Scientific evaluation of *Darvyadi Kvatha Curna* – A classical Ayurvedic compound formulation

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Ayurveda comprises of various types of medicines including *Asavas* (fermented infusions), *Arishtas* (fermented decoctions), *Curnas* (fine powder) and *Kvatha curnas* (coarse powder). These are regarded as valuable therapeutics due to their efficacy and desirable features. Identification and quality evaluation of crude drugs is a fundamental requirement of industry and other organizations dealing with natural health products. Thus, there is an urgent need to evaluate such parameters which can be adopted by the pharmaceutical industries. In present communication, attempt has been made to standardize *Darvyadi Kvatha Curna* an Ayurvedic compound formulation which is used to treat Pradara (Excessive vaginal discharge) and formulated by eight ingredients, viz. *Berberis aristata* DC. - stem, *B. aristata* DC. - solid extract stem, *Adhatoda zeylanica* Nees. - root, *Cyperus rotundus* L. - rhizome, *Swertia chirayita* (Roxb.) Buch.-Ham. ex C.B.Clarke - whole plant, *Aegle marmelos* Corr. - fruit pulp, *Semecarpus anacardium* L.f. - fruit and *Nymphaea alba* L. - flower. Three samples procured from different manufacturers were subjected to powder microscopic characterization, HPTLC fingerprinting and physico-chemical analysis. It was observed that the pharmacognostic and chromatographic analysis complements each other and can be implemented effectively for the identification of raw materials used in the compound formulation.

**Keywords:** *Darvyadi Kvatha Curna*, HPTLC fingerprint, Pharmacognosy, Scientific evaluation.

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**Introduction**

Ayurveda is a traditional Indian medical system which is being practiced for thousands of years. More than 1200 species of plants, nearly 100 minerals and over 100 animal products comprise the Ayurvedic system. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics of Ayurveda has been carried out and thereby numerous drugs have entered into the international pharmacopoeia. Ayurvedic system of treatment has been estimated to meet 70-80 % of the healthcare needs of India.

Present study was undertaken to develop methods for evaluation of *Darvyadi Kvatha Curna*, an Ayurvedic compound formulation which is formulated by 8 single ingredients, viz. Dārvī (*Berberis aristata* DC. - stem), Rasānjana (*B. aristata* DC.-solid extract stem), Vrsa (*Adhatoda zeylanica* Nees. - root), Abda (*Cyperus rotundus* L. - rhizome), Kirāta (*Swertia chirayita* (Roxb.) Buch.-Ham. ex C.B.Clarke - whole plant), Bilva (*Aegle marmelos* Corr. - fruit pulp), Bhallātaka (Śuddha) (*Semecarpus anacardium* L.f. - fruit) and Kairava (*Nymphaea alba* L. - flower). The *Kvatha curna* is formulated in Chitrakoot Rasshala Pharmacy, Chitrakoot which is very effective in Pradara (excessive vaginal discharge) and its ingredients are also used to cure several diseases and preparation of Ayurvedic compound formulations. The *Kvatha curna* was analysed following scientific parameters including organoleptic characters, microscopic characterization, physico-chemical analysis and chromatographic patterns.

**Materials and Methods**

Collection and authentication of raw materials

Dārvī (*B. aristata* DC. - stem), Rasānjana (*B. aristata* DC. - solid extract stem) and Bhallātaka (Śuddha) (*S. anacardium* L.f. - Fruit) were procured from Karwi, Chitrakoot (U.P.) during 2012. Other plants like Vrsa (*A. zeylanica* Nees – root), Bilva (*A. marmelos* Corr. - fruit pulp), Kairava (*N. alba* L. – flower) and Abda (*C. rotundus* L. – Rhizome) were collected during 2012 from Chitrakoot forest range.

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whereas Kirāṭa [S. chirayita (Roxb.) Buch.-Ham. ex C.B.Clarke -whole plant] was collected from Herbal garden, Chitrakoot, Arogyadham in 2012. All materials were authenticated with the help of taxonomist Dr. R L S Sikarwar at Deendayal Research Institute, Chitrakoot, Satna, Madhya Pradesh.

**Preparation of Kvatha Curna**

All the ingredients used were of pharmacopoeial quality. Treated Bhallataka to prepare Suddha-Bhallataka and passed through 710 µm IS Sieve (old sieve number 22). Cleaned, washed, dried and grinded the Dãrvî (Dãruharidrã), Rasãnjana, Vrsa (Vãsã), Abda (Mustã), Kirãṭa (Kirãtatikta), Bilva, Kairava (Kumuda) individually, passed through 710 µm IS Sieve (old sieve number 22) to obtain coarse powder. Weighed separately and mixed them in equal proportions (1:1:1:1:1:1:1:1) to ensure a homogenous mixture, these were stored in airtight containers to protect from light and moisture. Two samples were prepared at research laboratory Ayurveda Sadan, Chitrakoot Batch-A and Batch-B whereas Batch-C was prepared by Chitrakoot Rasshala Pharmacy, Chitrakoot.

**Microscopic examination**

For microscopic analysis about 2 g of Kvatha curna was washed thoroughly with water and water was poured out without loss of material; mounted a small portion in glycerine; warmed a few mg with chloral hydrate solution, washed and mounted in glycerine; treated a few mg with iodine in potassium iodide solution and mounted in glycerine. Heated a few mg in 2 % aqueous potassium hydroxide, then washed in water and mounted in glycerine. In 0.5 g of sample added 50 % conc. nitric acid in a test tube and warmed over water bath till brown fumes appeared. It was washed thoroughly with water and then mounted a small portion in glycerine which was subjected to microscopic examination.

**Physico-chemical tests**

Organoleptic characters, particle size and physico-chemical analysis of all the samples were carried out. Quantitative analysis was done for loss on drying at 105 °C, alcohol soluble extractive, water soluble extractive, total ash and acid insoluble ash.

**TLC profile**

For HPTLC, 2 g of each sample was extracted with 25 mL of methanol on boiling water bath for 25 min consecutively 3 times using fresh portion of 25 mL methanol, filtered and then concentrated. TLC of extracts of all the samples was carried out on Silica Gel 60 F$_{254}$ 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 Toluene: Ethyl acetate (7:3). The plate was developed over a distance of 9 cm in a saturated development chamber (Twin trough chamber 10x10 cm with SS lid) and visualized under visible light (254 and 366 nm) after spraying with 5 % methanolic sulphuric acid followed by heating at 110 °C for 5-10 min.

**Results and Discussion**

A coarse powder, grey in colour with an astringent odour and pungent taste was obtained. All particles passed through 710 µm IS Sieve (old sieve number 22) and not more than 10 % passed through 355 µm IS Sieve (old sieve number 22).

**Physico-chemical parameters and powder microscopic characteristics**

Physicochemical tests were done and results are given in Table 1. Powder microscopy was carried out:

<table>
<thead>
<tr>
<th>Name of Curna</th>
<th>Loss on drying (%) w/w</th>
<th>Total ash (%) w/w</th>
<th>Acid insoluble ash (%) w/w</th>
<th>Alcohol soluble extractive (%) w/w</th>
<th>Water soluble extractive (%) w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darvyadi Kvatha Curna (Batch A)</td>
<td>7.15</td>
<td>5.26</td>
<td>1.26</td>
<td>12.43</td>
<td>19.64</td>
</tr>
<tr>
<td>Darvyadi Kvatha Curna (Batch B)</td>
<td>7.13</td>
<td>5.36</td>
<td>1.40</td>
<td>12.55</td>
<td>19.73</td>
</tr>
<tr>
<td>Darvyadi Kvatha Curna (Batch C)</td>
<td>7.42</td>
<td>5.66</td>
<td>1.40</td>
<td>12.25</td>
<td>19.80</td>
</tr>
<tr>
<td>Dãrvî (Dãruharidrã)</td>
<td>5.56</td>
<td>8.26</td>
<td>2.56</td>
<td>9.86</td>
<td>19.6</td>
</tr>
<tr>
<td>Rasãnjana (Dãruharidrã)</td>
<td>3.93</td>
<td>8.26</td>
<td>1.56</td>
<td>5.93</td>
<td>36.6</td>
</tr>
<tr>
<td>Vrsa (Vãsã)</td>
<td>3.56</td>
<td>3.9</td>
<td>1.0</td>
<td>9.86</td>
<td>19.6</td>
</tr>
<tr>
<td>Abda (Mustã)</td>
<td>6.64</td>
<td>2.87</td>
<td>1.86</td>
<td>11.65</td>
<td>18.70</td>
</tr>
<tr>
<td>Kirãṭa (Kirãtatikta)</td>
<td>5.56</td>
<td>4.26</td>
<td>0.56</td>
<td>15.86</td>
<td>20.3</td>
</tr>
<tr>
<td>Bilva</td>
<td>3.56</td>
<td>2.63</td>
<td>0.56</td>
<td>9.86</td>
<td>52.6</td>
</tr>
<tr>
<td>Bhallataka (Suddha)</td>
<td>3.56</td>
<td>2.63</td>
<td>0.26</td>
<td>17.86</td>
<td>7.6</td>
</tr>
<tr>
<td>Kairava (Kumuda)</td>
<td>5.56</td>
<td>8.26</td>
<td>1.56</td>
<td>9.86</td>
<td>19.6</td>
</tr>
</tbody>
</table>
for individual ingredients present in the formulation. The following diagnostic characters are observed in the various mounts. Yellow coloured, short, lignified, thick walled phloem fibres with wide lumen and pointed ends, fibres associated with parenchymatous cells from medullary ray in radial view from phloem, cells containing prismatic crystals of calcium oxalate, debris from rhytidoma tissue fragments showing yellowish thin walled cork cells and cortical parenchyma with prisms of calcium oxalate crystals, as well as a few isolated stone cells confirm the presence of Dārvī; similarly, groups of rectangular stone cells having distinct pits and striations on the walls, cortical parenchymatous cells containing starch grains simple and compound having 2 to 3 components, round to oval 3 to 6 µ in diam. having concentric striations and hilum indicated the presence of Vrsā. Likewise, fibre sclerids from scale leaves in packed rows, beak shaped starch grains 6 to 28 µ confirm the presence of Abda; similarly, spongy parenchyma with minute acicular crystals, resin mass and mucilage cells from leaf, cortical

Plate 1—Powder microscopy of Darvāḍi Kvatha Cumna (Contd.)
Parenchyma with minute acicular crystals of calcium oxalate from root, unicellular to multicellular covering trichomes from leaf, lower epidermis in surface view showing anisocytic stomata, epidermis with striated cuticle and embedded with mucilage cells confirm the presence of Kirāta; in case of Bilva thick walled round to oval elongated parenchymatous cells containing oil globules and small prisms of calcium oxalate crystals, groups of round to oval stone cells with large lumen, elongated, pitted sclereids, endosperm cells filled with oil globules, prismatic crystals of calcium oxalate, simple and compound starch grains, testa in surface view and fibres were observed. Similarly the presence of Kairava can be detected by polygonal thin walled epidermal cells of the sepal with oil cells and anomocytic stomata, spherical or trigonal pollen grains measuring 11-24 μ and fragments of stellate sclereids (Plate 1).

**HPTLC fingerprint profile**

HPTLC fingerprint profiles of the formulations are depicted in Plate 2a-c. The presence of all the ingredients in proportional quantity in the formulations was ascertained. 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 366 nm, after derivatization 366 nm and visible light. The Rf values and colours of the bands obtained were recorded. It showed major spots at 366nm Rf 0.16, 0.28 (both blue), 0.37 (white), 0.52, 0.70, 0.76 (all blue), 0.83 (pink), 0.90 (red) and after derivatization the plate showed major spots at 366 nm Rf 0.17, 0.31, 0.37 (all brown), 0.58 (gray), 0.71 (red), 0.77 (brown), 0.97 (red) and visible light Rf 0.51 (blackish red), 0.71, 0.76, 0.97 (all brownish black).

The microscopic diagnostic features and physico-chemical tests of powder established the identity and strength of Darvyadi Kvatha Curna. The microscopical parameters can be used for checking the adulteration and purity of this compound formulation. HPTLC fingerprint profile helps in identification of various ingredients present in the Darvyadi Kvatha Curna thereby substantiating and authenticating of crude drug. These finding could be helpful in identification and authentication.
Plate 2—(a) HPTLC fingerprints profile at 366 nm (before derivatization), (b) HPTLC fingerprints profile at 366 nm (after derivatization) and (c) HPTLC fingerprints profile at visible light (after derivatization)

Track A: Batch A - Darvyadi Kvatha Curna; Track 1: Dārvī (Dāruharidrā); Track 2: Rasānjana (Dāruharidrā); Track 3: Vrsa (Vāsā); Track 4: Abda (Mustā); Track B: Batch B - Darvyadi Kvatha Curna; Track 5: Kirāta (Kirātatikta); Track 6: Bilva; Track 7: Bhallātaka (Śuddha); Track 8: Kairava (Kumuda); Track C: Batch C - Darvyadi Kvatha Curna.

Conclusion
The microscopic features, HPTLC fingerprint profiles and the physico-chemical parameters dealt within this paper may be used for standardization and quality evaluation of Darvyadi Kvatha Curna compound formulation. Comparison of these formulations with the different genuine ingredients further confirms the presence of individual components in them.

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