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STANDARDIZATION OF SIDDHA POLY HERBAL FORMULATION VILVATHY LEGIUM

A. Divya *, V. Priyadharshini, P. Arul Mozhi and M. Meenakshisundaram

Department of Kuzhandhai Maruthuvam, National Institute of Siddha, The Tamil Nadu Dr. M.G.R Medical University, Chennai - 600032, Tamil Nadu, India.

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Correspondence to Author:
A. Divya

PG Scholar,
Department of Kuzhandhai Maruthuvam, National Institute of Siddha, The Tamil Nadu Dr. M.G.R Medical University, Chennai - 600032, Tamil Nadu, India.

E-mail: divyabsms05@gmail.com

ABSTRACT: The standardization of Siddha formulations is a critical step for establishing consistent chemical profiles and ensuring quality control in the commercial manufacturing of herbal drugs. Vilvathy Legium is a Siddha polyherbal formulation in the Siddha Sastric textbook, *Athmarakshamirtham Ennum Vaidhiya Sarasangiragam*, authored by Kandhasamy Mudhaliyar and indicated for conditions such as gastrointestinal disorders and respiratory tract diseases. To ensure safety and therapeutic efficacy, the current study aimed to standardize Vilvathy Legium according to PLIM guidelines through organoleptic evaluation, physicochemical analysis, and safety testing. Safety assessments confirmed that heavy metals (Pb, Hg, As, Cd), microbial loads, and mycotoxins were well within permissible limits, while all targeted pesticide residues were below the limit of quantification. Preliminary HPTLC fingerprinting at 254 nm, 366 nm, and 580 nm identified distinct chemical peaks for phenolics, flavonoids, and saponins. This study provides quality-indicating physiochemical and chemical fingerprints necessary for the routine quality assurance of Vilvathy Legium.

INTRODUCTION: The Siddha System of Medicine is one of the oldest traditional healthcare systems in the world, originating from the ancient Tamil civilization of South India. The global resurgence of herbal medicine has sparked a renewed interest in the Siddha system of medicine and is now seeing large-scale commercial production. However, one of the impediments in the acceptance of the ancient systems of medicine preparation is the lack of standard quality control profiles [1]. Because Siddha pharmacology relies on a diverse range of herbal, mineral, and metallic preparations, the high demand for raw plant materials has led to frequent issues with adulteration.

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Establishing standard quality management guidelines is therefore critical; it ensures the safety, purity, and reproducibility of formulations. In Siddha system medicines are categorized into 32 internal and 32 external types. One notable internal medicine preparation is Legium, a semisolid medicinal paste created by blending powdered herbs with honey, ghee, or jaggery to enhance both its shelf life and therapeutic delivery [2].

Vilvathy legium is one of the herbal preparation mentioned in the Classic Siddha Sastric text book, *Athmarakshamirtham Ennum Vaidhiya Sarasangiragam*, Authored by *Kandhasamy Mudhaliyar.* Vilvathy legium is indicated for *Vayitru vali* (abdominal discomfort), *Kunmam* (gastric ulcer), *Neerettram* (sinusitis), *Irumal* (cough), *Eelai* (expectorant/asthma), *Kaasam* (respiratory tract diseases), *Nenjerivu* (dyspepsia), *Vaayukirani* (intestinal malabsorption), *Athisaaram* (diarrhea), *Pithakaasam* (bilious cough), *Thegakanthal* (burning sensation of thebody),

Vikkal (hiccups), *Vishapandu* (anemia), *Uppisam* (abdominal bloating*), Vayitruulaichal* (intestinal griping pain), *Vaanthi* (vomiting), *Pitham* (biliousness*),* and *Malakattu* (constipation) [3] in a dosage of *Kottaipakkalvu* which is 10 to 12 gram [4]. The plants *Milagu (Piper nigrum), Sevviyam (Piper nigrum* root*),* and *Thippili (Piper longum)* hold a major part in medicinal preparation of Vilvathy legium. These herbs are characterized by the presence of alkaloids (specifically piperine), steroids, sugars, phenolics, flavonoids, saponins, and tannins [5, 6]. They are collectively recognized as potent rejuvenating *(Karpam)* drugs, they work synergistically to enhance bioavailability, strengthen the digestive system, and clear the respiratory tract.

In recent years, the global demand for Siddha formulations has grown significantly. This surge in interest has brought the commercial manufacture of Siddha medicines into the spotlight both in domestic and international markets. To ensure widespread acceptance, it is imperative to establish rigorous standards for quality control and safety. The aim of this study is to analyse Vilvathy Legium according to PLIM (Pharmacopeial Laboratory of Indian Medicine) guidelines through detailed physicochemical analysis, Test for microbes, Test for heavy metals, Test for pesticides as well as High-Performance Thin Layer Chromatography (HPTLC) [7]. By establishing these parameters, this study aims to create a comprehensive fingerprint for Vilvathy Legium, providing a vital reference for future research and quality assurance.

MATERIALS AND METHODS:

Raw Drugs: All the ingredients of Vilvathy legium were purchased from reputed local raw drug supplier shop and authenticated by the Medicinal Botanist of National Institute of Siddha, Tambaram Sanatorium, Chennai.

Preparation of Vilvathy legium: Purification of raw drugs [8, 9] and preparation of Vilvathy Legium was done at the Department of Gunapadam [Pharmaceutical Laboratory], National Institute of Siddha, Chennai. Purification of raw drugs was done as mentioned in **Table 1.** Following the purification of raw drugs and the test drug Vilvathy legium was prepared with the ingredients in the ratio as mentioned in **Table 2.** Following purification, 87.5 grams of *Vilvaver* was crushed and placed in a pot filled with *Thoonineer* (10.75 litre), then boiled to create a 1/4-part decoction, which is subsequently filtered. To this decoction, 3 palam (105g) of sugar is added and mixed thoroughly. Then, finely ground *Elam, Ilavanagam, Chukku, Sirunagapoo, Thalisapathiri, Milagu, Sevviyam, Thippili, Sathipathiri, Kirambu* powders are incorporated. Then finally, 1/16 padi (81.25ml) of cow's ghee is introduced and thoroughly stirred, then 1/16 padi (81.25ml) of honey is added and thoroughly stirred until the concentrated herbal mass becoming completely non-sticky to the touch when rolled firmly between the fingers. The finished Legium is stored in a clean, air-tight glass container to maintain its quality and efficacy.

The preparation was carried out in using a precise half-measure scale of the formulation's baseline criteria as per reference textbook, yielding an initial raw drug batch size of 1005 g of dry ingredients (comprising 87.5 g *Vilvaver* root, 162.5 g *Milagu*, 162.5 g *Sevviyam*, 162.5 g *Thippili*, 17.5 g each of *Elam*, *Ilavangam*, *Chukku*, *Sirunagapoo*, and *Thalisapathiri*, 8.75 g each of *Saathipathiri* and *Kirambu*, and 105 g of *Sarkarai* sugar) along with 81.25 ml of cow's ghee and 81.25 ml of honey and the final yield of Vilvathy legium obtained was 1100grams.

To ensure reproducible batch processing in a modern pharmaceutical setting, classical Siddha units were converted to metric units in strict accordance with the standards of the *Siddha Pharmacopoeia of India* (SPI) and the standard metrics outlined in the authoritative text *Siddha Marunthakaviyal Vidhigalum Seimuraigalum* by Dr. I. Swarnamariammal (Published by the Department of Indian Medicine and Homeopathy, Chennai, p. 173).

TABLE 1: PURIFICATION OF RAW DRUGS

S. no.	Raw drug name	Botanical name	Purification process
1	Vilvam	*Aegle marmelos*	Washed in fresh water and dried in Sunlight
2	Milagu	*Piper nigrum*	Soaked in buttermilk for 3 hours and dried

3	Sevviyam	*Piper nigrum*	The outer layer is removed and made into small pieces and dried under sunlight
4	Thippili	*Piper longum*	Soaked in lemon juice and dried
5	Elam	*Elettaria cardamomum*	Cleaned and dried under sunlight
6	Ilavangam	*Cinnamomum verum*	Dried under sunlight
7	Chukku	*Zingiber officinale*	Soaked in Sunna neer and dried
8	Sirunagapoo	*Cinnamomum wightii*	Cleaned and dried under sunlight
9	Thalisapathiri	*Abies webbiana*	Cleaned and dried under sunlight
10	Saathipathiri	*Myristica fragrans*	Cleaned and dried under sunlight
11	Kirambu	*Syzygium aromaticum*	Cleaned and dried under sunlight

TABLE 2: INGREDIENTS OF THE VILVATHY LEGIUM

S. no.	Raw drug name	Botanical name	Part used	Quantity
1	Vilvam	*Aegle marmelos*	Root	175g
2	Milagu	*Piper nigrum*	Fruit	325g
3	Sevviyam	*Piper nigrum*	Root	325g
4	Thippili	*Piper longum*	Fruit	325g
5	Elam	*Elettaria cardamomum*	Fruit	35g
6	Ilavangam	*Cinnamomum verum*	Bark	35g
7	Chukku	*Zingiber officinale*	Dried Rhizome	35g
8	Sirunagapoo	*Cinnamomum wightii*	Flower	35g
9	Thalisapathiri	*Abies webbiana*	Leaf	35g
10	Saathipathiri	*Myristica fragrans*	Aril	17.5g
11	Kirambu	*Syzygium aromaticum*	Flower bud	17.5g
12	Sarkarai	*Saccharum officinarum*	Crystallized stem juice	210g
13	Cow's ghee			162.5 ml
14	Honey			162.5 ml

Quality Evaluation and Standardization Procedures: Standardization is a tool in the quality control process. As a preliminary work, the parameters mentioned in the Pharmacopeial Laboratory of Indian Medicine for Ilagam (Confectionary/ Semi solid) were studied [7]. All the following studies were accomplished at Interstellar testing Centre Pvt Limited, Haryana.

Physiochemical Analysis of Vilvathy Legium [7]:

Organoleptic Characters of Vilvathy Legium: Colour, Odour, and Texture were noted. Organoleptic evaluation of Vilvathy legium was carried out using standard techniques.

Loss on Drying: A sample of the test drug was accurately weighed into an evaporating dish. The sample was subsequently dried at 105ºC for 5 hours and then re-weighed to determine the loss on drying.

Determination of Total Ash: The test drug was accurately weighed into a silica dish and incinerated in a furnace at 400°C. The process was continued until the residue turned white, indicating the complete absence of carbon. The percentage of total ash was calculated with reference to the weight of the air-dried drug.

Determination of Acid Insoluble Ash: The ash obtained from the total ash determination was boiled with 25 ml of dilute hydrochloric acid for 6 minutes. The resulting insoluble matter was collected in a crucible, washed with hot water, and ignited to constant weight. The percentage of acid-insoluble ash was then calculated.

Determination of Alcohol Soluble Extractive: The test sample was macerated with 100 ml of alcohol in a closed flask for 24 hours. The mixture was shaken frequently during the first 6 hours and allowed to stand for the remaining 18 hours. After maceration, the solution was filtered rapidly to prevent solvent loss. A 25 mL portion of the filtrate was evaporated to dryness in a tared, flat-bottomed dish and dried at 105°C to a constant weight. Finally, the percentage of alcohol-soluble extractive was calculated based on the weight of the air-dried drug.

Determination of Water-Soluble Extractive: The test sample was macerated with 100 ml of chloroform water in a closed flask for 24 hours. The mixture was shaken frequently during the first 6 hours and then allowed to stand for the following 18 hours.

The solution was filtered rapidly, taking care to prevent the loss of solvent. A 25 ml aliquot of the filtrate was evaporated to dryness in a tared, flat-bottomed shallow dish and dried at 105°C to a constant weight. The percentage of water-soluble extractive was calculated based on the weight of the air-dried drug.

Determination of pH: One gram of the test drug was transferred into a 100 ml graduated cylinder containing approximately 50 ml of water. The cylinder was shaken vigorously for two minutes, and the resulting suspension was allowed to settle for one hour at a temperature between 25°C and 27°C. Subsequently, 25 ml of the clear aqueous supernatant was transferred into a 50 mL beaker, and the pH was measured using a digital pH meter.

Test for Heavy Metal [7]: The heavy metal analysis for the test sample was conducted using Inductively Coupled Plasma Mass Spectrometry (ICPMS). This assessment evaluated the concentration of four specific toxic metals: Arsenic (As), Cadmium (Cd), Lead (Pb), and Mercury (Hg). The sample is digested using 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample was digested with 1 mol/L of HNO_3. This process mineralizes the organic matrix, ensuring the metals are fully extracted into a liquid state for analysis. The resulting solution is then introduced into the Inductively Coupled Plasma Mass Spectrometry (ICPMS) for high-precision quantification.

Test for Specific Pathogen [7]: Testing for specified pathogens (*Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) was performed in accordance with PLIM and WHO guidelines. A 1:10 sample dilution was prepared using Buffered Peptone Water. For selective enrichment, homogenates were inoculated into Rappaport-Vassiliadis Broth (*Salmonella spp.*), MacConkey Broth (*E. coli*), and Fluid Soybean-Casein Digest Medium (*S. aureus* and *P. aeruginosa*), followed by incubation at target temperatures (35°C; 43–45°C for *E. coli*).

Enriched cultures were sub-cultured onto diagnostic selective agars: Xylose Lysine Deoxycholate (XLD) for *Salmonella* spp., MacConkey for *E. coli*, Baird-Parker for *S. aureus*, and Cetrimide for *P. aeruginosa*. To prevent false positives from visual inspection alone, suspected colonies underwent mandatory biochemical validation: Triple Sugar Iron and Urea tests (Salmonella spp.), the IMViC test battery (*E. coli*), Gram staining and Coagulase tests (*S. aureus*), and Oxidase testing (*P. aeruginosa*).

Test for Total Viable Aerobic Count [7]: The Total Viable Aerobic Count was determined using the Plate Count Method. The procedure involved the serial dilution of the sample and subsequent inoculation into a nutrient agar medium using the pour plate method, with the plates incubated at 37°C for 24 to 72 hours.

Test for Aflatoxin (B1, B2, G1, G2) [7]: A standard aflatoxin solution was applied to the surface of a pre-coated TLC plate in volumes of2.5 µL, 5 µL, 7.5 µL, and 10 µL. The test sample was also applied to the plate. After allowing the spots to dry, the chromatogram was developed in an unsaturated chamber containing a solvent mixture of chloroform, acetone, and isopropyl alcohol (85:10:5), until the solvent front had moved at least 15 cm from the origin. Once the plate was removed from the chamber, the solvent front was marked, and the plate was left to air dry. The spots were then located by examining the plate under UV light at 365 nm.

Test for Pesticides Residues [7]: The chromatographic screening program encompassed a comprehensive multi-residue analysis covering all target organochlorine, organophosphorus, and pyrethroid pesticide classes specified under regional regulatory frameworks. Quantitation was executed against multi-component reference standards to verify absolute baseline safety boundaries across all listed analytes.

High Performance Thin Layer Chromatography Analysis [7]:

Sample Preparation for HPTLC Fingerprinting: A test drug quantity of 1.082 g was dissolved and diluted in methanol to reach a final volume of 10.00 ml. This specific preparation created a standardized liquid extract suitable for chromatography. Once the solution was ready, it was loaded into a Linomat 5 applicator, which precisely deposited a 10.0 µl volume onto the

HPTLC plate at a dosage speed of 150nL/s. This process ensures that the chemical constituents of the Vilvathy legium are properly extracted and concentrated enough to produce a clear "fingerprint" across the various scanning wavelengths.

HPTLC Chromatogram Preparation: The chromatographic identification of the test drug sample is conducted using a specific combination of stationary and mobile phases to separate its herbal constituents. The stationary phase consists of Merck HPTLC Silica gel 60 F254 on a plate format of 50x100 mm. The mobile phase is a solvent mixture of Toluene: Ethyl acetate: Formic acid in a volume ratio of 5:4:1. The process begins with chamber saturation, a critical preparation step where the developing tank is lined with a saturation pad and filled with the mobile phase a mixture of Toluene, Ethyl acetate, and Formic acid (5:4:1) for 20 minutes. This ensures the air inside the tank is filled with solvent vapours, preventing the liquid from evaporating off the plate during the test and ensuring the herbal constituents migrate in straight, consistent lines. Once the environment is stabilized, the Merck HPTLC Silica gel 60 F_{254} plate (50 x 100 mm) is placed inside, and the solvent is allowed to travel up the silica "track" to a distance of 70 mm. After development, the plate is dried at room temperature for 5 minutes and scanned at wavelengths of 254 nm, 366 nm, and 580 nm to detect the unique chemical peaks that serve as the fingerprint for the test drug.

Chromatogram Scanning: After the plate is developed and dried, the final stage is the measurement and documentation of the chemical fingerprint through instrumental scanning. Using a TLC Scanner 4, the sample is analysed at three distinct wavelengths to capture a complete profile of its herbal constituents: 254 nm (Deuterium lamp) for absorbance, 366 nm (Mercury lamp) for fluorescence, and 580 nm (Tungsten lamp) for visible light detection following post-chromatographic derivatization by spraying uniformly with Vanillin-Sulfuric Acid reagent and heating at 105°C."During this process, the scanner moves across the plate at a speed of 20 mm/s with a data resolution of 100µm/step, converting the physical spots on the silica gel into electronic peaks on a chromatogram. These scans allow analysts to see compounds that are otherwise invisible to the naked eye, measuring the intensity (AU) and position (R_f) of each substance to confirm the identity. Given the qualitative scope of this baseline profiling, the analysis was executed without isolated reference standards, serving as a preliminary baseline chromatographic fingerprint.

RESULTS AND DISCUSSION: In the physicochemical testing, the test drug Vilvathy legium was characterized as a dark brown coloured semi-solid mass. The analysis revealed a Loss on Drying at 105°C of 52.19% w/w. This notably high value indicates a high moisture footprint within the formulation matrix. While a high moisture level is common in traditional legium due to the inclusion of liquid decoctions, honey, and ghee, it raises significant considerations regarding long-term storage stability and susceptibility to micro-environmental spoilage. High water availability typically drives enzymatic hydrolysis and chemical degradation of volatile bioactive compounds. Although this is structurally co-balanced by a Total Solid Content of 47.81% w/w which establishes a dense, concentrated herbal matrix supporting dosage standardization the elevated moisture baseline remains a critical variable for shelf-life management.

The total ash of 1.32% by mass and low acid insoluble ash of 0.88% w/w, a significant finding that indicates a high degree of purity and the absence of extraneous inorganic matter like sand or soil. Solubility markers for Vilvathy legium, including water soluble extractive (15.30% w/w) and alcohol soluble extractive (12.79% w/w), signify the concentration of active polar and non-polar constituents available for therapeutic action. Polar constituents found in the water extract typically include tannins, glycosides, and mucilage, which contribute to the drug's bioactivity. Conversely, the alcohol extract indicates the presence of non-polar compounds such as alkaloids, resins, and steroids. These markers together ensure that the formulation contains medicinal efficacy.

A pH of 5.03 and total acidity of 0.39 signify a slightly acidic environment, which is vital for maintaining the stability of herbal active ingredients and preventing the degradation of

sensitive phytochemicals. The quantification of total sugar (11.66% w/w) and reducing sugar (4.41% w/w) in Vilvathy legium serves as a vital chemical fingerprint for the traditional formulation. These sugars are significant as they act as natural preservatives by reducing water activity, which helps prevent microbial spoilage in the high-moisture semi-solid mass.

The results for the microbiological and mycotoxin analysis, as detailed in **Table 6**, demonstrate that the sample is in full compliance with safety requirements. All tested pathogens, including *Escherichia coli*, *Salmonella species*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were found to be absent, indicating high standards of hygiene and the absence of infectious agents.

The microbial safety evaluation revealed a Total Viable Aerobic Count of 60,000 CFU/g. While this complies with the maximum permissible limits (100,000 CFU/g) stipulated by PLIM and WHO guidelines, it represents a substantial baseline bioburden that warrants cautious interpretation given the formulation's high moisture profile (52.19% w/w). This elevated baseline creates a potential vulnerability to microbial proliferation and secondary fermentation during storage. Consequently, it should not be treated as an unqualified confirmation of long-term safety. To preserve product integrity, strict post-manufacturing controls are essential; the formulation requires packaging in airtight glass or high-density polyethylene (HDPE) containers to prevent ambient moisture absorption, alongside storage in cool, dry conditions.

Furthermore, the Total Yeast and Mould Count was exceptionally low at less than 10 cfu/g, far below the threshold of 1,000 cfu/g, suggesting excellent environmental control against fungal spoilage. Finally, the concentration of total aflatoxins (B1, B2, G1, and G2) was determined to be below the limit of quantitation (BLQ), staying safely beneath the maximum requirement of 2.0. Collectively, these findings confirm that the sample meets the necessary safety specifications for consumer use. Safety evaluation revealed that Lead (Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (As) were within the maximum permissible limits stipulated by PLIM and WHO guidelines. Specifically, while the detected concentration of Lead complied with regulatory thresholds, its presence is not entirely negligible. Because heavy metals can exhibit cumulative biological profiles over prolonged therapeutic windows, these results confirm that this specific batch strictly complies with the tested regulatory safety limits rather than establishing an absolute absence of toxicological risk. Continuous monitoring across manufacturing batches remains imperative to ensure uniform quality control

The analysis for pesticide residues confirms that the sample is highly compliant with established safety standards. All eleven targeted pesticide parameters yielded results that are Below the Limit of Quantification (BLQ).This consistent lack of detectable chemical contaminants across all tested variables demonstrates that the product is safe from pesticide-related hazards.

The HPTLC chemical fingerprint of Vilvathy Legium provides a characteristic visual map for baseline identification and quality control. Because this profiling was executed without isolated pure reference standards, the chromatogram is presented as a preliminary qualitative fingerprint rather than a fully validated quantitative standard. Under short-wave UV light at 254 nm, the sample shows a complex profile consisting of 10 to 11 distinct peaks.

This wavelength highlighted a dominant UV-absorbing marker at an R_f value of approximately 0.677, which made up 32.48% to 36.67% of the total area; this absorption band is characteristically associated with UV-absorbing phytochemical classes, such as phenolics and flavonoids. When analyzed under long-wave fluorescence at 366 nm, the pattern remained highly consistent, showing 9 to 11 peaks. The primary fluorescent peak appeared at Rf0.682–0.685 and accounted for 23.25% to 26.05% of the total area, which characteristically aligns with naturally fluorescent fractions like coumarins or alkaloids under long-wave UV light.

Following post-chromatographic chemical derivatization with Vanillin-Sulfuric Acid reagent, scanning under visible light at 580 nm simplified the fingerprint down to 6 distinct peaks.

At this wavelength, a single major component stands out near the start of the run at R_f 0.027–0.029, representing nearly 50% of the total area. This peak is tentatively suggestive of constituents like saponins or terpenoids that typically require a chemical spray reagent to react and become visible in the spectrum.

Without co-chromatography alongside pure reference standards, these broad constituent-class assignments remain tentative structural indications based on literature characteristics rather than definitive structural identifications.

However, the high degree of correlation between the two sample tracks across all scanned wavelengths demonstrates excellent operational repeatability,

TABLE 3: ORGANOLEPTIC CHARACTERS OF TEST DRUG

Parameter	Result	Method
Colour	Dark brown	Visual Examination
Odour	Characteristic	Organoleptic
Consistency	Semi solid mass	Chemically/In house

TABLE 4: PHYSIOCHEMICAL ANALYSIS OF TEST DRUG

Parameter	Result (with Unit)
Loss on drying at 105 degrees Celsius	52.19% w/w
Total ash	1.32% By Mass
Acid insoluble ash	0.88% w/w
Alcohol Soluble Extractive	12.79% w/w
Water Soluble Extractive	15.30% w/w
pH (10% w/v solution)	5.03
Total Solid content	47.81% w/w
Reducing Sugar	4.41% w/w
Total Sugar	11.66% w/w

TABLE 5: HEAVY METAL ANALYSIS OF TEST DRUG

Heavy Metal	Requirement (Max. Limit)	Result	Status
Arsenic (As)	Max. 3ppm	0.34 ppm	Within limit
Cadmium (Cd)	Max. 0.3ppm	BLQ (LOQ: 0.10 ppm)	Within limit
Lead (Pb)	Max. 10 ppm	4.51 ppm	Within limit
Mercury (Hg)	Max. 1 ppm	0.18 ppm	Within limit

TABLE 6: TEST FOR PATHOGENS, MICROBIAL LOAD AND MYCOTOXINS

Category	Parameter	Requirement (Max/Limit)	Result
Pathogens	*Escherichia coli*	Absent	Absent
	Salmonella species	Absent	Absent
	Staphylococcus aureus	Absent	Absent
	Pseudomonas aeruginosa	Absent	Absent
Microbial Load	Total Viable Aerobic Count	NMT 100,000	60,000 cfu/g
	Total Yeast and Mould Count	NMT 1,000	<10 cfu/g
Mycotoxins	Aflatoxin (B1+B2+G1+G2)	Max. 2.0	BLQ (LOQ: 1.0)

TABLE 7: TEST FOR PESTICIDE RESIDUES

Parameter	Limit (Max ppm)	Result
Azinphos-methyl	1.0	BLQ (LOQ: 0.01)
Chlorfenvinphos	0.5	BLQ (LOQ: 0.01)
Chlorpyriphos	0.2	BLQ (LOQ: 0.01)
Chlorpyrifos-methyl	0.1	BLQ (LOQ: 0.01)
Diazinon	0.5	BLQ (LOQ: 0.01)
Dichlorvos	1.0	BLQ (LOQ: 0.01)
Ethion	2.0	BLQ (LOQ: 0.01)
Fenitrothion	0.5	BLQ (LOQ: 0.01)
Malathion	1.0	BLQ (LOQ: 0.01)
Parathion	0.5	BLQ (LOQ: 0.01)
Parathion-methyl	0.2	BLQ (LOQ: 0.01)

BLQ: Below Limit of Quantification, LOQ: Limit of Quantification

TABLE 8: HPTLC CHROMATOGRAPHIC FINGERPRINT PROFILE UNDER ULTRAVIOLET LIGHT AT 254 NM ON TRACK 1

Peak #	Start Rf	Start H	Max Rf	Max H	Max %	End Rf	End H	Area A	Area %	Manual Peak
1	0.015	0.0000	0.056	0.5886	34.14	0.079	0.1362	0.01317	20.05	No
2	0.155	0.0308	0.171	0.0493	2.86	0.216	0.0057	0.00156	2.38	No
3	0.252	0.0086	0.279	0.0304	1.76	0.305	0.0055	0.00091	1.39	No

4	0.305	0.0055	0.321	0.0211	1.23	0.335	0.0000	0.00034	0.52	No
5	0.335	0.0000	0.368	0.0461	2.67	0.402	0.0055	0.00149	2.26	No
6	0.402	0.0055	0.434	0.0422	2.45	0.481	0.0043	0.00175	2.66	No
7	0.516	0.0004	0.553	0.0222	1.29	0.590	0.0000	0.00085	1.29	No
8	0.615	0.0150	0.677	0.4532	26.29	0.729	0.0771	0.02408	36.67	No
9	0.731	0.0769	0.752	0.0980	5.68	0.803	0.0341	0.00483	7.36	No
10	0.834	0.0367	0.874	0.2404	13.94	0.935	0.0044	0.01200	18.27	No
11	0.937	0.0038	0.973	0.1325	7.68	1.000	0.0003	0.00468	7.13	No

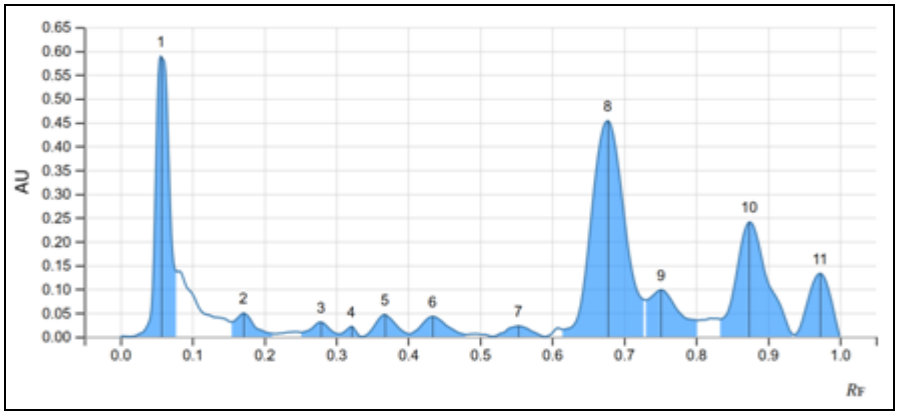

FIG. 1: HPTLC CHROMATOGRAPHIC FINGERPRINT PEAK PROFILE AT 254 NM ON TRACK 1

TABLE 9: HPTLC CHROMATOGRAPHIC FINGERPRINT PROFILE UNDER ULTRAVIOLET LIGHT AT 254 NM ON TRACK 2

Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %	Manual Peak
1	0.013	0.0000	0.053	0.5796	34.69	0.135	0.0266	0.01718	24.28	No
2	0.155	0.0183	0.174	0.0403	2.41	0.211	0.0018	0.00112	1.58	No
3	0.247	0.0043	0.279	0.0249	1.49	0.305	0.0025	0.00075	1.05	No
4	0.342	0.0000	0.373	0.0403	2.41	0.400	0.0048	0.00119	1.68	No
5	0.413	0.0074	0.439	0.0381	2.28	0.498	0.0015	0.00160	2.27	No
6	0.518	0.0000	0.561	0.0288	1.72	0.598	0.0033	0.00114	1.61	No
7	0.598	0.0033	0.677	0.4308	25.78	0.727	0.0848	0.02298	32.48	No
8	0.727	0.0848	0.748	0.1083	6.48	0.792	0.0509	0.00534	7.55	No
9	0.806	0.0485	0.869	0.2412	14.43	0.932	0.0183	0.01408	19.90	No
10	0.932	0.0183	0.969	0.1387	8.30	1.000	0.0002	0.00538	7.60	No

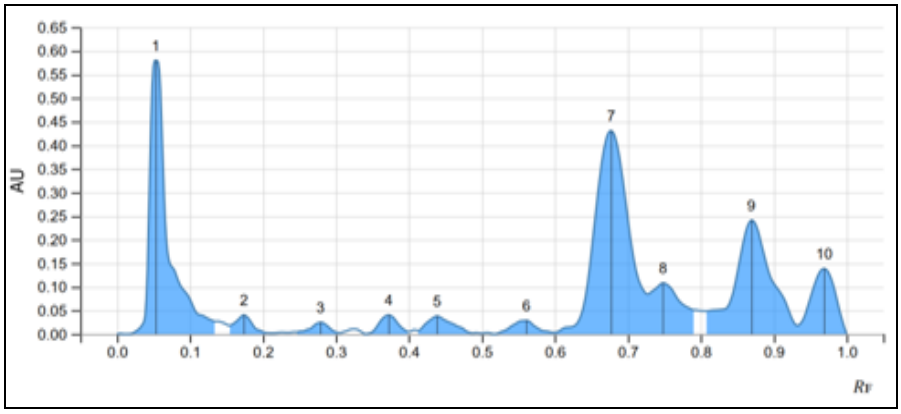

FIG. 2: HPTLC CHROMATOGRAPHIC FINGERPRINT PEAK PROFILE AT 254 NM ON TRACK 2

TABLE 10: HPTLC CHROMATOGRAPHIC FINGERPRINT PROFILE UNDER FLUORESCENCE LIGHTAT 366 NM ON TRACK 1

Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %	Manual Peak
1	0.000	0.0000	0.047	0.5486	27.89	0.094	0.0326	0.01173	17.28	No
2	0.113	0.0449	0.132	0.1305	6.63	0.155	0.0407	0.00320	4.71	No
3	0.184	0.0395	0.232	0.1311	6.67	0.252	0.0836	0.00540	7.95	No
4	0.252	0.0836	0.273	0.2238	11.38	0.298	0.0560	0.00649	9.56	No

5	0.298	0.0560	0.315	0.1133	5.76	0.326	0.0668	0.00224	3.29	No
6	0.326	0.0668	0.363	0.1847	9.39	0.403	0.0739	0.01006	14.82	No
7	0.403	0.0739	0.432	0.1150	5.85	0.463	0.0561	0.00552	8.13	No
8	0.463	0.0561	0.471	0.1486	7.55	0.489	0.0439	0.00205	3.02	No
9	0.655	0.2067	0.682	0.2603	13.23	0.735	0.0685	0.01578	23.25	No
10	0.737	0.0682	0.755	0.0900	4.57	0.808	0.0000	0.00398	5.87	No
11	0.808	0.0000	0.839	0.0212	1.08	0.906	0.0087	0.00142	2.10	No

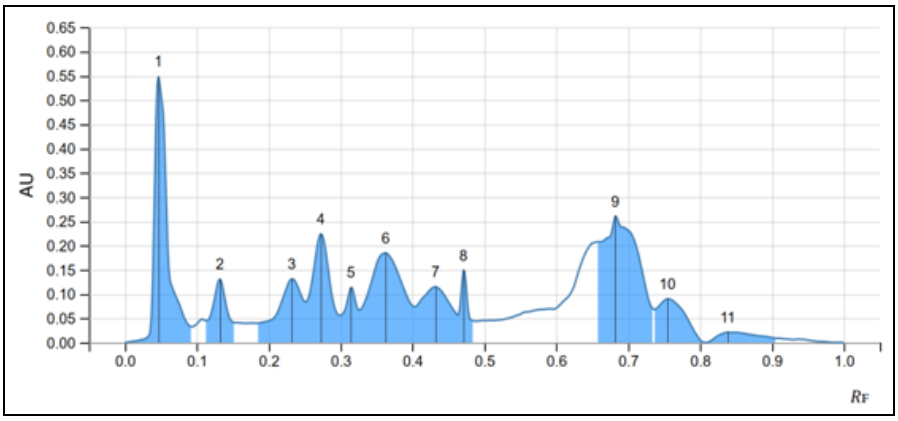

FIG. 3: HPTLC CHROMATOGRAPHIC FINGERPRINT PEAK PROFILE AT 366 NM ON TRACK 1

TABLE 11: HPTLC CHROMATOGRAPHIC FINGERPRINT PROFILE UNDER FLUORESCENCE LIGHTAT 366 NM ON TRACK 2

Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %	Manual peak
1	0.000	0.0000	0.037	0.5313	31.28	0.084	0.0327	0.01157	18.22	No
2	0.110	0.0402	0.131	0.1159	6.82	0.153	0.0392	0.00302	4.76	No
3	0.198	0.0441	0.234	0.1242	7.31	0.250	0.0906	0.00449	7.06	No
4	0.250	0.0906	0.271	0.2129	12.54	0.298	0.0544	0.00621	9.78	No
5	0.298	0.0544	0.316	0.0956	5.63	0.326	0.0681	0.00201	3.16	No
6	0.326	0.0681	0.360	0.1718	10.12	0.400	0.0779	0.00927	14.59	No
7	0.400	0.0779	0.427	0.1095	6.45	0.482	0.0432	0.00634	9.99	No
8	0.645	0.2085	0.685	0.2453	14.44	0.729	0.0751	0.01654	26.05	No
9	0.729	0.0751	0.747	0.0919	5.41	0.797	0.0000	0.00406	6.39	No

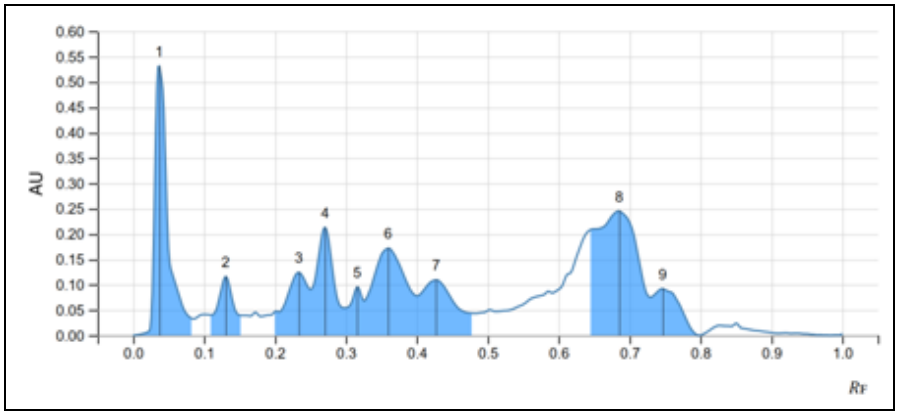

FIG. 4: HPTLC CHROMATOGRAPHIC FINGERPRINT PEAK PROFILE AT 366 NM ON TRACK 2

TABLE 12: HPTLC CHROMATOGRAPHIC FINGERPRINT PROFILE UNDER VISIBLE LIGHT SCANAT 580NM ON TRACK 1

Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %	Manual peak
1	0.000	0.0000	0.029	0.5588	49.02	0.158	0.0000	0.02392	41.80	No
2	0.318	0.0057	0.347	0.0659	5.78	0.384	0.0016	0.00213	3.73	No
3	0.385	0.0016	0.424	0.0433	3.80	0.460	0.0000	0.00150	2.62	No
4	0.547	0.0000	0.661	0.2895	25.40	0.716	0.0496	0.02052	35.87	No
5	0.716	0.0496	0.735	0.0643	5.64	0.816	0.0000	0.00316	5.52	No
6	0.818	0.0000	0.879	0.1182	10.36	0.939	0.0000	0.00598	10.46	No

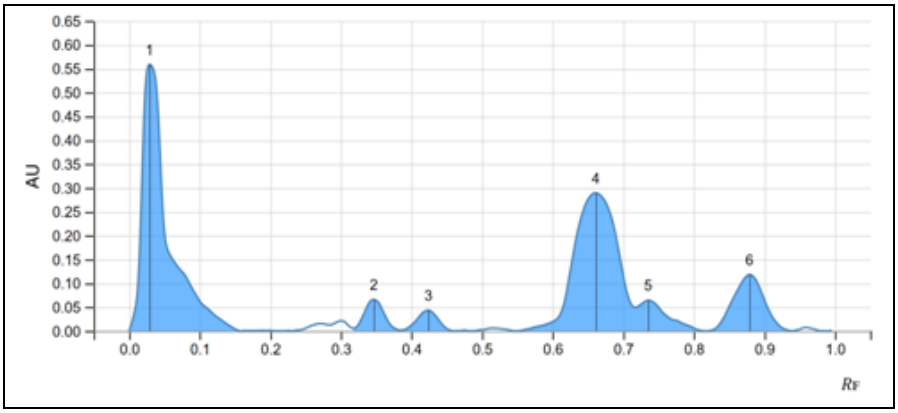

FIG. 5: HPTLC CHROMATOGRAPHIC FINGERPRINT PEAK PROFILE AT 580 NM ON TRACK 1

TABLE 13: HPTLC CHROMATOGRAPHIC FINGERPRINT PROFILE UNDER VISIBLE LIGHT SCANAT 580NM ON TRACK 2

Peak #	Start RF	Start H	Max RF	Max H	Max %	End RF	End H	Area A	Area %	Manual Peak
1	0.000	0.0000	0.027	0.5648	50.96	0.119	0.0000	0.02986	49.01	No
2	0.321	0.0100	0.348	0.0701	6.33	0.387	0.0002	0.00225	3.70	No
3	0.387	0.0002	0.423	0.0477	4.31	0.456	0.0000	0.00156	2.56	No
4	0.556	0.0063	0.656	0.2819	25.43	0.713	0.0560	0.02045	33.56	No
5	0.823	0.0000	0.876	0.1236	11.15	0.931	0.0089	0.00599	9.83	No
6	0.932	0.0087	0.961	0.0201	1.81	0.995	0.0010	0.00082	1.34	No

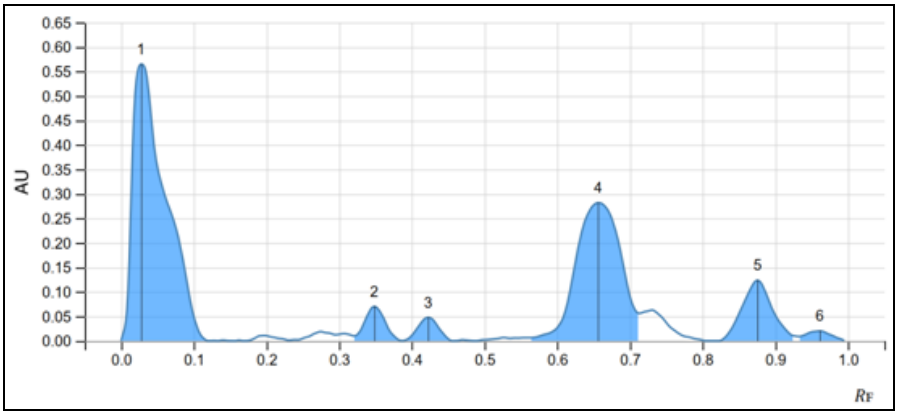

FIG. 6: HPTLC CHROMATOGRAPHIC FINGERPRINT PEAK PROFILE AT 580 NM ON TRACK 2

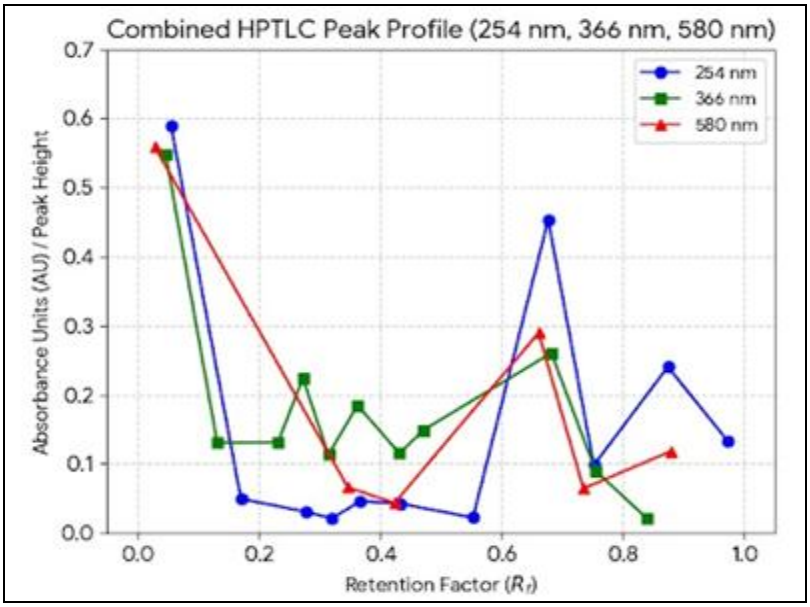

FIG. 7: COMBINED HPTLC PEAK PROFILE (254NM, 366NM, 580NM)

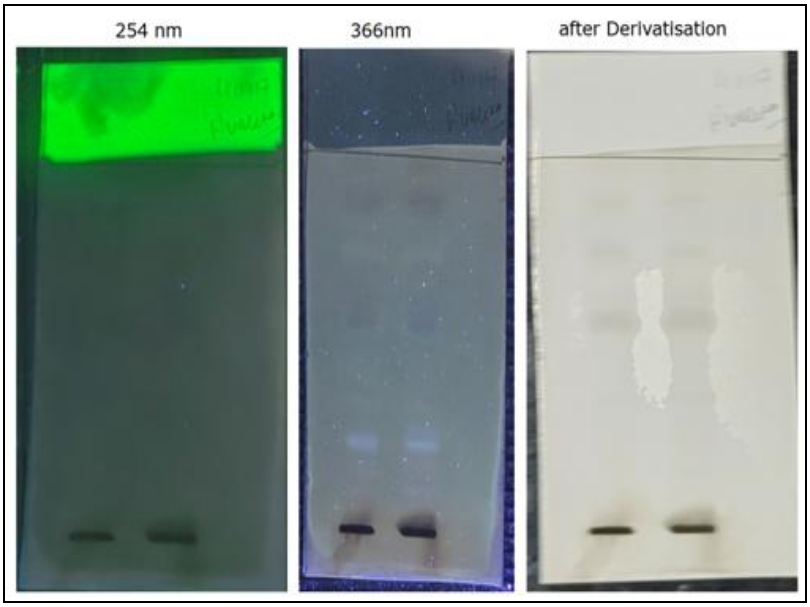

FIG. 8: HPTLC PEAK ANALYSIS AND CHROMATOGRAM VISUALIZATION

CONCLUSION: In conclusion, this study successfully establishes the first preliminary quality control and standardization baseline for Vilvathy Legium prepared on a precise laboratory scale. The formulation complies with all tested regulatory safety limits, demonstrating parameters within permissible microbial thresholds alongside conforming benchmarks for heavy metals, specific pathogens, mycotoxins, and pesticide residues.

While the detected heavy metal concentrations including Lead fully comply with established PLIM and WHO limits, their trace presence necessitates careful interpretation rather than broad assumptions of absolute safety. While the metrics defined here are bounded by a single-sample pilot design, the highly reproducible distribution of phytochemicals shown in the Preliminary HPTLC fingerprinting confirms a precise extraction process.

Ultimately, these baseline parameters provide an essential stepping stone for routine batch-to-batch quality checks, continuous toxicological monitoring, and future multi-batch industrial scale-up validation of this classical Siddha polyherbal matrix.

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