

Elucidation of Bioactive Compounds in Methanol Extract of *Mitracarpus Vilosus* Leaf using GC-MS analysis

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Abstract.

Mitracarpus vilosus is widely used among the indigenes of Ibeju-Lekki area of Lagos State for the treatment of skin lesions. This study elucidated the phytochemical constituents in 80% methanolic leaf extract of *Mitracarpus vilosus*. Phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analyses were carried out on the leaf extract. The qualitative analyses showed the presence of the following phytochemicals, tannins, terpenoids, saponins, cardiac glycosides and steroids. The phytochemical screen did not reveal any flavonoids. However, Squalene 14.27%, n-Hexadecanoic acid 11.60%, 2-Octylcyclopropene-1-heptanol 8.43%, Octadecanoic acid 5.45%, 1,4-Naphthalenedione 3.19% were the first five major bioactive compounds revealed by GC-MS in the leaf extract. The results of this study validated the bioactive constituents in *Mitracarpus vilosus* which may be the justification for its use for the treatment of skin lesions and other ailments.

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Introduction

Previous study has documented man's reliance on medicinal plants for the treatment of various ailments before the arrival of modern medicine. (1). With over 3.3 billion people in undeveloped countries depending on medicinal plants for cure. (2). The medicinal benefits of these plants have been linked to their phytochemical contents as well as their secondary metabolites. (3) Literature have also reported the use of medicinal plant in the treatment of diseases such as Hypertension, diabetes, inflammatory diseases and bacterial infections, skin infections. (4) *Mitracarpus* species, a plant of the Rubiaceae family have been documented as a medicinal plant and used in the treatment of various medical conditions such as fungal infections, liver diseases, hypertension, diabetes mellitus, cancer and stroke (5), Unfortunately, documentation on the genus *Mitracarpus* has not been exhaustive in view of claims on its medicinal benefits. *Mitracarpus vilosus* belongs to the Rubiaceae family, it is an annual plant which grows to a height of about 2 feet. This plant grows as weed on uncultivated soils in tropical countries such as Nigeria, Senegal and Liberia. In Nigeria, the Yorubas call it 'Irawo Ile' (6), while it is called 'Obuobwa' and 'Gududal' by Ibos and Sokoto Fulanis respectively (7). *Mitracarpus* species grows freely on uncultivated ground in Ibeju-Lekki area of Lagos State Nigeria and it has been classified as a weed with medicinal benefit at 0.7% frequency (8). The leaves of *Mitracarpus* species are widely used in traditional medicine for the treatment of various diseases (9). This study therefore elucidated the bioactive compounds in Methanol extract of *Mitracarpus vilosus* leaf growing on uncultivated ground in Ibeju-Lekki area of Lagos State, using GC-MS analysis.

MATERIALS AND METHODS

Plant collection

Fresh aerial parts of *Mitracarpus vilosus* leaves were collected from uncultivated farm land at Siriwon village of Ibeju-Lekki area of Lagos State, Southwest Nigeria. Plant sample was identified by Mr. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria.

Preparation of plant extract

The leaves were thoroughly cleaned under running tap water and air-dried on a clean laboratory bench at ambient temperature for 7 days. The dried leaves were separated from stalks and grinded to fine powder using porcelain mortar and pestle. Methanol extract was prepared by weighing 100 g dried powder, which was soaked in 2 L of 80% methanol for 72 h with intermittent mixing. This was thereafter filtered with a muslin cloth followed by Whatman No.1 filter paper. The filtrate was concentrated and evaporated at 40°C under reduced pressure using rotary evaporator to obtain the extract.

Phytochemical screening

Phytochemical tests were carried out on the methanol extract of *Mitracarpus vilosus* leaf to identify the

constituents using standard procedures (10, 11,12).

Tannins test

0.5 g of extract was boiled in 20 ml of water in a test tube and filtered. Few drops of 0.1% ferric chloride was added to the filtrate. The occurrence of blackish green coloration confirmed the presence of tannins.

Saponin test

2 g of extract was dissolved in 20 ml of distilled water, boiled in a water bath and then filtered. 5 ml of distilled water was thereafter mixed with 10 ml of the filtrate and vigorously shaken to give a consistent froth. 3 drops of olive oil were added to the froth. The presence of emulsion formation indicated the presence of saponin.

Flavonoids test

5 ml of dilute ammonia solution was added to 1 ml of methanol extract with a drop of conc. H₂SO₄. The absence of brownish precipitate indicated that flavonoid was absent.

Steroids test

0.5 ml of methanol extract was added to 2 ml of acetic anhydride with 2 ml H₂SO₄. The colour change from violet to blue confirmed the presence of steroids.

Cardiac Glycosides test (Keller-Killani test)

Five ml of methanol extract was mixed with a solution of 2 ml of glacial acetic acid with a drop of ferric chloride. 1 ml of concentrated H₂SO₄ was slowly and cautiously added. The presence of brown ring at interface demonstrated the presence of deoxysugar characteristic of cardenolides.

Test for terpenoids (Salkowski test):

Five ml of methanolic extract was mixed in 3 ml of concentrated H₂SO₄ was carefully added after 2 ml of chloroform was added. A reddish brown colouration at the inter face was formed to show positive results for the presence of terpenoids.

GC-MS analysis

The GC-MS analysis was carried out on metanol extract of *Mitracarpus vilosus* according to the method described below by Ajayi et al. (13, 14).

The GC-MS used comprised of a Gas Chromatograph (Model 6890 series), a product of Hewlett Packard which was equipped with flame ionization detector (FID) and coupled with 7683 series injector (Hewlett Packard) possessing 250°C temperature MS transfer line. The column of the GC was a fused silica capillary column-HP-5MS (30 x 0.25 mm) with film thickness of 1.0 µm. Oven temperature was initially set at 50°C for 5 min and then raised to 250°C at a rate of 2°C /min and carrier gas used was helium gas (99.99%). 1.0 µl of leaf extract was injected at a split ratio of 1:30. Agilent Technology Network Mass Spectrometer (Model 5973 series) equipped with NIST08 Library software database was used for the identification of compounds by comparing the mass spectrum of unknown compounds with the known compounds stored in the software database Library.

RESULTS

Qualitative phytochemical screening

The qualitative phytochemical screening of methanol extract of the leaf of *Mitracarpus vilosus* revealed the presence of tannins, terpenoids, saponins, cardiac glycosides and steroids. The phytochemical screen did not reveal any flavonoids. (Table 2).

GC-MS analysis

The chromatographic profile of the methanol extract of *Mitracarpus vilosus* is shown in Figure 1, while the list of phytochemical compounds is in Table 1. A total of 67 bioactive compounds were identified (Table 1) corresponding to the peaks shown in Figure 1. However, the first five highest bioactive compounds were our focus (Table 3), which included Squalene 14.27%, n-Hexadecanoic acid 11.60%, 2-Octylcyclopropene-1-heptanol 8.43%, Octadecanoic acid 5.45%, 1,4-Naphthalenedione 3.19%. The GC-MS mass spectrum and structures of these five major bioactive compounds are shown in Figures 2-6.

Peak#	R.Time	I.Time	F.Time	Peak Report TIC				A/H	Mark	Name
				Area	Area%	Height	Height%			
1	5.172	5.142	5.242	1549428	0.13	483365	0.14	3.21		3,4Dihydro-dl-proline
2	5.269	5.242	5.350	1627795	0.13	662372	0.20	2.46	V	6-Oxa-bicyclo[3.1.0]hexan-3-one
3	5.666	5.625	5.783	2353945	0.19	1022108	0.30	2.30		2-Furancarboxaldehyde, 5-methyl-
4	6.069	6.050	6.125	1239415	0.10	429571	0.13	2.89	V	trans-2,3-Epoxy-nonane
5	6.586	6.550	6.658	9017610	0.73	3964855	1.18	2.27	V	Thiophene, 2-methoxy-5-methyl-
6	7.026	6.983	7.125	4868343	0.39	1270692	0.38	3.83	V	Isosorbide Dinitrate
7	7.158	7.125	7.233	3671632	0.30	1241787	0.37	2.96	V	2,4-(1H,3H)-Pyrimidinedione, 5-hydroxy-
8	7.273	7.233	7.300	1676478	0.14	701699	0.21	2.39	V	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl-
9	7.474	7.400	7.492	1378651	0.11	372507	0.11	3.70	MI	1,2-Diazabicyclo[2.2.2]octan-3-one, 2-hydr
10	8.241	8.192	8.358	1552929	0.13	354809	0.11	4.38		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy
11	8.876	8.817	8.992	2921363	0.24	676882	0.20	4.32	V	Allyl heptanoate
12	9.350	9.267	9.350	1489134	0.12	543363	0.16	2.74	MI	Hydroquinone
13	9.377	9.350	9.550	7456397	0.60	1193217	0.35	6.25	MI	Benzofuran, 2,3-dihydro-
14	10.458	10.408	10.483	2775795	0.22	1606057	0.48	1.73		2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydr
15	10.629	10.567	10.725	11212743	0.91	2203042	0.65	5.09	V	2-Methoxy-4-vinylphenol
16	10.775	10.725	10.883	6714850	0.54	919405	0.27	7.30	V	2-Deoxy-D-galactose
17	11.009	10.950	11.058	2714788	0.22	536468	0.16	5.06	V	Trifluoroacetyl-.alpha.-terpineol
18	11.200	11.150	11.242	1662057	0.13	469050	0.14	3.54	V	8-Methyl-6-nonenoic acid
19	11.316	11.242	11.417	5820882	0.47	1112461	0.33	5.23	V	Octane, 1-(ethenylthio)-
20	12.002	11.975	12.058	1073567	0.09	494971	0.15	2.17	V	Diethylene glycol monododecyl ether
21	12.443	12.358	12.508	3196092	0.26	475754	0.14	6.72	V	cis-p-Mentha-2,8-dien-1-ol
22	12.600	12.558	12.642	2255925	0.18	690869	0.20	3.27	V	1,2,4-Oxadiazole, 3-methyl-5-phenyl-
23	12.672	12.642	12.725	3195266	0.26	1230267	0.37	2.60	V	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but
24	12.790	12.725	12.825	4395052	0.36	1248010	0.37	3.52	V	Nonanoic acid
25	13.052	13.008	13.075	8350855	0.68	2367051	0.70	3.53	V	3,4-Altrosan
26	13.355	13.333	13.392	3655679	0.30	1438148	0.43	2.54	V	11-(2-Cyclopenten-1-yl)undecanoic acid, (±
27	13.525	13.492	13.542	2790739	0.23	1303587	0.39	2.14	V	Dodecanoic acid
28	14.049	13.967	14.117	25485687	2.06	10764323	3.19	2.37	V	1,4-Naphthalenedione, 2,3-dimethyl-
29	14.811	14.742	14.883	12873841	1.04	2978765	0.88	4.32	V	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,
30	15.037	14.983	15.117	10133611	0.82	2020407	0.60	5.02	V	Propionic acid, 3-(1-hydroxy-2-isopropyl-2-
31	15.205	15.117	15.283	18413816	1.49	5271601	1.56	3.49	V	Tetradecanoic acid
32	15.317	15.283	15.417	7607681	0.62	1271316	0.38	5.98	V	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion
33	15.792	15.725	15.817	6987710	0.57	1568485	0.47	4.46	V	Butyl 9-decenoate
34	15.842	15.817	15.858	5649108	0.46	3152762	0.94	1.79	V	Cyclopentadecanone
35	15.883	15.858	15.908	13090830	1.06	8677358	2.57	1.51	V	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
36	15.933	15.908	16.017	13154226	1.07	3612155	1.07	3.64	V	Pentadecanoic acid
37	16.048	16.017	16.075	5417615	0.44	2395476	0.71	2.26	V	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
38	16.182	16.150	16.208	6667545	0.54	3489409	1.04	1.91	V	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
39	16.384	16.325	16.417	9292901	0.75	3478904	1.03	2.67	V	Cyclopentanetricadecanoic acid, methyl ester
40	16.453	16.417	16.475	7338137	0.59	2710250	0.80	2.71	V	Propanol, 1,1-bis(1-aza-4-oxabicyclo[4.3.0
41	16.589	16.517	16.608	13314943	1.08	5048119	1.50	2.64	MI	1H-Benzofuro[3,2-e]indole, 1-[2-(aminocar
42	16.699	16.608	16.833	143246726	11.60	31063806	9.22	4.61	MI	n-Hexadecanoic acid
43	17.102	17.042	17.217	24765923	2.01	3675254	1.09	6.74	V	Dodecanoic acid, 1-methylethyl ester
44	17.289	17.217	17.433	26077068	2.11	3539132	1.05	7.37	V	Eicosanoic acid
45	17.476	17.433	17.492	7856845	0.64	3313948	0.98	2.37	V	9,12-Octadecadienoic acid (Z,Z)-
46	17.531	17.492	17.592	17158495	1.39	5104869	1.51	3.36	V	14-Pentadecenoic acid
47	17.659	17.592	17.683	16721178	1.35	6265487	1.86	2.67	V	Phytol
48	17.800	17.683	17.800	104081666	8.43	27485111	8.15	3.79	MI	2-Octylcyclopropene-1-heptanol
49	17.820	17.800	17.883	72664220	5.89	31268202	9.28	2.32	MI	2-Octylcyclopropene-1-heptanol
50	17.951	17.883	18.000	48666196	3.94	18367877	5.45	2.65	MI	Octadecanoic acid
51	18.086	18.000	18.117	46134878	3.74	9352024	2.77	4.93	MI	Vitamin E
52	18.149	18.117	18.242	27570680	2.23	9659663	2.87	2.85	MI	2,3-Dihydro-5-hydroxy-4-methyl-2-oxonap
53	18.507	18.417	18.550	19594483	1.59	3550057	1.05	5.52	V	1-Heptatriacontanol
54	18.659	18.617	18.750	26457096	2.14	5507835	1.63	4.80	V	Benzene, 1-acetyl-4-(4-propylcyclohexyl)-
55	18.928	18.850	18.958	15815336	1.28	3573546	1.06	4.43	V	4(3H)-Pteridinone, 2-amino-7,8-dihydro-8-
56	18.988	18.958	19.050	25611655	2.07	7484061	2.22	3.42	V	9-Octadecenamide, (Z)-
57	19.076	19.050	19.117	9632218	0.78	3153012	0.94	3.05	V	Octadecanoic acid
58	19.144	19.117	19.258	17702069	1.43	3154309	0.94	5.61	V	Estra-1,3,5(10)-trien-17.beta.-ol
59	19.636	19.542	19.667	14371183	1.16	2638563	0.78	5.45	V	9,10-Secocholesta-5,7,10(19)-triene-1,3-dic
60	19.787	19.750	19.800	8234224	0.67	3324516	0.99	2.48	V	4-Hexyl-1-(7-methoxycarbonylheptyl)bicyc
61	19.873	19.800	19.983	31994306	2.59	4186087	1.24	7.64	V	Canpestrol
62	20.436	20.275	20.583	53073241	4.30	6640261	1.97	7.99	V	Stigmasterol
63	20.714	20.675	20.775	13809938	1.12	3164046	0.94	4.36	V	1-Hydroxy-3-methoxy-6-methylanthraquin
64	22.097	21.900	22.133	176146391	14.27	49383851	14.65	3.57	V	Squalene
65	22.207	22.167	22.258	11685451	0.95	3135010	0.93	3.73	V	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3
66	22.293	22.258	22.375	10080425	0.82	2034655	0.60	4.95	V	Methanesulfonic acid, 2-(3-hydroxy-4,4,10
67	22.481	22.417	22.567	9205942	0.75	1561213	0.46	5.90	V	Methanesulfonic acid, 2-(3-hydroxy-4,4,10

Table 1: Phytochemical compounds identified by GC-MS in Methanol extract of *Mitracarpus vilosus* leaf

S/N	PHYTOCHEMICAL	STATUS
1	Tannins,	Present
2	Terpenoids,	Present
3	Saponins,	Present
4	Cardiac glycosides	Present
5	Steroids	Present
6	Flavonoids	Absent

Table 2: Result of phytochemical screening of the Methanol extract of *Mitracarpus vilosus* leaf

S/N	Name of Compound	Molecular Weight	Biological uses
1	Squalene	410	A precursor for the synthesis of plant and animal sterols, including cholesterol and steroid hormones in the human body. Antifungal agent.
2	n-Hexadecanoic acid	256	An antioxidant and an anti-inflammatory substance
3	2-Octylcyclopropene-1-heptanol	266	Antibacterial Activity
4	1,4-Naphthalenedione	158	Potential inhibitor of monoamine oxidase and DNA topoisomerase activities. Inhibit the acetyltransferase activity.
5	Octadecanoic acid	284	it is used in hardening soaps, softening plastics and in making cosmetics, candles and plastics

Table 3: Shows the Molecular weight and biological activities of the 5 major phytochemical compounds found in the Methanol extract of *Mitracarpus vilosus* leaf identified by GC-MS

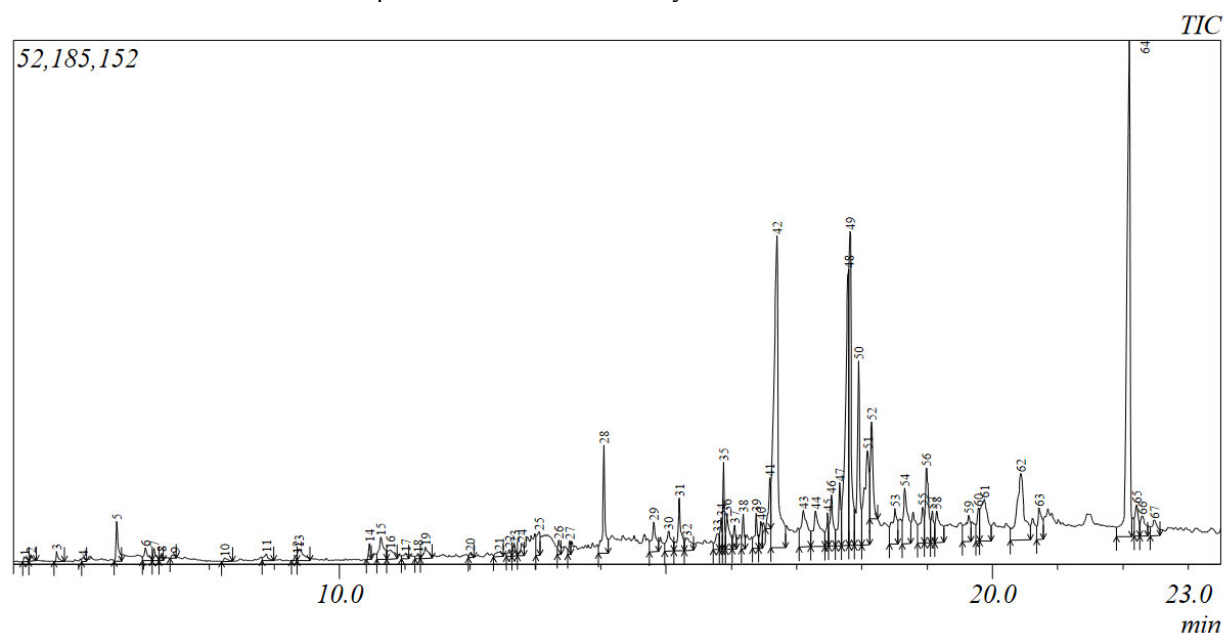


Figure 1. GC-MS chromatogram of Methanol extract of the leaf of *Mitracarpus vilosus*, showing different peaks of the phytochemical components identified.

SI:86 Formula:C30H50 CAS:111-02-4 MolWeight:410 RetIndex:2914
 CompName:Squalene \$\$ 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- \$\$ all-trans-Squalene \$\$ trans-Squalene \$\$ Spinacen \$\$ Spin

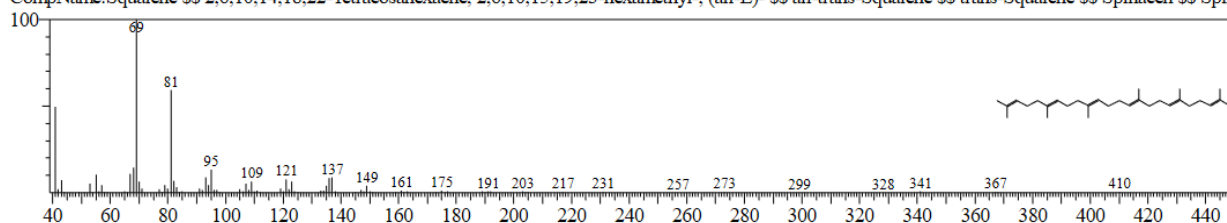


Figure 2. GC-MS mass spectrum and molecular structure of Squalene

SI:68 Formula:C16H32O2 CAS:57-10-3 MolWeight:256 RetIndex:1968
 CompName:n-Hexadecanoic acid \$\$ Hexadecanoic acid \$\$ n-Hexadecic acid \$\$ Palmitic acid \$\$ Pentadecanecarboxylic acid \$\$ 1-Pentadecanecarboxylic acid

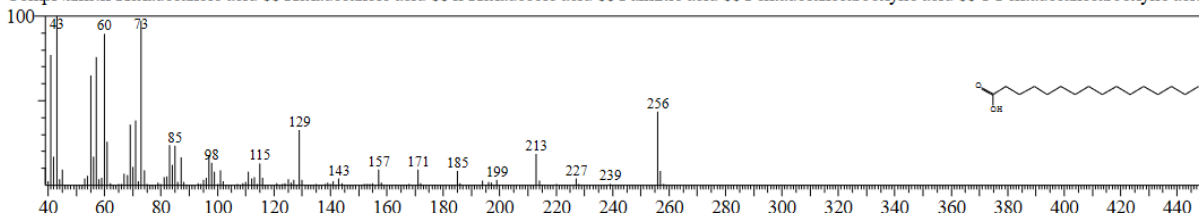


Figure 3. GC-MS mass spectrum and molecular structure of n-Hexadecanoic acid

SI:87 Formula:C₁₈H₃₄O CAS:54467-85-5 MolWeight:266 RetIndex:2056
CompName:2-Octylcyclopropene-1-heptanol \$\$ 7-(2-Octyl-1-cyclopropen-1-yl)-1-heptanol # \$\$

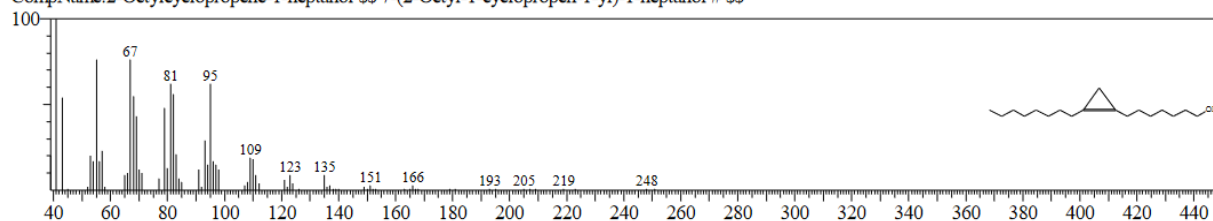


Figure 4. GC-MS mass spectrum and molecular structure of 2-Octylcyclopropene-1-heptanol

Hit#:23 Entry:106158 Library:NIST11.lib
SI:60 Formula:C₁₈H₃₆O₂ CAS:57-11-4 MolWeight:284 RetIndex:2167
CompName:Octadecanoic acid \$\$ Stearic acid \$\$ n-Octadecanoic acid \$\$ Humko Industrine R \$\$ Hydrofol Acid 150 \$\$ Hystrene S-97 \$\$ Hystrene T-70 \$\$ Hys

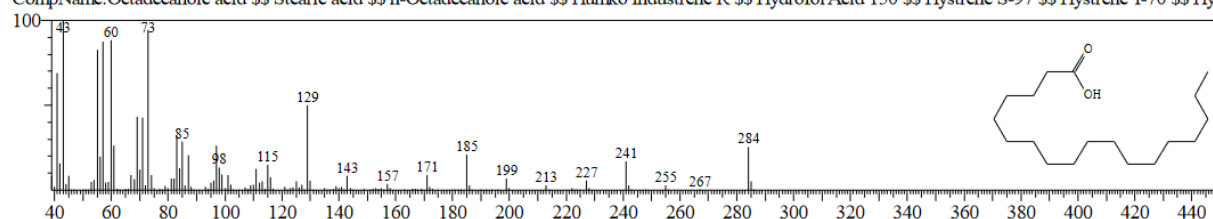


Figure 5. GC-MS mass spectrum and molecular structure of Octadecanoic acid

SI:85 Formula:C₁₂H₁₀O₂ CAS:2197-57-1 MolWeight:186 RetIndex:1670
CompName:1,4-Naphthalenedione, 2,3-dimethyl- \$\$ 1,4-Naphthoquinone, 2,3-dimethyl- \$\$ 2,3-Dimethyl-1,4-naphthoquinone \$\$ 2,3-Dimethylnaphthoquinone

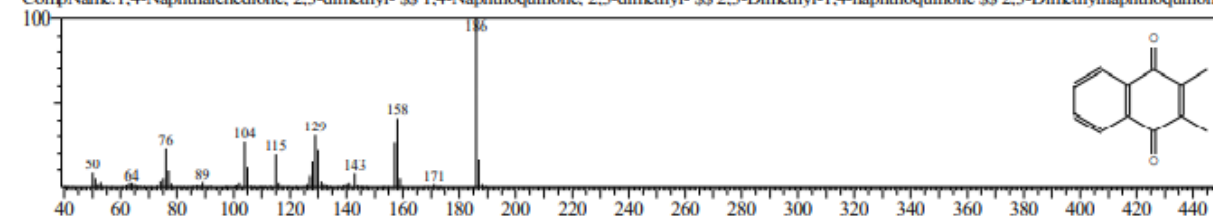


Figure 6. GC-MS mass spectrum and molecular structure of 1,4-Naphthalenedione

Discussion

This study evaluated and elucidated the bioactive constituents of *Mitracarpus vilosus*, a medicinal plant used traditionally for the treatment of different diseases.

Medicinal plants and their uses have been widely explored for the treatment of different ailments due to their rich phytochemical constituents, and have been essentially utilized as raw materials in the pharmaceutical industries for the production of drugs and also, as alternative treatments for diseases (15, 16). Of recent, medicinal plants are being used as nutraceuticals (herbal nutraceuticals) or as functional foods in the promotion of optimal health and prevention of diseases (17, 18). *Mitracarpus vilosus* is a plant rich in a variety of phytochemicals used indigenously for different purposes (19).

Phytochemicals are bioactive chemicals produced by plants that have been proven to have great antioxidant potential and therefore are beneficial to human health (20). Antioxidants are the quality control of the system also known as free radical scavengers, which help reduce the overproduction of reactive oxygen species also known as pro-oxidants in the system, hence preventing the system from being overwhelmed (21). Phytochemicals with potential health benefits are polyphenols, flavonoids, isoflavonoids, anthocyanins, phytoestrogens, terpenoids, carotenoids, and limonoids, phytosterols, glucosinolates, and fibers (22, 23).

Phytochemical screening of *Mitracarpus vilosus* carried out in our study revealed the presence of tannins, saponins, cardiac glycosides, reducing sugars, terpenoids, and alkaloids which are secondary metabolites known to exert pharmacological activities in the body system. Tannins which are known to be the main polyphenolic secondary metabolite are naturally biological chemicals broadly distributed in plants, and could also be found in fruits like grapes, strawberries, etc., and teas (24). This polyphenolic compound is broadly divided into two namely, condensed tannins which are composed mainly of flavonoids in the absence of a sugar core, and hydrolyzable tannins which consist of ellagitannins and gallic acid with a sugar core mainly glucose (25). The hydrolyzable tannins have been shown to have limited sources in nature in comparison with the condensed tannin (26). Although the result obtained from the phytochemical screening of *Mitracarpus vilosus* extract showed the absence of flavonoid in the extract, Pizzi (27) reported that out of 20,000 total number of tannins extracted annually, 90% was observed to be composed of condensed tannin. It could thus be deduced that the

extract contains mostly hydrolyzable tannins than condensed tannins. Hydrolyzable tannins are biologically important because of their beneficial effects on humans and have been shown to possess anticancer, anti-mutagenic, antibacterial, antimicrobial, and antioxidant properties (28). This shows a promising role of the extract in the prevention and treatment of diseases. More so, the absence of flavonoids in the plant extract could be a result of both internal and external factors, like climate change, soil chemistry, and plant variety. Meanwhile, terpenoids are plant metabolites known for their variety of biological functions (29). Studies have reported its pharmacological uses owing to its anti-parasitic, antiviral, anti-hepatitis, anti-allergic, anti-inflammatory, chemotherapeutic, anti-hyperglycemic, and antispasmodic action (30, 31, 32). Terpenoids have also been reported to elicit antimicrobial activities (33), and have also been shown to inhibit different cancer cells in humans (34). They have also been incorporated into the food industry, cosmetic industry, pharmaceutical industry, etc. (35). The presence of terpenoids in *Mitracarpus vilosus* leaf extract further provides validates its potential as a phytomedicine. In addition, saponins are an important group of secondary metabolites found in plants. Most of their biological effects have been associated with their specific abilities to form pores in membranes, and have been reported to have hypoglycemic activity and lower blood cholesterol (36). Their anti-inflammatory, antifungal, anticancer, and antibacterial actions have also been reported (37). A review article on *Mitracarpus* spp (38) reported studies on phytochemical screening of the leaf extract of *Mitracarpus vilosus* which revealed potent saponin, steroids, and cardiac glycoside constituents (39, 40). Ibrahim et al. who explored the antibacterial efficacy of *Mitracarpus vilosus* extract on some selected multidrug resistance clinical isolates also reported the presence of saponin, tannins alkaloids, terpenoids, steroids, anthraquinones, flavonoids, and phenols in the acetone extract (41). Moreso, studies have shown that the presence of alkaloids, saponins, cardiac glycosides, and tannins were present in the ethanol leaf extract of *M. scaber* zucc which is another family of *Mitracarpus* sp. (42) Literature showed that these phytochemicals elicit their biological roles due to their pharmacological properties such as antibacterial, anti-inflammatory, antifungal, antiviral, insecticidal, anticancer, antidiarrheal (43, 44, 45, 46, 47, 48). Recently, different techniques have been employed in the purification and isolation of bioactive compounds and elucidation of the chemical structures in plants. These techniques include chromatographic techniques namely, column chromatography, high-pressure liquid chromatography, which can speed up the purification process of the bioactive molecule, and spectroscopic techniques namely, UV-Visible, infrared, Nuclear Magnetic Resonance, and Mass Spectroscopy which aid in the identification of the purified compounds (49). In our study, Gas Chromatography-Mass Spectrometry (GC-MS) technique was utilized for the isolation and characterization of methanolic extract of *Mitracarpus vilosus*. We discovered 67 bioactive compounds present in the extract of which five are major constituents because of their amounts with respect to their high molecular weights in the extracts. These five bioactive compounds are Squalene (14.65%), n-Hexadecanoic acid (11.60 %), 2-Octylcyclopropene-1-heptanol (8.43%), Stigmasterol (4.30%), and Octadecanoic acid (3.94%). Literature has shown that the GC-MS analysis of different solvent extract of *Mitracarpus vilosus* also led to the isolation and characterization of 13 major bioactive compounds which includes octane, 3,5-dimethyl decane, nonane, 1-dodecane, dodecane, 5-butyl nonane, 1-hexadecane, 2-bromododecane, pentadecanoic acid, 7-hexadecanoic acid, tetracosanoic acid, and 20-methyl-heneicosanoate (50). Likewise, some of these bioactive compounds namely; octane, pentadecanoic acid, and 7-hexadecanoic acid though in smaller amounts, were observed to be present in the methanol extract of *Mitracarpus vilosus* used in our study. Meanwhile, our extract did not seem to contain some of the other bioactive compounds which are; 3,5-dimethyl decane, nonane, 1-dodecane, dodecane, 5-butyl nonane, 1-hexadecane, 2-bromododecane, tetracosanoic acid, and 20-methyl-heneicosanoate.

Conclusion

The results from this study showed that the methanol extract of *Mitracarpus vilosus* exhibited significant pharmacological properties which could be targeted and employed in the treatment of diseases, and as such should be considered for use as a novel therapeutic agent. This study also shows that Methanol extract of *Mitracarpus vilosus* leaf possess major phytochemicals compounds that corroborates the claims of the traditional medicine practioners.

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