IJP (2016), Vol. 3, Issue 1 **(Research Article)**

Received on 21 October, 2015; received in revised form, 26 November, 2015; accepted, 06 December, 2015; published 31 January, 2016

CHEMICAL STANDARDIZATION OF MUCAMPARAP PARRU

R. Shakila [*1], R Jayachandran [1], Shyamala Rajkumar [1], A. Kanagarajan[2] and P Sathiyarajeswaran[1]

Siddha Central Research Institute (Central Council for Research in Siddha) [1], Arignar Anna Hospital Campus, Arumbakkam, Chennai-600 106.Tamilnadu, India.
Siddha Clinical Research Unit [2], Palayamkottai, Tirunelveli-627 002, Tamilnadu, India.

Keywords:

Mūcāmparap Paṛṛu, Kuṇrip Paṛṛu, Karuvangam, Mercury, Inflammation, Edema.

Correspondence to Author:
R. Shakila

Siddha Central Research Institute (Central Council for Research in Siddha), Arignar Anna Hospital Campus, Arumbakkam, Chennai - 600 106.Tamilnadu, India
E-mail: shakilasiva@gmail.com

ABSTRACT: Aim of the present study is to analyze Mūcāmparap paṛṛu, a poly herbomineral Siddha formulation for physico-chemical parameters, TLC photo documentation and HPTLC finger print profile studies. The physico-chemical parameters such as ash contents, solubility in water and ethanol, pH, loss on drying and successive extraction with hexane, chloroform and ethanol were carried out. Hexane, chloroform and ethanol extracts were subjected to TLC photo documentation, HPTLC analysis. HPTLC finger print profile under UV 254 nm, hexane extract showed 13 peaks, chloroform showed 9 peaks and ethanol showed 10 peaks; under UV 366 nm, hexane showed 11 peaks, chloroform showed 15 peaks and ethanol showed 12 peaks; after derivatization with vanillin-sulphuric acid all the extracts showed 11 peaks. The results obtained in the study are specific and could be used as a reference in the quality control of the drug.

INTRODUCTION: Analytical chemists have broad scope in the field of herbal drug analysis due to recent increasing popularity of herbal drugs treatment. Siddha system of medicine is an indigenous system of India practiced in Southern states. Siddha Formulary of India is the collection of Siddha formulations gathered from the Siddha literatures and published by Government of India. Mūcāmparap paṛṛu is enlisted in the Siddha Formulary of India, the official publication for Siddha medicines. It is also known as Kuṇrip paṛṛu. It is one of the external medicines used for the treatment of inflammation, sprain, edema during pregnancy [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.3(1).35-42

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(1).35-42

It is a combination of six herbal drugs, two metallic drugs, egg and sesame oil. As there is no report on the standardization on this drug in literature, authors aim to analyze the drug for physico-chemical parameters, TLC photodocumentation and HPTLC finger print profile studies.

MATERIALS AND METHODS:

Procurement of Drug:

The drug was procured from M/s**.** SKM Siddha and Ayurveda Company (India) Limited, Erode, Tamil Nadu, India. The drug consists of ten raw materials including mercury and lead. The list of all raw materials and the composition are presented in the **Table 1**.

Chemicals and solvents:

Toluene, ethyl acetate, formic acid, hexane, chloroform, ethyl alcohol (Merck), sulphuric acid, hydrochloric acid and vanillin (SDFCL) of analar grade were used in the study. For visualization of

spots, vanillin-sulphuric acid reagent (1% vanillin in 5 ml of sulphuric acid mixed with 95 ml ethyl alcohol) was prepared and used as visualizing reagent.

Physicochemical analysis:
The loss on drying at 105^0C, total ash, water soluble ash, alkalinity of the water soluble ash, acid insoluble ash, water soluble extractives, alcohol soluble extractive and pH of 10 % aqueous solution were carried out as per the methods described in pharmacopoeial texts [2, 3].

TABLE 1: LIST OF RAW MATERIALS OF MUCAMPARAP PARRU

Sl.No	Regional Name of the Drug	Botanical/Chemical Name of the Drug	Quantity
1.	Iracam	Mercury	10 parts
2.	Karuvaṅkam	Lead	10 parts
3.	Mūcāmparam	*Aloe vera* (L.) Burm.f. dried juice	10 parts
4.	Kantupāraṅki	*Pygmaeopremna herbacea (Roxb.) Mold. root*	10 parts
5.	Kuṇṛi vittup paruppu	*Abrus precatorius* L. seed	10 parts
6.	Āli vittu	*Lepidium sativum* L. seed	10 parts
7.	Eṭṭi vittu	*Strychnous nux-vomica* L. seed	10 parts
8.	Peruṅkāyam	*Ferula foetida* Regel exudate	5 parts
9.	Kōḷimuṭṭai veṇkaru	Egg white	Sufficient quantity
10.	Nalleṇṇey	Sesame oil	Sufficient quantity

TLC Photo documentation / HPTLC finger print analysis:
Sample preparation: Four gram of the drug was successively extracted with hexane, chloroform and ethanol in a soxhlet extractor. The extracts were separately filtered, concentrated over water bath and made up to 10 ml in standard flasks.

TLC plate: Aluminium sheets precoated with 0.2 mm thick silica gel $60F_{254}$ (Merck) was used as TLC plate for the TLC photo documentation and HPTLC finger print analysis.

Solvent system: Various solvent systems with different solvent ratio were tried in order to attain improved separation of spots. Toluene: Ethyl acetate (6:1.5, *v/v*) was selected as the suitable solvent system. Chloroform: Methanol: Glacial acetic acid (10:0.5:0.1, v/v) was finalized for ethanol extract.

Instruments:
Twin trough chamber (CAMAG) of 10 x 10 size was used for the development of the plate. Automatic TLC Sampler 4 (ATS4) applicator, visualizer (CAMAG), scanner (CAMAG) equipped with WINCATS software were the instruments used for TLC photo documentation and HPTLC finger printing.

Procedure: Extracts (15 µl) were applied as 10 mm band and developed up to 8 cm in the above mentioned solvent system. The developed plate was air dried, visualized under UV 254, 366 nm for documenting the TLC chromatograms; Then scanned in both wavelengths for generating the finger print profiles. The photo documentation and finger printing was also done at 575 nm after dipping the plate in vanillin-sulphuric acid reagent, followed by heating in an oven till the appearance of color of the spots.

RESULTS AND DISCUSSION:
Physico-chemical analysis: All the results of physico-chemical parameters are presented in **Table 2**. The loss on drying was found to be 7.65 % which is indicating the safe storage of the drug from microbial contaminations. The total ash remained 21.73 % which indicates the total inorganic matter present in the drug; 1.64 % water soluble ash indicates the soluble salts present; acid insoluble ash was determined as 15.71 % which may be due to the lead added in the drug. Water and ethanol soluble extracts are nearly same (17 %) indicating the presence of polar phytoconstituents. The pH value shows the slight acidic nature of the drug. The successive extract value also indicates the total extractable phytoconstituents.

TLC Photo documentation / HPTLC finger prints: The R_f and colour of spots of TLC of hexane extract of the drug before and after derivatization are presented in **Table 3** and the corresponding photos in **Fig. 1**. Similarly the results of chloroform extract and ethanol extract are detailed in **Table 4, 5, Fig. 2** and **Fig.3**. All these results showed the presence of numerous phytoconstituents.

TABLE 2: PHYSICO-CHEMICAL RESULTS OF MUCAMPARAP PARRU

Sl.No.	Physico-chemical parameters	Mean (n=2)
1.	Loss on drying at 105^0 C (%, w/w)	7.65
2.	Total ash (%, w/w)	21.73
3.	Water soluble ash (%, w/w)	1.64
4.	Acid insoluble ash (%, w/w)	15.71
5.	Water soluble extractives (%, w/w)	16.70
6.	Ethanol soluble extractives (%, w/w)	17.70
7.	Alkalinity (ml of 0.1 N HCl/ g)	0.15
8.	pH (10% solution, w/v)	
9.	Extractive value	
	Hexane (%, w/w)	8.80
	Chloroform (%, w/w)	4.70
	Ethanol (%, w/w)	6.60

TABLE 3: R_f AND COLOUR OF SPOTS OF TLC OF HEXANE EXTRACT OF MUCAMPARAP PARRU

Sl. No	Under UV 254 nm	Under UV 366 nm		After Dipping in Vanillin-Sulphuric acid	
	R_f value	R_f value	Colour of the spot	R_f value	Colour of the spot
1	0.06*	-	-	0.06	Purple
2	-	0.10	Pink	0.10	Purple
3	0.17*	0.16	Blue	0.17	Purple
4	-	-	-	0.20	Purple
5	0.23*	0.23	Blue	0.23	Purple
6	0.57*	0.36	Blue	-	-
7	-	0.46	Blue	-	-
8	-	-	-	0.51	Purple
9	-	0.57	Blue	0.57	Violet
10	-	0.62	Pale blue	0.62	Purple
11	-	0.67	Pale blue	0.67	Purple
12	-	0.77	Blue	-	-
13	-	0.86	Blue	-	-
			*Green colour		

TABLE 4: R_f AND COLOUR OF SPOTS OF TLC OF CHLOROFORM EXTRACT OF MUCAMPARAP PARRU

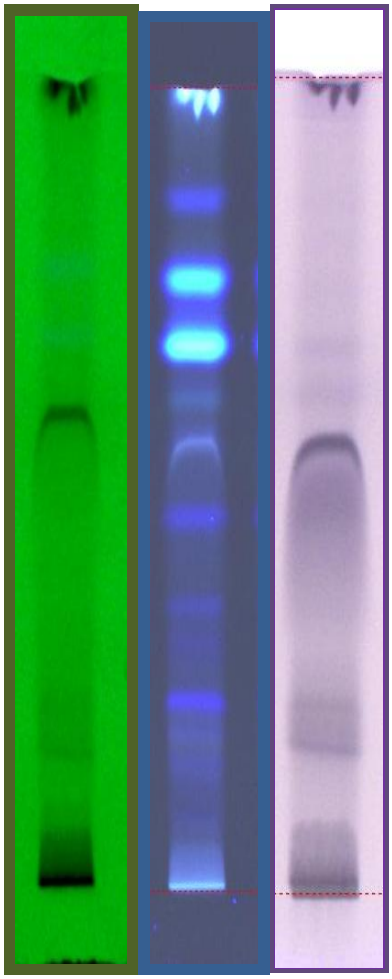
Sl. No	Under UV 254 nm	Under UV 366 nm		After Dipping in Vanillin-Sulphuric acid	
	R_f value	R_f value	Colour of the spot	R_f value	Colour of the spot
1	0.12*	0.12	Pink	-	-
2	-	0.19	Pale blue	-	-
3	0.22*	0.22	Pale blue	0.23	Purple
4	0.25*	0.25	Pale blue	0.25	Purple
5	-	0.31	Bluish white	-	-
6	0.37*	0.37	Blue	-	-
7	0.44*	0.43	Pale blue	-	-
8	-	0.52	Pale blue	0.55	Purple
9	0.57*	0.59	Bluish white	-	-
10	0.62*	0.62	Pale blue	0.62	Purple
11	-	0.66	Blue	-	Grey
12	-	0.75	Blue	-	Blue
13	-	0.87	Blue	-	Pink
			*Green colour		

TABLE 5: R_F AND COLOUR OF SPOTS OF TLC OF ETHANOL EXTRACT OF MUCAMPARAP PARRU

S. No	Under UV 254 nm	Under UV 366 nm		After Dipping in Vanillin-Sulphuric acid	
	R_f value	R_f value	Colour of the spot	R_f value	Colour of the spot
1	-	0.03	Pale blue	0.06	Purple
2	-	0.14	Blue	-	-
3	0.17*	0.17	Creamy white	-	-

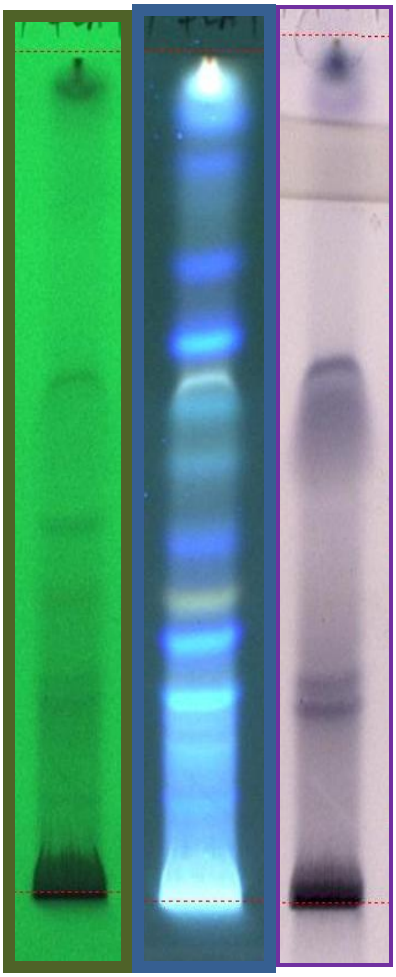
4	-	-	-	-	-
5	0.29*	-	-	0.29	Purple
6	-	0.34	Blue	0.32	Purple
7	0.38*	0.38	Blue	-	-
8	0.44*	0.42	Pale Blue	0.42	Purple
9	-	0.50	Blue	-	-
10	-	0.59	Blue	-	-
11	-	0.72	Blue	-	-
12	0.92*	0.92	Blue	-	-
13	-			-	-

*Green colour



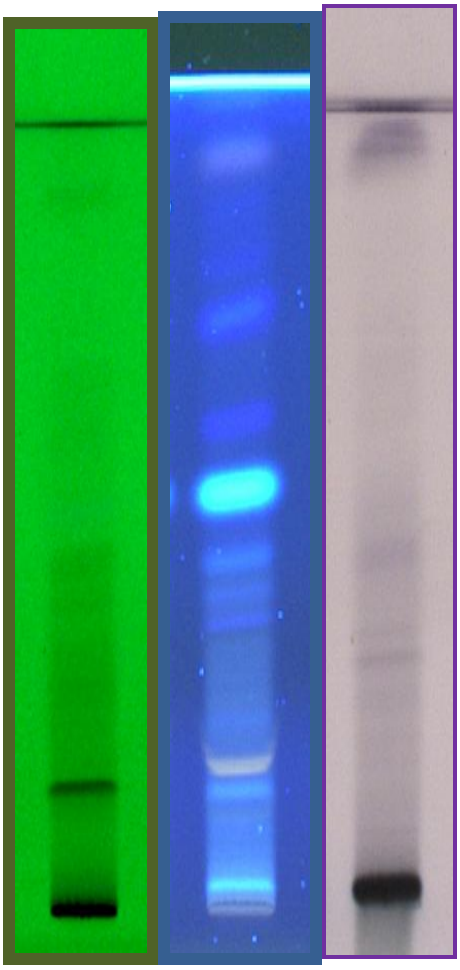
1 2 3

FIG.1: TLC PHOTO DOCUMENTATION OF HEXANE EXTRACT OF MUCAMPARAP PARRU



1 2 3

FIG.2: TLC PHOTO DOCUMENTATION OF CHLOROFORM EXTRACT OF MUCAMPARAP PARRU

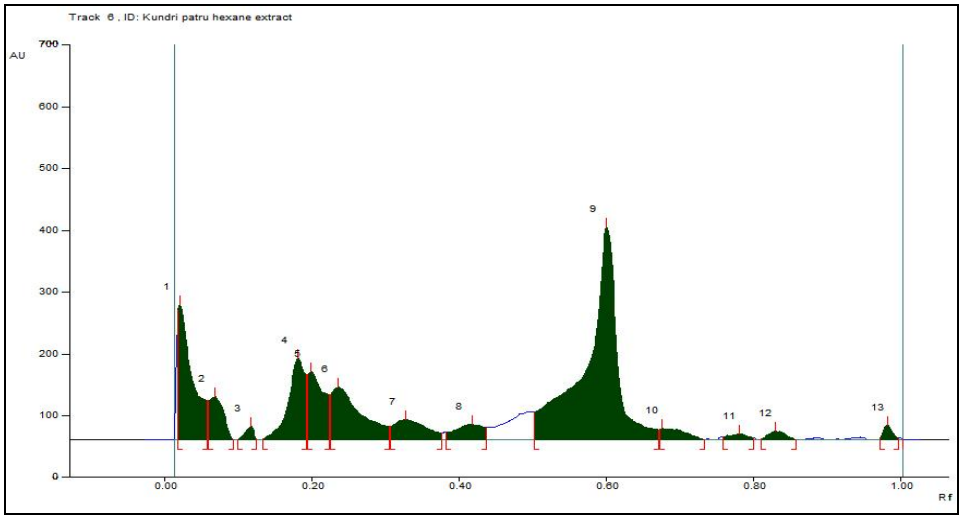


1 2 3

FIG. 3: TLC PHOTO DOCUMENTATION OF ETHANOL EXTRACT OF MUCAMPARAP PARRU

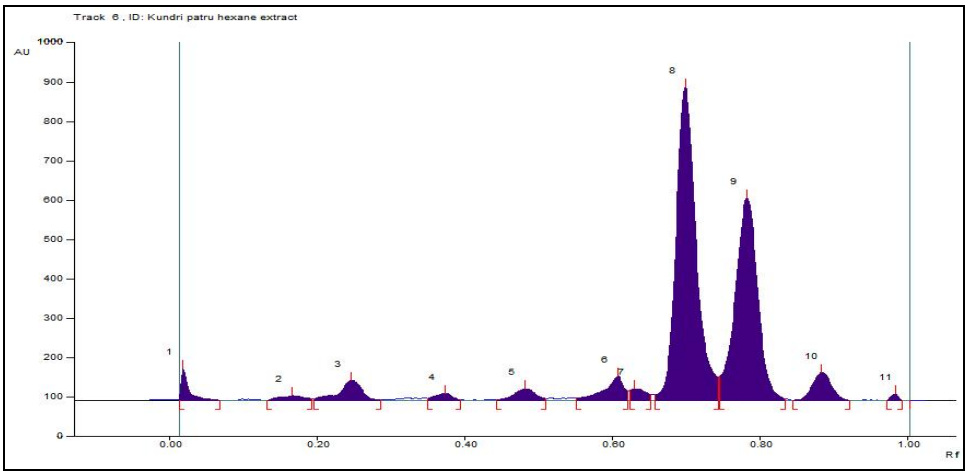
1. Under UV 254 nm; **2.** Under UV 366 nm;
3. White light after derivatization with vanillin-sulphuric acid

HPTLC finger print profile of hexane extract under UV 254 nm (**Fig. 4**) showed 13 peaks in which peak at R_f 0.60 contributes to a maximum of 43.43% and a peak at R_f 0.24 remains as the second largest peak and all other peaks are small; under UV 366 nm (**Fig. 5**) showed 11 peaks in which peak at R_f 0.70 contributes to a maximum of 47.33% and a peak at R_f 0.78 remains as the second largest peak with an area of 34.10% and all other peaks are small; under white light after derivatization at 575 nm (**Fig. 6**) also exhibited 11 peaks in which peak at R_f 0.53 contributes to a maximum of 51.47%, a peak at R_f 0.59 remains as the second largest peak with an area % of 18.41 and all other peaks are small



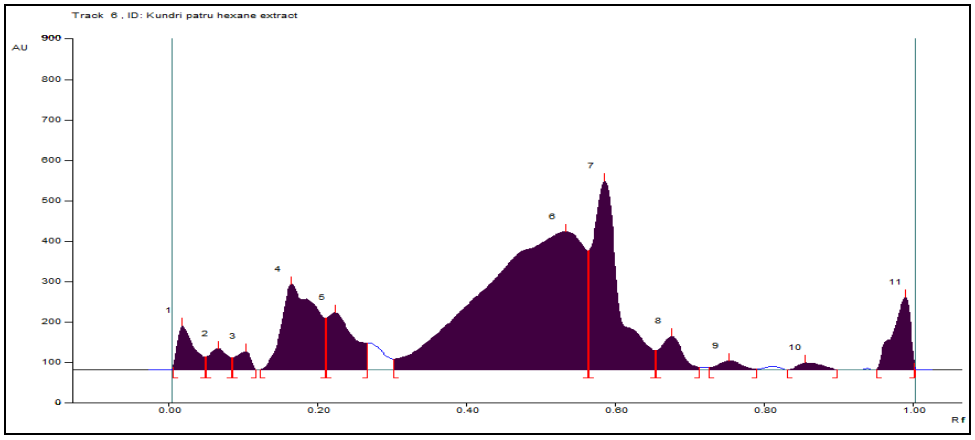
Max Position	Area %
0.02 Rf	12.79 %
0.07 Rf	4.14 %
0.12 Rf	0.73 %
0.18 Rf	9.01 %
0.20 Rf	7.58 %
0.24 Rf	10.48 %
0.33 Rf	4.39 %
0.42 Rf	2.92 %
0.60 Rf	43.42 %
0.68 Rf	1.92 %
0.78 Rf	0.76 %
0.83 Rf	1.00 %
0.98 Rf	0.86 %

FIG. 4: HPTLC FINGER PRINT PROFILE OF HEXANE EXTRACT UNDER UV 254 nm



Max Position	Area %
0.02 Rf	1.87 %
0.17 Rf	0.93 %
0.25 Rf	3.45 %
0.37 Rf	0.95 %
0.48 Rf	1.95 %
0.61 Rf	3.53 %
0.63 Rf	1.44 %
0.70 Rf	47.33 %
0.78 Rf	34.10 %
0.88 Rf	4.06 %
0.98 Rf	0.37 %

FIG.5: HPTLC FINGER PRINT PROFILE OF HEXANE EXTRACT UNDER UV 366 nm

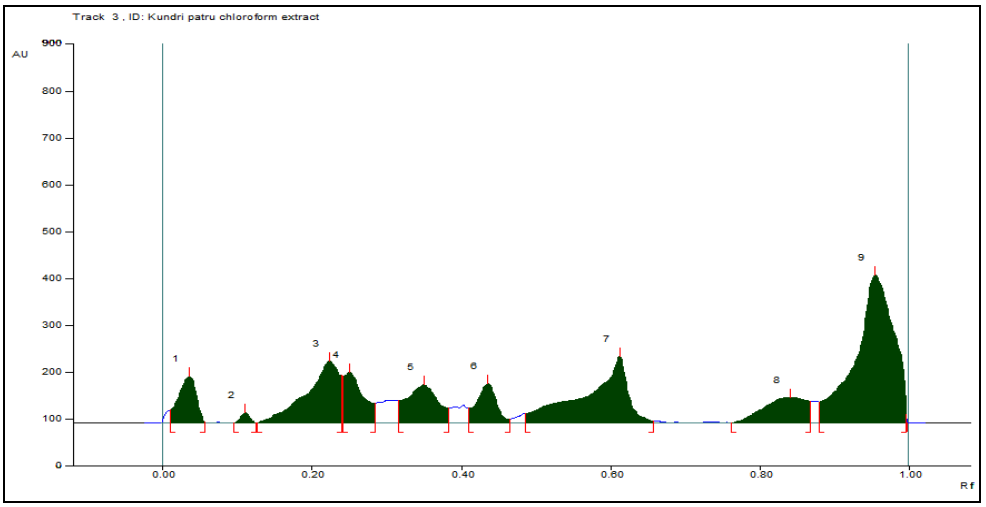


Max Position	Area %
0.02 Rf	2.64 %
0.07 Rf	1.44 %
0.10 Rf	0.99 %
0.16 Rf	10.59 %
0.22 Rf	5.74 %
0.53 Rf	51.47 %
0.59 Rf	18.41 %
0.68 Rf	2.66 %
0.75 Rf	0.76 %
0.86 Rf	0.62 %
0.99 Rf	4.69 %

FIG. 6: HPTLC FINGER PRINT PROFILE OF HEXANE EXTRACT UNDER WHITE LIGHT AFTER DERIVATIZATION

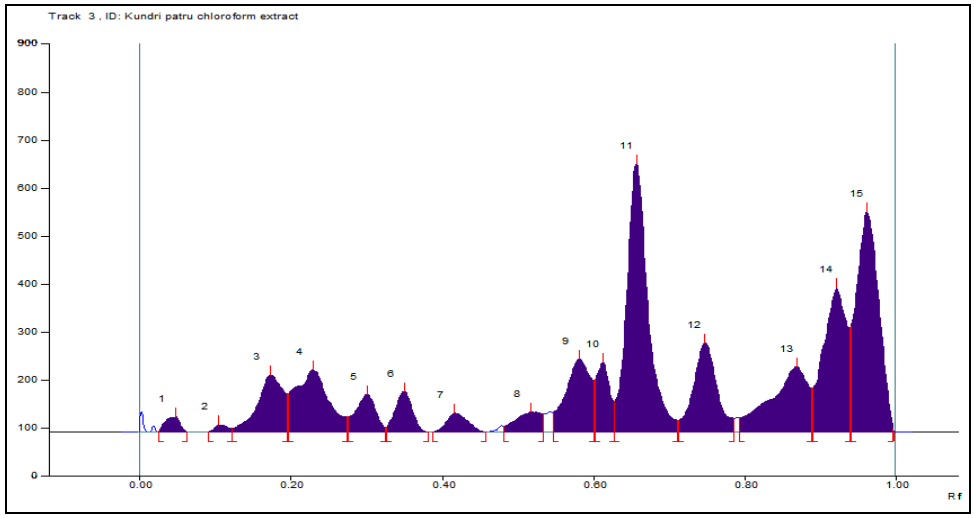
HPTLC finger print profile of chloroform extract under UV 254 nm (**Fig.7**) showed 9 peaks in which peak at R_f 0.96 was the major with 36.74% area, a peak at R_f 0.61 remains as the second largest peak, a peak at R_f 0.22 remains as the third major peak and all other peaks are small; under UV 366 nm (**Fig. 8**) showed 15 peaks in which peak at R_f 0.66 contributes to 20.52%, a peak at R_f 0.96 remains as the second largest peak with an area of 18.12%, a peak at R_f 0.92 was the third major peak with an area of 12.27% and all other peaks are small; under white light after derivatization at 575 nm (**Fig. 9**) showed 11 peaks in which peak at R_f 0.56 contributes to a maximum of 32.16%, a peak at R_f

0.25 remains as the second largest peak with an area % of 16.95, a peak at R_f 0.22 was the third largest peak with an area of 14.38% and all other peaks are small.



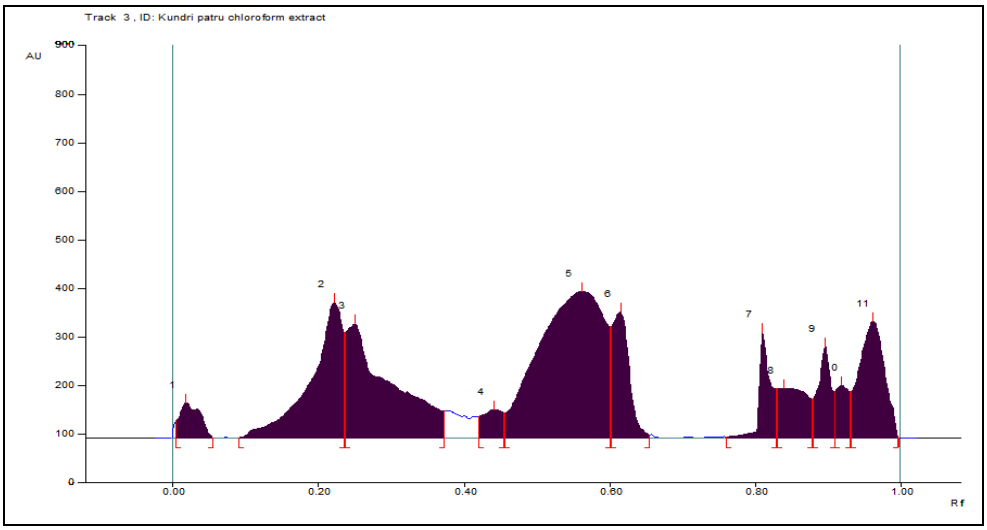
Max Position	Area %
0.04 Rf	5.42 %
0.11 Rf	0.61 %
0.22 Rf	13.06 %
0.25 Rf	6.37 %
0.35 Rf	7.70 %
0.44 Rf	5.02 %
0.61 Rf	17.84 %
0.84 Rf	7.23 %
0.96 Rf	36.74 %

FIG. 7: HPTLC FINGER PRINT PROFILE OF CHLOROFORM EXTRACT UNDER UV 254 nm



Max Position	Area %
0.05 Rf	0.84 %
0.10 Rf	0.35 %
0.17 Rf	5.34 %
0.23 Rf	7.51 %
0.30 Rf	2.74 %
0.35 Rf	2.53 %
0.42 Rf	1.45 %
0.52 Rf	1.90 %
0.58 Rf	6.62 %
0.61 Rf	3.42 %
0.66 Rf	20.52 %
0.75 Rf	7.59 %
0.87 Rf	8.82 %
0.92 Rf	12.27 %
0.96 Rf	18.12 %

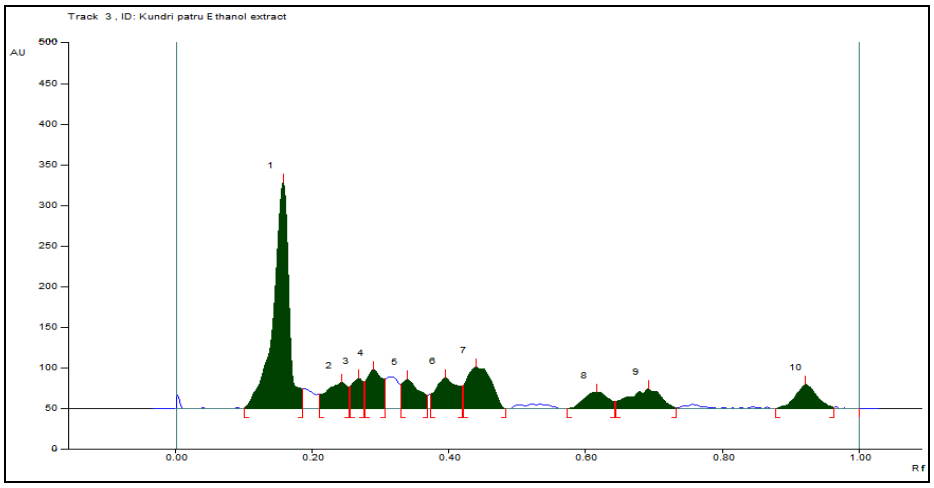
FIG. 8: HPTLC FINGER PRINT PROFILE OF CHLOROFORM EXTRACT UNDER UV 366 nm



Max Position	Area %
0.02 Rf	2.42 %
0.22 Rf	14.38 %
0.25 Rf	16.95 %
0.44 Rf	1.96 %
0.56 Rf	32.16 %
0.62 Rf	7.26 %
0.81 Rf	3.97 %
0.84 Rf	4.88 %
0.90 Rf	3.88 %
0.92 Rf	2.27 %
0.96 Rf	9.87 %

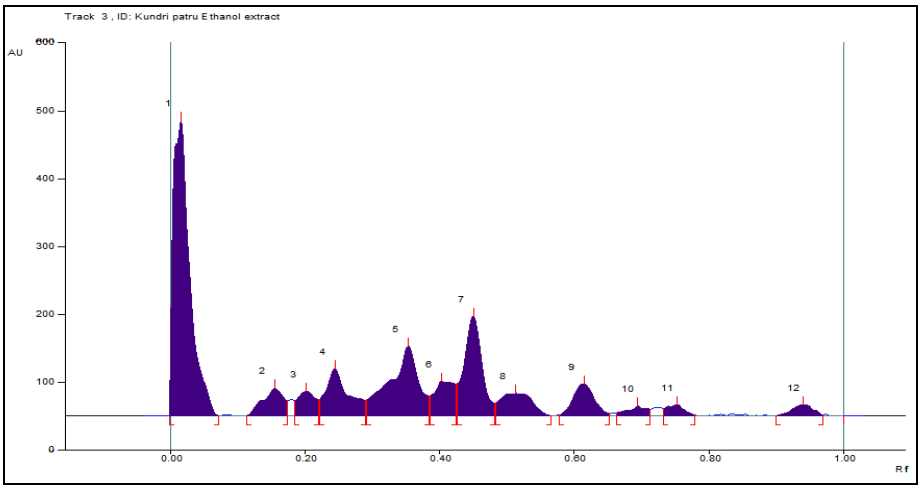
FIG.9: HPTLC FINGER PRINT PROFILE OF CHLOROFORM EXTRACT UNDER WHITE LIGHT AFTER DERIVATIZATION

HPTLC finger print profile of ethanol extract, under UV 254 nm (**Fig.10**) showed 10 peaks in which peak at R_f 0.16 was the major with 40.06 % area, a peak at R_f 0.44 remains as the second largest peak with the peak are of 11.65 % and all other peaks are minor peaks; under UV 366 nm (**Fig. 11**) showed 12 peaks in which peak at R_f 0.35 and 0.45 contributes to 14.78 % and 13.19 % respectively. A peak at R_f 0.02 with an area of 36.69 % need not be considered as major as it is very close to the application position; under white light after derivatization at 575 nm (**Fig. 12**) showed 11 peaks in which peak at R_f 0.44 contributes to 33 % and a peak at R_f 0.96 remains as the second largest peak with an area of 14.63%.



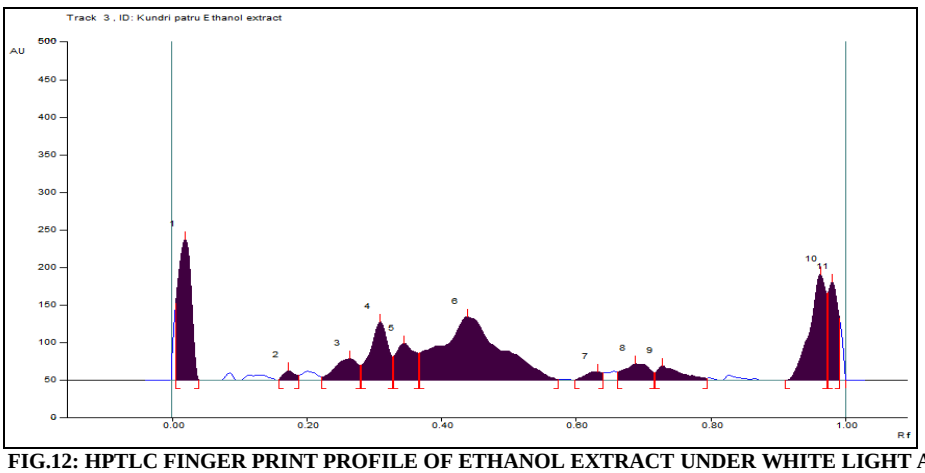
Max Position	Area %
0.16 Rf	40.06 %
0.24 Rf	6.16 %
0.27 Rf	4.04 %
0.29 Rf	7.02 %
0.34 Rf	5.98 %
0.40 Rf	7.54 %
0.44 Rf	11.65 %
0.62 Rf	4.58 %
0.69 Rf	7.10 %
0.92 Rf	5.87 %

FIG. 10: HPTLC FINGER PRINT PROFILE OF ETHANOL EXTRACT UNDER UV 254 nm



Max Position	Area %
0.02 Rf	36.69 %
0.16 Rf	4.27 %
0.20 Rf	3.27 %
0.25 Rf	7.75 %
0.35 Rf	14.78 %
0.40 Rf	5.03 %
0.45 Rf	13.19 %
0.51 Rf	5.41 %
0.62 Rf	5.07 %
0.70 Rf	1.38 %
0.75 Rf	1.43 %
0.94 Rf	1.72 %

FIG.11: HPTLC FINGER PRINT PROFILE OF ETHANOL EXTRACT UNDER UV 366 nm



Max Position	Area %
0.02 Rf	16.15 %
0.17 Rf	0.99 %
0.26 Rf	4.28 %
0.31 Rf	8.85 %
0.35 Rf	6.14 %
0.44 Rf	33.00 %
0.63 Rf	1.23 %
0.69 Rf	3.63 %
0.73 Rf	2.94 %
0.96 Rf	14.63 %
0.98 Rf	8.16 %

FIG.12: HPTLC FINGER PRINT PROFILE OF ETHANOL EXTRACT UNDER WHITE LIGHT AFTER DERIVATIZATION

CONCLUSION: From the results of the study, it is evident that all the physico-chemical parameters, TLC photo documentations of hexane, chloroform and ethanol extracts under different conditions and the HPTLC finger print profiles of these extracts under UV 254 nm, 366 nm and white light at 575 nm can be considered as a reference data for the quality assessment of the drug in future. Though the drug is an external medicine, needs to be standardized for confirming its efficacy.

ACKNOWLEDGEMENT: Authors are thankful to the Director General, CCRS for facilities and support.

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How to cite this article:
Shakila R, Jayachandran R, Rajkumar S, Kanagarajan A and Sathiyarajeswaran P: Chemical Standardization of Mucamparap Parru. *Int J Pharmacognosy* 2016; 3(1): 35-42:.doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(1).35-42.