PHYSICO-CHEMICAL, CHROMATOGRAPHIC, AND SPECTROPHOTOMETRIC MEASUREMENTS IN THE STANDARDIZATION OF “SEETHARAMA WATTEE” - A SRI Lankan HERBO-MINERAL FORMULATION

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ABSTRACT

“Seetharama watee” is widely used in the febrile illnesses. Although the standardization of this herbo-mineral drug is extremely difficult, it has now become essential for the drug industry. A study was carried out to verify whether its physico-chemical fingerprints could be used for its standardization. The minerals in purified forms were mixed with powdered herbs thoroughly and then were ground using different herbal juices oils. Authentically prepared drug was then comparatively assessed with five commercially available counterparts for several parameters related to its physical and chemical properties to evaluate the deviations from the standardized drug. The residue of the ethanol extraction dissolved in ethanol was used for UV spectrophotometry and HPLC fingerprint analysis. In terms of the acid-insoluble ash, loss on drying, dichloromethane extract and methanol extracts of standard samples were similar to all commercial samples. However, all the samples differed significantly at P < 0.05 level with regard to the weight of a pill, ash content, specific gravity, hexane extract, and ethyl acetate extract values. In the HPLC analysis of the authentically prepared samples, seven major peaks were identified. All samples of “Seetharama watee”, had a λ max value between 287 - 290 nm. in the spectrophotometric analysis. This study shows that, standardization by assessing the above physical and chemical properties and the HPLC fingerprint profiles and UV spectrophotometric measurements could be considered as a prudent way to achieve a consistent quality with optimal efficacy of “Seetharama watee” authentically prepared.

Keywords: High Performance Liquid Chromatography, Seetharama Watee, Standardization, UltraViolet Spectrophotometry, Watika Prakaranaya

INTRODUCTION

Ayurveda medicine and Sri Lankan indigenous medicine are popular medicine systems among Sri Lankans as well as foreign tourists. Although these medicines have been used for immemorial time, no attempt has been made for controlling the quality and achieving standardization. 1,2,3

“Seetharama Watee”, a popular and effective indigenous medicine used as pills. This preparation is used for various types of febrile illnesses and its complications. This preparation was first mentioned in the publication called “Watika Prakaranaya”, first published in 1879 by a Sri Lankan physician. Only a few physico-chemical research studies have been carried out on this preparation;3 no attempt has been made to standardized the drug. The objective of the present study was to explore the possibility of using physical, chemical spectrophotometric and chromatographic properties as quality control tools of “Seetharama Watee”.

MATERIALS AND METHODS


The raw materials were purchased from open market and terrestrial environment. They were washed (except minerals) dried and identified based on Ayurveda parameters “varna” (colour), “gandha”, (odour), “ruche”, (taste) “akruti” (shape) and “parimana” (size). The authenticity of the herbs were tested and confirmed. In macro morphological evaluation, the identified parts of the plants were arranged according to their morphological characteristics.

All herbal ingredients were washed, dried and pulverized separately except garlic and asafetida, and they were filtered through 100 mesh sieve. The nine minerals were purified using traditional methods described in the book “Watika Prakaranaya” and then pulverized separately and filtered through 100 mesh sieve, mixed thoroughly with powdered herbal ingredients and ground after adding garlic and asafetida using five herbal juices, (holy basil, leaves, neem leaves, Indian privet leaves, sour orange and fresh ginger) and two oils (peem and ghee) respectively for seven days. They were made into pills, of size of raw green gram fruit and dried under shade.

This drug was prepared in three stages during the year namely December – January, July –August and March –April months to account for seasonal variations if any. These preparations were prepared as per the “Watika Prakaranaya” and considered as an authentic standard sample (Control). The five commercial samples of the drug were purchased from the open market randomly. All six samples of the drug were (three prepared samples considered as one and five commercial samples) compared by using the parameters such as the weight of the pill, specific gravity, loss on drying, total ash content, acid insoluble ash content. Specific gravity was calculated using the specific gravity bottle method; loss on drying was determined using the dry oven method at 105 °C. Total ash content was determined using a muffle furnace at 450 °C.3,35,36,37

Sequential extractions were done with hexane, dichloromethane, ethyl acetate, and methanol respectively using the Soxhlet extraction method. Solvents were evaporated using a rotavaporator at 50 °C and calculated the final constant weight of the residue as chemical parameter.

A sample of 0.5 g of “Seetharama watee” was weighed and extracted using 95% ethanol in the Soxhlet apparatus using pre-
weighed 100 ml round bottom flask, for 3 hours. The extract was evaporated using a rotavapor (Buchi R-114) at the temperature not exceeding 60 °C. The residue was used for the High Performance Liquid Chromatography (HPLC) and Spectrophotometric analysis.

**HPLC analysis**

The residue was dissolved in HPLC grade methanol 10 ml vortexed for 30 seconds and filtered through 0.45µm teflon micro filters before introducing to the HPLC. C-18 Hypersil ODS column (250 x 4.6µm ID 5µm particle) was used. Flow rate was 1.0/min and the injection volume was 25µl. Acetonitril: Water (2:3 v/v) solution was used as mobile phase and the 254 nm wavelength was used.

**Ultra Violet spectrophotometry**

The residue was dissolved in 10ml of 95% ethanol and 0.5 ml was taken from it and again diluted up to 5ml using the same 95% ethanol. The samples were scanned over range of 200-500 nm using UV mini 1240 (Shimadzu) spectrophotometer equipped with quarts cuvettes of 10mm path length. Ethanol 95% was used as the reference.

SPSS package was used in the analysis of data. One way ANOVA statistical technique was used in the analysis of data followed by Dunnett t test to compare with the controller. P value ≤ 0.05 was considered as the level of significance.

**RESULTS AND DISCUSSION**

As a part of the standardization procedure, the mean values of the three standard samples (control-S0) were compared with five commercial samples (S1-S5 for relevant physical and chemical parameters.

The corresponding P values were significantly the same for all five commercial samples with regard to acid-insoluble ash value, loss on drying, dichloromethane extract and methanol extract percentages. Weight of a pill, Ash content, Specific gravity, hexane extract percentage, and ethyl acetate extract percentage values were significantly different at 0.05 level. (Table -1)

When comparing the prepared samples with commercial samples, the weight of the pill was significantly different in second, third and fourth samples while the first and fifth were the same. Ash value was significantly different in sample one, two and three while fourth and fifth was not different. Specific gravity was significantly different in second third and fourth samples while the first and fifth were the same. Hexane extract percentages in all commercial samples were significantly different presumably due to the less amount of oil content used. Ethyl acetate extraction percentage of the fifth sample was significantly different while other four samples were similar.

### Table 1: Physico-Chemical Properties of “Seetharama watee” Samples (S0, S1-S5)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average pill Wt</th>
<th>Ash value</th>
<th>Acid insoluble ash</th>
<th>Loss on drying</th>
<th>Specific gravity</th>
<th>Hexane extract %</th>
<th>Dichloro methane%</th>
<th>Ethyl acetate %</th>
<th>Methanol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>1.32±0.04</td>
<td>10.08±0.59</td>
<td>0.05±0.03</td>
<td>9.25±0.97</td>
<td>1.16±0.01</td>
<td>26.54±1.43</td>
<td>2.38±0.03</td>
<td>1.57±0.18</td>
<td>8.45±1.47</td>
</tr>
<tr>
<td>S1</td>
<td>1.44±0.24</td>
<td>12.66±0.01</td>
<td>0.02±0.00</td>
<td>7.19±0.57</td>
<td>1.14±0.03</td>
<td>13.04±1.32</td>
<td>0.000</td>
<td>0.000</td>
<td>12.1±3.16</td>
</tr>
<tr>
<td>S2</td>
<td>0.73±0.01</td>
<td>16.21±0.59</td>
<td>0.000</td>
<td>10.85±0.48</td>
<td>1.25±0.17</td>
<td>6.73±0.05</td>
<td>2.89±0.16</td>
<td>2.49±0.19</td>
<td>12.1±3.16</td>
</tr>
<tr>
<td>S3</td>
<td>0.75±0.11</td>
<td>12.35±0.19</td>
<td>0.047</td>
<td>9.2±0.92</td>
<td>1.06±0.02</td>
<td>12.2±0.45</td>
<td>2.33±0.34</td>
<td>1.96±0.49</td>
<td>11.34±1.9</td>
</tr>
<tr>
<td>S4</td>
<td>2.35±0.01</td>
<td>9.79±0.21</td>
<td>0.47</td>
<td>9.31±1.01</td>
<td>1.22±0.03</td>
<td>5.8±0.14</td>
<td>2.1±0.14</td>
<td>1.74±0.04</td>
<td>12.21±0.63</td>
</tr>
<tr>
<td>S5</td>
<td>1.5±0.01</td>
<td>11.41±0.09</td>
<td>0.402</td>
<td>11.1±0.11</td>
<td>1.09±0.02</td>
<td>3.03±0.59</td>
<td>1.45±0.71</td>
<td>3.03±0.59</td>
<td>15.55±1.8</td>
</tr>
</tbody>
</table>

S0 – Authentically prepared sample, S1-S5 – Commercial samples

**Fig. 1: HPLC finger print of authentically prepared sample “Seetharama watee”**
Seven major peaks can be identified in all authentically and commercially prepared samples. Therefore, the same chemical compounds present in these all preparations.

In the spectroscopic measurements, all prepared samples and commercial samples S1, S2, and S3 had a \( \lambda \) max value of 290 nm. The functional group of all prepared and most of commercial samples has \( \lambda \) max values around 287-290 nm. All the samples have almost equal \( \lambda \) max values, so the action groups are almost equal in all formulations. This suggests that the actions of all samples may have close relationships.

**CONCLUSION**

Standardization by assessment of physical and chemical properties that namely weight of pill, ash content value, acid insoluble ash content, loss on drying value, value of specific gravity and the sequential extraction values of hexane, dichloro methane, ethyl acetate, methanol can be used to assess the quality enabling to achieve the optimal efficacy of "Seetharama watee". Furthermore, HPLC finger print profiles and UV-Vis Spectrophotometric measurements also can be used as visible parameters to help standardize Sri Lankan formulations "Seetharama watee" prepared according to "Watika Prakaranaya."

**ACKNOWLEDGEMENTS**

Finance assistance given by University Grant Commission of Sri Lanka.

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