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## Pharmaceutico-Analytical Study of *Hingula*(Cinnabar) purified with *Ardraka Swarasa* (Juice of *Zingiber officinale* Rosc.)

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### ABSTRACT

*Hingula*, categorized under *Sadharana Rasa Varga* in most of the *Rasashastra* classics is a chief source of Mercury since ancient times to date. For therapeutic use, it is subjected to *Shodhana* (purification) by triturating with indicated liquid media. It is claimed that the drug transforms to safe and therapeutically usable form after *Shodhana*. For global acceptability, this is need of the hour to make it evidence based. In wake of this, *Hingula* was purified with *Ardraka Swarasa* following SOP and both the raw and purified *Hingula* were subjected to various physico-chemical analyses to see the changes occurring after *Shodhana*. It was seen that *Hingula* transformed to dark red coloured fine, smooth powder after purification. The pH changed from 5.82 to 6.11 after *Shodhana*. Also there were changes in LOD from 0.29% w/w to 0.58% w/w, in Total Ash content from 0.0079% w/w to 3.7237% w/w, in acid insoluble ash content from 0.00263% w/w to 2.4127% w/w. The Raw *Hingula* was insoluble where as the purified *Hingula* was partially soluble. The *Shodhita Hingula* got the classical *lakshanas* viz:- *Nischandrika*, *Rekhapurna* and *Varitara*. ED-XRF study showed the addition of new elements like Fe, K and Br with relative concentration of 0.220%, 0.185% and 0.709%, respectively and deletion of Cs in purified *Hingula*. No remarkable differences were seen in the d-spacing [ $\text{\AA}$ ] values at 2-Theta angle of both the samples in XRD study. The changes are seen due to treatment of raw *Hingula* with liquid media (*Ardraka Swarasa*) for trituration. The differences observed in *Hingula* before and after purification signify the principle of *Shodhana* and *Samskara* as described by the *Acharyas*.

### KEYWORDS

*Hingula Shodhana*, *Cinnabar*, *ED-XRF*, *XRD*, *Pharmaceutico-Analytical Study*



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## INTRODUCTION

*Hingula*(Cinnabar),the Red Sulphide of Mercury is categorized under *Sadharana Rasa Varga* in most of the *Rasashastra* classics. This is a chief source of mercury since ancient times to date. *Parada* extracted from it is said to be equivalent to *AstasamskaritaParada*. Besides this, it is used as an ingredient in many herbo-mineral formulations due to its action like *Sarvadoshahara*, *Rasayana* etc. But for therapeutic use, *Hingula* must be subjected to *Shodhana*(purification) by the pharmaceutical process named *Bhavana*(Wet Trituration Method) and *Prakshalana*. Many procedures are described in ancient treatise for *HingulaShodhanalike Bhavana* with *ArdrakaSwarasa*(ginger juice), *Lakucha* juice, lemon juice etc<sup>1</sup>.

It is claimed that *Hingula* becomes devoid of toxic and undesired materials and transforms to therapeutically safe and usable form after purification along with induction of some desired qualities to it. The concept behind this transformation has been hinted in ancient classics by *Acharya Charak* that enhancing the utility (pharmaco-dynamic action) of a relatively small quantity/quality of a substance (drug) or decreasing the utility of a relatively large quantity/quality of a substance is possible

by *Samyoga*(combination), *Vishlesha*(division/separation), *Kala* (time factor), *Samskara*(various pharmaceutical operations) and *Yukti*(intelligent planning)<sup>2</sup>. Here, *Acharya Charaka* indicated that potency of the drug can be raised by addition of synergistic substance, by subtraction of antagonistic substance, by favourable pharmaceutical treatment, by right way of administration and by prolongation of desired time factors and *Samskara*<sup>3</sup> is the sole fundamental principle of Ayurvedic pharmaceuticals, which is the process to incorporate much more qualities into the natural drug and to get the desirable therapeutic effect. Here, *Bhavana* is a unit procedure coming under *Samskara*.

To make this principle widely acceptable by the different scientific communities, interpretation in scientific and modern language is the need of hour. Here a small attempt has been made to carryout pharmaceutical, physico-chemical and analytical study of raw *Hingula* and purified *Hingula* with the following aims and objectives.

## AIMS & OBJECTIVES

1. Purification of *Hingula* by *Bhavana* with *Ardraka Swarasa* following SOPs.
2. Physico-chemical and instrumental analysis of raw *Hingula* and purified



*Hingula* to know the changes occurring due to *Shodhana* process and to revalidate the principle of *Shodhana*.

## MATERIALS AND METHODS

This study involved the following unit processes: -

1. Collection of basic raw material (*Hingula*) and materials needed for *Shodhana* (i.e. *Ardraka*)
2. *Ardraka Swarasa Nirman*
3. *Hingula Shodhana* by *Ardraka Swarasa*
4. Physico-chemical and instrumental analysis of *Hingula* before and after purification.

### 1. COLLECTION OF RAW MATERIALS

The raw drug (*Hingula*) was procured from the Govt. registered local supplier and the genuineness of the sample was confirmed by a specialist. The materials were also identified and confirmed through instrumental analysis like XRD and ED-XRF analysis. The *Ardraka* was purchased from local market and identified with the help of relevant literatures and was again authenticated and confirmed by the subject specialist in the pharmacognosy laboratory of the institute.

### 2. ARDRAKA SWARASA NIRMAN

**Aim:** To use as media in purification of *Hingula*

**Reference:** *Sharngadhara Samhita, Madhyama Khanda*1/2 (*Swarasa Nirman Vidhi*)<sup>4</sup>

**Date of Commencement** : 19.03.2018

**Date of Completion** : 19.03.2018

**Principle/ Method** : *Swarasa Nirman Vidhi (Vastra Nishpidita Method)*

**Ingredients:** Fresh *Ardraka (Ginger)*: 500 gm (*Zingiber officinale* Rosc.)

#### Equipments:

- Digital weighing machine
- Mortar & pestle (Stone)
- Knife
- Stainless steel vessel
- Glass container
- Clean cotton cloth
- Measuring cylinder
- Hand gloves and face mask

#### Procedure:

- *Ardraka* was freshly collected and washed with tap water to clean the mud.
- Then 500 gm was taken and cut into small pieces and was kept in a mortar.
- It was then pounded with pestle to disintegrate and convert it into paste.
- Then it was transferred to a clean cotton cloth and squeezed to obtain the juice.
- It was then measured by measuring



cylinder and kept in a glass container for further use in *Hingula Shodhana*.

- Each time, the whole procedure was repeated for obtaining *swarasa* required for 7 *Bhavana* during *Hingula Shodhana*.

#### Precautions:

- Care was taken not to take infested and damaged *Ardraka*.
- Properly cleaned equipment's were used so as to avoid mixing of foreign matters.
- All the procedures were performed wearing hand globes and face mask.
- Pounding was done carefully so that pieces of *Ardraka* would not come out and the juice would not spill over dress and face.

#### Observations:

- Due to pounding, the *Ardraka* pieces were converted to small pieces and finally creamish paste like consistency was obtained.

#### Results:

Sl. No.	Amount of <i>Ardraka</i> taken	Time Taken	Total Yield	Gain /Loss
1	500 gm	1 hr 35 min	114 ml	Loss

**Probable Cause of loss:** The major part of *Ardraka* was fibrous which was separated after pounding and squeezing.

### 3. HINGULA SHODHANA BY ARDRAKA SWARASA

**Aim:** For Physico-chemical and analytical study.

**Reference:** *Rasa Tarangin* 9/12<sup>5</sup>

**Date of Commencement** : 19.03.2018

**Date of Completion** : 27.03.2018

**Principle/ Method** : *Bhavana*(By *Mardana* or Wet Trituration Method) and *Prakshalana*.

#### Ingredients:

1. Unpurified *Hingula* : 250gm
2. *Ardraka Swarasa* : q.s.

#### Equipments:

- Digital weighing machine
- Mortar & pestle (Stone)
- Spatula
- Measuring cylinder
- Air tight glass container.
- Hand globes and face mask

#### Procedure:

- Impure *Hingula* was weighed and exactly 250 gms was kept in a stone mortar.
- First fine powder was made by churning with pestle.
- For first *Bhavana*, about 35 ml of *Ardraka Swarasa*, the quantity sufficient to immerse the *Hingula Churna* was added.
- The mixture was subjected to continuous and cautious trituration till *swarasa* dried up.
- When the powder was totally dried up, it was considered as the completion of first *Bhavana*.



- Then again sufficient quantity of *swarasa* was added and the mixture was triturated.
- The same process was repeated for 6 times and total 07 (Seven) *Bhabanas* were given.
- Every time, fresh *swarasa* was used.
- After completion of 7<sup>th</sup> *Bhavana*, it was collected from the Mortar (*Khalva*) and was washed thoroughly with water and allowed to settle in stainless steel vessel.
- Settling of *Hingula* at bottom of the pot took about 06 hours, after which water was decanted.
- Then it was dried and collected in an air tight glass container.

#### **Precautions:**

- Properly cleaned equipment's were used so as to avoid mixing of foreign matters.
- Care was taken to powder the impure *Hingula* so that it would not come out of mortar causing loss.
- The quantity of *Ardraka Swarasa* taken for every *Bhavana* was sufficient enough for the immersion of *Hingula Churna*.
- *Mardana* (trituration) was done continuously and cautiously so as to avoid the expulsion of material from the mortar as initially especially when the *swarasa* was added as the powder became wet and there was chance of easy expulsion from mortar.

- *Mardana* was carried out cautiously allowing pestle to move entire length of the mortar.
- Also at the end of every *Bhavana*, trituration was done slowly as the material became stickier.
- When the material was totally dried up by trituration, then it was considered as the completion of one *Bhavana* and fresh *swarasa* was added for next *Bhavana*.
- After washing with water and when the water settled, the supernatant water was drained carefully so as to avoid loss of *Hingula*.
- The mortar was kept covered when the process was not going on.
- All the procedures were performed wearing hand globes and face mask.

#### **Observations:**

- First *Hingula* was in solid form with glistening white/Mercurial lines.
- After half an hour of *Mardana*(Trituration), the shiny particles disappeared.
- For first *Bhavana* the quantity of *Ardraka swarasa* required was quite more than the subsequent *Bhabanas*.
- The colour of impure *Hingula* was shining dull red/ Brick red which kept changing in each subsequent *Bhavana* and became brighter and brighter.



- The shining nature of *Hingula* was lost after *Bhavana*.
- Time required for giving *Bhavana* was changing according to the surrounding temperature. Time required for *Bhavana* given in afternoon, was comparatively less than time required for *Bhavana* given in morning.
- At the end of each *Bhavana*, the material became stickier.
- Time required for completion of *Bhavana* also decreased as *Bhavana* continued.
- Particle size was seen reducing in subsequent *Bhavana*.
- The characteristic smell of *Ardraka* was appreciated during trituration.
- 
- After completion of 7<sup>th</sup> *Bhavana*, water was added to the Mortar (*Khalva*) and the *Hingula* was washed.
- After completion of 7<sup>th</sup> *Bhavana*, water was added to the Mortar (*Khalva*) and the *Hingula* was washed.
- Then the water along with the *Hingula* was transferred to a stainless steel vessel for proper washing and was then allowed to settle.
- Settling of *Hingula* at bottom of the vessel took about 06 hours, after which water was decanted.
- After decanting the supernatant water, *Hingula* was seen as bright red, smooth, sticky paste like substance along with slight brownish sticky strain.
- After drying, *Hingula* changed to fine powder from solid crystals.
- The stepwise observation has been depicted in Table No.01.
- The final result has been depicted in Table No.02.

**Table 1** Changes of *Hingula* during each *Bhavana*

No. of <i>Bhavana</i>	Date	Duration of <i>Mardana</i> in hrs	Quantity of <i>Swarasaad</i> ded	<i>Shabda</i>	<i>Sparsha</i>	<i>Roopa</i>	<i>Gandha</i>	<i>Chandrika</i>
1 <sup>st</sup>	19.03.18	2 hrs 42 min	35 ml	<i>Khara</i>	Rough	Bright Red	<i>Like Ardraka</i>	Present ++++
2 <sup>nd</sup>	20.03.18	2 hrs 40 min	30 ml	<i>Nishabda</i>	Smooth +	Bright Red	<i>Like Ardraka</i>	+++
3 <sup>rd</sup>	21.03.18	2 hrs 38 min	28 ml	<i>Nishabda</i>	Smooth +	Bright Red	<i>Like Ardraka</i>	++
4 <sup>th</sup>	22.03.18	2 hrs 35 min	25 ml	<i>Nishabda</i>	Smooth ++	Bright Red	<i>Like Ardraka</i>	+
5 <sup>th</sup>	23.03.18	2 hrs 30 min	25 ml	<i>Nishabda</i>	Smooth ++	Bright Red	<i>Like Ardraka</i>	Absent ( <i>Nischandra</i> )
6 <sup>th</sup>	24.03.18	2 hrs 24 min	23 ml	<i>Nishabda</i>	Smooth +++	Bright Red	<i>Like Ardraka</i>	Absent ( <i>Nischandra</i> )
7 <sup>th</sup>	26.03.18	2 hrs 19 min	23 ml	<i>Nishabda</i>	Smooth +++	Bright Red	<i>Like Ardraka</i>	Absent ( <i>Nischandra</i> )



**Table 2** The final result of practical

Sl.No.	Quantity of <i>Hingula</i> taken	Time Taken	Hours Taken	Quantity Obtained	Weight Gain/Loss
1	250 gms	09 Days	17 Hrs 48 min	276 gm	Gain, 26 gms

**Probable Cause of Gain:** Due to addition of solid contents (*Satwa*) of *Ardraka* in *Hingula*.

Both the experiments (*Ardraka Swarasa Nirman* and *Hingula Shodhana*) were carried out with the equipment and instruments of the mini pharmacy attached to PG Dept. of RS & BK, Gopabandhu Ayurveda Mahavidyalaya, Puri.

#### 4. PHYSICO-CHEMICAL AND INSTRUMENTAL ANALYSIS OF HINGULA BEFORE AND AFTER PURIFICATION.

The analysis of *Hingula* before and after purification was carried out on following parameters and the results have been depicted in Table. No. 03.

- a. Organoleptic parameters
  - i. Description
  - ii. Colour
  - iii. Odour
  - iv. Texture
  - v. Taste
- b. pH(1% Aqueous suspension)
- c. Loss on drying at 105<sup>0</sup>c (Moisture Content)
- d. Total Ash value
- e. Acid insoluble ash
- f. Solubility in Water
- g. Classical parameters(*Nischandra*, *Rekhapurna*, *Varitara*)
- h. Energy Dispersive X-Ray Fluorescence(ED-XRF)
- i. X-Ray Diffraction (XRD)

**Table 3** Physico-chemical analysis results

Sl. No.	Tests	Limits	Raw <i>Hingula</i>	Purified <i>Hingula</i>
1	Description	NA in API	Bright Red, Crystalline, Scarlet Streak, Translucent to Opaque	Brick Red Coloured Powder
2	Colour	NA in API	Bright Red	Brick Red
3	Odour	NA in API	Odourless	Odourless
4	Texture	NA in API	Hard, Rough	Fine, Smooth
5	Taste	NA in API	Tasteless	Tasteless
6	pH(1% Aqueous suspension)	NA in API	5.82	6.11
7	Loss on Drying at 105 °C	NA in API	0.29 % w/w	0.58 % w/w
8	Total Ash	NA in API	0.0079 % w/w	3.7237 % w/w
9	Acid Insoluble Ash	NA in API	0.00263 % w/w	2.4127 % w/w
10	Solubility in water	NA in API	Insoluble	Partially Soluble
11	<i>Nischandra</i>	NA in API	No	Yes
12	<i>Rekhapurna</i>	NA in API	-	Yes
13	<i>Varitara</i>	NA in API	-	Yes





It was seen from the physico-chemical analysis that *Hingula* was transformed to dark red coloured fine, smooth powder after purification. The pH changed from 5.82 to 6.11 after *Shodhana*. Also there were changes in LOD from 0.29% w/w to 0.58% w/w, in Total Ash content from 0.0079% w/w to 3.7237% w/w, in acid insoluble ash content from 0.00263% w/w to 2.4127% w/w. The Raw *Hingula* was insoluble where as the purified *Hingula* was partially soluble.

The *ShodhitaHingula* got the classical *lakshanas of Bhasma viz;-Nischandrika*-there was no metallic lusture, *Rekhapurna*-when taken between the index finger and thumb spread, it was so fine as to get easily into the finger lines, *Varitara*-when a small

quantity of sample sprinkled on water, it floated on the water surface.

This study was carried out at State Drug Testing & Research Laboratory(ISM), Bhubaneswar, Odisha.

### ENERGY DISPERSIVE X-RAY FLUORESCENCE(ED-XRF)

Energy Dispersive X-ray Fluorescence (ED-XRF) is one of two general types of X-ray Fluorescence techniques used for elemental analysis applications or chemical characterization of a sample.

The compositional analysis by ED-XRF was carried out at School of Electronics Engineering, KIIT Deemed-to-be University, Bhubaneswar, Odisha and the relative concentration of elements in percentage has been depicted in Table 04.

**Table 4** Compositional Analysis of Raw and purified *Hingula*

Sl. No.	Analyte	Relative Concentration (in %) of elements in Raw <i>Hingula</i>	Relative Concentration (in %) of elements in Purified <i>Hingula</i>
1	Hg	78.944	80.743
2	S	18.710	15.497
3	Si	1.252	1.402
4	Re	0.487	0.876
5	Cs	0.420	-
6	Ca	0.114	0.291
7	Cu	0.074	0.073
8	Fe	-	0.220
9	K	-	0.185
10	Br	-	0.709

It was seen from the above table that in purified *Hingula*, the concentration of Hg was seen increased to 80.743% from 78.944% and the concentration of S was

seen decreased to 15.497% from 18.710%. Concentrations of other elements were also seen changed though slightly. New elements like Fe, K and Br were seen added

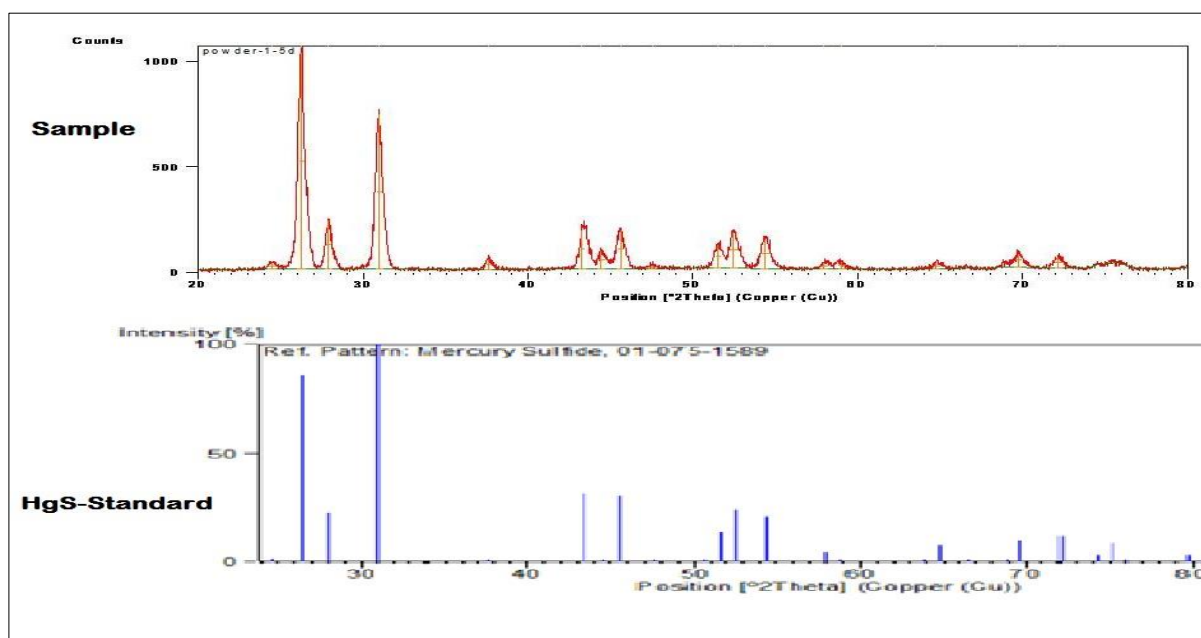


in purified *Hingula* with relative concentration of 0.220%, 0.185% and 0.709% respectively and there was absence of Cs in purified *Hingula* which was present in raw *Hingula* with 0.420%. The major elements were Hg & S and rest were found in trace amount.

### X-RAY DIFFRACTION (XRD) STUDY

X-ray diffraction (XRD) is a rapid analytical technique primarily used for

#### *Main Graphics, Analyze View: Raw Hingula*

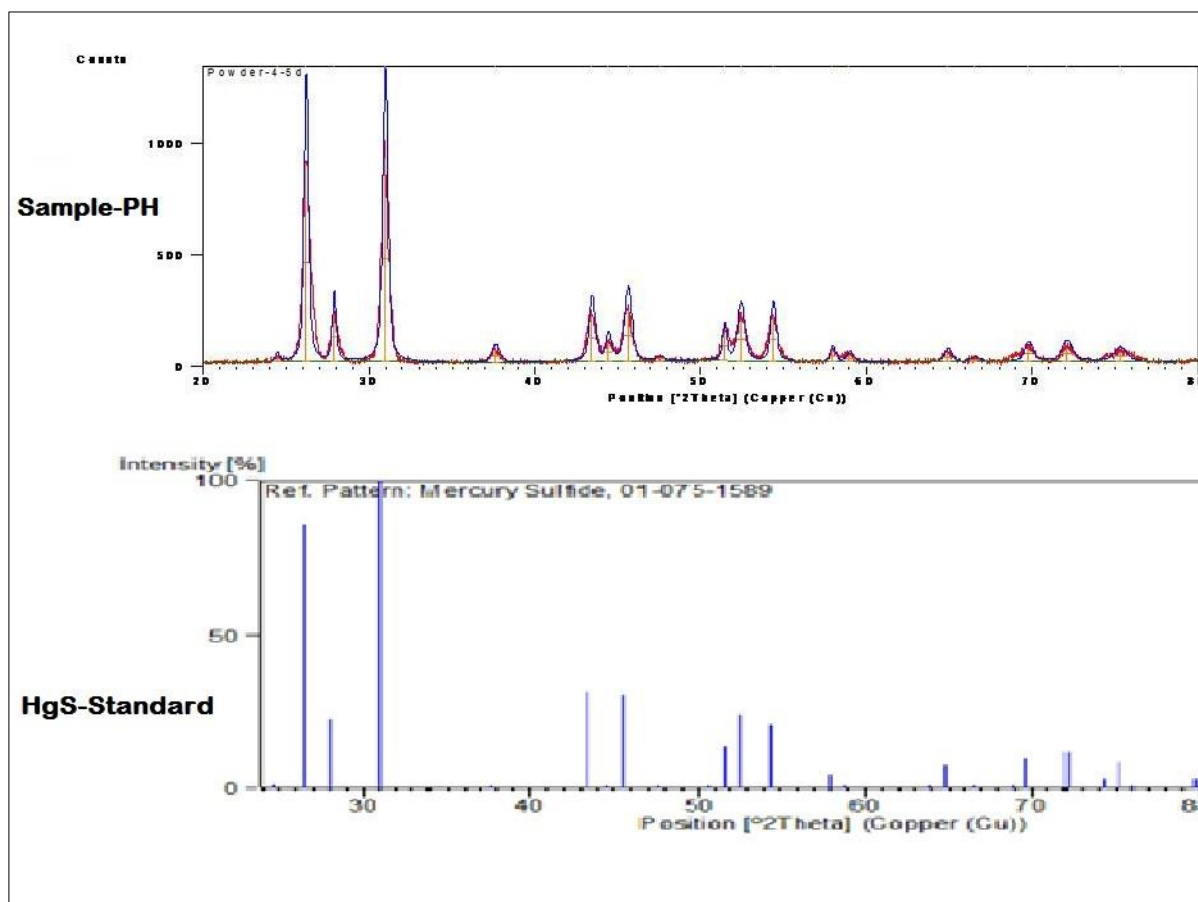


**Table 5** Peak list of Raw *Hingula*

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
26.233030	1027.781000	0.314880	3.39722	100.00
27.877250	233.171100	0.354240	3.20047	22.69
30.949840	732.224400	0.314880	2.88939	71.24
43.230880	181.856000	0.196800	2.09280	17.69
45.589120	182.763600	0.393600	1.98988	17.78
47.560520	16.695160	0.472320	1.91190	1.62
51.519660	110.915400	0.314880	1.77390	10.79
52.473660	170.792400	0.551040	1.74388	16.62
54.354640	140.966500	0.629760	1.68789	13.72
59.032550	35.361360	0.472320	1.56481	3.44
64.754890	26.403510	0.393600	1.43967	2.57
69.759780	66.881850	0.393600	1.34813	6.51
72.156530	52.352840	0.551040	1.30914	5.09



## Main Graphics, Analyze View: Purified Hingula



**Table 6** Peak list of Purified *Hingula*

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
26.194400	891.264600	0.314880	3.40214	97.28
27.878100	216.020000	0.275520	3.20038	23.58
30.951830	916.206900	0.354240	2.88921	100.00
43.400320	206.044600	0.393600	2.08503	22.49
45.577980	232.098900	0.472320	1.99034	25.33
51.436050	126.384600	0.275520	1.77659	13.79
52.424580	185.356700	0.472320	1.74539	20.23
54.373760	198.654800	0.354240	1.68734	21.68
57.949110	55.053540	0.314880	1.59146	6.01
64.889800	43.372780	0.472320	1.43701	4.73
69.746480	63.086970	0.551040	1.34835	6.89
72.063510	63.525650	0.708480	1.31060	6.93
75.262210	45.126920	0.787200	1.26264	4.93

In XRD Graph, d-spacing [Å] values of major peaks were compared with standard JCPDS card table for HgS. There were no remarkable differences seen in the d-spacing [Å] values at 2-Theta angle of both

the samples. The strongest peaks identified were of HgS. The crystal systems in these samples were trigonal and lattice system-hexagonal.



## DISCUSSION

After proper identification, the raw drugs were taken and processed for purification. The weight of *Hingula* increased after purification due to addition of starchy material found in *Ardraka swarasa*. Physico-chemical analysis of *Hingula* before and after purification was carried out through various parameters. It was observed that the Raw *Hingula* changed slightly to brick red and fine powder form after purification. The pH was seen changed to 6.11 and LOD was seen increased to 0.58% w/w after *Shodhana* which might be due to addition of some herbal constituents from media used for *Shodhana*, which validates principle of the addition during *Shodhana*.

Also increased value in parameters like total ash content (3.7237% w/w), acid insoluble ash content (2.4127% w/w), solubility (partially soluble) were seen in purified *Hingula*. It was probably due to addition of some inorganic constituents from the media used for *Shodhana*. The *Shodhita Hingula* shows the classical *lakshanas of Bhasma viz;-Nischandrika, Rekhapurna and Varitara* which indicated the reduction in particle size and weight due to trituration process.

The compositional analysis by EDXRF revealed the real changes that were brought

due to *Shodhana*. The purified *Hingula* was added with elements like Fe, K, Br and devoid of Cs. The relative concentration of Hg increased and S decreased in both the purified sample in comparison to raw one. The XRD study confirmed the presence of HgS and the strongest peaks identified were of HgS in both the samples. There were no remarkable differences seen in the d-spacing [ $\text{\AA}$ ] values at 2-Theta angle of both the samples. It needs more intensive study for better understanding on the differences.

## CONCLUSION

It is evident from the present study that the treatment of raw *Hingula* with liquid media (*Ardraka swarasa*) for trituration converts the sample into powder form and the consistency changes from rough to smooth and fine. It also brings the some chemical changes, though not much significant. The changes in physico-chemical parameters and presence of trace level extraneous elements in purified *Hingula* is due to the medium in which it is triturated and that probably help in enhancing its potency. The changes observed before and after purification signify the principle of *Shodhana* and *Samskara* as described by the *Acharyas*. Present work can be considered as a preliminary analysis and the first step towards the process of



revalidation of the principle of *Shodhana*. More intensive and thorough work is needed to be carried-out for better interpretation and understanding.

#### **ACKNOWLEDGEMENT**

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