IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 15, Issue 4 Ver. XIV (Apr. 2016), PP 09-15
www.iosrjournals.org

Physico Chemical Characterization of Linga Chendhuram

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Abstract: *Linga Chendhuram (LC) is a traditional Siddha Sastric formulation used in the management of fever at the dosage 65 mg with the adjuvant Honey. It is prepared through the special oxidation of purified Lingam (Cinnabar – Mercuric sulphide) using juice of Citrullus colocynthis as narrated in Siddha Materia Medica. Physicochemical characterization of LC has been carried out using analyses mentioned in the Chendhuram protocol of Pharmacopoeial Laboratory for Indian Medicine. Sophisticated Instrumentation such as X-ray Powder diffraction (XRPD), Scanned electron microscopy (SEM), Atomic Absorption Spectroscopy (AAS), Wavelength dispersive X-ray Fluorescent Spectroscopy (WDXRF) and Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) studies were done on LC. Physicochemical analysis showed the minimal value of moisture content, ash and extractive values. XRPD revealed the presence of HgS along with additional compounds. SEM showed the particles were in micrometer size. WDXRF showed the elements found in the LC were in oxides form. AAS and ICP-OES studies revealed the presence of heavy metals such as Lead and Mercury rather than Arsenic and Cadmium. The above results of LC will be considered as finger print for the standardization of the method of LC preparation.*

Keywords: *Cinnabar, Chendhuram, Fever, Lingam, Siddha*

I. Introduction

Lingam commonly known as Cinnabar is a naturally occurring mineral with mercury in combination with sulfur and is red in color so called red mercury sulfide. Cinnabar (an inorganic mercurial compound) contains more than 95% mercury sulfide (HgS) and has been used for thousands of years in traditional Siddha medicine and in Asian and Middle Eastern countries as a sedative and hypnotic[1,2,3]. *Linga Chendhura*m (LC) is a herbo-metallic preparation used in the therapeutic management of fevers, arthritis and anemia cited in the literature Siddha Materia Medica [4]. Generally there are two types of *Linga Chendhura*m viz '*Linga Chendhura*m' number 1 (Titration process - 'Churukku' process [4]) and '*Linga Chendhura*m' number 2 (burning in cow-dung cakes -'Pudam'process [4]) based on the preparation methods [5, 6]. Robert Saper reported the presence of heavy metals in Ayurvedic herbal medicine products which makes it essential to perform characterization and safety study in Indian system of medicine to validate its usage in community [7]. We know the Siddha medicines are safe and potential for treating diseases, but for universal acceptance, we are in need to explore Siddha medicine only by scientific manner by analyzing the physico chemical constituents.

II. Materials And Methods

2.1. Preparation of Linga Chendhuram

2.1.1. Procurement and collection of raw materials

The raw mineral *Lingam* (Cinnabar) was procured from M/s Gopal Aasan Country drug store, Nagercoil, Tamilnadu, India. Herbs such as *Atruthumatti* (entire part of *Citrullus colocynthis*) and *Kuppaimeni ilai* (Leaves of *Achalypa indica)* were collected from the field of Kancheepuram district, Tamil Nadu, India. Cow's milk and Lemon fruit were purchased from the local market at Chennai, Tamil Nadu, India.

2.1.2. Authentication of Raw materials:

Lingam has got authentication from Dr. M. Suresh Gandhi, Assistant professor, Department of Geology, University of Madras, Chennai, Tamil Nadu, India after studying its physicochemical properties. Herbs were authenticated by Dr. D. Aravindhan, Associate professor of Medicinal Botany, National Institute of *Siddha*, Chennai, Tamil Nadu, India.

2.1.3. Purification of Lingam

Lingam was purified by the process of *Surukku*. The juices of *Acalypa indica*, Citrus lemon and Cow's milk were mixed at equal proportion. Then *Lingam* was placed over the Mud pan (*Agal*) and heated at low flame

using LPG stove for the period of 3 h. On heating, simultaneously the mixed juice was added drop by drop over the *Lingam* constantly [4].

2.1.4. Preparation of *Linga Chendhuram*

35g of purified *Lingam* was placed over the mud pan (*Agal*) and started to heat at low flame using LPG stove. 1300ml juice of *Citrullus colocynthis* (*Atruthumatti*) was added drop by drop over the *Lingam* simultaneously during heating. After this process, *Lingam* was taken to *kalvam* and ground it into very fine powder until the disappearance of shines. The finished test drug was stored in an air tight sterile glass container and kept in a dark condition [4].

2.2. Physico chemical characterization of *Linga Chendhuram*

2.2.1. Qualititative analysis of *Linga Chendhuram*

2.2.1.1. *Siddha* classical method

The quality of *Linga Chendhuram* was accessed by the parameters cited in the Protocol for Testing Ayurvedic, Siddha & Unani Medicines [8]. The parameters are follows

- Red in colour without any shiny appearance
- Tasteless and odourless
- Did not regain luster on heating again at same temperature
- Sample floats on water. Did not immediately immersed in water
- Not translucent
- Impinged in the papillary ridges when the sample rubbed in between Index finger and Thumb

2.2.1.2. Organoleptic evaluation

Organoleptic evaluation was used for identification of sensory characteristics like colour, odour, taste, texture, etc [9]

2.2.1.3. Physico chemical analysis

The physico-chemical test was performed at Regional Research Institute of Unani Medicine, Chennai, India. The procedures recommended in WHO guidelines (Anonymous, 1998) were followed to determine loss on drying at 105°C, total Ash, total acid-insoluble ash and solubility in alcohol and water.

2.2.1.3.1. Loss on Drying

3 g of LC was kept and dried in the oven at 100 - 105°C for 6 h and constant weight was calculated.

2.2.1.3.2. Total Ash

3g of LC was incinerated in a crucible dish at a temperature not exceed 450^{o}C until free from carbon. Then it was cooled and weighed. % w/w of ash with reference to the air-dried powder was calculated.

2.2.1.3.3. Water Soluble Ash

The above obtained ash was boiled for 5minutes with 25ml water. The insoluble ash was collected using filter paper and washed with hot water and transferred to the silica crucible then ignites for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight was attained for determination of weight of insoluble ash. The weight of the water soluble ash was determined by subtracting the weight of insoluble ash from the weight of total ash.

2.2.1.3.4. Acid insoluble Ash

The total ash was obtained as the above method for preparation of total ash. The ash was boiled for 5minutes with 25ml 10% Hcl. The insoluble ash was collected using filter paper and washed with hot water and transferred to the silica crucible then ignites for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight was attained.

2.2.1.3.5. Alcohol Soluble Extractive Value

3g of LC was macerated with 100 ml of ethanol and kept in a closed container for 24 h. The resulting solution was shaken continuously for 6 hours kept in the shaking incubator and allowed to stand for 18 hours. The solution was filtered in a flat bottomed shallow dish and 25 ml of filtrate was evaporated and dried at 105^{o}C then cooled and weighed.

2.2.1.3.6. Water soluble Extractive value

3g of LC was macerated with 100 ml of chloroform water (2.5 ml chloroform in 1000 ml of purified water) and kept in a closed container for 24 h. The resulting solution was shaken continuously for 6 hours kept

in the shaking incubator and allowed to stand for 18 hours. The solution was filtered in a flat bottomed shallow dish and 25 ml of filtrate was evaporated and dried at 105^{o}C then cooled and weighed.

2.2.1.4. X ray diffraction analysis

Powder X-Ray diffraction (XRD) studies were carried out in at Madras University; Chennai, India. The powder XRD patterns of the LC were recorded on Bruker D8 Advance X-ray diffractometer using CuKα radiation at the wave length 1.5405A° and equipped with filter Nickel foil. The diffraction pattern was recorded for 2theta (angle) ranging from 5 to 80 degree (scanning rate 3 degree/second).

2.2.1.5. Microscopic analysis

Scanning Electron Microscopic study (SEM) on LC was carried out by using Carl Zeiss MA15/EVO 18 High Resolution Instrument done at SAIF, IIT Madras, Chennai-36. The nature of particle distribution and morphology in LC was analyzed.

2.2.2. Quantitative analysis of *Linga Chendhuram*

2.2.2.1. Atomic Absorption Spectroscopic study

The study was done under flame technique by using the instrument Thermo Fisher M Series, 650902 VI.27 model Atomic Absorption Spectrometer at Regional Research Institute of Unani Medicine, Chennai, India. The concentrations of Lead and Cadmium were estimated using hallow cathode lamps at the wave length of 217 and 228.8 nm respectively with a slit width of 0.5 mm. 4.0 and 3.0 mA Lamp current were used for Lead and Cadmium respectively. Air and Acetylene were used as Carrier gas at the flow rate of 1.1 L/min. The total flow rate was set as 2 mL/min. The procedures recommended in WHO guidelines, 1998 and AOAC, 2005.0000 were followed.

2.2.2.2. Wavelength dispersive X-ray fluorescence spectroscopic study

The study was performed using Bruker S8 Tiger Wavelength dispersive X-ray fluorescence spectrometer (WDXRF) under vacuum mode.

2.2.2.3. Inductively Coupled Plasma – Optical Emission spectroscopic study

The study was performed using Perkin Elmer Optima 5300 DV Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES). For digestion of 100 to 200 mg of LC, mixture of Nitric acid and Perchloric acid (2:1) was used. After the completion of digestion, the content was cooled and heated for the removal of acid. Then the solution was made into 50 mL using deionized water.

III. Results And Discussion

3.1. Siddha classical method

The TABLE 1 show LC satisfies all the quality parameters mentioned in the literature and proves that the LC prepared by the above process was well finished.

Table 1: Quality analysis of *Linga Chendhura*m

S.No	Siddha classical method	Results
1	Red in colour without any shiny appearance	Positive
2	Tasteless	Positive
3	Odourless	Positive
4	Did not regain luster on heating again at same temperature	Positive
5	Sample floats on water. Did not immediately immersed in water	Positive
6	Not translucent	Positive
7	Impinged in the papillary ridges when the sample rubbed in between Index finger and Thumb	Positive

3.2. Organoleptic characters of *Linga Chendhuram*

The Organoleptic characters of the *Linga Chendhuram* were as shown in TABLE 2. LC was brick red smooth fine powder with characteristic odour slightly pungent and no taste.

Table 2: Organoleptic characters of *Linga Chendhuram*

S.no	Parameter	Linga Chendhuram
1	Colour	Brick red
2	Taste	Nil
3	Odour	Slightly pungent
4	Magnetism	Nil
5	Reaction to HCL	No effervescence
6	Luminescence	Non fluorescent

3.3. Physico chemical analysis

Physicochemical analysis of LC was done and the results were presented in TABLE 3. The parameters such as Loss of drying at 105ºC (0.236±0.005), Total ash value (0.55±0.13), Acid insoluble ash value (0.016±0.007), Alcohol soluble Extractive (1.3±0.123) and Water soluble ash value (2.24±0.15).

Table 3: Physico chemical analysis of *Linga Chendhuram*

S.no	Parameter	Result % (Mean±S.D)
1	Loss on drying at 105°C	0.236±0.005
2	Total Ash Value	0.55±0.13
3	Acid Insoluble ash	0.016±0.007
4	Alcohol soluble extract	1.3±0.123
5	Water soluble extract	2.24±0.15

3.4. Estimation of Heavy Metals Concentration in *Linga Chendhuram*

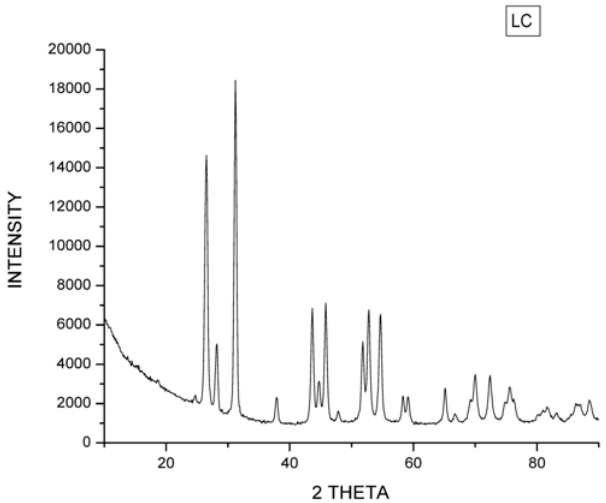
Heavy Metals Concentration LC was quantified by Atomic Absorption Spectrophotometer (Thermo Fisher M Series, 650902 VI.27 model of AAS). The results were presented in TABLE 4.

Table 3: Heavy Metals Concentration in *Linga Chendhuram*

S. No.	Heavy metal	Concentration	Methods/Instrument used
1	Lead	0.3098 ppm	WHO, 1998 & AOAC, 2005/Thermo Fisher AAS
2	Cadmium	Not detected	

3.5. X ray diffraction analysis

X-ray diffraction (XRD) is a versatile, nondestructive technique that reveals detailed information about the chemical composition and crystallographic structure of natural and manufactured materials. The basic principle of the phase analysis using powder XRD technique lies in the presence of diffraction peaks corresponding to various inter planar (dhkl) spacings which are the characteristics of a given material. The relative intensities of various peaks occurring at different'd' spacings are also different for different phases. The XRD patterns of the LC are shown in Fig. 1. LC was present in crystalline form with characteristics peaks near to 26°, 31°, 37°, 43°, 45°, 53° and 56° 2-Theta. XRD finger print of LC was found to be similar to the previous work of Sudha et al., 2009 [7]. The peak value in between 26° - 27° and 31°-32° corresponds to the standard peaks for HgS. The remaining peaks indicate the presence of other compound formed due to processing using herb.



Fiure.1- XRD image of LC

3.6. Microscopic analysis

SEM images of LC showed difference in size from 1 – 10 µm and agglomeration of the particles (Fig.2-5). Constant addition of herbal juice and grinding during process of LC made agglomeration of the particles.

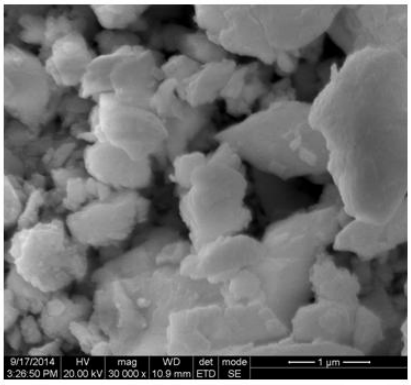


Figure.2- SEM image of LC 1 µm

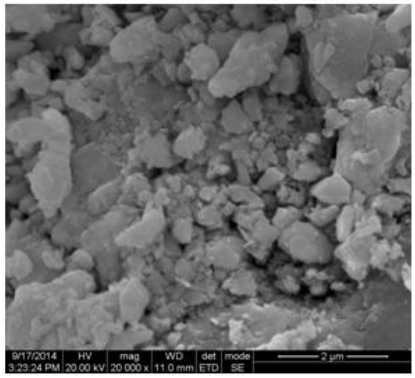


Figure.3- SEM image of LC 2 µm

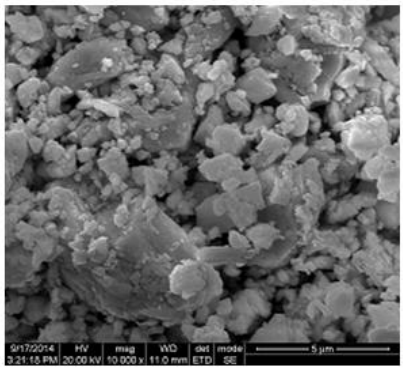


Figure.4- SEM image of LC 5 µm

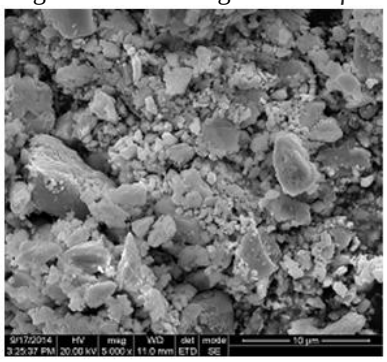


Figure.5- SEM image of LC 10 µm

3.7. Concentrations of elements present in the *Linga Chendhuram*

The concentration of elements in oxide form were observed by Wavelength dispersive X-ray fluorescence Spectroscopic study was shown in the TABLE 4 and 5

Table 4: Element in oxide form of *Linga Chendhuram*

Formula	Concentration (%)
Mercury	66.97
Sulphur trioxide	26.89
Arsenic trioxide	3.83
Magnesium oxide	0.45
Silicon dioxide	0.32
Lead oxide	0.28
Manganese oxide	0.22
Thallium	0.15
Iron(III) oxide	0.13
Calcium oxide	0.11
Sodium oxide	0.11
Chloride	0.11
Potassium oxide	0.11
Zinc oxide	0.10
Selenium dioxide	0.08
Aluminium oxide	0.07
Platinum	0.04
Copper(II) oxide	0.02

Table 5: Elemental form of *Linga Chendhuram*

Formula	Concentration (%)
Mercury	84.64
Sulphur	10.77
Arsenic	2.90
Magnesium	0.27
Lead	0.26
Manganese	0.17
Thallium	0.15
Silicon	0.15
Chloride	0.11
Iron	0.09
Potassium	0.09
Zinc	0.08
Sodium	0.08
Calcium	0.08
Selenium	0.06
Platinum	0.04
Aluminium	0.04
Copper	0.02

The concentration of trace elements including heavy metals were observed by Inductively Coupled Plasma Optical Emission Spectroscopic study was shown in the TABLE 6.

Table 6: Trace Elements of *Linga Chendhuram*

S.no	Elemental symbol	Wavelength (nm)	Concentration (ppm)
1	Arsenic	188.979	BDL
2	Cadmium	228.802	BDL
3	Chromium	267.716	0.023mg/L (0.023026273 ppm)
4	Mercury	253.652	3.021 mg/L (3.024450897 ppm)
5	Nickel	231.604	BDL
6	Lead	220.353	BDL
7	Sulphur	180.731	71.524 mg/L (71.60570208ppm)
8	Vanadium	313.07	BDL

IV. Conclusion

Cinnabar has the characteristics of insolubility in water and other solvent. The concentration of solubility is very less for Cinnabar and it shall not be used as therapeutic agent because of poor bioavailability. After oxidation of Cinnabar by the traditional Siddha procedure as *Chendhuram* form, *Lingam* has high efficacy

due to more bioavailability. It has been proved in SEM that LC has the particle size range below 10 µm and provides increased dissolution rate due to increased surface area. Further, XRD data can be used as the tool for finger print to analysis the quality of LC leads to SOP for *Linga Chendhuram* preparation.

Acknowledgement

The authors wish to thank Vice Chancellor of The Tamil Nadu Dr. MGR Medical University, Chennai and Director of National Institute of Siddha for their support.

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