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Pharmacognostical and Physicochemical Evaluation of Karpuradi Kuzhambu – An Ayurvedic Anjana Preparation

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Abstract

Ayurveda employs eye drops (Ashcotana) and eye salves (Anjana) for prevention and healing of eye diseases. Karpuradi Kuzhambu is an Anjana preparation containing Pippali (*Piper longum)*, Amalaki *(Emblica officinalis*), Marica (*Piper nigrum*), Pitakarohini *(Coptis teeta*), Karpura, Saindhava Lavana *(Sodium chloride, Rock salt*), Purana ghrta (Cow's ghee), and Madhu (*Honey*). It is used widely for eye disorders due to Pitta origin also helps to relieve burning sensation and itching. This study was undertaken to develop the physico chemical quality standards of Karpooradi kuzhambu. Organoleptic, physico - chemical, microscopical, microbiological, heavy metal analysis and HPTLC fingerprint studies were carried out. The data evolved can be adopted for laying down the Pharmacopoeial standards for Karpuradi kuzhambu.

Key-Words: *Karpooradi kuzhambu*, Pharmacopoeial, Physico-chemical, HPTLC

Introduction

Ayurveda is one of the oldest systems of healing. Today Ayurveda is the sum total of the accumulated wisdom and practical experiences of very many generations. This process of evolution will continue as long as Ayurveda remains a living tradition. Standardized Ayurvedic formulations of uniform quality are essential for therapeutic efficacy. Lack of quality standards in any product, can lead to batch to batch variations. 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 formulation procured from different sources. Awareness of quality standards of Ayurvedic medicines is increasing with the demand for these products.

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The growth and accessibility of these medicines depends up on the compliance with the quality standards and in process quality assurance in the manufacture of these medicines. Therefore it is essential to have scientific standards for identity, purity and strength of these medicines.

In Ayurvedic Classical texts potent formulations of the eye are prepared in the form of Vartti, Netra bindu and Anjana. The majority of Anjanas are very fine semi solids of drugs formulated in the aqueous medium and has since long been used to expel ocular problems which acts longer than any other topical ocular administrations[1]. Anjana forms of ocular applications are very effective and safe. For the present study *Karpuradi Kuzhambu* (Anjana form) is taken. *Karpuradi kuzhambu* is a dark brown semisolid suspension used for treatment of *Netra vrana* (Wounds in eye), *Arma* (Pterygium), *Timira* (Cataract), *Abhisyanda* (Conjunctivitis) etc.

The present study was undertaken with the objective to delineate the pharmacognostical and physicochemical analysis of *Karpuradi Kuzhambu* used as Anjana.

Material and Methods

Collection of Drug and herbs used.

Authentic sample of *Karpuradi kuzhambu* was procured from Sreedahreeyam Ayurvedic Eye Hospital & Research Centre, Koothattukulam. The identities of all herbs used in the formulation were confirmed by correlating their morphological and microscopical characters with those given in the literature.[2]

Method of Preparation

The formulation is prepared by grinding the ingredients Pippali (Piper longum), Amalaki (Emblica officnalis), Marica (Piper nigrum), Pitakarohini (Coptis teeta), Karpura (Cinnamomum camphora) and Saindhava lavana (Rock Salt), in Madhu (Honey)[3,4]. After it has attained the required consistency dilute with required quantity of cow's ghee and store in glass bottles. The quantities taken for the preparation are detailed in Table – 1.

Evaluation

Karpuradi kuzhambu was analysed for its pharmacognistic and physico chemical parameters in our chemical and instrumental laboratory. Parameters such as colour, odour and texture of the finished product were observed and recorded.

Powder microscopy

Powder microscopic studies were done by standard procedures [5]. Two to three drops of Karpuradi kuzhambu was taken on a clean glass slide along with sufficient quantity of chloral hydrate solution, heat mildly and mount with 10 % glycerin. Slides were also prepared with Phloro glucinol and iodine in potassium iodide solution. The characters were observed under Magnus Trinocular Microscope and the images were captured with Sony Digital camera.

Physico-chemical Studies

Physicochemical parameters like specific gravity, percentage of sodium chloride, pH, microbiology and heavy metal analysis were determined as per procedures described in the Ayurvedic Pharmacopoeia of India.

Thin Layer Chromatography (TLC)

Reference sample – 1, 2, 3 and 4 : Powdered 5 gm Pippali (*Piper longum),* Marica (*Piper nigrum)* , Amlaki (*Emblica officinalis and* Pitakarohini (*coptis teeta).* Shake the samples in different conical flasks with 25 ml methanol each and kept overnight. Refluxed with methanol, separated the methanol layer. Filtered methanol extract and concentrated to 5 ml. Apply 10 µl of each extract as a band of 8 mm length at the bottom of the plate. when tested for their microbial and heavy metal contaminant **Visualisation :** The plates were completely dried in air at room temperature and exposed under UV 254 and 366 nm [6].

Estimation of Berberine by High Performance Thin Layer Chromatography (HPTLC)

Reference sample – 1 & 2: Dissolve 1.0 mg Piperine and Berberine (Sigma Aldrich) respectively in 1.0 ml methanol. Dilute 0.1 ml of this solution to 1.0 ml . Apply 2 µl. **Sample preparation:** 10 g Karpuradi kuzhambu was refluxed with 25 ml *methanol* for 30 minutes; filtered and evaporated completely in a water bath and the residue was dissolved in 1 ml *methanol*. 5 µl of the sample were applied as bands of 8 mm length. Aluminum sheet pre coated with silica gel 60 F_{254} (E Merck) was used for thin layer chromatography. The solvent system used was a mixture of Toluene: Ethyl acetate: Formic acid: Methanol in the ratio 4:4:1:1. **Visualisation : .** After chromatography, the plates were dried in air at room temperature and exposed under UV 366 nm. The plates were derivatised by spraying with dragendorf reagent and visualized under visible light also.

Results and Discussion

Powder Microscopy

The diagnostic characters of microscopic analysis of *Karpooradi kuzhambu* showed the presence Fragments of pericarp cells (exocarp, mesocarp &endocarp cells), Thick and thin walled stone cells having different sizes, starch prizams, fragments of parenchymatous cells, simple rounded starch grains, aseptate elongated sclerid fibers, trichomes and fragments of xylem vessels.

The following characters were also observed. Polygonal hypodermal cells (a), stone cells (b) and oil globules (e) thin walled polygonal parenchyma cells (d) in Marica, Group of elongated spindle shaped wide lumened lignified stone cells (b) oil globules (e) in Pippali, stone cells (b), trichome (f) in Amalaki. Vessals (g), wide lumened lignified stone cells (b), trichomes (f) in Peetha. The data is given in Plate – 1.

Organoleptic Characters

Karpuradi kuzhambu is a dark brown semisolid suspension, characterized as fine homogenous thick liquid which was sticky and has camphor like smell. The Organoleptic parameters of *Karpuradi kuzhambu* are tabulated in Table - 2.

Physico-chemical, Microbiological evaluation and Heavy Metals determination

In Physicochemical parameters like Specific gravity, sodium chloride content and pH were determined. Microbial load and heavy metals were determined. The results are tabulated in Table – 3

Thin Layer Chromatography

The Chromatographic profile of the methanolic extract of the formulation showed the presence of the constituents as per the ingredients. (Fig - 2).

Estimation of Berberine by HPTLC

The HPTLC profile of the methanolic extract of the formulation showed the presence of the Piperine (0.85) and Berberine (0.62) in the formulation. Apply 2 µl of the solution. *(0.02 µg berberine)* on an Aluminum backed Silica gel 60 F 254 HPTLC Plate. Samples were developed using *Toluene : Ethyl acetate : Formic acid : Methanol* (4:4:1:1) as mobile phase. After development, the plate was scanned densitometrically at 366 nm in conjunction with that of reference solution to calculate *berberine* content. The spots were also prominently visible when derivatised by Dragendorff's reagent and observed under visible light. (Fig - 3). The plates were scanned densitometrically using win cats software (Fig – 4) and the amount of Berberine was estimated.

Conclusion

Standardization and development of reliable quality protocols for Ayurvedic formulations using modern techniques of analysis is extremely important[7]. In the present study microscopic characters of the formulation showed the presence of the identifying characters of the ingredients used. When tested for their microbial and heavy metal content the drug samples were found to be complying to AYUSH Guidelines. By HPTLC Studies the content of the active ingredient Berberine could also be estimated. So it can be concluded that these parameters can be used for the evaluation of *Karpuradi kuzhambu*. We hope that the present study will serve as the reference for the future works on *Karpuradi kuzhambu*.

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Table 1: Raw Materials and their quantities used

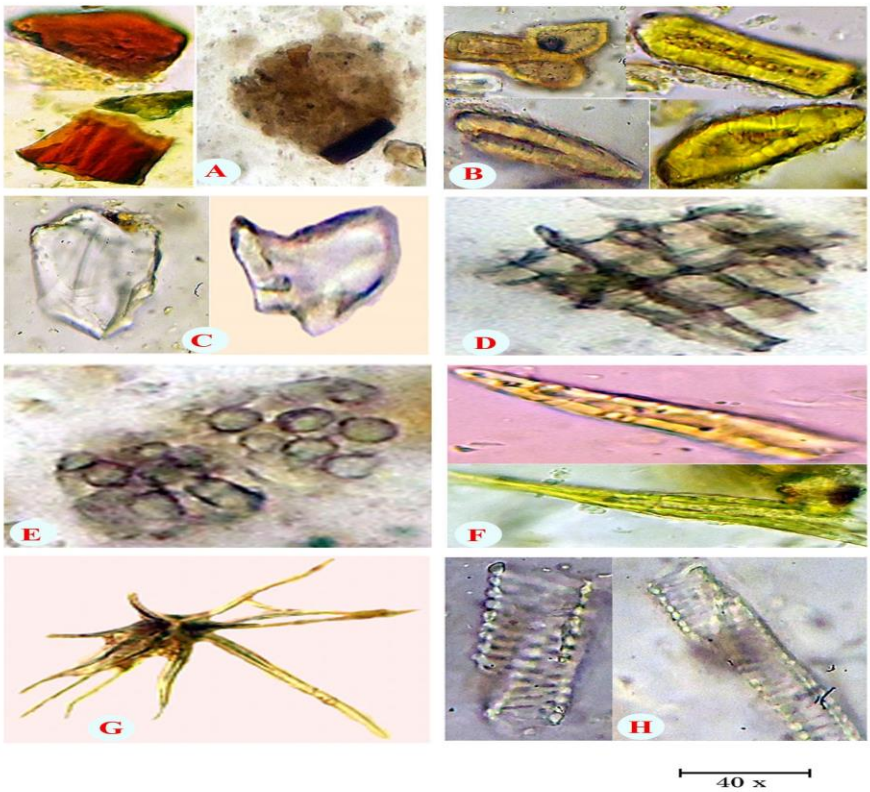
Sr. No.	Sanskrit name	*Botanical name*	Part used	Quantity
1	Upakulya (Pippali)	*Piper longum*	Fruit	10 g
2	Dhatri phala (Amalaki)	*Emblica officinalis*	Pericarp	10 g
3	Usana (Marica)	*Piper nigrum*	Fruit	10 g
4	Pitaka rohini	*Coptis teeta*	Rhizome	10 g
5	Jala	*Potable water*		As required
6	Saindhava Lavana	*Rock salt*	Mineral	10 g
7	Sasi (Karpura)	*Borneol camphor*	Dist.	10 g
8	Madhu	*Honey*		480 g
9	Purana ghrta (Go ghrta)	*Cow's ghee*		As required.

Table 2: Organoleptic Characters of Karpuradi Kuzhambu

Sr. No.	Parameters	Results
1	Colour	Brownish black
2	Odour	Aromatic camphor like
3	Taste	Bitter , Saltish , Astringent
4	Texture	Smooth

Table 3: Estimation of Physico chemical parameters, Microbial Load and Heavy Metals

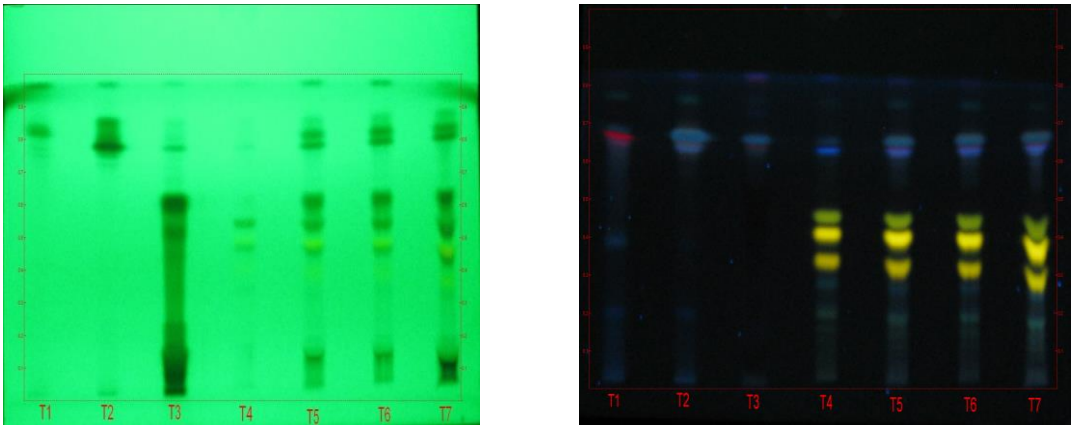
Sr. No.	Parameters		Results
1	Specific gravity at 25^0C		1.358
2	Sodium chloride content		1.83 %
3	Total soluble solids		7.20 %
4	pH		3.80
Sr. No.	**Parameters**	**AYUSH Limits**	**Result**
5	**Microbial Limits**		
	Aerobic bacteria	10^5 CFU / g	110 CFU/g
	Yeast and mould	10^3 CFU / g	20 CFU/g
6	**Test for specific pathogen**		
	Salmonella	Absent	Absent
	Pseudomonas	Absent	Absent
	E. coli	Absent	Absent
	S. aureus	Absent	Absent
7	**Heavy metals**		
	Arsenic	3.0 ppm	B D L
	Cadmium	0.3 ppm	B D L
	Mercury	1.0 ppm	B D L
	Lead	10.0 ppm	B D L



A- Fragments of pericarp cells, B- Thick walled stone cells, C- Starch prizams, D- Fragments ofparenchymatous cells, E- Starch grains. F- Elongated sclerid fibers, G- Trichome, H- Vessels

Fig. 1: Powder microscopy of Karpuradi kuzhambu

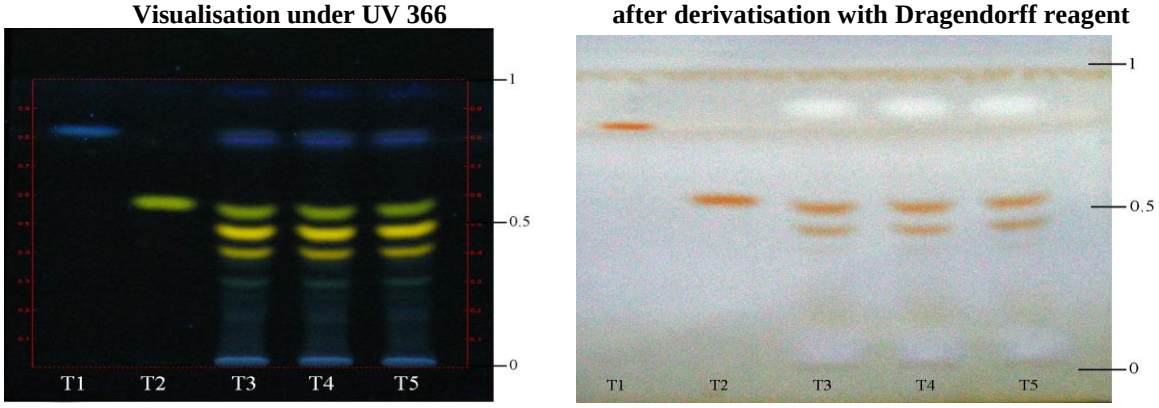
Visualisation under UV 254 **Visualisation under UV 366**



T1 : Piper longum , **T2** : Piper nigrum , **T3** : Emblica officinalis,
T4 : Coptis teeta, **T5** : Sample - 1 **T6** : Sample - 2 **T7** : Sample - 3

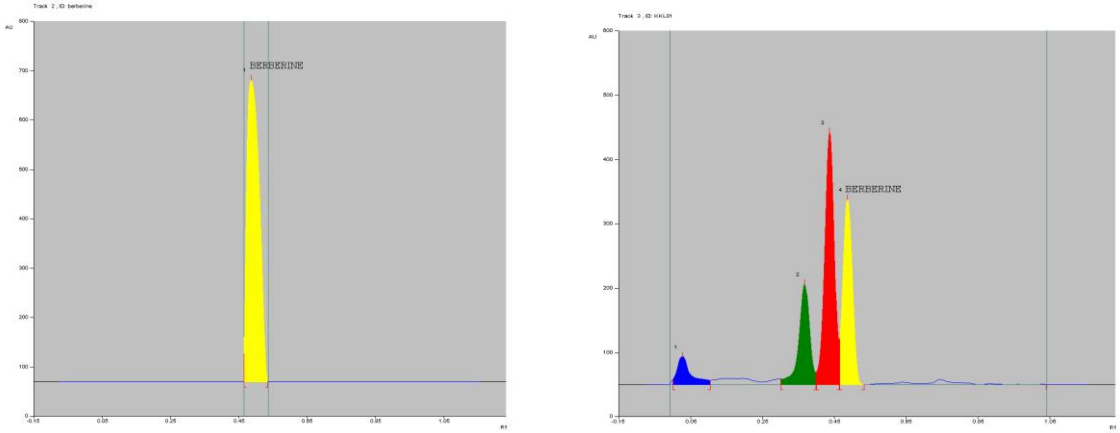
Fig. 2: TLC Finger print of Test solution of Karpuradi kuzhambu with ingredients

Visualisation under visible light
Visualisation under UV 366 **after derivatisation with Dragendorff reagent**



T1 : Piperine , **T2**: Berberine, **T3** : Sample – 1, **T4** : Sample - 2 **T5** : Sample - 3

Fig. 3: TLC Finger print of Test solution of Karpuradi kuzhambu with ingredients



Berberine standard **Berberine in Karpooradi kuzhambu**

Fig. 4: Densitogram of Berberine in Karpuradi kuzhambu by HPTLC

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