Estimation of Phytoconstituents from \textit{Citrullus colocynthis} (L.) Schrad Roots Extract by GC-MS Spectroscopy

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Abstract: \textit{Citrullus colocynthis} (L.) Schrad (family Cucurbitaceae) is widespread annual uncultivated plant grows fast in sandy soil. The present study was undertaken on the roots of this plant to investigate the possible bioactive phytoconstituents using gas chromatography-mass spectrometry (GC-MS) analysis. GC-MS analysis of hydro alcoholic extract from roots has led to the identification of 57 compounds by comparison of their retention indices and mass spectra fragmentation patterns with those stored in the GC-MS computer library. The main constituents identified as glycerol, 2-methoxy-4-vinphenol, 1-pentadecanol, pluchidiol, myristic acid, oleic acid, codein, beta-codein, morphine, thebaol, thebaine, dimethylmorphine, 1-(3,4-dimethoxybenzyl)-6,7-dimethoxysisquinoline, protopine, kryptopine, narcine, alpharnocotine, methyl-3-hydroxycholest-5-en-26-oate, sebacic acid, 2,7-dimethyllocta-7-en-5-yn-4-yl heptyl ester, verticil etc. In spite of its high medicinal value, the GC/MS analysis of its root part is not reported earlier.

Keywords: Gas Chromatography-Mass Spectroscopy (GC-MS), \textit{Citrullus colocynthis} (L.) Schrad, Cucurbitaceae, Phytoconstituents

1. Introduction

\textit{Citrullus colocynthis} (L.) Schrad (Family: Cucurbitaceae) is perennial herbs usually trailing[1]. It is commonly known as the colocynth[2], bitter apple[1,3], bitter cucumber, english colocynth, bitter gourd, wild gourd[4] etc. It resembles a common watermelon vine, but bears, small, hard fruit with a bitter pulp. \textit{Citrullus colocynthis} (L.) Schrad is commonly found wild in the sandy lands of North West, the Punjab, Sind, and Central and southern India, and coromandial coast[1,5]. Also found indigenous in Arabia, West Asia, and Tropical Africa and in the Mediterranean region[3,4]. \textit{Citrullus colocynthis} (L.) Schrad has the traditional use in remedy for cancer, carcinoma, endotheioma, leukemia, tumors of the liver and spleen, even the eye. A decoction of the whole plant, made with juice of fennel is said to help induations of the liver. Roots may also be used as a purgative against as cistes for jaundice, urinary diseases, rheumatism and for snake poison[3,4,6]. \textit{Citrullus colocynthis} (L.) Schrad is widely used in folk medicine for centuries and as an energy source also. e.g. oilseed and biofuel[6,7]. In spite of its high medicinal value, the GC/MS analysis of its root part is not reported earlier. Gas chromatography-mass spectrometry (GC-MS) is a technique that combines the features of gas-liquid chromatography and mass spectrometry to recognize different substances within a test sample [8,9].

2. Materials and Methods

\textit{Citrullus colocynthis} (L.) Schrad whole plants were collected from widely grown region of Southern Haryana in the month of June 2015. The plant was taxonomically identified and authenticated by Dr. Anjula Pandey, Principal Scientist Raw Materials, Herbarium and Museum Division, NISCAR, New Delhi, vide reference number NHCP/NBPR/2016-14 as (\textit{Citrullus colocynthis} (L.) Schrad) family Cucurbitaceae dated on 17 March, 2016. A voucher specimen of the same has been retained in the department for the future reference. \textit{Citrullus colocynthis} (L.) Schrad roots were used to carry out the experimental work.

Preparation of Extracts
The shade dried roots of \textit{Citrullus colocynthis} (L.) Schrad were crushed and coarsely grind[10]. Then sample was kept in a four necked round bottom flask with solvent ethanol (50%) and extracted in U-Wave 1000 Microwave synthesis reactor (SINEO Microwave Chemistry Technology, China) at Power-time mode[11]. The instrument operates at an input power of 2000W with operating frequency of 2450MHz and works at atmospheric pressure. The real time temperature was monitored by high precision platinum resistance temperature sensor. The flask was connected to outside condenser through a glass connecting tube (19mm U) and a X shaped tube. The pulverized drug was extracted at different operating conditions (Microwave power, ethanol concentration (%)) and different volume of solvent/g of drug) as suggested by experimental design. The extracts obtained by different techniques were cooled for 5 min before filtration. Further the extracts was filtered and concentrated under reduced pressure by a rotary evaporator at 60°C. The experiment was conducted in triplicate and percentage yield (w/w) was determined. The extracts were kept in a desiccator before further analysis[12].

GC-MS analysis
The extract was directly used for the analysis. GC-MS analysis was carried out on a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and
auto injector (AOC-20i), equipped with mass selective
detector, having ion source temperature of 230°C, interface
temperature of 270°C, a solvent cut time of 3.50 min, detector
gain mode relative, threshold of 1,000 and mass range of 40
to 650 m/z. Compounds were separated using a Rtx 5 MS
capillary column (Restek Company, Bellefonte, USA; crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having
dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 μm
(film thickness). The split mode was used at a ratio of 10:1.
The temperature of the injector was initialized to 260°C,
having a split injection mode, pressure 69.0 kPa. The
temperature was programmed from 50°C (3 min), then further
increased to 280°C at a rate of 10°C/min (24 min hold).
Helium (>99.999%) was used as the carrier gas at a linear
flow velocity of 39.9 cm/s with constant flow of 1.21 mL/min
and an injection volume of 1.0 μL was employed. The
chemical constituents were identified by comparison of their
retention indices (RI) relative to homologous alkane series
(purchased from Sigma, St. Louis, USA) and by comparison
of their mass spectral fragmentation patterns with those data
provided in WILEY8.LIB, NIST08.LIB, NIST08s.LIB and
NIST.LIB. Identification was assumed when a good match of
mass spectrum and RI was achieved [13,14].

3. Results

![Figure 1: GC-MS Chromatogram of Citrullus colocynthis L. Schrd. root extract](image)

<table>
<thead>
<tr>
<th>Peak</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area %</th>
<th>Name</th>
<th>Molecular Weight</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.760</td>
<td>4769572</td>
<td>5.54</td>
<td>Glycerol; 1,2,3-Propanetriol</td>
<td>92</td>
<td>C3H8O3</td>
</tr>
<tr>
<td>2</td>
<td>12.892</td>
<td>1421572</td>
<td>1.65</td>
<td>2-Methoxy-4-vinylphenol</td>
<td>150</td>
<td>C9H10O2</td>
</tr>
<tr>
<td>3</td>
<td>13.995</td>
<td>1427758</td>
<td>1.66</td>
<td>DL-Proline, 5-oxo-methyl ester</td>
<td>143</td>
<td>C6H11NO3</td>
</tr>
<tr>
<td>4</td>
<td>14.859</td>
<td>166312</td>
<td>0.19</td>
<td>Dodecane, 1-chloro-</td>
<td>204</td>
<td>C12H25Cl</td>
</tr>
<tr>
<td>5</td>
<td>14.924</td>
<td>274928</td>
<td>0.32</td>
<td>1-Dodecanol</td>
<td>186</td>
<td>C12H26O</td>
</tr>
<tr>
<td>6</td>
<td>15.448</td>
<td>93934</td>
<td>0.11</td>
<td>7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl-</td>
<td>226</td>
<td>C17H25O2</td>
</tr>
<tr>
<td>7</td>
<td>17.346</td>
<td>281388</td>
<td>0.33</td>
<td>1-Pentadecanol</td>
<td>228</td>
<td>C17H31O</td>
</tr>
<tr>
<td>8</td>
<td>18.325</td>
<td>1268482</td>
<td>1.47</td>
<td>Tetradecanoic acid; Myristic acid</td>
<td>228</td>
<td>C18H36O2</td>
</tr>
<tr>
<td>9</td>
<td>18.64</td>
<td>229405</td>
<td>0.27</td>
<td>6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a tetrahydrobenzofuran-2(4H)-one</td>
<td>196</td>
<td>C16H26O3</td>
</tr>
<tr>
<td>10</td>
<td>18.778</td>
<td>415778</td>
<td>0.48</td>
<td>Pluchadiol</td>
<td>208</td>
<td>C14H26O2</td>
</tr>
</tbody>
</table>
Dimethylmorphine; 7,8-dihydroxy-1,5,6-dimethoxyisoquinoline

Myristic acid

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Oleic acid

Codein

Thebaine

Hexadecanoic acid, methyl ester

Dimethylmorphine

cis-3,14-Clerodadien-13-ol

Beta Codein

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4. Conclusions

The GC/MS investigation led to the identification of 57 constituents components in the roots of plant *Citrullus colocynthis* L. Schrd. Table 1. revealed the important bioactive constituents presumed to be responsible for eliciting the traditional activity of this plant. The results revealed the major compounds are fatty acid esters and alkaloids which showed antioxidant, antimicrobial, anticancer, antineuropathic, anti-inflammatory activities of *Citrullus colocynthis* L. Schrd roots.

References


