Chemical composition of *Tetracarpidium conophorum* (African Walnut) seeds grown in Nigeria

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**ABSTRACT**

*Tetracarpidium conophorum* is one of the medicinal plants of importance belonging to the family Euphorbiaceae. Investigations aimed at identifying the major chemical compounds in dichloromethane seed extracts *T. conophorum* using Gas chromatography-Mass spectrometry (GC-MS) method was carried out. The amino acids were extracted using dichloromethane and analyzed with High Performance Liquid Chromatography (HPLC). A total of ten compounds were identified which amounted to 100% of the total oil composition. The major abundant compounds in the essential oil was Diethyl Phthalate (76.94%), followed by minute concentrations of 9,12-Octadecadienoic acid (Z,Z) (6.42%), Phthalic acid, decyl isobutyl ester (4.06%), Oleic acid (2.99%), 1-Eicosene (2.87%), Spiro[3.5]nonan-1-one, 5-methyl-, trans (1.75%), Cycloeicosane (1.44%), Hexadecanoic acid, methyl ester (1.34%), 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester (1.2%) and 8-Hexadecenal, 14-methyl-, (Z) (0.9%). A total of seven essential amino acids were identified. The major essential amino acid was Lycine (20.38 mg/100g) while the lowest was Isoleucine (5.24 mg/100g). The order depending on the contents of the essential amino acids in *T. conophorum* seeds was Lycine (20.38) > Threonine (18.58) > Methionine (16.21) > Phenylalanine (13.47) > Valine (13.30) > Leucine (6.60) > Isoleucine (5.24).

**Keywords:** Essential oils, Essential amino acids, *Tetracarpidium conophorum*, medicinal plants, herbal medicine.

**INTRODUCTION**

The dogma “Let food be thy medicine and medicine be thy food”, advocated almost 2,600 years ago by Hippocrates, is inheriting revived enthusiasm. Man’s relentless quest for medicinal plants that could serve as sufficient sources of nutrients, and alleviate diseases has always been a continuous endeavor (Abebe and Ayehu, 1993). Majority of the populace in Nigeria and other developing countries subscribe to the use of herbal medicines and formulation because of cost, accessibility and availability (Nwaichi and Osuoha, 2018) and African walnut is one of them.

African walnut or Nigerian walnut popularly known in the scientific world as *Tetracarpidium conophorum* is said to lower the risk of heart diseases, and numerous clinical (Edem et al., 2009) and scientific trials (Nwaichi et al., 2017) have confirmed the medicinal prowess of this plant. It is known in eastern part of Nigeria as “UKPA” and is prevalent in every part of the country. The seed has also been reported to improve numerous illnesses, promote weight loss and enhance man’s health generally (Nwaichi et al., 2017).

Furthermore, according to Soumya et al. (2009), the better appreciation of the plant derived medicine relies upon two factors that go hand in hand. One yardstick involves the identification of the active compound by means of the chemical analysis and the other is the proof to show that the formulated medicine does what it is claimed to do. Taking into consideration the high cost of drugs and side effects attached to them, there has been a strict diversion towards evaluating the essential oil, nutritive value and chemical composition of tropical plants, of which...
many are medicinal (Repetto and Llesuy, 2002).

MATERIALS AND METHODS

Sample collection

Fresh samples of *T. conophorum* was obtained from the famous Eke-Ukwu market located in Owerri metropolis Imo State, Nigeria and air dried after due identification.

Extraction of dried samples

Prior to extraction, *T. conophorum* was cooked for 2 h thirty minutes (Nwaichi et al., 2017) before grinding in order to simulate a real eating state. Milled sample (10 g) was extracted in 20 mL dichloromethane (DCM) after soaking for five days in a well stoppered bottle. The mixture was vigorously agitated and was left to stand for five days. The crude extract was gathered by sieving into a quartz beaker, the process was repeated two more consecutive times. The combined aliquot collected was concentrated on a steam bath to about 5 ml. This was purified by passing through a Pasteur pipette on a membrane and air dried to about 2 ml for gas chromatographic analysis.

Gas chromatography-mass spectrometry analysis

The extract of the sample was subjected to GC/MS analysis, this group of powerful instruments interfaced helped to characterize the various compositions. The gas chromatographic Model: 7890A (GC) analysis was performed on an Agilent Technologies interfaced with Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70 v with an ion source temperature at 250°C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5 (30 mm X 0.25mm X 0.320μm) was used as the stationary phase. The oven temperature was at 60°C held for 0.5 minute and ramped to 140°C at the rate of 4°C/minutes holding for a minute, then ramped to 280 degrees while holding for 5 minutes at the rate of 8°C/minutes. 1 μl was auto injected. The split ratio was 1:5. Retention indices for all components were determined according to the Van Den Dool method (Dool and Kratz., 1963), using n-alkanes as standard. Identification of the components was based on the comparison of their mass spectra with those of internal (computer) library, NIST libraries and some reference compounds and those described by Adams (1995).

Amino acid analysis

Ground sample (3 mg) was hydrolyzed with HCl 6 M at 150°C for 6 h. After hydrolysis, the acid was removed by rotary evaporation (RE500 Yamato Scientific America Inc.). Sample was resuspended on 2 ml of sodium citrate buffer pH 2.2. Sample derivation was achieved adding o-phthalaldehyde (OPA) 7.5 mM to the sample on citrate buffer (OPA reagent contains β-mercaptoethanol and Brij 35). The HPLC method precision and accuracy was evaluated using external and internal standards. The amino acid reference standard consisted on fifteen amino acids (0.05 μmoles mL-1 each amino acid) and was utilized to determine the retention times for each amino acid. As well, internal standard α-amino butyric (0.05 μmoles mL-1) was added to amino acid reference standard and each plant sample to normalize and quantify the amino acid content.

A gradient mobile phase of sodium acetate 0.1 M pH 7.2 and methanol (9:1) elute sample for amino acid separation trough C18 column reversed-phase octadecyl dymethylsilane particles (100 x 4.6 mm x 1/4” Microsorb 100-3C18). Fluorescence detection was realized using an excitation-emission wavelength of 360 and 455 nm respectively. Star Chromatography workstation (Varian version 5.51) software was used to achieve amino acid peak integration.

RESULTS

The specific compounds, their retention times in minutes, percentage total, molecular weights and structures of the ten volatile components of the seeds of *T. conophorum* are presented in Table 1a-d. Data from this study uncovered the presence of ten essential oils. From the results, Diethyl phthalate (74.88%) was the abundant essential oil constituents of *T. conophorum* seed oil, followed by 9,12-Octadecadienoic acid (Z,Z)- while 8-Hexadecenal, 14-methyl-, (Z)- (0.9%) was the least compound identified. The essential amino acid contents (Table 2) showed that lysine was the essential amino acid detected in the largest amount in this study (20.38 ± 0.15 mg/100g), followed by threonine and phenylalanine at the values of 18.58 ± 0.24 and 13.47 ± 0.41 mg/100g, respectively. Isoleucine had the lowest concentration at a value of 5.24 ± 0.09 mg/100g.

DISCUSSION

Plants serving as nutrients for man are progressively playing unrestrained and remarkable role in the maintenance of human health because of their medicinal prowess. The gigantic reliance on therapeutic plants in developing countries was highlighted by the World Health Organization where it was projected that about 80% of the populace in these countries depend heavily on herbal medicines for basic health care (WHO, 2001). The therapeutic and medicinal potentials of plants were attributed to the active compounds inherent in them.
Table 1a: Chemical constituents of the essential oil of the seeds of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Total (%)</th>
<th>Molecular formular</th>
<th>Molecular weight (gmol⁻¹)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl Phthalate</td>
<td>12.19</td>
<td>33.44</td>
<td>C₁₂H₁₄O₄</td>
<td>222.23</td>
<td><img src="mainlib" alt="Structure" /> Phthalic acid, isobutyl octadecyl ester</td>
</tr>
<tr>
<td></td>
<td>12.36</td>
<td>25.04</td>
<td>C₁₂H₁₄O₄</td>
<td>222.23</td>
<td><img src="mainlib" alt="Structure" /> Phthalic acid, isobutyl octadecyl ester</td>
</tr>
<tr>
<td></td>
<td>12.40</td>
<td>15.62</td>
<td>C₁₂H₁₄O₄</td>
<td>222.23</td>
<td><img src="mainlib" alt="Structure" /> Phthalic acid, isobutyl octadecyl ester</td>
</tr>
<tr>
<td></td>
<td>13.03</td>
<td>2.82</td>
<td>C₁₂H₁₄O₄</td>
<td>222.23</td>
<td><img src="mainlib" alt="Structure" /> Phthalic acid, isobutyl octadecyl ester</td>
</tr>
<tr>
<td>Phthalic acid, decyl isobutyl ester</td>
<td>16.08</td>
<td>4.06</td>
<td>C₂₂H₃₄O₄</td>
<td>362.50</td>
<td><img src="mainlib" alt="Structure" /> Phthalic acid, isobutyl octadecyl ester</td>
</tr>
<tr>
<td>Hexadecanoic acid, methyl ester</td>
<td>16.68</td>
<td>1.34</td>
<td>C₁₇H₃₄O₂</td>
<td>270.45</td>
<td><img src="mainlib" alt="Structure" /> Hexadecanoic acid, methyl ester</td>
</tr>
</tbody>
</table>

(Nwaichi et al., 2017).

The human body is supplied with these bioactive compounds (Tables 1a-d and 2) via consumption of spices, seeds, some dietary supplements and aromatic herbs. Lemon grass, dill, black pepper, caraway, cherry, citrus peels, to mention but a few, are some of the sources of essential oils in our diets. On this account, humans are unavoidably exposed to essential oils through our ecosystem and diet. Information on the accurate evaluation of essential oil ingestion in human is minimal. Most of the time, due to the nature of the size and lipophilic character of volatile compounds, they easily migrate across the blood
Table 1b: Chemical constituents of the essential oil of the seeds of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Total (%)</th>
<th>Molecular formular</th>
<th>Molecular weight (gmol⁻¹)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester</td>
<td>17.11</td>
<td>1.2</td>
<td>C₁₂₂₃₅O₄₂</td>
<td>362.50</td>
<td><img src="image1.png" alt="structure" /></td>
</tr>
<tr>
<td>8-Hexadecenal, 14-methyl-, (Z)-</td>
<td>18.25</td>
<td>0.95</td>
<td>C₁₇₂₈O₂</td>
<td>252.43</td>
<td><img src="image2.png" alt="structure" /></td>
</tr>
<tr>
<td>Spiro[3.5]nonan-1-one, 5-methyl-, trans</td>
<td>18.56</td>
<td>1.75</td>
<td>C₁₀₁₆O</td>
<td>152.23</td>
<td><img src="image3.png" alt="structure" /></td>
</tr>
</tbody>
</table>

brain barrier and are easily absorbed from the food matrix (Abdelouaheb and Amadou, 2012). The GC/MS characterization of cooked *T. conophorum* seeds showed the existence of ten compounds. The recognized compounds of the dried seeds of *T. conophorum*, their retention times, percentage composition, molecular formular, molecular weight and their structures are shown in Table 1a-d. These 10 identified compounds amounted to 86.2% of the total oil composition. The GC/MS chromatogram displayed in (Figure 1) demonstrates the retention time in minutes of the identified compounds on the X-axis and the corresponding percentage peaks and the intensity of various compounds at different retention times on the Y-axis.
Table 1c: Chemical constituents of the essential oil of the seeds of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Total (%)</th>
<th>Molecular formula</th>
<th>Molecular weight (gmol⁻¹)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycloeicosane</td>
<td>18.88</td>
<td>1.44</td>
<td>C₂₀H₄₀</td>
<td>280.53</td>
<td></td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid (Z,Z)-</td>
<td>19.68</td>
<td>2.757</td>
<td>C₁₈H₃₂O₂</td>
<td>280.44</td>
<td></td>
</tr>
<tr>
<td>9,12-Octadecadienoic acid (Z,Z)</td>
<td>19.90</td>
<td>2.945</td>
<td>C₁₈H₃₂O₂</td>
<td>280.44</td>
<td></td>
</tr>
<tr>
<td>1-Eicosene</td>
<td>19.86</td>
<td>2.87</td>
<td>C₂₀H₄₀</td>
<td>280.53</td>
<td></td>
</tr>
</tbody>
</table>

The major abundant compound in the essential oil was Diethyl Phthalate (76.94%), followed by minute concentrations of 9,12-Octadecadienoic acid (Z,Z)- (6.42%), Phthalic acid, decyl isobutyl ester (4.06%), Oleic acid (2.99%), 1-Eicosene (2.87%), Spiro[3.5]nonan-1-one, 5-methyl-, trans (1.75%), Cycloeicosane (1.44%), Hexadecanoic acid, methyl ester (1.34%), 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester (1.2%) and 8-Hexadecenal, 14-methyl-, (Z)- (0.9%). These identified compounds present in minute concentrations have numerous biological activities particularly in the pharmaceutical industry. For instance, Sermakkani and
**Table 1d**: Chemical constituents of the essential oil of the seeds of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Total (%)</th>
<th>Molecular formula</th>
<th>Molecular weight (g/mol)</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>19.97</td>
<td>1.261</td>
<td>C_{18}H_{34}O_{2}</td>
<td>282.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.32</td>
<td>1.734</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The individual essential oils and their structures were determined using GC/MS and their structures and molecular weight were confirmed using (NIST) National Institute of Standard Test database.

**Table 2**: Essential Amino acid composition of the seeds of *Tetracarpidium conophorum*.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Concentration (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>13.30 ±0.14</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.60 ±0.41</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>5.24 ±0.09</td>
</tr>
<tr>
<td>Methionine</td>
<td>16.21 ±0.57</td>
</tr>
<tr>
<td>Threonine</td>
<td>18.58 ±0.24</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>13.47 ±0.41</td>
</tr>
<tr>
<td>Lycine</td>
<td>20.38 ±0.15</td>
</tr>
</tbody>
</table>

Values are in means ±standard deviation (M±SD) of triplicate determinations.

**Figure 1**: GC/MS Chromatogram of the DCM extract of the dried seeds of *Tetracarpidium conophorum*.

Thangapandian (2012) reported that 9,12-Octadecadienoic acid (Z,Z) (Table 1c) possesses hypocholesterolemic, hepatoprotective, anti-inflammatory, anti-arthritic, nematicidal and anti-histaminic biological activities while
the compound Hexanedicarboxylic acid methyl ester (Table 1a) detected in T. conophorum has been reported by Hema et al. (2011) to possess in vivo and in vitro hypocholesterolemic, anti-oxidant, anti-fungal and nematicidal activities. Many fatty acids such as Oleic acid (Table 1d) identified as one of the phyto-compounds possesses anti-fungal and anti-bacterial potentials and has widely been touted as a healthy source of fat in diet by virtue of its saturation (Russel, 1991). Recently, Nwaichi et al. (2017) highlighted the hyperlipidemic potentials of cooked T. conophorum seeds and their findings may not be unconnected to the presence of Hexanedicarboxylic acid methyl ester (Table 1a) identified in the present study.

However, the heavy presence of diethyl phthalate (76.94%) (Table 1a), as the abundant compound in the extract along with phthalic acid, decyl isobutyl ester (Table 1a) and 1, 2-benzenedicarboxylic acid and butyl 8-methylnonyl ester (Table 1b), may pose great threat to humans despite their curative potentials. These compounds generally called phthalates are phthalic acid esters and are principally utilized to augment the flexibility of conventional polymers (Sears and Darby, 1982). To a reasonable degree, they are inevitable part of modern life and are utilized in so many consumer end products such as adhesives, building materials, lubricants, paints, electronics and medical devices (Shea, 2003; Horn et al., 2004). In humans, multiple biochemical processes have been affected by phthalates of which damage to sperm, infertility and effects on reproduction are some of them (Roza et al., 2002), allergies (Bornehag et al., 2004) and early puberty inception in females (Wolff et al., 2010). Oral administration of diethyl phthalate has been reported to induce hepatotoxicity (Heena and Ramtej, 2013).

More recently, Okon and Atai (2014) stated that although T. conophorum appears to promote the biosynthesis and secretion of fertility hormones in Wistar rats, there was a clear evidence of toxic damage to the spermatozoa and this may not be unconnected with the presence of diethyl phthalate and other phthalate esters reported in this study. If these results in rats can be extrapolated to man, there may be need for caution on the excessive consumption of T. conophorum especially in males with fertility challenges (Okon and Atai, 2014).

Furthermore, amino acids apart from forming the building blocks of proteins and serving as intermediates in cellular metabolism play a significant role is sustaining health and vitality. Essential amino acids are those amino acids that the body cannot synthesize and they must be obtained from our diets (Olusanya, 2008). Numerous diseases have been linked to the deficiency of essential amino acids.

The compositions of seven essential amino acids detected in T. conophorum seeds are shown in Table 2. The essential amino acids Valine, Leucine, Isoleucine, Methionine, Threonine, Phenylalanine, and Lycine, were detected in T. conophorum seeds. As demonstrated in Table 2, the highest content of essential amino acid was Lycine (20.38 mg/100g), while the lowest was Isoleucine (5.24 mg/100g). The order depending on the contents of the essential amino acids in T. conophorum seeds was Lycine (20.38) > Threonine (18.58) > Methionine (16.21) > Phenylalanine (13.47) > Valine (13.30) > Leucine (6.60) > Isoleucine (5.24).

Lysine is essential for both receptor-dependent pro-inflammatory and receptor-independent cytoytic activities (Cheung et al., 2014) and lysine is a precursor compound of histidine and arginine. Arginine is an essential amino acids in children because of its role in their development and growth. However, elevated concentration of lysine influences the absorption of arginine negatively in vivo (Wu and Meininger, 2002).

Methionine is a sulfur-containing amino acid which is needed in the human diet. Deficiency of methionine has been connected to greying of hair in humans and also the buildup of hydrogen peroxide in the follicles of human hair (Wood et al., 2009). Gomez et al. (2009) reported elevated production of reactive oxygen species and DNA oxidative damage in the mitochondrial of experimental rats as a result of supplementation of their diets with excess methionine. Therefore, care should be taken to control the level of consumption of methionine in order to avoid possible hepatotoxicity. Furthermore, the concentration of phenylalanine in this study is comparable to those reported by Pimentel et al. (2014). Phenylalanine is an essential amino acid that is essential for some anabolic metabolism in the body (Pimentel et al., 2014). Phenylalanine is utilized in the pharmaceutical industries as a nutritional supplement for the production of certain drinks and food because of its antidepressant and analgesic potentials. In addition, the enzyme phenylalanine hydroxylase is culpable for the conversion of dietary phenylalanine into tyrosine in the liver. On the other hand, when phenylalanine hydroxylase is undersupplied in the body, the individual might be predisposed to a disease condition known as phenylketonuria (PKU). For that reason, patients suffering from PKU need to follow a Phenylalanine restricted diet in order to attain safe concentrations of phenylalanine in the blood (Giovannini et al., 2007; Rocha et al., 2012). Valine, on the other hand, can accelerate wound healing, raise blood sugar levels, and increase the content of growth hormone (Zhai et al., 2014) and the concentration of valine in this study is comparable to those reported for other plant species (Zhai et al., 2014). As a dietary supplement, leucine has been reported to slow the degradation of muscle tissue by increasing the synthesis of muscle proteins in aged rats (Combaret et al., 2005).

**Conclusion**

Isolation of these pharmaceutically active compounds in T. conophorum may be a great step towards understanding...
the principles and mechanisms behind the application of this plant in traditional medicine, which may be helpful in the elucidation and production of novel drugs for treatment and management of various disease conditions.

REFERENCES


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