PHYTOCHEMICAL ANALYSIS OF SELECTED INDIAN MEDICINAL PLANTS BY HR-LCMS SPECTRA METHOD

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ABSTRACT

Due to the widespread use of medicinal plant products in the pharmaceutical and biotechnology field, phytochemical analysis of medicinal plants has become an important and challenging task. Analytical techniques including liquid chromatography coupled with mass spectrometry were found to be an important technique in the analysis of complex bioactive constituents. The present study was aimed at bioactive constituent analysis from select Indian medicinal plants viz., *Pongamia pinnata*, *Dodonea viscosa*, *Gardenia resinifera* and *Gymnospora emarginata* by (HR)-LCMS (High Resolution- Liquid Chromatography Mass Spectrometry) analysis. The present study confirms presence of important therapeutic secondary metabolites including alkaloids (Hydroxycotinine), Flavonoids (Apiin, Genkwanin, Kaempferol), Coumarin (Lomatin), Carboxylic acid (Quinic acid) and Phenolic compounds (Isoliquiritigenin) in the select Indian medicinal plants.

Keywords: HR-LCMS, Anthelmintic Activity, Antibacterial Activity, Phytochemicals.

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INTRODUCTION

The role of bioactive compounds extracted from medicinal plants in maintaining sustainable human health globally is well documented. The traditional medicinal practices including Rigveda (3700 B.C.), Ayurveda, Homeopathy and Unani mentioned the use of medicinal plant products for the cure of various human ailments.^{1,2} In the recent times, many widely used drugs with low side effects were sourced from medicinal plants. There is a great demand for the identification of novel, potent drug molecules from medicinal plant products that are safe with low side effects to treat infections caused by parasites and microbial pathogens.^{3,4}

In the biochemical analysis of plant products, the first step is the identification and isolation of bioactive compounds from the medicinal plants. In the present study, based on the literature survey, four Indian medicinal plants viz., *Pongamia pinnata*, *Dodonea viscosa*, *Gardenia resinifera*, *Gymnospora emarginata* were selected from Ananthagiri forest of Telangana⁵ and their extracts were analyzed using (HR)-LCMS (High-resolution liquid chromatography-mass spectrometry) for the identification of bioactive compounds.

Pongamia pinnata also known as karum tree belongs to the family *Fabaceae*. All the parts of this plant are shown to exhibit various medicinal properties. People suffering from bleeding piles are treated with *Pongamia pinnata* bark extract and fruits are used as antidyslipidemic. Oil extracted from seeds is used in the treatment of rheumatism, leucoderma and scabies. The extract of seeds and leaves is shown to have anthelmintic and insecticidal activities.^{6,7}

Dodonea viscosa commonly called hop bush belongs to the family *Sapindaceae*. This plant is widely used globally for treating various ailments. Leaf and bark extract shows antibacterial activity⁸, antidiabetic activity in rats,⁹ anticancer activity against lung and breast cancer cell lines. It is also found to be effective in treating rheumatism, malaria, and snake bites.¹⁰

Gardenia resinifera belongs to the family *Rubiaceae* is commonly called Indian boxwood. The various plant parts of it shown different medicinal properties and among them, gum extract from stems is found to show antihyperlipidemic activity and hepatoprotective effects. The leaf extract is used in treating pathogenic bacteria of the mouth. Methanolic extract of leaves is found to be anthelmintic and antispasmodic.^{11,12}

Gymnospora emarginata, commonly called Danthi belongs to the family *Celastraceae*. The leaf extract is found to be effective in controlling various gram positive and gram negative bacteria¹³, in treating different cancers in humans (Hep3b hepatocellular carcinoma, hela; cervical epithelial cancer and A549 human lung adrenal cancer)¹⁴ along with antioxidant activity.¹⁵

EXPERIMENTAL

Collection of Medicinal Plants

The leaves of *Dodonea viscosa*, *Gymnospora emarginata*, *Gardenia resinifera* and seeds of *Pongamia pinnata* were collected from Ananthagiri forest Vikarabad District Telangana, India. The plant specimens were submitted to Botany Department, Osmania University, Hyderabad and Botany Department, Kakatiya University, Warangal for the taxonomic identification of plants. One of the plant specimens was identified as *Dodonea viscosa* (L) Jacq (family *Sapindaceae*, Voucher number 0073) and deposited in the Department of Botany, Osmania University, Hyderabad. The other three plant specimens were identified as *Gymnospora emarginata* (family *Celastraceae*, Voucher number KUW 4529), *Gardenia resinifera* (family *Rubiaceae*, Voucher number KUW 4528) and *Pongamia pinnata* (family *Leguminosae*, Voucher number KUW4557) and deposited in the Department of Botany, Kakatiya University, Warangal.

Extraction of Bioactive Compounds

The leaves and seeds of select medicinal plants were finely powdered and bioactive compounds were extracted with methanol using a Soxhlet extractor.¹⁶ The methanol extracts of *Dodonea viscosa*, *Gymnospora emarginata*, *Gardenia resinifera* and *Pongamia pinnata* were filtered through Whatman filter paper no 42 individually and concentrated using an evaporator at 40°C and stored at 4°C.

High Resolution -Liquid Chromatography Mass Spectrometry(HR-LCMS) Methodology

The methanolic extracts of *Pongamia pinnata*, *Dodonea viscosa*, *Gardenia resinifera*, *Gymnospora emarginata* were subjected to (HR)-LCMS analysis individually and chemical fingerprints were prepared using high-resolution liquid chromatography and mass spectrometry (model-G6550A of Agilent technologies) with 0.01% mass resolution¹⁷ with following parameters:

1. MS- minimum range 150 (M/Z) and maximum 1000 daltons with scanning rate each per Second.
2. The source parameter for gas chromatography was maintained at 250 °C with a gas flow of 13 psi/minute.
3. The auxiliary draw speed was 100 µl/minute, eject speed at 100.0 µL/min, draw position offset 0.0 mm wait time after drawing 2.0 s, Sample flush out factor was 5.0.

The following solvent composition was used:

S.No.	Channel	Ch.1 Solvent	Name 1	Ch2 Solvent	Selected	Used	Percent
1	A	100.0% Water V.02	0.1% FA in water	100.0%Water V.02	Ch.2	yes	95.00%
2	B	100.0% AcetonitrileV.02	90% ACN +10% H2O+ 0.1% FA	100.0% Acetonitrile V.02	Ch.2	yes	5.00%

RESULTS AND DISCUSSION

The high resolution-liquid chromatography-mass spectrometry analysis (HR)-LCMS of methanolic extract of *Pongamia pinnata* seeds was found to contain 52 compounds of which, 35 major compounds were confirmed based on their retention time, mass, and molecular formula as shown in Table-1, chromatogram, Figure-1. The chromatogram gives information on the relative concentrations of various compounds eluted as a function of retention time.

The height of the peak indicates the relative concentrations of bioactive compounds. Mass Spectrometer analyses the structure of unknown compounds which are eluted at different times.

Fig-1 : (HR)-LCMS Spectrogram of *Pongamia pinnata*

The important phytoconstituents confirmed by (HR)-LCMS Analysis were Apiin, Juglone, P-Hydroxyphenylacetic acid, Betaxolol, Artelinic acid, Picrotoxinin, Dihydrodeoxystreptomycin, Isoliquiritigenin, Sterigmatocystin, Hydroxyflutamide, Formononetin, Genkwanin, Dicumarol, Vulpinic acid, Lomatin, Chryophanic acid, 9-anthrone Isoliquiritigenin, Dihydrosphingosine. The compounds were also found in other plants which have shown different pharmacological activities. Among them, Juglone a 1-4 naphtha quinone compound found in *Juglans nigra* plant has shown potent antibacterial activity against various species.^{18,19} Vulpinic acid which was also found in lichens has shown anticancer activity and antibacterial activity.^{20,21} Cucurbitacins have shown anticancer, antidiabetic, anti-inflammatory effects.²²

Table-1: Bioactive Compounds identified in Methanol Extract of *Pongamia pinnata*

S. No.	Name of the Compound	Formula	Mass	RT (min)	DBDiff (ppm)
1.	Mebeverine metabolite (veratric acid glucuronide)	C ₁₅ H ₁₈ O ₁₀	358.0855	1.013	12.46
2.	12a-hydroxy-5- deoxydehydromunduser one	C ₁₉ H ₁₈ O ₆	342.1122	1.018	-5.5
3.	6,3'-dimethoxyflavone	C ₁₇ H ₁₄ O ₄	282.0843	4.669	17.36
4.	galnacalpha(1-3)[fucalpha(1- 2)]galbeta(1-4)glcnacbeta- sp	C ₃₀ H ₅₁ N ₅ O ₂₀	801.3232	5.102	-13.06
5.	Apiin	C ₂₆ H ₂₈ O ₁₄	564.1434	5.786	7.92
6.	beta-erythroidine	C ₁₆ H ₁₉ N O ₃	273.1365	6.911	-0.01
7.	Juglone	C ₁₀ H ₆ O ₃	174.0306	7.218	6.3
8.	p-hydroxyphenylacetic acid	C ₈ H ₈ O ₃	152.048	7.949	-4.49
9.	betaxolol	C ₁₈ H ₂₉ N O ₃	307.2123	8.134	8
10.	3a-oh desogestrel	C ₂₂ H ₃₀ O ₂	326.2289	8.322	-13.35
11.	s-adenosylmethionine	C ₁₅ H ₂₃ N ₆ O ₅ S	399.1452	8.433	-0.46
12.	Artelinic acid	C ₂₃ H ₃₀ O ₇	418.1967	8.86	5.76
13.	Picrotoxinin	C ₁₅ H ₁₆ O ₆	292.0948	8.861	-0.42
14.	Dihydrodeoxystreptomycin	C ₂₁ H ₄₁ N ₇ O ₁₁	567.2856	9.305	1.48
15.	trans-3-hydroxycotinine glucuronide	C ₁₆ H ₂₀ N ₂ O ₈	368.1233	9.697	-3.74
16.	Sappanone a 7-methyl ether	C ₁₇ H ₁₄ O ₅	298.0818	9.755	7.8
17.	Isoliquiritigenin	C ₁₅ H ₁₂ O ₄	256.0741	10.145	-2.17
18.	Sterigmatocystin	C ₁₈ H ₁₂ O ₆	324.0612	10.635	6.77
19.	estradiol-17beta 3-sulfate	C ₁₈ H ₂₄ O ₅ S	352.129	10.726	15.46
20.	12a-hydroxy-5- deoxydehydromunduser one	C ₁₉ H ₁₈ O ₆	342.1081	10.798	6.41
21.	Deoxysappanone b trimethyl ether	C ₁₉ H ₂₀ O ₅	328.1277	10.831	10.37

22.	Hydroxyflutamide	C ₁₁ H ₁₁ F ₃ N ₂ O ₄	292.0718	11.044	-16.02
23.	deoxysappanone b trimethyl ether	C ₁₉ H ₂₀ O ₅	328.1313	11.204	-0.54
24.	8,13-dihydroxy-9,11-octadecadienoic acid	C ₁₈ H ₃₂ O ₄	312.2279	11.23	7.03
25.	Formononetin	C ₁₆ H ₁₂ O ₄	268.0741	11.24	-1.98
26.	n,n-Didemethylchlorpromazine	C ₁₅ H ₁₅ Cl N ₂ S	290.0594	11.299	17.38
27.	Cuneatin methyl ether	C ₁₈ H ₁₄ O ₆	326.0771	11.456	5.92
28.	Sterigmatocystin	C ₁₈ H ₁₂ O ₆	324.0615	11.462	5.96
29.	Genkwanin	C ₁₆ H ₁₂ O ₅	284.0693	11.613	-3.07
30.	5-o-methylvisamminol	C ₁₆ H ₁₈ O ₅	290.1153	11.647	0.51
31.	6,3'-dimethoxyflavone	C ₁₇ H ₁₄ O ₄	282.0877	11.675	5.48
32.	Chrysophanic acid 9- anthrone	C ₁₅ H ₁₂ O ₃	240.0799	11.736	-5.15
33.	Dalbergione, 4-methoxy- 4'-hydroxy-	C ₁₆ H ₁₄ O ₄	270.0902	11.783	-3.64
34.	Juglone	C ₁₀ H ₆ O ₃	174.0307	11.949	5.66
35.	4-hydroxyphenylethanol	C ₈ H ₁₀ O ₂	138.0699	11.956	-13.09
36.	Warfarin	C ₁₉ H ₁₆ O ₄	308.1033	11.957	5.01
37.	i-methyl-4-nitro-5-thio- imidazole	C ₄ H ₅ N ₃ O ₂ S	159.0082	11.963	13.17
38.	Dicumarol	C ₁₉ H ₁₂ O ₆	336.0618	11.966	4.83
39.	Hydroxyflutamide	C ₁₁ H ₁₁ F ₃ N ₂ O ₄	292.0723	12.27	-17.74
40.	Vulpinic acid	C ₁₉ H ₁₄ O ₅	322.0826	12.378	4.78
41.	Lomatin	C ₁₄ H ₁₄ O ₄	246.088	13.113	4.79
42.	Methyl robustone	C ₂₂ H ₁₈ O ₆	378.1087	13.513	4.21
43.	3'-hydroxy-e,e-dienoestrol	C ₁₈ H ₁₈ O ₃	282.1262	13.525	-2.17
44.	Propargite	C ₁₉ H ₂₆ O ₄ S	350.1497	13.608	15.66
45.	5,7-dimethoxyisoflavone	C ₁₇ H ₁₄ O ₄	282.0911	13.862	-6.78
46.	3,5-Pyridinedicarboxylic acid,1,4-dihydro-2,6-dimethyl-4-(3nitrophenyl)-, monomethyl ester	C ₁₆ H ₁₆ N ₂ O ₆	332.1032	13.906	-7.09
47.	Benzylbutylphthalate	C ₁₉ H ₂₀ O ₄	312.137	13.929	-2.59
48.	Isoliquiritigenin	C ₁₅ H ₁₂ O ₄	256.0746	13.944	-4
49.	5c-aglycone	C ₁₆ H ₁₆ O ₄	272.1056	14.318	-2.64
50.	Juglone	C ₁₀ H ₆ O ₃	174.0306	14.341	6.01
51.	Avocadene acetate	C ₁₉ H ₃₆ O ₄	328.2591	14.364	6.83
52.	Lomatin	C ₁₄ H ₁₄ O ₄	246.0878	14.875	5.66

The (HR)-LCMS High-Resolution Liquid Chromatography Mass Spectrometer analysis of methanolic extract of *Dodonea viscosa* spectrum profile (Fig.-2) shows 33 compounds of which 29 major compounds were confirmed based on their retention time, mass and molecular formula.

Fig -2: (HR)-LCMS Spectrogram of *Dodonea viscosa*

The Phytochemicals found in the extract including Fraxetin, Hieracin, Desmethylzopiclone, Kaempherol, Tamarixetin, Epothilone A, 4Hydroxyestrone, 4'Hydroxypenbutololglucuronide, Orthothymotinic acid, Dihydrodeoxystreptomycin, Piretanide, Rutin, Irbesartan, Eupatorin were shown in Table-2.

It was also reported that these compounds found in the different species of plants exhibit different pharmacological activities. Among them, Kaempherol was also found in tea, berries and apples exhibits antitumor activity and anti-inflammatory activity.

Fraxetin also found in *Fraxinus rhynchophylla* has shown antibacterial activity and antioxidant effect. Rutin also found in tea leaves and apples exhibited various pharmacological effects like antidiabetic, anti-inflammatory, antibacterial activity.²³⁻²⁵

Table -2: Bioactive Compounds in Methanol Extract of *Dodonea viscosa*

S. No.	Name of the Compound	Formula	Mass	RT (min)	DBDiff (ppm)
1.	Mebeverine metabolite (Veratric acid glucuronide)	C ₁₅ H ₁₈ O ₁₀	358.0848	1.035	
2.	12a-Hydroxy-5-Deoxydehydromunduser one	C ₁₉ H ₁₈ O ₆	342.1112	1.06	14.62
3.	Fraxetin	C ₁₀ H ₈ O ₅	208.0347	5.21	-2.49
4.	Hieracin	C ₁₅ H ₁₀ O ₇	302.0389	6.281	
5.	Desmethylzopiclone	C ₁₆ H ₁₅ Cl N ₆ O ₃	374.0807	6.446	12.35
6.	Kaempherol	C ₁₅ H ₁₀ O ₆	286.0446	6.546	23.31
7.	Tamarixetin	C ₁₆ H ₁₂ O ₇	316.0547	6.678	11.12
8.	Epothilone A	C ₂₆ H ₃₉ N O ₆ S	493.2499	6.78	11.36
9.	1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo-	C ₁₂ H ₁₆ O ₃	208.1099	7.707	
10.	4-Hydroxyestrone	C ₁₈ H ₂₂ O ₃	286.1539	7.709	0.43
11.	4'-Hydroxypenbutolol glucuronide	C ₂₄ H ₃₇ N O ₉	483.2426	7.71	10.3
12.	6,8,10,12-pentadecatetraenal	C ₁₅ H ₂₂ O	218.167	7.71	8.74
13.	Orthothymotinic acid	C ₁₁ H ₁₄ O ₃	194.0945	7.713	0.32
14.	17alpha-Estradiol 3-D- glucuronide	C ₂₄ H ₃₂ O ₈	448.2033	8.024	3.34
15.	4-Hydroxyestrone	C ₁₈ H ₂₂ O ₃	286.1573	8.433	14.35
16.	2,4,6-Heptatrienoic acid, 5- methyl-7-(2,6,6-trimethyl-3- oxo-1-cyclohexen-1-yl)-, (2E,4E,6E)-	C ₁₇ H ₂₂ O ₃	274.1568	8.438	-1.45
17.	2-Methylene-5-(2,5-Dioxotetrahydrofuran- 3-yl)-6-oxo--10,10- Dimethylbicyclo[7: 2: 0]Undecane	C ₁₈ H ₂₄ O ₄	304.1665	8.446	0.19
18.	thyrotropin releasing hormone	C ₁₆ H ₂₂ N ₆ O ₄	362.1699	8.455	3.26
19.	7-hydroxy Tetranor Iloprost	C ₁₈ H ₂₆ O ₅	322.1779	8.463	0.9
20.	17beta-Estradiol 3-(beta-D-glucuronide)	C ₂₄ H ₃₂ O ₈	448.2029	8.515	0.47
21.	16b-Hydroxyestradiol	C ₁₈ H ₂₄ O ₃	288.172	8.54	15.13
22.	Gibberellin A44 diacid	C ₂₀ H ₂₈ O ₆	364.1851	8.558	1.8
23.	2,9-heptadecadien-4,6-diyn- 1,8-diol	C ₁₇ H ₂₄ O ₂	260.1769	8.604	9.65
24.	5-hydroperoxy-7-[3,5- epidioxy-2-(2-octenyl)- cyclopentyl]-6-heptenoic acid	C ₁₉ H ₃₀ O ₆	354.2031	8.781	2.66
25.	4-Hydroxyestrone	C ₁₈ H ₂₂ O ₃	286.1545	9.123	-2.5
26.	Dihydrodeoxystreptomycin	C ₂₁ H ₄₁ N ₇ O ₁₁	567.2848	9.31	
27.	Gibberellin A15	C ₂₀ H ₂₈ O ₅	348.1908	9.444	2.8
28.	Piretanide	C ₁₇ H ₁₈ N ₂ O ₅ S	362.0975	9.857	8.12
29.	Alprazolam	C ₁₇ H ₁₃ Cl N ₄	308.0897	9.877	-10.73
30.	Rutin	C ₂₇ H ₃₀ O ₁₆	610.151	6.495	
31.	Irbesartan	C ₂₅ H ₂₈ N ₆ O	428.2403	8.661	-5.08

32.	Gibberellin A44 diacid	C ₂₀ H ₂₈ O ₆	364.1887	9.034	1.53
33.	18-Hydroxycortisol	C ₂₁ H ₃₀ O	378.2038	10.933	3.24

The (HR)-LCMS High-Resolution Liquid Chromatography-Mass Spectrometer analysis of *Gardenia resinifera* methanolic extract spectrum profile Fig.-3 shows 43 compounds of which 27 major compounds were confirmed based on their retention time, mass, and molecular formula.

Fig-3: (HR)-LCMS Spectrogram of *Gardenia resinifera*

The phytochemicals identified in the extract includes Chlorogenic acid, Ecgonine, Loganin, Kaempferol, Hieracin, Warfarin, Benzylbutylphthalate, Promazine sulfoxide, Quinic acid, Hydroxyhydroquinone, Khayanthone and Bergenin were shown in table -3. Various authors reported different pharmacological activities for these compounds. Among them, Loganin found in *Strychnos nux-vomica L* shows central nervous stimulant activity and anti-inflammatory activity²⁷. Quinic acid obtained from flowers of *Moringa olifera* exhibited various pharmacological activities including anticancer activity and antibacterial activity^{28,29}. Genkwanin found in *Callicarpa Americana* revealed various pharmacological activities including anti-inflammatory, antibacterial and anticancer properties.³⁰

The High-Resolution Liquid Chromatography-Mass Spectrometer (HR-LCMS) analysis of *Gymnospora emarginata* methanolic extract spectrum profile Fig.-4 shows 19 compounds of which 15 major compounds were confirmed based on their retention time, mass, and molecular formula.

Table-3: Bioactive Compounds in Methanol Extract of *Garedenia resinifera*

S. No.	Name	Formula	Mass	RT	DB Diff (ppm)
1.	Mebeverine metabolite (Veratric acid glucuronide)	C ₁₅ H ₁₈ O ₁₀	358.0856	1.042	12.29
2.	3,5-Pyridinedicarboxylic acid, 2,6-dimethyl-4-(3- nitrophenyl)-, mono(2- hydroxyethyl) ester	C ₁₇ H ₁₆ N ₂ O ₇	360.0993	1.043	-9.86
3.	Deoxyelephantopin	C ₁₉ H ₂₀ O ₆	344.1244	1.045	4.53
4.	12a-Hydroxy-5- Deoxydehydromunduser one	C ₁₉ H ₁₈ O ₆	342.1115	1.05	-3.28
5.	Bis (2-hydroxypropyl) amine	C ₆ H ₁₅ N O ₂	133.1103	1.14	-0.39
6.	4-Hydroxy-L-threonine	C ₄ H ₉ N O ₄	135.0524	1.363	5.47
7.	cis-3-(6-Hydroxy-7-methoxy- 5-benzofuranyl)acrylic acid glucuronide	C ₁₈ H ₁₈ O ₁₁	410.0763	2.862	20.95
8.	4-Amino-m-cresol	C ₇ H ₉ N O	123.0686	4.228	-1.41

9.	2-Methoxyresorcinol	C ₇ H ₈ O ₃	140.0475	4.897	-0.8
10.	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.0905	4.902	12.92
11.	Octopamine (p- Hydroxyphenylethanolamine)	C ₈ H ₁₁ N O ₂	153.0787	5.13	1.76
12.	Ecgonine	C ₉ H ₁₅ N O ₃	185.1048	5.414	2.37
13.	Octopamine (p- Hydroxyphenylethanolamine)	C ₈ H ₁₁ N O ₂	153.0789	5.42	0.28
14.	3-Methylindole	C ₉ H ₉ N	131.0718	5.424	12.69
15.	8S-hydroxy-2-Decene-4,6- diynoic acid	C ₁₀ H ₁₀ O ₃	178.0609	5.771	12.04
16.	2,4-heptadienal	C ₇ H ₁₀ O	110.074	5.772	-7.25
17.	3-Methylsuberic acid	C ₉ H ₁₆ O ₄	188.1046	5.774	1.32
18.	4-Hydroxyphenylethanol	C ₈ H ₁₀ O ₂	138.0683	5.774	-1.47
19.	2,2-dimethyl-4-pentenoic acid	C ₇ H ₁₂ O ₂	128.0841	5.775	-2.98
20.	3-heptenal	C ₇ H ₁₂ O	112.0892	5.782	-3.19
21.	Loganin	C ₁₇ H ₂₆ O ₁₀	390.148	5.794	11.89
22.	Kaempherol	C ₁₅ H ₁₀ O ₆	286.0448	6.096	10.33
23.	Hieracin	C ₁₅ H ₁₀ O ₇	302.0393	6.143	11.15
24.	Kaempherol	C ₁₅ H ₁₀ O ₆	286.0447	6.485	10.56
25.	Dihydrodeoxystreptomycin	C ₂₁ H ₄₁ N ₇ O ₁₁	567.284	9.304	4.21
26.	Esmolol	C ₁₆ H ₂₅ N O ₄	295.1751	10.478	11.12
27.	(-)-Usnic acid	C ₁₈ H ₁₆ O ₇	344.0862	11.344	9.88
28.	Cuneatin methyl ether	C ₁₈ H ₁₄ O ₆	326.0754	11.467	11.21
29.	Warfarin	C ₁₉ H ₁₆ O ₄	308.1015	11.979	10.87
30.	Dalbergione, 4-methoxy- 4'-hydroxy-	C ₁₆ H ₁₄ O ₄	270.0886	12.245	2.22
31.	Benzylbutylphthalate	C ₁₉ H ₂₀ O ₄	312.1351	13.894	3.53
32.	12-oxo-9-octadecynoic acid	C ₁₈ H ₃₀ O ₃	294.2158	13.997	12.44
33.	Promazine sulfoxide	C ₁₇ H ₂₀ N ₂ O S	300.1305	14.309	-2.91
34.	Hydroxyhydroquinone	C ₆ H ₆ O ₃	126.0324	14.324	-5.77
35.	Harderoporphyryn	C ₃₅ H ₃₆ N ₄ O ₆	608.2586	16.188	8.03
36.	Harderoporphyryn	C ₃₅ H ₃₆ N ₄ O ₆	608.2591	16.584	7.18
37.	Khayanthone	C ₃₂ H ₄₂ O ₉	570.2819	16.871	1.66
38.	Quinic acid	C ₇ H ₁₂ O ₆	192.063	4.984	1.94
39.	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	354.0945	5.56	1.67
40.	BML-190	C ₂₃ H ₂₃ C N ₂ O ₄	426.1291	5.944	13.03
41.	Loganin	C ₁₇ H ₂₆ O ₁₀	390.1525	5.945	0.14
42.	Rutin	C ₂₇ H ₃₀ O ₁₆	610.1526	6.46	1.21
43.	Madecassic acid	C ₃₀ H ₄₈ O ₆	504.344	11.081	2.12

Fig- 4: (HR)-LCMS Spectrogram of *Gymnospora emarginata*

The phytochemicals found in the extract are Proline, Thiabendazole, Disulfiram, Tamarixetin, Luteoline, Pargyline, Oxdemetonmethyl, Naphazoline, Dihydrodeoxystreptomycin (Table-4). These compounds were also shown to be present in other plants and exhibit antimicrobial, antioxidant, anticancer, anti-inflammatory activity.³¹

Table-4: Bioactive Compounds in Methanol Extract of *Gymnospora emarginata*

S. No.	Name	Formula	Mass	Rt	DB Diff (ppm)
1.	2a-hydroxy-5- deoxydehydromunduser one	C ₁₉ H ₁₈ O ₆	42.1115	1.047	-3.28
2.	Thiabendazole	C ₁₀ H ₇ N ₃ S	201.0333	4.728	13.87
3.	Disulfiram	C ₁₀ H ₂₀ N ₂ S ₄	296.0518	5.921	-2.89
4.	Luteoline	C ₁₅ H ₁₀ O ₆	286.0441	6.114	12.7
5.	Pargyline	C ₁₁ H ₁₃ N	159.1026	6.195	14.1
6.	Tamarixetin	C ₁₆ H ₁₂ O ₇	316.0541	6.198	13.25
7.	Luteoline	C ₁₅ H ₁₀ O ₆	286.0443	6.645	12.13
8.	Naphazoline	C ₁₄ H ₁₄ N ₂	210.115	8.706	3.51
9.	Dihydrodeoxystreptomycin	C ₂₁ H ₄₁ N ₇ O ₁₁	567.2839	9.323	4.44
10.	Uroporphyrinogen III	C ₄₀ H ₄₄ N ₄ O ₁₆	836.2714	12.105	4.54
11.	Promazine sulfoxide	C ₁₇ H ₂₀ N ₂ O S	300.1301	14.293	-1.49
12.	Hydroxyhydroquinone	C ₆ H ₆ O ₃	126.0322	14.306	-4.05
13.	1-Methylinosine	C ₁₁ H ₁₄ N ₄ O ₅	282.0993	14.699	-10.03
14.	Mometasone Furoate	C ₂₇ H ₃₀ C ₁₂ O ₆	520.1406	15.508	2.5
15.	Avobenzone	C ₂₀ H ₂₂ O ₃	310.1534	15.951	11.41
16.	12beta-Hydroxy-3-oxo-5beta- cholan-24-oic Acid	C ₂₄ H ₃₈ O ₄	390.2729	18.448	10.55
19	Hydroxyhydroquinone	C ₆ H ₆ O ₃	126.0322	18.489	-4.26

CONCLUSION

The Methanolic extract of *Pongamia pinnata*, *Dodonea viscosa*, *Gardenia resinifera*, *Gymnospora emarginata* revealed the presence of therapeutically important bioactive compounds like flavonoids, glycosides, alkaloids, coumarins, terpenoids, saponins using (HR)-LCMS high-resolution liquid chromatography-mass spectrometer analysis. These bioactive components possess important pharmacological activities and could be useful for treating various human ailments. Further, *invitro* and *insilico* studies were planned on these bioactive compounds to identify candidate drug molecules for treating various parasitic diseases including filariasis.

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