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Original Article

Antimicrobial Characteristics of Some Herbal Oils on *Pseudomonas aeruginosa* with Special Reference to their Chemical Compositions

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Abstract

Pseudomonas aeruginosa is an important opportunistic pathogen causing widespread infections by numerous virulence factors. Increasing resistance to antibiotics makes the *Pseudomonas* infections treatment further difficult. The purpose of this study was to evaluate antimicrobial characteristics of essential oils from *Matricaria chamomilla*, *Artemisia persica, Zataria multiflora, Myrtus communis, Ruta graveolens, Eucalyptus camaldulensis* and *Ferula gummosa* on *Pseudomonas aeruginosa* (ATCC 27853).

The selected essential oils were screened against *P. aeruginosa* using the disc diffusion method. The minimal inhibitory and bactericidal concentrations (MIC and MBC) of the active essential oils were tested using macrodilution method at concentrations ranging from 0.125 to 256 µg/ml. It was found by GC/MS analyses that *Z. multiflora, M. communis* and *E. camaldulensis* possess the most potent oils.

Three of the seven essential oils (*Z. multiflora, M. communis* and *E. camaldulensis*) were significantly active against *P. aeruginosa* exhibiting MIC/MBC of 64/128, 64/64 and 64/128 µg/ml, respectively. Gas chromatography mass spectrometry (GC/MS) analysis led to identification of 32, 21 and 22 components in *M. communis, E. camaldulensis* and *Z. multiflora* oils, respectively.

With a view to antibacterial activity of some oils against the tested bacterium, their safe antibacterial potentials can therefore be exploited as alternative agents in combating infections of *P. aeruginosa* origin.

Keywords: *Eucalyptus camaldulensis*; *Myrtus communis; Pseudomonas aeruginosa*; *Zataria multiflora.*

Introduction

The spread of drug resistant microbial pathogens is one of the most serious threats to successful treatment of infectious diseases. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes severe and life-threatening infections in immunocompromised patients such as these with respiratory diseases, burns, cancers undergoing chemotherapy and cystic fibrosis. Several studies have documented increasing resistance rates in *P. aeruginosa* to antibiotics (1). Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious

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diseases (2). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (3, 4). Some oils have been used in cancer treatment (5). Some other oils have been used in food preservation (6), aromatherapy (7) and fragrance industries. Essential oils are rich sources of biologically active compounds. There has been an increased interest in looking for antimicrobial properties of extracts from aromatic plants particularly essential oils (8). Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential (9). Little antibacterial activity was found against *P. aeruginosa* when *Ferula gummosa* essential oil was studied (10). The essential oil from *Artemisia douglasiana* leaf showed limited antimicrobial activity in vitro, so it was unclear if the oil exertes a direct antimicrobial effect in vivo, or plays some role in stimulation of host defenses (11). The essential oil of *Thymus numidicus* has been reported to have the strongest antibacterial activity against *P. aeruginosa* (12). Combinations of essential oils of *Thymus vulgaris* and *Pimpinella anisum* seeds methanol extracts showed an additive action against most tested pathogens especially *P. aeruginosa* (13). Oregano combined with marjoram, thyme or basil also had an additive effect against *P. aeruginosa* (14). Inhibitory effects of ethanol, methanol, chloroform and hexane extracts of *Zataria multiflora* Boiss. were investigated against two clinical isolates of multiple drug resistant *P. aeruginosa*. All the extracts showed activity against both the strains. Maximum antibacterial activity was observed in methanol extract. The combination of extracts had variable synergistic/ antagonistic effect (15). Antibacterial activity of various concentrations of aqueous extracts of leaves of *Myrtus communis* and Eucalyptus were evaluated with comparison to 6 antibiotics. The extracts showed an excellent effect on bacterial growth and their effects were observed within the limits of antibiotic effects. Most concentrations of the extracts of the studied plants showed a high antibacterial activity against *P. aeruginosa* and showed significant differences between susceptibility of *P. aeruginosa* isolated from each tetracycline covered burn and non-tetracycline covered burn (16). Tunisian *Ruta graveolens* L. essential oil had a moderate antimicrobial activity against *P. aeruginosa* (17). Reviewing the above mentioned repents, we designed this study to explore anti pseudomonas properties of *Matricaria chamomilla*. *Artemisia persica, Z. multiflora, Myrtus communis, R. graveolens, Eucalyptus camaldulensis* and *F. gummosa* essential oils.

Experimental

Microbial strain plants and oil isolation

Pseudomonas aeruginosa ATCC 27853 was grown on Mueller-Hinton agar and used as standard strain. The plants (*M. chamomilla*, *A. persica, Z. multiflora, M. communis, R. graveolens, E. camaldulensis* and *F. gummosa*) were identified and provided by Research Institute of Medicinal Plants (Tehran, Iran). The shadow dried plants were hydrodistilled for 90 min in full glass apparatus. The oil was isolated using a Clevenger type apparatus. The extraction was carried out for 2 h after 4-hour maceration in 500 ml of water. The oils so extracted were stored in dark glass bottles in a refrigerator until they were used.

Screening of antibacterial activity

Screening of essential oils for antibacterial activity was done by the disk diffusion method, which is normally a preliminary check to select efficient essential oils (3). It was performed by 18 h culture at 37°C in 10 ml of Mueller-Hinton broth. The cultures were adjusted to approximately 10^5 CFU/ml with sterile saline solution. Five hundred microliters of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The essential oils were dissolved in dimethylsulfoxide (with volume ratio of 50:50) and sterilized by filtration through a 0.45 µm membrane filter.

Under aseptic conditions, empty sterilized blank discs (6 mm diameter) were impregnated with 20 µl of the respective essential oils and placed on the agar surface. Blank disc moistened with dimethylsulfoxide was placed on the seeded petriplate as a vehicle control. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37 °C for 18 h (18 h was fixed as the optimum incubation time since there was no change in the inhibition up to 24 h). After the incubation period, the zone of inhibition was measured with a caliper. All studies were performed in triplicate and mean value was calculated. The results were expressed as mean±SD.

MIC and MBC assay

Based on the previous screening of seven essential oils, *Z. multiflora, M. communis* and *E. camaldulensis* were identified to have potent antibacterial activity and their Minimal Inhibitory (MIC) and Minimal Bactericidal Concentrations (MBC) were determined. The macrodilution method recommended by the National Committee for Clinical Laboratory Standards (18) was used with the following modification; measured quantities of the essential oil were added to each of Mueller-Hinton broth tubes to achieve final oil concentrations from 0.125- 512 µg/ml. Tubes without oil served as control. Measured quantities from each of the 24 hour old *P. aeruginosa* suspensions prepared in normal saline at 0.5 McFarland were added to each tube containing various oil concentrations so as to achieve final bacterial load of 10^6 CFU/ml. The tubes were then incubated at 37 °C for 24 h on a shaker incubator as to evenly disperse the oil throughout the broth in tubes. MIC determination was carried out by bacterial count instead of turbidometry to avoid misleading false turbidity caused by oil interference. The lowest concentration, showing no increased growth compared to that of the control tubes, was regarded as MIC. MBC was determined as the lowest concentration at which 99.9% bacterial death occurred on the plates. Bacterial counts were carried out at time zero both in control and test. All tubes including the control were run simultaneously. All the tests were carried out in triplicate.

Gas chromatography mass spectrometry (GC/MS)

The most potent antibacterial oils viz; *Z. multiflora, M. communis* and *E. camaldulensis* were analyzed by GC/MS. GC analyses were performed using a Shimadzu-9A gas chromaph equipped with a flame ionization detector and quantitation was carried out on Euro Chrom 2000 from Knauer by the area normalization method neglecting response factors. The analysis performed by a DB-5 fused-silica column (30 m × 0.25 mm, film thickness 0.25 µm, J & W Scientific Inc., Rancho Cordova, CA, USA). The operating conditions were as follows: injector and detector temperature, 250 °C and 265 °C, respectively; carrier gas, Helium. Oven temperature programme was 40-250 °C at the rate of 4 °C/min. The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used. The column was same as GC and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V. The identities of the oil components were established from their GC retention indices, relative to C7-C25 n-alkanes, by comparison of their MS spectra with those reported in the literature (19) and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results and Discussion

The antibacterial activity of selected essential oils against *P. aeruginosa* is summarized in Table 1. *Zataria multiflora, M. communis* and *E. camaldulensis* oils had antibacterial activities of varying magnitudes against *P. aeruginosa* in preliminary screenings exhibiting MICs/MBCs of 64/128, 64/64 and 64/128 µg/ml respectively (Table 1). *Matricaria chamomilla*, *A. persica, R. graveolens,* and *F. gummosa* did not have antimicrobial activity.

Gas chromatography mass spectrometry (GC/MS) analysis led to identification of 32, 21, and 22 components in *M. communis, E. camaldulensis* and *Z. multiflora* oils, respectively (Table 2-5). The major components of *M. communis* oil were α-pinene (29.4%),

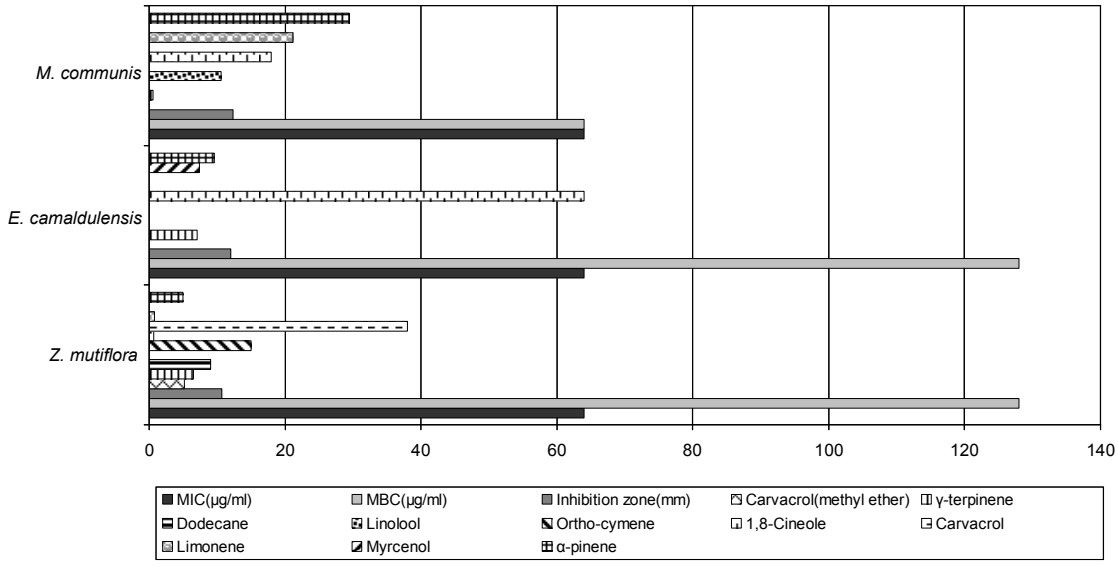

Figure 1. Antibacterial effectiveness of chemical compositions from essential oils.

limonene (21.2%), 1, 8-cineole (18%) and linalool (10.6%). *Eucalyptus camaldulensis* oil was characterized with prominent concentrations of 1,8-cineole (64%), α-pinene (9.6%), myrcenol (7.4%) and γ-terpinene (7%). *Zataria multiflora* oil was distinctive in its high concentrations of α-pinene (5%), carvacrol (37%), γ-terpinene (6.5%) and dodecane (9%). Anti bacterial effectiveness of chemical components from essential oils is graphically compared (Figure 1).

Plant essential oils and extracts have been used for thousands of years (20), in food preservation, pharmaceuticals, alternative medicine and natural therapies (21). It is necessary to scientifically investigate bioactivities of those plants that were traditional used in improving the quality of health. Essential oils are potential sources of novel antimicrobial compounds (22) especially against bacterial pathogens (23). In vitro studies in this work showed that some essential oils inhibited growth of *P. aeruginosa*, but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak (24). In our study, *Z. multiflora, M. communis* and *E. camaldulensis* oils exhibited moderate activity against *P. aeruginosa*. Several studies have shown that of *Z. multiflora, M. communis* and *E. camaldulensis* oils had strong and consistent inhibitory effects against various pathogens (25). Even earlier studies have reported better antimicrobial activity for eucalyptus oil (26, 27). Among all oils analyzed in this work, the essential oil of *M. communis* was the most effective one as an antibacterial agent.

The antibacterial activity of essential oils has been attributed to the presence of some active constituents in the oils. Our GC-MS study revealed α-Pinene to be the major constituent of

Table 1. Antimicrobial activity of selected essential oils against *P. aeruginosa*.

	Zataria multiflora	***Myrtus communis***	***Eucalyptus camaldulensis***
Diameter of inhibition zone (mm±SD)*	10.66±0.57	12.33±0.57	12±1.7
MIC (µg/ml)	64	64	64
MBC (µg/ml)	128	64	128

*Diameter of disk was 6 mm.

Table 2. Chemical composition of the essential oil of *Myrtus communis.*

No.	Oil compounds	RI	%
1	Isobutyl isobutyrate	892	0.7
2	α-Thujene	922	0.25
3	α-Pinene	931	29.4
4	Sabinene	971	0.6
5	Myrcene	981	0.3
6	δ-3-Carene	998	0.2
7	p-Cymene	1013	0.4
8	Limonene	1025	21.2
9	1,8-Cineole	1028	18.0
10	(E)- -Ocimene	1038	0.1
11	γ-terpinene	1051	0.6
12	Terpinolene	1082	0.3
13	Linalool	1089	10.6
14	α -Campholenal	1122	Trace
15	Trans-Pinocarveole	1130	Trace
16	δ-Terpineole	1154	Trace
17	Terpinene-4-ol	1169	0.5
18	α-Terpineole	1180	3.1
19	Trans-Carveole	1213	0.4
20	Cis- Carveole	1217	Trace
21	Geraniol	1242	1.1
22	Linalyl acetate	1248	4.6
23	Methyl geranate	1310	0.2
24	α-terpinyl acetate	1342	1.3
25	Neryl acetate	1351	Trace
26	Methyl eugenol	1369	1.6
27	β-Caryophyllene	1430	0.2
28	α-humulene	1463	0.2
29	Spathulenol	1562	Trace
30	Caryophylleneb epoxide	1586	0.1
31	Humulene epoxide II	1608	Trace

RI= Retention indices
Trace= Less than 0.1%

Table 3. Chemical composition of the essential oil of *Eucalyptus camaldulensis.*

No.	Oil compounds	RI	%
1	α-pinene	935	9.6
2	Sabinene	975	0.4
3	β-myrcene	991	0.4
4	α-phellandrene	1004	1.6
5	1,8-Cineole	1027	64
6	γ-terpinene	1055	7
7	Terpinolene	1084	0.7
8	Myrcenol	1111	7.4
9	Nopinone	1136	1.4
10	Pinocarvone	1161	0.4
11	Terpin-4-ol	1175	1.5
12	α-terpineole	1185	0.3
13	Dihydro carveol	1193	0.4
14	Neo-iso- Dihydro carveol	1227	0.3
15	Aromadendrene	1436	1.9
16	α-humulene	1457	0.7
17	Bicyclogermacrene	1492	0.5
18	Elemol	1556	0.6
19	Caryophyllene oxide	1578	3.7
20	Humulene epoxide II	1601	0.5
21	α-acorenol	1623	0.6

RI= Retention indices

M. communis oil. Pinene has antibacterial and antifungal remedy employed in both veterinary and human medicine (28). Limonene, 1, 8-cineole, linalool, myrcenol, carvacrol and dodecane are another important components that detected in mentioned oils. Earlier studies suggested that the antibacterial activity of these oils was probably due to their major component (29-35). A graphical comparison of the effectiveness of various chemical compounds on antimicrobial characteristics of the essential oils is shown in Figure 1. It is assumed that higher the effect of a compound lower is the amount of the oil required to achieve MIC/MBC. In this work, as seen in Figure 1, α-pinene has an ascending ratio from *Z. mutiflora* (5%), *E. camaldulensis* (9.6%) to *M. communis* (29.4%). This phenomenon is parallel to an increase in zone of growth inhibition. This effectiveness could be further explained by a descending amount of oil required to impart inhibitory or cidal effect by *Z. mutiflora*, *E. camaldulensis* and *M. communis* oils, respectively (Figure 1). α-pinene could therefore be thought of having significant contribution to the antimicrobial activities in the present study. Other chemical components do not seem to have significant antibacterial property as compared to α -pinene (Figure 1).

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane, disturbing the cell

Table 4. Chemical composition of the essential oil of *Zataria multiflora.*

No.	Oil compounds	RI	%
1	α-Thujene	925	0.9
2	α-pinene	933	5
3	Camphene	947	0.3
4	Sabinene	970	0.6
5	Myrcene	986	1.6
6	Decane	996	4
7	α-Terpinene	1013	1.4
8	Ortho Cymene	1018	15
9	Limonene	1026	0.8
10	1,8-Cineole	1027	0.7
11	γ-Terpinene	1057	6.5
12	Sabinene hydrate	1064	0.3
13	Terpinolene	1086	0.2
14	Undecane	1098	3.8
15	Dodecane	1198	9
16	Thymol (Methyl ether)	1232	0.5
17	Carvacrol (Methyl ether)	1242	5.2
18	Thymol	1289	3.3
19	Carvacrol	1298	37
20	Thymyl acetate	1349	0.2
21	Tetradecane	1394	2
22	β-Caryophyllene	1418	2

RI= Retention indices

structures and rendering them more permeable (36). Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (37). Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria (24). Among the Gram-negative bacteria, *Pseudomonas*, and in particular *P. aeruginosa*, appears to be least sensitive to the action of essential oils (38-40). *Pseudomonas* consistently shows high or often the highest resistance to the antimicrobials such as linalool/ chavicol (41) and terpenoids/carvacrol/thymol (42). From this study it can be concluded that some essential oils possess antibacterial activity against *P. aeruginosa.*

New antimicrobial agents against this bacterium are very valuable, especially in multi drug resistant strains. We believe that the present investigation together with previous studies provide support to the antibacterial properties of *Z. multiflora, M. communis* and *E. camaldulensis* oils. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agents. Additional in vivo studies and clinical trials will also be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral applications.

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References

(1) Streit JM, Jones RN, Sader HS and Fritsche TR. Assessment of pathogen occurrences and resistance profiles among infected patients in the intensive care unit: report from the SENTRY Antimicrobial

Table 5. Comparative common chemical compositions (%) of the essential oil of *Myrtus communis, Eucalyptus camaldulensis and Zataria multiflora.*

No.	Compound	*M. communis*	*E. camaldulensis*	*Z. multiflora*
1	Carvacrol (Methyl ether)	0	0	5.2
2	Dodecane	0	0	9
3	Ortho Cymene	0	0	15
4	Carvacrol	0	0	37
5	Myrcenol	0	7.4	0
6	γ-terpinene	0.6	7	6.5
7	Linalool	10.6	0	0
8	1,8-Cineole	18	64	0.7
9	Limonene	21.2	0	0.8
10	α-pinene	29.4	9.6	5

Surveillance Program. *Int. J. Antimicrob. Agents* (2004) 24: 111-18

(2) Tepe B, Daferera D, Sokmen M, Polissiou M and Sokmen A. *In vitro* antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigii* M. Zohary et P.H. Davis. *J. Agric. Food. Chem.* (2004) 52: 1132-7

(3) Burt SA. Essential oils: their antibacterial properties and potential applications in foods: a review. *Int. J. Food Microbiol.* (2004) 94: 223-53

(4) Kordali S, Kotan R, Mavi A, Cakir A, Ala A and Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, Artemisia santonicum,* and *Artemisia spicigera* essential oils. *J. Agric. Food. Chem.* (2005) 53: 9452-8

(5) Sylvestre M, Pichette A, Longtin A, Nagau F and Legault J. Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *J. Ethnopharmacol*. (2006) 103: 99-102

(6) Faid M, Bakhy K, Anchad M, Tantaoui-Elaraki A and Alomondpaste J. Physicochemical and microbiological characterizations and preservation with sorbic acid and cinnamon. *J. Food Prot*. (1995) 58: 547-50

(7) Buttner MP, Willeke K and Grinshpun SA. Sampling and analysis of airborne microorganisms. In: Hurst CJ, Knudsen GR, McInerney MJ, Stetzenbach LD and Walter MV. (Eds.) *Manual Environmental Microbiology*. ASM Press, Washington DC (1996) 629- 40

(8) Milhau G, Valentin A, Benoit F, Mallie M, Bastide J, Pelissier Y and Bessiere J. *In vitro* antimicrobial activity of eight essential oils. *J. Essen. Oil Res*. (1997) 9: 329-33

(9) Darokar MP, Mathur A, Dwivedi S, Bhalla R, Khanuja SPS and Kumar S. Detection of antibacterial activity in the floral petals of some higher plants. *Curr. Sci*. (1998) 75: 187

(10) Eftekhar F, Yousefzadi M and Borhani K. Antibacterial activity of the essential oil from *Ferula gummosa* seed. *Fitoterapia* (2004) 75: 758-9

(11) Setzer WN, Vogler B, Schmidt JM, Leahy JG and R Rives. Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. *Fitoterapia* (2004) 75:192-200

(12) Kabouche Z, Boutaghane N, Laggoune S, Kabouche A, Ait-Kaki Z and Benlabed K. Comparative antibacterial activity of five Lamiaceae essential oils from Algeria. *Int. J. Aromatherapy* (2005) 15: 129-33

(13) Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *J. Ethnopharmacol.* (2008) 116: 403-6

(14) Gutierrez J, Barry-Ryan C and Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int. J. Food Microbiol.* (2008) 124: 91-7

(15) Rahman MU, Gul S, Ul Haq MZ, Hafeez I and Azam M. Inhibitory effects of *Zataria multiflora* Boiss. on multiple drug resistant *Pseudomonas aeruginosa*. *J. Chem. Soc. Pakistan* (2008) 30: 626-9

(16) Al-Saimary IE, Bakr SS, Jaffar T, Al-Saimary AE, Salim H and Al-Muosawi R. Effects of some plant extracts and antibiotics on *Pseudomonas aeruginosa* isolated from various burn cases. *Saudi Med. J.* (2002) 23: 802-5

(17) Fredj MBH, Marzouk B, Chraief I, Boukef K and Marzouk Z. Analysis of Tunisian *Ruta graveolens* L. oils from Jemmel. *J. Food Agric. Environ.* (2007) 5: 52-5

(18) National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests of Bacteria that Grow Aerobically. In approved standard M100-S12 Wayne, PA (2002)

(19) Davies NW. Gas chromatographic retention index of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20 M phases. *J. Chromatogr.* (1998) 503: 1-24

(20) Jones FA. Herbs- useful plants. Their role in history and today. *Eur. J. Gastroenterol. Hepatol.* (1996) 8: 1227-31

(21) Lis-Balchin M and Deans SG. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. App. Bacteriol*. (1997) 82: 759-62

(22) Mitscher LA, Drake S, Gollapudi SR and Okwute SK. A modern look at folkloric use of anti-infective agents. *J. Nat. Prod.* (1987) 50: 1025-40

(23) Malek Jafarian M and Ghazvini K. *In vitro* susceptibility of *Helicobacter pylori* to licorice extract. *Iranian J. Pharm. Res.* (2007) 6: 69-72

(24) Zaika LL. Spices and herbs: their antibacterial activity and its determination. *J. Food. Safety* (1998) 23: 97-118

(25) Rasooli I. Food preservation- a biopreservative approach. *Food* (2007) 1: 111-36

(26) Cimanga KK, Kambu Tona L, Apers S, De Bruyne T, Hermans N, Totte J, Pieters L and Vlietinck AJ. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J. Ethnopharmacol.* (2002) 79: 213-20

(27) Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K and Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. *Oral Microbiol. Immunol*. (2002) 19: 61-4

(28) Dryden MS, Dailly S and Crouch M. A randomized controlled trial of tea tree topical preparations versus a standard topical regimen for clearance of MRSA colonization. *J. Hosp. Infect.* (2004) 56: 283-6

(29) Delaquis PJ, Stanich K, Girard B and Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol*. (2002) 74: 101-9

(30) Periago PM and Moezelaar R. Combined effect of nisin and carvacrol at different pH and temperature levels on the variability of different strains of *Bacillus cereus*. *Int. J. Food Microbiol*. (2001) 68: 141-8

(31) Periago PM, Palop A and Fernandez PS. Combined effect of nisin, carvacrol and thymol on the viability of *Bacillus cereus* heat-treated vegetative cells. *Food Sci. Technol. Int.* (2001) 7: 487-92

(32) Aligiannis N, Kalpoutzakis E, Mitaku S and Chinou IB. Composition and antimicrobial activity of the essential oils of two *Origanum* species. *J. Agric. Food Chem*. (2001) 49: 4168-70

(33) Vardar-Unlu G, Candan F, Sokmen A, Daferera D, Polissiou M, Sokmen M, Donmez E and Tepe B. Antimicrobial and antitoxic activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. var. *pectinatus* (Lamiaceae). *J. Agric Food. Chem.* (2003) 51: 63-7

(34) Baydar H, Sagdic O, Ozkan G and Karadogan T. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control* (2004) 15: 169-72

(35) Alzoreky NS and Nakahara K. Antimicrobial activity of extracts from some edible plants commonly consumed in Asia. *Int. J. Food Microbiol.* (2002) 80: 223-30.

(36) Sikkema J, De Bont JAM and Poolman B. Interactions of cyclic hydrocarbons with biological membranes. *J. Biol. Chem*. (1994) 269: 8022-8

(37) Denyer SP and Hugo WB. Biocide-induced damage to the bacterial cytoplasmic membrane. In: Denyer SP and Hugo WB. (Eds.) *Mechanisms of Action of Chemical Biocides*. Oxford Blackwell Scientific Publication. Oxford (1991) 171-188

(38) Ruberto G, Baratta MT, Deans SG and Dorman HJD. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. *Planta Med.* (2000) 66: 687-93

(39) Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, Chessa M, Cerri R and Casanova J. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. *Flav. Frag. J.* (2002) 17: 15- 9

(40) Wilkinson JM, Hipwell M, Ryan T and Cavanagh HMA. Bioactivity of *Backhousia citriodora*: antibacterial and antifungal activity. *J. Agric. Food Chem*. (2003) 51: 76-81

(41) Smith-Palmer A, Stewart J and Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbiol*. (1998) 26: 118-22

(42) Griffin SG, Wyllie SG, Markham JL and Leach DN. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flav. Frag. J*. (1999) 14: 322-32

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