

KHAGESWARA RASA- A KUPIPAKA RASAYAN W.S.R. TO PHARMACEUTICO-ANALYTICAL STUDY**Dr. Praveen Raghuwanshi*¹ and Dr. Nagendra Thakre²**¹Assistant Professor, Department of Rasa Shastra, Pt.KLS Government Ayurved College & Institute, Bhopal (M.P.).²Assistant Professor, Department of Kaumarbhritya/Balroga, SAM College of Ayurvedic Sciences and Hospital, Bhopal.***Corresponding Author: Dr. Praveen Raghuwanshi**

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ABSTRACT

Ayurveda places great emphasis on prevention and encourages the maintenance of health through close attention to balance in one's life, right thinking, diet, lifestyle and the use of herbs. Knowledge of Ayurveda enables one to understand how to create this balance of body, mind and consciousness according to one's own individual constitution and how to make lifestyle changes to bring about and maintain this balance. Rasa shastra is the branch deal with the knowledge which teaches us to purification, process, conversion of the drugs, irrespective of their nature into Rasa like form means in an absorbable, bioavailable form. Khageswara Rasa is Sagandha, Saagni Kupipaka Rasayana described in R.R.S. containing purified Mercury, Sulphur and Green Vitriol in equal proportions processed with Bhavana of Arjuna Kwatha than preparation of small Balls like pellets from the Bhavita mixture. Finally these pellets are subjected to Kupipaka for 36 hrs. with specific heating patterns, Analytical study of Khageswara Rasa shows weight loss on drying is 1.69 % which indicates the preparation has less moisture value, suggesting its long shelf life. Total Ash value is 22.41%, pH Value is 4.9, XRD report shows that all peaks are of Hg, S and Fe. In the SEM analysis particle size was Maximum 540nm and 440nm was Minimum at 5KX and 335nm, 281.nm at 7KX. This proves that the drug can be easily absorbed. EDX report shows Hg-72.34% and S-27.64% of product optioned from neck of the bottle and product optioned from bottom reports shows Fe-88.25% and S-11.75% of Khageswara Rasa.

KEYWORD: Kupipaka Rasayana, SEM, EDX.**INTRODUCTION**

Ayurveda, the Indian system of medicine uses drugs from three main sources i.e. plant, animal and mineral. Out of which Rasa Shastra is the pharmaceutical branch of Ayurveda which deals with preparation of drugs using metals and minerals. Several specialized pharmaceutical procedures like Shodhana, Jarana, Marana, Murchhana are adopted for these mineral materials to convert them into safe, nontoxic and efficacious forms. The Rasa aushadhies are highly potent, fast acting and capable of alleviating dreadful and incurable diseases. Rasa aushadhies have more shelf life as compared to herbal formulations. Gandhaka Jarana enhances the therapeutic qualities of Parada and Murchchhana converts it into medicinal form. In the context of Gandhaka Jarana, Jarana is a process and murchchhana is its result. Khageswara Rasa is an outcome of Gandhaka Jarana process and Kupipakwa process is its further advancement. Due to Gandhaka Jarana the drugs became more safe and nontoxic and useful in the management of skin disorders likes Kustha, Switra etc.

Pharmaceutical Study

Pharmaceutical study was conducted in several experiments to obtain the contents in desired form for preparation of Khageswara Rasa as per actual reference. The pharmaceutical procedures adopted in this study are Shodhana, Bhavana and Kupipaka procedure. Kajjali (Shuddha Parada, Shuddha Gandhaka in ratio of 1:1) and Shuddha Kasisa were taken in 2:1 proportion and processed together with Arjuna twak kwatha as Bhavana, then preparation of small Balls like pellets from this Bhavita mixture, finally these Balls are subjected to Kupipaka to prepare Khageswara Rasa.

Shodhana of Parada was carried out by doing mardana with Sudha churna in khalwa yantra for three days and filtered through cloth. Then Lasuna kalka was added in equal quantity and Saindhava lavana was added in half the quantity of Parada and mardana was carried out till the kalka became completely black. After completion of mardana washing of contents was done with hot water to obtain Shuddha Parada. Shodhana of Gandhaka was carried out by placing it in an iron ladle along with

sufficient quantity of Ghrita. It was heated up to melting and poured in a vessel of milk. The mouth of vessel was tied with cloth which was smeared with Ghrita. Then it was washed with hot water and powdered. This procedure was repeated for seven times to obtain Shuddha Gandhaka. Shodhana of Kasisa was carried out with Bhavana of Bhringaraja swarasa. The Shodhita Parada, Shodhita Gandhaka and Shodhita Kasisa were powdered separately. Kajjali was prepared by mixing equal quantity of Parada and Gandhaka. In this Kajjali, Kasisa was added in half amount of Kajjali and triturated to get fine homogenous mixture.

The mixture thus prepared was subjected to Bhavana with Arjuna twak kwatha for three days. This enhances the bala (Potency) of aushadhi dravya, helps in the particle size reduction. Now small Balls like pellets are prepared from this mixture. The purpose behind making of small Balls, to increase the total surface area of raw drug for proper agni sanskara during the process of Kupipaka. The Khageswara Rasa is prepared with the well dried, small Balls by Antardhum Kupipaka Rasayana method. These Balls is filled up to 1/3rd portion in the Kupa. After filling mouth of Kupa closed by cork and dried up. It is later placed in Valuka Yantra and again sealing of Valuka Yantra by a sarava is done. Heating procedure was continued in three phases for 36 hours. After the end of last stage of heating phase Kupa was kept for self cooling. Finally Ubhaythastha Khageswara Rasa was collected.

Analytical Study

Analytical study is essential of any scientific research. It tells us about the correlation between pre-determined hypothetical values and actual results obtained. It gives us valuable information about safety, efficacy, stability, and contra-indications etc of any formulation, it includes following tests:

1) **Organoleptic tests:** Organoleptic tests helps in providing basic information about drugs. This generally includes tests that can be done by one's sensory organs and quality of material can be inferred up to limited extent.

Table 1: Showing results of Organoleptic tests of KR.

S. No.	Parameter	Result
1.	Touch	Soft powder
2.	Colour	Reddish brown
3.	Taste	Tasteless
4.	Odour	Odourless

$$\frac{\text{weight of sample obtained}}{\text{weight of sample taken}} \times 100 = \% \text{ value of moisture content}$$

Table 3: Showing results of Moisture value of KS.

S. No	Test name	Values in % w/w			Arithmetic Mean of three attempts
		1 st time	2 nd time	3 rd time	
1.	Moisture value	1.60	1.78	1.69	1.69% w/w

Table 2: Showing the Classical Characteristics of KR.

S.No.	Parameter	Result
1	Rekhapurnatva	+ ve
2	Varitaratva	+ ve
3	Nischadtratva	+ ve

2) **Physico-Chemical tests:** Physico-Chemical tests deal with primary physical and chemical properties of a sample, which can hint about the internal molecular behaviours at different conditions.

A. Moisture Value

- **Definition:** It is the loss in weight of sample after heating at 105°C until attains constant weight.
- **Significance:** This value determines the quantity of moisture a sample contains. Stability, shelf-life, and microbiological safety depend on it.
- **Materials required:** Hot air oven, Digital balance, Porcelain crucibles.
- **Samples for analysis:** 5 gms of Khageswara Rasa powder.

❖ Procedure

The digital balance is calibrated to 0.00gms. Sample is weighed on balance to check accuracy of weight and is taken in a porcelain crucible. Hot air oven thermostat is adjusted to 105°C and left for certain time to get stabilised at that temperature. Porcelain crucible with sample is kept on oven tray with equidistant from four walls of oven. Sample is dried for one hour. Porcelain crucible is taken out and kept in desiccator to prevent any moisture absorption. After self-cooling porcelain crucible with sample is weighed to calculate the loss of weight on drying. The percentage content of moisture value is calculated in percentage (% w/w). Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

B. Total Ash Value

- **Definition:** It is the total quantity of ash obtained by igniting the sample at 450°C until it gets free form carbon.
- **Significance:** This value determines the percentage of non-volatile Inorganic content of the sample.
- **Materials required:** Muffle furnace, Digital balance, Silicon crucibles.
- **Samples for analysis:** 5gm of Khageswara Rasa powder

❖ Procedure

The digital balance is calibrated to 0.00gms. Sample is weighed on balance to check accuracy of weight and is placed in a silicon crucible. This crucible is kept on wire gauze and heated on a gas stove. It starts emitting fumes and heating is continued until fumes get stopped. Then

this crucible is kept in muffle furnace equidistant from four walls and furnace temperature is gradually raised up to 450°C. Burning is carried out for 6 hours. After complete incineration and after self-cooling, crucible is taken out and kept in a desiccator. The weight of ash with silica crucible is noted. Then the total ash is calculated in terms of percentage (% w/w).

$$\frac{\text{Weight of ash obtained}}{\text{Weight of sample taken}} \times 100 = \% \text{ value of total ash content}$$

Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

Table 4: Showing results of Total ash value of KR.

S. No	Test name	Values in % w/w			Arithmetic Mean of three attempts
		1 st time	2 nd time	3 rd time	
1.	Total Ash value	22.33	22.49	22.41	22.41% w/w

C. Acid insoluble Ash Value

- **Definition:** It is the percentage of inorganic content of the sample, which is insoluble in dilute acid.
- **Significance:** Ash contains inorganic radicals like phosphates, carbonates, and silicates of sodium, potassium, magnesium, calcium etc. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'total ash'. Such variables are then removed by treating with acid and this value determines the percentage of Inorganic content.
- **Materials required:** 100ml glass conical flask, 100ml glass beaker, whatman ash less filter paper, 2N HCl, glass pipette, Digital balance, Muffle furnace, Silicon crucibles.
- **Samples for analysis:** 5gms of Khageswara Rasa powder

❖ Procedure

The digital balance is calibrated to 0.00gms. Sample is weighed on balance to check accuracy of weight. Further Using 25 ml. of dilute hydrochloric acid, total ash is washed into a 100 ml beaker. Beaker is boiled for 5 minutes. Contents are filtered through an ash less filter paper and residue is washed twice with hot water. Filter paper placed in a silica crucible then, incinerated by gradually increasing the heat in a muffle furnace at 450° C for some hours. After complete incineration and after self-cooling, crucible is taken out and kept in a desiccator. The weight of ash with silica crucible is noted. Then the total ash is calculated in terms of percentage (% w/w). Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

$$\frac{\text{Weight of ash remained in crucible}}{\text{Weight of sample taken}} \times 100 = \% \text{ value of acid insoluble ash}$$

Table 5: Showing results of Acid insoluble Ash value of KR.

S. No	Test name	Values in % w/w			Arithmetic Mean of three attempts
		1 st time	2 nd time	3 rd time	
1.	Acid insoluble Ash value	20.22	17.86	15.50	17.86% w/w

D. Water soluble Ash Value

- **Definition:** It is the percentage of water-soluble content of the sample, which is soluble in non-ionic water.
- **Significance:** This procedure determines quantity of water-soluble content contributing to the weight of ash.

- **Materials required:** 100ml glass conical flask, 100ml glass beaker, whatman ash less filter paper, double distilled water, glass pipette, Digital balance, muffle furnace, silicon crucibles.
- **Samples for analysis:** 1gm of Khageswara Rasa ash

❖ Procedure

The digital balance is calibrated to 0.00gms. Sample is weighed on balance to check accuracy of weight. Further Using 25 ml. of double distilled water, total ash is washed into a 100 ml beaker. Beaker is boiled for 5 minutes. Filtered through an ash less filter paper, residue is washed twice with hot water. Filter paper placed in a silica crucible then, incinerated by gradually increasing the heat in furnace at 450° C for some hours. After complete incineration, it is kept in a desiccator to cool. The weight of ash with silica crucible is noted. Then

the loss of ash in water is calculated and water soluble ash value is quantified in terms of percentage (% w/w).

$$\frac{\text{Weight of ash dissolved in water}}{\text{Weight of sample taken}} \times 100 = \% \text{ value of water soluble ash}$$

Similar procedure is repeated for three times. Arithmetic mean is calculated from the values of three attempts to obtain the probable best accurate value.

Table 6: Showing results of Water soluble Ash value of KR.

S. No	Test name	Values in % w/w			Arithmetic Mean of three attempts
		1 st time	2 nd time	3 rd time	
1.	Water soluble ash value	11.12	9.56	8	9.56% w/w

E. Determination of pH value

The pH value of a liquid is determined by means of a glass electrode and a pH meter. Suitable glass electrode and pH meter of both potentiometer and deflection type are available.

The pH meter is an electronic digital voltmeter, scaled to read pH directly, and may range from a comparatively simple hand held instrument, suitable for use in the field, to more elaborate bench models, often provided with a scale expansion facility, with a resolution of 0.001 pH unit and an accuracy of +0.001 unit.

Materials

Glass electrode, pH meter, Buffer tablets, Beakers, Khageswara Rasa – 1 gram

Procedure

- Instrument is switched on and allowed to warm up.
- As the instrument is equipped with a manual temperature control, the temperature of the solutions is taken and the control is set to this value. The electrode assembly is inserted into the same beaker, the selector switch of the instrument is set and pH is noted. The "Set buffer" control is adjusted until the meter reading agrees with the known pH of the buffer solution.
- The electrode assembly is removed, rinsed in distilled water and placed into a small beaker containing the second buffer solution.
- Now 1 gram of Khageswara Rasa sample was put in 5 ml. of water and pH is determined for the solution.

Result: pH value of Khageswara Rasa is 4.90

3) X- Ray Diffraction Studies: Present study was carried out by two samples that were taken at three different stages of this formulation. Analysis was done to identify the components.

❖ Sample details

1. Khageswara Rasa I and II

❖ Procedure

- Sample is finely powdered in agate mortar to very fine powder.
- It is mounted in sample tray of machine.
- X-Ray beam bearing a wavelength of 1.540598 Å from copper source is passed on the sample. Detector was set to identify diffracted beams between 10 -70 degrees of 2θ range.
- Obtained values are plotted on graph with the help of inbuilt "Reyflex Software" for further analysis.
- Obtained soft files of XRD containing values of 2θ and intensity are plotted on a graph (2θ on X-Axis and Intensity on Y-Axis) using "Origin Pro 8 SRO" Data Analysis Software.
- Various compounds consisting similar diffraction pattern were identified by matching their peaks with corresponding JCPDS Crystallographic Cards.
- Powder diffraction file (PDF-2, 2003) database of International Center for Diffraction Data (ICDD) was used for this purpose.
- For even better accuracy and precision, XRD soft files were also analyzed for corresponding phase/entry matching with Crystallographic Open Database (COD-20120320) – USA, after plotting values in Match! Software (Version-2)

Table 7: Showing results of XRD for final drug powder sample I* & II**

Sample	Element/Molecule	JCPDS Ref.	2θ	Intensity	FWHM
I	HgS	01-089-0438	26.67	1000	0.1599
		00-001-0643	43.95	399.4	0.1599
II	FeS ₂	01-072-0409	48.16	291.7	0.1599

- ** Sample I belong to Kanthashta material of final drug.
 ** Sample II belongs to Talashta material of final drug.
 ❖ Khageswara Rasa (KR) is result of mixing of Sample I and II

4) Scanning Electron Microscope Studies (SEM)

In the present study was carried out on final product of Khageswara Rasa to obtain micro-structural films at two different magnifications.

Preparation of SEM specimen

Specimen of the sample be analysed is directly kept on the specimen holder for visualisation. As the sample employed has nonconductive nature. The sample surface is coated by carbon by arc melting technique. The special technique that is used for mounting the surface of fine-grained powders is described below.

Materials needed

Small amount of powder sample, Small round piece of metals specimen holder. Generally it is made of Aluminium or Copper, Double sided cello tape and conducting paste of Aluminium powder, Spreading and vapour sputtering unit.

Method

Dried powder sample was placed over the specimen holder and observed under the microscope at 5,000X and 7,000X. Micrographs were taken with the inbuilt camera.

OBSERVATION AND RESULTS

Scanning microscope images that were obtained from various regions of sample shows that at different magnifications the grain size was found to be ranging between 440.1nm and 540.4 nm at 5K magnification, and 281.1nm to 335.4nm at 7KX. Most of the Particles are found to be extremely agglomerated. Images clearly show that the bigger particles nothing but agglomerations of small particles. Percentage of particles that were found to be agglomerated is relatively less at 5 K (20 – 30%) magnifications than at magnification of 7K (70%). Particles shape is varying from spherical to irregular. Both smaller and larger particles are lacking well defined borders and are irregular with smooth surface. The smallest grain size is found to be 281.1nm at 7K magnification.

5) Energy-dispersive X-ray spectroscopy (EDX)

Energy Dispersive X-ray Analysis (EDX) technique is used for performing chemical analysis in conjunction with Scanning Electron Microscopy (SEM) and transmission Electron Microscopy (TEM) and is not a surface science technique. The analytical data are acquired in the form of digitized spectra, viewed during accumulation on a computer monitor, which display the number of x-rays, I, detected as a function of E, the x-ray energy.

Principle of EDS

The excess energy of the electron that migrates to an inner shell to fill the newly created hole can do more than emit an X-ray. Often, instead of X-ray emission, the excess energy is transferred to a third electron from a further outer shell, prompting its ejection. This ejected species is called an Auger electron, and the method for its analysis is known as Auger electron spectroscopy (AES).

OBSERVATION AND RESULTS

Table 8: Showing the Quantity of elements in Sample I*of final drug.

ELEMENT	WEIGHT %	ATOMIC%
Hg	72.34	29.50
S	27.66	70.49
Total	100.00	100.00

Table 9: Showing the Quantity of elements in Sample IIof final drug.**

ELEMENT	WEIGHT %	ATOMIC %
Fe	88.25	81.44
S	11.75	18.92
Total	100.00	100.00

CONCLUSION

Khageswara Rasa is a Saagni, Sagandha murchana of Kasisa and Kajjali in the ratio of 1:2 and prepared by Antardhuma Kupipaka method with gradually increasing heating pattern for 36 hrs. with chief reference of R.R.S. Final product i.e. Khageswara Rasa was subjected after Kupipaka is to analysis with highly sensitive analyzers like XRD and SEM with EDS for checking their identity, crystalline structure and particle size. KR shows weight loss on drying i.e. 1.69 % which indicates the preparation has less moisture value, suggesting its long shelf life. Total ash value of Khageswara Rasa is 22.41%. Acid insoluble Ash Value is 17.86% w/w, Water soluble Ash value of KR is 9.56% w/w, and the pH Value of Khageswara Rasa is 4.9 which means the drug is acidic in nature.

XRD was carried out for sample Khageswara Rasa. It was clear in the report that all peaks are of Hg, S and Fe means Cinnabar and Ferrous sulphide, In the SEM analysis particle size was Maximum 540.4nm and 440.1nm was Minimum at 5K X and 335.4nm (max), 281.1nm (mini) at 7K X. This proves that the drug can be easily absorbed. EDX report shows Hg-72.34% and S-27.64% in sample I of Khageswara Rasa i.e. final product optioned from neck of the bottle. Sample II report shows Fe-88.25% and S-11.75% i.e. final product optioned from bottom of the bottle. The results show that Hg, S and Fe present in Khageswara Rasa.

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