Findings of Gas chromatography - Mass Spectrometry (GCMA) Analysis of Eclipta Alba Plant Root

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Abstract:
Background: Eclipta alba L. Hassk. (Asteraceae), commonly known as Bhringaraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice. This study was designed to investigate the phytochemical compositions of methanol extract of Eclipta Alba root through GCMS analysis.

Material and methods: 5 grams of plant material was extracted with 250 ml 99% methanol. For methanolic extract dried powder samples were extracted in a Soxhlet apparatus with methanol until becoming colourless. The extract was filtered and centrifuged at 1500 rpm for 20 minutes to remove any plant debris. Supernatants were stored at 22 °C until assayed.

Results: In the Methenolonic extract of Eclipta alba plant root, we observed the presence of 25 medicinally important bioactive compounds among those 13,21,19,12,17,16 & 25 - showed lowest peak of 2(3H)-Furanone, 5-acetylhydrole-0.09%, Penetonic acid, 4-methyl-3-methylethenyl. isopropyl ester - 0.14%, Phenol, 4-ethyl-2-methoxy-0.27%, 4H-Pyran4-one, 3-hydroxy-2-methyl- (CAS) Maltol - 0.36, 2,3-DIHYDRO-BENZOFRAN - 0.92%, 1H-Imidazole, 4,5-dihydro-2-methyl- 1.53%, 1,2-Benzenediol (CAS) Pyrocatechol - 2.93% and D-Alloose - 10.11 identified during analysis.

Conclusions: The presence of various secondary metabolites such as glycosides, alkaloids, Saponins, and Flavanoids are believed to exhibit the, antiobeisty and Hepatoprotective properties of Eclipta Alba roots.

Keywords: Bhringraj (Eclipta Alba), GC-MS analysis, 2(3H)-Furanone, 5-acetylhydrole and D-Alloose

Date of Submission: 31-10-2017 Date of acceptance: 16-11-2017

I. Introduction

Eclipta alba L. Hassk. (Asteraceae), commonly known as Bhringaraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice [1]. The plant will be used for the treatment of liver cirrhosis and infective hepatitis [2]. The medicinal properties of plants have been investigated in the recent scientific studies throughout the world, because of their potent antioxidant activities, there will be no side effects and economic viability [3]. In Ayurveda, extract of the root powder is used for treating hepatitis, and enlarged spleen disorders. In most of the parts of India, it grows commercially as a medicinal crop. It is an annual, erect or prostate entirely pubescent herb, often rooting at nodes with opposite, sessile, usually oblong, 2.5–7.5 cm long leaves with appressed hairs. Floral heads 6–8 mm in diameter, solitary, white: achene compressed and narrowly winged. The aerial parts of the plant are used in medicine like Ayurveda, Siddha.

In another study, the areal parts and root of alcoholic extraction exhibited significant Hepatoprotective activity against CCl4-induced liver injury in rats. In yet another study, treatment with 50% ethanol extract of E.alba (100 & 250mg/100g body weight) was found to protect the mice from hepatotoxic action of paracetamol as evidenced by significant reduction in the elevated serum transaminase levels [4].

The total alcoholic extract of E. prostrata exhibited a dose-dependent activity in albino rats when compared to standard drugs. The activity was assessed by studying the lipid profiles of serum, liver and heart of the control and drug-treated animals Two studies reported efficacy of E.alba in the treatment of infective hepatitis in adults and jaundice in children, respectively [5-7]. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary
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healthcare needs [8]. Phytochemicals are naturally occurring biochemical compounds in plants for colour, flavour, smell and texture for pollination and define mechanism [9]. The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. This has led to their widespread adoption in a number of fields. In this context, our study was aimed to investigate the phytochemical compositions of methanol extract of Eclipta Alba root through GCMS analysis.

II. Material And Methods

Collection and extraction of plant materials

Fresh plant parts (Including roots) collected from adjoining village area of Chitradurga city and taxonomic identity of the plant was confirmed in the Department of Botany, Govt Science College, Davangere University, and Chitradurga. The mature plants were washed thoroughly with running tap water, then with deionised water (Fig 1). Plant material (Roots) was extracted with 250 ml (1:50, w/v) of solvent; 99% methanol. For methonolic extract dried powder samples were extracted in a Soxhlet apparatus for 5 – 6 hrs, until becoming colourless. The extract was filtered. The filtrate was centrifuged at 1500 rpm for 20 minutes to remove any plant debris. Supernatants were stored at 22 °C until assayed. It is packed in food grade, virgin, polythene bags.

GC-MS Analysis:

Sample preparation: About 1 g of Eclipta alba root extract was taken in vial and 5 ml of methanol added. The sample was sonicated for 15 mins and supernatant layer taken for GC-MS analysis (Fig 2).

Column: Restek Rtx-5 capillary column, length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 μm.

Column programming: Rate of heating - 10 °C/min, temperature - 60 °C & 330 °C and Hold time 0 min &10 min


III. Results

The identification of phytochemical compounds is based on their retention time (RT), molecular formula, molecular weight (MW), chemical structure and concentration (peak area %). GC-MS chromatogram of roots of E. alba analysis showed the presence of 25 Chemical compounds Table 1.

IV. Discussion

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species [10]. Hence, in the present study phytochemical screening of E.alba was carried out, qualitative phytochemical analysis of this plant confirms the presence of bio active compounds.

The results of the phytochemical analysis of the different alcohol and aqueous plant extract of Eclipta alba mostly contained higher quantities of glycosides, followed by flavonoids and alkaloids. Alkaloids and tannins were entirely absent in most of the tested aqueous extracts. The Phytochemical screening of the alcohol and aqueous extracts of Tylophora indica revealed the presence of glycosides, alkaloids, flavanoids and tannins in all the plant extracts studied. [11].

The metabolites are of various pharmacological im-portance. The presence of tannin in most of plant extract could be responsible for possible antitumor and anti oxidant activities [12]. Thenmozhi et al., (2011) identified the phytochemicals present in methanol extracts of Eclipta alba.

The results of methonolic extracts of E. alba leaves clearly implies that the strength of active principle depends upon the use of solvent besides the type of plant species to achieve the positive results. The identified phytochemical compounds have many biological properties. For instance, Oleic acid, eicosyl ester reported to contain anti-inflammatory, cancer preventive, dermatitigenic, Hypocholesterolemic and anemiagenic Insectifuge. 1-Heptatriacotanol is an alcoholic compound which showed antimicrobial activity.

Previous studies reported that the phytochemical studies of E. alba using methanol solvent yielded eleven bio active compounds, which are The active compounds shows the presence of eight possible bio active compounds Tridecanol, 2-ethyl-2-methyl, 1-Heptatriacotanol , c-Sitosterol, Oleic acid, eicosyl ester, 9,19-Cyclocholestan-3-ol-7-one,4a-dimethy-[20R], 10-Octadecenoic acid, methyl ester, 1,2 Benzenedicarboxylic
acid, butyl octy ester, Dodecanic acid, 10 methyl, methyl ester 11, whereas the current study showed seven compounds c-Sitosterol, Glycine, N[(3a,5a,12a)]-3,12-dihydroxy 24-oxocholan-24-yl]-, Oleic acid, eicosyl ester, Ethanol, 2-(9,12-octadecadienol)-, (ZZ), 10-Octodeconic acid, methyl ester, Pentadecanic acid,14methyl,1methyl ester, Diethyl Phthalate which are divergent. The identified phytochemical compounds have many biological properties. For instance, Oleic acid, eicosyl ester IS reported to contain anti-inflammatory, hepatoprotective, cancer preventive, dermatogenic, hypcholesterolemic properties [13].

In our Research we found the presence of 25 phytochemical compounds, which are 2(3H)-Furanone, 5-acetyldihydro-, -Pentenoic acid, 4-methyl-3-4methylene-, isopropyl ester, 2-Hexene, 2-methyl- (CAS) 2-Methyl-2-hexene, Phenol, 4-ethyl-2-methoxy-, Phenol, 2-methoxy- (CAS) Guaiacol, Acetic acid, pentyl ester (CAS) n-Amyl acetate, Pentanoic acid, 4-oxo-(CAS) Levulinic acid, Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 4H-Pyrane-4-one, 3-hydroxy-2-methyl- (CAS) Maltol, N,N'-Dimethylpiperazine, 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one, Phenol, 2,3,5-trimethyl- (CAS) 2,5-Trimethylphenol, Cyclopentane, 1-acetyl-1,2-epoxy-, Cyclopropyl carbinol, 2,3-DIHYDRO-BENZOFURAN, 2-Hydroxy-gamma-butyrolactone, Phenol (CAS) Izal, 4H-Pyrane-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1H-Imidazole, 4,5-dihydro-2-methyl-, Pentanoic acid, 4-oxo- (CAS) Levulinic acid, 2-Cyclopenten-1-one, 2-hydroxy, 2-Furancarboxaldehyde, 5-(hydroxymethyl, 1,2-Benzenediol (CAS) Pyrocatechol and D-Allose.

Through our study, the presence of various phytochemical compounds justifies the use of the A. alba root for various ailments by traditional practitioners. Isolation of these compounds was supportive for new drugs to treat various diseases. Therefore, it is recommended as a plant of phytopharmaceutical importance. Further investigation of the plant with various solvents can increase the isolation of the newer molecules which will be helpful for the study of the pharmacological activities and in discovering drugs from the plant which may prevent the human and the economic losses in the environment.

V. Conclusions

The presence of various bioactive compounds justifies the use of the plant leaves for various ailments by traditional practitioners. On the basis of the results obtained, the present work conclude that the roots of Eclipta Alba are also rich in phytochemical constituents even though the phytochemical screening of the root extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous and methanolic extracts of roots. The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, Saponins, phenols and flavanoids are believed to exhibit the antibiotic, antibioste and hepatoprotective properties in Eclipta Alba roots. The present work highlights the potential use of Eclipta Alba root extracts as a medicine with hepatoprotective and antibioste properties.

Acknowledgements

The authors sincerely thank to Mrs. N. Shankaramma, Associate Professor, Department of Botany, Govt Science College, Chitradora, Karnataka for authentication of the plant, Vittal Mallya Scientific Research Foundation, Bangalore, India, for providing the laboratory facilities for GC-MS Analysis of Eclipta alba and also faculty of the Department of anatomy for providing constant support.

References

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Figure 1: Eclipta alba plant

Figure 2: GCMS analysis equipment

Table 1: Phytochemical components of Eclipta alba root extract by GC-MS Analysis

<table>
<thead>
<tr>
<th>Peak</th>
<th>R. Time</th>
<th>L. Time</th>
<th>F. Time</th>
<th>Name of the Compound</th>
<th>Peak Area%</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>6.564</td>
<td>6.542</td>
<td>6.592</td>
<td>2(3H)-Furanone, 5-acetyldihydro- trioxide</td>
<td>0.09</td>
<td>128</td>
</tr>
<tr>
<td>21</td>
<td>10.291</td>
<td>10.250</td>
<td>10.325</td>
<td>Pentenoic acid, 4-methyl-3,4-methylene-, isopropyl ester</td>
<td>0.14</td>
<td>168</td>
</tr>
<tr>
<td>23</td>
<td>11.037</td>
<td>11.008</td>
<td>11.058</td>
<td>2-Hexene, 2-methyl- (CAS) 2-Methyl-2-hexene</td>
<td>0.24</td>
<td>98</td>
</tr>
<tr>
<td>19</td>
<td>9.233</td>
<td>9.192</td>
<td>9.283</td>
<td>Phenol, 4-ethenyl-2-methoxy-</td>
<td>0.27</td>
<td>150</td>
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<tr>
<td>10</td>
<td>6.067</td>
<td>5.958</td>
<td>6.108</td>
<td>Phenol, 2-methoxy- (CAS) Guaiacol</td>
<td>0.30</td>
<td>124</td>
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<tr>
<td>6</td>
<td>5.450</td>
<td>5.417</td>
<td>5.483</td>
<td>Acetic acid, pentyl ester (CAS) n- Amyl acetate</td>
<td>0.32</td>
<td>130</td>
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<tr>
<td>14</td>
<td>6.711</td>
<td>6.675</td>
<td>6.758</td>
<td>Pentanoic acid, 4-oxo- (CAS) Levulinic acid</td>
<td>0.33</td>
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<tr>
<td>20</td>
<td>9.710</td>
<td>9.675</td>
<td>9.750</td>
<td>Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol</td>
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<td>8</td>
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<td>5.683</td>
<td>2,5-Dimethyl-4-hydroxy-3(2H)-furane</td>
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<td>12</td>
<td>6.411</td>
<td>6.375</td>
<td>6.458</td>
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<td>0.36</td>
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<tr>
<td>5</td>
<td>3.381</td>
<td>3.342</td>
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<tr>
<td>24</td>
<td>11.449</td>
<td>11.417</td>
<td>11.483</td>
<td>4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one</td>
<td>0.42</td>
<td>190</td>
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<td>22</td>
<td>10.919</td>
<td>10.883</td>
<td>11.000</td>
<td>Phenol, 2,3,5-trimethyl- (CAS) 2,3,5-Trimethylphenol</td>
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</tr>
<tr>
<td>9</td>
<td>5.926</td>
<td>5.883</td>
<td>5.958</td>
<td>Cyclopentane, 1-acetyl-1,2-epoxy-</td>
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<tr>
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<td>6.108</td>
<td>6.192</td>
<td>Cyclopentanol</td>
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<td>7.725</td>
<td>7.908</td>
<td>2,3-DIHYDRO-BENZOFURAN</td>
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<tr>
<td>4</td>
<td>4.700</td>
<td>4.575</td>
<td>4.733</td>
<td>2-Hydroxy-gamma-butyrolactone</td>
<td>1.01</td>
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<tr>
<td>3</td>
<td>4.530</td>
<td>4.483</td>
<td>4.575</td>
<td>Phenol (CAS) Izal</td>
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<td>15</td>
<td>6.851</td>
<td>6.808</td>
<td>6.892</td>
<td>4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-</td>
<td>1.15</td>
<td>144</td>
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<tr>
<td>1</td>
<td>3.760</td>
<td>3.692</td>
<td>3.833</td>
<td>1H-Imidazol, 4,5-dihydro-2-methyl-</td>
<td>1.53</td>
<td>84</td>
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IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.