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ORIGINAL ARTICLE

Pharmacognostic, physicochemical, and TLC Characterisation of *Brhatyadi Kwatha* used in Kerala

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Abstract

Brihatyadi Kwatha (BK) a popular Ayurvedic polyherbal decoction for urinary problems is a formulation of five roots - *Salaparni, Prisniparni, Brihati, Kantakari* and *Gokshura*. Sahasrayoga, adopting Ashtanga Hrdaya describes the drug ratio as 1:1:1:1:2 respectively, but some formularies consider ratio of the last drug as 4 and 8. The source of *Prisniparni* is *Desmodium gangeticum* (L.) DC in Kerala where as it is *Uraria picta* (Jacq.)DC in north. This diversity in source plants and proportion of ingredients can create variability in product standards. Currently there is no specific standard prescribed for BK in Ayurveda Formulary of India. Hence, this study is taken up as step towards standardisation of *Brihatyadi Kwatha.* The study aims to determine characteristic Pharmacognostic and Physicochemical parameters and TLC profile of ingredients as well as the end product - two variants of *Brihatyadi Kwatha*, to set standards. Ingredient drugs of BK were subjected to macroscopic, microscopic, physicochemical analysis. Ingredients and products were subjected to Thin Layer Chromatography

Characteristic features of the analyses were recorded. R_f values in the TLC profile demonstrated presence of all ingredients of BK. The findings may be useful as standards for quality control of *Brihatyadi Kwatha*.

Introduction

Ayurveda, the most accepted and most ancient codified traditional medical system in India has drawn its resources from all regions of the nation as well as from abroad. Languages and culture in different regions of the nation being manifold and diverse, it is natural for any traditional pharmacopoeia or formulary to have minor differences from region to region. Standardisation of an Ayurveda formulation hence requires validation of formulation variants too.

Traditional, complementary, alternative, or non-conventional medications are utilised by 70–95 percent of the global population, primarily in developing countries, according to the World Health Organization (WHO) [1]. The growing use of Ayurveda system by the public is forcing moves to produce good quality medicines and to develop standards of quality and manu-

facture. For many disorders, Ayurveda has a wide number of helpful formulations. One of the roadblocks to standardisation is the lack of reference standards for compound compositions. .

The process of prescribing a set of standards or inherent features, consistent parameters, definitive qualitative and quantitative values that convey an assurance of quality, efficacy, safety, and repeatability for herbal medicines is known as standardisation. If the drug examined has not been validated and characterised, a herbal product cannot be regarded genuine. As a result, quality control in traditional medicine necessitates the development of a set of standards that connect Botany, Phytochemistry etc [2]. In light of this, standardisation is a critical step in establishing a consistent biological activity, a consistent chemical profile, or simply a quality assurance programme for herbal medication manufacture [3].

Polyherbal therapy is mostly adopted in Ayurveda than single drugs, so that herbs are consumed as formulations. Till the beginning of last century the production and distribution of medicine/ polyherbal combinations was focused in and around the Doctor's home or neighbourhood, but later turned to institutionalised bulk production and commercialization[4]. To cope with herbal drug technologies and wide marketing maneuver, in the transition of Ayurvedic medicine from Vaidya's kitchen to over the counter drugs, quality control and standardization has become inevitable [5]. Unfortunately, in the realm of Ayurvedic medicine manufacturing, still a gap exists in standardisation and quality control. The majority of Ayurvedic medicines lack specific quality control parameters and methods of evaluation.[6]

Brihatyadi Kwatha (BK) is a popular Ayurvedic polyherbal decoction for urinary problems such as urinary tract infections, dysuria, urinary stones etc. Currently BK is manufactured and marketed by many herbal pharmaceutical companies. *Brihatyadi Kwatha* formulation is a modification of *Laghupanchamoola*- a group of five roots as *Salaparni, Prisniparni, Brihati, Kantakari* and *Gokshura*. The proportion of *Gokshura* is modified in BK with a range from 2 to 8 parts. Most of the manufacturers claim the drug ratio of BK as per the reference document Sahasrayoga, which adopts it from Ashtanga Hridaya, where four herbs are taken one part each and the last one, *Gokshura* two parts. The source plants of BK with their ratio proportion is listed in the Table 1.

In Kerala *Pseudarthria viscida* (L.) Wight & Arn is the source plant of *Salaparni,* where as it is *Desmodium gangeticum* (L.) DC in many other states. *Uraria picta* (Jacq.)DC is the source plant of *Prisniparni* in many states, where as it is *Desmodium gangeticum* (L.) DC in Kerala. This diversity in source plants and proportion of ingredients create variability in standards by different manufactures. Currently there is no specific standard prescribed for BK in Ayurveda formulary of India.

Table .1. Source plants of ingredients of BK

No	Name of ingredient	Botanical source	Acronym used in this article	Part used	Quantity
1	*Salaparni*	*Pseudarthria viscida* (L.) Wight &Arn	PV	Root	1/6
		Desmodium gangeticum (L.) DC	DG		
2	*Prisniparni*	*Desmodium gangeticum* (L.) DC	DG	Root	1/6
		Uraria picta (Jacq.)DC	UP		
3	*Brihati*	*Solanum indicum*. Linn	SI	Root	1/6
4	*Kantakari*	*Solanum xanthocarpum* Schrad. and Wendl	SX	Root	1/6
5	*Gokshura*	*Tribulus terrestris*.Linn	TT	Root/fruit	2/6

The goal of this study was to establish a standard profile for *Brihatyadi Kwatha* produced with both of the source plants of *Salaparni* and *Prisniparni.* BK was prepared using authentic raw drugs and was subjected to pharmacognostical, physicochemical, phytochemical and chromatographic analysis as per standard protocol

Materials and Methods

1 Raw drug Collection

Fresh samples of roots of DG, UP, SI, SX and fruits of TT were collected from their natural habitat. Drugs were authenticated in the Department of *Dravyagunavigyana*, Govt. Ayurveda College, Thiruvananthapuram. The collected drugs were washed, cleaned, shade dried and stored in airtight containers.

2.Pharmacognostical Evaluation

Macroscopic and microscopic evaluation of roots of DG, PV,UP, SI, SX and fruit of TT were done.

3 Preparation of *Brihatyadi Kwatha*

Brihatyadi Kwatha with ingredients roots of DG, PV, SI, SX and fruit of TT in the proportion 1:1:1:1:2 was prepared as per standard procedure described in Sarangadhara Samhita and was named as BK-1. B K with ingredients roots of DG,UP, SI, SX and fruit of TT prepared in the ratio of 1:1:1:1:2 was named as BK-2.

4. Preliminary Physical and Phytochemical evaluation

Standard pharmacopoeial methods[7] were used to investigate the raw materials of *BrihatyadiKwatha.* Determination of alcohol soluble extractives, water soluble extractives, total ash, acid insoluble ash, loss on drying, and pH determinations were conducted.

5. Thin layer chromatography (TLC)

Sample Preparation

Separately weighed 1g powdered ingredient samples were dissolved in 20ml methanol and refluxed for 15 minutes on a water bath at 90–100°C. They were filtered and evaporated to a volume of 5 mL in a porcelain dish for TLC profiling. 1 ml of prepared decoctionsBK-1and BK-2 were refluxed separately with 40ml of methanol and extract was obtained.

Solvent System

The TLC plates were developed using the solvent system for each constituent, as well as BK1 and BK2, which were chosen by trial and error.

Development

Methanolic extracts were applied on a 0.2mm precoated Silica Gel 60 F254 plates (Merck KGaA) and developed in the solvent system..

Visualization

Under ultraviolet illumination at 254nm and 366nm, the produced TLC plates were inspected. The plates were then derivatized with anisaldehyde-sulphuric acid reagent, heated at 110°C till coloured spots appeared, and observed in daylight[8]. The colour and Rf values of spots were recorded.

Results

1 Preliminary pharmacognostical evaluation

Images of DG, PV,UP, SI, SX and TT are shown in Fig.1 and the officinal parts as root of DG, PV,UP, SI, SX and fruit of TT

is given in Fig 1a.Pictures of powdered officinal parts are shown in Fig. 2.The decoctions BK-1 and BK-2were brown in colour (Fig. 2.A), had a distinct odour, and tasted bitter during organoleptic evaluation. Microscopic characters of roots of DG, PV,UP, SI, SX and fruit of TT are shown in Fig. 3 and 4. Powder Microscopic characters of roots of DG, PV,UP, SI, SX and fruit of TT are shown in Fig. 5 and 6

Fig 1. Pictures of source plants of ingredients in *Brihatyadi Kwadha.* **A**-*Solanum indicum,* **B**-*Solanum xanthocarpum,* **C**- *Pseudarthria viscida,* **D**-*Desmodium gangeticum,* **E**-*Uraria picta,* **F**-*Tribulus terrestris*

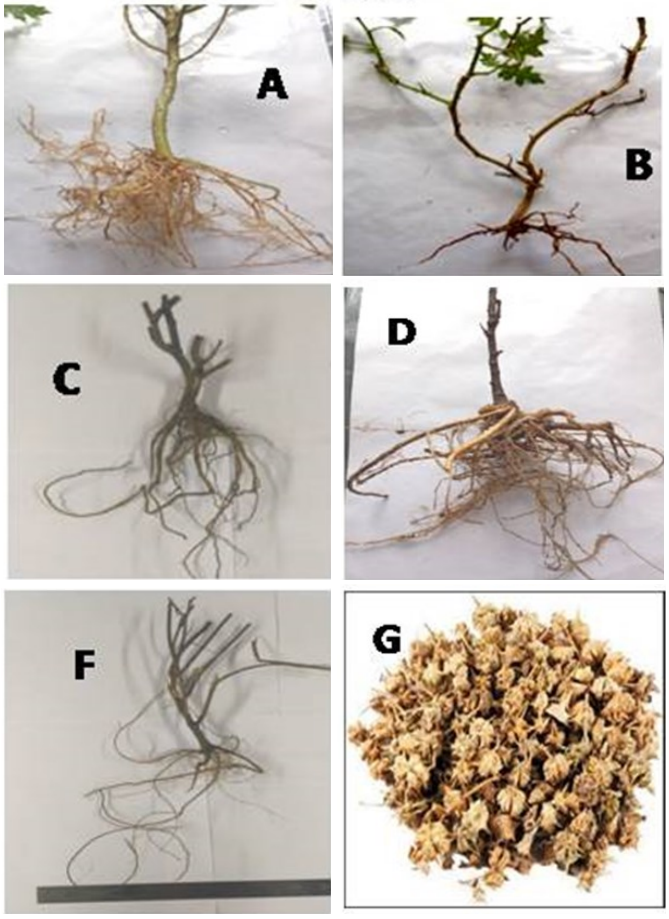


Fig 1A.. Root. **A**-*Solanum indicum*, **B**- *Solanum xanthocarpum,* **C** -*Desmodium gangeticum*, **D**-*Uraria picta*, **E**-*Pseudarthria viscida*, **F**-Fruit of *Tribulus terrestris*

Fig 2. Powder of officinal parts of drugs of *Brihatyadi Kwatha.* **A**-*Solanum indicum,* **B**-*Solanum xanthocarpum,* **C**-*Desmodium gangeticum,* **D**- *Uraria picta,* **E**-*Pseudarthria viscida,* **F**-*Tribulus terrestris*

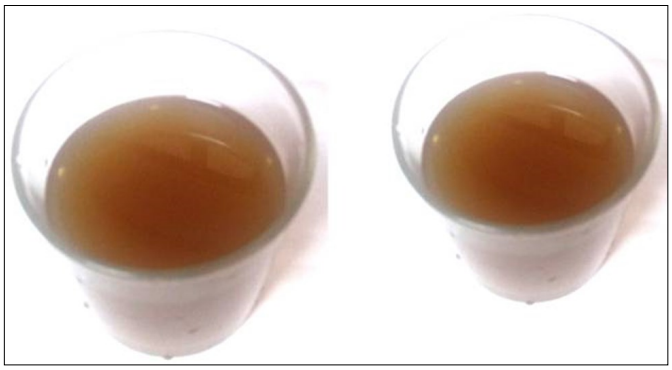
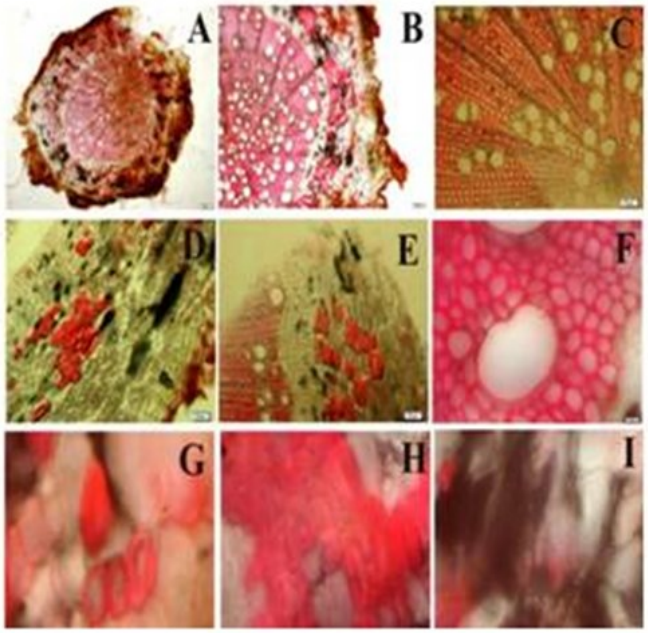
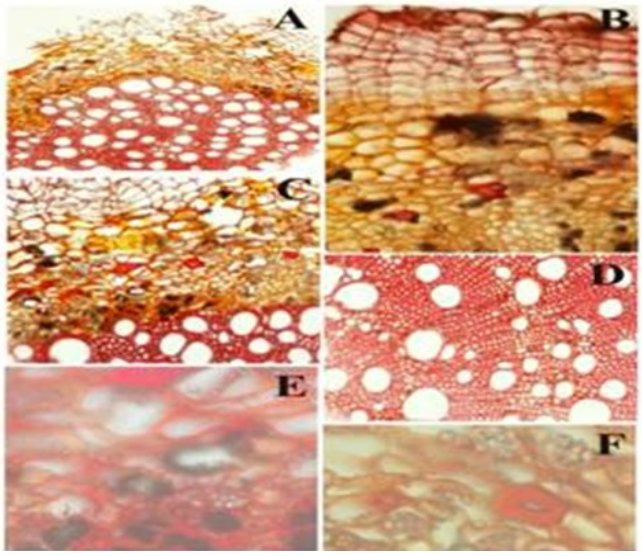


Fig 2A. Prepared BK-1 and BK2

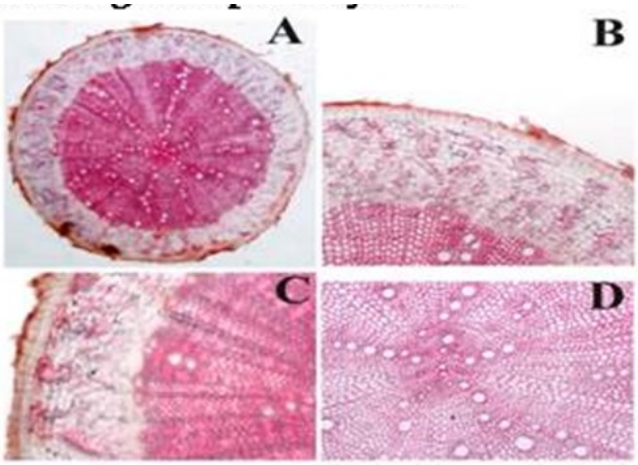
2 Preliminary Physical and phytochemical evaluation



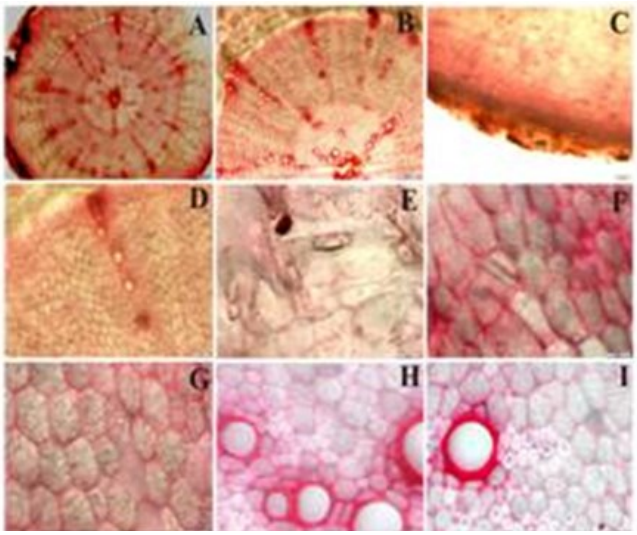
T S of root of *Solanum indicum.* **A**-2X, **B**.4X cork, cortex, vascular elements and medullary rays, **C**-10X vessels, parenchyma, fibers and medullary rays, **D**-10X cork and cortex with starch grains, stone cells and black powdery mass, **E**- 10X groups of stone cells, **F**- 40X vessels and fibers, **G,H**- 40X solitary and group of stone cells, **I**- 40X black powdery mass



T S of root of *Solanum xanthocarpum.* **A**-4X,**B**-10X cork cells, **C**-10X Parenchymal cells of cortex with starch grains and sclerids , **D**-10 X Vascular elements (xylem vessels, fibers, medullary rays), **E**- 40 X Cortical parenchymacells with starch grains and black powdery mass, **F**- 40 XSclerids,

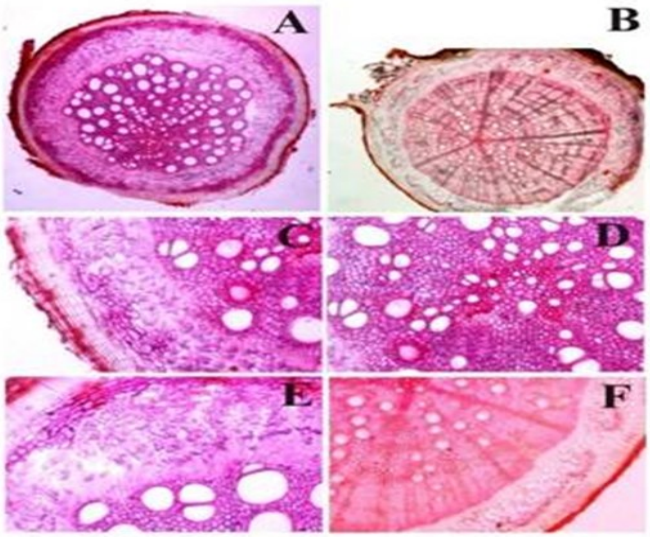


T S of root of *Desmodium gangeticum.* **A**-2X,**B**.10X cork cells,cortical parenchymatous cells with starch grains, **C**-Cortical parenchymal cells with sclerides, medullary rays and xylem vessels, **D**-Vascular bundles with xylem vessels, fibers

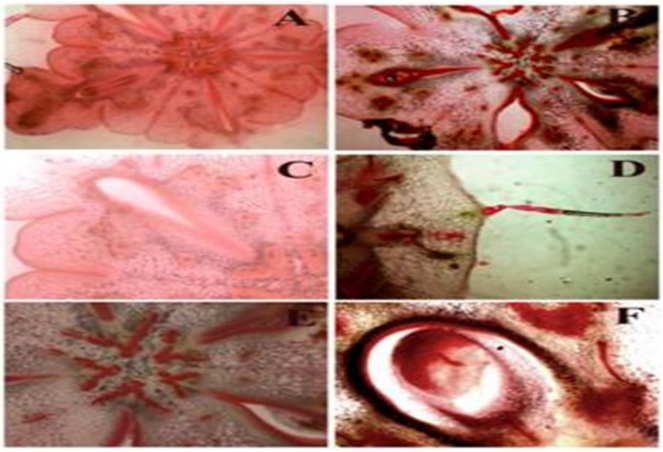


T S of root of *Uraria picta.* **A**-2X,**B**-4X, **C**-10X cork and cortex, **D** -10 x Vacular bundles, E parenchyma cells with crystals and starch grains, **F**- 40 X Medullary rays with starch grains and crystals, **G**- 40X Xylem parenchymal cells with starch grains, **H**- 40X Xylem vessels, **I**- 40 x parenchymal cells with crystals

Fig 3.Microscopic characters of roots of DG, PV,UP, SI, SX

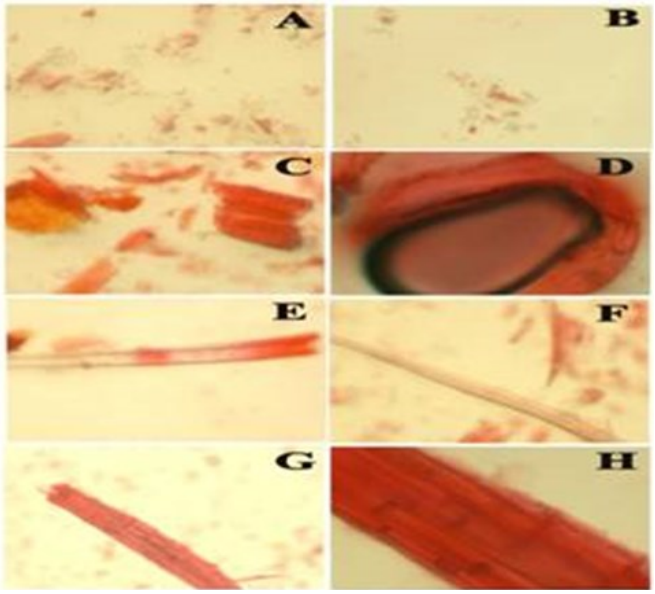


T S of root of *Pseudarthria viscida.* **A**-2X,**B**-4X, lenticels opening cork, cortex, vascular bundles and medullary rays, **C**-10X cork and cortex, **D**-10 X Vascular bundles and small pithE- Cells of cortex with starch grains, **F**- 10 X medullary rays, xylems vessels and fibers.

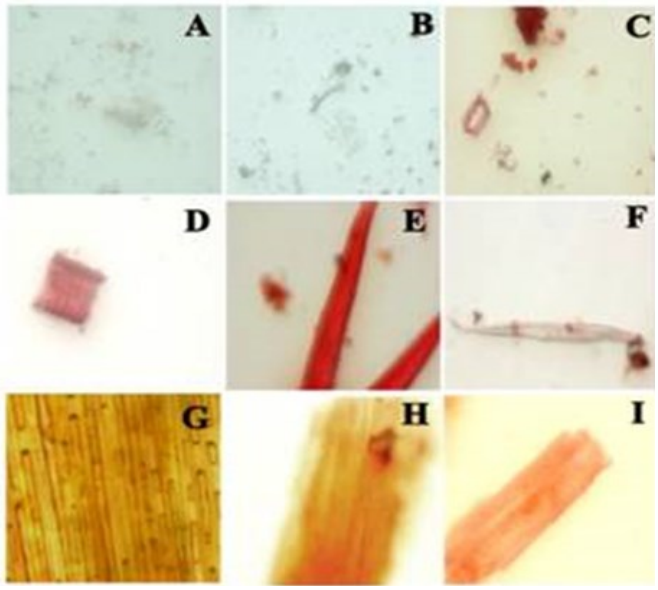


T S of fruit of *Tribulus terrestris.* **A**-2X showing 5 cocci, **B**-4X, **C**-10X showing mesocart, endocarp and vascular bundle, **D**-10 X showing trichomes, epicarp and mesocarp, **E**- 10X showing endosperm, **F**- 10X showing seed chamber with seed

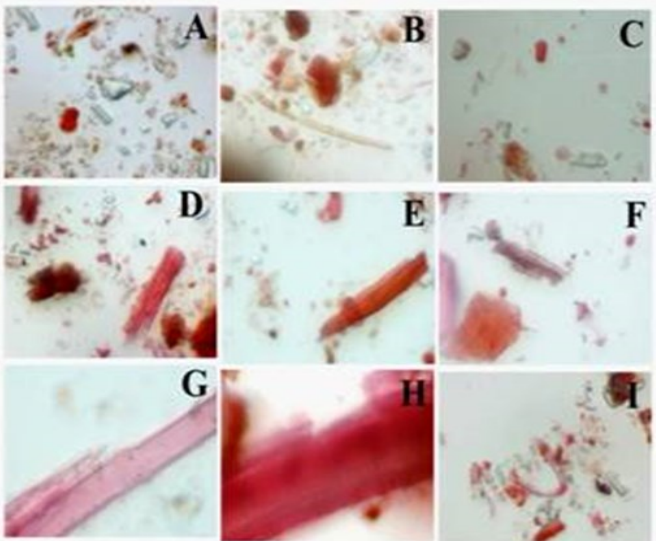
Fig 4.



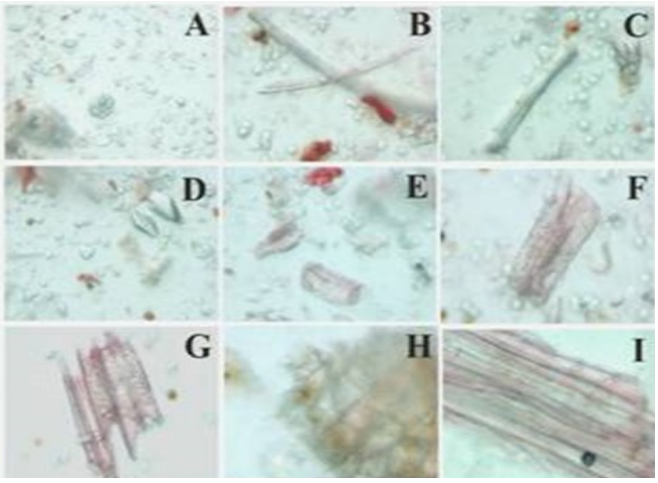
Root of *Solanum indicum*, **A-B**. Simple and compound starch grains, **C** – Pitted vessel, **D**– Stone cells, **E,F**– Vessel, **G.H**– Bunch of fibers



Root of *Solanum xanthocarpum*. **A,C**-Starch grains and crystals, **D**– Septed fibre, **E**– Vessel element, **F**– Trachiedal fibre, **G**– Vessels with crystals, **H**– Cortical cells with crystals, **I**– Pitted vessels



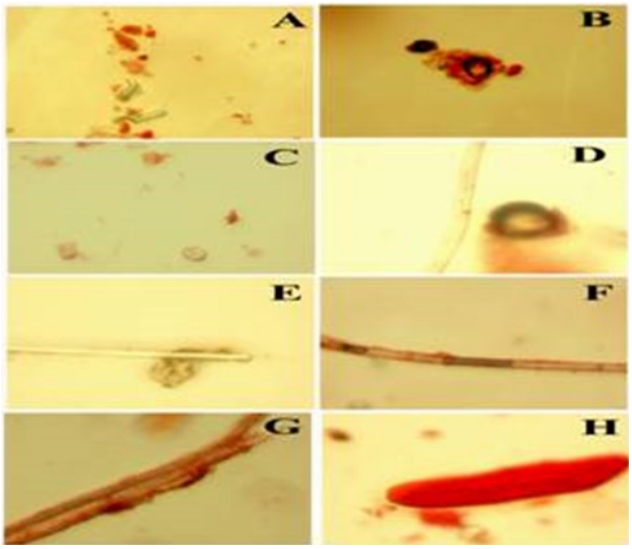
Root of *Desmodium gangeticum*. **A**-Prismatic crystals and starch grains, **B**-Simple fiber, **C**- Colouring pigment, **D**- Septed fiber, **E**-Vessesl element,



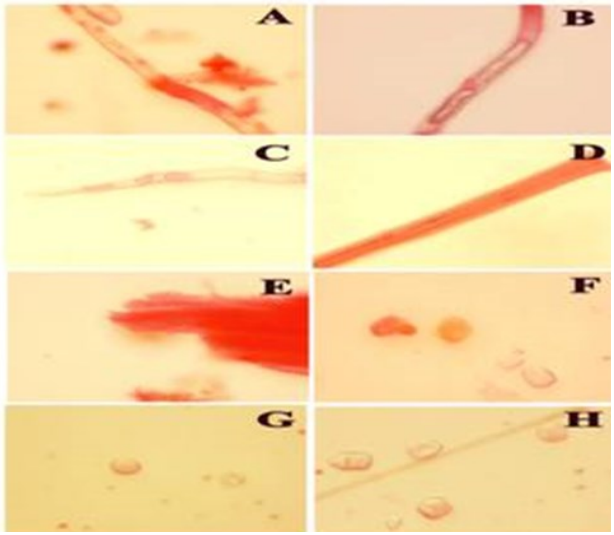
Root of *Uraria picta*. **A**-Simple and compound starch grains, **B**- fibers, **C**- Vessels, **D**- Prismatic crystals, **E**-Sclerids, **F**&**G** Pitted vessels, **H** -Parenchymal cells of cortex, **I** - fibers

Fig 5

Results of Physico-chemical evaluations of each plant mate-



Root of *Pseudathria viscida*. **A**- Prismatic crystals, **B**-Stone cells, **C**-Starch grain, **D**-Tracheids, **E**-Vessel, **F**-Vessel element with crystal, **G**-Fibers, **H**- Sclerids with crystals



Fruit of *Tribulus terrestris*. **A,B,C**-Trichomes, **D**-Vessel, **E**-Bunch of fibers, **F,H**- Crystals, **G**-Starch grain,

Fig.6

rial of *Brihatyadi Kwatha* are shown in Table .2. Percentages of extract obtained in successive solvent extraction of each plant material of BK are shown in Table .3. Table. 4 shows the results of qualitative phytochemical examination of plant materials, BK-1 and BK-2.

3. Thin layer chromatography (TLC)

The images of TLC plate of ingredients of BK and BK1 and BK 2are shown in Fig 7.

a. TLC Analysis of Solanum indicum. Linn

Subjected ethanolic extract of the drug to Thin Layer Chro-

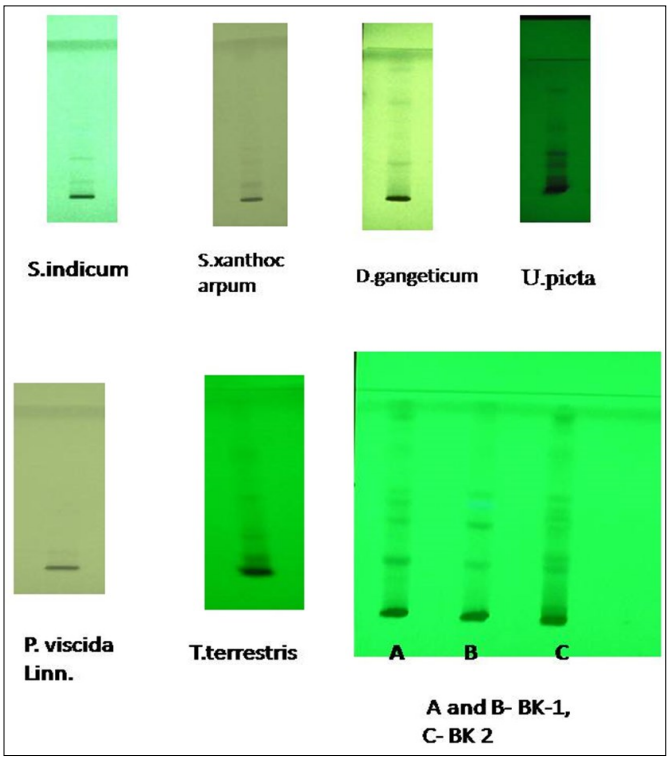


Fig. 7. The images of TLC profile of ingredients of BK, BK1 and BK2

Table . 2. Physico-chemical evaluation of each plant material of *Brihatyadi kwatha*

Sl. No	Experiment	*S. indicum*	*S. xantho-carpum*	*D. Gangeticum*	*Uraria picta*	*P. viscida*	*T. terrestris*
1	Foreign matter	Nil	Nil	Nil	Nil	Nil	Nil
2	Moisture Content (%)	8	6	6	8	6	8
3	Volatile oil content (%)	Nil	Nil	Nil	Nil	Nil	Nil
4	Total ash(%)	5	5.87	2.02	3.31	1.14	7.3
5	Water insoluble ash (%)	2	2	1.32	2.89	0.13	1.2
6	Acid insoluble ash (%)	0.07	0.07	0.07	0.07	0.07	0.69
7	Water soluble extractives (%)	7.19	7.19	13.17	10.48	5.29	10.9
8	Alcohol soluble extractives (%)	2.83	4	3.7	4	3.72	6.8
9	Fibre content (%)	35.27	88.27	62.60	96.56	69.72	38
10	Total Sugar (%)	0.83	Trace	2.04	Trace	1.52	0.66
11	Reducing Sugar (%)	0.66	Trace	1.52	Trace	1.52	0.33
12	Non-reducing sugar (%)	0.17	Trace	0.52	Trace	Nil	0.33

Table. 3. Successive Solvent Extraction of each plant material of BK

Sl.No	Experiment	Percentages of extract obtained					
		S. indicum	*S. xanthocarpum*	*D. Gangeticum*	*Uraria picta*	*P. viscida*	*T. terrestris*
1	Petroleum ether	0.892	1.224	0.006	1.4	1.33	1.2
2	Cyclohexane	0.528	0.7	0.17	0.4	0.094	0.01
3	Acetone	1.96	3.402	1.86	2.5	1.442	1.6
4	Methanol	5.75	3.018	12,02	4.18	1.462	2.02

Table 4. Results of qualitative phytochemical analysis

Sl. No	Experiment	*S. indicum*	*S. xanthocarpum*	*D. Gangeticum*	*Uraria picta*	*P. viscida*	*T. terrestris*	BK 1	BK 2
1	Alkaloids	+	+	+	+	+	+	+	+
2	Phenols	+	+	+	+	+	_	+	+
3	Steroids	++	++	++	++	++	+	++	++
4	Flavonoid	-	-	+++	+++	++	+	++	++
5	Tannin	+	+	+	+	+	+	++	++
6	Saponin	++	++	+	+	+	++	+	+

matography in the solvent system Toluene: Ethyl acetate: Formic acid in the proportion– 7:5:0.5. The plates were allowed to develop and then the spots were visualized in visible light and UV. The Rf values are 0.13, 0.29, 0.35

b. TLC Analysis of Solanum xanthocarpum Schrad. and Wendl

Ethanolic extracts was subjected to T L Chromatography in the same solvent system. The plates were allowed to develop and then the spots were visualized in visible light and UV. The Rf values are 0.13, 0.21 0.28, 0.37, 0.51.

c. TLC Analysis of Desmodium gangeticum (L.) DC

The ethanolic extracts of sample were subjected to Thin Layer Chromatography in the solvent system Toluene: Ethyl acetate: Formic acid in the proportion 35:8:1:1. The plates were allowed to develop and then the spots were visualized in visible light and UV. The Rf values are 0.178, 0.571, 0.714.

d. TLC Analysis of Uraria picta (Jacq.)DC

The ethanolic extracts of sample were subjected to Thin Layer Chromatography in the solvent system Dichloromethane: Toluene: Ethyl acetate: methanol in the ratio 3.4:5.6:0.5:0.3. The plates were allowed to develop and then the spots were visualized in visible light and UV. The Rf values are 0.16, 0.29, 0.45.

e. TLC Analysis of Pseudarthria viscida (L.) Wight &Arn

The ethanolic extracts of sample were subjected to Thin Layer Chromatography in the solvent system Chloroform:methanol:water:formic acid in the ratio 35:8:1:1. The plates were allowed to develop and then the spots were visualized in visible light and UV0. The Rf values are 0.385 and 0.642

f. TLC Analysis of Tribulus terrestris. Linn

The ethanolic extracts of sample of were subjected to Thin Layer Chromatography in the solvent system Toluene: Ethyl acetate in the proportion 8:1. The plates were allowed to develop and then the spots were visualized in visible light and UV. The Rf values are 0.78 and 0.21

g.TLC profiling of Brihatyadi Kwatha

BK-1 had ingredients roots of DG, PV,SI, SX and fruit of TT. The Solvent system was Toluene: Ethyl acetate: Formic acid – 7:5:0.5. The Rf values obtained are 0.16, 0.29, 0.45.

BK-2 had ingredients roots of DG,UP, SI, SX and fruit of TT. The Solvent system for BK2 was same. The Rf values observed are 0.16, 0.29, 0.45, 0.642, 0.714

Discussion

Brihatyadi Kwatha is an important polyherbal decoction used for urinary problems such as infection, stone etc. Currently there is no specific standard prescribed for BK in Ayurveda formulary of India. Hence, this study is taken up as step towards its standardisation. In order to address the controversy on two source plants, two decoctions with the different source drugs were prepared. BK-1had ingredients roots of DG, PV, S.I, SX and fruit of TT in the proportion of 1:1:1:1:2. BK-2was prepared with UP as an ingredient instead of PV.

Standardization guidelines of Ayurvedic Pharmaco-

poeia of India and the World Health Organization's were used to assess pharmacognostic, physicochemical, and chromatographic features of Both forms of *Brihatyadi Kwatha* (BK-1and BK-2)

Foreign matter such as other portions of the same plant or other plants, moulds or insects, including excreta and visible impurity such as sand and stones, dangerous and harmful foreign matter, and chemical residues should be avoided in herbal remedies. The evaluated foreign matter content of BK components was confirmed to be below the API limits in our investigation.

The features of food or other substances as perceived by the senses are known as organoleptic qualities. The difference in these qualities is a good indicator of quality variation. This method is used to determine the quality of many crude medications, and it is a significant metric in pharmacognostical evaluation. As a result, we report on the unique organoleptic features of BK's parent medicines.

Different staining reagents are employed to analyse the particular microscopic features of medicinal plants utilising microscopy and powder microscopy. These studies provide a useful diagnostic tool for determining standards and detecting adulterants. When elements in a polyherbal decoction are available in coarse powder form, this method is also highly beneficial for confirming their existence. The microscopic features found in BK ingredients match API parameters, according to our microscopic research. (See Fig 2-6).

Preliminary physicochemical criteria provide critical information for further inquiry and confirm the authenticity of raw materials. Because of the significance of these physicochemical properties, BK components were evaluated for water soluble extractive, ethanol soluble extractive, total ash content, acid insoluble ash, pH, and loss on drying at 105°C.

The major goal of TLC study of BK was to create a unique profile that could be used to identify each ingredient. A common solvent system Toluene: ethyl acetate: formic acid (7:5:0.5)was developed by experimenting with various solvent combinations. The presence of every constituent of BK in its ethanolic extract was established in TLC profile.

Conclusion

Characteristic pharmacognostic, physicochemical, and TL chromatographic parameters of *Brihatyadi Kwatha* prepared with two raw drug combination were determined in the present study. In terms of quality-based raw materials, the product's analytical standards were created. The TLC data revealed that all of the components were present in *Brihatyadi Kwatha* with their unique R_f values.

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