American Journal of Analytical Chemistry, 2015, 6, 604-609
Published Online June 2015 in SciRes. http://www.scirp.org/journal/ajac
http://dx.doi.org/10.4236/ajac.2015.67058

Comparative Assessment of Phytochemicals, Proximate and Elemental Composition of *Gnetum africanum* (Okazi) Leaves

I. O. Okerulu, C. T. Onyema*

Department Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria
Email: Isaacokerulu@yahoo.com, *ct.onyema@unizik.edu.ng

Received 21 May 2015; accepted 26 June 2015; published 30 June 2015

Abstract

The leaves of *Gnetum africanum* were studied for their proximate (nutrient), mineral (elemental) and phytochemical constituents. All analyses were done using standard analytical procedures and the results obtained showed a moisture content, crude protein, ash content, crude fibre, crude lipids and carbohydrates of 10.9%, 20.12%, 6.70%, 7.10%, 2.79% and 52.39% respectively. The mineral (elemental) analysis showed the presence of sodium, magnesium, calcium, iron, zinc, manganesse, potassium and copper in that order of decreasing concentration while notably absent was chromium and lead in the examined leaves. The phytochemicals detected in the leaves using various selected solvents were alkaloids, saponins, glycosides and tannins with various concentrations while flavonoids, phenols and steroids were totally absent. From the results of the aforementioned analyses, it could be concluded that the leaves of *Gnetum africanum* contain some beneficial nutrients, mineral elements and secondary metabolites justifying the medicinal status and possible potency of the plant part.

Keywords

***Gnetum africanum* Leaves, Phytochemicals, Proximate and Elemental Compositions**

1. Introduction

Gnetum africanum commonly called "Okazi" by Igbos and "Afang" by Efik of Nigeria is one of the most popular green leafy vegetables in Nigeria and is gaining popularity as a delicious food leaf in other African countries

*Corresponding author.

How to cite this paper: Okerulu, I.O. and Onyema, C.T. (2015) Comparative Assessment of Phytochemicals, Proximate and Elemental Composition of *Gnetum africanum* (Okazi) Leaves. *American Journal of Analytical Chemistry*, **6**, 604-609.
http://dx.doi.org/10.4236/ajac.2015.67058

such as Cameroon, Gabon, Congo, Angola etc. [1]. *Gnetum africanum* leaves are widely consumed in the South Eastern Nigeria due to its palatability and taste and often cooked with water leaves (*Talinium triangulare*) to give the soup a special flavour. The plant grows as a wild evergreen climbing plant in the rain forest of Nigeria where it is searched for and highly priced in the regional markets. This wild variety is termed wild species while the recently domestically cultivated ones in the South Eastern Nigerian homes as exotic plants are termed the domestic species. *Gnetum africanum* belongs to the family *Gnetacea* and order *Gnetales* [2]. The seed of *Gnetum africanum* is oval in shape, small in size, greenish in colour when unripe and reddish when ripe. The seeds but most especially the leaves which are our interest in this work have shown medical efficiency in the treatment of enlarged spleen, sore throats, reduction of pain during child birth, antidotes to some forms of poison and snake bites. The seeds are specially used as fungicide for dressing fresh and septic wounds and can also be chewed raw for the management of excessive urination [3].

Various chemical composition analyses have been done on these leaves to determine their proximate, phytochemicals, antinutrirional, mineral composition and other essential contents. Dike [4] carried out some analysis on the leaves of *Gnetum africanum* sourced from Umudike rainforest, Abia State while Ekpo and Eddy [5] studied the chemical composition of both wild and domestic species of *Gnetum africanum* collected from Urua Akpan Ndem in Uyo Akwa Ibom State and Akwa Ibom State University Obio Akpa Campus Teaching and Research Farms respectively. The results of the above works will be compared with those of our results obtained from species sourced from Ohianri Forest Umoli Village Mgbirichi on Ohaji Egbema L.G.A of Imo State Nigeria to discover any specific variations with respect to its chemical and elemental composition.

2. Materials and Methods

2.1. Plant Collection, Identification and Preparation

Random samples of the leaves of *Gnetum africanum* (Okazi) were obtained from Ohianri forest Umoli village Mgbirichi in Ohaji Egbema L.G.A Imo state, Nigeria and identified by Mr. P.O. Ugwuozor a taxonomist of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The leaves were removed from their stalks and air dried in the laboratory for twelve days. They were subsequently pulverized by pounding to ensure homogeneity and kept in an airtight container for further analysis. Leaves for moisture content determination were collected and analyzed *in-situ*.

2.2. Proximate Analysis

The sample was analysed for moisture content, Crude protein, Crude fibre and ash content. Crude protein was determined using the kjeldahl method. The moisture and crude fat were determined using standard methods [6] and the percentage calculated on dry weight basis. Ash was determined by incineration in a muffle furnance and the weight of ash was calculated from difference with the sample taken and calculated on a dry weight basis. The carbohydrates were determined by difference of the sum of all the proximate composition from 100%.

2.3. Elemental Analysis

2 g of the powerded sample was weighed into 50 ml beaker, 10 ml of conc. nitric acid (BDH analytical grade) and 10 ml of conc. perchloric acid (BDH analytical grade) were added to the sample. The mixture was put into a fume cupboard and heated until clear solution was obtained. The digested sample was filtered into a 250 ml volumetric flask using whatmann filter paper and made up to the mark with distilled water. The solution was subsequently analyzed for Ca, CU, Zn, Mg, K, Na, Pb and Cr using Atomic Absorption Spectrophotometer Varian AA 280 with appropriate hallow cathode lamps.

2.4. Preliminary Phytochemical Screening

Phytochemcial constituents of the leaves both qualitatively using five different solvents (water, ethanol, methanol, n-hexane and diethyl ether) and quantitatively were determined according to the Harbone method for plant analysis [7]. The 50 ml of each solvent was used to extract the leaves separately and the presence of the phytochemicals: alkaloids, saponins, flavonoids, phenol, steroids, glycosides and tannins were tested. The phytochemicals present were estimated quantitatively.

3. Results and Discussion

The results as presented in **Table 1** showed a relatively low moisture content (10.9%) for *Gnetum africanum* against some values (31.6%) for *Gnetum africanum* seeds reported by Ekop [8] and some vegetables like *Piper guineese* and *Gongromea latifolium* [9], 81.36% recorded in *Bressica oleraecea* [10], 83.75% in *Pterocarpus soyaubixii* and *Gnetum africanum* [11] but however is within the range recorded for other vegetables like 6.82% for *Cissus petiolate* [12] and 7.60% - 8.55% for some vegetables from Nigeria [13].

Moisture content which is an index of the water activity of many foods provides for greater activity of water soluble enzymes and coenzymes needed for the metabolic activities of these leaves. Higher moisture content indicates higher susceptibility to microbial attack during storage and shorter shelf life and also indicative of high total solids [14] [15]. The crude protein content of the leaves of *Gnetum africanum* (20.12%) was relatively high as in **Table 1** and compared favourably with 17.5% recorded by Epko [8] for the seeds of *Gnetum africanum* and 19.67% and 20.80% for the leaves of *Gnetum africanum* and *C. pepo* respectively [13]. It is however, lower than 32.95% recorded in undefatted leaves of *A. hybridus* [13]. Plant protein still remains a major source of food nutrient for the less priviledged population in developing countries including Nigeria such that protein content of the leaves makes it suitable for consumption and a rich source of vegetable protein [10].

The relatively high protein content suggests the high amount of essential acids which serve as an alternative source of energy when the carbohydrate metabolism is impaired via glucogenesis [13]. The carbohydrate content of the sample (52.39%) as presented in **Table 1**, could be said to be high for a vegetable material but this value was justified by the 87.62% for *Gnetum africanum* seeds [8], 52.32% reported for *Pachira glabra* and 45.92% for *A. Africana* seed flowers [14], 52.18% for *Amaranthus hybridus* [16]. According to Emebu and Anyika [10] most vegetables are generally not good sources of carbohydrates. As far as vegetables are concerned, some of them are rich sources while others contain traces of the nutrients. They provide the body with a source of fuel and energy for daily activities [17]. The crude fibre content of 7.1% in the leaves of *G. africanum* in **Table 1** is higher than 0.80% recorded for the seeds of *G. africanum* [8]. The composition of leaves with high crude fibre may contribute to a reduction in the incidence of certain diseases like colon cancer, coronary heart disease, diabetis, high blood pressure, obesity and other digestive disorders [18]. From **Table 1**, the low fat content (2.79%) indicated that the leaves contain low quantities of lipid biomolecules [13] and cannot serve as main source of these biomolecules that are important for body metabolism. Epko (2007) recorded a value of 3.15% for *G. africanum* seeds which is still in unison with our obtained value (2.79%). The ash content of 6.70% in the leaves was also in line with the low ash content (1.2%) obtained by Epko [8] for the seeds of *G. africanum*, 4.34% for *R. glabra* [14] and 4.03% for *A. Africana* but lower than some vegetables such as *P. mildbraedi* (20.6%) [19] and *Talinum triangulare* (20.05%) [20]. The ash content is an indication of the mineral contents of the leaves as low ash content suggests low mineral composition or high organic components a claim verified by the results of the Elemental composition (**Table 2**).

From the results in **Table 2**, the Major elements present in the leaves were Fe, Ca, Mg and Na in that order with Cr and Pb totally absent. The absent of Cr and Pb which are toxic metals shows that these leaves do not pose any health risks. The high concentration of Ca (11.20 mg/l), a vital element that helps in bone formation and blood coagulation whose deficiency may contribute to rickets, curvature of the spine and pelvic and thoracic deformities [21] was also noticed. Magnessium was equally in high concentration (12.00 mg/l) which is in line with the fact that an adult human body contains about 25 grams of Magnessium and that most vegetables are rich in magnesium (more than 500 mg/kg fresh weight) [22]. The presence of Mg in these leaves is also known to prevent cardiomyopathy, impaired spermatogenesis and bleeding disorders [23].

Iron, a key element was also present in high amount (7.23 mg/l) as in **Table 2** and this helps in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes [24] [25]. The iron content of the leaves was higher than the FAO/WHO (1988) recommend dietary allowance for males (1.37 mg/day) and females (2.94 mg/day) [26]. Iron as an essential trace metal plays numerous biochemical roles in the body, including oxygen blinding in haemoglobin and acting as an important catalytic centre in many enzymes [27]. The high level of Fe could be the reason behind the common use of these leaves in tackling iron deficiency associated disease (Anemia).

4. Phytochemcial Analysis

Phytochemicals are natural bioactive compounds found in plants that work with nutrients and dietary fiber for

disease protection [28]. The phytochemical results showed the absence of flavonoids, phenols and steroids in all the solvents used in the extraction as in **Table 3**. Alkaloids and saponins were present in moderate amount in ethanol extract with percentage composition of 4.56 and 1.47 respectively as in **Table 4**. Saponin was also equally

Table 1. Proximate Composition of *Gnetum africanum* leaves.

Parameters	Composition (%)
Moisture content	10.90
Crude protein	20.12
Ash content	6.70
Crude fibre	7.10
Lipids	2.79
Carbohydrate	52.39
Vit A.	0.129 mg/g
Vit C.	0.360 mg/l

Table 2. Elemental composition of *G. africanum* leaves.

Parameters	Compositon mg/l
Ca	11.20
Cu	0.30
Zn	2.40
Cr	-
Fe	7.23
Pb	-
Mg	12.00
K	0.43
Na	23.00
Mn	1.56

Table 3. Qualitative composition of the phytochemicals of *G. africanum* leaves.

Parameters/Solvent	Water	Diethylether	Ethanol	n-hexane	Methanol
Alkaloids	-	-	++	-	-
Saponins	+	+	++	-	++
Flavonoids	-	-	-	-	-
Phenols	-	-	-	-	-
Steroids	-	-	-	-	-
Glycosides	-	-	-	+	-
Tannins	-	-	-	++	++

Key : +: Slightly present; +++: Highly present; ++: Moderately present; -: Absent

Table 4. Quantitative composition of the phytochemicals of *G. africanum* leaves.

Parameter	Composition (%)
Alkaloids	4.56
Glycosides	0.25
Saponins	1.47
Tannins	4.23

present in water and diethyl ether in slight proportions. The valiue (1.47%) was higher than that (0.25%) previously obtained by Dike (2010) [4]. The presence supports its anti-inflammatory property. Glycosides was spotted in very low concentration only in n-hexane with quantitative compositon of 0.25% showing its medicinal property in prevention of heart diseases as in **Table 3** and **Table 4**. Glycosides and alkaloids have been reported to exert inhibiting activity against most bacteria [29] [30].

Tannins an agent for toning of vital organs such as kidney and liver were only substantially present in n-hexane and methanol as in **Table 3** and **Table 4**.

Each of these phytochemicals is known for various protective and therapeutic effects [31].

5. Conclusion

The present study has shown the safety of this plant part based on the absence of the heavy metals with adverse health effects and its nutritive composition given the presence of requisite elements like Ca, Fe, Mg, Na, food classes like proteins, carborhydrates, crude fibre etc. Its phytochemical composition determine the medicinal values of these edible vegetable leaves and also could serve as a stating materials for the synthesis of new drugs in pharmaceutical industries.

6. Recommendations

Further studies have to be carried out to isolate, characterise and elucidate the structures of the bioactive compounds from the plant for industrial drug formulation.

References

[1] Eyo, E. and Abel, U. (1983) Chemical Composition of Amino-Acid Content of *Gnetum africanum* Leaves. *Nig. J. Nutr. Sci.*, **4**, 52-57.

[2] Dutta, A. (1981) Botany for Degree Students. 5th Edition, Oxford University Press, Dellin, 286-288.

[3] Mailoundama, F. (1993) Nutritional and Socio-Economic Value in Central African Forest. In: Itladikom, *et al*., Eds., *Tropical Forest People and Food*: *Bio-Cultural Interactions and Applications to Development*, Parthenon Publishing Group, Carnforth.

[4] Dike, M.C. (2010) Proximate and Nutrient Compositions of Some Fruits, Seeds and Leaves of Some Plant Species at Umudike, Nigeria. *ARPN-Journal of Agricultural and Biological Science*, **5**, 7-16.

[5] Ekop, A.S. and Eddy, N.O. (2005) Comparative Studies of the Level of Toxicants in the Seed of Indian Almond (*Terminalia catappa*) and African Walnut (*Coula edulis*). *Chem. Class J.*, **2**, 74-76.

[6] AOAC (1990) Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemists, Washington DC, 200-210.

[7] Harbone, J.B. (1998) Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis. 3rd Edition, Chapman and Hall, London, 36-89.

[8] Ekop, A.S. (2007) Determination of Chemical Composition of *Gnetum africanum* (AFANG) Seed. *Pakistan Journal of Nutrition*, **6**, 40-43. http://dx.doi.org/10.3923/pjn.2007.40.43

[9] Mensah, J.K., Okoli, R.I., Ohaju-Obodo, J.O. and Eifidiyi, K. (2008) Phytochemical, Nutritional and Medicinal Properties of Some Leafy Vegetables Consumed by Edo People of Nigeria. *African Journal of Biotechnology*, **7**, 2305-2308.

[10] Emedu, P.K. and Anyika, J.U. (2011) Proximate and Mineral Composition of Kale (*Brassica oleracea*) Grown in Delta State, Nigeria. *Pakistan Journal of Nutrition*, **10**, 190-194.

[11] Ekumankama, I.O. (2008) Nutrient Composition of Indigenous Vegetables (*Pterocarpus soyanxii*, *Pterocarpus santalicides* and *Gnetum aficanum*). *Nigerian Journal of Nutritional Sciences*, **29**, 195-200.

[12] Omoyeni A.O. and Aluko, B.T. (2010) Qualitative Determination of Chemical and Nutritional Composition of *Cissus petiolata* Leaves. *Electronic Journal of Environmental*, *Agricultural and Food Chemistry*, **9**, 436-440.

[13] Iheanacho, K.M.E. and Udebuani, A.C. (2009) Nutritional Composition of Some Leafy Vegetables Consumed in Imo State Nigeria. *Journal of Applied Sciences and Environmental Management*, **13**, 35-38.

[14] Ogungbele, H.N. (2006) Chemical Composition Functional Properties and Amino Acid Composition of Some Edible Oil Seeds. *Rivista Italiana delle Sostanze Grasse*, **83**, 81-86.

[15] Adepoju, O.T., Onasanya, L.O. and Udoh, C.H. (2006) Comparative Studies of Nutrient Composition of Cocoyam (*Colocassia esculenta*) Leaf with Some Green Leafy Vegetables. *Nigerian Journal of Nutritional Sciences*, **27**, 40-43.

[16] Akubugwo, I.E., Obasi, N.A., Chinyere, G.C. and Ugbogu, A.E. (2007) Nutritional and Chemical Value of *Amaranthus hybridus* L. Leaves from Afikpo, Nigeria. *African Journal of Biotechnology*, **6**, 2833-2839.

[17] Yisa, J., Egila, J.N. and Darlinton, A.O. (2010) Chemical Composition of *Annona senegalensis* from Nupe Land, Nigeria. *African Journal of Biotechnology*, **9**, 4106-4109.

[18] Ogunlade, I.A., Ilugbiyin, A. and Osasona, A.I. (2011) A Comparative Study of Proximate Composition, Anti-Nutrient Composition and Functional Properties of *Pachira glabra* and *Afzelia Africana* Seed Flours. *African Journal of Food Science*, **5**, 32-35.

[19] Akinyeye, R.O., Oluwadunsi, A. and Omoyeni, A. (2010) Proximate, Mineral, Antinutrients, Phyto-Chemical Screening and Amino Acid Compositions of Leaves of *Pterocarpus mildbraedi* Harms. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **9**, 1322-1333.

[20] Akindahusi, A.A. and Salawu, S.O. (2005) Phytochemical Screening of Nutrients and Anti-Nutrient Composition of Selected Tropical Green Leafy Vegetables. *African Journal of Biotechnology*, **4**, 497-581.

[21] Wardlaw, G.M. and Smith, A.M. (2006) Contemporary Nutrition. McGraw-Hill Education, New York, 353-367.

[22] FAO/WHO (2001) Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation, Bangkok. Food and Nutrition Division, FAO, Rome, 223-224.

[23] Chaturvedi, V.C., Shrivastava, R. and Upreti, R.K. (2004) Viral Infections and Trace Elements: A Complex Trace Element. *Current Science*, **87**, 1536-1554.

[24] Beard, J.L. and Dawson, H.D. (1997) Iron. In: O'Dell, B.L. and Sunde, R.A., Eds., *Handbook of Nutritionally Essential Minerals*, Marcel Dekker, New York, 275-334.

[25] Fairbanks, V.F. (1999) Iron in Medicine and Nutrition. In: Shils, M., Olson, J.A., Shike, M. and Ross, A.C., Eds., *Nutrition in Health and Disease*, 9th Edition, Williams and Wilkins, Baltimore, 223-239.

[26] FAO/WHO (1988) Requirement of Vitamin A, Iron, Folate and Vitamin B12. Report of a Joint Expert Consultation. *WHO Technical Report Series*, Food and Agricultural Organization (FAO), Rome, 724.

[27] Geissler, C.A. and Powers, H.J. (2005) Human Nutrition. 11th Edition. Elsevier, Churchill Livingstone, 236-243.

[28] Oteng-Gang, K. and Mbachu, J.I. (1990) Changes in the Ascorbic Acid Content of Some Tropical Leafy Vegetables during Traditional Cooing and Local Processing. *Journal of Food Chemistry*, **23**, 9-17.

[29] Camacho-Corona, M.D.R., Ramirez-Cabrera, M.A., Gonzalez-Santigo, O., Garza-Gonzalez, E., Palacios, I.D.P. and Luna-Herrera, J. (2008) Activity against Drug Resistant Tuberculosis Strains of Plants Used in Mexican Traditional Medicine to Treat Tuberculosis and Other Respiratory Diseases. *Phytotherapy Research*, **22**, 82-85. http://dx.doi.org/10.1002/ptr.2269

[30] Al-Bayati, F.A. and Sulaiman, K.D. (2008) *In Vitro* Antimicrobial Activity of *Salvadora persica* L. Extracts against Some Isolated Oral Pathogens in Iraq. *Turkish Journal of Biology*, **32**, 57-62.

[31] Asaolu, M.F., Oyeyemi, O.A. and Olanlokun, J.O. (2009) Chemical Compositions, Phyto Chemical Constitutents and *in vitro* Biological Activity of Various Extracts of *Cymbopogon citratus*. *Pakistan Journal of Nutrition*, **8**, 1920-1922. http://dx.doi.org/10.3923/pjn.2009.1920.1922