Gas chromatography mass spectrophotometry analysis and insecticidal activities of essential oils of *Azadirachta indica*, *Citrus sinensis* and *Anacardium occidentale* on *Blattella germanica*’

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World Journal of Advanced Pharmaceutical and Life Sciences, 2022, 03(02), 001–010

Publication history: Received on 04 September 2022; revised on 09 November 2022; accepted on 12 November 2022

Article DOI: https://doi.org/10.53346/wjapls.2022.3.2.0038

Abstract

Essential oils are a viable alternative to conventional pesticides because of their natural insect resistance, as well as their environmental and human health implications. In this study, the chemical compositions of essential oils extracted from *Citrus sinensis* (peel), *Anacardium occidentale* (nuts) and *Azadirachta indica* (leaves) were characterized. The insecticidal activities of the oils and its constituents against *Blattella germanica* (German cockroach) were investigated. The essential oils of *A. occidentale*, *A. indica*, and *C. sinensis* were gotten from the respective plants using hydrodistillation methods and were screened for phytochemical components using Gas chromatography and mass spectrometer (GC-MS). Thirty-six (36) compounds were detected with the percentage composition of over 100% from *A. occidentale*, D-Limonene had the highest composition of about 34.68%. *C. sinensis* contained 11 compounds with D-Limonene having the highest value of 93.98% higher than that of *A. occidentale* and the total percentage composition is lesser than 100%. *A. indica* essential oil contained 8 compounds in which cyclotrisiloxane had the highest percentage composition of 58.52%. Concentrations of 0.1ml and 0.2ml of the essential oils of *A. occidentale*, *A. indica* and *C. sinensis* were tested on *Blattella germanica*. The essential oils of *C. sinensis* and *A. indica* caused 100% mortality of the cockroaches using continuous exposure method while the essential oil of *A. occidentale* was repelled by the cockroaches. At the concentrations of 0.1 ml and 0.2 ml of *C. sinensis* oil, all the cockroaches died within 24 hours, whereas the essential oil of *A. indica* at the same concentrations mortalized all the cockroaches after 24 hours.

Keywords: Mortality; Repelled; Phytochemicals; Essential oils; Insecticidal plants

1. Introduction

Essential oils, as secondary plant compounds responsible for the aromatic characteristics of plants, present the potential alternative to conventional insecticides, (Omara et al., 2013). Plant extracts and essential oils are reported to have a wide range of activity against insect and mite pests, plant pathogens, fungi and nematodes (Isman 2006). Recent reports have highlighted antimicrobial, antifungal, anti-cancer, and insecticidal properties of plant essential oils (Isman, 2006). They have fumigants, antifeedant and repellent effects as well as inhibiting the reproduction in cockroaches and other insects (Omara et al., 2013). They could be used in areas where chemical insecticides are prohibited. The repellent effect of essential oils has been reported against many insect pests such as cockroaches, termites, mosquitoes, ticks, ants, and houseflies (Chen et al., 2002).
Conventional insecticides are used as main tool to control cockroach infestations but there are many concerns about the harmful side-effects of these chemical compounds. Also, the use of insecticides is restricted in places such as food preparation areas, restaurants, storage buildings and apartments. These restrictions of chemical insecticide application increase demand for safer alternatives against cockroach infestations (Phillips and Appel 2010). Different level of resistance to many compounds of chemical insecticides including organochlorine, organophosphorus and carbamate insecticides have been documented in many field-collected strains of cockroaches from Iran. So, application of these insecticides should be stopped and replaced with other safer compounds (Nasirian et al., 2006).

Botanical insecticides had been in use for thousands of years. They have been reported to possess contact toxicity, systemic or repellent action against their target pests (Rajashekar et al., 2012). However, decades ago, the introduction and use of synthetic insecticides, which began in the 1940s, led to the neglect of botanical insecticides owing to their high efficiency, fast action, ease of use and low cost. However, about twenty years after introducing synthetic insecticides, several adverse side effects associated with their use that were not initially thought of at the time of their introduction began to surface. These include the development of insect resistance, pesticide food contamination, environmental pollution problems, the disruption of natural balance, toxicity to non-target organisms, and most importantly, negative impact on human health (Grdisa and Grsic 2013). The repellent action of plants and plant products against pests reported by Khater (2012) showed their roles as defensive phytochemicals (Isman and Akhtar 2007). The beauty about these compounds is that they are often easily decomposed by various common microbes in most soils. Consequently, the potential for environmental contamination is reduced (Khater 2012). These compounds are easily decomposed by UV light from the sun, making them disappear from the environment in a few days after their application in the field (Miresmailli and Isman 2006).

2. Material and method

2.1. Collection of Plant Materials

Fresh plant samples of *Anarcadium occidentale* (Cashew seeds), *Azadirachta indica* (Neem leaves) and *Citrus sinensis* (Orange peel) were collected from Ilorin, Kwara State; Lagos State University Botanical Garden and Okokomaiko Market in Ojo Local Government area of Lagos State respectively between March and May 2022.

![Figure 1](image_url) Extraction of the Essential Oil using Hydro distillation Method
2.3 Preparation of Plant Materials and Extraction of Essential Oils

The fresh plant samples were air dried on the laboratory benches for 3 weeks. Cashew seeds were further sun dried for additional 1 week to obtain minimal moisture content. The plant materials were then pulverized by blending into rough powdery substance. The pulverized plant materials were stored in labeled airtight container at ambient temperature and protected from sunlight for further use.

2.2 Extraction of Essential oil using hydro distillation methods

The method of Perveen et al. (2013) was used for the extraction of the essential oils from the plant samples. Each pulverized plant sample was weighed (100 g) and was transferred to the sealed compartment of the distillation set up. Water was added sufficiently, and the mixture was brought to a boil. The vapour mixture of water and oil was condensed by indirect cooling with water. Condensed mixture flowed from the condenser to a separator where oil and bioactive compounds were separated automatically from the water. Each plant sample was boiled for 4 hours at a temperature of 100 °C after which the oil was collected from the mixture of oil and water vapour in the separator.

2.3 GC-MS Determination of phytochemicals

Prior to analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of m/z 69, 219, 502 and other instrument optimal and sensitivity conditions.

Determination of the levels of phytochemicals in the sample was carried out using GC-MS by operating MSD in Scan mode to ensure all levels of detection of the target constituents.

Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies) was used. The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies).

The carrier gas was Helium used at constant flow of 1.4871 mL/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. 1µL of the samples were injected in splitless mode at an injection temperature of 300 °C. Purge flow to split vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min; gas saver mode was switched off. Oven was initially programmed at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). Run time was 32.667 min with a 5 min solvent delay.

The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate).

2.4 Insecticidal activities

![Figure 2](image)

*Figure 2 Insecticidal Activities of Essential Oils of A. occidentale, A. indica and C. sinensis*
Discs of filter paper of small triangular sizes were sprayed with essential oils of *A. occidentale*, *A. indica* and *C. sinensis* in the volumes of 0.1 ml and 0.2 ml. They were put into the beaker. Five small to medium sized cockroaches were introduced into the beakers containing the sprayed filter papers and were covered with cotton wool. Mortality was checked every 24 hours for a period of 5 days.

\[ \text{Mortality} \% = \frac{\text{Number of dead insect}}{\text{Total number of insects}} \times 100 \]

3. Results and discussion

3.1. Essential Oil yields from the Plant Samples through Hydro distillation Method

One hundred gram each of *Anarcadium occidentale*, *Azadirachta indica* and *Citrus sinensis* yielded 20, 3 ml, 0.9 ml, and 5.0 ml respectively. It was observed that *A. occidentale* produced the highest quantity of the essential oils. El Asbahani *et al.* (2015) reported that the disadvantages of the hydro-distillation method were long extraction time, possible chemical changes in the structures of terpenes, and the loss of some polar molecules owing to the applied heat. Bowes *et al.*, 2004 stated that extractable oil yields in plant samples can be affected by plant species, locations, plant tissue type being processed and the drying conditions.

3.2. GC-MS Analysis of the Essential Oils of *A. occidentale*, *A. indica* and *C. sinensis*

The essential oils of *A. occidentale*, *A. indica*, and *C. sinensis* gotten from the respective plant sample using hydro distillation method was screened for phytochemical components using Gas chromatography Mass Spectrophotometer (GC-MS). A total number of 36 compounds were detected with the percentage composition of over 100% from *A. occidentale*, D-Limonene has the highest composition of about 34.68% as shown in Table 1. The results in Table 2 shows the composition of *C. sinensis* which contained 11 compounds with D-Limonene having the highest value of 93.98 % higher than that of *A. occidentale* and the total percentage composition is lesser than 100%. From Table 3, *A. indica*, has the total number of 8 compounds in which cyclotrisiloxane has the highest percentage Composition of 58.52%. The results of the abundance composition of each component of *A. occidentale*, *A. indica*, and *C. sinensis* are represented in Fig 1, 2 and 3 respectively.

**Table 1** Result on the GC-MS analysis on *A. occidentale* essential oil

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compounds</th>
<th>Rate (RT)</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>alpha.-Pinene</td>
<td>6.801</td>
<td>10.23</td>
</tr>
<tr>
<td>2.</td>
<td>beta.-Myrcene</td>
<td>7.877</td>
<td>3.00</td>
</tr>
<tr>
<td>3.</td>
<td>D-Limonene</td>
<td>8.603</td>
<td>34.68</td>
</tr>
<tr>
<td>4.</td>
<td>gamma.-Terpinene</td>
<td>9.485</td>
<td>1.28</td>
</tr>
<tr>
<td>5.</td>
<td>Cyclohept-4-enol</td>
<td>9.593</td>
<td>0.48</td>
</tr>
<tr>
<td>6.</td>
<td>2-Carene</td>
<td>9.753</td>
<td>1.60</td>
</tr>
<tr>
<td>7.</td>
<td>Linalool</td>
<td>9.919</td>
<td>7.08</td>
</tr>
<tr>
<td>8.</td>
<td>1,3,8-p-Menthatriene</td>
<td>10.051</td>
<td>0.45</td>
</tr>
<tr>
<td>9.</td>
<td>p-Menth-8-en-1-ol</td>
<td>10.509</td>
<td>0.63</td>
</tr>
<tr>
<td>10.</td>
<td>Terpinen-4-ol</td>
<td>11.018</td>
<td>3.59</td>
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<tr>
<td>11.</td>
<td>alpha.-Terpineol</td>
<td>11.281</td>
<td>15.41</td>
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<tr>
<td>12.</td>
<td>Decanal</td>
<td>11.384</td>
<td>2.84</td>
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<tr>
<td>13.</td>
<td>Bicyclo[2.2.1]hept-2-ene</td>
<td>11.556</td>
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<tr>
<td>14.</td>
<td>trans-2-Caren-4-ol</td>
<td>11.613</td>
<td>2.73</td>
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<tr>
<td>15.</td>
<td>Citronellol</td>
<td>11.699</td>
<td>2.49</td>
</tr>
<tr>
<td>16.</td>
<td>2-Cyclohexen-1-ol</td>
<td>11.768</td>
<td>0.47</td>
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<tr>
<td>17.</td>
<td>(-)-Carvone</td>
<td>11.951</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Compound</td>
<td>Peak Area</td>
<td>Relative %</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------</td>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td>18</td>
<td>2,6-Octadien-1-ol</td>
<td>12.054</td>
<td>0.59</td>
</tr>
<tr>
<td>19</td>
<td>Cyclopropane</td>
<td>12.288</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>p-Mentha-1(7)</td>
<td>12.580</td>
<td>0.37</td>
</tr>
<tr>
<td>21</td>
<td>Copaene</td>
<td>13.742</td>
<td>0.80</td>
</tr>
<tr>
<td>22</td>
<td>Tridecanal</td>
<td>14.102</td>
<td>0.64</td>
</tr>
<tr>
<td>23</td>
<td>Caryophyllene</td>
<td>14.331</td>
<td>0.73</td>
</tr>
<tr>
<td>24</td>
<td>beta-copaene</td>
<td>14.446</td>
<td>0.67</td>
</tr>
<tr>
<td>25</td>
<td>gamma-Muurolene</td>
<td>15.023</td>
<td>0.39</td>
</tr>
<tr>
<td>26</td>
<td>8a-dimethyl-7-(1-methyleth enyl)-Naphthalene</td>
<td>15.247</td>
<td>0.46</td>
</tr>
<tr>
<td>27</td>
<td>Naphthalene</td>
<td>15.601</td>
<td>2.13</td>
</tr>
<tr>
<td>28</td>
<td>Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-</td>
<td>15.916</td>
<td>0.40</td>
</tr>
<tr>
<td>29</td>
<td>Caryophyllene oxide</td>
<td>16.368</td>
<td>0.38</td>
</tr>
<tr>
<td>30</td>
<td>Alloaromadendrene oxide-(1)</td>
<td>16.854</td>
<td>0.55</td>
</tr>
<tr>
<td>31</td>
<td>2-Naphthalenemethanol,</td>
<td>16.912</td>
<td>0.81</td>
</tr>
<tr>
<td>32</td>
<td>tau-Muurolol</td>
<td>17.015</td>
<td>0.39</td>
</tr>
<tr>
<td>33</td>
<td>5,6,8a-octahydro-2-Naphthalenemethanol, 1,2,3,4,4a,</td>
<td>17.163</td>
<td>1.04</td>
</tr>
<tr>
<td>34</td>
<td>2,6,9,11-Dodecatetraenal</td>
<td>17.587</td>
<td>0.39</td>
</tr>
<tr>
<td>35</td>
<td>beta-Ocimene</td>
<td>18.188</td>
<td>0.51</td>
</tr>
<tr>
<td>36</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>19.899</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Total %Composition</td>
<td></td>
<td>101.3</td>
</tr>
</tbody>
</table>

**Figure 3** Graph showing the abundance of each constituent of Anacadium oil on Mass spectrometer
Table 2 Result on the GC-MS analysis on *C. sinensis* essential oils

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compounds</th>
<th>Rate (RT)</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>alpha.-Pinene</td>
<td>6.657</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>beta.-Myrcene</td>
<td>7.785</td>
<td>2.29</td>
</tr>
<tr>
<td>3</td>
<td>alpha.-Phellandrene</td>
<td>8.008</td>
<td>0.10</td>
</tr>
<tr>
<td>4</td>
<td>D-Limonene</td>
<td>8.643</td>
<td>93.80</td>
</tr>
<tr>
<td>5</td>
<td>gamma.-Terpinene</td>
<td>8.931</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>Cyclohexene,4-Carene</td>
<td>9.467</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>Linalool</td>
<td>9.667</td>
<td>0.54</td>
</tr>
<tr>
<td>8</td>
<td>Terpinen-4-ol</td>
<td>10.897</td>
<td>0.31</td>
</tr>
<tr>
<td>9</td>
<td>alpha.-Terpineol</td>
<td>11.109</td>
<td>1.44</td>
</tr>
<tr>
<td>10</td>
<td>Decanal</td>
<td>11.292</td>
<td>0.20</td>
</tr>
<tr>
<td>11</td>
<td>1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene</td>
<td>15.590</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Total %Composition</td>
<td></td>
<td>99.99</td>
</tr>
</tbody>
</table>

![Graph showing the abundance of each constituent of *C. sinensis* oil on Mass Spectrometer](image)

**Figure 4** Graph showing the abundance of each constituent of *C. sinensis* oil on Mass Spectrometer

A total of 11 volatile compounds majorly monoterpenes were detected in the EO of *C. sinensis* peel. These compounds include limonene, terpineol, linalool, and citronellol. A varied amount and types of EO constituents in *C. sinensis* have been previously reported (Njoroge et al., 2009; Oboh et al., 2017; González-Mas et al., 2019). These variations compared to this study may be due to factors such as the vegetative age of the fruits, geographical and seasonal variations, species of the plant and type of equipment used for the extraction process.

Thirty-six (36) volatile compounds were detected from the essential oil of *A. occidentale* (nuts), with the percentage composition of over 100% in which D-Limonene had the highest composition of about 34.68%, which is in accordance with the findings of (Kossouoh et al., 2008) as one of the most prominent compounds. *A. indica* contained 8 compounds...
in which cyclotrisiloxane has the highest percentage composition of 58.52%. Some of the compounds identified in this study have been reported to be potential insecticides (Oyedeji et al., 2020).

Table 3 Result on the GC-MS analysis on A. indica essential oil

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compounds</th>
<th>Rate (RT)</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyclotrisiloxane</td>
<td>4.604</td>
<td>58.42</td>
</tr>
<tr>
<td>2</td>
<td>p-Xylene</td>
<td>5.937</td>
<td>1.27</td>
</tr>
<tr>
<td>3</td>
<td>Benzene, 1-ethyl-3-methyl-</td>
<td>7.842</td>
<td>1.22</td>
</tr>
<tr>
<td>4</td>
<td>Cyclotetrasiloxane, octamethyl-</td>
<td>7.962</td>
<td>25.65</td>
</tr>
<tr>
<td>5</td>
<td>2-Pyrrolidinone, 1-methyl-</td>
<td>8.970</td>
<td>3.04</td>
</tr>
<tr>
<td>6</td>
<td>Cyclopentasiloxane, decamethyl-</td>
<td>10.509</td>
<td>6.66</td>
</tr>
<tr>
<td>7</td>
<td>Cyclohexasiloxane, dodecamethyl-</td>
<td>12.986</td>
<td>2.44</td>
</tr>
<tr>
<td>8</td>
<td>Cycloheptasiloxane, tetradecamethy</td>
<td>15.207</td>
<td>1.30</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total % Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 5 Graph showing the abundance of each constituent of A. indica oil on Mass spectrometer

3.3. Insecticidal Activities

Table 4 shows the insecticidal activities of the essential oils of A. occidentale, C. sinensis and A. indica at concentrations of 0.1 ml and 0.2 ml tested on Blattella germanica. The essential oils of C. sinensis and A. indica caused 100% mortality of the cockroach nymphs while the essential oil of A. occidentale was repelled by the cockroaches. At the concentrations of 0.1 ml and 0.2 ml of C. sinensis, all the cockroaches died within 24 hours, whereas the essential oil of A. indica at the same concentrations mortalized all the cockroaches after 24 hours.

From this study, the essential oils of C. sinensis and A. indica caused 100% mortality of the cockroaches; while the essential oil A. occidentale, was repelled by the cockroaches. The fumigant toxicity of the EOs of C. sinensis and A. indica against Blattella germanica was comparable to Rosmarinus officinalis EO (Güdek and Çetin, 2017), greater than Petroselinum sativum EO (Massango et al., 2017) and lower than Eucalyptus cinerea EO (Rossi et al., 2015). However, A. occidentale EO was less toxic to Blattella germanica as compared to Murraya exotica EO (Li et al., 2010) and Lonicera japonica EO (Zhou et al., 2012).
Table 4 The Mortality Rates of *Blattella germanica* at Varying Concentrations of Essential Oils of *A. occidentale*, *C. sinensis* and *A. indica*

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Concentration</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. occidentale</em></td>
<td>0.1 ml</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>0.2 ml</td>
<td>0%</td>
</tr>
<tr>
<td><em>C. sinensis</em></td>
<td>0.1 ml</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>0.2 ml</td>
<td>100%</td>
</tr>
<tr>
<td><em>A. indica</em></td>
<td>0.1 ml</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>0.2 ml</td>
<td>100%</td>
</tr>
</tbody>
</table>

Plant-derived insecticides, otherwise referred to as botanical insecticides like their counterpart; the synthetic insecticides act on their targets in many ways. One of which is their repellent activity due to the presence of essential oils. (Chaudhary *et al.*, 2017). Essential oils are promising substitute for chemical pesticides with the inherent resistance by pests, environmental and health effects on humans (Oyedeji *et al.*, 2020). For thousands of years, man has exploited the repellent and fumigating actions of plants against insects. The simplest way is by hanging or burning plants in homes to drive away nuisance mosquitoes. Later as oil formulations applied to the skin or clothes, whereby it repels the insects. This practice is still widely used in developing countries (Moore and Debboun, 2006). Essential oils which are mixtures of terpenes have a wide spectrum of biological activities. They have been investigated and reported severally in the literatures for the insecticidal activities. Abdelgaleil *et al.* (2004) reported the contact and fumigant toxicity of eleven monoterpenes from the fruits of *Khaya senegalensis*. According to Marchese *et al.* (2016), the monoterpenoid thymol obtained from *Thymus vulgaris* exhibited significant toxicity against *Sitophilus litura*. Also, Cao *et al.* (2018) stated that Linalool, an essential oil extracted from the fruits of *Evodia lenticellata* exhibited both contact and fumigant toxicity against *Lasioderma serricorne* and *Liposcelis bostrychophila*. Batish *et al.* (2008) reported the natural insect repellent potential of essential oils from *Eucalyptus* species. Also, the essential oils of *Artemisia annua* was reported to show acute toxicity against the larvae and adults of *Tribolium castaneum* according to Deb and Kumar, 2020.

4. Conclusion

This study showed that *A. occidentale*, *C. sinensis* and *A. indica* contained essential oils with several volatile compounds with insecticidal properties. The essential oils of *C. sinensis* and *A. indica* showed strong insecticidal activities against the tested insects while *A. occidentale* EO was found to be less toxic to the tested cockroach which may be due to low impact of inhibition on the tested organisms. Further research is necessary to investigate the insecticidal property of the essential oils of *A. occidentale*, *C. sinensis* and *A. indica* against other stored products insects. This can help to develop the Eos from these plants into botanical pesticides for managing the stored products insects, which in turn reduces the use of conventional insecticides and ensures global food security.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the contribution of Nigerian Stored Products and Raw Materials Institute to this project.

Disclosure of conflict of interest

No conflicts of interest by the authors.

References


