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 (microbicidal) 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., 2003; 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 terpenes, 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 bactericide 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, monoterpen, 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 Hsoune 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 Hsoune 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, nigellicine-N-oxide, nigellicine, nigellidine, nigelmines-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. int 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 epidermidis and Staphylococcus aureus (ATCC 6538), six Gram-negative: Escherichia coli ATCC (8739), Salmonella Typhi, Klebsiella pneumonia, Psedumomas 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 (1.5×10⁶ 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>
<thead>
<tr>
<th>Compound</th>
<th>Retention index</th>
<th>Peak area (%)</th>
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<tbody>
<tr>
<td>α-Pinene</td>
<td>940</td>
<td>0.30</td>
</tr>
<tr>
<td>B-Myrcene</td>
<td>1002</td>
<td>0.59</td>
</tr>
<tr>
<td>Limonene</td>
<td>1083</td>
<td>39.58</td>
</tr>
<tr>
<td>α-Terpinene</td>
<td>1087</td>
<td>1.96</td>
</tr>
<tr>
<td>Nonanal</td>
<td>1110</td>
<td>0.48</td>
</tr>
<tr>
<td>2-(α)-nomen-1-al</td>
<td>1144</td>
<td>2.15</td>
</tr>
<tr>
<td>Cumin aldehyde</td>
<td>1238</td>
<td>3.74</td>
</tr>
<tr>
<td>Myristeryl acetate</td>
<td>1334</td>
<td>0.69</td>
</tr>
<tr>
<td>α-Neoclovine</td>
<td>1463</td>
<td>5.49</td>
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<tr>
<td>α-cadinen</td>
<td>1576</td>
<td>6.65</td>
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<thead>
<tr>
<th>Compound</th>
<th>Retention index</th>
<th>Peak area (%)</th>
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<tbody>
<tr>
<td>α-Pinene</td>
<td>940</td>
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</tr>
<tr>
<td>Myrcene</td>
<td>984</td>
<td>0.99</td>
</tr>
<tr>
<td>(E)-B-Limonene</td>
<td>1029</td>
<td>36.13</td>
</tr>
<tr>
<td>Ocimene</td>
<td>1048</td>
<td>10.44</td>
</tr>
<tr>
<td>Cis-Linalool</td>
<td>1058</td>
<td>9.84</td>
</tr>
<tr>
<td>Trans linalool oxide</td>
<td>1077</td>
<td>2.27</td>
</tr>
<tr>
<td>Trans rose oxide</td>
<td>1112</td>
<td>1.02</td>
</tr>
<tr>
<td>D-methene</td>
<td>1151</td>
<td>1.92</td>
</tr>
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<td>Isomenthone</td>
<td>1182</td>
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</tr>
<tr>
<td>Terpinen-4-oil</td>
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</tr>
<tr>
<td>α-Terpineol</td>
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<td>0.79</td>
</tr>
<tr>
<td>Nerol</td>
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<td>0.89</td>
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<td>Cotonellol</td>
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<td>Geranil</td>
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<tr>
<td>B-phenylethyl acetate</td>
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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 (50 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., 2005).

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


