Preliminary GC-MS analysis of an Ayurvedic medicine “Kulathadi Kashayam”

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ABSTRACT

Kulathadi kasahaym is an ayurvedic formulation used for treatment of Amenorrhoea. The main ingredients of this medicine seeds of horse gram, sesame, errand and garlic. This is a liquid formulation. GC MS analysis of this medicine is done in the present study to know the various bioactive compounds present therein. It was found that major peaks represented Benzoic acid, Diethyl Phtha late, Tetratricontane, Octadecanoic acid, n-Hexadecanoic acid, Heptacosane 1-Chloro, Eicosane, Phenol, 2,4-bis(1,1-dimethylethyl)-, Eicosane, 2-methyl-, Nonadecane, 2-methyl-, Hexadecane 2,6,11,15-tetramethyl-, Heptadecane, 2,6,10,15-tetramethyl- and Eicosane, 10-methyl- derivative. The medicinal values of most of these compounds indicate that this medicine does have a scientific indication as an effective cure for Ammenorrhorea. Further work is in progress to prove the validity of this medicine.

Key words: Kulathadi Kashayam, GC MS, horse gram, Garlic, Errand, Sesame, Ammenorrhorea, Benzoic acid.

INTRODUCTION

The use of contemporary and alternative medicines among the population the world over is time tested. Ayurveda and Sidha in India are age old systems of treatment. The Ayurvedic formulations in the form of solids, semi solids, liquids, pastes and powders are in use for more than 3000 years in India. But these medicinal practices are not getting their due in the international scenario of Medical practice due to lack of scientific evidence and proven efficacy. Some of the factors for this state of Ayurveda and Sidha medical system is discussed by Raj et al, 2011; Rao et al, 2015a, b; Ravi et al, 2015). [1, 2, 3, 4] Pordie’ and Gaudillie’re 2014, have discussed in details about the factors involved in polyherbal formulation in drug discovery and related issues pertaining to Ayurveda as an industry. [5] The present article tries to understand the presence and role of the bioactive compounds present in one such liquid Ayurvedic formulation, namely, Kukathadi Kashayam by GC MS analysis.

Kulathadi kashayam is an Ayurvedic formulation to treat Amenorrhoea and Cryptomenorrhoea. This formulation is made up the following ingredients:
1. Kulatha - Horse gram (*Dolichos biflorus*) seeds
2. Lahsuna - Garlic (*Allium sativum*)
3. Eranda - *Ricinus communis* (seeds)
4. Tila - *Sesamum indicum* (Seeds)

One part each of the above mentioned ingredients are made to coarse powder and mixed thoroughly. One part of this powder is boiled in 16 parts of water till the volume reduces to 4 parts. This is Kulathadi kashayam which is bottled, sealed and used as medicine. The dose is 5 to 10 ml before food in empty stomach once or twice or as advised by the medical practitioner. This formulation is prepared according to the Ayurvedic treatise, Yogagrantha. The manufacturer of this formulation is Arya Vaidya Sal, Kottakkal, Kerala State, India. The four plant ingredients which are used for the preparation of this formulation have many medicinal values. Some of the medicinal properties of each plant are discussed in the following paragraphs.

*Dolichos biflorus*
This is common vegetable of India belonging to Leguminosae family. The antilithiatic activity of the aqueous extracts of the seeds is a well known folklore medicine in India. The seeds contain a dimeric protein of 98 kDa molecular weight which is a crystal growth inhibitor. (Bijamia *et al.*, 2009). [6] Atodariya *et al.*, 2013 have reported that the aqueous fractions of the seeds have very good capacity to dissolve calcium oxalate crystals. [7] The hypolipidemic role of the seed extract is shown in rats by Menaka and Helitha, 2013. [8]The seed extract is antihistamic (Sularkar *et al.*, 2013 a, b) and the seed lecithin has lipooxygenase activity (Subbaiah *et al.*, 2009). [9, 10, 11]

*Garlic* (*Allium sativum*)
Garlic is one of the oldest medicinal having various beneficial activities. The organo-sulphur compounds present in it are known to treat bacterial and fungal infections, cardiovascular diseases and cancer (Raghu *et al.*, 2012). [12] In their review Thomson and Ali, 2003, have mentioned the role of garlic as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and as antitumor. [13]The anticancer activities of garlic are attributed to the organo-sulphur compounds present in Garlic (Karmakar *et al.*, 2011). [14]

3. Erand (Castor)
The oil of Erand is commonly used in India as purgative for children and also as lamp oil. The medicinal role of Erand has been reported by many researchers. Rachhadiya *et al.*, 2011, have reported the cytoprotective role of the oil of Erand on gastric mucosa thus reducing the risk of ulcer formation. [15]The castor oil is shown to have hypolytic (Lombard *et al.*, 2001), antidiabetic (Shokeen *et al.*, 2008), antibacterial (Verma *et al.*, 2011, Islam *et al.*, 2010), anti-inflammatory (Saini *et al.*, 2010; Valderramas *et al.*, 2008), wound healing (Prasad *et al.*, 2011) hepatoprotective Natu *et al.*, 1977), antioxidant (Singh *et al.*, 2010; Oloyede, 2012) activities. [16-25] Darmanin *et al.*, 2009, have reported the cytotoxic and apoptotic activity of castor. [26]

4. Sesame
The nutritional, medicinal and industrial use of Sesame is elaborately reviewed by Raghavan *et al.*, 2010. [27] It contains two compound sesamin and sesamolin, which control blood pressure by lowering cholesterol in the blood. Nigam *et al.*, 2015 have shown the antioxidant and antibacterial properties of sesame. [28] The anti-hyperlipidemic activity of sesam was demonstrated by Asgary *et al.*, 2010. [29]

**EXPERIMENTAL SECTION**

Kulathadi Kashayam were procured from a standard local Ayurvedic shop at Chennai.

The medicine which is available in liquid form was subjected to GC MS analysis as per standard procedure. (Figure 1)

The metabolites in the samples were identified using a P2010 gas chromatography with thermal desorption system TD20 coupled with mass spectroscopy (Shimadzu). The ionization voltage 70ev and GC was conducted in the temperature programming mode with a Restek column (0.25mm, 60m, XTI-5). The temperature in the initial column was 80°C for 1 min, and then increased linearly to 70°C to 220°C held for 3 min followed by linear increased temperature 100°C up to 290°C and held for 10min. The injection port temperature was 290°C and the GC/MS
interface was maintained at 290°C, the samples were introduced via an all glass injector working in the split mode with helium carrier gas low rate with 1.2 ml per minute. The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed with peak area normalization.

RESULTS AND DISCUSSION

Figure 1. The GC MS Graph of Kulathadi Kashayam

The GC MS data of Kulathadi Kashayam is shown in Table 1.
Table 1

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Retention Time (In Min)</th>
<th>Name of the Compound</th>
<th>Molecular formula</th>
<th>Molecular Weight</th>
<th>Peak (%)</th>
<th>(%) Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.594</td>
<td>Benzoic acid</td>
<td>C7H6O2</td>
<td>122</td>
<td>62.236</td>
<td>46.1</td>
</tr>
<tr>
<td>2</td>
<td>15.042</td>
<td>Heptadecane, 2,6,10,15-tetramethyl-</td>
<td>C21H44</td>
<td>296</td>
<td>0.630</td>
<td>7.23</td>
</tr>
<tr>
<td>3</td>
<td>15.292</td>
<td>Phenol, 2,4-bis(1,1-dimethylethyl)-</td>
<td>C14H22O</td>
<td>206</td>
<td>1.967</td>
<td>60.5</td>
</tr>
<tr>
<td>4</td>
<td>15.605</td>
<td>Hexadecane</td>
<td>C16H34</td>
<td>226</td>
<td>0.394</td>
<td>7.97</td>
</tr>
<tr>
<td>5</td>
<td>16.349</td>
<td>Diethyl Phthalate</td>
<td>C12H14O4</td>
<td>222</td>
<td>7.362</td>
<td>68.6</td>
</tr>
<tr>
<td>6</td>
<td>17.613</td>
<td>Hexadecane, 2,6,11,15-tetramethyl-</td>
<td>C20H42</td>
<td>282</td>
<td>0.665</td>
<td>7.53</td>
</tr>
<tr>
<td>7</td>
<td>18.094</td>
<td>Heptadecane</td>
<td>C17H36</td>
<td>240</td>
<td>0.337</td>
<td>7.86</td>
</tr>
<tr>
<td>8</td>
<td>19.890</td>
<td>Nonadecane, 2-methyl-</td>
<td>C20H42</td>
<td>282</td>
<td>0.782</td>
<td>6.48</td>
</tr>
<tr>
<td>9</td>
<td>20.259</td>
<td>n-Hexadecanoic acid</td>
<td>C16H32O2</td>
<td>256</td>
<td>2.882</td>
<td>75.4</td>
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<tr>
<td>10</td>
<td>20.609</td>
<td>Heptadecane, 9-hexyl-</td>
<td>C23H48</td>
<td>324</td>
<td>0.314</td>
<td>15.7</td>
</tr>
<tr>
<td>11</td>
<td>21.823</td>
<td>Octadecane, 1,1’-[1-methyl-1,2-ethanediyl]bis(oxy)]bis-</td>
<td>C24H48O2</td>
<td>496</td>
<td>0.401</td>
<td>7.79</td>
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<tr>
<td>12</td>
<td>21.941</td>
<td>Eicosane, 2-methyl-</td>
<td>C22H44</td>
<td>296</td>
<td>1.400</td>
<td>7.14</td>
</tr>
<tr>
<td>13</td>
<td>22.154</td>
<td>Octadecanoic acid</td>
<td>C18H36O2</td>
<td>284</td>
<td>3.519</td>
<td>73.3</td>
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<tr>
<td>14</td>
<td>23.799</td>
<td>Heptadecane, 9-hexyl-</td>
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<td>324</td>
<td>0.379</td>
<td>6.27</td>
</tr>
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<td>15</td>
<td>24.969</td>
<td>Eicosane, 10-methyl-</td>
<td>C21H44</td>
<td>296</td>
<td>0.564</td>
<td>10.5</td>
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<td>25.807</td>
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<td>10.2</td>
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<td>C20H42</td>
<td>282</td>
<td>2.120</td>
<td>13.5</td>
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<tr>
<td>19</td>
<td>29.310</td>
<td>Tetratriacontane</td>
<td>C34H70</td>
<td>478</td>
<td>5.141</td>
<td>6.72</td>
</tr>
<tr>
<td>20</td>
<td>31.024</td>
<td>Eicosane</td>
<td>C20H42</td>
<td>242</td>
<td>2.529</td>
<td>7.65</td>
</tr>
<tr>
<td>21</td>
<td>33.164</td>
<td>Tetratriacontane</td>
<td>C34H70</td>
<td>478</td>
<td>1.822</td>
<td>9.52</td>
</tr>
</tbody>
</table>

The important biomolecules present in Kulathadi kas hayam are represented by Figure no. 2 to 13.  
Figure 2. Benzoic Acid  

\[
\text{Name: Benzoic acid} \\
\text{Formula: C}_7\text{H}_6\text{O}_2 \\
\text{MW: 122}
\]

Figure 3. Diethyl Phthalate  

\[
\text{Name: Diethyl Phthalate} \\
\text{Formula: C}_{12}\text{H}_{14}\text{O}_4 \\
\text{MW: 222}
\]
Figure 4. Tetratriacontane

Name: Tetratriacontane
Formula: C_{34}H_{70}
MW: 478

Figure 5. Octadecanoic acid

Name: Octadecanoic acid
Formula: C_{18}H_{36}O_{2}
MW: 284

Figure 6. n-Hexadecanoic acid

Name: n-Hexadecanoic acid
Formula: C_{16}H_{32}O_{2}
MW: 256

Figure 7. Heptacosane, 1-chloro-

Name: Heptacosane, 1-chloro-
Formula: C_{27}H_{55}Cl
MW: 414

Figure 8. Eicosane

Name: Eicosane
Formula: C_{20}H_{42}
MW: 282
Figure 9. Phenol, 2,4-bis(1,1-dimethylethyl)-
OH
Name: Phenol, 2,4-bis(1,1-dimethylethyl)-
Formula: C₁₄H₂₂O
MW: 206

Figure 10. Eicosane, 2-methyl-

Name: Eicosane, 2-methyl-
Formula: C₂₁H₄₄
MW: 296

Figure 11. Nonadecane, 2-methyl-

Name: Nonadecane, 2-methyl-
Formula: C₂₀H₄₂
MW: 282

Figure 12. Hexadecane, 2,6,11,15-tetramethyl-

Name: Hexadecane, 2,6,11,15-tetramethyl-
Formula: C₂₀H₄₂
MW: 282

13. Heptadecane, 2, 6, 10,15-tetramethyl-
The GC MS analysis results have shown the presence of the following biomolecules in abundance as shown by their peak value percentages: Benzoic acid, Diethyl Phtha late, Tetratricontane, Octadecanoic acid, n-Hexadecanoic acid, Heptacosane 1-Chloro, Eicosane, Phenol, 2,4-bis(1,1-dimethylethyl)-, Eicosane, 2-methyl-, Nonadecane, 2-methyl-, Hexadecane 2,6,11,15- tetramethyl-, Heptadecane, 2,6,10,15-tetramethyl- and Eicosane, 10-methyl-.

Phenol, 2, 4-bis (1, 1-dimethylethyl) - derivative is present in various plants and is known for its antibacterial and anti-inflammatory activities. (Mujeeb et al, 2014, Costantino et al, 1993) [30, 31]

Octadecanoic acid is Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic , antiacne , 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticytotoxic and insectifuge (Dandekar et al, 2015). [32] n- Hexadecanoic acid is antioxidant, hypocholesterolemic, nematicide, pesticide, and lubricant, antiandrogenic, as flavoring agents, hemolytic and as 5-alpha reductase inhibitor. (Rajeswari et al, 2013). [33]

Eicosane, 2-methyl- derivative is a good antioxidant. Octadecanoic acid esters are reported to be antiviral, antibacterial and antioxidant activities. (Reagan et al, 2013, Sudharsan et al, 2010) [34, 35] Benzoic acid derivatives possess antibacterial, antifungal properties (Vidhu and Evans, 2015; Terreaux et al., 1998). [36, 37] Hexadecanoic acid and naphthalene are known to have antifeedant and insect-repellent activities (Senthilkumar et al 2012). [38] Phenolic compounds, esters, alkanes, aldehydes, alkenes and ketones are the major volatile compounds which have anti-inflammatory, antiarthritic, antiidiabetic, antiulcer, hypolipidemic, antiatherosclerotic, anti-HIV and cytotoxic activities (Safayhi and Sailer, 1997). [39] Heptacosane is anti corrosive/ antioxidant. Nonadecane, 2- methyl is antioxidant. Tetratricontane and other higher alkenes are antifungal particularly against fungal spores, germination and also are antioxidant and antitumour (Dandekaret al, 2015) [32].

REFERENCES