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# SURVEY, SELECTION, AND STUDY OF PROMISING FORMS OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) IN BURABAY NATIONAL PARK

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**Abstract**

This study aimed to conduct an ecological and biological survey of natural populations of sea buckthorn and select promising forms in the Burabai National Park of the Republic of Kazakhstan. The selection was based on the pomological and antioxidant properties of six forms (Hr-01, Hr-02, Hr-03, Hr-04, Hr-05, and Hr-06). The results showed that the highest fruit weight was found in form Hr-1 (21.7±0.8 g), and the lowest fruit weight was found in form Hr-5 (9.3 g±0.09). Fruit length ranged from 5.6 to 8.4 mm, diameter ranged from 4.1 to 6.1 mm, and weight ranged from 9.3 g to 21.7 g. The highest content of total polyphenol was found in forms Hr-04, Hr-02, and Hr-05 (13.60, 12.82, and 12.68 GAE/g, respectively). According to the results of DPPH and FRAP tests, four sea buckthorn forms had high antioxidant activity (Hr-06, Hr-05, Hr-02, Hr-01). The results revealed significant variation in fruit size, weight, and antioxidant activity among the forms. Forms Hr-01, Hr-02, and Hr-06 showed superior pomological characteristics, while Hr-05 and Hr-06 exhibited the highest antioxidant activities and polyphenol content. These selected forms demonstrate potential for further research under ex-situ conditions for applications in agriculture and health promotion.

**Key words**: Burabay National Park, *Hippophae rhamnoides* L., Antioxidant activities, Total polyphenols.

## Introduction

Burabay National Park is situated in the Akmola region of northern Kazakhstan. Its territory is on the Kokshetau Upland, which belongs to the Kazakh hilly area. The park has 14 lakes, including Burabai, Shabakty, Ainakol, Balpash Sor, Maibalyk, and Katarkol. The Kokshetau upland falls under the continental steppe region and has a sharply continental climate. The climate is characterized by a significant difference in winter and summer temperatures and day and night temperatures. The area has a high runoff coefficient due to deep freezing and rapid snowmelt. The average annual temperature in the park ranges from 1 to 20°C. The absolute minimum temperature is -51°C, while the maximum temperature is +41°C in July. The park receives an average annual precipitation of 400 mm (Sultangazina *et al.*, 2014).

The national park is located at the junction of steppe and forest-steppe zones, making it a favorable habitat for the growth of many different species of flowering plants. The park has various plant communities, where flowering plants of interest can be found in forests, steppes, and coastal water areas. Birch-pine forests in the park have a diverse undergrowth of plant species, including *Ribes nigrum* L., *Rosa acicularis* Lindl., *Rosa majalis* Herrm., *Cotoneaster melanocarpus* (Bunge) Fisch. ex Loudon, *Sorbus sibirica* (Hedl.) Prain, *Padus avium* Mill., *Rubus idaeus* L., *Cerasus fruticosa* (Pall.) Borkh., *Crataegus chlorocarpa* Gand., *Crataegus sanguinea* Pall., *and Crataegus altaica* (Loudon) Lange. The undergrowth of birch forests consists of species such as *Rosa acicularis* Lindl., *Rosa majalis* Herrm., *Cotoneaster melanocarpus* (Bunge) Fisch. ex Loudon, *Rubus idaeus* L., and *Ribes nigrum* L. In steppe communities, species of the genus rosehip, such as *Rosa spinosissima* L. and *R. Laxa* Retz., can be found. Sea buckthorn is a coastal-water plant that grows on the shores of forest and steppe lakes. It is an adventive plant, an agriophyte, which has naturalized and inhabited the shores of Lake Ainacol. Other species of agriophytes growing in the park include *Berberis vulgaris* L., *Malus baccata* (L) Borkh., *Cerasus tomentosa* (Thunb.) Loisel., *Elaegnus oxycarpa* Schltdl., and *Ribes aureum* Pursh (Sultangazina *et al.*, 2014; Anon., 2021).

*Hippophae rhamnoides* L. is a polymorphic tertiary-relict species that belongs to the family *Elaeagnaceae* Lineal. It has a fragmentary distribution in Europe and Asia (Rongsen, 1997; Lian *et al.*, 2000; Bartish *et al.*, 2000; Bartish *et al.*, 2002; Swenson & Bartish, 2002; Vdovina, 2011) and is naturally found in floodplains and river banks, on stony and rubbly soils of the eastern hilly area, Zaisan, Balkhash–Alakol, in the mountain systems of Altai, Tarbagatai, Zhongar, Ile, Kungei, Terskey, Kirghiz Alatau, Karatau, and Western Tien Shan (Dzhangaliev *et al.*, 2001; Vdovina, 2011). This plant has been the subject of many studies in the last few decades, focusing on its agricultural, nutritional, medicinal, and ornamental value (Süleyman *et al.*, 2001; Geetha *et al.*, 2002; Gupta *et al.*, 2005; Suomela *et al.*, 2006; Bal *et al.*, 2011).

Sea buckthorn (*Hippophae rhamnoides* L.) is unique in its diversity of practical uses. Biologically active substances in its fruits can increase human immunity, strengthen the cardiovascular system, and reduce cancer risk (Suomela *et al.*, 2006; Xu, *et al.*, 2011). Its fruits are used to make jam, pastilla, confit, puree, jelly, compotes, and a variety of alcoholic beverages. Sea buckthorn preparations are widely used in cosmetics and perfumery. This plant is also used for the reclamation of man-made landscapes and in anti-erosion plantations, as it enriches the soil with nitrogen and possesses a highly branched root system. The original color of leaves combined with densely arranged bright fruits, which remain on the branches almost throughout the winter, make this species promising for landscaping.

The aim of the research conducted in the national park "Burabay" was to survey, select, and study promising forms of sea buckthorn as source material for breeding.

## Material and Methods

The study was conducted on naturalized sea buckthorn plantings, where sea buckthorn forms were chosen from three coenopopulations on the southeastern shore of Lake Ainacol (Fig. 1, Table 1). The data was collected by taking route walks with sample plots established at each location (Ramensky, 1971; Koikov, 1978). These plantings were surveyed using geobotany methods, and the GPS coordinates of the plots were recorded (Sukachev,1957). The parameters measured for each bush included the bush height, crown diameter, age, sanitary condition, and yield score (Kondrashov, 1977). The approximate age of the plant was determined by the number of annual wood growth, starting from yearly shoots. The yield score was categorized on a scale of 1 to 5, based on the fruit yield of each plant. Fruits were harvested when fully ripe, and forms were named Hr-01, Hr-02, Hr-03, Hr-04, Hr-05, and Hr-06. The GPS coordinates and altitude of the selected sea buckthorn forms are presented in Table 1. One hundred fruits were collected from each form. The fruit samples were stored in a freezer at -18°C to analyze the sum of phenolic compounds and antioxidant activity.

**Morphological analyses:** The experiment involved weighing 100 fresh berries using a KERN EMB 200-2 analytical scale. The height and diameter of the berries were also measured using a high-precision electronic caliper. The Fruit Shape Index (FSI) and fruit shape were determined by the ratio of fruit length and diameter. The fetuses were classified into rounded (1.00-1.19) and oval (1.20-1.51) based on their FSI value. Three replicates were used for each measurement.

**Table 1. GPS coordinates and altitude of *Hippopaea ramnoides* forms.**

| Genotypes | Forestry | Cordinates | | Altitude (m) |
|---|---|---|---|---|
| | | North | East | |
| Hr-01 | Burabay | 53˚06’7709 | 070˚18’8757 | 181 |
| Hr-02 | Burabay | 53˚06’6853 | 070˚18’7375 | 174 |
| Hr-03 | Burabay | 53˚06’6881 | 070˚18’7243 | 174 |
| Hr-04 | Burabay | 53˚06’6856 | 070˚18’6866 | 175 |
| Hr-05 | Burabay | 53˚06’6725 | 070˚18’6727 | 174 |
| Hr-06 | Burabay | 53˚06’6966 | 070˚18’7961 | 175 |

The study also included measuring phenotypic characteristics such as bush age and height, shape, type, crown density and diameter, angle of skeletal branches, degree of damage by diseases and pests, presence of thorns, length of annual shoots, degree of bush fruiting, length of stalk, color, shape, size, weight, and taste of fruits, fruit shape index (height, and diameter of fruits), force of fruit detachment from the branch, color and shape of seeds.

The sea buckthorn fruits were categorized into three groups based on their size, small (<7 mm in length), medium (from 7 to 9 mm), and large (> 9 mm). The fruits were also classified into 5 groups based on their color, yellow, yellowish-orange, orange, reddish-orange, and red. The morphological characters were described using the method of Kondrashov (Kondrashov, 1977).

**Chemical analyses:** Extraction procedure: 5 g of sea buckthorn samples were extracted in 50 ml of 96 % ethanol, then evaporated to dryness in the water bath (40°C). The dry extract was used to determine total phenolic content and total antioxidant capacity (TAC) using DPPH and FRAP methods. The TAC and total phenolic content (TPC) Folin–Ciocalteu assays were performed in a 96-well round-bottom microplate in a Thermo Scientific Multiskan FC (Vantaa, Finland).

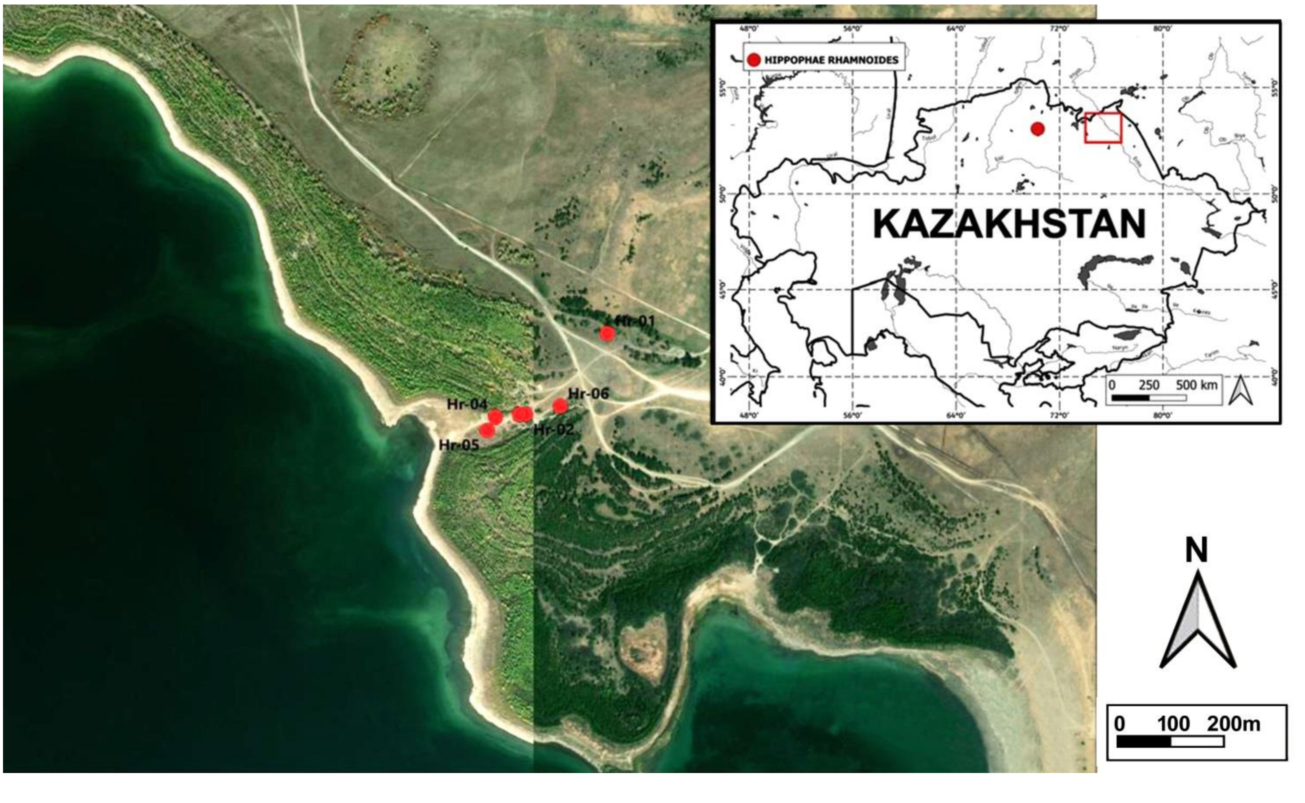


Fig. 1. Map of *Hippophae rhamnoides* forms’ locations (Hr-01, Hr-02, Hr-03, Hr-04, Hr-05, and Hr-06) in Burabay National Park.

**Total phenolic compounds** (TPC) were determined by the Folin–Ciocalteu procedure (Hagerman *et al.*, 2000) with some modifications. Aliquots (40µl) of ethanol extracts were mixed with 210µl of sodium carbonate solution -Na2CO3 (1:10), incubated for 10 min at 38°C, added 50 µl Folin-Ciocalteu reagent (1:6 with ethanol), and allowed to react for 15 min at room temperature before absorbance was measured at 690 nm. The amount of total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions (covering the concentration range between 0.1-0.005 mg/ml) and expressed as mg gallic acid/g dry extract. All measurements were done in triplicate.

**DPPH assay:** The free radical scavenging activity of the sea buckthorn fruits was measured following the method described by (Reis *et al.*, 2012) with some modifications. For the test, sea buckthorn extracts with the following dilutions (1-0.025). Three wells of the microplate were loaded with 20 µl of sample extract and 300µl of DPPH (1,1-diphenyl-2-picryl-hydrazyl) (only), another three wells were filled with 20 µl of solvent and 300µl of DPPH (control). The microplate was incubated at room temperature for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. All measurements were taken in triplicate.

**FRAP assay:** The ferric-reducing antioxidant power of the sea buckthorn extracts was evaluated as described by Benzie & Strain (1996) with some modifications. Briefly, 300 mM acetic acid buffer (pH 3.6), 20 mM $FeCl_3$, and 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (10:1:1, v/v/v) in 40 mM HCl were mixed and then warmed in water at 37°C for 5 min to prepare the working reagent of FRAP. 0.8 mM $FeSo_4$ (1:5, v/v) solution was used as a positive control. The sea buckthorn extract samples 35 µL were mixed with 265 µL of FRAP solution, and then the absorbance of the mixture was measured at 620 nm after reacting at 37°C for 4 min. The FRAP values of the sea buckthorn extracts were expressed as µM $FeSo_4$/g dry extract. All measurements were taken in triplicate.

**Statistical analysis:** The obtained data was processed using the *Excel* descriptive statistical program. The average of 100 fruits harvested from each form was expressed as the mean and standard deviation. The coefficient of variation was determined by the formula: CV = SD/X*100. Cophenetic correlation was calculated using the ultrametric distance method using the VARCOV module in the NTSYSpc program (Applied Biostatistics Inc., ABD). The dendrogram was reconstructed by an unweighted pairwise grouping using the arithmetic mean (UPGMA) as a clustering algorithm.

## Results and Discussions

Cenopopulation 1 is located on the northeastern lakeside terrace of Lake Ainacol. The site measured 20x20, and the community was a dry meadow consisting of birch trees (*Betula pendula* Roth). The tiers of shrubs and semi-shrubs included *Spiraea hypericifolia* L., and *Rosa majalis* Herrn. (20 bushes), *Crataegus sanguinea* Pall. (10 bushes), *Lonicera tatarica* L. (9 bushes), *Thymus asiaticus* Serg.

Cenopopulation 2 is a sea buckthorn community located along Lake Ainacol. The sample plot measured 10x15, and there were about 70 bushes of sea buckthorn. The community was a mixture of pine trees (*Pinus sylvestris* L. H-6-8 m.) and birch trees (*Betula pendula* Roth. H-6 m.), along with shrubs such as *Hippophae rhamnoides* L. and *Crataegus sanguinea* Pall.

Cenopopulation 3 was a birch-buckthorn community with excellent regeneration. The height of the sea buckthorn ranges from 1.5 to 2 meters, and the diameter of the species trunks is 8 to 15 cm.

All three cenopopulations contain plants were 12 to 20 years old, with individuals up to 10 years old accounting for almost 80% of all plants. The condition of the sea buckthorn was good, with an average score of 1.03 (excluding deadwood). The height of the sea buckthorn ranged from 0.9 to 2.6 meters, the trunk diameter from 1 to 10 cm, and the crown width from 0.6 to 1.5 meters.

**Morphological analyses of the sea buckthorn forms:** Table 2 presents data on 100 fruit weight, fruit shape, color of fruit skin, growth type, and shape of forms. The weight of 100 fruits ranged from 9.3 g (Hr-5) to 21.7 g (Hr-1), indicating a threefold difference between the Hr-5 and Hr-1 forms. Most of the forms had oblong fruit shapes, followed by cylindrical and round shapes. All selected forms from Hr-1 to Hr-6 were shrubs, free from thorns, diseases, and pests, and had distinct flavors (as described in Table 2). Further details on the forms can be found below. The shape of the fruits of the studied *Hippopaea ramnoides* forms is presented in Fig. 2.

Hr-1 was chosen from the first cenopopulation as listed in Table 1. This shrub was a height of 1.9 m, with an oval and compact crown that is medium dense, and a crown diameter of 0.75 m. The angle of departure of skeletal branches was up to 40-45, and the age of the plant was 18 years. The length of annual shoots was around 10-15 cm, and abundant fruiting. The fruits were yellow in color, oval in shape, and medium in size, measuring 0.85±0.02 cm long and 0.60±0.02 cm in diameter. The berries detach together with their pedicle, with a bitter-sour taste-sweet with tartness (The force required for berry detachment was measured using a dynamometer). The seed's color was light brown, and the seed was ovoid. The length of the stalk was 3.8 mm, and the weight of 50 fruits was around 21.7±0.8 g. The FSI is 1.37.

Hr-2 was chosen from the cenopopulation 2. The form was 1.7 m tall, with an oval and compact crown, and was of medium density. Its crown diameter was 0.70 m, and the angle of departure of its skeletal branches was up to 45. The shrub was 15 years old, and its annual shoots were 10-15 cm in length. The shrub had an abundant fruiting capacity, orange in color, oval in shape, medium in size (0.83±0.15 cm in length and 0.59±0.16 cm in diameter), FSI–1.40, and the weight of 100 fruits is 20.3±0.39 g. The berries detach together with their pedicle with a bitter-sour taste, with light brown, ovoid-long shaped seeds.

**Table 2. Variability of six sea buckthorn forms morphological characteristics.**

| Trait name | Hr-01 | Hr-02 | Hr-03 | Hr-04 | Hr-05 | Hr-06 |
|---|---|---|---|---|---|---|
| Fruit weight, g<br>X ± SD<br>Variations limit<br>CV %<br>Confidence level (95.0%) | 21.7 ± 0.8<br>20.2-22.9<br>3.68 %<br>0.23 | 20.3 ± 0.39<br>19.6-20.9<br>1.92 %<br>0.11 | 10.4 ± 0.13<br>10.2-10.7<br>1.25 %<br>0.03 | 15.6 ± 0.19<br>15.2-15.9<br>1.21 %<br>0.05 | 9.3 ± 0.09<br>9.2-9.6<br>0.96 %<br>0.02 | 20,3 ± 0.25<br>20.0-20.9<br>1.23 %<br>0.07 |
| Fruit height (mm)<br>X ± SD<br>Variations limit<br>CV %<br>Confidence level (95.0%) | 8.4 ± 0.16<br>8.2-8.9<br>1.9 %<br>0.04 | 8.3 ± 0.15<br>7.9-8.7<br>1.80%<br>0.04 | 6.4 ± 0.36<br>4.5-6.9<br>5.6%<br>0.10 | 5.7 ± 0.11<br>5.5-5.9<br>1.92%<br>0.03 | 5.6 ± 0.27<br>5.3-5.9<br>4.82%<br>0.07 | 8.2 ± 0.12<br>8.1-8.5<br>1.46<br>0.03 |
| Fruit diameter (mm)<br>X ± SD<br>Variations limit<br>CV %<br>Confidence level (95.0%) | 6.1 ± 0.29<br>5.3-6.4<br>4.75%<br>0.08 | 5.9 ± 0.16<br>5.7-6.3<br>2.71%<br>0.04 | 4.6 ± 0.19<br>4.2-4.9<br>4.13%<br>0.05 | 5.6 ± 0.08<br>5.4-5.7<br>1.42%<br>0.02 | 4.1 ± 0.14<br>4.0-4.3<br>3.41%<br>0.04 | 5.7 ± 0.06<br>5.6-5.8<br>1.05%<br>0.02 |
| FSI* | 1.37 | 1.40 | 1.39 | 1.0 | 1.36 | 1.43 |
| Fruit shape | Oval | Oval | Oval | Round | Cylindrical | Oval |
| Fruit skin color | Yellow | Orange | Reddish orange | Yellowish orange | Red | Orange |
| Thorns | Absent | Absent | Absent | Absent | Absent | Absent |
| Fruit flavor | BS with tartness | BS* | BSW* | S* | B* | BS* |

BS = Bitter sour, BSW = Bitter-sweet, S = Sour, B = Bitter; X = Mean; SD = Standard deviation; CV% = Coefficient of variation; FSI = Fruit Shape Index (fruit height/fruit diameter)

Hr-3 was chosen from the cenopopulation 2. The form was 2 m tall, with a medium-dense, rounded, and spreading crown, measuring 1.8 m in diameter. The angle of departure of skeletal branches was up to 60, and the plant was 18 years old. The length of annual shoots was between 7-12 cm, and the degree of fruiting of the plant was abundant. The length of the fruit stalk was 3.8 mm, and the color of the fruits was reddish-orange. The shape of the fruits was oval and small, measuring 0.64±0.36 cm in length and 0.46±0.19 cm in diameter, FSI-1.39. The fruit weight was 10.4±0.13 g per 100 fruits, and the berries detached together with their pedicle. The taste of the fruit was bittersweet, seeds were light brown and ovoid-long in shape.

Hr-4 was chosen from the cenopopulation 2. The form was 2.5 m tall, with a round and spreading crown that was of medium density. The diameter of the crown was 1.70 m, and the angle of departure of skeletal branches was up to 45. The plant was 20 years old. The length of the annual shoots was 10-15 cm. The degree of fruiting was abundant. The stalk was 3.7 mm, fruit was yellowish orange, round in shape, and small, measuring 0.57±0.11 cm in length and 0.56±0.08 cm in diameter. The Fruit Surface Index (FSI) was 1.0. The weight of 100 fruits was 15.6±0.19 g. The fruit detaches without the pedicle. The fruit tasted sour, while the seed was dark brown and round.

Hr-5, a shrub, was chosen from cenopopulation 2 (Table 1). It was 1.2 meters tall with an oval and compact crown, having a medium density. The crown's diameter was 0.60 m, and the angle of departure of skeletal branches was up to 70-80. The plant was 15 years old, and the annual shoots were 7-10 cm long. The plant produced an abundant amount of fruit, and the length of the stalk was 3.7 mm. The fruits were red, cylindrical in shape, and small in size, measuring 0.56±0.27 cm in length and 0.41±0.14 cm in diameter. The FSI-1.36, and the weight of 100 fruits was 9±0.02 g. The berries detached together with their pedicle, with a bitter taste. The seeds are light brown in color and ovoid-long in shape.

Hr-6 was selected from the cenopopulation 3 (Table 1). The form was 1.8 m high, crown oval and compact, medium dense, crown diameter -0.80 m, angle of departure of skeletal branches up to 40-45 $^{0C}$, age-28 years, and the length of annual shoots-12-18 cm, degree of fruiting of the plant - abundant, length of the stalk - 3.8 mm, fruits orange, oval, medium, 0.82±0.12 cm in length and 0.57±0.06 cm in diameter, FSI-1.43, weight of 100 fruits -20.3±0.25 g, the berry detaches together with their pedicle, fruit taste - bitter-sour, seed color - light brown, seed shape - ovoid-long.

According to the results, sea buckthorn forms were highly diverse in most morphological traits. This was consistent with previous studies conducted in different countries, which also reported high diversity in most of the morphological traits of sea buckthorn (Yadav *et al.*, 2006; Zheng *et al.*, 2012; Sezen *et al.*, 2015; Li *et al.*, 2020). For instance, Singh and Singh (2004) reported that most sea buckthorn genotypes had a shrub-type growth, although trees were also found (Singh & Singh, 2004). In a study on purposeful selection work conducted by Vdovina (Vdovina *et al.*, 2024), the weight of sea buckthorn fruits from natural populations in the East Kazakhstan region ranged from 27.2 g to 65.5 g. The selected forms of sea buckthorn also exhibited differences in fruit length and diameter, with lengths varying from 6.1 mm to 12.8 mm and diameters ranging from 5.8 mm to 7.5 mm. In an earlier study, Sezen *et al.*, (2015) found that most sea buckthorn genotypes have a low to medium number of thorns. Our selected forms, on the other hand, are thornless, which is important for berry harvesting.

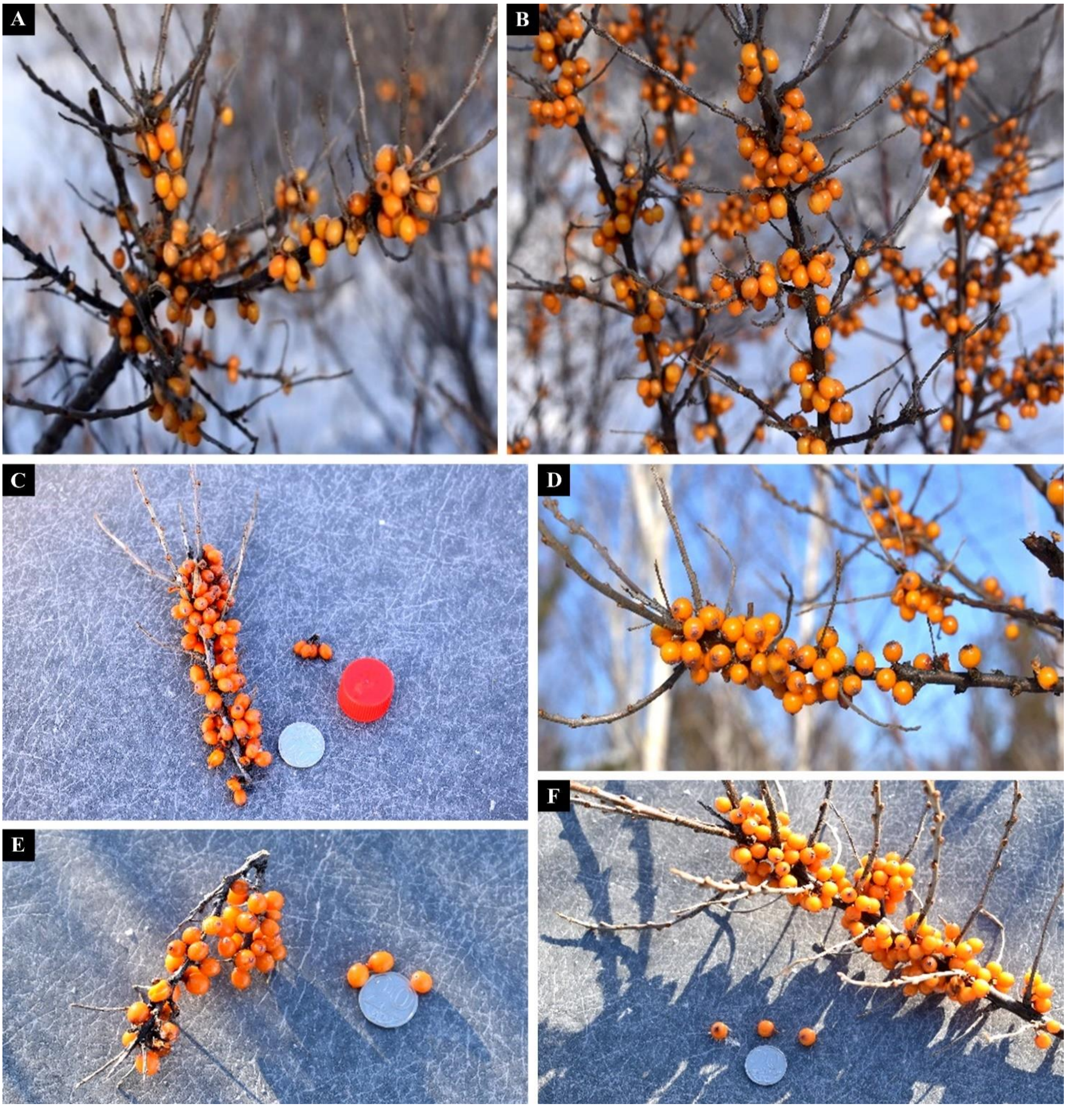


Fig. 2. The selected forms of the sea buckthorn in the Burabay National Park: A, Hr-01; B, Hr-02; C, Hr-03; D, Hr-04; E, Hr-05; F, Hr-06 (photo by Mukan G.S.).

Total polyphenols and antioxidant activity of the sea buckthorn forms: In recent decades, there has been a growing interest in healthy lifestyles and diets, leading to increased scientific and practical exploration of foods rich in natural antioxidants (Kumar *et al.*, 2011). In this context, sea buckthorn fruit (Hippophae rhamnoides L.) stands out as a "superfruit," packed with a variety of biologically active substances, including antioxidants such as ascorbic acid, carotenoids, and polyphenolic compounds (Chen *et al.*, 2013; Sytařová *et al.*, 2019). Polyphenolic substances exhibit a wide range of biochemical activities, demonstrating primarily antioxidant, antimutagenic, and anticarcinogenic properties, along with the ability to alter gene expression (Gao *et al.*, 2000; Ercisli *et al.*, 2007; Sytařová *et al.*, 2019). Numerous studies have indicated that sea buckthorn fruits possess the highest antioxidant activity compared to other plant materials (Miller *et al.*, 2000; Guo *et al.*, 2017).

The content of polyphenols and antioxidant capacity (measured by DPPH and FRAP values) were measured in sea buckthorn fruits, and all three parameters showed significant differences between the tested genotypes. The polyphenol content ranged from 5.3 to 13.60 mg GAE/g DW (Table 3). Five sea buckthorn genotypes (Hr-01, Hr-06, Hr-05, Hr-2, Hr-04) showed high polyphenol content (11.26, 11.98, 12.68, 12.82, 13.60 mg GAE/g DW, respectively). The total content of phenolic compounds in the selected forms of sea buckthorn fruits was comparable to that of European sea buckthorn varieties (Criste *et al.*, 2020). However, it significantly exceeded the values found in Russian sea buckthorn varieties (Zemtsova *et al.*, 2016).

**Table 3. Total polyphenols and antioxidant activity of sea buckthorn fruits of six different forms.**

| Chemical traits | Hr-01 | Hr-02 | Hr-03 | Hr-04 | Hr-05 | Hr-06 |
|---|---|---|---|---|---|---|
| Total Phenolic Content (mg GAE/1 g DW) | 11.26c | 12.82b | 5.31d | 13.60a | 12.68b | 11.98c |
| DPPH, % | 25d | 24d | 12e | 57c | 61b | 79a |
| FRAP (µM Ferrous/100 g DW) | 93c | 106b | 42f | 84d | 196a | 85d |

Different superscripts in each line indicate significant differences in the mean at p<0.05

Alcoholic extracts of six forms of sea buckthorn were examined for antioxidant properties using DPPH and FRAP methods, and total phenolic content was measured. Hr-04 had the highest value of 13.60 mg GAE/1 g DW. The descending order is Hr-04 (13.60) > Hr-02 (12.82) > Hr-05 (12.68) > Hr-06 (11.98) > Hr-01 (11.26) > Hr-03 (5.31) (Fig. 3). Form Hr-03 shows significantly lower phenolic content of 5.31 mg GAE/1 g DW. Hr-06 shows maximum antioxidant activity of 79%. The descending order is Hr-06 (79%) > Hr-05 (61%) > Hr-04 (57%) > Hr-01 (25%) ≈ , Hr-02 (24%) > Hr-03 (12%). The form Hr-06 has the highest activity (79%), and Hr-05 has the second highest activity (61%). Forms Hr-01, Hr-02, and Hr-03 have lower values, which may indicate that they are less efficient in trapping DPPH radicals.

For FRAP results, Hr-05 leads with 196 µM Ferrous/100 g DW. The descending order is: Hr-05 (196) > Hr-02 (106) > Hr-01 (93) > Hr-06 (85) ≈ Hr-04 (84) > Hr-03 (42) (Fig. 4). Notably, Hr-05 significantly outperforms the other forms in FRAP, although it is inferior to Hr-06 in DPPH. Only the Hr-03 form exhibited low antioxidant activity under the DPPH and FRAP assay methods.

Some studies have suggested that there is a direct correlation between the amount of phenolic compounds and antioxidant activity (Dlugosz *et al.*, 2006; Wojdyło *et al.*, 2007). However, other researchers have claimed that there is no such relationship between these two factors (Hassimoto *et al.*, 2005; Harish & Shivanandappa, 2006). It can be argued that, besides phenolic compounds, other chemical compounds may also impact antioxidant activity (Wojdyło *et al.*, 2007). Therefore, in our study, we concluded that the content of polyphenols in sea buckthorn forms alone might not be the main reason for their antioxidant activity. We found that the Pearson correlation coefficients (r) between the total polyphenol content of selected forms and antioxidant activity in two tests, DPPH and FRAP, were moderate (r = 0.624, 0.596, respectively) (Table 4).

Clustering analysis of the data revealed that *Hippophae rhamnoides* samples could be classified based on their polyphenol content and antioxidant activity, which differed depending on the form. The dendrogram we obtained showed common characteristics of the forms, which enabled us to identify samples with high polyphenol content and high antioxidant activity (as shown in Fig. 5). According to the dendrogram, forms Hr-1, Hr-2, Hr-4, and Hr-6 were grouped into clades, while forms Hr-3 and Hr-5 were exceptions. Form Hr-3 had the lowest polyphenol content (5.31 mgGAE/1 g dry matter) and low values of antioxidant activity (12% DPPH and 42 µM Ferrous equivalents/100 g). On the other hand, the Hr-5 form had high polyphenol content (12.68 mgGAE/1 g dry matter) and high antioxidant activity in both tests (62% DPPH and 196 µM Ferrous equivalents/100 g) compared to the other forms studied.

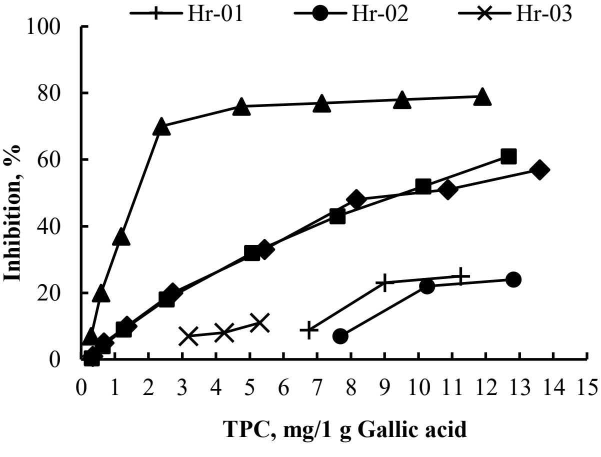


Fig. 3. DPPH radical scavenging activity of sea buckthorn forms. Data are presented as the percentage of DPPH radical scavenging.

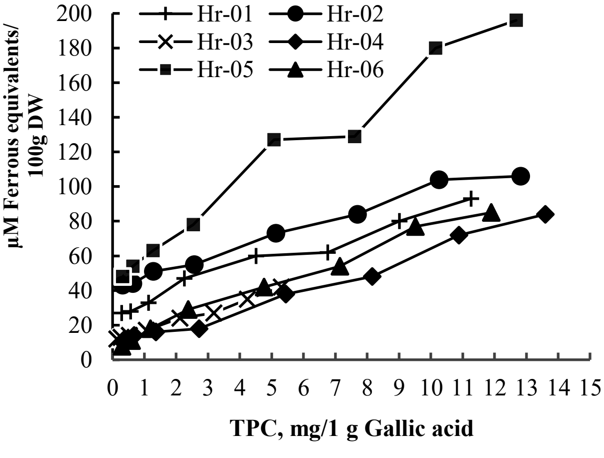


Fig. 4. Ferric reducing power of sea buckthorn forms. Data are presented as µM Ferrous equivalents/100g.

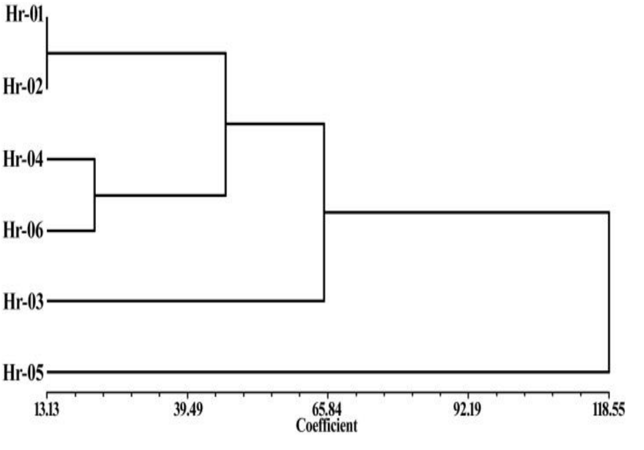


Fig. 5. Results of clustering of sea buckthorn forms based on the ratio of quantitative content of polyphenols and antioxidant activity.

**Table 4. Pearson correlations between morphological characteristics and biochemical properties (DPPH, FRAP, total polyphenols) of the 6 studied sea buckthorn forms.**

| Correlation matrix [Pearson (n)]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variables** | **ALT** | **FW** | **FH** | **FD** | **FSI** | **TPC** | **DPPH** | **FRAP** |
| ALT | 1 | 0,591 | 0,494 | 0,513 | 0,051 | 0,071 | -0,215 | -0,148 |
| FW | 0,591 | 1 | 0,833 | 0,953 | -0,004 | 0,388 | 0,026 | 0,030 |
| FH | 0,494 | 0,833 | 1 | 0,650 | 0,544 | -0,021 | -0,248 | 0,001 |
| FD | 0,513 | 0,953 | 0,650 | 1 | -0,282 | 0,485 | 0,048 | -0,084 |
| FSI | 0,051 | -0,004 | 0,544 | -0,282 | 1 | -0,597 | -0,387 | 0,056 |
| TPC | 0,071 | 0,388 | -0,021 | 0,485 | -0,597 | 1 | 0,624 | 0,596 |
| DPPH | -0,215 | 0,026 | -0,248 | 0,048 | -0,387 | 0,624 | 1 | 0,806 |
| FRAP | -0,148 | 0,030 | 0,001 | -0,084 | 0,056 | 0,596 | 0,806 | 1 |

Values in bold are different from 0 with a significance level alpha = 0.95, ALT = Altitude, FW = Fruit weight, FH = Fruit height, FD = Fruit diameter, FSI = Fruit shape index, TPC = Total phenolic content

## Conclusion

A study conducted on six different forms of sea buckthorn selected from Burabay National Park yielded promising results. All the forms exhibited significant variability in their polyphenol content, antioxidant activity, and pomological characteristics, making them highly valuable for future breeding programs aimed at various objectives.

The selected forms of sea buckthorn (*Hippophaë rhamnoides* L.) demonstrated a total polyphenol content comparable to European sea buckthorn varieties but significantly higher than that of Russian varieties. Two tests confirmed the antioxidant activity of the selected forms. In the FRAP test, which evaluated phenolic-type antioxidants, the sea buckthorn forms were ranked as follows: Hr-05 > Hr-02 > Hr-01 > Hr-06 > Hr-04 > Hr-03. The DPPH test, which also assessed antioxidant activity, ranked the varieties as: Hr-06 > Hr-05 > Hr-04 > Hr-01 > Hr-02 > Hr-03. Among the selected forms- Hr-01, Hr-02, Hr-04, Hr-05, and Hr-06, all except Hr-03 demonstrated pronounced pomological and antioxidant properties. Consequently, these forms will be introduced into *ex-situ* culture for further study.

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## References

Anonymous. 2021. Plants of the World Online. facilitated by the Royal Botanic Gardens, Kew. Published on the internet, http://www. plantsoftheworldonline.org/.

Bal, L.M., V. Meda, S.N. Naik and S. Satya. 2011. Sea buckthorn berries: a potential source of valuable nutrients for nutraceuticals and cosmeceuticals. *Food Res. Int.,* 44: 1718-1727.

Bartish, I.V., N. Jeppsson and G.I. Bartish. 2000. Inter and intraspecific genetic variation in *Hippophae* (*Elaeagnaceae*) investigated by RAPD markers. *Plant Syst. Evol*., 225(1-4): 85-101.

Bartish, I.V., N. Jeppsson and H. Nybom. 2002. Phylogeny of *Hippophae* (*Elaeagnaceae*) inferred from parsimony analysis of chloroplast DNA and morphology. *Syst Bot*., 27(1): 41-54.

Benzie, I. and J. Strain. 1996. The Ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power: The FRAP assay". *Anal. Biochem.,* 239: 70-76.

Chen, C., X.M. Xu, Y. Chen, M.Y. Yu, F.Y. Wen and H. Zhang. 2013. Identification, quantification, and antioxidant activity of acylated flavonol glycosides from sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*). *Food Chem*., 141(3): 1573-1579.

Criste, A., A.C. Urcan, A. Bunea and F.R.P. Furtuna. 2020. Phytochemical composition and biological activity of berries and leaves from four Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Molecules*, 25: 1170-1192.

Dlugosz, A., J. Lembas-Bogaczyk and E. Lamer-Zarawsko. 2006. Antioxidant increases ferric reducing antioxidant power (FRAP) even stronger than vitamin C. *Acta Pol. Pharm*., 63: 446-448.

Dzhangaliev A.D., T.N. Salova and R.M. Turechanova. 2001. Wild fruit plants of Kazakhstan. *KazgosINTI: Almaty, Kazakhstan*, p. 135.

Ercisli, S., E. Orhan, O. Ozdemir and M. Sengul. 2007. The genotypic effects on the chemical composition and antioxidant activity of sea buckthorn (*Hippophae rhamnoides* L.) berries grown in Turkey. *Sci Hort. Amsterdam,* 115: 27-33.

Gao, X., M. Ohlander, N. Jeppsson, L. Björk and V. Trajkovski. 2000. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *J. Agr. Food Chem*., 48(5): 1485-1490.

Geetha, S., M.S. Ram, V. Singh, G. Ilavazhagan and R.C. Sawhney. 2002. Antioxidant and immunomodulatory properties of Sea buckthorn (*Hippophae rhamnoides*) *In vitro* study. *J. Ethnopharmacol*., 79: 373-378.

Guo, R., X. Chang, X. Guo, C.S. Brennan, T. Li, X. Fu and R.H. Liu. 2017. Phenolic compounds, antioxidant activity, antiproliferative activity, and bioaccessibility of Sea buckthorn (*Hippophaë rhamnoides* L.) berries as affected by *In vitro* digestion. *Food Funct*., 8(11): 4229-4240.

Gupta, A., R. Kumar, K. Pal, P.K. Banerjee and R.C. Sawhney. 2005. A preclinical study of the effects of Sea buckthorn (*Hippophae rhamnoides* L.) leaf extract on cutaneous wound healing in albino rats. *Int. J. Low Extr. Wound.,* 4: 88-92.

Hagerman, A., I. Harvey-Mueller and H.P.S. Makkar. 2000. Quantification of tannins in tree foliage laboratory manual. FAO/IAEA, Vienna, pp. 4-7.

Harish, R. and T. Shivanandappa. 2006. Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri. Food Chem.,* 95: 180-185.

Hassimotto, N.M.A., M.I. Genovese and F.M. Lajolo. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agri. Food Chem.*, 53: 2928-2935.

Koikov, N.T. 1978. Features of taxation of natural thickets of sea buckthorn. Sea buckthorn.- Moscow: Forest Industry, 25-33.

Kondrashov, V.T. 1977. To the methodology of description of wild forms of Sea buckthorn. *Plant Resour*., 13(1): 140-144.

Kumar, M.S.Y., R. Dutta, D. Prasad and K. Misra. 2011. Subcritical water extraction of antioxidant compounds from Seabuckthorn (*Hippophae rhamnoides*) leaves for the comparative evaluation of antioxidant activity. *Food Chem*., 127(3): 1309-1316.

Li, H., C. Ruan, J. Ding, J. Li, L. Wang and X. Tian. 2020. Diversity in sea buckthorn (*Hippophae rhamnoides* L.) accessions with different origins based on morphological characteristics, oil traits, and microsatellite markers. *Plos One,* 15: e0230356.

Lian, Y.S., S.G. Lu, S.K. Xue and X.L. Chen. 2000. Biology and chemistry of the genus *Hippophae*. Gansu Science Technology Press, Lanzhou, pp. 1-226.

Miller, H.E., F. Rigelhof, L. Marquart, A. Prakash and M. Kanter. 2000. Antioxidant content of whole grain breakfast cereals, Fruits, and vegetables. *J. Amer. Coll. Nutr.*, 19(3): 312S-319S.

Ramensky, L.G. 1971. Selected works: Problems and methods of studying the vegetation cover. L.: Nauka, pp. 334.

Reis, S.F., D.K. Rai and N. Abu-Ghannam. 2012. Water at room temperature as a solvent for the extraction of apple pomace phenolic compounds. *Food Chem.,* 135: 1991-1998.

Rongsen, L. 1997. Eco-geographical distribution of sea buckthorn and prospects of international cooperation. In: (Eds.): Lu., S., M. Li., J. Hu. and S. Liu. *Worldwide Research & Development Seabuckthorn*. Beijing, Chine Science & Technology Press, pp. 11-22.

Sezen, I., S. Ercisli, O. Cakir, A. Koc, E. Temim and A. Hadziabulic. 2015. Biodiversity and landscape use of sea buckthorn (*Hippophae rhamnoides* L.) in the Coruh Valley of Turkey. *Erwerbs-Obstbau*, 57: 23-28.

Singh, V. and R.K. Singh. 2004. Morphobiochemical variations in seabuckthorn (*Hippophae* spp.) populations growing in Lahaul Valley, dry temperate Himalayas. *Ind. For*., 130: 663-672.

Sukachev, V.N. 1957. Methodological guidelines for the study of forest types. M.: ANS SSSR., p. 116.

Süleyman, H., L.O. Demirezer, M.E. Buyukokuroglu, M.F. Akcay, A. Gepdiremen, Z.N. Banoglu and F. Gocer. 2001. Antiulcerogenic effect of *Hippophae rhamnoides*. *Phytother. Res.*, 33: 77-81.

Sultangasina, G.J., I.A. Chrustaleva, A.N. Cuprijanov and C.M. Adekenov. 2014. Flora of Burabay National Nature Park. Novosibirsk. p. 242.

Suomela, J.P., M. Ahotupa, B. Yang, T. Vasankari and H. Kallio. 2006. Absorption of flavonols derived from sea buckthorn (*Hippophaë rhamnoides* L.) and their effect on emerging risk factors for cardiovascular disease in humans. *J. Agri. Food Chem.,* 54: 7364-7369.

Swenson, U. and I.V. Bartish. 2002. Taxonomic synopsis of *Hippophae* (*Elaeagnaceae*). *Nord. J. Bot.*, 22: 369-374.

Sytařová, I., J. Orsavová, L. Snopek, J. Mlček, L. Byczyński and L. Mišurcová. 2019. Impact of phenolic compounds and vitamins C and E on antioxidant activity of sea buckthorn (*Hippophaë rhamnoides* L.) berries and leaves of diverse ripening times. *Food Chem*., 310: 125784.

Vdovina T.A. 2011. Intraspecific diversity of wild-growing sea buckthorn in the East Kazakhstan region and prospects of its introduction. rider, p. 196.

Vdovina, T.A., E.A. Isakova, O.A. Lagus and A.A. Sumbembayev. 2024. Selection assessment of promising forms of natural *Hippophae rhamnoides* (*Elaeagnaceae*) populations and their offspring in the Kazakhstan Altai Mountains. *J. Biol. Divers*., 25(4): 1809-1822.

Wojdyło, A., J. Oszmian and R. Czemerys. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.*, 105: 940-949.

Xu, Y.J., M. Kaur, S.R. Dhillon, S.P. Tappia and S.N. Dhalla. 2011. Health benefits of sea buckthorn for the prevention of cardiovascular diseases. *J. Fun. Foods,* 3: 2-12.

Yadav, V.K., V.K. Sah, A.K. Singh and S.K. Sharma. 2006. Variations in morphological and biochemical characters of seabuckthorn (*Hippophae salicifolia* D. Don) populations growing in Harsil area of Garhwal Himalaya in India. *Trop. Agric. Res. Ext*., 9*:* 1-7.

Zemtsova, A.Y., Y.A. Zubarev, A.V. Gunin and J.T. Merzel. 2016. Total content of phenolic substances in the fruit varieties of sea buckthorn (*Hippophaë rhamnoides* L.) of different ecological and geographical origin. *Problems of Botany of South Siberia and Mongolia*, 15: 478-479.

Zheng, J., B. Yang, M. Trepanier and H. Kallio. 2012. Effects of genotype, latitude, and weather conditions on the composition of sugars, sugar alcohols, fruit acids, and ascorbic acid in sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) berry juice. *J. Agri. Food Chem*., 60: 3180-3189.

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