

# Analytical profile of *Kukkutanda Tvak Bhasma* (incinerated hen egg shells) prepared by two different methods

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## Abstract

**Background:** *Kukkutanda Tvak Bhasma* (KTB) (incinerated hen egg shells) is one of the important calcium-rich medicines used to treat leukorrhea, urinary tract infections, etc. Ancient scholars suggested that KTB prepared with processed *Hingula* (cinnabar) is more potent than *Bhasma* prepared without *Hingula*. Hence, in the present study, an attempt has been made to prepare incinerated hen egg shells using two different methods with and without cinnabar and their analytical profiles have been developed. **Aims and Objectives:** To develop analytical profile of KTB prepared by two different methods. **Materials and Methods:** Two samples of KTB were prepared. Sample KTB-A was prepared by *Kumari Swarasa* (juice of *Aloe vera* Tourn. Linn.) and sample KTB-B was prepared in the presence of *Hingula* as a medium for *Marana* using electric muffle furnace. The final product of both the samples of *Bhasma* were analyzed by organoleptic characteristics, physicochemical parameters and advanced sophisticated instrumental technologies such as particle size detection, inductively coupled plasma-atomic emission spectroscopy, Fourier transfer infrared spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy. **Observations and Results:** 22.75% and 41.16% of Calcium was detected in samples KTB-A and KTB-B, respectively. 0.29% and 0.15% of magnesium was found in samples KTB-A and KTB-B respectively. Both the samples of *Bhasma* were found to contain calcium hydroxide  $\text{Ca}(\text{OH})_2$ . **Conclusions:** Minimum four *Putra* (incineration cycles) with average 800°C temperature is required to prepare KTB through electric muffle furnace using *Kumari Swarasa* and processed *Hingula* as a medium. An average particle size was found as 9.35  $\mu\text{m}$  and 9.97  $\mu\text{m}$  in samples KTB-A and KTB-B, respectively. XRD study reveals that raw *Kukkutanda Tvak* is  $\text{CaCO}_3$  (calcite) and  $\text{CaCO}_3$  (calcium carbonate) whereas both the *Bhasma* contain  $\text{CaH}_2\text{O}_2$  (portlandite syn) and  $\text{Ca}(\text{OH})_2$ .

**Keywords:** *Hingula*, *Kukkutanda Tvak Bhasma*, *Kumari Swarasa*

## Introduction

Ayurveda is one of the ancient Indian traditional systems of medicine practiced over thousands of years. *Rasashastra* (an ancient Indian pharmaceutical science dealing with metals, minerals and ores) is one of the parts of Ayurveda that deals with metals/nonmetals/herbomineral preparations called as *Bhasma*.<sup>[1]</sup> *Bhasma*, literally means ‘ash’ are inorganic preparations produced by alchemic process, which converts a metal or mineral into its compounds such as carbonates and oxides.<sup>[2]</sup> The advantages of these preparations over plant preparations are their stability, lower therapeutic dose and potency.<sup>[3]</sup> The lack of validation of traditional medicines raises concerns on their authenticity. The *Kukkutanda Tvak Bhasma* (KTB) (incinerated hen egg shells) is one of the calcium-rich mineral medicinal formulations mentioned

in Ayurveda.<sup>[4]</sup> It is used to treat ailments such as *Shweta Pradara* (leukorrhea), *Rakta Pradara* (menorrhagia), *Prameha* (diabetes mellitus), *Mutraroga* (urinary tract infection) and *Manasika Daurbalya* (mental disorders). It also has the properties such as *Rasayana* (immunomodulation) and *Balya* (strength). The *Bhasma* were administered with milk as an *Anupana*.<sup>[5]</sup> It is also used as a key ingredient in many patent drugs and proprietary.<sup>[6,7]</sup> There are different methods of preparation for KTB mentioned in classical texts based on the media used during *Marana* (incineration) process.<sup>[8-11]</sup> *Kumari*

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*Swarasa* (juice of *Aloe vera* Tourn. Linn.) and processed *Hingula* (cinnabar) are frequently used during preparation of KTB as a medium.<sup>[11]</sup> To find out the analytical profile of Ayurvedic medicine is the need of hour to identify nature of final product. By considering this fact, the present work was carried out to establish analytical profile of KTB prepared by *Kumari Swarasa* and processed *Hingula* as media by evaluating physicochemical parameters and using sophisticated analytical techniques such as particle size detection, inductively coupled plasma-atomic emission spectroscopy (ICP, AES), Fourier transfer infra-red spectroscopy (FTIR), X-ray diffraction (XRD) and scanning electron microscopy (SEM).

## Materials and Methods

### Collection of raw materials

Hen eggshells (*Kukkutanda Tvak* [KT]) were collected from local market of Jamnagar. *Hingula* (cinnabar) and *Saindhava Lavana* (rock salt) were procured from the Pharmacy, Gujarat Ayurved University, Jamnagar and authentication was done as per *Grahyalakshana* (acceptable quality of the drug) mentioned in the Ayurvedic classical texts.<sup>[12]</sup> *Kumari* (*A. vera* Tourn. Linn.) was collected from botanical garden, Gujarat Ayurved University, Jamnagar and authentication was done at Pharmacognosy Laboratory, IPGT and RA, Jamnagar.

### Shodhana of raw materials

Purification of KT was done by following classical guidelines.<sup>[13]</sup> Raw KT was taken and washed with potable water. Purification was carried out by *Swedana* (boiling) in saltwater<sup>[14]</sup> for 3 hr. and then, it was left for self-cooling. After self-cooling, it was rubbed with hands and washed with hot water until separation of the inner layer from KT. Purification of cinnabar was carried out as per the reference of *Rasatarangini*.<sup>[15]</sup> Three kilograms cinnabar was made into powder and levigated with sufficient quantity of *Nimbu Swarasa* (lemon juice) for 6 h. The same procedure was repeated for six times more.

### Preparation of *Kukkutanda Tvak Bhasma*

#### *Marana* (incineration) of *Shodhita Kukkutanda Tvak* without cinnabar (*Kukkutanda Tvak Bhasma-A*)

For first incineration, purified *Shodhita* KT powder was taken in *Sharava* (earthen saucer) and covered with another earthen saucer. The junctions of the two earthen saucers were sealed with cotton cloth, which was smeared with clay. Sealed saucer was allowed to dry under sunlight. These earthen saucers were subjected for incineration in electric muffle furnaces (EMFs) at 800°C temperature. Temperature at 800°C was maintained for 15 min. Next day, after self-cooling, earthen saucers were withdrawn and KT powder was collected. For second incineration cycle, collected KT powder was levigated with juice of *Kumari* for 6 h, followed by preparation of pellets and subjected for sun drying. The dried pellets of KT powder were taken in *Sharava* (earthen saucer) and covered with another earthen saucer sealed with clay-smeared cloth. After sun drying, it was subjected to incineration at 800°C for 15 min. The same procedure was repeated to obtain desired quality of KTB.<sup>[11]</sup>

#### *Marana* of *Shodhita Kukkutanda Tvak* with Cinnabar (*Kukkutanda Tvak Bhasma-B*)

First incineration cycle was carried out same as that of KTB-A. For second incineration, one part of first incinerated powder of KT was triturated with purified cinnabar (¼<sup>th</sup> part) in *Khalva Yantra* (mortar and pestle) till homogenous mixing. Then, mixture was levigated with juice of *Kumari* and it was followed by preparation of pellets and subjected for sun drying. The dried pellets were placed in *Sharava* (earthen saucer) and covered with another earthen saucer sealed with clay-smeared cloth. After sun drying it was subjected for incineration at 800°C for 15 min. The same procedure was repeated to obtain desired quality of KTB.<sup>[11]</sup>

### Analytical study

Analytical studies of both the samples of *KT Bhasma* were carried out by classical parameters such as *Varna* (color), *Sparsha* (touch), *Rasa* (taste), *Gandha* (odor), *Shabda* (sound), *Rekhpurnatwa* and *Nishchandratwa* (incinerated powder of KT should enter between the lines of finger and should not glitter), *Sukshmatwa* (sufficient fineness), *Mridutwa* (softness) and *Nirgandhatwa* (having no specific smell).<sup>[16]</sup> Modern physicochemical parameters such as determination of pH,<sup>[17]</sup> specific gravity,<sup>[18]</sup> loss on drying,<sup>[19]</sup> total solid contents,<sup>[20]</sup> ash value,<sup>[21]</sup> acid-insoluble ash,<sup>[21]</sup> water-soluble extractives,<sup>[19]</sup> alcohol-soluble extractives<sup>[19]</sup> and acid-neutralizing capacity were also done.<sup>[22]</sup> In addition, particle size distribution, atomic emission spectroscopy with inductively coupled plasma, FTIR, XRD, and SEM were carried out.

## Results

During purification of KT, average 10 l of saltwater was required to complete purification of 2 kg of KT. The pH of saltwater increased after purification and the solid contents of salt water also increased from 6.3 to 6.9% [Table 1]. A total of 629 g, i.e., 31.45% weight loss, was observed during purification process of KT [Table 2 and Figure 1a, b]. After purification of *Hingula*, no significant changes in weight were observed, but color changed from reddish brown to brick red [Figure 1c and d]. For complete conversion into *Bhasma* of desired quality, minimum four incineration cycles were required to prepare KTB-A and KTB-B [Figure 2]. Average weight loss of 183 g, (36.6%) and 118.4 g, (23.68%) was found in incineration process during preparation of KTB-A and KTB-B respectively [Table 3]. The pH of KTB-A and KTB-B was 10.5 and 10 respectively [Table 4].

**Table 1: Physicochemical analysis of saltwater before and after purification of *Kukkutanda Tvak***

Media	pH	Specific gravity	Total solid content (% w/w)
Before purification	7.39	1.18	6.30
After purification	8.04	1.14	6.9

**Table 2: Observations made during *Shodhana* of *Kukkutanda Tvak***

Media	Quantity (l)	Initial weight (g)	Final weight (g)	Loss (↓) (g)	Actual percentage change
Salt water	10,000	2000	1371	629↓	31.45↓

**Figure 1:** *Shodhana* of raw materials. (a) Raw *Kukkutanda Tvak* (b) *Shodhita Kukkutanda Tvak* (c) Raw *Hingula* (d) *Shodhita Hingula*

### Acid-neutralizing capacity

There was no significant change in pH of KTB-A till 1 h, while testing acid-neutralizing capacity, but in pH of KTB-B, there was comparatively significant increase. 0.1 N NaOH was required slightly more in quantity for neutralization of KTB-A in comparison to KTB-B. The results of acid-neutralizing capacity are summarized in Tables 5 and 6.

Comparatively better acid neutralizing capacity of KTB-B is supported with less alkalinity and presence of polysulphides (FTIR) due to cinnabar.

### Particle size distribution

It was observed that the particle size of KTB-B is less than KTB-A. The detailed results of particle size distribution are depicted in Table 7.

### Inductively coupled plasma-atomic emission spectroscopic analysis

22.706 ppm and 4383.5 ppm of arsenic were detected in sample KTB-A and KTB-B, respectively, whereas 0.0591 ppm and 82.572 ppm of cadmium were detected in Sample KTB-A and KTB-B, respectively. 88547.0 ppm of mercury was detected in only sample KTB-B. Lead was not detected in sample of KTB-A. The results of ICP-AES analysis are tabulated in Table 8.

### Scanning electron microscopic analysis

As per the stoichiometry of the compounds identified in sample KTB-A and KTB-B, the percentages of calcium were 22.75 and 41.16%, respectively. Magnesium and sulfur were 0.29% and 12.45% and 0.15% and 1.93% in KTB-A and KTB-B, respectively. When XRD reports matched with SEM, it revealed that there is a formation of multiple compounds. The detailed results of SEM analysis are given in Tables 9 and 10.

**Table 3: Observations made during *Marana* of *Shodhita Kukkutanda Tvak* powder**

Media	Initial weight (g)	Final weight (g)	Loss (↓) (g)	Actual percentage change
<i>Kumari Swarasa</i>	500	317 (after 4 <i>Puti</i> )	183↓	36.6↓
<i>Hingula</i>	500	382 (after 8 <i>Puti</i> )	118↓	23.68↓

**Table 4: Results of physicochemical parameters of *Kukkutanda Tvak Bhasma***

Parameter	KTB-A	KTB-B
Loss on drying (%)	1.54	1.29
Ash value (%)	74.34	96.41
Acid-insoluble ash (%)	0.67	0.37
Water-soluble extractive (%)	7.51	7.38
Alcohol-soluble extractive (%)	0.004	1.44
pH	10.5	10

\*KTB: *Kukkutanda Tvak Bhasma*

**Table 5: Average alkali requirement for *Kukkutanda Tvak Bhasma* to adjusting pH to 3.5 and for neutralization**

Samples	0.1 N HCl required in ml		0.1 N NaOH required in ml	
	Adjusting pH to 3.5	Adjusting pH to 3.5	Adjusting pH to 3.5	Neutralization
KTB-A	37	-	-	9
KTB-B	-	24	-	4

\*\*KTB: *Kukkutanda Tvak Bhasma*

**Table 6: Average comparative acid-neutralizing capacity of two samples of *Kukkutanda Tvak Bhasma***

Time (min)	KTB-A	KTB-B
0	7.5	2.0
10	7.5	2.0
20	7.5	2.0
30	7.5	2.0
40	7.5	2.0
50	7.5	2.0
60	7.5	2.0

\*KTB: *Kukkutanda Tvak Bhasma*

### Fourier transform infrared spectroscopy

FTIR spectra of both samples were taken in the region of 400–4000  $\text{cm}^{-1}$ . General overview indicates the presence of large number of functional groups. In KTB-A, fairly sharp peaks were obtained at and around 3440.41, 1500–1400,





**Figure 2:** Marana of Shodhita Kukkutanda Tvak. (a) Kukkutanda Tvak Churna after first Puta, (b) Kumari Swarasa, (c) Levigation, (d) Kukkutanda Tvak Chakrika before second Puta, (e) Kukkutanda Tvak Bhasma A after second Puta, (f) Kukkutanda Tvak Bhasma A Chakrika after fourth Puta, (g) Kukkutanda Tvak Bhasma B after fourth Puta, (h) Kukkutanda Tvak Bhasma B Chakrika after fourth Puta

1446.56, 1056.50 and 875.56. These peaks indicate the presence of organic compounds such as OH, Ca, sulfate, CO<sub>2</sub> and carbonation. In sample KTB-B, fairly sharp peaks were obtained at and around 3643–3441.95, 2924–2854.04, 2135.24, 1632.24–1558.62, 1453.84, 1122.32, 875.81, 674.88–612.88 and 416.36. These peaks indicate the presence of organic compounds such as OH, methyl, cyanide, Ca, sulfate, carbonate and polysulfide ion [Tables 11 and 12].

### X-ray diffraction analysis

Raw KT was found to be in calcite (calcium carbonate [CaCO<sub>3</sub>]), and the crystal structure was rhombohedral in nature. Both the samples of KTB were found to contain portlandite and calcium hydroxide Ca(OH)<sub>2</sub> and hexagonal in nature [Tables 13-17 and Figure 3].

### Discussion

KTB is one of the important calcium-rich medicines mentioned under *Sudha Varga/Shukla Varga* in different classics of Ayurveda.<sup>[4]</sup> It is used to treat ailments such as *Rakta Pradara* (menorrhagia), *Shweta Pradara* (leucorrhoea), *Shwasa* (bronchial asthma), *Kasa* (cough), *Napunsakata* (impotency) and *Prameha* (diabetes).<sup>[9-11]</sup> For preparation of KTB, it is necessary to purify KT as it contains impurities such as excreta and tissues of creatures, which are of heterogeneous nature. Purification of KT was carried out by applying boiling process

**Table 7: Results of particle size distribution analysis of Kukkutanda Tvak Bhasma**

Results	KTB-A	KTB-B
VMD	16.98 μm	17.19 μm
SMD	9.35 μm	9.97 μm
SV	0.64 μm	0.60 μm
SM	2368.79 cm <sup>2</sup> /g	2220.57 cm <sup>2</sup> /g
X10	4.58 μm	5.30 μm
X16	6.29 μm	6.88 μm
X50	14.51 μm	14.47 μm
X84	27.91 μm	27.60 μm
X90	33.11 μm	33.22 μm
X99	53.96 μm	57.71 μm

\*KTB: Kukkutanda Tvak Bhasma, VMD: Volumetric mean diameter, X10: 10% of the material is below the mentioned micron value, X16: 16% of the material is below the mentioned micron value, X50: 50% of the material is below the mentioned micron value, X84: 84% of the material is below the mentioned micron value, X90: 90% of the material is below the mentioned micron value, X99: 99% of the material is below the mentioned micron value, SMD: Steered molecular dynamics, SV: Surface area per unit volume, SM: Surface area per unit mass

**Table 8: Results of inductively coupled plasma-atomic emission spectroscopic analysis of Kukkutanda Tvak Bhasma**

Element	Wavelength	Instrument detection limit (ppm), mg/L	KTB-A mg/kg (ppm)	KTB-B mg/kg (ppm)
Arsenic (As)	193.696	0.0530	22.706	4383.5
Lead (Pb)	220.353	0.0420	Not detected	5.8388
Mercury (Hg)	253.652	0.0610	Not detected	88547.0
Cadmium (Cd)	228.802	0.0027	0.0591	82.572
Sulfur (S)	181.975	-	1839.6	Not detected

\*KTB: Kukkutanda Tvak Bhasma

**Table 9: Result of scanning electron microscopy of Kukkutanda Tvak Bhasma-A (full scale 3484 cts)**

No	Element	Weight percentage	Atomic percentage
1	C K	19.76	30.97
2	O K	42.62	50.15
4	Mg K	0.29	0.22
5	S K	12.45	7.31
7	Cl K	0.35	0.18
8	Ca K	22.75	10.69
10	Fe K	0.38	0.13
13	As L	1.39	0.35

in salt water. It was prepared in the ratio of 1:4 of rock salt and portable water.<sup>[13,14]</sup> Average 10 l salt water was required to complete purification process of 2 kg KT. Average 629 g, i.e., 31.45%, weight loss was observed during the purification process of KT [Table 2]; it may be due to removal of impurities and inner side albumin layer of eggshell. The average pH of saltwater before and after purification was 7.36 and 8.25 respectively. The calcium component present in KT causes

its basic nature; hence, during steam heating process due to dissociation of solid contents of the KT in the media, the alkalinity may be increased. It is also evident by observing the increase in total solid content of the media after purification. Total solid contents of purification media increased from

**Table 10: Result of scanning electron microscopy of *Kukkutanda Tvak Bhasma-B* (full scale 7145 cts)**

Number	Element	Weight percentage	Atomic percentage
1	C K	3.35	5.95
2	O K	53.13	70.70
3	NaK	0	0
4	Mg K	0.15	0.13
5	S K	1.93	1.28
7	Ca K	41.16	21.87

**Table 11: Results of Fourier transfer infra-red spectroscopy analysis of *Kukkutanda Tvak Bhasma-A***

Peak position $\text{cm}^{-1}$	Assigned functional group
3440.41	-OH stretching vibration
1500-1400	Ca
1446.56	$\text{CO}_2$ molecule
1056.50	Sulfate ion
875.56	Carbonate ion

**Table 12: Results of Fourier transfer infra-red spectroscopy analysis of *Kukkutanda Tvak Bhasma-B***

Peak position $\text{cm}^{-1}$	Assigned functional group
3643-3439.01	-OH stretching vibration
2924.00-2854.04	Methyl stretching vibration (organic region)
2135.24	Cyanide related ion
1632.13-1558.62	Ca region
1453.84	Carbonate ion
1122.32	Sulfate ion
875.81	Carbonate ion
674.88-612.88	Sulfate ion
416.36	Polysulfite ion

**Table 13: X-ray diffraction peak report of raw *Kukkutanda Tvak***

Pos. [ $^{\circ}2\theta$ ]	d-spacing [ $\text{\AA}$ ]	FWHM [ $\text{\AA}^22\theta$ ]	H	K	L
23.074	3.85150	9.7	0	1	2
29.476	3.02791	100	1	0	4
31.605	2.82867	2.1	0	0	1
35.959	2.49550	13.8	1	1	0
39.434	2.28323	18.5	1	1	3
43.161	2.09432	14.5	2	0	2
47.156	1.92575	6.1	0	2	4
47.716	1.90447	18.1	0	1	8
48.614	1.87135	19.1	1	1	6
56.548	1.62617	3.3	2	1	1
57.393	1.60423	8.8	1	2	2

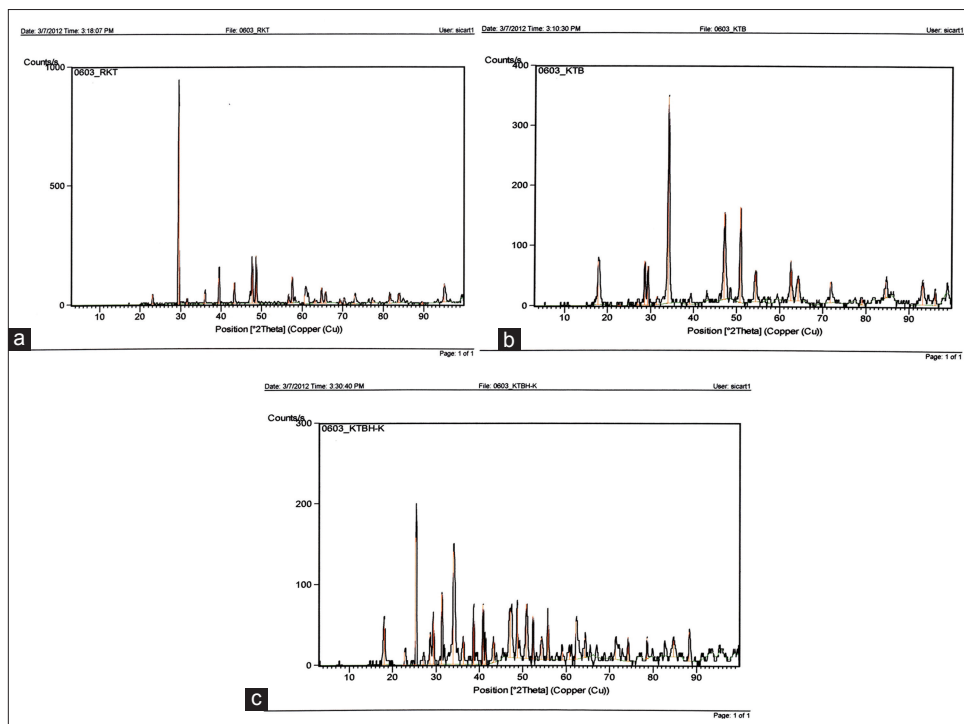
6.30 to 6.90; after purification, it may be due to addition of protein matter from KT besides simple concentration due to heating [Table 1].

Incineration of KT was carried out as per the reference of *Bhasma Vigyaneyam*.<sup>[11]</sup> For incineration process, conventional heating method of EMF was used due to convenience in temperature regulation and recording. For both incineration methods, the first incineration process was given *Putra* without levigation at  $800^{\circ}\text{C}$ , which was maintained for 15 min. From the second incineration, *Kumari Swarasa* (KTB-A) and processed *Hingula* (KTB-B) were used as media for levigation and the same temperature was given as that of first *Putra*. For KTB-A and KTB-B, minimum 4 and 8 incineration cycles (*Putra*) were required to obtain desired quality of *Bhasma*, respectively [Figure 4]. An average weight loss was found 183 g, i.e., 36.6%, and 118.4 g, i.e., 23.68%, during preparation of KTB-A and KTB-B, respectively [Table 3]. This loss may be due to evaporation of water and burning of some organic or organo-inorganic materials. Both the samples were smooth, tasteless and lusterless without any specific odor and produced no perceptible sound during chewing, indicating that they passed all the classical tests of *Bhasma Pariksha*.<sup>[16]</sup> *Varitara Pariksha* (floating over water surface) was negative in KTB due to hygroscopic nature of the KTB.

The physicochemical parameters of all the batches of KTB revealed that pH of KTB-A and KTB-B were 10.5 and 10 respectively [Table 4]. It is evident from previous research work that KTB is in  $\text{CaCO}_3$ ,  $\text{CaO}$ , or  $\text{CaPO}_4$  form.<sup>[23]</sup> Hence, calcium compounds are inherently basic in nature. The ash values were found 74.34% and 96.41% in samples KTB-A and KTB-B respectively [Table 4]. It is suggestive of remnant unburnt and evaporative substances in the prepared samples. These tests revealed that among two samples, KTB-B has least unburnt and evaporative constituents. [Table 4] Juice of *Kumari* was used as a medium for preparing KTB-A, whereas in other sample KTB-B cinnabar was used as a medium during incineration; hence, inorganic compound of cinnabar might have increased the ash value of sample KTB-B as compared to KTB-A. An average loss on drying observed in KTB-A and KTB-B was 1.54 and 1.29 respectively, which indicates the presence of least amount of moisture content. The acid-insoluble ash value was carried out to evaluate the percentage of insoluble inorganic content of KTB in dilute acid, which provides a step toward the bioavailability of the *Bhasma*. The acid-insoluble ash of KTB-A (0.67%) was higher in comparison to KTB-B (0.37%), but both *Bhasma* are highly soluble in acidic media suggestive of good bio-availability. It can be inferred that bioavailability of some constituents of KTB-A might be comparatively less than KTB-B. Water-soluble extractives were 7.51% in KTB-A and 7.38% in KTB-B and alcohol-soluble extractives were 0.004% in KTB-A and 1.44% in KTB-B [Table 4]. Less alcohol-soluble extractive indicates less extraction of polar organic matter. In acid-neutralizing capacity, there was no significant change in pH of KTB-A, whereas pH of KTB-B was comparatively less alkaline [Table 4]. It may indicate the KTB prepared without

**Table 14: X-ray diffraction report of *Kukkutanda Tvak Bhasma-A***

Compound name	Chemical formula	a/b/c (angstrom)	Crystal system	Bravais lattice	JCPDF
Portlandite, Syn	CaH <sub>2</sub> O <sub>2</sub>	3.5899/3.589/4.9160	Hexagonal	Primitive (P)	00-044-1481
Calcium hydroxide	Ca (OH) <sub>2</sub>				

**Figure 3:** X-ray diffraction image of *Kukkutanda Tvak Bhasma*. (a) Raw *Kukkutanda Tvak*, (b) *Kukkutanda Tvak Bhasma A*, (c) *Kukkutanda Tvak Bhasma B***Figure 4:** *Kukkutanda Tvak Bhasma*. (a) *Kukkutanda Tvak Bhasma A*, (b) *Kukkutanda Tvak Bhasma B*

cinnabar (KTB-A) has faster and better acid-neutralizing capacity [Tables 5 and 6]. It was observed that the particle size of KTB-B is less than KTB-A [Table 7]. Small particle size enhances the absorption,<sup>[17]</sup> hence, the bioavailability and potency of the drug increases resulting in to decrease in its therapeutic dose. This eventually leads to decrease in dose related side effects of the drug. Normal particle size distribution is found in KTB-A, while it shows minor skewness at the particle size of 3–4  $\mu\text{m}$  in KTB-B [Table 7]. SEM analysis showed the presence of calcium, magnesium and sulfur in both

the samples of KTB [Tables 9 and 10]. Various other elements such as Cl and Fe might be due to juice of *Kumari* used for levigation. It is evident from previous research work that raw KT contains Mg and S in trace amounts, which were found in the analysis of end product. FTIR study revealed the presence of large number of functional groups such as OH, Ca, sulfate and CO<sub>2</sub> in both the samples of KTB, which might be because of herbs used during preparation. In KTB-B, methyl and cyanide groups were observed, which may be due to the use of cinnabar [Tables 11 and 12]. In XRD study, KT was found contain calcite (CCaCO<sub>3</sub>) form whereas both the samples of KTB were found to have Ca(OH)<sub>2</sub> form [Tables 13 to 17 and Figure 3].

## Conclusions

For KTB preparation, minimum four incineration cycles are required using EMF with an average 800°C temperature without and with cinnabar as a medium. The average particle size of KTB are 9.35  $\mu\text{m}$  (KTB-A) and 9.97  $\mu\text{m}$  (KTB-B). XRD reveals that raw KT is rhombohedral in nature with CCaCO<sub>3</sub> (calcite) and CaCO<sub>3</sub> form, whereas both the *Bhasma* contain hexagonal CaH<sub>2</sub>O<sub>2</sub> (portlandite syn) and Ca(OH)<sub>2</sub> form. SEM, FTIR, and XRD reports suggest that both samples are crystalline in nature with mixture of different organic and inorganic compounds.



**Table 15: X-ray diffraction peak report of *Kukkutanda Tvak Bhasma-A***

Pos [°2Th]	d-spacing [Å]	FWHM [°2Th]	H	K	L
18.008	4.92200	72	0	0	1
28.672	3.11100	27	1	0	0
34.102	2.62700	100	1	0	1
36.527	2.45800	1.0	0	0	2
47.121	1.92710	30	1	0	2
50.813	1.79540	31.0	1	1	0
54.358	1.68640	14.0	1	1	1
56.092	1.63830	1.0	0	0	3
59.426	1.55410	3.0	2	0	0
62.634	1.48200	9.0	2	0	1
64.233	1.44890	7.0	1	0	3

**Table 16: X-ray diffraction report of *Kukkutanda Tvak Bhasma-B***

Compound name	Chemical formula	a/b/c (angstrom)	Crystal system	Bravais lattice	JCPDF
Portlandite, Syn	CaH <sub>2</sub> O <sub>2</sub>	3.5918/3.5918/4.9063	Hexagonal	Primitive (P)	01-084-1263
Calcium hydroxide	Ca (OH) <sub>2</sub>				

**Table 17: X-ray diffraction peak report of *Kukkutanda Tvak Bhasma-B***

Pos. [°2Th]	d-spacing [Å]	FWHM [°2Th]	H	K	L
18.066	4.90630	71.3	0	0	1
28.676	3.11059	18.1	1	0	0
34.101	2.62709	100	0	1	1
34.101	2.45315	0.8	0	0	2
47.144	1.92621	41.9	0	1	2
50.798	1.79590	27.6	1	1	0
54.356	1.68647	15.0	1	1	1
56.199	1.63543	1.3	0	0	3
59.376	1.55529	2.2	2	0	0
62.606	1.48259	10.9	2	0	1
64.300	1.44755	11.5	1	0	3

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### Conflicts of interest

There are no conflicts of interest.

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