Identification of Chemical Compounds from the *Cassia sophera*

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**ABSTRACT**

*Cassia sophera* Linn. (Caesalpinaceae), an important drug in Unani medicine, act as a blood purifier, carminative, purgative, digestive and diaphoretic. A number of compounds have been reported from plants belonging to genus Cassia. The chemical analysis of *Cassia sophera* Linn seed revealed the presence of ascorbic acid, dihydroascorbic acid and β-sitosterol. Novel anthraquinone sopheranin was isolated from the heartwood of *Cassia sophera* along with β-sitosterol, chrysophenol, physcion and emodin. A new cycloartane triterpene glycoside named cyclosphoroside A was isolated from the seeds of *Cassia sophera*. The present study aim to identify the constituents present in the *Cassia sophera* leaves. Seven chemical compounds were isolated from the leaves of *Cassia sophera* Linn and analysed by GC-MS. The identified compounds are (1) Butanedioic Acid, (2) 1, 2, 4-Butanetriol Triacetate, (3) 7-Hexadecene, (4) E-15-Heptadecenal, (5) 1, 2-Benzenedicarboxylic acid, (6) 3-Eicosene, and (7) 10-Heneicosene with retention time are 15.99, 13.763, 19.93, 18.03, 11.4, 16.70 and 9.54 respectively.

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Introduction

Cassia sophera Linn. (Caesalpinaceae), known as ‘Kasondi’, is used as a drug in Unani medicine. ‘Kasondi’ is described in Unani literature as able to combat morbid humors (specially phlegm), act as a blood purifier, carminative, purgative, digestive, and diaphoretic and has also been reported as being useful for the treatment of epilepsy, ascites, skin disorders, piles, jaundice, fever, articular pain and palpitation [1,2]

Cassia sophera Linn. is known as “Kasmard” in Ayurvedic literature it means cough suppressant (Kas - cough; Mard - to protect) [3]. In folk literature it is used in asthma as expectorant, GIT disorder, and rheumatic disorders. It is reported to be used in homoeopathy; decoction of plant is used as antidiuretic. The juice made with the paste of sandal wood and lime juice is considered specific for ring worm. An infusion of leaves is given with sugar in jaundice, and in sub acute stage of gonorrhea. It is used to as febrifuge in rheumatic and inflammatory fever. However it is also used in some immunomodulatory preparations of homeopathy and Ayurveda [4].

Cassia sophera Linn has been reported for anti-inflammatory and analgesic activity [5]. Aqueous extract of leaves of Cassia sophera showed significant hepatoprotective activity against ranitidine induced hepatic damage [6]. The chemical analysis of Cassia sophera Linn seed revealed the presence of ascorbic acid, dihydroascorbic acid and β-sitosterol. A number of compounds have been reported from plants belonging to genus Cassia. Novel anthraquinone sopheranin has been isolated from the heartwood of Cassia sophera along with β-sitosterol, chrysophenol, physcion and emodin [7]. A new cycloartane triterpene glycoside named cyclosphoroside A was isolated from the seeds of Cassia sophera [8]. Chemical examinations of the flowers of Cassia sophera was carried out [9].

Materials and Methods

Preparation of methanolic extract of Cassia sophera leaves

The leaves of Cassia sophera were collected from Valsad, India. A herbarium specimen (Voucher specimen No.12) was authenticated by Prof. (Dr.) M. Parabia, Veer Narmad South Gujarat University, Surat. The dried and powdered leaves of C. sophera were soaked in methanol for 12 h. To remove the sediments and traces of water, the leaves methanol solution filtered through whatmann filter paper in 2 gm of sodium sulphate. Before filtration, the filter paper along with sodium sulphate wetted with ethanol. The filtrate was concentrated by bubbling nitrogen gas into the solution. The extract contains both polar and non polar phytocomponents. A weighed quantity of extract was suspended in 1% CMC (carboxy methyl cellulose) for oral administration to the animals to evaluate the acute toxicity.
Experimental/ Methodology

Acute oral toxicity

Acute oral toxicity study was investigated as per the OECD-423 (2001) guidelines, i.e. the acute toxic class method. Female Wistar rats (n =3) selected by a random sampling technique were used for the study [10, 11, 12]. All animal experiments were carried out in accordance with the guidelines of CPCSEA and were approved by the Institutional Animal Ethical Committee. The animals were acclimatized for 5 days and fasted overnight with access only to water, after which the methanol extract of Cassia sophera leaves was administered orally at a dose of 2000 mg/kg body using a feeding needle and observed for 14 days. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose [13].

Phytochemical evaluation

A phytochemical evaluation of methanol extract of the Cassia sophera leaves was carried out by qualitative chemical testing [14, 15].

Separation of volatile components

Methanol extract of Cassia sophera leaves was subjected to column chromatography over silica gel and eluted with a gradient of hexane: ethyl acetate (10:0). Collected volatile components are further separated and analyzed by GC-MS.

GC-MS analysis

GC MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following condition:-

Column Elite-1 fused silica capillary column (30 X 0.25 mm ID X 1 um df, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70 eV; helium (99.99%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 μl was employed (split ratio 10:1) injector temperature 250° C; ion source temperature 280° C, ending with a 9 min isothermal at 280° C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40-450 Da. Total GC running time is 30 min.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared
with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and discussion

Acute toxicity

The extracted compound from *C. sophera* were analysed for the toxicity. Acute toxicity studies revealed the non-toxic nature of the methanol extract of *C. sophera*. There were no deaths and no toxic reactions in the female rats following administration of the extract at a dose of 2000 mg/kg body weight.

Isolation and Identification of chemical constituents by GC-MS

Seven compounds were identified in *Cassia sophera* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Figure 1).

Table 1: Components identified in hexane fraction of *Cassia sophera* leaves

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>RT</th>
<th>Name of the Compound</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.54</td>
<td>Butanedioic Acid, Hydroxy-Diethyl ether (+/-)</td>
<td>C₈H₁₄O₅</td>
<td>190</td>
<td>1.16</td>
</tr>
<tr>
<td>2</td>
<td>11.4</td>
<td>1,2,4-Butanetriol, Triacetate</td>
<td>C₁₀H₁₆O₆</td>
<td>232</td>
<td>7.56</td>
</tr>
<tr>
<td>3</td>
<td>13.76</td>
<td>7-Hexadecene (z)-</td>
<td>C₁₆H₃₂</td>
<td>224</td>
<td>2.88</td>
</tr>
<tr>
<td>4</td>
<td>15.99</td>
<td>E-15-Heptadecenal</td>
<td>C₁₇H₃₂O</td>
<td>252</td>
<td>5.92</td>
</tr>
<tr>
<td>5</td>
<td>16.70</td>
<td>1,2-Benzenedicarboxylic acid, Butyl octyl ester</td>
<td>C₂₀H₃₀O</td>
<td>334</td>
<td>7.69</td>
</tr>
<tr>
<td>6</td>
<td>18.03</td>
<td>3-Eicosene (E)-</td>
<td>C₂₀H₄₀</td>
<td>280</td>
<td>6.9</td>
</tr>
<tr>
<td>7</td>
<td>19.94</td>
<td>10-Heneicosene (C, T)</td>
<td>C₂₁H₄₂</td>
<td>294</td>
<td>2.95</td>
</tr>
</tbody>
</table>
The prevailing compounds were (1) E-15 Heptadecenal (15.99), (2) 7 Hexadecene (13.763), (3) 10-Heneicosene (19.93), (4) 3-Eicosene (18.03), (5) 1,2,4-Butanetriol Triacetate (11.4), (6) 1,2-Benzene dicarboxylic acid (16.70) and (7) Butanedioic acid (9.54).

**Figure 1:** GC-MS spectrum of hexane fraction of *Cassia sophera* leaves

**Table 2:** Profile of qualitative phytochemical evaluation of methanol extracts of *Cassia sophera*.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Phytochemical test</th>
<th>ME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>i) Anthraquinone</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>ii) Cardiac</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>iii) Coumarins</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>iv) Cyanogenetic</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponin glycosides</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids and Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Tannins and Phenolics</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Mucilage</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Gums</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Fixed oils</td>
<td>-</td>
</tr>
</tbody>
</table>
Preliminary phytochemical investigation

A phytochemical analysis was carried out to identify the presence of bioactive substances. The results obtained are summarised in Table 2. The methanol extract under investigation contained mainly tannins, flavonoids volatile oil and carbohydrates.

Conclusion

The present investigation has helped to identify the compounds present in the leaves of Cassia sophera Linn an uninvestigated species. The presence of large number of long chain hydrocarbons is common in the leaves of tropical plants, which to some extent lower the rate of transpiration.

Authors’ Statements

The authors declare no conflict of interest.

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References


