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USE OF PHYSICO-CHEMICAL PROPERTIES, CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC MEASURMENTS IN THE STANDADIZATION OF SRI LANKAN POLY HERBAL FORMULATION "MAHA VARTHIKAVA WATEE"

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ABSTRACT
The efficacy and quality of commercially manufactured "Maha Varthikava watee" preparations may differ from its authentic preparation prepared as per original recipe. Therefore, a study was carried out to investigate whether the physico-chemical properties, HPLC and UV chromatographic and spectrophotometric measurements can be used for standardization of the above drug.
Purified herbals used in the original recipe of "Maha Varthikava watee", were finely powdered and mixed. The mixture was ground together with three juices extracted from leaves of three different species and bee's honey to make pills. Five commercial samples purchased from the market were compared with the authentically prepared samples. Physical properties, chemical properties and chromatographic and spectrophotometric measurements were considered as tools for standardization. For the HPLC and UV spectrophotometry, ethanol extraction was used. Data were analyzed at 0.05 significant levels. No significant differences were observed in any commercial samples with regard to fiber content, acid insoluble ash, dichloromethene extract, ethyl acetate extract and methanol extract while the weight of pills was significantly different. The pH values and loss on drying were significantly different only in two commercial samples. Specific gravity and ash values were found to be different only in one sample. Hexane extract value was significantly different in one sample.
In HPLC chromatograms, one main peak was more prominent in three commercial samples. In UV spectrophotometric measurement, two commercial samples had a λ max of 287-290 nm while other three samples had a λ max of 340-345 nm. Hence, these measurements can be used for the standardization of "Maha Varthikava watee".
Key words: Maha Varthikawa,Watika Prakaranaya, HPLC, Spectrophotometry ,Sri Lankan medicine.

INTRODUCTION

In Ayurvedic and Indigenous medicinal systems, great emphasis was given to complete knowledge of drugs including identification, procurement, processing and applications. In ancient times physicians used to prepare drugs by themselves according to the need of different patients. Therefore, there were no concerns over the drug standardization[1]; however, the situation has changed very fast with time. The growing population and their life style, industrialization etc, have forced physicians to depend mainly on market preparations, which on the other hand affected the quality of these formulations drastically[2]. Among the factors that affect the quality of drugs, the authenticity of raw materials and ingredients, process standards, proper storage practices are of primary significance. Therefore, standardization of drugs has become a long felt need in the herbal drug industry[3].

Quality assessment and standardization of complex herbal formulations are multifaceted due to the synergistic effect of constituent components. The main problem in poly herbal formulation is that the presence of each ingredient in desired condition has to be ensured. Modern techniques based on physical, chemical, spectrophotometric and biological parameters can be used to achieve the prerequisites of standardization.

"Maha Varthikava watee" is an effective and widely used poly herbal specific formulation in Sri Lanka, mentioned long ago in the authenticated book called "Watika Prakaranaya" which was first published in 1879.This poly herbal formulation consists of twenty nine herbal ingredients in pill form[4]. It has multifaceted action in all type of gastro intestinal tract disorders. Due to lack of proposed modern pharmacopeia standards for processing of "Maha Varthikava watee" using traditional methods, the drug prepared may not have the desired quality and it may vary from batch to batch. The standardization guidelines for herbal products provided by World Health Organization (WHO) and the Indian pharmacopeia standards have been followed in this study.

MATERIALS AND METHODS

"Maha Vartikawa Watee" is a poly herbal formulation that contains 29 herbal ingredients[5]. They are *Cuminum cyminum* L. [Fr.][6], *Nigella sativa* L. [Sd.][7], *Allium satium* L. [Bl.][8], *Trachyspermum ammi* (L.) Sprague. [Fr.][9], *Trigonella foenum-graecum* L. [Fr.][10], *Zingiber officinale* Rosc. [Rz.][11], *Ferula assa-foetida L.* [Ex.][12], *Piper nigrum* L. [Fr.][13], *Piper longum* L. [Fr.][14], *Solanum virginianum* L.[Rt][15], *Terminalia chebula* Retz [P.][16], *Terminalia bellarica* (Gaertn) [P][17], *Phyllanthus emblica* L. [P][18], *Syzygium aromaticum* (L.) Merr. & Perry. [Fl.bd][19], *Myristica fragrans* Houtt. [Ar.][20], *Myristica fragrans* Houtt. [Sd.][20], *Fumaria parviflora,* Lamk. Sow [Pl.][21], *Caesalpinia bonduc* (L.) Roxb. [Sd.][22], *Carum cavi* L. [Fr.][23], *Cinnamomum verum* J.Presl [St.Bk.][24], *Acorus calamus* L. [Rz.][25], *Seasamus indicum* L. [Sd.][26], *Brassica juncea* (L.) Czern.[Sd.][27], *Embelica ribes* Burm.f. [Sd.][28], *Santalum album* L. [Ht.Wd.][29], *Acacia chundra* Willd.

[Sl.Ex.][30], *Mesua ferrea* L. [Stmn.][31], *Piper betel* L. [Lf.][32], *Vitex nigundo* L. [Lf.][33]. All plants materials were collected from herbal gardens and identified as correct plants by Dr. M.H.A. Tissera, Professor of Dravyaguna, Department of Dravyaguna, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya. Dried raw materials were purchased from open market in Colombo-Sri Lanka and authenticated at the same Institute as the correct ingredients of the formulation according to the Sri Lankan Ayurveda Pharmacopeia. Samples of all ingredients (MVW29) have been kept in the museum and herbarium, Department of Dravyaguna G.W.A.I, University of Kelaniya. This formulation was prepared in three consecutive batches as mentioned in the "Watika Prakaranaya" during December-January, July-August, and March-April periods to minimize the seasonal changes. These three authentically prepared sample batches were taken as the controls.

All raw materials were washed in running tap water and air-dried at 60^0C. All the ingredients were powdered separately, passed through No 125 sieve and then mixed together in equal portions. Then the powder was grounded thoroughly using Betel leaf juice, Indian privet juice, ginger juice and bee honey respectively. Then the pills were made in the size of raw green gram grain as mentioned in the text "Watika Prakaranaya", dried under sunshade, and stored in hermetically sealed containers. Five market samples were purchased randomly from the open market. The authentically prepared samples and the five market samples were standardized using the physical characters, physicochemical properties and spectrophotometric analysis. The physical characteristics such as weight of a pill, total ash, acid insoluble ash, pH value, fiber content, loss on drying at 105^0C, were determined. As physiochemical characteristics, sequential extractability with hexane, dichloromethane, ethyl acetate and methanol were carried out in triplicates, for all three controls and the all five market samples.

Weight comparison of pills was done by using randomly selected ten pills from each eight samples. pH of the 10% solution was determined. Fiber content was calculated as mentioned in Ayurveda Pharmacopeia[34]. Loss on drying was determined using the oven drying method. Specific gravity was determined by using the specific gravity bottle method. Total ash and acid insoluble ash were determined by using the method mentioned in Indian Ayurveda Pharmacopeia.

Sequential extractions with n-hexane, dichloromethane, ethyl acetate and methanol were done using Soxhlet apparatus. Extractions were done for two hours for each solvent respectively. After extractions, the solvents were evaporated by using Buchi type Rota -evaporator at 50^0C and calculated the final constant weight of the residue.

In the Spectrophotometric analysis, 0.5g of "Maha Varthikava 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 a temperature not exceeding 60^0C. The residue was used for the HPLC and Spectrosphotometric 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 it was introduced to the HPLC. C-18 Hypersil ODS column (250x4.6µm ID 5µm particle) was used. Flow rate was 1.0 ml/min and the injection volume was 25µl. Acetonitrile : Water (2:3 v/v) was used as mobile phase and the 254 nm wave length was used.[35-37]

UV spectrophotometry

The residue was dissolved in 10 ml of 95% ethanol and 0.5 ml was taken from it and again diluted up to 5 ml with 95% ethanol. The samples were scanned over range of 200-500 nm using UV mini 1240 (Shimadzu) spectrophotometer equipped with quarts cuvettes of 10 mm path length. Ethanol 95% was used as the reference[38].

Statistical analysis

Results are expressed as mean ± S.E. Analysis of one way ANOVA followed by Dunnett's t-test was use to evaluate the significance of the results. The difference is considered significant when p value is < 0.05.

Table 1: Physico-Chemical properties of "Maha Varthikava Watee" samples (V_0-V_5)

	Weight of pill	pH value	Fiber Content	loss on drying	specific gravity	Ash Content	Acid insoluble ash content	Hexane ext. %	Dichloro Methane ext. %	Ethyl acetate ext. %	Methanol ext.%
V_0-M	1.18 ±0.062	4.57 ±0.074	8.42 ±1.8	12.3 ±0.478	1.24 ±0.035	6.87 ±0.166	0.088 ±0.071	6.12 ±0.84	1.17 ±0.197	2.13 ±0.52	15.29 ±4.36
V_1-M	0.933 ±0.008	4.14 ±0.127	11.4 ±5.02	7.01 ±0.467	1.09 ±0.019	7.42 ±0.006	0.088 ±0.071	5.83 ±1.57	2.66 ±0.34	2.18 ±0.316	5.78 ±1.03
P.Value	**0.034**	0.079	0.884	**0.003**	0.09	0.931	0.965	1.000	0.560	1.000	0.497
V_2-M	2.33 ±0.005	4.43 ±0.0202	14.46 ±2.46	7.63 ±1.08	0.952 ±0.016	7.5 ±0.1	0.024 ±0.008	10.96 ±0.643	1.94 ±0.28	6.92 ±4.88	13.16 ±0.55
P.Value	**0.000**	0.916	0.340	**0.010**	**0.001**	0.887	0.626	**0.035**	0.998	0.125	0.998
V_3-M	0.81 ±0.03	4.27 ±0.219	10.25 ±1.14	10.46 ±1.23	1.148 ±0.063	3.7 ±1.76	0.026 ±0.016	4.64 ±0.5	1.73 ±0.84	3.34 ±0.65	2.99 ±0.399
P.Value	**0.001**	0.324	0.984	0.568	0.492	**0.002**	0.636	0.87	1.000	0.975	0.257
V_4-M	0.94 ±0.027	3.74 ±0.055	11.4 ±1.21	10.036 ±1.039	1.112 ±0.026	5.93 ±0.035	0.033 ±0.02	6.31 ±2	1.63 ±0.42	1.89 ±0.19	9.86 ±4.53
p.Value	**0.043**	**0.000**	0.888	0.364	0.186	0.635	0.685	1.000	1.000	1.000	0.894
V_5-M	1.43 ±0.018	3.94 ±0.1	8.87 ±2.02	12.99 ±2.16	1.11 ±0.049	5.43 ±0.032	0.01 ±0.006	10.12 ±1.39	1.8 ±1.2	3.75 ±0.32	10.52 ±3.87
P.Value	**0.041**	**0.006**	1.000	0.985	0.161	0.242	0.526	0.1000	1.000	0.922	0.934
P,Value (over roll)	0.000	0.001	0.589	0.002	0.003	0.002	0.549	0.020	0.795	0.303	0.426

P Value (over roll) - One way ANOVA, P value- Dunnett's t, V_0-Authentically prepared sample, V_1,V_2,$V_3$$V_4$,$V_5$-Commercial Samples, M-Mean, P.-Significance

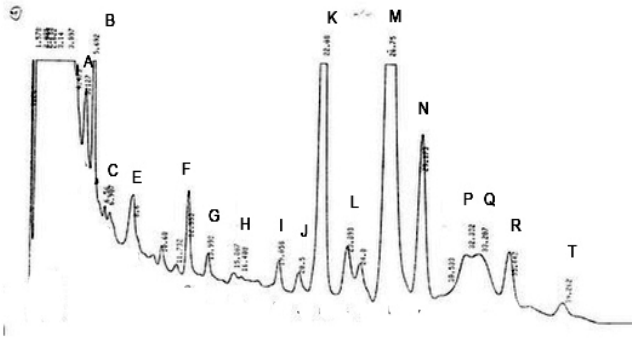

Figure 1: HPLC finger print of authentically prepared sample; Maha Varthikava watee

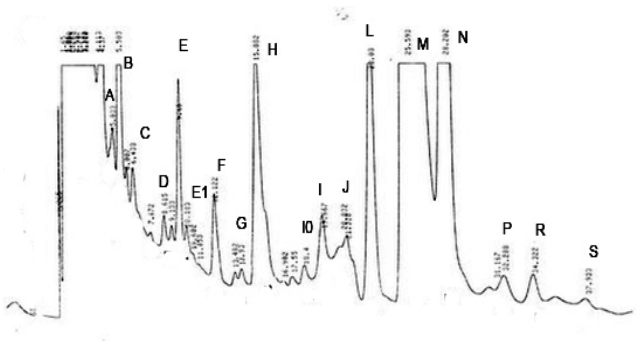

Figure 2: HPLC fingerprints commercial sample; Maha Varthikava watee

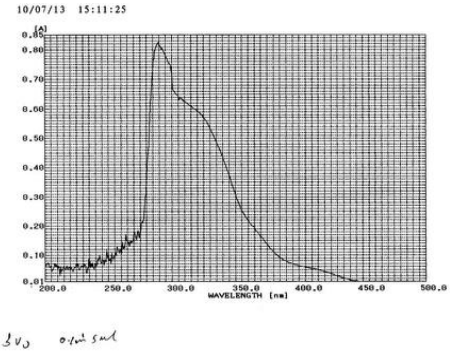

Figure 3: Spectrophotometric pic. Authentically prepared; Maha Varthikava watee

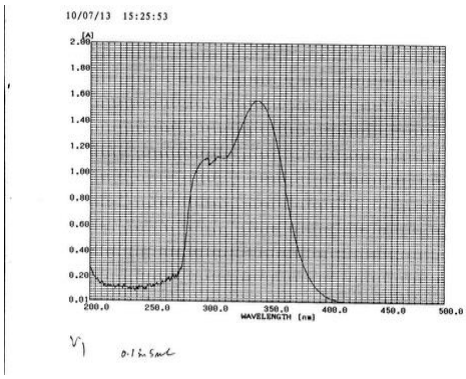

Figure 4: Spectrophotometric pic. Commercially prepared Maha Varthikava watee

RESULTS AND DISCUSSION

It was observed that physiochemical parameters, such as fiber content, acid insoluble ash content, dichloromethane extract percentage values, ethyl acetate extract percentage values, methanol extract percentage values of the commercial drug samples and the authentically prepared samples are not significantly different, while the weight of pill was significantly different, when compared with authentically prepared samples. However, the pH value was significantly different in first, second and third commercial samples, while in fourth and fifth were not significantly different from the prepared samples. Loss on drying was significantly different in first and second while other three samples were not significantly different, the Specific gravity was different only in second sample while other four samples were not significantly different when comparing with the authentically prepared samples at the 5% level. The mean of ash values was significantly different only in the fourth sample while other four samples were not significantly different; the mean of hexane extract percentage values were not significantly different except in the second sample. (Table 1)

HPLC is a popular method for analysis of herbal medicines because of its easy handling. Its usage is not limited by volatility of stability of the compound. Peak M is more prominent in commercial samples V_1, V_2 and V_3 (concentration 67.2, 63.5 and 46.8) and not in any of the prepared samples and V_2, V_5 commercial samples. Spectrophotometry is one of the branches of spectroscopy to measure the absorption of light by molecules that are in gas or in vapour state or dissolved molecules/ions. (Figure 1and 2)

In the spectrophotometric measurement all prepared samples and commercial samples V_2 and V_5 had a λ max of 287-290 nm while the V_1, V_2 and V_4 samples had a λ max 340-345 nm. There was a functional group in λ max of 287-290 nm in all prepared and two commercial samples and different functional group in λ max of 340-345 in other three commercial samples. Therefore the authentically prepared and V_2 and V_5 commercial samples have the same functional group while the other three commercial samples may have different functional group. (Figure 3 and 4) When the functional groups are different, the actions of these preparations may also different.

CONCLUSION

"Maha Vartikava Watee" can be standardized using physical, chemical, chromatographic and spectrophotometric parameters as modern scientific quality control tools. In addition, biological measurements and the presence of toxins can also be used as standardization parameters in the preparation of "Maha Varthikava Watee" in order to obtain the optimal efficacy of the medicine.

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