Analysis On Chemical Composition Of Natural And Synthetic Essentials Oils Of Pelargonium Graveolens (Geranium) By GC-MS And Their Antimicrobial Activity Against Human Pathogenic Bacteria And Fungi

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ABSTRACT
The natural and synthetic essential oil from the plant Pelargonium graveolens were selected for anti bacterial and anti fungal activity on selected bacterial and fungal pathogens. The natural and synthetic oil was prepared separately with Dimethyl sulfoxide (DMSO) at 25µl, 50µl and 75µl concentration for the sensitivity test. The antimicrobial activity of plant essential oil was tested against clinical isolates. The results showed that natural Pelargonium graveolens oil showed very high effective activity than the synthetic oil at all the three different concentrations. GC/MS analysis was done to know the chemical comparison of Pelargonium graveolens natural and synthetic essential oils. The chemical compound geraniol present in Pelargonium graveolens natural oil may be showed high activity against the bacterial and fungal isolates.

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INTRODUCTION
Essential oils are volatile, natural, complex compounds that are produced by plants as secondary metabolites for protection against bacteria, viruses, fungi and pests (Rota et al., 2008). They also have an important role in dispersion of pollens and seeds by attracting some insects.

In Middle Ages essential oils were used for preservation of foods and as flavoring antimicrobial, analgesic, sedative, anti-inflammatory, spasmylytic and locally anesthetic remedies (Baydar et al., 2004; Demirci et al., 2008; Sagdi and Ozcan, 2003). But characterizing these properties in laboratory dated back to the early 1900s.

At present, about 3000 essential oils are known and 300 of them are used commercially in different industries such as pharmaceutical, agronomic, food, sanitary, cosmetic and perfume (Bakkali et al., 2008). Today, antioxidiant, antitumor and antiviral, antifungal and antibacterial activity of P. graveolens essential oils and their constituents are widely studied (Lee et al., 2012; Shanab et al., 2011; Zanetti et al., 2010; Crespo-ortiz and Wei, 2012; Campelo et al., 2011; Astani et al., 2010; Vukovic et al., 2007; Zeng et al., 2011; Furletti et al., 2011). In the other hand, Antimicrobial resistance (AMR) is now a global concern.

The Pelargonium (Geraniaceae) genus is represented by many essential oil producing species sinter alia: P. graveo lens, P. odoransissimum, P. zonaleand and P. roseum. Geranium oil is obtained from leaves, flowers and stalks by steam or hydro distillation. The therapeutic effects of the oil find application in the treatment of dysentery, diarrhoea, biliary conditions, gastric ulcers, diabetes, cancer and skin diseases. The main constituents responsible for biological activity are citronellol, geraniol, linalool, isomenthone, nerol and citronellylformate (Lis-Balchin and Geranium, 2004; Verma et al., 2011; Shal et al., 2006). Due to these components the essential oil from P. graveolens has a strong antibacterial and antifungal effect.

The overuse of antimicrobial chemotherapeutic agents, unfortunately typical of modern medicine, is evident and cannot be glossed over in silence.

Thus the search for effective and safe medicines that could be used to treat staphylococcal infections is on. We have decided to determine if the essential oil derived from Pelargonium graveolens and it has antimicrobial properties against clinical S. aureus and A. flavus isolates, what could make it an alternative or complementary to antibiotics therapy.

MATERIALS AND METHODS
Essential oils
The natural and synthetic essential oil geranium was purchased from Commercial center Aromax Trading Company, Chennai, Tamil Nadu (India). GC-MS technique was done to know the composition of Pelargonium graveolens natural and synthetic geranium essential oils.

Chemicals and microorganisms
All chemicals with the highest purity available and culture media were purchased from Himedia Mumbai, Maharashtra (India). Aspergillus niger, Aspergillus flavus, Candida albicans, Candida tropicalis, Candida kefyr, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli (Clinical isolates) was used as test organisms.

The bacteria and fungi were obtained from Microlabs Institute of Research and Technology Arcot, Tamil Nadu (India).

GC/MS analysis of Pelargonium graveolens, natural and synthetic essential oils
GC-MS was done at South Indian Textile Research
Association Coimbatore, Tamil Nadu (India) and analysis was carried out using a Hewlett-Packard 5890/5971A system fitted with a HP1 column (50 m × 0.20 mm fused silica capillary column; film thickness, 0.5 μm). GC oven initial temperature was 60°C and was programmed to 220°C at a rate of 2°C/min and 220°C during 120 min under the following operation conditions: vector gas, He; injector and detector temperatures, 250°C; injected volume: 0.2 μl, with a ratio split of 1/100. Retention indices were determined with Hexane standards as reference. The mass spectra were performed at 70 eV of the mass range of 35 – 400 amu. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial (Wiley, MassFinder 2.1 Library, NIST98) and home-made libraries and MS literature data (McLafferty and Stauffer, 1989; Adams, 1995; Joulain and König, 1998; Joulain et al., 2001).

**Determination of antimicrobial activity**

In this study standard agar well diffusion method was followed (Perez et al., 1990; Perez et al., 1999; Erdemoglu et al., 2003; Bagamboula et al., 2004). The fungal isolate was suspended in Potato Dextrose broth and the bacterial isolate in nutrient broth. The isolate was added to the broth. The fungal isolate was also added to sterile cork-borer, and 15 l of the samples solutions were delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Dyme thol sulfa oxide was used as solvent control. Amphotericin B was used as reference antifungal agent for molds, flucanazole was for yeast-like fungi and ciprofloxacin for bacteria. The tests were carried out in triplicate.

**Statistical analysis**

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at P < 0.05 using Duncan’s multiple range test (by SPSS software)Version 9.1.

**RESULTS**

**Chemical composition of natural and synthetic Pelargonium graveolens essential oils from GC-MS**

Chemical compositions of natural and synthetic Pelargonium graveolens essential oils are shown in (Tables 1 and 2). As seen, major components of natural and synthetic Pelargonium graveolens essential oil were: Geraniol (0.36%), Geranial formate (9.31%), farnesol (0.34%), trans-Geranion (0.41%), Citronellyl acetate (3.79%), Geranyl acetate (6.54%) respectively.

**Table 2 Chemical composition of synthetic Pelargonium graveolens oil**

<table>
<thead>
<tr>
<th>Compound Name</th>
<th>Retention time(min)</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geranyl acetate</td>
<td>9.21</td>
<td>6.94</td>
</tr>
<tr>
<td>Lavanduly acetate</td>
<td>11.33</td>
<td>0.73</td>
</tr>
<tr>
<td>Geranyl tiglate</td>
<td>16.69</td>
<td>3.96</td>
</tr>
<tr>
<td>Farnesyl acetate 3</td>
<td>19.02</td>
<td>0.38</td>
</tr>
<tr>
<td>Benzyln benzoate</td>
<td>18.08</td>
<td>5.53</td>
</tr>
<tr>
<td>Geranyl vinyl ether</td>
<td>21.93</td>
<td>0.19</td>
</tr>
<tr>
<td>2,4-Octadienoic acid</td>
<td>23.68</td>
<td>0.17</td>
</tr>
<tr>
<td>Citronellyl acetate</td>
<td>24.89</td>
<td>0.26</td>
</tr>
<tr>
<td>Guaiol</td>
<td>16.14</td>
<td>1.76</td>
</tr>
<tr>
<td>Neryl acetate</td>
<td>10.31</td>
<td>1.40</td>
</tr>
</tbody>
</table>

**In vitro antimicrobial activity**

The natural and synthetic geranium oil showed effective antimicrobial activity against the bacterial and fungal isolates. In agar well diffusion method the selected essential oils were effective against all forms such as yeast like fungi and molds and bacteria. (Table 3).

The Pelargonium graveolens natural oil 75μl mixed with 25μl of DMSO showed high activity against Escherichia coli (13.06±0.40) least against Bacillus subtilis (4.00±0.10) and in Pelargonium graveolens synthetic oil 75μl mixed with DMSO 25μl at showed higher activity in Klebsiella Pneumoniae (7.06±0.30) and low activity against Staphylococcus aureus (3.00±0.00). (Table 4) The Pelargonium graveolens natural oil 50μl mixed with DMSO at 50μl showed high activity against Staphylococcus aureus (12.50±0.50) least against Bacillus subtilis (9.00±0.30) and in Pelargonium graveolens synthetic oil 50μl mixed with DMSO 50μl at showed higher activity in Klebsiella Pneumoniae (8.00±0.30) and low activity against Staphylococcus aureus (3.96±0.05). (Table 5) The geranium natural oil 25μl mixed with DMSO at 75μl showed high activity against Bacillus cereus (11.00±0.50) and low activity against Bacillus subtilis (4.00±0.00) and in Pelargonium graveolens synthetic oil 25μl mixed with DMSO 75μl at showed higher activity in Klebsiella Pneumoniae (7.03±0.25) and low activity against Escherichia coli (4.00±0.00). (Table 6) The Pelargonium graveolens natural oil 75μl mixed with DMSO at 25μl showed high activity against Aspergillus niger (18.00±0.50) least against Aspergillus flavus (4.00±0.00) and in Pelargonium graveolens synthetic oil 75μl mixed with DMSO 25μl showed higher activity in Aspergillus niger (10.96±0.35) and low activity against Candida kefyr (6.00±0.20). (Table 7) The geranium natural oil 50μl mixed with DMSO at 50μl showed high activity against Candida kefyr (11.00±0.36) least against Candida albicans (6.00±0.10) and in geranium synthetic oil 50μl mixed with DMSO 50μl at showed higher activity in Aspergillus niger (11.10±0.36) and low activity against Candida tropicalis (4.00±0.00). (Table 8) The geranium natural oil 25μl mixed with DMSO at 75μl showed high activity against Aspergillus flavus (14.96±0.45) least against Candida kefyr (5.03±0.15) and in geranium synthetic oil 25μl mixed with DMSO 75μl at showed higher activity in Aspergillus flavus (10.96±0.45) and low activity against Candida kefyr (3.00±0.00).

All bacteria and fungi were found to be sensitive to all test essential oils and mostly comparable to the standard reference antifungal drug Amphotericin B, flucanazole and bacteria for ciprofloxacin to higher extent.
The essential oils of the plant were active against all tested strains. The activity is due to the high content of alcoholic compounds with antibacterial properties such as citronellol and geraniol, which account for over 40% of the ingredients of the geranium oil (Lis-balchin and Deans, 2007; Fabi et al., 2007).

The plant essential oil *Pelargonium graveolens* was also sensitive to the control antibiotics (Ciprofloxazin and Amphoterecin -B). The essential oils of the plant were active against all of the bacteria and fungi the susceptibility of the strains changed with the dilution of essential oils in DMSO. The pure and neat essential oils showed the most extensive inhibition zones and they were very effective antimicrobial agents for most of the microorganisms tested. High proportions of citronellol and caryophyllene oxide in our oils make them sensitive to the control antibiotics (Ciprofloxazin and Amphoterecin-B). The activity of the oils would be expected to relate to the composition of the plant essential oils and possible synergistic interaction between components. High proportions of β-citronellol and carophyllene oxide in our oils make them interesting and valuable subjects for food, medicine, aromatherapy and cosmetics industries where an antiseptic, clean and fresh characteristics flavor and fragrance is desired. The food protective and antimicrobial properties of β-citronellol as prominent part of *P. graveolens* volatile oil.
In our investigation it has been found that geranium essential oil is effective against bacterial and fungal strains with different mechanisms of drug resistance. Geranium oil can be applied not only in the treatment of dysentery, urinary tract and skin infections, but also in inflammation of the mouth, larynx, pharynx caused by bacterial and fungal pathogens. It can be used as an effective air disinfectant and as an additive to antiseptic preparations, and be used in the hospitals, nursing homes and clinics. The application of essential oils in the treatment of human diseases, particularly infectious diseases caused by multidrug resistant bacterial strains, may be an interesting alternative to synthetic drugs.

### Table 6 Antifungal activity of *Pelargonium graveolens* natural and synthetic oil

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Geranium natural oil</th>
<th>Geranium synthetic oil</th>
<th>Geranium natural Oil 75 µl &amp; DMSO25 µl</th>
<th>Geranium synthetic Oil 75 µl &amp; DMSO25 µl</th>
<th>Antifungal agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>12.00±0.50^a</td>
<td>6.00±0.10^b</td>
<td>18.00±0.50^c</td>
<td>10.96±0.35^d</td>
<td>10.00±0.20^e</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>9.03±0.25^c</td>
<td>5.00±0.10^d</td>
<td>4.00±0.00^e</td>
<td>7.03±0.15^f</td>
<td>(Amphotericin B) 11.03±0.25^g</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>6.96±0.35^f</td>
<td>5.03±0.05^g</td>
<td>4.00±0.10^h</td>
<td>6.03±0.15^i</td>
<td>12.00±1.00^j</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>9.10±0.36^h</td>
<td>6.03±0.15^i</td>
<td>4.06±0.11^j</td>
<td>7.00±0.20^k</td>
<td>10.50±0.50^l</td>
</tr>
<tr>
<td>Candida kefyr</td>
<td>7.03±0.15^j</td>
<td>4.03±0.05^k</td>
<td>4.00±0.10^l</td>
<td>6.00±0.20^m</td>
<td>9.96±0.45^n</td>
</tr>
</tbody>
</table>

The values are represented as the Mean ± SD of two essential oils. These essential oils have significant effect at 0.05 level

### Table 7 Antibacterial activity of plant essential oils

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Geranium natural oil</th>
<th>Geranium synthetic oil</th>
<th>Geranium natural Oil 50 µl &amp; DMSO50 µl</th>
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<td>11.10±0.36^d</td>
<td>4.00± 0.00^e</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>8.00±0.20^f</td>
<td>3.00±0.00^g</td>
<td>7.00±0.20^h</td>
<td>5.00±0.10^i</td>
<td>(Amphotericin B) 3.96±0.05^j</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>7.03±0.15^i</td>
<td>4.00±0.00^k</td>
<td>6.00±0.10^l</td>
<td>8.03±0.15^m</td>
<td>17.00±1.00^n</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>12.00±0.50^o</td>
<td>6.03±0.25^p</td>
<td>11.03±0.55^q</td>
<td>4.00±0.00^t</td>
<td>19.00±1.00^u</td>
</tr>
<tr>
<td>Candida kefyr</td>
<td>14.00±0.50^p</td>
<td>6.03±0.15^p</td>
<td>11.10±0.36^q</td>
<td>7.03±0.15^r</td>
<td>20.00±1.00^v</td>
</tr>
</tbody>
</table>

### Table 8 Inhibition of growth of selected fungi by chemical compounds of two essential oils

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Geranium natural oil</th>
<th>Geranium synthetic oil</th>
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<td>7.03±0.15^d</td>
<td>7.96± 0.25^e</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>11.96±0.45^h</td>
<td>10.10±0.26^i</td>
<td>14.96± 0.45^j</td>
<td>10.96±0.45^k</td>
<td>(Amphotericin B) 9.10±0.36^l</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>8.03±0.15^l</td>
<td>5.03±0.15^m</td>
<td>7.06±0.20^n</td>
<td>4.00± 0.00^o</td>
<td>18.00±1.00^p</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>7.03±0.15^l</td>
<td>4.00±0.00^o</td>
<td>5.03±0.15^m</td>
<td>7.03±0.25^n</td>
<td>19.00±1.00^q</td>
</tr>
<tr>
<td>Candida kefyr</td>
<td>10.93±0.40^h</td>
<td>7.03±0.25^p</td>
<td>5.03±0.15^m</td>
<td>3.00±0.00^q</td>
<td>21.00±1.00^r</td>
</tr>
</tbody>
</table>

In our investigation it has been found that geranium essential oil is effective against bacterial and fungal strains with different mechanisms of drug resistance. Geranium oil can be applied not only in the treatment of dysentery, urinary tract and skin infections, but also in inflammation of the mouth, larynx, pharynx caused by bacterial and fungal pathogens. It can be used as an effective air disinfectant and as an additive to antiseptic preparations, and be used in the hospitals, nursing homes and clinics. The application of essential oils in the treatment of human diseases, particularly infectious diseases caused by multidrug resistant bacterial strains, may be an interesting alternative to synthetic drugs.
acquired infections. (Dorian et al., 2009) have tested the antibacterial activity of geranium essential oil against bacterial and fungal pathogens.

CONCLUSION

Essential oils obtained from leaves, stems, and flowers of different plant species exhibited antimicrobial activities because they were able to kill or inhibit the growth of medically important bacteria and fungi used in the present study. The essential oil from *P. graveolens* natural and synthetichow show good antibacterial and anti-fungal activities and could be used in further pharmacological and phytochemical analysis. Additional studies in vitro and clinical trials would be needed to further characterize the active principles and evaluate the potential activity of these oils.

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