PRELIMINARY INVESTIGATION, PHYTOCHEMICAL PROFILE AND PROXIMATE COMPOSITION OF Cola lepidota (Karl SCHUM) SEED.

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Received 28 August 2019; accepted 18 October 2019, published online 07 February 2020

ABSTRACT
Medicinal plants are rich sources of some active ingredients used in drugs synthesis and development. Monkey cola is a common name given to some edible wild relatives of West African kola nut. The physico-chemical and phytochemical profile of Cola lepidota seeds were assessed using standard chemical methods. The proximate analysis showed that the seed contained moisture (9.40±0.02 %), ash (4.57±0.03 %), fibre (4.58±0.01 %), fat (16.70±0.02 %), protein (23.78±0.04 %) and carbohydrate (40.97±0.05 %). The Fourier Transform Infra-Red (FTIR) spectrometer analysis revealed the presence of hydroxyl (−OH), nitrile (C−N) and carboxylic acid (COOH) in the seed. Elemental analysis using Micro Particle induced X-ray emission/Rutherford backscattering spectrometry (PIXE/RBS) revealed the presence of major and trace elements like sodium, magnesium, calcium, zinc and manganese. The percentage composition of carbon was 57.53, oxygen 39.96 and nitrogen 0.84. The phytochemical contents (mg/100g) obtained in the sample were as follows; alkaloids 4239.37±0.29, flavonoids 10.72±0.14, saponins 22.5±0.02, tannins 288.44±27 and terpenoids 1032.0±41.70. This result shows that C. lepidota has some medicinal values and contains essential elements needed for human body growth and development.

KEYWORDS: Physico-chemical properties, Phytochemical profile, Fourier transform infra-red (FTIR) spectrometer, Cola lepidota

INTRODUCTION:

Monkey cola is a common name given to a number of minor relatives of the Kola species that produce edible tasty fruits [1]. They include the C. pachycarpa K.Schum (White Monkey cola), C. lateritia K.Schum (Red Monkey cola) and C. lepidota K.Schum (Yellow Monkey cola). All these yield fruits of varying characteristics and sweetness [1]. The species are known in southern Nigeria, where they are common sights in local markets during its peak fruiting season by June to November. All of the species are identified by various local names in southeastern Nigeria: “Achicha” or “Ochiricha” in Igbo and Ndiyah in Efik as well as Ibibio. However, the nutritional value of the fruits have been evaluated and quantified by the authors [2]. Monkey cola fruit is consumed by men, women and children alike because of their natural tasty pulp, especially that of the species C. lepidota and C. pachycarpa [3]. The aril (waxy mesocarp) form the edible portion of the follicle and varied in colour, with the Cola. rostrata having whitish aril, while Cola. lepidota is characterized by yellowish aril. Monkey cola is an under-utilized fruit found in South Eastern Nigeria [2]. The value of this underutilized indigenous fruit tree in meeting the micro nutrient needs of local people, in alleviating food insecurity and as a source of income for
resource-poor farmers cannot be over stressed [4]. *C. lepidota* is reported to be employed in Nigerian folk medicine as for pulmonary problems and cancer related ailments [5]. In Nigeria about twenty three species are known and some are used in traditional medicine as stimulant, to prevent dysentery, and to suppress sleep [6] . There is scanty research and information on the monkey cola species. The seeds of the monkey cola species are obliquely ovoid with two flattered surfaces, rough and reddish brown or green; but not edible unlike the seeds of cola nut (*Cola nitida*). Literature search reveals that there is paucity of information as regards the antioxidant potential of *C. rostrata* and *C. lepidota* seeds and fruit aril coupled with the increase in demand for herbs and the urgent need to evaluate nature's repository of chemicals in plants for their potential value in health care[7]. Most of the analysis were carried out on the membrane of these fruit while the seeds are considered as waste and discarded. The aim of this study is to provide more information and find new uses for *C. lepidota* seeds.

**MATERIALS AND METHODS**

**SAMPLE COLLECTION**

Fresh fruits of *C. lepidota* were purchased from Watts market in Calabar, Cross River State in Nigeria. The plants were identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Ibadan, Oyo state, Nigeria. The seeds were separated from the fruits, dried at room temperature, sorted out and pulverized with mill machine of 0.5mm sieve size, stored in a clean and dry container with tight lid. Some of its physical parameters like texture, colour, solubility and pH were immediately determined.

**Proximate Analysis**

Standard methods by Association of official analytical chemists (AOAC) [8] were used to determine the carbohydrate content, crude fats, crude fibres, protein, ash and moisture content of the sample.

**Gross Energy**

The formula used for gross energy is as follows:

\[
GE (\text{Kcal/g}) = 5.72 \times \text{(protein)} + 9.5 \times \text{(fat)} + 4.79 \times \text{(fibre)} + 4.03 \times \text{(carbohydrate)}
\]

**ELEMENTAL ANALYSIS**

**Preparation of Sample for FTIR Analysis**

Approximately 1 mg of the powdered sample of the seed of *C. lepidota* was put in a ceramic crucible and 120 mg of potassium bromide was measured into it and mixed together for proper homogeneity, after that it was put into a potassium bromide disc to form a pellet and later compressed, the disc and the pellet formed were put in the sample holder, tightened well with its bolts and then introduced into the spectrometer.

**Preparation of Sample For Micro PIXE Analysis**

Approximately 1 mg of the powdered sample of the seed of *C. lepidota* were ground to 200 mesh and pressed into pellets for analysis. Samples were sent to centre for energy and research development, Obafemi Awolowo University, Ile Ife.

**Phytochemical screening:** Phytochemical Screening of *C. lepidota* were performed using standard procedure as described by [11], [12],[13] for the presence of tannins, saponins, flavonoids, terpenoids, anthraquinones, cardiac glycosides and alkaloids.

**Quantitative Phytochemical analysis**

Quantitative Phytochemical analysis were performed using the method described by [11], [13], [14], [15], [16], [17].

**Determination of flavonoid**

Colorimetric method was used with some modifications to determine flavonoid content.
1mL of seed extract was mixed with 3mL of methanol, 0.2mL of 10% aluminium chloride, 0.2mL of 1M potassium acetate and 5.6 mL of distilled water and was allowed to remain standing at room temperature for 30 minutes. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/mL). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/100g of extracted compound).

**Determination for Antraquinone**

Fifty milligram (50 mg) of the sample was soaked in 50 mL of distilled water for 16 h. The suspensions of the samples were heated in water bath at 70 °C for one h. After the suspensions were cooled, 50mL of 50% methanol was added to the sample, followed by filtration. The spectrophotometric value of the filtrate was read at a wavelength of 450 nm and compared with those of standard solutions containing 1mg/100mL of alizarin and purpurin respectively.

**Determination for Saponins**

Fifty milligram (50 mg) of the sample was weighed into a conical flask and 100 cm$^3$ of 20% aqueous ethanol was added to the sample. The sample was heated over a hot water bath for 4 h with continuous stirring at about 550°C. The mixture was filtered and the residue re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 900°C. The concentrated filtrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The resultant solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the weight of saponin was determined in the samples.

**Determination for Tannins**

Fifty milligram (50mg) of the sample was weighed into a 250 mL beaker. 50 mL of distilled water was added and stirred for 1 hour on a mechanical shaker. The sample was filtered into a 50 mL volumetric flask and made up to the mark. Five (5 mL) of the filtered sample was measured into test tube containing 2 mL of 0.1 M FeCl$_3$ in 0.1 M HCl and 0.008 M K$_3$Fe(CN)$_6$ (1:1). The absorbance was measured with a spectrophotometer at 120 nm wavelength within 10 Minutes.

**Determination for Alkaloids**

Five hundred milligram (500mg) sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added to it, covered and allowed to stand for 4 h. This was then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide solution and then filtered, dried and weighed.

**Determination for Terpenoids**

Five gram (5 g) of the sample was weighed into a conical flask, 20 ml distilled water was added with 5ml of ethanol The sample mixture was kept in a water bath for 1 hour then filtered with
15 ml of petroleum ether and read at an absorbance of 420nm. [19]

Statistical Analysis
All determinations were conducted in triplicate and statistical analysis was performed using SPSS software 16.0 (SPSS Inc., Chicago, IL). Results were recorded as Mean ± SD

Results
The seeds exhibited brown coloration and smooth texture with a pH of 4.78 ± 0.01 (Table 1)

Table 1: Physical Parameters of C. lepidota seed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>Smooth</td>
</tr>
<tr>
<td>Colour</td>
<td>Brown</td>
</tr>
<tr>
<td>Solubility</td>
<td>Not soluble in polar solvent but soluble in nonpolar solvent</td>
</tr>
<tr>
<td>pH</td>
<td>4.78 ± 0.01</td>
</tr>
</tbody>
</table>

Results are means of triplicate determinations ± standard deviation

C. lepidota seed contains a lot of nutrients which include sodium, magnesium, aluminium and other elements as listed on the table below.

The results below show that C. lepidota seed contains a large amount of potassium and a very small amount of nitrogen.

Table 2 showing contents of Kola lepidota

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein%</td>
<td>23.78 ± 0.04</td>
</tr>
<tr>
<td>Fibre %</td>
<td>4.58 ± 0.01</td>
</tr>
<tr>
<td>Fat %</td>
<td>16.70 ± 0.02</td>
</tr>
<tr>
<td>Ash %</td>
<td>4.57 ± 0.03</td>
</tr>
<tr>
<td>Moisture %</td>
<td>9.40 ± 0.02</td>
</tr>
<tr>
<td>Carbohydrate %</td>
<td>40.97 ± 0.05</td>
</tr>
</tbody>
</table>

Results are means of triplicate determinations ± standard deviation

C. lepidota seed contains a lot of phytochemical component with flavonoid having the least value of 10.72 ± 0.14 and alkaloids having a large value of 4239.37±0.13 as shown in Table 3.

Table 3: Phytochemical Components (mg/100g) of C. lepidota (monkey kola)

<table>
<thead>
<tr>
<th>Phytochemical Component</th>
<th>Amount (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoids</td>
<td>10.72 ± 0.14</td>
</tr>
<tr>
<td>Total terpenoids</td>
<td>1032.00 ± 41.70</td>
</tr>
<tr>
<td>Total saponins</td>
<td>22.51 ± 0.02</td>
</tr>
<tr>
<td>Total antraquinone</td>
<td>356.81 ± 0.017</td>
</tr>
<tr>
<td>Total tannins</td>
<td>288.44 ± 21.40</td>
</tr>
<tr>
<td>Total alkaloids</td>
<td>4239.37 ± 0.13</td>
</tr>
</tbody>
</table>

Results are means of triplicate determinations ± standard deviation

C. lepidota seed contains a lot of nutrients which include sodium, magnesium, aluminium and other elements as listed on the table below.

The results below show that C. lepidota seed contains a large amount of potassium and a very small amount of nitrogen.

Table 4: Elemental quantification of C. lepidota

<table>
<thead>
<tr>
<th>Elements</th>
<th>Quantity (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na)</td>
<td>103.7 ± 25.29</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>1724.2 ± 26.55</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>102.1 ± 10.37</td>
</tr>
<tr>
<td>Silicon (Si)</td>
<td>160.3 ± 9.20</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>1917.9 ± 12.27</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>1900.8 ± 11.02</td>
</tr>
<tr>
<td>Chlorine (Cl)</td>
<td>526.4 ± 8.58</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>15648.6 ± 25.04</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>1553.2 ± 36.03</td>
</tr>
<tr>
<td>Mangenese (Mn)</td>
<td>13.0 ± 8.83</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>80.7 ± 7.46</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>5.6 ± 4.70</td>
</tr>
<tr>
<td>Bromine (Br)</td>
<td>17.6 ± 7.94</td>
</tr>
<tr>
<td>Carbon (C)</td>
<td>57.5 ± 8.81</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>9.9 ± 4.01</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>0.84 ± 2.00</td>
</tr>
</tbody>
</table>

Results are means of triplicate determinations ± standard deviation
The Standard curve for determination of Total Flavonoid found in *C. lepidota* is shown in figure 1 below.

![Standard curve for determination of Total Flavonoid](image)

Figure 1 : *Standard curve for determination of Total Flavonoid*

The FTIR spectrum for determination of the functional groups found in *C. lepidota* seed is shown in figure 2 below. It revealed the presence of functional groups like Hydroxyl (-OH), Nitrile (C-N) and carboxylic acid (COOH) in the seed.

![The FTIR spectrum of C. lepidota seed](image)

Figure 2 : The FTIR spectrum of *C. lepidota* seed

**DISCUSSION**

The texture, colour, odour, solubility and pH of the seed of *C. lepidota* were assessed. The texture was smooth while the colour was brown. It was odourless and the pH was 4.78 ± 0.04, it showed that seed is slightly acidic as some of our foods like milk and yoghurts, beans and lentils and some fruits. It acidic nature is not as high as that of the stomach which is 3.5 [20]. These acidic food or fruit aids digestion and some tends to become alkaline in the body. The proximate analysis revealed that the seed contained protein (23.78 ± 0.04%) . Most foods with large amount of protein are slightly acidic [21]. Protein renews cells in all ages and builds new life. The presence of fibre, fats, carbohydrate, ash and moisture was also shown in the seed analysis. Fibre is an important component of human and animal nutrition. Fat is useful in the secretion of oil on the body and also keeps the body warm. The carbohydrate content of the seed was the highest with a value of 40.97 ± 0.05, it is a good source of energy to both humans and animals. The FTIR interpretation of the spectrum of *C. lepidota* seed showed some functional groups like alcohol, nitrile, alkynes, carboxylic acids, methyl group, aromatic compounds etc, due to
the bands (wave numbers) and the bend and stretch spectra. The alcohol and carboxylic acid are good source found in beverages (ethyl alcohol) and cell metabolism while nitrile, alkynes, and methyl groups are source of structure formation.

The micro PIXE/RBS results revealed that some major mineral elements and trace elements were present in the seed of *C. lepidota*. Some major mineral elements like magnesium (Mg), calcium (Ca), sulphur (S) and Iron (Fe) as the essential elements were seen. Sodium is important in the food of animals because it helps transmit nerve impulses and also maintain osmotic balance of the cells. Magnesium is important in diet as it aids muscle contraction, it is needed for utilization of iron and it is present in the teeth & bone. Silicon plays a vital role in the prevention of atherosclerosis, as it increases overall benefit of vitamin D glucosamine and calcium. Phosphorus is important in the food of animal and man, for it stirs development of teeth and bones, it also form part of DNA and RNA, it is also aids in respiration. Carbon is a good source of fuel, when combine with oxygen it helps in photosynthesis for plants to manufacture their food.

Medicinal plants are rich sources of ingredient used in drug synthesis and development. They are backbone of traditional medicines and over 3 billion people in developing countries utilize phyto-medicine on regular basis [22]. Some species cola have been reported to be of great medicinal use. Phytochemical property of *C. lepidota* was accessed using standard protocols. Analysis of the plant extracts revealed the presence of phytochemicals such as phenolic, tannins, flavonoids, saponins, glycosides, terpenoids, and alkaloids.

The phenolic compounds found in *C. lepidota* to have a value of 477.2 ± 5.05 mg/100g. The presence of phenolic compounds will impose some useful biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [23]. Studies have described the antioxidant properties of medicinal plants which are rich in phenolic acids, [24]. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc [14]. Flavonoids were also found in *C. lepidota* seed with a value of 10.72 ± 0.14 mg/100g, flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [25]. They also are effective antioxidant and show strong anticancer activities [24]. Plant extracts also contained saponins which are known to produce inhibitory effect on inflammation [23]. Saponins has the property of precipitating and coagulating red blood cells. Iron plays an important role in the formation of hemoglobin in red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [24]. Saponins was found in *C. lepidota* seed. Saponins contain glycosides and some glycosides have been reported to lower blood pressure according to many reports [24]. Tannins were also found in *C. lepidota* seed at a value of 288.44±21.40 mg/100g, tannin bind to proline rich protein and interfere with protein synthesis. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhea. 4239.37±0.13 mg/100g of alkaloids was found in *C. lepidota* seed which showed that monkey
Kola is a good source of alkaloid. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [26]. Several workers have reported the analgesic [27], antispasmodic and antibacterial properties of alkaloids [28]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this seed is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

CONCLUSION

C. lepidota seed could serve as food and drug to both man and animals due to its rich nutrients and high phenolic contents. It is a good source of alkaloid and phenolic compounds which are notable sources of modern formulated therapeutic drugs. C. lepidota has some medicinal values and contains essential elements needed for human body growth and development

REFERENCES

15. Sahu, R. and Saxena., J.( 2013). Screening of total phenolic acid and flavonoid content in conventional and non-conventional species of Curama. International Journal of pharmaceutical review and research. 21(2); 24-26