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Research Article

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Evaluation of Analytical Specifications of Siddha Herbal Formulations Thippili Lehiyam

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Abstract

Siddha's system of medicine is unique for its physical, psychological, spiritual and social well being of an individual. This system also plays a role in improving the quality of life by prevention and rejuvenation Various siddha paediatric text books explains medications for Iraipu noi. Symptoms of ***Balar Iraipu noi*** is compared with **Bronchial Asthma.** This paper explain about the analytical specification of ***Thippili lehiyam*** as per PLIM guidelines are evaluated.

Keywords: Siddha system, Pillai pini maruthuvam, Iraipu noi, analytical specification.

Introduction

Thippili lehiyam is a polyherbal formulation,mainly used for the treatment of Iraipu noi (bronchial Asthma) mainly for the paediatric age groups. In our Siddha literature 32 types of internal medicines are explained, lehiyam is one among the type. It is a semi solid compound drug preparation with addition of jaggery, sugar, and boiled with prescribed drug juice or decoction. Self life for the lehiyam is 6 months as per our literature. Iraipu is defined as inflammation of lung and its airways when exposed to certain triggers like pollen, cold etc,. symptoms like coughing, wheezing, chest tightness and shortness of breath. Pillai pini maruthuvam shows exposure to unhygienic environment. Thus the analytical specification of Thippili lehiyam is evaluated for scientific validation and standardization formulary drug.

Materials and Methods

2.1 Drug selection

The siddha formulation drug Thippili lehiyam selected from the siddha text of – *Gunapadam mooligai* and this medication is indicated for treating Iraipu noi.

2.2 Ingredients of Thippili lehiyam

This poly herbal formulation contains raw drugs and the ingredients of the drug and its quantity are listed below in **Table 1.**

Table 1

S. No	Name	Botanical name	Family	Part used	Quantity
1.	Thippili	*Piper longum*	Piperaceae	Fruit	70 grm
2.	Chirakam	*Cuminum cyminum*	Apiaceae	Seed	8 ½ grm
3.	Chukku	*Zingiber officinale*	Zingiberaceae	Rhizome	8 ½ grm
4.	Elam	*Elettaria cardamomum*	Zingiberaceae	Seed	8 ½ grm
5.	Thippili ver	*Piper longum*	Piperaceae	Root	8 ½ grm
6.	Vaivilangam	*Embelia ribes*	Primulaceae	Seed	8 ½ grm
7.	Kadukkai	*Terminalia chebula*	Combretaceae	Fruit	8 ½ grm
8.	Milagu	*Piper nigrum*		Seed	8 ½ grm
9.	Honey	*Mel*	-	-	Needed quantity
10.	Sarkari	*Saccharum officinarum*	Poaceae	-	Needed quantity

2.3 Collection of raw materials

Raw drugs were bought from well reputed raw drug store in Tirunelveli town.

2.4 Identification and authentication o the drug

Raw drugs were identified and authenticated by the head of the department of post graduate department of Gunapadam, Government Siddha Medical College, and Palayamkottai. The sample specimen of each raw material is stored in the PG Department of Gunapadam for further reference.

2.5 Purification of the raw drugs

Purification of the raw drugs was done as per classical *Siddha* literature.

2.6 Preparation of the drug

The above mentioned purified raw drugs are finely powdered. Honey and jaggery are taken into equal ratio then it is allowed to boil and taken into syrup consistency. Then fine powdered raw drugs were added into it, mixed well and taken into lehiyam consistency. Allowed it to cool and stored in air tight container.

Picture 1 – Sample description

2.7 Administration of the drug

Form of the medicine: Lehiyam
Route of administration: Oral
Dose: 2grm(Twice a day after food)
Indication: Balar Iraipu noi

2.8 Analytical specification of lehiyam

As per the guidelines of PLIM (The Pharmacopoeial Laboratory of Indian Medicine), analytical specification of lehiyam includes physiochemical description, test or heavy metals, sterility test (detecting microbial contamination) identification TLC/HPTLC, Test for specific pathogen, pesticide residue and test for alpha toxins. In this article all the analytical specifications are evaluated. The analysis was done by Noble research solutions Pvt.Ltd., Chennai, India.

2.9 Physiochemical analysis of Thippili Lehiyam

Physiochemical analysis of Thippili Lehiyam includes about sample Description, Solubility Test, Loss on Drying, Determination of Total Ash. Water Soluble Ash, Acid Insoluble Ash, Water and Alcohol Soluble Extract. The Analysis was done at noble research solutions Pvt. Ltd., Chennai, India. Each analysis is done three times and mean value is calculated.

3. Results and Discussion

3.1.1 Sample Description

Table 2

State	Semi solid
Odour	Aromatic
Touch	Greasy
Flow Property	Non free flowing
Appearance	Brownish black

3.1.2 Solubility Profile

Table 3

S.No	Solvent Used	Solubility / Dispersibility
1	chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
5	DMSO	Soluble

3.1.3 Percentage Loss on Drying

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

3.1.4 Determination of Total Ash

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

3.1.5 Determination of Acid Insoluble Ash

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

3.1.6 Determination Extractive of Alcohol Soluble

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. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

3.1.7 Determination Extractive Water Soluble

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. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.The test report of the above analysis is mentioned in the Table 4.

Table 4

S. No	Parameter	Mean(n=3) SD
1.	*Loss on Drying at 105 °C (%)*	18.2+2.066
2.	*Total Ash (%)*	0.3347+0.1816
3.	*Acid insoluble Ash (%)*	0.3457+0.4454
4.	*Water soluble Extractive (%)*	7.433+0.9504
5.	*Alcohol soluble extractive (%)*	12.17+0.4933

3.2 Test for heavy metals

Heavy Metal Analysis evaluated by Atomic Absorption Spectrometry (AAS). It is a very common and reliable technique for detecting metals and metalloids in environmental samples.

The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item

Sample Digestion:

For the determination of lead and cadmium the sample were digested with 1mol/L ofHNO3.

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

The analysis report is detailed in **Table 5**

Table 5

Name of Heavy metal	Absorption max	Results Analysis	Maximum Limit
Lead	217.0 nm	10 ppm	10 ppm
Arsenic	193.7 nm	3 ppm	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.4 ppm	1 ppm

3.3 Sterility test (Microbial contamination)

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10minutes). Plates were then inverted and incubated at 37° C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU. The result shown in Table 6 and it was observed that no growth/colonies in any of the plates inoculated with test sample.

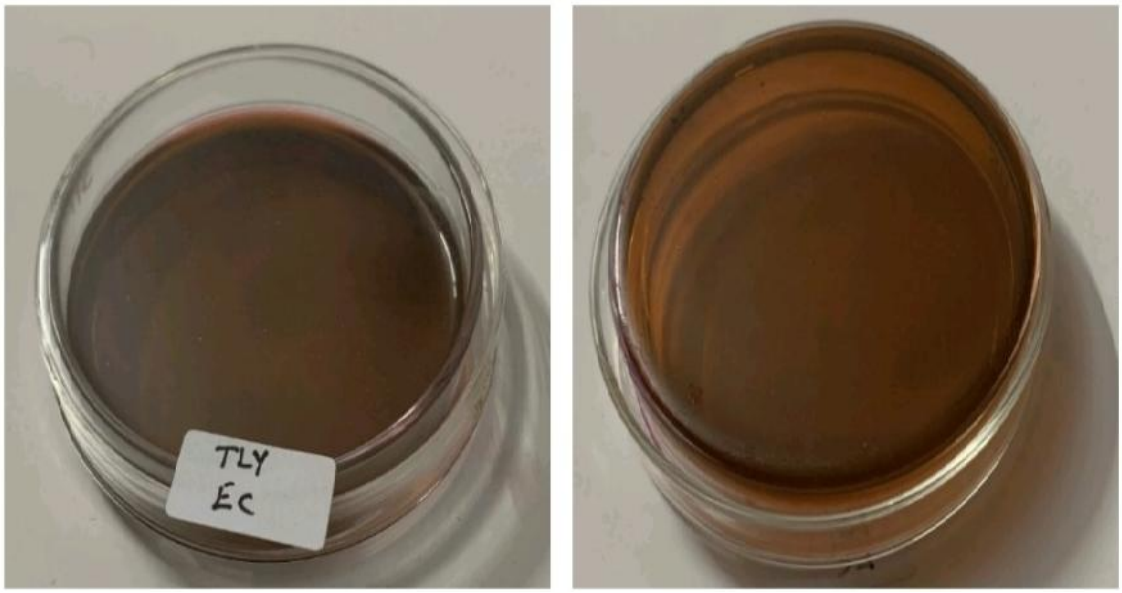
Table 6

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5 CFU/g	As per AYUSH specifications
Total Fungal Count	Absent	NMT 10^3 CFU/g	

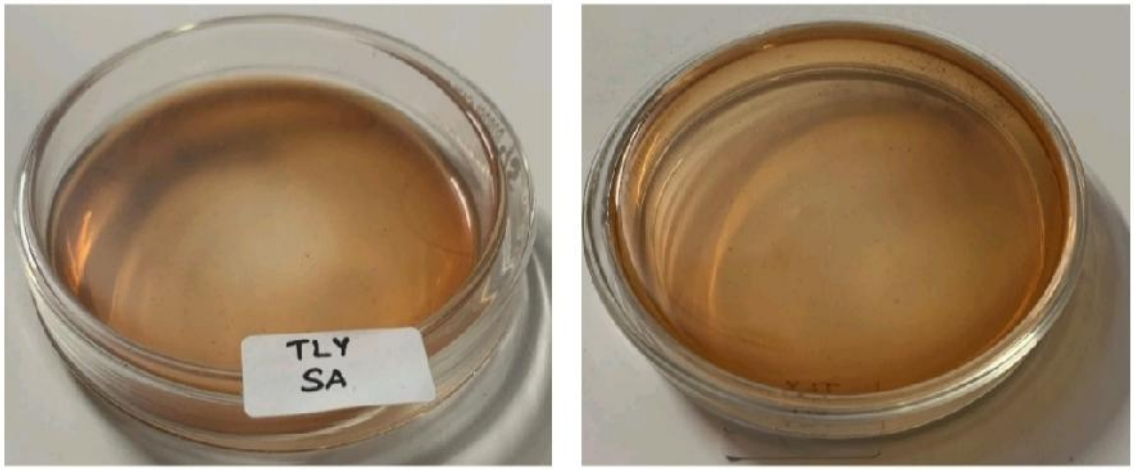
3.4 Test for specific pathogen

Test sample was directly inoculated in to the specific pathogen medium (Eosin Methylene Blue Agar- *E.coli*, Deoxycholate Agar - Salmonella, Mannitol salt Agar- *Staphylococcus aureus*, Cetrimide Agar- *Pseudomonas aeruginosa*) by pour plate method. The plates were incubated at37°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. It was observed that there is no growth after inoculation reveals that the absence of specific pathogen. (Shown in picture 1.1, 1.2, 1.3, 1.4)

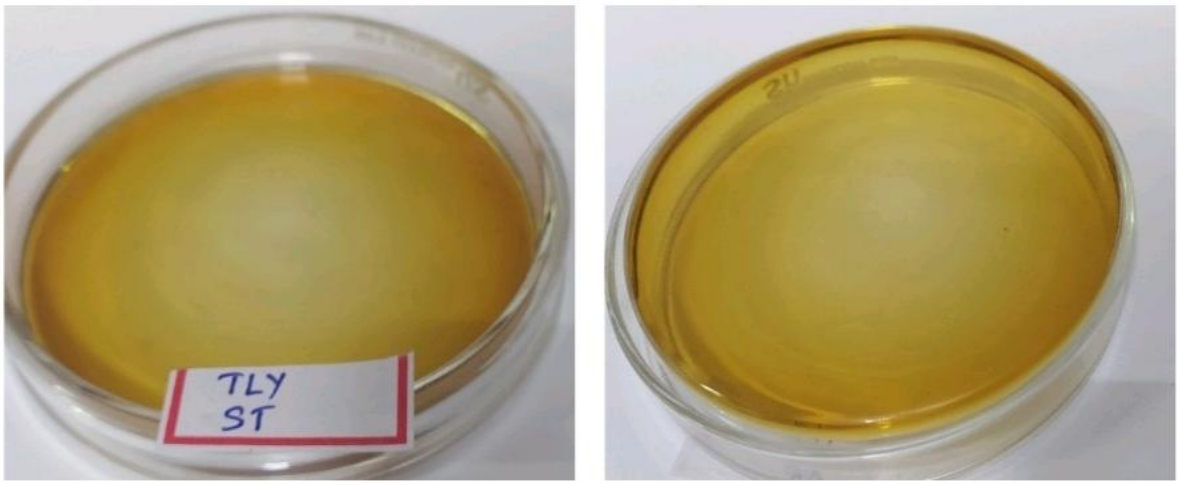
1.1 culture plate with *E.coli (EC) specific medium*



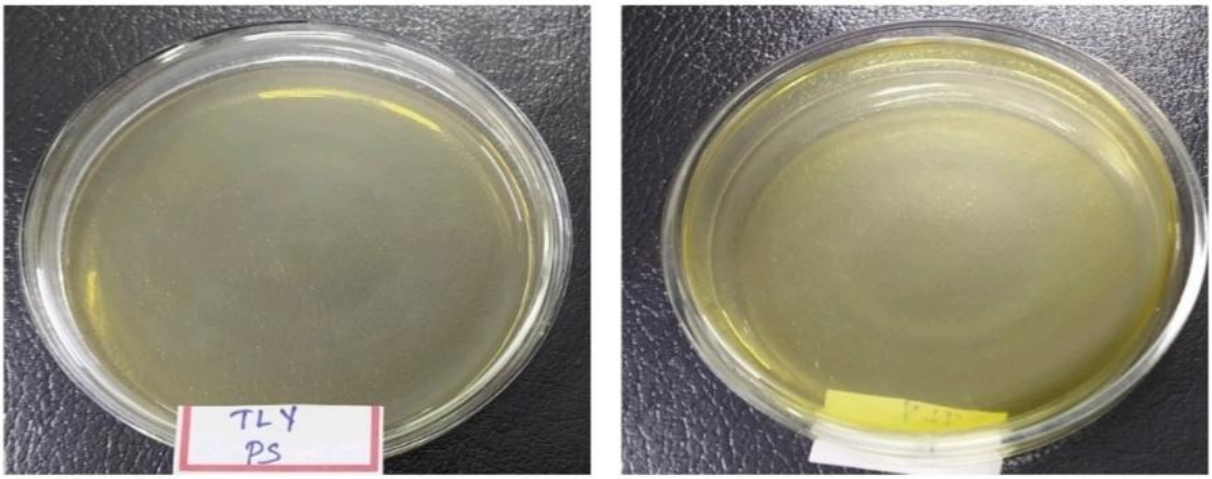
1.2 culture plate with *Salmonella* (SA) specific medium



1.3 culture plate with *Staphylococcus aureus* (ST) specific medium



1.4 Culture plate with *Pseudomonas aeruginosa*(PC) specific medium



3.5 Pesticide residue

Test sample were extracted with 100ml 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 40°C until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter. Result analysis of drug detailed in Table 7and it showed that there were no traces of pesticides residues such as Organochlorine, Organophosphorus, Organocarbamates and pyrethroids in the sample provided for analysis.

Table 7

Pesticide residue	**Sample TLY**	**AYUSH Limit (mg/kg)**
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II. Organo phosphorus Pesticides		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1 mg/kg
III. Organocarbamates		
Carbofuran	BQL	0.1mg/kg
III. pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL-Below Quantification Limit

3.6 Test for Aflatoxins

Standard aflatoxin was applied on to the surface to precoated TLC plate in the volume of 2.5 µL, 5µL, 7.5µL 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 15mm from the origin. Remove the plate from the developing chamber, mark the solvent form and allow the plate to air dry. Locate the spots on the plate by examination under UV light at 365 nm. The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compared to the standard which indicates that the sample free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 and it is detailed in Table 8.

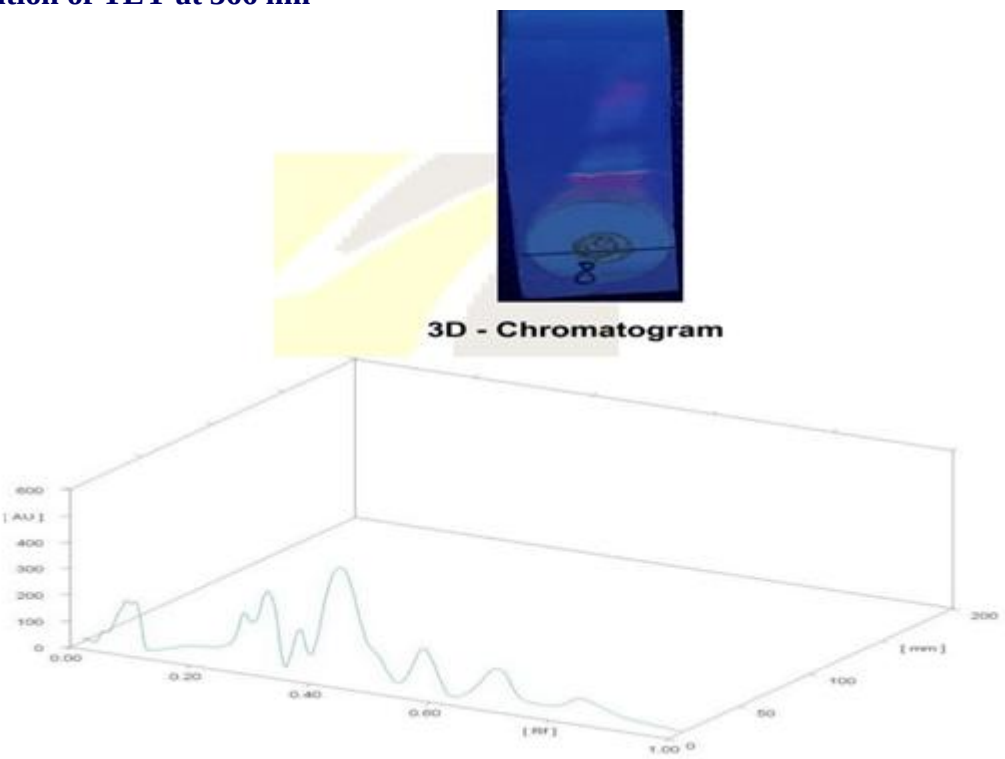
Table 8

Aflatoxin	**Sample TLY**	**AYUSH Specification Limit**
B1	Not Detected-Absent	0.5 ppm
B2	Not Detected-Absent	0.1 ppm
G1	Not Detected-Absent	0.5 ppm
G2	Not Detected-Absent	0.1 ppm

3.7 TLC and HPTLC 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 were used to spot the sample for TLC applied sample volume 10-micro litter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

TLC Visualization of TLY at 366 nm



High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemical which is suitable for confirming the identity and purity of phytotherapeutics.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated. Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated. Test report analysis the Table no 9

HPTLC finger printing of sample TLY

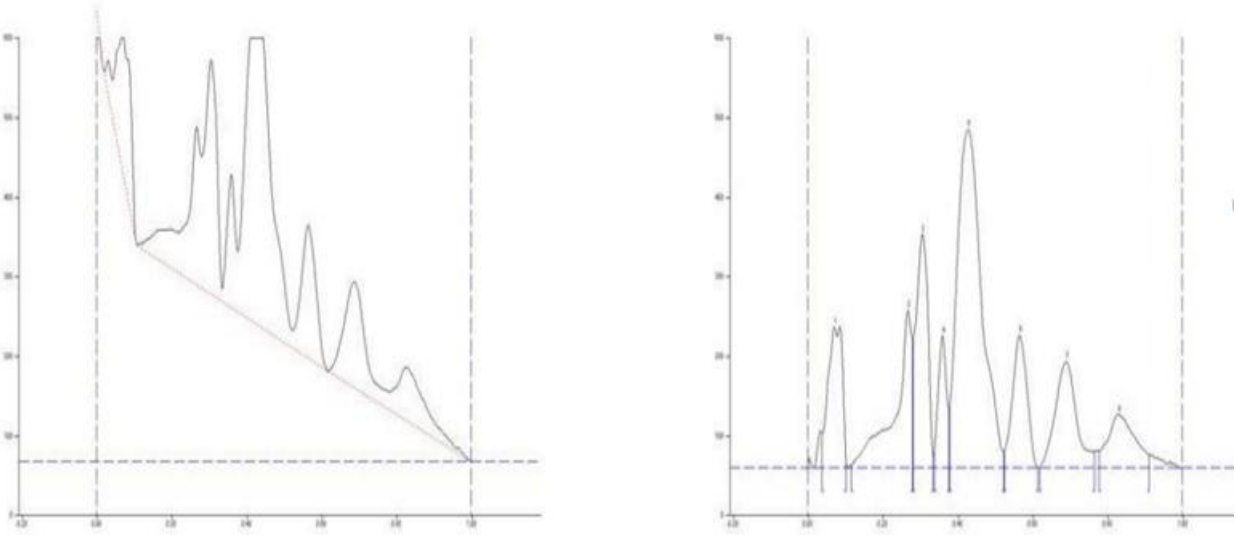


Table 9

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.04	44.3	0.07	178.0	10.92	0.10	5.7	3408.6	9.04
2	0.12	4.1	0.27	198.7	12.19	0.28	164.1	4429.1	11.75
3	0.28	164.8	0.30	293.4	18.00	0.33	15.2	4692.7	12.45
4	0.34	20.1	0.36	166.8	10.24	0.38	75.6	1893.1	5.02
5	0.38	79.1	0.43	425.4	26.10	0.52	21.4	13856.5	36.77
6	0.53	22.6	0.57	167.2	10.26	0.61	0.6	3234.9	8.58
7	0.62	0.1	0.69	133.2	8.17	0.77	20.8	3766.0	9.99
8	0.78	22.0	0.83	67.2	4.13	0.91	16.9	2407.8	6.39

HPTLC finger printing analysis of the sample reveals the presence of eight prominent peaks corresponds to presence of eight phytocomponents present within it. Rf value of the peaks ranges 0.04 to 0.78.

On evaluating the given sample of Thippili Lehiyamtest for heavy metals shows that the results was less than the maximum limit, sterility test indicates there is no microbial contamination, test for Pesticide residue shows there is no trace for pesticide compound, test for Aflatoxins indicates it is free from Aflatoxins compounds, analysis of HPTLC and TLC methods are adopted for identifying Phytotheraputics in that HPTLC finger printing analysis shows the Rf value peak ranges from 0.04 to 0.78

Conclusion

Standardizing the drug on evaluation of analytical specifications of lehiyam as per PLIM guidelines evaluating the test for heavy metal sterility, pesticide residue, aflatoxin and HPTLC results shows the safe to use and it assures to improve the quality of life. with the help of this modern analytical tools widens the acceptance and scope of siddha drugs. Hence, this evaluation method will be helpful for analysing other siddha formulation.

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