

Standardization and quality control of *Darvyadi Pravahi Kvatha* - An Ayurvedic formulation

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The present study deals with the standardization and quality control of the *Ayurvedic* formulation *Darvyadi Pravahi Kvatha* following quality control procedures for the finished product. The results of physico-chemical parameters, viz. specific gravity (1.02), total solids (4.83%) and pH (5.54) were found. Microbiological limit test and heavy metals Pb, Cd, As, Hg were also found within the limits set by *Ayurvedic Pharmacopoeia of India* (API). The obtained values can be adapted to lay down new pharmacopoeial standards with batch to batch consistency. The phytochemical constituents found in the raw material used for the preparation of *Darvyadi Pravahi Kvatha* facilitate the desirable therapeutic efficacy of the medicinal formulations a whole in elements and also could help in understanding the underlying mechanism of pharmacological action.

Key words: *Ayurvedic* formulation, *Darvyadi Pravahi Kvatha*, Standardization, Quality control

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Ayurveda is an indigenous Indian system of medicine that is mainly plant-based and has gained worldwide attention due to safety and efficacy. However, due to lack of proper quality control methods there are variations in the same product obtained from different sources. Standardization is important for insuring of good quality products as standardized drugs of well defined consistent quality are needed for reliable beneficial therapeutic uses. Thus, there is an urgent need to develop parameters for quality control which are cost effective and can be easily adopted by the manufactures. Efforts are being made in this area that have led to the development of analytical protocols both for single herbal drugs as well as for compound herbal formulations¹⁻⁹ that can be used as valuable analytical tools in the routine standardization of *Ayurvedic* drugs and formulations. Standards such as identity, purity and potency of single drugs as well as formulations are important for ensuring therapeutic efficacy of herbal medicines. If the raw materials to be used in a medicine and stage by stage processes of manufacturing are standardized the final product namely, the compound formulation could be expected to conform to uniform standards. The study was

undertaken to develop methods for evaluation of *Darvyadi Pravahi Kvatha* an *Ayurvedic* compound formulation which is formulated by eight single ingredients¹⁰, viz. *Dārvi* (*Berberis aristata* DC., stem), *Rasānjana* (*Berberis aristata* DC., solid extract)¹¹, *Vrsa* (*Adhatoda zeylanica* Nees., root), *Abda* (*Cyperus rotundus* L., rhizome), *Kirāta* (*Swertia chirata* Buch Ham., whole plant), *Bilva* (*Aegle marmelos* Corr., fruit pulp), *Bhallātaka* (*Śuddha*) (*Semecarpus anacardum* L. f., fruit) and *Kairava* (*Nymphaea alba* L., flower) prepared as per AFI¹⁰ (Table 1). The *Pravahi Kvatha* is formulated in house and Chitrakoot Rasshala Pharmacy, Chitrakoot which is very effective in *Pradara* (excessive vaginal discharge)¹⁰ and its ingredients are also used to cure several diseases and preparation of *Ayurvedic* compound formulations. The *Darvyadi Pravahi Kvatha* were analysed following scientific parameters including organoleptic characters, physico-chemical analysis and chromatographic patterns. The work was undertaken is the trust as part of a program of testing and validation of traditional practices of using the *Ayurvedic* medicine. In this connection, standardization and quality control of *Darvyadi Pravahi Kvatha* becomes imperative. This paper dealt with standardization followed according to GMP

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Table 1—Ingredients of *Darvyadi Pravahi Kvatha*

S. No.	Sanskrit name	Botanical name	Part used	Portion
1.	Dārvī (<i>Dāruharidrā</i>)	<i>Berberis aristata</i> DC.	Stem	1 Part
2.	Rasānjana (<i>Dāruharidrā</i>)	<i>Berberis aristata</i> DC.	Solid extract	1 Part
3.	Vrsa (<i>Vāsā</i>)	<i>Adhatoda zeylanica</i> Nees.	Root	1 Part
4.	Abda (<i>Mustā</i>)	<i>Cyperus rotundus</i> L.	Rhizome	1 Part
5.	Kirāta (<i>Kirātatikta</i>)	<i>Swertia chirata</i> Buch Ham.	whole plant	1 Part
6.	Bilva	<i>Aegle marmelos</i> Corr.	Fruit pulp	1 Part
7.	Bhallātaka (<i>Śuddha</i>)	<i>Semecarpus anacardum</i> L. f.	Fruit	1 Part
8.	Kairava (<i>Kumuda</i>)	<i>Nymphaea alba</i> L.	Flower	1 Part

guideline. Standardization guidelines to be followed for herbals products provided by World Health Organization¹², and *Ayurvedic* pharmacopoeia of India have been considered.

Methodology

Traditional method for the preparation of *Rasanjana*

Rasant (also called *Rasaunt* or *Rasanjana*) is a crude, concentrated extract prepared from the stem bark and root of *Daruharidra* (*Berberis aristata* DC.). It is generally used for the preparation of *Rasant* in Himanchal Pradesh¹¹.

For the preparation of *Rasanjana*, root and stem bark of *Daruharidra* are collected from well grown plants. The collected root and stem bark of *Daruharidra*, are cleaned and washed in tap water to remove soil particles. They are chopped into small pieces and boiled in water in an aluminum vessel under low heat for 5-6 hrs. While boiling, care should be taken to boil the extract over low heat with continuous stirring to avoid burning of the extract. The watery extract is constantly stirred until the extract has a syrupy consistency. Then, the extract is filtered to remove the root samples and the impurities, and the extract is boiled again for an hour and cooled in the open air. After cooling, the extract becomes semi-solid and is called *Rasant*.

Collection and authentication of raw materials

Dārvī (*Berberis aristata* DC., stem), *Rasānjana* (*Berberis aristata* DC., solid extract) and *Bhallātaka* (*Śuddha*) (*Semecarpus anacardum* L. f., fruit) were procured from Karwi, Chitrakoot (UP) during 2012. Other plants like *Vrsa* (*Adhatoda zeylanica* Nees., root), *Bilva* (*Aegle marmelos* Corr., fruit pulp), *Kairava* (*Nymphaea alba* L., flower) and *Abda* (*Cyperus rotundus* L., rhizome), were collected during year 2012 from Chitrakoot forest range.

Whereas *Kirāta* (*Swertia chirata* Buch Ham., whole plant) was collected from herbal garden, Chitrakoot, Arogyadham in 2012. All the materials were authenticated with the help of Taxonomist Dr RLS Sikarwar at Deendayal Research Institute, Chitrakoot, Satna, MP.

Preparation of the *Darvyadi Pravahi Kvatha*

All the ingredients used were of pharmacopoeial quality¹³. Treated *Bhallātaka* to prepare *Śuddha-Bhallātaka*¹⁴. Cleaned, washed, dried and grind the *Dārvī* (*Dāruharidrā*), *Rasānjana*, *Vrsa* (*Vāsā*), *Abda* (*Mustā*), *Kirāta* (*Kirātatikta*), *Bilva*, *Kairava* (*Kumuda*) individually, crushed all the ingredients into pieces of 1-3 cm except 4 and 7. Weighed separately and mixed them in equal proportions (1:1:1:1:1:1:1) to ensure a homogenous mixture. Boiled the crush of all the ingredients together in 16 times water and reduce to 1/8th, filtered through muslin cloth and heated further to concentrate it to 1/4th, added approved preservatives, these were stored in an airtight containers to protect from light and moisture. Two samples were prepared at research laboratory Ayurveda Sadan, Chitrakoot Batch-A and Batch-B where Batch-C was prepared by Chitrakoot Rasshala Pharmacy, Chitrakoot.

Physicochemical tests

Organoleptic characters, specific gravity and physico-chemical analysis of all the samples were carried out. Quantitative analysis for total solids, pH of filtrate of 10% w/v aqueous solution was checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia¹⁵⁻¹⁷.

TLC profile

For HPTLC, 1 ml *Pravahi Kvatha* with 10 ml methanol (10 ml x 3), filtered each of the extracts and combined, add 5 gm of *anhydrous sodium sulphate*, filtrate and concentrated. TLC of extracts of all the samples were carried out on Silica Gel 60 F₂₅₄

precoated plates (0.2 mm thickness; from Merck India Limited Mumbai). A TLC applicator from Camag Linomat-5 (Camag Switzerland 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used was Ethyl acetate: Acetonitrile: Water (7:5:1). The plate was developed over a distance of 9 cm in a saturated development chamber (Twin trough chamber (10 x 10 cm with SS lid, and visualized under visible light, 254nm and 366 nm. After spraying with *Vanillin - sulphuric acid* followed by heating at 110 °C for 5-10 min¹⁸⁻¹⁹.

Test for microbial limits

Following tests were carry out as per WHO to determine the microbial load^{14,20} in three batches of *Darvyadi Pravahi Kvatha*, a formulated compound drug powder of pharmaceutical substances.

- (1) Enumeration of *Staphylococcus aureus*/gm
- (2) Enumeration of *Salmonella* sp./gm
- (3) Enumeration of *Pseudomonas aeruginosa*/gm
- (4) Determination of *E.coli*
- (5) Determination of total bacterial count (TBC)
- (6) Determination of yeast and mould

The microbiological tests were determined using specified agar and enrichment media from Himedia and Pvt. Ltd., Mumbai.

Heavy metal

Heavy metal analysis^{14,21} (lead, cadmium, arsenic and mercury) were carried out using Atomic absorption spectrophotometry (Shimadzu-Model-AA-7000). All samples were digested with concentrated HNO₃: HClO₄ (4:1). Standards solutions were made for different dilution to get linear calibration (Merck). Pb and Cd were performed using graphite oven method, while As (Arsenic) were determined as hydride method and Hg were determined using cold absorption method.

Results and discussion

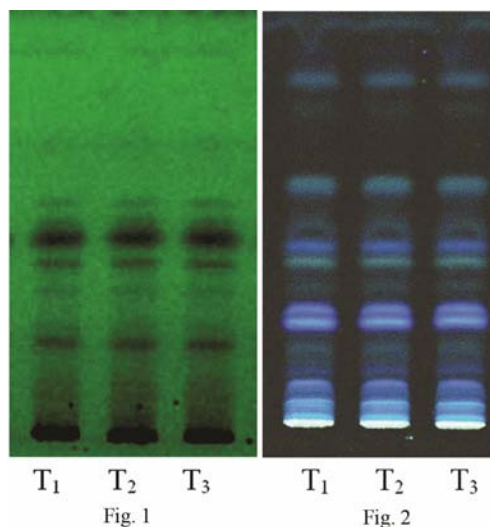
Light brown coloured clear liquid with a slight bitter taste; any sedimentation on standing shall be to a reasonable extent.

Physico-chemical tests were done and results are given in Table 2.

HPTLC fingerprint profile of the formulations are depicted in (Figs. 1-4) indicates the presence of all the ingredients in proportional quantity in

Table 2—Physico-chemical results of *Darvyadi Pravahi Kvatha*

Name of <i>Pravahi Kvatha</i>	Specific gravity	Total solids (%) w/w	pH
<i>Darvyadi Pravahi Kvatha</i> (Batch A)	1.02	4.90	5.57
<i>Darvyadi Pravahi Kvatha</i> (Batch B)	1.02	4.87	5.56
<i>Darvyadi Pravahi Kvatha</i> (Batch C)	1.02	4.73	5.52
Average value	1.02	4.83	5.54



Figs. 1-2, Fig. 1—TLC profile of *Darvyadi Pravahi Kvatha* observed under 254 nm Track T1: Batch A, Track T2: Batch B, Track T3: Batch C; Fig. 2— TLC profile of *Darvyadi Pravahi Kvatha* observed under 366 nm

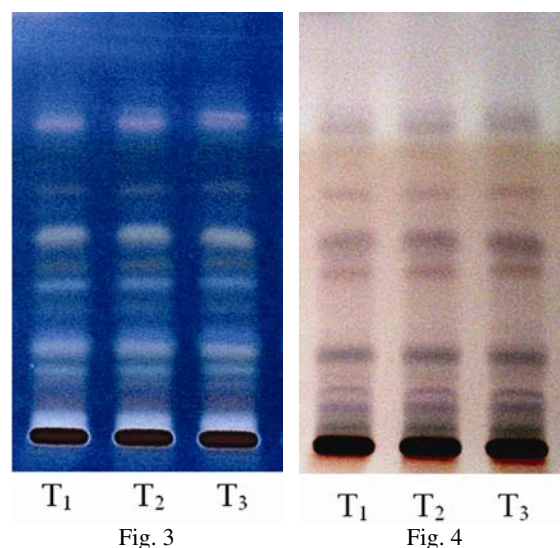


Fig. 3-4; Fig. 3—TLC profile of *Darvyadi Pravahi Kvatha* after spraying with *Vanillin-sulphuric acid* reagent observed under 366 nm, Fig. 4—TLC profile of *Darvyadi Pravahi Kvatha* after spraying with *Vanillin-sulphuric acid* reagent observed under visible light

Table 3—Heavy metal content of *Darvyadi Pravahi Kvatha*

Parameter	<i>Darvyadi Pravahi Kvatha</i> (Actual concentration unit is ppm)			WHO & API limites
	Batch A	Batch B	Batch C	
Lead (Pb)	0.1865	0.1867	0.1822	10 ppm
Cadmium (Cd)	ND	ND	ND	0.3 ppm
Arsenic (As)	0.013	0.013	0.014	03 ppm
Mercury (Hg)	0.012	0.012	0.012	01 ppm

the formulations. This confirms the batch- to- batch consistency of the finished products. Development of fingerprint profile would serve as a reference standard of the formulation. The TLC plate was examined under 254nm, 366nm, after derivatization 366nm and visible light. The R_f values and colours of the bands obtained were recorded. It shows major spots at 245 nm R_f 0.36, 0.45, 0.83(all spots were seen as black). It shows major spots at 366 nm R_f 0.09 (red), 0.16, 0.28 (both spots were seen as blue), 0.37(white), 0.52, 0.70, 0.76 (all spots were seen as blue), 0.83(pink), 0.90(red). And after derivatization the plate shows major spots at 366 nm R_f 0.17, 0.31, 0.37 (all spots were seen as brown), 0.58 (gray), 0.71(red), 0.77(brown), 0.97(red) and visible light R_f 0.51, 0.71, 0.76, 0.97 (all spots were seen as brownish red).

Total bacterial count (103-108 cfu/gm), Yeast and Moulds counts (25-28 cfu/gm) were reported to be less than the limit set by API (API limit total bacterial count -10^5 cfu/gm and yeast and mould -10^3 cfu/gm). Pathogenic bacteria, i.e., *Salmonella*, *Pseudomonas*, *Staphylococcus* and *E.coli* were not detected in samples. Heavy metals if present in the drug may carcinogenic and toxic. In the present study the level of heavy metals viz. Pb, Cd, As, Hg are within limit set by WHO (Table 3).

Conclusion

The present study reveals that sufficient quality control parameters were followed during the preparation of formulation *Darvyadi Pravahi Kvatha*. Physicochemical analysis, heavy metal analysis, and microbial overload analysis were carried out as per the norms of WHO guidelines. Generated physicochemical data can be assured its purity and strength. Heavy metal analysis shows that all the metals are within limit set by WHO. The absence of microbes in the finished product indicates the genuineness of final product. HPTLC profile generated in this particular study can be considered as a preliminary tool ascertaining the authenticity of *Darvyadi Pravahi Kvatha*.

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