PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF DRYPETES SEPPIARIA (WIGHT & ARN.) PAX. & HOFFIM. FROM SILAMBUR SACRED GROVE, TAMILNADU

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ABSTRACT
Drypetes sepiaria (Wight & Arn.) Pax. & Hoffim a medium sized tree member of Euphorbiaceae was investigated to determine the phytochemical constituents present in various extracts of the leaves through GC-MS analysis. Powdered leaf plant materials were subjected to successive extraction with organic solvents such as methanol by Soxhlet extraction method. In the present study, GC-MS analysis revealed that a total of 23 different compounds identified by using methanol extract and all the identified compounds were medicinally valuable for the treatment of various human ailments. In addition, all the phytochemical compounds were needed for further investigations on toxicological aspects for the development of new lead of therapeutic interest.

Keywords: Drypetes sepiaria preliminary screening, GC-MS analysis.

1. INTRODUCTION

Plants have been a rich source for drug discovery (Mishra and Tiwari, 2011). Plants and plant parts have been provide a good source of pharmaceutical active compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, saponin and some other secondary metabolites, which are rich in valuable bioactivities of antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic (Maridass et al., 2008). The genus Drypetes (Putranjivaceae (Euphorbiaceae) comprises nearly 160 species which has been used in the folk medicine of many cultures for many years (Nganga et al., 2008). Even though the species was different, they used to treat similar disorders. Among the members of the genus Drypetes earlier phytochemical studies on some species including D. parrifolia, D. laciniata, D. inaequalis, D. armoracia, D. gossweileri, D. molunduana, D. roxburghii have yielded flavonoids, chalcone glycosides, saponins, tripterpenoids, phenolics, alkaloids, etc.

Drypetes sepiaria (Wight & Arn.) Pax. & Hoffim. an ever green tree locally known as Kalvirai (Tamil) commonly grown in foothills and shrub jungles and some sacred groves of Tamil Nadu. Drypetes sepiaria is traditionally used to treat pain and inflammation and seeds are used as a wild edible food and their root paste can be used as an antidote for scorpion bite. Decoction of leaves and seeds is also noted for reducing rheumatic inflammation (Arinathan et al., 2007; Bharath Kumar and Suryanarayana, 2011). As per earlier literature, there is no scientific investigations found in D. sepiaria on phytoconstituents present. In ethnomedicinal point of view as described above, the GC-MS analysis was carried out with methanolic leaf extract of D. sepiaria to investigate the chemical constituents present in it.

2. MATERIALS AND METHODS

2.1. Collection of plant materials and preparation of the extract

The fresh leaves of D. sepiaria was collected from the sacred grove of Silambur (Lat, 11.35 °N; Long, 79.31°E), Ariyalur District, Tamil Nadu, India. The specimen was botanically identified and confirmed by Rapinat Herbarium, St. Joseph’s College, Tiruchirappalli. The preserved plant specimens were submitted to the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu for further reference. The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The powdered leaf were then subjected to successive extraction with organic solvents such as hexane chloroform and ethanol by Soxhlet method (Catherine et al., 1997). The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed and stored at 4°C. They were used for GC-MS analysis.

2.2. Preparation of extract

The powdered leaf of D. sepiaria (500 g) was extracted with methanol (95%) and double distilled water separately in a soxhlet extractor. The extract was evaporated to dryness at 60°C under reduced pressure in a rotary evaporator and kept in refrigerator at 4°C till used. The extracts were
dissolved in dimethylsulphoxide to make the final concentrations at the time study.

2.3. Preliminary phytochemical screening

A small portion of the dry extracts were subjected to preliminary phytochemical screening to detect the presence of various phytoconstituents present in the leaves of D. sepiaria (Harborne, 1973; Evans, 2003).

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with GC-MS Clarus 500 Perkin Elmer Equipment. Compounds were separated on Elite-5 capillary column (Crossbond 5% Phenyl 95% dimethylpolysiloxane). Oven temperature was programmed as follows: isothermal temperature at 60°C then increased to 200°C at the rate of 10°C/min., then increased up to 280°C at the rate of 5°C/min. held for 9 min. Ionization of the sample components was performed in the Electron energy (70 eV). The helium was used as gas carrier (1ml/min.), and 1.0 μL of sample was injected. The detector was Mass detector Turbomass gold Perkin Elmer. The total running time for GC was 36 min. and software Turbomass 5.2.0 was used in this GC-MS study (Manjamalai et al., 2010).

2.5. Identification of compounds

All the compounds were identified from methanol extracts based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, NIST (Version year 2005).

### Table 1. Preliminary phytochemical screening of Drypetes sepiaria leaves.

| S.No. | Phytochemicals | PE | Chl | AC | E |
|-------|----------------|----|-----|----|---|
| 1.    | Alkaloids      | +  | -   | +  | - |
| 2.    | Steroids       | -  | +   | +  | + |
| 3.    | Terpenoids     | -  | -   | -  | - |
| 4.    | Flavonoids     | +  | -   | +  | + |
| 5.    | Tannins        | -  | +   | -  | - |
| 6.    | Saponins       | +  | +   | +  | + |
| 7.    | Glycosides     | -  | -   | -  | - |
| 8.    | Total phenol   | +  | -   | +  | + |

PE: Petroleum ether, Chl: Chloroform, AC: Acetone, E: Ethanol, + present, - absent

The chemical constituents identified by the GC-MS analysis on methanolic leaf extract of D. sepiaria were enumerated along with Molecular Formula (MF), Molecular Weight (MW), Retention Time (RT), and peak area and peak area (%) is presented in Table-2.

### Table 2. Compounds identified in methanolic leaf extract of Drypetes sepiaria.

| S.No. | Peak Name                                      | Retention Time (min) | Peak Area | % Peak area |
|-------|------------------------------------------------|----------------------|-----------|-------------|
| 1     | Name: Propanoic acid, 2-oxo-, methyl ester    | 2.83                 | 352644    | 2.8966      |
|       | Formula: C₆H₁₀O₃                               |                      |           |             |
|       | MW: 102                                        |                      |           |             |
| 2     | Name: 2-Furanmethanol                          | 3.69                 | 526502    | 0.4325      |
|       | Formula: C₅H₆O₂                                 |                      |           |             |
|       | MW: 98                                         |                      |           |             |
| 3     | Name: 2-Cyclopenten-1-one, 2-hydroxy-          | 4.82                 | 251654    | 2.0671      |
|       | Formula: C₅H₆O₂                                 |                      |           |             |
|       | MW: 98                                         |                      |           |             |
| 4     | Name: Benzaldehyde                             | 5.46                 | 253243    | 2.0801      |
|       | Formula: C₇H₆O                                  |                      |           |             |
|       | MW:106                                        |                      |           |             |
| 5     | Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-| 5.69                 | 232379    | 1.9087      |
|       | one                                          |                      |           |             |
|       | Formula: C₈H₁₀O₄                               |                      |           |             |
|       | MW: 144                                        |                      |           |             |
| 6     | Name: 2-Hydroxy-gamma-butyrolactone            | 6.25                 | 174726    | 1.4352      |
|       | Formula: C₆H₆O₁                                 |                      |           |             |
|       | MW: 102                                        |                      |           |             |
| Name                                                      | Formula                     | MW       | 8.2  | 341872 | 2.8081 |
|-----------------------------------------------------------|-----------------------------|----------|------|--------|--------|
| 3-Acetylthymine                                           | C₇H₈N₂O₃                   | 168      | 8.73 | 621868 | 0.5108 |
| Pyrimidine-4,6-diol, 5-methyl                            | C₅H₆N₂O₂                   | 126      | 9.53 | 284421 | 2.3362 |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl        | C₆H₆O₄                     | 144      | 10.04| 247028 | 2.0291 |
| 2-Propenal, 3-(2-furanyl)-                                | C₇H₆O₂                     | 122      | 10.52| 546851 | 0.4492 |
| 2(1H)-Pyrimidinethione, 4,6-diamino-                      | C₄H₄N₄S                   | 142      | 10.9 | 96626  | 0.0794 |
| 1,6,3,4-Dianhydro-2-O-acetyl-á-d-allopyranose             | C₈H₁₀O₅                    | 186      | 11.01| 318631 | 2.6172 |
| Dianhydromannitol                                         | C₆H₁₀O₄                    | 146      | 11.59| 284490 | 2.3368 |
| 2-Furancarboxaldehyde, 5-(hydroxymethyl)-                 | C₅H₆O₃                     | 126      | 12.64| 544630 | 0.4474 |
| 2H-Pyran-5-carboxylic acid, 2-oxo-, methyl ester         | C₇H₈O₄                     | 154      | 12.95| 218384 | 0.1794 |
| 2-EthylN-hydroxyacetimidate                              | C₄H₉NO₂                    | 103      | 13.32| 950045 | 7.8036 |
| 2-Methoxy-4-vinylphenol                                   | C₈H₁₀O₂                    | 150      | 13.79| 644626 | 0.5295 |
| 5-Formylsalicylaldehyde                                   | C₈H₆O₃                     | 150      | 14.15| 505296 | 0.415  |
| Phenol, 3,4-dimethoxy-                                    | C₆H₁₀O₃                    | 154      | 14.45| 513228 | 4.2156 |
| Benzoic acid, 4-formyl-, methyl ester                    | C₈H₈O₃                     | 164      | 16.39| 405797 | 3.3332 |
| Benzeneethanol, 4-hydroxy                                  | C₈H₁₀O₂                    | 138      | 16.7 | 346247 | 2.844  |
| Phenol, 2-methoxy-4-(1-propenyl)-                         | C₁₀H₁₂O₂                   | 138      | 18.18| 655066 | 5.3806 |
| Cyclohexane, 1-methylene-4-(1-methylethenyl)              | C₁₀H₁₆                     | 164      | 19.5 | 665066 | 5.3806 |
| No. | Name                                                                 | Formula                  | MW  | %     | 1/1000000 | 1/1000000 |
|-----|-----------------------------------------------------------------------|--------------------------|-----|--------|------------|------------|
| 24  | 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)                            | C₁₀H₁₂O₃                 | 180 | 18.68  | 689038     | 0.566      |
| 25  | 3',5'-Dimethoxyacetophenone                                           | C₁₀H₁₂O₃                 | 19.6| 116518 | 0.9571     |
| 26  | Benzeneacetic acid, 3,4-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)       | C₆H₆O₄                   | 23.49| 151320 | 12.4293    |
| 27  | Benzeneacetic acid, 4-hydroxy-3-methoxy-2-methyl ester                | C₁₀H₁₂O₄                 | 24.19| 120783 | 0.9921     |
| 28  | (R)-( )-4,4a,5,6,7,8-Hexahydro-4a-methyl-2(3H)-naphthalenone         | C₁₀H₈N₂O                 | 24.42| 197221 | 1.62       |
| 29  | 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol                         | C₅H₈N₂O₂                 | 24.62| 432379 | 3.5515     |
| 30  | Benzoic acid, 3-formyl-4,6-dihydroxy-2,5-dimethyl-2-methyl est         | C₄H₈N₂O                  | 25.57| 144845 | 1.1897     |
| 31  | 3,4-Dihydrocoumarin-7-ol                                             | C₅H₈O₂                   | 25.82| 940934 | 7.7287     |
| 32  | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol                                | C₁₅H₃₂O₈                 | 26.53| 531514 | 4.3658     |
| 33  | Undecanoic acid, 2-methyl                                            | C₁₁H₂₀O                  | 28.79| 111426 | 0.9152     |
| 34  | n-Hexadecanoic acid                                                  | C₁₂H₈O                   | 30.13| 161403 | 13.2575    |
| 35  | 9,12-Octadecadienoic acid, methyl est                                | C₁₀H₁₆O₃                 | 32.86| 154532 | 1.2693     |
| 36  | 10-Octadecenoic acid, methyl est                                     | C₁₀H₁₆O₃                 | 32.99| 246271 | 2.0228     |

In the methanolic leaf extract of *D. sepiaria*, a total of 36 compounds were identified, of which n-Hexadecanoic acid (13.25%), was found as major compound followed by other compounds namely, Benzeneacetic acid, 3,4-dihydroxy-(12.92%), 2-Methoxy-4-vinylphenol (7.80%), 3,4-Dihydrocoumarin-7-ol (7.72%), and Cyclohexane, 1-methylene-4-(1-methylethenyl)-(5.38%).

Phenolic compounds have antimicrobial properties. Phenol and phenolic compounds have
been extensively used in disinfections. Thus the reported antimicrobial properties of both plants may be attributed to phenolic compounds. Plants with tannins are used for healing of wounds, varicose ulcers and burns (Nafiu et al., 2011). Among the identified phytochemicals, n-hexadecanoic acid has the property of antioxidant activity and it justifies with the earlier work in Alstonea venenata (Sutha, 2012).

4. CONCLUSION

The present investigation through the present study revealed that the species D. sepiaria is a reliable source of bioactive compounds like fatty acid esters, alcohols, hydrocarbons, alkanes, amines, terpenes, and sugars that justify the traditional usage of this species by the local healers in Tamil Nadu, India, for various ailments. As GC-MS is the first step towards understanding the nature of active principles. Further investigation in this species is suggested for the development of novel drugs.

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