**GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF A SIDDHA POLY HERBAL DRUG ADATHODAI CHOORANAM**

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**ABSTRACT**

Adathodai chooranam is a Siddha Sastric poly herbal formulation which contains seventeen herbal ingredients including *Adathoda vasica* root bark and inflorescence. It is indicated for the treatment of Eraippu Noi (Bronchial Asthma) in Siddha Classical Text. The aim of the present study was to identify the bioactive compounds of the drug using the instrument Agilant 6890N Gas Chromatography equipped with JEOLGC MATE-II HR - mass spectrometer. Under GC-MS Study, the Adathodai Chooranam found to have 1,2 - benzenedicarboxylic acid, butyl 2-methyl propyl ester, 8-octadecenoic acid, methyl ester (E), Heptadecanoic acid, 16-methyl, methyl ester, (E) - 9 - octadecenoic acid ethyl ester, methyl tetradecanoate. The components possess antioxidant, anti microbial and anti inflammatory property. These properties of the compounds in Adathodai chooranam contributes its therapeutic effect in Bronchial Asthma.

**Keywords:** Siddha Medicine, poly herbal drug, Adathodai Chooranam, Bronchial Asthma, Gas Chromatography - Mass Spectroscopy.

**INTRODUCTION**

Plants are a rich source of Secondary Metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. Volatiles play a vital role in health care systems of Medicinal Plants. Volatiles can be identified by GC-MS analysis. Gas Chromatography (GC/MS) is normally used for analysis of components existing in traditional medicines and medicinal plants. Recently GC-MS Studies have been increasingly applied for the analysis of Medicinal Plants as this technique has proved to be a valuable tool in biological and chemical studies. The present study is carried out to find out the bioactive chemical constituents from the Adathodai chooram by GC-MS analysis which is an advanced and accurate one to find out the bioactive compounds.

**MATERIALS AND METHODS**

The drug Adathodai Chooranam was prepared as per the Siddha Sastric Text. The GC-MS Analysis was conducted at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Chennai. The GC-MS Analysis was performed using the instrument Agilant 6890N gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. Mass Spectrometry in conjunction with Gas Chromatography has been reported to be a powerful tool in biological and chemical studies. The JEOL GC MATE II GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI).

**Applications**

1. Structural elucidation of organic compounds.
2. Mechanistic study of fragmentation process under mass spectrometric condition.
3. Molar mass and structural analysis of small biomolecules.

**GC-MS Procedure**

Gas chromatography (GC) analysis was done using Agilant 6890N gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted in DB 5 MS capillary column (30 m ×0.25 mm i.d., film thickness 0.25 µm). The injection temperature was set at 280 °C, and the oven temperature was 45 °C then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas in the flow rate of 1.0 ml/min. One microlitre of the sample (diluted with acetone 1:10) was injected to the split mode in the ratio of 1:100. The percentage of composition of the essential oil was calculated using the GC peak areas. GC-mass spectrometry (GC-MS) analysis of essential oil was conducted using Agilant gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. GC conditions were the same as reported to GC analysis and the same column was used. The mass spectrometer was started in the electron impact mode at 70 eV. Ion source and transfer line temperature was set at 250 °C. The mass spectra were obtained by centroid scan of the mass ranges from 40 to 1000 amu. The compounds were found based on the comparison of their retention indices (RI), retention time (RT), mass spectra...
RESULTS AND DISCUSSION

In the present study five chemical compounds have been identified. They were 1,2-benzenedicarboxylic acid, butyl 2-methyl propyl ester, 8-octadecenoic acid, methyl ester (E), Heptadecanoic acid, 16-methyl, methyl ester, (E)-9-Octadecenoic acid ethyl ester, Methyl tetradecanoate. The active principles with their retention time and peak area\% were tabulated in Table 1. The Gc-MS graph is given in Graph 1. 1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester showed α-Glucosidase inhibition and the in-vivo hypoglycemic effect and antimicrobial activity. 8-Octadecenoic acid, methyl ester, (E) showed Antioxidant and Antimicrobial Activity. Heptadecanoic acid, 16 methyl; methyl ester possess the property of antioxidant and anti microbial. Heptadecanoic acid also had the hypocholesterolemic lubricant, anti androgenic, hemolytic 5 - Alpha reductase inhibitor activity. (E)-9-Octadecanoic acid ethyl ester showed anti inflammatory, Cancer preventive, dermogenic hypocholesterolemic, 5-Alha reductase inhibitor and anti androgenic activity. Methyl tetradecanoate showed Antioxidant, Cancer preventive, hypocholesterolemic, lubricant, Nematinocide.

Table 1: Result of Gc-MS Study of Adathodi Chooranam

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Formula</th>
<th>Molecular weight</th>
<th>Retention time</th>
<th>% Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester</td>
<td>C₁₆H₂₂O₄</td>
<td>278.3435</td>
<td>15.89</td>
<td>12.93</td>
</tr>
<tr>
<td>8-Octadecanoic acid, methyl ester, (E)</td>
<td>C₁₉H₃₆O₂</td>
<td>296.49</td>
<td>17.13</td>
<td>43.10</td>
</tr>
<tr>
<td>Heptadecanoic acid, 16-methyl, methyl ester</td>
<td>C₁₉H₃₆O₂</td>
<td>298</td>
<td>17.36</td>
<td>8.62</td>
</tr>
<tr>
<td>(E)-9-Octadecanoic acid ethyl ester</td>
<td>C₂₀H₃₈O₂</td>
<td>310</td>
<td>17.75</td>
<td>17.24</td>
</tr>
<tr>
<td>Methyl tetradecanoate</td>
<td>C₁₅H₃₀O₂</td>
<td>242.3975</td>
<td>15.39</td>
<td>18.10</td>
</tr>
</tbody>
</table>

Figure 1: 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester

Figure 2: 8-Octadecenoic acid, methyl ester, (E)
Figure 3: Heptadecanoic acid, 16-methyl, methyl ester

Figure 4: (E)-9-Octadecenoic acid ethyl ester

Figure 5: Methyl tetradecanoate
CONCLUSION

The bioactive compounds identified by GC-MS in the trial drug Adathodai Chooranam are 1, 2 - benzenedicarboxylic acid, butyl 2-methyl propyl ester, 8-octadecenoic acid, methyl ester (E), Heptadecanoic acid, 16-methyl, methyl ester, (E) - 9 - octadecenoic acid ethyl ester, methyl tetradecanoate. These components possess antioxidant, anti microbial and anti inflammatory behavior. So the results indicate the poly herbal formulation Adathodai Chooranam has potential antioxidant, antimicrobial and anti inflammatory properties for the treatment of Bronchial Asthma.

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