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Pharmacognostic evaluation and HPTLC fingerprinting of Pirantai Vatakam, a Siddha formulation

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ABSTRACT

The present study is an attempt to evaluate the pharmacognostic parameters and HPTLC fingerprint profiles for a Siddha compound formulation, Pirantai vatakam in which *Cissus quadrangularis* is the main ingredient. Powder microscopy studies were carried out and different microscopic characters were distinguished. Physico-chemical parameters such as loss on drying at 105ºC, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive AND volatile oil were determined. High performance thin layer chromatographic (HPTLC) study of Pirantai vatakam was performed and the chromatograms were documented. The observations laid down a platform for the standardization of Pirantai vatakam and will help us to determine the genuineness of the drug from the adulterants and substitutes.

Keywords: Pirantai vatakam, *Cissus quadrangularis*, Powder microscopy, Physico-chemical, HPTLC.

INTRODUCTION

Standardization of herbal formulation is essential in order to assess the quality of drugs. It is based on the concentration of their active principles, physical, chemical, phyto-chemical and pharmacological parameters. Lack of standardization is a major concern regarding use of medicinal herbal medicines. Herbal medicines are complex mixtures of chemical constituents in which the active ingredient may not be known or may be only a small percentage of the total product. Hence, the standardization of drugs is a key factor in assessing their quality control for establishing them in a valuable mode. Standardization of drug is essential to exhibit conformation of its identity and determination of its purity, quality and quantity [1].

Methods of standardization should take into consideration all aspects that contribute to the quality of the herbal drugs, namely correct botanical identification of the sample, organoleptic, pharmacognostic, physic-chemical and phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing, and biological activity. Of these, the phytochemical profiling is of special significance since it has a direct relation on the activity of the herbal drugs [2].

Vatakams are generally prepared from finely powdered drugs, being made into a bigger size of pill after the cooking process. It retains their potency for atleast three months, when kept in clean, dry and air-tight glass containers. Pirantai vatakam is a brown colored compound formulation made of the several ingredients of which *Cissus quadrangularis* is the important one and it is prepared as balls, in which rice water and curd helps to retain its shape. Pirantai vatakam is mainly used to relieve abdominal pain, flatulence, indigestion, anorexia, ascites etc. It also helps to strengthen the body as described in Siddha Formulary of India.

The present study is an attempt to evaluate the quality of the Siddha polyherbal formulation, Pirantai vatakam based on its botanical, physico-chemical and HPTLC finger printing analysis.

MATERIALS AND METHODS

Standard Operational Procedure

Ingredients

1. *Cissus quadrangularis* Linn. (Stem) - 350 gm
2. *Terminalia chebula* Retz.(Fruit) - 35 gm
3. *Zingiber officinale* Rosc.(Dried rhizome) - 35 gm

4. *Macrotyloma uniflorum* Lam. (Verdc) (Seed)- 35 gm
5. *Vigna mungo* L. Hepper (Seed) - 35 gm
6. *Curcuma longa* L. (Rhizome) - 35 gm
7. *Piper nigrum L.*(Dried Fruit) - 35 gm
8. *Allium sativum* L. (Bulb) - 35 gm
9. *Cuminum cyminum* L. (Dried Fruit) - 35 gm
10. *Embelia ribes* Burm. f. (Fruits) - 35 gm
11. Rock salt - 35 gm
12. Curd - Sufficient quantity
13. Rice water - Sufficient quantity

Figure 1: The ingredients of Pirantai vatakam

Method of preparation

The ingredients of Pirantai vatakam is given in Fig. 1. First of all, remove the skin from the Item 1 by scraping, split the pieces and put in a container. Pour enough of item 13 to submerge the pieces. Keep in sun for two days and dry so that the slime disappears. This is the purification step of *C. quadrangularis*. Pound it finely, grind drugs 2 to 11 with item 12 add with more of item 13 and dry in sun. Mix into the pounded Pirantai and make small lumps, (10 gms). This preparation may be consumed as such or after frying in ney.

Microscopical studies

The powdered form of Pirantai vatakam was mounted in glycerin at room temperature for 24 h and observed under 10X and 40X objective of bright field microscope for powder characteristics.

Extraction for HPTLC studies

Chloroform extract of Pirantai vatakam was taken by refluxing the material at a temperature of 60°C for 10 minutes. The extract was filtered and concentrated to desired volume.

Physico-chemical studies

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 105ºC were determined as per standard protocol [3].

HPTLC fingerprinting

The chloroform extract of Pirantai vatakam was subjected to HPTLC analysis. The instrument employed was CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4 with win CATS software version 1.4.4. Volume of sample applied were Track 1- 4 µl; Track 2 – 8 µl. The plate was developed using solvent systems (Toluene: Ethyl acetate: Formic acid (5: 1: 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, 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 spots 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 AND DISCUSSION

Microscopical studies

Powder microscopy revealed the presence of several characters such as stone cells, perisperm cells, bundle fibres, xylem vessel with pitted thickening, starch grains with hilum, part of testa, sclereids etc. All these cellular characteristics derived from the ingredients of the Pirantai vatakam (Fig. 2).

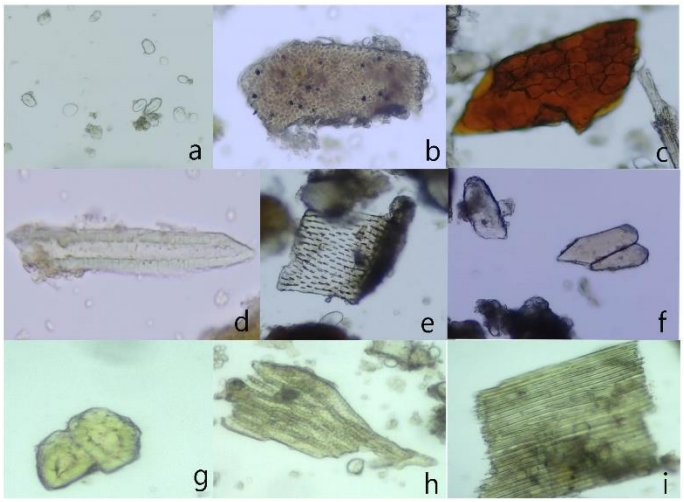


Figure 2: a. Starch grains with hilum, **b.** surface view of testa, **c.** part of fruit, **d.** sclereid, **e.** vessel with pitted thickening, **f.** perisperm cells, **g.** stone cells, **h.** tracheidal fibre, **i.** fibre bundle

Physico-chemical evaluation

The physico-chemical parameters such as loss on drying at 105˚C, ash content, acid insoluble ash, extractive values (water soluble extractive and alcohol soluble extractive) and volatile oil were evaluated and results were tabulated (Table 1).

Ash values may be effective parameter to assess the degree of purity of a given drug. Total ash value of plant material or drug indicates the amount of minerals and other earthy materials present. The total inorganic content (Calcium, Chloride, Sulphate, Iron, etc.) present in the drug is measured via Total ash value and its value is 12.26% for Pirantai vatakam. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing [4]. Acid insoluble ash value of the Pirantai vatakam represents the quantity of siliceous matter present in the drug. The quality of the drug is better if the acid insoluble value is low. It is 1.63% for Pirantai vatakam.

The percentage of soluble matters present in the drug is established by the values of water extractive and alcohol extractive. Based on the extractive value suitable solvent can be selected [5]. For the extractive values, water soluble extractive was found to be higher than alcohol soluble extractive. It hints the high ability of water to extract the maximum components of Pirantai vatakam into it.

Loss on Drying at 105°C indicates that only 12.53% of water and

volatile components have been lost when 1g of Pirantai vatakam was kept at 105°C. This moisture content helped to prevent deprivation of efficacy and degeneration of the formulation. High moisture content can adversely affect the active principles of the drug. It may possibly get early infection of the drug. Thus, low moisture content could get maximum stability and long shelf life [6].

Volatile oils are characterized by their odour, oil like appearance and ability to volatilize at room temperature. Chemically they are usually composed of mixtures of monoterpenes, sesquiterpenes and their oxygenated derivative. Aromatic compounds predominate in certain volatile oils. Because they are considered to be the "essence" of the plant material and often biologically active, they are also known as "essential oils" [7]. In Pirantai Vatakam, volatile oil content is found to be 1.00%.

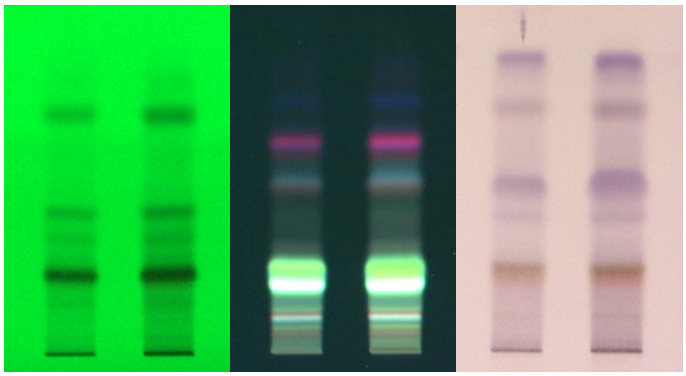
Table 1: Physico-chemical parameters of Pirantai vatakam

Sl. No.	Parameter	Result (%)
1.	Loss on Drying at 105˚C	12.53
2.	Ash Content	12.26
3.	Acid Insoluble Ash	1.63
4.	Water Soluble Extractive	20.52
5.	Alcohol Soluble Extractive	13.02
6.	Volatile oil	1.00

HPTLC documentation

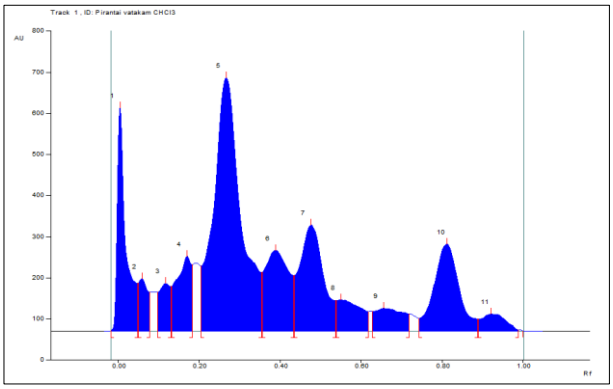
HPTLC profile of Pirantai vatakam was shown in Fig. 3. The solvent system of Toluene: Ethyl acetate: Formic acid (5: 1: 0.1) resolved various bands on the chromatogram which indicates diverse phytochemicals present in the formulation.

At 254 nm, the dark green band of R_f value 0.26 possess high area percent of 22.47% concentration. At 366 nm, high area, 42.32 % corresponds to R_f value of 0.22 of Fluorescent white colour. In derivatized plate of 575 nm, maximum area percentage of 34.72 % corresponds to R_f value of 0.58 having purple colour. The developed chromatogram at different wavelength shows the presence of various bands at particular R_f values indicating the presence of various phytocompounds at various concentrations. It was apparent that the R_f values were comparatively different irrespective of peaks for HPTLC analysis. The similarity in few R_f values at different wavelengths evidence the presence of specific compounds in this drug. The difference in R_f values in most of the appeared peaks reflected qualitative variation in the phytocompounds [8].

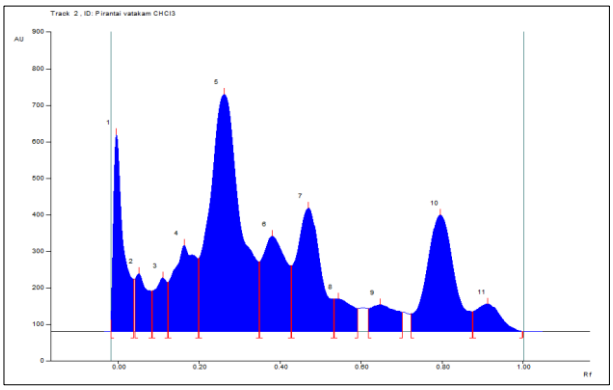


Under UV short | Under UV long | Under white light after derivatisation

Figure 3a: HPTLC profile of chloroform extract of Pirantai vatakamViewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light; Solvent system: Toluene: Ethyl acetate: Formic acid (5: 1: 0.1)

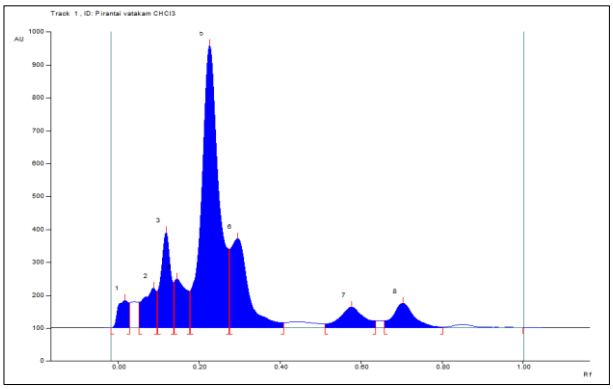


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	1.1 AU	0.01 Rf	542.7 AU	22.36 %	0.05 Rf	16.2 AU	8053.6 AU	9.94 %
2	0.05 Rf	117.0 AU	0.06 Rf	128.3 AU	5.29 %	0.08 Rf	95.1 AU	2045.9 AU	2.52 %
3	0.10 Rf	95.2 AU	0.12 Rf	116.0 AU	4.78 %	0.13 Rf	08.2 AU	2263.7 AU	2.79 %
4	0.13 Rf	108.9 AU	0.17 Rf	182.8 AU	7.53 %	0.19 Rf	61.6 AU	4702.7 AU	5.80 %
5	0.20 Rf	159.0 AU	0.27 Rf	616.5 AU	25.40 %	0.35 Rf	42.5 AU	28909.5 AU	35.67 %
6	0.36 Rf	142.8 AU	0.39 Rf	196.5 AU	8.10 %	0.44 Rf	35.5 AU	8046.8 AU	9.93 %
7	0.44 Rf	135.7 AU	0.48 Rf	258.2 AU	10.64 %	0.54 Rf	74.0 AU	10323.4 AU	12.74 %
8	0.54 Rf	74.3 AU	0.55 Rf	76.6 AU	3.16 %	0.62 Rf	47.5 AU	3190.1 AU	3.94 %
9	0.63 Rf	47.6 AU	0.66 Rf	55.5 AU	2.29 %	0.72 Rf	42.3 AU	2742.5 AU	3.38 %
10	0.75 Rf	31.5 AU	0.81 Rf	212.1 AU	8.74 %	0.89 Rf	29.8 AU	9031.0 AU	11.14 %
11	0.89 Rf	29.8 AU	0.92 Rf	41.7 AU	1.72 %	0.99 Rf	3.7 AU	1731.9 AU	2.14 %

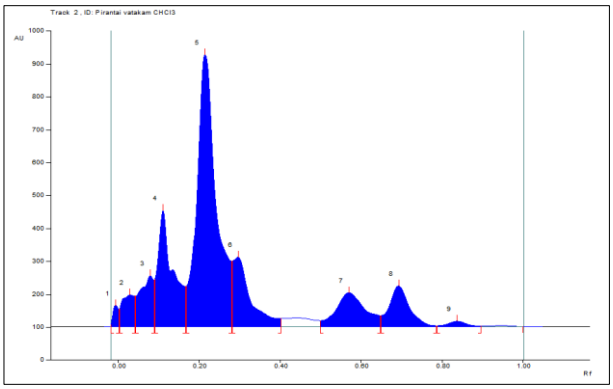


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	31.0 AU	-0.00 Rf	538.4 AU	18.66 %	0.04 Rf	43.6 AU	9444.2 AU	8.80 %
2	0.04 Rf	144.3 AU	0.05 Rf	159.2 AU	5.52 %	0.08 Rf	11.0 AU	3433.1 AU	3.20 %
3	0.09 Rf	111.2 AU	0.11 Rf	146.9 AU	5.09 %	0.12 Rf	34.0 AU	3122.6 AU	2.91 %
4	0.13 Rf	134.6 AU	0.17 Rf	235.7 AU	8.17 %	0.20 Rf	99.7 AU	8730.7 AU	8.14 %
5	0.20 Rf	200.6 AU	0.26 Rf	648.8 AU	22.49 %	0.35 Rf	90.1 AU	34322.8 AU	31.99 %
6	0.35 Rf	190.4 AU	0.38 Rf	260.8 AU	9.04 %	0.43 Rf	79.7 AU	10676.6 AU	9.95 %
7	0.43 Rf	180.1 AU	0.47 Rf	337.6 AU	11.70 %	0.53 Rf	88.6 AU	13559.6 AU	12.64 %
8	0.54 Rf	88.7 AU	0.55 Rf	90.1 AU	3.12 %	0.59 Rf	63.2 AU	2833.6 AU	2.64 %
9	0.62 Rf	63.1 AU	0.65 Rf	73.4 AU	2.54 %	0.70 Rf	52.8 AU	3284.3 AU	3.06 %
10	0.73 Rf	48.3 AU	0.80 Rf	319.1 AU	11.06 %	0.88 Rf	54.7 AU	14738.6 AU	13.74 %
11	0.88 Rf	55.1 AU	0.92 Rf	75.4 AU	2.61 %	1.00 Rf	0.4 AU	3138.7 AU	2.93 %

Figure 3b: HPTLC finger print profile of 4 µl and 8 µl of chloroform extract of Pirantai vatakam at 254 nm

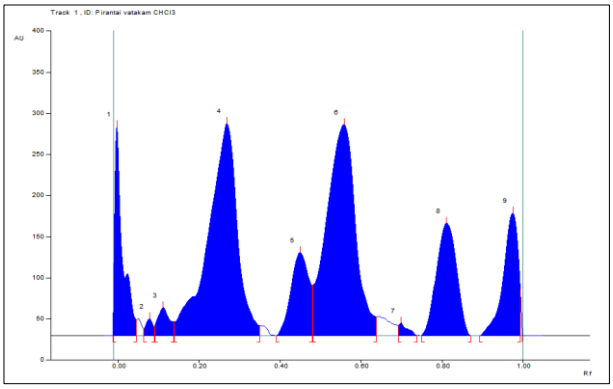


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.2 AU	0.02 Rf	82.8 AU	4.34 %	0.03 Rf	76.2 AU	1561.9 AU	3.06 %
2	0.05 Rf	76.9 AU	0.09 Rf	121.0 AU	6.34 %	0.10 Rf	09.9 AU	2767.3 AU	5.42 %
3	0.10 Rf	110.9 AU	0.12 Rf	289.5 AU	15.16 %	0.14 Rf	37.4 AU	4914.4 AU	9.62 %
4	0.14 Rf	139.5 AU	0.15 Rf	147.9 AU	7.74 %	0.18 Rf	11.7 AU	3108.4 AU	6.08 %
5	0.18 Rf	112.6 AU	0.23 Rf	858.7 AU	44.98 %	0.28 Rf	38.9 AU	25031.6 AU	48.99 %
6	0.28 Rf	239.2 AU	0.30 Rf	271.3 AU	14.21 %	0.41 Rf	15.5 AU	8414.0 AU	16.47 %
7	0.51 Rf	11.4 AU	0.58 Rf	63.0 AU	3.30 %	0.64 Rf	21.3 AU	2607.0 AU	5.10 %
8	0.66 Rf	21.0 AU	0.70 Rf	74.8 AU	3.92 %	0.80 Rf	2.3 AU	2690.6 AU	5.27 %

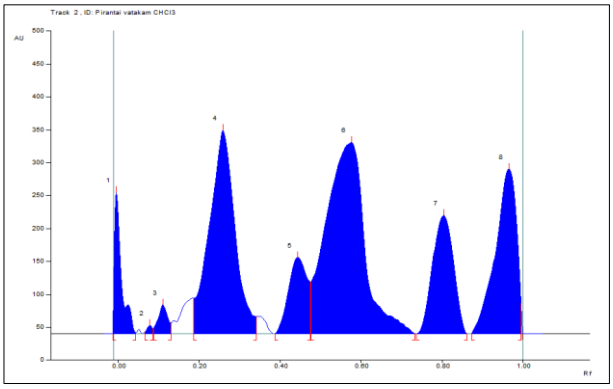


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	6.1 AU	-0.00 Rf	65.1 AU	3.33 %	0.00 Rf	53.2 AU	624.3 AU	1.05 %
2	0.00 Rf	55.1 AU	0.03 Rf	96.6 AU	4.94 %	0.04 Rf	93.0 AU	1999.9 AU	3.35 %
3	0.04 Rf	93.3 AU	0.08 Rf	155.3 AU	7.94 %	0.09 Rf	41.2 AU	3648.2 AU	6.12 %
4	0.09 Rf	144.4 AU	0.11 Rf	353.5 AU	18.08 %	0.17 Rf	22.0 AU	9242.9 AU	15.51 %
5	0.17 Rf	122.8 AU	0.22 Rf	827.3 AU	42.32 %	0.28 Rf	99.3 AU	27507.1 AU	46.14 %
6	0.28 Rf	199.6 AU	0.30 Rf	212.0 AU	10.85 %	0.40 Rf	24.4 AU	6529.2 AU	10.95 %
7	0.50 Rf	18.3 AU	0.57 Rf	103.9 AU	5.32 %	0.65 Rf	33.8 AU	5184.8 AU	8.70 %
8	0.65 Rf	33.9 AU	0.69 Rf	124.3 AU	6.36 %	0.79 Rf	2.8 AU	4330.7 AU	7.26 %
9	0.79 Rf	2.9 AU	0.84 Rf	16.7 AU	0.85 %	0.90 Rf	1.9 AU	544.1 AU	0.91 %

Figure 3b: HPTLC finger print profile of 4 µl and 8 µl of chloroform extract of Pirantai vatakam at 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.0 AU	-0.00 Rf	254.6 AU	20.75 %	0.05 Rf	19.4 AU	3415.1 AU	8.18 %
2	0.06 Rf	8.7 AU	0.08 Rf	20.7 AU	1.69 %	0.09 Rf	10.9 AU	256.1 AU	0.61 %
3	0.09 Rf	12.3 AU	0.11 Rf	34.3 AU	2.80 %	0.14 Rf	16.8 AU	689.6 AU	1.65 %
4	0.14 Rf	16.8 AU	0.27 Rf	258.3 AU	21.05 %	0.35 Rf	12.1 AU	12791.9 AU	30.65 %
5	0.39 Rf	0.0 AU	0.45 Rf	101.0 AU	8.23 %	0.48 Rf	61.2 AU	3122.5 AU	7.48 %
6	0.48 Rf	61.4 AU	0.56 Rf	256.6 AU	20.91 %	0.64 Rf	22.8 AU	12701.7 AU	30.44 %
7	0.69 Rf	12.3 AU	0.70 Rf	15.9 AU	1.29 %	0.74 Rf	0.4 AU	238.8 AU	0.57 %
8	0.75 Rf	0.5 AU	0.81 Rf	136.6 AU	11.13 %	0.87 Rf	0.1 AU	4724.9 AU	11.32 %
9	0.89 Rf	0.1 AU	0.98 Rf	149.1 AU	12.15 %	1.00 Rf	55.7 AU	3789.4 AU	9.08 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.0 AU	-0.00 Rf	214.8 AU	15.16 %	0.04 Rf	2.0 AU	2605.4 AU	4.70 %
2	0.07 Rf	2.6 AU	0.08 Rf	12.5 AU	0.88 %	0.09 Rf	7.8 AU	111.7 AU	0.20 %
3	0.09 Rf	8.0 AU	0.11 Rf	43.9 AU	3.10 %	0.13 Rf	16.1 AU	712.2 AU	1.28 %
4	0.19 Rf	54.0 AU	0.26 Rf	309.2 AU	21.82 %	0.34 Rf	26.0 AU	13592.9 AU	24.53 %
5	0.39 Rf	0.1 AU	0.44 Rf	116.1 AU	8.19 %	0.48 Rf	79.1 AU	3745.7 AU	6.76 %
6	0.48 Rf	79.1 AU	0.58 Rf	290.8 AU	20.52 %	0.74 Rf	0.2 AU	19239.6 AU	34.72 %
7	0.74 Rf	0.1 AU	0.81 Rf	179.7 AU	12.68 %	0.86 Rf	0.2 AU	6425.9 AU	11.59 %
8	0.88 Rf	0.7 AU	0.97 Rf	250.1 AU	17.65 %	1.00 Rf	45.9 AU	8987.9 AU	16.22 %

Figure 3c: HPTLC finger print profile of 4 µl and 8 µl of chloroform extract Pirantai vatakam at 575 nm after derivatisation

Table 2: R_f values and colour of bands

Wavelength	R_f value	Colour of band
254 nm	0.06	Light green
	0.27	Dark green
	0.39	Light green
	0.48	Light green
	0.81	Dark green
366 nm	0.09	Light brown
	0.12	Green
	0.15	Light brown
	0.23	Fluorescent white
	0.30	Fluorescent green
	0.58	Greenish blue
	0.70	Magenta
	0.85	Violet
575 nm	0.11	Light purple
	0.27	Brown
	0.45	Light purple
	0.56	Purple
	0.81	Light purple
	0.98	Purple

CONCLUSION

The pharmacognostic, physico-chemical and HPTLC fingerprint profile lays down a platform to establish the Pharmacopeial standards for Pirantai vatakam. Thereby, quality, safety and efficacy of Pirantai vatakam could be ensured and also could reveal its phytochemical components by HPTLC.

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