Original Research

Analysis of *Rasa Parpati* through Advanced Analytical Techniques

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ABSTRACT

Introduction: Ayurveda advocates drugs originated from herbal, minerals, metallic and marine resources. Safety concerns are being raised day by day on traditional formulations at different levels. It may be due to non-compliance of pharmaceutical code of conduct as described in Ayurvedic classics. There is a need to provide safety, quality and efficacy aspects in current scenario. *Rasa Parpati* (RP) is one among the mercurial preparations frequently being used in Ayurveda for different ailments. Physicochemical fingerprinting profile of *Rasa Parpati* is not reported till date. **Materials and methods:** Considering this, *Rasa Parpati* prepared with *Hingulottha Parada*, *Shodhita Gandhaka* (HRP) and *Ashodhita Parada*, *Ashodhita Gandhaka* (ARP) was analyzed through advanced analytical techniques like X-Ray Diffraction, Fourier Transform Infra-Red Spectroscopy, Scanning Electron Microscopy, Inductive Coupled Plasma Atomic Emission Spectroscopy and Particle Size Distribution. **Results and conclusion**: Results revealed that both samples of *Rasa Parpati* are organo-metalic compounds with presence of organic matter as coating material on the surface of inorganic matter, combination of mercury and sulphur as major elements and magnesium, iron as trace elements. Mercuric sulphide (HgS) is present in cubic and hexagonal form, while free Sulphur is present in ortho-rhombic form in crystal lattice of both samples. Different analytical tests reveal insignificant differences in the profiles of both samples of *Rasa Parpati.*

Keywords : *Gandhaka*, Mercury, *Rasa Parpati*, *Shodhana.*

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Introduction

Ayurveda is a traditional medical system used by majority of population in Indian sub continent which uses processed natural resources of herbal, metallic, mineral and animal in origin. In the era of modernization and scientific validation, it becomes mandatory to match the benefits of the age old remedies by following the existing protocols or strategies. Ayurveda needs to undergo hardcore scientific validation in the current scenario. The current study is an attempt to verify the need for the use of modern analytical technique in the finding of quality assurance of such potent drugs. Concerns are being raised about quality and safety of ayurvedic medicines in recent past.[1],[2],[3],[4] Particularly, *Rasoushadhies,* which may contain heavy metals and even poisonous herbs have become main target about safety aspects. Classics emphasized on *Shodhana*[5]of metals and minerals before their therapeutic use. They emphasized that metals and minerals become devoid of toxicity after *Shodhana* and can be administered safely.[6] On this circumstances, quality assurance of respective medicines can be achieved through good manufacturing practice, regulatory control, Physico-chemical fingerprinting and proper documentation for further authentication. In this

age of scientific evolution, many advanced techniques like X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Inductive Coupled Plasma Analysis (ICP-AES) and Particle Size Distribution (PSD) are available for physico-chemical and characterization of metals and minerals. Present study is aimed to provide analytical profile of *Rasa Parpati* prepared by using *Hingulottha Parada,*[7] *Shuddha Gandhaka*[8] (HRP) and *Ashuddha Parada*, *Ashuddha Gandhaka* (ARP). This study is attempted to support rational behind concept of *Shodhana* in preparation of Ayurvedic formulations before their therapeutic use.

Materials and Methods

Preparation of *Rasa Parpati*

Two samples of *Rasa Parpati* one with *Hingulottha Parada*, *Shuddha Gandhaka* and another with *Ashuddha Parada*, *Ashuddha Gandhaka* were prepared by following classical guidelines as described in *Rasa* classics.[9] They were coded as HRP and ARP respectively. *Ashuddha Hingula*, *Ashuddha Parada* and *Ashuddha Gandhaka* were procured from Pharmacy, Gujarat Ayurved University, Jamnagar. Identification and authentication of raw materials were done in Rasa Shastra and Bhaishajya Kalpana Dept. including drug research, IPGT&RA, Gujarat Ayurved University, Jamnagar. Platform of even surface was prepared by fresh cow dung. Fresh *Kadali Patra* (Banana leaves) was placed over the cow dung platform. In preparation of HRP, 25 gm of prepared *Samaguna Kajjali* was taken in a *Darvi* (stainless Steel) smeared with ghee and melted over the heat source (LPG Stove) maintaining 125^0C temperature. Heat was given till the contents became semisolid (*Pankawat*). To bring uniformity in melting, the compound was stirred periodically with the help of spatula (stainless steel). *Pankawat Kajjali* was immediately poured over the smooth surface of banana leaf and covered with another banana leaf. These leaves were immediately compressed gently by using flat surface of steel plate. After cooling, solidified flakes of *Rasa Parpati* were collected. Same procedure was followed for ARP.

Analysis of samples

Samples of HRP and ARP were analyzed for XRD, FTIR, ICP-AES, FEG-SEM and PSD Analysis.

Morphological analysis

The surface morphology of samples was observed using a cold Field Emission Scanning Electron Microscope (JSM 7600F) at an acceleration voltage of 0.1 to 30 kV.

Elemental Analysis

Assessment of metallic constitution was made by ICP-AES analysis using ARCOS, Simultaneous ICP Spectrometer, SPECTRO Analytical Instruments GmbH, Germany.

Powder X-ray diffraction analysis

A powder X-ray diffractometer (Panalytical MRD system for bulk texture and residual stress management) is used for XRD analysis.

Spectroscopic analysis

Fourier Transform Infrared (FTIR) spectra of the samples were recorded between 3800 to 600 cm^{-1} in FTIR spectrometer (3000 Hyperion Microscope with Vertex 80 FTIR System, Bruker, Germany).

Particle Size distribution Analysis

A Laser Particle Size Analyzer Symantec Helos BF (Germany) Range: 0.1 to 875 microns; 31 Multi element detector was used for Particle size distribution Analysis.

Results and Discussion

Fig. 1(a) and 1(b) illustrates SEM images of both the samples, which showed several amorphous particles agglomerated in a particle due to the forms of flakes with the aid of heat as the physical agent. There was characteristic difference in the apparent morphology due to the dissimilarity raw materials taken as final *Rasa Parpati* formulation. They also interconnected well with the particle size dissemination of nano and sub micro sized

particles. Fig. 1(c) and 1(d) depicts EDS analysis data for the samples. EDS delivered useful evidence on the distribution and the chemical form of the elements founding the sample. The elemental composition of sample HRP and ARP by EDS is presented in [Table- 1 and Table 2].

EDS data shows the presence of mercury, sulfur and carbon in both the samples. Though Carbon is not in the basic composition, SEM analysis showed presence of Carbon in HRP (43.13%) and ARP (21.08%). That might be because of burning of Ghee used in the preparation of HRP and ARP. On the other hand presence of other element as a traces which would have been incorporated due to process realm of Ayurveda to enhancing the bioavailability of the drug.

Results of ICP-AES analysis [Table- 3] showed presence of mercury and sulfur in both samples in different percentage. Cadmium, Arsenic and Lead were observed less than 0.01 ppm in both samples. The iron content present in both samples in different amount due to surface contact of iron vessel on going processing *RasaParpati* preparation.

In XRD, total 29 and 27 peaks were found in HRP and ARP respectively. (Fig2a, 2b) Major peaks were identified and interpreted. Crystalline nature of both the samples is clear by sharp peaks exhibited in XRD graph. Samples contain 1:1 proportions of Mercury and Sulfur. [Table- 4] Peaks identified 2è value of diffraction at HRP and ARP. XRD pattern reveals HgS along with free sulphur in crystalline phase present in both samples. HgS is present in cubic and hexagonal form, while free Sulphur is present in ortho-rhombic form. HRP and ARP have maximum peaks at same 2è values showing both samples have similar crystalline structure. Despite these, six peaks were obtained in HRP on different 2è values than ARP while four peaks were obtained in ARP on different 2è values than HRP [Table-5]. This shows some minute differences in structural level in both the samples.

Though XRD analysis showed presence of HgS and sulphur in crystalline phase but SEM images depicts amorphous morphology of both samples. It revealed that, crystalline nature of HgS and sulphur are enclosed by organic materials present in the sample.

The infrared spectrum of HRP and ARP are shown in Fig. 3(a) and 3(b). Absorption bands 3000 – 2800 cm^{-1} assigned to aliphatic C–H stretching.[10] This region is intense, hence indicating the presence of C–H groups in both samples. Methylene groups show asymmetric stretching at 2930 cm^{-1} and symmetric stretching at 2850 cm^{-1}. Both samples depicts absorption bands in this region, confirms the presence of methylene group in both samples.[11] Distinct C=O and C-O stretching modes at 1750 – 1730 cm^{-1} and 1300 - 1100 cm^{-1} respectively appear at wave numbers characteristic of esters.[12] Absorption bands in this region confirms the presence of ester group in both samples. Esters possibly present due to addition of ghee in *Parpati* during in preparation. Free Sulfur is detected in the form of S-S stretching vibrations at 610.67 and represents disulfides in HRP sample.[13] The FTIR analysis strongly suggests organo-mineral nature of both HRP and ARP. Presence of organic matter acts as coating material on the surface of the metallic component that preferably acts as the carrier of organic matter derived from ghee used during the pharmaceutical processing.

Fig. 4(a) and 4(b) shows the particle size distribution of HRP and ARP. Particle size is one of the factors, which will affect dissolution and absorption of drug. Particle size and surface area of a solid drug is inversely related to each other. Smaller the drug particle greater will be the surface area available for chemical reaction and thus more will be the activity of drug. Range of particle size in HRP (16.93 µ - 288.33 µ) and ARP (16.11 µ - 236.93 µ) is almost similar. Particle size distribution curve of HRP and ARP reveals that distributions of particles are more even in ARP than HRP.

ICP-AES and PSD are the techniques that quantify, while SEM-EDX, XRD and FTIR are the techniques which qualify the characters related to attribute. So ICP-AES and PSD can be used for measurement while SEM-EDX, XRD and FTIR can be used for identification and authentication for both samples of *Rasa Parpati*.

Conclusion

It can be concluded that both the samples of *Rasa Parpati* are organo-metalic compounds with presence of organic matter as coating material on the surface of inorganic matter. They represent combination of mercury and sulphur as major elements and magnesium, iron as trace elements. Mercuric sulphide (HgS) is present in cubic and hexagonal form, while free Sulphur is present in ortho-rhombic form in crystal lattice of *Rasaparpati*. Different analytical tests reveal insignificant differences in the profiles of both samples of *Rasa Parpati.*

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Source of Support : Nil
Conflict of Interest : None

Figure Legends

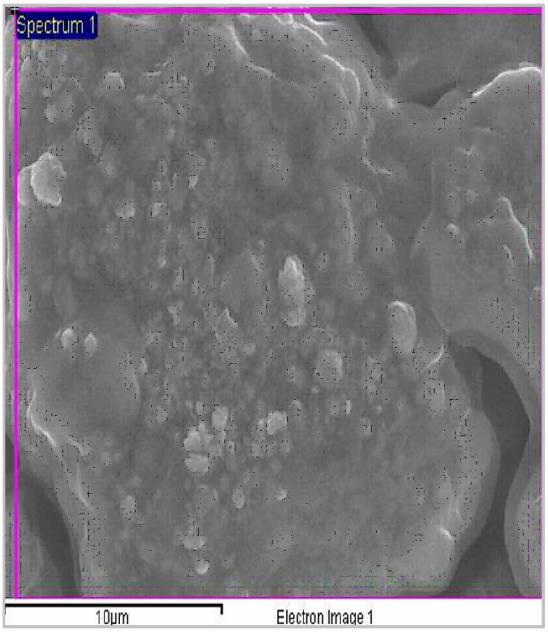

Figure 1(a): SEM image of HRP

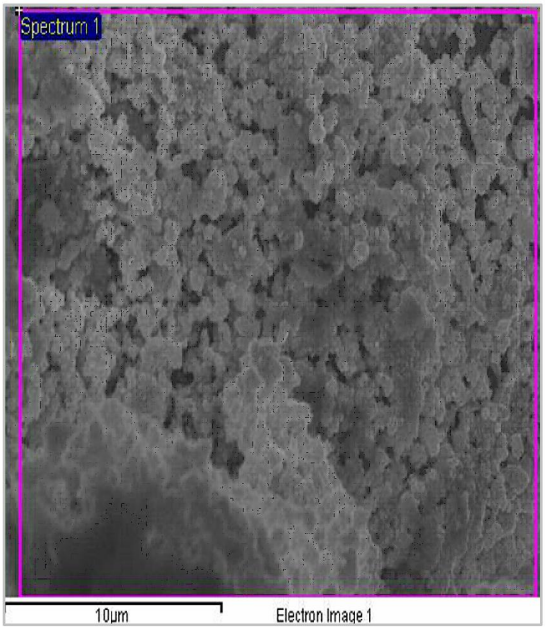

Figure 1(b): SEM image of ARP

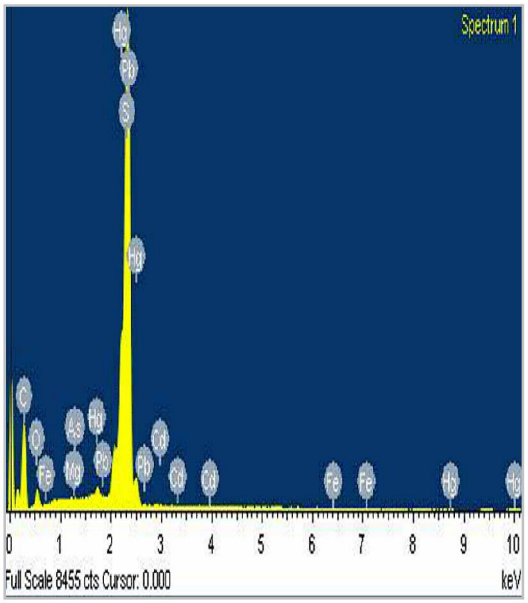

Figure 1(c): EDX analysis graph of HRP

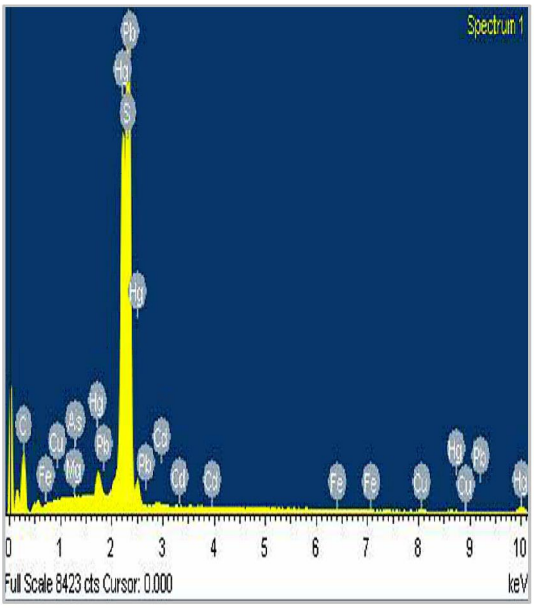

Figure 1(d): EDX analysis graph of ARP

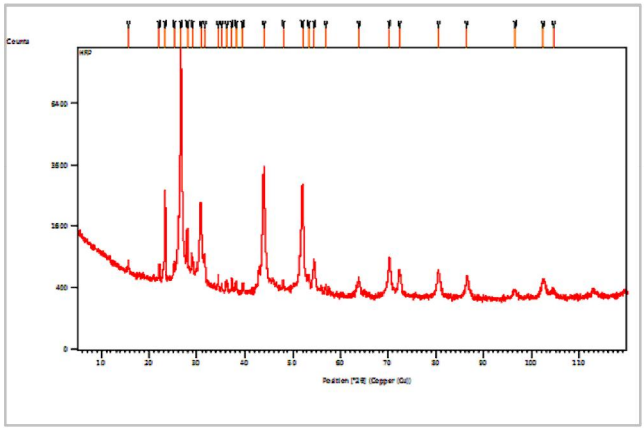

Figure 2(a): XRD pattern of HRP

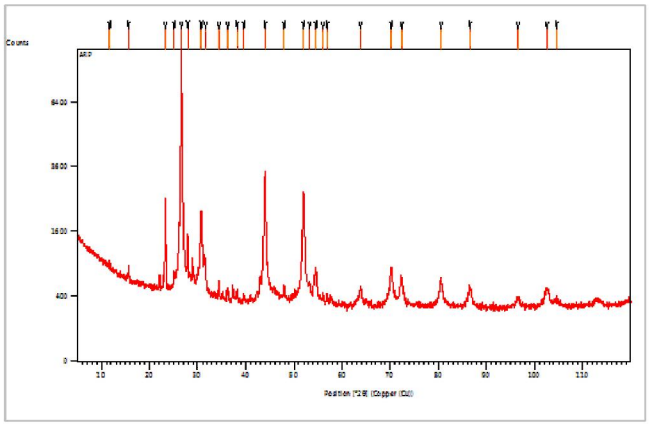

Figure 2(b): XRD pattern of ARP

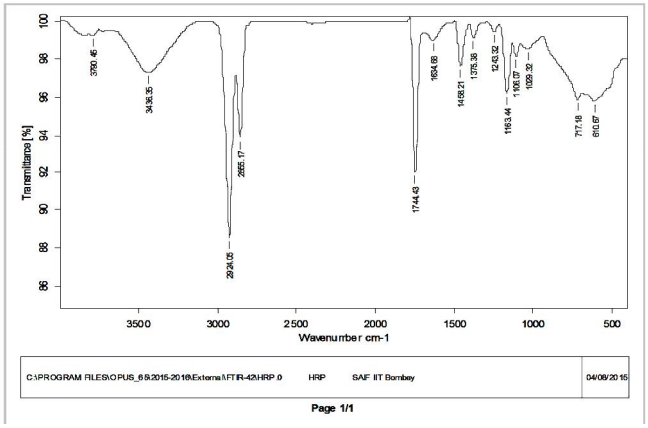

Figure 3(a): FTIR spectrum of HRP

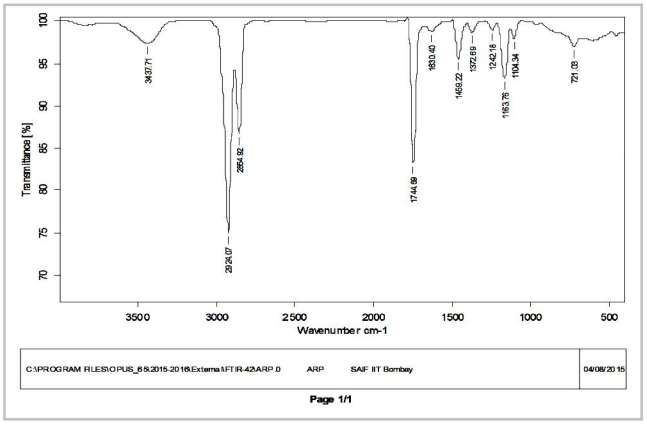

Figure 3(b): FTIR spectrum of ARP

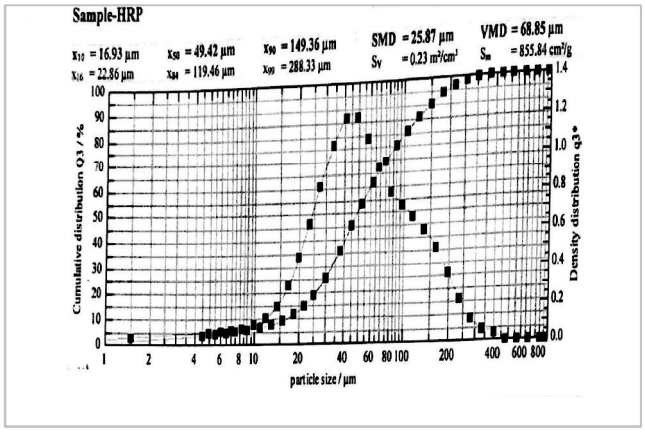

Figure 4(a): Particle size distribution curve of HRP

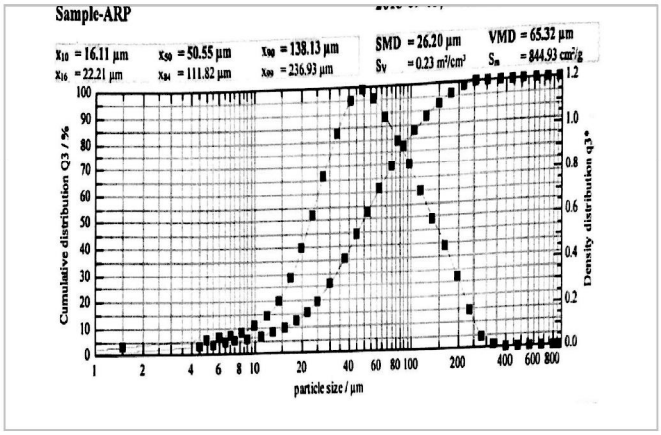

Figure 4(b): Particle size distribution curve of ARP

Table 1: FEG-SEM analysis of HRP

Element	Weight%	Atomic%
C K	43.13	73.73
O K	3.83	4.92
Mg K	0.00	0.00
S K	29.59	18.95
Fe K	0.00	0.00
As L	0.00	0.00
Cd L	0.00	0.00
Hg M	23.44	2.40
Pb M	0.00	0.00
Totals	100.00	100

Table 2: FEG-SEM analysis of ARP

Element	Weight%	Atomic%
C K	21.08	66.45
Mg K	0.00	0.00
S K	18.80	22.20
Fe K	0.00	0.00
Cu L	0.00	0.00
As L	0.00	0.00
Cd L	0.00	0.00
Hg M	60.12	11.35
Pb M	0.00	0.00
Totals	100.00	100.00

Table 3: Showing results of ICP-AES analysis of HRP and ARP samples

Element	HRP(ppm)	ARP(ppm)
Mercury (Hg)	4.18	3.34
Sulphur (S)	24.08	21.36
Iron (Fe)	0.044	0.0058
Magnesium (Mg)	0.00052	0.0041
Lead (Pb)	ND	0.0035
Arsenic (As)	0.0012	0.0017
Cadmium (Cd)	ND	ND

ND (Not Detected) – means less than 0.01 ppm

Table 4: 2è values of identified peaks in XRD of HRP and ARP

HRP			ARP		
2è value	**d-spacing**	**Phase of compound**	**2è value**	**Rel. intensity**	**Phase of compound**
23.3089	3.81635	S- orthorhombic	23.2828	3.82058	S- orthorhombic
26.6170	3.34907	HgS- hexagonal	26.5883	3.35263	HgS- hexagonal
27.9555	3.19169	S- orthorhombic	27.9250	3.19511	S- orthorhombic
29.0074	3.07830	HgS- hexagonal	- -	-	
30.7930	2.90375	HgS- cubic	30.7298	2.90957	HgS- cubic
31.6477	2.82725	HgS- hexagonal	31.6272	2.82904	HgS- hexagonal
43.9755	2.05908	HgS-hexagonal/ cubic	43.9569	2.05991	HgS- hexagonal/ cubic
52.0053	1.75847	HgS- hexagonal/ cubic	51.9804	1.75926	HgS- hexagonal/ cubic
54.5067	1.68354	HgS- hexagonal	54.4921	1.68396	H-gS- hexagonal

Table 5: Showing dissimilarities in 2è values of identified peaks in XRD of HRP and ARP

Sr No	HRP			ARP		
	Pos. [°2Th.]	**d-spacing [Å]**	**Rel.Int. [%]**	**Pos. [°2Th.]**	**d-spacing[Å]**	**Rel.Int. [%]**
1.	22.1094	4.02062	2.12	11.7183	7.55206	1.11
2.	29.0074	3.07830	4.26	47.9453	1.89745	1.33
3.	35.1563	2.55272	0.76	51.9804	1.75926	27.13
4.	37.2959	2.41104	1.91	56.8973	1.61835	0.99
5.	48.0111	1.89501	1.36	-	-	-
6.	52.0053	1.75847	27.79			