Infrared and Ultraviolet Spectroscopic Analysis of Methanol Extract of Phyllanthus Muellerianus Root

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Research Article

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ABSTRACT

The roots sample of *phyllanthus muellerianus* was extracted with Hexane and Methanol respectively, given the percentage yield of 2.37% and 10.18% respectively. Column chromatography of the Methanol extract afforded 3 crystals which were labeled as M, N, Q. IR and The UV analysis shows that M gave absorption peaks at 238nm and 326nm, Q gave absorption peaks at 240nm and 326nm while N gave peaks at 220nm and 240nm. The IR analysis shows absorption for M at 1378.5cm⁻¹ and Q at 1378.5cm⁻¹, also, there are other absorption band for M, N and Q at 1465.9cm⁻¹, 1475.6cm⁻¹ and 1470.9cm⁻¹ respectively which suggest C-H bending of saturation. It was also shown that there are absorption of M, N and Q at 1704.3cm⁻¹, 1699.4cm⁻¹ and 1699.4cm⁻¹ respectively. Other absorption for M and Q at 1704.3cm⁻¹ and 1699.4cm⁻¹ which suggest C-H stretching and absorption at 2939.8cm⁻¹ and 2934.8cm⁻¹ which indicate the aldehyde functional group. Furthermore, absorptions were shown for M, N and Q at 3446.5cm⁻¹, 3440.7cm⁻¹ and 3438.2cm⁻¹ respectively which suggest O-H stretching.

Keywords: column chromatography, extract, crystal, absorption peaks

INTRODUCTION

A medicinal plant is described as a plant which has in its parts bioactive agents which are used for therapeutic purposes or precursor for the synthesis of useful drugs,(Sofowora., 1993). The utilization of medicines produced from medicinal plants is increasing (Li et al., 2009; Hall and Nair, 2005). This probably led to the revival of interest in the use and importance of African medicinal plants as well as an intensified research and effort to document scientific evidence for the claimed therapeutic efficacy (Lawal et al., 2010). Over the last decade, interest in drugs of plant origin and their uses in various diseases management have increased in many developed countries since plants used in traditional medicine are more likely to yield pharmacologically active compounds than developing new drugs synthetically (WHO,1990). In Nigeria today, necessary framework has been put in place to incorporate

*Phyllanthus Muellerianus* is commonly known as fula-puaar, Mamoti in Senegal, Akan-Asante aobe in Ivory Coast, akan-Asante awobe in Ghana, and in Nigeria Igbehen=1horns of fish(Edo), nkana(Efik), Kuma= athicket, from the habit(hausa), Anya nnumi=birds eye (Igbo), egu eza (igbo), Arunjera or Arunyeran (Yoruba), *phyllanthus muellerians* belongs to the family of Ephorbiaceae. It is a shrub or climber, occasionally arborescent, of deciduous and secondary forest from Guinea-Bissau and Mali to West African. In Bendel, it is reported as weed of rice-field, by lack of timely cultivation35. The stem seldom becomes large, clear potable water may be obtained from the cut stem and this sap is used in Sierra Leone to relieve ophthalmia, and in Nigeria for pain in the eyes or to remove foreign body. In Nigeria twigs are used as chew-stick after removal of the surface spines (Isawumi, 1978).
The roots are widely used for intestinal troubles. The root is cooked with maize meal for severe dysentery in Ghana, powdered root charcoal with palm-oil is taken in Congo for stomach-upsets and as antemetic. In Nigeria a root-decoction is used as a febrifuge and an infusion of roots and leaves is given to children in Togo suffering from eruptive fevers. In Nigeria, the young root with young leafy twigs, is given for jaundice and as a mild purgative and to treat dysentery and urethra discharge (Alnslie, 1937).

Leaves are an occasional supplement, cooked with food or in soup in Sierra Leone and South Nigeria. In Ghana and in Nigeria, leaves boiled with palm fruit are given as a soup to women after delivery (Alnslie, 1937), this preparation is also a general tonic. Freshly pounded leaves are used for wound-dressing and leaf-sap is used as wash for fever and skin-eruptions (Dalziel, 1937).

Leaf sap is widely used in instillation for eye-troubles. Also in Ivory Coast the leaves made into an eye-pad on the lids, leafy twigs prepared with a pulp are rubbed topically on the body in Ivory Coast to cure paralysis. A leaf-decoction serves in Ubangi as a mouth-wash for toothache after which the cooked leaves are applied to the gums.

For cough, the root in Ghana is cut into small pieces with those of Psychotria calva (Rubiaceae) and Harrisonia Abyssinica (Simaroubaceae) and decocted and the liquid drunk. This prescription is also given for whooping cough. An infusion of the flowers is cooling and gently aperient. The fruits are edible and are eaten by some people. The pulp provides a hair fixative used in Ubangi. Literature information are scanty on the biological activities, Phyllumuellerianus. In vitro antimicrobial properties of leaf chloroform and methanol extract of Phyllanthus amarus and Phyllanthus muellerianus using human pathogenic microorganisms were evaluated respectively. The leaf methanol extract of P. amarus and the leaf chloroform extract of P. muellerianus exhibited antimicrobial properties. The chloroform extract of P. muellerianus displayed sensitivity higher than the P. amarus candida albicans but inhibited the growth of only staphylococcus aureus (gram positive) and Escheria coli (gram negative) (Onocha, et al., 2003).

MATERIALS AND METHODS

The roots of Phyllanthus muellerianus were collected at the Federal Research Institute of Nigeria (FRIN) and identified by Mr Felix Usang of the Herbarium Department. FRIN.

Preparation of Sample

The root sample were air dried for about two weeks to reduce the moisture content and then ground, this was to prevent as far as possible physiological changes taking place before the extraction by making use of a grinding machine.

Sterilization of Apparatus and Precautions

In Organic chemical investigation, prevention of interference and contamination from unwanted materials is very important. To achieve this aim, the following precautions were observed:

- All glass ware were thoroughly washed with detergent and water, rinsed with distilled water and dried in an oven.
- All solvents used were redistilled and stored in a clean glass bottles.
- Plastics containers were not allowed to come in contact with solvent.
- Drying of sample extract as well as fractions was carried out at ambient condition to reduce loss of low molecular weight components.
- Chromatographic plates coated with silica gel were activated in an oven at 120°C for two hours.

Extraction of the Sample

A weighed amount of the dry/ground root of Phyllanthus muellerianus (3.5kg) was packed into 20L aspirator bottle in which the lower opening has been blocked with glass wool, and connected to
a 5L flask on a hot water-bath. Hexane (the extracting solvent) was poured over the sample into the flask. Antibumping granules were placed in the flask to enhance even boiling and a reflux condenser was connected to the top of the aspirator bottle. The Hexane solvent was heated gently with a water bath. The vapour passed through a condenser and the condensed solvent dropped into the top of the *Phyllanthus muellerianus* sample and slowly filled it, this removes the components that had to be extracted. The set up was heated continuously and the process was repeated automatically. The extraction took place for about 15 hours.

The crude extract was collected into a pre-weighed sample bottle after distilling off the solvent by a process called steam distillation. The solvent (Hexane) was drained from the root sample in the aspirator bottle and the plant allowed to dry. The extraction was repeated using methanol. The weight of the crude methanolic extract was also taken.

**Column Chromatography**

This is a form of adsorption chromatography in which the stationary phase is a solid and the mobile phase is a liquid. It is normally employed for routine separation and purification of products.

The column of about 97cm long and 4cm in diameter was thoroughly washed with detergent, rinsed with distilled water and then allowed to dry. When the column has been fully dried, small piece of glass wool was inserted into the lower part of the column and the column was supported using a clasp and retort stand. A funnel is attached to the open end and little clean white sand was poured on top of the glass wool already inserted, after this some quantity of the solvent (Hexane) was poured down the column. The silical gel used was activated in the oven at 120°C for 2 hours. The slurry of the silical gel was made and the slurried silical gel was poured in a thin stream into the tube (column) which was about one third filled with hexane solvent. Gradual setting was arranged by maintaining a gentle agitation while there was solvent flow through the column in order to obtain a homogenous packing.

7.4g of the methanolic extract of the sample was thoroughly mixed with silical gel and placed on steam bath until the methanol solvent remaining in the extract was totally removed which turned to powder form. The plant extract sample was applied to the top of the column as evenly as possible and distortion of the column packing avoided as this would lead to distorted bands. The top of the column was there protected with a thin layer of clean sand and more hexane was allowed to run with replacement. 100% hexane was first used to run the column and later, 2-5% gradual increase of ethyl acetate in hexane. This was carried out in order to increase the adsorption of different component contained in the plant extract. A more polar solvent than the eluent was used to wash down the column to obtain the tightly held component of the extract.

**Determination of Melting Point**

The melting point of a substance is the temperature at which the solid phase changes to the liquid phase under 1 atmospheric pressure.

Melting point is one of the physical properties of a substance that is useful for characterizing and identifying the substance.

To determine the melting point, the crystal/solids were put in a capillary tube with one end sealed. This was tied to a thermometer held in position by clamp. The thermometer was dipped in paraffin oil inside a 100ml beaker placed on tripod stand with bursen burner under it. The paraffin oil was stirred continuously with glass rod to allow even distribution of heat and the melting point was taken immediately the crystal changed to liquid.

**Spectroscopic Analysis**

UV spectroscopic analysis was carried out using Unicam Aurora for Helios scan software VII. And chloroform used to dissolve the crystals.

IR analysis was equally carried out using Buck scientific spectrophotometer M500.KBr salt was used.
RESULT AND DISCUSSION

Percentage Yields

The percentage yield of the crude hexane and Methanol extracts of the *phyllanthus muellerianus* root was calculated thus:

Hexane extract:
Weight of extract=83g
Weight of the root sample=3500g
%yield=83/3500 x 100
=2.37%

Methanol extract:
Weight of extract=356.20
Weight of root sample=3500g
%yield=356.20/3500 x 100
=10.18%

From the above percentage yields, it can be seen that the yield of Methanol extract is more than that of hexane and this is because Methanol is a more polar solvent, in which most organic compounds will dissolve while the less polar compounds particularly liquid/oil compounds are extracted by hexane.

Chromatographic Separation

Column chromatography of 7.4g of Methanol extract *phyllanthus muellerianus* root afforded 320 fractions, but were reduced to 27 by thin layer chromatography analysis i.e. the fractions with the same TLC picture were pooled together. The result of the chromatographic separation is tabulated below.

<table>
<thead>
<tr>
<th>NO</th>
<th>FRACTIONS</th>
<th>ELUENTS</th>
<th>NATURE OF FRACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-25</td>
<td>Pure hexane</td>
<td>Colourless</td>
</tr>
<tr>
<td>2</td>
<td>26-38</td>
<td>2%EtOAC in hexane</td>
<td>Colourless</td>
</tr>
<tr>
<td>3</td>
<td>39-50</td>
<td>2%EtOAC in hexane</td>
<td>Light green</td>
</tr>
<tr>
<td>4</td>
<td>51-59</td>
<td>5%EtOAC in hexane</td>
<td>Light green</td>
</tr>
<tr>
<td>5</td>
<td>60-62</td>
<td>8%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>6</td>
<td>63-64</td>
<td>8%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>7</td>
<td>65-69</td>
<td>8%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>8</td>
<td>70-92</td>
<td>8%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>9</td>
<td>93-101</td>
<td>8%EtOAC in hexane</td>
<td>Greenish black</td>
</tr>
<tr>
<td>10</td>
<td>102-115</td>
<td>10%EtOAC in hexane</td>
<td>Brick red</td>
</tr>
<tr>
<td>11</td>
<td>116-126</td>
<td>15%EtOAC in hexane</td>
<td>Brick red</td>
</tr>
<tr>
<td>12</td>
<td>127-139</td>
<td>15%EtOAC in hexane</td>
<td>Brick red</td>
</tr>
<tr>
<td>13</td>
<td>140-154</td>
<td>15%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>14</td>
<td>155-175</td>
<td>18%EtOAC in hexane</td>
<td>Orange</td>
</tr>
<tr>
<td>15</td>
<td>176-195</td>
<td>18%EtOAC in hexane</td>
<td>Orange</td>
</tr>
<tr>
<td>16</td>
<td>196-216</td>
<td>20%EtOAC in hexane</td>
<td>Orange</td>
</tr>
<tr>
<td>17</td>
<td>217-228</td>
<td>25%EtOAC in hexane</td>
<td>Dirty green</td>
</tr>
<tr>
<td>18</td>
<td>229-235</td>
<td>30%EtOAC in hexane</td>
<td>Light yellow</td>
</tr>
<tr>
<td>19</td>
<td>236-245</td>
<td>30%EtOAC in hexane</td>
<td>Light yellow</td>
</tr>
<tr>
<td>20</td>
<td>246-260</td>
<td>35%EtOAC in hexane</td>
<td>Light yellow</td>
</tr>
<tr>
<td>21</td>
<td>261-269</td>
<td>35%EtOAC in hexane</td>
<td>Green</td>
</tr>
<tr>
<td>22</td>
<td>270-280</td>
<td>50%EtOAC in hexane</td>
<td>Orange</td>
</tr>
<tr>
<td>23</td>
<td>281-287</td>
<td>50%EtOAC in hexane</td>
<td>Orange</td>
</tr>
<tr>
<td>24</td>
<td>288-299</td>
<td>75%EtOAC in hexane</td>
<td>Orange</td>
</tr>
<tr>
<td>25</td>
<td>300-308</td>
<td>75%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>26</td>
<td>309-313</td>
<td>75%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
<tr>
<td>27</td>
<td>314-320</td>
<td>100%EtOAC in hexane</td>
<td>Yellow</td>
</tr>
</tbody>
</table>
Fractions 60-62 were combined on the basis of TLC and the two crystals obtained labeled M and N. Fractions 300-308 were also combined and the crystal obtained labeled as Q. The weights of M, N, and Q were taken and their melting point determined as shown below.

<table>
<thead>
<tr>
<th>CRYSTALS</th>
<th>MELTING POINT(°C)</th>
<th>WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>126-130</td>
<td>1.2 X 10^{-4}</td>
</tr>
<tr>
<td>N</td>
<td>122-126</td>
<td>6.0 X 10^{-5}</td>
</tr>
<tr>
<td>Q</td>
<td>260-263</td>
<td>1.0 X 10^{-4}</td>
</tr>
</tbody>
</table>

**Spectroscopic Analysis Result**

The UV analysis result showed that M gave absorption Peaks at 238nm and 326nm, Q gave absorption peaks at 240nm and 326nm while N gave peaks at 220nm and 249nm. It can be inferred that the compounds in the three crystals has chromophore and hence absorption can take place to allow transition. The IR absorption of C-H bending (symmetry) for M at 1378.5cm^{-1} and Q at 1378.5cm^{-1} indicate the presence of saturation in the compound. This is supported by the I.R. absorption bending (asymmetric) for M, N, and Q at 1465.9cm^{-1}, 1475.6cm^{-1} and 1470.9cm^{-1} respectively. In Q, C=C stretching of 1645.0cm^{-1} indicate the presence of conjugated unsaturation in the compound which is supported by the UV absorbance of 240nm. The I.R absorption of M, N, and Q at 1704.3cm^{-1}, 1699.4cm^{-1}, 1699.4cm^{-1} respectively indicate the C=O stretching of either ketone, aldehydes, acids or esters which may be alter by the absorption of 326nm.

The absorption of M, N, and Q with carbonyl (C=O) absorption at 1704.3cm^{-1} and 1699.4cm^{-1} respectively, and C-H stretching of M and Q at 2939.8cm^{-1} and 2934.8cm^{-1} respectively indicates the presence of aldehyde in the compounds, this was not found in N. The I.R absorption of M at 2370.8cm^{-1} and Q at 2365.8cm^{-1} indicate the presence of unsaturation with triple bond. In M, N, and Q, O-H stretching of 3446.5cm^{-1}, 3440.7cm^{-1} and 3438.2cm^{-1} were obtained respectively. Also the I.R. absorption at 1115.6cm^{-1}, 1110.8cm^{-1} and 1115.8cm^{-1} for M, N, and Q respectively indicate the C-O stretching of ester.

The absorption at 819.2cm^{-1}, 843.4cm^{-1}, 828.9cm^{-1} for M, N, and Q respectively indicate the presence of substituted benzene in the compounds. The major difference in the three crystals is that the C-H stretching absorption in M and Q which support the C=O stretching for the presence of aldehyde in them is not present in N. Also, the C=C stretching for conjugated unsaturation that is present in Q is not found in M and N.

**CONCLUSION**

The UV absorption of M at 238nm, Q at 240nm and N at 220nm is in agreement with IR result for the presence of benzene ring in all the crystals. Also, the UV absorption of M at 326nm and Q at 326nm agrees with the IR analysis for the presence of C=O (carbonyl) of aldehyde in the two crystals. Finally, UV and IR analysis are not enough to elucidate structure of compounds, therefore, other instrumentation techniques such as mass spectrometry and H and C nuclear magnetic resonance spectrometer must be employed for the structure of the compound to be fully elucidated which can be supplemented by chemical method if need be.

**REFERENCES**


Isawumi MA. Nigeria Cwewins Sticks, Nigeria Field 43: 50-58.


