



Received on 19 July 2025; received in revised form, 29 August 2025; accepted, 30 August 2025; published 01 January 2026

SOPHISTICATED INSTRUMENTAL ANALYSIS OF NOVEL SIDDHA FORMULATION NAVAMANI CHENDURAM THROUGH MODERN TECHNIQUES

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Keywords:

FTIR, ICP-OES, Instrumental analysis, Navamani Chenduram, SEM, XRD

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ABSTRACT: Navamani Chenduram (NC) is one of the metallic preparations mentioned in the classical Siddha literature “Sikitcha Rathina Deepam” which is indicated mainly to treat female reproductive diseases like amenorrhea, ovarian tumor and ascites. Preparing the Trial drug in accordance with the standard operating procedure and put through a number of studies to ensure its safety, effectiveness, purity, and quality. WHO has highlighted the necessity of employing contemporary methods to guarantee quality control of Indian medicines. The aim of the study was to standardize the NC through Sophisticated instrumentation techniques like Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES), Fourier Transform Infra-Red (FTIR), Scanned Electron Microscopy with Energy Dispersive X-Ray Analysis (SEM EDAX) and X-ray Diffraction (XRD) for the detection of Functional group, Chemical, Elemental analysis, Particle size, Heavy metals analysis and Minerals present in NC. Also identified the drug substance forms and determine its molecular structure. The results showed that under ICP OES Heavy metals like Aluminum, Arsenic, Copper, Magnesium, and Mercury were found below the detection level. The stretches and bonds present in the FTIR analysis indicates that the presence of functional groups Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Alkyne which may be the reason for the therapeutic potency of the drug. SEM EDAX analysis indicated the existence of nanoparticles. XRD results revealed the good crystallinity after calcinations process. Therefore, it seems an evident that the safety of NC has been validated using modern scientific method.

INTRODUCTION: The Siddha system of medicine is enhanced with unique features and a wealth of hidden scientific knowledge. The Siddha system asserts a wide range of therapeutic methods, including the treatment of infectious and non-infectious ailments with drugs that promote internal healing, rejuvenation, and disease prevention.

The three humors that form the basis of the Siddha system symbolize contemporary scientific theories of diseases, such as those involving glandular, endocrine, and metabolic processes and the disruption of these processes ¹.

This classical Siddha drug Navamani Chenduram (NC) is yet remained unexplored for its potent activities, physiochemical, pharmacological actions in terms of scientific research. The World Health Organization (WHO) has created and released a number of guidelines, including Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines and Guidelines for the

QUICK RESPONSE CODE



DOI:

10.13040/IJPSR.0975-8232.17(1).308-14

This article can be accessed online on
www.ijpsr.com

DOI link: [https://doi.org/10.13040/IJPSR.0975-8232.17\(1\).308-14](https://doi.org/10.13040/IJPSR.0975-8232.17(1).308-14)

Assessment of Herbal Medicines². Standardization of Siddha formulation means that their identity, quality, and purity are verified at every stage of its life cycle, including distribution, metabolism, absorption, storage, shelf life and elimination³. There appears to be very little scientific research on Siddha remedies to support these claims. To fill these scientific lacunae, the present work was undertaken to identify the chemical characterization of the Siddha classical drug Navamani Chenduram (NC) by analyzing its safety, therapeutic potency, waying its heavy metals and functional groups through modern instrumental analysis such as FT-IR, XRD, SEM, and ICP-OES⁴. In Indian alchemy, chendhuram were the formulations of metals or their own salts such as gold, silver, tin, stannum, lead, zinc, mercury. Similarly, organic macromolecules made from herbal juices through alchemical processes are biologically equivalent. These Herbo mineral compositions, which are consumed with butter, honey, milk, or ghee and by regularly

burning metals or their salts with medicinally beneficial herbs or extracts to remove the toxicological effects of the drug⁵.

This analytical method promotes efforts to standardize ancient medicines for wider acceptability and in corporation into contemporary healthcare practices, and also helps to comprehend the chemical complexity of these formulations. Thus, the particle size, chemical properties and functional group changes were identified through the analysis. The obtained results help to provide the composition of test drug NC by proving it scientifically and improving interest for further more studies.

MATERIALS AND METHODS:

Drug Selection: *Navamani Chendhuram* (NC) is a traditional Siddha mineral formulation described in *Sigitcha Rathinadeepam*. This drug was specifically indicated for the management of *Soodhaga katti* (PCOS), *Soodhaga Sikkal* (Amenorrhea) and *Peruvayiru* (Ascites).

Ingredients of Navamani Chendhuram Table 1:

TABLE 1: INGREDIENTS OF NAVAMANI CHENDHURAM

Sl. no.	Ingredient	Chemical name
1	<i>Veeram</i>	Hydrargyrum perchloride
2	<i>Pooram</i>	Calomel
3	<i>Lingam</i>	Cinnabar
4	<i>Kandhagam</i>	Sulphur
5	<i>Padigaram</i>	Alum
6	<i>Vediuppu</i>	Potassium nitrate
7	<i>Navacharam</i>	Ammonium chloride
8	<i>Vengaram</i>	Borax
9	<i>Ayam</i>	Ferrum

Raw Drug Collection: All the above-mentioned raw drugs were procured from a well reputed country shop in Parrys Corner, Chennai. All the ingredients were purified as per the standard protocol and the medicine was prepared in the *Gunapadam* laboratory at the National Institute of Siddha.

Identification and Authentication of the Drug:

The metal and mineral drugs were identified by Pharmacologist, Dept. of *Gunapadam*, NIS, Tambaram sanatorium, Chennai. (CN: Gun/Aut/026/21)

Purification of the Drugs: All the drugs mentioned here were purified as per the Siddha literature.

Preparation of the Trial Drug: The mentioned drugs were purified and powdered (except ayam), grind the powder in lemon juice for two *saamam* (6 hours) and then add purified *ayam* in it. This mixture was undergone calcification process by using 50 cow dung cakes.

The calcified drug was grinded by using lemon juice for four *saamam* (12 hours) made into 16 *villai* and dried under sunlight.

After drying, *chenduram* were incinerated with 30 cow dung cakes, repeated this procedure for 6 times then the final product is obtained.



FIG. 1: NAVAMANI CHENDHURAM

Sophisticated Instrumental Analysis:

FT-IR (Fourier Transform Infra-Red): FT-IR is an important and more advanced technique to identify the functional group. The spectrum which indicates the absorption and transmission of molecules. It generates the sample's molecular fingerprint. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wave number and the peaks seen in the spectrum indicates the amount of material present.

In FT-IR, an infrared beam from a source was sent through a sample. According to the sample's chemical makeup, some of the infrared was absorbed and some was transferred. The spectrum that shows the absorption and transmission of molecules. It creates the sample's molecular fingerprint. There were no two distinct chemical configurations that produced the same infrared spectrum, similar to the finger print. It was noted because the wavelength and peaks in the spectrum show the amount of material present in it ⁶⁻⁹.

SEM (Scanning Electron Microscope): In a scanning electron microscope, a high-energy electron beam was directed at the sample material through a probe. Interaction with the sample's surface leads to the production of a variety of signals. This causes the emission of electrons or photons, which were then captured by a suitable detector.

The morphology and elemental composition of the sample NC can be determined by Environmental SEM (FEI Quanta). In order to get a higher quality electron image for SEM examination, a portion of each sample must be sprinkled on a double side carbon tape and could be mounted on aluminum stubs. This provides information about the sample, which includes its shape, texture, external

morphology, crystalline structure, and its chemical composition ¹⁰.

EDX Analysis: The energy dispersive X-ray microanalysis is a technique of elemental analysis based on the generation of X-rays that reveals the presence of elements present in the sample.

EDX can detect major and minor elements with concentrations higher than 10 wt.% and minor concentrations less than 10 wt% ¹¹.

ICPOES (Inductively Coupled Plasma Optic Emission Spectrometry):

Equipment: ICP-OES (Perkin Elmer Optima 5300 Dv).

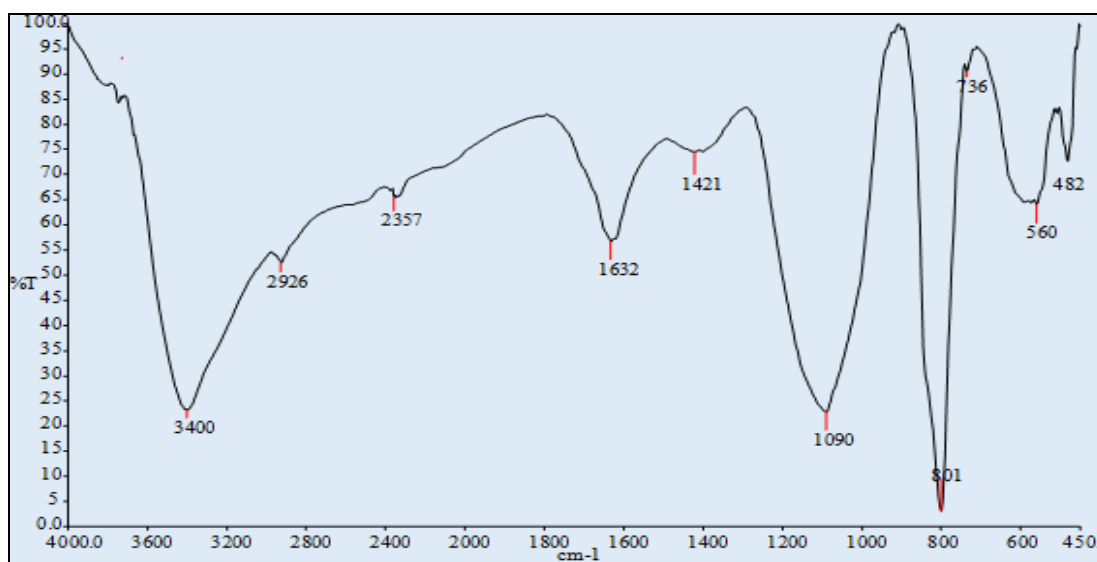
Principle: Inductively Coupled Plasma or ICP, is one technique for optical emission spectrometry. The component elements (atoms) of an analytical sample are stimulated when plasma energy is applied from the outside. The emission rays (spectrum rays) that match to the photon wavelength are released when the excited atoms return to their low energy positions. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray's intensity ^{12, 13}.

Application: The major and minor elements in the sample solution were analyzed.

X-Ray Diffraction (XRD) Analysis: The XRD powder diffraction pattern of the drug can be recorded on X-ray diffractometer (Siemens D500d Diffractometer) using Cu Ka radiation, $\lambda = 1.5406 \text{ \AA}$ over the range of $10.0 - 80.0^\circ$.

Application:

- Characterize the crystallinity
- Fine grained minerals that are hard to identify optically, also clays and mixed layer clays can be identified, sorted and counted using advanced superior angular resolution.
- Measure the super lattices in multilayered epitaxial structures to characterize thin film samples.
- Using glancing incidence X-ray reflectivity measurements to obtain the film's thickness, roughness and density ¹⁴.

RESULTS:**FTIR:****FIG. 2: VIBRATIONAL MODES AND FUNCTIONAL GROUP OF NAVAMANI CHENDHURAM IN FTIR**

In the FTIR analysis of NC exhibits the peak at the wave number of 3400, 2926, 2357, 1632, 1421, 1090, 801, 736, 560, 482 having the stretch of O-H stretch, O-H stretch, H-C-H stretch, C=O stretch,

N-H stretch, C-C=C symmetric stretch, H-C-H bend, C-O stretch, C -H bend and C-C stretch respectively.

TABLE 2: VIBRATIONAL MODES AND FUNCTIONAL GROUP OF NAVAMANI CHENDHURAM IN FTIR

Wave number (cm-1)	Vibrational mode of Navamani chendhuram in IR region	Functional group
3400	O-H stretch	Amide
2926	O-H stretch	Phenols and Alcohols
2357	H-C-H stretch	Alkanes
1632	C=O stretch	Aldehyde
1421	N-H stretch	Amine
1090	C-C=C symmetric stretch	Alkenes
801	H-C-H bend	Alkanes
736	C-O stretch	Ester
560	C -H bend	Ether
482	C-C stretch	Alkyne

Interpretation: The FTIR results shows the observed water O-H stretch, O-H stretch, H-C-H stretch, C=O stretch, N-H stretch, C-C=C symmetric stretch, H-C-H bend, C-O stretch, C -H

bend, C-C stretch which indicates that the presence of functional groups Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Alkyne.

ICP-OES for Minerals:**TABLE 3: WAVELENGTH AND ITS CONCENTRATION OF NAVAMANI CHENDHURAM IN ICP-OES**

S. no.	Elements	Wavelength in nm	Concentration (mg/L) in Navamani chendhuram (wt:0.431710g)
1	Aluminum	Al 167.020	12.021 mg/L
2	Arsenic	As 188.979	BDL
3	Boron	B 182.520	05.123 mg/L
4	Iron	Fe 238.204	50.256 mg/L
5	Mercury	Hg 253.652	03.101 mg/L
6	Manganese	Mn 257.610	BDL
7	Sodium	Na 589.592	171.320 mg/L
8	Potassium	K 766.491	94.821 mg/L
9	Lead	Pb 220.353	BDL

10	Phosphorus	P 213.617	06.341 mg/L
11	Sulfur	S 180.731	211.254 mg/L
12	Magnesium	Mg 285.213	00.342 mg/L

BDL-Below detection limit

Interpretation: The heavy metals like Arsenic, Manganese and Lead were found below detection limits. The presence of other elements shows the therapeutic value of NC. Sodium and potassium are indirectly related to reproduction. The normal physiology of reproduction can be impacted by sodium deficiency because it prevents the use of protein and energy. Potassium deficiency results in muscle weakness, which in turn affects the musculature of the female genital canal, impairing

the normal reproductive process. Thus, the therapeutic effects of NC are enhanced by the presence of these minerals.

HR- SEM Analysis: Determination of particle size of Navamani Chendhuram: Particle size and chemical elements were assessed by Scanning Electron Microscope (SEM) is one of the most widely used instruments in research areas. Results of NC is shown in figs.

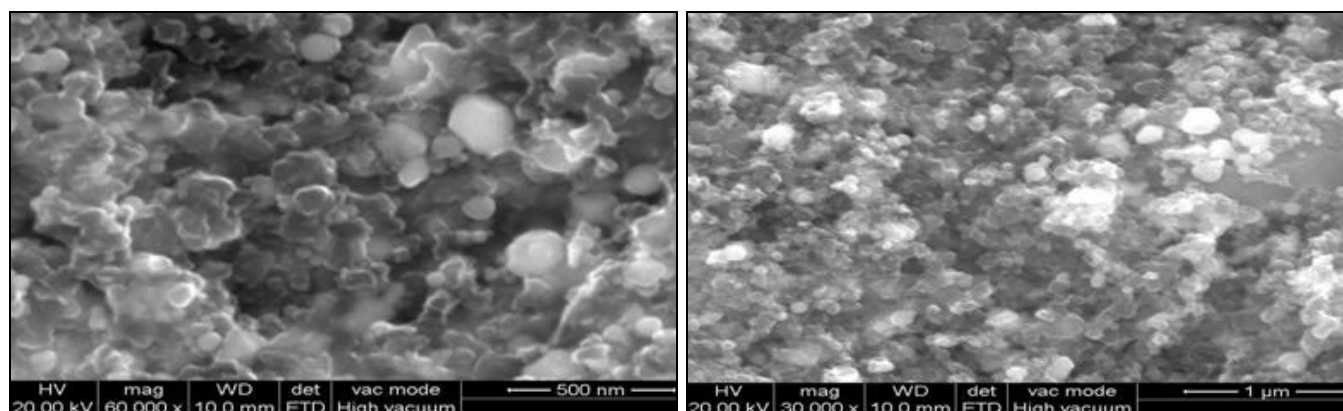


FIG. 3: SEM ANALYSIS

Interpretation: SEM analysis of the test drug *Navamani chendhuram* revealed the presence of nano particles of size 128 nm, 130 nm, 330 nm, 250 nm and 480 nm.

Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. Particles with a high positive surface charge like chitosan are usually attracted by the intestinal mucosa which helps in increasing the intestinal absorption of the encapsulated drug. However, the strong electrostatic interaction between the positively charged particles and the negatively charged glycocalyx may slow down the progression and penetration of these particles towards the epithelial cell surface reducing their uptake. Also, it has been shown that non-ionized particles have a greater affinity for M cells than for ionized particles and positively charged particles that are nano and near-nano in size demonstrate how a medicine can quickly and easily enter cells at the molecular level to treat a disease and boost therapeutic impact.

EDX of NC: The energy dispersive X-ray microanalysis is a technique of elemental analysis based on the generation of X-rays that reveals the presence of elements present in the sample. No result is shown below.

TABLE 4: WEIGHT AND ATOMIC PERCENTAGE OF NAVAMANI CHENDHURAM

Elements	Wt%	At%
C K	06.64	11.43
O K	13.44	10.51
Al K	01.27	02.12
Fe K	22.04	20.10
Hg K	05.10	03.32
K K	01.10	00.10
Na K	28.12	27.54
S K	22.90	19.10
Matrix	Correction	ZAF

Interpretation: EDX can detect the major and minor elements with concentrations higher than 10 Wt% and concentrations less than 10 Wt%. From this analysis SEM, we can observe

➤ Nano Particles exist in NC medicine.

- Nano emulsions can be synthesized, as there is abundant use of Oils.
- Nutraceuticals available in the foods can be explored to higher level.

If achieved, can be used for higher absorption, less toxicity and cost efficacy.

X-Ray Diffraction (XRD) Analysis:

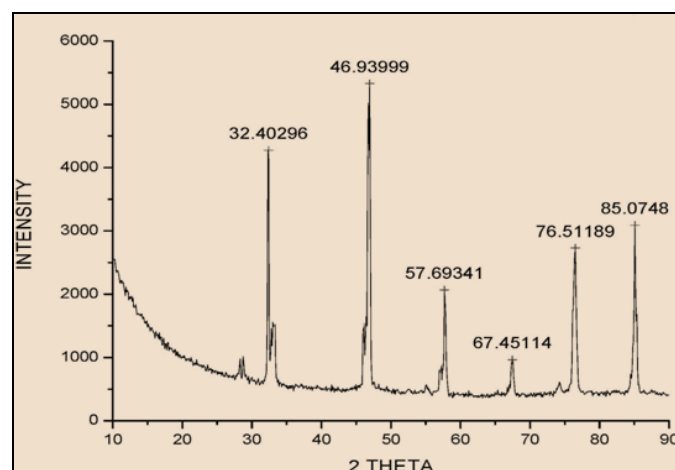


FIG. 4: POWDER XRD PATTERN OF NC

XRD Interpretation:

- The X-ray diffraction pattern of a pure substance is, therefore, like a fingerprint of the substance. It is based on the scattering of X-ray by crystals
- XRD was used to identify drug substance forms and determine the molecular structure of the sample.
- It well monitoring the crystal morphology of drugs. Results of XRD: There are three strongest peaks present in the XRD analysis such as 32.40296, 46.93999 and 27.69341.
- XRD pattern of NC shows the good crystallinity after calcinations process.

DISCUSSION: Indian herbal product exports are low for a number of reasons among that quality being the crucial one, according to a report by Group II of a task force on “Pharmaceuticals and Knowledge Based Industries” at 1999¹⁵. Standardization is the tool to aid this and aims to proven the quality potency and safety of the herbs and many traditional formulations. The classical siddha drug ‘Navamani Chendhuram’ (NC) was

selected from “sigitcha Rathinadeepam” the ancient Siddha literature indicated for PCOS and Amenorrhea. NC has been undergone standardization by using instrumental analysis to determine its functional group, particle size, crystalline nature and the level of heavy metals present in it.

The Instrumental analysis report reveals that in FTIR results shows that water O-H stretch, O-H stretch, H-C-H stretch, C=O stretch, N-H stretch, C-C=C symmetric stretch, H-C-H bend, C O stretch, C -H bend, C-C stretch which indicates that the presence of functional groups Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Ether, Alkyne respectively. The results of SEM- EDX analysis revealed that particle size of the sample NC ranged from 1µm to 10µm and end product ranged from 500 nm to 5µm. This demonstrated that the particle size was decreased to nano size during incineration. Particles that are nano and near-nano in size demonstrate how a medicine can quickly and easily enter cells at the molecular level to treat a disease and boost therapeutic impact.

ICP-OES results showed that Heavy metals like Aluminum, Arsenic, Copper, Magnesium, and Nickel were found below the detection level. It also shows the presence of physiologically important minerals like sodium, potassium, iron, zinc, Calcium, and phosphorus. The presence of other elements shows the therapeutic value of NC. Indirectly, Sodium and potassium were related to reproduction. The normal physiology of reproduction can be impacted by sodium deficiency because it prevents the use of protein and energy. Potassium deficiency results in muscle weakness, which in turn affects the musculature of the female genital canal, impairing the normal reproductive process. Thus, the therapeutic effects of NC are enhanced by the presence of these minerals. A pure substance's X-ray diffraction pattern can be related to the substance's fingerprint. It is based on the scattering of X-ray by crystals. It was well monitoring the crystal morphology of the drugs. There are three strongest peaks present in the XRD analysis of NC such as 32.40296, 46.93999 and 27.69341 showed the good crystallinity after calcinations process.

CONCLUSION: *Navamani Chendhuram* (NC), a classical formulation was prepared as per the Siddha literature. The globalization of traditional medicines was frequently coexisted with the development of novel methods and the optimization of existing purification methodologies. Result showed that the instrumental analysis of this study ensures that the drug NC may possess increased functional groups evident by FTIR. The particle size, active compounds with concentration and identification of *Navamani chendhuram* was done by HR-SEM, EDX. Metals and minerals were identified through ICP-OES, FTIR and X-Ray Diffraction (XRD). The *Navamani chendhuram* showed the evidence for the presence of Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes, Ester, Ether, Alkyne. The report reveals that the heavy metals like Arsenic, Manganese and Lead are within the permissible limits and consist of Iron, Sodium, Potassium and Magnesium as its active composition. Thus, the findings were investigated in order to set guidelines for the management of emerging health issues.

ACKNOWLEDGEMENT: We are greatly thankful to the Director, National Institute of Siddha, Chennai - 47 for providing necessary facilities to carry out this work. I'm also thank full to the Indian Institute of Technology, Madras for helping in Lab Analysis. Last but not the least heart full thanks to my colleagues of our Department of Gunapadam, National Institute of Siddha and DR. T. N. MGR Medical University, Tamil Nadu, India for their kind support and cooperation.

CONFLICTS OF INTEREST: Nil

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How to cite this article:

Ayyanarappan PM and Visweswaran S: Sophisticated instrumental analysis of novel siddha formulation navamani chenduram through modern techniques. Int J Pharm Sci & Res 2026; 17(1): 308-14. doi: 10.13040/IJPSR.0975-8232.17(1).308-14.

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