ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

INSTRUMENTAL ANALYSIS OF NOVEL SIDDHA FORMULATION *POONAGA PARPAM*

V.Elakkiyaa[1]*, S. Visweswaran[2] , N. J. Muthu Kumar[3]

*[1]Resident Medical Officer, [2] HOD i/c, Department of Gunapadam, [3]Director, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India.

ABSTRACT

Poonaga parpam (PNP) is an animal origin Siddha formulation mentioned in the Siddha text "*Sikicha Rathina Deepam"*. The trial drug was prepared as per the standard operative procedure and subjected to various studies to reveal its potency. **Materials and methods:** The aim of the study was to standardize the PNP by modern instrumental techniques such as Fourier Transform Infra-Red Spectroscopy (FTIR), Inductively Coupled Plasma Optical Emission Spectroscopy (ICPOES), Scanning Electron Microscope with Energy Dispersive X-Ray Analysis (SEM with EDAX), X-Ray Diffractometer (XRD), Thermo Gravimetric Analysis (TGA). **Results and Discussion:** ICP-OES revealed the heavy metals like As, Cd, Pb, Hg and trace element like Ni were below detectable level. The stretches and bonds present in the FTIR analysis indicated the presence of some organic functional groups such as alcohol, aromatic, alkane, carbonyl, ketone, alkane, amine and alkyl halide which may be the reason for the therapeutic potency of the drug. XRD results revealed that the copper and iron may be the key ingredients present in the test drug PNP and the SEM picture indicated the existence of nanoparticles. TGA results revealed that the test drug PNP seems to be stable at varying temperature ranges from 50 to 400°C. Based on the results, *Poonaga Parpam* is preferably non-toxic to human in its therapeutic dose.

KEYWORDS: Inductively Coupled Plasma Optical Emission Spectrometry, *Poonaga parpam*, Scanning Electron Microscopy, X-Ray Diffractometer, Thermo Gravimetric Analysis.

INTRODUCTION

Siddha System from Tamil Nadu is widely regarded as one of the ancient healthcare system in the world. This system has deep roots in the cultural heritage and history of South India and Tamil Nadu more specifically. The origin of the Siddha Medical system is based on mythology, tradition and religion. Siddha system has 64 different varieties of medication. All these 64 medications are brought under broad headings 'Internal medicine' (*Ul Marunthu*) and 'External medicine' (*Veli Marunthu*). *Parpam* comes under the category of internal medicines. Other names of *Parpam* are *Neeru* or *Venneeru.*

The materials that go into the preparation of '*Parpam*' should first be cleaned and taken through the specified processes of purification that are recommended for each component. Then they are ground with juices of leaves, distillates or extractives and then subjected to frying or calcinations or suitably heating in the manner recommended in the recipe till the product is satisfactorily calcined.

Standardization of Siddha formulations could be an important section for the institution of a regular biological activity and chemical profile, or just a top quality assurance program for production and producing of Siddha formulations. It also denotes confirmation of its identity, quality and purity throughout all phases of its cycle i.e. shelf-life, storage, absorption, metabolism, distribution and elimination [1].

The present study deals with the Standardization of Siddha formulation *Poonaga parpam* (PNP) mentioned in the classical Siddha literature "*Sikicha Rathina Deepam"*. The formulation was evaluated using modern instrumental techniques such as FTIR, ICPOES, SEM with EDAX, XRD, TGA and proved the refinement of the classical literature.

MATERIALS AND METHODS

Selection of Drug

The drug PNP was selected from the classical Siddha literature, "*Sikicha Rathina Deepam"*. The ingredients of *Poonaga Parpam* (PNP) are.

1.	**Purified *Poonagam* (Earthworm)**	**1 *Veesai* (1400gm)**
2.	Juice of *Aduthinna paalai* (*Aristolochia bracteata*)	1 litre
3.	Butter milk	3 litres

Collection and Authentication of the Drug

The raw materials included in the formulation are Purified *Poonagam* (Earthworm), juice of *Aduthinna paalai* (*Aristolochia bracteata*) and butter milk which was collected from in and around Thanjavur district, Tamil Nadu. They were identified and authenticated by the faculty and experts members of *Gunapadam* (Siddha Pharmacology) Department and Botanist at the National Institute of Siddha.

Purification and Preparation of *Poonaga Parpam* [2,3,4]

The purification process was done according to the procedures mentioned in the classical Siddha literature, *Sarakku suthi muraigal* and Siddha Materia *Medica* (*Gunapadam Thathu Jeeva Vaguppu*). *Poonagam* was soaked in buttermilk. When it repels the sand it was taken out and dried, then it was ground. *Aduthinnapaalai* was washed in the running tap water to remove the soil and impurities.

Purified *Poonagam* was ground well using mortar and pestle and juice of *Aduthinna Paalai* (*Aristolochia bracteolata*) was added little by little to it for one day and made into pellet and dried. The pellet was then placed in between two earthen saucers and it was covered by mud sealed cloth. Then it was subjected into *Pudam* by using 100 cow dung cakes. The above mentioned procedure was repeated for 9 times and finally the *Parpam* was powdered well and stored in an air tight container.

Ingredients of *Poonaga Parpam*

Fig.No.1 *Poonagam* (Earthworms)

Fig.No.2 *Aaduthinna Paalai* (*Aristolochia bracteata*)

Fig.No.3 *Poonaga Parpam*

Instrumental Analysis

The above mentioned elemental analyses have been done at IIT Madras, SAIF and Madurai Kamaraj University.

Fourier Transform Infrared Spectroscopy (FTIR) [5,6]

FT-IR spectra were recorded at SAIF, IIT Madras, India. Fourier Transform Infrared Spectroscopy (FTIR) is a spectrographic analysis used to determine principally organic materials. FTIR analysis ends up in absorption spectra which offer data regarding the chemical bonds and molecular structure of a material. The FTIR spectrum is comparable to the "fingerprint" of the material and can be compared with cataloged FTIR spectra to identify the material.

The Perkin Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of PNP in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution 4cm-1 in the wave number region 450-4000cm-1. The samples were grounded to fine powder using agate motor and pestle and then mixed with KBr. They were then pelletized by applying pressure to prepare the specimen (the size of specimen about 13mm diameter and 0.3mm in thickness) to record the FT- IR Spectra under Standard conditions. FT- IR Spectra were used to determine the presence of the functional groups and bands in the PNP. The recorded spectrum shown in Fig.No.1

ICPOES (Inductively Coupled Plasma Optic Emission Spectrometry) [7]

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was used in the determination of heavy metal elements present in the test drug PNP and the procedure was done in SAIF, IIT Madras, Chennai-36 using Perkin Elmer Optima 5300 DV.

0.37g of test sample was weighed and transferred into a liner provided with instrument. Slowly 9ml of Nitric acid or Sulphuric acid is added such that no piece of sample sticks on the slide. Then mixed thoroughly and allowed to react for few minutes. Placed the liner in the vessel jacket and closed the screw cap hand- tight in clockwise direction. Sealed the vessel and placed in the rotor fixed in microwave. Temperature was set to 180°C for 5 minutes, held at 180°C for least 10 minutes.

Allowed the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor. The digested sample was made up to 100ml with Millipore water. If visible insoluble particles exist, solution could be filtered through Whatmann filter paper. Transferred the digested solution into plastic containers and label them properly.

Scanning Electron Microscopy (SEM) With Energy Dispersive X-Ray Analysis (EDX) [8]

To evaluate the size of the particle and surface topography of PNP, SEM analysis was carried out using VEGA TESCAN 3 instrument at Kamaraj University, Madurai. All samples must be of an appropriate size to suit within the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. For conventional imaging in the SEM, specimens should be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface.

In the present study, a representative portion of each sample was sprinkled onto a double side carbon tape and mounted on aluminum stubs in order to get a high quality secondary electron image for SEM examination. The sample is coated with gold in vacuum chamber by adopting suitable reaction conditions. The gold coated is further used to record SEM images. The SEM instrument is also connected with EDX tool for analyzing the presence of atomic elements in the sample.

SEM picture and EDX results of PNP was shown in Fig.No.2 and 3

EDX results of PNP was represented in Table.3

X-Ray Diffractometry (XRD) [9]

The x-ray diffraction technique is used as a basic characterization tool for different materials. The XRD pattern is the fingerprint of a crystalline material as this technique gives information on the phase, purity, and structure of a material.

PNP were characterized using a X-ray diffractometer (D8 Focus, Bruker) equipped with a photo scintillation detector at IIT Madras, SAIF. Angular range (2θ=10-60°), rate 0.01°/sec. 200mg of sample was taken. Sample was grinded to fine powder. Powder less than ~10µm (or 200-mesh) in size is most preferred. The powder was mounted on sample holder. Then by using a glass slide gently pressed the powder with the sample holder. Removed excess powder from the sample holder edges and carefully placed in the appropriate XRD slot and subjected for reading.

Thermo Graviometric Analysis (TGA) [10]

Thermo Gravimetric Analysis (TGA) is a thermal analysis used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs when a specimen is heated.

Thermo Gravimetric analysis was carried out using the model STA 449 F3 Jupiter at IIT madras, SAIF. Good thermal contact between the sample and heat- flux sensor is an important requirement for optimum results. 12.4mg of the sample is evenly distributed in the bottom of the sample crucible. When filling the crucible, no sample material may remain on the edge of the crucible. The sample crucible was placed on the front- hand sample support and subjected for reading.

RESULTS AND DISCUSSION

FTIR

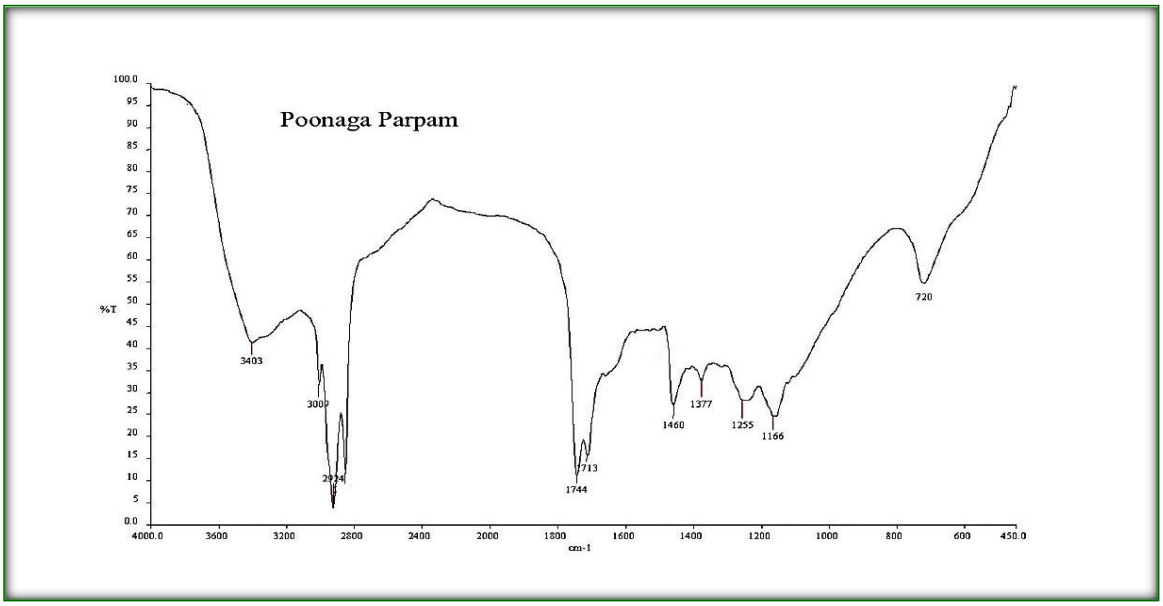

Fig.No.1 PNP has following functional groups

Table1: Interpretation of FTIR spectrum

Wave number (cm-1)	Vibration modes of PNP in IR region	Functional groups
3403	O-H Stretch	Alcohol
3009	C-H Stretch	Aromatic
2924	C-H Stretch	Alkane
1744	C=O Stretch	Carbonyl
1713	Acyclic Stretch	Ketone
1460	-C-H Bending	Alkane
1377	-C-H Bending	Alkane
1255	C-N Stretch	Amine
1166	C-F Stretch	Alkyl halide
720	C-Cl Stretch	Alkyl halide

- In the FT-IR Spectra analysis, this PNP sample exhibits the peak value shows in Table.1 at the wave number of 3403, 3009, 2924, 1744, 1713, 1460, 1377, 1255, 1166,720 having O-H stretch, C-H stretch, C=O stretch, acyclic stretch, -C-H bending, C-N stretch, C-F stretch, C-Cl stretch.
- This indicates the presence of some organic functional groups such as alcohol, aromatic, alkane, carbonyl, ketone, alkane, amine and alkyl halide.
- Stretching and bending modes of PNP shows the vibrational frequencies in the IR region. It confirms the dipole moment of the sample.
- From the observed FTIR Spectra the presence of some organic compounds are identified such as copper phosphate, potassium bicarbonate, copper selenite, ammonium thiosulfate, ammonium chloride, ammonium sulfate, ammonium chromate, sodium selenate and zinc chromate.

ICP-OES (Inductively Coupled Plasma Optic Emission Spectrometry)

Table 2: ICP-OES of PNP

S.No	Elements	Wavelength (nm)	*Poonaga Parpam* (0.37010g)
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	12.080 mg/L
4.	Cd	228.802	BDL
5.	Cu	27.393	21.561 mg/L
6.	Fe	238.204	01.306 mg/L
7.	Hg	253.652	BDL
8.	K	766.491	03.021 mg/L
9.	Mg	285.213	01.204 mg/L
10.	Na	589.592	01.300 mg/L
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	16.381 mg/L
14.	S	180.731	01.204 mg/L

Interpretation

ICP-OES reveals high concentration of Cu in PNP (21.561 mg/l). It also has physiologically important minerals like Ca, Fe, K, Mg, Na, P and S. From the above results, the heavy metals such as Arsenic, Cadmium, Lead, Mercury were observed as BDL and those are within the WHO permissible limits. Hence the safety of the drug PNP is ensured for clinical use.

SEM (Scanning Electron Microscope with Energy Dispersive X-Ray Analysis)

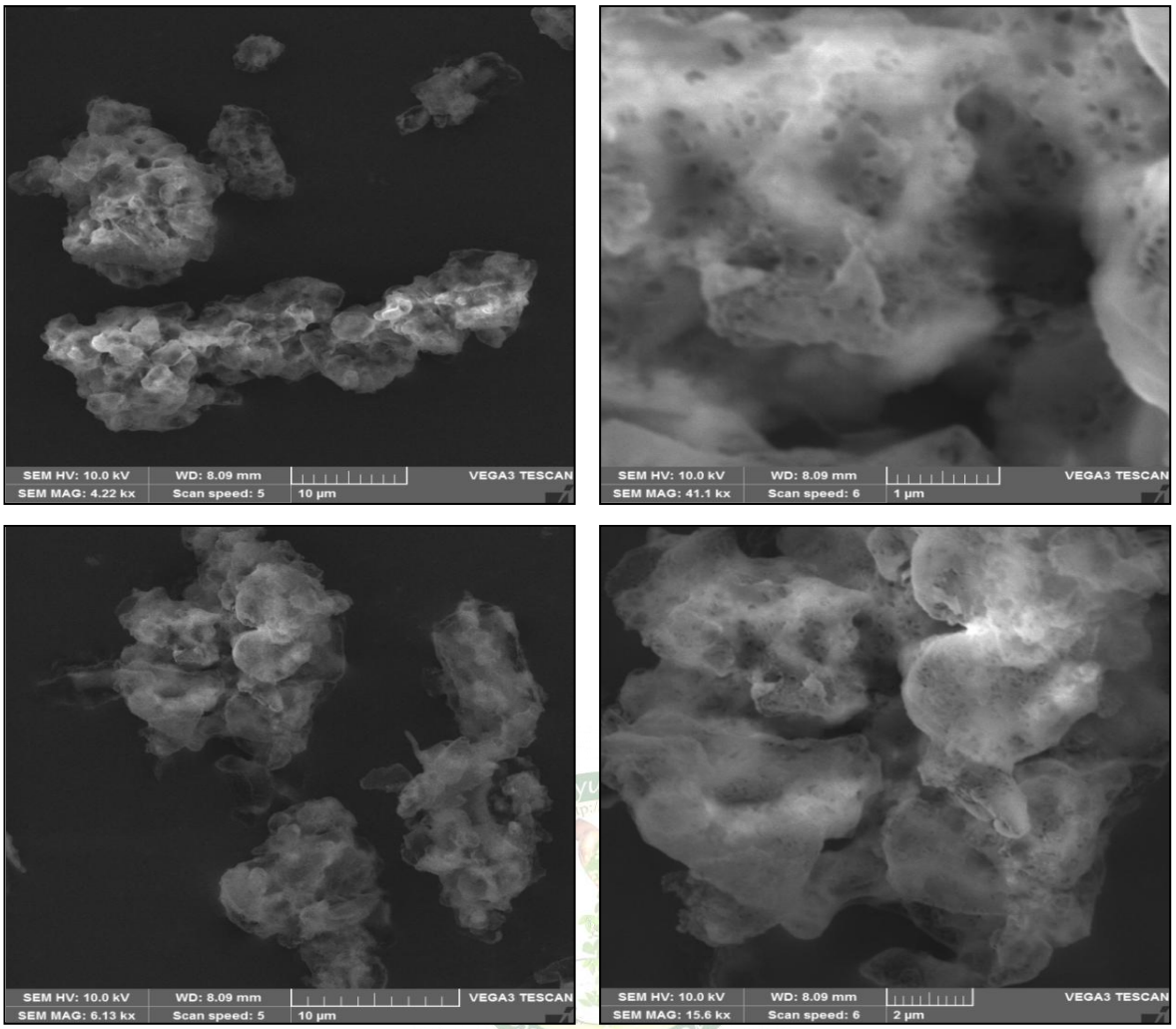

Fig.No.2. SEM image shows nano particles in PNP

EDAX

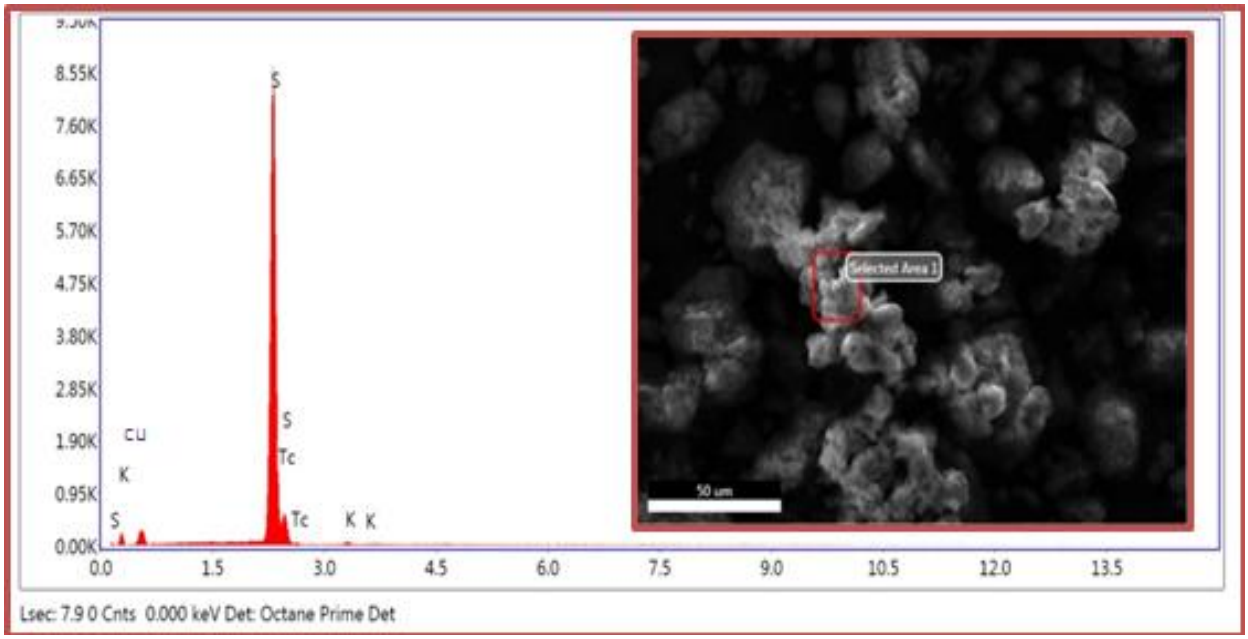

Fig.No.3 EDAX analysis of PNP

Table 3: Representing the weight and atomic percentage of elements present in PNP

Element	Weight %	Atomic %	Net Int.	Error %
Cu K	44.62	69.99	163.17	13.36
S K	48.65	28.59	9323.94	1.26
TcL	6.28	1.21	533.86	5.63
K K	0.45	0.22	48.41	21.64

Interpretation

The SEM imaging of the PNP shows that the particles are in nano size and have pores in it as shown in Fig 8. They are nano particles having a size of 100 to 120 nm. The particles aggregate and individual particles are seen on the top of the clusters. The particle size is low because of the grounding for more than 12 hours and the particle aggregation is due to the calcination process. The extremely tiny size of nanoparticles allows them to penetrate cells and interact with cellular molecules.

Nanoparticles have significant properties that can be used to enhance drug delivery. As the particle is in nano size, a low dose of the drug is enough to treat diseases. Hence PNP which is prepared biologically contains nanoparticles to enhance fast pharmacological action in target site. EDAX analysis shows the elements present in the sample as shown in Fig.no.3. The table represents the weight and atomic percentage of sample. The quantitative estimation of copper and Sulphur in the test drug PNP is 44.62 Wt % and 48.65 Wt% respectively which may be due to the presence of earthworms. The presence of Tc and K is very small which may be contributed by the presence of extract of *Aduthinna Paalai* in the sample.

(XRD) X-Ray Diffractometer

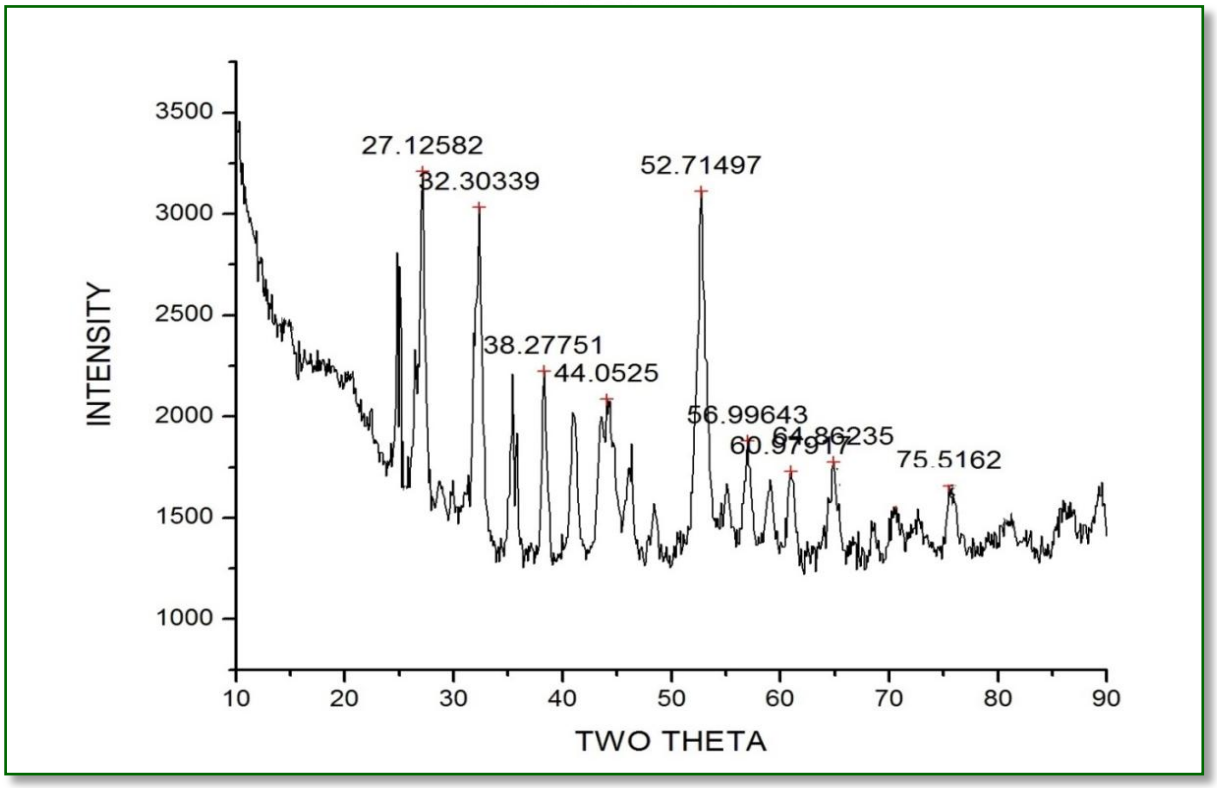

Fig.No.4 XRD image of PNP

- The X-Ray Diffraction pattern of the drug PNP reveals the presence of major peak with two theta value of 27.12 with the intensity of 3250.
- The particles of PNP show some crystalline in nature.
- Major peaks observed in test sample with two theta values of 27.12 and their corresponding intensities matching with the material copper.
- The X-Ray Diffraction pattern of the test drug PNP reveals the presence of major peak with two theta value of 32.30 with the intensity of 3100 corresponds to iron.

- Further from this observation it was concluded that copper and iron may the key ingredients present in the test drug PNP.

Thermo Gravimetric Analysis (TGA)

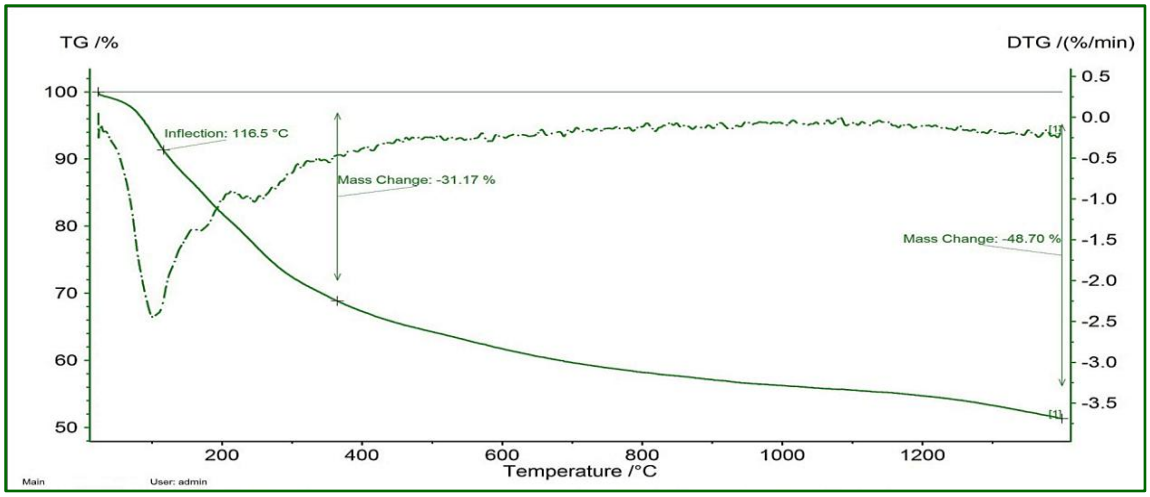

Fig.No.5 Thermal chemical characterization of *Poonaaga Parpam*

- Thermo Gravimetric analysis of PNP carried out at the maximum of 1300°C. The main objective of the study is to evaluate the decomposition and stability limit of the test drug PNP.
- The test drug PNP seems to be stable at the temperature varying from 100°C to 380°C.
- Point of decomposition begins when the temperature increases beyond 400°C.
- Weight of the final residual matter was observed with 48.70% of residual volume.
- From the result of the present investigation it was concluded that the test drugs PNP seems to be stable at varying temperature ranges from 50 to 400°C.

CONCLUSION

Based on the results, PNP is preferably non-toxic to human in its therapeutic dose. The standardization of the drug was evaluated by chemical characterization with heavy metal analysis, functional group analysis, elemental analysis, thermal analysis and determination of particle size by ICP-OES, FTIR, XRD, TGA and SEM EDAX respectively. The FTIR result indicates the presence of some organic functional groups such as alcohol, aromatic, alkane, carbonyl, ketone, alkane, amine and alkyl halide. ICP-OES reveals reveals the safety of the drug. The SEM picture shows that PNP is a kind of nano medicine which favours the advantages of bio availability, better absorption and non-toxic with minimal dose level. XRD results revealed that the copper and iron may be the key ingredients present in the test drug PNP. TGA results revealed that the test drug PNP seems to be stable at varying temperature ranges from 50 to 400°C. Point of decomposition begins when the temperature increases beyond 400°C.

ACKNOWLEDGEMENT

The authors sincerely thank the Head, SAIF, Indian Institute of Technology Madras, Chennai-600 036 and Kamaraj University, Madurai for providing the laboratory facility. The authors sincerely express their gratitude to the Director and Faculties of *Gunapadam* Department, National Institute of Siddha, Tambaram, Chennai – 600047 for their guidance and support.

REFERENCES

1. Rohit Kumar Bijauliya, Shashi Alok, Dilip Kumar Chanchal and Mayank Kumar. A Comprehensive Review on Standardization of Herbal Drugs. International Journal of Pharmaceutical Sciences and Research. 2017; 6(2), Vol. 8(9): 3663-3677.
2. Sarakku suthi muraigal. Published by Siddha Maruthuva nool Veliyitu Pirivu; Indian medicine and Homoeopathy department; First edition, 2008.
3. Dr.R.Thiagarajan, L.I.M. Gunapadam thathu jeeva vaguppu. Indian Medicine and homeopathy Department Chennai-106; p.725-727.
4. Kannusamy pillai. Sikicha Rathina Deepam, B.Rathna Nayakar & sons, Thirumagal achagam; First Edition, 2007. p.28-40, 232,233.
5. Michal H. et al. Application of infrared spectrophotometry to the identification of inorganic substances in dosage forms of antacid group. Acta pooloniae pharmaceutica- Drug Research, 2000, Vol.57 No. 2, 83-91.
6. Murali G. & Krishna, et al. A Critical Review On Fundamental And Pharmaceutical Analysis of Ft-Ir Spectroscopy. Int J Pharm 2013; 3(2): 396-402

7. Charles B. Boss and Kenneth J. Fredeen. Concepts, Instrumentation and Techniques in Inductively Coupled Plasma Optical Emission Spectrometry. U.S.A. Perkin Elmer. 2nd edition. 1997: 4-16.
8. Sathiyathilaga B et.al. Chemical Characterization of Siddha Herbo mineral formulation sangu chunnam by using modern techniques. Indo American Journal of Pharmaceutical research, 2014, 3662-3667.
9. Madhavan R et al. Standardization of Sangu Parpam a Herbo marine Siddha Drug. International Journal of current research in chemistry and pharmaceutical sciences. 2016; 3(6); 77-84.
10. Akila B et al. Analytical study on Siddha Herbomineral Formulation: Chandrakanthi Chooranam. International Journal of Pharma and Bio Sciences. 2014; 5(4): 175-185.

Cite this article as:
V. Elakkiyaa, S. Visweswaran, N. J. Muthu Kumar, Instrumental Analysis of Novel Siddha Formulation Poonaga Parpam. International Journal of Ayurveda and Pharma Research. 2019;7(7):47-54.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. V. Elakkiyaa
Resident Medical Officer,
National Institute of Siddha,
Tambaram Sanatorium, Chennai-47,
Tamil Nadu, India.
Email: elakiya92@gmail.com