

In-process Profiling of Herbomineral Formulation from Marine Origin

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ABSTRACT

Objectives: To prepare Shankha Bhasma, an antiulcer formulation from marine origin and to standardize the formulation by two different methods of preparation and to compare with marketed formulation by modern scientific tools

Methods: Shankha Bhasma was prepared from conch shell by open and closed methods using adjuvants Kumari Swarasa and water and subjected to characterization by conventional method and AAS, XRD, EDAX, SEM, TEM, BET, TGA, IR spectroscopy. The effectiveness was checked by animal studies, toxicity by histopathology and cytotoxicity studies.

Results: Open method takes few steps and less time and shows crystalline nature (XRD). TGA shows the decomposition temp of calcium oxide. The closed method produces smoother particle (SEM, TEM and BET) with no peaks for specific organic components (IR), elements within the prescribed limits (AAS) of Ayurveda. The adjuvant's effectiveness was shown by in-process standardization and showed promising pharmacological effects without any toxic effects the dose selected was 25mg to 50mg

Conclusions: The size, smoother surface and therapeutic effectiveness of the herbominerals prepared by the two methods can be attributed to the calcination steps and adjuvants. Thus, maybe dose reduction and rapid effect can be achieved.

Keywords: Shankha Bhasma, Herbomineral, Standardization, Ayurveda, Calcination.

1 INTRODUCTION

The herbomineral formulations coming under the Rasoushadi branch of the Ayurvedic system of medicine fundamentally encompass metals and minerals as an integral part, but not in elemental form. These are normally found in form of compounds. Once in the body, they behave very differently than the elemental form of heavy metals. Huge changes occur to minerals, once they are exposed to the refined manufacturing process of Shodhana and Marana¹. The outcome of all these methods is obtained after the treatment with several organic and inorganic

herbal materials. All these exposure and methodologies may alter the toxic metal characteristics. Thus therapeutical effectiveness with high-grade safety is made possible². As per the traditional physicians, the ashing process (Calcination) incorporates the depth and personality of the herbal juices in the metal². The distinctive abilities of Rasausadhies like rapid action, smaller dosage, flavorlessness, lengthy shelf-life, and improved delectableness have facilitated to surmount the demands of these drugs

1.1 Incinerated Conch Shell (Shankha) - An Ayurvedic Miracle Formulations

Shankha Bhasma is prepared from the Conch (Shankha) shell of the family Gastropoda of Class Mollusca, in ash form. In Ayurveda, it is used to treat many

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diseases. Conch is known to have many properties like alkaline (kshariya), adsorbent (*grahi*) and cooling (*sheetal*). The Bhasma preparations have importance in maintaining optimum alkalinity for maintaining better health, and neutralizing harmful acids that leads to illness³

Since Bhasma is stable and does not metabolize, the chances of producing harmful metabolite⁹ is less. It further breaks down the heavy metals present in the body. The prepared Bhasma are Micro to Nano-sized and are stable for a long period⁹. Because of their minute size, the absorption of drugs in to the circulation is fast, and thereby the dose required for producing the desired effect is small. So, Shankha Bhasma can be considered to be a better cure for stomach issues⁴. Thus, the present research work is focused on preparing Shankh Bhasma, which is an antiulcer formulation from marine source. This preparation is being done by two different methods with different adjuvants. Further, the Shankh Bhasma will be standardized and the Bhasma prepared by two methods will be compared each other and with the marketed formulation with the help of modern scientific tools.⁵

2 MATERIALS AND METHODS

2.1 Formulation of Bhasma⁶⁻⁸

Shankha was acquired and authenticated from The Government Ayurveda College Parasinikadavu, Kannur. The traditional preparation of the Shankha Bhasma was done as per the Ayurvedic Formulary of India, under

Rasoushadi branch of Ayurveda. The steps involved in the preparation are^{9,10}

- Samanya Shodhana (normal purification)
- Visheshha Shodhana (special purification)
- Bhavana (heating in the sun after trituration)
- Putapaka (calcination)

The raw materials required for the preparation of the Shankha Bhasma are as follows

Table 1: Raw material used for the formulation of shankha Bhasma

S. No.	Ingredients	Quantity
1	Ashudha shankha	315 ±00g
2	Kanji	1600±00ml
3	pH of Kanji	3.4±0.2
4	Shankha after shodhana	303±0.3g
	% yield	95.87±0.64%

2.1.1 Open Method preparation of Bhasma

The calcination method used is Uthputheleekarana. The purified and triturated shankha was taken in three separate samples weighing 100gm each in a vessel known as sharava. These were then kept in a pit of coconut husk fuel and were exposed to intense heat. The process was repeated two times for all three samples (Fig.1). Changes for shankha during uthputhelekarana given in Table 2.

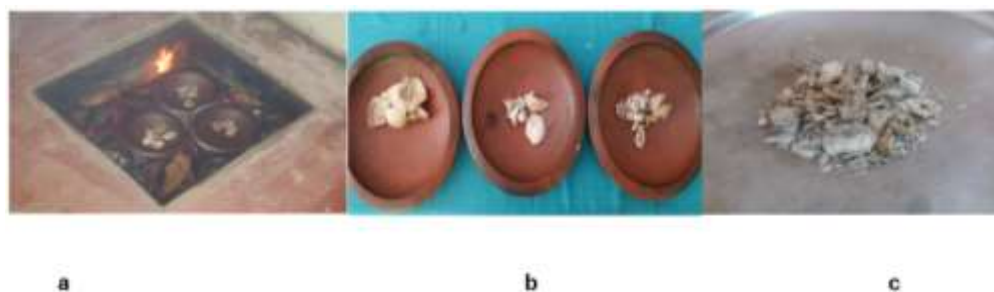


Figure 1: (a) Uthputhelekarana process. (b) After 1st uthputhelekarana. (c) after 2nd uthputhelekarana

Table 2: Changes for raw Shankha during open Method

Particulars	OBSERVATIONS	
	1 st Utphulleekarana	2 nd Utphulleekarana
Wt taken	100 g	100 g
Wt after Utphulleekarana	100 g	98.83g
Color changes	Appearance of blackish tinge	Dull white
Hardness	Became brittle	Brittle

2.1.2 Closed Method preparation of Bhasma

In the closed method, calcination was done by Puta (Repeated heating) method. Herein two adjuvants have been used.

1st Puta- Six samples each weighing 100gms of triturated Shankha were taken and kept in a Sharava. The Sharava was closed by a lid. The edges of the Sharava were sealed by a paste of mud. The three Sharava were then subjected to intense heat in Gaja Puta.

2nd Puta- Out of the six samples of triturated Shankha, three were powdered and triturated with Kumari Swarasa.

These were then made in to pellets of a specific dimension Measured with the help of scale having Diameter: 1.5 – 2 cm and thickness: 0.5 cm and dried. After drying, they were again kept in Sharava and closed by a lid. The heating process was repeated. The remaining three samples were powdered and triturated with water to make the paste and the above procedure was followed

3rd Puta- The procedure of 2nd Puta was repeated ¹⁴ Changes for raw Shankha during Closed Method have been presented in (Table: 3)

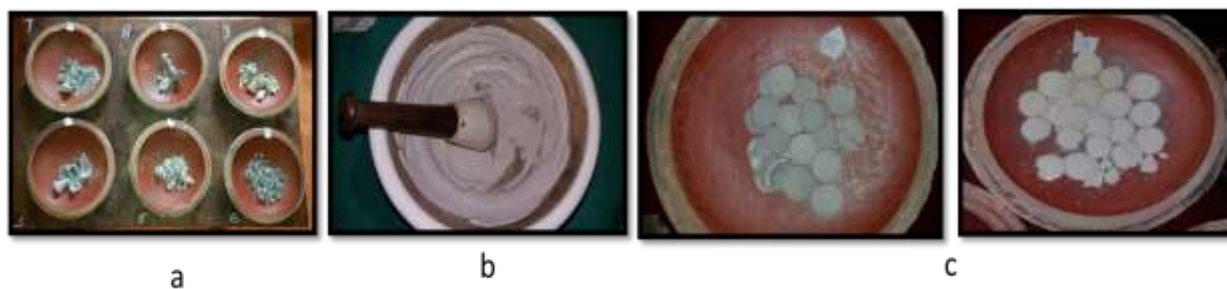


Figure 2: (a) Purified Shankha after 1st Puta (b) Trituration with adjuvants (Kumari warasa/Water) (c) Purified Shankha after 2nd Puta

Table 3: Changes for raw Shankha during Closed Method

Order of Puta	Weight of Shankha taken	Weight after Puta	SEM
First Puta	100 g	98.08±.584	0.238
Second Puta	98g	93.83±2.38	0.972
Third Puta	93g	89.666±4.916	2.007

2.2 Characterization of Shankha Bhasma Preparations

2.2.1 The preliminary test -are done as per traditional books.

2.2.2 Physico Chemical Standardization –

Ash Value-The inorganic residue after the heating effect removes organic matter and water is called ash. This helps in assessing the overall quantity of minerals within the drug¹⁵. The ash value has been determined on the based on following aspects

Total Ash- Taken a tared container, 1gm of the sample was added and heated. Heating was continued until a constant weight was obtained. The total ash was then calculated using the following formula-

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

Acid Ash- The total ash obtained was treated with 25 ml of dilute hydrochloric acid. It was then boiled gently for 5 minutes. After cooling, it was filtered using a filter paper. An insoluble matter was retained which was then ignited and weight was taken. The percentage of acid- insoluble ash was calculated concerning the total ash

Determination of Loss on Drying- The sample was crushed into fine powder and heated at 105° C for one hour. Loss on drying was calculated using following formula-

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

Solubility- Solvents of declining polarity were supplemented to definite quantity of formulation. It was found soluble in dilute HCl

Determination of pH- pH value facilitates in analysing the acidic and basic phases of adrug¹⁶. In the present research, the acid-basic properties of the Bhasma were tested a using shaken for 5 minutes and then allowed to settle until a clear supernatant solution was obtained. This solution was taken

for valuation via DIGISUN digital pH meter

2.2.3 Instrumental Analysis-

Elemental analysis of the Bhasma by Atomic Absorption Spectroscopy- Atomic Absorption Spectroscopy is used to analyse metals at very low concentrations, typically in the parts per million (ppm) or parts per billion (ppb) ranges¹⁶⁻¹⁷. The sample was digested in dilute HCl and appropriate dilutions are made with distilled water. For conducting the spectroscopy, Varian.Make-240 has been used. Each element emits light at its characteristic wavelength, and based on the intensity of the light, the concentration of the element was calculated

IR Spectroscopy- This method is used to access the presence of organic impurities. It was carried out for the intermediates after each step of calcination and for the final preparation of Bhasma. IR spectrum was determined using Bruker FTIR spectroscopy using the ATR sampling technique. IR Spectrum of intermediate and the final product was accessed to recognize the alterations happening during the preparation of Shankha Bhasma by both open and closed methods

Thermo gravimetric curve/Differential Scanning Colorimetry-Thermo gravimetric curve has been used for checking the purity and decomposition temperature of the final products. The reduction in weight of substances has been plotted on y- axis while the temperature has been plotted along the X-axis. The process has been carried out under the atmosphere of air by exposing the sample to a controlled temperature. The test has been conducted using model FDTQ600, Make-DA instrument, USA

X-ray Diffraction (XRD)- X-ray powder diffraction (XRD) “is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions”¹⁸. In the present research, XRD analysis was conducted for identifying the crystalline phase and quantification of intermediate and the final preparation. The crystalline size was calculated using Scherrerequation

$$t = \frac{\lambda}{\beta \cos \theta}$$

SEM-EDAX- SEM (Scanning Electron Microscopy) has been used in the present research to study the size and morphology of particles of Shankha Bhasma. EDAX (Energy Dispersive X-ray spectroscopy) analysis was conducted to study the composition of Bhasma. These tests were carried out by using Model- EVO 18 Research SEM mode, Make-USA

BET (Brunauer, Emmett and Teller) Surface Imaging- BET analysis is used to measure surface area of a sample. It can also analyse the pore size distribution. The analysis is used to calculate the dissolution rate. Thus, it can be used to predict bioavailability¹⁸⁻¹⁹. In the present research, BET analysis was conducted using Autosorb iQ Station 1, at temperature of 77.350K

Table 4: Relative Pressure and Volume Table

Relative Pressure	Volume @ STP
1.05E-01	0.1632
1.55E-01	0.1983
2.05E-01	0.2284
2.55E-01	0.2626
3.05E-01	0.303

Transmission Electron Microscopy (TEM) - The Bhasma prepared by closed method underwent TEM analysis to assess the size of the particle²⁰⁻²³

Pharmacological Evaluation by Animal experiments- Pharmacological activity and toxicity evaluation was conducted. For this study, Wistar albino rats of both sexes were selected weighing between 150-175g. The animals were kept in polypropylene cages and food and water were given as per the requirement. The institutional Animal Ethical Committee approved the study protocols (Reg.no.1195/Re/S/08/CPCSEA). The dose was given by oral route

Acute oral toxicity study: The dose was selected from the previous study conducted by (Pandit et al.,2000) ²⁴. The animals were observed for 72 hours

2.2.4 Aspirin-induced antiulcer study for Shankha Bhasma- Six groups of rats containing six members in each group were selected for the study. These animals were pre-treated with a 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)

Table 5: Doses for Different Groups of Rats

Group 1:	1% CMC.
Group 2:	Ranitidine 50mg/kg.
Group 3:	Positive Control(Aspirin 500 mg/kg)
Group 4:	Shankha Bhasma open method 25mg/kg.
Group 5:	Shankha Bhasma open method 50mg/kg.
Group 6:	Shankha Bhasma closed method SB (b) 25mg/kg.
Group 7:	Shankha Bhasma closed method SB (b) 50mg/kg

The animals were kept fasting overnight before the study. On the day of the study, the animals were treated with Aspirin ulcerogen 500mg/kg body weight one hour after the treatment with the routine dose of the drug ²⁵⁻²⁶.

After six hours of ulcerogenic treatment, the animals were sacrificed. The abdomen was opened and the stomach was incised along the greater curvature and examined for ulcers. Ulcer lesions were counted and ulcer index was calculated.

3 ANALYSIS AND RESULTS

3.1 Formation of Shankha Bhasma- The temperature changes during each step of the preparation were noted as presented in (Table: 4).The result shows that the colour of the preparation changes from Pale white to Ash. The size of the particles was reduced by the calcination step. At high temperature, the particles get activated and the absorption is increased. This leads to reduction in the dose requirement. The assimilation of micronutrient by the added adjuvants increases the effectiveness of the Bhasma.

Table.6: The temperature changes during each step of the preparation

Open Method			
steps	Time taken	Max.Temp attained	SD
Utphuleekarana - 1 st	1.15hrs	311.67±2.887	1.667
Utphuleekarana – 2nd	1.15hrs	325±50	2.887
Closed Method			
steps	Time taken	Max.temp attained	SD
Putta 1 st	1hr	359.83±15.88	6.483
Putta 2 nd	1hr	371±11.08	4.524
Putta 3 rd	1hr	364.83±18.22	7.441

The values are average of three readings

3.2 Characterization of Shankha Bhasma Preparations-

Preliminary tests for standardization presented that the Shankha Bhasma prepared showed fineness. It was further

found that the Bhasma is light and without any lustre and taste.

3.2.1 Physicochemical Standardization-

Physicochemical Standardization of the sample prepared by two methods has been presented in (Table: 7).

Table 7: Physicochemical Standardization of Open and Closed Methods

Physical constants	Open Method	Closed method	
Total Ash	89%	80%	82.1%
Acid insoluble ash	9.2%	8.3%	9.5%
Water-insoluble ash	15%	14%	14%
Loss on Drying	0.11%	0.09%	0.15%
pH	10.9	11.3	9.8
Solubility	Soluble in dil HCl	Soluble in dil HCl	Soluble in dil HCl
Acid neutralizing capacity	12.3mEq/gm	13mEq/gm	12.5mEq/gm

Physicochemical Standardization has been presented in Appendix 1 (Table: 8). Ash value shows the presence of carbonaceous materials in the Shankha Bhasma. The prepared Bhasma by both the method contains more than 80% ash values and loss on drying was within the limit showing that the moisture content

is less in these preparations.

3.2.2 Instrumental Analysis²⁸⁻³¹

Results of Atomic Absorption Spectroscopy showed that elements are present within the permissible limits. The Elemental composition of Shankha Bhasma is presented in (Table: 8).

Table 8: The Elemental composition of Shankha Bhasma

Element	Open Method	Water
Lead	2.75ppm	3.0 ppm
Copper	0.107ppm	0.721ppm
Cadmium	0.336ppm	0.299ppm
Cobalt	1.806ppm	1.223ppm
Arsenic	Within limits	Within limits
Mercury	Within limits	Within limits
Chromium	0.286ppm	0.239ppm

IR Spectroscopy by FTIR analysis of Shankha Bhasma was conducted. The peaks due to O-H stretching (3200-3600) showed the presence of moisture. Peak due to C=O stretching shown at 1603 is becoming sharp. As the Puta number is increased, C-O stretching peak is shown at 1250-1050. The absence of peaks in the functional group region indicates that the sample does not contain any major constituents. In the open method, the organic matter is

getting reduced after each heating process. On comparing with raw Shankha and the peak due to C=O stretching is prominent in the final sample. In the closed method, as the number of Puta was increased, the peak due to functional group are getting diminished and C=O stretching peak became more clear. The Extra peaks shown in (b) sample are due to the added micronutrients from the adjuvant used.

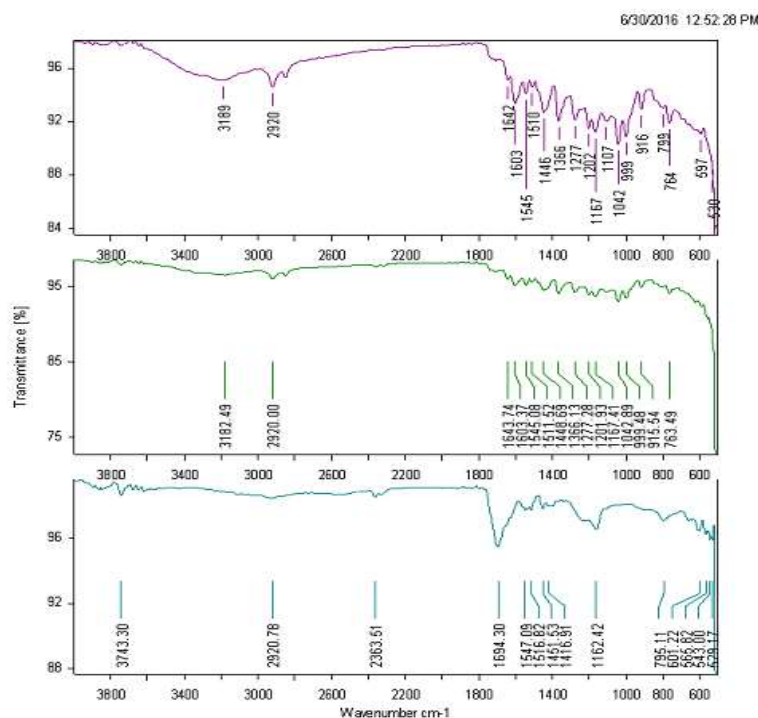


Figure 3: IR Spectrum for Shankha raw Shankha after 1st and 2nd Uthputhelekarana by Open method

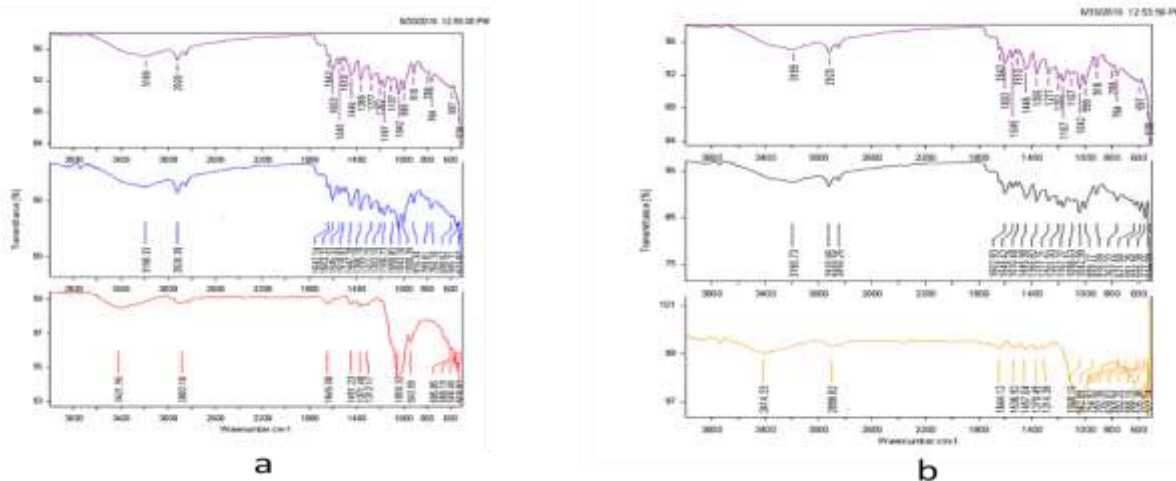


Figure 4: IR spectrum of Shankha after 1st second and third Puta by Closed Method (a) using Water (b) using Kumari Swarasa

In the TGA/DSC method, the decomposition temperature was shown as 734.5°C for the open method and 731.2°C, 729.9°C for the closed method with Kumari Swarasa and water respectively. The analysis shows the

purity of the compound. This temperature is close to the decomposition temperature of calcium carbonate to calcium oxide. Both the methods produced pure calcium oxide in the final product.

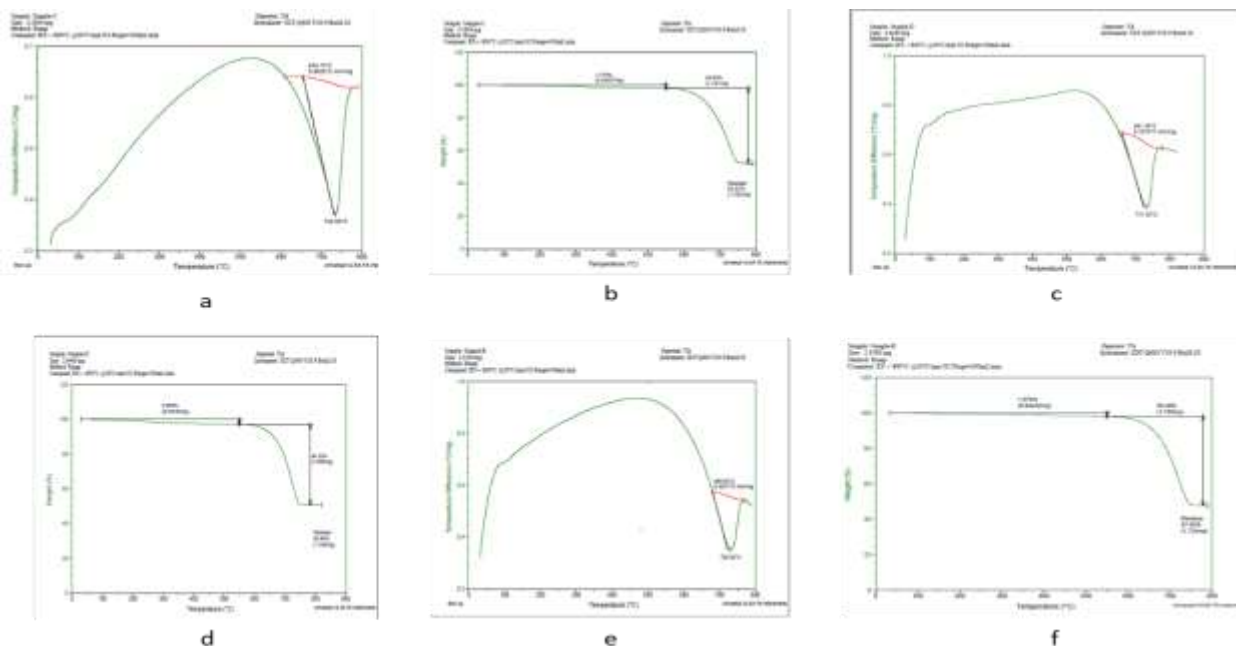


Figure 5: TGA and DSC analysis for Shankha Bhasma (a) & (b) Open method (c) & (d) Closed method with Kumari Swarasa; (e) & (f) Closed method with water

The images of the SEM analysis of Shankha Bhasma revealed that the size of the particle is reduced by the preparation technique. SEM analysis has done to determine the surface morphology and shape of particles. In the open method the temperature attained during heating process is 311°C after first uthputheleekarana (Calcination in open condition) and 325°C after second uthputheleekarana (Second calcination). SEM photographs shows that the particle has changed to more fine and smooth after second calcination of Open method. The

surface of the particles was smooth and clustered. In Closed method the effect of heating with the adjuvants water and kumariswarasa on particle size and surface were evaluated. Closed method with water shows smoother and clearer particles than with open method. With kumariswarasa adjuvant more smooth and finer particles are produced. The heating temperature reached in closed method after Puta (calcination in closed vessel) is 359°C - 365°C. The high temperature attained in closed method may be attributed to more fineness of particles.

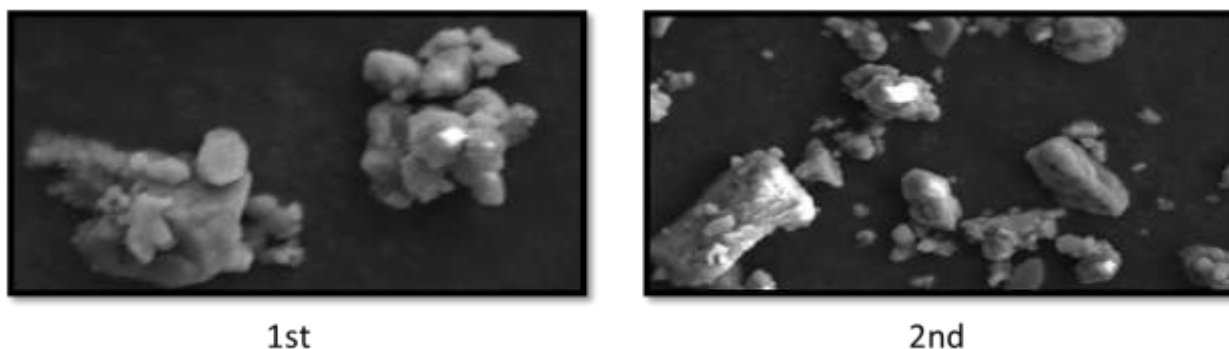


Figure 6: SEM Images for Open Method

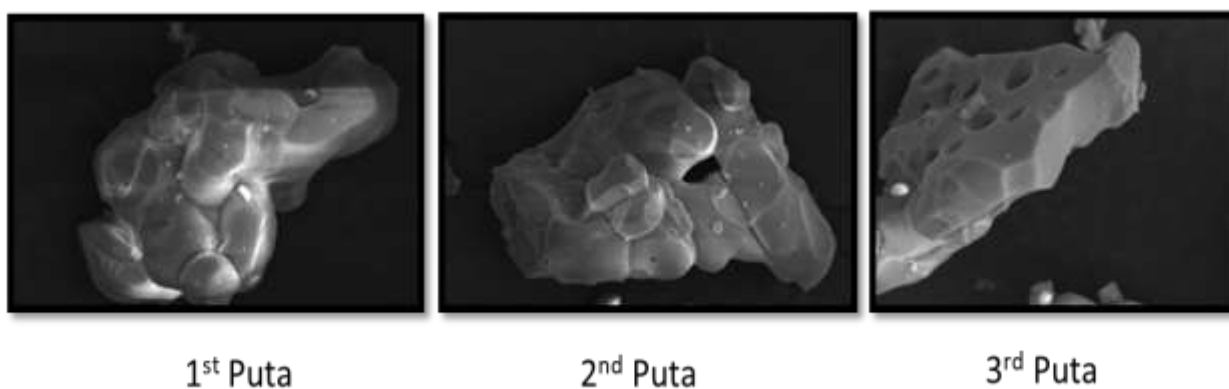


Figure 7: SEM Images for Closed Method with Water



Figure 8: SEM images for closed method with Kumari Swarasa

It can be inferred from the SEM images Fig.6, Fig.7 and Fig 8, that the heating process in the closed method

makes the particle surface smooth and fluffy and in the open method the particles appear in clusters.

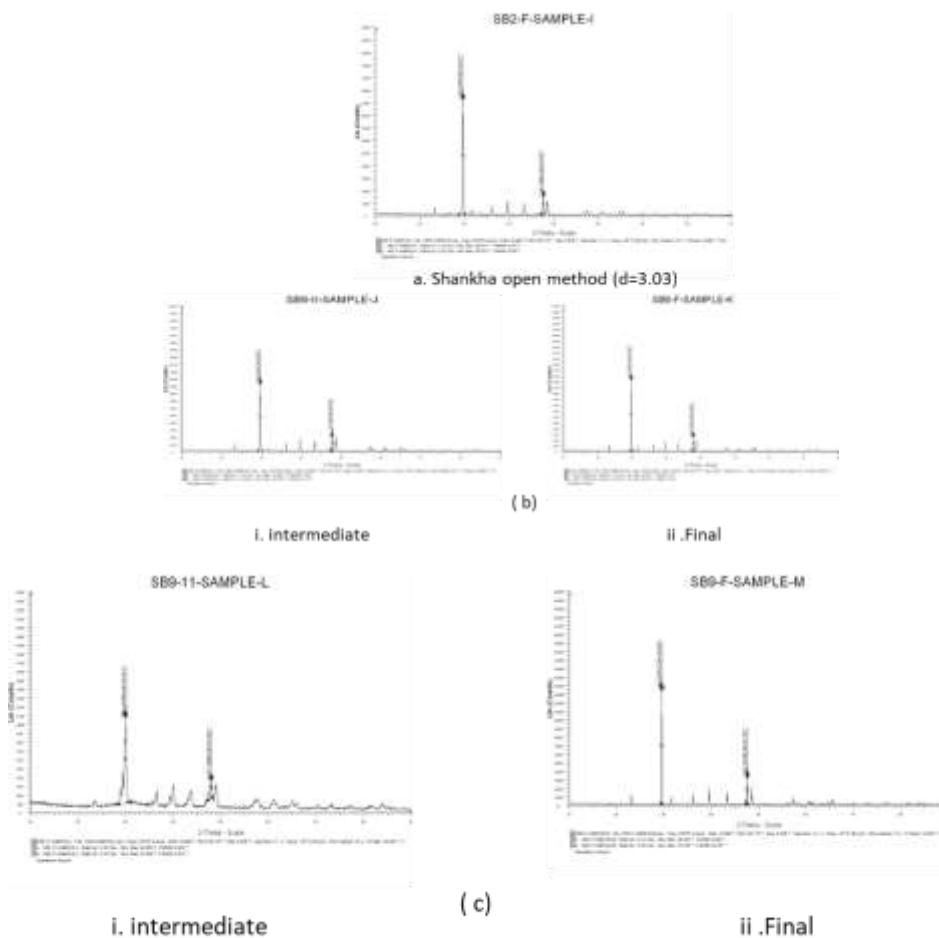


Figure 9: XRD analysis for Shankha Bhasma; b. Shankha Closed method with water (d=3.01); c. Shankha closed method with Kumari Swarasa (d=3.026)

The sharp peaks in XRD analysis (Figure 09) shows the crystalline nature of the powder and the d value was calculated using Scherrer formula. The low intensity peaks were due to the presence of trace elements or due to the

micronutrients. During the preparation of Bhasma, the amorphous nature of the material is changed to crystalline nature. Both the methods produced crystals with comparable d values.

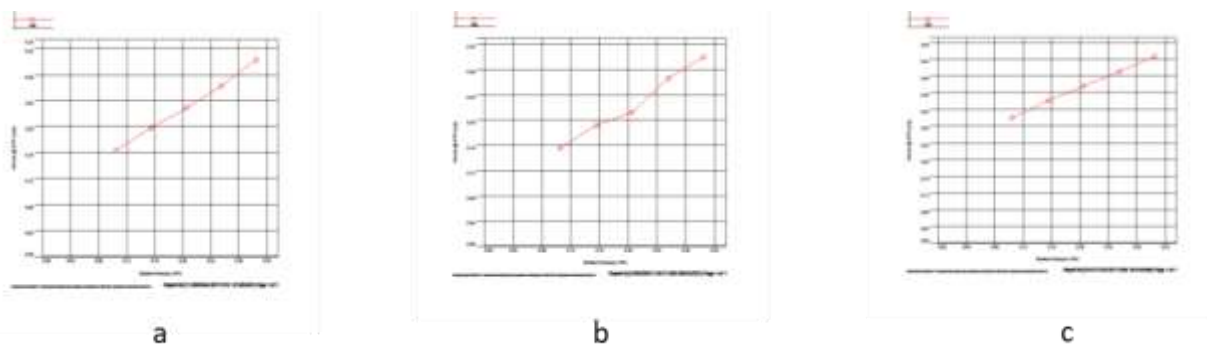


Figure 10: BET Surface Imaging (a) Open Method (b) Closed Method with Kumari Swarasa and (c) Closed Method with Water

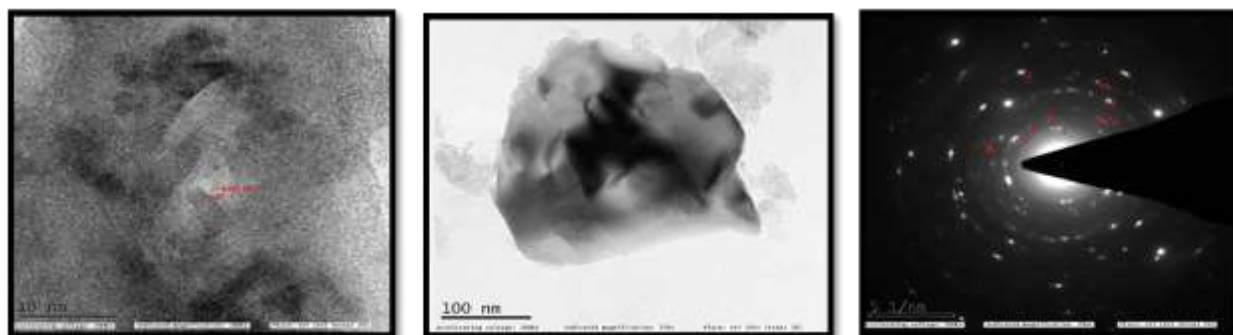


Figure 11: Particle characterization using Transmission Electron Microscopy (TEM)- Closed method

BET (Brunauer–Emmett–Teller)surface imaging of the shankha bhasma formulations(Figure 10) showed that the particle size is smaller for closed method than the open method formulation. To confirm the crystalline nature and nano size the shankha formulations prepared by closed

method were characterized by TEM Analysis.TEM analysis of the closed method for preparation of Shankha Bhasma showed results presented in Appendix 1 (Table: 9).The results shows the d spacing and the number of spots indicating that the particles are of nanorange.

Table 9: TEM analysis of closed method

Spot#	d-Spacing (nm)	Rec. Pos.(1/nm)	Degrees to Spot 1	Degrees to x-axis	Amplitude
1	0.3928	2.546	0	92.16	3364.67
2	0.3317	3.015	46.8	138.97	1090.06
3	0.3089	3.238	33.58	125.74	1368.68

Spot#	d-Spacing (nm)	Rec. Pos.(1/nm)	Degrees to Spot 1	Degrees to x-axis	Amplitude
4	0.2874	3.479	10.72	102.89	1011.31
5	0.2524	3.963	35.32	56.84	1372.83
6	0.2311	4.327	44.43	47.73	1433.02
7	0.2318	4.314	71.63	163.79	1109.78
8	0.2116	4.725	70.25	162.42	665.66
9	0.191	5.235	34.27	57.89	6725.28
10	0.1645	6.078	22.72	69.44	1754.82
11	0.1471	6.799	17.85	110.01	6359.89

3.2.3 Pharmacological Evaluation- Shankha Bhasma was prepared by two different methods which were then analyzed for its pharmacological activities. The acute toxicity studies were carried as per OECD 425 guidelines for fixing the dose and checking the toxicity of the formulations under study. The Bhasma preparations were found to be safe up to a maximum dose of 2000 mg/kg body weight in acute toxicity studies. Aspirin induced method was selected for antiulcer study. The control group, standard ranitidine treated and the formulation treated animals were sacrificed and the ulcer index were calculated. Shankha Bhasma demonstrated significant protection in Aspirin induced ulcers by ulcer index calculation ($p < 0.001$) (Table 10). The results suggest that this Ayurvedic preparation possess significant gastro protective activity in lower doses of the therapeutic range. Shankha Bhasma prepared by the Open method and Closed method were showed marked antiulcer activity. Open method has got reduced steps without adding any additional ingredients and finishes within a short time. While Method II (Closed method) has used two different adjuvants namely water and Kumari Swarasa (aloe vera juice) for the trituration and Puta (calcination). The thiobarbituric acid reacting substances (TBARS) in gastric tissue is determined to assess the free radical scavenging activity and lipid peroxidation. The reduction in TBARS value for the Bhasma formulation shows that there exists a free radical scavenging activity their by gastric mucosa protection.

Serum calcium level is also determined and it shows that there is no change in serum calcium level although there exists reduction in TBARS value and thereby antiperoxidative effect. The slightly increased activity produced by Closed method shankha bhasma may be attributed to the adjuvants added. [Prasanta Kumar Sarkar, *et.al.*, 2010]. The adjuvant used in closed method, Kumari swarasa itself has got wound healing activity which may be an added advantage for antiulcer effect.

The histopathological studies were done for toxicological purpose. The histopathological results in the figure below revealed the normal structure of cells of the stomach, liver and kidney and the structure of cells after administration of Shankha Bhasma. A detailed histopathological examination was carried out for the liver and kidney. The liver sections of the normal group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. Disarrangement of normal hepatic cells with cellular necrosis, Congested portal veins, vacuolization of cytoplasm and fatty degeneration was observed in Aspirin treated rats. Kidney Sections of the control group showed normal renal glomeruli, collecting tubules intestinal tissues and internal structures whereas aspirin treated rats showed infiltrated cellular structure and slightly congested blood vessels. The liver sections of the group 4 and 6 rats treated with SB (a) Method I and SB (b) method II at the dose of 25 mg/kg showed a sign of significant protection as it was evident by

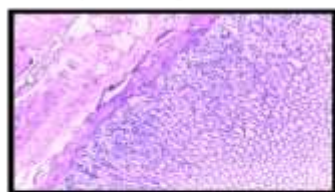
the low accumulation of fatty lobules, absence of necrosis and vacuoles in a dose dependent manner which is shown in Figure 12 below. Almost similar sign of protection was

shown in the liver sections of ranitidine at a dose of 50 mg/kg treated rat group.³²⁻³⁴

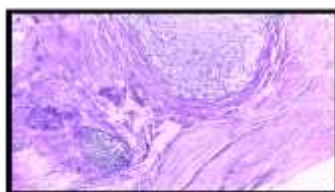
Table 10: Aspirin induced antiulcer study shankha for closed and open method

S.No	Groups	Dose (mg/kg)	Ulcer Index	SerumCalcium	TBARS (nmol/mg protein)
1	Control	1% CMC	55.80±.702	9.66±0.153	14.10±0.185
2	Ranitidine	20mg/kg	19.50±.363	10.43±0.199	9.91±0.028
3	Shankha test (open method)	25mg/kg	13.78±0.631	10.25±0.631*	8.80±0.401
4	Shankha test (openmethod)	50mg/kg	10.03±0.176	10.29±0.327*	8.29±0.344
5	Shankha test (closed method)	25mg/kg	14.08±0.135	10.65±0.221	9.83±0.370
6	Shankha test (closed method)	50mg/kg	10.16±0.158	10.81±0.3446	8.71±0.380

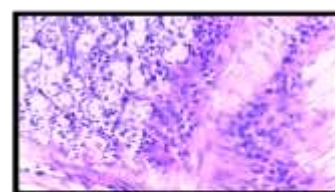
Histopathological sections of Stomach



Control



SB Method I

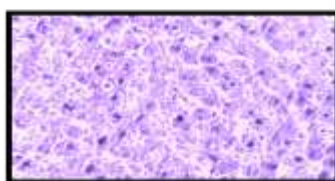


SB Method II

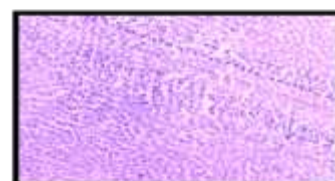
Histopathological sections of Liver



Control

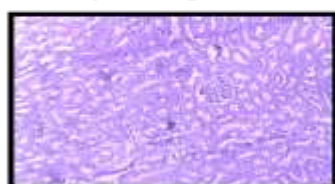


SB Method I

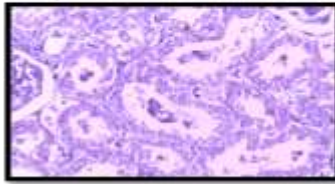


SB Method II

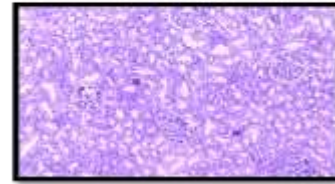
Histopathological sections of Kidney



Control



SB Method I



SB Method II

Figure 12: Histopathological images of Method I (Closed Method) and Method II(Open Method)

4 CONCLUSION

Shankha Bhasma, the herbomineral formulation prepared from the shell of marine organism contains calcium carbonate as the major component. The in process standardization for the formulation of Bhasma are done by adopting two methods that incorporates different adjuvants and which may influence, the particle size, efficacy and dose. The peculiar method of formulation technique involving Shodhana, Bhavana and Puta was used which reduced the particle size to Nano size. The method I involves heating directly, has the advantage of reduced steps without adding any additional ingredients and finishes within a short time. On the other hand, method II used two different adjuvants namely water and Kumari Swarasa (aloe vera juice) for the trituration and Puta (calcination). It was repeated four times. The physicochemical evaluation gives the absence of

carbonaceous matter and alkalinity of shankha bhasma. The evaluation parameters by AAS, XRD, TGA, SEM, TEM revealed that although the particle size and chemical constituents are almost same for two methods, the crystalline nature, surface morphology and pharmacological effect is comparatively good for method II when compared with method I. This effectiveness may be attributed to the adjuvant added and the repeated heating process. Cytotoxic studies revealed that the toxicity of this particular Shankha Bhasma prepared on stomach cell was nil and does not have any toxic effect in liver and kidney cell.

Shankha Bhasma has evaluated by WHO Guidelines and the quality control Evaluation has done for in process and for final product. A standardization parameter for the Shankha Bhasma has been established and the method can be included in the official monograph.

REFERENCES

- [1] Chaudhary A., Singh N. Herbo Mineral Formulations (Rasaoushadhies) of Ayurveda an Amazing Inheritance of Ayurvedic Pharmaceutics. *Anc Sci Life*. 2010;30:10.
- [2] Garg M., Das S., Singh G., Comparative physicochemical evaluation of a marketed herbomineral formulation: Naga bhasma. *Indian J Pharm Sc*. 2012.;74:6.
- [3] Chavan S., Tayade S., Gupta V. et al. Pharmaceutical Standardization and Physicochemical Characterization of Traditional Ayurvedic Marine Drug: Incinerated Conch Shell (Shankha Bhasma). *Multidiscip Digit Publ Inst*. 2018.;16:11.
- [4] Nadkarni KM. *Indian Materia Medica*. New Delhi: Popular Prakashan; 1996.
- [5] Shah NC., Vaidya G., *Bharat Bhaishajya Ratnakar*: Jain Publishers Pvt. Ltd; 1958.
- [6] Sarkar P., Chaudhary A. Role of Milk in Shodhan (Detoxification) with special reference to Nux Vomica., *Aryavaidyan*; 2007.
- [7] Sharma S. *Rasa Tarangini (Ayurveda Pharmaceutics and Indian Alchemy)*. Chaukhamba Surbharati Prakashan; 1979.
- [8] NCHS. National Center for Health Statistics. Centers for disease control and prevention.
- [9] Rasheed S., Shivashankar M. Evaluation of Herbo Mineral Formulations (Bhasma): An Overview. *Int J Res Ayurveda Pharm*. 2015;6:3.
- [10] Krishnamachary B., Purushothaman AK., Pemiah B., et al., Bhanupaka: A Green Process in the Preparation of an Indian Ayurvedic Medicine. Lauha Bhasma. *J Chem*. 2013.
- [11] Dhiman S., Upadhyaya A., Sharma N., et al. Comparative study of kumari swarasa and kanyasara in management of kashtartava. *Anc Sci Life*. 2012;32;1.
- [12] Awate S., Babiuk L.A. and Mutwiri G. Mechanisms of Action of Adjuvants. *Front Immunol*. 2013;4.
- [13] Parmar D.K., Patgiri B.J and Prajapati P.K. Standardization of Gaja Puta and Ardha Gaja Puta in the preparation of Vanga Bhasma. *Ayu*. 2010.;31:4.
- [14] Pandit S., Biswas T., Debnath P. et al., Chemical and pharmacological evaluation of different ayurvedic preparations of iron. *J Ethnopharmacol*. 1999;65;2.
- [15] Bhargava V V., Saluja A K., Dholwani K. K. Detection of Heavy Metal Contents and Proximate Analysis of roots of *Anogeissus latifolia*. *J Pharmacogn Phytochem*. 2013;1:6.

- [16] Manallack D T., Pranker R J., Yuriev E. et al. The Significance of Acid/Base Properties in Drug Discovery. *Natl Cent Biotechnol Inf.* 2014;42:2.
- [17] Atomic Absorption Spectrometer. IIT Kanpur. https://www.iitk.ac.in/dordold/index.php?option=com_content&view=category&layout=blog&id=218&Itemid=237. Published 2019.
- [18] Dutrow BL, Clark CM. X-ray Powder Diffraction (XRD). Geochemical Instrumentation and Analysis. https://serc.carleton.edu/research_education/geochemsheets/techniques/XRD.html. Published 2019
- [19] Tan YH, Davis JA., Stine K.J, et al. Surface area and pore size characteristics of nanoporous gold subjected to thermal, mechanical, or surface modification studied using gas adsorption isotherms, cyclic voltammetry, thermogravimetric analysis, and scanning electron microscopy. *J Mater Chem.* 2012.;22:14.
- [20] Mukkavall S., Chalivendra V. and Singh BR. Physicochemical analysis of herbally prepared silver nanoparticles and its potential as a drug bioenhancer. *OpenNano.* 2017; 2.
- [21] Elahi A., Jairajpuri D.S. and Khan F. Characterization of Calcined Jade and its immunomodulatory effect on macrophage isolated from Swiss albino mice. *J Tradit Complement Med.* 2017;7:4.
- [22] Sharma R., Bhatt A. and Thakur M. Physicochemical characterization and antibacterial activity of Rajata Bhasma and silver nanoparticle. *Ayu.* 2016; 37:1.
- [23] Singh R., Kumar S., Aman A., et al. Study on physical properties of Ayurvedic nanocrystalline Tamra Bhasma by employing modern scientific tools. *J Ayurveda Integr Med.* 2019.;10:2.
- [24] Pandit S., Sur T K., Jana U. et al. Anti-Ulcer Effect Of Shankha Bhasma In Rats : A Preliminary Study. *Indian J Pharmacol.* 2000;32.
- [25] Sur TK., Pandit S., Sen S., et al. Anti-ulcer activity of Shankha Bhasma (calcined conch-shell). *Explor Anim Med Res.* 2013;3:1.
- [26] Saha S., Goswami G. Study of anti-ulcer activity of Ficus religiosa L. on experimentally induced gastric ulcers in rats. *Asian Pac J Trop Med.* 2010;3:10.
- [27] Rai R.K., Jha C. et al. Assessment of antihyperlipidaemic action of Tamra Bhasma. *Indian J Tradit Knowl.* 2008;7:2.
- [28] Rasheed A., Naik M., Pillanayil K., et al. Formulation, characterization and comparative evaluation of Trivangabhasma: A herbo-mineral Indian traditional medicine. *Pak J Pharm Sci.* 2014;27:4.
- [29] Mandi S., Reddy B., Belapu V. et al. Comparative Pharmaceutical and Analytical Study of Badarashma Bhasma and Pishti. *J Ayurvedic Herb Med.* 2016.;4:1.
- [30] Verma A., Bedarakar P., Galib BJP et al. Standardization of Naga Bhasma prepared by two different Bhavana Dravya. *J Phytopharm.* 2016.;5:5.
- [31] Kzar H., Al-Gazally E., Wtw A. Everolimus loaded NPs with FOL targeting: preparation, characterization and study of its cytotoxicity action on MCF-7 breast cancer cell lines. *Jordan j. pharm. sci.* 2022; 15: 1.
- [32] Osanloo, M., Yousefpoor, Y., Alipanah, H., et al. In-vitro Assessment of Essential Oils as Anticancer Therapeutic Agents: A Systematic Literature Review. *Jordan j. pharm. sci.* 2022; 15:2.173–203. <https://doi.org/10.35516/jjps.v15i2.319>
- [33] Al-Awar A. Acute and Sub-Acute Oral Toxicity Assessment of Mixed Extract of Trigonella Foenum-Graecum Seeds and Withania Somnifera Root in Rats. *Jordan j. pharm. sci.* 2022;15; 4.
- [34] Hossain E., Aziz A., Vabna N. et al. Phytochemical Screening and Pharmacological Evaluation of the Methanolic Extract of Cissus Elongata Roxb. Leaves. *Jordan j. pharm. sci.* 2022; 15:4.

في عملية تحديد ملامح صياغة هيربومينرال من أصل بحري

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ملخص

يستخدم النظام الأيورفيدا مشنقات نباتية وحيوانية لقيمتها الطبية. وجود الآثار الجانبية للأدوية المتاحة لقرحة المعدة أدى لتعزيز وزيادة الميول لإستخدام الطرق التقليدية للعلاج .

الهدف: لإعداد شانخا بهاسما (Shanka Bhasma) ، وهي صياغة مضادة للقرحة من أصل بحري بواسطة طريقتين مختلفتين ومقارنتها بالأدوات العلمية الحديثة.

الأساليب: تم إعداد شانخا بهاسما من قوقعة المحارة بطرق مفتوحة ومغلقة باستخدام المواد المكملة (adjuvants) مثل كوماري سواراسا (Kumari Swarasa) والماء وتعرض للتوصيف بالطريقة التقليدية و AAS و XRD و EDAX و SEM و TEM و BET و TGA والتحليل الطيفي IR. تم تحقق الفعالية من خلال الدراسات الحيوانية، وتحقق السّميّة بواسطة الدراسات الهستوباثولوجيا والسّميّة الخلويّة.

النتائج: الأسلوب المفتوح يأخذ خطوات قليلة وأقل من الوقت ويظهر الطبيعة البلورية TGA. (XRD) يظهر درجة الحرارة التحلل من أكسيد الكالسيوم. الأسلوب المغلق ينتج جسيمات أكثر سلاسة (SEM) ، (TEM) و (BET) مع عدم وجود مواد عضوية (IR) ، مع عناصر ضمن الحدود المنصوص عليها (AAS) من الأيورفيدا. وقد أظهرت فعالية المواد المكملة (adjuvants) من خلال توحيد العمليات وحصل على التأثير الدوائي الكافي دون أي تأثير سام.

الاستنتاج: الحجم والسطح الأكثر سلاسة والفعالية العلاجية لهيربومينرال (herbominerals) التي أعدتها من خلال الطريقتان يمكن أن يعزى إلى خطوات التكليس و المواد المكملة. وبالتالي يمكن تحقّق خفض الجرعة و تأثير سريع.

الكلمات الدالة: شانخا بهاسما، هيربومينرال، التوحيد القياسي، الأيورفيدا، التكليس.

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