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# INDIAN DRUGS

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# PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTI-MICROBIAL ACTIVITY OF AN AYURVEDIC FORMULATION NISHAKATAKADI KASHAYAM

Radha A. \*, Prabhakaran M., Paul M., Sreevalsan A., Sebastian J.,  
Verghese J., and Jolly C.I.

(Received 31 January 2013) (Accepted 26 April 2013)

## ABSTRACT

The objective of the present study was to develop quality parameters to evaluate and standardize the polyherbal formulation and also to study the eight crude drugs viz. *Curcuma longa* - rhizome (*Manjal*), *Salacia oblonga*- root bark (*Ekanayakam*), *Embllica officinalis*- pericarp (*Nellika*), *Strychnos potatorum* - seed (*Thettanparal*), *Ixora coccinea* - root (*Chethi*), *Vetiveria zizanioides* - root (*Ramacham*), *Aerva lanata* - root (*Cherula*) and *Symplocos racemosa* - bark (*Pachotti*). The studies included physic-chemical parameters, HPTLC studies, heavy metal analysis and standardization of formulation using chemical markers. Antimicrobial activity of different fractions of kashayam was studied and found to be highest for ethanolic extract of kashayam. The pharmacognostical studies include microscopical, macroscopical examinations, ash values and extractive values, mineral elemental analysis, fluorescence analysis and FTIR analysis.

**Keywords:** High Performance Thin Layer Chromatography (HPTLC), Inductively Coupled Plasma Mass Spectroscopy (ICPMS).

## INTRODUCTION

Ayurveda utilises many dosage forms in the treatment of diseases. Nishakathakadi kashayam is a decoction popularly used for curing diabetes mellitus. It is prepared by boiling coarse powders of *Curcuma longa* - rhizome (*Manjal*), *Salacia oblonga*-root bark (*Ekanayakam*), *Embllica officinalis*-pericarp (*Nellika*), *Strychnos potatorum*-seed (*Thettanparal*), *Ixora coccinea* -root (*Chethi*), *Vetiveria zizanioides* - root (*Ramacham*), *Aerva lanata*-root (*Cherula*) and *Symplocos racemosa* -bark (*Pachotti*). Care Keralam undertook an extensive study on this Kashayam, focusing on pharmacognosy (Fig. 5-8), phytochemical screening (Table I), antimicrobial activity (Fig. 1),

HPTLC analysis (Fig. 2) and estimation of heavy metals and other minerals (Table II). The FTIR profiling of kashayam powder was done and it was found to contain amines, carboxylic acids esters and phenols (Fig. 4).

## MATERIALS AND METHODS

### EXPERIMENTAL

#### Plant collection

All the eight raw materials were collected and authenticated by the botanist from the raw drug division of Kerala Ayurveda Ltd, Athani. These raw materials were further authenticated by established pharmacognostical evaluations vis-a-vis free hand sectioning and powder analysis. The raw materials were air dried, pulverized, homogenized to fine powder and stored in airtight bottles.

#### Kashayam Preparation

Coarse powders of eight raw materials at the rate of 100 g were mixed thoroughly and boiled with 1600mL distilled water. This was further reduced to 400mL under low heat. The decoction thus obtained was strained through a clean cloth, allowed to cool and kept in airtight bottle in refrigerator.

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## Phytochemical Screening of Kashayam

25 mL of this kashayam was evaporated to dryness using a water-bath. Methanolic extract of this sample filtrate was dissolved in dilute hydrochloric acid and filtered. The above filtrate was subjected to

raw materials. Derivatized of developed plates was performed using vanillin sulphuric acid reagent, ferric chloride reagent and Libermann Burchard reagent to re-confirm the presence of bitters, tannins and sterols respectively<sup>1,3</sup>.

**Table I: Phytochemical screening of eight raw materials**

SI No:	Raw Material	Tannins	Alkaloids	Sterols	Phenols	Saponins
1	<i>Curcuma longa</i>	+	+	-	+	-
2	<i>Aerva lanata</i>	-	+	-	-	+
3.	<i>Ixora coccinea</i>	+	+	-	+	-
4	<i>Phyllanthus emblica</i>	+	+	-	+	-
5	<i>Salacia reticulata</i>	+	+	-	+	-
6	<i>Strychnos potatorum</i>	+	-	+	+	-
7	<i>Symplocos racemosa</i>	-	+	+	-	+
8.	<i>Vetiveria zizanioides</i>	-	+	+	-	-

**Table II: Heavy metal analysis of formulation and its ingredients**

SI No:	Raw Material	Mercury(ppm)	Arsenic (ppm)	Cadmium (ppm)	Lead (ppm)
1	<i>Curcuma longa</i>	BDL	0.070	0.273	0.49
2	<i>Aerva lanata</i>	ND	0.075	0.058	2.55
3.	<i>Ixora coccinea</i>	BDL	ND	ND	0.75
4	<i>Phyllanthus emblica</i>	0.103	BDL	0.36	1.32
5	<i>Salacia reticulata</i>	BDL	ND	ND	1.64
6	<i>Strychnos potatorum</i>	0.069	BDL	BDL	1.53
7	<i>Symplocos racemosa</i>	BDL	ND	ND	0.41
8.	<i>Vetiveria zizanioides</i>	1.27	BDL	0.082	2.17

Mayer's test, Dragendorff's test to check the presence of alkaloids. Presence of tannins and sterols was checked using ferric chloride reagent and Liebermann-Burchard reagent and these classes of compounds were quantified as per standard methods cited in IP2010, 2.6.4.

## HPTLC Analysis of Class of Compounds in Kashayam

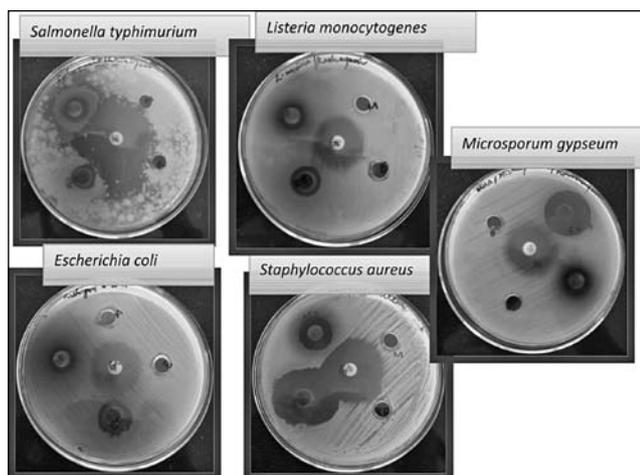
HPTLC of Methanolic extract of kashayam powder was carried out to substantiate the presence of all eight

**Table III: Heavy metal analysis in Kashayam**

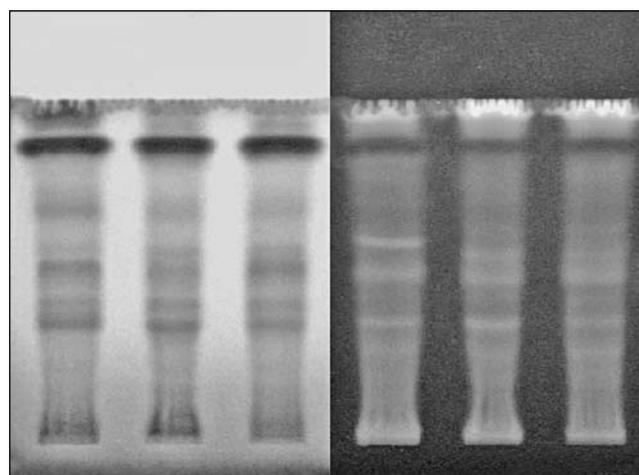
SI No	Metals analysed	Results(ppm)
1.	Arsenic	BDL
2.	Sodium	1679.54
3.	Potassium	9422.07
4	Cadmium	BDL
5	Mercury	BDL
6	Lead	BDL

**Table IV: The antimicrobial activity of Kashayam**

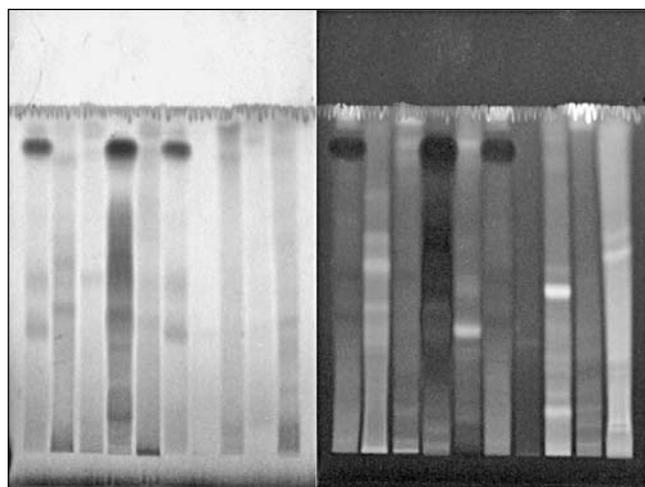
Extracts	Microorganisms				
	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Staphylococcus aureus</i>	<i>Listeria monocytogenes</i>	<i>Microsporium gypseum</i>
Methanol	14mm	15 mm	19 mm	13 mm	15 mm
Ethanol	18 mm	15 mm	31 mm	15 mm	25 mm
Water extract	-	-	16 mm	-	15 mm
Gentamycin	21 mm	20 mm	27 mm	21 mm	21 mm



**Fig. 1: Plates showing the antimicrobial activity of Kashayam**



**Fig. 3: Batch comparison of three batches of kashayam**



**Fig. 2: Developed plates of kashayam and its raw materials at 254nm and 366nm Track details:Track 1-Kashayam 2-Symplocus racemosus 3-Salacia oblonga 4-Emblica officinalis 5-Ixora coccinea 6-Kashayam 7-Strychnos potatorum 8-Vetiveria zizanioides 9-Curcuma longa 10-Aerva Lanata**

### HPTLC Comparison of Three Batches of Kashayam

Three batches of kashayam were prepared and compared the HPTLC to check any difference in the chemistry and manufacturing process<sup>1,3</sup> (Fig. 3).

### FTIR Profiling of Kashayam Powder

Finely powdered methanolic extract of kashayam powder was subjected to FTIR analysis to reconfirm the presence of phenols, sterols and tannins in kashayam (Fig. 4).

### Heavy Metal Analysis of Kashayam using ICPMS

25 mL of kashayam was evaporated to dryness and 1g of the sample kashayam powder was digested in microwave digestive system (MDS) for thirty minutes. This sample was analysed for the presence of heavy metals, inorganic minerals and elements like

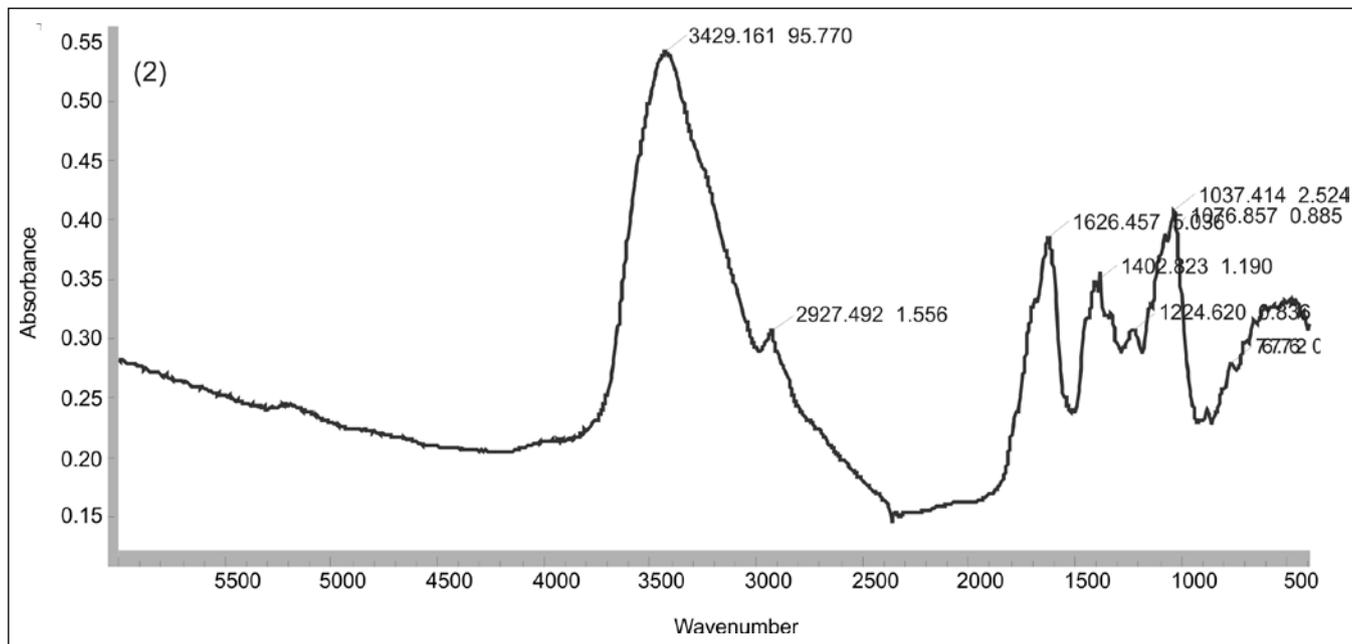


Fig. 4: FTIR profile of Kashayam

**Peak Details**

3400-3200-Phenols

2927.492-Alkanes

1626.457-Arenes

1402.823-Carboxylic acid (C-O-H bending)

1224.620-Carboxylic acid (med-str O-C)

1037.414-Amines med (C-N)

1076.857- Amines med (C-N)

767.7-S-OR esters

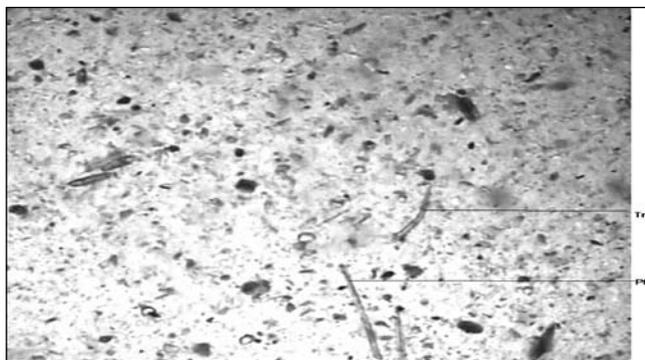


Fig. 5: Trichomes and Phloem fibers of Kashayam powder

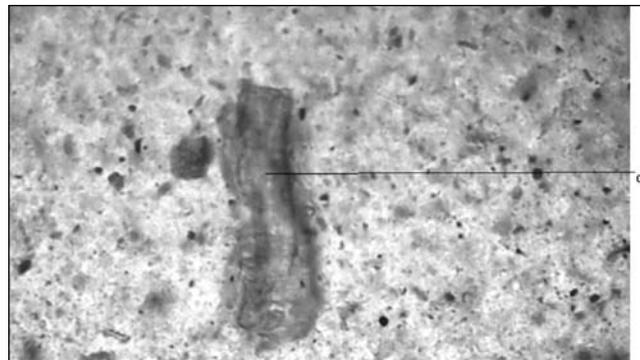


Fig. 6: Oleoresin cells of Kashayam Powder

chromium, calcium, potassium, sodium and vanadium were also analysed using ICPMS<sup>1</sup>.

**Powder Analysis of Kashayam**

Powder microscopy of kashayam filtrate was done to establish the presence of phloem fibres, oleoresin cells, oil cells, trichomes and to ensure absence of

starch<sup>3-5</sup>. The kashayam powder was also subjected to aflatoxin analysis. Microbial load in kashayam powder was performed. Total viable plate count, Yeast and mould count, specific organisms including *E.coli*, *Salmonella sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* according to<sup>1,7</sup> was also carried out<sup>5-8</sup> (Fig. 1, 5-8).

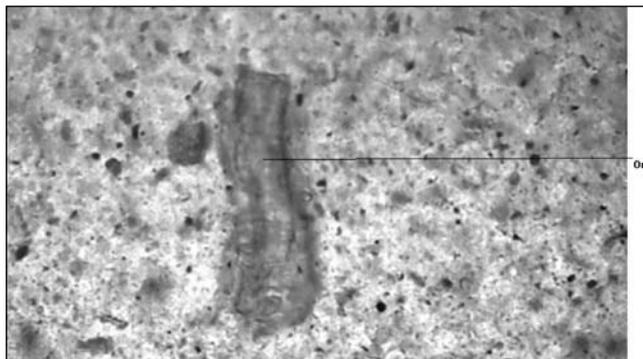


Fig. 7: Stone cells and Phloem fibers

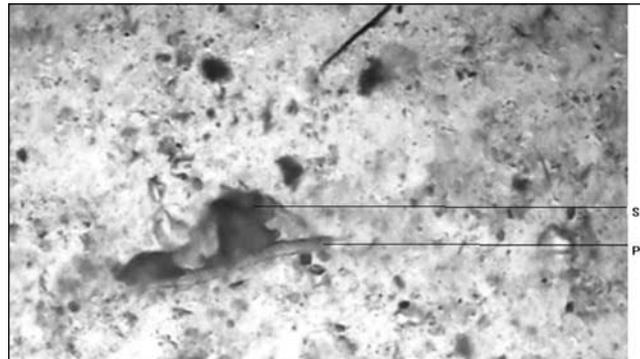


Fig. 8: Kashayam filtrate powder microscopy

### Antimicrobial Activity of Kashayam

Five microbial cultures were analysed. The bacteria include *Escherichia coli* (MTCC 433), *Salmonella typhimurium* (MTCC 3231), *Listeria monocytogenes* (MTCC 657), *Staphylococcus aureus* (MTCC 9886) and fungal species of *Microsporum gypseum* (MTCC 2819), which were taken from IMTECH culture collection (MTCC), Chandigarh. Concentrated methanolic, ethanolic and aqueous extract of the powdered kashayam in four different concentrations (50 $\mu$ L, 100 $\mu$ L, 150 $\mu$ L, 200 $\mu$ L) were employed for the study. Well-diffusion assay method<sup>1,7</sup> (CCRAS 2010) was used to check the antimicrobial activity (Fig. 1).

### RESULTS AND CONCLUSION

The alkaloids, tannins, sterols and bitters were found to be present in Nishakathakadi kashayam and furthermore the percentage of alkaloids, tannins, and bitters were estimated to be 0.32%, 0.65%, and 0.24% respectively<sup>4-6,8,9</sup>. HPTLC analysis revealed the presence of all eight raw materials in kashayam, which was confirmed by the Rf values of individual raw materials (Fig. 2). Arsenic, cadmium, mercury and lead were found to be under acceptable limits during heavy metal analysis (Table II, III). It was found to be highest for ethanolic extract of Kashayam powder whilst methanolic extract exhibited some antimicrobial activity and aqueous extract showed no antimicrobial activity (Fig.1, Table IV). Three batches of kashayam was compared using HPTLC to check any variations in the chemistry during kashayam preparation. The HPTLC finger print of three batches was exactly the same by comparing

the Rf values (Fig. 3). FTIR profile confirmed the presence of sterols, Phenols and tannins in Kashayam (Fig. 4).

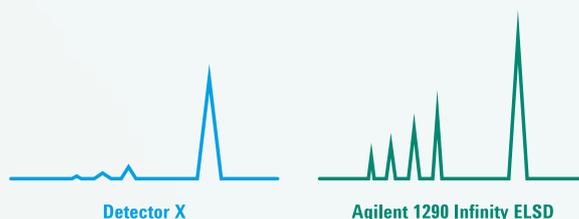
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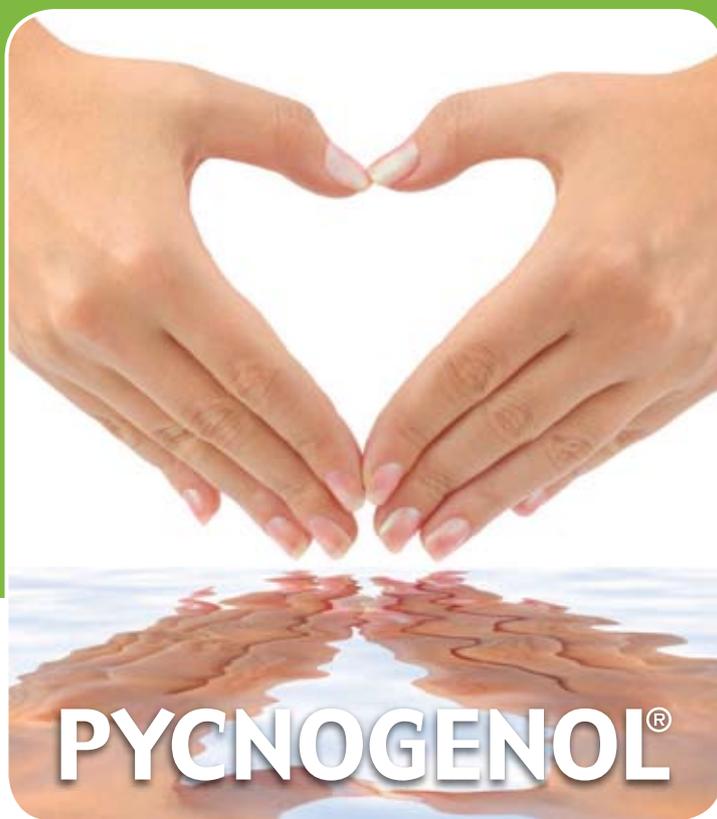
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- ✓ Best suitable for all regulatory requirements



Ethernet communication



Controller n Data logging



Alarm alert



Safety



Software

Manufacturers of: Walk-In Chambers, Humidity (Stability) Chambers, Photostability Chambers, Cooling Chambers, Incubators, Deep Freezers, Pharma Refrigerators, Ovens, Data Logger

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