

Proximate Composition, Mineral Content, Phytochemical Screening and Anti-Nutritional Constituents of Walnut (*Tetracarpidium conophorum* OR *Plukenetia conophora*) Seeds.

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Abstract: This study was designed to evaluate the phytochemical screening, proximate composition, mineral content and anti nutritional constituents of cooked and raw African walnut (*Tetracarpidium conophorum* or *Plukenetia conophora*) seeds. The phytochemical screening of the seed reveals the presence of alkaloids, glycosides, steroids and polyphenols. The result of the proximate analysis was shown to be moisture (17.5±0.03%), crude protein (4.506±0.01%), lipid (20.0±0.05%), crude fibre (20.0±0.02%), ash (15.5±0.05%), carbohydrate (22.49±0.01%), vitamin C (11.15±0.1mg/kg) for cooked seeds and moisture (18.0±0.02%), crude protein (13.13±0.01%), lipid (22.50±0.025%), crude fibre(18.0±0.01%), ash (14.25±0.08%), carbohydrate (14.12±0.01%) and vitamin C (11.0±0.1mg/kg) for the raw seeds. The result of the mineral content of the seed was shown to be Cu (0.079±0.003PPM), Zn (0.1507±0.01PPM), Mn (0.124±0.01PPM), and Fe (0.124±0.01PPM) for the cooked seeds and Cu (1.08±0.1PPM), Zn (2.26±0.1PPM), Mn (0.064±0.001PPM) and Fe (0.079±0.002PPM) for the raw seeds. The anti nutrients content of the cooked sample was shown to be oxalate (0.0207± 0.01mg/100ml), phytate (0.114±0.01mg/100ml), hydrocyanide (0.011±0.10mg/100ml) and that of the raw seeds was shown to be oxalate (0.204±0.10mg/100ml), phytate (0.123±0.02mg/100ml) and hydrocyanide (0.112±0.10mg/100ml). This analysis showed that African walnut seeds are a rich source of lipid, fibre, carbohydrate, vitamin C, alkaloids, Polyphenols, glycosides, Cu, Zn, Mn and Fe. The concentration of some of the analytes were lower in the cooked sample especially the anti-nutritional constituent, while carbohydrate, fibre and ash were higher in the cooked sample; this implies that processing of food results in the reduction of anti-nutritional factors. This seed could be eaten frequently by diabetic and hypertensive patients because of its constituents.

Keywords: Proximate composition, phytochemical screening, Anti nutrient, Mineral, Walnut.

1. INTRODUCTION

Walnuts are edible seeds that are widely cultivated for their delicacy. Prominent species include *Juglans regia* (L.), commonly known as the English walnut and belonging to the family *Juglandaceae*^[4]. The tropical African walnut, known as *Tetracarpidium conophorum* or *Plukenetia conophora*^[18], belongs to the family *Euphorbiaceae*^[7] is the seed of interest in this research. ^[1] stated that some walnut species are found in the family *Oleaceae*. The walnut is generally referred to as the conophor tree or conophor nut^[12]. The plant is popularly known as African walnut, black walnut and Nigerian walnut^{[8][16]}. However, lack of storage facilities has hampered the market value of the walnut and the nuts must be consumed within 1–2 days when cooked or else it will become foul smelling and unpleasant for sale and consumption^[13]. The seeds are consumed as snacks for refreshments. During its season, hawkers relate their walnuts quality to kola nut maturity. The buyers also shake each seed to ensure that the seed is intact in the

hard epicarp, and most times the quality of the seed can be seen from the nut colour and size. It is a perennial cash crop and an economic tree that is widely grown for its edible seeds^{[5][7][9]}; it is also used as wood in the timber industry.

This research work was designed to evaluate the proximate composition, mineral content, anti-nutrients and the phytochemistry of African walnut (*Tetracarpidium conophorum*) seeds to ascertain the nutritional value of the seeds.

2 MATERIALS AND METHODS

2.1 Sample collection and identification

Tetracarpidium conophorum or *Plukenetia conophora* (Walnut) seeds were obtained from watt market, Calabar North Local Government Area, Cross River State and were identified by a herbarium in Department of Biological Sciences, Cross River University of Technology, Nigeria.

2.2 PROXIMATE ANALYSIS

The proximate analysis was carried out using standard analytical methods as described by[3].

2.3 Determination of Moisture Content

2g of each of the samples were crushed and put into a dried weighed crucible. The samples were put in a moisture extraction oven at 105°C and heated for 3 hours. The dried samples were then put into desiccator and allowed to cool and reweighed. The difference in weight was calculated as a percentage of the original sample.

$$\% \text{ moisture} = \frac{\text{weight of original sample} - \text{weight of dry} \times 100}{\text{weight of original sample}}$$

2.4 Determination of Ash Content

2g of each of the samples were weighed into a crucible and were heated in a moisture extraction oven for 3 hours at 100°C before it was transferred into a muffle furnace at 550°C. This continued until the samples turned white and free of carbon. The samples were then removed from the furnace, allowed to cool in the desiccator at ambient temperature and were reweighed immediately. The weight of the residual ash was then calculated as ash content.

$$\% \text{ ash} = \frac{\text{weight of ash} \times 100}{\text{weight of original sample}}$$

2.5 Determination of Fat Content

25g of the samples were wrapped with a filter paper and put into a thimble which was fitted to a clean round bottom flask which has been cleaned, dried and weighed. 120ml of petroleum ether was used as extraction solvent. The samples were heated with a heating mantle and allowed to reflux for 5 hours. The reflux was recovered and evaporated. The weight of the fat was then determined.

$$\% \text{ oil content or } \% \text{ fat} = \frac{\text{weight of flask and oil extracted} - \text{weight of empty flask} \times 100}{\text{weight of original sample}}$$

2.6 Estimation of Crude Fibre Content

Crude fibre content was determined using the method reported by^[11] with the following processes; acid and base digestions.

$$\% \text{ crude fibre} = \frac{\text{weight of sample before incineration} - \text{weight of sample after incineration} \times 100}{\text{weight of original sample}}$$

2.7 Determination of crude protein content (Modified Kjeldahl Method)

Crude protein content was determined using Modified Kjeldahl Method.

$$\% \text{ Nitrogen} = \frac{\text{ml of HCl (sample)} - \text{ml of HCl (blank)} - \text{molarity of HCl}}{\text{weight of sample} \times \text{ml of digest} \times 1000}$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times \text{Protein factor.}$$

2.8 Determination of Carbohydrate Content

The carbohydrate content was calculated as the difference between the dry weight and the summation of other proximate parameters as Nitrogen free extract (NFE) percentage carbohydrate as shown below.

$$\text{NFE} = 82.5 - (\% \text{protein} + \% \text{fat} + \% \text{Ash} + \% \text{crude fibre}) \text{ for cooked sample.}$$

$$\text{NFE} = 82 - (\% \text{protein} + \% \text{fat} + \% \text{Ash} + \% \text{crude fibre}) \text{ for raw sample.}$$

2.9 Determination of Mineral content.

The mineral content was determined using Atomic Absorption Spectrophotometer (AAS) and the values for Cu, Zn, Mn and Fe were obtained.

2.10 Estimation of vitamin C Content

Vitamin C content was determined using titrimetric method described by^[19].

3. RESULTS AND DISCUSSION

The proximate composition of the walnut seeds is shown in table I. The moisture content of the raw sample was higher than that of the cooked sample (18±0.002 and 17.5±0.03% respectively). This is attributed to the hydrolysis of some of the minerals during the boiling process, this account for why the raw sample has a longer shelf life compared to the cooked sample. The ash content of the raw (14.25±0.08%) was lower than the cooked sample (15.5±0.05%). This increase in ash in the cooked sample is absurd. The crude fat content of the walnut seed was higher in the raw sample (22.50±0.025%) compared to the cooked sample (20.0±0.05%). This result agrees with 52.1% and 21.1% for both raw and cooked walnut seeds^[21]. This shows that the raw seeds contain higher fat than the cooked sample.

Table 1: Proximate Composition of Walnut seeds

	COOKED % DRY WT	RAW % DRY WT
Moisture content	17.5±0.03	18.0±0.02
Ash	15.5±0.05	14.25±0.08
Lipid	20.0±0.05	22.50±0.025
Fibre	20.0±0.02	18.0±0.01
Protein	4.506±0.01	13.13±0.01
Carbohydrate	22.49±0.01	14.12±0.01
Vitamin C	11.15±0.1(mg/kg)	11.0±0.1(mg/kg)

Values are mean ± standard deviations of triplicate determinations

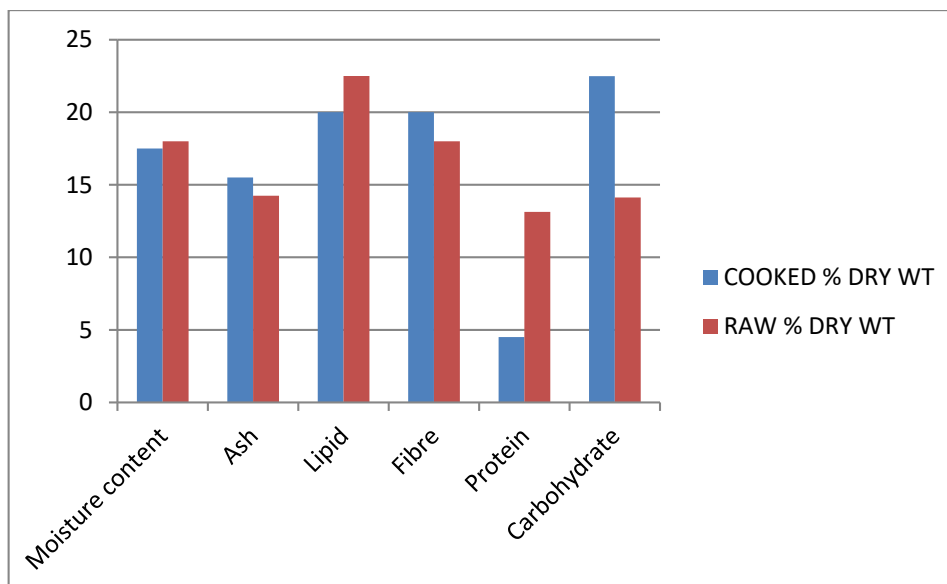


Fig. 1(a) Bar chart for proximate composition of cooked and raw samples of walnut seeds

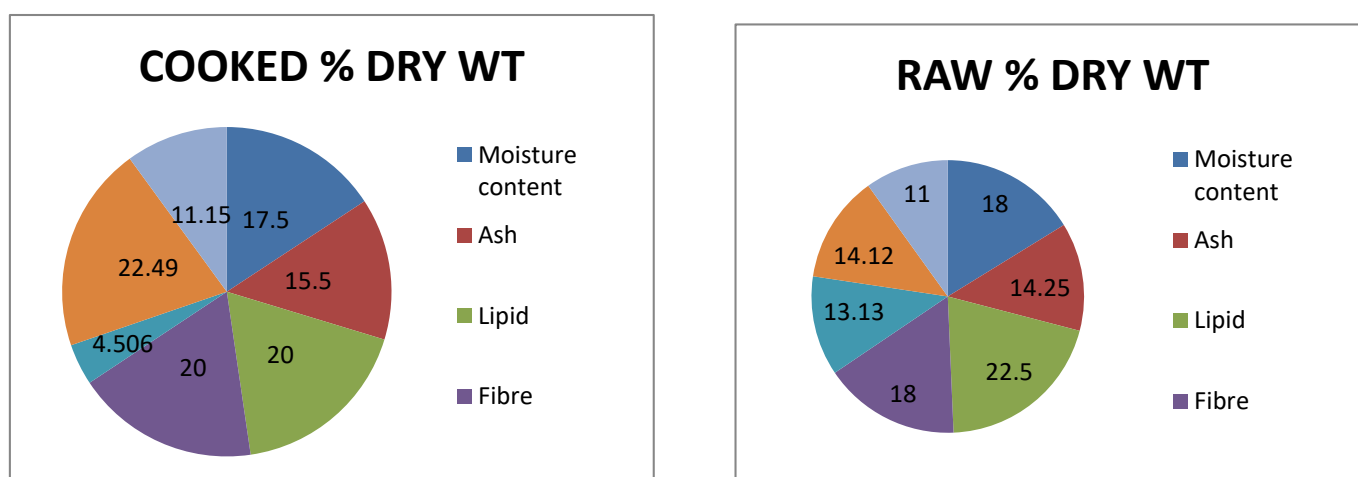


Fig. 1(b): Pie charts for proximate composition of cooked and raw samples of walnut seeds

The crude fibre content for the cooked seeds ($20.0 \pm 0.02\%$) was higher than the fibre content of the raw seed ($18.0 \pm 0.01\%$). These values are higher than that obtained from *Chrysophyllum albidum* seed ($16.00 \pm 0.13\%$)^[15]. Fibre in food plays an important role in providing roughage that aids digestion and reduces the accumulation of carcinogens in the body. The crude protein was found to be ($4.506 \pm 0.01\%$) for the cooked seed and ($13.13 \pm 0.01\%$) for the raw seed. This is lower than ($25.76 \pm 0.45\%$) for *Chrysophyllum albidum* seed^[15]. However, any plant food that provides more than 12% of its energy from protein is considered a good source of energy^[10]. Therefore, *Tetracarpidium conophora* is a good source of protein. The carbohydrate content for cooked walnut seed ($22.49 \pm 0.01\%$) was higher than that of the raw seed (14.12 ± 0.01). The major function of carbohydrate is to provide the body with energy.

Table 2: Phytochemical screening of walnut seeds

COMPONENTS	PRESENCE BOILED	PRESENCE UNBOILED
Alkaloids	+	++
Saponins	BDL	BDL
Flavonoids	BDL	BDL
Tannins	BDL	BDL
Glycosides	+	+++
Anthraquinons	BDL	BDL

Steroids	+	+
Reducing compound	BDL	BDL
Polyphenols	+++	+++
Phlobatannins	BDL	BDL

+ slightly present, ++ moderately present, +++ heavily present, BDL below detection limit.

The result of the phytochemical screening of walnut (*Tertracarpidium conophora*) seeds is shown in table 2. From the result obtained, alkaloids were slightly present in the cooked seeds and moderately present in raw seed. Alkaloids belong to a class of mainly basic nitrogenous compounds that have significant pharmacological and physiological importance and are not widely distributed in nature^[14]. Glycosides were slightly present and heavily present in both cooked and raw samples respectively. Glycoside have specific characteristics and powerful action exerted on cardiac muscles and therefore is used in congestive heart failure due to determination of work capacity per unit weight of mycroches tissues, medicinal interest on cardiac glycosides is because of their stimulant effect in the heart^[17]. Steroids were slightly present in both cooked and raw seeds. Polyphenol was heavily present in both cooked and raw samples. Phenols are structurally similar to alcohols but are much stronger acids^[20]. It helps in contracting the blood capillaries and so prevents certain hemorrhages. Saponins, flavonoids, tannins, anthraquinones, reducing compound and phlobatannins were below detection limit (BDL) in this study.

Table 3: Mineral content of Walnut seeds

ELEMENTS	COOKED COMPOSITION (PPM)	RAW COMPOSITION (PPM)
copper (Cu)	0.079±0.003	1.08±0.1
Zinc (Zn)	0.1507±0.01	2.26±0.1
Manganese (Mn)	0.124±0.01	0.064±0.001
Iron (Fe)	0.040±0.002	0.079±0.002

Values are mean ± standard deviations of triplicate determinations

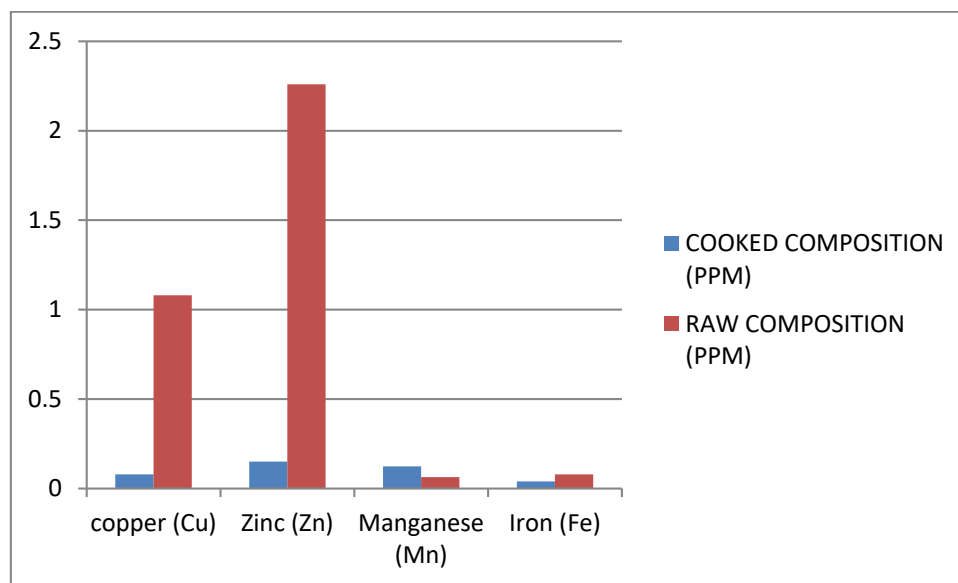
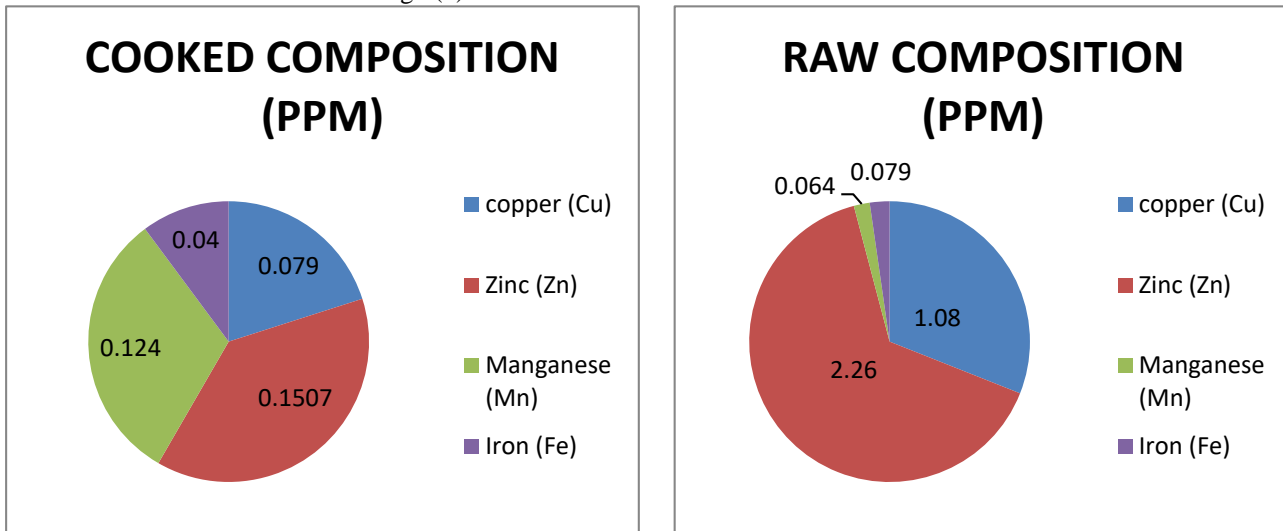


Fig. 2(a): Bar charts for mineral content of cooked and raw walnut seeds

Fig.2(b):Pie charts for mineral content of cooked and raw walnut seeds



The result of the mineral content of walnut seed shows that Cu, Zn and Fe were higher in the raw seeds compared to the cooked seeds with Mn slightly higher in the cooked seeds (Table 3 and pictorially in fig. 2(a) and 2(b) i.e. Bar and Pie charts). These minerals are essential for a healthy body especially Fe and Zn. Iron is needed for red blood cell formation. When iron is deficient in the body, it will manifest itself as iron-deficiency anemia^[15]. Zinc is require for the metabolic activity of about 300 of the body enzymes and is needed for meiosis and mitosis^{[6][15]}.

Table 4: Anti Nutritional composition of walnut seeds

SAMPLE	Composition (mg/100ml)	
	Cooked	Raw
Oxalate	0.0207± 0.01	0.204±0.10
Phytate	0.114 ± 0.01	0.123±0.02
Hydrocyanide	0.011±0.10	0.112±0.10

Values are mean ± standard deviations of triplicate determination.

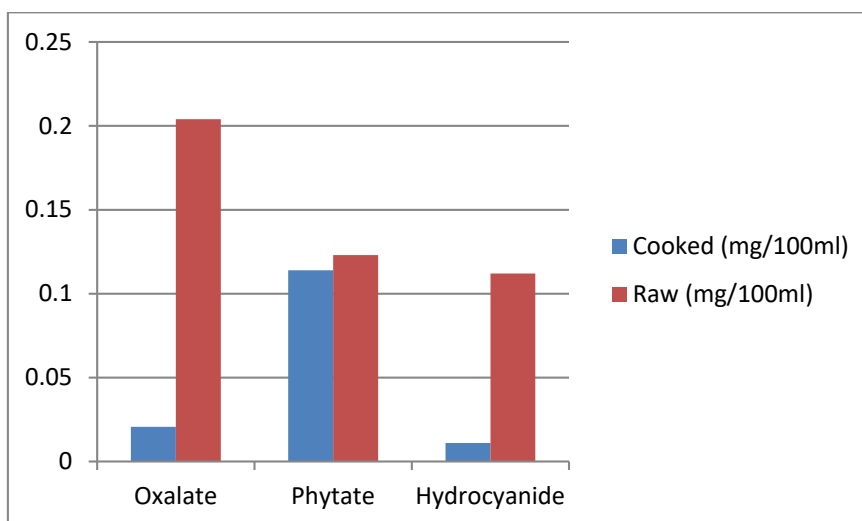


Fig. 3(a): Bar chart of anti-nutritional constituents of walnut seeds

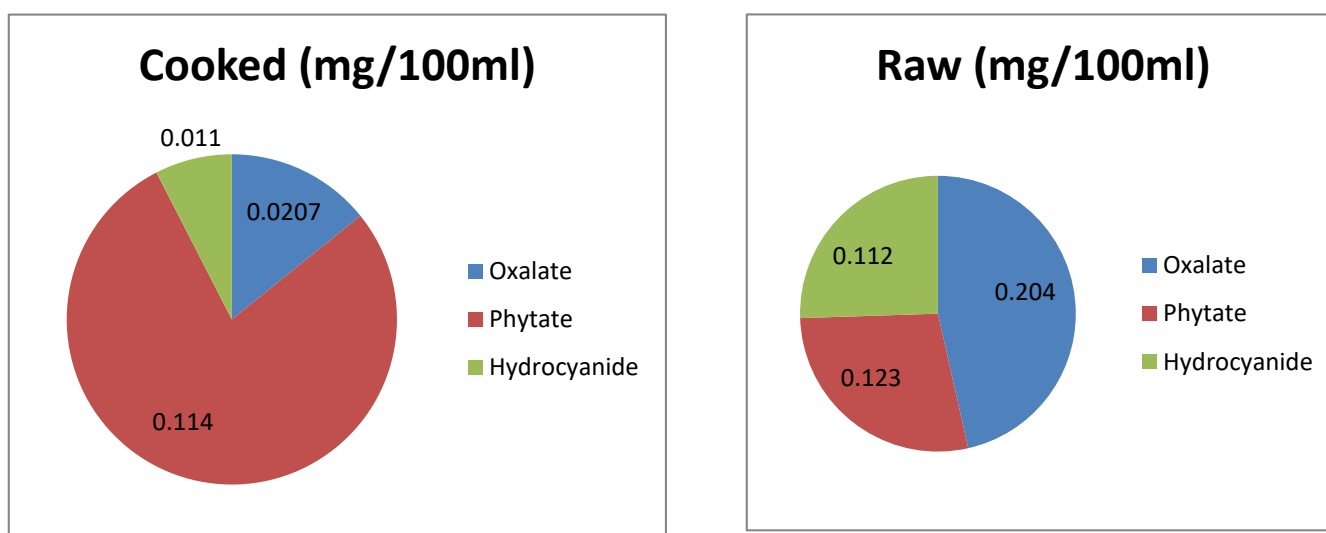


Fig.3(b):Pie charts of anti-nutritional constituents of walnut seeds

The anti-nutritional content of African walnut seed is shown in table 4 and pictorially in fig. 3(a) and 3(b) i.e. Bar and Pie charts. The values of oxalate, phytate and hydrocyanide in both the cooked and raw samples are lower than the values (oxalate; 1.43 ± 0.61 mg/l, phytate; 9.15 ± 2.31 mg/l, hydrocyanide, 0.15 ± 0.1 respectively) reported for some local edible vegetables^[2].

4. CONCLUSION

Phytochemical screening of African walnut (*Tetracarpidium conophora*) samples shows that it contains potent phytochemical substances such as alkaloids, glycosides, steroids and polyphenols which could be employed in the pharmaceutical industry for manufacture of drugs. The African walnut seed contains high level of carbohydrate, lipid and fibre but relatively low level of protein and vitamin C for the cooked seed. The seeds are rich in minerals such as Cu, Zn, Mn, and Fe. This seed could be eaten frequently by diabetic and hypertensive patients because of its constituents. Intensive research should be carried out on the samples and related members of the same family on the use of the seed for mineral, energy and medicinal supplements. The concentration of some of the analytes were lower in the cooked sample especially the anti-nutritional constituent, while carbohydrate, fibre and ash were higher in the cooked sample; this implies that processing of food results in the reduction of anti-nutritional factors. It is recommended that isolation, characterization and structural elucidation of the phytochemicals in the leaf and stem bark of the walnut plant should be assayed in view of pharmaceutical application.

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AUTHORS CONTRIBUTION

Sample collection, preparation and analysis were carried out by Mr. Agwupuye, John Akwagiobe of University of Calabar while the interpretation of results and writing of manuscript was done by Dr. Neji, Peter Amba of Cross River University of Technology, Nigeria.

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