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Health promoting compounds and *in vitro* antioxidant activity of raw and decoctions of *Gnetum africanum* Welw.

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PEER REVIEW

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Comments

A great deal of work was done to determine the chemical composition of the leaves of *G. africana*. Appropriate materials and methods were employed and elaborate literature review was carried out. However, there were unnecessary theoretical literature details in the discussion over chemical constituents that decreased in level after cooking. Generally, the paper was well written-up and I recommend it for publication.

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ABSTRACT

Objective: To investigate the effect of *Gnetum africanum* on its pharmacopotency as well as its nutritional properties.

Methods: The total chlorophyll, carotenoids, proximates, phytochemicals, minerals, vitamins as well as antioxidant assays were performed using standard techniques.

Results: The raw leaves of the vegetable (*Gnetum africanum*) possessed significant amounts of proximates, phytochemicals, minerals, vitamins, chlorophyll as well as antioxidant activity, but had low levels of carbohydrates and energy value. The cooking method adopted resulted in insignificant loss of lipids, carbohydrates, vitamins, phytochemicals, chlorophyll, significant loss of energy and carotenoids ($P < 0.05$), insignificant increase in moisture, crude fibre, crude protein, Ca, Fe, Zn, but significant increase in ash, Mg and P ($P < 0.05$).

Conclusions: The conventional method of cooking the raw *Gnetum africana* in Nigeria results in insignificant loss of its lipids, carbohydrates, vitamins, phytochemicals, chlorophyll, significant loss of its energy and carotenoids ($P < 0.05$), insignificant increase in its moisture, crude fibre, crude protein, Ca, Fe, Zn, but significant increase in its ash, Mg and P contents as well as its antioxidant capacity which confers high pharmaco potency to the cooked form of this vegetable, justifying its usage in the dietary management of a wide array of diseases in Nigeria. The results obtained are discussed from a biochemical point of view.

KEYWORDS

Vegetables, *Gnetum africanum*, Cooking, Raw, Fresh, Leaf

1. Introduction

Vegetables are important sources of protective foods^[1]. They have also been reported to be good sources of minerals as well as vitamins^[2].

Gnetum africanum (*G. africanum*) is one of the most popular green leafy vegetables in Nigeria, and it is gaining equal popularity as a delicious vegetable in other African countries such as Cameroun, Gabon, Congo and Angola^[3]. It is known as “Eru or Kok” in Cameroon, “Koko” in the Republic of Central Africa, “Ntoumou” in Gabon, “Afang or Okazi” in Nigeria and eru in English. The leaves are edible

and used in the treatment of enlarged spleen, boils, nausea, sore throat, pain at child birth, snake poisoning, diabetes mellitus, cataracts, and worm expeller^[4,5]. *G. africanum* belongs to the Kingdom Plantae; Division Gnetophyta; Class Gnetopsida; Order Gnetales and Family Gnetaceae^[6].

The leaves are widely consumed in the southern eastern parts of Nigeria where it is mostly used in the preparation of soups “ukazi” for the Ibos, Ero (Cameroun), Saka-saka (Congolese), Koko (French) or Nkoko (Portugese) and “Afang” for the Efiks/Ibibios^[7]. It is also put to minor use by the Ibos and the Efiks/Ibibios where it's eaten as a salad mixed with dry fish or meat, certain spices and palm oil.

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Due to the importance of *G. africanum* in the southern eastern parts of Nigeria such as Akwa Ibom, Cross River and Abia states, the vegetable is cooked as a soup (where it is added in hot boiling water that contains all the needed ingredients of the desired soup and allowed to steam for approximately 5 min) in almost all the hotels, restaurants and bars on daily basis. In Akwa Ibom, Cross River and Abia States of Nigeria, there is hardly any restaurants, hotels or bars where this soup (afang or okazi soup) is not served on a daily basis.

Despite the pharmacological importance and the wide spread usage of this vegetable, it is surprising that there is paucity of information in literature on the residual constituents of this vegetable when cooked. This is because, most vegetables in Nigeria are commonly cooked before being consumed and generally, the vegetables are prepared at home on the basis of convenience and taste preference rather than the retention of nutrient and health-promoting compounds.

In most homes in Nigeria, the most commonly used method of cooking involves the use of stoves or gas cookers for the rich while those in rural settlements resort to the use of firewood unlike in the developing countries where cooking with microwave is very common and widely adopted.

Since *Gnetum africanum* (*G. africanum*) is mostly eaten when cooked, and cooking could induce significant changes in the chemical composition, decrease the nutritive quality, antioxidant activities and chemo-preventive compounds in vegetables, so as to decrease their bio-availability when consumed[8]. We decided to investigate the effect of domestic cooking of *G. africanum* on its residual composition and *in vitro* antioxidant capacity.

2. Materials and methods

Fresh leaves of *G. africanum* were bought from Umuahia main market, Abia State, Nigeria and transported to our laboratory for processing and analysis within 10 min. The leaves were properly washed before being then chopped into homogeneous pieces. Some samples of the vegetable were taken to the department of Botany, Michael Okpara University of Agriculture, Umudike, Nigeria where they were identified and authenticated. DPPH (2,2-diphenyl-1-picrylhydrazyl), standard tocopherol and quercetin were products of Sigma and Aldrich Chemical Company (UK) and were of analytical grade. Other chemicals were bought from Hoslab, Umuahia, Abia State, Nigeria and were of analytical grade.

2.1. Processing treatments

In the boiling method adopted, 200 g of homogeneous pieces of *G. africanum* were immersed in a pot, containing 500 mL of boiling water and the pot was covered with a lid. The leaves were removed after after 5 min of boiling.

2.2. Determination of chlorophyll

The raw and cooked leaves of *G. africanum* (0.5 g) were ground to slurry and extracted in 10 mL of 80% (v/v) acetone after 30 seconds of grinding. The extract was centrifuged at 1500 g for 10 min at room temperature and the residue was removed. The total chlorophyll content was determined by recording the absorbance at 645 and 663 nm with a UV spectrophotometer (Genesys 10 VIS Thermo Electron Corporation) against the reagent blank that contained 80% acetone. The total chlorophyll contents of the samples (raw and cooked) were calculated as the sum of chlorophyll a and chlorophyll b, where chlorophyll a = $12.7 \times \text{Absorbance}_{663} - 2.69 \times \text{Absorbance}_{645}$, and chlorophyll b = $22.9 \times \text{Absorbance}_{645} - 4.68 \times \text{Absorbance}_{663}$, and results were expressed as mg/g fresh weight[9].

2.3. Determination of total carotenoid

The carotenoid content of the samples (raw and cooked) were determined using the method of Rodriquez-Amaya and Kimura[10]. Results were expressed as $\mu\text{g/g}$ on fresh weight basis.

2.4. Cyanide assay

The cyanide contents of the samples (raw and cooked) were determined using the alkaline picrate method[11].

2.5. Qualitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay on thin layer chromatographic (TLC)

TLC screening of the antioxidant activity of the extracts of the raw and cooked leaves of *G. africanum* was determined using the DPPH method as proposed by Mensor *et al.* with minor modifications[12]. With the aid of a capillary tube, stock solutions (100 mg/mL) (instead of 1 mg/mL) of the methanolic extracts were spotted on a silica gel TLC plate and developed with a solvent system of ethanol: methanol (50:50) instead of (90:10). After development, the chromatograms were dried and sprayed with a 0.3 mmol/L solution of the stable DPPH free radical. The plates were visualized for the presence of yellow spots and the degree of activity was determined qualitatively from the observation of the yellow colour intensity. Yellow spot formed (within 30 min of spraying) against a purple background were taken as positive results. Quercetin was used as the positive control for this assay.

2.6. DPPH radical scavenging assay

The DPPH method as described by Omale and Okafor was used with modifications[13]. A measured amount (5 g) of each sample (raw and cooked) was dissolved in 100 mL of methanol to give a concentration of 50 mg/mL and the mixture was filtered with Whatman No. 1 Filter Paper

in a vacuum pump. Then, 0.1, 0.2, 0.3, 0.4 and 0.5 mL of each filtrate was diluted with methanol to give final concentrations of 62.5, 125.0, 187.5, 250.0 and 312.5 µg/mL respectively. Finally, 0.1 mL of 0.3 mmol/L DPPH in methanol was added to each of the reaction mixtures and the whole setup was well shaken and left in the dark for 30 min before the absorbance was read spectrophotometrically at 517 nm against the DPPH control that contained 1 mL of methanol only in place of the extract. The radical scavenging activity was calculated as:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.7. Mineral assay

The atomic absorption spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) was used in the analysis of Fe, Mg and Ca while the molybdate method was used for the analysis of the phosphorous content of the raw and cooked vegetables^[11].

2.8. Proximate analysis

The proximate composition analysis of the raw and cooked vegetables was determined using the Association of Official Analytical Chemists (AOAC) methods^[14].

2.9. Phytochemical analysis

The gravimetric method was used in the determination of the total alkaloid contents of both the raw and cooked vegetables^[15]. The AOAC method was used in the determination of the saponins, tannins and flavonoid contents of the raw and cooked vegetables^[14].

2.10. Determination of riboflavin

Five gram of each sample (raw and cooked) was extracted with 100 mL of 50% ethanol solution and shaken for 1 h. It was filtered into a 100 mL flask, and 10 mL of the extract was pipetted into a 50 mL volumetric flask. A measured volume (10 mL) of 5% potassium permanganate and 10 mL of 30% H₂O₂ were added and allowed to stand over a hot water bath for about 30 min. A measured amount (2 mL) of 40% sodium sulphate was added. This was made up to the 50 mL mark and the absorbance was measured at 510 nm with a spectrophotometer^[15].

2.11. Determination of thiamin

A measured amount (5 g) of each sample (raw and cooked) was homogenized with ethanolic sodium hydroxide (50 mL) and filtered into a 100 mL flask. Then, 10 mL of the filtrate was pipetted and the colour was developed by the addition of 10 mL of potassium dichromate, and the absorbance of the resulting solution was read at 360 nm against the

reagent blank^[15].

2.12. Determination of niacin

Five gram of each sample (raw and cooked) was treated with 50 mL of 0.5 mol/L sulphuric acid and the set up was shaken for 30 min, after which 3 drops of ammonia solution were added to the solution and filtered. About 10 mL of the filtrate was pipetted into a 50 mL volumetric flask and 5 mL of potassium cyanide was added. This was acidified with 5 mL of 0.01 mol/L H₂SO₄ and the absorbance was measured with a UV spectrophotometer at 470 nm^[15].

2.13. Determination of ascorbic acid (vitamin C) and tocopherol (vitamin E)

The titrimetric method using 2,6 dichlorophenolinophenol was used to determinate the content of ascorbic acid^[11]. The tocopherol contents of both the raw and cooked vegetables were determined using the method of Tsen as described by Petrus *et al*^[16,17]. Results were expressed as mg/100 g on fresh weight basis.

2.14. Statistical analysis

Results are reported as the means±standard deviations of triplicate experiments. Student *t*-test was used for statistical comparison while One-way analysis of variance was used for statistical analysis of DPPH antioxidant assays. Results were considered to be significant at *P*<0.05.

3. Results

The specie of *G. africanum* that was used in this study is shown in Figure 1.



Figure 1. Cross section of the leaves of *Gnetum africanum*

Table 1

Proximate composition of raw and cooked *G. africanum* (%).

Sample	Moisture content	Ash	Crude fibre	Lipid	Crude protein	Carbohydrates	Energy value
Raw	82.37±0.12	1.49±0.05	4.14±0.06	1.21±0.03	4.90±0.06	10.04±0.19	70.63±0.28
Cooked	83.39±0.08	1.94±0.11*	5.11±0.04	1.16±0.05	5.05±0.04	7.95±0.10	62.45±0.21*

Values are expressed as mean±SD. Energy value (Kcal/100 g). *P<0.05 in comparison with raw within the column.

Table 2

Vitamin composition of raw and cooked *G. africanum* (mg/100 g)

Sample	Thiamine	Riboflavin	Niacin	Tocopherol	Vitamin C	Carotenoid
Raw	0.51±0.00	0.12±0.00	0.41±0.01	25.95±0.04	52.49±0.16	316.78±0.11
Cooked	0.47±0.02	0.24±0.03	0.52±0.00	27.17±0.10	45.61±0.06	254.83±0.04*

Values are expressed as mean±SD. Carotenoid (µg/100 g). *P<0.05 within the group (column).

The percentage decreases in lipids, carbohydrates and energy value were 4.13%, 20.82% and 11.58% respectively in the cooked leaves of *G. africanum*, while 1.24%, 30.20%, 23.43% and 3.06% increases were observed in moisture, ash, crude fibre and crude protein contents of the cooked vegetable respectively (Table 1).

We observed 7.85%, 13.11% and 19.56% decreases in thiamin, vitamin C and carotenoid contents when the raw vegetable was cooked, while we observed 100% increase riboflavin, 26.83% increase in niacin and a corresponding 4.7% increase in tocopherols respectively (Table 2).

Analysis of the phytochemical constituents of the vegetable indicated that the raw vegetable had (1.37±0.02)% tannin, (1.86±0.06)% alkaloid, (1.04±0.03)% flavonoid, (3.82±0.11)% cyanogenic glucoside, (1.09±0.00)% saponin and (0.05±0.00)% sterols, while the cooked vegetable had (1.18±0.00)% tannin, (1.67±0.04)% alkaloid, (0.79±0.00)% flavonoid, (2.82±0.04)% cyanogenic glucoside, (0.85±0.01)% saponin and (0.054±0.010)% sterol. There were no significant differences in the phytochemical composition of the raw vegetable compared with the cooked one. In addition, we had 13.87% loss of tannins, 10.22% loss of alkaloids, 24.04% loss of flavonoids, 26.18% loss of cyanogenic glucosides as well as 22% loss of saponins, while we recorded 8% increase in sterols when the raw vegetable was cooked (Figure 2).

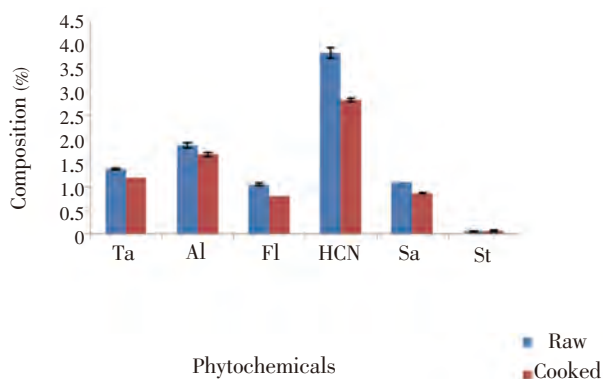


Figure 2. Phytochemical composition of raw and cooked samples of *G. africanum*. Each parameter is not significantly different. Ta: Tannin; Al: Alkaloid; Fl: Flavonoid; Sa: Saponin; St: Sterol. HCN (mg/kg).

The TLC screening of the methanolic/ethanolic extracts of the raw and cooked vegetables indicated that both possessed strong antioxidant activities (Table 3).

Table 3

Free radical scavenging activities of the methanolic/ethanolic extracts of uncooked and cooked *G. africanum*.

Sample	Reaction speed	Intensity of spots
Raw	Fast	+++
Cooked	Fast	+++
Quercetin	Fast	+++

+++; Strong activity.

Analysis of the scavenging activities of the methanolic extracts of the raw and cooked leaves of *G. africanum* on 2,2 diphenyl-1-picrylhydrazyl radical indicated that the raw vegetable had a mean scavenging activity of (94.82±1.47)%, while the cooked vegetable had a mean scavenging activity of (93.54±2.60)% (Figure 3).

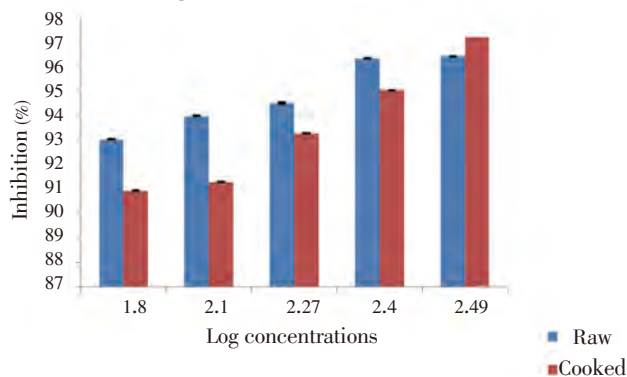


Figure 3. Total antioxidant capacity of raw and cooked *G. africanum*. Raw: $Y=5.126x+83.58$ ($R^2=0.902$); Cooked: $Y=8.652x+74.38$ ($R^2=0.839$).

We recorded an 80.74% increase in Ca, 94.40% increase in Mg, 68.19% increase in P, 73.29% increase in Fe and 65.06% increase in Zn (Figure 4).

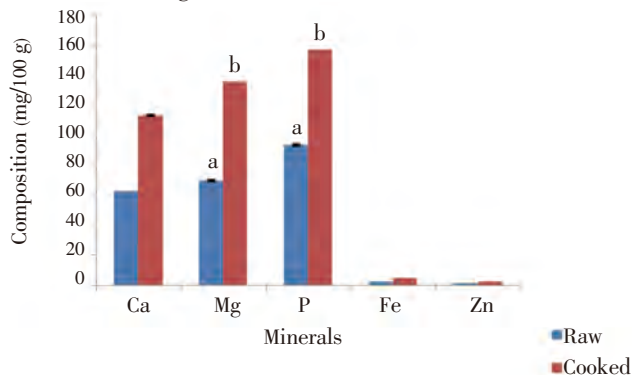


Figure 4. Mineral composition of raw and cooked samples of *G. africanum*. Means with different superscript for each parameter are significantly different ($P<0.05$).

Analysis of the chlorophyll contents of the raw and cooked vegetable of the *G. africanum* showed that the raw vegetable had a total chlorophyll content of (16.12±0.01) mg/g fresh weight while the cooked vegetable had a chlorophyll content of (11.44±0.05) mg/g fresh weight. In addition, we recorded a 29.28% loss of chlorophyll but there was no significant difference in the chlorophyll content of the raw vegetable compared with the cooked one (Figure 5).

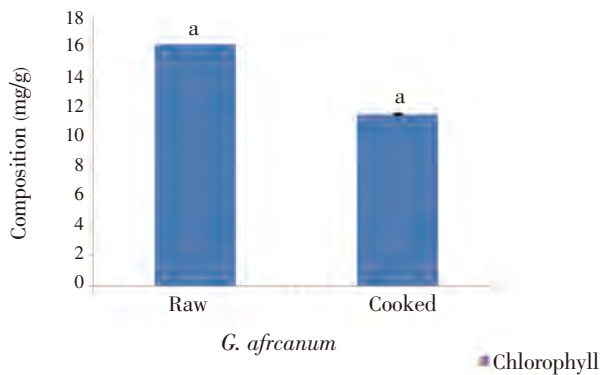


Figure 5. Total chlorophyll content of raw and cooked samples of *G. africanum*.

4. Discussion

The choice of analysis of the leaves of *G. africanum* which was cooked for 5 min stemmed from the fact that in the usage of this vegetable in soups, it is added after all the desired ingredients of the required soup which will be allowed to boil for 5 min or less.

The increase in the moisture content of the leaves of *G. africanum* after cooking was expected as cooking in water tends to soften the cell wall of the vegetable. In addition, more water may have entered the vegetable by osmosis as a result of its membrane permeability.

The ash content of the raw vegetable fell within the acceptable range of edible vegetables in Nigeria[18]. The increased ash content of the cooked leaf of *G. africanum*, which is a reflection of the total mineral content of the cooked vegetable, is a significant finding in this present study. Our results are consistent with previous reports of Mepba *et al*[18]. The implication is that the conventional method of cooking this vegetable increases its mineral content which is of immense benefit to its consumers.

One of the medicinal properties of *G. africanum* is in the management of diabetes mellitus. Dietary fibre decreases the absorption of cholesterol from the gut in addition to delaying the digestion and conversion of starch to simple sugars, an important factor in the management of diabetes[19]. Thus, the increased fibre content of the cooked vegetable could be one explanation for the usage of the cooked form of this vegetable by the traditional medical practitioners in Nigeria, in the dietary management of

diabetes mellitus.

The decrease in the lipid content of the cooked *G. africanum* could be attributed to some lipids that may have leached into the boiling water[20].

The increased protein content of the cooked *G. africanum* as observed in this study, is attributed to increased release of nitrogen during the digestion process.

The decreased carbohydrate content of the cooked form of *G. africanum* is assumed to be as a result of some low molecular weight carbohydrates (monosaccharides and disaccharides) in the vegetable that may have leached into the processing water[20].

The energy value of the raw vegetable as obtained in this study was within the range obtained by previous researchers[21]. Results indicate that both the raw and cooked forms of *G. africanum* can not be recommended solely to vegetarians due to their low energy values. Thus consumption of other food substances that are rich in energy content alongside the vegetable may be needful.

The results obtained for the vitamin assay showed the raw vegetable to be a rich source of water soluble as well as fat soluble vitamins, and this is not surprising as most of these vitamins are abundant and synthesized from plant tissues. The decreased quantity of thiamine (Vitamin B1) in the cooked vegetable could be attributed to the fact that thiamine is partially destroyed by cooking[22,23].

The increased quantities of riboflavin, niacin and tocopherol in the cooked vegetable is another significant finding in this present study.

Riboflavin is involved in the regulatory functions of some hormones that are connected with carbohydrate metabolism. Niacin (Vitamin B3) is essential for the normal functioning of the skin, intestinal tract and the nervous system. Tocopherol as a lipophilic vitamin, is the most powerful antioxidant[23]. Tocopherol protects the red blood cell from hemolysis, boosts the immune response, reduces the risk of myocardial infarction by reducing the oxidation of low density lipoprotein as well as acting as an anti-mutagen. Although riboflavin and niacin are heat stable, the tocopherol contents of foods decrease with cooking. Therefore, the increased release of riboflavin, niacin and tocopherol in the cooked form of *G. africanum* is an important finding in this present study. Our findings on tocopherol are similar to the previous reports of some other scientists who reported an increased release of tocopherol in cooked broccoli[24]. Vitamin E is a lipophilic antioxidant that functions synergistically with other antioxidants like vitamins A and C, selenium[25]. Vitamin E is also involved in the metabolism of all cells, protects vitamin A and essential fatty acids from oxidation in the body cells and prevents breakdown of body tissues. Results indicate that the conventional method of cooking of this vegetable in Nigeria could impact strong antioxidant potentials on it.

Vitamin C functions as a water soluble antioxidant. The loss of this vitamin in the cooked vegetable is attributed to leaching.

The carotenoids have been extensively studied for their potential protection against numerous cancers. The loss of carotenoid contents of the cooked vegetable is attributed to the softening of the plant tissue, leading to the release of the carotenoids^[24]. In addition, the study showed that the conventional cooking method for *G. africanum* in Nigeria retained Vitamin C better than carotenoids.

Flavonoids, alkaloids and tannins are polyphenolic compounds with antioxidant properties that have been associated with hypoglycemic activity by inhibiting brush boarder enzymes^[26]. Although, the cooked form of the vegetable lost some of these polyphenolic constituents, the quantity retained was not significantly different from that of the raw vegetable which confers significant antioxidant potentials to both the raw and cooked vegetable. This finding could be another explanation for the usage of this vegetable in the traditional management of diabetes. Results indicate that both the raw and cooked vegetable possess good potency and could be useful in the management of diseases.

The amount of cyanide in plants are always taken into consideration when such plants are to be consumed since cyanogenic glycosides are present in most plants and the quantities available could make the plant to be either toxic, non-toxic or lethal when eaten bearing in mind that cyanide is an effective cytochrome oxidase inhibitor in the electron transport chain pathway. The lethal dose of cyanide in humans has been reported by several authors as ranging between 50 to 300 mg/kg body weight^[27]. Therefore, the low level of cyanogenic glycosides in both the raw and cooked vegetable makes it very safe for consumption even in the raw state.

Sterols are a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring. They are amphipathic lipids synthesized from acetyl-coenzyme A via the HMG-CoA reductase pathway. Sterols of plants, known as phytosterols, have been shown in clinical trials to block cholesterol absorption sites in the human intestine, thus helping to reduce cholesterol levels in humans^[9]. However, in large quantities, they may block absorption, not only of cholesterol, but also of other important nutrients as well^[28]. Thus, the range of sterols obtained in both the raw and cooked leaves was within the range 0.01% to 10% of sterols in plants.

The DPPH assay measures the electron-donating ability of the compounds in a mixture and thus provides an estimate of the antioxidant activity due to free radical scavenging. Reaction of DPPH with hydroxyl groups involves a homolytic substitution of one of the phenyl rings of DPPH yielding 2-(4-hydroxyphenyl)-2-phenyl-1-picryl hydrazine as

a major product, whilst 2-(4nitrophenyl)-2-phenyl-1-picrylhydrazine is also formed via a series of secondary processes. The qualitative rapid TLC screening for the antioxidant activity of methanolic/ethanolic extracts of both the raw and cooked forms of the vegetable was positive, as the colour of the DPPH changed from deep purple to yellowish spots, indicating that both the raw and cooked forms of the vegetable possessed free radical scavenging activity, and could be considered very good sources of antioxidants.

The high scavenging activities of both the raw and cooked forms of the vegetable on 2,2 diphenyl-1-picrylhydrazyl radical could be attributed to their polyphenolic and tocopherol contents which have varying levels of antioxidant activity, as well as the hydrogen donating ability of the OH-groups of the phenolic compounds. This finding justifies the traditional usage of this vegetable in the management of diseases that have free radical origin.

Minerals are inorganic elements which function as co-factors in enzyme catalyzed reactions, regulation of acid-base balance, nerve conduction, muscle irritability and structural elements of the body. Fruits, vegetables and cereals are the chief sources of mineral elements in the diet. Unlike vitamins, minerals are not destroyed by light, heat and oxygen but only removed from the food by leaching or physical separation^[25]. However, the bioavailability of some minerals like iron may be increased by cooking.

Calcium, Magnesium and Phosphorous fall under macro-nutrients or the principal mineral elements, and they form important constituents of the bones and teeth. Calcium functions as calmodulin binding regulatory protein, and mediates the excitation and contraction of the muscle fibers. Magnesium functions as an activator of many adenosine triphosphate requiring enzymes such as alkaline phosphatase, hexokinase, fructokinase, phosphofructokinase, adenyl cyclase, *etc.*, as well as playing a role in insulin sensitivity. Phosphorous functions in the production of high energy compounds such as adenosine triphosphate, cytidine triphosphate, guanosine triphosphate, creatine phosphate, *etc.*, as well as phosphate buffer system in the blood.

Fe and Zn function as essential trace elements (micronutrients). Fe functions mainly in the transport of oxygen to the tissues, and it's involved in cellular respiration. It also influences glucose metabolism, insulin action as well as interferes with insulin inhibition of glucose production by the liver. Zn functions as an essential constituent of many enzymes such as carbonic anhydrase and alkaline phosphatase. It is also concerning with the healing of wounds as well as playing a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells. The higher quantities of these minerals in the cooked vegetable

compared with the raw vegetable were expected since the cooked vegetable had higher quantities of ash (reflection of total mineral content) than the raw vegetable. This could also be another explanation for the hypoglycemic action of the cooked form of this vegetable.

Chlorophylls are of great importance in plants because of their role in photosynthesis with the formation of carbohydrates. The degree of greenness, due to chlorophyll content, is important in determining the final quality of green vegetables^[29]. Chlorophyll and its derivatives exert beneficial effects such as anti-carcinogenic and anti-mutagenic activities^[30,31]. Many green vegetables contain volatile acids that are partially given off during cooking. Covering the cooking vessel during cooking leads to the volatile acids being dissolved in the steam generated from condensation and this leads to a reduction of the pH of the cooking water. Chlorophyll is very sensitive to any pH below 7 and is converted to pheophytin. This could be one explanation for the reduced chlorophyll content of the cooked vegetable. In addition, chlorophylls which exist in the chloroplast as protein complexes are very unstable. Thus when the cell is killed by heating, the protein gets denatured with the corresponding release of the chlorophyll. These explain the reduction in the chlorophyll content of the cooked vegetable. However, findings from this study show that there were no significant differences in the chlorophyll content of the cooked vegetable compared with the raw vegetable.

From the foregoing, it is clear that the wide usage of both the raw and cooked forms of *G. africanum* could exert very high pharmacological/medicinal properties. Moreover, boiling, which incidentally is a common practice of traditional medical practitioners in Nigeria, potentiated the riboflavin, niacin, tocopherol, total proteins, crude fiber and mineral contents of this vegetable. Finally, the antioxidant potentials of both the raw and boiled vegetable indicate that both forms of the vegetable could be useful in the management of diseases that implicate free radicals.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

G. africanum is one of the most popular green leafy vegetables in Nigeria and other African countries and has nutritive and medicinal values. Generally, it is cooked before consumption, and this method of heat processing could bring about significant changes in the chemical composition of the vegetable which may diminish its nutritive and medicinal values. There is paucity of information on this aspect of *G. africanum* research, hence the authors decided to investigate it.

Research frontiers

The research was carried out to determine the effect of cooking the leaves of *G. africanum* for 5 min on its chemical composition. The increases in the levels of the following were total ash (minerals), crude fibre, crude protein, riboflavin, niacin and tocopherol. Although similar results have been obtained by other researchers working on other plant materials with respect to total ash (minerals), crude fibre, tocopherol and crude proteins, none has been reported for *G. africanum*. In addition, the increases in riboflavin and niacin contents with cooking has not been reported in literature.

Related reports

The reports on increases in the contents of minerals, crude fibre and crude protein of *G. africanum* leaves after cooking are respectively in agreement with those of Mepha *et al.* (2007), Monago and Uwakwe (2009), and Lilian (2002), working on other leafy vegetables. There is no report in the literature on increases in niacin, riboflavin and tocopherol levels as a result of cooking the leafy vegetable.

Innovations & breakthroughs

Boiling the leaves of *G. africanum* for 5 min before consumption can enhance intake of minerals, crude fibre, crude protein, niacin, riboflavin and tocopherol.

Applications

1. Use of the cooked *G. Africanum* leaves as a good source of niacin, riboflavin and tocopherol in diets.
2. Use of the cooked leaves in the diets of diabetics on account of its reasonable level of crude fibre.
3. Use of both raw and cooked leaves of the crop to manage diseases that implicate free radicals.

Peer review

A great deal of work was done to determine the chemical composition of the leaves of *G. africanum*. Appropriate materials and methods were employed and elaborate literature review was carried out. However, there were

unnecessary theoretical literature details in the discussion over chemical constituents that decreased in level after cooking. Generally, the paper was well written–up and I recommend it for publication.

References

- [1] Nnamani CV, Oselebe HO, Agbatutu A. Assessment of nutritional values of three underutilized indigenous leafy vegetables of Ebonyi State, Nigeria. *Afr J Biotechnol* 2009; **8**(9): 2321–2324.
- [2] Adenipenkun CO, Oyetunji OJ. Nutritional values of some tropical vegetables. *J Appl Biosci* 2010; **35**: 2294–2300.
- [3] Eyo E, Abel U. Chemical composition and amino acid content of *Gnetum africanum* leaves. *Nig J Nutr Sci* 1983; **4**: 52–57.
- [4] Lucas EO. The potential of leaf vegetable in Nigeria. *Outlook Agr* 1998; **17**(4): 163–168.
- [5] Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestry database: A tree reference and selection guide version 4.0. Nairobi: World Agroforestry Centre; 2009.
- [6] Dutta AC. *Botany for degree students*. 5th ed. Oxford: Oxford University Press; 2000, p. 37–42.
- [7] Domenyang PF, John CA, Paul DF. *Traditional medicines of Congo (Brazaville)*. Paris: ORSTOM; 2001, p. 114.
- [8] Yuan GF, Sun B, Wang QM. Effects of different cooking methods on health–promoting compounds of broccoli. *J Zhejiang Univ Sci B* 2009; **10**(8): 580–588.
- [9] Wellburn AR. The spectral determination of chlorophyll a and chlorophyll b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Phys* 1994; **144**: 307–313.
- [10] Rodriguez–Amaya DB, Kimura M. *Harvestplus handbook for carotenoid analysis*. Washington, DC: International Food Policy Research Institute (IFPRI); 2004, p. 34–36.
- [11] Onwuka GI. *Food analysis and instrumentation. Theory and practice*. Lagos: Naphthali Prints; 2005, p. 140–146.
- [12] Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical methods. *Phytother Res* 2001; **15**: 127–130.
- [13] Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr J Biotechnol* 2008; **7**(17): 3129–3133.
- [14] Association of the Analytical Chemists. *Official methods of analysis*. 17th ed. Gaithersburg: Association of the Analytical Chemists; 1920.
- [15] Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. *Afr J Biotechnol* 2006; **5**(4): 357–361.
- [16] Petrus AJA, Bhuvaneshwarri N, Alain JAL. Anti–oxidative constitution of *Mukia maderaspatana* (Linn). M. Roem leaves. *Indian J Nat Prod Resour* 2011; **2**(1): 34–43.
- [17] Tsen CC. An improved spectrophotometric method for the determination of tocopherols using 4,7 diphenyl–1, 10–phenanthroline. *Anal Chem* 1961; **33**(7): 849–851.
- [18] Mepba HD, Eboh L, Banigo DEB. Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *Afr J Food Agr Nutr Dev* 2007; **7**(1): 1–18.
- [19] Monago C, Uwakwe A. Proximate composition and *in vitro* anti–sickling property of Nigeria *Cyperus esculentus* (tiger nut sedge). *Trees Life J* 2009; **4**(2): 1–6.
- [20] Lilian HMC. *Food chemistry*. New York: Reinhold Publishing Corporation; 1960, p. 137.
- [21] Chinma CE, Igyor MA. Micronutrients and anti–nutritional contents of selected tropical vegetables grown in southeast, Nigeria. *Niger Food J* 2007; **25**(1): 111–116.
- [22] Deb AC. *Concepts of biochemistry, theory and practice*. Calcutta: Books and Allied (P) Ltd.; 2004, p. 132–137.
- [23] Vasundev V. *Fundamentals of biochemistry, textbook of biochemistry*. 2nd ed. New York: John Wiley & Sons Inc.; 2006, p. 281–289.
- [24] Simone B, Elmar S. Impact of different cooking methods on food quality: Retention of lipophilic vitamins in fresh and frozen vegetables. *J Food Eng* 2006; **77**: 327–333.
- [25] Wagner KH, Kamal–Eldin A, Elmadfa I. Gamma–tocopherol–an underestimated vitamin? *Ann Nutr Metab* 2004; **48**: 169–188.
- [26] Karau GM, Njagi ENM, Machocho AK, Wangai LN, Kamau PN. Hypoglycemic activity of aqueous and ethylacetate leaf and stem bark extracts of *Pappea capensis* in alloxan–induced diabetic BALB/c mice. *Br J Pharmacol Toxicol* 2012; **3**(5): 251–258.
- [27] Akiyama H, Toida T, Sakai S, Amakura Y, Kondo K, Sugita–Kunishi Y, et al. Determination of cyanide and thiocyanate in Sugihirata Ke mushroom using HPLC method with fluorimetric detection. *J Health Sci* 2006; **52**: 73–77.
- [28] Ostlund RE, Racette SB, Stenson WF. Inhibition of cholesterol absorption by phytosterol–replete wheat germ compared with phytosterol–depleted wheat germ. *Am J Clin Nutr* 2003; **77**(6): 1385–1389.
- [29] Nisha P, Singhal RS, Pandit AB. A study on the degradation kinetics of visual green colour in spinach (*Spinacea oleracea* L.) and the effect of salt therein. *J Food Eng* 2004; **64**(1): 135–142.
- [30] Turkmen N, Poyrazoglu ES, Sari F, Velioglu YS. Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables. *Int J Food Sci Technol* 2006; **41**(3): 281–288.
- [31] Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH Jr., Murphy RC, et al. A comprehensive classification system for lipids. *J Lipid Res* 2005; **46**(5): 839–861.