Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(9); 1239-1248
doi: 10.25258/phyto.v9i09.10312
ISSN: 0975-4873

Research Article

Qualitative and Quantitative Analytical Studies on *Poora parpam*- A Siddha Medicine

Kabilan N[1*], Murugesan M [2], Balasubramanian T[1], Geethalakshmi S[1]

[1]*The Tamil Nadu Dr.M.G.R. Medical University, Chennai, Tamil Nadu, India.*
[2]*National Institute of Siddha, Chennai-600 047*

Received: 11th Aug, 17; Revised 21st Aug, 17, Accepted: 16th Sept, 7; Available Online:25th Sept, 17

ABSTRACT
Siddha system of medicine is a potent and unique indigenous system of medicine, which deals with the diseases of human being efficiently with the knowledge of both subtle and also the gross material body. This study was to prepare the *Poora Parpam* a *Siddha* medicine which contains herbo-mineral compounds responsible for the therapeutic activity and find out the compound by qualitative and quantitative analysis by different methods. Literature review reveals many herbo–mineral formulations available in market which is useful in anemia, diabetes, cancer, liver diseases, skin diseases etc. Therefore our aim of the study was to determine the compound by qualitative and quantitative analysis of ICP-MS, FTIR, MS, SEM and TEM. The study found some compound by above studies which were responsible for different activity.

Keywords: *Poora Parpam, Siddha*, ICP-MS, FT-IR, SEM, TEM.

INTRODUCTION

Siddha medicine means medicine that is perfect. *Siddhars* spend their lifetime in experimenting the gifts of Mother Nature the herbs, the minerals and the animals. As a result of their experiments, they could formulate so many valuable medicines which include small herbal preparations to the potent medicines. *Siddha* medicine is claimed to alleviate the root cause of the diseases by maintaining the ratio of *Vatham, Pitham* and *Kapham*[1].

Herbo-mineral formulation has the metals and minerals uses for chronic disorders in various combinations, dosage forms and at various levels of purities. Hence it is very essential to prepare it in a proper way. Literature review reveals many herbo–mineral formulations available in market which is useful in anemia, diabetes, cancer, liver diseases, skin diseases etc. Review of literature for present work was done by referring various national and international journals, published articles in various official standard books and referring to various websites on the internet[2].

The Present study was to find out the qualitative and quantitative analysis of herbo-mineral compound ***Poora parpam***.

MATERIALS AND METHODS

Procurement and authentication of raw drugs

The Natural and Synthetic *Pooram* were appropriately collected from country / Chemical drug merchant shop, Chennai and were authenticated by Department of Geology, V.O. Chidambaram College, Tuticorin, Tamil Nadu[6,7].

Purification of Pooram

Pooram (raw-purifed)	–	35 g
Vettrilai (*Piper betle*) leaves	–	8.75g
Milagu (*Piper nigrum*)	–	8.75g

Method of purification

Piper betle leaves and *Piper nigrum* seeds were ground together and made into a poultice. Then one liter of water was taken in a mud pot and the poultice was mixed in that water. *Pooram* (raw) was covered with a piece of clean dry cloth, so that it was not exposed outside. One end of the cloth was tied to a bamboo stick and placed horizontally over the opening of the mud pot. The raw drug *Pooram* in cloth was suspended in the above decoction. The vessel was constantly heated till decoction reduced by three fourth of its volume. Finally the *Pooram* was taken out from the cloth, washed with clean water and dried in sunlight[8].

Preparation of Poora parpam

Ingredients

Purified Natural *Pooram* / Synthetic *Pooram*(Calomel)

Method of Preparation

Take 50 g of the purified natural *Pooram* and put in the *kalvam*. Ground for seven days continuously. Then collect into the container. This was the study drug "Natural *Poora parpam"*.

The above method of preparation was also followed for synthetic *Pooram* (Calomel).

Route of Administration

Oral

Dosage

1/2 *ulundu edai* (32 mg) to 3 *ulundu edai* (195 mg)

Anubanam

Karumbu Vellam (Cane sugar)

**Author for Correspondence: kabilan.n@tnmgrmu.ac.in*

Duration of Treatment

Twice a day for 7 days after morning and night meal.

Qualitative Analysis

Test for Mercurial compound[3]

Identification test for mercurial compound in which sample added with 1 N sodium hydroxide and the characteristic color change was observed.

Identification test for mercurial compound in which sample added with hydrogen sulfide

Test for Chloride[4]

Identification test for chloride in which sample added with 1 N sodium hydroxide and appearance of white precipitate indicates the presence of chloride.

Sophisticated Instrumental Analysis

Inductively Coupled Plasma Mass Spectrometry (ICP–MS)

ICP–MS is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentration below one part in 1012 (parts per trillion). Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios. It is an automated, simple and unique quantitative and qualitative analysis. It measures elemental isotopes ratio[5,6].

Digestion of sample is carried out by transforming 0.5 g of the test drug Natural and Synthetic *Poora parpam* into a closed beaker and 5 ml of concentrated HNO_3 was added and digested to near dryness. 16 M HNO_3 was further added each time to the sample and digested until the clear solution was obtained. 5ml of 12 M HCl was added to ensure complete digestion. The digested solution was cooled to room temperature and made to the final volume of 100 ml with deionized water. Sample solutions were then filtered through membrane (0.45 µ) filter. Finally, the digested samples were used for metal analysis using ICP–MS (Perkin Elmer DRC-e Model). Each sample was digested in triplicate. A blank solution was also prepared in a similar manner and analyzed.

Fourier Transform – Infra Red Spectroscopy[7]

Fourier Transform – Infra Red Spectroscopy Study (FT–IR) The data acquired was with FT – IR spectrometer [FT-IR – 4100 –Jascoasia portal]. About 20 mg of the sample Natural and Synthetic *Poora parpam* was taken on a micro spatula and grounded well with required quantity of KBr salt. Sample admixed with KBr with trituration aided by mortar and pestle until to get a uniform fine powder of sample- KBr mixture. Further mixture was loaded in pellet die and subjected to 5000-10,000 psi in pelletizer. Resulting pellet was placed in FT-IR sample holder and exposed to IR radiation to get the spectra.

Mass spectrometry (MS)[8]

Mass spectrometry (MS) is a destructive analytical technique used for measuring the characteristics of individual molecules. The basic information obtained from Mass Spectrometric Analysis is the molecular mass of a compound, which is determined by measuring the mass to charge ratio (m/z) of its ion. ESI–LC–MS Mass spectrometer [FLEX–PC Bruker Daltonics – MALDI TOF / TOF] was used for our study. Sample injected to septum inlet probe by injector syringe that was introduced to electron spray ionization chamber. The stream of ions was transferred to the analyzer, where they were sorted and separated according to m/z. ion signals after reaching detector and separated based on the mass of the ions. Solvent used for sample preparation was 0.1% DMSO in which the x-axis was: mass-to-charge ratio (m/z) and y-axis Intensity / ion abundance in %.

Scanning Electron Microscope Analysis (SEM)[9]

The surface morphology of Natural and Synthetic *Poora parpam* was analyzed with a Zeiss Gemini Supra 55 – Oxford instrument X-act. The copper disc was pasted with carbon tape and the sample was dispersed over the tape. The disc was coated with gold in ionization chamber before microscopic analysis. The sample was mounted on specimen stub, placed inside the microscope's vacuum column evaporator and a beam of electrons passed from an electron gun, traveled through a series of magnetic lenses. The electrons are counted by the detector and the signals are sent to the amplifier. The number of electrons dispersed from each spot of the sample builds up the resultant image. The micro graphs obtained gave sufficient data about the topography of the sample. Energy used for SEM analysis is 0.5 – 15 kV with magnification range of 5000 to 6, 00,000X and Spatial resolution of 200 nm – 2000 nm.

Transmission Electron Microscopy (TEM)

TEM was utilized to monitor the surface morphology of the sample Natural and Synthetic *Poora parpam*. Samples of Natural and Synthetic *Poora parpam* put on Formvar – coated copper grids were examined using the Hitachi HD-2000 transmission electron microscope

TEM specimen stage designs include airlocks to allow for insertion of the specimen holder into the vacuum with minimal increase in pressure in other areas of the microscope. The specimen holders are adapted to hold a standard size of grid upon which the sample is placed or a standard size of self-supporting specimen. Standard TEM grid sizes are a 3.05 mm diameter ring, with a thickness and mesh size ranging from a few to 100 µm. The sample was placed onto the inner meshed area having diameter of approximately 2.5 mm. usual grid materials are copper, molybdenum, gold or platinum. This grid was placed into the sample holder, which was paired with the specimen stage. A wide variety of designs of stages and holders exist, depending upon the type of experiment being performed. In addition to 3.05 mm grids, 2.3 mm grids are sometimes, if rarely, used. These grids were particularly used in the mineral sciences where a large degree of tilt can be required and the specimen material may be extremely rare. Electron transparent specimens have a thickness around 100 – 500 nm[10,11].

RESULTS AND DISCUSION

Qualitative Analysis of Pooram and Poora parpam

The results obtained from Qualitative Analysis of *Pooram* (Before and after purification process) and *Poora parpam*[Final formulations of both source] showed the presence of Mercury and chloride in all forms. The results were tabulated in Table 01.

Table 1: Qualitative Analysis of *Pooram* and *Poora parpam* A. Test for mercurial compound.

Sample	Test	Observation	Inference
Natural *Pooram* Before Purification	Sample added with 1 N sodium hydroxide	A black colored solution	Shows the presence of Mercurous compound
Natural *Pooram* After Purification	Sample added with 1 N sodium hydroxide	A black colored solution	
Synthetic *Pooram* Before Purification	Sample added with 1 N sodium hydroxide	A black colored solution	
Synthetic *Pooram* After Purification	Sample added with 1 N sodium hydroxide	A black colored solution	
Natural *Poora parpam* [Final formulation]	Sample added with 1 N sodium hydroxide	A black colored solution	
Synthetic *Poora parpam* [Final formulation]	Sample added with 1 N sodium hydroxide	A black colored solution	

B. Test for Mercurial compound.

Sample	Test	Observation	Inference
Natural *Pooram* Before Purification	Sample added with hydrogen sulfide	Black precipitate	Shows the presence of Mercurous compound
Natural *Pooram* After Purification	Sample added with hydrogen sulfide	Black precipitate	
Synthetic *Pooram* Before Purification	Sample added with hydrogen sulfide	Black precipitate	
Synthetic *Pooram* After Purification	Sample added with hydrogen sulfide	Black precipitate	
Natural *Poora parpam* [Final formulation]	Sample added with hydrogen sulfide	Black precipitate	
Synthetic *Poora parpam* [Final formulation]	Sample added with hydrogen sulfide	Black precipitate	

C. Test for Chlorides.

Sample	Test	Observation	Inference
Natural *Pooram* Before Purification	Sample added one drop of diluted nitric acid and 0.5 ml of silver nitrate	Appearance of white, curdy precipitate	Shows the presence of Chlorides
Natural *Pooram* After Purification	Sample added one drop of diluted nitric acid and 0.5 ml of silver nitrate	Appearance of white, curdy precipitate	
Synthetic *Pooram* Before Purification	Sample added one drop of diluted nitric acid and 0.5 ml of silver nitrate	Appearance of white, curdy precipitate	
Synthetic *Pooram* After Purification	Sample added one drop of diluted nitric acid and 0.5 ml of silver nitrate	Appearance of white, curdy precipitate	
Natural *Poora parpam* [Final formulation]	Sample added one drop of diluted nitric acid and 0.5 ml of silver nitrate	Appearance of white, curdy precipitate	
Synthetic *Poora parpam* [Final formulation]	Sample added one drop of diluted nitric acid and 0.5 ml of silver nitrate	Appearance of white, curdy precipitate	

Elemental Analysis of Natural and Synthetic Poora parpam [Final formulation]

The results obtained from qualitative elemental analysis of Natural *Poora parpam* [Final formulation] showed the presence of Arsenic (As), Lead (Pb), Mercury (Hg),Cadmium (Cd), Iron (Fe), Calcium (Ca), Potassium (K), Phosphorus (P), Sulphur (S) and Sodium (Na). Elemental analysis of Synthetic *Poora parpam* [Final formulation] showed the presence of Arsenic (As), Lead (Pb), Mercury (Hg),Cadmium (Cd), Calcium (Ca), Potassium (K), Phosphorus (P), and Sulphur (S). The results were tabulated in Table 02.

FT–IR Analysis of Natural and Synthetic Poora parpam

The results obtained from FT–IR analysis of Natural *Poora parpam* showed the presence of O-H bending, alkenes, amides and alkyl halides. The FT–IR spectra were illustrated in Figure 01 and results were tabulated in Table 03.

FT-IR analysis of Synthetic *Poora parpam* showed the presence of O-H stretching, alkynes, amides and alkyl halides. The FT–IR spectra were illustrated in Figure 02 and results were tabulated in Table 04.

Mass spectral Analysis of Natural and Synthetic Poora parpam

The results obtained from mass spectral analysis of Natural *Poora parpam* reveals that m/z ion peaks on 26, 28, 35, 39, 70, 72 and 74 indicates the presence of chloride ions. m/z ion peaks on 195,198,199,200,201 and 205 indicates the presence of mercury ions. The spectra were illustrated in Figure 03.

The results obtained from mass spectral analysis of Synthetic *Poora parpam* reveals that m/z ion peaks on 30, 32, 34, 38, 39, 70, 72 and 74 indicates the presence of chloride ions. m/z ion peaks on 195, 199, 200, 201, 202 and 204 indicates the presence of mercury ions. The mass spectra were illustrated in Figure 04.

SEM Analysis of Natural and Synthetic Poora parpam

SEM analysis of Natural and Synthetic *Poora parpam* clearly projects that overall particle size distributions of the sample are in nano size range. Further several crystalline particles are aggregated as a cluster form.

Table 2: Elemental Analysis of Natural and Synthetic *Poora parpam* [Final formulation].

Element	Concentration (mg/L)	
	Natural *Poora parpam* [Final formulation]	Synthetic *Poora parpam* [Final formulation]
Arsenic (As)	1.6	1.43
Lead (Pb)	0.172	0.124
Mercury (Hg)	0.518	0.716
Cadmium (Cd)	0.551	0.311
Aluminum (Al)	BDL	BDL
Copper (Cu)	BDL	BDL
Magnesium (Mg)	BDL	BDL
Iron (Fe)	8.365	BDL
Zinc (Zn)	BDL	BDL
Calcium (Ca)	1.254	BDL
Potassium (K)	15.121	5.321
Phosphorus (P)	8.341	5.541
Sulphur (S)	1.124	BDL
Sodium (Na)	4.210	BDL

BDL- Below Detectable Level

Table 3: IR Spectral Analysis of Natural Pooraparpam Final formulation.

Wavelength(cm-1)	Vibrations/Functional Group
3422.98	O-H bond stretching
3391.16	O-H bond stretching
3334.84	O-H bond stretching
3240.58	Amine
3026.15	Alkenes
2928.92	Acid derivatives
2501.78	Acid derivatives
2208.33	Alkynes
2198.39	Alkynes
1894.34	Amides
1848.16	Amides
1791.01	C=O stretching
1721.84	C=O stretching
1622.38	Aldehydes C-H
1508.27	Amides
1303.43	O-H bond bending
1183.69	C-N
904.25	C-H bending
846.77	C-H bending
760.18	Cl stretching- Alkyl halides
540.12	Br- Stretching -Alkyl halides

From the image of SEM analysis it was clear that cluster image of both the formulation reveals spongy like structure with well-defined boundaries. SEM analysis of Natural *Poora parpam* reveals the presence of particle with the size range of 59.08 nm to 137.7 nm. SEM analysis of Synthetic *Poora parpam* reveals the presence of particles with the size range of 40.71 nm to 176.1 nm. The results were illustrated in Figure 05 and 06.

TEM Analysis of Natural and Synthetic Poora parpam

Results of TEM clearly indicated that both the formulation contains nano particles which may be responsible for the clinical efficacy of the drug. Clustered image of both formulations showed the presence of agglomeration of particle. TEM analysis Natural *Poora parpam* revealed that most of the particles are in nano size range between 56.08 nm to 98.79 nm. The results were illustrated in Figure 07.

TEM analysis Synthetic *Poora parpam* revealed that most of the particles were in nano size range between 41.19nm to 150.7 nm. The results were illustrated in Figure 08.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) ICP-MS is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentration below one part in 1012 (parts per trillion). Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios. It is an automated, simple and unique quantitative and qualitative analysis. It measures elemental isotopes ratio[12].

The results obtained from qualitative elemental analysis of Natural *Poora parpam* showed the presence of Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Iron (Fe), Calcium (Ca), Potassium (K), Phosphorus (P), Sulphur (S) and Sodium (Na). Elemental analysis of Synthetic *Poora parpam* final formulation showed the presence of Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Calcium (Ca), Potassium (K), Phosphorus (P), and Sulphur (S).

Infrared (IR) spectroscopy is one of the most important and widely used analytical techniques available to scientists working on *Siddha* formulations. It is based on the vibrations of the atoms of a molecule. The infrared spectrum is commonly obtained by passing infrared electromagnetic radiation through a sample that possesses a permanent or induced dipole moment and determining what fraction of the incident radiation is absorbed at a particular energy[13]. The energy of each peak in an absorption spectrum corresponds to the frequency of the vibration of a molecule part, thus allowing qualitative identification of certain bond types in the sample.

The FT-IR results of Natural and Synthetic *Poora parpam* showed the presence of O-H Stretching and bend,

Table 4: IR Spectral Analysis of Synthetic *Poora parpam* [Final formulation].

Wavelength(cm-1)	Vibrations/Functional Group
3491.17	O-H bond stretching
3412.66	O-H bond stretching
2826.08	C-H Aldehydes
2691.25	C-H Aldehydes
2494.43	Acid derivatives
2380.77	Acid derivatives
2231.21	Alkynes
2010.59	C=C Alkenes
1703.12	C=O stretching
1624.78	Aldehydes C-H
1494.23	Amides
1362.11	O-H bond bending
1094.13	C-N
1008.81	C-N
796.03	Alkyl halide

C-H Stretching and bend, C=O Stretching as functional groups. The shift of C=O stretching frequency indicates a bounding of the nano particles through this group. Prominent stretching for alkyl halide indicates the presence of chloride that justifies the identity of calomel.

Mass spectrometry (MS) is a powerful characterization technique used for the identification of a wide variety of chemical compounds. At its simplest, MS is merely a tool for determining the molecular weight of the chemical species in a sample. However, with the high resolution obtainable from modern machines, it is possible to distinguish isomers, isotopes, and even compounds with nominally identical molecular weights. Libraries of mass

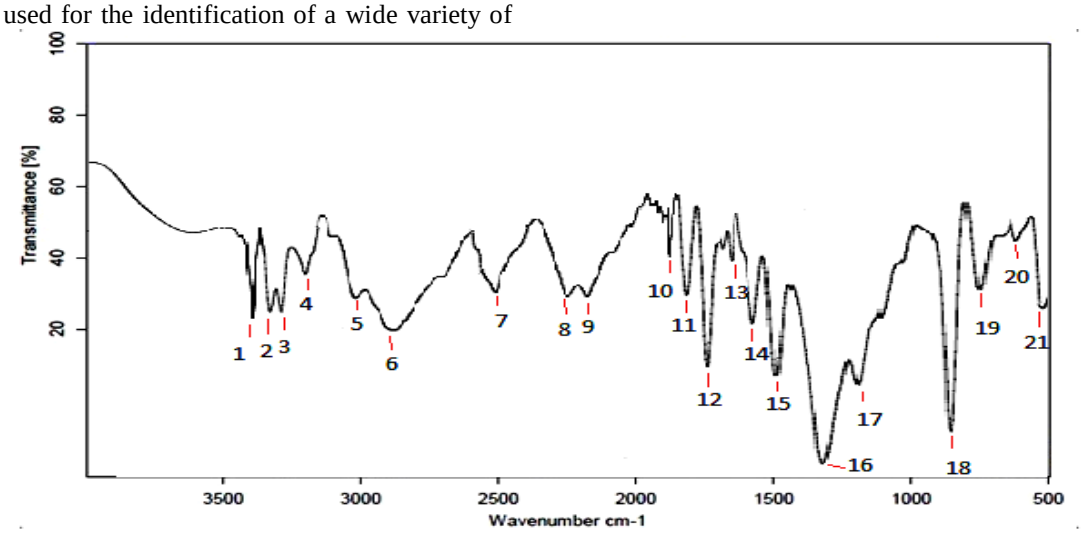


Figure 1: IR Spectrum of Natural *Poora parpam* [Final formulation].

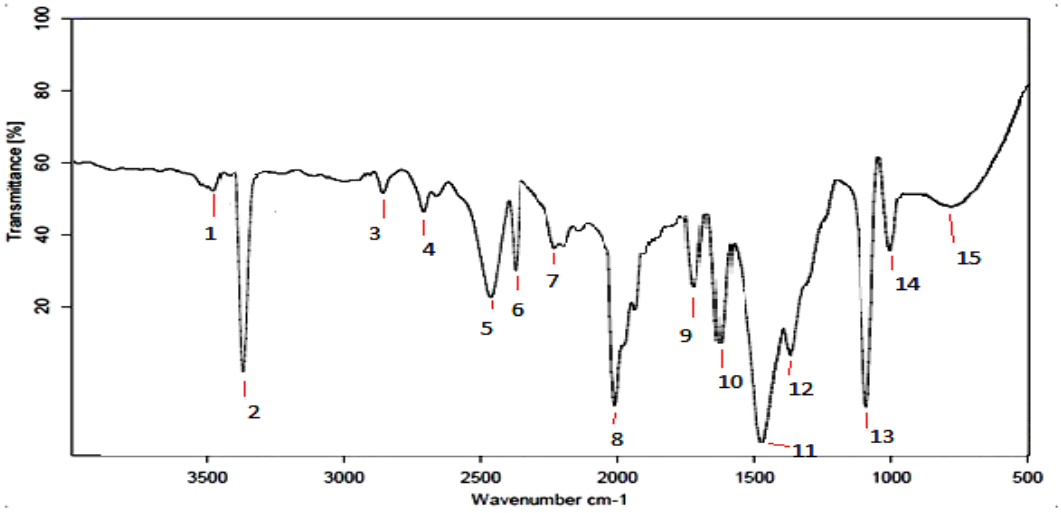


Figure 2: IR Spectrum of Synthetic *Poora parpam* [Final formulation].

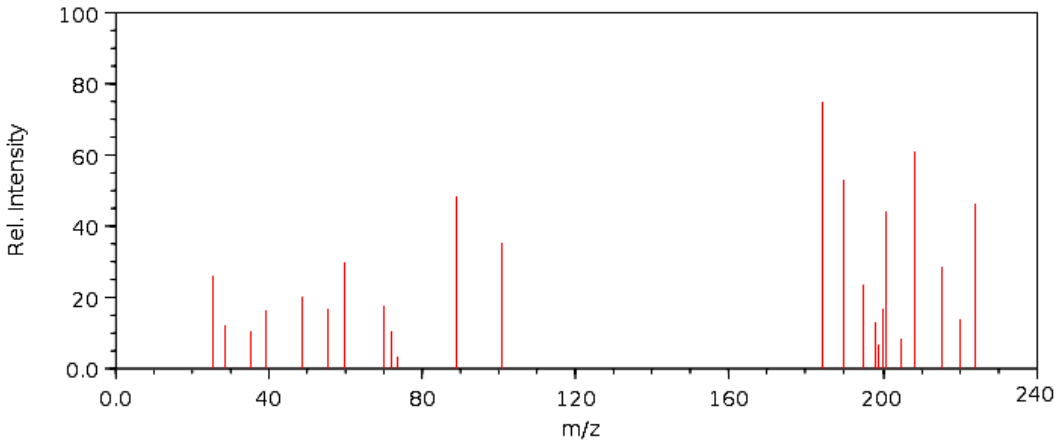


Figure 3: Mass Spectrum of Natural *Poora parpam* [Final formulation]

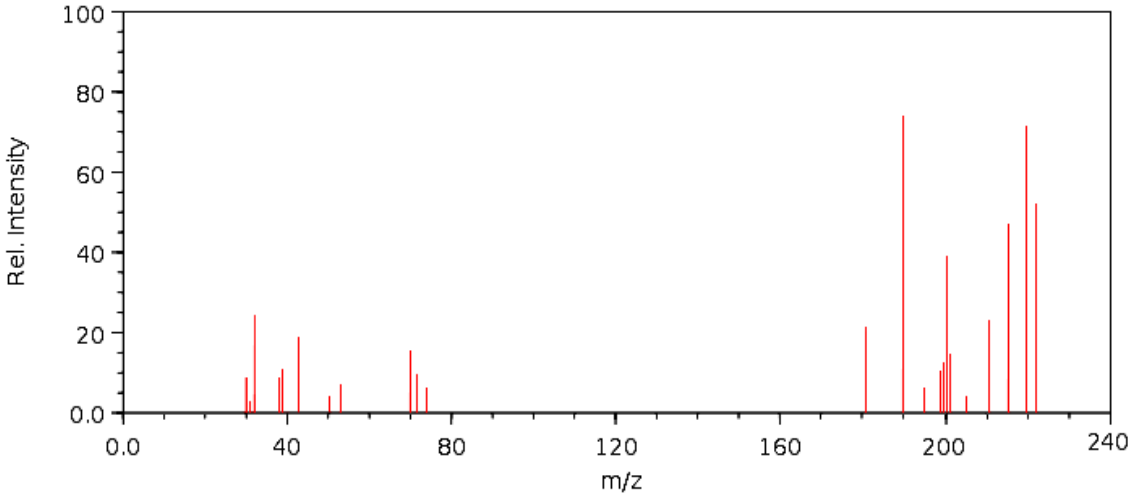
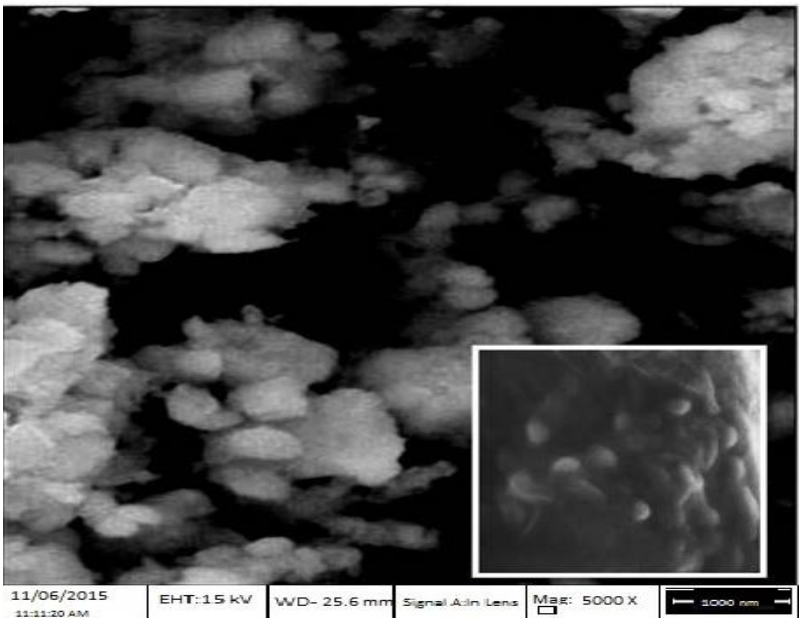
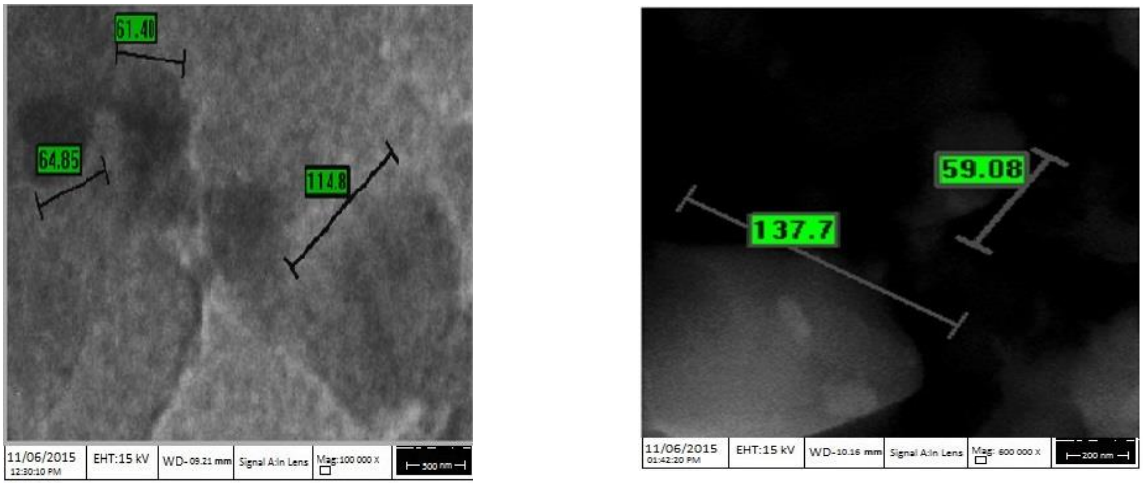


Figure 4: Mass Spectrum of Synthetic *Poora parpam* [Final formulation].

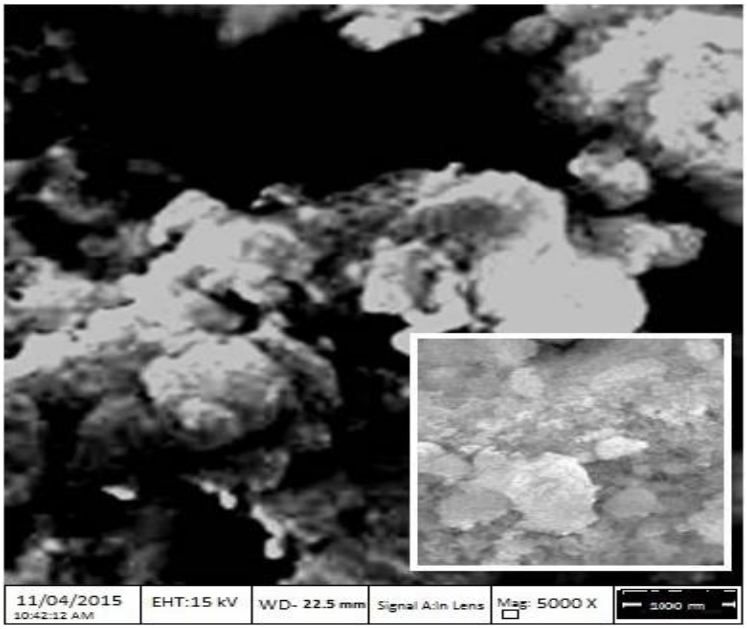


SEM Image of Natural *Poora parpam* [Final formulation] – Clustered View

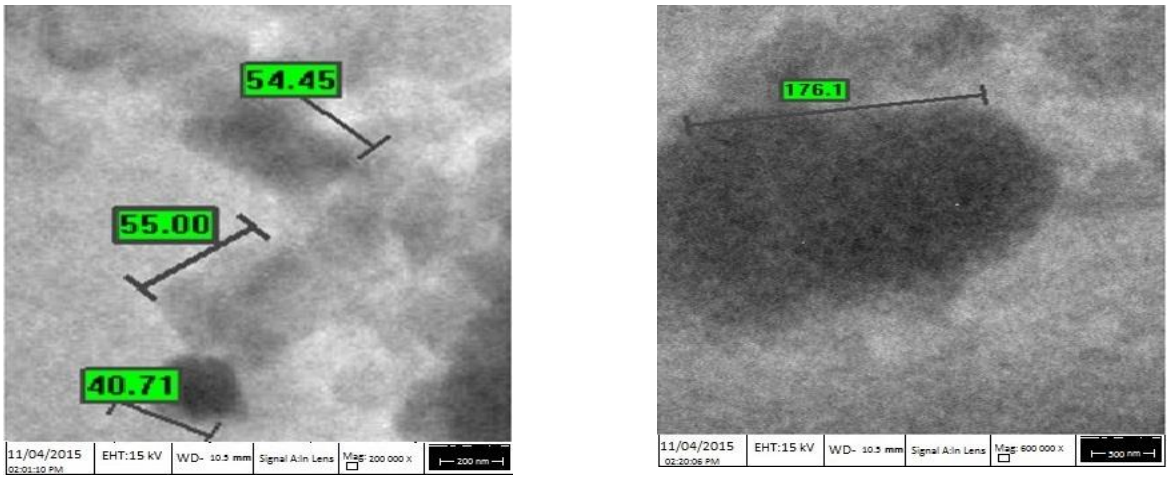


SEM Image of Natural *Poora parpam* [Final formulation] - Isolated view

Figure 5: SEM Image Analysis of Natural *Poora parpam* [Final Formulation].



SEM Image of Synthetic *Poora parpam* [Final formulation] – Clustered View



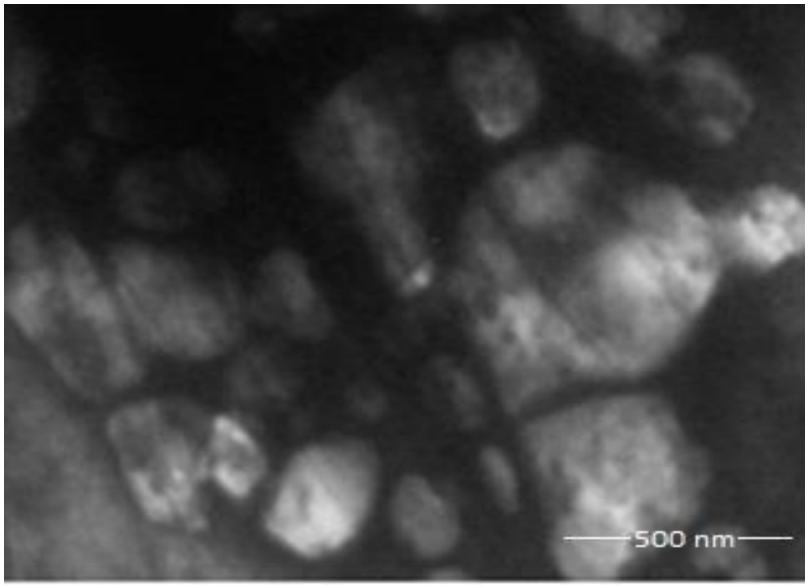
SEM Image of Synthetic *Poora parpam* [Final formulation] -Isolated view

Figure 6: SEM Image Analysis of Synthetic *Poora parpam* [Final formulation].

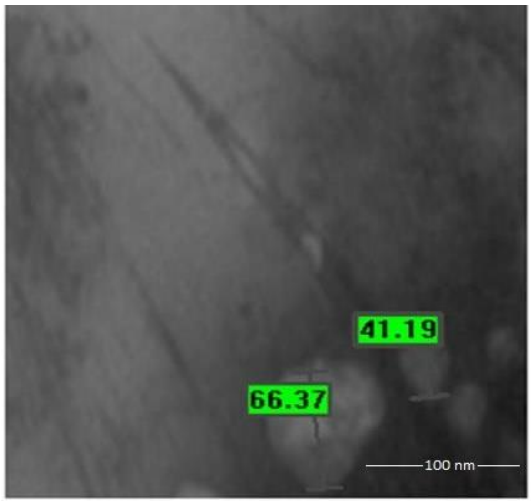
spectra have been compiled which allow rapid identification of most known compounds, including herbo mineral formulations[14,15]. The results obtained from mass spectral analysis of Natural and Synthetic *Poora parpam* reveals the presence of prominent m/z ion peaks on from 26-74 indicates the presence of chloride ions. m/z ion peaks ranges from 195- 205 indicates the presence of mercury ions.

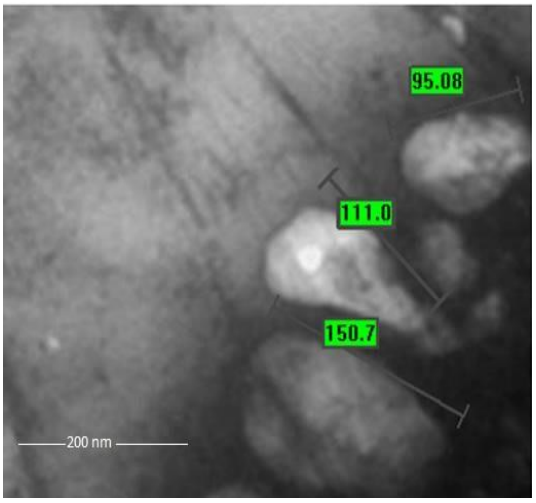
SEM is an extremely useful method for the visual confirmation of the morphology and physical state of the surface[16]. SEM has been used, for example, for determining the surface morphology of products derived from natural source and also formulates as per the prescribed standards[17]. SEM analysis of Natural and Synthetic *Poora parpam* showed the presence of nano and micro sized particles. The particle size varies between 40.71 nm to 176.1 nm. The extremely small size of nano particles allow them to penetrate the cells and interact with cellular molecules. As the particle is in nano size, a low dose of the drug is enough to treat diseases. Hence the drug Natural and Synthetic *Poora parpam*, which is prepared according to vedic literature contains nano particles enhancing fast pharmacological action at target sites[18] .

Transmission electron microscopy (TEM) has been found to be an excellent tool for characterizing the size of nanoparticles[19]. TEM can be used to directly image nanoparticles at scales approaching a single atom. However, the advantage gained by being able to see these nanoparticles comes with several tradeoffs that must be addressed and balanced. Results of TEM clearly indicated that both the formulation contains nano particle ranges from 41.19nm to 150.7 nm which may be responsible for the clinical efficacy of the drug. Clustered image of both formulations shows the presence of agglomeration of particle.



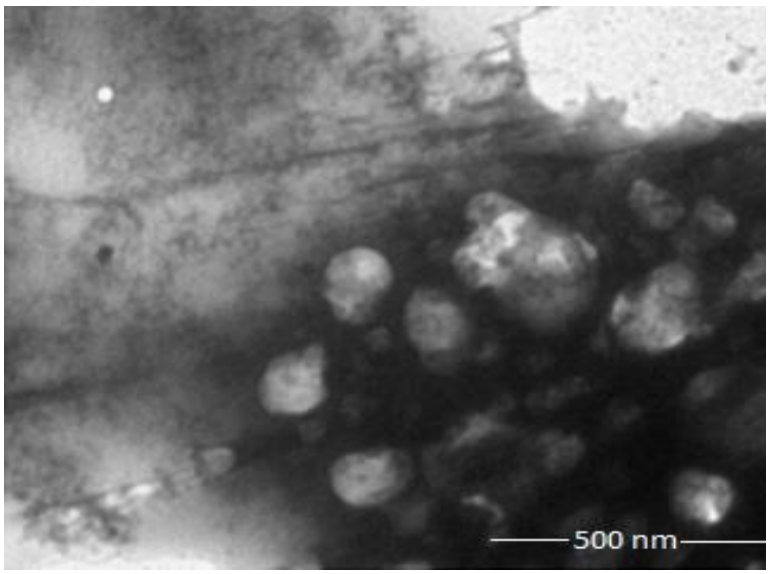
TEM Image of Natural *Poora parpam* [Final formulation] – Clustered View



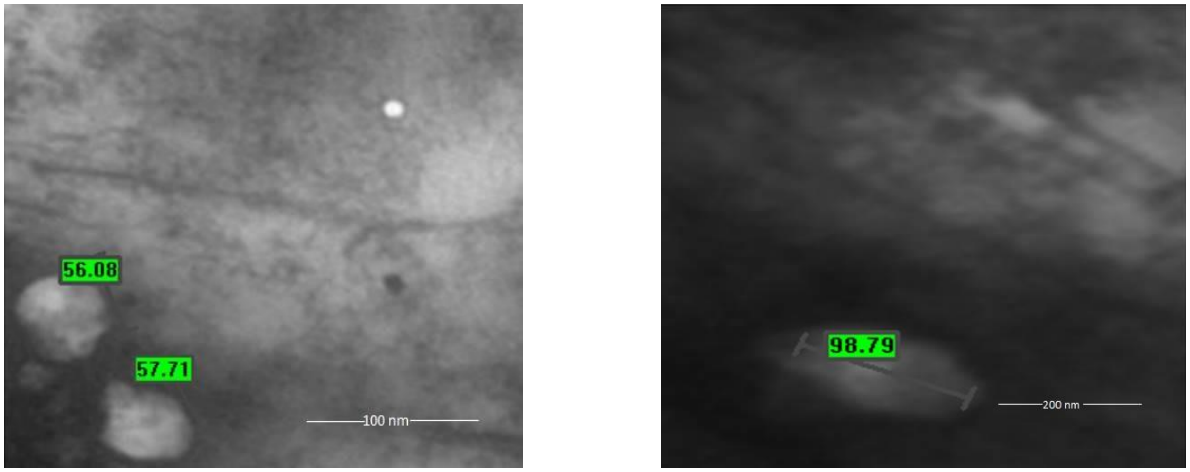


TEM Image of Natural *Poora parpam* [Final formulation]- Sorted view

Figure 7: TEM Image Analysis of Natural *Poora parpam* [Final formulation].



TEM Image of Synthetic *Poora parpam* [Final formulation] – Clustered View



TEM Image of Synthetic *Poora parpam* [Final formulation] - Sorted view
Figure 8: TEM Image Analysis of Synthetic *Poora parpam* [Final formulation]

CONCLUSION

Characterization of both Natural and Synthetic *Poora parpam* carried out with modern sophisticated instrumental methods. The results obtained from qualitative elemental analysis of Natural and Synthetic *Poora parpam* showed the presence of heavy metals within the prescribed range. Results of FT-IR and Mass spectral analysis showed the characteristic peaks for the presence of Mercury and Chloride and other relative functional groups which was responsible for the biological activity of the drug.

SEM and TEM analysis of both Natural and Synthetic *Poora parpam* revealed the evidence based result for the presence of nano particles within the formulation. Nano particles have multiple mechanisms in biological environment such as cellular penetration, alteration in physiology, regulation of enzyme function, restoring cell environment and functional property etc. Further studies were planned for preclinical studies.

ACKNOWLEDGEMENT

Authors are thankful to Vice chancellor and Registrar of The TN Dr.M.G.R. Medical University, Chennai for permitting to do this study. We acknowledge gratitude to Sathyabama University and Dr. Purushoth Prabhu.T, C.L. Baid Metha College of Pharmacy, Chennai for providing Laboratory facilities to carry out this study.

CONFLICT OF INTEREST

Nil

REFERENCES

1. Rajalakshmi P "Analytical studies on Muthucippi parpam" J pharm research; 2010;3(10):2366-2370.
2. Grimes IC, Einarsson S, Spier BJ"Mercury ingestion retrieved by colonoscopy" Gastrointest Endosc 2009; 70:559–60.
3. Partington JRA., "Text-Book of Inorganic Chemistry" MacMillan and Co Limited: London: 1950; 858-859.
4. Smille TJ, Khan IA., "A Comprehensive approach to Identifying and authenticating Botanical Products" Clinical Pharma therapeutics;2010; 87(2): 175-186.
5. Limmatvapirat CJ, Manjunath and Sannappa., "Simultaneous analysis of eleven heavy metals in extracts of Sonneratiacaseolaris (L.) Engl. By ICP-MS" Res J Pharm Biol Chem Sci; 2012 ;2(3): 744-750.
6. Chamberain J., "The determination of refractive index spectra by fourier spectrometry" Infrared Physics; 1969; 9 (4): 189–209.
7. Stobiecki M "Application of mass spectrometry for identification and structural studies of flavonoid glycosides" *Phytochemistry;*2000;54: 237-256.
8. Goldstein J "SEM and X-Ray microanalysis" 3rd ed. New York: Springer Science:2003; 690.
9. Santosh S Kulkarini., "Bhasma and Nanomedicine" Int Res J Pharm;2013; 4(4):10-16.
10. Annop Austin., "Chemical Characterisation of gold and mercury based siddha sasthric preparation-Poornachandrodayam" American J drug discovery and development;2012;01:1-14
11. Mukherhee PK., "Integrated approach towards drug development from Ayurveda and other system of medicines" J Ethanopharmacology;2006 ; 103: 25-35.
12. Mehta A ., "The dynamics of sand" Reports on Progress in Physics; 1994; 57 (4): 383.
13. Henderson W "Mass Spectrometry of Inorganic, Coordination, and Organometallic Compounds" John Wiley & Sons Ltd., Chichester; 2005.
14. Barshick CM "Inorganic Mass Spectrometry Fundamentals and Applications" New York; 2000.
15. Varma AJ.,"Metal complexation by chitosan and its derivatives: a review". Carbohydr Polym; 2004; 55: 77–93.
16. Yen MT., "Physicochemical characterization of chitin and chitosan from crab shells" Carbohydr Polym; 2009; 75, 15–21.
17. Sharon Sagnella.,"Drug Delivery: A Nanomedicine Approach" Australian Biochemist; 2012; 43 (3): 5-20.

18. Jung KY., "Measurement of 100-nm polystyrene sphere by transmission electron microscope" Powder Technology;2002;126:112-120.
19. William D., "Particle Size Determination Using TEM: A Discussion of Image Acquisition and Analysis for the Novice Microscopist" Langmuir; 2008; 24 (20):11350–11360.