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Chemical Analysis of *Gnetum africanum* roots

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Abstract

The use of medicinal plants as complementary therapies has lately grown in popularity. The aim of this study was to determine the chemical composition of the aqueous root extract of *Gnetum africanum* using GC-MS and GC-FID analysis. Fresh roots of *G. africanum* Welw used in this study were collected, cut into pieces, washed and air-dried. The dried plant materials were ground into powder and kept in an airtight container. GC-MS analysis of the aqueous root extract revealed 20 bioactive compounds with the following being most abundant 2,3-Butanediol as the predominant phytochemical at 46.02%, followed by Benzene, 1,4-dichloro (3.57%) and Oxalic acid, allyl octadecyl ester (3.45%). The quantitative GC-FID determination of the phytochemicals of *Gnetum africanum* aqueous root extract presented common phytochemicals such as, alkaloids, flavonoids, steroids, phenols and glycosides. Phytochemicals in higher proportions include; sapogenin (26.2645 µg/ml), anthocyanins (17.4986 µg/ml), cyanogenic glycosides (15.3616 µg/ml), kaempferol (13.7621 µg/ml) and flavan-3-ol (13.0446 µg/ml). It has been discovered that the root of *Gnetum africanum* contains an array of phytocompounds with a variety of therapeutic properties.

Keywords: GC-MS; GC-FID; *Gnetum africanum*; Phytochemicals

1. Introduction

Gnetum africanum, a widely cherished leafy green across Nigeria, Cameroun, Gabon, the Democratic Republic of Congo, and Angola, holds significant nutritional and therapeutic value [1]. Known as "eru" or "kok" in Cameroun, "koko" in Central Africa, "ntoumou" in Gabon, and "ukazi" or "afang" in Nigeria, this plant from the Gnataceae family has drawn attention for its diverse applications [1][2].

Exploration of *G. africanum* has revealed a rich phytochemical composition in its leaves and seeds, including alkaloids, saponins, glucosides, and tannins, with documented anti-inflammatory, anti-carcinogenic, and antioxidant properties [3][4][5][6]. Traditionally, the leaves have been employed for medicinal purposes, treating conditions such as enlarged spleen, piles, high blood pressure, and sore throats. They have also served as antidotes to poison and snake bites, as enemas for constipation, and in the management of diabetes and fungal infections [1][5].

Gnetum grows all year in the wild via rhizomes or new shoots, and it creates subterranean roots or tubers in which it stores its food reserves. Its edible tuber could boost food sustainability and security in areas of the world plagued by famine while simultaneously serving medicinal purposes. Even after the foliage and *Gnetum* vines above have been destroyed and the soil surface has been exposed, these tubers can live for many years. According to the authors, several native communities in eastern Cameroon and the Democratic Republic of the Congo use these tubers as wild yams, particularly during the dry season [6][7].

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While the leaves are commonly consumed raw in local salads and southeastern Nigerian soups, the roots of *Gnetum africanum* have been less explored in terms of their chemical composition. Recent studies on ethanol and aqueous root extracts indicate antioxidant potentials, suggesting effectiveness against oxidative damage and potential benefits for cholesterol metabolism [1]. This underlines the importance of further chemical analysis of G. africanum roots, holding promise for the discovery of novel compounds with potential medicinal applications.

2. Materials and Methods

2.1. Collection and Preparation of Root Samples

The fresh root of *G. africanum* Welw used in this study was obtained from cultivated farmland in Emii, Owerri North Local Government Area of Imo State of Nigeria on 22nd February 2022. It was authenticated by Mr. Francis Iwueze, a taxonomist in the Department of Forestry and Wildlife Technology at the Federal University of Technology, Owerri (FUTO). The root parts were cut into pieces, washed, and air-dried at ambient temperature of 28 ± 2 ℃ for ten days. The dried plant materials were ground into powder and kept in an airtight container.

2.2. Extraction

Equal portions of the pulverized roots of *G. africanum* Welw were immersed in aqueous solution, in the ratio of 1:4 (1.0 g of plant sample to 4.0 mL of the solvents). The mixtures were placed on an electronic shaker to agitate for three (3) days and then filtered with No 1 Whatman filter paper. The filtrates were subjected to soxhlet extraction to remove the solvents used for the extraction of the active components. The crude extracts were transferred into a container and then placed in a water bath set at 50 °C for complete evaporation of the solvents. The resulting grey sticky viscous extracts (21% yield) were placed in an air-tight container, wrapped in aluminum foil, and stored at 4 °C for further use.

2.3. Determination of Phenolic Phytochemicals using Gas Chromatography (GC-FID)

2.3.1. Preparation of Sample

One gram (1g) of ground sample was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50%m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60 ℃ for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the separating funnel. This extract was combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis.

2.3.2. Quantification with GC-FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280 °C with split-less injection of 2ul of sample and a linear velocity of 30cms^{-1}, Helium 5. 0pa.s was the carrier gas with a flow rate of 40ml/min. The oven operated initially at 200 °C, it was heated to 330 °C at a rate of 3 °Cmin^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320 °C.

Phenolic compounds were determined by the ratio between the area and mass of internal standard and the area of the identified compounds. The concentration of the different bioactive ingredients is expressed in ug/ml and percentage.

2.4. Determination of Volatile Phytochemicals using Gas Chromatography-Mass Spectrophotometry (GC-MS)

One microliter (1ul) of the concentrated sample was injected into the GC column for analysis. The GC (Agilent 6890N) and MS (5975B MSD) is equipped with DB-5ms capillary column (30 m×0.25 mm; film thickness 0.25 µm). The initial temperature was set at 40 °C which increased to 150 °C at the rate of 10 °C/min. The temperature was again increased to 230 °C at the rate of 5 °C/min. The process continued till the temperature reached 280 °C at the rate of 20 °C/min which was held for 8 minutes. The injector port temperature remained constant at 280 °C and detector temperature was 250 °C. Helium was used as the carrier gas with a flow rate of 1 mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively.

2.5. Identification of chemical constituents

To identify and quantify the target active compounds present in the extracted sample, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014[8]; followed by obtaining the percent report from the equipment. The percent report shows the exact amount at which the targeted compounds were present.

3. Results

The results of the phytochemical analysis are presented in Table 1, where twenty compounds were identified within the *Gnetum africanum* root extract. Notably, Table 1 highlights 2,3-Butanediol as the predominant phytochemical at 46.02%, followed by Benzene, 1,4-dichloro (3.57%) and Oxalic acid, allyl octadecyl ester (3.45%).

Table 2 displays the amounts of phenolic compounds derived from Gas Chromatography with Flame Ionization Detector (GC-FID) study. Interestingly, sapogenin (26.26 µg/ml), anthocyanin (17.49 µg/ml), and cyanogenic glycoside (15.36 µg/ml) were the three most prevalent phenolic acids in the *Gnetum africanum* aqueous root extract.

Table 1 Phytocompounds Present in Aqueous root extract of *Gnetum africanum* by GC-MS Analysis

PK	R.T.	Bioactive compound	M.F	% composition
1	2.602	2,3-Butanediol	$C_4H_{10}O_2$	46.02%
2	4.219	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone	$C_8H_9N_5O_2$	2.58%
3	4.751	Bicyclo[10.8.0]eicosane, cis-	$C_{20}H_{38}$	2.41%
4	5.054	Pentafluoropropionic acid, undecyl ester	$C_{14}H_{23}F_5O_2$	2.54%
5	5.362	2-Heptenal, (Z)-	$C_7H_{12}O$	2.83%
6	5.494	Fumaric acid, (p-nitroanilino)-, diethyl ester	$C_{14}H_{16}N_2O_6$	2.50%
7	6.391	2-Nitrobenzyl alcohol, trifluoroacetate	$C_9H_6F_3NO_4$	3.32%
8	7.346	Diallyl disulphide	$C_6H_{10}S_2$	2.46%
9	8.014	1H-4-Azacycloprop[cd]indene, octahydro-4-methyl-	$C_9H_{15}N$	3.00%
10	8.351	L-Methionine, N-(1-methylethyl)-, 1-methylethyl ester	$C_{11}H_{23}NO_2S$	2.40%
11	9.58	3-Nitrophthalhydrazide	$C_8H_5N_3O_4$	2.71%
12	10.512	9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]-	$C_{13}H_{26}BN$	2.43%
13	10.889	(p-Methoxyphenyl)-acetonyl-dimethylsilane	$C_{12}H_{18}O_2Si$	2.42%
14	12.381	4-Chloro-8-fluoroquinoline	C_9H_5ClFN	2.80%
15	12.672	Oxalic acid, allyl octadecyl ester	$C_{23}H_{42}O_4$	3.45%
16	13.764	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	3.57%
17	14.855	Benzamide, N-[(1,5-dimethyl-1H-pyrrol-2-yl)methyl]-4-methoxy-	$C_{15}H_{18}N_2O_2$	3.29%
18	15.41	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	2.65%
19	15.747	4-Hydroxyphenyl pyrrolidinyl thione	$C_{11}H_{13}NOS$	3.39%
20	15.89	5,6-Dicarbadecaborane(12), 5,6-dimethyl-	$C_4H_{16}B_8$	3.24%

Table 2 Phenolic profile of aqueous root extract of *Gnetum africanum*

Component	Concentration (ug/ml)	Concentration (%)
Kaempferol	13.7621	9.6953
Sapogenin	26.2645	18.5032
Flavanones	6.8832	4.8491
Anthocyanin	17.4986	12.3277
Flavan-3-ol	13.0446	9.1898
Epihedrine	8.6474	6.0921
Naringenin	7.7926	5.4898
Catechin	6.3953	4.5054
Phytate	10.7300	7.5592
Cyanogenic glycoside	15.3616	10.8222
Steroid	6.3954	4.5055
Phenol	9.1697	6.4600

4. Discussion

Plants are increasingly being used as traditional medicinal cures and as sources of conventional medications around the world. Medicinal plants are used to support and improve health, an attribute that is closely linked to the wide spectrum of phytochemicals they synthesize and accumulate. The quantitative GC-FID determination of the phytochemicals of *Gnetum africanum* aqueous root extract presented common phytochemicals such as, alkaloids, flavonoids, steroids, phenols and glycosides. These compounds are all known to be the main constituents responsible for the antioxidant activity of plants [9]. Phytochemicals in higher proportions include; sapogenin (26.2645 µg/ml), anthocyanins (17.4986 µg/ml), cyanogenic glycosides (15.3616 µg/ml), kaempferol (13.7621 µg/ml) and flavan-3-ol (13.0446 µg/ml).

The roots of *Gnetum africanum* presented significant amount of flavonoids which are secondary metabolites, consisting a benzopyrone ring bearing a phenolic or polyphenolic group at different position. Flavonoids are applied extensively as anticancer, antimicrobial, antiviral, antioxidant and anti-proliferating agent [10]. The root extract of *Gnetum africanum* recorded appreciable number of alkaloids which play vital pharmacological activities, acting as human therapeutic arsenal, such as antioxidant compounds, antitumoral drugs, analgesics, anti-inflammatories and stimulants [11]. Steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [12]. They are often used in medicines due to their well-known biological activities. Furthermore, some antinutrient compounds such as phytate (10.7300 µg/ml) and Cyanogenic glycosides (15.3616 µg/ml). These compounds interfere with intake, absorption and utilization of nutrients. Antinutrients may further elicit very harmful biological responses while some are used as pharmacologically active agents.

The GC-MS analysis of the volatile phytochemical constituents within the aqueous root extract of *Gnetum africanum* revealed a diverse array of compounds, as detailed in Table 1. Notably, the aqueous root extract exhibited a rich content of bioactive compounds, with 2,3-Butanediol being the most prominent (46.02%). Additionally, other compounds were present in lower quantities, including Benzene, 1,4-dichloro- (3.57%), Oxalic acid, allyl octadecyl ester (3.45%), 4-Hydroxyphenyl pyrrolidinyl thione (3.39%), 2-Nitrobenzyl alcohol, trifluoroacetate (3.32%), Benzamide, N-[(1,5-dimethyl-1H-pyrrol-2-yl) methyl]-4-methoxy- (3.29%), 5,6-Dicarbadecaborane(12), 5,6-dimethyl- (3.24%), and 1H-4-Azacycloprop[cd]indene, octahydro-4-methyl- (3.00%). Further constituents were detected in trace amounts, such as 2-Heptenal, (Z)- (2.83%), 4-Chloro-8-fluoroquinoline (2.80%), 3-Nitrophthalhydrazide (2.71%), Benzene, 1,4-dichloro- (2.65%), Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone (2.58%), Pentafluoropropionic acid, undecyl ester (2.54%), Fumaric acid, (p-nitroanilino)-, diethyl ester (2.50%), Diallyl disulphide (2.46%), 9-Borabicyclo[3.3.1]nonane, 9-[3-(dimethylamino)propyl]- (2.43%), (p-Methoxyphenyl)-acetonyl-dimethylsilane (2.42%), Bicyclo[10.8.0]eicosane, cis- (2.41%), and L-Methionine, N-(1-methylethyl)-, and 1-methylethyl ester (2.40%).

5. Conclusion

GC-MS and GC-FID analysis of *Gnetum africanum* aqueous root extracts revealed a rich presence of bioactive phytochemicals. It has shown that *Gnetum africanum* aqueous root extract is a significant source of phenolic compounds with antioxidant and free-radical scavenging abilities. This groundbreaking study is the first to profile *Gnetum africanum* aqueous root extract, providing solid scientific evidence of its antimicrobial compound richness in 2,3-Butanediol, phytol, fumaric acid, and Benzene. These findings shed light on the plant's potential as a natural cure, with significant implications for the pharmaceutical and healthcare sectors.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interest exist in the work.

References

[1] Iheme CI, Nwufo CK, Obasi UK, Igwe CU, Ujowundu CO, Chukwudoruo CS, Ibeh RC, Ukwueze TO, & Haruna MA. Antioxidant property and lipid profile effects of aqueous and ethanol root extracts of *Gnetum africanum* welw. *Tropical Journal of Natural Product Research*, 2022; 6(4), 615 – 20.

[2] Ogbonnaya EC, & Chinedum, E.K. Health promoting compounds and in vitro antioxidant activity of raw and decoctions of *Gnetum africanum* Welw. *Asian Pacific Journal of Tropical Disease*, 2013; 3(6), 472 – 9.

[3] Okerulu IO, & Onyema CT. Comparative Assessment of Phytochemicals, Proximate and Elemental Composition of *Gnetum africanum* (Okazi) Leaves. *American Journal of Analytical Chemistry*; 2015; 6: 604 – 9

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

[5] Ezekwe A, Ugwuezumba P, Nwankpa P, Egwurugwu J, Ekweogu C, Emengaha F, & Akukwu D. Qualitative Phytochemical Screening, GCMS Studies and In-Vitro Anti-Oxidative Properties of Aqeuous Leaf Extract of *Gnetum africanum*. *Journal of Drug Delivery and Therapeutics*, 2020; *10*(1), 11-7.

[6] Ali F, Assanta MA, & Robert C. *Gnetum africanum*: A Wild Food Plant from the African Forest with Many Nutritional and Medicinal Properties. *Journal of Medicinal Food*, 2011; *14*(11), 1289 – 97. https://doi.org/10.1089/jmf.2010.0327

[7] Bahuchet S. "The Akwa pygmies: Hunting and Gathering in the Lobaye Forest." In: Food and Nutrition in the African Rain Forest. *Food Anthropology,* 1990; Unit 263, UNESCO."

[8] National Institute of Science and Technology Policy (NISTEP). 2014; Retrieved from https://www.nistep.go.jp/en/?m=201407

[9] Brukum MF, Kpegba K, Evenamede, KS, Agbonon A, & Simalou O. Antioxidant and anti-sickling activity of extracts from the stem and roots barks of strychnos innocua (del.). *International Journal of Pharmacy and Pharmaceutical Sciences*, 2023; *15*(9), 18–23. https://doi.org/10.22159/ijpps.2023v15i9.48625

[10] Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, & Jaremko M. Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules (Basel, Switzerland)*, 2020; *25*(22), 5243. https://doi.org/10.3390/molecules 25225243

[11] Bhambhani S, Kondhare KR, & Giri AP. Diversity in Chemical Structures and Biological Properties of Plant Alkaloids. *Molecules (Basel, Switzerland)*, 2021; *26*(11), 3374. https://doi.org/10.3390/molecules26113374

[12] Bagrov AY, Shapiro JI, & Fedorova OV. Endogenous Cardiotonic Steroids: Physiology, Pharmacology, and Novel Therapeutic Targets. *Pharmacological Reviews*, 2009; *61*(1), 9–38. https://doi.org/10.1124/pr.108.000711