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Elisaveta Sandulachi , [Natalia Netreba](#) \* , Artur Macari , Iuliana Sandu , [Olga Boestean](#) , Irina Dianu , [Sergiu Popa](#)

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Keywords: sea buckthorn; chemical compositions; carotenoids; antimicrobial activity; Bacillus pumilus



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Article

# The Correlation between Chemical Indicators and Antimicrobial Properties of Sea Buckthorn

Elisaveta Sandulachi <sup>1</sup>, Natalia Netreba <sup>1,\*</sup>, Artur Macari <sup>1</sup>, Iuliana Sandu <sup>1</sup>, Olga Boeștean <sup>1</sup>, Irina Dianu <sup>1</sup> and Sergiu Popa <sup>2</sup>

<sup>1</sup> Faculty of Food Technology, Technical University of Moldova, 168 Ștefan cel Mare Street, MD 2004, Chișinău, Republic of Moldova

<sup>2</sup> State Agrarian University of Moldova, Chisinau, 42 Mircesti Street, Republic of Moldova

\* Correspondence: Natalia Netreba natalia.netreba@tpa.utm.md; Tel.: +37369353017

**Abstract:** Due to the content of biologically active substances, sea buckthorn (*Hippophae rhamnoides* L.) is of growing interest to scientists, the food industry, pharmaceuticals, cosmetology and consumers. The aim of this study is to investigate the correlation between the chemical composition (Carotenoid content (CC), Ascorbic acid content (AAC), Total dry matters (TDM), Titratable acidity (TA), pH, Organic acids (OA)) and the Antibacterial effect (AA) (Diameter of the inhibition zone in mm of *Bacillus pumilus*) of four species of sea buckthorn (Clara, Dora, Cora, Mara), cultivated in the Republic of Moldova. The sea buckthorn species tested was found to have a different Carotenoid content (1.79±0.43 ... 48.92±0.61 mg/100g), Ascorbic acid content (74.36±0.60 ... 373.38±2.29 mg/100g), Organic acids (malic acid 5.8±0.02 ... 13.4±0.01 mg/100g, citric acid 0.08±0.00 ... 0.32±0.01 mg/100g, succinic acid 0.03±0.00 ... 1.1±0.00 mg/100g), Total dry matters (16.71±0.05 ... 24.54±0.09 %), Total acidity (2.15±0.05 ... 8.76±0.00 %), and pH value (2.73±0.02 ... 3.00±0.07). The microbial activity of sea buckthorn, evaluated by the diameter of the inhibition zone, constituted for *Bacillus pumilus* (3.70...15.91mm/g<sup>-1</sup> for whole sea buckthorn fruits and respectively 13.33...26.67 mm/g<sup>-1</sup> for sea buckthorn puree).

**Keywords:** sea buckthorn; carotenoids; antimicrobial activity; *Bacillus pumilus*

## 1. Introduction

Sea buckthorn is a relevance source of vitamins (the most important being C and E vitamins are also present B1, B2, K and bioflavonoids), macro, microelements (nitrogen, phosphorus, iron, manganese, boron, calcium, aluminum, silicon and others), antioxidants (catechin, myricetin, quercetin, p-coumaric acid, caffeic acid, L-ascorbic acid, and gallic acid) [1–4]. Currently, papers containing experimental data obtained by various international researchers supporting the consumption of sea buckthorn fruit as functional foods [5–7], the use of fruit as therapeutic remedies [8–10] and their use as sources of natural antioxidants with antimicrobial properties by the food industry [11–14]. Sea buckthorn fruits have a great potential as antimicrobial compounds against microorganisms [15,16]. So, these berries can be used to control the stability of stored food [17,18].

The antioxidant and antimicrobial activity of sea buckthorn depends on the chemical composition of the berries [19,20]. Published experimental data [21,22] support the consumption of sea buckthorn fruits as functional foods and their use as sources of natural antioxidants by the food industry. The content of biologically active substances and the level of antioxidant activity of sea buckthorn depend on growing conditions, agricultural technology and climate [23,24].

The aim of this study was to determine the relationship between the antimicrobial properties of sea buckthorn fruits and the chemical parameters (Carotenoid content (CC), Ascorbic acid content (AAC), Organic acids (OA), Total dry matters (TDM), Titratable acidity (TA), pH) of the berries.

## 2. Results

To determine the interdependence between the antimicrobial properties and biologically active substances of sea buckthorn, in berries of 4 species, physicochemical parameters were studied: the content of carotenoids, ascorbic acid, organic acids, total dry matters, titratable acidity, pH.

Table 1 shows the average values and limits of the content of carotenoids (CC), ascorbic acid (AA) and organic acids (OA) for the investigated sea buckthorn varieties Clara, Dora, Cora and Mara.

**Table 1.** Average levels and range (mg/100g) for carotenoids, ascorbic acid (Vitamin C) and organic acids in tested sea buckthorn fruit.

Sea buckthorn species	Samples	Chemical indicators (CI), mg/100g						
		Carotenoid content (CC),		Ascorbic acid content (AAC),		Organic acids (OA),		
		mean	min-max	mean	min-max	malic	citric	succinic
Clara	42	7.88±0.54	5.72±0.56 - 11.13±0.53	151.63±0.88	99.08±0.41 - 193.85±1.36	11,90±0.004	0,20±0.001	1,1±0.001
Dora	42	23.16±0.52	18.50±0.43 - 48.92±0.61	254.16±1.95	198.68±1,64 - 322.11±2.26	5,80±0.002	0,08±0.001	0,36±0.001
Cora	42	7.13±0.50	1.79±0.43 - 4.92±0.57	113.14±0.81	74.36±0,60 - 241.83±1.02	9,60±0.002	0,09±0.001	0,72±0.001
Mara	42	13.93±0.15	7.37±0.17 - 18.77±0.13	278.71±2.03	248.52±1.78 - 373.38±2.29	13,40±0.002	0,32±0.01	0,03±0.001
Average fruit values		17,03±0.43	1.79±0.43- 48.92±0.61	199.34±1.42	74.36±0,60- 373.38±2.29	10,18±0.002	0,17±0.002	0,55±0.002

Table 2 shows the average values and limits of the physical indicators TDM, TA and pH for the investigated sea buckthorn varieties Clara, Dora, Cora and Mara.

**Table 2.** Average levels and range of limits for physical indicators in tested sea buckthorn fruits.

Indicator tested	Sea buckthorn species			
	Clara	Dora	Cora	Mara
<b>TDM,%</b>				
Min-Max	16.22±0,02...20.37±0.08	14.19±0.09... 20.50±0.03	17.22±0.07...24.54±0.09	16.71±0.05...24.20±0.06
Mean	17.34±0.04	16.74±0.06	17.22±0.08	18.78±0.05
<b>TA,%</b>				
Min-Max	2.57±0,04... 4.73±0.25	2.15±0,05...5.55±0,00	3.59±0,23...8.76±0,00	4.52±0,10...7.21±0.14
Mean	3.25±14	3.22±0.03	4.94±0.12	5.92±0.12
<b>pH</b>				
Min-Max	2.77±0.03 ... 3.00±0.07	2.87±0.02...2.99±0.03	2.66±0.01..2.87±0.05	2.73±0.02...2.98±0.02
Mean	2.88±-.0.05	3.00±0.02	2.76±0.03	2.84±0.02

The sea buckthorn species tested was found to have a different Carotenoid content (1.79±0.43 ... 48.92±0.61 mg/100g), Ascorbic acid content (74.36±0.60 ... 373.38±2.29 mg/100g), Organic acids (malic acid 5.8±0.02 ... 13.4±0.01 mg/100g, citric acid 0.08±0.00 ... 0.32±0.01 mg/100g, succinic acid 0.03±0.00 ... 1.1±0.00 mg/100g), Total dry matters (16.71±0.05 ... 24.54±0.09 %), Total acidity (2.15±0.05 ... 8.76±0.00 %), and pH value (2.73±0.02 ... 3.00±0.07).

The antimicrobial activity (inhibition zone diameter in mm) of the samples was also evaluated against *Bacillus pumilus*.

Table 3 shows the interdependence between the antibacterial activity (AA) of fruit and sea buckthorn puree and the content of Total dry matters (TDM), Carotenoid content (CC), Ascorbic acid content (AAC), Titratable acidity (TA) and pH.

**Table 3.** Pearson coefficient values between chemical indicators and antimicrobial properties of sea buckthorn.

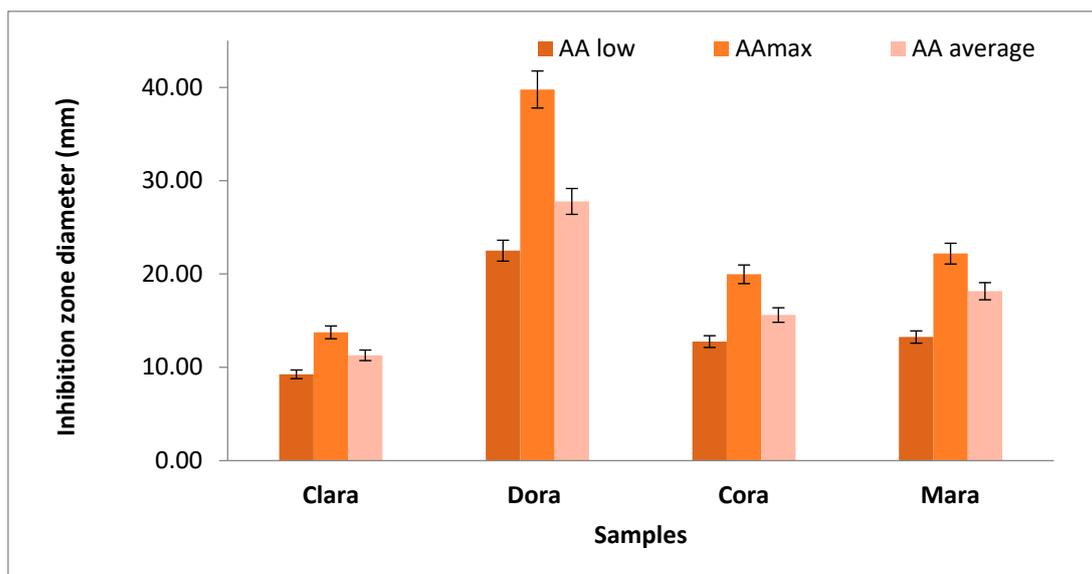
Sea buckthorn species	Fruit mass, g	Antibacterial activity (AA) (Diameter of inhibition zone in mm)		Pearson coefficient $P_c = f(\text{AA}_{B. pumilus} \text{ and PCI})$				
		mean	min-max	TDM	CC	AAC	TA	pH
<b>Whole sea buckthorn fruits</b>								
<b>Clara</b>	0.28 ...0.30	13.10	7.60 ...15.40					
	0.10*	4.52	3.70 ...5.50	0.8520	0.8488	0.5738	0.9762	- 0.9524
	0.25*	11.29	9.25 ...13.75	0.8525	0.8473	0.5727	0.9766	- 0.9534
<b>Dora</b>	0.16 ... 0.19	13.33	10.80 ...17.50					
	0.10*	11.11	9.00 ...15.91	0.9791	0.9791	0.9791	1.0000	- 0.9628
	0.25*	27.78	22.50 ...39.77	0.9758	0.9758	0.9758	1.0000	- 0.9758
<b>Cora</b>	0.16 ...0.19	11.03	9.70 ...11.90					
	0.10*	6.25	5.11 ...7.19	0.7154	0.7179	0.9689	0.9689	- 0.9952
	0.25*	15.61	12.76...17.97	0.8417	0.8443	0.9766	0.9927	- 0.8429
<b>Mara</b>	0.16 ... 0.20	12.83	10.60 ...14.20					
	0.10*	7.26	5.30 ...8.88	0.9261	0.9209	0.8714	0.9706	- 0.9280
	0.25*	18.16	13.25 ...22.19	0.9254	0.9202	0.8704	0.9704	- 0.9273
<b>Sea buckthorn puree</b>								
<b>Clara</b>	0.14...0.15	21.50	20.00...23.60					
	0.10*	15.00	13.33...16.86	0.8232	0.8526	0.5174	0.9883	- 0.9588
	0.25*	37.50	33.33...42.14	0.8233	0.8528	0.5177	0.9888	- 0.9886
<b>Dora</b>	0.07 ...0.12	18.07	17.60...17.60					- 0.9582
	0.10*	17.48	15.00...26.14	0.9577	0.9577	0.9577	1.000	- 0.9582
	0.25*	43.71	37.50...62.86	0.9578	0.9578	0.9578	1.000	- 0.9583
<b>Cora</b>	0.09 ...0.17	24.10	20.80...27.50					
	0.10*	19.54	16.18...26.67	0.7174	0.7552	0.7178	0.7061	- 0.7720
	0.25*	48.85	40.44...66.67	0.7176	0.7554	0.7781	0.7062	- 0.7720
<b>Mara</b>	0.10	19.30	17.20...21.40					
	0.10*	19.30	17.20...21.40	0.9035	0.9940	0.9035	0.7780	- 0.9047
	0.25*	48.25	44.75...53.50	0.9035	0.9940	0.9035	0.7780	- 0.9450

\* recalculated.

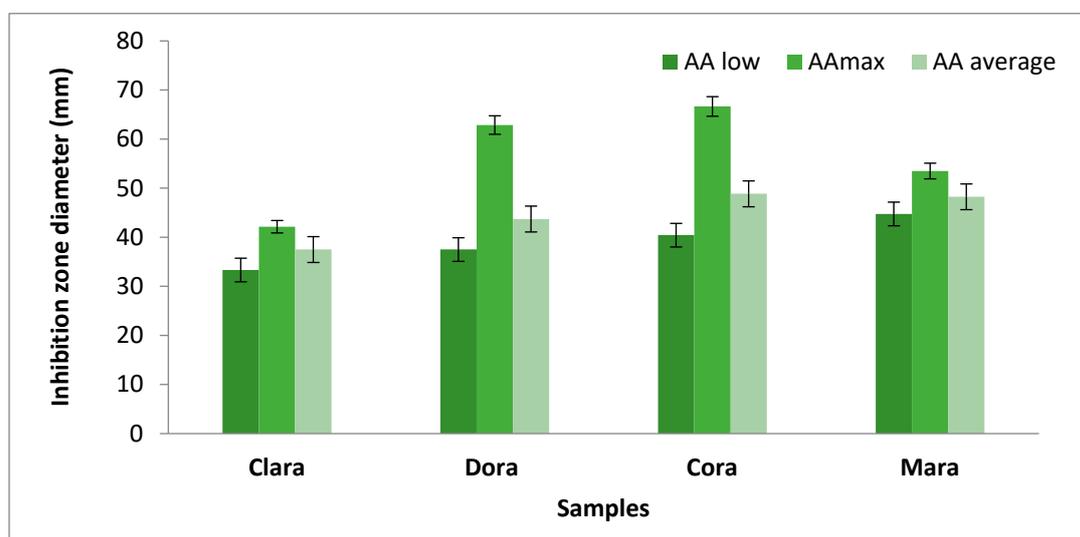
The antimicrobial activity of sea buckthorn, evaluated by the diameter of the inhibition zone, constituted for *Bacillus pumilus* (3.70 ... 15.91mm/g<sup>-1</sup> for whole sea buckthorn fruits and respectively 13.33 ... 26.67 mm/g<sup>-1</sup> for sea buckthorn puree).

It is known that the antimicrobial activity of fruits depends on their antioxidant activity. Xiangqun Gao et al. [25] investigated the antioxidant activity of sea buckthorn and found that it depends on the content of phenolic substances and ascorbic acid, as well as the content of carotenoids. The authors mention that when sea buckthorn fruits ripen, their antioxidant activity changes depending on the correlation of these substances in berries.

Figures 1 and 2 show the areas of inhibition of *Bacillus pumilus* by sea buckthorn fruits and puree.



**Figure 1.** Areas of inhibition of the bacterium *Bacillus pumilus* by sea buckthorn fruits.



**Figure 2.** Areas of inhibition of the bacterium *Bacillus pumilus* by sea buckthorn puree.

Beta-carotene content analysis, of ascorbic acid, the total content of drying substances, the titratable acidity and pH of the berries gave the possibility to identify the correlation between these components and the values of the diameter of the area of inhibition of bacteria that cause food poisoning.

The antiviral, antibacterial and antifungal properties of sea buckthorn are reported in several studies (Table 4).

**Table 4.** The antiviral, antibacterial and antifungal properties of sea buckthorn.

Properties	Investigation method	Target microorganisms	Source
Antibacterial activity	Standard disc diffusion method	<i>Staphylococcus aureus</i>	Muhammad Imran Qadir et al. [26]
Antimicrobial		<i>Streptococcus gordonii</i> , <i>Porphyromonas gingivalis</i> , <i>Actinomyces viscosus</i> and <i>Candida albicans</i> .	Smida et al. [27]
antimicrobial activity		<i>Candida albicans</i> , <i>Pichia jadinii</i> , <i>Bacillus subtilis</i> and <i>Staphylococcus aureus</i>	Jeong, J.H et al. [28]
Antiviral, antibacterial activity, fungal strains	Inhibition zone diameter, IS(50)	<i>Staphylococcus aureus</i> , <i>Haemophilus influenzae</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i>	<u>Heikki Kallio</u> . [18]
Antibacterial property	Diffusion method, Minim inhibition concentration (MIC)	<i>Escherichia coli</i> , <i>Salmonella enterica</i> . <i>Yersinia enterocolitica</i> , <i>Bacillus thuringiensis</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i>	Ivanišová, E. et al. [29]
Antibacterial property and fungal strains		gram positive ( <i>Bacillus cereus</i> , <i>Enterococcus durans</i> , <i>Enterococcus faecalis</i> , <i>Staphylococcus aureus</i> ), gram negative ( <i>Aeromonas hydrophila</i> , <i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas fluorescens</i> , <i>Pichia jadinii</i> , <i>Salmonella enterica</i> , <i>Salmonella typhimurium</i> , <i>Yersinia enterocolitica</i> ) bacteria and yeast ( <i>Candida albicans</i> ).	Jeong, J.H et al. [28]; Michel et al. [30]; Upadhyay et al. [31]; Arora et al. [32];
Antimicrobial activity		<i>Escherichia coli</i>	Giedre Kasparaviciene [33]
Antimicrobial activity	Inhibition zone, Minim inhibition concentration (MIC)	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Salmonella Typhimurium</i> , <i>Escherichia coli</i> , <i>Candida albicans</i>	Sandulachi et al. [34]

### 3. Discussion

#### 3.1. Chemical composition of the sea buckthorn varieties analysed

Michalak, M et al. [35] notes that sea buckthorn is a rich source of carotenoids including  $\beta$ -carotene, they can be arranged as follows: sea buckthorn oil > carrot oil > marigold oil > pumpkin seed oil. Another study conducted by Andrea Mendelová et al. [36] reported that the total carotenoid content expressed as  $\beta$ -carotene content in sea buckthorn juice ranged from 50.63 mg/100g to 93.63 mg/100g, the highest content was in the Askola variety and the lowest in Terhi. Beveridge [37] reported a total carotenoid concentration of 22.2 mg/100 g seed oil. 41.1 mg/100 g sea buckthorn juice et a wide range of total carotenoids from 330 to 1000 mg/100 g pulp oil, depending on plant subspecies or cultivar. A comparison of total carotenoids in seed oil showed substantial variation in the carotenoid content between solvent extraction [38].

Other research and scientific studies report that carotenoid content in sea buckthorn is in the range of 11-26.6 mg/100g [39], 6-28 mg/100g [39], 19.7 mg/100g [40], 242.0-325.0 mg/100g [40,41].

Minimum value of ascorbic acid content ( $26.70 \pm 0.01$  mg/100g) was found in the AGG variety, and maximum values ( $134.56 \pm 7.55$  mg / 100g) - in the six variety. The study [41] reports a very wide range of vitamin C in sea buckthorn (502-2600 mg/100g).

Bujinlkham, B. et al. [42] notes that the organic acid of sea-buckthorn fruits were composed of malic acid (6.30 mg/100 g), tartaric acid (5.55 mg/100g), quinic acid (1,67 mg/100g), citric acid (0.31 mg/100g), and succinic acid (0.29 mg/100g). Malic acid and tatraic acid were the major organic acids in sea-buckthorn fruits which accounted for about 90% of total organic acids. This is also confirmed by studies of the varieties tested.

The total acidity and pH of the samples studied were also different. The Titratable acidity in our investigated sea buckthorn varieties was  $3.22 \pm 0.03$  for Dora varieties (lowest value) and  $5.92 \pm 0.12$  for Mara (maximum value). The pH values for the investigated samples were within  $2.84 \pm 0.02$ ...  $3.00 \pm 0.02$ .

D. Munkhbayar et al. [41] reports that the pH of sea buckthorn was within 2.3 2.1-2.5.

Organic acids in the juice of seabuckthorn have been identified as oxalic, citric, tartaric, malic, quinic and ascorbic acid [42,43].

The organic acid content of fruit pulp depends on environmental factors and cultivation practices (temperature, light intensity, variety, rootstock, mineral nutrition, water availability, fruit loading/cuttings). When the total amount of organic acids per fruit is considered, an increase in their content is also observed during fruit development and ripening [44,45]. This increase is observed in the Mara variety.

In the researched varieties, there is a tendency to increase the concentration of malic and citric acids (responsible for the sour taste of the fruits), as the fruits ripen. These values regarding the concentration of malic acid reach: Dora variety – 5.8 mg/100g, Cora – 9.6 mg/100g, Clara – 11.9 mg/100g and Mara – 13.4 mg/100g. The concentration of citric acid is in the same increasing order: Dora variety - 0.08 mg/100g, Cora - 0.09 mg/100g, Clara - 0.2 mg/100g and Mara - 0.32 mg/100g These observations suggest a less sour taste for buckthorn harvested in the highlighted period.

At the same time, the insignificant increase in the concentration of succinic acid can be observed, at the beginning of September, especially in the Clara variety. Probably, the formation of succinic acid is related to the degree of fruit maturity. In all varieties (except the Cora variety) the concentration of acetic acid also increases insignificantly. The concentration of acetic acid is higher in the Clara variety, it increased in parallel with the concentration of succinic acid.

This fact denotes the beginning of ester hydrolysis processes, oxidation, decarboxylation and transformation of acids with a higher molecular weight: malic (hydroxy-butanedioic), citric (2-hydroxy-propane-1,2,3-tricarboxylic) and .in low molecular weight acids (acetic, etc.). The respective processes take place after fruit maturity and affect the quality of the fruit.

Authors of other studies report that malic and quinic acids are the main organic acids in sea buckthorn fruits, which make up about 90% of all berry acids. In Russian sea buckthorn varieties, the

total acidity is (2.1-3.2 g/100 ml), in the Finnish varieties (4.2 - 6.5 g/100 ml), and in Chinese genotypes 3.5-9.1 g/100 ml [46,47].

### 3.2. Antimicrobial activity of sea buckthorn fruits and puree

Calculating the Pearson coefficient, it was found that the antimicrobial activity of sea buckthorn fruits and puree is largely influenced by the chemical composition of the berries. Although different results have been obtained between varieties, this correlation is quite high. Was found a very high correlation between chemical indicators and sea buckthorn antimicrobial activity:

for whole sea buckthorn fruits:

Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and CC}) = 0.7179 \dots 0.9791$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and AAC}) = 0.5738 \dots 0.9791$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and TDM}) = 0.7154 \dots 0.9791$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and TA}) = 0.9689 \dots 1.000$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and pH}) = - 0.9280 \dots - 0.9952$ .

for sea buckthorn puree:

Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and CC}) = 0.7552 \dots 0.9940$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and AAC}) = 0.5174 \dots 0.9577$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and TDM}) = 0.7174 \dots 0.9577$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and TA}) = 0.7061 \dots 1.000$ ; Pearson coefficient  $P_c = f(\text{AA Bacillus pumilus and pH}) = - 0.7720 \dots - 0.9582$ .

The Pearson coefficient ( $P_c = f(\text{AA Bacillus pumilus and pH})$ ) has negative values, which shows us that the interdependence between these indicators is inversely proportional.

Carotenoids are among the most common natural pigments, and more than 600 different compounds have been characterized until now, with  $\beta$ -carotene as the most prominent [48]. Various scientific researches and studies show that sea buckthorn has remarkable antimicrobial antioxidant properties [49]. Information on the antioxidant properties of sea buckthorn carotene is reported by researchers Oguz Merhan [50], Andrea Mendelová [36]. Carotenoids can inhibit active radicals by transferring electrons, giving hydrogen atoms to radicals or attaching to radicals [51]. The activity of carotenoids as antioxidants also depends on their interaction with other antioxidants, such as vitamins E and C [52]. There are three isomers of carotene, alpha, beta and gamma, with the beta isomer being most active [53]. The mechanism of microbial action of berries is diverse: by destroying the cell membrane, inhibiting DNA and preventing protein biosynthesis, etc. [48]. The antioxidant activity is more potent with extracted seabuckthorn oil because of higher carotenoid levels. The researchers' results [54] indicate that the antioxidant activity of sea buckthorn oil depends on the extraction methods and heat treatments used. Sea buckthorn can be used as natural replacements for synthetic additives and for food products with functional properties [54,55].

## 4. Materials and Methods

### 4.1. Materials

The plant material used in this study consisted of four species of sea buckthorn Clara, Dora, Cora, Mara, harvested during August 2022 from Dubossary district, Pohrebea village of the Republic of Moldova (47°10'34" N 29°10'04" E). Sea buckthorn fruits were harvested at the stage of full ripening. Berry samples were frozen at minus 25°C. In this work, frozen sea buckthorn fruits stored for 6 months under these conditions were studied.

Figure 3 show the sea buckthorn varieties analyzed.



**Figure 3.** Sea buckthorn varieties of sea buckthorn grown in the Republic of Moldova.

#### 4.2. Chemical Materials

The methanol (>99,9%), ethyl acetate, ( $\geq 99,9\%$ ), were provided by Honeywell (Charlotte, North Carolina US); petroleum ether puriss. p.a., ACS reagent, reag. ISO, low boiling point hydrogen treated naphtha,  $b_p > 90\%$  40-60 °C ( $\geq 90\%$ ), hydrochloric acid solution, sodium 2,6-dichloroindophenolate hydrate ACS reagent, sodium hydroxide for the preparation of dilute volumetric solutions or for direct use, phenolphthalein indicator ACS, Reag. Ph Eur, hydrochloric acid for the preparation of dilute volumetric solutions or for direct use, benzoic acid (ACS reagent,  $\geq 99,5\%$ ), cetrionium bromide (grade pharmaceutical primary standard), diethalonamine reagent ( $\geq 98,0\%$ ) were provided by Sigma-Aldrich (Schnelldorf, Germany); the MPW-380R centrifuge was purchased from IKA®-Werke GmbH & Co (Germany/Deutschland); Shimadzu UV-1900 UV-VIS spectrophotometer was obtained from Shimadzu Europa GmbH (Duisburg, Germany); capillary electrophoresis system KAPEL-105M was purchased from LUMEX, Russia.

#### 4.3. Content of biologically active substances

##### 4.3.1. Ascorbic acid content

Ascorbic acid content was determined by potentiometric titration [56].

The method is based on vitamin C extraction with hydrochloric acid solution followed by titration with 2,6-dichlorophenolindophenolate sodium.

A solution of hydrochloric acid with a mass fraction of 2% is used as an extraction solution. Solution for titration 0.05 g of 2,6-dichlorophenolindophenolate is dissolved in pre-boiled water, 150 ml, for 30 min, cooled and the solution is brought to 200 ml. The shelf life of the solution is not more than 10 days. The titer of the 2,6-dichlorophenolindophenolate sodium solution is established by a standard ascorbic acid solution with a concentration of 1.0 and 0.1 g/dm, on the day of the test.

Extraction. A 5 g sample weight is weighed with an accuracy of  $\pm 0.01$  g, homogenized for not more than 2 min with a small amount of extraction solution and transferred into a 100 cm<sup>3</sup> volumetric flask, washing the homogenizer with a small amount of extraction solution until the volume reaches the mark. The contents are incubated for 10 min, stirred and filtered. Pipette 0.5 to 1.0 ml of extract into a 50 cm<sup>3</sup> beaker, add extraction solution to a volume of 30 cm<sup>3</sup> and immerse pH electrodes of the millivolt meter so that when stirring they do not touch the magnetic stir bar. Then they are titrated potentiometrically from a microburette with sodium 2,6-dichlorophenolindophenolate solution. Sodium 2,6-dichlorophenolindophenolate solution is added in portions of 0.1-0.2 cm<sup>3</sup> with constant stirring. The volume of sodium 2,6-dichlorophenolindophenolate solution corresponding to the

equivalence point and, therefore, consumed for the titration volume is determined by the maximum difference ("jump") of two neighboring readings.

The mass fraction of ascorbic acid as a percentage is calculated by the formula:

$$X = \frac{(V1-V2)*T*V3*100}{V4*m} \quad (1)$$

V1 - volume of sodium 2,6-dichlorophenolindophenolate solution consumed for the titration of the sample extract, cm<sup>3</sup>;

V2 - volume of sodium 2,6-dichlorophenolindophenolate solution consumed for control test, cm<sup>3</sup>;

T - titer of sodium 2,6-dichlorophenolindophenolate solution, g/cm<sup>3</sup>;

V3 - volume of extract obtained during extraction of vitamin C from a product sample, cm<sup>3</sup>;

V4 - volume of extract used for titration, cm<sup>3</sup>;

m - mass of a portion of the product, g.

The final result is the arithmetic mean of the results of two parallel determinations.

#### 4.2.2. Organic acids

The determination of organic acids was carried out by the method of capillary electrophoresis using the KAPEL105M system, the modern of the certified models in the Kapelseries. Along with the latest electronic database, the KAPEL105M system implements complete instrument control, data collection, and processing using its software, and the ability to record the absorption spectra of the components of the analyzed sample during analysis [57–59].

Was used an unmodified quartz capillary with an inner diameter of 50 μm and a total length of 64.5 cm (effective length of 56 cm). The capillary was thermostatically controlled at 20 °C. Conditions of the research: phosphate buffer was used as the main electrolyte in the work, capillary  $L_{\text{eff}}/L_{\text{tot}}=40/50$  cm, ID=50 μm. The sample inlet is hydrodynamic at 300 mbar\*s. Voltage 17 kV. Indirect detection: UV-detector was used at a wavelength of 190 nm (±1.0 nm). Reagents of ACS grade (≥95%) were used for the analysis. Before working, the capillary was successively washed with 0.1 M NaOH solution for 60 s, then twice for 1 min with deionized water, 5 min with background electrolyte solution, between analyzes – with background electrolyte solution for 5 min. The calculation of all quantitative characteristics was carried out in pereschete on absolutely dry raw material.

#### 4.2.3. Carotenoid content

The carotenoid content in sea buckthorn (*Hippophaë rhamnoides* L.) was determined by UV/VIS spectrophotometry [42,60].

Extraction was performed by extracting carotenoids from 2 g of the sample using 25 mL extraction solvent [methanol: ethyl acetate: petroleum ether, v/v/v, 1:1:1] by homogenization at 10000 rpm for 10 min. Then the separated lipophilic part was extracted. The absorbance of the extract was recorded at 450 nm.

#### 4.4. Physicochemical Analysis

Total dry matter content (TDM) was determined according to gravimetric method, based on the weight loss of the analyzed sample to constant mass, due to water evaporation by heating in an oven at a temperature of up to (105±2)°C and atmospheric pressure [61]. Total acidity (TA) was determined according to potentiometric titration with a standard volumetric solution of sodium hydroxide in the presence of phenolphthalein as indicator [61]. The pH was measured with a Titrator SI Analytics TitroLine® 5000 (Xylem Analytics, Letchworth, UK), at 20°C.

#### 4.5. Antimicrobial activity

The antimicrobial activity of sea buckthorn was evaluated by the agar diffusion method. In this study we used inhibition zone test, also called Kirby-Bauer Test [62], against reference bacterial

strains *Bacillus pumilus* ATCC 7061 - Gram-positive aerobic spore-forming bacteria, cultivated in the microbiology laboratory of the Department of Food Technology, Technical University of Moldova.

Wells with a diameter of  $4\pm 0.1$  mm were made on Müeller-Hinton agar plates and the distances between neighboring wells and to the edge of the plate were equal. The plates were inoculated with a fruits and puree of equal weight, was weighed to the nearest 0.01 g, were left at 18-22 °C for 1-2 hours, then thermostated at 37°C for 48 hours. The total inhibition area diameter for microbial culture growth was measured with the Carbon Fiber Composites Digital Caliper at a 0,1 mm accuracy [63,64]. All assays were performed in triplicate.

#### 4.6. Statistical analysis

The variance analysis of the results was carried out by least square method with application of Student test and Microsoft Office Excel program version 2010 (Microsoft, Redmond, WA, USA). The differences were considered statistically significant if probability was greater than 95% (p-value <0.05). All assays were performed in triplicate. The experimental results are expressed as average  $\pm$  SD.

### 5. Conclusions

In this study was evaluated the content of carotenoids and ascorbic acid in 4 varieties of sea buckthorn grown in the Republic of Moldova (Clara, Dora, Cora, Mara), as well as the total content of dry matter, titratable acidity and pH of berries. Antimicrobial activity of sea buckthorn fruit and puree on *Bacillus subtilis* was also investigated. There was a very high interdependence between the chemical indicators of sea buckthorn and their antibacterial activity.

The Pearson coefficient ( $P_c = f(\text{AA } \textit{Bacillus pumilus} \text{ and PCI})$ ) for the tested sea buckthorn fruits formed the following sequence: Dora> Mara> Clara> Cora, and for the respective sea buckthorn puree Dora> Mara> Clara> Cora.

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### References

1. Bal, L.M.; Meda, V.; Naik, S.N.; Satya, S. Sea Buckthorn Berries: A Potential Source of Valuable Nutrients for Nutraceuticals and Cosmeceuticals.
2. Dong, R.; Su, J.; Nian, H.; Shen, H.; Zhai, X.; Xin, H.; Qin, L.; Han, T. Chemical Fingerprint and Quantitative Analysis of Flavonoids for Quality Control of Sea Buckthorn Leaves by HPLC and UHPLC-ESI-QTOF-MS. *Journal of Functional Foods* **2017**, *37*, 513–522. <https://doi.org/10.1016/j.jff.2017.08.019>.

3. Tian, Y.; Liimatainen, J.; Alanne, A.-L.; Lindstedt, A.; Liu, P.; Sinkkonen, J.; Kallio, H.; Yang, B. Phenolic Compounds Extracted by Acidic Aqueous Ethanol from Berries and Leaves of Different Berry Plants. *Food Chemistry* **2017**, *220*, 266–281. <https://doi.org/10.1016/j.foodchem.2016.09.145>.
4. Sharma, U. K.; Sharma, K.; Sharma, N.; Sharma, A.; Singh, H. P.; Sinha, A. K. Microwave-Assisted Efficient Extraction of Different Parts of *Hippophae Rhamnoides* for the Comparative Evaluation of Antioxidant Activity and Quantification of Its Phenolic Constituents by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). *J. Agric. Food Chem.* **2008**, *56* (2), 374–379. <https://doi.org/10.1021/jf072510j>.
5. Beveridge, T.; Li, T. S. C.; Oomah, B. D.; Smith, A. Sea Buckthorn Products: Manufacture and Composition. *J. Agric. Food Chem.* **1999**, *47* (9), 3480–3488. <https://doi.org/10.1021/jf981331m>.
6. Jaśniewska, A.; Diowksz, A. Wide Spectrum of Active Compounds in Sea Buckthorn (*Hippophae Rhamnoides*) for Disease Prevention and Food Production. *Antioxidants* **2021**, *10* (8), 1279. <https://doi.org/10.3390/antiox10081279>.
7. Tudor, C.; Bohn, T.; Iddir, M.; Dulf, F. V.; Focșan, M.; Rugină, D. O.; Pinte, A. Sea Buckthorn Oil as a Valuable Source of Bioaccessible Xanthophylls. *Nutrients* **2019**, *12* (1), 76. <https://doi.org/10.3390/nu12010076>.
8. Ercisli, S.; Orhan, E.; Ozdemir, O.; Sengul, M. The Genotypic Effects on the Chemical Composition and Antioxidant Activity of Sea Buckthorn (*Hippophae Rhamnoides* L.) Berries Grown in Turkey. *Scientia Horticulturae* **2007**, *115* (1), 27–33. <https://doi.org/10.1016/j.scienta.2007.07.004>.
9. Zhang W.; Yan J.; Duo J.; Ren B.; Guo J. Preliminary study of biochemical constitutions of berry of sea buckthorn growing in Shanxi province and their changing trend; Proceedings of the International Symposium on Sea Buckthorn (*H. rhamnoides* L.); Xi'an, China. 19–23 October 1989; pp. 96–105.
10. Solà Marsiñach, M.; Cuenca, A. P. The Impact of Sea Buckthorn Oil Fatty Acids on Human Health. *Lipids Health Dis* **2019**, *18* (1), 145. <https://doi.org/10.1186/s12944-019-1065-9>.
11. Sandulachi, E.; Bulgaru, V.; Ghendov-Mosanu, A.; Sturza, R. Controlling the Risk of Bacillus in Food Using Berries. *FNS* **2021**, *12* (06), 557–577. <https://doi.org/10.4236/fns.2021.126042>.
12. Sandulachi, E.; Cojocari, D.; Balan, G.; Popescu, L.; Ghendov-Moșanu, A.; Sturza, R. Antimicrobial Effects of Berries on *Listeria Monocytogenes*. *FNS* **2020**, *11* (09), 873–886. <https://doi.org/10.4236/fns.2020.119061>.
13. Sturza, R.; Sandulachi, E.; Cojocari, D.; Balan, G.; Popescu, L.; Ghendov-Mosanu, A. ANTIMICROBIAL PROPERTIES OF BERRY POWDERS IN CREAM CHEESE. **2019**. <https://doi.org/10.5281/ZENODO.3464222>.
14. Cojocari, D.; Sturza, R.; Sandulachi, E.; Macari, A.; Balan, G.; Ghendov-Mosanu, A. INHIBITING OF ACCIDENTAL PATHOGENIC MICROBIOTA IN MEAT PRODUCTS WITH BERRY POWDERS. **2019**. <https://doi.org/10.5281/ZENODO.2640056>.
15. Kohli, K. New Insights towards Implications of Sea Buckthorn Oil in Human Health: A Review. *IPCM* **2018**, *2* (3). <https://doi.org/10.23880/IPCM-16000135>.
16. Mohan Gupta, S.; K Gupta, A.; Ahmed, Z. Antibacterial and Antifungal Activity in Leaf, Seed Extract and Seed Oil of Seabuckthorn (*Hippophae Salicifolia* D. Don) Plant. *J Plant Pathol Microbiol* **2011**, *02* (02). <https://doi.org/10.4172/2157-7471.1000105>.
17. Schubertová, S.; Krepsová, Z.; Janotková, L.; Potočnáková, M.; Kreps, F. Exploitation of Sea Buckthorn Fruit for Novel Fermented Foods Production: A Review. *Processes* **2021**, *9* (5), 749. <https://doi.org/10.3390/pr9050749>.
18. Kallio, H.; Yang, W.; Liu, P.; Yang, B. Proanthocyanidins in Wild Sea Buckthorn (*Hippophaë Rhamnoides*) Berries Analyzed by Reversed-Phase, Normal-Phase, and Hydrophilic Interaction Liquid Chromatography with UV and MS Detection. *J. Agric. Food Chem.* **2014**, *62* (31), 7721–7729. <https://doi.org/10.1021/jf502056f>.
19. Teleszko, M.; Wojdyło, A.; Rudzińska, M.; Oszmiański, J.; Golis, T. Analysis of Lipophilic and Hydrophilic Bioactive Compounds Content in Sea Buckthorn (*Hippophaë Rhamnoides* L.) Berries. *J. Agric. Food Chem.* **2015**, *63* (16), 4120–4129. <https://doi.org/10.1021/acs.jafc.5b00564>.
20. Criste, A.; Urcan, A. C.; Bunea, A.; Pripon Furtuna, F. R.; Olah, N. K.; Madden, R. H.; Corcionivoschi, N. Phytochemical Composition and Biological Activity of Berries and Leaves from Four Romanian Sea Buckthorn (*Hippophae Rhamnoides* L.) Varieties. *Molecules* **2020**, *25* (5), 1170. <https://doi.org/10.3390/molecules25051170>.
21. Gâtlan, A.-M.; Gutt, G. Sea Buckthorn in Plant Based Diets. An Analytical Approach of Sea Buckthorn Fruits Composition: Nutritional Value, Applications, and Health Benefits. *IJERPH* **2021**, *18* (17), 8986. <https://doi.org/10.3390/ijerph18178986>.
22. Terechuk, L.; Starovoytova, K.; Ivanova, S.; Sergeeva, I. Obtaining Functional Products from Sea Buckthorn Berries. In *Proceedings of the 2nd International Conference on Education Science and Social Development (ESSD 2019)*; Atlantis Press: Changsha, China, 2019. <https://doi.org/10.2991/essd-19.2019.90>.
23. Yang, B.; Kallio, H. P. Fatty Acid Composition of Lipids in Sea Buckthorn (*Hippophaë Rhamnoides* L.) Berries of Different Origins. *J. Agric. Food Chem.* **2001**, *49* (4), 1939–1947. <https://doi.org/10.1021/jf001059s>.

24. Yue, X.-F.; Shang, X.; Zhang, Z.-J.; Zhang, Y.-N. Phytochemical Composition and Antibacterial Activity of the Essential Oils from Different Parts of Sea Buckthorn (*Hippophae Rhamnoides* L.). *Journal of Food and Drug Analysis* **2017**, *25* (2), 327–332. <https://doi.org/10.1016/j.jfda.2016.10.010>.
25. Gao, X.; Ohlander, M.; Jeppsson, N.; Björk, L.; Trajkovski, V. Changes in Antioxidant Effects and Their Relationship to Phytonutrients in Fruits of Sea Buckthorn (*Hippophae Rhamnoides* L.) during Maturation. *J. Agric. Food Chem.* **2000**, *48* (5), 1485–1490. <https://doi.org/10.1021/jf991072g>.
26. Qadir, M. I.; Abbas, K.; Younus, A.; Shaikh, R. S. Report - Antibacterial Activity of Sea Buckthorn (*Hippophae Rhamnoides* L.) against Methicillin Resistant Staphylococcus Aureus (MRSA). *Pak J Pharm Sci* **2016**, *29* (5), 1711–1713.
27. Smida, I.; Pentelescu, C.; Pentelescu, O.; Sweidan, A.; Oliviero, N.; Meuric, V.; Martin, B.; Colceriu, L.; Bonnaure-Mallet, M.; Tamanai-Shacoori, Z. Benefits of Sea Buckthorn (*Hippophae Rhamnoides*) Pulp Oil-based Mouthwash on Oral Health. *J Appl Microbiol* **2019**, *126* (5), 1594–1605. <https://doi.org/10.1111/jam.14210>.
28. Jeong, J. H. Antioxidant and Antimicrobial Activities of Extracts from a Medicinal Plant, Sea Buckthorn. *J. Korean Soc. Appl. Biol. Chem.* **2009**, *53* (1), 33–38. <https://doi.org/10.3839/jksabc.2010.006>.
29. Ivanišová, E.; Blašková, M.; Terentjeva, M.; Grygorieva, O.; Vergun, O.; Brindza, J.; Kačániová, M. Biological Properties of Sea Buckthorn (*Hippophae Rhamnoides* L.) Derived Products [Pdf]. *Acta Sci Pol Technol Aliment* **2020**, *19* (2), 195–205. <https://doi.org/10.17306/J.AFS.2020.0809>.
30. Michel, T.; Destandau, E.; Le Floch, G.; Lucchesi, M. E.; Elfakir, C. Antimicrobial, Antioxidant and Phytochemical Investigations of Sea Buckthorn (*Hippophaë Rhamnoides* L.) Leaf, Stem, Root and Seed. *Food Chemistry* **2012**, *131* (3), 754–760. <https://doi.org/10.1016/j.foodchem.2011.09.029>.
31. Upadhyay, N. K.; Yogendra Kumar, M. S.; Gupta, A. Antioxidant, Cytoprotective and Antibacterial Effects of Sea Buckthorn (*Hippophae Rhamnoides* L.) Leaves. *Food and Chemical Toxicology* **2010**, *48* (12), 3443–3448. <https://doi.org/10.1016/j.fct.2010.09.019>.
32. Richa Arora. Antimicrobial Activity of Seed, Pomace and Leaf Extracts of Sea Buckthorn (*Hippophae Rhamnoides* L.) against Foodborne and Food Spoilage Pathogens. *Afr. J. Biotechnol.* **2012**, *11* (45). <https://doi.org/10.5897/AJB11.4150>.
33. Kasparaviciene, G.; Briedis, V.; Ivanauskas, L. [Influence of sea buckthorn oil production technology on its antioxidant activity]. *Medicina (Kaunas)* **2004**, *40* (8), 753–757.
34. Sandulachi, E.; Macari, A.; Cojocari, D.; Balan, G.; Popa, S.; Turculet, N.; Ghendov-Mosanu, A.; Sturza, R. ANTIMICROBIAL PROPERTIES OF SEA BUCKTHORN GROWN IN THE REPUBLIC OF MOLDOVA. *JES* **2022**, *29* (1), 164–175. [https://doi.org/10.52326/jes.utm.2022.29\(1\).15](https://doi.org/10.52326/jes.utm.2022.29(1).15).
35. Michalak, M.; Paradowska, K.; Zielińska, A. Selected plant oils as a source of carotenoids for the applications in cosmetology *Postepy Fitoterapii* **2018** No.1 pp.10-17
36. Mendelová, A.; Mendel, L.; Czako, P.; Mareček, J. Evaluation of Carotenoids, Polyphenols Content and Antioxidant Activity in the Sea Buckthorn Fruit. *Potr. S. J. F. Sci.* **2016**, *10* (1), 59–64. <https://doi.org/10.5219/551>.
37. Beveridge, T. Chemical composition and some physical properties. In Sea Buckthorn (*Hippophaë rhamnoides* L.): *Production and Utilization*, **2003**, 79–88. Ottawa, ON: NRC Research Press.
38. R. Yakimishen; S. Cenkowski; W. E. Muir. OIL RECOVERIES FROM SEA BUCKTHORN SEEDS AND PULP. *Applied Engineering in Agriculture* **2005**, *21* (6), 1047–1055. <https://doi.org/10.13031/2013.20021>.
39. Lõugas, T. *Study on Physico-Chemical Properties and Some Bioactive Compounds of Sea Buckthorn (Hippophae Rhamnoides L.)*; Thesis on natural and exact sciences; TUT Press: Tallinn, 2006.
40. Kumar, R.; Kumar, G. P.; Chaurasia, O.; Bala Singh, S. Phytochemical and Pharmacological Profile of Sea buckthorn Oil: A Review. *Research J. of Medicinal Plant* **2011**, *5* (5), 491–499. <https://doi.org/10.3923/rjmp.2011.491.499>.
41. Munkhbayar, D.; Ariuntungalag, J.; Delgersuuri, G.; Badamkhand, D. Enzymatic Technology for Sea Buckthorn Oil Extraction and Its Biochemical Analysis. *Mong. J. Chem.* **2014**, *15*, 62–65. <https://doi.org/10.5564/mjc.v15i0.325>.
42. Beveridge, T.; Harrison, J. E.; Drover, J. Processing Effects on the Composition of Sea Buckthorn Juice from *Hippophae Rhamnoides* L. Cv. Indian Summer. *J. Agric. Food Chem.* **2002**, *50* (1), 113–116. <https://doi.org/10.1021/jf010369n>.
43. Yong Hoon Lee, Hee Joo Jang, Kun Hee Park, Seon-Hee Kim, Jung Kyu Kim, Jin-Chul Kim, Tae Su Jang, Ki Hyun Kim Phytochemical Analysis of the Fruits of Sea Buckthorn (*Hippophae rhamnoides*): Identification of Organic Acid Derivatives *Plants* **2021**, *10*(5), 860; <https://doi.org/10.3390/plants10050860>
44. Famiani, F.; Battistelli, A.; Moscatello, S.; Cruz-Castillo, J. G.; Walker, R. P. The Organic Acids That Are Accumulated in the Flesh of Fruits: Occurrence, Metabolism and Factors Affecting Their Contents – a Review. *rchsh* **2015**, *XXI* (2), 97–128. <https://doi.org/10.5154/r.rchsh.2015.01.004>.
45. Famiani, F.; Bonghi, C.; Chen, Z.-H.; Drincovich, M. F.; Farinelli, D.; Lara, M. V.; Proietti, S.; Rosati, A.; Vizzotto, G.; Walker, R. P. Stone Fruits: Growth and Nitrogen and Organic Acid Metabolism in the Fruits and Seeds—A Review. *Front. Plant Sci.* **2020**, *11*, 572601. <https://doi.org/10.3389/fpls.2020.572601>.

46. Ma, Z.; Cui, Y.; Feng, G. Studies on the fruit character and biochemical compositions of some forms within Chinese sea buckthorn (*Hippophae rhamnoides* subsp. *sinensis*). In Proceedings of the International Symposium on Sea Buckthorn (*H. rhamnoides* L.), Xi'an, China, 19–23 October 1989; pp. 106–113.
47. Kallio, K.; Yang, B.R.; Tahvonen, R.; Hakala, M. Composition of sea buckthorn berries of various origins. In Proceedings of the International Symposium on Sea Buckthorn (*Hippophae rhamnoides* L.), Beijing, China, 29 August 1999.
48. Stahl, W.; Sies, H. Antioxidant Activity of Carotenoids. *Molecular Aspects of Medicine* **2003**, *24* (6), 345–351. [https://doi.org/10.1016/S0098-2997\(03\)00030-X](https://doi.org/10.1016/S0098-2997(03)00030-X).
49. Roshan Kumar Shah, Aniket Idate, Poorva Sharma Review on Antimicrobial Properties of Sea Buckthorn *Hippophae Rhamnoides* L International Journal of All Research Education and Scientific Methods (IJARESM), ISSN: 2455-6211 Volume 8, Issue 12, December-2020
50. Merhan, O. The Biochemistry and Antioxidant Properties of Carotenoids. In *Carotenoids*; Cvetkovic, D. J., Nikolic, G. S., Eds.; InTech, 2017. <https://doi.org/10.5772/67592>.
51. Kopsell, D.; Kopsell, D. Accumulation and Bioavailability of Dietary Carotenoids in Vegetable Crops. *Trends in Plant Science* **2006**, *11* (10), 499–507. <https://doi.org/10.1016/j.tplants.2006.08.006>.
52. Gammone, M.; Riccioni, G.; D'Orazio, N. Marine Carotenoids against Oxidative Stress: Effects on Human Health. *Marine Drugs* **2015**, *13* (10), 6226–6246. <https://doi.org/10.3390/md13106226>.
53. Stephen Clark, in xPharm: The Comprehensive Pharmacology Reference, 2007
54. Kasparaviciene, G.; Briedis, V.; Ivanauskas, L. [Influence of sea buckthorn oil production technology on its antioxidant activity]. *Medicina (Kaunas)* **2004**, *40* (8), 753–757.
55. Šnē, E.; Galoburda, R.; Segliņa, D. Sea Buckthorn Vegetative Parts – A Good Source of Bioactive Compounds. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences* **2013**, *67* (2), 101–108. <https://doi.org/10.2478/prolas-2013-0016>.
56. Siriangkhawut, W. Electrochemical Analysis of Ascorbic Acid in Commercial Fruit Juices and Drinks. *Asian J. Chem.* **2014**, *26* (19), 6487–6491. <https://doi.org/10.14233/ajchem.2014.16468>.
57. Ferey, L.; Delaunay, N. Food Analysis on Electrophoretic Microchips. *Separation & Purification Reviews* **2016**, *45* (3), 193–226. <https://doi.org/10.1080/15422119.2015.1014049>.
58. Wang, M.; Qu, F.; Shan, X.-Q.; Lin, J.-M. Development and Optimization of a Method for the Analysis of Low-Molecular-Mass Organic Acids in Plants by Capillary Electrophoresis with Indirect UV Detection. *Journal of Chromatography A* **2003**, *989* (2), 285–292. [https://doi.org/10.1016/S0021-9673\(03\)00026-8](https://doi.org/10.1016/S0021-9673(03)00026-8).
59. Rovio, S.; Sirén, K.; Sirén, H. Application of Capillary Electrophoresis to Determine Metal Cations, Anions, Organic Acids, and Carbohydrates in Some Pinot Noir Red Wines. *Food Chemistry* **2011**, *124* (3), 1194–1200. <https://doi.org/10.1016/j.foodchem.2010.07.044>.
60. Khoo, H.-E.; Prasad, K. N.; Kong, K.-W.; Jiang, Y.; Ismail, A. Carotenoids and Their Isomers: Color Pigments in Fruits and Vegetables. *Molecules* **2011**, *16* (2), 1710–1738. <https://doi.org/10.3390/molecules16021710>.
61. *Official Methods of Analysis of AOAC INTERNATIONAL*, 22nd ed.; Latimer, G. W., Ed.; Oxford University Press: New York, 2023. <https://doi.org/10.1093/9780197610145.001.0001>.
62. Hudzicki, J. (2016) Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology, Washington DC, 23
63. Michel, T.; Destandau, E.; Le Floch, G.; Lucchesi, M. E.; Elfakir, C. Antimicrobial, Antioxidant and Phytochemical Investigations of Sea Buckthorn (*Hippophaë Rhamnoides* L.) Leaf, Stem, Root and Seed. *Food Chemistry* **2012**, *131* (3), 754–760. <https://doi.org/10.1016/j.foodchem.2011.09.029>.
64. Valgas, C.; De Souza, S.M.; Smânia, E.F.A. Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* **2007**, *38*, 369–380.

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