

Standardization and quality control of *Kutajastaka Kvatha Ghana Vati*: An *Ayurvedic* formulation

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Quality assurance of herbal medicine is an important factor and basic requirement for herbal drug industry and other drug development organizations. *Kutajastaka Kvatha Ghana Vati* is one of the ancient, most commonly used *Ayurvedic* formulations which are made in combination of nine plant ingredients. *Ayurvedic* literature reveals that the formulation was used for the treatment of *Daha* (burning sensation), *Raktatisara* (Diarrhoea with blood), *Sula* (pain), *Amadosa* (products of impaired digestion and metabolism) and *Sarvatisara* (all types of diarrhoea). Due to the lack of quality standards, there are batch to batch variations. The present study was thus undertaken to develop standards for the quality control of the *Vati*. All the ingredients were procured locally; identified by a taxonomist and an *Ayurvedacharya*; *Vati* was prepared in laboratory scale using GMP; and analyzed as per standard methods. All the samples were subjected to physico-chemical analysis, HPTLC finger printing, heavy metals analysis, and microbial load. The data obtained can be adopted for laying down the pharmacopoeial standards for *Kutajastaka Kvatha Ghana Vati*.

Keywords: HPTLC fingerprint, *Kutajastaka Kvatha Ghana Vati*, Pharmacognosy, Quality control, Standardization.

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Introduction

Kutajastaka Kvatha Ghana Vati is an *Ayurvedic* compound formulation, formulated with nine ingredients (Table 1) viz. *Kutaja* (*Holarrhena antidysenterica* Wall.- stem bark), *Ativisha* (*Aconitum heterophyllum* Wall.- root), *Musta* (*Cyperus rotundus* L.- rhizome), *Balaka* (*Coleus vettiveroides* K.C. Jacos.- root), *Lodhra* (*Symplocos racemosa* Roxb.- stem bark), *Raktacandana* (*Pterocarpus santalinus* L.f - heart wood), *Dhataki* (*Woodfordia fruticosa* Kurz.- flower), *Dadima* (*Punica granatum* L.- dried fruit) and *Patha* (*Cissampelos pareira* L.- root).

Classical literature reveals that the *Vati* is used for the treatment of *Daha* (burning sensation), *Raktatisara* (Diarrhoea with blood), *Sula* (pain), *Amadosa* (products of impaired digestion and metabolism) and *Sarvatisara* (all types of diarrhoea)¹. Standardized *Ayurvedic* formulations of uniform quality are essential for beneficial therapeutic use.

Due to lack of standards and quality control methods, there are batch to batch variations in the same formulation as well as variation amongst the same formulation procured from different sources. The study was undertaken in order to develop standards for the quality control of the *Vati* and the quality control parameters for standardization of the *Vati* have been taken with official methods².

Materials and Methods

Collection and authentication of raw materials

All the ingredients used in the preparation of *Vati* were collected by using good collection practices. *Ativisha* (*Aconitum heterophyllum* Wall.- root), *Balaka* (*Coleus vettiveroides* K.C. Jacos.- root), *Lodhra* (*Symplocos racemosa* Roxb.- stem bark), *Raktacandana* (*Pterocarpus santalinus* L.f.- heart wood) and *Dadima* (*Punica granatum* L.- dried fruit) were procured from Karwi, Chitrakoot (U.P.) during 2012. Other plants like *Kutaja* (*Holarrhena antidysenterica* Wall.- stem bark), *Musta* (*Cyperus rotundus* L.- rhizome), *Dhataki* (*Woodfordia fruticosa* Kurz. - flower) and *Patha* (*Cissampelos pareira* L. - root)³ were collected during the year 2012 from

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Table 1—Ingredients of *Kutajastaka Kvatha Ghana Vati*

Sanskrit /Hindi name	Botanical name	Parts used	Portion
<i>Kutaja</i>	<i>Holarrhena antidyssentrica</i> Wall.	Stem bark	1 part
<i>Ativisha</i>	<i>Aconitum heterophyllum</i> Wall.	Root	1 part
<i>Musta (Mustā)</i>	<i>Cyperus rotundus</i> L.	Rhizome	1 part
<i>Bālaka (hrīvera)</i>	<i>Coleus vettiveroides</i> K.C. Jacos.	Root	1 part
<i>Lodhra</i>	<i>Symplocos racemosa</i> Roxb.	Stem bark	1 part
<i>Candana (Raktacandana)</i>	<i>Pterocarpus santalinus</i> L.f.	Heart Wood	1 part
<i>Dhātakī</i>	<i>Woodfordia fruticosa</i> Kurz.	Flower	1 part
<i>Dadīma</i>	<i>Punica granatum</i> L.	Dried Fruit	1 part
<i>Pāthā</i>	<i>Cissampelos pareira</i> L.	Root	1 part

Chitrakoot forest. All the ingredients were authenticated with the help of taxonomist Dr. RLS Sikarwar, *Ayurvedacharya* and available literature³.

Preparation of the *Kutajastaka Kvatha Ghana Vati*

The authenticated crude drugs were crushed to a coarse powder separately (180 µm IS sieve or sieve number 85) and then mixed thoroughly with equal proportion of each ingredient (1:1: 1:1: 1:1:1) and 8 parts of water in a stainless steel container and then continuous mild heat was supplied until it was reduced to one-fourth of its initial quantity. During the heating process, continuous stirring was done to facilitate the evaporation and avoid any deterioration due to burning of materials. After a desirable reduction in volume was achieved, the *Kvatha* was filtered through single folded cotton cloth and collected in a separate vessel^{1,2,4,5}.

Kvatha was boiled again over low flame on a gas stove, maintaining the temperature between 90-95 °C till a semisolid consistency was obtained. As the water evaporates, viscosity of the extract increases, resulting in *Ghana*⁵ form. Then, the *Ghana* was mixed with the *Curnas* of *Kutajastaka* (up to 10 % of extract) further forming a solid mass.

The solid mass (*Ghana*) was forced through a number of 16 sieve and granules were prepared. The rounded *Vatis* were then dried in a tray-dryer at a temperature not exceeding 50-60 °C for 10-12 h. The formulation was then compressed in a single-punch press with a target weight of 250 mg. The *Vatis* were then stored in containers and packed in air-tight condition to protect them from light and moisture^{6,7}.

Two samples (Batch-A and Batch-B) were prepared in research laboratory at Ayurveda Sadan, Chitrakoot, whereas, Batch-C was prepared at Chitrakoot Rasshala Pharmacy, Chitrakoot.

Physico-chemical and quantitative parameters

Organoleptic characters, weight, loss on drying at 105 °C, total phenolics, and physico-chemical analysis of all the samples were carried out. Quantitative analysis for friability test (Digital friability test apparatus, Model 902, Electronic India, New Delhi) and disintegration time (Disintegration test apparatus, Model 1901, Electronic India, New Delhi) were checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia^{1,7,8}.

HPTLC profile

For HPTLC^{9,10}, 0.25 g of each dried samples and powdered *Ghana Vati* macerated with 10 mL ethanol were taken. Each extract was filtered and combined together. Added 5 g of anhydrous sodium sulphate, kept it for 10 min, filtered, and concentrated. HPTLC of extracts of all the samples were carried out on silica gel 60 F₂₅₄ precoated plates. Toluene: ethyl acetate: methanol: water (5: 3.5: 1: 0.5) were used as mobile phase. The plate was developed and visualized under ultraviolet at 366 nm, visible light, and after spraying with 5 % methanolic- sulphuric acid reagent followed by heating at 105 °C for 5 min.

Heavy metal

Heavy metal analysis⁷ (lead, cadmium, arsenic and mercury) was carried out using Atomic Absorption Spectrophotometry (Shimadzu-Model-AA-7000). All the samples were digested with concentrated HNO₃: HClO₄ (4:1). Standard solutions were made of different dilutions to get linear calibration (Merck). Lead (Pb) and cadmium (Cd) were performed using graphite oven method, while arsenic (As) was determined as hydride method and mercury (Hg) was determined using cold absorption method⁸.

Test for microbial limits

Following tests were carried out as per standard methods^{7,8,11} to determine the microbial load in three batches of *Kutajastaka Kvatha Ghana Vati*: i) Enumeration of *Staphylococcus aureus*/g, ii) Enumeration of *Salmonella sp.*/g, iii) Enumeration of *Pseudomonas aeruginosa*/g, iv) Determination of *E. coli*, v) Determination of total bacterial plate count (TBC), and vi) Determination of yeast and mould.

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

Results and Discussion

The observed organoleptic characters of *Kutajastaka Kvatha Ghana Vati* were: dark brown in colour, bitter in taste with a characteristic odour due to the specific properties of the various ingredients and consistency/ texture of was smooth (Table 2).

Physico-chemical parameters

Loss on drying in *Ghana Vati* was 6.6 %, weight of the *Vati* was 4.6 % and total phenolics were 2.03 % (Table 3).

Quantitative parameters

The friability and disintegration time of *Kutajastaka Kvatha Ghana Vati* was found to be 0.10 % and 30 min.

HPTLC finger print profile

HPTLC fingerprint profiles of the formulations are depicted in Plates 1. The TLC plate was examined under 366 nm, after derivatization 366 nm, and in visible light. The R_f values and colour of the bands obtained were recorded. It showed major spots at 366 nm R_f 0.08, 0.39 (both spots were seen as brick red), 0.17, 0.29, 0.46, 0.53, 0.65, 0.72, 0.79 (all spots were seen as blue), and 0.89 (fluorescent). After derivatization, the plate showed major spots at 366 nm R_f 0.22 (grey), 0.33 (blue), 0.47 (white), 0.68, 0.74, 0.82, 0.92 (all spots were seen as blue) and in visible light major spots were seen at R_f 0.45, 0.80, 0.98 (all spots were seen as brick red).

Table 2—Comparative organoleptic characters of *Kutajastaka Kvatha Ghana Vati*

Parameter	<i>Kutajastaka Kvatha Ghana Vati</i>		
	Batch A	Batch B	Batch C
Colour	Dark brown	Dark brown	Dark brown
Taste	Bitter	Bitter	Bitter
Odour	Characteristic	Characteristic	Characteristic
Consistency/ texture	Smooth	Smooth	Smooth

Table 3—Physico-chemical parameters of *Kutajastaka Kvatha Ghana Vati*

Parameter	<i>Kutajastaka Kvatha Ghana Vati</i>			Average Results
	Batch A	Batch B	Batch C	
Loss on drying at 105 °C (% w/w)	6.5	6.6	6.7	6.6
Average weight (% w/w)	4.5	4.6	4.7	4.6
Friability (% w/w)	0.10	0.11	0.10	0.10
Disintegration time (min)	30	30	30	30
Estimation of total phenolics (% w/v)	2.02	2.03	2.04	2.03

Heavy metals, if present in the drug may be carcinogenic and toxic. In the present study also, the level of heavy metals tested were found to be within the limit set by the Ayurvedic Pharmacopoeia of India (Table 4). These results thus revealed that the formulation develop is safe for consumption.



Plate 1-a) HPTLC fingerprints profile at 366nm (before derivatization), b) HPTLC fingerprints profile at 366nm (after derivatization), and c) HPTLC fingerprints profile at visible light (after derivatization)

Track A: Batch A- *Kutajastaka Kvatha Ghana Vati*; Track B: Batch B- *Kutajastaka Kvatha Ghana Vati* (2 samples were prepared at research laboratory Ayurveda Sadan, Chitrakoot Batch-A and Batch-B); Track C: Batch C- *Kutajastaka Kvatha Ghana Vati* (Batch-C was prepared by Chitrakoot Rasshala Pharmacy, Chitrakoot.)

Table 4—Determination of heavy metals in *Kutajastaka Kvatha Ghana Vati*

Parameters	<i>Kutajastaka Kvatha Ghana Vati</i>			Average results	Actual concentration unit	API limits
	Batch A	Batch B	Batch C			
Lead	2.15912	1.973	2.1833	2.1799	ppm	10 ppm
Cadmium	ND	ND	ND	ND	ppm	0.3 ppm
Arsenic	0.01910	0.0194	0.0194	0.0193	ppm	03 ppm
Mercury	0.01900	0.0204	0.0193	0.0195	ppm	01 ppm
ND- Not detected						

The microbial profile of the *Kutajastaka Kvatha Ghana Vati* was found satisfactory. TBC was an average of 83/g, while the yeast and moulds counts showed an average of 92/g. The recorded levels were far less than the limits set by the Ayurvedic Pharmacopoeia of India.

Pathogenic bacteria, i.e. *Salmonella sp.*, *P. aeruginosa*, *S. aureus* and *E. coli* were found to be absent in the formulation. These results thus revealed that the formulation developed is safe for consumption.

Conclusion

The study revealed that sufficient quality control parameters were followed during the preparation of formulation. Organoleptic parameters, physico-chemical analysis, heavy metal analysis, and microbial load analysis were carried out as per the norms of WHO/API guidelines and the absence of heavy metals and microbes in the finished product indicates the genuineness of final product. All the above physico-chemical, microbial load, and heavy metals data can be useful as diagnostic tool for identification and play an important role in quality control for further research.

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