

## PHYSICO-CHEMICAL, CHROMATOGRAPHIC, AND SPECTROPHOTOMETRIC MEASUREMENTS IN THE STANDARDIZATION OF "SEETHARAMA WATEE" -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 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 fingerprints. 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  $\lambda$  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.<sup>1,2,3</sup>

"Seetharama Watee", a popular and effective indigenous medicine used as pills. This preparation is used for various types of febrile illnesses and its complications.<sup>4</sup> 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;<sup>5,6</sup> no attempt has been made to standardize 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

"Seetharama Watee" is a polyherbo-mineral preparation that contains 28 herbs<sup>7</sup> and 9 minerals<sup>8</sup>. *Cuminum cyminum* L. [Fr.]<sup>9</sup> *Nigella sativa* L. [Sd.]<sup>9</sup>, *Foeniculum vulgare* Miller<sup>10</sup>, [Fr.] *Trachyspermum ammi* (L.) Sprague<sup>11</sup>, [Fr.] *Anethum graveolens* L.<sup>12</sup>, [Fr.] *Zingiber officinale* Roscoe<sup>13</sup>, [Rz.] *Piper nigrum* L.<sup>14</sup>, [Fr.] *Piper longum* L.<sup>15</sup>, [Fr.] *Myristica fragrans* Houtt.<sup>16</sup>, [Fr.] *Myristica fragrans* Houtt.<sup>16</sup>, [Ar.] *Syzygium aromaticum* (L.) Merr. & Perry<sup>17</sup> [Fl.bd.] *Aconitum palmatum* D.Don<sup>18</sup>, [Rt.] *Saussurea costus* (Falc)<sup>19</sup> [Rt.] *Glycyrrhiza glabra* L.<sup>20</sup>, [St.Rt.] *Allium sativum* L.<sup>21</sup>, [Bl.] *Coriandrum sativum* L.<sup>22</sup>, [Fr.] *Holarrhena antidysenterica* <sup>23</sup>[Roxb.] [St.Bk.] *Pterocarpus santalinus* L.F.<sup>24</sup>[H.Lwd.] *Aconitum heterophyllum* Wall<sup>25</sup>, [Rt.] *Picrorhiza kurrooa* Royle ex Benth<sup>26</sup>, [Rz.] *Ferula asafoetida* L.<sup>27</sup>, [Ex.] *Ocimum teniflorum* L.<sup>28</sup>, [Lf.] *Vitex negundo* L.<sup>29</sup>, [Lf.] *Toddalia asiatica* (L.) Lam.<sup>30</sup>, [Lf.] *Leucas zeylanica* (L.) R.Br.<sup>31</sup> [Lf.] *Cleome gynandra* L.<sup>32</sup> [Lf.] *Azadirachta indica* A.Juss.<sup>33</sup> [Lf.] *Acorus calamus* L.<sup>34</sup> [Rz.] are these herbs and Yellow arsenic, Calamine, Copper sulphate, Rock salt, Cinnabar, Alum, Realgar, Borax, Gypsum are the minerals.

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), "ruchi", (taste) "akrut", (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 (neem 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.<sup>35</sup> Total ash content was determined using a muffle furnace at 450 °C.<sup>35,36,37</sup>

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

A sample of 0.5g of "Seetharama watee" was weighed and extracted using 95% ethanol in the Soxhlet apparatus using pre-