



Research Article

Pharmacognostical and Physicochemical Standardization of *Pun Sudar Thylam*: A Traditional Siddha Medicinal Oil

Sanjana S^{1*}, Lavanya M², Jayaveeran T³, Mahadevan M V⁴

1. Lecturer, Department of Aruvai Maruthuvam, Sri Sairam siddha Medical College and Research Centre, Chennai, Tamil Nadu. India. 600132. ORCID: 0009-0009-9671-9063
2. PG Scholar, Department of Pura Maruthuvam, National Institute of Siddha, Chennai, Tamil Nadu. India. 600047.
3. Assistant Professor, Department of Gunapadam-Marunthiyal, Santhigiri Siddha Medical College, Pothencode, Thiruvananthapuram, Kerala. India. 695589.
4. Professor, Department of Pura Maruthuvam, National Institute of Siddha, Chennai. India. 600047.

Received: 24-06-2025

Accepted: 02-03-2026

Published: 31-03-2026

Abstract

Pun Sudar Thylam (PST) is a classical Siddha formulation that has been traditionally used for the management of chronic skin and soft tissue disorders such as anorectal fistula, non-healing ulcers, lymphedema, and abscesses. The formulation consists of purified *Nellikaai*, *Gandhagam* (sublimated sulphur), *Erukam paal* (latex of *Calotropis gigantea* (L.) W.T. Aiton), and castor oil, all of which are known in traditional practice for their antimicrobial, anti-inflammatory, and wound-healing properties. Despite its long-standing therapeutic use, PST requires scientific validation and standardization for broader acceptance in modern healthcare. The present study aimed to prepare and standardize *Pun Sudar Thylam* using modern analytical techniques in accordance with AYUSH-Pharmacopoeial Laboratory for Indian Medicine (PLIM) guidelines to ensure its quality, safety, and efficacy. *Pun Sudar Thylam* was prepared following the traditional *Sudar Thylam* method using authenticated and purified raw materials. The formulation was evaluated for various physicochemical parameters, including specific gravity, viscosity, acid value, peroxide value, saponification value, and refractive index. Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC) were used for phytochemical fingerprinting, while Atomic Absorption Spectroscopy (AAS) and microbial limit tests were conducted to assess heavy metal content and microbiological safety, respectively. The results confirmed the stability and consistency of the formulation, with acceptable levels of physicochemical parameters. Phytochemical profiling revealed the presence of key constituents, while heavy metals and microbial counts were within permissible limits. Overall, the study supports the quality and safety of *Pun Sudar Thylam* and provides scientific evidence reinforcing its traditional ethnomedical relevance.

Keywords: *Pun Sudar Thylam*, Physico-chemical, Siddha, Fistula- in- Ano.

Access this article
online

Website:
<https://ijam.co.in>



DOI: <https://doi.org/10.47552/ijam.v17i1.6279>

Introduction

The Siddha system of Medicine, an ancient Indian holistic approach, offers a rich legacy of therapeutic interventions for a diverse array of illnesses. Its formulations, uniquely crafted from herbal, mineral, and metal-based raw ingredients, stand in contrast to the protracted conventional drug development timeline, which typically spans 12-15 years for market availability. Recognizing this disparity, the reverse pharmacology approach, rooted in traditional medicine knowledge, has emerged as a cost-effective

and time-saving alternative to modern drug discovery and development by leveraging existing clinical observations from traditional systems to identify bioactive compounds and mechanisms of action (22).

Crucially, standardization forms the bedrock of drug development, ensuring the quality, safety, and efficacy of pharmaceutical products. For Siddha formulations, this assessment of quality is paramount before proceeding with *in vivo* and *in vitro* studies, and subsequent clinical trials (25). Among the myriad Siddha preparations, the herbo-mineral formulation *Pun Sudar Thylam* holds significant therapeutic value. This *thylam*-based medicine is commonly prescribed for a spectrum of conditions including fistula in ano, chronic ulcers, lymphedema, and abscesses (12). While the specific details of the "*Sudar Thylam* method" for *Pun Sudar Thylam* are often part of traditional Siddha pharmacological texts, the general principle of *thylam* (medicated oil) preparation involves boiling decoctions, juices, milk, and pastes of herbal and

* Corresponding Author:

Sanjana S

Lecturer, Department of Aruvai Maruthuvam,
Sri Sairam siddha Medical College and Research Centre,
Chennai, Tamil Nadu. 600132.

Email Id: drsanjana1603@gmail.com

mineral drugs with base oils until dehydration, ensuring the transfer of active principles (2).

In a pivotal step towards ensuring quality control and consistency, the Pharmacopeial Laboratory for Indian Medicine (PLIM) published comprehensive guidelines in Volume 1 in 2018, specifically addressing the standardization of Siddha Preparations (16). These guidelines meticulously outline parameters for the standardization of *thylam* preparations, encompassing physicochemical evaluation, heavy metal assessment, microbial contamination, and other quality control parameters (6). Consequently, this study aims to evaluate the quality of *Pun Sudar Thylam* by rigorously applying the established PLIM guidelines, thereby contributing to the evidence-based validation and wider acceptance of this traditional medicine.

Materials and Methods

Selection of the Test Formulation

The test formulation, *Pun Sudar Thylam* (PST), is a herbo-mineral Siddha preparation traditionally indicated for conditions such as *Pouthiram*, *Pillavai*, and *Pungal*, as described in the classical Siddha text *Anuboga Vaithiya Navaneetham – Part 10*,” written by Hakkim Pa. Mohammad Abdula Sayubu

Procurement and Authentication of Raw Materials

The raw materials required for the preparation of PST were procured from a certified country shop in Chennai, Tamil Nadu. Botanical authentication was performed by experts from the Department of *Gunapadam*, National Institute of Siddha, Chennai, and a certificate of authentication was obtained (Certificate No. NISMB6732024, Gun/Aut/14/24).

Composition of Pun Sudar Thylam

Pun Sudar Thylam was prepared using the following ingredients in accordance with classical Siddha literature:

- *Nellikaai Gandhagam* (Purified sublimated sulphur) – 17 g
- *Erukam paal* (latex of *Calotropis gigantea* (L.) W.T. Aiton) – sufficient quantity for impregnation
- *Chitramanakku ennai* (*Ricinus communis* L., castor oil) – sufficient quantity for moistening and extraction

The proportions and method followed were as described in *Anuboga Vaithiya Navaneetham – Part 10*, ensuring adherence to traditional Siddha pharmaceutics

Purification of Nellikaai Gandhagam (Sublimated Sulphur)

A paste (*karkam*) prepared from *Maruthondri* (*Lawsonia inermis*) was mixed with cow's curd and placed inside a mud pot. The mouth of the pot was covered with a cotton cloth, over which *Gandhagam* (sulphur) was placed. The pot was then closed with a lid and completely sealed using a cloth coated with mud paste. The sealed pot was buried in the ground up to the level of its mouth, and cow dung cakes were arranged over it and ignited. The heat generated caused the *Gandhagam* placed above the cloth to melt and collect at the bottom of the pot. The processed sulphur was removed after cooling. This entire procedure was repeated seven times, each cycle using a fresh preparation of *Maruthondri* paste and cow's curd (17).

Preparation Method of Pun sudar thylam

A clean cotton cloth (*seelai*) was soaked in *Erukam paal* (latex of *Calotropis gigantea*(L) W.T.Aiton), dried, and the process was

repeated three times. Subsequently, 17 g of purified *Nellikaai Gandhagam* (purified sublimated sulphur) was placed on the dried cloth and rolled onto the tip of an iron rod. The cloth was then moistened with castor oil and ignited. The resultant oil extract was collected in a sterile, dark-colored glass bottle. Prior to use, the cloth (*seelai*) was dipped in the prepared *thylam* to maintain procedural consistency.

Quality Evaluation (8)

Physicochemical Analysis

As per the guidelines of AYUSH-Pharmacopeial Laboratory for Indian Medicine (PLIM), physicochemical parameters of the formulation were analysed. The analyses were carried out at International Testing Centre Lab (ITC), Panchkula, Haryana. The tests included organoleptic evaluation, specific gravity, loss on drying, congealing temperature, refractive index, total ash, viscosity, iodine value, saponification value, acid value, peroxide value, free fatty acids, total fatty matter, and mineral oil content.

Specific Gravity

The specific gravity of PST was determined using a pycnometer by comparing the weight of a fixed volume of the sample at 25 °C with the weight of an equal volume of water at the same temperature.

Loss on Drying

Approximately 10 g of the test sample was weighed without prior drying and placed in a pre-weighed evaporating dish. The sample was dried to constant weight and the percentage loss in weight was calculated.

Refractive Index

The refractive index (*n*) was measured using a refractometer, where the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a light beam passing from air into the test substance was determined.

Total Ash

Approximately 2–3 g of the powdered drug was incinerated at a temperature not exceeding 450 °C until a carbon-free ash was obtained. If necessary, the charred mass was extracted with hot water, filtered, dried, and re-incinerated. The total ash was expressed as a percentage of the air-dried sample.

Viscosity

Viscosity was measured using a U-tube viscometer. The time taken for the liquid meniscus to pass between two calibration marks was recorded, and kinematic viscosity (in centistokes) was calculated using the formula:

$$\text{Kinematic viscosity} = kt$$

where *k* is the viscometer constant and *t* is the flow time in seconds.

Iodine Value

The iodine value was determined using the Iodine Monochloride method, which measures the amount of iodine absorbed by 100 g of the test substance. After reaction with iodine monochloride and potassium iodide, the liberated iodine was titrated against 0.1 N sodium thiosulphate using starch as an indicator. Calculate the iodine value from the formula:

$$\text{Iodine value} = \frac{(b - a) \times 0.01269 \times 100}{W}$$

Where 'W' is the weight in g of the substance taken.

Saponification Value

Approximately 2 g of the test sample was refluxed with alcoholic KOH for one hour. After cooling, the excess alkali was titrated with 0.5 N HCl using phenolphthalein as an indicator. The saponification value was calculated using the formula:

$$\text{Saponification Value} = \frac{(b - a) \times 0.02805 \times 1.000}{W}$$

where b and a are the titration volumes of the blank and sample respectively, and W is the weight of the sample in grams.

Unsaponifiable Matter

Unsaponifiable matter was determined by saponifying the sample with alkali, followed by extraction of the non-saponifiable residue using organic solvents. The residue was quantified gravimetrically.

Acid Value

The acid value was calculated by titrating the sample dissolved in ethanol-ether mixture against 0.1 N KOH, using phenolphthalein as an indicator. The value is expressed as mg of KOH required to neutralize free acids in 1 g of substance. Calculate the acid value from the following formula:

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{W}$$

Peroxide Value

Peroxide value was determined by reacting the test sample with potassium iodide in an acetic acid-chloroform mixture and titrating the liberated iodine with 0.01 M sodium thiosulphate using starch as an indicator. The result was expressed in milliequivalents of peroxide per kg of sample.

Fatty acid

The free fatty acid (FFA) content in *Pun Sudar Thylam* was measured using a standard titration method based on the Ayurvedic Pharmacopoeia of India. About 10 g of the oil sample was weighed and mixed with a 1:1 solution of ethanol and diethyl ether. The mixture was gently warmed to fully dissolve the oil. This solution was then titrated with 0.1 N potassium hydroxide (KOH) using phenolphthalein as an indicator. The endpoint of the titration was noted when a pale pink color appeared and remained for 30 seconds.

The free fatty acid percentage, expressed as oleic acid, was calculated using the formula

$$\text{FFA (\%)} = \frac{V \times N \times 28.2}{W}$$

Where:

- V = Volume of KOH used (mL)
- N = Normality of KOH
- W = Weight of the sample (g)
- 28.2 = Equivalent weight of oleic acid

Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) was performed to identify the phytochemical constituents present in the formulation. The process utilizes the distribution of a solute between two phases: a

stationary phase, acting through adsorption, and a mobile liquid phase. A silica gel 60F254 TLC plate (Merck), cut to 7 × 6 cm dimensions, was used as the stationary phase. The sample (10 μL) was applied using a micropipette across five tracks, each spaced 1 cm apart. The plate was developed in a one-dimensional ascending method within a CAMAG Twin Trough chamber, utilizing a designated solvent system. Post development, the plate was air-dried and visualized under visible light, short-wave UV (254 nm), and long-wave UV (366 nm) to detect spot formation. Rf values were calculated to aid in compound identification by comparing spot position, color, and intensity with standard references (18).

High-Performance Thin Layer Chromatography (HPTLC)

HPTLC was employed for precise profiling of phytoconstituents. The CAMAG Twin Trough chamber was used under controlled conditions. Samples were eluted based on the differential adsorption capacity of components. After development, plates were air-dried and scanned under UV light at 366 nm using a CAMAG TLC scanner. Chromatographic data were analysed using CAMAG software to generate fingerprint profiles, and Rf values were recorded for each constituent (18).

Atomic Absorption Spectroscopy (AAS)

The presence and concentration of heavy metals in the formulation were determined using Atomic Absorption Spectroscopy (AAS). AAS Model AA 240 Series was employed for the quantification of metals such as lead, cadmium, arsenic, and mercury. Sample digestion and analysis were conducted in accordance with standard procedures specified in the Ayurvedic Pharmacopoeia of India (11).

Sterility Test – Pour Plate Method

The sterility of the formulation was assessed using the pour plate method. A sterile Petri dish was inoculated with the test sample, followed by the addition of ~15 mL of molten agar (cooled to 45°C). After swirling and solidification (approx. 10 min), the plates were incubated in an inverted position at 37°C for 48 hours to monitor bacterial growth. Plates were then exposed for an additional 72 hours to detect fungal contamination. Colony-forming units (CFUs) were recorded to evaluate microbial load (8).

Test for Specific Pathogens

To assess contamination with specific pathogens, the test sample was inoculated into selective media including Eosin Methylene Blue (EMB) for *Escherichia coli*, Mannitol Salt Agar for *Staphylococcus aureus*, Cetrimide Agar for *Pseudomonas aeruginosa*, and Deoxycholate Citrate Agar (DCC) for *Salmonella spp.* Plates were incubated at 37°C for 24–72 hours. The appearance of characteristic colonies and color changes in the media indicated the presence of specific organisms (2).

Results

Organoleptic Evaluation

The organoleptic assessment of *Pun Sudar Thylam* (PST) revealed it to be a black to dark brown semisolid oil with a smoky, sulphur-like characteristic odour. The formulation possessed a greasy touch, which is typical of lipid-based Siddha preparations, and exhibited free-flowing properties. These attributes align with traditional expectations for *thylam*-type Siddha drugs, and suggest the presence of both herbal extracts and mineral components in a homogenized base. (Table 1.)

Physicochemical Analysis

Physicochemical standardization of PST was conducted following the AYUSH-PLIM guidelines, with results indicating strong compositional integrity. The specific gravity of the formulation was found to be 1.185, which is significantly higher than that of pure castor oil (0.945–0.966), suggesting a dense matrix rich in phytochemicals and mineral content. The loss on drying was minimal at 0.44%, indicating low moisture content and reduced risk of microbial spoilage. The refractive index measured at 1.494, and a congealing temperature of 33°C was observed, consistent with a semi-solid nature at ambient temperature. The total ash content, indicative of inorganic residue, was found to be 0.13%, reflecting the purity of the formulation. Viscosity, measured at 730 mPas (25°C), demonstrated an optimal consistency for external application. The iodine value, a marker of unsaturation in fatty acids, was determined as 97.81, which is marginally above the range seen in plain castor oil, suggesting mild unsaturation that may influence oxidative stability. The saponification value of 212.79 suggests the presence of shorter-chain fatty acids, contributing to the formulation's lipidic texture and skin penetration potential. The unsaponifiable matter was 0.59%, likely representing bioactive compounds not bound in triglycerides. Additionally, the acid value was found to be 2.52, within acceptable limits, indicating controlled hydrolysis and good product stability. A peroxide value of 5.19 mEq/kg was noted, which confirms minimal lipid oxidation, further supporting the formulation's shelf stability. The presence of free fatty acids (0.98%) and total fatty matter (8.70%) also reinforce the presence of functionally active lipid constituents. Notably, mineral oil was absent, confirming the absence of adulteration. (Table 2.)

Heavy Metal Analysis

The safety profile of PST was further validated by its heavy metal content, assessed through ICP-MS. The levels of lead (Pb), cadmium (Cd), and mercury (Hg) were all reported as below the limit of quantification (BLQ), reflecting excellent raw material quality and processing standards. Arsenic (As) was detected at 1.93 ppm, a value that remains within permissible limits as per AYUSH guidelines. The trace presence of arsenic is likely attributable to the inclusion of purified sulphur (Nellikkaai *Gandhagam*) in the formulation, a common feature in traditional *Sudar Thylam* preparations. (Table 3.)

Microbiological Evaluation

Microbial safety testing confirmed that PST meets pharmaceutical-grade microbial quality standards. The total viable aerobic count was limited to 500 CFU/g, and the total fungal count was less than 10 CFU/g, both of which fall well within the acceptable range for topical herbal formulations. No contamination by pathogenic bacteria—including *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Enterobacteriaceae*, or *Pseudomonas aeruginosa*—was detected. These findings affirm that the formulation is microbiologically safe for external application and adheres to current AYUSH safety norms. (Table 4)

High Performance Thin Layer Chromatography (HPTLC) Analysis

The HPTLC analysis of *Pun Sudar Thylam* was performed to develop a phytochemical fingerprint profile and assess its chemical complexity and reproducibility. Chromatograms were obtained under three different detection wavelengths—254 nm, 366 nm, and 580 nm—across multiple sample tracks. The analysis revealed a total of four prominent peaks at 254 nm, and eight well-resolved peaks each at 366 nm and 580 nm, signifying a chemically diverse matrix with a wide range of constituents. Table 5 presents the peak characteristics extracted from chromatographic plots (b, c, and d), highlighting the R_f values, peak areas, and their approximate percentage contributions to total peak intensity. Among the peaks observed, the spot at R_f 0.782 in plot (b) displayed the highest peak area (~45.35%), indicating the dominance of a specific constituent, possibly a major bioactive compound. Likewise, peaks at R_f 0.000 appeared consistently in both plots (b) and (c), contributing substantially to the total area (~28.73% and ~31.82%, respectively), thus qualifying as reliable marker compounds for standardization purposes. Additionally, a significant peak was observed at R_f 0.476 in plot (c), accounting for ~26.01% of the total area. This mid-R_f peak suggests the presence of medium polarity phytochemicals. Further peaks at R_f 0.285, 0.336, 0.429, and 0.717 (plot d) also demonstrated notable area percentages (ranging from ~17% to ~21%), highlighting the complexity and richness of the chemical composition of the formulation. The consistency of key peaks across different detection wavelengths and sample tracks supports the formulation's reproducibility and robustness. These well-resolved peaks can serve as diagnostic reference markers for future quality control studies, offering a validated fingerprint for *Pun Sudar Thylam* under the AYUSH standardization framework.

Table 1: Organoleptic characters of PST

| S. No | Organoleptic characters | Observations |
|-------|-------------------------|---------------------------------|
| 1 | Description | Black coloured semisolid oil |
| 2 | Odour | Smoky characteristic odour |
| 3 | Touch | Greasy like texture |
| 4 | Flow property | Free flowing in nature |
| 5 | Appearance and colour | Black and dark brown appearance |

Table 2: Chemical Parameters of PST

| S. No | Chemical Parameters | Inst. Used | Method | Result |
|-------|-----------------------|-----------------------|--------|----------|
| 1 | Specific gravity | Chemically | API | 1.185 |
| 2 | Loss on drying | Vacuum Oven | API | 0.44% |
| 3 | Congeeing Temperature | Chemically | API | 33°C |
| 4 | Refractive index | Chemically | API | 1.494 |
| 5 | Total ash | Chemically | API | 0.13% |
| 6 | Viscosity at 25°C | Brookfield Viscometer | API | 730 mPas |

| | | | | |
|----|------------------------|------------|-----|-------------|
| 7 | Iodine value | Chemically | API | 97.81 |
| 8 | Saponification Value | Chemically | API | 212.79 |
| 9 | Unsaponification Value | Chemically | API | 0.59 |
| 10 | Acid value | Chemically | API | 2.52 |
| 11 | Peroxide Value | Chemically | API | 5.19 mEq/kg |
| 12 | Free fatty acids | Chemically | API | 0.98% |
| 13 | Total fatty matter | Chemically | API | 8.70% |
| 14 | Mineral Oil | Chemically | API | Absent |

Table 3: Heavy metal analysis of PST

| S.no | Heavy metals | Instd.used | Method | Results |
|------|-----------------|------------|--------|---|
| 1 | Lead (as Pb) | ICPMS | API | BLQ (LOQ: 0.10) ppm |
| 2 | Arsenic (as As) | ICPMS | API | 1.93 ppm |
| 3 | Mercury (as Hg) | ICPMS | API | BLQ (LOQ: 0.10) ppm BLQ (LOQ: 0.10) ppm |
| 4 | Cadmium (as Cd) | ICPMS | API | BLQ (LOQ: 0.10) ppm |

Table 4: Microbiological test of PST

| S.no | Microbiological test | Inst.used | Method | Results |
|------|----------------------------|-----------------|--------|-------------|
| 1 | Total viable aerobic count | Microbiological | API | 500 cfu/gm |
| 2 | Total fungal count | Microbiological | API | < 10 cfu/gm |
| 3 | Enterobacteriaceae | Microbiological | API | Absent/gm |
| 4 | E. coli | Microbiological | API | Absent/gm |
| 5 | Salmonella | Microbiological | API | Absent/gm |
| 6 | Staphylococcus | Microbiological | API | Absent/gm |
| 7 | Pseudomonas aeruginosa | Microbiological | API | Absent/gm |

Table 5: Peak Characteristics of *Pun Sundar Thylam* from HPTLC Analysis at 254 nm, 366 nm, and 580 nm, Highlighting Rf Values, Peak Height, and Peak Area Across Sample Tracks

| Plot | Peak # | Rf | Area | % of Total Area (approx) | Remarks |
|------|--------|-------|---------|--------------------------|----------------------|
| (b) | 4 | 0.782 | 0.00759 | ~45.35% | Dominant, sharp |
| (b) | 1 | 0.000 | 0.00481 | ~28.73% | Consistent |
| (c) | 1 | 0.000 | 0.00790 | ~31.82% | High contribution |
| (c) | 5 | 0.476 | 0.00645 | ~26.01% | Strong mid-Rf peak |
| (d) | 5 | 0.285 | 0.01760 | ~21.17% | Major mid-range peak |
| (d) | 6 | 0.336 | 0.01640 | ~19.73% | Broad support |
| (d) | 7 | 0.429 | 0.01490 | ~17.91% | Still strong |
| (d) | 8 | 0.717 | 0.01483 | ~17.85% | High Rf dominant |

Figure 1: Chromatogram visualized at 254 nm, showing four major peaks with Rf values ranging from 0.000 to 0.924, indicating the UV-active compounds in PST

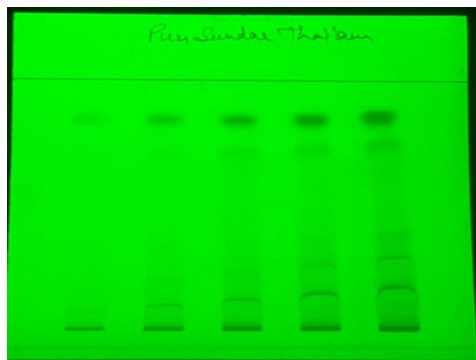


Figure 2: Chromatogram visualized at 366 nm, showing eight prominent peaks distributed between Rf 0.000 to 0.846, highlighting compounds with fluorescence under long-wave UV

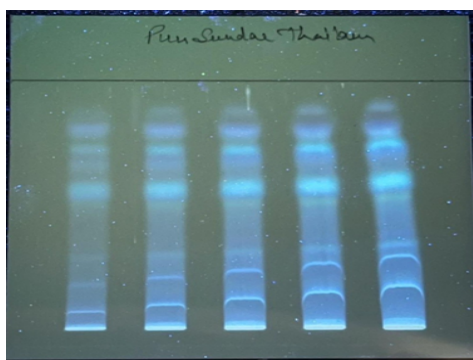
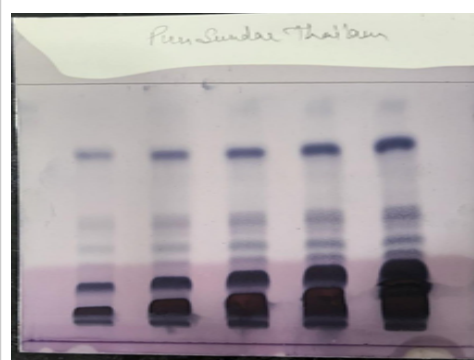


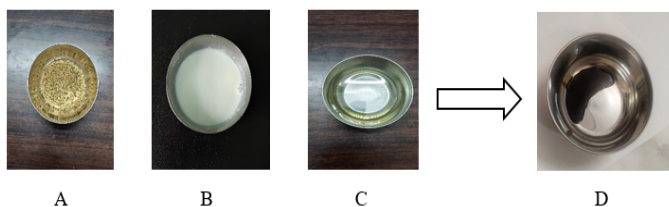
Figure 3: Post-derivatization scan at 580 nm, revealing eight well-defined peaks from Rf 0.000 to 0.964, capturing secondary metabolite presence with chromogenic response



Discussion

The standardization of traditional Siddha formulations like *Pun Sudar Thylam* (PST) is essential for ensuring quality, safety, and therapeutic efficacy. This study involved the detailed evaluation of PST using classical pharmacopoeial tests, heavy metal analysis, microbiological screening, and HPTLC fingerprinting. The findings are discussed below in relation to each assessment parameter.

The formulation process starts with (A) *Nellikai Gandhagam*, (purified sublimated sulphur). sulphur plays a key role in skin regeneration and antimicrobial activity (Kumar et al., 2011) *Erukam Paal* (B), the latex of *Calotropis gigantea*(L) *W.T.Aiton*, which has been widely documented in ethnomedicine for its wound healing, anti-inflammatory, and antimicrobial properties (21). The latex contains bioactive compounds such as calotropin and uscharin that contribute to its therapeutic efficacy. Castor oil (C) (*Ricinus communis* L.), which acts as the base oil in this formulation. Castor oil has emollient, anti-inflammatory, and analgesic properties and is commonly used as a carrier oil in Siddha and Ayurvedic preparations (3). These three ingredients are subjected to traditional Siddha pharmacopoeial techniques involving controlled heating and continuous stirring to ensure homogenization and activation of therapeutic constituents. The final product, (D) *Pun sudar thylam*, is shown in panel D. The darkened oil indicates the completion of the preparation process, where active phytochemicals have been infused into the oil matrix. This polyherbal oil is primarily applied externally for managing chronic wounds, ulcers, skin infections, and inflammatory skin conditions. Its efficacy is attributed to the synergistic effects of its ingredients, each contributing specific bioactive properties. The integrative approach of combining herbal and mineral ingredients is a hallmark of Siddha medicine, aiming to balance bodily humors and promote holistic healing (23). (Figure .4)



Organoleptic Characters (Table 1)

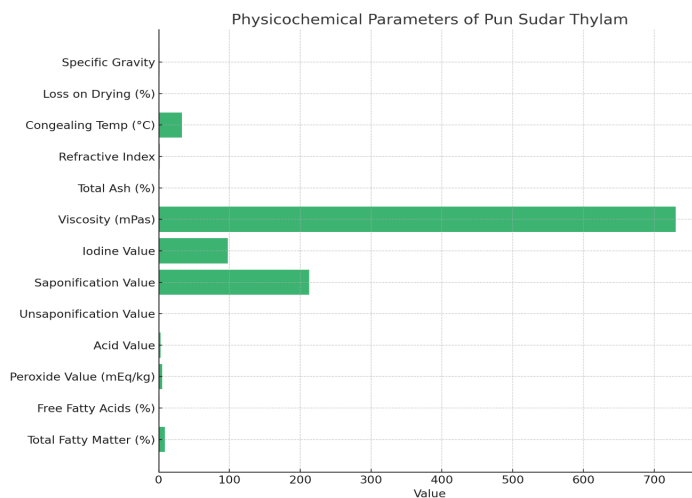
The organoleptic properties of PST, including its black to dark brown semisolid consistency, smoky sulphur-like odor, and greasy texture, are in line with classical Siddha literature descriptions of *Sudar Thylam* preparations, which involve sulphur processing and herbal decoctions in castor oil base (20). The free-flowing nature supports its ease of application on the skin. These observations are important for traditional medicine where sensory characteristics often guide preliminary quality checks (8).

Physicochemical Parameters (Table 2)

The specific gravity of 1.185 observed for PST is considerably higher than that of plain castor oil (0.945 to 0.966), suggesting enrichment with dissolved phytoconstituents from processed herbs and minerals. Refractive index (1.494) and viscosity (730 mPas at 25°C) values also support the complex nature of the formulation. The low moisture content (Loss on Drying = 0.44%) indicates reduced risk of microbial contamination and prolonged shelf life (Indian Pharmacopoeia Commission, 2014). The iodine value of

97.81, slightly above that of pure castor oil, implies unsaturation in fatty acids, which could affect oxidation stability (14). However, the peroxide value (5.19 mEq/kg) is within acceptable limits, indicating low oxidative rancidity (9). The acid value (2.52) and saponification value (212.79) suggest a significant presence of short-chain fatty acids and minimal hydrolytic degradation (18). These values reflect a stable, chemically active formulation suitable for therapeutic use.

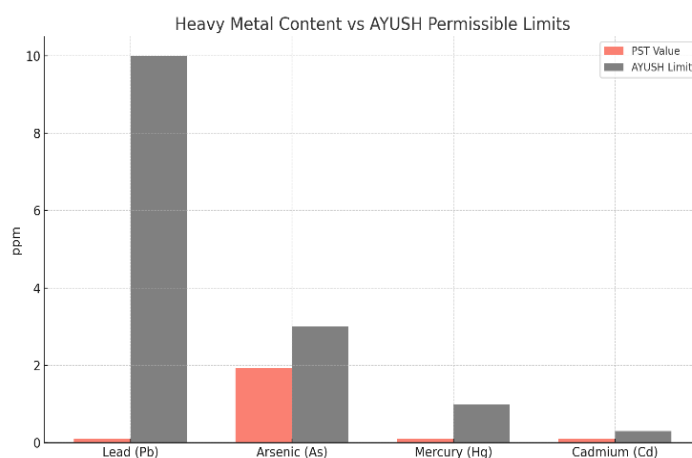
Figure 5: This horizontal bar chart displays various physicochemical properties of *Pun sudar thylam*. Viscosity has the highest value among all parameters, followed by saponification and iodine values, while other values like peroxide value and total fatty matter are comparatively low



Heavy Metal Analysis (Table 3)

Safety assessment of Siddha formulations must include toxic metal screening due to the use of mineral ingredients. In this study, PST was found to have lead, mercury, and cadmium below the quantification limit, while arsenic was detected at 1.93 ppm. This is within the AYUSH permissible limits (<10 ppm for arsenic), ensuring the formulation's safety (10). The trace arsenic may be attributed to the inclusion of purified sulphur (*Nellikai Gandhagam*), a common Siddha component known to carry trace elements (1).

Figure 6: This bar chart compares the heavy metal content (Pb, As, Hg, Cd) in *Pun Sudar Thylam* with AYUSH safety limits. The values for all tested metals are within permissible limits, with Arsenic nearing its threshold while others remain significantly lower



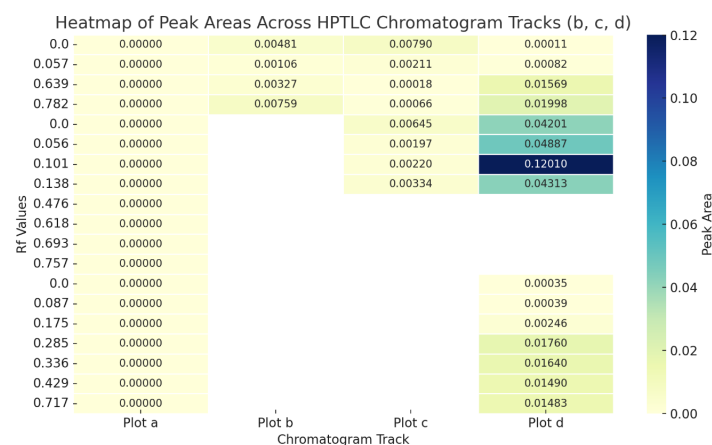
Microbiological Screening (Table 4)

Microbial quality control is vital for topical preparations. PST showed a total aerobic count of 500 CFU/g and a fungal count of less than 10 CFU/g, both of which fall within AYUSH acceptable ranges (7). Additionally, no pathogenic bacteria such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Enterobacteriaceae*, or *Pseudomonas aeruginosa* were detected. These results confirm that the formulation is safe for external application without risk of infection.

HPTLC Fingerprinting (Table 5)

HPTLC profiling serves as a diagnostic tool for phytochemical fingerprinting and standardization. In PST, distinct peaks were observed at Rf values 0.000, 0.476, and 0.782, which represented the most abundant constituents. Peaks in the mid and high Rf regions (0.285 to 0.717) further confirmed the chemical complexity and reproducibility of the formulation. These findings support the use of these Rf values as marker zones for quality control in future batch preparations (2). HPTLC methods are widely accepted for herbal drug authentication and have been endorsed by the AYUSH-PLIM standards (11).

Figure 7: This heatmap illustrates the peak areas across different HPTLC chromatogram tracks (b, c, and d) for various Rf values. Darker shades indicate higher peak intensities, with the highest peak (0.12010) observed in Plot d at an Rf value of 0.101, suggesting strong compound presence at that point. Plot a shows negligible peak activity across all Rf values.



Conclusion

PST exhibits robust physicochemical characteristics that underscore its stability and quality. The formulation's specific gravity indicates a rich concentration of phytoconstituents, while its moderate unsaturation level suggests a balanced composition. Controlled acid and peroxide values further enhance its shelf life and resistance to rancidity. The absence of microbial contaminants and minimal presence of heavy metals ensure its safety and purity. These attributes collectively position PST as a dependable product suitable for therapeutic applications. Thus, this study represents progress towards scientifically validating *Pun sudar thylam*.

Future scope

Given these findings, PST might be the focus of toxicity tests in the future, both in vitro and in vivo. The outcomes show the quality of the medicine PST and could serve as a fingerprint for further PST investigation.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Funding

The authors do not have any direct financial relation with the commercial identities mentioned in the paper.

Author's contribution

In this work, Dr. Sanjana. S involved in research concept, design, collection of data, data analysis, interpretation and writing the article. Dr. Lavanya. M involved in collection of data, data analysis, interpretation and writing the article. Dr. Jayaveeran. T involved in collection of data, data analysis, interpretation and writing the article.

Acknowledgements

The authors gratefully acknowledge Dr. M. V. Mahadevan, Head of the Pura Maruthuvam Department, Dr. D. Periyasami, Head of Varma Maruthuvam Department, Dr. R. Keerthika, Assistant Professor, Department of Pura Maruthuvam for providing the necessary facilities. I am thankful to Dr. Vigneshwaran. K Ph.D., (Agri) for the technical support.

References

- Adithya RS, Manikgandan EM, Natarajan K, Kanimozhi S. Standardization of Ghandhaga Thailam—A traditional Siddha formulation for skin disorders. SSRN. 2024; Preprint; 1–15. DOI: 10.2139/ssrn.4723600
- Arunadevi R, Susila R, Murugammal S, Divya S. Preparation and standardization of Mathan Tailam: A classical Siddha formulation for diabetic ulcerative wound healing. Journal of Ayurveda and Integrative Medicine. 2020; 11(1); 10–15. DOI: 10.1016/j.jaim.2017.08.011
- Burdock GA. Review of the biological properties and toxicity of bee propolis (propolis). Food and Chemical Toxicology. 1998; 36(4); 347–363. DOI: 10.1016/S0278-6915(97)00145-2
- Deore AB, Dhurane JR, Wagh R, Sonawane R. The stages of drug discovery and development process. Asian Journal of Pharmaceutical Research and Development. 2019; 7(6); 62–67. DOI: 10.22270/ajprd.v7i6.616
- Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Vol. 1. Ghaziabad; Ministry of Health and Family Welfare, Government of India; 2014.
- Jothikaviyaran G, Duraichi A. Qualitative and quantitative analysis of Karunkozhi Thylam prepared as per Siddha literature. International Journal of Ayurveda and Pharma Research. 2024; 12(9); 12–18. DOI: 10.47070/ijapr.v12i9.3391
- Kamaliya S, Vaghela B, Harisha C, Shukla V. Quality control assessment of an Ayurvedic medicine – Durvadi Ghrita. International Journal of Ayurvedic Medicine. 2020; 11(4); 754–758. DOI: 10.47552/ijam.v11i4.1712
- Lohar DR. Quality control manual for Ayurvedic, Siddha and Unani medicines. Ghaziabad; Pharmacopoeial Laboratory for Indian Medicine, Ministry of AYUSH; 2008. p. 69–74.
- Menkudale BS, Pawar M. Physico-chemical analysis of Brahmi Ghrita prepared from Puran Ghrita and fresh Go-Ghrita. Asian Journal of Pharmacy and Pharmacology. 2018; 4(3); 271–274. DOI: 10.31024/ajpp.2018.4.3.5
- Ministry of AYUSH. The Ayurvedic Pharmacopoeia of India. Part II, Vol. I. New Delhi; Government of India; 2007. p. 63–74.

11. Ministry of Health and Family Welfare. Ayurvedic Pharmacopoeia of India. Part I, Vol. 4. New Delhi; Government of India; 2011. Appendix 3/1.3.
12. National Institute of Siddha. Standard Siddha treatment guidelines. Chennai; Ministry of AYUSH, Government of India; 2018.
13. Neha K, Haider MR, Pathak A, Yar MS. Medicinal prospects of antioxidants: A review. *European Journal of Medicinal Chemistry*. 2019; 178; 687–704. DOI: 10.1016/j.ejmech.2019.06.010
14. Pal RS, Mishra A. Standardization of Dhatriyadi Ghrita: A herbal ghee-based Ayurvedic medicinal preparation. *The Open Medicine Journal*. 2018; 5(1); 1–7. DOI: 10.2174/1874220301805010047
15. Patwardhan B, Vaidya AD, Chorghade M. Ayurveda and natural products drug discovery. *Current Science*. 2004; 86(6); 789–799.
16. Pharmacopoeial Laboratory for Indian Medicine. Ayurvedic Pharmacopoeia of India. Part II, Vol. I: Formulations. Ghaziabad; Ministry of AYUSH, Government of India; 2018.
17. Ra. Thiyagarajan, Gunapadam thathu jeevam vaguppu, 8th edition, Indian medicine and homeopathy, the nadar press limited, Sivakasi 2013.
18. Rajeswari K, Muthu M, Vennila K, Malayappan MS, Meenakumari R. Physicochemical standardisation of Siddha tablet preparation Jathikaai Mathirai. *International Journal of Research in Ayurveda and Pharmacy*. 2021; 12(4); 87–93. DOI: 10.7897/2277-4343.1204112
19. Salti G, Ghersetich I. Advanced botulinum toxin techniques against wrinkles in the upper face. *Clinics in Dermatology*. 2008; 26(2); 182–191. DOI: 10.1016/j.clindermatol.2007.09.008
20. Sayubu HPM. Anuboga Vaithiya Navaneetham. Part-10. Chennai; Traditional Siddha Publication; n.d. p. 64.
21. Sharma A, Shanker C, Tyagi LK, Singh M, Rao CV. Herbal medicine for market potential in India: An overview. *Academic Journal of Plant Sciences*. 2008; 1(2); 26–36.
22. Surh YJ. Reverse pharmacology applicable for botanical drug development—Inspiration from the legacy of traditional wisdom. *Journal of Traditional and Complementary Medicine*. 2011; 1(1); 5–10. DOI: 10.1016/S2225-4110(16)30051-7
23. Thas JJ. Siddha medicine—Background, principles and application for skin diseases. *Clinics in Dermatology*. 2008; 26(1); 62–78. DOI: 10.1016/j.clindermatol.2007.11.010
24. Willcox M, Bodeker G, Rasoanaivo P, Addae-Kyereme J. Traditional medicinal plants and malaria. Boca Raton; CRC Press; 2004. p. 1–384. DOI: 10.1201/9780203502327
25. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva; WHO Press; 2011.
