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DETERMINATION OF THE CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF FRUIT EXTRACTS FROM *HIPPOPHAE RHAMNOIDES* L. AND *CRATAEGUS SANGUINEA* L.

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Abstract. The article presents data on the chemical composition and antioxidant activity of the studied plant samples. Wild-growing berries of *Hippophae rhamnoides* L. (sea buckthorn) and *Crataegus sanguinea* L. (hawthorn) served as raw materials for extraction. Elemental analysis of the plant material samples was performed to determine the content of nitrogen, sulfur, total carbon, and organic carbon. The ICP-MS (Inductively Coupled Plasma Mass Spectrometry) method was used to determine the concentrations of various chemical elements. Liquid extracts were obtained from sea buckthorn and hawthorn fruits using 70% ethanol (1:10 ratio). The extracts were examined for their total flavonoid content and antioxidant activity. The article details the results of flavonoid quantification and antioxidant capacity analysis. Total flavonoid content in the extracts, expressed as quercetin (%) using a spectrophotometric method:

Hippophae rhamnoides L. – 0.37±0.03, *Crataegus sanguinea* L. – 0.41±0.05. Antioxidant activity was evaluated by the FRAP (Ferric Reducing Antioxidant Power) method, using ascorbic acid as the reference antioxidant. The study revealed that increasing extract concentration from 0.01 to 0.05 mg/mL enhanced antioxidant activity. The antioxidant potential of *Crataegus sanguinea* L. was higher than that of *Hippophae rhamnoides* L. The findings broaden understanding of these species and support their potential use in antioxidant cosmeceutical formulations.

Keywords: antioxidant activity, plant extract, bioactive substances, flavonoid, FRAP method

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HIPPOPHAE RHAMNOIDES L. ЖӘНЕ *CRATAEGUS SANGUINEA* L. ӨСІМДІК ЖЕМІСТЕРІНІҢ ЭКСТРАКТТАРЫНЫҢ ХИМИЯЛЫҚ ҚҰРАМЫН ЖӘНЕ АНТИОКСИДАНТТЫҚ БЕЛСЕНДІЛІГІН АНЫҚТАУ

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Аннотация. Мақалада зерттелетін өсімдік үлгілерінің химиялық құрамы мен антиоксиданттық белсенділігі туралы мәліметтер келтірілген. Экстракт алу үшін өсімдік шикізаты ретінде жабайы өсімдіктердің жидектері *Hippophae rhamnoides* L. (шырғанақ) және *Crataegus sanguinea* L. (долана) пайдаланылды. Өсімдіктерден алынған шикізаттың элементтік құрамы зерттеліп, оның құрамындағы маңызды

химиялық элементтер, атап айтқанда, азот, күкірт, жалпы көміртек және органикалық көміртек мөлшері анықталды. Индуктивті байланысқан плазмалық масс-спектрометрия (ICP-MS) әдісі қолданылып, үлгілердегі түрлі химиялық элементтердің мөлшері сандық түрде анықталды. 70%-дық этил спиртін қолдана отырып, шырғанақ пен долана жемістерінен 1:10 қатынасында сұйық экстрактылар алынды. Экстрактылардың негізгі параметрлері ретінде олардың флавоноидтар құрамының жалпы мөлшері анықталып, антиоксиданттық белсенділігі сандық түрде бағаланды. Бұл мақалада экстрактылардың флавоноидтар жиынтық мөлшерін және олардың антиоксиданттық белсенділігін анықтау нәтижелері ұсынылған. Экстрактылардағы флавоноидтардың жиынтық мөлшері спектрофотометриялық әдіспен зерттеліп, алынған нәтижелер кверцетинге шаққандағы пайыздық (**%**) мөлшерде берілді: *Hippophae rhamnoides* L. - 0.37±0.03, *Crataegus sanguinea* L. - 0.41±0.05. Экстракттардың антиоксиданттық белсенділігін зерттеу FRAP (Ferric Reducing Antioxidant Power) әдісімен жүргізілді, анықтамалық антиоксидант аскорбин қышқылы болып табылады. Зерттеулер нәтижесінде экстракт концентрациясы 0,01-ден 0,05 мг/мл-ге дейін артқанда антиоксиданттық белсенділіктің де жоғарылайтыны анықталды. *Crataegus sanguinea* L. жемістеріндегі антиоксиданттық белсенділігі *Hippophae rhamnoides* L. жемістеріне қарағанда жоғары екені анықталды. Бұл зерттеулер зерттеліп отырған өсімдік түрлері туралы деректерді кеңейтеді және олардың негізінде антиоксиданттық белсенділігі бар космецевтикалық препараттарды әзірлеуге мүмкіндік береді.

Түйін сөздер: антиоксидантты белсенділік, өсімдік экстрактысы, биологиялық белсенді заттар, флавоноид, FRAP әдісі

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ОПРЕДЕЛЕНИЕ ХИМИЧЕСКОГО СОСТАВА И АНТИОКСИДАНТНОЙ АКТИВНОСТИ ЭКСТРАКТОВ ПЛОДОВ РАСТЕНИЙ *HIPPOPHAE RHAMNOIDES L.* И *CRATAEGUS SANGUINEA L.*

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Аннотация. Статья содержит сведения о химическом составе и антиоксидантной активности исследуемых растительных образцов. В качестве растительного сырья для получения экстрактов использовали ягоды дикорастущих растений *Hippophae rhamnoides L.* (облепиха) и *Crataegus sanguinea L.* (боярышник). Элементный анализ образцов растительного сырья проводился для определения содержания таких элементов, как азот, сера, общий углерод и органический углерод. Методом масс-спектрометрии с индуктивно связанной плазмой (ICP-MS) определено содержание различных химических элементов. Из плодов облепихи и боярышника с использованием 70%-ного этилового спирта получены жидкие экстракты в соотношении 1:10. Экстракты охарактеризованы такими параметрами, как суммарное содержание флавоноидов, приведена количественная оценка антиоксидантной активности. В статье описаны результаты количественного определения суммы флавоноидов и определения антиоксидантной активности экстрактов. Содержание суммы флавоноидов в экстрактах в пересчете на кверцетин (%) спектрофотометрическим методом: *Hippophae rhamnoides L.* - 0.37±0.03, *Crataegus sanguinea L.* - 0.41±0.05. Исследования антиоксидантной активности экстрактов проводили методом FRAP (Ferric Reducing Antioxidant Power), эталонным антиоксидантом служит аскорбиновая кислота. В результате исследований было установлено, что при увеличении концентрации экстракта от 0,01 до 0,05 мг/мл антиоксидантная активность повышается. Антиоксидантная активность у плодов *Crataegus sanguinea L.* оказалась выше, чем у плодов *Hippophae rhamnoides L.* Эти исследования расширяют данные об изучаемых видах и могут быть применимы, в том числе для разработки на их основе космецевтических средств с антиоксидантной активностью.

Ключевые слова: антиоксидантная активность, растительный экстракт, биологически активные вещества, флавоноид, метод FRAP.

Introduction. Currently, antioxidant activity (AOA) is one of the main and most significant characteristics of substances, and its study receives considerable attention in works dedicated to biologically active compounds. The primary function of antioxidants is to neutralize free radicals formed in the cells of living organisms during various processes. These radicals can damage important molecular targets if the natural mechanisms for their neutralization are insufficiently effective. To prevent such processes, natural antioxidants found in vegetables, fruits, berries, vegetable oils, extracts, and other natural products can be used. Antioxidants are also widely used in cosmetics to combat skin damage associated with the effects of free radicals, which helps slow down the aging process and maintain a healthy skin condition. However, for the safe and effective use of antioxidants in cosmetics and nutrition, it is crucial to precisely determine their content and evaluate their antioxidant activity (Pang, et all., 2018).

The overall antioxidant effect of biologically active compounds (BACs) contained

in plant extracts is determined by the presence of various natural compounds and their interactions. These compounds work together, creating effective systems of redox reactions and synergistic cycles that enhance their overall antioxidant effect (Stagos, 2020). Flavonoids have been identified as one of the key components exhibiting antioxidant activity (Chrzaszcz, et all., 2021). They are capable of inhibiting free radical oxidation processes, thereby preventing damage to cells and tissues caused by free radicals. Thus, flavonoids play a crucial role in protecting the body from oxidative stress, making them an important component of plant extracts and other natural remedies (Li, et all., 2014).

The purpose of this study is to assess the quantitative content of flavonoids and the antioxidant activity of plant extracts.

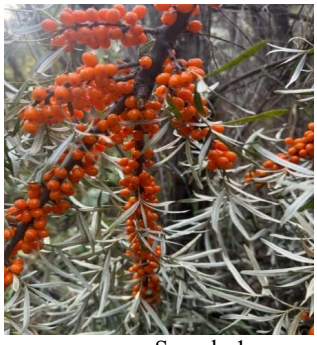
Materials and methods

Objects of the study

The objects of the study are the fruits of *Hippophae rhamnoides* L. (sea buckthorn) and *Crataegus sanguinea* L. (hawthorn) growing in the Abai region (Figure 1).

The fruits of sea buckthorn contain a wide range of components, including minerals (micro- and macroelements), sugars (glucose, fructose, and xylose), organic acids (malic and succinic acids), as well as amino acids. The fruits are rich in vitamins (provitamin A, vitamins B, C, K, and E), carotenoids (beta-carotene, lycopene, lutein, zeaxanthin), and flavonoids (isorhamnetin, quercetin, kaempferol, and others) (Munteanu, et all., 2020) and contain essential fatty acids. Unlike other plants, sea buckthorn accumulates lipids in all morphological parts of the fruit, including the mesocarp, pericarp, and seeds (Zielińska, Nowak, 2017).

According to the literature, the fruits of hawthorn are rich in biologically active compounds, such as triterpene acids (oleanolic, ursolic, and crataegic acids), choline and acetylcholine, quercetin, tannins, phytosterols, tartaric and citric acids, (Kumar, et all., 2012) as well as vitamins A, C, and P. The total acid content is relatively low, ranging from 0.26% to 0.93%, with higher concentrations observed in juicier fruits. The fruits also contain pectin, which not only facilitates gel formation during processing but also aids in the removal of heavy metal salts and other harmful substances from the body (Jurikova, et all., 2012).



Sample 1

Sample 2

Figure 1. Sample 1 - fruits of Hippophae rhamnoides L., Sample 2 - fruits of Crataegus sanguinea L.

Collection and preparation of Raw Materials for Analysis

The sea buckthorn fruits were collected in the Karagaily district of the Abai region in September 2024. The hawthorn fruits were collected in the Aksuat district of the Abai region in late August 2024. Appropriate species identification keys were employed to identify the wild species *C. sanguinea* L. and *H. rhamnoides* L. Frozen fruits were dried using a lyophilizer (SCIENTZ-12N Freeze Dryer, China) for 48 hours, and the dried fruits were ground to a particle size of 1 mm.

Elemental analysis

Elemental analysis of the dried raw material for nitrogen, sulfur, total carbon, and organic carbon content was performed using a VarioMax elemental analyzer (Elementar Analysensysteme GmbH, Germany).

Inductively coupled plasma mass spectrometry

Extractable content of elements in samples were determined by inductively coupled plasma mass spectrometry with Integrated Collision Reaction Cell HPLC-ICP(iCRC) MS Plasma Quant MS Q (Analityk Jena, Germany). Samples were firstly lyophilized for 24h and then ground manually to homogenize. The samples (0.250 ± 0.01 g) were extracted in 5 ml of nitric acid in closed PTFE containers in the Mars 6 Microwave Digestive System (CEM, USA). The program included 20 min of heating to 180°C, 20 min of holding the temperature and 30 min of cooling. Afterwards, samples were filtered through previously washed filter papers (with 200 ml of deionized water) to the polypropylene Falcon tubes and filled up to 15 ml with water.

Extraction process

The extraction of 20 g of ground plant fruit material was performed using a Soxhlet apparatus with 70% ethanol as the solvent at a ratio of 1:10. The process was conducted at a temperature of 80°C for 6 hours. The obtained extracts were concentrated using a rotary evaporator (IKA RV 10, China) to remove the solvent. The resulting extracts appeared as clear or slightly turbid, intensely colored liquids with a characteristic fragrant aroma. The liquid extracts were subsequently filtered and subjected to analysis.

The quantitative determination of flavonoids in the extracts was carried out and expressed in terms of quercetin equivalents.

A 2 mL aliquot of the extract was transferred into a 25 ml volumetric flask, followed by the addition of 2 mL of a 1% aluminum chloride solution in 90% ethanol. The volume was then adjusted to the mark with the same ethanol. After 30 minutes, the optical density of the resulting solution was measured using a spectrophotometer at a wavelength of 426 nm in a cuvette with a 10 mm path length. Simultaneously, the optical density of a quercetin standard solution was measured. For this purpose, 2 ml of a 0.02% standard solution was placed into a 25 ml volumetric flask, followed by the addition of 2 ml of a 1% aluminum chloride solution, and the volume was adjusted to the mark with 90% ethanol. The flavonoid content, expressed as a percentage (%) in terms of quercetin, was calculated using a specific formula:

$$X = \frac{D \cdot m_o \cdot 50 \cdot 50 \cdot 1 \cdot 100 \cdot 100}{D_o \cdot m \cdot 1 \cdot 25 \cdot 25 \cdot (100 - W)}$$

D – optical density of the test solution;
Do – optical density of the standard solution;
m – mass of the raw material (g);
m_o – mass of the standard sample (g);
W – moisture loss percentage during drying

The antioxidant activity of the extracts was determined using a spectrophotometer (SPECORD-210 Plus, Analytik Jena, Germany) according to the FRAP method (Ferric Reducing Antioxidant Power) (Madhuranga, et all., 2023).

The extracts were diluted with ethanol to various concentrations ranging from 0.01 to 0.05 mg/mL. As a standard for comparison, ascorbic acid solutions with identical concentrations (0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml) were used. Ascorbic acid serves as a reference antioxidant, providing a basis for evaluating the antioxidant activity of the tested samples. To the samples and reference standards, 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of a 1% solution of $K_3[Fe(CN)]_6$ were added. The mixture was vortexed for 5 minutes using a vortex shaker and then incubated at 50°C for 20 minutes. Following incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The supernatant obtained after centrifugation (2.5 ml) was collected, followed by the addition of 2.5 ml of deionized water and thorough mixing. Subsequently, 0.5 ml of a 0.1% ferric chloride solution was added to the mixture to produce a colored solution. The absorbance of the reference standard solutions and samples was measured using a UV-visible spectrophotometer at a wavelength of 711 nm.

The reducing power of the extract was evaluated based on the reaction:

$$K_3[Fe(CN)]_6 + e^- \rightarrow K_4[Fe(CN)]_6$$
$$Fe^{3+} + e^- \rightarrow Fe^{2+}$$

The FRAP value was expressed as milligrams of ascorbic acid equivalents per milliliter of extract (AAE).

Results and Discussion. According to the results of elemental analysis, the studied samples contained the highest levels of total carbon (48.38; 45.28) and organic carbon (48.22; 44.57), while sulfur content was the lowest (0.23; 0.07). The element concentrations were higher in *Hippophae rhamnoides* L. (Table 1).

Table 1. Elemental Analysis Results of Plant Samples: Sample 1 (*Hippophae rhamnoides* L.) and Sample 2 (*Crataegus sanguinea* L.)

Sample	Element Content, wt.%			
	Nitrogen	Sulfur	Carbon (Total)	Carbon (Organic)
Sample 1	2,07 ± 0,04	0,23 ± 0,02	48,38 ± 0,07	48,22 ± 4,20
Sample 2	0,51 ± 0,02	0,07 ± 0,03	45,28 ± 0,32	44,57 ± 0,64

Table 2. Results of Plant Analysis by ICPM Spectrometry: Sample 1 (*Hippophae rhamnoides* L.) and Sample 2 (*Crataegus sanguinea* L.)

Element	Al	Mn	Co	Ni	Cu	Zn	Cd	La	Ce	Na	Mg
Sample 1, mg/kg	23,1	7,05	0,0178	0,990	4,24	17,4	0,0099	<DL	<DL	571	528
Sample 2, mg/kg	42,9	5,59	0,102	0,432	2,45	9,00	0,0310	0,0171	0,032	5,46	776
Element	P	K	Ca	Fe	B	Ba	Pb	Cr	As	Se	
Sample 1, mg/kg	1270	8670	915	31,1	11,6	1,21	0,663	0,138	0,0065	0,051	
Sample 2, mg/kg	1530	9306	2472	41,6	14,1	5,24	0,388	0,167	0,0326	0,063	

*DL-Detection Limit

The data on the flavonoid content expressed as quercetin equivalents in the obtained extracts are provided in Table 3.

Table 3. Total Flavonoid Content in Samples (as %, in Quercetin Equivalents): Sample 1 (*Hippophae rhamnoides* L.) and Sample 2 (*Crataegus sanguinea* L.)

Sample	Total flavonoid content expressed as quercetin equivalents, %.
Sample 1	0.37±0.03
Sample 2	0.41±0.05

The FRAP method has significant advantages over other methods for determining antioxidant activity, including its rapid analysis speed, low cost per sample, simplicity of the required equipment, and high reproducibility of results. Evaluates antioxidants capable of reducing iron (Fe^{3+} to Fe^{2+}). (Munteanu, et all., 2021; Madhuranga, et all, 2023). Ascorbic acid was used as the standard solution, as it acts as a secondary antioxidant, scavenging free radicals and preventing the propagation of chain reactions.

During the experiment, changes in the intensity or hue of the solution's color were observed, which were correlated with the amount (concentration) of antioxidants present in the samples (Figure 2).

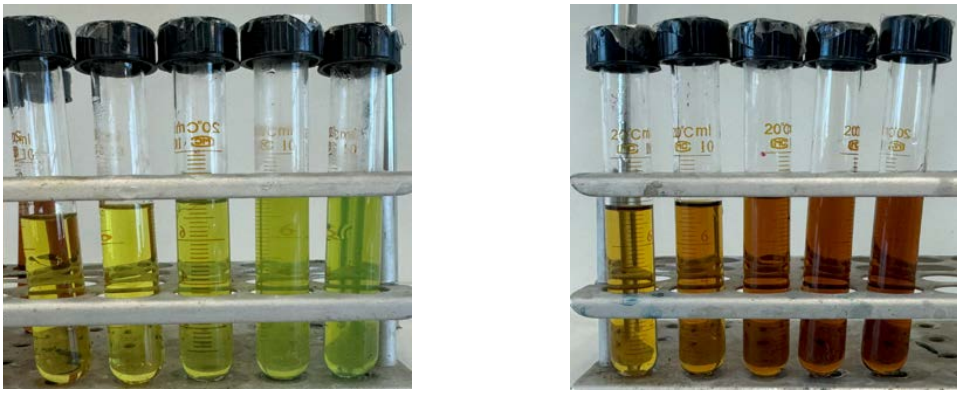


Figure 3. Color changes in the solution as a function of sample concentrations: Sample 1 (*Hippophae rhamnoides*),
Sample 2 (*Crataegus sanguinea*)

The regression curve was plotted based on the following parameters: y-represents the optical density, while x-denotes the concentration (mg/ml) (Figure 4). The calibration curve for the standard concentrations is described by the equation: y=1.6920□x+0.0803. The R^2 (coefficient of determination) value is approximately 0.9998, demonstrating an exceptionally high degree of linearity and accuracy within the specified concentration range.

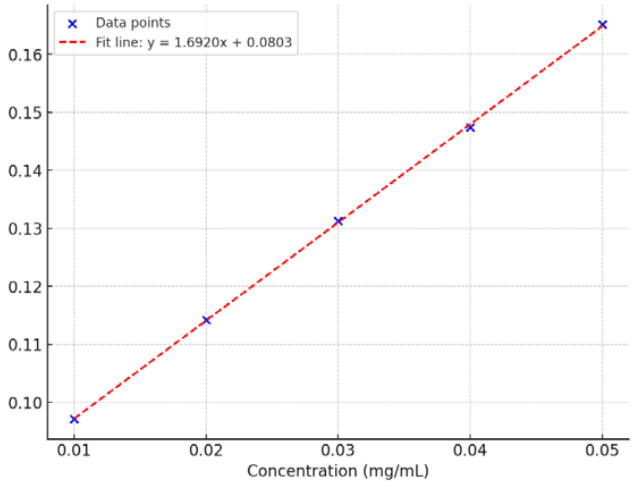


Figure 4. Calibration curve of the ascorbic acid

The calculation of antioxidant activity was based on the optical density (absorbance) values of the analyzed samples (Table 4). The measured absorbance values were applied to the equation derived from the calibration curve. This enabled the quantification of antioxidant activity (Figure 5) by relating the sample's absorbance to the known antioxidant concentration, fulfilling the objective of the analysis.

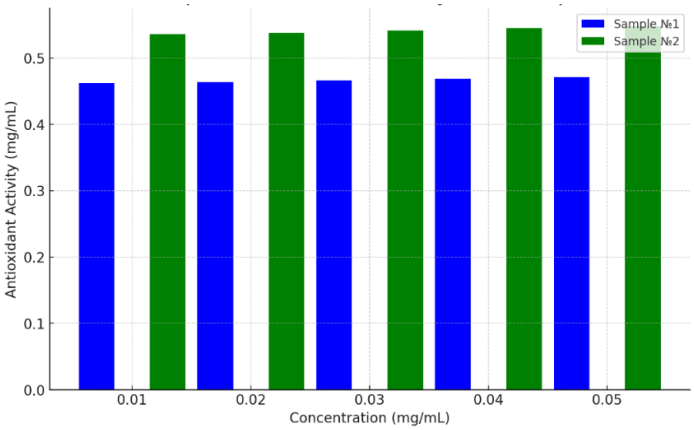


Figure 5. Comparative Antioxidant Activity: Sample 1 (*Hippophae rhamnoides* L.) and Sample 2 (*Crataegus sanguinea* L.)

Table 4. Results of Antioxidant Activity Determination of Extracts: Sample 1 (*Hippophae rhamnoides* L.) and Sample 2 (*Crataegus sanguinea* L.)

Concentration (mg/ml)	Absorbance (711 nm)		Antioxidant activity (mg AAE/ml extract)	
	Sample 1	Sample 2	Sample 1	Sample 2
0.01	0.8623	0.9864	0.4622	0.5355
0.02	0.8651	0.9901	0.4639	0.5377
0.03	0.8686	0.9963	0.4659	0.5414
0.04	0.8733	1.0023	0.4687	0.5449
0.05	0.8776	1.0067	0.4712	0.5475

As shown in Table 4, an increase in extract concentration from 0.01 to 0.05 mg/mL results in higher antioxidant activity. The fruits of *Crataegus sanguinea* L. exhibit greater antioxidant activity compared to the fruits of *Hippophae rhamnoides* L.

Conclusion.

The analyzed plant fruit samples revealed the presence of various essential macro- and microelements, as well as flavonoids with antioxidant activity. The flavonoid content, and consequently the antioxidant activity, was slightly higher in the fruits of *Crataegus sanguinea* L. compared to those of *Hippophae rhamnoides* L.

Extracts from medicinal plants represent an accessible and cost-effective source of biologically active compounds (Donkor, et all., 2023). Flavonoids exhibit anti-inflammatory effects and support lipid and carbohydrate metabolism. Currently, flavonoids are considered a crucial component of various cosmetic, pharmaceutical, and medicinal products. Quercetin has been shown to possess antioxidant, antifungal, anticancer, hepatoprotective, and cytotoxic activities (Batiha, et all., 2020).

Plant-based antioxidants reduce oxidative stress and prevent DNA damage (Shubayr, 2023).

The results obtained indicate that the extracts of *Hippophae rhamnoides* L. and *Crataegus sanguinea* L. fruits have potential applications in the development of therapeutic, preventive, and cosmetic products (Silva, et all., 2023).

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