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Physico-chemical analysis of *Haratala* w.s.r. to its

various *Shodhana* procedures

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ABSTRACT

Kalpana is the process through which a substance can be transformed into the form of medicine according to the need. Among all these pharmaceutical processes Shodhana is one of them. For a single drug many process of Shodhana have been mentioned. Arsenic compounds are being popularly used in Ayurveda therapeutics since centuries, Haratala being important among them. It is commonly used in treating the diseases like Sleshmaroga, Raktapitta, Vatarakta, Kustha etc. Haratala is called orpiment of yellow arsenic with two molecules of Arsenic and three molecules of Sulphur (AS₂S₃). Patra Haratala is Srestha and used for the present study. Haratala consumed without proper Shodhana shortens the life span, causes diseases of Kapha and Vata, Prameha, Santapa, Spotha, Snayu Sankocha. Hence Shodhana of Haratala is essential. There are different Medias explained in literature for Shodhana of Haratala. According to the media of purification the quality and pharmacological properties of Haratala will vary. Depending on the change in properties the therapeutic effect may also vary. The present study includes Shodhana of Patra Haratala as per Classical reference of Rasa Ratna Samucchaya where Shodana of Patra Haratala is done by Kushmanda Swarasa, Tila Kshara Jala and Churnodaka. Standard Operative Procedure of the process is done in the pharmaceutical study. The analytical study reveals the standards which can be given for Ashuddha Haratala and Shuddha Haratala of various Samples. The differences in the parameters reveal that there are some changes which give us the idea regarding role of a particular media in purification of a substance, where it adds some properties of the media used.

Key words: Shodhana, Haratala, Kushmanda Swarasa, Tila Kshara Jala, Churnodaka.

INTRODUCTION

There is a lot of discussion in the global scenario regarding the toxicity of arsenic compounds. Arsenic compounds are being popularly used in Ayurveda therapeutics since centuries, and *Manahshila* being

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important among them.^[1] Among them *Haratala* is commonly used in treating the diseases like *Sleshmaroga, Raktapitta, Vatarakta, Kustha* etc.^[2]

Haratala is called orpiment of yellow arsenic with two molecules of Arsenic and three molecules of Sulphur (AS₂S₃). Haratala consumed without proper Shodhana shortens the life span, causes diseases of Kapha and Vata, Prameha, Santapa, Spotha, Snayu Sankocha.^[3] Hence Shodhana of Haratala is essential. Shodhana is the process of removal of physical, chemical impurities and potentiating of the drugs.^{[4],[5]} Generally Shuddha Haratala is not given alone. It is administered along with herbal drugs or in the form of Rasamanikya or also as a main ingredient in most of the popular formulations like Samirapannaga Rasa, Vatagajankusha Kasturibhairava Rasa, Rasa, Talakeshwara Rasa etc.

There are various *Shodhana* procedures explained for *Haratala* in *Rasa Granthas* like *Rasa Ratna Samuchaya*,^[6] *Ayurveda Prakasha*^[7] and *Rasa Tarangini*.^[8] Various works on *Haratala* has been carried out like *Haratala Bhasma*, its preparation, toxicity, antimicrobial study and experimental evaluation by using single *Shodhana* procedure.

Till today no work has been carried out on various *Shodhana* procedures of *Haratala*, intention behind them and complete structural validation of the same is yet to be established. Hence for the present study the various *Shodhana* procedures mentioned in *Rasa Ratna Samuchaya*^[6] and AFI.^[9]

All the constituents used for *Shodhana* will be collected from local market area and our college Herbal garden. Good manufacturing practice will be followed for preparing the various medias and *Shodhana* of *Haratala* as per Classical reference^[6] mentioned below.

Here scientific evaluation of various *Shodhana* procedures and Standard Operating Procedure (S.O.P) will be done by considering suitable physico- chemical parameters and possible instrumental methods which may add considerable input to the existing knowledge.

OBJECTIVES OF THE STUDY

- 1. Authentification of Patra Haratala.
- 2. Physico chemical analysis of *Haratala*, before and after *Shodhana* procedures.
- 3. An attempt will be made to establish Standard Operating Procedure (S.O.P) for Shodhana procedures of Haratala by Kushmanda Swarasa, Tila Kshara Jala and Churnodaka.

Analytical Study

Analytical study of *Ayurvedic* drugs has become the need of present hour. In ancient days, the drugs were prepared by the physicians himself, with the help of experienced, assistants in their own pharmacies attached to their clinics. Now a days the trends have been entirely changed. The demand of *Ayurvedic* drugs have been increased by many folds and

availability of raw materials are limited. So, there are of chances of production of low quality drugs for the commercial benefits.

The increasing demand for *Ayurvedic* drugs have made it necessary that some sort of uniformity in the manufacturing of *Ayurvedic* medicine should be brought out. The need has also been felt for statutory control to ensure standards of *Ayurvedic* drugs.

The quality of final products depends on the raw material used, intermediate process as well as on the pharmaceutical procedure adopted. Intermediate process also include the *Shodhana* procedure, where in different *Shodhana* media have different property which may result in mode of absorption, assimilation and action of the main drug. Various methods have also been prescribed for *Shodhana* of different drugs.

Chemical analysis of any drug should be known well before experimental and clinical trials. Chemical study ensures not only chemical constituents but also suggests us standards of any preparation. It not only gives standards of the products but indirectly gives suggestions for further advancement if required.

To evaluate the quality of finished products, it becomes necessary to subject the drugs for various analytical studies. The drugs should be understood and interpreted in the light of advanced chemistry to provide scientific background. For *Haratala*, which is an important drug of *Ayurveda*, *Shodhana* has been prescribed in various media and different methods are also available. For the present study, *Shodhana* of *Haratala* as per Classical reference of *Rasa Ratna Samucchaya*.^[6] was followed for preparing the various medias and *Shodana* of *Haratala* mentioned below.

Sam	Raw	Media	Process/Appara	Duratio
ple	Drug		tus	n
1	Patra Haratala	Kushman da Swarasa	Swedana/Dola Yantra	3 hours

Table 1: Showing different processes adopted forHaratala Shodhana.

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2	Patra Haratala	Tila Kshara Jala	Swedana/Dola Yantra	3 hours
3	Patra Haratala	Churnoda ka	Swedana/Dola Yantra	3 hours

Analysis were carried out at Central Laboratory, Bhagavathi Ana Labs Pvt. Ltd., Industrial Estate, Sanathnagar, Hyderabad.

The analytical study was undertaken with an aim to suggest suitable parameters and their expected values for routine quality control of the below samples.

- Sample 1. Raw Patra Haratala
- Sample 2. Shuddha Haratala (By Kushmanda Swarasa)
- Sample 3. Shuddha Haratala (By Tila Kshara Jala)
- Sample 4. Shuddha Haratala (By Churnodaka)

Analytical Parameters

The 4 samples were analyzed by using the following parameters:

A. Organoleptic characters

- Colour Rupa
- Odour Gandha
- Consistency Sparsha
- Taste Rasa
- **B.** Physico-chemical parameters
- Determination of Foreign Matter of Ashuddha Haratala
- Loss on drying at 110^o c
- Ash Value (Water insoluble)
- Ash Value (Acid insoluble)
- Water Soluble Extractive
- C. Inductively coupled Plasma Mass spectroscopy (ICPMS)
- D. Phase identification by diffract gram using x ray diffraction method

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A) Organoleptic parameters

The Sparsha (Consistency), Rupa (Colour), Rasa (Taste) and Gandha (Odour) of all the 4 samples were noted. These characters correspond to the Panchagyanedriya Pariksha of Ayurveda. These various organoleptic characters provides an idea regarding the genuinely of the sample both to the physician and patient. These give a primary idea about the quality of different formulations without using any chemical tests.

B) Physico-chemical parameters

1) Determination of foreign matter^[10]

Raw drugs should be free from moulds, insects, animal fecal matter and other contaminations such as earthen, stones and extraneous material. Any matter not covered by the description of the drug in the monograph shall be regarded as a non- extraneous foreign matter.

Foreign matter is material consisting of any or all of the following:

- In particular, parts of the organ or organs from which the drug is derived other than the parts named in the definition or for which a limit is prescribed in the individual monograph.
- Any organ or part of organ, other than those named in the definition and description.

It was determined by taking the 100gm weighed quantity of Sample 1 i.e. *Ashuddha Patra Haratala* and it was spread in a thin layer. Foreign mater or foreign organs was separated out and weighed and percentage was calculated out.

2) Loss on drying at 110°C^[11]

This test was conducted to find out the moisture content in the samples. About 1g. accurately weighed samples 1,2,3,4 were taken in a previously dried and weighed dish and heated in a hot air oven at 110°C till constant weight. It was cooled and the weight was noted. Difference between the weights was calculated and taken as the loss on drying. The loss on drying of the sample was expressed as % w/w.

3) Determination of Total Ash^[12]

Incinerate about 2 to 3g. accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°. Calculate the percentage of ash with reference to the air-dried drug.

4) Determination of Acid Insoluble Ash^[13]

Boil the ash obtained in (2.2.3) for 5 minutes with 25ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

5) Determination of Water Soluble Ash^[14]

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

6) Determination of Water soluble extractive^[15]

This test was carried out to evaluate the water-soluble principles of the samples. 5g. of sample was weighed accurately, 100 ml of distilled water was added to it and it was kept overnight. Next day, it was filtered. 20ml of the filtrate was transferred to a dried and weighed evaporating dish. The solvent was evaporated on a water bath, dried till constant weight, cooled and weighed immediately. From the weight of the residue, the percentage of watersoluble extractive was calculated and expressed as %w/w.

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7) Determination of Alcohol Soluble Extractive^[16]

Macerate 5g. of the air dried drug, coarsely powdered, with 100ml of Alcohol of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

8) Determination of Sulfur as S^[17]

Extract a suitable quantity of the sample with carbon disulphide. Filter the carbon disulphide solution and evaporate off the solvent. To the residue add 10ml of 10% alcoholic potash and boil until the sulfur has dissolved. Dilute with water, oxidize by adding hydrogen peroxide solution in excess and heat on a water bath for ½ hour. Acidify with hydrochloric acid, filter and to the filtrate add barium chloride solution. White precipitate of BaSO₄ shows the presence of sulfur.

C) Inductively coupled Plasma – Mass spectroscopy (ICPMS)^[18]

Among the various digestion procedures microwave digestion in the most modem reliable, sensitive method as it retains all the volatile metal ions and can be done with a small volume of sample.

In the present investigation, Microwave closed digestion technique has been adopted as it is not only rapid procedure for digestion of samples but protects all volatile metal ions (Pb, Cd, Mg, As, Se) (Figure 8)

Materials required

Reagents

- 1. Sub-boiled Nitric acid
- 2. De ionised water (Milli-Q)
- 3. Rhodium (2 ppm) Internal standard (Purchased from High purity standards, cat no 100044-2).
- Multi-Element reference standard. Supplied by National institute of Standard Technology (NIST – Standard Reference material – 3171a and 3172b).

- a. NIST A
- b. NIST B

Table 2: Certified concentrations of constituentelements.

Elements	Elements Source, Purity Conce % ug/ml	
Aluminum	Metal, (99.99)	100.1 +/- 0.5
Antimony	Metal, (99.99)	100.0 +/- 0.5
Beryllium	Metal, (99.99)	10.0 + /- 0.1
Cadmium	Metal, (99.99 +)	100.3 + /- 0.5
Chromium	Metal, (99.96 +)	100.0 + /- 0.5
Iron	Metal, (99.96)	100.1 + /-0.5
Magnesium	Metal, (99.98)	100.0 + /- 0.5
Manganese	Metal, (99.76)	99.8 +/-0.5
Molybdenum	Metal, (99.96)	100.0 + /- 0.5
Nickel	Metal, (99.99)	100.1 +/- 0.5
Potassium	Kcl, (99.98)	499.8 + /-2.5
Sodium	Nacl, (99.9)	100.0+ /- 0.5
Vanadium	Metal, (99.97)	100.0 +/- 0.5

Equipment

- 1. Microwave oven (Domestic): The microwave oven is placed in fume hood having exhaust facility to fulfill the safety criteria.
 - Microwave oven details
 - Bajaj Microwave oven Frequency 2450 MHZ;
 - Power input Voltage 220-240 Volts
 - Current 8 Amp (Max)

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- Frequency 50 Hz
- Type Single phase 3 wire grounded
- Power output Variable level 10 levels (1 to H) 140-700 watts
- Parr microwave Acid digestion Vessel (Model 4782 with PTFE cup, cover and O-ring) preferably 45ml capacity which is obtained from parr instrument company, USA is used.
- 3. ICPMS Model VG elemental Plasma Quad 3

A complete profile of the required elements is obtained after calibrating the equipment.

Procedure

- The preserved samples at 80° C have been taken out from deep freezer and kept at room temperature for 1hr before digesting the samples.
- 300ul of sample is mixed with 2ml of sub boiled nitric acid for digestion in Teflon lined Parr bomb which are cleaned thoroughly by Nitric acid.
- The sample containing Parr bombs are subjected to closed microwave digestive system at medium power (level 5) for 3 minutes.
- 4. The par bomb is removed from microwave and allowed to cool for 45-60 minutes to release the pressure built up.
- The clear digested sample is carefully transferred to Nitric acid cleaned Poly propylene tubes / Standard volumetric flasks and diluted to 10ml with Demonized water ICP-MS analysis.
- 20ppb of Rhodium, NIST A and NIST B is added to the digested sample before subjecting to ICPMS analysis.
- 7. The above prepared sample (50ul) is passed in to ICPMS after calibrating the equipment.

Calculations

The values in ppb levels i.e. ng/ml obtained are converted into μ g/dl by applying the dilution factor.

Elemental concentration = $\frac{\text{Value obtained in ppb (A) X Dilution factor (B)}}{\text{Amount of Sample taken (C)}}$

Sr. No.	Elements	Sensitivity (ppb-ng/ml)	Normal values (µg/dl)
1	Lead	Ppt to sub part per billion	> 20
2	Cadmium	Ppt to sub part per billion	0.3-7.0
3	Arsenic*	Ppt to sub part per billion	2-23
4	Mercury*	Ppt to sub part per billion	0.6-5.9

Phase identification by diffract gram using x ray diffraction method^[19]

It is categorized as a special and sophisticated technique, conducting the analysis in a nondestructive fashion. A variety of X-Ray techniques and methods are in use. The main three categories in which all the methods are classified are.

- a. X-Ray Absorption Methods
- b. X-Ray Fluorescence Methods
- c. X-Ray Diffraction Methods

As we have adopted the X-Ray Diffraction method, we will go into the essential details of this method only.

Principle: X-Ray Diffreaction Methods

When a beam of X-Radiation is incident upon a substance, the electrons constituting the atoms of the substances become as small oscillators. These oscillate at the same frequency as that of incident X-radiation. These scattered waves come from electrons which are arranged in a regular manner in a crystal lattice and then travel in certain directions. If these waves undergo constructive interference they are said to be diffracted by the crystal place. Every crystalline substance scatters the X-rays in its own unique diffraction pattern producing a finger print of its atomic and molecular structure. The following methods are used in the X-Ray diffraction Technique.

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- Laue Photographic Method
- Bragg X-ray Spectrometer Method
- Rotating Crystal Method
- Powder Method

We have adopted the Bragg X-Ray spectrometer method. When X-rays fall on a sample, they get diffracted as per the Bragg's equation

 $N\lambda = 2d \sin \theta$ (depending upon arrangement of atoms)

Where, λ = Wavelength of X-rays

- θ = Spacing between the layers of atoms
- d = Angle of incident X-rays

MATERIALS AND METHODS

X-ray Diffraction (XRD) patterns were obtained using a Shimadzu XRD-6000 diffract meter with Cu-K α as target with 40 KV voltages and 30 MA current.

Sample Preparation

The powdered sample was placed in a sample holder and analysis was carried out in a static position with the detector moving through $2 \theta 3$ to 70.

OBSERVATIONS AND RESULTS

Table 3: Showing Organoleptic Parameters of allSamples

Hartala	Colour	Odour	Consistency
Sample 1	Yellowish with brown tinge and shiny	Peculiar	Crystalline, Smooth
Sample 2	Bright yellowish shiny	Aromatic	Crystalline, Smooth
Sample 3	Yellowish dull	Peculiar	Crystalline, Smooth
Sample 4	Bright yellowish shiny	Peculiar	Crystalline, Smooth

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Table 4: Showing Physico-chemical parameters of allsamples

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Determination of Foreign Matter % w/w	2%			
L.O.D at 110 ⁰ C w/w	0.01	0.02	0.05	0.02
Water Soluble Ash % w/v	8.4	1.6	3.1	3.4
Acid Insoluble Ash % w/v	2.7	<0.1	0.8	0.9
Water Soluble Extractive % w/v	0.4	0.5	0.6	1.4
Alcohol Soluble Extractive % w/v	0.7	0.6	1.1	1.0
Determination Of Sulfur as S % w/w	31.14	35.04	35.16	36.12
Arsenic as As (ICPMS) mg/kg (ppm)	8.89	8.12	9.2	8.89

Phase identification by diffract gram using x ray diffraction method^[19]

Sample*	As 3d (FWHM) ev	S 2d (FWHM) ev	As:S Atomic %	Auger Parameter
Sample – 1 As 2S 3	43.4 (1.7)	162.5 (2.2)	40:60	1266.1
Sample – 2	43.0 (1.85) Trace Oxide at surface	162.0 (2.3)	40:60	1266.2

Table 5: Showing X ray diffraction.

	(19 at%)			
Sample – 3	43.0 (1.76)	162.7 (2.7)	32:68	1265.8
Sample – 4	43.1 (1.8)	162.4 (2.3)	37:63	1266.2

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Figure 1: Pictures depicting raw drugs used for *Haratala Shodhana*.



DISCUSSION

The present research work was planned with an aim to establish Standard Operating Procedure (S.O.P) for *Shodhana* procedures of *Ashuddha Patra Haratala* by *Kushmanda Swarasa, Tila Kshara Jala* and *Churnodaka*. To find out the effect of different *Shodhana* medias on the physico-chemical properties of *Haratala*. Went through the whole literature on *Haratala* available from *Vedic* period to the advancement of present time.

Analysis was carried out at Central Laboratory, Bhagavathi Ana Labs Pvt. Ltd., Industrial Estate, Sanathnagar, Hyderabad. The analytical study was undertaken with an aim to suggest suitable Bandeppa Sangolgi et.al. Physico-chemical analysis of Haratala w.s.r. to its various Shodhana procedures

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parameters and their expected values for routine quality control.

Figure 2: Pictures depicting *Shodhida Haratala* by *Kushmanda Swarasa*.



Figure 3: Pictures depicting *Shodhida Haratala* by *Tila Kshara Jala*.



Figure 4: Pictures depicting *Shodhida Haratala* by *Churnodaka*.



Table no 3 reveals that Sample 1 i.e. Ashuddha Patra Haratala is having yellowish with brown tinge with shiny, peculiar odor with crystalline smooth surface. Sample 2 i.e. Shuddha Haratala (Kushmanda Swarasa Shodhita) was bright yellowish shiny, aromatic, crystalline smooth. Sample 3 i.e Shuddha Haratala (Tila Kshara Jala Shodhita) was yellowish dull color, peculiar odor, and crystalline smooth texture. Sample 4 i.e Shuddha Haratala (Churnodaka Shodhita) was bright yellowish shiny, peculiar odor and crystalline smooth texture. It was observed that after *Shodhana* the bluish tinge got disappeared and the samples turned bright yellowish and shiny.

Table no. 4 reveals that in Ashuddha Patra Haratala there is 2% of foreign matter, which reveals the purity of the raw drug. Loss on drying was found less in Ashuddha Patra Haratala and more in Shuddha Haratala (Tila Kshara Jala Shodhita). Water soluble ash was found less in Shuddha Haratala (Kushmanda Swarasa Shodhita) and more in Ashuddha Patra Haratala. Acid insoluble ash was found least in Shuddha Haratala (Kushmanda Swarasa Shodhita) and more in Ashuddha Patra Haratala. Water soluble extractive was found less in Ashuddha Patra Haratala and most in Shuddha Haratala (Churnodaka Shodhita).

Alcohol soluble extractive was found less in *Shuddha Haratala (Kushmanda Swarasa Shodhita)* and more in *Shuddha Haratala (Tila Kshara Jala Shodhita)*. Determination of Sulfur reveals that it is less in *Ashuddha Patra Haratala* and more in *Shuddha Haratala (Tila Kshara Jala Shodhita)*. Arsenic as As is less in *Shuddha Haratala (Kushmanda Swarasa Shodhita)* and more in *Shuddha Haratala (Tila Kshara Jala Shodhita)*.

By performing *Shodhana* procedure, moisture content was increased. Ash value was reduced, water soluble ash was reduced. Acid insoluble ash was increased. Water soluble extractive and alcohol soluble extractive were increased compared to *Ashuddha Haratala*. Sulfur as S was increased. Arsenic as As was equal and slight increase was found.

From Auger parameter (AP) values it appears that the samples are As2S3. AP values for AS-O are much lower than that for sulphide. For example AP:As2O3 = 1263.3 and AP: As2O5 = 1263.6. We tried to get at% of As and S on the surface. However XRD can get the exact phase. Trace of oxide is found in sample 2 and sample 4. This shows the role of different media in deciding the absorption, assimilation, effect and excretion of the drug. So due to these there may be

changes in mode of action and also disease and disease condition.



CONCLUSION

Shuddha Haratala (Kushmanda Swarasa Shodhita) was bright yellowish shiny, aromatic, crystalline smooth. Shuddha Haratala (Churnodaka Shodhita) was bright yellowish shiny, peculiar odor and crystalline smooth texture. All relevant analytical data of samples of Ashuddha and Shuddha Haratala are showing difference in their physical and chemical values. It shows the importance of process of Shodhana, which is probably responsible for safe therapeutic uses of Haratala. This shows the role of different media in deciding the absorption, assimilation, effect and excretion of the drug. So due to these there may be changes in mode of action and also disease and disease condition. The properties of liquid media embedded into the *Haratala* during the process of *Shodhana* may augment the effect of *Haratala*.

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