PREPARATION AND ANALYTICAL STUDY OF VATA GAJENDRA SINGH RASA

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ABSTRACT

Rasaushadhi’s (Herbomineral preparations) are unique, acts in low dose, highly potent, don’t have annoying taste and gives quick results, hence have edge over the herbal medicines. In the present trend of commercialization, there are many doubts raised regarding authenticity of Ayurvedic products. Hence analysis and assessment of raw material, different processes of drug preparation and final product is necessary to maintain authenticity, quality and purity of drug. At present time Amavata and Vatavyadhi are most common problem due to lifestyle change, changed food habits, lack of exercise etc. Vata Gajendra Singh Rasa is a Kharaliya Rasayana mentioned in Bhaishajyaratnavali under Amavata Rogadhikara. The main ingredients of Vata Gajendra Singh Rasa are Abhraka Bhasma, Loha Bhasma, Tamra Bhasma, Naga Bhasma, Kajjali and Vatsanabha. In this study Vata Gajendra Singh Rasa is prepared as per text of Bhaishajyaratnavali and assessment of drug done as per Ayurvedic and modern criteria. Chemical analytical studies carried out at government approved quality control laboratory. Analysis of main ingredients like Abhraka, Loha, Tamra, Naga, Vatsanabha, Parada and Gandhaka done by methods mentioned Ayurvedic Pharmacopoeia of India. In process analytical studies like analysis of the Bhasma’s carried out as per methods of Ayurvedic Pharmacopoeia of India and evaluated with the standards mentioned in Pharmacopoeial Standards for Ayurvedic Formulations of Central Council for Research in Ayurveda and Siddha. Analytical tests of the final product (Vata Gajendra Singh Rasa pills) carried out as per reference of Ayurvedic Pharmacopoeia of India and Indian pharmacopoeia. Results of the raw material study justifies authenticity of raw material, results of in process study of the Bhasma are compared with standards, fulfills assessment criteria. Results of the final product discussed and conclusions are drawn, explained in full paper. Further studies are required to develop standards for the formulation.

KEYWORDS: Vata Gajendra Singh Rasa, Rasaushadhi, Preparation, Analytical Study.

INTRODUCTION

The great alchemist Nagarjuna first time processed Maharasa, Dhatu etc. (metals and minerals) with various plant juices, Kwathana etc. (decoction) e.g. Dashamool Kwatha etc. by different mechanisms like Shodhana, Jarana, Marana, Satvapata (standard procedures for Bhasma preparation) etc. and converted these once highly toxic inorganic substances into qualified lifesaving medicines.[1] These medicines are now known as Rasashastra (Herbomineral preparations) and science is known as Rasashastra i.e. Alchymy of Indian Medicine. This is said to be the ancient medicinal alchemy, Rasashastra (Herbomineral compounds) acts as a weapon for Ayurvedic practitioners as they are highly potent even in low dose, don’t have annoying taste, can give quick results and can be preserved for longer period.[2]

Now a day there is a huge debate all over regarding the toxicity of Rasashastra. There is no doubt that the basic principles established by Rasacharaya have concrete scientific background, the questions are raised because of faulty methods of preparation and assessment. Hence development of SOP’s, analysis of different processes of drug preparation with the help of well-developed modern science and technology is important. Department of AyUSH (Ayurveda, Yoga, Unani, Siddha, and Homeopathy) and C.C.R.A.S. (Central Council of Research in Ayurveda and Siddha) developed standards for some Ayurvedic drugs and formulations in “Ayurvedic Pharmacopoeia of India”. Here attempts are made for the preparation and analysis of Vata Gajendra Singh Rasa in terms of Ayurvedic as well as modern science and technology.

There are several formulations available in the text of Ayurveda for the treatment of Vatavyadhi. Mahavatavidhwansa Rasa is one of the famous formulations for Vatavyadhi[3], but every formulation has its own features and limitations, like this Mahavatavidhwansa Rasa cannot be used in Pitta Prakruti, and patient with heart diseases because it contains ‘Vatsanabha’ in more quantity. The main ingredients of Mahavatavidhwansa Rasa and Vata Gajendra Singh Rasa...
are nearly same but reduction in the quantity of Vatsanabha and few other changes makes the formulation so beautiful that it can be used in all types of Jirna/ Kaphanubandhi Vatavadyadi and Amavata. [4]

**MATERIAL AND METHODS**

**Parada Shodhana**

Equal amount of Sudha (slaked lime) was mixed with Parada and the mixture is triturated for 3 days. It was then strained through twofold cloth and again taken into the Khalsa. This time mixed with Lasuna Kalka (peeled garlic cloves) ½ part and ¾ the part Saindhava (Rock salt). It was triturated for such a time, till the mixture become black in colour then washed with hot water to get Parada back.[5]

**Gandhaka Shodhana**

Ghrita with equal quantity of Gandhaka added to pan and subjected to heat. As soon as Gandhaka was liquefied the mixture as whole is poured in another pot containing cold Godugdha, through a permeable muslin cloth. Then Gandhaka was drawn out and washed with hot water. This procedure was repeated for 3 times. [6]

**Preparation of Kajjali**

Samaguna Kajjali prepared by triturating equal quantity of Parada and Gandhaka in Khalvayantar till fulfill Siddhilakshana.

**Abhraka Bhasma Nirmana**

**A) Shodhana of Abhraka:** First blocks of Krishna Abhraka subjected to heating on gas stove. Then Abhraka quenched (Nirvapa) into Triphalakwatha when it turned red hot. Procedure repeated for 7 times.[8]

**B) Dhanyabhraka Nirmana:** Shuddha Abhraka and Dhana in proportion 4:1 tied in jute cloth then it was dipped into Kanji for 72 hrs. Thereafter it was rubbed by sole to get coarse powder of Abhraka.[9]

**C) Abhraka Marana:** for Nischandikarana of Abhraka Bhasma Kalami Sora and Guda (Jaggery) used for first 2 Putas. Arkapatra Swarasa, Dashamoola Swarasa and Ghritakumari Swarasa were used as Bhavana Dravya, 40 Gajaputas given.[10]

**Loha Bhasma Nirmana**

**A. Samanya Shodhana of Loha:** Red hot Loha immersed seven times each into Taila, Takra, Gomutra, Kanji and Kutilattha Kwatha respectively.[11]

**B. Vishesha Shodhana of Loha:** red hot Loha crusts immersed into Triphala Kwatha and same procedure repeated seven times. [12]

**C. Marana of Loha:** Bhanupaka - first Shuddha Loha along with Triphala Kwatha kept in sunlight till dehydration. Same procedure repeated 7 times. Sthalipaka- then Loha Choorna was taken in iron pan and Triphala Kwatha added, intense heat given till it became dry. Putapaka - Shuddha Loha and 1/12th part Hingula Choorna triturated with Triphala Kwath, then Chakrika prepared, then it was packed in Sharava Samputa and 16 Gajaputa given. Hingula added upto 4th puta.[13]

**Tamra Bhasma Nirman**

**A) Samanya Shodhana of Tamra** – same as Loha Samanya Shodhana.

**B) Vishesha Shodhana of Tamra** - Swedana of Tamra done with Gomutra for 3 hrs by Dolayantra method. [14]

**C) Tamra Marana:** Shuddha Tamra 1 part and Samaguna Kajjali 1/4th part triturated in Khalvayantar with Jambir Swarasa and circular cakes of coin shaped prepared, dried cakes kept into Sharavasamputa and subjected to Pata, after 19 Putas Tamra Bhasma fulfilled Bhasma Pariksha. 1/4th part Kajjali added upto 4th Pata.[15]

**Naga Bhasma Nirmana**

**A) Shodhana of Naga:** Samanya Shodhana of Naga done as per reference of Rasaratnasamuchaya 5/13.

**B) Vishesha Shodhana of Naga:** Rods of Naga subjected to heat in iron pan on gas stove. Then melted Naga poured into Choornodaka by using Pithara Yantra. Same procedure repeated 7 times.[16]

**C) Naga Marana:** Shuddha Naga 1 part and Shuddha Manahshila 1/12th part triturated in Khalvayantar with Vasa Swarasa and circular cakes of coin shaped prepared, dried cakes kept into Sharavasamputa and subjected to Kapot Pata, the procedure repeated upto 40 Pata. Manahshila added upto 10 Pata[17].

**Vatsanabha Shodhana**

Chanaka shaped pieces of Vatsanabha were kept immersed in Gomutra (cow’s urine) placed in earthen pot for three days. Gomutra was changed every day. Later on pieces were taken out washed with water, external layer was separated and dried. [18]

**Tankana Shodhana**

Tankana is taken in an iron pot and started heating as soon as it melts, it was stirred by spoon. When it was completely free from water it was taken out. This is the Shuddha Tankana which can be used for medicinal purpose.[19]

**Preparation of Vata Gajendra Singh Rasa**

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abhraka Bhasma</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Loha Bhasma</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Tamra Bhasma</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Naga Bhasma</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Shuddha Parada</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Shuddha Gandhaka</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Shuddha Tankana</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Sh. Vatsanabha</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Saindhava</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Lavanga</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Shuddha Hingu</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Jatipatha</td>
<td>1 part - 10 gm</td>
</tr>
<tr>
<td>Twaka</td>
<td>½ part - 5 gm</td>
</tr>
<tr>
<td>Tejpatra</td>
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</tr>
<tr>
<td>Sookshma Ela</td>
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</tr>
<tr>
<td>Haritaki</td>
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</tr>
<tr>
<td>Bibhitaki</td>
<td>½ part - 5 gm</td>
</tr>
<tr>
<td>Amalaki</td>
<td>½ part - 5 gm</td>
</tr>
</tbody>
</table>

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Procedure
First all the ingredients measured accurately and sieved through the cloth. Then Kumari Sara was crushed in a mixture grinder and juice was kept in a measuring cylinder. Then Kaijali was triturated for 3 hour then Abhraka, Tamra, Loha, Naga Bhasma were added and triturated for 1 hour. Thereafter the other ingredients were added and Kumari Swarasa was added gradually, till the mixture became wet. The mixture was triturated till it became thick and able to form Vati. Then Vati each of weight 3 Ratti (360 mg average) was formed dried and packed in air tight container.[20]

Analytical Study (According to Modern Science)
The Analytical tests were carried out at FDA and Government authorized, Agmark certified Chemical Laboratory. The Analytical tests are divided in 3 steps – 1) Raw material study 2) In process study and 3) Final product study. 1) Raw material study – It includes analysis of Ashuddha Parada, Ashuddha Gandhaka, Ashuddha Abhraka, Ashuddha Loha Churna, Ashuddha Copper wire, Ashuddha Naga and Ashuddha Vatsanabha. 2) In process study –It includes Physio-chemical analysis of Abhraka Bhasma, Loha Bhasma, Tamra Bhasma, Naga Bhasma and Shuddha Vatsanabha. 3) Final product study - It includes physio-chemical analysis of Vata Gajendra Singh Rasa. The following tests were carried out at Laboratory.

1) Raw material study
A) Determination of mercury (Assay for Hg) -(By complexometric Titration method).
0.2 gm sample was taken in volumetric flask and dissolved in HNO₃. 50 ml of distilled water was added. PH was maintained at 6 by adding excess of Hexamine. Mixture in volumetric flask was then titrated with 0.05 N EDTA, by using xylinon orange as indicator. Each ml of EDTA = 0.0104 gm of Mercury.[21]

B) Determination of Sulphur (Assay for S) –(Gravimetric assay)
Solutions: 1) Carbon tetrachloride saturated with Bromine, 2) Barium Chloride – 10% solution in purified water.
Procedure: 1 gm of sample was taken in 250 ml beaker. Then 10 ml carbon tetrachloride saturated with bromine added and kept in fume chamber overnight and 15 ml nitric acid added, digested on water bath then 10 ml hydrochloric acid added, digested it to expel NO₂ fumes till syrupy mass was obtained. Allowed to cool and extracted with hydrochloric acid, made the volume up to 100 ml, boiled and filtered through whatman 40 No. filter paper, then residue washed with hot purified water. Filtrate treated with ammonia solution for R₂O₃ precipitations (R stands for Fe and Al). Filtered through whatman 41 No. filter paper in 500 ml beaker, filtrate acidified with hydrochloric acid and 20 ml of 10% barium chloride solution added, digested on burner. Then precipitate allowed to settle for overnight. Then filtered through whatman 42 No. filter paper and washed with purified water, precipitate kept in muffle furnace in pre weighed platinum crucible upto 850°. Weighed after cooling.

Calculation: % of Sulphur = Weight of the precipitate x 0.13734 x 100/ Weight of sample. [22]

D) Determination of Iron (Assay for Fe) -(By Dichromate method)

Solutions: 1) Stannous chloride solutions – 5 mg of SnCl₂ dissolved in 25 ml hydrochloric acid and diluted to 100 ml (5% solution), 2) Mercuric chloride – saturated solution in purified water. 3) Sulphuric acid + orthophosphoric acid mixture – 60 ml purified water taken, 15 ml sulphuric acid and 15 ml phosphoric acid added, then diluted to 100 ml. 4) Diphenyleamine barium sulphonate – 0.25 g dissolved in 100 ml water. 5) 0.1N standard potassium dichromate solution.

Procedure: An aliquot from the stock solution was taken into 250 ml conical flask and diluted to 100 ml with purified water then 2 drops of methyl red indicator followed by 2 gm of ammonium chloride added, dilute ammonia solution added till brown precipitate appears and solution with precipitate was boiled for 5 minutes the content cooled and filtered through whatman no. 41 filter paper. Residue washed with hot purified water. Then dissolved in dilute hydrochloric acid in 250 ml beaker and made the volume upto 100 ml. Then solution boiled on burner to reduce the Fe³⁺ to Fe²⁺ by adding stannous chloride solution drop wise till solution became colorless. Then 2 drops of stannous chloride, 15 ml 10% solution of mercuric chloride, 25 ml acid mixture and 3 drops of biphenyl amine barium sulphonate indicator added. Thereafter purified water added, titration done against potassium dichromate solution; appearance of violet colour shows end point.

Calculation: 1 ml 1N K₂Cr₂O₇ = 0.05585 g Fe / = 0.7985 Fe₂O₃
% Fe = 0.0558 X normality of K₂Cr₂O₇ X aliquot X ml K₂Cr₂O₇ X 100/Weight of sample X total volume.[23]

E) Determination of Copper (from copper wire) - (By Iodometric titration)

Solutions: 1) Standard 0.1 sodium thiosulphate solution, 2) Potassium Iodide 3) Starch 1% solution- 1 gm dissolved in purified water and boiled to make up to 100 ml.

Procedure: An aliquot of sample from the stock solution was taken in a beaker and 1 gm sodium fluoride added. Then ammonia solution added till precipitation occurs and acetic acid added to dissolve the precipitate, boiled and cooled in water bath. Then potassium 1 gm added titrated the liberated iodine against 0.1N sodium thiosulphate solutions by adding starch solution as indicator in iodine flask. The colour changes from blackish brown to white indicated end point. Calculation of copper value done against 1 ml sodium thiosulphate solution titrating against standard 1000 ppm copper solution.

Calculations: 1 ml Na₂S₂O₃ = 0.06354 g of Cu
%Cu = 0.0636 X Normality of Na₂S₂O₃ X ml of Na₂S₂O₃ X Aliquot X 100/Weight of sample X Total volume.[24]

E) Determination of Aluminum in Mica

Preparation of solution: A weighed quantity of well mixed sample was transferred to long necked flask of 250 ml capacity. 10 ml of saturated solution of bromine was added in carbon tetrachloride. Flask was covered and
allowed to stand for about 30 minutes, stirring several times. Then 15 ml of conc. Nitric Acid was added to flask was covered and was allowed to stand for 30 minutes, stirring several times. Flask was given heat over a low flame. Small quantity of conc. Nitric Acid was added from time to time until the solution was clear and didn’t darken on standing. Solution was transferred quantitatively to 250 ml beaker with the aid of water evaporated on hot plate to about 5 ml. 20 ml of sample solution was pipetted out and 20 % excess of oxine solution (1 ml will precipitate 0.001g of Aluminium was added. When the complex Al (CaH4.0N) was formed precipitation was completed by addition of solution 4g of ammonium acetate in the minimum quantity of water mixture was stirred and allowed to cool. Granular precipitate was filtered through a sintered glass crucible of porosity No. 4 and was washed with warm water. The complex was dissolved in warm conc.HCL. Solution was collected in 250 ml reagent bottle. Few drops of indicator (0.1% methyl orange solution) were added and 0.5 to 1 gm of pure potassium bromide was also added until colour become pure yellow. To determine exact end point it was better to add a slight excess of standard bromide solution, dilute solution considerably with hydrochloric Acid then 10 ml of 10% potassium iodide solution was added. The liberated iodine was titrated with standard 0.1 N sodium thiosulphate using starch as indicator 0.1 ml of NKBro3 = 0.002249 g Al.[25]

F) Determination of Magnesia in Mica - This was done by Complexometric Titration Method.[26]

G) Determination of Silica in Mica - Determination of silica as SiO2 was done by Hydrofluorisation.[27]

H) Determination of K2O content in Mica - This was done by flame photometer.[28]

I) Determination of Fe in Mica - was done by Dichromate method as explained before in Determination of Fe.[29]

J) Determination of Lead (Assay for Pb) -(By Complexometric Titration Method)

**Solutions**: 1) Acetic acid – ammonium acetate buffer 5.5 – 6.0 pH, 200 g ammonium acetate taken – 30 ml glacial acetic acid added and made up to 1000 ml. 2) EDTA solution 0.05 M – 18-6120 g of sodium salt of ethylene diamine tetraacetic acid (EDTA) in purified water and made up to 1000 ml. 3) Xylenol orange indicator – 0.2 g indicator dissolved in 100 ml purified water and 2 drops of acetic acid added. 40 Thio urea 5) Ascorbic acid, 6) Urea and 7) Sodium fluoride.

**Procedure**: 1 gm of powdered sample taken in a beaker and 30 ml aquaregia and digested it on hotplate. After 15 minutes 15 ml sulphuric acid added and solution evaporated to dryness. The residue along with filter paper taken in a beaker and 50 ml acetic acid-ammonium acetate buffer added. Boiled and filtered through whatman no. 40 filter paper, residue washed with hot purified water. A pinch of thiourea and ascorbic acid and 3 drops of xylenol orange indicator added. Solution titrated against 0.05 M ethylene diamine tetraacetic acid (EDTA) solution. Purple content from the ethylene diamine tetraacetic acid used up in titration. Lead value calculated against 1 ml of ethylene diamine tetraacetic acid solution against standard 1000 ppm lead solution. Calculation: 1ml 0.05 N EDTA = 10.3605 mg of Pb. %Lead = 10.3605 X Normality X ml of EDTA X ml of EDTA X Aliquot X100/ Weight of the sample X Total volume.[30]

k) Total alkaloids from impure Vatsanabha - The sample was extracted repeatedly using (3 x 50ml) 0.1 N H2SO4 in an ultrasonic bath. The solution was filtered the mixed acid solution was washed with 4 successive quantities of 25 ml chloroform. The chloroform washing was then rejected acid solution basified with dilute ammonia solution and extracted with (20 ml x 5) diethyl ether. The combined diethyl ether extracts were then washed with 5 ml distilled water and other evaporated to dryness in a weighed beaker on a water bath. Residue dried to constant weight at 105°C and the total alkaloid content was estimated.[31]

2) In process study

a) Loss on Drying: About 2 gm sample was taken in a tarred china dish, dried at 105 °C, cooled it in desiccator and weighed. Again the procedure of heating, cooling and weighing was repeated until the constant weight was obtained. The loss on drying was calculated.[32]

b) Loss on Ignition: Loss on ignition test is used to determine the relative percentage by weight organic portion to inorganic portion of formulate.1 gm of Bhasma sample was taken in porcelain crucible and dried at 110°C. Then after heated to 950°C allowed volatile substance to escape and organic matter to destroy until its mass ceases to change, mass was redetermined and process was repeated until it shows that mass change was complete. Loss on ignition =100 x (loss in weight)/Wt. of sample taken for test.[33]

c) Acid Insoluble Ash: 1 gm sample was taken in silica dish. Incinerated at a temperature not exceeding 450°C until become free carbon, cooled in desiccator and weighed samples were heated, cooled and weighed till constant weight was obtained. Thus obtained ash of sample was treated with dil. HCL. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate becomes neutral. Then this filter containing insoluble matter was transferred to original crucible dried on hot plate and ignited at 900 °C to constant weight. Acid Insoluble Ash =100x wt. of acid insolubles ash/Wt. of sample taken.[34]

d) Determination of Iron (in Loha and Abhraka Bhasma): (By Dichromate method) – by same method as Mentioned in raw Loha analysis.[35]

e) Determination of Copper from Tamra Bhasma - (By Ideometric titration) –as mentioned in raw Copper analysis.[36]

f) Determination of lead from Naga Bhasma - By Complexometric Titration Method as described in analysis of raw lead.

g) Total alkaloids from Purified Vatsanabha -The method was same as mentioned for total alkaloids from impure Vatsanabha in Raw material study.

3) Final product study

a) Disintegration time: Pills of sample were placed the tube of disintegration apparatus, the tube was raised and lowered in such a manner that the complete up and down
movement repeated 30 times per minute. The time required to disintegrate the pills was calculated.\[37\]

b) Loss on Drying -Method same as given for Bhasma in 'In process study'.

c) Total Ash: Method as per mentioned for Bhasma, in 'In process study'.

d) Acid Insoluble Ash: Method as per mentioned for Bhasma, in 'In process study'.

e) Water Soluble Ash: Ash boiled with 25ml of water for 5 minutes; insoluble matter collected in Gooch crucible, washed with hot water, and ignited for 15 minutes at the temperature not exceeding 450°C. The weight of the insoluble matter subtracted from the weight of the ash; the difference in the weight represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug calculated.\[38\]

f) Alcohol Soluble Extractive: 5 g of the air dried drug coarsely powdered, with 100 ml of alcohol the specified strength kept in closed flask for 24 hours, rapidly filtered by taking precautions against loss of solvent. Evaporated 25ml of the filtrate to dryness in a tared flat bottomed shallow dish and dried at 105°C, to constant weight and weighed. The percentage of alcohol soluble extractive calculated with reference to the air dried drug.\[39\]

g) Water Soluble Extractive: Proceeded as directed for the alcohol soluble extractive, used chloroform water instead of ethanol.\[40\]

h) Assay for Pb: Method - By Atomic Absorption Spectrophotometer.

Solutions: 1) 1000 ppm lead standard solution prepared from Pb. 2) 1 ppm, 5 ppm and 10 ppm standard Pb solution prepared. 3) Wavelength 213.9 nm.

Procedure: 0.10 g powered sample taken in 250 ml beaker and 25 ml aqua regia added, digested on hot plate till syrupy mass was obtained. Filtered through whatman 40 no. filter paper in 100 ml volumetric flask. 1% acidity maintained with nitric acid and washed with hot water made volume to 100 ml. Observation taken on Atomic Absorption Spectrophotometry on appropriate wavelength.

Calculations: ppm (metal) = Reading (Conc.) X original volume/Weight of sample.\[41\]

i) Assay for Fe: Method was same as mentioned for Bhasma in 'In process study'.


Reagents and Standards: 1) Stannous chloride solution (20% w/v), 2) Potassium dichromate Solution (1% w/v), 3) Potassium permanganate solution (5% w/v), 4) Sulphuric acid (1%), 5) Nitric acid (10% w/v), 6) Sodium hydroxide (20% w/v), 7) Sulphuric acid (1:1 H₂SO₄). 8) Standard Mercury solution – 0.1354 g of mercuric chloride dissolved in 25 ml of 5% nitric acid. 1 ml potassium dichromate solution added and made up to 100 ml with 5% nitric acid. 1 ml of this solution = 1.0 mg Hg/ml = 1000 ug Hg/1 ml = 1000 ppm Hg.

Procedure: 5 g dry fine powder of sample weighed in a conical flask, 15 nitric acid and 5 ml sulphuric acid added and left flask in ice bath for 90 minutes. Heated on purified water bath for 30 minutes and 150 g potassium permanganate and 3 ml mercury free hydrochloric acid added. Boiled gently for 5 minutes, cooled and transferred into a 50ml plastic volumetric conical tube and made up to volume. After centrifugation clear solution used for determination of mercury content. 10 ml of aliquot pipette out into reaction vessel R2 of mercury analyzer. Procedure followed for reading mercury content as described in operation manual of the instrument.

Calculation: Mercury content (ng) = Mercury content in aliquot/Weight of the sample (g) X Total volume of the aliquot (50ml)/ Volume of aliquot for measurement (10ml).\[42\]

K) Assay for Cu- by same method as mentioned for Bhasma in 'Raw material study'.

l) Total Sulphur as S- Method – by same method as mentioned in 'Raw material study.'

OBSERVATIONS AND RESULTS

Observations

Abhraka Bhasma Nirmana

During Marana procedure, the 85-90% of Nishchandratava was achieved after third Pata. After first Pata Abhraka was swollen and cake like in appearance. After 40th Pata Abhraka was Sookshma, Shlakshna, and Ishtikabha and fulfilled all the Bhasma Pariksha.

Loha Bhasma

After Vishesh Shodhana, it became more brittle, black in appearance and Triphala Gandhi. After Bhanupaka, it became so brittle that it was easily converted into powder form. The Sookshmatva of Loha Bhasma was depend on the Mardana of Bhasma after every Pata. Loha Bhasma was triturated and filtered through cloth and subjected to next Pata. After 16th Pata Jambuphala Varna, Sookshma, Shlakshna Nishchandra, Varitara Bhasma was formed.

Tamra Bhasma

Vishesh Shodhana of Tamra was done in Gomutra there after it became more brittle, brown in colour with blackish coarse particles of Gomutra Kshara, which removed after Tamra Prakshalana in hot water. The colour of Tamra was dark black up to adding the Kajjali (till 4th Pata) then it became lighter. After 19th Pata Sookshma, Shlakshna, Nishchandra and Varitara Bhasma of Tamra was formed.

Naga Bhasma

During Pata procedure, the Chakrika of Naga was converted into uniform thick layer which was stuck to Sharava after first Pata, and then gradually the Agnipramana was reduced. It is observed that Naga is very sensitive to Agni even 300 gms of increase in cow dung cakes leads to increase in hardness of Chakrika. After 40 Pata, Niruttha, blackish, Sookshma, Shlakshna Nishchandra and Varitara Naga Bhasma was formed.
Results

A) ANALYTICAL STUDY (According To Ayurveda)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abhraka Bhasma</th>
<th>Loha Bhasma</th>
<th>Tamra Bhasma</th>
<th>Naga Bhasma</th>
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<tbody>
<tr>
<td>Shabda</td>
<td>Dantagrekachakachabhava</td>
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<tr>
<td>Sparsha</td>
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<td>Shlakshna, Mrudu, Laghu, Sookshma</td>
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<tr>
<td>Rupa</td>
<td>Ishitikavarna, Nischandra, Rekhapurnatva, Varitara, Unama, Nirdhuma</td>
<td>Jambuphalavarna, Nischandra, Rekhapurnatva, Varitara, Unama, Nirdhuma</td>
<td>Krishna Varna, Nischandra, Rekhapurnatva, Varitara, Unama, Nirdhuma</td>
<td>Brownish black, Nischandra, Rekhapurnatva, Varitara, Unama, Nirdhuma</td>
</tr>
<tr>
<td>Rasa</td>
<td>Nhiswadu (tasteless)</td>
<td>Nhiswadu (tasteless)</td>
<td>Nhiswadu (tasteless)</td>
<td>Nhiswadu (tasteless)</td>
</tr>
<tr>
<td>Gandha</td>
<td>Nirgandha (odorless)</td>
<td>Nirgandha (odorless)</td>
<td>Nirgandha (odorless)</td>
<td>Nirgandha (odorless)</td>
</tr>
</tbody>
</table>

II) Chemical Analysis

A) Apunarbhava Pariksha - Bhasmas retained their original form even after Apunarbhava Pariksha confirming their stability. They didn’t regain their metallic state, it means test is positive.

B) Nirittha Parikshana - Consistency in the weight of silver leaf is the rule of passing the test. Results indicate that the Bhasmas has passed through the Nirittha Pariksha successfully.

F) Dadi Pariksha- It was done for Tamra Bhasma. The test was positive i.e. there was no change in colour of Dadhi after 72hrs.

G) Amala Pariksha - After sprinkling Loha Bhasma over cut surface of fresh fruit of Amalaki. There was no change in colour of Loha Bhasma. This means test is positive.

II) Analytical Study (According to Modern Science)

Results of Raw material study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>Assay as Hg</td>
<td>99.56 w/w%</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Carbon disulphide insoluble matter</td>
<td>0.88% w/w</td>
</tr>
<tr>
<td>Mica</td>
<td>Aluminum as Al₂O₃</td>
<td>8.62% w/w</td>
</tr>
<tr>
<td>Iron</td>
<td>Assay as Fe</td>
<td>99.28% w/w</td>
</tr>
<tr>
<td>Copper</td>
<td>Assay as Cu</td>
<td>99.18% w/w</td>
</tr>
<tr>
<td>Lead</td>
<td>Assay for Pb</td>
<td>99.98% w/w</td>
</tr>
<tr>
<td>Impure Vatsanabha</td>
<td>Total alkaloids</td>
<td>0.36% w/w</td>
</tr>
</tbody>
</table>

Results of In Process Study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Value of Abhraka Bhasma</th>
<th>Value of Loha Bhasma</th>
<th>Value of Tamra Bhasma</th>
<th>Value of Naga Bhasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on Drying (% w/w)</td>
<td>0.32</td>
<td>0.35</td>
<td>1.06</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>Loss on Ignition (% w/w)</td>
<td>0.47</td>
<td>0.71</td>
<td>3.10</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>Acid Insoluble Ash (% w/w)</td>
<td>51.25</td>
<td>0.68</td>
<td>1.05</td>
<td>82.37</td>
</tr>
<tr>
<td>4</td>
<td>Iron as Fe (% w/w)</td>
<td>15.29</td>
<td>61.61</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Copper as Cu (% w/w)</td>
<td>-</td>
<td>-</td>
<td>63.47</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Lead as Pb (% w/w)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>64.57</td>
</tr>
</tbody>
</table>

Vishadravya

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Alkaloids</td>
<td>0.08%</td>
</tr>
</tbody>
</table>

Available online at: [http://ijapr.in](http://ijapr.in)
Results of Final Product study

Table 5: Results of Analysis Vata Gajendra Singh Rasa (as per analytical study of final product)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Disintegration Time (min.)</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>Loss on drying (% w/w)</td>
<td>19.14</td>
</tr>
<tr>
<td>3</td>
<td>Total Ash (% w/w)</td>
<td>33.51</td>
</tr>
<tr>
<td>4</td>
<td>Acid Insoluble Ash (% w/w)</td>
<td>1.25</td>
</tr>
<tr>
<td>5</td>
<td>Acid Soluble Ash (% w/w)</td>
<td>32.26</td>
</tr>
<tr>
<td>6</td>
<td>Water Insoluble Ash (% w/w)</td>
<td>18.26</td>
</tr>
<tr>
<td>7</td>
<td>Water Soluble Extractive (% w/w)</td>
<td>23.45</td>
</tr>
<tr>
<td>8</td>
<td>Alcohol soluble Extractive (% w/w)</td>
<td>6.39</td>
</tr>
<tr>
<td>9</td>
<td>Assay for Pb (% w/w)</td>
<td>3.02</td>
</tr>
<tr>
<td>10</td>
<td>Assay for Fe (% w/w)</td>
<td>4.26</td>
</tr>
<tr>
<td>11</td>
<td>Assay for Hg (ppm)</td>
<td>6.82</td>
</tr>
<tr>
<td>12</td>
<td>Assay for Cu (% w/w)</td>
<td>3.12</td>
</tr>
<tr>
<td>13</td>
<td>Total Sulphur as S</td>
<td>1.27</td>
</tr>
</tbody>
</table>

DISCUSSION

Discussion about Preparation of Vata Gajendra Singh Rasa

Abhraka Bhasma

For the Abhraka Bhasma preparation Krishnavajrabhraka was selected which didn’t lose its properties on Agnipariksha. The cow dung cakes & coal was used as fuel for the Puta and its proportion was taken according to weight of Abhraka. For the Nishchandrikarana of Abhraka first two Putas given with Guda and Kalamisora (Anubhata) and Chandrika reduced to almost 80-90% after 4th Puta. The temperature was increased slowly according to Agnisahatva of Abhraka. For the Bhavana procedure Arkapatra, Kumari Swarasa and Dashamooli Kwatha were used to achieve Vataghna property. The Ishtika Varna of Abhraka Bhasma is because Iron Oxide is a chief constituent of Abhraka Bhasma which believed to be causative factor of that typical brick red colour.

Loha Bhasma

During Shodhana when Loha heated up to red hot stage then some of iron was converted into ferroso-ferric oxide by reacting with atmospheric oxygen which form black layer on the surface which was removed after quenching. Vishesa Shodhana of Loha was done in Triphala Kwatha. Six liquid Medias used during Shodhana of Loha. The alternate heating and quenching in the acidic and basic liquid media may leads to the corrosive changes and removal of acid and alkali soluble impurities.

Loha Marana

Hingula is said to be best Maraka Dravya of Loha hence 1/12 quantity of Hingula was added up to 4th Puta. Cow dung cakes and coal was used as fuel in Putapaka according to weight of Loha. The colour of Loha Bhasma was purple (Pakva Jambuphala Varna). Loha Bhasma may be considered as mixture of ferrous oxide, ferric oxide, ferrous sulphate and trace elements. Out of this ferrous sulphate is black and ferric oxide is red in colour. Hence combination of all these compounds makes Loha Bhasma purple in colour.

Tamra Bhasma

For the Tamra Bhasma Nirmana the copper wire of diameter 0.36 mm selected as raw material. As it is said to be the pure form of Tamra expect the insulator Vernix which was removed by heating and dipping into water. It is said that Rasabhasma Marita Bhasmas posses good quality. But there are doubts about Rasabhasma hence Kajjali is selected as Maraka Dravya for Tamra Bhasma. Tamra is soft metal as compared to Loha therefore it requires less heat than Loha hence only cow dung cakes are selected as fuel. Tamra Bhasma Nirmana require specific temperature pattern. The no. of cow dung cakes for first Puta was 25 (each of wt. average 280gms) which were gradually reduced to 5 for the 19th Puta for 1340 gm of Tamra. The black colour of Tamra Bhasma may because of cupric oxide (black to brown colour) and copper sulphide (blue-black colour).

Naga Bhasma

Raw Naga was purchased from local market as lead-triangular straight rod. As Naga is highly sensitive to temperature initially 15 cow Dung cakes (each of weight 280gm) were used as fuel for 1763 gm of mixture, but after first Puta the Chakrika were converted into the thick layer was stuck to the Sharava, it means that proportion of Agni was more than the required quantity and orange colour turns to black. Then the no. of cow dung cakes gradually reduced to 12,6,4,3. For 8, 9, 10th Puta 3 cow dung cakes (average wt. 900 gms.) was used as fuel. After 10th Puta the Chakrika were not so hard and could be breakable. Therefore in case of Puttilohas initially the proportion of Agni should be less as possible and should be increase gradually according to Agnisahatva.

Vatsanabha Shodhana

According to modern science the toxic content of Vatsanabha is Alkaloids it varies from 0.63 - 4.7%. The total Alkaloid in Ashudhha Vatsanabha was 0.36% w/w and after Shodhana it was reduced to 0.085% w/w which is 4½ times less than Ashudhha Vatsanabha, it means that although Shodhana of Vatsanabha is looks simple process but the results were significant. It justifies the Vishagna Prabhava of Gomutra.

Discussion about Analytical Study

1) Discussion about Raw drug study

The percentage of Mercury in Parada was 99.56% w/w, Sulphur in Gandhaka was 99.28% w/w, Copper in Tamra was 99.18% w/w. and Lead in Naga was 99.98%
w/w. It means that the selected raw material was of good quality. In the Analysis of Raw Abhraka - Aluminum was 8.62% w/w, Iron -11.71% w/w, Silica -50.54% w/w, Magnesium -2.43% w/w and potassium -5.26% w/w was found. These contents are naturally present in Abhraka; the analysis was justified for preparation of Abhraka. The total alkaloids in Ashuddha Vatsanabha were found 0.36% w/w, usually it are present in the range of 0.63-0.47%. The analytical test was carried out to check authentication of Vatsanabha. Above results shows that raw material selected was authentic.

ii) Discussion about ‘In process study’

A) Bhasma: The results of Analytical study of Bhasma are compared with the standards of Bhasma mentioned in the “Pharmacopoeial standards for Ayurvedic formulations”.

### Table 6: Assessment of Bhasmas

<table>
<thead>
<tr>
<th>Test</th>
<th>Abhraka Bhasma[^43]</th>
<th>Loha Bhasma[^44]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on Drying</td>
<td>&lt; 0.5</td>
<td>Loss on Drying</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Loss on Ignition</td>
<td>&lt; 1</td>
<td>Loss on Ignition</td>
</tr>
<tr>
<td></td>
<td>0.47</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Acid Insoluble Ash</td>
<td>40-65</td>
<td>Acid Insoluble Ash</td>
</tr>
<tr>
<td></td>
<td>51.25</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Iron as Fe</td>
<td>14-16</td>
<td>Iron as Fe</td>
</tr>
<tr>
<td></td>
<td>15.29</td>
<td>65-70</td>
</tr>
</tbody>
</table>

The value indicates that results of Analytical study of Bhasma were nearly matched with the standard parameters means Bhasma prepared are of good quality.

### iii) Discussion about ‘End Product’

The Disintegrate time of Vata Gajendra Singh Rasa was 65 minute which is quite high. It may because starch content of Bhavana Dravya Kumari. Assay for lead - 3.02% w/w, Assay for Iron - 4.26% w/w, Assay for Mercury - 6.82% w/w, Assay for Copper - 3.12% w/w, Total Sulphur - 1.2% w/w. Presence of these values in final product is applicable for authentication and standardization Vata Gajendra Singh Rasa. To develop standards further studies are required, because the standards for Vata Gajendra Singh Rasa are not mentioned in the “Pharmacopoeial standards for the Ayurvedic formulations” of C.C.R.A.S.

**CONCLUSION**

**Conclusion regarding Preparation of drug**

For the Nishchandrikarana of Abhraka first 2 Putas given with Guda and Kalamisora after this process there was marked reduction in Chandrika. In Trividha Lauhapa Loha became so brittle that its powder was formed easily after Bhanupaka and Sthoilpak it concludes Trividha Lauhapa is essential for Marana of Loha. It is said that salts of Naga (powder form) are stable for Putapaka. Even if, initially the Agnipramana should be minimum and it should be increase gradually according to Agnisahatva of Naga. The Mardana of Dhatu Bhasma enhances the Bhasma formation. If Dhatu Bhasma triturated properly after each Puta the Bhasma will be formed easy and easily.

**Conclusions regarding Analytical Study**

Analysis of Bhasma fulfills the criteria of Bhasma Pariksha. Dantagrekachakachabhava of all the Bhasma (Abhraka Loha Tamra, and Naga) indicates that there was absence of free metal particles in Bhasma. Sookshmatva, Rekkapurnatva, Varitara, Unama Pariksha of Bhasma indicates its micro fineness. Nirghanda and Nirdhuma indicate that there were no free Gandhaka present in the Bhasma. Chemical analysis as per Ayurveda i.e. Apunarbharva Pariksha, Niruttha Pariksha was carried out to check the stability of Dhatu Bhasma. Naga, Loha, Tamra, Bhasma passed through these test, it concludes that Bhasma formed were stable i.e. Marana of Dhatu was achieved. Dadhi Pariksha of Tamra Bhasma is authentic to check its toxicity. Amla Pariksha of Loha Bhasma is easy method to check free Iron content in the Bhasma.

**Analysis as per Modern Science**

The total alkaloids in Shuddha Vatsanabha was 0.085% which is 4½ times less than found in Ashuddha Vatsanabha (0.36% w/w) it concludes that the principle of Vishadravya shodhana is to reduce its poisonous properties up to extent, where it does not show any unwanted effect therapeutically. Conclusion drawn about the final product i.e. Vata Gajendra Singh Rasa is- The facts observed in the analysis of Vata Gajendra Singh Rasa are as follow. The Disintegration time of drug was 65 min. it was quite high it may happen because Kumari Swarasa was used for Bhavana contain starch leads to tight bonding of ingredients. Acid insoluble Ash in Vata Gajendra Singh Rasa was found 1.25% w/w it conclude that 98.75% of the drug is available in the body for absorption and action.

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46. Ibid, p. 57.


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