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Research Article

Phytochemical Profiling and Quality Control of *Sadaveri khirutham*: A PLIM-Based Standardization Study

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ABSTRACT

Background: Sadaveri Khirutham (SK) is a classical Siddha polyherbal medicated ghee indicated for gastrointestinal and dermatologic disorders. **Objectives:** The present study aimed to standardize SK as per Pharmacopoeial Laboratory for Indian Medicine (PLIM) guidelines through physicochemical, phytochemical, chromatographic, and safety evaluations. **Materials and Methods:** The formulation was prepared using authenticated raw materials following classical procedures. Physicochemical analysis indicated good stability and quality of the formulation with characteristic lipophilic nature. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, tannins, and steroids. HPTLC fingerprint analysis showed distinct peaks, confirming the presence of diverse phytoconstituents and serving as a characteristic profile for identification. Heavy metal analysis demonstrated values within permissible limits, and safety studies confirmed the absence of microbial contamination, pesticide residues, and aflatoxins. **Results:** Physicochemical analysis indicated good stability and quality of the formulation with characteristic lipophilic nature. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, tannins, and steroids, suggesting therapeutic potential. HPTLC fingerprint analysis showed distinct peaks, confirming the presence of diverse phytoconstituents and serving as a characteristic profile for identification. Heavy metal analysis demonstrated values within permissible limits, and safety studies confirmed the absence of microbial contamination, pesticide residues, and aflatoxins. **Conclusion:** The findings validate the quality, purity, and safety of SK and support its standardization for future research and therapeutic application.

Keywords: Sadaveri Khirutham, Siddha medicine, Standardization, Quality control, PLIM guideline.

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INTRODUCTION

The Siddha system is a traditional medical practice widely followed in Tamil Nadu, South India. Siddha medicines are classified into internal and external types, each consisting of 32 dosage forms (Ramasamy *et al*., 2025). Among the internal medicines, Khirutham (medicated ghee) is an important preparation used for various therapeutic purposes. Sadaveri Khirutham (SK) is a classical Siddha polyherbal formulation indicated for the treatment of dyspepsia, parasitic infections, giddiness, and skin diseases such as eczema (Sambasivampillai, 1931; Velusamy *et al*., 1997). Although this formulation is used in clinical practice, there is limited scientific evidence available regarding its quality and standardization. Standardization is essential to ensure the quality, purity, and safety of Siddha medicines (Abarna *et al*., 2024). It helps to establish consistent parameters for the preparation and evaluation of the drug. Therefore, the present study aims to standardize Sadaveri Khirutham by following the guidelines of the Pharmacopoeial Laboratory for Indian Medicine (PLIM). The study includes evaluation of physicochemical parameters and other quality control measures specific to medicated ghee formulations.

MATERIALS AND METHODS

Choice of the Drug

Sadaveri Khirutham is a classical Siddha polyherbal preparation mentioned in Agathiyar Vaithiya Pooranam-205 and is used in the treatment of atopic dermatitis.

Collection and Authentication of Raw Materials

The raw materials required for the preparation of Sadaveri Khirutham were procured from a local herbal market in Chennai. The identity of the ingredients was authenticated by experts from the "PG and Research Department of Botany, Pachaiyappa's College, Chennai, India.

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Composition of Sadaveri Khirutham

The formulation components are provided in Table 1.

Purification of Raw Drugs

Thanneervittan Kizhangu

The tubers were thoroughly washed, and the outer skin and fibrous strands were removed (Pillai, 1951).

Chukku

Chukku was soaked in lime water for 3 hr, then dried, and the outer layer was removed (*"Murukesa mudhaliyar K. Su. Gunapadam, part 1* (Mooligai vaguppu)", 2013).

Milagu

Soaked in sore buttermilk for 1 to 1 1/4 hr and then fried ("Murukesa mudhaliyar K. Su. Gunapadam, part 1 (Mooligai vaguppu)", 2013).

Thippili

The Thippili was soaked in Kodiveli juice for 24 min, then removed and dried (Pillai, 1951).

Kadukkai

Soak Kadukkai in kazhuneer to remove the yellowish impurity, remove the seeds, and dry well (Pillai, 1951).

Nellikai

Boil in milk, remove the seeds (kottai), and dry well (Pillai, 1951).

Thandrikai

Soak in Thazhai vizhuthu juice for 3 hr, remove the seeds, and dry in sunlight (Pillai, 1951).

Preparation of Sadaveri Khirutham

First, 1.3 L of juice was extracted from Thannervittan tubers. The remaining ingredients were dried and made into a fine powder. Then, the powder was mixed with 1.3 L of cow ghee and the extracted juice. The mixture was heated until a ghee-like consistency was obtained. Finally, the preparation was filtered and stored in an airtight container (Velusamy *et al*., 1997).

Physicochemical Analysis Following PLIM Standards

SK was evaluated for physicochemical parameters and active components as per AYUSH-PLIM guidelines (Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008). The analysis was performed at "Noble Research Solutions, Chennai."

Organoleptic Characters of SK

Organoleptic characters of SK were evaluated as per PLIM guidelines.

Physicochemical Evaluation

Determination of the solubility of SK

A pinch of sample was placed in a dry test tube, mixed with "chloroform, ethanol, water, ethyl acetate, and DMSO" then shaken for a minute. The results were observed individually (Indian Pharmacopoeia Commission, 2014).

Determination of Iodine Value

Iodine value was determined by standard titrimetric method as per PLIM guidelines.

Determination of Saponification Value

Saponification value was determined as per standard procedures (*Indian Standard Methods of Sampling and Test for Oils and Fats Indian Standard Institution New Delhi*, 1964; Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008).

Determination of Viscosity Value

Viscosity was determined using an Ostwald viscometer by measuring the time taken for a fixed volume of the sample to flow through a capillary (*Indian Standard Methods of Sampling and Test for Oils and Fats Indian Standard Institution New Delhi*, 1964; Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008).

Determination of Refractive Index

Determination of RL was carried out using Refractometer (*Indian Standard Methods of Sampling and Test for Oils and Fats Indian Standard Institution New Delhi*, 1964; Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008).

Determination of wt/mL

wt/mL was determined using a comparative calibration method by measuring the weight of 1 mL of the base and the final formulation, and calculating the difference (*Indian Standard Methods of Sampling and Test for Oils and Fats Indian Standard Institution New Delhi*, 1964; Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008).

Determination of Acid Value

Acid value was determined using standard titration method (*Indian Standard Methods of Sampling and Test for Oils and Fats Indian Standard Institution New Delhi*, 1964; Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008).

Determination of Peroxide Value

Peroxide value was estimated using standard iodometric method (*Indian Standard Methods of Sampling and Test for Oils and Fats Indian Standard Institution New Delhi*, 1964; Indian Pharmacopoeia Commission, 2014; *ResearchGate*, 2008).

Analytic Studies

Qualitative analysis of acid and basic radicals

Qualitative analysis of acid and basic radicals was performed using standard chemical tests (Indian Pharmacopoeia Commission, 2014).

Phytochemical Analysis

Preliminary phytochemical screening was carried out using standard qualitative tests to identify the presence of alkaloids, coumarins, saponins, tannins, glycosides, flavonoids, phenols, steroids, triterpenoids, anthocyanins, carbohydrates, and proteins (Indian Pharmacopoeia Commission, 2014).

Instrument-Based Analysis of SK

SK evaluation in high-performance thin layer chromatography

HPTLC is an advanced, automated technique derived from TLC, used for precise and sensitive separation of compounds. Precoated HPTLC plates and an autosampler were used to achieve accurate qualitative and quantitative analysis. It is a reliable and cost-effective method for quality assessment of herbal materials, providing a characteristic chromatographic fingerprint for identification and purity evaluation. Chromatogram development was carried out in a CAMAG twin trough chamber based on the adsorption properties of the components. After development, the plates were removed and dried. The plates were then scanned under UV light at 366 nm using CAMAG software, and the obtained data were used to generate chromatographic fingerprints. The R_f values of the detected phytoconstituents were recorded (Hapuarachchi *et al.*, 2020; Wagner, 2002).

Heavy Metal Analysis of SK by AAS

Standard solutions of arsenic, lead, cadmium, and mercury were used. Heavy metals were analyzed using an Atomic Absorption Spectrometer (AA 240 Series) after acid treatment of the sample with HCl and HNO_3, and 100 parts per million standards were prepared (Mohammed *et al.*, 2018).

Safety/Contamination Studies

SK sterility testing using the pour plate method

Sterility of SK was tested using the pour plate method. The sample was mixed with agar, incubated at 37°C, and colonies were counted as CFU after 48-72 hr (Li and Deng, 2003).

Specific Pathogen Identification in SK

The sample was cultured on selective media and incubated at 37°C. Pathogens were identified based on colony characteristics and specific media reactions (*Pour plate method: Procedure, uses,* (dis)advantages, 2002).

Pesticide Residue Analysis of SK

The sample was extracted with acetone, concentrated below 40°C, and treated with toluene. The final solution was filtered and used for pesticide residue analysis (Kiehlbauch *et al.*, 2000; World Health Organization, 2007).

Aflatoxin Analysis of SK by TLC

Aflatoxin analysis of SK was carried out using TLC. The sample and standards were developed in a suitable solvent system and observed under UV light at 365 nm (de Castro and Vargas, 2001).

RESULTS

Organoleptic Characters of SK

The organoleptic characters of SK are presented in Table 2.

Physicochemical Evaluation

Solubility profile of the SK

The solubility profile of the test sample SK is presented in Table 3.

Physicochemical Parameters

The physicochemical parameters of the formulation SK were determined using standard analytic methods. The observed values are summarized in Table 4.

Table 1: Composition of *Sadaveri khirutham*.

Sl. No.	Local name of the ingredients	Botanical name	Quantity
1.	Thanneervittan Kizhangu (juice)	Asparagus recemosus	1.3 L
2.	Chukku	Zingiber officinale	5.1 g
3.	Milagu	Piper nigrum	5.1 g
4.	Thippili	Piper longum	5.1 g
5.	Kadukkai	Terminalia chebula	5.1 g
6.	Nellikkai	Phyllanthus emblica	5.1 g
7.	Thaandrikkai	Terminalia bellarica	5.1 g

Qualitative Inorganic Analysis

Acid radicals

The qualitative analysis of acid radicals in SK revealed the presence of carbonates and sulfates, while other radicals were absent (Table 5).

Basic Radicals

The qualitative analysis of basic radicals indicated the presence of lead, while all other tested radicals were absent (Table 6).

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of the formulation SK revealed the presence of flavonoids, phenols, tannins, and steroids, while other constituents were absent (Table 7).

Instrument-Based Analysis of SK

The HPTLC fingerprint and peak values of SK are presented in Figures 1, 2.

Heavy Metal Analysis

The results indicated that lead was present within the permissible limit, while arsenic, cadmium, and mercury were below detection limits. The findings are presented in Table 8.

Sterility Test (Pour Plate Method)

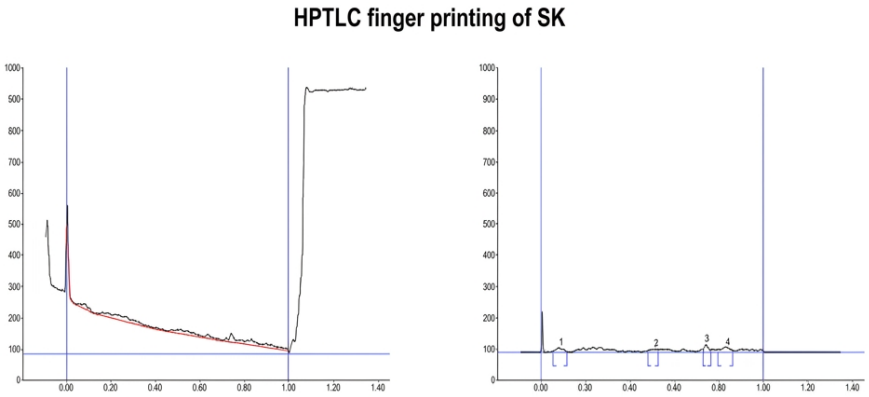
The formulation SK showed no detectable bacterial or fungal growth and complied with the specified microbial limits (Figure 2, Table 9).

Detection of Specific Pathogens

No pathogenic microorganisms were detected in the formulation SK, indicating compliance with the required microbiological standards (Figure 3, Table 10).

Pesticide Residue Analysis

The formulation SK was examined for pesticide contaminants, and no detectable residues were identified (Table 11).



Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	1.1	0.08	16.2	22.10	0.12	0.2	271.9	23.63
2	0.48	4.3	0.51	11.6	15.82	0.53	9.9	221.7	19.28
3	0.73	4.5	0.74	26.2	35.89	0.76	6.9	263.5	22.91
4	0.80	8.0	0.83	19.1	26.19	0.86	5.0	393.2	34.18

Figure 1: (A) HPTLC fingerprint (B) Peak values.

Table 2: Organoleptic character of SK.

Sl. No.	Parameter	Results
1.	Physical state	Semisolid form
2.	Nature	Viscous consistency
3.	Odor	Characteristic smell
4.	Consistency	Greasy texture
5.	Flow property	Nonflowing
6.	Appearance	Yellowish color

Table 3: Solubility profile of the SK.

Sl. No.	Solvent used	Solubility/dispersibility
1.	Chloroform	Soluble
2.	Ethanol	Insoluble
3.	Water	Insoluble
4.	Ethyl acetate	Soluble
5.	DMSO	Insoluble

Aflatoxin Analysis

The formulation SK was evaluated for aflatoxin contamination, and no detectable levels of aflatoxins were observed (Table 12).

DISCUSSION

The present study focused on the standardization and safety evaluation of *Sadaveri Khirutham* (SK) using physicochemical, phytochemical, and contamination analyses. The solubility profile indicated that SK is predominantly lipophilic, as it was soluble in nonpolar solvents such as chloroform and ethyl acetate. This behavior is characteristic of ghee-based formulations and supports enhanced bioavailability of lipid-soluble constituents (Reich and Schibli, 2007). The physicochemical parameters, including iodine and saponification values, suggest the presence of unsaturated fatty acids and stable lipid components. The low acid and peroxide values further indicate minimal oxidation and good stability, which are consistent with standard medicated ghee formulations (*ResearchGate*, 2008). Phytochemical analysis revealed the presence of flavonoids, phenols, tannins, and steroids. These compounds are known for their antioxidant and anti-inflammatory activities, which are relevant in the management of dermatologic conditions. Similar findings have been reported in *Asparagus racemosus* and *Terminalia* species (Baliga *et al.*, 2011; Pandey and Rizvi, 2009). The HPTLC fingerprint profile of SK revealed multiple well-defined peaks at R_f values of 0.08, 0.51, 0.74, and 0.83, indicating the presence of diverse phytoconstituents. The variation in peak intensity reflects differences in concentration, suggesting a complex phytochemical composition. Such chromatographic patterns serve as characteristic fingerprints for identification, purity assessment, and batch consistency, as reported in herbal drug standardization studies (Reich and Schibli, 2007). Inorganic analysis showed the presence of carbonate and sulfate radicals. Among heavy metals, lead was detected at 2.51 parts per million, which is within permissible limits. Previous studies indicate

Table 4: Physicochemical parameters of the formulation SK.

Sl. No.	Parameter	SK
1.	viscosity at 50°C (Pa s)	79.16
2.	Refractive Index	1.46
3.	wt/mL (g/mL)	0.720
4.	Iodine Value (mg I^2/g)	89.789
5.	Saponification Value (mg KOH/g of fat)	177.63
6.	Acid Value (mg KOH/g)	0.897
7.	Peroxide value (mEq/kg)	4.87

Table 5: Acid radical analysis of SK.

Sl. No.	Radical tested	Results	Inference
1.	Carbonates	Positive	Present
2.	Chlorides	Negative	Absent
3.	Sulfates	Positive	Present
4.	Sulfides	Negative	Absent
5.	Phosphates	Negative	Absent
6.	Fluoride and oxalate	Negative	Absent
7.	Borates	Negative	Absent
8.	Nitrates	Negative	Absent

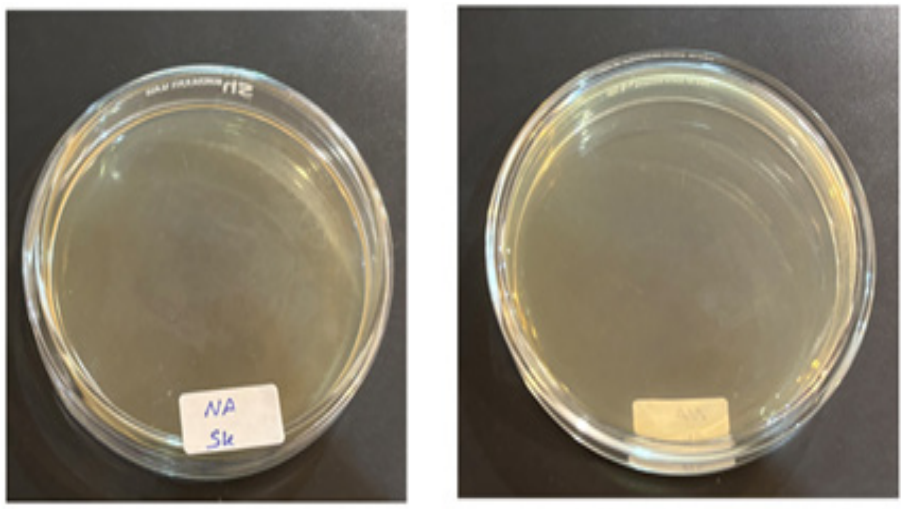


Figure 2: Results of sterility testing for SK.

Table 6: Basic radical analysis of SK.

Sl. No.	Radical tested	Results	Inference
1.	Lead	Positive	Present
2.	Arsenic	Negative	Absent
3.	Mercury	Negative	Absent
4.	Copper	Negative	Absent
5.	Ferric	Negative	Absent
6.	Ferrous	Negative	Absent
7.	Zinc	Negative	Absent
8.	Silver	Negative	Absent
9.	Magnesium	Negative	Absent

Table 7: Preliminary phytochemical screening of SK.

Sl. No.	Phytochemical constituent	Results	Inference
1.	Alkaloids	Negative	Absent
2.	Flavonoids	Positive	Present
3.	Glycosides	Negative	Absent
4.	Steroids	Positive	Present
5.	Triterpenoids	Negative	Absent
6.	Coumarins	Negative	Absent
7.	Phenols	Positive	Present
8.	Tannins	Positive	Present
9.	Saponins	Negative	Absent
10.	Proteins	Negative	Absent
11.	Sugars	Negative	Absent
12.	Anthocyanins	Negative	Absent
13.	Betacyanins	Negative	Absent

Table 8: Heavy metal analysis of SK.

Sl. No.	Metal	Wavelength (nm)	Value	Limit
1.	Lead (Pb)	217.0	2.51 parts per million	≤10 parts per million
2.	Arsenic (as)	193.7	Not detected	≤3 parts per million
3.	Cadmium (Cd)	228.8	Not detected	≤0.3 parts per million
4.	Mercury (Hg)	253.7	Not detected	≤1 parts per million

Table 9: Microbiological quality of SK.

Sl. No.	Test parameter	Results	Limit (CFU/g)
1.	Total Aerobic Count	Not detected	NMT 10^5
2.	Total Fungal Count	Not detected	NMT 10^3

Table 10: Specific pathogen analysis of SK.

Sl. No.	Test organism	Requirement	Results
1.	Escherichia coli	absent	absent
2.	*Salmonella* spp.	absent	absent
3.	Staphylococcus aureus	absent	absent
4.	Pseudomonas aeruginosa	absent	absent

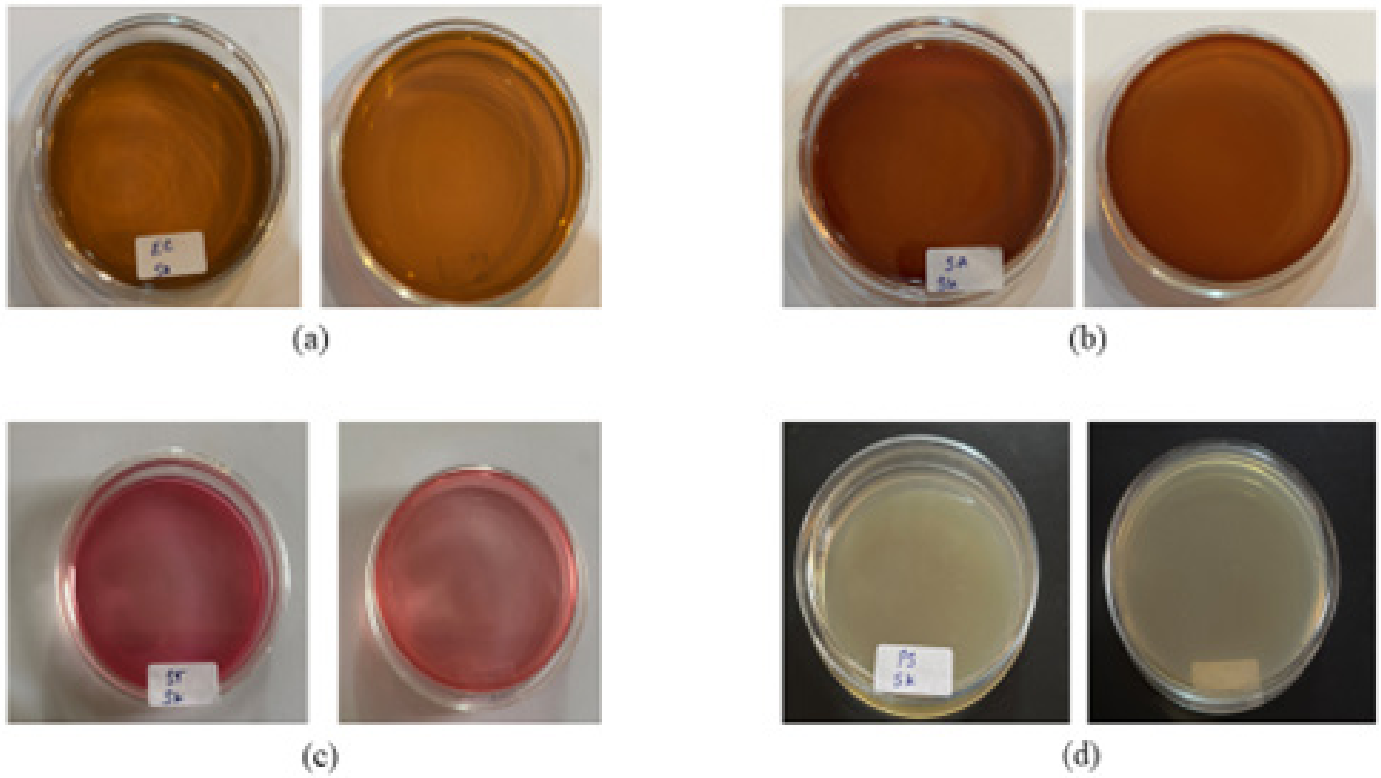


Figure 3: Culture plates of SK on organism-specific media-(A) EC, (B) Salmonella, (C) SA, and (D) PA.

Table 11: Pesticide residue screening of SK.

Category	Representative compounds	Results
Organochlorines	BHC isomers, DDT, endosulfan	Not detected
Organophosphates	Malathion, chlorpyrifos, dichlorvos	Not detected
Carbamates	Carbofuran	Not detected
Pyrethroids	Permethrin, cypermethrin	Not detected

Abbreviations: Not detected, Below quantification limit.

Table 12: Aflatoxin profile of SK.

Component	Status	Limit (mg/kg)
B1	Not detected	0.5
B2	Not detected	0.1
G1	Not detected	0.5
G2	Not detected	0.1

that trace levels within regulatory limits do not pose significant toxicity risks (Saper *et al.*, 2004). Comparable findings have been reported in Siddha formulations such as Vaalai Rasa Mezhugu, supporting safety and standardization (Ramasamy *et al.*, 2025).

Microbial evaluation demonstrated the absence of bacterial and fungal contamination, and no specific pathogens such as *Escherichia coli, Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were detected. These findings comply with WHO guidelines and indicate good microbiological stability and suitability of the formulation for safe therapeutic use (World Health Organization, 2007). Furthermore, pesticide residues and aflatoxins were not detected in SK. The absence of these contaminants confirms the purity and safety of the formulation and its compliance with standard quality requirements (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012).

CONCLUSION

The present study successfully established the standardization profile of *Sadaveri khirutham* (SK) in accordance with PLIM guidelines. The formulation demonstrated consistent quality characteristics with a well-defined analytic profile and the presence of bioactive constituents. The safety evaluation confirmed compliance with permissible limits and absence of contamination. These findings provide scientific validation for the quality, safety, and reproducibility of the formulation and serve as a reference for future quality control and research.

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ABBREVIATIONS

SK: Sadaveri Khirutham; **PLIM:** Pharmacopoeial Laboratory for Indian Medicine; **HPTLC:** High-Performance Thin Layer Chromatography; **AAS:** Atomic Absorption Spectrometer; **R_f:** Retardation factor; **CFU:** Colony Forming Units; **UV:** Ultraviolet; **DMSO:** Dimethyl sulfoxide; **Pa s:** Pascal-second; **mEq/kg:** Milliequivalents per kilogram; **ppm:** Parts per million; **NMT:** Not More Than; **EC:** *Escherichia coli*; **SA:** *Staphylococcus aureus*; **PA:** *Pseudomonas aeruginosa*; **BHC:** Benzene hexachloride; **DDT:** Dichlorodiphenyltrichloroethane.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTION

Conceptualization: G. S.; Medicine Preparation: G. S.; Data collection and compilation: G. S.; Manuscript Writing: G. S.; Proofreading and editing: G. S. and C. S.

ETHICAL APPROVAL

As the present study is an *in vitro* investigation, it does not involve the use of human or animal subjects and therefore, Ethical clearance is not applicable.

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