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PHYTOCHEMICAL AND GC-MS ANALYSIS OF *GNETUM AFRICANUM*: A POTENTIAL CHEMOPREVENTIVE NIGERIAN VEGETABLE

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ABSTRACT

Cancer is a health burden of global dimensions as the disease is the second leading cause of death worldwide, claiming 9.6 million lives in 2018. The burden from the disease is enormous and there is an immense increase in the cancer diagnosis statistics report for African, Asian, Central and South American populations; where about 70% of the cancer deaths reportedly occurred in these regions. Thus, the need for chemopreventive agents has arisen. *Gnetum africanum* popularly known as Okazi/Afang in Nigeria is a tropical wild vegetable with high consumption rate in Nigeria. The plant has a robust ethnopharmacological data of its use in various parts of Africa. Recently, the anticarcinogenic, antioxidant and antiinflammatory activities of *G. africanum* were reported. There is need to identify the bioactive compounds in the leaves of *G. africanum*. Hence, this study is focused on investigating the phytochemical constituents and GC-MS profile of *G. africanum*. The results of the phytochemical analysis identified alkaloids, flavonoids, lipids, phenolic compounds, tannins, saponins and triterpenoids/steroids as the classes of compounds contained in the leaves. The GC-MS evaluation indicated the following fourteen bioactive compounds: Mepindolol, Butanoic acid, 2-methyl, methyl ester, .beta. –Eudesmol, Benzene, 1,3-bis(1,1-dimethylethyl)-, Trimethyl-, phosphate, Heptafluorobutyric acid, pentadecyl ester, Benzoic acid, 4-ethoxy-, ethyl ester, 2-(2-Hydroxyethylamino)-1,4-naphthoquinone, Phytol, acetate, 4-Isopropyl-1,3-cyclohexanedione, Tributyl Citrate, Stigmasterol, 13-Docosenamide,(Z)- and .beta.-Sitosterol. Stigmasterol and .beta.-Sitosterol are two highly bioactive phytosterols with numerous bioactivities. The findings of this study have demonstrated the chemopreventive potentials of *G. africanum*. The plant

merits further studies to justify its recommendation as a functional food or use as a dietary supplement.

Keywords: *Gnetum africanum,* Chemoprevention, GC-MS, Phytochemical

INTRODUCTION

Popular cruciferous vegetables like broccoli, cauliflower, cabbage, carrot and tomatoes have been shown to exhibit cancer chemopreventive activities [1]. Chemoprevention could be defined as the use of natural or synthesized compounds for the prevention of cancer, inhibition of its spread and total elimination on a long-term basis. Studies have shown that leafy vegetables, such as spinach, watercress and kale, are beneficial in cancer chemoprevention [2].

Chemoprevention is considered the way forward in minimizing the incidence of new cancers and also beneficial in the treatment of existing cancers. This is necessary as this approach is cost-effective, low risk, easy to cope with and contributes to the overall well-being of individuals. This approach supports a healthy lifestyle which reduces the burdens on the healthcare providers and institutions.

Gnetum africanum belongs to the Family Gnetaceae [3]. The plant is native to tropical Africa and is found in countries such as Nigeria, Gabon, Angola, Democratic republic of Congo and Central African Republic. The plant is a perennial climbing wild vegetable that grows to a length of 10 m [4]. The leaves occur in groups of three and are very thick; posing difficulties when shredded with bare hands. At maturity, some cone-shaped reproductive-structures are formed. *G. africanum* is called wild spinach in English, okazi in the Eastern part of Nigeria, Afang by the Efik & Ibibios [5], ajaabaje in Yoruba and yala in ogoja [6]. The leaves of *G. africanum* are used in the preparation of a delicious soup consumed very regularly in Rivers, Imo, Abia, Akwa Ibom and Cross Rivers states of Nigeria. There is a growing popularity in the consumption of this vegetable even in some other African countries and in the developed nations. The ethnopharmacological uses of the plant includes the treatment of a vast array of diseases such as high blood pressure, diabetes, spleen enlargement, sore throat, piles and as an antipoison [6,7,8,9]. The antioxidant, anti-inflammatory and anticarcinogenic activities of *G. africanum* has been reported [2]. Also, the Phytochemical screening of *G. africanum* leaves identified saponin, alkaloids, tannins and glycosides as the classes of compounds in the plant [10,11,12] . This research work analyzed the leaves of *G. africanum* phytochemically and by GC-MS to identify the potential bioactive compounds that could support the use of this plant in chemoprevention.

MATERIALS AND METHOD

Materials Collection, Identification and Authentication
G. africanum (leaves) was sourced from a bio reserve in Nigeria. The plant was authenticated by a botanist, Dr. Suleiman Mikailu of the Department of Pharmacognosy & Phytotherapy, University of Port Harcourt.

EQUIPMENT AND INSTRUMENTS

Electronic weighing balance (model WT6002A), Maceration jars, Thermostat bath (HH-6; Techmel and Techmel, USA), Lypholiser (Harvest right scientific freeze dryer), Beakers, Glass funnels, Measuring cylinders, Conical flask, Rotary evaporator (R-205), Desiccator, Spatula, Crucibles, Filter papers and Syringes.

REAGENTS

Methanol, Dichloromethane of Analytical grade (SigmaAldrich). Chloroform, Diethyl Ether, Acetic Anhydride, Glacial acetic acid, Sodium picrate, 2% 3,5-Dinitrobenzoic acid, Picric acid, Iodine solution, Dimethyl sulfoxide, (JHD company, Guangdong. GuanghuaSci-Tech. Co. Ltd. China), Hydrochloric acid, Million's reagent, Benedict's solution, Wagner's reagent, Sodium Hydroxide, Ferric chloride solution, Saturated lead acetate solution, Dragendorff's reagent, Kedde reagent, Ammonia solution, 7.5% Potassium Hydroxide, Fehling's solution A and B (Sigma Aldrich Chemicals, St Louis, USA), Distilled water, Deionized water (Pharmaceutical Chemistry Lab, University of Port Harcourt).

METHODS

EXTRACTION OF THE PLANT MATERIALS

The plant materials were extracted according to the American National Cancer Institute (NCI) method of extraction [13].
250g of the pulverized plant material was macerated in a 1: 1 mixture of 500ml of dichloromethane and 500ml of methanol for 24 h.The obtained solution containing the extracts was decanted off and 500ml of methanol was added to the residue and allowed to stand for another 24 h. The solution of the extract was collected by filtration. The methanol extraction was combined with the 1:1 dichloromethane and methanol extraction to yield the organic extract. This extraction solution was evaporated to dryness on a rotary evaporator at a temperature of 40˚C. The obtained dry extracts were further dried in a desiccator to remove any trace of solvent.

PHYTOCHEMICAL SCREENING

The plant extract (leaves) was subjected to preliminary analysis using the method described by Trease and Evans [14].

GC-MS ANALYSIS OF THE ORGANIC EXTRACTS

The gas chromatography mass spectrometry (GC-MS) analysis of the DSE was quantitatively determined using an Agilent 7890B GC system coupled with an Agilent 5977A MSD with a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.025μm) (5%-phenylmethylpolysiloxane). The GC-grade helium served as the carrier gas at a constant flow rate of 2 mL/min. The DSE was dissolved with ethanol and filtered before use. The column temperature was maintained at 60°C and gradually increased at 10°C per minute until a final temperature of 300°C was reached.

The time taken for the GC-MS analysis was 30 min. The compounds were identified based on computer matching of the mass spectra with the NIST 11 MS library (National Institute of Standards and Technology library).

RESULTS

The results of the phytochemical screening are presented in Table 1.

Table 1. The Phytochemical Screening Results

TEST	PRESCENCE
1. Triterpenoids/Steriods	
a. Liebermann-Buchard Test	+
b. Salkwoski's Test	+
2. Phenolics test	
a. $FeCl_3$ test	+
3. Tannin test	
a. Phlobatannins test	+
4. Flavonoids	
a. Shinoda Reduction Test	+
b. $AlCl_3$ test	+
5. Alkaloids	
a. Dragendorff's (orange colour)	+
b. Mayer's test (cream)	+
c. Hager's test (yellow ppt)	+
6. Saponin	
a. Frothing Test	+
b. Emulsion Test	+
7. Fats and Oils	
a. Oil Stain test	+

The results presented in Table 1 showed that the organic extract of *G. africanum* contained alkaloids, flavonoids, phenolic compounds, tannins, saponins, triterpenoids/steriods, and fats & oils. This study is in consonance with the work of other researchers who have investigated the phytochemical constituents of *G. africanum.* These earlier researchers identified flavonoids, alkaloids, saponins, glycosides, and tannins as secondary metabolites in *G. africanum* [10,11,12,15].

The results of the GC-MS analysis of the organic extract of *G. africanum* are presented in Table 2. A total of 14 peaks were recorded.

Table 2: The identified Compounds from the GC-MS analysis of *G. africanum*.

Peak	Retention Time	Compound	Chemical Formula	Molecular Weight	Documented Bioactivity
1	6.9	Mepindolol	$C_{15}H_{22}N_2O_2$	262	Treatment of glaucoma [16]
2	8.6	Butanoic acid, 2-methyl, methyl ester	$C_6H_{12}O_2$	116	
3	9.3	.beta. –Eudesmol	$C_{17}H_{28}O_2$	264	
4	10.5	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{15}H_{24}O$	190	Antimicrobial [17]
5	10.9	Trimethyl-, phosphate	$(CH_3)_3PO_4$	140	
6	12.22	Heptafluorobutyric acid, pentadecyl ester	$C_{19}H_{31}F_7O_2$	424	Antimicrobial, antioxidant and anticancer[18,19,20]
7	14.4	Benzoic acid, 4-ethoxy-, ethyl ester	$C_{11}H_{14}O_3$	194	Antioxidant and Antibacterial [21]
8	17.51	2-(2-Hydroxyethylamino)-1,4-naphthoquinone	$C_{12}H_{11}NO_3$	217	Antibacterial [22]
9	18.1	Phytol, acetate	$C_{22}H_{42}O_2$	338	Antinociceptive and Antioxidant [23]
10	20.68	4-Isopropyl-1,3-cyclohexanedione	$C_9H_{14}O_2$	154	
11	22.3	Tributyl Citrate	$C_{18}H_{32}O_7$	360	Anti-inflammatory [24]
12	26.1	Stigmasterol	$C_{29}H_{48}O$	412	Antioxidant, Antiiflammatory, Anticancer, Analgesic, Antidiabetic, Cytotoxic and Hypocholesterolemic [25]
13	26.73	13-Docosenamide,(Z)-	$C_{22}H_{43}NO$	337	Antidiarrheal, Antiiflammatory and Anthelmintic [26]
14	27.2	.beta.-Sitosterol	$C_{29}H_{50}O$	414	Antioxidant, Antiiflammatory, Anticancer, Analgesic, Antidiabetic, Immunomodulatory and Hypocholesterolemic [27]

The GC-MS chemical characterization of the organic extract of *G. africanum* was carried out and the results presented in Table 2. The interpretation of GC-MS mass-spectra was based on the NIST library of the equipment. The individual spectrum were matched with that of the library and the following parameter; molecular weight, structure, retention time and fragmentation patterns compared. Fourteen bioactive compounds were ascertained from the spectral match. Ten of these compounds have pharmacological activities documented from previous studies. 4-Isopropyl-1,3-cyclohexanedione, has been reported as one of the constituents identified in the

GC-MS analysis of *Putranjiva roxburghii* Wall. Fruit Peel which has analgesic, antipyretic and anti-inflammatory activities [28,29].

DISCUSSION

The formation of reactive oxygen species (ROS) is the root cause of most degenerative disease conditions in the human body as a result of the oxidation of DNA, lipids and protein [30]. The demand for antioxidants is ever increasing as the need to counter the generated ROS in the body is unavoidable, since the preventive role of antioxidants is required to militate against cancer, cardiovascular diseases and many other conditions. Most cancer-related occurrences are preventable from a dietary point of view. The concept of chemoprevention, is fast gaining popularity and global attention owing to its numerous advantages. Firstly, the approach is cost-effective, low risk, adaptable, leads to increase in well-being, enhances healthy lifestyles and reduces the healthcare burdens of healthcare providers and institutions. This would result in huge savings both for individuals and governments. Vegetables have been shown to inhibit the proliferation of cancer cells. For example tomato lycopene and the S-ally cysteine of garlic in combination inhibited the progression of gastric cancer induced chemically [2]. Phytosterols are considered highly beneficial, given their pharmacological properties. More studies on this class of compounds are needed. Currently, campesterol, β- sitosterol and stigmasterol are the most consumed phytosterols [31]. This study revealed that *G. africanum*, is rich in phytosterols of significant biological importance. The combination of these bioactive compounds in the plant further establishes the chemopreventive potentials of *G. africanum*. However, what remains is whether the process of cooking of the leaves of *G. africanum* undermines the efficacy of these compounds; resulting in diminished bioactivities.

CONCLUSION

In conclusion, the phytochemical screening and GC-MS analysis of *G. africanum* has been carried out. The results showed that eight classes of phytochemicals were identified. The GC-MS analysis revealed the presence of fourteen bioactive compounds. Two of which; Stigmasterol and .beta.-Sitosterol have numerous bioactivities. This work demonstrates that *G. africanum* possess chemopreventive potentials and warrants further investigations.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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