed. *International Journal of Food Science and Nutrition*, 63(6), 730–736.
25. Fatima, T., Nazir, A., Naseer, B., Hussain, S. Z. (2018). Seabuckthorn (*Hippophaë rhamnoides*): A repository of phytochemicals. *International Journal of Pharmaceutical Science and Research*, 3, 9–12.
26. Guo, R., Guo, X., Li, T., Fu, X., Liu, R. H. (2017). Comparative assessment of phytochemical profiles, antioxidant and antiproliferative activities of (*Hippophaë rhamnoides* L.) berries. *Food Chemistry*, 221, 997–1003.
27. Teleszko, M., Wojdylo, A., Rudzinska, M., Oszmianski, J., Golis, T. (2015). Analysis of lipophilic and hydrophilic bioactive compounds content in sea buckthorn (*Hippophaë rhamnoides* L.) berries. *Journal of Agriculture and Food Chemistry*, 63, 4120–4129.
28. He, N., Wang, Q., Huang, H., Chen, J., Wu, G. et al. (2023). A comprehensive review on extraction, structure, detection, bioactivity, and metabolism of flavonoids from sea buckthorn (*Hippophaë rhamnoides* L.). *Journal of Food Biochemistry*, 2023, 4839124.
29. Wang, S. L. (2008). Study on the extraction technology of flavones from leaves of *Hippophaë rhamnoides* L. *Food Research and Development*, 29, 110–113.
30. Li, S. Z., Wu, F., Chen, Y. L., Yang, Y. (2015). The optimization of purification technics of total flavonoids from leaves of *Hippophaë rhamnoides* L. *Journal of Anhui Normal University*, 38, 567–571.
31. Hui, R. J., Feng, J., Lin, M. H., Feng, B. N. (2017). Optimization of extraction technology for flavonoids in leaves and seeds of *Hippophaë rhamnoides* L. by multiindex-orthogonal test. *China Pharmacy*, 28, 4856–4859.
32. Guliyev, V. B., Gul, M., Yildirim, A. (2004). *Hippophaë rhamnoides* L.: Chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects. *Journal of Chromatography B*, 812, 291–307.
33. Li, G., Zhang, J., Liu, E., Wang, F. H., Qi, S. et al. (2016). Effects of different sea buckthorn leaf tea processing technologies on nutrient level and fecal microflora *in vitro*. *Journal of Food and Nutrition Research*, 55, 205–213.
34. Ma, Q. G., He, N. X., Huand, H. L., Fu, X. M., Zhang, Z. L. et al. (2023). *Hippophaë rhamnoides* L.: A comprehensive review on the botany, traditional uses, phytonutrients, health benefits, quality markers, and applications. *Journal of Agricultural and Food Chemistry*, 71, 4769–4788.
35. Wang, K., Xu, Z., Liao, X. (2022). Bioactive compounds, health benefits and functional food products of seabuckthorn: A review. *Critical Reviews in Food Science and Nutrition*, 62, 6761–6782.
36. Šne, E., Ruta Galoburda, R., Dalija Segliņa, D. (2013). Sea buckthorn vegetative parts—A good source of bioactive compounds. *Proceedings of the Latvian Academy of Sciences. Section B*, 67, 101–108.
37. Michel, T., Destandau, E., Le Floch, G., Lucchesi, M. E., Elfakir, C. (2012). Antimicrobial, antioxidant and phytochemical investigations of seabuckthorn (*Hippophaë rhamnoides* L.) leaf, stem, root and seed. *Food Chemistry*, 131, 754–760.
38. Koshovyi, O. N., Vovk, G. V., Akhmedov, E. Y., Komissarenko, A. N. (2015). The study of the chemical composition and pharmacological activity of *Salvia officinalis* leaves extracts getting by complex processing. *Azerbaijan Pharmaceutical and Pharmacotherapy Journal*, 15(1), 30–34.
39. Raal, A., Jaama, M., Utt, M., Püssa, T., Žvikas, V. et al. (2022). The phytochemical profile and anticancer activity of *Anthemis tinctoria* and *Angelica sylvestris* used in Estonian ethnomedicine. *Plants*, 11, 994.
40. Chaika, N., Koshovyi, O., Raal, A., Kireyev, I., Zupanets, A. et al. (2020). Phytochemical profile and pharmacological activity of the dry extract from *Arctostaphylos uva-ursi* leaves modified with phenylalanine. *Sciencise: Pharmaceutical Science*, 6(28), 74–78.
41. Brand-Williams, W., Cuvelier, M. E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25–30.

42. Huzio, N., Grytsyk, A., Raal, A., Grytsyk, L., Koshovyi, O. (2022). Phytochemical and pharmacological research in *Agrimonia eupatoria* L. herb extract with anti-inflammatory and hepatoprotective properties. *Plants*, *11*, 2371.
43. Dinis, T. C. P., Madeira, V. M. C., Almeida, M. L. M. (1994). Action of phenolic derivates (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry and Biophysics*, *315*(1), 161–169.
44. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. et al. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237.
45. Justi, M., Silva, C. A., Rosa, S. D. (2022). Organic acids as complexing agents for iron and their effects on the nutrition and growth of maize and soybean. *Archives of Agronomy and Soil Science*, *68*(10), 1361–1384.