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RESEARCH ARTICLE

Comprehensive Characterization of the Siddha Herbo-mineral Formulation Vaalai Rasa Mezhugu using X-ray Diffraction and Fourier Transform Infrared Spectroscopy Techniques

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Abstract

Background: "Vaalai Rasa Mezhugu" is a herbo-mineral formulation widely used in Siddha medicine prepared according to the process found in Siddha marunthu sei perumuraigal. Traditionally the chosen formulation has been prescribed for the management of Soolai (Throbbing pain), Mudakku vaatham (Arthritis), Kandamaalai (Cervical lymphadenitis), Pauttiram (Fistula), Araiyappu (Lymphnode related neoplasia). **Objective:** The aim of the research work was to characterize the chemical composition of the drug using XRD and FTIR. **Method:** The formulation was prepared as per the literature and then subjected to quantitative analysis with XRD and FTIR. **Findings:** The XRD analysis showed the presence of sylvine and zinc sulfide and the FTIR analysis showed the presence of hydroxyl, carboxylic acids, amines, aromatic compounds, hydrocarbons, and carbonyl groups. **Novelty:** This study provides a novel chemical characterization of the Siddha formulation "Vaalai Rasa Mezhugu" using XRD and FTIR, revealing its potential antioxidant, antimicrobial, and anti-inflammatory properties. It bridges traditional knowledge with modern scientific validation, encouraging further pharmacological research.

Keywords: Vaalai Rasa Mezhugu; XRD; FTIR; Siddha medicine; Internal medicine

1 Introduction

Siddha system of medicine is one of the most traditional systems of medicine. Herbs, minerals, and animal products are the major components used in the Siddha medical system to treat a variety of illnesses since ancient times [1]. The internal medicines are classified into 32 types in this system one among them is mezhugu (Medicated wax) [2]. This type of medicine is prepared by grinding the raw drugs to mezhugu pakkuvam or waxy state. This has a shelf life of 5 years [2]. Medicated wax is categorized into two types based on the preparation procedure, Surukku mezhugu and Araippu mezhugu. As the name suggests, Surukku mezhugu is obtained by heating them by adding an oily substance, and araippu mezhugu is obtained by grinding. One such Araippu Mezhugu is Vaalai Rasa Mezhugu [3]. Vaalai Rasa Mezhugu has been mentioned in Siddha texts for the management of Soolai (Throbbing pain), Mudakku vaatham (Arthritis), Kandamaalai (Cervical lymphadenitis), Pauttiram (Fistula), Araiyappu (Lymphnode related neoplasia) [4]. Despite the traditional usage of VRM, it is not scientifically validated for its chemical characterization thus; VRM has been opted for this study. The characterization of a herbo-mineral formulation determines its therapeutic activity. The characterization is done using XRD and FTIR, which are analytical techniques that can be used to identify and characterize the chemical composition of the formulation [5]. XRD is used to analyze the structural properties and crystalline nature and FTIR is used to identify the functional group and molecular structure of the drug [6]. These methods aid in standardizing traditional formulations by providing objective data about the composition, purity, and stability of the medicine. This is crucial for maintaining batch-to-batch consistency, which is a common challenge in traditional medicine. This characterization not only supports the traditional knowledge of Siddha medicine but also enhances the credibility and quality control of VRM as a potent drug for treating various diseases.

2 Methodology

2.1 Selection of the test drugs

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 Pg No. 21 [3].

2.2 Collection of Raw drugs

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

Table 1. Ingredients of Vaalai Rasa Mezhugu

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

2.3 Extraction of Vaalai rasam

1-part purified Cinnabar (Lingam) powdered and added to 4 parts *Plumbago zeylanica* (Chitramoolam) root bark powder. Then, burnt in the sublimation apparatus, and collected mercury residue (Linga Rasam) from the upper portion [2,3].

2.4 Purification of Veeram

Camphor was mixed with tender coconut water and placed in a mud pot. Perchloride of mercury was tied with a cloth and hung within the pot without touching the water and the pot was burnt for half an hour until the 1/3rd of water content was reduced. [2,3]

2.5Purification of Pooram

Betel leaf and pepper are made into a paste and mixed in 1.3L water. The calomel is immersed and heated until $\frac{3}{4}$ volume remains, then washed and dried [2,3].

2.6Purification of Elam (*Elettaria cardamomum*):

The cardamom was gently fried and the seeds (cardamom rice) were separated from the seed coat [7,8].

2.7Purification of Kirambu (*Syzygium Aromaticum*):

The bud of the clove was removed [7,8].

2.8Preparation 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 shallow fried and powdered then added to Varagarisi flour mixed with the above and ground well. A small proportion of castor oil was added and blended till it reached the mezhugu (semisolid/wax) form. Then the medicine is stored in an airtight container [3].

- **Dose:** 500mg
- **Adjuvant/Vehicle:** Palm Jaggery
- **Route of administration:** Oral
- **Duration:** 3 days
- **Indications:** Soolai (Throbbing pain), Mudakku vaatham (Arthritis), Kandamaalai (Cervical lymphadenitis), Pauttiram (Fistula), Araiyappu (Lymphnode related neoplasia) [3,4].
- **Reference:** Siddha marunthu sei perumuraigal, authored by V. Balarammaiya, Pg. No:21

2.9Instrumental analysis of VRM

2.9.1 XRD analysis

Diffraction data were collected by Aeris Analytical diffractometer (Netherlands) with Ni-filtered copper radiation in Bragg-Brentano geometry. Fine powder of the sample was taken in a thin layer on silicon zero background holders. The sample was recorded for the angle 2θ in the range of 10-90 degrees at a scanning rate of 4 degrees/sec with CuKα (λ 1.5418 A°) [9].

2.9.2 FTIR Analysis

Sample processed using Bruker Alpha-E by ATR module (attenuated total reflectance). Sample positioned on the Crystal platform with perfect alignment of keeping anvil in up position. To ensure that the sample makes a good contact angle with the crystal before start of the IR radiation exposure. Spectra measurement was achieved with the desired wavelength and the corresponding observational peaks/ waves were recorded with wave number and were subjected to further interpretation. The software used for the analysis is OPUS version 7 for functional group analysis. Signal detection is processed through the DTGS detector. Baseline correction is adjusted as per the requirement [10].

3 Results and Discussion

3.1 XRD analysis

The XRD results for VRM are illustrated in Figure 1 , with the pattern listed in Table 2.

Table 2. Pattern list of XRD for the analysis of VRM

Visible	Ref. code	Score	Compound Name	Displ. [°2θ]	Scale Fac.	Chem. Formula
*	98-005-3841	60	Sylvine	0.000	0.348	Cl1 K1
*	98-004-2821	40	Zinc Sulfide	0.000	0.491	S1 Zn1

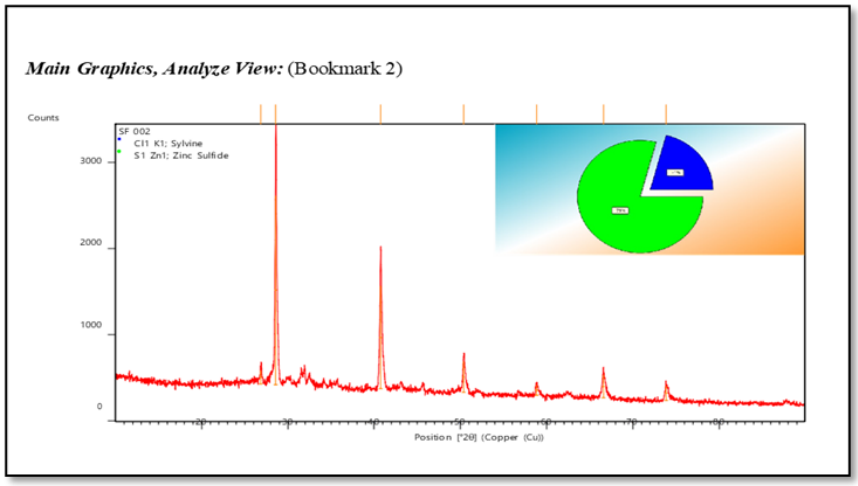

Fig 1. XRD Analysis of VRM

3.2 FTIR analysis

The FTIR analysis results for VRM are shown in Figure 2, highlighting the peak values, while the corresponding functional groups are listed in Table 3.

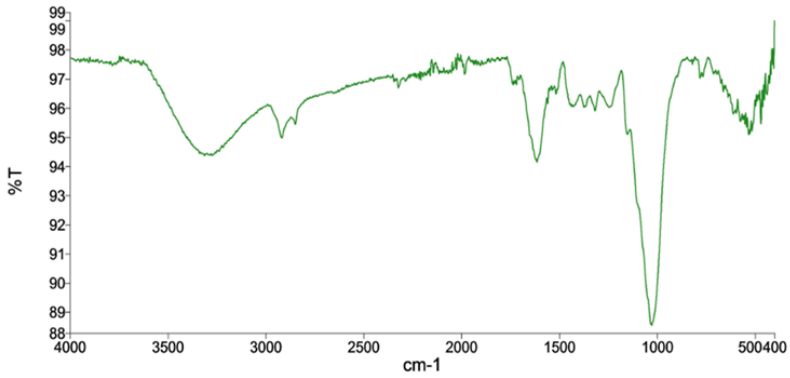

Fig 2. FTIR Analysis of VRM

Table 3. Interpretation of FTIR Peaks obtained for the analysis of VRM

Peak Number	Range (cm^{-1})	Functional Group
1.	4000 – 3200	O-H stretching (alcohols, carboxylic acids)- Broad peaks here suggest hydroxyl groups (O-H), which could indicate the presence of alcohols or acids.
2.	3300 – 3250	N-H stretching (amines)- Sharp peaks here may indicate the presence of amines (N-H).
3.	3000 – 2850	C-H stretching (alkanes) - Peaks in this region suggest the presence of alkyl groups (C-H) typically found in hydrocarbons or lipid components.
4.	2250 – 2100	C≡N, C≡C (nitriles, alkynes) - A peak here could indicate triple bonds such as nitriles (C≡N) or alkynes (C≡C).

Continued on next page

Table 3 continued

5.	1750 – 1650	C=O stretching (carbonyls: ketones, aldehydes, esters, carboxylic acids)- A sharp peak in this range is indicative of carbonyl groups (C=O), common in esters, aldehydes, and carboxylic acids.
6.	1600 – 1500	C=C stretching (aromatics)- Peaks here may suggest aromatic rings or alkenes.
7.	1450 – 1350	C-H bending (alkanes)- This region often shows bending vibrations of alkyl groups.
8.	1300 – 1000	C-O stretching (alcohols, ethers, esters)- Strong peaks here indicate C-O bonds, typically found in alcohols or esters.
9.	900 – 650	C-H out-of-plane bending (aromatics) - Peaks in this area are associated with aromatic ring vibrations.

X-ray diffraction analysis (XRD) is a non-destructive technique that provides detailed information about the crystallographic structure, chemical composition, and physical properties of a material [9]. The X-ray diffraction data indicates a highly crystalline structure with seven distinct peaks. The most intense peak at 28.59° 2θ (100% relative intensity, d = 3.119 Å) suggests the presence of a dominant phase with well-ordered atomic planes. Peaks at 40.75° 2θ and 50.39° 2θ show significant intensities (54.53% and 16.28%, respectively), indicating secondary phases or additional crystalline planes contributing to the structure. The narrow FWHM (Full Width at Half Maximum) values across the peaks reflect good crystallinity, though minor broadening suggests small crystallites or slight structural disorder in some regions. The varying d-spacing values (1.28–3.31 Å) suggest a mixture of closely packed and more open planes, indicating a complex material with diverse atomic arrangements. This points to a well-defined crystalline structure with multiple phases. The major peaks correspond to sylvine (KCl) and zinc sulfide. The strong alignment between the observed data and reference codes indicates that the sample contains both potassium chloride and zinc sulfide in well-defined forms, with ZnS possibly being more prevalent. This formulation is a testament to the alchemy of traditional medicine, where precise preparation methods and carefully chosen ingredients create the foundation for synergistic transformations. While the direct formation of sylvine (KCl) and zinc sulfide (ZnS) may not be immediately apparent, traditional processes often involve gradual chemical evolution. The interaction between metals, minerals, and herbs in this mixture could yield unique complex salt formation under specific conditions such as controlled maturation or environmental exposure. KCl is used to treat hypokalemia in various conditions, including diabetic ketoacidosis, kidney disease, hyperaldosteronism, medication-induced hypokalemia, and gastrointestinal diseases like vomiting and diarrhea. Additionally, KCl induces cardioplegia during cardiac surgery. Untreated hypokalemia can lead to life-threatening cardiac arrhythmias, making prompt correction essential. KCl replenishes potassium, helping maintain adequate serum levels and preventing complications. Its use requires careful monitoring and individualized dosing [11]. ZnS is incorporated into topical formulations in dermatology for its antimicrobial properties and used in sunscreens to block UV radiation. Research also explores ZnS nanoparticles in photodynamic therapy (PDT) for cancer treatment and as antimicrobial agents in wound dressings and medical devices to prevent infections [12].

The FT-IR spectrum analysis indicates the presence of alcohols suggesting the existence of hydroxyl (-OH) groups, which are often associated with solubility in water and can participate in hydrogen bonding, enhancing biological activity [13]. Similarly, the presence of carboxylic acids implies an acidic nature. The identification of amines highlights the presence of nitrogen-containing groups that may impart basic properties to the compound [13]. Amines are known to play critical roles in biological systems, often acting as ligands in enzyme reactions or as components in drug design due to their ability to interact with biological targets [14]. The presence of aromatic compounds and unsaturated hydrocarbons in the spectrum suggests the presence of delocalized electrons, which can enhance the stability and reactivity of the compound. Aromatic compounds are frequently recognized for their antioxidant properties, helping to neutralize free radicals and reduce oxidative stress in living organisms. The detection of saturated hydrocarbons, particularly alkanes or lipid components, points to a potential for energy storage or structural roles in biological membranes [15]. Lipids are essential for maintaining cell membrane integrity and can also serve as signaling molecules. The presence of carbonyl groups (C=O), which are characteristic of ketones, aldehydes, or esters, adds another layer of complexity to the sample. Carbonyl compounds are often associated with varied reactivity, playing pivotal roles in organic synthesis and exhibiting unique biological activities [16]. For instance, esters are commonly used in pharmaceuticals and fragrances, highlighting their importance in both medicinal chemistry and sensory applications [17]. Overall, these functional groups reveal a complicated chemical composition, where aliphatic and aromatic structures coexist. The complexity of VRM indicates potential biological activities such as antioxidant, antimicrobial, and anti-inflammatory effects. These properties align with its traditional use in treating conditions like Soolai (throbbing pain), Mudakku Vaatham (arthritis), Kandamaalai (cervical lymphadenitis), Pauttiram (fistula), and Araiyappu (lymph node-related neoplasia).

X-ray diffraction and FT-IR analysis reveal a complex material comprising the crystalline structure of sylvine (KCl) and zinc sulfide (ZnS), alongside various functional groups [18]. These include hydroxyl, carboxylic acids, amines, aromatic compounds,

unsaturated and saturated hydrocarbons, and carbonyl groups. This diverse chemical composition suggests potential biological activities, such as antioxidant, antimicrobial, and anti-inflammatory effects. Veera mezhugu is a similar formulation which is studied for FT-IR analysis and showed the presence of organic and inorganic groups [19].

4 Conclusion

This present study represents a pioneering effort to scientifically validate VRM by employing advanced analytical techniques like XRD and FTIR. These methods provide a unique fingerprint of the drug establishing a baseline for future research like in-vitro and in-vivo studies. Apart from this, standardization can be done to further evaluate the quality parameters for future research. The novelty of this work lies in its approach to bridging the gap between traditional knowledge and modern science. This study contributes to a valuable foundation for the global acceptance of Siddha medicines.

Abbreviations

VRM: Vaalai Rasa Mezhugu; **XRD**: X-Ray Diffraction; **FTIR**: Fourier Transform Infrared Spectroscopy; **ATR:** Attenuated Total Reflectance**; FWHM**: Full Width at Half Maximum.

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