ORIGINAL ARTICLE

Preparation and characterization of an ayurvedic metallo mineral preparation – *Mallasindura*

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Abstract

Background: Mercurial preparations known by the name of *Kupipakwa Rasayana* are prepared in a glass bottle by adopting gradual temperature gradient. **Aims and Objectives:** To standardize the manufacturing process and develop an analytical profile for *Mallasindura*. **Materials and Methods:** *Mallasindura* was prepared by heating a mixture of *Parada* (mercury), *Gandhaka* (sulfur), and *Malla* (arsenic) in a *Kupi* (glass bottle covered with mud smeared cloth) placed in an electric muffle furnace. Final product deposited at the neck of bottle was collected as *Mallasindura* and analyzed on various physiochemical parameters along with scanning electron microscopy and energy dispersive X-ray (EDAX), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) analysis. **Results and Conclusion:** *Mallasindura* was prepared by adopting gradient temperature pattern for 24 h, i.e., 8 h of mild (120-250°C), moderate (250-450°C) and strong heating (450-600°C) each. Average yield of *Mallasindura* is 151.33 g (37.83%) using 400 g *Kajjali* in each batch. XRD analysis showed that *Mallasindura* is mercury sulfide having an empirical formula of HgS and hexagonal crystal structure. Elemental composition of *Mallasindura* by EDAX showed the presence of 80.85%, 11.06% and 6.13% of mercury, sulfur and arsenic, respectively along with other trace elements. FTIR analysis revealed presence of a large number of functional groups.

Key words: Characterization, *Kupipakwa Rasayana*, *Mallasindura*

INTRODUCTION

Rasashastra, a branch of Ayurveda has been evolved on the principles of converting metals and minerals into their bio-available forms and thus making them therapeutically viable. Recently, *Rasashastra* formulations *viz*. mercurial preparations have been in limelight for concerns over their quality and safety.[1] Pharmaceutical standardization of these formulations is one among the measures to ensure their safety and efficacy. In general, polyherbal formulations have multi-molecules acting simultaneously. Similarly, metallic preparations also act by the virtue of their physical structure and chemical properties. To know the exact mode of action of a particular drug, it is necessary to know the accurate chemical structure of the active molecule. Therefore, it becomes necessary to identify the moiety involved in various physiological processes.

Mercurial preparations known by the name of *Kupipakwa Rasayana* are prepared in a glass bottle by adopting gradual temperature gradient. *Rasasindura*,[2] *Makaradhwaja*,[3] *SwarnaVanga*,[4] and *Mallasindura*[5] are few often used medicaments prepared by above method. *Mallasindura* is a well-known drug for its use in various diseases like bronchial asthma, bronchitis, rheumatoid arthritis, diabetes mellitus,

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etc.[5] As far as treatment with *Rasaushadhis* is concerned, along with *Rasasindoora*, it forms an indispensible part of ayurvedic repertory for physicians. *Mallasindura* is prepared by heating a combination of *Parada* (mercury), *Gandhaka* (sulfur) and *Malla* (arsenic trioxide) in a glass bottle. Very few published literature is available regarding its standardization and characterization. Hence, here an attempt is made to lay down standard manufacturing process as well as to analyze its physicochemical properties.

MATERIALS AND METHODS

Materials

Raw materials *viz*. *Parada*, *Gandhaka* and *Mall* including required equipment such as *Kupi* (glass bottle coated with seven layers of mud-smeared cloth), vertical electric muffle furnace (EMF), iron spoke, and big iron rod were collected from Pharmacy, Gujarat Ayurved University, Jamnagar

Method

The preparation of *Mallasindura* was divided into three stages, namely, preparatory stage, principal stage and concluding stage.

Preparatory stage

Purification of raw materials

Parada was purified by triturating it with lime for 3 days and then again triturating it with garlic paste and rock salt followed by washing with hot water.[6] *Gandhaka* was purified by subjecting it to *Dhalana* (melting and pouring in media) process in *Godugdha* (cow's milk) for 3 times.[7] Purification of *Malla* was done by *Swedana* (steaming under media) method in a *Dolayantra* (heating under liquid media), whereby small pieces of *Malla* were tied in a four folded cotton cloth and suspended over an earthen vessel containing *Karavellaka Swarasa* (expressed juice of *Momordica charantia* Linn. fruits) for two *Yama* (6 h). After self-cooling of the pot, pieces of *Malla* were obtained and washed carefully with hot water and dried.[8]

Preparation of Kajjali (fine black powder)

Shuddha Parada and *Shuddha Gandhaka* were taken in 1:1 ratio and triturated in an iron mortar until the whole mixture was converted into *Kajjali*. Then *Shuddha Malla* was added half the quantity of *Parada* to the *Kajjali* and again it was triturated. This *Kajjali* was filled in the *Kupi* and placed in the center of EMF in such a way that it could receive equal distribution of heat.

Principal stage

Mallasindura was prepared in three batches of 400 g *Kajjali* by following *Kramagni* (gradual intermittent increase in temperature) pattern. Temperature pattern adopted for *Mallasindura* was 8 h of mild (120-250°C), 8 h of moderate (250-450°C), and lastly 8 h of intense (450-600°C) heating [Figure 1]. Different observations like mild white fumes, thick yellow fumes, blue flame, etc. were recorded during the process. Accumulated sulfur at the neck was burnt by rubbing *Tapta Shalaka* (red-hot iron rod) repeatedly with the neck of bottle. Confirmative tests like cessation of flame, red hot appearance of the bottom, etc. were ascertained before corking (600°C) and the temperature was increased to around 50°C to facilitate sublimation of the final product at the neck. The bottle was removed from the furnace after adequate cooling.

Concluding stage

Kupi was cautiously removed from EMF. The layers of wrapped cloth were carefully scraped, bottle was broken and the product deposited at the neck was judiciously collected. [Figures 2-5]. The whole process was repeated in three batches.

Analytical Profile

Chemical characterization of finished sublimated product *Mallasindura* was subjected to various organoleptic and quantitative parameters such as texture, color, taste, odor, pH, ash value, acid-insoluble ash, water soluble ash, loss on drying, and percentages of mercury, sulfur and arsenic. Advanced analysis like scanning electron microscope with energy dispersive X-ray spectroscopy (SEM-EDAX),

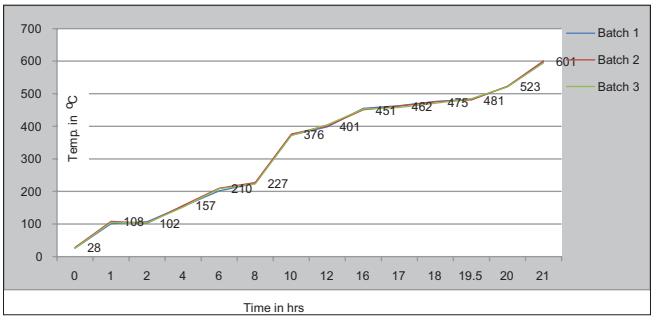

Figure 1: Temperature pattern of *Mallasindura* of three batches

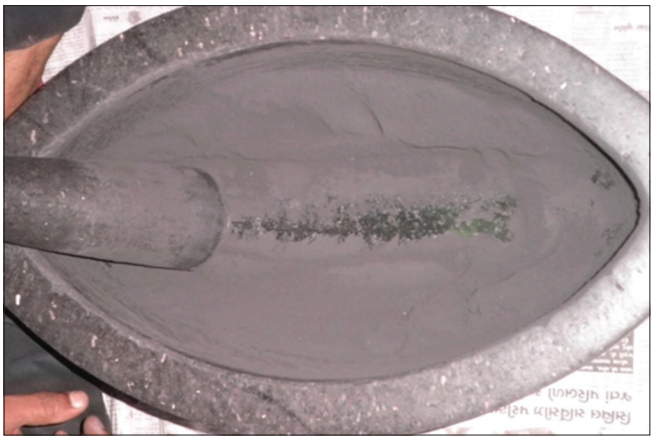

Figure 2: Trituration of *Kajjali*

Figure 3: Filling of *Kajjali* in *Kupi*

Figure 5: Finished product, i.e., *Mallasindura*

Figure 4: *Kupipakwa* in electric muffle furnace

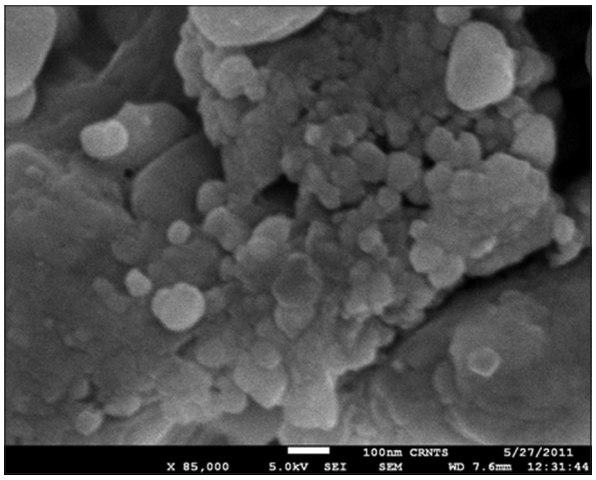

Figure 6: Scanning electron microscope images of *Mallasindura*

crystallography by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were also carried out to confirm the composition of *Mallasindura*.

OBSERVATIONS AND RESULTS

Different stages during the process like fuming, melting of *Kajjali*, flaming, confirmative test for completion of product formation (flame disappearance, *Shita Shalaka* test, copper coin test, red hot appearance of the bottom, etc.) were observed and recorded [Table 1]. *Mallasindura* collected at the neck of the *Kupi* in all three batches were weighed and calculated for the percentage of yield [Table 2]. Results of various organoleptic and physicochemical analyses are shown in Tables 3 and 4. Photomicrographs of SEM-EDAX, crystallography by XRD and FTIR studies have been shown in Figures 5-11.

DISCUSSION

Mallasindura is a *Kupipakwa Rasayana* formulation which is prepared by heating a mixture of few essential materials in a glass bottle with graded pattern of mild, moderate and intense heat in an EMF.[9] Various references of *Mallasindura* are found in classical texts of *Rasashastra*. The reference[5] selected for present study is from one of the scheduled books of Drugs and Cosmetics Act, 1945.[10] Furthermore, studies on pharmaceutical standardization and characterization of other *Kupipakwa Rasayanas viz*. *Rasasindoora*,[11] *Makaradhwaja*[12] and *Swarna Vanga*[13] have been reported by various scholars. Therefore, it is the need of hour to generate analytical profile for at hand reference of *Mallasindura*.

The following classical guidelines, *Shodhana* (purification) of *Parada*, *Gandhaka* and *Malla* was done as a preliminary mandatory process. *Shodhana* ads to therapeutic activity and curtails potential adverse effects of the purified drug.[14] All the ingredients of the formulation were triturated to form an admixture of fine powder, i.e., *Kajjali*. Seven layers of cloth soaked in clay were wrapped over the bottle to strengthen the bottle and protect it from breaking during the process. *Kajjali* was filled up to 1/3rd of the bottle which was 400 g, and kept constant for all the three batches.

Table 1: Observation during *Kupipakwa*

Time in h	Observations	First batch temperature (°C)	Second batch temperature (°C)	Third batch temperature (°C)
0	EMF started	26	28	26
1	No specific observations	101	108	105
2	Mild sulfur smell is felt	107	102	103
4	Whitish fumes seen. Partial melting of *Kajjali*	154	157	152
6	*Kajjali* completely melted	202	210	208
8	Dense fumes with sulfur smell	225	227	223
10	Dense fumes with sulfur smell	373	376	372
16	Dense yellow fumes seen	455	451	453
17	White fumes observed	463	462	458
18	Hot iron rod probing done to remove *Kupi* neck obstruction due to sublimated *Gandhaka*. Flame appears at the neck which is of 2-3 inches in length	476	475	471
19.5	Flame decreases in height. Hot iron rod probing done after every 5 min	483	481	485
20	Flame 1 inch in height outside the *Kupi*	522	523	521
21	Coin test positive. No sulfur seen in coin test. Few particles of mercury seen. Cold *Shalaka* probing test positive. Corking done	597	601	595
24	EMF stopped	613	608	611

EMF: Electric muffle furnace

Table 2: Weight and yield of *Mallasindura*

Weight of *Kajjali* (g)	Weight of product (g)	Yield (%)
400	152	38.00
400	156	39.00
400	146	36.50
Average	151.33	37.83

Table 3: Results of organoleptic tests

Parameters	Texture	Color (after trituration)	Taste	Smell
Mallasindura	Compact	Vermillion	Tasteless	Indistinct

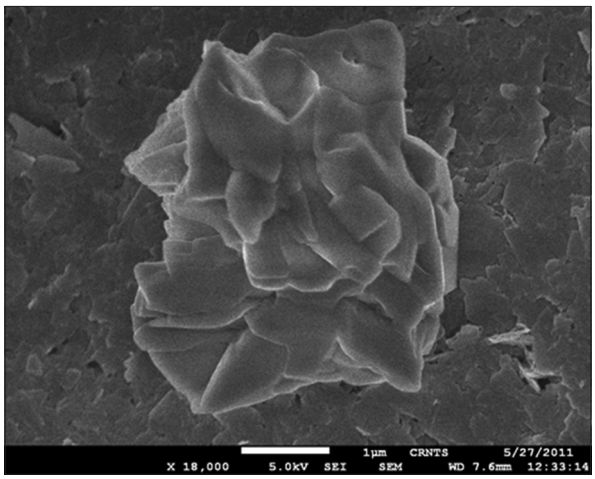

Figure 7: Scanning electron microscope images of *Mallasindura*

Graded heating pattern in *Kupipakwa* is essential, as the transformation of *Kajjali* to *Mallasindura* is dependent on time and temperature pattern. In *Mrudu Agni* (120-250°C) stage, light white fumes due to sublimation of *Malla* as well as light sulfur fumes are observed followed by melting of *Kajjali* at around 200-210°C. During *Madhyama Agni* (250-450°C) profuse sulfur fumes are seen followed by constant boiling of *Kajjali*. In the stage of *Teevra Agni* (45t00°C), profuse white fumes are observed at around 460°C, *Malla* normally vaporises when heated and escapes along with sulfur during *Kupipakwa*.

Flame appears at the neck of *Kupi* indicating burning of excess sulfur at around 470°C. Deposition of sulfur around neck of *Kupi* was cleared by insertion of hot iron rod repeatedly. Once the flame subsides, observations like complete cessation of sulfur fumes, *Sheeta Shalaka* test (insertion of iron spoke in the bottle to confirm complete burning of free sulphur) were undertaken. When *Sheeta Shalaka* appears sticky and black, indicates the presence of sulfur, but when it is dry and reddish orange in color, test is said to be positive indicating completion of the process. Copper coin test (copper plate is placed at the mouth to detect the free sulfur or mercury) is done to test the absence of free sulfur. If, mercury particles adhere to copper coin, it decolorizes coin and is said to be positive.

Table 4: Results of physicochemical tests

Tests	pH value	Ash value (%)	Acid insoluble ash	Loss on drying at 110°C (%)	% Mercury	% Sulfur	% Arsenic
Batch 1	6.2	0.17	0.54	0.7	80.15	11.37	6.3
Batch 2	6.3	0.13	0.61	0.4	82.62	10.72	5.7
Batch 3	6.2	0.22	0.32	0.5	79.80	11.11	6.4
Average	6.23	0.17	0.49	0.53	80.85	11.06	6.13

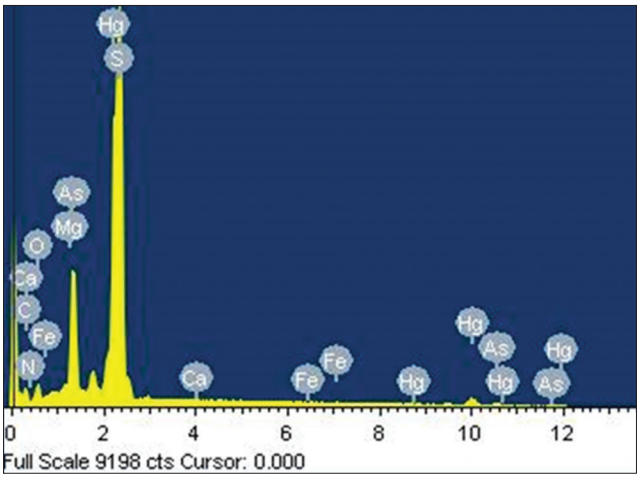

Figure 8: Energy dispersive X-ray profile of *Mallasindura*

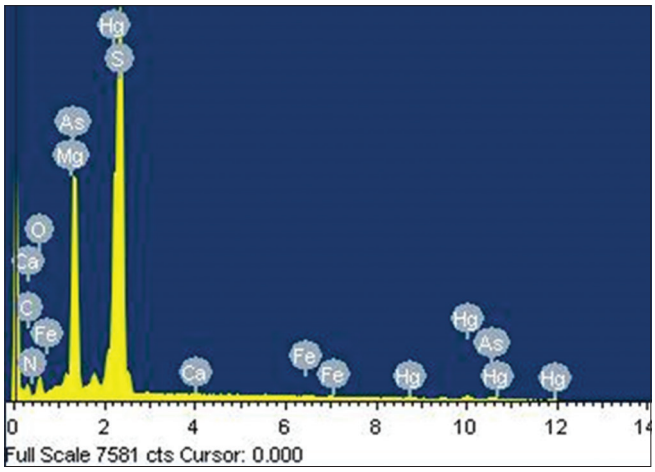

Figure 9: Energy dispersive X-ray profile of *Mallasindura*

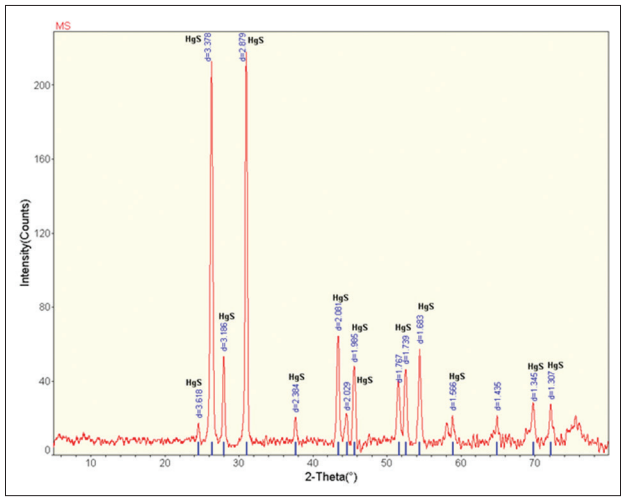

Figure 10: X-ray diffraction pattern of *Mallasindura*

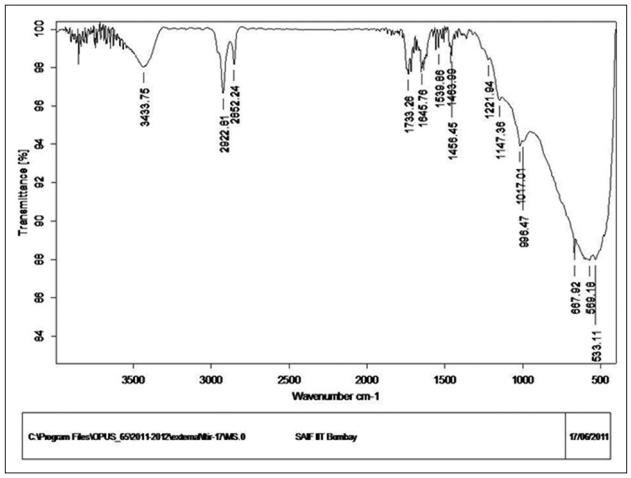

Figure 11: Interferogram of *Mallasindura*

Red hot appearance of the bottom is also a confirmative test as an indication of complete formation of compound. Thereafter, corking over the mouth of *Kupi* is done to ensure the sublimation of product followed by increasing the temperature [Table 1]. Optimum heat is necessary at later stages of *Kupipakwa* because low temperatures reduce chances of sublimation whereas high temperature can lead to dissociation of compound. Proper precautions are also to be taken by covering eyes and nose because arsenic and sulfur fumes produced during *Kupipakwa* can prove hazardous if inhaled.

Dark red colored *Mallasindura* collected at the neck of *Kupi* was collected judiciously after self-cooling of EMF on the following day. The complete process was repeated in two more batches for the development of standard operating procedure. On an average 37.83% of *Mallasindura* was obtained in three batches [Table 2]. On trituration, *Mallasindura* turned into bright red color and was tasteless [Table 3].

Chemical tests show that *Mallasindura* is slightly acidic in nature with traces of organic components. Low percentages of ash value, acid insoluble ash and loss on drying indicate the inorganic nature of *Mallasindura* with almost negligible amount of trace elements and moisture content. Gravimetric analysis for three major elements showed their presence in different percentages [Table 4]. Chemically *Mallasindura*

is a by-product of mercury, sulfur and arsenic trioxide. Therefore, these elements were analyzed in the finished product. An SEM is used to get the images of samples by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that produces the signals which contain information about the sample's surface topography, composition, and other properties such as electrical conductivity. This study revealed the particle size of *Mallasindura* in a range of 10-15 µ. EDAX is a popular technique used nowadays for the estimation of macro-elements in herbo-mineral formulations. An EDAX spectrum plot not only identifies the element corresponding to each of it speaks, but the type of X-ray to which it corresponds as well. In the present work, this technique was effectively applied for estimation of gross elements present in *Mallasindura* [Table 5]. This analysis showed that elements present in *Mallasindura* were mercury (44.45-57.70%), sulfur (19.79-23.77%), arsenic (12.26-21.17%), carbon (6.51-6.88%), and oxygen (3.37-4.11%). Topography is found to be same in all samples. The images show a spongy structure with particle size lying in the micro range. From the images, it is clear that several crystallites are agglomerated in a single particle giving rise to micro crystalline structure with loss of grain boundaries [Figures 6-9].

Crystallography by XRD was carried out to chemically characterize the sublimated part as well as to identify the major phase present in the compound. XRD pattern of *Mallasindura* showed peaks only due to mercury sulfide. It has an empirical formula of HgS and hexagonal crystal structure. Arsenic is also present in very less amount [Figure 10].

FTIR was performed to detect the presence of functional groups or organic ligands in *Mallasindura*. FTIR spectrum was taken in the region of 400-4000/cm. A general overview of the sample indicates the presence of large number of functional groups [Figure 11]. Fairly sharp peaks were obtained at and around 500, 900, 1400-1700, 2800, and 3400/cm. These peaks indicate the presence of organic compounds in *Mallasindura*.

Arsenic has a boiling point of 465°C. It sublimes at 193°C and melts at 312°C. So by the time complete sublimation of HgS is achieved, most of the arsenic is lost in form of vapors and left in traces in the finished product which is also the optimum quantity of arsenic required for desired therapeutic effect. This not only significantly reduces the toxicity of *Mallasindura* but also make it useful in treating a majority of ailments.

Table 5: Results of EDAX study

Element	Weight %	Atomic %	Weight %	Atomic %
C K	6.88	30.93	6.51	26.50
O K	3.37	11.38	4.11	12.57
S K	19.79	33.32	23.77	36.27
As L	12.26	8.83	21.17	13.82
Hg M	57.70	15.53	44.45	10.84
Total	100		100	

EDAX: Energy dispersive X-ray

CONCLUSION

Mallasindura was prepared by adopting gradient temperature pattern for 24 h, i.e., 8 h of mild, moderate and severe heating each. Average yield of *Mallasindura* is 151.33 g (37.83%) using 400 g *Kajjali* in each batch. XRD analysis showed that *Mallasindura* is mercury sulfide having an empirical formula of HgS and hexagonal crystal structure. Elemental composition of *Mallasindura* by EDAX showed the presence of 80.85%, 11.06% and 6.13% of mercury, sulfur and arsenic respectively along with other trace elements. FTIR analysis revealed presence of a large number of functional groups. Hence, it can be concluded that process not only significantly reduces the toxicity of *Mallasindura* but also make it useful in treating a majority of ailments.

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