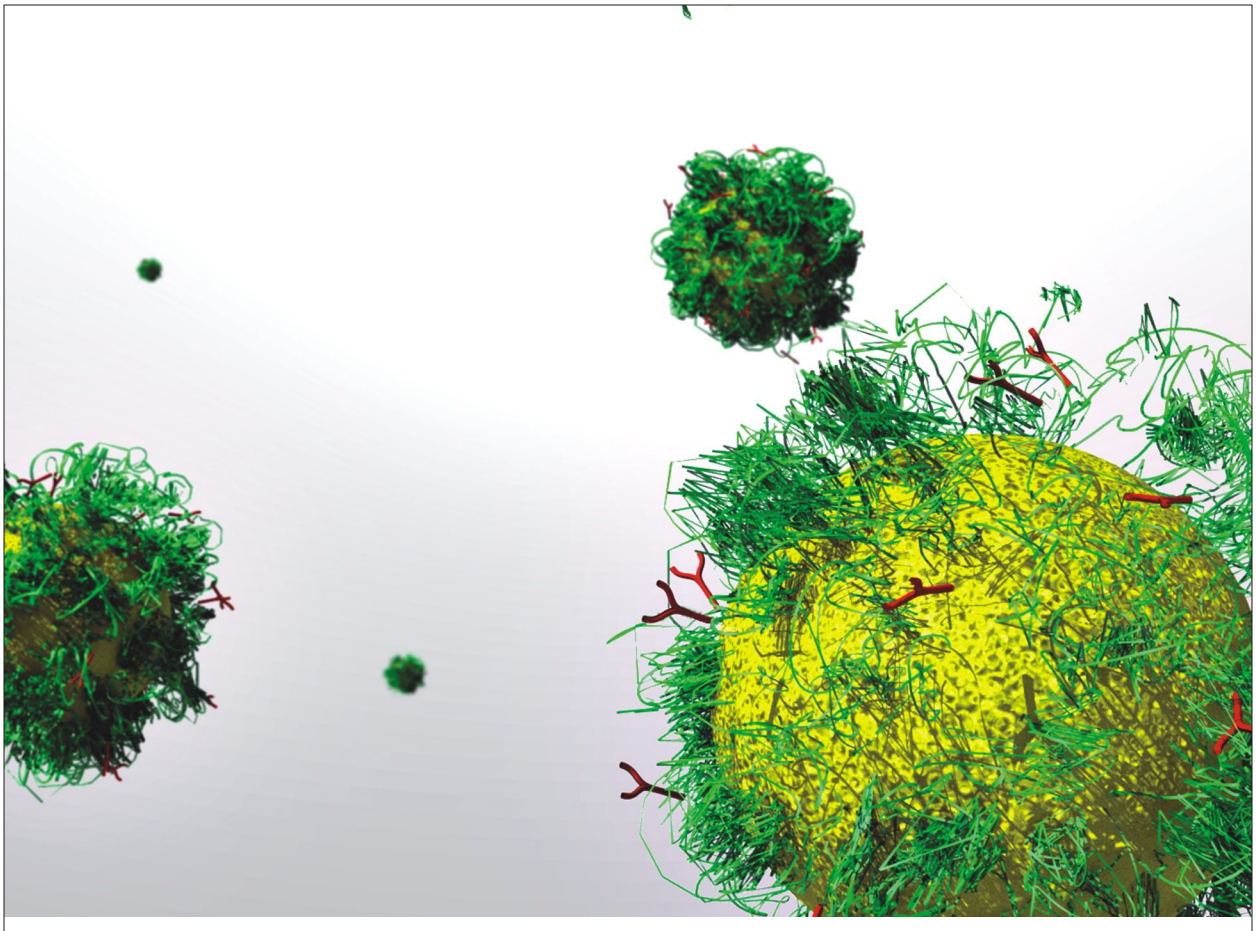

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In vitro Antimicrobial Activities of Some Egyptian Plants' Essential Oils with Medicinal Applications

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ABSTRACT

The resistance of microorganisms issue to conventional antibiotics has necessitated the search for efficient and cost effective ways for treating infectious diseases. Plant essential oils are potential sources of novel antimicrobial compounds. In addition, the antimicrobial susceptibility testing provides an information aids in selecting and developing the appropriate antimicrobial agent. In the current study, the chemical compositions of the essential oils of *Nigella sativa*, *Menthe piperita* and *Pelargonium graveolens* (Geranium oil) collected in Egypt were characterized by GC-. In addition, the *in vitro* antimicrobial activity of these essential oils were tested, using agar diffusion method, against different eleven pathogenic microbial species including three Gram-positive, six Gram-negative and two fungi; a yeast like *Candida albicans* and a filamentous like *Aspergillus niger*. Inhibition zones showed that the essential oils of the two plants were active against all Gram-positive studied bacteria and fungi. The susceptibility of the strains changed with the dilution of essential oils in DMSO. The pure essential oils showed the most wide inhibition zones and they were very effective compared to standard drugs ciprofloxacin and/or nystatin. However, the activity against Gram-negative bacteria varied. Thus, this study indicates that the essential oils of *Nigella sativa*, *Menthe piperita* and *Pelargonium graveolens* have antimicrobial activity against different species of human pathogenic microorganisms and research should continue to examine the activity in experimental animals.

Key words: *Menthe piperita* leaves oil, *Pelargonium graveolens* leaves (Geranium oil), *Nigella sativa* seeds oil, antimicrobial activity, medicinal plants, essential oils

INTRODUCTION

Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses and fungi. These diseases are the important cause of morbidity and mortality in immune compromised patients in developing countries (Mallam *et al*., 2012). An anti-microbial is a substance that kills (microbiocidal) or inhibits the growth (microbiostatic) of microorganisms, such as bacteria and fungi (Jagtap *et al*., 2012). The antimicrobial substances of plant origin have enormous potential uses as alternative remedies for the treatment of many infectious diseases (Prabuseenivasan *et al*., 2006; Jagtap *et al*., 2012). In addition, they are effective in treating these diseases without the diverse side effects which are often associated with synthetic antimicrobials (Prabuseenivasan *et al*., 2006). Furthermore, in the past two decades, the emergence of resistant pathogens to various antibacterial and antifungal drugs is one of the most serious threats to successful treatment of microbial diseases.

Recently, azole-resistant *Candida* and *Aspergillus* species are the top pathogens responsible for nosocomial or food-borne infections. Thus, plants' essential oils and extracts have evoked interest as sources of natural antimicrobial products (Prabuseenivasan *et al*., 2006; Saharkhiz *et al*., 2012). Although, the antimicrobial activities of plants' extracts are mainly attributed to some of the compounds such as terpens, essential oils, coumarines and flavonoids (Calvo *et al*., 2012). The exact mechanism of these properties is not well known, however it has been proved that their bacteriostatic or bacteriocide activity is due to the loss of control and integrity of bacterial cell wall (Calvo *et al*., 2012).

Mentha piperita (family Lamiaceae) is a species found in many parts of the world which has an economical value and therapeutic properties (Saharkhiz *et al*., 2012). *M. piperita* leaves contains about 0.5-4% volatile oil that is composed of 50-78% free menthol, monoterpene, menthofurane and traces of jasmine (0.15%) to improve the oils quality remarkably (Bupesh *et al*., 2007; Kizil *et al*., 2010). In addition, peppermint (*M. piperita* oil) is one of the most popular and widely used essential oils, because of its main components, menthol and menthone (Derwich *et al*., 2010; Kizil *et al*., 2010). Notably, in many earlier studies, *M. piperita* essential oil has shown antiviral, antibacterial, antifungal. In addition to inhibition of biofilms formation which protects the microbial cells within biofilms from the host immune defenses and increase the drug resistance (Sokovic *et al*., 2009; Saharkhiz *et al*., 2012).

The Pelargonium (Geraniaceae family) genus has been found to possess significant pharmacological and biological activities, including antioxidant, anti-neuroinflammatory, antiinfluenza, anticancer, antimicrobial and antifungal activity. *Pelargonium graveolens* is the most important species of its genus used in several foods, remedies and cosmeceuticals (Ghannadi *et al*., 2012). *Pelargonium*, *Pelargonium* distillates and absolutes, commonly known as "geranium oil", are sold for aromatherapy and massage therapy applications. In addition, they are also sometimes used to supplement or adulterate more expensive rose oils. Analyses of Indian geranium oils showed that the major constituents, in term of % composition, were citronellol, nerol and geraniol (Rana *et al*., 2012). In addition, Ghannadi *et al*. (2012) study has found that the main constituents of *P. graveolens* volatile oil were B-citronellol (36.4%) and citronellyl formate (12.1%). Ghannadi *et al*. (2012) and Ben Hsouna and Hamdi (2012) study have proved that *P. graveolens* essential oil have a potential as antimicrobial agent and natural preservative in different products (Ghannadi *et al*., 2012; Ben Hsouna and Hamdi, 2012).

Seeds of *Nigella sativa* L. (Ranunculaceae), known commonly as "black cumin" have been used for thousands years as a spice and food preservative (Haloci *et al*., 2012). The seed extracts and essential oil have shown potential medicinal properties including: Immune stimulation, antioxidant, anti-inflammatory, anticancer (Aljabre *et al*., 2005; Haloci *et al*., 2012). In addition to antibacterial and antifungal activities have been confirmed by many research groups (Ara *et al*., 2005; Aljabre *et al*., 2005; Salman *et al*., 2008; Haloci *et al*., 2012; Shohayeb and Halawani, 2012; Hasan *et al*., 2013). These pharmacological activities are attributed to many active components have been isolated from N. sativa including: Thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, nigellimine-x-oxide and alpha-hederin (Aljabre *et al*., 2005; Haloci *et al*., 2012). A recent acute and chronic toxicity studies have confirmed the safety of *N. sativa* oil and its most abundant active component, thymoquinone, particularly when given orally (Randhawa and Alghamdi, 2011).

Essential oils especially with known antimicrobial effects have the potential to be used in food industry as preservatives and to increase the shelf life of products. Therefore, determining the antimicrobial properties of essential oils might help to overcome microbial resistance to antibiotics and antifungals and prevent food spoilage (Burt, 2004; Saharkhiz *et al*., 2012). In addition, many

of infectious microorganisms are resistant to synthetic drugs, hence an alternative therapy is very much needed (Mallam *et al*., 2012). Thus, the present study is aimed to investigate the chemical composition and *in vitro* antibacterial and antifungal activities of the essential oils of *M. piperita*, *Pelargonium graveolens* and *Nigella sativa* collected in Egypt on some common bacterial and fungal pathogens.

MATERIALS AND METHODS

Collection and preparation of samples: Samples were collected from Cairo University Farm, Cairo, Egypt. Apparently healthy plants were collected, washed thoroughly in tap water and dried at dark room temperature for 15 days. The leaves was powdered and extracted by hydro-distillation following the previous published procedures with N. Sativa seeds oil extract slight modification as standardized in Department of Medicinal Plant, NRC, Egypt by Dr. S. Mahfouz (Eweis and Gad, 2011; Eweis *et al*., 2012).

Identification of its constituents: Conditions of essential oil on GC Instrument Gies-agilent technolo.6890 n network GC system USA. Oven initial temp. 70°C, initial time,1 min, rate c min^{-1} 4, final temp. 190°C, final time 13 min. intel temp. 250°C, detector temp. 280°C (FID) flame ionization detector flow 2 mL min^{-1}, carrier gas n230 mL min^{-1}, H2, 30 mL min^{-1}, air, 300 mL min^{-1}, capillary column HP-5 (5% phenyl methy siloxane) length 30 m, diameter 320 μm film thickness, 0.25 μm NRC-Dokki-Egypt.

Test organisms: Identified pure culture of test organisms representing different species were obtained by sub-culture from glycerol stock cultures from the Department of Microbiology and Immunology Department, Faculty of Pharmacy, Al-Azhar University. The bacteria studied, standard strains and clinical isolates, included three Gram-positive: *Bacillus cereus*, *Staphylococcus epedrmidis* and *Staphylococcus aureus* (ATCC 6538), six Gram-negative: *Escherichia coli* ATCC (8739), *Salmonella Typhi, Klebsiella pneumonia, Psedumonas aeruginosa* (ATCC 27853), *Proteus mirabilis* and *Shigella flexneri*. The clinical isolates were resistant to several antibiotics. In addition, two fungi, one yeast like and one filamentous, were used in this assay *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404), respectively. The bacteria were stored by culturing on nutrient agar slants; however fungi were cultured on Sabouraud dextrose agar.

Assay for antimicrobial activity: The antimicrobial activities of the extracted essential oils were systematically performed against 11 different bacterial and fungal species by agar cup plate diffusion method based on the methodology used by Bauer *et al*. (1966) with some modifications. The sterile Müeller-Hinton agar (Oxoid Ltd, UK) (for bacteria) or Sabouraud dextrose agar (for fungi) was poured into sterile Petri plates aseptically and allowed to solidify at room temperature. Both media were poured into Petri plates on a horizontal surface to give a uniform depth of approximately. All Petri plates were flooded with 400 μL mL^{-1} of the microbial suspension in sterilized saline equivalent to McFarland 0.5 standard solution (1.5×10^{8} CFU mL^{-1}) and uniformly distributed by sterile glass rod, then allowed to dry for 20 min with lid in place. A loop full of fresh bacterium suspended in sterilized distilled water and its optical density adjusted to OD = 0.13 using spectrophotometer at 600 nm wavelength. In each plate, 6 wells of 6 mm diameter were made with a sterile borer and the agar plugs were taken out carefully so as not disturb the surrounding medium. In addition, a disc of standard drug ciprofloxacin (5 μg $disc^{-1}$) served as reference standard for bacteria and nystatin (30 μg $disc^{-1}$) for fungi. Precisely 100 μL of

the test solution was added to the cups aseptically and labeled accordingly as well as DMSO. Test activities of *Menthe piperita*, *Pelargonium graveolens* (Geranium) and *Nigella sativa* essential oils were carried out by agar diffusion technique, in particular, cup plate method. The antibacterial solutions are the crude oil and different dilutions (1:1, 1:5, 1:10 and 1:20) in Dimethyl sulfoxide (DMSO). After holding the plates at room temperature for 1 h to allow diffusion of test samples into the agar, the plates were incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for fungi. The diameter of the clear zone of inhibition surrounding each well was measured twice at right angles and the average of the two readings was recorded to the nearest mm. The inhibition effects were compared with that of standard drug ciprofloxacin (5 μg $disc^{-1}$) for bacteria and nystatin (30 μg $disc^{-1}$) for fungi. Negative controls were set up with equivalent quantities of DMSO. All the tests were performed in triplicate. Values are presented as means of three parallel measurements. The zone of inhibition above 7 mm in diameter was taken as positive result.

RESULTS

The chemical compositions of the essential oils of *Nigella sativa* oil, *Pelargonium graveolens* (Geranium oil) and *Menthe piperita* oil were characterized using GC analyses, listed in Table 1, 2 and 3, respectively, according to their elution order. In addition, the antimicrobial

Table 1: Main chemical constituents of *Nigella sativa* essential oil

Compound	Retention index	Peak area (%)
α-Pinine	940	0.30
B-Myrecene	1002	0.59
Limonene	1083	39.58
ϒ-Terpinene	1087	1.96
Nonianal	1110	0.48
2-(z)-nonen-1-al	1144	2.15
Cumin aldehyde	1238	3.74
Myertenyl acetate	1334	0.69
α-Neoclovine	1463	5.49
α-cadenine	1576	6.65

Table 2: The main chemical constituents of *Pelargonium graveolens* (Geranium) oil

Compound	Retention index	Peak area (%)
α-Pinene	940	5.95
Myrecene	984	0.99
(E)-B-Limonene	1029	36.13
Ocimene	1048	10.44
Cis-Linalool	1058	9.84
Trans linalool oxide	1077	2.27
Trans rose oxide	1112	1.02
D-menthone	1131	1.92
Isomenthone	1152	1.22
Terpinen -4-ol	1167	1.68
α-Terpineol	1186	0.79
Nerol	1227	0.89
Citonellol	1235	0.94
Geraniol	1249	1.80
Citronelllyl formate	1270	4.14
Geranyl formate	1285	2.13
B-phenylethyl acetate	1302	0.30

Table 3: The main chemical constituents of *Mentha piperitta* essential oil

Compound	Retention index	Peak area (%)
Camphene	946	19.62
Sabinene	975	12.26
Limonene	1030	40.64
1, 8-cineol	1041	2.86
α-terpinene	1055	1.06
z-sabinene hydrate	1071	0.16
Terpinolene	1081	4.23
Limonene oxide	1131	0.33
B-Terpineol	1152	0.46
L-Menthone	1172	0.56
Menthol	1186	0.41
α-Terpineol	1199	0.09
Trans-dihdrocarvone	1214	0.11
Cis-Carveol	1237	0.08
Pulegone	1247	0.04
Menthyl acetate	1275	0.31

Table 4: Antimicrobial activity profile of the extracted essential oils by agar well diffusion method

	Zone of inhibition[1] of oil 1					Zone of inhibition[1] of oil 2					Zone of inhibition[1] of oil 3						
Micro-organism	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	Zone of inhibition[1] of Ciprofloxacin (5 µg mL^{-1})	Zone of inhibition[1] of DMSO
Gram-positive																	
B. cereus	29	27	21	18	16	28	26	18	16	-	25	18	12	10	-	25	-
S. aureus	22	18	12	-	-	25	19	16	10	-	28	15	10	-	-	26	-
S. epidermidis	25	16	-	-	-	26	21	15	8	-	28	23	15	11	-	26	
Gram-negative																	
E. coli	-	-	-	-	-	25	21	15	12	-	25	17	-	-	-	27	-
K. pneumonia	-	-	-	-	-	23	16	12	-	-	22	22	15	-	-	23	-
S. typhi	25	18	12	8	-	28	21	14	-	-	23	16	-	-	-	28	-
S. flexneri	-	-	-	-	-	22	16	13	-	-	-	-	-	-	-	29	-
P. aeruginosa	-	-	-	-	-	21	14	-	-	-	17	-	-	-	-	22	-
P. mirabilis	24	16	-	-	-	-	-	-	-	-	26	18	-	-	-	28	-
Fungal strains																Zone of inhibition of Nystatin 30 µg disc^{-1}	
A. niger	13	-	-	-	-	16	12	9	-	-	11	-	-	-	-	16	-
C. albicans	12	8	-	-	-	15	10	-	-	-	12	-	-	-	-	18	-

[1]zone size, mm; oil 1, stands for *Menthe piperitta* oil; oil 2, stands for *Pelargonium graveolens* (geranium oil); oil 3, stands for *Nigella sativa* oil, a, crude oil; b, c, d, e, 1:1, 1:5, 1:10, 1:20 dilutions in DMSO, respectively; [1] average diameter in mm of three measures in inhibition zone; "-" indicates no inhibition

activity, compared with standard drug ciprofloxacin (5 µg disc^{-1}), was assessed against three Gram-positive bacteria, six Gram-negative bacteria. In addition, the antifungal activity of the oils, compared with standard drug nystatin (30 µg disc^{-1}) was assessed against two fungi. The zone of inhibition above 7 mm in diameter was considered as positive result. Presence or absence of growth inhibition zones and zones diameter produced by the three essential oils are summarized in Table 4. Generally, the results revealed that the selected essential oils showed good antibacterial

and antifungal activities against the specific organisms tested with varying magnitudes. Most of the tested microorganisms were sensitive to the three essential oils, especially Gram-positive bacteria and fungi; however, some Gram-negative bacteria were sensitive to one or two of the tested oils. The three essential oils showed strong antimicrobial activity against *C. albicans* and *A. niger* when compared to standard drug nystatin (30 μg $disc^{-1}$). In addition, the size of zone of inhibition indicates increasing in the antimicrobial activity with increasing in concentration of oils as evident by the size of zone of inhibition of crude oil compared with the diluted ones in DMSO. The *Pelargonium graveolens* (Geranium oil) showed maximum activity against tested organisms compared with the other two. There was no inhibition of growth with the negative control DMSO.

DISCUSSION

Infectious diseases, also called as communicable diseases, are disorders caused by pathogenic microorganisms like bacteria, viruses and fungi, however the highest percentage are commonly caused by bacteria (Khosravi *et al.*, 2007; Mallam *et al.*, 2012). These illnesses were found to be the most significant cause of morbidity and mortality in developing countries, especially in immune compromised patients. Importantly, many of these infectious microorganisms are resistant to synthetic drugs, in addition to the side effects of synthetic antibiotics, thus medicinal plants are gaining attractiveness over these drugs as an alternative therapy (Al-Bari *et al.*, 2006). Indeed, the results of different studies have provided evidences that some medicinal plants might be potential sources of new antimicrobial and/or antifungal agents (Kone *et al.*, 2004; Mallam *et al.*, 2012). Above all, the antimicrobial properties of the essential oils and their components are established and exploited in such diverse commercial products as antiseptics and preservatives (Burt, 2004). Thus it is required to investigate those plants which have been used in traditional medicine to improve the quality of healthcare (Prabuseenivasan *et al.*, 2006).

The chemical compositions of the essential oils of *Nigella sativa* oil, *Pelargonium graveolens* (Geranium oil) and *Menthe piperita* oil, characterized using GC- analyses, showed variations in comparison to compositions of oils collected from other countries. These differences may be attributed to climate conditions and types and methods of distillation (Ghannadi *et al.*, 2012).

The *in vitro* antimicrobial activity of the three essential oils was carried out in this study by cup plate diffusion method which is mostly used for investigation of antimicrobial activity of essential oils. There is no a standard methodology to evaluate the inhibitory activity of plant extracts, thus most of the methods are based on the ones used to evaluate the resistance and/or susceptibility to antibiotics (Calvo *et al.*, 2012). However, since the outcome of this test can be affected by factors such as the method used to extract the essential oil from plant material, the volume of inoculum, growth phase, culture medium used, pH of the media and incubation time and temperature, comparison of published data is complicated (Friedman *et al.*, 2002; Burt, 2004).

In vitro studies in this study showed that the essential oils of *Nigella sativa, Menthe piperita* and *Pelargonium graveolens* inhibited both the fungal and bacterial growth although their effectiveness varied. This is consistent with the finding that antimicrobial activity of many essential oils were previously investigated and classified as strong, medium or weak (Zaika, 1988). In addition, it was found that the three essential oils exhibited better inhibitory effects and/or greater zone of inhibition to *S. aureus, S. epidermidis* and *B. cereus* species (Gram-positive) in comparison

to Gram-negative ones. This finding is consistent with Al-Bayati (2008) and Haloci *et al.* (2012) findings that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria. That could be explained that tolerance of Gram negative bacteria to essential oils is due to the presence of a hydrophilic outer membrane that obstruct the penetration of hydrophobic essential oils into target cell membrane (Al-Bayati, 2008). The essential oil of *Menthe piperita* showed an inhibitory effect on the growth of *S. aureus*, *S. epidermidis*, *B. cereus*, *S. typhi* and *P. mirabilis*. The essential oil of *Pelargonium graveolens* inhibited the growth of *S. aureus*, *S. epidermidis*, *B. cereus*, *S. typhi*, *K. pneumonia*, *E. coli*, *P. aeruginosa* and *S. flexneri*. The essential oil of *Nigella sativa* showed growth inhibition of *S. aureus*, *S. epidermidis*, *B. cereus*, *S. typhi*, *K. pneumonia*, *E. coli* and *P. mirabilis*. In addition, the three essential oils showed good inhibition of the growth of the two tested fungi *C. albicans* and *A. niger*. In addition to the crude oil, four different dilutions 1:1, 1:5,1:10,1:120 were used against the tested microorganisms. Among them the crude oil and 1:1 dilution showed more inhibition to the growth of all organisms; indicates increasing in the antimicrobial activity with increasing in concentrations of oil constituents. This finding is consistent with results of Ara *et al.* (2005) study.

CONCLUSION

In the present study we report the main chemical compositions of the Egyptian essential oils of *Nigella sativa, Menthe piperita* and *Pelargonium graveolens*. In addition, this study concluded that the essential oils of these traditional medicinal plants have antimicrobial activity against different species of pathogenic microorganisms. Thus, our results are a preliminary scientific validation for the use of these essential oils for antimicrobial activity against human pathogens. It is essential that research should continue to isolate and purify the active components of these oils and testing in experimental animals is warranted.

REFERENCES

Al-Bari, M.A., M.A. Sayeed, M.S. Rahman and M.A. Mossadik, 2006. Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis* a novel specis collected in Bangladesh. Res. J. Med. Sci., 1: 77-81.

Al-Bayati, F.A., 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J. Ethnopharmacol., 116: 403-406.

Aljabre, S.H.M., M.A. Randhawa, N. Akhtar, O.M. Alakloby, A.M. Alqurashi and A. Aldossary, 2005. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. J. Ethnopharmacol., 101: 116-119.

Ara, N., S.A.R. Choudhury and R. Amin, 2005. *In vitro* antimicrobial activity of the volatile oil of *Nigella sativa* Linn seeds. J. Teach. Assoc., 18: 109-112.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Ben Hsouna, A. and N. Hamdi, 2012. Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from *Pelargonium graveolens* growing in Tunisia. Lipids Health Dis., Vol. 11. 10.1186/1476-511X-11-167

Bupesh, G., C. Amutha, S. Nandagopal, A. Ganeshkumar, P. Sureshkumar and K. Saravana, 2007. Antibacterial activity of *Mentha piperita* L. (peppermint) from leaf extracts: A medicinal plant. Acta Agriculturae Slovenica, 89: 73-79.

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol., 94: 223-253.

Calvo, M.A., E.L. Arosemena, C. Shiva and C. Adelantado, 2012. Antimicrobial Activity of Plant Natural Extracts and Essential Oils. In: Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, Mendez-Vilas, A. (Ed.). Formatex Research Center, Spain, pp: 1179-1185.

Derwich, E., Z. Benziane, R. Taouil, O. Senhaji and M. Touzani, 2010. Aromatic plants of Morocco: GC/MS analysis of the essential oils of leaves of *Mentha piperita*. Adv. Environ. Biol., 4: 80-85.

Eweis, M. and A.S. Gad, 2011. Control of *Beta vulgaris* pathogens using *Lantana camara* Linn. essential oil *in vitro*. Int. J. Bot., 7: 289-294.

Eweis, M., A.A. Immhemmed and Amber S. Gad, 2012. Influence of *Thymus serpyllum* essential oil on Aspergillus parasiticus morphology and aflatoxins production. Res. J. Pharm. Biol. Chem. Sci., 3: 322-332.

Friedman, M., P.R. Henika and R.E. Mandrell, 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica*. J. Food Prot., 65: 1545-1560.

Ghannadi, A., M.R. Bagherinejad, D. Abedi, M. Jalali, B. Absalan and N. Sadeghi, 2012. Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L. Her and Vitex agnus-castus L. Iran. J. Microbiol., 4: 171-176.

Haloci, E., S. Manfredini, V. Toska, S. Vertuani, P. Ziosi, I. Topi and H. Kolani, 2012. Antibacterial and antifungal activity assesment of *Nigella sativa* essential oils. World Acad. Sci. Eng. Technol., 66: 1198-1200.

Hasan, N., M.Z. Nawahwi and H.A. Malek, 2013. Antimicrobial activity of *Nigella sativa* seed extract. Sains Malaysiana, 42: 143-147.

Jagtap, S.G., R.S. Shelar, N.M. Munto, M.R. Ghante and S.D. Sawant, 2012. Antimicrobial activity of *Ficus glomerata* Linn. bark. Int. Res. J. Pharm., 3: 281-284.

Khosravi, P., M.K. Jafarkhani, A.G. Nematzadeh and R.M. Bihamta, 2007. A protocol for mass production of *Rosa hybrid* cv. Iceberg through *in vitro* propagation. Iranian J. Biotechnol., 5: 100-104.

Kizil, S., N. Hasimi, V. Tolan, E. Kilinc and U. Yuksel, 2010. Mineral content essential oil components and biological activity of two *Mentha* species (*M. piperita* L., M. spicata L.). Turk. J. Field Crops, 15: 148-153.

Kone, M.W., K.A. Kamanzi, C. Terreaux, K. Hostettmann, D. Traore and M. Dosso, 2004. Traditional medicine in North: Screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacol., 93: 43-49.

Mallam, A., S. Angothu, S. Gurajala and G.A. Khuddus, 2012. Antimicrobial activity of *Sarcostemma acidum* Voigt. (Apocynaceae) Stem. Int. J. Biol. Pharm. Res., 3: 752-757.

Prabuseenivasan, S., M. Jayakumar and S. Ignacimuthu, 2006. *In vitro* antibacterial activity of some plant essential oils. BMC Complem. Alternative Med., 6: 39-39.

Rana, V.S., J.P. Juyal and M.A. Blazquez, 2012. Chemical constituents of essential oil of *Pelargonium graveolens* leaves. Int. J. Aromather., 12: 216-218.

Randhawa, M.A. and M.S. Alghamdi, 2011. Anticancer activity of *Nigella sativa* (black seed): A review. Am. J. Chin. Med., 39: 1075-1091.

Saharkhiz, M.J., M. Motamedi, K. Zomorodian, K. Pakshir, R. Miri and K. Hemyari, 2012. Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. Int. Scholarly Res. Network Pharmaceut. 10.5402/2012/718645

Salman, M.T., R.A. Khan and I. Shukla, 2008. Anti-microbial activity of *Nigella sativa* Linn: Seed oil against multi-drug resistant bacteria from clinical isolates. Nat. Prod. Radiance, 7: 10-14.

Shohayeb, M. and E. Halawani, 2012. Comparative antimicrobial activity of some active constituents of *N. sativa* L. World Applied Sci. J., 20: 182-189.

Sokovic, M.D., J. Vukojevic, P.D. Marin, D.D. Brkic, V. Vajs and L.J.L.D. van Griensven, 2009. Chemical composition of essential oils of *Thymus* and *Mentha* speciesand their antifungal activities. Molecules, 14: 238-249.

Zaika, L.L., 1988. Spices and herbs: Their antimicrobial activity and its determination. J. Food Saf., 23: 97-118.