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THE PHYSICO-CHEMICAL, CHROMATOGRAPHIC AND SPECTROSCOPIC EVALUATION OF KUPPAIMENI CHOORANAM (*Acalypha indica* Linn)

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ABSTRACT

The Siddha System of medicine is one of the most ancient traditional systems of India. Based on WHO guidelines, herbal products standardization is most important.The **Kuppaimeni Chooranam**(*Acalypha indica* Linn) coded as KC, is a classical siddha mono herbal formulation chosen from 'Gunapadam – Mooligai Vaguppu' textbook, It is indicated to **Iraippu irumal** which can be correlated with modern medicine as **Bronchial Asthma.** The study was performed to evaluate the Physico chemical, chromatographic and spectroscopy of Kuppaimeni Chooranam(KC). The raw drug was collected and authenticated by department of Medicinal botany. The ingredient was purified as mentioned in siddha classical literatures.The leaves were dried well in shade and made into fine powder. The physicochemical parameters include LOD at 105^{0}C,Total ash,Acid insoluble ash,Water soluble ash,Sulphated ash,pH,Alcohol soluble extractives and Water soluble extractives.The alcohol extracts of KC was subjected to HPTLC and Ultra violet - visible spectroscopic analysis.The physico-chemical parameters observed were LOD at 105^{0}C to be 8.6%, total ash 20.99%,Acid insoluble ash 7.15%,Water soluble ash 15.58%, Sulphated ash 24.67%, pH 5.8%,Alcohol soluble extractives 8.73% and Water soluble extractives 15.15%. The HPTLC fingerprinting pattern of alcohol extract of Kuppaimeni Chooranam have shown peak of Rf value 0.76 at 254nm, Rf value 0.76 at 366nm and Rf value 0.86 at 575nm after derivatisation. The qualitative UV-Vis spectrum of the extract was recorded from wave length 200-1100nm.

KEY WORDS:Kuppaimeni chooranam,*Acalypha indica* Linn, Iraippu irumal, Bronchial asthma, Physicochemical parameters, HPTLC,UV visible spectroscopy

INTRODUCTION

The Siddha system of medicine is mainly practiced mainly in southern parts of India. **Kuppaimeni Chooranam** (*Acalypha indic*a Linn) is a mono herbal formulation.[7,8]Herbs are used as a natural remedy for the management of Bronchial asthma.*Acalypha indica* Linn(Family:Euphorbiaceae) is a weed widely distributed throughout the plains of India.It has been reported to be useful in treating Asthma and respiratory disorders.[1,2,3,6,9] Seven cyanopyridone derivatives (Acalyphin, Epiacalyphin, Noracalyphin, Epinoracalyphin, Acalyphin amide,Epiacalyphin amide cycloside,ar-Acalyphidone and one coreesponding seco compound (seco-Acalyphin) have been isolated from methanolic extract of *Acalypha indica* Linn leaves.[4]The physicochemical analysis is useful to establish the authenticity of crude drug.The High performance thin layer chromatographic technique(HPTLC) is sensitive and reliable and this method has been developed for qualitative determination of pharmacologically important active constituents.[5]

MATERIALS AND METHODS

The plant *Acalypha indica* Linn were collected from in and around Palayamkottai and authenticated by the Department of Medicinal botany, Government Siddha Medical College,Palayamkottai. The Leaves of the plant were dried in shade, powdered and stored in air tight containers.

PHYSICO-CHEMICAL ANALYSIS

The physico chemical parameters like determination of LOD at 105^{0}C,Total ash,Acid insoluble ash,Water soluble ash,Sulphated ash,pH,Volatile oil,Alcohol soluble extractives and Water soluble extractives were carried out by standard methods.[10]

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) STUDIES

Developing solvent system:

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the specified solvent system.

Sample application :

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram :

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation :

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry :

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were

recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation:
The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

ULTRA VIOLET-VISIBLE (UV-VIS) SPECTROSCOPY

The alcohol extract of the drug was subjected to Ultra Violet-Visible spectroscopic analysis. The extract was scanned at wave length ranging from 200 to 1100 nm using UV/VIS spectrophotometer (Model: UV3120) and the characteristic peaks were detected and recorded.

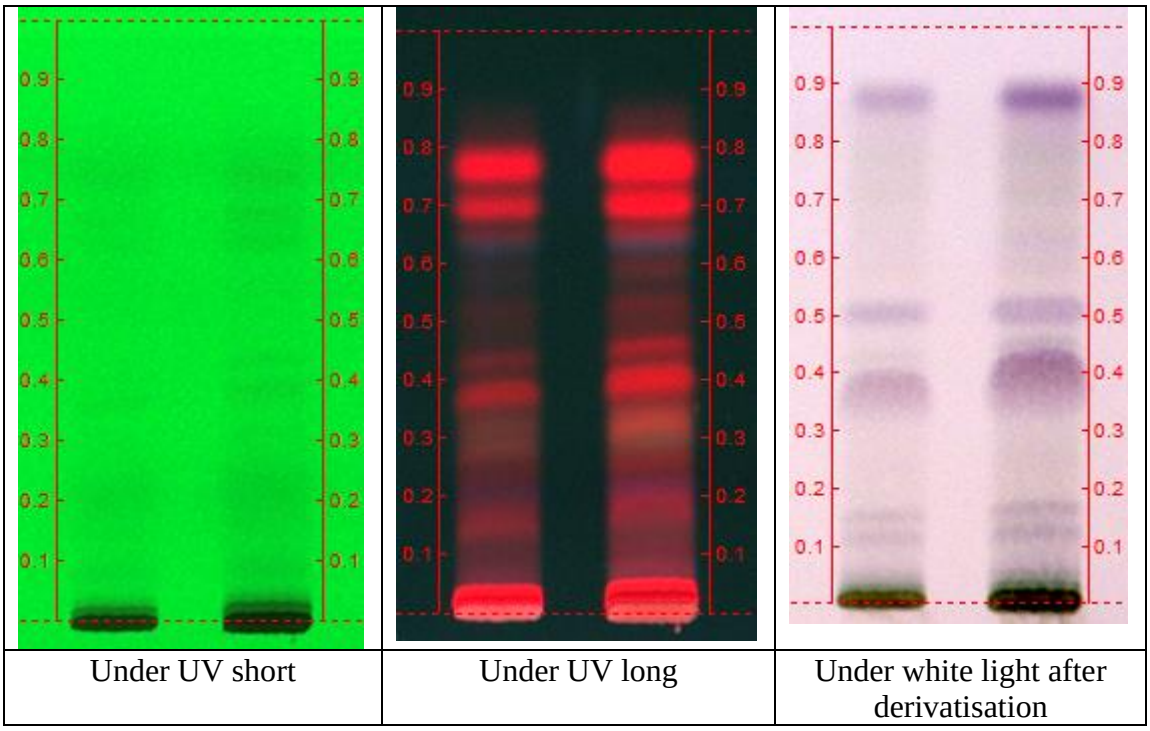
RESULTS AND DISCUSSION
The physico-chemical data obtained for the Kuppaimeni Chooranam are given in Table 1.

Table 1:**Physico- chemical parameters of Kuppaimeni Chooranam**

Sl. No.	Tests	Result in %
1	LOD at 105^0C	8.6
2	Total Ash	20.99
3	Acid insoluble ash	7.15
4	Water soluble ash	15.58
5	Sulphated ash	24.67
6	pH (4 % water extract)	5.8
7	Volatile oil	Nil
8	Alcohol soluble extractives	8.73
9	Water soluble extractives	15.15

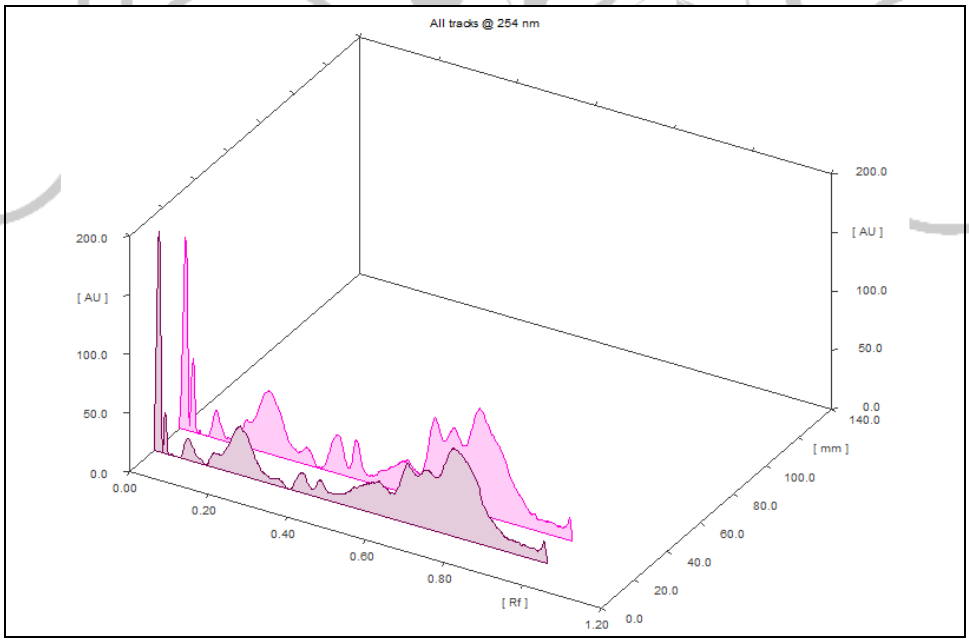
The physico-chemical parameters observed in Table 1 were LOD at 105^0C to be 8.6%, total ash 20.99%,Acid insoluble ash 7.15%,Water soluble ash 15.58%, Sulphated ash 24.67%, pH 5.8%,Alcohol soluble extractives 8.73% and Water soluble extractives 15.15%.

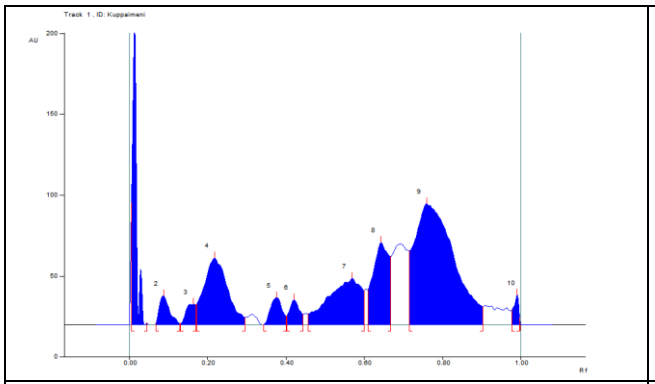
HPTLC study can be considered as an important tool in routine drug analysis . In the present study HPTLC finger printing is used as a parameter for standardisation of the samples.



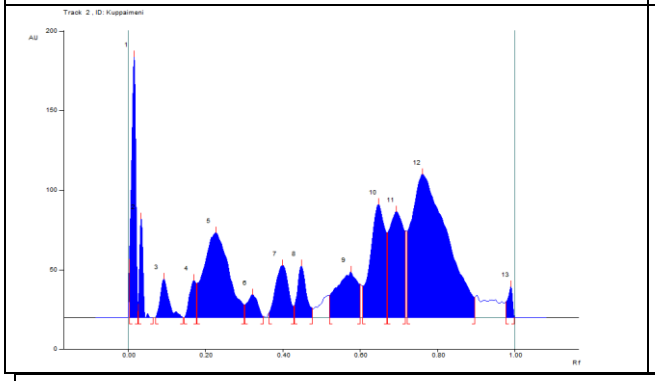
Under UV short	Under UV long	Under white light after derivatisation

Fig. 1 :HPTLC profile of Alcohol extract of Kuppaimeni chooranam viewed in UV short; viewed in UV long; viewed in visible light after derivatisation using vanillin-sulphuric acid; Solvent system: Toluene: Ethyl acetate - 5:2; Volume applied; Track 1- 5 µl: Track 2 – 10 µl.





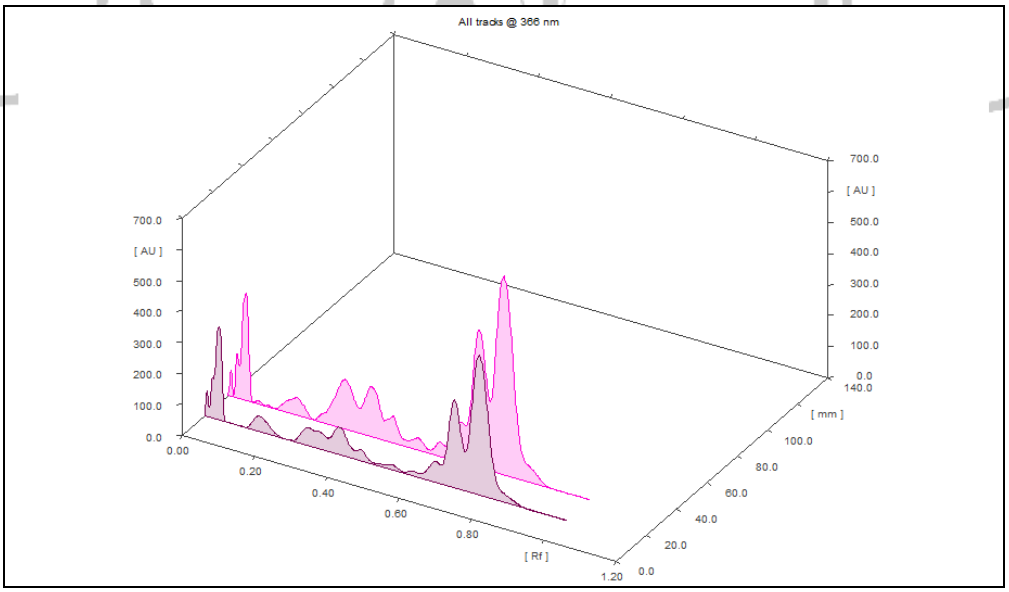
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	75.2 AU	0.01 Rf	187.2 AU	40.38 %	0.05 Rf	1.2 AU	1361.0 AU	10.73 %
2	0.07 Rf	0.6 AU	0.09 Rf	18.0 AU	3.88 %	0.13 Rf	0.7 AU	326.5 AU	2.57 %
3	0.13 Rf	0.7 AU	0.16 Rf	12.7 AU	2.73 %	0.17 Rf	12.3 AU	214.9 AU	1.69 %
4	0.17 Rf	12.6 AU	0.22 Rf	41.3 AU	8.91 %	0.29 Rf	4.4 AU	1729.3 AU	13.63 %
5	0.34 Rf	0.1 AU	0.38 Rf	16.9 AU	3.64 %	0.40 Rf	5.4 AU	348.4 AU	2.75 %
6	0.40 Rf	5.6 AU	0.42 Rf	15.4 AU	3.32 %	0.44 Rf	6.3 AU	262.6 AU	2.07 %
7	0.46 Rf	6.6 AU	0.57 Rf	28.5 AU	6.14 %	0.60 Rf	21.1 AU	1638.6 AU	12.92 %
8	0.61 Rf	21.5 AU	0.64 Rf	50.8 AU	10.95 %	0.67 Rf	42.1 AU	1441.1 AU	11.36 %
9	0.72 Rf	45.6 AU	0.76 Rf	74.6 AU	16.10 %	0.90 Rf	11.0 AU	5203.3 AU	41.01 %
10	0.98 Rf	9.2 AU	0.99 Rf	18.3 AU	3.95 %	1.00 Rf	4.2 AU	161.0 AU	1.27 %

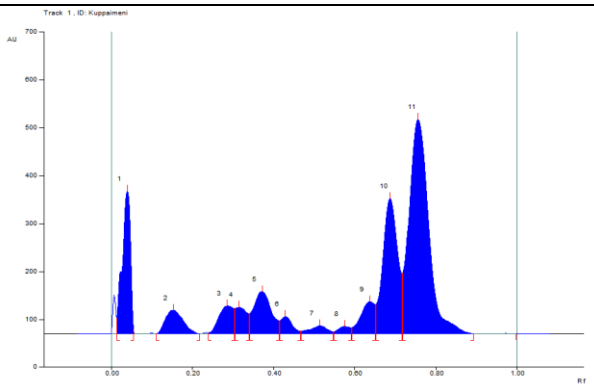


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	36.5 AU	0.01 Rf	163.9 AU	24.02 %	0.03 Rf	4.1 AU	1257.0 AU	7.48 %
2	0.03 Rf	8.2 AU	0.03 Rf	62.0 AU	9.09 %	0.06 Rf	0.0 AU	368.8 AU	2.20 %
3	0.07 Rf	0.2 AU	0.09 Rf	24.2 AU	3.55 %	0.14 Rf	0.1 AU	372.2 AU	2.22 %
4	0.14 Rf	0.1 AU	0.17 Rf	23.5 AU	3.44 %	0.18 Rf	21.5 AU	276.8 AU	1.65 %
5	0.18 Rf	21.6 AU	0.23 Rf	53.2 AU	7.80 %	0.30 Rf	7.9 AU	2378.1 AU	14.16 %
6	0.30 Rf	8.0 AU	0.32 Rf	14.5 AU	2.12 %	0.35 Rf	0.7 AU	273.9 AU	1.63 %
7	0.37 Rf	2.9 AU	0.40 Rf	32.7 AU	4.79 %	0.43 Rf	7.2 AU	762.2 AU	4.54 %
8	0.43 Rf	7.4 AU	0.45 Rf	32.5 AU	4.77 %	0.48 Rf	6.0 AU	494.9 AU	2.95 %
9	0.52 Rf	13.7 AU	0.58 Rf	28.6 AU	4.19 %	0.60 Rf	20.9 AU	1061.8 AU	6.32 %
10	0.61 Rf	19.7 AU	0.65 Rf	71.3 AU	10.45 %	0.67 Rf	53.1 AU	1895.4 AU	11.28 %
11	0.67 Rf	53.2 AU	0.69 Rf	66.5 AU	9.75 %	0.72 Rf	54.3 AU	1746.2 AU	10.40 %
12	0.72 Rf	54.2 AU	0.76 Rf	90.0 AU	13.18 %	0.90 Rf	12.8 AU	5743.5 AU	34.19 %
13	0.98 Rf	10.1 AU	0.99 Rf	19.4 AU	2.84 %	1.00 Rf	-0.0 AU	166.5 AU	0.99 %

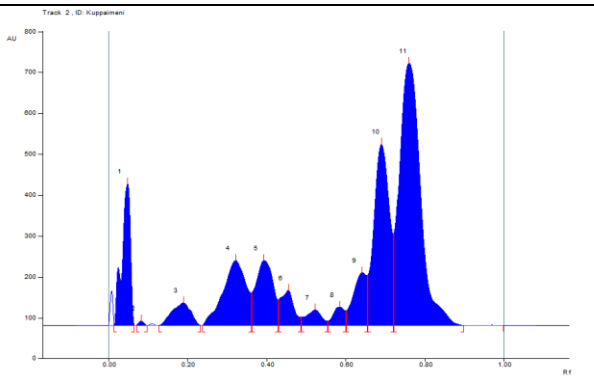
At 254 nm

Fig .2.a. HPTLC finger print profile of 5 µl and 10 µl of Alcohol extract of Kuppaimeni chooranam at 254 nm.





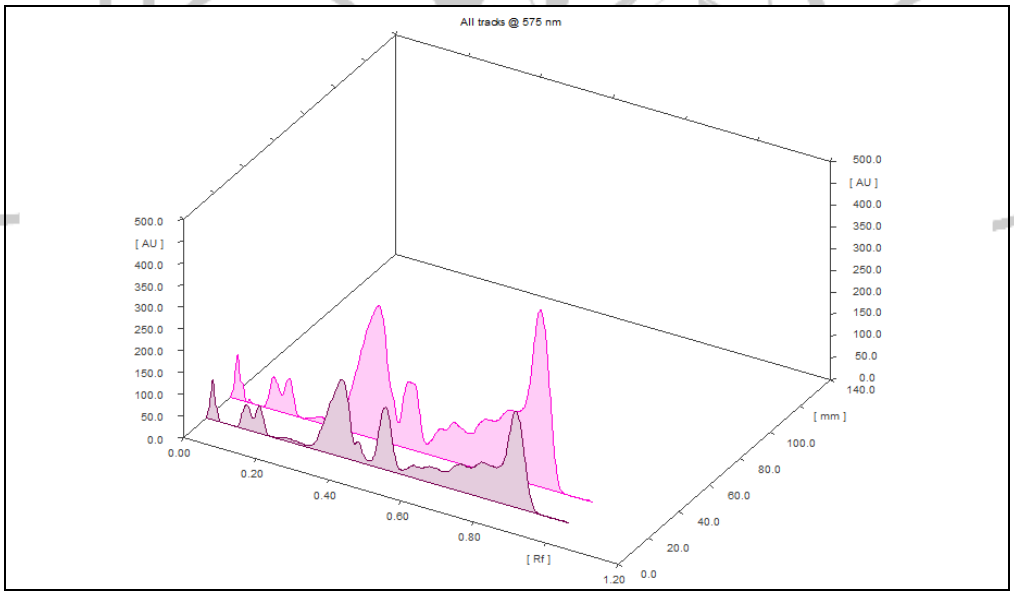
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	32.2 AU	0.04 Rf	297.9 AU	21.06 %	0.05 Rf	8.3 AU	4273.1 AU	11.78 %
2	0.11 Rf	0.1 AU	0.15 Rf	49.2 AU	3.48 %	0.22 Rf	0.0 AU	1476.9 AU	4.07 %
3	0.24 Rf	2.1 AU	0.29 Rf	58.1 AU	4.11 %	0.30 Rf	53.1 AU	1404.3 AU	3.87 %
4	0.31 Rf	53.2 AU	0.31 Rf	55.1 AU	3.90 %	0.34 Rf	42.0 AU	1111.2 AU	3.06 %
5	0.34 Rf	42.5 AU	0.37 Rf	88.2 AU	6.23 %	0.41 Rf	27.0 AU	2681.3 AU	7.39 %
6	0.42 Rf	27.3 AU	0.43 Rf	36.1 AU	2.56 %	0.47 Rf	5.0 AU	678.7 AU	1.87 %
7	0.47 Rf	5.1 AU	0.51 Rf	16.6 AU	1.17 %	0.55 Rf	4.4 AU	499.8 AU	1.38 %
8	0.55 Rf	4.6 AU	0.58 Rf	15.5 AU	1.10 %	0.59 Rf	12.5 AU	299.9 AU	0.83 %
9	0.59 Rf	12.8 AU	0.64 Rf	67.3 AU	4.76 %	0.65 Rf	60.7 AU	1616.0 AU	4.45 %
10	0.65 Rf	61.1 AU	0.69 Rf	282.7 AU	19.99 %	0.72 Rf	25.6 AU	7246.4 AU	19.97 %
11	0.72 Rf	126.5 AU	0.76 Rf	447.9 AU	31.66 %	0.89 Rf	0.2 AU	14997.8 AU	41.33 %

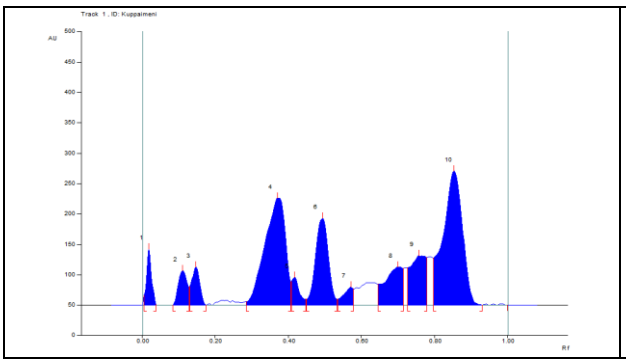


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	10.9 AU	0.05 Rf	346.9 AU	16.42 %	0.06 Rf	1.8 AU	5251.4 AU	8.56 %
2	0.07 Rf	1.2 AU	0.08 Rf	10.5 AU	0.50 %	0.10 Rf	0.3 AU	98.1 AU	0.16 %
3	0.13 Rf	0.0 AU	0.19 Rf	55.0 AU	2.60 %	0.23 Rf	0.3 AU	1853.7 AU	3.02 %
4	0.24 Rf	0.5 AU	0.32 Rf	158.7 AU	7.51 %	0.36 Rf	79.9 AU	6397.3 AU	10.43 %
5	0.36 Rf	80.6 AU	0.39 Rf	159.0 AU	7.53 %	0.43 Rf	62.2 AU	4865.3 AU	7.93 %
6	0.43 Rf	63.1 AU	0.46 Rf	85.8 AU	4.06 %	0.49 Rf	20.1 AU	1943.0 AU	3.17 %
7	0.49 Rf	20.1 AU	0.52 Rf	38.2 AU	1.81 %	0.56 Rf	10.5 AU	1042.1 AU	1.70 %
8	0.56 Rf	10.7 AU	0.58 Rf	44.9 AU	2.13 %	0.60 Rf	36.1 AU	872.8 AU	1.42 %
9	0.60 Rf	36.4 AU	0.64 Rf	129.2 AU	6.12 %	0.65 Rf	22.2 AU	3069.2 AU	5.00 %
10	0.66 Rf	123.2 AU	0.69 Rf	443.0 AU	20.97 %	0.72 Rf	22.5 AU	12192.1 AU	19.87 %
11	0.72 Rf	224.4 AU	0.76 Rf	641.5 AU	30.36 %	0.90 Rf	0.5 AU	23765.9 AU	38.74 %

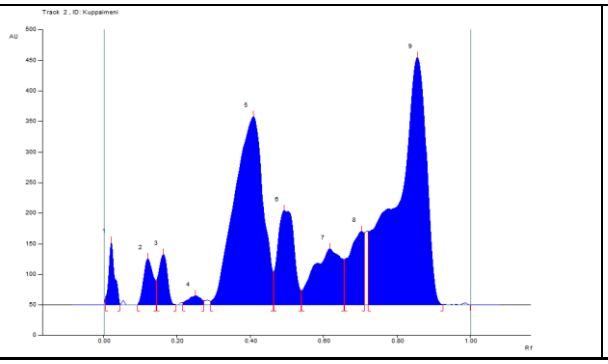
At 366 nm

Fig .2.b.HPTLC finger print profile of 5 µl and 10 µl of Alcohol extract of Kuppaimeni chooranam at 366 nm.





Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	16.4 AU	0.02 Rf	92.2 AU	9.49 %	0.04 Rf	0.9 AU	865.9 AU	3.18 %
2	0.09 Rf	0.4 AU	0.11 Rf	56.6 AU	5.82 %	0.13 Rf	30.0 AU	962.1 AU	3.53 %
3	0.13 Rf	30.7 AU	0.15 Rf	63.1 AU	6.50 %	0.17 Rf	0.3 AU	928.5 AU	3.41 %
4	0.29 Rf	5.7 AU	0.37 Rf	176.4 AU	18.16 %	0.41 Rf	38.8 AU	7000.5 AU	25.70 %
5	0.41 Rf	39.0 AU	0.42 Rf	45.7 AU	4.71 %	0.45 Rf	9.5 AU	663.0 AU	2.43 %
6	0.45 Rf	9.7 AU	0.50 Rf	142.7 AU	14.70 %	0.54 Rf	10.2 AU	3582.9 AU	13.15 %
7	0.54 Rf	10.2 AU	0.57 Rf	29.5 AU	3.04 %	0.58 Rf	26.8 AU	551.3 AU	2.02 %
8	0.65 Rf	34.3 AU	0.70 Rf	63.0 AU	6.49 %	0.72 Rf	60.8 AU	2141.8 AU	7.86 %
9	0.73 Rf	61.4 AU	0.76 Rf	81.2 AU	8.36 %	0.78 Rf	78.6 AU	2384.3 AU	8.75 %
10	0.80 Rf	78.0 AU	0.86 Rf	220.8 AU	22.74 %	0.93 Rf	0.1 AU	8163.1 AU	29.96 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	11.5 AU	0.02 Rf	102.7 AU	7.57 %	0.05 Rf	1.5 AU	1134.1 AU	2.01 %
2	0.09 Rf	0.3 AU	0.12 Rf	75.6 AU	5.58 %	0.14 Rf	39.7 AU	1363.6 AU	2.42 %
3	0.14 Rf	40.8 AU	0.16 Rf	82.3 AU	6.07 %	0.20 Rf	0.4 AU	1377.3 AU	2.44 %
4	0.22 Rf	3.6 AU	0.25 Rf	15.4 AU	1.14 %	0.28 Rf	6.7 AU	370.7 AU	0.66 %
5	0.29 Rf	5.8 AU	0.41 Rf	308.3 AU	22.74 %	0.46 Rf	53.6 AU	16598.2 AU	29.46 %
6	0.47 Rf	53.7 AU	0.49 Rf	154.4 AU	11.39 %	0.54 Rf	23.6 AU	4849.1 AU	8.61 %
7	0.54 Rf	23.6 AU	0.62 Rf	91.8 AU	6.77 %	0.66 Rf	74.8 AU	4708.3 AU	8.36 %
8	0.66 Rf	74.8 AU	0.70 Rf	120.4 AU	8.88 %	0.71 Rf	18.1 AU	3399.4 AU	6.03 %
9	0.72 Rf	119.8 AU	0.86 Rf	404.8 AU	29.86 %	0.93 Rf	0.5 AU	22531.7 AU	40.00 %

At 575 nm after derivatisation

Fig .2.c.HPTLC finger print profile of 5 µl and 10 µl of Alcohol extract of Kuppaimeni chooranam at 575 nm after derivatisation.

The HPTLC fingerprinting pattern of alcohol extract of Kuppaimeni Chooranam was developed at 254 nm,366nm and at 575nm after derivatisation with vanillin-sulphuric acid. The solvent system- Toluene:Ethyl acetate- 5:2 effectively resolved the chemical constituents in the alcohol extract of KC. HPTLC photo documentation profile of alcohol extract of leaf of the plant at 254nm,366nm and after derivatisation is given in Fig.1 and the finger printing profile and the Rf value and percentage area of the peaks are shown in Fig.2.a, Fig.2.b. and Fig.2.c. From Fig 2.a. it can be observed that 12 bands appeared at 254 nm with Rf 0.03,0.09, 0.17, 0.23,0.32,0.40, 0.45,0.58,0.65,0.69,0.76 and 0.99 out of which Rf value at 0.76 has maximum area of 34.19% indicating the presence of highest concentration of the phytoconstituent. From Fig 2.b. it can be observed that 10 bands appeared at 366 nm with Rf 0.08,0.19,0.32,0.39,0.46,0.52,0.58,0.64,0.69 and 0.76 out of which Rf value at 0.76 has maximum area of 38.74% . From Fig 2.c. it can be observed that 8 bands appeared at 575nm after derivatisation with Rf 0.12,0.16,0.25,0.41,0.49,0.62,0.70 and 0.86 out of which Rf value at 0.86 has maximum area of 40.00%. These results implies that the chemical constituents were present in significant quantity in Kuppaimeni chooranam.

UV- Visible Spectroscopy

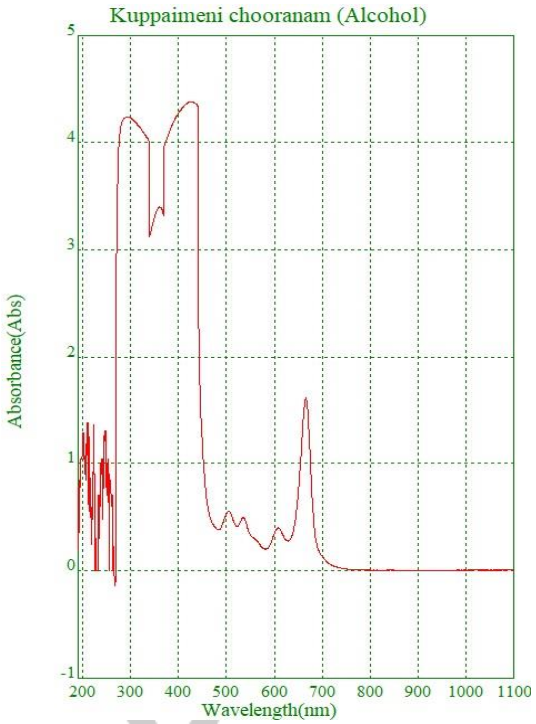


Fig.3:Ultra Violet-Visible Spectrum of alcohol extract of Kuppaimeni Chooranam (KC)

The UV-Vis spectrum of alcohol extract of Kuppaimeni Chooranam (KC) are shown in Fig.3. The qualitative UV-Vis spectrum of the extract was recorded from wavelength 200-1100nm. The spectrum obtained can be considered unique for alcohol extract of KC.

CONCLUSION

The plant *Acalypha indica* Linn is having an important role in siddha system of medicine for the treatment of Iraippu irumal(Bronchial Asthma).The physico-chemical parameters observed were LOD at 105^{0}C to be 8.6%, total ash 20.99%,Acid insoluble ash 7.15%,Water soluble ash 15.58%, Sulphated ash 24.67%, pH 5.8%,Alcohol soluble extractives 8.73% and Water soluble extractives 15.15%. The HPTLC fingerprinting pattern of alcohol extract of Kuppaimeni Chooranam have shown peak of Rf value 0.76 at 254nm, Rf value 0.76 at 366nm and Rf value 0.86 at 575nm after derivatisation. The qualitative UV-Vis spectrum of the extract was recorded from wavelength 200-1100nm. Thus, present study provides information about the concentration of phytoconstituents present. The physicochemical parameters,HPTLC chromatogram and UV visible spectrum obtained from this study helps in the correct identification, standardization and quality control.

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