

Pharmaceutical Standardization

Pharmacognostical and phytochemical evaluation of *Vara Asanadi Kwatha*

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Access this article online

Website: www.ayujournal.org

DOI: 10.4103/0974-8520.100330

Quick Response Code:



Abstract

Vara Asanadi Kwatha (VAK) is a compound Ayurvedic formulation prescribed in the management of obesity. Pharmacognostical study counting both macroscopic and powder microscopy of raw drug exposed the quality and genuineness of all the constituents of VAK. Organoleptic features of coarse powder made out of the crude drugs were within the standard range. Specific gravity of the decoction was 1.0185 and pH was 5.5. Total solid content present in the *Kwatha* was 4.525% w/v, total ash 0.949% w/v, and acid insoluble ash was 0.052% w/v. Iron assay showed the presence of Fe₂O₃ as 0.065% w/v. Qualitative scrutiny demonstrated the presence of flavonoids and tannis. Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) were carried out after organizing appropriate solvent system in which maximum three spots were distinguished in TLC and nine spots in HPTLC and most of the R_f values were identical when done with different sample extractive methods. This shows the presence of certain definite constituents in the decoction and is helpful for the easy separation of these constituents.

Key words: HPTLC, Pharmacognosy, TLC, *Vara Asanadi Kwatha*

Introduction

The vital responsibility played by herbal medicine in serving the therapeutic requirements of major fraction of human populace worldwide is identified since ancient times. But the quality control and standardization facets of these herbal drugs stay as a herculean task even in the 21st century. Accurate identification and guarantee of purity through pharmacognosy and pharmaceutical chemistry measures is inescapable ladder needed for the quality assurance and standardization of any of the herbal medicine whether it is single drug or formulation. *Vara Asanadi Kwatha* is one of the most potential herbo-mineral compound preparations mentioned in *Sahasra Yoga*^[1] which is claimed to be extremely successful in the management of obesity and overweight. But till now no pharmacognostical, pharmacological or phytochemical evaluation of this compound

is been conducted. Obesity is considered as a global epidemic and stands as the most established risk factor for number of serious disorders like Cardio Vascular Diseases (CVD), diabetes mellitus, metabolic syndrome etc.^[2] Ayurveda, the Indian traditional system of medicine can offer much in this regard, if thousands of unexplored combinations are brought into limelight. *Vara Asanadi Kwatha* is one among them. Ingredients of this decoction are Haritaki (*Terminalia chebula* Retz.), Bibhitaka (*Terminalia belerica* Roxb.) Amalaki (*Emblica officinalis* Gaertn.), Asana (*Pterocarpus marsupium* Roxb.), Chitraka (*Plumbago zeylanica* Linn.), Haridra (*Curcuma longa* Linn.), and Loha Patra (Thin iron foil/flake).

Materials and Methods

Collection

Dried specimens of mature fruits of *Triphala*, heart wood of *Asana*, rhizome of *Haridra*, roots of *Chitraka* and *Loha Patra* were collected from natural environment of Kannur, Kerala. The samples were preserved and transported by placing in separate high quality air tight containers avoiding any kind of infectivity. The samples were made into coarse powder from Dept of Bhaishajya kalpana I P G T and R A, GAU, Jamnagar. Later, the

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organoleptic and powder microscopy of all the individual drugs were carried out in Pharmacognosy Laboratory of I P G T and R A Jannagar. All the drugs were confirmed to be authentic and of good quality. The test drug *Vara Asanadi* decoction was prepared as per the classical reference and phytochemical analysis of the final product was carried out in Pharmaceutical chemistry Laboratory of IPGT and RA.

Microscopy

Powder microscopy of each raw drug was made with the coarse powder of the dried samples before and after staining. Phloroglucinol and HCl were used for the staining purpose.

Physicochemical parameters and qualitative analysis

Kwatha was made out of the ingredients in equal proportion and it was subjected to physicochemical parameters like refractive index, specific gravity, pH value, total solid content, total ash and acid insoluble ash. The percentage of iron present in the *Kwatha* was assessed by Iron assay method. In qualitative analysis, presence of tannins and flavonoids were assessed. TLC and HPTLC were carried out after making appropriate solvent system with ethyl acetate extract and methanolic extract of VAK.

Thin layer chromatography

Thin layer chromatography (TLC) and high-performance thin layer chromatography (HPTLC) were performed for the normal phase separation of components of ethyl acetate and methanol extracts of VAK. Solvent system was prepared by taking toluene, ethyl acetate, formic acid, and glacial acetic acid in a proportion of 5:5:1:1, respectively.^[3] The spots obtained from both the extracts were examined under ultra violet light of wave length 254 nm and 366 nm.

- Sample preparation: Track 1: Ethyl acetate extract of VAK (E.A)
- Track 2: Methanol extract of VAK(Me-OH)

- Solvent system -Toluene:Ethyl acetate:Formic acid:Glacial acetic acid(5:5:1:1)
- Stationary phase- Silica gel G
- Visualization-Under long U.V (366nm) and short U.V (254nm)

High-performance thin layer chromatography

HPTLC study of the ethyl acetate extract and Methanol extract was also carried out by using the same solvent system of Toluene+Ethyl acetate+Formic acid+Glacial acetic acid (5:5:1:1).^[4] After completion of HPTLC; post chromatographic deprivation was done with methanolic sulphuric acid.

Track 1: Ethyl acetate extract of VAK

Tack 2: Me-OH extract of VAK

Solvent system: Toluene+Ethyl acetate+Formic acid+Glacial acetic acid (5:5:1:1)

Observations and Results

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of VAK. For that, coarse powder of all the ingredients/drugs was subjected for organoleptic feature estimation.

Detailed pharmacognostical evaluation was carried out for all the ingredients of VAK. Macroscopic characters of the each drug observed were as mentioned in Table 1. The powder microscopy of the roots of *Plumbago zeylanica* revealed presence of lignified pitted parenchyma [Figure 1], sclerieds, lignified fibres thick walled cork cells [Figure 2], and reticulate vessels [Figure 3]. Phloem fibers [Figure 4], pitted stone cells [Figure 5] and bordered pitted vessels [Figure 6] were present in the Ht.wood of *Pterocarpus marsupium*. When microscopy of coarse powder of mature fruit of *Terminalia chebula* was done it showed features like parenchyma [Figure 7] simple and compound starch grains [Figure 8], sclerieds [Figure 9], tannin

Table 1: Macroscopic Characters

	<i>Chitraka</i>	<i>Asana</i>	<i>Haritaki</i>	<i>Amalaki</i>	<i>Bibhitaka</i>	<i>Haridra</i>
Part used	Root	Ht.wood	Pericarp of dried mature fruit	Pericarp	Pericarp	Rhizome
Nature of powder	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse
Color	Yellowish brown	Brownish red	Brownish grey	Grey	Grey	Yellowish red
Taste	Bitter	Bitter - astringent	Bitter - astringent	Bitter	Astringent	Bitter
Odor	Not specific	Not specific	Aromatic	Aromatic	Slight aromatic	Highly aromatic



Figure 1: Pitted parenchyma

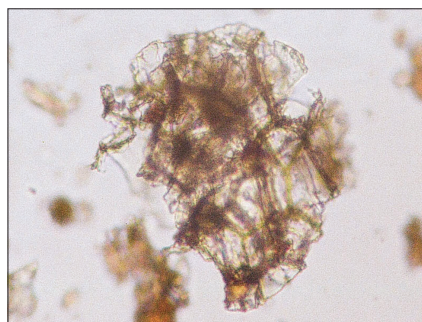


Figure 2: Sclerieds, lignified fibres thick walled cork cells



Figure 3: Reticulate vessels

contents, fibres etc. Features seen in the powder microscopy examination of *Emblica officinalis* were tannin [Figure 10] oleoresin, mesocarpal cells [Figure 11], fibres [Figure 12] pitted stone cells, and prismatic crystals. Whereas *Terminalia belerica* presented with abundant number of trichomes [Figure 13], tannins [Figure 14], starch grains, pitted stone cells.

[Figure 15], sclerieds etc. *Curcuma longa* on microscopy mainly consist of annular vessels [Figure 16], scleriform vessels.

[Figure 17], and epidermal cells [Figure 18]. The diagnostic features obtained by powder microscopy was compared with the standards mentioned in Ayurvedic Pharmacopoeia of India (API).^[5] The details of results obtained in pharmacognostical study are enlisted in the Table 2.

Organoleptic parameters: The prepared test drug VAK is liquid in form, brownish – black in color, astringent in both taste and odor.



Figure 4: Phloem fibers



Figure 5: Pitted stone cells



Figure 6: Bordered pitted vessels



Figure 7: Parenchyma

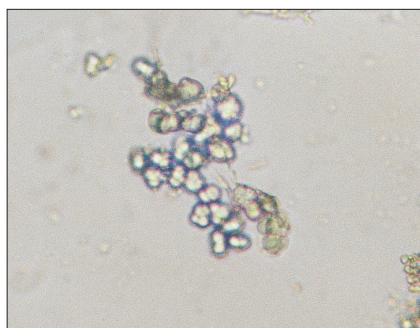


Figure 8: Simple and compound starch grains



Figure 9: Sclerieds



Figure 10: Tannin

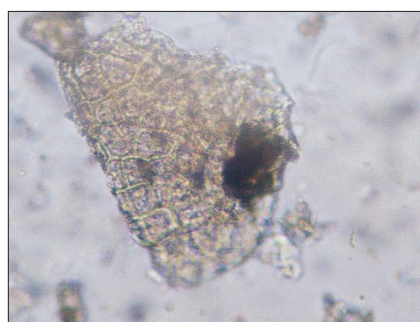


Figure 11: Oleoresin, mesocarpal cells



Figure 12: Fibres



Figure 13: Trichomes

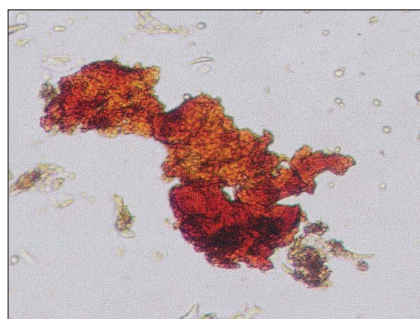


Figure 14: Tannins



Figure 15: Starch grains, Pitted stone cells



Figure 16: Annular vessels

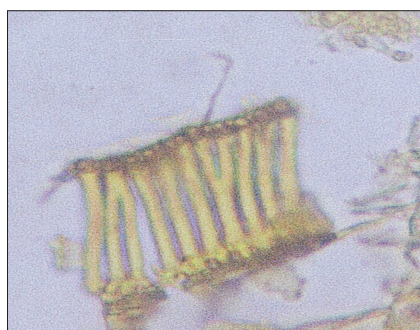


Figure 17: Scleriform vessels



Figure 18: Epidermal cells

Table 2: Results of powder microscopy

Features identified	<i>Chitraka</i>	<i>Asana</i>	<i>Haritaki</i>	<i>Amalaki</i>	<i>Bibhitaka</i>	<i>Haridra</i>
Yellowish coloring agent	++	—	—	—	—	—
Prismatic calcium oxalate crystals	++	++	—	++	—	—
Lignified parenchyma cells	++	—	++	—	—	++
Pitted vessels	++	++	++	—	++	—
Thick walled cork cells	++	—	—	—	—	—
Lignified fibres	++	—	++	++	++	—
Sclereids	++	—	++	++	++	—
Starch grains	—	++	++	++	++	++
Pitted stone cells	—	++	++	++	++	—
Brownish colouring agent	—	++	—	—	—	—
Phloem fibres	++	++	++	—	—	—
Fibres passing through medullary rays	—	++	—	—	—	—
Brownish tannin content	—	++	++	—	—	—
Mesocarpal cells	—	—	—	++	—	—
Oleoresin	—	—	—	++	—	++
Rossete crystals	—	—	—	—	++	—
Trichomes	—	—	—	—	++	—
Scleriform vessels	—	—	—	—	—	++
Annular vessels	—	—	—	—	—	++
Epidermal cells	—	—	—	—	—	++

++ Present --Absent

Table 3: Physicochemical parameters

Test	Result
Specific gravity	1.0185
pH Value	5.5 (by P ^H indicator paper)
Total solid content	4.525% w/v
Total Ash	0.949% w/v
Acid insoluble ash	0.052% w/v
Iron as Fe ₂ O ₃	0.065% w/v

Table 4: Qualitative analysis

Test for flavonoids	Positive
Test for tannins	Positive

Physicochemical parameters: The results observed after experiments are mentioned in the Table 3.

Preliminary qualitative tests revealed the presence of flavonoids and tannins in the test drug [Table 4].

Discussion

The specific gravity of the current sample was 1.0185 showing the sample is denser than water. In the present sample, pH was detected by using pH indicator paper and it was 5.5 showing the mild acidic nature of the solution. In present study the total solid content was 4.525% w/v. *Vara Asanadi Kwatha* the total ash determined was 0.949%w/v and

Table 5: Results of TLC

Track	Visualization				R _f value
	No of spots	366nm	No of spots	254 nm	
E.A	2	Green Bluish green	1	Green	0.90 0.35
Me-OH	3	Green Bluish green Blue	1	Green	0.90 0.35 0.1

Table 6: Results of HPTLC

Track	254 nm		366 nm	
	No of spots	R _f	No of spots	R _f
E.A	4	0.29,0.58,0.77,0.83	2	0.76,0.83
Me- OH	7	0.06,0.20,0.35,0.55,0.66, 0.72,0.78	9	0.02, 0.07, 0.22, 0.35, 0.55,0.65, 0.70,0.74,0.83

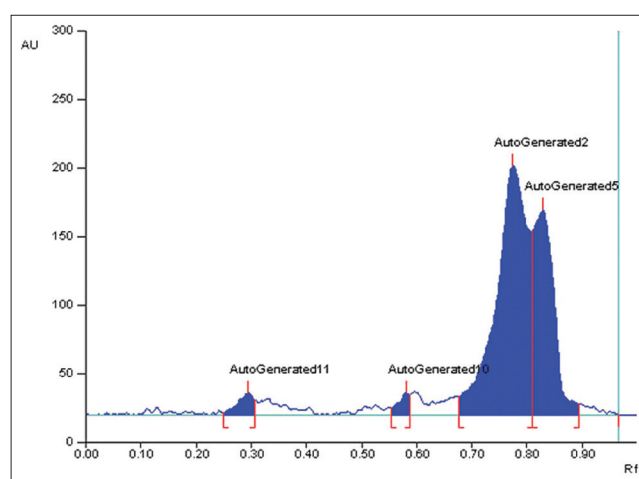
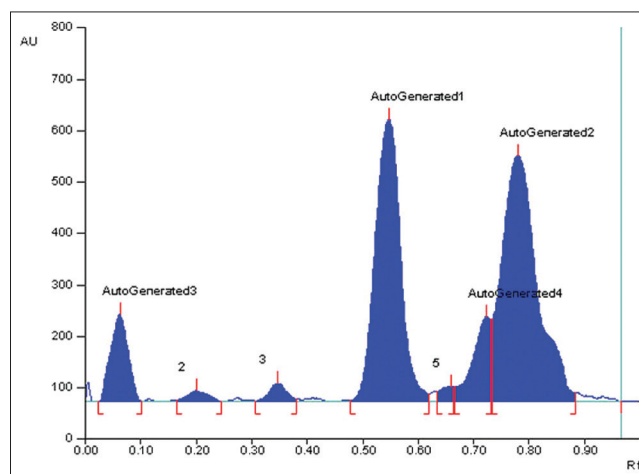
Table 7: After post chromatographic deprivation with methanolic sulphuric acid Solvent front -6

Track	No of spots	R _f
E.A	1	0.85
Me-OH	6	0.05,0.15,0.23, 0.65, 0.68, 0.85

percentage of acid insoluble ash was 0.052% w/v. The test showed Iron assay result as 0.065% w/v.^[6]

Qualitative test was performed mainly to detect the presence of tannins and flavonoids in the sample. The presence of tannins was confirmed by adding 5% of FeCl₃ solution to 2-3 ml of aqueous extract of sample resulting in the formation of deep blue – black color showing the abundance of tannin content in the test drug. Methanol extract of VAK was used for the purpose of detection of flavonoid and test was carried out by two methods by adding lead acetate and sodium hydroxide respectively. Both the experiments showed positive result for the presence of flavonoids.

Mobile phase of TLC was Toluene+Ethylacetate+Formic acid+Glacialaceticacid in the proportion of 5:5:1:1, respectively. The sample tracks and mobile phase remained the same for all the experiments related to TLC and HPTLC. The spots produced by TLC were observed in day light, short UV and long UV and R_f value was calculated. Track 1 showed two spots with R_f 0.90 and 0.35 and three spots were available in track 2 with R_f 0.90, 0.35, and 0.1 [Table 5]. The matter of significance is that in both the tracks the R_f value of two compounds are same. So this implies the definite presence of certain constituents in the sample. HPTLC is a more convenient and simple procedure in which finger printing profile is available in the form of graph and densitogram.^[7] In the present HPTLC study, four spots obtained in track 1 and 9 spots in track 2 in which once again most of the R_f values were matching when compared between both the tracks [Tables 6,7 and Figures 19,20,21]. After completion of chromatographic procedure spraying of the plate was done with methanolic sulphuric acid and the spots obtained were observed in day light.

**Figure 19: Densitogram of ethyl acetate extract of VAK at 254 nm****Figure 20: Densitogram of methanolic extract at 254 nm**

Conclusion

Preliminary Organoleptic features and results of powder microscopy were compared with the parameters mentioned in API and all the ingredients were proved to be authentic.

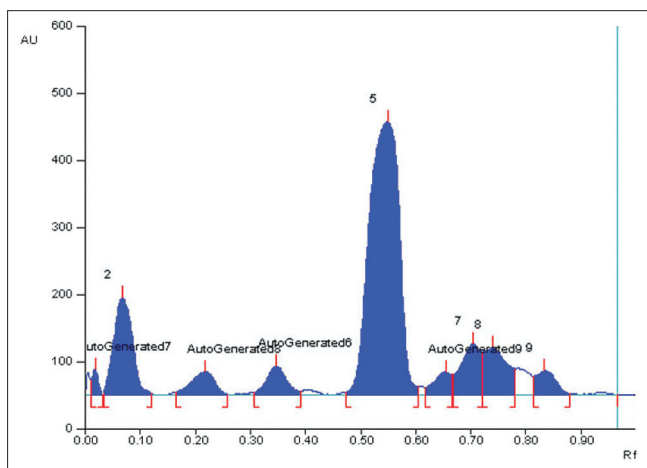


Figure 21: Densitogram of methanolic extract at 366 nm

In phytochemical analysis specific gravity, pH, total ash, acid insoluble ash, and total solid content were assessed. The percentage of amount of Iron present in the sample was detected and it was within the acceptable range. Qualitative analysis revealed the presence of tannins and flavonoids in the decoction. Though the base work requisites for the

standardization of VAK are covered in the current study, additional analysis and investigations are required for the identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy.

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हिन्दी सारांश

वरा असनादि क्वाथ का फार्माकोग्नोस्टिकल और फायटो-कैमिकल मूल्यांकन

अंजु पी. रामचन्द्रन्, श्याम प्रसाद एम., एस. एम. एस. समरकून, एच. एम. चन्दोला,
सी. आर. हरीशा, वी.जे. शुक्ला

वरा असनादि क्वाथ, स्थौल्य की चिकित्सा में वर्णित शास्त्रीय योग है। वरा असनादि क्वाथ का फार्माकोग्नोस्टिक अध्ययन, स्थूल तथा सूक्ष्मदर्शी परीक्षण द्वारा करने पर, प्रस्तुत योग के सब घटक प्रमाणित पाये गए। अपरिष्कृत घटक द्रव्यों के खुरदरे चूर्ण के ऑर्गनोलेप्टिक फिचर्स प्रमाणित रेंज में पाये गए। वरा असनादि क्वाथ का विशिष्ट गुरुत्व १.०१८५, पी.एच. ५.५ पाया गया। क्वाथ में प्रस्तुत कुल घनांश ४.५२५ % W/v, कुल राख ०.९४९% W/v और अम्ल अघुलनशील राख ०.०५२% W/v पाया गया। लौह परीक्षण करने पर लौह की Fe_2O_3 के रूप में ०.०६५ % W/v उपस्थिति पाई गई। गुणवाचक परीक्षण करने पर फ्लेवेनोइड्स और टैनिन की उपस्थिति पाई गई। योग्य विलायक प्रणाली पद्धति निर्मित करने के पश्चात् भिन्न भिन्न प्रतिदर्श निष्कर्ष पद्धति से TLC और HPTLC करने पर, TLC में महत्तम ३ बिन्दु तथा HPTLC में महत्तम ९ बिन्दु पाये गए, जो कि क्वाथ में किसी निश्चित घटकांशों की उपस्थिति को दर्शाता है।