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ORIGINAL PAPER

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BIOLOGICAL PROPERTIES OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.) DERIVED PRODUCTS

Eva Ivanišová[1], Martina Blašková[1], Margarita Terentjeva[2], Olga Grygorieva[3]✉, Olena Vergun[3], Jan Brindza[4], Miroslava Kačániová[5,6]

[1]Department of Technology and Quality of Plant Products, Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 94976 Nitra, **Slovakia**
[2]Institute of Food, Environmental Hygiene, University of Life Sciences and Technology
Jelgava, 8 K. Helmaņa iela, 3004 Jelgava, **Latvia**
[3]Department of Fruit Plants Acclimatisation, M.M. Gryshko National Botanical Garden of Ukraine of National Academy of Sciences
Timiryazevska 1, 01014 Kyiv, **Ukraine**
[4]Institute of Biological Conservation and Biosafety, Slovak University of Agriculutre in Nitra
Tr. A. Hlinku 2, 94976 Nitra, **Slovakia**
[5]Department of Fruit Growing, Viticulture and Enology, Slovak University of Agriculture in Nitra
Tr. A. Hlinku 2, 94976 Nitra, **Slovakia**
[6]Department of Bioenergetics and Food Analysis, University of Rzeszow
Zelwerowicza 4, 35-601 Rzeszów, **Poland**

ABSTRACT

Background. Sea buckthorn is a good and possible source of a wide range of bioactive compounds with a positive effect on the human body, especially polyphenols and carotenoids.

Materials and methods. The present study aimed to evaluate the antioxidant and antimicrobial activities of *Hippophae rhamnoides* L. products – 100% oil, 100% juice, dry berries, and tea (dry berries, leaves, and twigs). The antioxidant activity was determined by DPPH radical scavenging and molybdenum reducing antioxidant power methods. The content of phytochemicals (polyphenols, flavonoids, carotenoids) was measured by colorimetric methods. The detection of antimicrobial activity was carried out using the disc diffusion method and an evaluation of minimal inhibition concentration against three species of Gram-negative bacteria: *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, and three Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subsp. *aureus* CCM 2461.

Results. All the tested sea buckthorn products had a high antioxidant activity. The highest content of total polyphenols (204.26 mg GAE/g), flavonoids (30.00 mg QE/g), and carotenoids (0.34 mg/g) were identified in 100% sea buckthorn juice. Maximal values of the antioxidant activity were found using the DPPH method for 100% oil (8.75 mg TE/g) and molybdenum reducing power for tea (196.41 mg TE/g). All the tested products showed strong antimicrobial activity against the tested bacteria, confirmed by both methods – disc diffusion (especially for *Yersinia enterocolitica* CCM 5671) and minimal inhibitory concentration.

Conclusion. The pronounced antioxidant and antibacterial properties of sea buckthorn products indicate the importance of sea buckthorn application in health promotion and agricultural practice.

Keywords: *Hippophae rhamnoides*, polyphenols, flavonoids, carotenoids, antimicrobial activity

✉olgrygorieva@gmail.com, https://orcid.org/0000-0003-1161-0018

INTRODUCTION

Sea buckthorn (genus *Hippophae* L.) is a berry-bearing, thorny, nitrogen-fixing deciduous shrub from the *Elaeagnaceae* family. It includes 6 species and 12 subspecies, of which *Hipphophae rhamnoides* L., also known as sea buckthorn, sandthorn or, nowadays, seaberry, is of particular interest from a nutritional and medicinal point of view (Suryakumar and Gupta, 2011). Sea buckthorn grows widely in various regions of Asia, Europe, and North America. All parts of the plant are considered to be a good source of a large number of bioactive compounds with medicinal and nutritional properties (Cho et al., 2017). Among them, ascorbic acid, tocopherols, carotenoids, and flavonoids exhibit antioxidant activity (Christaki, 2012).

The plant has been used extensively, mainly in folk medicine for the treatment of cough, skin diseases, gastric ulcers, asthma, and lung disorders (Balkrishna et al., 2019). However, many studies have been undertaken to investigate the biological activity of sea buckthorn berry and leaf extracts in recent years, such as anti-inflammatory, immunomodulatory, radioprotective, adaptogenic, anticancer, etc. (Geetha et al., 2005; Ji et al., 2020; Olas et al., 2018).

Dienaitė et al. (2020) identified 28 compounds in the pomace of *H. rhamnoides*, among which the most abundant were flavonols with the structures of isorhamnetin, quercetin, kaempferol glycosides and catechin.

Sea buckthorn seed oil possesses significant wound healing properties and has no associated toxicity or side effects (Upadhyay et al., 2009). The aqueous leaf extract of sea buckthorn promotes wound healing (Upadhyay et al., 2011). Sea buckthorn oil from the plant's fruit can be used as an anti-psoriatic nutraceutical (Balkrishna et al., 2019). In the studies of Smida et al. (2019), mouthwash with sea buckthorn oil presented as a suitable alternative for a preventive agent for periodontal inflammation. In addition, the anti-inflammatory effects of *H. rhamnoides* oil were studied by Dubey et al. (2018). The positive properties of sea buckthorn usually correlate to flavonoids and phenolic acid derivative content in a different part of the shrub. It is important to stress that in the seeds, pulp, fruit, and juice of the sea buckthorn more than 190 compounds can be found, including water and fat-soluble vitamins, fatty acids, organic acids, carotenoids, carbohydrates, amino acids, and polyphenols that indicate the health-promoting properties of sea buckthorn (Bonesi et al., 2016; Zielińska and Nowak, 2017). Different kinds of products – jams, jellies, juices, orange pigments, and seed oils – can be prepared from sea buckthorn berries (Guo et al., 2017). Juice extraction of the berries leads to a residual press cake which can be used after purification for the extraction of oil.

The antimicrobial activity of sea buckthorn berries (Qadir et al., 2016), seeds (Chauhan and Varshneya, 2012), leaves (Upadhyay et al., 2011; Qadir et al., 2016) and oil (Yu et al., 2017) has also been reported. The aqueous and hydroalcoholic leaf and berry extracts showed a growth-inhibiting effect against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Enterococcus faecalis* (Chauhan and Varshneya, 2012; Upadhyay et al., 2011). It is important to study the antioxidant and antimicrobial effects of this plant in order to elaborate on the new application method and to revise the existing knowledge on sea buckthorn.

This study aimed to investigate the antioxidant and antimicrobial properties, as well as the phytochemical profile of sea buckthorn plants from Slovakia and their products to determine their potential as antioxidant and antimicrobial agents.

MATERIALS AND METHODS

Sampling and sample preparation

Sea buckthorn products made from the *Leicora* variety – 100% oil, 100% juice, dry berries, and tea (a mixture of dry berries, leaves, and twigs) – were purchased from the Slovak farm, Tvrdošovce (120 m a.s.l.; latitude: 48.1; longitude: 18.067). Analyses from each sample were carried out in three replications. Sea buckthorn juice and oil were used in the original form provided by the manufacturer.

The dry berries and tea were used in the preparation of ethanolic extract and an amount of 0.2 g of each sample was extracted with 20 mL of 80% ethanol for 24 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurements with the DPPH and phosphomolybdenum methods for the detection of total phenolic content and total flavonoid content.

An amount of 0.5 g of sample (except oil because this method is not suitable for oil) was homogenized in mortar with sea sand and repeatedly extracted with 10 mL acetone until the sample became colorless. The extract was filtered using Whatman filter paper and used for the detection of total carotenoid content.

For the detection of antimicrobial activity, after drying the plant's fruit, the sea buckthorn was crushed, weighed out to 5 g (dried fruit and tea), and soaked separately in 50 mL of ethanol p.a. (99.9%) for two weeks at room temperature. Exposure to sunlight was avoided to prevent the degradation of active components. Then, ethanolic plant extracts were filtered through Whatman No. 1 filter paper. The obtained extracts were subjected to evaporation under reduced pressure at 40°C to remove the ethanol (Stuart RE300DB rotary evaporator, Bibby Scientific Limited, UK, and vacuum pump KNF N838 KNF, Germany). For the antimicrobial assays, the crude fruit and tea extracts were dissolved in dimethyl sulfoxide (DMSO) to 102.4 mg/mL as a stock solution. The stock solutions of the plant extracts were stored at –16°C in the refrigerator until use (Hleba et al., 2014). The oil and juice of sea buckthorn were tested directly.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia), Penta (Czech republic) and Sigma Aldrich (USA).

Detection of antioxidant activity

Free radical scavenging activity. The free radical scavenging activity of the samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchés-Moreno et al., 1998). The sample (0.4 mL) was carefully mixed with 3.6 mL of DPPH solution using a vortex at 1500 rpm (IKA Vortex 3, Germany) (0.025 g DPPH in 100 mL methanol). The absorbance of the reaction mixture was determined using a Jenway spectrophotometer (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; $R^2 = 0.989$) was used as the standard, and the results were expressed in mg/g Trolox equivalents.

Molybdenum reducing antioxidant power. The molybdenum reducing antioxidant power of the samples was determined using the method of Prieto et al. (1999) with slight modifications. The mixture of the sample containing (1 mL), monopotassium phosphate (2.8 mL, 0.1M), sulfuric acid (6 mL, 1M), ammonium heptamolybdate (0.4 mL, 0.1M) and distilled water (0.8 mL) was incubated at 90°C for 120 min, then rapidly cooled and detected by monitoring the absorbance at 700 nm using a Jenway spectrophotometer (6405 UV/Vis, England). Trolox (10–1000 $mg \cdot L^{-1}$; $R^2 = 0.998$) was used as the standard, and the results were expressed in mg/g Trolox equivalents.

Total polyphenol content. The total polyphenol content of the samples (except oil because this method is not suitable for oil) was measured using the method of Singleton and Rossi (1965) using a Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with a Jenway spectrophotometer (6405 UV/Vis, England). Gallic acid (25–300 mg/L^{-1}; $R^2 = 0.998$) was used as the standard, and the results were expressed in mg/g gallic acid equivalents.

Total flavonoid content. Total flavonoid content was determined using the modified method of Willett (2002). A quantity of 0.5 mL of the sample (except oil because this method is not suitable for oil) was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using a Jenway spectrophotometer (6405 UV/Vis, England). Quercetin (1–400 mg/L; $R^2 = 0.9977$) was used as the standard, and the results were expressed in mg/g quercetin equivalents.

Total carotenoid content. Petroleum ether was pipetted into a separating funnel with a teflon stopcock. The acetone extract of the sample and distilled water were added by flowing them along the walls of the funnel. The mixture was allowed to separate into two phases, and the aqueous phase was discarded. The petroleum ether phase was washed 2 times with distilled water to remove any residual acetone. The petroleum ether phase was collected in a 50 ml volumetric flask

by passing the solution through a small funnel containing 5 g of anhydrous sodium sulfate to remove residual water.

The volumetric flask was then made up to volume with petroleum ether, and the total carotenoid content was determined from the molar absorption coefficient of β-carotene (Ball, 1988; STN, 1986). The concentration (mg/g) of carotenoids was calculated according to the following formula:

$$\text{TCC, mg/g} = \frac{A \cdot r \cdot V \cdot 10}{E \cdot n}$$

where:

A – absorbance at 445 nm,
r – sample dilution,
E – molar absorption coefficient $E^{1\%}_{1cm} = 2620$,
n – sample weight,
TCC – total carotenoid content.

Detection of antimicrobial activity

Altogether, six strains of microorganisms were tested in the study, including three Gram-negative bacteria (*Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671), and three Gram-positive bacteria (*Bacillus thuringiensis* CCM 19, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subsp. *aureus* CCM 2461). All the tested strains originated from the Czech Collection of Microorganisms. The bacterial suspensions were cultured in a nutrient broth (Imuna, Slovakia) at 37°C for 24 h.

Sample preparation

Disc diffusion method. A suspension of 0.1 mL of the tested microorganism with a density of (of 10^5 cfu per mL) was spread on a Mueller Hinton Agar (MHA, Oxoid, Basingstoke, United Kingdom). Filter paper discs of 6 mm in diameter were impregnated with 15 µL of the plant material extract and placed on the inoculated plates. The plates were left at 4°C for 2 hours and then incubated aerobically at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters after incubation. All the tests were performed in duplicate.

Minimal inhibitory concentration. The minimal inhibitory concentration (MIC) is the lowest concentration of the sample that will inhibit the visible growth of microorganisms. The MICs were determined by the micro broth dilution method according to the Clinical and Laboratory Standards Institute recommendation, 2009 (CLSI, 2009), in a Mueller Hinton broth (Biolife, Italy) for bacteria. Plant material extracts dissolved in DMSO were prepared to a final concentration of 102.4 mg/mL. The DMSO plant material extract solutions were prepared as serial two-fold dilutions to obtain a final concentration ranging between 5–10240 µg/mL. Oil and juice were prepared without being dried and tested in the same concentration as the dry material. Then each well was inoculated with the microbial suspension at a final density of 0.5 McFarland, and the plates were incubated at 37°C for 24 h. After incubation, the inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader, Biotek EL808 with a shaker (Biotek Instruments, USA). The 96-microwell plates were measured before and after the experiment. The differences between both measurements were evaluated as growth, and any values exceeding 450 nm from the mean absorbance were considered to be an error. Wells without plant extracts were used as the positive control, while the pure DMSO was used as the negative control. This experiment was done in eight-replicates for higher accuracy of the MICs of used medical plant extracts (Hleba et al., 2014).

Statistical analysis

For antioxidant activity, the SAS program was used (The SAS system v. 9.2). The data were submitted and differences between the means compared through the Tukey-Kramer test ($\alpha = 0.05$). Correlation coefficients were calculated by CORR analysis (SAS, 2009). For antimicrobial activity, the coefficient of variation and frequency of size of the inhibition zones were calculated with Statgraphic (STATPOINT TECHNOLOGIES, WARRENTON, VA, USA). The MIC absorbance values taken before and after incubation were used as a set of binary values to assign the exact concentrations. The following formulations were used: value 1 (inhibitory effect) was assigned to absorbance values lower than 0.05 nm, while value 0 (no effect or stimulant effect) was assigned to absorbance values higher than 0.05 nm. For this, a probit analysis in Statgraphics software was used.

RESULTS AND DISCUSSION

Antioxidant activity

DPPH scavenging activity is one of the most widespread methods to determine antioxidant activity based on the reduction of the color of the radical solution from violet to yellow due to the power of hydrogen donating ability (Dontha, 2016). The scavenging effects of the sea buckthorn extracts (Table 1) on the DPPH radical expressed as mg TE/g decreased in this order: 100% oil > 100% juice > dry berries > tea.

Table 1. Antioxidant activity of selected *Hippophae rhamnoides* L. products evaluated by two methods

Sample	DPPH method mg TE/g	Reducing power mg TE/g
100% oil	8.75 ±0.11^{a}	111.59 ±5.25^{c}
100% juice	7.53 ±0.13^{b}	138.62 ±0.71^{b}
Dry berries	7.42 ±0.06^{bc}	146.35 ±0.84^{ab}
Tea	6.83 ±0.05^{c}	196.41 ±8.03^{a}

Mean ±standard deviation.
TE – Trolox equivalent.
Different superscripts in each column indicate significant differences in the mean at $P < 0.05$.

These results indicated that all the extracts had a noticeable effect on free radical scavenging. Sea buckthorn oil had the best antioxidant activity on the DPPH radical. It is known that the strong antioxidant activity of oil is attributed to a high level of fatty acid, mainly palmitoleic and omega-7 monounsaturated fatty acid, and vitamin E and C. Ito et al. (2014) reported that sea buckthorn oil from the *Mongolica* variety is rich in omega-7 monounsaturated fatty acid with a concentration of about 48%. As well as macadamia oil, sea buckthorn oil was recognized as a major source of omega-7 monounsaturated fatty acid and, therefore, is increasingly used in the pharmaceutical industry for skin protection. Ting et al. (2011) found that sea buckthorn oil had strong inhibitory effects on CCl_4--induced oxidative damage in mice, led to the increased activity of antioxidant enzymes, and decreased lipid peroxidation in the liver. The tested 100% juice also exhibited a strong antioxidant effect on the DPPH radical. This finding could be linked to the high content of biologically active compounds in juice. The DPPH scavenging activity of the methanolic extracts of *H. rhamnoides* increased with a high concentration, and 30 µl/ml of extract corresponds to 68% of inhibition (Gill et al., 2012).

According to Muzykiewicz et al. (2018), antioxidant activity by the DPPH method of *H. rhamnoides* leaves was 3.41–4.48 mg TE/g, while that of unripe fruits was 0.44–2.07 mg TE/g and of ripe fruits 0.75–1.16 mg TE/g.

The specific sensory properties of sea buckthorn berries, namely that it has a bitter and acidic taste, do not promote their use among consumers and especially for children. However, the consumption of sea buckthorn products can enrich the human diet, and can also help protect the human body before free radicals are released. Since free radicals are involved in the process of lipid oxidation and are the causative agents of numerous chronic diseases, the importance of their neutralization is evident. For this reason, the methods for improving the sensory attributes of sea buckthorn products are in process, and the addition of honey and sweeteners of plant origin are among the most promising.

For a measurement of reductive ability, the Mo^{6+} – Mo^{5+} transformation in the presence of sea buckthorn samples was investigated (Table 1). The increase in absorbance of the reaction mixture indicated the reducing power of the samples. The reducing power of the sea buckthorn samples was expressed in mg TE/g and increased in the following order: tea > dry berries > 100% juice > 100% oil. The best reducing power was detected in sea buckthorn tea (a mixture of leaves, berries, and twigs) and sea buckthorn berries. Varshneya et al. (2012) determined the strong reducing ability of a 70% methanol extract of sea buckthorn pomace without seeds. In the study of Geetha et al. (2005), an alcoholic extract of sea buckthorn berries showed significant cytoprotection effects against sodium nitroprusside induced oxidative stress in lymphocytes. Sea buckthorn leaves have very good adaptogenic effects on the human body and could be a very interesting and promising material for application in medicine and the food industry.

Total polyphenol and flavonoid content. Polyphenol compounds in fruit and leaves of *Hippophae* species represent mainly flavonoids and phenolic acids, which are the most important active agents contributing to antioxidant capacity (Ji et al., 2020). The total polyphenol content was determined by Folin-Ciocalteau assay. The results are presented in Table 2.

Table 2. Total polyphenol and flavonoid content of selected *Hippophae rhamnoides* L. products

Sample	Total polyphenol content mg GAE/g	Total flavonoid content mg QE/g
100% juice	204.26 ±1.07[a]	30.01 ±1.19[a]
Dry berries	32.18 ±5.52[c]	9.18 ±0.55[b]
Tea	56.77 ±2.73[b]	6.59 ±0.64[c]

Mean ±standard deviation.
GAE – gallic acid equivalent.
QE – quercetin equivalent.
Different superscripts in each column indicate significant differences in the mean at $P < 0.05$.

The total polyphenol content of the sea buckthorn samples in mg GAE/g decreased in this order: 100% juice > tea > dry berries, and the highest content was found in 100% juice. A determination of the Pearson correlation coefficients between the total polyphenol content and reducing power of juice ($r = 0.811$) indicated that there was a correlation between the antioxidant activity and the content of polyphenols. Chauhan and Varshneya (2012) determined the total polyphenol content in various sea buckthorn dried pulp extracts, and the best results were found in 70% ethanol (14.41 ±0.16 mg GAE/g). Methanolic extracts of buckthorn had 34.6 mg GAE of polyphenols and 18.1 mg QE of flavonoids per 100 g, as reported by Gill et al. (2012). According to Kuhkheil et al. (2017), dry fruits of *H. rhamnoides* contained 20.78–34.60 mg GAE/g of polyphenol compounds and 0.98–2.80 mg QE/g of flavonoids. As reported by Criste et al. (2020), the total polyphenol content in the leaves of this species was higher than in the berries and ranged from 41.60–48.12 and 10.12–18.66 mg GAE/g respectively. The content of flavonoids in the leaves and berries in the above study was identified as 31.53–36.58 and 6.57–9.01 mg QE/g respectively. These results concerning the flavonoid content of berries were similar to ours, while the total content of polyphenols was higher in our study. Muzykiewicz et al. (2018) found the polyphenol content in leaf extracts to be 17.83–28.37 GAE/g, in ripe fruits of 1.01–7.14 mg GAE/g depending on the concentration of the investigated extracts.

The total flavonoid content in mg QE/g in the sea buckthorn samples is shown in Table 2. A higher concentration was found in the juice and decreased in this order: dry berries > tea. Similar to polyphenols, the Pearson correlation coefficient was $r = 0.960$, indicating that antioxidant activity correlated with the flavonoid content. Ma et al. (2016) found that isorhamnetin and quercetin are the major flavonoids in berries. The content and profile of flavonoids in sea buckthorn berries are strongly influenced by the variety. According to Xing (2018) and Criste et al. (2020), flavonoid compounds can be found in all parts of sea buckthorn – root, stem, leaves, berries, and seeds.

Total carotenoid content. Carotenoids receive a lot of interest because they can act as antioxidant, antimutagenic, and antitumor agents (Pop et al., 2014). The content of total carotenoids (mg/g) in the samples was determined and expressed in terms of β-carotene.

In the samples, the total carotenoid content decreased in this order: 100% juice (0.34 ±0.02 mg/g) > dry berries (0.16 ±0.01 mg/g) > tea (0.13 ±0.03 mg/g). Pop et al. (2014) determined that the content of total carotenoids varied between 53 and 97 mg/100 g of dry weight in berries, and between 3.5 and 4.2 mg/100 g in leaves. The main fraction of carotenoids among berries was represented by carotenoid diester zeaxanthin dipalmitate, while the leaves contained only free carotenoids like lutein, β-carotene, violaxanthin, and neoxanthin. It was previously reported that the content of total carotenoids (calculated as β-carotene) in fresh berries was from 1 to 120 mg/100 g (Yan and Kallio, 2002). According to Kuhkheil et al. (2017), the content of carotenoids in dry fruits of *H. rhamnoides* was from 0.80 to 1.17 mg/g.

Antibacterial activity

Nowadays, there is growing attention in search of natural products, which may serve as a safer, and effective

alternative to pesticides, antimicrobial agents, and a source of new compounds for the promotion of human well-being and health. Among the numerous biological activities of *H. rhamnoides* extracts is an antimicrobial capacity that makes the raw plant more valuable as a medicinal plant (Krejcarova et al., 2015).

Disc diffusion method. The antibacterial activity of the sea buckthorn tea, berries, oil, and juice against Gram-positive and Gram-negative bacteria detected with the disc diffusion method is shown in Table 3.

The zones of inhibition of bacteria ranged from 2.33 ±0.58 mm in the sea buckthorn oil to 10.67 ±4.93 mm in the sea buckthorn tea. The antibacterial activity of the sea buckthorn tea ranged from 3.00 ±1.00 mm against *S. aureus* subsp. *Aureus*, to 10.67 ±4.93 mm against *Y. enterocolitica*. This parameter for the sea buckthorn dry berries ranged from 4.00 ±1.00 against *S. enterica* subsp. *enterica*, and *S. aureus* subsp. *aureus* to 8.67 ±1.53 mm against *L. monocytogenes*. The sea buckthorn oil showed the lowest antibacterial activity against *S. enterica* subsp. *enterica* (2.33 ±0.58 mm), and the highest antibacterial activity against *E. coli* (8.00 ±2.00 mm). The sea buckthorn juice revealed the lowest antibacterial activity of 3.33 ±1.53 to *B. thuringiensis*, and *L. monocytogenes*, but the highest against *Y. enterocolitica* (6.00 ±2.00 mm). Sea buckthorn exhibited antimicrobial activity against Gram-negative bacteria, which was in agreement with a study of Michel et al. (2012). In their study, Yogendra Kumar et al. (2013) showed the marked antibacterial activity of sea buckthorn leaf extract (SLE) and a phenolic rich fraction (PRF) against Gram-positive and Gram-negative bacteria including *E. coli*, *S. typhi*, *S. dysenteriae*, *S. pneumoniae*, and *S. aureus*. The SLE had the maximum zone of inhibition (15.23 ±0.84 mm) against *S. dysenteriae*, and the minimum zone of inhibition (8.33 ±1.12 mm) for *S. pneumoniae* while the PRF had the maximum zone of inhibition (20.67 ±1.54 mm) for *S. dysenteriae*, and the minimum zone of inhibition (8.0 ±0.47 mm) for *S. typhi*.

The extract of sea buckthorn berries and leaves was found to possess antibacterial activity against methicillin resistant *Staphylococcus aureus* (Qadir et al., 2016). Also, the aqueous and hydroalcoholic leaf extracts of sea buckthorn showed a growth-inhibiting effect against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis* (Upadhyay et al., 2010).

Minimal inhibition concentration. The results on antimicrobial activity of four extracts from *Hippophae rhamnoides* against various strains of bacteria are summarized in Table 4.

E. coli was found to be the most susceptible to sea buckthorn tea, and the sea buckthorn berries extract with a MIC 50 value of 1086.37 μg/mL, and a MIC 90 value of 1687.93 μg/mL. *S. enteritidis* subsp.

Table 3. Antibacterial property of *Hippophae rhamnoides* L. extracts using disc diffusion method, mm

Sample	EC	SE	YE	BT	LM	SA
Tea	7.67 ±1.53^{ab}	4.00 ±1.73^{ab}	10.67 ±4.93^{a}	6.67 ±1.53^{a}	5.67 ±2.08^{b}	3.00 ±1.00^{b}
Dry berries	5.00 ±1.00^{b}	4.00 ±1.00^{ab}	8.33 ±3.06^{a}	5.33 ±1.53^{ab}	8.67 ±1.53^{a}	4.00 ±1.00^{ab}
100% oil	8.00 ±2.00^{a}	2.33 ±0.58^{b}	4.67 ±1.53^{a}	3.67 ±1.53^{ab}	5.00 ±1.00^{b}	5.33 ±1.53^{a}
100% juice	4.33 ±1.53^{b}	5.67 ±2.08^{a}	6.00 ±2.00^{a}	3.33 ±1.53^{b}	3.33 ±1.53^{b}	4.00 ±1.00^{ab}

EC – *Escherichia coli* CCM 3988.
SE – *Salmonella enterica* subsp. *enterica* CCM 3807.
YE – *Yersinia enterocolitica* CCM 5671.
BT – *Bacillus thuringiensis* CCM 19.
LM – *Listeria monocytogenes* CCM 4699.
SA – *Stapylococcus aureus* subsp. *aereus* CCM 2461.
Mean ±standard deviation.
Different superscripts in each column indicate significant differences in the mean at $P < 0.05$.

Table 4. Minimal inhibition concentration (MIC) in μg/mL of microorganisms against different *Hippophae rhamnoides* L. products

TO	Tea		Dry berries		100% oil		100% juice	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
EC	1 086.37	1 687.93	1 086.37	1 687.93	>10 2040	>10 2040	>10 2040	>10 2040
SE	1 583.90	2 860.52	1 583.90	2 860.52	>10 2040	>10 2040	>10 2040	>10 2040
YE	1 583.90	2 860.52	1 583.90	2 860.52	>10 2040	>10 2040	>10 2040	>10 2040
BT	7 660.07	8 142.60	7 660.07	8 142.60	>10 2040	>10 2040	>10 2040	>10 2040
LM	7 660.07	8 142.60	7 660.07	8 142.60	>10 2040	>10 2040	>10 2040	>10 2040
SA	7 660.07	8 142.60	7 660.07	8 142.60	>10 2040	>10 2040	>10 2040	>10 2040

TO – tested microorganisms.
EC – *Escherichia coli* CCM 3988.
SE – *Salmonella enterica* subsp. *enterica* CCM 3807.
YE – *Yersinia enterocolitica* CCM 5671.
BT – *Bacillus thuringiensis* CCM 19.
LM – *Listeria monocytogenes* CCM 4699.
SA – *Staphylococcus aureus* subsp. *aureus* CCM 2461.

enteritidis, and *Y. enterocolitica* were the least susceptible to sea buckthorn tea, and the sea buckthorn berries with MIC 50 value of 1583.90 μg/mL, and MIC 90 value of 2860.52 μg/mL. *B. thurigiensis*, *L. monocytogenes*, and *S. aureus* subsp. *aureus* were the least susceptible to sea buckthorn tea and the sea buckthorn berry extract with the highest MIC 50 values of 7660.07 μg/ml, and MIC 90 value of 8142.60 μg/mL. The sea buckthorn oil and sea buckthorn juice didn't show antimicrobial activity against Gram-positive or Gram-negative bacteria higher than 10.240 μg/mL. *E. coli*, *S. enteritidis* subsp. *enteritidis*, and *Y. enterocolitica* were found to be more susceptible to *Hippophae rhamnoides* L. in comparison to the other microorganisms that were tested. The results of MIC depended on the extract applied for the study, and *B. subtilis* and *S. aureus* were the most resistant to all the extracts (Saikia and Handique, 2013).

Our study shows that *B. thurigiensis* and *S. aureus* were among the most resistant to sea buckthorn action, and all of the tested extracts showed greater potent antibacterial activity against Gram-positive bacteria than Gram-negative. This is attributable to differences between the outer membrane structure among Gram-negative and Gram-positive bacteria. Gram-negative bacteria have an outer membrane consisting of lipoprotein and lipopolysaccharide, which is selectively permeable and thus regulates access to the underlying structure making Gram-negative bacteria generally less susceptible to plant extracts than Gram-positive bacteria (Koohsari et al., 2015).

The antimicrobial activity of the sea buckthorn extracts is probably due to their ability to form a complex with extracellular and soluble proteins, and bacterial cell walls by nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial actions may be related to their ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins. Therefore, the results of the present study suggests that *H. salicifolia* is a potential source of bioactive antimicrobial agents, which could be used as a natural preservative and for nutraceutical formulations (Gupta et al., 2011).

CONCLUSION

Sea buckthorn products are a prospective product for application in medical, pharmaceutical, and cosmetic based industries. The investigated raw parts of the plant are rich in biologically active compounds with strong antioxidant and antibacterial effects. These

compounds could have practical applications, and compounds of different sea buckthorn parts may be used as functional foods and nutraceuticals to increase antioxidant status and strengthen the immune system for better resistance of the body to multiple stresses. Carotenoids obtained from berries can be used as a natural colorant in foods where they can replace synthetic colorants because they are safer to humans in comparison to synthetic additives. Further studies for the identification of bioactive compounds are needed for a comprehensive evaluation of the antioxidant and antimicrobial properties of sea buckthorn.

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REFERENCES

Balkrishna, A., Sakat, S. S., Joshi, K., Joshi, K., Sharma, V., Ranjan, R., ..., Varshney, A. (2019). Cytokines driven anti-inflammatory and anti-psoriasis like efficacies of nutraceutical sea buckthorn (*Hippophae rhamnoides*) oil. Front. Pharm., 10. https://doi.org/10.3389/fphar.2019.01186

Ball, G. F. M. (1988). Fat soluble vitamin assays in food analysis. London: Elsevier.

Bonesi, M., Loizzo, M. R., Provenzano, E., Menichini, F., Tundis, R. (2016). Anti-psoriasis agents from natural plant sources. Curr. Med. Chem., 23, 12, 1250–1267. https://doi.org/10.2174/0929867323666160321121819

Chauhan, S., Varshneya, C. (2012). The profile of bioactive compounds in sea buckthorn berries and oil. Int. J. Theor. Appl. Sci., 4, 216–220.

Cho, C. H., Jang, H., Lee, M., Kang, H., Heo, H. J., Kim, D.-O. (2017). Sea buckthorn (*Hippophae rhamnoides* L.) leaf extracts protect neuronal PC-12 cells from oxidative stress. J. Microbiol. Biotechnol., 27(7), 1257–1265. https://doi.org/10.4014/jmb.1704.04033

Christaki, E. (2012). *Hippophae rhamnoides* L. (sea buckthorn): a potential source of nutraceuticals. Food Public Health, 2, 69–72. https://doi.org/10.5923/j.fph.20120203.02

CLSI (2009). Performance standards for antimicrobial susceptibility testing; 20th informational supplement. CLSI document M100-S19. CLSI, Wayne, PA: Clinical and Laboratory Standard Institute.

Criste, A., Urcan, A. C., Bunea, A., Furtuna, F. R. P., Olah, N. K., Madden, R. H., Corcionivoschi, N. (2020). Phytochemical composition and biological activity of berries and leaves from four Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. Molecules, 25(5), 1170. https://doi.org/10.3390/molecules25051170

Dienaitė, L., Pukalskas, A., Pukalskienė, M., Pereira, C. V., Matias, A. A., Venskutonis, P. R. (2020). Phytochemical composition, antioxidant and antiproliferative activities of defatted sea bukhtorn (*Hippophae rhamnoides* L.) berry pomace fractions concequtively recovered by pressurized ethanol and water. Antioxidants, 9, 274. https://doi.org/10.3390/antiox9040274

Dontha, S. (2016). A review on antioxidant methods. Asian J. Pharm. Clin. Res., 9, 14–32. https://dx.doi.org/10.22159/ajpcr.2016.v9s2.13092

Dubey, S., Ramana, M. V., Mishra, A., Gupta, P. S., Awasthi, H. (2018). Seabuckthorn (*Hippophae rhamnoides* and *Hippophae salicifolia*) seed oil in combating inflammation: a mechanistic approach. Pharm. Res., 10, 404–407.

Geetha, S., Singh, V., Ram, M. S., Ilavazhagan, G., Banerjee, P. K., Sawhney, R. C. (2005). Immunomodulatory effects of seabuckthorn (*Hippophae rhamnoides* L.) against chromium (VI) induced immunosuppression. Molecul. Cell. Biochem., 278, 101–109. https://doi.org/10.1007/s11010-005-7095-9

Gill, N. S., Sharma, R., Arora, R., Bali, M. (2012). Antioxidant and antibacterial activity of *Hippophae rhamnoides* methanolic leaf extracts from dry temperate agroclimatic region of Himahal Pradesh. J. Plant Sci., 7, 194–200. https://doi.org/10.3923/jps.2012.194.200

Guo, R., Chang, X., Guo, X., Brennan, Ch. S., Li, T., Fu, X., Liu, R. H. (2017). Phenolic compounds, antioxidant activity, antiproliferative activity and bioaccessibility of sea buckthorn (*Hippophae rhamnoides* L.) berries as affected by in vitro digestion. Food Funct., 8, 4229. https://doi.org/10.1039/C7FO00917H

Gupta, S. M., Gupta, A. K., Ahmed, Z., Kumar, A. (2011). Antibacterial and antifungal activity in leaf, seed extract and seed oil of seabuckthorn (*Hippophae salicifolia* D. Don) plant. J. Plant Pathol. Microbiol., 2, 105. https://doi.org/10.4172/2157-7471.1000105

Hleba, L., Vuković, N., Horská, E., Petrová, J., Sukdolak, S., Kačániová, M. (2014). Phenolic profile and antimicrobial activities to selected microorganisms of some wild medical plant from Slovakia. Asian Pacific J.

Trop. Dis., 4, 269–274. https://doi.org/10.1016/S2222-1808(14)60571-5

Ito, H., Asmussen, S., Traber, D. L., Cox, R. A., Hawkins, H. K., Connelly, R., Traber, L. D., ..., Enkhbaatar, P. (2014). Healing efficacy of sea buckthorn (*Hippophae rhamnoides* L.) seed oil in an ovine burn wound model. Burns, 40, 511–519. https://doi.org/10.1016/j.burns.2013.08.011

Ji, M., Gong, X., Li, X., Wang, C., Li, M. (2020). Advanced research on the antioxidant activity and mechanism of polyphenols from *Hippophae* species – a review. Molecules, 25, 917. https://doi.org/10.3390/molecules25040917

Koohsari, H., Chaemi, E. A., Sadesh Sheshpoli, M., Jahedi, M., Zahiri, M. (2015). The investigation of antibacterial activity of selected native plants from North of Iran. J. Med. Life, 8, 38–42.

Krejcarova, J., Straková, E., Suchy, P., Herzig, I., Karáskova, K. (2015). Sea buckthorn (*Hippophae rhamnoides* L.) as a potential source of nutraceutics and its therapeutic possibilities – a review. Acta Vet. Brno, 84, 257–268. https://doi.org/10.2754/avb201584030257

Kuhkheil, A., Naghdi Badi, H., Mehrafarin, A., Abdossi, V. (2017). Chemical constituents of sea buckthorn (*Hippophae rhamnoides* L.) fruit in populations of central Alborz Mountains in Iran. Res. J. Pharmacog., 4(3), 1–12.

Liu, Q., Yao, H. (2007). Antioxidant activities of barley seeds extracts. Food Chem., 102, 732–737. https://doi.org/10.1016/j.foodchem.2006.06.051

Ma, X., Laaksonen, O., Zheng, J., Yang, J., Trepanier, M., Kallio, H. (2016). Flavone glycosides in berries of two major subspecies of sea buckthorn (*Hippophaë rhamnoides* L.) and influence of growth sites. Food Chem., 200, 189–198. https://doi.org/10.1016/j.foodchem.2016.01.036

Michel, T., Destandau, E., Le Floch, G., Lucchesi, M. E., Elfakir, C. (2012). Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (*Hippophaë rhamnoides* L.) leaf, stem, root and seed. Food Chem., 131, 754–760. https://doi.org/10.1016/j.foodchem.2011.09.029

Muzykiewicz, A., Zielonka-Brzezicka, J., Klimowicz, A. (2018). Antioxidant potential of *Hippophae rhamnoides* L. extracts obtained with green extraction technique. Herba Pol., 64(4), 14–22. https://doi.org/10.2478/hepo-2018-0022

Olas, B., Skalski, B., Ulanowska, K. (2018). The anticancer activity of sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson). Front. Pharmacol., 9, 232. https://doi.org/10.3389/fphar.2018.00232

Pop, R., Weesepoel, Y., Socaciu, C., Pintea, A., Vincken, J. P. (2014). Carotenoid composition of berries and leaves from six Romanian Sea buckthorn (*Hippophae rhamnoides* L.) varieties. Food Chem., 147, 1–9. https://doi.org/10.1016/j.foodchem.2013.09.083

Prieto, P., Pinera, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem., 269, 334–337. https://doi.org/10.1006/abio.1999.4019

Qadir, M. I., Abbas, K., Younus, A., Shaikh, R. S. (2016). Report – Antibacterial activity of sea buckthorn (*Hippophae rhamnoides* L.) against methicillin resistant *Staphylococcus aureus* (MRSA). Pakistan J. Pharm. Sci., 29(5), 1711–1713.

Saikia, M., Handique, P. J. (2013). Antioxidant and antibacterial activity of leaf and bark extracts of Seabuckthorn (*Hippophae salicifolia* D Don) of North East India. Int. J. Life Sci. Biotechnol. Pharma Res., 2, 81–91.

Sánchés-Moreno, C., Larrauri, A., Saura-Calixto, F. (1998). A procedure to measure the antioxidant efficiency of polyphenols. J. Sci. Food Agric., 76, 270–276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

SAS (2009). Users guide. Version 9.2. SAS/STAT (r). Cary, NC, USA: SAS Institute.

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult., 6, 144–158.

Smida, I., Pentelescu, C., Pentelescu, O., Sweidan, A., Oliviero, N., Meuric, V., ..., Tamanai-Shacoori, Z. (2019). Benefits of sea buckthorn (*Hippophae rhamnoides*) pulp oil based-mouthwash on oral health. J. Appl. Microbiol., 126, 1594–1605. https://doi.org/10.1111/jam.14210

Suryakumar, G., Gupta, A. (2011). Medicinal and therapeutical potential of sea buckthorn (*Hippophae rhamnoides* L.). J. Ethnopharm., 138(2), 268–278. https://doi.org/10.1016/j.jep.2011.09.024

STN 56 0053 (1986). Determination of vitamin A and its provitamins. Praha: ÚNN.

Ting, H., Hsu, Y., Tsai, C., Lua, F., Chou, M., Chen, W. (2011). The *in vitro* and *in vivo* antioxidant properties of seabuckthorn (*Hippophae rhamnoides* L.) seed oil. Food Chem., 125, 652–659.

Upadhyay, N. K., Kumar, M. S. Y., Gupta, A. (2010). Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (*Hippophae rhamnoides* L.) leaves. Food Chem. Toxicol., 48, 3443–3448. https://doi.org/10.1016/j.fct.2010.09.019

Upadhyay, N. K., Kumar, R., Mandotra, S. K., Meena, R. N., Siddiqui, M. S., Sawhney, R. C., Gupta, A. (2009). Safety and healing efficacy of sea buckthorn (*Hippophae rhamnoides* L.) seed oil in experimental rats. Food Chem. Toxicol., 47, 1146–1153. https://doi.org/10.1016/j.fct.2009.02.002

Upadhyay, N. K., Kumar, R., Siddiqui, M. S., Gupta, A. (2011). Mechanism of wound healing activity of *Hippophae rhamnoides* L. leaf extract in experimental burns. Evidence-Based Compl. Altern. Med., art. ID 659705. https://doi.org/10.1093/ecam/nep189

Varshneya, C., Kant, V., Mehta, M. (2012). Total phenolic contents and free radical scavenging activities of different extracts of seabuckthorn (*Hippophae rhamnoides*) pomade without seeds. Int. J. Food Sci. Nutr., 63, 153–159. https://doi.org/10.3109/09637486.2011.608652

Veluri, R., Weir, T. L., Bais, H. P., Stermitz, F. R., Vivanco, J. M. (2004). Phytotoxic and antimicrobial activities of catechin derivatives. J. Agric. Food Chem., 52, 1077–1082. https://doi.org/10.1021/jf030653+

Willett, W. C. (2002). Balancing life-style and genomics research for disiase prevention. Science, 292, 695–698. https://doi.org/10.1126/science.1071055

Xing, J. X. (2018). Comparative study on total flavonoids content in different parts of *Hippophae rhamnoides* Linn. Shanxi For. Sci. Technol., 47, 4–5.

Yan, G. B., Kallio, H. (2002). Composition and physiological effects of sea buckthorn (*Hippophaë*) lipids. Trends Food Sci. Technol., 13, 160–167. https://doi.org/10.1016/S0924-2244(02)00136-X

Yogendra Kumar, M. S., Tirpude, R. J., Maheshwari, D. T., Bansal, A., Misra, K. (2013). Antioxidant and antimicrobial properties of phenolic rich fraction of seabuckthorn (*Hippophae rhamnoides* L.) leaves *in vitro*. Food Chem., 141, 3443–3450. https://doi.org/10.1016/j.foodchem.2013.06.057

Yu, X.-F., Shang, X., Zhang, Zh.-J., Zhang, Y.-N. (2017). Phytochemical composition and antibacterial activity of the essential oils from different parts of sea buckthorn (*Hippophae rhamnoides* L.). J. Food Drug Anal., 25(2), 327–332. https://doi.org/10.1016/j.jfda.2016.10.010

Zielińska, A., Nowak, I. (2017). Abundance of active ingredients in sea-buckthorn oil. Lipids Health Dis., 16, 95. https://doi.org/10.1186/s12944-017-0469-7