

STANDARDIZATION, PHYTOCHEMICAL PROFILING, HEAVY METAL SAFETY AND MICROBIAL QUALITY EVALUATION OF VETRILAI THYLAM: A SIDDHA HERBAL OIL FORMULATION

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ABSTRACT

Background: Standardization of Siddha formulations is essential to ensure quality, safety, and reproducibility. *Vetrilai Thylam* is a traditional herbal oil widely used in Siddha practice. **Aim:** To evaluate the physicochemical parameters, heavy metal safety, and phytochemical constituents of Vetrilai Thylam. **Materials and Methods:** The formulation was analyzed using PLIM protocols for ASU drugs. Physicochemical parameters were assessed as per standard procedures. Heavy metal analysis was performed according to WHO guidelines. Phytochemical screening and sterility testing were carried out using standard microbiological and phytochemical methods. **Results:** The formulation exhibited acceptable physicochemical parameters. Heavy metals were within permissible limits. No microbial growth was observed in sterility testing, confirming the absence of contamination. Phytochemical evaluation indicated the presence of bioactive principles supporting therapeutic potential. **Conclusion:** The

study confirms that Vetrilai Thylam meets safety standards and contains significant bioactive compounds, supporting its therapeutic potential and validating its traditional use.

KEYWORDS: Siddha medicine, Vetrilai Thylam, standardization, physicochemical analysis, phytochemical screening, heavy metals.

1. INTRODUCTION

Siddha formulations are complex herbal preparations requiring systematic standardization to ensure quality, safety, and efficacy.^[1] Herbal oils such as Vetrilai Thylam play an important role in clinical practice due to their anti-inflammatory and antimicrobial properties, as described in classical Siddha texts such as *Balavatha Nidhanam*.^[2]

Modern analytical approaches, including physicochemical evaluation, heavy metal testing, and phytochemical screening, are essential for validating traditional medicines and ensuring compliance with regulatory standards.^[3,5]

2. MATERIALS AND METHODS

2.1 Sample Details

- **Drug Name:** Vetrilai Thylam (VT)
- **Source:** Govt. Siddha Medical College, Palayamkottai
- **Protocol:** PLIM – ASU formulations.^[3]
- **Form:** Herbal oil

2.2 Physicochemical Analysis

The physicochemical parameters were analyzed as per standard procedures.^[4]

OBSERVED RESULTS

Parameter	Result
Description	Light green coloured oil
Refractive Index	1.22
Acid Value	11.22
Saponification Value	90.07
Iodine Value	98.73
Mineral Oil	Absent

2.3 Heavy Metal Analysis

Heavy metal estimation was carried out according to WHO guidelines.^[5]

Heavy Metal	Result	Permissible Limit
Lead (Pb)	< 5 ppm	Not more than 10 ppm
Arsenic (As)	< 1 ppm	Not more than 3 ppm
Mercury (Hg)	< 0.5 ppm	Not more than 1 ppm
Cadmium (Cd)	< 0.1 ppm	Not more than 0.3 ppm

Interpretation: All heavy metals are within WHO permissible limits → **SAFE**^[5]

2.4 Phytochemical Analysis

Qualitative phytochemical screening was performed using standard methods described in phytopharmaceutical evaluation literature.^[6,7]

Test for alkaloids

Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for coumarins

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

Test for saponins

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids

Alkaline reagent test. Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Test for phenols

Lead acetate test: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

Test for Cyanins**A. Aanthocyanin**

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

Test for Carbohydrates - Benedict's test

To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

S.No	Phytochemical	Result
1.	Alkaloids	+
2.	Flavonoids	+
3.	Glycosides	+
4.	Steroids	+
5.	Triterpenoids	+
6.	Phenols	+
7.	Tannins	+
8.	Sugars	+
9.	Coumarins	-
10.	Saponins	-
11.	Proteins	-
12.	Anthocyanin	-
13.	Betacyanin	-



Figure 1: Phytochemical analysis of Vetrilai thylam.

2.5 Sterility Test

Sterility testing was carried out using the pour plate method to evaluate microbial contamination.^[8] The test sample was inoculated into sterile petri dishes, followed by the addition of molten agar at 45°C. The plates were incubated at 37°C for 24–48 hours and extended up to 72 hours for fungal growth observation. Colony formation was monitored and expressed as Colony Forming Units (CFUs).

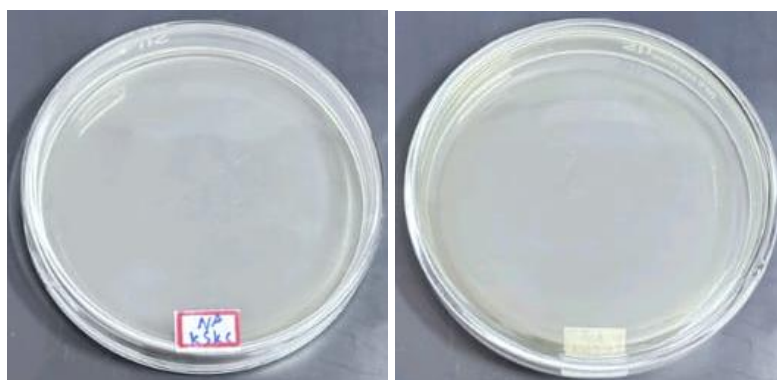


Figure 2: Sterility test of the formulated sample Vetrilai thylam showing no microbial growth.

3. RESULTS AND INTERPRETATION

3.1 Physicochemical Significance

- **Acid value (11.22):** Indicates acceptable level of free fatty acids → good stability.^[4]
- **Saponification value (90.07):** Confirms presence of fatty acids suitable for therapeutic oil.^[4]
- **Iodine value (98.73):** Suggests unsaturated fatty acids → beneficial for absorption.^[4]

3.2 Heavy Metal Safety

All toxic metals were within permissible limits confirming safety and compliance with WHO standards.^[5]

- Non toxic
- Safe for therapeutic use
- Meets regulatory standards

3.3 Phytochemical Significance

- **Flavonoids & Phenols:** Antioxidant activity
- **Alkaloids:** Antimicrobial effects
- **Triterpenoids & Steroids:** Anti-inflammatory action
- **Tannins:** Wound healing.^[6,7]

This confirms multi-target therapeutic potential.

3.4 Sterility Test Results

No microbial growth was observed in any of the inoculated plates, indicating the absence of bacterial and fungal contamination. The total bacterial count and total fungal count were found to be within acceptable limits as per AYUSH/WHO standards.^[8]

4. DISCUSSION

The integration of physicochemical, phytochemical, and heavy metal analysis provides a comprehensive evaluation of Vetrilai Thylam.

- Physicochemical parameters confirm **identity and purity**
- Heavy metal analysis ensures **safety compliance**.^[5]
- Phytochemical screening validates **therapeutic efficacy**.^[6]
- The absence of microbial growth in sterility testing further confirms the safety, quality, and suitability of the formulation for therapeutic use.^[8]

This aligns with Siddha principles of **holistic drug action and quality assurance**.

5. CONCLUSION

The present study establishes that Vetrilai Thylam:

Meets standard physicochemical criteria

- Is free from toxic heavy metal contamination
- Is microbiologically safe

- Contains pharmacologically active phytoconstituents

Thus, the formulation is **safe, standardized, and therapeutically valuable**.

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