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Chemical composition and antibacterial effects of some essential oils individually and in combination with sodium benzoate against methicillin-resistant *Staphylococcus aureus* and *Yersinia enterocolitica*

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Abstract

Side effects of chemical preservatives and drug resistance have raised interest in use of herbal products. This study aimed to examine the chemical composition and antibacterial effects of *Cuminum cyminum*, *Mentha spicata,* and *Mentha longifolia* essential oils individually and in combination with sodium benzoate against methicillin-resistant *Staphylococcus aureus* and *Yersinia enterocolitica.* The essential oils were analyzed by gas chromatography-mass spectrometry. Disc diffusion and microdilution assays were used for *in vitro* antimicrobial screening. The main components were cumin aldehyde, carvone, and pulegone in *C. cyminum*, *M. spicata,* and *M. longifolia* essential oils, respectively. Antibacterial data analysis showed significant differences between different antibacterial effects of essential oils individually and in combination with sodium benzoate. In terms of individual effects, antibacterial effect of *M. longifolia* and *C. cyminum* essential oils were the highest against methicillin-resistant *S. aureus* and *Y. enterocolitica,* respectively. The antibacterial effects of sodium benzoate combined with essential oils showed significant differences with the individual effect of sodium benzoate in most cases. The results indicated that the combined effect of these essential oils with sodium benzoate could reduce the use of sodium benzoate as an antimicrobial agent, which could decrease its possible side effects. Thus, for more significant effects, these essential oils could be combined with other agents for the preservation of drug and food products.

Introduction

Natural products provide a great opportunity for the development of new drugs, particularly antimicrobials. Essential oils can be a valuable source for discovering their antibacterial attributes against multidrug-resistant pathogens.[1] Essential oils are volatile aromatic components that are obtained from different parts of plants including buds, seeds, roots, leaves, stems, woods, barks, and flowers.[2] Since ancient times, essential oils have been known for their biological effects and have been extensively appraised against different biological purposes and other pharmaceutical applications.[1,3] The mechanism of action of the essential oils appears to be mainly on the cell membrane, disrupting its structure and causing cytoplasmic leakage leading to cell death.[4]

Cuminum cyminum L. is an aromatic herb from the Apiaceae family and is used as flavorants, fragrances, and medical products.[5] It has great antioxidant, antibacterial, antifungal, and analgesic properties.[6,7] *Mentha spicata* (spearmint, Lamiaceae family) grows throughout the world and this herb is commonly employed as a flavoring agent in foods and as an herbal medicine in folk remedies.[8] The wild mint (*Mentha longifolia* L.) is another medicinal herb belonging to the Lamiaceae family which is widely used in the food industry, pharmaceutical, and especially in cosmetology. Various biological activities such as antibacterial, antifungal, and insecticidal properties have been shown for some species of *Mentha*.[9] These medicinal herbs and their essential oils are widely used and safe.[5-9] Sodium benzoate is commonly used as a chemical preservative and antimicrobial agent in liquid

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pharmaceutical preparations and the food industry. Benzoates have been suspected to cause asthma, allergies, and skin rashes.[10] With regards to the harmful effects of artificial preservatives, the usage of natural preservatives for better remedial efficacy, safety, and preservation of substances are recommended. Natural substances such as essential oils are considered an alternative approach.[11]

Methicillin-resistant *Staphylococcus aureus* was initially reported as a nosocomial pathogen in human hospitals and frequently causes abscesses, bloodstream infections, post-surgical infections, and sometimes deaths.[12] *Yersinia enterocolitica* as a worldwide pathogen is an important cause of food and water-borne gastrointestinal infections.[13] Nowadays, essential oils have demonstrated strong synergistic effects when used in combination with artificial and less effective preservatives.[14] Therefore, the objective of this study was to evaluate the effects of the essential oils individually and combined with sodium benzoate against methicillin-resistant *S. aureus* and *Y. enterocolitica.*

Materials and Methods

Essential oils and microbial strains. The essential oils were purchased from Barij Essence Company (Kashan, Iran). Lyophilized bacterial strains of methicillin-resistant *S. aureus* (ATCC: 33591) and *Y. enterocolitica* (ATCC: 23715) were obtained from the Pasture Institute of Iran (Tehran, Iran). The lyophilized culture was grown in a tube containing 10.00 mL of brain and heart infusion broth (Merck, Darmstadt, Germany) twice and incubated at 37.00 ˚C for 24 hr.

Analysis of essential oils. Analysis of the essential oil was performed by gas chromatography-mass spectrometry (GC/MS). The chromatograph (7890S/5975C; Agilent, London, UK) was equipped with a capillary column (HP-5MS; 30.00 m × 0.25 mm ID × 0.25 µm film thickness). The carrier gas was helium, and the split ratio was 1.00 mL min^{-1}. The column temperature was at first 40.00 ˚C, and then gently increased to 300 ˚C at a 5.00 ˚C per min rate and kept for 1 min. The mass spectrometer operated in electronic impact ionization mode and the energy of the electrons was kept at 70.00 eV. Identification of the major constituents of the essential oil was attained based on a comparison between their retention index, computer library information, and standard mass spectral fragmentation pattern (Wiley/NBS). The percentage of each essential oil compositions was calculated from GC peak areas.[15]

Agar disk diffusion assay. The antibacterial activity of essential oils and sodium benzoate was examined by agar disk diffusion assay.[16] Sterile filter paper disks (6.00 mm diameter; Padtan Teb, Tehran, Iran) were impregnated with 30.00 µL of essential oil and sodium benzoate, equivalent to minimum inhibitory concentration (MIC), aseptically placed on the surface of inoculated Mueller-Hinton agar (MHA; Quelab, Montréal, Canada) medium with adjusted suspension (0.5 McFarland standard turbidity) of the bacteria. The dimethyl sulfoxide (DMSO; Samchun Chemical Co. Seoul, South Korea) as the solvent of essential oils only was considered as a negative control. Erythromycin (15.00 µg), streptomycin (10.00 µg), gentamicin (10.00 µg), and ciprofloxacin (5.00µg) per disk were used as a positive control. The inoculated plates were incubated overnight at 37.00 ˚C for 24 hr. Antimicrobial activity was evaluated by measuring the zone of growth inhibition in millimeters. Each experiment was tested in triplicate.

Micro-well dilution assay. For the preparation of bacterial inoculants (5.00 × 10^6 CFU per mL), one isolated colony of each bacteria were inoculated into a tube containing 5.00 mL of Mueller-Hinton broth (MHB; Quelab) medium and incubated at 37.00 ˚C for 20 hr. The second transfer of 0.10 mL of cultures into 5.00 mL of MHB was grown in 37.00 ˚C for 20 hr. After twice incubation, the number of bacterial colonies was enumerated by serial dilution and plating on MHA in triplicate. The broth microdilution method was used to determine the MIC and the minimum bactericidal concentration (MBC) according to the guideline of the Clinical and Laboratory Standards Institute with some modifications.[17,18] First, 10.00% (v/v) DMSO as an emulsifier of the essential oils was added in MHB medium. Different concentrations of the essential oils were set up in MHB medium containing DMSO. The sterile 96-well microplates were prepared and briefly 160 µL of MHB containing DMSO, 20.00 µL of different concentrations of the essential oils (final concentration in each well: 0.01 to 4.40%) or sodium benzoate (the equivalent of 0.01 to 4.40% in each well) and 20.00 µL of inoculum diluted in MHB with 5.00 × 10^6 CFU per mL of the bacteria were inoculated into each well to achieve a concentration of 10^5 CFU per well. The last well as a positive control contained 180 µL of MHB containing DMSO and 20.00 µL of bacterial inoculum without essential oil or sodium benzoate. For negative control, an un-inoculated MHB containing DMSO was designed. The plates were prepared in triplicates. Then, contents of each well were mixed on a plate shaker at 300 rpm for 20 sec and incubated at 37.00 ˚C for 24 hr. The plates were placed in a wet container to ensure that the bacteria did not get dehydrated during incubation. For combination effects of sodium benzoate with essential oils, the procedure presented above was used, however, 140 µL of MHB containing DMSO, 20.00 µL of different concentrations sodium benzoate (final concentration in each well: 0.01 to 4.40%) with 20.00 µL of the equivalent of half MIC of the essential oils in a well for each bacteria and 20.00 µL of the bacterial inoculum were added into each well. The concentration of the first well without turbidity was considered as MIC. The MIC was determined as the lowest concentration of the essential oil or sodium benzoate that inhibited the growth of the bacteria. To detect MBC, the

contents of the non-growth or clear wells were cultured on MHA plates and incubated at 37.00 ˚C for 24 hr. Afterward, the colonies were counted. The MBC was defined as the lowest concentration in which no bacterial growth was detectable on the plates. These procedures were repeated for three times and the average of the three values was calculated to provide the MIC and MBC values.

Statistical analysis. The data were presented as means ± standard deviations (SD) of triplicate measurements. Statistical analysis of data was performed using one-way ANOVA by SPSS (version 16.0; IBM Corp. Armonk, USA), and multiple comparisons of means were done through the Duncan test. Differences were considered significant when $p \leq 0.05$. Also, independent sample *t*-test was used to compare the results of *S. aureus* and *Y. enterocolitica* with each other.

Results

Chemical components of essential oils. The essential oils were liquid at room temperature. Their odors were acceptable. As the results of the GC-MS analysis of the essential oils, their chemical components were different. The components identified in the essential oils are listed in Table 1. The main components were cumin aldehyde (28.24% of the total essential oil), carvone (58.89%), and pulegone (66.95%) in *C. cyminum*, *M. spicata,* and *M. longifolia* essential oils respectively.

Antibacterial effect of essential oils. The results of the antibacterial effect of the essential oils and sodium benzoate on methicillin-resistant *S. aureus* and *Y. enterocolitica* by disk diffusion method are shown based on the diameter of inhibition zone (Table 2). The results of the broth microdilution test as means and SD of MIC and MBC of the essential oils individually and combined with sodium benzoate are expressed in Tables 3 and 4 respectively. Statistical analysis by analysis of variance showed a significant difference between different antibacterial effects of essential oils individually and combined with sodium benzoate. Antibacterial effect of *Mentha longifolia* essential oil against methicillin-resistant *S. aureus* was the highest and then *Cuminum cyminum* and *Mentha spicata* essential oils and sodium benzoate indicated antibacterial activity. These data were completely consistent with the disk diffusion test. Also, the combined effect of sodium benzoate with *M. longifolia* essential oil was the strongest against methicillin-resistant *S. aureus*. The effects of sodium benzoate combined with the essential oils on *S. aureus* showed significant differences with sodium benzoate effect individually ($p < 0.05$). Antibacterial effect of *C. cyminum* essential oil on *Y. enterocolitica* was the highest and then *M. longifolia*, *M. spicata* and sodium benzoate indicated antibacterial activity. Antibacterial effect of *C. cyminum* essential oil was consistent with the disk diffusion test. Data inconsistency about other factors might be due to less precision of the disk diffusion test. On the other hand, sodium benzoate placed on the disk was obtained from MIC dilution, however, the oils were placed directly. The combined effect of sodium benzoate with essential oils on *Y. enterocolitica* showed significant differences than it's an individual effect ($p < 0.05$). The effect of sodium benzoate combined with *M. longifolia* was the highest against *Y. enterocolitica*, indicating that the combined effect might be different from the individual effect of essential oils. Data analysis of two bacteria using independent sample *t*-test showed that *Y. enterocolitica* was more susceptible to *C. cyminum* essential oil, sodium benzoate, and sodium benzoate combined with *M. longifolia* and *M. spicata* rather than *S.* aureus ($p < 0.05$). However, there was no significant difference between the two bacteria in effects of *M. longifolia*, *M. spicata,* and sodium benzoate combined with *C. cyminum* essential oil ($p > 0.05$). The MBC results of sodium benzoate individually and combined with *M. longifolia* did not confirm any bactericidal effect against methicillin-resistant *S. aureus*.

Table 1. Chemical composition of (A) *Cuminum cyminum*, (B) *Mentha longifolia*, and (C) *Mentha spicata* essential oils.

Components	KI	Composition (%)		
		A	B	C
Sabinene	975	-	0.38	0.35
β-Pinene	979	9.14	0.70	0.73
β-Myrcene	991	0.51	0.25	0.47
α-Terpinene	1017	0.17	-	-
o-Cymene	1025	13.78	-	-
Limonene	1029	-	1.07	27.34
β-phellandrene	1030	0.60	-	-
1, 8-Cineole	1031	0.04	4.06	0.13
γ-Terpinene	1060	21.39	0.04	0.04
3, 8-Menthadien	1073	-	0.42	-
Menthone	1163	-	2.71	-
Menthofuran	1164	-	10.89	-
Borneol	1169	-	0.69	-
Cis-isopulegon	1172	-	1.30	-
4-Terpineole	1177	0.10	0.11	0.10
Dihydrocarvone	1193	-	-	1.07
Trans-Pulegone	1215	-	0.19	-
Cic-Pulegone	1229	-	0.20	-
Pulegone	1237	-	66.95	2.81
Cumin aldehyde	1242	28.24	-	-
Carvone	1243	-	0.63	58.89
Cic-Piperitenone oxide	1254	-	0.54	-
Carvacrol	1299	0.17	-	-
Piperitenone oxide	1369	-	1.20	-
Trans-Caryophyllene	1419	0.20	0.84	1.85
α-Humulene	1455	-	-	0.30
Trans-β-Farnesene	1457	0.27	-	-
β-Acoradiene	1471	1.68	-	-
Germacrene-D	1485	-	0.30	0.11
Caryophyllene-oxide	1583	0.12	-	0.15
Bicyclogermacrene	1500	-	0.17	-
Carotol	1595	0.43	-	-

KI: Kovats index on HP-5MS column.

Table 2. Inhibition zone diameter (mm) of essential oils, sodium benzoate (MIC), and standards antibiotic disks by disk diffusion assay.

Bacteria	*Cuminum cyminum*	*Mentha longifolia*	*Mentha spicata*	Sodium benzoate	Erythromycin (15.00 µg)	Streptomycin (10.00 µg)	Ciprofloxacin (5.00 µg)	Gentamicin (10.00 µg)	Dimethyl sulfoxide
S. aureus	16.00	18.00	12.00	13.00	Resistant	Resistant	32.00	17.00	Resistant
Y. enterocolitica	14.00	8.00	10.00	12.00	Resistant	21.00	34.00	25.00	Resistant

MIC: Minimum inhibitory concentration.

Table 3. Means and standard deviation (SD) of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils and sodium benzoate against methicillin-resistant *Staphylococcus aureus* and *Yersinia enterocolitica.* Data are presented as mean ± SD.

Bacteria	*Cuminum cyminum* (%)	*Mentha longifolia* (%)	*Mentha spicata* (%)	Sodium benzoate (%)
S. aureus				
MIC	1.46 ± 0.11	1.20 ± 0.20	1.66 ± 0.11	3.40 ± 0.20
MBC	2.46 ± 0.11	4.00 ± 0.00	2.53 ± 0.11	Not observed
Y. enterocolitica				
MIC	0.46 ± 0.11	1.06 ± 0.11	1.40 ± 0.20	1.53 ± 0.11
MBC	2.40 ± 0.00	3.06 ± 0.11	3.20 ± 0.00	4.06 ± 0.11

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sodium benzoate individually and combined with the essential oils against methicillin-resistant *Staphylococcus.* Data are presented as mean ± SD.

Bacteria	Sodium benzoate (%)	Sodium benzoate (%) + ½ MIC of *Cuminum cyminum*	Sodium benzoate (%) + ½ MIC of *Mentha longifolia*	Sodium benzoate (%) + ½ MIC of *Mentha spicata*
S .aureus				
MIC	3.40 ± 0.20	1.33 ± 0.11	1.13 ± 0.11	1.53 ± 0.11
MBC	Not observed	4.06 ± 0.11	Not observed	4.20 ± 0.00
Y. enterocolitica				
MIC	1.53 ± 0.11	1.13 ± 0.11	0.20 ± 0.00	1.13 ± 0.11
MBC	4.06 ± 0.11	3.20 ± 0.00	2.86 ± 0.11	3.66 ± 0.11

Discussion

Plant essential oils are possible sources of new antimicrobial agents. Different antibacterial properties of essential oils depend on their chemical composition. Variation in the chemical profile of essential oils in different plant species and even within the same species could be different depending on geographic region, harvest time, age of the plant, growing conditions, drying and extraction method.[19] Therefore, despite the various studies on the antimicrobial effect and chemical composition of the essential oils, it was important to determine the chemical composition of the essential oils to correlate with their antimicrobial activities.[20] The chemical profiles of the studied essential oils almost were in agreement with previous researches.[19,21,22] For example, in chemical analysis of the original fresh leaf of *M. longifolia* oil by Asekun *et al*., pulegone (35.00%) was the major compound.[21] Also in research, cumin aldehyde has been reported as the major compound (29.02%) in *C. cyminum* essential oil, and in a study by Snoussi *et al*., the main components of *M. spicata* essential oil were carvone (40.80%) and limonene (20.80%).[19,22] In this study, monoterpenes in *C. cyminum* and oxygenated monoterpenes in *M. spicata* and *M. longifolia* essential oils were the main compounds. The lipophilic character of monoterpenes causes membrane fluidity and permeability, disturbance of membrane-embedded proteins, and alteration of ion transport processes. The results of previous studies have established that these compounds have a degree of antibacterial activity. Kotan *et al.*, in the evaluation of inhibitory effects of 21 pure oxygenated monoterpenes on the growth of 63 bacterial strains showed that alcohol derivatives of oxygenated monoterpenes were more active than their acetate derivatives.[23] Antibacterial activity could be influenced by terpene, however, other components can contribute to this activity as well.[23,24] It seems pulegone and cumin aldehyde, the main component of the *M. longifolia* and *C. cyminum*, respectively, are predominantly responsible for most of their antibacterial activity and pharmacological effects.[19,21] The antimicrobial activity of the *M. spicata* essential oil could be associated with the presence of carvone and limonene. Although other compounds in essential oils that are not abundant can contribute to this activity. The mechanism of carvone is related to the destabilization of the phospholipid bilayer structure, interaction with enzymes and proteins of the membrane, and its act as a proton exchanger reducing the pH among the membrane.[25] Antibacterial activities of some medicinal plants such as *Althaea officinalis*, *M. longifolia* and *C. cyminum* extracts against methicillin-resistant *S. aureus* have been reported.[26,27] Furthermore, Bokaeian *et al*. have reported that *C. cyminum* essential oil has a strong antimicrobial effect against multidrug-resistant *Escherichia coli* strains.[28] Due to undesirable flavor changes of essential oils which these preservatives cause in food and oral drugs, their application is currently limited. Food and drug preservation by multiple

preservatives in small amounts are better than preservation by a large amount of a single preservative for secure microbial stability, safety, and maintaining of the sensory properties.[29] Nowadays, extensive efforts have been performed to use less concentrations of essential oils with other antimicrobial agents to receive stronger synergistic effects. The synergistic effect between essential oils and other antimicrobial contents has been conclusively demonstrated.[14,18] For example in a study, the synergistic effect of *Zataria multiflora* essential oil with nisin on *S. aureus* and *S. typhimurium* was observed.[30] In this research, we found the significant inhibitory effect of the combined use of the essential oils with sodium benzoate on the studied bacteria. There is evidence that gram-positive bacteria more than gram-negative bacteria are vulnerable to plant oils and extracts.[31] Based on our results, *Y. enterocolitica* was more sensitive in the presence of studied essential oils individually and combined with sodium benzoate, therefore, it could be concluded that the essential oils did not have selective antimicrobial activity based on the cell-wall differences of the bacteria as reported previously. Previous studies have also shown that these essential oils were active against different gram-negative bacteria.[32-34] Therefore, the sensitivity of a microorganism to essential oils not only depends on the properties of essential oil but also on the nature of the microorganism (morphology, structure, metabolic type, nutritional, pathogenicity).[35] The results of this study demonstrated that the antimicrobial effect of sodium benzoate was higher when used in combination with the essential oils. A high concentration of sodium benzoate leads to the accumulation of toxic products and side effects.[10,36,37] Thus, for a significant effect on these bacteria, a combination of sodium benzoate with other antimicrobial agents such as essential oils is recommended to reduce sodium benzoate concentration.

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Conflicts of interest

The authors declare no conflict of interest.

References

1. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils—present status and future perspectives. Medicines (Basel). 2017;4(3):58, doi: 10.3390/medicines4030058.
2. Burt S. Essential oils: their antibacterial properties and and potential applications in foods - a review. Int J Food Microbiol 2004;94(3):223-253.
3. Mimica-Dukic N, Popovic M, Jakovljevic V, et al. Pharmacological studies of *Mentha longifolia* phenolic extracts. II. Hepatoprotective activity. Pharm Biol 1999;37(3):221-224.
4. Gill AO, Holley RA. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. Int J Food Microbiol 2006; 108(1):1-9. doi: 10.1016/j.ijfoodmicro. 2005.10.009.
5. Iacobellis NS, Lo Cantore P, Capasso F, et al. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J Agric Food Chem 2005;53(1):57-61.
6. Škrovánková S, Mišurcová L, Machů L. Antioxidant activity and protecting health effects of common medicinal plants. Adv Food Nutr Res 2012;67:75-139.
7. Wanner J, Bail S, Jirovetz L, et al. Chemical composition and antimicrobial activity of cumin oil (*Cuminum cyminum*, Apiaceae). Nat Prod Commun 2010; 5(9):1355-1358.
8. Hussain AI, Anwar F, Shahid M, et al. Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (*Mentha spicata* L.) from Pakistan. J Essent Oil Res 2010;22(1):78-84.
9. Mikaili P, Mojaverrostami S, Moloudizargari M, et al. Pharmacological and therapeutic effects of *Mentha Longifolia* L. and its main constituent, menthol. Anc Sci Life 2013;33(2):131-138.
10. Boukarim C, Jaoude SA, Bahnam R, et al. Preservatives in liquid pharmaceutical preparations. Drug Test Anal 2009;1(3):146-148.
11. Anand SP, Sati N. Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. Int J Pharm Sci Res 2013;4(7):2496-2501.
12. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 2007;298(15):1763-1771.
13. Falcão JP, Brocchi M, Proença-Módena JL, et al. Virulence characteristics and epidemiology of *Yersinia enterocolitica* and *Yersiniae* other than *Y. pseudotuberculosis* and *Y. pestis* isolated from water and sewage. J Appl Microbiol 2004; 96(6):1230-1236.
14. Tavakoli HR, Mashak Z, Moradi B, et al. Antimicrobial activities of the combined use of *Cuminum cyminum* L. essential oil, nisin and storage temperature against *Salmonella typhimurium* and *Staphylococcus aureus in vitro*. Jundishapur J Microbiol 2015;8(4): e24838. doi: 10.5812/jjm.8(4)2015.24838.
15. Jalali-Heravi M, Zekavat B, Sereshti H. Use of gas chromatography-mass spectrometry combined with resolution methods to characterize the essential oil components of Iranian cumin and caraway. J Chromatogr A 2007;1143(1-2):215-226.

16. CLSI. Performance standards for antimicrobial susceptibility testing. 19th ed. Pennsylvania, USA: Clinical and Laboratory Standards Institute 2009; 29.
17. CLSI. Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. 8th ed. Pennsylvania, USA: Clinical and Laboratory Standards Institute 2009.17-18.
18. Rohani SMR, Moradi M, Mehdizadeh T, et al. The effect of nisin and garlic (*Allium sativum* L.) essential oil separately and in combination on the growth of *Listeria monocytogenes*. LWT-Food Sci Technol 2011;44(10):2260-2265.
19. Sadeghi E, Akhodzadeh-Basti A, Noori N, et al. Effect of *Cuminum cyminum* L. essential oil and *Lactobacillus acidophilus* (a probiotic) on *Staphylococcus aureus* during the manufacture, ripening and storage of white brined cheese. J Food Process Preserv 2013;37(5): 449-455.
20. Lin KH, Yeh SY, Lin MY, et al. Major chemotypes and antioxidative activity of the leaf essential oils of *Cinnamomum osmophloeum* Kaneh. from a clonal orchard. Food Chem 2007;105(1):133-139.
21. Asekun OT, Grierson DS, Afolayan AJ. Effects of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. subsp. *Capensis*. Food Chem 2007;101(3):995-998.
22. Snoussi M, Noumi E, Trabelsi N, et al. *Mentha spicata* essential oil: Chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of *Vibrio* spp. strains. Molecules 2015; 20(8):14402-14424.
23. Kotan R, Kordali S, Cakir A. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. Z Naturforsch C J Biosci 2007;62(7-8):507-513.
24. Zengin H, Baysal AH. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules 2014;19(11):17773-17798.
25. Kumar P, Mishra S, Malik A, et al. Insecticidal properties of *Mentha* species: A review. Ind Crops Prod 2011;34(1):802-817.
26. Mandal S, DebMandal M, Saha K, et al. *In vitro* antibacterial activity of three Indian spices against methicillin-resistant *Staphylococcus aureus*. Oman Med J 2011;26(5):319-323.
27. Abu-Shanab B, Adwan G, Jarrar N, et al. Antibacterial activity of four plant extracts used in Palestine in folkloric medicine against methicillin-resistant *Staphylococcus aureus.* Turk J Biol 2006;30(4):195-198.
28. Bokaeian M, Shiri Y, Bazi S, et al. Antibacterial activities of *Cuminum cyminum* Linn. essential oil against multi-drug resistant *Escherichia coli*. Int J Infect 2014;1(1). e18739, doi: 10.17795/iji-18739.
29. Leistner L, Gorris LGM. Food preservation by hurdle technology. Trends in Food Sci Technol 1995;6:41-46.
30. Moosavy MH, Akhondzaseh Basti A, Misaghi A, et al. Effect of *Zataria multiflora* Boiss. essential oil and nisin on *Salmonella typhimurium* and *Staphylococcus aureus* in a food model system and on the bacterial cell membranes. Food Res Int 2008;41(10): 1050-1057.
31. Mann CM, Cox SD, Markham JL. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). Lett Appl Microbiol 2000;30(4):294-297.
32. Stefanini MB, Figueiredo RO, Ming LC, et al. Antimicrobial activity of the essential oils of some spice herbs. Acta Horticulturae 2003; doi: 10.17660/Acta Hortic.2003.597.30.
33. Shahbazi Y. Chemical composition and *in vitro* antibacterial activity of *Mentha spicata* essential oil against common food-borne pathogenic bacteria. J Pathog 2015;2015. doi: 10.1155/2015/916305.
34. Nikšić H, Kovač-Bešović E, Makarević E, et al. Chemical composition, antimicrobial and antioxidant properties of *Mentha longifolia* (L.) Huds. essential oil. J Health Sci 2012;2(3):192-200.
35. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003;10(10):813-829.
36. Zengin N, Yüzbaşioğlu D, Unal F, et al. The evaluation of the genotoxicity of two food preservatives: Sodium benzoate and potassium benzoate. Food Chem Toxicol 2011;49(4):763-769.
37. Beezhold BL, Johnston CS, Nochta KA. Sodium benzoate-rich beverage consumption is associated with increased reporting of ADHD symptoms in college students: a pilot investigation. J Atten Disord 2014;18(3):236-241.