Antimicrobial activity of Origanum heracleoticum L. essential oil from Serbia

Ivana Čabarkapa¹*, Marija Škrinjar², Ivan Milošanović¹, Dragana Plavšić¹, Dragan Palić¹, Bojana Kokic¹, Ivana Arsić³

*Corresponding author
1. University of Novi Sad Institute of Food Technology, Bulevar cara Lazara 1, Novi Sad, 21000, Serbia
2. University of Novi Sad Faculty of Technology, Bulevar cara Lazara 1, Novi Sad, 21000, Serbia
3. Institute for Medicinal Plant Research “Dr. Josif Pančić”, Tadeuša Košćuška 1, Belgrade, 11000, Serbia

KEYWORDS: Antimicrobial activity; essential oil Origanum heracleoticum L.; Origanum L.

ABSTRACT: The modern trends in nutrition suggest the limitation of synthetic food additives or the substitution with natural ones. Aromatic herbs are probably the most important source of natural antimicrobial agents. The aim of this study was to investigate antibacterial effects of various concentrations of Origanum heracleoticum essential oil on the food-borne bacteria. The antimicrobial activity of Origanum heracleoticum essential oil was evaluated using laboratory control strains Escherichia coli ATCC /10536/, Salmonella choleraesuis ATCC /11070/, Salmonella enteritidis ATCC/13076/, Proteus mirabilis ATCC /12453/, Pseudomonas aeruginosa ATCC/10145/, Staphylococcus aureus ATCC /11632/, Bacillus cereus ATCC/10876/ and Enterococcus faecalis ATCC /14560/, obtained from the American Type Culture Collection. The antibacterial activity was determined using disk diffusion method and more precise broth microdilution method. Using the broth microdilution method essential oil of Origanum heracleoticum L. showed antimicrobial activity against all tested strains of microorganisms with exception of the test strain of Pseudomonas aeruginosa. The tested oil had antibacterial effect on gram-positive bacteria in the range of MIC/MBC=0.2-0.39/0.78 μl/ml. The essential oil was active in the range from MIC/MBC=0.39 to 50/0.78 to 50 μl/ml against the tested gram-negative bacteria. P. aeruginosa ATCC/10145/ showed the lowest sensitivity of MIC/MBC=50/50 μl/ml.

INTRODUCTION

The modern trends in nutrition suggest the limitation of synthetic food additives or the substitution with natural ones. Aromatic herbs are probably the most important source of natural antimicrobial agents.

Among the aromatic plant species from family Lamiaceae (Labiatae), genus Origanum occupies a special position. In Europe and, in general, all over the world, the most commonly found oregano species belong to the botanical genus Origanum.

Within this genus, Ietswaart (1) recognised three groups, 10 sections, 38 species, 6 subspecies and 17 hybrids based on morphological criteria (2).

Origanum heracleoticum L. (Origanum vulgare L. ssp. hirtum) is widely distributed in the Mediterranean basin and is used as a spicy herb under the name “Greek oregano”. It is generally accepted that Greek oregano is of the highest quality (3).

Oregano is of great economic importance but this is not only related to its use as a spice.

Chemical analysis of the oregano essential oil (EO) revealed the presence of several ingredients, most of which have important antioxidant, antibacterial and antifungal properties (4, 2, 5, 6). The major antibacterial components of these oils are carvacrol and its isomer thymol (7, 8).

Both are approved food flavourings in the United States and Europe (9, 10) and have potential as antibacterial additives in food and feed (7, 11).

A number of food additives and food preservatives containing essential oils or carvacrol are already commercially available (12). p - Cymene is also a constituent of oregano but is less effective against food related pathogens (8, 13) and is thought to be a precursor to carvacrol and thymol in the plant (14).

The precise targets of the antibacterial action of EOs and their components have not yet been fully established. Changes in the fatty acid composition of bacterial cell membranes (an increase in unsaturated fatty acids) have been observed when cells are exposed to sub-lethal concentrations of EO components (15). Carvacrol and thymol damage the outer membrane of gram-negative bacteria and increase the general permeability of the cytoplasmic membrane leading to leakage of ATP (16, 17). Carvacrol possesses ATPase inhibiting activity (16, 17), in any case it appears to dissipate the proton motive force (14, 16). p-cymene has been shown to have lipolytic properties (13). The aim of this study was to investigate antibacterial effects of various concentrations of Origanum heracleoticum L. essential oil on the food-borne bacteria.

MATERIALS AND METHODS

Plant material

Aerial parts of Origanum heracleoticum L. were collected during blooming stage (August 2009) from the locality Kamendol near Smederevo, Serbia. The plant material was dried under laboratory conditions (20-25°C). Institute of Medicinal Plant Research Dr. Josif Pančić identified the plants and voucher specimens were stored in the herbarium of the Institute of Medicinal Plant Research Dr. Josif Pančić.

Isolation of the essential oil

The essential oil was isolated from dried plant material by hydro-distillation according to the standard procedure reported in the Sixth European Pharmacopeia (18). Distillation was performed using Clevenger type apparatus, for 2.5 hours. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4°C.
The MBC is defined as the lowest concentration of the essential oil at which ≥ 99.9 percent of the inoculated microorganisms were killed. According to MBC definition, the presence of ≤2 cfu per inspected plate was tolerated.

RESULTS AND DISCUSSION

It is well known that both environmental and genetic factors are effective in observed variations among Origanum heracleoticum L. accessions with high accuracy (3). Because of this, yield and chemical composition of essential oil can vary among the populations of the same species from different localities. From the collected plant material of Origanum heracleoticum L. total of 2.05 percent (v/w) of essential oil has been isolated by the process of hydro-distillation. In our previous investigation (4), twenty six components (92.86 percent) were identified as constituents of this essential oil by Gas Chromatography-Mass Spectroscopy (GC-MSD) analysis. The major components were carvacrol (69.00 percent), p-cymene (10.50 percent), thymol (7.94 percent) and γ-terpinene (2.86 percent). Except β-caryophyllene (1.53 percent) and β-bisabolene (1.01 percent) the amount of all remaining oil components was less than 1 percent. Aromatic alcohol carvacrol was also dominant compound in Origanum heracleoticum oil analyzed by other authors (6, 20, 21). The predominant group of compounds in the oil were monoterpenes (95.77 percent), with significantly more oxidized compounds (79.21 percent) than hydrocarbons (16.56 percent). Sesquiterpenes were present at a low percentage in the oil (3.48 percent).

Numerous studies have demonstrated that the essential oils of Origanum species are among the most potent essential oils with regard to antimicrobial properties (2, 5, 6). This was confirmed in the present study.

Figure 1. Antimicrobial activity of various concentrations of Origanum heracleoticum L. essential oil against gram-positive bacteria obtained by disk diffusion method.

Figure 2. Antimicrobial activity of various concentrations of Origanum heracleoticum L. essential oil against gram-negative bacteria obtained by disk diffusion method.
According to the results of preliminary testing, disc diffusion method indicated generally strong antimicrobial activity of the oil against all tested strains of bacteria with the exception of the test strain of *Pseudomonas aeruginosa*. Using disk diffusion method according to the standard conditions (composition and thickness of the substrate, inoculum size, pH of the substrate, incubation time, etc.) the diameter of inhibition zone is proportional to the logarithm of the concentration of the substance studied. The results obtained with all tested bacteria show that the inhibition zone diameter was proportional to the logarithm of the concentration of tested oil at a concentration of 500 μl/ml to 31.2 μl/ml, while it was not proportional at lower concentrations (Figure 1 and 2). The disk diffusion method applied can be used only for preliminary screening of antimicrobial substances, since easily volatile components of essential oils evaporate over a period of incubation together with the solvent, while poorly dissolved components do not pass through the medium. Because of that, during further investigation, we used more precise, broth micro-well dilution method. Using the broth microdilution method, the essential oil of *Origanum heracleoticum* L. showed antimicrobial activity against all tested strains of microorganisms. The tested oil had antibacterial effect on gram-positive bacteria in the range of MIC/MBC=0.2-0.39/0.78 μl/ml (Figure 3). The oil exhibited the highest activity against *S. aureus* ATCC /11632/ [MIC/MBC=0.2/0.78 μl/ml]. The obtained value for the MIC of the tested essential oil with 69 percent carvacrol against *S. aureus* is in agreement with previous studies in which the MIC of carvacrol against *S. aureus* ranged from 0.2 to 0.9 μl/ ml (22, 23).

The minimal inhibitory concentration of tested oil against *B. cereus* ATCC/10876/ was 0.3 μl/ml and the MBC was 0.78 μl/ ml. The obtained value for the MIC of the tested essential oil with 69 percent carvacrol against *B. cereus* is in agreement with previous studies in which the MIC of carvacrol against *B. cereus* ranged from 0.2 to 0.9 μl/ ml (26, 27). *E. faecalis* ATCC/14506/ showed the lowest sensitivity of the tested gram-positive bacteria to tested oil at MIC/ MBC=0.78/0.78 μl/ml. The oil showed bacteriostatic and bactericidal effects against *E. faecalis* at the same concentration of MIC / MBC = 0.78 / 0.78 μl / ml.

The essential oil was active against tested gram-negative bacteria in the range from MIC/MBC=0.39 to 50/0.78 to 50 μl/ ml (Figure 4). The oil showed the highest activity against strains of *E. coli* ATCC/10536/ and *P. mirabilis* ATCC/12453/ MIC/MBC=0.39/0.78 μl/ml. The obtained value for the MIC of the tested essential oil with 69 percent carvacrol against *E. coli* is in agreement with the results of Oussalah et al. (28) who showed the essential oil of *Origanum heracleoticum* L. with 54 percent carvacrol exhibited MIC against *E. coli* at a concentration of 0.25 μl/ml.
In previously published studies of carvacrol against E. coli, MIC ranged from 0.2 to 5 μl/ml (22, 27). The effect of the tested oil was uniform, against S. enteritidis ATCC/13076/ and S. choleraesuis ATCC/10708/ at MIC/MBC = 0.78/1.56 μl/ml. The obtained value of MIC of the tested essential oil of Origanum heracleoticum L. is in agreement with previously published results of antimicrobial activity of essential oil of oregano against S. typhimurium in which the essential oil of oregano exhibited MIC concentrations ranging from 0.5 to 1.2 μl/ml (29, 28). In the research of Cosentino et al. (27) MIC value of carvacrol against S. typhimurium was 0.2 μl/ml. P. aeruginosa ATCC/10145/ showed the lowest sensitivity at MIC/MBC=50/50 μl/ml. Very low sensitivity of P. aeruginosa to the tested essential oil is a result of its outer hydrophobic membrane, which prevents the passage and effects of hydrophobic oils (30).

The surface of its cells forms selectively permeable membrane, through which small hydrophylic molecules can pass, while hydrophobic macromolecules (such as those included in the composition of essential oil) remain on the outer side of the membrane. The present results demonstrate high activity of essential oil of Origanum heracleoticum L. against tested bacterial strains with exception of the test strain of Pseudomonas aeruginosa.

High antimicrobial activity is explained firstly by the fact that the aromatic alcohol carvacrol is the main constituent of the oil, present in very high percentage (20, 23).

CONCLUSION

Origanum heracleoticum L. collected in Serbia is found to be (2.05 percent) of essential oil. The oil exhibited very high antibacterial activity, owing to high content of monoterpenic carvacrol, which is well known antimicrobial compound. The tested oil had antibacterial effect on gram-positive bacteria in the range of MIC/MBC=0.2-0.39/0.78 μl/ml. The essential oil was active in the range from MIC/MBC=0.39 to 50/0.78 to 50 μl/ml against the tested gram-negative bacteria. P. aeruginosa ATCC/10145/ showed the lowest sensitivity with MIC/MBC=50/50 μl/ml. These values, together with high yield and lack of toxicity economically justify the use of essential oil derived from Origanum heracleoticum L for many purposes such as food preservation, active and intelligent packaging systems and also for the treatment of different human diseases.

Future research will be focused on application of the essential oil in food systems. The most interesting area of application for EOs is the inhibition of growth and reduction in numbers of the more serious food borne pathogens.

Extension of shelf-life and improvement of organoleptic qualities of meat and meat products may also be interesting from a commercial point of view. In view of their organoleptic properties, EOs could most readily be incorporated in the manufactured foods that are traditionally associated with herbs or with spices.

ACKNOWLEDGMENTS

These results are part of a research on the Project III046009, financed by the Ministry of Education and Science, Republic of Serbia.

REFERENCES AND NOTES

2. L. de Martino, V. de Feo et al., Molecules, 14, pp. 2735-2746 (2009).
The leading ingredients exhibition for nutrition + wellness

NuW: the 360° perspective

Health ingredients & Natural ingredients Europe will join forces with NuW in Frankfurt, Germany to create a powerhouse that will provide a complete 360 perspective of the Nutritional + Wellness Solutions industry. Using our database of over 150,000 contacts, NuW will attract a targeted audience of senior managers with defined objectives and the power to purchase.

Sell, see, network, inspire. Contact us today to discuss how you can be a part of the leading event for health ingredients, dietary supplements and nutraceuticals in Europe while showcasing alongside the largest and most innovative companies in the health ingredients, natural ingredients and nutritional sectors. As we are booking stands in combination with Hi/NI 2012, we strongly advise you to contact us early to secure a prime location, only 15% of the floor space is left!

13th to 15th November
Messe Frankfurt, Germany
hieurope.ingredientsnetwork.com