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Observations on the Quality of commercially manufactured Ayurvedic Decoction, *Maharasnadi Kvatha*

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ABSTRACT

Plan: *Ayurveda makes use of many Kvatha or hot infusions. In olden days the physician provided a handwritten recipe of the Kvatha in question and patients prepared these medicines at home. However, this tradition ended with the advent of commercial manufacture of Ayurvedic medicines in Kerala since 1902. Considering the growing popularity of Ayurveda, an attempt was made to compare the quality of seven brands of Maharasnadi Kvatha, a formulation that is widely recommended by physicians.*

Methodology: *Seven brands of Maharasnadi Kvatha available in the local market were procured. Their pH, total dissolved solids, sodium benzoate content, HPTLC profiles and microbial load were measured. Additionally, the presence of alkaloids, tannins, phenols, and sterols was detected qualitatively. Total tannins, alkaloids, phenols and sterols were estimated.*

Outcome: *The colour of the seven brands, their pH, total dissolved solids, sodium benzoate content, HPTLC profiles, microbial load and content of compound classes showed wide variation. HPTLC fingerprinting can serve as a useful technique to assess the quality of Ayurvedic medicines. The number of bands and their area percentages can serve as quality indices. Such methods can be used for improving the quality of Ayurvedic medicines.*

Keywords: *Ayurveda, Quality control, hot infusion, HPTLC*

1. INTRODUCTION

Ayurveda makes use of several classes of formulations. *Kvatha* or hot infusion is an important one among them. The standard procedure to prepare a *kvatha* is described in *Sarngadhara Samhita*, according to which 1 part of coarsely powdered herbs is to be boiled in 16 parts of water and the volume reduced by 1/8[1]. According to traditions of Ayurveda, the physician provides a handwritten recipe of the *kvatha* in question and the patient prepares the medicine at home, utilizing herbs procured locally. However, this tradition virtually disappeared ever since P.S. Variar founded Aryavaidya Sala at Kottakkal, Kerala, in 1902. Commercially manufactured *kvatha* are now widely used.

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More than 800 companies are engaged in the commercial production of Ayurvedic medicines in the province of Kerala. In the backdrop of growing popularity of Ayurvedic medicine, an attempt was made to compare the quality of several brands of a particular commercially manufactured formulation. *Maharasnadi Kvatha* was chosen for the study as it is the formulation that is maximally prescribed by physicians.

2. MATERIALS AND METHODS

Samples of *Maharasnadi Kvatha* manufactured by Aryavaidya Pharmacy (Cbe) Ltd. (Sample 1, Batch No. 74310), Aryavaidya Sala (Sample 2, Batch No. 508052), E.T.M. Oushadhasala (India) Ltd. (Sample 3, Batch No. 1241), Chyavana Ayurvedics (Sample 4, Batch No. 239), AMPIC Pharmacy Ltd. (Sample 5, Batch No. 8651),Bhuvaneswari Ayurvedics (Sample 6, Batch No. 193) and Vaidyaratnam Oushadhasala (P) Ltd (Sample 7, Batch No. 1042) were purchased from Ayurveda pharmacies in Chalakkudy town.

50 ml of each brand of *Kvatha* were taken in a glass beaker and the pH measured using a digital pH meter (Lab India Model No. PN 11280704). 50 ml of stirred *kvatha* was filtered through Whatman filter paper (No. 44) into a tared beaker and washed thrice with 30 ml of distilled water. The pooled filtrate was evaporated to dryness, weighed and the content of total dissolved solids (TDS) calculated[2]. The presence of alkaloids, tannins, phenols and sterols was detected qualitatively using methanol extract of the *kvatha*[3].Total alkaloids were estimated by gravimetry[4].Total tannins were estimated by titration against N/10 potassium permanganate solution in the presence of indigosulphonic acid[5].The content of total phenols was estimated using Folin-Ciocalteau reagent as described by Singleton and Rossi[6].Total sterols were extracted with ethyl acetate and measured spectrophotometrically, using cholesterol as standard[7].The content of sodium benzoate in the seven brands was estimated by titrimetry[8].

2.1. Microbiology

10 ml of each brand of *Maharasnadi Kvatha* were withdrawn and diluted serially to 10^{-6} using 90 ml of Butterfield's phosphate buffer. One ml of each diluted sample was pour plated using plate count agar for bacteria and Rose Bengal Chloramphenicol agar for yeast and mould. Incubation period for bacteria was 24 hrs at 37^{o}C. For yeast and mould the plated samples were incubated for 5-6 days at 20-25^{o}C[9, 10]. The samples were also tested for specific organisms like *E. coli*, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*[11].

2.2. HPTLC Profiling

2g sample of each brand of *Maharasnadi Kvatha* was dried in a water bath at 80^{0}C and extracted in methanol. 5µl aliquot of the extract was spotted on precoated silica gel HPTLC plate (10 x 10 cm, 0.2 mm thickness, 5-6 mm particle size) as band of 6 mm width using a Linomat 5 sample applicator fitted with 100µl Hamilton syringe. Seven samples were spotted on the same HPTLC plate (Tracks 1-7). After spotting the plate was dried and developed to a distance of 80 mm using a mobile phase composed of toluene-ethyl acetate-acetone-formic acid (20:4:2:1), in a CAMAG (Muttenz, Switzerland) twin-trough development chamber (20 x 10 cm), lined with filter paper and pre saturated with 30 ml of mobile phase for 20 minutes.

The developed plate was dried using a hair dryer and scanned through scanner 171019 at 254 nm and 366 nm. A spectro densitometer (CAMAG) equipped with win CATS planar chromatography manager (Version 1.4.6) software was used for densitometric measurements, spectra recording and data processing. Spectrum of each *kvatha* was recorded and the corresponding λ max noted. The plate was photographed at 254 nm and 366 nm using CAMAG documentation system DigiStore 2.

3. RESULTS

The colour of the seven brands showed wide variation. They were brick red, light brown, dark brown and black. pH of the samples varied from 4.18 to 5.02. TDS showed great variation. The lowest value was 1.69% and the highest was 27.84%. The content of sodium benzoate in the samples varied from 0.02% to 0.59% (Table 1). Similarly, the content of tannins, alkaloid, phenols and sterols also showed wide variation (Tables 2 and 3).

Table 1: Colour, pH, TDS and sodium benzoate content of the seven brands

Sample	*Colour*	*pH*	*TDS (%)*	*Sodium benzoate (%)*
Sample 1	Black	4.74	27.84	0.40
Sample 2	Brick red	5.02	24.05	0.59
Sample 3	Brick red	4.84	11.05	0.54
Sample 4	Dark brown	4.19	8.61	0.21
Sample 5	Light brown	4.18	1.69	0.02
Sample 6	Black	4.70	10.12	0.08
Sample 7	Dark brown	4.95	13.97	0.41

Table 2: Qualitative analysis of the seven brands

Sample number	*Alkaloids*	*Tannins*	*Phenols*	*Sterols*
Sample 1	+	+	+	+
Sample 2	+	+	+	+
Sample 3	-	-	-	+
Sample 4	+	+	+	+
Sample 5	-	-	-	+
Sample 6	+	+	+	+
Sample 7	-	+	+	+

Table 3: Content of compound classes in the seven brands

Sample number	*Tannins*	*Alkaloids*	*Phenols*	*Sterols*
Sample 1	0.19%	0.35%	1.20%	1.43%
Sample 2	0.25%	0.31%	0.53%	1.20%
Sample 3	0.13%	0.14%	0.22%	0.78%
Sample 4	0.12%	0.10%	0.38%	0.59%
Sample 5	0.06%	0.08%	0.19%	0.42%
Sample 6	0.14%	0.13%	0.67%	0.39%
Sample 7	0.23%	0.25%	0.93%	1.63%

Microbiology of the samples showed some consistency. Total plate count in all samples was $<10^5$ cfu/g and yeast and fungi were totally absent. Similarly, *E.coli*, *Salmonella* sp. and *Pseudomonas* sp. were also completely absent in all the samples. However, *Staphylococcus* sp. was detected in four samples (Table 4).

Table 4: Microbial load on the seven brands

Samples	*Total plate count*	*Specific Pathogens*			
		E.coli	*Salmonella* sp.	*Staphylococcus* sp.	*Pseudomonas* sp.
Sample 1	$<10^5$cfu/g	Nil	Absent	Present	Absent
Sample 2	$<10^5$ cfu/g	Nil	Absent	Present	Absent
Sample 3	$<10^5$cfu/g	Nil	Absent	Absent	Absent
Sample 4	$<10^5$cfu/g	Nil	Absent	Present	Absent
Sample 5	$<10^5$cfu/g	Nil	Absent	Absent	Absent
Sample 6	$<10^5$cfu/g	Nil	Absent	Present	Absent
Sample 7	$<10^5$ cfu/g	Nil	Absent	Absent	Absent

3.1. HPTLC of the seven brands

HPTLC of the seven brands present interesting data. All the seven brands show different bands and some similarity is shown by samples 1 and 7 at 254 nm as well as 366 nm (Figures 1 and 2).

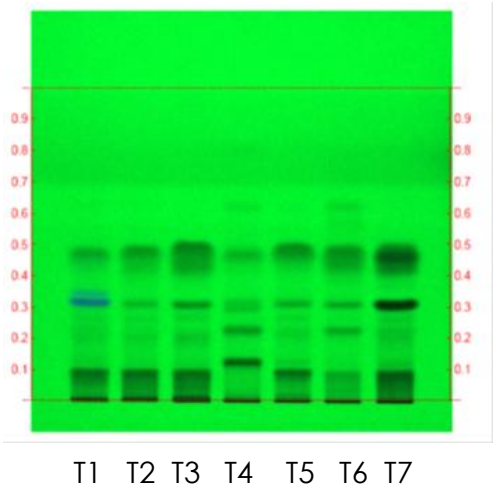

Figure 1: Chromatogram at 254 nm
Tracks T1-T7 represents respectively samples 1-7.

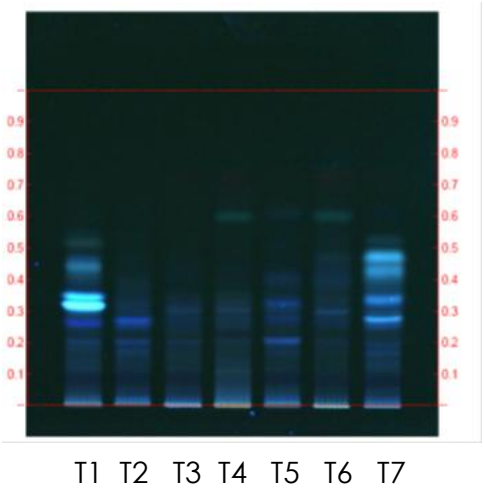

Figure 2. Chromatogram at 366 nm
Tracks T1-T7 represents respectively samples 1-7.

It was assumed that sample 1 is the best among the seven. The Rf and percentage area of the various bands present in track 1 were compared with corresponding bands in the remaining six tracks. The common peaks and their Rf at 254 nm and 366 nm are shown in Tables 5 and 6.

Table 5: List of common peaks and their Rf at 254 nm

Parameter	*Track 1*	*Track 2*	*Track 3*	*Track 4*	*Track 5*	*Track 6*	*Track 7*
Max. Rf	0.08	0.08	0.08	--	0.09	0.08	0.09
Area %	19.19	21.28	14.59	--	18.62	7.69	12.72
Max. Rf	0.26	0.26	0.26	--	0.27	0.26	0.26
Area %	3.70	3.84	3.40	--	3.71	1.79	2.87
Max. Rf	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Area %	18.27	9.46	9.55	5.79	10.97	9.16	19.55
Max. Rf	0.34	--	--	--	--	--	--
Area %	6.82	--	--	--	--	--	--
Max. Rf	0.47	0.48	0.49	0.47	0.49	0.48	0.47
Area %	29.19	52.74	51.45	27.10	46.66	47.32	47.99
Max. Rf	0.72	0.72	0.72	0.72	0.72	0.73	0.73
Area %	9.57	5.38	14.39	6.01	4.05	6.53	3.76
Max. Rf	0.80	--	--	0.81	0.80	0.80	0.81
Area %	7.30	--	--	13.35	8.91	8.00	7.22

Table 6: List of common peaks and their Rf at 366 nm

Parameter	*Track 1*	*Track 2*	*Track 3*	*Track 4*	*Track 5*	*Track 6*	*Track 7*
Max. Rf	0.08	0.09	0.09	--	0.09	0.09	0.09
Area %	18.28	25.29	46.76	--	39.21	28.22	32.45
Max. Rf	0.22	0.22	0.22	0.23	0.22	--	0.22
Area %	12.28	19.20	28.70	58.39	9.44	--	21.13
Max. Rf	0.31	0.31	0.30	--	0.31	0.31	--
Area %	28.22	10.67	8.91	--	8.18	5.80	--
Max. Rf	0.34	--	--	0.33	--	--	--
Area %	11.55	--	--	9.41	--	--	--
Max. Rf	0.38	0.38	--	--	0.39	0.39	0.38
Area %	6.00	37.79	--	--	32.26	65.98	38.97
Max. Rf	0.44	--	0.44	--	--	--	--
Area %	17.6	--	15.63	--	--	--	--

4. DISCUSSION

Maharasnadi Kvatha is a formulation widely used in the treatment of neurological diseases. The original formula is believed to have been designed by Prajapati[12].There is a need for standardizing this formulation, considering its widespread use. The results of the present study indicate that there is wide variation in the characteristics of the seven samples studied. The colour, pH, TDS and content of sodium benzoate showed variation. The pH of the samples varied from 4.18 to 5.02. Ayurvedic *kvatha* are generally acidic in nature. *Dasanga Kvatha, Vara Asanadi Kvatha* and *Guduchi Kvatha* are reported to have pH of 4.5, 5.5 and 5.7 respectively[13-15].

One major concern arising from the present study is the wide variation in TDS, which is an indicator of the total inorganic and organic substances present in a decoction. The lowest value was 1.69% and highest was 27.84% (Table 1). This has direct bearing on the medicinal quality of the samples studied. The high TDS of samples 1 and 2 is substantiated by the content of tannins, alkaloids, phenols and sterols (Table 3).

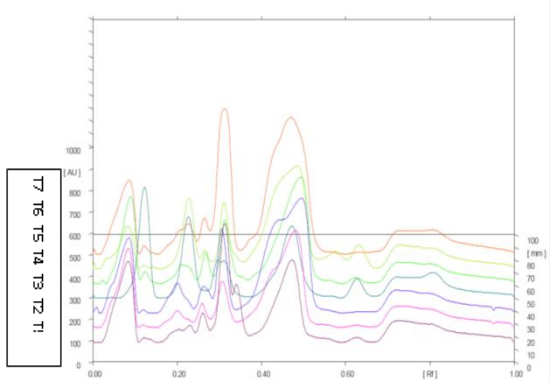

Figure3: Overlain chromatograms of the seven samples are 254 nm

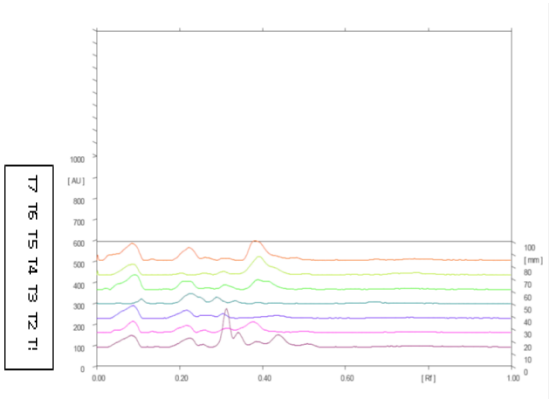

Figure 4: Overlain chromatograms of the seven samples are 366 nm

The overlain chromatograms of the seven samples at 254 nm and 366 nm are given in Figures 3 and 4.

Sodium benzoate is primarily used as an antimycotic agent in liquid pharmaceutical products. It is used in low-pH acidic food and used with a maximum limit of 0.1%. In most countries the maximum permissible use concentration is 0.15-0.25%[16]. However, in the present study, the samples showed wide variation in the content of sodium benzoate (0.02- 0.59).Though considered a GRAS (Generally Regarded As Safe) substance so far, it is now known that benzene can be formed in parts per billion level in beverages that contain both benzoate salts and ascorbic acid (Vitamin C)[17]. As benzene is a carcinogen[18], the high levels of sodium benzoate found in the samples tested in the present study are alarming.

Microbiological profiles of the seven brands showed consistency. However, four samples contained *Staphylococcus* sp. This finding emphasizes the need to check the microbial load of raw materials used in the manufacture of Ayurvedic medicines. Contamination of herbal medicine with potentially pathogenic bacteria is already reported in literature. In a study of bacterial contamination of powdered herbal medicinal preparations conducted in Nigeria, the products were found to be contaminated with *Salmonella typhii*, *Shigella* sp., *E. coli* and *Staphylococcus aureus*[19].

Gamma irradiation is now getting accepted all over the world as a phytosanitary treatment of herbal materials. It enhances the hygienic status of herbal materials and curbs losses due to microbial and insect infestation[20]. Such methods can be introduced into Ayurvedic industry also. In addition to this, production staff should be trained in Good Manufacturing Practices, Good Harvesting Practices and the safe handling and storage of Ayurvedic products[21].

HPTLC fingerprinting provides valuable information on the quality of the samples studied. The samples show great variation in the number of bands and their area percentages. This indicates that the samples are of differing quality.

The present study also shows that HPTLC fingerprinting is a reliable method to assess the quality of Ayurvedic medicines. Quality of a formulation can be confirmed by preparing a standard formulation using authentic crude herbs and comparing its HTLC fingerprint with that of the formulation in question. The study demonstrates that market samples of a popular Ayurvedic formulation like *Maharasnadi Kashayam* are of varying quality. This also partially explains the poor clinical performance of many commercially manufactured Ayurvedic medicines. Research and development efforts need to be initiated urgently to improve the situation.

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