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Research Article

SIGNIFICANCE OF PHYTOCHEMICALS IN THE PROCESS OF *LINGA CHENDOORAM* PREPARATION

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ABSTRACT

Objectives: We know that most of the metals are toxic that raised a great concern among people. The siddhars' knowledge of iatro-chemistry, minerals, metals, and plants was stupendous. Hence, in this project, attempts are made to standardize *Linga chendooram* (LC) and to prove that the end product is free from free metals. LC is a herbo-metallic preparation, having mercury as its chief constituent. The Siddhars have devised the preparation in such a way that makes it bio-compatible and non-toxic.

Methods: To prove this research works, select two methods for preparation and carry out standardization and validation studies on Zeta sizer, gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), atomic absorption spectroscopy (AAS), thermogravimetric-differential thermal analysis (TG-DTA), and scanning electron microscopy (SEM) analysis.

Results: Standardization results indicate that it preparation was fully completed as per literatures. GC-MS and FTIR results observed that the amine group present in the final product have complex with cinnabar. AAS results point out that medicines were free from toxic metals. The average size of the both products determined by Zeta sizer Nano size and was found to be 832.7 nm and 676.7 nm, respectively. The TG-DTA and Zeta-sizer results conclude that the product is stable and can be used for biological system infusions. The SEM images confirmed the transformations in the morphology of the starting material which is due to the preparation process. The final product is coagulated which serves as evidence of complex formation.

Conclusion: This research work concludes that the toxic mercury may be converted into biocompatible form due to the chemical transformations and surface morphology changes that occurred during the process.

Keywords: *Linga chendooram*, Zeta sizer, Gas chromatography-mass spectrometry, Fourier transform infrared spectroscopy, Atomic absorption spectroscopy, Thermogravimetric-differential thermal analysis and Scanning electron microscopy analysis.

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INTRODUCTION

The Siddha system of medicine, one of India's oldest traditional medical sciences, has been practiced since ancient times and is believed to have originated in the Lemurian civilization before being propagated among the early Dravidian population [1]. Classical Siddha pharmaceutics includes an extensive repertoire of herbal, mineral, and metal-based formulations, which are traditionally categorized into Metals, Minerals, Toxins, and Hydrochemicals [2]. Although such formulations have been used therapeutically for centuries, public apprehension persists due to the widespread perception that all metals are toxic. This generalization has led to skepticism regarding herbo-mineral preparations, despite longstanding textual descriptions of processing and detoxification procedures meant to render these substances safe.

Linga chendooram (LC) is a classical Siddha herbo-mineral formulation containing lingam (cinnabar; HgS) as its chief constituent [3]. Cinnabar has been used in Indian and Chinese systems of medicine for more than a millennium and is chemically distinct from elemental mercury, consisting predominantly of mercury sulfide (HgS), which is far less soluble and toxic than Hg or organo mercurials [4]. Traditional texts emphasize that cinnabar must be subjected to meticulous detoxification before therapeutic use, during which plant-based media such as lemon juice, milk, mother's milk, *Acalypha indica*, and *Alangium salviifolium* are used to remove surface impurities and modify mineral characteristics [5]. Among these, *Citrus limon* juice is one of the most widely recommended purifying agents. Beyond its therapeutic value, Siddha literature specifies that it aids in cleansing minerals and grinding metal-based preparations effectively [6,1]. However, the scientific basis for this detoxification step – particularly how phytochemicals interact with cinnabar – remains poorly understood.

LC is traditionally prescribed for fever, vitiated vata–kapha disorders, skin diseases, venereal conditions, abdominal complaints, and delirium [7]. LC's dose level is 50–100 mg, twice a day honey used as an adjuvant [8,9]. Lethal dose fixation study suggests acute toxicity level 4000mg/kg and subacute level 200 mg/kg [10]. Modern studies have explored its anti-urolithiatic, antipyretic, anti-inflammatory, antiviral, and antifungal properties [11-14]. These findings support its therapeutic relevance, but they do not address the fundamental scientific question of how traditional processing transforms cinnabar into a biocompatible form. The existing literature largely focuses on pharmacological activity, leaving a major knowledge gap on the physicochemical changes occurring during detoxification and preparation.

Therefore, the present study aims to scientifically evaluate two classical methods of LC preparation with emphasis on:

1. Understanding phytochemical interactions during the detoxification of cinnabar using *C. limon* juice;
2. Characterizing physicochemical transformations that occur across preparation steps;
3. Structural, chemical, and morphological changes that distinguish raw cinnabar from purified and processed samples. and
4. Demonstrating that mercury remains in a non-elemental, non-amalgamating, and biocompatible form, rather than being eliminated from the formulation.

METHODS

Raw cinnabar and other plant materials were procured from Siddha medicine dealers, Trichy, Tamil Nadu. The *Citrullus colocynthis*, *C. limon*, *Morinda tinctoria* plant used in this process is identified using Flora of the Presidency of Madras and authenticated with herbarium specimen deposited at RHT (ACC): 1854 Raphinet Herbarium, Department of Botany, St. Joseph's College, Trichirapalli, Tamil Nadu, India [15].

Method of detoxification

Before the preparation, cinnabar should be processed with lemon juice which is called detoxification. For detoxification, take two parts of lemon juice, one part of cinnabar in small pieces. The weighed cinnabar is taken in a glass container which is filled with lemon juice such that it is completely immersed. The cinnabar is soaked in lemon juice for 24 h (Fig. 1) [2]. Then, the cinnabar-soaked lemon juice was subjected to gas chromatography mass spectroscopic analysis.

Fig. 1: Detoxification process of cinnabar (a) raw cinnabar, (b) lemon, (c) lemon fruit juice, (d) processed cinnabar

LC preparation

Preparation method.1 (LC.1)

Ingredients are purified cinnabar and colocynth plant juice. 40 g of powder cinnabar gently heat in a pan. Take 2 L of colocynthis juice, add small quantities and mix thoroughly. Continue the process till all the juice is spent. Then heat just till perfectly dry. Cool and grinding are done for 4 h and 30 min [5]. The *Chendooram* should be bright orangish red (Fig. 2). Both cinnabar and plant juice were collected immediately after adding 100 mL, 700 mL, and 1400 mL, respectively. Then, raw *citrullus* juice and collected samples were subjected to elemental analysis. This plant has the highest medicinal value and also biochemical reactions [16].

Preparation method.2 (LC.2)

Second method take each 30 gm of purified cinnabar, gum benzoin, camphor and sufficient quantity of morinda bark powder. Benzoin and camphor were triturated into a waxy mass. Cover the cinnabar mass with this mass smeared on a cloth and make a small bundle. Burn this till the covering is carbonized. Take the treated cinnabar and repeat this 6 more times. Finally, place the cinnabar hidden in *morinda* bark powder in an earthen disc capsule and calcine with five cow dung cakes. Powder the product and store [5]. The *chendooram* should be bright orangish red (Fig. 3).

Physicochemical standardization

Physicochemical analysis of loss on drying, total ash, acid-insoluble ash, and water-soluble extractive was performed according to standard procedures given in Ayurvedic Pharmacopeia of India [17] and quality control methods for medicinal plant materials [18]. This type of physicochemical work was carried out previously by Sreelakshmi *et al*., 2022 [19].

Siddha methods of standardization [5]

Luster test: Final product should not have any glitter or shine. Fingerprints test: If a small quantity is pinched and rubbed between

Fig. 2: *Linga chendooram* (LC.1) preparation (a) colocynthis plant, (b) colocynthis juice, (c) cinnabar heated with juice on a pan, (d) chendooram preparing, (e) chendooram grinding, (f) LC.1

Fig. 3: *Linga chendooram* (LC.2) preparation (a) camphor, (b) benzoin, (c) cinnabar burning with camphor and benzoin, (d) treated cinnabar, (e) morinda bark, (f) morinda bark powder, (g) moringa powder with cinnabar, (h) sealed earthen disc, (i) cinnabar burning with cowdung cakes, (j) earthen disc after burning, (k) chendooram grinding, (l) LC.2

the thumb and index finger, the particles should be so fine as to enter and reside in the furrows and folds. Test for floatability: It is called sprinkle test. If a pinch of *chenthooram* is gently put on the surface of water kept in a container, the material should not shrink, but it should float. Irreversible test: One gram of LC was mixed with 1 g of silver separately. Test was carried out at two different temperatures 300°C and 400°C. After cooling, the weight changes were noted. This traditional standardization method was explained in Chandrasekar *et al*., 2023 [20].

Instrumental analysis

ZETA SIZER analysis

This is from Malvern instruments allow the accumulation of volume of particle size from 0.6 nm to 6000 nm and its zeta potential. Zeta sizer results will help in concluded particle or agglomeration of particles, if any.

Gas chromatography-mass spectrometry (GC-MS) analysis

Methanol extract was tested by a gas chromatographic system coupled with mass spectrometry (MS) (Perkin Elmer, Model: Clarus-500). Silica capillary pillar (30 m × 0.25 mm, 0.25 µm layer thicknesses, Elite-5 MS non-polar fused) was applied. Furnace heat was listed with raise of 6°C/min to 150°C; injector heat was 280°C. Transporter gas was helium with a run rate of 1 mL/min. Sample (1.4 µL) was injected with a split ratio of 1:10. Ionization energy 70 ev was employed in the electron ionization mode; ion source heat was set at 160–200°C, mass was scanned in the range of 40–450 amu. The resulting mass spectrum was compared with inbuilt NIST library database, and fragments of various compounds present in the extracts were recognized.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR is a spectroscopic technique that employs lesser energy emission to induce pulsation and revolving excitation of atoms and groups of atoms surrounded by molecules (Spectrum100, Perkin Elmer, USA). It also knows how to be used in considering the complex arrangement of various metals with phytoconstituents. Sample-to-KBr ratio: 1 mg sample: 100 mg KBr.

Mixing method: Manual grinding in an agate mortar for 10 min.

Atomic absorption spectroscopy (AAS) analysis

AAS (A Analyst 400 (for flame), HGA 900 (for graphite furnace), Perkin Elmer). AAS is a quantitative method to estimate the concentration of trace elements and heavy metals according to Beer-Lambert's law. The instrument consists of a particular light source for each element, a sample compartment in which the sample is aspirated into the flame and a detector. When the sample is aspirated into the flame, a much larger number of gaseous metal atoms will remain in the ground state which is capable of absorbing radiant energy of their own specific resonance wavelength. The extent of absorption will be proportional to the number of ground-state atoms present in the flame.

Each sample (100 mg) was digested with 5 mL of aqua regia (HCl: HNO_3 = 3:1) and heated at 120°C for 2 h on a hot plate. It was then diluted to 50 mL with deionized water and filtered with a 0.45 µm filter. Calibration was done using Hg, Pb, and Cd standards.

Thermo gravimetry-differential thermal analyzer (TG-DTA) analysis

TG-DTA find modify in weight with respect to raise in temperature. It is applied to conclude thermal stability, sublimation, or evaporation temperature and presence of moisture content in samples.

- Heating rate: 10°C/min
- Temperature range: RT to 800°C
- Atmosphere: Nitrogen purge at 50 mL/min.

Scanning electron microscopy (SEM)

A SEM is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with electrons in the sample, producing various signals that can be detected and that contain information about the sample's surface topography and composition. The electron beam is generally scanned in a raster scan pattern, and the beam's position is combined with the detected signal to produce an image.

- Coating: Gold sputter-coated (10 nm thickness)
- Vacuum: 10^{-5} Torr
- Acceleration voltage: 15 kV.

RESULTS AND DISCUSSION

Siddha methods of standardization

Fig. 4 shows the siddha standardization results. The floatability test indicates that the process completion, a pinch of sample is suspended in stagnant water. Both the batches of LC completely float on water. This is due to the surface tension of the particle and the water. As the size of particles is small, it has large surface area, this property allows it to float on water. This proves that the drug is complete. The fingerprint test – appearance of LC shows the fine enough to enter the crevices of the finger. Image D proves that the final product should not have any glitter or shine. The metal irreversibility test was carried out at two different temperatures. The result is tabulated as follows in Table 1.

At 300°C, the composition of drug and silver taken was in a ratio of 1:1. At this temperature, silver is in solid state; this could be inferred from the silver-mercury phase diagram. As there is no weight change, it infers that there is no amalgam formation. At 400°C in the mercury–silver phase diagram, the silver is in solid state. There is no significant weight change in silver, and this confirms the absence of free mercury in the drug. These marks conclude that the preparation followed the instructions according to siddha texts. This traditional method of standardization work was carried out previously for *Swarna makshika bhasma* [21].

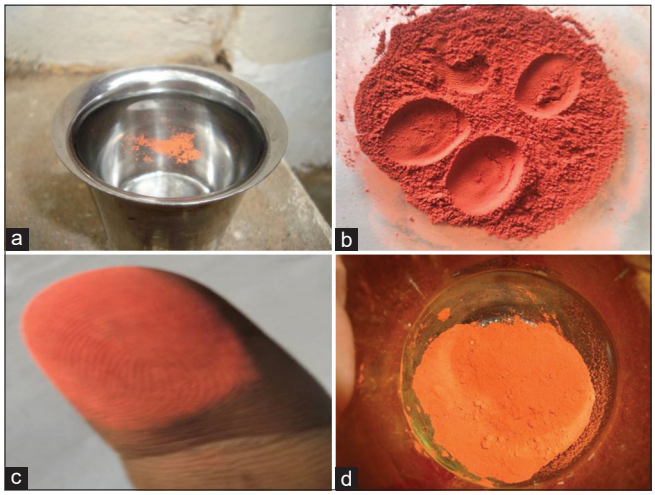

Fig. 4: Siddha methods of standardization: (a) *Linga chendooram* (LC) Floated in water, (b) Finesse of LC, (c) Appearance of fingerprint in LC, (d) Absence of glitter or shine in LC

The above Table 2 data show that the color of LC was found to be orangish red and does not possess any characteristic odor. From the above tabulation, it can be inferred that there is no moisture content in LC.1 and negligible in LC.2. There is very less percentage of ash in LC.1 and LC.2. The above results indicate that the ash is almost insoluble in acid. There is only a negligible amount of loss in ash. Importance of this parameters was explained previously in a mercury based siddha medicine Poora Parpam [22]. The average size of the LC.1 and LC.2 was determined by Zeta sizer Nano size and was found to be 832.7 nm and 676.7 nm, respectively. Nano-sized particles will enhance smooth flowing within the digestive tract without producing any irritation [23].

GC-MS

Fresh lemon juice and cinnabar-soaked lemon juice were lyophilized. One gram of both lyophilized lemon powder sample were dissolved in 10 mL of methanol extract separately. GC-MS analysis of methanol extract of lemon juice and processed juice were presented in Table 3. About 49 compounds are reported in raw lemon juice, and 42 compounds in cinnabar-soaked lemon juice. 20 compounds are present in both raw and purified lemon juice. Identified compounds with high percentage were non-volatile oil and recorded at peak area percentage of 69.8248, 9.0305, and 1.8195 whose names have been identified as 3-methyl, 2,5 furandione, pyrazole-1-(2-hydroxyethyl)-3-methyl-5-ethoxy, dimethyl dl-malate, and citric acid-trimethyl ester. Retention time is 7.60, 15.31, 11.56, and 20.46. This peaks shown in Fig. 5.

29 compounds were not found reported in the lemon juice after the detoxification process, which suggests that those compounds might have been absorbed by cinnabar to form a complex, e.g., 1-(2-hydroxyethyl)-3-methyl-5-ethoxy pyrazole with peak area of 9.0981% and retention time of 13.94. There were appearance of 22 new compounds, e.g.,

Table 1: Change in weight of silver in % at 300°C and 400°C

Name	300°C	400°C
LC.1	0	0.4979
LC.2	0	0.1675

LC: *Linga chendooram*

Table 2: Physicochemical parameters of LC.1 and LC.2

Parameter	LC.1	LC.2
Color	Orangish red	Orangish red
Odor	None	None
Moisture content %	0	0.1939
Ash %	0.6228	0.7492
Acid insoluble ash %	0.4358	0.6554
Size	832.7 nm	676.7 nm

LC: *Linga chendooram*

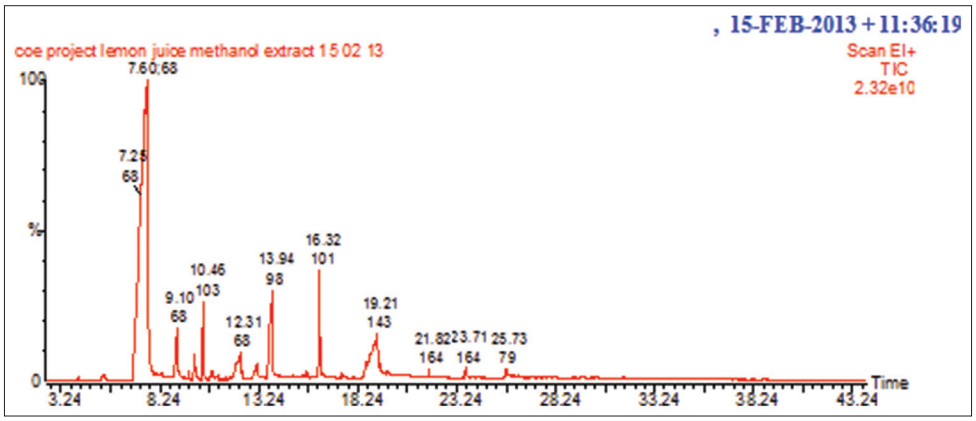

Fig. 5: Gas chromatography-mass spectrometry chromatogram of lemon juice

4-hydroxyl-1,6-dimethyl, 2(1H)-pyridione with peak area 11.657% and retention time 14.75. This peaks shown in Fig.6.

3- methyl, 2,5 furandione is chemically called citraconic anhydride. The highest peak area compound retention time is changed in both samples data. That is shown in Table 1. It is identified previously from pomegranate peel, in that research paper mentioned that it has anticancer activity [24].

FTIR analysis

FTIR spectra of raw cinnabar, purified cinnabar, LC.1, and LC.2 were obtained in the range of 4000–400 cm^{-1} in KBr pellets and are as follows. They are shown in Figs. 7-10. Functional groups detected in the samples are tabulated in Table 3.

The groups present in raw cinnabar are O-H, Amide, Aromatic,C-H. The groups present in purified cinnabar are O-H, Methyl, Aromatic, Amide, C=C, Carboxyl group. The groups present in *C. Colocynthis* are Amine, Amide, Aliphatic C-H, C-N,C=C,C-C. The Functional groups present in LC.1 and LC.2 are O-H, Amine, Amide, Carboxyl group, C=C, Aromatic group (Table 4).

O-H group is present in all the samples; this is due to the moisture content in KBr. The appearance of methyl, Aromatic, Carboxyl group could be from the lemon juice during the detoxification process. Amine and Amide peaks were found in LC.1 and LC.2 are imbibed from *C. colocynthis* juice during the preparation process. *C. colocynthis* contains Amine and Amide peak which is obtained because of Citrulline, a component of *C. colocynthis* [25]. From these results, it could be hypothesized that there is a complex formation between mercury sulfide of cinnabar and the organic functional groups of *C. colocynthis*. The same type of metallic medicine spectrum work was done for siddha medicine *Naga C*henthooram [26].

AAS RESULTS

This data is shown in Table 4. The results confirmed that mercury content was slightly reduced from raw to purified but increased in the final products. Concentration of mercury is high in LC.2 compared to other which means that the metal quantity present depends upon the preparation method. The toxic metals lead and cadmium are present only in below the detectable limit (BDL). It indicates that all the samples were free from toxic metals. Previously, one manuscript explained the importance of heavy metal toxic analysis in 2019 [27].

Table 4: AAS Report

Sample code	Hg (in%)	Lead (in ppm)	Cadmium (in ppm)
Cinnabar raw	33.8	BDL	BDL
Cinnabar purified	33.4	BDL	BDL
LC.1	34.30	BDL	BDL
LC.2	36.68	BDL	BDL

LC: *Linga chendooram*, AAS: Atomic absorption spectroscopy, BDL: Below detectable limit

Zeta sizer

Zeta sizer results of raw cinnabar, purified cinnabar, LC-1, and LC-2 are graphically represented as in Fig. 11. The size distribution of raw cinnabar varied. About 86.7% posses 448 nm and 13.3% posses 106.1 nm. The polydispersity index (PDI) of 0.667 shows that the distribution of sample

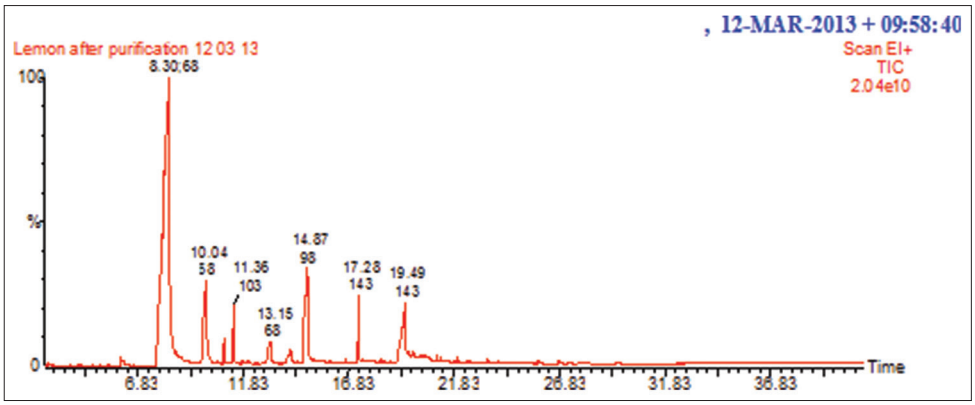

Fig. 6: Gas chromatography-mass spectrometry chromatogram of lemon juice after detoxification of cinnabar

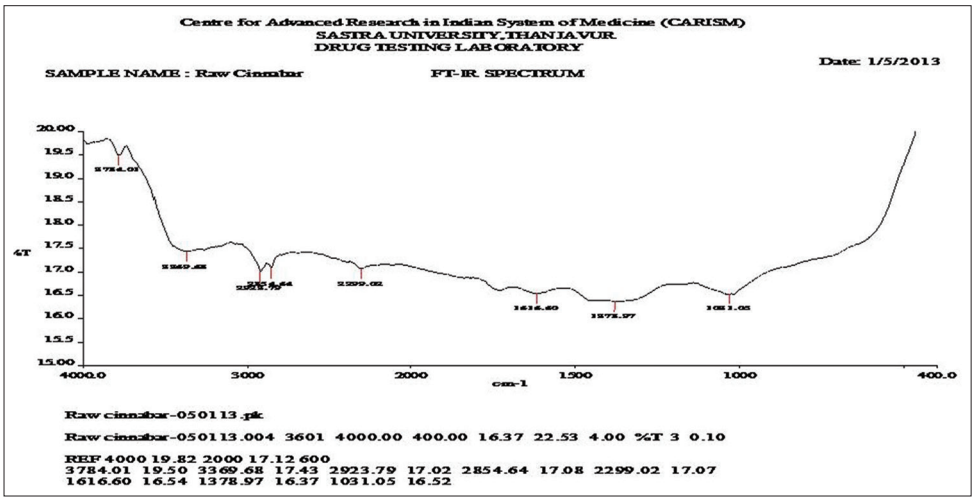

Fig. 7: Fourier transform infrared spectroscopy spectrum of raw cinnabar

size is vast. In the purified cinnabar, 60.5% posses 4103 nm and 39.5% posses 607 nm, and this shows an increase in particle size. The PDI is similar to raw cinnabar. LC-1 shows that 100% posses 865.9 nm which may be due to the aggregation of nanoparticles which are much reduced which is confirmed by PDI of 0.028. In LC-2, confirmed 91.8% of particles possess 676.7 nm but the PDI of 0.497 shows broad sample distribution compared to that of LC-1. This same type of work was carried out for a Ayurvedic medicine Yasatha Bhasma [28].

Table 3: Peak area difference between fresh and processed juice

Sample	Retention time	Peak area %
Fresh juice	7.60	69.8248
Processed juice	8.30	63.4365

Table 4: Functional groups detected in the samples

Raw cinnabar	Purified cinnabar	LC
O-H	O-H	O-H
Aromatic group	$-CH_3$	Amine
Amide	$-OCH_3$	Carboxyl
C-H	Amide	Carboxy
C-H	Amide	C=C
	C=C	
	Carboxy	

LC: *Linga chendooram*

LC-1 shows a monodisperse distribution (~865 nm) in contrast to the polydisperse raw and purified cinnabar, indicating formation of aggregated nano-scale particles post-processing.

Zeta potential

Raw cinnabar shows a zeta potential of −32.7 mV; hence, the particles are stable in colloidal solution and can be used for biological system infusion. The charge of purified cinnabar has reduced to −17.8 mV, and the particles are moderately stable. The LC-1 shows a zeta potential of −22.0 mV, and LC-2 shows a zeta potential of −33.5 mV. This shows that particles are stable in colloidal solution. Therefore, it is said to be biocompatible. This results shown in Fig. 12.

Values more negative than ±30 mV (as observed in raw cinnabar and LC-2) suggest good electrostatic stabilization and a stable colloidal suspension.

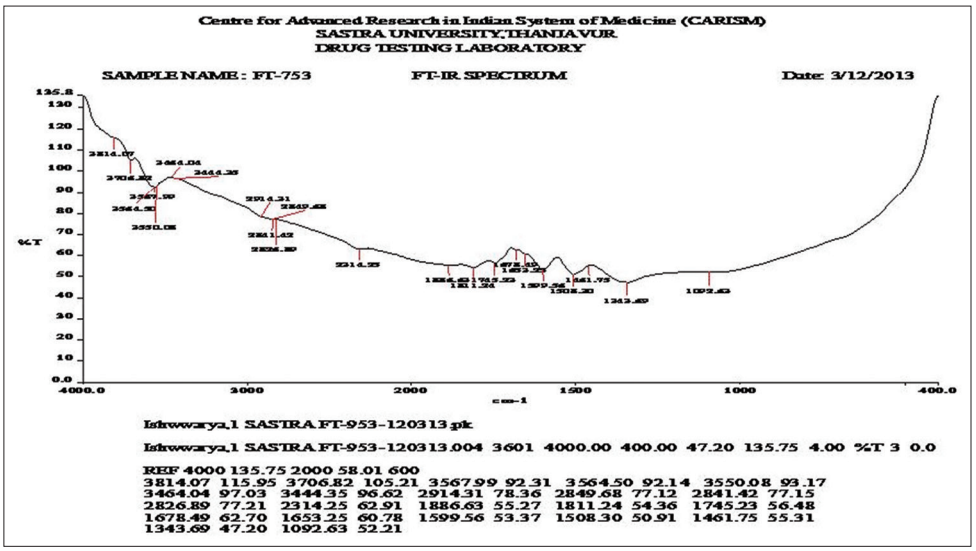

Fig. 8: Fourier transform infrared spectroscopy spectrum of purified cinnabar

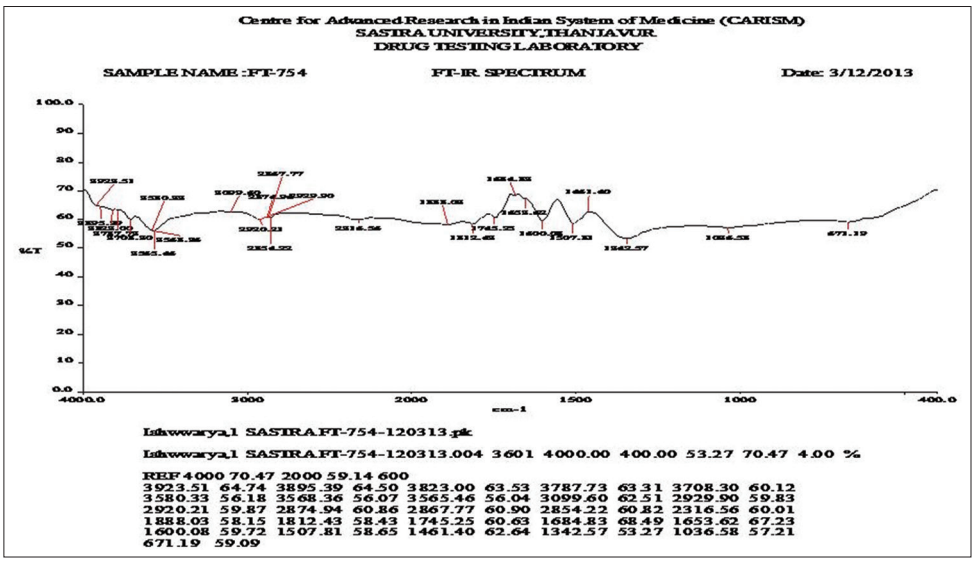

Fig. 9: Fourier transform infrared spectroscopy spectrum of *Linga chendooram*-1

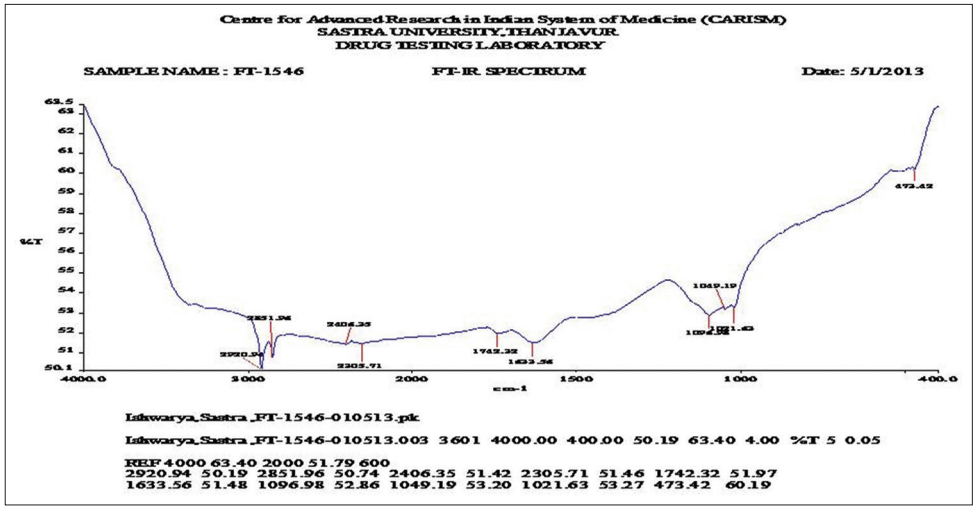

Fig. 10: Fourier transform infrared spectroscopy spectrum of *Linga chendooram*-2

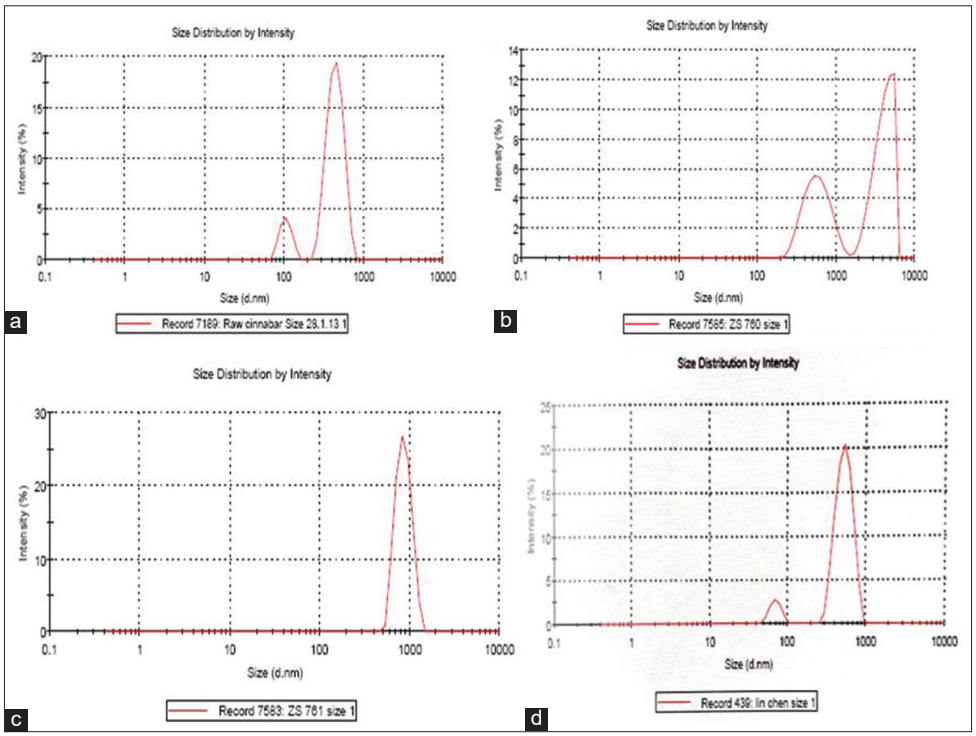

Fig. 11: (a-d) Particle size distribution by dynamic light scattering

TG-DTA

The TG-DTA analysis was done for purified cinnabar and the final product of LC. The intermediate samples for TG-DTA were taken to understand possible phase changes occurring during the process. The following are the graphical representations of the data.

Pure cinnabar phase diagram shows that there is degradation only at 433.8°C and absence of intermediate peaks indicates that no phase transformations have taken place. This results shown in Fig. 13. There is no free sulfur present in the compound as melting point of sulfur is 119°C and no peaks have been obtained at that range. After each stage, the degradation temperature of the product decreased. Impurities were not found in the sample. The treatment process has reduced the thermal stability from 433.8°C of purified cinnabar to 396°C of the final product. No phase transition curves have been observed apart from the degradation curve. No degradation and no phase change render the product to be stable. The increase in stability increases the bioavailability of the drug. The importance of this test was explained in one manuscript in 2017 [29].

The reduction in degradation temperature in LC samples in comparison to purified cinnabar suggests structural modification or organic association resulting from traditional preparation.

SEM

The images of raw cinnabar and final product (Fig. 14) were obtained at different resolutions. These micrographs serve to understand the influence of preparation process on the morphology of the starting material. The particles are observed to be more spherical. The image of LC shows coagulation of particles which is a result of complex formation which formation was received after the burning process. Previously, Ayurvedic metallic medicines explained the importance of

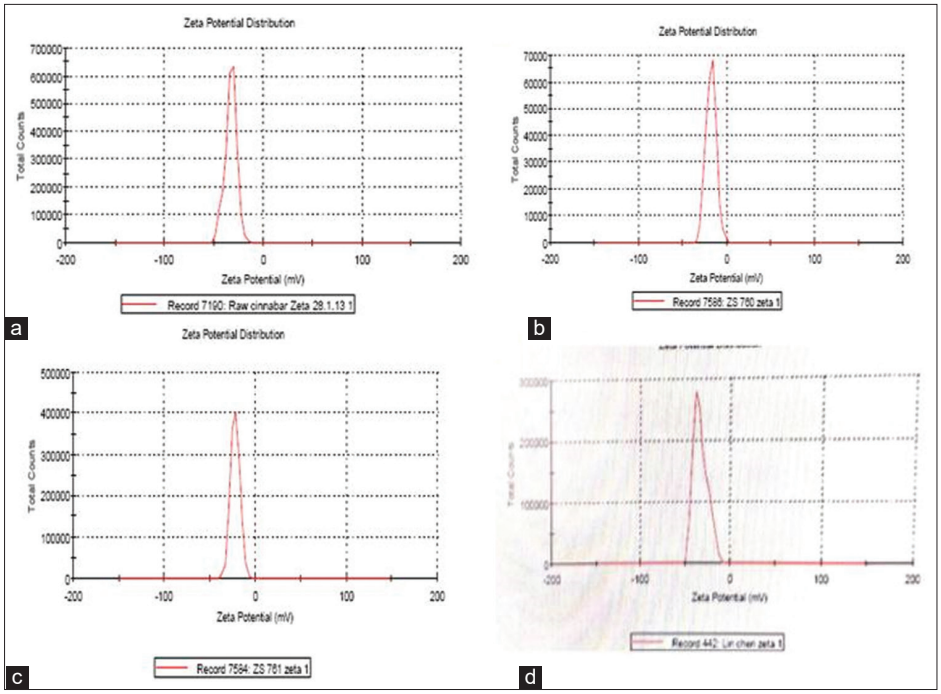

Fig. 12: (a-d) Zeta potential analysis of raw cinnabar, purified cinnabar, *Linga chendooram* (LC)-1, and LC-2

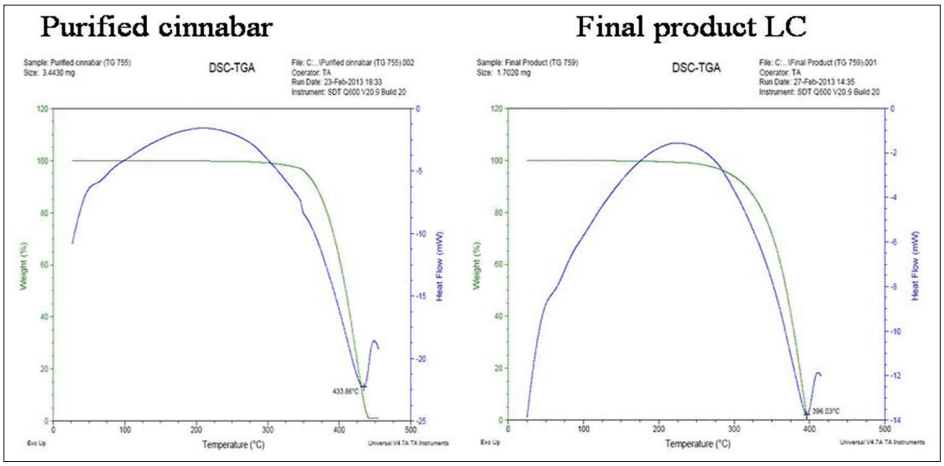

Fig. 13: Thermogravimetric-differential thermal analysis thermograms of purified cinnabar and final *Linga chendooram* products

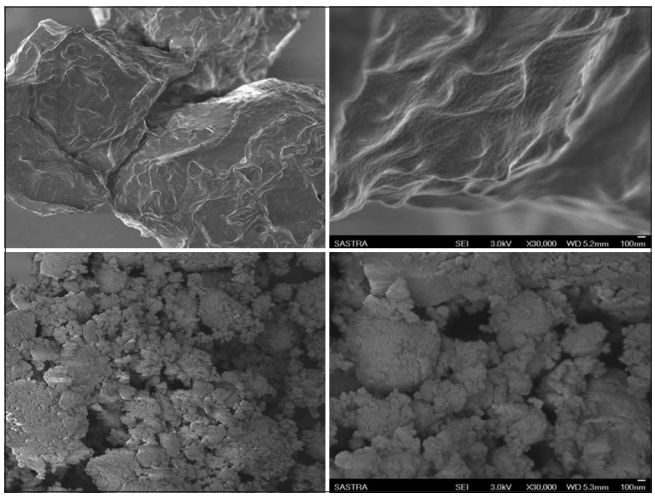

Fig. 14: Scanning electron micrographs showing morphological transition

the burning process [30,31]. The complex formed could be between the main HgS and the organic group of the herb used during the preparation process.

Scanning electron micrographs showed morphological transitions during processing. (A–B) Raw and purified cinnabar exhibit irregular and heterogeneous particle morphologies. (C–D) LC samples (LC-1 and LC-2) display aggregated, more spherical, and compact structures, reflecting morphological changes consistent with the formation of a processed herbo-mineral matrix during traditional calcinations.

CONCLUSION

LC is a very popular and well-therapeutic metallic medicine in Siddha system of medicine. In detoxification part, the impurities were removed and enhanced the mercury content. Compounds from lemon juice could have imbibed by the cinnabar, resulting in disappearance and appearance of new compounds. This observation can be hypothesized from GC-MS report. Physicochemical standardization data confirm the absence of free mercury in the *chenthooram*. The TG-DTA and Zeta-

sizer results conclude that the product is stable and can be used for biological system infusions. The fall in temperature of the final product predicts that some impurities would have been removed during the process. From FTIR results, it is observed that the amine group present in the final product indicate that phyto-chemicals might have formed a complex with cinnabar. AAS data indicate that all the samples were free from toxic metals. The SEM images confirmed the transformations in the morphology of the starting material which is due to the preparation process. The final product is coagulated which serves as evidence of complex formation. The present study contributed to proving the non-toxic nature and bio-compatibility of mercurial *siddha* preparations. This is because of the various treatment and preparation processes. The toxic mercury may be converted into a biocompatible form due to the chemical transformations and surface morphology changes that occurred during the process.

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AUTHOR'S CONTRIBUTION

Sriram S- Instrumental analysis. Aishwarya S Aier- Project student. Rajalakshmi P- Medicine preparation, Manuscript writing. Brindha P- Guide to the project.

CONFLICT OF INTEREST

Nil.

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