Synthesis and characterization of Shanku bhasma-an anti-ulcer herbomineral formulation

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Synthesis and characterization of Shanku bhasma-an anti-ulcer herbomineral formulation

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Abstract. The traditional systems of Medicines are considered as a safer therapy. This context urges the need of Characterization of traditional medicines for their worldwide acceptance and for the safer, efficacious use. Bhasma the traditional Herbo mineral Ayurvedic formulation prepared by bhasmikaran process. Shanka Bhasma is prepared from the shell of a marine organism is a well-known herbo mineral formulation used for the treatment of peptic ulcer. In the present study, shanka bhasma was prepared and its characterization was done by traditional methods and by modern analytical Parameters like IR, X-ray, EDAX TGA and atomic absorption methods. Its antiulcer effect was also evaluated by animal studies.

1. Introduction

Bhasmas are formulations coming under the traditional system of medicines. In Ayurveda they belong to the class *Rasoushadi*; herbo mineral formulations containing metals and mineral as major therapeutic ingredients. Although metals were used from Samhita period (600 to 1000 BC) itself, their acceptance was less because of the chance of toxicity. With the introduction of Rasashastra, the new class of drugs with a specific method of preparation, the metals are converted into an easily absorbed, with a small dose and having much more therapeutic effectiveness ie Bhasma [1-3].

The use of metals as medicines was not widely recommended for the fear of toxic effects. But Bhasma the herbometallic formulation is prepared by a special process known as *Bhasmikaran* by which the toxicity is reduced or diminished to a negligible amount. The process involves mainly three steps Shodana, Bhavana, Marana and Putapaka, which includes repeated cycles of heating and size reduction by trituration of metal with the proper adjuvant. The metal containing sample on trituration with herbal juices and repeated heating will be subjected to chemical conversion and get converted to their compound form and finally higher oxides of metal having the particle size up to nano size are obtained. Special apparatus known as Sharava and mortar and pestle are used for this purpose and the fuel used for heating is cow dung. There is a temperature rise of about 800^{o} to 1000^{o} C.

Shanka Bhasma is a traditional medicine used for peptic ulcer, piles, cough and for some types of gastrointestinal disorders.Shanka Bhasma is of marine origin having the major role in treating H.pylori infection which has a 70% prevalence in third world countries. But the acceptance of these medicines by worldwide population requires proper standardization to meet the specified criteria. I n the present study, an attempt has been made to formulate and standardize by Physiochemical parameters, Instrumental methods and for Pharmacological activity [4-5].

2. Experimental

2.1. Formulation of Bhasma

The formulation of Shanka Bhasma was carried out as per the procedure in Rasatharangini, The traditional Ayurvedic text book. Raw Materials: Shanka (The outer covering of conch shell coming under family Gastropoda) and nimbu juice are obtained from local market of Nilambur, Malappuram, Kerala.

Cow dung-The fuel for Bhasma preparation is obtained from local people

2.2. Methodology

1) **Authentication** of raw material-Shankha: The outer covering of marine organism (Conch shell), family Gastropoda, Class Mollusca were collected from local market

2) Shanka Bhasma Nirmanam.

Shankha shodhana-50gms of Shankha is taken which is authenticated. It is subjected to shodhana (purification) as per the reference [3-4].

Shodhana- Shanka is powder into small pieces and then it is tied into two folded cloth and it is made to hang on a stick placed across a pot which is filled with nimbu swarasam (fresh juice of Citrus Limonam1.5 L). Then it is subjected to heat for 3 hrs at 70-80^{o} C. After 3 hrs, it is taken out and washed in warm water and dried. Shankha must immerse fully into the kanji (Extract prepared from paddy) while heating.

Marana (Bhasma Nirmana)- Purified shankha is taken and it is kept in mud vessel then this is covered by another vessel .The mouth of the vessel is covered tightly by a cloth which is smeared with gopichandanam (A type of mud).Cow dung cakes are used for fuel. The vessel must be placed over the cow dung cakes and set the fire. After the combustion, the vessel was taken and charred shankha must be collected. The same procedure is done in same way for 2 times after powdering.4, 5 (Figure 1)

3. Standardization [7-8]

Acceptance of a product depends upon the assurance of quality. The prepared Shanka Bhasma has to be standardized by traditional and by instrumental Analysis. The pharmacological action is verified by animal studies.

3.1. Physical Standardization Traditional method of characterization has done by following methods

Verna: The colour was found as ash by the naked eye. Niswadhutha: By keeping a pinch of bhasma on tongue taste was checked and it was found tasteless. Nischandratvam: Lustrousness was checked by observation of bhasma under bright light and was found without lusture. Rekhapurnatwa or fineness was checked by rubbing bhasma in between the fingers. It was found entering into the furrows of the fingers. Floating test showed bhasma over the surface of water as floating which infers the lightness of bhasma.

3.2. Physio Chemical Standardization

3.2.1 Ash values

a) Total ash: Take a previously tare crucible and add 1gm of accurately weighed Shanka formulation and was heated and incinerated at a temperature, not more than450^{o}C. Carbonaceous matters get removed. Weighed the crucible after cooling. Check the weight of the crucible in every half hour during the heating. Heating should be continued until constant weight was obtained.

$$\% \text{ of total ash} = \frac{\text{Weight of the ash obtained}}{\text{Weight of crude drug taken}} \times 100$$

b) Acid insoluble Ash: To the total ash obtained added 25 ml of dilute hydrochloric acid and was boiled gently for 5 minutes. cooled and the insoluble matter retained in the ash less filter paper was washed and ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the total ash.

c) Determination of loss on drying-Crushed the sample into fine powder. To a previously weighed crucible and heated at 1050 C for one hour.

$$\text{Loss on Drying} = \frac{\text{Weight difference of sample}}{\text{Weight of the sample taken}} \times 100$$

3.2.2. Solubility: Solvents of decreasing polarity are added to specified amount of formulation and it was found soluble in dil HCl

3.2.3. pH: The acidity-basicity characters of the prepared Bhasma was checked by using pH meter. A solution of Bhasma was prepared by adding 2 gm of bhasma into 100ml of water and it was shaken for 5 minutes. Allow to settle and a clear supernatant solution was taken for assessment using DIGISUN digital pH meter and it is found as 9.3.

3.2.4. XRD Analysis

The crystalline nature and powder characteristics are determined by Xray diffraction techniques. The crystalline size was calculated from the pattern by Scherrer equation $t=ʎx0.9/\beta x \cos\theta$.

3.2.5. SEM Analysis

The morphological character and Nanosize of particles are studied by Scanning electron Microscopy. A representative sample was mounted on alumina stubs using double adhesive tape Model- EVO 18 Research SEM mode, Make-USA. The image gives spherical shaped particle in agglomerates.

3.2.6.TGC

Thermo Gravimetric curve is plotted with the reduction in weight of substances as the function of temperature by allowing the sample to undergo a controlled temperature program in an air atmosphere. The equipment used is Model FDTQ600, Make-DA instrument USA. Figure 6.

3.2.7. EDAX Analysis

EDAX analysis (Energy Dispersive X-ray spectroscopy) was done for the morphological characterization of the sample. The instrument used was Model OXFORD, Make-USA. Figure 7.

3.2.8. Atomic Absorption Spectroscopy

Evaluation of the formulation for the concentration of elements is done by AAS. The instrument used acetylene and air as the flame. The sample was digested in dilute HCl and appropriate dilutions are made with distilled water. Model: Varian.Make-240.The presence of heavy metals is within the limits.

3.2.9. Pharmacologic Evaluation

Animals: Male Wistar rats (150-175g) were taken for the study. The animals were kept in polypropylene cages and food and water were given *ad libitum*. The institutional animal ethical committee approved the study protocols. (Reg.no.1195/Re/S/08/CPCSEA).Acute oral toxicity study: The dose for the present study was selected from the previous study (Pandit *et al.*2000) and 0.001, 0.01, 0.1 and 1g per kg by the oral route. The animals were observed for a period of 72 hrs.

3.2.10. Aspirin-induced antiulcer study for Shankha bhasma

Four groups of rats containing six animals, each was selected for the study and they were pretreated with vehicle, test drug, and standard drug for 14 days by the oral route. The dose was selected from the previous study (Pandit *et al.*2000). Group 1:1% CMC.

Group 2: Ranitidine 50mg/kg. Group 3: Shankha Bhasma 25mg/kg. Group 4: Shankha Bhasma 50mg/kg. The animals were fastened overnight before the study. On the day of study, the animals were treated with aspirin ulcerogen 500mg/kg body weight[8]. One hour after the treatment with the routine dose of the drug.

After six hours of ulcerogenic treatment, the animals were sacrificed under ether anesthesia by cervical dislocation. The abdomen was opened and stomach was incised along the greater curvature and examined for ulcers. Ulcer lesions were counted and ulcer index was calculated. (Vogel)

***4*. Result and Discussion**

Shanka Bhasma was formulated as per the Rasatharangini.[3-4]. The official textbook in Ayurveda. The special process used for the formulation which involves repeated heating and incineration with adjuvants. This process produces a synergic effect with reduced toxicity and potent and bio-safe product. Figure 1.The traditional standardization shows results as follows: - Nischandratvam: showed the product without Metalic lusture. Rekhapurnatwa: Fineness. The sample was so fine that it entered into the furrows of the fingers Floating test: lightness of bhasma was revealed by floating of the sample on the surface of water Figure 2 and Figure 3.

Figure 1: Stages of Bhasmikaran

Figure 2: Physical properties for Shanka Bhasma

Figure 3: Fineness test for Shanka bhasma

4.1. **Physiochemical Standardization**.[5]

The Ash values are calculated as per the procedure and Tabulated in Table 1.The values indicate that organic matter are less and mineral are present in appreciable amounts. Acid insoluble ash and loss on drying values indicate that the impurities are less. The pH values show the alkaline nature. Table 1

Table 1: Phyico Chemical parameters of Shanka Bhasma

S.No	Parameters	Value
1	pH	9.3
2	Total Ash	79.80%
3	Acid Insoluble Ash	10.1%
4	Loss on Drying at 105^0C	0.11%

XRD *4.1.1*.Analysis

Crystalline form was revealed by the X- ray photographs. The size of the crystals are calculated by Scherrer formula and the size lies in the range of 80nm. Figure 4

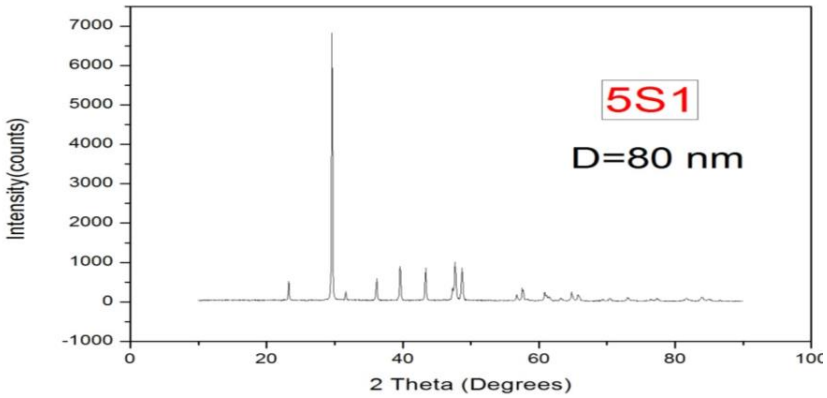

Figure 4: XRD Analysis of shanka Bhasma

***4.2*. SEM**

The studies show the particle size 600nm.The shape of the particle was oval and smooth and gives inference that the method of preparation determines the size of the particle. Calcination method, the no of calcination and the temperature should be proper for the uniqueness of the product. Deposition of small dusty particles over the calcium oxide crystals causes agglomeration of particle which is seen in the image. Figure 5

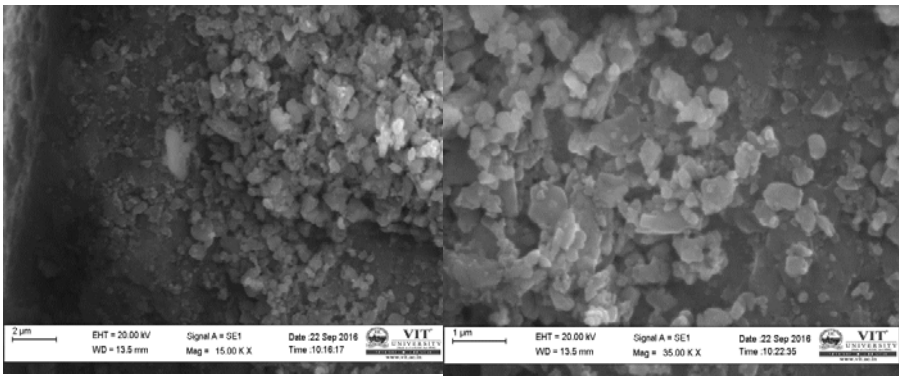

Figure 5: SEM images of Shanka Bhasma

4.3. TGA

The decomposition temperature of 726.13 ^{0}C is obtained from TGA curve shows that the Shanka Bhasma has got pure calcium oxide as the major component. Figure 6.

Figure 6: TGC curve for Shanka Bhasma

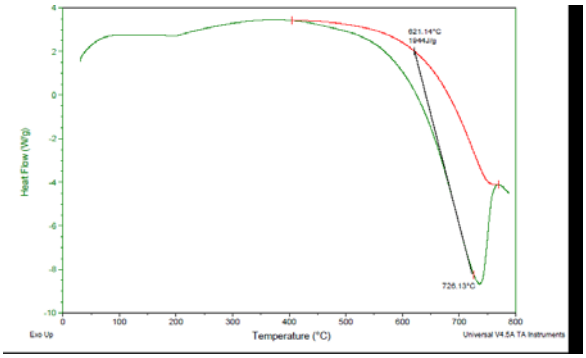

Figure 6: TGC curve for Shanka Bhasma

4.4. EDAX Analysis

The Figure 7 of EDAX analysis gives the stoichiometry of particles. Table 2

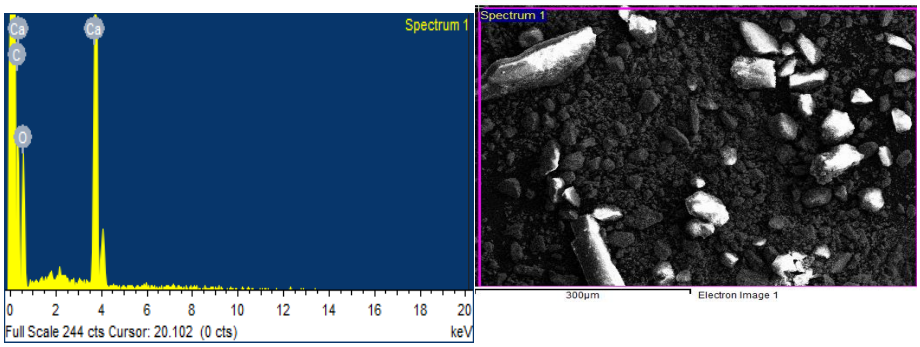

Figure 7: EDAX images of Shanka Bhasma

Table 2-Results of EDAX analysis

SNo	Element	Weight	Atomic%
1	Carbon	-10.63	-11
2	Calcium	37.82	20.47
3	oxygen	72.82	98.74

4.5. Atomic Absorption Spectroscopy

The concentration of metals is determined by AAS. The results are given in Table 3

Table 3-Results of Atomic Absorption Spectroscopy

SNo	Element	Weight	Atomic%
1	Carbon	-10.63	-11
2	Calcium	37.82	20.47
3	oxygen	72.82	98.74

The standardization parameters confirm the particle nature of the Shanka bhasma and this oxygen-deficient state improves the therapeutic activity.

4.6. Pharmacological activity

The anti-ulcer effect of Shankha Bhasma was evaluated by aspirin-induced model and the effect was found good. Figure 8.

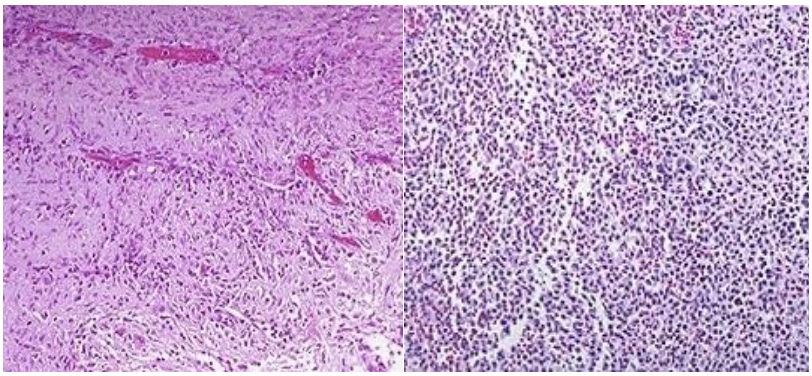

Figure 8: Anti-ulcer effect for Shanka Bhasma

5. Conclusion

Shanka bhasma was prepared by traditional method and their possible standardization parameters have done. Organoleptic characters and physical parameters showed that sample is prepared in the proper way so that possible chances of impurities are avoided. From the TGA study the chemical form of shanka bhasma was identified as calcium oxide and XRD and EDAX showed the characteristic morphology of particles as oval shaped crystals with smooth surface and micro to nano size. An AAS study reveals the possible heavy metals present in the final product. The antiulcer effect produced can be attributed the synergestic effect of calcium and other adjuvants. Pharmacological studies are done for standardization of ulcer effect and it was found have good anti-ulcer effect in aspirin induced study.

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Reference

[1] Chavare,a., *et.al*. 2016 Safety and bioactivity studies of jasad bhasma and its in-process Intermediate in swiss mice, *The journal of ethnopharmacology.***197** 75-86.
[2] Thakur Rakesh Singh. Laxmi Narayan Gupta.Neeraj Kumar. 2015.Standard Manufacturing Procedure Of Teekshana Lauha Bhasma *Journal of Ayurveda And Integrative Medicine*. **Aug 7** 100-108
[3] Shebina P.Rasheed Murugesh Shivashankar, 2015 Evaluation of Herbomineral Formulations Bhasma -An Overview *I J.of R A.* **6** 3
[4] Sadananda sharma.2014 Rasa tarangini (trans: eng:Dr,Ravindra angadi*) Chaukambha surbharathi prakashan, Varanasi.*
[5] Sirisha Mukkavalli,, Vijay Chalivendra Bal Ram Singh.2017 Physico-chemical analysis of herbally prepared silver nanoparticles and its potential as a drug bio enhancer. *Open Nano* **2.**19–27.
[6] Sanjoy Kumar Pal 2015 The Ayurvedic Bhasma: The Ancient Science of Nano medicine,

Recent Patents in Nanomedicine. **5**, 12-18.
[7] Vogel, Drug Discovery and Evaluation: Pharmacological Assays, Springer, third edition 256-259.

[8] Sumithra, P.Raghavendra Rao, A. Nagaratnam,Y.Aparna, 2015 Characterization of SnO2 Nanoparticles in the traditionally prepared Ayurvedic medicine, *Science Direct*. **2** 4636-4639.
[9] Santhosh B Raghuveer Jadar PG and VNageswara Rao 2013. Analytical Study of Yasada bhasma (Zinc based Ayurvedic Metallic Preparation) with reference to Ancient and Modern Parameters, *Ancient and Modern Parameters*.**2 1**1-4.
[10] Raju.D, Ilango.K, Chitra.V, Ashish.K. 2009 Evaluation of Anti-ulcer activity of methanolic extract of *Terminaliachebula* fruits in experimental rats, *J. Pharm. Sci. & Res.* **Vol.1(3)**

101-107.
[11] Swapnil Y. Chaudhari a, Mukesh B. Nariya b, R. Galib a, Pradeep K. Prajapati 2016 Acute and subchronic toxicity study of Tamra Bhasma (incinerated copper) prepared with and without Amritikarana*, Journal of Ayurveda and Integrative Medicine* **7** 23-29.