International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 3(5): September-October 2013

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 14-11-2012; Revised; Accepted: 25-10-2013

GC/MS SPECTROSCOPIC ANALYSIS OF SOME DIFFERENT *IN VIVO* METHANOLIC PLANT EXTRACTS OF MAYTENUS *EMARGINATA* (WILLD.): AN IMPORTANT MEDICINAL PLANT

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Keywords:

GC/MS spectroscopy, volatile organic compounds, Saturated fatty acid, Phytosterols, Aromatic compound, Sesquiterpenes

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ABSTRACT

Maytenus emarginata (Willd.) is one of the medicinally important plants belonging to the family *Celastraceae*, commonly known as "Malkangni"in Hindi and "Thorny staff tree"in English. In the present study the Methanolic extract of root, stem, leaves and fruits of Maytenus emarginata has been subjected to GC-MS analysis. A total of 34, 29, 39 and 56 compounds were identified from leaf, root, fruit and stem have been identified, respectively. A variety of volatile organic compounds have been detected in this plant species including pentadecanoic acid (Saturated fatty acid), cholesterol, stigmasterol, β-sitosterol (phytosterols), tridecane, dodecane (alkane hydrocabon), glycerin (alcohol), myo-inositol (aromatic compound), evonine (alkaloid), hexadecen-1ol (terpene alcohol), hexadecanoic acid (palmitic acid), phytol (diterpene), octadecadienoic acid (linoleic acid), tocopherol (vitamin-E) and squalene, lupeol, betulin (tri-terpene), valerenol, alpha- caryophyllene, sclareolide (sesquiterpenes) with various proportions.

INTRODUCTION

In the plant kingdom, there are thousands of plants known and unknown that yield medicine or drugs use to man. These plants are known as medicinal or drug plants, which are the chemical gold mines existing in the ecosystems to get the disease of men and animals cured in the natural way[1]. Plants are the traditional sources for many chemicals used as pharmaceutical biochemicals, fragrances, food colours and flavours [2]. Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds.

Maytenus emarginata (Family:*Celastraceae*), is a traditionally valuable plant. The celastraceae family, commonly known as bittersweet family, comprising about 50 genera and 800 species, is indigenous to tropical and sub-tropical regions of the world, including North Africa, South America, and many parts of East Asia, particularly China [3,4]. Plants of this family generally grow as small trees, bushes or lianas and have resinous stems and leaves. They have been valued since antiquity because their extracts have useful medicinal properties [5]. The crude plant extracts of the celastraceae in traditional medicine and agriculture is astonishing, and includes stimulant, restorative, male contraceptive, anti-tumor, anti-leukemic, anti-bacterial, insecticidal and insect repellent activities[6]. Traditionally species of *Maytenus* has been used for fever, asthama, rheumatism and gastrointestinal disorders worldwide. Recently some biomolecules from *Maytenus* species has been reported to be active against HIV-Protease[7] Carcinoma and leukemia[8]. Ulcers and MDR (Multi Drug Resistance) [9]. Roots of Maytenus emarginata used in gastrointestinal troubles, especially dysentery [10]. Tender shoots of the plant help for mouth ulcer. The bark is ground to a paste and applied with mustard oil to kill lice in the hair. Pulverized leaves of *Maytenus emarginata* are given in milk to children as a vermifuge[11].

A decoction of the leafy twigs is used as a mouthwash to relieve toothache. Ash of leaves used to heal up sores and wound gives cooling effect. The leaves are burnt and mixed with ghee to form an ointment used to heal sores [12]. The tender leaves are chewed raw in the treatment of jaundice. The fruits are used in medicines to purify blood [13]. There is no report of chemical constituents isolated from this plant. The objective of this study was to evaluate the phytochemical compounds (quantitative method) using GC-MS analysis.

MATERIAL AND METHOD

(i) Collection of plant material

Plant of *Maytenus emarginata* was collected from the campus of University of Rajasthan. Specimens were compared with the voucher specimens at Herbarium of Department of Botany, University of Rajasthan, Jaipur.

(ii) Preparation of plant material

The fresh plant samples (root, stem, leaf, fruits) were collected and washed individually under running tap water to remove soil particles and other dirt. All the samples were dried firstly at 60°C for 2 days in an oven after that live it on room temperature. They were then macerated to powder form with a mixer grinder. The powder was stored in air sealed polythene bags at room temperature before extraction.

(iii) Preparation of sample for GC/MS study

About 20 grams of the each plant part powdered were soaked in 100 ml methanol individually. It was left for 24 hours so that alkaloids, terpenoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove the traces of moisture.

(iv) Gas chromatography – Mass Spectrum analysis

GC Programme	
Column: Elite-1 (100% Dimethyl poly siloxane), 30 × 0.25mm × 1 mdf Equipment: QP 2010 Plus SHIMADZU Carrier gas: 1ml per min, Split: 10:1 Detector: Mass detector: Turbo mass gold-Perkin Elmer Software: Turbomass 5.2 and Sample injected: 2 µl	
Oven temperature programme	**MS Programme**
110° C-2 min hold Up to 200° C at the rate of 10° C / min- 1 min hold Up to 280° C at the rate of 5° C / min-9 min hold Injector temperature: 250° C and Total GC running time: 36 min	Library used: NIST Library / Wiley Library Inlet line temperature: 200° C Source temperature: 200° C Electron energy: 70 eV Mass scan: (m/z): 45-450 Total MS running time: 40 min

RESULTS AND DISCUSSION

Photochemistry is the study of phytochemicals, which are secondary metabolic substances found in plants. Many of these are known to provide protection against insect attacks and plant diseases. Phytochemical analysis mainly applies to the quality control of Chinese medicine or herbal medicine to determine the various chemical components, such as saponins, alkaloids, volatile oils, flavonoids and anthraquinones.

The present study carried out on the *Maytenus emarginata* the presence of medicinal active constituents. In the GC-MS analysis, phytochemical compounds were identified in the methanolic extract of root, stem, leaf and fruits of this plant. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. The results are presented in Table 1 to 4.

Table-1: Activity of phytocomponents identified in the methanolic root extract of *M. emarginata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	9.770	Tridecane	0.68	$C_{13}H_{28}$	184.35	Alkane hydrocarbon
2.	10.654	Pentadecane	0.92	$C_{15}H_{32}$	212	Fragrance agents
3.	11.425	1-hexadecanol	0.18	$C_{16}H_{34}O$	242	Fatty alcohol
4.	11.475	Trichloroacetic acid, dodecyl ester	0.56	$C_{14}H_{25}C_{l3}O_2$	330	
5.	12.653	1,2-Dimethyl-3,5-divinylcyclohexane	0.52	$C_{12}H_{20}$	164	
6.	12.725	Tetradecanoic acid (Myristic acid)	0.46	$C_{14}H_{28}O_2$	228.37	Saturated fatty acid
7.	13.613	Diisobutyl Phthalate	0.74	$C_{16}H_{22}O_4$	278.35	
8.	13.866	Pentadecanoic acid, 14-methyl-, methyl ester	0.67	$C_{17}H_{34}O_2$	270	Fatty acid
9.	14.113	n-Hexadecanoic acid (Palmitic acid)	8.16	$C_{16}H_{32}O_2$	256	Fatty acid
10.	14.518	Eicosanoic acid, methyl ester	0.39	$C_{21}H_{42}O_2$	326	Fatty acid
11.	15.016	9-Hexadecenoic acid, methyl ester, (Z)-	1.12	$C_{17}H_{32}O_2$	268	Fatty acid
12.	15.139	Octadecanoic acid, methyl ester	0.55	$C_{19}H_{38}O_2$	298	Fatty acid
13.	15.265	9-Octadecenoic acid (Z)- (Oleic Acid)	11.93	$C_{18}H_{34}O_2$	282	Unsaturated fatty acid
14.	15.367	Octadecanoic acid (Stearic acid)	4.32	$C_{18}H_{36}O_2$	284	Saturated fatty acid
15.	17.025	Hexadecanal	0.35	$C_{16}H_{32}O$	240	Aldehyde
16.	17.250	2-Ethyl-9,10-anthracenediol	0.78	$C_{16}H_{14}O_2$	238	
17.	17.635	Palmitic acid .beta.-monoglyceride	2.52	$C_{19}H_{38}O_4$	330	
18.	17.700	n-Octadecanal	0.51	$C_{18}H_{36}O$	268	Aldehyde
19.	17.927	Diisooctyl phthalate	0.62	$C_{24}H_{38}O_4$	390.56	
20.	18.808	Cyclocarbosilane	0.80	$C_3H_{12}Si_3$	132	
21.	19.224	alpha.-Monostearin	17.63	$C_{21}H_{42}O_4$	358	
22.	20.232	Squalene	0.70	$C_{30}H_{50}$	410.72	Triterpene
23.	25.148	Cholesterol	1.50	$C_{30}H_{50}$	410.72	Phytosterol
24.	29.381	B-Sitosterol	7.11	$C_{29}H_{50}O$	414.71	Phytosterol
25.	30.109	Methyl commate C	1.84	$C_{31}H_{50}O_4$	486	Triterpenes glycoside
26.	30.814	Methyl commate A	26.56	$C_{32}H_{52}O_4$	500	Triterpenes glycoside
27.	31.332	Lupeol	3.18	$C_{30}H_{50}O$	426.73	Triterpenoid
28.	31.995	Farnesyl bromide	2.42	$C_{15}H_{25}Br$	284	
29.	32.418	Longifolenaldehyde	1.91	$C_{15}H_{24}O$	220.35	

Table-2: Activity of phytocomponents identified in the methanolic stem extract of *M. emarginata*.

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	9.770	Tridecane	0.37	$C_{13}H_{28}$	184.35	Alkane hydrocarbon
2.	10.654	Pentadecane	0.56	$C_{15}H_{32}$	212	Fragrance agents
3.	11.475	Trichloroacetic acid, dodecyl ester	0.48	$C_{14}H_{25}Cl_3O_2$	330	
4.	11.596	Sorbitol	1.18	$C_6H_{14}O_6$	182.17	Sugar alcohol
5.	13.613	Diisobutyl Phthalate	0.37	$C_{16}H_{22}O_4$	278.35	
6.	13.867	Pentadecanoic acid, 14-methyl-, methyl ester	0.72	$C_{17}H_{34}O_2$	270	
7.	14.123	n-Hexadecanoic acid (Palmitic acid)	10.48	$C_{16}H_{32}O_2$	256	Saturated fatty acid
8.	15.018	9-Octadecenoic acid (Oleic acid)	1.07	$C_{18}H_{34}O_2$	282.46	Unsaturated fatty acid
9.	15.141	Octadecanoic acid, methyl ester	0.43	$C_{19}H_{38}O_2$	298	Unsaturated fatty acid
10.	15.282	9-Octadecenoic acid (Z)-(Oleic acid)	19.86	$C_{18}H_{34}O_2$	282	Unsaturated fatty acid
11.	15.378	Octadecanoic acid (Stearic acid)	5.87	$C_{18}H_{36}O_2$	284	Saturated fatty acid
12.	17.425	1-Heneicosyl formate	0.93	$C_{22}H_{44}O_2$	340	Volatile and semivolatile organic compound
13.	17.637	Palmitic acid .beta.-monoglyceride	1.65	$C_{19}H_{38}O_4$	330	
14.	17.931	1,2-Benzenedicarboxylic acid, dioctyl ester	0.46	$C_{24}H_{38}O_4$	390	
15.	18.106	Pentadecane, 8-hexyl-	0.34	$C_{21}H_{44}$	296	
16.	18.933	1-Eicosanol	0.35	$C_{20}H_{42}O$	298	
17.	19.105	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	1.80	$C_{17}H_{13}N_3O_2$	291	
18.	19.227	alpha.-Monostearin	13.47	$C_{21}H_{42}O_4$	358	
19.	19.736	1-Hentetracontanol	0.71	$C_{41}H_{84}O$	592	
20.	20.767	n-Tetratetracontane	0.94	$C_{44}H_{90}$	619.19	
21.	24.399	4,6-Cholestadien-3.beta.-ol	0.65	$C_{27}H_{44}O$	384.64	
22.	25.152	Cholesterol	1.18	$C_{30}H_{50}$	410.72	Triterpene
23.	27.451	n-Tetracosano (Lignoceric alcohol)	1.25	$C_{24}H_{50}O$	354	
24.	29.387	Beta.-Sitosterol	4.98	$C_{29}H_{50}O$	414	Phytosterol
25.	30.814	Methyl commate A	12.95	$C_{32}H_{52}O_4$	500	Triterpenes glycoside
26.	31.349	Lupeol	2.94	$C_{30}H_{50}O$	426.73	Triterpenoid
27.	32.012	Betulin	3.72	$C_{30}H_{50}O_2$	442.72	Triterpene
28.	36.652	Friedelin	6.15	$C_{30}H_{50}O$	426	Triterpene
29.	37.261	Alpha.-Bisabolol	4.15	$C_{15}H_{26}O$	222	Unsaturated sesquiterpene alcohol

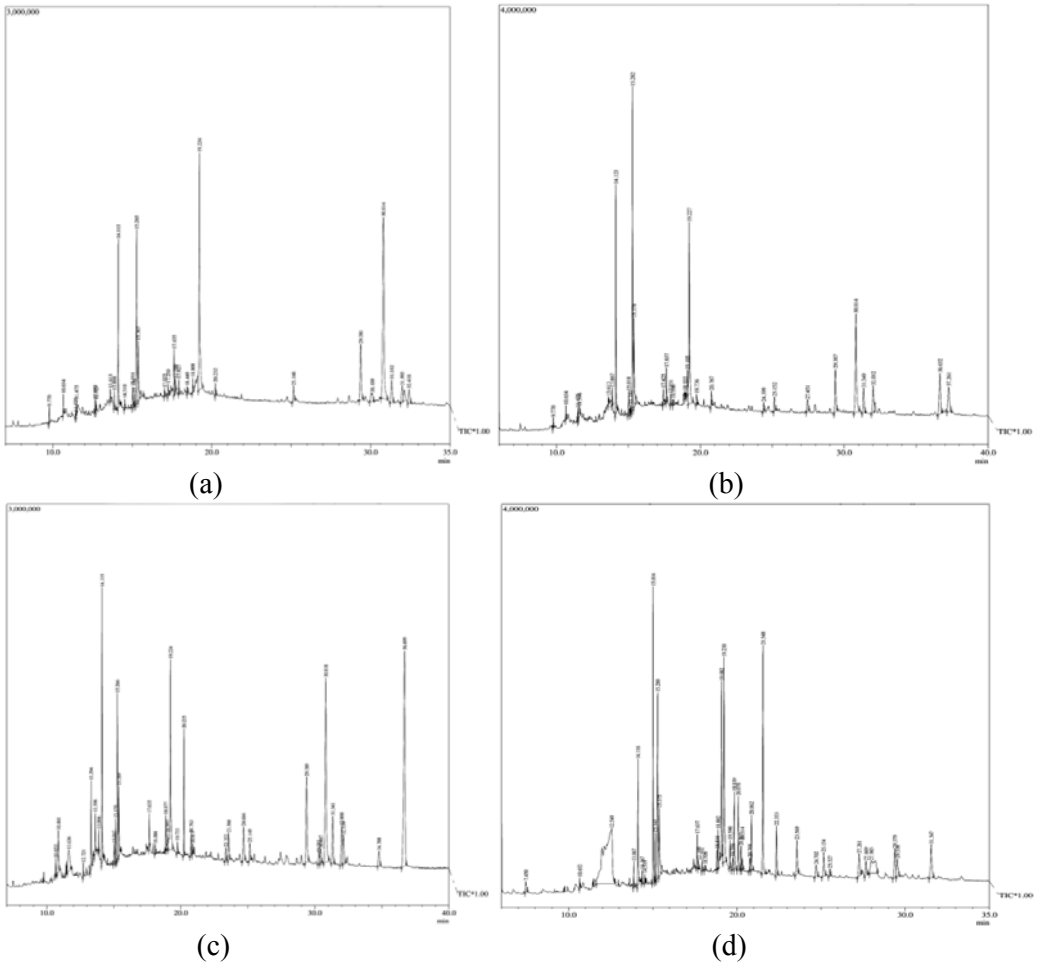
Table-3: Activity of phytocomponents identified in the methanolic leaf extract of *M. emarginata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	10.653	Pentadecane	0.38	$C_{15}H_{32}$	212	Fragrance agents
2.	10.861	Levoglucosan	2.41	$C_6H_{10}O_5$	162.14	Organic compound
3.	11.636	1,4-Anhydro-d-galactitol	2.73	$C_6H_{12}O_5$	164.14	
4.	12.721	Tetradecanoic acid (Myristic acid)	0.34	$C_{14}H_{28}O_2$	228.37	Saturated fatty acid
5.	13.294	Neophytadiene	2.26	$C_{20}H_{38}$	278.51	
6.	13.598	Linoleic acid	1.38	$C_{18}H_{32}O_2$	280.45	unsaturated fatty acid (carboxylic acid)
7.	13.866	Pentadecanoic acid, 14-methyl-, methyl ester	0.43	$C_{17}H_{34}O_2$	270	fatty acid
8.	14.119	n-Hexadecanoic acid (Palmitic acid)	7.16	$C_{16}H_{32}O_2$	256	fatty acid
9.	15.017	Palmitoleic acid methyl ester	0.72	$C_{17}H_{32}O_2$	268.43	unsaturated fatty acid
10.	15.120	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	1.26	$C_{20}H_{40}O$	296.53	Diterpene alcohol
11.	15.266	9-Octadecenoic acid (Oleic Acid)	6.08	$C_{18}H_{34}O_2$	282	unsaturated fatty acid
12.	15.369	Octadecanoic acid (Stearic acid)	2.18	$C_{18}H_{36}O_2$	284	Saturated fatty acid
13.	17.635	Palmitic acid .beta.-monoglyceride	0.80	$C_{19}H_{38}O_4$	330	
14.	18.086	n-Hexadecane sulfonyl chloride	0.34	$C_{16}H_{33}ClO_2S$	324	
15.	18.877	N-Hexacosane	0.83	$C_{26}H_{54}$	366.71	
16.	18.993	Octadecanedioic acid	0.70	$C_{18}H_{34}O_4$	314	
17.	19.224	Alpha.-Monostearin	8.17	$C_{21}H_{42}O_4$	358	Saturated fatty acid
18.	19.733	1-Hentetracontanol	0.45	$C_{41}H_{84}O$	592	
19.	20.235	Squalene	3.52	$C_{30}H_{50}$	410.72	Triterpene
20.	20.763	Nonacosane	0.82	$C_{29}H_{60}$	408	Hydrocarbon
21.	20.877	9-Tricosene, (Z)	0.40	$C_{23}H_{46}$	322	
22.	23.353	gamma.-Tocopherol	0.69	$C_{28}H_{48}O_2$	416.68	vitamin E
23.	23.566	1-Triacontanol	1.29	$C_{30}H_{62}O$	438.81	Fatty alcohol
24.	24.684	dl-.alpha.-Tocopherol	2.19	$C_{29}H_{50}O_2$	430	vitamin E
25.	25.149	Cholesterol	0.94	$C_{27}H_{46}O$	386.65	Phytosterol
26.	29.389	β-Sitosterol	5.95	$C_{29}H_{50}O$	414	Phytosterol
27.	30.283	Lupenyl acetate	0.93	$C_{32}H_{52}O_2$	468	Tri- terpenes
28.	30.447	Ursodeoxycholic acid (Ursodiol)	0.89	$C_{24}H_{40}O_4$	392	Secondary bile acids
29.	30.818	Methyl commate A	14.21	$C_{32}H_{52}O_4$	500	Triterpenes glycoside
30.	31.341	Lupeol	3.12	$C_{30}H_{50}O$	426.73	Triterpenoid
31.	32.008	Betulin	2.93	$C_{30}H_{50}O_2$	442.72	Triterpene
32.	32.139	alpha.-Amyrin acetate	2.32	$C_{32}H_{52}O_2$	468	Phytosterol
33.	34.788	Sclareolide	1.28	$C_{16}H_{26}O_2$	250.38	Sesquiterpene
34.	36.699	Friedelin	19.91	$C_{30}H_{50}O$	426.73	Pentacyclic triterpene

Table-4: Activity of phytocomponents identified in the methanolic fruit extract of *M. emarginata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	7.450	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.49	$C_6H_8O_4$	144	
2.	10.652	Pentadecane	0.22	$C_{15}H_{32}$	212	Fragrance agent
3.	12.549	1,2,3,4,5-Cyclohexanepentol	23.17	$C_6H_{12}O_5$	164	
4.	13.867	Pentadecanoic acid, 14-methyl-, methyl ester	0.51	$C_{17}H_{34}O_2$	270	Fatty acid
5.	14.116	n-Hexadecanoic acid	3.22	$C_{16}H_{32}O_2$	256	Fatty acid
6.	14.367	6,6-Dimethylcycloocta-2,4-dienone	0.28	$C_{10}H_{14}O$	150	
7.	14.519	Eicosanoic acid, methyl ester	0.19	$C_{21}H_{42}O_2$	326	Fatty acid
8.	15.014	8-Octadecenoic acid, methyl ester	7.12	$C_{19}H_{36}O_2$	296	Fatty acid
9.	15.141	Octadecanoic acid, methyl ester	0.99	$C_{19}H_{38}O_2$	298	Fatty acid
10.	15.280	9-Octadecenoic acid (Oleic Acid)	7.75	$C_{18}H_{34}O_2$	282	Unsaturated fatty acid
11.	15.375	Octadecanoic acid (Stearic acid)	2.72	$C_{18}H_{36}O_2$	284	Saturated fatty acid
12.	17.637	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.97	$C_{19}H_{38}O_4$	330	Fatty acid
13.	17.708	n-Octadecanal	0.28	$C_{18}H_{36}O$	268	
14.	17.931	1,2-Benzenedicarboxylic acid, dioctyl ester	0.28	$C_{24}H_{38}O_4$	390	
15.	18.106	Pentadecane, 8-hexyl-	0.17	$C_{21}H_{44}$	296	
16.	18.816	1H-Indole-3-ethanamine	0.62	$C_{10}H_{12}N_2$	160	
17.	18.882	n-Heneicosane	1.10	$C_{21}H_{44}$	296	
18.	19.082	Valerenol	5.51	$C_{15}H_{24}O$	220	Sesquiterpenoid
19.	19.230	Alpha.-Monostearin	7.80	$C_{21}H_{42}O_4$	358	
20.	19.590	1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol	0.82	$C_{13}H_{22}O_2$	210	
21.	19.759	N-Nonadecane	0.48	$C_{19}H_{40}$	268	Saturated aliphatic hydrocarbon
22.	19.839	Germacrene A	2.89	$C_{15}H_{24}$	204.3	Volatile organic hydrocarbons (sesquiterpene)
23.	20.076	Seychellene	2.42	$C_{15}H_{24}$	204.3	
24.	20.237	Squalene	0.70	$C_{30}H_{50}$	410.7	Triterpene
25.	20.314	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-azabenzocyclohepten-8-one	0.38	$C_{10}H_{12}N_2O_2$	192.2	
26.	20.768	Nonacosane	0.40	$C_{29}H_{60}$	408	Hydrocarbon
27.	20.862	Perillaldehyde	2.05	$C_{10}H_{14}O$	150.2	Monoterpenoid
28.	21.548	Isovellerdiol	8.56	$C_{15}H_{24}O_2$	236	Sesquiterpenes
29.	22.353	Andrographolide	1.84	$C_{20}H_{30}O_5$	350.4	diterpenoid
30.	23.569	1-Triacontanol	1.77	$C_{30}H_{62}O$	438.8	Fatty alcohol
31.	24.702	A-Tocopherol	0.80	$C_{29}H_{50}O_2$	430.7	Vitamin- E
32.	25.154	Cholesterol	1.40	$C_{30}H_{50}$	410.7	Triterpene
33.	25.527	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis- (Widdrol)	0.35	$C_{15}H_{26}O$	222.3	Odorous compound
34.	27.261	alpha.-Tocopherol	1.58	$C_{29}H_{50}O_2$	430	Vitamin- E
35.	27.669	Trilostane	0.87	$C_{20}H_{27}NO$	329.4	3β-hydroxysteroid dehydrogenase inhibitor
36.	27.985	Evonine	3.36	$C_{36}H_{43}NO_{17}$	761	Alkaloid
37.	29.379	B-Sitosterol	2.11	$C_{29}H_{50}O$	414.7	Phytosterol
38.	29.538	1,4,8-Cycloundecatriene, 2,6,6,9-tetramethyl-, (E,E,E)-(alpha- Caryophyllene)	1.36	$C_{15}H_{24}$	204.35	sesquiterpenes
39.	31.547	Cycloartenol	2.48	$C_{30}H_{50}O$	426.72	Phytostanol

In methanol root extract of *M. emarginata* the highest peak area (%) of 26.56 was obtained by methyl commate A (triterpenes glycoside) (retention-time 30.814) and the lowest peak area (%) of 0.18 was obtained by 1- hexadecanol (retention-time 11.425) (Table-1, Fig. A). Whereas, in methanol stem extract the highest peak area (%) of 19.86 was obtained by 9- octadecenoic acid (Z)- (retention-time 15.282) and the lowest peak area (%) of 0.34 was obtained by pentadecane, 8-hexyl- (retention-time 18.106) (Table-2, Fig. B).In methanolic leaf extract *M. emarginata* the highest peak area (%) of 19.91 was obtained by friedelin (pentacyclic triterpene) (retention-time 36.699) and the lowest peak area (%) of 0.34 was obtained by n- hexadecane sulfonyl chloride (retention-time 18.086) (Table-3, Fig. C). However, in fruit extract of this plant the highest peak area (%) of 23.17 was obtained by 1,2,3,4,5-cyclohexanepentol (retention-time 12.549) and the lowest peak area (%) of 0.17 was obtained by pentadecane, 8-hexyl- (retention-time 18.106) (Table-4), Fig D.



(a) (b)

(c) (d)

Fig. A-D: GC/MS spectrogram for the methanolic (a) root (b) stem (c) leaf and (d) fruit extract of *Maytenus emarginata*

In the present study, methanolic extract of the different plant parts of the *M. emarginata* samples were analyzed for the first time. The comparison of the mass spectrum with the NIST database library gave more than 90% match as well as a confirmatory compound structure match. This work will help to identify the compounds, which may be used in body products, drugs, pharmaceutical and therapeutic value.

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