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KHAGESHWARA RASA – METHODICAL PHARMACEUTICAL AND ANALYTICAL STUDY

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ABSTRACT

*Kupipakwa rasayana*s have been much venerated pharmaceutical preparations because of its nano particle size, long shelf life, faster action, and minimum dose**.** *Khageshwara rasa* is a unique *Kupipaka* preparation containing *Kaseesa* which is indicated in conditions of *Shwitra*, *shwasa, Prameha and pittaja kushta. Kajjali* was prepared from *shuddha parada, shuddha gandhaka* and *Shuddha Kaseesa,* taken in equal quantities and subjected to *Kupi paka* in VMF, by giving *kramagni*, to prepare *Khageshwara rasa.* The analytical standards of the preparation have not been established yet. Hence organoleptic, physico-chemical and instrumental analyses were carried out for *Khageshwara rasa*. An XRD report of *Khageshwara rasa* showed peaks of Cinnabar and Fe embedded in it, product at the base of the kupi showed peaks coinciding with Iron oxide. In SEM analysis, the particle size of *Khageshwara rasa* was 315.4 nm. EDX analysis revealed Hg and S in significant percentage and also showed Fe and O in minor percentage. FTIR analysis showed –OH bonding present in the formulation, suggesting functional group of alcohols present and S=O bonding suggested presence of sulphate.

Keywords: *Khageshwara rasa, Kupi pakwa rasa, shuddha parada, shuddha gandhaka, Shwitra, shwasa*

INTRODUCTION

Kupipakwa rasayanas are distinctive, having a specific method of preparation, wherein mercury along with other minerals and metals are sublimed by subjecting it to a gradual increase in temperature for a specific period of time. They are unique due to their quick action, higher therapeutic value, long shelf life, minimum dose, easy administration and more potency. *Khageshwara rasa*, one such *sagandha saagni kupipakwa rasayana*, mentioned in *Rasa Ratna Samucchaya,* is indicated in *Shwitra* along with *Kasa and shwasa*[1]. Numerous works are being carried out for coming up with a solution to *Shwitra* (Vitiligo), as the present methods of treatment are not upto the mark. Owing to its therapeutic importance, no traces of scientific works done and to provide a scientific analytical basis of the formulation for further research, the present study was carried out.

Aim and Objectives:

This study aims at providing a scientific base for pharmaceutical and analytical study of *Khageshwara*

rasa. (1) To prepare Khageshwara rasa (2) to carry out analytical study of Khageshwara rasa by subjecting it to various physico-chemical tests and instrumental analysis – XRD, SEM-EDX and FTIR

MATERIALS AND METHODS:

Materials:

The raw materials *Hingula*, *Gandhaka*, *Kaseesa* and other associated drugs were purchased from authentic drug vendors in local market after eliciting the *Graahya lakshanas*. Physico-chemical analysis was carried out at Drug testing laboratory, Government Central Pharmacy, Bengaluru and instrumental analysis at IISC, Bengaluru.

Methods: Pharmaceutical Study

Khageshwara rasa mainly contains *Parada*, *Gandhaka and Kaseesa* with *Arjuna twak kashaya* as the *Bhavana dravya*. The pharmaceutical study was carried out under the following steps.

1. *Hingulottha parada* and *shodhana*: *Hingula* was subjected to *Shodhana* by *bhavana* in *Ardraka swarasa* for 7 times[2] and then *Parada* was extracted from it by *urdhwapatana* method. The extracted *Parada* was trichurated with 1/16th part of *Haridra churna* for a period of 2 days, filtered through cloth several times and was stored[3].
2. *Gandhaka shodhana*[4]: It was done using modified *bhudhara* method, by using *Vidyut patana yantra*. Instead of keeping it under ground and igniting *Vanopalas*, *Vidyuth yantra* (heating coil) was placed on the *sharava* and heating was done till the entire *gandhaka* over the cloth melted and collected in the pot containing milk.
3. *Kaseesa shodhana*[5]: *Kaseesa* was given *bhavana* with *Bhringaraja swarasa* for 3 times.
4. Preparation of *Kajjali* for *Khageshwara rasa*: 1 part of *Shuddha Parada* and 1 part of *Shuddha gandhaka* were taken in a *Khalwa yantra and Kajjali* was preparated by mardana for a period of 120 hours[6]. To this *Kajjali*, 1 part of *Shuddha Kaseesa* was added and mardana was done for another 20 hours to obtain the homogenous mixture of *Khageshwara rasa Kajjali*
5. *Bhavana* to *Kajjali*: *Arjuna twak Kashaya* was prepared by adding 8 parts of water to *arjuna twak* and reduced to 1/8th for *bhavana*[7] of the above prepared *kajjali*.
6. Preparation of *Khageshwara rasa*[8]: Classified under 3 headings:

- ***Purva karma*** (prepreparatory phase):- *Kupi* was prepared by applying khora cloth and multani mitti for 7 successive layers, after drying of each layer. The *kajjali* was filled into the bottle upto a maximum limit of 1/3rd the capacity of bottle with the help of a funnel and glass rod, placed inside VMF and ceramic wool was used to close the opening of the furnace.
- ***Pradhana karma*** (Main pharmaceutical procedure):- The furnace was switched on and heated for 36 hrs (23 hrs before corking and 13 hrs after corking) in three stages of graded heating i.e. *Mruduagni, Madhyamagni and Tivragni.* The temperature was recorded with the help of a non-contact infrared pyrometer in the wall of furnace, outer surface of kupi and inside *kupi* every half hourly. The neck of the *kupi* was cleared now and then with the help of red hot iron rod, to avoid choking of neck of bottle by fumes of sulphur. After the cessation of fumes and flames, the *Kupi* mouth was closed with cork and sealed with mud smeared cloth. After corking, the ceramic wool around the neck was widened to facilitate deposition of the product by condensation. *Teevragni* was given after corking the *kupi. Kupi* was allowed for self-cooling in VMF and then removed.
- ***Pashchat karma*** (Post pharmaceutical procedures):- The outer layer of the *kupi* was scrapped with the help of knife to remove the layer of cloth and *multani mitti*. The bottle was then wiped properly with a clean cloth. Thread dipped in kerosene was tied to the bottle below the point where product could be visualized. Then the thread was burnt till the bottle made a cracking sound of breaking. A wet cloth was wrapped around the burning thread and when the bottle cracks, it was separated into 2 halves. Cut neck portion of bottle was held over a tray; a gentle tap was given to the side walls of neck of bottle so that product disassociates itself from bottle and

falls down. The product was collected carefully such that it was devoid of glass pieces and stored in air tight container.

- **Analytical Study**
- **Physico – chemical analysis:** pH value[9], Total ash[10], Acid insoluble ash, Loss on drying at 110°C[11], alcohol and water soluble extractives[12] were determined as per the standard procedure for 3 samples each to minimize error in determination.
- **Instrumental analysis:** X-ray Diffraction study[13] and Scanning electron Microscopy with EDX study[14] was carried out for *Hingula, Kajjali and Khageshwara rasa.* Fourier transform infrared spectroscopy[15] was carried out for *Khageshwara rasa*

Observations:

1. *Hingulottha parada* and *shodhana*

After removal of *Sandhi bandana*:

It was observed that the inside surface of the upper pot was covered by a layer of black soot with a silvery white circle in the centre having silver globules of Mercury. Three filtrations were required to obtain clear silver mercury devoid of soot.

Table 1: Showing observations of *Nishkasana* of *Parada* From *Hingula*

No of Batch	*Weight of Hingula*	*Parada Extracted*	*Agni given in hours*	*Parada Obtained In %*	*Soot*
1	*113 gms*	*68.26 gms*	*9 hours*	*60.4%*	*18 gms*
2	*175 gms*	*99.35 gms*	*9 hours*	*56.77%*	*27 gms*

On trituration with *Parada,* the colour of *Haridra churna* turned to mustard colour in 30 minutes. On further trituration, small globules of *Parada* mixed with *Haridra churna* turning its colour to brownish yellow. After 3 filtrations with double folded kora cloth, the obtained *Parada* was bright silvery white.

2. *Gandhaka shodhana*

   It took about 20 mins for the entire *gandhaka* to melt and collect in the pot below. During heating, the sound of *Gandhaka* droplets falling into the milk was heard and characteristic odour could be appreciated.

3. *Kaseesa shodhana:*

   Metallic odour was appreciated while pounding. On addition of *Bhringaraja swarasa*, it turned to dark green and on complete drying it turned to dull white in colour.

4. Preparation of *Kajjali*:

Kajjalabha Lakshanas like *Rekhapurnata, Laghuta* and *Shlakshnata* started appearing after 50 hours of trituration, but few shiny particles were seen, which completely reduced at the end of 120 hours.

After adding *Kaseesa* to the *kajjali*: Initially after adding *Kaseesa,* colour of *Kajjali* became Greyish in colour. After complete *mardana* for 20 hours, *kajjali* became black in colour.

5. *Bhavana* to *kajjali*

   It took 5 mins for complete wetting of *Kajjali*. During *bhavana*, colour of *Kajjali* looked brownish black in colour and after complete drying; it was dull black in colour

6. Preparation of *Khageshwara rasa*

Table 4: Showing observation & temperature pattern of *Khageshwara rasa*:

Time	Set temp. in Furnace in °C	Display temp. in °C	Temp inside *Kupi* in °C	Temp outside *kupi* in °C	Temp of wall of furnace	Observations
0 hrs	100	21				Furnace was switched on
15 mins	150	104	34	84	172.2	---
45 mins	165	154	69.2	143.5	196.2	Slight smell of *gandhaka,* mild

						white fumes started
1 hr	175	170	75.5	150.6	201	----
1hr 30 min	175	180	83.5	174.5	220	----
2 hrs	185	193	90.1	185	225	----
2 hr 30 mins	185	192	90.7	195	242	----
3 hrs	195	207	96.5	203.5	254	Fumes became a bit denser
3 hr 30 mins	200	204	97.3	213.1	257	Small globules of sulphur seen around the mouth of the *Kupi*
4 hrs	200	210	97.9	219.9	260.4	----
4hr 30 mins	205	211	98.4	231.2	263.5	Fumes were slightly yellow in colour and became more denser
5 hrs	210	215	101.7	238.2	263.9	----
5hr 30 min	215	215	107.5	240.3	264.2	----
6 hrs	240	220	112.2	242	274.5	----
6 hr 30 mins	260	251	120.5	267.3	279.7	----

Madhyamagni started

7 hrs	280	270	125	271	287.7	Cleared small globules of sulphur around the mouth with *tapta shalaka*
7 hr 30 mins	300	290	130.5	275.9	301	Dense fumes, base of *Kupi* not visible
8 hrs	320	312	132.7	289.2	320.4	Inserted *sheeta shalaka*, melting of *kajjali* observed
8 hr 30 mins	340	331	135	293.1	347.4	----
9 hrs	360	351	137.8	320	360.7	----
9 hr 30 mins	380	341	139.5	355.3	372.4	----
10 hrs	400	371	142.2	358.4	402.5	----
10 hr 30 mins	420	388	144.5	361.5	421.2	Cleared neck of *Kupi* with *Tapta shalaka*
11 hrs	450	430	146.2	378.5	439.5	Thick yellow fumes. Yellowish deposition in neck
12 hrs	475	460	148.9	395.5	460.1	----
13 hrs	500	498	149.4	425.4	506.9	----
14 hrs	525	509	150.7	445.5	553.4	Golden yellow fumes near the base, fumes are dense and choking
15 hrs	550	530	151	465	573	----
16 hrs	575	557	151.9	492.5	591	Yellow coloured flame on probing – about ½ inch above the neck
17 hrs	600	585	175	520	615	----

Teevragni Started

18 hrs	625	605	184.8	524	627	Orange base seen, but fumes are dense
19 hrs	650	628	195	529.8	645.8	Orange brown flames appear and disappear inside the bottle near the neck and body junction
19 hr 30 mins	675	654	218.7	530.4	653.2	*Sheeta shalaka* inserted showed upper 1/3 adhering; lower 2/3 dry powder; Product on rubbing showed *sindoora varna*
20 hrs	675	686	239.2	533	668	On probing, bluish flame persisted for about 10 secs, occlusion near the neck became harder, orange base seen.
21 hrs	675	691	248	538.5	678.2	*Suryodaya lakshana* ++
22 hrs	675	689	252	543	693	Fumes and flames reduced on insertion of *tapta shalaka*. Copper coin test performed; surface of coin turned greyish with minute particles of mercury adhering to mouth of *kupi.*
22 hrs 45 min						**Corking done**

Table 5: showing Temperature pattern after corking

Time	Set temp in ^{0}C	Read temp in ^{0}C	Temp outside *Kupi* ^{0}C	Temp of wall ^{o}C
23 hrs	700	703	474	536.8
24 hrs	725	704	510	642
25 hrs	750	735	602	705
26 hrs	775	755	620	775
27 hrs	800	794	639	800
28 hrs	800	734	678	805
29 hrs	800	745	687	810
30 hrs	700	702	697.4	790
31 hrs	600	594	645	750
32 hrs	500	500	527	732
33 hrs	400	400	532	690
34 hrs	300	310	510	630
35 hrs	200	205	463	578.3
36 hrs	150	155	423	415
36 hrs	Furnace turned off			

RESULTS:

Pharmaceutical study:

✦ **Preparation of *Khageshwara rasa* :**

- Weight of *kajjali*: 120gm
- Product *Khageshwara rasa* : 37.049 gms
- Total yield: 30.87%

Analytical study:

Table 6:- Showing results of Physico-chemical analysis of Khageshwara rasa

Parameter	***Khageshwara rasa***
pH	5.19
Total ash	0.3077%
Acid Insoluble Ash	0.049 %
Loss on Drying	0.273%
Loss on ignition	99.6923%
Water soluble extractive	0.2569 %
Alcohol soluble extractive	13.904%

Table 7: Showing results of XRD

Sl. no	**Sample**	**Standard**	**Crystal system**
1	*Hingula*	HgS – Mercury sulphide	Hexagonal
2	*Kajjali*	HgS – Mercury sulphide	Cubic
3	*Khageshwara rasa*	(Hg0.89 Fe0.11) S- Mercury Sulphide	Hexagonal
4	Product at base of *Kupi*	Fe2O3 – Iron oxide	Rhombohedral

Table 8:- SEM EDX results

1. ***Hingula:*** S:- Wt% = 13.88, At% = 38.12; Hg:- Wt% = 81.37, At% = 35.73
2. ***Kajjali:*** S:- Wt% = 30.98, At% = 50.95; Hg:- Wt% = 56.36, At% = 14.82; Fe:- Wt% = 3.19, At%=3.02; O:- Wt%=9.47, At%=31.22

3. ***Khageshwara rasa:*** S:- Wt% = 11.52, At% = 33.16; Hg:- Wt% = 82.70, At% = 38.06; Fe:- Wt% = 0.75, At%=1.34; O:- Wt% = 3.16, At% = 19.74

Mean Particle size:

1. *Hingula* :1.8 µm
2. *Khageshwara rasa kajjali*: 2.7 µm
3. *Khageshwara rasa* : 315.4nm

Table 8:- Showing results of FTIR analysis of *Khageshwara rasa*

Peak (cm^{-1})	Absorbance range	Bond	Functional class
3633.91	3580 – 3650	O-H (free), usually sharp	Alcohols, phenols
3202.31	3200 – 3550	O-H (H bonded), usually broad	Alcohols, phenols
1157.33	1190 – 1120	S=O stretching	SO_4, adsorbed sulphate groups

DISCUSSION:

When mercury is processed with other ingredients in controlled heating procedure, high pressure and high temperature, it leads to formation of an intermediate product, which on heating leads to a chain of reactions inside the Kupi, giving rise to the sublimation and condensation of the final product with strong bonds. Hence *Kupipakwa rasayanas* are ancient pharmaceutical innovation of greater shelf life, lower dose, high stability and great therapeutic value. The Mendeleev's periodic table states that Mercury is the most non-reactive element because of complete d and s orbital, but can be transformed to the highest ligand forming source. These co-ordination complexes consist of central atom or ion, which is usually metallic and a surrounding array of bound molecules or ions that are known as ligands or complex agents. This could be the reason for incorporating mercury as core ingredient in many rasa yogas[16].

Studies conducted have shown that there is formation of complex ligands between the impurities of Mercury – Zinc and Cadmium, with curcumin thereby chelating these toxic metals, thus potentially reducing neurotoxicity and tissue damage[17].

When *Gandhaka* first melts, the poisonous materials present in Sulphur like arsenic will be removed in the ghee. When it passes through the cloth, it leaves behind silica impurities on the cloth. Pouring into the milk causes change in allotropic form from Rhombic to Monoclinic with higher porosity[18].

Continuous trituration of *kajjali* may reduce the particle size, thereby bioavailability increases.

The system of applying *kramagni* or ladder step heating procedure is recommended to give uniform, slow and steady rise in temperature. By this, the ingredients are given enough time at each range of temperature allowing probable reactions to take place.

The odour of Sulphur started around 165 ^{o}C and appearance of fumes were observed at around 195 ^{o}C after 3 hrs of heating. The sulphur might have combined with oxygen and escaped in the form of SO_2, giving rise to the white fumes and odour. Thick yellow fumes are observed at around 250 ^{o}C as sulphur starts forming long polymer chains and thus its viscosity increases.

At 250 – 300 ^{o}C melting of *kajjali* started. Melting of *kajjali* denotes intermediate stage between transitions from solid to vapour. In *teevragni* stage, *Kajjali* tends to attain its boiling point and start to sublime. This is the stage where in the *Kajjali* from semisolid phase attains the gaseous stage and tends to move towards cooler part of *Kupi*. To clear chocking of sulphur at neck of the *Kupi* hot *shalaka* was inserted. This process involves burning of excess of sulphur, which produced a blue flame.

To check escaping of mercury, copper coin was placed on mouth of the *Kupi* and kept for two minutes and coin was seen for white discoloration of copper by mercury vapours. Corking was done before the escape of the compound. To facilitate sublimation and collection of HgS at the cooler part, the ceramic wool was pushed further down and the neck of the kupi was seen to that it was outside the furnace. At this moment ingredients convert into gaseous form and form a complex.

The furnace was not switched off immediately after heating, but temperature was lowered slowly to mimic the valuka yantra heating pattern. It is during the

swanga sheeta period that the product condenses at the neck of the Kupi.

The physico-chemical parameters were determined to test the genuinity of the sample and to test for contaminations.

XRD study was carried out on *Hingula, kajjali, Khageshwara rasa*, Product obtained at the base of the *kupi* after *Kupi pakwa* preparation and to identify the structure and chemical composition of each one of them. Even though JCPDF data file contains thousands of standard compounds, they lack in standards for mixture of metals & metalloid which are mainly concerned for *Rasoushadhis*. Hence, we can only compare our sample with standard but precise identification cannot be done.

22 peaks of *Hingula* matched with standard peaks of Mercury Sulphide, the crystal system being Hexagonal. 8 peaks of *Kajjali* coincided with mercury sulphide, but the crystal system changed from hexagonal structure in *Hingula* to Cubic system after trituration to form *Kajjali*, indicating formation of metacinnabar from cinnabar. 22 peaks of *Khageshwara rasa* matched with Mercury sulphide, with the crystal system Hexagonal. Here the chemical formula was found to be (Hg0.89 Fe 0.11) S, suggesting Fe has combined to HgS in a small quantity, matching with one peak. This phase of transition from metacinnabar to cinnabar is crucial to understand the changes in *Kupipakwa* stage under controlled temperature, pressure crystallinity, bonding level and therapeutic implications. 10 peaks of product at the base of *Kupi* matched with the standard of Iron oxide, having Rhombhedral crystal system. This could be suggestive of formation of *Kaseesa bhasma* in the base of the *Kupi*, but needs further processing to obtain all the *bhasma siddha lakshanas*.

In SEM-EDX study, as the samples were not electrically conductive, they were first subjected to sputtering of gold ions. The Wt % of Sulphur is 13.88 and that of Mercury is 81.37. The compound formation of HgS takes place with the fixed ratio of 6:1 Mercury: Sulphur as per the law of definite proportion, which could be well elicited from the values obtained. At% of sulphur is 38.12 and Mercury if 35.73. The values are almost equal; depicting that 1 atom of mercury combines with 1 atom of sulphur to form the compound and some free sulphur is present in the compound.

In kajjali, the Wt% of Sulphur is more when compared to *Khageshwara* Rasa. The S % might be more due to presence of SO_4 in *Kaseesa*. The % might have reduced in *Khageshwara* rasa as the excess of sulphur is burnt during the *Kupi paka*. Similarly the Wt% of Fe has also reduced. The temperature subjected during *Kupi paka* is not sufficient for Fe to sublimate and collect at the neck region, hence reduction is seen in the percentage. The Wt% of Mercury has increased from *Kajjali* to *Khageshwara* rasa and is almost 6 times to that of wt% of Sulphur, showing formation of mercury sulphide. The At% of mercury has also increased and is almost equal to that of Sulphur.

Mean particle size of Kajjali was 2.7µm and 315.4 nm in *Khageshwara* rasa, indicating reduction in particle size and conversion to nano particles in the end product.

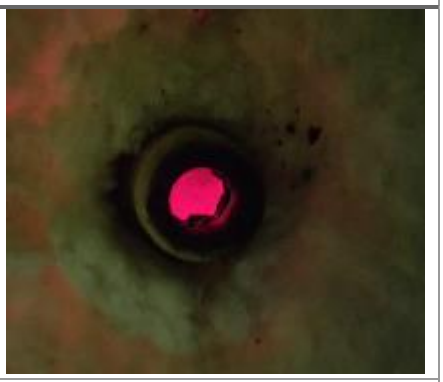
FTIR provides special information about chemical bonding and molecular structures. Molecular bonds vibrate at various frequencies depending on the elements and type of bonds, which is specific for any given bond. The peaks indicate the absorption of a region of Infra-red spectrum. The IR band at 3633.91 is attributed to OH stretching, surface hydroxyl groups and H_2O. Absorption in this region indicates that in OH bonding, H is free and this range belongs to Alcohols and phenols. The peak is narrow, suggesting that OH group is in vapour phase and no hydrogen bonds. The IR band at 3202.31 can be assigned to stretching modes of surface H_2O molecules or to an envelope of hydrogen- bonded surface OH groups. The band is broad indicating many different bonding states of OH groups. Also while using KBr pellet method, KBr is hygroscopic in nature and might alter the measurement sometimes. 1157.33 band suggest S=O stretching. It could be attributed to adsorbed sulphate groups (SO_4).

CONCLUSION

Methodical Pharmaceutical study revealed many observations similar to other *Kupi pakwa* preparations, but the time of appearance of lakshanas depends on

the ingredients used and the temperature pattern followed. As the standards of the Physico-chemical parameters of *Khageshwara rasa* is unavailable in the literature, an attempt was made to know the moisture content, ash values etc. Analytical studies are useful for the characterization of *Khageshwara rasa*, but researches have to be made to determine its Pharmacological activity- Pharmacokinetics and Pharmacodynamics.

Preparation of Khageshwara rasa

		
Observation of fumes	Observation of flame	Suryodaya lakshana
		
Thread dipped in kerosene, tied and ignited	Product obtained at the neck of the Kupi	Khageshwara rasa

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