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STANDARDIZATION OF *SHUDHA HINGUL* W.S.R TO ITS PURIFICATION METHOD

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ABSTRACT

In *Ayurveda*, minerals and their many Poly formulations are explained but in present scenario standardization of minerals after their purification are most important. *Hingul* is widely used entity in Herbomineral formulation, hence there is need to standardize it, after purification and before using in formulation by using different analytical tests. *Kshalan* is one of the unique procedures done after *Shodhan* of *Hingul* with *Nimbu swaras*. This study carried out with the focus on the necessity of the *Kshalan* process, whether it makes the *Shodhit Hingul* more potent for internal use or not. In this study we did ICP MS, XRD and HPTLC. In ICP MS we found percentage of Mercury was increased, In XRD no any structural changes were found after purification of *Hingul.* In HPTLC, white band were seen which shows no physical impurities in purified *Hingul.*

Key words: *Hingul, Kshalan, Shodhan,* ICP-MS, XRD, HPTLC.

INTRODUCTION

Rasashastra is one of the important branch of *Ayurveda*, more inclined towards pharmaceutical and pharmacological angles of different methods of preparations, processing and therapeutical utilization of Mercury, mercurial compounds, metals, minerals, herbo-mineral and metallo-mineral compounds. Improper *Shodhan* of *Rasa aushadhi* have delirious toxic effects on the human body. It is essential to Validate, Standardize in details the various aspects of pharmaceutics with the structural changes taking place before, during and after the *Shodhan* procedure mentioned in our *Rasashastra* texts. *Hingul* is one of the most widely used entities, used in preparation of various formulations. Just as it is highly efficacious similarly it is toxic as well. Hence there is a need to standardize the *Hingul*

Material and Method:

Process of *Hingul shodhan* was carried out in two steps namely trituration of *Ashudha Hingul* (impurified cinnabar) with *nimbuk swaras* (lemon juice) and repeated *kshalan* (washing) of triturated *Hingul*. The *Hingul* was procured from genuine dealer and Lemon for lemon juice was obtained from market.

Equipment: Mortar and pestle, Measuring Cylinder, glass jar, stirrer, Deionized water,

Procedure: At first the *Hingul* was powdered in a mortar with a pestle. Then this Hingul powder was triturated with Lemon juice seven times. After completion of the seventh times triturated *Hingul* turned crystallized to powder form its pH was highly acidic. Then do seven times *kshalan* with DI water of this triturated *Hingul*.[1] After *kshalan Hingul* was done become very smooth dark red in color, lusterless and pH came near neutral. The process was completed in 14 days and the final product called *shudha Hingul* was obtained.

The raw *Hingul*, seven times triturated *Hingul*, *kshalan* liquid and *shudha Hingul* obtained from the above process were taken for different analysis.

Modern parameters for analysis

ICP MS was performed in Texan laboratory at Rabale Thane, for percentage of mercury in *Hingul* samples. XRD was performed in IIT Bombay for the phase identification. HPTLC was performed in Anchrom laboratory at Thane.

A) ICP MS:

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique used for elemental determinations. Thermo Scientific ELEMENT XR, a high performance, double focusing magnetic sector field ICP-MS.[2]

- Three samples for this test were.

1) First sample: Raw *Hingul* (H)
2) Second sample: Seven times triturated sample. (HB7)
3) Third sample: Seven times washed *Hingul* powder sample. (G)

1) First Sample: Raw *Hingul* (H)

- Method:

Test for the detection of total mercury content. Sample preparation as per EPA 3050b using microwave digestion detection and quantification by ICP-MS.[2]

- Procedure :

1. 0.2 gm sample was taken in to the microwave container. Then 10 ml of concentrated nitric acid and 1ml of 50% hydrogen peroxide were added.
2. The tube was placed in the microwave oven at 220ºc for 1 hour.
3. The container was cooled and slowly the pressure was released and the content was transferred into a conical flask.
4. If the solution is not clear filter in to the container and again transfer filtered solution quantitatively 50 ml standard volumetric flasks.
5. The container was rinsed with ICP grade water.
6. Simultaneously prepare blank solution by taking 10 ml nitric acid and 1ml hydrogen peroxide and was treated as above.

7. Both the solutions were subject to ICP-MS.
8. The same procedure was followed for all samples.

- Result :

Sr.no	Name of Heavy metal	Concentration (mg/kg)	Detection limit (mg/kg)
1.	Mercury	2.9%	0.02

Note: 1% = 10000 mg/kg (ppm)

2) Second sample: Seven times triturated sample of *Hingul.* (HB7)

- Result :

Sr.no	Name of heavy metal	Concentration (mg/kg)	Detection limit (mg/kg)
1.	Mercury	754.0	0.02

Note: 1% = 10000 mg/kg (ppm)

3) Third sample: Seven times washed *Hingul* powder sample. (G)

- Result:

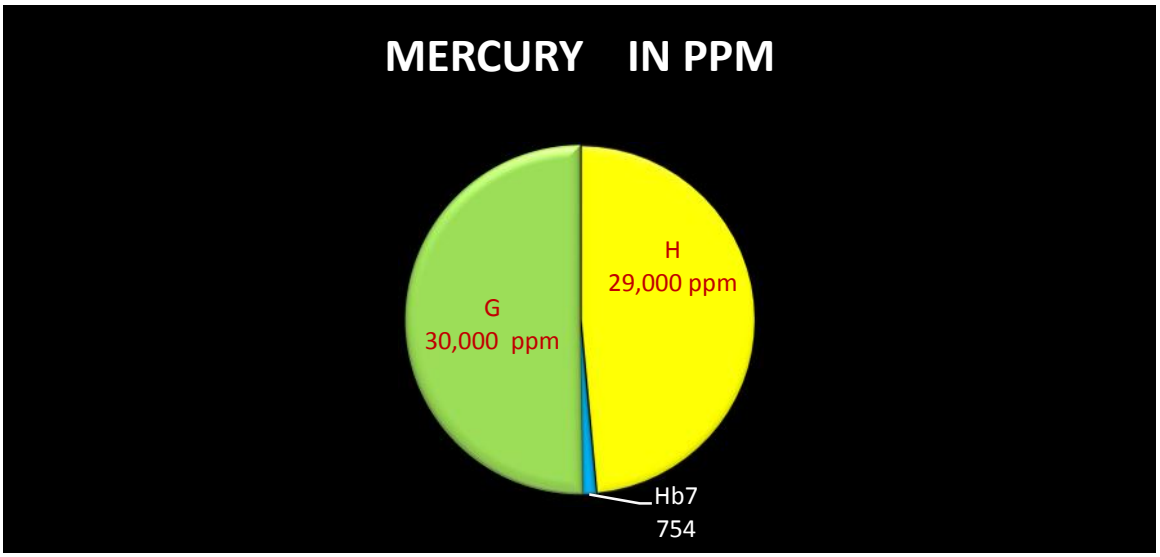
Sr.no	Name of heavy metal	Concentration (mg/kg)	Detection limit (mg/kg)
1.	Mercury	3.0	0.02

Note: 1% = 10000 mg/kg (ppm)

Observation table of ICP-MS result:

Sr.no	Name of sample	Name of heavy metal	Concentration (mg/kg)	Detection limit (mg/kg)
1.	*AshudhaHingul* (H)	Mercury	2.9 %	0.02
2.	Seven times triturated *Hingul* (HB7)	Mercury	745.0	0.02
3.	After seven times *ksha-lan* (G)	Mercury	3.0 %	0.02

Note: 1% = 10000 mg/kg (ppm)



B) XRD: X-ray powder diffraction analysis (XRD) is perhaps the most widely used X-

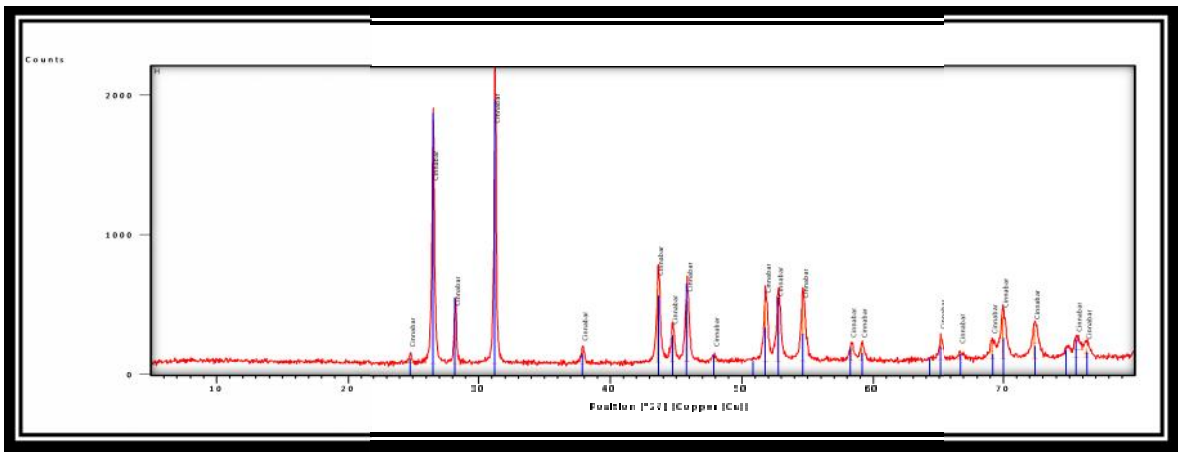
ray based analytical techniques for characterizing materials. Phase identification is important because the material properties are highly dependent on structure. XRD analysis has a wide range of applications.

- Material and Method :

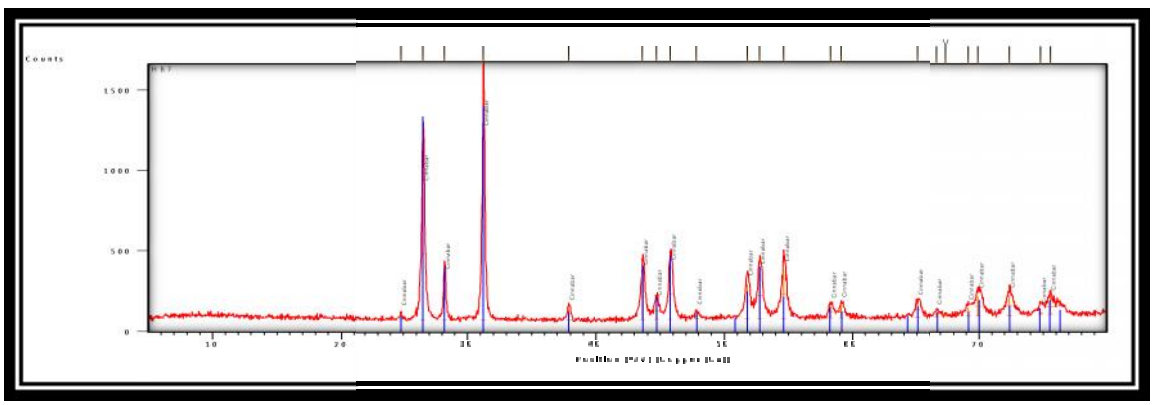
Three samples were run for XRD (powder)[3]:

1. First sample: Raw *Hingul* (H)
2. Second sample: Seven times triturated sample. (HB7)
3. Third sample: seven times washed *Hingul* powder sample. (G)

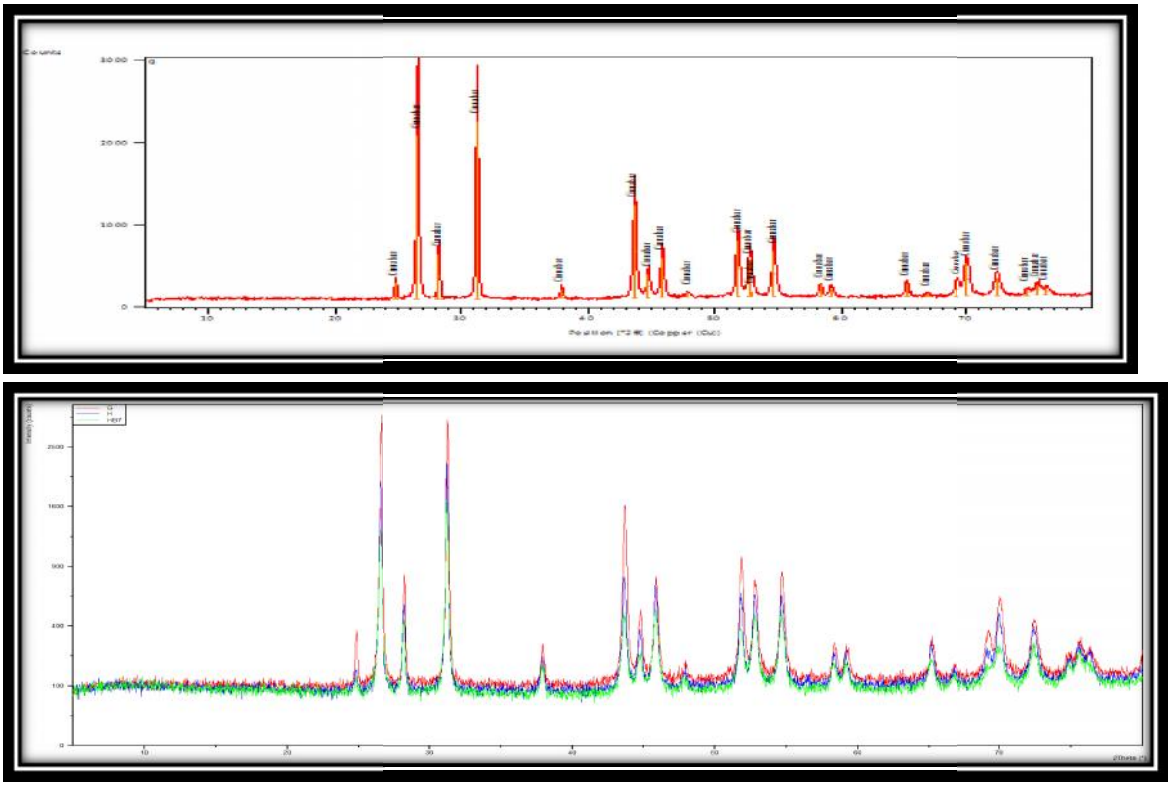
1) First sample: Raw *Hingul* (H)[3]
File name: D:\XRD Data\Year 2014\POWDER DIFFRACTION DATA
Diffractometer system=EMPYREAN
Measurement program = C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp,
Operator: Indian Institute
Raw Data Origin: XRD measurement (*.XRDML)



2) Second sample: Seven times triturated sample. (HB7)
File name: D:\XRD Data\Year 2014\POWDER DIFFRACTION
Diffractometer system=EMPYREAN
Measurement program=C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp
Operator: Indian Institute



3) Third sample: Seven times washed *Hingul* powder sample. (G)
File name: D:\XRD Data\Year 2014\POWDER DIFFRACTION DATA
Diffractometer system=EMPYREAN
Measurement program=C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp.
Operator: Indian Institute



This graph shows the intensity of peak of *Ashudha Hingul* (H), Triturated *Hingul* (HB7) and Seven times washed *Hingul*

Result:
1) XRD report shows, the structure of cinnabar is not changed in raw, triturated and seven times washed *Hingul* sample.
2) No D' spacing changes occurs in Raw, Triturated and Seven times washed *Hingul* sample.
3) Intensity of peak of cinnabar was increased after completion of *kshalan* of *Hingul.*
4) Atomic spacing or structure is not affected in Raw, Triturated and Seven times washed *Hingul* sample.

C) HPTLC:
In the current work HPTLC technique was adopted for deriving the finger print patterns of the samples. Procedure:
The dried materials were powdered and sieved through ASTM sieve (85/BS sieve) separately and were kept in separate air-tight containers. Samples for HPTLC fingerprinting were prepared by using optimized conditions for extraction.[4]

1) Preparation of raw *Hingul*: (H)

Step 1: Accurately weighed 500 mg of raw *Hingul* powder was transferred to 20 cm^3 test tube and 5.0 cm^3 of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

2) Preparation of seven times triturated *Hingul* in *Nimbuk swarasa*: (HB7)

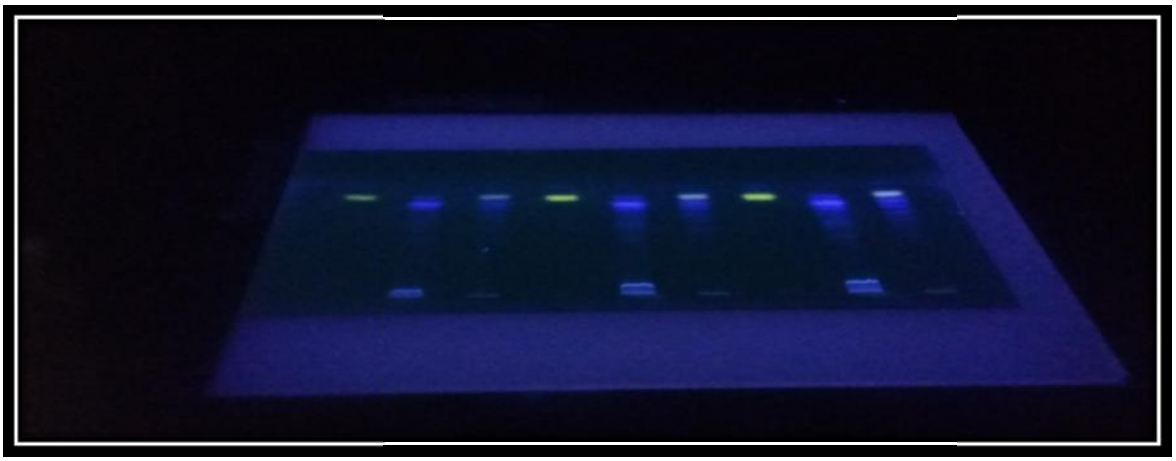
Step 2: Accurately weighed 500 mg of seven times triturated *Hingul* powder was transferred to 20 cm^3 test tube and 5.0 cm^3 of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

3) Preparation of *Hingul* powder after seven *kshalan* : (G)

Step 3: Accurately weighed 500 mg of *Hingul* powder after seven *kshalan* was transferred to 20 cm^3 test tube and 5.0 cm^3 of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

- Chromatography :

High Performance Thin Layer Chromatography was used to establish the fingerprint of the sample extracts. In TLC (>90%) silica is used mainly for the separation of pharmaceuticals and drugs. Normal phase mode of separation was employed. The stationary phase used was Silica gel 60 F_{254}. A mobile phase was developed in such a way that the maximum separation was achieved on a TLC plate useful for phytochemical analysis.



- Result:

The proposed HPTLC method can be used to develop a fingerprint for the identification of samples.

1) In this study we found the difference between three samples in the fluorescence we got chromatographic separation of our organic and inorganic substances in the sample of H, HB7 and G.

2) In sample of raw *Hingul* we saw the color of spectra in fluorescence was yellow and in next sample of seven times trituarated *Hingul* in *nimbuk swaras* the color of spectra in fluorescence was blue with multiple bands and in last sample of *Hingul* powder after *kshalan* the color of spectra in fluorescence was white.

Hence according to the result found in these three samples we conclude that may

be the impurities are removed after the completion of *shodhan* process, mean may be the impurities are removed in the sample of *Hingul* powder after doing seven times *kshalan.*

HPTLC Fingerprints are suitable for monitoring the identity and purity of crude drugs or processed drug for detecting adulterations. With the help of fingerprints, we can identify the active components of selected disease which is causing it. As the technique is simple and rapid it is a highly used technique. It has benefits of being highly portable methods and can be practiced at every place without any difficulty. The analysis is quick and the fingerprints can easily evaluate to give the analyst an overview of the research.

The proposed HPTLC methods demonstrated in this study are simple, economical and reliable. The fingerprints which are established are representatives of a given species. These methods are suitable for monitoring the identity and purity of crude and processed drugs and for detecting adulteration. These methods can also be used to check the batch to batch consistency of marketed herbal and Herbomineral formulation.

DISCUSSION

ICP MS: In this test three samples were examined i.e. H, HB7 and G. We have analyzed crude cinnabar for mercury content. Where the analysis was about 2.9 % means 29,000 mg/kg (parts per million) of mercury in the crude sample.

The third sample was treated for purification by applying with trituration with lemon juice and later removing the material applied for purification by *kshalan* process. In the process other impurities also got removed (wash out). And crude cinnabar got purified.

Where in mercury content level went up in the purified sample, and this was substantiated by analyzing purified sample for mercury content and found to be more than 3% means 30,000 mg/kg (parts per million).

We have also analyzed the seven times triturated sample and observed that the proportion of mercury in the triturated sample obviously got reduced due to addition of this material of trituration in practice it was observed 754 only.

The above observation indicates that pure form of mercury is on higher parameter in sample of *Hingul* powder after *kshalan* compared to the other two samples.

XRD: XRD report shows, the structure of cinnabar were not changed in raw, triturated and seven times washed *Hingul* sample. Intensity of peak of cinnabar was increased after completion of *kshalan* of *Hingul.* Atomic spacing or structure is not affected in Raw, Triturated and Seven times washed *Hingul* sample.

HPTLC:

In this study we found the difference between three samples. In the fluroscence we got chromatographic separation of our organic and inorganic substances in the sample of H, HB7 and G. In sample of raw *Hingul* we saw the color of spectra in fluroscence was yellow and in next sample of seven times trituarated *Hingul* in *nimbuk swaras* the color of spectra in fluorescence was blue with multiple bands and in last sample of *Hingul* powder after *kshalan* the color of spectra in fluroscence was white. It indicates that the last sample which

shows white band i.e. *Hingul* powder after *kshalan* indicates the absence of any organic traces i.e *nimbuk swarasa* is absent.

CONCLUSION

After tituration of *Hingul* with a *Nimbuk swaras* for seven times, the acidic nature increases. Later when repeated *kshalan* of same sample was done with DI water the acidic nature was reduced and the pH became neutral.

The removal of acidic nature of triturated *Hingul* by the process of *kshalan*, supports the concept that substances with neutral nature are less gastric irritant which supports the aim of increasing the therapeutic efficacy of the drug after *shodhan*.

In HPTLC study, the final sample i.e. (G) *Hingul* after seven *kshalan* shows white band. Hence we can conclude that the purified *Hingul* is devoid of any organic impurities as is evident from band color.

To this, supportive result is of XRD and ICP MS tests, which indicates high intensity of cinnabar and high percentage of mercury, which supports the standards that mercury is present 86.2% and 13.8% sulphur in Hingul, according to result of XRD study. Shows that the intensity of peak was increased but atomic structure was not changed which supports the *sidhant* of *gunantaradhan*.

Kshalan process helps to remove unwanted substances, which is the primary aim of *Shodhan* i.e *Dravyadoshanivaranam*. Hence it can be concluded that the study or the process of *kshalan* of *shodhit Hingul* supports the fundaments of *Shodhan* i.e. purification of substance, eradication of impurities and enhancement in their qualities for potent result in therapeutic use.

REFERENCES

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2. ICP MS (heavy metal): Sample preparation as per EPA3050b using microwave digestion detection and quantification by ICP/MS.
3. Powder XRD were done by using Diffractometer system-EMPYREAN C:\PANalytical\Data Collector\Programs\ROCK POWDER GENERAL METHOD-ADS.xrdmp
4. Instrument CAMAG HPTLC, Stationary Phase Silica gel 60 F254 HPTLC pre-coated plates, Sample Applicator CAMAG LINOMAT V, Measurements Mode Absorbance & Florescence & visible, Photo Documentation CAMAG TLC Visualizer.

Source of Support: Nil
Conflict Of Interest: None Declared

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