Preparation and Standardization of Egg Shell Bhasma

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Abstract **- Egg shell bhasma was prepared by using eggshell as a raw material. It is the rich source of calcium and shows more bioavailability along with maximum therapeutic effect with minimum side effects as compared to synthetic calcium sources. Egg shell bhasma was prepared by using standard classical methods. Egg shell bhasma was analysed by ancient methods like nirgandh, Verna, varitwaratva Rekhapurnatva, unama and some advanced methods of analysis like X-Ray Diffraction, FTIR, Namburi Phased Spot Test etc. Amount of calcium present in it was determined by using EDTA titration.**

Index Terms **- Egg shell, standardization, bhasma, calcium.**

INTRODUCTION

Ayurveda is one of the leading and popular traditional Indian systems of medicine. Bhasma is unique formulation belonging to Ayurveda. This group of medicines can work even in smaller dose as well as controls incurable diseases effectively. Products obtained from mineral metals, are supposed to be unfavourable to human body. But it is very shocking to know that in rasa-shasatra text the side effects are previously mentioned if we utilize these medicines if they are not prepared appropriately. Most of animal derivatives such as feathers, horns, shells, metallic and non-metallic minerals are normally administered in the form of bhasma.

Egg shell bhasma [4-8]

Egg shell is converted to their bhasma forms which reduces side effects of crude shell and convert them into fine powder which increases absorption of that particular drug. Egg shell bhasma is not only rich in calcium but also contains other trace minerals which promotes absorption. It is the natural novel dietary supplement contains richest source of calcium, proteins and elements like strontium, magnesium, selenium and fluorine etc. Egg shell bhasma shows positive effect in increasing bone mineral density and stimulates chondrocyte differentiation and cartilage growth. Assimilation of Egg shell bhasma which is not present in synthetic calcium supplements. It is used for the treatment of diseases like leucorrhoea i.e. vaginal white discharge, menorrhagia, gonorrhoea, diabetes mellitus, urinary tract infection, mental disorder. It also has the properties like rasayana i.e. immunomodulation and balya i.e. strength. Egg shell bhasma is used for the treatment of some health conditions disorders related to muscle, one and joints like low mineral density, osteoporosis, osteopenia, osteomalacia, osteoarthritis, low backache (in case of women), vaginitis, and frequent urination. It is also utilise in the treatment of hair care by promoting hair growth and delays hair fall.

Synonym: - In Ayurveda hen's eggshell is also known as kukkutanda tvak, kukkutanda tvak bhasma, kukkutanda dala, shvetanda bhasma, dakshanda tvak, niyodha, dahar, yamnadi etc.

Class: - Sudhaverga.

MATERIALS AND METHODS

Hens' eggshell, aloe vera juice was obtained from our botanical garden, citric acid was purchased from Meher chemie B203, Mumbai-67

PREPARATION OF EGG SHELL BHASMA [9-12]

Egg shell bhasma is prepared by using following methods

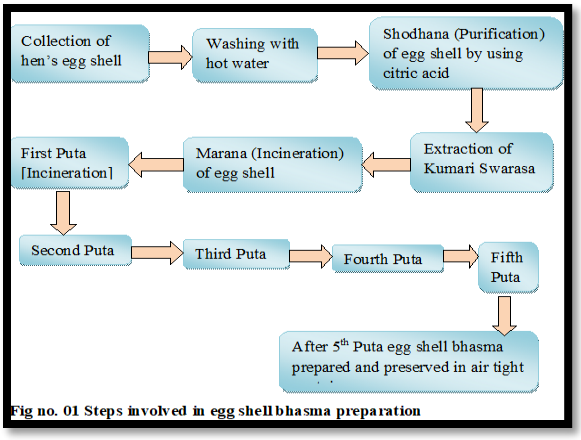

Fig no. 01 Steps involved in egg shell bhasma preparation

STANDARDISATION OF EGG SHELL BHASMA [13- 20]

Standardisation of eggshell bhasma was carried out for identification as well as determination of its quality and purity. Standardisation also determines the nature of adulteration along with safety and efficacy. Standardisation of eggshell bhasma was carried out by using both Ayurvedic and advanced methods of analysis.

Ancient Ayurvedic methods of analysis

I. Physical characteristics of bhasma

a) Organoleptic characters: Colour, Taste, Odour, Touch, Appearance.

b) Solubility test: - The sample was subjected for solubility test with following solvents Distilled water, Methanol, Chloroform, Propylene glycol, Ethyl alcohol, 0.1 N HCL.

1. Verna: - Colour of the preparation is depending on parent material. Any changes in specific colour suggest that bhasma is not prepared properly.

2. Nisvadutam: - This test is used for the detection of taste. A pinch of bhasma is placed on the tongue and it taste should perceive to be tasteless.

3. Nirgandha: - This test was used to determine the odour or smell of the bhasma.

4. Nishchandratvam:- Bhasma must be nishchandandra i.e. lustreless before therapeutic application.

5. Rekhapurnatva:- Particles of bhasma should be of minimum size because decreases particle size, surface area increases and ultimately absorption and assimilation in the body also increases. It was used to study the particle size of bhasma.

6. Varitaratva :- Bhasma should be light and fine. This test is totally based upon the law of surface tension. Properly incinerated bhasma will float on surface of water which states that prepared bhasma is light and fine.

7. Unama test: - It is somewhat same to varitaratva test. If the grains remain on the surface, than bhasma can be considered as excellent.

8. Slakshnatvam: - This test is carried out to study tactile sensation produced by bhasma by simple touch with fingers tips.

II. Chemical characteristics

1. Apurnabhavavta: - Apurnabhavta means incapability to retain its original metallic form. Lusterous particles in it show presence of free metals which is inactive after incineration.

2. Amla pariksha: - A pinch of prepared bhasma was mixed with little amount of dahi (curd) in clean and dry Petridish and is observed for any colour change. No colour change of dahi should be observed. The same colour of lemon juice taken in test tube and the same results should be observed.

B) Advanced methods for analysis of bhasma

I) Physicochemical test

1) Determination of pH value of the sample

pH meter was calibrated by using standard buffer of known pH- 4.0 and 9.2 at 30°C. The reference electrode was thoroughly washed with distilled water every time. Water was drained by using filter paper. 1% egg shell bhasma (1 gm of egg shell bhasma add 10 ml of distilled water) was prepared then tip of electrode was dipped and reading was recorded.

2. Loss on drying

Gravimetric method: - loss on drying was calculated by using following formula,

$$\text{Loss on drying (\%)} = \frac{\text{Initial wt of sample} - \text{wt of sample after drying} \times 100}{\text{Initial weight of sample}}$$

3. Determination of Total ash value

It is used to determine quality and purity of crude drug and to establish the identity. Some inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, calcium, etc are present in ash. These are present in definite amount in a particular crude drug hence quantitative determination in terms of various ash values helps in their standardisation. The percentage of total ash was calculated as follows,

Total Ash value = W2 – W1

% of total ash = W2 – W1 ×100

Where, W1= Wt. of empty crucible.

W2= Wt. of crucible + ash

4. Determination of acid insoluble ash

Total ash obtained above was treated with 25 ml of dilute hydrochloric acid and boiled. Ash less filter paper was used to collect insoluble matter. The residue was repeatedly washed with hot water, dried well, ignited in electric burner, cooled and weighed. The percentage of acid insoluble ash was calculated as follows

Acid insoluble ash = (W2 – W1) ×100

Where, W1 = Wt. of empty crucible

W2 = Wt. of crucible + acid insoluble ash

5. Determination of Water soluble ash

Total ash obtained was boiled with 25 ml of distilled water and filtered through ash less filter paper. The residue was washed with hot water and dried well, ignited on burner in a crucible, once again converted into ash and weight was noted (i.e. water insoluble ash). The weight of water-soluble ash was calculated by subtracting the weight of water insoluble ash from the weight of total ash.

Water soluble ash = total ash – W3

% of water soluble ash = (total ash – W3) ×100

% of water insoluble ash = W3 × 100

Where, W1 = Wt. of empty crucible

W2 = Wt. of crucible + water insoluble ash

W3 = Wt. of water insoluble ash = W2 - W1

W4 = Wt. of water soluble ash = total ash – W3

Analytical Methods

1) X- Ray Diffraction study (XRD)

XRD is a technique in which the special arrangement of structural units of a substance in the crystalline state is known the distance between each set of atomic planes is determined with the help of wavelength of X-ray beam and angle of diffraction applying Bragg's law. Results show the characterization of the crystallographic structure and heterogeneous solid mixture. Egg shell bhasma was analyzed by XRD.

2) Infrared Spectrum:- Infrared spectrum is the technique based upon the simple fact that the substance shows marked selective absorption in the infrared region. After absorption of IR radiation, the molecules of the chemical substance vibrate at many rates of vibration, giving rise to close- packed absorption bands, called as IR spectrum which extends over a wide wavelength range. IR spectrum shows various bands which will correspond to the characteristic functional groups and bonds present in the chemical substance. This help to establish the structure of unknown compound as well as analysis of functional group. The sample of eggshell bhasma was analysed between 4000- 600 cm^{-1}.

3) Namburi Phased Spot Test (NPST)

The bhasma solution was prepared by adding 1 gm of eggshell bhasma in 10 ml of distilled water. Then one drop of clear solution of sample was examined by putting it on reacting haridra paper using dropper. Immediately characteristic spot begins to form and changes continuously for some time. During this time the colour and the pattern of spot at three different phases was studied.

First phase: - 0 to 5 min, Second phase: - 5 min to 20 min, Third phase: - 20 min to 1 day.

RESULT AND DISCUSSION

Physicochemical characterisation of eggshell bhasma according to ancient Ayurvedic methods

9.1.1 Physical characterisation of drug

a) Organoleptic characters:

Colour: - White

Taste: - Tasteless

Odour: - Odourless

Touch: - Soft and fine

Appearance: - Amorphous powder.

b) Solubility study: -

The sample was subjected for solubility test with following solvents

Table no. 01 Solubility study of eggshell bhasma in different solvents

Sr. No	Solvents	Results
1	Distilled water	Sparingly soluble
2	Acetic acid	Soluble
3	Chloroform	Insoluble
4	Propylene glycol	Insoluble
5	Ethyl alcohol	Insoluble
6	0.1 N HCL	Soluble

Table no. 02 Ayurvediya parikshan of eggshell bhasma

Sr. No	Parameter	Observation	Results
1	Verna	White	Positive
2	Nisvadutam	Tasteless	Positive
3	Nirgandha	No smell	Positive
4	Nishchandratvam	Lustreless	Positive
5	Rekhapurnatva	Some portion of eggshell bhasma remain in	Positive

		crevices of figures	
6	Varitaratva	Egg shell bhasma float on water surface	Positive
7	Unama test	Rise grains sink in water	Negative
8	Slakshnatvam	tactile sensation produced	Positive

Table no.03. Chemical characterisation of eggshell bhasma

Sr. No	Parameter	Observation	Results
1	Apurnabhavavta	Non lusterous particle	Free from free metal
2	Amla pariksha	No colour change	Positive

Table no.04. Physicochemical test according to advanced method of analysis

Sr. No	Parameter	Results
1	pH	9
2	Loss on drying	0.89%
3	Total ash value	11.56%
4	Acid insoluble ash	5.4%
5	Water soluble ash	4.61 %

Table no. 05 Qualitative analysis of egg shell bhasma

Sr. No	Test	Observation	Results
1	Bhasma solution + ammonium carbonate solution, boil and cool	White precipitate	Calcium is present
2	Bhasma solution+ dilute HCL acid, liberating Co_2	White precipitate produced in calcium hydroxide solution	Calcium carbonate is present.

Table no. 06 Identification test for calcium carbonate

Sr. No	Test	Observation	Results
1	For calcium Reaction A	white crystalline precipitates	Calcium present
2	Reaction B	white precipitate	Calcium present
3	For calcium carbonate	white precipitate	Calcium carbonate is present.
4	Flame test	Blue/ orange coloured flame	Calcium carbonate present.

9.1.2 Determination of the percentage of calcium present in eggshell bhasma

Firstly solution of egg shell bhasma was prepared by using hydrochloric acid. Then 25 ml of these solutions was taken and then pH was adjusted to 7 by using sodium hydroxide solution and add 10 ml of 8.5 M NH_3- NH_4Cl buffer to adjust the solution to pH 10. 2-3 drop of EBT i.e. Erichrome Black T indicator was added to the Erlenmeyer flask and titrates with 0.01 M EDTA, Perform this analysis in triplicate.

Percentage of calcium present in eggshell bhasma was found to be 94.21 %.

Fig no.02 Observation of titration.

9.2 Analytical Methods

9.2.1 X- Ray Diffraction study (XRD)

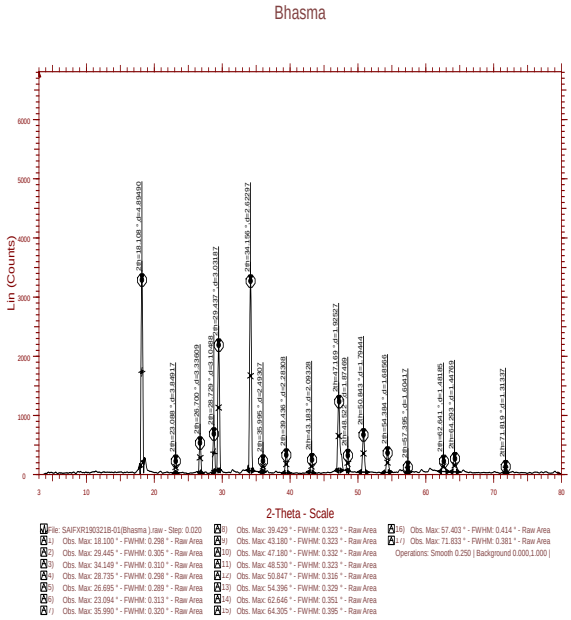

Fig no. 03 XRD peak of eggshell bhasma

Egg shell bhasma was prepared by using eggshell as raw material and this bhasma was identified by matching d- spacing obtained after XRD study. Diffraction pattern indicates calcite as the major

crystalline phase present in eggshell bhasma. Standard calcite was also analysed with XRD and diffraction pattern as peaks at d= 3.0488 Aº (2Ɵ= 29.437), d=2.283 Aº (2Ɵ=39.43), d=1.92Aº (2Ɵ=47.16), d=1.87Aº (2Ɵ=48.62), d=1.44Aº (2Ɵ=64.29) confirms the presence of calcite as major crystalline phase in sample. Lines with low intensity indicates presence of calcium hydroxide at d=2.62Aº (2Ɵ=34.15), d=4.89Aº (2Ɵ=18.10), d=1.92Aº (2Ɵ=47.16). This may be attributed to the hydrolysis of calcium oxide formed due to partial decomposition of calcite during calcinations cycles the mean crystal size of eggshell bhasma particles was calculated from XRD pattern (2Ɵ for 100 % intensity peaks) by following Scherer's Equation. The mean crystal size of eggshell bhasma was 40.03nm. Whereas mean crystal size of standard calcite was 39.80 nm. Egg shell bhasma particle have smaller crystal size as that of standard calcite.

9.2.2 FTIR Spectrum

FTIR study was done by using Perkin Elmer IR Series model no 21 spectrometers. Some peaks found in FTIR study of eggshell bhasma are as follows,

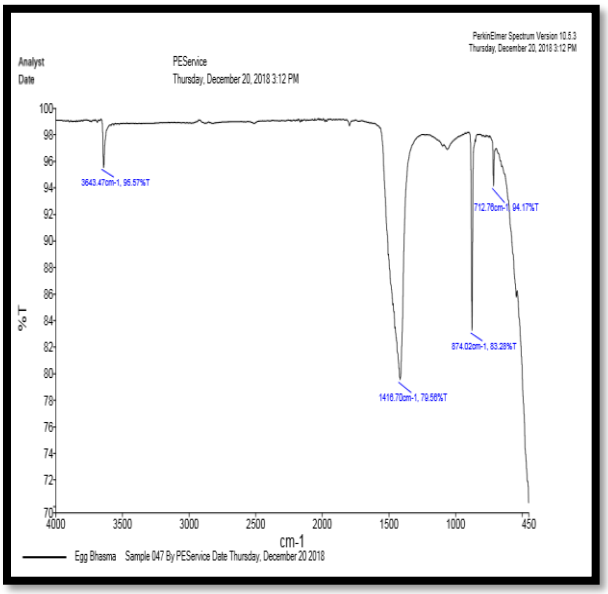

Fig No. 04 FTIR Spectrum of eggshell bhasma.

Table no. 07 Interpretation of FTIR study of eggshell bhasma.

Sr. No.	Assigned Functional group	Standard peak cm^{-1}	Observed peak cm^{-1}
1	OH stretching vibration	3643 – 3439.01	3643
2	Ca	1500 - 1400	1416
3	Carbonate ion	875.56	874.02
4	C- H stretching	860- 680	712

9.2.3 Namburi Phased Spot Test (NPST)

Table no. 08 Observation of NPST

Sr. No	Criteria	Egg shell bhasma	
1	Changes on heating	Liberation of fumes	Not present
		Odour	Not present
2	Changes on wetting	Solvent	Distilled water
		Colour of solution	Grayish
		Exothermic reaction	Not present
		Endothermic reaction	Not present
		Absorption	More in water
		Settling time	Slow
3	Colour pattern of first phase (after 5 min)	Orange	
4	Colour pattern of second phase (after 20 min)	Pinkish	
5	Colour pattern of third phase (after one day)	Pink	

CONCLUSION

Most of animal derivatives such as feathers, horns, shells, metallic and non-metallic minerals are used as medicines but if these are not used in the proper form they show side effects. According to rasashatra they are normally administered in the form of bhasma to reduces their side effects and convert them to nontoxic form. Egg shell is the rich source of calcium and converted to suitable form of administration in the form of bhasma. Standardisation of eggshell was carried out to determine quality, purity as well as safety and efficacy of eggshell. It is also used to determine the nature of adulteration. From the research work it is concluded that eggshell in the form of bhasma was safe and nontoxic form of drug and safe form of administration.

ACKNOWLEDGEMENTS

Authors are thankful to Channabasweshwar Pharmacy College, Latur, Late Babruwan Vitthalrao Kale Ayurved Medical College and Hospital, Latur and Shivlingeshwar College of Pharmacy for providing necessary facility to carry out this research work.

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