Chemical Composition and Bioactivity of the Essential Oil of Cassia singueana Flowers Growing in Nigeria

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Abstract

Cassia singueana (Delile) Lock from the family Fabaceae is a well-known medicinal plant that grows abundantly in Nigeria and other African countries, and has long been used in the treatment of various ailments including malaria and other infectious diseases. The present study aimed at assessing the composition, and bioactivity of the essential oil of the flowers of C. singueana collected from Nigeria. The essential oil was extracted by hydrodistillation and the chemical composition was analyzed by gas chromatography (GC) coupled with a flame ionization detector (GC-FID) and GC coupled to mass spectrometry (GC-MS). The bioactivity of the oil was determined using the brine shrimp lethality assay, agar diffusion antimicrobial test, the 2, 2-diphenylpicrylhydrazyl, metal chelation, and superoxide anion antioxidant assays. The essential oil yield and the percentage of identified compounds were 1.58% and 97.91%, respectively. More than 20 compounds were identified. The major component was geranyl acetone (36.82%) followed by phytol (18.12%). The essential oil showed lethality against the brine shrimp larvae with LC50 value of 187 μg/ml, and antimicrobial activity with largest inhibition zones of 32-33 mm against Candida albicans, Streptococcus pneumoniae, and Staphylococcus aureus. The oil also exhibited considerable antioxidant activity as evident from its ability to scavenge free-radicals such as DPPH, superoxide anions, and metal-chelation.

Keywords: Cassia singueana, Fabaceae, Essential oils, Antimicrobial, Antioxidant, Brine shrimp lethality assay

Introduction

Cassia singueana (Delile) Lock from the family Fabaceae—also called Senna singueana, and commonly known as scrambled egg, sticky pod, winter cassia, or winter-flowering cassia—is a woody annual herb or under shrub with 1.2 to 1.5 m height and small yellow flowers; it is widely distributed in India and tropical Africa including Western and Northern Nigeria (1, 2). The flowers of C. singueana have long been used traditionally in the treatment of typhoid, malaria, respiratory tract infections and as an antidiarrheal, antispasmodic, and anti-inflammatory agent by Yoruba, Fulani, and Hausa herbal medicine practitioners in Nigeria. Previous bioactivity studies on C. singueana showed its antidiabetic, antipyretic, antinociceptive, antioxidant, anticancer, antiplasmodial/antimalarial, hypolipidemic, and hepatoprotective properties (2-10), whilst the phytochemical investigations revealed the presence of anthraquinones, proanthocyanidins, other phenolics, fatty acids, amino acids, and triterpenoids in various parts of this plant (6, 10, 11). However, to the best of authors’ knowledge, there was no report on the chemical composition and bioactivity of the essential oil of the flowers of C. singueana growing in Nigeria. As a part of authors ongoing phytochemical and bioactivity studies on African medicinal plants (12-18), the results of the GC-MS and GC-FID analyses of the essential oil of the flowers of C. singueana, and its antioxidant and antimicrobial properties and brine shrimp...
lethality/toxicity were reported for the first time in the present paper.

**Materials and methods**

**Plant material**: Fresh flowers of *C. singueana* (Delile) Lock were collected from the premises of Ahmadu Bello University Zaria, Nigeria. The taxonomic identification of the plant was carried out at the herbarium section of the Department of Biological Science Ahmadu Bello University Zaria, Nigeria, where a voucher specimen (No. 1242) of this collection has been retained.

**Extraction of essential oil**: Fresh flowers of *C. singueana* (1 kg) were subjected to hydrodistillation for 3.5 hours in a 5000 mL round bottom flask using a modified Gévenger-type hydrodistillation apparatus as described by the British Pharmacopoeia (19). The oil was condensed and collected in *n*-hexane and rinsed with *n*-hexane to dissolve any oil particles adhering to the glass and kept in a freezer to allow separation of water from the essential oil. The concentrated oil was collected using a syringe, then dried over anhydrous sodium sulfate. The pure oil kept at 4 °C in the dark for further analysis (20).

**Gas chromatography-mass spectrometry analysis**: The GC-MS analysis of the essential oil was performed on an Agilent 7890A GC coupled to a 5973 MSD mass spectrometer using an HP-5MS capillary column (30 m x 0.25 µm x 0.25 µm) and helium as a carrier gas at a flow rate of 3.3245 ml/minute. The GC oven was initially programmed at 50 °C for one minute and ramped at 80 °C/minute finally to 300 °C for five minutes. Electron impact ionization (EI) was achieved with ionization energy of 7 ev. The essential oil was diluted with *n*-hexane and 2 µL of the diluted sample was injected. The data was acquired using GC-MS Analytical Chem-station software NIST-MS Library. Individual constituents were identified by comparing their retention data spectra with those stored in NIST08/Database/Kovat index and using analytical condition similar to that of GC-FID in reference with those of the chemical compounds gathered from the Adam table (21). The fragmentation patterns were also analyzed and compared with those stored in NIST-MS 08/Database (20).

**Gas chromatography coupled to flame ionization detector**: The GC-FID analysis was performed on an Agilent 7890 series filter with a flame ionization detector (FID) using the operating conditions as stated above. The FID was maintained at 260 °C. Split injection ratio was 1:20 (20).

**Brine shrimp lethality assay**: The brine shrimp lethality of the essential oil was determined using brine shrimp larvae (*Artemia salina*) in accordance with the method described by Oloyede *et al.* (22).

In this assay, a drop of dimethyl sulfoxide (DMSO) was added to both test and control vials to enhance the solubility of the essential oil. Brine shrimp eggs (70 g) were hatched in a beaker containing 250 mL of seawater. The beaker was placed beside a window under sunlight for proper ventilation at room temperature. After 48 hours the brine shrimp larvae were collected by dropping pipette. About 1 mL of the essential oil was dissolved in 2 mL of *n*-hexane. Then, 50, 5, and 1 µg/L of the solution were drawn into vials; two drops of DMSO were added, and made up to 2 mL with distilled water corresponding to concentrations of 1000, 100, and 10 µg/mL, respectively. Each dosage was prepared in triplicates including the control. Ten shrimp larvae were added to each vial. The number of the surviving shrimp at each dosage and the control was recorded after 24 hours and the LC50 was computed using Finney Probit Analysis computer program (23).

**Antimicrobial activity**: Four bacterial strains, *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (NCTC10662), *Staphylococcus aureus* (NCTC6571), *Streptococcus pneumoniae* (NCTC746S) and one fungal strain, *Candida albicans* (NCMB 3179), were used in the in vitro antimicrobial assay. DMSO was used to dissolve essential oil/drug, and the solution was diluted with sterile water to achieve the final concentration of 10%, which was used as the negative control. Levofloxacin, ofloxacin, and pefloxin were used as the positive controls. The agar diffusion method using Muller-Hilton agar (MHA) (24) was used to determine the antimicrobial activity of the essential oil at different concentrations (1.0, 0.5, and 0.25 µg/ml). Agar (35 g) was dissolved in 1 L of distilled water and autoclaved at 121 °C for 15 minutes, cooled and poured into sterile Petri-dishes to solidify. A sterile cork-borer was used to make holes on each seeded agar plate for each concentration of the essential oil and the control. Plates count agar (PCA) plates were inoculated with 100 µL of standardized inoculum (1.5 x 106 CFU/ml) of each selected microbe (in triplicates) and spread with sterile cotton swab. The essential oil solutions (10 µg/ml) and the control were introduced into the holes containing the bacterial/ fungi inoculum and the plates were incubated microaerobically at 37 °C for 24 hours. The diameters of inhibition zones were recorded after 24 hours using a transparent ruler. The minimum inhibitory concentration (MIC) and MBC (minimum bactericidal concentration) of the essential oil were determined by microdilution agar method as described in the literature (24, 25). Active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks, inoculated in MHA, and incubated at 37 °C for 24 hours. A 2-fold serial dilution of the essential oil was prepared in sterile distilled water to achieve a decreasing concentration
 ranging from 160 to 1.25 mg/ml in nine sterile tubes labelled 1 to 9.

Sterile cork-borer of 8 mm diameter was used to bore well in the presolidified MHA plates and 100 µL of each dilution was added aseptically into the wells in that had microbe isolate seeded with the standardized inoculum (1.5 x 10^6 CFU/ml) and incubated at 37 °C for 24 hours. The lowest concentration of an extract showing a clear zone of inhibition after the macroscopic evaluation was considered as the MIC. In the determination of MBC, a 100 µL aliquot from the tube showing MIC was placed on MHA plate and spread over the plate and also incubated. After incubation at 37 °C for 24 hours, the plates were examined for bacterial growth to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved.

**Antioxidant activity:** The antioxidant property of the essential oils of *C. singueana* flowers was determined by three different assays as outlined below:

**DPPH free-radical scavenging activity:** DPPH (the 2,2-diphenyl-1-picryl hydrazyl radical) free-radical scavenging activity of the essential oil was assessed using the method described by Lugasi et al. (26) and Marijana et al. (27). DPPH (39.4 mg) was dissolved in 100 mL of methanol to give a 1M solution. The solution was allowed to stand for 10 minutes and the absorbance was measured at 517 nm. About 2 mL of the oil extract solution (0.25, 0.50, 1.00, and 1.50 mg/mL) was prepared in the n-hexane, and 2 mL of 1M DPPH was added to 0.5 mL of each of the test solution. The mixture was shaken and left to stand for 10 minutes and the absorbance at 517 nm of the solutions was measured. Ascorbic acid, BHA, and α-tocopherol were used as positive controls. The percentage inhibition of DPPH free radical scavenging effect of superoxide anions was calculated using the Equation 2.

\[
\text{Inhibition} = \left( \frac{A_{\text{DPPH }} - A_s}{A_{\text{DPPH }}} \right) \times 100%
\]

Where \(A_{\text{DPPH}}\) and \(A_s\) are the respective absorbance of the neat DPPH and test solutions, respectively.

**Superoxide scavenging activity:** The scavenging effect of the essential oil towards superoxide anion radicals were measured using the method published by Nishimiki et al. (28). However, 1 mL of nitroblue tetrazolium solution (156 µM in 100 mM phosphate buffer, pH 7.4), 1 mL of nicotine amide dinucleotide solution (468 µM in 100 mM phosphate buffer, pH 7.4), and 1 mL of sample solution (0.25, 0.5, and 1.0 mg/mL) were mixed in n-hexane. The reaction started with the addition of 100 µL of phenozone methosulfate solution (60 µM in 100 mM phosphate buffer, pH 7.4) to the mixture and after five minutes at room temperature, the absorbance was measured at 560 nm. BHA, α-tocopherol, and ascorbic acid were used as positive controls. The percentage inhibition of scavenging effect of superoxide anions was calculated using the Equation 2.

\[
\text{Inhibition} = \left( \frac{A_{\text{blank }} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100%
\]

Where \(A_{\text{blank}}\) is the absorbance of the blank in the absence of sample and \(A_{\text{sample}}\) is the absorbance in the presence of the sample.

**Metal chelating activity:** The chelation of ferrous ions by essential oil was estimated by the method described by Dinis et al. (29). The reaction mixture contained 0.5 mL of the oil in n-hexane (0.25, 0.5, 1.0, and 1.5 mg/mL), 1.5 mL of deionized water, and 0.5 mL of 2 mM FeCl₂ solution. After 30 minutes, 1.0 mL of 5 mM ferrozine solution was added. After 10 minutes of incubation at room temperature, the absorbance at 562 nm was measured. Ascorbic acid, BHA, and α-tocopherol were used as positive controls. The percentage inhibition of ferrozine-Fe²⁺ complex formation was calculated using the Equation 3.

\[
\% \text{ activity} = \left( \frac{A_{\text{control }} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100%
\]

Where \(A_{\text{control}}\) is the absorbance of the blank in the absence of the sample and \(A_{\text{sample}}\) is the absorbance in the presence of the sample.

**Results**

**Extraction of essential oil and its composition:** The average yield of the essential oil form the fresh flowers of *C. singueana* was 1.58%. The composition of the essential oil of this plant is presented in Table 1. The retention profile is presented in Figure 1. A total of 25 compounds making up 97.91% of the total essential oil, were identified. The major component was geranyl acetone (36.82%), followed by phytol (18.12%), squalene (10.84%), calarene (9.32%), α-terpineol (4.25%), menthol (3.51%), α-copaene (3.31%), α-bergamotene (2.19%), neomenthol (2.71%), geranyl acetate (1.67%), isomenthone (1.52%), p-mentha-1,5-dien-8-ol (1.47), limonene (1.34 %), and menthofuran (1.10%).

**Bioactivities:** Brine shrimp toxicity of the essential oil of the flowers of *C. singueana* was determined by the brine shrimp lethality assay and the LD₅₀ value was calculated 18.7 µg/mL after 24 hours. The essential oils displayed concentration-dependent antimicrobial activity against all of the test organisms (Table 2), with largest inhibition zones of 32-33 mm against *C. albicans*, *S. pneumoniae*, and *S. aureus*. The broadest activity of the essential oil against most Gram-negative bacteria was 1.563 µg/mL that was considered as MIC, while the MBC was 6.250 µg/mL. The essential oil showed considerable antioxidant activity as determined by all three assays.
The scavenging of the free-radicals by the essential oil increased with the increase in the concentration. In the DPPH assay, at a concentration of 1.5 µg/mL, the scavenging capacity of the essential oil was 93.3%, which was comparable to that of the positive control BHA (96.7%) and higher than those of ascorbic acid and α-tocopherol (75.8% and 17.3%, respectively). In the superoxide anion assay, at a concentration of 1.5 µg/mL, the percent scavenging by the essential oil was the same as that of BHA (82.6%) and higher than those of ascorbic acid and α-tocopherol (64.8% and 9.3%, respectively). The effectiveness of the antioxidants as superoxide anion scavenger ranged in the following descending order: BHA > α-tocopherol. In the metal chelation assay, the highest scavenging capacity of the essential oil was the same (96.7%) and higher than those of ascorbic acid and α-tocopherol (82.6% and 17.3%, respectively). In the metal chelation assay, the highest scavenging capacity of the essential oil was the same (96.7%) and higher than those of ascorbic acid and α-tocopherol (82.6% and 17.3%, respectively). In the metal chelation assay, the highest scavenging capacity of the essential oil was the same (96.7%) and higher than those of ascorbic acid and α-tocopherol (82.6% and 17.3%, respectively).

The yield (1.58%) of essential oils from the hydrodistillation of fresh flowers of C. singueana collected from Nigeria was higher than the yields reported for other plants industrially exploited as sources of essential oils, e.g., lavender (0.8%-1.8%), mint (0.5%-1%), neroli (0.5%-1%), laurel (0.1035%), and Lippia rotundifolia (0.01%) (20). However, the yield was similar to the values reported for other Cassia species, e.g., C. senna-alata (1.43%), C. sophera (1.07%) and C. arrerehdel (1.47%) (30, 31).

This is the first report on the GC-MS and GC-FID analyses leading to the identification and quantification of the constituents of the essential oil of the flowers of C. singueana collected from Nigeria. However, reports on composition of the essential oils from other Cassia species—e.g., C. senna-alata, C. sophera, and C. arrerehdel are available to date (30, 31). The essential oil composition of C. singueana as determined in the current study showed that geranyl acetone (36.82%), phytol (18.12%), squalene (10.84%), calarene (6.23%), and menthol (3.51%) were the major components (Table 1). These major components were also found in the essential oils of other Cassia species. Phytol was reported as one of the major compounds in the essential oil in another Cassia species (30).

Brine shrimp lethality of the essential oils of C. singueana flowers (LD50 = 18.7 µg/ml) indicated that the oil was more toxic than the extracts obtained from the leaves, stems, and roots (LD50 = 36.67, 39.78, and 20.9 µg/ml, respectively) (32). The higher cytotoxicity of the essential oil of the flowers might be partly attributed to various volatile components (20, 33). For example, phytol, one of the major compounds in the essential oil of the flowers of C. singueana is an antitumor and anti-inflammatory agent (34).

The essential oil was active against all tested microbial strains (Table 2), and the activity increased with the increase in concentrations. At a concentration of 1.0 µg/ml, the maximum zone of inhibition of 32 and 33 mm were recorded against C. alibicans and S. aureus and 30 mm against S. pneumonia, which were comparable to those observed with the reference standards (positive controls) at the same concentration for these microbes. These results were in agreement with the observations made by Usman et al. (35) and Esmaeili et al. (36) for the essential oil of other plant species, e.g., Euphorbia species and Tamarix boveana. However, Usman et al. (35) reported that the essential oil of Euphorbia species was more active against Gram-negative bacteria than Gram-positive ones, while Esmaeili et al., (36) observed that the essential oil of T. boveana was more active against Gram-positive bacteria than Gram-negative ones.

### Table 1: Chemical Composition of the Essential Oil of the Flowers of Cassia singueana

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Kovat Index</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Limonene</td>
<td>9.76</td>
<td>1033</td>
<td>1.34</td>
</tr>
<tr>
<td>2 Phytol</td>
<td>12.01</td>
<td>784</td>
<td>18.12</td>
</tr>
<tr>
<td>3 α-Copaene</td>
<td>13.61</td>
<td>1022</td>
<td>3.31</td>
</tr>
<tr>
<td>4 α-Bergamoten</td>
<td>13.90</td>
<td>983</td>
<td>2.19</td>
</tr>
<tr>
<td>5 Caryophyllene oxide</td>
<td>21.22</td>
<td>987</td>
<td>0.03</td>
</tr>
<tr>
<td>6 Squalene</td>
<td>24.41</td>
<td>1054</td>
<td>10.84</td>
</tr>
<tr>
<td>7 α-Terpineol</td>
<td>23.55</td>
<td>1175</td>
<td>4.25</td>
</tr>
<tr>
<td>8 Calarene</td>
<td>25.65</td>
<td>1420</td>
<td>6.23</td>
</tr>
<tr>
<td>9 Cadinene</td>
<td>26.34</td>
<td>1529</td>
<td>0.51</td>
</tr>
<tr>
<td>10 Neophytadiene</td>
<td>28.57</td>
<td>1223</td>
<td>1.04</td>
</tr>
<tr>
<td>11 Neomenthol</td>
<td>29.50</td>
<td>1159</td>
<td>2.71</td>
</tr>
<tr>
<td>12 Geranyl acetone</td>
<td>30.43</td>
<td>1237</td>
<td>36.82</td>
</tr>
<tr>
<td>13 β-Caryophyllene</td>
<td>31.01</td>
<td>1415</td>
<td>0.45</td>
</tr>
<tr>
<td>14 Isomenthone</td>
<td>32.02</td>
<td>1192</td>
<td>1.52</td>
</tr>
<tr>
<td>15 Menthofuran</td>
<td>32.50</td>
<td>1155</td>
<td>1.10</td>
</tr>
<tr>
<td>16 Menthol</td>
<td>33.82</td>
<td>1171</td>
<td>3.51</td>
</tr>
<tr>
<td>17 1-Octen-3-ol</td>
<td>34.50</td>
<td>969</td>
<td>0.06</td>
</tr>
<tr>
<td>18 Germacrene D</td>
<td>35.90</td>
<td>1474</td>
<td>1.05</td>
</tr>
<tr>
<td>19 Geranyl acetate</td>
<td>36.50</td>
<td>1352</td>
<td>1.67</td>
</tr>
<tr>
<td>20 Terpinolene</td>
<td>37.20</td>
<td>1080</td>
<td>0.53</td>
</tr>
<tr>
<td>21 α-Caryophyllene</td>
<td>39.03</td>
<td>1230</td>
<td>1.02</td>
</tr>
<tr>
<td>22 α-Terpineene</td>
<td>40.01</td>
<td>1057</td>
<td>0.08</td>
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<tr>
<td>23 Menthol</td>
<td>42.76</td>
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<td>1.03</td>
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<td>24 Terpinen-4-ol</td>
<td>43.14</td>
<td>1159</td>
<td>0.03</td>
</tr>
<tr>
<td>25 p-Mentha-1,5-dien-8-ol</td>
<td>45.00</td>
<td>1182</td>
<td>1.47</td>
</tr>
</tbody>
</table>

Total Constituents Identified: 97.91
Yield (%): 1.58

The yield (1.58%) of essential oils from the hydrodistillation of fresh flowers of C. singueana was higher than the yields reported for other plants industrially exploited as sources of essential oils, e.g., lavender (0.8%-1.8%), mint (0.5%-1%), neroli (0.5%-1%), laurel (0.1035%), and Lippia rotundifolia (0.01%) (20). However, the yield was similar to the values reported for other Cassia species, e.g., C. senna-alata (1.43%), C. sophera (1.07%) and C. arrerehdel (1.47%) (30, 31).
Figure 1 GC Retention Profile of the Essential Oil of *C. singueana*

Table 2 Antimicrobial Activity of the Essential Oil of the Flowers of *C. singueana*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>Zones of Inhibition (mm)</th>
<th>SA</th>
<th>EC</th>
<th>PA</th>
<th>SP</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>1.0</td>
<td>33 ± 0.03</td>
<td>27 ± 0.02</td>
<td>28 ± 0.21</td>
<td>30 ± 0.30</td>
<td>32 ± 0.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>17 ± 0.50</td>
<td>13 ± 0.11</td>
<td>16 ± 0.03</td>
<td>14 ± 0.07</td>
<td>12 ± 0.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>10 ± 0.01</td>
<td>10 ± 0.03</td>
<td>12 ± 0.04</td>
<td>10 ± 0.15</td>
<td>10 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Levoxin®</td>
<td>1.0</td>
<td>31 ± 0.02</td>
<td>27 ± 0.04</td>
<td>28 ± 0.23</td>
<td>30 ± 0.03</td>
<td>30 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>17 ± 0.03</td>
<td>13 ± 0.31</td>
<td>16 ± 0.41</td>
<td>14 ± 0.13</td>
<td>12 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>10 ± 0.30</td>
<td>10 ± 0.04</td>
<td>12 ± 0.32</td>
<td>10 ± 0.20</td>
<td>10 ± 0.24</td>
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<tr>
<td>Ofloxaxin®</td>
<td>1.0</td>
<td>31 ± 0.23</td>
<td>27 ± 0.12</td>
<td>28 ± 0.31</td>
<td>32 ± 0.04</td>
<td>30 ± 0.11</td>
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</tr>
<tr>
<td></td>
<td>0.5</td>
<td>17 ± 0.12</td>
<td>13 ± 0.03</td>
<td>16 ± 0.51</td>
<td>14 ± 0.33</td>
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<td></td>
<td>0.25</td>
<td>10 ± 0.21</td>
<td>10 ± 0.31</td>
<td>12 ± 0.22</td>
<td>10 ± 0.41</td>
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<tr>
<td>Peflotab®</td>
<td>1.0</td>
<td>32 ± 0.04</td>
<td>27 ± 0.22</td>
<td>28 ± 0.08</td>
<td>30 ± 0.06</td>
<td>31 ± 0.03</td>
<td></td>
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<td></td>
<td>0.5</td>
<td>17 ± 0.03</td>
<td>13 ± 0.04</td>
<td>16 ± 0.05</td>
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<td></td>
<td>0.25</td>
<td>10 ± 0.06</td>
<td>10 ± 0.03</td>
<td>12 ± 0.51</td>
<td>10 ± 0.41</td>
<td>10 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

SA = *Staphylococcus aureus*; EC = *Escherichia coli*; PA = *Pseudomonas aeruginosa*; SP = *Streptococcus pneumonia*; CA = *Candida albicans*
The essential oil of C. singueana was found to be a better metal chelating agent than radical scavenger (DPPH and superoxide anion). Overall, the essential oil displayed significant antioxidant property comparable or better than the positive controls used in this work. Iron is the most important lipid oxidation pro-oxidant among the transition metals (37). Lipid oxidation is accelerated by breaking down of hydrogen peroxide and lipid peroxides by the ferrous state of iron to reactive free radicals through the reaction shown below:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot \text{OH} + \cdot \text{OH} + (\text{Fenton reaction})
\]

Radicals from peroxides are also produced by Fe\(^{3+}\) ion. These can lead to lipid oxidation, modification of protein and damage to DNA. Metal ions could be inactivated by chelating agents and the metal-dependent processes could potentially be inhibited (38). In the metal chelating assay, ferrozine can quantitatively form complexes with Fe\(^{2+}\). Terpenoids, which are major constituents of this essential oil generally act as antioxidants by trapping free radicals, scavenge free radicals, and chelate metals as well (39). Three main ways of antioxidant actions of terpenoids are quenching of singlet oxygen, hydrogen transfer, or electron transfer. The present study established that the essential oil had proton donating ability and could serve as free-radical inhibitor. The antimicrobial and antioxidant properties, and brine shrimp toxicity of the essential oil of the flowers of C. singueana as observed in the present study might provide some scientific rationale behind some of the traditional medicinal uses of this plant; particularly, the high level of antioxidant property of the essential oil could contribute to its traditional uses as an anti-inflammatory agent.

**Conclusion**

This is the first report on the analysis of the essential oil of the flowers of C. singueana growing in Nigeria, and geranyl acetone (36.82%) was identified as the major compound of this oil. Bioactivity studies on this oil established its potential as an antioxidant and antimicrobial agent. However, considerable level of toxicity towards brine shrimps indicated that caution should be taken before using this oil for any medicinal purposes.

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**Authors’ Contributions**

Bilkisu Adedoyin carried out the lab work, all other authors contributed equally in coming up with the concept, project planning and editing the first draft submitted by Bilkisu Adedoyin.

**Conflict of interest**

Authors declared no conflict of interest.

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