Screening of Antibacterial Activities of Twenty-One Oxygenated Monoterpenes

Recep Kotan[a,b], Saban Kordali[c], and Ahmet Cakir[d,*]

[a] Ataturk University, Biotechnology Research and Application Center, 25240-Erzurum, Turkey
[b] Ataturk University, Oltu Vocational Training School, Nutrition Technology Programme, 25400-Oltu/Erzurum, Turkey
[c] Ataturk University, Faculty of Agriculture, Department of Plant Protection, 25240-Erzurum, Turkey
[d] Ataturk University, Kazım Karabekir Education Faculty, Department of Chemistry, Campus, 25240-Erzurum, Turkey. Fax: +90 442 236 09 55. E-mail: cakira@atauni.edu.tr

* Author for correspondence and reprint requests

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Plant essential oils are widely used as fragrances and flavours in the cosmetic, perfume, drug and food industries. Oxygenated monoterpenes are widespread components of the essential oils, usually occurring in high amount. In this paper, the antibacterial activities of twenty-one oxygenated monoterpenes (borneol, borneol acetate, camphor, carvone, 1,8-cineole, citronellal, β-citronellol, dihydrocarvone, fenchol, fenchone, geraniol acetate, isomenthol, limonene oxide, linalool, linalool acetate, nerol, nerol acetate, terpinen-4-ol, α-terpineol, menthol and menthone) and penicillin (standard antibiotic) were determined using a disc diffusion method (*in vitro*) against 63 bacterial strains, belonging to 37 different genera and 54 species (plant, food and clinic origins). The results showed that the oxygenated monoterpenes exhibited a variable degree of antibacterial activities. These compounds also inhibited the growth of bacterial strains by producing a weak zone of inhibition from 7 to 11 mm in diameter, depending on the susceptibility of the tested bacteria. Among the tested compounds, nerol, linalool α-terpineol, fenchol and terpinen-4-ol showed antibacterial activity at a broad spectrum. However, their antibacterial activities were lower than those of penicillin. In contrast to these compounds, camphor and 1,8-cineole exhibited no inhibition effects on the growth of all tested bacteria.

Key words: Antibacterial, Essential Oils, Oxygenated Monoterpenes

Introduction

Commercial antibiotics are widely used to control infections and diseases in humans and plants; on the other side they may cause lethal hypersensitivity reactions (Davis, 1994; Service, 1995). It is, therefore, necessary to develop alternative natural and safe methods for controlling infections of humans and plants (Clark, 1996; Yao and Tian, 2005). Thus, there has been a growing interest in research concerning the possibility to use plant extracts, essential oils and/or their components that are relatively less damaging to the environment (Rebenhorst, 1996; Misra and Pavlostathis, 1997; Isman, 2000).

It has been postulated that the use of spices in food processing was adopted due to their taste and preventive effects on the spoilage of food. Plant essential oils are widely used as fragrances and flavours in the cosmetic, soap, perfume, drug and food industries. Plant essential oils contain numerous compounds grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acides and alcohols). Many essential oils isolated from various plant species belonging to different genera contain relatively high amounts of oxygenated monoterpenes. Antibacterial activities of numerous essential oils and their major components have extensively been studied (Kim *et al.*, 1995; Sivropoulou *et al.*, 1995; Lis-Balchin *et al.*, 1998; Hammer and Carson, 1999; Iscan *et al.*, 2002; Oumzil *et al.*, 2002; Gulluce *et al.*, 2003; Friedman *et al.*, 2004; Kim and Shin, 2004; Sokmen *et al.*, 2004). However, in these reports, a limited number of their pure major components

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was tested for antibacterial activities against a limited number of microorganisms. In this respect, the action of monoterpenes as antimicrobial agents has not been elucidated in detail.

Thus, the objective of this study was to evaluate the inhibitory effects of 21 pure oxygenated monoterpenes, obtained commercially, on the growth of 63 bacterial strains (plant, food and clinic origins).

Experimental

Chemicals

The pure compounds were purchased from Fluka, Sigma, Merck, Aldrich and Alfa. The compounds tested for antimicrobial activities were borneol (Fluka), borneol acetate (Sigma), camphor (Fluka), carvone (Fluka), 1,8-cineole (Sigma), citronellal (Sigma), β-citronellol (Fluka), dihydrocarvone (Alfa), fenchol (Fluka), fenchone (Fluka), geraniol acetate (Alfa), isomenthol (Alfa), nerol (Sigma), nerol acetate (Alfa), linalool (Fluka), linalool acetate (Fluka), limonene oxide (Aldrich), menthol (Fluka), menthone (Fluka), terpinen-4-ol (Aldrich), α-terpineol (Merck).

Antibacterial activity assays

Twenty-one oxygenated monoterpenes (Fig. 1) obtained commercially were individually tested against a total of 63 bacterial strains belonging to 37 different genera and 54 species (plant, food and clinic origins). The list of used bacterial strains is given in Table I. Microorganisms were provided from the Department of Clinical Microbiology, Faculty of Medicine and Plant Diagnostic Laboratory, Faculty of Agriculture, Ataturk University, Erzurum, Turkey. The identities of the microorganisms were confirmed by a microbial identification system (MIS) (Roy, 1988) in the Biotechnology Application and Research Center of Ataturk University. Phytopathogenic bacterial organisms have been isolated from some fruits and vegetables exhibiting typical bacterial disease symptoms on host plants. It was observed that hypersensitivity reactions (HR) test results of some phytopathogenic bacterial strains were positive on tobacco plants (*Nicotiana tabacum* L. var. Samsun) based on the method previously described (Table I) (Klement *et al.*, 1964; Agrios, 1997). Bacterial cultures were preserved in Luria Broth and 15% glycerol solution at −80 °C prior to use.

Antibacterial activity assays of the compounds and penicillin were carried out by a disc diffusion method (Olson and McDade, 1995) on NA (Difco) medium. Suspensions (100 μl) of the bacteria, adjusted to 10^8 cfu/ml final cell concentration, were added to flasks containing 25 ml sterile NA medium and then poured into Petri dishes and spread by a sterile swab (9 cm). 30 mg of each of the compounds were dissolved in 1 ml of methanol and these solutions were sterilized in 0.45 μm milipore filters. Sterilized discs (5 mm) were soaked with 10 μl of each compound solution. These discs were put in the middle of plates containing NA medium. Penicillin was used as a positive control. For this purpose, 1 mg of penicillin was added into 1 ml sterilized water, and a sterilized disc was soaked with 10 μl of this solution. Bacterial cultures of plant origins were incubated at (27 ± 2) °C, whereas the bacterial cultures of clinic and food origins were incubated at (35 ± 2) °C for 6 d. At the end of six-day-periods, inhibition zones were measured in mm. All the tests were made in triplicate.

Results and Discussion

In the present study, inhibitory effects of 21 oxygenated monoterpenes (Fig. 1) on the growth of 63 bacterial strains of plant, food and cilinic origins were evaluated. The results are given in Table I. The tested compounds showed various degrees of antibacterial activities, depending on tested bacterial strains. They inhibited the growth of bacteria by producing a zone of inhibition from 7 to 11 mm in diameter. However, the compounds had lower antibacterial activities in comparison to penicillin showing potent antibacterial activities against 63 bacterial strains by producing a zone of inhibition from 7 to 65 mm in diameter.

On the basis of the results given in Table I, it is possible to conclude that nerol, linalool, α-terpineol, fenchol, terpinen-4-ol, β-citronellol as well as menthol among the used compounds had a broader antibacterial spectrum. Nerol, linalool, α-terpineol, fenchol, terpinen-4-ol and β-citronellol were found to be effective against 45, 42, 40, 40, 35 and 33 bacterial strains, respectively. In contrast to these compounds, camphor and 1,8-cineole did not show any antibacterial activity against all of the tested bacterial strains. Some compounds also showed antibacterial activities against penicillin-resistant bacterial strains (Table I). As can be seen

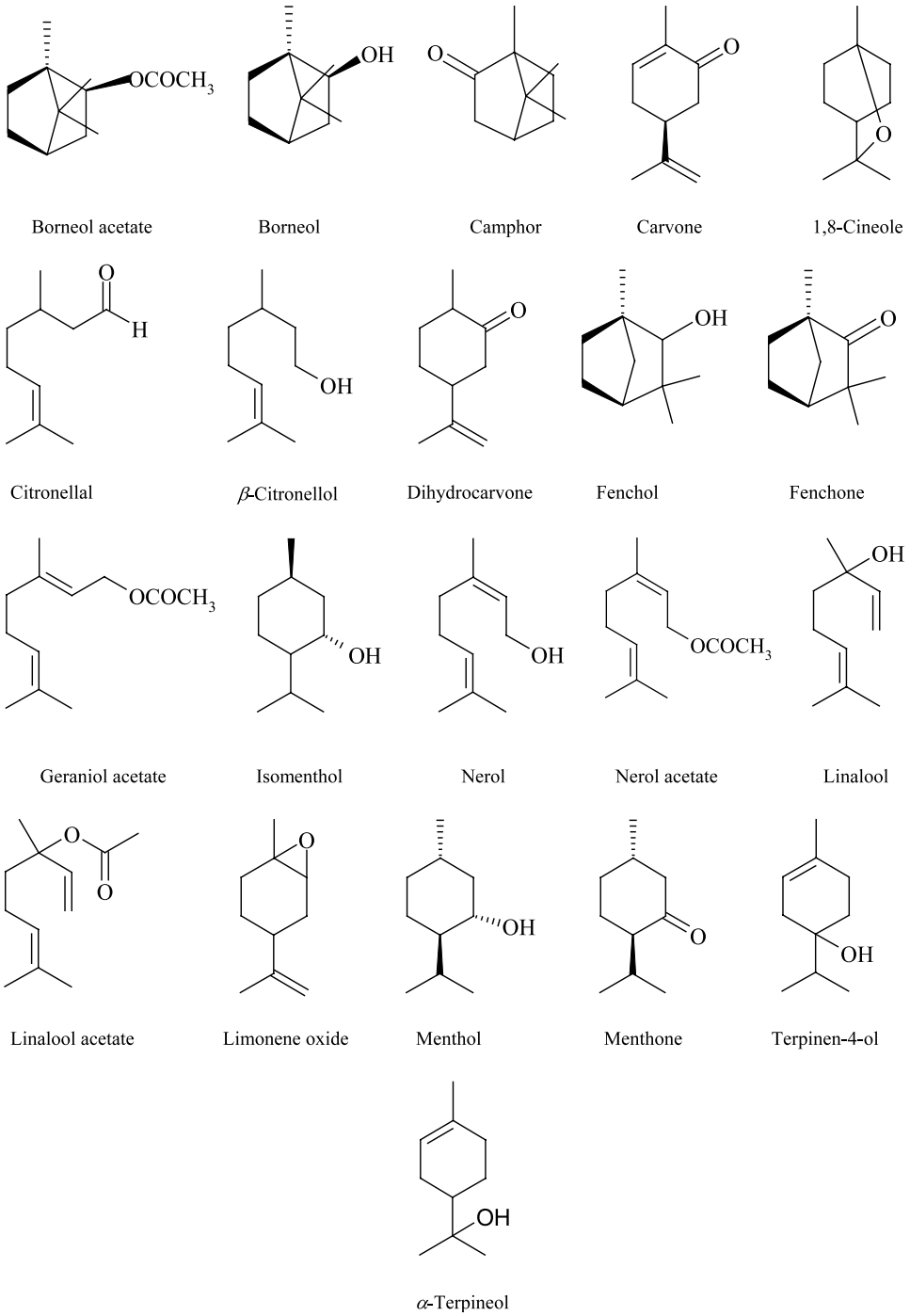

Fig. 1. Chemical structures of oxygenated monoterpenes tested for their antibacterial activity.

Table I. Antibacterial activities of oxygenated monoterpenes as diameter of average inhibition zone (mm).

Bacterial species	Citronellal[a]	β-Citronellol[a]	Linalool[a]	Linalool acetate[a]	Nerol[a]	Nerol acetate[a]	Geraniol acetate[a]	Terpinen-4-ol[b]	α-Terpineol[b]	Isomenthol[b]	Menthol[b]	Menthone[b]	1,8-Cineole[b]	Limonene oxide[b]	Carvone[b]	Dihydrocarvone[b]	Borneol[c]	Borneol acetate[c]	Camphor[c]	Fenchol[c]	Fenchone[c]	Penicillin
Plant origin																						
Gram-positive																						
Bacillus subtilis ATCC 6633	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26
Aerococcus viridans	7	7	8	–	8	–	–	8	9	–	8	–	–	–	–	–	–	–	–	9	–	17
Brevibacillus brevis	–	–	7	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11	–	40
Brevibacterium casei	9	–	9	–	7	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	39
Brevundimonas diminuta	8	–	8	–	9	–	–	–	8	–	–	–	–	–	–	–	–	–	–	8	–	46
Clavibacter michiganense[*]	–	7	7	11	7	–	–	8	12	–	8	9	–	–	–	–	7	–	–	8	11	40
Curtobacterium flaccumfaciens	–	–	9	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–	60
Kocuria rosea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	10	–	–	–	–	–	57
Kocuria varians	7	9	–	–	7	–	–	–	–	–	9	–	–	–	–	–	–	–	–	8	–	23
Microbacterium saperdae	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	65
Gram-negative																						
Agrobacterium tumefaciens[*]	7	8	7	–	7	–	–	–	8	–	9	–	–	7	–	–	7	–	–	10	–	12
Burkholderia pyriocinia	7	7	7	–	8	–	–	8	8	–	8	–	–	–	–	–	–	–	–	9	–	9
Chromobacterium violaceum	–	–	8	–	–	–	–	8	9	–	–	–	–	–	–	–	–	–	–	8	–	21
Chryseobacterium indologenes	8	8	8	–	–	8	–	8	–	–	–	–	–	–	7	7	–	–	–	–	–	18
Citrobacter freundii	7	7	10	–	8	–	–	8	9	–	–	–	–	–	7	–	–	–	–	7	–	12
Enterobacter intermedius	8	7	–	–	7	–	–	7	8	–	7	–	–	–	–	–	–	–	–	10	–	26
Erwinia amylovora[*]	–	7	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	–	31
Erwinia ananas[*]	–	8	–	–	9	–	–	7	8	–	8	–	–	7	–	–	–	–	–	9	–	30
Erwinia carotovora[*]	–	–	11	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	54
Erwinia chrysanthemi[*]	–	8	7	–	8	–	–	8	7	–	7	–	–	7	–	–	–	–	–	10	–	22
Leclercia adecarboxylata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	17
Neisseria subflava	7	9	8	–	7	–	–	8	8	–	10	–	–	–	–	–	–	–	–	10	–	13
Pseudomonas aeruginosa	–	–	8	–	8	–	–	7	8	–	–	–	–	–	–	–	–	–	–	7	–	41
Pseudomonas savastonoi pv. *fraxinus*[*]	–	–	7	–	–	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	26
Pseudomonas syringae pv. *glycinea*[*]	–	7	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	7	–	24
Pseudomonas syringae pv. *maculicola*[*]	–	–	8	–	7	–	–	7	7	–	8	–	–	–	–	–	–	–	–	8	–	–
Pseudomonas syringae pv. *populans*[*]	8	–	8	–	9	–	–	8	8	–	8	–	–	–	–	–	–	–	–	8	–	26
Pseudomonas syringae pv. *syringae*[*]	–	–	7	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	20
Pseudomonas syringae pv. *tabaci*[*]	–	–	–	–	8	–	–	–	7	–	–	–	–	–	–	–	–	–	–	8	–	40
Pseudomonas syringae pv. *tomato*[*]	7	–	7	–	8	–	–	8	8	–	–	–	–	–	–	–	–	–	–	8	–	–
Serratia grimesii	–	8	7	–	7	–	–	7	7	–	7	–	–	–	–	–	–	–	–	8	–	46
Xanthomonas campestris pv. *rhapontici*[*]	8	8	9	–	9	–	–	8	7	–	9	7	–	–	–	8	–	7	–	8	–	43
Sphingomonas capsulata	7	8	8	–	–	–	–	7	7	–	7	–	–	–	7	7	–	–	–	–	–	13
Xanthomonas campestris pv. *campestris*[*]	8	–	7	–	9	7	7	7	–	–	7	–	–	–	–	7	7	–	–	–	–	59
Xanthomonas pelargonii[*]	–	9	–	–	9	–	–	–	7	–	8	–	–	7	–	–	–	–	–	10	–	23
Ralstonia pickettii	–	8	8	–	9	–	–	9	9	–	–	–	–	–	–	–	–	–	–	–	–	35

Tabble I (continued).

Bacterial species	Citronella[a]	β-Citronellol[a]	Linalool[a]	Linalool acetate[a]	Nerol[a]	Nerol acetate[a]	Geraniol acetate[a]	Terpinen-4-ol[b]	α-Terpineol[b]	Isomenthol[b]	Menthol[b]	Menthone[b]	1,8-Cineole[b]	Limonene oxide[b]	Carvone[b]	Dihydrocarvone[b]	Borneol[c]	Borneol acetate[c]	Camphor[c]	Fenchol[c]	Fenchone[c]	Penicillin
Clinic and food origins																						
Gram-positive																						
Arthrobacter spp.	8	8	8	–	9	–	–	7	8	–	10	–	–	–	–	–	–	–	–	–	–	41
Bacillus coagulans	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	50
Bacillus mycoides	7	7	11	–	8	–	–	7	8	–	7	–	–	–	–	–	–	–	–	7	–	12
Enterococcus fecalis ATCC 29122	7	–	–	–	7	–	–	7	8	–	–	–	–	–	7	–	–	7	–	–	–	–
Micrococcus luteus	–	–	7	–	7	–	–	–	8	–	–	–	–	–	–	–	–	–	–	7	–	59
Micrococcus lylae	–	–	–	–	10	–	–	–	8	–	7	–	–	–	–	–	–	–	–	8	–	56
Staphylococcus aureus ATCC 29213	7	7	–	–	–	–	–	7	7	–	–	–	–	7	–	–	–	–	–	–	–	36
Staphylococcus hominis	–	–	–	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	–	–	39
Streptococcus pyogenes ATCC 176	–	7	–	–	–	–	–	–	7	–	–	–	–	–	7	–	–	–	–	–	–	27
Acinetobacter baumannii	–	7	9	–	10	–	–	9	–	–	8	–	–	–	–	–	–	–	–	10	–	34
Acinetobacter calcoaceticus	–	9	–	–	8	–	–	–	7	8	–	–	–	–	7	–	–	–	–	9	–	25
Acinetobacter johnsonii	–	–	–	–	8	–	–	–	7	–	–	–	–	–	–	–	–	–	–	8	–	30
Acinetobacter radioresistens	7	8	8	–	10	–	–	9	–	–	–	–	–	7	–	–	–	–	–	7	–	32
Enterobacter cloacae	8	7	7	–	7	–	–	7	8	–	–	–	–	7	–	–	7	–	–	9	–	–
Escherichia coli	–	–	8	–	–	–	–	7	–	–	–	–	–	–	–	–	–	–	–	10	–	–
Hafnia alvai	–	–	8	–	7	–	–	7	7	–	8	–	–	8	–	–	–	–	–	8	–	18
Klebsiella pneumoniae	–	8	8	–	9	–	–	7	9	–	9	–	–	–	–	7	–	–	–	8	–	16
Klebsiella trevisanii	–	–	10	–	8	8	8	8	8	–	–	8	–	–	–	–	–	–	–	7	–	15
Klebsiella planticola	7	7	7	–	7	–	–	7	8	–	–	–	–	–	–	–	–	–	–	8	–	–
Proteus vulgaris	–	8	–	–	10	–	–	7	–	7	–	–	–	–	7	–	–	–	–	8	–	21
Pseudomonas aeruginosa ATCC 9027	7	–	–	–	–	–	–	–	7	–	–	7	–	–	–	–	–	–	–	–	–	26
Pseudomonas aeruginosa ATCC 27859	–	7	–	–	7	–	–	–	7	–	–	–	–	–	7	–	–	–	–	–	–	29
Salmonella enteritidis ATCC 13076	–	–	7	–	8	–	–	–	8	–	–	–	–	–	7	–	–	–	–	–	–	8
Salmonella typhimurium	7	9	10	–	11	–	–	7	8	–	7	–	–	–	–	–	–	–	–	–	–	20
Stenotrophomonas maltopholia	–	7	–	–	7	–	–	–	7	–	7	–	–	–	–	–	–	–	–	7	–	32
Vibrio alginolyticus	–	8	10	–	–	–	–	9	9	–	–	–	–	–	–	–	–	–	–	8	–	19
Vibrio hollisae	–	–	7	–	–	–	–	–	–	–	–	–	–	–	7	–	–	–	–	–	–	16

[a] Acyclic oxygenated monoterpenes. [b] Monocyclic oxygenated monoterpenes. [c] Bicyclic oxygenated monoterpenes. – Not active. * HR (hypersensitivity reactions) test results were positive on *Nicotiana tabacum* L. var. Samsun.

from this table, the growth of *E. coli*, which is a penicillin-resistant bacterium was inhibited by fenchol, terpinen-4-ol and linalool.

Based on the present data, it can be also concluded that alcohol derivatives of oxygenated monoterpenes had greater antibacterial activities than those of ketone derivatives. For instance, menthol had inhibitory effects on the growth of 24 bacterial strains, whereas menthone showed activity only against four bacteria. Similar results were also found for fenchol and fenchone (Table I). The present results also showed that nerol, linalool and borneol were more active than their acetate derivatives (nerol acetate, linalool acetate and borneol acetate). According to these results, it can be concluded that alcohol derivatives of oxygenated monoterpenes were more active than their acetate derivatives.

Recently, a large number of investigations has been performed on the antibacterial activities of plant essential oils, but not of the major components (Kim *et al.*, 1995; Sivropoulou *et al.*, 1995; Pattnaik *et al.*, 1997; Lis-Balchin *et al.*, 1998; Dorman and Deans, 2000; Friedman *et al.*, 2002, 2004; Iscan *et al.*, 2002; Oumzil *et al.*, 2002; Gulluce *et al.*, 2003; Karaman *et al.*, 2003; Sahin *et al.*, 2003; Kim and Shin, 2004). On the other hand, this is the first time that the antibacterial activities of pure oxygenated monoterpenes have been screened toward bacterial species of plant, food and clinic origins.

Antibacterial activities of pure oxygenated monoterpenes, citral, carvacrol, cinnamaldehyde, citral, linalool, linalool acetate, eugenol, geraniol and terpinen-4-ol, against *Escherichia coli* and *Salmonella enterica* have been evaluated by Friedman *et al.* (2004). They found, that carvacrol, cinnamaldehyde, geraniol, eugenol, citral were more effective than linalool and terpinen-4-ol against *E. coli*. Contrarily, linalool acetate did not exhibit any inhibition effect against *E. coli*. Our results showed the similar behaviour for terpinen-4-ol, linalool and linalool acetate against *E. coli* as well. These researchers also reported that carvacrol, terpinen-4-ol, geraniol, citral and linalool were more active against *S. enterica* (Friedman *et al.*, 2004). In another report, the same researchers found that α-terpineol was active against *S. enterica*, however, carvone and geranyl acetate were not found to be active against *E. coli* and *S. enterica* (Friedman *et al.*, 2002). These findings are compatible with our results. The data presented in Table I show that α-terpineol was active against *S. enteritidis* ATCC 13076. It has been also reported that linalool, menthol and α-terpineol were less potent against *E. coli* and *Salmonella typhimurium* (Sivropoulou *et al.*, 1995; Kim and Shin, 2004). The similar results for these compounds were found in the present study. However, α-terpineol did not exhibit any activity against *E. coli* in the present study. Furthermore, 1,8-cineole, geraniol, linalool, menthol and citral were tested for their antibacterial activity against 17 bacteria by Pattnaik *et al.* (1997). These researchers reported that linalool possessed a wide antibacterial spectrum inhibiting 17 bacteria, followed by 1,8-cineole, geraniol, menthol and citral. On the basis of our results, linalool, inhibiting 42 bacterial strains, was most effective as compared with the antibacterial spectra of menthol and 1,8-cineole. Contrarily, 1,8-cineole showed no antibacterial activity. As shown in Table I, borneol and carvone exhibited a weak and low antibacterial spectrum against the tested bacteria. Similar results were obtained by several researcher groups (Dorman and Deans, 2000; Oumzil *et al.*, 2002), who found that these compounds had weak antibacterial activities.

Based on the present results, it is possible to conclude that alcohol derivatives of oxygenated monoterpenes have a stronger activity and broader spectrum than their ketone derivatives. These findings are in agreement with a previous report (Iscan *et al.*, 2002) in which menthol has a stronger activity and broader spectrum than menthone.

Food-borne diseases are still one of the major problems in the world (Mead *et al.*, 1999). A variety of microorganisms such as *E. coli*, *Klebsiella pneumoniae*, *S. typhimurium*, *Enterobacter* sp., *Pseudomonas* sp. and *Staphylococcus* sp. have been reported as the causal agents of food-borne diseases and/or food spoilage (Deak and Beuchat, 1996). In the present study, the pure compounds were also tested for their antibacterial activities against some food-borne pathogens such as *Bacillus coagulans*, *Enterobacter cloacae*, *Enterococcus fecalis*, *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. Some of the tested compounds showed antibacterial activities against food-borne pathogens (Table I). *Agrobacterium tumefaciens*, *Clavibacter michiganense*, *Erwinia amylovora*, *Erwinia carotovora*, *Pseudomonas* sp. and *Xanthomonas* sp. are causal bacterial agents of plant diseases. Citronellal, β-citronellol, linalool, nerol, terpinen-4-ol, α-terpineol, menthol and fenchol also showed a broad spectrum of antibacterial avtivity against those plant pathogens.

In conclusion, the development of natural antimicrobials will help to decrease the negative effects (residues, resistance and environmental pollution) of synthetic drugs. In this respect, natural antimicrobials may be also effective, selective, biodegradable and less toxic to the environment. The present results demonstrate that nerol, linalool, α-terpineol, fenchol and terpinen-4-ol have a wide antibacterial spectrum against the tested bacteria, although their inhibitory effects are low in comparison to penicillin. In the view of our results, it was concluded that these oxygenated monoterpenes and essential oils rich in these components can be used as antimicrobial agents for food preservation. However, the safeties and toxicities of these

compounds will need to be addressed. Our results would also be useful in research aiming at the antibacterial properties of essential oils and their major components.

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