Research Article

Standardization of Siddha Poly Herbal Formulation Kadukkai Legium

***M.Shanmugeshwari[1], *S.Sarathi[2], *K.Ramya[3] ,* P. Arul Mozhi[4], *M. Meenakshi Sundaram[5]**

[1,2,3]PG Scholar, Department of Kuzhandhai Maruthuvam, National Institute of Siddha, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, Tamilnadu, India.

[4]Associate Professor and Guide, Department of Kuzhandhai Maruthvam, National Institute of Siddha, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, Tamilnadu, India.

[5]Professor and HOD, Department of Kuzhandhai Maruthvam, National Institute of Siddha, Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, Tamilnadu, India.

***Corresponding author's E-mail:** shanmubsms@gmail.com

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ABSTRACT

Siddha system of medicine is one of the oldest traditional healing practices, originating in South India founded by the Siddhars, ancient sages who combined medicine, spirituality, and lifestyle for holistic health. Siddha emphasizes balance between the body's three humors—Vatham, Pitham, and Kabham—for maintaining well-being. Herbal, mineral, and animal-based preparations form the core of its therapeutic methods. Beyond treating disease, it focuses on prevention, longevity, and spiritual advancement. Legium is one of the favourable internal forms of medicine and it facilitates greater absorption. Kadukkai Legium which is indicated for Malakattu, Akkini Mantham, Soolai, and Vaayumoolam as described in the textbook Athmarakshamirthamenum Vaithyasarasangarakam. Kadukkai Legium was prepared in Gunapadam laboratory of National Institute of Siddha. Standardization of Kadukkai Legium was carried out at Interstellar Testing Centre Pvt. Ltd, Panchkula, Haryana. This study includes organoleptic characters, physiochemical, phytochemical and biochemical analysis of Kadukkai Legium. The results obtained from this study confirm the trial drug Kadukkai Legium is of standard quality, and it will be helpful in further research to ensure the quality, safety and acceptability of the drug.

Keywords: Kadukkai Legium, Standardization, Siddha.

INTRODUCTION

The Siddha system of medicine is one of the oldest traditional systems of healing, with its origins rooted in ancient Tamil culture. The system emphasizes the maintenance of health and prevention of disease through a holistic approach that integrates diet, lifestyle, herbal remedies, minerals, and therapeutic procedures. Siddha medicine is based on the concepts of the five elements (Aimpootham) and the three humors (Mukkutram) – Vali (Vatha), Azhal (Pitha), and Iyyam (Kaba) – which are considered fundamental in maintaining the equilibrium of the human body. Disease is understood to result from the derangement of these humors, and treatment is aimed at restoring balance. Kuzhandhai Maruthuvam is a specialized branch within the Siddha system that is dedicated to the treatment of children up to the age of 12. Malakattu, a classification within pediatric Siddha medicine, falls under the category of purakarananoigal, or ailments attributed to external factors. As per the Siddha literature, Malakattu is associated with the symptoms like decreased appetite, indigestion, abdominal discomfort, constipation, flatulence, cough, and fever. Malakattu exhibits significant clinical similarities to functional constipation, a prevalent pediatric issue associated with both physical and psychological distress, ultimately affecting the quality of life. Childhood constipation develops in distinct phases—during weaning in infants, toilet training in toddlers, and throughout school-age years. Kadukkai Legium, which is recommended for the treatment of Malakattu in the Siddha literature titled "Athmarakshamirthamenum Vaithyasarasangarakam[1.] It is indicated for the treatment of indigestion, abdominal discomfort, constipation and flatulence. Standardization of siddha medicines will help to follow a protocol for usage of siddha medicine in a safe and effective way around worldwide. The aim of the study is to standardize the Kadukkai Legium by PLIM guidelines though organoleptic characters, physicochemical, phytochemical, biochemical analysis. The outcome of the study may help to use as a standard reference for further studies performed using this trial drug.

MATERIALS AND METHODS

Source of the sample

The essential raw materials required for the production of *Kadukkai legium* were purchased from a reputable supplier specializing in raw medicinal ingredients and were authenticated by medicinal botanist of NIS. After proper purification the medicine was prepared in the Gunapadam Laboratory of National Institute of Siddha.

Ingredients:[1,2]

1. Kadukkai [*Terminalia chebula* Retz] - 100 balam (3500 grams)
2. Milagu [*Piper nigrum*] -1 balam (35 grams)
3. Thippili [*Piper longum*] -1 balam (35 grams)
4. Omam [*Carum copticum*] -1 balam (35 grams)
5. Inji [*Zingiber officinale* Rosc] -1 balam (35 grams)

6. Sivathai [*Operculina turpethum* Linn] -1 balam (35 grams)
7. Vaivilangam [*Embelia ribes*] -1 balam (35 grams)
8. Sarkarai -10 balam (350 grams)
9. Cow's ghee -1 padi (1520 ml)

PHYSIOCHEMICAL EVALUATION:[3,4,5]

Organoleptic characters:

The organoleptic characters such as colour, odour and consistency of the drug were noted.

Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105ºC for 5 hours and then weighed.

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400oC until it turns white in colour which indicates absence of carbon. Percentage of total ash was calculated with reference to the weight of air-dried drug.

Determination of Acid Insoluble Ash

The ash obtained by total ash test was boiled with 25 ml of dilute hydrochloric acid for 6 mins. Then the insoluble matter is collected in crucible and was washed with hot water and ignited to constant weight. Percentage of acid insoluble ash was calculated with reference to the weight of air-dried ash.

Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105oC, to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of Water-Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, and dried at 105oC, to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Determination of pH

One gram of the test drug was taken into a 100ml graduated cylinder containing about 50 ml of water. The cylinder was shaken vigorously for two minutes, and the suspension was allowed to settle for hour at 25ºC to 27ºC, then 25 ml of the clear aqueous solution was transferred into a 50 ml beaker and tested for pH using digital pH meter.

High Performance Thin Layer Chromatography Analysis (HPTLC) [6]

HPTLC method is a sophisticated and automated selection technique derived from TLC. Pre coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. HPTLC is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. It offers high degree of selectivity, sensitivity and rapidity combined with single step sample preparation. In addition, it is a reliable method for the quantitation of nano grams level of samples. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprints of phytochemicals which is suitable for confirming the identity purity of medicinal plant raw materials.

Chromatogram development

It was carried out in CAMAG twin trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366 nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint ewes developed for the detection of Phyto constituents present in each extract and Rf value were tabulated.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litre by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system. Toluene: Ethyl Acetate: Acetic Acid (1.5:1:0.5). After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365nm

Test for Heavy metal Analysis

Standard: Hg, As, Pb and Cd – Sigma.

Methodology

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample KN was performed by Atomic Absorption

Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test sample.

Sample Digestion

Test sample digested with 1mol/L HCl for determination of arsenic and mercury. Similarly for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO_3.

Test for specific pathogen

Methodology

0.5 ml of the test sample was directly inoculated into the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

Details of specific pathogens and their Abbreviation:

Organism	Abbreviation	Medium
E-coli	*EC*	*EMB Agar*
Salmonella	*SA*	*Deoxycholate agar*
Staphylococcus Aureus	*ST*	*Mannitol salt agar*
Pseudomonas aeruginosa	*PS*	*Cetrimide Agar*

Test for Organochlorine pesticide, organophosphorus pesticide and pyrethroids

About 10 g of test substance were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Aflatoxin Assay by TLC [6]

Solvent

Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of aflatoxin B1 and aflatoxin G1 and 0.1 µg per ml each of aflatoxin B2 and aflatoxin G2.

Test solution: Concentration 1 µg per ml

Procedure

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5µL, 5 µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Physio chemical analysis:

Physio chemical analysis of the trial drug Kadukkai Legium was done at Interstellar testing centre Pvt.Ltd, Panchukula (Haryana).

Table 1: Organoleptic Character

Organoleptic Character	Results
Colour	Brown
Odour	Characteristic
Consistency	Thick paste

Table 2: Chemical parameters

Parameters	Unit	Instrument	Method	Results
Loss on drying at 105°C/Moisture content	%w/w	Hot Air Oven	Inhouse	13.83%
Total ash	-	Chemically	Inhouse	2.38%
Acid-insoluble ash	-	Chemically	Inhouse	1.02%
Alcohol Soluble Extractive	%w/w	Chemically	Inhouse	46.73%
Water Soluble Extractive	%w/w	Chemically	Inhouse	38.82%
PH	-	pH Meter	Inhouse	3.75
Total acidity	-	Chemically	Inhouse	29.1 ml of 0.1N Sodium hydroxide used to neutralize 10g sample
Specific Gravity	-	Chemically	Inhouse	NA
Total Solid content	-	Chemically	Inhouse	86.17%
Fat Content		Chemically	Inhouse	13.81%
Reducing Sugar/Non-reducing sugar	-	Chemically	Inhouse	Absent
Total Sugar	-	Chemically	Inhouse	NA
Assay for major ingredients	-	Chemically	Inhouse	Absent

Table 3: Microbiological Tests

Total viable aerobic count	**cfu / g**	**Microbiological**	**API**	**NMT 100**	**<10 cfu/g**
Enterobacteriaceae	/ g	Microbiological	API	-	Absent/g
Total fungal count	cfu / g	Microbiological	API	NMT 1000	<10 cfu/g
E.coli	-	Microbiological	API	Absent	Absent/g
Salmonella	/ g	Microbiological	API	Absent	Absent/g
Staphylococcus aureus	/ g	Microbiological	API	Absent	Absent/g
Pseudomonas aeruginosa	/ g	Microbiological	API	Absent	Absent/g

Table 4: Heavy Metals

Lead (as Pb)	Ppm	ICPMS	ITC/STP/AY/001	Max. 10	0.33
Arsenic (as As)	Ppm	ICPMS	ITC/STP/AY/001	Max. 3	0.42
Mercury (as Hg)	Ppm	ICPMS	ITC/STP/AY/001	Max. 1	0.53
Cadmium (as Cd)	Ppm	ICPMS	ITC/STP/AY/001	Max. 0.3	BLQ(LOQ:0.10)

Table 5: Aflatoxins

Aflatoxin B1	Ppb	HPLC	STP/ITC/AY/003	BLQ(LOQ:1)
Aflatoxin B2	Ppb	HPLC	STP/ITC/AY/003	BLQ(LOQ:1)
Aflatoxin G1	Ppb	HPLC	STP/ITC/AY/003	BLQ(LOQ:1)
Aflatoxin G2	Ppb	HPLC	STP/ITC/AY/003	BLQ(LOQ:1)

Table 6: Microbiological Tests

Total viable aerobic count	cfu / g	Microbiological	API	NMT 100	<10 cfu/g
Enterobacteriaceae	/ g	Microbiological	API	-	Absent/g
Total fungal count	cfu / g	Microbiological	API	NMT 100	<10 cfu/g
E.coli	-	Microbiological	API	Absent	Absent/g
Salmonella	/ g	Microbiological	API	Absent	Absent/g
Staphylococcus aureus	/ g	Microbiological	API	Absent	Absent/g
Pseudomonas aeruginosa	/ g	Microbiological	API	Absent	Absent/g

Table 7: Pesticidal Residue

Bromopropylate	Ppm	GCMSMS	ITC/STP/AY/002	NMT 3.0	BLQ(LOQ:0.01)
Chlordane (Sum of cis, trans and oxythlordane)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.05	BLQ(LOQ:0.01)
Cypermethrin (and isomers)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)
DDT (sum of p,p'-DDT, o,p'-DDT, p,p-DDE and p,p'-TDE)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)
Deltamethrin	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.5	BLQ(LOQ:0.01)
Dichlorvos	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)
Dithiocarbamates (as CS2)	Ppm	GCMSMS	ITC/STP/ AY/002	NMT-2.0	BLQ(LOQ:0.01)
Endosulfan (Sum of isomers and Endosulfan sulphate)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-3.0	BLQ(LOQ:0.01)
Endrin	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.05	BLQ(LOQ:0.01)
Ethion	Ppm	GCMSMS	ITC/STP/AY/002	NMT-2.0	BLQ(LOQ:0.01)
Fenitrothion	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.5	BLQ(LOQ:0.01)
Fenvalerate	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.5	BLQ(LOQ:0.01)
Fonofos	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.05	BLQ(LOQ:0.01)
Heptachlor (Sum of heptachlor and heptachlorepoxide)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.05	BLQ(LOQ:0.01)
Hexachlorobenzene	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.1	BLQ(LOQ:0.01)
Hexachlorocyclohexane isomers	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.3	BLQ(LOQ:0.01)
Malathion	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)
Methidathion	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)

Parathion	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.5	BLQ(LOQ:0.01)
Parathion methyl	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.2	BLQ(LOQ:0.01)
Permethrin	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.2	BLQ(LOQ:0.01)
Phosalone	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.1	BLQ(LOQ:0.01)
Piperonyl butoxide	Ppm	GCMSMS	ITC/STP/AY/002	NMT-3.0	BLQ(LOQ:0.01)
Primiphos-methyl	Ppm	GCMSMS	ITC/STP/AY/002	NMT-4.0	BLQ(LOQ:0.01)
Pyrethrins (sum of)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-3.0	BLQ(LOQ:0.01)
Quintozene (sum of quintozene, pentachloroaniline and methylpentachlorophenyl sulphide)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)
Alachlor	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.02	BLQ(LOQ:0.01)
Aldrin & Dieldrin	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.05	BLQ(LOQ:0.01)
Azinphos-methyl	Ppm	GCMSMS	ITC/STP/AY/002	NMT-1.0	BLQ(LOQ:0.01)
Chlorfenvinphos	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.5	BLQ(LOQ:0.01)
Chlorpyriphos	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.2	BLQ(LOQ:0.01)
Chlorpyrifos-methyl	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.1	BLQ(LOQ:0.01)
Lindane (y hexachlrocyclohexane)	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.6	BLQ(LOQ:0.01)
Diazinon	Ppm	GCMSMS	ITC/STP/AY/002	NMT-0.5	BLQ(LOQ:0.01)

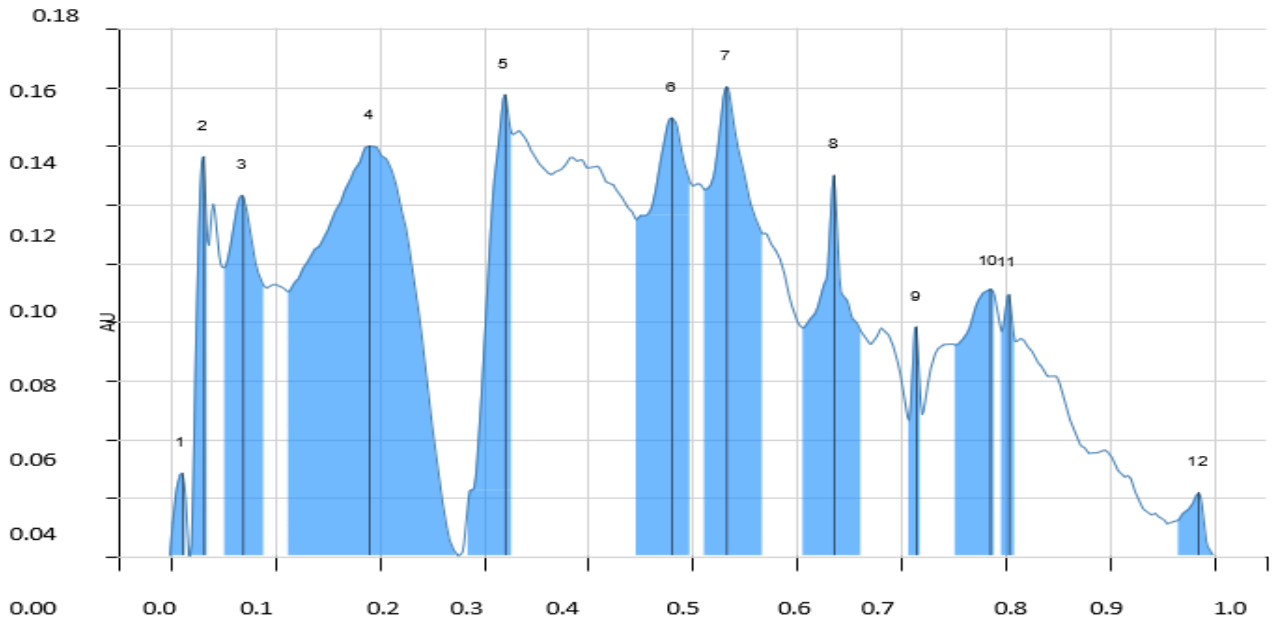

Figure 1: HPTLC chromatogram

#	R_F	H	R_F	H	%	R_F	H	A	%	Peak
1	0.000	0.0000	0.011	0.0281	2.16	0.018	0.0001	0.00033	0.63	No
2	0.019	0.0000	0.032	0.1361	10.45	0.037	0.1058	0.00159	3.01	No
3	0.051	0.0981	0.069	0.1227	9.43	0.093	0.0913	0.00445	8.44	No
4	0.114	0.0900	0.190	0.1397	10.73	0.276	0.0000	0.01582	29.98	No
5	0.283	0.0085	0.321	0.1572	12.08	0.329	0.1437	0.00423	8.01	No
6	0.447	0.1145	0.481	0.1492	11.46	0.501	0.1261	0.00711	13.47	No
7	0.512	0.1247	0.533	0.1599	12.28	0.569	0.1098	0.00758	14.36	No
8	0.607	0.0776	0.636	0.1296	9.96	0.671	0.0722	0.00564	10.69	No
9	0.708	0.0462	0.715	0.0780	5.99	0.721	0.0482	0.00078	1.48	No
10	0.753	0.0719	0.786	0.0908	6.98	0.796	0.0780	0.00355	6.74	No
11	0.797	0.0765	0.804	0.0889	6.83	0.811	0.0730	0.00113	2.14	No
12	0.957	0.0109	0.986	0.0214	1.65	1.000	0.0003	0.00055	1.04	No

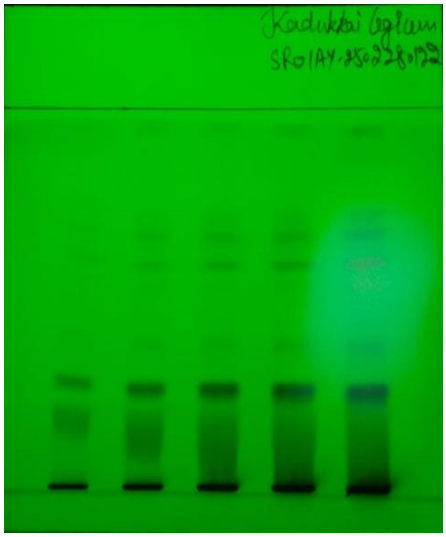

Figure 2: HPTLC Plate at 254nm

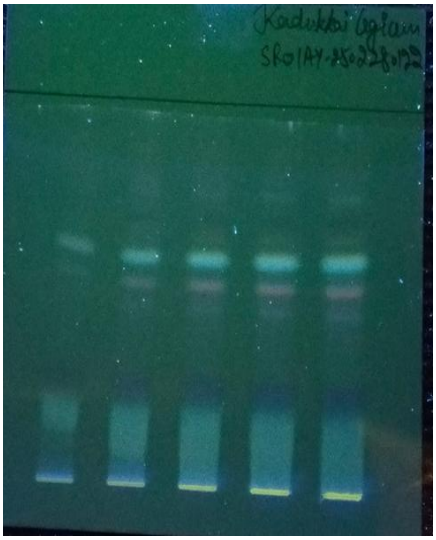

Figure 3: HPTLC Plate at 366nm

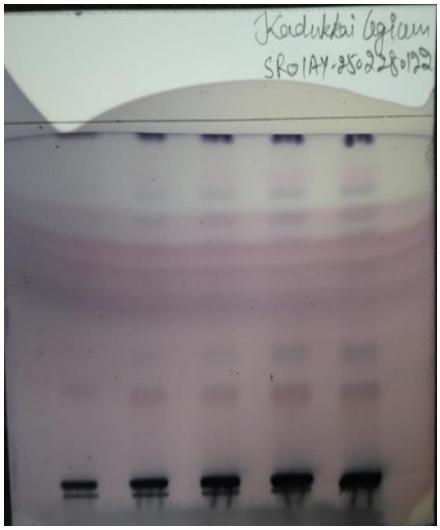

Figure 4: HPTLC Plate after derivatization

DISCUSSION

The physicochemical analysis of *Kadukkai Legium*, a traditional Siddha herbal drug, reveals important insights into its quality and standardization. This analysis is crucial for ensuring the efficacy and safety of Kadukkai Legium especially given its use for irregular bowel habits in children. The organoleptic properties of Kadukkai Legium, which include its brown in colour, characteristic odour and thick paste in consistency (as mentioned in the table.1) These characteristics are essential for identifying the drug and ensuring its authenticity.

The total-ash value of 2.38% suggests a moderate level of inorganic material, which is typical for herbal preparations. The loss on drying value of 13.83% indicates a relatively high moisture content, which is important for understanding the stability and shelf-life of the drug. The acid-insoluble ash percentage of 1.02% is minimal, reflecting the low content of siliceous matter, which is beneficial for ensuring the purity of the formulation. The water-soluble extractive of 38.82% and alcohol-soluble extractive of 46.73% highlight the presence of significant quantities of soluble phytoconstituents. This information is crucial for evaluating the drug's therapeutic potential, as these extracts often contain the active principles responsible for the drug's medicinal properties. The pH value of 3.75 suggests that Kadukkai Legium is slightly acidic, which is consistent with many herbal preparations and may influence its stability and compatibility with biological systems (as mentioned in the table.2). Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) analyses reveal the presence of twelve prominent peaks, indicating the presence of various phytoconstituents with Rf values ranging from 0.000 to 0.957 (as mentioned in the fig.1) This chromatographic fingerprint is essential for the standardization and quality control of Kadukkai Legium, as it provides a unique profile for the drug and helps in identifying its key chemical components. This study also reveals that the drug was sterile and free of bacteria, fungi and specific pathogen like Salmonella, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and pesticide residues (as mentioned in table 6). In heavy metal analysis there is no traces of mercury, arsenic, lead and cadmium in the sample seems to be Below Quantification limit (as mentioned in table 4).

There were no spots of Aflatoxin like B1, B2, G1, G2 (as mentioned in table 5).

CONCLUSION

The result obtained from this study providing the fingerprint of the drug formulation Kadukkai Legium ensures the identity, purity and quality of the prepared medicine. The result can be used as reference for further researches in this drug formulation.

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