

# Quality Assessment of a Traditional Siddha Drug “Mupoora Chendurum”

Mahalakshmi Gopal<sup>1</sup>, Sathish Raja Thinakaran\*<sup>2</sup> and Poorna Pushkala Venkat subramanian<sup>3</sup>

<sup>1</sup>Siddha Physician, Sri Sairam Siddha Medical College & Research Centre, Chennai, India

<sup>2</sup>Dept.of Toxicology, Sri Sairam Siddha Medical College & Research Centre, Chennai, India

<sup>3</sup>Dept.of Microbiology, Sri Sairam Siddha Medical College & Research Centre, Chennai, India

## Address for Correspondence

Lecturer, Dept. of Toxicology, Sri Sairam Siddha Medical College & Research Centre, Chennai, India.

E-mail: [Sathizkannan@gmail.com](mailto:Sathizkannan@gmail.com)

## ABSTRACT

Mupoora chendurum was prepared as per the method mentioned in the Siddha literature. In the present study Raw and purified drugs of Lingam (Cinnabar–Red sulphide of Mercury), Pooram (Mercury subchloride), Rasa chendurum (Mercury sulphide) and the final product mupoora chendurum were characterized by using the modern techniques FTIR, ICPOES, SEM and Anti microbial study. FTIR analysis showed the broad peaks at 3584, 3524 and 1612 cm<sup>-1</sup>. Peak at ~2319 cm<sup>-1</sup> and ~2395 cm<sup>-1</sup> was assigned to C-H and C-H (methoxy compounds) stretching vibration correspondingly. Absence of Organic contaminations indicates the proper incineration process. Trace element analysis by ICP-OES indicated, absence of heavy metals Lead, Copper, Cadmium and Arsenic. Mercury concentration was under acceptable limit (0.97 ppm) at prescribed dose. Microbial load of the preparation has the highest antibacterial activity against *Bacillus*, *Streptococcus* and *Vibrio* species, medium activity against *Salmonella typhi* and *Staphylococcus* and considerably low activity against *E. coli*. SEM analysis indicates agglomeration of the particles and aggregated particle size of 83.3 nm.

**Keywords:** Mupoora chendurum, Siddha, FTIR, ICPOES, SEM, Antimicrobial activity.

## INTRODUCTION

Holistic Siddha System of Medicine is an ancient Indian System of Medicine. It consist of medicaments prepared by materials obtained from nature viz: plant products, animal products and metals/minerals. Converting the metals & minerals into acceptable form (ie. Parpam and

Chendurum preparations) for its internal administration is done by various pharmaceutical processing methods which is mentioned in various Siddha literatures. At the end of processing this micro fine medicinal product has easy digestive power and quick reaction with the bile juices<sup>1</sup>. It is very clear and evident from long

history of usage of herbomineral and metallic preparations in Ayurveda and Siddha medical system that properly processed herbomineral preparation can contribute significantly to the health care of the society<sup>2</sup>. Of these “Mupoora chendurum” is a vital preparation which is indicated for Suram (All types of fever), Gunmam (Peptic Ulcer), Mega noigal, Soolai Noi, Soothagavayu. Mercury is the major ingredient of Mupoora chendurum. Siddha system has immense faith in the miracles of Mercurial drugs and in the prolongation of life through rejuvenating treatments and intense yogic practice<sup>3</sup>. In the present day scenario there is a need of more sophisticated testing methods and pharmacological, Toxicological and Microbiological activities for determining quality of the Chendurum. Standardized products provide more security and increase the level of trust that people have in herbal drugs<sup>4</sup>. It is, therefore, essential that definite and accurate analytical protocols be available to ascertain consistency and quality of herbal formulations, and more so these should be adhered to so that these products exhibit the desired medicinal effects<sup>5</sup>. WHO specific guidelines for the assessment of the safety, efficacy and quality of herbal medicines as a prerequisite for global harmonization are of utmost importance<sup>6</sup>. In the present research work, the drug “Mupoora chendurum” was prepared according to the method mentioned in Siddha literatures and taken up for sophisticated tests and Antimicrobial study.

## MATERIALS & METHODS

### Preparation of the test drug

The Siddha drug “Mupoora Chendurum” was prepared as per the standard procedure mentioned in Siddha formulary literature Anupoga Vaithiya Navaneetham<sup>7</sup>. All the ingredients were bought from authorized Siddha raw material

shop in Chennai, India. Lingam (Cinnabar–Red sulphide of Mercury), Pooram (Mercury subchloride), Rasa chendurum (Mercury sulphide) were taken in the quantity of 35gms each. All the four inorganic raw materials were further processed individually with selective liquids for purification process (Suddhi muraigal). The above four purified inorganic materials were added one by one in Kalvam (Stone mortar) and ground with *Ficus Religiosa* leaf extract for 12 hours. Then required amount of *Ficus Religiosa* leaf was ground with water and made into semisolid paste and covered the material which was obtained from 12 hours of grinding. Then it was taken into Pudam process (calcinations process). Then the drug was taken and made into powdered form.

### Estimation of Inorganic elements and infrared spectra in raw, purified drugs & Mupoora chendurum

Inorganic elements of all raw and purified materials and Mupoora chendurum were quantified by inductively coupled plasma emission spectroscopy (Optima 5300 DV ICP-OES) equipped with a Sea spray concentric nebulizer (Glass expansion, Pocasset, MA) and cyclonic spray chamber. Following procedure were followed: nebulizer flow, 0.8/min<sup>-1</sup>: radiofrequency power, 1450 W: sample introduction, 1.5 ml min<sup>-1</sup>: flush time, 20 s: delay time, 10 s: read time, 10 s: wash time, 30 s: and replicates, three. Standards were prepared by dilution of 1000 mg l<sup>-1</sup> stock solutions and the calibration curve was obtained using five to ten points including the blank<sup>8</sup>.

Raw, purified drugs and Mupoora chendurum were powdered and the infrared spectral characterization was obtained by using Perkin-Elmer FTIR Spectrophotometer in the region (4000-450 cm<sup>-1</sup>) by KBr Pellet method<sup>8</sup>.

### Evaluation of Powder properties of Mupoora Chendurum

JEOL ASM 3500 SEM was used for the analysis. A representative portion of each sample was sprinkled onto a double side carbon tape and mounted on aluminium stubs in order to get a higher quality secondary electron image for SEM examination<sup>9</sup>.

### Antimicrobial assay<sup>10</sup>

#### Preparation of the solvent

1gm of the sample was mixed with ethanol. It was kept in a rotary shaker for few minutes till the sample gets dissolved completely.

#### Preparation of the Culture

The antimicrobial activity of the sample was tested against both gram positive & gram negative organisms. The standard bacterial cultures were obtained from IMTECH, Chandigarh, India. Gram positive organisms used were *Staphylococcus aureus*, *Streptococcus feacalis* and *Bacillus cereus* and Gram negative organisms used were *E. coli*, *Salmonella typhi* and *Vibrio cholerae*. 100ml of Nutrient broth was prepared and suspended in six separate tubes equally and the bacterial cultures were inoculated in each tube separately. The tubes were incubated at 37 °C for 24 hrs for bacterial growth and turbidity.

#### Antimicrobial Activity Testing

150ml of Mueller Hinton Agar was prepared and sterilized. 20ml of the agar was suspended in seven sterile Petri Plates each and the seventh plate was kept as Control. Once the agar was solidified, the broth cultures were inoculated on the plates as a lawn culture and the plates were named according to the culture inoculated. Once the

culture is inoculated, wells were punched in all the seven plates.

The ethanol dissolved sample was taken. 10 microlitre of the sample was added in the wells punched on the first six plates. In the control plate, 10 microlitre of ethanol was added instead of the sample. The plates were then incubated at an incubation temperature of 37 °C for 24 hrs.

## RESULTS AND DISCUSSION

### Changes of inorganic elements by ICP-OES Analysis

Test reveals that the Heavy metals Arsenic, Lead, Cadmium and Copper were below detectable limit. And the Major ingredient Mercury was reduced from its concentration by every purification processes and Mercury concentration of Mupoora chendurum was within the permissible limit (0.97ppm)<sup>11</sup>. Calcium was below detectable limit and Phosphorus level was increased in the final product Mupoora chendurum. Iron, Magnesium and Sulphur were tested in the final product only. Results indicate absence of Iron and magnesium and the sulphur level was 50.5 ppm. Thus the results indicate the drug is safety from mercurial toxicity.

### Changes of Infrared spectra in Raw and Purified ingredients and Mupoora chendurum

Result indicates that the presence of a peak at ~2319 cm<sup>-1</sup> and ~2395 cm<sup>-1</sup> was assigned to C-H and C-H (Methoxy compounds) stretching vibration correspondingly, additional proven with the absorption band of hydroxyl (3500–3480 cm<sup>-1</sup>), ester carbonyl (1270–1150 cm<sup>-1</sup>) and phenyl (1600, 1420 cm<sup>-1</sup>). There are no significant difference in the characteristic absorption bands but the intensity of certain wavelength do differ from each other

especially at the fingerprint region (3584–1035 cm<sup>-1</sup>).

The three different bands showed the broad peaks at 3584, 3524 and 1612 cm<sup>-1</sup> in all the samples which are due to the characteristic OH stretching (g-OH) H OH bending (d-OH) vibrational bands due to adsorbed water in the sample. FT-IR spectra of the drug samples demonstrate that they do not hold organic compounds (Fig. 1-7). The absence of organic matter is further proof of proper incineration during the preparation of these medicines and the un-appearance of any external organic contamination.

#### Powder property of Mupoora chendurum by HR SEM analysis

Agglomeration of the particles can be viewed, due to repeated calcination process in the preparation<sup>12</sup>. Some aggregated particle size of the drug was 83.3nm. Thus the repeated calcination process can be used to produce the nanoparticles of Mupoora chendurum to improve its quality and reactivity<sup>13</sup>. Surface of the particles were smooth and cumulatively distributed (Fig.8). Powder property indicates the possible flow ability and easy absorption of the drug Mupoora chendurum.

#### Anti microbial activity of Mupoora chendurum

From table 2, it is evident that with 50% concentration of the drug prepared, the zone of inhibition by the Well Diffusion method was upto 0.4 cm for *E. coli* (Fig.9), 1cm for *Staphylococcus aureus* (Fig.10), 2.5 cm for *Bacillus cereus* (Fig.11), 1cm for *Salmonella typhi* (Fig.12), 1.7 cm for *Vibrio cholerae* and 2.5 cm for *Streptococcus faecalis*. The figures also shows that the drug Mupoora chendurum has the highest antibacterial activity against *Bacillus*, *Streptococcus* and *Vibrio* species, medium activity against *Salmonella typhi* and

*Staphylococcus* and considerably low activity against *E. coli*.

#### CONCLUSION

In the present study Mupoora chendurum was prepared as per the Siddha textual references. Sophisticated tests reveal the absence of heavy metals like Arsenic, Lead, Cadmium and Copper. Mercury was within the permissible limit. The Particle size of the drug was 83.3 nm. In spectra by visual gratitude, there are no significant difference in the characteristic absorption bands but the intensity of certain wavelength do differ from each other especially at the fingerprint region (3584–1035 cm<sup>-1</sup>). Overall spectra result indicates that the organic material was not found in Mupoora chendurum. This report is a fingerprint for future references in analysis of Mupoora chendurum. And the drug has antibacterial activity against *Bacillus*, *Streptococcus* and *Vibrio*.

Further detailed studies are required to standardize the drug. Pharmacological and toxicological studies are required to evaluate the safety and importance of the drug. Though it is a difficult task to decide the standards for metal and mineral drugs, a step has been made here to lay down standards for quality of the drug Mupoora chendurum.

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**Table 1.** Analysis of inorganic elements by ICP-OES in raw materials before and after purification process and in final product Mupoora chendurum

S. No.	Name of the drug	Inorganic elements in Parts per Million (PPM)									
		Arsenic	Lead	Cadmium	Mercury	Copper	Phosphorus	Sulphur	Ca <sup>2+</sup>	Fe	Mg <sup>+</sup>
1.	Lingam (Raw)	BDL	BDL	BDL	282.4	BDL	25.4	-	BDL	-	-
2.	Linga (Purified)	BDL	BDL	BDL	164.0	BDL	14.2	-	BDL	-	-
3.	Pooram (Raw)	BDL	BDL	BDL	250.7	BDL	20.5	-	BDL	-	-
4.	Pooram (Purified)	BDL	BDL	BDL	148.5	BDL	11.8	-	BDL	-	-
5.	Rasa Chendurum (Raw)	BDL	BDL	BDL	255.7	BDL	18.4	-	BDL	-	-
6.	Rasa Chendurum (Purified)	BDL	BDL	BDL	172.9	BDL	13.2	-	BDL	-	-
7.	Mupoora Chendurum	BDL	BDL	BDL	0.97	BDL	25.4	50.5	BDL	BDL	BDL

BDL- Below detectable limit

**Table 2.** Measurements of the zone of inhibition of Mupoora chendurum against the growth of organisms around the well

S. No.	Name of the organism	Zone of Inhibition (in cm)
1.	Staphylococcus aureus	1
2.	Bacillus cereus	2.5
3.	Salmonella typhi	1
4.	E. coli	0.4
5.	Vibrio cholera	1.7
6.	Streptococcus feacalis	2.5

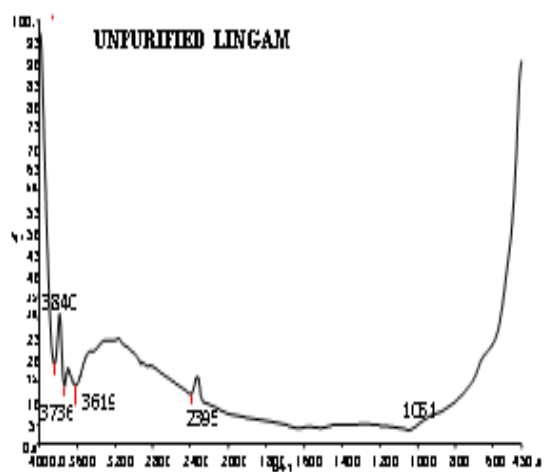


Figure 1. IR Spectra of Lingam before Purification

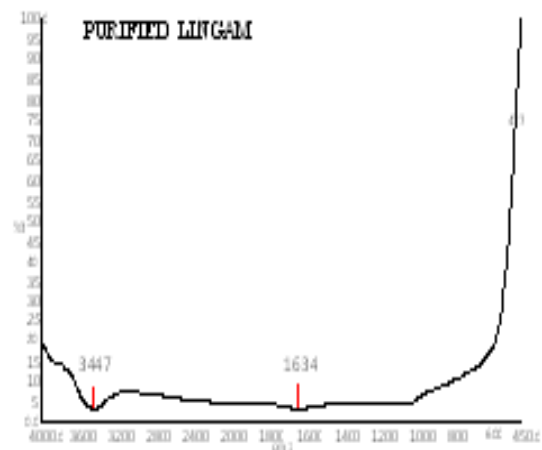


Figure 2. IR Spectra of Lingam after Purification

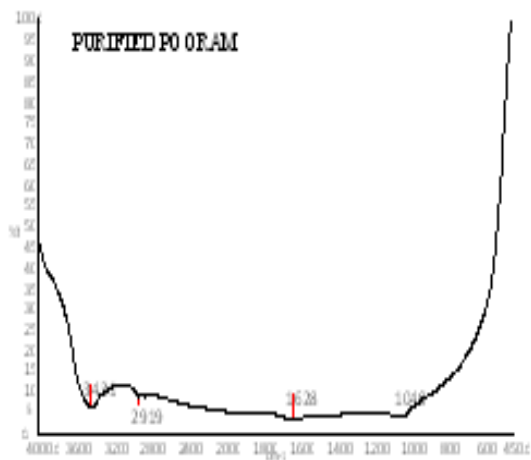


Figure 3. IR Spectra of Pooram after Purification

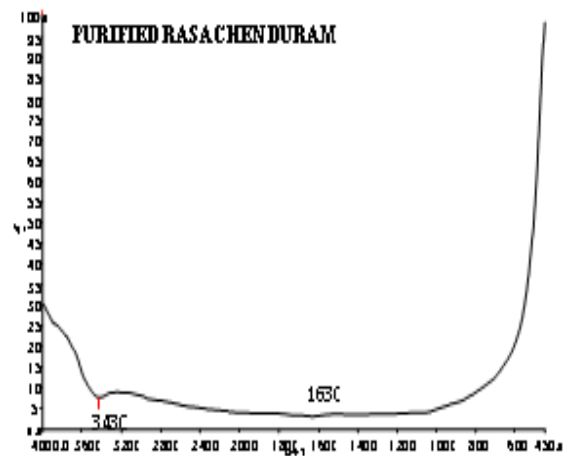
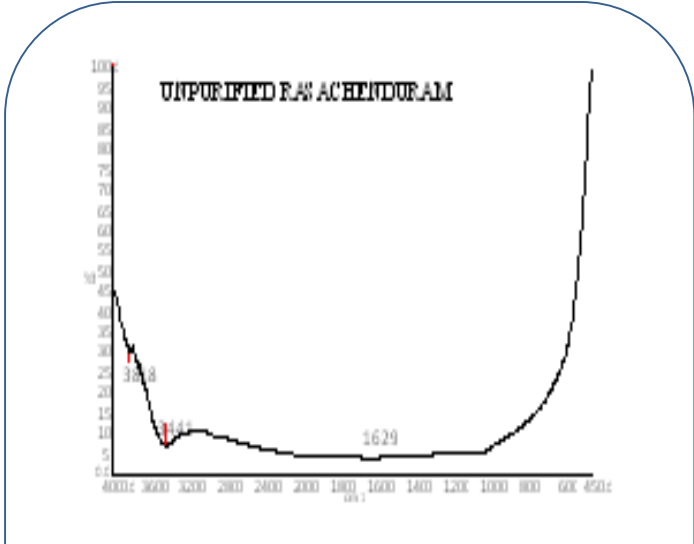
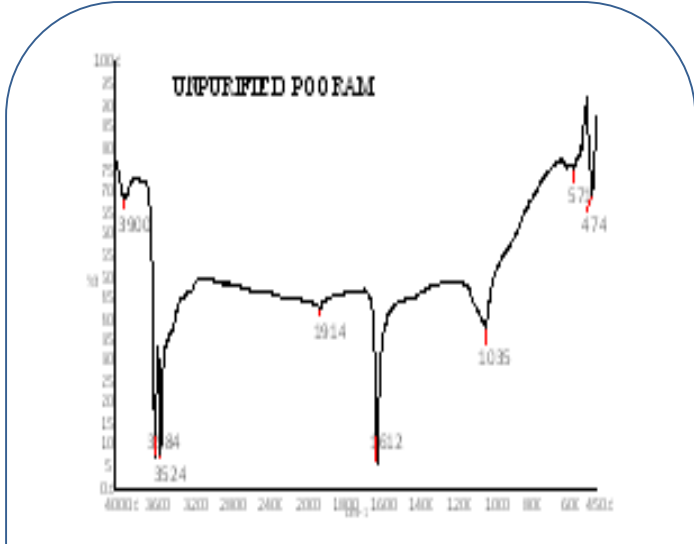


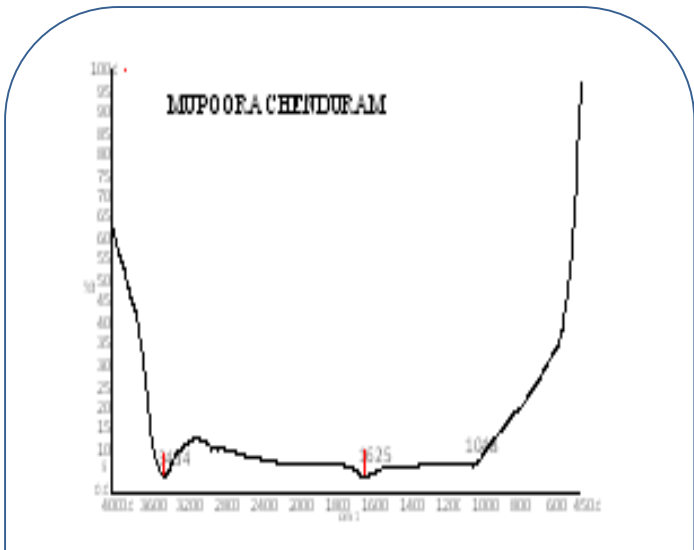
Figure 4. IR Spectra of Rasa chenduram after Purification



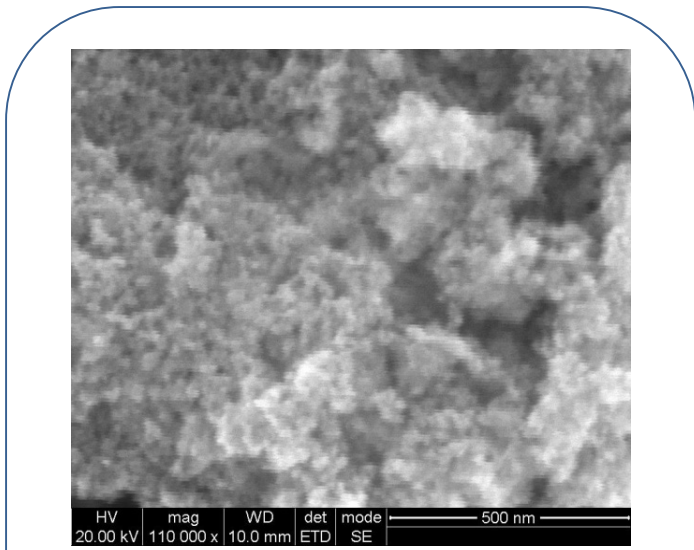
**Figure 5.** IR Spectra of Rasa chendurum before Purification



**Figure 6.** IR Spectra of Pooram before Purification



**Figure 7.** IR Spectra of Mupoora chendurum



**Figure 8.** HR SEM Image of Mupoora Chendurum





**Figure 9.** Zone of inhibition against *E. coli*



**Figure 10.** Zone of inhibition against *Staphylococcus aureus*



**Figure 11.** Zone of inhibition against *Bacillus spp*



**Figure 12.** Zone of inhibition against *Salmonella spp*