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Preliminary Standardization of *Rasna Kashaya* and *Erandamoola Kashaya* – Two Herbal Formulations

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ABSTRACT

The lifetime incidence of low back pain including Sciatica is 30-90% with worldwide prevalence ranging from 5 to 25%, and this condition escalates with age and lifestyle factors. Radiating leg pain and related disabilities are observed in Sciatica which can very well be analogized with *Gridhrasi*. *Gridhrasi* is one of the most prevalent of *Vatavyadhi's* in the current age and time. *Charakacharya* has mentioned a special class of drugs called *Agreya dravyas* which states the best of drugs along with its expected action. Out of these best mentioned modalities *Rasna* and *Erandamool* are the most suitable *Vatahara* drugs. *Gridhrasi* being '*Vataja nanatmaja vyadhi*', '*Vataprakopa*' and '*Vata-Kapha prakopa*' are the two courses of manifestation of this disease. The drugs chosen for this study, *Rasna* and *Erandmool* are both *Vata- kaphahara* in nature.

Lack of standardization of herbal formulations creates a non- uniformity in validation of the efficacy and maintaining quality of the product. In the present study an attempt was made at setting up a standard profile which was prepared using pharmacognostically authenticated drug *Rasna* (Pluchea lanceolate) and *Erandamool* (Ricinus communis), followed by subjecting it to detailed physico-chemical analysis as per standard protocol. So, an attempt has been made here to study *Rasna kashaya* and *Erandamoola kashaya* by analyzing through qualitative and quantitative physiochemical parameters and to develop fingerprints of High performance thin layer chromatography (HPTLC). Major peaks with R_f values – 0.73 of *Rasna* and 0.77 of *Erandamool* were found at wavelength 215 nm and 366 nm respectively. The data obtained in the present study will help for the use of this formulation.

KEYWORDS

Rasna Kashaya, *Erandamoola Kashaya*, *Gridhrasi*, Physico-chemical analysis, TLC, HPTLC

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INTRODUCTION

The lifetime incidence of low back pain including sciatica is 30-90% with worldwide prevalence ranging from 5 to 25%[1]. And this condition escalates with age and lifestyle factors. Radiating leg pain and related disabilities are observed in Sciatica which can very well be analogized with *Gridhrasi*[2]. *Gridhrasi* is one of the most prevalent of *Vatavyadhi's* in the current age and time. *Charakacharya* has mentioned a special class of drugs called *Agreya dravyas*, which states the best of drugs along with its expected action[3]. Out of these best mentioned modalities *Rasna* and *Erandamool* are the most suitable *Vatahara* drugs. *Gridhrasi* being '*Vataja nanatmaja vyadhi*', '*Vataprakopa*' and '*Vata-Kapha prakopa*' are the two courses of manifestation of this disease[4-5]. The drugs chosen for this study, *Rasna* and *Erandamool* are both *Vata- kaphahara* in nature[6].

To get the desired benefits in a particular diseased condition, various poly-herbal drugs are utilized in treatment. A single herb often contains more than one phytochemical constituent which act synergistically with each other to produce desired pharmacological action. With the pertinent use of modern technology, studies are being carried out which yield pharmacologically active ingredients of the herbal medicines as well as their usefulness in drug therapy[7]. The raw drugs used here, *Rasna* (Pluchea lanceolata) belonging to Asteracea family contains secondary metabolites like quercetin, beta sitosterol and pluchine[8-9]. *Erandamool* (Riccinus communis) belonging to Euphorbiacea family contains flavonoids having the potentiality to scavenge the free radicals which have been reported to have anti-inflammatory, antiarthritic activity[10]. Both these drugs are popularly useful in numerous inflammatory conditions like rheumatism, arthritis, bronchitis and neurological diseases[11]. Most of these principles are water soluble and are utilized in the manufacturing of decoctions. Hereby, an attempt was made to standardize the single drug formulations '*Rasna Kashaya*' and '*Erandamool Kashaya'* using High Performance Thin Layer Chromatography technique. To understand the specificity and utility of a single drug formulation and to establish quality parameters, in a given disease is main the aim of this study.

AIMS AND OBJECTIVES

Pharmacognostical and analytical study of *Rasna Kashaya* and *Erandamool Kashaya* in *Gridhrasi*.

MATERIALS AND METHODS

Collection, Identification and authentication of raw drugs:

Market samples of the raw drugs *Rasna* and *Erandmool* were procured from Navi Mumbai, Maharashtra. The ingredients and the parts used are given in table No. 1. The raw drugs are identified and authenticated as Pluchea lanceolata belonging to Asteracea family and Riccinus communis belonging to Euphorbiacea family, by Alarsin Pharmaceuticals, Andheri, Mumbai as mentioned in figure No. 1.

Table 1 Raw drug Macroscopic and Microscopic characters

Sr. No	Drug	Botanical Name	Family	Parts Used
1	***Rasna***	Pluchea lanceolate	Asteracea	Root
2	***Erandamool***	Ricinus communis	Euphorbiacea	Root

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Date : 25/09/2018

AUTHENTICATION CERTIFICATE

Drug Samples provided by ***Dr. Sneha Nambiar*** of ***Kayachikitsa Dept., Dr. D.Y.Patil College of Ayurveda, Pimpri Pune*** are studied exomorphologically and microscopically.

We hereby Authenticate that the samples belongs to.

No.	Drug	Latin name	Family
1	RASNAMOOL	*Pluchea lanceolata*	Asteraceae
2	ERANDMOOL	*Ricinus communis*	Euphorbiaceae.

The above Drugs are Authenticated.

This certificate is provided on demand of ***Dr. Sneha Nambiar*** , for her research.

J.P. Vasavada
ALARSIN

Fig 1 Authentication Certificate

Preparation of the Decoction (*Kashaya*):

Both the procured drugs *Rasna* and *Erandmool* are made into coarse powder (*bharad)* form in a mass mixer. The mixture is converted into fresh decoction (*Kashaya*) as per reference of *Sharangdhara Samhita*[3]. Decoction is prepared by mixing 20 gms *bharad* with 16 times water i.e, 320 ml and reducing it to 1/8 part i.e, 40 ml.

Phytochemical Analysis of Compound Drugs:

The prepared decoction was analysed for organoleptic parameters as shown in table no. 2 A and 2 B.

Table 2A Organoleptic parameters- *Rasna* (Pluchea lanceolata)

Test	**Raw Drug Sample**	***Kashaya***
Appearance	Dry Root	Clear Decoction
Colour	Yellowish brown	Dirty Brown
Odour	Strong Acidic	Herbaceous
Taste	Slightly Sour	Bitter
Texture	Rough	-

Table 2B: Organoleptic parameters- *Erandamool* (Riccinus communis)

Test	**Raw Drug Sample**	***Kashaya***
Appearance	Dry root	Clear Decoction
Colour	Light brown	Light reddish Brown
Odour	Faint	Herbaceous
Taste	Bitter	Bitter
Texture	Rough	-

Phytochemical Parameters:

Both the drugs *Rasna Kashaya* and *Erandamool Kashaya* were evaluated for various physico- chemical analysis like loss on drying, total ash, acid insoluble ash,

alcohol soluble extract, water soluble extract. The results are shown in table no. 3 A and table no. 3 B. The samples of *Rasna and Erandmool Kashaya* were authenticated microscopically and findings are noted in figure no. 2 A and 2 B.

Table 3A Phytochemical parameters- *Rasna* (Pluchea lanceolata)

Test	Raw Drug Sample	Test	Kashaya
Foreign Matter	NIL	**Foreign Matter**	NIL
Total Ash	4.36%	**pH**	6
Acid Insoluble Ash	1.23%	**Specific Gravity**	0.9965%
Alcohol Soluble Extract	7.12%	**Refractive Index**	1.345
Water Soluble Extract	10.11%	**Loss on Drying**	99.23%
		Total Dissolved Salts	0.42%
		Total Suspended solids	0.56%

Table 3 B Phytochemical parameters- *Erandmool* (Riccinus communis)

Test	Raw Drug Sample	Test	Kashaya
Foreign Matter	NIL	**Foreign Matter**	NIL
Total Ash	5.84%	**pH**	6.3
Acid Insoluble Ash	1.52%	**Specific Gravity**	0.9971%
Alcohol Soluble Extract	6.12%	**Refractive Index**	1.248
Water Soluble Extract	20.18%	**Loss on Drying**	98.56%
		Total Dissolved Salts	0.58%
		Total Suspended solids	0.69%

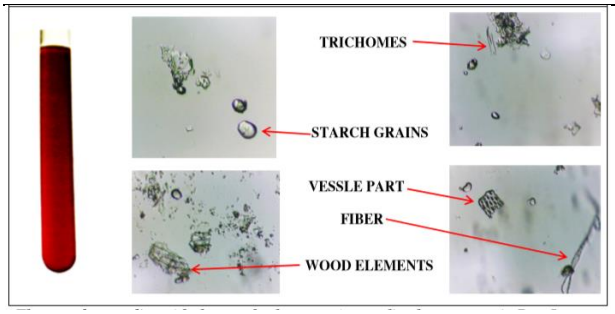

Fig 2A *Rasna Kashaya* (Pluchea lanceolata)- Microscopy

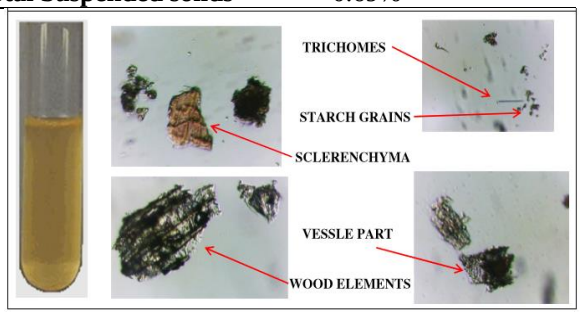

Fig 2 B *Erandamool Kashaya* (Riccinus communis)

Thin Layer Chromatography:

The thin layer chromatography profiling of 50% Ethanolic extract of dried root of *Rasna* and Chloroform extract of dried root of *Erandamool* was performed using silica gel plate. The three TLC plates were taken and 50 µl crude extracts applied on 1 centimetre above the TLC plate with the help of micro-pipette. After sample application the plates were dried and kept in the chamber equipped with solvent system; Ethyl Acetate: Methanol: Water (10:2.5:2 v/v) and Hexane: Diethyl ether: acetic acid (70:140:1 v/v) for *Rasna* and *Erandamool* respectively as mentioned in figure no. 3 A and 3 B. **IR Spectroscopy:**

The dried powder of plants *Rasna* and *Erandamool* were kept in oven for drying the moisture and then triturated with the help of mortal and pestle along with dried KBr. The blank reading of KBr analysed in FTIR (Fourier transform infrared) to avoid the interference of it in sample. The triturated extract kept in sample cell of

THIN LAYER CHROMATOGRAPHY
RASNA (PLUCHEA LANCEOLATA) 50%
Ethanloic extract of Dried Root Pieces
Mobile phase = Ethyl Acetate: Methanol: Water (10:2.5:2v/v)
Staining: Iodine Vapour

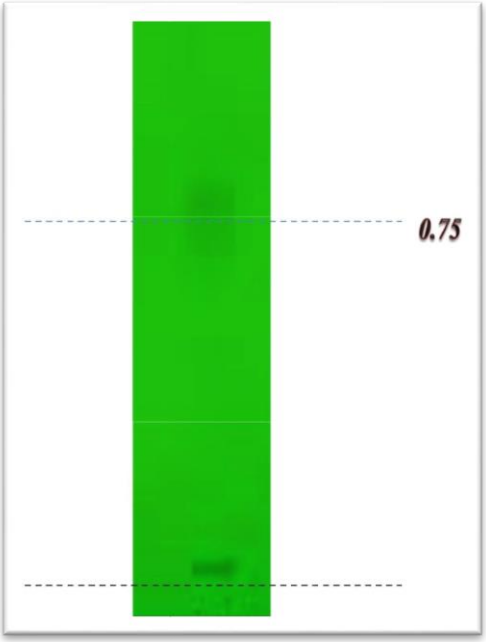

Fig 3A *Rasna* (Pluchea lanceolata) (R_f value): 0.75

THIN LAYER CHROMATOGRAPHY
ERAND MOOL (RICINUS COMMUNIS)
Chloroform extract of Dried Root Pieces
Mobile phase = Hexane: Diethyl Ether: Acetic Acid (70:140:1 v/v)
Staining: Iodine Vapour

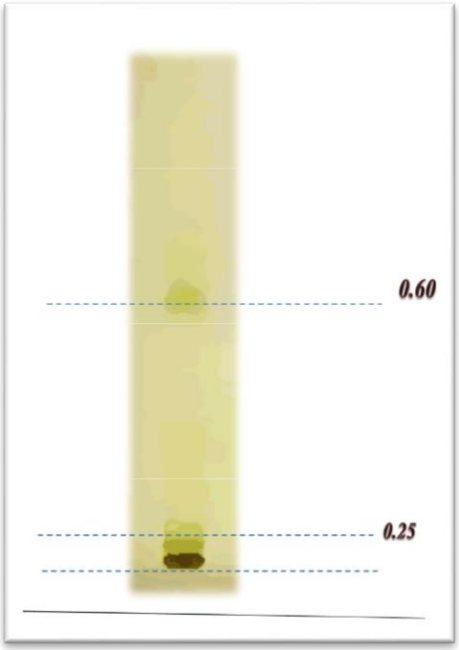

Fig 3B *Erandamool* (Riccinus communis) (R_f value): 0.25 Glycerol, 0.60 Fatty Acid

FTIR and instrument allowed to run spectra which generated peaks of functional groups as shown in figure no. 4 A and 4 B.

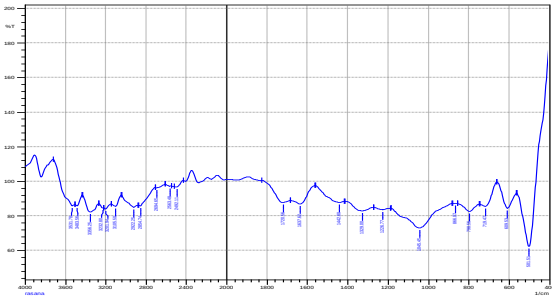

Fig 4A IR Spectra- *Rasna* (Pluchea lanceolata)

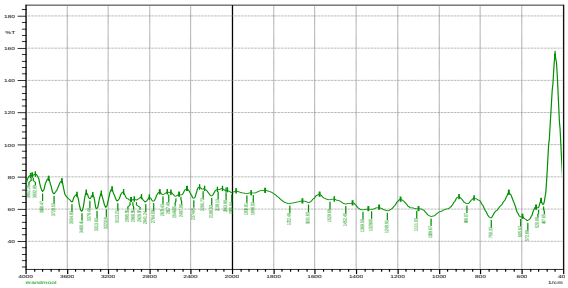

Fig 4A IR Spectra- *Erandamool* (Riccinus communis)

High performance Thin Layer Chromatography:

Preparation of test solution (T): The freshly prepared aqueous extract was filtered well with Whatman filter paper no. 1.

Method of the HPTLC profiles of *Rasna Kashaya* and *Erandamool Kashaya* are given in table no. 4 A and 4 B and their HPTLC spectra at figure no. 5 A and 5 B.

Table 4A Details of HPTLC profile- *Rasna Kashaya*

Chromatographic conditions	
Application Mode	CAMAG Linomat 5, 10, 15
Stationary Phase	MERCK-TLC/HPTLC silica gel 60 F_{254} on Aluminium sheets
Application (Y axis) start position	10mm

Application (Y axis) End position	90mm from plate base
Bottom edge	20mm
Space between band	6mm
Sample application volume	5, 10, 15 µL
Development mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation time	15 mins
Mobile phase (MP)	Ethyl Acetate: Methanol: Water (10:2.5:2)
Wavelength Detection	366nm
Drying mode, Temp and Time	TLC plate heater preheated at 100± 5º C for 10 mins
Observation:	Plate was examined for appearance of major peak R_f value 0.77

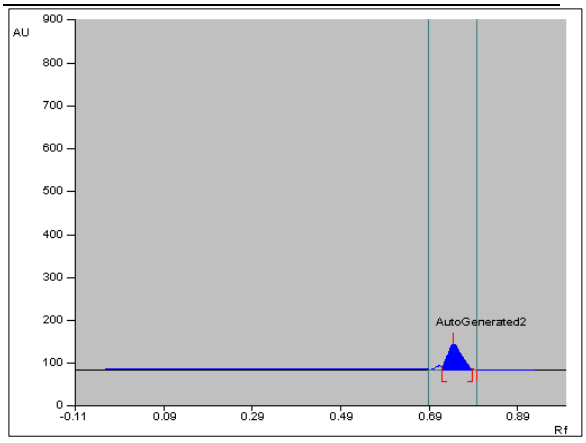

Fig 5 A Details of HPTLC profile- *Rasna Kashaya*

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.74 Rf	11.1 AU	0.77 Rf	38.9 AU	100.00 %	0.80 Rf	0.2 AU	954.5 AU	100.00 %

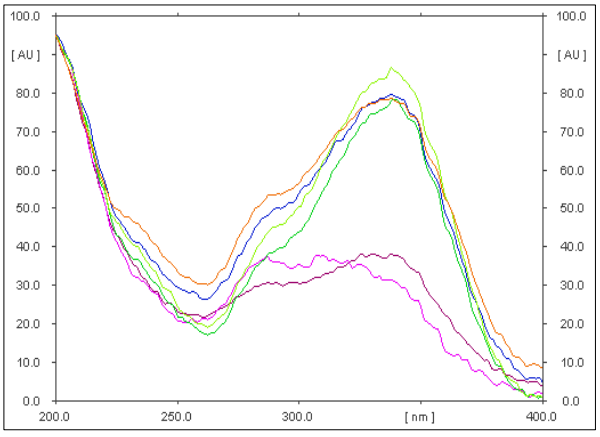

Fig 6 A UV spectra- *Rasna Kashaya*

Table 4B *Erandamool Kashaya*- Details of HPTLC profile

Chromatographic conditions	
Application Mode	CAMAG Linomat 5, 10, 15
Stationary Phase	MERCK-TLC/HPTLC silica gel 60 F_{254} on Aluminium sheets
Application (Y axis) start position	10mm
Application (Y axis) End position	90mm from plate base
Bottom edge	20mm
Space between band	6mm
Sample application volume	5, 10, 15 µL
Development mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation time	15 mins
Mobile phase (MP)	Hexane: Diethyl Ether: Acetic acid (2:7:1)
Wavelength Detection	215nm
Drying mode, Temp and Time	TLC plate heater preheated at 100± 5º C for 10 mins
Observation:	Plate was examined for appearance of major peak R_f value 0.73

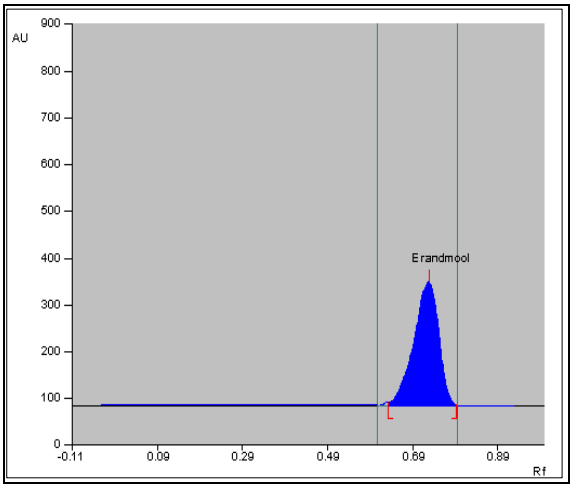

Fig 5B *Erandamool Kashaya*: HPTLC

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.63 Rf	7.4 AU	0.73 Rf	265.4 AU	100.00 %	0.79 Rf	2.5 AU	13054.2 AU	100.00 %

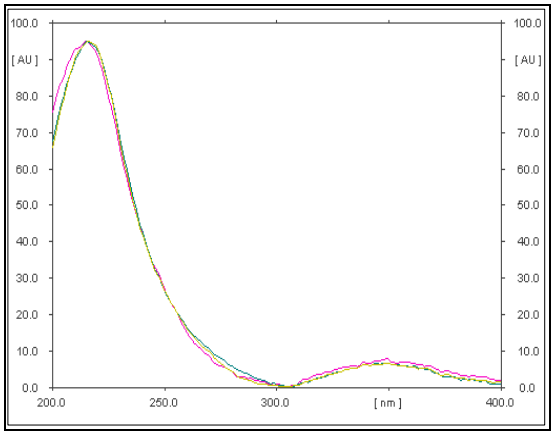

Fig 6B UV spectra- *Erandamool Kashaya*

RESULTS AND DISCUSSION

The plant sources were widely used for medicinal purpose since ancient times due to active chemical constituents present in it. The results of phytochemical screening are compiled in table no. 2 A and 2 B. The pH was measured for noting the acidity and alkalinity of the sample which helps in understanding the pharmacological activity of drug absorption and metabolism. In the sample of *Rasna* and *Erandamool*, pH was found to be 6 and 6.3 with their loss on drying 99.23% and 98.56% respectively.

Total ash value indicates contamination substation and adulteration. In this sample it is 0.21% and 0.35% for *Rasna* and *Erandamool* respectively. Low total ash signifies low levels of inorganic matter and silica content. Specific gravity of *Rasna* was found to be 0.9965% and *Erandamool* was 0.9971% with the refractive index 1.345 and 1.248, respectively. The TLC was performed using mobile phase of ethyl acetate: methanol: water for *Rasna* and hexane: diethyl ether: acetic acid for *Erandamool*. The glycerol was present at R_f value 0.25 and fatty acids at R_f value 0.60 in the chloroform extract of *Erandamool*.

The FTIR technique was used to identify functional groups present in the extract of *Rasna*, represent narrow -NH group peak obtained at 3531 cm^{-1}, stretch -OH peak at 3232 cm^{-1}, aromatic group -C-H peak at 3105 cm^{-1}, -C-H peak at 1442 cm^{-1}, C=O peak at 1637cm^{-1} and CHO peak at 1720 cm^{-1}. The functional groups present in the extract of *Erandamool*, represent -NH group peak obtained at 3460 cm^{-1}, aromatic group C-H peak at 3100 cm^{-1}, CHO =C-H peak at 2841cm^{-1}, N-O peak at 1529 cm^{-1}, C=O peak at 1722 cm^{-1}. The investigation confirmed presence of important phytochemicals in the plant extracts which are known for its therapeutic value.

The chromatography was carried out using Merck TLC aluminum sheets of silica gel 60 F_{254}, (10 X 10 cm) with the mobile phase for *Rasna* being Ethyl acetate: Methanol: Water (10:2.5:2, v/v/v) and Hexane: Diethyl Ether: Acetic acid (2:7:1) for *Erandamool*. Figure 5 A shows the chromatogram of *Rasna* at starting R_f value 0.74, max. R_f value 0.77, end R_f value 0.80 with starting height 11.1 AU, maximum height 38.9 AU and area 954.5 AU. Figure 5 B shows chromatogram of *Erandamool*

with starting R_f value 0.63, max. R_f value 0.73, end R_f value 0.79 with starting height 7.4 AU, max. height 265.4 AU and area 13054.2 AU. The densitometric scanning was performed at 366 nm and 215 nm for *Rasna* and *Erandamool* respectively. Figure 6 A and 6 B shows the UV spectra of *Rasna* and *Erandamool* respectively. The HPTLC fingerprinting performed revealed major peaks visualized at R_f value 0.77 for *Rasna* and 0.73 R_f value for *Erandamool*. The presence of Glycerol and fatty acid was confirmed in the extract of *Erandamool* at the above mention R_f. Similarity is seen in TLC and HPTLC results of *Rasna* and *Erandamool*. The phytochemical analysis of these plant extracts reveals the presence of chemical constituents like flavonoids, carbohydrates, saponins, amino acids, alkaloids.

CONCLUSION

In modern medicine this condition is routinely managed by NSAID's, analgesic drugs, steroids which may have untoward effects. The raw drugs used here, *Rasna* (Pluchea lanceolata) belonging to Asteracea family contains secondary metabolites like quercetin, beta sitosterol and pluchine and *Erandamool* (Riccinus communis) belonging to Euphorbiacea family contains flavonoids having the potentiality to scavenge the free radicals which have been reported to have anti-inflammatory, antiarthritic activity. Both these drugs are popularly useful in numerous inflammatory conditions like rheumatism, arthritis, bronchitis and neurological diseases. From *Ayurvedic* perspective, *Vata* is the main *dosha* responsible for causing pain. The drugs used in this study are mainly *Vatahara* in nature and are commonly used in many Ayurvedic formulations. *Vatahara* property of these drugs are responsible for pain relieving action.

Kashaya kalpanas are the basic formulations in *Ayurvedic* pharmaceutics from which various secondary preparations can be made. *Kashaya* formulations are prepared in such a way that the drug and its active principles are extracted into it. The purpose of preliminary phytochemical screening is qualitative chemical evaluation, which indicate the presence of wide range of chemical constituents in the chosen drug. The phytochemical analysis of these plant extracts reveals the presence of chemical constituents like flavonoids, carbohydrates, saponins, amino acids, alkaloids.

TLC and HPTLC results show the identification of chemical constituent with the help of finger printing using the solvent system as mentioned in Table 4 A and 4 B.

The R_f values and percentage peak area determined by HPTLC for both *Erandamool* and *Rasna* were noted at 0.60 and 0.77 respectively.

The IR spectroscopy of both the extracts represent various functional groups like acid(-COOH), alcohol (-OH), ester (R-O-R), carbonyl (-CO-), alkanes and alkenes. The major component of the extracts are hydrocarbons.

This detailed chemical profile may be useful in the identification and determination of quality of the drugs. The phytochemical tests carried out reveal that it is safe for further pharmacological evaluation and patient trials. The finger printing technique developed here could be useful for researchers to carry out further researches. Research work with larger sample for a longer period of time should be carried out to prove its efficacy as a single drug therapy.

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