ejpmr, 2016,3(7), 270-274.

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PURIFICATION PROCESS OF *LINGAM* (CINNABAR)-A COMPARATIVE PHYSICOCHEMICAL ANALYZES

Thanigavelan V.[1]*, Mathukumar S.[2] and Pitchiah Kumar M.[3]

[1]Assosciate Professor, Sairam Advanced Centre for Research, Sri Sairam *Siddha* Medical College and Research Centre, Chennai, India / Part Time Research Scholar, The Tamil Nadu Dr. MGR Medical University, Chennai, India.
[2]Professor cum Principal, Sairam Advanced Centre for Research, Sri Sairam *Siddha* Medical College and Research Centre, Chennai, India.
[3]State Drug Licensing Authority (Indian Medicine), Post Graduate Department of Gunapadam, Government *Siddha* Medical College, Chennai, Tamil Nadu, India.

***Corresponding Author: Thanigavelan V.**
Assosciate Professor, Sairam Advanced Centre for Research, Sri Sairam *Siddha* Medical College and Research Centre, Chennai, India / Part Time Research Scholar, The Tamil Nadu Dr. MGR Medical University, Chennai, India.

Article Received on 22/04/2016 **Article Revised on 12/05/2016** **Article Accepted on 01/06/2016**

ABSTRACT

Natural ore *Lingam* (Cinnabar) is grouped under the category of *Paashanams* (toxicants) used in traditional Siddha medical system in formulating herbo-mineral drugs for treating chronic ailments. *Siddha* concept is to subject Lingam to *Suddhi* (detoxification) before formulating into medicines. The study deals with evaluation of quality among the two purification methods of *Lingam* commonly adopted among *Siddha* Practitioners through physicochemical characterization analyses. *Lingam* was purified by *Erippu* (Burning) and *Surukku* (Consolidation) methods and purified samples were analyzed and compared with unpurified. No characterization difference in physical properties was observed among the samples except in hardness and loss of weight. Raman shifts were observed among the samples revealed that purified sample done by *Surukku* method deviated from certain functional groups. Mercury and Sulphur contents were more reduced in purified samples of *Surukku* method. It is concluded that *Lingam* purified by *Surukku* method is better in quality than purified by *Erippu* method.

KEYWORDS: *Siddha, Lingam,* Cinnabar, Purification and Quality.

INTRODUCTION

Siddha system of medicine uses certain mineral preparations which are effective in chronic ailments even in very small doses and are used without loss of potency for many years.[1] In Siddha system, *Thathu* materials (Minerals) are classified into metals, salts, toxicants and secondary minerals.[1] *Siddhars* have converted potentially toxic heavy metals and its salts such as elemental mercury and its compounds, arsenic, copper sulphate, etc., into good medicines by removing their toxicity by a special process of *Suddhi* purification.[1] *Lingam* commonly known as Cinnabar is an insoluble form of Mercuric sulphide used extensively in *Siddha*, *Ayurveda*, *Unani* and *Chinese* Medicine. Many preparations used *Lingam* as one of the ingredients to treat Vatha diseases (Diseases occurs due to aggravated wind humour, chronic inflammatory and infectious diseases).[2] Before using *Lingam*, it should be purified completely to remove the toxicity. Improper purification and processing of cinnabar will result in toxicity such as inflammation of Gastro intestinal tract leading to ulcerations in the buccal floor, uvula, and inner portion of tongue, larynx and large intestine. There may be loss of taste, difficulty in eating, drinking water and speaking, foul odour from mouth, whitish viscous salivary secretion and burning sensation over the body.[2] The antidote for the *Lingam* toxicity is also mentioned in the literature as the aqueous decoction made with 4 g *Myristica fragrans* (Nut meg), 4 g *Piper cubeba* (Cubeb), 4 g Root bark of *Gossypium arboretum* (Red Cotton) with 35 g rock candy.[1] In Ayurveda, it is cited that *Lingam* which has not undergone *Suddhi* is likely to cause mental disorders, blindness, weakness, fatigue, giddiness, delusion and urinary disorders.[3] The current research on Cinnabar reported on using ten folds of human therapeutic dose (10 times more than 80 mg/kg day) results in neurotoxicity particularly in cerebellum and leads to dysfunction of Vestibulo Ocular reflex system due to accumulation of Mercury.[4] The Pharmacopoeia of China (2010) illustrate that Cinnabar shall be administered at the doses of 0.1-0.5 g/day up to 10 days.[5] Chronic administration of cinnabar at its effective dosage causes anxiolytic effects due to decrease in brain Serotonin level.[6]

Numerous purification processes were cited in the Siddha literature and in traditional practice having complicated and laborious methods. In this study, we analyzed the physicochemical characters of two purified *Lingam* samples derived by two different purification

processes viz., *Erippu* (Burning) and *Surukku* (Consolidation) and evaluated as to which purified *Lingam* is optimum for formulating the medicines.

MATERIALS AND METHODS

Procurement and collection of raw materials

Lingam (Cinnabar) was procured from M/S Gopal Aasan Country drug store, Nagercoil, Tamilnadu, India. *Lingam* coded as sample R "Fig. 1" has been authenticated by the department of Geology, University of Madras, Chennai, Tamil Nadu, India after studying its physicochemical properties. Neem oil, Cow's milk and Lemon fruits were purchased from the local market at Chennai, Tamil Nadu, India.

Figure 1: The raw *Lingam* purchased from country drug store to be subjected for purification processes (Sample R).

Purification and detoxification of *Lingam* by *Erippu* process

This method was adopted from a traditional *Siddha Vaidhiyar (*Practitioner). Raw *Lingam* 40 g was separated into eight equal parts (5 g) without powdering. Each part was covered by small piece of cotton gauze and tied by a thread to make into a pouch like structure. 200 mL Neem oil was poured into an *Agal* (Earthenware lid). All pouches were immersed in the oil leaving the tied part above and ignited by the match stick. All pouches were burnt until the oil is used up for burning. After completion of the burning, pouches were opened and the *Lingam* samples were secured. The soot found coated over the *Lingam* was scrapped out and washed with water and dried. This purified *Lingam* was coded as sample E "Fig. 2".

***Erippu* process**

Figure 2: A denotes the raw *Lingam* was purified by *Erippu* method and
B denotes the purified lingam done by this process (Sample E)

Purification and detoxification of *Lingam* by *Surukku* process

This is the common routine method employed for the purification of *Lingam* which is cited in the text of Gunapadam (*Siddha* Materia Medica).[2] Cow's milk 150 mL, Lemon juice 150 mL and *Acalypha indica* juice 150 mL were mixed well in a glass jar. A single piece of *Lingam* weighed 57.83 g was placed on a mud plate and heated over hot plate mounted on it. The juices in the glass jar were instilled over the *Lingam* drop by drop for 3 h continuously. After 3 h, the *Lingam* was allowed to cool and washed out with water and dried. This purified *Lingam* was coded as sample S "Fig. 3".

***Surukku* process**

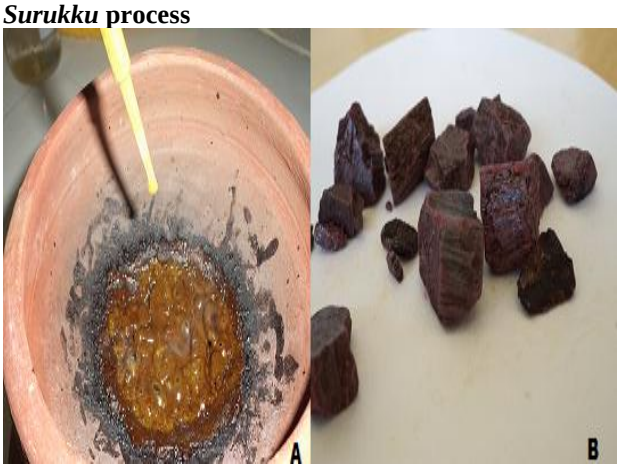

Figure 3: A denotes the raw *Lingam* was purified by *Surukku* method and
B denotes the purified *Lingam* done by this process (Sample S)

Physical parameters analyses

The raw and purified *Lingam*s coded as sample R, A and B was observed for Cleavage, Colour, Crystal form, Fracture, Hardness, Lustre, Magnetism, Reaction to HCl, Streak, Luminescence and Loss of weight. Colour was examined under visible light and the form of crystal was examined by the observation of geometric shape such as trigonal, cubic, hexagonal, etc. Fracture denotes the areas

where the sample is broken (irregular or concoidal). Hardness was observed by scratching the samples using Mohs Hardness kit and Mohs scale was recorded. Lustre, was observed by the appearance of sample in metallic and non-metallic states. Magnetism was done to analyse the presence of electromagnetic force using magnet. Reaction to HCl was done to know the presence of Calcium carbonate in the sample confirmed by the formation of effervescence. Streak was evaluated by determining the colour of the sample on grinding the sample into powder on a porcelain streak plate. Luminescence was done by using UV lamp to analyse the fluorescence property. Loss of weight in percentage was estimated in the purified samples to determine the reduction of metallic concentration by the formula [(Weight of the sample before purification - Weight of the sample after purification) / Weight of the sample before purification] x 100.

Functional group analyses

The study was carried out using BRUKER RFS27 Stand-alone FT-Raman Spectrometer having scan range from 50 to 4000 cm^{-1} done at Sophisticated Analytical Instrumentation Facilities, Indian Institute of Technology - Madras, Chennai, Tamil Nadu, India. The functional groups present among Sample R, E and S were analysed by correlating with the standard Raman Spectroscopy data.[7]

Estimation of Mercury and Sulphur

The concentrations of Mercury and Sulphur were observed between Sample R, E and S by Perkin Elmer Optima 5300 DVInductive coupled plasma optical emission spectrometer (ICP-OES). The study was performed at Sophisticated Analytical Instrumentation Facilities, Indian Institute of Technology - Madras, Chennai, Tamil Nadu, India.

RESULTS AND DISCUSSION

Table 1: Physical parameters of *Lingam* before and after purification processes

Parameters	Sample R*	Sample E*	Sample S*
Cleavage	Perfect	Perfect	Perfect
Colour	Cochineal red towards brownish red	Blackish red	Blackish red colour
Crystal form	Trigonal	Trigonal	Trigonal
Fracture	Brittle	Brittle	Brittle
Hardness	Finger nail 2.5	Finger nail 2.5	Penny 3
Luster	Adamantine	Dull, having a plain looking surface that is not submetallic	Adamantine but decreased on compared to Sample A
Magnetism	Not attracted	Not attracted	Not attracted
Reaction to HCl	No effervescence	No effervescence	No effervescence
Streak	Bright red	Brownish red	Bright red
Luminescence	Non fluorescent	Non fluorescent	Non fluorescent
Loss of weight	-	10.26 g %	10.34 g %

**Sample R – Raw Lingam before purification, Sample E – Purified Lingam by Erippu method, Sample S - Purified Lingam by Surukku method*

The result of table 1 shows the similarities and differences in the physical observations among the raw and purified samples. It was observed that Sample S differs in its lustre (decreased adamantine), hardness (increased – Penny) and change in colour (Blackish red) on compared with Sample R. After purification, sample S loss its 10.34 g% weight and qualitatively indicated that the concentrations of mercury and sulphur was reduced better on compared with Sample E.

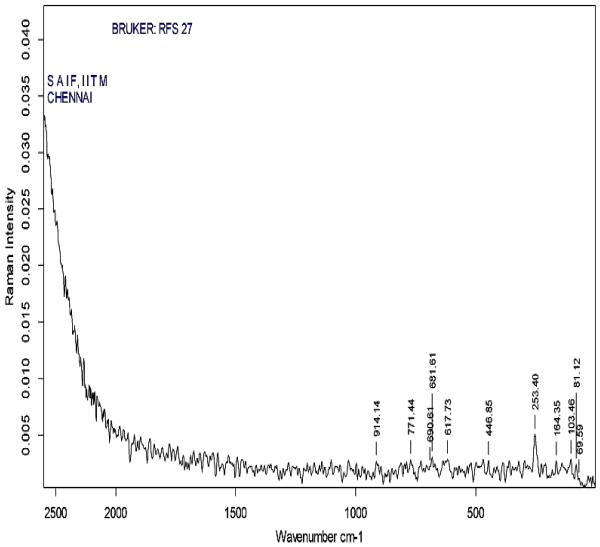

Figure 4: Raman shifts of raw *Lingam* before purification (Sample R) shows eleven peaks

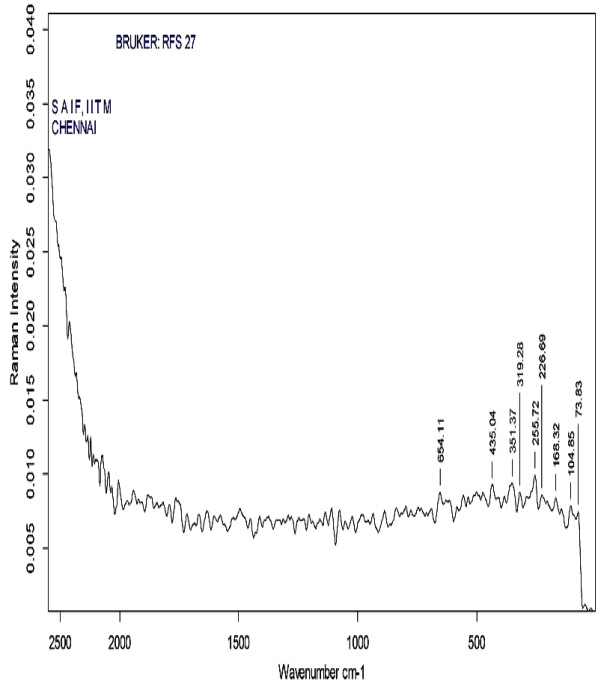

Figure 5: Raman shifts of purified *Lingam* (Sample E) shows nine peaks

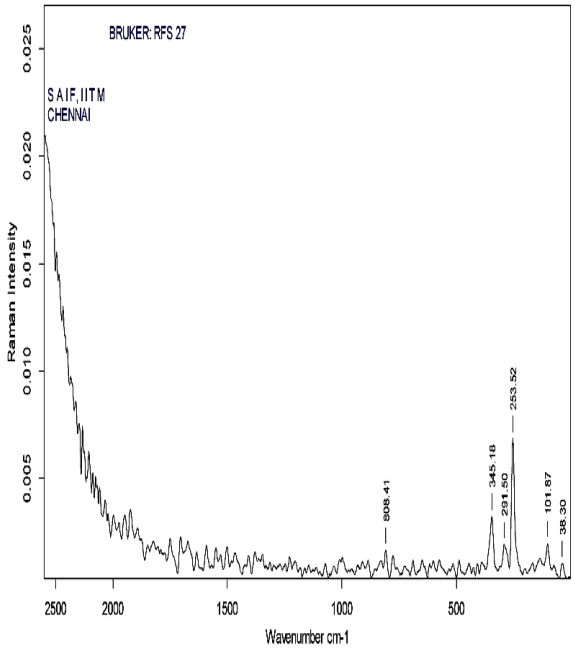

Figure 6: Raman shifts of purified *Lingam* (Sample S) shows six peaks

Table 2: Analyzes of functional groups present in *Lingam* before and after purification processes done by Raman spectroscopic studies

Functional Group/Vibration	Region	Sample R* (Band cm^{-1})	Sample E* (Band cm^{-1})	Sample S* (Band cm^{-1})
Lattice vibrations in crystals	10-200 cm^{-1}	69.59, 81.12, 103.46, 164.35,	73.83, 104.85, 168.32,	38.30, 101.87
υ(S-S)	430 -550 cm^{-1}	446.85	435.04	-
υ(Xmetal-O)	150-450 cm^{-1}	253.40,	226.69, 255.72, 319.28, 351.37,	253.52, 291.50, 345.18
υ(C-S) aliphatic	630 - 790 cm^{-1}	771.44, 690.61, 681.61,	654.11	-
υ(C-O-C)	800 -970 cm^{-1}	914.14	-	808.41
υ(CC) alicyclic, aliphatic chain vibrations	600 - 1300 cm^{-1}	617.73	-	-

**Sample R – Raw Lingam before purification, Sample E – Purified Lingam by Erippu method, Sample S - Purified Lingam by Surukku method*

The results of table 2 show the functional groups present in the samples. By Raman Spectroscopy of Sample R, Raman shifts have been observed at 11 peaks "Fig. 4". Among 11 peaks, prominent band at 253.40 cm^{-1} was observed representing strong υ(Xmetal-O) group and has other bands representing the presence of lattice vibrations in crystals indicating storage of heat in the form of oscillatory energy, strong υ(S-S), strong υ(C-S) aliphatic, medium υ(C-O-C) and medium υ(CC) alicyclic groups aliphatic chain vibrations. Nine peaks were observed in Sample E "Fig. 5" having prominent band at 255.72 cm^{-1} representing strong υ(Xmetal-O) group. The other bands in Sample E indicate the presence of lattice vibrations in crystals, strong υ(S-S) and strong υ(C-S) aliphatic groups. In Sample S, Raman shifts have been observed at 6 peaks "Fig. 6" and prominent bands are seen at 253.52 and 345.18 cm^{-1} representing the presence of strong υ(Xmetal-O) group. On compared with Sample E and R, no υ(S-S) and υ(C-S) aliphatic groups were not found in Sample S. υ(CC) alicyclic group with aliphatic chain vibration was eliminated in both purified samples. Sample S has two bands representing lattice vibrations in crystals and stretching of medium vibrations of υ(C-O-C).

Table 3: Estimation of Mercury and Sulphur content in *Lingam* before and after purification processes.

Element	Wave length (nm)	Sample R*	Sample E*	Sample S*
Mercury	253.652	150.250 mg/L (150.422 ppm)	100.215 mg/L (100.329 ppm)	25.247 mg/L (25.275 ppm)
Sulphur	180.731	450.024 mg/L (450.538 ppm)	400.245 mg/L (400.702 ppm)	150.024 mg/L (150.195 ppm)

**Sample R – Raw Lingam before purification, Sample E – Purified Lingam by Erippu method, Sample S - Purified Lingam by Surukku method.*

The concentrations of mercury and Sulphur estimated using ICP-OES in different samples were shown in the table 3. 83.20% of mercury was reduced in sample S and 33.30% of mercury was reduced in sample E on compared with sample R. 66.66% of sulphur was reduced in sample S and 11.06% of sulphur was reduced in sample E on compared with sample R. From the results of table 1, 2 & 3, it is inferred that purified *Lingam* by *Surukku* method (Sample S) is superior in quality than purified *Lingam* by *Erippu* method.

CONCLUSION

Even though, there are many methods of purification process illustrated for *Lingam*, under this study, it is concluded that purification of *Lingam* done by *Surukku* method (Sample S) is superior in quality than *Erippu* method. Further animal toxicity studies need on these purified *Lingams* warrant which method is suitable for better therapeutic usage.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors wish to thank Vice Chancellor and Department of *Siddha*, The Tamil Nadu Dr. MGR Medical University, Chennai and the Management of Sairam Group of Institutions for their support.

REFERENCES

1. Jayaprakash Narayanan P. Siddha Medicine Vol. II. Chennai; Tamil Valarchi Kazhagam, 2011.
2. Thiyagarajan R. Siddha Materia Medica Mineral & Animal section. Chennai; Department of Indian Medicine and Homoeopathy, 2008.
3. Himsagar Chandra Murthy P. Rasasastra The Mercurial system. 3rd ed., Varanasi; Chowkhamba Sanskrit Series Office, 2013.
4. Yi-Ho Young, Jiunn-Jye Chuu, Shing-Hwa Liu, Shoei-Yn Lin-Shiau. Neurotoxic mechanism of Cinnabar and Mercuric sulfide on the Vestibulo-Occular Reflex system of Guinea Pig. Toxicological Sciences, 2002; 67(2): 256-63.
5. Wang Q, Yang X, Zhang B, Yang X, Wang K. Cinnabar is Different from Mercuric Chloride in Mercury Absorption and Influence on the Brain Serotonin Level. Basic & Clinical Pharmacology & Toxicology, 2013; 112(6): 412–7.
6. Wang Q, Yang X, Zhang B, Wang K. The anxiolytic effect of cinnabar involves changes of serotonin levels. Eur J Pharmacol, 2007; 65(1-3): 132–7.
7. Raman Data and Analysis, http://www.horiba.com/fileadmin/uploads/scintific/Documents/Raman/bands.pdf.