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Pharmacognostic Investigation and HPTLC Fingerprinting of a Siddha Polyherbal Drug, Padai chankaran

Neethu Kannan B*, Gayathri Devi V, Anitha John, Lekha GS, Natarajan M, Kanagarajan A

Siddha Regional Research Institute, Thiruvananthapuram, Kerala, India

ABSTRACT

The present study aims to establish the quality and purity of a Siddha formulation, Padai chankaran by laying down various pharmacognostic parameters, physico-chemical constants and HPTLC fingerprint profiles. Padai chankaran is a Siddha polyherbal preparation comprised of *Catunaregum spinosa* – root bark, *C. spinosa* – seed and *Alangium salvifolium* – root bark as the ingredients. The formulation is used as an external application, having astringent, anthelmintic and antiseptic activities that supports in healing of ulcers and dermatological diseases. Powder microscopy studies and physico-chemical analysis were carried out. Also, an attempt has been made to develop a HPTLC method for phytochemical fingerprinting and the mobile phase Toluene: Ethyl acetate: Formic acid (5: 2: 0.1) gave the best resolution for various components. Hence, the aforesaid analyses confirmed the purity and quality of the Siddha formulation for their future applications.

Keywords: Padai chankaran, powder microscopy, physico-chemical, HPTLC studies

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***Address for Correspondence:**

Neethu Kannan B., Siddha Regional Research Institute, Thiruvananthapuram, Kerala, India

INTRODUCTION

Many herbal drugs or herbal products have found to play a vital role in the treatment of various ailments. Although, modern medicines are available, herbal medicines have often retained popularity for historical and cultural reasons. Since the usage of these herbal medicines has increased, the issues regarding their safety, quality, and efficacy in industrialized and developing countries are cropped up[1]. Hence, to ensure desired quality, efficacy and batch to batch consistency of traditional medicines, standardization techniques are mandatory. Standardization guidelines provided by international bodies like World Health Organization (WHO), European Agency for the evaluation of Medicinal Products (EMEA) and United States Pharmacopeia (USP) should be followed for herbal products[2]. In this study, Padai chankaran, a Siddha polyherbal formulation was selected and screened for standardization studies.

Padai chankaran is a formulation which belongs to a class of Siddha medicine, Poochu. Poochu is an external application of liquid formulation which can be used locally and it is the application of medicated oils or herbal juices or mixtures of powdered. Padai chankaran is light reddish-brown in colour in powdered form and it becomes sticky in texture when lime juice is added into it. It consists of *Catunaregum spinosa* – root bark, *C. spinosa* – seed and *Alangium salvifolium* – root bark as the ingredients. The formulation can be used for internal or external application in the treatment of skin diseases and taenial infection as described in the Siddha Formulary of India.

*C. spinosa is a s*mall tree (Rubiaceae) with small straight axillary spines grows upto 4m. The decoction of *C. spinosa* root bark (15-30 mL) is beneficial in the treatment of dysentery and diarrohea. The regular intake of root bark powder in the dose of 65-130 mg is useful in the treatment of pain and inflammation. Their seeds have astringent action useful for healing the ulcers and other skin lesions. It is also useful for external application in contusion, oedema, etc. *A. salviifolium,* commonly known as *sage-leaved* alangium, is a flowering plant in the Cornaceae family. It is mostly found in dry regions in plains and low hills and also found on roadsides. The root bark of *A. salvifolium* has anthelmintic and febrifuge activity useful in the treatment of fever, syphilis, scabies and certain other infectious diseases. It is a best antidote used for the treatment of toxic bites like scorpion bite, snake bite, etc. The oil extracted from the root bark of *A. salvifolium* is anti-inflammatory and cures severe arthritic ailments. The fine powder of the root bark can be applied externally to cure leprosy, syphilitic ulcers, scabies, wounds and taenial infection. The Gastro-intestinal ailments like indigestion and diarrhea can be treated by giving 4-8 grams of root bark grinded and mixed with honey[3].

The present study aims to evaluate the pharmacognostic parameters as well as the HPTLC fingerprint patterns of the Siddha formulation, Padai chankaran.

MATERIALS AND METHODS

2.1. Standard Operational Procedure

Ingredients

1. *Catunaregum spinosa* (Thunb.) Tirveng. – Root bark
2. *Catunaregum spinosa* (Thunb.) Tirveng. – Seed
3. *Alangium salvifolium* (L. f.) – Root bark
4. Lime juice – Sufficient quantity

Fig. 1: Padai chankaran

Method of preparation

The formulation was prepared by Clinical Department, Siddha Regional Research Institute, Thiruvananthapuram. The raw drugs namely root bark of *A. salvifolium*, root bark and seeds of *C. spinosa* were collected from SMPG, Mettur and authenticated. These raw drugs were cleaned well and equal quantity of each drug was powdered into fine particles by using stone mortar. Then the powder was mixed well and grinded with lemon juice of sufficient quantity till a waxy consistency obtained (**Fig. 1**). Foreign matter, if any, was removed from the collected raw drugs.

2.2. Organoleptic evaluation

Organoleptic evaluation refers to evaluation of the formulation and its ingredients by colour, odor, taste, texture, etc. which are determined by standard protocol.

2.3. Microscopical studies

The powdered form of Padai chankaran and its powder ingredients were mounted in glycerin at room temperature for 24 h and observed under 10X and 40X objective of bright field microscope for powder characteristics.

2.4. Extraction for HPTLC studies

Chloroform extract of Padai chankaran and its powder ingredients were taken by refluxing 1g each of the material with 10 mL chloroform at a temperature of 60°C for 10 minutes. The extracts were filtered and concentrated to desired volume.

2.5. Physico-chemical evaluation

Physico-chemical constants like total ash value, acid insoluble ash value, water soluble extractive value, alcohol soluble extractive value, volatile oil content and loss on drying at 105 °C were determined as per standard protocol[4].

2.6. HPTLC fingerprinting

The chloroform extracts of Padai chankaran and its powder ingredients were subjected to HPTLC analysis. The instrument employed was CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC auto sampler 4 with win CATS software version 1.4.4. Each extract was applied as two tracks of volume 15 µL and 20 µL. The plate was developed using the solvent system, Toluene: Ethyl acetate: Formic acid (5: 2: 0.1 v/v) in a twin trough chamber. The plate was developed up to 7 cm, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. at λ_{max} 254 nm using deuterium light source, at λ_{max} 366 nm with mercury light source and the slit dimensions were 8.00 mm × 0.40 mm. Densitometric documentation was done. After scanning, the plate was observed under 254 nm and 366 nm and TLC chromatograms were recorded. Then the plate was dipped in vanillin-sulfuric acid reagent and dried at 105 °C on a hot plate till the colour of the bands appears. The plate was visualized under white light and scanned at 575 nm. TLC chromatograms, R_f values and fingerprint data were recorded by win CATS software.

RESULTS & DISCUSSION

Organoleptic evaluation

Organo leptic characters of Padai chankaran and its powder ingredients are given in **Table 1.**

Table 1: Organoleptic characters of Padaichankaran and its ingredients. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

Sample	Appearance	Colour	Taste	Odour
PC	Waxy	Brown	Pungent	-
ASR	Homogenous	Brown	Bitter	-
CSR	Fibrous	Wheatish	Pungent	-
CSS	Homogenous	Light brown	Pungent	-

Powder microscopic studies

The powder of Padai chankaran is appeared to be fibrous in texture. It revealed various characters like stone cells, pitted vessel, starch grains, fragment of tracheid, (comes from *C. spinosa*- root bark) testa, sclereids of endocarp, (*C. spinosa*- seed), cork cells, rosette crystals (*A. salvifolium*- root bark) etc. by powder microscopy (**Fig. 2**). The presence of these unique cellular characters indicates the presence of the above said ingredients in the formulation.

Powder microscopy of single ingredients of the formulation was also done individually so as to ensure the genuineness of the formulation, Padai chankaran. ***C. spinosa* (Root bark):** Powder greyish brown in colour without any characteristic smell and taste, showed sclereids, stone cells, medullary rays, parenchyma and starch (**Fig. 3**). ***C. spinosa* (Seed):** Powder is fine, homogenous and brownish in colour. Sclereids and testa were the main characteristics revealed by powder microscopy (**Fig. 4**). ***A. salvifolium* (Root bark):** Brownish in colour and tastes bitter, showed cork cells in

surface view, parenchyma cells from the cortex, starch grains and rosette crystals (**Fig. 5)**.

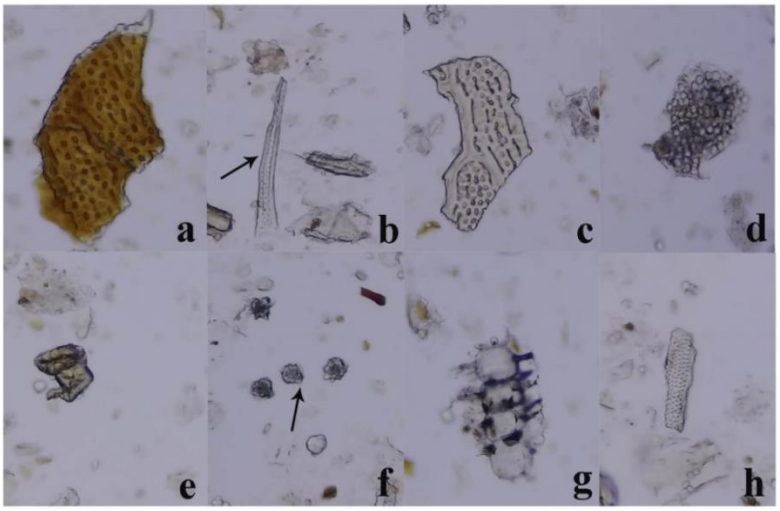

Fig. 2: Powder microscopy of Padai chankaran: a: testa**, b:** fragment of tracheid**, c:** sclereid of endocarp**, d:** clumps of starch grains, **e:** stone cells, **f:** rosette calcium oxalate crystals**, g:** cork cells, **h:** pitted vessel.

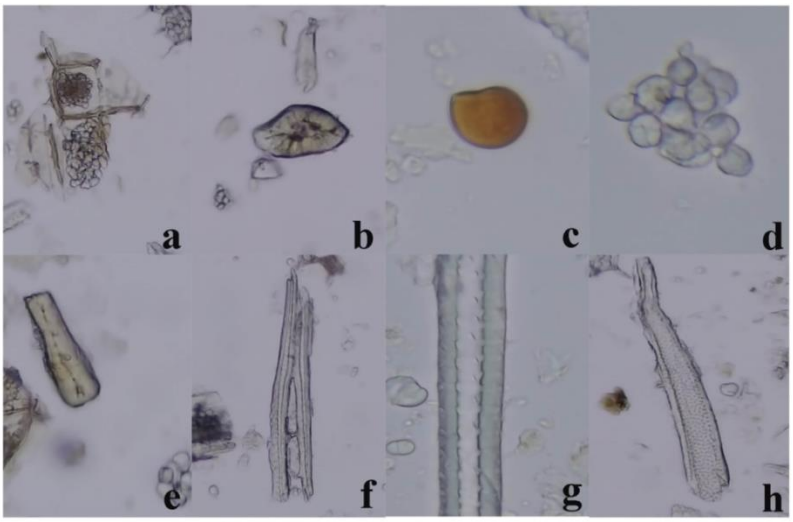

Fig. 3: Powder microscopy of *C. spinosa* (root bark)*: a:* parenchyma cells with starch grains**, b:** stone cell, **c:** oil globule, **d:** starch grains, **e:** sclereid, **f**: medullary ray, **g:** fragment of tracheid with narrow lumen, **h:** vessel with pitted thickening.

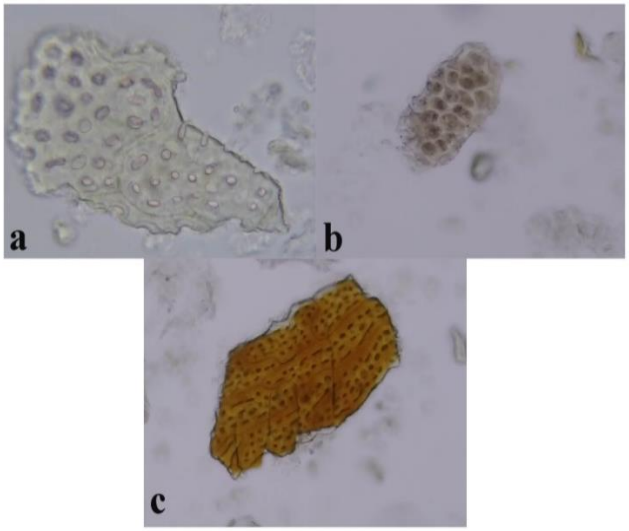

Fig. 4: Powder microscopy of *C. spinosa* (seed): a: sclereids of endocarp, **b:** filamentous layer, **c:** testa.

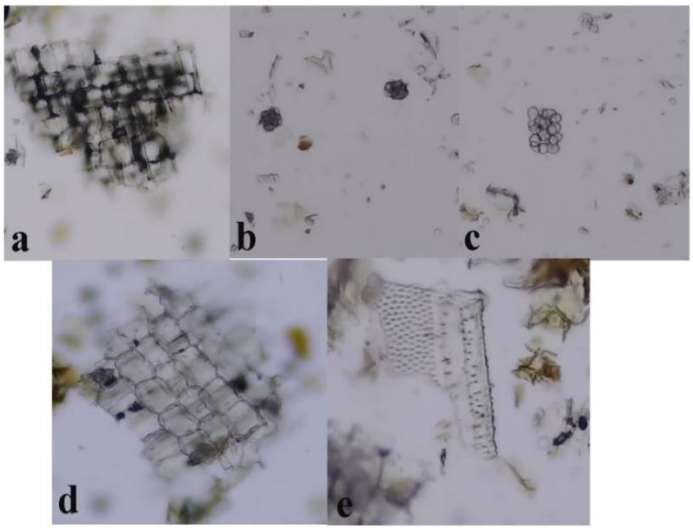

Fig. 5: Powder microscopy of *A. salvifolium* (root bark)*: a:* cork cells, **b:** rosette calcium oxalate crystals, **c:** starch grains, **d:** thin walled parenchyma cells**, e:** vessel with bordered pitted thickening.

Physico-chemical evaluation

The physico-chemical parameters such as loss on drying at 105 ˚C, total ash content, acid insoluble ash, extractive values (water soluble extractive and alcohol soluble extractive) and volatile oil were evaluated and results are tabulated **(Table 2)**.

Table 2: Physico-chemical parameters of Padaichankaran and its ingredients. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

Sl. No.	Parameters	PC	CSR	CSS	ASR
	Loss on Drying at 105 ˚C %	69.17	10.83	9.15	15.37
	Total Ash Content %	1.49	2.94	3.76	8.43
	Acid Insoluble Ash %	0.20	1.42	0.055	1.21
	Water Soluble Extractive %	14.20	12.47	48.24	21.61
	Alcohol Soluble Extractive %	11.40	15.18	2.00	29.62
	Volatile oil %	Nil	Nil	Nil	Nil

Loss on drying shows the presence of moisture content and volatile oil in the samples. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. The loss on drying value of the formulation obtained is 69.17 % and this high value is due to its waxy consistency obtained by adding lime juice during the preparation. This preparation should be used freshly and the powdered form before adding lime juice can be stored for further use. In this study, loss on drying values of the single ingredients is found to be low which signifies that the drugs were properly dried and it has a low possibility of deterioration due to fungus[5]. Loss on drying can serve as a valuable source of information and provide appropriate standards to establish the quality of the crude drug/formulation in future study or application.

The ash value was determined by two different forms viz., total ash and acid insoluble ash. The total ash and acid insoluble ash values were found be low for Padai chankaran and its single ingredients. Total ash estimates the presence of various inorganic components. The acid insoluble ash measures the amount of silica present; especially sand and it indicate contamination with earthy material[6]. Low levels of these two parameters indicate that the inorganic matter and silica were less in the selected drugs.

The extractive values are useful to evaluate the chemical constituents present in the crude drugs and the formulation. These values help in estimation of specific constituents soluble in a particular solvent[7]. Water extraction gave a better yield for Padai chankaran and *C. spinosa* (seed), whereas alcohol was able to extract more constituents in the case of *C. spinosa* (root) and *A. salvifolium* (root).

Volatile oil content was found to be absent in this formulation and its ingredients. Generally, odour of the drug depends upon the type of odorous principles (volatile oils) present and it possibly influences the activity of the drug[8].

Herbal drug should be free from visible signs of contamination by moulds or insects, and other animal contamination, including animal excreta. Presence of contamination may also be due to faulty collection of crude drug or due to deliberate mixing[9]. Hence, the foreign matter, if any, should be separated from the single drugs during the preparation of the formulation so that results obtained from analysis gives accuracy. In the present study, Padai chankaran and its single ingredients are devoid of foreign matter which indicates the purity of the formulation and its ingredients[10,11]. The establishment of physical constants viz; moisture content, ash value, acid-insoluble ash value and extractive values has equal importance in the evaluation and identification of inorganic impurities present in the crude drugs[12].

HPTLC fingerprinting

In the present study, the patterns of phytochemical constituents were identified based on the colour zone obtained during the HPTLC analysis under UV short, UV long and white light after derivatization in the chromatogram. HPTLC fingerprints obtained can be used for the correct identification of ingredients and its phytoconstituents. HPTLC chromatogram of Padai chankaran and its ingredients are shown in **Fig. 6.** HPTLC fingerprinting profile and their corresponding R_f values are given in **Fig. 7, 8 &9.** 3D densitogram of the formulation and ingredients are given in **Fig. 10**. The HPTLC fingerprinting results showed several peaks with different R_f values. Toluene: Ethyl acetate: Formic acid (5: 2: 0.1) was the most effective solvent system which resolved various bands on the chromatogram which indicates plethora of phytochemicals present in the formulation as well as in their ingredients. Colour of bands and their corresponding R_f values of Padai chankaran and its powder ingredients are given in **Table 3.**

At 254 nm, R_f value of 0.35 showed a prominent band of light green colour with maximum area percentage for Padai chankaran formulation and the ingredients. At 366 nm, 0.35 and 0.88 were the R_f positions of fluorescent blue and blue colours respectively at which maximum concentration of phytochemical constituents in the form of thick band is observed. In a derivatized plate of 575 nm, maximum area percentage corresponds to the R_f value of 0.67 having purple colour. The bands with similar or almost similar R_f values were obtained for all samples which were marked as bold in **Table 3.** The presence of a band at same R_f position in all samples may substantiate the presence of specific compound present[13]. Along with the prominent bands observed, all other colour bands also indicate the presence of various compounds which could attribute the bio efficacy of the formulation and their ingredients in a synergistic manner[14,15].

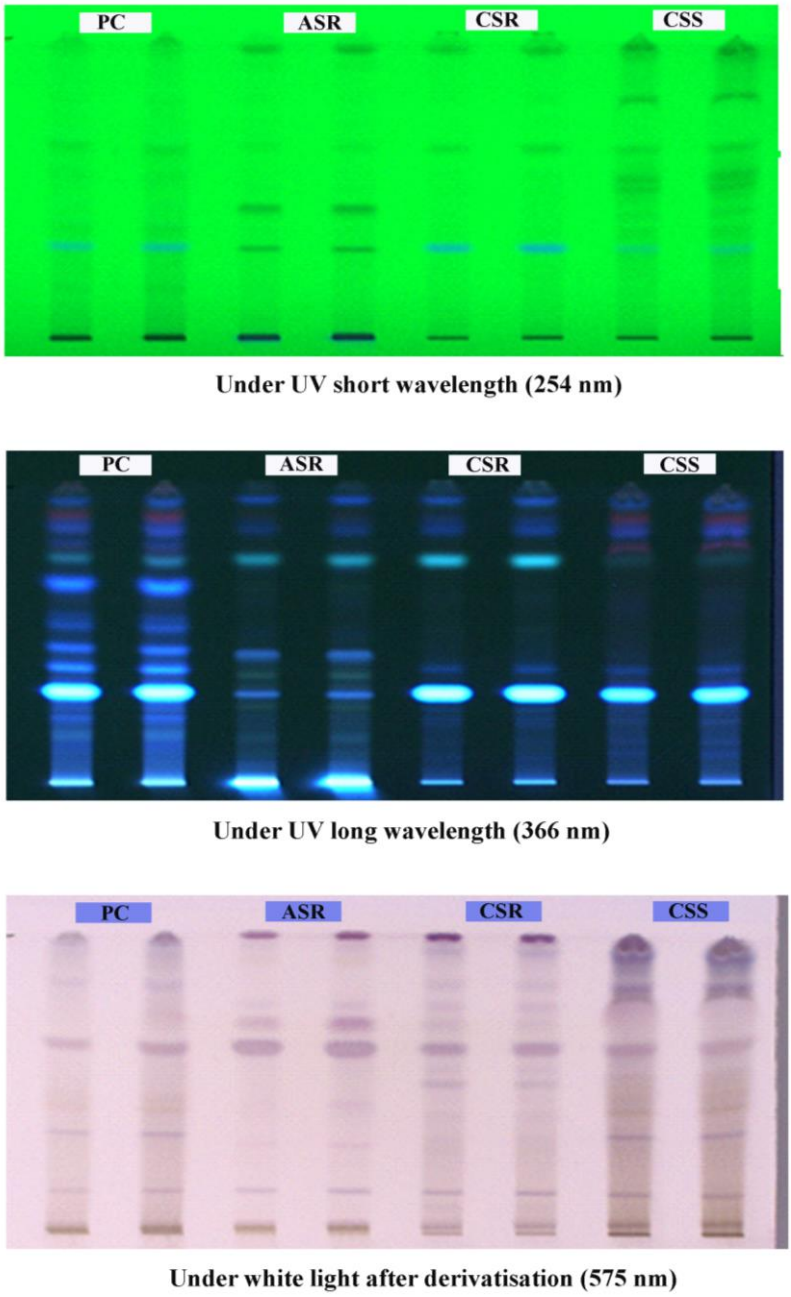

Fig. 6: HPTLC chromatogram of chloroform extract of Padai chankaran and its ingredients. Solvent system: Toluene: Ethyl acetate: Formic acid (5: 2: 0.1), Volume applied: Track 1: 15 µL, Track 2: 20 µL. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

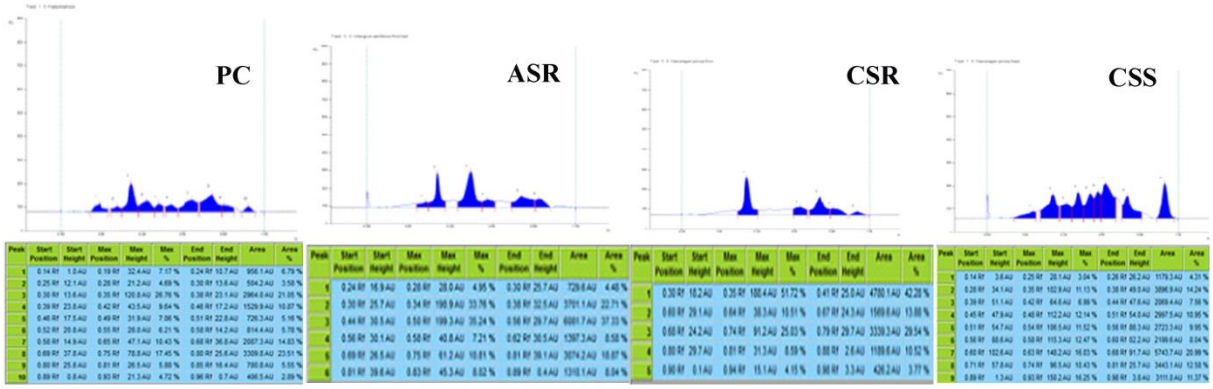

Fig. 7: HPTLC fingerprint profile and R_f values of Padai chankaran and its ingredients at 254 nm. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

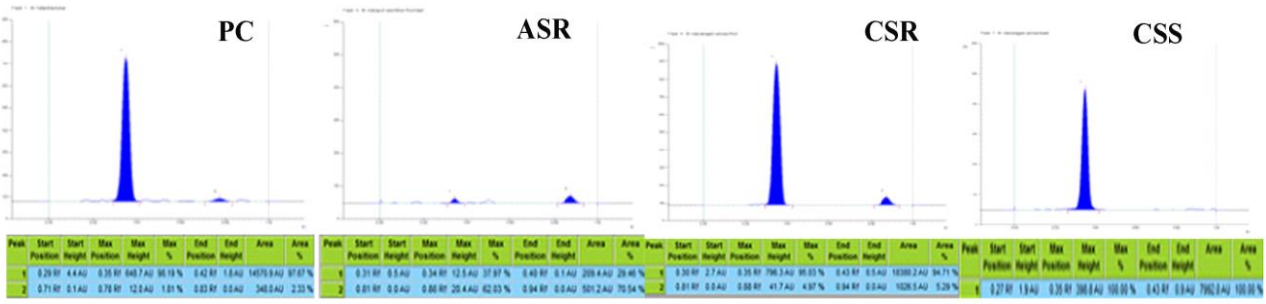

Fig. 8: HPTLC fingerprint profile and R_f values of Padai chankaran and its ingredients at 366 nm. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

Table 3: R_f values and colour of bands of Padai chankaran and its ingredients. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

Sample	Wavelength (254 nm)		Wavelength (366 nm)		Wavelength (575 nm)	
	R_f value	Colour of band	R_f value	Colour of band	R_f value	Colour of band
PC	0.17	Light green	0.17	Light blue	0.16	Light purple
	0.28	Blue	0.23	Light blue	**0.34**	Light purple
	0.35	Light green	**0.35**	Fluorescent blue	0.42	Light yellow
	0.55	Light green	0.40	Blue	0.52	Light yellow
	0.65	Dark green	0.46	Blue	0.63	Purple
	0.76	Light green	0.54	Blue	0.83	Light purple
	0.94	Dark green	**0.68**	Blue	0.98	Purple
			0.77	Bluish green		
			0.87	Blue		
			0.92	Cherry red		
			0.98	Blue		
ASR	0.28	Dark green	0.17	Light blue	0.13	Light purple
	0.42	Dark green	0.27	Light green	0.29	Light purple
	0.60	Light green	**0.34**	Blue	0.42	Light purple
	0.95	Dark green	0.38	Green	0.64	Purple
			0.44	Blue	0.72	Purple
			0.67	Light green	**0.77**	Light purple
			0.78	Bluish green	**0.88**	Light yellow
			0.88	Blue	0.98	Purple
			0.98	Blue		
CSS	0.28	Light blue	0.10	Light blue	0.13	Light purple
	0.35	Light green	0.16	Light blue	**0.31**	Light purple
	0.42	Light green	0.23	Light blue	0.45	Light yellow
	0.48	Light green	**0.35**	Fluorescent blue	0.50	Light yellow
	0.54	Light green	0.39	Blue	0.60	Purple
	0.77	Dark green	**0.77**	Light green	0.80	Purple
	0.93	Dark green	0.81	Cherry red	0.83	Yellow
			0.86	Blue	**0.89**	Blue
			0.92	Cherry red	**0.95**	Purple
			0.96	Blue		
CSR	0.28	Blue	**0.35**	Fluorescent blue	0.13	Light purple
	0.61	Light green	0.40	Light blue	0.29	Light yellow
	0.94	Dark green	0.52	Light green	0.42	Light purple
			0.78	Bluish green	0.50	Light purple
			0.88	Blue	0.55	Light purple
			0.97	Blue	0.62	Purple
					0.68	Light purple
					0.78	Light purple
					0.86	Light purple
					0.97	Purple

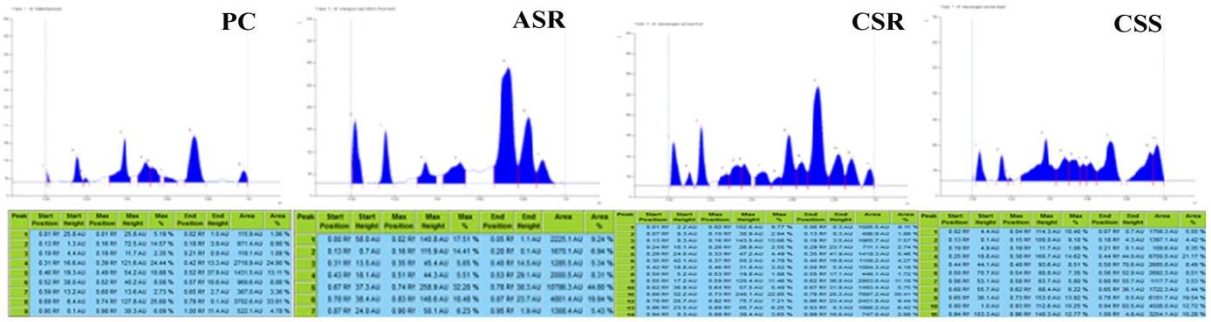

Fig. 9: HPTLC fingerprint profile and R_f values of Padai chankaran and its ingredients at 575 nm. PC- Padai chankaran, ASR- *A. salvifolium* (root bark), CSR- *C. spinosa* (root bark), CSS- *C. spinosa* (seed).

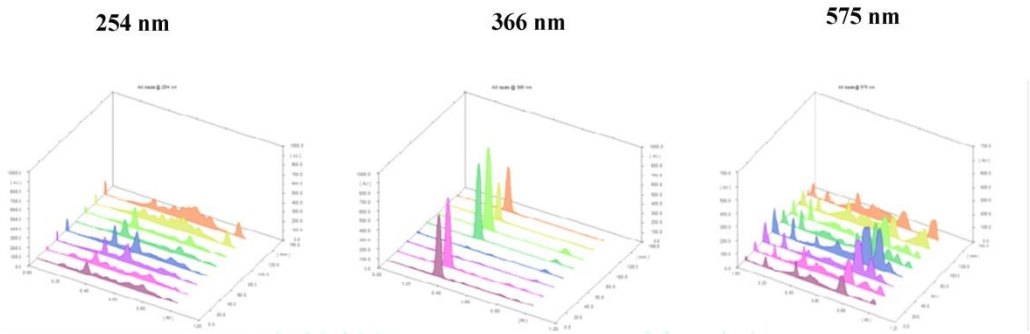

Fig. 10: 3D Densitograms of chloroform extract of Padai chankaran and its ingredients at different wavelengths.

CONCLUSIONS

The study suggests the quality assurance of the Siddha formulation, Padai chankaran and its ingredients by evaluating their organoleptic properties, powder characteristics, physico-chemical parameters and HPTLC profiles. The present HPTLC-fingerprinting profile along with other analysed parameters can be used as a diagnostic tool for the identity and to determine the quality and purity of the formulation in future studies. Also, the chromatographic fingerprint pattern will represent pharmacologically active and chemically characteristic components in the formulation and the single drugs.

CONFLICT OF INTEREST

No conflicts declared

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