

## ANALYTICAL STUDY OF *KUKKUTANDTWAK BHASMA*

Meenakshi Sharma<sup>1</sup>, Gaurav Garg<sup>2</sup>

<sup>1,2</sup>Ayurvedic Medical officer, Haryana

Email: [dr.mani2008@gmail.com](mailto:dr.mani2008@gmail.com)

### ABSTRACT

*Kukkutandtwak bhasma* is incinerated powder of hens egg shell, a rich source of calcium derived from animal origin finds place in *sudha varga* of Ayurveda texts. Ayurved has richest and most exhaustive compendium of formulations. Notably for a single calcium, it prescribes around eight sources used according to nature of disease and condition of patient. Amongst them *kukkutandtwak bhasma* was selected owing its easy availability, abundant resources and free of cost supply. Therapeutically it is indicated mainly in *shukra vikar* and *shweta pradar*<sup>1</sup>. Bhasma was prepared as per classics and detailed analytical study was carried out with the help of fine sophisticated instrumentation techniques like X-ray diffraction, F.T.I.R, I.C.P spectrometry and particle size analysis to reveal complex structure, chemical bonding and any heavy metal contamination.

**Keywords:** *kukkutandtwak bhasma*, *sudha varga*, *shukra vicar*, *shweta pradar*

### INTRODUCTION

Human inquisitiveness has unleashed many hidden truths. The classical methods for *Bhasma* preparation have been modified now due to advent of increased commercialization of the Ayurvedic pharmacies, unavailability of sufficient cow-dung cakes, Use of newer instruments and equipments, lack of identification of authentic raw material and majorly due to the lack of S.O.Ps. Further due to the raised doubts on the safety of Mineral based preparation, serious concerns are expressed about the presence of Heavy Metals, about the particle size of *Bhasma*, thus the Physico-chemical study was conducted on Modern Scientific parameters by using Sophisticated Instrumentation techniques. Thus present study was conducted on animal origin based calcium preparation *kukkutandtwak bhasma*.

### AIM AND OBJECTIVES

- To perform *shodhan* and *maaran* of *kukkutandtwak*.
- To analyze the Physico-chemical properties of *kukkutandtwak bhasma* for knowing the structure, elements present and particle size.

### MATERIALS AND METHODS

Egg shells i.e. *Kukkutand twak* were procured from a local dairy and subjected to *shodhan* and *maaran* in accordance with classical reference as mentioned in schedule 1 of drugs and cosmetics act.<sup>2</sup>

*Kukkutand twak Shodhan*<sup>3</sup>

**Reference:** R.T.S.S.P.S Vol 1

**Ingredients:** *Kukkutandtwak* 800g *Saindhav*  
*Lavana* 1/8<sup>th</sup> :100g  
*Navsacara* 1/8<sup>th</sup>:100g

**Apparatus Required:** Stainless steel Containers,  
L.P.G Stove, Match Box, Cloth

#### Procedure

800 g *Kukkutandtwak Twak* was washed with warm water and was dipped in 10 Litres water containing 100g *Saindhava lavana* and 100g *Navsadar* for 6 days. Thereafter they were taken out, washed and egg membranes were separated carefully.

#### Observations:

*Kukkutandtwak Twak* was free from visible impurities

Weight: 780g.

Color: pure white.

#### Result:

End product obtained: *Purified Kukkutandtwak Twak*

*Kukkutandtwak Marana* <sup>4</sup>

**Reference** : R.T.S.S.P.S. vol2

**Ingredients** : *Shudh Kukkutandtwak Twak* 800 g  
: *Neembu* 8kg {Average juice extracted per kg lemon 400ml

: Purified *Hingul* 100g

#### Procedure

Purified *Kukkutandtwak Twak* was grounded in Pestle and 100 g. *Shudh Hingul* was added to *jarit Vang* and was triturated with *neembu swaras* Q.S in Pestle for six hours, till it dried up. The semisolid paste was spread over ghee smeared tray and cut into cubes using knife and kept for drying. These cakes were placed uniformly in earthen plate and were covered with another inverted kept earthen plate and the margins were sealed with the help of *Gachani mitti* smeared wet piece of cloth and again allowed to dry. As the plates dried they were subjected to *Gajaputa*. After cooling plates with the material were removed carefully from the pit of *Gajaputa* and the observations were recorded.

**Table 1:** Observations during Marana of *Kukkutand Twak Bhasma*

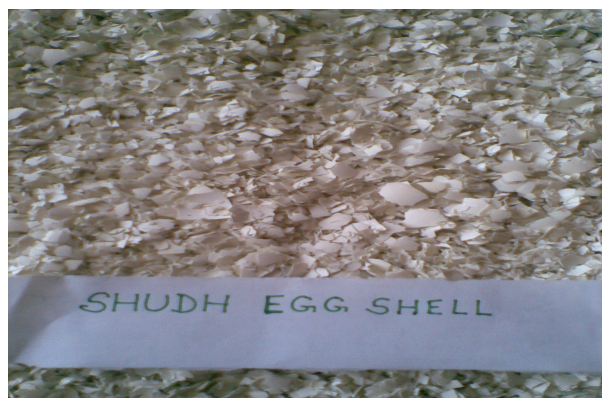
No. of <i>Putra</i>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
Wt. of <i>Hingul</i> added	100g	(-)	(-)	(-)	(-)
Wt. of lemon juice added	800ml	650ml	600ml	550ml	550ml
Duration of trituration	6hrs	6hrs	6hrs	6hrs	6hrs
Wt. of cakes after the <i>Putra</i> (g)	730	720	710	700	700
Amount of cow dung used	30kg	30kg	30kg	30kg	30kg
Color of material	50% cakes white, rest dark grey	70% cakes white, rest grayish	80% whitish	90% whitish	95% whitish
<i>Varitaratva</i> Test	Negative	Positive 95%	Positive 99%	Positive 100%	Positive 100%
<i>Rekhapurnata</i> test	Negative	Positive	Positive	Positive	Positive

#### Result

Final product obtained: *Kukkutandtwak bhasma*

Wt. of final product: 700g

Color of final product: Whitish grayish



## RESULTS AND DISCUSSION

Whitish grey coloured *kukkutand twak bhasma* weighing 700g was prepared.

### Organoleptic Parameters of Assessment

- Varna/Colour - grayish
  - Rasa/taste - tasteless
  - Sparsh/Touch - Soft
  - Gandha/Odour - Odourless
- *Varitaratva*<sup>5</sup>: When the *Bhasma* is steadily sprinkled over steady surface of water, it floats over it indicating the fineness that the combined force created due to its weight and gravitation is less than the surface tension of water. Picture couldn't be found
- *Rekhapooranta*<sup>6</sup>: When rubbed in between the index finger and thumb the pattern of ridges on the fingertips can be clearly seen, again indicative of fineness of particle size of *Bhasma*.  
Statement deleted

### MODERN PARAMETERS

The following Sophisticated Instrumental methods of analysis were selected:

- 1) Fourier Transform Infrared Spectrometry (FT-IR)

### Result and Interpretation

Functional class and Assignment at various vibrations and stretching

Table 1

Stretching/Bending	Criteria	Expected Bond/Functional Group
2848.70,2922.32	2800-3300cm <sup>-1</sup>	C-H stretching
2922.32	2850-2960cm <sup>-1</sup>	Saturated Acyclic Hydrocarbon

- 2) X-Ray Diffraction
  - 3) Particle Size Analysis
  - 4) Inductively Coupled Plasma Spectrometry (I.C.P.)
1. **Fourier Transform Infra Red Spectrometry (F.T.I.R.)**<sup>7</sup>

FTIR relies on the fact that most of the molecules absorb light in the infrared region of the electromagnetic spectrum and this absorption corresponds specifically to the bonds present in the molecule. With infrared spectrometers absorption spectra of compounds is obtained that are a unique reflection of their molecular structure. The resulting spectrum represents the molecular absorption & transmission, creating a Molecular fingerprint of the sample Fourier Transmission is a mathematical technique performed by the computers for decoding and analyzing the frequency spectrum. The most commonly used region of IR spectrum in organic chemistry is the region corresponding to 4000-400cm<sup>-1</sup> FTIR offers quantitative and qualitative analysis for organic and inorganic samples. FTIR detects functional groups and characterizes covalent bonding information.

1144.70,1116.33	1000-1280cm-1	C-O, Acid,Ether,Alcohol,Ester
2848.70	2720-2820cm-1	CH=O,Aldehyde
3424.52	3300-3500cm-1	N-H,Amide,Amine
34.24	3350-3700cm-1	O-H stretching
957.83	800-1200cm-1	C-C,Alkanes

Inferences were made with FTIR study that the end compound contains the stretching/bending range of various organic functional groups ranging from, 544.12 to 3424.52 in *bhasma* sample indicating conversion of inorganic calcium in crude *kukkutandtwak* to organic calcium in its *bhasma* due to processing techniques, making its bioavailability higher.

## 2. X-Ray Diffraction

X-Ray scattering techniques are non-destructive analytical techniques which reveal information about the crystallographic structure, chemical composition, and physical properties of materials and thin film. Powder X-RD is used to identify any new chemical transformation occurred after *Shodhana* process. Powder X-RD (X-Ray Diffraction) is perhaps the most widely used X-Ray diffraction technique for characterizing materials. The data usually includes mineral (common) name of the substance, chemical formula, crystalline system, and reference pattern number from the ICDD International database. Every crystalline substance scatters the X-rays in its own

unique diffraction pattern and produces a finger print of its atomic and molecular structure.

As per comparison with ICDD International database, Calcium oxide was found the major phase in *Kukkutand twak bhasma*. It may be due to repeated heating, quenching and further incinerating the material and exposing it to oxidation.

## 3. Particle Size Analysis.

The purpose of particle size analysis is to obtain quantitative data on the mean size, particle size distribution (PSD) and shape of the compounds to be used in pharmaceutical formulation. The particle size analysis is also required to assure the quality of the final dosage forms and drug delivery systems. Laser Diffraction is a preferred standard method for particle sizing in the pharmaceutical industry, due to its short analytical time, robustness, high precision, reproducibility, wide measurement range and flexibility of operation using liquid, spray and dry dispersion attachments & it gives Effective Measuring Range : 0.5–1,000µm & Most Representative PSD is Volume-weighted.

**Table 3:** Particle Size Distribution

S. No.	% Below	Size (In µm)	Volumetric Mean Diameter (In µm)
1	10	9.01	41.7
2	16	12.29	
3	50	33.24	
4	84	74.43	
5	90	88.21	
6	99	134.16	

## Result and Interpretation

PSA report showed that the particle size of *Kukkutand twaka* was 9.01 µm at x10 and 134.16 µm at x99 respectively. Size at 99 x indicates 99% particles of *Bhasma* were below that size. . The volumetric mean diameter was 41.7µm. PSA result des-

ignates that the small particle size can be achieved by proper levigation, trituration and incineration.

## 4. Inductively Coupled Plasma Spectrometry (I.C.P.)

It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms

and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The sample, which must be in a liquid form, is pumped at 1 mL/min into a nebulizer, where it is converted into a fine aerosol with argon gas at about 1 L/min. The fine droplets of the aerosol, which represent only 1 - 2% of the sample, are separated from larger droplets using a spray chamber. The fine aerosol then emerges from the exit tube of the spray chamber and is transported into the plasma torch via a

sample injector. The plasma torch is used to generate positively charged ions rather than photons. An ion detector converts the ions into an electrical signal. This electronic signal is then processed by the data handling system and converted into analyte concentration using ICP-MS calibration standards. Samples are decomposed to neutral elements in high temperature argon plasma and analyzed based on their mass to charge ratios.

**Table 4:** Results and interpretation

Sample ID	Element	Wavelength	Instrument Detection Limit (mg/L)	Sample Results (mg/kg)
Sample	Mercury	253.62	.0610	102.36
	Lead	220.353	.0420	Not detected
	Tin	189.927	-	Not detected

In Kukkutand twaka *Bhasma*, 102.36 mg/Kg mercury was detected. The reason behind was addition of 100g Hingula in first *puta*.

## CONCLUSION

The purpose of *Shodhana* and *Marana* is to reduce the particle size and to convert inorganic metals and minerals into organic vital form which is evident in the light of modern sophisticated instrumentation techniques. *Kukkutand twak Bhasma* is odourless, tasteless, *Varitar*, *Rekhapurna* and without any *Chandrika*. F.T.I.R revealed C-H, O-H, N-H, C-O, C=HO aldehyde linkage, presence of saturated acyclic hydro carbon and alkane nature. Particle size analysis revealed *Bhasma* having 50% particle of size 33.24 $\mu$ m with VMD 41.7 $\mu$ m. Mercury was 102.36mg/kg and Tin and lead were below detection limits. This study established the fact that *bhasmas* prepared with classical methods always yield best results in terms of safety.

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