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Phytochemical profiling of aqueous extract of *Gnetum africanum* stem bark using Gas Chromatography–Mass Spectrometry (GC-MS)

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Abstract

The aqueous extract of stem bark of Gnetum africanum, was subjected to phytochemical screening using gas chromatography equipped with mass spectrometry (GC-MS). In this study gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of remarkable natural bioactive components in methanol extract of Gnetum africanum. The major constituents are propofol, trimethylsilyl ether (9.72%), phenol, 2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl)- (7.25), -4'-dimethylamino-2'-(trimethylsilyl) acetanilide (5.69%), 2,2,2-trifluoro-N-(3-(methylthio)phenyl)acetamide (5.48%), 4-(4-bromophenyl)pyridine (5.35%), 17.beta.-hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester (5.17%), methaqualone (5.07%). The bioactive components in the aqueous extract of the stem bark of Gnetum africanum have favourable medicinal qualities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, properties and also serve as an anesthetic and a sedative used in treatment of and neurological disorders. The phytochemical profiling of aqueous extract of Gnetum africanum using gas chromatography–mass spectrometry (GC-MS) revealed the presence of bioactive compounds with important therapeutic properties.

Keywords: Vegetable; Gymnosperms; Bioactive components; Medicinal; Extracts; Analysis

1. Introduction

Gnetum africanum is a vine gymnosperm species found native throughout tropical Africa. Though bearing leaves, the genus Gnetum are gymnosperms, related to pine and other conifers. *Gnetum africanum*, is a climbing vine in the tropical rainforest of West and Central Africa. This vine will grow in all seasons and typically spreads along forest floors. The vine grows in two ways: through rhizomes, or through new shoots that grow where the stem has been cut (Chaw, et al., 2000; Besong et al., 2001).

Gnetum africanum tends to grow best in shaded areas and through the years been domesticated and grown for subsistence and commercial use among the people of the southern part of Nigeria. The leaves are highly valued as a nutritious green vegetable and today, as an article of considerable cross-border trade, extending to Europe and America. (Tekwe et al., 2003)

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. Traditional medicinal plants play an important role in medical system in Nigeria and plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Feachem et al., 2010).

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The *Gnetum africanum* plant may further be used as a remedy for nausea, sore throats, or as a dressing for warts. The stem of the plant may also be eaten for medicinal purposes, including the reduction of pain during childbirth. (Soltis et al., 2002)

Gnetum africanum is a good source of protein and is rich in essential and non-essential amino acids. It is high in glutamic acid, leucine, and aspartic acid, with low levels of histidine, and cysteine, while there appears to be trace amounts of tryptophan in the plant. The content of amino acids found in *Gnetum africanum* is similar to recommended levels by the FAO. It has also been found that the levels of iodine are also high in the vine. Fibre levels average approximately 33.4 g/100 g of dried *Gnetum africanum* leaves, while recommended daily intake of fibre is 30g. Medicinally, *Gnetum africanum* is used 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 (Ndam et al., 2000). *Gnetum africanum* has been noted to be anti-inflammatory, anticarcinogenic and antioxidant.(Ali, et al*.,* 2011) .

The present study, therefore seeks to examine the phytochemical composition *Gnetum africanum* as to identify the bioactive materials responsible for some of these medicinal properties mentioned.

2. Materials and Methods

2.1. Identification/Collection of Samples

The plants G*netum africanum* were harvested in large quantities from Trans Egbu, Owerri Municipal L.G.A respectively and were identified by a plant taxanomist Dr. D. I Edet from the Department of Forestery and Wildlife, Federal University of Technology, Owerri, Nigeria and deposited at the University herbarium with voucher number FUTO/FW/HERB/2022/075 respectively, for reference purposes.

2.2. Plant Extraction Process

The *Gnetum africanum* stem bark were harvested in large quantities in June, 2024; washed thoroughly in running tap water. The stem bark was cut into pieces (about 3cm) and dried separately under shade at room temperature for about seven weeks before grinding into a powder form using an electric blender. Using a soxholet extraction, 30 g of the powdered plant sample was extracted in deionised water, for 6 hours. Aqueous filtrates were concentrated using water bath at 100^{o}C and stored at -4^{o}C until required. However, the percentage yield of the extract was determined.

2.3. Phytochemical Studies

The phytochemical constituents of the aqueous extracts of the stem bark *Gnetum africanum* was determined using GC-MS.

GC–MS analysis of compounds in the extracts was carried out using Agilent Technologies GC systems with a 7000C GC/MS Triple Quad model (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS column (30 m x 0.25mm; 0.25 µm). Spectroscopic detection by GC–MS involved an electron ionization system with a high ionization energy of 70 eV, ion source temperature of 250^{o}C and mass scanning range of 33–600 amu in full scan. Pure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 ml/min, and the injector temperature was maintained at a constant of 250^{o}C. The initial column temperature was set to 60^{o}C for 2 min and increased to 150^{o}C with an increasing rate of 10^{o}C/min. Finally, the temperature was increased to 300^{o}C at 5^{o}C/min. One microliter of the sample in ethanol was injected in split mode with a split ratio of 20:1. The identification of the phytochemical compounds in the test samples was performed by comparing their mass spectra with the spectral database of known compounds in the National Institute of Standards and Technology (NIST2011) structural library. Only selected peaks with 80% similarity and above with NIST libraries were chosen and identified.

2.4. Percentage Yield

Percentaage yield was calculated as:

$$\% \text{ Yield } = \frac{\text{Total weight of extract}}{\text{Total weight of plant sample}} \text{x} \frac{100}{1}$$

3. Results and Discussion

An analysis of the percentage yield of the aqueous extracts of the stem bark of *Gnetum africanum* showed a good amount of extract was produced by the plant's stem bark in the aqueous solvents.

Analysis of the percentage yield of plant showed that the aqueous extracts of *Gnetum africanum* stem yielded (40.86%) of the sample as shown in table 1. below.

Table 1 Percentage Yield of Extracts from Different Parts of *Gnetum africanum* Using Deionized Water

Samples	Deionised H_2O (ml)	Sample weight(g)	Weight of extract (g)	Percentage yield of extract (%)
G. africanum stem	500	50	20.39	40.78

The *Gnetum africanum* plants have been shown to have presented with phytochemicals such as, flavonoids, alkaloids, steroids, and glycosides, and pharmacological activities Okerulu et al., (2015).

In this study of the aqueous extract of *Gnetum africanum*, the gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of twenty remarkable natural bioactive components these compounds were identified and characterized The most abundant compound was o-Xylene (44.37%) with a retention time of 2.365 min, followed by 2,3-Heptadien-5-yne, 2,4-dimethyl- (20.60%) with a retention time of 3.268 min, 1,3-Cyclopentadiene, 5-(1-methylpropylidene)- (9.24%), Cyclohexane, (2-methylpropyl)- (6.60%), 3,3a epoxydicyclopenta [a,d]cyclo octan-4.beta.-ol, and 9,10a-dimethyl- 6-methylene-3.beta.-isopropyl- (3.32%). Other compounds were found in low amounts of less than 3.0% as shown in Table 2.

Table 2 GC-MS results of Phytochemical Composition of *Gnetum africanum* Stem Aqueous Extract

S/No	Retention time	Name of chemical	Composition (%)	Molecular weight	Molecular formular
1	1.742	Cyclohexane, 1,4-dimethyl-, trans- Cyclohexane, 1,3-dimethyl-, trans-	3.90	352	C_8H_{16}
2	1.976	Cyclohexane, (2-methylpropyl)- 1-Hexyn-3-ol Cyclohexanone, 4-methyl- 2-Tridecene, (E)-	6.60	250	$C_{10}H_{20}$
3	2.125	2-(Prop-2-enoyloxy)pentadecane Cyclohexanone, 4-methyl- 2-Tridecene, (E)-	1.05	336	$C_{18}H_{34}O_2$
4	2.365	Benzene, 1,3-dimethyl- o-Xylene	44.37	235	C_8H_{10}
5	2.799	9-Decen-1-ol, pentafluoropropionat 1-Heptafluorobutyryloxy-10-undecen Chloromethyl 6-chloroundecanoate	3.11	250	$C_{13}H_{19}F_5O_2$
6	3.268	2,3-Heptadien-5-yne, 2,4-dimethyl- Cyclohexane, 1,2,4-tris(methylene) 1,3-Cyclopentadiene, 5-(1-methylpropylidene)-	20.60	250	C_9H_{12}
7	3.531	2,3-Heptadien-5-yne, 2,4-dimethyl- 1,3-Cyclopentadiene, 5-(1-methylpropylidene)-	9.24	250	C_9H_{12}

		Benzene, 1,2,3-trimethyl-			
8	3.788	1,3-Cyclopentadiene, 5-(1-methylpropylidene)- 2,3-Heptadien-5-yne, 2,4-dimethyl- Benzene, 1,2,3-trimethyl-	2.72	250	C_9H_{12}
9	4.085	Cycloheptane, 1,3,5-tris(methylene)- Ethanone, 1-(2-methylphenyl)- Ethanone, 1-(4-methylphenyl)-	1.58	250	C_9H_{12}
10	4.382	Propanamide, N-(2,1,3-benzothiadiazol-4-yl)-2,2-dimethyl- Benzoic acid, 2-[(2,2-dimethyl-1-oxopropyl)amino]-, methyl ester 3,4-Methylenedioxyphenyl-2-propanone semicarbazone	1.06	233	$C_{11}H_{13}N_3OS$
11	4.736	2,5-di-tert-Butyl-1,4-dimethoxybenzene 3,5-Di-t-butyl-4-methoxy-1,4-dihydrobenzaldehyde Ethanone, 1,1'-(9H-fluorene-2,7-diyl)bis-	0.37	206	$C_{16}H_{26}O_2$
12	5.491	Naphthalene 1H-Indene, 1-methylene- Azulene	0.87	390	$C_{10}H_8$
13	6.560	1,4-Methanonaphthalene, 1,4-dihydro- Naphthalene, 1-methyl-	1.44	250	$C_{11}H_{10}$
14	6.702	Silane, 9-anthracenyltrimethyl- 3,5-Di-t-butyl-4-methoxy-1,4-dihydrobenzaldehyde Pyrazolo[5,1-c][1,2,4]-triazine-3- carbonitrile, 4-methyl-7-phenyl-	0.48	250	$C_{17}H_{18}Si$
15	7.554	Methylarsine dibromide Thiophene-4-carboxylic acid, 2-methyl-3-sulfonamide-, methyl ester Methaqualone	0.43	235	CH_3AsBr_2
16	7.703	Ethanone, 1,1'-(9H-fluorene-2,7-diyl)bis- Propofol, trimethylsilyl ether 2,5-di-tert-Butyl-1,4-dimethoxybenzene	0.58	235	$C_{17}H_{14}O_2$
17	12.212	2,3,6,7-Tetrahydro-5H-selenazolo[3,2-a]-S-triazine-5-one-7-thione Didesmethylimipramine Anthraquinone-1-carboxylic acid	0.39	375	$C_5H_5N_3OSSe$
18	13.967	2,3-Dihydro-2-methyl-4-(4-methylphenyl)-1H-1,5-benzodiazepine N-(3-Diethylamino-4-ethoxy-phenyl) -acetamide 2-Hydroxyimino-4-methyl-4-nitro-1-phenylpentan-1-one	0.39	250	$C_{17}H_{18}N_2$
19	14.332	N-Allyl-N-cyano-N',N'-dimethyl-6-methylthio-1,3,5-triazine-2,4-diamine	0.45	250	$C_{10}H_{14}N_6S$

		Ethanone, 1-(3,4-dihydro-7-hydroxy -5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-yl)- 3,4-Pentadien-2-ol, 2-methyl-5,5-diphenyl-			
20	14.933	N-(3-Diethylamino-4-ethoxy-phenyl) -acetamide Propofol, trimethylsilyl ether Pyridine, 2,3,4,5-tetrachloro-6-fluoro-	0.45	250	$C_{14}H_{22}N_2O_2$

The gas chromatograph - mass spectrometry result of the *Gnetum africanum* stem aqueous extracts presented ten major phytochemical compositions with favourable medicinal characteristics such as anti-cancer, anti-inflammatory, and antimicrobial properties and also serve as antioxidant, anesthetic, sedative and hypnotic used in neurological disorders as shown in Table 3.

Table 3 Major phytochemical compositions of *Gnetum africanum* leaf aqueous extract analysed by GC-MS and its biological activities

S/No	Retention time	Name of chemical	Composition (%)	Biological Activity	Reference
1	4.570	Propofol, trimethylsilyl ether Methaqualone	9.72	Used as an anesthetic and a sedative.	Haeseler et al., 2008
2	4.730	17.beta.-Hydroxy-6-oxo-4,5-secoandrostan-4-oic acid methyl ester Methaqualone N-(4-Hydroxyphenyl)-2-naphthylamin	5.71	Used as Vasoconstrictor Agents and Platelet Aggregation Inhibitors	Duran-Frigola, et al.,2020
3	5.645	2,2,2-Trifluoro-N-(3-(methylthio)phenyl)acetamide Benzoxazole, 2-(2-benzimidazolyl)- Methaqualone	5.48	Used as anti-cancer, anti-inflammatory, and antimicrobial.	Mohamed, 2017
4	6.130	Methaqualone Silane, 9-anthracenyltrimethyl-	5.31	Used as as a sedative and hypnotic.	Waghmare et al., 2023
6	6.999	-4'-Dimethylamino-2'-(trimethylsilyl)acetanilide 4,4-Diphenyl-3-buten-2-one, p-tosylhydrazone Benzothiazole-2-thiol, 5-trifluoromethyl-	5.69	Used as an anesthetic and a sedative.	Haeseler et al., 2008
7	7.588	4-(4-Bromophenyl)pyridine p,p'-DDT	5.35	Used in the treatment of cancer, inflammation, and neurological disorders.	Sinan et al., 2015.
8	9.594	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl)- Acetic acid, 4-methanesulfonylamino-2,2,6,6-tetramethylpiperidin-1-yl ester Propofol, trimethylsilyl ether	7.25	Used as an antioxidant	William, 2004
9	10.360	Propofol, trimethylsilyl ether 4-Oxo-3-(phenylamino)-3,4-dihydroquinoline-2-carboxylic acid	5.64	Used as an anesthetic and a sedative.	Haeseler et al., 2008

		Pyridazine-3,5-dicarbonitrile, 1,6 -dihydro-4-amino-6-imino-1-(2-nitr ophenyl)-			
10	15.898	Methaqualone 1H-Pyrazole-1-acetamide, N-cycloheptyl-4-iodo-3,5-dimethyl	5.07	Used as a sedative and hypnotic.	Waghmare et al., 2023

Aqueous solvent was used in this study because the natives who use this plant for medicinal purposes majorly use this solvent. On the other hand the *Gnetum africanum* plant was used for the study because it is being used as food and has been shown to possess medicinal properties. Additionally, *Gnetum africanum* have high contents of vitamin A, dietary fiber, minerals (potassium, calcium, iron, zinc, phosphorus) and high chlorophylls, which are related to chlorophyllin synthesis, antioxidant activity, anti-diabetes activity, and health benefits against various diseases (Anisong et al., 2022).

In recent years, substantial interest has been placed on the chemical and pharmacological properties of herbal plants, especially in traditional medicine (Igwe et al., 2007).

Phytochemicals occur in various parts of plants. Their functions are diverse which include provision of strength to plants, attraction of insects for pollination and feeding, defense against predators, provision of colour, while some are simply waste products (Ibegbulem et al., 2003).

The extraction method presented is simple, rapid and affordable, with reduced solvent consumption. Gas Chromatography mass spectrometry method used for the analysis of the *Gnetum africanum* aqueous stem extract can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry, environmental and forensic applications (Uma et al., 2009)

The GC–MS analyses of the *Gnetum africanum* stem aqueous extract showed some bioactive compounds which were identified and characterised. The most abundant compound was Benzene, 1,3-dimethyl- (44.37%) with a retention time of 2.365 min, followed by 2,3-Heptadien-5-yne, 2,4-dimethyl- (20.60%) with a retention time of 3.268 min, 1,3-Cyclopentadiene, 5-(1-methylpropylidene)- (9.24%), Cyclohexane, (2-methylpropyl)- (6.60%), 3,3a epoxydicyclopenta [a,d]cyclo octan-4.beta.-ol, and 9,10a-dimethyl- 6-methylene-3.beta.-isopropyl- (3.32%). Other compounds were found in low amounts of less than 3.0%. The compound benzene, 1,3-dimethyl- has been applied *as an anti-cancer agent (*Daniel, 2017*),* 2,3-Heptadien-5-yne, 2,4-dimethyl- on the other hand is used as antibacterial agent (Safara et al., 2022). Methaqualone has been used as anesthetic, sedative, hypnotic and also in neurological disorders (Sinan et al., 2015; Waghmare et al., 2023).

4. Conclusion

The phytochemical profiling of *Gnetum africanum* stem aqueous extracts using gas chromatography – mass spectrometry (GC-MS) revealed the presence of bioactive compounds. The bioactive components in *Gnetum africanum* stem aqueous extracts have been known to manifest favourable medicinal characteristics such as anti-cancer, anti-inflammatory, and antimicrobial properties and also serve as antioxidant, anesthetic, sedative and hypnotic used in neurological disorders.

In view of these bioactive components in the aqueous stem bark of *Gnetum africanum* which possess medicinal properties further research should be carried out on this extract, *in vivo,* to ascertain its replicability in living organisms and the toxicity of this plant extract should be further evaluated to know its interactions with the liver, kidney, heart and other organs of the end users

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that we have no conflict of interest.

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