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ANALYTICAL FINGERPRINTING OF THE SIDDHA POLYHERBAL REMEDY LAVANGADHI VADAGAM (LV) USING FTIR, TLC/HPTLC AND ICP-OES –A QUALITY CONTROL APPROACH

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ABSTRACT

Siddha polyherbal formulations, rooted in traditional medicine, offer promising holistic management due to their synergistic bioactive constituents. However, standardization and quality control remain critical challenges for their wider acceptance. The present study focuses on the analytical fingerprinting of a Siddha polyherbal remedy Lavangadhi Vadagam (LV) using advanced instrumental techniques, namely Fourier Transform Infrared Spectroscopy (FTIR), High Performance Thin Layer Chromatography (HPTLC), and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). FTIR analysis was employed to identify functional groups and characterize major phytochemical classes present in the formulation. HPTLC profiling provided a comprehensive chromatographic fingerprint, enabling the detection and comparison of key bioactive compounds. ICP-OES analysis was conducted and to assess the presence of heavy metals, ensuring safety and compliance with pharmacopeial standards. The integration of these analytical techniques establishes a robust quality control framework for the Siddha formulation LV.

Keywords: Lavangadhi Vadagam (LV), Siddha, Instrumental analysis, HPTLC, FT-IR, ICP-OES

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INTRODUCTION

The Siddha system of medicine is one of the popular traditional holistic health care systems of south India which comprises 32 forms of internal medicine. One among them is “Vadagam” which is correlated with Lozenges in modern medical system. The drug Lavangadhi Vadagam (LV) is known to cure indications like Neeradaippu, Kalladaippu, Soothagavayu, Yeppam, Kandhal, Varatchi as per the Siddha classical text “Aaviyalikkum Amudhamurai Surukkam”. Despite its extensive traditional use, scientific validation and quality standardization have become increasingly important in the modern healthcare system. In

addition, concerns regarding adulteration, contamination, and heavy metal toxicity necessitate the implementation of reliable analytical techniques to ensure the safety, efficacy, and reproducibility of traditional medicines. Therefore, the present investigation was undertaken to scientifically validate and standardize the Siddha polyherbal formulation LV using FTIR, HPTLC, and ICP-OES analytical technique to establish a reliable phytochemical and elemental fingerprint profile that can support the quality control, authenticity, safety evaluation, and wider acceptance of Siddha formulations in contemporary healthcare and pharmaceutical research.

MATERIALS AND METHODS

Drug Profile [1]

Drug name: LavangadhiVadagam(LV)

Dosage: Kottaipaakkalavu (6.022g)

Route: Oral Administration

Indications: Neeradaippu, Kalladaippu, Soothagavayu, Yeppam, Vayu, Soolai, Kandhal, Varatchi.

Table 01: Ingredients of LV

S.NO	TAMIL NAME	BOTANICAL NAME	QUANTITY	PARTS USED
1.	Lavangam	<i>Syzygium aromaticum</i>	100 varagan (420g)	Dried Flower bud
2.	Sirunaga poo	<i>Mesua ferrea</i>	100 varagan (420g)	Dried Flower bud
3.	Vilamichu ver	<i>Coleus vettiveroides</i>	100 varagan (420g)	Dried Root
4.	Chukku	<i>Zingiber officinale</i>	100 varagan (420g)	Dried Rhizome
5.	Milagu	<i>Piper nigrum</i>	100 varagan (420g)	Dried Fruit
6.	Thippili	<i>Piper longum</i>	100 varagan (420g)	Dried Fruit
7.	Vellam	<i>Saccharum officinarum</i>	120 varagan (504g)	Jaggery

COLLECTION OF RAW DRUGS

All the above ingredients were bought from an authentic country drug store.

IDENTIFICATION AND AUTHENTICATION

All the drugs were identified and authenticated by Botanist and Gunapadam experts of Government Siddha Medical College, Arumbakkam, Chennai. The identified product Samples were maintained in the PG Gunapadam laboratory for future references.

PURIFICATION OF THE DRUG

Purification process was made according to the procedures mentioned in the classical Siddha literature 2 (Sarakkugalin Suthi Sei Muraigal).

METHOD OF PREPARATION OF LAVANGADHIVADAGAM

- The above drugs are taken dried, finely powdered and subjected to *vasthirakayam*.
- Jaggery was added to it and mixed.
- Then the mixture was purified by *pittaviyal* method.
- Then the mixture in the muslin doth will be cooled.
- Finally the mixture rolled into pills of the size of *kottaipaaku* (6.022g)

ANALYTICAL INSTRUMENTATION TECHNIQUES - METHODOLOGY

Sample Preparation for TLC One gram sample was sonicated in 10 ml of ethanol for 15 minutes and then filtered. This solution was used for TLC.

TLC/HPTLC Methodology

Applied 15, 20 µl of sample extract on TLC plate using Camag's ATS4 applicator and developed by the Mobile phase: Toluene: Ethyl acetate: Acetic acid (9:1:0.5, v/v) up to 8 cm distance. After development, the plate was photo documented using Camag's TLC Visualizer under UV 254 nm and UV 366 nm. Scanned the plate using Camag's Scanner 4 at UV 254 nm (D 2 lamp/Absorption mode) finger print profiles of the extract were documented. Then the plate was dipped in vanillin-sulphuric acid reagent followed by heating at 105 °C till development of coloured spots. The plate was then photo documented in white light and scanned at 520nm (W lamp/Absorption mode)

FTIR METHODOLOGY [3 & 4]

Fourier Transform Infrared (FTIR) analysis was carried out to identify the functional groups present in the sample following standard procedures reported in similar studies. The sample was dried, finely powdered, and a small quantity was mixed with potassium bromide (KBr) and compressed into a thin pellet (or analyzed directly using ATR mode). The spectrum was recorded using an FTIR spectrophotometer over the range of 4000–400 cm⁻¹ at a resolution of about 4 cm⁻¹ with multiple scans to ensure accuracy. A background spectrum was obtained prior to analysis. The resulting absorption peaks were interpreted based on characteristic wave numbers corresponding to different functional groups such as hydroxyl, carbonyl, alkane, alkene, and amine groups, and the obtained spectral data were compared with standard references to determine the chemical constituents present in the sample.

ICP-OES METHODOLOGY [5]

The heavy metals in the sample were analyzed using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES) following AOAC Official Methods of Analysis (22nd Ed., 2023). Approximately 0.5–1.0 g of the sample was accurately weighed and subjected to acid digestion using concentrated nitric acid and hydrogen peroxide in a microwave digestion system until a clear solution was obtained. The digested sample was cooled, diluted to a known volume with deionized water, and filtered if necessary. Calibration standards were prepared from multi-element standard solutions, and a blank was used for baseline correction. The prepared solutions were introduced into the ICP-OES instrument, where elements such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) were quantified based on their characteristic emission wavelengths. Quality control measures including blanks and standard checks were employed to ensure accuracy. Results were expressed in mg/kg, and values below the limit of quantification were reported as BLQ, with limits of quantification set

at 2.0 mg/kg for As, 0.1 mg/kg for Cd, 1.0 mg/kg for Pb, and 0.5 mg/kg for Hg.

RESULTS

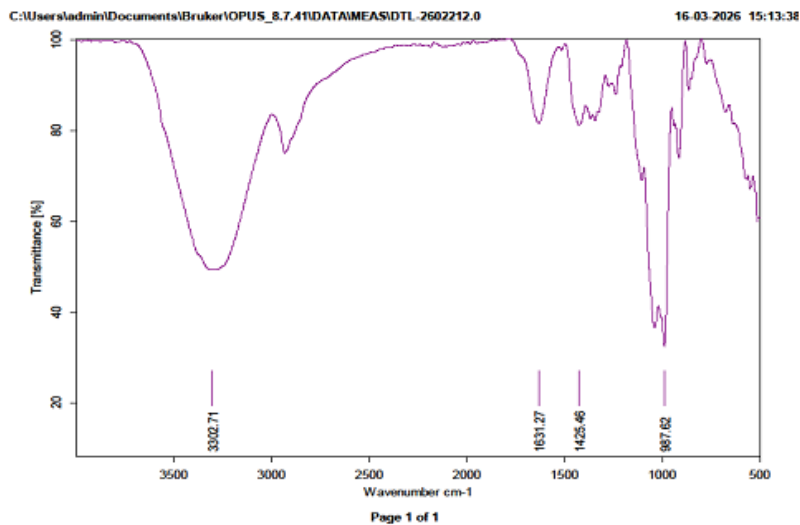


Figure 01: FTIR peak spectrum of LV

FTIR INTERPRETATION

Table 01: FTIR spectral peaks and their corresponding functional groups with indications in the formulation

S. NO.	WAVENUMBER (CM ⁻¹)	FUNCTIONAL GROUP	INDICATION
1	3302.71	O–H stretching	Indicates the presence of hydroxyl (–OH) groups, adsorbed moisture, alcohols, phenols, or hydrated inorganic constituents. Suggests hydrogen-bonded hydroxyl groups commonly found in herbo-mineral formulations.
2	1631.27	C=O stretching / H–O–H bending	Indicates carbonyl-containing compounds such as amides or ketones and may also represent bending vibrations of bound water molecules. Suggests the presence of organic residues or proteinaceous materials.
3	1425.46	C–H bending / Carbonate vibration	Suggests aliphatic C–H groups or carbonate ions. Indicates carbonate-containing mineral phases and organic constituents within the formulation.
4	987.62	C–O stretching / Si–O / PO ₄ vibration	Indicates C–O functional groups and inorganic components such as silicates, phosphates, or metal–oxygen bonds. Suggests the presence of mineral oxides or phosphate-containing structures.

UV 254 nm		UV 366 nm		Derivatized	
R _f value	Color	R _f value	Color	R _f value	Color
0.18	Green	0.23	Blue	0.44	Blue

0.23	Green	0.36	Blue	0.55	Green
0.44	Green	0.40	Red	0.64	Blue
0.48	Green	0.42	Blue	0.76	Yellow
0.55	Green	0.51	Red	0.85	Pink
0.61	Green	0.55	Green	0.98	Purple
0.67	Green	0.56	Green		
0.78	Green	0.60	Green		
0.93	Green	0.73	Blue		
		0.80	Blue		
		0.88	Red		

Figure 02: TLC Photo documentation of LV

TLC INTERPRETATION:

The TLC profile of the drug revealed the presence of multiple phytochemical constituents with different polarities and fluorescence characteristics. Under UV 254 nm, several green quenching spots were observed, indicating UV-active compounds. At UV 366 nm, blue, red, and green fluorescent bands confirmed the presence of diverse fluorescent constituents, while derivatization produced distinct blue, green, yellow, pink, and purple bands, suggesting chemically varied metabolites. Prominent spots observed at R_f values around 0.40, 0.51, 0.85, and 0.98 indicate the possible presence of major flavonoid, phenolic, or terpenoid-type compounds. Overall, the TLC fingerprint demonstrates successful separation and confirms the complex phytochemical nature of the drug sample.

At UV 254 nm

The chromatogram showed several green fluorescent bands with different R_f values ranging from **0.18 to 0.93**, indicating the presence of compounds with conjugated double bonds and aromatic structures.

Observed major bands

Table 02: TLC Interpretation at UV 254nm

RF VALUE	OBSERVATION	INTERPRETATION
0.18–0.23	Green bands	Polar phytoconstituents such as phenolics or glycosides
0.44–0.67	Multiple intense bands	Presence of flavonoids, tannins, or alkaloidal fractions
0.78–0.93	Distinct upper bands	Less polar volatile or resinous compounds

At UV 366 nm

Under long-wave UV, blue, red, and green fluorescent bands were observed at R_f values from **0.23 to 0.88**

Key observations

Table 03: TLC Interpretation at UV 366nm

RF VALUE	FLUORESCENCE	POSSIBLE INDICATION
0.23, 0.36, 0.42	Blue	Coumarins, phenolics, flavonoids
0.40, 0.51, 0.88	Red	Polyphenolic or highly conjugated compounds
0.55–0.60	Green	Terpenoids or chlorophyll-related compounds

Post-Derivatization Profile

After derivatization, colored bands appeared at R_f values between 0.44 and 0.98, including blue, green, yellow, pink, and purple zones.

Table 04: TLC Interpretation at Post derivatization

RF VALUE	COLOR	INTERPRETATION
0.44–0.64	Blue/Green	Phenolic and flavonoid compounds
0.76	Yellow	Terpenoids or essential oil components

0.85	Pink	Alkaloidal constituents
0.98	Purple	Highly non-polar compounds/resins

Derivatization enhances visualization of phytochemical groups, suggesting the formulation contains a broad spectrum of secondary metabolites.

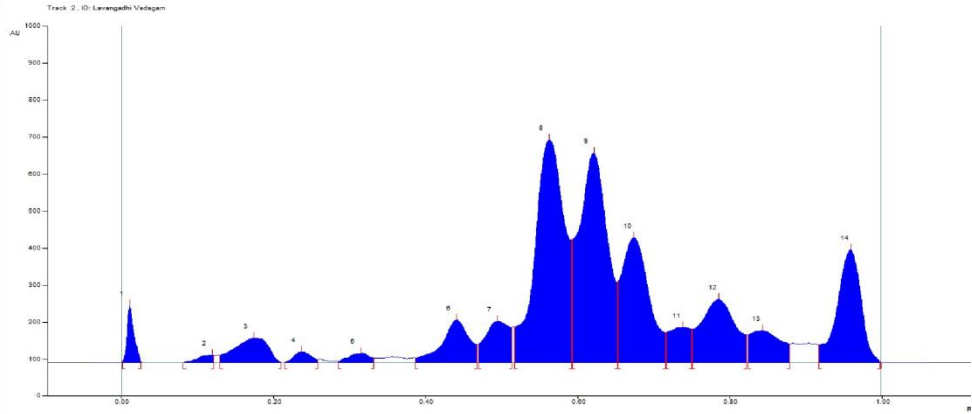


Figure 03: HPTLC Finger print profile at UV 254 nm

Track 2, ID: Lavangadhi Vadagam									
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	5.7 AU	0.01 Rf	154.9 AU	5.76 %	0.03 Rf	2.0 AU	1297.1 AU	1.46 %
2	0.08 Rf	0.7 AU	0.12 Rf	20.0 AU	0.74 %	0.12 Rf	19.6 AU	383.3 AU	0.43 %
3	0.13 Rf	20.6 AU	0.17 Rf	66.8 AU	2.48 %	0.21 Rf	0.2 AU	2796.8 AU	3.14 %
4	0.21 Rf	0.6 AU	0.24 Rf	28.7 AU	1.07 %	0.26 Rf	7.6 AU	620.0 AU	0.70 %
5	0.29 Rf	3.8 AU	0.31 Rf	24.9 AU	0.93 %	0.33 Rf	13.2 AU	670.5 AU	0.75 %
6	0.39 Rf	12.4 AU	0.44 Rf	115.4 AU	4.29 %	0.47 Rf	49.2 AU	3897.4 AU	4.37 %
7	0.47 Rf	49.7 AU	0.50 Rf	112.3 AU	4.18 %	0.51 Rf	95.8 AU	3369.7 AU	3.78 %
8	0.52 Rf	96.0 AU	0.56 Rf	602.3 AU	22.41 %	0.59 Rf	32.2 AU	22292.8 AU	25.01 %
9	0.59 Rf	333.1 AU	0.62 Rf	566.3 AU	21.07 %	0.65 Rf	16.7 AU	19916.8 AU	22.35 %
10	0.65 Rf	218.0 AU	0.67 Rf	338.4 AU	12.59 %	0.72 Rf	81.4 AU	11563.9 AU	12.97 %
11	0.72 Rf	81.9 AU	0.74 Rf	95.4 AU	3.55 %	0.75 Rf	91.1 AU	2542.4 AU	2.85 %
12	0.75 Rf	90.8 AU	0.79 Rf	171.1 AU	6.36 %	0.82 Rf	74.5 AU	7389.8 AU	8.29 %
13	0.82 Rf	75.0 AU	0.84 Rf	86.4 AU	3.21 %	0.88 Rf	50.6 AU	3345.5 AU	3.75 %
14	0.92 Rf	48.1 AU	0.96 Rf	304.9 AU	11.34 %	1.00 Rf	1.7 AU	9043.8 AU	10.15 %

Figure 04: HPTLC Peak table at UV 254nm

HPTLC Profile at 254 nm

The densitogram revealed multiple peaks, confirming the presence of several constituents in the formulation.

Major peaks observed

Table 05: HPTLC Interpretation at UV 254nm

Rf	AREA %	INTERPRETATION
0.52	25.01%	Major bioactive constituent
0.65	12.97%	Significant phytochemical fraction
0.93	16.15%	Non-polar major constituent
0.47	3.78%	Moderate constituent

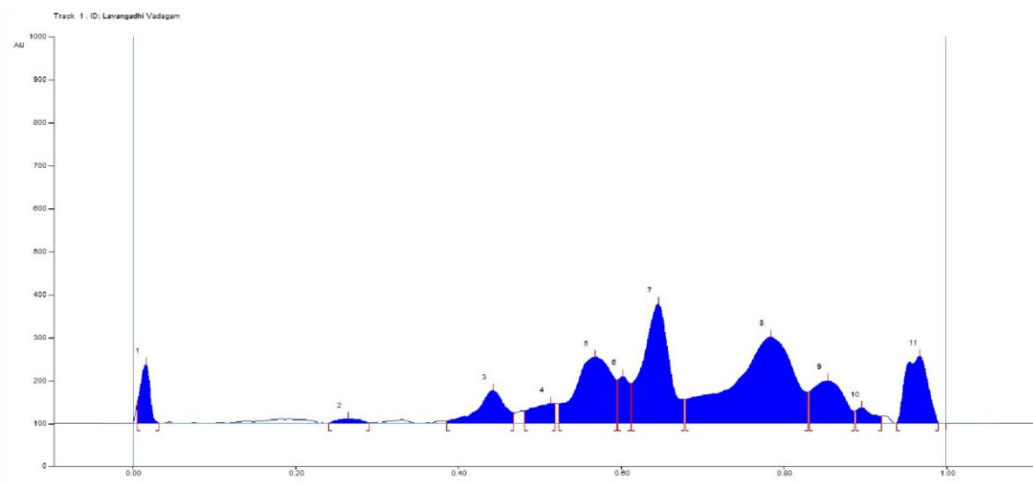


Figure 05: HPTLC Finger print profile at 520 nm

Track 1, ID: Lavangadhi Vadagam

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	47.0 AU	0.02 Rf	137.0 AU	10.48 %	0.03 Rf	0.6 AU	1523.1 AU	3.43 %
2	0.24 Rf	0.8 AU	0.26 Rf	10.8 AU	0.82 %	0.29 Rf	0.6 AU	290.4 AU	0.65 %
3	0.39 Rf	5.8 AU	0.44 Rf	77.0 AU	5.89 %	0.47 Rf	24.4 AU	2406.0 AU	5.43 %
4	0.48 Rf	28.7 AU	0.51 Rf	46.1 AU	3.53 %	0.52 Rf	45.1 AU	1262.8 AU	2.85 %
5	0.52 Rf	46.0 AU	0.57 Rf	155.1 AU	11.87 %	0.60 Rf	00.5 AU	6496.0 AU	14.65 %
6	0.60 Rf	101.0 AU	0.60 Rf	109.6 AU	8.39 %	0.61 Rf	92.3 AU	1434.5 AU	3.23 %
7	0.61 Rf	93.1 AU	0.65 Rf	277.8 AU	21.26 %	0.68 Rf	55.3 AU	8404.5 AU	18.95 %
8	0.68 Rf	55.4 AU	0.78 Rf	201.4 AU	15.41 %	0.83 Rf	73.4 AU	14207.7 AU	32.04 %
9	0.83 Rf	73.9 AU	0.85 Rf	99.4 AU	7.61 %	0.89 Rf	27.7 AU	3604.4 AU	8.13 %
10	0.89 Rf	28.4 AU	0.90 Rf	36.5 AU	2.79 %	0.92 Rf	16.4 AU	694.6 AU	1.57 %
11	0.94 Rf	0.2 AU	0.97 Rf	156.1 AU	11.95 %	0.99 Rf	3.0 AU	4020.2 AU	9.07 %

Figure 06: HPTLC Peak table at UV 520nm

HPTLC PROFILE AT 520 NM

The densitometric scan at 520 nm also showed characteristic peaks.

Major peaks

Table 06: HPTLC Interpretation at UV 520nm

Rf	AREA %	INTERPRETATION
0.78	32.04%	Predominant compound
0.61	18.95%	Major secondary metabolite
0.52	14.65%	Important phytochemical constituent
0.94	9.07%	Less polar compound

HEAVY METAL ANALYSIS

Table 07: Heavy metal report of LV

S.NO	DRUG RECEIVED	ELEMENT	RESULTS (MG [KG])
1.	LavangadhiVadagam	Arsenic(As)	BLQ
2.		Cadmium (Cd)	BLQ
3.		Mercury (Hg)	BLQ
4.		Lead (Pb)	BLQ

BLQ: Below Limit of Quantification

DISCUSSION

The FTIR spectrum indicates the coexistence of both organic and inorganic constituents in the sample. The broad hydroxyl peak at 3302.71 cm^{-1} , confirms the presence of hydrogen-bonded compounds and adsorbed moisture, while the bands around 1631 cm^{-1} and 1425 cm^{-1} suggest organic functional groups and carbonate-related vibrations. The intense band near 987 cm^{-1} indicates mineral-associated bonds such as phosphate, silicate, or metal–oxygen linkages, supporting the presence of stabilized inorganic constituents. The TLC fingerprint profile of the drug LV demonstrates the presence of multiple phytochemical constituents with good separation under different visualization conditions (UV 254 nm, UV 366 nm, and post-derivatization). The chromatographic profile indicates a chemically complex herbal formulation containing several bioactive compounds. The HPTLC densitogram recorded at UV 254 nm revealed the presence of multiple phytochemical constituents with varying concentrations and polarities. The chromatographic profile showed several well-resolved peaks distributed across the R_f range, indicating a chemically complex herbal formulation. Among the detected peaks, the major constituents were observed at R_f 0.56 and R_f 0.62, showing the highest peak area percentages of approximately 25.01% and 22.35%, respectively. These peaks represent the dominant UV-active compounds present in the formulation. Another significant peak was observed at R_f 0.67 with an area percentage of about 12.97%, followed by peaks at R_f 0.79 and R_f 0.96, indicating moderately abundant constituents. The presence of peaks at lower R_f values suggests relatively polar compounds, whereas peaks at higher R_f values correspond to less polar constituents. The strong absorption at UV 254 nm indicates the presence of conjugated or aromatic compounds such as flavonoids, phenolics, tannins, or alkaloidal constituents. The overall densitometric pattern can serve as a reference fingerprint for indicating the presence of diverse bioactive compounds such as flavonoids, phenolics, alkaloids, and terpenoid constituents. The reproducible fingerprint profile confirms the identity, purity, and phytochemical complexity of the formulation LV. Heavy metal analysis shows that the drug LV is free of the contamination of toxic metals like Lead, Cadmium, Arsenic and Mercury as they are below the detectable quantity.

CONCLUSION

The present study scientifically validated the *Siddha* polyherbal formulation LV through comprehensive analytical fingerprinting using FTIR, HPTLC, and ICP-OES techniques. FTIR analysis confirmed the presence of important functional groups such as hydroxyl, carboxyl, C-O bending, and C-H stretching vibrations, indicating the occurrence of biologically active

phytoconstituents. HPTLC profiling demonstrated the presence of major secondary metabolites including flavonoids, phenolics, terpenoids, and alkaloids, which are known for their antioxidant, anti-inflammatory, antimicrobial, diuretic, and protective pharmacological properties. ICP-OES analysis further established the safety profile of the formulation by confirming that toxic heavy metals such as cadmium (Cd), arsenic (As), mercury (Hg), and lead (Pb) were below detectable limits. The generated analytical fingerprint profile provides a valuable scientific basis for the authentication, standardization, and quality control of the *Siddha* formulation. These findings support the traditional therapeutic claims of the drug and enhance its reliability, reproducibility, and acceptability in evidence-based traditional medicine research. The identified phytochemical constituents may contribute to the therapeutic efficacy of the formulation in the management of *Siddha* indications such as Neeradaipu (urinary tract infections), Kalladaipu (urinary calculi/stones), Soothaga Vayu (PCOS-related disorders), Vayu disorders, Kandhal, and Varatchi. The presence of phenolics and flavonoids may help reduce oxidative stress and inflammation, while alkaloids and terpenoids may contribute to antimicrobial, analgesic, diuretic, and metabolic regulatory activities associated with these conditions, thus supporting the traditional therapeutic claims of the drug LV and enhance its reliability, reproducibility, and acceptability in evidence-based traditional medicine research. Furthermore, the analytical data revealed in this study can serve as a reference standard for future pharmacological, toxicological, phytochemical isolation and clinical studies on the formulation LV. The established fingerprint profile may also facilitate batch-to-batch consistency, formulation validation, and development of standardized *Siddha* herbal products for wider clinical and pharmaceutical applications. Thus, the study contributes significantly toward bridging traditional *Siddha* knowledge with modern scientific validation and promotes the global acceptance of *Siddha* medicine in integrative healthcare research.

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Not Applicable

INFORM CONSENT

Not Applicable

AUTHOR CONTRIBUTION

All are contributed equally

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