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Antimicrobial activity of Thyme (*Tymus vulgaris*) and Oregano (*Origanum vulgare*) essential oils against some food-borne microorganisms

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Abstract

The aim of this study was to investigate antibacterial effects of oregano and thyme essential oils (EOs) on some food-borne bacteria. GC-MS analysis of EOs was performed in order to determine their composition and phenols were predominant constituents. The investigation of the antibacterial effects of EOs was performed on *Salmonella* Enteritidis, *Salmonella* Thyphimurium, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus*, and MICs were determined by broth microdilution method. EOs exhibited antibacterial activity against all tested microorganisms.

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Keywords: EO composition; carvacrol; thymol; pathogens; antibacterial properties

1. Introduction

Meat consumption is important for human growth, development and maintenance of health, which is why safety of meat and meat products is of growing concern in modern society[1]. A major issue related to meat consumption is

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the presence of pathogens, among them the causative agents of food borne diseases[2], for which raw meat provides an ideal growth medium. *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli, Staphylococcus aureus* are most common pathogens which cause a million episodes of illness every year on global level[2,3]. The overuse of antibiotics in order to reduce these pathogens has led to the phenomenon of multi-drug resistant bacteria[3]. Moreover, meat industry is challenged by the new trend of producing all-natural food, where is no place for artificial preservatives which may have some carcinogenic and toxic properties or may cause food allergies or sensitivities[4]. Essential oils, as plant extracts that exhibit antimicrobial activity, and also possess antioxidative properties, can be considered as healthy ingredients for meat products. If used in meat products, these aromatic oily liquids obtained from a variety of plant materials can reduce the incidence of food-borne diseases and retard lipid oxidation as well[3,4,5]. A number of studies have been performed in order to determine the antimicrobial activity of many essential oils, and oregano and thyme essential oils are those which exhibit the great effects.

2. Materials and methods

2.1. Essential oils

Oregano and thyme essential oils were purchased from the manufacturer Herba doo (Belgrade, Serbia).

2.2. GC-MS analysis of essential oils

EOs were analysed by gas chromatography with electron-ionization mass-selective detector (Agilent Technologies 6890N + 5975B). EO (1 µl) was injected into a split/splitless inlet at 250°C, with a split ratio 1:10. Helium (purity 99.999%) was used as a carrier, with a constant flow of 1 mL/min. The separation was achieved on a 30 m×0.25 mm×0.25 m semipolar HP-5ms capillary column (Agilent Technologies) made of polydimethylsiloxane with 5% of phenyl groups, using the following temperature program: start at 50°C, 8°C/min to 120°C, 15°C/min to 230°C, 20°C/min to 270°C and hold for 16.9 min. Elute was delivered to the mass spectrometer (Agilent Technologies series 5975) via a transfer line held at 280°C. Ion source temperature was 230°C, electron energy 70 eV, and quadrupole temperature 150°C. Data were acquired in Scan mode. Data were analyzed by Agilent MSD ChemStation software and AMDIS (Automated Mass Spectral Deconvolution and Identification System) in conjunction with NIST MS Search software. The compounds were identified by mass spectra comparison with libraries (Wiley Registry of Mass Spectral Data 7th ed., and NIST/EPA/NIH Mass Spectral Library 05 (NIST/EPA/NIH, 2005) and confirmed by comparison of Kovats retention indices (KI) with literature data. Diesel oil was used as a standard for determination of retention indices. Relative amounts of components, expressed in percentages, were calculated by normalization measurement according to peak area in total ion chromatogram.

2.3. Antibacterial assay

The investigation of the antibacterial effects of oregano and thyme essential oils was performed on *Salmonella* Enteritidis ATCC 13076; *Salmonella* Thyphimurium ATCC 14028; *Staphylococcus aureus* ATCC 25923; methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Escherichia coli* ATCC 25922 and *Bacillus cereus* ATCC 11778. Susceptibility of bacterial strains to essential oils was investigated by broth microdilution method[6,7.] Broth microdilution method was performed in sterile U-bottom microtiter plates (Spektar, Serbia). The inoculum density was set to 0.5 McFarland, diluted 10 times in sterile saline and 5 µl of this suspension was inoculated in 0.1 ml of CAMHB-Cation Adjusted Mueller-Hinton Broth (Becton, Dickinson and Company, Sparks, USA) to reach final inoculum of 5×10^4 cfu/well. Active substance was diluted in DMSO (Serva, Heidelberg, Germany) and added to CAMHB from 2560 µg/ml to 1.25µg/ml by two-fold dilution in 96-well microtiter plates. After inoculation, plates were incubated at 37°C for 24 hours. Minimal inhibitory concentration (MIC) was determined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in broth dilution susceptibility test[6]. From wells without visible growth 10 µl were subcultivated to on CAMHA and incubated at 37°C for 24 h. Less than 5 colonies were taken as minimal bactericidal concentration as it represents kill ratio of over 99.9%[7]. For control amikacin (Sigma-Aldrich, USA) was used in range of 64 – 0.03 µg/ml.

3. Results and discussion

Sixteen compounds which together accounted for 100% were identified, in both oregano and thyme EO. Major components of oregano EO were carvacrol (77.6%) followed by *p*-cymene (5.14%), *trans-β*-caryophyllene (2.45%), linalool (2.44%), γ-terpinene (2.35%) and thymol (2.11%), while other compounds were present under 2%. In the study conducted by Govaris et al.[8] predominant components in oregano EO were carvacrol (80.15%), p-cymene (5.18%), thymol (4.82%) and γ-terpinene (0.77%), so the composition of EO used in that study was similar to that used in the current study. Composition of EOs depends on number of factors including harvesting seasons and geographical sources. These can explain differences between results obtained from different studies, in which variation in carvacrol amount is significant, as this chemical compound can be found in traces or make up to 80% of the EO composition[5,8]. Results of study conducted by De Falco et al. [9] on three biotypes of *O. vulgare* showed variations of carvacrol amount not only between biotypes, but between the same biotype during different stages of plant growth. In their study the highest content of carvacrol were obtained from oregano during full bloom, when carvacrol content was 70.1% and total phenol content peaked at 76.1%. According to specifications from the manufacturer, EOs used in present study were obtained from plants collected during flowering, which can explain the high amount of phenol compounds. Also it has been demonstrated that EOs produced from herbs harvested during or immediately after flowering possess the strongest antimicrobial activity[5]. The most dominant of all identified compounds of thyme EO were thymol (50.48%), followed by p-cymene (24.79%), linalool (4.69%), γ-terpinene (4.14%) and 1,8-cineole (4.35%). Similar results were obtained by Nikolić et al. [10]. In their study EO extracted from *T. vulgaris*, thymol also was the major constituent (49.10%), along with p-cymene (20.01%). In addition, the chemical profile of thyme EO sample in the present study is also in agreement with report of Ghasemi[11]. The results from the antimicrobial activity tested by microdilution method are presented in Table 1.

Table 1. Minimal inhibitory (MIC) and minimal bactericidal concentration (MBC) of essential oils (μg/ml).

	MIC			MBC		
	A	Oregano	Thyme	A	Oregano	Thyme
Salmonella Enteritidis ATCC 13076	0.5	320	320	0.5	320	320
Salmonella Thyphimurium ATCC 14028	0.5	160	320	4	320	640
Escherichia coli ATCC 25922	0.5	320	320	0.5	320	320
Staphylococcus aureus ATCC 25923	0.25	640	640	0.5	≥2560	1280
Methicillin resistant *Staphylococcus aureus* ATCC43300	1	320	320	4	1280	640
Bacillus cereus ATCC 11778	0.5	160	320	≥4	1280	≥2560

Legend: A-amikacin

EOs possess the strongest antibacterial properties when contain a high percentage of phenolic compounds[5]. EOs used in this study contained a high concentration of phenols, especially oregano EO, which exhibited a greater antimicrobial effect compared to thyme EO. Antimicrobial mechanism of carvacrol and thymol, which are the two major constituents of the EOs used, is based on their ability to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP[12]. Among Gram-negative bacteria which continue to present major human public health and economic problems are *Salmonella* spp[13]. *S.* Enteritidis and *S.* Typhimurium are the most frequently reported serotypes in the incidence of human salmonellosis in both EU and United States[14]. Both EOs exhibited excellent antibacterial activity against *Escherichia coli* and *Salmonella* spp used in this test, although *S.* Thyphimurium showed to be more sensitive than *S.* Enteritidis when oregano EO was used. According to literature data, the MIC of *T. vulgaris* and *O. vulgare* EOs for *S.* Typhimurium ranges from 0.45–720 µl/ml and 0.12-3.12 µl/ml, while for *S.* Enteritidis it is between 66.7–320 µg/ml and 66.7–160 µg/ml respectively, indicating that *S.* Enteritidis is more sensitive to EOs which is in agreement with results obtained in this study[15]. Although generally EOs are more affective against Gram-positive bacteria[16], in this study both EOs showed moderately strong activity against *S. aureus* with an obtained MIC value of 640 µg/mL. Oregano EO cause phosphate ion leakage in *S. aureus*, and thus exhibit antibacterial activity[12]. MRSA is the cause of severe infections mainly in hospital settings, but this bacterium is also found in raw pork, poultry and beef meat[17]. An interesting result obtained in this study is that EOs showed stronger antibacterial activity against MRSA than against *S. aureus*, even though amikacin exhibited four times weaker inhibitory activity and eight times weaker bactericidal effect on MRSA than on *S. aureus*. It has long been thought that the diarrheal syndrome caused by *B. cereus* was a classical intoxication but recent data show that disease is rather caused by ingested *B. cereus* cells that grow and produce enterotoxin within the intestinal tract[18]. In *B. cereus*, carvacrol depletes the intracellular ATP pool, changes the membrane potential, and increases the permeability of the cytoplasmic membrane to potassium ions[19]. EOs showed great inhibitory activity against this microorganism, but higher concentration of these were needed to achieve bactericidal effects. Although oregano and thyme EOs exhibited strong antibacterial activity, further researches are needed in order to determinate their antibacterial effects on pathogens in meat model media.

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