Chemical evaluation of mosquito repellent formulation prepared from the essential oil of plants

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ABSTRACT
The essential oil of leaves and peels of Ocimum gratissimum, Cymbopogon citratus, Hyptis suaveolens, Eucalyptus globulus, Azadirachta indica and Citrus sinensis were extracted and then formulated in a complex solution. Constituent analysis of the repellent formulation was conducted using Gas Chromatography (GC). A total of seven compounds were detected representing 99.92% of the essential oil components of the repellent formulation. The major component present was limonene (34.91%) and the other main components were salanine (4.34%), citral (22.64%), 1,8 – cineole (7.00%), citronella (17.49%), caryophyllene (11.25%) and azadirachtin (2.29%). The present study showed the potential of volatile compounds of plants as possible constituents of repellent products.

Keywords: Essential oil; Gas Chromatography; Repellent; Volatile constituent.

INTRODUCTION
Over two billion people, primarily in tropical countries, are at risk from mosquito-borne diseases such as Malaria, Arboviral Encephalitis, Dengue Fever, Rift Valley Fever and Yellow Fever (Service, 1993). Resistance to antimalarial drugs as well as synthetic repellents have been noted in all the vectors causing these diseases. Although, the development of resistance by these vectors cannot be stopped however, appropriate action will reduce morbidity and mortality rate. One of these methods is by the use of repellents of plant origin. The search for phytochemicals of plant origin having repellency activities against the malaria causing vectors and other diseases like Malaria, Dengue Fever, Japanese Encephalitis have been mainly stimulated by the fact that some of the major repellents like DEET and DDT have considerable drawback in term of resistance and toxicity. Phytochemicals obtained from plants with proven mosquito control potential can therefore be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Essential oil has been the active principle of most important herbal remedies.
since ancient times. The repellency properties of essential oils are well recognized for many years and used in some of the repellent products as active ingredients (Chang, et al., 2001; 2002). Phytochemical studies have also confirmed that traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harbone, et al., 1995). Previous research (Lawal, et al., 2012) on this study showed 10% formulation to possess more repellency activity amongst other formulations (6%, 8%, and 12%) as compared with the standard (Odomos® mosquito repellent cream). There is therefore need to establish the chemical constituents of this 10% formulation using the appropriate analytical procedures. In view of this and in line with the previous study carried out on this project, 10% formulation was prepared in a complex solution to evaluate the chemical composition in order to establish and ascertain the active chemical compositions being established by previous studies (Van Duong, et al., 1993; Peerzada, 1997; Oyedele, et al., 2002; Olawore, et al., 2003; Sammeeh, et al., 2004) on the essential oils used in this formulation. This work therefore investigated the active constituents of an herbal mosquito repellent formulated from the essential oil of six Nigerian medicinal and aromatic plants.

**MATERIALS AND METHOD**

**Plant Materials:** Fresh leaves and peels of the plant samples (Ocimum gratissimum, Cymbopogon citratus, Hyptis suaveolens, Eucalyptus globulus, Azadirachta indica and Citrus sinensis) were collected in June 2011 at the Nigeria Natural Medicine Development Agency’s Botanical Garden, Epe, Lagos, identified and voucher in the Botany Department, University of Lagos, Nigeria. Voucher specimens (LUH4685, LUH4686, LUH4687, LUH4688, LUH4689 and LUH4690) deposited at the herbarium of the Botany Department, University of Lagos, Nigeria.

**Extraction of Essential Oil:** Essential oil from the fresh leaves and peels of the plant samples were obtained using Hydrodistillation method by Clevenger type apparatus (BP, 1980). The oils were separately collected in airtight containers, dried over anhydrous sodium sulphate and stored at 4°C for further use in the formulation.

**Repellent Formulation:** Graded concentration (10%) of the essential oil extract from the six medicinal plant samples was formulated in a complex solution of Polyethylene glycol, Ethanol and Water. This was then transferred into a screwed vial and stored at 4°C in the refrigerator for further study and phytochemical analysis.

**GC Analysis:** The phytochemical analysis was carried out using Gas Chromatographic instrumentation method of analysis. GC analysis was conducted on HP ChemStation Software equipped with a less polar capillary column SPB 5 (30m x 0.53mm x 0.50µm film thickness using Shimadzu CC-RI (FID) gas chromatograph. The GC operating conditions are as follows: oven temperature programmed from 40°C (4min) to 300°C at 10°C/min, injection temperature (250°C), detector temperature (270°C), the carrier gas was Nitrogen at a flow rate of 0.9ml/min and a pressure of 30.0psi. Hydrogen pressure was 22psi and component Air pressure was 28psi. Identification of the components was performed by comparison of their retention times with those of pure authentic samples used as standards.
RESULT

The Gas Chromatograph of the essential oil components of the herbal mosquito repellent formulation is shown in figure1.

Table 1 show the components identified in the herbal mosquito repellent formulation in order of elution from SPB 5 column. Seven compounds were identified accounting for 99.92% of the total essential oil components of the herbal mosquito repellent formulation.

DISCUSSION

Essential oils constitute mainly monoterpenes and sesquiterpenes. These constituents have been shown to have repellency, insecticidal and larvicidal activities (Chavasse, et al., 1997; Oyedele, et al., 2000; Omobuwajo, et al., 2005; Sarah, 2007). Previous work- Van Duong, et al.,1993; Peerzada, 1997; Oyedele, et al., 2002; Olawore, et al., 2003; Sammeh, et al., 2004) carried out on the essential oil extract from the selected aromatic plants used in this study confirmed the presence of some of the compounds obtained from the GC analysis of herbal repellent formulation. It therefore showed that formulation of the herbal mosquito repellent product by the incorporation of the essential oil from plants into a complex solution may not necessarily alter the nature of constituents present in the individual plant essential oil used for this study. The major constituents in the herbal repellent formulation – limonene (34.91%) and citral (22.64%) are suspected to be the most active components of the herbal repellent formulation, however, it is also possible that the components in lower percentage might be involved in some type of synergism with the two other active major components in the repellency activity of the herbal repellent formulation

CONCLUSION

 Constituents of the essential oil in the herbal mosquito repellent formulation has potential as natural mosquito repellent and may therefore serve as an alternative to commercially available synthetic repellents.

REFERENCES


Table 1: Chemical Composition of Mosquito Repellent Formulation.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Chemical constituents*</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.322</td>
<td>Salanin</td>
<td>4.34</td>
</tr>
<tr>
<td>9.743</td>
<td>Limonene</td>
<td>34.91</td>
</tr>
<tr>
<td>11.341</td>
<td>Citral</td>
<td>22.64</td>
</tr>
<tr>
<td>12.824</td>
<td>1,8-cineole</td>
<td>7.00</td>
</tr>
<tr>
<td>14.153</td>
<td>Citronellal</td>
<td>17.49</td>
</tr>
<tr>
<td>15.501</td>
<td>Caryophyllene</td>
<td>11.25</td>
</tr>
<tr>
<td>17.773</td>
<td>Azadirachtin</td>
<td>2.29</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>SEVEN</strong></td>
<td><strong>99.92</strong></td>
</tr>
</tbody>
</table>

*Chemical Compounds listed in order of elution from SPB 5 column.

Figure 1: Gas chromatogram of the Mosquito Repellent Formulation.