Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 4, 2012

Research Article

PHYSICO- CHEMICAL, CHROMATOGRAPHIC, AND SPECTROPHOTOMETRIC MEASUREMENTS IN THE STANDARDIZATION OF "SEETHARAMA WATEE" -A SRI LANKAN HERBO- MINERAL FORMULATION

TISSA HEWAVITHANA[1], K.K.D.S.RANAWEERA[2], M.H.A.TISSERA[1], P.A.J YAPA[3]

[1]Department of Dravyaguna,Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya Yakkala. Sri Lanka. [2]Department of Food Science and Technology, Faculty of Applied Science, University of Sri Jayewardenepura, Sri Lanka. [3]Department of Botany, Faculty of Applied Science, University of Sri Jayewardenepura, Sri Lanka.

Received: 09 Jun 2012, Revised and Accepted: 18 July 2012

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 λ max value between287 - 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.[4]This preparation was first mentioned in the publication called **"Watika Prakaranaya"**, first published in 1879 by a Sri Lankan physian. Only a few physico-chemical research studies have been carried out on this preparation; [5,6] 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

"Seetharama Wattee" is a polyherbo-mineral preparation that contains 28 herbals and 9 minerals[7]. *Cuminum cyminum* L ,[Fr.][8] *Nigella sativa* L [Sd.][9],*Foeniculum valgare* Miller[10],[Fr.] *Trachyspermum ammi* (L) Sprague[11],[Fr.] *Anethum graviolens* L[12],[Fr.] *Zingiber officinale* Roscoe[13],[Rz.] *Piper nigrum* L[14].,[Fr.] *Piper longum* L.[15][Fr.], *Myristica fragrans* Houtt[16].,[Fr.] *Myristica fragrans* Houtt[16].,[Ar.] *Syzygium aromaticum* (L.) Merr. & Perry[17] [Fl.bd.] *Aconitum palmatum* D.Don[18],[Rt.] *Saussurea costus* (Falc)[19] [Rt.],*Glycyrrhiza glabra* L[20].[St.Rt.], *Allium sativum* L.[21][Bl], *Coriandrum satium* L[22].[Fr.], *Holarrhena antidysenterica* [23](Roxb), [St.Bk.] *Pterocapus santalinus* L.F.[24][Ht.wd.], *Aconitum heteropyllum* Wall[25],[Rt.] *Picrorhiza kurrooa* Royle ex Benth[26],[Rz.] *Ferula assa-foetida* L[27].,[Ex.] *Ocimum teniflorum* L.[28],[Lf.] *Vitex negundo* L.[29],[Lf.] *Toddalia asiatica* (L.) Lam.[30],[Lf.] *Leucas zeylanica* (L.) R.Br.[31] [Lf.], *Cleome gynandra* L.[32] [Lf.], *Azadirachta indica* A.juss.,[33][Lf.] *Acorus calamus* L.[34][Rz.] are these herbals 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),**"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 (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 ºC;[35] Total ash content was determined using a muffle furnace at 450 ºC.[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 rota-evaporater at 50 ºC 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-

weighed 100 ml round bottom flask, for 3 hours. The extract was evaporated using a rota - evaporator (Buchi R-114) at the temperature not exceeding 60 ºC. The residue was used for the High Performance Liquid Chromatography (HPLC) and Spectrosphotomeric 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 (250x4.6µm 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.[38, 39, 40]

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.[41]

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-S_0) were compared with five commercial samples (S_1-S_5) 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 (S_0, S_1-S_5)

	Mean±SE S_0	**Mean±SE S_1**	**Mean±SE S_2**	**Mean±SE S_3**	**Mean±SE S_4**	**Mean±SE S_5**
1.Average pill Wt.	1.32±0.04	1.44±0.24	0.73±0.01	0.75±0.11	2.35±0.01	1.5±0.01
P value	-	0.785	**0.000**	**0.000**	**0.000**	0.403
2.Ash value	10.08±0.59	12.66±0.01	16.21±0.59	12.35±0.19	9.79±0.21	11.41±0.09
P value	-	**0.021**	**0.000**	**0.047**	0.994	0.402
3.Acid insoluble ash	0.05±0.03	0.03±0.01	0.03±0.00	0.7±0.04	0.05±0.01	0.31±0.31
P value	-	**0.042**	1.000	1.000	1.000	0.178
4.Loss on drying	9.25±0.97	7.19±0.57	10.85±0.48	9.2±0.92	9.31±1.01	11.1±0.11
P value	-	0.546	0.756	1.000	1.000	0.636
5.Specific gravity	1.16±0.01	1.14±0.03	1.25±0.17	1.06±0.02	1.22±0.03	1.09±0.02
P value	-	0.900	0.009	0.003	0.000	0.055
6.Hexane extract %	26.54±1.43	13.04±1.32	6.73±0.05	12.22±0.45	5.8±0.14	13.08±0.28
P value	-	0.000	0.000	0.000	0.000	0.000
7.Dichloro methane%	2.38±0.03	1.22±0.02	2.89±0.16	2.33±0.34	2.1±0.14	1.45±0.71
P value	-	0.696	0.984	1.000	0.999	0.003
8.Ethyl acetate %	1.57±0.18	1.16±0.07	2.49±0.19	1.96±0.49	1.74±0.04	3.03±0.59
P value	-	0.000	0.083	0.774	0.991	0.003
9.Methanol %	8.45±1.47	12.1±3.16	15.2±0.74	11.34±1.9	12.21±0.63	15.55±1.8
P value	-	0.553	0.072	0.75	0.525	0.055

S_0 –Authentically prepared sample **S_1-S_5** –Commercial samples

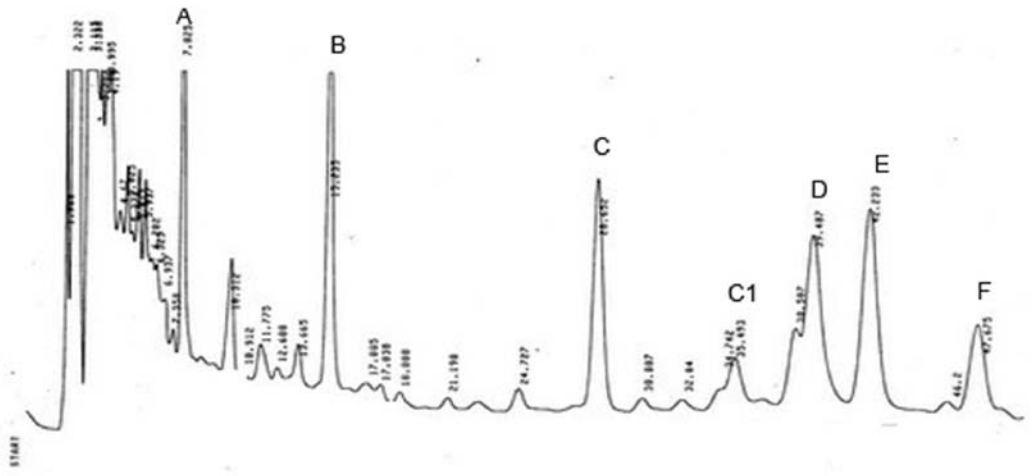

Fig. 1: HPLC finger print of authentically prepared sample "Seetharama watee"

Seven major peaks can be identified in all authentically and commercially prepared samples. Therefore, same chemical compounds present in these all preparations.

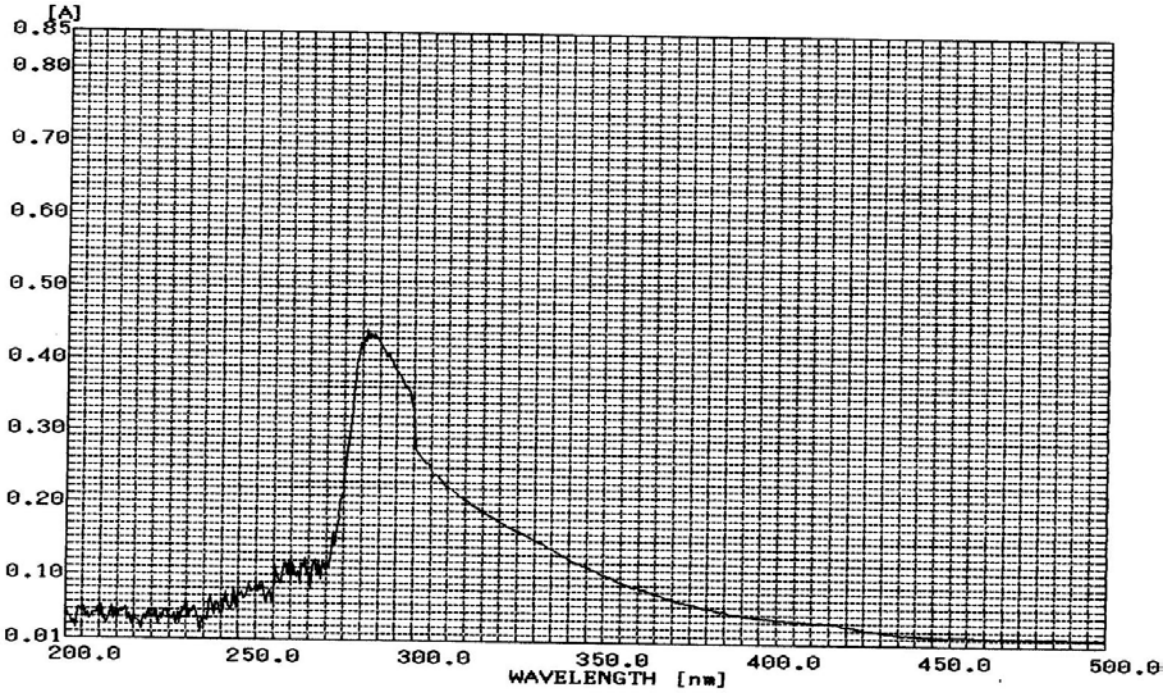

Fig. 2: Spectrophotometric absorption pattern of authentically prepared "Seetharama watee"

In the spectroscopic measurements, all prepared samples and commercial samples S_1, S_2 and S_5 had a λ max value of 290 nm. The functional group of all prepared and most of commercial samples has λ max values around 287-290 nm. All the samples have almost equal λ 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.

REFERENCES

1. Simha K R G, Laxminarayana V, Prasad S V L N , Shahjahan K, Standardization of *Yogaraja guggulu*-An Ayurvedic polyherbal formulation. *Indian Journal of Traditional Knowledge*, 2008; 7(3):389-96.
2. Simha K R G Laxminarayana V, Standardization of *Navaka Guggulu* – An Auyrvedic polyherbal formulation. *Indian Journal of Traditional Knowledge*, 2008; 7(4): 542-47.
3. Pattanaya P, Jena R K, Panda S K, HPTLC Finger printing in the Standardization of *Sulaharan yoga* an Ayurvedic tablet formulation. *International Journal of Pharmaceutical Science Review and Research*, 2010; 3: 33-36.
4. Anonymous, Ayurveda Pharmacopeia *(Sinhalese)*, Department of Ayurveda Sri Lanka.1976; 146.
5. Karunanayake E H, Weerakoon S N, Tillainathan A S, Sivasamy S.*Ayurveda Pradeepika,* Department of Ayurveda Sri Lanka. 1971; 3:15-21.
6. Rodrigo A N N, Samarathunga U *Ayurveda Sameeksha* Department of Ayurveda Sri Lanka. 2008; 2: 49-52.
7. Ilapperuma S S I A. *Watika Prakaranaya* –Colombo Press Sri Lanka.1879; 70.
8. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:139.
9. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:243.
10. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:145.
11. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:133.
12. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:149.
13. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:217.
14. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:209.
15. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:207.
16. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:109.

17. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:115.
18. Nadkarni K M, Indian Meteri Medica Revised Nadkarni A K Popular Prakashan Private Ltd. 35c Tarde Rd, Bombay, India ; 2005;43
19. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 2:67.
20. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 3:211.
21. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 1:57.
22. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:137.
23. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 1:103.
24. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 3:235.
25. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:237.
26. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:76.
27. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:141.
28. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 3:105.
29. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:181.
30. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 5:37.
31. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 2:17.
32. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 3:93.
33. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 4:53.
34. Jayaweera D.M. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. The National Science Foundation Sri Lanka.2006; 1:121.
35. Anonymus, Ayurvedic Pharmacopeia of India. Ministry of Health and Welfare New Delhi India,Vol 1,1990;143.
36. Chithra B, Ramaswamy R S, Pharmacological Analysis of a Siddha formulation Karuncheeraga Churanam; International Journal of Pharmacy and Pharmaceutical Sciences, 2012 (4) 2: 104-107.
37. 37.Sudha V, Lavanya R, Niraimathi K L, Savariraj S C ,Brindha P Chemical Standardization Studies on Rasaganthi Mezhugu; International Journal of Pharmacy and Pharmaceutical Science, 2012 (4) 2: 21-26
38. Jie Y,Li S L, Li P, Simultaneous determination of seven active components of Fufang Danshen tablet by high performance liquid chromatography. *Biomedical Chromatography*, 2007; (21):1-9.
39. Chen Y,Yan Y,Xie M Y,Nie S P, Liu W,Gong X F, Wang Y X, Development of a Chromatographic fingerprint for the chloroform extracts of *Ganoderma lucidum* by HPLC and LC-MS. *Journal of Pharmaceutical and Biomedical Analysis*,2008 ;(47): 469-77.
40. Aeri V,Khan M I, Alam S, A validated HPLC method for quantification of Oleanolic acid in the root of *Achyranthes aspera* Linn. and marketed formulation. *International Journal of Pharmacy and Pharmaceutical Science,*2010; 2: 74-78.
41. Shaila D, Santhosh M K, Chandrakumar T, Rao I S, Standardization study of Grithas. EJournal of Chemistry, 2004 (1):151-57. (Cited on 20th January 2012)