ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS FROM SERBIA

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Abstract: The aim of this work was to evaluate antimicrobial properties of ethanolic extracts of plants (*Mentha x piperita* L., *Carum carvi* L., *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill, *Betula pendula* Roth. and *Rhamnus frangula* L.) originated from Serbia. The antimicrobial activity was tested by paper disc diffusion method and by microdilution technique against six pathogenic bacteria (*Bacillus Cereus* ATCC 10876, *Enterococcus Faecalis* ATCC 14506, *Salmonella Choleraesuis* ATCC 10708, *Staphylococcus Aureus* ATCC 11632, *Proteus Mirabilis* ATCC 12453 and *Escherichia Coli* ATCC 10536).

B. cereus was the most susceptible to the extracts of *M. piperita*, *R. frangula* and *B. pendula* among tested microorganisms. Ethanolic extracts of *Betula pendula* Roth., *Mentha x piperita* L. and *Rhamnus frangula* L. have been shown to possess the strongest antimicrobial activity against *Bacillus Cereus*, where the minimum inhibitory concentrations were 10 $mg \cdot ml^{-1}$ (*Betula pendula* Roth.) and 50 $mg \cdot ml^{-1}$ (*Mentha x piperita* L. and *Rhamnus frangula* L.)

The highest antibacterial potential was exibited by ethanolic extract of *Mentha x piperita* L., followed by *Rhamnus frangula* L. and *Betula pendula* Roth. Contrary to this, the extracts of *Carum carvi* L. and *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill did not show significant antimicrobial effects towards investigated bacteria.

Key words: Antimicrobial activity, plant extract, *Rhamnus frangula* L., *Carum carvi* L., *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill, *Mentha x piperita* L., *Betula pendula* Roth.

INTRODUCTION

The application and research for drugs and food and feed supplements obtained from plants have increased in recent years (Frankič, 2009). Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Frankič, 2009). Being a rich source of secondary biomolecules which exhibit significant pharmacological effects, spices and herbs appeal to many consumers who question the safety of synthetic food additives (Craig, 1999).

Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and the favorable effect on the animal intestinal system (Al-Kassien, 2009). Spices and herbs can have a great influence on the function and reactivity of the immune system of the farm animals (Craig, 1999). The growth promoting active feed suplements improve stability of feed and auspicious impact the digestive micropopulation mostly through inhibition of pathogenic microorganisms growth. In consideration of promoted health status of intestinal tract, farm animals are less exposed to the toxins

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produced by different microorganisms (Frankič et al., 2009). Windisch et al. (2008) reported that spices and herbs have beneficially effect the stress resistance of the animals and amplify the absorption of essential nutrient. There is also, one other important advantage of using plant extract of herbs and spices or their essential oils instead synthetic drugs in feed: synthetic drugs residues in animal meat and eggs can cause health problems in people who consume them, esspecialy due to increasing resistance of pathogens present in the human body as a result of prolonged use of synthetic drugs (Barbour et al., 2010).

The objective of this work was to evaluate and compare the antibacterial effects of ethanolic extracts of *Mentha x piperita* L., *Carum carvi* L., *Petroselinum crispum* (Mill.) A.W. Nym. ex Hill, *Betula pendula* Roth. and *Rhamnus frangula* L. against six pathogenic bacteria.

MATERIAL AND METHODS

Test bacteria

Antibacterial investigations were carried out against *E. Coli* ATCC 10536, *S. Choleraesuis* ATCC 10708, *Staph. Aureus* ATCC 11632, *P. Mirabilis* ATCC 12453, *E. Faecalis* ATCC 14506 and *B. Cereus* ATCC 10876.

Plant samples

Herbal drugs used in this study (Table 1) are the products of the Institute for Research of Medicinal Plants "Dr Josif Pančić" from Belgrade. Herbal drugs were in the form of powder with granulation of up to 3 mm.

Table 1.
Plants used in experiments.

Botanical name	Sample	Drug
Mentha x piperita L.	Mint leaves	*Menthae piperitae folium*
Carum carvi L.	Caraway fruits	*Carvi fructus*
Petroselinum crispum (Mill.) A.W. Nym. ex Hill,	Parsley fruits	*Petroselini fructus*
Rhamnus frangula L.	Buckthorn bark	*Frangulae cortex*
Betula pendula	Birch leaves	*Betula pendula folium*

Preparation of plant extracts

Crude plant extracts were obtained by maceration with ethanol/water mixture (80:20, v/v), with the ratio of raw materials to ethanol solution of 1:10, for 24 h at room temperature and subsequently extracted in a ultrasonic bath at room temperature for 10 min. After filtration through a filter paper (Whatman, Grade 4 Chr, UK) and vacuum-evaporation of the solvent at 40 °C, yield of the extract was measured The combined extracts were stored at -4 °C until further use.

Testing of antibacterial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA) obtained by Torlak, Serbia. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Test Bacteria were grown in Mueller Hinton broth (Torlak, Serbia). Bacterial suspension was diluted with sterile physiological solution to 10^8 cfu· ml^{-1} (turbidity = McFarland standard 0.5). One hundred microlitres of bacterial suspension were swabbed uniformly on surface of MHA and the inoculums was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts (500 $mg \cdot ml^{-1}$, 250 $mg \cdot ml^{-1}$, 100 $mg \cdot ml^{-1}$ and 50 $mg \cdot ml^{-1}$) in 80% ethanol (Merck-Darmstadt, Germany). 80% ethanol was used as a negative control. The inoculated plates were stored at 4 °C for 2 h and then incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured (diameter of paper disc, 6 mm is included). Studies were performed in triplicate and the results are expressed as means along with the standard deviaton (SD) of three parallel measurements.

Determination of minimum inhibitory concentration (MIC)

A broth microdilution susceptibility assay was performed using National Committee for Clinical Laboratory Standards Guidelines methods for the determination of the MIC (NCCLS, 2000). Stock solution of the dry plant extract was prepared in 80% ethanol and then serial dilutions were made with

sterile physiological solution in a concentration range from 5.0 to 50.0 mg·ml^{-1}. The 96-well plates were prepared by dispensing into each well 160 µl of Mueller Hinton broth (MHB), 20 µl of the plant extract and 20 µl of the inoculum. The inoculum of microorganisms was prepared using 24 h cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The final volume in each well was 200 µl. A positive control (containing 20 µl inoculum and 180 µl MHB) and negative control (containing 20 µl of plant extract and 180 µl MHB without inoculum) were included on each microplate. The contents of the wells were mixed and the microplates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. The experiment was carried out in triplicate.

Minimum bactericidal concentration (MBC) of plant extracts

MBC was determined by subculturing the 5 µl of test dilution from each well on to a nutrient agar (Torlak, Belgrade, Serbia) plates and incubating further at 37 °C for 24 h. The complete absence of growth at applied concentration was considered as the minimum bactericidal concentration.

RESULTS AND DISCUSSION

Paper disc difusion method

The effects of three different concentrations of five plant extracts on the growth of test microorganisms by the paper disc diffusion method are presented in Tables 2 – 7.

Table 2.
Antibacterial properties of plant extracts against *B. Cereus* ATCC 10876 at three dilutions (inhibition zone diameter in mm; diameter of paper disc, 6 mm, included).

Plant extracts	Dilution of plant extracts (mg.ml^{-1})				
	500	250	100	50	Control (80% ethanol)
M. piperita	20.3 ± 0.6	20.0 ± 0.0	18.7 ± 1.15	18.7 ± 1.15	n.d.
R. frangula	20.3 ± 0.6	19.0 ± 1.0	18.3 ± 0.6	15.3 ± 0.6	n.d.
B. pendula	23.5 ± 0.7	17.3 ± 2.1	17.0 ± 2.0	14.5 ± 0.7	n.d.
P. crispum	n.d.	n.d.	n.d.	n.d.	n.d.
C. carvi	n.d.	n.d.	n.d.	n.d.	n.d.

Note: n.d. – not detected

Table 3.
Antibacterial properties of plant extracts against *E. Faecalis* ATCC 14506 at three dilutions (inhibition zone diameter in mm; diameter of paper disc, 6 mm, included).

Plant extracts	Dilution of plant extracts (mg.ml^{-1})				
	500	250	100	50	Control (80% ethanol)
M. piperita	18.3 ± 0.6	18.3 ± 0.6	17.3 ± 1.52	17.0 ± 2.0	n.d.
R. frangula	18.3 ± 2.1	11.0 ± 1.0	10.3 ± 0.6	10.3 ± 0.6	n.d.
B. pendula	15.0 ± 0.0	13.7 ± 1.5	12.3 ± 2.5	12.3 ± 1.5	n.d.
P. crispum	n.d.	n.d.	n.d.	n.d.	n.d.
C. carvi	n.d.	n.d.	n.d.	n.d.	n.d.

Note: n.d. – not detected

Table 4.
Antibacterial properties of plant extracts against *S. Aureus* ATCC 11632 at three dilutions (inhibition zone diameter in mm; diameter of paper disc, 6 mm, included).

Plant extracts	Dilution of plant extracts (mg.ml^{-1})				
	500	250	100	50	Control (80% ethanol)
R. frangula	18.3 ± 2.1	15.7 ± 0.6	12.7 ± 0.6	12.3 ± 0.6	n.d.
B. pendula	11.3 ± 1.2	10.7 ± 1.2	10.3 ± 1.2	9.3 ± 0.6	n.d.
P. crispum	10.7 ± 0.6	9.7 ± 0.6	10.0 ± 1.0	n.d.	n.d.
M. piperita	n.d.	n.d.	n.d.	n.d.	n.d.
C. carvi	n.d.	n.d.	n.d.	n.d.	n.d.

Note: n.d. – not detected

Table 5.
Antibacterial properties of plant extracts against *P. Mirabilis* ATCC 12453 at three dilutions (inhibition zone diameter in mm; diameter of paper disc, 6 mm, included)

Plant extracts	Dilution of plant extracts (mg.ml^{-1})				
	500	250	100	50	Control (80% ethanol)
R. frangula	15.3 ± 0.6	14.7 ± 0.6	14.0 ± 0.0	12.3 ± 0.6	n.d.
M. piperita	14.0 ± 0.0	13.7 ± 0.6	11.3 ± 1.15	n.d.	n.d.
B. pendula	12.3 ± 2.5	12.7 ± 1.2	10.3 ± 0.6	10.3 ± 0.6	n.d.
C. carvi	15.0 ± 0.0	n.d.	n.d.	n.d.	n.d.
P. crispum	10.7 ± 1.6	n.d.	n.d.	n.d.	n.d.

Note: n.d. – not detected

Table 6.
Antibacterial properties of plant extracts against *S. Choleraesuis* ATCC 10708 at three dilutions (inhibition zone diameter in mm; diameter of paper disc, 6 mm, included)

Plant extracts	Dilution of plant extracts (mg.ml^{-1})				
	500	250	100	50	Control (80% ethanol)
B. pendula	14.7 ± 0.6	12.7 ± 1.2	12.0 ± 2.0	10.7 ± 0.6	n.d.
M. piperita	15.7 ± 1.15	16.3 ± 1.15	16.0 ± 1.0	13.3 ± 1.5	n.d.
R. frangula	15.3 ± 0.6	13.0 ± 1.0	11.7 ± 0.6	11.0 ± 1.0	n.d.
C. carvi	n.d.	n.d.	n.d.	n.d.	n.d.
P. crispum	n.d.	n.d.	n.d.	n.d.	n.d.

Note: n.d. – not detected

Table 7.
Antibacterial properties of plant extracts against *E. Coli* ATCC 10536 at three dilutions (inhibition zone diameter in mm; diameter of paper disc, 6 mm, included)

Plant extracts	Dilution of plant extracts (mg.ml^{-1})				
	500	250	100	50	Control (80% ethanol)
M. piperita	14.7 ± 0.6	14.0 ± 1.7	13.3 ± 1.5	13.3 ± 0.6	n.d.
R. frangula	14.0 ± 1.7	12.0 ± 1.0	11.3 ± 0.6	10.7 ± 0.6	n.d.
B. pendula	11.7 ± 1.2	11.3 ± 1.5	11.3 ± 1.15	9.7 ± 0.6	n.d.
C. carvi	n.d.	n.d.	n.d.	n.d.	n.d.
P. crispum	n.d.	n.d.	n.d.	n.d.	n.d.

Note: n.d. – not detected

According to the results of antimicrobial screening given in Tables 2-7, the strongest antimicrobial activities against tested microorganisms were obtained for extracts of *M. piperita* and *R. frangula*. The ethanolic extract of *B. pendula* has been shown to possess the strongest antimicrobial effect against *B. Cereus*. This plant extract exhibited moderate antimicrobial activity towards the others investigated bacteria, except in the case of *E. Faecalis* and *S. Choleraesuis*, where at the higest working concentration (500 mg·ml^{-1}) it has been shown to possess the intermediate effect. The extracts of the *C. carvi* and *P. crispum* at the concentration of 500 mg·ml^{-1} exhibited intermediate and moderate antibacterial effects, respectively. *C. carvi* and *P. crispum* did not show antibacterial activity towards tested bacteria at lower concentrations (250 mg·ml^{-1}, 100 mg·ml^{-1} and 50 mg·ml^{-1}). *B. cereus* was the most susceptible to the extracts of *M. piperita*, *R. frangula* and *B. pendula* among tested microorganisms.

MIC of the extract of *B. pendula* varied from 10 mg·ml^{-1} (*B. cereus*) to 50 mg·ml^{-1} (other tested bacteria), while the MBC was from 25 mg·ml^{-1} (*B. cereus*) to 50 mg·ml^{-1} (other tested bacteria). MIC of the extracts of *M. piperita* and *R. frangula* were 50 mg.ml^{-1} (*B. cereus*) and 50 mg·ml^{-1} (other tested bacteria). MBC of the extracts of *M. piperita* and *R. frangula* were 50 mg·ml^{-1} for other investigated strains. Obtained results of the investigations of the antimicrobial activity of mint EOs are in accordance with literature data (Deans & Baratta, 1998, Bupesh et al., 2007, Sharafi et al., 2010). This results point out that EOs of mint from Serbia could be useful

in controling the development of tested bacteria (*B. Cereus*, *E. Faecalis*) in different food and feed. The biological activity of *B. pendula* against *Bac. Coagulans* reported by Lindberg et al., 2004. Izhaki (2002) noticed the antibacterial effect of *R. frangula*. According to Ulate-Rodriquez, 1997, ethanolic extracts of dried parsley can decrease the number of viable micropopulations of *E. Coli*, but in this investigation ethanolic extract of parsley did not showed antibacterial activity. There is no literature data of the application of *B. pendula* and *R. frangula* in food and feed.

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