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Original Article

The antibacterial activity of some essential oils against clinical isolates of *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is categorized as a red alert pathogen that is increasingly associated with a high mortality rate in infected patients due to its resistance to extensive antibiotics. In this study, we evaluated the antibacterial activities of some essential oils (*Oliveria decumbens*, *Pelargonium graveolens*, *Eugenia caryophyllata*, *Ziziphora tenuir* and *Trachyspermum copticum* oils) against 32 clinical isolates of *A. baumannii*. The antibacterial evaluations and chemical composition of essential oils was determined. Thymol, eugenol, α-terpineol, α-citronellol and thymol were the chief portions of *T. copticum*, *E. caryophyllata*, *Z. tenuir*, *O. decumbens* and *P. graveolens* oils, respectively. The MIC values of oils against these clinical isolates revealed the three subsets of oils including 1- *T. copticum*, *E. caryophyllata* and *O. decumbens,* 2- *Z. tenuir* and 3- *P. graveolens* oils. These oils showed the synergistic activity with amikacin, the lower Fractional Inhibitory Concentration Index (FICI) was for *P. graveolens* oil (0.23) and the higher FICI was for *E. caryophyllata* (0.325).

Keywords: essential oils, antibacterial activity, isobologram curve*, Acinetobacter baumannii*

1. Introduction

Acinetobacter species are opportunistic gram negative bacteria that are primarily associated with hospital acquired infections; bacteriemia, urinary tract infection, surgical-site infections, and nosocomial and ventilator-associated pneumonia especially in intensive-care-unit (ICU) hospitalized patients. The members of genus *Acinetobacter* are very resistant to drying and disinfectants, therefore their outbreak is very fast. Todays, *Acinetobacter baumannii* has been defined as red alert pathogen due to its resistance to extensive antibiotic (Howard *et al*., 2012). Furthermore, multidrug resistant isolates of *A. baumannii* have been reported from all over the world (Peleg *et al*., 2008). *A. baumannii* causes high mortality rate in patients with *Acinetobacter* infections (Sunenshine *et al*., 2007).

Persistence of *A. baumannii* isolates in hospital environments and the appearance of isolates that are resistant to extensive antibiotics is one of the most significant concerns for many scientists. They are looking for new sources of agents with more efficacy and low side effects. Among many different sources of materials with antiseptic activity, medicinal plants as a natural source of agents with multifunctional properties including antimicrobial activity are very interesting for many of these investigators.

Many investigations have evaluated the antibacterial activity of plant essential oils against *A. baumannii* (Costa *et al*., 2009; Lysakonska *et al*., 2011; Salman *et al*., 2008; Duarte *et al*., 2012; Damjanovic-Vratnica *et al*., 2011; Tan *et al*., 2009; Jazani *et al*., 2011; Rosato *et al*., 2010).

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Therefore, due to importance of genus *Acinetobacter* as significant nosocomial infections, we identified the clinical isolates of *A. baumannii* from infected patients. Then we evaluated the efficacy of some new essential oils (*Oliveria decumbens*, *Pelargonium graveolens*, *Eugenia caryophyllata*, *Ziziphora tenuir* and *Trachyspermum copticum* oils) with folkloric uses as antiseptic for the first time against 32 clinical isolates of *A. baumannii* and their synergistic effects with amikacin by drawing isobologram curves.

2. Materials and Methods

2.1 Plant materials and extraction of essential oils

Eugenium caryophyllata, *Oliveria decumbens*, *Pelargonium graveolens*, *Ziziphora tenuir*, and *Trachyspermum ammi* essential oils were prepared from Barij Essence Pharmaceutical Company under Batch numbers 2100746, 21007434, 21007439, 21007433 and 21007440, respectively. The oils were analyzed using GC-FID and GC-MS. The GC-FID and GC-MS apparatus were conducted on an HP 6890 GC system coupled with 5973 network mass selective detectors with a capillary column of HP-5MS (30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 60°C, held for 1 min, then raised to 245°C at a rate of 3°C/min held for 10 min. Helium was used as the carrier gas at a flow rate 1.5 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectral fragmentation with those in the stored Wiley 7n.1 mass computer library. Quantization of major constituents of the oils was performed by the area normalization method (Adams, 2001).

2.2 *A. baumannii* isolates and antimicrobial susceptibility testing

A total of 32 isolates cultured from wounds, trachea, blood, CSF, catheter and other samples of patients at hospitals from Tehran were the subject of this investigation. Antimicrobial susceptibility testing was evaluated using disc diffusion (NCCLS, 2012) and micro broth (CLSI, 2009) dilution assays. This inoculate of microorganism was adjusted to 0.5 McFarland (1×10^7 - 1×10^8 CFU/ml) and using a sterile cotton swab, the microbial suspensions were cultured on appropriate media. Subsequently, sterile blank discs (6 mm in diameter) were saturated with 5 μl of oil and were put on the cultured media. The plates were incubated at 37°C for 24 h. The inhibition zones (IZ) diameters were measured in millimeters (mm) and the average IZ was recorded as mean ± SD (standard deviation).

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of oils were determined by micro broth dilution assay. The oil was two-fold serially diluted (8-0.0125 μl/ml of oil). Cation adjusted Muller Hinton broth was used as broth media. After shaking, 100 μl of oil was added to each well. The above microbial suspensions were diluted to 1×10^6 and then 100 μl were added to each well and incubated at 35±2 °C. MIC was defined as the lowest concentration of oil that inhibits bacteria after 24 h. MBC value was the first tube that showed no growth on suitable media. All experiments were performed in triplicates. Statistical data analysis was performed using SPSS software (version 17, Chicago, Illinois, USA). Statistical analysis (ANOVA) was used to define the differences ($P<0.05$). Significant differences between the essential oils and microorganisms were determined by Tukey test.

2.3 Check board titer test

Amikacin was purchased from Sigma and dissolved in water. The dilutions were prepared in water in concentrations 64-0.0125 μg/ml and antimicrobial susceptibility testing was performed (CLSI, 2009).

Eight serial twofold dilutions of oils and amikacin were used. Fifty μl of each dilution of essential oil was added to the wells of 96-well plates in vertical orientation and 10 μl of amikacin dilution was added in horizontal orientation. 50 μl of *A. baumannii* reference strains NCTC 13304 suspensions (10^6 CFU/ well) was added to each well and incubated for 24 h. Fractional inhibitory concentrations (FICs) were calculated as the MIC of the combination of oil and amikacin divided by the MIC of oil or amikacin alone. The FIC index (FICI) was interpreted as a synergistic effect when it was $\leq$0.5, as additive or indifferent when it was >0.5-2 and as antagonistic when it was>2.0. The synergic effect is demonstrated graphically by applying the isobole method (Rosato *et al*., 2007).

3. Results and Discussion

3.1 Chemical composition of essential oils

The chemical compositions of different oils are reported in Table 1. Thymol (50.6%), p-cymene (27.2%) and γ-terpinene (18.6%) were the major components of *T. copticum* oil. Thymol, p-cymene, and γ-terpinene were reported as the main components of *T. copticum* oil by others (Rasooli *et al*., 2008; Khajeh *et al*., 2004; Mahboubi & Kazempour, 2011). P-cymene was the second main component of *T. copticum* oil from the study of Rasooli *et al.* (2008) while other studies (Khajeh *et al*., 2004; Mahboubi & Kazempour, 2011) have been reported γ-terpinene as the second main component of *T. copticum* oil. In this study, p-cymene was the second main component of *T. copticum* oil in spite of the results of our previous study (Mahboubi & Kazempour, 2011).

Eugenol (78.4%), eugenol acetate (10.1%) and β-caryophyllene (8%) were found as the main components of the *E. caryophyllata* oil. Eugenol was reported as the first major component of the *E. caryophyllata* oil (Fichi *et al*., 2007; Alma *et al*., 2007; Chaieb *et al*., 2007). Trans caryo-

Table 1. Chemical composition of essential oils by GC and GC-MS

Components	Retention Index	*T. ammi*	*E. caryophyllata*	*Z. tenuir*	*P. graveolens*	*O. decumbens*
α-Thujene	853	-	-	-	-	0.2
α-Pinene	866	0.3	-	3.1	0.512	0.4
Camphene	872	-	-	1.6	0.512	0.4
β-Pinene	896	1.0	-	1.6	-	-
β-Myrcene	919	-	-	2.1	-	0.3
α-Terpinene	941	-	-	0.25	-	-
p-Cymene	942	27.2	-	-	0.3	-
Limonene	951	-	-	0.7	0.1	0.84
Benzene methyl-1-methylethyl	950	-	-	8.8	-	-
1,8-Cineole	953	-	-	4.1	-	-
Sabinene	957	-	-	0.5	-	1.5
cis-Ocimene	973	-	-	2.8	-	-
γ-Terpinene	982	18.6	-	2.5	-	20.7
cis-β-Terpineol	991	-	-	2.7	-	-
α-Terpinolene	1005	-	-	0.2	1.4	0.1
cis-Rose Oxide	1022	-	-	-	1.5	-
Linalool	1025	-	-	3.8	-	-
Phenylethyl Alcohol	1035	-	-	2.4	-	-
Camphor	1043	-	-	1.3	-	-
trans-Menthan-3-one	1057	-	-	-	2.9	-
p-Menthone	1065	-	-	-	2.7	-
borneol	1077	-	-	2.1	-	-
4-Terpineol	1086	0.1	-	2.3	-	-
α-Terpineol	1115	0.1	-	19.5	-	-
1-Undecene	1116	-	1	-	-	-
Farnesol	1137	-	-	1.5	0.6	-
Isothymol methyl ether	1142	-	-	1	-	-
β-Citronellol	1174	-	-	-	39.3	-
Geraniol	1176	-	-	5.8	23.6	-
Nerol	1197	-	-	-	11.7	-
Carvacrol	1225	0.05	-	2.6	-	-
Thymol	1238	50.6	-	13.9	-	50.1
Eugenol	1242	-	78.4	-	-	-
Geranyl acetate	1298	-	-	-	0.4	-
trans-Caryophyllene	1319	-	-	3.4	0.7	-
β-Caryophyllene	1324	-	8	-	-	-
Citronellyl propionate	1339	-	-	-	1.2	-
α-Amorphene	1357	-	-	0.2	0.1	-
Alloaromadendrene	1357	-	-	0.1	0.1	-
Eugenol acetate	1375	-	10.1	-	-	-
Germacrene B	1375	-	-	0.7	0.3	-
Croweacin	1388	-	-	-	-	5.3
δ-Cadinene	1399	-	-	0.1	0.2	-
Spathulenol	1441	-	-	1.2	-	0.1
Carotol	1505	-	-	-	46	-
5,6-Dimethoxy-1-indanone	1513	-	-	-	46	-
Citronellyl n-butyrate	1527	-	-	-	2.6	-

phyllene was the second major component in one report (Fichi *et al*., 2007); while Eugenol acetate was reported as the second main components by others (Alma *et al*., 2007; Chaieb *et al*., 2007).

Z. tenuir oil had α-terpineol (19.5%), thymol (13.9%), p-cymene (8.8%) and geraniol (5.8%) as the major chemical components. There are two different chemotypes for *Z. tenuir* oil, including pulegone chemotype (Sezik *et al*., 1991)

and α-terpineole- thymol chemotype (Mahboubi *et al*., 2012).

B-citronellol (39.3%), geraniol (23.6%) and nerol (11.7%) were in *P. graveolens* oil as the main components. Citronellol (45%) and geraniol (10-12%) were reported as two main components of *P. graveolens* oil (Guenther, 1982).

Thymol (50.1%), γ-terpinene (20.7%) and p-cymene (17.6%) were found in *O. decumbens* oil. Thymol, carvacrol, γ-terpinene and p-cymene were reported as the principal components of *O. decumbens* oil (Mahboubi *et al*., 2007; Amin *et al*., 2007). Thymol, p-cymene, and γ-terpinene were three major components of the oil in this study while carvacrol was not found as a component of *O. decumbens* oil.

Therefore, there are many varieties in chemical composition of oils that may affect their biological activities. Therefore, it is very important to determine the chemical composition of the oils before evaluation of their biological activities.

3.2 The antibacterial activity of oils against *A. baumannii*

The antibacterial activity of oils against clinical isolates of *A. baumannii* revealed that the antibacterial activity of essential oils increased dose dependently in the disc diffusion method. There were four subsets of essential oils according to their antibacterial activities in the disc diffusion method. They include *O. decumbens* and *T. copticum* oils, *Z. tenuir* oil, *E. caryophyllata* oil and *P. graveolens* oil. The MIC evaluation of different oils against clinical isolates of *A. baumannii,* showed 3 subsets of essential oils including *O. decumbens*, *E. caryophyllata* and *T. copticum* oils; *Z. tenuir* oil and *P. graveolens* oil, while the MBC values of oils revealed that *T. copticum* oil had the lowest MBC values against *A. baumannii* followed by *O. decumbens* and *E. caryophyllata* oils. *Z. tenuir* and *P. graveolens* oils had the highest MBC values for *A. baumannii* (Table 2). Hence the nature of the oils was different in solid or broth media, and it may be a result of the hydrophobic or hydrophilic nature of oils or components in these media. So, we have different subsets of antimicrobial oils according the method and medium.

Although there are many studies on the chemical compositions of these oils (Mahboubi *et al*., 2007; Amin *et al*., 2007; Alma *et al*., 2007; Chaieb *et al*., 2007), no study has evaluated the antibacterial activity of these oils against clinical isolates of *A. baumannii*. Furthermore, there are many studies that have evaluated the antimicrobial activity of other essential oils against *Acinetobacter* spp. These include *Abies alba* (Yang *et al.*, 2009), different chemotypes of basil (Koba *et al.*, 2009), tea tree oil (Sienkiewicz *et al.*, 2011), *Bixa orellana* (Tamil Selvi *et al*., 2011), *Artemisia dracunculus* (Jazani *et al*., 2011) and many others (Lysakowska *et al*., 2011; Mikaili *et al*., 2011). Only one study has evaluated the antibacterial activity of *P. graveolens* oil against *A. baumannii* ATCC 19606. In this study, Rosato and his colleagues (2010) reported the MIC value 0.5 mg/ml against *A. baumannii* ATCC 19606 (Rosato *et al*., 2010).

Our results have shown that the essential oils such as *O. decumbens*, *E. caryophyllata* and *T. copticum* oils possess the best antibacterial activity against clinical isolates of *A. baumannii.* Inspection of the chemical composition of these oils showed that the effective components against *A. baumannii* are phenolic compounds, including thymol and eugenol. There is one report that shows the antibacterial activity of thyme oil containing thymol (38.1%) as the main component against *Acinetobacter* spp. with MIC values in the ranges between 0.25-1 µl/ml (Lysakowska *et al.*, 2011). Thus, A. *baumannii* like other Gram negative bacteria is sensitive to thymol and other phenolic compounds. Thymol damages the cytoplasmic membrane and cell membrane and change the cellular uptake (Xu *et al*., 2008; Di Pasqua *et al*., 2010). Eugenol also increases the transport of potassium ions and ATP from the cells and its hydroxyl group binds to proteins and inhibits the activity of enzymes (Walsh *et al.,* 2003; Gill & Holley, 2004). Therefore, essential oils with phenolic compound can be used as an alternative treatment of *A. baumannii* infected patients or disinfectant for hospital environments.

3.3 Synergistic activity of essential oils with amikacin

The FICs for essential oils were 0.03 and 0.125 for *P. graveolens* and *E. caryophyllata* oils and were 0.25 for *T. copticum*, *Z. tenuir* and *O. decumbens* oils. The FICs for amikacin were 0.2, 0.025, 0.0125, 0.05 and 0.2 for *P. graveolens*, *E. caryophyllata*, *T. copticum*, *Z. tenuir* and *O. decumbens*

Table 2. The antimicrobial activity of oils against clinical isolates of *Acinetobacter baumannii.*

Essential oil	IZ (mm±SD)			Broth dilution		
	0.5(µl)	1(µl)	2(µl)	Subset*	MIC(µl/ml)	MBC(µl/ml)
P. graveolens	6.4±0.12	8.3±0.27	14.5±0.35	9.7 ± 0.35^{d}	1.1 ± 0.51^{c}	1.4 ± 0.75^{d}
T. copticum	12.9±0.29	18.0±0.45	25.9±0.45	18.9 ± 0.59^{a}	0.16 ± 0.06^{a}	0.25 ± 0.13^{a}
Z. tenuir	7.1±0.2	10.5±0.24	16.1±0.58	11.2 ± 0.4^{b}	0.7 ± 0.29^{b}	0.91 ± 0.54^{c}
O. decumbens	13.9±0.37	19.78±0.57	28.8±0.85	20.8 ± 0.6^{a}	0.23 ± 0.34^{a}	$0.31\pm0.38^{a,b}$
E. caryophyllata	9.7±0.2	13.3±0.21	20.3±0.47	14.4 ± 0.4^{c}	0.28 ± 0.11^{a}	0.49 ± 0.17^{b}

*Post hoc Duncan $^{a,\cdots}$ Anova test

Table 3. Fractional Inhibitory Concentration (FIC) and FIC indices (FICI)

Combinations	*P. graveolens*		*T. copticum*		*Z. tenuir*		*O. decumbens*		*E. caryophyllata*	
	FIC	FICI	FIC	FICI	FIC	FICI	FIC	FICI	FIC	FICI
Oil	0.03	*0.23*	0.25	*0.275*	0.25	*0.26*	0.25	*0.3*	0.125	*0.325*
amikacin	0.2		0.025		0.0125		0.05		0.2	

FIC of oil = MIC in combination with amikacin / MIC of oil alone; FIC of amikacin = MIC in combination with oil / MIC of amikacin alone

FICI = FIC of oil + FIC of amikacin

oils, respectively. The FICIs were 0.23, 0.275, 0.26, 0.3 and 0.325 for *P. graveolens*, *T. copticum*, *Z. tenuir*, *O. decumbens* and *E. caryophyllata* oils, respectively, and isobologram showed a convex diagram (Table 3, Figure 1). Rosato *et al.* (2010) evaluated the synergistic effect of gentamicin and *P. graveolens* oil with FICI 0.11 for *A. baumannii* ATCC 19606 (Rosato *et al.*, 2010). The synergistic activity of *Citrus limon* with amikacin (FICI 0.37) and gentamicin (FICI 0.5) (Guerra *et al.,* 2011); and of *Coriandrum sativum* with chloramphenicol (FICI 0.47), cefoperazone (FICI 0.75), ciprofloxacin (FICI 0.281), gentamicin (FICI 0.25) and tetracycline (FICI 0.312) for *A. baumannii* LMG 1041 (Duarte *et al*., 2012) have been described. The FICIs for *P. graveolens* and *Z. tenuir* oils were lower than that of *T. copticum* oil and followed by *O. decumbens* and *E. caryophyllata* oils. Thus, although the antibacterial activity of *P. graveolens* and *Z. tenuir* was weaker than the others, the synergistic activities with amikacin were higher than the stronger essential oils such as *T. copticum*, *O. decumbens* and *E. caryophyllata* oils. Therefore, according to FICI of these oils with amikacin and their isobologram curves, these oils showed a synergistic activity with amikacin and can be used as an alternative treatment with amikacin for lowering the duration of treatment or decreasing the dose of treatment.

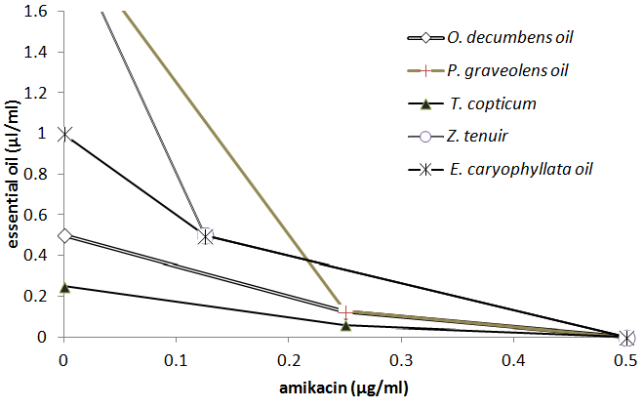

Figure 1. The isobologram curve of amikacin with essential oils against *A. baumannii*

4. Conclusion

The antibacterial activity of essential oils against 32 clinical isolates of *A. baumannii* showed that the oils with phenolic compounds showed the higher antibacterial activities. The *Oliveria decumbens*, *Pelargonium graveolens*, *Eugenia caryophyllata*, *Ziziphora tenuir* and *Trachyspermum copticum* oils were categorized into subsets, including 1: *O. decumbens*, *E. caryophyllata* and *T. copticum* oils 2: *Z. tenuir* oil and 3: *P. graveolens* oil. Indeed, *Pelargonium graveolens* oil has the lower activity than the others, but it has the lower FICI and higher synergistic activity with amikacin than the other oils.

However, due to high variability in chemical composition of essential oils from different regions, and the influence of chemical composition of oils on their antibacterial activity, it is important to standardize the essential oil before administrating it as an alternative treatment. As the limitation of essential oils for control of *Acinetobacter* related infections is administration route as intravenous injection. Taking any type of oil by intravenous injection can cause serious effects, including death; therefore, the essential oils can be used as vapor in environment of hospitals for control of these infections, because *Acinetobacter* related infections are primarily associated with hospital acquired infections.

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References

Adams, R.P. 2001. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy, Allured Publishing Corp., Carol Stream, Illinois, U.S.A.

Alma, M.H., Ertas, M., Nitz, S. and Kollmannsberger, H. 2007. Chemical composition and content of essential oil from the bud of cultivated Turkish clove (*Syzygium aromaticum* L.). BioResources. 2(2), 265-269.

Amin, G.H., Salehi Sourmaghi, M.H., Zahedi, M., Khanavi, M. and Samadi, N. 2005. Essential oil composition and antimicrobial activity of *Oliveria decumbens*. Fitoterapia. 76,704-7.

Chaieb, K., Zmantar, T., Ksouri, R., Hajlaoui, H., Mahdouani, K., Abdelly, C. and Bakhrouf, A. 2007. Antioxidant properties of the essential oil of *Eugenia caryophyllata* and its antifungal activity against a large number of clinical *Candida* species. Mycoses. 50, 403-406.

Costa, A.C., Santos, B.H., Filho, L.S. and Lima, E. 2009. Antibacterial activity of the essential oil of *Origanum vulgare* L. (Lamiaceae) against bacterial multi-resistant strains isolated from nosocomial patients. Brazilian Journal of Pharmacognosy. 19(1B), 236-241.

Damjanović-Vratnica, B., Đakov,T., Šukoviæ, D. and Damjanović, J. 2011. Antimicrobial Effect of Essential Oil Isolated from *Eucalyptus globulus* Labill from Montenegro. Czech Journal of Food Sciences. 29(3), 277-284.

Di Pasqua, R., Mamone, G., Ferranti, P., Ercolini, D. and Mauriello, G. 2010. Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sub lethal concentrations of thymol. Proteomics. 10, 1040-1049.

Duarte, A., Ferreira, S., Silva, F. and Domingues, F.C. 2012. Synergistic activity of coriander oil and conventional antibiotics against *Acinetobacter baumannii*. Phytomed. 19,236-238.

Fichi, G., Flamini, G., Giovanelli, F., Otranto, D. and Perrucci, S. 2007. The efficacy of an essential oil of *Eugenia caryophyllata* against *Psoroptes cuniculi*. Experimental Parasitology. 115,168-17.

Gill, A.O. and Holley, R.A. 2004. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *Listeria monocytogenes* and *Lactobacillus sakei*. Applied and Environmental Microbiology. 70, 5750-5755.

Guenther, E. 1982. The Essential oils. Robert E Krieger Publishing company Malabar, Florida, edition 5, Volume IV, p. 671.

Howard, A., O'Donoghue, M., Feeney, A. and Sleator, R.D. 2012. *Acinetobacter baumannii*. Virulence. 3(3), 243-250.

Jazani, N.H., Zartoshti, M., Babazadeh, H. and Ali-daiee, N. 2011. Antibacterial effects of *Artemisa dracunculus* essential oil on multi-drug resistant isolates of *Acinetobacter baumannii*. Journal of Bacteriology. 1,31-36.

Khajeh, M., Yamini, Y., Sefidkon, F. and Bahramifar, N. 2004. Comparison of essential oil composition of *Carum copticum* obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chemistry. 86, 587-591.

Koba, K., Poutouli, P.W., Raynaud, C., Chaumont, J.P. and Sanda, K. 2009. Chemical composition and antimicrobial properties of different basil essential oils chemotypes from Togo. Bangladesh Journal of Pharmacology. 4, 1-8.

Łysakowska, M., Denys, A. and Sienkiewicz, M. 2011. The activity of thyme essential oil against *Acinetobacter* spp. Central European Journal of Biology. 6 (3), 405-413.

Mahboubi, M. and Kazempour, N. 2011. Chemical composition and antimicrobial activity of *Satureja hortensis* and *Trachyspermum copticum* essential oil. Iranian Journal of Microbiology. 3 (4), 194-200.

Mahboubi, M., Mohammadi Yeganeh, S., Bokaee , S., Dehdashti, H. and Feizabadi, M.M. 2007. Antimicrobial activity of essential oil from *Oliveria decumbens* and its synergy with vancomycin against *Staphylococcus aureus.* Herba Polonica Journal, 53(4), 69-76.

Mahboubi, M., Kazempour, N. and Hosseini, H. 2012. Chemical composition, antimicrobial activity of essential oil from *Ziziphora tenuir* L aerial. Journal of essential oil-bearing plants. 15(4), 545-549.

Mikaili, P., Jazani, N.H., Shayegh, J., Haghighi, N., Aghamohammadi, N. and Zartoshti, M., 2011. The aerial parts of *Stachys schtschegleevii* Sosn as hydroalcoholic extract has antibacterial activity on multi-drug resistant bacterial isolates in comparison to ciprofloxacin. Journal of American Science. 7(8), 694-699.

NCCLS. 2009. Methods For dilution Antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A8, Eighth Edition, and Wayne, Pennsylvania.

NCCLS. 2012. Performance Standards for Antimicrobial Disc Susceptibility Test. Tentative standard, M02-A11 Vol. 32. Eleventh Edition, Wayne, Pennsylvania, U.S.A.

Peleg, A.Y., Seifert, Ç. and Paterson, D.L. 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clinical Microbiology Reviews. 21, 538-582.

Rasooli, I., Fakoor, M.H., Yadegarinia, D., Gachkar, L., Allameh, A. and Rezaei, M.B. 2008. Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils. International Journal of Food Microbiology. 122,135-139.

Rosato, A., Vitali, C., De Laurentis, N., Armenise, D. and Milillo, M.A. 2007. The antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. Phytomed. 14, 727-732.

Rosato, A., Piarulli, M., Corbo, F., Muraglia, M., Carone, A., Vitali, M.E. and Vitali, C. 2010. *In Vitro* synergistic action of certain combinations of gentamicin and essential oils. Current Medicinal Chemistry. 17, 3289-3295.

Salman, M.T., Khan, R.A. and Shukla, I. 2008. Antimicrobial activity of *Nigella sativa* Linn. seed oil against multidrug resistant bacteria from clinical isolates. Natural Product Radiance. 7(1), 10-14.

Sezik, E., Tümen, G. and Baser, K.H.C. 1991. *Ziziphora tenuir* L., a new source of pulegone. Flavour and Fragrance Journal. 6(1), 101-103.

Sienkiewicz, M., Denys, P. and Kowalczyk, E. 2011. Antibacterial and immunostimulatory effect of essential oils. International Review of Allergology and Clinical Immunology. 17(1-2), 40-44.

Sunenshine, R.H., Wright, M.O., Maragakis, L.L., Haris, A.D., Song, X., Hebden, J., Cosgrove, S.E., Anderson, A., Carnell, J., Jernigan, D.B., Kleinbaum, D.G., Perl, T.M., Standiford, H.C. and Srinivasan, A. 2007. Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. Emerging Infectious Diseases. 13, 97-103.

Tamil Selvi, A., Dinesh, M.G., Satyan, R.S., Chandrasekaran, B. and Rose, C. 2011. Leaf and Seed extracts of *Bixa orellana* L. exert anti-microbial activity against bacterial pathogens. Journal of Applied Pharmaceutical Science. 1(9), 116-120.

Tan, H.T., Rahman, R.A., Gan, S.H., Halim, A.S., Hassan, S.H., Sulaiman, S.A. and Kirnpal-Kaur, B.S., 2009. The antibacterial properties of Malaysian Tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complementary and Alternative Medicine. 9, 34-42.

Walsh, S.E., Maillard, J.Y., Russell, A.D., Catrenich, C.E., Charbonneau, D.L. and Bartolo, R.G. 2003. Activity and mechanisms of action of selected biocidal agents on Gram- positive and-negative bacteria. Journal of Applied Microbiology. 94, 240-247.

Xu, J., Zhou, F., Ji, B.P., Pei, R.S. and Xu, N. 2008. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Letters in Applied Microbiology. 47, 174-179.

Yang, S.A., Jeon, S.K., Lee, E.J., Im, N.K., Jhee, K.H., Lee, S.P. and Lee, I.S. 2009. Radical scavenging activity of the essential oil of Silver Fir (*Abies alba*). Journal of Clinical Biochemistry and Nutrition. 44, 253-259.