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ANTIMICROBIAL PROPERTIES OF SEA BUCKTHORN GROWN IN THE REPUBLIC OF MOLDOVA

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Abstract. This study deals with the antibacterial activity of Sea buckthorn (SB) (*Hippophae rhamnoides* L.) grown in the Republic of Moldova. Eight sea buckthorn species were investigated: R1, R2, R4, R5, C6, AGG, AGA, Pomona, 2020 harvest, from Dubasari district, Pohrebea village of the Republic of Moldova. The sea buckthorn fruit was harvested during the complete sweeping phase. The antibacterial efficacy of Sea buckthorn on different microbial cultures (*Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella Typhimurium*, *Escherichia coli, Candida albicans*) causing infections/diseases was investigated by agar disc diffusion method. The inhibition zones ranged from 12 to 30 mm: *Staphylococcus aureus* ATCC 25923 (21-30 mm); *Bacillus subtilis* ATCC6633 (19-29 mm); *Salmonella Typhimurium* ATCC 14028 (13-18 mm); *Escherichia coli* ATCC 25922 (12-18 mm). In the case of *Candida albicans* ATCC 10231, this fungal pathogen was resistant to SB. It was found that antimicrobial efficacy of SB depends on the species, concentration, and form of use (fruit puree, extracts with different solvents and powder). Results suggested that SB might be a valuable ingredient for the development of safe products for consumption.

Keywords: *Hippophae rhamnoides L., Gram-positive bacteria, gram-negative bacteria, inhibition zone, food safety.*

Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) (SB) is well known as a multifunctional plant, which is widely grown in Asia, Europe, and Canada. In recent years, great attention has been paid to the cultivation of this crop in the Republic of Moldova, to be used in various industries. Both fruits and sea buckthorn leaves, having a relevant composition in biologically active substances, are good for health [1]. Sea buckthorn has a great potential for use in maintaining the local landscapes, in the food industry (manufacture of juices, drinks, jams, powders, candies, natural dyes, etc.), in the pharmaceutical and cosmetics industry [2 - 3]. Sea buckthorn products are delicious, have an attractive appearance, a relevant content of antioxidants, vitamins, minerals and are beneficial to consumer health [4 - 5].

Currently, great attention is being paid to the use of berries, as therapeutic remedies and with a high content of polyphenols, used as natural antioxidants and as nutraceutical supplements, with antimicrobial, anticancer, antiallergic and other properties [6 - 8]. Sea buckthorn fruits have all these miraculous properties and can be used successfully in a healthy diet. In this direction, various research studies are carried out, SB being in the top of investigated berries. There are many studies [9 - 11], which report the relevance of SB for consumption. The authors note SB as a relevant source of dietary antioxidants belonging mainly to the class of phenolic compounds. The antioxidant and antimicrobial activity of berries depends largely on their chemical composition.

In the published bibliographic sources, the chemical composition of SB is quite varied, depending on several factors: variety, methods and time of cultivation, climatic conditions, etc. The authors of the sources [12 - 13] reported a content of vitamin C in SB in the range of 0.98 - 5.14 mg/g and a large number of phenolic substances, the largest share belonging to phenolic glycosides. The authors of two other studies [14 - 15] report that the vitamin C content in SB varies in the range of 0.59 to 4.07 mg/g and in the berries is predominant isorhamnetin and quercetin glycosides. Carotenoids are present mainly in form of β-carotene [16 - 17], vitamin E in form of α-tocopherol [18 - 19] and niacin is the most abundant of the B vitamins. Sea buckthorn has antibacterial properties, due to the high content of polyphenols in its composition. The authors of the studies [20-25] reported the inhibitory effect on the growth of gram-positive (*Streptococcus pneumoniae* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae*) pathogenic bacteria.

Hippophae rhamnoides L. has a relevant chemical composition that possesses antioxidant and antimicrobial properties. The chemical composition of SB is found in many research studies [26 - 31]. The berries are rich in both hydrophilic and lipophilic phytochemicals, including ascorbic acid, tocopherols, carotenoids, essential fatty acids. and various phenolic compounds [32 - 34]. In recent years, more and more research is being done to determine the antioxidant properties of SB [35 - 39]. The study authors [34], [40 - 41] in their research found that flavonoids were a class of major bioactive components in SB fruits, leaves and seeds, which have been shown to have anti-inflammatory, immunomodulatory, antioxidant properties, cardiovascular, anti-cancer, etc.

Dienaitė et al. [42] reported that SB pulp is a relevant source of biologically active substances with antioxidant properties, which inhibit the development of cancer cells. The authors mention that the main role in this regard belongs to galloylated flavonols and tanshinlactone derivatives. Phenolic compounds in plants they exhibit anti-inflammatory activity *in vitro* and *in vivo*, their mechanism of action is the inhibition of enzymes, by binding with hydrosulphide groups and inactivating bacterial proteins [43 - 44].

The authors of the studies [36, 45] have correlated the antimicrobial properties with the content of phenolic compounds in sea buckthorn.

The antimicrobial activity of sea buckthorn fruits and leaves is also found in the study conducted by Qadir et al. [46], showing the inhibitory effect of berries on *Staphylococcus aureus* (MRSA), using the standard method of disc diffusion. Another study [47] found that aqueous and hydroalcoholic sea buckthorn leaf extracts inhibited the growth of *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*.

There is currently a tendency to replace synthetic preservatives with natural ones, using berries in any form in food manufacturing. Many researchers in his work have shown positive results showing that sea buckthorn can be used for medicinal and preservation purposes. The antioxidant and antimicrobial effects of berries imply its natural conservation potential [20, 48 - 54]. The results presented by the authors showed that fruits, extracts and powders of sea buckthorn can serve as relevant ingredients to obtain quality products, stable in storage and safe for consumption. Sea buckthorn samples showed microbial activity against Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 11778 and Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853 [22].

The antibacterial activity of SB has a fairly wide spectrum and can be evaluated by different methods. Tables 1, 2 and 3 include some international research results published in recent years.

Table 1

Minimum inhibitory concentration (mean) of sea buckthorn and positive control on different bacteria

Sea buckthorn	Bacteria								Source
	Bacillus subtilis	*Bacillus cereus*	*Bacillus coagulans*	*Staphylococcus aureus*	*Escherichi coli*	*Listeria monocytogenes* EGDe	*Listeria monocytogenes* sp.	*Yersinia enterocolitica*	
	Minimum inhibitory concentration, (mg/mL)								
Seed	1.52	24.39	6.10	12.20	6.10				[23]
Pulp	0.19	3.05	0.10	12.20	12.20				
Leaf	3.05	48.78	1.52	12.20	12.20				
Tetracycline hydrochloride	0.76	0.76	3.05	1.52	0.76				
Methanol extract	0.3	0.2	0.3				0.3	0.35	[24]
Seeds aqueous extract							0.75	1.00	[49]
Leaves extract							0.125		[37]
Hydroalcoholic concentrates C_1						2.6			[50]
C_2						5.2			
Hydroalcoholic extract H_1							2.6		[50]

Table 2

Antibacterial and antifungal activity of sea buckthorn

Bacteria	Diameter of inhibition zone, (mm)	Source	Bacteria	Diameter of inhibition zone, (mm)	Source
Klebsiella pneumoniae	8.0 ± 0.4	[55]	*Shigella dysenteriae*	20.67-15.23	[20]
Salmonella enterica	11.0 ± 0.3		*Agrobacterium tumefaciens*	*12±0.4 **13±0.2	[57]
Pseudomonas aeruginosa	20.0 ± 0.2		*Arcobacter butzleri*	18.0 ± 2.0	[58]
Acinetobacter baumannii	16.0 ± 0.1		*Arcobacter cryaerophilus*	28.0± 0.0	
Proteus mirabilis	20.0 ± 0.2		*Staphylococcus aureus*	10−14	[20]
Bacillus subtilis	*11.0±0.6 **12.0±0.5	[57]	*Proteus mirabilis*	20.0 ± 0.2	[55]
Bacillus thuringiensis	*13.0±0.5 **14.0±0.6		*Enterococcus faecalis*	18.0 ± 0.3	
Mucor indicus	**14±0.2		*Tilletia indica*	**11±0.4	[57]

*Fresh crude leaf extract. **Concentrated crude leaf extract

The results in Table 2 show different antimicrobial and antifungal influences of sea buckthorn depending on the genus of pathogenic bacteria.

Table 3

Inhibitory activities of sea buckthorn extracts on arcobacters [58]

Extract	*Arcobacter butzleri* CCUG 30484	*Arcobacter butzieri* UPa 2015/6	*Arcobacter cryaerophilus* CCM 3934	*Arcobacter cryaerophilus* UPa 2015/16
	Diameter of inhibition zone (mm)			
EtOH extract	28.5 ± 2.4	24.5 ± 2.1	23.0 ± 1.0	25.5 ± 0.7
Extract in PBS*	18.0 ± 2.0	20.5 ± 0.7	28.0 ± 0.0	20.3 ± 1.5
EtOH 96% (v/v)	16.0 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.5 ± 0.1

*PBS- phosphate buffered saline.

The data in Table 3 show that the sea buckthorn extracts had an inhibitory activity on *Arcobacter* bacteria and may be recommended as an antimicrobial agent.

Materials and methods

Eight sea buckthorn species were investigated: R1, R2, R4, R5, C6, AGG, AGA, Pomorancevaia, 2020 harvest, from Dubasari district, Pohrebea village of the Republic of Moldova. The sea buckthorn fruit was harvested during the complete sweeping phase. Sea buckthorn samples were frozen at -25°C. For testing antimicrobial properties, berries kept frozen for 6 months were used.

The antimicrobial activity of SB was evaluated by the agar diffusion method. In this study we used inhibition zone test, also called Kirby-Bauer Test [59]. It is a qualitative method used to measure and compare the inhibitory activity levels of the tested substances (different fractions of eight sea buckthorn species: pulp, extracts, powder). The diameter of the inhibition zone which marks the absence of microbial growth was measured with the shubler ruler. Bacterial cultures namely: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Salmonella Typhimurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231 were obtained from the Microbiology and Immunology Department, ”Nicolae Testemitanu” State University of Medicine and Pharmacy.

All calculations were done using Microsoft Office Excel 2007 (Microsoft, USA). Data obtained in this study are presented as mean values ± the standard error of the mean calculated from 3 parallel experiments.

Results and Discussions

The antimicrobial activity of the eight SB species was evaluated by the agar diffusion method. Areas of inhibition of the development of the tested microorganisms were estimated. The results obtained are included in Table 4.

Table 4

Antimicrobial activity of sea buckthorn species grown in the Republic of Moldova

Sea buckthorn species	Diameter of inhibition zone (mm)				
	Staphylococcus aureus ATCC 25923	*Bacillus subtilis* ATCC 6633	*Salmonella typhimurium* ATCC 14028	*Escherichia coli* ATCC 25922	*Candida albicans* ATCC 10231
R1	22.0 ± 0.1	19.0 ± 0.1	R	12.0 ± 0.2	R
R2	24.0 ±0.1	22.0 ± 0.2	13.0 ± 0.1	13.0 ± 0.1	R
R4	26.0 ±0.1	24.0± 0.1	15.0 ± 0.1	15.0 ± 0.1	R
R5	26.0 ±0.1	25.0 ± 0.1	14.0 ± 0.2	15.0 ± 0.1	R
C6	24.0 ± 0.2	26.0 ± 0.1	14.0 ± 0.1	17.0 ± 0.1	R
AGG	29.0 ± 0.2	28.0 ± 0.1	18.0 ± 0.1	18.0 ± 0.1	R
AGA	30.0 ± 0.1	29.0 ± 0.1	18.0 ± 0.1	18.0 ± 0.1	R
Pomorancevaia	21.0 ± 0.2	22.0 ± 0.1	R	12.0 ± 0.1	R

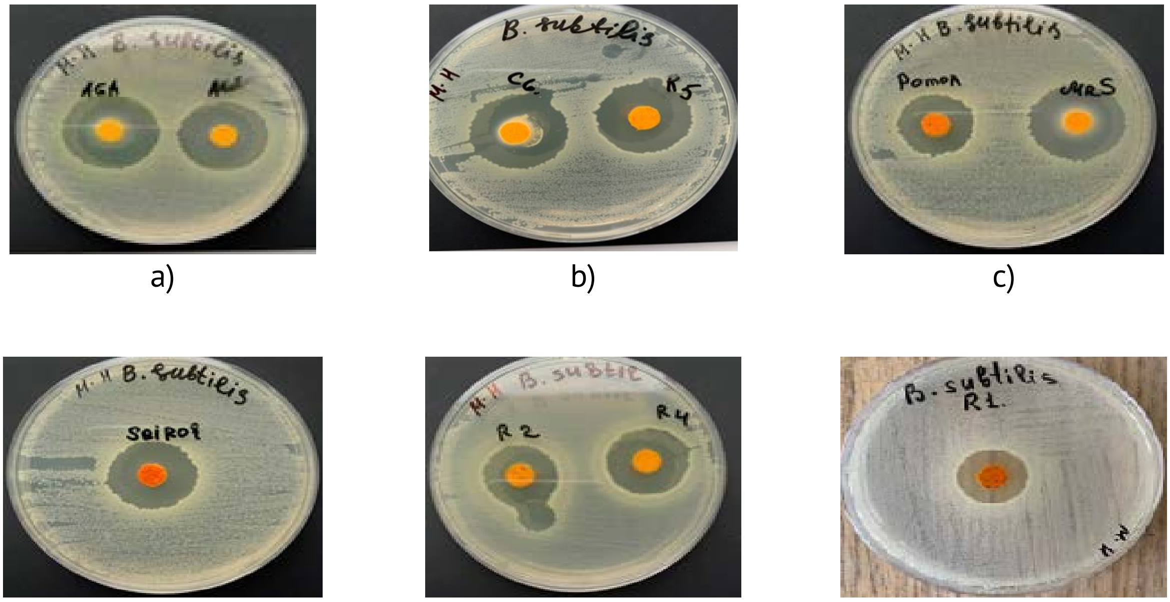
R- resistant

Obtaining different zones of inhibition for the same microorganism can be explained by the different chemical composition of the tested sea buckthorn species (content of antioxidants, organic acids, etc.). The results obtained by us correlate with the results reported by other authors. Kumar et al. [20], by the agar diffusion method, found that an area of inhibition for *Escherichia coli* and *Staphylococcus aureus* was respectively, 10.2 mm and 12.3 mm. In this paper the authors also present the evaluation of the inhibitory effect of SB with the help of the percentage reduction test. A result of 96.0% and 93.0% is reported for *Staphylococcus aureus* and *Escherichia coli*, respectively. Another study by Arora et al. [37] reported a high total phenolic content (278.80 mg GAE/g extract, methanolic extract from SB leaves) with a minimum inhibitory concentration (MIC) value of 125 μg/mL against *Listeria monocytogenes*. Scalbert A. [60] described the mechanism of inactivation and destruction of microorganisms. The researcher mentioned that the microbicidal activity of SB extracts is probably due to their ability to form complexes with extracellular proteins of bacteria, covalent bond formation, hydrophobic effects, and inactivating transport enzymes. A study by Chouhan et al. [61] reveals that the antimicrobial effect of sea buckthorn is due to the phenolic compounds that are present in the berries. The authors reported that SB had an antimicrobial effect against *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Streptococcus pneumoniae* and *Staphylococcus aureus* [62].

Reporting different results of sea buckthorn microbial activity can be justified by the difference between the chemical composition of the sea buckthorn varieties and the sea buckthorn samples tested. For example, in the study by Zheng et al. [63] shows the difference between the organic acid content present in different sea buckthorn species. The authors found that the content of malic acid and quinic acid (main acids) was higher in the Pertsik variety and in Vitaminaya, respectively. The highest share of ascorbic acid was in the Oranzhevaya variety and the lowest in Vitaminaya. The antimicrobial activity of sea buckthorn also depends on the climatic conditions, the region in which the berries are grown. Confirmation of this can be found in published scientific papers. For example, the study authors [64] reported that SB in northern Finland had a higher content of quinic acid, glucose, L-quebrachitol and ascorbic acid than SB in Canada. The data [65] are also significant, includes the fatty acid composition of sea buckthorn lipids of different origins.

The antimicrobial activity of sea buckthorn is directly proportional to the antioxidant activity. These properties of berries depend on the degree of ripening of the fruit and the period of harvest. The results of the study [17] this aspect. The authors found that, for different periods of growth, the antioxidant activity of SB fruits proved to be different. The varieties of late and medium maturation had the highest antioxidant activity than in other periods. The authors argue this with the different content of vitamin C in the fruit. The authors of another study [48] reported the presence in SB of acids: neochlorogenic, chlorogenic, *trans-p*-caffeic, *trans-p*-coumaric, *trans-p*-synaptic, *trans-p*-ferulic and rosmarinic acid. So the presence of other acids in sea buckthorn characterizes the antimicrobial properties of the fruit.

Figure 1 shows the images of the inhibition zones of different sea buckthorn species on the tested microorganisms.



a) b) c)

d) e) f)

Figure 1. Antimicrobial activity of different species of sea buckthorn grown in the Republic of Moldova on pathogenic bacteria - *Bacillus subtilis* ATCC 6633: a) AGA and AGG; b) C6 and R5; c) Pomorancevaia and Mr. Sandu; d) Seirol; e) R2 and R4; f) R1.

The results from Table 5 demonstrate the antimicrobial activity of powder and hydroethanolic extract from sea buckthorn fruits.

Table 5

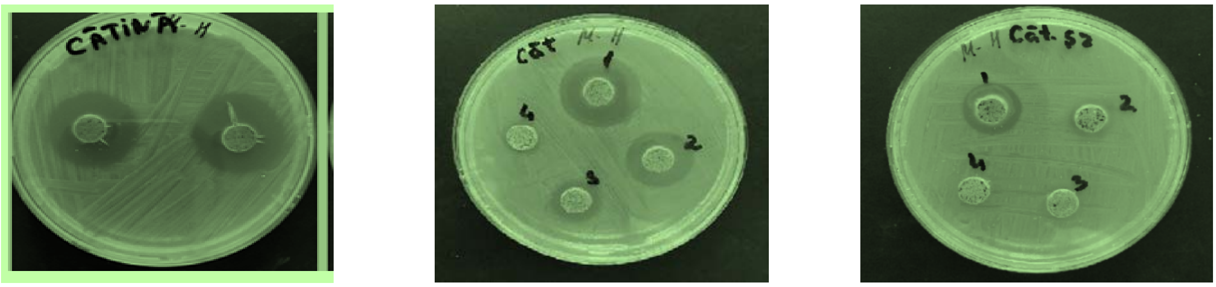
The diameter of the growth inhibition zone of different species of pathogenic microorganisms under the action of sea buckthorn

Sea buckthorn	Diameter of inhibition zone (mm)						
	Staphylococcus aureus ATCC 25923	*Bacillus subtilis* ATCC 6633	*Salmonella typhimurium* ATCC 14028	*Escherichia coli* ATCC 25922	*Listeria monocytogenes* ATCC 19118	*Listeria monocytogenes* EGDe	*Candida albicans* ATCC 10231
Powder	22 - 30	19 - 29	13 - 19	12 - 18	16.07 - 16.59	30 - 32	R
Hydroethanolic extract	-	-	-	-	22.25 - 22.75	30 - 32	-

R- resistant.

As a result of the tests performed, it was found that SB powder was a pronounced antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*, the zone diameter of inhibition varied respectively, between 22-30 mm and 19-29 mm. Powder showed lower antimicrobial activity for *Salmonella typhimurium* – 13-19 mm, *Escherichia coli* – 12-18 mm and *Listeria monocytogenes* (Table 5). *Candida albicans* has shown resistance to sea buckthorn powder. Bibliographic sources attest that the sensitivity of pathogenic microorganisms to sea buckthorn powders is probably due to the structure of the cell wall and outer membrane [66]. Gram-positive bacteria are differed by significant differences in the outer layers, do not have an outer membrane and a periplasmic space [67]. In Gram-negative bacteria, the permeability of the cell wall is reduced due to the high level of phospholipids in the cell wall. Probably, the resistance of Gram-negative bacteria to sea buckthorn powder is related to the hydrophilic surface of their outer membrane, which consists of lipopolysaccharide molecules, presenting a barrier for the penetration of numerous compounds that have antimicrobial activity [68].

Figure 2 shows the influence of antimicrobial activity of sea buckthorn (hydroalcoholic extract, fruit and powder) at different concentrations of test substances on the growth of *Listeria monocytogenes* ATCC 19118.



Sea buckthorn hydroalcoholic extract | Sea buckthorn fruit | Sea buckthorn powder

Figure 2. Inhibition Zone for *Listeria monocytogenes* ATCC 19118 using sea buckthorn. Concentration of test substances: 1 - 250 mg/mL; 2 -125 mg/mL; 3 - 62.5 mg/mL; 4 - 31.25 mg/mL.

Sea buckthorn has been shown to have antimicrobial properties on *Listeria monocytogenes*. These properties are probably due to the chemical composition of the berries, especially organic acids (citric, ascorbic, malic, succinic, lactic, acetic, etc.), tannins, flavonoids, and vitamins. The antimicrobial properties of certain classes of polyphenols and carotenoids are a means of developing new natural food preservatives due to increasing consumer pressure for food without the addition or with minimal use of harmful synthetic additives. However, this activity will always depend on the species of bacteria.

The antimicrobial mechanisms of polyphenols are based on their effects on cytoplasmic membrane destabilization, cell membrane permeability, extracellular microbial enzyme inhibition, direct action on microbial metabolism or deprivation of microbial growth substrates, especially essential minerals [69].

Furthermore, research of berries found that elagitanins showed varying degrees of antimicrobial activity in the growth of selected Gram-negative intestinal bacteria (*Salmonella*, *Staphylococcus*, *Helicobacter*, *Escherichia coli*, *Clostridium*, *Campylobacter*, and *Bacillus* strains), but are not active against beneficial probiotic lactic acid (Gram-positive bacteria). Galotanins have shown antibacterial activity against food bacteria, and Gram-positive bacteria have generally been more sensitive than Gram-negative bacteria. The activity of galotanins is attributed to their strong affinity for iron and is also linked to the inactivation of membrane-bound protein.

Carotenoids have antioxidant and antimicrobial activity against pathogenic bacteria such as colorless *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*, as well as toxigenic fungi such as *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus carbonarius* and *Aspergillus ochraceus*. Several studies investigating the action of carotenoids and essential oils against food spoilage organisms and food pathogens believe that they are more active against Gram-positive than Gram-negative bacteria, as evidenced in this research [70]. Gram-negative organisms are thought to be less susceptible to antibacterial action because they have an outer membrane that surrounds the cell wall, which restricts the diffusion of hydrophobic compounds through the lipopolysaccharide coating.

Conclusions

The paper tested the antimicrobial action of 8 varieties of sea buckthorn original from the Republic of Moldova on Gram-positive pathogenic bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Salmonella typhimurium*). The antifungal action on *Candida albicans* was also tested.

Various inhibition zones for the same microorganism were obtained for different varieties of SB. This can be explained by the different chemical composition of the SB species tested (content of antioxidants, organic acids, etc.). The antimicrobial activity of SB is directly proportional to the antioxidant activity. The microbicidal activity of SB extracts is due to their ability to form complexes with bacterial extracellular proteins, covalent bonds, hydrophobic effects and inactivating transport enzymes.

Following the tests, it was found that SB powder had a pronounced antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*, the diameter of the inhibition zone varied, respectively, between 22-30 mm and 19-29 mm. The powder showed lower antimicrobial activity for *Salmonella typhimurium* - 13-19 mm, *Escherichia coli* - 12-18 mm.

SB exhibits antimicrobial properties on *Listeria monocytogenes*, which are dependent on the SB species. *Candida albicans* has shown resistance to sea buckthorn powder.

The results of this research show that SB powder or extract, grown in the Republic of Moldova could be used as natural preservatives for food, thus expanding the category of food without or with a minimum of synthetic additives. Obviously, further studies are needed to evaluate the matrix effect of these foods on the antimicrobial activity of SB.

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