ABSTRACT

Objective: In the present study, phytochemical constituents of crude water extract of Wrightia tinctoria (WT) bark is done using GC-MS technique. Wrightia tinctoria is an important medicinal herb being used in tribal areas of Chhattisgarh since long but chemical constituents of its bark responsible for the activities are still not studied in depth.

Methods: Dried bark powder was successively extracted with petroleum ether, ethyl acetate and methanol using soxhlet apparatus and lastly material was dissolved in distilled water for 10 hrs for extraction. Water extract was selected for the further analysis using Agilent 7890A GC with 5975MS.

Results: As per the GC-MS analysis, twelve different compounds namely benzene 1, 2, 4, 5-tetramethyl (2.85%), benzene 1, 2, 3, 5-tetramethyl (1.16%), 1-decanol, 2, 2-dimethyl (4.38%), phenol 2, 4-bis [1, 1-dimethyl ethyl] (7.78%), heptadecane (3.60%), 3-hexadecanoic (3.30%), 1-propyl tetradecanol (3.64%), benzo (h) quinoline (3.66%), n-hexadecanoic acid (6.54%), octadecanoic acid methyl ester (0.81%), phytane (1.95%) and pentadecane (2.25%) were characterized. Analysis and identification of presence of the compound in these extracts were done using the database of NIST library.

Conclusion: Study confirms the presence of biologically active phyto-constituent in water extract of Wrightia tinctoria bark those may be the key chemical of natural origin in new drugs designing against major disease those are being treated in tribal areas using this plant bark. Further confirmation of in-vitro bioactivity using cell line culture is required which is planned as the future prospect of current study.

Keywords: Wrightia tinctoria, Phytochemical, GC-MS analysis, Apocynaceae.
For the analysis of the extracts, Agilent 5975C TDA series gas chromatography/mass spectroscopy with selective detector system was used which offer high performance and flexibility with many options. Crude water extract (1 µl) of WT bark was used in GC-MS analysis with Agilent (5975C MS) 5% poly siloxane column of 30×250 µm×0.25 µm size. Oven temperature was programmed as: Isothermal temperature was 5ºC/min and held for 1.75 min then increased to 275 °C at the rate of 8ºC/min and kept constant for 5 min. The run time was 25 min. Ionization of sample components were performed on EI mode (70eV).

Interpretation of GC-MS spectrum was done using the database of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Name, molecular weight and structure of compounds of the water extract were ascertained.

![Fig. 2: Mass Spectrum of Phenol 2, 4-bis (1, 1-dimethyl ethyl)](image)

Results showed the presence of benzene 1, 2, 4, 5-tetramethyl (2.85%), benzene 1, 2, 3, 5-tetramethyl (1.16%), 1-decanol, 2, 2-dimethyl (4.38%), phenol 2, 4-bis (1, 1-dimethyl ethyl) (7.78%), heptadecane (3.60%), 3-hexadecanol (3.30%), i-propyl tetradecanol (3.64%), benzo (h) quinoline (3.66%), n-hexadecanoic acid (6.54%), octadeanoic acid methyl ester (0.81%), phytane (1.95%), pentadecane (2.25%).

List of 12 compounds detected in crude water extract of WT bark with their retention indices, percentage composition, chemical structure and activities are given in table-1 and fig. 1-3.

![Fig. 3: Mass Spectrum of n-Hexadecanoic acid](image)

Among the identified phyto chemicals, n-hexadecanoic acid and Phenol 2, 4-bis (1, 1-dimethyl ethyl) has a role in antioxidant effect [17-18] present in maximum percentage. Phenol 2, 4-bis (1, 1-dimethyl ethyl) have good antibacterial activity [19]. Previously no results are discussed in available literature about the chemical composition of WT bark but a same compound, n-hexadecanoic acid was found already reported in leaves of WT plant. 3-O-Methyl-d-glucose (51.44%) was reported as major compound along with 21 minor compounds identified in WT leaves with GC-MS analysis [20].

**Table 1:** Shows compounds identified in crude waster extract of WT using GC/MS.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RT (in minutes)</th>
<th>Name of the isolated compound</th>
<th>Molecular formula</th>
<th>MW (amu)</th>
<th>Peak % area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.48</td>
<td>Benzene 1, 2, 4, 5-tetramethyl</td>
<td>C₃₄H₈₂</td>
<td>134</td>
<td>2.85</td>
</tr>
<tr>
<td>2</td>
<td>5.70</td>
<td>Benzene 1, 2, 3, 5-tetramethyl</td>
<td>C₃₄H₈₂</td>
<td>134</td>
<td>1.16</td>
</tr>
<tr>
<td>3</td>
<td>7.32</td>
<td>1-decanol 2, 2-dimethyl</td>
<td>C₁₇H₃₄O₂</td>
<td>186</td>
<td>4.38</td>
</tr>
<tr>
<td>4</td>
<td>7.43</td>
<td>Pheno12, 4-bis (1, 1-dimethyl ethyl)</td>
<td>C₁₄H₂₀O₂</td>
<td>206</td>
<td>7.78</td>
</tr>
<tr>
<td>5</td>
<td>8.18</td>
<td>Heptadecane</td>
<td>C₁₇H₃₄O₂</td>
<td>240</td>
<td>3.60</td>
</tr>
<tr>
<td>6</td>
<td>8.51</td>
<td>3-hexadecanol</td>
<td>C₁₇H₃₄O₂</td>
<td>242</td>
<td>3.30</td>
</tr>
<tr>
<td>7</td>
<td>8.57</td>
<td>i-Propyl tetradecan</td>
<td>C₁₇H₃₄O₂</td>
<td>270</td>
<td>3.64</td>
</tr>
<tr>
<td>8</td>
<td>8.82</td>
<td>Benzo (h) quinoline</td>
<td>C₁₇H₃₄N</td>
<td>179</td>
<td>3.66</td>
</tr>
<tr>
<td>9</td>
<td>9.01</td>
<td>n-hexadecanoic acid</td>
<td>C₁₇H₃₄O₂</td>
<td>256</td>
<td>6.54</td>
</tr>
<tr>
<td>10</td>
<td>9.86</td>
<td>Octadecanoic acid methyl ester</td>
<td>C₁₇H₃₄O₂</td>
<td>298</td>
<td>0.81</td>
</tr>
<tr>
<td>11</td>
<td>10.20</td>
<td>Phytane</td>
<td>C₂₀H₃₂O₂</td>
<td>282</td>
<td>1.95</td>
</tr>
<tr>
<td>12</td>
<td>11.33</td>
<td>Pentadecane</td>
<td>C₁₇H₃₂O₂</td>
<td>212</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Hexadecanoic acid and 1, 5-methyl ester (58.30%) were reported as major compound along with minor 10 compounds in ethanolic extract of WT flowers by GC-MS analysis [22]. No similar compound was found in crude water extract of WT bark as reported in flower. In this study, presences of twelve components resolved by GC-MS are being reported. This study provided with the new knowledge for the upcoming research in recent future.

**CONCLUSION**

In the present study twelve chemical constituents have identified from the water extract of WT bark by GC-MS analysis those are not reported in bark of this plant in already published literate. Some of the peaks could not be identified which may be the next step for further investigation along with the detailed bioactivity of compounds identified.

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**ABBREVIATION**

WT - Wrightia tinctoria; WE - Water Extract; NIST - National Institute of Standards and Technology; TDA - Toluene-diamine; EI - Electron Ionization; PCI - Positive Chemical Ionization; NCI - Negative Chemical Ionization; RT - Retention Time; MF - Molecular Formula; MW - Molecular Weight; Amu - Atomic mass unit; MS - Mass spectroscopy/mass spectrum.

**CONFLICT OF INTERESTS**

Declared None
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