Chemical Fingerprinting of *Nauclea latifolia*, an Antidiabetic Plant, Using GC-MS

Bob I. A. Mgbeje¹* and Caroline Abu¹

¹Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria.

Authors’ contributions

This work was carried out in collaboration between both authors. Author BIAM managed the project conception and design, coordination, interpretation of data and preparation of manuscript. Author CA managed the experimentation, acquisition and analysis of data, statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

**Aim:** Earlier studies have established the antidiabetic activity of *Nauclea latifolia*. The aim of this study was to obtain chemical fingerprints of the ethanolic extracts of *Nauclea latifolia* leaf using Gas chromatography-Mass spectrometry (GC-MS), in order to assure proper identification of the plant and to evaluate its phytochemical composition using GC-MS. It was also aimed at unravelling the phytochemical constituents responsible for its antidiabetic activities.

**Methodology:** The phytochemicals in the plant leaves were extracted by cold maceration in ethanol and subjected to GC-MS analysis.

**Results:** Chromatograms showed 51 peaks of identified phytochemical compounds. The GC-MS analysis also revealed sixteen (16) active antidiabetic phytochemicals namely: Pentanoic acid 4-oxo- ethyl ester, 2-Methoxy-4-vinylphenol, Triethyl citrate, Quinic acid, 3-O-Methyl-d-glucose, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, Phytol, Hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, Octadecanoic acid, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, Vitamin E, Campesterol, Stigmasterol, Gamma-sitosterol.

*Corresponding author: E-mail: bobmgbeje@yahoo.com;*
Conclusion: The GC-MS profile provides a suitable chemical fingerprint to assure proper identification of the *Nauclea latifolia* plant. Sixteen (16) active phytochemicals of the plant leaf are known to have antidiabetic activities; these could be used as a basis for standardization of the plant preparations for diabetes therapy.

**Keywords:** *Nauclea latifolia*; antidiabetic; chemical fingerprinting; gas chromatography; mass spectroscopy.

1. INTRODUCTION

Medicinal plants constitute a source of raw materials for both traditional systems of medicine and modern medicine. Most rural populations, especially in the developing world, depend on medicinal plants as their main source of primary healthcare ostensibly because of its relatively low cost, availability and limited access to modern medicine [1,2]. It is estimated that about 80% of the world’s population living in Africa, Asia and Latin-America, use herbal medicine as their source of primary health care [2-6]. Over the years there has been increasing acceptance and public interest in natural therapies in developed countries because of the belief that it will result in healthier living. Herbal medicines are also often viewed as a balanced and moderate approach to healing [7] more so as adverse effects of most herbal drugs are minimal and relatively less frequent, when the drugs are used properly.

However, the increasing popularity of the use of medicinal plants globally has brought concerns and fears over quality, efficacy and safety of the products from medicinal plants used in health care [8-11]. These fears has limited the rational use of herbal medicine and have been responsible for the rather slow integration into western medicine [9,10].

An important issue in the Quality Assurance of medicinal plant products is the improper plant identification. Correct identification of the starting material is an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy [5,12-14]. Wrongly identified species, subspecies or species varieties are either therapeutically less active or inactive, or may even contain poisonous ingredients [9]. This is particularly pertinent as many medicinal plants are harvested in the wild, where conditions for growth and cultivation have not been optimized [15].

To ensure proper identification of medicinal plants the classical botanical identification of the plant is now being supplemented with chemical tests and chromatographic techniques as well as DNA based technologies which produce unique chemical and DNA fingerprint respectively for the plant. A chemical fingerprint can be defined as a characteristic profile reflecting the complex chemical composition of the analyzed plant sample that indicates the presence of multiple chemical markers within a sample and can be obtained by spectroscopic, chromatographic or electrophoretic techniques [5,9,16]. Chemical fingerprinting has been demonstrated to be a powerful technique for the quality control of medicinal plant extracts. Chromatographic fingerprinting analysis (chemo profiling) are known to disclose the detectable ingredients composition and concentration distribution [16-18]. Apart from qualitative and quantitative data, chemical fingerprints enable the screening of plant samples for the presence of active compounds from a myriad of compounds present in herbal samples [19]. Fingerprint analysis has been accepted by the World Health Organization (WHO) as a methodology for the quality control of herbal samples [3,4,16].

One of those ailments for which plants are continuously being scrutinized for its remedy is diabetes mellitus [20,21] a metabolic disorder characterized by hyperglycaemia and resulting from defects in insulin secretion or decreased sensitivity of tissues to insulin or both [22]. One of the medicinal plants that have been used in ethno medicine for the treatment of diabetes is *Nauclea latifolia* [23,24]. It is in ensuring the proper identification of this medicinal plant preparation by providing its chemical profiles, as part of assuring the quality of the plant preparation that this work is being carried out. It will also enable the screening of the plant preparation for the presence of antidiabetic compounds from the myriad of compounds present in the plant preparation.

*Nauclea latifolia*, commonly known as Pin cushion tree, belongs to the family Rubiaceae. It is a deciduous flowering, straggling evergreen, multi-stemmed shrub or small tree possessing an open canopy and not exceeding a height of 10 m
(32 ft). It grows well in tropical environments and is native to tropical Africa and Asia [25]. Its antidiabetic activity has been established in our laboratory [24] and elsewhere [26-28]. It has also been shown to have cardiovascular [29], antihypertensive [30], anticonvulsant, anxiolytic and sedative [31] antiviral [32], antimalarial [33], analgesic [34] and antiulcer [35] activities. Other ailments that have been proven to be improved by extracts of *Nauclea latifolia* include anxiety, destruction of intestinal parasites, boost of male libido and potency as well as the management of menstrual issues [36].

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh mature leaves of *Nauclea latifolia*, were harvested from the University of Calabar botanical farm, Calabar, Cross River State, Nigeria. The plant was authenticated at the Department of Botany, University of Calabar and was in accordance with voucher specimen Herb/Bot/ucc/017 already deposited in the department’s herbarium.

2.2 Preparation of Plant Extracts

The harvested *Nauclea latifolia* leaves were thoroughly washed with clean water, rinsed with distilled water and allowed to drain. The leaves where then dried away from direct sunlight for seven (7) days, after which they were grounded into powder with a manual blender. The pulverised leaves were weighed and then soaked in 80% ethanol solution in the ratio 1:4 (sample: ethanol) for 48 hours with intermittent agitation. The plant homogenate was doubly filtered using cheese cloth followed by Whatman filter paper. The filtrates concentrated in a water bath at a temperature of 40°C. The dark brown gummy residual mass was stored at a temperature of 4°C in a refrigerator until ready for use.

2.3 Determination of Chromatographic Chemical Profile Using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was performed as previously described [21]. A Shimadzu GC-MS QP-2010 comprising a gas chromatograph hyphenated to a mass spectrophotometer (MS) and an auto sampler was used for this analysis. A 25 m x 0.25 mm fused silica capillary column coated with CP-Sil5 and film thickness at 0.15 μm was fitted to the gas chromatograph. The carrier gas was helium at 1.2 ml/min. Operating conditions of the MS was: ion source temperature 230°C, ionization voltage 70ev. The MS data obtained was processed online with desktop computer fitted with disk memory. The identification of the components was accomplished by comparison of the retention indices, fragmentation pattern and mass spectra with spectrum of known components stored in the database of National Institute of Standard and Technology (NIST) library (http://chemdata.nist.gov).

3. RESULTS AND DISCUSSION

3.1 Gas Chromatography - Mass Spectroscopy (GC-MS) Profiling

The GC-MS chromatograms of ethanol leaf extract of *Nauclea latifolia* is shown in Fig. 1. A complete list of the identified compounds, retention times and percentage peak areas are shown in Table 1. The chromatogram revealed the presences of fifty-one (51) phytochemical compounds. Hexadecanoic acid, ethyl ester was the most abundant compound (15.80%) and 2-Hexanethiol the least abundant (0.06%).

Similar GC-MS analysis have been carried out on *Nauclea latifolia* leaf, by other workers, after steam distillation [37], cold maceration in purified re-distilled methanol [37], ethanol and aqueous extraction [38]. GC-MS analysis have also been performed on the methanol extract of the stem [37], chloroform extract of the pulverized ripe fruits [39] and the dry water extract of the root of the plant [40]. The GC-MS profile of the ethanol leaf extract by Iheagwam et al. [38] showed the presence of 47 peaks while the same profiling in this study resulted in 51 peaks. Even more striking was that only fourteen (14) of the phytochemicals (taking into account the derivatives) were common to both profiles emphasizing the need for chemical profiling as a means of unambiguously identifying plant preparations. The cause of the variability in the profile of the two ethanol extract could be Intrinsic factors like difference in subspecies or species varieties, age of the plant, genetic variability and/or extrinsic factors like environment (climate, altitude, temperature, light exposure, water and nutrient availability), geographic location, diurnal and seasonal variations and method of cultivation [11,14,41].
### Table 1. Phytochemical compounds identified for various peaks in *Nauclea latifolia* ethanolic leaf extract (GC-MS)

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention time</th>
<th>Area%</th>
<th>Height%</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.048</td>
<td>0.06</td>
<td>0.13</td>
<td>2-Hexanethiol</td>
</tr>
<tr>
<td>2</td>
<td>3.157</td>
<td>0.11</td>
<td>0.11</td>
<td>2-Decanynoic acid</td>
</tr>
<tr>
<td>3</td>
<td>3.324</td>
<td>1.42</td>
<td>2.53</td>
<td>Pentanoic acid, 4-oxo-, ethyl ester</td>
</tr>
<tr>
<td>4</td>
<td>4.431</td>
<td>0.25</td>
<td>0.33</td>
<td>Butanedioic acid, diethyl ester</td>
</tr>
<tr>
<td>5</td>
<td>4.685</td>
<td>0.23</td>
<td>0.15</td>
<td>Pentanoic acid, 3-hydroxy-, ethyl ester</td>
</tr>
<tr>
<td>6</td>
<td>4.825</td>
<td>0.11</td>
<td>0.10</td>
<td>Isosorbide</td>
</tr>
<tr>
<td>7</td>
<td>5.083</td>
<td>0.74</td>
<td>0.78</td>
<td>Pentanoic acid, 4,4-dimethoxy-, ethyl ester</td>
</tr>
<tr>
<td>8</td>
<td>5.583</td>
<td>0.63</td>
<td>0.79</td>
<td>Butanedioic acid, hydroxy-, diethyl ester</td>
</tr>
<tr>
<td>9</td>
<td>6.206</td>
<td>0.38</td>
<td>0.17</td>
<td>Cyclohexanone, 2-(hydroxymethyl)</td>
</tr>
<tr>
<td>10</td>
<td>6.442</td>
<td>0.32</td>
<td>0.18</td>
<td>2-Methoxy-4-vinylphenol</td>
</tr>
<tr>
<td>11</td>
<td>6.880</td>
<td>0.13</td>
<td>0.13</td>
<td>2-Ethyl-3-hydroxy-2-methyl-succinic acid, 1-ethyl ester</td>
</tr>
<tr>
<td>12</td>
<td>7.593</td>
<td>0.06</td>
<td>0.09</td>
<td>Nonanoic acid, 9-oxo-, ethyl ester</td>
</tr>
<tr>
<td>13</td>
<td>7.853</td>
<td>0.18</td>
<td>0.17</td>
<td>alpha.-D-Glucopyranoside, O.-alpha.-D-glu</td>
</tr>
<tr>
<td>14</td>
<td>8.117</td>
<td>0.43</td>
<td>0.27</td>
<td>1-Pyrrolid-2-one, N-carboxyhydrazide</td>
</tr>
<tr>
<td>15</td>
<td>8.635</td>
<td>0.55</td>
<td>0.65</td>
<td>3-Furanacetic acid, 4-hexyl-2,5-dihydro-2,5-dioxo</td>
</tr>
<tr>
<td>16</td>
<td>8.829</td>
<td>0.76</td>
<td>0.45</td>
<td>alpha.-D-Glucopyranoside, O.-alpha.-D-glu</td>
</tr>
<tr>
<td>17</td>
<td>9.072</td>
<td>0.16</td>
<td>0.18</td>
<td>9-Octadecenoic acid (Z)-, methyl ester</td>
</tr>
<tr>
<td>18</td>
<td>9.479</td>
<td>0.21</td>
<td>0.17</td>
<td>3-Decenoic acid, (E)-</td>
</tr>
<tr>
<td>19</td>
<td>9.745</td>
<td>0.62</td>
<td>0.70</td>
<td>Ethyl tridecanoate</td>
</tr>
<tr>
<td>20</td>
<td>10.011</td>
<td>1.03</td>
<td>0.59</td>
<td>Decanoic acid, silver(1+) salt</td>
</tr>
<tr>
<td>21</td>
<td>10.314</td>
<td>3.66</td>
<td>1.54</td>
<td>Triethyl citrate</td>
</tr>
<tr>
<td>22</td>
<td>10.453</td>
<td>4.96</td>
<td>2.04</td>
<td>Quinic acid</td>
</tr>
<tr>
<td>23</td>
<td>10.526</td>
<td>1.92</td>
<td>1.29</td>
<td>Azelaic acid</td>
</tr>
<tr>
<td>24</td>
<td>11.394</td>
<td>9.79</td>
<td>1.78</td>
<td>3-O-Methyl-d-glucose</td>
</tr>
<tr>
<td>25</td>
<td>11.757</td>
<td>0.59</td>
<td>0.87</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
</tr>
<tr>
<td>26</td>
<td>11.811</td>
<td>0.38</td>
<td>0.27</td>
<td>[1,1’-Bicycloprenyl]-2-octanoic acid, 2’-hexyl</td>
</tr>
<tr>
<td>27</td>
<td>11.931</td>
<td>0.84</td>
<td>0.91</td>
<td>Hexadecanal</td>
</tr>
<tr>
<td>28</td>
<td>12.062</td>
<td>0.63</td>
<td>0.91</td>
<td>Phytol, acetate</td>
</tr>
<tr>
<td>29</td>
<td>12.118</td>
<td>0.20</td>
<td>0.36</td>
<td>Hexadecanoic acid, ethyl ester</td>
</tr>
<tr>
<td>30</td>
<td>12.218</td>
<td>0.39</td>
<td>0.66</td>
<td>3,7,11,Trimethyl-2,4-dodecadiene</td>
</tr>
<tr>
<td>31</td>
<td>12.342</td>
<td>1.77</td>
<td>2.99</td>
<td>Hexadecanoic acid, methyl ester</td>
</tr>
<tr>
<td>32</td>
<td>12.657</td>
<td>1.24</td>
<td>1.02</td>
<td>Ethyl 9-hexadecenoate</td>
</tr>
<tr>
<td>33</td>
<td>12.753</td>
<td>1.22</td>
<td>1.46</td>
<td>Ethyl 9-hexadecenoate</td>
</tr>
<tr>
<td>34</td>
<td>12.823</td>
<td>15.80</td>
<td>20.29</td>
<td>Hexadecanoic acid, ethyl ester</td>
</tr>
<tr>
<td>35</td>
<td>13.444</td>
<td>1.37</td>
<td>1.83</td>
<td>Ethyl 14-methyl-hexadecanoate</td>
</tr>
<tr>
<td>36</td>
<td>13.488</td>
<td>1.62</td>
<td>2.66</td>
<td>Cyclopropanoic acid, 2-[(2-(2-ethyl cylo propyl)</td>
</tr>
<tr>
<td>37</td>
<td>13.642</td>
<td>0.74</td>
<td>1.05</td>
<td>Methyl stearate</td>
</tr>
<tr>
<td>38</td>
<td>13.781</td>
<td>2.41</td>
<td>1.30</td>
<td>cis-9-Hexadecenal</td>
</tr>
<tr>
<td>39</td>
<td>13.871</td>
<td>6.46</td>
<td>7.69</td>
<td>9,12-Octadecadienoic acid, ethyl ester</td>
</tr>
<tr>
<td>40</td>
<td>13.922</td>
<td>13.62</td>
<td>18.26</td>
<td>9,12,15-Octadecatrienoic acid, ethyl ester,</td>
</tr>
<tr>
<td>41</td>
<td>14.055</td>
<td>4.93</td>
<td>7.19</td>
<td>Octadecanoic acid, ethyl ester</td>
</tr>
<tr>
<td>42</td>
<td>14.627</td>
<td>0.55</td>
<td>0.56</td>
<td>Ethyl 9-hexadecanoate</td>
</tr>
<tr>
<td>43</td>
<td>15.183</td>
<td>1.41</td>
<td>2.05</td>
<td>Hexadecanoic acid, ethyl ester</td>
</tr>
<tr>
<td>44</td>
<td>15.795</td>
<td>1.54</td>
<td>1.28</td>
<td>Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
</tr>
<tr>
<td>45</td>
<td>17.803</td>
<td>1.04</td>
<td>1.04</td>
<td>Methyl 2-hydroxy-pentacosanoate</td>
</tr>
<tr>
<td>46</td>
<td>18.816</td>
<td>1.53</td>
<td>1.27</td>
<td>Stigmast-5-en-3-ol, oleate</td>
</tr>
<tr>
<td>47</td>
<td>18.898</td>
<td>4.32</td>
<td>3.45</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>48</td>
<td>19.594</td>
<td>1.48</td>
<td>1.09</td>
<td>Campesterol</td>
</tr>
<tr>
<td>49</td>
<td>19.807</td>
<td>0.68</td>
<td>0.64</td>
<td>Stigmastrol</td>
</tr>
<tr>
<td>50</td>
<td>20.239</td>
<td>4.11</td>
<td>3.39</td>
<td>gamma.-Sitosterol</td>
</tr>
<tr>
<td>51</td>
<td>20.348</td>
<td>0.41</td>
<td>0.21</td>
<td>Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β…)</td>
</tr>
</tbody>
</table>
Of the 51 phytocomponents in the ethanol extract of *Nauclea latifolia*, at least sixteen (16) phytochemicals namely: Pentanoic acid 4-oxo-ethyl ester, 2-Methoxy-4-vinylphenol, Triethyl citrate, Quinic acid, 3-O-Methyl-d-glucose, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, Phytol, Hexadecanoic acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, Octadecanoic acid, Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester, Vitamin E, Campesterol, Stigmasterol and Gamma-sitosterol are known to have anti-diabetic properties. These 16 components will be discussed further only as they affect diabetes.

**Pentanoic acid**, 4-oxo-, ethyl ester is Hepatoprotective [42]. 2-Methoxy-4-vinylphenol has anti-inflammatory and antioxidant activities [43-45] while Triethyl citrate is an anti-inflammatory agent [46]. Quinic acid has been shown to reduce hyperglycemia and oxidation by increasing c-peptide as well as insulin levels [47]. 3-O-Methyl-d-glucose inhibits glucose-stimulated insulin release under experimental condition [48]. 3,7,11,15-tetramethyl-2-hexadecen-1-ol has been reported to have, among other properties, insulin/antidiabetic, antiarthritic [49] and anti-inflammatory [50] properties. Phytol is important in the processing of glucose and can activate enzymes within the body that have strong positive effects on insulin level implying that it could possibly help restore the metabolic functions of those with type-2 diabetes [21,51]. Phytyric acid (PA) a chlorophyll metabolite from phytol has potentials in regulating glucose metabolism by regulating hepatic glucose homeostasis [52]. Hexadecanoic acid, otherwise called palmitic acid, has anti-inflammatory [53], antioxidant, hypocholesterolemic, anti-androgenic [54] activities. It has also been shown to stimulate glucose uptake in skeletal muscle cells [55].

**9,12-octadecadienoic acid**: (linoleic acid) has, among other attributes, been shown to have hypcholesterolemic, hepatoprotective, anti-androgenic [54] and antioxidant [56] properties. 9,12,15-octadecatrienoic acid also called α-linolenic acid (ALA) possesses anti-cardiovascular, anti-inflammatory, hypocholesterolemic, hepatoprotective and antiarthritic properties [57-59] via inhibition of reactive oxygen species (ROS) generation and

![GC-MS full spectrum analysis of Nauclea latifolia ethanolic leaf extract](image)
modulation of NF-kB signal transduction [57]. Octadecanoic acid (Stearic acid), as it concerns diabetes, has been shown to lower LDL cholesterol, the ratio of total to HDL cholesterol [60] and to dramatically reduce visceral fat as well as lowering blood glucose and leptin concentrations [61]. Reducing excess visceral fat may be very beneficial for type 2 diabetes, and possibly other disease states. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester has antioxidant and anti-inflammatory activities [46,62]. Vitamin E is a potent antioxidant, hypocholesterolemic, anti-inflammatory, hepatoprotective and antidiabetic agent [50,59,63]. Campesterol has antioxidant and hypocholesterolemic activity [63,64]. The campesterol molecules are thought to compete with cholesterol, thus reducing the absorption of cholesterol in the human intestine [64]. Stigmasterol has been shown to have anti-hepatotoxic, antioxidant, Cancer preventive, hypocholesterolemic [63] and antidiabetic [65] activity. Gamma-sitosterol has anti-inflammatory, antidiabetic, properties [66,67]. Administration of Gamma-sitosterol resulted in significant decreases in glycosylated hemoglobin and blood glucose with a significant improvement in plasma insulin levels, body weight and also food intake and further showed antihyperlipidemic and hepatoprotective activities [66].

4. CONCLUSION AND SUMMARY

The GC-MS profile provides a suitable chemical fingerprint that will ensure proper identification of the Nauclea latifolia plant. Sixteen (16) active phytochemicals of the plant leaf are known to have antidiabetic activities; these could be used as a basis for standardization of the plant preparations for diabetes therapy.

In summary earlier studies has validated the use of Nauclea latifolia leaf extract for treatment of diabetes. To assure property identification of the plant, a GC-MS chemical profile has been established for the plant. Of the fifty-one (51) phytochemical constituents of the plant, as revealed by GC-MS analysis, at least sixteen (16) are known to have antidiabetic activities. This work represents a step towards “true” standardization of the plant preparation, based on constituents of known therapeutic or pharmacological activity, for presentation in diabetes therapy. It also is a step towards isolating the active diabetic principles in the plant.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


47. Arya A, Al-Obaidi M, Shahid N, Bin N, Loo C, Wong W, Khating S, Mustafa M. Synergistic effect of quercetin and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of...


58. Kim K-B, Nam YA, Kim HS, Hayes AW, Lee B-M. α-linolenic acid: Nutraceutical, pharmacological and toxicological evaluation. Food and Chemical Toxicology. 2014;70:163–178. DOI: 10.1016/j.fct.2014.05.009


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