INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

# STANDARDIZATION OF SIDDHA HERBO-MINERAL FORMULATION *SANDHANATHY CHOORANAM*

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**ARTICLE INFO**

**Article history**
Received 19/05/2019
Available online
31/05/2019

**Keywords**
Siddha Drug,
*Sandhanathy Chooranam*,
FTIR,
ICPOES,
XRF,
SEM.

**ABSTRACT**

Standardization of a Siddha herbo-mineral formulation is essential in order to assess the quality of drugs based on the concentration of their active principles, physical, chemical, phytochemical, in-vitro, in-vivo parameters. All the plant and mineral materials were identified and authenticated by the Botanist and *Gunapadam* (Siddha Pharmacology) experts in Government Siddha Medical College, Arumbakkam, Chennai - 106. FTIR is used to identify the functional group, to determine the quality and consistency of the sample. SEM gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample. ICPOES helps to analyze the major and minor elements in solution sample. FTIR shows the presence of alcohol, alkanes, alkenes, alkynes, amide, amine, aromatic, ester, ether and nitro groups. SEM results reveal the presence of micro particles ranging from 238nm, 280nm, 306nm. ICPOES shows the presence of Ca, Fe, K, Na, P and S has physiologically important. In *Sandhanathy Chooranam*, the heavy metals like As, Cd, Hg, Pb and Ni were below detectable level. This reveals the safety of the drug. Based on the results *Sandhanathy Chooranam* is a safe drug.

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*Please cite this article in press as **Punithavalli. V** et al. Standardization of Siddha Herbo-Mineral Formulation Sandhanathy Chooranam. Indo American Journal of Pharmaceutical Research.2019:9(05).*

## INTRODUCTION

Standardization is a code of conduct that ensures the correct substance in correct amount for desired therapeutic effect (Safety, Quality and Efficacy) is known as standardization [1, 2]. Standardization confirms the drug identify (Authentication) and determines the quality and purity [3]. Traditional medicines are widely used for various human ailments and significantly more popular all over the globe because of the effective and curative nature for chronic disease with less toxicity. Herbal medicines are not a simple task since many factor influence the biological efficacy and reproducible the therapeutic effect [4, 5]. Standardization of a siddha herbo-mineral formulation is essential in order to assess the quality of drug based on the concentration of their active principles, physical, chemical, phytochemical, in-vitro, in-vivo parameters. The quality assessment of herbo-mineral formulation is paramount in order to justify their acceptability in modern era [6]. So we aimed to Standardize "*sandhanathy chooranam"* a Siddha herbo-mineral formulation based on the Instrumental analysis.

## MATERIALS AND METHODS

### DRUG SELECTIONS

The purified and prepared trial drug "*Sandhanathy Chooranam"* was taken for the purpose of standardization. This formulation taken from the Siddha literature *"Agasthiyar Vaithiya Sindhamani Venba 4000 Ennum Mani 4000".pg no: 185-186.*

### IDENTIFICATION AND AUTHENTICATION OF THE DRUG

All the plant materials were identified and authenticated by the Botanist and *Gunapadam* experts in Government Siddha Medical College, Arumbakkam, Chennai – 106. The specimen sample of all the herbs have been preserved in PG *Gunapadam* department individually for future reference.

### PREPARATION OF THE DRUG

All the ingredients were purified and dried in the shade until complete evaporation of the moisture content. It was finely powdered and kept in an air tight container. It was labeled as "*Sandhanathy Chooranam".*

### FOURIER TRANSFORM INFRA-RED SPECTROSCOPY

Model : Spectrum one: FT-IR Spectrometer
Scan Range : MIR 450-4000 cm-1
Resolution : 1.0 cm-1
Sample required : 50 mg, solid or liquid.

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample. It is an excellent tool for quantitative analysis [7].

In FT-IR infrared was passed from a source through the sample (*sandhanathy chooranam*). This infrared was absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample.

Like the finger print there was no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

### SEM (SCANNING ELECTRON MICROSCOPE

In scanning electron microscope high-energy electron beam was focused through a probe towards the sample (*sandhanathy chooranam*) material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample [8].

### ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

**Manufacture**r: Perkin Elmer
**Model:** Optima 5300 DV ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy)

**Application**:

- The analysis the major and minor elements of solution sample.
- Probes the outer electronic structure of atoms.
- Determine elemental concentrations of different metals.

**Mechanism:**

In optic emission spectroscopy (OES), a sample (*sandhanathy chooranam*) solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all the elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values.

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV [9].

**SAMPLE PREPARATION:**

Inductively Coupled Plasma Spectroscopy techniques are so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg *sandhanathy chooranam* was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Sterile water, was added to prepare solution. The digested sample solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

**RESULTS AND DISUSSION**

**FT-IR (FOURIER TRANSFORM INFRA RED SPECTROSCOPY)**

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material. The results of Table no: 1 and Fig no: 1 shows the presence of functional group and inorganic compounds of *Sandhanathy Chooranam.*

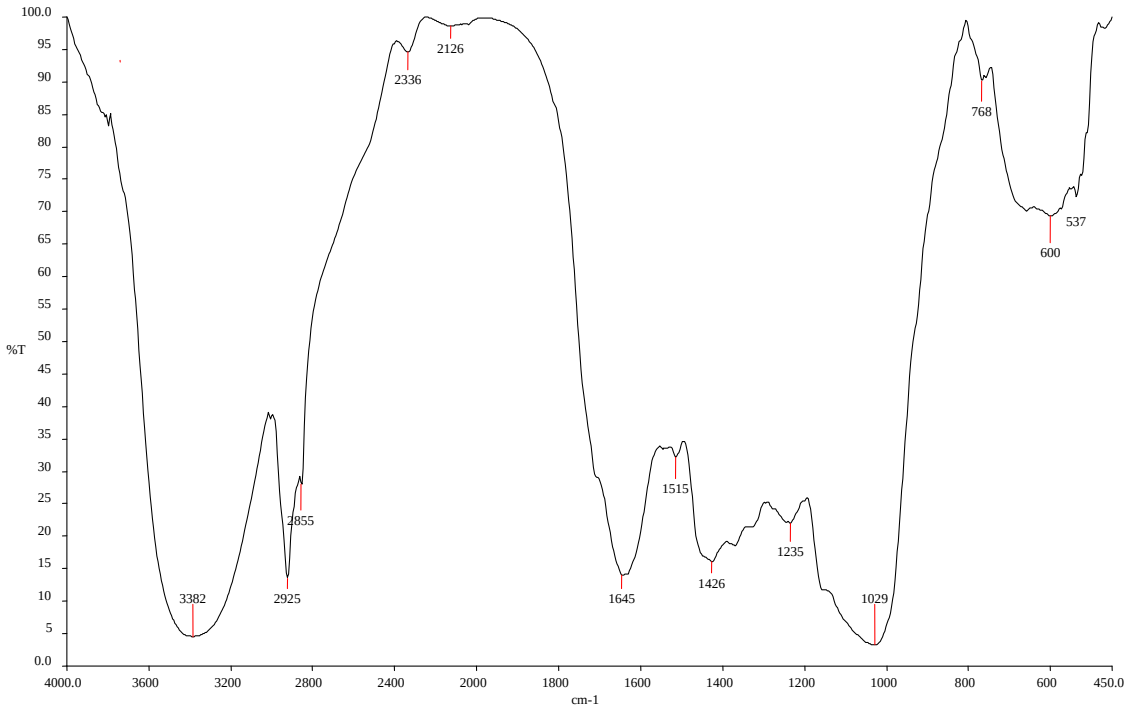


**Fig no.1 FT-IR (Fourier Transform Infra Red.**

**Table no.1 FTIR-INTERPRETATION.**

| Absorption peak $cm^{-1}$ | Stretch | Functional group |
|---|---|---|
| 3382 | O-H Stretch | Alcohol |
| | N-H Stretch | Amine |
| | N-H Stretch | Amide |
| 2925 | C-H Stretch | Alkane |
| | O-H Stretch | Acid |
| 2855 | C-H Stretch | Alkane |
| | O-H Stretch | Acid |
| 2126 | -C≡C- Stretch | Alkyne |
| 1645 | C=O Stretch | Amide |
| | C=C Stretch | Alkene |
| 1515 | N-O Stretch | Nitro |
| | C=C Stretch | Aromatic |
| 1426 | -C-H Bending | Alkane |
| | C=C Stretch | Aromatic |
| 1235 | C-F Stretch | Alkylhalide |
| | C-N Stretch | Amine |
| | C-O Stretch | Ether |
| | C-O Stretch | Acid |
| 1029 | C-F Stretch | Alkylhalide |
| | C-O Stretch | Ether |
| | C-O Stretch | Ester |
| 768 | C-Cl Stretch | Alkylhalide |
| | =C-H Bending | Alkene |
| 600 | C-Cl Stretch | Alkylhalide |
| | C-Br Stretch | Alkylhalide |
| 537 | C-Br Stretch | Alkylhalide |

FTIR instrumental analysis was done. The test drug was identified to have 12 peaks. They are the functional groups present in the trial drug "*Sandhanathy Chooranam.* The above table shows the presence of alcohol, alkanes, alkenes, alkynes, amide, amine, aromatic, ester, ether and nitro groups which represents the peak value.

- OH group has higher potential towards inhibitory activity against microorganisms.
- Phenols possess highly Anti-Oxidant property which enhances the drug effect against the disease.
- Amines enhance the drug effect against the disease.

**SEM: (SCANNING ELECTRON MICROSCOPE)**

The particle size and the chemical elements were assessed by Scanning Electron Microscope. SEM is one of the most widely used instruments in research side. The SEM picture of *Sandhanathy Chooranam* is shown in Fig no: 2 and 2.1.

The SEM studies of microscopic resolution of 1.00kx and examining surface area of 800x800µm$^2$, showed objects of sizes ranging from 238nm to 409nm. The surface of the sample grains are uniformly arranged in agglomerates. There are micro particles ranging from 238nm, 280nm, 306nm.

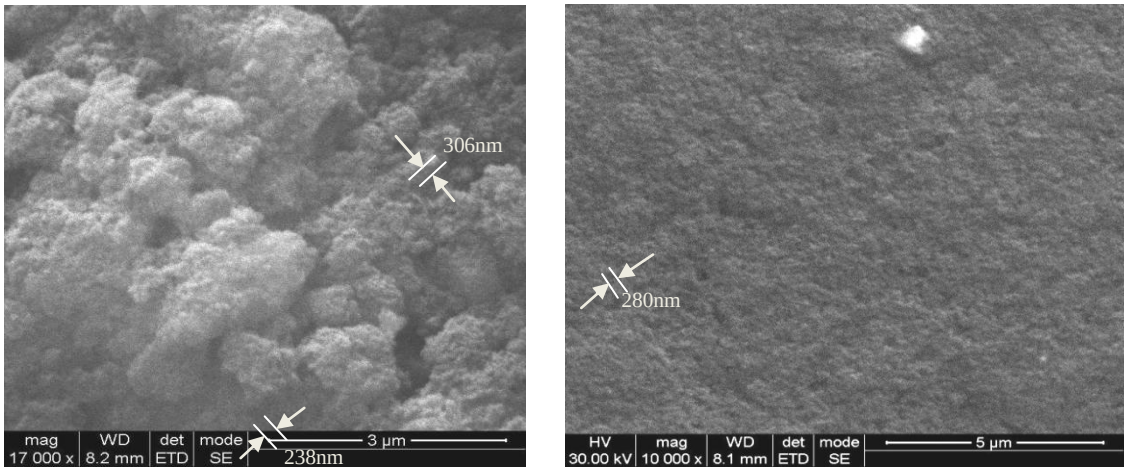


**Fig no.2 and 2.1 SEM PICTURE.**

- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000nm in diameter.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control and sustain the release of drug during the transportation and at the site of localization, alter drug distribution in the body and subsequent clearance of the drug so as to achieve increased drug therapeutic efficacy thereby increase bio-availability and reduced side effects.

Hence *Sandhanathy Chooranam* which is prepared biologically contains near nano size particles to enhance the pharmacological action in the target sites.

**ICP-OES (INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY):**

The drug sample *Sandhanathy Chooranam* was analyzed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given on the Table No.2.

**Table No.2 Results of ICPOES.**

| S. no | Elements | Detected levels |
|---|---|---|
| 1. | Calcium | 14.180 mg/L |
| 2. | Iron | 10.370 mg/L |
| 3. | Potassium | 70.881 mg/L |
| 4. | Sodium | 51.110 mg/L |
| 5. | Phosphorus | 68.571 mg/L |
| 6. | Sulphur | 01.324 mg/L |
| 7. | Arsenic | BDL |
| 8. | Cadmium | BDL |
| 9. | Mercury | BDL |
| 10. | Nickel | BDL |
| 11. | Lead | BDL* |

*BDL:Below Detectable Limit.

- The results of ICP OES shown in Table No.2 indicates that the trial drug is extremely safe as it contains heavy metals within specified limits.
- The presence of Ca, Fe, K, Na, P and S has physiologically important. In *Sandhanathy Chooranam,*the heavy metals like As, Cd, Hg, Pb and Ni were below detectable level. This reveals the safety of the drug.

**CONCLUSION**

Instrumental techniques one the greatest techniques used for identification, qualitative, quantitative analysis of chemical substances, that is useful in standardization and new chemical entity in clinical purpose. In the present study, the results of *Sandhanathy chooranam* were observed. The FT-IR shows the presences of various functional groups such as OH group has higher potential towards inhibitory activity against microorganisms. Phenols possess highly Anti-Oxidant property which enhances the drug effect against the disease. Amines enhance the drug effect against the disease and posses various pharmacological actions. The SEM result shows, the drug *sandhanathy chooranam* possess micro particle, pharmacologically these particles provides better absorption and enhances therapeutic effect by their bioavailability. The ICPOES shows the presence of elements Ca, Fe, K, Na, P and S and heavy metals As, Cd, Hg, Pb and Ni within normal limits (below detectable level), that reveals the safety of drug. Currently, we standardize *Sandhanathy chooranam* through instrumental analysis. In future, we should evaluate the toxicity of the drug at various levels through acute, sub acute and chronic toxicity studies.

**ACKNOWLEDGMENT**

The authors are very much thankful to the Principal and HOD, Government Siddha Medical College, Arumbakkam, Chennai106. Dr. R. Murugesan, SAIF, IIT, Chennai-36. HOD, Department of Mech.Engineering, Anna University, Chennai-25.

***Competing Interests***

The authors declare no conflict of interest.

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