Journal of Global Biosciences
ISSN 2320-1355
Volume 4, Number 2, 2015, pp. 1366-1372
Website: www.mutagens.co.in
E-mail: submit@mutagens.co.in
researchsubmission@hotmail.com

Research Paper

STUDIES ON THE PHYTOCHEMICAL AND NUTRITIONAL PROPERTIES OF *TETRACARPIDIUM CONOPHORUM* (BLACK WALNUT) SEEDS

Ojobor Charles Chijioke[1], Anosike Chioma A.[1] and Ani Chijiokes Collins[2]

[1]Department of Biochemistry,
University of Nigeria, Nsukka, Nigeria
[2]Department of Biological Sciences,
University of Agriculture,
Makurdi, Benue State, Nigeria.

Abstract

This study evaluated the phytochemical, proximate, vitamin and mineral element composition of *Tetracarpidium conophorum* (black walnut) seeds. The proximate analysis of the seed extract revealed 31.40±0.01% moisture; 3.77±0.02% crude fats; 28.85±0.01% crude protein; 6.01±0.02% ash; 8.66±0.02% fibre; 21.30±0.04% carbohydrate. Phytochemical analysis of the seed extract also revealed the presence of alkaloids 2.29±0.02 mg/100g, saponins 8.07±0.01mg/100g, flavonoids 0.02±0.02mg/100g, terpenoids, tannins 0.89±0.02mg/100g, glycosides 2.19±0.01mg/100g, reducing sugar 4.10±0.11mg/100g but no resins. The seeds are also rich sources of mineral elements like calcium 44.99±0.14mg/100g, potassium 24.08±0.25mg/100g, sodium 9.59±0.08mg/100g, magnesium 59.77±0.78mg/100g, phosphorus 265.92±0.32mg/100g, iron 2.89±0.02, zinc 6.78±0.08mg/100g, manganese 3,20±0.02 mg/100g and copper 1.87±0.02mg/100g. The seeds also contain some appreciable amount of some vitamins: vitamin A 2.24±0.06mg/100g, vitamin C 5.08±0.01 and vitamin E 70.00±0.82mg/100g. Other vitamins found in trace amounts were vitamin D, K, B_1, B_2, B_3, B_5, B_6, B_9 and B_{12}.

Key words: Black walnut seed, Phytochemical screening, Proximate analysis, Mineral content, Vitamin analysis.

INTRODUCTION

Tetracarpidium conophorum otherwise known as walnut is an edible seed of the tree which belongs to the genus juglans and the family juglandaceae. It is a large deciduous tree attaining the height of 25-35m and a trunk up to 2m diameter with a short trunk and broad crown (Caglarimark, 2003). It is light-demanding specie, requiring full sun to grow well (Brinkman, 1974). Walnut comprises such families as Juglandaceae (English walnut), Euphorbiaceae and Olacaceae (African walnut). It is an economic plant widely cultivated for the production of nuts and it is used as delicacies (Adebona, 1988). The English walnuts are called *Juglan regia* while the black walnuts are known as *Juglans nigra*. Each family has its own peculiar characteristics but they have some things in common such as the nuts. *Tetracarpidium conophorum* is known in the Easthern Nigeria as ukpa (Igbo), Western Nigeria as awusa or asala (Yoruba) and Northern Nigeria as gawudi bairi (Hausa). It is cultivated principally for its nuts which are cooked and consumed. Black walnut grows best on moist, deep, fertile, well-drained, loamy soils; although it

also grows quite well in silty clay loam soils (Williams, 1990; Cogliastro *et al.*, 1997). These sites include coves, bottomlands, abandoned agricultural fields, and rich woodlands. *Tetracarpidium conophorum* is known in the littorial and the western Cameroon as kaso or ngak and serves as an edible nut eaten between meals. The leaf extract of *T. conophorum* possesses antibacterial and antifungal activities. The root extract of the plant also displays antibacterial activity (Ajaiyeoba and Fadare, 2006). Walnuts extract possess anticancer property (Herbert *et al*., 1998) and reduce diabetic complications (Kaneto *et al*., 1999). Also, the presence of oxalates, phytates, tannins as well as proteins, fibres, oil and carbohydrate in *Tetracarpidium conophorum* has been reported (Enujiugha, 2003). The phytochemical and nutrient evaluation of *Tetracarpidium conophorum* (Nigerian walnut) root has also been reported (Ayoola *et al.,* 2011). Therefore, the objective of this work was to evaluate the proximate, phytochemical, mineral and vitamin constituents of the seed sample of *Tetracarpidium conophorum.*

MATERIALS AND METHODS

Collection and preparation of plant material: Healthy seeds of walnut were obtained from Nkwo-Ibagwa, Igbo Eze South L.G.A., Enugu State. The seeds were identified and authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka (UNN). The freshly collected seeds of *Tetracarpidium conophorum* were dehulled, chopped, sun-dried to constant weight, and milled to powder using the hand mill. Portions of the powdered sample were then used for the various analyses.

Qualitative phytochemistry: Qualitative phytochemical analysis of the samples for alkaloids, glycosides, saponins, flavonoid, tannins, terpenoid, reducing sugar and soluble carbohydrates were carried out by methods described by Harborne (1973).

Quantitative phytochemistry: Quantitative phytochemical screening of the samples for relative abundance or absence of alkaloids, glycosides, saponins, flavonoids, tannins, terpenoid, reducing sugar and soluble carbohydrates were carried out by method outlined by Pearson (1976).

Proximate analysis: The proximate analysis of the seed extract for crude protein, crude fiber and fat contents were determined using the methods described by Pearson (1976). Crude protein determination was done using Kjedhal's method, while crude fibre determination was done using acid and alkaline digestive method. Fat content was determined using continuous solvent extraction method. Total ash content was determined by ignition at 550^{o}C in a muffle furnace for 4hr. Moisture and carbohydrate contents were determined using the methods described by AOAC (1990).

Mineral analysis: The methods outlined by AOAC (2000) were used for the determination of minerals in the test sample. Calcium, sodium, potassium, magnesium were determined by flame photometric method while iron, zinc, manganese and copper were determined by atomic absorption spectrophotometric method. The sample (2g) was weighed and put into a clean dried crucible. Then it was transferred to a muffle furnace, ashed at 700^{o}C for 3 hours and cooled in a dessicator. 30% HCl (5 ml) was added, with 10 ml 0f distilled water and diluted to 50 ml with distilled water. The resulting solution was use in the analysis of iron, zinc, manganese and copper respectively.

Vitamin analysis: The composition of the water soluble vitamins such as thiamine, niacin, pantothenic acid, pyridoxine, cobalamin, folate were determined by the method of Association of Official and Analytical Chemists (AOAC, 1990). Vitamins A, C, D, E and K contents were determined by the method described by Okwu (2004)

RESULTS AND DISCUSSION

The result obtained from the phytochemical tests *Tetracarpidium conophorum* seeds. These are as follows;

Table 1: Qualitative Phytochemicals Results of *Tetracarpidium conophorum* seeds.

Phytochemicals	Walnut
Alkaloid	+++
Flavonoid	+
Saponins	+++
Glycoside	+++
Tannins	+
Soluble Carbohydrate	++
Hydrogen Cyanide	ND
Terpenoid	ND
Reducing sugar	+++

KEY: + Trace amount present
++ Moderate amount present
+++ Appreciable amount
ND Not detected.

The qualitative phytochemical constituents of walnut (*Tetracarpidium conophorum*) are shown in table 1. Analyses revealed high abundance of alkaloid saponins, glycoside and reducing sugar in *Tetracarpidium conophorum* seeds. There were also moderate amounts of soluble carbohydrate observed in the sample. However, phytochemicals such as flavonoid, and tannins were observed in trace amounts while hydrogen cyanide and terpenoid were not detected in the samples.

Table 2: Quantitative Phytochemical results of *Tetracarpidium conophorum* seeds

Phytochemical	Mean Composition (mg/100g dry weight)
Alkaloid (mg/100g)	2.29 ± 0.02
Flavonoid (mg/100g)	0.02 ± 0.02
Saponins (mg/100g)	8.07 ± 0.01
Glycoside (mg/100g)	2.19 ± 0.01
Tannins (mg/100g)	0.89 ± 0.02
Soluble Carbohydrate(mg/100g)	1.06 ± 0.01
Hydrogen Cyanide (mg/100g)	0.02 ± 0.01
Terpenoid (mg/100g)	0.01 ± 0.02
Reducing Sugar (mg/100g)	4.10±0.11

Values are means of three Determinations ± S.D.

Table 2 shows the phytonutrients present in the seed sample analysed. The results obtained from the phytochemical analyses show high concentration of alkaloid (2.29 ± 0.02 mg/100g); saponin (8.07 ± 0.01 mg/100g); glycoside (2.19 ± 0.01 mg/100g); reducing sugar (4.10 ± 0.11 mg/100g), moderate concentration of soluble carbohydrate (1.06 ± 0.01 mg/100g) and trace amount of flavonoid (0.02 ± 0.02 mg/100g); tannins (0.89 ± 0.02 mg/100g). Phytochemicals are biologically active compounds, found in trace amounts, which are not established nutrients but which nevertheless contribute significantly to protection against degenerative diseases (Dreosti, 2000). The results of phytochemical analysis revealed appreciable amount of alkaloids, saponins, glycosides and reducing sugar. However, other phytonutrients were also quantified. Flavonoids have protective effects including anti-inflammatory, anti-oxidants, antiviral, and anti-carcinogenic properties. They are generally found in a variety of foods, such as oranges,

tangerines, berries, apples and onions (Middleton *et al.,* 2000). The presences of tannins in the sample suggest that it could be used for healing of haemorrhoids and varicose ulcers in herbal medicine (Igboko, 1983; Maduiyi, 1983). Alkaloids are heterogeneous group of naturally occurring compounds found in the leaves, bark, roots or seeds of plants. They are the most effective plant substance used therapeutically as analgesic, antimicrobial and bacterial properties. This may probably be the reason walnut is believed to stop asthma (not acute asthma) and also a constipation cure for elderly. However, the high amount of saponins in the walnut samples is in closed conformity with the earlier report of Ayoola *et al.,* 2011, for walnut root and it is an indication that walnut has a cytotoxic effect such as permealisation of the intestine. Saponin also gives the plant its bitter taste; saponin has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk (Okwu and Okwu, 2004).

Table 3: Proximate Composition of *Tetracarpidium conophorum* seeds.

Proximate	Mean Composition
Moisture (%)	31.40 ± 0.01
Ash (%)	6.01 ± 0.02
Fats (%)	3.77 ± 0.02
Fibre (%)	8.66 ± 0.02
Protein (%)	28.85 ± 0.01
Carbohydrate (%)	21.30 ± 0.04
Energy Value (kcal)	234.57 ± 0.02

Values are means of three Determinations ± S.D.

Table 3 shows the percentage proximate composition of the samples. The proximate compositions of *Tetracarpidium conophorum* seed were moisture (31.40±0.01%), fats (3.77±0.02%), protein (28.85±0.01%), ash (6.01±0.02%), fibre (8.66±0.02%), carbohydrates (21.30±0.04%) and energy values (234.57±0.02 kcal) respectively**.** These values were fairly in agreement with earlier reports on proximate compositions of these seeds by Ayoola *et al.,* 2011 and Okwu, 2004. The moisture content is an important parameter as it affects the percentage yield of the seed oils during extraction (Mansor, *et al*., 2012). This indicates why the walnut seed has low oil yield judging from the high amount of its moisture content. The fibre and protein contents of the sample show that it is nutritionally potent and also, could be regarded as valuable sources of dietary fibre in human nutrition. Adequate intake of dietary fibre can lower cholesterol level, risk of coronary heart diseases, hypertension, constipation, diabetes, colon and breast cancer (Ishida *et al.,* 2000; Rao *et al.,* 1998).

Table 4: Mineral Analysis of *Tetracarpidium conophorum* seeds.

Mineral Elements	Mean Composition (mg/100g dry weight)
Copper (mg/100g)	1.87 ± 0.02
Manganese (mg/100g)	3.20 ± 0.02
Zinc (mg/100g)	6.78 ± 0.08
Iron (mg/100g)	2.89 ± 0.02
Phosphorus (mg/100g)	265.92 ± 0.32
Magnesium (mg/100g)	59.77 ± 0.78
Calcium (mg/100g)	44.99 ± 0.14
Potassium (mg/100g)	24.08 ± 0.25
Sodium (mg/100g)	9.59 ± 0.08

Values are means of three Determinations ± S.D.

The results of the mineral constituents of the seeds as shown in Table 4 revealed copper (1.87±0.02mg/100g), manganese (3.20±0.02mg/100g), zinc (6.78±0.08mg/100g), iron (2.89±0.02mg/100g), phosphorus (265.92±0.03mg/100g), magnesium (59.77±0.77mg/100g), calcium (44.99±0.14mg/100g), potassium (24.08±0.25mg/100g) and sodium (9.59±0.08mg/100g). Our results were found higher when compared with mineral values reported by Caglarirmak (2003) for English walnut (*Juglans regia*) kernel. The result indicates that walnut seeds are rich source of some minerals and it becomes so important when the usefulness of such nutrients like Ca, Mg, K, Na in the body are considered. The minerals play important roles in health and nutrition. However, the Na and K content of *Tetracarpidium conophorum* is an added advantage because of the direct relationship of sodium intake with hypertension in human (Dahl, 1972). This could be the reason for earlier report by James, 2000 which states that walnut contains active agent for prevention and control of high blood pressure. Calcium and phosphorus are very essential for bone metabolism and assist in teeth development. Calcium is also a cofactor of three important enzymes; pyruvate dehydrogenase complex, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex in the citric acid cycle. The presence of copper may be responsible for the absorption of iron, it is therefore, often seen with iron naturally. Copper is also important for cellular defense and protection of the mucous membrane, antianaemic and essential for the formation of haemoglobin from iron (Claude and Paule, 1979). Iron plays important roles in many proteins and enzymes, notably in haemoglobin to prevent anaemia. The amount of Manganese determined on walnut shows that the plant can be used to protect bone disease (James, 2000). The activity of this element is noticeable in the metabolism of food incorporated into the bone. Manganese is also necessary for the functioning of the pituitary gland, the pineal gland and the brain (Claude and Paule, 1979), it promotes hepatorenal function, combat anaemia and also essential for growth. The amount of zinc found in walnut sample is an indication that the seed may have some effect on the nerve function and male fertility. It is also important for normal sexual development, especially for the development of testes and ovaries, it is essential for reproduction. Zinc stimulates the activity of vitamins, formation of red and white corpuscles (Claude and Paule, 1979), healthy functioning of the heart and normal growth (Elizabeth, 1994). Zinc is also an antioxidant, as it is a cofactor for many antioxidant enzymes such as glutamate dehydrogenase, alcohol dehydrogenase, lactate dehydrogenase, DNA and RNA polymerase, superoxide dismutase etc. Magnesium is a cofactor of many enzymes such as kinases, phospatase, transketolase, ribonuclease, carboxylase, peptidase and adenylyl cyclase. A good example is seen in glycolytic pathway were it serves as a cofactor of the enzymes that catalyse steps one, two, three, seven, eight and ten of the pathway.

Table 5: Vitamins analysis of *Tetracarpidium conophorum* seeds

Vitamin	**Mean Composition (mg/100g dry weight)**
VitaminA	2.24±0.06
VitaminC	5.08±0.01
VitaminD	0.43±0.02
VitaminE	70.00±0.82
VitaminK	0.08±0.01
VitaminB_1	0.20±0.01
VitaminB_2	0.89±0.06
VitaminB_3	0.14±0.01
VitaminB_5	0.02±0.01
VitaminB_6	0.06±0.04
VitaminB_{12}	0.38±0.02
Folate	0.07±0.01

Values are means of three Determinations ± S.D.

The results of the vitamins analyses proved that the sample have preponderance amount of vitamin A (2.24±0.06 mg/100g), vitamin C (5.08±0.00 mg/100g), vitamin E (70.00±0.08mg/100g) respectively. As a result of the presence of ascorbic acid in both seeds, the plants can be used in herbal medicine for the treatment of skin conditions, including eczema, pruritus, psoriasis and parasitic skin conditions (D'Amelio, 1999). This vitamin can also be used for the treatment of common cold and other diseases like prostate cancer (Okwu and Okwu, 2004; Okwu and Okeke, 2003). There is also an interesting ability of ascorbic acid as an antioxidant, to prevent or at least minimises the formation of carcinogenic substances from dietary material (Hunt *et al.,* 1980). Deficiency of ascorbic acid is associated with pains in the joint and defect in skeletal calcification, anaemia, manifestation of scurvy haemorrhage from mucous membrane of the mouth and gastrointestinal track (Hunt *et al.,* 1980). Report also indicates that walnut could be used in treatment of indigestion, constipation and diarrhea (Ayoola *et al.*, 2011). The vitamin E content of walnut was exceptionally high and this supports its use in Southern Nigeria ethno-medicine as a male fertility agent (Ajaiyoba and Fadare, 2006). Other vitamins determined in this work (table 5) though observed in trace amount are essential for body metabolism.

CONCLUSION

The present study has shown the proximate, vitamins, minerals and phytochemical compositions of *Tetracarpidium conophorum* (Nigerian walnut) seed. 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-inflamatory property. This also proves that the seed may be helpful in asthma, rheumatoid and arthritis. High content of ascorbic acid also indicates that the plant can be used to prevent or at least minimize the formation of carcinogenic substances from dietary material. Further studies have to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the seed for industrial drug formulation. More so, extensive works should be carried out to search for the effectiveness of the seed in male reproductive organ and also its cardiovascular functions.

REFERENCE

1. Adebona M. B., Ogunsua A. O., Ologunde M. O., 1988. Development of conophor nut-basedcereal snack food I- Biscuits. *Journal of Food and Agriculture*, 2: 123-136.
2. Ajaiyeoba, E.O. and Fadare, D.A., 2006. Antimicrobial potential of extracts and fractions of the African walnut *Tetracarpidium conophorum*. *African Journal of Biotechnology,* 5(22): 2322-5.
3. AOAC. 1990. Official method of analysis 15th Edn. Association of Official Analytical Chemist, Washington D.C. 1: pp. 600-792.
4. AOAC. 2000. Method of Analysis of Official Analytical Chemists. 17th Edn. Peroxide Value in Oils and Fats/ Pearson Composition and Analysis of Foods 9th Edn. p. 641.
5. Ayoola, P.B., Onawumi, O.O. and Faboya, O.O.P., 2011. Chemical evaluation and nutritive values of *Tetracarpidium conophorum* (Nigerian walnut) seeds. *Journal of Pharmaceutical and Biomedical Sciences,* Vol. 11(15)
6. Brinkman KA (1974) Juglans L. Walnut. In: Schopmeyer CS (tech coord) Seeds of woody plants in the United States, USDA For Serv Agric Handb 450, Washington, pp. 454-459
7. Caglarirmak N., 2003. Biochemical and physical properties of some walnut genotypes *(Juglans regia*). *Nahrung Food,* 47: 28–32.
8. Claude, B. and Paule, S., 1979. The Manual of Natural Living. 1sted.Biddles Limited Guildford Surrey. pp. 98-99,101.
9. Cogliastro, A., Gagnon, D. and Bouchard A., 1997. Experimental determination of soil characteristics optimal for the growth of ten hardwoods planted on abandoned farmland. For Ecol Manage 96 (1-2): pp.49-63. Goodell, E. (1984). Walnuts for the northeast. Arnoldia, 44 (1): pp. 3-19.

10. D,Amelio F.S., 1999. Botanical; A phytocosmetic Desk Reference, Boca Raton, FL,CRC Press. p. 209.
11. Dahl, L.K., 1972. Salt and Hypertension. *American Journal of Clinical Nutrition*, Vol.25, 231-238.
12. Dreosti, I.E., 2000. Recommended dietary intake levels for phytochemicals: Feasible or Fanciful? *Asia Pacific Journal of Clinical Nutrition* 9: 119-122.
13. Elizabeth, K., 1994. Text book on immense help from Natures workshop. 1sted. Elikaf Health Services Limited, p. 207.
14. Enujiugha, V.N., 2003. Chemical and Functional Characteristics of Conophor Nut. *Pakistan Journal of Nutrition*. 2(6): 335-338.
15. Harborne, J.B., 1973. Textbook of Phytochemical Methods. 1st Edn. Chaperon and Hall Limited, London. pp. 110-113.
16. Hunt, S., Goff, J.L. and Holbrook, J., 1980. Nutrition Principles and Chemical Practices. John Wiley and Sons. New York, pp. 49-2.
17. Igboko, D.O., 1983. Phytochemical studies on Garcinia kola Heckel. M.sc Thesis. University of Nigeria Nsukka, p. 202.
18. Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., Tadokoro, T. and Maekawa, A., 2000. Nutritional evaluation of chemical component of leaves stalks and stems of sweet potatoes (*Ipomoea batatas poir*). *Food Chemistry,* 68: 359-367.
19. James, N.R., 2000."Volatile Components of Green Walnut Husks. *Journal of Agricultural and food Chemistry,* 48(7): 2858-2861.
20. Maduiyi, I., 1983. Biochemical and pharmacological studies of active principles of the seeds of Garcinia Kola Heckel.M.sc Thesis University of Nigeria, Nsukka, p. 108.
21. Mansor, T. S. T., Che Man, Y. B., Shuhaimi, M., Abdul Afiq, M. J. and Ku Nurul, F. K. M., 2012. Physicochemical properties of virgin coconut oil extracted from different processing methods. *International Food Research Journal*, 19(3): 837-845.
22. Middleton, E. Jr., Kandaswani, C. and Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells, implication for inflammation, heart disease and cancer. *Journal of Pharmacology,* 52: 673-751.
23. Okwu, D.E. and Okeke, O., 2003. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global Journal of Pure Applied Science*, 9: 235-38.
24. Okwu, D. E., 2004. Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. *Journal of Sustainable Agriculture and Environment*, 1: 30-37.
25. Okwu, D.E. and Okwu, M.E., 2004. Chemical Composition of *Spondiamombin plants. Journal of Sustainable Agricultural Environment,* 6: 140-147.
26. Pearson, D., 1976. Chemical Analysis of Foods. 7th Edn. Church Hill Livingstone, London, UK. pp. 72-73, 138-143, 488-496.
27. Rao, C.V. and Newmark, H.L. and Reddy, B.S., 1998. Chemo-preventive effect of Squalene on colon cancer. *Carcinogenesis,* 19: 287-290.
28. Williams, R.D., 1990. Juglans nigra L., Black walnut. In: Burns RM, Honkala BH (tech coords) Silvics of North America, Vol 2. Hardwoods. USDA For Serv Agric Handb 654, Washington, pp. 386-390.