CARACTERÍSTICAS BIOLÓGICAS DA PLANTA MEDICINAL *ELAEAGNUS RHAMNOIDES* CULTIVADA NO SUDESTE DO CAZAQUISTÃO

BIOLOGICAL FEATURES OF MEDICINAL PLANT *ELAEAGNUS RHAMNOIDES* GROWING AT SOUTH-EAST OF KAZAKHSTAN

БИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ ОБЛЕПИХИ (*ELAEAGNUS RHAMNOIDES*), ПРОИЗРАСТАЮЩЕЙ В ЮГО-ВОСТОЧНОМ КАЗАХСТАНЕ

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RESUMO

As bagas de *Elaeagnus rhamnoides* contêm quantidades significativas de vitaminas bioativas, lipídios, carotenóides, compostos fenólicos. São amplamente utilizadas como ingrediente em produtos funcionais, cosméticos e formulações farmacêuticas para a prevenção e tratamento de doenças cardiovasculares, estomacais, cutâneas e hepáticas. As populações naturais de espinheiro marítimo são comuns no Cazaquistão. Os dados sobre indicadores fitoquímicos e de diagnóstico estão ausentes para *E. rhamnoides* cultivados no Cazaquistão. Neste trabalho, as bagas do mar do Cazaquistão foram pesquisadas quanto a indicadores de diagnóstico, compostos lipofílicos e hidrofílicos. As análises foram conduzidas para os principais indicadores diagnósticos de folhas e frutos por microtécnicas padrão; perfis de ácidos graxos por cromatografia em fase gasosa; β -caroteno por HPLC-PDA; vitamina C e B por eletroforese capilar e substâncias de pectina por titulação. Os principais indicadores diagnósticos das folhas foram o complexo anômocítico estomático; pêlos corimbósicos e estrelados; feixe condutor - colateral fechada; frutas - passagens secretoras e feixes condutores do tipo espiral no parênquima pulpar e grande quantidade de óleo graxo e grãos de aleuron no embrião. No óleo de polpa / casca de baga, os ácidos graxos dominantes foram os ácidos palmitoléico e palmítico (28,53 e 30,03%, respectivamente). O óleo de semente de espinheiro marítimo, com seus altos níveis de α -linolênico juntamente com uma proporção próxima de 1: 1 de ácidos graxos ω -6: ω -3, representou uma fonte muito equilibrada de ácidos graxos poliinsaturados para a saúde e nutrição humana. O conteúdo de β -caroteno foi de 7,75 mg por kg, substâncias de pectina - 3,27%. Além disso, as bagas eram ricas em vitaminas do complexo B (0,0035-0,014 mg/100g) e vitamina C (0,21 mg/100g). Este trabalho constitui a primeira abordagem do conhecimento sobre o perfil fitoquímico de frutas de *E. rhamnoides* do Cazaquistão e fornece argumentos para o uso múltiplo de *Elaeagnus rhamnoides* tanto para consumo fresco quanto para preparações industriais na forma de compotas e produtos relacionados (alimentos multivitamínicos funcionais) assim como subprodutos (sementes) como matéria-prima para a produção de óleos adequados para serem comercializados pelas indústrias farmacêutica, cosmética e alimentícia.

Palavras-chave: *Elaeagnus rhamnoides*, Plantas medicinais, Fitoquímica, Anatomia, ácidos graxos poliinsaturados.

ABSTRACT

Elaeagnus rhamnoides berries contain significant amounts of bioactive vitamins, lipids, carotenoids, and phenolic compounds. They are widely used as an ingredient in functional products, cosmetics, and pharmaceutical formulations to prevent and treat cardiovascular, stomach, skin, and liver diseases. Natural sea buckthorn populations are widespread in Kazakhstan. Data on phytochemical and diagnostic indicators are absent

for *E. rhamnoides* growing in Kazakhstan. In this work, seaberry from Kazakhstan was surveyed for diagnostic indicators, lipophilic and hydrophilic compounds. Analyses were conducted for main diagnostic indicators of leaves and fruits by standard microtechniques; fatty acid profiles by gas-chromatography; β -carotene by HPLC-PDA; vitamin C and B by capillary electrophoresis, and pectin substances by titration. The main diagnostic indicators of leaves were stomatal anomocytic complex; corymbose and stellate hairs; conductive bundle - closed collateral; fruits - secretory passages and conducting bundles of a spiral type in the pulp parenchyma, and a large amount of fatty oil and aleuron grains in the embryo. In the oil from berry pulp/peel, the dominating fatty acids were palmitoleic and palmitic (28.53 and 30.03 %, respectively). Sea buckthorn seed oil, with its high α -linolenic levels and a near 1:1 ratio of ω -6: ω -3 fatty acids, represented a very balanced source of polyunsaturated fatty acids for human health and nutrition. β -Carotene content was 7.75 mg per kg, pectin substances – 3.27 %. In addition, the berries were rich in vitamins B complex (0.0035-0.014 mg/100g) and vitamin C (0.21 mg/100g). This work constitutes the first approach on knowledge about the phytochemical profile of *Elaeagnus rhamnoides* fruits from Kazakhstan and provides arguments multiple using of *E. rhamnoides*, both for fresh consumption and for industrial preparations in the form of jams and related products (functional multivitamin food) as well as byproducts (seeds) as raw materials for the production of oils suitable to be marketed by the pharmaceutical, cosmetic and food industries.

Keywords: *Elaeagnus rhamnoides*, Medicinal plants, Phytochemistry, Anatomy, polyunsaturated fatty acids.

АННОТАЦИЯ

Ягоды облепихи *Elaeagnus rhamnoides* содержат значительные количества биологически активных веществ - витаминов, липидов, каротиноидов, фенольных соединений и широко используются в функциональных продуктах, косметических средствах и фармацевтических составах для профилактики и лечения заболеваний сердечно-сосудистой системы, желудочно-кишечного тракта, кожи и печени. Естественные популяции облепихи широко распространены в Казахстане. Данные о фитохимических и диагностических показателях для *E. rhamnoides* в Казахстане отсутствуют. В данной работе изучены диагностические показатели, липофильные и гидрофильные соединения облепихи, произрастающей в Казахстане. Основные диагностические показатели листьев и плодов определяли по стандартной микротехнике; профили жирных кислот - методом газовой хроматографии; β -каротин с помощью ВЭЖХ-КПК; витамин С и В - методом капиллярного электрофореза и пектиновые вещества с помощью титрования. Основными диагностическими показателями листьев были: устьичный аппарат аномоцитного типа; щитковидные и звездчатые волоски; закрытый проводящий пучок; плодов - проводящие пучки спирального типа в паренхиме пульпы, а также большое количество жирного масла и алейроновых зерен в зародыше. В масле из мякоти / кожуры доминирующими жирными кислотами были пальмитолеиновая и пальмитиновая (28,53 и 30,03% соответственно). Масло облепихи с высоким содержанием α -линоленовой кислоты и соотношением жирных кислот ω -6: ω -3 около 1: 1 представляет собой очень сбалансированный источник полиненасыщенных жирных кислот для здоровья и питания человека. Содержание β -каротина составило 7,75 мг на кг, пектиновых веществ - 3,27%. Кроме того, ягоды были богаты комплексом витаминов группы В (0,0035-0,014 мг / 100 г) и витамином С (0,21 мг / 100 г). В этой работе впервые изложены данные о фитохимическом профиле плодов *Elaeagnus rhamnoides* из Казахстана и приведены аргументы в пользу их комплексного использования, как для потребления в свежем виде, так и в виде джемов и сопутствующих продуктов (функциональных поливитаминных продуктов питания), а также возможности использования побочных продуктов (семян) в качестве источника масел для фармацевтической, косметической и пищевой промышленности.

Ключевые слова: *Elaeagnus rhamnoides*, облепиха, фитохимия, анатомия, полиненасыщенные жирные кислоты

1. INTRODUCTION:

The properties of *Elaeagnus rhamnoides* L. (Elaeagnaceae) known as sea buckthorn, or Siberian pineapple as a medicinal plant were known even since ancient times, and in 1977 the species received the status of pharmacopeia in China (The State of Pharmacopoeia Commission of People's Republic of China, 1997). The first clinical studies on the medicinal use of *E. rhamnoides* were initiated in Russia during the 1950s (Ding *et al.*, 2016). Now fruits and other

organs of *E. rhamnoides* have been used in traditional medicine, especially in Tibet, Mongolia, China, and Central Asia Luchowski, Pecio, Marciniak, Kontek, and Stochmal, 2019). Sea buckthorn is grown almost worldwide, but in the most significant volumes in Russia and in China (Olas, Skalski, and Ulanowska, 2018).

The popularity of sea buckthorn has been growing, mainly because of the high nutritional value and medicinal properties of its fruit. Sea buckthorn is reported to be a natural reservoir of

many nutrients including vitamins, essential polyunsaturated fatty acids, carotenoids, phytosterols, essential volatiles, flavonoids, phenolics, organic acids (e.g., hydroxybenzoic acid derivatives), and amino acids. Sea buckthorn berries, known as seaberry, or Siberian pineapple have a range of bioactive chemicals (elements and vitamins (especially A, C, and E), lipids, carotenoids, amino acids, unsaturated fatty acids, and phenolic compounds). These compounds exhibit a wide range of anti-inflammatory, anticancer, antioxidant, and anti-atherosclerotic activities. Nowadays, extracts from *Elaeagnus rhamnoides* different parts use as cosmetics, functional food, and pharmaceutical formulation for diseases of the gastrointestinal tract, heart, liver, and skin (Olas *et al.*, 2018).

Simultaneously, raw fruits exhibit biological activity and jams, tinctures, marmalades, liquors, and other food products prepared from them. This makes sea buckthorn an indispensable component of a healthy diet and an ingredient in functional products.

E. rhamnoides in ecological respect to a very ductile appearance grows on different types of soil, drought-resistant, and salt-tolerant withstanding both high and low temperatures (vibration amplitude - 90 ... 95 ° C), which makes it well adapted to growth in different climatic zones, such as Kazakhstan. It is common in floodplains and along river banks, on sandy stony-crushed mountain soils. Natural sea buckthorn populations in Kazakhstan occupy about 680 hectares in the Eastern Shallow Hills, Zaysan, Balkhash, Altai, the Alatau Mountains, Karatau, and the Western Tien Shan. As a result of the work of a group of Kazakhstani botanists, various varieties and promising forms placed in leshozes of Almaty, Taldy-Kurgan and Dzhambul regions: "Gift of Katun", "News of Altai", "Golden Ear", "Vitamin", "Scherbinka-1", "Chuyskaya", "Golden Siberia", "Orange" *et al.*

Despite the widespread use of sea buckthorn in Kazakhstan, scientific research is limited by ecology and fruit processing technology. Research works showed that almost the spectrum of vital microelements is contained in the vegetative organs of sea buckthorn, which is growing in the Zailiysk Alatau. In contrast, the primary accumulation of microelements occurs not in the stem, but in the sea, buckthorn leaves. This circumstance highlights the value to the local sea buckthorn as a medicinal plant and raw material for the manufacture of medicines and biologically active supplements (Kumar, Kumar, Chaurasia, and Bala Singh, 2011).

Considering the bioactive and unusual fatty acid composition of sea buckthorn berry, and its potential as a health product, this study aimed to analyze lipophilic and hydrophilic bioactive compounds in sea buckthorn (*Hippophae rhamnoides* L.) berries collected from the south-east side of Kazakhstan.

2. MATERIALS AND METHODS:

2.1. Plants materials

E. rhamnoides was collected from Mountains Alatau, Almaty Region South-East side of Kazakhstan, ca 950 m 30 Sep 2018 (43°10'13" N, 76°41'53" E). The herbarium materials have been deposited at the Herbarium of the Department of Biology, Kazakh National Women's Teacher Training University. Manual harvesting fruits were picked, rinsed, and were brought to the laboratory in polythene bags. The powdered samples were stored in a refrigerator at 4°C until further analysis.

2.2. Methods of anatomical and morphological study

The plants were identified according to Flora of Kazakhstan and illustrated the keys to the plants of Kazakhstan. Morphological characters were compared from five randomly-selected plants (in triplicate for each population), and mean values were taken for analysis using the ANOVA method. The results of the analysis were expressed as a mean of three determinations $\pm$ SD. The material was fixed in a mixture of alcohol, water, and glycerol with ratio 1:1:1, according to Strasburger and Flemming. Anatomical and morphological studies were conducted according to the methods of M.N. Prozina (1960).

The anatomic slices were made by microtome MZP-01 "Technom" (Ekaterinburg, Russia). The temporary preparations were immersed in glycerol. The thickness of the anatomic slices ranged from 10 to 15 micrometers. For microscopic analysis of fruits, dried ground fruits were placed in a container with hexane for a day, then treated with a solution of chloral hydrate—over 1000 temporary and permanent preparations made for microphotography and morphometric analysis. For quantitative analysis, morphometric indicators were measured by MCX 100 Micros microscope (Austria) with camera adapter (with lens4x/0.10, and multiplication ratio EW 10x/20). The statistical analysis of

morphometric indicators performed according to the methods of Lakin (1990).

2.3. Lipophilic compounds analysis

2.3.1 Determination of β -Carotene

β -Carotene extraction and HPLC-PDA analysis were carried out according to Biehler, Mayer, Hoffmann, Krause, and Bohn (2010) with modifications. Samples were extracted (0.1 g/ml) with ice-cold hexane: acetone (1:1, v/v). The mixture was centrifuged at 2000 rpm for 2 min after vortexing for 2 min. The organic phase was decanted into a tube with a saturated sodium chloride solution and placed on ice. The residue was extracted until the extract was discolored, and the organic phase was combined in a test tube with a saturated solution of sodium chloride. Then, the obtained organic phase was filtered through a 0.45-mm membrane filter. β -Carotene was analyzed using a Prominence-i HPLC-PDA model system equipped with sample cooler LC-2030C (Shimadzu, Japan) and a C18 Luna® column (150 $\times$ 4.6 mm, 5 μ). The column temperature was set at 35 °C, and the injection volume was 20 μ L. The compound was analyzed with an isocratic mobile phase using acetonitrile: dichloromethane: methanol (7:2:1) as eluents at ambient temperature. A flow rate of 1.0 mL/min was set. Peaks were monitored at 450 nm and identified by congruent retention times compared with pure β -carotene standard. Quantification of the compound was made using calibration curves obtained from pure standards in the HPLC system.

2.3.2 Method for determination of the fatty acid composition

A Soxhlet apparatus extracted the oil for four hours using petroleum ether as a solvent. This was evaporated under reduced pressure, using a rotary evaporator at 50 °C, and then were hydrolyzed by 10% KOH in methanol at 60°C for 30 minutes. The product of hydrolysis was extracted with chloroform. Methyl esters were prepared via methylation using diazomethane (Fieser and Fieser, 1967).

The fatty acid composition was analyzed by GC (Crystallux-4000M "Meta-Chrom", Russia) equipped with a flame ionization detector (FID) and a capillary column DB-5ms (5% diphenyl and 95% dimethylpolysiloxane, phase thickness 0.25 μ m) with a size of 30 m $\times$ 0.25 mm. The initial temperature of the thermostat is 40 °C; holding at initial temperature - 1 min; temperature programming - from 40 to 210 °C with a speed of

15 °C / min, from 210 to 280 °C with a speed of 5 °C / min. Holding at the final temperature - 20 min. Carrier gas-helium, 1 cm / min. Sample 0.2 μ L, the evaporator - 280 °C. The temperature of the evaporator is 280 °C.

2.4. Hydrophilic compounds analysis

2.4.1 Method for determination of vitamin C and B

Vitamin C and B were determined by method M 04-41-2005 "Determination of the mass fraction of free forms of water-soluble vitamins in samples of premixes, vitamin supplements, concentrates and mixtures by the method of capillary electrophoresis using the system of capillary electrophoresis "Drop-105" (Certificate on certification of measurement procedure No. 224.04.17.035/2006).

2.4.2 Pectin substances determination

The carbazole test carried out substantially, as described by Filipov and Vlasheva (1973). The sample sizes were 20-25 g fresh weight of fruits. Per 10ml of solution or suspension, 1-2 ml of 1 N NaOH was added and thoroughly mixed. De-esterification occurred for 20 min at 20-25°C. Subsequently, the suspension was acidified by the addition of 1 N HCl, 1-5 x the volume of 1 N NaOH added before, and mixed thoroughly. To precipitate the pectin, 50 ml of 0.1 N HCl added. The suspension stirred and kept for 5 min at room temperature to equilibrate the medium and pectin flakes concentrations. The final volume or weight of each sample was measured. The mixture filtered through a wide pore filter (Whatman). 10-20ml of this filtrate pipetted into a 250 ml volumetric flask. The residue of the filter was pooled with the remaining filtrate. Funnel and vial washed twice with distilled water and the wash solutions added to the residue filtrate mixture and thoroughly stirred. The filtrate in the flask and the mixture were separately titrated with 0.1 N NaOH using Hinton's indicator. From the result of the titration of the 10-20ml filtrate, the HCl content of the original volume was calculated.

2.5. Statistical analysis

All determinations were obtained from triplicate measurements, and results were expressed as means $\pm$ standard deviations. The data were analyzed using a one-way analysis of variance (ANOVA) for mean differences.

3. RESULTS AND DISCUSSION:

3.1. Morphological and microscopic characterization of *E. rhamnoides*

3.1.1 Macroscopic characters

E. rhamnoides is a small tree 2m tall with black bark, have strong spines, 2-7 cm in length. Shoots of different ages create a rounded, pyramidal crown. The sea buckthorn root system develops close to the surface, no deeper than 40 cm, spreading over a wide area. The root system consists of skeletal, semi-skeletal, weakly branching roots, on which are formed nodules that contain nitrogen-fixing bacteria.

Sea buckthorn is a dioecious plant; on some bushes grow female flowers, from which developed fruits; on other bushes grow male flowers, which pollinate female flowers with wind help. The flowers are regular, with a simple perianth cup-shaped.

Leaves are alternate, simple, linear-lanceolate 4-8 cm long. Above of leaves grayish-green, below side grayish-white and have stellate hairs. The smell is weak, peculiar, taste slightly bitter. Fruits are oval or round 0,5-1 cm long, smooth pitted yellowish-golden, red or orange, with one bone, shiny, juicy, with a peculiar taste and smell, reminiscent the scent of pineapple (Figure 1).

3.1.2. Microscopic study

In the cross-section of the leaf on both sides, have epidermis, columnar and spongy mesophyll in a single row. In the center of a leaf, there is collateral, larger conductive fascicle with well-developed xylem vessels (Figure 2).

The transverse section of a leaf of sea buckthorn shows dorsal ventral characters. The mesophyll is divided into two layers: the upper, palisade layer, and the lower spongy layer. Palisade parenchyma consists of 2-3 elongated rows of cells, located under the cuticle and the upper epidermis on the inner side of the leaf. Isodiametric cells of the spongy parenchyma are located under the epidermal tissue of the outer side of the leaf plate. The chlorophyll-bearing cells of the palisade parenchyma are oriented perpendicular to the leaf; they differ in size from chlorenchyma cells of the spongy parenchyma. There are rather large intercellular cavities in the spongy mesophyll, which connect and stomata of the dorsal side of the leaf. The cuticle is even but wrinkled in the region of vein on the underside of

the leaf. On the ventral side of the leaf, stomata are not available, and the lower part is densely covered with gray, multicellular stellate hairs. There is collantern on the center of the leaf and a larger conducting bundle with well-developed xylem and phloem (Figure 2).

Thickness of lamina 528 microns length. Cells of the upper epidermis have straight walls. The length is 20, 33 microns. Cells of the lower epidermis more thin-walled than cells of the upper epidermis; walls are straight—length 18.33 microns. The stomata are found only on the lower epidermis, an anomocytic type (4-8 cells), with a frequency of 345-603 per 1 mm. The leaf mesophyll is protected on both sides by a single layer of rounded epidermal cells. The epidermal cells of the upper side of the leaf are cutinized with a waxy fat-like substance. The length of the xylem is 69.41 microns; the length of the phloem is 56.66 microns (Table 1).

3.1.3 Sea buckthorn fruits diagnostic indicators

Macroscopic characters. Fruits (Figure 3) are 6–10 mm long, 4–8 mm in diameter, oblong, highly wrinkled, oval, soaked, with one bone, with a peduncle. The pulp of the fetus is formed from the receptacle. The stone in outline is elongated obovate, dark brown, the surface is smooth shiny, with a clearly visible longitudinal line, up to 5 mm long. Fruits are yellow with a characteristic odor; the taste is sour.

Figure 3. Fruits of *E. rhamnoides*

Trichomes with a diameter of 197.8 - 291.8 microns were found. The cells of the epidermis are polygonal in shape, 18–48 μm long, 3–40 μm wide irregular stony cells with a length of 8.98 μm and a width of 2.87 μm (Figure 4a). In the pulp parenchyma, secretory passages and conducting bundles of a spiral type are found. Seabuckthorn seed consists of palisade cells. Behind it, there is a perisperm and many cells of the aleuron layer

and an embryo composed of tight-fitting cells, a large amount of fatty oil, and aleuron grains (Figure 4b, c, d).

3.2. Analysis of lipophilic bioactive compounds

3.2.1 Fatty acid composition

Both seeds and flesh (pulp) of sea buckthorn berries are rich in lipids (Table 2). Oil can be extracted from the seeds and the pulp (Zeb and Ullah, 2015). The seeds contain 22.5% oil, the fruit pulp - about 24.32%, the berries—27.89%, which is consistent with data from other researchers (Kumar *et al.*, 2011). The fatty acid profiles in pulp oil and seed oil were different. The predominant fatty acids in seed oil were linoleic acid (C18:2 ω -6, 35.34%) and α -linolenic acid (C18:3 ω -3, 25.46%), in the oil from berry pulp/peel – palmitoleic (C16:1 ω -7, 30.03%) and palmitic acids (28.53%). The saturated fatty acids (SFA) fraction ranged between 11.13 and 32.45% of the total FA. The main SFA in all samples investigated was palmitic acid (PA, hexadecanoic acid, 16:0), which is the most common SFA found in animals and plants.

Seed oil was richer in total polyunsaturated fatty acids (PUFA), because of higher linoleic and linolenic acids (the major fatty acid components of the PUFA fraction). Linoleic acid was found in the greatest proportion of PUFA in the seed and soft parts oils. α -linolenic acid content was at the highest level in the seed oil (25.46%) but was found to be at the lowest level in pulp/peel oil (3.82 %). It should be noted, that the seed oil contains linoleic acid (18:2cisD9,12) (35.34 %) and α -linolenic acid (18:3cisD9,12,15) (25.46%) in close to 1:1 ratio. This ratio is unique to plant lipids (Ursin, 2003).

Because the whole fruit oil is mostly made up of pulp oil, the features of these two oil kinds were very similar. Pulp and whole fruit oils were richer in individual monounsaturated fatty acids (both in palmitoleic acid, and berries in oleic acid), as well as in total monounsaturated fatty acids (MUFA). The analyzed oil is attracting attention because of the increasing interest in the physiological role of the monounsaturated fatty acids (Ranalli *et al.*, 2002) and given the fact that a high level of palmitoleic acid is not common in the plant kingdom. High levels of palmitoleic acid are present in only a few plants, for example, in Nuez Australiana (*Macadamia integrifolia* Maiden and Betcher). *Macadamia* oil contains ~17% palmitoleic acid (Parveez *et al.*, 2012). Palmitoleic acid is low in seed oils but is characteristic of the fruit pulp (Saeidi, Alirezalu, and Akbari, 2016). The

monounsaturated fatty acid palmitoleate (palmitoleic acid) is one of the most abundant fatty acids in serum and tissues, particularly adipose tissue and liver (Frigolet and Gutiérrez-Aguilar, 2017). Palmitoleic acid is an omega-7 monounsaturated fatty acid essential for pharmaceutical applications. Palmitoleic acid has been reported to have anti-thrombotic effects, favorable effects on insulin sensitivity, cholesterol metabolism, and hemostasis and averts beta-cell apoptosis persuaded by glucose or saturated fatty acids (Hernandez, 2016). This fatty acid is less susceptible to oxidation than polyunsaturated fatty acids, which is especially important when used in the food industry with frying and baking processes. Also, palmitoleic acid is widely used in cosmetics.

LA and ALA obtained from foods furnished by the diet is the starting point for synthesizing a variety of other unsaturated fatty acids. After ingestion, ALA is converted to very-long-chain PUFAs, i.e., readily to eicosapentaenoic acid, 20:5 ω -3, and more slowly to docosahexaenoic acid, 22:6 ω -3. Using the same pathways (the same enzymes) and in competition with ALA, LA is converted into arachidonic acid, 20:4 ω -6, which is, in a competition (again) with eicosapentaenoic acid, the starting point for the synthesis of eicosanoids and prostaglandins that are important mediators in many inflammatory diseases and in particular in cardiovascular diseases (de Lorgeril, Salen, Laporte, and de Leiris, 2001).

It has been previously found that a diet rich in fish and seafood significantly reduces the risk of heart disease due to the high eicosapentaenoic acid (EPA) and docosahexaenoic acid [DHA] content of these foods (Blondeau *et al.*, 2015). With growing concerns regarding the accumulation of environmental pollutants in fish oil as well as the sustainability of marine fish stocks, plants rich in n-3 fatty acids may offer a sustainable source of these beneficial fatty acids.

Except for flaxseed oil, which contains 50% α -linolenic acid, such high levels of n-3 fatty acids are different in seed oils. Research is currently underway to find and produce n-3 fatty acid-enriched products, both for human consumption and use as animal feed. Sea buckthorn seed oil, growing at South-East of Kazakhstan, with its high α -linolenic acid content and 1:1 ratio of ω -6: ω -3 fatty acids may be a new balanced source of polyunsaturated fatty acids.

3.2.2 Carotenoids content

The orange-yellow color of sea buckthorn berry reflects the existence of carotenoids.

Typically, carotenoids content is much lower in seeds than in pulp (Yang and Kallio, 2002). Therefore, the carotene content was determined in the fruit (7.75 mg/kg, Table 3).

Table 3. β -Carotene content

Name	Result, mg/kg
β -Carotene	7.75 $\pm$ 0.84

Note: Values are means of triplicate determinations ($n = 3$) $\pm$ standard deviations.

Even though some authors showed a significantly higher content of β -carotene (between 4 and 7.5 mg/100 g) (Pop *et al.*, 2014), our results are consistent with the results of Yang's (2002). They showed the concentration of β -carotene ranged between 0.2 and 17 mg/100 g and that of total carotenoids from 1 to 120 mg/100 g of sea buckthorn berries (Teleszko, Wojdyło, Rudzińska, Oszmiański, and Golis, 2015). Among biologically active substances, β -carotene is a member of the carotene family with no polar functional groups and can be cleaved in the liver into two vitamins A molecules. β -Carotene can be supplemented in many individuals with a reduced concern of toxicity (Hammond and Renzi, 2013). Carotenoids receive much interest, as they have multiple activities such as antioxidant, anti-mutagenic, and anti-tumour (Pop *et al.*, 2014).

Carotenoids are biologically active compounds in residual pomace after juice extraction from sea buckthorn berries consisting of pulp, seed, and skin. Interestingly, Corbu *et al.* concluded that the carotenoids from dried sea buckthorn byproducts might be safely used as a source of natural carotenoids for the enrichment of vegetable oils for their coloring effect and for the appeal to enhance the acceptability and valorization of the edible oils (Corbu, Rotaru, and Nour, 2019). The β -carotene is served as a quality checkpoint for sea buckthorn oil in some countries (Suryakumar and Gupta, 2011).

3.2.3 Analysis of hydrophilic bioactive compounds

The berries of sea buckthorn were rich in vitamins B complex (content ranged from 0.0035 to 0.014 mg/100g), vitamin C (0.21 mg/100g) and pectin (3.27 %) (Table 4). These compounds have high biological activity.

So, pectins are the polysaccharides found in plant cell walls and the outer skin and rind of fruits and vegetables. They are soluble in hot water and then form gels on cooling, hence gelling

and thickening agents in various food products such as jams and jellies. Cholesterol-lowering effects of pectin is due to its gel-forming capacity too. Pectin lowers cholesterol by binding the cholesterol and bile acids in the gut and promoting their excretion (Mudgil and Barak, 2013).

Vitamin C has antioxidant, immunomodulatory, anti-infectious, antimicrobial, antibacterial, antiviral, antiparasitic, and antifungal effects (Mousavi, Bereswill, and Heimesaat, 2019). Vitamin C content in analyzed sea buckthorn berries samples was lower than showed (27.8 mg/100 g). However, vitamin C content depends on the geographical location and related environmental parameters. For example, fresh sea buckthorn fruits from coastal dunes of Europe contain 120–315 mg% of vitamin C, while from Alps – 405– 1100 mg% (Zielińska and Nowak, 2017). Besides, in sea buckthorn, extensive chemical composition variations have been revealed among populations, subspecies, or cultivars. For example, seven wild berries of *subsp. sinensis*, native to China, contained 5–10 times more vitamin C in the juice fraction than the berries of *subsp. rhamnoides* from Europe and of *subsp. mongolica* from Russia. The content of ascorbic acid among Russian cultivars (*subsp. mongolica*) may range from 0.5 to 3.3 g/kg, whereas in berries of *subsp. turkistanica* ranged from 2.52 to 4.19 g/kg (Teleszko *et al.*, 2015). The lower vitamin C concentration in our samples could be due to the specific geographical nature of the area where the short growing season prevails (Shi, Ho, and Shahidi, 2010).

As for vitamin B, other researches also showed the vitamin B complex group in sea buckthorn berries (B1 (0.035 mg%), B2 (up to 0.056 mg%), and B6 (Zielińska and Nowak, 2017). The B vitamins are cofactors for many enzymes for biosynthetic processes in mammalian cells, including the de novo production of RNA and DNA (Lai, 2013).

4. CONCLUSIONS:

In short, sea buckthorn growing in Kazakhstan is a unique plant with high medical and nutritional value. *Hippophae* fruits are appreciated due to their multiple-use, both for fresh consumption and for industrial preparations in jams and related products (functional multivitamin food), and may use to obtain MUFAs-rich functional oils from industrial byproducts; i.e., the seeds. Juice extraction from sea buckthorn berries leads to a residual pomace consisting of pulp, seed, and skin, rich in biologically active

compounds with antioxidant properties, such as vitamins C, B, pectin, β -carotene, and unsaturated fatty acids. A diet rich in MUFAs may be a choice to a low-fat diet, which may lower blood cholesterol levels, modulate immune function, decrease susceptibility of oxidation of low-density lipoprotein and improve the fluidity of high-density lipoprotein. The PUFAs enriched diet may also be necessary for the structure and function of many membrane proteins, including receptors, enzymes, and active transport molecules. Thus, sea buckthorn from Kazakhstan can be widely used for the production of functional foods and in the pharmaceutical industry due to the rich composition of biologically active compounds with a broad spectrum of biological activity.

5. CONFLICT OF INTEREST STATEMENT:

The authors declare that they have no conflicts of interest.

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Table 1. Morphometric indicators of the anatomical structure of leaf *E. rhamnoides*

Thickness of lamina, μm	Upper Epidermis thickness, μm	Lower Epidermis thickness, μm	Columnar mesophyll thickness, μm	Spongy mesophyll thickness, μm	Xylem, μm	Phloem, μm
528 $\pm$ 0.04	20.33 $\pm$ 0.58	18.33 $\pm$ 0.89	127.75 $\pm$ 0.35	115,83 $\pm$ 0.75	69.41 $\pm$ 1.66	56.66 $\pm$ 2.07

Table 2. Oil content and fatty acid composition of oils from seeds, whole berries and pulp/peel of sea buckthorn berries

Indicator	Seeds	Soft parts (pulp/peel)	Berries
Oil content, %	22.55 $\pm$ 1.31	24.32 $\pm$ 0.54	27.89 $\pm$ 2.41
16:0	8.80 $\pm$ 0.32	28.53 $\pm$ 0.44	32.45 $\pm$ 1.32
16:1	1.49 $\pm$ 0.02	30.03 $\pm$ 2.51	33.05 $\pm$ 4.21
18:0	2.33 $\pm$ 0.01	2.28 $\pm$ 0.34	-
18:1 ω 9	14.97 $\pm$ 0.87	6.45 $\pm$ 0.98	28.65 $\pm$ 2.2
18:1 ω 7	1.41 $\pm$ 0.03	5.76 $\pm$ 0.31	9.63 $\pm$ 0.34
18:2 ω 6	35.34 $\pm$ 1.31	10.01 $\pm$ 0.02	3.48 $\pm$ 0.01
18:3 ω 3	25.46 $\pm$ 1.23	3.82 $\pm$ 0.04	13.62 $\pm$ 1.78
SFA	11.13	30.81	32.45
MUFA	17.87	42.24	71.33
PUFA	60.80	13.83	17.10
ω-6 / ω -3 ratio	1.39	2.62	0.26

Table 4. Hydrophilic compounds contents

No.	Compound	Content
1	B ₂ , mg/100g	0.0035 $\pm$ 0.0015
2	B ₃ , mg/100g	0.033 $\pm$ 0.007
3	B ₅ , mg/100g	0.037 $\pm$ 0.007
4	B ₆ , mg/100g	0.014 $\pm$ 0.003
5	B _c , mg/100g	0.0077 $\pm$ 0.0015
6	C, mg/100g	0.21 $\pm$ 0.07
7	Pectin substances, %	3.27 $\pm$ 0.43

Figure 1. Leaves (a) and fruits (b) shape of *E. rhamnoides*

Figure 2. A: General anatomical view of *H. rhamnoides*, B and C: Cross-section of leaf from upper to lower (c: cuticle, ue: upper epiderma, cc: chlorenchymal cell, pc: parenchyma cell, pp: palisade parenchyma, xy: xylem, tr: trachae, ph: phloem, le: lower epiderma)

Figure 4. The fruits of *E. rhamnoides* (a: the epidermis of the fetus; b: drops of sea buckthorn fruit oil; c: inner layer - the pulp of the fetus; d: cross section of the peel of the seed)
[with 10x / 0.25 lens and EW factor 10x / 20]