Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(1): 149-154
Received: 31-03-2015
Accepted: 01-05-2015

Dandekar R
Gahlot Institute of Pharmacy, Plot no-59, Sector 14, Koparkhairane, Navi Mumbai-400709

Fegade B
Gahlot Institute of Pharmacy, Plot no-59, Sector 14, Koparkhairane, Navi Mumbai-400709

Bhaskar VH
Gahlot Institute of Pharmacy, Plot no-59, Sector 14, Koparkhairane, Navi Mumbai-400709

Correspondence:
Dandekar R
Gahlot Institute of Pharmacy, Plot no-59, Sector 14, Koparkhairane, Navi Mumbai-400709

GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves

Dandekar R, Fegade B, Bhaskar VH

Abstract
Epiphyllum oxypetalum plant is very unique plant which belongs to family cactaceae and very scanty research work has been reported. In India it is commonly known as Bramhakamal and is treated as a sacred plant but it is found to have many medicinal values. The GC-MS analysis of alcohol extract of *Epiphyllum oxypetalum* leaves dissolved in ethanol revealed the presence of some compounds. The compounds include: ethanone, 1-(2-hydroxy-5-methylphenyl)-; 4-Hydroxy-2-methylacetophenone; Megastigmatrienone; Cycloocta-1,3,6-triene,2,3,5,5,8,8,-hexamethyl; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; 2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one; n-Hexadecanoic acid; Octadecanoic acid; Phytol; 6-octen-1-ol,3,7-dimethyl; Stigmasterol; Cholesta-22,24-dien-5-ol,4,4-dimethyl; 22-stigmasten-3-one. The GC-MS analysis of alcohol extract dissolved in mixture of solvents revealed few other compounds. These compounds include: Allyldimethyl(prop-1-ynyl)silane; Sulfurous acid, cyclohexylmethyl hexyl ester; Heptacosane; Nonadecane, 2-methyl-; Hexadecane, 2,6,10,14-tetramethyl-; Octadecane, 2-methyl-; Eicosane, 2-methyl-; Spinasterone; 4,22-Stigmastadiene-3-one; Tetracosane; Hentriacontane; Stigmast-4-en-3-one; Testosterone cypionate. Majority of the phytoconstituent present in both the extracts were found to be steroids. From the results it could be concluded that the *Epiphyllum oxypetalum* leaves contain various bioactive compounds which have various medicinal properties.

Keywords: *Epiphyllum oxypetalum* leaves, Gas chromatography-mass spectrometry (GC-MS) analysis, phytoconstituents

1. Introduction
Epiphyllum oxypetalum is a species of cactus and one of the most cultivated species in the genus. It is a variety of night blooming Cereus. Oxypetalum (Lat.) = with acute petals, refers to the acute petals of this species[1]. In India it is known as 'Bramhakamal' and considered as a sacred plant. The plant resembles cactus in habit. It is also known as Orchid cactus as the flower has orchid like beauty[2]. It is known by different common names in different parts of the world, like Night blooming Cereus, Queen of the Night, Lady of night as its beautiful lotus like flower blooms late night, WijayaKusuma (Indonesian), Nishagandhi in Hindi, Jungle cactus, Dutchman's pipe [1, 2].

Epiphyllum oxypetalum plant blooms rarely and that too in late night. These plants have wide distribution and grow wild in tropical rain forest. Epiphyllum are indeed true cacti, but are more often labeled as "Epicacti" or "Jungle cacti" to differentiate them from related desert flora [2].

The phylloclades contain some active ingredients and show anti-bacterial activity. The stem is also used medicinally to cure dropsy and cardiac affections. Vietnamese people use petals of the faded blooms to make soups which are supposed to have tonic and aphrodisiac medicinal properties [2]. Flower also has a power that can speed up the cook wound abscesses. Also it is used in bloody phlegm and cough, uterine bleeding and shortness of breath [3]. The chemical content of Epiphyllum oxypetalum in plants has a potent power to stifle the pain and is able to neutralize blood clotting [4].

The phytochemical investigation of alcohol extract of *Epiphyllum oxypetalum* leaves revealed the presence of steroids, phenolic compounds, tannins, saponins, resins, glycosides. The objective of present study is to identify the phytochemical constituents in alcohol extract of *Epiphyllum oxypetalum* leaves using GC-MS analysis.

Materials and Methods

Collection of plant material

The fresh leaves of *Epiphyllum oxypetalum* were collected from the nusery and local garden in Ratnagiri, Maharashtra. The collected leaves were identified and authenticated by Dr. Harshad Pandit from Guru Nanak Khalsa College, Matunga, Mumbai, Maharashtra.

Preparation of powder and extract

The fresh leaves were washed under running tap water, dried in oven at temperature 40 °C and coarsely powdered in a grinder. The powder was extracted with absolute ethanol in soxhlet extractor at temperature 40-50 °C. The extract was dried on water bath at 60 °C. GC-MS analysis of this dried ethanol extract was further carried out.

Gas chromatography-Mass spectrometry (GC-MS) analysis

The extract was dissolved in ethanol and mixture of solvents and then subjected to GC-MS analysis. Mixture of solvents involved toluene, chloroform, ethanol and ethyl acetate. The GC-MS of sample was carried out in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. Agilent 7890 instrument for GC and Joel Accu TOF GCV instrument for MS were used. The inert gas helium (99.999%) was used as carrier gas with the flow rate of 1 ml/min. HP5 column with specification length 30 mm, internal diameter 0.32 mm, film of 0.25 mm and temperature limit -60 °C to 325 °C (350 °C) was used. The total run time of GC was 35 minutes. The oven temperature raised from 70 °C upto 280 °C with the rate of 8 °C per min rise in temperature. The sample size of 4µl was injected through the injector. The MS was taken at 70eV. The identification of compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in their library and the name, molecular weight and structure were probably determined.

Result and Discussion

GC-MS analysis of alcohol extract of *Epiphyllum oxypetalum* leaves dissolved in ethanol and mixture of solvents showed respectively 9 and 6 major peaks indicating the presence of various phytochemical constituents. On comparison of the mass spectra of constituents with the main library all these compounds were charachterised and probably identified.

Table 1: Phytoconstituents identified in alcohol extract of *E. oxypetalum* leaves dissolved in ethanol by GC-MS analysis

Peak	Retention time (min)	Name of compound	Molecular formula	Molecular weight	Peak area (Intensity *sec)
1	14.02	Megastigmatrienone	$C_{13}H_{18}O$	190.2814	77345.10
2	14.02	Cycloocta-1,3,6-triene, 2,3,5,5,8,8-hexamethyl	$C_{14}H_{22}$	190.32	77345.10
3	14.09	Megastigmatrienone	$C_{13}H_{18}O$	190.2814	677094.33
4	14.09	Cycloocta-1,3,6-triene, 2,3,5,5,8,8-hexamethyl-	$C_{14}H_{22}$	190.32	677094.33
5	14.79	Megastigmatrienone	$C_{13}H_{18}O$	190.2814	2328396.34
6	14.79	Cycloocta-1,3,6-triene, 2,3,5,5,8,8-hexamethyl-	$C_{14}H_{22}$	190.32	2328396.34
7	16.37	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.20	1094647.26
8	16.37	2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one	$C_{10}H_{12}O_3$	180.20	1094647.26
9	31.32	Stigmasterol	$C_{29}H_{48}O$	412.69	30585799.32
10	31.32	Cholesta-22,24-dien-5-ol, 4,4-dimethyl	$C_{29}H_{48}O$	412.69	30585799.32
11	32.45	22-Stigmasten-3-one	$C_{29}H_{48}O$	412.69	631195.47
12	32.45	Stigmasterol	$C_{29}H_{48}O$	412.69	2631195.47

There were few smaller peaks in the chromatogram whose peak area was not calculated but the MS was carried out. Give below are the peaks and name of predicted compound.

Table 2: Phytoconstituents identified in alcohol extract of *E. oxypetalum* leaves dissolved in ethanol by GC-MS analysis without peak area

Peak	Retention time (min)	Name of compound	Molecular formula	Molecular weight
1	9.86	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	$C_9H_{10}O_2$	150.17
2	9.86	4-Hydroxy-2-methylacetophenone	$C_9H_{10}O_2$	150.17
3	19.59	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
4	19.59	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48
5	22.45	Phytol	$C_{20}H_{40}O$	296.53
6	22.45	6-Octen-1-ol, 3,7-dimethyl-, ()-	$C_{10}H_{20}O$	156.2652

Table 3: Phytoconstituents identified in alcohol extract of *E. oxypetalum* leaves dissolved in mixture of solvents by GC-MS analysis

Peak	Retention time (min)	Name of compound	Molecular formula	Molecular weight	Peak area (Intensity *sec)
1	26.54	Heptacosane	$C_{27}H_{56}$	380.74	3027128.72
2	26.54	Octadecane, 2-methyl-	$C_{19}H_{40}$	268.52	3027128.72
3	28.11	Heptacosane	$C_{27}H_{56}$	380.74	2978531.10
4	28.11	Eicosane, 2-methyl-	$C_{21}H_{44}$	296.57	2978531.10
5	31.76	Heptacosane	$C_{27}H_{56}$	380.74	4152787.03
6	31.76	Hentriacontane	$C_{31}H_{64}$	436.84	4152787.03
7	31.93	-	-	-	124830.50
8	32.54	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.69	15921447.15
9	32.54	Testosterone cypionate	$C_{27}H_{40}O_3$	412.61	15921447.15
10	33.00	Heptacosane	$C_{27}H_{56}$	380.74	2970718.83
11	33.00	Hentriacontane	$C_{31}H_{64}$	436.84	2970718.83

The alcohol extract also showed few smaller peaks in the chromatogram whose peak area was not calculated but the MS was carried out. Give below are the peaks and name of predicted compound.

Table 4: Phytoconstituents identified in alcohol extract of *E. oxypetalum* leaves dissolved in a mixture of solvent by GC-MS analysis without peak area

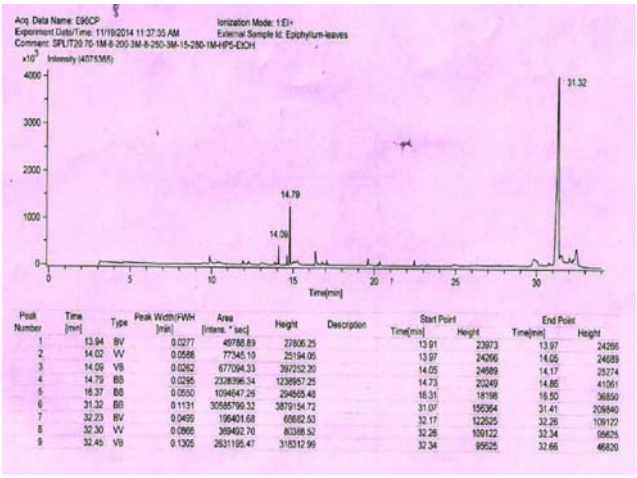
Peak	Retention time (min)	Name of compound	Molecular formula	Molecular weight
1	14.59	Allyldimethyl(prop-1-ynyl)silane	$C_8H_{14}Si$	138.282
2	14.59	Sulfurous acid, cyclohexylmethyl hexyl ester	$C_{13}H_{26}O_3S$	262.409
3	23.96	Heptacosane	$C_{27}H_{56}$	380.74
4	23.96	Nonadecane, 2-methyl-	$C_{20}H_{42}$	282.5475
5	25.23	Heptacosane	$C_{27}H_{56}$	380.74
6	25.23	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282.5475
7	28.66	Spinasterone	$C_{29}H_{46}O$	410.67
8	28.66	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	410.67
9	30.04	Heptacosane	$C_{27}H_{56}$	380.74
10	30.04	Tetracosane	$C_{24}H_{50}$	338.65

Table 5: Activity of the phytoconstituents found in alcohol extract of *E. oxypetalum* leaves by GC-MS analysis

1	Name of compound	Nature of compound	Reference number	Activity
(1)	4-Hydroxy-2-methylacetophenone	Phenolic compound	5	Anticancer, vasodilator, analgesic, anaesthetic, antioxidant, antiseptic, antiviral, fungicide, rodenticide, emetic
(2)	Megastigmatrienone	C13 nor-isoprenoid derived from carotenoids (terpene)	6	Aroma
(3)	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Phenolic compound	7	Antimicrobial, Antioxidant, Anti inflammatory
(4)	n-Hexadecanoic acid	Palmitric acid (saturated fatty acid)	8,9	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-alpha reductase inhibitor, antipsychotic
(5)	Octadecanoic acid	Stearic acid	8,9,10	Antifungal, Antitumour, Antibacterial
(6)	Phytol	Diterpene	8,9,10	Antimicrobial, Anti inflammatory, Anticancer, Diuretic, Antifungal against *S. typhi*, resistant gonorrhea, joint dislocation, headache, hernia, stimulant and antimalarial
(7)	Cholesta-22,24-dien-5-ol, 4,4-dimethyl	Steroid	9	Antibacterial, trypanocidal activity
(8)	6-Octen-1-ol, 3,7-dimethyl-, (±)-	Natural acyclic mono terpenoid	11,12, 13	Used in perfumes, insect repellant and mite attractant, raw material for production of rose oxide, antibacterial, antifungal
(9)	Stigmasterol	Steroid	14	Antioxidant, hypoglycemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer, antiarthritic, antiasthama, anti inflammatory, diuretic
(10)	22-Stigmasten-3-one	Steroid	15	Antimicrobial, anticancer, antiarthritic, antiasthama, diuretic

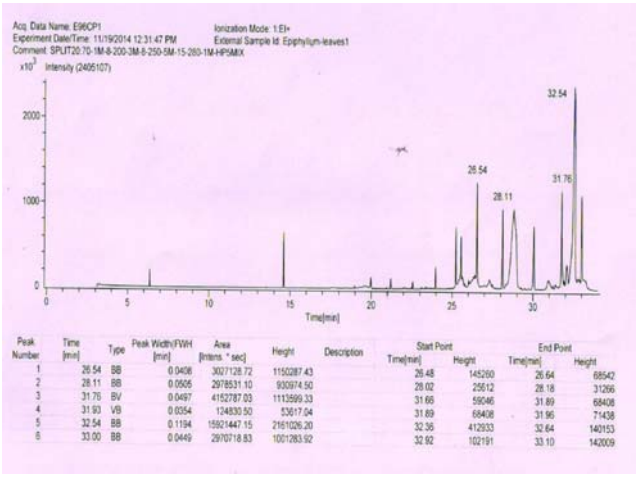
Table 6: Activity of the phytoconstituents found in alcohol extract of *E. oxypetalum* leaves dissolved in mixture of solvent by GC-MS analysis

Sr. no.	Name of the compound	Nature of compound	Reference number	Activity
(1)	Heptacosane	Higher alkane	10,16	Anti corrosive/ Antioxidant
(2)	Nonadecane, 2-methyl-	Volatile heterocyclic hydrocarbon	17	Antioxidant
(4)	Spinasterone	Steroid	18	Antifungal
(5)	4,22-Stigmastadiene-3-one	Steroid	19,20	Antimicrobial
(6)	Tetracosane	Higher alkane	16	Anti corrosive/ Antioxidant
(7)	Hentriacontane	Higher alkane	10	Antifungal against fungal spores germination, Antioxidant, Antitumour, Antibacterial
(8)	Stigmast-4-en-3-one	Steroid	20	Hypoglycemic
(9)	Testosterone cypionate	Steroid	21	Replacing or supplementing the testosterone that is naturally made in body



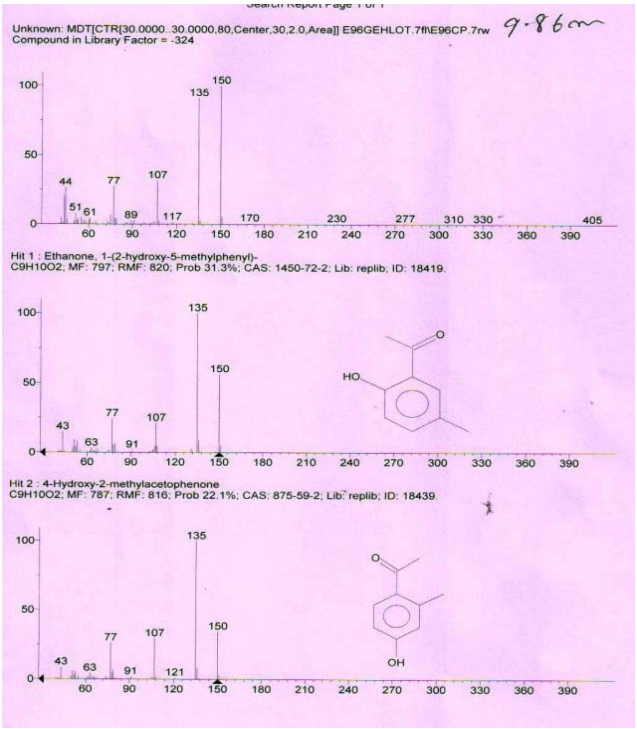
Peak Number	Time [min]	Type	Peak Width/FWH [min]	Area [Intens. * sec]	Height	Description	Start Point Time[min]	Start Point Height	End Point Time[min]	End Point Height
1	13.94	BV	0.0277	49788.89	27806.25		13.91	23973	13.97	24266
2	14.02	VV	0.0588	77345.10	25194.05		13.97	24266	14.05	24689
3	14.09	VB	0.0262	677094.33	397252.20		14.05	24689	14.17	25274
4	14.79	BB	0.0295	2326996.34	1238957.25		14.73	20249	14.86	41061
5	16.37	BB	0.0550	1094647.26	294565.48		16.31	18198	16.50	36850
6	31.32	BB	0.1131	30585799.32	3879154.72		31.07	156364	31.41	208840
7	32.23	BV	0.0499	196401.68	65682.53		32.17	122525	32.26	109122
8	32.30	VV	0.0866	369492.70	80388.52		32.26	109122	32.34	95625
9	32.45	VB	0.1305	2631195.47	318312.99		32.34	95625	32.66	46820

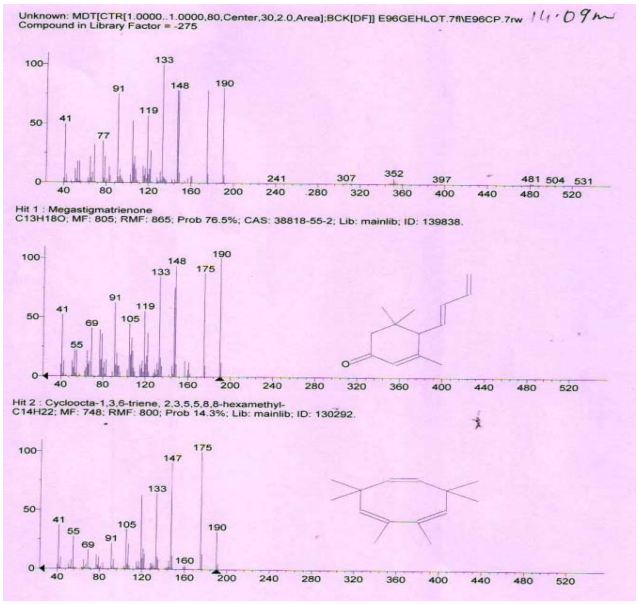
Fig 1: GC-MS chromatogram of alcohol extract of *E. oxypetalum* leaves dissolved in ethanol

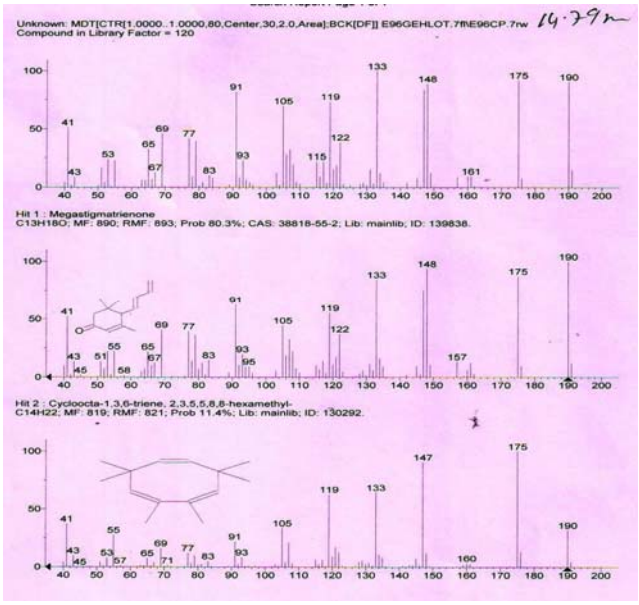


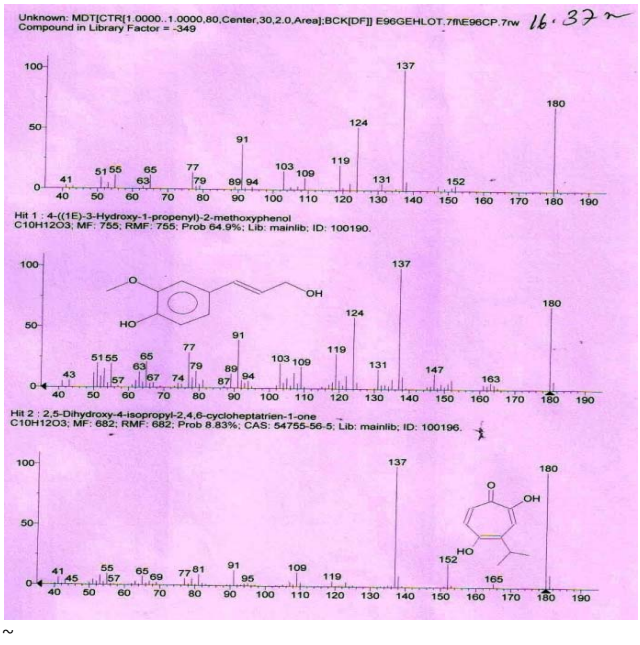
Peak Number	Time [min]	Type	Peak Width/FWH [min]	Area [Intens. * sec]	Height	Description	Start Point Time[min]	Start Point Height	End Point Time[min]	End Point Height
1	26.54	BB	0.0408	3027128.72	1150287.43		26.48	145260	26.64	68542
2	28.11	BB	0.0505	2978531.10	930974.50		28.02	25612	28.18	31266
3	31.76	BV	0.0497	4152787.03	1113599.33		31.66	59046	31.89	68408
4	31.93	VB	0.0354	124830.50	53617.04		31.89	68408	31.96	71438
5	32.54	BB	0.1194	15921447.15	2161026.20		32.36	412933	32.64	140153
6	33.00	BB	0.0449	2970718.83	1001283.92		32.92	102191	33.10	142009

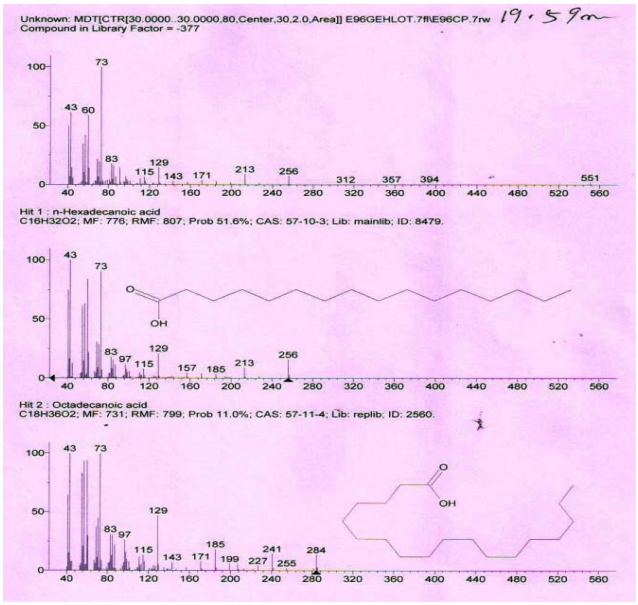
Fig 2: GC-MS chromatogram of alcohol extract of *E. oxypetalum* leaves dissolved in mixture of solvents











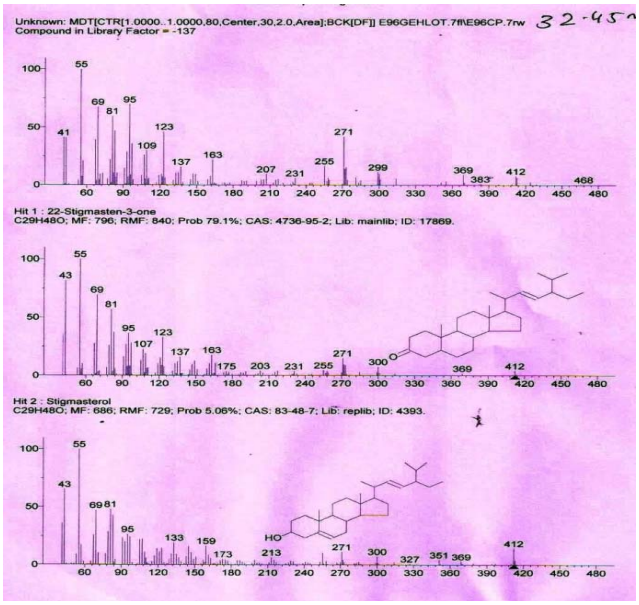
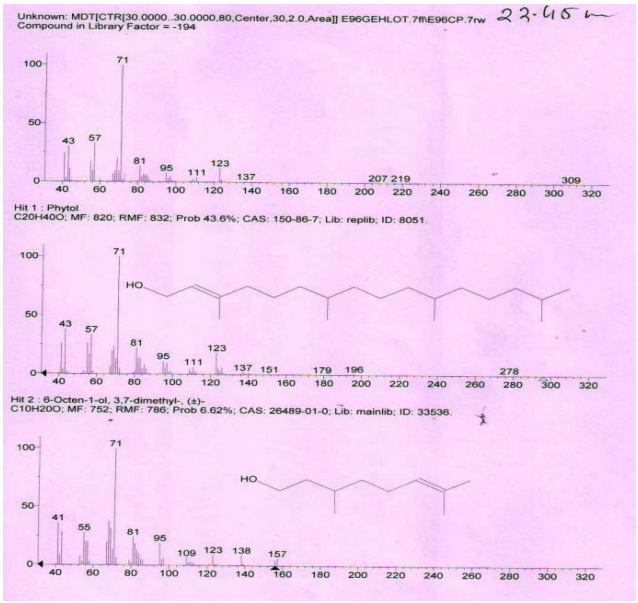
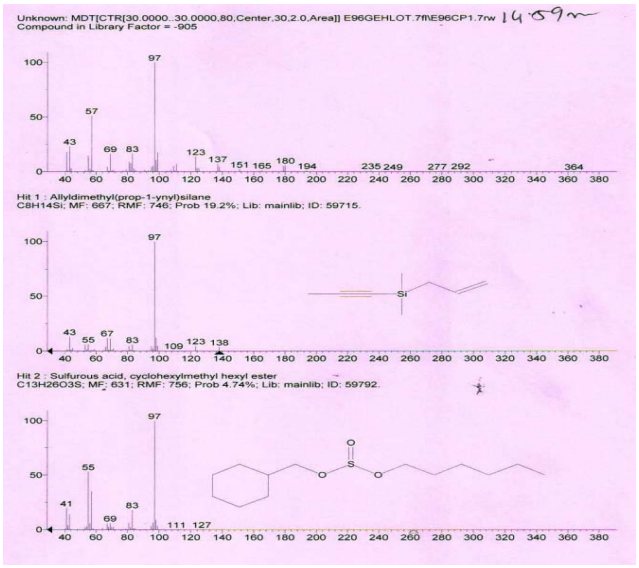
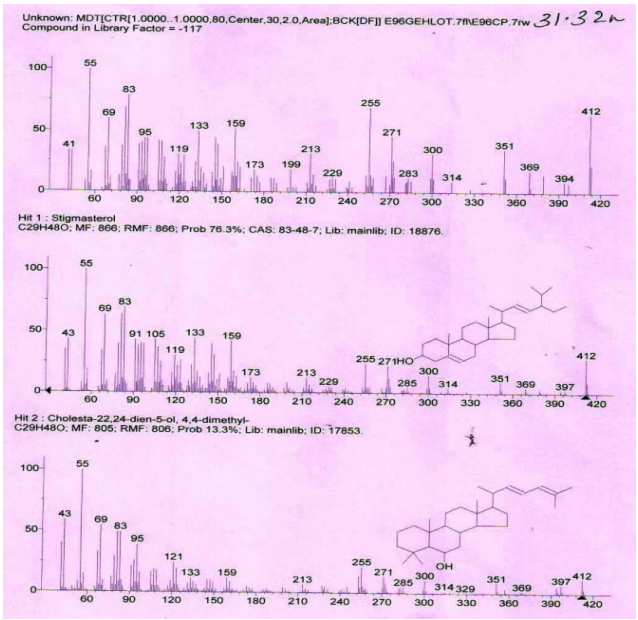
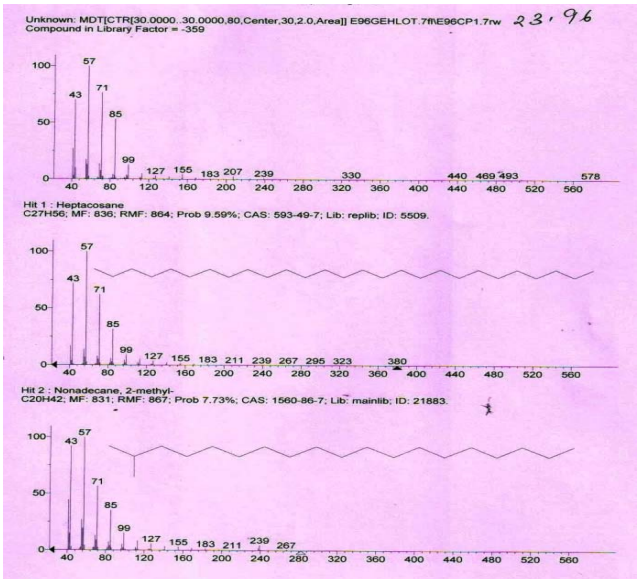


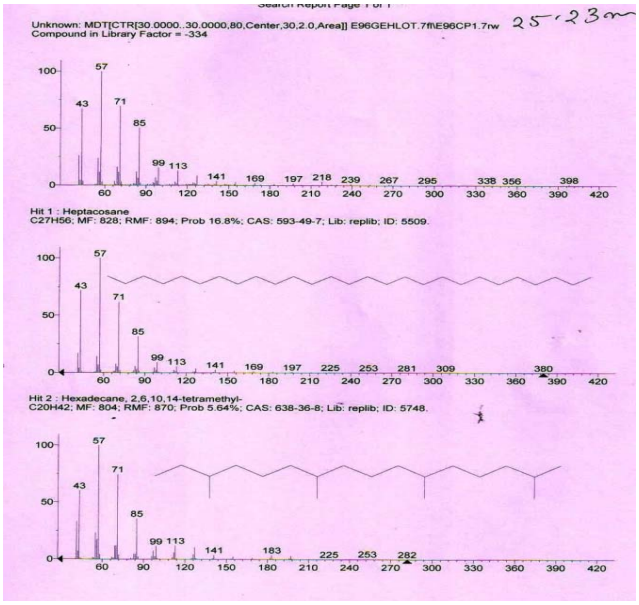
Fig 3: Components identified in alcohol extract of *E. oxypetalum* leaves dissolved in ethanol

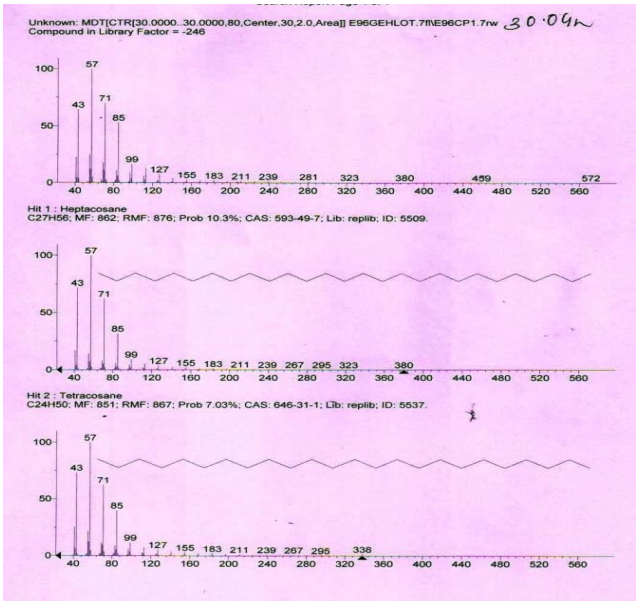


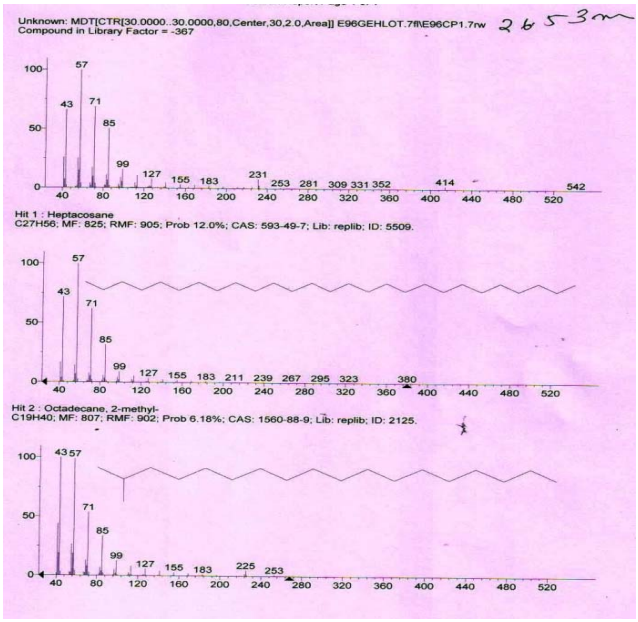


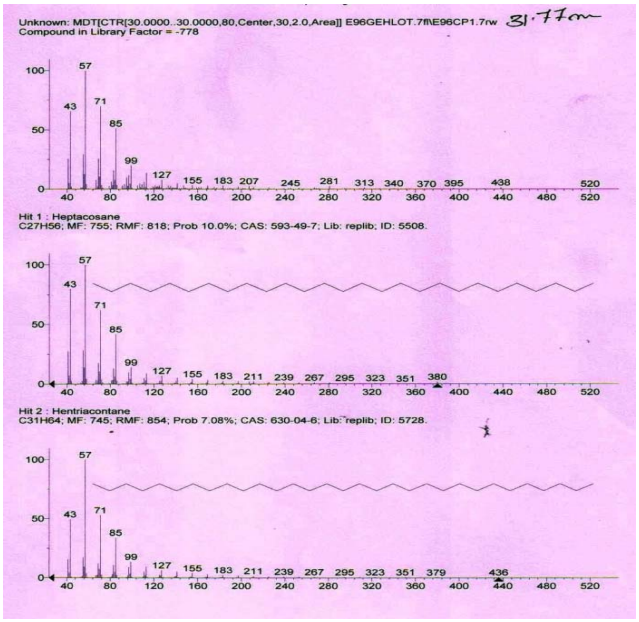


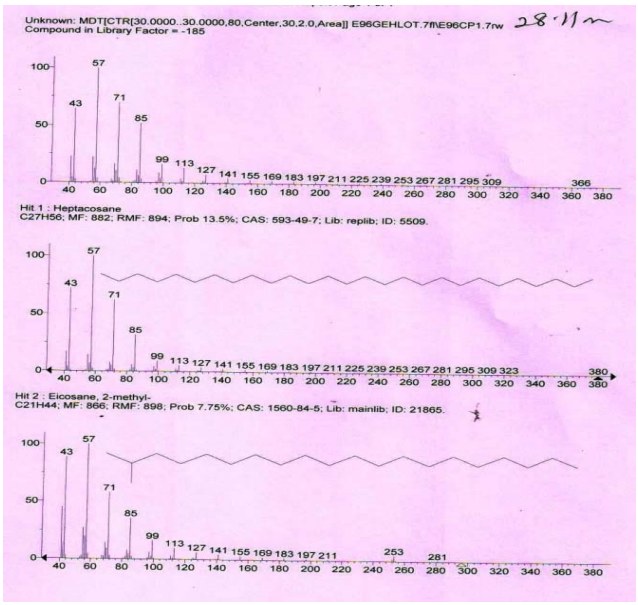


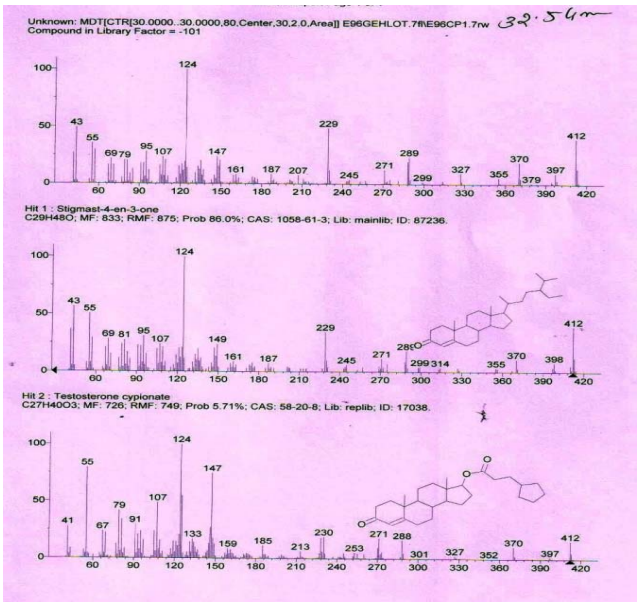

Unknown: MDT[CTR[30.0000..30.0000,80,Center,30,2.0,Area]] E96GEHLOT.7fl\E96CP1.7rw 25.23m
Compound in Library Factor = -334
Hit 1 : Heptacosane
C27H56; MF: 828; RMF: 894; Prob 16.8%; CAS: 593-49-7; Lib: replib; ID: 5509.
Hit 2 : Hexadecane, 2,6,10,14-tetramethyl-
C20H42; MF: 804; RMF: 870; Prob 5.64%; CAS: 638-36-8; Lib: replib; ID: 5748.


Unknown: MDT[CTR[30.0000..30.0000,80,Center,30,2.0,Area]] E96GEHLOT.7fl\E96CP1.7rw 30.04m
Compound in Library Factor = -246
Hit 1 : Heptacosane
C27H56; MF: 862; RMF: 876; Prob 10.3%; CAS: 593-49-7; Lib: replib; ID: 5509.
Hit 2 : Tetracosane
C24H50; MF: 851; RMF: 867; Prob 7.03%; CAS: 646-31-1; Lib: replib; ID: 5537.


Unknown: MDT[CTR[30.0000..30.0000,80,Center,30,2.0,Area]] E96GEHLOT.7fl\E96CP1.7rw 26.53m
Compound in Library Factor = -367
Hit 1 : Heptacosane
C27H56; MF: 825; RMF: 905; Prob 12.0%; CAS: 593-49-7; Lib: replib; ID: 5509.
Hit 2 : Octadecane, 2-methyl-
C19H40; MF: 807; RMF: 902; Prob 6.18%; CAS: 1560-88-9; Lib: replib; ID: 2125.


Unknown: MDT[CTR[30.0000..30.0000,80,Center,30,2.0,Area]] E96GEHLOT.7fl\E96CP1.7rw 31.77m
Compound in Library Factor = -778
Hit 1 : Heptacosane
C27H56; MF: 755; RMF: 818; Prob 10.0%; CAS: 593-49-7; Lib: replib; ID: 5508.
Hit 2 : Hentriacontane
C31H64; MF: 745; RMF: 854; Prob 7.08%; CAS: 630-04-6; Lib: replib; ID: 5728.


Unknown: MDT[CTR[30.0000..30.0000,80,Center,30,2.0,Area]] E96GEHLOT.7fl\E96CP1.7rw 28.11m
Compound in Library Factor = -185
Hit 1 : Heptacosane
C27H56; MF: 882; RMF: 894; Prob 13.5%; CAS: 593-49-7; Lib: replib; ID: 5509.
Hit 2 : Eicosane, 2-methyl-
C21H44; MF: 866; RMF: 898; Prob 7.75%; CAS: 1560-84-5; Lib: mainlib; ID: 21865.


Unknown: MDT[CTR[30.0000..30.0000,80,Center,30,2.0,Area]] E96GEHLOT.7fl\E96CP1.7rw 32.56m
Compound in Library Factor = -101
Hit 1 : Stigmast-4-en-3-one
C29H48O; MF: 833; RMF: 875; Prob 86.0%; CAS: 1058-61-3; Lib: mainlib; ID: 87236.
Hit 2 : Testosterone cypionate
C27H40O3; MF: 726; RMF: 749; Prob 5.71%; CAS: 58-20-8; Lib: replib; ID: 17038.

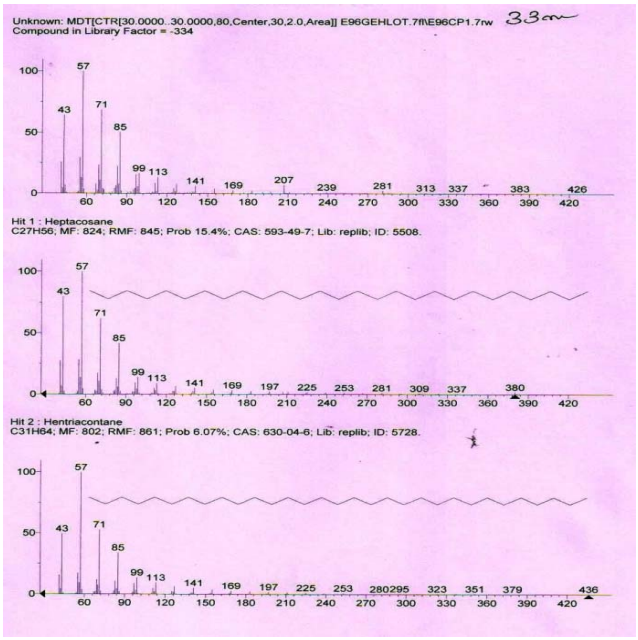


Fig 4: Components identified in alcohol extract of *E. oxypetalum* leaves dissolved in mixture of solvents

Conclusion

The result of GC-MS analysis specifies that the alcohol extract of *Epiphyllum oxypetalum* leaves contains various bioactive compounds which have various medicinal properties that can be useful for the treatment of various diseases.

References

1. Upendra RS, Assessment of nutritive values, phytochemical constituents and biotherapeutic potentials of Epiphyllum oxypetalum, Int. J Pharm and Pharm Sci 2012, 4(5):421- 425
2. Dr. Purak I. Epiphyllum oxypetalum (Bramhakamal) Orchid cactus – an interesting plant, Indian Botanist Interdisciplinary Multi-column Plant Sciences Portal, April 24, 2013.
3. http://en.wikipedia.org/wiki/Epiphyllum_oxypetalum
4. Doprops.com
5. http://webbook.nist.gov/cgi/cbook.cgi?ID=C875592&Mask=200A
6. Slaghenaufi D, Perello MC, Marchand-Marion S, Tempere S, Revel G. Quantitative solid phase microextraction – Gas chromatography mass spectrometry analysis of five megastigmatrienone isomers in aged wine, Analytica Chemical Acta 2014, 813:63-69.
7. http://en.wikipedia.org/wiki/Coniferyl_alcohol
8. Vijisaral ED and Subramanian A, GC – MS Analysis of Ethanol Extract of *Cyperus rotundus* Leaves, International Journal of Current Biotechnology 2014, 2(1):19-23.
9. Sermakkani M, Thangapandian V. GC-MS Analysis of *Cassia italica* leaf methanol extract, Asian J Pharm Clin Res 2012, 5(2):90-94
10. Akpuaka A, Ekwenchi MM, Dashak DA, Dildar A. Biological Activities of Characterized Isolates of n-Hexane Extract of Azadirachta Indica A. Juss (Neem) Leaves, Nature and Science 2013, 11(5):141-147.
11. Rajendra kumar N, Vasantha K, Mohan VR. GC-MS Analysis of Bioactive Components of Tubers of *Ruellia tuberosa* L. (Acanthaceae), American Journal of Phytomedicine and Clinical Therapeutics 2014, 2(2):209-216.
12. http://webbook.nist.gov/cgi/cbook.cgi?ID=1117-61-9
13. Suman PS Khanuja. Comparative antifungal activity of essential oils and constituents from three distinct genotypes of *cymbopogon* spp., Current Science 2001, 80(10):1264-1266
14. Léopold TN, Antifungal properties of essential oils and some constituents to reduce food borne pathogen, Journal of Yeast and Fungal Research 2010, 1(1):001-008
15. Jenecius A, Ramasamy Mohan V. GC-MS analysis of bioactive components on stem extract of Bacolepis Nervosa (Wight & arn.) Decne. Ex Moq. (Periplocaceae), World Journal of Pharmacy and Pharmaceutical Sciences 2014, 3(4):1044-1059.
16. http://en.wikipedia.org/wiki/Higher_alkanes
17. Radhamani T, Britto John S. GC-MS analysis of *Polygala arillata* Buch.-Ham Ex D. Don, Annals of Biological Research 2013, 4(11):70-75.
18. Singariya P *et al.*, An Activity Guided Isolation and Evaluation of Various Solvent Extracts Of the Leaves of Anjan Grass, Hygeia journal for drugs and medicines, October, 2012-March, 2013.
19. Singariya P *et al.*, Antimicrobial Activity and Identification of 4, 22-Stigmastadiene-3-one and Some Other Compounds in Motha Dhaman Grass Cenchrus setigerus from Tribal Area of Western Rajasthan, Proceedings of the National Academy of Sciences, India Section B:Biological Sciences ISSN 0369-8211.
20. http://www.sciencedirect.com/science/article/pii/0308814695926565
21. http://www.drugs.com/cdi/testosterone-cypionate.html