Bioactivity evaluation study of phytochemicals in *Gouania longipetala* ethanol leaf extract using GC-MS analysis.

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ABSTRACT: *The bioactive phytochemicals in Gouania longipetala was determined using GCMS analysis. The extract was prepared using Soxhlet`s extraction method and concentrated at 35^{o}C in hot air oven. GCMS analyzes phytochemicals in plant by demonstrating the structures of the chemical compounds in it. The gas chromatogram showed the presence of eight phytochemicals. The molecular mass of the phytochemicals were established based on the molecular ion in the mass spectra. Identification of the phytochemicals was based on comparison with National Institute of Standards and Technology (NIST) database. The identified phytochemicals with their peak area percentages are 11,14-octadecadienoic acid (1.72%), Hexadecanoic acid also known as Palmitic acid (19.86%), 9,11-octadecadienoic acid (1.33%), 9,12,15-Octadecatrien-1-ol (2.92%), 9-Octadecenoic acid (56.40%), Ethyl palmitate (9.42%), 17-carboxyheptadec-9-en-1-ylium (1.70%) and Glutaric acid, isobutyl 2-nitrophenyl ester (6.65%). These identified compounds exhibited the following bioactivities; inhibition of uric acid, urine acidifiers, amino acid decarboxylase activity, arachidonic acid inhibitor, oligosaccharide provider, decrease endothelial leukocyte and platelet adhesion . Gouania longipetala therefore contain active phytochemicals that may be beneficial in pharmacognosy. We recommend further work to be done on its isolation and synthesis.*

Keywords*: GC-MS, Gouania longipetala, Extract, Bioactivity.*

I. INTRODUCTION

Gouania longipetala is from the family Rhamnaceae [1]. The family Rhamnaceae is composed of 52 genera and 900 to 950 species [2]. This family is made up of trees, shrubs and a few herbs. Their flowers have a hypanthium with a disc of nectar with valrate calyx lobes in the inner surface. It grows up to 10m tall reaching to the top of the trees. The apex is curled and hairy at first and later turns woody as it becomes part of the base. It has simple alternate stipulate leaves that are toothed and with strong parallel veins and tertiary venation [3, 4]. The stems are used as thread and fibres in sewing. They are used in producing household items.Traditionally in Africa, it is used to manage menopausal complaints, abdominal pains, gonorrhoea, conjunctivitis and rickets [5]. It is also used to treat edema, heart diseases, malaria and diabetes mellitus.In this research, we want to evaluate the bioactivity of phytochemicals in the ethanolic leaf extract of *Gouania longipetala* using GC-MS analysis which has also been used to analyze various plant extracts by several authors [6 – 10]. The pictorial view of Gouania longipetala is presented in Figure 1.

Figure 1 Pictorial view of *Gouania longipetala*

II. MATERIALS AND METHODS

A *Plant materials*

Fresh leaves of *Gouania longipetala* were harvested from natural habitat at Ohafia town in Abia State, Nigeria. The plant leaves were identified at the Taxonomy section of College of Natural and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

B *Preparation of plant extract*

The plant material of *Gouania longipetala* were collected from wild, shade dried for 8 days at room temperature and pulverized to powder using electric grinder. The plant extract was prepared using Soxhlet method described by [11]. Twenty grams (20g) of powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as solvent. Temperature was maintained at 70^0C throughout the extraction period of 48 hrs. The sample was concentrated using hot air oven at 35^0C to obtain dried extract which was used for GCMS analysis.

C *GCMS analysis of Gouania longipetala*

The characterization of the Phytochemicals in *Gouania longipetala* was done using GC-MS QP2010 Plus (Shimadzu, Japan).

The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5).The initial column temperature was 80^0C for 1min, and then increased linearly at 70^0C min-1 to 220^0C, held for 3 min followed by linear increased temperature 10^0C min-1 to 290^0C for 10 min. The temperature of the injection port was 290^0C and the GC-MS interface was maintained at 290^0C .The sample was introduced through an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min-1. The identification of compounds was accomplished by comparison of retention time and fragmentation pattern, as well as with mass spectra of the GC-MS.

D *Identification of* phytochemicals *in Gouania longipetala*

GC-MS Chromatogram of *Gouania longipetala* revealed eight peaks showing that eight different phytochemicals were present. Identity of the active phytochemicals in the extract was done by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored in the database of National Institute of Standards and Technology (NIST) and also with published literature, NIST08.LIB [12], WILEY8.LIB [13], PESTEI-3.LIB and FA-ME.LIB library sources were used for matching the identified phytochemicals from the plant material. The name, molecular weight, formula, structure and bioactivities of the phytochemicals were ascertained.

III. RESULTS AND DISCUSSION

A *Results*

GCMS chromatogram of the ethanolic extract of *Gouania longipetala* is presented in Figure 2. It showed eight peaks which indicated the presence of eight phytochemicals.

Figure 3 shows the mass spectra of *Gouania longipetala* corresponding to the molecular weight of the phytochemicals of *Gouania longipetala*.

Table 1 shows the names, retention time (RT), peak area percentage, molecular weight, molecular formula and bioactivities of the suggested phytochemicals in the ethanolic extract of *Gouania longipetala*.

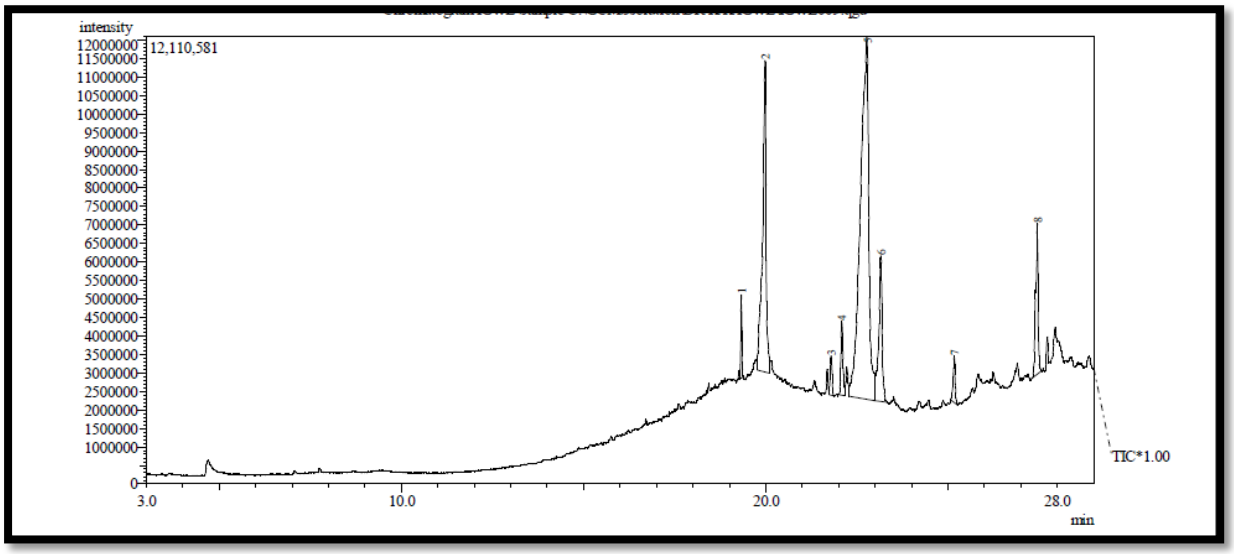
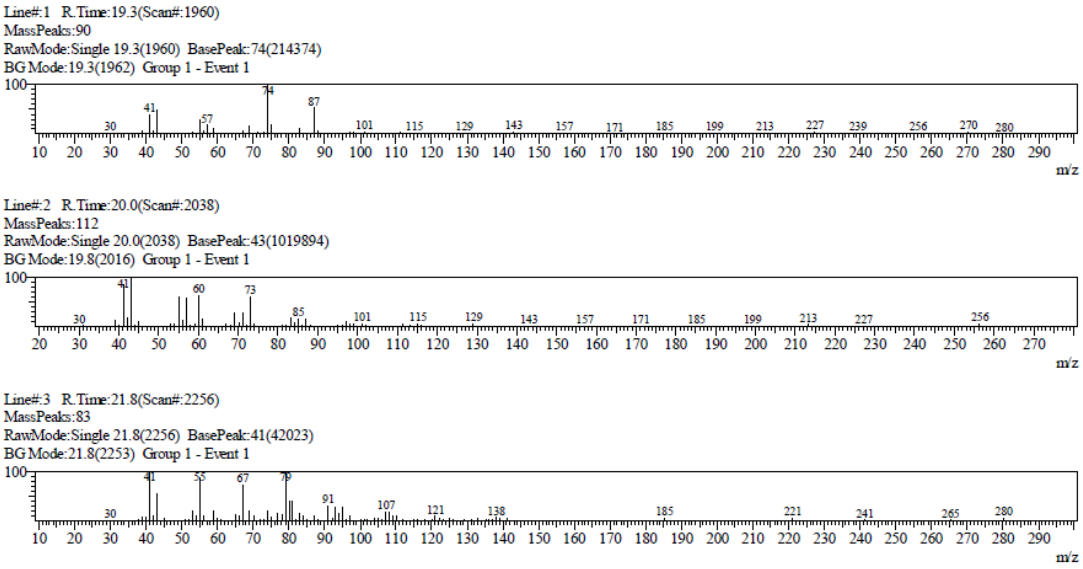


Figure 2 Gas chromatogram of ethanol extract of *Gouania longipetala* leaves



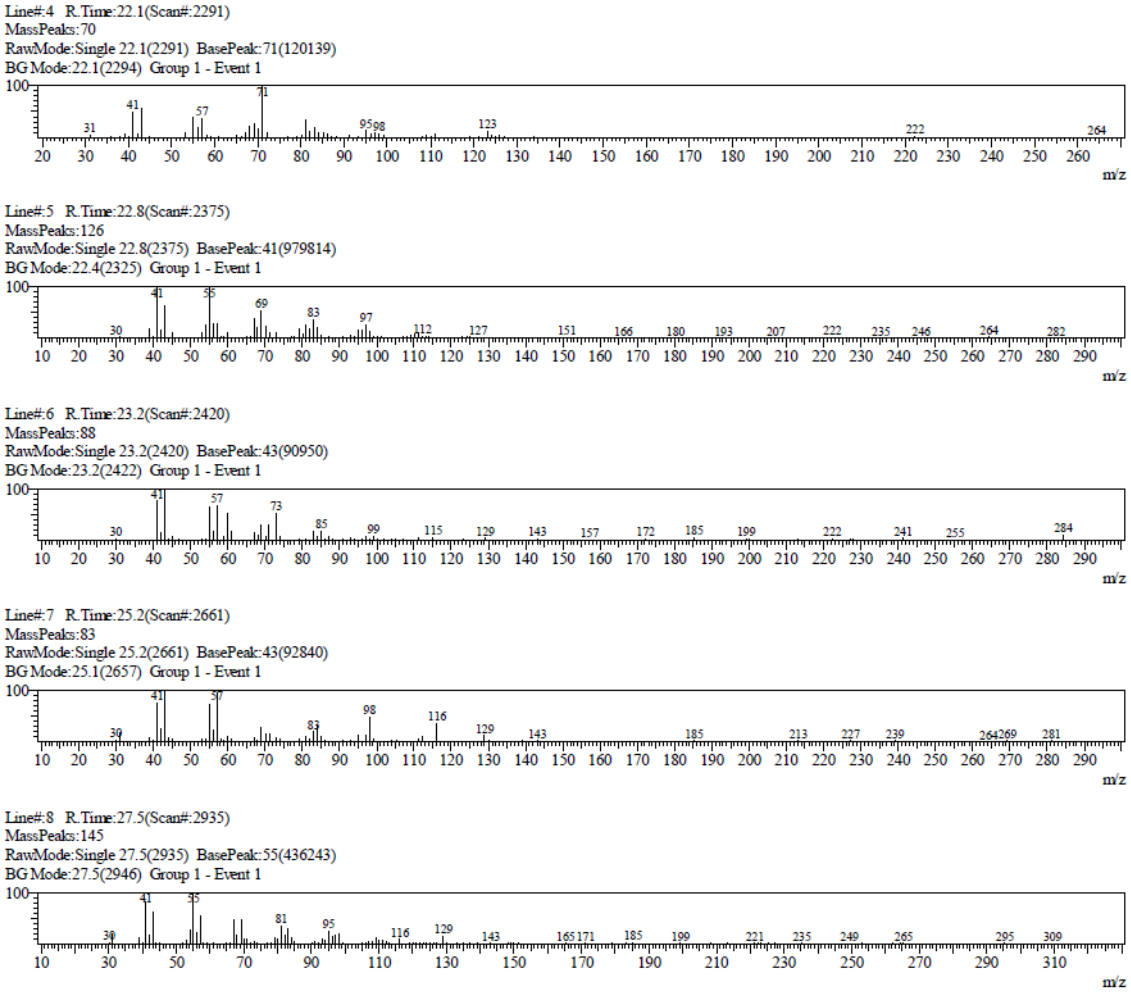
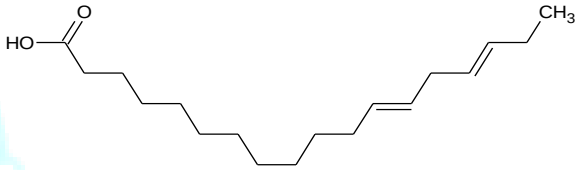
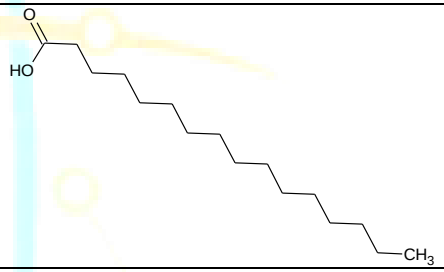
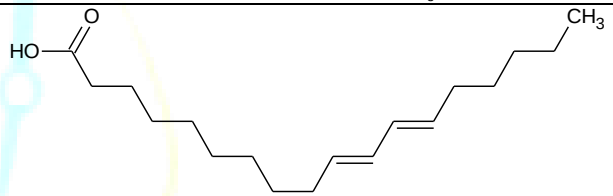
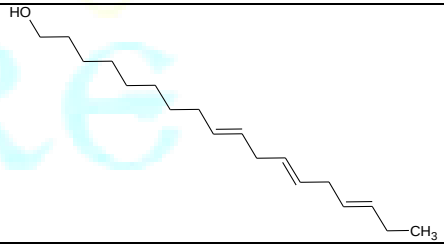
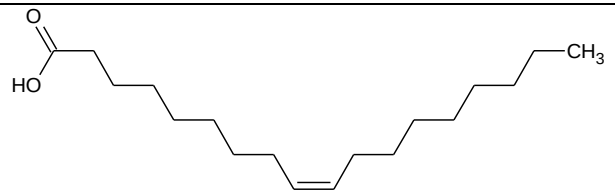
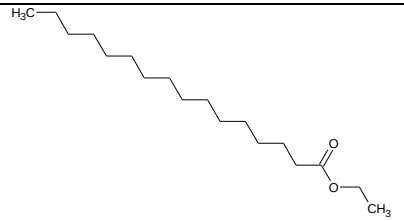
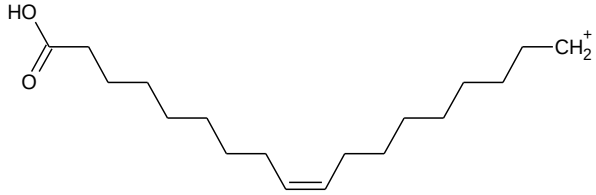


Figure 3 Mass spectra of the eight phytochemicals in ethanolic extract of *Gouania longipetala*

S/No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formular	Molecular structure	Bioactivity
1	11,14-octadecadienoic acid	19.326	1.72	280.44	$C_{18}H_{32}O_2$		Inhibit Production of Uric Acid , Increase Aromatic Amino Acid Decarboxylase Activity , Arachidonic-Acid-Inhibitor ,Urine-Acidifier, Urinary-Acidulant
2	Hexadecanoic acid also known as Palmitic acid	19.979	19.86	256.42	$C_{16}H_{32}O_2$		Acidifier, Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
3	9,11-octadecadienoic acid	21.791	1.33	280.44	$C_{18}H_{32}O_2$		Inhibit Production of Uric Acid , Increase Aromatic Amino Acid Decarboxylase Activity , Arachidonic-Acid-Inhibitor ,Urine-Acidifier, Urinary-Acidulant
4	9,12,15-Octadecatrien-1-ol	22.084	2.92	264.44	$C_{18}H_{32}O$		Oligosaccharide Provider,

5	9-Octadecenoic acid	22.771	56.40	282.46	$C_{18}H_{34}O_2$		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic-Acid-Inhibitor,
6	Ethyl palmitate	23.156	9.42	284.47	$C_{18}H_{36}O_2$		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic-Acid-Inhibitor,
7	17-carboxyheptadec-9-en-1-ylium	25.169	1.70	281.45	$C_{18}H_{33}O_2$		Decrease Endothilial Leukocyte Adhesion, Decrease Endothilial Platelet Adhesion, Encephalopathic, Endoanesthetic, Endocrinactive, Ergotamine-Enhancer, Enterotoxic, Enterotonic, Enteromotility-Enhancer, Enterodepressant, Enkephalinogenic, Energizer

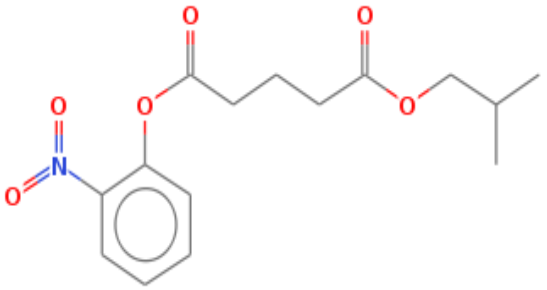
8	Glutaric acid, isobutyl 2-nitrophenyl ester	27.449	6.65	309.31	$C_{15}H_{19}NO_6$		Urine-Acidifier, Urinary-Acidulant, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic-Acid-Inhibitor

Table1. Name, retention time, peak area, molecular formula, molecular weight and bioactivity of phytochemicals in ethanol extracts of *Gouania longipetala*

B *Discussion*

Eight phytochemicals were identified in *Gouania longipetala* ethanolic leaf extract, showed by gas chromatogram as eight peaks (Figure 2). The molecular ions were identified using mass spectroscopy (Shimadzu). The name,retention time, peak area percentage, molecular weight, molecular formula and bioactivity of the ethanol extracts of *Gouania longipetala* are shown in Table 1. 9-Octadecanoic acid with the highest peak area % of 56.40; 11,14-octadecadienoic acid with peak area % of 1.72; Hexadecanoic acid with peak area % of 19.86; 9,11-octadecadienoic acid with peak area % of 1.33;Ethyl palmitate with peak area % of 9.42 and Glutaric acid with peak area % of 6.65 ,all demonstrated bioactivity of urine acidifier, aromatic acid decarboxylase activity and arachidonic acid inhibitor. Urine acidifiers increase the level of acid in the urine making them useful in controlling the pH of urine [14]. They are often given to animals with some types of urinary or bladder stones since most bladder stones are formed at a neutral or alkaline pH. Magnesium – ammonium- phosphate stones ($MgNH_4PO_4.6H_2O$) or calcium- phosphate stones can be treated with such urine acidifiers to aid in the health of urinary tracts [15]. Arachidonic acid is a polysaturated omega – 6 fatty acid which resembles arachidic acid structurally [16]. They are mostly present in the cell membranes of the body in the phospholipid portion where they serve as an intermediate in inflammatory response and in vasodilation [17]. The phytochemicals identified in this extract inhibit arachidonic acid, thus this extract can be used to inhibit inflammations and pain [18]. Pain is one of the cardinal points of inflammation. Aromatic amino acid decarboxylase is a lyase enzyme seen in different decarboxylation reactions that produce different neurotransmitters like serotonin, histamine and catecholamines. Increase in the aromatic amino acid decarboxylase can worsen some diseases like Parkinson disease [19] while its deficiency can lead to severe movement disorders and neurological impairments. Other phytochemicals identified from ethanolic leaf extract of *Gouania longipetala* include; 9,12,15- octadecatrien-1-ol with peak area % of 2.92 showed activity as an oligosaccharide provider.Oligosaccharide is a saccharide polymer containing approximately three to ten simple sugars[20], so ethanolic leaf extract of *Gouania longipetala* can to some extent serve as a source of energy due to the presence of 9,12,15- octadecatrien-1-ol.The compound , 17-carboxyheptadec-9-en-1-ylium with peak area % of 1.70 demonstrated bioactivity that include decreased endothelial leukocyte adhesion, decreased endothelial platelet adhesion, encephalopathic, endoanesthetic, endocrinactive, ergotamine – enhancer, enterodepressant, enkephalinogenic energizer. Endothelial leukocyte adhesion is a hallmark of inflammatory process through leukocyte adhesion receptors and their ligands on activated endothelial cells [21] but 17-carboxyheptadec-9-en-1-ylium decreases this effect thus reduces inflammatory process. Endothelial platelet adhesion is a process by which the body responds to vascular injury whereby the platelets bind through the action of specific membrane receptors to the matrix of the endothelium for thrombi formation to arrest heamorrhage and initiate wound healing[22] but in the ethanolic extract of *Gouania longipetala*, 17-carboxyheptadec-9-en-1-ylium decreased this activity thus it can serve as an anticlotting factor that could be used to prevent emboli or thrombi in thus enabling free blood flow in the body but can have a negative effect in reducing wound healing on injured vasculature. Encephalopathic enhancers tend to potentiate degenerative disease that affects the function and structure of the brain which can be present from birth or caused by infections, drugs, toxins, trauma or physiologic changes [23]. Endocrinative substances mimic the action of hormones in the body and can alter the normal growth and functioning of the exposed organism even at very low quantities [24]. Enterotoxins are chromosomally encoded or plasmid encoded exotoxins that are produced and secreted from several bacterial organisms [25]. They are often heat-stable, and are of low molecular weight and water-soluble. Enterotoxins are frequently cytotoxic and kill cells by altering the apical membrane permeability of the mucosal (epithelial) cells of the intestinal wall. They are mostly spore-forming toxins, secreted by bacteria, that assemble to form spores in cell membranes which causes the cells to die.

IV. CONCLUSION

Gouania longipetala showed the presence of 8 phytochemicals using GC-MS analysis. The bioactivity of these phytochemicals showed that Gouania longipetala is a urinary acidifier, increases aromatic amino acid decarboxylase activity, inhibits arachidonic acid, decreases endothelial leukocyte and platelet adhesion, enterodepressant and energizer. These phytochemicals in Gouania longipetala could be isolated and further research done for drug production and treatment of diseases.

V Acknowledgment

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