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PHARMACEUTICAL AND ANALYTICAL STUDY OF KUKKUTAND TWAK BHASMA WITH SPECIAL REFERENCE TO AYURVED SAAR SANGRAHA AND VRUDDHA VAIDYA PARAMPARA

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ABSTRACT

Kukkutand Twak (Eggshell) has been described in the *Sudha* (Calcium) *Varga* (Category) *dravyas* in Rasashastra. In this category, *dravyas* having Calcium as its main constituent are described. Kukkutand Twak is an excellent source of organic form of Calcium and has more bioavailability than that of inorganic form. In present study, Kukkutand Twak Bhasma has been prepared by two different methods i. e. as per Ayurved Saar Sangraha (ASS) and VruddhaVaidya Parampara (VVP). Both of these methods required two no. of *Putas* (Method of heating) to obtain the *bhasma*. X-Ray Diffraction (XRD) analysis of the prepared *bhasma* from both the methods shows presence of $CaCo_3$.

Keywords: *Sudha Varga, Kukkutand Twak, Bhasma, Puta*

INTRODUCTION

Rasashastra is the science which deals with the study of metals, minerals, *Sudha varga* and *Visha* (poison) *dravyas*. It describes the identification, purification, incineration and the properties of these *dravyas* along with their medicinal preparations. In *Sudha Varga, dravyas* having Calcium as the main constituent have been described.

As per *Sudha Varga* is concerned, in ancient texts like Rasarnava and Rasaratnakar, the term *Shukla Varga* is found to be used for it. In *Shukla Varga*, the drugs like *Sudha* (Lime), *Shankha* (Conch Shell), *Shukti* (Pearl Oyster) and *Varatika* (Cowry) are included. The texts like Rasamruta and Ayurved Saar Sangraha, may be due to the influence of modern chemistry, have developed a separate view of *Sudha Varga* as a group of calcium containing group.

Use of KT (Kukkutand Twak) is seen since *Samhita kala* for the external application on the body. Use of KT in its *bhasma* form is found to be started in Rasashastra kala. For the preparation of *bhasma, maran* (incineration) of the *shodhit* (purified) metal / mineral has to be done. Maran is the process in which *shodhit* metal / mineral is triturated with specific plant juice and then whole mixture is subjected to *Agni sanskara*, which yields *bhasma* i. e. very fine

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powder. [1]

In the different drugs of *Sudha Varga*, calcium is found to be present in different forms of its salts e.g. calcium silicate in Badarashma, calcium sulphate in Godanti, calcium phosphate in Ajasthi, calcium hydroxide in Sudha , calcium carbonate in Kukkutand Twak, etc. In majority of the drugs, calcium is present in the form of calcium carbonate e.g. in *Shankha, Shukti, Kapardika, Praval, Mukta*, etc.

Calcium carbonate supplements have the highest percentage of elemental calcium among the calcium salts.[2]

KT contains 95% Calcium carbonate and 5% Calcium phosphate, magnesium carbonate, proteins, etc.[3]. Use of Calcium in the form of Calcium salts may be useful to prevent or to correct Calcium deficiencies, to treat osteoporosis, as an antacid, as a Phosphate binder or for acute treatment of Tetani, Lead colic, etc.[4]. KTB (Kukkutand Twak Bhasma) is found to be very effective in the conditions like *Shwetapradara* (Leucorrhoea), *Vatavikaar*, *Prameha* (Diabetis), etc.[5] It is useful to improve bone density, since it is a good source of calcium. Hence used in arthritis, osteoporosis etc. [6]. There are no significant side effects reported with the regular use of eggshell calcium (KTB). Therefore we can consider that it is safe for long-term and regular use.[7] But the scientific validation of KTB found is inadequate. Effective use of KTB also helps to adequate utilization of the egg shells which are otherwise disposed as a waste.

Most common textual reference of KTB is from Ayurved Saar Sangraha (ASS). But in this reference *shodhan* (Purification) of KT and *bhavana* (levigation) procedure is not found mentioned. In the reference from Vruddha Vaidya Parampara (VVP) procedure for *shodhan* and *bhavana* of Kumari (Aloe Vera) is found mentioned.

Shodhana means processing of the substance along with the specific indicated *shodhan dravya* through the procedures like *peshan* (triturating), etc. to remove the impurities from the substance. [8]

The procedure of *bhavana* is grinding of the powder of any substance like *Dhatu* (metal), *Maharasa*, etc. with specific liquid till it soaks the liquid completely and becomes dry.[9] For enhancing the *bala* (power) of *aushadhi dravya*, *Bhavana* of *swaras* of specific *dravya* is given. Thus, the *subhavit dravya* i.e. *dravya* after satisfactory levigation can work sufficiently even in small quantity. [10]

Hence, by taking into consideration the importance of *shodhan* and *bhavana*, present study has been done using both the above references. *Bhasma* obtained from both the methods has been analysed using Ayurvedic and modern analytical tests.

MATERIALS AND METHOD:

Preparation of KTB was done using following two references-

1. Ayurved Saar Sangraha (ASS)
2. Vruddha Vaidya Parampara (VVP)

1. Preparation of KTB as per ASS[11]-

Raw drugs(Figure1):

1. Kukkutand Twak .....….....500 gm
2. Changeri swaras (juice of Oxalis Corniculata) .....… 140ml

Procedure:

KT was collected and the adherent dirt was properly scrapped off. Then KT was immersed in water, the inner membranous layer of KT was removed. Such KT was subjected for *Puta*.

I Puta:

Coarse powder of KT was made in a *khalvayantra* (Mortar and pestle). Leaves of Changeri were snapped into small fragments. Changeri swaras was prepared us-

ing a mixer. It was filtered through a cotton cloth. The coarse powder of KT was taken in a *sharava* (Earthen pot) and Changeri swaras was added to it **(Figure 2)**. The *sharava* was then covered with another *sharava*, joint of the sharava was sealed with Multani clay. After drying the seal, the *sharava samputa* was subjected to Puta (heating). 40 no. of cow dung cakes were arranged in a pit. The *sharava samput* was placed on it. It was covered with 20 no.of cow dung cakes. Then the cow dung cakes were ignited **(Figure 3).**

After self-cooling of *Puta*, the *Sharav samputa* was taken out and it was opened carefully. The output obtained was observed for the *bhasma parikshas*. But it was not fulfilling the pariksha. Therefore, it was again subjected to Puta.

II Puta:

Raw drugs-

KTB obtained after I Puta……380 gm

Changeri swaras……..…..……100 ml

II Puta was given similarly as I Puta using 60 no. of cow dung cakes. KTB bhasma obtained after II Puta **(Figure 4)** was fulfilling the *bhasma parikshas***.**

2. Preparation of KTB as per VVP[12]-

Raw drugs(Figure 5):

1. Kukkutand Twak ……. 500 gm
2. Takra(Buttermilk)……..… 400 ml
3. Kumari swaras (Juice of Aloe Vera) .. 240 ml

Procedure:

Shodhan of KT:

Kukkutand Twak was washed thoroughly with water. Then it was kept dipped in takra for 24 hrs **(Figure 6)**. After that, it was again washed with water. Thus shodhan of KT was performed.

Bhavana Procedure:

After *shodhan*, KT was taken in a *khalvayantra* and triturated to prepare its coarse powder. Kumari swaras was added to this powder and the mixture was triturated until it becomes homogeneous **(Figure 7).**

I Puta:

After *Bhavana* procedure, *chakrika* (small pellets) were prepared of the homogenous mixture. These *chakrika* were placed in a sharava **(Figure 8)** and it was covered with another *sharava. Sharav samputa* was prepared by sealing the joint of the sharava with Multani clay. After drying, the samputa was subjected to Puta. Total 60 no. of cow dung cakes were used for the Puta. After self-cooling of the Puta, sharava was taken out and the KTB was collected. In this method also after I Puta, the bhasma was not fulfilling the parikshas. So it was subjected to II Puta.

II Puta:

Raw drugs-

1. KTB obtained after I Puta …. 390 gm
2. Kumari swaras ……...….. 180 ml

II Puta was given similarly as I Puta using 60 no. of cow dung cakes. KTB bhasma obtained after II Puta **(Figure 9)** was fulfilling the *bhasma parikshas*.

Recording the temperature of Puta (Figure 10):

When the *Puta* was ignited, a pyrometer was placed into the pit to record the temperature. The temperature was recorded during the *Puta* procedures of both the methods.

Methodology of the Analytical techniques:

Moisture Content:

2 gm of sample was accurately weighed and taken in a weighed Petri dish with a fitting cover (dried in 100º C before use) and kept in hot air oven for one hour maintained at 105º C. After one hour the Petri dish was taken out and cooled in desiccators and the weight was noted. The proce-

dure was repeated till Constant weight did not differ by 2 mg.

The moisture content of the sample was calculated using the following equation:

%W =A−B/B× 100

Where:

%W = Percentage of moisture in the sample,

A = Weight of wet sample (grams), and

B = Weight of dry sample (grams)

Ash value:

About 2 gram of accurately weighed sample was taken in a silica dish and incinerated. After cooling it was weighed. The carbon-free ash was not obtained so the charred mass was mixed with hot water; the residue was collected and incinerated at a low temperature in a silica dish until free from carbon. The percentage of ash is calculated with reference to the moisture free drug.

Determination of pH:

The meter and electrodes were standardized with 0.05H Sodium borate while measuring an alkaline solution. At the end of a set of measurements, a reading of the Solution used to standardize the meter and electrodes was taken. This reading should not differ by more than 0.02 from the original value at which the apparatus was standardized. Now in 5ml of water 1gm of sample was put and pH was determined for the solution

Determination of acid insoluble ash:

The method of determination of acid insoluble ash is as follows: The ash was boiled for five minutes with 25 ml of dilute hydrochloric acid (6 N). The insoluble matter was collected in a crucible. It was then washed with hot water and then ignited till it got constant weight at a low temperature. The percentage of ash was calculated with reference to the moisture free drug.

X- Ray diffraction:

When a beam of x-rays are incident upon a substance, the electrons of the constituent atoms of the substance become as small oscillators. These oscillate at the same frequency as that of incident x-rays. These scattered waves come from electrons, which are arranged in a regular manner in a crystal lattice and then travel in certain directions. If these waves undergo constructive interference, then these are diffracted from that crystal plane. Every crystalline substance scatters the X-rays in its own unique diffraction pattern producing a fingerprint of its atomic and molecular structure. The following methods used in the x-ray diffraction technique.

Powder method:

When a monochromatic beam of x-ray is allowed to fall on the powder of a crystal, then the following possibilities may happen.

i) There will be some particles out of the random orientation of small crystals in the powder, which lie within a given set of lattice planes (making the correct angle with the incident beam) for reflection to occur.

ii)While another fraction of the grains will have another set of planes in the correct position for the reflections to occur and so on.

iii) Also reflections are possible in different order for each set.

All the like orientations of the grains due to reflection for each set of planes and for each order will constitute diffraction cone whose interaction with a photographic plate gives rise to trace. The crystal structure can be obtained from the arrangement of the traces and their relative's intensities. If the angle of incidence is Q, the angle of reflection will be 2Q. If the film radius is r, the circumference 2 r corresponds to scattering angle of 360.

SEM EDAX: (Scanning electron microscopy with Energy dispersive X-ray analysis):

Accelerated electrons in a scanning electron microscopy carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons, photons and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples.The x-ray is produced by inelastic collisions of the incident electrons with electrons in discrete shells of atoms in the sample. As the excited electrons return to lower energy states, they yield x-rays that are of a fixed wavelength. Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. This analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyse the same materials repeatedly.

Sample preparation

Sample preparation can be minimal or elaborate for analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples. Most electrically insulating samples are coated with a thin layer of conducting material, commonly carbon, gold, or some other metal or alloy. The choice of material for conductive coatings depends on the data to be acquired; carbon is most desirable if elemental analysis is a priority, while metal coatings are most effective for high resolution electron imaging applications.

OBSERVATIONS AND RESULT:

Temperature Pattern during the Puta Procedure:

The details of the temperature are given in **Table 1**

Bhasma Parikshas:

Following *Bhasma Parikshas* were carried out for the *bhasma* obtained from both the above methods.

Rekhapurnatvam- A pinch of *bhasma* was taken in between the thumb and index finger and rubbed. It was observed that the *bhasma* entered into the grooves of the finger and could not be easily removed out.

Varitartvam- A small amount of *bhasma*- was sprinkled over the still water surface in a beaker. It was found that the *bhasma* particles floated over the surface of water.

Gandha (Smell)- Smell of the prepared *bhasma* was taken, it was having no smell i.e. it was *Nirgandha*.

Rasa (Taste)- A small pinch of the *bhasma* was tasted, it was tasteless i.e. it was *Niswadu*.

KTB obtained from both the methods was observed for its organoleptic characteristics. It is described in **Table 2.**

Analytical Observations:

Raw Kukkutand Twak and Bhasma obtained from both the methods were observed analytically. The findings are shown in **Table 3.**

X-Ray Diffraction:

Raw KT and the KTB obtained from both the methods were subjected to the X-RD analysis. From the X-Ray diffraction pattern obtained, it was found that the majority peaks indicates the presence of Calcium Carbonate. Maximum intensity was observed at 29.77(2 Q position).No remarkable change was seen among all the samples.

SEM-EDAX Analysis:

The findings are shown in **Table 4.**

DISCUSSION

Rasashastra has given an immense contribution to the field of Ayurvedic therapeutics. In present study, pharmaceutical and analytical study of KTB has been done by taking into consideration its therapeutic *efficacy*.

Pharmaceutical study- For the preparation of KTB, KT having white colour were selected. Specially, unfertilized hen's eggs were collected so as to ensure that the calcium present in the shell was not utilized by the growing chick. The adherent dirt was properly scrapped off to avoid contaminations if any. After the immersion in water, the KT become brighter, its odour was reduced which was completely diminished after drying in sunlight.

In the first method from ASS, Changeri swaras was used. Changeri, being a dravya from *Amla varga*, is having *kshalana* (purification by washing) *guna* (property) and *bhedan* (breaking), *deepan* and *paachan* (digestive) *karma* (function). It is slightly acidic which is probably helping in reducing the alkalinity of KT. It also helps KT for breaking into fine particles. Some properties of changeri swaras may be incorporating in the KTB and it may have role in reducing the levels of heavy metals in KTB. Recent studies have established that changeri possesses antimicrobial activities against bacillus septillus, Staphylococcus aurigenosa. It has also antifungal and wound healing activities. These properties are expected to be infused in KTB.

Processing egg shell (KT) with Changeri juice eliminates impurities and toxins, which increases its suitability for human consumption. Calcination process increases its bioavailability in human body.[13]

In the method from VPP, KT was kept immersed in Takra (buttermilk). Takra, being an *amla dravya*, may be helping in its *kshalana*. Kumari swaras is used for *bhavana* (levigation). Since Kumari is rich in calcium, its use for *bhavana* purpose can be helpful to increase the calcium percentage in *bhavya dravya* i.e. KT. Hence it may be enhancing the therapeutic property of the KTB.

The KTB prepared using both the methods was passing all the *Bhasma Parikshas*, indicating its fineness due to which it could be easily absorbed and assimilated in the body on its consumption.

Analytical study-

Moisture content: Moisture content of the KTB was less, indicating its more stability.

Ash value:

Ash value is the direct indicator of organic and inorganic content of the material. Ash is the residue of substance remaining after its complete incineration. The proportion of Ash remains constant for that particular substance. *Bhasma*, literally means Ash. KT, being inorganic in nature, its Ash value should be high. Ash value of raw KT was 53.46% while that of KTB obtained from ASS method was 55.87% and that-from VVP was 55.94%, which is comparatively high.

pH value:

Bhasma obtained from both the methods showed an alkaline pH. Higher alkalinity of the *bhasma* is probably due to higher calcium content indicating its higher acid neutralizing capacity.

SEM-EDAX:

This study showed that a portion of calcium present in the KT changed from carbonate form to its oxide form during the process of *marana*. This study also revealed that the percentage of heavy metal was decreased in bhasma, may be due to bhavana and marana procedures. The percentage of Calcium was found more in the

bhasma from VVP method than that of ASS method, probably due to the *shodhan-* and *bhavana* procedure.

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Table 1-Details of the recording of the temperature:

ASS method			VVP method		
Time	Temperature in degree Celsius		Time	Temperature in degree Celsius	
	I Puta	II Puta		I Puta	II Puta
8.00 am	20	35	8.00 am	22	36
9.00 am	108	140	9.00 am	128	144
10.00 am	223	222	10.00 am	223	220
11.00 am	456	435	11.00 am	450	440
12.00 am	540	574	12.00 am	545	570
1.00 pm	520	509	1.00 pm	521	510
2.00 pm	508	489	2.00 pm	508	480
3.00 pm	470	458	3.00 pm	470	450
4.00 pm	389	370	4.00 pm	370	370
5.00 pm	290	305	5.00 pm	280	321
6.00 pm	204	190	6.00 pm	200	198
7.00 pm	160	148	7.00 pm	140	150

Table 2 - Organoleptic characteristics:

Organoleptic parameter	KT (Raw Sample)	KTB from ASS method	KTB from VVP method

Rupa	*Ghana, Laghu, Shweta* (White)	*Laghu, Kapotvarni* (Grey coloured)	*Laghu, Kapotvarni* (Grey coloured)
Rasa	*Niswadu*	*Niswadu*	*Niswadu*
Gandha	*Visragandha* (Purtid smell)	*Nirgandha*	*Nirgandha*
Sparsha	*Kathin*(Hard)	*Mrudu*	*Mrudu*
Shabda	*Bhangur* (Fragile)	-	-

Table 3 - Analytical Observations:

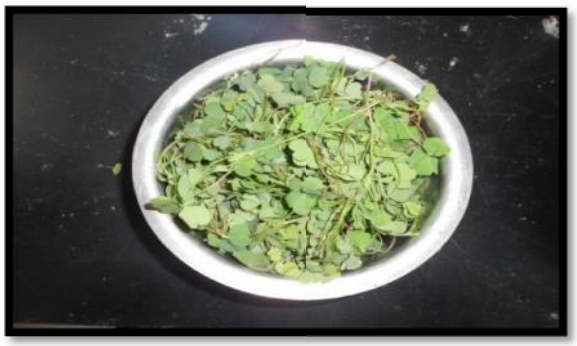
Analytical Test	Raw KT	KTB from ASS method	KTB from VVP method
pH	8.96	9.42	9.64
Moisture content	1.05	0.018	0.044
Ash Value	53.46	55.87	55.94
Acid insoluble ash	1.37	1.45	0.63

Table 4 - SEM-EDAX Analysis:

Element	Raw KT	KTB from ASS method	KTB from VVP method
C	53.83	24.55	22.01
O	35.54	58.26	56.14
Mg	0.23	0.27	0.26
S	1.48	0.03	0.10
Ca	8.00	16.89	21.17
Fe	0.06	0.04	0.08
As	0.08	0.02	0.02

Figure 1: Raw materials-ASS method

Kukkutandatwak



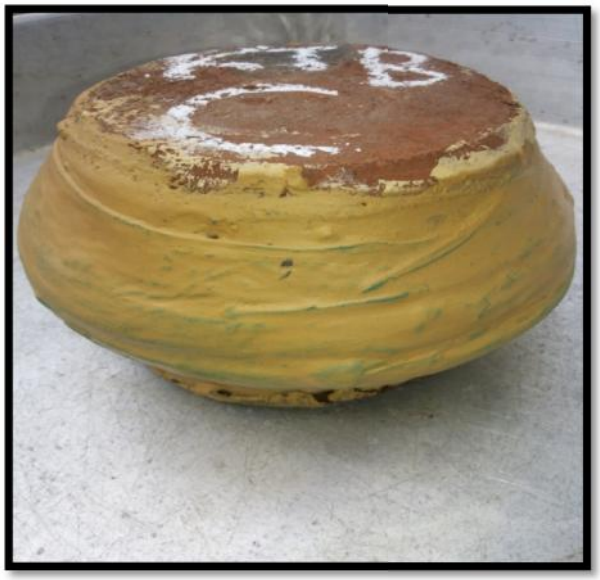
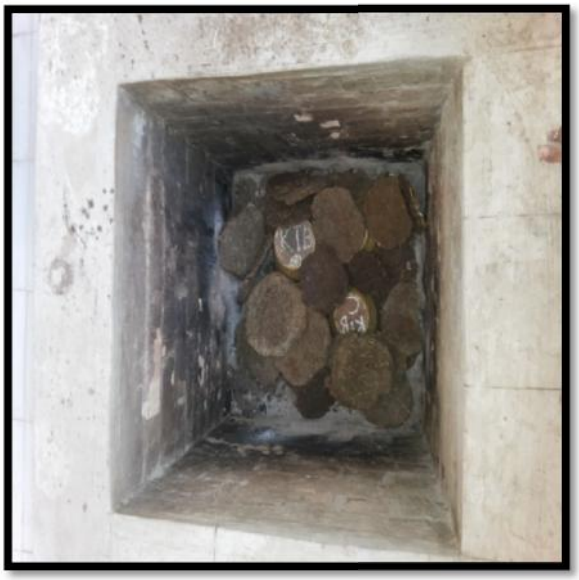
Leaves of Changeri

Powder of Kukkutandatwak

Changeri swara


Powder of Kukkutandatwak Changeri swaras poured into sharava Containing powder of KT

Figure 3: Puta Procedure

Sharav samputa **Sharav samput kept in Puta yantra**

Figure 4:KTB obtained from ASS method

Figure 5: Raw Materials- VVP method

KT

Takra

Kumari

Figure 6: KT dipped in Takra

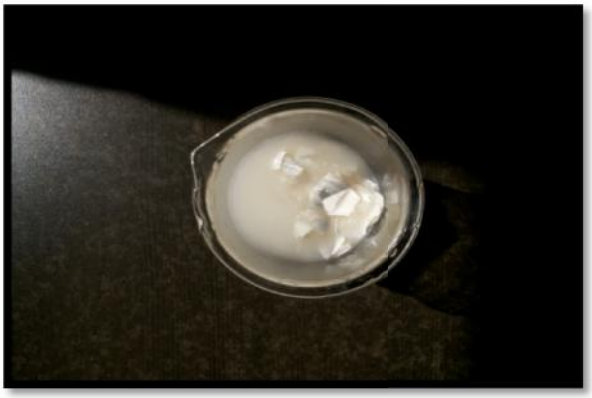


Figure 7: Bhavana of Kumari Swaras

Figure 8: Chakrika placed in sharava

Figure 9: KTB obtained from VVP method

Figure 10: Recording of the temperature using Pyrometer

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