Antimicrobial activity of Origanum heracleoticum L. essential oil from Serbia

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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 /10708/, Salmonella enteritidis ATCC/13076/, Proteus mirabilis ATCC /1243/ Salmononas aeruginosa ATCC/10451/, Staphylococcus aureus ATCC /11632/, Bacillus cereus ATCC/10876/ and Enterococcus faecalis ATCC /14506/, obtained from the American Type Culture Collection. The antimicrobial 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/10451/ 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 (Labiaceae), 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 feed 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).

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.
Antimicrobial activity assessment

The antimicrobial activity of essential oil was evaluated using laboratory control strains, Escherichia coli ATCC /10536/, Salmonella choleraesuis ATCC /10708/, Salmonella enteritidis ATCC/13076/, Proteus mirabilis ATCC /12453/, Pseudomonas aeruginosa ATCC/10145/, Staphylococcus aureus ATCC /11632/, Bacillus cereus ATCC/10876/ and Enterococcus faecalis ATCC /14506/, obtained from the American Type Culture Collection.

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 [6] twenty six components (92.86 percent) were identified as constituents of this essential oil byGas 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 L. 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 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 (22, 23).

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 obtained value for the MIC of the tested essential oil with 69 percent carvacrol against E. faecalis at the same concentration of MIC / MBC = 0.78 / 0.78 μl / ml.

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 (22, 23).

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 0.78 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.
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.

REFERENCES AND Notes

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