ESSENTIAL OIL COMPOSITIONS AND ANTIOXIDANT ACTIVITY OF THE FRESH LEAVES OF TALINUM TRIANGULARE (JACQ.) WILLD FROM NIGERIA


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Received: 01-06-2023
Accepted: 20-07-2023

ABSTRACT
Essential oils from the fresh leaves of Talinum triangulare (100 g) growing along Ijesha road, Ago Iwoye, Ijebu north local government area of Ogun State, Nigeria were obtained by hydrodistillation in an all-glass clevenger type apparatus and the chemical constituents determined by gas chromatography coupled with mass spectrometry (GC-MS). The antioxidant activity of the essential oils was tested with 1,1 diphenyl 2 picrylhydrazyl (DPPH). The yield of the essential oils was 0.060% (v/w). A total of thirteen constituents representing 100% of the essential oil were identified from the GC-MS analysis. The major components of T. triangulare essential oil were γ-terpinene (26.34%), d-limonene (16.08%), β-pinene (13.91%), β-bisabolol (12.62%), cyclotrisiloxane hexamethyl (8.18%) and caryophyllene (5.65%). Monoterpenes (61.30%), sesquiterpenoids (14.14%), sesquiterpenes (11.72%) and organosilicon (12.84%) were the classes of compounds identified from the essential oil. The leaves essential oil of T. triangulare displayed potent antioxidant activity with percentage inhibition values of 51.886, 54.209, 58.447, 62.137 and 66.648 at varying concentrations. T. triangulare exhibited the highest inhibition (66.648) at concentration of 0.5 mg/mL as against the standard of 70.36 at similar concentration which makes it a good antioxidant agent.

Keywords: Talinum triangulare, hydrodistillation, essential oil, GC-MS, γ-terpinene, antioxidant activity, monoterpenes.

INTRODUCTION
The genus Talinum belongs to the family Portulacaceae, consisting of an erect perennial herb with swollen roots and succulent stems, 30-100 cm tall. The branches have two lateral basal buds with leaves which are spirally arranged to nearly opposite and often crowded at the top of the stem. Talinum flowers early year-round, is mainly self-pollinating, flowers are pink in color and open in the morning. The genus Talinum consists of 50 species of semi succulent herbs or sub shrubs distributed in the tropics and sub tropics of America, Africa and Asia McNeil (1974). According to Veselova et al., (2012) members were initially part of Portulacaceae but later segregated to the family Talinaceae.

Nigeria has a rich germplasm of Talinum and five species were identified namely: T. triangulare (Jacq.) Willd, T. fruticosum (L)
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Talinum triangulare (Jacq.) commonly called water leaf is an herbaceous perennial plant, having a well developed stem above ground and widely grown in tropical regions as a leaf vegetable (Ezekwe et al., 2001). It is called ‘gbure’ in Yoruba land, ‘nte oka’ or ‘ofe bekee’ in Ibo land and largely consumed as vegetables which can easily reseeds itself once established (Adodo, 2004).

The plants contain vitamins, essential amino acids, minerals, high total lipids, essential oils, \( \alpha \)-tocopherols, \( \beta \)-tocopherols and antioxidants (Sridhar and Lakshminarayana, 1993; Fasuyi, 2006). Preliminary phytochemical studies on T. triangulare revealed the presence of omega-3 fatty acids, high levels of essential nutrients like minerals (such as calcium, potassium and magnesium), soluble fibers (such as pectin), \( \beta \)-carotene, vitamin C, \( \alpha \) and \( \beta \)-tocopherols (Ezekwe et al., 2002). Qualitative phytochemical screening also revealed the presence of alkaloids, tannins, phenols, flavonoids, terpenoids, and saponins while the leaf extract was devoid of glycosides, steroids and carbohydrates (Ameh and Eze, 2010; Ilodibia and Igboabuchi, 2017). Monoterpenes found in plants are also used as antioxidant, anticancer, antidiabetic, food flavours, food additives, cosmetics, perfumes and pharmaceuticals (Lota, 2002; Marie et al., 2007).

Similarly, the gas chromatography analysis of an aqueous leaf extract of T. triangulare screened for the presence of bioactive molecules revealed the presence of limonene (65.1%), camphor (5.0%), 1,8-cineole (3.7%), terpinen-4-ol (2.9%), borneol acetate (2.4%), geranyl acetate (2.2%), neral (1.9%), borneol (1.6%), \( \beta \)-pinene (1.5%), camphene (1.5%), sabinene (1.4%), neryl acetate (1.1%), citronellol (1.1%), and \( \beta \)-amyrin (1.0%) as the main constituents of the terpenoid fraction (Ikewuchi et al., 2017). In addition, ten known carotenoids (mainly 50.42% carotene and 33.30% lycopene), nine benzoic acid derivatives (mainly 84.63% ferulic acid and 11.92% vanillic acid), and six hydroxycinnamates (mainly 55.44% p-coumaric acid and 44.46% caffeic acid) were detected. Also identified were eight lignans (mainly 88.02% retusin) and thirty flavonoids (mainly 50.35% quercetin and 39.36% kaempferol) (Ikewuchi et al., 2017).

Similar report revealed that Gas Chromatography Mass Spectrometric (GC-MS) analysis of ethanolic extract from T. triangulare is composed of fifteen compounds which the major constituents are; Stigmasta-5,22-dien-3-ol (3.BETA.,22E) (44.4), campesterol (30.1), 1,54-dibromo tetrabromocamphane (5.89), glycidyl oleate (3.67), Oleic acid (2.98), 9-octadecenoic acid(E)- (2.78), oxirane, hexadecyl-(1.74) and 9-octadecenoic acid (Z)-, methyl ester (1.27). And among other constituents at <1% are ([1Z]-1,3-diphenyl-1-pentenyl]oxy)-trimethyl) silane and cyclopentasiloxane decamethyl- (Ananthi and Pravina, 2021).

According to traditional medicine the leaves of T. triangulare are used to treat polyuria, inflammations, diuretic, stomach troubles, sore throat (Burkill, 1994; Khare, 2007), gastrointestinal disorders (Mensah et al., 2008), bacterial infections, diabetes mellitus (Aja et al., 2010; Eleazu and Eleazu, 2013), antidiabetic, hypoglycaemic, antiulcer, antimicrobial, antioxidant activities (Fasuyi, 2006), anti-inflammatory properties, high cholesterol, high kaempferol content, hypertension (Teugwa et al., 2013; Ijomone and Ekpe, 2016; Olorunnisola et al., 2016; Oguntibeju and Okaiyeto, 2021), hepatic ailments and cancer (Liang et al., 2011). It is also used to treat shistosomiasis, scabies, fresh cuts, high blood pressure, anemia, stroke, obesity and can function as immune-stimulant...
In spite of the reported local use of the *Talinum triangulare* in the management of diseases, there is still a paucity of information on the essential oil constituents. Therefore, the present study was designed to investigate the chemical composition and antioxidant activity of essential oil from *Talinum triangulare* (Jacq.).

**MATERIALS AND METHODS**

**Collection of the leaves of *T. triangulare* and extraction of essential oils**

Large quantities of the leaves of *T. triangulare* were obtained from Ijesha Road, Ago Iwoye, Ijebu north local government area, Ogun State, Nigeria. The plant was taxonomically identified at the Department of Botany, Olabisi Onabanjo University Ago Iwoye.

A 100 g of the fresh leaves was pulverized and subjected to hydrodistillation experiment using an all-glass Clevenger-type apparatus. The sample was introduced into a 5 L flask after which distilled water was added until it covered the sample completely. The distillation time of the essential oil was 3 h and conducted at normal pressure according to an established procedure (British Pharmacopoeia, 1980). The volatile oils which distilled over water were collected separately into clean and previously weighed sample bottles. The oils were kept under refrigeration (4°C) until the moment of analyses. The essential oil yield (%) was calculated by mass (g) of the essential oils divided by the mass (g) of the leaves of the plants.

**GC-MS Analysis of the essential oils of *T. triangulare***

The analysis of the chemical constituents of the leaves essential oil of *T. triangulare* was achieved using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with an FID and fitted with HP-5MS column (Agilent Technologies, Santa Clara, California, USA) of dimension 30 m × 0.32 mm with a film thickness of 0.25 μm. The analytical conditions employed in the GC analysis were: carrier gas He with flow rate of 2 mL/min, while both the injector temperature (PTV: programmable temperature vaporization) and detector temperature were maintained at 250°C and 260°C, respectively. The column was temperature programmed from 80°C, with a 2 min hold, to 240°C (6 min hold) at a rate of 8°C/min. The essential oil (1.0 μL; 10% n-hexane solution) was injected using a split mode with a split ratio of 10:1, at inlet pressure was 6.1 kPa. Quantification was done using the calibration curves generated from the analyses of representative standard compounds from each class.

**Identification of the constituents of the essential oils of *T. triangulare***

The identification of constituents of essential oils from the GC-MS spectra of *T. triangulare* was performed on the basis of comparison of retention indices with reference to a homologous series of n-alkanes (C₆-C₄₀), under identical experimental conditions. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition in literature (Joulain and Koenig 1998; NIST 2019) as described recently (Dung et al., 2021).

**Antioxidant activity assays**

The antioxidant activity was determined using methanol solution of 1, 1- diphenyl-2-
picrylhydrazyl (DPPH) reagent. The stock solution of the plant was prepared in methanol to achieve a concentration of 10mg/mL. Dilutions were made to obtain concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml. Ascorbic acid was used as a standard in 1-100 µg/mL concentration. The dilutions each were mixed with 1mL of DPPH. After 30 min in darkness at room temperature, the samples were analyzed using the T90+UV/VIS spectrometer. The absorbance was recorded at 517 nm.

The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

$$\text{AA} = \frac{A_c - A_s}{A_c} \times 100$$

Where

$A_c$ = the absorbance of control.

$A_s$ = the absorbance of the test (sample).

$\text{AA}$ = the antioxidant activity.

**RESULTS**

**Chemical composition of T. triangulare essential oil**

The hydrodistillation of the leaves produced light-yellow colored essential oil with percentage yield of 0.060% (v/w). The chromatogram of the various components and compounds identified by GC-MS were presented in Fig. 1 and Table 1. The result revealed that T. Triangularare is composed of thirteen constituents representing 100% of the essential oil. The composition of the essential oil was dominated by monoterpenes (61.3%). Other classes of compounds present are; sesquiterpenoids (14.14%), sesquiterpenes (11.72%) and organosilicon (12.84%). The main constituents of T. triangulare were; γ-terpinene(26.34%), d-limonene (16.08%), β-pinene (13.91%), β-bisabolol (12.62%), cyclohexamethyl (8.18%) and caryophyllene (5.65%). In addition, (+)-4-carene (4.97%), thymol, TMS derivative (4.66%), humulene (2.49%), (E)-phytol (1.52%), cis-gamma-bisabolene (1.44%), cadina-1(6)-4-diene (1.24%), were the other compounds present in amount >1% while the least of the component identified was copaene (0.90%).

![Chromatogram of identified compounds of essential oils from T. triangulare](image-url)
Antioxidant activity of *T. triangulare* essential oil

The investigated essential oil of *T. triangulare* determined on the basis of scavenging activity of the free radical DPPH demonstrated good antioxidant abilities to reduce DPPH (Table 2) with the highest inhibition of 66.648% at concentration of 0.5 mg/mL.

**DISCUSSION**

Since this is the first report on the essential oil based on available information from any parts of *T. triangulare*, the present data could not be compared with other analyzed samples of the same species. However, GC analysis of an aqueous leaf extract of *Talinum triangulare* showed the presence of limonene (65.1%), β-pinene (1.5%) and other terpenes which are also found in the essential oil constituents but with varying percentage (Ikewuchi *et al.*, 2017).

Furthermore, the quantitative amount of γ-terpinene (26.34%) in the oils is noteworthy, since it has not been previously reported as a major constituent of *T. triangulare* essential oil.

In addition, fifteen compounds were identified in the ethanolic extract by GC-MS from *T. triangulare* and among its constituents are organosilicon which were also found in the essential oil though with varying quantity (Ananthi and Pravina, 2021).

Essential oil from plants is predominated by terpene compounds and this is also confirmed by the present study (Ikewuchi *et al.*, 2017). Monoterpenes (61.3%) which is the major class of compound are generally used as antioxidant, anticancer, antidiabetic, food flavours, food additives, cosmetics and pharmaceuticals (Lota, 2002; Marie *et al.*, 2007). Similarly, γ-terpinene (26.34%) which is the main constituents is used as flavours, cosmetics, pharmaceuticals, perfumes, and antioxidant. The identified monoterpenes and γ-terpinene may be responsible for the use of the plants as anti diabetic, hypoglycaemic, antiulcer, antioxidant activities and anti-cancer (Fasuyi, 2006; Aja *et al.*, 2010; Liang *et al.*, 2011).

### Table 1: Percentage composition of essential oils from the fresh leaves of *Talinum triangulare*

<table>
<thead>
<tr>
<th>Compounds (common name)</th>
<th>Kovat index</th>
<th>Retention time</th>
<th>Percentage</th>
<th>Class</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. β- pinene</td>
<td>828</td>
<td>3.528</td>
<td>13.91</td>
<td>Monoterpene</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
</tr>
<tr>
<td>2. D-Limonene</td>
<td>1000</td>
<td>4.260</td>
<td>16.08</td>
<td>Monoterpene</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
</tr>
<tr>
<td>3. Gamma-terpinene</td>
<td>1000</td>
<td>4.706</td>
<td>26.34</td>
<td>Monoterpene</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
</tr>
<tr>
<td>4. (+) 4- careen</td>
<td>1046</td>
<td>5.130</td>
<td>4.97</td>
<td>Monoterpene</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;</td>
</tr>
<tr>
<td>5. Copaene</td>
<td>1438</td>
<td>9.267</td>
<td>0.90</td>
<td>Sesquiterpene</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
</tr>
<tr>
<td>6. Caryophyllene</td>
<td>1500</td>
<td>9.850</td>
<td>5.65</td>
<td>Sesquiterpene</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
</tr>
<tr>
<td>7. Humulene</td>
<td>1500</td>
<td>10.308</td>
<td>2.49</td>
<td>Sesquiterpene</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
</tr>
<tr>
<td>8. Cis-gamma-bisabolene</td>
<td>1500</td>
<td>11.143</td>
<td>1.44</td>
<td>Sesquiterpene</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
</tr>
</tbody>
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<p>| | | | | | |</p>
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</thead>
<tbody>
<tr>
<td>9.</td>
<td>Cadina-1(6),4-diene</td>
<td>1500</td>
<td>11.212</td>
<td>1.24</td>
<td>Sesquiterpene</td>
</tr>
<tr>
<td>10.</td>
<td>β-bisabolol</td>
<td>1500</td>
<td>13.032</td>
<td>12.62</td>
<td>Sesquiterpenoid</td>
</tr>
<tr>
<td>11.</td>
<td>Phytol</td>
<td>1255</td>
<td>18.582</td>
<td>1.52</td>
<td>Sesquiterpenoid</td>
</tr>
<tr>
<td>12.</td>
<td>Cyclotrisiloxane, hexamethyl</td>
<td>607</td>
<td>33.408</td>
<td>8.18</td>
<td>Organosilicon</td>
</tr>
<tr>
<td>13.</td>
<td>Thymol, TMS derivative</td>
<td>600</td>
<td>34.089</td>
<td>4.66</td>
<td>Organosilicon</td>
</tr>
</tbody>
</table>

| Monoterpenes |   | 61.30 |
| Sesquiterpenes |   | 11.72 |
| Sesquiterpenoids |   | 14.14 |
| Organosilicon |   | 12.84 |
| **Total** |   | **100** |

Table 2: Free radical scavenging activity of essential oils from the fresh leaves of *Talinum triangulare*

<table>
<thead>
<tr>
<th>Ascorbic acid</th>
<th>TTWL</th>
<th>Absorbance at 517 nm</th>
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</thead>
<tbody>
<tr>
<td>Concentration (mg/ml)</td>
<td>Mean/SD</td>
<td>% inhibition</td>
</tr>
<tr>
<td>0.1</td>
<td>0.130±0.006</td>
<td>68.67</td>
</tr>
<tr>
<td>0.2</td>
<td>0.123±0.014</td>
<td>70.36</td>
</tr>
<tr>
<td>0.3</td>
<td>0.131±0.002</td>
<td>68.43</td>
</tr>
<tr>
<td>0.4</td>
<td>0.126±0.004</td>
<td>69.64</td>
</tr>
<tr>
<td>0.5</td>
<td>0.123±0.006</td>
<td>70.36</td>
</tr>
</tbody>
</table>

The free radical scavenging power of the leaf essential oil of *T. triangulare* was found to be dose dependent (Table 2) as evident in the rapid reduction of the stable DPPH radical and thus, may be considered good sources of antioxidants for nutrition, medicine and commercial use (Ayoola *et al.*, 2008). The result also revealed that the essential oil of *T. triangulare* possesses fairly good radical scavenging activities when compared to that of ascorbic acid at all the concentrations. The report of Anyasor *et al.*, (2010) is in agreement with current study in terms of moderate antioxidant activity of *T. triangulare*.
plant. It was also found that the interesting antioxidant effect may be attributed to the presence of flavonoids and other phenolic compounds (Ameh and Eze, 2010; Meghashri et al., 2010; Ramalingam et al., 2013; Ilobia and Igboabuchi, 2017).

In most such studies, phenolics, due to their chemical structures that allow them to donate hydrogen to free radicals, were introduced as the major factor contributing to the antioxidant activity of the species (Ang et al., 2015). Moreover, essential oils consisting of phenolic monoterpenes and/or sesquiterpenes have been recognized for their higher antioxidative capacity (Mancini et al., 2015). This method was also considered easy, simple and valid to evaluate the scavenging activity of any compound as a result of its ability to donate hydrogen (Maizura et al., 2011; Ademola et al., 2014; Atewolara-Odule et al., 2020).

CONCLUSIONS

The GC-MS analysis of the essential oil revealed significant quantity γ-terpinene (26.34%), d-limonene (16.08%), β-pinene (13.91%), β-bisabolol (12.62%), cyclotrisiloxane hexamethyl (8.18%) and caryophyllene (5.65%), while copaene (0.9%) is the least component. The essential oils also displayed varying degree of antioxidant activity. The essential oil of T. Triangulare exhibited the highest percentage inhibition (66.648) at concentration of 0.5 mg/mL while the least is 51.886 at 0.1 mg/mL. Therefore, the essential oils of T. triangulare may have potential application as antioxidant agents.

Acknowledgment

Authors are grateful to Ogunmade Ilemobayo for his assistance in the extraction of the oil sample.

Conflict of interest

Authors declare that there is no conflict of interest related to this study.

REFERENCES


