Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (4), 2022; 853-858

Standardization and quality control parameters evaluation on the Siddha mineral preparation: *Linga chenduram*

Research Article

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Abstract

Standardization of herbal or herbo-mineral formulations is an important factor to assess the quality, purity, safety and efficacy of drugs. WHO has emphasized the need to ensure quality control of Indian Medicines including Siddha formulations by using modern techniques and by applying suitable parameters for Standardization of *Linga Chenduram* where *Lingam* (Cinnabar) is the primary constituent was selected for evaluation of Standardization and quality control parameters. Analysis of the drug showed that *LC* was dark brown in colour, odourless, tasteless, fine powder in appearance and fine to touch which indicates the completion of calcination process. In physicochemical analysis values of the drug such as loss on drying, total ash, water-soluble ash and acid insoluble ash were found to be 1.19%. 3.73%, 0.19% and 1.49% respectively. The total ash value indicated the absence of impure substances in LC. The XRD Analysis of *Linga Chenduram* showed that it was a highly crystalline drug. SEM analysis of *Linga Chenduram* showed that most of the particles present in the sample were Nano size and the results showed that the values of Mercury (51.21) and Carbon (43.63) were present in larger amount compared to Oxygen and Magnesium. The ICP-OES result showed that the toxic heavy metals such as Lead, Cadmium, Cupper, Potassium, Magnesium, Iron, Zinc, Nickel and Sodium were below the detection limit (BDL). Thus it is evident that the safety of siddha medicine had been proved in the modern scientific way. The end of the study concluded that the end product Linga Chenduram yields standard and safety.

Key Words: *Linga Chenduram*, XRD, ICP-OES, SEM, EDAX*, Lingam,* Cinnabar.

Introduction

The Siddha system of Medicine is an ancient medical system, providing preventive, promotive, curative, rejuvenative and rehabilitative health care by adopting scientific and holistic approach.(1) Raw drugs were used as a medicine directly or after they had undergone some processes or modifications. It may be of plant or animal or metal and mineral origin.(2-5) Ancient physicians prepared medicines by themselves for treating their patients. These physicians were well experienced in identifying the raw drugs and in the processes of medicine preparation. They were at liberty to modify the composition of any formula depending on the need and availability of raw materials. In course of time, variation in composition became an established practice, though the names of the formulations remained the same.(6)

Standardization of herbal formulations is an important factor to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles. It is very important to establish a system of standardization for every end product of the medicine since the scope for variation in different batches of medicine is enormous.(7)

WHO has emphasized the need to ensure quality control of Siddha formulations by using modern techniques and applying suitable parameters. It was the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication with purity, safety, potency, and efficacy. As prescribed by the WHO, evaluations of physicochemical and phytochemical properties were essential to standardize the various Siddha formulations.(6)(8)(9)

Linga Chenduram one of the herbo-mineral preparation was mentioned in *Anuboga Vaiithiya Navaneetham*, Volume 4.(10). The aim of this study is to standardize *Linga Chenduram* as per WHO Guidelines.

Aim

To evaluate the standardization parameters of *Linga Chenduram* as per siddha literature preparation.

Objective

- Preparation of *Linga Chenduram* as per siddha literature
- Evaluation of Organoleptic and Physicochemical parameters

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- Evaluation of characters using modern instrumental analysis

Methodology

Linga Chenduram had been selected from classical Siddha literature *Anuboga Vaithiya Navanitham,* By author Hakkim Mohammad Abdullah Sahib, volume No 4, Page No. 51.

Collection of the drug

The raw drug *Lingam* was purchased from authorized drug store in Parrys Corner, Chennai. The herbal drugs *Thirugukalli* Latex was collected from Aandimadam, Ariyalur district. *Erukkam* flowers and *Utthamani* flowers were collected in and around Tambaram, Kanchipuram district.

Ingredients

Purified *Lingam* (Cinnabar)	17.5g (5 varaganedai)
Thirugukalli Latex (*Euphorbia Tortilis* Rottler ex Ainslie)	Sufficient
Utthamani Flowers (*Pergularia Daemia* (forsk.) Chiov.	70g (2 *Palam*)
Vellaierukkam Flowers (*Calotropis Procera* (Aiton) W. T. Aiton)	70g (2 *Palam*)

Identification and Authentication of drugs

The raw materials were identified and authenticated by the experts of Gunapadam Department, National Institute of Siddha, Chennai 47.

Purification of *Lingam* (Cinnabar)

Placed the desired quantity of the *Lingam* sample on the stone motor, added sufficient quantity of lemon juice little by little and ground well for about 6 hours. Then collected the sample, made them as a single round disc and covered in a thin muslin cloth. It was then suspended in a mud pot 4 inches from the base without touching the bottom, added desired amount of lemon juice then ignited the pot in low flame and heated well until the lemon juice got dried off. Finally the sample was ground and stored in an airtight glass jar (Figure No: 1)

Preparation process

Purified *Lingam* was measured and made into powder form with mortar and pestle. *Euphorbia tortilis (Thirugukalli)* latex poured into it and ground well by stone motor and pestle for 12 hours (*4 saamam*). The mixture of *Lingam* was then made into small disc (*villai*) and spread in a suitable pot for drying in sun light. Flowers of *Pergularia daemia (Utthamani)* and *Calotropis procera* (*Vellarukkam*) were ground together and made into paste (*karkam*). Dried disc of *Lingam* was covered with prepared *karkam* then placed into pot with lid and sealed with clay smeared cloth (*seelai mann*). Weight of clay pot with lid containing mixture was measured. Then it was subjected into incineration process (P*udam*) by cow dung cake (4 times the weight of the measured clay pot weight). After the incineration process clay pot was allowed to cool itself. Processed medicine was taken from the clay pot and ground into fine powder. Finally, it was stored in an airtight glass container and labelled as **LC** (Sample - LC).

Standardization of drug

This was done as per the protocol for testing of Ayurveda, Siddha and Unani medicines by WHO guideline.

Organoleptic Evaluation (11)(12)

Colour: The *Linga Chenduram* was taken into watch glass and placed against white back ground in tube light. It was observed for its colour by naked eye.

Odour: The *Linga Chenduram* was smelled at two intervals. The time interval between them was about 2 minutes to nullify the effect of previous smelling.

Physicochemical Analysis (11)(12)

Determination of moisture content (Loss on drying)

Accurately weigh 4 g of the drug. Place it in a pre-weighed beaker and dried at 105º C constantly for 5 hours. Now weigh the drug. Continue drying and weighing every one hour. This process has to be continued until the two corresponding weights don't exceed 0.25 percent. Constant weight is said to be obtained when drying and cooling for 30 minutes in a desiccator didn't show a difference more than 0.01 g.

$$\text{Percentage of loss on drying at 105ºC} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample taken}} \times 100$$

Determination of total ash

Incinerate about 2 - 3 g accurately weighed of the ground drug in a pre - weighed silica dish. Calculate the percentage of ash with reference to the air dried drug.

$$\text{Percentage of total ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample taken}} \times 100$$

Determination of Water soluble ash

Boil the ash obtained from above test for few minutes with 25 ml of distilled water; repeat the process for one more time. Filter the insoluble matter on an ash less filter paper and ignite in a silica crucible to constant weight. Calculate the percentage of water soluble ash with reference to the air dried drug.

$$\text{Percentage of water soluble ash} = \frac{\text{Weight of the total ash weight of water insoluble residue}}{\text{Weight of the sample}} \times 100$$

Determination of acid insoluble ash

Boil the ash obtained from the above test for 5 min with 25 ml of dilute hydrochloric acid. Filter the insoluble matter on an ash less filter paper, wash with hot water and ignite in a silica crucible to constant weight. Calculate the percentage of acid insoluble ash.

$$\text{Percentage of Acid - insoluble ash} = \frac{\text{Weight of the acid insoluble residue}}{\text{Weight of the sample}} \times 100$$

Determination of Ph

10 gram of sample was weighed and mixed with 90 ml of distilled water. The mixture was stirred for three hours of time using rotary shaker. Then the pH was measured using pH meter.

As Per Siddha Classical Literature (11)(12)

Floating on Water

A pinch of *Linga chenduram* was sprinkled over the water in a glass container.

Finger Print Test

A pinch of *Linga chenduram* was taken and rubbed in between the thumb and index finger.

Lustre

The *Linga chenduram* was taken in a Petri dish and observed for any lustrous particle in day light via magnifying glass.

Taste

A small amount of *Linga chenduram* was kept in the tip of the tongue.

Modern instrumental analysis

X ray diffraction (XRD) analysis

The powder x-ray diffraction patterns of the solid samples were recorded on x ray diffractometer (Rigaku MiniFlex-II Desktop X-ray Diffractometer). The range of diffraction angle used to analyses the sample was 20-80°. The wavelength of the radiation used was 1.5405 A°. (13)

SEM and EDX analysis

The SEM images of the drug were taken by TESCAN CLARA, Ultra-High Resolution Scanning Electron Microscope (SEM) The Samples LC was placed separately on a carbon tape inside an airtight chamber. High-energy electron beam was focused through a probe towards the samples. Variety of signals were produced, on interaction with the surface of the samples. The element present in the drug was analyzed by Energy Dispersive X-ray analysis (EDX) attached to the above instrument. (14)(15)

Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis

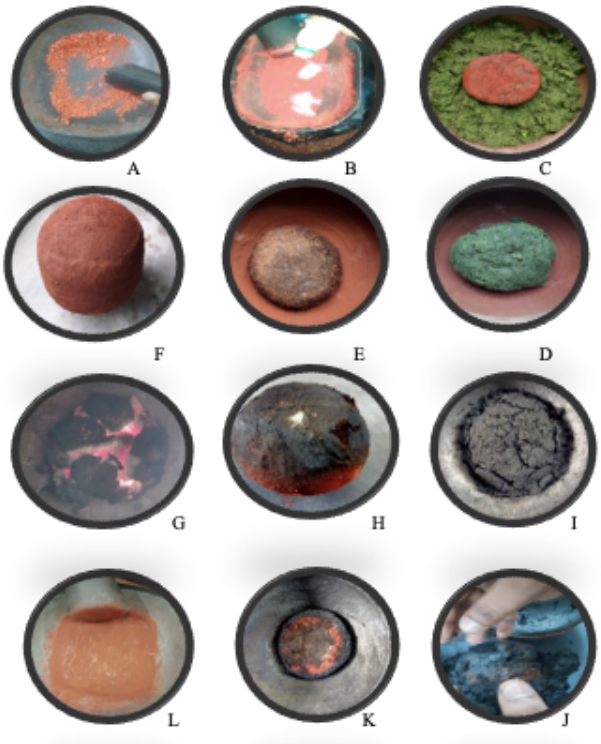
Assessment of heavy metallic constitution was made by Inductively coupled plasma optical emission spectrometer (Perkin-Elmer 5300 DV ICP-OES). (16)

Results

Preparation of the drug

Figure No 1: Process of *Lingam* purification A:Raw *Lingam*, B: Powdering the *Lingam*, C: Grinding with lemon juice, D: Making *Villai*, E: Pouring of lemon juice, F: Making *Kizhi*, G: Immersed and boil with lemon juice and H: Purified *Lingam*

Figure No 2: Preparation process of *Linga Chenduram* A: Powdering the purified *Lingam*, B: Grinding with *Thirugukali* Latex, C: Making *Villai*, D: Making *Kavasam* with *Uthagamani* and *Vellarukku* flowers, E: Dried *Villai*, F : Sealed with mud pots, G : Incineration, H: After Incineration of the mud pots, I: After Separations of the Mud pots, J: Separations of *Kavasam*, K : After Separations of the *Kavasam* and L: Grinding the *Villai (LC)*



Organoleptic Evaluation

The following characters have been noted in *Linga Chenduram*.

Table 1: Organoleptic Evaluation

S. No	Parameters	Interpretation
1	Colour	Dark in Brown
2	Odour	Odorless
3	Taste	Taste less
4	Texture	Fine powder
5	pH Value (5% aq. solution)	7.38

The Organoleptic characters of the LC was Dark Brown in colour, tasteless, fine powder appearance and nice to touch which is indicated the complete preparation through calcination process.

The pH of the trial drug was 7.38 it shows the alkalinity of the drug. According to pharmacokinetics, alkaline drugs are absorbed in alkaline environment.

Physicochemical Analysis

Table 2: Chemical standardization of *Linga Chenduram*

S. No	Parameters	Interpretation
1	Loss on drying	1.16%
2	Total Ash Value	3.87%
3	Acid insoluble Ash	1.49%
4	Water Soluble Ash	0.19%

According to chemical standardization parameters, the loss of drying at 105^{o}C of *LC* was found to be 1.16%. Loss of drying indicates the moisture content. It indicates the long shelf life. The ash values of *LC* such as total ash, water-soluble ash and acid insoluble ash were found to be 3.87 %, 0.19% and 1.49% respectively. The total ash of LC is 3.87 % which indicates the absence of impure substances and the drug also meets the specifications as per WHO guidelines. Acid insoluble ash indicated the absence of silicate and water-soluble ash under the limit.

As Per Siddha Classical Literature:

Siddhars used these following standardization methods to ensure the safety and efficacy of the *chenduram*. It shows the effectiveness of the drug.

Table 3: Results of Siddha Standardization

S.No	Parameter	Results Of *Linga Chenduram*	Interpretation
1	Floating on Water	Floats on water	Lightness of drug.
2	Finger Print Test	Impinged in the furrow of Fingers	Indicates fine particles of powder.
3	Luster	Lusterless	Change of specific character of raw material after incineration
4	Taste	No specific taste, Mild Irritation is felt	Change of specific character of raw material after incineration

Floating on the water indicates lightness of drug lesser specific gravity than the water. Lusterless indicates no free form of metals present in that drug. Impinged in the furrow of fingers indicates fine particles of powder.

X-Ray Diffraction (XRD)

The XRD Analysis of *Linga Chenduram* shows that it was a highly crystalline drug. The XRD pattern correlated with that of standard Mercury sulfide which was shown in Figure No.4. Hence the *Linga Chenduram* prepared as per the classical siddha text was the sulphide form of mercury.

Figure 3: XRD Analysis of *Linga Chenduram*

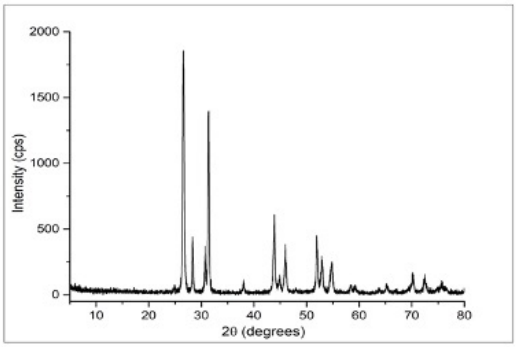
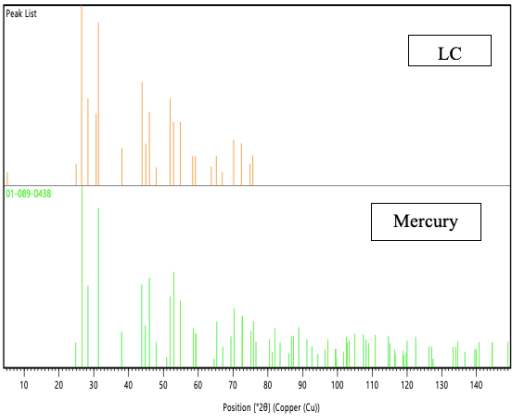
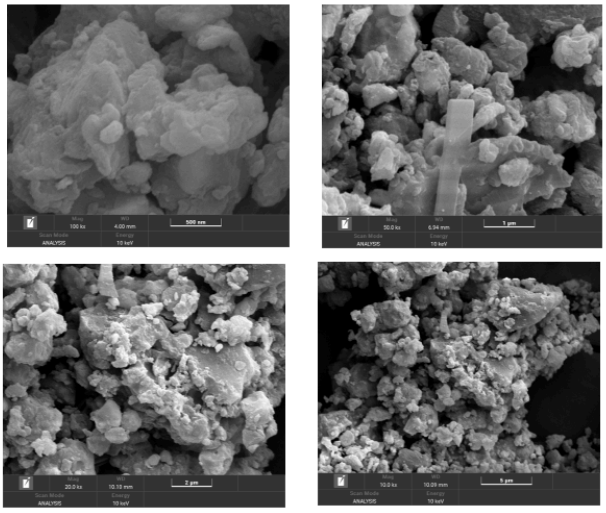


Figure 4: XRD pattern correlated with that of standard Mercury sulfide



Scanning Electron Microscope (SEM)

Figure 5: Scanning Electron Microscope images of LC



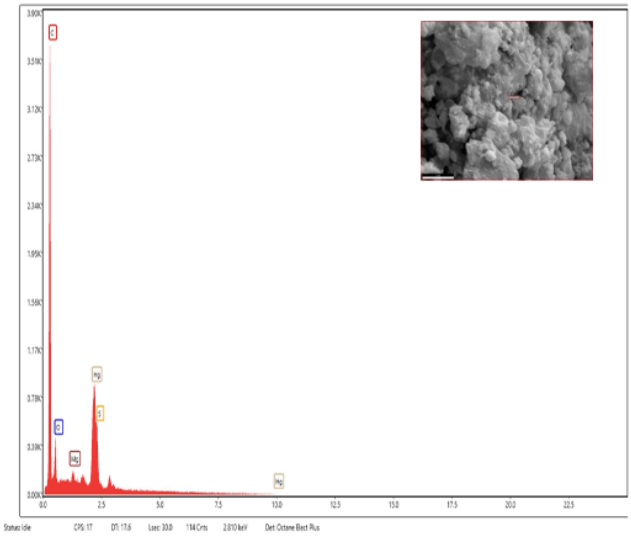
The morphology of the *Linga Chenduram* samples can be determined by SEM. The SEM photographs revealed that particle sizes were in the range 500 nm to 5µm (Figure No 5). The SEM analyses of the ***Linga Chenduram*** shows most of the particles present in the sample are Nano size.

The EDX analysis shows the elements present in the *Linga Chenduram a*s shown in Figure No. 6 and the Table No. 4 the mass percentage of Carbon, Oxygen, Magnesium and Mercury were found to be 43.63, 4.01, 1.15 and 51.2 percentages respectively. The results showed that Mercury (51.21) and Carbon (43.63) were present in larger amount compared to Oxygen and Magnesium.

Table 4: Weight and atomic percentage of LC

Element	Weight %	Atomic %	Net Int.
C K	43.63	86.78	776.13
O K	4.01	5.99	58.26
MgK	1.15	1.13	31.95
HgM	51.21	6.1	390.23
S K	0	0	0.03

Figure 6: EDAX analysis of LC



Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The drug *Linga Chenduram* sample was analyzed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. Heavy metals was analyzed by ICP-OES, results had been tabulated.

The results indicated that the formulation contains heavy metals in below detectable level. The presence of heavy metals such as Lead, Cadmium, Copper, Potassium, Magnesium, Iron, Zinc, Nickel and Sodium is Below Detection Limit. The amount of Sulphur - 352.254mg/dl, Mercury - 281.345 mg/ dl, Arsenic - 2.723mg/dl and Calcium - 1.180 mg/dl were present in *Linga Chenduram*. Cinnabar was the main ingredient in *Linga Chenduram* also cinnabar was the naturally occurring mineral of mercury in combination with Sulphur, therefore Sulphur and Mercury's levels were increased in the sample.

Table No. 5: Elements detected in ICP-OES analysis

S.No	Elements	Wavelength	*Linga Chenduram*
1	Arsenic	As 188.979	02.723 mg/L
2	Calcium	Ca 315.807	01.180 mg/L
3	Cadmium	Cd 228.802	Below Detection Limit
4	Cupper	Cu 327.393	Below Detection Limit
5	Iron	Fe 238.204	Below Detection Limit
6	Mercury	Hg 253.652	281.345 mg/L
7	Potassium	K 766.491	Below Detection Limit
8	Magnesium	Mg 279.077	Below Detection Limit
9	Sodium	Na 589.592	Below Detection Limit
10	Lead	Pb 220.353	Below Detection Limit
11	Nickel	Ni 231.610	Below Detection Limit
12	Sulphur	S 180.731	352.254 mg/L
13	Zinc	Zn 206.200	Below Detection Limit

Discussion

The preparation of trial drug was standardized primarily by the Organoleptic characters. Analysis of the drug shows that *LC* was dark brown in colour, odourless, tasteless, fine powder of appearance and fine to touch. This indicated the completion of calcination process. In physicochemical analysis values of the drug such as loss on drying, total ash, water-soluble ash and acid insoluble ash were found to be 1.19%. 3.73%, 0.19% and 1.49% respectively. The total ash value indicated the absence of impure substances in LC.

The XRD Analysis of *Linga Chenduram* showed that it is a highly crystalline drug. The XRD pattern was correlated with that of standard Mercury sulfide. Hence the *Linga Chenduram* prepared as per the classical siddha text is the sulphide form of mercury. SEM analysis of *Linga Chenduram* showed that most of the particles present in the sample were Nano size. Average particle size 500 nm to 5µm and the EDAX results showed that the values of Mercury (51.21) and Carbon (43.63) were present in larger amount compared to Oxygen and Magnesium.

In instrumental analysis, ICP-OES results showed that the toxic heavy metals such as Lead, Cadmium, Cupper, Potassium, Magnesium, Iron, Zinc, Nickel and Sodium were below the detection limit (BDL). It was evident that the safety of siddha medicine had been proved by the modern scientific way. Cinnabar was the main ingredient in *Linga Chenduram* also cinnabar was the naturally occurring mineral of mercury in combination with Sulphur, therefore Sulphur and Mercury's levels were increased in the sample.

Conclusion

The standardization methods such as the Physicochemical analysis, *Siddha* standardization, Chemical analysis and Instrumental analysis such as X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), Energy Dispersive X ray spectrometry (EDAX) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) results shows the principles of the trial drug *Linga Chenduram* was safer to consume even for a longer duration.

Acknowledgement

We are greatly thankful to the Director, National Institute of Siddha, Chennai - 47 for providing necessary facilities and permission to carry out this work. I express my sincere gratitude to the Head of the CATERS, Central Leather Research Institute, Adayar, Interstellar Testing Center Private Limited and 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.

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