

STANDARDISATION OF INCI VATAKAM BY PHARMACOGNOSTIC AND CHROMATOGRAPHIC METHODS

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ABSTRACT

In Indian systems of traditional medicine, Siddha system is considered to be one of the oldest traditional healing therapies known to the mankind since several centuries. Siddha system of medicine has versatile formulations in curing indigestion and diseases of liver. Inji and Chukku (Fresh and dried ginger – *Zingiber officinale*) play very important role in Siddha preparations. The role of ginger both in diet and preparation of medicine is well documented in age old Siddha texts and manuscripts. The present study is proposed to carry out the standardization of a Siddha formulation, *Inci vatakam* based on its organoleptic, physicochemical and HPTLC fingerprinting aspects. *Inci vatakam* was prepared by the method described in Teraiyar Taru. Botanical characters such as macroscopic and powder microscopic features of *Inci vatakam* was carried out to ensure the quality of the raw drug used. Physico-chemical characters of *Inci vatakam* and the ingredients were determined using standard methods. High performance thin layer chromatographic (HPTLC) study of the formulation was performed and the chromatograms were documented. Thus, physico-chemical standardisation and HPTLC fingerprinting helped to ensure the quality of formulation and to identify the presence of different phyto components based on its R_f values.

KEYWORDS: *Inci vatakam*, Physicochemical analysis, Powder microscopy, HPTLC Study.

1. INTRODUCTION

With the growing demand and popularity of Indian systems of medicines, it becomes very essential to provide quality medicines. Government of India as well as consumers are very much concerned with the quality of medicines for human healthcare. For any quality medicine, the quality control methods are important. Standardization of herbal medicines ensures the quality of medicines and gives authenticity to the medicines prepared by the manufacturers, satisfaction of the prescribing physicians and relief to the consumers. This paper deals with the standardization of a Siddha formulation, *Inci vatakam* by carrying out a detailed analysis based on the WHO and FDA guidelines.^[1,2]

One or more drugs of plant, animal or mineral origin are powdered, sieved, mixed with prescribed liquids and triturated till attains the consistency suitable for making *vatakam*. These are dried under shade or sun and stored in airtight containers.

Siddha medicines mainly comprises of plant parts, metals, minerals and some animal substances. *Inci vatakam* is commonly used for sluggish liver, hepatomegaly, diseases of vitiated vaatha, piththa and kaphadoshams, indigestion and diarrhoea. It promotes good appetite in post-operative convalescence.^[3-6] *Inci vatakam* consists of eight herbal ingredients. The formulation taken for the study was prepared by the Clinical Department, Siddha Regional Research Institute, Thiruvananthapuram with reference to the Siddha formulary of India.^[3] The physico-chemical parameters of prepared *Inci vatakam* and its ingredients were determined. Fingerprint analysis by HPTLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant. In combination with microscopic investigations, the fingerprint provides the means for a convenient identity check. It can also be used to detect adulterations in raw materials.^[7]

2. MATERIALS AND METHODS

2.1 Ingredients

Inci vatakam comprises of the following ingredients.^[3]

Table 1: Ingredients of *Inci vatakam*.

Sl.No.	Tamil name of the drug	Botanical name	Parts/products used	Quantity used
1.	Inji	<i>Zingiber officinale</i> Rosc.	Rhizome	8 parts
2.	Chukku	<i>Zingiber officinale</i> Rosc.	Rhizome (dried)	1 part
3.	Katukkaitol	<i>Terminalia chebula</i> Retz	Fruit rind	1 part
4.	Milaku	<i>Piper nigrum</i> L.	Fruit	1 part
5.	Tippali	<i>Piper longum</i> L.	Fruit	1 part
6.	KotamalliVitai	<i>Coriandrum sativum</i> L.	Fruit	1 part
7.	Ilavankam	<i>Syzygium aromaticum</i> (L.) Merr. et Perry	Flower bud	1 part
8.	Omam	<i>Trachyspermum ammi</i> (L.) Sprague	Fruit	1 part
9.	Uppu	Salt		1 part
10.	Tair	Curd		Sufficient Quantity
11.	Neyy	Ghee		Sufficient Quantity

2.2. Source of raw drug

The authentic ingredients were procured from the local market of Thiruvananthapuram, Kerala and were botanically identified by Dept. of Pharmacognosy, Poojappura, Thiruvananthapuram.

2.3. Method of preparation of *Inci vatakam*

The items 1 to 9 are fried with item 11 and ground with item 10. It was dried suitably and made to pill making consistency. It was made into 1 gm pills and dried^[3].

2.4. Organoleptic evaluation

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

2.5. Macroscopy of the ingredients of *Inci vatakam*

Macroscopic studies of the ingredients were carried out and were compared with that in literature^[7] for their proper identification.

2.6. Powder microscopy of *Inci vatakam*

The prepared vatakam was powdered and subjected to powder microscopy. Powder microscopy was carried out as per standard protocol^[9]. Powder microscopy was carried out to identify the presence of genuine ingredients in the formulation. The formulation was then observed under 10X and 40X objective of microscope. A few quantity of powder was mounted in glycerine for 2 hours to observe the diagnostic characters of the ingredients.

2.7. Physico-chemical studies

Physico-chemical studies of *Inci vatakam* and other eight ingredients were carried out as per standard protocol^[1,10-11]. The physico-chemical parameters determined for *Inci vatakam* and other eight ingredients are foreign matter, loss on drying at 105°C, total ash, acid insoluble ash, solubility in alcohol and water and volatile oil. Analysis of physico-chemical constants of the ingredients were done to evaluate the quality and purity of the raw drugs.

2.8. Preliminary phytochemical analysis

Preliminary phytochemical screening of the vatakam was carried out by standard methods^[12-14] to detect the presence of different secondary metabolites such as terpenoids, flavonoids, phenols, steroids, alkaloids, tannins, glycosides, carbohydrates etc.

2.9. HPTLC fingerprinting

Sample preparation

The extracts for the HPTLC studies was prepared by taking 4 g of the *Inci vatakam* and added 40 ml of ethanol to the flask and kept overnight. The solution was boiled for 10 minutes and filtered. The filtrate was concentrated and made up to 10 ml in standard flask. This ethanol extract was used for chromatographic studies.^[15]

Developing solvent system

A number of solvent systems were tried to obtain an appropriate mobile phase. The solvent system Toluene: Ethyl acetate: Formic acid (5:2:0.1) which gave a satisfactory resolution was opted.

Sample application

Sample application was performed on a 5x10 cm silica gel 60 F₂₅₄ pre-coated aluminium sheet. The chloroform extracts of the *Inci vatakam* applied on two tracks as bands of width 10 mm using CAMAG microliter syringe with Automatic TLC Sampler 4 (ATS4).^[16]

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: Formic acid (5:2:0.1). 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 254 and 366nm.

Densitometry

The densitometric scanning was performed by using TLC Scanner 4. The plate was scanned at 254, 366nm 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°C 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.

3. RESULTS AND DISCUSSION

Inci vatakam is an indigenous herbal formulation containing eight herbal ingredients which claims to have the potential in the treatment of hepatomegaly, diseases of vitiated vaatha, piththa and kapha doshams, indigestion and diarrhoea. *Inci vatakam* also prescribed in diseases of liver and spleen. It promotes good appetite in post-operative convalescence.^[17] *Inci vatakam* and its ingredients are shown in Figure 1.



Figure 1: *Inci vatakam* and its ingredients.

3.1. Organoleptic characters

Inci vatakam is brown in colour having characteristic odour and astringent taste.

3.2. Macroscopical characters of the ingredients

a. *Zingiber officinale* (Rhizome)

Z. officinale (Zingiberaceae) (English – Ginger; Hindi – Adrak; Malayalam – Inchi; Sanskrit – Adraka; Tamil – Inji) is a rhizomatous perennial herb, reaching up to 90 cm in height (Plate: 1a). Rhizomes are aromatic, 2-3 cm thick, palmately lobed, grayish-yellow within, pungent; leaves sessile, simple, alternate, distichous, narrow oblong lanceolate; ligule 2-4 mm long, membranous, slightly bilobed, base hairy; lamina 25-30 × 1.5-2 cm, narrowly lanceolate, acuminate, base attenuate, lower surface hairy, upper glabrous. Inflorescence: radical, 15-25 cm long; peduncle leafless, enclosed by 3-5 cm long,

minutely pubescent sheaths; spike 4-7 × 2-3 cm long. Bracts 2.5 × 1.5-2cm, green with a paler membranous margin, the lower ones usually mucronate, turning to red at maturity. Bracteoles 2.5 × 1.2 cm, ovate-oblong, almost equal to the bracts, white, membranous, glabrous. Calyx 1- 1.2 cm, tubular, unilaterally split. Corolla tube 2.5 cm long; corolla lobes almost equal, pale yellow (Plate: 1d); dorsal lobe 2 × 0.8 cm, narrowed at apex; lateral lobes 2 × 0.5 cm, labellum 1.4 × 1.1 cm, more or less round, dark purple, blotched creamy- yellow; lateral staminodes smaller, 8 × 6 mm, free, almost to the base. Anther-thecae parallel, connective yellow, crest 5-8 mm long, dark purple. Stigma white, prolonged above the crest, margin ciliate. Epigynous glands 4 mm long, linear, free from each other. The pollen grains of the ginger are exineless, possessing a structurally complex intine.

b. Terminalia chebula (Fruit rind)

T. chebula (Combretaceae) (English - Black myrobalan; Malayalam – Kadukka; Tamil - Kadukkai; Hindi - Haritak) is a medium-sized deciduous tree with a height of up to 30 m, wide spreading branches and a broad roundish crown. It grows in the altitude of 1500 to 2000 m in mostly clayey as well as shady soils. The leaves are elliptic rhombus, with an acute tip, cordate at the base, outskirts entire, glabrous above a yellowish pubescence below. The flowers are monoecious, monotonous white to yellow, with a strong unlikable odor, born in terminal prickles or short panicles. The fruit are glabrous, ellipsoids ovoid drupes, yellow to orange brown in colour, encloses a single angle stone.

c. Piper nigrum (Fruit)

P. nigrum (English - Black pepper; Malayalam – Kurumulaku; Tamil – Milaku; Hindi - Kali mirchi) is a glabrous climber belongs to Piperaceae. Leaves to 16 x 10 cm, ovate, acuminate at apex, acute, rounded or truncate at base, 7-nerved, nervules subparallel; petiole to 2.5 cm long. Spikes to 12 cm long, slender; peduncles to 2.5 cm long; bracts united forming a cup below the flower. Flowers closely placed; stamens 2. Drupes 5 mm across, globose, smooth, deep red; stigmas 3-5, recurved.

d. Piper longum (Fruit)

P. longum (Piperaceae) (English - Long pepper; Malayalam – Tippali; Tamil – Tippli; Hindi – Pippli; Gujarathi – Pipari) is a scandent or straggling shrubs, sometimes ascending and climbing. Leaves to 8 x 4 cm, ovate, acute at apex, cordate and strongly oblique at base, 7-nerved, membranous, shining above; nerves impressed; petiole 1-3 cm long. Female spike stout, erect; peduncle 1.5 cm long; bracts peltate, orbicular. Male spike to 7 cm long, erect, slender; stamens 2. Berry 2 mm across, glabrous, black or deep red.

e. Trachyspermum ammi (Fruit)

T. ammi (Apiaceae) (Bengali – Jowan; Gujarati – Yavano; Hindi – Ajwain; Kannada - ajamoola, oma, omu, ajamoda; Marathi - Ova, Sanskrit – Ajamoda) is an erect, hairless or minutely pubescent, branched annual herb. The stems are grooved, the leaves are rather distant, 2-3-pinnately divided in narrow linear segments. Flowers are borne in terminal or seemingly-lateral stalked, compound umbels, white and small. The fruits are ovoid, aromatic, greyish brown. The mericarps, which are the components of the fruit, are compressed, with distinct ridges and tubercular surface, 1-seeded. This is what is used as the spice Ajwain, in cooking.

f. Coriandrum sativum (Fruit)

C. sativum (Apiaceae) (Hindi – Dhaniya; Manipuri – Phadigom; Tamil – Kotthumalli; Malayalam – Kothamalli) is a slender, solid-stemmed annual reaching 60 cm in height. The upper leaves are divided into narrow linear segments, the lower leaves into broad

leaflets. The small flowers are white or pink. The middle flowers in each umbel are infertile, while the outer flowers are slightly larger and fertile. The globose fruits are around 3 mm in diameter, ridged, and yellowish brown. Plants 15-60 cm tall, branched. Basal leaves ternately lobed; segments oval to ovate; margin toothed; cauline leaves pinnately dissected; segments linear. Rays 3-6, unequal. Calyx teeth prominent, unequal. Petals purplish-white, the outer larger, bilobed. Styles slender, spreading, 1.5-2.5 mm long. Fruit sub-globose, c. 4 mm long; vittae in furrows obscure; commissure 2-vittate. Inner seed face concave.

g. Syzygium aromaticum (Flower bud)

S. aromaticum (Myrtaceae) (Hindi - Lavang; Kannada - Lavanga; Marathi – Lavang; Malayalam - Grampu, karayampu; Tamil - Ilavangam; Sanskrit - Badrasriya, lavangais a bushy, evergreen tree with a medium-sized crown, growing 8-20 metres tall. Several parts of the tree are aromatic, including the leaves and bark, but it is most valued for the aromatic flower buds, which are usually harvested by hand. Leaves are simple, bright green and glossy. The lower surface is covered in aromatic oil glands. Elliptical leaves with petioles (leaf stalks) grow up to 13cm long in opposite pairs along numerous short branches. Flower buds develop in small clusters. Flower buds are initially pale with a glossy, fleshy appearance, turning green and then bright red as they mature. They consist of a long, narrow ovary, approximately 1.5–2.0 cm in length, with four small triangular sepals projecting outwards at one end. The sepals surround a small ball of four overlapping petals that protect the developing flower parts inside. It is in this form that the clove is harvested and dried for use as a spice.

3.3. Powder microscopy of Inci vatakam

Powder microscopic studies of *Inci vatakam* was carried out and the results are given in Fig. 2. Powder microscopy study of the vatakam revealed various characters such as starch grains, perisperm cell, fibre bundles, cork cells, stone cells, sclerenchymatous layer of mesocarp, xylem vessel with spiral thickening and sclerenchyma cells.

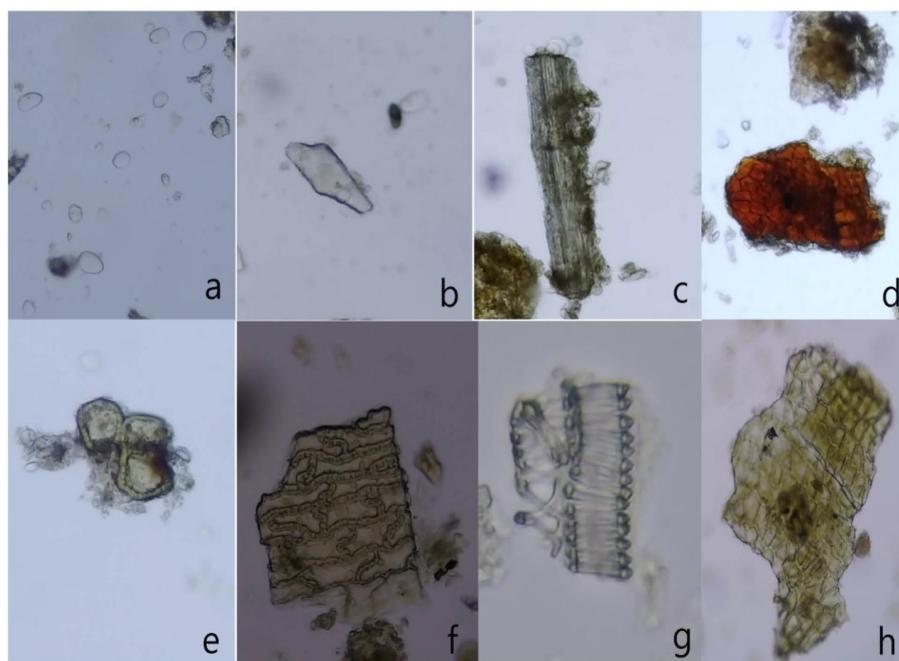


Figure 2 a. Starch grains, b. perisperm cell, c. fibre bundles, d. cork cells, e. stone cells, f. sclerenchymatous layer of mesocarp, g. xylem vessel with spiral thickening, h. sclerenchyma cells.

3.4. Physico-chemical properties of the ingredients

Standardisation of the drug is more essential to derive the efficacy and potency of the drug by analysing it by various studies. Following are the results of physio

chemical and phytochemical analysis of *inci vatakam* and its ingredients. Table 2. Shows the Physico-chemical properties of the ingredients and the Table 3 shows the physico-chemical properties of *Inci vatakam*.

Table 2: Physico-chemical properties of the ingredients.

Tests	Results							
	<i>Z. officinale</i> (fresh ginger)	<i>Z. officinale</i> (dried ginger)	<i>T. chebula</i>	<i>P. nigrum</i>	<i>P. longum</i>	<i>C. sativum</i>	<i>S. aromaticum</i>	<i>T. ammi</i>
Loss on drying at 105°C (%)	64.80	12.82	12.10	51.61	14.88	10.39	11.20	11.28
Total ash (%)	1.78	4.50	2.47	4.54	10.09	2.15	4.27	5.7
Acid insoluble ash (%)	0.16	0.60	0.18	0.10	2.52	0.65	0.65	0.008
Water soluble extractive (%)	75.71	11.46	53.35	8.56	12.97	20.52	24.29	17.55
Alcohol soluble extractive (%)	42.31	3.54	42.27	7.93	4.05	19.26	10.24	26.52
Volatile oil (%)	3.50	1.00	Nil	3.50	2.50	2.00	2.00	5.00

Table 3: Physico-chemical properties of *Inci vatakam*.

Sl.No.	Parameter	Result
1.	Loss on Drying at 105°C %	23.76
2.	Total Ash Content %	10.70
3.	Acid Insoluble Ash %	1.05
4.	Water Soluble Extractive %	20.98
5.	Alcohol Soluble Extractive %	23.26
6.	Volatile oil %	4.00

Loss on drying at 105°C indicates the amount of volatile substance and moisture present in the drug. This also indicates the stability and shelf life of the drug. The loss on drying percentage of *inci vatakam* is 23.76%. According to the literature vatakam retains their potency upto 3 months. Total ash value determines the amount of

minerals and earthy materials present in the drug. The total ash value of *inci vatakam* is 10.70 % which determines the presence of inorganic content. The acid insoluble ash value of the drug denotes the amount of siliceous matter (dust, sand etc.) present in that drug. The quality of the drug is better if the acid insoluble ash value is low. Here, acid insoluble ash value of *Inci vatakam* is 1.05 %. Hence, it represents the good quality of the *inci vatakam*. Table 3 shows the physico-chemical properties of *Inci vatakam*.

3.5. Preliminary phytochemical analysis

The preliminary phytochemical analysis was carried out to determine the group of constituents present in the *vatakam* and the results are tabulated in Table 4.

Table 4: Phytochemical constituents of *Inci vatakam*.

Sl. No.	Natural products	Test performed	Present/ Absent
1	Acids	With dil. alkali	Absent
2	Alkaloids	Mayer s reagent test	Present
3	Amino acids	Ninhydrin test	Present
4	Chalcones	Antimony trichloride test	Absent
5	Anthraquinones	Borntrager's test	Present
6	Coumarins	Test with alkali	Present
7	Flavonoids	Shinoda test	Present
8	Furanoids	Para dimethyl amino Benzaldehyde test	Present
9	Glycosides	Anthrone test	Present
10	Lignans	Phloroglucinol test	Present
11	Phenols	Neutral FeCl ₃ test	Present
12	Quinones	Test with alkali	Present
13	Steroids	Liebermann s test	Present
14	Tannins	Lead acetate test	Present
15	Terpenoids	Leibermann test	Present
16	Saponins	Froth test	Absent

3.6. HPTLC fingerprinting of *Inci vatakam*

Ethanol extract was applied in TLC aluminium sheet silica gel 60 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate: Formic acid (5:2:0.1). After development, the plate is allowed to dry

in air and examined under UV short (254 nm), UV long (366 nm) and 575 nm (white light) after derivatisation using Vanillin - Sulphuric acid reagent. The representative chromatograms are shown in Figure 3.

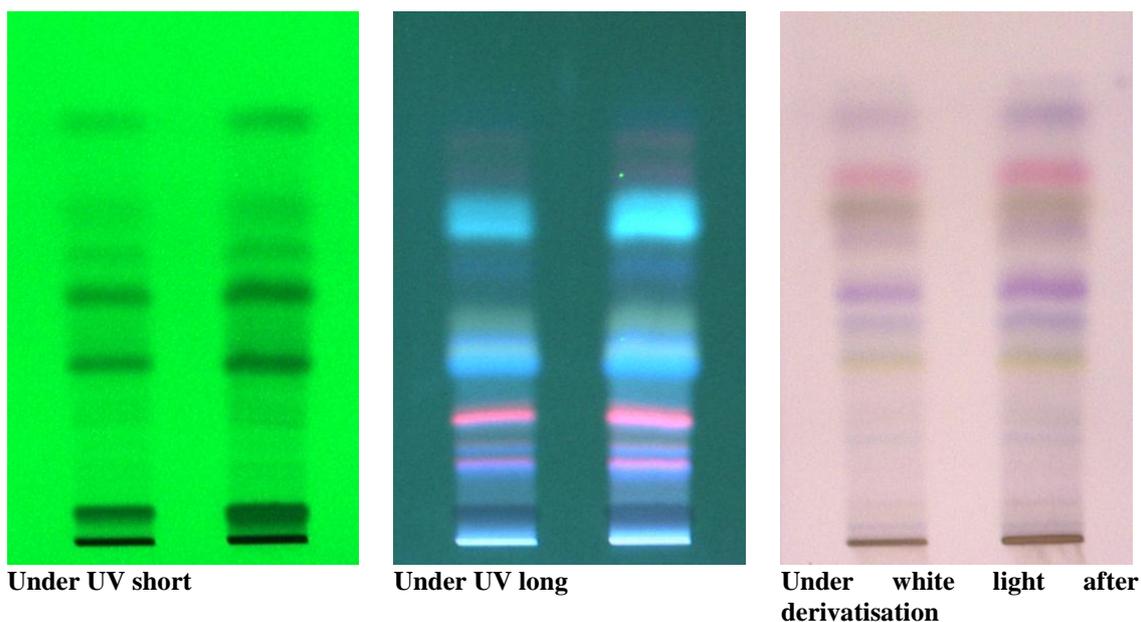


Figure 3: HPTLC profile of alcohol extract of *Inci vatakam* viewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light; Solvent system – Toluene: Ethyl acetate: Formic acid (5: 2: 0.1).

The R_f values and peak area percentages of the observed bands for 4 μ l and 8 μ l of ethanol extract of *Inci vatakam* under UV short (254 nm), UV long (366 nm) and 575 nm (white light) after derivatisation are represented in Figure 4, 5 and 6 respectively.

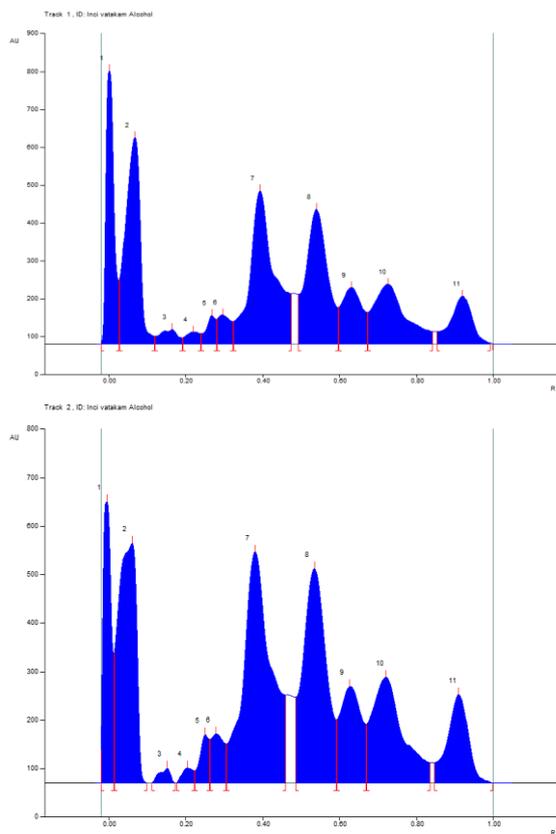


Figure 4. HPTLC finger print profile of 4 µl and 8µl of alcohol extract of *Inci vatakam* at 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	3.8 AU	0.00 Rf	720.8 AU	26.87 %	0.03 Rf	67.2 AU	10677.9 AU	13.10 %
2	0.03 Rf	171.3 AU	0.07 Rf	545.1 AU	20.32 %	0.12 Rf	20.9 AU	14333.6 AU	17.59 %
3	0.12 Rf	20.9 AU	0.17 Rf	38.3 AU	1.43 %	0.19 Rf	16.5 AU	1226.4 AU	1.51 %
4	0.19 Rf	16.7 AU	0.22 Rf	31.8 AU	1.18 %	0.24 Rf	27.7 AU	788.4 AU	0.97 %
5	0.24 Rf	27.7 AU	0.27 Rf	75.9 AU	2.83 %	0.28 Rf	65.1 AU	1330.6 AU	1.63 %
6	0.28 Rf	65.9 AU	0.30 Rf	77.1 AU	2.88 %	0.32 Rf	59.3 AU	1806.3 AU	2.22 %
7	0.33 Rf	59.5 AU	0.40 Rf	403.7 AU	15.05 %	0.48 Rf	33.2 AU	17913.4 AU	21.98 %
8	0.49 Rf	130.7 AU	0.54 Rf	356.1 AU	13.27 %	0.60 Rf	96.6 AU	14017.4 AU	17.20 %
9	0.60 Rf	97.2 AU	0.63 Rf	149.4 AU	5.57 %	0.67 Rf	83.4 AU	5348.4 AU	6.56 %
10	0.68 Rf	83.5 AU	0.73 Rf	158.0 AU	5.89 %	0.84 Rf	33.0 AU	9159.9 AU	11.24 %
11	0.86 Rf	33.1 AU	0.92 Rf	126.3 AU	4.71 %	0.99 Rf	3.0 AU	4880.2 AU	5.99 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	68.4 AU	-0.00 Rf	580.1 AU	20.32 %	0.01 Rf	65.1 AU	8772.8 AU	8.84 %
2	0.02 Rf	271.6 AU	0.06 Rf	494.5 AU	17.32 %	0.10 Rf	0.2 AU	15991.2 AU	16.11 %
3	0.11 Rf	0.1 AU	0.15 Rf	30.4 AU	1.07 %	0.17 Rf	0.0 AU	612.9 AU	0.62 %
4	0.18 Rf	0.6 AU	0.20 Rf	30.8 AU	1.08 %	0.22 Rf	24.9 AU	607.3 AU	0.61 %
5	0.22 Rf	25.2 AU	0.25 Rf	99.7 AU	3.49 %	0.26 Rf	89.7 AU	1657.6 AU	1.67 %
6	0.26 Rf	89.8 AU	0.28 Rf	101.5 AU	3.55 %	0.31 Rf	80.7 AU	2415.7 AU	2.43 %
7	0.31 Rf	80.9 AU	0.38 Rf	476.8 AU	16.70 %	0.46 Rf	81.0 AU	23773.4 AU	23.95 %
8	0.49 Rf	177.2 AU	0.54 Rf	441.9 AU	15.48 %	0.59 Rf	30.3 AU	18789.1 AU	18.93 %
9	0.60 Rf	130.5 AU	0.63 Rf	199.3 AU	6.98 %	0.67 Rf	20.9 AU	7482.8 AU	7.54 %
10	0.67 Rf	120.9 AU	0.72 Rf	217.5 AU	7.62 %	0.84 Rf	41.9 AU	12333.6 AU	12.42 %
11	0.85 Rf	42.0 AU	0.91 Rf	182.2 AU	6.38 %	1.00 Rf	0.0 AU	6838.5 AU	6.89 %

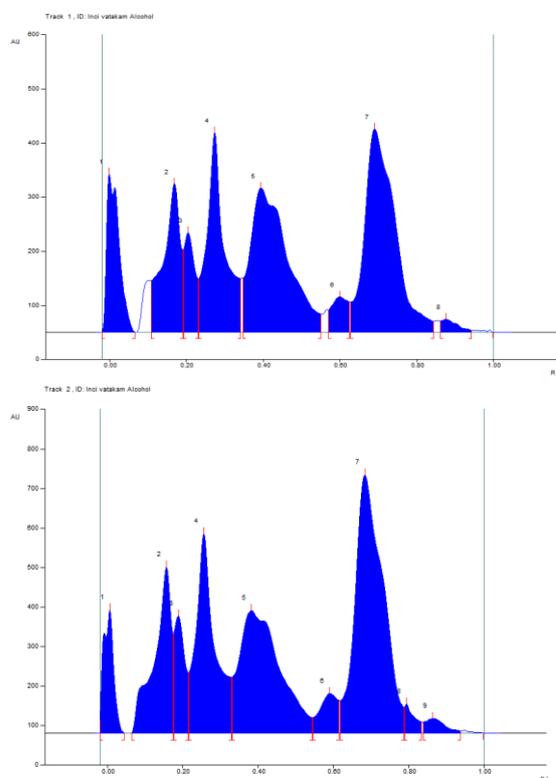


Figure 5. HPTLC finger print profile of 4 µl and 8µl of alcohol extract of *Inci vatakam* at 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	2.0 AU	0.00 Rf	293.1 AU	15.82 %	0.07 Rf	0.4 AU	6679.1 AU	9.30 %
2	0.11 Rf	95.9 AU	0.17 Rf	274.9 AU	14.84 %	0.19 Rf	51.2 AU	8268.3 AU	11.52 %
3	0.19 Rf	152.7 AU	0.20 Rf	184.4 AU	9.95 %	0.23 Rf	99.1 AU	3554.2 AU	4.95 %
4	0.23 Rf	99.2 AU	0.28 Rf	369.2 AU	19.93 %	0.34 Rf	99.3 AU	11703.1 AU	16.30 %
5	0.35 Rf	100.0 AU	0.40 Rf	266.3 AU	14.37 %	0.55 Rf	34.0 AU	18173.9 AU	25.31 %
6	0.57 Rf	41.4 AU	0.60 Rf	65.4 AU	3.53 %	0.63 Rf	56.2 AU	1949.2 AU	2.71 %
7	0.63 Rf	55.5 AU	0.69 Rf	375.5 AU	20.27 %	0.85 Rf	20.3 AU	20746.8 AU	28.90 %
8	0.86 Rf	21.0 AU	0.88 Rf	24.0 AU	1.30 %	0.94 Rf	3.9 AU	720.0 AU	1.00 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	32.8 AU	0.01 Rf	312.2 AU	11.54 %	0.05 Rf	0.5 AU	5945.3 AU	5.63 %
2	0.06 Rf	1.3 AU	0.16 Rf	420.4 AU	15.54 %	0.18 Rf	50.3 AU	13257.5 AU	12.55 %
3	0.18 Rf	252.3 AU	0.19 Rf	295.3 AU	10.92 %	0.22 Rf	53.0 AU	5428.6 AU	5.14 %
4	0.22 Rf	153.3 AU	0.26 Rf	504.0 AU	18.64 %	0.33 Rf	41.2 AU	17756.0 AU	16.81 %
5	0.33 Rf	141.7 AU	0.38 Rf	309.5 AU	11.44 %	0.55 Rf	39.4 AU	23412.9 AU	22.16 %
6	0.55 Rf	39.4 AU	0.59 Rf	100.0 AU	3.70 %	0.62 Rf	83.6 AU	3293.0 AU	3.12 %
7	0.62 Rf	82.8 AU	0.69 Rf	652.8 AU	24.14 %	0.79 Rf	64.9 AU	33851.5 AU	32.04 %
8	0.79 Rf	65.5 AU	0.80 Rf	73.8 AU	2.73 %	0.83 Rf	28.7 AU	1329.6 AU	1.26 %
9	0.84 Rf	28.7 AU	0.87 Rf	36.5 AU	1.35 %	0.94 Rf	5.7 AU	1368.6 AU	1.29 %

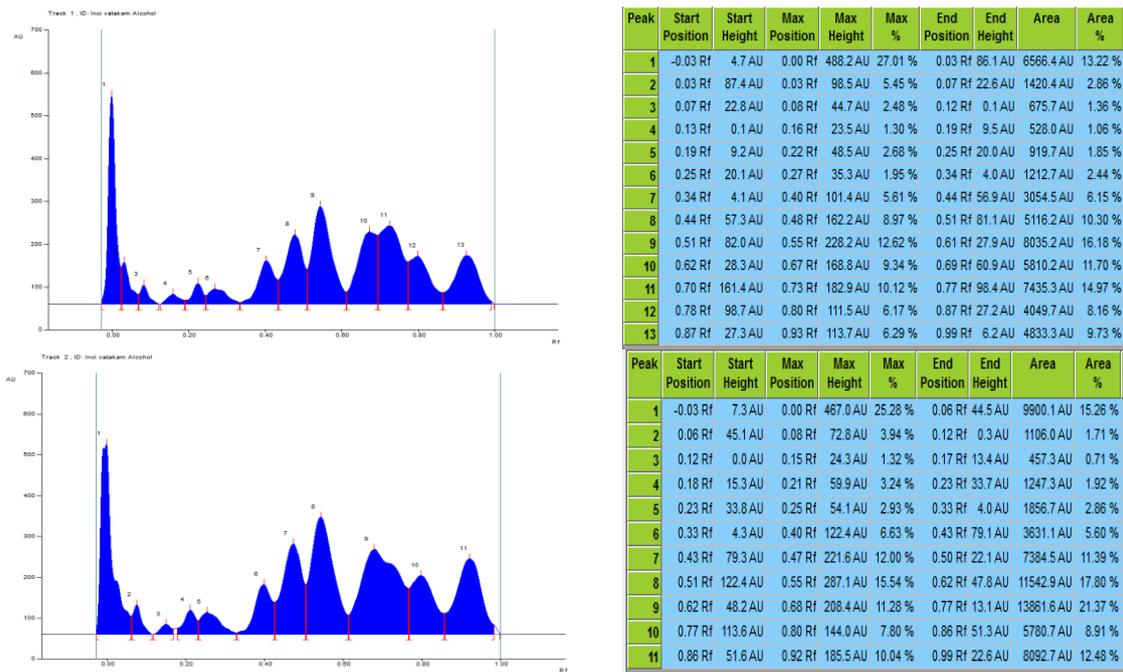


Figure 6. HPTLC finger print profile of 4 µl and 8 µl of alcohol extract of *Inci vatakam* at 575 nm after derivatisation.

The R_f values and colour of bands obtained at different wavelengths for *Inci vatakam* and the area of spots obtained for the *vatakam* are represented in Table 5.

Table 5: R_f values, colour of bands and area of spots obtained at different wavelengths for *Inci vatakam*.

Wavelength (nm)	<i>Inci vatakam</i>		
	R_f values	Colour	Area of spots (AU)
254 nm	0.07	Dark green	14333.6
	0.17	Dark green	1224.4
	0.22	Light green	788.4
	0.27	Light green	1330.6
	0.30	Light green	1806.3
	0.40	Light green	17913.4
	0.54	Dark green	14017.4
	0.63	Dark green	5348.4
	0.73	Light green	9159.9
366 nm	0.17	Light blue	8268.3
	0.20	Light blue	3554.2
	0.28	Pink	11703.1
	0.40	Pink	18173.9
	0.60	Light blue	1949.2
	0.69	Light blue	20746.8
	0.88	Light blue	720.0
575 nm	0.08	Light purple	1106.0
	0.15	Light purple	457.3
	0.21	Light purple	1247.3
	0.25	Dark purple	1856.7
	0.40	Yellow	3631.1
	0.47	Light purple	7384.5
	0.55	Dark purple	11542.9
	0.68	Yellow	13861.6
	0.80	Pink	5780.7
0.92	Light purple	8092.7	

HPTLC is an important quality assessment tool for the evaluation of herbal samples and is the simplest separation technique today available to the researchers and scientists. HPTLC is a micro analytical separation and determination method which has a wide application in herbal drug analysis. HPTLC fingerprinting profile helps very much in standardization for the proper identification of medicinal plants and is suitable for rapid decisive authentication of plant materials. The chemical fingerprints developed in HPTLC study are able to ensure the quality of the formulation and provide referential information for standardisation

4. CONCLUSION

The above cited parameters can be considered as pharmacopoeial standards and will help us to determine the genuineness of *Inci vatakam* and its ingredients. These values can be used in turn to check and ensure the quality of the medicine. The results of HPTLC studies further help in fulfilling the purpose of standardization.

CONFLICT OF INTEREST

No conflicts declared.

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REFERENCES

1. Anonymous. World Health Organization (WHO). Quality control Methods of Medicinal Plant Materials, Geneva, 1998; 8(28-34): 45-46.
2. CDER/USFDA. Guidance for Industry, Botanical Drug Products, draft guidance, Center for Drug Evaluation and Research. U.S. Food and Drug Administration, 2000.
3. Anonymous. The Siddha Formulary of India: Part I. 1st Ed., Ministry of Health and Family Welfare, Govt. of India, 1992; 146.
4. Teraiyar Taru, 1975; 44-45.
5. Khare, C.P. Indian Medicinal Plants: An Illustrated Dictionary. New Delhi; Springer: 2007.
6. Dr. Murugesu Muthaliyar, *Siddha Materia Medica (Vegetable section)*, I. 4th Ed., Chennai; Tamilnadu Siddha Medical Council, 1988.
7. Siddiqui A, Hakim MA. Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of unani drugs, (appendix), New Delhi; Central council for research in unani medicine, 1995; 24-25.
8. Gamble J S. Flora of Presidency of Madras (Repr.). Botanical survey of India, 1957; 1-3: 1925.
9. Johansen, D.A. Plant Microtechnique. New York; McGraw-Hill Book Co., 1940.
10. Anonymous, The Siddha Pharmacopoeia of India Part-1, Volume II, First Ed., Govt. of India, Ministry of Health and Family Welfare, AYUSH, New Delhi, 242-246.
11. Anonymous, The Ayurveda Pharmacopoeia of India Part II (Formulations), Vol.1, First Edition, Govt. of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy, New Delhi, 2007; 199-202.
12. Vogel, AI. Vogel's Text Book of Practical Organic Chemistry. 4th ed., London; Longman Group Limited, 1978.
13. Raman. Phytochemical Techniques. New Delhi; New India Publishing Agency, 2006.
14. Harbone, JB. Phytochemical Methods, A guide to modern techniques of plant analysis. London; Chapman and Hall Ltd., 1984.
15. Wagner, H. Bladt, S. Plant drug analysis - A Thin Layer Chromatography Atlas, Berlin; Springer - Verlag, 1996.
16. Camag: Application Notes on instrumental thin layer chromatography, 1996.
17. Anonymous, Formulary of Siddha medicines. Madras; The Indian Medical Practitioner's Co-operative Pharmacy and Stores Ltd., 1993; 119.