Evaluation of Proximate, Sugar and Fatty Acids Compositions of African Walnut (Plukenetia conophora Mull. Arg.) Root Bark

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**ABSTRACT**

African walnut (\textit{P. conophora} Mull. Arg.) root is used traditionally as antitussive and expectorant. The decoction and infusion of the root is often taken without any standard measurement, hence the need to investigate the proximate, sugar compositions and fatty acids content of its root bark with a view to establish its industrial and pharmacological importance. Proximate, sugar, and fatty acids were determined using standard methods as described by Association of Official Analytical Chemists. The proximate analysis showed that the root bark contained high amount of moisture (71.70 ± 0.01)\%, followed by crude fiber (18.80 ± 0.01)\% while protein and ash contents were (3.42 ± 0.01)\%, and (2.84 ± 0.02)\%, respectively, and crude fat was (1.34 ± 0.01)\%. Sugar analysis revealed that the root contained sucrose (4.37 ± 0.02) \%, glucose (3.28 ± 0.02) \% and maltose (0.86 ± 0.02)\%. The fatty acids analysis showed that the root bark contained saturated, mono unsaturated and poly unsaturated fatty acids. The prominent saturated fatty acid was stearic acid with the value of 4.10\% while oleic acid (33.88\%) was the highest for mono unsaturated fatty acid. Linolenic acid with the values of 35.57\% was the prominent among the polyunsaturated fatty acids. The study has revealed the presence of some essential nutrients in the root bark of African walnut in moderate proportion which suggest the potential utilization of this plant part as nutrient, addictive, or supplement in drug and cosmetics formulation.

**KEYWORDS**

African walnut; fatty acid; sugar; proximate; root bark

**Introduction**

Walnut is a common name used for a small family of flowering plant. It is important because of the nuts and timber most of them produced. The walnut was so-called because it was introduced from Gaul and Italy. The former Latin name for walnut was Nux gallic “Garlic nut” (Brinkman, 1974). This family contains 59 species, most of them found in Europe and Asia are trees (Marshall, 2009). The walnuts found in Africa are climbing shrubs except \textit{Coula edulis} which is referred to as “false walnut” and it is a tree (Agroforestry, 2009). The walnut family is placed in an order with a family containing a single species, an aromatic deciduous tree confined to China and Vietnam (Marshall, 2009) in which the resemblances are also found in Africa (Agroforestry, 2009). Plant families that are referred to as walnuts are: Juglandaceae (\textit{Juglans regia}), Olaceae (\textit{Coula edulis}) and Euphorbiaceae (formerly called \textit{Tetracarpidium conophorum} currently called \textit{Plukenetia conophora} Mull. Arg.). \textit{Plukenetia conophora} Mull. Arg. (\textit{Euphorbiaceae} family) is a species of walnut found in Nigeria and Cameroon while species found in other African continent and Asian are called by different names. \textit{P. conophora} is a west
equatorial woody perennial climber and is mostly found growing in the moist forest zones of sub-Saharan Africa and it is widely distributed in the southern part of Nigeria and plenteous in all cocoa-producing states in Nigeria, such as Abak, Uyo, Etinan, Akankpa akpa buyo, Lagos, Kogi, Ife, Ondo, Ogbomoso and Ibadan (Nwaichi et al., 2017). Plukenetia conophora is called with different name ranging from ukpa in Igbo, awusa or asala in Yoruba, arinsa in Igbira, kporo in Efiks. Plukenetia conophora plant is cultivated principally for the nuts which are cooked and consumed as snacks (Oke, 1995). Ndukwu and Ejirika (2016) considered physical properties of the African walnut from Nigeria and found that the various properties are affected by increase in moisture contents and change in nut mass. Oke et al. (2020) reviewed the economics, nutritional, and medicinal values of African walnut from Nigeria and gave information on the values of African walnut monetarily and for national developments.

Many works had been reported on the proximate composition, amino acids, phytochemicals, fatty acids, vitamin, and mineral compositions of nuts and leaves of this plant (Ayoola et al., 2013; Onawumi et al., 2013) but scanty report had been read about the root bark of this plant which is essential in traditional medicine. Therefore, this work aimed to investigate the nutritional potential of the root bark of Plukenetia conophora Mull. Arg and so the proximate, sugar and fatty acids compositions of the plant are determined.

**Materials and Methods**

Fresh Plukenetia conophora root sample was collected from a private farmland situated at Osupa ile area of Ajaawa, Ogo-Oluwa Local Government Area, Ogbomoso, Oyo State, Nigeria (Figure 1) The root bark was removed from the root of a matured plant using a stainless knife, washed, cut into smaller pieces to facilitate dryness and dried in the open air for seven days (7 days) and finally oven dried at 105°C for 12 h. The dried sample was crushed and ground into fine powder using mechanical grinder before stored in an airtight bottle prior to analyses.
**Determination of the Proximate Composition**

Moisture content was determined by drying to a constant weight at 100°C in an oven. The ash content was determined by igniting at 550°C in a muffle furnace for 4 h. The oil content was determined by Soxhlet extraction with n-hexane for 8 h. Protein was determined by the Kjeldahl method, and crude fiber was determined by the acid and alkaline digestion methods. All the methods used were as described by AOAC (2005). The carbohydrate content was estimated by difference.

**Isolation of Oil**

The powdered sample of the root bark was weighed (50 g) into a 2.5 L bottles and 1.5 L of n-hexane was added into the extraction bottle and the mixture was left for 72 hours with intermittent shaking (extraction by maceration). The mixture was filtered using glass wool and the filtrate was exposed to air for the evaporation of n-hexane. Green colored liquid (oil) was left as residue and its weight was determined.

**Gas Chromatography (GC)**

The extracted oils were subjected to GC analyses on GC 2010 instrument. Column oven temperature was 60°C with injection temperature of 250°C. Split injection mode, at 100, 2k Pa; Column flow of 1.61 ml/min and total flow of 6.2 ml/min; 1.0 split ratio; oven temperature programming is 60°C for 5 min and at the rate of 5°C/min to 140°C, and at 15°C/min to 280°C.

**Gas chromatography-Mass Spectrometry**

The GC-MS analyses were performed on GC-MS QP2010 Plus ion, source temperature 200°C; interface temperature 250°C; solvent cut time 2.5 min; with relative detector gain mode and threshold 3000; scan MS ACQ mode; detector FTD; mass range of m/z 40–400.

**Identification of Components**

Identification of the oil components were based on their retention indices (determined with a reference to a homologous series of n-alkanes), their mass spectral were compared with fragmentation patterns in computer matching against in built data and commercials such as Joulain and Koenig (1998), Adams (1995) and Massada (1976) libraries as well as in-house “Baser library of essential oil constituents” built up by genuine compounds and components of known oils.

**Determination of Fatty Acids Profile of the Root Bark of Plukenetia conophora Plant Using Spectrophotometric Method**

Two grams (2 g) each of the samples was weighed into 100 ml conical flask and 20 ml of benzene was added, shaking thoroughly to extract all the fatty acids. The mixture was transferred into a 250 ml separating funnel to separate the benzene extract from the aqueous extract. Then, 5 ml aliquot of the benzene extract was pipetted into a 15 ml test tube and 2 ml of 10% copper acetate was added to develop blue color. Standard solutions of each fatty acid were prepared in the range 0–10 ppm from 100 ppm stock solution of each fatty acid. Absorbance or optical density of sample extract as well as standard solutions of different concentrations were read on a spectrophotometer at a wavelength defined for each of the fatty acid as listed below:

- Lauric acid (640 nm), stearic acid (650 nm), palmitic acid (630 nm), arachidonic acid (690 nm), oleic acid (670 nm), linoleic acid (660 nm), linolenic (680), ricinoleic acid (610 nm), and dihydroxystearic acid (655 nm).

The % of each fatty acid (AOAC, 2005) was obtained using the formula: % fatty acid = \( \frac{\text{Absorbance of sample} \times \text{gradient}}{\text{factor of a specific fatty acid} \times \text{dilution factor}} \times \frac{10000}{\text{Weight of sample}} \)
Determination of Tocopherol (Vitamin E) (AOAC, 2005)

Into a 250 ml conical flask 1 g of the sample was weighed and filtered with a reflux condenser into the flask. 10 ml of absolute alcohol and 20 ml of 1 M alcoholic sulfuric acid were added. The condenser and flask were wrapped in aluminum foil and refluxed for 45 min and cooled for 15 min. Thereafter, 50 ml of distilled water was added to the mixture and transferred to a 250 ml separating funnel covered with aluminum foil. The unsaponifiable matters in the mixture were extracted with 150 ml dimethyl ether. The combined extracts were washed free of acid, dried and evaporated at a low temperature (25°C) and the residues obtained were immediately dissolved in 10 ml absolute alcohol. Solutions of the root bark sample and the standard were each transferred into 20 ml volumetric flasks; 5 ml absolute alcohol was separately added, followed by a careful addition of 1 ml conc. HNO₃. The flasks were placed on a water bath at 90°C for exactly 3 min from the time the alcohol began to boil; it was cooled rapidly under running water and adjusted to volume with absolute alcohol. The absorbance was measured at 470 nm against a blank containing 5 ml absolute alcohol and 1 ml conc. HNO₃ treated in a similar manner.

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\text{Vitamin E (μg/100g)} = \frac{\text{Absorbance} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample}}
\]

Determination of Sugars

Extraction of sugars

The root bark powder sample was weighed (1.0 g) into a boiling tube with analytical balance. 25 ml of hot ethanol was added into the boiling tube and mixed on a vortex mixer. The mixture was allowed to settle for 30 min and then filtered through what man No.41 filter papers into a beaker. The steps were repeated 3 times for complete extraction of glucose. The extract was heated at 50°C to achieve complete evaporation of ethanol. 10 ml water was added to dissolve the content and transferred into 100 ml volumetric flask. The content of the beaker was washed three times and added to the volumetric flasks and made up with distilled water to 100 ml mark. Anthrone method was used in the determination of sucrose, maltose and glucose (Brown and Zerbon, 1981)

Results and Discussion

The results of proximate analysis are as shown in Table 1. The presence of all the nutrients that constitute balance diet for human in the root bark is a proof that it can be used for nutritional purposes. The moisture content (71.67 ± 0.02) of the root bark is relatively high compared to the amount found in the seed and leaf of the nuts (Ayoola et al., 2013) which is typical characteristic of a rhizome. The percentage of ash is lower in the root (2.84) when

| Parameter  | % Composition |
|------------|---------------|
| Moisture   | 71.70 ± 0.01  |
| Ash        | 2.84 ± 0.02   |
| Crude fiber| 18.80 ± 0.01  |
| Crude fat  | 1.34 ± 0.01   |
| Crude protein | 3.42 ± 0.01 |
| Carbohydrate          | 1.70 ± 0.02   |

Values are mean of triplicate determination (±SD).
compared with the value obtained for seed and the leaves, 5.27 ± 1.35% and 12.89 ± 0.02%, respectively. The samples with high percentage of ash content are expected to have high concentration of various mineral elements, which are expected to speed up metabolic process and improve growth and development (Oluymomi et al., 2006). The percentage of fiber was a bit higher (18.78 ± 0.02) compared to other nutritive parameters except moisture. Fiber helps in the maintenance of human health by reducing cholesterol level in human body. Fiber diets promote peristaltic movement, the wave-like contraction that moves food through the intestine. Presence of high crude fiber improves glucose tolerance which is beneficial in treating the onset of diabetics (Eromosele and Eromosele, 1993). Thus, the incorporation of this plant into human diets would increase the level of fiber intake and could be of tremendous benefit to the diabetic patients.

The percentage of crude fat and carbohydrate were low, thus making the root bark to be low in energy given nutrient but could be medicinal. Food with low carbohydrate content had been reported to be ideal for diabetics and hypertensive patients (Gbolamali et al., 2007). The percentage of the crude protein is moderate (3.67 ± 0.02%) and could support in healing, growth, and repair of worn-out tissues. However, the protein content obtained was lower compared to the values of crude protein value reported for walnut seeds (26.3%, 29.09% and 35.22%) by Stevens (2003), Victor (2005) and Edem et al. (2009), but relatively closer to the percentage of crude protein (20–24%) in the P. conophora seed reported by Ogunsua and Adebona (1983). The fatty acids profile of the root bark of P. conophora contained high level of oleic and linolenic acids (unsaturated fatty acid) with minute amount of other fatty acids (Table 2). The amount of oleic acid present in the root bark (33.88%) is closer to that reported for palm oil and tallow oil, respectively (36.6% and 36.0%). While the amount reported by Osagie and Odutuga (1986) for P. conophora seed was 23.9%. However, walnut oil of Juglan regia (seed oil) has been reported to contain 52.9% of linolenic acid (James, 1997) while the seed oil of P. conophora has also been reported to contain 49.9% of linolenic acid. Asturia and Spain samples of virgin walnut oil were reported to contain palmitic acid (saturated fatty acid), oleic, linoleic, and linolenic acid (unsaturated fatty acid), the oil also contained tocopherol

| Type of Fatty acid | Fatty acid | Carbon and bond | Root bark oil (%) |
|-------------------|-----------|-----------------|------------------|
| Saturated fatty acid | Caproic acid | 6.0 | 0.40 |
| | Caprylic acid | 8.0 | 1.41 |
| | Capric acid | 10.0 | 1.55 |
| | Lauric acid | 12.0 | 1.18 |
| | Myristic acid | 14.0 | 1.59 |
| | Palmitic acid | 16.0 | 3.36 |
| | Stearic acid | 18.0 | 4.10 |
| | Behenic acid | 22.0 | 0.00 |
| | Lignoceric acid | 24.0 | 0.00 |
| Monounsaturated fatty acids (MUFA) | Palmitoleic | 16.1 | 0.51 |
| | Oleic acid | 18.1 | 33.88 |
| | Erucic acid | 22.1 | 0.05 |
| Polyunsaturated fatty acids (PUFA) | Linoleic acid | 18.2 | 0.02 |
| | Linolenic acid | 18.3 | 35.57 |
| | Arachidonic (Omega 6) | 20.4 | 0.05 |

Table 3. Quantity of Vitamin E in the Plukenetia conophora root bark.

| Vitamin Tocopherol(E) | Content(µg/100 g) | 0.64 ± 0.01 |
|-----------------------|-------------------|-------------|

Values are mean of triplicate determination (±SD).
(Bada et al., 2010). When hexane chloroform/methanol were used for extraction and GC used for the analysis of some nuts (almonds, Brazil, hazel, pecans, pine, and walnut) the result revealed that oleic acid was the predominant fatty acid present in all the samples subjected to the extraction except the walnut oils which contained high amount of linoleic acid and tocopherol. The lipid class composition of walnut oils (Juglan regia L) has been reported to affect the stability of the oil (Miraliakbari and Shahidi, 2008).

The walnut oil extracted with compressed carbon dioxide was not different from those obtained with n-hexane, the fatty acids present were linoleic acid, followed by oleic acid and linolenic acid while the amount of tocopherol obtained was larger in the n-hexane extracted oil (Rui Oliveira et al., 2002). Therefore, the root bark of P. conophora is now being reported to contained moderate level (35.57%) of the linolenic acid. It is a known fact that polyunsaturated fatty acids are good for human health, especially in cardiovascular diseases, and with the presence of omega-3 and omega-6 polyunsaturated fatty acids which show positive effect on cardiovascular diseases and cancers. It is also used in cosmetics (Conner, 1997), Uauy and Carlos (2003). It has also been reported that polyunsaturated fatty acids can regulate prostaglandin synthesis and hence induce wound healing (Bowman and Rand, 1980). The polyunsaturated fats in walnuts are easily oxidized therefore it is usually stored in a refrigerator or freezer in an airtight container. Fatty acids are the main ingredient of lipids (triglycerides, cholesterol esters, and phospholipids) which are tissue components and are lipid fraction par excellence for infant’s functions and requirement). The total tocopherol contents present in the root bark was found to be 0.64 ± 0.01 µg/100 g while the tocopherol content of J. regia L walnut was reported to be in the range of 268.5 to 436.0 µg/g oil (John, 2019). Lavedrine et al. (1997) reported that significant differences related to variety and geographical origin could be observed with decrease in the amount of tocopherols present.

The sugars present in the root bark were sucrose, glucose, and maltose (Table 3 and 4). They serve as the primary energy source for the brain and nervous system and can be used by many other tissues. Therefore, because of the presence of these sugars the root bark can be used as sources of energy for recuperating patients. Sucrose is water soluble and can easily be transported through the circulatory system of the plant. High concentrations of sucrose (sugar) produce a high osmotic pressure which inhibits the growth of microorganisms, so it could be used as preservative (Denniston et al., 2004). This could be the reasons behind the high level of antimicrobial properties of the P. conophora plant (Ajaiyeoba and Fadare, 2006).

### Conclusion

The study has shown that essential fatty acids (polyunsaturated fatty acids), sugars, and nutrients that are essential for the body systems are present in the root bark of the studied plant. Therefore, the root bark of the plant could be used nutritionally, medicinally, and fully utilized for economic growth of the continent.
Plate 1. *Plukenetia conophora* plant climbing a kolanut tree displaying the stem.

Plate 2. *Plukenetia conophora* root bark cut into smaller pieces. Source: Ayoola et al. 2015

Disclosure statement

No potential conflict of interest was reported by the authors.

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