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Original Article

Standardization of Vaalai Rasa Mezhugu (VRM): A Siddha Formulation

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ABSTRACT

Background: The Siddha system, widely practiced in Tamil Nadu, incorporates plant, mineral and animal components in its medicinal formulations. Vaalai Rasa Mezhugu (VRM) is one such herbo-mineral preparation indicated for conditions like throbbing pain, arthritis and lymphadenitis. **Objectives:** This study aims to standardize VRM according to the guidelines established by the Pharmacopeia Laboratory of Indian Medicine (PLIM), addressing the urgent need for modern quality control in traditional medicine. **Materials and Methods:** A comprehensive quality control protocol was implemented, involving physico-chemical analysis, instrumental assessments such as HPTLC (High-Performance Thin-Layer Chromatography) and evaluations for heavy metals, microbial contamination, specific pathogens, pesticide residues and aflatoxins. **Results:** The physico-chemical analysis indicated a pH of 6.25, loss on drying at 6.68% and a total ash content of 1.735%. HPTLC fingerprinting identified eleven phytocomponents at 254 nm, 17 at 366 nm and 12 at 520 nm, demonstrating a complex phytochemical profile. Importantly, VRM was found to be free from microbial contaminants, heavy metals, specific pathogens, pesticide residues and aflatoxins below quantification limits. **Conclusion:** These findings affirm the efficacy of Vaalai Rasa Mezhugu and establish a valuable framework for quality assurance in traditional medicine, ensuring the safety and effectiveness of this herbo-mineral formulation.

Keywords: Standardization, Vaalai Rasa Mezhugu, Physico-chemical analysis, HPTLC, PLIM Guidelines, Internal Medicine.

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INTRODUCTION

The Siddha system is a prominent traditional medicinal practice in South India. It characterizes medicine as a substance that enhances physical strength normalizes bodily functions and helps reduce or eliminate diseases. The Siddha approach includes two categories of medicines: internal and external, each containing 32 distinct types. One internal medicine example is Mezhugu, which is prepared by finely grinding various ingredients with specific juices or extracts until a soft, waxy texture is achieved. This research aims to standardize the Siddha herbo-mineral formulation known as Vaalai Rasa Mezhugu (VRM), indicated for treating conditions such as tumors, throbbing pain, cervical lymphadenitis, fistulas and rheumatoid arthritis (Sambasivampillai, 1931; Thiyagarajan and Jeevam, 2013). Standardization is crucial today as it develops a detailed quality profile while ensuring safety monitoring and comprehensive quality assurance for herbal medicines. Our focus on establishing a comprehensive national regulatory framework and safety monitoring systems for Siddha medicine is the need of the hour (Abarna *et al*., 2024). Currently, there is a lack of sufficient documentation regarding its standardization and investigative aspects. This study systematically standardizes Vaalai Rasa Mezhugu using PLIM guideline-based analytical methods, including evaluations of organoleptic properties, HPTLC profiling, physicochemical tests and instrumental analyses for heavy metals, microbial contamination, specific pathogens, pesticide residues and aflatoxins.

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MATERIALS AND METHODS

Selection of the test drug

The test drug "Vaalai Rasa Mezhugu" is one of the Herbo mineral formulations for arthritis and tumor which is indicated in the Siddha literature "Siddha marunthu sei perumuraigal" written by V. Balarammaiya (Balarammaiya *et al.*, 1980).

Collection of raw drugs

The raw drug was purchased in a country drug store, in Chennai and authenticated by the "Department of Gunapadam, National Institute of Siddha, Chennai". (Certified No. NISMB6652024, GUN/AUT/07/24).

Ingredients of Vaalai Rasa Mezhugu

The ingredients are depicted in Table 1.

Purification of Raw Drugs

Purification of Vaalai rasam

Cinnabar (Lingam) powder and *Plumbago zeylanica* (Chitramoolam) root bark are powdered in the ratio 1:4. Then Burnt in the sublimation apparatus, resultant mercury residue (Linga Rasam) is collected from the upper portion.

Purification of Veeram

Camphor was mixed with tender coconut water in a clay pot. mercuric chloride, it was tied in cloth and hung in the pot, ensuring it did not contact the surface of water. The pot was boiled for 30 min until one-third of the water evaporated.

Purification of Pooram

Betel leaf and pepper are ground and mixed in 1.3 L water, calomel is immersed in water and heated till 3/4th volume of water remains. It is then washed and dried.

Preparation of Vaalai Rasa Mezhugu

First, the Rasam is ground with the juice of betel leaves till they completely blend. After that, Veeram and Pooram are added to it. Then Clove (Kirambu) and Cardamom (Elam) were dry roasted and powdered. Add both powders and Varagarisi flour to the above mixture and grind them well. A small proportion of castor oil is added and triturated until it reaches the mezhugu (semisolid/wax) consistency. Then the medicine is stored in an airtight container.

Physico-chemical analysis according to PLIM Guidelines

Following AYUSH-PLIM guidelines, Vaalai Rasa Mezhugu (VRM) was evaluated for its physicochemical properties and active components using HPTLC (Indian Pharmacopeia, 2014; LOHAR *et al.*, 2008). Various tests including pH, loss on drying at 105°C, total ash, water-soluble ash, acid-soluble ash, acid-insoluble ash and both water and alcohol-soluble extracts were conducted at the "Siddha Central Research, Anna Govt. Hospital Campus, Arumbakkam, Institute in Chennai". Results were noted in (Tables 3 and 4).

Physicochemical Evaluation

Determination of Loss on Drying in VRM

Weigh 2 g of VRM in an evaporating dish, heat at 105°C for 5 hr, then weigh again to determine moisture content.

Determination of pH Value of VRM

Dissolve 5 g of VRM in 25 mL of distilled water, filter and measure pH after letting it sit for 30 min.

Analysis of Total Ash Content in VRM

Incinerate 2 g of VRM in a silica dish at 400°C until it turns white, indicating carbon's absence. The % of total ash was then calculated concerning the weight of the air-dried drug.

Analysis of Water-Soluble Ash Content in VRM

The ash was boiled in 25 mL water for 5 min and then filtered to separate the insoluble residue. The residue was washed with hot water, incinerated at 450°C for 15 min and weighed. The water-soluble ash percentage was calculated by subtracting the residue weight from the total ash weight and then expressed as a % of the air-dried sample.

Table 1: Ingredients of Vaalai Rasa Mezhugu.

Sl. No.	The vernacular name of the ingredients	Botanical name/Chemical name	Quantity
1.	Purified Veeram	Hydrargyrum perchloride	10 g
2.	Purified Vaalai rasam	Hydrargyrum	10 g
3.	Purified Pooram	Hydrargyrum subchloride	10 g
4.	Elam	*Elettaria cardamomum.* (L.) Maton	10 g
5.	Kirambu	*Syzygium Aromaticum.* Linn	10 g
6.	Varagarisi	*Pasplum scrobiculatum.* Linn	35 g

Analysis of Acid Insoluble Ash Content in VRM

The total ash sample was treated with 25 mL of hydrochloric acid and boiled for 6 min. The resulting mixture was filtered using ash-free filter paper. Residual insoluble material was rinsed with hot water and then incinerated in a muffle furnace until constant weight was achieved. The weight of the air-dried ash was subsequently used to determine the percentage of acid-insoluble ash content.

Analysis of Water-Soluble Extractive Content in VRM

5 g of VRM were soaked in 100 mL chloroform-water (1:1) for 24 hr with intermittent shaking (6 hr) and settling (18 hr). The filtered solution (25 mL) was evaporated to dryness at 105ºC and weighed. The % of the water-soluble extract was calculated based on the initial air-dried VRM weight.

Analysis of Alcohol Soluble Extractive Content in VRM

VRM was subjected to solvent extraction using 100 mL of alcohol. The sealed mixture underwent intermittent agitation for 6 hr, followed by 18 hr of stagnation. The filtered extract (25 mL) was evaporated and dried at 105ºC to constant weight. The % of alcohol-soluble extract was then calculated relative to the air-dried drug's initial weight.

Instrument-Based Analysis of Vaalai Rasa Mezhugu

The Vaalai Rasa Mezhgu test drug underwent High-Performance Thin Layer Chromatography (HPTLC) techniques to produce its unique fingerprint.

VRM Evaluation in High-Performance Thin Layer Chromatography

The HPTLC method is a sophisticated and automated separation technique that has evolved from traditional TLC. Using autosamplers and specially coated HPTLC plates enables precise and sensitive separation of components on both qualitative and quantitative levels. High-Performance Thin-Layer Chromatography (HPTLC) is a valuable tool for cost-effective and efficient analysis of plant materials. It offers high selectivity, sensitivity, speed and a streamlined sample preparation process, making it ideal for routine quality control checks. This method generates a chromatographic fingerprint of phytochemicals, helping verify herbal products' identity and purity.

Chromatogram development was conducted in CAMAG Twin Trough chambers. The elution method was based on how well the sample components adhere to the stationary phase. After the elution, the plates were taken out and dried. They were then scanned at 366 nm using UV light. CAMAG software was employed to analyze the scanned data, resulting in a chromatographic fingerprint for each sample, with the respective R_f values recorded (Hapuarachchi *et al.*, 2020; Subramanian *et al.*, 2023). The HPTLC of VRM is shown in Figure 1.

VRM Analysis in Heavy Metal Assessment Using Atomic Absorption Spectrometry (AAS)

Sigma standards for Arsenic (As), Lead (Pb), Cadmium (Cd) and Mercury (Hg) were utilized in this analysis. Atomic Absorption Spectrometry (AAS), specifically the AA 240 Series model, was selected for its reliability in detecting metals and metalloids in

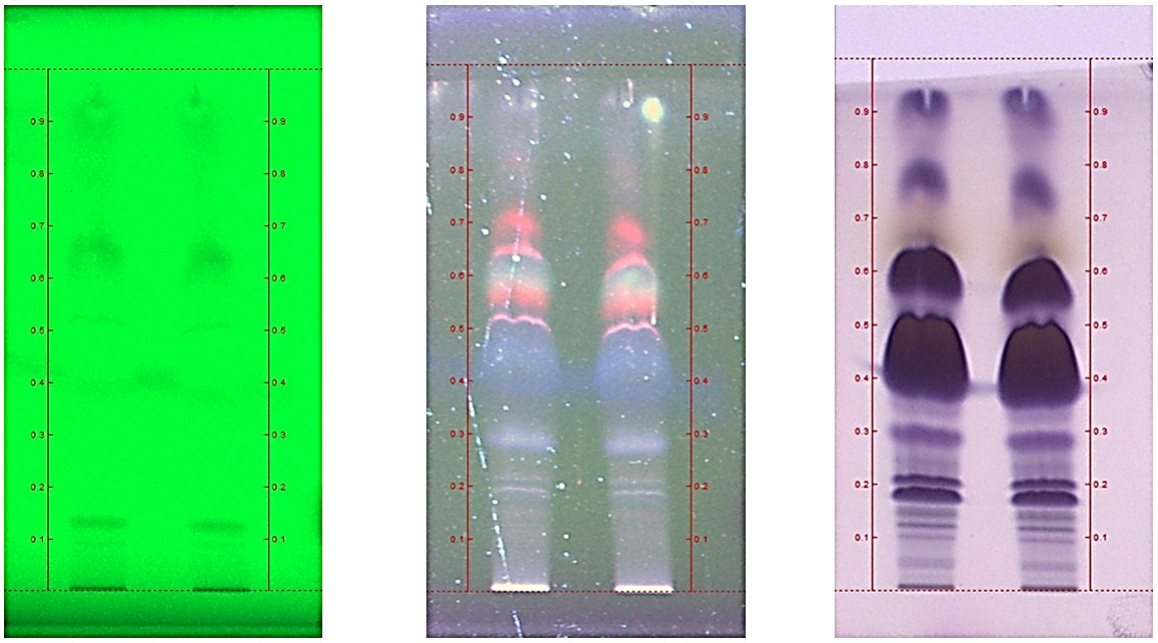


Figure 1: HPTLC Results Demonstrating the Fingerprint of Vaalai Rasa Mezhugu (VRM).

environmental samples. This method was applied to determine the overall concentrations of heavy metals-cadmium, lead, mercury and arsenic in the test sample. For sample preparation, digestion with 1 mol/L HCl was performed to measure mercury and arsenic levels, while 1 mol/L HNO_3 was used for lead and cadmium. Standard solutions of 100 ppm for As and Hg were prepared in 1 mol/L HCl, with Cd and Pb prepared in 1 mol/L HNO_3 (Mohammed *et al.*, 2018).

VRM Sterility Testing Using the Pour Plate Method

The product's sterility was assessed using the pour plate procedure. When a contaminated or non-sterile sample (formulation) comes into touch with a medium rich in nutrients, it encourages the growth of the organism. After the required amount of time, the organism's growth is recognized by a distinctive pattern of colonies. The colonies are known as CFUs or colony-forming units. About 15 mL of melted agar was added to a sterile petri dish that had been inoculated with the test sample at 45°C. The dish was tilted and swirled to mix the agar and sample properly. Till the agar completely gelled, it was kept undisturbed (about 10 min). After that, plates were turned over and incubated for 48 hr at 37°C before being left open for 72 hr to observe fungal development. The number of grown organism colonies was then determined for CFU (Li *et al.*, 2003).

VRM Evaluation for Specific Pathogen Identification

The test sample was inoculated into selective media "(Eosin Methylene Blue, Deoxycholate Citrate, Mannitol Salt and Cetrimide Agar)" using the pour plate technique. Plants exhibited visible growth after 24-72 hr of incubation at 37°C. Distinct colony morphology and coloration patterns, specific to each medium, indicated the presence of targeted pathogens (Pour plate Method, 2022). Detailed methods of specific pathogen testing for VRM are summarized in Table 2.

VRM Analysis of Pesticide Residues

Test samples were extracted using acetone and then mixed thoroughly for a short time. The liquid was allowed to filter before adding more acetone. The sample was heated in a rotary evaporator at a temperature not exceeding 40°C until most of the solvent had evaporated. A few milliliters of toluene were then added to the remaining residue and it was heated again until all the acetone was eliminated. The toluene was used to dissolve the resulting residue and a membrane filter was employed to filter the solution (Kiehlbauch *et al.*, 2000; WHO, 2007).

VRM Aflatoxin Analysis Using Thin Layer Chromatography (TLC) for B1, B2, G1 and G2

A standard solution of aflatoxins B1, G1, B2 and G2 was prepared at concentrations of 0.5 µg/mL for B1 and G1 and 0.1 µg/mL for B2 and G2 by dissolving the standards in a 9.8:0.2 chloroform-acetonitrile mixture. For the analysis, aflatoxin standards (2.5-10 µL) were applied to a pre-coated TLC plate. The test sample was then subjected to a solvent mixture of acetone, chloroform and isopropyl alcohol (85:10:5) in an unsaturated chamber until the solvent front reached 15 cm. After drying, the chromatogram was developed and examined under 365 nm UV light to visualize the separated spots (Luciana *et al.*, 2001).

RESULTS

Organoleptic characters

The organoleptic characters of VRM were done and noted in Table 3.

Physico-chemical parameters

The physicochemical analysis of VRM was done and noted in Table 4.

Analytical Techniques for Vaalai Rasa Mezhugu (VRM) Using Instrumentation

HPTLC fingerprinting can be employed to create quality standards for polyherbal compositions. For the sample, the VRM fingerprint is mentioned in Figures 1-4.

Table 2: Detailed of Specific Pathogen Testing for VRM.

Micro-organism	Growth medium
Salmonella	Deoxycholate Citrate
Escherichia coli	Eosin Methylene Blue
Pseudomonas Aeruginosa	Cetrimide Agar
Staphylococcus Aureus	Mannitol Salt

Table 3: Results of organoleptic characters of VRM.

Sl. No.	Parameters	Results
1.	Colour	Grey in colour
2.	Taste	Sweet taste
3.	Odour	Aromatic and characteristic
4.	Consistency	Wax-like semisolid mass

Table 4: Results of Physicochemical Analysis of VRM.

Sl. No.	Name of the Experiment	Mean value
1.	pH	6.25
2.	Loss on drying	6.68%
3.	Total ash	1.735%
4.	Water soluble ash	0.88%
5.	Acid insoluble ash	0.43%
6.	Water soluble extractive	2.26%
7.	Alcohol soluble extractive	26.085%

HPTLC fingerprinting analysis of VRM included the generation of 3D chromatograms at wavelengths of 254, 366 and 520 nm. The HPTLC fingerprints were scanned at these wavelengths, with peaks at 254, 366 and 520 nm highlighted. Figures 2-4 display the respective chromatograms and noted peak details.

VRM Analysis in Heavy Metal Assessment Using Atomic Absorption Spectrometry (AAS)

The results of this investigation (Table 5) indicate that the sample contains no detectable traces of heavy metals, including mercury, arsenic, lead and cadmium.

VRM Sterility Testing Using the Pour Plate Method

Following the incubation period, no growth or colony formation was detected on any plates inoculated with the test sample. The results of the sterility testing for bacteria and fungi in VRM, as shown in Table 6 and Figure 5 revealed the following findings.

VRM Evaluation for Specific Pathogen Identification

In any of the plates the test material was put into, no growths or colonies were visible (Table 7 and Figure 6).

VRM Analysis of Pesticide Residues

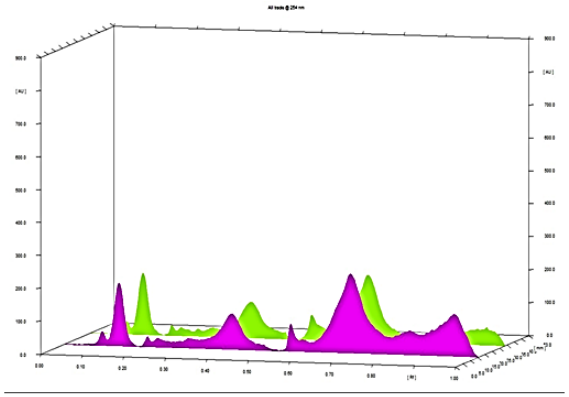
The results showed (Table 8) no traces of pesticide residues such as Organochlorine, Organophosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

Table 5: **Results of Heavy Metal Analysis in VRM (BDL-Below Detection Limit).**

Heavy Metal	Wavelength (nm)	Result	Maximum limit
(VRM) Lead	217.0 nm	BDL	10 ppm
(VRM) Arsenic	193.7 nm	BDL	3 ppm
(VRM) Cadmium	228.8 nm	BDL	0.3 ppm
(VRM) Mercury	253.7 nm	BDL	1 ppm

Table 6: **Results of Sterility Testing for VRM.**

Test	Results	Specification	Reference
Total Bacterial Count	Nil	Not more than 10^5 CFU/g.	Complies with AYUSH.
Total Fungal Count	Nil	Not more than 10^3 CFU/g.	Complies with AYUSH.



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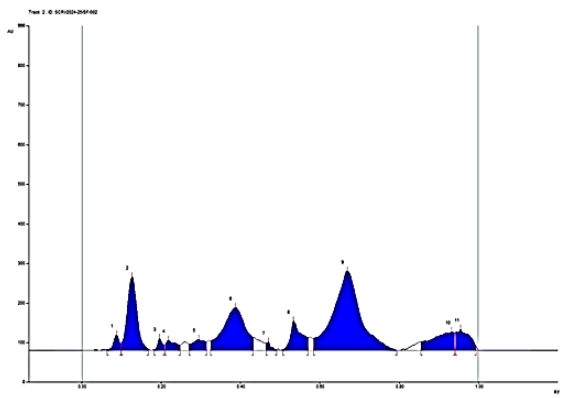


(b)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	2.0 AU	0.09 Rf	38.8 AU	4.78 %	0.10 Rf	18.3 AU	515.3 AU	1.76 %
2	0.10 Rf	19.4 AU	0.13 Rf	185.4 AU	22.86 %	0.17 Rf	3.4 AU	4088.0 AU	13.94 %
3	0.18 Rf	2.9 AU	0.20 Rf	29.9 AU	3.69 %	0.21 Rf	14.4 AU	333.1 AU	1.14 %
4	0.21 Rf	14.9 AU	0.22 Rf	25.3 AU	3.11 %	0.25 Rf	13.8 AU	565.7 AU	1.93 %
5	0.27 Rf	16.0 AU	0.29 Rf	27.7 AU	3.42 %	0.31 Rf	22.2 AU	772.9 AU	2.64 %
6	0.32 Rf	23.5 AU	0.39 Rf	108.3 AU	13.35 %	0.43 Rf	32.7 AU	5246.8 AU	17.89 %
7	0.46 Rf	18.2 AU	0.47 Rf	21.2 AU	2.61 %	0.49 Rf	1.4 AU	220.4 AU	0.75 %
8	0.51 Rf	1.5 AU	0.53 Rf	74.1 AU	9.13 %	0.57 Rf	32.6 AU	1861.2 AU	6.35 %
9	0.58 Rf	31.0 AU	0.67 Rf	200.3 AU	24.69 %	0.79 Rf	0.3 AU	11882.9 AU	40.53 %
10	0.85 Rf	21.7 AU	0.93 Rf	47.0 AU	5.79 %	0.94 Rf	45.1 AU	2262.1 AU	7.71 %
11	0.94 Rf	45.5 AU	0.95 Rf	53.2 AU	6.56 %	0.99 Rf	12.6 AU	1572.8 AU	5.36 %

(c)

Figure 2: HPTLC Finger Printing Analysis of VRM (a) 3D chromatogram of 254 nm, (b) HPTLC fingerprints scanning of 254 nm, (c) peak 254 nm.

Aflatoxin assay by Thin Layer Chromatography (TLC) (B1, B2, G1, G2)

The results indicated (Table 9) no spots on the TLC plates for the test sample when compared to the standards, confirming the absence of aflatoxin G1, G2, B1 and B2 in the sample.

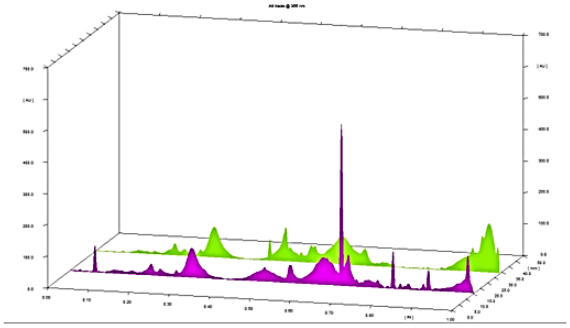
DISCUSSION

In this study, the experimental drug Vaalai Rasa Mezhugu was prepared following established Siddha texts and included several purification processes as described in the literature. Physicochemical parameters were defined to evaluate the quality of the final product. The organoleptic characteristics of Vaalai Rasa Mezhugu verify the authenticity of both the raw materials and the final product, which is noted for its grey color, aromatic fragrance, sweet taste, semi-solid consistency, non-free-flowing nature and non-greasy texture. The moisture content of VRM is 6.8%, indicating its stability and shelf life. Additionally, the total ash value of VRM is 1.735%, representing the inorganic residue remaining after combustion, which is an important aspect of assessing the quality of crude drugs.

Table 7: **Results of Specific Pathogen Testing for VRM.**

Organism	Results	Method
Salmonella	Not Detected	Complies with AYUSH Standards.
Escherichia coli	Not Detected	Complies with AYUSH Standards.
Pseudomonas aeruginosa	Not Detected	Complies with AYUSH Standards.
Staphylococcus aureus	Not Detected	Complies with AYUSH Standards.

This analysis primarily ensures the quality of powdered medications. Assessing ash values helps confirm the absence of mineral contaminants such as earth, sand, or floor sweepings, as well as plant parts that should not be included, potential adulterants and degraded material (Shyamala *et al.*, 2021). The acid-insoluble ash value of VRM is 0.43%, while the water-soluble ash is 0.88%. A low acid-insoluble ash indicates minimal adulteration with siliceous substances (Shyamala *et al.*, 2021). The pH of the VRM is 6.25, reflecting its acidic nature. This acidity facilitates rapid absorption in the stomach when taken orally, making it suitable for oral administration. Solubility is essential for effective drug absorption in the gastrointestinal tract, as low oral bioavailability is often linked to poor solubility and permeability (Adithya *et al.*, 2024). The alcohol-soluble and water-soluble extractive values for the VRM formulation are 2.26% and 26.085%, respectively. The lower water-soluble extractive value suggests that water is less effective than ethanol in extracting active components of the drug. The HPTLC fingerprinting analysis of Vaalai Rasa Mezhugu (VRM) at 254 nm identifies eleven versatile phytocomponents, with R_f values spanning from 0.06 to 0.94. The peak with an R_f value of 0.58 shows the highest area percentage of 40.53%, indicating a high abundance of that compound. At 366 nm, there are 17 versatile



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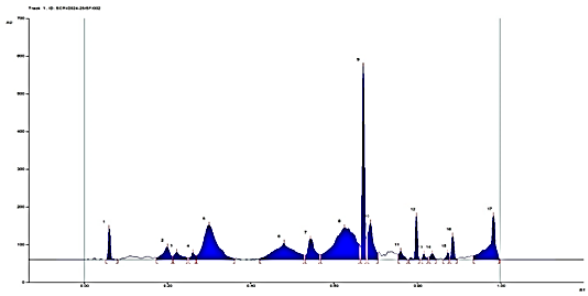
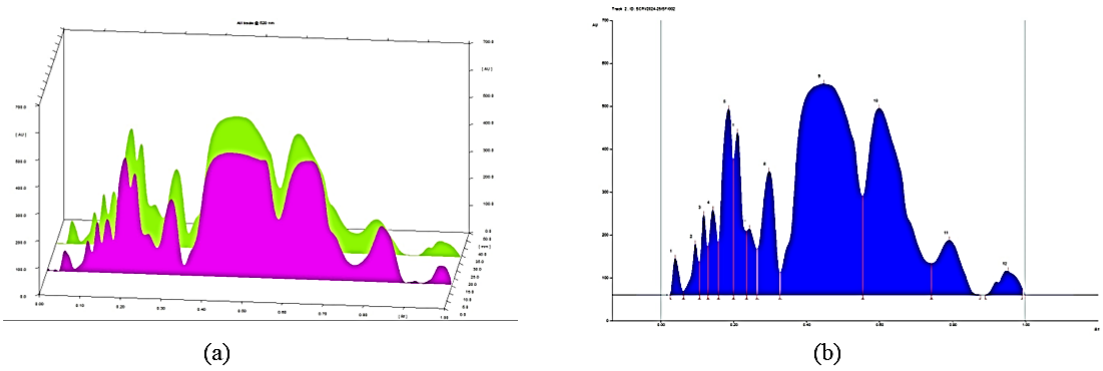


(b)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.2 AU	0.06 Rf	83.0 AU	5.88 %	0.08 Rf	0.9 AU	358.3 AU	2.14 %
2	0.17 Rf	5.4 AU	0.20 Rf	34.0 AU	2.41 %	0.21 Rf	9.8 AU	517.6 AU	3.09 %
3	0.21 Rf	10.2 AU	0.22 Rf	20.0 AU	1.41 %	0.25 Rf	6.0 AU	315.1 AU	1.88 %
4	0.25 Rf	6.1 AU	0.26 Rf	19.3 AU	1.37 %	0.27 Rf	10.7 AU	153.4 AU	0.92 %
5	0.27 Rf	11.1 AU	0.30 Rf	91.4 AU	6.47 %	0.36 Rf	3.3 AU	2618.8 AU	15.63 %
6	0.42 Rf	2.7 AU	0.48 Rf	44.3 AU	3.14 %	0.53 Rf	13.2 AU	1801.0 AU	10.75 %
7	0.53 Rf	12.6 AU	0.54 Rf	54.8 AU	3.88 %	0.57 Rf	14.8 AU	814.0 AU	4.86 %
8	0.57 Rf	14.8 AU	0.63 Rf	84.7 AU	6.00 %	0.66 Rf	42.5 AU	3757.8 AU	22.42 %
9	0.66 Rf	31.6 AU	0.67 Rf	512.9 AU	36.34 %	0.68 Rf	54.9 AU	2425.2 AU	14.47 %
10	0.68 Rf	71.6 AU	0.69 Rf	98.2 AU	6.96 %	0.71 Rf	19.0 AU	1061.9 AU	6.34 %
11	0.75 Rf	14.1 AU	0.76 Rf	22.6 AU	1.60 %	0.78 Rf	0.1 AU	207.0 AU	1.24 %
12	0.78 Rf	0.9 AU	0.80 Rf	116.1 AU	8.23 %	0.81 Rf	2.4 AU	537.6 AU	3.21 %
13	0.81 Rf	1.6 AU	0.82 Rf	15.6 AU	1.11 %	0.83 Rf	7.9 AU	95.6 AU	0.57 %
14	0.83 Rf	7.7 AU	0.84 Rf	16.2 AU	1.15 %	0.85 Rf	1.6 AU	126.0 AU	0.75 %
15	0.86 Rf	0.6 AU	0.87 Rf	18.4 AU	1.30 %	0.88 Rf	12.9 AU	95.3 AU	0.57 %
16	0.88 Rf	2.7 AU	0.89 Rf	63.2 AU	4.48 %	0.90 Rf	1.9 AU	315.8 AU	1.88 %
17	0.93 Rf	10.3 AU	0.98 Rf	116.6 AU	8.26 %	1.00 Rf	0.7 AU	1558.2 AU	9.30 %

(c)

Figure 3: HPTLC Finger Printing Analysis of VRM 3D chromatogram of 366 nm, (b) HPTLC fingerprints scanning of 366 nm, (c) peak 366 nm.



(a) (b)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.9 AU	0.04 Rf	84.9 AU	2.88 %	0.06 Rf	9.7 AU	1240.7 AU	0.83 %
2	0.06 Rf	10.0 AU	0.09 Rf	118.8 AU	4.02 %	0.11 Rf	77.7 AU	1917.1 AU	1.28 %
3	0.11 Rf	80.5 AU	0.12 Rf	186.9 AU	6.33 %	0.13 Rf	12.8 AU	2498.7 AU	1.67 %
4	0.13 Rf	117.0 AU	0.14 Rf	198.0 AU	6.70 %	0.16 Rf	24.0 AU	3425.4 AU	2.29 %
5	0.16 Rf	126.3 AU	0.19 Rf	433.4 AU	14.67 %	0.20 Rf	14.8 AU	10393.5 AU	6.96 %
6	0.20 Rf	314.9 AU	0.21 Rf	378.6 AU	12.82 %	0.23 Rf	48.8 AU	7301.3 AU	4.89 %
7	0.24 Rf	149.1 AU	0.24 Rf	154.2 AU	5.22 %	0.26 Rf	07.7 AU	2992.4 AU	2.00 %
8	0.27 Rf	108.3 AU	0.30 Rf	287.7 AU	9.74 %	0.33 Rf	52.8 AU	8988.8 AU	6.02 %
9	0.33 Rf	52.8 AU	0.45 Rf	492.2 AU	16.66 %	0.55 Rf	31.2 AU	63453.0 AU	42.47 %
10	0.55 Rf	231.5 AU	0.60 Rf	435.0 AU	14.73 %	0.74 Rf	73.9 AU	37410.1 AU	25.04 %
11	0.74 Rf	74.0 AU	0.79 Rf	127.8 AU	4.33 %	0.88 Rf	0.1 AU	7220.8 AU	4.83 %
12	0.89 Rf	0.0 AU	0.95 Rf	55.9 AU	1.89 %	0.99 Rf	15.2 AU	2566.6 AU	1.72 %

(c)

Figure 4: HPTLC Finger Printing Analysis of VRM 3D (post derivatized) chromatogram of 520 nm, (b) HPTLC fingerprints scanning of 520 nm, (c) peak 520 nm.

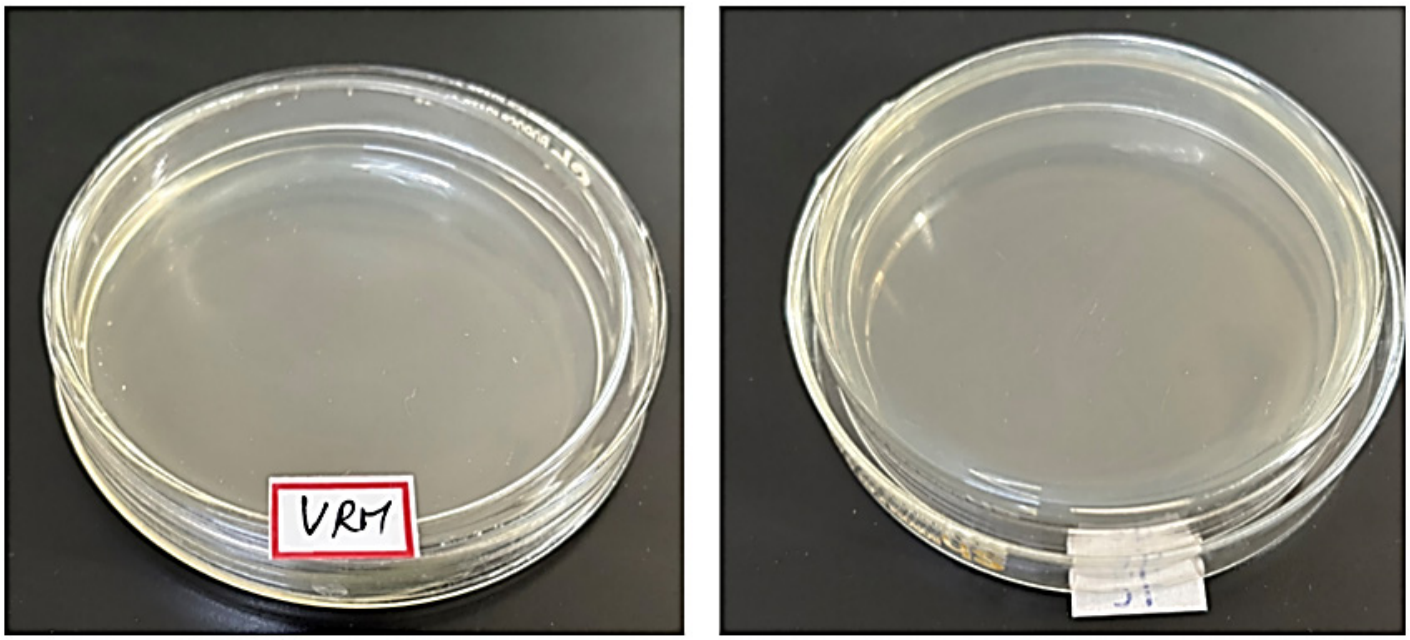


Figure 5: Results of Sterility Testing for VRM.

phytocomponents present, with R_f values from 0.05 to 0.93 and the peak at 0.57 has the highest area percentage of 22.42%, reflecting the corresponding compound's abundance. Finally, at 520 nm, twelve versatile phytocomponents are observed, with R_f values ranging from 0.03 to 0.89 and the peak with an R_f value of 0.33 has the highest area percentage of 42.47%, indicating a significant presence of that compound. The investigation results confirm that VRM is free from heavy metals such as arsenic and cadmium, ensuring the safety of the drug. Moreover, the levels of mercury and lead are below permissible limits, further ensuring safety. Microbiological analysis demonstrated no growth or colonies in plates inoculated with VRM, confirming the absence of viable microorganisms. The absence of pesticide residues such as organo-chlorine, organo-phosphorus and pyrethroids in VRM underscores its quality control.

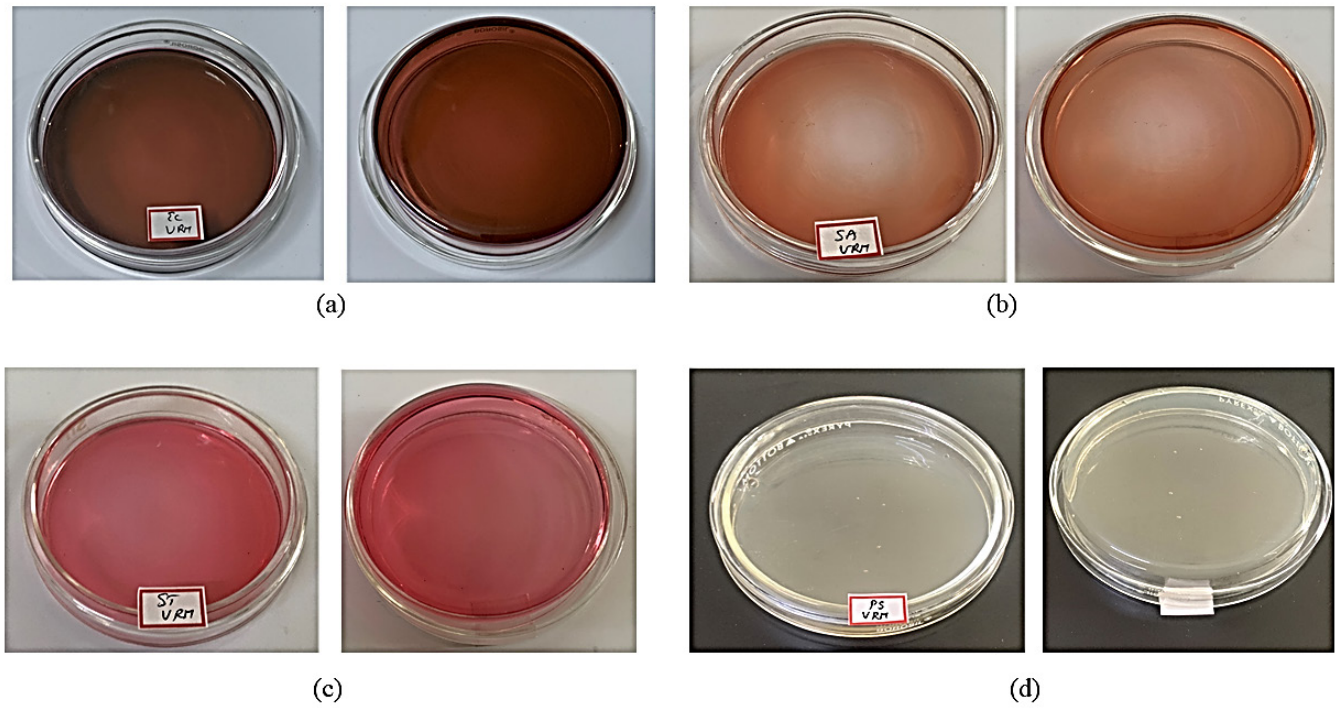


Figure 6: Culture plate with specific pathogen medium (a) *E. coli*, (b) *Salmonella*, (c) *Staphylococcus aureus*, (d) *Pseudomonas aeruginosa.*

Table 8: Results of pesticide residue test for VRM (BQL- Below Quantification Limit).

Pesticide Residue	Sample VRM	AYUSH Limit (mg/kg)
Organo chlorine pesticides		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1 mg/kg
Gamma BHC	BQL	0.1 mg/kg
Delta BHC	BQL	0.1 mg/kg
DDT	BQL	1 mg/kg
Endosulfan	BQL	3 mg/kg
Organo phosphorus pesticides		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1 mg/kg
Organo carbamates		
Carbofuran	BQL	0.1 mg/kg
Pyrethroid		
Cypermethrin	BQL	1 mg/kg

Table 9: Results of Aflatoxin Testing for VRM.

Aflatoxin	VRM	AYUSH Specification Limit
B1	Nil	0.5 ppm
B2	Nil	0.1 ppm
G1	Nil	0.5 ppm
G2	Nil	0.1 ppm

CONCLUSION

In conclusion, the study on Vaalai Rasa Mezhugu (VRM) demonstrates the adherence to established Siddha practices and quality standards. These findings support the efficacy and safety of Vaalai Rasa Mezhugu, providing a solid foundation for its use in traditional medicine and encouraging further research into its pharmacological applications. Also this study will be serve as a reference in compiling the monograph of Vaalai Rasa Mezhgu in future.

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CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

ABBREVIATIONS

VRM: Vaalai Rasa Mezhugu; **PLIM:** Pharmacopeia Laboratory of Indian Medicine; **HPTLC:** High Performance Thin Layer Chromatography; **TLC:** Thin Layer Chromatography; **AAS:** Atomic Absorption Spectrometry; **As:** Arsenic; **Pb:** Lead; **Cd:** Cadmium; **Hg:** Mercury; **HCl:** Hydrochloric acid; **HNO_3:** Nitric acid; **CFU:** Colony Forming Units; **AYUSH:** Ayurveda, Yoga naturopathy, Unani, Siddha and Homeopathy; **BHC:** Benzene Hexachloride; **DDT:** Dichlorodiphenyltrichloroethane; **BQL:** Below Quantification Limit.

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.

AUTHOR CONTRIBUTION

Conceptualization: SR; Medicine Preparation: SR; Data collection and compilation: SR; Manuscript Writing: SR, SSG, KAP, SA, AB, NM and SS; Proofreading and editing: SR, SSG, KAP, SA, AB, NM and SS.

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