Bio-active compounds analysis and characterization in Ethanolic plant extracts of *Justicia tranquebariensis* L. (Acanthaceae) – using GC-MS

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Abstract: Phytocompounds in Ethanolic extract of *Justicia tranquebariensis* was elucidated using Gas chromatography – Mass spectrometry method. Fifteen compounds were identified. The major constituents are Hexadecane, Dibutyl phthalate, Dotriacontane and Hexadecanoic acid, and ethyl ester. From this study it is obvious that *Justicia tranquebariensis* plant extracts contains many biologically active compounds, such as Antimicrobial activity, antioxidant, antiviral and Cytotoxic activity.

Introduction

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors and fragrances. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.

In recent years, Gas Chromatography and Mass Spectrum (GC–MS) has been applied unambiguously to identify the structures of different phytoconstituents from plant extracts and biological samples with great success.

*Justicia tranquebariensis* is a small shrub, which is widely distributed in southern parts of India. It is a perennial plant, leaves opposite, oval, entire. Therapeutic value of this plant ranges from anti tumor, anti viral, analgesic to anti-inflammatory activities. The juice is used as cooling and aperients, and is prescribed for the children to cure smallpox, and bruised leaves are also applied to external injuries.

Some species of the genus *Justicia* have been used in the traditional system of medicines for the treatment of fever, pain, inflammation, diabetes, diarrhoea and liver diseases. They also possess anti-
inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities. The leaf juice of *J. tranquebariensis* has been used to treat jaundice and leaf paste is applied over affected area to treat skin diseases.

**Materials and Methods**

**Collection of samples**

*Justicia tranquebariensis* were selected based on their ethno medical importance. Healthy disease free leaves, stems of *Justicia tranquebariensis* were collected from Semmalai, Karur district, Tamilnadu, India. The plant materials were shade dried, pulverized and stored at 4°C. Until further use.

**Preparation of extracts**

30g of air dried powder of plant material were infused in ethanol (100ml) until complete exhaustion. The infusion was filtered with four layered muslin cloth and stored at 4°C.

**Gas chromatography and Mass spectrum analysis**

Gas chromatography analysis was carried out at South Indian Textile Institute (SITRA), Coimbatore. It is one of the key techniques generally used for screening / identification of many groups of plant phytochemicals. Often irreplaceable tool in the phytochemical analysis even at trace level of plant chemical compounds. 5ml of ethanol extract was evaporated to dryness and reconstituted in 2µl ethanol. The extracts were then subjected into GC-MS analysis. Chromatographic separation was carried out with CE GC 8000 top MSMD 8000 Fyson instrument with D6 35mr column (10m x 0.5mm, 0.25 µm film thicknesses). Heating programs were executed from 100 - 250°C at 3 minutes by using the helium as a carrier with the injector heater at 250°C. Injection temperature at 250°C, interface temperature at 200°C, quadruples temperature at 230°C were maintained. Injection was performed in split less mode. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70eV and the detector operated in scan mode from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST.

**Results and discussion**

**Ethnomedicinal Uses**

Local people use this plant against inflammations. Leaf is used as expectorant, in cold, cough and nasal disorders. Leaf juice, about15-20 ml, is administered orally for every one hour up to half of the day and keeping of leaf paste externally on the sight of snake bite work as an antidote for Cobra bite. Leaf juice is given orally to treat jaundice and leaf paste is applied over affected area to treat skin disease. The active principles with their retention time (RT), Molecular formula, molecular weight (MW), peak area in percentage are presented below (Table 1).

This typical gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the compounds present in the plant. The numbers at various peaks are the retention time in minutes. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds.

The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. The GC-MS analysis of 4 compounds and its biological activity were presented in (Table 2). Major compounds are Tetradecanal, Hexadecanoic acid, ethyl ester (CAS), Dotriacontane, and Pentatriacontane (Figure -1).

Tetradecanal compounds have the property of Antibacterial activity. The identified compounds possess many biological properties for instance Hexadecanoic acid, ethyl ester (CAS) has antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, ant androgenic, flavor, hemolytic 5-Alpha reductase.
Dotriacontane and Pentatriacontane have antimicrobial, antioxidant, antispasmodic, antibacterial, and antiviral properties.

Table 1: Phytoconstituents present in *Justicia tranquebariensis* as identified by GC-MS

<table>
<thead>
<tr>
<th>S. no</th>
<th>Retention time (RT)</th>
<th>% of Peak Area</th>
<th>Compound Name</th>
<th>Molecular formula (MF)</th>
<th>Molecular weight (MW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.80</td>
<td>3.73</td>
<td>Dodecane</td>
<td>C12H26</td>
<td>170</td>
</tr>
<tr>
<td>2.</td>
<td>10.79</td>
<td>4.57</td>
<td>Tetradecane</td>
<td>C14H30</td>
<td>198</td>
</tr>
<tr>
<td>3.</td>
<td>14.90</td>
<td>2.98</td>
<td>Hexadecane</td>
<td>C16H34</td>
<td>226</td>
</tr>
<tr>
<td>4.</td>
<td>15.18</td>
<td>3.37</td>
<td>Tetradecanal (CAS)</td>
<td>C14H28O</td>
<td>212</td>
</tr>
<tr>
<td>5.</td>
<td>19.14</td>
<td>1.67</td>
<td>Octadecane</td>
<td>C18H38</td>
<td>254</td>
</tr>
<tr>
<td>6.</td>
<td>22.22</td>
<td>5.61</td>
<td>Dibutyl phthalate</td>
<td>C16H22O4</td>
<td>278</td>
</tr>
<tr>
<td>7.</td>
<td>23.01</td>
<td>1.37</td>
<td>Hexadecanoic acid, ethyl ester (CAS)</td>
<td>C18H36O2</td>
<td>284</td>
</tr>
<tr>
<td>8.</td>
<td>24.15</td>
<td>1.48</td>
<td>Triacetone</td>
<td>C30H62</td>
<td>422</td>
</tr>
<tr>
<td>9.</td>
<td>24.61</td>
<td>9.55</td>
<td>Tetratriacontane</td>
<td>C34H70</td>
<td>478</td>
</tr>
<tr>
<td>10.</td>
<td>29.82</td>
<td>1.21</td>
<td>Hexatriacontane (CAS)</td>
<td>C36H74</td>
<td>506</td>
</tr>
<tr>
<td>11.</td>
<td>30.99</td>
<td>1.8</td>
<td>Pentacosane (CAS)</td>
<td>C25H52</td>
<td>352</td>
</tr>
<tr>
<td>12.</td>
<td>31.36</td>
<td>3.57</td>
<td>Di-(2-ethylhexyl)phthalate</td>
<td>C24H38O4</td>
<td>390</td>
</tr>
<tr>
<td>13.</td>
<td>32.05</td>
<td>22.30</td>
<td>Dotriacontane</td>
<td>C32H66</td>
<td>450</td>
</tr>
<tr>
<td>14.</td>
<td>33.86</td>
<td>2.09</td>
<td>Pentatriacontane</td>
<td>C35H72</td>
<td>492</td>
</tr>
<tr>
<td>15.</td>
<td>35.78</td>
<td>2.82</td>
<td>Octacosane (CAS)</td>
<td>C28H58</td>
<td>394</td>
</tr>
</tbody>
</table>

Table 2: Biological properties of GC-MS compounds

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the compound</th>
<th>Biological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tetradecanal</td>
<td>Antibacterial activity.</td>
</tr>
<tr>
<td>2.</td>
<td>Hexadecanoic acid, ethyl ester (CAS)</td>
<td>Antioxidant, Hypcholesterolemic, Nematicide, Pesticide, Lubricant, Ant androgenic, Flavor, Hemolytic 5-Alpha reductase inhibitor</td>
</tr>
<tr>
<td>3.</td>
<td>Dotriacontane</td>
<td>Antimicrobial, antioxidant, antispasmodic</td>
</tr>
<tr>
<td>4.</td>
<td>Pentatriacontane</td>
<td>Antibacterial, Antiviral</td>
</tr>
</tbody>
</table>

Figure -1 Gas chromatography and mass spectroscopy (GC-MS) analysis of Ethanolic sample of *Justicia tranquebariensis*

**Conclusion**

The GC-MS analysis of the Ethanolic extract of *J. tranquebariensis* reveals the presence of phytoconstituents belonging to the, esters, alcohols, ethers and the compounds are Tetradecane, Hexadecane, Tetradecanal (CAS), Octadecane, Dibutyl phthalate, Hexadecanoic acid, ethyl ester (CAS), Triaccontane, Tetratriacontane, Hexatriacontane (CAS), Pentacosane (CAS), Di-(2-ethylhexyl) phthalate, Dotriacontane, Pentatriacontane, and Octacosane (CAS). The plant holds promise for the production of novel pharmaceuticals as well as a nutraceutical. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for their therapeutic roles.

Here we report the presence of some important compounds in this plant isolated by GC-MS analysis. Thus, this type of study may give information on nature of active principles present in the medicinal plants. These identified phytoconstituents presumed to be responsible for eliciting the traditional activity of *Justicia tranquebariensis*. 
Acknowledgement

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References

12. Yoganarasimhan, S.N, Medicinal Plants of India, Regional Research Institute, Bangalore, 2000, 2.

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