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## PHYSICO-CHEMICAL PARAMETERS AND HPTLC FINGERPRINTING OF KUNTHIRIKKAM TAILAM

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### Abstract

Kunthirikkam tailam is an herbal preparation in Siddha system of medicine. The ingredients of the formulation are resin of *Pistacia lentiscus* L. (Punaikkan Kunkiliyam) and Gingelly oil. *Pistacia lentiscus* L. (Anacardiaceae family) commonly known as mastic tree or mastagi, has been used in traditional system of medicines for treatment of various kinds of diseases since long-standing time. Gingelly oil is an edible oil derived from sesame seeds and is well known for its health benefits. In the present study, the standardization of Kunthirikkam tailam based on its organoleptic, physico-chemical and HPTLC fingerprinting aspects is carried out. The tailam was prepared by the method described in Siddha Formulary of India, Part I. Botanical characters such as macroscopic and microscopic features of Punaikkan Kunkiliyam was carried out to ensure the quality of the raw drug used.

Physico-chemical characters of the tailam and the ingredients were determined using standard methods. High performance thin layer chromatographic (HPTLC) studies of the formulation and the ingredients were 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.

**Key Words:** Kunthirikkam tailam, Powder microscopy, Physico – chemical analysis, HPTLC study.

## Introduction

Kunthirikkam tailam is a herbal preparation in Siddha system of medicine for osteoarthritis and joint function - particularly for osteoarthritis of the knee, wound healing and in pains, especially in rheumatic pains. It is also used in contusion and abrasions. It is used as an external application. The ingredients of the formulation are resin of *Pistacia lentiscus* L. and gingelly oil.

*Pistacia lentiscus* L. an aromatic member of the Anacardiaceae family, is a shrub or small tree indigenous to Mediterranean areas especially Spain, Portugal, Morocco, Italy, Greece, Turkey and southern France<sup>1,2,3</sup>. Its various parts contain a variety of chemical constituents which are medicinally important such as resin, essential oil, gallic acid, anthocyanins, flavonol glycosides, nortriterpenoids,  $\alpha$ -tocopherol and arabino-galactan proteins. It has antiatherogenic, antimicrobial, antimutagenic, antioxidant, antifungal, lipid lowering, hepatoprotective, anticancer, anthelmintic, wound healing, hypotensive, antiarthritic and antigout activities. It is also used in the treatment of functional dyspepsia.

It has been used both as dietary supplement and as medicine since centuries. Its vernacular names<sup>2</sup> are Bengali - Rumi Mastungi; English - Mastic, Mastiche, Mastictree; Gujrati - Rumi Mastagee; Hindi - Rumi Mastagi, Rumi mastiki, Mastagi; Marathi - Rumaa Mastakee; Tamil - Punaikkan kungiliyam; Telugu – Jeevakamu and Urdu - Rume Mastagee. Punaikkan Kunkiliyam is the resin obtained from *Pistacia lentiscus* Linn. which is one of the well-known drugs of Siddha system of medicine used for the treatment of various diseases. Several pharmacological studies also reported that essential oil from *P. lentiscus* possesses appreciable biological properties such as antifungal, antibacterial and antimicrobial activities<sup>4</sup>. Apart from this, many experimental studies proved its anti-inflammatory, antioxidant, antiatherogenic and wound healing activities<sup>5,6</sup>. Recent reports show that orally administered mastic oil (resin of *P. lentiscus*) induces tumor-suppressing effects against experimental colon cancer<sup>7</sup>. Unani physicians have been using this drug since centuries for the treatment of many ailments like gastrointestinal disturbances, hepatobiliary disorders, gynaecological diseases, fractures, wounds, ENT problems etc<sup>8,9</sup>. Chemical analysis reveals that it contains resins, volatile oils, terpenoids and fatty acids<sup>2,3</sup>. Gingelly oil is an edible oil derived from sesame seeds. AYUSH systems recognize the health benefits of gingelly oil. The health benefits of the oil include its ability to improve hair and skin health, help in

bone growth, reduce blood pressure, maintain good heart health, manage anxiety and depression, cure dental problems, prevent cancer, improve the digestive process and lower inflammation. It is used for massaging as its chemical structure gives it a unique ability to penetrate the skin easily, nourishing and detoxifying even the deepest tissue layers. It is also highly nutritious, rich in vitamins A, B and E as well as the minerals iron, calcium, magnesium, copper, silicic acid and phosphorus<sup>10</sup>.

The present study aims to ensure the standardization of Kunthirikkam tailam with respect to its organoleptic, macroscopical, microscopical, physico-chemical and HPTLC fingerprinting aspects.

## **Materials & Methods**

### **Ingredients of Kunthirikkam tailam**

- 1). Punaikkan Kunkiliyam – 1 part
- 2). Gingelly oil – 10 part

Punaikkan Kunkiliyam was procured from raw drug store at Chennai. Gingelly oil was procured from raw drug store at Thiruvananthapuram. The identity and authenticity of the drugs was confirmed by Dept. of Pharmacognosy, SRRI, Thiruvananthapuram.

### **Method of Preparation**

The tailam was prepared by the methods mentioned in Siddha Formulary of India<sup>11</sup>. Item 1 was finely powdered, mixed with item 2 and boiled till the powder was completely dissolved. Filter and stored in airtight glass containers for further studies.

### **Organoleptic evaluation**

Organoleptic characters such as colour, odour and taste of the tailam and the ingredients were noted.

### **Macroscopical studies of Punaikkan Kunkiliyam**

The macroscopical characters of the drug Punaikkan Kunkiliyam analysed using Stereomicroscope.

### **Powder microscopy of Punaikkan Kunkiliyam**

The powdered resin of *P. lentiscus* was mounted in glycerin for 1 h and observed under 10X and 40X objectives of bright field microscope to determine the characteristics.

### **Physico-chemical studies**

Physico-chemical studies of Kunthirikkam tailam, Gingelly oil and Punaikkan Kunkiliyam were carried out as per standard protocol<sup>11,12,13</sup>.

Physico-chemical constants like specific gravity, acid value, peroxide value, saponification value, unsaponifiable matter and iodine value for Kunthirikkam tailam and gingelly oil were determined. The physico-chemical parameters determined for Punaikkan Kunkiliyam were foreign matter, loss on drying at 105°C, total ash, acid insoluble ash, solubility in alcohol, solubility in water and volatile oil. Analysis of physico-chemical constants of the ingredients were done to evaluate the quality and purity of the raw drugs.

### **HPTLC fingerprinting**

The unsaponifiable matter of Kunthirikkam tailam and gingelly oil was subjected to HPTLC analysis<sup>14</sup>. The unsaponifiable matters of both drugs were prepared by standard methods<sup>15</sup>.

The instrument employed was CAMAG HPTLC system (Muttensz, Switzerland) equipped with a sample applicator TLC auto sampler 4 with win CATS software version 1.4.4. Volume of sample applied were Track 1- 15 µl: Track 2 – 20 µl. The plate was developed using the solvent system, Toluene: Ethyl acetate: Formic acid (5: 0.8: 0.1) (mobile phase) in a twin trough chamber. The plate was developed up to 8 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  $\lambda_{\text{max}}$  254 nm and 366 nm using deuterium light source. The chromatograms were recorded. After scanning, the plate was dipped in vanillin-sulphuric acid reagent and dried at 105°C by placing on CAMAG TLC plate till the color of the spots appeared. The  $R_f$  values and fingerprint data were recorded by win CATS software.

### **Results and Discussion**

Kunthirikkam tailam is an indigenous herbal formulation containing *P. lentiscus* L. and *Sesamun indicum* (gingelly oil) which claims to have the potential in the treatment of wounds, burns, cuts etc. It is being practiced by Siddha practitioners for the treatment of wounds and burns. Kunthirikkam tailam and its ingredients - Punaikkan Kunkiliyam and Gingelly oil - are shown in Figure 1.



**Figure 1: Kunthirikkam tailam and its ingredients.**

### Organoleptic characters

Organoleptic characters such as colour, smell and taste of Punaikkan Kunkiliyam, Gingelly oil and Kunthirikkam tailam are given in Table 1.

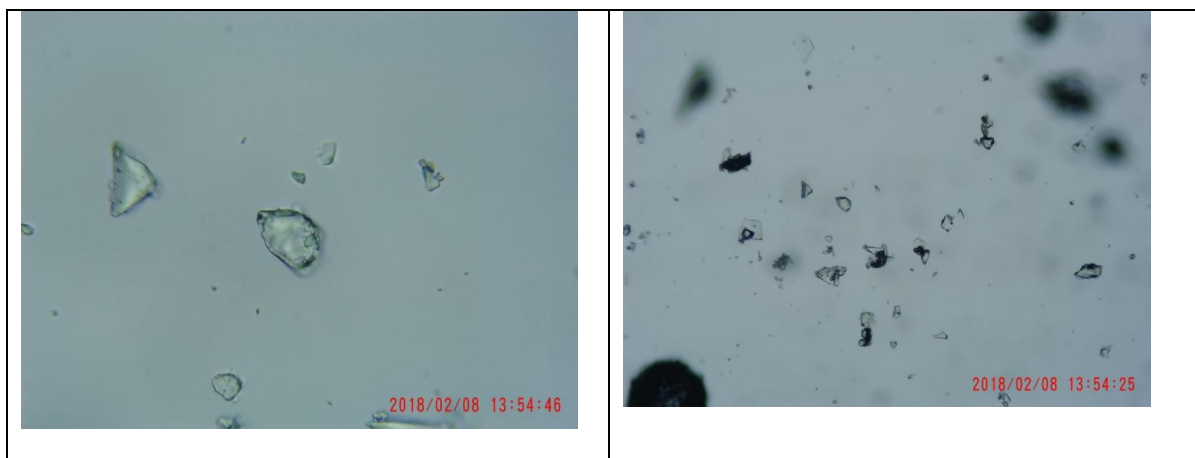
**Table-1: Organoleptic characters of Kunthirikkam tailam and its ingredients.**

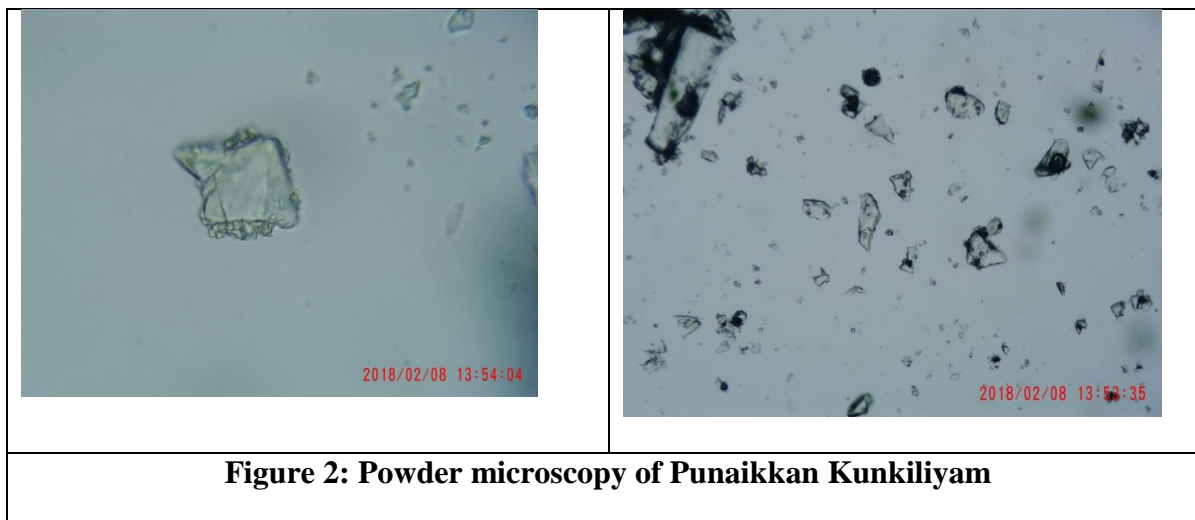
Sl.No.	Organoleptic character	Punaikkan Kunkiliyam	Gingelly oil	Kunthirikkam tailam
1.	Colour	Pale yellowish brown	Pale yellow	Dark brown
2.	Smell	Aromatic	Odourless	Pleasant
3.	Taste	Slightly bitter acid	Agreeable	Bitter

### Macroscopical characters of Punaikkan Kunkiliyam

Punaikkan Kunkiliyam is pale yellowish brown in colour, brittle, breaking into clear glossy fracture, interior transparent and crushing to a sandy powder. The resin of *P. lentiscus* L. has brittle pieces, rough having a faint resinous, whitish brown, freely flowing in the surface of water.

### Powder microscopy of Punaikkan Kunkiliyam





In the Powder microscopy of Punaikkan kunkiliyam crystalline structures of various shape and size were observed and are shown in Figure 2.

### Physico-chemical properties

The physico-chemical parameters of the resin of *P. lentiscus* Linn. are tabulated in Table 2 and Kunthirikkam tailam and gingelly oil in Table 3.

**Table 2: Physico-chemical characters of Punaikkan Kunkiliyam.**

Sl. No.	Test conducted	Result	API/SPI values
1.	Foreign matter	Nil	NMT 2 % w/w
2.	Total ash	1.35% w/w	NMT 2.6 % w/w
3.	Acid insoluble ash	0.38% w/w	NMT 0.34 % w/w
4.	Loss on drying at 105 °C	18.34 % w/w	--
5.	Alcohol soluble extractive	94.74 % w/w	NLT 94 % w/w
6.	Water soluble extractive	0.9 % w/w	NLT 0.5 % w/w

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash, which is derived from the plant tissue itself, and non-physiological ash, which is the residue of the extraneous matter such as sand and soil adhering to the plant surface. The ash value of Kunthirikkam is only 1.35% suggesting the presence of less amount of inorganics in the plant. The acid insoluble ash indicates the presence of less residue of the adhering material to the plant surface such as sand and soil. Loss on drying is a measure of the moisture content and volatile oil present in the sample. High alcohol

soluble extractive value and low water soluble extractive value indicate the presence of high amount of organic constituents. All the Pharmacognostical characters were found to be in agreement with the data available in The Ayurvedic Pharmacopoeia of India Part- I Volume V.

**Table 3: Physico-chemical characters of Gingelly oil and Kunthirikkam tailam.**

Sl. No.	Test	Gingelly oil	Kunthirikkam tailam
1	Specific gravity	0.943 g/mL	0.93 g/mL
2	Acid value	2.918	2.62
3	Peroxide value	10.16	9.26
4	Saponification value	183.727	163.73
5	Unsaponification matter	2.59%	2.19%
6	Iodine value	112.1	106.2

The acid value is defined as the number of milligram of potassium hydroxide required to neutralize the free acid present in 1 g of oil or fat. The iodine value of a substance is the weight of halogens expressed as iodine absorbed by 100 parts by weight of the substance.

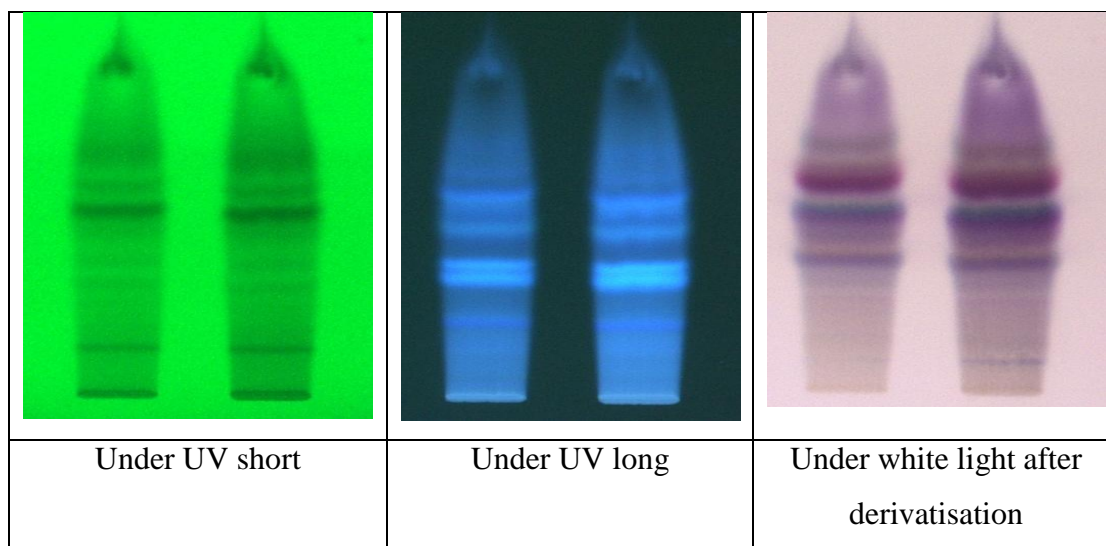
The quantity of substance used in the determination should be such that at least 70% of the iodine added, as provided in the recommended procedure, is not absorbed. The acid and iodine values depend on the extraction of acidic and unsaturated compounds into the gingelly oil from the ingredient during the process of preparation. The saponification value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralize the free acid and to saponify the ester present in 1 g of fat or oil. saponification value for Gingelly oil was found to be 183.727 whereas that for Kunthirikkam tailam was 163.73.

Unsaponification value consists of substance present in oils and fats which are not saponifiable by alkali and are determined by extraction with an organic solvent of a solution of the saponified substance under examination. The values obtained for Gingelly oil was 2.59% and that for Kunthirikkam tailam was 2.19%. The gingelly oil acts as base in the Kunthirikkam tailam for the extraction of active principles from the resin.

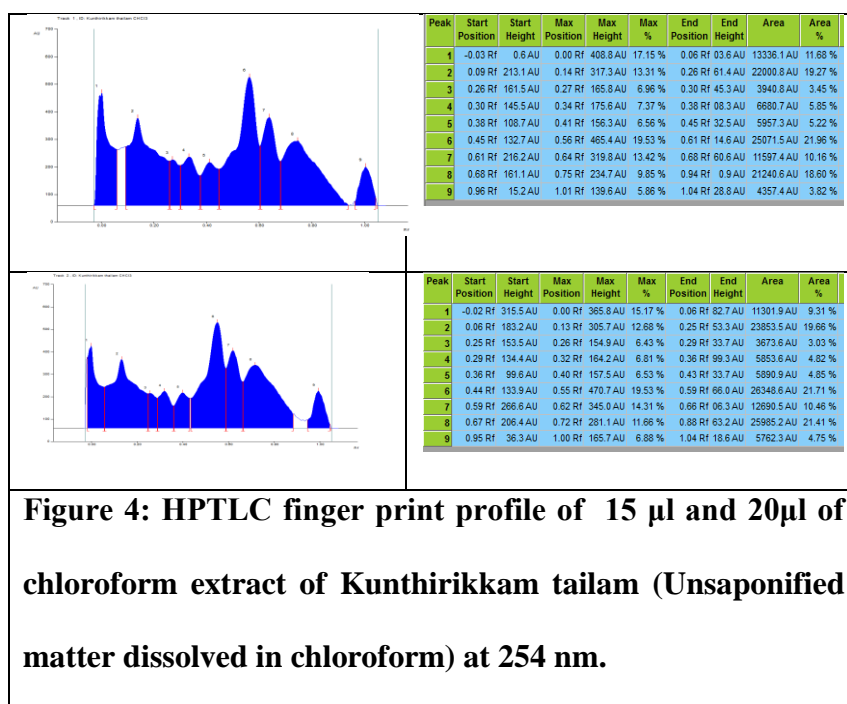
These parameters may be used as standards for Kunthirikkam tailam. The values obtained for gingelly oil are in agreement with the reported values ensuring the authenticity of the oil which is used for the preparation of the tailam.

### HPTLC fingerprinting of Kunthirikkam tailam

The results showed various bioactive compounds in the chloroform extract of Kunthirikkam tailam. The developed HPTLC plate at 254 nm, 366 nm and 575 nm after derivatisation using vanillin-sulphuric acid reagent represented various bands having different band intensities. The representative chromatograms are shown in Figure 3.



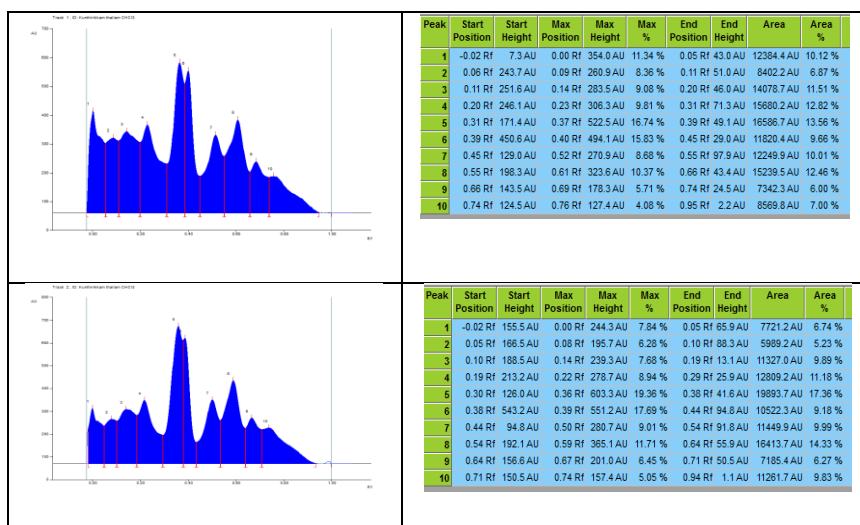
**Figure 3: HPTLC profile of chloroform extract of Kunthirikkam tailam (Unsaponified matter dissolved in chloroform) Viewed in UV short; Viewed in UV long; After derivatisation using vanillin-sulphuric acid viewed in visible light; Solvent system – Toluene: Ethyl acetate: Formic acid (5: 0.8: 0.1).**



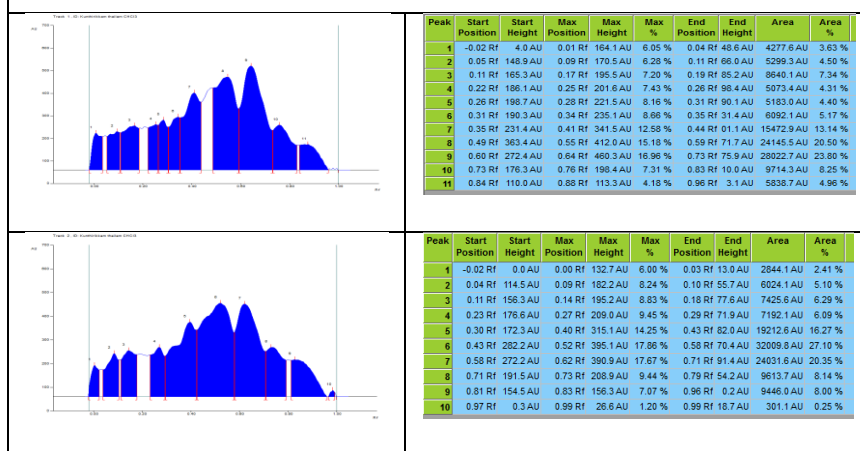
**Figure 4: HPTLC finger print profile of 15 µl and 20µl of chloroform extract of Kunthirikkam tailam (Unsaponified matter dissolved in chloroform) at 254 nm.**



The  $R_f$  values and peak area percentages of the observed bands for 15  $\mu$ l and 20 $\mu$ l of chloroform extract of Kunthirikkam tailam (Unsaponified matter dissolved in chloroform) at 254 nm, 366 nm and 575 nm after derivatisation are represented in Figures 4, 5, and 6 respectively.



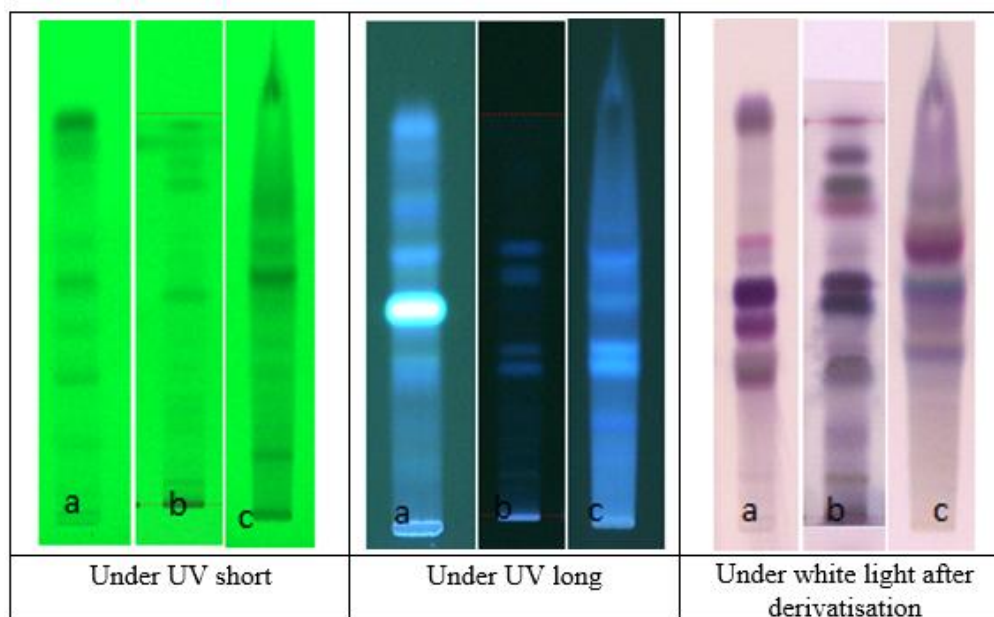
**Figure 5: HPTLC finger print profile of 15  $\mu$ l and 20 $\mu$ l of chloroform extract of Kunthirikkam tailam (Unsaponified matter dissolved in chloroform) at 366 nm.**



**Figure 6: HPTLC finger print profile of 15  $\mu$ l and 20 $\mu$ l of chloroform extract of Kunthirikkam tailam (Unsaponified matter dissolved in chloroform) at 575 nm after derivatisation.**

A comparative HPTLC study of unsaponified matter of Gingelly oil and Kunthirikkam tailam and Punaikkan Kunkiliyam was carried out. The chromatograms were viewed under UV short (254 nm), UV long (366 nm)

and white light (575 nm) after derivatisation using vanillin-sulphuric acid reagent. Solvent system used was Toluene: Ethyl acetate: Formic acid (5: 0.8: 0.1). The results obtained are given in figure 7.



**Figure 7: HPTLC profile of unsaponified matter of Gingelly oil (a), Punaikkan Kunkiliyam (b) and unsaponified matter of Kunthirikkam tailam (c)**

The  $R_f$  values and colour of bands obtained at different wavelengths for Kunthirikkam tailam and its ingredients and the area of spots obtained for the tailam are represented in **Table 4**.

**Table 4:  $R_f$  values and colour of bands obtained at different wavelengths for Kunthirikkam tailam and its ingredients and the area of spots obtained for the tailam**

Wave length (nm)	Gingelly oil		Punaikkan Kunkiliyam		Kunthirikkam tailam		
	$R_f$ values	Colour	$R_f$ values	Colour	$R_f$ values	Colour	Area of spots (AU)
254 nm	0.20	Lightgreen	0.22	Light green	0.14	Light green	22000.8
	0.32	Light green	0.32	Light green	0.27	Light green	3940.8
	0.44	Light green	0.52	Dark green	0.34	Dark green	6680.7
	0.56	Dark Green	0.81	Light green	0.41	Dark green	5957.3
	0.65	Light green	0.89	Light green	0.56	Light green	25071.5
	0.86	Dark green	0.92	Light green	0.64	Light green	11597.4

	0.93	Dark green	---	---	0.75	Light green	21240.6
366 nm	0.37	Blue	0.37	Light blue	0.09	Light blue	8402.2
	0.50	Fluorescent blue	0.41	Light blue	0.14	Light blue	14078.7
	0.63	Blue	0.51	Dark blue	0.23	Light blue	15680.2
	0.72	Light blue	0.60	Dark blue	0.37	Light blue	16586.7
	0.92	Blue	0.68	Dark blue	0.40	Light blue	11820.4
	---	---	---	---	0.52	Light blue	12249.9
	---	---	---	---	0.61	Yellow	15239.5
	---	---	---	---	0.69	Dark blue	7342.3
	---	---	---	---	0.76	Dark blue	8569.8
575 nm	0.10	Light purple	0.10	Light purple	0.09	Light purple	5299.3
	0.33	Grey	0.23	Light brown	0.17	Light purple	8640.1
	0.45	Purple	0.38	Light purple	0.25	Light purple	5073.4
	0.52	Dark purple	0.53	Brown	0.28	Dark purple	5183.0
	0.61	Grey	0.59	Dark purple	0.34	Dark purple	6092.1
	0.64	Pink	0.67	Light purple	0.41	Dark purple	15472.9
	0.93	Greyish purple	0.78	Brown	0.55	Light purple	24145.5
	---	---	0.84	Greyish purple	0.64	Light purple	28022.7
	---	---	0.90	Light purple	0.76	Light purple	9714.3
---	---	---	---	0.88	Light purple	5838.7	

From the HPTLC studies, it is observed that some of the spots obtained for the single drugs are identical with those in the prepared medicine. The spots missing in the finished product may be due to the overlapping with one another and hence cannot be identified. The additional spots observed in the tailam may be due to the formation of new entities during the process of preparation.

### Conclusions

The above cited parameters can be considered as pharmacopoeial standards and will help us to determine the genuineness of Kunthirikkam tailam 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.

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**Conflict of Interest:** No conflicts declared

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