Phytochemical Compositions of *Gnetum africanum* (Okazi) Root

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

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 *Gnetum 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, 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. 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.

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1. Introduction

Gnetum 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 *Gnetumafricanum* 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, *Gnetum 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. *Gnetum 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 *Gnetumafricanum* (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).

Fig. 1: Leaves of *Gnetum africanum*

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 (Oboh 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; Oboh 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 and 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 inplants, animals, bacteria and fungi, with a large diversity structures and diverse pharmacological potentials; antibacterial, antiviral, anti-inflammatory and antitumoreffects (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 eukaryoticcells, 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, orsteroidal 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-hexa*kis*phosphate, or as inorganic pi in plants' seeds yet humans and nonruminant livestocks 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 chelatesthese 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 were 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 ^{o}C and 100 ^{O}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.

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

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

5mlof 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 H_2SO_4. 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. Results And Discussion

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
Kaempterol	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

4.1 Qualititative 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, antivirus, 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, antiinflammatory, 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, antiinflammatory, 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 toquercetin, an antioxidant with antimicrobial activity. Kaempferol has also been implicated with anti-microbialactivity (Quarenghi *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). Flavanoids biological functionsrange 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.

Corresponding author:
Ihedimbu, C.P.
Department of Biochemistry, Faculty of Science, University of Port Harcourt, East-West Road, P.M.B. 5323, Choba, 500004 Port Harcourt, Nigeria.
Telephone: +234835492927
E-mail: amakaihedimbu@yahoo.com

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