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Anatomical studies and nutritional analysis of the leaf extract of *Plukenetia conophora*

C.E.C. Ugwoke[1]*, SPG Anze[1], C.V. Obisike[1], A.E. Nweze[2]

[1]Department of Pharmacognosy and Environmental Medicines, University of Nigeria Nsukka, Nigeria

[2]Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Nigeria

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Abstract

Background and aim: *Plukenetia conophora* Mull. Arg. (African walnut) belongs to the Euphorbiaceae family. The significance of wood anatomy in relation to taxonomy is by no means new or recent. **Aim:** The work evaluates the anatomy of the leaf, stem, root and the nutritional values of the leaves of *Plukenetia conophora*. **Methodology:** Leaf epidermal strips were made by Impression technique and all sectionings (TS, TLS and RLS) were made with Reichert sliding microtome. Standard stains were used to differentiate the tissues. The proximate and mineral analyses were carried out using standard methods. **Results:** Anatomical studies showed hypostomatic leaves with anomocytic stomata and no trichomes. Concentric vascular bundles were observed in the transverse sections of the leaf and petiole. The various sections of the stem anatomy revealed diffuse-in-aggregate vessels with scanty axial parenchyma in the transverse section, both uniseriate and biseriate rays in the transverse longitudinal section, and procumbent rays in the radial longitudinal section. Nutritional analysis showed presence of all the tested proximate and minerals. The leaves contained proximate: carbohydrates (25.57 ± 0.05), protein (18.02 ± 0.02), ash (29.36 ± 0.33), moisture (18.06 ± 0.03), crude fibre (9.83 ± 0.17) and fat (7.41 ± 0.11) and minerals: calcium (19.18 ± 0.00), potassium (14.01 ± 0.00) and sodium (7.97 ± 0.03), Zinc (0.70 ± 0.01), magnesium (1.91 ± 0.01), iron (4.93 ± 0.04) and manganese (0.91 ± 0.00). **Conclusion:** The anatomical results are quite useful in the delimitation of this species and nutritional results suggest the use of the leaves as a healthy food.

Keywords: *Plukenetia conophora, Anatomical sectioning, Staining, Proximate analysis*.

1. Introduction

Plukenetia conophora Mull. Arg. (African walnut) is a member of the Euphorbiaceae family. It is a woody climber about 6–18 m long on attainment of reproductive phase with a stem found in the wet parts of Eastern and Western Nigeria, and Western Africa in general (Udedi *et al*., 2013). Conophor plants are cultivated principally for the nuts which are usually cooked and consumed as snacks (Enujiugha and Ayodele, 2003). The fruits are four winged ridged between wings and up to 3 inches in diameter with four round seeds (usually brown) in each fruit (Nuhu *et al*., 2000)

The Euphorbaiceae is one of the most interesting and economically important plant families. The family is of much importance from the point of view of producing a number of useful products, for example, natural rubber is produced from *Hevea* sp; biodiesel from *Jatropha*; starch from *Manihot* and castor oil from *Ricinus*. Some of the families are also used as ornamentals. The plant is known in Africa especially in the Eastern and Western parts of Nigeria for its antibacterial efficacy (Okerulu and Ani, 2001). Decoction of leaves and seeds serve as beverage which relieves abdominal pains and fever. Dried walnuts can be ground and turned into flour which can be used as composite flour during baking or in-place of milk in tea preparation (Malu *et al*., 2009). Phytochemical analysis of the nuts, leaves and roots indicates that it contains bioactive compounds such as oxalates, phytates, tannins, saponins, alkaloids, flavonoids and terpenoids (Ayodele, 2003; Kalu, 2010; Ojobor *et al*., 2015). It possesses wound healing, antibacterial, antioxidant and immune stimulating activities (Animashun *et al*., 1994). It is a healthy food for cardiological patients due to its ability to reduce cholesterol and triglyceride in rats compared with the control group fed with standard diets (Kanu *et al*., 2015). Results from the study conducted by Onwuli *et al.*

Corresponding author
Full Address :
Department of Pharmacognosy and Environmental Medicines, University of Nigeria Nsukka, Nigeria
E-mail: ***christopher.ugwoke@unn.edu.ng***

(2014) suggest that *Plukenetia conophora* nut have antihyperglycaemic effect and can be encouraged as food for diabetic patients. The anti-inflammatory and antioxidant nutrients found in African walnut show prospects for its anticancer benefits.

2. Materials and methods

2.1 Collection of Plant Specimen

The leaf, stem and root of *Plukenetia conophora* used for the anatomical work were collected from a farm land at Afor Edem in Nsukka Local Government Area, Enugu State, Nigeria in April 2015. The materials were authenticated by Mr. A.O. Ozioko, a plant taxonomist with the Internation Centre for Ethnomedicine and Drug Development (InterCEDD) Aku Road, where the voucher specimen was deposited and numbered INTERCEED/Euph./032015. The plant materials were then taken to the Plant Anatomy Laboratory of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka for anatomical sectioning.

2.2 Anatomical Studies

Sectioning: Transverse sections (TS) of the leaf, petiole, stem and root, and the transverse longitudinal sections (TLS) and radial longitudinal sections (RLS) of the stem were made using a Reichert sledge microtome. Formalin acetic acid (FAA) and alcohol were used to preserve the specimens for further anatomical work. The sections were collected in different Petri dishes. About 70 % of alcohol was poured into Petri dishes which served as preservatives.

Staining: The sections were transferred into different staining jars stained with Safranin for 5 minutes and counter stained with Fast green. After the 5 minutes, the Safranin was allowed to drain off and then the sections were washed three times in distilled water. 97% alcohol was used to wash the sections twice and then washed again with absolute alcohol for proper dehydration. Then sections were mounted on the microscope using Canada balsam and then observed under an Olympus Standard Microscope. Photomicrographs were taken using a digital camera.

Foliar epidermal study: Impression technique was used to study the foliar epidermis of adaxial (upper) and abaxial (lower) surfaces of the leaf. The leaf samples were cleared by washing with water and allowed to dry. The epidermal strips of the leaves were peeled gently with the aid of a pair of forceps, and then placed on a clean slide and covered with a cover – slip. The slide was viewed under the light microscope at different magnifications but the photomicrograph was taken at x400 magnification.

Temporary Slide Preparation: The sample for study was mounted in I – 2 drops of aqueous iodine. The result indicated the presence of starch, that is, the tissue stained blue – black in the presence of iodine. Additionally, two drops of phloroglucinol and 2 drops of concentrated hydrochloric acid were added. Glycerin was used as the mountant. This test confirmed the presence of lignin.

Permanent Slide Preparation: The sections were transferred into staining jars and stained with 1% Safranin for 5 minutes. The Safranin was rinsed off and the sections were rinsed in 97% alcohol twice. The sections were counter stained in 1% Fast green for 5 minutes and washed 3 times with absolute alcohol. Fast green and Safranin served to differentiate lignified and unlignified tissues. The sections were transferred into staining jars containing 50/50 alcohol and xylene in the ratio of 1:1 and washed until they became clear. Pure xylene was finally used to clear the sections further and the specimen was eventually mounted on a standard microscope slide using Canada balsam as mountant and each was covered with a cover slip and allowed to dry. The prepared sections were viewed under Olympus light microscope at different magnifications. Photomicrographs of the specimen were taken.

2.3 Nutritional Analysis

Fresh leaves of *P. conophora* were harvested and air-dried under shade to prevent loss of active constituents. The dried leaves were ground into powder and used for nutritional analysis viz.

2.3 Proximate Analysis

Crude Protein: The crude protein content of foods or plant sample was determined using the micro kjedahl method (Pearson, 1976). The method involves digestion of samples, distillation of digests and titration of distillate using the formula below:

Carbohydrate: Carbohydrate content was determined by difference (AOAC, 1995). The percentage protein, fat, ash, moisture and crude fibre were summed up and subtracted from 100.

Determination of crude fats: The percentage of the crude fibre was achieved using the formula below:

Fibre Determination: The fibre percentage was calculated using the formula below:

Moisture Determination Using Oven Method: Similarly, the percentage moisture content of the sample was determined using the formula below:

Ash Determination Using Muffle Furnace: Ash content was calculated as follows:

2.4 Mineral Composition

Calcium: Calcium was determined using Pearson (1976) method. A 25 ml of the sample was pipette into a conical flask, a pinch of EBT was added, 2 ml of the NaOH solution was also added and the mixture with standard

EDTA solution.

$$Ca\ (mg/100g) = \frac{T \times N \times E \times 1000}{\text{Volume of sample used}}$$

Where T = Titre value; M= Molarity of EDTA; E = Equivalent weight of calcium

Magnesium: Magnesium was determined using Pearson (1976) method. A 25 ml of the sample was pipette into a conical flask and a pinch of EBT was added and then shaken. This was followed by the addition of 2 ml buffer. The mixture was then titrated using 0.01 M EDTA.

$$Mg\ (mg/100g) = \frac{T \times N \times E \times 1000}{\text{Volume of sample used}}$$

Where T = Titre value; M= Molarity of the standardized EDTA; E = Equivalent weight of magnesium

Potassium Determination Using Flame Photometer: Potassium was determined using Pearson (1976) method. The instrument was switched on and allowed for about 20 minutes to stabilize. The gas was then turned on, distilled water was aspirated through the siphon in order to zero the instrument and the samples were aspirated and the emission recorded.

The concentrations were calculated using sodium and potassium calibration curve for sodium and potassium readings respectively.

Iron Determination: Iron was determined using Pearson, (1976) method. A 10 ml of the sample was added into a 100 ml flask and made up to 50 ml with de-ionized water. A 20 ml of conc. HCI was added followed by the addition of 1.0 ml of hydroxylamine solution. About 0.5 g glass beads was added and heated to boiling point till the volume reduced to 2.0 ml, 10.0 ml of ammonium acetate buffer solution was added, 2.0 ml of phenanthroline was added and the content made up to 100 ml mark with de ionized water.

2.5 Statistical Analysis

Statistical package (SPSS) software version 20 was used to compute means of replicate data from nutritional analysis.

3. Results

Leaf epidermal study showed many anomocytic stomatal types – with ordinary epidermal cells surrounding the guard cells – no subsidiary (Figure I). Stomatal distribution was hypostomatic – stomata distributed only on the abaxial surface (Figures II). The epidermal cell walls are irregular in shape. The stomata are scattered within the epidermal cells. Epidermal hairs (trichomes) were absent.

3.1 Anatomy of the Leaf T

The transverse section (T. S.) of the leaf of *P. conophora* as viewed under X100 objectives is shown in Figure III. There was presence of single-layered upper epidermis and the lower epidermis. The palisade mesophylls are compactly arranged immediately after the epidermis. This is followed by the scattered spongy mesophyll cells, interrupted by the intercellular air spaces. A single vascular bundle consisting the xylem and phloem was observed. The xylem and the phloem are separated by meristematic cells called cambium.

3.2 Anatomy of the Petiole

The transverse section of the petiole as shown in Figure IV below revealed the internal structures of the petiole. Here there is a single layer of epidermal tissues covering the internal structures and prevent excessive loss of water. The epidermis is followed by layers of collechyma and parenchyma cells making up the cortex. There is also presence of about six vascular bundles (xylem and phloem) arranged in concentric manner.

3.3 Anatomy of the Stem

The transverse section of the stem of *P. conophora* is shown in Figure V. Here the internal tissues are protected by thin layer of epidermal tissues which would be replaced by the periderm in the mature stem as a sign of secondary growth. This is followed by the wide space of cells called the cortex and then the vascular bundles. The xylem vessels were diffuse-in-aggregate and associated

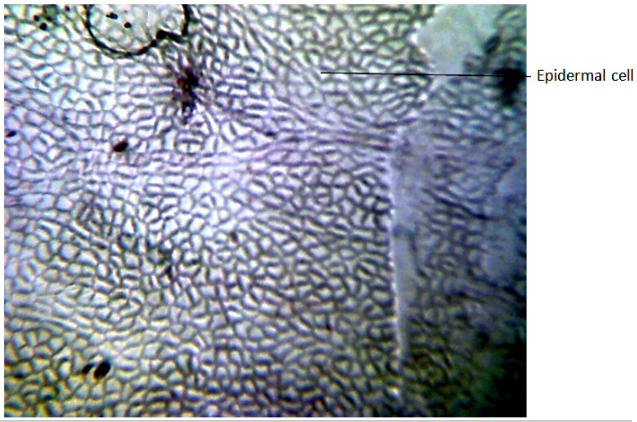

Figure I. Adaxial surface of the leaf of *P. conophora* (X100)

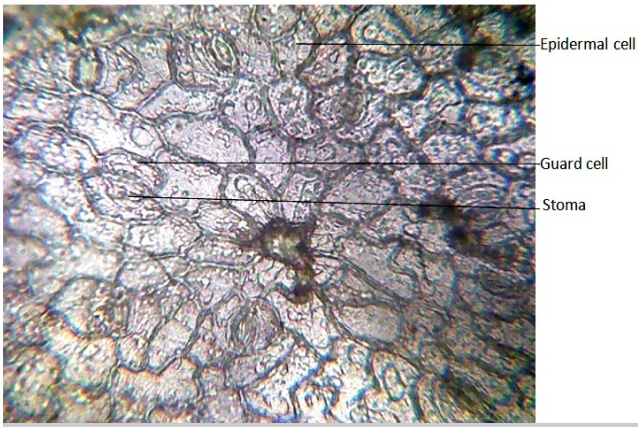

Figure II. Abaxial surface of the leaf of *P. conophora* (X400)

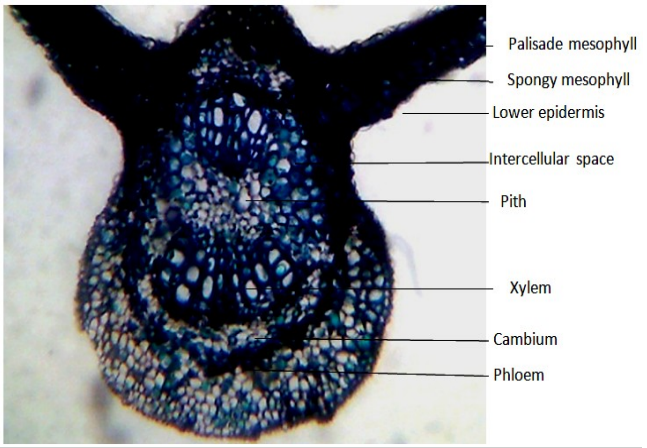

Figure III. Transverse section of the leaf of *P. conophora*

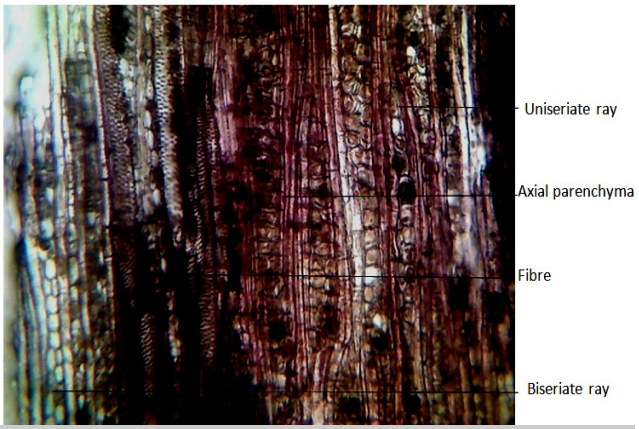

Figure VI. Tangential longitudinal section of the stem of *P. conophora* (X400)

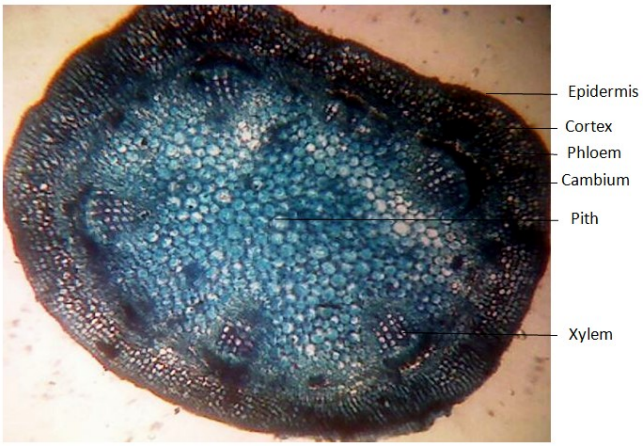

Figure IV. Transverse section of the petiole of *P. conophora*

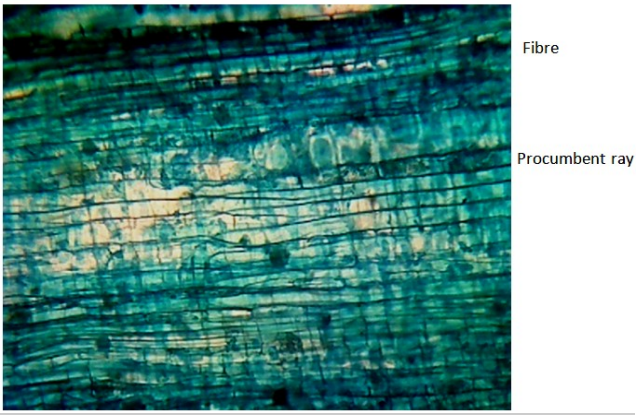

Figure VII. Radial longitudinal section of the stem of *P. conophora* (X400)

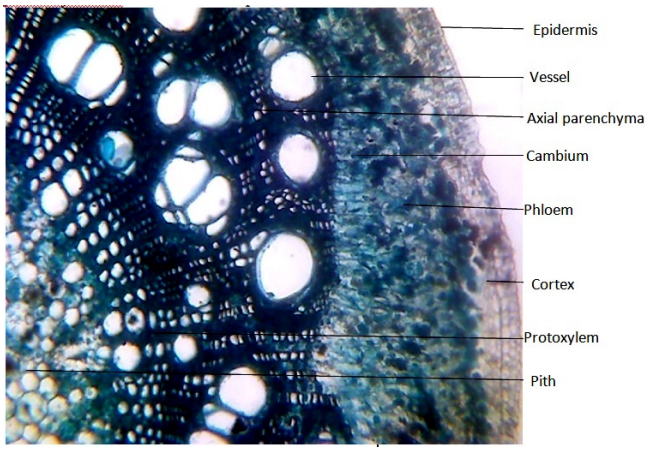

Figure V. Transverse section of the stem of *P. conophora*

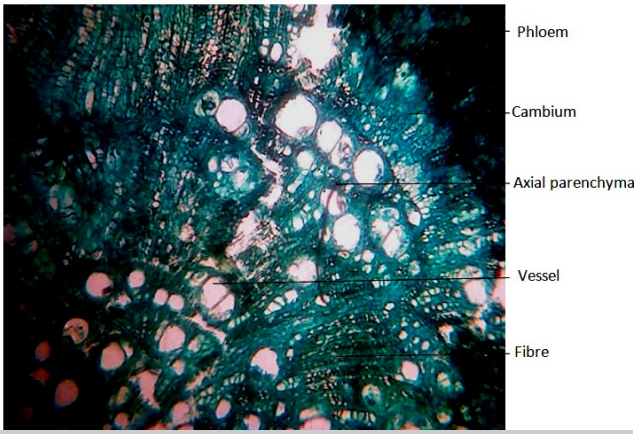

Figure VIII. Transverse section of the root of *P. conophora* (X400)

with scanty axial parenchyma. Within the centre of the section lies mass of parenchymatous tissue called the pith.

The tangential longitudinal section (TLS) of the stem wood revealed the presence of heterocellular multiseriate (uniseriate and biseriate) rays. The fibre cells are seen along the section, stacked on top of one another within the regions of the axial parenchyma (Figure VI).

The radial longitudinal section (RLS) revealed presence of homocellular (procumbent) rays (Figure VII).

3.4 Anatomy of the Root

The transverse section of the root of the plant as shown in Figure VIII revealed same vessel types as in the stem. The xylem vessels are diffuse-in-aggregate and associated with scanty axial parenchyma.

3.5 Nutritional Analysis of the leaf of Plukenetia conophora

Table I and Table II show the results of the qualitative and quantitative analyses of the proximate and mineral constituents of the leaves of *Plukenetia conophora*

Table I. Proximate composition of the leaves of *Plukenetia conophora*

Proximate	Qualitative composition	Quantitative composition (g/100g)
Carbohydrate	+++	25.57 ± 0.05
Protein	++	18.02 ± 0.02
Crude fibre	+	9.83 ± 0.17
Moisture	+	18.06 ± 0.03
Ash	+++	29.36 ± 0.33
Fat	+++	7.41 ± 0.11

N = 3; Values expressed as means ± standard error of means
+ = present; ++ = highly present; +++ = very highly present

Table II. Mineral composition of the leaves of *Plukenetia conophora*

Mineral	Qualitative composition	Quantitative composition (g/100g)
Zinc	+	0.70 ± 0.01
Sodium	+	7.97 ± 0.03
Potassium	++	14.01 ± 0.00
Magnesium	+	1.91 ± 0.01
Iron	+	4.93 ± 0.04
Calcium	++	19.18 ± 0.00
Manganese	+	0.91 ± 0.00

N = 3; Values expressed as means ± standard error of means
+ = present; ++ = highly present

respectively. The qualitative analysis showed the presence of all the tested constituents. Quantitative analysis result showed that the leaves contained very high amounts of carbohydrates (25.57 ± 0.05), protein (18.02 ± 0.02), ash (29.36 ± 0.33) and moisture (18.06 ± 0.03) while crude fibre (9.83 ± 0.17) and fat (7.41 ± 0.11) were in lower quantities. Result of the quantitative analysis showed appreciable quantities of the essential minerals such as calcium (19.18 ± 0.00), potassium (14.01 ± 0.00) and sodium (7.97 ± 0.03).

4. Discussions

Even though phylogenetic studies have been employed in the delimitation of Euphorbiaceae clades, several anatomical characters represent good diagnostic tools and provide further support to clades identified in molecular phylogenetic studies (Pace *et al.*, 2014). The descriptions of the anatomical features of *P. conophora* were presented to enhance its correct identity and as a tool for its conservation. This is because Taxonomic studies have been proven as one of the major ways to conserve biodiversity as forest resources to be conserved must have to be properly identified (Pandey and Misra, 2008). Foliar epidermal characters such as the epidermal cells, stomata and trichomes have been very useful in the classification of the members of Euphorbiaceae (Baranova, 1992). The result of this study showed that the epidermal cells are polygonal in shape with straight or curved walls. The leaf was hypostomatic, which is an adaptation to reduce the rate of transpiration. The stomata were anomocytic; this agrees with findings of Metcalfe and Chalk (1950) who reported that the mature stomata of Euphorbiaceae are anomocytic, paracytic or anisocytic. They further documented that these stomata are usually confined to the lower leaf surface and most rarely on both surfaces.

Wood anatomy (of both stem and root) is another important tool in delimitation of *taxa* because of the wide range of forms and distribution of their tissues (Oladipo and Oyaniran, 2014). Porous woods (woods with vessels) are hardwoods, which have two main types of growth rings, and one intermediate form (Metcalfe and Chalk, 1950; Carlquist, 2001). The result obtained from the transverse section of *P. conophora* stem and root showed the presence of diffuse-in-aggregate vessels. There was also presence of paratracheal axial parenchyma cells that are more or less scanty.

The types of rays found in angiosperm woods help in their identification. Rays in hardwoods are composed of ray parenchyma cells that are either procumbent or upright. As the name implies, procumbent ray cells are horizontal and the upright ray cells are ray parenchyma cells turned so that their long axis is vertical. Rays that have only one type of ray cell, typically only procumbent cells, are called homocellular rays. Those that have procumbent and upright cells are called heterocellular rays (Metcalfe and Chalk, 1950; Carlquist, 2001). The results from the TLS and RLS of the wood showed that both multiseriate (uniseriate and biseriates) and homocellular (procumbent) rays types are found in *P. conophora*.

Plant nutrients such as protein, fats, carbohydrate, minerals and vitamins are essential for life and contribute to caloric content of our diet (Underwood, 1994). The results from the nutritional analysis showed that the values for all the nutrients tested are within the reported values for some other important vegetables (Iheanacho and Udebuani, 2009; Olaposi and Adenni, 2010). The protein content reported in this study is comparable with those reported in *Cucurbita pepo* and *Gnetum africanum* (Iheanacho and Udebuani, 2009) and higher that those reported in *Cnidoscolus chayamansa*, *Solanum nodiflorum* and *Senecio biafrae* (Olaposi and Adunni, 2010). The result revealed the leaves to be rich in carbohydrates. Carbohydrate has the highest composition by percentage. This level is favourably compared with the acceptable range of mean values for vegetables. The carbohydrate content gave an indication that the leaves of *P. conophora* can be considered as a rich source of energy and is able to supply the daily energy requirements of the body in children and adults (Aranda *et al*, 2001; Balogun and Olatidoye, 2012).

It is well known that the dietary fibre and moisture content in vegetables play an important role in digestion and maintenance of the intestinal tracts (Iheanacho and Udebuani, 2009). The leaves studied proved that this plant is rich in fibre and therefore a good source of dietary fibre.

This study revealed that leaves of *Plukenetia conophora* are rich in mineral elements including calcium, phosphorus, potassium, magnesium and iron. These minerals are necessary for cell formation, transmission of nerve impulse and fluid balance (Ezeagu and Ologbodo, 1995). Calcium, phosphorus, magnesium are involved in bone formation. It also revealed that the leaves contain appreciable amounts of these mineral elements that could sufficiently meet the human mineral requirement (NRC/ NAS, 1980).

5. Conclusion

The findings from this study have shown anatomical characters of the leaves and wood, which are useful in distinguishing *Plukenetia conophora* from other inter-tribal and intra-tribal members of the family Euphorbiaceae. Furthermore, the high nutritional constituents reported in this study on the leaves of *P. conophora* are comparable with those of other vegetables and suggest that the leaves are a source of basic human nutritional requirements for healthy living.

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Conflict of interest

The author's declares none.

References

Animashun T, Togun RA and Hughes C R (1994). Characterization of isolectins in *Tetracarpidium conophorum* seeds (Nigerian walnut). *Glycoconjugation Journal* **11** 299 –303.

Aranda P, Dostalova J, Frias J, Lopez-jurado M, Koozhowska H and Pokerny J (2001). Nutrition. In: C. L. hedley (ed.). *Carbohydrate in Grain Legume Seeds. Improving Nutritional Quality and Agronomic Characteristics*. CAB International Wallingford, UK. p. 61 – 87.

Ayodele OB (2003). Nutrition in Nigeria. Ibadan Catoon Publishers.

Balogun, A. M. and Olatidoye, O. P. (2012). Chemical composition and nutritional evaluation of velvet bean seeds *(Mucuna utilis*) for domestic consumption and industrial utilization in Nigeria. *Parkistan Journal of Nutrition*, 11: 116-122.

Baranova, M. (1992). Principels of comparative stomatographic studies of flowering plants. *The Botanical Review,* 58: 49-99.

Carlquist, S. (2001). *Comparative Wood Anatomy (2nd ed.)*. Springer-Verlag, New York, 448 pp.

Essiett, U. A., Illoh, H. C. and Udoh, U. E. (2012). Leaf epidermal studies of three species of *Euphorbia* in Akwa Ibom State. Advances in Applied Science of conophor nut. *Pakistan Journal of Nutrition,* 2(6): 335 – 338. Research, 3 (4): 2481-2491.

Ezeagu, I. E. and Ologhobo, A. D. (1995). Proximate composition of unfamiliar plant seeds in Nigeria. *Polish Journal of Food and Nutrition Sciences,* 4: 79-83.

Iheanacho, K. M .E. and Udebuani, A. C. (2009). Nutritional composition of some leafy vegetables consumed in Imo State, Nigeria. *Journal of Applied Science and Environmental Management*, 13(3): 35 – 38.

Kanu, A. M., Kalu, J. E. and Okorie, A. C. (2015). Nutritional and health values of African walnut (*Tetracarpidium conophorum*). *International Journal of Scientific and Technology Research*, 4(9): 2277 – 8616.

Malu, S. P., Obochi, G. O., Edem, C. A. and Nyong, B. E. (2009). Effects of methods of extraction on phytochemical constituents and antibacterial properties of *Tetracarpidium conophorum* seeds. *Global Journal of Pure and Applied Sciences,* 15(3): 373 – 376.

Metcalfe C, Chalk L (1950). *Anatomy of the Dicotyledons*. Clarendon Press, Oxford, 634 pp.

NRC/NAS. (1980). *Nutritional Research Council Committee on Dietary Allowances*. Nutritional Academic of Science press. Washington, DC, U.S.A., 1120 pp.

Nuhu, A. M., Mshelia, M. S. and Yakubu, Y. (2000). Antimicrobial screening of the bark extract of *Pterocarpus erinaceous* tree. *Journal Chemical Society of Nigeria,* 25: 85 – 92.

Ojobor, C. C., Anosike, C. A. and Ani, C. C. (2015). Studies on the phytochemical and nutritional properties of *Tetracarpidium conophorum* (black walnut) seeds. *Journal of Global Bioscience,* 4(2): 1366–1372.

Okerulu, I. O. and Ani, C. J. (2001). The phytochemical analysis and antibacterial screening of extracts

of *Tetracarpidium Conophorum*. *Journal of Chemical Society of Nigeria,* 26(1): 53 – 55.

Oladipo, O. T. and Oyaniran, A. O. (2014). Taxonomic study of the wood anatomy of the genus *Ocimum* L. in Nigeria. *Ife Journal of Science,* 15(2): 295 – 302.

Olaposi, A. R. and Adunni, A. O. (2010). Chemical composition of three traditional vegetables in Nigeria. *Pakistan Journal of Nutrition*, 9(9): 858 – 860.

Onwuli, D. O., Brown, H. and Ozoani, H. A. (2014). Antihyperglycaemic effect of *Tetracarpidium conophorum* nuts in alloxan induced diabetic female albino rats. *Endocrinology*, 20(14): 1 – 4.

Pace, M. R., Lohman, L. G., Olmstead, R. G. and Angyalossy, V. (2014). Wood anatomy of major Bignoniaceae clades. Plant Systematics and Evolution, 45(2): 1129 – 1159.

Pandey, S. N. and Misra, S. P. (2008). *Taxonomy of Angiosperms*. Ane Books Pvt. Ltd.,New Delhi, India, 620 pp.

Pearson, D. (1976). *The Chemical Analysis of Foods.* (7th ed.). Churchill, Livingstone, 493 pp.

Udedi, S. C., Ani, O. N., Anajekwu, B. N., Igwilo, I. O., Ononamadu, C. J. Adindu, C. S. and Okafor, U. M. (2013). Comparative proximate analyses of raw and cooked *Tetracarpidium conophorum* (African walnut) found in Awka, Anambra State, Nigeria. *Bioscience,* 1(2): 114 –118.

Underwood, E. J. (1994). *Trace elements in Human and Animal Nutrition. (3rd ed.)*. Academic press, New York, London, 478 pp.