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Analytical Profile Of Calcium-Enriched Ayurvedic Preparation: Kukutanda Twaka Bhasma

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Abstract

Kukutanda Twaka Bhasma (KTB) is a traditional Ayurvedic medicinal preparation and one of the important calcium- enriched medicines used to treat various ailments such as Shweta Pradara (leuchorrhea), Rakta Pradara (menorrhagia), Kasa (cough), Shwasa (bronchial asthama) and Prameha (diabetes). Kukkutanda is jangama dravya and included under Sudha Varga with Sudha, Kurma prishta, Varatika etc. In this study *Kukutanda Twaka Bhasma* was prepared by using *Kukkutanda Twaka* (KT) as per the classical Ayurvedic text and using EMF for completion of incineration process at 850^0C to prepare good quality KTB. The physicochemical characterization was carried out by using modern tools and techniques such as XRD, SEM, EDAX and ICP-OES. The study showed that the raw material *Kukutanda Twaka* is a mineral organic matrix containing $CaCO_3$ and *Kukutanda Twaka Bhasma* containing $CaCO_3$ in calcite form.

Keywords: *Kukutanda Twaka Bhasma*, Sudha Varga, XRD, SEM, EDAX, ICP-OES.

Introduction

Ayurveda is the most ancient system of life and health care in the world, its antiquity going back to the Vedas. However, with the changing needs and trends of health care system Ayurveda is again resurging and getting globalised with the hope that it will effectively complement the mainstream of medicine to develop a safe and effective modality of medicine.

As for as traditional Indian system of medicine Ayurveda and Siddha are concered, metals and minerals have been used mainly as bhasma. Rasa Shastra (an ancient Indian pharmaceutical science dealing with metals, minerals and ores) is one of the Ayurveda that deals with metals/nonmetals/herbomineral preparations called as Bhasma[(1)]. 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 [(2)]. The advantage of these preparations over plant preparations are their stability, lower therapeutic dose and potency [(3)]. The *Kukutanda Twaka Bhasma* is one of the calcium-enriched mineral medicinal formulations mentioned in Ayurvedic classics [(4)]. This biomedicine is synthesized through special calcination of eggshell. It is used to treat ailments such as Shweta Pradara (leuchorrhea), Rakta Pradara (menorrhagia), Kasa (cough), Shwasa (bronchial asthama), Prameha (diabetes), Mutraroga (urinary tract infection) and Manasika

Daurbalyata (mental disorders). It also has the properties such as Rasayana and Balya (strength) [5]. It is well known to increase the intestinal absorption of calcium which is beneficial for bone mineralization and also used in treatment of bone metabolic disorders associated with calcium deficiency[6]. It is also used as a key constituent in many patent drugs and proprietary [7]. There are different methods of preparation of KTB mentioned in our classical text (Table 1 & 2).

Table 1. Shodhana of *Kukutanda Twaka* in different Ayurvedic Classics

S. No.	Media	Process	Time	Reference
01.	Ushnodaka (warm water)	Nimmajjana	-	Ayurveda Sara Sangraha[8]
02.	Ushnodaka (warm water)	Nimmajjana	1 day	Siddha Bheshaja Sangraha [9]
03.	*Saindhava Lavana* + *Nausadara* + Udaka (Water mixed with *Saindhava Lavana* and *Nausadara*)	Nimmajjana	4-6 days	Rasa Tantra Sara va Siddha Prayoga Sangraha [10]
04.	Takra	Nimmajjana	24 hours	Vriddha Vaidya Parampara [11]

Table 2. Marana of Shodhita *Kukutanda Twaka* in different Ayurvedic Classics

S.No.	Bhavana Dravya	Puta	Reference
01.	Changeri (*Oxalis corniculata*) Swarasa	3 Gaja Puta	Ayurveda Sara Sangraha[12]
02.	Changeri (*Oxalis corniculata*) / Ghritakumari (*Aloe barbendensis*) Swarasa	4 Gaja Puta	Siddha Bheshaja Sangraha [13]
03.	Changeri (*Oxalis corniculata*) Swarasa	4 Gaja Puta	Rasa Tantra Sara va Siddha Prayoga Sangraha [14]
04.	Ghritakumari (*Aloe barbendensis*) Swarasa	2 Gaja Puta	Vriddha Vaidya Parampara [15]

Considering all these facts, to find out the analytical profile of Ayurvedic medicine is the need of hour to identify nature of final product. The present work was carried out to establish analytical profile of KTB prepared by *Changeri* (*Oxalis corniculata*) Swarasa as a media by evaluating physicochemical characterization and using sophisticated modern tools and techniques such as X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy – Dispersive X-ray Spectroscopy Analysis (EDAX) and Inductively Coupled Plasma Optical Emission Spectroscopic Analysis (ICP-OES).

Materials and Methods

Collection of raw materials

Kukkutanda Twak (KT) was collected from local market of shopkeeper of Lanka, Varanasi. *Nausadar* and *Saindhava Lavana* (rock salt) were procured from the local market of Gola Dinanath, Varanasi and authentication was done by experts of Rasa Shastra & Bhaishajya Kalpana, Faculty of Ayurveda, IMS, BHU, Varanasi. Fresh Changeri (*Oxalis corniculata*) panchanga (whole plant) was collected from the garden of Ayurvedic Pharmacy, BHU and authenticated by experts of Department of Dravya Guna.

Shodhana of raw materials [16]

Shodhana of KT was done by following classical guidelines. Raw KT was taken and washed with filled with tap water and add *Nausadar* and *Saindhava Lavana* and heated till the water boils up to 100^0C and kept for 3 days and it becomes soft and the inner layer separated easily.

Marana (Incineration) of Kukkutanda Twak [17]

In the first marana, shodhita KT was kept in earthen Sharava and covered with another Sharava. Joint of both Sharava was sealed by clay smeared cotton cloth up to seven times and every coating allowed to dry in sunlight. This Sharava samputa was subjected to heat in EMF at 850^0C and the temperature was maintained upto 3 hours. Next day, after self-cooling, Sharava samputa withdrawn from EMF and after removing clay smeared cotton, KT powder was collected. For second Marana process, collected KT powder was triturated with Changeri Swarasa for two hours, followed by preparation of small, round, flat pellets and subjected for sun drying. The dried pellets were taken in Sharava and covered with another Sharava, sealed with clay smeared cloth and after drying subjected to heat in EMF at 850^0C for three hours. The same procedure was repeated up to three times and finally desired quality of KTB obtained.

Organoleptic Analysis

Organoleptic Analysis of the prepared sample of KTB was carried out by classical parameters such as Shabda (sound), Sparsha (touch), Roop (color), Rasa (taste), Gandha (odor), Varitara test, Unam test, Rekhapurnatwa and Niswadatva test [18]. Physicochemical Analysis was also carried out by following the standard methods for estimation of loss on drying [19], water soluble ash [19], total ash [20], acid-insoluble ash [20], and determination of pH [21]. In addition, XRD, SEM, EDAX and Determination of heavy metals through inductively coupled plasma optical emission spectroscopy (ICP-OES) were carried out.

Results

KTB is a smooth, tasteless, white colored fine powder & with no specific odor. During the pharmaceutical processing like Shodhana of KT, 20 ltrs of tap water (Water+*Nausadar*+*Saindhav Lavana*) was required to complete the Shodhana of 800 gms of KT and total 75 gms i.e. 09.37% weight loss observed during the process (Table 3). After Shodhana of KT and finally it converted into KTB of desired quality, minimum three Putas was required and total loss during the whole process was 185 gms (25.51%) (Table 4).

Table 3. Observation during Shodhana of *Kukkutanda Twaka*

Media	Duration of Shodhana	Quantity of Media	Initial Weight	Final Weight	% Loss
Water + *Nausadar* (28.75 gm) + *Saindhava Lavana* (71.25 gm)	3 days	20 liters	800 gms	725 gms	09.37%

Table 4. Observation during Marana of *Kukkutanda Tawaka Bhasma*

Media	Initial Weight	Final Weight	% Loss
Changeri (*Oxalis corniculata*) Swarasa	725 gms	540 gms	25.51

Physicochemical analysis of KTB was carried out and detailed results are depicted in Table 5.

Table 5. Physicochemical analysis of *Kukkutanda Tawaka Bhasma*

S.No.	Parameters	Results
01.	Loss on Drying at 105^0C	5.21%
02.	Total Ash	10.99%
03.	Acid Insoluble Ash	02.15%
04.	pH	12.50

The X-ray diffraction analysis

The raw KT X-RD patterns match with the Calcium Carbonate ($CaCO_3$), and the crystal structure was Rhombohedral in nature and the KTB found to contain Calcite variety of $CaCO_3$ also Rhombohedral in nature. The detailed results of XRD analysis are depicted in Table 6 & 7.

Table 6. XRD analysis of Raw *Kukkutanda Twaka*

2-theta (degree)	d- spacing (^{0}A)	FWHM (degree)	Height	Size	Rel. Int.
27.041	3.2948	0.235	207	364	0.99
29.650	3.010	0.62	1919	139	20.85
29.932	2.98271	0.242	25065	355	100.00
31.984	2.7959	0.350	772	247	4.82
39.727	2.2671	0.23	226	381	1.04
39.949	2.2549	0.19	615	463	2.35
43.601	2.0742	0.28	358	323	2.44
47.900	1.89755	0.26	4555	347	23.92
48.048	1.89207	0.098	2099	926	4.51
48.881	1.8617	0.360	2209	253	14.00
49.063	1.85527	0.103	1730	886	3.16

Table 6. XRD analysis of Raw *Kukkutanda Twaka Bhasma*

2-theta (degree)	**d- spacing (^{0}A)**	**FWHM (degree)**	**Height**	**Size**	**Rel. Int.**
18.091	4.8995	0.451	3625	186.4	42.48
28.733	3.1045	0.293	2248	292	18.29
29.417	3.0338	0.16	131	534	0.58
34.140	2.6242	0.474	7664	183.1	100.00
47.093	1.9282	0.612	2298	148	39.73
50.846	1.7943	0.393	2697	234	25.32
54.409	1.6849	0.478	1180	195	14.73
59.39	1.5549	0.34	231	277	2.29
62.618	1.48233	0.556	745	175	11.21
64.274	1.4481	0.793	508	123.5	10.91
71.885	1.3123	0.643	453	159	9.16

Scanning electron microscope (SEM) analysis and Energy – dispersive X-ray spectroscopy Analysis (EDAX)

The SEM observation about raw *Kukkutanda Twaka* particle size ranges from 20um to 200um whereas *Kukkutanda Twaka Bhasma* particle size ranges from 2um to 10um and as per the EDAX analysis the percentages of Ca in KT & KTB were 52.08% & 35.49% respectively. O in KT & KTB were 37.78 % & 46.68% respectively whereas C detected in KT & KTB 10.14% & 17.83& respectively. The detailed results of EDAX analysis are depicted in Table 7 & 8.

Table 7. EDAX of Compositions of Raw *Kukkutanda Twaka* with elemental %

Element	Weight %	Atomic %
C	10.14	18.74
O	37.78	52.41
Ca	52.08	28.85

Table 8. EDAX of Compositions of Raw *Kukkutanda Twaka Bhasma* with elemental %

Element	Weight %	Atomic %
C	17.83	28.08
O	46.68	55.18
Ca	35.49	16.74

Inductively Coupled plasma optical emission spectroscopic analysis (ICP-OES)

The ICP-OES of KTB shows the presence of Arsenic in the sample was 1.8 ppm i.e. within the permissible limit. The other heavy metals like Cadmium, Lead and Mercury were not detected in the sample. The result of ICP-OES analysis is tabulated in Table 9.

Table 9. ICP-OES analysis of *Kukkutanda Twaka Bhasma*

Elements	Results mg/ltr
Arsenic (As)	1.8 ppm
Cadmium (Cd)	Not detected
Mercury (Hg)	Not detected
Lead (Pb)	Not detected

Discussion

KTB is an animal origin significant drug in Ayurvedic literature, obtained from Hen's egg shell. Due to the rich source of Calcium in their bhasma, mentioned under Sudha Varga of different Ayurvedic classics and is used to treat various ailments such as Shweta Pradara (leuchorrhea), Rakta Pradara (menorrhagia), Kasa (cough), Shwasa (bronchial asthama), Napunsakata (impotency), Prameha (diabetes) etc. For the preparation of KTB, it is necessary to purify raw KT as it contains impurities such as excreta and tissues of creatures, which are of heterogeneous nature. KT is mainly composed of Calcium carbonate, Calcium phosphate, organic matter and Magnesium carbonate.

During the Shodhana procedure cleaning of raw KT filled with tap water and adds Nausadar & Saindhava Lavana and initially this mixture heated till the water boils after that it kept aside for three days. Therefore 20 ltrs tap water, *Nausadar* (28.75 gms) & *Saindhava Lavana* (71.25 gms) was required to complete the Shodhana of 800 gms of KT and total 75 gms i.e. 09.37% weight loss observed during the process (Table 3); it may be due to removal of impurities and inner side albumin layer of egg cell.

In the Marana procedure, Shodhita KT was kept in earthen Sharava and covered with another Sharava. After proper sealing with clay smeared cotton cloth up to seven times and every times coating was allowed to dry in sunlight and this Sharava samputa was subjected to transfer in EMF at heat it upto 850^0C and this temperature was maintained till three hours, the first puta process was given without trituration with liquid media (*Changeri Swarasa*). Next day, after self-cooling, Sharava samputa withdrawn from EMF and after removing clay smeared cotton cloth, KT powder was collected and triturated with *Changeri* (*Oxalis corniculata*) *Swarasa* for two hours, followed by preparation of small, round, flat pellets and subjected for sun drying. These pellets kept into Sharava samputa and the same process at the same temperature repeated upto two times (total three Puta) finally white colored desired quality of KTB obtained and average weight loss was found 185 gms, i.e. 25.51% during the preparation of KTB. This loss may be due to evaporation of water content and burning of organic materials associated with the shell. The finally collected sample of KTB was smooth, tasteless without any specific odor and produced no any perceptible sound during chewing, indicating that they passed all the classical tests of Bhasma Parishsha like Varitara test, Rekhapurnata test, Niswadatva test and Unam test.

The physicochemical analysis of KTB exposed that the pH of KTB was 12.5 hence it indicate that Calcium compounds are naturally basic in nature and mainly it contains $CaCO_3$, CaO and $CaPO_4$, it is marked from previous research work [22]. The details of other physicochemical analysis value of KTB are mentioned in Table 5. The XRD study exposed the presence of $CaCO_3$ in raw KT and in KTB Calcite variety of $CaCO_3$ present (Table 5,6 & Figure 1,2) whereas SEM analysis showed the presence of Ca, C & O in KT & KTB and detail quantities are mentioned (Table 7). Quality assured traditional medicines are imperative for their global acceptance and rational use. Several herbal or metal-mineral based drugs are being developed in the past decades apart from classical medicines [23-37]. Future studies are required to investigate these formulations on analytical as well as therapeutic grounds.

Conclusion

Kukkutand Twaka Bhasma (KTB) preparation includes two major steps such as Shodhana and Marana. It is essential to follow all the procedures as per our classical texts for getting a good quality of bhasma. The minimum four Puta are required using EMF with an average of 850^0C temperature. This procedure assist a reduction in the particle size which facilitates better absorption and assimilation into the human physiological system and enhance the therapeutic utility of drug, This study revealed that a temperature of 850^0C maintained for three hours duration in three puta is sufficient to obtained good quality of *Kukkutand Twaka Bhasma*.

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Figure 1. X- Ray Diffraction Graph of Raw *Kukkutanda Twaka*

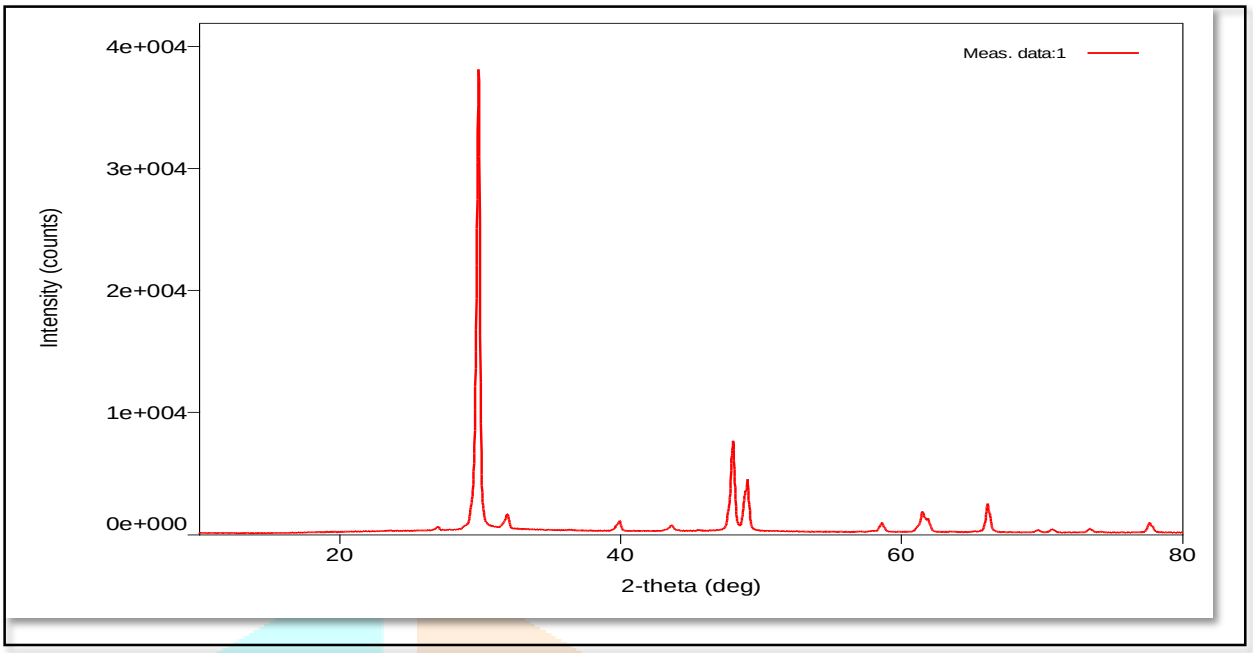

Figure 2. X-Ray Diffraction Graph of Raw *Kukkutanda Twaka Bhasma*