



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading

Comparative evaluation in the efficacy of peppermint (*Mentha piperita*) oil with standards antibiotics against selected bacterial pathogens

Ebenezer Jeyakumar*, Rubina Lawrence, Tripti Pal

Department of Microbiology & Fermentation Technology, Sam Higginbottom Institute of Agriculture Technology & Sciences, Allahabad–211007, Uttar Pradesh, India

ARTICLE INFO

Article history:

Received 10 August 2011

Received in revised form 7 September 2011

Accepted 28 September 2011

Available online 15 October 2011

Keywords:

Peppermint oil

Antibacterial activity

Minimum inhibitory concentration

Minimum bactericidal concentration

Multi–drug resistance

Mentha piperita

Bacterial pathogen

Susceptibility

Organism

ABSTRACT

Objective: To find the efficacy of peppermint oil against selected bacterial pathogens and compare with their susceptibility towards antibiotics. **Methods:** Peppermint oil was evaluated for activity against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The antibacterial assay was evaluated using agar well diffusion method and the viability of the organisms (MIC and MBC) was determined at different concentrations using broth dilution method. **Results:** Peppermint oil was found to be effective against all the gram positive and gram negative organisms tested. A progressive effect of antibacterial activity with increase in concentration of oil was observed. The test organisms were found to be inhibited by peppermint oil at lower concentration in broth dilution method as compared with agar diffusion method. When comparing the assessment of the inhibitory effect of peppermint oil, broth dilution was found to be more effective as compared with agar diffusion method. Except *S. aureus*, the remaining organisms tested in the present study were found to possess multiple drug resistance. However, peppermint oil was found to be effective against these bacterial strains studied. **Conclusions:** Hence, with such broad spectrum activity of peppermint oil, it can be further recommended in the treatment of the infections caused by these multi–drug resistant bacteria.

1. Introduction

Mentha piperita (*M. piperita*) is a medicinally important plant that grows throughout North America, Asia, and Europe. It is primarily cultivated for its oil which is extracted from the leaves of the flowering plant[1]. Peppermint oil is used for flavoring pharmaceuticals and oral preparations, such as toothpastes, dental creams, and mouth washes. Higher and aromatic plants have traditionally been used in folk medicine as well as to extend the shelf life of food, showing inhibition against bacteria, fungi and yeast. Most of their properties are due to essential oils produced by their secondary metabolites[2–4].

Peppermint oil is chemo–preventive and anti–mutagenic. It is helpful in symptomatic relief of the common cold. It is also used topically as an analgesic and to treat headache. It is also used to treat many ailments of the skin,

circulatory system, respiratory system, digestive system, immune system and nervous system. The principal active constituents of peppermint are the essential oils, which comprise about 1% of the herb. Essential oils are dominated by monoterpenes, mainly menthol, menthone and their derivatives (*e.g.* isomenthone, neomenthone, acetylmenthol, pulegone, menthofuran). These essential oil dilates blood vessels and inhibits bacteria, especially menthol has a broad spectrum antibacterial activity[5]. Peppermint oil is found to be strongly effective against *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Enterococcus faecium* (*E. faecium*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Escherichia coli* (*E. coli*)[6]. Peppermint is also found to have antiviral and fungicidal activity. It is virucidal against influenza, herpes and other viruses[7]. Antifungal activity of the essential oil of *M. piperita* was also reported[8].

Medicinal plant oil is a natural alternative to synthetic drugs particularly against microbial agents. In recent years, multiple drug resistance developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious disease. In addition to this, antibiotics are associated with adverse effects. Therefore, there is a need to develop alternative antimicrobial medicines for the

*Corresponding author: Dr. Ebenezer Jeyakumar, Assistant Professor, Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad–211007, India.

Tel: +91–532–3297818

Fax: +91–532–2684394

E–mail: ebenezerjeyakumar@rediffmail.com, ebenezer_74@yahoo.co.uk

treatment of the infectious diseases from other sources such as plant. Essential oil derived from plant is safe and dependable, compared with costly synthetic drugs that have adverse effect. Hence, peppermint oil was evaluated in the present study against some multi-drug resistant bacteria.

2. Materials and methods

2.1. Procurement of oil

The oil of peppermint (*M. piperita*) was obtained from Central Institute of Medicinal and Aromatic Plant (CIMAP), Lucknow, India.

2.2. Test organisms

The gram positive and gram negative bacteria viz., *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae* and *Pseudomonas aeruginosa* (*P. aeruginosa*) obtained from the Microbial Culture Collection Bank, Department of Microbiology and Fermentation Technology, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India were used in the present study. The test organisms were periodically streak inoculated on Muller–Hinton agar slants and incubated overnight at $(37\pm 1)^\circ\text{C}$ and maintained under refrigerated conditions.

2.3. Preparation of concentration of peppermint oil

The different concentrations (v/v) of peppermint oil viz., 5% (T1), 10% (T2), 15% (T3), 20% (T4), 25% (T5), 30% (T6) were prepared aseptically in sterilized Tween 80.

2.4. Antibacterial activity assay

The testing of the bacterial cultures for the inhibitory effect of essential oil of peppermint for different concentrations (5%, 10%, 15%, 20%, 25%, and 30%) was performed by using agar well diffusion method^[9]. The Muller–Hinton agar media containing 0.5% Tween 80 was melted and 20 mL of media was poured on Petri plate separately and allowed to solidify. The active cell suspension (1 mL) was spread with the help of sterile swab on the agar surface uniformly. Three wells of 5 mm diameter each was made in agar Petri plate of the solidified agar medium using sterilized cork borer. Measured quantity (0.03 μL) of each concentration was pipette out with sterilized pipette and filled in the wells aseptically. In the control plate, the well was added only with Tween 80. The oil was allowed to diffusing in the well for a period of 1 h and the plates were incubated at $(37\pm 1)^\circ\text{C}$ for 24 h. The zone of inhibition was measured with graduated scale after the period of incubation.

2.5. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC is defined as the lowest concentration of the test compound to inhibit the growth of microorganisms while MBC is defined as the lowest concentration of the test compound to kill the test microorganism. The determination of MICs of the different concentration of the essential oil of

peppermint on the test bacterial strain was done by using broth dilution method^[10] with different concentrations of oil. The test bacterial culture was inoculated onto tryptic soy broth and incubated overnight at $(37\pm 1)^\circ\text{C}$ for 24 h and the inoculum concentration of Mcfarland standard (0.5) was used for the assay. The culture tube containing 10 mL of sterilized tryptic soy broth with 0.5% (v/v) Tween–80 was inoculated with different concentrations of peppermint oil ranging from (5–0.15) $\mu\text{L}/\text{mL}$. Tryptic soy broth with 0%–5% (v/v) Tween–80 without oil was used as positive growth control. An aliquot of bacterial suspension (25 μL) to each test tube was added uniformly and incubated at $(37\pm 1)^\circ\text{C}$ for 24–48 h and observed. The lowest concentration at which no visible growth occurs in either culture tubes was taken as initial MIC. After 48 h of incubation, the lowest concentration at which no visible growth occurs was taken as final MIC. Then the tubes showing no increase in turbidity at each time interval 24–48 h were streaked onto Muller–Hinton agar plates to check the growth. Each trial was repeated thrice and compared with the control tubes.

2.6. Antibiotic susceptibility test

Antibiotic susceptibility of the test organisms were performed using agar disc diffusion method^[11]. For this, 1 mL of overnight broth culture of the test organism was swabbed on the solidified Muller–Hinton agar plates and the antibiotic discs (azithromycin 15 μg ; ceftriaxone 30 μg ; chloramphenicol 30 μg ; ciprofloxacin 5 μg ; clindamycin 2 μg ; co-trimoxazole 25 μg ; erythromycin 15 μg ; gentamycin 10 μg ; ofloxacin 5 μg ; penicillin–G 10 units) of Hi–media Laboratories, Mumbai, India were impregnated onto the surface plates. The plates were then incubated at $(37\pm 1)^\circ\text{C}$ for 24 h to 48 h. The zone of inhibition was recorded in mm diameter and the data were interpreted using CLSI standards.

2.7. Statistical analysis

The data on the zone of inhibition of peppermint oil obtained against different bacterial pathogens were statistically analyzed by three way classification and tested with F–test and significance was evaluated at 5% level. The correlation coefficient followed by T–test was applied to analyze the significance between the values of MIC and MBC and the conclusion was drawn^[12].

3. Results

3.1. Antibacterial activity of peppermint oil against selected pathogenic bacteria

Peppermint oil was found to effectively inhibit the organisms tested. Gram positive organisms (*S. aureus*, *B. subtilis* and *B. cereus*) were found more susceptible than gram negative organisms (*E. coli*, *K. pneumoniae*, *P. aeruginosa*). Among gram positive organisms, *S. aureus* and *B. subtilis* were found more susceptible to peppermint oil as compared with *B. cereus*. Among gram negative organisms, *E. coli* was more sensitive as compared with *K. pneumoniae* and *P. aeruginosa*. The antibacterial activity was found progressively increasing with the increase in concentration

of oil. The maximum effect was found at 30% concentration and minimum effect was observed at 5% concentration of oil (Table 1). Among the gram positive bacteria, peppermint oil showed maximum activity against *S. aureus*, producing the maximum zone of inhibition (24.33 mm), followed by *B. subtilis* (21.66 mm) and *B. cereus* (20.66 mm). Among the gram negative bacteria tested, *E. coli* (16 mm) was found to be the most susceptible to peppermint oil as compared with *K. Pneumoniae* (14.66 mm) and *P. aeruginosa* (10.33 mm) ($P<0.05$).

3.2. MIC and MBC of peppermint oil

The bacterial pathogens were found to be inhibited by peppermint oil in broth dilution method at very low concentration as compared with the higher concentration required in agar well diffusion method. The diffusion of oil was found to be less as compared with broth dilution method, this is due to the inefficient penetration of oil in agar medium and hence a higher concentration of oil was required to inhibit the growth of pathogenic bacteria in agar well diffusion method.

Gram positive organisms were more susceptible against peppermint oil as compared with gram negative organisms. *S. aureus* and *B. subtilis* were found to be most sensitive among test organisms and were inhibited by peppermint oil at a concentration of 0.03 μ L/mL (initial MIC) then at 0.06 μ L/mL (final MIC) with a similar final MIC and MBC. *B. cereus* was found inhibited by peppermint oil at 0.06 μ L/mL concentration initial and final MIC. MBC was found at 0.012 μ L/mL. *E. coli* was found inhibited at 0.12 μ L/mL (initial MIC) and 0.25 μ L/mL (final MIC) and MBC was recorded at 0.50 μ L/mL. *K. pneumoniae* was found inhibited at 0.25 μ L/mL (initial MIC). Final MIC and MBC were recorded at the concentration of 0.50 μ L/mL. *P. aeruginosa* was found

inhibited at a higher concentration of 50 μ L/mL initial. However, the final MIC and MBC were recorded at 100 μ L/mL. A positive significant correlation of the initial MIC and MBC was observed ($P<0.05$) i.e., as the initial MIC increased there was simultaneous increase in MBC. However, between the final MIC and MBC no correlation in the data was observed.

3.3. Antibiotic susceptibility pattern against test organisms

Varying degree of antibiotic susceptibility was recorded against various antibiotics tested in the present study. Gram positive organisms were found to be more susceptible as compared with gram negative organisms. *S. aureus* was found to be sensitive to all the antibiotics except penicillin and with chloramphenicol intermediate resistance was recorded. *B. subtilis* was also found sensitive to all the antibiotics except azithromycin and erythromycin and with penicillin it was found to show intermediate resistance. *B. cereus* was found resistant against azithromycin, chloramphenicol, gentamycin and intermediate against penicillin was recorded (Table 2). *S. aureus* exhibited minimum antibiotic resistance (10%) while *B. subtilis* and *B. cereus* exhibited antibiotics resistance of 20% and 30%, respectively. And *E. coli*, *K. pneumoniae* and *P. aeruginosa* showed antibiotics resistance of 50%, 60% and 60%, respectively.

Gram negative organisms showed maximum resistance to antibiotics with *E. coli* being resistant to azithromycin, clindamycin, co-trimoxazole, erythromycin and penicillin. *K. pneumoniae* was found to be resistant to all antibiotics except ciprofloxacin, chloramphenicol with ofloxacin and co-trimoxazole an intermediate resistance was observed. *P. aeruginosa* exhibited maximum antibiotics resistant except being sensitive to ciprofloxacin, and ofloxacin. Similarly with chloramphenicol and gentamycin an intermediate

Table 1
Inhibitory activity of peppermint oil against selected pathogenic bacteria (Mean \pm SD) (mm*).

Test organisms	Zone of inhibition					
	5%	10%	15%	20%	25%	30%
Gram positive <i>S. aureus</i>	12.00 \pm 0.81	12.66 \pm 0.46	15.33 \pm 0.46	17.66 \pm 0.46	19.00 \pm 0.81	24.33 \pm 1.70
<i>B. subtilis</i>	11.00 \pm 0.81	12.33 \pm 0.46	14.33 \pm 1.24	16.66 \pm 0.47	18.66 \pm 0.46	21.66 \pm 0.46
<i>B. cereus</i>	9.66 \pm 0.46	11.33 \pm 0.46	13.66 \pm 0.94	16.33 \pm 2.05	18.33 \pm 1.24	20.66 \pm 0.46
Gram negative <i>E. coli</i>	9.33 \pm 0.46	11.00 \pm 0.81	13.00 \pm 0.00	13.66 \pm 0.47	14.33 \pm 0.46	16.00 \pm 0.81
<i>K. pneumoniae</i>	7.33 \pm 0.47	9.00 \pm 0.81	10.00 \pm 0.81	11.00 \pm 0.81	12.00 \pm 0.81	14.66 \pm 0.46
<i>P. aeruginosa</i>	6.00 \pm 0.00	6.33 \pm 0.46	8.00 \pm 0.00	8.66 \pm 0.46	9.66 \pm 0.46	10.33 \pm 0.47
Results	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

*: Includes well size of 5 mm; $P<0.05$.

Table 2
Effect of peppermint oil in comparison with antibiotics against selected pathogenic bacteria.

Organisms	Antibiotics										<i>M. piperita</i> oil
	Azithromycin (15 μ g)	Ceftriaxone (30 μ g)	Ciprofloxacin (5 μ g)	Chloramphenicol (30 μ g)	Clindamycin (2 μ g)	Co-trimoxazole (25 μ g)	Erythromycin (15 μ g)	Gentamycin (10 μ g)	Ofloxacin (5 μ g)	Penicillin (100 units)	
<i>S. aureus</i>	++	++	++	+	++	++	++	++	++	-	++
<i>B. subtilis</i>	-	++	++	++	++	++	-	++	++	+	+
<i>B. cereus</i>	-	++	++	++	++	++	++	-	++	+	++
<i>E. coli</i>	-	++	++	++	-	-	-	++	++	-	++
<i>K. pneumoniae</i>	-	-	++	++	-	+	-	-	++	-	++
<i>P. aeruginosa</i>	-	-	++	+	-	-	-	+	++	-	+

++: Sensitive; +: Intermediate; -: Resistant.

resistance was observed. Among gram negative organisms, *P. aeruginosa* (60%) and *K. pneumoniae* (60%) exhibited maximum antibiotic resistant followed by *E. coli* (50%). Except *S. aureus*, the remaining gram positive and gram negative organisms tested in the present study were found to show multiple drug resistance (Table 2).

3.4. Comparative analysis of peppermint oil and antibiotics against the test organisms

A comparative study was done between the antibiotics and *M. piperita* oil and it was observed that *M. piperita* oil showed higher pattern of sensitivity against the test pathogens. Minimum percentage of resistance was found against *S. aureus* followed by *B. subtilis*, *B. cereus* and *E. coli*. *K. pneumoniae* and *P. aeruginosa* exhibited maximum percentage of resistance towards antibiotics. The tested organisms particularly gram negative organisms had shown high resistance towards different antibiotics whereas they were found to be inhibited by peppermint oil even at low concentration in broth dilution method. Peppermint oil was found to be effective against even the multi-drug resistant strains. In broth dilution method, peppermint oil inhibited all the organisms at $<5 \mu\text{L/mL}$ except *P. aeruginosa* which was found to be less sensitive.

4. Discussion

The antibacterial activity of *M. piperita* oil observed in the present study is comparable with the reports of Gupta *et al*[13]. Further, the present study findings with respect to the data on *E. coli*, *K. pneumoniae*, *P. aeruginosa* are highly in accordance with the reports of Saeed and Rusenova *et al*[2,14]. However, reports of Saeed *et al*[15] on the effectiveness of peppermint oil against *P. aeruginosa* (18.11 mm) followed by *K. pneumoniae* (17.00 mm) and *E. coli* (16.38 mm). Slight variations in the effectiveness of peppermint oil against *P. aeruginosa*, *K. pneumoniae* and *E. coli* was reported in the studies of Saeed and Tariq[15].

The antimicrobial activity of peppermint oil is due to the presence of terpenoides menthol, menthone, 1–8–cineole, methyl acetate, menthofuran, isomenthone, limonene, b–pinene, germacerene–d, trans–sabinene hydrate and pulegone[6]. The inhibition zone among the observation of difference workers may differ due to many factors. First, the composition of plant oils is known to vary according to local climate and environmental conditions[16,17]. Furthermost, some oils with the same common name may be derived from different plant species. Secondly, the method used to assess antimicrobial activity, strains and the choice of test organisms, varies between different studies. The usefulness of this method is limited to the generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils prevents the uniform diffusion of these substances through the agar medium[4]. Hence these variations in the activity between different organisms were observed.

The results MIC observed in the present study are in fair correlation with the studies of Fabio *et al*[3] where the MIC values of peppermint oil was reported to range between $1.25 \mu\text{L/mL}$ (*E. coli*) and $3.125 \mu\text{L/mL}$ (*K. pneumoniae*). The present study results are also in accordance with those of Sartoratto *et al*[6] who have also reported that gram positive

organisms were found to be inhibited by peppermint oil at very low concentration. However, a contrast in the MIC values of peppermint oil for *E. coli* was 0.5% and for *S. aureus* 0.4% was reported in the studies of Mohsenzadeh[7]. The MBC of peppermint oil reported for *E. coli* and *S. aureus* is comparable with the findings of Rusenova and Parvanov[14]. Similarly, the MIC values reported for *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* were in highly in agreement with the present study findings.

In the present study, gram positive bacteria are known to be more susceptible to essential oils than gram negative bacteria. The weak antibacterial activity against gram negative bacteria could be ascribed to the presence of an outer membrane which possessed hydrophilic polysaccharide chains as a barrier to hydrophobic essential oil. According to Kim *et al*[18], the mode by which microorganisms are inhibited by essential oils and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents and impairment of bacterial enzymes systems.

The results obtained by each of these methods may differ as many factors vary between assays. These include differences in microbial growth, exposure of microorganisms to plant oil, the solubility of oil or oil components and the use and quantity of an emulsifier[4]. These and other elements may account for the large differences in MIC's obtained by the agar and broth dilution methods in this study.

The antibiotic susceptibility pattern of the test organisms observed in the present study was in agreement with the studies of Brooks *et al*[19] where a difference in the sensitivity pattern of *E. coli*, *P. aeruginosa*, *S. dysenteriae* and *S. typhi* was reported. In a similar study of Ates and Erdogru[20] *B. cereus*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa* were reported to show resistance against cefuroxime, oxacillin, erythromycin, azithromycin and sensitive to ofloxacin, tobramycin and vancomycin, which is comparable with the present study. However, the findings of Fabio *et al*[3] demonstrated that *S. aureus* exhibited resistance to ofloxacin, gentamycin, tobramycin and erythromycin which is in contrast with the present study findings. The results of present study are in fair correlation with the study of Hashemi *et al*[21] where *E. coli*, *S. aureus* and *B. subtilis* were reported to show sensitive against gentamycin, clindamycin, cephalothin and resistance against tetracycline, penicillin and erythromycin.

Gram negative bacteria are more resistant to antibiotics than gram positive bacteria due to permeability barrier provided by the cell accumulation mechanism. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structure and rendering them more permeable[22,23]. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death[24]. The study of Bupesh *et al*[25] showed the antibacterial activity of *M. piperita* along with chloramphenicol against pathogenic bacteria like *B. subtilis*, *P. aeruginosa*. Only synergistic studies are found in this aspect. However, no comparative study with antibiotics was found, hence the data could not

be compared.

In conclusion, the present study confirms the broad-spectrum antibacterial activity of peppermint oil with potent activity against even the multi-drug resistant strains. Considering the attribute and broad spectrum activities, successful development of antimicrobial substance would not only provide a potent tool for the control of various diseases, but also could promise success in multipurpose biorational alternatives to antibiotics for the management of diseases caused by these organisms. Some of these pathogens are also related to food-borne diseases. Hence, a potential application of this oil as food preservative could also be recommended.

However, this study further requires *in vivo* extension to find appropriate doses of essential oils showing both antimicrobial activity and very low detrimental effect on eukaryotic cells. The investigation on the incorporation of peppermint oil into appropriate food formulations and evaluation of flavor, chemical changes and antimicrobial effect in the whole food system for extension of shelf life as well as prevention of food deterioration is also a pre-requisite. Although the present study provides enough evidence of peppermint oil with broad-spectrum activity, there is the need to address the issues of safety and toxicity before it could be recommended for preservation or medicinal purposes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors would like to sincerely thank the support of Sam Higginbottom Institute of Agriculture, Technology and Sciences extended towards this study.

References

- [1] Scavroni J, Boaro CSF, Marques MOM, Ferreira LC. Yield and composition of the essential oil of *Mentha piperita* L. (Lamiaceae) grown with biosolid. *Braz J Plant Physiol* 2005; **17**: 345–352.
- [2] Saeed S, Naim A, Tariq P. *In vitro* antibacterial activity of peppermint. *Pak J Bot* 2006; **38**(3): 869–872.
- [3] Fabio A, Cermelli C, Fabio G, Nicoetti P, Quaglio P. Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infection. *J Phytother Res* 2007; **21**: 374–377.
- [4] Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against pathogenic *Aspergillus fumigatus* and *A. niger*. *J Med Sci* 2008; **3**(2): 81–88.
- [5] Pattnaik S, Subramanyam VR, Kole C. Antibacterial and antifungal activity of ten essential oils *in vitro*. *Microbios* 1997; **86**: 237–246.
- [6] Sartoratto A, Machado MAL, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz J Microbiol* 2004; **35**: 275–280.
- [7] Mohsenzadeh M. Evaluation of antibacterial activity of selected Iranian essential oils against *Staphylococcus aureus* and *Escherichia coli* in nutrient broth medium. *Pak J Biol Sci* 2007; **10**(20): 3693–3697.
- [8] Barrera–Necha LL, Garduno–Pizana C, Garcia–Barrera LJ. *In vitro* antifungal activity of essential oils and their compounds on mycelial growth of *Fusarium oxysporium* f.sp. *gladioli* (Massey) Synder and Hansen. *Plant Pathol J* 2009; **8**: 17–21.
- [9] Vivek S, Nisha S, Vijaylata P, Reyaz MA, Bikram S, Raghbir GC. GC–MS analysis and anti–microbial activity of essential oil of *Mentha piperita* L. from Kullu—a North Indian region of higher altitude Himalayas. *Int J Drug Dev Res* 2010; **2**(4): 40–46.
- [10] Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity of berry and leaf essential oils of *Piper nigrum* L. *Int J Biol Med Res* 2010; **1**(4): 215–218.
- [11] Mayrhofer S, Domig KJ, Mair C, Zitz U, Huys G, Kneifel W. Comparison of broth microdilution, etest, and agar disk diffusion methods for antimicrobial susceptibility testing of *Lactobacillus acidophilus* group members. *Appl Environ Microbiol* 2008; **74**(12): 3745–3748.
- [12] Paulson DS. *Biostatistics and microbiology: a survival manual*. New York: Springer Science–Business Media Publication; 2008, p. 63–81.
- [13] Gupta C, Garg AP, Uniyal RC, Kumari A. Antimicrobial activity of some herbal oils against common food borne pathogens. *J Microbiol Res* 2008; **2**: 258–261.
- [14] Rusenova N, Parvanov P. Antimicrobial activities of twelve essential oils against microorganisms of veterinary importance. *Trak J Sci* 2009; **7**: 37–43.
- [15] Saeed S, Tariq P. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pak J Bot* 2005; **37**(4): 997–1001.
- [16] Janssen AM, Scheffer JJC, Baerheim A, Svendren A. Antimicrobial activity of essential oils. *J Plant Med* 1987; **53**: 395–398.
- [17] Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M. Antibacterial activity of mint essential oils. *J Agric Food Chem* 1995; **43**: 2384–2388.
- [18] Kim J, Wei CI, Marshall MR. Antibacterial activity of some essential oil components against five food-borne pathogens. *J Agric Food Chem* 1995; **48**: 2839–2845.
- [19] Brooks JT, Ochieng JB, Kumar L, Okoth G, Shapiro RL, Wells JG, et al. Surveillance for bacterial diarrhea and antimicrobial resistance in rural Western Kenya. *J Clin Infect Dis* 2006; **43**(4): 393–401.
- [20] Ates DA, Erdogru OT. Antimicrobial activities of various medicinal and commercial plant extracts. *Turk J Biol* 2003; **3**: 157–162.
- [21] Hashemi SR, Zulkifli L, Zunita Z, Somchit MN. The effects of selected sterilization methods on antibacterial activity of aqueous extracts of herbal plants. *J Biol Sci* 2008; **8**(6): 1072–1076.
- [22] Knobloch K, Weigand H, Weis N, Schwarm H–M, Vigneschow H. Action of terpenoids on energy metabolism. In: Brunke E–J. (ed.) *Progress in essential oil research*. Berlin, Germany: Walter de Gruyter and Co.; 1986, p. 429–445.
- [23] Sikkema J, De Bont JAM, Poolman B. Interaction of cyclic hydrocarbons with biological membranes. *J Biol Chem* 1994; **269**: 8022–8028.
- [24] Denyer SP, Hugo WB. Biocide–induced damage to the bacterial cytoplasmic membranes. In: Denyer SP, Hugo WB. (eds.) *Mechanisms of action of chemical biocides: their study and exploitation*. Oxford: Blackwell Scientific Publications; 1991, p. 171–187.
- [25] Bupesh G, Amutha C, Nandagopal S, Ganeshkumar A, Sureshkumar P, Murali SK. Antibacterial activity of *Mentha piperita* L. (peppermint) from leaf extracts—a medicinal plant. *Acta Agric Slov* 2007; **89**: 73–79.