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, Kaempherol), Coumarin (Lomatin), Carboxylic acid (Quinic acid) and Phenolic compounds (Isoliquiritigenin) in the select Indian medicinal plants.

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

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. 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.

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 Telangana 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 antidiyslipidemic. 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.

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 activity, antidiabetic activity in rats, anticancer activity against lung and breast cancer cell lines. It is also found to be effective in treating rheumatism, malaria, and snake bites.
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 bacteria 13, 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 ºC 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.

The important phytoconstituents confirmed by (HR)-LCMS Analysis were Apiin, Juglone, P-Hydroxyphenylacetic acid, Betaxolol, Artenlic acid, Picrotoxinin, Dihydrodeoxystreptomycin, Isoliquiritigenin, Sterigmatocystin, Hydroxyflutamide, Formononetin, Genkwain, 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. Vulpinic acid which was also found in lichens has shown anticancer and antibacterial activity. Cucurbitacins have shown anticancer, antidiabetic, anti-inflammatory effects.

### 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-deoxydehydromunduserone                    | 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_{26}O_{14} | 564.1434   | 5.786    | 7.92         |
| 6.     | beta-erythroidine                                         | C_{16}H_{16}N_{3}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_{20}N_{3}O_{3} | 307.2123 | 8.134    | 8            |
| 10.    | 3a-oh desogestrel                                         | C_{22}H_{32}O_{2}  | 326.2289   | 8.322    | -13.35       |
| 11.    | s-adenosylmethionine                                     | C_{15}H_{23}N_{6}O_{3}S | 399.1452 | 8.433    | -0.46        |
| 12.    | Artenlic acid                                             | C_{23}H_{32}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_{21}N_{7}O_{11} | 567.2856 | 9.305   | 1.48         |
| 15.    | trans-3-hydroxycoptine glucuronide                       | C_{16}H_{23}N_{3}O_{8} | 368.1233 | 9.697    | -3.74        |
| 16.    | Sappanone a 7-methyl ether                               | C_{17}H_{16}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_{25}O_{3}S | 352.1292  | 10.726   | 15.46        |
| 20.    | 12a-hydroxy-5-deoxydehydromunduserone                    | 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        |
| #  | Compound                        | Molecular Formula | Retention Time | Mass  |
|----|--------------------------------|-------------------|----------------|-------|
| 22 | Hydroxyflutamide               | C_{11}H_{13}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_{3} | 268.0741 | 11.24  | -1.98  |
| 26 | n,n-Dideethylchlordpromazine    | C_{15}H_{12}C_{1}N_{2}S | 290.0594 | 11.299 | 17.38  |
| 27 | Cuneatin methyl ether          | C_{18}H_{12}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_{3} | 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_{8}O_{1}  | 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_{3}N_{2}O_{3}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_{13}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 | Lomatinit                      | C_{14}H_{14}O_{4} | 246.088  | 13.113 | 4.79   |
| 42 | Methyl robustone               | C_{22}H_{22}O_{6} | 378.1087 | 13.513 | 4.21   |
| 43 | 3'-hydroxy-e,e-dienoestrol     | C_{18}H_{16}O_{3} | 282.1262 | 13.525 | -2.17  |
| 44 | Propargite                     | C_{19}H_{20}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-uglycone                    | C_{16}H_{16}O_{4} | 272.1056 | 14.318 | -2.64  |
| 50 | Juglone                        | C_{10}H_{8}O_{3}  | 174.0307 | 14.341 | 6.01   |
| 51 | Avocadene acetate              | C_{19}H_{14}O_{4} | 328.2591 | 14.364 | 6.83   |
| 52 | Lomatinit                      | C_{14}H_{14}O_{4} | 246.0878 | 14.875 | 5.66   |

The (HR)-LCMS High-Resolution Liquid Chromatography Mass Spectrometry 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*](image-url)
The phytochemicals found in the extract including Fraxetin, Hieracin, Desmethylzopiclone, Kaempferol, Tamarixetin, Epothilone A, 4-Hydroxyestrone, 4'-Hydroxypentololglucuronide, 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, Kaempferol 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 (ppm) | RT (min) | DBDiff (ppm) |
|--------|----------------------|---------|------------|----------|--------------|
| 1.     | Mebeverine metabolite (Veratric acid glucuronide) | C15 H15 O10 | 358.0848 | 1.035 |              |
| 2.     | 12a-Hydroxy-5-Deoxydehydroenduplusone | C19 H18 O6 | 342.1112 | 1.06 | 14.62 |
| 3.     | Fraxetin | C10 H4 O4 | 208.0347 | 5.21 | -2.49 |
| 4.     | Hieracin | C15 H16 O7 | 302.0389 | 6.281 |              |
| 5.     | Desmethylzopiclone | C16 H15C6N6 O3 | 374.0807 | 6.446 | 12.35 |
| 6.     | Kaempferol | C15 H10 O6 | 286.0446 | 6.546 | 23.31 |
| 7.     | Tamarixetin | C16 H12 O7 | 316.0547 | 6.678 | 11.12 |
| 8.     | Epothilone A | C26 H30 N6 O6 | 493.2499 | 6.78 | 11.36 |
| 9.     | 1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo- | C12 H16 O3 | 208.1099 | 7.707 |              |
| 10.    | 4-Hydroxyestrone | C18 H22 O3 | 286.1539 | 7.709 | 0.43 |
| 11.    | 4'-Hydroxypentolol glucuronide | C24 H17 N O9 | 483.2426 | 7.71 | 10.3 |
| 12.    | 6,8,10,12-pentadecatetraenal | C15 H22 O | 218.167 | 7.71 | 8.74 |
| 13.    | Orthothymotinic acid | C11 H14 O3 | 194.0945 | 7.713 | 0.32 |
| 14.    | 17alpha-Estradiol 3-D-glucuronide | C24 H32 O8 | 448.2033 | 8.024 | 3.34 |
| 15.    | 4-Hydroxyestrone | C18 H22 O3 | 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)- | C17 H22 O3 | 274.1568 | 8.438 | -1.45 |
| 17.    | 2-Methylene-5-(2,5-Dioxotetrahydrofurane-3-yl)-6-oxo-(10,10,10-Dimethylbicyclo[7:2:0][Undecane] | C18 H24 O4 | 304.1665 | 8.446 | 0.19 |
| 18.    | thrytropin releasing hormone | C16 H22 N6 O4 | 362.1699 | 8.455 | 3.26 |
| 19.    | 7-hydroxy Tetranor Iprost | C18 H26 O5 | 322.1779 | 8.463 | 0.9 |
| 20.    | 17beta-Estradiol 3-(beta-D-glucuronide) | C24 H32 O8 | 448.2029 | 8.515 | 0.47 |
| 21.    | 16b-Hydroxyestradiol | C18 H22 O3 | 288.172 | 8.54 | 15.13 |
| 22.    | Gibberellin A44 acid | C20 H28 O6 | 364.1851 | 8.558 | 1.8 |
| 23.    | 2,9-heptadecadien-4,6-diyne-1,8-diol | C17 H24 O2 | 260.1769 | 8.604 | 9.65 |
| 24.    | 5-hydroperoxy-7-[3,5-epidioxy-2-(2-octenyl)-cyclopentyl]-6-hetpenoic acid | C19 H30 O6 | 354.2031 | 8.781 | 2.66 |
| 25.    | 4-Hydroxyestrone | C18 H22 O3 | 286.1545 | 9.123 | -2.5 |
| 26.    | Dihydrodeoxystreptomycin | C21 H24 N7 O11 | 567.2848 | 9.31 |              |
| 27.    | Gibberellin A15 | C20 H32 O5 | 348.1908 | 9.444 | 2.8 |
| 28.    | Piretanide | C17 H16 N2 O2S | 362.0975 | 9.857 | 8.12 |
| 29.    | Alprazolam | C17 H18 Cl N4 | 308.0897 | 9.877 | -10.73 |
| 30.    | Rutin | C27 H30 O16 | 610.151 | 6.495 |              |
| 31.    | Irbesartan | C25 H24 N6 O | 428.2403 | 8.661 | -5.08 |
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.

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 activity 27. 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- Deoxydehydrodumunduser one                             | C_{19}H_{18}O_{6} | 342.1115 | 1.05    | -3.28         |
| 5.    | Bis (2-hydroxypropyl) amine                                           | C_{6}H_{13}N_{3}O_{2} | 133.1103 | 1.14    | -0.39         |
| 6.    | 4-Hydroxy-L-threonine                                                 | C_{4}H_{6}N_{4}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_{6}N_{3}O_{2} | 123.0686 | 4.228   | -1.41         |
|   |   |
|---|---|
| 9. | 2-Methoxyresorcinol | C_{10}H_{16}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_{6}H_{11}O_{3} | 153.0787 | 5.13 | 1.76 |
| 12. | Ecgonine | C_{9}H_{15}N_{2}O_{3} | 185.1048 | 5.414 | 2.37 |
| 13. | Octopamine (p-Hydroxyphenylethanolamine) | C_{6}H_{11}N_{2}O_{2} | 153.0789 | 5.42 | 0.28 |
| 14. | 3-Methylindole | C_{9}H_{9}N | 131.0718 | 5.424 | 12.69 |
| 15. | 88-hydroxy-2-Decene-4,6-diyinoic acid | C_{16}H_{10}O_{3} | 178.0609 | 5.771 | 12.04 |
| 16. | 2,4-Heptadienial | C_{7}H_{10}O | 110.074 | 5.772 | -7.25 |
| 17. | 3-Methylsuberic acid | C_{6}H_{16}O_{4} | 188.1046 | 5.774 | 1.32 |
| 18. | 4-Hydroxyphenylethanol | C_{6}H_{10}O_{2} | 138.0683 | 5.774 | -1.47 |
| 19. | 2,2-dimethyl-4-pentenoic acid | C_{7}H_{13}O_{2} | 128.0841 | 5.775 | -2.98 |
| 20. | 3-heptenal | C_{7}H_{12}O | 112.0892 | 5.782 | -3.19 |
| 21. | Logannin | C_{17}H_{26}O_{10} | 390.148 | 5.794 | 11.89 |
| 22. | Kaempferol | 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. | Kaempferol | 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_{2}O_{4} | 295.1751 | 10.478 | 11.12 |
| 27. | (3)-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_{16}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_{3} | 312.1351 | 13.894 | 3.53 |
| 32. | 12-oxo-9-octadecenoic 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. | Harderoporphyrin | C_{35}H_{36}N_{4}O_{6} | 608.2586 | 16.188 | 8.03 |
| 36. | Harderoporphyrin | C_{35}H_{36}N_{4}O_{5} | 608.2591 | 16.584 | 7.18 |
| 37. | Khayanthone | C_{12}H_{42}O_{6} | 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_{15}H_{18}O_{8} | 354.0945 | 5.56 | 1.67 |
| 40. | BML-190 | C_{25}H_{32}C_{N_{2}}O_{4} | 426.1291 | 5.944 | 13.03 |
| 41. | Logannin | 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, Oxendetonmethyl, Naphazoline, Dihydrodeoxystreomptycin (Table-4). These compounds were also shown to be present in other plants and exhibit antimicrobial, antioxidant, anticancer, anti-inflammatory activity.

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            | C₁₀H₁₈O₆          | 42.1115 | 1.047  | -3.28         |
| 2.     | Thiabendazole                                    | C₁₀H₇N₃S         | 201.0333| 4.728  | 13.87         |
| 3.     | Disulfiram                                        | C₁₀H₂₀N₂S₄       | 296.0518| 5.921  | -2.89         |
| 4.     | Luteoline                                         | C₁₅H₁₆O₆         | 286.0441| 6.114  | 12.7          |
| 5.     | Pargyline                                         | C₁₁H₁₃N          | 159.1026| 6.195  | 14.1          |
| 6.     | Tamarixetin                                       | C₁₆H₁₂O₇         | 316.0541| 6.198  | 13.25         |
| 7.     | Luteoline                                         | C₁₅H₁₀O₆         | 286.0443| 6.645  | 12.13         |
| 8.     | Naphazoline                                       | C₁₄H₁₄N₂         | 210.115 | 8.706  | 3.51          |
| 9.     | Dihydrodeoxystreptomycin                          | C₂₁H₄₁N₇O₁₁      | 567.2839| 9.323  | 4.44          |
| 10.    | Uroporphyrinogen III                             | C₄₀H₄₄N₄O₁₆      | 836.2714| 12.105 | 4.54          |
| 11.    | Promazine sulfoxide                               | C₁₇H₂₀N₂O S      | 300.1301| 14.293 | -1.49         |
| 12.    | Hydroxyhydroquinone                               | C₆H₆O₃           | 126.0322| 14.306 | -4.05         |
| 13.    | 1-Methylinosine                                   | C₁₁H₁₄N₄O₅      | 282.0993| 14.699 | -10.03        |
| 14.    | Mometasone Furoate                                | C₂₇H₃₀C₂₂O₆      | 520.1406| 15.508 | 2.5           |
| 15.    | Avobenzone                                        | C₂₀H₂₂O₃         | 310.1534| 15.951 | 11.41         |
| 16.    | 12beta-Hydroxy-3-oxo-5beta- cholan-24-oic Acid   | C₂₄H₂₈O₄         | 390.2729| 18.448 | 10.55         |
| 17.    | Hydroxyhydroquinone                               | C₆H₆O₃           | 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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