Proximate Composition, Phytochemical and Mineral Analysis of Cooked and Raw Walnut (*Tetracarpidium conophorum*)

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Authors’ contributions

This work was carried out in collaboration among all authors. Author OE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OLO and NCM managed the analyses of the study. Authors OR and EJC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This work attempts to determine the bioactive agents’ groups probably responsible for the diverse growth characteristics and medicinal uses of (*Tetracarpidium conophorum*) Nigerian walnut.

Methodology: The following analyses were carried out in the study proximate, phytochemical and mineral were analyzed.

Results: The result for proximate analysis of the raw and cooked walnut seed extract contained the following parameters 42.57\% and 40.91\% moisture content, 20.54\% and 23.03\% protein, 4.51\% and 4.27\% Fat content, 7.26\% and 7.05\% crude fiber, 5.02\% and 5.16\% Ash content, 20.12\% and 19.59\% carbohydrate respectively. The phytochemical analysis results obtained revealed the presence of bioactive compounds containing 0.92 mg/100 g Tannin, 1.69 mg/100 g Saponin, 2.78 mg/100 g Flavonoid, 0.41 mg/100 g Alkaloid for cooked walnut while raw walnut contained 1.10 mg/100 g Tannin, 3.09 mg/100 g Oxalate, 2.63 mg/100 g Phytate, 3.43 mg/100 g

*Corresponding author: Email: solozospalace@yahoo.co.uk, ojokoheroms@hotmail.com;
Saponin, 3.19 mg/100 g Flavonoid and 1.34 mg/100 g Alkaloid. This study has shown the phytochemical, mineral and proximate compositions of boiled *Tetracarpidium conophorum* seeds.

**Conclusion:** The study revealed that the boiled seeds contain concentrations of alkaloids and also contain moderate quantities of tannins. These bioactive compounds could be responsible for the reported medicinal properties of *Tetracarpidium conophorum* seeds. This partly shows the use of this seed in herbal medicine.

**Keywords:** African walnut seed; phytochemical screening; proximate; analysis; mineral content.

1. **INTRODUCTION**

Nigerian walnut (*Tetracarpidium conophorum*) is an organic seasonal fruit mainly grown in West African Countries. It is gotten from the mother tree, African walnut tree. It has black coloured pericarp covering the milk coloured round nut inside. In Edo language it is called Okhue or Okwe; Asala or Awwusa in Yoruba, Ukpa in Igbo, Arinsa in Igbira and Kaso or Ngak in Cameroon. The entire plant compounds are nutritious and are associated with potential health benefits to humans. African walnuts are good to be consumed on daily basis to help take off some life threatening disease. They have been known as a good source of nutrients such as vitamins and minerals, antioxidants and some plant compounds that the body requires daily for over all maintenance and development. African walnuts help to prevent some mineral and vitamins deficiencies. Eating this fruit helps to protect body from certain chronic diseases. *Tetracarpidium conophorum* is among the list of lesser known foodstuff [1]. Recently in order to unravel the numerous benefits of the different parts of the plant more researches are being conducted regularly. One of the important benefits is its medicinal value. Previous and present reports have shown that all the parts of the walnut (leaves, bark, roots, hull and nuts) possess antimicrobial effect especially the leaves. However, polar solvents and soxhlet extracted extracts revealed more antimicrobial activity than the aqueous extract. Such organisms include (Staphylococcus aureus, Bacillus subtilis Gram negative (Pseudomonas aeruginosa and Escherichia coli) as well as fungi (Candida albicans) and mold (Aspergillus flavus) strains. Their susceptibility was concentration-dependent [2]. This justifies why the plant parts are used for the treatment of various ailments. Oyenuga [3] reported on the amino acid and fatty components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups. The edible nut or kernel is used to strengthen the kidney functions, and therefore used to strengthen the lumber region of the back, the legs and organs. It is commonly combined with other kidney tonics to enhance this action. Also, walnuts are said to be beneficial to the brain since the brain is believed to be controlled by the kidney function. Olabinri et al., [4] reported that African walnut has been known to possess antioxidant property which is as a result of its constituent bioactive polyphenols. There is evidence that phenolic substances act as antioxidant by preventing the oxidation of LDL-lipoprotein, platelet aggregation, and damage of red blood cells [5]; this explains the reason that made the nut to be listed among the plant foods that are of great health benefit to human body. Ogunsua and Adebona [1] have also reported on the high nutritional potential of the nut. According to Oyenuga [3] the nuts of *T. conophorum* are good sources of ascorbic acid and the heavy metal content of the nut is also shown to be below WHO permissible limit which makes the nut safe for public consumption without any fear of heavy metal pollution. Nwokolo [6] also reported on the impact of traditional processing on the nutrient and sensory qualities of the nut. Okpero [7] reported on the methods of processing the *T. conophorum* nuts while, Okafor [8], reported on the use of *T. conophorum* seeds and processing of waste in livestock feed formulation [9].

Walnuts are rich in vitamins, minerals, antioxidants and phytochemicals needed for the health improvement such nutrient include thiamine, Vitamin C, folates, riboflavins, cyanocobalamin, niancin, vitamin E and B vitamins. They are actually the powerhouse of nutrition containing essential nutrients such as tocopherol, pantothenic acid, healthy fats, plant sterol and pyridoxine. Besides the nutritional, medicinal, agricultural etc. benefits of *T. conophorum*, several industrial benefits have been reported as well. The oil from the nut has been reported to be used in the formulation of wood varnish, stand oil and vulcanized oil [2]. Heating of the oil with powdered sulphur at a high temperature of about 150 - 160°C for 30
minutes can give rise to the production of vulcanized oil [10]. Also, wood vanish was made from boiled conophor oil with other additives. The nuts contain uncommon fatty acids that are been used industrially, they are currently used in protective coatings, dispersants, pharmaceuticals, cosmetics and a variety of synthetic intermediates as stabilizers in plastic formulation [11]. The aim of carrying out this is study is to determine the content of the nutritional composition of raw and cooked walnut by investigating the proximate composition, to identify the mineral content present in raw and cooked walnut and to quantitatively determine the difference in phytochemical contents of cooked and raw walnut.

2. METHODOLOGY

Walnut (seed) was purchased from Ngwa Market Aba, in Abia State. The seeds were sorted and washed with tap water to remove flesh residues and other contaminations and divided into two lots. The first lot was boiled for 1½ h and the second lot was used as raw. The walnut both cooked and uncooked samples were deshelled and the edible part was mashed with mortar and pestle and then kept aside prior to analysis. The chemicals were of analytical reagent grade and were obtained from Research Institute Umudike, Abia State and the analysis was also carried out at Research Institute Umudike, Abia State. Distilled water was used for all the washing, cleaning and preparation of solutions.

2.1 Phytochemical analysis

The phytochemical screening was done on the sample using methods as described by [12]. Alkaloids were extracted using a slightly modified method [13]. The dried sample was homogenized and the alkaloid extracted from 10g of the sample for 4h using 20% v/v acetic acid in ethanol. The extract was filtered to remove cellulose debris and then concentrated to about one quarter of the original volume. One percent NH₄OH was added drop wise until a precipitate occurred. The crude alkaloid was dried to constant weight in an oven and the percentage alkaloid calculated.

2.2 Proximate analysis

Moisture content was determined by drying to constant weight at 60-80°C in an oven, ash content by ignition at 550°C in a muffle furnace for 4hr, oil content by soxhlet extraction with hexane as solvent, protein by the kjeldahl method, and crude fibre by the acid and alkaline digestive methods [12]. The carbohydrate content was estimated by difference, subtracting the sum of water, protein, fat, crude fibre and ash percentages from one hundred.

2.3 Mineral analysis

The method of [12] was used for the determination of minerals in the test sample. Calcium and manganese were determined by flame photometric method while iron and zinc were determined by atomic absorption spectrophotometric method.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

From the results obtained in this study, proximate analysis of raw and cooked walnut had moisture content of 42.57% and 40.91% respectively. This value obtained was slightly higher than the values obtained in a previous work reported by [14]. The result for crude protein content of raw and cooked walnut had 20.54% and 23.03% respectively. These values are comparable to the findings of [15,14]. The Fat content of the walnut was low it recorded 4.51% and 4.27% respectively for both raw and cooked walnut samples. The values obtained are comparable to the findings of [14]. Crude fiber content recorded 7.26% and 7.05% respectively for raw and cooked walnut. This value falls within the range of values obtained by [15]. Ash content for raw and cooked walnut samples were 5.02% and 5.16% respectively. Total carbohydrate content obtained in this study for raw walnut was 20.12%, while that of the cooked was 19.59%. The result is in line with the findings of [14]. From the overall result obtained in the proximate analysis, it shows that the walnut has potentials for food and is obviously a good source of protein when cooked before eating. It is safer to eat the nuts when cooked.

3.2 Phytochemical Analysis

Phytochemical composition of raw and cooked walnut is shown in Table 2. From the result findings, Tannin recorded 1.01 and 0.92mg/100g respectively. The result obtained in this study is in accordance with the report of Chijioke et al. [16]. Flavonoids content recorded 3.19 and 2.78 mg/100 g respectively for raw and cooked
walnut. The value obtained is in accordance with that of [15]. Alkaloids content had 1.34 and 0.41 mg/100 g for raw and cooked walnut respectively. The values obtained in this study were lower when compared with that of [16]. Saponin content had 3.43 mg/100 g and 1.69 mg/100 g for raw and cooked walnut samples respectively. Oxalate content was 3.09 mg/100 g and 2.99 mg/100 g respectively for raw and cooked walnut. Phytate content had 2.63 and 2.37 for raw and cooked walnut respectively. Results obtained from this study shows that walnut is used in the treatment of indigestion, constipation and diarrhoea [16]. Walnut is a good source of vitamins. Alkaloids are the most efficient plant substances used therapeutically. Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal agent because of their analgesic, antispasmodic and bacterial properties. This is why the seed is believed to stop asthma and is prescribed to be taken between bouts of asthma, but not for acute asthma, it is used by the elderly as a constipation cure. The presence of tannins in the seed of *Tetracarpidium conophorum* can support its strong use for healing of haemorrhoids, frost bite and varicose ulcers in herbal medicine [16].

The result obtained for raw walnut is as follows calcium (Ca) recorded 74.06 mg/g, iron (Fe), 20.39 mg/g zinc (Zn), 0.93mg/g and manganese (Mn), 219.00 mg/g. While cooked walnut recorded 53.95 mg/g for calcium, 20.83 mg/g for iron, 0.37 mg/g for zinc which was low when compared with the raw nuts and 203.15 mg/g for manganese. *Tetracarpidium conophorum* has shown to be a good source of manganese and iron, two elements that are very useful to mankind. Manganese is used in the management of diabetes which is a plus medically.

**Table 1. Proximate composition of raw and cooked walnut (T. conophorum)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>Raw walnut: 42.57± 0.01 b Cooked walnut: 40.19 ± 0.01 a</td>
</tr>
<tr>
<td>Crude protein</td>
<td>Raw walnut: 20.54 ± 0.01 b Cooked walnut: 23.03 ± 0.02 a</td>
</tr>
<tr>
<td>Crude fat</td>
<td>Raw walnut: 4.51 ± 0.01 a Cooked walnut: 4.27 ± 0.00 b</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>Raw walnut: 7.26 ± 0.01 a Cooked walnut: 7.05 ± 0.01 b</td>
</tr>
<tr>
<td>Ash</td>
<td>Raw walnut: 5.02 ± 0.01 b Cooked walnut: 5.16 ± 0.01 a</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Raw walnut: 20.12 ± 0.04 a Cooked walnut: 19.59 ± 0.01 b</td>
</tr>
</tbody>
</table>

Mean values are of triplicate determination. Different superscript along the same column shows significant difference (p < 0.05)

**Table 2. Phytochemicals analysis of raw and cooked walnut (T. conophorum)**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Concentration in mg/ 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw walnut</td>
</tr>
<tr>
<td>Tannin</td>
<td>1.10 ± 0.00 a</td>
</tr>
<tr>
<td>Oxalate</td>
<td>3.09 ± 0.01 a</td>
</tr>
<tr>
<td>Phytate</td>
<td>2.62 ± 0.01 a</td>
</tr>
<tr>
<td>Saponin</td>
<td>3.43 ± 0.05 a</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>3.19 ± 0.01 a</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>1.34 ± 0.1 ^a</td>
</tr>
</tbody>
</table>

Mean values are of triplicate determination. Different superscript along the same column shows significant difference (p < 0.05)

**Table 3. Mineral profile of raw and cooked walnut (T. conophorum)**

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Concentration in mg/ 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw walnut</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>74.06 ± 0.64 a</td>
</tr>
<tr>
<td>Iron(Fe)</td>
<td>20.39 ± 0.01 ^a</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.93 ± 0.01 ^a</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>219.49 ± 0.53 ^a</td>
</tr>
</tbody>
</table>

Mean values are of triplicate determination. Different superscript along the same column shows significant difference (p < 0.05)
4. DISCUSSION

Findings from the present study revealed that phytochemical screening of the various parts of the *Tetracarpidium conophorum* nuts indicated the presence of some bioactive compounds such as alkaloids, tannins, oxalate, phytate, flavonoids, saponin, and trace amount of some other phytochemicals like phenols. Alkaloids augment human respiration and perform primarily on the central nervous system [17]. The foliage of the *T. conophorum* contains the highest concentration of alkaloid thus can probably be used as a healthier stimulant of human respiration than other parts of the conophor seedling. The leaves of the conophor seedling are considered a cure for headache according to Burkill [18]. Alkaloids in excess present in plants serve as poisons, feeding deterrents, antimicrobial defenses and germination inhibitors to reduce competition from other plants allelopathic agents [17]. Hence, the need to cook walnuts before consumption is of great importance in reducing the high amount of alkaloids present in plants. On the other hand, tannins can be used to treat diarrhoea (in the absence of fever or inflammation), it can also be used as antimicrobial, antioxidant and antidepressants agents [19,20] and thus the non-regenerative hypocotyl of the *T. conophorum* seedling could be used compared to other parts of the seedling because it contains the highest concentration of tannin. Tannins may provide protection against microbiological degradation of dietary proteins in the semen according to Abulude et al. [21]. The amount of saponin was highest in the phytochemical analysis of *T. conophorum* during this study, thus the foliage of the *T. conophorum* seedling can probably be used effectively to control human cardiovascular disease, as human immune system stimulator and as contraceptives for women [17]. Phenolic compounds has the tendency to reduce the risk of cardiovascular disease and certain types of [22,23]. The *T. conophorum* is considered as anti-venom most likely because of the presence of cardiac glycoside, alkaloids, saponin and phenol which are the active substances responsible for detoxification of the venoms in the human system. The presence of the cardiac glycoside will improve cardiac output and the reduce heart disension [23]. The result obtained in this study also revealed that the boiled *Tetracarpidium conophorum* seeds contain high quantities of crude protein. This is in line with the findings of [24]. They are very important especially due to their relationship with nutritional value of the plant [25]. Ajaiyeoba and Fadare [2] reported that the nut has been shown to cure male infertility problems. Some other researchers confirmed that the plant steroids are also useful when consumed by human beings, because their presence decreases the amount of cholesterol in the blood stream [26].

In all, the result of phytochemical screening obtained suggests that the identified phytochemical compounds present in the boiled *Tetracarpidium conophorum* seeds could be the bioactive compounds that confer medicinal qualities to the seeds. These observations support the use of *Tetracarpidium conophorum* seeds in herbal cure remedies or traditional medicines. Therefore the seeds have good nutritional value. The main nutritional value of legumes lies in their supply of cheap, dietary protein and calories in the developing countries of the world [27]. Therefore, *Tetracarpidium conophorum* seeds could play a role, considering its crude protein and carbohydrate contents. Previous reports by [28], have also shown that the *Tetracarpidium conophorum* seeds contain rich sources of mineral elements, such as; Calcium, Magnesium, Potassium, Sodium, Iron, Zinc, Manganese, and Copper, which are very useful in the body. The sodium and potassium content of *Tetracarpidium conophorum* seeds is an added advantage, because of the direct relationship of sodium intake with hypertension in human. This may be the reason why the plant is used to prevent and control high blood pressure according to the report of [29]. Manganese is necessary for the functioning of the pituitary gland, the pineal gland and the brain, it promotes hepatorenal function, combats anemia and is also essential for growth [30]. Manganese is used in the management of diabetes [31,32].

5. CONCLUSION

This study has shown the phytochemical, mineral and proximate compositions of boiled *Tetracarpidium conophorum* seeds. It revealed that the boiled seeds contain concentrations of alkaloids and also contain moderate quantities of tannins. These bioactive compounds could be responsible for the reported medicinal properties of *Tetracarpidium conophorum* seeds. This partly shows the use of this seed in herbal medicine. As a rich source of alkaloids, coupled with the presence of the essential vitamins and minerals, *T. conophorum* can be seen as a potential...
source of useful food and drugs. The presence of tannin supports its anti-inflammatory property. The study has also shown that the boiled Tetracarpidium conophorum seeds contain moderate quantities of crude protein and carbohydrate. Thus, the seed is a source of cheap dietary protein and calories. The Tetracarpidium conophorum seeds also contain some essential vitamins and is a rich source of mineral elements. Therefore, this study reveals that the boiled Tetracarpidium conophorum seeds contain some bioactive compounds and have good nutritional value. They could be useful in pharmaceutical formulations and as food.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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