

Phytochemical Compositions of *Gnetium africanum* (Okazi) Root

*Ihedimbu, C.P., Ayalogu, E.O. and Essien, E.B.

¹ Department of Biochemistry, Faculty of Science, University of Port Harcourt, East-West Road, P.M.B. 5323, Choba, 500004 Port Harcourt, Nigeria.
amakaihedimbu@yahoo.com

Abstract: The phytochemical compositions of *Gnetium africanum* (Okazi) root were evaluated. Proximate analyses of the raw dry root sample revealed carbohydrate content of 37.50±0.02% as the highest followed by ash content of 18.70±0.06%, fibre content of 9.53±0.06%, lipid content of 7.06±0.06%, moisture content of 5.52±0.03% and protein content of 2.80±0.02% as the least. The percentage quantitative phytochemical composition of *G. africanum* root ranged from sparteine 0.1±0.01 as the least, Phytate 0.67±0.01, Epicartechin 1.35±0.02, Rutin 3.13±0.03, Anthocyanin 3.54±0.01, Ribalinidine 3.68 ± 0.06, Phenol 4.84 ± 0.05, Kaempterol 6.26 ± 0.02, Lunamarine 7.66 ± 0.02, Naringerin 18.76 ± 0.03 and Catechin 25.23 ± 0.04 with the highest phytochemical quantitatively. The qualitative phytochemical composition of *G. africanum* root showed that alkaloid, carbohydrate, saponins and cardiac glucoside were moderately present expressed as (++) , flavonoid, tannin, protein and resins were present in low concentration expressed as (+) while terpenoids and steroid were completely absent expressed as (-). The phytochemical components were high enough to contribute to the antioxidant need from the diet.

[Ihedimbu, C.P., Ayalogu, E.O. and Essien, E.B. **Phytochemical Compositions of *Gnetium africanum* (Okazi) Root.** *Biomedicine and Nursing* 2018;4(1): 53-61]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). <http://www.nbmedicine.org>. 9. doi: [10.7537/marsbnj040118.09](https://doi.org/10.7537/marsbnj040118.09).

Key words: *Gnetium africanum*, Phytochemical, Phytate, flavonoid, tannin, antioxidant.

1. Introduction

Gnetium africanum (Gnetaceae) is a traditionally wild perennial vine, known by a number of local and trade names; Afang in Efik, Okazi among the Ibos, Eru in Western Cameroon and Koko in French (Bahuchet, 1990). Leaves of *Gnetum africanum* have high nutritional and medicinal values (Ali, 2011). A highly valued house hold vegetable, collected more from the wild (rather than farmed) across the tropics, nutritionally, *Gnetium africanum* is very rich in proteins and minerals. The leaves are highly nutritious containing eight (8) essential amino acids (Mialoundama, 1993). The plant generates income for many rural women and unemployed youths. *Gnetium spp.* forms ectomycorrhiza (EM) (Onguene and Kuyper, 2001), a symbiotic association between some soil inhabiting fungi and the roots of higher plants where the fungus receives photosynthetically derived carbon compounds from the plant which in turn benefits by increased uptake of mineral nutrients, possibly improved disease and toxin resistance as well as water absorption (Smith and Read, 1997). The aqueous extract from *Gnetum africanum* (fresh and dry) inhibited the growth of diarrhoeagenic bacteria isolated from children's stool (Enyi-Idoh *et al.*, 2013). Medicinally, okazi is efficient in the treatment of a variety of illnesses. In Nigeria, the leaves are used for the treatment of enlarged spleen, for sore throat and as a cathartic (Burkill, 1994). The leaves are used as local delicacy and spice in food and also can cure enlarged spleen, boils, nausea, sore throat, and pain at child

birth, snake poisoning, diabetes mellitus, cataracts, and as worm expeller (Lucas, 1998). According to Iweala *et al.*, (2009), the plant extract has antifungal and antibacterial properties due to the presence of these phytochemicals; tannin, flavonoid, terpene, alkaloid, saponin, phenol. Under wild conditions, both species grow and form underground root-tubers that resemble cassava tubers found 3 to 5 feet that store plant food which can stay alive for many years underground when the vegetation and the *Gnetum* vines above ground are cleared and the soil surface is laid bare and have potential of regenerating after damage as the leaves are being harvested (Shiembo 1994). It has been reported that some local tribes in East Cameroon and the Congo eat these tubers as wild yams, particularly during lean seasons (Bahuchet, 1990). Many chemical compounds are contained in foods which are needed to nourish the body. Some of these food components are useful nutrients such as water, protein, lipid, carbohydrate, minerals and vitamins. Plants are also the largest repository of phytochemical constituents (anti nutrients) which naturally when present in human food, animal feed or water reduces the availability of one or more nutrients (Tan-Wilson *et al.*, 1987), but also at certain conditions take part in relieving many health problems (Heck, 2000). It is important to have knowledge of antinutritional factors because they can adversely affect the health of your poultry flock. Most plant foods consist of natural compounds or anti-nutrients that appear to function generally in defense against

herbivores and pathogens. Anti-nutrients are potentially harmful and have been implicated in the pathogenesis of several ailments, prevent digestion and absorption of nutrients seen as a great health threat to humans and animals. Anti-nutrients may not be toxic themselves, but potentially reduce the nutritional value of a plant by causing a deficiency in essential nutrients or preventing thorough digestion and absorption when consumed (Chavan and Kadam, 1989). Some commonly popular and most studied anti-nutritional factors in roots and tubers include cyanogenic glycosides, saponin, phytate, oxalate, enzyme inhibitors and total alkaloids. Through selective traditional processing/ preparation processes of food like fermentation, cooking and malting, which may reduce certain antinutrients such as phytic acid, polyphenols, and oxalic acid, the nutritive quality of plant foods may be improved (Hotz and Gibson, 2007). Such processing methods are generally employed where cereals and legumes often serve as their major diet (Chavan and Kadam, 1989; Phillips, 1993), such as fermentation of cassava in cassava flour production, which reduces the levels and activities of toxins as well as antinutrients cyanide, present in the tuber (Obboh and Oladunmoye, 2007). They must be inactivated or removed from the plant source before they are considered fit to be used as food (Phillips, 1993; Obboh and Oladunmoye, 2007). In the last few decades or more, large numbers of scientific data have emerged, linking diet and food selection patterns to the maintenance of health and the prevention of some chronic diseases (Oyewole and Atinmo, 2008). Phytosterols are the type of sterols found in plants which be either saturated or unsaturated or both. Unsaturated phytosterols are mostly from plant source, found in abundance in foods like green and yellow vegetables, pumpkins, rice, yam and vegetable oil (Patel & Thompson, 2006). Phytosterols has been shown to decrease hypercholesterolemia development and accumulation of cholesterol in liver hence, inhibiting the development of atherosclerosis in alimentary tract of experimental animals (Lees, *et al* 1977; Peterson *et al.*, 1953; Pollak, 1953a). Plant sterols (phytosterols) exhibit hypocholesterolemic effect by reducing the intestinal absorption of both dietary and endogenous cholesterol and for this, the nutritional status of many processed food products, like margarines, have been upgraded by incorporation phytosterols to effectively manage moderate hypercholesterolemia. Because. Phytosterols may at high doses interference with the absorption of fat-soluble vitamins, higher doses are not recommended. They are good in reducing risk in cardiovascular disease through lowering cholesterol and triglyceride effect (Nguyen, 1999). Some classes include sitosterol, campesterol and stigmasterol, sterol esters

(Weingartner, *et al.*, 2008). Alkaloids, the largest family of nitrogen-containing phytochemicals commonly found in plants, animals, bacteria and fungi, with a large diversity structures and diverse pharmacological potentials; antibacterial, antiviral, anti-inflammatory and antitumor effects (Wink, 2007; Wink, 2010; Lu *et al.*, 2012, Kittakoop *et al.*, 2014). Alkaloids exhibit their biological actions by targeting directly various cellular molecules; cell membranes, proteins, DNA topoisomerase, nucleic acids, neuroreceptors and ion channels, with cytoskeleton as one of the targeted cell (Wink, 2007; Wink and Schimmer, 2010), specifically the microtubules and actin filaments, the two vital components of the cytoskeleton in eukaryotic cells, that mainly carry out cellular processes (the microtubules that see to the spatial distribution of membrane-enclosed organelles and give rise to mitotic spindles during the cell cycle and the actin filaments form the cell shape and in conjunction with microtubules are maintain the cell motility (Alberts, 2014; Fletcher and Mullins 2010). Saponins are glycosylated triterpenoid, steroid, or steroidal alkaloid molecules with an oligosaccharide chain and with soap-like foaming nature in aqueous solution which occurs constitutively in abundant in a variety of plants (Hostettmann and Marston, 1995b; Price *et al.*, 1987; Morrissey and Osbourn, 1999). They are one of the extensively studied bioactive phytochemicals with a wide range of pharmacological activities; antimicrobial, cytotoxic, anti-inflammatory, and immune-stimulatory (Hostettmann and Marston, 1995; Francis *et al.*, 2002) and strong hypoglycemic agents (Harinantenaina *et al.*, 2006; Tan *et al.*, 2006). Flavonoids are a group of plant phenolic phytochemicals with well-known wonderful antioxidant and metallic ions chelating activities. They give plants their attractive colors of the flowers, fruit, and leaves (Brouillard 1988), found in various vegetables, fruits, seeds, nuts, grains, spices, beverages and wine (especially red wine), tea and at lower concentration in beer (Nisakorn and Ampa, 2013). Ecologically, flavonoids play a variety of roles in plants; protective effects due to their capacity to transfer electrons free radicals, chelate metal catalysts (Ferrali *et al.*, 1997), activate antioxidant properties (Elliott *et al.*, 1992), reduce alpha-tocopherol radicals (Hirano *et al.*, 2001), and inhibit oxidases (Cos *et al.*, 1998). Most importantly, flavonoids protect plants from solar UV radiation and scavenge UV formed ROS (Shirley, 1996) by direct UV rays absorption, direct and indirect antioxidant properties, and modulation of many signaling pathways. Phosphate is mostly stored as Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate, or as inorganic pi in plants' seeds yet humans and nonruminant livestock cannot digest it (Guttieriet *et al.*, 2004). In humans, phytate rich

diets can significantly reduce essential micronutrients absorption; Ca (Kies, 1985), Fe (Brune *et al.*, 1992), and Zn (Sandstrom *et al.*, 1987). Phytate chelates these divalent minerals, hence reducing their bioavailability to humans (Jacobsen and Slotfeldt-Ellingsen, 1983).

2. Materials and Methods

2.1 Plant material collection and authentication

The plant samples (leaves and root-tubers) were collected from Obokwe in Ngokpala in Imo State from an unpolluted plantation between September and October, 2014 (rainy season), identified and authenticated at the Plant Science Department of the University of Port Harcourt, Nigeria, with voucher specimen number (UPH/V/1,142) and was preserved at the herbarium. The samples were washed and air dried, was ground into powder using an electric blender (Blender/Miller III, model MS-223, Taiwan, China).

2.2 Extraction procedure of the extracts:

Two portions (900 g) each of the pulverized roots of *G. africanum* was soaked in 5L of ethanol and aqueous solutions respectively then placed on electronic shaker GFL shaker (no. 3017GBh, Germany), for three (3) days according to the method of (Nworgu *et al.*, 2008). The mixture was filtered using No.1 Whatman filter paper.

2.3 Recovery of solvent (soxhlet) (Sukhdev *et al.*, 2008):

The filtrate was poured into a clean round bottom flask at a volume that will not allow the filtrate to siphon into the extraction chamber. The temperature is adjusted in accordance with the boiling point of ethanol and water (78.4 °C and 100 °C) respectively. The solvent evaporated and drip into the extraction chamber where it was collected. The active component left behind in the flask was dried in water bath to completely evaporate the remaining solvent. The percentage yield was calculated and then preserved in sample bottle. The percentage yield was obtained with the calculation as shown below.

$$\% \text{ purity} = \frac{\text{weight of extract}}{\text{weight of raw sample}} \times 100\text{s}$$

2.4 Preliminary Phytochemical Screening

The condensed extracts were used for preliminary screening of phytochemicals such as cholesterol, alkaloid, flavanoids, saponin, cardiac glycosides and terpenoid.

2.5 Test for Flavanoids

A few drops of concentrated HCL and Mg were added to 1 ml of ethanol extract. Appearance of pink or magenta-red colour indicates the presence of flavanoids (Odebiyi and Sofoworang, 1978).

2.6 Test for Cholesterol

To 2 ml of the extract 2 ml of the chloroform was added in a dry test tube. Then 10 drop of acetic anhydride and 2 to 3 drops of con. H₂SO₄ was added. A red colour changed to blue green colour (Deb 2002).

2.7 Test for Cardiac Glycoside

5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of con. H₂SO₄. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might form just gradually throughout thin Layer (Ayoola *et al.*, 2008).

2.8 Test for Alkaloids

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendorff reagent. Any organic precipitate indicated the presence of alkaloids in the sample (Kumar *et al.*, 2009).

2.9 Test for Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H₂SO₄ to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids (Ayoola *et al.*, 2008).

2.10 Test for Steroids

2 ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2ml of H₂SO₄. The colour change from violet to blue or green indicated the presence of steroids (Edeoga, *et al.*, 2005).

2.11 Test for Saponins

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes.

The formation of 1cm layer of foam indicated the presence of saponins (Kumar *et al.*, 2009).

3. Result

Results of the quantitative and qualitative phytochemical composition of *G. africanum* root are presented in Tables 1 and 2. From Table 1, the percentage quantitative phytochemical composition of *G. africanum* root ranges from sparteine 0.1±0.01 as the least, Phytate 0.67±0.01, Epicartechin 1.35±0.02, Rutin 3.13±0.03, Anthrocyanin 3.54±0.01, Ribalinidine 3.68 ± 0.06, Phenol 4.84 ± 0.05, Kaempterol 6.26 ± 0.02, Lunamarine 7.66 ± 0.02, Naringerin 18.76 ± 0.03 and Catechin 25.23 ± 0.04 with the highest phytochemical quantitatively. Also, from the results in Table 2, alkaloid, carbohydrate, saponins and cardia glucoside were moderately present, flavonoid, tannin, protein and resins were present in low concentration while terpenoids and steroids were completely absent.

Table 1: Quantitative Phytochemical Composition of *G. africanum* Root

Phytochemicals	Amount (%)
Sparteine	0.1±0.01
Epicartechin	1.35 ± 0.02
Anthrocyanin	3.54 ± 0.01
Tanninn	13.41 ± 0.11
Phytate	0.67 ± 0.01
Phenol	4.84 ± 0.05
Lunamarine	7.66 ± 0.02
Naringerin	18.76 ± 0.03
Ribalinidine	3.68 ± 0.06
Catechin	25.23 ± 0.04
Rutin	3.13 ± 0.03
Kaempferol	6.26 ± 0.02

Table 2: Qualitative Phytochemical Composition of *G. africanum* Root

Parameters	Result
Alkaloid	++
Flavonoid	+
Tannin	+
Protein	+
Resins	+
Carbohydrate	++
Saponins	++
Cardiac Glucoside	++
Terpenoids	-
Steroid	-

++ = Moderate concentration

+ = Low concentration

- = Absent

4. Discussion

4.1 Qualitative Phytochemical Composition of *G. africanum* Root

Report has shown that plants used for medicinal purposes are very rich in variety of bioactive compounds (Lewis and Ausubel, 2006). Alkaloids play a lot of important metabolic functions in living cells, leading to some physiological alterations. They play some protective roles and exhibit important pharmacological functions; anticancer, psychedelics and antimalarial, analgesic, antispasmodic and bactericidal (Okwu and Okwu, 2004), antioxidant and stimulating activities (Ross and Brain, 1977). Tannins, a class of polyphenols, are well known for their pharmacological potentials exhibiting antibacterial, antioxidants, antimicrobial, anti-inflammatory, antitumor, antiviral, antidiarrheal, antihaemorrhoid, and antimalarial activities (Mori *et al.*, 1987; Vattern *et al.*, 2005). Saponins are also well known to exhibit broad range of pharmacological activities, ranging

from ability to heal wounds to inflamed mucous membranes (Okwu and Okwu, 2004), anti-hyper cholesterol and haemolytic effects (Reddy *et al.*, 2007). The extract contained flavonoids in moderate concentration (+), which are the most common polyphenols found in human diet which is implicated as a strong antioxidant in many human diseases; lipid lowering, hepatoprotective, anti-inflammatory, antioxidant, antimalarial and antimicrobial activities (Okwu and Josiah, 2006; Sodipo *et al.*, 2000; Okwu, 2006; Wegener and Fintelmann, 1996). Anthocyanins are flavonoid found in virtually all vegetables, fruits and other plant parts and are reported to show antioxidant properties as well as anti-inflammatory activity (Kowalczyk *et al.*, 2001; Seeram *et al.*, 2002); play a beneficial role in visual acuity, treatment of cancer, heart disease, age-related neurodegenerative disorders and in angiogenesis (Roy *et al.*, 2009) are commonly found in plants and have diverse physiological functions, including anti-inflammatory, antioxidant and antimalarial activities (Han *et al.*, 2007; Khahkonen *et al.*, 1999; Ovenden *et al.*, 2011). Spartein, lunamarine and ribalinidine are quinoline alkaloids. Quinoline alkaloids are pharmacologically active compounds with biological activities such as antimalarial, anti-inflammatory, and antimicrobial (Kinghorn and Balandrin, 1984; Marella *et al.*, 2013). The quinoline alkaloids also have anti-protozoal, antioxidant and metal chelating activities (Franck *et al.*, 2004; Bachiller *et al.*, 2010a). Lunamarine and ribalinidine have been reported to have free radical scavenging potentials (Rahmani and Sukari, 2010). Lunamarine is also said to possessed anti-amoebic activity (Rahmani and Sukari, 2010). The flavonoid epicatechin is a strong antioxidant, while catechin the major constituent of the extract is hemostatic in nature (Taylor, 2000). Rutin is digested in the body to quercetin, an antioxidant with antimicrobial activity. Kaempferol has also been implicated with antimicrobial activity (Quarengi *et al.*, 2000; Urbano *et al.*, 2000). Phytate has been shown to exhibit anti-inflammatory and cholesterol lowering effects (Urbano *et al.*, 2000). It also acts as an antioxidant and metal chelator (Ooma *et al.*, 2008; Frontela *et al.*, 2009; Gibson *et al.*, 2010).

4.2 Quantitative Phytochemical Composition of *G. africanum* Root

Recently, the dependence on medicinal plants as herbal remedy as well as food sources has increased as the cost of raw material for orthodox medicine preparations and the quest for self-medication in large populations increase all over the world. The result of the preliminary phytochemical screening from the root tuber of *Gnetum africanum* reveals the presences of different phytochemicals at different concentrations. Quantitative assessments of the different

phytochemicals detected during investigation were graded as -ve for absent, +ve for low concentration and ++ve for moderate concentration. Alkaloid, carbohydrate, saponins and cardiac glucoside were present in moderate concentration, but flavonoid, tannin, protein and resins were also found but in low concentration (+ve) while terpenoids and steroid were completely absent (-ve). Root tubers like yams have been well respected by the herbalist community for generations due to their medicinal potency like fertility enhancement in males due to the presence of steroidal drug e.g diosgenin extracted from yam tubers normally used as precursors for the synthesis of male fertility hormones and corticosteroids (Odebiyi, and Sofowora, 1978., Oliver-Bever 1989). The presence of alkaloid in moderate concentration showed the root sample up as a good antibiotic. Among many multitarget properties shown by alkaloids as a competent antibiotic, usually with several molecular targets interactions (Wink, 2007), whose biological potency lies in their interactions with varieties of molecular targets; biomembranes, proteins, DNA topoisomerase, nucleic acids, neuroreceptors and ion channels, and cytoskeleton (Wink, 2007; Wink and Schimmer, 2010) and such may exhibit a wide range of potential in the future development of anticancer therapies (Xiaojuan *et al.*, 2016). Saponins which have the natural ability to defend plants against microbial attack qualify the sample as anti-fungal and yeast infections agents. Saponines exhibit natural antibacterial property, defending the body against microbial attacks (Sodipo *et al.*, 2000). Flavonoids biological functions range from antioxidant properties, protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Barakat *et al.*, 1993). The presence of flavonoid in this roots (+) suggests its antioxidant, antimicrobial etc potentials. Cardiac glycosides content was found to be moderately present. Cardiac glycosides have been shown to stimulate the heart in case of cardiac failure (Olayinki *et al.*, 1992., Trease and Evans, 1998). Antioxidants are very important nutraceuticals because of their many health benefits. Normal physico-chemical of the body processes generates reactive oxygen species (ROS). Excessive ROS production overcomes cellular antioxidant defenses leading to oxidative stress condition. This in turn results to the progression of millions of degenerative diseases; aging related diseases, cancer, cardiovascular diseases, diabetes mellitus, and various neurodegenerative diseases, through DNA mutation, protein oxidation, and/or lipid peroxidation. Hence, the role of antioxidants in either preventing or delaying the oxidative damage caused by ROS in various ways cannot be over emphasized and most medicinal plants are reservoirs of many

potent antioxidant found relevant in treating such chronic diseases (Amessis-Ouchemoukha *et al.*, 2014; Chiang *et al.*, 2015). Medicinal plants are of great interest now to researchers because of these antioxidants and active phytochemical content, like phenol compounds, alkaloids, terpenoids, and vitamins, for their implications in diseases prevention as well as use as nutraceuticals and/or food additives (Craig, (1999). Dietary polyphenols exert various biological functions; free-radical scavenging, metal chelation, enzymatic activity modulation, alteration of signal transduction pathways and this made them beneficial for human health (Sato *et al.*, 2011). The potent antioxidant activity of the root may be linked to presence of phenolics, tannins, and flavonoids etc which were also found in considerable high amounts in most plant extracts (Souza *et al.*, 2008).

5. Conclusion

The phytochemical profiles reveal many potent antioxidant and antimicrobial activity of okazi root owing to the phytocomponents and their quantity present.

References

1. Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K. and Walter, P. (2014). *Molecular Biology of the Cell*, 6th ed.; Garland Science: New York, NY, USA.
2. Ali, F., Assanta, M.A., and Robert, C. (2011). *Gnetum Africanum*: A wild food plant from the African forest with many nutritional and medicinal purposes. *Journal of Medicinal Food* 14:11 1289-1297.
3. Amessis-Ouchemoukha, N., Abu-Reidahb, I.M., Quirantes Pine, R., Madani, K. and Segura-Carretero, A. (2014). Phytochemical profiling, in vitro evaluation of total phenolic contents and antioxidant properties of Marrubium vulgare (horehound) leaves of plant growing in Algeria. *Indian Crop Production*; 61:120e9. *Annual Clinical Biochemistry* 6: 245-246.
4. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello A.A., Obaweya, K. and Ezennia, E.C. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*. 7(3):1019-1014.
5. Bachiller, M.I.F., Perez, C., Munoz, G.C.G., Conde, S., Lopez, M.G. and Villarroja, M. (2010a). Novel tacrine-8hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and

- copper complexing properties. *Journal of Medicinal Chemistry*. 53:4927-4937.
6. Bahuchet, S. (1990). The Akwa pygmies: Hunting and Gathering in the Lobaye Forest. In Food and Nutrition in the African Rain Forest. Food Anthropology Unit 263, UNESCO.
 7. Barakat, M.Z., Shahab, S.K., Darwin, N. and Zahemy, E.I. (1993). Determination of ascorbic acid from plants. *Annal of Biochemistry*; 53:225-245.
 8. Brouillard, R. and Cheminant, A. (1998). Flavonoids and Plant Color. In: Cody V, Middleton E and Harborne JB eds. *Plant Flavonoids in Biology and Medicine: Biochemical, Cellular and Medicinal Properties*. New York: Alan R. Liss, Inc. 93–106.
 9. Brune, M., Rossander-Hulten, L., Hallberg, A., Gleerup, A. and Sandberg.A.S. (1992). Iron absorption from bread in humans: Inhibiting effects of cereal fiber phytate and inositol phosphates with different numbers of phosphate groups. *Journal of Nutrition*. 122:442– 449.
 10. Burkill, H.M. (1994). *The Useful Plants of West Tropical Africa*. Volume 2: Families E-I. Kew.
 11. Chavan, J.K. and Kadam, S.S. (1989). "Nutritional improvement of cereals by fermentation". *Critical Review of Food Science and Nutrition*. 28 (5): 394–400.
 12. Chiang, H.M., Chen, H.C., Wu, C.S., Wu, P.Y. and Wen, K.C. (2015). Rhodiola plants: chemistry and biological activity. *Journal of Food and Drug Analysis*;23:359e69.
 13. Cos, P., Ying, L., Calomme, M., Hu, J.P., Cimanga, K., Van, P.B., Pieters, L., Vlietnck, A.J. and Vanden, B.D. (1998). Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Products*. 61:71–76.
 14. Craig, W.J. (1999). Health-promoting properties of common herbs. *American Journal of Clinical Nutrition*;70:491se9s.
 15. Deb, A.C. (2002). *Fundamental of Biochemistry*. Edit 8, New Central Book Agency, Kolkata.
 16. Edeoga, H.A., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituent of some Nigerian medicinal plants. *African Journal of Biotechnology*; 4(7):685-688.
 17. Elliott, A.J., Scheiber, S.A., Thomas, C. and Pardini, R.S. (1992). Inhibition of glutathione reductase by flavonoids. *Biochemical Pharmacology*. 44:1603–1608.
 18. Enyi-Idoh, K. H., Ikpeme, E. M. and Iwuh, G. C. (2013). Antibacterial activity of *Gnetum africanum* and *Heinsiacrinite* on diarrhoeagenic bacteria stool isolates from children in Calabar South Iga, Cross River State, Nigeria. *Transnational Journal of Science and Technology*. 3(3): 1-9.
 19. Ferrali, M., Signorini, C., Caciotti, B., Sugherini, L., Ciccoli, L., Giachetti, D. and Comporti, M. (1997). Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to iron chelating activity. *Febs Letter*. 416:123–129.
 20. Fletcher, D.A. and Mullins, D. (2010). Cell mechanics and the cytoskeleton. *Nature*:463, 485– 492.
 21. Francis, G., Kerem, Z., Makker, H.P.S. and Becker, K. (2002). The biological action of saponins in animal systems: a review. *British Journal of Nutrition*.88:587–605.
 22. Franck, X., Fournet, A., Prina, E., Mahieux, R., Hocquemiller, R. and Figaderre, B. (2004). Biological evaluation of substituted quinolines. *Bioorganic and Medical Chemistry Letter*.14:3635-3638.
 23. Frontela C, Scarino, ML, Feruzza S, Ros G, Martinez C. (2009). Effect of dephytinization on bioavailability of iron, calcium and zinc from infant cereals assessed in the CaCo-2 cell model. *World Journal of Gastroenterology*. 28-15(16):1977-1984.
 24. Gibson RS, Bailey KB, Gibbs M, Ferguson EL. (2010). A review of phytate, iron, zinc and calcium concentrations in plantbased complementary foods used in low-income countries and implications for bioavailability. *Food Nutrition Bulletin*. 31(suppl 2):S134-146.
 25. Han, X., Shen, T. and Lou, H. (2007). Dietary polyphenols and their Biological significance. *International Journal of Molecular Sciences*. 8(9):950-988.
 26. Harinantenaina, L., Tanaka, M., Takaoka, S., Oda, M., Mogami, O., Uchida, M. and Asakawa, Y. (2006). *Momordica charantia* constituents and antidiabetic screening of the isolated major compounds. *Chemistry and Pharmacology Bulletin*.54:1017–1021.
 27. Heck, A.M., Amy, Yanovski, J.A. and Calis, K.A. (2000). "Orlistat, a new lipase inhibitor for the management of obesity". *Pharmacotherapy*. 20 (3): 270–9.
 28. Hostettmann, K. and Marston, A. (1995). *Chemistry and Pharmacology of Natural Products: Saponins*. Cambridge University Press; Cambridge: pp. 233–283.
 29. Hotz, C. and Gibson, R.S. (2007). Traditional food-processing and preparation practices to enhance bioavailability of micronutrients in plant- based diets. *Journal of Nutrition*. 137 (4): 1097–100.

30. Iwuala-Emeka, E.J., Uhegbu F.O. and Obidoa, O. (2009). Biochemical and Histological Changes Associated with Long Term Consumption of *Gnetum africanum* Welw. Leaves in Rats. *Science Alert*, 10 (3923): 125-132.
31. Jacobsen, T. and Slotfeldt-Ellingsen, K.D. (1983). Phytic acid and metal availability: A study of Ca and Cu binding foodstuffs. *Cereal Chemistry*. 60:392-395.
32. Khahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K. and Kujala, T.S. (1999). Antioxidant activity of plants extracts containing phenolic compounds. *Journal of the Agricultural and Food Chemistry*. 47:3954-3962.
33. Kies, C. (1985). Effect of dietary fat and fiber on calcium bioavailability. absorption in humans from meals based on rye, barley, oatmeal, ACS Symp. Series 12:175-187.
34. Kinghorn, A.D. and Balandrin, M.F. (1984). Alkaloids: Chemical and biological perspectives, WS. Pelletier (ed.), Wiley, New York.
35. Kittakoop, P., Mahidol, C. and Ruchirawat, S. (2014). Alkaloids as important therapeutic drug for treatment of cancer, tuberculosis and smoking. *Current Tropical Medicine Chemistry*. 14 (2):239- 259.
36. Kowalczyk, E., Niedworok, J., Andrykowski, G. and Jankowski, A. (2001). Effect of anthocyanins from *Aronia melanocarpa* Elliot. on oxidative stress biomarkers in animals chronically exposed to lead acetate or cadmium chloride. *Circulation of Elements in Nature*. 262-267.
37. Kumar, A., Ilavarasan, R., Jayachandran, T., Decaraman, M., Aravindhan, P. and Padmanaban, N. (2009). Phytochemical investigation on tropical plant. *Pakistan Journal of Nutrition*. 8(1):83-85.
38. Lees, A.M., Mok, H.Y., Lees, R.S., McCluskey, M.A. and Grundy, S.M. (1977). Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. *Atherosclerosis* 28:325-338.
39. Lewis, K. and Ausubel, F, M. (2006). Prospects for plant-derived antibacterials. *Nature Biotechnology*. 24(12):1504-1507.
40. Lu, J.J., Bao, J.L., Chen, X.P., Huang, M. and Wang, Y.T. (2012). Alkaloids isolated from natural herbs as the anticancer agents. *Evidence Based Complementary Alternative Medicine*. 28:225-229.
41. Lucas, E.O. (1998). The potential of leaf vegetable in Nigeria. *Outlook of Agriculture*. 17(4):163- 168.
42. Marella, A., Tanwar, O.P., Saha, R. Ali., M.R. Srivastava, S. and Akhter, M. (2013). Quinoline: A versatile heterocyclic. *Saudi Pharmaceutical Journal*. 21:1-12.
43. Mialoundama, F. (1993). "Nutritional and Socio-Economic Value of Gnetum Leaves in Central African Forest". In Hladik, C.M. Tropical forests, people and food: biocultural interactions and applications to development. Man and the biosphere. Paris: UNESCO: Carnforth, UK; Pearl River, N.Y.: Parthenon Pub. Group. pp. 177-181
44. Mori, A., Nishino, C., Enoki, N. and Tawata, S. (1987). Antibacterial activity and mode of action of plant Flavonoids against *Proteus vulgaris* and *Staphylococcus aureus*. *Phytochemistry*. 26(8):2231-2234.
45. Nguyen, T.T. (1999). The cholesterol-lowering action of plant stanol esters. *Journal of Nutrition*. 129, 2109- 2112.
46. Nisakorn, S. and Ampa, J. (2013). Photoprotection of natural flavonoids. *Journal of Applied Pharmaceutical Science* 3 (09), pp. 129-141.
47. Nworgu, Z.A., Onwukaeme, D.N., Afolayan, A.J., Ameachina, F.C. and Ayinde, B.A. (2008). "Preliminary studies of blood pressure lowering effect of *Nauclea latifolia* in rats". *Journal of Pharmaceutical Pharmacology*, 2(2): 037-041.
48. Oboh, G. and Oladunmoye, M.K. (2007). "Biochemical changes in micro-fungi fermented cassava flour produced from low- and medium-cyanide variety of cassava tubers". *Nutrition and Health*. 18 (4): 355-67.
49. Odebiyi, O.O. and Sofowora, E.A. (1978). Phytochemical screening of Nigerian medicinal plant II. *Lloydia* 41:234-246
50. Okwu, D.E. (2004). Phytochemicals and vitamins content of indigenous species of south eastern Nigeria. *Journal of Sustainable Agriculture and the Environment*. 6(1):30-37.
51. Okwu, D.E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medical plants. *African Journal Biotechnology*. 5(4):357-361.
52. Okwu, D.E. and Okwu, M.E. (2004). Chemical composition of *Spondias mombin* Linn. Plant parts. *Journal of Sustainable Agriculture and the Environment*. 6(2):140-147.
53. Olayinki, A.O., Onuruvwe, O., and Lot, T.Y. (1992). Cardiovascular effects of the methanolic extract of the stem bark of *Khaya sengaensis*. *Phytotherapy Research* 6(5):282-284.
54. Oliver-Bever, B. (1989). Medicinal plants in Tropical West Africa. Cambridge Uni Cambridge.
55. Onguene, N.A. and Kuyper, T.W. (2001). Mycorrhiza associations in the rain forest of

- South Cameroon. *Forest Ecology and Management*. 140: 277-287.
56. Oomah BD, Blanchard C, Balasubramanian P. (2008). Phytic acid, Phytase, Minerals, and Antioxidant Activity in Canadian Dry Bean (*Phaseolus vulgaris* L.) cultivars. *Journal of Agricultural and Food Chemistry*. 56(23):11312-11319.
 57. Osbourn, A. E. (1996). Saponins and plant defence a soap story. *Trends in Plant Science*. 1:4-9.
 58. Ovenden, S.P., Cobbe, M., Kissell, R., Birrell, G.W., Chavchich, M. and Edstein, M.D. (2011). Phenolic glycosides with antimalarial activity from *Grevillea poorinda* Queen. *Journal of Natural Products*. 28(1):74-78.
 59. Oyewole, O.E. and Atinmo, T. (2008). Nutrition education in medical training: the need to reconsider the sacrosanctity of medical education in Nigeria. *African Journal of Medicine and Medical Sciences*. 37: 219-224.
 60. Patel, M.D. and Thompson, P.D. (2006). Phytosterols and vascular disease. *Atherosclerosis*, 186, 12–19.
 61. Peterson, D.W., Shneur, E.A. and Peek, N.F. (1953) Dietary constituents affecting plasma and liver cholesterol in cholesterol-fed chicks. *Journal of Nutrition*. 50, 191–201S.
 62. Phillips, R.D. (1993). "Starchy legumes in human nutrition, health and culture". *Plant Foods and Human Nutrition*. 44 (3): 195–211.
 63. Pollak O.J., (1953a). Successful prevention of experimental hypercholesterolemia and cholesterol atherosclerosis in the rabbit. *Circulation*, 7, 696–701.
 64. Qiu, S., Sun, H., Zhang, A.-H., Xu, H.-Y., Yan, G.-L., Han, Y. and Wang, X.-J. (2014). Natural alkaloids: Basic aspects, biological roles, and future perspectives. *Chinese Journal of Natural Medicine* 12: 401–406.
 65. Quarenghi MV, Tereschuk ML, Baigori MD, Abdala LR. (2000). Antimicrobial activity of flowers from *Anthemis cotula*. *Fitoterapia*.
 66. Rahmani, MB, Sukari MAB. (2010). New Lignum and other Chemical Components from *haplophyllum villosum* and *H. leaviusculum* and their Antioxidant activity. Proceedings of the 16th Malaysian Chemical Congress, Malaysia.
 67. Reddy, M.K., Gupta, S.K., Jacob, M.R., Khan, S.I. and Ferreira, D. (2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. *Planta Medica*. 73:46146.
 68. Ross, M.S.T. and Brain, K.R. (1977). An Introduction to Phytopharmacy. Pitman Medical Publishing Coy. Tunbridge Wells, Kent, 7-49.
 69. Roy, H.J., Lundy, S., Eriksen, C. and Kalicki, B. (2009). Anthocyanins. *Pennington Nutrition series*. 6:34-39.
 70. .
 71. Sandstrom, B., Almgren, A., Kivisto, B. and Cederblad, A. (1987). Zinc absorption in humans from meals based on rye, barley, oatmeal, triticale and whole wheat. *Journal of Nutrition*. 117:1899–1902.
 72. Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., Sugawara, M. and Iseki, K. (2011). In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *International Journal of Pharmacology*; 403:136e8.
 73. Scheiner, G. (2012). Latest and Greatest in Diabetics self-care. *Spry Publishing*. 978(1): 13-3.
 74. Seeram, N., Schutzki, R., Chandra, R. and Nair, M.G. (2002). Characterization, Quantification, and Bioactivities of Anthocyanins in *Cornus* species. *Journal of Agricultural and Food Chemistry*. 50:2519-2523.
 75. Shiemo, P.N., (1994). The sustainability of Eru (*Gnetum africanum* and *G. buchholzianum*): Over-exploited Non-wood Forest Product from the Forest of Central Africa: In T. C. H. Sunderland & L.E. Clark (eds.). *The non-wood Forest Products of Central Africa: Current Research Issues and Prospects for Conservation and Development*. Food and Agricultural Organisation. Rome.
 76. Smith, S.E. and Read, D.J. (1997). *Mycorrhiza Symbiosis* (2nd edn). Academic Press: London.
 77. Sodipo, O.A., Akiniyi, J.A. and Ogunbamosu, J.U. (2000). Studies on certain characteristics of extracts of bark of *Pausinystalia macroceras* (K. Schemp) Pierre Exbeille. *Global Journal of Pure and Applied Science*. 6:83-87.
 78. Sofowonag, K. (1993). Using medicinal plants to enhance the efficiency of skin care. *Journal of Australian Traditional Medicine and Sociology*. 1:17-19.
 79. Sukhdev, S.H., Suman, P.S., Gennaro, L and Dev, D.R. (2008). Extraction techniques for medicinal and aromatic plants. *International Center for Science and High Technology*. 1: 2-4.
 80. Talalay, P., and Talalay, P. (2001). The importance of using scientific principles in the development of medicinal agents from plants, *Academic Medicine*, 76: 238.
 81. Tan, M.J., Ye, J.M., Turner, N., Hohnen, B. C., Ke, C.Q., Tang, C.P., Chen, T., Weiss, H.C.,

- Gesing, E.R., Rowland, A. and James D.E. (2008). Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. *Chemistry and Biology*. 15:263–273.
82. Tan-Wilson, A.L., Chen, J.C., Duggan M.C., Chapman, C., Obach, R.S. and Wilson, K.A. (1987). "Soybean Bowman-Birk trypsin isoinhibitors: classification and report of a glycine-rich trypsin inhibitor class". *Journal of Agriculture and Food Chemistry*. 35 (6): 974.
83. Taylor, L. (2003). Technical Data report for Chance Piedra "Stone Breaker" (*Phyllanthus amarus*) in Herbal Secrets of Rain Forest. 2nd Edition, Sage Press Inc. Austin Texas.
84. Trease, G. E. and Evans, W.C. (1998). Pharmacology. Edn 11, Brailliere Tindall Ltd., London.
85. Urbano G, Lopez-Jurado M, Aranda P, Vidal-Valverde C, Tenorio E, Porres J. (2000). The role of phytic acid in legumes: antinutrient or beneficial function? *Journal of Physiology and Biochemistry*. 56(3):283-294.
86. Vattern, D.A., Ghaedian, R. and Shetty, K. (2005). Benefits of berries through phenolics antioxidant enrichment focus on cranberry. *Asia Pacific Journal of Clinical Nutrition*. 14(2):120-130.
87. Wegener, T. and Fintelmann, V. (1999). Flavonoids and Bioactivity. ~ 286 ~ *Weininger Medizinische Wochenschrift*.; 149:241-247.
88. Wink, M. (2007). Molecular modes of action of cytotoxic alkaloids: From DNA intercalation, spindle poisoning, topoisomerase inhibition to apoptosis and multiple drug resistance. *Alkaloids Chemistry and Biology*. 64 – 71.
89. Wink, M. and Schimmer, O. (2010). Molecular Modes of Action of Defensive Secondary Metabolites. In Annual Plant Reviews: Functions and Biotechnology of Plant Secondary Metabolites, 2nd ed.; Wiley-Blackwell: Oxford, UK, 39, pp. 21–161.
90. Xiaojuan, W., Mine, T., Sonja, K., Herbenya, S. P. and Michael, W. (2016). The Interference of Selected Cytotoxic Alkaloids with the Cytoskeleton: An Insight into Their Modes of Action. *Molecule* 906-918.

3/24/2018