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Phytochemical Composition, Proximate Analysis and Vitamin Content of (*Tetracarpidium conophorum*) Wall Nut Seed

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Abstract This study investigates the phytochemical composition, proximate analysis and vitamin content of *Tetracarpidium conophorum* (black walnut) seed. Standard procedures were used for the analyses, the result of the phytochemical composition of the walnut seed reveals the presence of the following which increase in this order; tannins < cyanides < terpenoids < saponins < steroids < cardiac glycosides < flavonoids < alkaloids. Proximate composition of the walnut seed was found to be; protein 18.42 ± 0.04%, ash 2.86 ± 0.02%, fat 4.88 ± 0.04% and fibre 6.6 ± 0.08%, Moisture 32.63 ± 0.02% and carbohydrate 34.59 ± 0.02%. The seeds also contain some appreciable amount of vitamins; vitamin A 9.64 ± 0.01 mg/100g, vitamin C 6.98 ± 0.02 mg/100g, vitamin E 96.42 ± 0.02. The B-group vitamins were also found in trace amounts. The presence of these bioactive and nutritive compounds in the seed has made it a veritable source for good health conditions.

Keywords Phytochemical, proximate, vitamins, walnut seeds

Introduction

Plants have been a great of source nutrients to human and animals. Humans have relied mostly on plants for nutritional and medicinal needs. Some plant seeds have nutritive and calorific values which make them necessary components of diets [1]. The use of plants by people for treatment of diseases and ailments has being in practice for a long time [2]. Nutritional discoveries of the 1990s showed that frequent eating of nuts greatly lower the risk of heart diseases [3].

Black walnut *Tetracarpidium conophorum* plant is a fine hardwood specie in the family of Juglandaceace occurring in North America and South America, West India, Asia, Japan and Nigeria [4]. Walnut plant is a large deciduous tree attaining a height of 25-35m with a trunk up to 2m in diameter and broad crown. It is draught demanding specie requiring full sun to grow well [5]. It is economic plant widely cultivated for the production of nuts and is used as delicacies [6]. The fruit of the walnut tree is a fleshy green drupe in which the nut is encased. The kernel of the nut is protected by a corrugated wood shell. The seed is large, with a relatively thin shell and edible with rich flavour [7].

Walnuts seed are known for their content of omega-3-fatty acids which are good in maintaining a healthy heart as they have low saturated fats. The seeds have potentials health benefits in the area of memory and cognitive function and promotes brain health. Low omega-3-fatty acid intake has been linked to depression and decline in cognitive

function [8]. Walnut seed contains melatonin a hormone used in the body to regulate sleep. Melatonin is a powerful anti-oxidant that fights free radical which is responsible for the development of cancer cells [9]. Black walnut seeds also contain allergic acids that neutralize cancer causing substances [10]. Therefore consumption of black walnut seeds may help to reduce the growth of cancer cells, heart disease and high blood pressure as it lower the level of bad cholesterol [11]. Hence this work seeks to evaluate the phytochemical composition, proximate analysis and vitamin content of the plant to encourage its consumption as a functional food.

Materials and Methods

Plant Material

The fresh *Tetracarpidium conophorum* (black walnut) seed used for this study were purchased from Eke market, Afikpo North Local Government Area of Ebonyi State, Nigeria. The seeds were cleaned, air dried and carefully ground into a coase form by the use of a mechanical blender.

Preparation of Sample

The dehulled sample was dried and ground to a fine powder using a milling machine 2 g of the powdered sample was defatted with 100ml of diethyl ether using a soxhlet apparatus for 2 hours.

Phytochemical Analysis

Alkaloids Determination

Five grams of the sample was weighed into 250 ml beaker and 200 ml of 20 % acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the extract was concentrated to one quarter of the original volume. Then concentrated ammonium hydroxide was added drop wise to the extract to precipitate the alkaloid. This was done until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, dried and re-weighed. The percentage alkaloid was calculated as the difference in weight [12-13].

$$\frac{W_2 - W_1}{W} x100$$

W= weight of the sample

W_1= weight of empty filter paper

W_2= weight of sample + empty filter paper

Flavonoids Determination

Ten grams of the test sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to constant weight [14].

$$\% \text{ Flavonoid } = \frac{\text{Weight of dried sample}}{\text{Weight of sample}} \text{x } 100$$

Saponins Determination

Twenty grams of ground sample was dispersed in 200 ml of 20 % ethanol, the suspension was heated on a water bath for 4 hours with continuous stirring at about 55 ^{o}C. The mixture was filtered and residue re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{o}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ethanol layer was discarded. The purification process was repeated. 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was oven-dried to a constant weight. The saponins content was calculated in percentage as difference in weight [15].

Tannins Determination

Five hundred milligram of sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. Then 5 ml of the filtrate was pipetted out into a tube and mixed with 3 ml of 0.1 M $FeCl_3$ in 0.1N HCl and 0.008 M potassium ferricyanide added. The absorbance was measured in a spectrophotometer at 120 nm wavelength within 10minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured [16].

Total Phenols

The extraction of the phenolic content: The fat free sample was boiled with 50 ml of ether in 15 min. 5 ml of the extract was pipetted into a 50 ml volumetric flask. Then 10ml of distilled water added. 2 ml of ammonium hydroxide solution and 5 ml of amyl alcohol were also added. The mixture was made up to mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelength [12].

Proximate Analysis of Walnut Seed

This was carried out according to the method of A.O.A.C. [17].

Moisture Content Determination

Two grams of the sample was weighed into a dried weighted crucible. The sample was put into a moisture extraction oven at 105 ^oC and heated for 3 hours. The dried sample was put into a desiccator and allowed to cool and reweighed. The process was repeated until constant weight was obtained. The difference in weight was calculated as the percentage moisture content.

Ash Content Determination

Two grams of the sample was weighed into a crucible heated in a moisture extraction oven for 3 hours at 100 ^oC before being transferred into a muffle furnace at 550 ^oC until it turned white and free of carbon. The sample was then removed from the furnace and cooled in a desiccator to room temperature and reweighed immediately. The weight of the residual ash was then calculated as Ash content.

$$\% \text{ Ash content} = \frac{\text{Wt of Ash}}{\text{Wt of original sample}} \text{x } 100$$

Crude Protein

The micro kjeldahl method described by A.O.A.C. [17] was used. Two grams of the sample was mixed with 10ml of H_2SO_4 acid in a heating tube. One tablespoon of selenium catalyst was added to the tube and mixture heated inside a fume cupboard. The digest was transferred into distilled water. Ten milliliter (10 ml) portion of the digest was mixed with equal volume of 45 % NaOH solution and poured into kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 4% boric acid solution containing 3 drops of methyl red indicator. A total of 50ml distillate was collected and titrated as well. The titration was duplicated and the average value taken. The nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein.

$$\% \text{ Nitrogen} = \frac{100 \text{ x N x } 14 \text{ x Vf) T}}{100 \text{ x Va}}$$

N = Normality of titrate (0.1 N)

Vf = total volume of the digest = 100 ml

T = titre value

Va = Aliquot of volume distilled

Fat Content Determination

Two grams of the sample was loosely wrapped with a filter paper and put into a thimble which was fitted to a dried weighed clean round bottom flask. The flask contained 120ml of petroleum ether. The sample was heated with heating mantle and allowed to reflux for 5hours. The heating was then stopped and spent sample kept and later weighed. The difference in weight was taken as the mass of fat and expressed as percentage fat content of the sample.

$$\% \text{ Fat or Oil content} = \frac{W_2 - W_1}{W_3}\ x\ 100$$

W_1 = Weight of the empty extraction flask
W_2 = Weight of the flask and oil extracted
W_3 = Weight of the sample

Crude Fibre Determination

Two grams of sample and one gram of asbestos were put into 200 ml of 1.25 % of H_2SO_4 and boiled for 30minutes. The solution and the content were then poured into Buchner funnel equipped with muslin cloth and secured with elastic band. This was allowed to filter, the residue was then put into 200 ml boiled NaOH and boiling continued for 30 minutes. It was then transferred to the Buchner funnel and filtered and later washed twice with petroleum ether. The residue obtained was put in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. The difference in weight (*i.e.* loss in ignition) was recorded as crude fibre and expressed as the percentage crude fibre.

$$\% \text{ Crude fibre} = \frac{W_1 - W_2}{W_3}\ x\ 100$$

W_1 =Weight of sample before incineration
W_2= Weight ofsample after incineration
W_3 = Weight of original sample

Cabohydrate Content Determination

The nitrogen free method by A.O.A.C. [17] was used. The carbohydrate content is calculated as weight difference between 100 % and the summation of other proximate parameters as nitrogen free extract (NFE) percentage carbohydrate.

% carbohydrate (NFE) = 100- (M + P + F + A + F_2)

M =moisture, P = protein, F_1 = Fat , A = Ash and F_2 = Crude fibre

Vitamin Content Determination of Walnut Seed

Determination of Vitamin C (Ascorbic Acid)

This was determined using the usual titration method. Five grams of the sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solutions were mixed and the mixture shaken for 30 min. This was transferred into a centrifuge and centrifuged at 3000 rpm for about 20 min. It was later transferred into a volumetric flask and made up to mark with the extraction solution. 20 ml of the extract was pipetted into a volumetric flask and 1 % starch indicator added. These were added and titrated with 20 % $CuSO_4$ solution to a dark end point [18].

Determination Riboflavin

Five grams of sample was extracted with 100 ml of 5 % ethanol solution and shaken for 1 hour.

This was filtered into a 100 ml flask. 10ml of the extract was pipetted into 50 ml volumetric flask. 10 ml of 5 % $KMnO_4$ and10 ml of 30 % H_2O_2 were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40

% $NaSO_4$ was added. This was made up to mark and the absorbance measured at 510 nm in a spectrophotometer [15].

Determination of Thiamin

Five grams of the sample was homogenized with 50 ml ethanolic solution of sodium hydroxide. It was filtered into100 ml flask, 10 ml of filtrate was pipetted and the colour developed by addition of potassium dichromate and read at 360 nm. A blank was prepared and the colour also developed and read at the same wavelength [15].

Determination of Niacin

Five grams of the sample was treated with 50 ml of 1 N H_2SO_4 and shaken for 30 min, 3 drops of ammonia solution (0.1 N) were added to sample and filtered. 10 ml of the filtrate was pipetted into a volumetric flask and 50 ml of potassium cyanide was added. This was acidified with 5 ml of 0.02 N H_2SO_4 and absorbance measured in the spectrophotometer [15].

Determination of Vitamin A

One gram (1 g) of the sample was weighed and percolated with 20 ml of petroleum ether. It was evaporated to dryness and 0.2 ml of $CHCl_3$ in acetic anhydride was added and 2 ml of TCA chloroform was also added and the absorbance read at 620 nm. The concentration of vitamin A was extrapolated from the standard curve.

Results

Table 1: Quantitative Phytochemical Result of Walnut Seeds

Phytochemicals	Mean Composition mg/100g dry in weight
Alkaloids	1.77± 0.02
Flavonoids	1.64±0.04
Steroids	1.44±0.02
Phenols	1.48 ±0.02
Tannins	0.96±0.02
Saponins	0.86±0.01

Values are triplicate determinations ± SEM

Table 2: Proximate Composition of Walnut Seeds

Proximate Composition	Mean Composition (%)
Protein	18.42±0.04
Moisture	32.63±0.02
Carbohydrate	34.59±0.03
Fat	4.88±0.44
Ash	2.86±0.02
Fibre	6.62±0.08

Value are triplicate determination ±SEM

Table 3: Vitamin Content of Walnut Seeds

Vitamin	Mean Composition mg/100g
Vitamin A (carotene)	9.64±0.01
Vitamin (ascorbic acid)	6.98±0.02
Vitamin E (tocopherol)	96.42±0.02
Vitamin (Vit B_1)	0.04±0.02
Niacin (Vit B_3)	0.02±0.01

Riboflavin (Vit B_2)	0.08±0.02

Values are triplicate determination ± SEM

Discussions

Plants have become a source of important bioactive compounds that are beneficial to human and animal, through their ability to affect metabolic and physiological activity.

Table I shows the result of phytochemical composition of walnut seeds. Alkaloid content was found to be the highest 1.77 ± 0.02 mg/100g among others. Generally alkaloids have medicinal and physiological function, pure isolated alkaloids from plants and their derivatives possess analgesic, anti-spasmodic and anti- bacterial effects [19]. Alkaloids are the effective plant substances used therapeutically as analgesic, anti-microbial and antibacterial properties. Consumption walnut seeds help the body in fighting this microorganism.

Apart from alkaloids, the walnut seeds contain an appreciable amount of flavonoids 1.64 ± 0.04 mg/100g. Flavonoids have been reported to have anti-oxidative property, free radical scavenging capacity, coronary heart disease preventions and anti-cancer activity [20-21].

With antioxidant and anti-inflammatory nutrients found in walnut it is not surprising to see that the nut shows measurable anti-cancer benefit. The anti-oxidant property of walnut helps to lower risk of chronic inflammation and precisely these two types of risk when combined pose the greatest threat for cancer development. Consumption of large amount of walnut seed has helped to reduce the risk of breast and prostate cancer [9].

Flavonoids have been known to have high hypoglycemic activity which may account for it been used to treat diabetes [22]. Walnut are beneficial in handing and treatment of types 2 diabetes which is a problem primarily related to blood sugar control and insulin metabolism [9].

The tannin content was found to be 0.96 ± 0.02 mg/100g while saponins was detected to be 0.76±0.01 mg/100g. Plant saponins help humans to fight fungal infection, combat microbes and reduce the risk of heart diseases by lowering blood cholesterol [23-24].

The seed also contain a high amount of phenols 1.48±0.02 mg/100g. Phenols and phenolic compounds have anti-microbial and anti-oxidant protective effects and are anti-tumor agents [25].

The proximate composition of walnut seed is shown in Table 2. Protein content was found to be 18.42 ± 0.04%. The nuts are a good source of protein. Walnuts are high in protein food and are excellent addition to vegetarian diet. Plant proteins still remain a veritable source of food nutrient for the less privilege population in developing countries including Nigeria where cost of animal protein is beyond their per capita income [26].

Walnut seeds contain an appreciable amount of fat 4.88 ± 0.44%. Fats of walnut not only taste great but are rich source of healthy mono-saturated fats and an excellent source of omega-3-fatty acid [27]. Black walnut is good source arachonic fatty acid which some studies show to relieve bursitis symptoms [27].

Fiber content of walnut seeds of amounts to 6.62 ± 0.08%. The walnut seed serve as a valuable intake of dietary fiber which can lower cholesterol level, risk of coronary diabetes, colon and breast cancer [28].

The moisture content is an important parameter as it affects percentage yield of the seed oils during extraction. This indicates why walnut seed has low oil yields judging from the high moisture content.

Table 3 gives the result of the vitamins which show preponderance of vitamin A 9.64 ± 0.01 mg/100g. Vitamin A helps to provide good vision, healthy immune system and cell growth. Vitamin A fights cancer by inhibiting the production of DNA in cancerous cells. It also slow down tumor growth and may keep leukemia cells from dividing [29]. The high content of vitamin A in the seed has revealed the nutrient and medicinal value of the seed.

The content of vitamin C amounts to 6.98 ±0.0. With the presence of ascorbic acid (vitamin C) in the seed, the seeds can be used in herbal medicine for the treatment of skin condition including ezcema, psorasis and scurvy [30]. Vitamin C as an antioxidant helps to prevent or minimize the formation of carcinogenic substances from dietary materials [31].

The vitamin E. content of the walnut was exceptionally high 96.42 ± 0.02 mg/100g, and this supports the use of the plant in southern Nigeria medicine as a male fertility agent [32]. Vitamin E is a powerful anti-oxidant which is good

in the fight against cancer and heart disease. Vitamin E promotes mental wellbeing and ease arthritis pain. The vitamin E found in seed comes in the form of beta -tocopherol and this has been found to provide significant protection from health problem [27].

The B-group vitamins were found in trace amounts. Riboflavin vitamin B_2 helps in production of red blood cells and is important for growth and healthy body skin, eye and nervous system.

Niacin (vitamin B_3) helps to control cholesterol [33]. The consumption of walnut seed goes a long way to augment their deficiency since our body do not synthesis them.

Conclusion

This study has shown that walnut *Tetracarpidium conophorum,* seeds contain substantial amount of phytochemicals and phytonutrients. The proximate analysis shows high content of protein, carbohydrate, fibre and fat. More so, the nutrient content of seed reveals the high content of vitamin C and vitamin E which are powerful antioxidants. These go a long way to support the claims that the plant provide anti-cancer and cardio-protective agents. Consumption of the walnut seeds will help to relieve the trauma and pains of people suffering from these debilitating ailments.

References

1. Lawhorn, T. and Carter U. (1971).The International Diary Food Association: Microbiological and Nutritional foods from meat, fish and diary products. Pp 30-32.
2. Bada, S. O. (1997). Preliminary Information on the ecology of *Chrosophyllum albidun* G. Don is Work and central African proceeding of National workshop on the potentials of star Apple in Nigeria, Pp 16.25.
3. Anderson, K. J. and Gobielle, A. (2001). Wallnut polyphenol inhibit in vitro human plasma low density lipoprotein (LDL) oxidation. *Journal of Nutrition.* 131:2837-2844.
4. Manning, W.E. (1978). The classification within Juglandaceae. *Annuals of Missiouri Botannical Garden* 66: 1058-1087.
5. Caglarimark, N. (2003). Biochemical and physical properties of some walnut genotypes. (*Juglansreigia*). *Nahrung Food.* 47:28-32.
6. Adebona, M. B. (1988). Development of Conophorum nut-based cereal snack food biscuits. *Journal of Food and Agriculture*, 2: 123-135.
7. Willis J.C. (1966). A dictionary of flowering plants and ferns 7thedn, Cambridge University Press, Cambridge. P 1108
8. Claude, B. and Paule, S. (1979). The manual of natural living, 1st edn. Biddies Limited, Guild Ford Surrey. Pp 98-99.
9. Mercola, J. (2014). 7 benefits of walnuts. www.mercola.com retrieved 11/4/16.
10. Okwu, D.E and Okeke, O. (2003). Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global Journal Pure and Applied Science* 9: 235-238.
11. James, N.R. (2000). Volatile components of green Walnut Husks. *Journal of Agriculture and food chemistry* 48(7): 2858-2861.
12. Obadomi, B.O. and Ochuko, P. O. (2001). Phytochemical studies and efficacy of crude extracts of some homeostatic plants in Edo and Delta State of Nigeria. *Global Journal of Pure and Applied Science*. 8: 203-208.
13. Harbone J.B. (1973). Phytochemical Methods. Chapman and Hall, London. Pp: 110-113.
14. Boham, B. A. and Kocipai A. C. (1994), Flavonoids and condensed tannins from leaves of Hawaina *Vaccinium vaticulum* and *V. calycinium*, *Pacific Sci.,* 48: 458-463.
15. Okwu, D.E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigeria Medical plants, *African Journal of Biotech*. 5(4): 357-361.
16. Van–Burden, T.P. and Robinson, W.C. (1981). Formation of complexes between protein and tannin acid. Agric*Food Chem*. 1:77-82.

17. A.O.A.C. (1990). Official Method of analysis, 15th edn, Association of Official Analytical Chemist, Benjamin Franklin Station, Washington D.C., U.S.A.
18. Baraket, M. Z., Shehab, S.K., Darwish, N.andZahemy E.Z. (1973). Determination of Ascorbic acid from plants. *Analytical Biochem*., 53: 225-245.
19. Okwu, D. E. and Emelike, I. N. (2006). Evaluation of the phytonutrients and vitamin contents of citrus fruits. *Int'l J. Mol. Med AdvSci*, 2 (1): 1-6.
20. Salah, N., Miller N. J, Pangaga G., Tiburg L., Bolwell G.P, Rice, E and Evans C (1995). Polyphenolic flavonoids as scavengers of aqueous phase radicals as chain breaking antioxidants. *Arch BiochemBioph*2: 339-346.
21. Del-Rio, A., Obdulio, B.G., Castilo, J., Marin, F.R. and Ortuno, A. (1997). Uses and properties of citrus flavonoids. *Journal of Agriculture and Food Chemistry*, 45:4505-4515.
22. Ghamba, P.E, Agbo, E. B., Umar,A. F., Bukbuk, D.N. and Goje L,N. (2012). In vitro antibacterial activity of extracts of crude ethanol, acetone and aqueous *Garcinia kola* seed extracts on selected clinical isolates. *African J. Biotechnology*11, (6): 1478-1483.
23. Oliver- Bever B. (1986). Medicinal plants in tropical West Africa Cambridge Press, Cambridge pp.123 - 125.
24. Omoboyowa, D. A., Otu-Christian, G., Danladi J. G., Igara C. E., Ngobidi, K. C. Okon, U. O. Agbo F. A. (2012). Evaluation of chemical compositions of *Citruluslanatus* seed and *Cocosnucifera* stem bark. *African Journal of Food Science and Technology*. 6(3): 75-83.
25. Urquiaga, I. and Leighton, F. (2000). Plant polyphenol anti-oxidants and oxidative stress, *Biological Research* 33: 159-160.
26. Obasi, N. A., Ukadilonu, Joy, Eze E., Akubugwo, E. I. and Okorie, U. C. (2012). Proximate composition, extraction, chacterisation and comparative assessment of Coconut (*Cocos nutrifera*) and Melon (*Colocynthis citrullus*) seeds and seed oils. *Pakistan J. of Bio. Science*15: 1-9.
27. Mateljan, G. (2015). The world's healthcost foods. www.whfoo.com retrieved on 11/4/16.
28. Ishida, H., Suzuno, H., Sugiyama, N.,Innami, S., Tadokoro, T. and Maekawa, A. (2000). Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (Ipomoea batatapoir). *Food Chem*, 68: 359-368.
29. Brett, J. (2013). Health/ vitamins and supplements facts. www.howstuffworks.com. Retrieved 14/03/16.
30. Damelio F.S. (1999). Botannical: A phytocosmetic Desk Reference. Raton press. P. 209.
31. Hunt, S., Golf, J. and Holbrook, J. (1980). Nutrition principles and chemical practices. John Wiley and sons. Pp: 49-52.
32. Ayaiyoba, E. O. and Fadare, D. A. (2006). Antimicrobial potential of extracts and fractions of walnut *Tetracarpidium conophorm*. *African Journal of Biotechnology*, 5(22): 2322-2325.
33. Anonymous (2015). www.disabled.worldvitamin: List of types and health benefits, reviewed retrieved 27/01/2016.