THAUMATOCOCCUS DANIELLII LEAVES: ITS CHEMICAL COMPOSITIONS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES.


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The preliminary phytochemical investigation of n-hexane, ethyl acetate and methanol extracts of Thaumatococcus daniellii leaves revealed the presence of fats and oils, terpenoids, flavonoids, steroids and glycosides. The antimicrobial tests against some strains of bacteria and fungi showed inhibitions at moderate to high concentrations. Methanol extract of the plant exhibited low 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with IC₅₀ of 615.14 µg/ml. Gas chromatography-mass spectrometry (GC-MS) characterization of n-hexane, ethylacetate and methanol extracts of T. daniellii leaves identified ten, thirteen and fifteen compounds, with tetracontane (28.76%) and L-ascorbic acid (15.07%); hexadecanoic acid (21.62%) and γ-sitosterol (11.06%); and naphthalene-1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R(1.alpha.,7.beta.,8a.alpha.) (26.90%) and hexadecanoic acid (12.60%) being the major compounds respectively. The GC-MS analysis revealed various peaks of bioactive compounds of which the antioxidant and antimicrobial activities of the plant have been attributed to the prominent compounds in synergy with all the other compounds present in smaller quantities in the extracts.

Keywords: Thaumatococcus daniellii, Antimicrobial activity, Antioxidant activity, GC-MS analysis, L-ascorbic acid, γ-sitosterol

INTRODUCTION

Thaumatococcus daniellii (Benn.) Benth which belongs to the family of Marantaceae, is a plant species from West Africa known for being a natural source of thaumatin, an intensely sweet protein which is of interest in the development of sweeteners. It is a forest under-storey herb in the Marantaceae native to equatorial Africa, where its leaves are locally used to wrap food and as material for roofing and woven mats (Abbiw, 1990). It grows throughout the hot, humid, tropical rain forest and coastal zone of West Africa. Its natural habitat is the twigs of forest trees. Thaumatococcus daniellii grows three to four meters in height, and has large, papery leaves up to 46 centimeters long. It bears pale purple flowers and a soft fruit containing a few shiny black seeds. The sturdy leaf petioles are used as tools, building materials and as wrapper for food (Chinedu, 2014). The leaves and seeds have a number of traditional uses. The fruit of the plant is used as a laxative and the seeds used as an emetic and for treating pulmonary problems. The leave sap is used in traditional medicine as antidote against venoms, stings, and bites. Leave and root sap are used as sedative and for treating psychiatric problems (Bentham et al., 1883). Large quantities of the fruits are collected by local people to sweeten over fermented palm wine and sour foods (Franke and Thieme, 1985). The plant is significant such that the leaves have locally been used for wrapping and boiling foods in Ghana and Nigeria (Yeboah, 2002; Yeboah et al., 2003).

The banning of the artificial sweetener, sodium cyclamate in the USA in 1969 has provoked extensive analytical and agronomic research on thaumatin and T. daniellii (Adansi, 1970). This research included glass house experiments in the UK (Summerfield et al., 1977; Most et al., 1978) and field plantations in Ghana, Liberia, Nigeria, and Malaysia (Onwueme et al., 1979; Witty and Higginbotham, 1994). There has been surprisingly little research on the ecology and distribution of the species or the local knowledge of people who
currently utilize it (Wojciech et al., 2005), while little pharmacological and phytochemical properties of the plant have been reported. The antibacterial, antioxidant and insecticidal activities of essential oil of the plant have been reported (Adeola et al., 2015; Adeyemi et al., 2014; Anthony et al., 2013). Hence, we report the chemical composition, antimicrobial and antioxidant properties of non-volatile extracts of T. denielli leaves.

MATERIALS AND METHODS

Sample preparation: The plant was collected from Oyo town, Oyo state, Nigeria and was identified and authenticated by a plant taxonomist, Mr. Bolu of the Department of Plant biology, University of Ilorin with a voucher number of (UILH/006/1237). The leaves of the plant were sun dried, weighed and extracted using serial exhaustive solvent extraction with three solvents, namely; n-hexane, ethyl acetate and methanol which are of dissimilar polarities.

Phytochemical screening: The screening was carried out using the modified method of Prashant et al., 2011

Antimicrobial assay: Cultures of 10 human pathogenic bacteria and fungi made up of both gram negative and gram positive were used for the assay. The bacteria species used include; Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas Aeruginosa, Salmonella typhi, Klebsiella pneumonia and the fungi species are; Candida albicans, Aspergillus niger, Penicillium notatum and Rhizopus stolon. The screening was carried out at the Department of Microbiology, University of Ibadan. Nutrient agar, sabouraud dextrose agar, nutrient broth and tryptone soya agar were used as the media in which the assays were prepared. n-Hexane, ethyl acetate and methanol were used in dissolving the extracts. The antimicrobial standard reference drugs used in this study are Gentamycin (10µg/ml) and Tioconazole (0.7 mg/ml).

Determination of antimicrobial activity

Agar diffusion pour-plate method (Bacteria): An overnight culture of each organism was prepared appropriately from its stock and inoculated each into the sterile nutrient broth of 5 ml, each incubated for 18-24 hrs at 37 °C. From overnight culture, 0.1 ml of each organism was taken and put into 9.9 ml of sterile distilled water to get (1:100) 10⁻³ M inoculum concentration of the organism. From the diluted organism (10⁻² M), 0.2 ml was taken into the prepared sterile nutrient agar, cooled to about 40-45 °C, poured into sterile petri-dishes and allowed to solidify for about 45-60 minutes. Using a sterile cork-borer of 8 mm diameter, wells were made according to the number of the test tubes for the experiment. For this work, eight wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2 hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were afterwards incubated for 18-24 hrs at 37 °C.

Agar diffusion-surface plate method (Fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in duplicates, then left to solidify properly. 0.2 ml of (1:100) 10⁻² M inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish lid to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were put into the wells accordingly including the controls. All the plates were left on the bench for 2 hrs to allow the extract diffuse properly into the agar i.e.pre-diffusion. The plates were incubated at 25 °C for 72 hrs (Collins et al., 1970).

Antioxidant activity: The ability of the samples to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed by a standard method (Tekao et al., 1994), adopted with suitable modifications (Kumarasamy et al., 2007). The stock solution of extracts were prepared in methanol to achieve the concentration of 1mg/ml. Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 and 1.99 µg/ml. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. The absorbance was measured in duplicate at varying concentrations and the mean absorbance was determined. Parallel
to examination of the antioxidant activity of plant extracts, the value for the standard/control compound (ascorbic acid) was obtained and compared to the values of the antioxidant activity and the percentage inhibitions of the serial concentrations of the methanol DPPH extracts. The percentage inhibitions at different concentrations were determined using the formula (Sies, 1997).

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

The IC_{50} values were estimated from the plot of % inhibition against concentration, using a non-linear regression algorithm.

Gas chromatography-Mass spectrometry (GC-MS) analysis of the extract: GC-MS analysis of the three plant extracts was performed with Agilent 19091GC plus automatic sampler system coupled with a quadruple Mass Spectrometer 433HP-5MS. Compounds were separated in HP5MS column fused with phenyl methyl silox (length; 30m x 250µm; film thickness 0.25µm). Samples were injected at a temperature of about 250°C with a split ratio of 10:1 with a flow rate of helium 1ml/min.

RESULTS AND DISCUSSION
The preliminary phytochemical screening of the crude extracts of *Thaumatococcus daniellii* revealed the presence of fats and oils, terpenoids, steroids, tannins, flavonoids, glycosides, saponins as shown in table 1 below. The presence of these metabolites is an indication of the pharmacological activity of the plant.

The antimicrobial results of *n*-hexane extract showed antimicrobial activity on all the test organisms (bacteria and fungi) at different concentrations with relatively high zones of inhibition compared to ethyl acetate and the methanol extracts. Ethyl acetate extract shows no zone of inhibition against two fungi, *Aspergillus niger* and *Penicillium notatum* and is active against other organisms. On the other hand, methanol extract of the plant revealed no antibacterial activity against *Klebsiella pneumoniae* and *Salmonella typhi* and antifungal activity against *Rhizopus stolonifera* (Table 2). Furthermore, the minimum inhibitory results recorded the highest inhibition for *n*-hexane extract at the concentration range of 25-200 mg/ml compared to the inhibition results of ethyl acetate and methanol extracts. These inhibitions indicate that the leaves of *T. daniellii* exhibited antibacterial and antifungal activities and hence can be used for the treatment of various infections caused by these species of bacteria and fungi.

Table 1: Phytochemical screening of hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Hexane extract</th>
<th>Ethyl acetate extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>-</td>
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<tr>
<td>Terpenoids</td>
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<td>Steroids</td>
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<td>Flavonoids</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>Fats and oils</td>
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<td>Glycosides</td>
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<td>Phenols</td>
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<td>Protein</td>
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<td>Anthraquinones</td>
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<td>Tannins</td>
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Key: + = present; - = absent
Antioxidant activity: The antioxidant activity indicates the presence of certain compounds with structural features that are able to trap and retain free radicals so as to convert them to less toxic compounds in the plant extracts. The extracts' ability (n-hexane, ethyl acetate and methanol) to scavenge DPPH radicals and reducing their effects was analyzed (Table 3 and Figure 1).

The antioxidant activity of *T. daniellii* leaves showed that the percentage inhibition of n-hexane extract ranges from high to medium values at high concentrations. For ethyl acetate, there is high inhibition at high concentrations, but medium inhibition of DPPH radicals at low concentrations. The inhibition of methanol has an anomalous value (20 %) at the highest concentration after which it increases to medium inhibition and there is no significant change afterwards. Furthermore, by comparison of the IC<sub>50</sub> of the three extracts (hexane, ethyl acetate and methanol) with the IC<sub>50</sub> of the control (ascorbic acid), it was revealed that only methanol extract showed antioxidant activity with IC<sub>50</sub> value of 615.14 mg/ml. Therefore, only the extract of methanol can effectively reduce the concentration of DPPH radical to 50%.

Table 2: Antimicrobial activity of n-hexane, ethyl acetate and methanol extracts of *Thaumatococcus daniellii* leaves

<table>
<thead>
<tr>
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<th>B.sub</th>
<th>Ps.a</th>
<th>Sal</th>
<th>K.p</th>
<th>Ca</th>
<th>A.n</th>
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Key: S.a = Staphylococcus aureus; E.c = Escherichia coli; B.sub = Bacillus subtilis; Ps.a = Pseudomonas aeruginosa; Sal = Salmonella typhi; K.p = Klebsiella pneumoniae; C.a = Candida albicans; A.n = Aspergillus niger; Pe.n = Penicillium notatum; Rhiz = Rhizopus stolonifer; +ve = Gentamycin 10 µg/ml (bacteria); Tioconazole 70% (fungi); -ve = Solvent of dilution
Table 3: DPPH Antioxidant activity and % inhibition of \textit{n}-hexane, ethyl acetate and methanol extracts of \textit{Thaumatococcus daniellii} leaves

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>MEAN</th>
<th>% I H</th>
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<tbody>
<tr>
<td>1000</td>
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<td>0.302</td>
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<td>500</td>
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<td>0.175</td>
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<tr>
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<td>0.326±0.0030</td>
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<tr>
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<td>0.366</td>
<td>0.367</td>
<td>0.367±0.0010</td>
<td>67.26</td>
</tr>
<tr>
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<td>0.427</td>
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<td>0.426</td>
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<td>61.97</td>
</tr>
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Ethyl acetate extract. Absorbance of control = 0.330

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<tr>
<th>Conc. (µg/ml)</th>
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<th>% I H</th>
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<td>59.94</td>
</tr>
<tr>
<td>31.25</td>
<td>0.387</td>
<td>0.390</td>
<td>0.390</td>
<td>0.389±0.0010</td>
<td>58.42</td>
</tr>
<tr>
<td>15.81</td>
<td>0.425</td>
<td>0.421</td>
<td>0.420</td>
<td>0.422±0.0010</td>
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<tr>
<td>7.93</td>
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<td>0.440±0.0010</td>
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<tr>
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<td>0.419</td>
<td>0.417±0.0010</td>
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<tr>
<td>1.95</td>
<td>0.430</td>
<td>0.430</td>
<td>0.433</td>
<td>0.431±0.0010</td>
<td>57.17</td>
</tr>
</tbody>
</table>

Methanol extract. Absorbance of control = 1.265

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>MEAN</th>
<th>% I H</th>
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</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.378</td>
<td>0.382</td>
<td>0.384</td>
<td>0.381±0.0010</td>
<td>20.72</td>
</tr>
<tr>
<td>500</td>
<td>0.203</td>
<td>0.194</td>
<td>0.198</td>
<td>0.198±0.0030</td>
<td>58.77</td>
</tr>
<tr>
<td>250</td>
<td>0.178</td>
<td>0.173</td>
<td>0.170</td>
<td>0.173±0.0010</td>
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<tr>
<td>125</td>
<td>0.170</td>
<td>0.169</td>
<td>0.169</td>
<td>0.169±0.0000</td>
<td>64.80</td>
</tr>
<tr>
<td>62.5</td>
<td>0.195</td>
<td>0.191</td>
<td>0.192</td>
<td>0.192±0.0020</td>
<td>59.94</td>
</tr>
<tr>
<td>31.25</td>
<td>0.198</td>
<td>0.201</td>
<td>0.201</td>
<td>0.200±0.0010</td>
<td>58.42</td>
</tr>
<tr>
<td>15.62</td>
<td>0.197</td>
<td>0.196</td>
<td>0.196</td>
<td>0.196±0.0020</td>
<td>59.18</td>
</tr>
<tr>
<td>7.8</td>
<td>0.211</td>
<td>0.214</td>
<td>0.219</td>
<td>0.215±0.0010</td>
<td>55.37</td>
</tr>
<tr>
<td>3.9</td>
<td>0.201</td>
<td>0.194</td>
<td>0.194</td>
<td>0.196±0.0020</td>
<td>59.18</td>
</tr>
<tr>
<td>1.95</td>
<td>0.207</td>
<td>0.203</td>
<td>0.208</td>
<td>0.206±0.0000</td>
<td>57.17</td>
</tr>
</tbody>
</table>

KEYS:

- % ITD H: Percentage Inhibition of Hexane extract,
- % ITD EA: Percentage Inhibition of Ethyl acetate extract,
- % ITD M: Percentage Inhibition of Methanol extract
- % IA: Percentage Inhibition of Ascorbic Acid (control)

Figure 1: IC\textsubscript{50} Antioxidant activity of \textit{n}-hexane, ethyl acetate and methanol extracts of \textit{Thaumatococcus daniellii} leaves
GC-MS characterization: This analysis characterizes and determines the number of constituents present in the extracts and their relative abundance with retention time for each sample. Each extract component was eluted at different retention time from the gas chromatograph and the mass spectrometer captured, ionized, accelerated, deflected and detected each constituent separately (Gohlke and McLafferty, 1993).

The GC-MS characterization of n-hexane extract of *T. daniellii* leaves showed a total of eleven chemical constituents (Table 4). The abundant compounds from the extract are 2-methyloctacosane (% abundance = 15.99), L-ascorbic acid (% abundance = 15.07) and tetracosane (% abundance = 28.76). Hexadecanoic acid (% abundance = 21.62), 9-octadecenamide (% abundance = 17.41), γ-sitosterol (% abundance = 11.06) and urs-2-ene (% abundance = 6.66) are the major constituents from the thirteen chemical compounds of ethyl acetate extract of the plant (Table 5). Methanol extract of *T. daniellii* leaves revealed the principal constituent as: 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, hexadecanoic acid, β-sitosterol and naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methyleneyl) {-1 R (1.alpha.,7.beta.,8a.alpha.)} with their percentage abundance of 22.12, 12.60, 4.91 and 26.90% respectively (Table 6).

### Table 4: GC-MS Analysis of the hexane Extract of *Thaumatococcus daniellii* leaves

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Retention time</th>
<th>% Abundance</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3,7 –dimethylnonane</td>
<td>C₁₇H₃₄</td>
<td>8.760</td>
<td>2.73</td>
<td>520</td>
</tr>
<tr>
<td>2</td>
<td>Phthalic acid</td>
<td>C₄H₄ClO₂</td>
<td>11.22</td>
<td>0.01</td>
<td>270</td>
</tr>
<tr>
<td>3</td>
<td>6,10,14-trimethyl-2-pentadecanone</td>
<td>C₂₀H₃₀O</td>
<td>15.259</td>
<td>2.53</td>
<td>268</td>
</tr>
<tr>
<td>4</td>
<td>Stearic acid</td>
<td>C₁₇H₃₆O₂</td>
<td>16.699</td>
<td>2.17</td>
<td>284</td>
</tr>
<tr>
<td>5</td>
<td>Phytol</td>
<td>C₁₇H₃₆O</td>
<td>18.299</td>
<td>5.99</td>
<td>296</td>
</tr>
<tr>
<td>6</td>
<td>2,6,10,14-pentamethyl-2,6,10,14-</td>
<td>C₂₀H₃₂</td>
<td>24.182</td>
<td>6.59</td>
<td>342</td>
</tr>
<tr>
<td></td>
<td>eicosapentaene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2-methyloctacosane</td>
<td>C₂₀H₄₀</td>
<td>24.525</td>
<td>15.99</td>
<td>408</td>
</tr>
<tr>
<td>8</td>
<td>L-ascorbic acid</td>
<td>C₉H₈O₂</td>
<td>25.375</td>
<td>15.07</td>
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</tr>
<tr>
<td>9</td>
<td>Chloroacetic acid</td>
<td>C₉H₉O₂</td>
<td>25.758</td>
<td>10.72</td>
<td>318</td>
</tr>
<tr>
<td>10</td>
<td>Tetracosane</td>
<td>C₄₀H₆₀</td>
<td>26.583</td>
<td>28.76</td>
<td>561</td>
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</tbody>
</table>

### Table 5: GC–MS analysis of ethyl acetate extract of *Thaumatococcus daniellii* leaves.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Retention Time</th>
<th>% abundance</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dodecanoic acid</td>
<td>C₁₀H₂₀O₂</td>
<td>10.896</td>
<td>2.56</td>
<td>200</td>
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<tr>
<td>2</td>
<td>6,10,14-trimethyl-2-pentadecanone</td>
<td>C₁₄H₃₀O</td>
<td>15.29</td>
<td>2.99</td>
<td>268</td>
</tr>
<tr>
<td>3</td>
<td>n-hexadecanoic acid</td>
<td>C₁₄H₂₉O₂</td>
<td>16.779</td>
<td>21.62</td>
<td>256</td>
</tr>
<tr>
<td>4</td>
<td>Phytol</td>
<td>C₂₀H₄₀</td>
<td>18.300</td>
<td>2.44</td>
<td>296</td>
</tr>
<tr>
<td>5</td>
<td>7-hexadecenal (Z)</td>
<td>C₁₄H₂₉O</td>
<td>18.566</td>
<td>5.96</td>
<td>238</td>
</tr>
<tr>
<td>6</td>
<td>Hexadecanamide</td>
<td>C₁₄H₂₉NO</td>
<td>18.897</td>
<td>4.27</td>
<td>255</td>
</tr>
<tr>
<td>7</td>
<td>9-octadecanamide</td>
<td>C₁₄H₂₉NO</td>
<td>20.536</td>
<td>17.41</td>
<td>281</td>
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<tr>
<td>8</td>
<td>Bis (2-ethylhexyl)phthalate</td>
<td>C₂₂H₂₆O₆</td>
<td>22.057</td>
<td>1.78</td>
<td>390</td>
</tr>
<tr>
<td>9</td>
<td>Urs-2-ene</td>
<td>C₂₀H₄₀</td>
<td>22.716</td>
<td>11.06</td>
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<tr>
<td>10</td>
<td>Squalene</td>
<td>C₂₀H₄₀</td>
<td>23.374</td>
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<tr>
<td>11</td>
<td>Squalene</td>
<td>C₂₀H₄₀</td>
<td>24.178</td>
<td>4.66</td>
<td>410</td>
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<tr>
<td>12</td>
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<td>13</td>
<td>Tetracosane</td>
<td>C₄₀H₆₀</td>
<td>26.660</td>
<td>5.80</td>
<td>562</td>
</tr>
</tbody>
</table>
The authors declare no conflict of interest.

**CONCLUSION**

The leaves of *T. daniellii* have been investigated in this research and found to contain interesting metabolites like fats and oils, flavonoids, saponins, steroids, terpenoids and glycosides. The findings of this work showed that the leaves of *T. daniellii* exhibited antimicrobial activity which supports the previous work of Noda et al., 2000. The observed antimicrobial effect may be attributed to the presence of flavonoids as it has been reported that flavonoid has anti-allergic, anti-inflammatory, anti-cancer and anti-microbial effect. This supports the ethno-medicinal uses of *T. daniellii* as antidote against venoms, stings and bites. The GC-MS analyses reveals various peaks of bioactive compounds of which the activity of the plant against bacteria and fungi have been attributed to the prominent compounds in synergy with all the other compounds present in smaller quantities in the extracts.

**Conflict of interest.**
The authors declare no conflict of interest.

**REFERENCES**


Adansi, M. A. 1970. Indigenous plants in Ghana with taste-modifying properties or sweetening principles. Ghana


