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In vitro antibacterial effect of various berries on *Listeria monocytogenes* as food borne patogen

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Listeria monocytogenes is a food borne pathogen and causes illnesses with a high mortality rate in susceptible populations. It is often incriminated in outbreaks of human listeriosis. Increasing interest in the health benefits of various berries has led to investigation of their antibacterial activity. Causative agent can multiply at refrigerator temperatures, is resistant to disinfectants, and adheres to various surfaces. Native berries were assessed for their ability to inhibit the growth of bacteria *L. monocytogenes*. Extracts and powder berries – sea buckthorn (*Hippophae rhamnoides* L.), rosehip (*Rosa canina* L.), black chokeberry (*Aronia melanocarpa* (Michx.) Elliott), grape marc (*Vitis vinifera* L.) and hawthorn (*Crataegus oxyacantha* L.) were used. All plant materials come from the Rudi-Arionești Natural Complex in the Republic of Moldova in 2017–2019. In previous studies it has been found that sea buckthorn, rosehip, black chokeberry, and hawthorn have antimicrobial effects on pathogenic microorganisms responsible for food alteration. Bacteria showed varying susceptibilities to the berry fruits. Antimicrobial properties were evaluated using well diffusion method and broth dilution method. According to the results obtained, sea buckthorn was found to have the most pronounced effect on *Listeria monocytogenes*, the diameter of the growth inhibition zone being 32 mm, followed by rosehip samples 26 mm. The minimum inhibition concentration (MIC) and the minimum bactericidal (MBC) were determined.

Keywords: chemical compositions, leaves, antioxidants, antibacterial activity

Introduction

Food borne illness is a common, costly, sometimes life-threatening disease, but largely preventable and are public health problem. Many disease-causing agents can contaminate aliments, causing food poisoning. Researchers have identified more than 250 food borne illnesses. Most of them are infections, caused by a variety of bacteria, viruses, and parasites (Lakshmi and Rajendran, 2013). It is estimated that 48 million people get a food borne illness (or food borne infection) each year, 128,000 are hospitalised, and 3000 die. According to the WHO, an estimated 600 million people get sick each year, or almost one in 10 people on the planet, from food contaminated with microorganisms or chemicals, and 420000 die, resulting in the loss of 33 million years of healthy life (WHO, 2015).

Listeriosis is a life-threatening disease, especially for immunocompromised people and pregnant women.

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In 2019, 2621 confirmed invasive human cases were reported. The population groups that suffered the most from listeriosis were those over 64 years of age. The most common foods associated with listeriosis are ready-to-eat products, being rich in protein and low microflora and moderate water activity.

Listeria monocytogenes is a member of the *Listeria* genus, which also includes other species: *L. ivanovii*, *L. seeligeri*. *L. monocytogenes* is a Gram-positive non-spore-forming rod. Optimum temperature of bacteria is range from 30–37 °C. Microorganism is one of the leading foodborne pathogens and the causative agent of the disease listeriosis. The organism can withstand freezing, but it is inactivated by heating at 60 °C for 30 min (Batt, 2014). Causative agent can multiply at refrigerator temperatures, is resistant to disinfectants, and adheres to various surfaces. Once introduced into the processing plants, it is able to survive and remain for a long period under adverse conditions. *L. monocytogenes* is able to form biofilm which can act as a potential source of contamination and this property is dangerous for food industry (Batt, 2014; Jamshidi and Zeinali, 2019). Bacteria is well known for its tolerance of low pH, high salt conditions (10% NaCl), low temperature (-1 °C), and acid tolerance response, which contribute to its common contamination of food. *Listeria* contamination is habitually reported in dairy products, ready-to-cook fish, and meat products such as smoked salmon and sausage; therefore, they are considered as high-risk foods. Inspite of the conditions of food storage and processing, such as high salt and low temperature, infectious agent can survive and multiply because of its halotolerance and psychrotolerance ability (Jamshidi and Zeinali, 2019; Yap et al., 2021). A sustainable future requires control of antimicrobial resistance (Jeong et al., 2010; Akinduti et al., 2019; Wu et al., 2022).

Inspection of *L. monocytogenes* in food production remains henceforward important not only for producers, but also for consumers. Therefore, efficient antimicrobial approaches for preventing food contamination or the occurrence of listeriosis are urgently needed. Today, consumers desire to choose more natural, healthy, and safe food because inappropriate use of antimicrobial agents in food production might result in undesirable residues in food and the emergence of antimicrobial-resistant microorganisms (Wu et al., 2022). Excessive use of antibiotics has become a modern epidemic. These drugs have destroyed our natural immunity. They killed the beneficial bacteria in our gut and led to the creation of super bacteria that proved to be resistant to almost any form of prescription drugs. Consuming synthetic food preservatives may cause health concerns, including potential side effects and increased cancer risks. Thereby, the use of natural antimicrobials in food preservation has attracted increasing attention from scientists, food manufacturers, and consumers (Wu et al., 2022). Increasing interest of natural foods and their antibacterial activity, our research focused of various forms of native berries and ability to inhibit the growth of bacteria *L. monocytogenes*.

Material and methodology

Extracts and powder berries

The objects of research were the berries of sea buckthorn (*Hippophae rhamnoides* L.), wild rosehip (*Rosa canina* L.), black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) and hawthorn (*Crataegus oxyacantha* L.), as well as grape marc (*Vitis vinifera* L.). These berries contain biologically active compounds (carotenoids, polyphenols) and antioxidant activity, which are presented in many fields of research. All plant materials come from Rudi-Arionești Natural Complex in the Republic of Moldova. To obtain the extracts, the berries have been frozen at -18 °C, dried at room temperature (20.0 ±2.0 °C), and at a temperature of thermal agent 65.0 ±1.0 °C. Dry vegetable matter have moisture of 8.0 ±1.0%. For the experiments, the powder was obtained from berries (sea buckthorn, hawthorn, rosehip, black chokeberry), dried at a temperature of 55 ±1 °C to a final humidity of 6.8 ±0.5%. For extraction, dry matter has been ground and sieved in powder. To obtain the extracts, a different amount of solvent was dosed depending on the raw material according to the following ratio: for sea buckthorn powder – 1 (solid): 12 (solvent); rosehip – 1 : 15; hawthorn – 1 : 20, grape pomace – 1 : 8. The hydroethanolic solution (EtOH, 50% v/v) was used as solvent. The extraction process was performed by two methods: agitation and ultrasound, respecting two temperature regimes: 20.0 ±1.0 °C and 45.0 ±1.0 °C and 3 time periods: 0.5 h, 1.0 h and 1.5 h.

Microbial strain

The antimicrobial properties of plant extracts were tested against Gram-positive *Listeria monocytogenes* Strain EGDe, overnight culture standardised according to Mc Farland 0.5 (105) standard. Culture media – Triptone Soya Broth (Oxoid) 6%, TSA – Tryptone Soy Agar (Oxoid).

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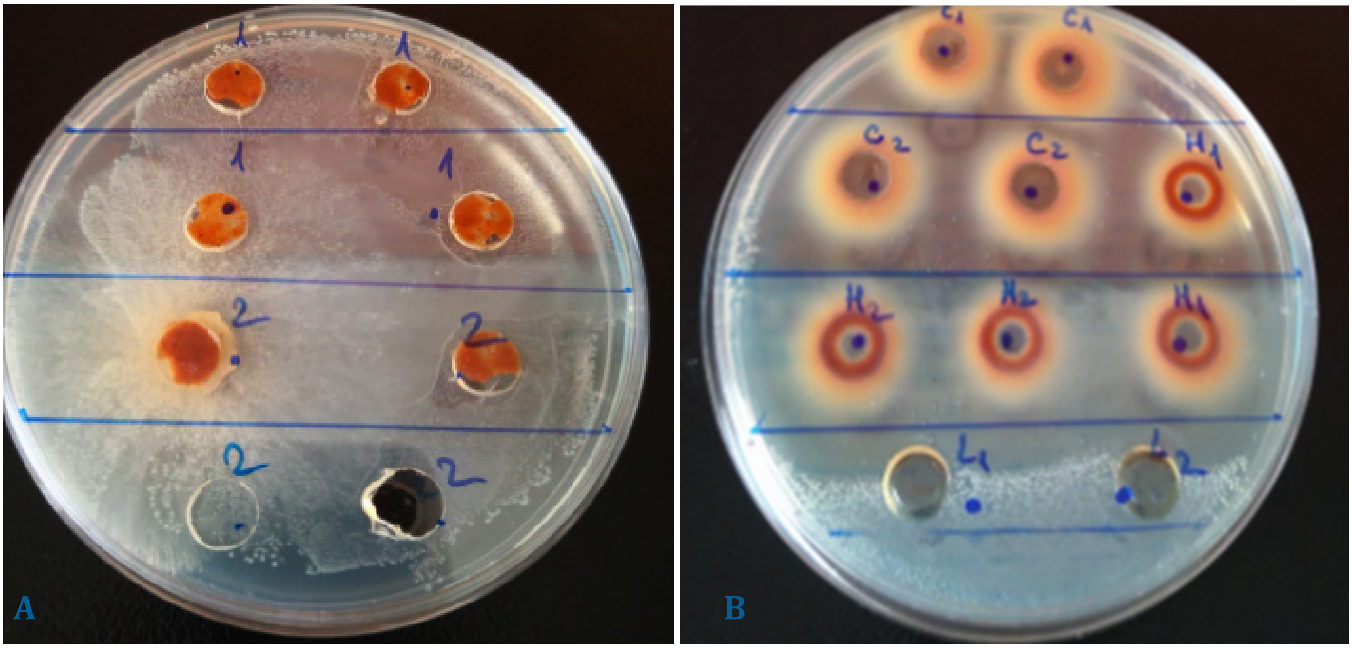

Figure 1 Well diffusion method
A – activity of *C. oxyacantha* extract on *L. monocytogenes*; B – activity of *H. rhamnoides* extract on *L. monocytogenes*

Antibacterial activity. Well diffusion method

\sAgar well diffusion method was done to evaluate antibacterial activity of berry extract and powder as describe by Bauer et al. (1966), Rovná et al. (2015), Olejar et al. (2019), Zhang et al. (2020), and Stefanowski et al. (2021a, b). The strains were grown on nutrient agar slants at 37 °C for 24 h. After the incubation, the cells were washed off the surface of agar and suspended in sterile physiological solution. One mL of fresh bacterial culture was pipetted in the centre of sterile Petri dish with optimum nutrition medium. Then wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 100 µL of each extract was added to respective wells. The plates were placed in the refrigerator for 30 min to let the extracts diffusion well into the agar. Then, the plates were incubated at 37 °C for 18–20 h. Antimicrobial activity was detected by measuring the zone of inhibition (including the wells diameter) appeared after the incubation period (Figure 1).

Determination of minimum inhibitory concentrations (MIC)

Almost all tested extracts exhibited antimicrobial activity. The basic 18 h culture suspension of *L. monocytogenes* was repared. One colony was dissolved in 10 mL of 0.6% TSB (Tryptic Soy Broth) + YE (broth culture medium). Initially the optical density corresponding to 108 was determined, two dilutions were made to obtain 105. For determination of minimum inhibitory concentration, 50 µL of culture (10^5) was inoculated into each well of the microtiter plate. To each well was added 50 µL TSB + YE 0.6% (nutrition medium for *Listeria*) and then 50 µL of each test extract (each of a certain concentration) was added. Binary dilutions were made from the basic solution of each extract: rosehip – 66.7 mg.mL^{-1}, sea buckthorn – 83 mg.mL^{-1}, grape marc – 125.0 mg.mL^{-1}, black chokeberry 55.5 mg.mL^{-1}. The microtiter plates thus prepared were incubated on a thermostat at 37 °C/overnight (18 h). Then the MIC was determined using a spectrophotometer, the OD (Optical Density) measured at λ = 600 nm. Anything above 0.1 at OD is considered microbial growth. The "Tecon" spectrophotometer was used.

Statistical analysis

All the experiments were repeated three times and the data were calculated as means ±SD. One-way ANOVA was used to determine the differences in yields of different extracts.

Results and discussion

Over the last few years, there has been an increasing global trend toward the use of natural antioxidants present in fruits and green leafy vegetables due to fact that consumers are more concerned regarding the safety of using synthetic compounds in convenient food products (Arora et al., 2012; Criste et al., 2020; Vågsholm et al., 2020). For this reason, probably, the diseases caused by psychotrophic bacteria such as *L. monocytogenes* increased. So, all possible strategies to prevent the proliferation of the pathogen in food, especially those using natural bioactive compounds, may contribute to the maintenance of human health (Puupponen-Pimiä et al., 2005). The antimicrobial activity of plant extracts depends on the type and amount of phenolics present in the plant tissue and the pathogen's inherent resistance (Romha et al., 2018; Arora et al., 2012).

According to presence or absence of inhibition zones and zone diameters we determined *in vitro* antimicrobial activity of sea buckthorn extracts and powders against the tested *L. monocytogenes*. In previous studies it has been found that sea buckthorn, rosehip, black chokeberry, and hawthorn have antimicrobial effects on pathogenic microorganisms (Efenberger-Szmechtyk et al., 2020; Sandulachi, et al., 2020; Shah et al., 2020; Efenberger-Szmechtyk et al., 2021). According to the data obtained (Table 1), we observed that *H. rahmnoides* has the most pronounced effect on *L. monocytogenes*, especially concentrated extract 1 and 2 followed by *R. canina* and grape marc of *V. vinifera*. The diameter of inhibition zone for sea buckthorn has shown 30 mm and 32 mm. *A. melanocarpa* indicate weak activity on *Listeria*. The *C. oxyacantha* does not show any activity on the tested bacteria. *Listeria* is resistant to all types of hawthorn preparations (water-soluble, fat-soluble concentrates and powders).

Above mentioned diffusion tests are widely used to determine the susceptibility of bacteria but have their limitations as result of "susceptible and resistant". This method is considered qualitative method. But a precise assessment is to determine the minimum inhibitory concentration (MIC) of the natural product or antibiotic against the organisms concerned. Dilution methods are used to determine the minimal concentration of antimicrobial that can inhibit or kill the microorganism. This can be achieved by dilution of natural product or antimicrobial agent in either agar or broth media by making serial dilutions (usually in two folds) (Tiwari et al., 2009). MIC was reported as the lowest concentration of the extract causing complete inhibition of the growth of the bacteria. The Figure 2 and 3 below shows the MIC determination.

According to the data presented above (Figure 2, 3), we can see that sea buckthorn has a more pronounced action on *L. monocytogenes*, followed by rosehips. *H. rhamnoides* concentrated extract 1 and aqueous preparations 1 are very active. Values of minimum inhibitory concentration are 2.6 ±1.45 and 5.2 ±1.22 $mg.mL^{-1}$. All *H. rhamnoides* extract had shown marked antibacterial activity against *L. monocytogenes* (Upadhyay et al., 2010). *A. melanocarpa* and *V. vinifera* have a lower effect on *Listeria*. For *A. melanocarpa* MIC is 7.8 ±0.88 $mg.mL^{-1}$ for concentrated extracts. Tescovina has a lower effect activity, the minimum amount of inhibition is 15.6 ±5.22 $mg.mL^{-1}$ for concentrated extracts (undiluted).

Previous research (Ghendov-Moșanu et al., 2018) analysed rosehip and hawthorn powder and their minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and bactericidal effect against *S. aureus* ATCC 25923 (3.91 ±0.15 – MIC, 7.81 ±0.21 – MBC and 41.66 ±1.35 – MIC,

Table 1 Determination of antibacterial activity by well diffusion method

Liquid extracts	***Listeria monocytogenes* Strain EGDe**				
	zone inhibition (mm)				
	Hippophae rhamnoides	***Rosa canina***	***Vitis vinifera***	***Aronia melanocarpa***	***Crataegus oxyacantha***
C1	32	20	20	12	R
C2	30	21.5	20	9.5	R
H1	29	21	–	15.5	R
H2	30	22	–	16	R
P1	27	26	–	–	R
P2	27	25	–	–	R
L1	R	R	–	R	R
L2	R	R	–	R	R
P1'	–	–	–	R	R
P2'	–	–	–	R	R

Notes: C – concentrated extract 1 and 2; H – hydroalcoholic extract – 1 and 2; L – liposolubile extract – 1 and 2; P – powder 1 and 2, 1'and 2'; "–" no samples, R – resistant

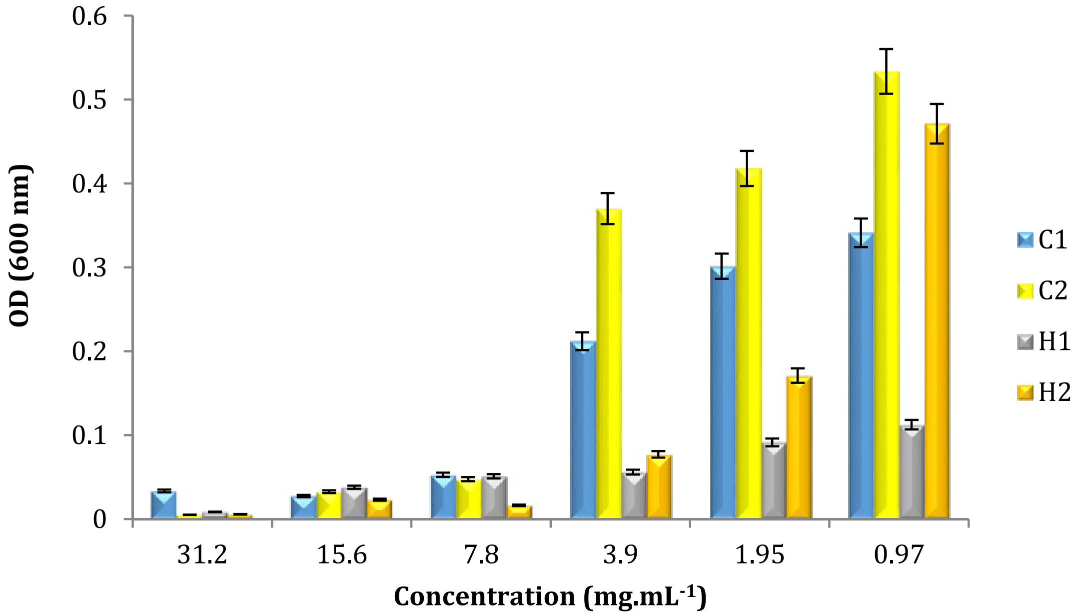

Figure 2 Determination of minimum inhibitory concentration *Aronia melanocarpa* (Michx.) Elliott (mg.mL^{-1})
C – concentrated extract 1 and 2; H – hydroalcoholic extract 1 and 2; 1, 2 – powders 1 and 2

83.33 ±2.47 – MBC, respectively), *E. coli* ATCC 25922 (31.25 ±0.98 –MIC, 62.5 ±1.8 – MBC and 62.5 ±2.2 – MIC, 125 ±5.0 – MBC, respectively) and *K. pneumoniae* ATCC 13883 (62.5 +2.1 – MIC, 125 ±5.0 – MBC for rosehip).

Negi et al. (2005) determined antibacterial activity of sea buckthorn seeds. The MIC values, with respect to MeOH extract for *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Listeria monocytogenes*, *Yersinia enterocolitica*, were found to be 200, 300, 300, 300, and 350 ppm, respectively. These results indicated the possibility of using sea buckthorn seeds for medicinal uses and food preservation.

Kim et al. (2018) showed results with antibacterial activity of various concentration of *Aronia melanocarpa* powder against *Bacillus cereus* ATCC10876,

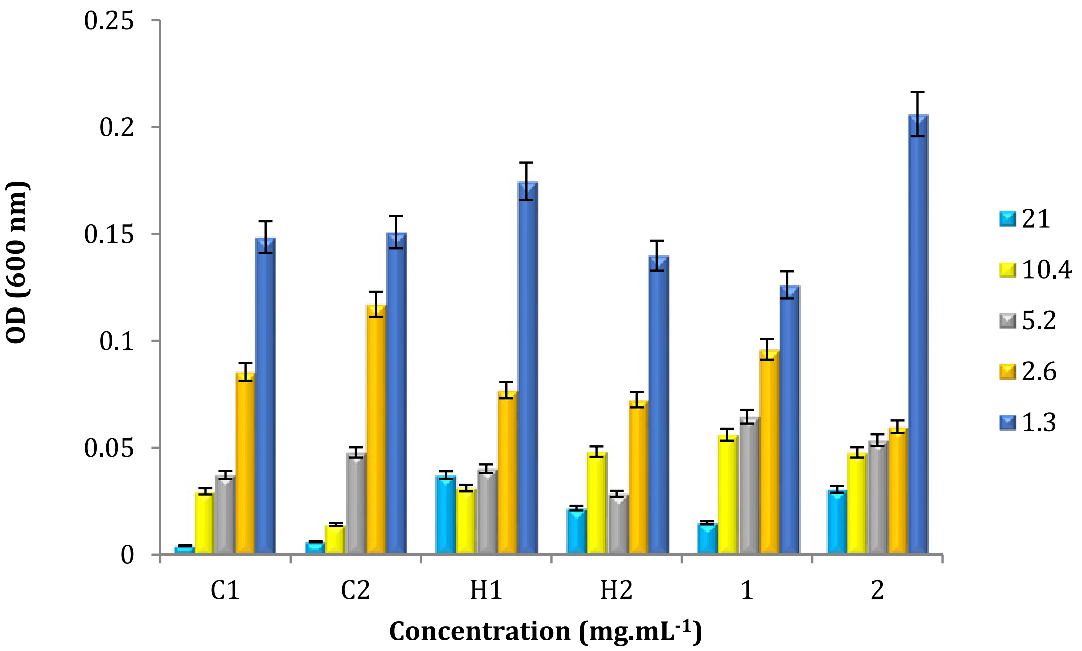

Figure 3 Determination of minimum inhibitory concentration *Hippophae rhamnoides* L. (mg.mL^{-1})
C – concentrated extract 1 and 2; H – hydroalcoholic extract 1 and 2; 1, 2 – powders 1 and 2

Staphylococcus aureus ATCC6538, *Cronobacter sakazakii* KCTC2949, and *Salmonella enteritidis* 110 tested by the spot-on-lawn assay with some modifications (Cadirci and Citak, 2005). The culture broth was diluted using MHB to 0.5 McF and spread onto Mueller-Hinton agar (MHA; Difco) and incubated at 37 °C ±0.5 for one day. This study demonstrated the potential of *A. melanocarpa* to inhibit the growth of *Bacillus cereus* and *S. aureus* as antimicrobial activity, except for *C. sakazakii* and *S. enteritidis*.

In another work, researchers analysed the rose fruits (*Rosa rugosa*) aqueous extract and determined the highest inhibitory activity against 5 strains of Gram-positive bacteria (*B. cereus* ATCC 11778, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *L. innocua* SGGW) and 5 strains of Gram-negative bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATCC13883, *P. mirabilis* ATCC 35659, *P. aeruginosa* ATCC 27853 *S. enteritidis* ATCC 13076) bacterial strains tested (Cendrowski et al., 2020). The reduction in the number of bacterial cells in matrices imitating protein food depended on the concentration of the extract used. The obtained test results confirm the possibility of using rose extracts to extend the microbiological stability of food.

Radulescu et al. (2020) found favourable antagonistic activities against the tested common bacteria strains which were exhibited by the hydroalcoholic extracts from the grapes (seeds) of the organic varieties, respectively the skin of the tested conventional varieties.

Conclusions

Due to the current fast pace of life, and the profit-oriented industries which minimize production and distribution costs by using preservatives, additives, antibiotics, hormones, and people eat a lot of fast food and quick meals, their health is often severely affected, and the general population immunity is much more weakened. The trend of organic food has been growing more and more over the past decades. In this context, coming up with reliable natural alternatives to the synthetic compounds is imperative. With a future hope to decrease the use of synthetic additives and antibiotics as preservatives. In conclusion, the antibacterial activity, give a scientific support to the modern studies which reported the positive influence of berries extracts. Our experiments determined *in vitro* antimicrobial activity of extracts and powders of berries against the tested *L. monocytogenes* food pathogen. The well diffusion method determined that the most pronounced effect on *L. monocytogenes* has *Hippophae rhamnoides*, with a diameter of 32 mm inhibition zone, for alcoholic *H. rhamnoides* extracts (C1, C2), followed by *Rosa canina*. *L. monocytogenes* is resistant to *Crataegus oxyacantha* action. MIC (minimum inhibitory concentration) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested. The smallest concentration of *H. rhamnoides* that can inhibit the growth and multiplication of tested *Listeria* is 2.6 $mg.mL^{-1}$. From the results we had concluded that the most active substances we can found in *H. rhamnoides* species.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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