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EVALUATION OF PUṄKAM PŪ CŪRAṆAM AND IDENTIFICATION OF PUṄKAM PŪ BY HPTLC METHOD

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ABSTRACT

Siddha medicines mainly comprises of plant parts, metals, minerals and some animal substances. Nowadays, the purity and quality of drugs were hampered by adulteration. So, it is mandatory to ensure the quality and safety of drugs. The aim of the present study is to standardize a Siddha formulation, Puṅkam pū cūraṇam and its herbal ingredient *Pongamia pinnata* (L.) Pierre (Puṅkam pū) by applying suitable standards and using modern techniques. The identification of the herbal ingredient in the cūraṇam is also performed with these methods. Puṅkam pū cūraṇam and Puṅkam pū were subjected to powder microscopical studies, physico-chemical analysis, preliminary phytochemical studies and HPTLC analysis. Physico-chemical parameters such as total ash, acid insoluble ash, solubility in alcohol and water and loss on drying at 105ºC of the cūraṇam and the ingredient were determined by standard methods. Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, steroids, tannins, carbohydrates, phenols and terpenoids in the cūraṇam and in the ingredient. Organoleptic characters (colour, odour and taste) of the cūraṇam were noted. TLC chromatogram & HPTLC finger print profiles were documented at 254 nm and 575 nm after derivatisation. The present study ensures the quality of the Puṅkam pū cūraṇam and its ingredient Puṅkam pū by evaluating the powder characteristics, physico-chemical constants and HPTLC fingerprints.

KEYWORDS: Puṅkam pū cūraṇam, *Pongamia pinnata* (L.) Pierre, Physico-chemical parameters, HPTLC studies, Powder microscopy.

INTRODUCTION

Siddha is one of the traditional and ancient systems of Indian medicine. Siddha aims in promotion and preservation of physical and mental health. The treatment includes both curative and preventive aspects. Siddha medicines are mainly prepared from the parts of the plants, metals, minerals and some animal substances. The system of quality, efficacy and safety has been put in place with scientific database generated through basic and applied research. The success of any system of medicine may be attributed to scientific research and database, validated methods, procedures and system of quality control, quality assurance and safety. In order to ensure all the above aspects, standardization of drugs will have to be done. Herbal products are mostly based on either the extracts or the dried and powdered form of herbs. The major issue that makes the Indian herbs and the herbal preparation unacceptable in Western countries is the lack of proper standardization of the drug. This paper presents information on standardization of an important Siddha compound formulation, Puṅkam pū cūraṇam and its major ingredient Puṅkam pū (Flower of *Pongamia pinnata* (L.) Pierre.).

Pongamia pinnata (L.) Pierre. belongs to the family Fabaceae, distributed in India, Myanmar, Nepal, Thailand, Bangladesh, China, Japan, Malaysia, Australia and Pacific islands. The vernacular names of the plant are Sanskrit- karanj, karanja; Hindi - karanja, kanji, karanji; Bengali – karanj; Tamil - punku, punkam, ponkam; English – Indian Beach tree. *P. pinnata* is a medium-sized evergreen or briefly deciduous, glabrous shrub or tree 15-25 m high, with straight or crooked trunk 50-80 cm or more in diameter and broad crown of spreading or drooping branches. Bark grey-brown, smooth or faintly vertically fissured Branchlets hairless with pale stipule scars. Inflorescence raceme-like, axillary, 6-27 cm long, bearing pairs of strongly fragrant flowers. Flower clusters at base of and shorter than leaves, to 15 cm long, slender, drooping. Flowers 2-4

together, short-stalked, pea-shaped, 15-18 mm long Calyx campanulate, 4-5 mm long, truncate, finely pubescent; corolla white to pink, purple inside, brownish veined outside, 5- toothed, standard rounded obovate 1-2 cm long, with basal auricles, often with green central blotch and thin silky hairs on back; wings oblong, oblique, slightly adherent to obtuse keel. Flowers are useful for biliousness and diabetes.[1]

Cūraṇam is a finely powdered form of one or more single drugs. The drugs free from foreign matter are dried, separately powdered and sieved through a fine mesh or cloth. Then the required quantities by weight are taken and thoroughly mixed to uniformity. Puṅkam pū cūraṇam is used for treating urogenital disorders. The dose of the drug is 1-2 g once a day. The period of treatment is 45 days. The formulation used for the study was prepared according to the method described in Siddha Formulary of India, Part II.[2] The present study aims to evaluate the formulation and the herbal ingredient by determining its organoleptic characters, powder characteristics, phytochemical components, physico-chemical properties and HPTLC finger printing profile. HPTLC and powder microscopical studies were carried out to detect the presence of the major ingredient Puṅkam pū in the formulation.

MATERIALS AND METHODS

Puṅkam pū cūraṇam and its ingredients (1) flower of *Pongamia pinnata* (L.) Pierre (Puṅkam pū) and (2) Cow's ghee are shown in Fig.1.

Fig.1: Puṅkam pū cūraṇam and its ingredients.

Preparation of Puṅkam pū cūraṇam

The cūraṇam was prepared in the pharmacy of Siddha Central Research Institute (SCRI), Chennai as per the procedure given in The Siddha Formulary of India, Part II.[2] Equal quantities of dried Puṅkam pū and Cow's ghee were taken, fried the drug in ghee and powdered. The prepared drug was storedin a clean and air tight container.

Organoleptic evaluation

Organoleptic evaluation refers to the characters of the cūraṇam by colour, odour and taste.

Powder microscopy

The cūraṇam is sieved to get a fine powder and Powder microscopy was carried out as per standard protocol.[3] The preparation was then observed under 10X and 40X objective of microscope.

Physico-chemical analysis

Physico-chemical analysis is an important analytical technique developed for quality control of the traditional medicines. Physico-chemical parameters of the Puṅkam pū cūraṇam and flower of *Pongamia pinnata* (L.). Pierre were carried out to ensure the quality of the drugs. Physico-chemical parameters such as total ash, acid insoluble ash, solubility in alcohol and water, pH of water extract and loss on drying at 105ºC were determined by standard methods[4,5]

Successive Soxhlet extraction

The cūraṇam and the ingredient, Puṅkam pū were subjected to successive Soxhlet extraction using hexane, chloroform and alcohol separately.

Preliminary phytochemical analysis

Preliminary phytochemical screening of the cūraṇam and Puṅkam pū were carried out by standard methods.[6,7] to detect the presence of different secondary metabolites such as terpenoids, flavonoids, phenols, steroids, alkaloids, tannins, glycosides, carbohydrates etc.

High performance thin layer chromatographic (HPTLC) studies

Sample preparation

The extracts for the HPTLC studies were prepared by taking 4 g each of the cūraṇam and Puṅkam pū in two different flasks and added 40 ml of chloroform to each flask and kept overnight. The solutions were boiled for 10 minutes and filtered. The filtrates were concentrated and made up to 10 ml in two different standard flasks. These chloroform extracts were used for chromatographic studies.[8,9]

Developing solvent system

A number of solvent systems were tried to obtain an appropriate mobile phase. The solvent system Toluene: Ethyl acetate (5:3) which gave a satisfactory resolution was opted.

Sample application

Sample application was performed on a 5x10 cm silica gel 60 F_{254} pre-coated aluminium sheet. The chloroform extracts of the cūraṇam and Puṅkam pū were applied on two tracks as bands of width 8 mm using CAMAG microlitre syringe with Automatic TLC Sampler 4 (ATS4).

Development of chromatogram
After sample application the plate was introduced vertically in a twin-trough plate development glass chamber pre-saturated with the mobile phase Toluene: Ethyl acetate (5:3). The plate was developed horizontally in Camag horizontal developing chamber (10 cm × 10 cm) at room temperature.

Documentation
The developed chromatogram was air dried to evaporate solvents from the plate. The developed plate was kept in CAMAG visualizer and the images were captured under UV light at 254nm.

Densitometry
The densitometric scanning was performed by using TLC Scanner 4. The plate was scanned at 254nm and the R_f values and the finger print profiles were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation
The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and scanned at 575nm and the results were documented.

RESULTS

Organoleptic evaluation
The Puṅkam pū cūraṇam is a coffee brown powder with no characteristic taste having odour of ghee.

Powder microscopy
Powder microscopy study of Puṅkam pū cūraṇam revealed various characters, which are of *P. pinnata* flower. The characters noted were pollen grains with smooth exine, anther wall and embedded pollen grains, stone cells, sclereids, oil globules, trichome, tracheids with spiral thickeining and shown in Fig. 2.

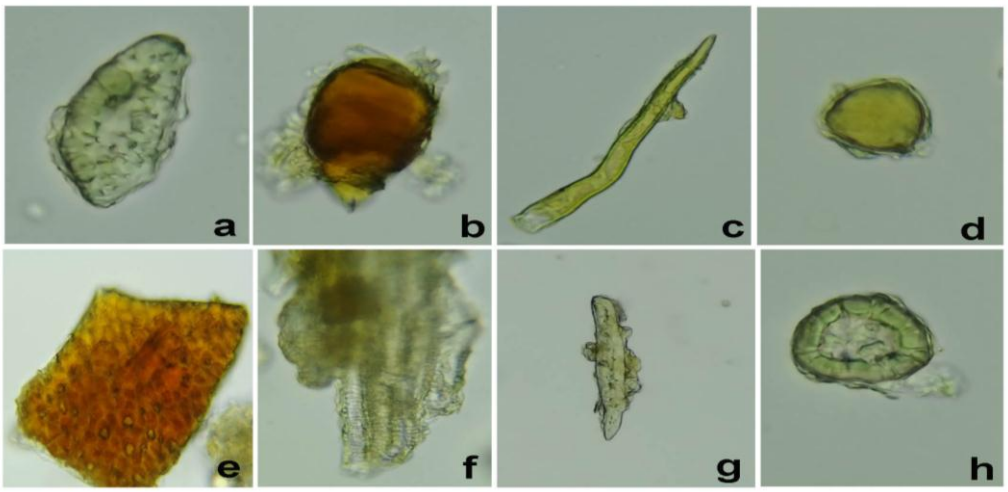


Fig. 2: Powder characteristics of Puṅkam pū cūraṇam. a: Isodiametric stone cell, b: Oil globule, c: Trichome, d: Pollen grain, e: Anther wall embedded with pollen grains, f: Tracheid with spiral thickeining, g: Sclereid, h: Ovoid stone cell.

Physico-chemical analysis
The physico-chemical parameters of Puṅkam pū cūraṇam and Puṅkam pū are given in Table 1.The cūraṇam and Puṅkam pū were subjected to successive Soxhlet extractions with hexane, chloroform and alcohol separately and the results are also given in Table 1.

Table 1: Physico-chemical parameters of Puṅkam pū cūraṇam and Puṅkam pū.

Sl. No.	Parameters	Results	
		Puṅkam pū cūraṇam	***Pongamia pinnata* (L.). Pierre –flower (Puṅkam pū)**
1.	Loss on Drying at 105^0C %	9.28	13.03
2.	Total Ash Content %	6.74	6.28
3.	Acid Insoluble Ash %	1.91	0.52
4.	Water Soluble Extractive %	25.18	28.62
5.	Alcohol Soluble Extractive %	29.17	13.55
6	pH	4.8	6.7
7	Successive Extraction % Hexane Chloroform Alcohol	 27.93 1.95 12.75	 2.17 5.94 12.22

Preliminary phytochemical analysis

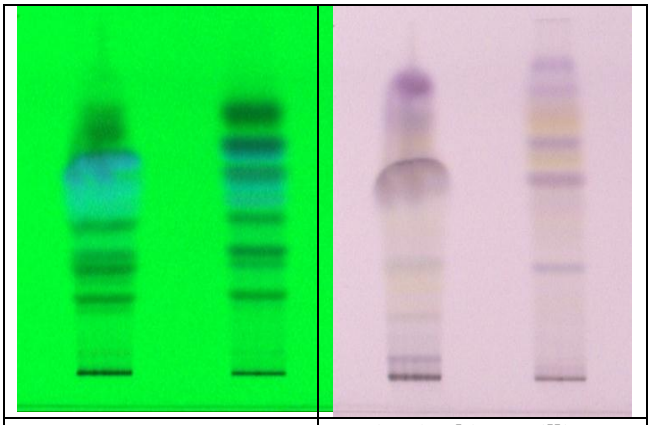
The preliminary phytochemical screening of Puṅkam pū cūraṇam and Puṅkam pū were carried out and the results are tabulated in Table 2. The results of the tests indicated the presence of alkaloids, flavonoids, steroids, tannins, glycosides, carbohydrates, phenols and terpenoids in both the samples. Saponins are present only in the single drug. Also it has been found that fixed oils & fats are present only in the cūraṇam.

Table 2: Preliminary phytochemical studies of Puṅkam Pū Cūraṇam and Puṅkam Pū.

Sl. No.	Phytochemicals	Observation	
		Puṅkam pū cūraṇam	*Pongamia pinnata* (L.) Pierre –flower (Puṅkam pū)
1	Terpenoids	+ve	+ve
2	Phenols	+ve	+ve
3	Steroids	+ve	+ve
4	Flavonoids	+ve	+ve
5	Alkaloids	+ve	+ve
6	Tannins	+ve	+ve
7	Glycosides	+ve	+ve
8	Carbohydrate	+ve	+ve
9	Saponins	-ve	+ve
10	Fixed oil & fats	+ve	-ve

High performance thin layer chromatographic (HPTLC) analysis

TLC photo documentation profiles of the chloroform extracts of Puṅkam pū cūraṇam (Track 1) and Puṅkam pū (Track 2) under UV short and under white light after derivatisation are given in Fig. 3**.** The R_f values and colour of the bands are shown in Table 3. 3D densitometric chromatogram of the chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū are given in Fig. 4 and the HPTLC fingerprinting patterns at 254 nm and 575 nm are shown in Fig. 5 and Fig. 6 respectively. The R_f values and their relative peak areas of Puṅkam pū cūraṇam and Puṅkam pū at the above mentioned wave lengths are tabulated in Table 4 and Table 5 respectively.

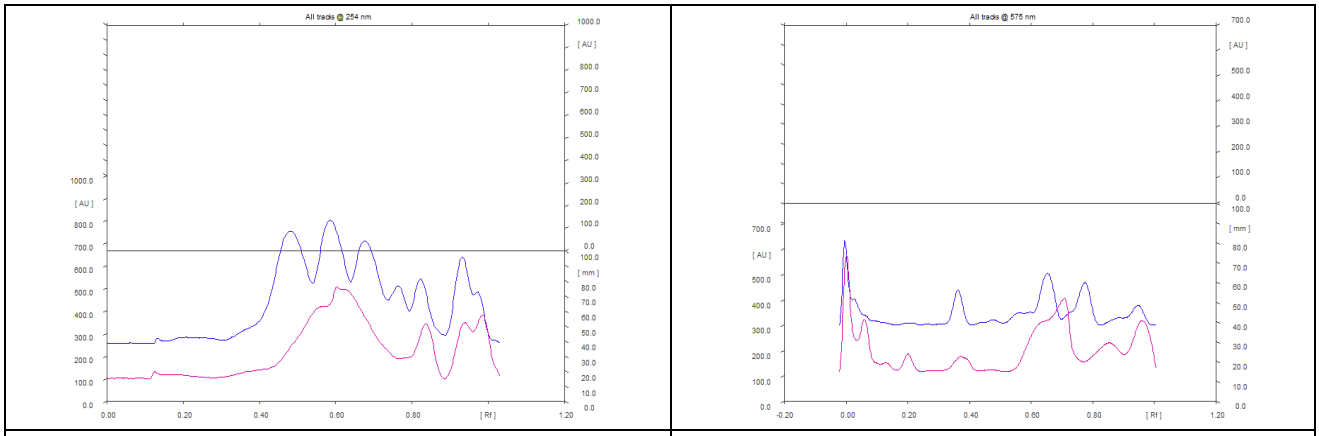


Under UV short	Derivatised in vanillin-sulphuric acid and viewed under white light

Fig 3: HPTLC photo documentation profile of the chloroform extract of Puṅkam pū cūraṇam (Track1) and Puṅkam pū (Track 2)

Table 3: R_f values and colour of visible major bands of chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū.

Drugs	Under UV 254 nm		After derivatisation under white light	
	R_f values	Colour	R_f values	Colour
Puṅkam pū cūraṇam	0.21 0.31 0.35 0.44 0.56 0.64 0.72	Brown Brown Brown Brown Bluish brown Blue Brown	0.06 0.20 0.35 0.62 0.71 0.86	Purple Purple Purple Purple Purple Purple
Puṅkam pū	0.21 0.31 0.35 0.44 0.56 0.64 0.72	Brown brown brown brown bluish brown blue brown	0.35 0.62 0.66 0.71 0.78 0.86 0.95	Purple Purple Yellow Purple Yellow Purple Purple



at 254 nm	at 575 nm

Fig. 4: 3D densitometric chromatogram of chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū.

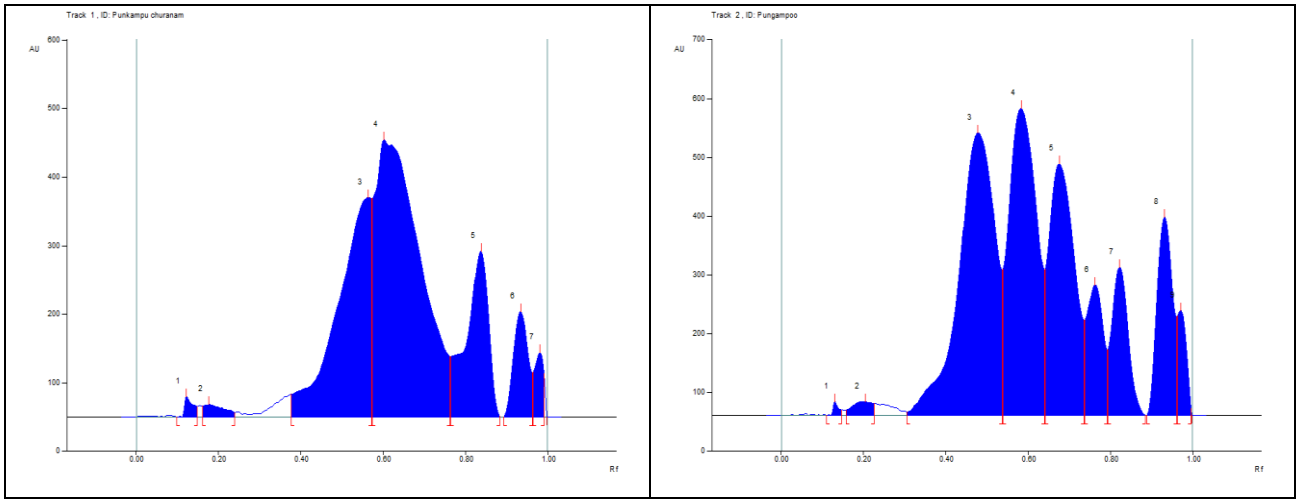


Fig 5: HPTLC finger print profile of chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū at 254 nm.

Track 1, ID: Punkampu churanam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	0.0 AU	0.12 Rf	30.1 AU	2.39 %	0.15 Rf	15.4 AU	427.6 AU	0.67 %
2	0.16 Rf	16.0 AU	0.18 Rf	18.2 AU	1.44 %	0.24 Rf	6.5 AU	653.6 AU	1.02 %
3	0.38 Rf	32.9 AU	0.56 Rf	320.4 AU	25.39 %	0.57 Rf	18.8 AU	17856.3 AU	27.97 %
4	0.57 Rf	319.1 AU	0.60 Rf	404.2 AU	32.03 %	0.76 Rf	88.2 AU	30678.3 AU	48.06 %
5	0.76 Rf	88.2 AU	0.84 Rf	241.6 AU	19.14 %	0.88 Rf	0.7 AU	9036.6 AU	14.16 %
6	0.89 Rf	0.4 AU	0.94 Rf	153.7 AU	12.18 %	0.96 Rf	64.9 AU	3805.4 AU	5.96 %
7	0.96 Rf	65.3 AU	0.98 Rf	93.7 AU	7.43 %	0.99 Rf	63.7 AU	1374.8 AU	2.15 %

Track 2, ID: Pungampoo

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	0.3 AU	0.13 Rf	23.0 AU	0.94 %	0.15 Rf	9.3 AU	212.7 AU	0.22 %
2	0.16 Rf	9.4 AU	0.21 Rf	23.4 AU	0.95 %	0.23 Rf	19.6 AU	807.6 AU	0.82 %
3	0.31 Rf	6.3 AU	0.48 Rf	480.2 AU	19.48 %	0.54 Rf	47.3 AU	29542.4 AU	30.10 %
4	0.54 Rf	248.4 AU	0.58 Rf	522.0 AU	21.18 %	0.64 Rf	47.3 AU	24511.2 AU	24.97 %
5	0.64 Rf	248.8 AU	0.68 Rf	427.7 AU	17.35 %	0.74 Rf	62.1 AU	18535.0 AU	18.88 %
6	0.74 Rf	162.2 AU	0.76 Rf	221.9 AU	9.00 %	0.79 Rf	11.1 AU	6150.1 AU	6.27 %
7	0.80 Rf	112.0 AU	0.82 Rf	251.3 AU	10.20 %	0.88 Rf	0.2 AU	6799.1 AU	6.93 %
8	0.89 Rf	0.3 AU	0.93 Rf	336.7 AU	13.66 %	0.96 Rf	67.6 AU	8910.0 AU	9.08 %
9	0.96 Rf	168.6 AU	0.97 Rf	178.3 AU	7.23 %	1.00 Rf	12.0 AU	2684.4 AU	2.73 %

Table 4: R_f table of chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū at 254 nm.

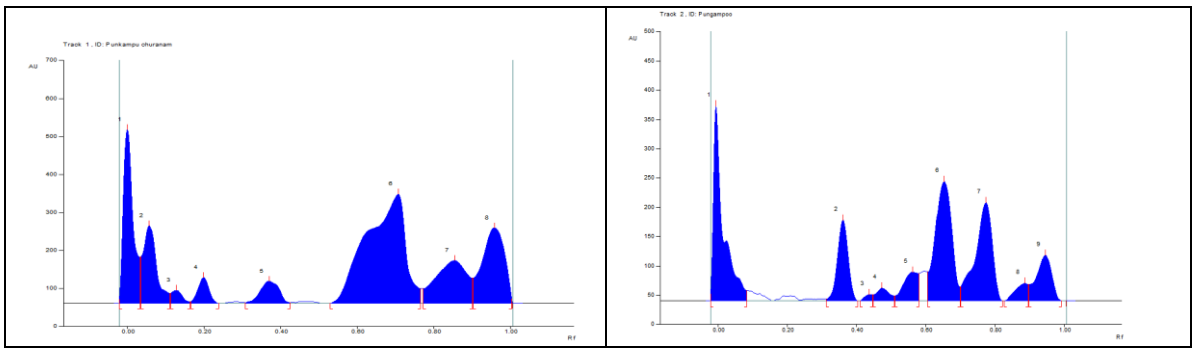


Fig. 6: HPTLC finger print profile of chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū at 575 nm after derivatisation.

Table 5: R_f table of chloroform extract of Puṅkam pū cūraṇam and Puṅkam pū at 575 nm after derivatisation.

Track 1, ID: Punkampu churanam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	10.8 AU	0.00 Rf	456.9 AU	32.18 %	0.04 Rf	21.5 AU	8164.4 AU	15.84 %
2	0.04 Rf	122.2 AU	0.06 Rf	204.5 AU	14.40 %	0.11 Rf	26.5 AU	4822.8 AU	9.36 %
3	0.11 Rf	26.7 AU	0.13 Rf	34.0 AU	2.40 %	0.17 Rf	4.3 AU	682.2 AU	1.32 %
4	0.17 Rf	4.7 AU	0.20 Rf	67.9 AU	4.78 %	0.24 Rf	0.0 AU	1352.8 AU	2.62 %
5	0.31 Rf	2.1 AU	0.37 Rf	58.5 AU	4.12 %	0.43 Rf	2.0 AU	2012.4 AU	3.90 %
6	0.53 Rf	0.2 AU	0.71 Rf	287.1 AU	20.22 %	0.77 Rf	38.4 AU	20087.0 AU	38.97 %
7	0.77 Rf	38.4 AU	0.86 Rf	112.3 AU	7.91 %	0.90 Rf	66.2 AU	6324.4 AU	12.27 %
8	0.90 Rf	66.5 AU	0.96 Rf	198.8 AU	14.00 %	1.00 Rf	12.0 AU	8092.2 AU	15.70 %

Track 2, ID: Pungampoo

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	11.0 AU	-0.00 Rf	332.2 AU	32.26 %	0.08 Rf	17.3 AU	6493.5 AU	23.93 %
2	0.31 Rf	2.9 AU	0.36 Rf	137.8 AU	13.38 %	0.41 Rf	0.1 AU	2950.9 AU	10.87 %
3	0.41 Rf	0.1 AU	0.44 Rf	10.7 AU	1.04 %	0.45 Rf	10.0 AU	153.7 AU	0.57 %
4	0.45 Rf	10.2 AU	0.47 Rf	21.4 AU	2.08 %	0.51 Rf	8.0 AU	556.9 AU	2.05 %
5	0.51 Rf	8.2 AU	0.56 Rf	49.0 AU	4.76 %	0.58 Rf	46.9 AU	1452.3 AU	5.35 %
6	0.61 Rf	49.2 AU	0.65 Rf	203.6 AU	19.77 %	0.70 Rf	23.6 AU	6714.1 AU	24.74 %
7	0.70 Rf	24.0 AU	0.77 Rf	167.6 AU	16.27 %	0.82 Rf	0.0 AU	5662.0 AU	20.87 %
8	0.83 Rf	0.0 AU	0.89 Rf	30.0 AU	2.91 %	0.90 Rf	27.6 AU	753.8 AU	2.78 %
9	0.90 Rf	27.7 AU	0.95 Rf	77.6 AU	7.53 %	0.99 Rf	0.0 AU	2398.8 AU	8.84 %

DISCUSSION

The standardization of the herbal medicines is a necessary prerequisite to assure the quality of the drug because substitute or counterfeit herbal materials are often found in the market. This analysis will help to ensure the identity, quality, purity and safety of drug for the human use. Powder microscopic analysis is one of the reliable methods to correctly identify the particular drug and the surety of raw material. The results of all type analysis are helping in establishing quality control standards and purity assurance of drugs. In the powder microscopy study of Puṅkam pū cūraṇam, the sole ingredient is the Puṅkam pū and therefore the unique characters of flower such as pollen grains, anther wall etc. could be identified.

Physico-chemical parameters help to a great extent for the purpose of standardisation. Ash values are useful in determining the purity of drugs. Acid insoluble ash value indicates contamination with silicaceous material e.g. earth and sand. Acid- insoluble ash values obtained in the study (1.91 % for Puṅkam pū cūraṇam and 0.52 % for Puṅkam pū) suggest less content of silicaceous matter in the cūraṇam and in the Puṅkam pū. The test for loss on drying determines both water and volatile matter. The less value of moisture content of the cūraṇam (9.28 %) than that of the ingredient (13.03 %) may be due to the process of frying and could prevent bacterial, fungal or yeast growth. Alcohol is an ideal solvent for extraction of various polar chemicals. Alcohol soluble extractive values (29.17 % & 13.55 %) revealed the presence of polar chemical constituents. The tests for preliminary phytochemicals also supported the presence of polar chemical constituent such as glycosides of flavonoids, steroids and terpenoids; tannins and fats & oils in the ingredients. The high alcohol soluble extractive value obtained for cūraṇam may be due to the presence of fat from the ingredient ghee. Water soluble extractive values were high due to the presence of carbohydrates and tannins present in the plant ingredient.The pH value of the cūraṇam (4.8) revealed that it is more acidic than the plant drug (6.7). The difference in the pH value may be due to the presence of ghee in the cūraṇam. The values obtained for successive extraction may also be used as a parameter for standardization. The greater value of hexane extract indicates the presence of fixed oil & fat in the cūraṇam.

The results obtained for preliminary phytochemical analysis indicates the class of chemical constituents present in the cūraṇam and Puṅkam pū. The higher amount of fixed oil & fat present in the cūraṇam may be the constituents of ghee. These chemical constituents may be possibly responsible for the biological activities of the formulation.

From the results of HPTLC studies, it has been found that the solvent system, Toluene: Ethyl acetate (5:3) efficiently resolved the components present in the extracts. Both the cūraṇam and flower of *Pongmia pinnata* have the same R_f values (0.21, 0.31, 0.35, 0.44, 0.56, 0.64, 0.72) and colour at 254 nm. The same bands seen in both the samples indicated the presence of Puṅkam pū in the cūraṇam. The R_f values and colour of the chromatogram of the cūraṇam and Puṅkam pū under white light after derivatisation were also compared. The track 1 belonging to the cūraṇam showed six major bands at R_f 0.06, 0.20, 0.35, 0.62, 0.71 and 0.86 and the track 2 of Puṅkam pū showed seven visible bands at R_f 0.35, 0.62, 0.66, 0.71, 0.78, 0.86, 0.95. The similar bands (R_f 0.35, 0.62, 0.71 and 0.86) present in both tracks assured the presence of Puṅkam pū in the cūraṇam. The additional bands seen in the cūraṇam may be due to the presence of ghee. The constituents corresponding to extra bands seen in the track of Puṅkam pū may be lost during the frying process. The bands with R_f value at 0.64, 0.72 in track 1could not be seen much distinct. This may be due to the presence of fat from ghee in the cūraṇam.

From Fig. 5, it could be seen that there are seven peaks indicating the occurance of atleast seven components in the cūraṇam and nine components in the Puṅkam pū. The components with R_f values 0.56, 0.60, 0.84 and 0.94 for cūraṇam and the components with R_f values 0.48, 0.58, 0.68, 0.82 and 0.93 for Puṅkam pū were found to be more predominant as the percentage areas are more. As the percentage area for other minor peaks are less, the

concentration of the components in the extracts corresponding to these peaks are less.

After scanning in UV short, post-chromatogram derivatisation was used to obtain more information about the samples. In the result obtained from Fig. 6, it was found that there are several peaks for both the samples. Five peaks with R_f values 0.06, 0.37, 0.71, 0.86 and 0.96 for the cūraṇam and four peaks with R_f 0.36, 0.65, 0.77 and 0.95 for Puṅkam pū showed large peak areas than the other peaks. The area of each peak is in proportion to the amount of the particular component present in the samples. The peaks with greater area were more prominant and the concentrations of the corresponding components in the samples were high. These results provide quantitative information about the main constituents present in the herbal drug.

CONCLUSION

The results of physico-chemical analysis, preliminary phytochemical analysis, TLC photo documenation, HPTLC fingerprinting profile will be helpful to authenticate and standardise the Puṅkam pū cūraṇam and flower of *Pongamia pinnata* (L.) Pierre. TLC photo documentation, HPTLC finger prints and powder Characteristics can be used as a diagnostic tool to detect the single drug, Puṅkam pū in the compound formulation. Briefly, the aspects described here can be considered as characteristic to identify and authenticate this drug.

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