Research Article

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Comparative physico-chemical validation between Arsenic-based Indian traditional drugs Haratal Bhasma and Rasamanikya

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ABSTRACT

Background: Arsenic containing drugs Rasamanikya (RM) and Haratal Bhasma (HB) are used in Ayurveda for the treatment of several ailments. They are prepared from raw Haratal (RH) by the distinct Ayurvedic procedure. Hence, proper scientific validation by physico-chemical studies is needed for their acceptability to the modern scientific community. **Methods:** RM and HB were prepared from RH. Namburi Phased spot test (NST) study was done to check the quality of prepared drugs. Loss on drying, extractive values, ash values was performed over the said two arsenic containing drugs. Sophisticated instrumental analysis like XRD, TEM, TGA, DTA, EDAX, AAS, etc. were studied to understand the crystal profiles, particle size, thermo stability, chemical microanalysis, trace elemental analysis of the drugs respectively. **Results:** XRD analysis of both RM and RH showed that they were comparatively amorphous in their structure. RH contains trace amount of lead which was confirmed by AAS analysis. TEM Image of HB showed that average particle size is 100nm. It is highly irregular in shape and is homogeneously distributed. While in the case of RM, images revealed that it is highly agglomerated to form small globules. Particles size is about 200nm. EDAX analysis revealed that RH contains Arsenic and Sulphur with wt% of Arsenic is 72.04% and sulphur is 27.96%. In HB wt % of Arsenic is 58.69% and sulphur is 11.69%. RM contains 41.77% wt % of Arsenic and 15.81% of sulphur. Both RM and HB also contain oxygen, carbon, silicon, etc. The DTA plot showed two endothermic peaks in the range of 300 to 600oc in the three samples of RH, HB and RM. **Conclusion:** Thus an attempt has been made for creating a comparative database of two such drugs by the incorporating modern analytical methods. It can be concluded that there were minimal comparative differences found in the HB and RM but HB showed better results from the standardization point of view.

Keywords: Haratal, Haratal Bhasma, Rasamanikya.

INTRODUCTION

Ayurveda makes use of several poisonous plants and minerals in the treatment and management of diseases. *Haratal* is an arsenic based mineral used in Ayurveda for preparing herbo-mineral medicines. Several Ayurvedic medicines like *Talkeswar Ras, Haratal bhasma, Tal Sindoor, Malla Sindoora, RasaManikya*, etc. are used as Ayurvedic medicines. Impure *Haratal* gets converted to purified *Haratal* after proper *Sodhana* (~Ayurvedic purification technique).

Bhasmikarana (calcinations) process is a very popular technique in Ayurvedic system of medicine which converts the metal into its specially desired chemical compound known as *Bhasmas* by at first triturating the purified metal with desired herbs and then subjected to heat through the process of repeated heating in an enclosed container. It eliminates the toxicity of the metal as well as has the significant medicinal benefits. There are certain traditional qualitative tests in Ayurvedic doctrine to prove the qualitative efficacy of the prepared *bhasma*. However, these traditional tests do not provide any quantitative information about the composition and structure of the *bhasma*. For any drug to be properly acceptable to international scientific community, proper validation is very necessity [1].

Haratal Bhasma and *Rasamanikya* both are the products of *Haratal,* but both therapeutic actions and mode of preparation are different. Both *Rasamanikya* and *Haratal bhasma* are derived from Raw *Haratal*. They are used in the treatment of cough, chronic fever, sinus, skin disorders, etc. The clinical use of *Haratal Bhasma* is comparatively less in comparison to *Rasamanikya*. One of the reasons for low acceptability of *Haratal Bhasma* in clinical practice may be related to scarce scientific reports available in

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support of *Haratal bhasma*. The basic goal of the present study was to characterize the *Haratal Bhasma* on elemental and structural basis and to compare it with *Rasa Manikya*. Qualitative analysis like Loss on drying, Total ash, water soluble extractive, etc were done as normal protocol of physico-chemical evaluation. Namburi phased spot test(NST) did chromatographic qualitative analysis of our drugs of interest. X-ray diffraction (XRD) and transmission electron microscopy (TEM) were used for the detection of compound type, crystalline/ amorphous nature and the crystal size present in *Haratal Bhasma* and *Rasamanikya.* Due to heating during the processing of *Haratal Bhasma,* the *possibility* of organic compounds is rare. Different elements present in the raw *Rasamanikya* and *Haratal Bhasma* were measured using energy dispersive X-ray analysis (EDAX) and atomic absorption spectroscopy (AAS). Thermo Gravimetric Analysis (TGA) and (Differential thermal Analysis (DTA) were also performed which provide information about second-order phase transitions, including vaporization, sublimation, and degradation of the two products.

MATERIALS AND METHODS

Method 1: Preparation of *Haratal Bhasma:*

Step 1:

Collection of *Raw Haratal (*Sample-RH***):** *Haratal* was collected from Ayurvedic Pharmacy, Institute of Medical Sciences, Banaras Hindu University.

Purification of *Haratal:* Purification of *Haratal* was carried out according to the reference of ancient book of *Rasa Ratna Samuchhaya* (R.R.S-3/70). 100 grams of crude *Haratal* was crushed into small pieces. Fresh juice of the fruit of *Kushmanda* (*Benincasa Hispida)* was collected and the crushed *Haratal* was tied within a cloth and boiled within the *Kushmanda* juice for three hours through a process known as *Swedana* (~boiling). It was then allowed to cool and *Haratal* was removed and washed with lukewarm water. After drying it was weighed.

Step 2:

Preparation Of *Haratal Bhasma* (Sample-HB): The accurately weighed purified *Haratal* was taken in mortar and pestle. Then the purified *Haratal* was triturated with the decoction of *Palash* roots (*Butea monosperma*) decoction for 3 days continuously for duration of 8 hours per day until the preparation get dried up. Then trituration was done with buffalo's urine. Finally pellets were prepared and made dried and kept in *Sharava samputa* (earthen casserole) and heated with 10 cow dung cakes. The whole process was repeated for 12 times. Finally grey coloured *Haratal bhasma* was obtained [2, 3].

Method 2: Preparation of Rasamanikya(Sampel-RM) By Mica sheets (*Abhrakpatra*)

10 gms *Haratal* was kept on one thin mica sheet and covered with another sheet of same size. *Abhraka Patras* was sealed properly with the help of U-pins from all around and with the help of proper instrumental arrangement, mild heat was applied. During heating when the color changes to black then to ruby red, the heating was stopped. After self cooling, *Rasamanikya* from inside was obtained carefully.

Physico-chemical characterization

Sample HB and RM were evaluated for physico-chemical properties and characterization of its chemical nature by using standard methods [4,5].

Loss on drying (LOD): The amount of water or volatile matters present in a sample is investigated by Loss on Drying Test. About 1gm of the air-dried HB and RM were taken in a Petri dish and weighed accurately. It was then placed in Electronic over at 110°C for 1 hour. After removing from over it is allowed to cool down to room temperature in a desiccator and then weighed to the constant weight.

Water soluble extractive: Water soluble extractive value is applied to the drugs which contain water soluble constituent by using chloroform water.

Alcohol soluble extractive: 1gm of air dried HB and RM was mixed with 20 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during 6hrs and allowing standing for 18hrs. It was then rapidly filtered. Taking precautions against loss of solvent, 10 ml of the filtrate was evaporated to dryness in a tarred flat bottomed of alcohol shallow dish, and dried to 105°C, to constant weight.

Total ash: 2 to 3 gm of HB and RM were incinerated at a temperature upto 450^0C, in a tarred platinum dish until free carbon, was obtained. It was then cooled and weighed, and total ash was calculated.

Acid insoluble ash: Both the ash of HB and RM was boiled for 5 mins with 25 ml of dilute HCL, the insoluble matter was collected in a cashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated.

Namburi phase spot test: Whatman filter paper no:1 were taken and impinged with 10% potassium iodide solution and shade dried. 0.25 gms HB and RM individually was treated with 0.5 ml Aqua regia in centrifuge tube. One set was heated and other was not heated. Both the samples were allowed to stay for 72 hours, by shaking now and then. Samples were shaken for every now and then for 72 hours. It was then allowed to settle to form a clear supernatant liquid in the centrifuge tube. One drop from each solution was carefully placed with the help of a dropper at the centre on previously treated and dried 10% potassium iodide filter paper. Then the colour changes were observed and recorded at different phases of time. Important observations were done at 2 different intervals, 90 mins after first phase and other 74 hours after the first phase [6].

X-ray diffraction (XRD) study: X-ray powder diffraction (XRD) is a fast analytical tool used for phase identification of a crystalline material. The analyzed material is finely ground, homogenized, and bulk average composition is determined. Powder of HB and RM were characterized by powder X-ray diffraction (XRD) using high Resolution powder Diffractometer XRD (Rigaku) based on a 12 kw rotating anode X-Ray generator with curved crystal monochromater fitted with a high temperature attachment. Ray diffractmeter scans were made randomly oriented sample from 2ϕ value: 20-80° with a step of 0.02° and 1 second time per step. It was carried out school of material science and technology, I.I.T, BHU.

About 1 gm of RM and HB were kept in the groove of sample holder of the X-ray diffractometer. Surface of the samples were made flat to avoid any error due to rough surface specimen.

Transmission Electron Microscopy (TEM): The transmission electron microscope forms an image accelerating a beam of electrons that pass through the specimen. The image was built up from the number of electrons emitted from each spot on the sample.

Energy dispersive x-ray Spectroscopy: Energy Dispersive X-Ray Spectroscopy (EDAX) is a technique used in conjunction with scanning electron microscopy (SEM). The EDAX technique detects x-rays emitted during bombardment by an electron beam from the sample to characterize the elemental composition. FEI Quanta 200 instrument was used for the study of chemical nature of sample RH, HB and RM based on ZAF quantification standardless.

Trace Elemental Analysis By A.A.S: Atomic Absorption Spectroscopy is a Spectro analytical technique for the quantitative investigation of elements mainly heavy metals employing the absorption of optical radiation by free atoms of gaseous state.

The instrument by SCIMADZU was used to determine the presence of trace element in the *sample* RH.

TGA/DTA Analysis: In Thermo gravimetric analysis (TGA), physical and chemical properties of materials were measured as a function of increasing temperature (with constant heating rate), or as a function of time (with a constant temperature and/or constant mass loss). Likewise DTA is a technique for recording the difference in temperature between a sample of interest and a reference material as a function of time or temperature. Two samples are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. Analysis was carried out with the help of the instrument made by Mettler and the heat was applied in range 40^{o}-600^{o}Cat 20^{o}C/ min [7].

By Analytical study it can be concluded that there were minimal differences found in the *Haratal Bhasma* and *Rasamanikya* but if the two samples are compared together it can be said that *Haratal Bhasma* showed better results from the standardization point of view.

The LOD, Water and Alcohol soluble extractive, Total ash, Acid insoluble ash, PH were under normal consideration and were comparatively studied at Table 1. The result of NST study was expressed in Table 2 and figure 3. The color of the spot revealed in NST revealed that the obtained product of HB and RM are of standard quality.

Table 1: Physico-chemical analysis of HB and RM

S. No.	Parameters	Values	
		HB	RM
1.	LOD (Loss on Drying)	0.05 %	0.06%.
2.	Water soluble extractive	0.08 %	0.07 %.
3.	Alcohol soluble extractive	8.5%	5.8%.
4.	Total ash	14%	13.5 %.
5.	Acid insoluble ash	2.5%	3 %.
6.	pH	6	6.5.

RESULTS AND DISCUSSION

Table 2: Namburi phase spot test of Haratal Bhasma

Sample for testing	Phase-1 (0- 5 min)	Phase-2 (90 minutes)	Phase-3 After 74 hours
Haratal Bhasma with heat treatment	There is light brown periphery with wide margin around the central spot.	Continues the same.	Central light chocolate color spot. Inner periphery light brown color and dark brown color periphery.
Haratal Bhasma without heat treatment	There is light brown periphery with wide margin around the central spot.	Continues the same.	Central light chocolate color with a brown ring followed by white circle, then by light brown inner periphery and brown outer periphery after that fading brown zone

Tabel 3: Namburi Phased Spot Test of Rasamanikya

Sample for testing	Phase-1 (0- 5 min)	Phase-2 (90 minutes)	Phase-3 After 74 hours
Rasamanikya with heat treatment	Central grey solid spot followed by light periphery then dark grey ring and brown periphery.	Continues the same.	Light brown colored central spots then white periphery surrounded by brown ring then creamish brown thin ring and followed by fading brown zone.
Rasamanikya without heat treatment	There is light brown periphery with wide margin around the central spot.	Continues the same.	Light brown colored central spot followed by white circle surrounded by light brown ring and fading brown zone.

XRD analysis of RH display that it was is crystalline and monoclinic system pattern by using powder diffraction. It showed that graph showing prominent peaks due to arsenic trisulphide. Certain peaks was corresponding to free sulphur were also present. In case of HB and RM, certain peaks are still unidentified. XRD analysis of both sample showed that it was comparatively amorphous. There is a probability that the samples may be converted to low crystalline shape. Due to amorphous nature of compound and zig-zag pattern of the graph, there were no peaks confirm that exact compound may contain arsenic trioxides or arsenic trisulphide (Fig:1 fig: 2).

TEM Image of HB showed that average particle size is 100nm. It is highly irregular in shape and is homogeneously distributed. While in case of RM, images revealed that it is highly agglomerated to form small globules. Particles size is about 200nm, and is homogeneously distributed. Both *Haratal bhasma* and *Rasamanikya* showed the presence of micro fine particle. Repeated tritruration and heating during preparation of HB causes significant size reduction to the level of nano scale. (Fig:3, fig:4).

Whereas EDAX is used to investigate the elements which are present in considerable amount in the sample, AAS confirms the presence of elements which are present in trace amount. Sample RH was found to be devoid of cadmium and chromium but it contain trace amount of lead. Investigation of Cadmium, Chromium and Lead were performed by standard protocol described in Ayurvedic Pharmacopeia of India.

The differential thermal analysis plot showed two endothermic peaks in the range of 300 to 600^{o}C in the three samples (RH, HB and RM). Melting point of *Haratal* is 310^{o}C. In case of RH at 350^{o}C the sample gets degraded randomly. In case of HB at 450^{o}C and RM at 550^{o}c the sample gets degraded randomly, which is proved by both of the graphs. Gradual decrease in weight in TGA graph from 450^{o}C, 550^{o}C and distinct peaks after 350°C in DTA graph supports the fact. TGA and DTA report confirms the physical nature of both HB and RM. (Fig:6,fig:7)

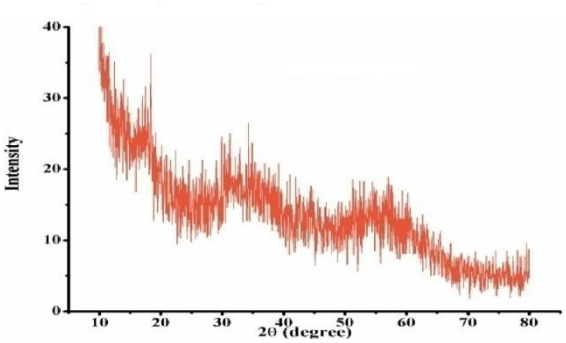

Figure 1: X-Ray diffractogram pattern of Haratal Bhasma

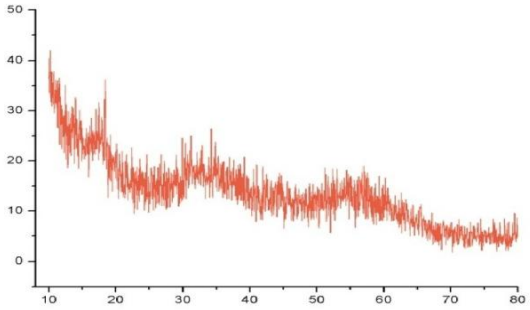

Figure 2: X-Ray diffractogram pattern of Rasamanikya

Figure 3: NST spot test on whatman filter paper

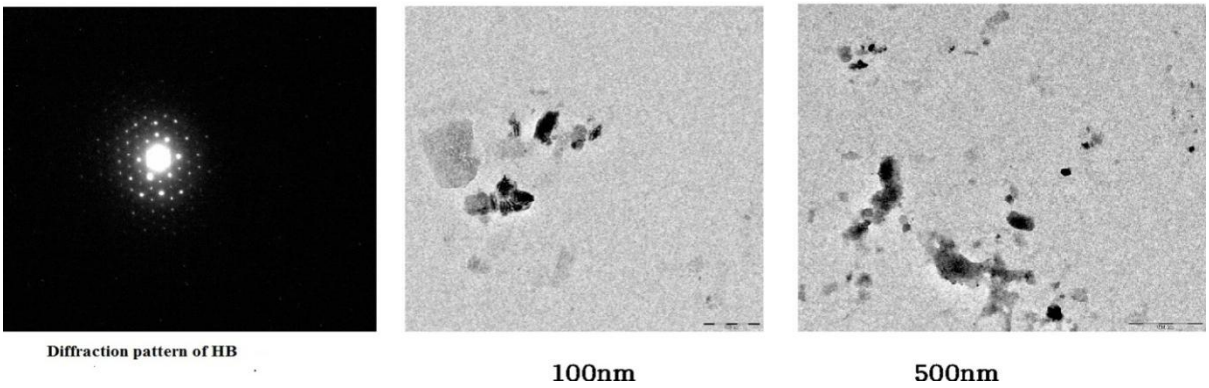

Figure 4: Images of Haratal Bhasma obtained after magnification through TEM

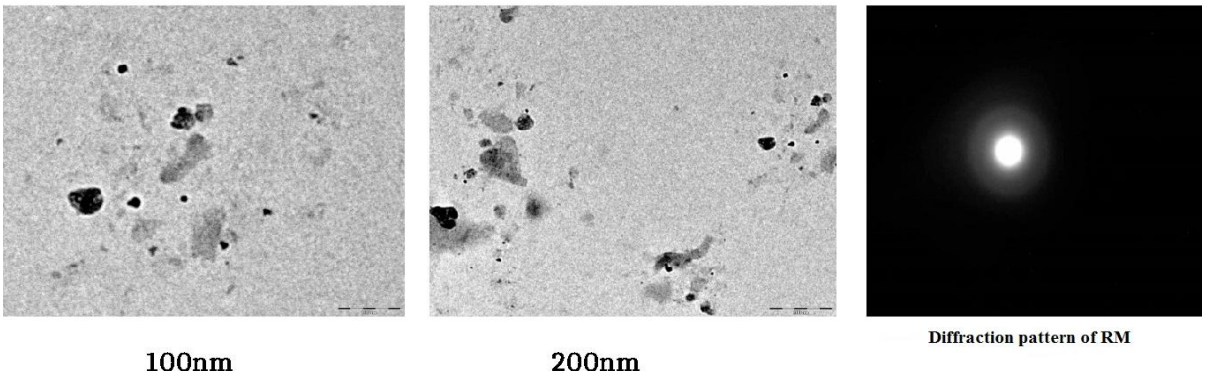

Figure 5: Images of Rasamanikya obtained after magnification through TEM

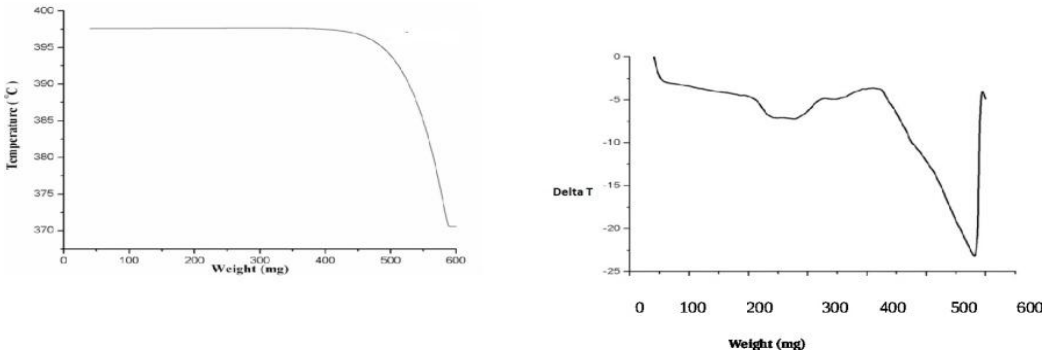

Figure 6: STA analysis of Haratal bhasma

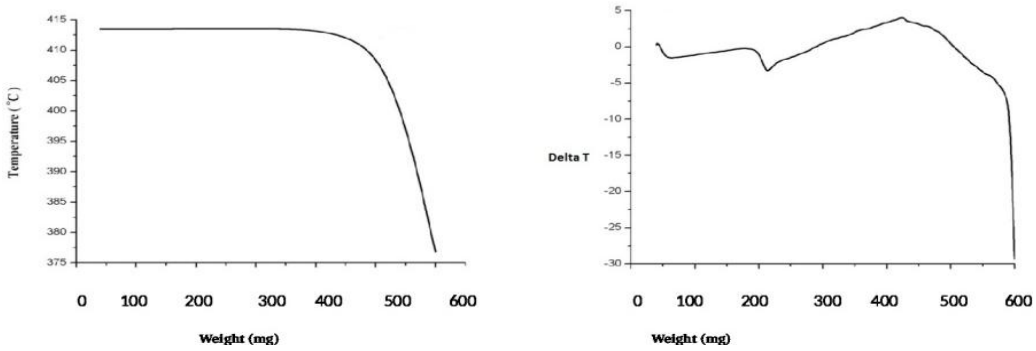

Figure 7: STA analysis of Rasamanikya

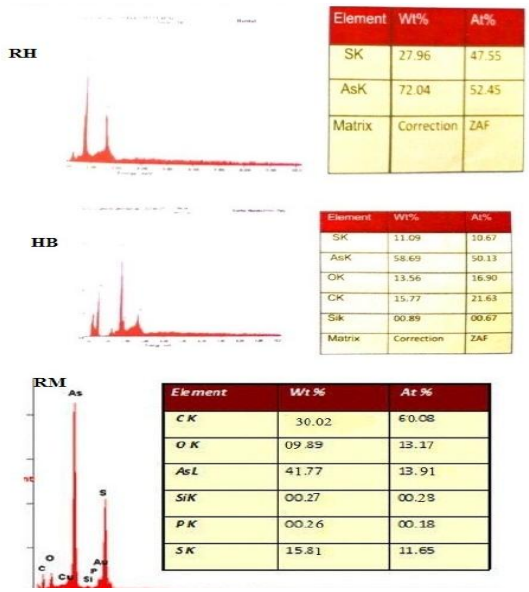

Figure 8: EDAX graph of RH, HB, RM

EDAX analysis revealed that RH contains Arsenic and Sulphur with wt% of Arsenic is 72.04% and sulphur is 27.96%. As the number of heat cycles increases the amount of Oxygen as an element increases which signify that Arsenic trioxide is being formed due to calcination process. As the number of heating cycles increases the amount of sulphur as an element decreases which signify that sulphur get vaporizes during *bhasmikaran* process from *Haratal*, hence we can conclude that during the said process *Haratal* partly converted to Arsenic trioxide. *Rasamanikya* is the drug which is derived out from only one single drug *Haratal* which is prepared by Mica leaves~*Abhraka patra* method for preparation of *RasaManikya* is comparatively easy but the negative point is that only a little amount of the product is prepared. In the sample of HB, wt % of Arsenic is 58.69% and sulphur is 11.69%. Apart from sulphur and arsenic HB also contains Oxygen with wt% of 13.56, due to the formation of oxides of arsenic.It also contains traces of carbon and silicon. During *bhasmikarana* (calcinations) process additional elements were added as impurities that mainly came from the plant extract by which the process of *bhasmikarana* had performed. In the sample of RM, wt % of Arsenic is 41.77% and sulphur is 15.81%. Apart from sulphur and arsenic RM also contains Oxygen with wt% of 9.89, due to the formation of oxides of arsenic. Like HB it also contains traces of carbon and silicon. The amount of arsenic in the RM is less than HB. This makes RM less toxic than HB. Also the amount of oxygen in HB is less than RM. We know arsenic trisulphide is less toxic than arsenic trioxide, hence RM is less toxic than HB. The popularity of RM over HB supports the fact.

CONCLUSION

The poisonous substances are used as medicine in traditional and integrative medicines. Proper scientific database is very necessary for validation of such drugs. Arsenic containing drugs are used extensively in Ayurveda. Hence an attempt has been made for creating a comparative database of two such drugs by the incorporating modern analytical methods. More research is welcomed in the field of validation of poisonous herbo-mineral Ayurvedic drug.

Conflict-of-Interest: There is no conflict of interest

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