STANDARDIZATION OF AN AYURVEDIC FORMULATION “JATYADI GHRITA” BY RP-HPLC

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ABSTRACT
In traditional Indian medicinal practice there are several Ayurvedic formulations mentioned which have been claimed as potential wound healing agents like Madhu Ghrita and Jatyadi ghrita. Jatyadi ghrita (JG) is a medicated ghee formulation popularly used in the treatment of various topical wounds. It is a polyherbal preparation containing eleven ingredients. Though JG has its composition recorded in ancient Ayurvedic texts, there have been minimal attempts to standardize its. In this research paper an attempt has been made to develop standardization method for some of the ingredients of Jatyadi Ghrita. Quantitative estimation was done by reported RP-HPLC methods using markers glycyrrhetinic acid, ursolic acid, karanjin, curcumin, berberine and kutkin which are major constituents of formulation. A standard laboratory reference sample of Jatyadi Ghrita and four marketed samples were evaluated as per methods. Data has been provided to demonstrate applicability of the methods to standardization of Jatyadi Ghrita.

Keywords: Jatyadi Ghrita, RP-HPLC, Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine, Kutkin.

INTRODUCTION
Herbal medicines are generally available as a mixture of more than one plant constituents. It is important to quantify the maximum possible numbers of markers in such herbal formulations through which the formulation may be assessed. Jatyadi Ghrita, a poly herbal preparation containing eleven ingredients, was found in authentic books like Ashtanga Hrudaya Uttarasthana 25/67 and used as a topical product prescribed for the treatment of all kind of wounds of the body. [1] As it is difficult to estimate each and every ingredient for its chemical constituents, few of the main ingredients of Jatyadi Ghrita have been identified and standardized. The formulation describes the presence of Glycyrrhiza glabra, Jasminum grandiflorum, Pongamia pinnata, Curcuma longa, Berberis aristata and Picrorhiza kurroa the principal constituents of which are Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin respectively [2-7]. Separate HPLC methods were available for analysis of Glycyrrhetic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin [8-11].

MATERIALS AND METHODS
Selection and authentication of medicinal plants
All the plants, viz. Jasminum grandiflorum Linn.[leaf], Azadirachta indica A.Juss.[leaf], Berberis aristata D.C.[stem], Curcuma longa Linn.[Rhizome], Picrorhiza kurroa Royle.[Root], Trichosanthes dioica Roxb.[leaf], Pongamia pinnata Linn.[seed], Hemidesmus indicus Linn.[Root], Glycyrrhiza glabra Linn.[Root], Rubia cordifolia Linn.[Root] and Vetiveria zizanioides Linn.[Root] were collected from Mankarnika Aushadhlay, Pune. All these plants were authenticated by Botany group, Plant Sciences Division, Agharkar Research Institute, (No.3/187/2010/Adm. 367), Pune.

Preparation of Jatyadi ghrita
In laboratory formulation of Jatyadi ghrita was prepared as per Ayurvedic Formulary of India [1]. All the plant parts, viz. Jasminum grandiflorum, Azadirachta indica, Berberis aristata, Curcuma longa, Picrorhiza kurroa, Trichosanthes dioica, Pongamia pinnata, Hemidesmus indicus, Glycyrrhiza glabra, Rubia cordifolia, and Vetiveria zizanioides were dried in oven at 45°C until they were free from moisture. They were then powdered and sieved through 60# and stored in airtight container for further use. All the drugs were taken in the equal amount that is 1.47 gm for 100 gms. Dection of each drug and 1.47 gm of copper sulphate in 10 ml water were mixed together and passed through muslin cloth. Bees wax 1.47 gm and 76.8 gm Cow’s ghee was heated on water bath at 100°C. The decoction and copper sulphate solution was mixed in it in melted condition with propeller mixer at 100 RPM for 15 min. A reference sample of Jatyadi Ghrita (JGL) prepared in the laboratory and four marketed formulations JGV, JGA, JGR and JGN were chosen for the standardization.

HPLC analysis:
HPLC analysis was performed by using Waters (2487) with Empowers-3 software with different chromatographic conditions for different constituents as mentioned in Table 1.

Table 1: Chromatographic Conditions

<table>
<thead>
<tr>
<th>Chromatographic Parameter →</th>
<th>Column</th>
<th>Mobile Phase</th>
<th>Flow Rate (ml/min)</th>
<th>Wavelength (nm)</th>
<th>Run Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycyrrhetinic acid</td>
<td>Hypercil BDS C₂₅0x4.6 mm,5μ</td>
<td>A] 1% Acetic acid in Water. B] 1% Acetic acid in Acetonitrile.</td>
<td>1.0</td>
<td>249</td>
<td>60</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>Hypercil BDS C₂₅0x4.6 mm,5μ</td>
<td>Methanol: Phosphate buffer (pH-3.0) (90:10v/v)</td>
<td>0.8</td>
<td>203</td>
<td>20</td>
</tr>
<tr>
<td>Karanjin</td>
<td>Microbondapack 300x3.9mm,10μ</td>
<td>Methanol: Water (80:20v/v)</td>
<td>1.0</td>
<td>217</td>
<td>15</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Hypercil BDS C₂₅0x4.6 mm,5μ</td>
<td>Acetonitrile: Water (1:90v/v)</td>
<td>1.0</td>
<td>423</td>
<td>8</td>
</tr>
<tr>
<td>Berberine</td>
<td>Hypercil BDS C₂₅0x4.6 mm,5μ</td>
<td>Acetonitrile: Water (1:90v/v)</td>
<td>0.6</td>
<td>265</td>
<td>20</td>
</tr>
<tr>
<td>Kutkin</td>
<td>Inertsil ODS-3, C₂₅0x4.6 mm,5μ</td>
<td>0.1%Orthophosphoric acid in Water: Acetonitrile (75:25v/v)</td>
<td>1.0</td>
<td>265</td>
<td>15</td>
</tr>
</tbody>
</table>
Preparation of standard solutions

Stock solutions of all markers were prepared separately in methanol. Final concentration was made up by further diluting with methanol which was mentioned in Table 2.

Preparation of sample solutions

Accurately weighed 5 gm formulations were extracted with equal amount of hexane and methanol (20 ml each) by means of separating funnel. It was shaken vigorously and allowed to stand for 5 min for separating the two layers. Methanolic layer was again treated with 10 ml hexane till it was free from fat. Hexane layers were discarded. The volume was made with methanol up to 25 ml by using volumetric flask and filtered through 0.22 micron filterpaper. This preparation was used as stock solution. Final concentration was made up by further diluting with methanol which was mentioned in Table 2.

Comparison was done between reference laboratory formulation (JGL) and four marketed formulations JGAV, JGA, JGR and JGN.

Table 2: Concentration of Standard and Sample solutions

<table>
<thead>
<tr>
<th>Analysed Constituents</th>
<th>Glycyrrhetinic acid (µg/ml)</th>
<th>Ursolic acid (µg/ml)</th>
<th>Karanjin (µg/ml)</th>
<th>Curcumin (µg/ml)</th>
<th>Berberine (µg/ml)</th>
<th>Kutkin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard solution</td>
<td>150</td>
<td>60</td>
<td>60</td>
<td>600</td>
<td>600</td>
<td>60</td>
</tr>
<tr>
<td>Test solution</td>
<td>150</td>
<td>60</td>
<td>60</td>
<td>600</td>
<td>600</td>
<td>60</td>
</tr>
</tbody>
</table>

Inject the prepared standard and sample solutions and quantitative analysis was carried out by comparison with respective peak areas.

RESULTS AND DISCUSSIONS

HPLC finger printing profile:

HPLC study of methanolic extracts of the in-house formulation and marketed formulations were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in all the formulations. Inject the prepared standard and sample solutions and quantitative analysis was carried out by comparison with respective peak areas. The HPLC fingerprint profiles of the formulations are presented in Fig 1-5.

![HPLC Finger Printing](image_url)

Fig. 1: HPLC Fingerprinting of Standard solutions and Sample solution of Glycyrrhetinic acid.
Fig. 2: HPLC Fingerprinting of Standard solutions and Sample solution of Ursolic acid.
Fig. 3: HPLC Fingerprinting of Standard solutions and Sample solution of Karanjin.
Fig. 4: HPLC Fingerprinting of Standard solutions and Sample solution of Curcumin.
Fig. 5: HPLC Fingerprinting of Standard solutions and Sample solution of Berberine.
Fig. 6: HPLC Fingerprinting of Standard solutions and Sample solution of Kutkin.

The percentage of concentration for each constituents were found in the reference laboratory formulation (JGL) and four marketed formulations JGAV, JGA, JGR and JGN mentioned in Table 3.

Table 3: Concentration of Constituents

<table>
<thead>
<tr>
<th>S. No</th>
<th>Formulations Constituents</th>
<th>JGL</th>
<th>JGAV</th>
<th>JGA</th>
<th>JGR</th>
<th>JGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycyrrhetinic acid</td>
<td>1.75</td>
<td>1.38</td>
<td>1.3</td>
<td>1.46</td>
<td>1.33</td>
</tr>
<tr>
<td>2</td>
<td>Ursolic acid</td>
<td>1.46</td>
<td>1.42</td>
<td>1.38</td>
<td>1.32</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>Karanjin</td>
<td>1.23</td>
<td>1.22</td>
<td>1.15</td>
<td>1.17</td>
<td>1.16</td>
</tr>
<tr>
<td>4</td>
<td>Curcumin</td>
<td>1</td>
<td>1.03</td>
<td>1.02</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>5</td>
<td>Berberine</td>
<td>1.01</td>
<td>1.1</td>
<td>1.06</td>
<td>0.9</td>
<td>1.07</td>
</tr>
<tr>
<td>6</td>
<td>Kutkin</td>
<td>0.89</td>
<td>0.82</td>
<td>0.45</td>
<td>0.50</td>
<td>0.54</td>
</tr>
</tbody>
</table>
There was no more difference in the concentration of Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin in laboratory and marketed formulations.

HPLC study of formulations was calculated the presence ingredient. The marker compound Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin were estimated by HPLC in Jatyadi ghrita samples. The chromatogram shows the presence of these components in the formulation. HPLC fingerprint profile of the Jatyadi ghrita formulations are depicted in figure 1-5 indicates the presence of all the ingredients in proportional quantity in the formulations.

CONCLUSION
This study was focused on quantitative estimation of Glycyrrhetinic acid, Ursolic acid, Karanjin, Curcumin, Berberine and Kutkin which are principal constituents of Jatyadi Ghrita by using modern methods of analysis. From this research paper it can be concluded that there was no more difference in the concentration of Glycyrrhetinic acid, ursolic acid, karanjin, curcumin, berberine and kutkin in laboratory reference and marketed formulations, which will go a long way for prescribing a dependable standard to these preparations.

REFERENCES
1. Anonyms. The ayurvedic formulary of India. 2nd ed. Ministry of health and family welfare. Dept. of Indian systems of medicine and homeopathy: New delhi; Part-I: p.84.