Comparative Evaluation of Phytoconstituents By GC-MS Of Four Selected Herbal Drugs Used In Abakaliki Ebonyi State, Nigeria.

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Abstract: The present study deals with the investigation of bioactive components in four selected medicinal plants identified in Ebonyi State Nigeria using GC/MS. The fresh leaves of Dodder esculenta, Vernonia cinerea, Erythrina senegalensis and Blighia unijugata were collected and dried in Biotechnology laboratory, Ebonyi State University, Abakaliki at room temperature, for two weeks and the dried leaves were grinded into fine powder using mechanical blender and further sieved with 2mm size sieve. This was further extracted with methanol and used for phytochemical identification by GC-MS analysis. The result revealed the presence of the following phytochemicals: fifteen in Erythrina senegalensis, eleven in Dodder esculanta, eight in Blighia unijugata, and seven in Vernonia cinerea respectively. Thus, our results revealed that the selected plants possess important phytocomponents such as nonadecenoic acid hexadecanoic acid, n-pentyl acetate, and methyl caprate. Medicinal use of these plants may be attributed to these bioactive components.

Keywords: Blighia unijugata, Dodder esculanta, Erythrina senegalensis, Vernonia cinerea, GC-MS analysis, medicinal plants, phytoconstituents

I. Introduction

Plants are used medicinally in different countries and they are the source of many potent and powerful drugs. Plants have been an important source of medicine with qualities for thousands years. Normally on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. Plants are capable of synthesizing low molecular weight organic compounds known as secondary metabolites, usually with a unique complex structure [1]. Many metabolites have been found to possess interesting biological activities such as bactericidal, fungicide, hepatoprotective and muscle relaxant [2].

The plant Dodder esculenta is a perennial parasite herbs, commonly known as akashbella or amarbel, ‘ogbaramborogwu yeleli’ in Ezzamgbo. It is an unusual parasitic vine belongs to the convolvulaceae family. The parasitism of dodder is by rapping itself around the host plants after attaching to it [3]. If the host contains food beneficial to Dodder, it will produce the haustoria inserting themselves into vascular system of the host [4]. Dodder esculenta is a valuable medicinal herb. Stem of the plant is antibacterial and used externally to treat itch and internally in fever [5]. It is useful in the treatment of androgen induced alopecia [6]. It also gives anti-inflammatory and anti cancer activity [7]. The aqueous and alcoholic extract of Dodder esculenta has diuretic activity [8].

On the other hand, the plant Vernonia cinerea belonging to the family asteraccae is an annual plant widely distributed in most tropical and subtropical countries, and have long been used in traditional medicine to treat various types of diseases [9]. It is called ‘ogwuoka’ in Ezzamgbo in Ebonyi State. In recent years, the interest in the plant-based medicine had increased noticeably worldwide. V. cinerea have many therapeutic uses in the practice of traditional medicine. Every part of the plant can be used medicinally [10]. This herbs has been used to treat a number of disorder including inflammation, malaria, fever, worms, pain, diuresis, cancer, abortion and various gastro intestinal disorder [11]. The roots of the plant are used traditionally for the treatment of all types of eruptive boils and the juice is used for quicker healing of accidental wound and toxic viral fever [12]. The young leaves of this plant are used for the treatment of tonsillitis. The leaf juice extract is used to treat skin diseases and also for treating dysentery in children [12]. Beside these, the plant is used in smoking cessation, cough, Arthritis, Urinary calculi, and leprosy [13]. The plant posses antimicrobial, antibacterial, antioxidant, antihelmentic, anti-inflammatory, analgesic, antiflaautulent, antispasmodic and antidepressive properties [14]. Some of the phytochemicals compounds present are sterols, flavonoids, sesquiterpane lactones and a terpenoid, leupeol acetate which shows antihypoglycaemic and antilucrel properties [15]. Leaves from this plant serve as vegetable and culinary herb in soup in traditional Nigerian homes, extracts of the plant are used as tonic, in the control of tick and treatment of constipation and hypertension [16].

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Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems [17].

*Erythrina senegalensis* also known as “coral tree” is a thorny shrub or small tree with bright red flowers arranged in panicles at the ends of the branches [19]. *Erythrina senegalensis* are also widely used in the tropics and subtropics as street and parks trees, especially in drier areas [20]. The fruit pods are thin-walled, strongly curved or coiled, 8 - 15 cm long and its seeds are bright red and ovoid [21]. It is commonly called ‘ukwaka’ in Ezzamgbo in Ohaukwu Local Government Area Ebonyi State Nigeria, grown in West Africa as an ornamental plant and one of the oldest known African medicinal plants [22].

*Blighia unijugata* is widespread in tropical Africa, extending from Guinea Bissau eastwards to Ethiopia and Kenya, and through DR Congo southwards to Angola, Zimbabwe and Mozambique and South Africa [23]. *Blighia unijugata* is a tree planted as shade tree in Nigeria [24]. It is commonly called ‘ukpocha’ in Ezzambo, Ngbo, Izzi, Ezza in Abakaliki in Ebonyi State Nigeria. It is a tree, 3-18 metres high, flowers whitish; very fragrant; calyx-lobes about 1 mm long; petals 1-1.5 mm long; ripe fruits red or pinkish red with three (3) shining black seed, each with a yellow aril [25].

II. Materials And Methods

Biological materials

Fresh leaves of *Dodder esculenta*, *Vernonia cinerea*, *Erythrina senegalensis* and *Blighia unijugata* were collected from their natural habitat in Mgboagbaja Izzi L.G.A Ebonyi State of Nigeria and Amovu-Amike Ezzangbo in Ohaukwu L.G.A, Ebonyi State South east Nigeria respectively. They were authenticated and identified by Prof. S.E Okafor, a plant taxonomist of the Department of Applied Biology, Ebonyi State University, Abakaliki.
Sample Preparation
The sample were washed, air-dried under room temperature in the laboratory and pulverized to powder in a mechanical grinder. This was sieved with 2 mm size sieve and packaged in an air tight glass and stored at 4°C until it was used. One hundred gram portion of the ground leave of each of the ground powdered plants were weighed, dissolved in 300 ml of methanol respectively. The system was allowed to stand for 48 hours and filtered with the white filter cloth. The filtrate was allowed to evaporate to dryness. The resulting extracts were stored into sterile container covered, stored in the refrigerator and used for the analysis.

GC-MS Analysis Method
Various phytoconstituents were tested for in the methanol extract of the plant. Methods employed were standard analytical GC/MS as outlined by [26].

III. Results

Legend: 1-15 are peaks of phytoconstituents
Plate 5: The Photo Microgram Of *Erythrina Senegenlensis*
Table 1: Result of Gas Chromatography Mass Spectrometry Contents of *Erythrina senegalensis*

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-pentylacetate</td>
<td>C₇H₁₴O₂</td>
<td>130</td>
<td>8.405</td>
<td>15.19</td>
</tr>
<tr>
<td>2</td>
<td>Isopropyl butane</td>
<td>C₉H₁₄NO₂</td>
<td>140</td>
<td>10.02</td>
<td>1.33</td>
</tr>
<tr>
<td>3</td>
<td>2-nitrobenzyl alcohol</td>
<td>C₇H₆N₂O₂</td>
<td>153</td>
<td>10.82</td>
<td>0.47</td>
</tr>
<tr>
<td>4</td>
<td>1-12 nitroalcohol</td>
<td>C₁₂H₁₄N₂O₂</td>
<td>270</td>
<td>14.46</td>
<td>3.99</td>
</tr>
<tr>
<td>5</td>
<td>2-methylbutyl ester</td>
<td>C₈H₁₈O₂</td>
<td>158</td>
<td>14.98</td>
<td>4.99</td>
</tr>
<tr>
<td>6</td>
<td>4-isopropenyl-1- methylcyclohexanol</td>
<td>C₁₀H₁₈O</td>
<td>154</td>
<td>15.11</td>
<td>0.66</td>
</tr>
</tbody>
</table>

7. Palmitaldehyde                     C₁₆H₃₂O          | 240              | 17.47          | 10.64          |

8. 1-octyne                           C₁₀H₁₈          | 124              | 18.25          | 1.33           |

9. pentadecanoic acid                C₁₅H₂₆O₂          | 270              | 19.23          | 1.71           |

10. octadecanoic acid                 C₁₆H₃₂O₂          | 284              | 20.85          | 11.97          |

11. methyl 11-octadecenoate          C₁₆H₃₀O₂          | 296              | 22.46          | 9.49           |

12. 3,7,11,15-tetramethyl-2-hexadecen-1-ol | C₁₆H₃₀O          | 296              | 22.74          | 5.70    |

13. E-9-tetradecenal                  C₁₄H₂₆O          | 210              | 23.75          | 17.09          |

14. n-nonadecanoic acid               C₁₉H₃₈O          | 298              | 23.89          | 12.92          |

15. 2,3-dihydroxypropyl ester        C₁₉H₃₈O₄         | 330              | 27.51          | 6.84           |

Legend: 1-11 are peaks of phytoconstituents

Plate 6: Photo microgram of *Dodder esculenta*

Table 2: Phyto Components Identified in *Dodder Esculenta*

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>% yield</th>
<th>Base peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-octadecyne</td>
<td>C₈H₁₆</td>
<td>250</td>
<td>17.47</td>
<td>18.6</td>
<td>43.00</td>
</tr>
<tr>
<td>2</td>
<td>2,6,10-trimethylundeca-1,3-diene</td>
<td>C₁₅H₂₆</td>
<td>194</td>
<td>17.9</td>
<td>2.76</td>
<td>66.05</td>
</tr>
<tr>
<td>3</td>
<td>2-tetradecyne</td>
<td>C₁₉H₃₈</td>
<td>194</td>
<td>18.25</td>
<td>4.1</td>
<td>57.05</td>
</tr>
<tr>
<td>4</td>
<td>Tridecanoic acid</td>
<td>C₁₉H₃₈O₂</td>
<td>228</td>
<td>19.22</td>
<td>2.4</td>
<td>74.05</td>
</tr>
<tr>
<td>5</td>
<td>Octadecanoic acid</td>
<td>C₁₉H₃₈O₂</td>
<td>284</td>
<td>20.74</td>
<td>9.76</td>
<td>43.05</td>
</tr>
<tr>
<td>6</td>
<td>11-ocadecanoic acid</td>
<td>C₁₉H₃₈O₂</td>
<td>296</td>
<td>22.45</td>
<td>8.7</td>
<td>55.05</td>
</tr>
<tr>
<td>7</td>
<td>2-hexadecen-1-ol</td>
<td>C₂₀H₄₀O</td>
<td>296</td>
<td>22.78</td>
<td>4.97</td>
<td>71.05</td>
</tr>
<tr>
<td>8</td>
<td>Oleic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>23.58</td>
<td>18.8</td>
<td>55.05</td>
</tr>
<tr>
<td>9</td>
<td>Nonadecanoic acid</td>
<td>C₁₉H₃₈O₂</td>
<td>298</td>
<td>23.80</td>
<td>7.92</td>
<td>43.05</td>
</tr>
<tr>
<td>10</td>
<td>9-decen-2-one</td>
<td>C₁₀H₂₀O</td>
<td>166</td>
<td>25.88</td>
<td>8.83</td>
<td>43.00</td>
</tr>
<tr>
<td>11</td>
<td>10-undecenal</td>
<td>C₁₀H₂₀O</td>
<td>168</td>
<td>26.91</td>
<td>13.3</td>
<td>55.05</td>
</tr>
</tbody>
</table>
Comparative Evaluation Of Phytoconstituents By GC-MS Of Four Selected Herbal Drugs Used In...

**Plate 7:** Photo microgram of *Vernonia cinerea* indicating the various peaks representing bioactive components

**Table 3:** Phyto Components Identified In *Vernonia Cinerea*

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>% yield</th>
<th>Base peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-Octadecyne</td>
<td>C₁₈H₃₄</td>
<td>250</td>
<td>17.46</td>
<td>0.7</td>
<td>43.05</td>
</tr>
<tr>
<td>2</td>
<td>Pentanoic acid</td>
<td>C₇H₁₄O₂</td>
<td>130</td>
<td>19.23</td>
<td>8.1</td>
<td>74.75</td>
</tr>
<tr>
<td>3</td>
<td>11-Octadecanoic acid</td>
<td>C₁₉H₃₆O₂</td>
<td>284</td>
<td>20.75</td>
<td>20.0</td>
<td>43.05</td>
</tr>
<tr>
<td>4</td>
<td>6-octadecanoic acid</td>
<td>C₁₈H₃₆O₂</td>
<td>296</td>
<td>22.45</td>
<td>20.2</td>
<td>55.05</td>
</tr>
<tr>
<td>5</td>
<td>Hexadecanoic acid</td>
<td>C₁₉H₃₆O₂</td>
<td>284</td>
<td>22.79</td>
<td>8.7</td>
<td>74.05</td>
</tr>
<tr>
<td>6</td>
<td>4-Tridec-1-ene</td>
<td>C₁₃H₂₆</td>
<td>182</td>
<td>23.58</td>
<td>28.8</td>
<td>41.05</td>
</tr>
<tr>
<td>7</td>
<td>Octadecanoic acid</td>
<td>C₁₈H₃₆O₂</td>
<td>284</td>
<td>23.81</td>
<td>13.8</td>
<td>43.05</td>
</tr>
</tbody>
</table>

**Legend:** 1-7 The peaks of various phytoconstituents

**Plate 8:** The Photo Microgram of *Blighia unijugata*

**Table 4:** The results of Gas Chromatography Mass Spectrometry Contents of *Blighia unijugata*

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-methylmannoside</td>
<td>C₇H₁₄O₆</td>
<td>194</td>
<td>17.85</td>
<td>44.09</td>
</tr>
<tr>
<td>2</td>
<td>Methyl 6-methyl heptanoate</td>
<td>C₉H₁₈O₂</td>
<td>158</td>
<td>19.23</td>
<td>20.47</td>
</tr>
<tr>
<td>3</td>
<td>n-octadecanoic acid</td>
<td>C₁₉H₃₆O₂</td>
<td>284</td>
<td>20.75</td>
<td>8.25</td>
</tr>
<tr>
<td>4</td>
<td>10-octadecenoic acid</td>
<td>C₂₀H₄₀O₂</td>
<td>296</td>
<td>22.45</td>
<td>6.93</td>
</tr>
<tr>
<td>5</td>
<td>Methyl caprate</td>
<td>C₁₁H₂₂O₂</td>
<td>186</td>
<td>22.79</td>
<td>3.31</td>
</tr>
<tr>
<td>6</td>
<td>11-hexadecenoic acid</td>
<td>C₁₁H₂₂O₂</td>
<td>254</td>
<td>23.58</td>
<td>8.03</td>
</tr>
<tr>
<td>7</td>
<td>n-hexadecanoic acid</td>
<td>C₁₉H₃₆O₂</td>
<td>256</td>
<td>23.80</td>
<td>3.67</td>
</tr>
<tr>
<td>8</td>
<td>Pentanoic acid</td>
<td>C₁₁H₂₂O₂</td>
<td>234</td>
<td>26.92</td>
<td>9.24</td>
</tr>
</tbody>
</table>

**Legend:** 1-8 The peaks of phytoconstituents
IV. Discussion

Medicinal plant have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases [27]. According to [28], more than 80% of the world population relies on traditional medicine for their primary healthcare needs.

Dodd er esculenta, Vernonia cinerea, Bligha unijugata and Erithrina senegal ais as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemo-therapeutants, these are non-phytotoxic, more systemic and easily biodegradable [29]. Hence, a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and product [30]. This is because several of the phytochemicals present in these plants have complementary and overlapping mechanism of action [31].

The compounds identified were fifteen (15) in Erythrina senegalensis among the major compounds include : n-pentylacetate (15.19%), palmi tialdehyde (10.64%), 2-methylbutylester (4.99%), 1-(2-nitromethyl (3.99%) are presented in (Plate 5 and Table 1). Literature have shown that these compounds have medicinal merits [32].

The GC-MS analysis of Dod der esculenta showed the presence of 15 compounds. The result revealed that 1-octadecene (18.6%), oleic acid (18.8%), 10-undecenal (13.3%), and octadecanoic acid (9.76%) were found as the major components of Dod der esculenta (Plate 6 and Table 2). Among the identified phytoconstituents, 1-octadecene posses antimicrobial and antifungal activity [33]. While, 10-undecenal also posses antimicrobial, antifungal, anti-inflammatory and antitumor activity [34]. Oleic acid posse’s anticancer activity, tumor-suppressing effect as reported in clinical studies. Numerous studies have reported an inhibition in cell proliferation induced by oleic acid in different tumor cell lines [35]. Oleic acid could suppress the over expression of cancerous gene (oncogene), which play a key role in the etiology, invasive, progression and metastasis in several human cancers [36]. Furthermore, Octadecanoic acid which is regarded as linolic acid in nature, posses anti-inflammatory, nematicide, insectifuge, hypcholesteromic, cancer preventive, hepatoprotective, antiacne, antihistaminic and antiarthritic [37].

However, the GC-MS analysis of Vernonia cinerea leaf methanol extract identified the existence of seven different compounds; 4-tridecycne (28.8%), 6-octadecanoic acid (20.2%), 11-octadecanoic acid (20.0%) and octadecanoic acid (13.8%) were found as the major components in methanol extract (Plate 7 and Tabble 3). However, 4-tridecyn (28.8%) posses analgesic activity, antibacterial and antiviral activity according to [38]. Whereas, 6-octadecanoic acid and 11-octadecanoic acid which are stearic acid has been reported to posses antibacterial, antiardox, antitumor, cancer preventive, immunostimulant and chemo preventive [39].

Other compounds identified in Vernonia cinerea have been reported to posses anti-inflammatory, antioxidant, nematicide, pesticide, lubricant, antiandrogenic and haemolytic activity [40]. Pentanoic acid (8.1%) according to [41], posses antioxidant activity and anti-inflammatory.

Among the major constituents identified in Blighia unijugata includes:3-methylmannoside (44.09%), methyl 6-methyl heptanoate (20.47%), pentanoic acid (9.24%), 10-octadecenoic acid (6.93%) (Plate 8 and Table 4). Reports have shown that 3-methylmannoside enhance innate immunity [42], by aiding the molecular mechanisms by which organisms defend themselves against parasites. In vertebrates, such defense mechanisms against microbes and parasites include anti-glycan antibodies as well as a vast army of innate immune receptors, many of these receptor systems are required to eliminate infectious organisms [43], and they do so by either promoting their direct killing or through uptake and signaling to bring additional cell-mediated effector mechanisms to eliminate the threat [44]. Methyl 6-methylheptanoate functions as a potent antifungal active against yeasts, mold and more active against gram positive bacteria, than gram-negative bacteria [45]. Whereas [46], reported that Pentanoic acid serves as antifungal and antimicrobial agent [47]. Furthermore, 10-Octadecenoic acid methyl ester enhances the immunity of hydroxy unsaturated fatty acid [48]. Other compounds identified in Blighia unijugata such as n-Hexadecanoic acid had properties which are essential in forming cosmetic products and even act as soap releasing agents [49].

It is also a saturated fatty acid and just like other fatty acids, it has antibacterial and antifungal properties [50]. Fatty acids can modulate immune responses by acting directly on T cells [51]. The dietary, conjugated linoleic acid exerts anti-inflammatory effect by decreasing production of the inflammatory mediators such as prostaglandin E2, IL-6, IL-1b, TNFα, and nitric oxide [52]. It also serves as antioxidant, hypocholesterolomic, nematicide, anti-androgenic flavour, antimalarial, haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial agent, [53].

The study confirm the presence of bioactive components which are known to exhibit medicinal value coupled with physiological activities. Hence, it may be inferred that the results obtained suggest that the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of phytocomstituents of substantial medicinal merit. Therefore, the data generated

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from this experimental has provided the chemical basis and confirm the folkloric wide use of this plant as therapeutic agent for treating various ailments. Hence, it offer a platform of using *Erithrina senegalensis*, *Dodder esculenta*, *Vernonia cinerea* and *Blighia unijugata* leaves as herbal alternative for various diseases. The study was set out to investigate and compare the medicinal value of four selected medicinal plants commonly used in Ebonyi State. Our results have shown that all the four plants have phytochemical components of medicinal value, ranging from *Blighia unijugata*, *Vernonia cinera*, *Erithrina senegalis* and *Dodder esculanta*. Hence, the use of these plants in this locality for treatment of diseases may be attributed to these bioactive components and should be encouraged.

References