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Comparative phytochemical analysis of bioactive constituents present in *in vitro* and *in vivo* plant parts of *Merremia aegyptia* and *Merremia dissecta*

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Abstract
GC-MS is a highly effective and versatile analytical technique with numerous scientific applications to cater the field of applied Sciences and Technology. It is a very useful technique for quality control, analytical research, impurity profiling and maintenance for human welfare and development. The bioactive constituents (alkaloids, tannins, flavonoids and phenolics) present in the plants aid in their medicinal properties and can be analysed and detected by Gas Chromatography- Mass Spectroscopy technique. The *in vivo* plant part (leaf) and *in vitro* callus tissues of *Merremia aegyptia* and *Merremia dissecta* were tested for their phytochemical constituents. Methanol extracts of these parts were prepared through soxhlet extraction. A wide range of fatty acids, heterocyclic compound with antibacterial activity, antispasmodic activity, antihyperglycemic, hypolipidemic, anti inflammatory or anti cancerous properties were observed. Other metabolites such as Lauric acid, Oleamide, Dibutyl phthalate, 1,2benzenedicarboxylic, Stigmasterol, Alpha-Amyrin, ßeta Amyrin, Mandelic acid, Cyclopentasiloxane, Phloroglucinol were reported from the samples. Further research work is needed on these identified compounds to analyse their mode of action and usefulness in phytopharmaceutical industries.

Keywords: *in vitro*, *in vivo*, flavonoids, GC-MS, *Merremia* spp

Introduction
Medicinal plants have been playing a decisive role in sustenance of health and happiness of mankind since time immemorial [1]. Probably, medicinal plants were in use for ritual magical powers as well as for medicinal virtues. There are various plant drugs for which synthetic ones are still not available. Although they have been directly used as food, fiber and medicine, yet medicinal plants are the most important source of life saving drugs for the majority of the world's population [2].

Phytochemicals are, in the strictest sense of the word, chemicals produced by plants. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc in them [3]. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development from these plants.

Plants are champion synthetic chemists; they take advantage of their anabolic prowess to produce volatiles, which are used to protect themselves against biotic and abiotic stresses and in providing information or disinformation — to mutualists and competitors alike. The majority of plant volatiles are derived from four biosynthetic classes: terpenoids, fatty acid catabolites, aromatics, and amino-acid derived products. Plant volatiles serve humankind as perfumes and aroma compounds, natural flavour constituents, food additives/preservatives, chemotherapeutics and anaesthetics [4].

India has a rich treasure of medicinal plants due to diversity in its agroclimatic conditions [5]. There are estimated to be around 2500 effective plant based formulations used in folk medicine and known to rural communities all over India and around 10,000 designed formulations are available in the indigenous medical texts.

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and is the most commonly used technique for the identification and quantification purpose. It has long been the method of choice for identifying volatile compounds in complex mixtures as polar, typically non-volatile compounds (sugars or hydroxy-carboxylic acids) are easier to analyse by LC/MS (HPLC coupled to MS) while apolar, volatile compounds (terpenes etc.) are ideally analysed by GC/MS. In recent years, gas chromatography–mass spectrometry (GC–MS) has been applied unambiguously to identify the structures of different phytoconstituents in plant extracts and biological samples with great success. The unknown

organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [7-8].

Preparation of plant extracts

Fresh leaves and about 9 weeks old calli of both the test plants were taken and shade dried. The dried masses were then powdered for extraction. Each of the dried powdered samples (10gms) was then soxhlet extracted in 95% methanol on a water bath for 24 hours. The methanol extracts were then concentrated.

Before analysing, known amount of concentrate was dissolved in 5ml methanol which was filtered initially with filter paper Whatmann no. 1 and then through 0.45 micron syringe filter. The collected sample was used for analysis.

GC-MS Analysis

The GC-MS analysis of methanolic extracts of plant samples was carried out on Shimadzu QP-2010 plus with thermal desorption system TD 20. This system included auto sampler and a gas chromatograph interfaced to a mass spectrophotometer. The column size of this system was 30m × 0.25mm i.d × 0.25 µm with a film thickness of 0.25mm, composed of 5MS (5% diphenyl/ 95% dimethyl poly siloxane). Helium gas (99.999%) was used as a carrier gas at constant flow rate of 1ml/min. The 2µl injection volume of sample was utilized with split ratio of 10:1. The injector temperature was programmed initially at 280 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C, with an increase of 10 °C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280 °C. Mass spectra were analyzed using electron impact ionization at 70 eV. The total running time for each sample was 45 min.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with the database patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Result and Discussion

Observations of GC-MS analysis of *in vivo* (leaf) and *in vitro* (callus) samples of *M. aegyptia and M. dissecta* are shown in spectrograms and the pertaining results are given in the following tables (1-4). *In vitro* and *in vivo* plant parts of *Merremia* species showed presence of phenols, sugars, phytosterols, carboxylic acids, fatty acids, amino acids, vitamins and esters etc.

The spectrogram of methanolic extract of *M. aegyptia* leaf listed 32 compounds and callus presented 34 different peaks whereas spectrogram of methanolic extract of *M. dissecta* leaf showed 40 different compounds and its callus showed presence of 26 phytoconstituents.

1. GC-MS chromatogram of *M. aegyptia* methanolic leaf extract.

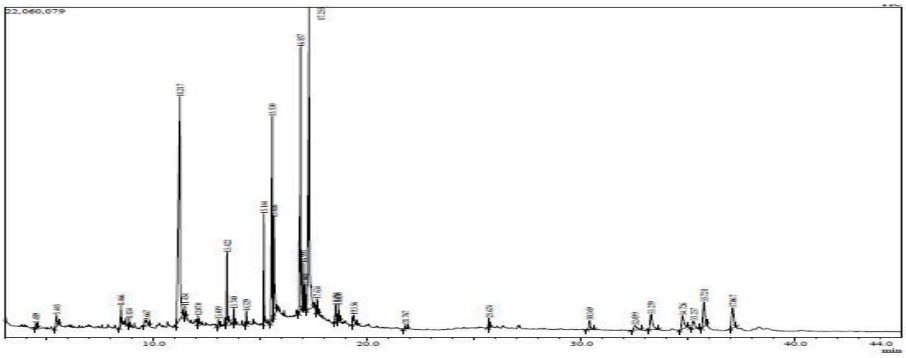

Table 1: Phytocomponents identified from methanolic extracts of *M. aegyptia* Leaf

Peak#	R.Time	Area%	Name
1	4.489	0.25	Oxetane, 2-Propyl-
2	5.445	0.99	Octanoic Acid
3	8.466	1.04	Decanoic Acid
4	8.834	0.20	1-Tetradecene
5	9.667	0.81	Cyclohexyl 6-Hydroxycaproate
6	11.217	22.42	Dodecanoic Acid
7	11.454	0.28	1-Octadecanol
8	12.070	0.27	Megastigmatrienone
9	13.019	0.39	Methyl Tetradecanoate
10	13.422	2.85	Tetradecanoic Acid
11	13.740	0.50	Trifluoroacetic Acid, Pentadecyl Ester
12	14.329	0.36	2-Pentadecanone, 6,10,14-Trimethyl-
13	15.144	3.31	Hexadecanoic Acid, Methyl Ester
14	15.530	9.76	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate
15	15.616	5.36	1,2-Benzenedicarboxylic Acid, Dibutyl Ester
16	16.857	11.92	9-Octadecenoic Acid, Methyl Ester
17	16.993	1.62	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-, [R-[R*,R*-(E)]]-
18	17.068	0.53	Methyl Stearate
19	17.255	21.23	Cyclopentadecanone, 2-Hydroxy-

20	17.634	0.72	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester
21	18.494	0.89	Cyclopentadecanone
22	18.630	0.79	9-Octadecensaeure,12-Hydroxy-,Methylester, (Ricinolsaeuremet
23	19.356	1.01	9-Octadecenamide, (Z)-
24	21.767	0.27	1,2-Benzenedicarboxylic Acid
25	25.674	0.40	Squalene
26	30.369	0.77	Vitamin E
27	32.499	0.74	Ergost-5-En-3-Ol, (3.Beta.,24r)-
28	33.259	2.10	Stigmasta-5,22-Dien-3-Ol, (3.Beta.,22e)-
29	34.726	1.81	Stigmast-5-En-3-Ol, (3.Beta.)-
30	35.237	1.05	Longifolen-V2
31	35.728	2.72	Beta.-Amyrin
32	37.067	2.65	Alpha.-Amyrin

2. GC-MS chromatogram of *M. aegyptia* methanolic callus extract

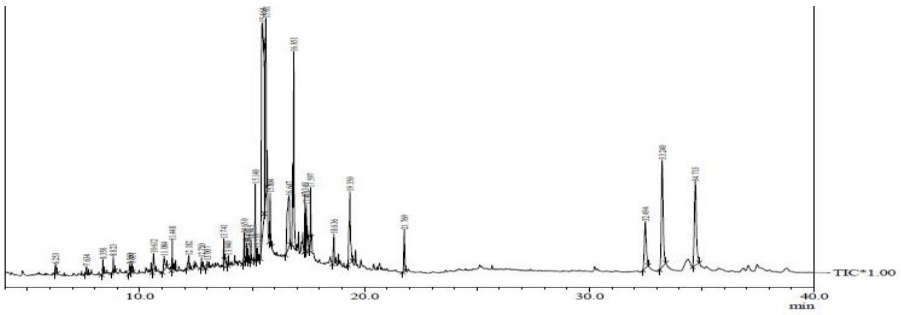

Table 2: Phytocomponents identified from methanolic extracts of *M. aegyptia* Callus

Peak#	R.Time	Area%	Name
1	6.253	0.46	2,4,4,6-Tetramethyl-6-Phenyl-2-Heptene
2	7.634	0.35	1h-Pyrazole, 4,5-Dihydro-3,5,5-Trimethyl-
3	8.358	0.34	Pyridine, 3-(1-Methyl-2-Pyrrolidinyl)-, (S)-
4	8.823	0.29	1-Tetradecene
5	9.560	0.34	Ethanone, 1-(4-Hydroxyphenyl)-
6	9.651	0.25	2-Octanol, 8,8-Dimethoxy-2,6-Dimethyl-
7	10.612	1.08	1,3,5-Triazine-2,4,6-Triamine
8	11.084	0.97	Dodecanoic Acid
9	11.448	0.761	1-Nonadecene
10	12.182	0.71	3,5,7,8-Tetrahydro-4,6-Pteridinedione
11	12.750	0.35	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-Pentaene
12	13.007	0.34	Methyl Tetradecanoate
13	13.741	0.52	1-Octadecene
14	13.940	0.24	Dehydroaromadendrene
15	14.650	0.94	1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester
16	14.753	0.38	5-(Isopropylamino)-1,6-Dimethyl-2(1h)-Quino
17	14.814	0.28	2,7(1h,8h)-Pteridinedione
18	14.945	0.50	9-Hexadecenoic Acid, Methyl Ester, (Z)-
19	15.140	2.25	Hexadecanoic Acid, Methyl Ester
20	15.235	0.33	Succinamic Acid
21	15.464	25.33	1,2-Benzenedicarboxylic Acid, Dibutyl Ester
22	15.612	13.09	Butyl 2-Methylpropyl Ester
23	15.804	1.50	Hexadecanoic Acid, Ethyl Ester
24	16.647	5.98	13-Hexyloxacyclotridec-10-En-2-One
25	16.851	8.82	9-Octadecenoic Acid (Z)-, Methyl Ester
26	17.349	1.36	Monomethyl Monobutyl "Capped" Tetraethylene Glycol
27	17.413	0.87	N-Propyl 9,12-Octadecadienoate
28	17.597	1.26	Hexadecanamide
29	18.636	1.30	Hexadecanoic Acid, 1-(Hydroxymethyl)-1,2-Ethanediyl Ester
30	19.350	4.83	9-Octadecenamide, (Z)-
31	21.769	1.94	Bis(2-Ethylhexyl) Phthalate
32	32.494	4.38	Ergost-5-En-3-ol, (3.Beta.,24r)-
33	33.240	9.42	Stigmasterol
34	34.713	8.23	Stigmast-5-En-3-ol, (3.Beta.)-

3. GC-MS chromatogram of *M. dissecta* methanolic leaf extract

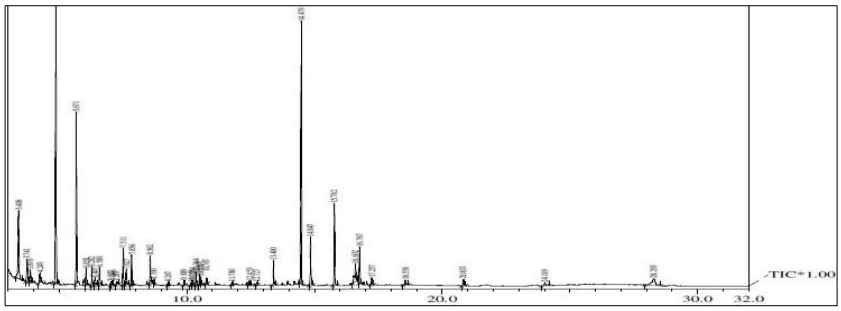

Table 3: Phytocomponents identified from methanolic extracts of *M. dissecta* Leaf

Peak#	R.Time	Area%%	Name
1	3.408	6.56	3-Methoxy-4-[(Trimethylsilyl)Oxy]-Benzalde
2	3.741	1.80	Benzoic Acid, 2,4-Bis(Trimethylsiloxy)-, Methyl Ester
3	3.876	0.81	Cyclotetrasiloxane, Octamethyl-
4	4.243	0.86	Benzaldehyde,2,5-Bis[(Trimethylsilyl)Oxy]-
5	4.873	24.09	Cyclopentasiloxane, Decamethyl-
6	5.671	11.48	4b,5a-Dihydro-5h-Dibenz[3,4 : 5,6]Anthra[1,2-B]
7	6.058	0.93	Silane, [(Methoxymethylene)Di-2,1-Phenylene]Bis[Trimethyl-
8	6.282	1.25	Benzeneacetic Acid, Alpha.,4-Bis[(Trimethylsilyl)Oxy]-, Methyl Ester
9	6.407	0.58	1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(Trimethylsiloxy)Hexasiloxane
10	6.580	1.30	3,5-Diisopropoxy-1,1,1,7,7,7-Hexamethyl-3,5-Bis(Trimethylsiloxy)Tetrasiloxane
11	7.027	0.22	Heptalen, 7,7'-Dihydro-6,6'-Bis(Trimethylsilyl
12	7.096	0.15	3,5-Bis[Dimethyl(Tert-Butyl)Silyloxy]Styrene
13	7.267	0.32	Mandelic Acid-Methyl Ester
14	7.511	2.29	Cyclohexasiloxane, Dodecamethyl-
15	7.627	1.31	Benzeneacetic Acid, Alpha.-Methoxy-, Methyl Ester
16	7.836	1.76	4-Hydroxymandelic Acid, Ethyl Ester,
17	8.562	2.01	Bis(Trimethylsilyl)-2-[Hydroxy-(Ethoxy)Phenyl]-2-Hydroxyphenylpropane
18	8.700	0.20	3,3,5-Triethoxy-1,1,1,7,7,7-Hexamethyl-5-(Trimethylsilyloxy)Tetrasiloxane (Trimethylsilyloxy)Tetrasiloxane
19	9.267	0.16	Heptamethyl-Phenyl-Cyclotetrasiloxane
20	9.889	0.30	3-Ethoxy-1,1,1,7,7,7-Hexamethyl-3,5,5-Tris(Trimethylsiloxy)Tetrasiloxane
21	10.168	0.18	Silane, Trimethyl-2-Propenyl-
22	10.234	0.23	Dimethyl2-[1-(Phenylthio)Butyl]Pyrimidine-4, 5-Dicarboxylate
23	10.364	0.86	1,2-Bis(.Gamma.-Trimethylsilypropoxy)Ethane
24	10.501	0.56	Naphthalene, 1,2,3,4-Tetrahydro-1,6-Dimethyl-4-(1-Methylethyl)-
25	10.551	0.26	2,2,4,4,6,6,8,8-Octamethyl-1,3,5,7,2,4,6,8-Tetraoxatetrasilocane
26	10.767	0.29	Silane, [(3-Methoxy-1-Methylene-2-Propenyl)Oxy]Trimethyl-,
27	11.780	0.14	1,4-Cyclohexadien, 1,3,6-Tris(Trimethylsilyl
28	12.453	0.31	3,5,6-Tris(Trimethylsilyl)Cyclohexa-1,3-Diene
29	12.727	0.17	Heptadecanoic Acid, Methyl Ester
30	13.400	1.47	1,3-Diphenyl-1,3,5,5-Tetramethyl-Cyclotrisiloxane
31	14.479	20.46	1,3,5-Tris(Trimethylsiloxy)Benzene
32	14.847	3.40	Pentadecanoic Acid, 14-Methyl-, Methyl Ester
33	15.782	4.65	Nonamethyl, Phenyl-, Cyclopentasiloxane
34	16.602	2.25	9-Octadecenoic Acid (Z)-, Methyl Ester
35	16.767	2.30	Methyl Stearate
36	17.237	0.54	Hexadecane, 1,1-Bis(Dodecyloxy)-
37	18.558	0.37	Eicosanoic Acid, Methyl Ester
38	20.833	0.67	Tetracosanoic Acid, Methyl Ester
39	24.019	0.41	Tricosanoic Acid, Methyl Ester
40	28.293	2.13	Diisobutyl 2,2-Dihydroxymalonate

4. GC-MS chromatogram of *M. dissecta* methanolic callus extract

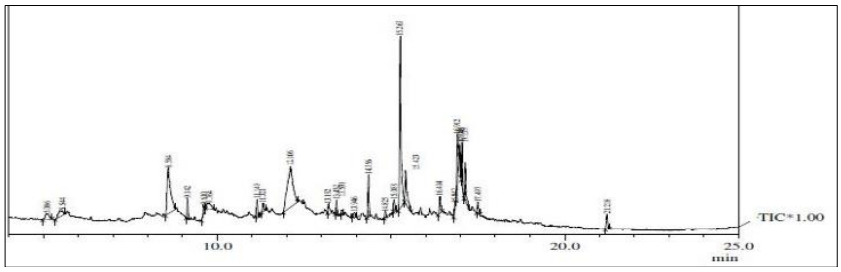

Table 4: Phytocomponents identified from methanolic extracts of *M. dissecta* Callus

Peak#	R.Time	Area%%	Name
1	5.086	1.99	Acetic Acid,3 Methyl Pentyl Ester-
2	5.544	2.07	Octanoic Acid
3	8.584	13.35	DL-Proline, 5-Oxo-, Methyl Ester
4	9.142	1.52	Caryophyllene <(E)->
5	9.610	0.75	Bicyclo[2.2.1]Heptane, 7,7-Dimethyl-2-Methylene-
6	9.764	2.03	Cyclopentanol
7	11.149	1.54	1-Hexadecene
8	11.321	1.11	8,11,14-Eicosatrienoic Acid
9	12.106	20.07	1,4,6,9-Nonadecatetraene
10	13.192	1.72	Hexadecanoic Acid
11	13.432	1.02	Hexadecanol <N->
12	13.591	0.46	Cyclopentanone <2-Acetyl->
13	13.946	0.60	Trans-2,3-Epoxy Cyclo Hexane-1-Methanol
14	14.356	3.69	1,2-Benzenedicarboxylic Acid,
15	14.825	0.03	Stearic Acid Methyl Ester
16	15.088	1.44	Cis-Vaccenic Acid
17	15.263	21.81	N-Hexadecanoic Acid
18	15.423	5.43	Dihydroatlantone<(E)-10,11->
19	16.404	2.12	2,6,10-Dodecatrien-1-Ol, 3,7,11-Trimethyl-, (Z,E)-
20	16.842	0.58	Thiourea, N-(3-Methoxyphenyl)-N'-(2-Propenyl)-
21	16.912	5.23	9,12-Octadecadienoic Acid (Z,Z)-
22	16.979	1.14	Linolenate <Methyl>
23	17.045	3.08	Monomethyl Monobutyl "Capped" Tetraethylene Glycol
24	17.133	4.04	9-Octadecenoic Acid (Z)-
25	17.497	1.41	Tricyclo[4.2.1.1(2,5)]Deca-3,7-Diene-9,10-Diol, 9-
26	21.218	1.78	1,2-Benzenedicarboxylic Acid, Dioctyl Ester

Conclusion

GC-MS analysis of the two species viz *M.aegyptia* and *M. dissecta* of Genus *Merremia* showed the presence of various useful secondary metabolites in their *In vitro* and *In vivo* tissues.

These species are source of various useful metabolites such as dodecanoic acid (Lauric acid), Tetradecanoic acid (Myristic acid), Hexadecanoic acid(Palmitic acid), Ascorbic acid, Phytosterols (Ergosterol and Stigmasterol), Triterpenes (alpha and beta amyrins) and Dibutyl Phthalate which were recorded from leaf and callus samples of *M. aegyptia* while *M. dissecta* plant samples showed the presence of Mandelic acid Pentadecanoic acid, Heptadecanoic acid, 9-Octadecanoic acid, Phloroglucinol, Hexadecanoic acid, 1-Hexadecene and Caryophyllene etc.

These samples also showed similarity in presence of compounds in *in vitro* and *in vivo* plant parts and few similar phytochemicals were also reported from both the tested species of *Merremia* genus. 1-tetradecene, Dodecanoic acid, Hexadecanoic acid, methyl ester, 9-Octadecenoic acid(Z)- ,methyl ester,1,2-Benzenedicarboxylic acid,9-Octadecenamide,(Z)- were some common phytochemicals present in more than one sample extracts of these plants.

The spectogram of methanolic extract of *M. aegyptia* leaf showed 32 different compounds in it while the callus extract showed 34 compounds.

Dodecanoic acid(Lauric acid) has Antibacterial, Antiviral, Antioxidant, Candidicide, Hypercholesterolemic activity and also have possible use in cosmetic industry. 9-Octadecenoic acid has Cancer preventive, Anti-inflammatory activity [9]. 9-Octadecenamide (Oleamide) could be used in mood and sleep disorders and also in cannabinoid regulated depression. 1,2benzenedicarboxylic acid <Dibutyl ester> may be used as plasticizer [10]. *M. aegyptia* leaf and callus extracts observed Stigmasterol,also known as Wulzen antistiffness factor, may be useful in prevention of certain cancers, such as ovarian, prostate, breast and colon cancer. It also possess potent antinflammatory, antioxidant, hypoglycemic and thyroid inhibiting properties. Alpha and ßeta-Amyrin reported from *M. aegyptia* leaf extract possess antihyperglycemic and hypolipidemic properties.

40 different compounds were detected from leaf extracts of *M. dissecta* and 26 phytocompounds were observed from its callus extract out of which Mandelic acid possesses antibacterial activity [11], cyclopentasiloxane, decamethyl has possible use in cosmetic industry and Phloroglucinol has antispasmodic activity and can preferably be used in pharmaceuticals and explosive industries [12]. 1-Hexadecene also known as "Cetene" is widely used as a surfactant in lubricating fluid, a drilling fluid in the boring and drilling industry, and in paper sizing. Caryophyllene is a sesquiterpene which has anti-tumor, analgesic**,** antibacterial, anti-inflammatory, sedative and fungicide activity [13].

Due to presence of such large number of useful metabolites in these species, these plants are recommended as phytopharmaceutically important. Also, this type of GC-MS analyses is the first step towards understanding the nature of active principles in *in vitro* and *in vivo* parts of these medicinal plants and this type of study would be helpful for large scale isolation of such pharmaceutically important compounds from the species.

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