Research article

Standard manufacturing procedure and characterisation of *Rasasindoora*

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Abstract:

Rasasindoora is widely prepared and clinically practised by *Ayurvedic* fraternity. It is a mercurial preparation prepared by treating specific ratio of mercury and Sulphur in a glass bottle in traditional furnace by graded heating pattern over stipulated duration. In the present paper, by laying standard procedures an attempt has been made eliminate controversies and technical hitches for its preparation on pharmacy scale. Results of 3 batches concluded that maximum 13 hrs of heating in a graded pattern of 2, 4 and 7 hrs for mild, moderate and stern heating is sufficient for preparing an average amount of 1.9 kg *Rasasindoora* from 3.5 kg of *Kajjali* with utilization of 21.67 kg of fuel in terms of coal and charcoal in a traditional furnace. Analysis of finished product for its characterization on refined instrumentations like XRD, EDAX and FTIR revealed that it is essentially a mineral compound having organic ligands and is chemically mercuric sulphide (HgS) with particle range between 10-50 μm and having mercury as 79.41 % and Sulphur as 20.59 % by weight.

Keywords: *Rasasindoora*, *Kajjali*, Mercuric sulphide

Introduction:

Rasashastra (Ayurvedic Pharmaceutics) is a blend of modern chemistry and pharmaceutical science. It aims at designing novel drugs with better therapeutic attributes at minimum doses. Due to lack of standardization, quality control and chemical characterization, the herbo-mineral or metallo-mineral formulations were overlooked and a lot of chaos was made pertaining to their toxicity.

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Rasasindoora is widely prepared and clinically practised by *Ayurvedic* fraternity. It is used as a single drug or in conjugation with other metallic *Bhasmas* (incinerated metals) and herbal drugs. The therapeutic repertory ranges from treating diseases like syphilis, genital disorders, respiratory infections, alleviating aging, in form of aphrodisiac and rejuvenator (1). It is a mercury based preparation prepared by treating specific ratio of mercury and Sulphur in a glass bottle in traditional furnace by graded heating pattern over stipulated duration. Laying standard procedures will eliminate controversies and technical hitches for its preparation on large scale. Chemical characterization has been undertaken by various authors (2). Therefore, in the present work, the finished product was analysed for its characterization on refined instrumentations to validate previous works.

Shodhana (purification) and *Bhavana* (levigation) are integral mandatory prerequisites for preparation of any metallic formulation in *Rasashastra*. In the present study, preparation of *Rasasindoora* was done from purified mercury and Sulphur following the guidelines of Ayurvedic Formulary of India (3).

Methodology:

Preparation of *Rasasindoora*:

To lay standard guidelines, a proforma was prepared and all the observations were recorded therein. 3 batches of *Rasasindoora* sample was prepared from raw materials obtained from the pharmacy of Gujarat Ayurved University, Jamnagar. Mercury so obtained was purified by triturating it with equal quantities of lime and garlic paste and half the quantity of rock salt (4). For purification of the Sulphur, the traditional method using cow's milk and ghee (a milk preparation) was employed. In this method, Sulphur mixed with ghee was heated up to its melting temperature and the resulting liquid is poured through a ghee smeared cotton cloth into a vessel containing boiled milk. Sulphur was on the bottom of this vessel. This process was repeated three times and the final deposited product was taken out, washed with hot water and dried (5).

Composition of *Rasasindoora* has been shown in Table 1. Mercury and Sulphur thus purified in the ratio (1:1) were levigated in decoction of *Vatajata* (aerial roots of *Ficus benghalensis* Linn.) for 3 times. This mixture was placed in an iron mortar and triturated till the whole mixture was converted into a fine black, lusterless powder (*Kajjali*). After complete drying, it was filled in a glass bottle (*Kupi*), previously coated with 7 layers of mud smeared cloth (Fig. 1) and heated on a traditional furnace ignited with charcoal in a controlled intermittent manner with gradually increasing temperature till the blue flame emerging (Fig. 2) from the pot disappeared and the bottom of the bottle becomes red hot. A red hot iron rod was repeatedly inserted in the neck of the bottle so as to burn any accumulated Sulphur at the neck of the bottle. After adequate cooling, the sublimate deposited at the neck of the bottle was collected (Fig. 3). A pyrometer was employed to record the temperature of traditional furnace at regular intervals. *Kajjali* was subjected to DSC (Differential Scanning Calorimetry) analysis. Chemical characterization of finished sublimated product, *Rasasindoora*, collected from the neck of *Kupi* was subjected to various organoleptic and quantitative parameters and also by SEM – EDAX (Scanning Electron Microscope with Energy dispersive X-ray spectroscopy), Crystallography by XRD (X-ray Diffraction) and FTIR (Fourier Transform Infrared Spectroscopy).

Discussion:

Traditional furnace was chosen to prepare *Rasasindoora* as it is difficult to do the same in an Electric Muffle Furnace for a large scale production. Moreover, even today many pharmacies employ the traditional *Valuka Yantra* (Iron pot filled with sand) and furnace for preparing *Rasasindoora*. *Kupi* was inserted into the sand of *Valuka Yantra* and placed inside the furnace in such a way that neck of *Kupi* remains just outside the sand. The furnace was ignited from below. Rod of pyrometer was inserted into the sand of the *Valuka Yantra*. A blower was also utilized to facilitate the increase in temperature. Procedure of *Paka* was initiated with 4 kg of coal and 2 kg of charcoal. Thereafter at definite intervals, certain fixed amount of fuel was added for maintaining and increment of temperature.

Observations recorded have been tabulated in Table 2. Around 150 – 250 °C, light whitish fumes are observed around the neck of bottle. This is the stage where whole of the *Kajjali* melts. After 2 hrs, the fumes turns more dense and at around 350-400 °C, mercury sulphide melts. At around 450 – 500 °C, fumes turn dense yellow in colour and molten *Kajjali* begins to boil. There are chances of spillage at this stage, so utmost care must be taken. Around same temperature, flame appears at the neck of Kupi (Fig. 2). Flame indicates burning of excess Sulphur from the compound. It goes on increasing with time and can go upto 4-5 feet in height and lasts around 1½ - 2 hrs (Table 4).

It is necessary to gradually increase the temperature during this duration to ease out the burning of excess Sulphur. Concurrent use of hot iron rod is also advised to clear the neck of *Kupi* from time to time to avoid chocking. Once the flame subsides, necessary tests are performed. Bottom of *Kupi* becomes red hot. Particles of compound (HgS) can be seen in brownian motion inside the bottle through a torch. They form an appearance like that of honeycomb. On placing a cold thin iron rod inside the *Kupi* for 1 minute, it gets coated with the compound and turns greyish. If it remains black, indicates that unburnt Sulphur is still in the finished product. On placing a copper coin over the mouth of *Kupi*, it turns white on inner side due to deposition of mercurial compound. These signs signify sublimation and dissociation of product into its components. Around 650 – 700 °C, flame ceases with presence of aforesaid signs. Thereafter, a cork is to be placed over the mouth of *Kupi* and sealed with mud smeared cloth. Simultaneously, furnace should be loaded with fuel and with the help of blower, temperature is increased. It ensures proper sublimation of finished product around neck of Kupi. For better yield, some amount of sand around neck of *Kupi* should be displaced.

On an average, 6 kg of charcoal and 15.67 kg of coal was utilized for complete process. Total fuel amounted to 21.67 kg (Table 3).

Mild temperature can be considered between 150-250 °C and maintained for 2 hrs. Similarly, moderate temperature of 250-450 °C can be maintained around 3-4 hrs and stern temperature of 450-700 °C for 6-8 hrs (Table 4).

Total duration of 11-13 hrs is sufficient for preparing *Rasasindoora* from a *Kajjali* of 3.5 kg. Average weight of sublimated part was 54.29 % amounting to 1.9 kg. However, as per law of definite proportions, it was found to be 93.14 % (Table 5). After self cooling, the area of sublimated was marked and *Kupi* was broken accordingly.

Analysis of *Kajjali* revealed total mercury as 57.72 % w/w and carbon disulphide soluble extractive as 38.61 % w/w. Nearly, half amount of mercury indicates that *Kajjali* had equal proportions of mercury and Sulphur. Carbon disulphide soluble extractive indicates unbound Sulphur in *Kajjali* (Table 6).

Organoleptic analysis of sublimated part of all the three batches showed that *Rasasindoora* was compact in nature, crystalline, gritty on feel, tasteless on flavour and brick red in colour (Table 7).

Quantitative analysis sublimated part i.e. *Rasasindoora* revealed total mercury as 81.36 %. Loss on drying (at 110°C), ash value (at 650 °C) and water soluble extractive were 3.9 %, 6.5 % and 5.3 % w/w respectively. Carbon disulphide soluble extractive was 0.08 % w/w indicative of relatively no free Sulphur in the finished product (Table 8). Earlier works on quantitative analysis of *Rasasindoora* has shown total mercury between 83 – 84 %. This is very much in accordance with the present work (6).

Differential Scanning Calorimetry (DSC) is unsurpassed for understanding the stability of biological systems. DSC directly measures heat changes that occur in bio-molecules during controlled increase or decrease in temperature, making it possible to study materials in their native state (7). DSC analysis was carried out to evaluate the melting point of *Kajjali*. The data shows two peaks between at 112.5 and 116.68^{o}C. This could possibly be the temperature range of melting of *Kajjali*. It slightly corresponds with that of Sulphur (Fig. 4). A scanning electron microscope (SEM) is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity (7). In the present research work, SEM was carried out to evaluate the average particle size of *Rasasindoora*. Spot magnification photographs revealed the particle size of *Rasasindoora* was found ranging between 10-50 μm. The images show 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 microcrystalline structure with loss of grain boundaries (Fig 5).

X-ray scattering techniques are a family of non-destructive analytical techniques which reveal information about the crystallographic structure, chemical composition, and physical properties of materials. These techniques are based on observing the scattered intensity of an X-ray beam hitting a sample as a function of incident and scattered angle, polarization, and wavelength or energy (7). Crystallography by XRD (X-ray Diffraction) was carried out to chemically characterize the sublimated part as well as to identify the major phase present in the compound. XRD pattern of *Rasasindoora* showed peaks only due to mercury sulphide. This was confirmed after matching the obtained pattern with standard catalogue and further by EDAX analysis. It had an empirical formula of HgS and hexagonal crystal structure. No extra diffraction peaks were observed confirming that while in the initial stages of processing of the medicine; mercury oxide and free Sulphur are present in significant amount while after *Kupipaka*, only mercury sulphide remains in the finished product. The observations confirm that heat treatment of *Kajjali* helps in formation of mercury sulphide and at the same time increase the crystallinity in the sample. Absence of sulphates is also an important finding. Presence of trace elements was not reflected in XRD pattern. This shows that they are possibly not present on the surface of crystal (Fig 6). These results are in accordance with previous work, where *Rasasindoora* is shown to contain mercury sulphide (crystalline in nature with crystallite size ranging from 25 to 50 nm) associated with several organic macromolecules (8).

EDAX (Energy dispersive X-ray spectroscopy) is a popular technique used nowadays for the estimation of macro-elements in a herbo-mineral formulation. An EDAX spectrum plot not only identifies the element corresponding to each of its peaks, but the type of X-ray to which it corresponds as well (7). In the present work, this technique was effectively applied for estimation of gross elements present in *Rasasindoora* (Fig 7). Mercury was found to be 79.41 % and Sulphur, 20.59 % (Table 9).

FTIR (Fourier Transform Infrared Spectroscopy) was performed to detect the presence of functional groups or organic ligands in *Rasasindoora*. FTIR spectrum was taken in the region of 400-4000 cm^{-1}. General overview of the sample indicates presence of large number of functional groups (Table 10). Fairly sharp peaks were obtained at and around 783, 1380, 1382, 1630, 2344 and 3400.

These peaks indicate the presence of organic compounds in the drugs. These arise probably from the herbs used in *Parada Shodhana* and levigation of *Kajjali*. The sharp absorption peak around 1630 are assigned to C=O stretching vibration in carbonyl compounds which may be characterized by the presence of high content of terpenoids and flavanoids in the complex mixture of these metallic compounds. The presence of peaks around 2800-2900 was assigned as alkyl group. It was further assigned as $–CH_2$, $-CH_3$, -CH=CH stretching vibration indicates presence of methoxy compounds. The present of diterpenes were further proven with the absorption band of hydroxyl (3400 – 3500) and phenyl (1630 cm-1) (9) Symmetric stretching was observed at 1380 and indicated presence of - CH_3 group. Similarly, presence of ions like Cl^-, F^- and I^- were found at 782. To summarize, alkane, alkene, alkyl, acetyl, hydroxyl, carboxyl and organic acids were present in the sublimated sample of *Rasasindoora* (Table 10) (Fig. 8).

The presence of organic moiety gives the compound a lipophilic character, so that it can easily pass through the biological lipid membrane (10). Thus, it can enter the systemic circulation or cell structure and directly act on the desired site by binding to an enzyme. It is also to be noted that more number of functional groups indicates towards the multifaceted action of single compound. Generally, it is seen that a single *Bhasma* or *Kupipakwa Rasayana* is attributed to versatile action by just altering the *Anupana* and *Sahapana*. This dynamic action may be possible due to large number of functional groups present in the parent element enabling it to work differently in different diseases. Further, the mercury is tightly bound to carbon so that the molecule is not easily degraded and it can sustain its action for a long time. It is well accepted fact that organometallic compounds are more poisonous than simple inorganic compounds, this appears probably due to their higher lipid solubility and they can easily cross the blood brain barrier. Moreover, the presence of organic matter on the surface of drug suggests that these organic matter acts as coating material on the surface of metallic compound present in the drug and metal compound acts as the carrier of the organic matter (just like the concept of novel drug delivery in the modern medicine) derived from the herbs/plants used during the pharmaceutical processing. Thus, presence of these organic functional groups indicated towards their potent action as well potential hazards in case of injudicious use. However, still, a systemic study for their pharmacological activity would be desirable to definitely illicit their role in therapeutics.

Conclusion:

Maximum 13 hrs of heating in a graded pattern of 2, 4 and 7 hrs for mild, moderate and stern heating is sufficient for preparing an average amount of 1.9 kg *Rasasindoora* from 3.5 kg of *Kajjali* with utilization of 21.67 kg of fuel in terms of coal and charcoal in a traditional furnace. Final sublimated product i.e. *Rasasindoora* is deposited in the neck of *Kupi* is brick red in colour, essentially a mineral compound having organic ligands and is chemically mercuric sulphide (HgS) with particle range between 10-50 µm and having mercury as 79.41 % and Sulphur as 20.59 % by weight.

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Table 1: Formulation composition of *Rasasindoora*:

Sr.no.	Ingredient	Generic name	Part used	Ratio	Quantity (kg)
1.	Purified *Parada*	Hydrargium	Element	1 part	1.75
2.	Purified *Gandhaka*	Sulphur	Element	1 part	1.75
3.	Vata decoction (for levigation)	*Ficus benghalensis* Linn.	Aerial roots	Q.S.	3 *l*

Table 2: Consolidated temperature pattern and observations of three batches:

Sr.no.	Temperature range (°C)	Duration (hr)	Observations
1.	150 – 250	1 – 2	Light fumes are seen around neck of bottle
2.	250 – 450	2 – 4	Dense whitish fumes, *Kajjali* melts
3.	450 – 500	4 – 6	Dense yellowish fumes, *Kajjali* boils
4.	500 – 550	6 – 8	Bluish flame appears with extensive burning of Sulphur, hot iron rod is used to remove the excess Sulphur and blocked neck of bottle
5.	550 – 600	8 – 10	Flame increases in height, Sulphurous smell, continuous use of iron rod
6.	600 – 700	10 – 13	Flame ceases, corking done after confirmatory tests (copper coin test and honeycomb appearance) with addition of fuel

Table 3: Utilization of fuel in three batches:

Sr.no.	Batch	Fuel for ignition (kg)		Total (kg)
		Charcoal	Coal	
1	I	5	17	22
2	II	7	14	21
3	III	6	16	22
	Average	**6**	**15.67**	**21.67**

Table 4: Graded temperature pattern of three batches:

Sr.no.	Grade (°C)	Batch I (hrs)	Batch II (hrs)	Batch III (hrs)
1.	Mild (150-250)	2	2	2
2.	Moderate (250-450)	3	3	4
3.	Stern (450-700)	6	8	7
	Total	**11**	**13**	**13**
4.	Duration of flame	2	2	2

Table 5: Yield of batches:

Sr.no.	Batch	Weight of *Kajjali* (kg)	Weight of sublimated part (kg)	Weight of residue (g)	% of product obtained	% of product obtained (as per law of definite proportion)
1.	I	3.5	1.9	190	54.29	93.14
2.	II	3.5	2.0	150	57.14	98.04
3.	III	3.5	1.8	210	51.43	88.24
	Average	**3.5**	**1.9**	**183.33**	**54.29**	**93.14**

Table 6: Analysis of *Kajjali*:

Sr.no.	Parameter	Result (% w/w)
1.	Total mercury	57.72
2.	Carbon disulphide soluble extractive	38.61

Table 7: Organoleptic characters of *Rasasindoora*:

Sr.no.	Parameters	Batch I	Batch II	Batch III
1.	Appearance	Compact	Compact	Compact
2.	Texture	Crystalline, hard	Crystalline, hard	Crystalline, hard
3.	Colour	Brick red (vermillion)	Brick red	Brick red
4.	Taste	Gritty, tasteless	Gritty, tasteless	Gritty, tasteless
5.	Smell	Indistinct	Indistinct	Indistinct

Table 8: Quantitative analysis of *Rasasindoora*:

Sr.no.	Parameter	Results
1.	Loss on drying (at 110°C)	3.9
2.	Ash value (at 650 °C)	6.5
3.	Water soluble extractive	5.3
4.	Carbon disulphide soluble extractive	0.08
5.	Total mercury	81.36

Table 9: EDAX analysis showing ratio of Hg:S in finished product:

Elem	Wt %	At %	K-Ratio	Z	A	F
S	20.59	61.86	0.1585	1.1753	0.6551	1.0000
Hg	79.41	38.14	0.7357	0.9130	1.0148	1.0000
Total	**100.00**	**100.00**				

Table 10: Showing results of FTIR analysis:

Sr. No.	Peak position cm^{-1}	Assignment
1.	3400.00-3500.00	-OH stretching vibration
2.	2800.00-2900.00	-alkyl group stretching vibration (-CH_2, -CH_3, -CH=CH-
3.	2344.00	-CO_2 molecule
4.	1630.00, 1631.00	-CO, -C=O, -COOH group
5.	1382.00	-CH_2 group
6.	1470.23	-CH_3 group
7.	1380.00	-CH_3 symmetric stretching
8.	782.00	-Cl, F, I Ion presence

Figures:

Fig. 1: Filling of *Kupi*

Fig. 2: Flaming stage

Fig. 3: Sublimated *Rasasindoora*

Fig. 4: DSC analysis of *Rasasindoora*

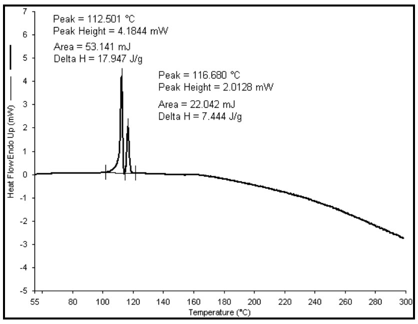


Fig. 5: SEM of *Rasasindoora*

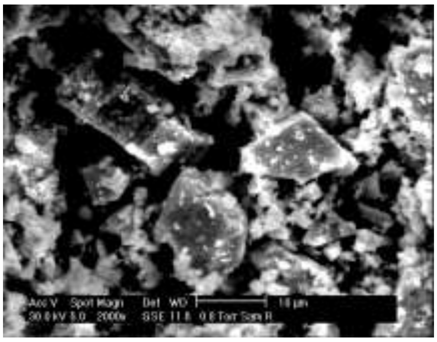


Fig. 6: XRD pattern of *Rasasindoora*

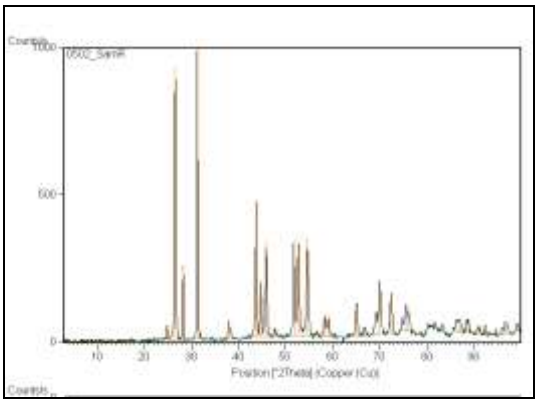


Fig. 7: EDAX analysis of *Rasasindoora*

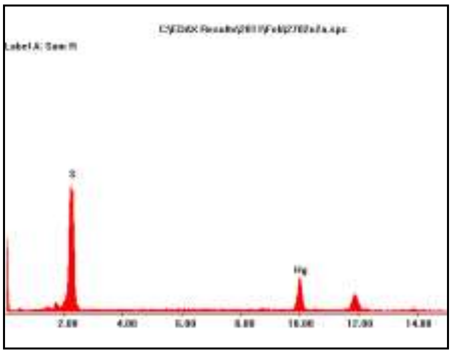
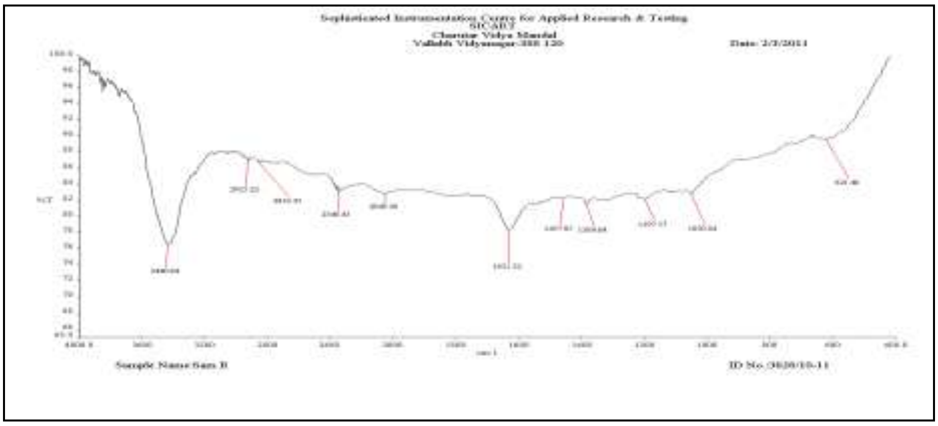


Fig. 8: FTIR spectrum of *Rasasindoora*



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