Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 2748-2752
Received: 14-11-2018
Accepted: 18-12-2018

Mahalakashmi R
PG and Research, Department of Botany, Kongunadu Arts and Science College Autonomous, Coimbatore, Tamil Nadu, India

Thangapandian V
PG and Research, Department of Botany, Kongunadu Arts and Science College Autonomous, Coimbatore, Tamil Nadu, India

Correspondence
Mahalakashmi R
PG and Research, Department of Botany, Kongunadu Arts and Science College Autonomous, Coimbatore, Tamil Nadu, India

Gas chromatography and mass spectrometry analysis of bioactive constituents of *Maytenus heyneana* (Roth) Roju & Babu (Celestraceae)

Mahalakashmi R and Thangapandian V

Abstract
The aim of the present study was to screen the bioactive components present in the methanol leaf extract of *Maytenus heyneana* using GC-MS analysis, which revealed a total of 29 bioactive constituents. Major peak area percentages observed were 18.35%, 10.39% and 9.50% that indicated the presence of Squalene, 1,3,3,5- Tetra cyanopentane and 2-Hexadecen-1-01, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]- (CAS) components respectively. Though the biological activities of some of the components are yet to be discovered, other components have certain entailed biological activities such as antiaging, antimicrobial, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic and anticoronary.

Keywords: Bioactive compounds, GC-MS, Maytenus heyneana

Introduction
The number and diversity of medicinal plants greatly differ in various regions, countries and continents. Human welfare and healthcare herbal medicines are being globally recognized on the basis of their enormous curative responses. In India, different traditional medicinal systems such as Ayurveda, Siddha and Unani have been practicing since time immemorial and in addition innumerable folk medicinal practices are also being adopted by the local people without knowing the importance of the phytochemicals. Phytochemicals or secondary metabolites of the plants are non-nutritive chemical compounds that are synthesized during critical period through special physiological process and they have protective or disease preventive properties.

M. heyneana, belongs to Celestraceae family and commonly known as the bitter sweet family, was collected at Chitteri Hills that are higher hills in the northern Tamil Nadu State of the Eastern Ghats located on the north of the Kaveri River. Though the Eastern Ghats are not as high as the Western Ghats, it has its own habits and habitats of flora and fauna (Sandhyarani *et al*., 2007) [10]. The local people of the Chitteri Hills has been traditionally using the leaves of *M. heyneana* to treat the inflammation (of insect bites) and dysentery (due to microbial infections) without knowing antioxidant, antiarthritis, antitumor, antineoplastic, anticoronary, antihypercholesterolemic, antirheumatoid arthritis and antiosteoarthritis activities (Dubravkova *et al*., 1998) [2]. As the leaves of *M. heyneana* are commonly used by the local people since ancient times due to their useful medicinal properties (Crombie, 1990) [1], an attempt has been made to analyze the bioactive phytocomponents present in this plant.

Gas chromatography - Mass spectrometry (GC-MS) is a commonly used technique to analyze the phytocomponents of the medicinal plants. It is the technique, where the Mass spectrometry is coupled with the Gas chromatography to separate the components of the medicinal plants. Furthermore, GC-MS technique is said to be a valuable method for plant material analyses, as it requires only small quantities to evaluate the non-polar components such as volatile essential oil, fatty acids, lipids, alkaloids, pytol, ether, hydrocarbon and alkane hydrocarbon (Robertson, 2005; Karthishwaran, 2012) [3, 4]. Knowledge on phytocomponents is not only desirable to get therapeutic agents from the plants; it has also great value for disclosing and synthesising medicinally important complex substances to derive from the folkloric remedies (Milne, 1993) [9].

Material and Methods
Study area
The present study area, Chitteri hills, is located in Dharmapuri district, Eastern Ghats of Tamil Nadu, India. The study area lies between 87°0' to 89°0' longitude and 28°0' to 37° 0° latitude.

The altitude of Chitteri hills falls between 240-1266 msl. The mean annual temperature in the study area ranges from 12°C to 35°C during March to June and averages temperature varies between 10°C and 25°C during October to January. An average rainfall of this area is 200 mm. The different forest vegetations of the Chitteri hills are the Scrub forest, Dry deciduous forest, Thorn and Semi evergreen forests.

Collection of Plant material

The leaves of *M. heyneana* were collected from Chitteri hills, Eastern Ghats of Dharmapuri district, Tamil Nadu. The plant specimen was verified and identified by the Botanical Survey of India (Southern circle, Coimbatore, Tamilnadu, India) and voucher specimens were deposited at Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Extraction of plant material

30 g leaves of *M. heyneana* were shad dried and ground into fine powder that was packed with Whatmann No.1 filter paper. Packed powdered material was kept in Soxhlet apparatus to extract the bioactive components of the plant. The extracted phyto components were allowed to evaporate at room temperature (30˚C) and the remaining dried residues were collected and weighed, which were taken for further analyses.

Gas Chromatography - Mass Spectrometry (GC-MS) analysis

5 ml methanol extract was evaporated to dryness to get into 2 ml. The extract was then subjected to GC-MS analysis. Chromatographic separation was carried out with Thermo GC - Trace Ultra Version 5.0, Thermo MS - DSQ II with Db 35.0 - MS Capillary Standard Non-Polar Column (30.0 m x 0.25 mm ID x 0.25µm thickness). The initial oven temperature was programmed at 70 °C for 2 minutes and then it was increased up to 150 °C with an increase of 10 °C/minute, where the holding time maintained was 2 minutes and the temperature raising was continued up to 220 °C with 5 °C/minute with 1 minute holding time. Finally, the temperature was increased up to 260 °C with an increase of 10 °C/minute and it was isothermal for 10 minutes. Mass spectra were taken at 70 eV; a scan interval of 0.5 and Full Mass ranged from 50 - 650 m/z. Helium was used as carrier gas at 99.999 % pressure with flow 1.0 ml/minute and the volume taken was 1µl. Samples were dissolved in methanol and injected automatically. Total GC running time was 37.51 minutes. The mass spectra derived from the GC-MS analyses were compared with NIST Library (2005) database and fragments of various compounds present in the extracts were also identified based on the molecular weight, molecular formula, retention time and peak area percentage.

Results and Discussion

Compound identification

Twenty nine biologically active compounds were screened from the methanol leaf extracts of *M. heyneana* using GC-MS analysis. These biologically active compounds of their peak area %, molecular formula (MF), molecular weight (MW) and retention time (RT) are presented in (Table - 1 & 2 and Figure – 1).

The prevalent chemical components identified with respective of their peak area percentages of the methanol leaf extracts of *M. heyneana* were Squalene (18.35%), 1,3,3,5-Tetracyanopentane (10.39%) (Fig-2) 2-Hexadecen-1-01, 3, 7, 11, 15-tetramethyl-,[R-[R*,R*-(E)]]-(CAS) (9.50%) (Fig-3), Hexadecanoic acid, methyl ester (CAS) (6.33%), Trans-3-methyl-2-(1-methyl-3-butynyl) oxirane (6.00%), Pseduosarsasapogenin-5,20-dien methyl ether (4.45%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS) (4.15%), Dodecane (CAS) (3.63%), (Z)1-Bromo-3, 3-dimethoxy- 2-methyl- 1-propene (3.28%), Neophytadiene (3.14%), 4-(2',6'-Dichlorophenylmethylene)-1,2,3,4-tetraisoquinoline-1,3-dione (2.30%), Tetradecanol-018 (1.56%), Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate (1.54%), Docosane (CAS) (1.53%), 1,1,2,3-Tetrachloro-1,2-dimethoxy-6-methoxymethyl-3-methylbenzoic acid (1.43%), Methyl 3-(Acetylthio)bicycle[1.1.1]pentane-1-carboxylate (1.32%), N-[2-(Hydroxymethyl)cyclohex-1-yl]methyl]methane sulphonamide (1.26%), 3-Carbamoyl-4-(p-chlorophenyl)-5,6-dihydrobenzo[h]thiochroman (1.12%), 1H-Pyrazole, 3-methyl-(CAS) (1.08%), 4,5-Dimethoxybenzocyclobutenol (1.08%), Tetradecanoic acid, methyl ester (CAS) (0.99%), 1,1-Bis(trimethylsily)-2-oxa-1-silaindan (0.92%), Phytol, acetate (0.85%), Octadecanoic acid, methyl ester (CAS) (0.83%), Dichloroacetic acid, decyl ester (0.77%), Dodecanedioic acid, dimethyl ester (CAS) (0.76%), (1S,5S,6s)-exo-5-Methyl-3-oxabicyclo[4.1.0]heptan-2-one-8-d3 (0.70%) and 3-Acetyl-4-(methylthio)pyrido[1,2-a]pyrimidin-2-one (0.66%).

The chemical component with maximum peak area% identified from the methanol leaf extracts of *M. heyneana* was Trans-3-methyl-2-(1-methyl-3-butynyl) oxirane that belong to ether and has anticarcinogenic and anti-inflammatory activities. It is also used as ophthalmic drug. Similar result was published by Nazneen Bobby *et al*. (2015) [17] from ethyl acetate extract of *Albizia lebbeck* Beneth belong to Fabaceae. It had been also stated that the ethyl acetate extract had anti carcinogenic, anti-inflammatory, ophthalmic drug activities. However, the chemical component reported by them was (2R, 3S)-2, 3-dimethyl-2-vinylcyclobutanones, which had similar molecular formula ($C_8H_{12}O$) and molecular weight (124), but differ in peak area percentage (0.61%). Further, they explained that this chemical compound had antifungal activity that was able to cure dandruff.

Another bioactive component identified with high peak area percentage (18.35%) was Squalene followed by 1,3,3,5- Tetra cyanopentane with peak area percentage (10.39) and 2-Hexadecen-1-01,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-(CAS) with 9.50%. Squalene is triterpene in nature and it was reported to have antiaging, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antiumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, antocoronary activities by Sudha *et al*. (2013), Vembarasi *et al*. (2013), Olena konovalova *et al*. (2013), Gomathi Rajashyamala and Elango (2015), Parthipan *et al*. (2015) and Karthikeyan and Baskaran (2016) [7, 11, 16, 12, 13, 14] in *Fluggea leucopyrus* (Euphorbiaceae), *Asparagus racemosus* (Asparagaceae), *Shepherdia argentea* (Elaeagnaceae), *Evolvulus alsinoides* (Convolvulaceae), *Pleiospermium alatum* (Rutaceae) and *Barleria acuminate* (Acanthaceae) respectively. 2-Hexadecen-1-01,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-(CAS), diterpene in nature, represented at 9.50% peak area and has antimicrobial, anti-inflammatory, anticancer and diuretic activities that were reported by Jagadeeswari *et al*. (2012), Sudha *et al*. (2013), Gomathi Rajashyamala and Elango (2015), Parthipan *et al*. (2015) and Rajalakshmi and Mohan (2016) [15, 7, 12, 13, 18].

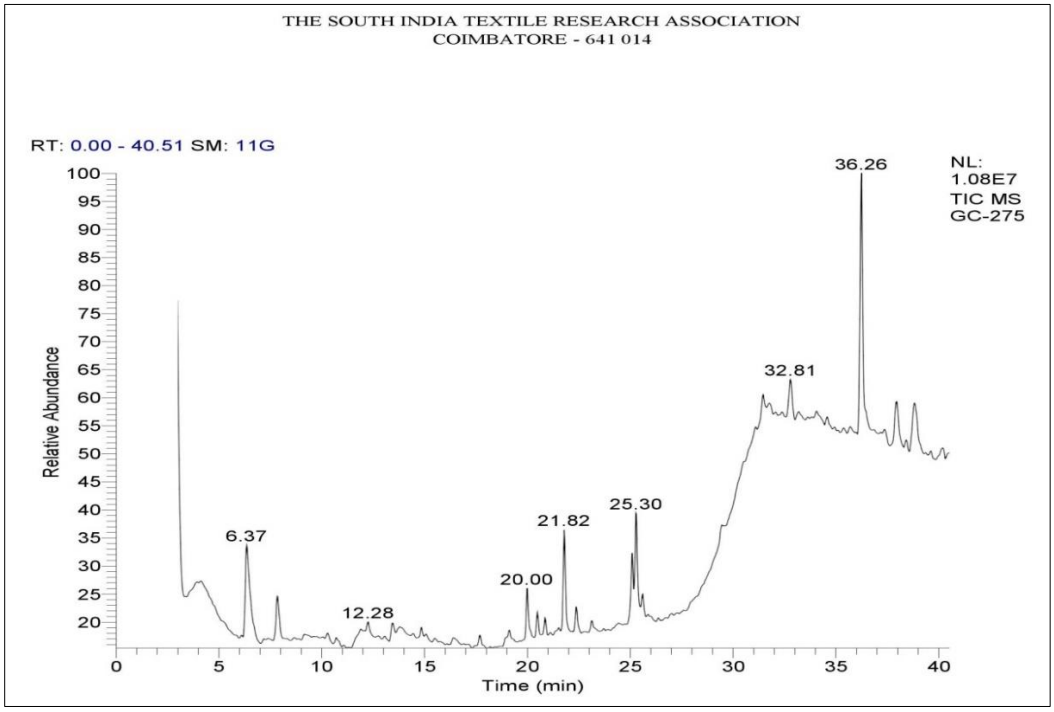

Fig 1: GC-MS spectrum derived from the methanol leaf extract of *Maytenus heyneana*.

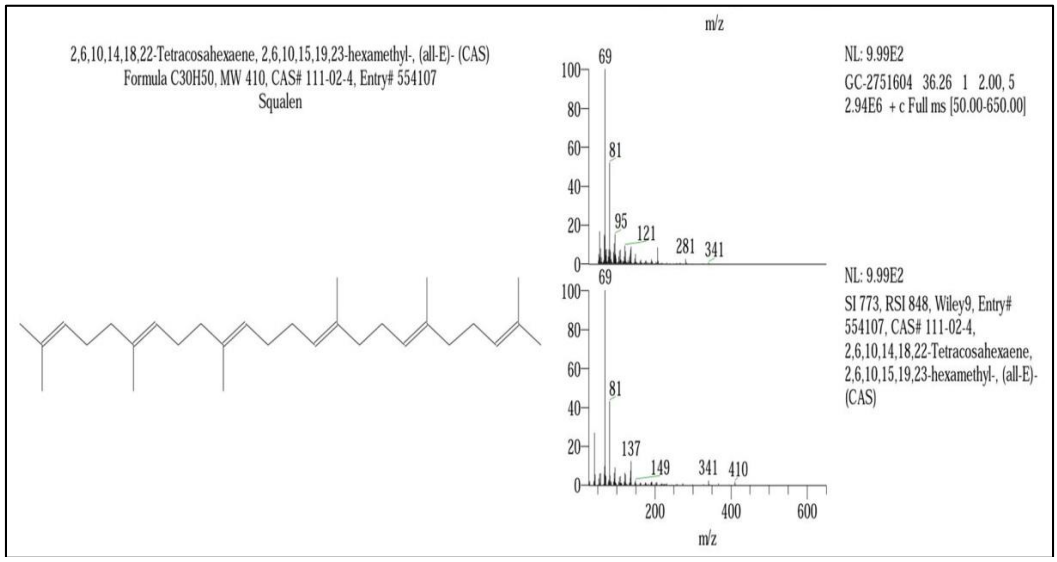

Fig 2: Mass Spectrum of Squalene compound identified methanol leave extract of *M.heyneana*

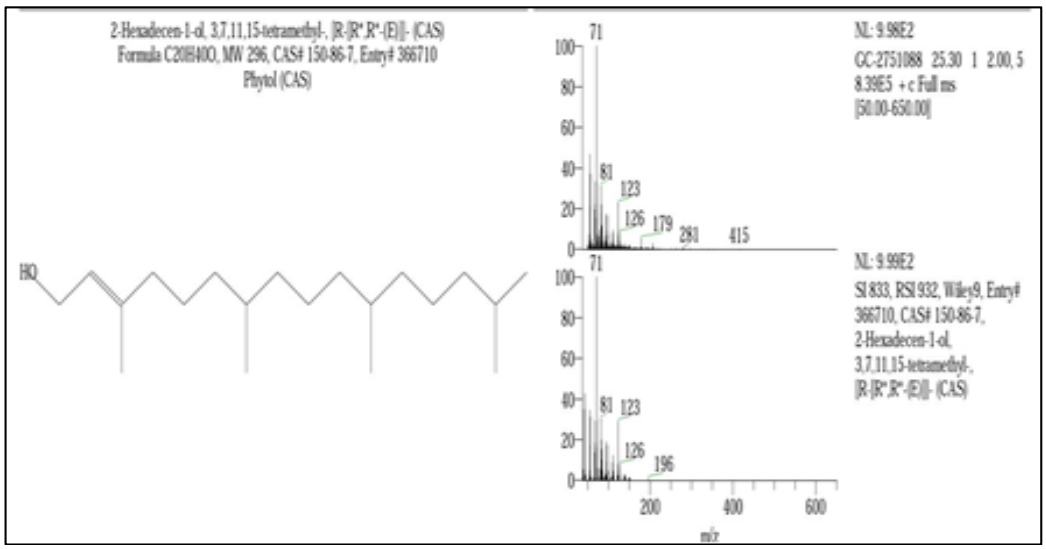

Fig 3: Mass-Spectrun of Diterpene compound identified methanol leave extract of *M.heyneana*

Table 1: Bioactive components identified in the methanol leaf extract of *Maytenus heyneana*.

S. No	Retention Time (RT)	Name of the component	Molecular formula (MF)	Molecular weight (MW)	Peak Area (%)
1	6.37	1,3,3,5- Tetra cyanopentane	$C_9H_8N_4$	172	10.39
2	7.86	Dodecane (CAS)	$C_{12}H_{26}$	170	3.63
3	11.91	4-(2',6'-Dichlorophenylmethylene)-1,2,3,4-tetraisoquinoline-1,3-dione	$C_{16}H_9C_{12}NO_2$	317	2.30
4	12.26	1,1,2,3-Tetrachloro-1,2-dimethoxy-6-methoxymethyl-3-methylbenzoic Acid	$C_5H_8C_{14}O_2$	240	1.43
5	13.46	Methyl 3-(Acetylthio)bicycle[1.1.1]pentane-1-carboxylate	$C_{10}H_{16}O_2S$	200	1.32
6	13.81	Docosane(CAS)	$C_{22}H_{46}$	310	1.53
7	16.44	3-Acetyl-4-(methylthio)pyrido[1,2-a]pyrimidin-2-one	$C_{11}H_{10}N_2O_2S$	234	0.66
8	17.70	Tetradecanoic acid, methyl ester (CAS)	$C_{15}H_{30}O_2$	242	0.99
9	19.15	TETRADECANOL-018	$C_{14}H_{30}O$	214	1.56
10	20.00	Neophytadiene	$C_{20}H_{38}$	278	3.14
11	20.51	N-[2-(Hydroxymethyl)cyclohex-1-yl]methyl]m ethanesulfonamide	$C_9H_{19}NO_3S$	221	1.26
12	20.88	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.85
13	21.82	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	6.33
14	22.41	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	$C_{10}H_{10}O_3$	178	1.54
15	23.16	Dichloroacetic acid, decyl ester	$C_{12}H_{22}C_{12}0_2$	268	0.77
16	25.30	2-Hexadecen-1-01,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-(CAS)	$C_{20}H_{40}O$	296	9.50
17	25.63	Octadecanoic acid, methyl ester (CAS)	$C_{19}H_{38}O_2$	298	0.83
18	29.46	4,5-Dimethoxybenzocyclobutenol	$C_{10}H_{12}O3$	180	1.08
19	30.50	1,1-Bis (trimethylsily)-2-oxa-1-silaindan	$C_{13}H_{24}OSi_3$	280	0.92
20	31.08	Dodecanedioic acid, dimethyl ester (CAS)	$C_{14}H_{26}O_4$	258	0.76
21	31.48	1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS)	$C_{24}H_{38}O_4$	390	4.15
22	32.81	(Z)1-Bromo-3, 3-dimethoxy- 2-methyl- 1-propene	$C_6H_{11}BrO_2$	194	3.28
23	33.21	(1S,5S,6s)-exo-5-Methyl-3-oxabicyclo[4.1.0]heptan-2-one-8-d3	$C_7H_7D_3O_2$	126	0.70
24	34.10	5-methoxy-15-nitro-2,3,7,8,12,13,17,18-octaet hylporphyrin	$C_{37}H_{47}N_5O_3$	609	1.43
25	36.26	Squalene	$C_{30}H_{50}$	410	18.35
26	37.40	3-Carbamoyl-4-(p-chlorophenyl)-5,6-dihydrobenzo[h]thiochroman	$C_{20}H_{18}ClNOS$	355	1.12
27	37.97	Pseduosarsasapogenin-5,20-dien methyl ether	$C_{28}H_{44}O_3$	428	4.45
28	38.85	Trans-3-methyl-2-(1-methyl-3-butynyl) oxirane	$C_8H_{12}O$	124	6.00
29	40.22	1H-Pyrazole, 3-methyl-(CAS)	$C_4H_6N_2$	82	1.08

Table 2: Biological activities of the bioactive components of the methanol leaf extract of *M. heyneana*.

S. No	Name of the component	Nature of the component	Biological activities of the component
1	1,3,3,5- Tetracyanopentane	Unknown	Not yet identified
2	Dodecane (CAS)	Hydrocarbon	Not yet identified
3	4-(2',6'-Dichlorophenylmethylene)-1,2,3,4-tetraisoquinoline-1,3-dione	Ether	Not yet identified
4	1,1,2,3-Tetrachloro-1,2-dimethoxy-6-methoxymethyl-3-methylbenzoic Acid	Ethane	Not yet identified
5	Methyl 3-(Acetylthio) bicycle[1.1.1]pentane-1-carboxylate	Fatty acid	Not yet identified
6	Docosane(CAS)	Hydrocarbon	Not yet identified
7	3-Acetyl-4-(methylthio) pyrido[1,2-a]pyrimidin-2-one	Alkaloids	Not yet identified
8	Tetradecanoic acid, methyl ester (CAS)	Fatty acid	Antioxidant, cancer preventive, hypercholesterolemic, nematicide activities
9	Tetradecanol-018	Cypionic acid	Not yet identified
10	Neophytadiene	Alkaloid	Antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant, antiprolerative
11	N-[2-(Hydroxymethyl)cyclohex-1-yl]methyl]m ethanesulfonamide	Unknown	Not yet identified
12	Phytol, acetate	Diterpene	Antimicrobial, anticancer, anti- inflammatory, diuretic, hypocholesterolemic, nematicide, anticoronory, antiarthritic, hepatoprotective, anti-androgenic
13	Hexadecanoic acid, methyl ester (CAS)	Fatty acid	Antibacterial, antifungal, antioxidant, hypocholestrolemic, nematicide, pesticide, antiantrogenic flavour, haemolytic, 5-Alpha reductase inhibitor
14	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	Fatty acid	Antimicrobial
15	Dichloroacetic acid, decyl ester	Fatty acid	Not yet identified
16	2-Hexadecen-1-01,3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-(CAS)	Diterpene	Antimicrobial, anti-inflammatory, anticancer, diuretic
17	Octadecanoic acid, methyl ester (CAS)	Fatty acid	Antimicrobial, antifungal, antibacterial, hypocholesterolemic
18	4,5-Dimethoxybenzocyclobutenol	Fatty acid	Antimicrobial, anti-inflammatory, antioxidant, analgesic
19	1,1-Bis(trimethylsily)-2-oxa-1-silaindan	Inorganic ether	Not yet identified
20	Dodecanedioic acid, dimethyl ester (CAS)	Fatty acid	Antimicrobial, antiulcer, cytotoxic
21	1,2-Benzenedicarboxylic acid, diisooctyl ester (CAS)	Ether	Antifouling, antimicrobial
22	(Z)1-Bromo-3, 3-dimethoxy- 2-methyl- 1-propene	Fatty acid	Not yet identified
23	(1S,5S,6s)-exo-5-Methyl-3-oxabicyclo[4.1.0]heptan-2-one-8-d3	Ether	Not yet identified
24	5-methoxy-15-nitro-2,3,7,8,12,13,17,18-octaet hylporphyrin	Ether	Not yet identified

25	Suqalene	Triterpene	Antiaging, analgesic, antidiabetic, antioxidant, antidermatitic, antileukemic, antiumor, anticancer, hepatoprotective, vasodilator, hypocholesterolemic, antiulcerogenic, antispasmodic, antibronchitic, anticoronary, anti-inflammatory,
26	3-Carbamoyl-4-(p-chlorophenyl)-5,6-dihydrobenzo[h]thiochroman	Amaide	Not yet identified
27	Pseduosarsasapogenin-5,20-dien methyl ether	Ether	Not yet identified
28	trans-3-methyl-2-(1-methyl-3-butynyl) oxirane	Ether	Anticarcinogenic, anti-inflammatory, ophthalmic drug
29	1H-Pyrazole, 3-methyl-(CAS)	Heteocyclic base	Not yet identified

Conclusion

GC-MS analysis of methanol leaf extract of *Maytenus heyneana* revealed 29 bioactive components with various important biological activities that are to be entailed to recommend for pharmaceutical industries to formulate new drugs from this plant by involving it in further investigation to validate its bioactive principles in relevance to its toxic qualities.

References

1. Crombie L, WML Crombie, DA Whiting. The Alkaloids. 1990; 39:139.
2. Dubravkova L, Voticky Z, Tomko J. Acta Fac. Pharm. Univ. Lomenianae. 1998; 42:141.
3. Robertson DG. Metabonomics in toxicology: A review. Toxicol Sci. 2005; 85:809-22.
4. Karthishwaran K, Muthukkumarasamy S, Sankaran M. GCMS analysis of methanolic extract of aerial parts of *Pergularia daemia*. Journal of Life Science. 2012; 1(1):50-55.
5. Velanganni J, Kadamban D. Phytoconstituents of ethanol extract of *Mallotus philippensis* (Lam.) Mull. Arg.var. philippensis (Euphorbiaceae). Int. J of. Pharm. re. and Dev. 2011; 3(8):73-76. 19.
6. Sridharan S, Meenaa V, Kavitha V, Agnel Arul John N. GC-MS study and phytochemical profiling of Mimosa pudica Linn. J Pharm. Res. 2011; 4(3):741-742. 20.
7. Mohan VR, Sudha T, Chidambarampillai S. GC-MS analysis of bioactive components of aerial parts of Kirganelia reticulata Poir. (Euphorbiaceae). J Curr. Chem. Pharm. Sc. 2013; 3(2):113-122.
8. Sudha T, Chidambarampillai S, Mohan VR. GC-MS Analysis of Bioactive components of aerial parts of Fluggea leucopyrus Willd. (Euphorbiaceae). Journal of Applied Pharmaceutical Science. 2013; 3(5):126-130.
9. Milne A. Inhalational and local anesthetics reduce tactile and thermal responses in *Mimosa pudica* Linn. Masui, 1993, 1190-1193.
10. Sandhyarani SK, Sri Rama Murthy, T Pullaiah. Tree Flora in Eastern Ghats of Southern Peninsular India. 2007; 2(4):176-185,
11. Vembarasi G, Velavan S, Mahadevan K. Identification of bioactive components and its biological activities of Asparagus racemosus Rhiomes through the Gas-chromatography and Mass spectrometry. International journal of natural products Research. 2013; 2(1):17-19.
12. Gomathi Rajashyamala L, Elango V. Identification of bioactive components and its biological activities of *Evolvulus alsinoides* Linn. -- A GC-MS study. International Journal of Chemical Studies. 2015; 3(1):41-44.
13. Parthipan B, Suky MGT, VR Mohan. GC-MS Analysis of Phytocomponents in Pleiospermium alatum (Wall. ex Wight & Arn.) Swingle, (Rutaceae). Journal of Pharmacognosy and Phyto Chemistry. 2015; 4(1):216-222
14. Karthikeyan V, A Baskaran, C Sebastian Rajasekaran. Gas Chromatography -Mass Spectrometry (GC-MS) Analysis of Ethanolic Extracts of Barleria acuminate Nees. Nternational Journal of Pharmacological Research. 2016; 6(02):55-61.
15. Jagadeeswari P, Nishanthini A, Muthukumarasamy S, Mohan VR. GC-MS analysis of bioactive components of Aristolochia Krysagathra (Aristolochiaceae). J Current Chem. Pharmaceu. Sci. 2012; 2(4):226-232.
16. Olena Konovalova, Evgenia Gergel, Vitaliy Herhel. GC-MS analysis of bioactive components of Shepherdia argentea (Pursh.) Nutt. From Ukrainian Flora. Thepharma innovation. 2013; 2(6):7-12.
17. Nazneen Bobby, Wesely Edward Gnanaraj, Johnson Marimuthu Alias Antonisamy, Anto Arockia Raj Adaikalam, Vinnarasi Jamesraj. GC-MS analysis of Albizia Lebbeck Benth. 2013; 4(11):1284-1304.
18. Rajalakshmi K, V Mohan. Determination of Bioactive components of Myxopyrum serratulum A.W. Hill (Oleaceae) Stem by GC-MS analysis, 2016.