RESEARCH ARTICLE

COMPARATIVE PHARMACEUTICO-ANALYTICAL STUDY OF SWARNA MAKSHIKA BHASMAS.

Dr. Sushant Kumar\textsuperscript{1}, Dr. (Mrs) Prabha Kumari\textsuperscript{2}, Dr Amarendra Kumar Singh\textsuperscript{3}, Dr. Prabhat Kumar Dwivedi\textsuperscript{4} and Dr. Ajay Kumar Singh\textsuperscript{5}.

1. Asst. Prof, Dept of RS & BK, Sri Sai Ayurvedic P.G. Medical College & Hospital, Aligarh (UP).
2. Asso. Prof., Dept of Prasuti Tantra & Śrī Roga, Govt. Ayurvedic College & Hospital, Chaukaghat, Varanasi (UP).
3. Reader, Dept of Roga Nidana & Vikriti Vigyana, Govt. Ayurvedic College & Hospital, Patna.
4. Reader, P.G. Dept of Rasa Shastra & Bhaishhya Kalpana, Govt. Ayurvedic College & Hospital, Patna.
5. Prof. & HOD, P. G. Dept of Rasa Shastra & Bhaishhya Kalpana, Govt. Ayurvedic College & Hospital, Patna.

Manuscript Info

Manuscript History

Received: 28 July 2017
Final Accepted: 30 August 2017
Published: September 2017

Key words:-
IDA; Swarnamakshika Bhasma; Shodhana; Marana.

Abstract

Swarnamakshika has been described in the treatment of Panduroga in almost all the classics of Ayurveda. Presently India is leading in the world in the context of iron deficiency anaemia because more than 50% of its population is suffering from it. Swarnamakshika Bhasmas prepared by different shodhana (RT 21/7-11, RRS 2/83 and RT 21/18) and marana processes (RT 21/21-22, RT 21/19-20 & RT 21/23-25) were analyzed and compared on physico chemical parameters, XRD, SEM, ICP-MS etc conducted at IIC, IIT, Roorkee and Science College, Patna. It could be a drug of choice for the treatment of anaemia due to easy availability and cheaper price.

Introduction:

Presently India is leading in the world in the context of iron deficiency anaemia because more than 50% of its population is suffering from it\textsuperscript{1}. This silent killer is very common among Indian rural population due to poor socioeconomic status, inadequate dietary habits, worms infestation and GI bleeding\textsuperscript{2}. The IFA programme was launched way back in 1970. Despite this incidence of anaemia in India during pregnancy has been noted as high as 40-80\%. 15-22\% of maternal mortality has been estimated due to anaemia during pregnancy \textit{(Source- Health Information of India, 2004)}. A 2007 Indian government “12 by 12 initiative”, aimed at ensuring that all Indian adolescents have 12 g/dL haemoglobin by 2012, listed the main causes of anaemia in India as low dietary intake, poor availability of iron, chronic blood loss due to hookworm infestation, and malaria\textsuperscript{3}. Iron deficiency causes defective haemoglobinisation leading to Microcytic Hypochromic Anaemia\textsuperscript{4}.

\textit{Rasa Shastra}, as rightly called the “Back Bone of Ayurveda” deals with the knowledge of alchemical and pharmaceutical processes. Here, \textit{Rasaushadhis} (herbomineral drugs) made of metals, minerals and different poisonous herbal substances are used for treating diseases. These are preferred over \textit{Kashtaushadhis} (herbal drugs) because of their certain innate qualities like instantaneous therapeutic action even when used in low doses, tastelessness (not unpalatable)\textsuperscript{5}.

\textit{Swarnamakshika} (Chalcopyrite or CuFeS\textsubscript{2}), mentioned in the \textit{Maharasa Varga} in \textit{Rasa Shastra}, is a compound of copper, iron and sulphur. It has been described for the treatment of Pandu Roga i.e. Iron Deficiency anaemia in...
various classics of Ayurveda. It contains extrinsic factors such as copper and iron required for the formation of haemoglobin. Copper is an essential trace element exists in the diet and is needed to absorb and utilize iron. The importance of copper as a supplement to iron for haemoglobin regeneration in anaemic rats has already been established. This discovery revealed the utility of small amount of copper in the treatment of Microcytic Hypochromic Anaemia. Being a Saumyakalpa of iron it is easily digestible and hence widely used in infants, pregnant, lactating women and frail old people. Easy availability and cheaper price makes it a drug of choice for the treatment of Pandu Roga i.e. IDA.

In Rasa Shastra, Shodhana (Purification/ Detoxification/ Potentiation) is the process of removing the impurities and toxicities of metals and minerals by means of Swedana, Bharjana, Bhawana, Mardana, Prakshalana, Avapa, Nirvapa etc and potentiating the efficacy by adding the qualities of liquid media in which it is done. So in this study, two different methods of Shodhana (i.e. Swedana in Kadlikanda Swarasa and Bharjana in lemon juice) have been done.

Marana or Bhasmikarana (Incineration/ Calcination) is the process of converting the already purified metals and minerals into ashes i.e. Bhasma. The whole process of Marana can be summarised in the following manner. The purified material is subjected to Bhawana (Wet levigation in the specified liquid media) → formation of Chakrika (pelletization) → Sharava Sampootikarana (Drying and closing in between two earthen lids)→ Putapaka (specialised conventional heating system) → Bhasma formation. Through the process of Marana, the purified material becomes detoxified, easily digestible, absorbable, assimilable and finally excreted if consumed in extra quantity which are quintessential for any drug. Incineration of metals with mercury results in uttama (top quality) bhasma, with herbs results in madhyama (middle quality) bhasma, with sulphur and other substances results in adhama (low quality) bhasma. Incineration with arilohas and other substances results in durguna (causing harmful effect) bhasma. Keeping this in mind, three different ways of marana were adopted here to evaluate their comparative pharmaceutical and analytical study.

Pharmaceutical study:-
Aims and Objectives:
The prime objective of the present study was to develop a SOP (Standard Operative Procedure) for the preparation of Swarnamakshika Bhasma by adopting different methods of shodhana and marana. Further it was also aimed to produce a safe, effective and quality drug for the treatment of Pandu Roga i.e. Iron Deficiency Anaemia. The materials used and the methods adopted was decided on the basis of references in the classical literatures, easy availability of raw materials, cost feasibility of the methods, traditional experiences as well as expert opinions.

Material and Methods:-
Procurement- Raw Swarnamakshika was provided by the P.G. department of Rasa Shastra & Bhaishyja Kalpana, Government Ayurvedic College & Hospital, Patna.

Physical characteristics of Swarnamakshika-
Colour- Brownish black
Odour- Nil
Texture- Crystalline

The whole process has been divided in two parts- 1) Shodhana & 2) Marana

1) Shodhana of Swarnamakshika by two methods-
- Swedana (boiling under liquid bath) in Kadlikanda Swarasa
- Bharjana (roasting) in lemon juice

2) Marana of Swarnamakshika by three methods-
Here, three different methods of marana of Swarnamakshika were adopted as mentioned in the book Rasa Taringini. These three methods were selected on the basis of different processes of marana of metals and their quality/efficacy given in Rasa Ratna Samuccaya (Ref- RRS 5/14). After final incineration comparative pharmaceutical study will be done-
- RT 21/ 21-22 (Kanishtham gandhakadibhih)
- RT 21/ 23-25 (lohanam maranam shreshtham sarvesham rasabhasmana)
- RT 21/ 19-20 (moolibhih madhyamam prahuh)
### Observation and Results

**Table no- 1: Comparative study of the two processes of Shodhana of raw Swarnamakshika**

| S. No | Methods adopted for shodhana | Weight of Makshika (in gm) | %age Change | Time taken |
|-------|------------------------------|---------------------------|-------------|------------|
|       | Before shodhana              | After shodhana             | %age gain   | %age loss  |
| 1     | Swedana with kadalikand swarasa (Ref - RT 21/18 and RRS 2/83) | 1250 | 1110 | 88.8 | 11.2 | 2hrs×3 times = 6hrs |
| 2     | Bharjana with nimbu swarasa (Ref- RT 21/7-11) | 1250 | 1090 | 87.2 | 12.8 | 2 hrs |

**Table no- 2: Showing Marana of Swarnamakshika (Sample-I) Ref- RT 21/21-22**

| No. of Puta | Swarnamakshika (wt in gm) | Shuddha Gandhaka | Nimbu Swarasa (in ml) | Wt of Chakrika (before puta) | Wt of Chakrika (after puta) | Colour |
|-------------|---------------------------|-------------------|-----------------------|------------------------------|----------------------------|--------|
| 1           | 250                       | 250               | 100                   | 520                          | 223                        | Brownish red |
| 2           | 223                       | 223               | 100                   | 500                          | 205                        | Brownish red |
| 3           | 205                       | 205               | 80                    | 440                          | 190                        | Brownish red |
| 4           | 190                       | 190               | 80                    | 400                          | 182                        | Red     |
| 5           | 182                       | 182               | 80                    | 380                          | 176                        | Red     |
| 6           | 176                       | 176               | 60                    | 350                          | 170                        | Red     |

**Table no- 3: showing Marana of Swarnamakshika (Sample-II) Ref- RT 21/21-22**

| No. of Puta | Swarnamakshika (wt in gm) | Shuddha Gandhaka | Nimbu Swarasa (in ml) | Wt of Chakrika (before puta) | Wt of Chakrika (after puta) | Colour   |
|-------------|---------------------------|-------------------|-----------------------|------------------------------|----------------------------|----------|
| 1           | 250                       | 250               | 100                   | 520                          | 220.8                      | Brownish red |
| 2           | 223                       | 223               | 100                   | 500                          | 205                        | Brownish red |
| 3           | 205                       | 205               | 80                    | 440                          | 190                        | Brownish red |
| 4           | 190                       | 190               | 80                    | 400                          | 182                        | Red      |
| 5           | 182                       | 182               | 80                    | 380                          | 176                        | Red      |
| 6           | 176                       | 176               | 60                    | 350                          | 170                        | Red      |

**Table no- 4: showing Marana of Swarnamakshika (Sample-III) Ref- 21/23-25**

| No. of Puta | Swarnamakshika (wt in gm) | Shuddha Hingula (wt in gm) | Nimbu Swarasa (in ml) | Wt of Chakrika (before puta) | Wt of Chakrika (after puta) | Colour   |
|-------------|---------------------------|---------------------------|-----------------------|------------------------------|----------------------------|----------|
| 1           | 750                       | 93.75                     | 250                   | 890                          | 670.8                      | Reddish Brown |
| 2           | 670.8                     | 84                        | 220                   | 810.2                        | 648.9                      | Reddish Brown |
| 3           | 648.9                     | 81                        | 200                   | 780.4                        | 621.5                      | Brownish red |
| 4           | 621.5                     | 77                        | 180                   | 743                          | 595.6                      | Brownish red |
| 5           | 595.6                     | 75                        | 180                   | 710                          | 575.1                      | Brownish red |
| 6           | 575.1                     | 72                        | 150                   | 662                          | 543.2                      | Brownish red |
| 7           | 543.2                     | 68                        | 150                   | 630                          | 521                        | Brownish red |
| 8           | 521                       | 65                        | 150                   | 598                          | 505                        | Brownish red |

**Table no- 5: Showing Marana of Swarnamakshika (Sample-IV) Ref- 21/19-20**

| No. of Puta | Swarnamakshika (wt in gm) | Nimbu Swarasa (in ml) | Wt of Chakrika (before puta) | Wt of Chakrika (after puta) | Colour |
|-------------|---------------------------|-----------------------|------------------------------|----------------------------|--------|
| 1           | 750                       |                       | 890                          | 670.8                      | Reddish Brown |
| 2           | 670.8                     |                       | 810.2                        | 648.9                      | Reddish Brown |
| 3           | 648.9                     |                       | 780.4                        | 621.5                      | Brownish red |
| 4           | 621.5                     |                       | 743                          | 595.6                      | Brownish red |
| 5           | 595.6                     |                       | 710                          | 575.1                      | Brownish red |
| 6           | 575.1                     |                       | 662                          | 543.2                      | Brownish red |
| 7           | 543.2                     |                       | 630                          | 521                        | Brownish red |
| 8           | 521                       |                       | 598                          | 505                        | Brownish red |
Flow Chart showing Pharmaceutical study of Swarnamakshika bhasma

Raw Swarnamakshika
↓
↓
Shodhana by Swedana
(Ref- RT21/21-22)
Shodhana by Bharjana
(Ref- RT21/23-25)

(Ref- RT21/19-20)

Shuddha Swaranamakshika obtained and used for Marana through

\((\text{with Nimbu swarasa for } 2\text{hrs})\) \((\text{with Nimbu swarasa for } 2\text{hrs } \times 3\text{times})\)

Shuddha Swaranamakshika obtained and used for Marana through

\((\text{with Kadalikanda swarasa for } 2\text{hrs})\)

\((\text{with Nimbu swarasa for } 2\text{hrs } \times 3\text{times})\)

Bhawana
Bhawana
Bhawana

(Ref- RT21/19-20)

\((\text{with Nimbu swarasa})\) \((\text{with Nimbu swarasa})\) \((\text{with Nimbu swarasa})\)

Added-
Added-
Added-

(Shuddha Hingula 1/8\text{th} part) \((\text{only Nimbu Swarasa})\)

\((\text{Equal amount of Shuddha Gandhaka})\)

Pelletized and dried
Sharava Samputikarana

Subjected to \text{Puta} (6)
Subjected to \text{Puta} (8)
Subjected to \text{Puta} (10)

\((\text{By conventional method})\)

Sample I & II
Sample III
Sample IV

Red
Red
Brownish Red

Swarnamakshika Bhasma obtained

Analytical study:-

Analytical study of a product provides some standards to judge its quality as well as interprets the pharmacokinetics and pharmacodynamics of the same. Main aim of this study is to find out the physico-chemical changes and interpret the effect of different samskaras (shodhana, marana etc) during the pharmaceutical processing.

In Ayurveda, use of metallic and herbomineral preparations for therapeutic purposes were mainly based on clinical observations. Ancient scholars had scientific vision regarding safe use of these preparations. So different parameters for examining the purity of bhasma have been mentioned in Textbooks of Rasa Shastra e.g. Varitara, Rekhapurnatwa, Apanarbhava, Uttama, Niruttha, Mrtaloha etc\textsuperscript{viii}.

Mere efficacy without safety is of no use. In this scientific era, people have become quite concerned regarding the safety of the drugs consumed by them. Hence it is necessary to know the nature of the drug, which we prescribe to our patients. For knowing whether it contains any harmful substance or not, analytical study of the metallic or herbomineral drugs is mandatory. Detection of the presence of any free metal in Bhasma is quite essential as free metals are considered highly toxic for the vital organs of the body.

Plan of study:-
The samples were evaluated on classical as well as modern analytical parameters. For modern analytical study, parameters mentioned in Protocol for Testing (Ayurvedic, Siddha & Unani Drugs, published by department of AYUSH, Government of India in collaboration with Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad),
were followed. Swarnamakshika Bhasma prepared by two different Shodhana (RT 21/7-11, RRS 2/83 and RT 2/18) and three different Marana processes (RT 21/21-22, RT 21/19-20 & RT 21/23-25) were analyzed and compared on the basis of physico-chemical parameters, XRD, SEM, ICP-MS studies etc conducted at Institute Instrumentation Centre, Indian Institute of Technology, Roorkee and Science College, Patna.

**Table no-6: Organoleptic Characters of Swarnamakshika Bhasma**

| S. No | Parameters | Textual References |
|-------|------------|--------------------|
|       |            | RT 21/21-22       |
| 1     | Varna      | RT 21/19-20       |
| 2     | Sparsha    | RT 21/23-25       |
| 3     | Rasa       |                    |
| 4     | Gandha     |                    |

**Table no-7: Classical analytical tests of different samples of Swarnamakshika Bhasmas**

| S. No | Parameters      | Textual References |
|-------|-----------------|--------------------|
|       |                 | RT 21/21-22       |
| 1     | Gatarasatva     | RT 21/19-20       |
| 2     | Rekhapurnatva   | RT 21/23-25       |
| 3     | Varitara        |                    |
| 4     | Slakshnatva     |                    |
| 5     | Dantagre kachkachabhawam | |

**Table no-8: Showing the pH of different samples of Swarnamakshika Bhasmas**

| pH value | Sample I | Sample II | Sample III | Sample IV |
|----------|----------|-----------|------------|-----------|
|          | 6.1      | 6.0       | 5.8        | 6.2       |

**Table no-9: Showing the analytical data on physicochemical parameters of different samples of Swarnamakshika Bhasmas**

| Samples                                      | Loss on drying (% W/w) | Ash Value (% w/w) | Acid insoluble ash (%w/w) |
|----------------------------------------------|------------------------|-------------------|---------------------------|
| Swarnamakshika (Raw drug)                    | 4.5                    | 74.8              | 24.2                      |
| Shodhita Swarnamakshika (by Sweedana)         | 2.2                    | 88.6              | 19.5                      |
| Shodhita Swarnamakshika (by Bharjana)         | 2.1                    | 90.6              | 18.3                      |
| Swarnamakshika Bhasmas (RT 21/21-22) Sample I| 0.88                   | 97.2              | 19.0                      |
| Swarnamakshika Bhasmas (RT 21/21-22) Sample II| 0.82                  | 98.2              | 14.6                      |
| Swarnamakshika Bhasmas (RT 21/23-25) Sample III| 0.45                  | 96.5              | 23.2                      |
| Swarnamakshika Bhasmas (RT 21/19-20) Sample IV| 0.56                  | 98.8              | 22.3                      |

**X-Ray diffraction study (XRDS):**

X-ray diffraction is a tool for investigation of the fine structures of the crystals. When a focussed X-ray beam interacts with planes of atoms, some part of the beam is transmitted, some is absorbed, some is refracted and some part is diffracted. X-rays are diffracted by each mineral differently, depending upon the atoms that make the crystal lattice and how these atoms are arranged.

This resulting analysis is described graphically as a set of peaks with one percent intensity on the Y-axis and goniometer angle on X-axis. The exact angle and the intensity of a set of peaks are unique to the crystal structure which is under examination. XRD characterisations of different samples of Swarnamakshika Bhasma are given below-.
• **Figure no- 1**: Showing the XRD pattern of **Sample III** i.e. *Kadalikanda swarasa shodhita Swarnamakshika Bhasma* in which trituration is done with *Hingula & Nimbu Swarasa* (Ref- RT21/23-25)

• **Figure no- 2**: Showing the XRD pattern of **Sample IV** i.e. *Nimbu swarasa shodhita Swarnamakshika Bhasma* in which triturition is done with only *Nimbu Swarasa* (Ref- RT21/19-20)

• All peaks of XRD results were identified Fe$_2$O$_3$ which shows strong peaks with high intensity in both the samples.

**Results:** *Swarnamakshika* which is chalcopyrite basically shows different complex compounds which completely get turned into oxides of iron and copper (Fe$_2$O$_3$) finally and this comparison observed in XRD results of final *Bhasma* in both the samples.

**Field Emission Scanning Electron Microscopy (FESEM) study:-**
It provides topographical and elemental information at magnification of 10x to 3,00,000x, with virtually unlimited depth of field. Compared with Scanning Electron Microscopy (SEM), it produces a clearer less electrostatically distorted images with spatial resolution down to 1.5 nm. It is 3-6 times better than the conventional SEM. So it is used for ultra high magnification imaging. The high resolution reached by FESEM (~ 2nm) allows the study of very small structural details. There is various application of this instrument but as far as *Rasa Shastra* is concerned, it is mainly used in the morphological analysis i.e. in knowing the shape and size of particles and qualitative elemental analysis. Besides this it is used in the localization of boundaries between regions of different atomic number.

**Results:**
The results of SEM of the two samples of *Swarnamakshika Bhasmas* (prepared by two different Shodhana & Marana processes) are shown in the Plate no. 1 & 2. These samples were analyzed at IIC, IIT, Roorkee.
Plate no 1 & 2: Showing Comparative SEM Study of Swarnamakshika Bhasma of Sample- III (Ref- RT 21/21-23) & Sample- IV (Ref- RT 21/19-20)

Figure no-1 SMB at 100× magnification
Figure no-2 SMB at 500× magnification
Figure no-3 SMB at 1000× magnification
Figure no-4 SMB at 2500× magnification

After going through the SEM study of both the samples (as received by the IIC, IIT, Roorkee) it was observed that-

- Most of the particles in all the photographs are in the range of 100nm-5µ.
- There is large amount of 2-5µ sized homogeneous particles.
- There are also very fine particles observed which are in the range of nano particle size.
- Most of the particles are in the nano range less than 80nm.
- There is agglomeration of fine nano particles.

Result:
The methodology employed for the preparation of Swarnamakshika Bhasma by different methods yields nano particles in general.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS):
It is considered as the most reliable and latest method for the quantitative elemental analysis available till date. The primary goal of ICP is to get elements to emit characteristic wavelength specific light which can then be measured. The intensity of wavelength specific light is compared to previous measured intensities of the known concentration of elements and the concentration is computed. This method is used to determine Arsenic, Cadmium, Lead, Mercury, Copper etc in any herbomineral or metallic drug.

Observation and Results:
The concentration of different elements (in %age) in the two samples of Swarnamakshika Bhasmas are given here. This analytical study through ICP-MS was done at IIC, IIT, Roorkee.

Table no-10: Showing the comparative data of %age of different elements present in the two samples of Sarnamakshika Bhasma as obtained through ICP-MS studies.

| Elements      | Swarnamakshika Bhasma (RT 21/23-25) Sample III | Swarnamakshika Bhasma (RT 21/19-20) Sample IV |
|---------------|-----------------------------------------------|-----------------------------------------------|
| Sodium (Na)   | 0.178                                         | 0.156                                         |
| Potassium (K) | 0.267                                         | 0.245                                         |
| Arsenic (As)  | 0.128                                         | 0.690                                         |
Ultimate 505 gm puta. Same process was repeated after each puta. After drying of puta, it was observed that 11.2% weight loss was observed. Total time taken was approximately 6 hrs. In the 2nd method of Shodhana, 1090 gm shuddha swarnamakshika was obtained from 1250 gm ashuddha one. Here 12.8% weight loss was observed. The loss might have occurred due to the hammering of ashuddha Swarnamakshika to make it coarse powder before the initiation of the process of Shodhana. More loss was observed in the Bharjana process. The cause of it might be the total time taken in the procedure is more compared to the Swedana procedure. Further the acidic media of lemon juice in might have caused more loss in the Bharjana process. Some amount of the material might have been lost during the repetition of the process.

In sample I (Ref- RT21/21-22), 250 mg gm of Shuddha Swarnamakshika (Swedita with Kadlikanda Swarasa) was mixed with 250 gm of Shuddha Gandhaka and triturated with sufficient quantity of Nimbu Swarasa. In sample II (Ref- RT21/21-22), 250 mg of shuddha Swarnamakshika was mixed with 250 gm of Shuddha Gandhaka triturated with sufficient quantity of Nimbu Swarasa. In both the samples, trituration was done for two hours till it became thick paste to prepare pellets (Chakrikas). These Chakrikas were made having diameter 2cm and thickness 1cm so that every particle of it get equal and adequate amount of heat for incineration.

After drying of chakrikas, sharava sampootikarana was done and subjected to Puta. Conventional method of heating was done to prepare all the samples. 10-12 kgs of Vanyopal were taken for Puta. Bhasma collected after each puta was subjected to triturition with Nimbu Swarasa. Chakrikas prepared after triturition were subjected to puta. Total 6 putas were given in both the samples.

After 1st puta, it was observed that Swarnamakshika chakrikas were brownish red in colour and soft. The brownish red colour of chakrikas was observed after 2nd & 3rd puta but the hardness comparatively decreased. After 4th, 5th & 6th puta Swarnamakshika chakrikas became red like vermillion in colour. 170 gm in Sample I and 163.7 gm in Sample II Swarnamakshika Bhasma was obtained from 250 gm of shuddha Swarnamakshika. It meant average yield of Swarnamakshika Bhasma was 68% in Sample I and 65.48% in Sample II (Table no-2 & 3).

As per Table no- 4, 750 gm of shuddha Swarnamakshika (kadalikand swarasa shodhita) was taken and 1/8th part of it shuddha hingula was added in it. This mixture was triturated by adding sufficient quantity of lemon juice in into it. Same process was repeated after each puta for total 7 times and total 8 putas were given in this method. Ultimately, 505 gm bhasma obtained from 750 gm of shuddha swarnamakshika. It meant the yield was 67.33% through this process.

| Elemental (Metal) | Sample I | Sample II |
|------------------|----------|-----------|
| Iron (Fe)        | 18.899   | 16.534    |
| Copper (Cu)      | 0.031    | 0.031     |
| Lead (Pb)        | 0.040    | 0.461     |
| Zinc (Zn)        | 0.318    | 0.252     |
| Chromium (Cr)    | 0.013    | 0.016     |
| Manganese (Mn)   | 0.034    | 0.039     |
| Magnesium (Mg)   | 0.292    | 0.192     |

It can be concluded from the above data that %age of heavy metals are within the permissible limit as per the GMP guidelines in both the samples of Swarnamakshika Bhasma.

Discussion:

In the pharmaceutical study, a SOP (standard operative procedure) for the preparation of Swarnamakshika Bhasmas by adopting different methods of purification and incineration has been taken. Here two different methods of purification (Shodhana) and three different processes of incineration (Marana) have been undertaken for the final preparation of Bhasma. The hypothesis behind the selection of these different methods was made on the basis of easy availability of raw materials, minimum labour involvement, less total time in the whole procedure, cheap cost of processing. There was also a thought to increase the iron content in the manufactured drug. Finally its easy absorption through the gut was also kept in mind behind adopting these Shodhana and Marana procedures.

In the present study, two methods of purification were adopted (Table no-1). In the first method of Shodhana through Swedana (heating under liquid bath), total 1110 gms of shuddha Swarnamakshika was obtained from 1250 gms of ashuddha one. In this method 11.2% weight loss was observed. Total time taken was approximately 6 hrs. In the 2nd method of Shodhana, 1090 gm shuddha swarnamakshika was obtained from 1250 gm ashuddha one. Here 12.8% weight loss was observed. The loss might have occurred due to the hammering of ashuddha Swarnamakshika to make it coarse powder before the initiation of the process of Shodhana. More loss was observed in the Bharjana process. The cause of it might be the total time taken in the procedure is more compared to the Swedana procedure. Further the acidic media of lemon juice in might have caused more loss in the Bharjana process. Some amount of the material might have been lost during the repetition of the process.

Marana (Incineration) of Swarnamakshika (Sample I, II, III & IV):

In sample I (Ref- RT 21/21-22), 250 mg gm of Shuddha Swarnamakshika (Swedita with Kadlikanda Swarasa) was mixed with 250 gm of Shuddha Gandhaka and triturated with sufficient quantity of Nimbu Swarasa. In sample II (Ref- RT21/21-22), 250 mg of shuddha Swarnamakshika was mixed with 250 gm of Shuddha Gandhaka triturated with sufficient quantity of Nimbu Swarasa. In both the samples, trituration was done for two hours till it became thick paste to prepare pellets (Chakrikas). These Chakrikas were made having diameter 2cm and thickness 1cm so that every particle of it get equal and adequate amount of heat for incineration.

After drying of chakrikas, sharava sampootikarana was done and subjected to Puta. Conventional method of heating was done to prepare all the samples. 10-12 kgs of Vanyopal were taken for Puta. Bhasma collected after each puta was subjected to triturition with Nimbu Swarasa. Chakrikas prepared after triturition were subjected to puta. Total 6 putas were given in both the samples.

After 1st puta, it was observed that Swarnamakshika chakrikas were brownish red in colour and soft. The brownish red colour of chakrikas was observed after 2nd & 3rd puta but the hardness comparatively decreased. After 4th, 5th & 6th puta Swarnamakshika chakrikas became red like vermillion in colour. 170 gm in Sample I and 163.7 gm in Sample II Swarnamakshika Bhasma was obtained from 250 gm of shuddha Swarnamakshika. It meant average yield of Swarnamakshika Bhasma was 68% in Sample I and 65.48% in Sample II (Table no-2 & 3).

As per Table no- 4, 750 gm of shuddha Swarnamakshika (kadalikand swarasa shodhita) was taken and 1/8th part of it shuddha hingula was added in it. This mixture was triturated by adding sufficient quantity of lemon juice in into it. Same process was repeated after each puta for total 7 times and total 8 putas were given in this method. Ultimately, 505 gm bhasma obtained from 750 gm of shuddha swarnamakshika. It meant the yield was 67.33% through this process.
In preparation of Sample no IV (Table no- 5), 750 gm of shuddha Swarnamakshika Bhasma (bharjita in lemon juice) was taken and triturated with adding 250 ml lemon juice in it. Pellets prepared after trituritation, were dried, kept in shrava samputa and finally subjected to puta. Same process was repeated for 9 times and total 10 putas were given in similar fashion. After 10th puta, Swarnamakshika bhasma thus obtained, passed all the classical parameters and colour of bhasma finally changed to red. 470 gm bhasma obtained from 750 gm shuddha Swarnamakshika that meant the average yield in this process was 62.66%. So as far as yield is concerned, methods adopted in Sample III is better than in Sample IV. This proves that marana through adding Rasa Bhasma (as done with adding hingula in sample III) is best method of marana.

As per Table no-6 and Table no-7, it can be said that Swarnamakshika Bhasma prepared by all the three methods were fulfilled the organoleptic and classical analytical parameters. pH of all sample was in slightly acidic (Table no-8) due to use of lemon juice in levigation, purification and incineration stages. Table no- 9 depict the LOD, ash value and acid insoluble ash of different samples which was done in the Dept. of Chemistry, Science College, Patna. Chemically, Bhasma of Swarnamakshika is a mixture of Copper and Iron Oxide in both the samples as proved by the XRD study shown in figure no-1 & figure no-2. Further The methodology employed for the preparation of Swarnamakshika Bhasma by different methods yields nano particles in general as shown by the FESEM study in Plate no-1 & Plate no-2. ICP-MS study proved that percentages of Iron, Sodium, Potassium, Magnesium and Zinc were more in the Sample III as compared to Sample IV. Percentages of Arsenic, Lead, Cromium and Manganese like heavy were less in Sample III as compared to Sample IV. Percentage of Copper was found to be equal in both the samples. Percentage of Mercury could not be detected in both the samples (Table no-10). Swarnamakshika Bhasma (Oxides of Iron and Copper) if taken in empty stomach (increased amount of gastric acids) with Amalaki Churna (richest source of Vitamin C) would convert ferric iron to soluble and easily absorbable ferrous form. This will lead to increased uptake by membrane transferrin receptors which will increase iron absorption and distribution in body. This increased Haemoglobinisation will ultimately cure IDA i.e. Microcytic Hypochromic Anaemia.

**Conclusion:**

It can be concluded from the present study that Shodhana by Swedana in kadakikand Swarasa) and Marana by Rasa Bhasma i.e. higula & lemon juice is ideal way of making Swarnamakshika Bhasma. Bhasma obtained through above method (in sample III) will be quite effective in treating iron deficiency anaemia because it contains higher percentage of iron and negligible percentages of As, Pb, Mn, Cr like heavy metals. If used with amalaki churna, this could be the ideal drug of treating IDA because of easy availability and cheaper price. It is further suggested to evaluate haematological effect of these drugs by conducting Serum Ferritin and TIBC investigations.

**Acknowledgements:**

Authors are thankful to Dr R. K. Choudhary (HOD, Institute Instrumentation Centre, Indian Institute of Technology, Roorkee) and all the staff members for their support and help during the analytical study of this work. I most sincerely pay homage to my Supervisor Late Dr. Dinesh Chandra Sir (Reader, P. G. Dept of Rasa Shastra & Bhaishhya Kalpana, Govt. Ayurvedic College & Hospital, Patna) whose affectionate behaviour, helpful suggestions and support throughout this research work are impossible to reciprocate in words. This work is a tribute to the departed soul.

**References:**

1. P.J. Mehta’s Practical Medicine by The National Book Depot, 17th ed
2. Davidson’s Principle and Practice of Medicine, By Churchill Livingstone 19th International Edition
3. Ministry of Health and Family Welfare, Government of India, Addressing Iron Deficiency Anaemia among Indian Adolescents—12 by 12 Initiative, Ministry of Health and Family Welfare, New Delhi, India, 2007.
4. Henry’s Clinical Diagnosis and Management by Laboratory Methods, by Richard A. McPherson and Matthew R. Pincus, 23rd edition published by Elsevier, Part-4, Chapter-32, page 560-561
5. Rasendra Sara Samgrha of Krishna Gopal Bhatta, commented by Dr. Indra Dev Tripathi, Chaukamba Orientalia, 2003
6. Rasa Taringini by Sadanand Sharma, commented by Kashi Ram Shastri, Motilal Banarasidas, 11th edition 1979, Reprinted 2009, Verse 21/26-28, page 524
7. Agrawal’s Textbook of Biochemistry (Physiological Chemistry), by Dr GR Agrawal, Dr Kiran Agrawal and Dr OP Agrawal, published by Goel Publishing House a unit of Krishna Prakashan Media (P) Ltd, Meerut, 14th Edition 2007, Page 553
8. Jones AA, Disilvescro RA, Coleman M, Wanger TL. Copper supplementation of adult man: effect on blood copper enzyme activities and indicators of cardiovascular disease risk. Metabolism. 1997; 46:1380–3. doi: 10.1016/S0026-0495(97)90135-9. [PubMed] [Cross Ref]
9. Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., J. Biol. Chem., 77, 797 (1928).
10. Ayurveda-Sarasamgraha by Sri Baidyanath Ayurveda Bhawan Ltd, Naini, Allahabad
11. Rasa Taringini by Sadanand Sharma, commented by Kashi Ram Shastri, Motilal Banarasidas, 11th edition 1979, Reprinted 2009, Verse 02/ 52
12. Rasendra Chudamani by Acharya Somadev, commented by Dr. S.N. Mishra, Chaukhamba Orientalia, verse 01/33
13. Rasa Ratna Sammuccaya, with ‘Vigyanbodhini’ Hindi commentary by Duttatraya Ananat Kulkarni, Meharchanda Lachchhamandas Publication, Reprint 1998, Vol-1, 5/14
14. Rasa Taringini by Sadanand Sharma, commented by Kashi Ram Shastri, Motilal Banarasidas, 11th edition 1979, Reprinted 2009, Verse 21/18
15. Rasa Ratna Sammuccaya, with ‘Vigyanbodhini’ Hindi commentary by Duttatraya Ananat Kulkarni, Meharchanda Lachchhamandas Publication, Reprint 1998, Vol-1, 2/83
16. Rasa Taringini by Sadanand Sharma, commented by Kashi Ram Shastri, Motilal Banarasidas, 11th edition 1979, Reprinted 2009, Verse 21/7-11
17. Rasa Taringini by Sadanand Sharma, commented by Kashi Ram Shastri, Motilal Banarasidas, 11th edition 1979, Reprinted 2009, Verse 21/21-22, 21/23-25 & 21/19-20, page 523-524
18. RRS, verse 8/26-30
19. Harrison’s Principle of Internal Medicine, 17th edition, Vol-1, Chapter- 103