Chemical constituents and bioactivity of steam distilled oil of *Monodora myristica* (Gaertn.) Dunal against some plant pathogenic fungi

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ABSTRACT

The chemical analysis of the essential oil of the dried seed nuts of *Monodora myristica* (Gaertn.) Dunal was investigated by GC and GC-MS. The results showed predominantly of hydrocarbon monoterpenes: p-cymene (31.5%), α-phellandrene (18.1%), α-pinene (6.1%), and β-pinene (5.1%) as major constituents. The bioactivity of the essential oil was evaluated against six plant pathogens by Micro broth dilution method. The essential oil suppressed the growth of these plant pathogenic fungi in culture and shows high to low significant potency.

Keywords: *Monodora myristica*, essential oil chemical analysis, hydrocarbon monoterpenes, Micro broth dilution method, plant pathogens, fungi.

INTRODUCTION

*Monodora myristica* (Gaertn.) Dunal is known as *Calabash Nutmeg*, African or Jamaican nutmeg, belongs to the family Annonaceae, a tropical to subtropical plant family that is widely distributed from Liberia, in West Africa to Cameroon, Central Africa, Asia, Central and South America and Australia (Ekundayo, 1989). The seeds are sold in the markets in rural areas all over West Africa and are used as condiment in soup and stimulating addition to other medicines and snuff (Iwu, 1993). 
*M. myristica* oil are used whole for flavouring food products and to cure various gastro-intestinal complaints, psychological disorders and urinary diseases (Usmanghani *et al.*, 1997 and Adegoke *et al.*, 1998). The volatile constituents of *M. myristica* has been extensively studied (Ikedia *et al.*, 1962; Ekundayo and Hammerschmidt, 1988; Onyenekwe *et al.*, 1992; Olajide *et al.*, 1999; Simpson and Jackson, 2002 and Olawore, *et al.*, 2002). Report have it that the main constituents established have been α-phellandrene, α-pinene, β-pinene and terpinen-4-ol. There are considerable variations in the content of the major components within the species. Because of the significant difference in geographical location (Lawrence, 1988) culture and environmental conditions (Charles and Simon, 1990) and different chemotypes in the oil composition, we have focused this study to investigate the chemical constituents responsible for the characteristic odour and bioactivity of steam distilled oil of *M. myristica* against some plant pathogenic fungi from Lagos in comparison with the one from Imo state as reported by (Onyenekwe *et al.*, 1992) and the one from Oyo state all in Nigeria as reported by(Olawore *et al.*, 2002).
MATERIAL AND METHODS

Plant materials

The dried seed nuts of *M. myristica* were obtained from a local market in Lagos, Nigeria in August 2006. The nuts were identified by Mr. O.K. Oluwa from the Botany Department, Lagos State University, where a specimen was deposited. The dried seed nuts (350g) were crushed into coarsed in a grinder and subjected to hydrodistillation for 4hrs in an all glass Clevenger-type apparatus (British Phamacopoeia, 1980). The oil obtained was dried over anhydrous sodium sulphate and stored in the refrigerator until it was sent to DDU Gorakhpur University, Gorakhpur, India, (Chemistry Department) for analysis.

Identification of the essential oil components

The volatile constituents were analysed by gas chromatography coupled to mass spectrometry (GC-MS). GC-MS analysis was carried out on a Hewlett -Packard HP 6890 equipped with Hewlett Packard mass Detector (model 5973) and a HP-5 Column 30m x 0.25mm i.d (cross linked 5% phenylmethylsiloxane). The injector, GC-MS inter phase, ion source and selected mass detector temperatures were maintained at 270oC, 280oC and 150oC respectively. Helium was used at the carrier gas and the oven temperature was programmed as follows: 60oC for 5 mins, rising at 1oC/min to 140oC, then at 10oC/min to 270oC and held for 5min. Chemical components of the oil were identified by comparing their mass spectra and retention indices ((RI) with those of standard included in the library NBS 75k (Adams, 1989) and the results were reported in Table 1.

Antifungal Activity

The micro-organisms screening were performed according to (Smith et al., 2002) using certain fungi pathogens obtained from Microbiology Department, Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria. *Fusarium sambucinum*, *Rhizoctonia solani*, *Aternaria solani* *Aspergillus niger*, *pencillium sclerotigenum* and *Rhizopus nodosus*. Micro broth dilution method (Smith et al., 2002) with slight modification was performed as follows. The fungi were cultured on Potato dextrose agar (PDA) medium at 28°C for one

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>RI*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>α-Thujene</td>
<td>928</td>
<td>3.4</td>
</tr>
<tr>
<td>2.</td>
<td>α-Pinene</td>
<td>932</td>
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<td>958</td>
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<td>4.</td>
<td>Sabine</td>
<td>973</td>
<td>0.4</td>
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<td>5.</td>
<td>α-Pinene</td>
<td>976</td>
<td>5.1</td>
</tr>
<tr>
<td>6.</td>
<td>2-Carene</td>
<td>1001</td>
<td>1.0</td>
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<td>(E)-β-Ocimene</td>
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<tr>
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<td>0.2</td>
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<tr>
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<td>γ-Terpinene</td>
<td>1059</td>
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<td>15.</td>
<td>Linalool</td>
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<td>Naphthalene</td>
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<td>α-Terpinol</td>
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<td>25.</td>
<td>cis-Piperitol</td>
<td>1194</td>
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<td>cis-Sabinol</td>
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<td>27.</td>
<td>Trans-Piperitol</td>
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<td>Carvatanacetone</td>
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<td>Geraniol</td>
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</tr>
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<td>32.</td>
<td>Carvacrol</td>
<td>1306</td>
<td>2.0</td>
</tr>
<tr>
<td>33.</td>
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<td>34.</td>
<td>Eugenol</td>
<td>1357</td>
<td>0.6</td>
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<td>35.</td>
<td>α-Copaene</td>
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<tr>
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<td>γ-Murolene</td>
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<td>epi-Cubebol</td>
<td>1491</td>
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<td>44.</td>
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<td>45.</td>
<td>α-Cadinene</td>
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<td>46.</td>
<td>Cubebol</td>
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<td>0.2</td>
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<tr>
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<td>δ-Cadinene</td>
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<td>48.</td>
<td>Germacrene D-4-ol</td>
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<td>49.</td>
<td>1,10-di-epi-Cubebol</td>
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<tr>
<td>51.</td>
<td>α-Cadinol</td>
<td>1651</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Total 95.24

HMT 66.55 OMT 23.79 HST 4.9

* Elution order and retention indices on a HP-5 Column (see Experimental) compared with authentic sample
week before the experiment. The Nutrient agar medium was prepared and distributed on sterile Petri dishes (80 mm diameter) which were aseptically placed in a chamber without lid. The essential oil was emulsified at the ratio 1:9 (v/v) in 0.1% water agar and separately introduced into each of the Petri dish and tightly covered. Incubation in the Petri dish was performed at 28°C for 3 to 5 days the period for colony formation. Control experiment was set up without the addition of essential oil and the period required for colony formation on the control Petri dish was determined. For each, three replicates were carried out and the average was determined. The fungi toxicity was determined by measuring the diameter of the zone of inhibition and its percentage colony inhibition was calculated according to the formula of (Pandey et al., 1982) with reference to the negative control. The results of the antifungal activity were shown in Table 2.

Growth inhibition (%) = \( \frac{(C - T)}{C} \times 100 \)

Where C = average zone inhibition of control, and T = average zone inhibition of fungal colony with essential oil.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Activity</th>
<th>Growth inhibition(mm)</th>
<th>Growth inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium sambucinum</td>
<td>++</td>
<td>9</td>
<td>47.4</td>
</tr>
<tr>
<td>Rhizoctania solani</td>
<td>+</td>
<td>3</td>
<td>15.8</td>
</tr>
<tr>
<td>Alternaria solani</td>
<td>++</td>
<td>8</td>
<td>42.1</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>+++</td>
<td>12</td>
<td>63.2</td>
</tr>
<tr>
<td>Penicillium sclerotigenum</td>
<td>+++</td>
<td>13</td>
<td>68.4</td>
</tr>
<tr>
<td>Rhizopus nodosus</td>
<td>+++</td>
<td>12</td>
<td>63.2</td>
</tr>
</tbody>
</table>

+++ strongly activity; ++ moderately activity; + weak activity.
The results are the average of three readings

RESULTS AND DISCUSSION

The obtained essential oil after 4hrs of hydrodistillation yielded 1.4 % (v/w). Table 1 shows the constituents identified by GC-MS in the oil together with their Retention indices and percentage compositions. Quantitatively, the oil was characterized by high amount of hydrocarbon monoterpenes (HMT) (66.55%). The predominant hydrocarbon monoterpenes were p-cymene (31.5%), α-phellandrene (18.1%), α-pinene (6.1%), α-pinene (5.1%), β-Thujene (3.4%). Hydrocarbon sesquiterpenes (HST) (4.9%) were also detected. The detection of p-cymene and α-phellandrene as the most abundant constituents makes it similar to an earlier report (Ekundayo and Hammerschmidt 1988 and Olawore et al., 2002). Furthermore, oxygenated monoterpenes were also identified comprising of eighteen alcohols (22.35%), two aldehydes (0.62%) three ketones (0.32%) and three esters (0.5%). The result showed that a number of other constituents which were not reported by (Ekundayo and Hammerschmidt 1988; Onyenekwe et al., 1992 and Olawore et al., 2002) were also identified. Some of these constituents included cis sabinol (8.9%), α-pinene (5.1%), carvacrol (2.0%), 2-carene (1.0%), eugenol (0.6%), (E)-β-ocimene (0.5%), epi-cubeol (0.4%) and α–cadinol (0.4%). This result showed that there were variations in the chemical constituents of this plant; some constituents were present as found in Imo and Oyo States and not found in Lagos State vice versa, these variations could probably be due to geographical origin (Lawrence, 1988), genetic factors, culture and environmental conditions (Charles and Simon, 1990), different chemotypes, and nutritional status of the plant as well as other factors that can influence the oil constituent. The reported concentration of p-cymene, α-pinene, linalool and α-thujene in the oil M.myristica is in conformity with previous work on nutmeg (Ikedia et al., 1962; Baldry et al., 1976 and Olawore et al., 2002).
The essential oil of *M. myrsitica* exhibited strong biological activity against tested pathogens. The highest activities were obtained with *Pencillium sclerotigenum*, *Aspergillus niger* and *Rhizopus nodosus*. Also, the oil was moderately effective against *Alternaria solani* and *Fusarium sambucinum* while slightly potent promise was observed against *Rhizoctania solani*. The variation of the biological activity could be correlated to chemical composition variability (Burt, 2004 and Lahlon, 2004). The control experiment showed uninhibited growth of the plant pathogens. It has also been reported that some volatile components from aromatic plants especially essential oil of *M. myrsitica* have been demonstrated to possess high antimicrobial activity (Dorman and Deans 2002; Tatsadjieu et al., 2003; Nguefack et al., 2004 and Odoh et al., 2004) and insecticidal (Okonkwo and Okoye, 1996) properties. The interactions between essential oil components play an important role in the determination of their biological activities.

Studies have shown that high concentration of *p-cymene* in the oil makes it potentially useful in the cream and food industries (Ullte et al., 2000 and Nguefack et al., 2004), and could possibly be applied as food preservatives by rural dwellers that frown at the use of synthetic chemicals that are hazardous to the health. The study of the stability of the bioactivity of the essential oil showed enhancement of storage life up to 5 days. In order to achieve a longer preservation using this oil, it may be necessary to consider reapplication after an expiration of 5 days of the first use, since the activity of essential oils decreases with time because of their high volatility. Therefore the oil could be recommended as a potential source of eco-friendly botanical fungicide, after long term and wide ranging trials.

ACKNOWLEDGMENTS

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