Phytochemical Profiling of Leaf of *Glinus lotoides* (Molluginaceae) Using GC-MS

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ABSTRACT

Glinus lotoides Linn. is a plant used in Nigerian traditional medicine for treating many diseases notably abdominal disorders. Decoction of leaf of the plant is mostly used in this case. This study was carried out in order to determine the bioactive compounds present in the leaf acetone–hexane extract of G. lotoides by using the gas chromatography-mass spectrometry (GC–MS) machine. G. lotoides leaves was extracted in acetone-hexane by cold maceration and concentrated in vacuo. The GC–MS analysis revealed the presence of the following phyto-compounds: 9-Octadecenoic acid, (E) (Z; 242.3975, RT: 16.663; 49.11%), n-Hexadecanoic acid (Z; 256.42, RT: 15.030, 25.58%), Octadecanoic acid (Z; 284.47, RT: 16.785; 6.80%), Stigmasterol (Z; 412.69, RT: 20.929; 5.25%) and Ergost-5-en-3-ol, (3.beta) (Z; 400.68, RT: 19.962; 2.72%) among others. These compounds were identified from leaves of G. lotoides for the first time, and unarguably play very vital roles in the health care system especially in abdominal disorders treatment and other diseases. The study showed that the presence of these compounds in the leaves of G. lotoides might be responsible for its biological activities in traditional medicine. It is therefore a promising important plant of medical and pharmaceutical significance from which drug can be discovered.

Key words: Pytochemical, *Glinus lotoides*, GC-MS, acetone–hexane extract

INTRODUCTION

Since ancient times, mankind has relied on herbs as medicine for treatment of various ailments and diseases[1]. Phytochemicals used in contemporary medicine such as morphine, atropine, digoxin, quinine, reserpine and ephedrine serve as evidence of drug discovered through examination of native medical practices[2]. However, due to paucity of local expertise and resources, the potential therapeutic value and profile of bioactive compounds of many African medicinal plants are still under explored. One of such plants, which have wide variety of uses as herbs for treatment of ailment, is *Glinus lotoides* Linn. It belongs to the family Molluginaceae under the order Caryophyllales. It is known as carpet weed or lotus sweet juice. It grows in the tropics and subtropics, especially in Nigeria, Egypt, Sudan and South Africa. *Glinus lotoides* is a

prostate to spreading annual herb up to 40 cm in length, with diverse parts woolly. Leaves are 0.6 – 2.0 cm long, 0.5 – 1.8 cm wide, petiolate. Leaves shape is round or elliptic, often with a sharp pointed apex and acute to obtuse at the base. Flowers are in axillary clusters of 3 – 15, sub-sessile. Flower stalk are up to 1.5 mm long, sepals 4 – 4.5 mm long. It bears flowers between February and May. Fruit, persistent, ovate to ovate-oblong, about 6 mm in length, membranous, enclosed in the sepals. Seeds are numerous, tuberculate, less than 1 mm long[3, 4].

Glinus lotoides possess myriads medicinal and nutritional values. Its tender shoots are eaten as pot herb. It is also used for the treatment of diarrhea, boils and abdominal disorders[5]. The seeds of *G. lotoides* are used traditionally in the treatment of tapeworm infestation in Ethiopia[6]. The juice of the plant is given to weak children for strength. A study conducted by Abdel-Hameed *et al*., 2008[7] shows antimicrobial potency of *G. lotoides*. The antihelmintic, antitumor, anti-spasmodic and antiviral properties of *G. lotoides* has also been evaluated in previous studies[8, 9, 10].

GC-MS is one of the modern analytical techniques used to determine and identify compounds present in plant samples. GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants. We present herein the phyto-constituents of *G. lotoides* leaf extract detected using GC-MS. The aim of this study is to provide profile of bioactive compounds in *G. lotoides* leaf for onward pharmacological research and drug development.

MATERIALS AND METHODS

Plant Material

Leaves of *G. lotoides* were collected from Ologuneru area in Ido local government, Oyo state. The plant was identified and authenticated in Forest Herbarium Ibadan (FHI) with voucher number FHI 113411. The leaves were air dried till all the moisture contents were removed and pulverized using anelectric grinder prior to extraction.

Extraction

Adequate mass (5g) of the pulverized leaves of *G. lotoides* was weighed, transferred into a 250mL conical flask with lid.40 mL of acetone-hexane (1:1) added to the sample in the conical flask and ultrasonicated at 27ºC for 15 min. The suspension was filtered and the filtrate concentrated *in vacuo* with a rotary evaporator.1µL of the sample was employed for GC-MS analysis of different compounds.

GC-MS Analysis

The GC-MS analysis of the acetone-hexane extract of *G. lotoides* was carried out using an Agilent 7820A gas chromatograph fixed to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies) at the Department of Chemistry, University of Lagos, Akoka, Nigeria. Capillary column (HP-5) coated with 5% Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25 μm film thickness) was the stationary phase of separation of the compounds. Helium was used as the carrier gas at constant flow of 1.4871 mL/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. At an injection temperature of 300 °C, an injection volume of 1μL of the sample was introduced in splitless mode. While the gas saver mode was turned off, the purge flow to spilt vent was 15 mL/min at 0.75 min with a total flow of 16.654 mL/min. Oven was initially auto regulated at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min). The run time was 32.667 min with a 5 min solvent delay. The mass spectrometer was utilized in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was through Scan mode (scanning from *m/z* 45 to 550 amu at 2.0s/scan rate)[11, 12].The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. National Institute Standard and Technology (NIST) 14.L library (2018) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C: \ Database \ NIST14.L).

RESULTS AND DISCUSSION

The GC-MS total ion chromatogram of acetone-hexane extract of *Glinus lotoides* leaf showed numerous peaks (Figure 1) which correspond to different compounds as shown in the analysis in (Table 1). The nature of compounds identified from acetone - hexane extract of *Glinus lotoides* leaf by GC-MS analysis with their corresponding peak areas (%) is shown in figure 2.

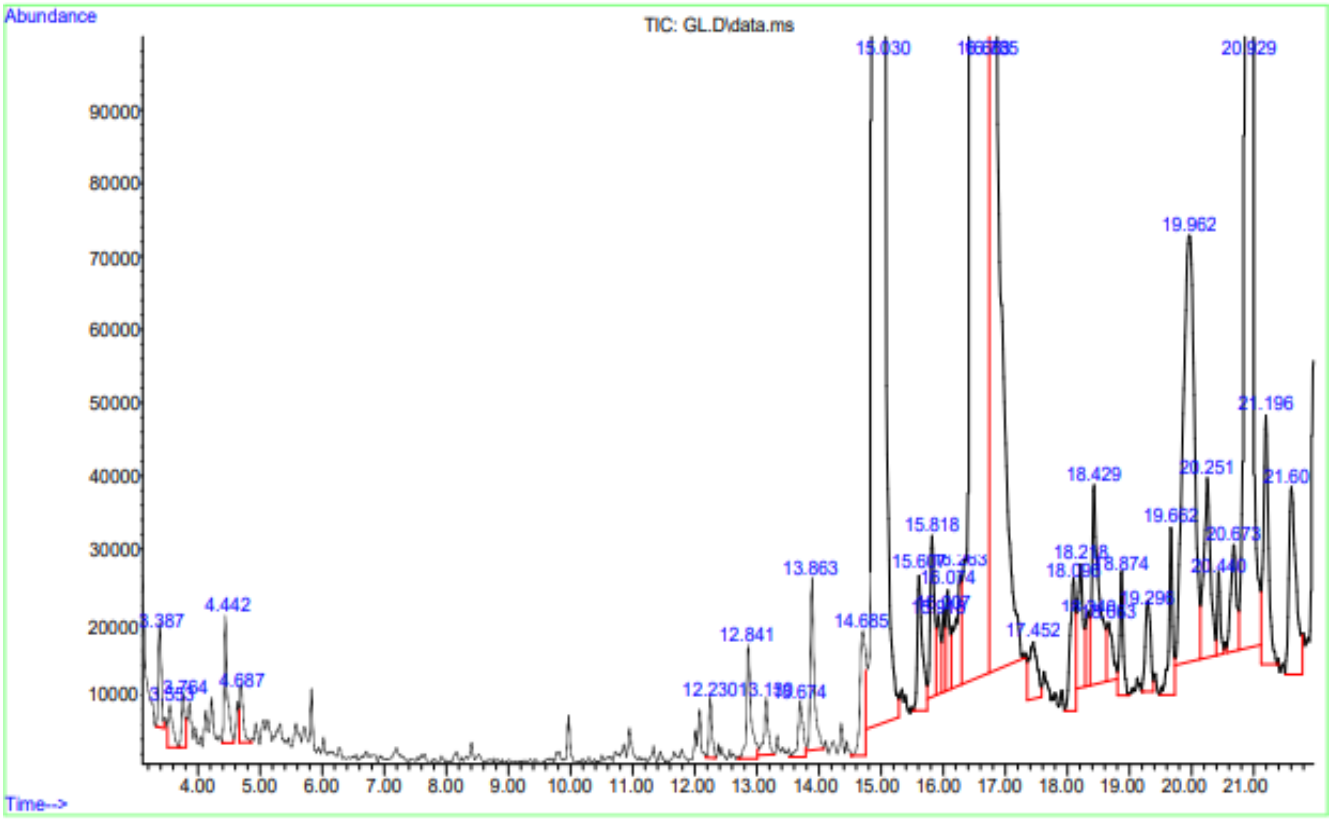


Figure 1: GC–MS chromatography of acetone-hexane extract of *Glinus lotoides* leaf

Table 1: Compounds identified from acetone - hexane extract of *Glinus lotoides* leaf by GC-MS analysis

S/n	Retention time(Min)	Name of compound	Molecular formula	Nature of compound	Mol. weight (g/mol)	Peak area (%)
1	3.387	Mesitylene	C_9H_{12}	Benzene	120.19	0.17
2	3.553	2-Heptafluorobutyroxydodecane	$C_{17}H_{27}F_7O_2$	Alkane	396.38	0.10
3	3.764	Benzene-1-methyl-3-propyl-	$C_{10}H_{14}$	Benzene	134.22	0.10
4	4.442	Undecane	$C_{11}H_{44}$	Alkane	156.31	0.10
5	4.687	1,3-Cyclopentadiene, 1,2,3,4-tetra methyl-5-methylene-	$C_{10}H_{14}$	Alkene	134.22	0.23
6	12.230	(E)-Dodec-2-en-1-yl propyl carbonate	$C_{16}H_{30}O_3$	Carbonic acid derivative	270.40	0.10
7	12.841	Tetradecanoic acid	$C_{14}H_{28}O_2$	Saturated fatty acid	228.37	0.29
8	13.130	Octadecane,1-chloro-	$C_{18}H_{37}Cl$	Alkyl halide	288.94	0.13
9	13.674	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-	$C_{12}H_{17}NO_2$	Aromatic piperidine	207.12	0.16
10	13.863	Pentadecanoic acid	$C_{15}H_{30}O_2$	Saturated	242.40	0.37

				fatty acid		
11	14.685	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	Ester	268.43	0.44
12	15.030	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Saturated fatty acid	256.42	25.58
13	15.607	9-Cycloheptadecen-1-one, (Z)-	$C_{17}H_{30}O$	Cyclic ketone	250.42	0.38
14	15.818	Heptadecanoic acid	$C_{17}H_{34}O_2$	Saturated fatty acid	270.45	0.39
15	15.918	Ergost-4,7,22-trien-3.alpha.-ol	$C_{28}H_{44}O$	Sterol	396.60	0.17
16	16.007	2-Hydroxychalcone	$C_{15}H_{12}O_2$	Chalcone	224.26	0.14
17	16.263	Ergosta-4,6,22-trien-3.beta.-ol	$C_{28}H_{44}O$	Sterol	396.6	0.39
18	16.663	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	Saturated fatty acid	282.46	49.11
19	16.785	Octadecanoic acid	$C_{18}H_{36}O_2$	Saturated fatty acid	284.47	6.80
20	17.452	2(1H)-Naphthalenone, octahydro-4a- methyl-7-(1-methylethyl)-, (4a.alp ha.,7.beta.,8a.beta.)-	$C_{14}H_{24}O$	Naphthalene	208.34	0.26
21	18.096	E,E-10,12-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	Fatty ester	280.4	0.40
22	18.218	Z,Z-10,12-Hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	Fatty ester	280.4	0.38
23	18.663	Cyclododecanol, 1-aminomethyl-	$C_{17}H_{27}NO$	Alcohol	213.36	0.18
24	18.874	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	Fatty aldehyde	264.4	0.24
25	19.296	Naphthalene, 1,2,3,4-tetrahydro-5-nitro-	$C_{10}H_{11}NO_2$	Naphthalene	177.20	0.27
26	19.662	1,22-Docosanediol	$C_{22}H_{46}O_2$	Alcohol	342.6	0.35
27	19.962	Ergost-5-en-3-ol, (3.beta.)- Campesterol	$C_{28}H_{48}O$	Sterol	400.68	2.72
28	20.251	Dihydrotachysterol	$C_{28}H_{46}O$	Sterol	398.7	0.68
29	20.440	1-Heptadecanamine	$C_{19}H_{14}N$	Amine	283.5	0.14
30	20.673	1H-Indene, 2-butyl-5-hexyloctahydro-		Alkene		0.35
31	20.929	Stigmasterol	$C_{29}H_{48}O$	Sterol	412.69	5.25
32	21.196	Stigmasta-3,5-diene	$C_{29}H_{48}$	Terpenoid sterol	396.7	0.71
33	21.607	Cholesta-3,5-diene	$C_{27}H_{44}$	Terpenoid sterol	368.64	0.76

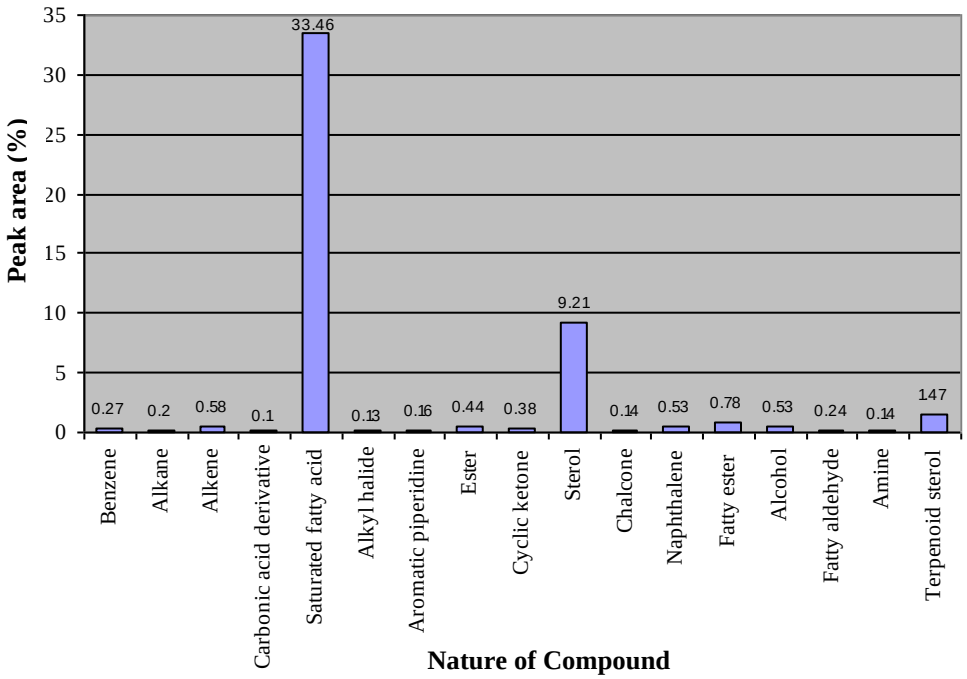
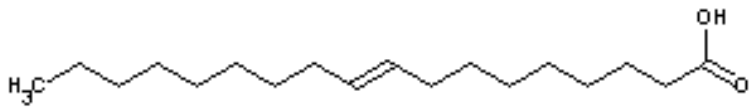
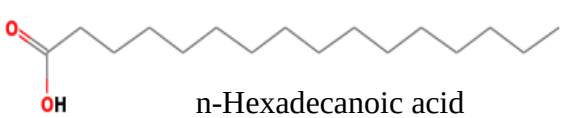


Figure 2: Peak areas (%) of the nature of compounds identified from acetone - hexane extract of *Glinus lotoides* leaf by GC-MS analysis

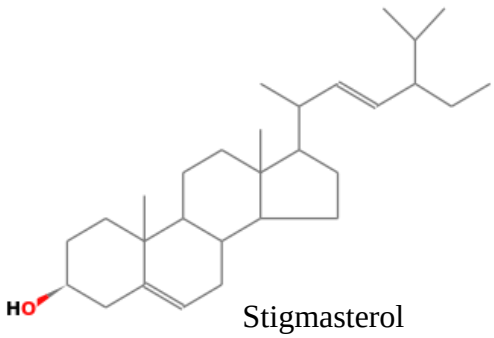


9-Octadecenoic acid, (E)-

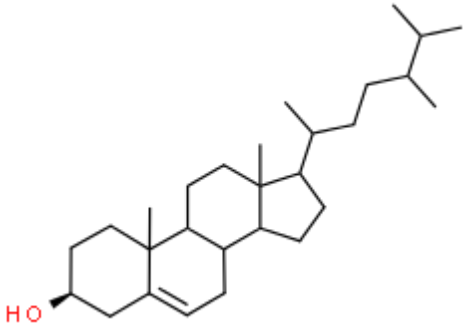


n-Hexadecanoic acid

Octadecanoic acid



Stigmasterol



Ergost-5-en-3-ol, (3.beta)

Figure 3: Chemical structures of some compounds identified in *Glinus lotoides* leaf

Gas chromatography-mass spectrometry characterization of *Glinus lotoides* leaf acetone - hexane extract revealed the presence of thirty-three compounds as shown in Table 1 and Fig. 1, many of which are saturated fatty acids as well as their esters (Fig. 2). From the results, of the GC–MS spectra, 9-Octadecenoic acid, (E) (49.11%), n-Hexadecanoic acid (25.58%), Octadecanoic acid (6.80%), Stigmasterol (5.25%) and Ergost-5-en-3-ol, (3.beta) (Campesterol) (2.72%) are the most abundant in occurrence while 2-Heptafluorobutyroxydodecane, benzene,1-methyl-3-propyl, (E)-Dodec-2-en-1-yl propyl carbonate and undecane are the least. These compounds have been reported to play vital roles in disease and general metabolisms of humans. For example, 9-Octadecenoic acid, (E) (oleic acid) detected, has been documented for its antibacterial and antibiofilm activities against methicillin-resistant *Staphylococcus aureus*[13, 14]. It was also reported for its antioxidant property[15, 16]. Also, the n-Hexadecanoic acid identified is a phytoconstituent of *Pentanisia prunelloides* and *Feronia limonia* leaves, and known to possess antimicrobial[17], anti-inflammatory[18] and larvicidal[19] properties. Octadecanoic acid was known for its ability to lower LDL cholesterol in humans[20] and as anticancer bioactive compound of rodent tuber[21]. The stigmasterol identified has been investigated for its larvicidal and repellant activities[22], antimicrobial[23], antioxidant, anti-inflammatory, antimutagenic and antitumor effects[24, 25]. Identified 2-Hydroxychalcones, has been reported as anti-*Trichomonas vaginalis* and anticancer agent[26, 27]. The fatty acids detected possess immune modulatory and anticancer activities[28]. Heptadecanoic acid act against the skin cancer protein (Hsp90) with an effect that is superior to standard drug, dyclonine[29]. Identified compound 9,17-Octadecadienal, (Z) has been found to exhibit anti-flammatory activity[30] and antimicrobial property[31]. Cholesta-3,5-diene identified has earlier been found in *Psidium guajava* leaves extract and reported to bind DNA gyrase of *Salmonella enteric serovar* Typhi more efficiently than, ciprofloxacin, the standard drug used for the treatment of typhoid fever[32]. The identified ergost-5-en-3-ol (3 beta) (campesterol) is known to have cholesterol lowering and anti-carcinogenic effects[33]. Campesterol could prevent carcinogenesis in lung[34], gastric[35] and ovarium[36]. Stigmastan-3,5-diene present in *G. lotoides*is frequently found in vegetable oil, this compound is reported to have antifungal and antibacterial activities[37]. Dihydrotachysterol identified has been used in clinical practice as treatment for several renal and endocrine conditions[38]. This compound is also used to prevent the osteogenic effects of long-term treatment with corticosteroids and in various vitamin D resistance disorders[39, 40, 41].The compound methyl hexadec-9-enoate detected has been used to produce bioethanol, biodiesel and biohydrogen[42]. Undecanes have shown remarkably high antitumor activity[43]. The tetradecanoic acid identified has been

investigated for its larvicidal and repellant activities[44].

CONCLUSION

GC-MS analysis of the acetone - hexane extract suggests that numerous medicinally important bioactive constituents are present in *Glinus lotoides* leaf. This justifies the use of the plant for therapeutic and treatments of various ailments by traditional practitioner. *G*. lotoides possess some bioactive components which could be effective as anti-bacterial, anti-fungal anti-inflammatory, antioxidant and anti-cancer agents. Isolation of individual bioactive compounds may pave way for development of new drugs and can therefore contribute to the effective treatment of the diseases.

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