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An evaluation of physico chemical, phyto chemical and biochemical analysis of siddha herbal formulation "Sarakkondrai Ilai Chooranam"(SIC)

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Abstract

Background: *Cassia fistula* is an important plant used in the Indian system of medicine. It is a medium sized deciduous tree with long and cylindrical fruits containing pulp and also with a bright yellow coloured flower. It belongs to the family Caesalpiniaceae. **Aim:** The aim of the study is to standardize the herbal formulation Sarakkondrai ilai chooranam. The chooranam is made with leaf of *Cassia fistula*Linn. **Objective:** This study is a pre-clinical Phytochemical ,Physicochemical, Biochemical and HPTLC analysis on *Cassia fistula Linn.* **Methodology:** The herb was purified according to the procedure methods described in Siddha classical literature and processed to obtain fine powder and then Phytochemical, Physicochemical , Biochemical, HPTLC analysis was carried out. **Results and Conclusion**: The standardization of this drug phytochemical screening revealed the presence of Tannin, Terpenoids, Alkaloids, Flavanoides, Steroids, Glycosides, Carbohydrates, Quinines and absence of Saponins, Phenolic compounds and Proteins. Physicochemical parameters found such as Ash values, Loss on dry at 105^0F(6.64%), Acid insoluble ash(0.05%),Volatile oil(0.5%), Foaming index (<100) and Swelling index(4ml), pH(6.78%). HPTLC fingerprints of Sarakkondrai ilai chooranam were also prepared to evaluate it's quality. These set of parameters were found to be sufficient to evaluate the authenticity of the Sarakkondrai ilai chooranam and can be used as reference standards for the preparation of a standardized pharmaceutical product and further quality control researches.

Keywords: Siddha Medicine, Standardization, Sarakkondrai ilai chooranam.

Introduction

The Siddha system of medicine is primarily practised in parts of southern India. According to the WHO more than 70%of the world population use traditional medicine to satisfy their principal health needs. *Cassia fistula*Linn. also known as the Golden shower tree[9,11].In Siddha literature it is mentioned as Sarakkondrai.The present study focuses in the management of first five Madhumega Avathaigal(Diabetes mellitus and it's complications) with the aid of Sarakkondrai ilai chooranam. It explained in the book named Sarabendra vaithya muraigal (Pg no.40 and 41).In the book of theriyar karisal urinary diseases are classified into 2 types.[13]

1.Neerinai perukkal noi
2. Neerinai arukkal noi

Neerizhivu or Mathumegam coming under Neerinai perukkal noi. Madhumegam is a chronic metabolic disorder characterized by increased and frequent urination which is sweet in odour, resulting in gradual diminution of udalthathuvam. Diabetes mellitus is a group of metabolic disorders characterized by high blood sugar level over a prolonged period of time. Symptoms often include frequent urination, increased thirst and increased appetite.If left untreated diabetes leads to many health complications such as cardiovascular disease, stroke, chronic kidney disease, foot ulcers, Neuropathy and Retinopathy. [14]

According to sarabendra vaithya muraigal (Neerzhivu chikitchai) page no.40 and 41 Sarakkondrai ilai chooranam is made up of leaf of *Cassia fistula* Linn.It is monoherbal formulation. The Golden shower tree is a medium sized tree, growing to 10-20(33-66 ft.)tall with fast growth. The leaves are deciduous 15-60 cm (6-24 in) long and pinnate with three to eight pairs of leaflets, each leaflet 7-21 cm(3-8 in)long and 4-9 cm broad.The Golden shower tree is not a nitrogen fixer.

Kingdom : Plantae
Order : Fabales
Family : Fabacea
Subfamily :Caesalpinoidae
Genus : Cassia
Species : C.fistula[6]

Vernacular Names:

Bengali	:	Bundaralti,Sonalu, Soondali, Sonda
English	:	Golden shower
Gujarati	:	Garmala
Hindi	:	Sonhali,Amultus
Kannada	:	Kakkemara
Marathi	:	Bahava
Tamil	:	Sharakonnai,Konai, Irjviruttam
Malayalam	:	Vishu konnai
Telugu	:	Kondrakkayi, Raelachettu, Aragvadhamu
Sanskrit	:	Nripadruma
Arab	:	Khayarsambhar
Oriya	:	Sunaari
Punjabi	:	Amaltaas,kaniyaar, Girdnalee[1]

Fig:1 Cassia fistula Plant

Materials and Methods

This study was designed to screen the physicochemical, biochemical phytochemical analysis of sarakkondrai ilai chooranam and the work was carried out in Siddha Research Regional Institute, Thiruvanathapuram, Kerala and Biochemical analysis in Lab in the Government Siddha Medical College, Palayamkottai, Tirunelveli Tamilnadu. The leaf

was collected from in and around places of Palayamkottai, Tirunelveli district, Tamilnadu. The leaf was authenticated by the experts from Department of Gunapadam,GSMC Palayamkottai.

Fig:2 SIC

It was shade dried and grind into a powder of coarse particles and filtered to get chooranam. The chooranam is stored in air tight container and labelled as "Sarakkondrai Ilai chooranam" which was used for experimental purposes.

Table:1

Drug	Botanical name	Part used	Family
Sarak kondrai	*Cassia fistula linn*	leaves	Caesalpiniaceae

2.1 Physicochemical analysis

The physico chemical analysis includes number of parameters such as Loss on drying,total ash,Acid insoluble ash,alcohol soluble extractive,water soluble extractive,pH(10% aqueous solution),Determination of volatile oil,estimation of fiber content.Thin Layer chromatography(TLC) as per standard method.The samples were analyzed.The information collected from these tests are used for standardization.[2]

2.2 Preliminary phyto chemical analysis

Phytochemical analysis of was carried out for saponins, flavonoids, terpenoids, steroids, tannins. Wagner's and Hegar's reagents were used to alkaloid foam test for saponins,Mg-Hcl and Zn-HCL for flavanoids, Salkonoski test for terpenoids, acetic anhydride and sulphuric acid for steroids, chloride for phenol hexane and diluted ammonia for anthra quinine test. All these experiments were carried out for three solvent extracts (aqueous, methanol and petroleum ether)of dried leaf individually.[3]

1. Test For Tannin:

Treat the substance in alcohol with lead acetate solution, a bulky precipitate show the presence of Tannins.

2. Test For Terpenoids:

Noller's test-Warmed the substance with tin bit and Thionyl chloride. A Magenta colour indicates the presence of Terpenoides.

3. Test For alkaloids: It react with Dragendroff's reagent, Mayer's reagent indicates the presence of alkaloids.

4.Test for Flavones:

Shinado's Test: Magenta colour indicates the presence of flavones. To the substance in alcohol add 10% NaOH or NH a dark yellow colour indicates the presence of Flavones.

5. Test for Steroids:

Libermann Burchard Test: An appearance of Blur green colour indicates the presence of steroids.

6. Test for Glycosides:

A Dark green colouration indicates the presence of glycosides.

7. Test for Quinones:

To the test substance add NaOH. A Red colour indicates the presence of Quinones.

2.2.1. High Performance Thin Layer Chromatography:

In the herbal medicinal research, HPTLC is widely used to analyse the presence of phytochemicals. More HPTLC analysis integrated with digital scanning and profiling offers precise measurable assessments of R_f values of herbal samples into chromatogram peaks with defined parameters as Scanning densitometry documentation, counting observance of intensity with corresponding R_f values.

Procedure:

Developing solvent system

A number of solvent system 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 solvent system.

Sample application

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

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing champer (10cm x10 cm) presaturated 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 Visualizer and the images were captured under UV light 254 nm and 366 nm.

Densitometry

The plate was scanned at 254nm 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 105^0C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatogram were documented. The plate was scanned at 575nm and R_f values and finger print data were documented.[5]

2.3 Qualitative bio chemical analysis:

Preparation of the Extract:

5 gms of the drug was weighed accurately and placed in a 250 ml clean beaker then 50 ml of distilled water is added and dissolved well.Then it is boiled well for about 10 minutes.It is cooled and filtered in a 100 ml with distilled water.This fluid is taken for analysis.

1. Test for Calcium:

Taken 2ml extract and added 2ml of Ammonium oxalate solution with drug.

2.Test for Sulphate:

Taken 2ml extract and added 5% Barium chloride solution.

3.Test for Chloride:

The extract is treated with Silver nitrate solution.

4.Test for Carbonate:

The substance is treated with concentrated Hcl.

5.Test for Starch:

The extract is added with weak iodine solution.

6.Test for Ferric Iron:

The extract is acidified with Glacial acetic acid and Potassium ferro cyanide.

7.Tesst For Ferrous Iron:

The extract is treated with concentrated Nitric acid and Ammonium thiocyanate solution.

8.Test for Phosphate:

The extract is treated with Ammonium Molybdate and concentrated nitric acid.

9.Test for Albumin:

The extract is treated with Esbach's reagent.

10.Test for Tannic acid:

The extract is treated with ferric chloride.

11.Test for Unsaturation:

Potassium permanganate solution is added to the extract.

12.Test for Reducing sugar:

5 ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8-10 drops of the extract and again boil it for 2 minutes.

13.Test for Amino acid:

One or two drops of the extract is placed on a filter paper and dried well. After drying 1% Ninhydrin is sprayed over the same and dried it well.

14.Test for Zinc:

The extract is treated with Potassium Ferro cyanide

Results

Table 2: Physicochemical Analysis of Sic

Parameters	Results
1.LOD at 105^{0}C	6.64%
2.Total Ash	7.11%
3.Acid insoluble Ash	0.05%
4.Water insoluble Ash	2.88%
5.Sulphated Ash	11.12%
6.Water soluble extract	15.62%
7.Alcohol soluble extract	14.56%
8.PH	6.78
9.Volatile oil	0.5%
10.Foaming index	<100
11.Swelling index	4.0ml

Discussion:

The determination of physicochemical parameter is important in determination of adulterant and proper handling of drugs. The physicochemical screening revealed that the extract values of Methanol and water extracts were found to be 14.56% and 15.65 respectively. More water soluble ash value denotes this powder is more soluble to water compared to other. Total ash value of plant material indicated the identity and purity of drug in powder form and it's value was calculated to be 7.11%.The amount of the acid insoluble siliceous matter present in the plant was 0.05%.The value of residue on ignition was 11.2%.The value for loss on drying was found to be 6.64%.Loss value of moisture content could prevent bacterial, fungal and yeast growth. The Ph value of 4% aqueous solution was found to be 6.78.The volatile oil content was found to be 0.5%.

Phytochemical analysis:

Table 3:Phytochemical Analysis of SIC

S.no	Phytochemicals	Result
1	Saponins	-
2	Tannins	**Present**
3	Phenol	-
4	Terpenoids	**Present**
5	Alkaloids	**Present**
6	Flavanoids	**Present**
7	Steroids	**Present**
8	Glycosides	**Present**
9	Carbohydrates	**Present**
10	Quinones	**Present**
11	Proteins	-

Discussion:

Qualitative phyto chemical investigation of methanolic extract of SIC revealed the presence of tannins, terpenoids, alkaloids, flavanoides, steroids, glycosides, carbohydrates and quinones. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They posses biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, anti atherosclerosis, cardio

vascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities.

Natural antioxidant mainly come from plants in the form phenolic compound such as flavanoids, Phenolic acids, tocopherol etc.

Tannins:

It bind to proline rich protein and interfere with protein synthesis.

Flavanoids:

Are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection. They also are effective antioxidant and show strong anticancer activities.

Steroids:

Have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as are hormones.

Alkaloides:

Have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity, it also have analgesic, antispasmodic, antibacterial properties.

Glycosides:

Are known to lower the blood pressure according to many reports.

Terpenoides:

Are one of the secondary plant metabolites have therapeutic activities of antiinflammatory, analgesics, antipyretic, hepatoprotective, cardiotonic and sedative.[8]

Quinones:

Play an important role in oxidative stress .Quinones have a diverse role in medicine including anti cancer agents, anti aging and arteriosclerosis.[10,7]

Carbohydrates:

Act as an energy source. It helps to control blood glucose and insulin metabolism. It participates in cholesterol and triglyceride metabolism.[15]

Table:4 Biochemical Analysis:

Qualitative biochemical analysis of sic

s.no	Compound	Result
1	Calcium	-
2	Sulphate	**present**
3	Chloride	-
4	Carbonate	-
5	Starch	**present**
6	Ferric Iron	-
7	Ferrous Iron	**present**
8	Phosphate	-
9	Albumin	-
10	Tannic acid	**present**
11	Unsaturatedcompound	**present**
12	Reducing sugar	-
13	Amino acid	**present**
14	Zinc	-

Discussion:

Biochemical analysis of Sarakkondrai ilai chooranam indicated it's richness in mineral element Sulphate, Starch, Ferrousiron, Tannic acid, Unsaturated compound and Amino acid.(Table 4).

Sulfur:

Sulfur is third most vital mineral that is present in abundance in the human body after Calcium and Phosphorus. Sulfur is highly essential for shielding the body against cellular damage and oxidative stress, retaining nitrogen balance and boosting immune function. When we taking sulphur containing supplements deals with skin problems, prevents liver anomalies and reduces the risk of different types of cancer.[16]

Starch:

Starch play a crucial role. They provide the body with glucose which is the main energy source for every cell. They also provide a range of vitamins, minerals, fiber and other nutrients.

Ferrous iron:

Ferrous sulphate replenishes iron, an essential component in haemoglobin myoglobin and various enzymes. Iron precipitate in oxygen transport and storage, electron transport and energy metabolism, antioxidant and tissue proliferation and growth as well as DNA replication and repair.[17]

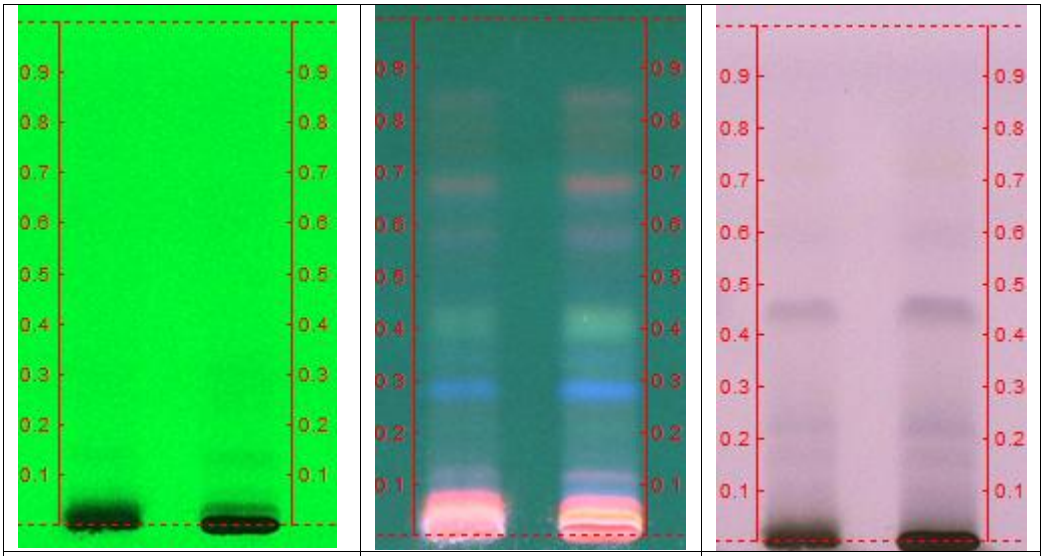
Amino acid:

Amino acids are molecules that combine to form proteins. Amino acids and proteins are the building blocks of life. Amino acid oxygenases also play vital metabolic roles such as in prevention of diseases .as a result, amino acids and their oxygenases isolated from various organisms are potent candidates in treatment of diseases which include cancers, inflammation as well as antibacterial agents.[4,18]

III. High Performance Thin Layer Chromatography:

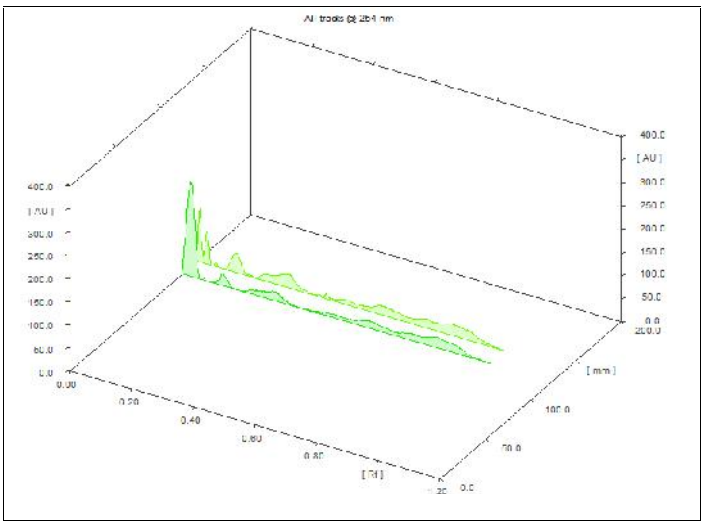
Solvent system: Toluene: Ethyl acetate: (5:2)

Track 1,2: Volume applied: 5, 10 µl each

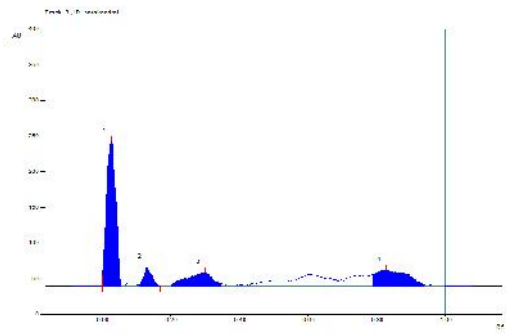


Under UV short	Under UV long	Under white light

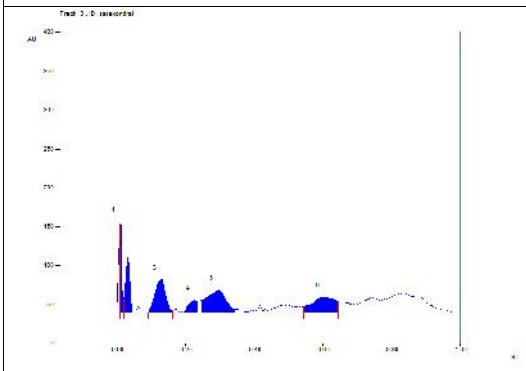
Fig 3: HPTLC profile of alcohol extract of Sarakkondrai ilai chooranam viewed in UV short; UV long; White light after derivatisatisation using vanillin –sulphuric acid



254nm

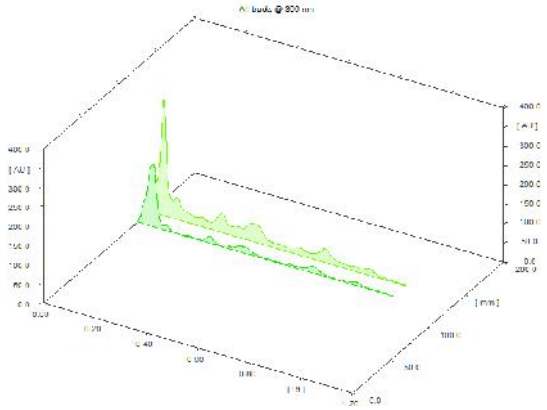


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	14.5 AU	0.03 Rf	203.6 AU	74.96 %	0.05 Rf	1.2 AU	3821.8 AU	57.24 %
2	0.11 Rf	1.7 AU	0.13 Rf	26.5 AU	9.74 %	0.17 Rf	0.2 AU	437.3 AU	6.55 %
3	0.20 Rf	1.4 AU	0.30 Rf	19.1 AU	7.02 %	0.35 Rf	3.1 AU	975.3 AU	14.61 %
4	0.79 Rf	15.3 AU	0.83 Rf	22.5 AU	8.28 %	0.94 Rf	1.9 AU	1442.4 AU	21.60 %

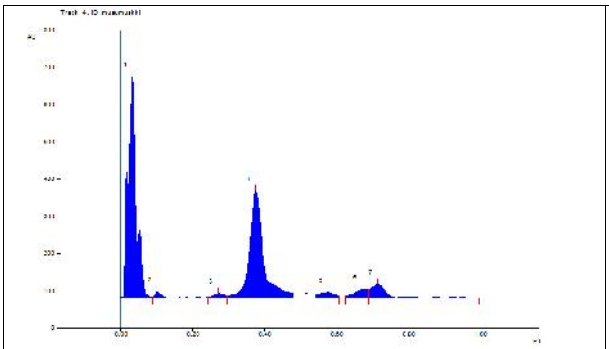


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	109.9 AU	0.01 Rf	116.5 AU	39.38 %	0.02 Rf	14.0 AU	551.5 AU	12.53 %
2	0.02 Rf	15.8 AU	0.03 Rf	72.4 AU	24.49 %	0.05 Rf	0.0 AU	610.5 AU	13.87 %
3	0.09 Rf	0.0 AU	0.13 Rf	42.4 AU	14.32 %	0.16 Rf	2.9 AU	916.4 AU	20.82 %
4	0.19 Rf	1.2 AU	0.23 Rf	15.5 AU	5.25 %	0.24 Rf	15.0 AU	277.1 AU	6.30 %
5	0.25 Rf	16.1 AU	0.30 Rf	28.7 AU	9.71 %	0.34 Rf	4.0 AU	1125.2 AU	25.57 %
6	0.55 Rf	7.4 AU	0.61 Rf	20.2 AU	6.84 %	0.64 Rf	14.2 AU	920.2 AU	20.91 %

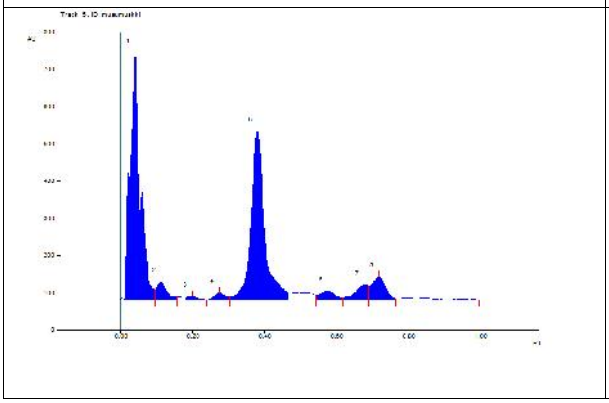
Fig:4 HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Sic25after derivatisation 254nm



366nm

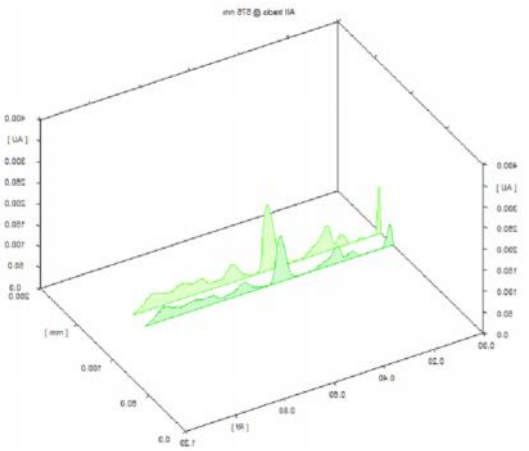


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Are %
1	0.01 Rf	4.7 AU	0.04 Rf	597.5 AU	60.03 %	0.09 Rf	2.4 AU	9496.2 AU	47.79
2	0.09 Rf	2.9 AU	0.10 Rf	17.4 AU	1.75 %	0.13 Rf	0.1 AU	212.9 AU	1.07
3	0.24 Rf	1.5 AU	0.27 Rf	12.8 AU	1.29 %	0.30 Rf	6.4 AU	263.2 AU	1.32
4	0.30 Rf	6.4 AU	0.38 Rf	287.6 AU	28.90 %	0.48 Rf	14.7 AU	7755.4 AU	39.03
5	0.54 Rf	9.0 AU	0.58 Rf	16.2 AU	1.63 %	0.61 Rf	5.8 AU	489.8 AU	2.46
6	0.63 Rf	6.7 AU	0.68 Rf	25.3 AU	2.54 %	0.69 Rf	23.7 AU	647.2 AU	3.26
7	0.69 Rf	23.9 AU	0.72 Rf	38.4 AU	3.86 %	0.77 Rf	0.6 AU	1006.1 AU	5.06

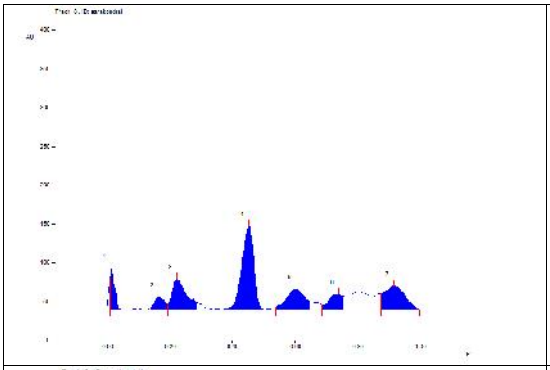


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.2 AU	0.04 Rf	664.2 AU	50.05 %	0.10 Rf	28.7 AU	12736.0 AU	42.75
2	0.10 Rf	29.4 AU	0.11 Rf	48.9 AU	3.69 %	0.16 Rf	8.6 AU	960.1 AU	3.22
3	0.18 Rf	6.8 AU	0.20 Rf	10.3 AU	0.77 %	0.23 Rf	1.2 AU	203.6 AU	0.68
4	0.24 Rf	0.1 AU	0.28 Rf	20.6 AU	1.55 %	0.30 Rf	9.9 AU	437.1 AU	1.47
5	0.31 Rf	9.9 AU	0.38 Rf	454.4 AU	34.24 %	0.47 Rf	21.9 AU	11997.7 AU	40.27
6	0.55 Rf	14.2 AU	0.58 Rf	25.4 AU	1.92 %	0.62 Rf	6.2 AU	760.7 AU	2.55
7	0.62 Rf	6.2 AU	0.68 Rf	40.8 AU	3.07 %	0.69 Rf	38.3 AU	1030.7 AU	3.46
8	0.69 Rf	38.3 AU	0.72 Rf	62.6 AU	4.72 %	0.77 Rf	3.6 AU	1667.1 AU	5.60

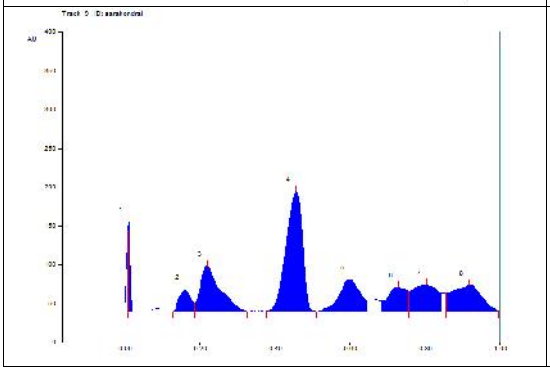
Fig:5: HPTLC fingerprint of 5µl and 10µl of alcohol extract of Sic at 366nm after derivatisation.



575nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	40.1 AU	0.01 Rf	53.4 AU	18.13 %	0.04 Rf	0.4 AU	575.6 AU	7.05 %
2	0.14 Rf	0.2 AU	0.17 Rf	16.7 AU	5.69 %	0.19 Rf	7.6 AU	365.5 AU	4.48 %
3	0.19 Rf	7.7 AU	0.22 Rf	39.4 AU	13.38 %	0.29 Rf	8.8 AU	1259.0 AU	15.43 %
4	0.38 Rf	0.9 AU	0.45 Rf	107.8 AU	36.62 %	0.50 Rf	0.3 AU	2879.6 AU	35.29 %
5	0.54 Rf	3.3 AU	0.61 Rf	26.6 AU	9.02 %	0.65 Rf	9.9 AU	1065.1 AU	13.05 %
6	0.69 Rf	5.6 AU	0.74 Rf	19.7 AU	6.69 %	0.75 Rf	17.1 AU	601.8 AU	7.37 %
7	0.88 Rf	20.8 AU	0.92 Rf	30.8 AU	10.47 %	1.00 Rf	0.4 AU	1413.3 AU	17.32 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	104.3 AU	0.01 Rf	115.6 AU	23.11 %	0.03 Rf	0.0 AU	585.5 AU	4.09 %
2	0.13 Rf	0.0 AU	0.16 Rf	28.7 AU	5.73 %	0.19 Rf	11.5 AU	612.1 AU	4.27 %
3	0.19 Rf	11.9 AU	0.22 Rf	59.0 AU	11.79 %	0.33 Rf	0.0 AU	2142.3 AU	14.96 %
4	0.38 Rf	0.9 AU	0.46 Rf	155.4 AU	31.07 %	0.51 Rf	0.1 AU	4738.2 AU	33.08 %
5	0.52 Rf	0.9 AU	0.60 Rf	41.2 AU	8.24 %	0.65 Rf	14.9 AU	1618.1 AU	11.30 %
6	0.69 Rf	13.6 AU	0.73 Rf	31.2 AU	6.24 %	0.76 Rf	26.9 AU	1119.8 AU	7.82 %
7	0.76 Rf	26.8 AU	0.81 Rf	34.9 AU	6.98 %	0.85 Rf	24.3 AU	1621.7 AU	11.32 %
8	0.86 Rf	23.6 AU	0.92 Rf	34.2 AU	6.84 %	1.00 Rf	0.5 AU	1886.6 AU	13.17 %

Fig:6: HPTLC fingerprint profile of 5µl and 10µl of alcohol extract of Sic at 575nm after derivatisation

The HPTLC fingerprints of alcohol extracts of Sarakkondrai ilai chooranam was developed at 254nm,366nm and after derivatisation with vanillin sulphuric acid at575nm.The solvent system,toluene:Ethyl acetate (5:2) efficiently resolved the components. The results from HPTLC chromatogram for alkaloids can be distinguished at 254 nm before derivatization. The methanolic extract evidenced 10 spots with their corresponding ascending order of R_f 0.01, 0.02,0.09,0.19,0.2 and 0.55 respectively.HPTLC pattern at 254nm showed the peak at R_f 0.25 having the maximum area 25.57% indicating the presence of highest concentration of phytoconstituents.

HPTLC pattern at 366nm showed the peak at 0.01 R_f having the maximum area of 42.57%.HPTLC pattern at 575 nm after derivatisation showed 8 bands with R_f 0.01,0.13,0.19,0.38,0.52, 0.69,0.76,0.86 respectively. out of which R_f value at 0.38 that the maximum area 33.08%..Each band indicates the presence of phytoconstituents present in the extract.

Conclusion

Through the results of this study, it has been concluded that the Sarakkondrai ilai chooranam has biologically active compounds and will act therapeutically in treating diseases.

The phytochemical analysis of provide data on pharmacologic properties of the drug. In future these data can be used as reference for the drug standardization.

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