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THE EXTRACTION AND GC-MS CHARACTERIZATION OF LEAF EXTRACT OF *PIPER GUINEENSE* (UZIZA LEAF)

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ABSTRACT: *The extraction and GC-MS characterisation of leaf extract of Piper guineense (uziza leaf) were carried out. The leaves were collected, washed, shade dried and powdered. N-hexane extracts were prepared using the soxhlet extraction method. All the extracts were concentrated and analysed using Gas Chromatography-Mass Spectroscopy for the identification of biochemical components present in the leaves of Piper guineense and the results showed these results: alkaloids (+), flavonoids (+), cardiac glycosides (+), steroids (+), saponins (+) and tannins (-). In the GC-MS analysis of bioactive compounds, more than twenty-eight (28) bioactive compounds were discovered in the leaf extract of P. guineense. These compounds according to studies have medicinal values, which include β-sitosterol (C29H50O), MW(414), RT(9.404), Stigmasterol (C29H48O), MW(412), RT(9.542), Vitamin E (C29H50O2), MW (430), RT(10.095), Phytol (C20H40O), MW(296), RT(16.079), Cis-α-bisabolene (C15H24), MW (204), RT (18.863), 1- Heptatriacotanol (C37H76O), MW (536) and RT(8.128) among others, which are some of the essential bioactive compounds found in the GC-MS leaf extract. Consequently, the above bioactive compounds and others give the leaf a high potency of nutrients with some medicinal benefits, which make it good for consumption.*

KEYWORDS: Chromatography, Spectrometry, Soxhlation, Guineense, Peak.

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INTRODUCTION

Piper guineense is an erect herbaceous climbing liana native to tropical Africa of the Magnoliophyta division belonging to the order Piperacae. The fruits of *P. guineense* are widely applied externally as a counter-irritant or in a stimulating ointment internally as a stomachic and carminative. The leaves are effective in the treatment of wounds, while the stems and twigs are for the treatment of coughs and bronchitis (Owolabi, Lawal, Ogunwande, Hauser & Setzer, 2013). According to Imo, Uhegbu, Ifeanacho and Azubuike (2015), *Piper guineense* seeds have a higher percentage of dry matter (94.03±0.21), crude lipid (4.06±0.12) and carbohydrates (65.46±0.85) than the leaves, while the leaves have higher percentage moisture (6.11±0.01), protein (15.17±0.39), crude fibre (20.99±0.16) and ash (11.98±0.03) than the seeds. Several extracts of *Piper guineense* have been found to exhibit pharmaceutical and medicinal values. For example, the methanol extract of *Piper guineense* was found to protect against infection comparable to that of Livolin forte and even with better efficacy when pre-treated with 400 mg/kg for 14 days prior to CCl_4 exposure (Oyinloye, Osunsanmi, Ajiboye, Ojo & Kappo, 2017). Ekundayo, Laakso, Adegbola, Oguntimein, Sofowora and Hiltunen (1988) identified elemicin as the major essential oil constituent of the plant and stated that the plant has significant medicinal applications. Olonisakin, Oladimeji and Lajide (2006) identified (1s)-(-1)-ÃŽÂ²- pinene (43.9%), D-Limonene (7.7%), caryophyllene (6.9%), car-2-ene (5.4%) and 1,6,10-dodecetrien-zol, 3, 7, 11-trimetyl (2.9%) and found that they did not display any antimicrobial activity against *Escherichia coli*, *Serratia*, *Salmonella typhi*, *Klebsiella sp.*, *Citrobacter* and *Pseudomonas aeruginosa* due to the solvent he used. Chinwendu, Ejike, Ejike, Oti and Nwachukwu (2016) identified alkaloids (0.86%), HCN (8.87%), saponins (1.87%) and phenols (0.66%) in ethanol extract of *Piper guineense* leave while Ebenso, Eddy and Odiongenyi (2008) also identified the presence of alkaloids, tannins, saponins, flavonoids, hydrogen cyanides and phenols in ethanol extract of the leaves. They observed that *Piper guineense* (Uziza leaf) contains some considerable amounts of anti-nutrients, which have medicinal benefits. GC-MS analysis of plant extract has been found to be one of the most powerful tools that are useful for identifying chemical constituents of plants (Eddy, Ameh, Gimba & Ebenso, 2011). Ojinnaka, Ubbor, Okudu, and Uga (2016) identified 22 peaks from the GC-MS spectrum of ethanol extract of *Piper guineense* leaves and found that acids and hydrocarbon dominated the spectrum while alcohol and ester were the least constituents. A recent study conducted by Usman, Mohammed, Muhammed and Zakari (2020) indicated that the component extracted from plant parts depends on the type of solvent. However, most studies on *Piper guineense* leaves are done with aqueous and ethanol extract.

The antimicrobial ability of plant extracts and oils has established a platform for the processing and transforming these plant products into pharmaceuticals, and preservatives, especially the "uziza" fruit and leave and natural medicine. *P. guineense* 'Uziza' is of the family of Piperaceae and contains over 700 species worldwide. According to Dada, Ifesan, and Fashakin, (2013). Piper. guineensis 'Uziza' is used locally as a spice that comprises dillapiol, 5-8% of piperine, elemicin, 10% of myristicin and safrole, and these chemicals exhibit bactericidal and antimicrobial effects on certain microorganisms. These effects have been associated with the presence of phytonutrients such as flavonoids, alkaloids, glycosides, essential oils, tannins, saponins, peptides and phenols in this spectacular plant. Sumathykutty, Rao, Padmakumari and Narayan (1999) agreed that *P. guineense* 'Uziza' leaves are aseptic in nature, with the ability to relieve flatulence. They are also useful for treating intestinal diseases, cough, bronchitis and rheumatism. According to the research carried out by Nwachukwu, Ume, Obasi, Nzewuihe,

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and Onyirioha (2010) on the uses of some medicinal plants, they found out that *Piper guineense* 'Uziza' is suitable for treating Infertility in women and low sperm count in men. The authors agreed that women with infertility problems could boil *Piper guineense* 'Uziza' together with *Xylopia spp* (Uda), lime juice, honey, *Gongronema latifolium* (utazi) and *Capsicum spp* pepper in 1 litre of water and then take 1 glass cup on a daily basis only during menstruation (for women). Men, on the other hand, can take ½ bottle of lime juice mixed with 1 bottle of honey and take 1 shot twice on a daily basis for low sperm count treatment. According to Ashok and Upadhyaya (2012), tannins are phenolic compounds with proline-rich proteins that help to inhibit the absorption of iron when present in the gastrointestinal lumen. Igile, Iwara, Mgbaje, Uboh and Ebong (2013) also studied Uziza Leaf and found that in addition to the above-mentioned, it also contains a high amount of ash. This implies high mineral content such as calcium, zinc, magnesium, copper and potassium in the vegetables Ndamitso, Yisa, Dauda, and Jacob (2011). The crude fibre content of Uziza leaf was also found to be high, so consumption of this leaf could aid digestion, absorption of water from the body, bulky stool and prevent constipation, Udousoro and Ekanem (2013). According to Morufu, Serges, Ogochukwu. Besong, Mbamalu and Obimma (2016), the fat content of Uziza Leaf was found to be low, and so could be used to reduce overweight in humans. The GC-MS study of plant extract has been found to be one of the most powerful tools in identifying chemical constituents of plants, Eddy, Ameh, Gimba and Ebenso, (2011). Ojinnaka *et al*. (2016) identified 22 peaks from the GC-MS spectrum of ethanol extract of *Piper guineense* leaves and found that acids and hydrocarbon dominated the spectrum while alcohol and ester were the least constituents. A recent study conducted by Usman *et al*. (2020) indicated that the component extracted from plant parts depends on the type of solvent. However, most studies on *Piper guineense* leaves are done with aqueous and ethanol extract.

Experimental

This study was carried out from the month of September to November 2021, when the samples were obtained in Nkwerre, Imo state, Nigeria. *Piper guineense* leaves were purchased at Eke-ego market in Umoko Okwelle, Ideato LGA of Imo state, Nigeria and identified by a botanist. The leaves were sorted for healthy ones and washed thoroughly with de-ionized water and air-dried at room temperature (inside the laboratory) to avoid the loss of any volatile organic compounds (VOCs) and as well reduce the moisture contents. Then, when the leaves were dried, the dried sample was milled using a Victoria grinding machine. A recent study conducted by Usaman, Maryam, Samiratu, Suleiman, Raihana and Faiza (2020) indicated that the components extracted from plant parts depend on the type of solvent used. However, most studies on *Piper guineense* leaves are done with aqueous and ethanol extract. Therefore, this study aimed to identify the chemical constituents of the n-hexane extract of *Piper guineense* leaves using a Soxhlet extractor and characterise the extract using GC-MS analytical method. The grounded sample was loaded into the thimble and placed into the main chamber of the soxhlet extractor. 100 ml of n-hexane was added into a round bottom flask and placed into a heating mantle. A soxhlet extractor was attached above the round bottom flask, and a reflux condenser was attached above the soxhlet extractor. The cold water (water inlet) was connected, and the water outlet (warm water) was connected too. The power source was turned on, which supplied heat to the reflux. When the heated water meets the cold one, it condenses, while the temperature was maintained at 50 ºC and was allowed to extract for two (4) hours. The sample, which was initially dark green, turned a whitish-ash colour on complete extraction. The extract solution was evaporated using a rotary evaporator, and the solvent was recovered.

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Then the sample was again re-extracted with chloroform so as to obtain a purer sample that contains only the organic compounds (the hydrocarbons) and was sent to the University of Portharcourt, Department Of Chemical Sciences for GC-MS analysis on the 4th of September 2021. However, some quantity of the grounded leave was used for the phytochemical screening. The aqueous and ethanolic extract was used to analyse phytochemicals. The phytochemicals include alkaloids, flavonoids, steroids, tannins, cardiac glycosides and saponins. The presence of alkaloids was determined using a freshly prepared *Meyer's (potassium-mecuric iodide)* as described by Akpuaka (2009). 5.0 g of the powdered sample was soaked in 10 ml of water for 30 minutes, the solution was filtered with Watman filter paper No.1 and 2 ml of the filtrate was added to 2 ml of the Meyers reagent**.** The formation of a creamy precipitate was observed, indicating the presence of alkaloids. Test for Steroids 5.0 cm^3 of the water extract was treated with concentrated H_2SO_4 in acetic anhydride. The formation of a blue-green colour indicates the presence of steroids, Iwu, (2018). 5.0 g of the sample was weighed into a 250 cm^3 beaker, and 150 cm^3 of water was added and allowed to stand for 4 hours and then filtered. 10 cm^3 of the filtrate was measured into a 50 cm^3 and drops of ammonia and 3.0 cm^3 of concentrated H_2SO_4 was added. A yellow precipitate, which disappears on storage, indicates the presence of flavonoids. Iwu, (2018). The frothing test is based on the ability of the saponins to produce froth in aqueous solution. 5.0 g of the plant extract was weighed into a test tube, and 100 cm^3 of water was added and extracted after 4 hours. The water extract was shaken vigorously in a conical flask. The production of a stable froth indicates the presence of saponins in the sample, according to Iwu (2018). Also, the test for Steroids was conducted according to Iwu (2018), who described the test for steroids. 5.0 cm^3 of the water extract was treated with concentrated H_2SO_4 in acetic anhydride. The formation of a blue-green colour indicates the presence of steroids. A qualitative test for the presence of Glycosides was conducted by treating 20 cm^3 of the water extract with Fehling solutions of A and B in equal amounts and boiled. A brownish-red precipitate indicates the presence of glycoside. The reddish-brown colouration, which appeared on boiling, keeps getting highly intense and permanent on cooling. In the determination of the presence of tannins, 5.0 g of the sample was weighed into a beaker and 50 cm^3 of water was added and allowed to stand for 4 hours and extracted. The extract was treated with drops of ferric chloride. The initial colour remained unchanged (no blue-black colourations, which should indicate the presence of tannins). Therefore, tannins are absent.

200 g of sample was soaked in n-hexane for 48 hours and then extracted using a soxhlet extractor. The n-hexane extract was re-extracted using chloroform to obtain chloroform soluble extract. GC-MS analysis was carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) with the following conditions: Temperature programming from 80 – 200 ^oC held at 80 ^oC for 1 minute, the rate is 5^oC/min and at 200 ^oC for 20 minutes. FID Temperature of 300 ^oC, injection temperature of 250 ^oC, and the carrier gas is Nitrogen at a flow rate of 1cm^3/min and a split ratio of 1:75. GC-MS Gas chromatography, Mass spectrum analysis were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector temperature at 230 ^oC and carrier gas pressure of 100 Kpa. The column length was 30 m with a diameter of 0.25 mm and a flow rate of 50 m/min. The eluents were automatically passed into the Mass Spectrometer with a detector voltage set at 1.5 kv and a sampling rate of 0.2 seconds. The Mass Spectrometer was also equipped with a computer-fed Mass Spectra data bank and HERMCE Z 233 M-Z centrifuge Germany was used. Reagents and solvents such as Ethanol, Chloroform, Diethyl ether, and n-

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hexane, all of the analytics grade was obtained from Merck Germany Iwu, Onu, Chijioke-okere, Ukaoma and Azorji. (2016) and Iwu, Chijioke-okere, Onu and Uchegbu, (2018).

RESULTS AND DISCUSSION

The results obtained from the analysis of the leaf extract of *Piper guineense* based on the phytochemical analysis are summarised in the tables and figures below. Initial phytochemical analysis of the plant revealed the presence of alkaloids, glycosides, steroids and saponins, while tannins were absent.

Table 1.0. qualitative analysis results of leave extract of *P. guineense.*

S/N	Phytochemical	Results
1	Alkaloid	+
2	Steroid	+
3.	Flavonoid	+
4.	Saponin	+
5.	Cardiac glycosides	+
6.	Tannins	_

Key: present(+) absent(-)

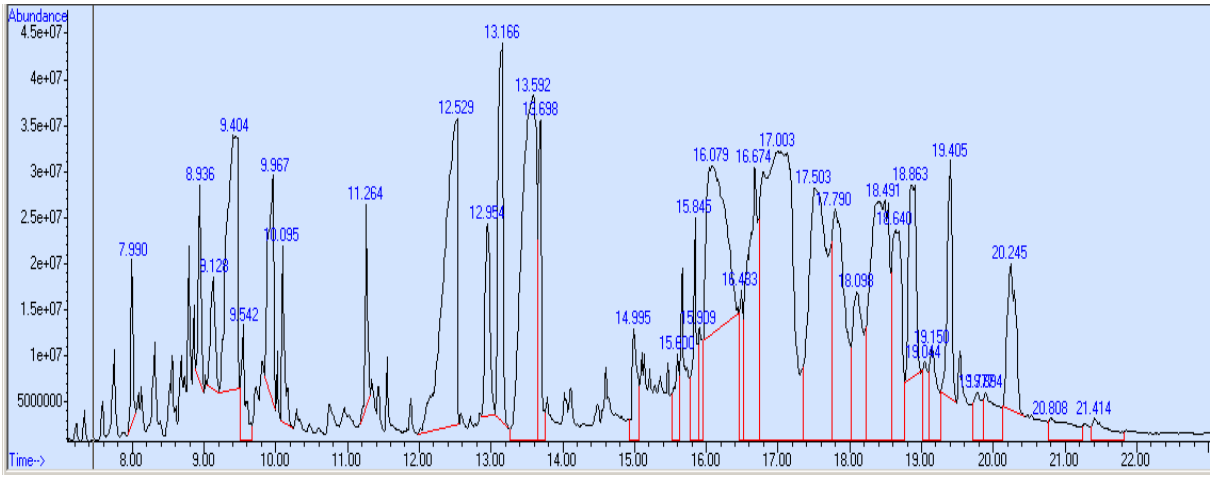

Plate 1.0.* Chromatogram(GC) analysis and mass spectral(MS) data of *P. guineense.

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Table 2.0. P. guineense showing a molecular formula, molecular weight, retention time and the highest 10 base peaks.

Peak	Compound	Molecular Formula	Molecular Weight	Retention Time	Highest 10 Peaks
1	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	7.990	43(999),55(914),41(867),57(797),69(609),81(507),44(492),29(476),17(469),83(460).
2	i-propyl l9,12-octadecenadienoate	$C_{21}H_{38}O_2$	322	8.936	67(999),54(949),81(939),95(769),69(569),82(529),96(429),109(419),68(409),279(389).
3	1-Heptatriacotanol	$C_{37}H_{76}O$	536	8.128	43(999),55(907),69(878),41(750),81(675),57(567),95(556),69(537),91(525),91 (474)
4	β-sitosterol	$C_{29}H_{50}O$	414	9.404	43(999),55(639),41(561),57(447),81(427),105(392),195(387),107(318),91(377),69(348)
5	Stigmasterol	$C_{29}H_{48}O$	412	9.542	55(999),83(869),81(737),255(634),41(455),95(447),43(445),97(427),133 (409),279(395).
6	Crinamidine,3-oxo-	$C_{17}H_{17}NO_5$	315	9.967	42(999),230(976),315 (961),56(883),231(252),77(709),115(635),203(597),57 (596),51 (587).
7	Vitamin E	$C_{29}H_{50}O_2$	430	10.095	165(999),43(419),57(410),164(326),67(67),71(226),55(185),41(171),85(135),166 (117).
8	Androstan-3-one,17-methoxy-3-methoxime,(5a,17 β)-	$C_{21}H_{35}NO_2$	333	11.264	302 (999), 69 (845), 55 (701), 79(694), 67 (678), 93 (629), 105 (617), 70 (591), 91 (579), 81 (568).
9	Bis-(3,4-dimethoxy phenyl)hydroxyacetic acid methyl ester	$C_{19}H_{22}O_7$	362	12.529	169(999),303(239),15(141),166 (107),77 (99),137 (83),79 (75),122 (59),18 (47),92 (47).
10	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	12.954	67 (999),81 (849),41 (803),55 (728),95(589),29(507),69(422),82 (421),68 (406),79 (383).
11	2-[4-methyl-6-(2,6,6trimethylhex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-carboxaldehyde	$C_{23}H_{32}O$	324	13.166	43(999),41(590),55(500),91(460),135(430),69 (420),79 (370),105 (360),95 (350),81 (340).

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12	2HBenzo(f)Oxireno[2,3-E]benzofuran-8-(9H)-one,9[[(1,3-benzodioxol-5-ylmethyl)amino] methyl] octadione	$C_{23}H_{29}NO_5$	399	13.952	135(999),136(269),77(178),68 (93),105 (85),55 (73),202 (66),79 (59),264 (59),41 (55).
13	Octanoic acid, 6-ethyl-3-octyl ester	$C_{18}H_{36}O_2$	284	13.698	127(999),84 (467),69 (406),55 (337),43 (315),70 (280),57 (274),111 (245),41 (196),85 (180).
14	Pyrrolidine,1-(1-oxo-10-octadecynyl)-	$C_{22}H_{39}NO$	333	14.995	113(999),55 (428),43 (318),41 (311),70 (304),126 (256),67 (223),81 (190),72 (175),71 (150).
15	Butanoic acid,2-(1-adamatyl)-3-oxo,ethyl ester	$C_{16}H_{24}O_3$	264	15.800	135(999),43 (149),79 (113),136 (111),93 (101),91 (87),41 (65),92 (64),77 (49),222 (48).
16	Octadecanoic acid	$C_{18}H_{36}O_2$	284	15.845	43 (999),73 (942),60 (881),57 (875),55 (827),41 (688),129 (500),69 (432),71 (429),83 (317).
17	9,12-octadecadienoic acid (ZZ)	$C_{18}H_{32}O_2$	280	15.909	67 (999),55 (954),81 (793),41 (708),69 (560),95 (545),68 (530),54 (486),43 (480),82 (479).
18	Phytol	$C_{20}H_{40}O$	296	16.079	71(999),43 (381),57 (334),41 (260),55 (259),69 (239),81 (223),68 (199),123 (184),56 (169).
19	1-Eicosanol	$C_{20}H_{42}O$	298	16.483	43 (999),55 (893),41 (799),57 (743),83 (620),69 (617),97 (542),56 (358),82 (350),71 (330).
20	n-hexanoic acid	$C_{16}H_{32}O_2$	256	16.674	60(999),73 (980),57 (840),43 (817),55 (767),41 (574),129 (435),71 (373),69 (351),83 (267).
21	3,7,11,15 tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	17.003	81 (999),82 (986),43 (965),95 (962),123(892),55(852),41(811),57 (748),71 (748),68 (728).
22	3,5-dimethoxy cinnamic acid	$C_{11}H_{12}O_4$	208	17.503	208(999),77 (221),161 (173),163 (159),177 (149),91 (142),51 (141),133 (138),148 (131),105 (126).

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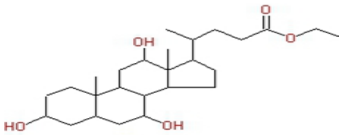
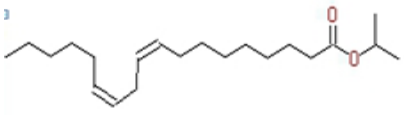
23	Apiol	$C_{12}H_{14}O_4$	222	17.790	222(999),207(239),149(158),177 (156),223 (137),195 (108),77 (102),39 (96),65 (95),91 (83).
24	1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadine	$C_{15}H_{26}O$	222	18.098	81 (999)43 (766),41 (394),123 (207),80 (196),55 (194),161 (189),105 (172),79 (168),67 (166).
25	Benzene-1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	208	18.491	208 (999),193 (569),209 (137),91 (93),105 (85),133 (84),79 (81),77 (80),177 (69),105 (63).
26	Naphthalene1,2,3,5,6,8a hexahydro-4,7-dimethyl-1-(1-methyl)-,1S-cis)-	$C_{15}H_{24}$	204	18.640	161(999),119 (899),105 (726),41 (754),91 (709),134 (617),204 (399),81 (370),77 (352),93 (324).
27	Cis-α-Bisabolene	$C_{15}H_{24}$	204	18.863	93(999),41 (747),91 (948),67 (344),79(344),39(342),77(303),27 (290),53 (272),29 (241).
28	Caryophyllene	$C_{15}H_{24}$	204	19.150	41(999),69 (976),93 (937),133 (646),79 (614),91 (551),55 (432),81 (389),107 (389),105 (372).



Compound 1



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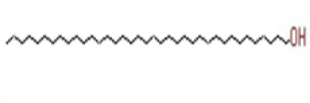

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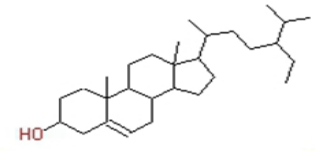

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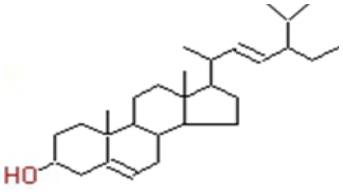

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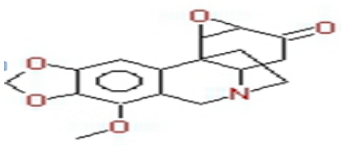

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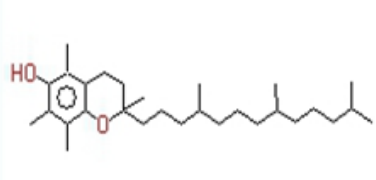

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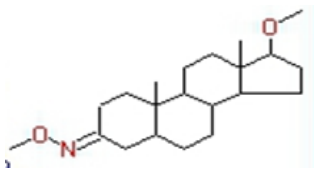
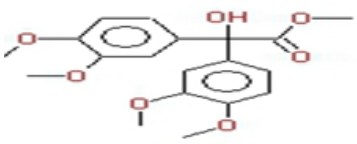


Compound 8



Compound 9

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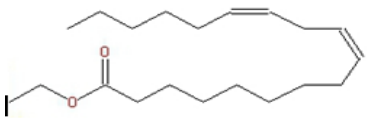

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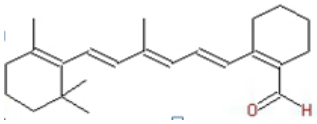

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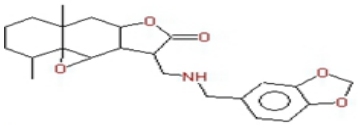

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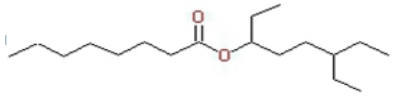

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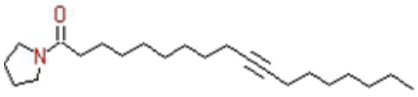

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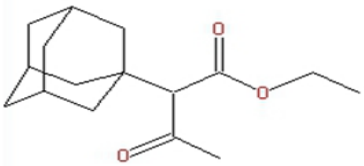

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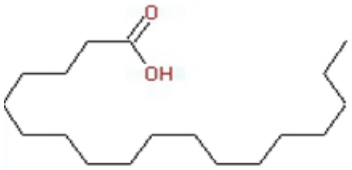

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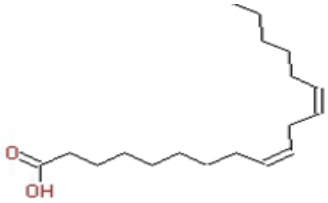

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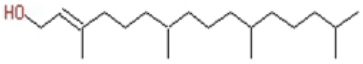

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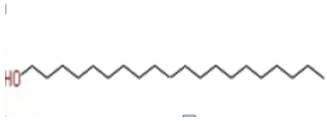

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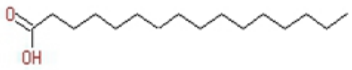

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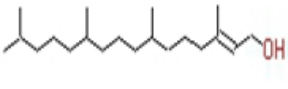

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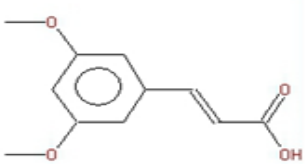

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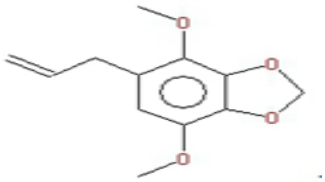

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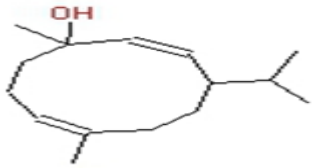

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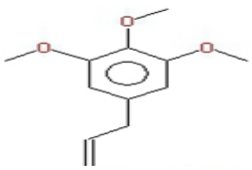

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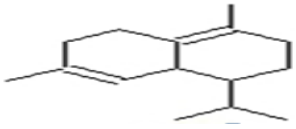

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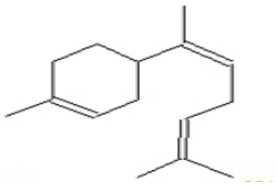

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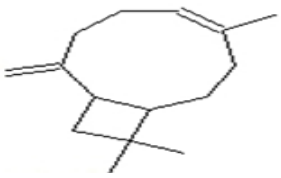

Compound 28

Fig. 1. Structures of the 28 isolated compounds were as follows;

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DISCUSSION

The quantitative phytochemical tests on the leave extract of *p. guineense* (uziza leave) carried out in this study are summarised in Table 1.0. The results showed the presence of alkaloids, steroids, saponins, flavonoids and cardiac glycosides, whereas tannins are absent in the leaf extract of *Piper guineense*. The presence of alkaloid shows that the leaf has some pharmacological activities, and steroids have some reproductive activities since it dominates in mostly sex hormones. Saponins have anti-carcinogenic properties in accordance with Adesokan and Akanji (2010) and may play an important role in the antimalarial activity of plants. The use of n-hexane as a solvent in GC-MS analysis of piper guineense leave is very rare; however, a recent study conducted by Usaman *et al.* (2020) requires that component extracted from plant parts is solvent dependent. Therefore, this study used n-hexane as a solvent, using the soxhlation method. However, the extract was re-extracted with chloroform to concentrate the crude and purer organic compounds (the hydrocarbons). In the GC-MS analysis, thirty-five (35) different compounds were observed, of which a higher percentage of them have medicinal and pharmacological benefits. Plate 1.0 shows the GC-MS data of *P. guineense*. The spectrum contains 35 peaks, each peak signifies a particular compound, and the spectrum contains 35 different compounds. The GC-MS is a fusion of gas chromatography and mass spectroscopy. The percentage abundance, molecular mass, fragmentation pattern and as well the functional group of each compound detected by the analyzer are shown in the spectrum. The functional group of each compound are indicated in red colour. The retention time is also indicated on the x-axis of the spectrum ranging from (8.00-22.00). The mass spectroscopy part of it is the molecular mass of each detected compound. The percentage abundance is on the Y-axis of the spectra, while the mass-to-charge ratio (m/z) is on the X-axis of the spectra. The smallest peak, which is always the last peak on the spectra, is known as the molecular ion peak, it shows the molecular mass of the compound. Table 2.0 summarises the molecular formula, molecular weight, retention time and the highest 10 base peaks of each compound detected by the GC-MS. Some compounds shown by the GC-MS analysis have been studied on their medicinal and non-medicinal benefits to man; β-sitosterol with the molecular formula $C_{29}H_{50}O$ and molecular weight 414 is similar to cholesterol, it reduces the cholesterol level by limiting the amount of cholesterol that is able to enter the body. It is also bound to prostrate, thereby reducing inflammation. Stigmasterol, with the molecular formula $C_{29}H_{48}O$ and molecular weight 412, is a phytosterol which maintains the structure and physiology of cell membranes. Vitamin E, which was also found in the leaf extract, is responsible mainly for vision and reproduction as well as the health of the brain and skin. Phytol ($C_{20}H_{40}O$) acts as a terpene, which works in a similar way to piperine. Piperine is responsible for the sweet scent of the leave. Phytol produces fragrance. Consequently, the fragrance industry uses it in cosmetics, shampoo, toilet soaps etc. It acts as an antioxidant and anti-ageing in cosmetics. C is-α-bisabolene ($C_{15}H_{24}$) is a kind of sesquiterpene, which has been described to have antiseptic and antibacterial properties. 1-Heptatriacotanol possesses an anti-hypercholesterolemic effect. Therefore, this work is an investigative analysis of the *P. guineense* leaves on new bioactive compounds when an unusual solvent is used and as well as knowing their functions and benefits to the human body system. Fig. 1 also shows the structures of the isolated compounds.

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DEDUCTIONS AND CONCLUSIONS

Piper guineense leave has a high coefficient of medicinal and pharmacological values. This study has revealed that it contains several bioactive compounds, which show high potency in tackling several diseases like rheumatism, cancer, and arthritis, it was also discovered that it contains anti-hyper cholesterol, anti-ageing, anti-oxidant and lots more. This work is a kind of proof of the work conducted by Usaman *et al.* (2020), who said that the kind of compounds shown by the GC-MS analysis depends on the solvent used. There are other works, which have been done on *Piper guineense* which showed some bioactive compounds, but mainly on the use of ethanol, acetone etc as solvents, but this work used n-hexane as a solvent, and it showed several new bioactive compounds, unlike others. In comparison with the work done by Nduche, Egbucha and Amakwe, (2018), based on the phytochemicals aspect, the sample preparation differs with this work. In their work, the leaf extract of p. guineense was oven dried for five (5) hours, milled and stored before the phytochemical screenings. In their work, all phytochemicals were present, including tannins. The sample preparation method could influence the presence of tannins. Here, the samples were shade-dried at room temperature, milled and screened immediately, resulting in the absence of tannins compared with the report of Nduche *et al.* (2019). In conclusion, much scientific research has been carried out to investigate the uses of this particular leave, both pharmacological and non-pharmacological uses, and all came out proving that *P. guineense* leave has several medicinal and non-medicinal benefits. This work has successfully achieved its aim by using an unusual solvent to discover some new bioactive compounds in the leave using standard procedures.

RECOMMENDATIONS

Since a study conducted by Usaman *et al*. (2020) revealed that the bioactive compounds obtained in GC-MS analysis are dependent on the type of solvent used, We, therefore, recommend that different solvents other than n-hexane (which was used in this study), should be used by researchers to quest more on the medicinal values of *P.Guineense*. We also recommend that, since this leaf extract contains some pharmacologically active compounds, which could be used to treat several impairments in human beings, those bioactive compounds should be extracted and used alternatively with other similar synthetic drugs in the treatment of diseases.

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