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INSTRUMENTAL STANDARDIZATION OF *SIDDHA* FORMULATION *CHITHIRAMOOLA RASAYANAM* USING FTIR, SEM AND HEAVY METAL ANALYSIS

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ABSTRACT

Objective: The present study aims to standardize a *Siddha* Polyherbal formulation (*Chithiramoola Rasayanam*) using modern analytical techniques to ensure its quality, safety, and efficacy.

Methods: The formulation was prepared using purified raw drugs including *Chithiramoolam (Plumbago zeylanica), Nilappanai (Curculigoorchioides), Thannirvittan (Asparagus racemosus), Parangichakkai (Smilax china), Serangkottai (Semecarpus anacardium)*, along with honey, ghee, and sugar, following classical *Siddha* procedures. The prepared drug was subjected to Fourier Transform Infrared Spectroscopy (FTIR) for identification of functional groups, Scanning Electron Microscopy (SEM) for surface morphology and particle size analysis, and Heavy Metal Analysis to assess safety by detecting toxic metals such as lead, cadmium, arsenic, and mercury.

Results: FTIR analysis revealed characteristic peaks corresponding to hydroxyl, aliphatic, carbonyl, and ether groups indicating the presence of phytoconstituents such as phenols, flavonoids, and glycosides. SEM analysis showed irregular, agglomerated particles with rough surface morphology, suggesting effective trituration and enhanced surface area. Heavy metal analysis confirmed that all toxic metals were within WHO permissible limits.

Conclusion: The findings confirm that the formulation possesses significant physicochemical characteristics and meets safety standards. This study provides scientific validation and supports the standardization of the Siddha formulation.

Keywords: *Siddha*, *Chithiramoola Rasayanam*, FTIR, SEM, Heavy metal analysis, Standardization

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INTRODUCTION

The *Siddha* system is one of the oldest traditional systems of medicine practiced mainly in South India [1-3]. Siddha medicines are prepared from herbs, metals, minerals, and animal products and are widely used for the management of chronic diseases. Among the various dosage forms, *Rasayanam* preparations are commonly used because of their rejuvenating and therapeutic properties.

Nowadays, scientific validation of traditional medicines has become important to ensure their quality, safety, and consistency [4-9]. Variations during collection of raw drugs, purification methods, and preparation procedures may affect the final quality of the medicine [10]. Therefore, standardization using modern analytical techniques plays an important role in evaluating traditional formulations [5].

FTIR analysis helps in identifying important functional groups present in the drug [7], while SEM analysis provides information regarding particle size and surface morphology [17]. Heavy metal analysis is also essential to ensure that toxic metals are within safe limits prescribed by WHO guidelines [1,2].

In the present study, *Chithiramoola Rasayanam* was prepared according to classical *Siddha* literature [8,10] and subjected to FTIR, SEM, and ICP-OES for instrumental standardization.

MATERIALS AND METHODS

Ingredients

- Purified Chithiramoolam (*Plumbago zeylanica*) – 30 palam (1050 g)

- Purified Nilappanai (*Curculigoorchioides*) – 30 palam (1050 g)
- Purified Thannirvittan (*Asparagus racemosus*) – 30 palam (1050 g)
- Purified Parangichakkai (*Smilax china*) – 30 palam (1050 g)
- Purified Serangkottai (*Semecarpus anacardium*) – 40 palam (1400 g)
- Honey – 80 palam (2800 g)
- Ghee – 80 palam (2800 g)
- Vellai Sarkarai (*Saccharum officinarum*) – 160 palam (5600 g)

Standard Operating Procedure

All raw drugs were authenticated by experts from the Department of Gunapadam, Government Siddha Medical College, Chennai. The ingredients were purified according to classical *Siddha* literature *Sarakugalinsuthi sei muraigal*,The purified drugs were dried, finely powdered using an iron mortar and pestle, and sieved through a silk cloth (vasthrakaayam procedure). The powdered ingredients were mixed thoroughly with sugar, honey, and ghee to obtain the *Chithiramoola Rasayanam*, which was stored in an airtight glass container.

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to determine the functional groups present in the formulation using a Shimadzu IR Affinity-1 spectrophotometer at the Captain Srinivasa Murthy CCRAS, Chennai [14]. The sample was completely dried and mixed with spectroscopic grade potassium bromide (KBr) in a 1:100 ratio, followed by pellet formation using a hydraulic press [15]. The prepared pellet was scanned over the range of 4000-400 cm^{-1}. The resulting spectrum was analyzed, and absorption bands were assigned to specific functional groups using standard reference data [16]. This technique facilitated the identification of chemical bonds and major constituents present in the formulation.

SEM Analysis

The surface characteristics and particle morphology of the formulation were evaluated using Scanning Electron Microscopy (SEM) with a Zeiss Gemini SEM 300, available at the SAIF facility, IIT Madras, Chennai [17]. A small amount of the powdered sample was fixed onto an aluminium stub using conductive carbon tape. To enhance conductivity, the sample was coated with a thin layer of gold under vacuum conditions [18]. The specimen was then examined under appropriate operating conditions, and micrographs were captured at different magnifications to study particle shape, size distribution, and aggregation behaviour.

Heavy Metal Analysis

The safety profile of the formulation was assessed by estimating heavy metal content using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [12]. Prior to analysis, the sample underwent acid digestion to obtain a clear solution suitable for elemental estimation [13]. The levels of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) were quantified and compared with established WHO permissible limits [2]. The results were used to evaluate the absence of toxic metal contamination and ensure the formulation's safety for therapeutic use.

RESULTS

FTIR Analysis

The FTIR spectrum of *ChithiramoolaRasayanam* showed characteristic peaks indicating various functional groups.

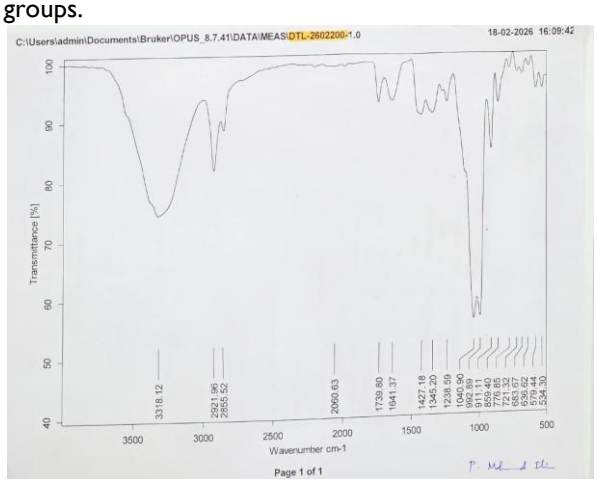

Figure 01:

Table 01: FTIR Peak Interpretation

S.NO	Wavenumber (cm^{-1})	Functional Group	Interpretation
1	3318	O–H / N–H stretching	Alcohols, phenols, amines
2	2922	C–H stretching	Alkanes
3	2855	C–H stretching	Alkanes
4	1739	C=O stretching	Carbonyl compounds (esters/acids)
5	1641	C=C stretching	Alkenes / amide groups
6	1427	C–H bending	Aliphatic compounds
7	1345	C–H bending	Methyl groups
8	1238	C–O stretching	Esters / ethers
9	1040–1028	C–O stretching	Alcohols / glycosides
10	911–816	=C–H bending	Aromatic/alkene compounds

Interpretation

The FTIR spectrum of *chithiramoolarasayanam* confirms the presence of hydroxyl, carbonyl, aliphatic and ether functional groups, indicating a complex mixture of

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phytoconstituents such as phenols, flavonoids and glycosides.

SEM Analysis

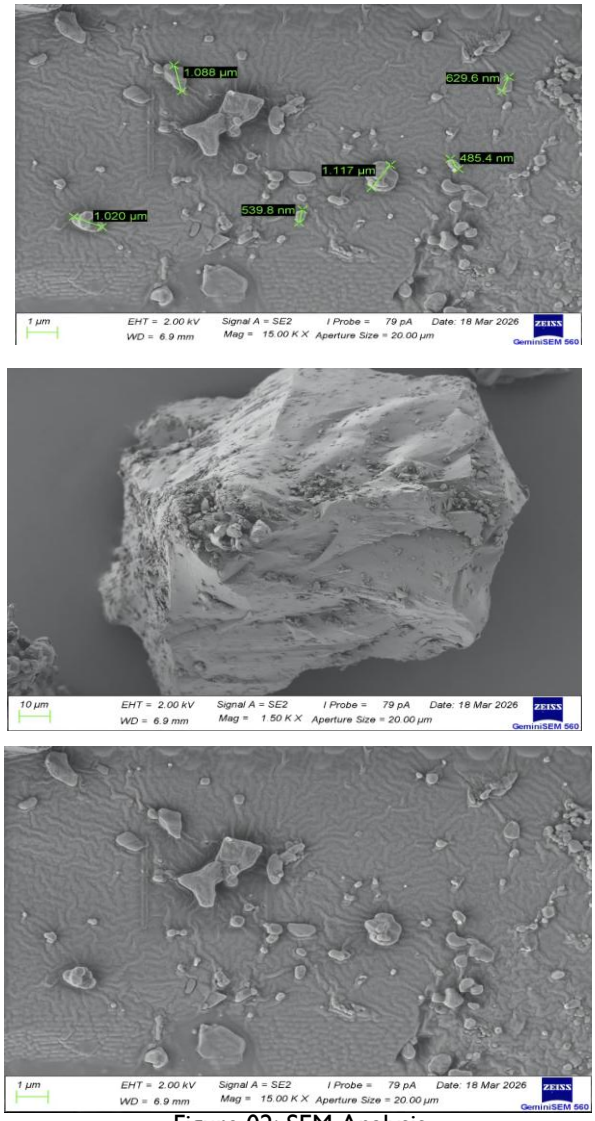

Figure 02: SEM Analysis

SEM images of *chithiramoolarasayanam* at different magnifications showed irregular, heterogeneous and agglomerated particles with rough surface morphology (Figure 2–4), indicating effective trituration and increased surface area.

Table 02: Heavy Metal Analysis

S.NO	DRUG RECEIVED	ELEMENT	RESULT
1	*Chithiramoola Rasayanam*	Arsenic (As)	2.5
2		Cadmium (Cd)	BLQ
3		Mercury (Hg)	BLQ
4		Lead (Pb)	BLQ

Heavy metal analysis showed that Pb, Cd, As and Hg were within WHO permissible limits.

DISCUSSION

The FTIR study of *Chithiramoola Rasayanam* showed different absorption peaks corresponding to hydroxyl, carbonyl, aliphatic, and ether functional groups. These functional groups may be related to the presence of phytochemical constituents such as phenols, flavonoids, glycosides, and other organic compounds naturally present in the herbal ingredients [7,11].

SEM analysis showed particles of irregular shape with aggregation and rough surface texture. Such morphological features may increase the surface area of the formulation and could help in better dissolution and absorption of the drug [17]. The particle distribution also indicates proper grinding and processing during preparation [5].

Heavy metal analysis showed that the levels of arsenic, lead, cadmium, and mercury were within the permissible limits recommended by WHO [2]. This finding indicates that the formulation is comparatively safe with respect to toxic metal contamination.

The analytical observations obtained from the present study may serve as supportive data for the standardization and quality assessment of *Chithiramoola Rasayanam* [4].

CONCLUSION

The present study was carried out to evaluate *Chithiramoola Rasayanam* using FTIR, SEM, and heavy metal analysis. FTIR analysis confirmed the presence of various functional groups associated with phytochemical constituents [7,11]. SEM study showed irregular and aggregated particles with rough surface morphology [17]. Heavy metal analysis indicated that the toxic metals were within WHO permissible limits [2].

The findings of this study provide useful preliminary data for the standardization and quality evaluation of the formulation [4]. The results also support the safety and acceptable quality of *Chithiramoola Rasayanam* prepared according to classical Siddha procedures [8,10].

CONFLICT OF INTEREST

None

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