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Original Article
Standardisation and Quality Control - Formulation

Quality standards for Bhūnimbādi Kvātha Cūrṇa

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ABSTRACT

Introduction: Bhūnimbādi Kvātha cūrṇa (BKC) is a polyherbal formulation, compounded from parts of *Swertia chirata* Buch.Ham, *Aconitum heterophyllum* Wall. ex. Royle, *Symplocos racemosa* Roxb., *Cyperus rotundus* Linn., *Holarrhena antidysenterica* Wall., *Coleus vettiveroides* K. C. Jacob, *Coriandrum sativum* Linn., and *Aegle marmelos* Corr. as per formula composition in Ayurvedic Formulary of India. It is used in the treatment of asthma, cough, fever due to pitta dosa, bleeding disorder and fever. It is a popular medicine for cure of digestive impairments in fevers of different types, and many other clinical conditions. Though herbal medicines are effective, becoming popular, but they lack standardization. Standardization of herbal formulation is essential in order to assess the quality, purity, safety and efficacy of the drug. ***Methods*:** In the present study BKC was subjected to organoleptic, macro-microscopic, physicochemical, HPTLC and ^{1}H NMR characterization employing standard methodology mentioned in API and specified protocols. ***Results:*** Organoleptic characters, macro – microscopy, physico-chemical studies *viz.* total ash, water soluble ash, acid insoluble ash, water, alcohol and hydro – alcohol soluble extractive, loss on drying at 105°C, pH successive extractive values by Soxhlet extraction method and HPTLC and ^{1}H NMR were recorded. ***Conclusion*:** A monograph on quality standards for BKC is proposed from the data obtained. The results of the present investigation would serve as a document to control the quality of the multi-herb formulation BKC.

KEYWORDS

HPTLC, ^{1}H NMR fingerprint, monograph, polyherbal medicines, standardization.

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Ayurveda, the herb-based system of medicine is now well recognized not only in India, but also in other countries. As the need for safer drugs is increasing, attention has been drawn to the quality, efficacy, and standards of Ayurvedic formulations.[1] Because of the lack of quality profile and documentation there is a difficulty in the approval of Ayurveda medicines in the developed countries. All medicines of synthetic or plant origin should fulfil the basic requirements of being safe and effective. [2-9] Several problems, which are not applicable to synthetic drugs, often influence the quality of herbal drugs. Due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameter.[10] The World Health Organization has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety, and efficacy.[11] Hence every herbal formulation in the Ayurvedic Formulary needs standardisation studies.

Bhūnimbādi Kvātha Cūrṇa (BKC) of Bṛhannighaṇṭu-ratnākara, Pittajvara-prakaraṇa mentioned in Ayurvedic Formulary of India (AFI) for diseases like asthma, cough, fever due to pitta dosa, bleeding disorder and fever.[12] BKC is composed of coarse powder of ingredients *viz*. Bhūnimba (*Swertia chirata* Buch. Ham. - plant), Ativiṣā (*Aconitum heterophyllum* Wall. ex. Royle - tuber), Lodhra (*Symplocos racemosa* Roxb. – stem bark), Mustaka (*Cyperus rotundus* Linn. - rhizome), Indrayava (*Holarrhena antidysenterica* Wall. - seed), Bālaka (*Coleus vettiveroides* K. C. Jacob - root), Dhānyaka (*Coriandrum sativum* Linn. - fruit), and Bilva (*Aegle marmelos* Corr. - fruit pulp) in equal proportions. In the present study we made an attempt to develop the quality control parameters for BKC using organoleptic, macro-microscopic, physico – chemical, HPTLC fingerprint and ^{1}H – NMR spectroscopy analysis.

MATERIALS AND METHODS

Collection and identification of plant samples

Dry raw samples were collected from the raw drug section of SDM Ayurveda pharmacy, Udupi as well as some authentic raw material suppliers. The samples were authenticated using macro-microscopic examination, voucher specimens (No. SDM/VGST-SMYSR/BKC/01-08) have been deposited in the crude drug museum of Pharmacognosy department of SDMCRAAS, Udupi, Karnataka.

Method of Preparation of Bhūnimbādi Kvātha Cūrṇa

So far, there is no protocol for the preparation of kvātha cūrṇa (coarse powder) in Ayurvedic Pharmacopoeia of India (API) though there is protocol for preparation of cūrṇa (fine powder). BKC was prepared as per procedure detailed for cūrṇa in API,[13] with modification of sieve size. All the ingredients of Pharmacopoeial quality were washed separately to have no microbial load,[14] dried, and coarsely powdered. The individual raw drug powders were passed separately through sieve number 10. Each ingredient was weighed separately and mixed together in equal proportions as per AFI.[12] The mixture was passed through sieve number 10 to obtain a homogenous blend and packed in an air-tight container.

Organoleptic examination, macro – microscopy, physico-chemical studies *viz.* total ash, water soluble ash, acid insoluble ash, water, alcohol and hydro – alcohol soluble extractive, loss on drying at 105°C, pH successive extractive values by Soxhlet extraction method were carried out as per the standard procedures mentioned in API.[15]

TLC/HPTLC

Sample preparation

Ingredients- 5 g each of Bhūnimba, Ativiṣā, Lodhra, Mustaka, Indrayava, Bālaka, Dhānyaka and Bilva were extracted exhaustively with methanol using Soxhlet apparatus. The filtrate was concentrated to dryness and 100 mg of dried residue was dissolved in 5 ml of methanol in a standard flask individually.

Kvātha Chūrṇa - 40 g of BKC was extracted exhaustively with methanol using Soxhlet apparatus. The filtrate was concentrated to dryness and 100 mg of dried residue was dissolved in 5 ml of methanol in a standard flask.

Method

Extracts of ingredients and BKC were applied on aluminium plates precoated with silica gel 60 F_{254} of 0.2 mm thickness (Merck, Germany) using LINOMAT 5 applicator.[16] The plate was developed in CAMAG glass twin trough chamber previously saturated with mobile phase toluene: ethyl acetate: formic acid (7: 2: 0.2 *v/v*). The developed plate was visualized in CAMAG visualizing chamber and scanned in CAMAG SCANNER 4 under 254, 366. Derivatisation was done by dipping in vanillin-sulphuric acid (VS) followed heating at 105 °C till the spots appeared.[17, 18] The derivatized plate was scanned at 620 nm. CAMAG WinCATS software was used to draw R_f values and densitograms.

^{1}H NMR spectroscopy

Sample preparations

The method for the extraction was developed from a standard method,[19] with slight modification. Approximately 20 mg of all the samples were weighed into a 1.5 ml reaction tube and 1.5 ml of deuterated DMSO-D6 containing 0.05% TMS was added. One millilitre of the supernatant was added to a 5 mm diameter NMR spectroscopy tube and the samples were submitted for NMR analysis.

Operation parameters

^{1}H NMR spectra of the formulation was recorded at 28 °C on a Bruker Avance 500 MHz spectrophotometer (Bruker Analytik, Rheinstetten, Germany) equipped with a 5 mm cyroprobe head and operating at proton frequency of 500.13 MHz. The spectra were acquired with 256 scans, requiring approximately 10 min acquisition time. The following parameters were used during the acquisition: ≈0.16 Hz/point, pulse width (PW) 30° and relaxation delay = 1 s. FIDs were Fourier transformed with LB = 0.30 Hz. The spectra were referenced to the internal TMS peak. TOPSPIN version 1.3 software was used for spectra acquisition and processing of the ^{1}H NMR spectra which were manually corrected for phase and baseline distortions. The residual solvents signals for DMSO (2.65–2.45 ppm) and water (3.60–3.10 ppm) were excluded.

RESULTS AND DISCUSSION

Macroscopic features of ingredients of BKC are shown in (Figure 1). Microscopic features of powder of individual ingredient along with BKC are presented in (Figure 2 and 3); physico – chemical constants for all the ingredients used in the preparation of BKC is analysed and given in (Table 1). HPTLC profile has been generated for BKC with ingredients is given in (Figure 4 and 5) and R_f values are presented in (Table 2, 3 and 4). Methanolic extract of BKC was analysed using ^{1}H NMR spectroscopy is shown in (Figure 6). There is no monograph on standardization of BKC in API (Part II - Formulations). This study includes macro-microscopy features, organoleptic, physico – chemical, HPTLC fingerprinting and ^{1}H NMR spectroscopy of BKC. Data derived from the present study can be used as a monograph for standardization of BKC for the academia and industry.

Macroscopic features of ingredients of BKC recorded in the present study would help in identification of the ingredients using naked eye. *Andrographis paniculata* Nees is sometimes used as substitute for Bhūnimba, but it is the source of Kirātatikta API. Root stock of Bālaka is often found as adulterant to increase weight. In Bilva pieces of pericarp of fruit and plenty of seeds were found as adulterant. Detailed macroscopic examination will help in differentiating these adulterants/ substitutes from official drug. Texture of the Bhūnimbādi Kvātha Cūrṇa was coarse. Colour was yellowish brown, with bitter taste and characteristic odour due to the specific properties of a variety of ingredients.

Powder of Bhūnimba showed characters like thick walled polygonal cells, pitted parenchyma, tracheidal fibres, thin walled fibres and fragments of pitted vessels (Figure 2.1); fragments of leaf lamina with chlorophyll and epidermis in surface view are the characters of Bhūnimba detected in BKC (Figure 3.1). Powder of Ativiṣā showed characters like

Figure 1 Macroscopy of ingredients of Bhūnimbādi Kvātha Cūrṇa

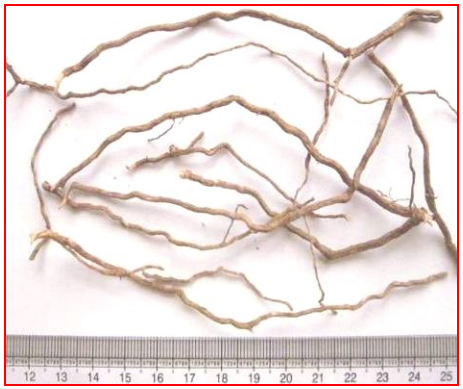

Bhūnimba – Whole Plant


Ativiṣā - Tuber


Lodhra - Bark


Mustaka – Rhizome


Indrayava - Seed


Bālaka – Root


Dhānyaka - Fruit

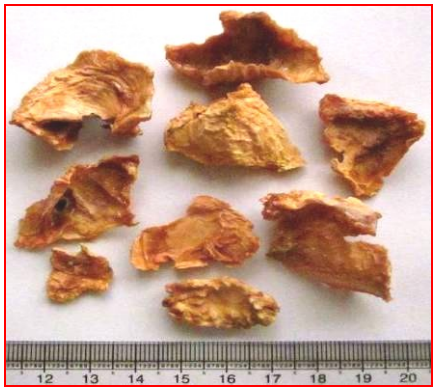
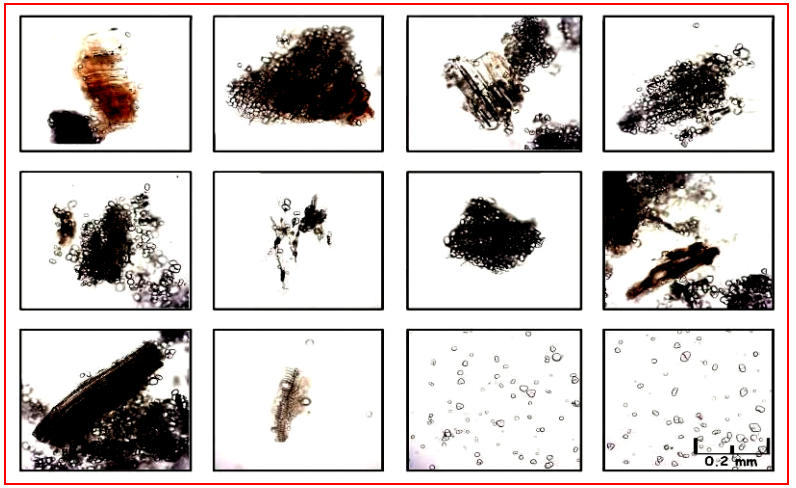
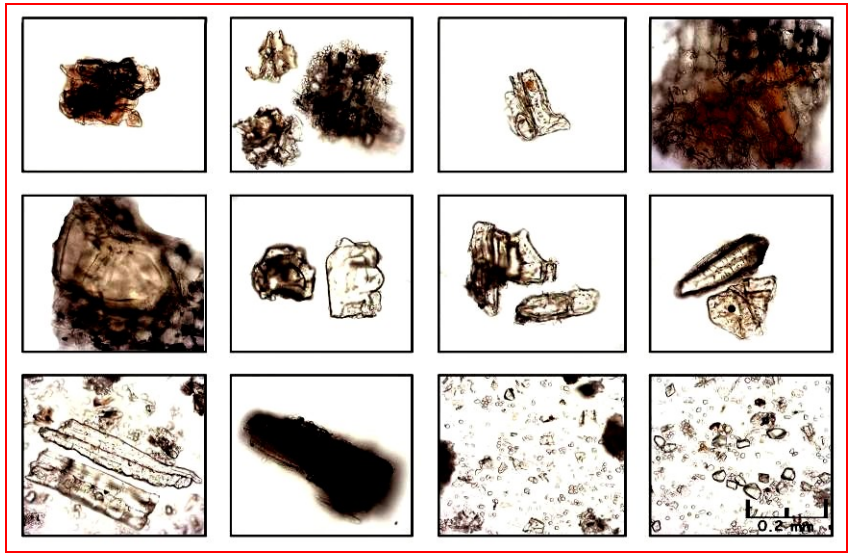

Bilva – Pulp

Figure 2 Microscopy of ingredients of Bhūnimbādi Kvātha Cūrṇa

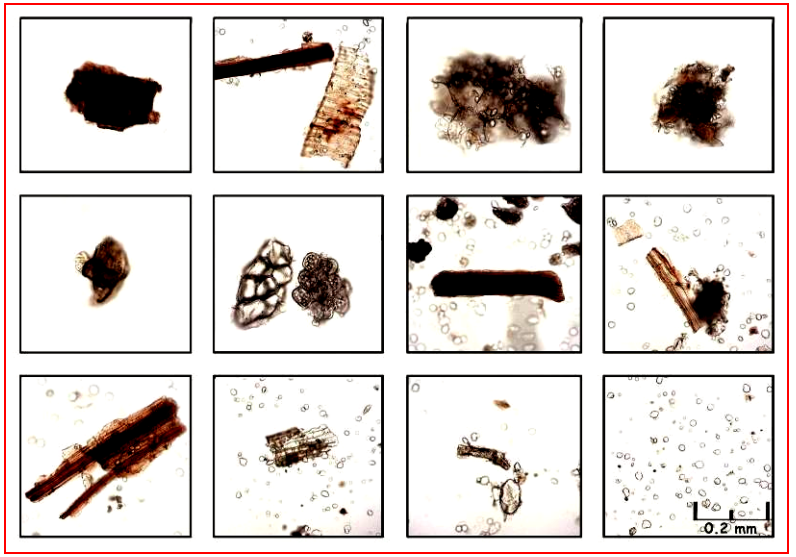
2.1 Bhūnimba



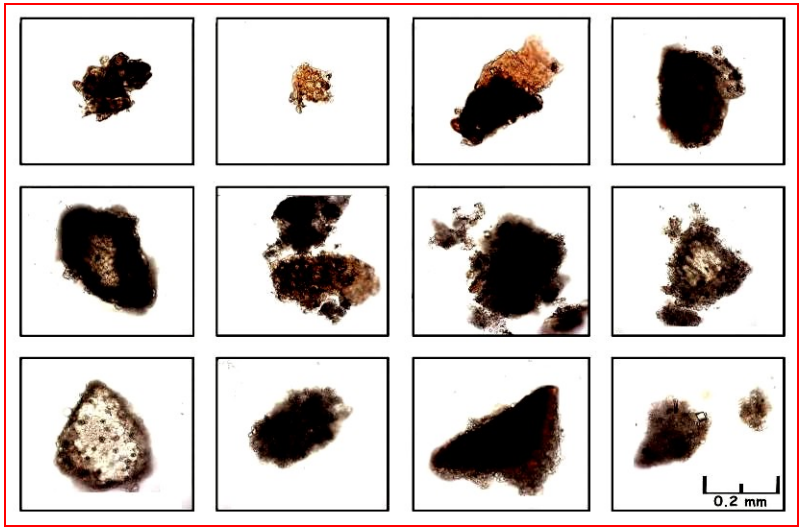
2.2 Ativiṣā



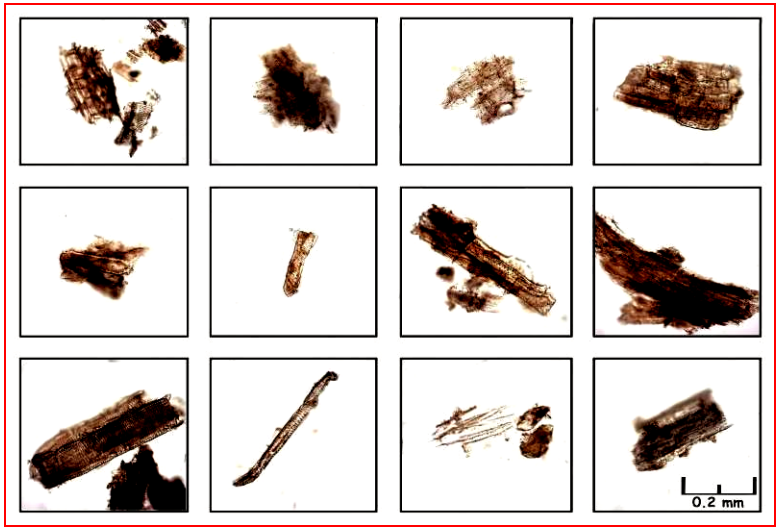
2.3 Lodhra



2.4 Mustaka

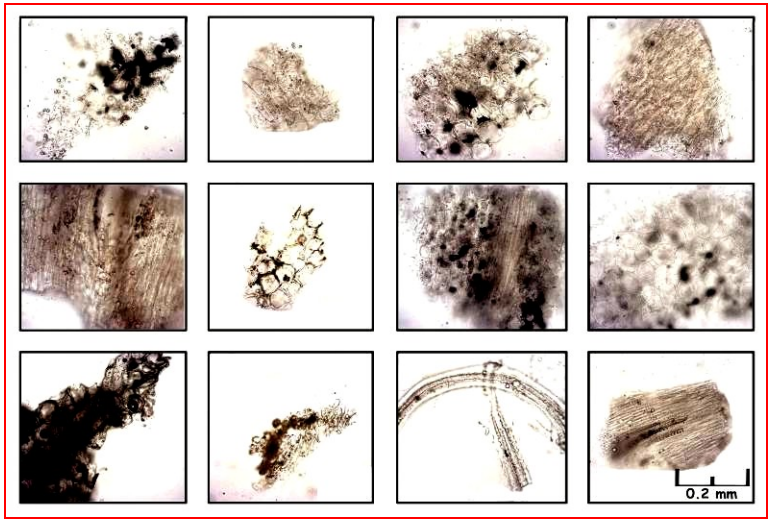


2.5 Indrayava



2.6 Bālaka

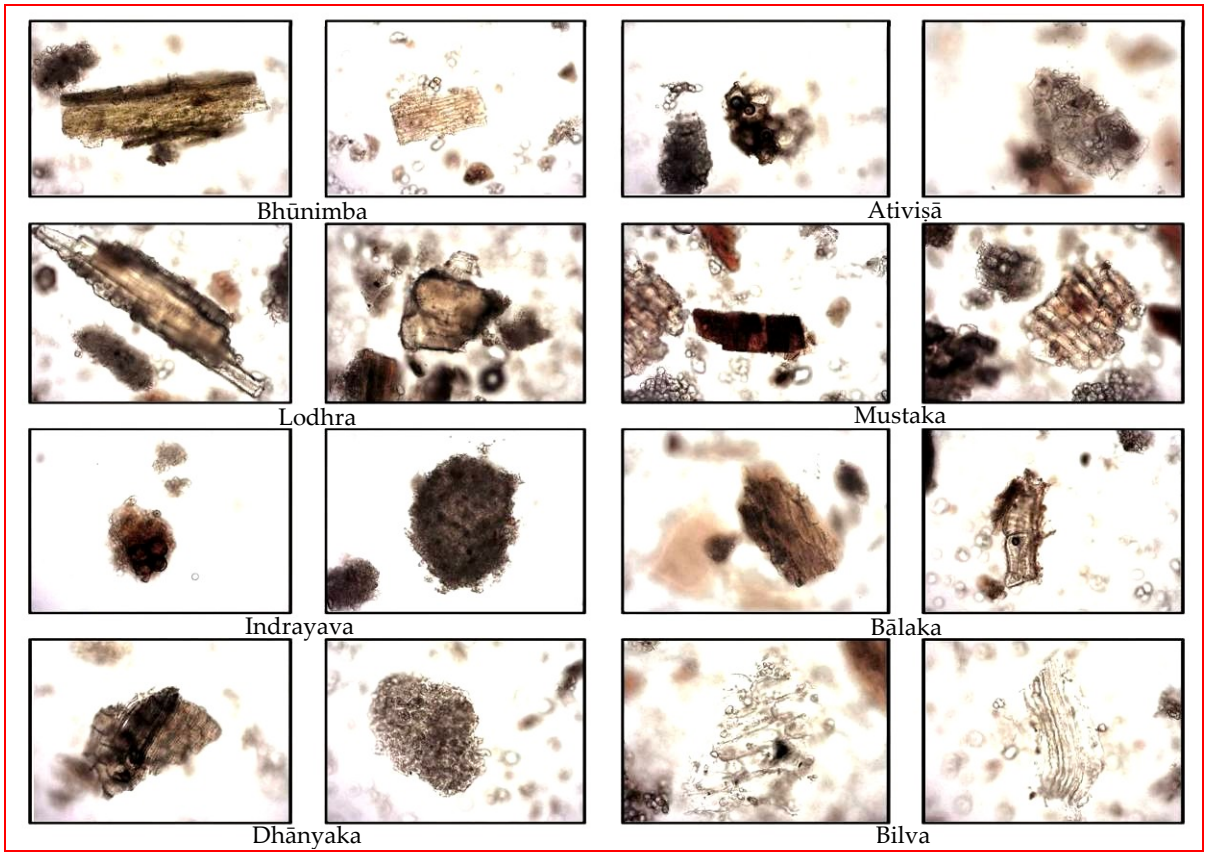
2.7 Dhānyaka



2.8 Bilva

thick walled polygonal cells with yellowish contents, thin and thick walled parenchyma with plenty of starch grains, fragments of spiral and pitted vessels, plenty of scattered simple to 3-compound starch grains (Figure 2.2);fragments of cork cells in surface view and parenchyma with plenty of starch grains are the characters of Ativiṣā detected in BKC (Figure 3.2). Powder of Lodhra showed characters like fragments of cork cells in surface view, parenchyma with few starch grains, groups of pitted parenchyma, plenty of pitted thick-walled sclereids, fragments of scereidal fibres often sheath of parenchyma with prismatic crystals in it (crystal fibre), plenty of scattered simple to 4-compound tiny starch grains and prismatic crystals of calcium oxalate (Figure 2.3); fragments of thick-walled crystal fibres, pitted stone cells and sclereids are the characters of Lodhra detected in BKC (Figure 3.3). Powder of Mustaka showed mostly reddish-orange coloured cells of cork in surface view, thick-walled rounded to elongated parenchyma with brown content of tannin or pale oleo-resin and starch grains, closely packed fibre-like sclerified cells, spiral and simple pitted vessels, round, oval to elongated starch grains and few rosette crystals of calcium oxalate are scattered throughout (Figure 2.4); fragments of thick-walled cells with reddish orange colour, and thick-walled polygonal parenchyma with oval to elongated starch grains and reddish orange resin content are the characters of Mustaka detected in BKC (Figure 3.4). Powder of Indrayava showed epidermis of testa in surface with reticulately thickened outgrowths, parenchyma with prismatic crystals, parenchyma with reticulate thickening, cells of cotyledon with rosette crystals (Figure 2.5); fragments of testa with reticulate balloon shaped outgrowths, and parenchyma with rosettes and prismatic crystals are the characters of Indrayava detected in BKC (Figure 3.5). Powder of Bālaka showed mostly reddish-brown coloured cells of cork in surface view, group of thick-walled pitted sclereids, thin-walled parenchyma framents, pitted and scalariform vessels, thin walled fibres (Figure 2.6); fragments of reddish brown parenchyma with contents, and thick-walled pitted sclereids in groups are the characters of Bālaka detected in BKC (Figure 3.6). Powder of Dhānyaka showed mostly yellowish coloured polygonal cells of epicarp in surface view, cells of pericarp forming parquetry arrangement, fragments of vittae, thick-walled cells with aleurone grains, oil drops and micro-rosette crystals, thin walled fibres in groups (Figure 2.7); fragments of parquetry cells of pericarp, and cells with micro-rosette crystals and oil drops are the characters of Dhānyaka detected in BKC (Figure 3.7). Powder of Bilva showed mostly colourless cells of pulp in surface view, many with simple starch grains, fragments of spiral vessels (Figure 2.8); parenchyma cells with inter cellular space containing simple starch grains, and fragments of spiral vessels are the characters of Bilva detected in BKC (Figure 3).

Figure 3 Microscopy of Bhūnimbādi Kvātha Cūrṇa



Loss on drying reveals the moisture content; total ash is the indication of total inorganic content; acid insoluble ash is the acid insoluble part of total ash, mainly silica; water soluble ash is the water soluble part of total ash indicating inorganic content without water insoluble inorganic salts like silica; alcohol and water soluble extractive is indicative of percentage active constituents soluble in ethanol and water; and pH is the indicative of acidity of sample. The above physico – chemical constants for all the ingredients used in the preparation of BKC is analysed (Table 1).

Table 1 Physico-chemical constants of raw materials used for the preparation of Bhūnimbādi Kvātha Cūrṇa

Ingredient	Results expressed as % w/w (n=3) Mean ± SD						
	LOD	TA	AIA	ASE	WSE	HASE	pH
Bhūnimba	12.21 ± 0.03	3.75 ± 0.08	0.75 ± 0.06	6.22 ± 0.38	9.94 ± 0.20	10.99 ± 0.74	5.93 ± 0.06
Ativiṣā	11.59 ± 0.14	2.44 ± 0.07	0.15 ± 0.07	1.94 ± 0.03	14.97 ± 1.01	18.24 ± 0.02	5.85 ± 0.05
Lodhra	9.79 ± 0.19	12.17 ± 0.01	0.95 ± 0.06	7.705 ± 0.22	24.66 ± 0.28	22.80 ± 0.06	5.10 ± 0.02
Mustaka	11.35 ± 0.02	6.94 ± 0.07	3.60 ± 0.14	1.74 ± 0.02	7.40 ± 0.42	7.34 ± 0.14	5.15 ± 0.01
Indrayava	7.52 ± 0.03	5.58 ± 0.10	0.495 ± 0.01	23.90 ± 0.40	21.47 ± 0.58	14.43 ± 0.32	4.65 ± 0.05
Bālaka	15.35 ± 0.32	10.84 ± 0.08	0.996 ± 0	4..33 ± 0.10	8.84 ± 0.03	6.65 ± 0.57	5.88 ± 0.04
Dhānyaka	8.20 ± 0.09	6.10 ± 0	0.65 ± 0.07	8.68 ± 0.27	11.85 ± 0.22	9.80 ± 0.16	4.94 ± 0.08
Bilva	14.69 ± 0.14	9.96 ± 0.1	0.30 ± 0	8.69 ± 0.02	52.87 ± 0.76	49.69 ± 0.22	6.04 ± 0.04

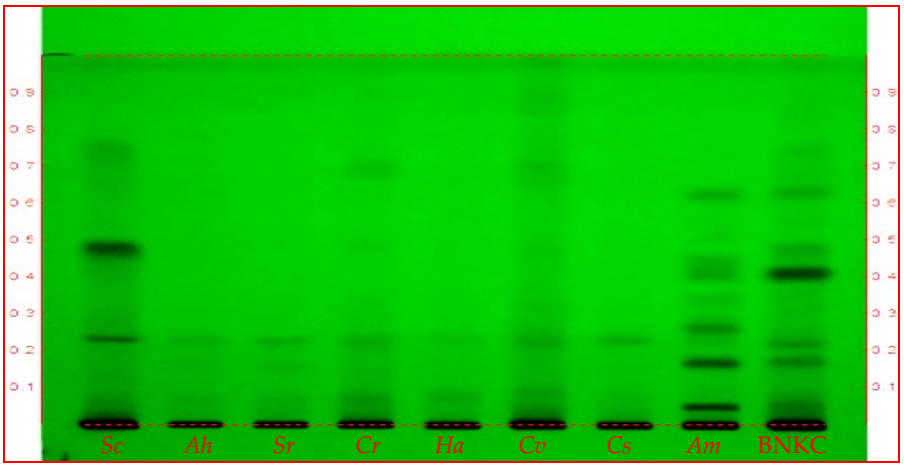
LOD – Loss on drying at 105º; FM - Foreign matter; TA - Total ash; AIA – Acid Insoluble ash; ASE – Alcohol soluble extractive; WSE – Water soluble extractive; HASE – Hydro alcohol soluble extractive

HPTLC profile has been generated for BKC with ingredients using toluene: ethyl acetate: formic acid (7: 2: 0.2 *v/v*) as solvent system. Fingerprint pattern of methanolic extract of BKC and its ingredients were documented under short UV, long UV and after derivatisation with vanillin sulphuric acid. Under short UV, Bhūnimba, Ativiṣā, Lodhra, Mustaka, Indrayava, Bālaka, Dhānyaka, Bilva and BKC showed 3, 2, 3, 5, 2, 8, 1, 8, 8 spots (all green) respectively. Eight spots occurred in BKC were from 8 of ingredients used for the formulation. Out of them, spot with R_f 0.05, 0.08 and 0.46 were detected in Bālaka and Bilva. R_f 0.16 was found in Lodhra, Mustaka and Bilva. R_f 0.41, 0.46 and 0.63 were found in Bilva. R_f 0.74 was found in Bhūnimba. R_f 0.23 was observed in all the tracks (Figure 4.1 and Table 2).

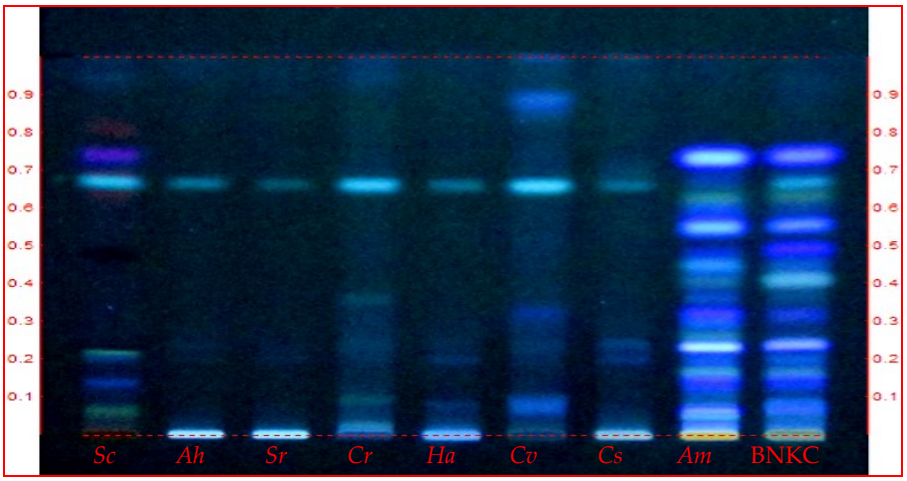
At 366 nm Bhūnimba , Ativiṣā , Lodhra, Mustaka, Indrayava, Bālaka , Dhānyaka , Bilva and BKC showed 7, 3, 3, 6, 4, 4, 3, 13 and 14 spots (of different fluorescent colours) respectively. Fourteen spots occurred in BKC, of them 13 were from 8 of ingredients used for the formulation. Spot with R_f 0.49 was formed after compounding of the ingredients to BKC. Spot with R_f 0.06, 0.14 and 0.74 were observed in Bhūnimba and Bilva. Spot with R_f 0.17, 0.20, 0.41, 0.45, 0.55, 0.63 were observed in Bilva. Spot with R_f 0.24 was observed commonly in Ativiṣā, Mustaka, Indrayava, Bālaka, Dhānyaka and Bilva. Spot with R_f 0.31was observed in Bālaka and Bilva. Spot with R_f 0.36 was seen in Mustaka and Bilva. Spot with R_f 0.67 has occurred in all the samples except in Bilva (Figure 4.2 and Table 3).

After derivatisation with VSA, Bhūnimba , Ativiṣā , Lodhra, Mustaka, Indrayava, Bālaka , Dhānyaka , Bilva and BKC showed 6, 1, 1, 4, 4, 4, 4, 1 and 7 spots (of different colours) respectively. Out of seven spots occurred in BKC, 6 were from 8 of ingredients used for the formulation, a spot with R_f 0.12 was formed after compounding of the ingredients to BKC. Spot with R_f 0.27 was observed in Bhūnimba and Dhānyaka. Spot with R_f 0.47 was observed in Bhūnimba. Spot with R_f 0.55 was observed in Mustaka, Indrayava, Bālaka and Dhānyaka. Spot with R_f 0.63 was observed in Indrayava, Bālaka and Dhānyaka. Spot with R_f 0.71 was observed in Indrayava and Dhānyaka. Spot with R_f 0.93 was observed in Bālaka (Figure 4.3 and Table 4).

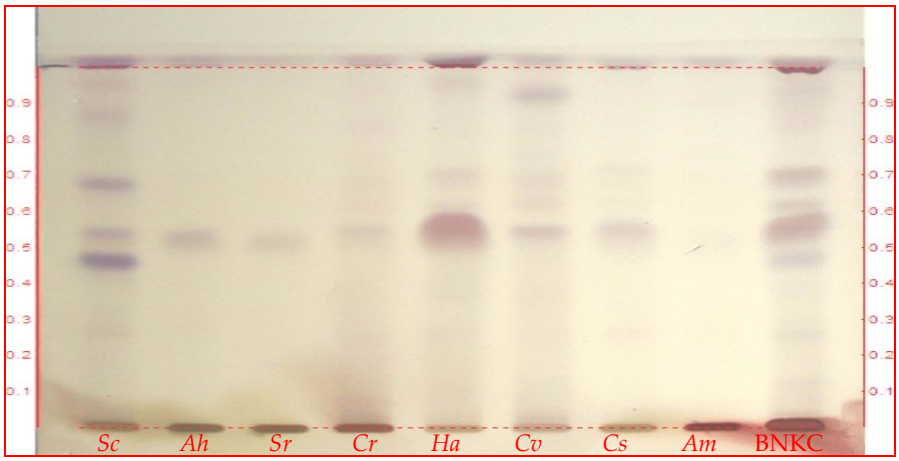
Figure 4 TLC Photo documentation of Bhūnimbādi Kvātha Cūrṇa & its Ingredients



4.1 Under short UV



4.2 Under long UV



4.3 Post derivatiation with VS

Solvent system - Toluene: Ethyl Acetate: Formic Acid 7: 2: 0.2

Sc – Kirātatikta (*Swertia chirata*) – 5 µl; *Ah* – Ativiṣā (*Aconitum heterophyllum*) - 5 µl; *Sr* – Lodhra (*Symplocos racemosa*) – 5 µl; *Cr* – Mustaka (*Cyperus rotundus*) - 5 µl; *Ha* – Indrayava (*Holarrhena antidysenterica*) – 5 µl; *Cv* – Bālaka (*Coleus vettiveroides*) - 5 µl;*Cs* – Dhānyaka (*Coriandrum sativum*) – 5 µl;*Am* – Bilva (*Aegle marmelos*) - 5 µl; BKC – Bhūnimbādi Kvātha Cūrṇa – 15 µl; VS – Vanillin Sulphuric acid spray reagent.

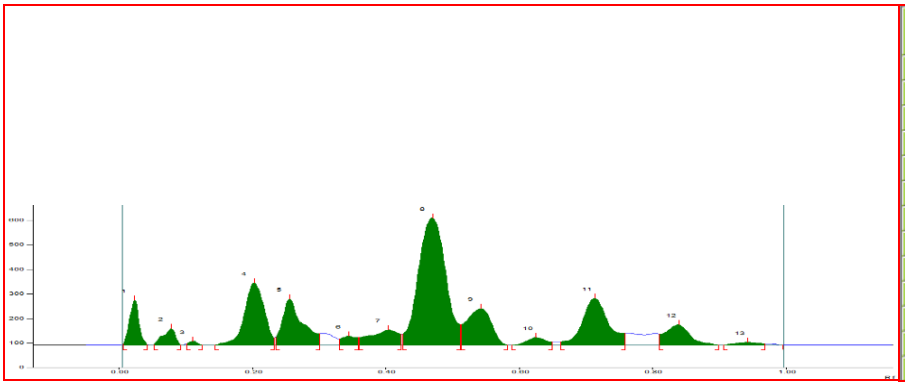
Table 2 R_f values of Bhūnimbādi Kvātha Cūrṇa (BKC) & its Ingredients at 254 nm

Bhūnimba	Ativiṣā	Lodhra	Mustaka	Indrayava	Bālaka	Dhānyaka	Bilva	BKC
-	-	-	-	-	0.05 L Green	-	0.05 D Green	0.05 L Green
-	0.06 L Green	0.06 L Green	0.06 L Green	0.06 L Green	-	-	-	-
-	-	-	-	-	0.08 L Green	-	-	0.08 L Green
-	-	0.16 L Green	0.16 L Green	-	-	-	0.16 Green	0.16 L Green
0.23 Green	0.23 L Green	0.23 L Green	0.23 L Green	0. 23 L Green	0. 23 L Green	0.23 L Green	0. 23 L Green	0. 23 L Green
-	-	-	-	-	-	-	0.26 Green	-
-	-	-	-	-	0.32 L Green	-	-	-
-	-	-	-	-	-	-	0.34 L Green	-
-	-	-	-	-	-	-	0.41 L Green	0.41 D Green
-	-	-	-	-	0.46 L Green	-	0.46 L Green	0.46 Green
0.48 Green	-	-	0.48 L Green	-	-	-		-
-	-	-		-	-	-	0.63 L Green	0.63 L Green
-	-	-		-	0.65 L Green	-	-	-
-	-	-	0.69 L Green	-	0.69 L Green	-	-	-
0.74 L Green	-	-		-	-	-	-	0.74 L Green
-	-	-	-	-	0.87 L Green	-	-	-
***3**	**2**	**3**	**5**	**2**	**8**	**1**	**8**	**8**

*Number of spots; Texts in highlight are spots with corresponding R_f values in BKC and ingredients. L – Light, D – Dark

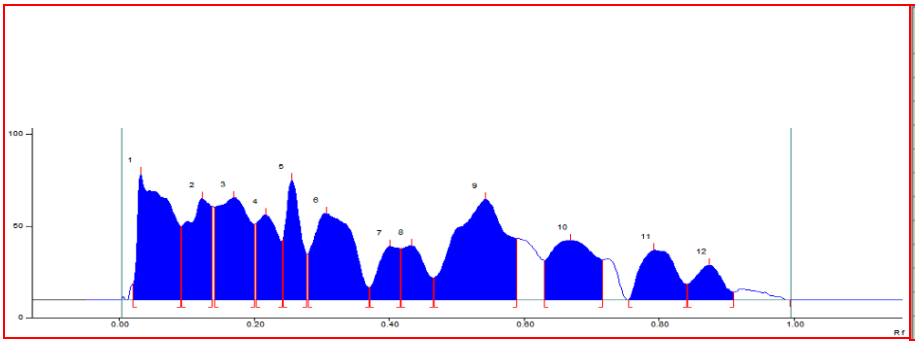
Densitometric scan of BKC showed 13, 12 and 10 peaks at 254 nm, 366 nm and 540 nm after derivatisation with vanillin sulphuric acid spray reagent (Figure 5).
Methanolic extract of BKC was analysed using ^{1}H NMR spectroscopy. The fingerprint showed peaks at 5.4, 3.6, 3.5, 2.1, 1.6, 1.3 and 0.9 ppm (Figure 6). ^{1}H NMR has been used as fingerprint for polyherbal formulation for the first time (Figure 6).

Figure 5 Densitometric scan of Bhūnimbādi Kvātha Cūrṇa



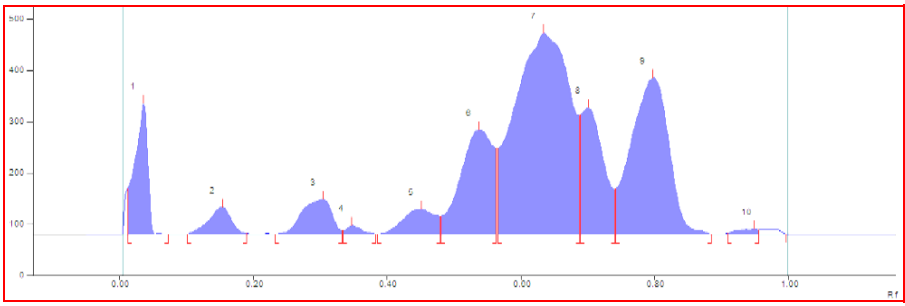
Peak	Max Position	Area %
1	0.02 Rf	4.32 %
2	0.08 Rf	2.09 %
3	0.11 Rf	0.34 %
4	0.20 Rf	12.66 %
5	0.26 Rf	9.60 %
6	0.35 Rf	1.42 %
7	0.40 Rf	4.41 %
8	0.47 Rf	35.98 %
9	0.54 Rf	9.21 %
10	0.63 Rf	1.48 %
11	0.71 Rf	12.61 %
12	0.84 Rf	5.18 %
13	0.94 Rf	0.70 %

5.1 At 254 nm



Peak	Max Position	Area %
1	0.03 Rf	13.50 %
2	0.12 Rf	8.13 %
3	0.17 Rf	11.29 %
4	0.22 Rf	6.07 %
5	0.26 Rf	6.18 %
6	0.31 Rf	11.51 %
7	0.40 Rf	3.80 %
8	0.43 Rf	4.11 %
9	0.54 Rf	17.27 %
10	0.67 Rf	9.06 %
11	0.79 Rf	5.89 %
12	0.88 Rf	3.20 %

5.2 At 366 nm



Peak	Max Position	Area %
1	0.04 Rf	6.15 %
2	0.15 Rf	2.24 %
3	0.30 Rf	3.74 %
4	0.35 Rf	0.46 %
5	0.45 Rf	2.97 %
6	0.54 Rf	12.34 %
7	0.64 Rf	40.15 %
8	0.70 Rf	10.30 %
9	0.80 Rf	21.24 %
10	0.95 Rf	0.40 %

5.3 At 540 nm (Post derivatisation)

Figure 6 ^{1}H-NMR spectroscopy of Bhūnimbādi Kvātha Cūrṇa

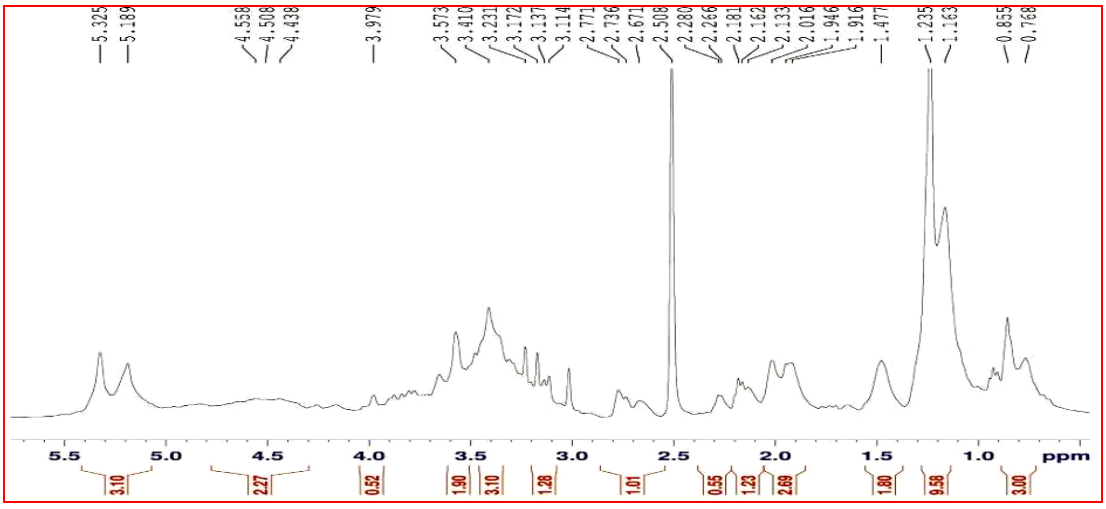


Table 3 R_f values of Bhūnimbādi Kvātha Cūrṇa (BKC) & its Ingredients at 366 nm

Bhūnimba	Ativiṣā	Lodhra	Mustaka	Indrayava	Bālaka	Dhānyaka	Bilva	BKC
0.06 F Green	-	-	-	-	-	-	0.06 F Purple	0.06 F Purple
-	-	-	0.08 F L Green	0.08 F L Blue	0.08 F Blue	-	-	-
0.14 F Purple	-	-			-	-	0.14 F Purple	0.14 F Purple
-	-	-			-	-	0.17 F L Blue	0.17 F Blue
-	0.19 F L Blue	0.19 F L Blue	0.19 F L Blue	0.19 F L Blue	-	0.19 F L Blue	-	-
-	-	-	-	-	-	-	0.20 F Blue	0.20 F L Blue
0.22 F Green	-	0.22 F L Blue	-	-	-	-	-	-
-	0.24 F L Blue	-	0.24 F L Blue	0.24 F L Blue	0.24 F L Blue	0.24 F L Blue	0.24 F Purple	0.24 F Purple
-	-	-	-	-	-	-	0.26 F L Green	-
-	-	-	-	-	0.31 F Purple		0.31 F Pink	0.31 F Purple
-	-	-	0.36 F L Green	-	-	-	0.36 F L Green	0.36 F L Green
-	-	-	-	-	-	-	0.41 F L Green	0.41 F Green
-	-	-	-	-	-	-	0.45 F Blue	0.45 F L Blue
-	-	-	-	-	-	-	-	0.49 F Pink
-	-	-	-	-	-	-	0.55 F Blue	0.55 F Purple
-	-	-	-	-	-	-	0.63 F G Pink	0.63 F Y Green
0.67 F M Blue	0.67 F M Blue	0.67 F M Blue	0.67 F M Blue	0.67 F M Blue	0.67 F M Blue	0.67 F M Blue	-	0.67 F M Blue
0.74 F Pink	-	-	-	-	-	-	0.74 F M Blue	0.74 F Purple
0.81 F Red	-	-	-	-	-	-	-	-
0.95 F L Blue	-	-	0.95 F L Blue	-	-	-	-	-
7*	**3***	**3***	**6***	**4***	**4***	**3***	**13***	**14***

*Total number of spots; Texts in highlight are spots with corresponding R_f values in BKC and ingredients. F – Fluorescent, L – Light, M – Medium, Y – Yellowish, G – Greenish

Table 4 R_f values of Bhūnimbādi Kvātha Cūrṇa (BKC) & its Ingredients post derivatisation with VSA

Bhūnimba	Ativiṣā	Lodhra	Mustaka	Indrayava	Bālaka	Dhānyaka	Bilva	BKC
-	-	-	0.07 L Brown	-	-	-	-	-
-	-	-	-	-	-	-	-	0.12 Grey
0.27 L Brown	-	-	-	-	-	0.27 Grey	-	0.27 Grey
0.47 Violet	-	-	-	-	-	-	-	0.47 Purple
-	-	0.51 Grey	-	-	-	-	-	-
0.53 Purple	0.53 Purple	-	-	-	-	-	0.53 Grey	-
-	-	-	0.55 Purple	0.55 Brown	0.55 Violet	0.55 Violet	-	0.55 Brown
-	-	-	-	0.63 Grey	0.63 Violet	0.63 Grey	-	0.63 Brown
0.68 Purple	-	-	0.68 Grey	-	0.68 Violet	-	-	-
-	-	-	-	0.71 Grey	-	0.71 Grey	-	0.71 Brown
-	-	-	0.84 Purple	-	-	-	-	-
0.87 Purple	-	-	-	-	-	-	-	-
-	-	-	-	-	0.93 Violet	-	-	0.93 Grey
0.95 Purple	-	-	-	0.95 Grey	-	-	-	-
6*	**1***	**1***	**4***	**4***	**4***	**4***	**1***	**7***

*Total number of spots; Texts in highlight are spots with corresponding R_f values in BKC and ingredients. L – Light

CONCLUSION

A comprehensive monograph on standards for quality of Bhūnimbādi Kvātha Cūrṇa of Ayurvedic Formulary of India[12] (AFI, 2011) has been proposed.

Definition:

Bhūnimbādi Kvātha Cūrṇa is a coarse powder preparation made with the ingredients in the Formulation Composition given below:

Formulation composition[12]

Bhūnimba (Kirātatikta)	*Swertia chirata* Buch. Ham.	Pl.
Ativiṣā	*Aconitum heterophyllum* Wall. ex. Royle	Rt.
Lodhra	*Symplocos racemosa* Roxb.	St. Bk.
Mustaka (Musta)	*Cyperus rotundus* Linn.	Rz.
Indrayava	*Holarrhena antidysenterica* Wall.	Sd.
Bālaka (Hrivera)	*Coleus vettiveroides*	Rt.
Dhānyaka	*Coriandrum sativum* Linn.	Fr.
Bilva	*Aegle marmelos* Carr.	Fr. Pp.

Method of preparation

- All the ingredients of Pharmacopoeial quality were washed properly.
- Dried raw drugs of were coarsely powdered separately.
- The individual powders were passed separately through sieve number 10 (1700 μm IS Sieve).
- Each ingredient was weighed separately and mixed together in equal proportions.
- Passed through sieve number 10 to obtain a homogenous blend.
- Packed it air-tight container and stored away from direct sunlight.

Characteristics and preservation

Kvātha Cūrṇa retains potency for two year and should be kept in an air tight container. They are also called Śṛta, Niryūha and Kaṣāya. Kvātha Cūrṇa can be used for preparing Kaṣāya, Hima, Phānṭa, etc[12]. (AFI, 2011).

Description

Yellowish brown coloured, coarse powder with characteristic odour and bitter taste.

Identification

Microscopy

The powder shows fragments of leaf lamina with chlorophyll and epidermis in surface view (Bhūnimba); fragments of cork cells in surface view and parenchyma with plenty of starch grains (Ativiṣā); fragments of thick-walled crystal fibres, pitted stone cells and sclereids (Lodhra); fragments of thick-walled cells with reddish orange colour, and thick-walled polygonal parenchyma with oval to elongated starch grains and reddish orange resin content (Mustaka); fragments of testa with reticulate balloon shaped outgrowths, and parenchyma with rosettes and prismatic crystals (Indrayava); fragments of reddish brown parenchyma with contents, and thick-walled pitted sclereids in groups (Bālaka); fragments of parquetry cells of pericarp, and cells with micro-rosette crystals and oil drops (Dhānyaka); and parenchyma cells with inter cellular space containing simple starch grains, and fragments of spiral vessels (Bilva).

Physico-chemical Parameters:

Loss on drying at 105º	:	Not more than 9.23 %
Total ash	:	Not more than 5.67 %
Acid-insoluble ash	:	Not more than 0.60 %
Water-soluble ash	:	Not more than 1.49 %
Alcohol -soluble extractive	:	Not less than 5.54 %
Water - soluble extractive	:	Not less than 13.39 %
Hydro – alcohol soluble extractive	:	Not less than 15.44 %
*p*H *(10% aqueous solution)*	:	Not more than 5.25

Thin Layer Chromatography

Under 254 nm 8 spots with R_f values of 0.05, 0.08, 0.16, 0.23, 0.41, 0.46, 0.63, 0.74 (All Green colour) were seen.

Under 366 nm 14 spots with R_f value of 0.06, 0.14 (Fluorescent Purple), 0.17, 0.20 (Fluorescent Blue), 0.24, 0.31 (Fluorescent Purple), 0.36, 0.41 (Fluorescent Green), 0.45 (Fluorescent Blue), 0.49 (Fluorescent Pink), 0.55 (Fluorescent Purple), 0.63 (Fluorescent Green), 0.67 (Fluorescent Blue), 0.74 (Fluorescent Purple) were seen.

After derivatisation with vanillin-sulphuric acid 7 spots with R_f value of 0.12 (Grey), 0.27 (Grey), 0.47 (Purple), 0.55 (Brown), 0.63 (Brown), 0.71 (Brown), 0.93 (Grey) were seen.

^{1}H-NMR

The ^{1}H NMR fingerprint shows peaks at 5.4, 3.6, 3.5, 2.1, 1.6, 1.3 and 0.9 ppm.

Important therapeutic uses

Asthama, cough, fever due to pitta dosa, bleeding disorder, fever.[12]

Substitutes and adulterants

Plant of *Andrographis paniculata* Nees is used as substitute for Bhūnimba. Pieces of stem in Bālaka and pericarp pieces and seeds in Bilva are found to be adulterants; these can be detected by macroscopic examination.

Dose

24 to 48 g twice a day.[12]

Sahapana

Honey.[12]

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CONFLICTS OF INTEREST

Nil

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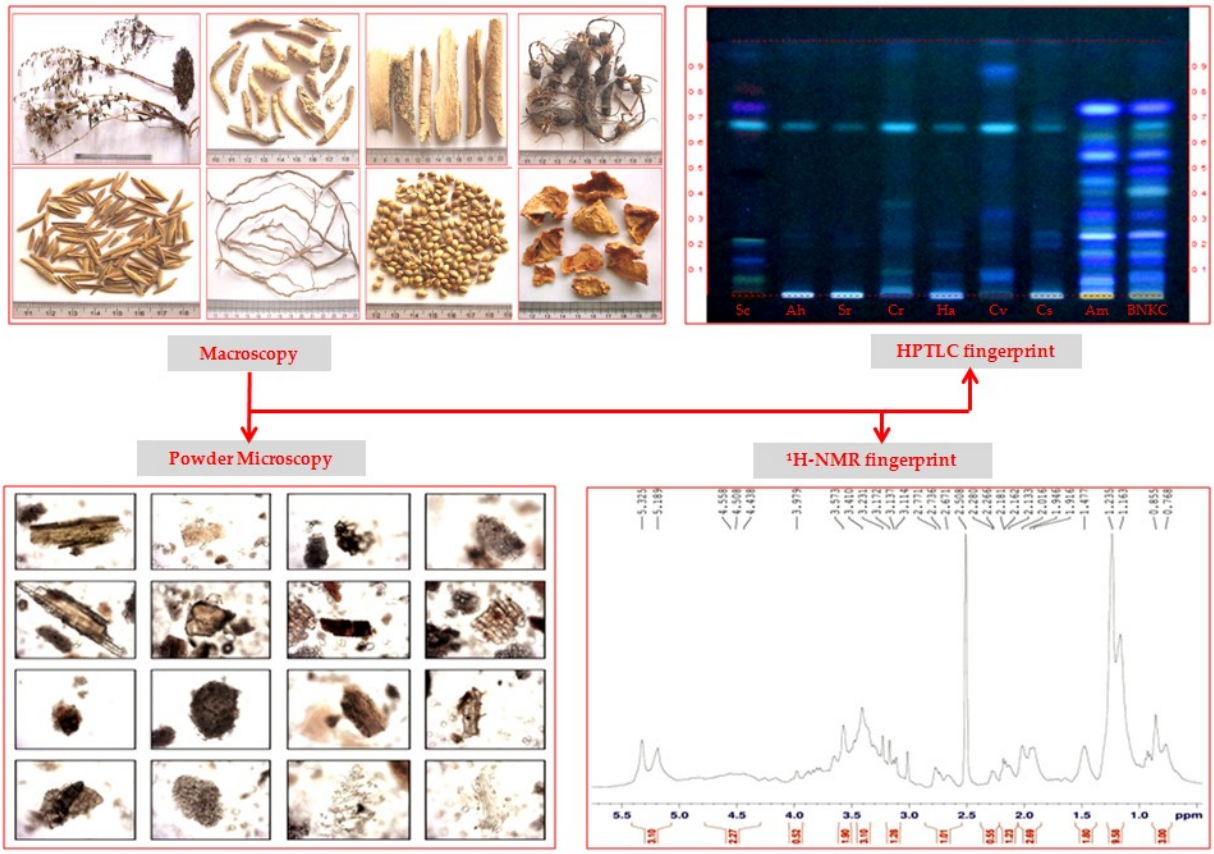
GRAPHICAL ABSTRACT



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