Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.12, No.14, 2022

Elucidation of Phytochemicals in Mitracarpus Vilosus Flower Extract using GC-MS

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Abstract
Mitracarpus villosus which belongs to the family of Rubiaceae is one of the important medicinal plants widely known for its broad spectrum of pharmacological activities and biological uses which includes antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-ulcer, and antibacterial activities amongst others. It is used in the treatment of various ailments such as ulcers, and skin-related infections like dermatitis, eczema, psoriasis, and acne. This study elucidated the phytochemical properties of Mitracarpus villosus flower. This was achieved through the methanolic extraction of M. villosus flower, and further phytochemical analysis of the methanol extract such as the tannins, saponins, flavonoids, steroids, terpenoids, and cardiac glucoside contents. Moreso, the use of Gas Chromatography-Mass Spectrophotometry (GC-MS) technique was employed on the methanolic extract to isolate and characterize the different bioactive compounds present and to further validate the qualitative data obtained from the phytochemical analysis. Results obtained exhibited the presence of tannins, saponins, flavonoids, steroids, and cardiac glucoside, with a noticeable absence of terpenoids. Also, the GC-MS analysis showed spectra of 52 bioactive compounds present in the extract with five specific compounds having the highest composition such as oleic acid (14.76%), 9,12-Octadecadienoic acid (11.25%), n-Hexadecanoic acid (10.92%), octadecanoic acid (9.21%), and squalene (8.87%) having the highest composition. The presence of these phytochemicals with their numerous biological activities in the methanolic extract of M. vilosus flower makes it a promising pharmaco-therapeutic agent, and thus should be employed in medicine for the treatment of diseases and also as an active agent in the pharmaceutical and non-pharmaceutical industries.
Keywords: Mitracarpus vilosus flower, Phytochemicals, Methanol extracts, GC-MS
DOI: 10.7176/JBAH/12-14-05
Publication date: July 31st 2022

1. Introduction

The use of medicinal plants for the treatment of different types of diseases is as old as human existence and a countless number of medicinal plants are variously distributed in West Africa. *Mitracarpus vilosus* (*M. vilosus*) is popular in Africa and tropical Asia for its use in traditional medicine to treat eczema, ring worm (1), headache, wounds, boils, sore throats (2), and liver diseases (3). In Nigeria for example, *M. vilosus* is known by different names. It is called obuobwa in Igbo, irawo ile in Yoruba, and gogamasu in Hausa (4).

The wide application of medicinal plants in traditional medicine and as potential drug candidates against various infectious agents is attributed to the phytochemicals they contain (5, 6). Extensive studies have been done to characterize the bioactive components of the different parts of the plant. Extracts of the whole plant and leaves of *M. vilosus* contain phytochemicals including phenols, alkaloids, flavonoids, tannins, resins, garlic acid, 4-methoxy-acetophenone, stigmasterol, and 4,5-trimethoxybenzoic acid (7, 2, 8, 9). These phytochemicals elicit anti-oxidant (10), anti-inflammatory (11), anti-fungal (2), anti-bacterial (9), and the hepatoprotective (12) effects against noxious agents and diseases. Extracts of *M. vilosus* also showed insecticidal potentials, which make the plant a biofumigant against weevils and beetles (13). In addition, the abundant phytochemicals and their over-lapping activities in *M. vilosus* is responsible for the health-promoting benefits of the plant. Whereas the phytochemicals in the upper parts and leaves of *M. vilosus* have been extensively characterized and reported, literature is limited regarding the bio-constituents in *M.vilosus* flower. This study is thus designed to elucidate and characterize the phytochemical components in the methanolic extract of *Mitracarpus vilosus* flower using the GC-MS technique.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grades.

Plant collection

Fresh flowers of Mitracarpus vilosus were collected from an uncultivated farm located in the Ibeju-Lekki area of

Lagos State, Southwest Nigeria. The 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 the plant extract
Following identification, the flowers were carefully washed under clean running tap water and air-dried on the bench in the laboratory at ambient temperature for 7 days. The dried flowers were then separated from the individual stalks and blended into a fine powder using porcelain mortar and pestle.

Extraction of Mitracarpus vilosus flower
The Methanol extract was prepared by weighing 100 g of the dried and finely grounded flower of Mitracarpus vilosus, which was soaked in 2 L 80% methanol for 72 h and mixed at regular intervals. This mixture was initially filtered with a muslin cloth, then with Whatman No.1 filter paper. The filtrate obtained was concentrated and evaporated at 40ºC with reduced pressure using a rotary evaporator to obtain the desired extract.

Qualitative analysis of methanolic extract of M.vilosus
Phytochemical screening
Phytochemical analysis was carried out on the methanol extract of the flower of Mitracarpus vilosus using standard methods (14, 15).

Evaluation of Tannins content
A tannin test was carried out on the methanol extract using the methods mentioned above. Briefly, 0.5 g extract of Mitracarpus vilosus flower was added to 20 ml of water in a test tube and boiled. The solution was filtered and a few drops of 0.1% ferric chloride was added to the filtrate. The appearance of a brownish-green coloration shows the presence of tannin.

Evaluation of Saponin content
The methanol extract was tested for saponin using a published method as previously mentioned. Briefly, 2 g extract of Mitracarpus vilosus flower was added to 20 ml of distilled water and boiled. The resulting solution was filtered. 10 ml of the filtrate was vigorously mixed with 5 ml of distilled water to obtain a froth. 3 drops of olive oil was added to the froth and shaken repeatedly. A visible emulsion indicates the presence of saponin.

Assessment of Flavonoid contents
Flavonoid content in the methanol extract was assessed using published methods. Succinctly, 5 ml of dilute ammonia solution was added to a 0.5 g of Mitracarpus vilosus flower extract accompanied by a gentle addition of conc. H_2SO_4. An observable brownish precipitate indicated the presence of flavonoids in the extract.

Steroids test
The presence of steroids in the methanol extract was evaluated using standard methods. Briefly, 2 ml of acetic anhydride was added to 0.5 g of Mitracarpus vilosus flower extract followed by the addition of 2 ml H_2SO_4. An observable change in color from violet to blue shows the presence of steroids in the extract.

Terpenoids test (Salkowski test)
The methanol extract of M. vilosus was screened for terpenoids using published methods. Briefly, 5 ml methanol extract of Mitracarpus vilosus flower was added to 2 ml of chloroform mixed, and 3 ml concentrated H_2SO_4 was cautiously added to form a layer. The formation of reddish-brown interphase coloration shows the presence of terpenoids.

Cardiac Glycosides test (Keller-Killani test)
Briefly, 5ml extract of Mitracarpus vilosus flower was mixed with 2 ml of glacial acetic acid after which a drop of ferric chloride was added. 1 ml of concentrated H_2SO_4 was cautiously added. The appearance of a brown ring at interface shows the deoxysugar characteristic of cardenolides.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYSIS
Gas Chromatography/Mass Spectrometry (GC-MS) analysis was carried out using a Shimadzu QP-5050A (SHIMADZU, JP) instrument, equipped with a PTETM-5 column (30 m, 0.25 mm, 0.25 µm, Supelco, USA), with helium gas as the carrier. The Helium was calibrated at 22.3 mL/min; injector temperature was maintained at 230ºC; while the oven was at an initial temperature of 80ºC for 3 minutes and then heated up to 300ºC at 7ºC/min, this was maintained for 5 min at 300ºC. The split valve was closed during the initial first minute of injection and later opened, at a 1:10 ratio. The mass detector was adjusted to scan from 50 to 500 *m/z*, at a rate of

2 scans per second. Data collection and handling were done using CLASS 5000 Shimadzu software.

RESULTS

Phytochemical constituents of Mitracarpus vilosus flower extract

Data in **Table 1** shows the phytochemical components of the methanol extract of M. vilosus flower. The methanol extract obtained was found to be rich in phytochemicals like tannins, saponins, flavonoids, and cardiac glucoside, although an absence of terpenoids. Different components of the phytochemicals obtained and their peaks are shown in the chromatogram (**Figure 1**).

Bioactive components of M. vilosus flower extract using GC-MS analysis

Results from the GC-MS analysis showed the presence of 52 bioactive compounds which include: 3,4Dehydro-dl-proline,6-Oxa-bicyclo[3,10]hexan-3-one,Imidazole,1,4,5-trimethyl-2-propyl-tetrahydropyran-3-ol, Carbamic acid, methyl-, phenyl ester, 5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl, isosorbide Dinitrate, 5-hydroxyuridine, 9-Acetoxynonenal, Allyl heptanoate, catechol, 2-methoxy-4-vinylphenol; hexadecenoic acid, trifluoroacetyl-alpha-terpineol, methylene-(3-trimethylsilylphenyl)amine, stevioside, 3,4-altrosan, 22-Deoxycarpesterol, methyl ester, 9,12-Octadecanoic acid, oleic acid, octadecanoic acid, 9-octadecenamide, eicosanoic acid, squalene amongst others as shown in **fig. 2**. Among the 52 phytochemicals identified, five were remarkably outstanding in terms of their percentage composition, as shown in **Table 2**. Moreso, the different components (**Fig. 2**).

Table 1. Relative phytochemical component of M. vilosus flower extract

PHYTOCHEMICALS	STATUS
Tannins	Present
Saponins	Present
Flavonoids	Present
Steroids	Present
Terpenoids	Absent
Cardiac glycoside	Present

Table 2. Major bioactive compounds of M. vilosus flower extract identified using GC-MS

Bioactive compounds	Molecular weight	Chemical formula	Percentage composition (%)
Oleic Acid	282	$C_{18}H_{34}O_2$	14.76
9,12-Octadecadienoic Acid	280	$C_{18}H_{32}O_2$	11.25
Hexadecanoic Acid	256	$C_{16}H_{32}O_2$	10.92
Octadecanoic acid	284	$C_{18}H_{36}O_2$	9.21
Squalene	410	$C_{30}H_{50}$	8.87

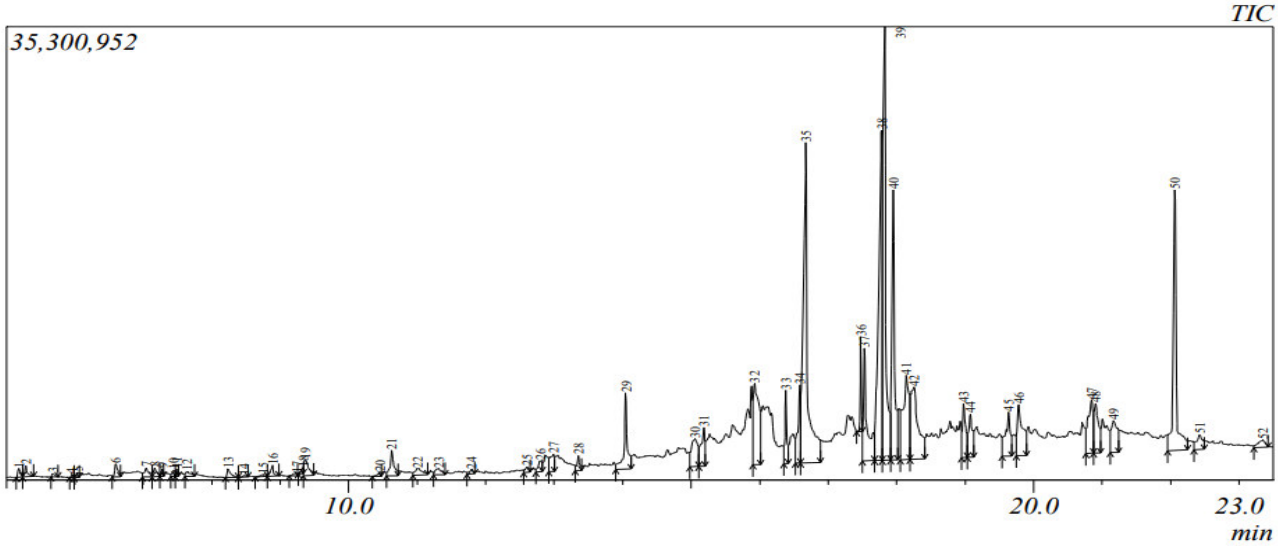

Figure 1: GC-MS chromatogram of Methanol extract of the flower of Mitracarpus vilosus showing different peaks of the phytochemical components identified.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.183	5.150	5.242	1931328	0.23	700185	0.31	2.76		3,4Dehydro-dl-proline
2	5.282	5.242	5.400	2673066	0.31	883537	0.39	3.03	V	6-Oxa-bicyclo[3.1.0]hexan-3-one
3	5.683	5.650	5.758	792558	0.09	266908	0.12	2.97		Imidazole, 1,4,5-trimethyl-
4	5.958	5.925	5.992	414680	0.05	217101	0.10	1.91		2-Propyl-tetrahydropyran-3-ol
5	6.051	5.992	6.067	838247	0.10	364364	0.16	2.30	V	Carbamic acid, methyl-, phenyl ester
6	6.596	6.550	6.667	3438244	0.40	994422	0.44	3.46	V	5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl
7	7.041	6.992	7.133	3218759	0.38	682226	0.30	4.72	V	Isosorbide Dinitrate
8	7.166	7.133	7.242	2256833	0.26	694362	0.30	3.25	V	5-Hydroxyuridine
9	7.279	7.242	7.300	1312459	0.15	602764	0.26	2.18	V	.alpha.-Methyl-.alpha.-[4-methyl-3-penteny
10	7.442	7.400	7.467	1433900	0.17	459777	0.20	3.12	V	1,2-Diazabicyclo[2.2.2]octan-3-one, 2-hydr
11	7.483	7.467	7.517	1225099	0.14	575339	0.25	2.13	V	9-Acetoxynonanal
12	7.636	7.608	7.750	1877233	0.22	357903	0.16	5.25	V	1R-Ethoxy-3-trans-methoxy-2-cis-methylcy
13	8.237	8.200	8.392	2609812	0.30	661410	0.29	3.95		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy
14	8.477	8.392	8.533	583918	0.07	148529	0.06	3.93	V	trans-2,3-Epoxynonane
15	8.742	8.633	8.817	1101643	0.13	160220	0.07	6.88		Hexanediamide, N,N'-di-benzoyloxy-
16	8.889	8.817	8.983	2762103	0.32	835187	0.37	3.31	V	Allyl heptanoate
17	9.241	9.133	9.258	934979	0.11	195328	0.09	4.79	MI	Cyclohexanone, 2-(hydroxymethyl)-
18	9.342	9.267	9.342	1125260	0.13	467047	0.20	2.41	MI	Catechol
19	9.365	9.342	9.492	5733751	0.67	1232720	0.54	4.65	MI	Benzofuran, 2,3-dihydro-
20	10.460	10.342	10.475	1111114	0.13	331673	0.15	3.35		8-Methyl-6-nonenoic acid
21	10.627	10.550	10.717	7569166	0.88	1981752	0.87	3.82	V	2-Methoxy-4-vinylphenol
22	11.000	10.933	11.150	3941962	0.46	552847	0.24	7.13	V	Trifluoroacetyl-.alpha.-terpineol
23	11.314	11.233	11.408	3140600	0.37	480047	0.21	6.54	V	Octane, 1-(ethenylthio)-
24	11.790	11.725	11.842	1252942	0.15	344398	0.15	3.64	V	Methylene-(3-trimethylsilylphenyl)amine
25	12.602	12.550	12.650	1687942	0.20	423135	0.19	3.99	V	1,2,4-Oxadiazole, 3-methyl-5-phenyl-
26	12.794	12.733	12.825	3402276	0.40	859302	0.38	3.96	V	Stevioside
27	12.986	12.917	13.008	6425360	0.75	1345517	0.59	4.78	V	3,4-Altrosan
28	13.350	13.308	13.400	3138704	0.37	1155196	0.51	2.72	V	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-
29	14.044	13.900	14.125	18802004	2.19	5918789	2.59	3.18	V	1,4-Naphthalenedione, 2,3-dimethyl-
30	15.055	14.975	15.108	14146423	1.65	2171141	0.95	6.52	V	22-Desoxycarpesterol
31	15.183	15.108	15.208	12111486	1.41	3012644	1.32	4.02	V	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dion
32	15.923	15.900	16.017	36306237	4.24	6349915	2.78	5.72	V	22-Desoxycarpesterol
33	16.383	16.350	16.417	10760502	1.26	5712227	2.50	1.88	V	Hexadecanoic acid, methyl ester
34	16.584	16.525	16.600	17010356	1.99	6098147	2.67	2.79	V	1H-Benzofuro[3,2-e]indole, 1-[2-(aminocar
35	16.675	16.600	16.883	111306653	12.99	24944876	10.92	4.46	V	n-Hexadecanoic acid
36	17.476	17.417	17.500	9551923	1.11	7375982	3.23	1.30	MI	9,12-Octadecadienoic acid, methyl ester
37	17.528	17.500	17.675	31788535	3.71	8777589	3.84	3.62	MI	9-Octadecenoic acid, methyl ester, (E)-
38	17.780	17.675	17.792	100087679	11.68	25714912	11.25	3.89	MI	9,12-Octadecadienoic acid (Z,Z)-
39	17.822	17.792	17.908	94902414	11.08	33736619	14.76	2.81	MI	Oleic Acid
40	17.950	17.908	18.017	55175357	6.44	21048481	9.21	2.62	V	Octadecanoic acid
41	18.139	18.050	18.192	41406487	4.83	6547208	2.87	6.32	V	Phenanthrene, 1,2,3,4,5,6,7,8-octahydro-
42	18.252	18.192	18.408	43609415	5.09	5619489	2.46	7.76	V	Tetracyclo[6.1.0.0(2,4).0(5,7)]nonane, 3,6,9
43	18.977	18.950	19.033	13299969	1.55	4222827	1.85	3.15	V	9-Octadecenamide, (Z)-
44	19.072	19.033	19.125	13274965	1.55	3358856	1.47	3.95	V	Eicosanoic acid
45	19.634	19.542	19.675	15893882	1.86	3438544	1.50	4.62	V	1,2-15,16-Diepoxyhexadecane
46	19.780	19.742	19.892	21638759	2.53	3964596	1.73	5.46	V	Hexanoic acid, octadecyl ester
47	20.841	20.758	20.875	21669303	2.53	4149308	1.82	5.22	V	4-Hexyl-1-(7-methoxycarbonylheptyl)bicycl
48	20.902	20.875	20.975	16951958	1.98	3855462	1.69	4.40	V	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hyd
49	21.167	21.117	21.242	15287704	1.78	2497986	1.09	6.12	V	Aromadendrene oxide-(1)
50	22.061	21.958	22.250	62409431	7.28	20276753	8.87	3.08	V	Squalene
51	22.422	22.342	22.492	7067718	0.82	1162754	0.51	6.08	V	Cholest-22-ene-21-ol, 3,5-dehydro-6-metho
52	23.347	23.217	23.425	4402407	0.51	593165	0.26	7.42	V	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy
				856795505	100.00	228521466	100.00			

Figure 2: Phytochemical compounds identified by GC-MS in Methanol extract of the flower of Mitracarpus vilosus

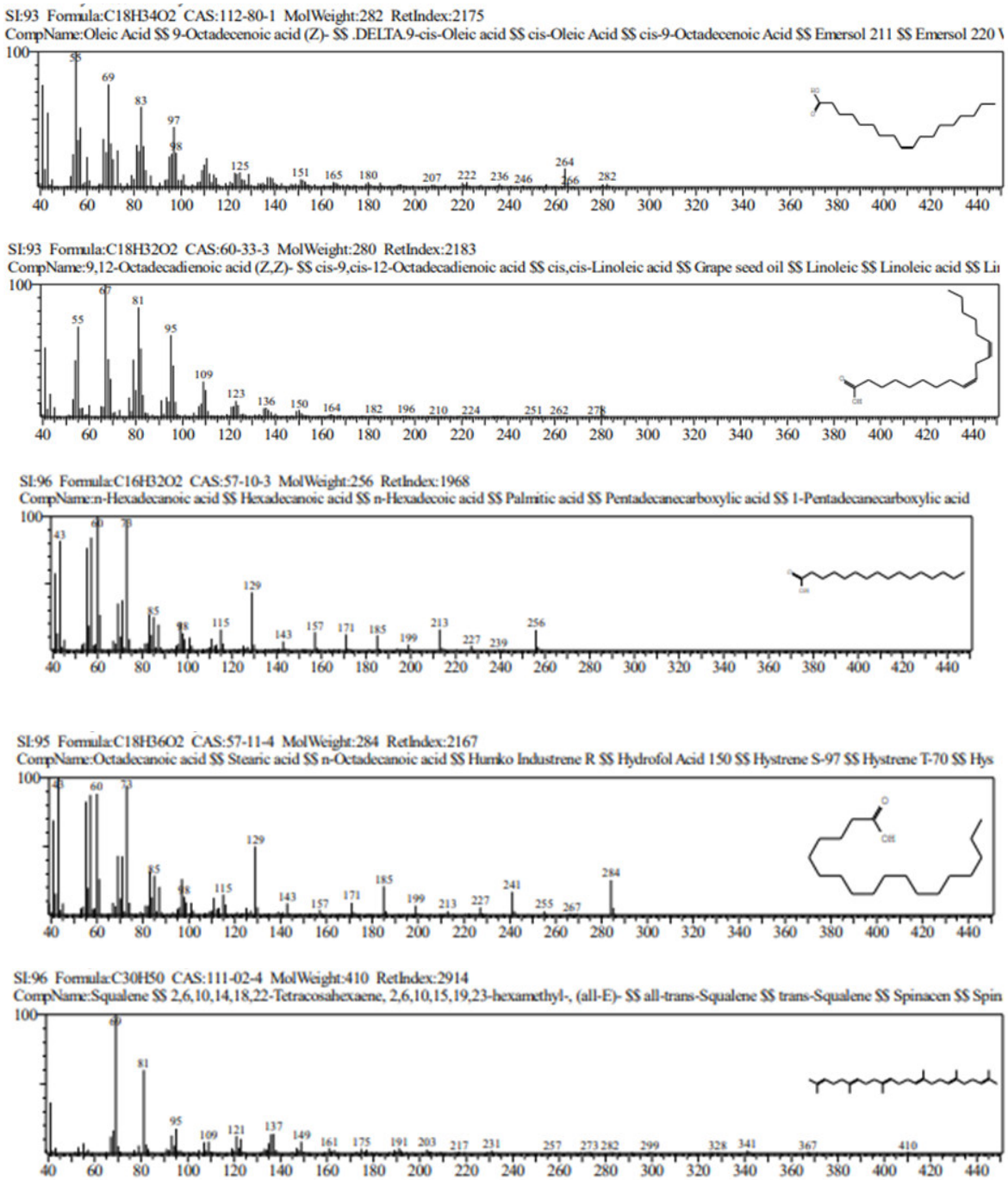

Figure 3: GC-MS mass spectrum and molecular structure of oleic acid, 9,12-octadecadenoic acid, n-hexadecanoic acid, Octadecanoic acid, and squalene.

DISCUSSION

Mitracarpus vilosus is a rich plant with diverse pharmacological properties such as antibacterial, antioxidant, antifungal, antidiabetic, anticancer, and anti-inflammatory which is beneficial to health (16, 17, 18, 19, 20, 21, 22). These pharmacological properties of M.villosus have been reported to be a result effect of the rich bioactive compounds present in the plant of which different parts have been well studied for their robust bioactive compounds eliciting the aforementioned pharmacological activities. For instance, studies have shown that the leaf extract of M. vilosus using different solvent fractions like methanol, acetone ethanol, n-hexane, and ethyl acetate yielded secondary metabolites like terpenoids, phenols, tannins, cardiac glycosides, and carbohydrates (23, 24). John-Africa and colleagues investigated the effect of the sedative properties of M.villosis leaves in mice, and observed the presence of psychoactive substances that are sedative in nature, then concluded that M.villosus leaf can be used as a therapeutic agent in the management of stress-related diseases (25). The crushed leaves of

M. villosus have also been shown to be beneficial in the treatment of ulcers, ringworms, eczema, and fresh wounds which could be due to its antibacterial and anti-ulcerative properties (24). Moreso, the aerial part of M. villosus have been employed in both in-vivo and in-vitro studies to treat various diseases (26, 27). However, this study explored the possible phytochemicals and bioactive compounds present in the methanol extract of the Mitracarpus vilosus flower using the Gas Chromatography-Mass Spectroscopy technique. Phytochemical analysis carried out on the flower extract revealed the presence of secondary metabolites which include tannins, saponins, flavonoids, steroids, and cardiac glycoside, with the absence of terpenoids. These secondary metabolites are beneficial to man because of their diverse biological activities in the body (28, 29, 30, 31, 32). Interestingly, a previous investigation carried out in our laboratory on the methanol plant extract of Mitracarpus vilosus revealed the presence of these potent phytochemicals in the plant. The presence of these phytochemicals in the methanol extract of M. villosus flower thus potentiates its efficacy in the treatment of different diseases like coronary heart disease (CHD), neurodegenerative diseases, hypertension, etc.

To further elucidate the different bioactive compounds, present in plants with their molecular weights and structures, different techniques are being employed, one of which is the Gas Chromatography-Mass Spectroscopy technique (33). This technique is based on the synergistic combination of two analytical methods to separate and identify different substances within a test sample. The use of GC-MS in the screening of bioactive compounds is essential in phytochemistry to isolate and characterize the different compounds present in plants. The GC-MS analysis carried out on the methanol extract of M. vilosus flower revealed the presence of 52 different phytochemical components of which five are most prominent due to their high percentage composition and these are listed in descending order; hexadecenoic acid, 9,12-octadecanoic acid, oleic acid, octadecanoic acid, and squalene. Some of these bioactive compounds are known to have so many biological functions, for instance, oleic acid is a cardioprotective and hypotensive agent. It also plays a role in the regulation of cholesterol, and it is therefore incorporated as a cholesterol-lowering agent in pharmaceuticals. In the industrial sector, it can be used as an emulsifying agent which is present in aerosol products. Oleic acid has also been shown to boost memory (34). Moreso, the presence of 9,12-octadecadienoic acid potentiates its antihistamine, anticoronary, antiandrogenic, antieczemic, likewise hypocholesterolemic, hepatoprotective antiarthritic, and nematicide properties and therefore could be used in the management of diseases. Octadecanoic acid present in the M. vilosus methanol flower extract makes it a good agent for the hardening of soaps, softening of plastics, and production of cosmetics and candles, thus could be employed in the industries. Most abundant is squalene which is used as an emollient. It can also act as an antioxidant with antitumor activities (35).

Collectively, results from this study further elucidate the different phytochemical components of Mitracarpus villosus flower which could shed more light on the particular phytochemical constituent responsible for the different pharmacological activities elicited. Thus, the use of M. vilosus flower should be employed in both industrial and pharmacological industries and in the management of health challenging diseases.

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