IJPSR (2023), Volume 14, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 August 2022; received in revised form, 29 September 2022; accepted 03 November 2022; published 01 April 2023

PHARMACEUTICAL PROCESSING AND ANALYTICAL CHARACTERIZATION OF METAL-BASED AYURVEDIC DRUG "*TAMRA BHASMA*"

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Keywords:

Amritikarana, Calcination, Copper, Nada Yantra, Puta, Tamra Bhasma

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ABSTRACT: The preparation of metal-based Indian traditional drugs involves the conversion of pure metal into its therapeutically effective form. "*Tamra Bhasma*" (calcined copper) is one such herbometallic preparation used for various medical conditions like anaemia, obesity, and skin disease, disorders of the liver and spleen, hyper-acidity, rhinitis, hemorrhoids. A detailed evaluation of the changes occurring during each step of pharmaceutical processing, factors affecting the type of *Puta* and its physicochemical characterization has been examined in the present work. The pharmaceutical preparation of "*Tamra Bhasma*" (TB) includes quenching in liquid media, then boiling in liquid, followed by calcination and finally to remove the leftover blemishes, potentiation is carried out. Traditional *Puta* (the process of controlled heating and cooling) system has been used for its calcinations and *Kajjali* and *Gandhaka* have been used as media for calcinations of *Tamra*. TB has been subjected to different physicochemical characterization using XRD and SEM-EDXA. The result suggests that *Bhasma Parikshas* (confirmatory tests) are the most reliable criteria to authenticate the formation of a good quality final product. TB was prepared in 19 *Putas*, maintaining a temperature range of 500ºC - 600ºC. It is chemically Copper (II) sulphide, the compound formed is covellite as per XRD analysis and its particle size ranges between 10-50 µm as per SEM analysis. Cu and S are in major amounts 61.2 % wt. and 36.3 % wt. respectively; apart from that Si and Fe are also present in very fewer amounts as per EDXA findings.

INTRODUCTION: Ayurveda is an ancient traditional system of medicine in India that evolved and was practiced over thousands of years. Plants, animals and metals/minerals are used to prepare Ayurvedic drugs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(4).1940-53

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(4).1940-53

The Rasashastra is one of the parts of Ayurvedic pharmaceutics that deals with using metals/ minerals for therapeutic purposes.

Bhasmas (calx) are herbo-metallic ashes in which the metal is calcined along with various herbal/ mineral ingredients to form complexes [1]. They play a significant role in Ayurvedic therapeutics compared to herbal drugs because of their lesser dosage, palatability, and quick action [2]. These are the exclusivity of a properly prepared herbo-mineral/ metallic preparation, which is also safe,

readily absorbable, compliant and assimilable to the human body [3]. *Bhasmas* of copper, iron, silver, gold, tin, lead and zinc are commonly used to manage various ailments as a single drug with suitable *Anupana* (adjuvant) or as an ingredient in any formulation.

Copper is the only essential one in all organisms living under aerobic conditions, it plays a crucial role in several biochemical processes. Copper is a catalytic cofactor and a structural component for proteins; it is present in proteins and metalloenzymes implicated in both antioxidative activity and energy metabolism (cytochrome c oxidases); it takes part in the growth and development of bones, heart and brain, controls the metabolism of sugar and cholesterol, formation of erythrocytes, iron metabolism, synthesis of connective tissues, pigment formation, boosting immunity, free radical scavenging action, oxygen transport and cell signalling [4].

The calcined copper preparation of Ayurveda is known as TB. It is important amongst the metallic *Bhasmas* used for the treatment of various diseases like *Pandu* (Anaemia), *Sthaulya* (Obesity), *Kushtha* (Skin disease), *Yakrita-Pleeharoga nashaka* (disorders of liver and spleen), *Amlapitta* (Hyper-acidity), *Pinasa* (Rhinitis), *Arsha* (Haemorrhoids) *etc* [5]. Recent research explored its anti-hyperlipidemic action [6,] free radical scavenging activity [7], anti-oxidant [7], anti-inflammatory [8] and antimicrobial activities [9]. Since the Samhita period, *Tamra* (copper) was used in the form of "*Ayaskriti*" (fine powder) for therapeutic purposes [10].

After the emergence of RasaShastra, there was a detailed description of the pharmaceutical processing of metals and minerals to make them free from all blemishes. Preparation etiquette plays a major role in deciding the therapeutic efficacy as well as the toxic effects of *Bhasmas*. Moreover, *Apakwa* (improperly prepared) TB has been quoted as poison because of its hazardous effects on the body. To indicate its toxic potential, Ayurveda Prakasha has quoted *Ashtamahadoshas* (eight major ill effects) of TB [11]. Therefore, pharmaceutical procedures should be adopted stringently as per the Rasashastra classics to prepare good quality TB. Although various methods of TB preparations are described in Rasa literature, its preparation has always been a practical problem. In most methods, Gandhaka (sulphur) and Parada (mercury) are used. Different media used for the preparation of *Bhasma* plays a key role in the pharmaceutical and therapeutic potential of the final product. In the present study, pharmaceutical processing of TB was done as per Rasaratna samucchya [12], an authoritative textbook of RasaShastra mentioned in the Drug and Cosmetic Act 1940, Schedule I [13].

The pharmaceutical procedure of *Tamra Bhasma* incorporates *Samanya Shodhana* (generalized purification), *Vishesha Shodhana* (specific purification of *Tamra*), *Marana* (calcination) and *Amritikarana* (potentiation) using Traditional *Puta* [14] (*Puta* refers to a process of controlled heating and cooling of the herbo-mineral mixture to achieve *bhasmikarana*) system using *Parada* (mercury) and *Gandhaka* (sulphur) as media for *Marana*.

This study also attempts to exemplify the physicochemical properties of TB through conventional methods for testing the quality of *Bhasma*: *Rekhapurnatva* (particles of *Bhasma* enter in the furrows of a finger), *Nischandratva* (lusterless), *Varitaratva* (floating test), *Unama* (floating of a rice husk over the *Varitara Bhasma*), *Slakshanatva* (smoothness), *Amla pariksha* (curd test), *Avaami* (not producing nausea), *Apunarbhava* (metal irreversibility test) [15]. Along with this, detailed information on the elemental composition and particle size of TB has been evaluated using modern analytical instruments like X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray Analysis (EDAX).

MATERIAL AND METHODS:

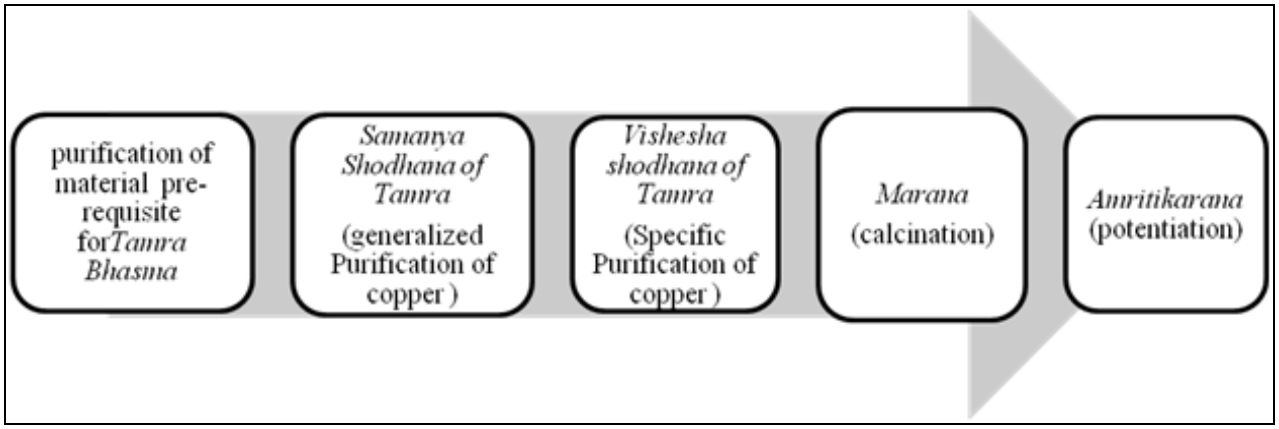
Procurement of Raw Material: Cu turnings 99.80% pure of Himedia (GRM6827-500G) laboratories private limited Mumbai, India. *Gandhaka* (Sulphur) and *Hingula* (Cinnabar) were purchased from the raw drug market in Varanasi. *Tila taila* (Sesamum edible oil, pasumark, *fssai* approved), *Kulattha* (seeds of *Dolichos biflorus Linn*.), *Goghrita* (clarified butter, Amul, *fssai* approved) *Nimbu* (*Citrus lemon Linn*.), *Surankanda* (the corm of *Amorphophallus campanulatus Linn*.) procured from the local market, Varanasi. *Gomutra*

(Cow's urine) was collected from a Dairy farm at the Institute of Agricultural Sciences, BHU, Varanasi.

Authentication of Raw Materials: Authentication of the raw drugs (*Tamra*, *Hingula*, *Gandhaka*) was done by the experts of the Department of RasaShastra and Bhaishajya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences, BHU, Varanasi. Herbal drugs were authenticated by the experts of the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, BHU, Varanasi and Uttar Pradesh, India.

Pharmaceutical Processing: The preparation of TB was carried out in the Laboratory of the Department of Rasa Shastra, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, in the year 2019-2020 by following the procedure as mentioned in Rasaratna Samucchya, authoritative textbook of Rasa Shastra, in Drug and Cosmetic Act 1940, Schedule I.

The pharmaceutical preparation of TB can be divided into the following steps:



Sulphur (*Gandhaka*) *Shodhana*: A stainless vessel with an 8 l capacity was filled with 4 l cow's milk and its mouth was fixed with a cloth. Cow's ghee was smeared on the cloth and then 1000 g of powdered *Gandhaka* was spread on the cloth. This vessel was kept in another stainless steel vessel having a volume of 10 l its mouth was covered with a convex-shaped pan and both were sealed airtight to prevent the escape of sulphur in the form of sulphur dioxide. Then the vessel containing milk was buried, and the upper surface was heated using 2kg of cow dung cakes (14 cow dung cakes). Finally, the melted sulphur was dropped into the milk and after the vessel got *Swangasheeta* (self-cooled), it was taken off and washed properly with warm water [16]. Washing in warm water was done thrice, to remove adhered milk stuck over it. Finally, purified *Gandhaka* was obtained **Fig. 1**.

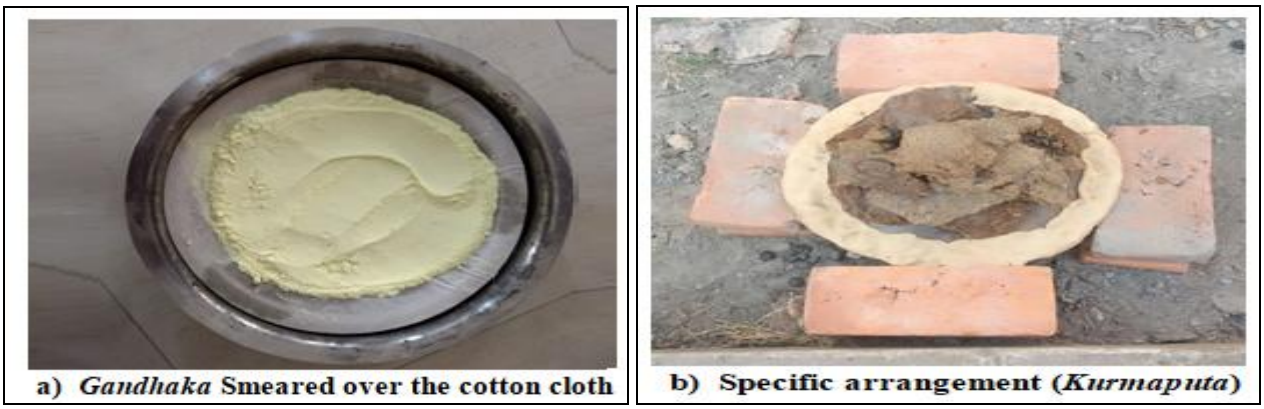


FIG. 1: SHOWING THE STEPS OF GANDHAKA SHODHANA

Cinnabar (*Hingula*) *Shodhana*: 1000 g of *Hingula* was taken in clean *Khalva Yantra* made up of granite (mortar having length: 36 cm, breadth: 21 cm, thickness: 3 cm, depth: 11 cm; pestle with length: 13 cm and diameters: 7 cm) and was pounded well to form a coarse powder. After that,

Bhavana (wet grinding) was done with *Nimbu Swarasa* until the whole material dried up and attained a paste-like consistency. A similar process was repeated seven times and finally, the *Hingula* was washed and dried up well in sunlight [17].

Extraction of *Parada* (mercury) from *Hingula* (cinnabar) by using *Nada Yantra* (Traditional Earthen Apparatus): In the Rasa classics, it was mentioned that *Parada* extracted from *Shodhita Hingula* is equivalent to *Shodhita Parada* [16]. 1000 g of *Shodhita Hingula* was taken, and an equal quantity of paper and cloth was taken in total. The paper was arranged in a square and *Shuddha Hingula* powder was uniformly sprinkled on it. The whole arrangement was made into bolus form and cloth was wrapped over it. Later it was kept in *Sharava* (an earthen vessel having a length of 7 cm and diameter of 15 cm) and coal (2.5 kg) was ignited. After proper ignition, the bolus wrapped in cloth was placed over the *Sharava*.

It was covered with *Nada* (dome-shaped earthen vessel), so there was no air entry between *Nada* and *Sharava.* The entire arrangement was left overnight for the process to be completed. The next morning, *Parada* was collected on the inner side of *Nada* with black soot deposited on it and was carefully collected with the help of a brush and cloth [17]. The arrangement of the traditional earthen apparatus (*Nada Yantra*) was shown in **Fig. 2.**

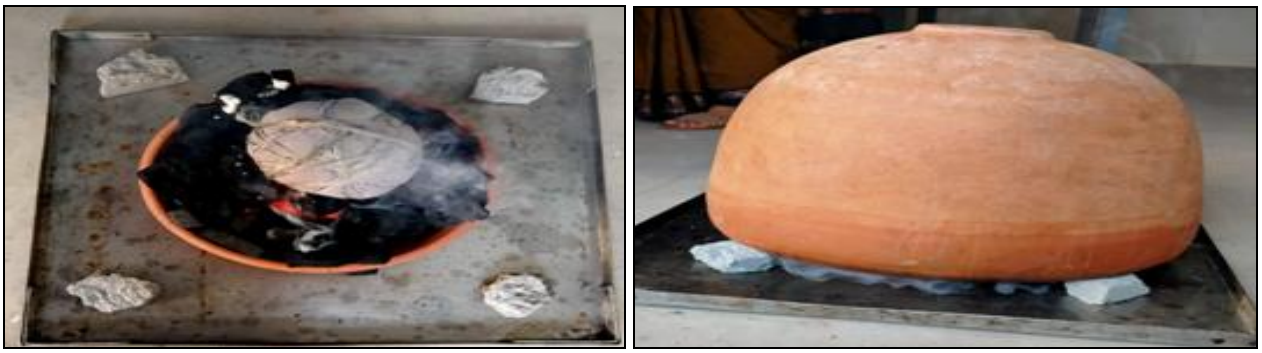


FIG. 2: EXTRACTION OF *PARADA* FROM *HINGULA* BY USING *NADA YANTRA*

***Samanya Shodhana* of *Tamra* (Cu turnings):** In this procedure, *Nirvapa* (heating and quenching) was done, it was executed as per the classical reference [18]. Raw *Tamra* (Cu turnings) weighing 500 g taken for *Samanya Shodhana* was heated in Electric Muffle Furnace (EMF) till red hot condition (750-800˚C) and immersed in 500ml of each medium viz. *Tila taila* (sesame oil), *Takra* (buttermilk), *Gomutra* (cow's urine), *Kanji* (rice gruel), and *Kulattha kwatha* (decoction of horse gram). This quenching process was repeated seven times in *Tila taila* followed by seven times consecutively in *Takra*, *Gomutra*, *Kanji*, and *Kulattha kwatha* by using fresh media every time, as shown in **Fig. 3**. After completion of the process, the material was filtered by cotton cloth and dried under sunlight. The material obtained at this stage was called *Samanya Shodhita Tamra* (Cu turnings).

FIG. 3: SHOWING THE STEPS INVOLVED IN *SAMANYA SHODHANA* OF *TAMRA*

***Vishesha Shodhana* of *Tamra* (Cu Turnings):** *Vishesha Shodhana* of *Tamra* (Cu turnings) was done as per the reference mentioned in Rasa Ratna Sammucchya [19]. *Samanya Shodhita Tamra* was

taken and placed on the Cora cloth. All the cloth edges were joined and tied with a thick thread to convert it into the form of *'Pottali'* as shown in **Fig. 4**. The '*Pottali'* was suspended using an iron spatula in a stainless steel vessel. Cow urine (*Gomutra*) was added to the vessel so that the entire Pottali was immersed. This apparatus was heated on mild/moderate heat for 3 h. The *Pottali* was removed and dried under sunlight, Tamra was taken, and the final weight was noted. The total quantity of *Gomutra* required during the whole process was 3.35 l.

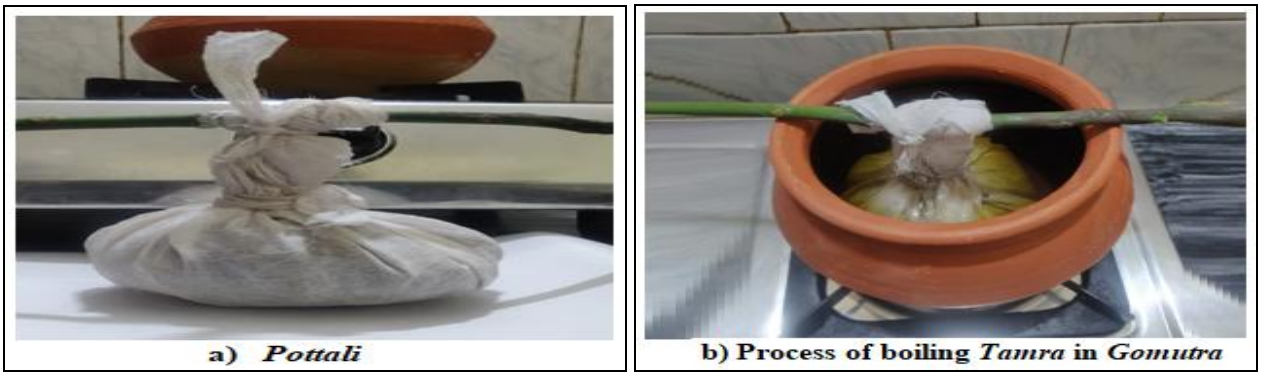


FIG. 4: SHOWING THE "*POTTALI*" AND PROCESS OF *VISHESHA SHODHANA* OF *TAMRA*

***Marana* (Calcination):** *Marana* means repeated cycles of high-temperature calcination in a sealed earthen pot[20]. During this process, the first step was *Bhavana* with certain herbal juices and further subjected to a specific amount of heat to convert them into the form of *Bhasma*.

In the present study 150 g of *Shodhita Tamra* was wet ground with an equal quantity of *Kajjali* (black sulphide of mercury obtained from trituration of *Shodhita Parada* and *Shodhita Gandhaka* until got lustreless), and *Nimbu swarasa* in sufficient quantity until all liquid media got dried. Then *Chakrikas* (pellets) were prepared from that dried material and kept in sunlight for drying.

After the complete drying of *Chakrikas*, these were arranged in *Sharava* and covered with another inverted *Sharava*. The space between the two *Sharavas* was covered with clay-smeared cloth; this specific process was known as *Sharava Samputikarana* (sealed earthen pot) [21]. After that, it was subjected to Traditional *Puta* in a pit measuring (57 X 57) cm using cow-dung cakes **Fig. 5**. In this study, a total of nineteen *Putas* were given to form TB, passing all completion tests as specified in Rasa classics.

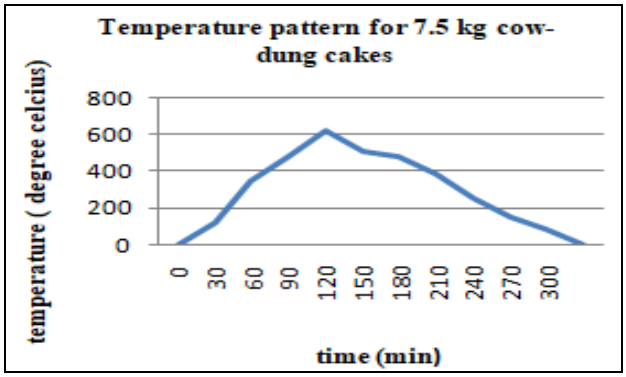
The heating pattern was followed per the reference of Rasamrita [22], i:e decreasing order of heating pattern. In the first six *Putas*, 7.5 kg cow-dung cakes were used; in further six *Putas*, 5 kg cow-dung cakes were used; in added seven *Putas*, 2.5 kg of cow-dung cakes and last two *Putas* 1.5 kg were used. The temperature range and its maintenance period were illustrated in **Table 1,** and **Graphs 1** and **2, 3, 4** given below.

After 19 *Putas*, *Bhasma* formed was *Krishna varna* (blackish), soft, lustreless, passing all the *Bhasma Parikshas* (confirmatory-tests) such as *Rekhapurna, Nishchandra, Varitara, Unama, Anunarbhava, Avami, Amla Pariksha* etc. prescribed in classical texts [15].

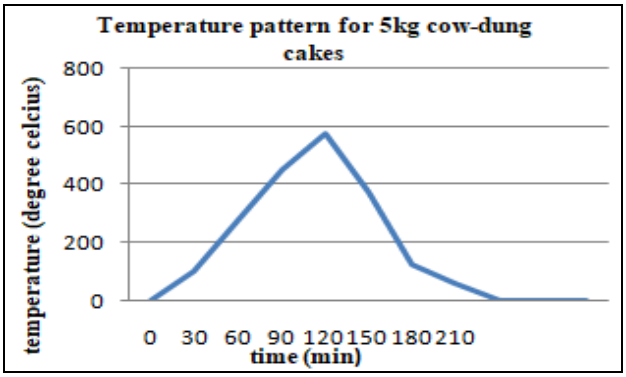
FIG. 5: SHOWING STEPS INVOLVED IN THE MARANA OF TB.

TABLE 1: RELATION BETWEEN FUEL QUANTITY AND MAINTENANCE OF TEMPERATURE RANGE

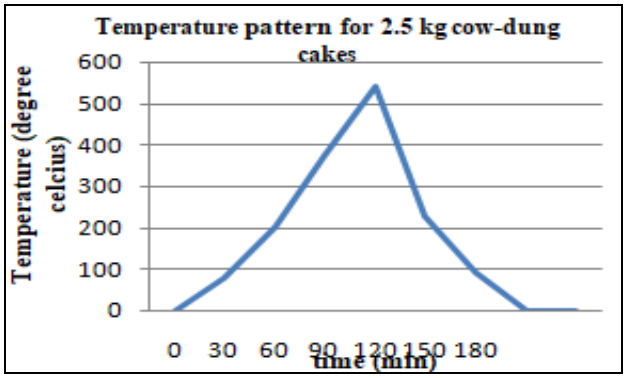
Weight of cow dung cakes in kg	Duration of time up to which temp between 500° C to 600° C is maintained in min
7.5	50
5	25
2.5	15
1.5	10



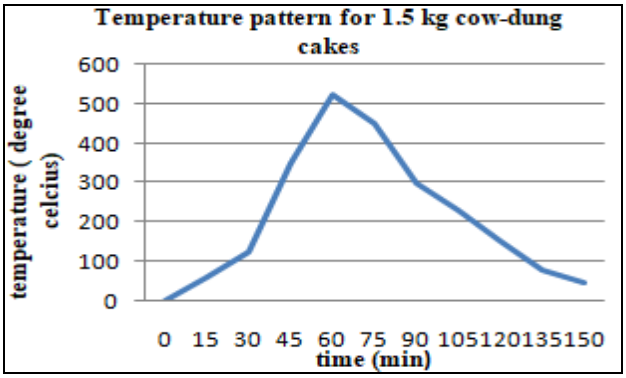
GRAPH 1: TEMPERATURE PATTERN FOR 7. KG COW-DUNG CAKES



GRAPH 2: TEMPERATURE PATTERN FOR 5KG COW-DUNG CAKES



GRAPH 3: TEMPERATURE PATTERN FOR 2.5 KG COW-CAKES



GRAPH 4: TEMPERATURE PATTERN FOR 1.5 KG COW DUNG CAKES

***Amritikarana* (Potentiation of Calcined Copper):** After *Marana*, a special procedure called *Amritikarana* was done to remove any residual blemishes leftover in the calcined *Bhasmas*.

It potentiates the qualities of *Bhasmas* and makes it more potent and free from the *Doshas* (flaws). TB was triturated with ½ part of *Shuddha Gandhaka* and *Nimbu Swarasa* was added in sufficient quantity for proper trituration; a round bolus was prepared and kept for drying in sunlight. *Surana Kanda* was cut into two halves horizontally, the bolus was kept inside and later the two halves were joined together. A thick layer of fuller earth with cloth was wrapped over it. After drying, it was kept in traditional *Puta*, using 7.5kg of cow-dung cakes [23] **Fig. 6.**

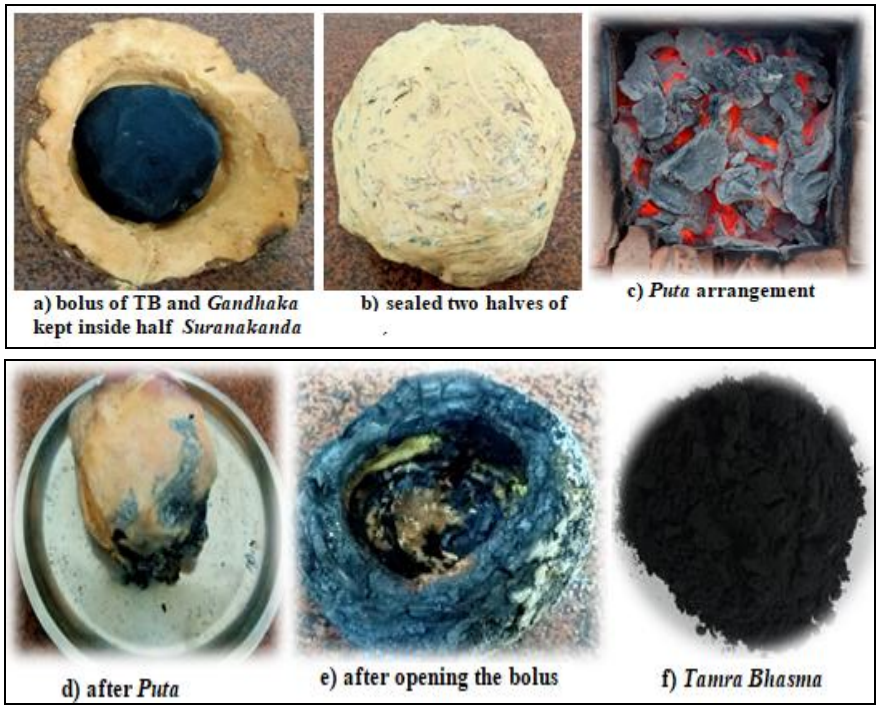


FIG. 6: SHOWING THE STEPS OF *AMRITIKARANA* OF TB.

Characterisation of TB: The prepared *Bhasma* was characterized by both the traditional and modern analytical methods. Different physical tests mentioned in the literature such as *Nishchandratvam* (lusterless), *Rekhapurnatvam* (particle size enters in the furrows of a finger), *Varitara* (floating of the product on water), *Unama* (floating of rice husk over the Bhasma floating on water), *Avaami* (not producing nausea/ vomiting on consumption), *Amla Pariksha* (curd test), *Apunarbhava* (metal irreversibility test) were examined. Various physicochemical parameters were analyzed for colour, taste, texture, loss on drying, ash value, acid insoluble ash and water-soluble ash [24].

The Prepared TB was Analyzed using Various Sophisticated Instruments: The XRD patterns of the solid samples were recorded on Rigaku Ultima IV powder X-ray diffractometer using Cu K radiation filtered by a nickel foil over the range of diffraction angle 3.0–50.0°. The wavelength of the radiation used was 1.5405A°.

Surface analysis of particles was done using a Scanning electron microscope (SEM) (Carl- Zeiss-EVO 18 research model), *Bhasma* was coated by ultrathin electrically conducting gold and palladium mixture alloy deposited on the sample by sputter coating with Hummer V sputter coater. The micrographs were obtained with an applied 10.00 kV electron beam, and the distance between the sample and electron source was 4.5 mm with a magnification of 50 kx. Quantitative elemental composition was carried out by EDAX (Zeiss EVO 18 EDAX Instrument).

RESULTS: *Samanya Shodhana* of *Tamra* involved the sequential quenching method in different media by following conventional order. After quenching in *Til taila*, the colour of copper turnings became dull and some blackish deposition was observed over the surface of Cu turnings. A burping sound was produced as the red hot material was immersed in *Takra*, it turned to curdle and the appearance of a few copper turnings was like a thin sheet.

After quenching in *Gomutra* peculiar smell was observed, and the colour of Cu turnings was changed to greyish black. A pungent smell and fussing were observed when the material was engrossed in *Kanji*. The maximum part was in powder form after quenching in *Kulattha kwatha*. Metallic lustre was lost after each step of quenching and the material became brittle, which will facilitate further processing. After *Samanya Shodhana*, a total of 4.6% weight gain was observed in the sample **Table 2,** and a change in the pH after quenching in each liquid media was

also observed **Table 3**. For the process of *Vishesha Shodhana*, 450 g of *Samanya Shodhita Tamra* was taken. After this process, brittleness and weight were increased and it was converted to small particles having black colour **Table 4.**

TABLE 2: VARIATION IN WEIGHT AFTER *SAMANYA SHODHANA* OF *TAMRA*

Media	Wt. Before *Shodhana* (gms)	Wt. After *Shodhana*(gms)	Loss/ Gain (gms)	%Loss/ Gain
Til Taila	500	511	11gain	2.2
Takra	511	522	11 gain	2.1
Gomutra	522	513	09 loss	1.72
Kanji	513	518	05 gain	0.97
Kulattha Kwatha	518	523	05 gain	0.96

TABLE 3: PH BEFORE AND AFTER *SAMANYA SHODHANA* OF *TAMRA*

Media Used	pH Before *Shodhana*	pH After *Shodhana*
Til Taila	6.5	6.1
Takra	3.42	3.51
Gomutra	7.44	8.0
Kanji	3.0	2.5
Kulattha Kwatha	6.09	8.0

TABLE 4: WEIGHT AND PH AFTER *VISHESHA SHODHANA* OF *TAMRA*

Particulars	Sample
Initial Wt. of *Samanya Shodhita* sample	450gms
Final wt of sample	453gms
% gain	0.6%
the pH of cow's urine before *Vishesha Shodhana*	7.5
the pH of cow's urine after *Vishesha Shodhana*	8.3

During the *Marana* process, the initial weight of *Shodhita Tamra* was 150 g and in the first three *Putas* (cycles of calcinations), an equal amount of *Kajjali* was added and *Shodhita Tamra* was triturated with *Nimbu swarasa* to form *Chakrikas*. In the first *Puta*, it took 3 h to get converted into the paste form, and it was difficult to make *Chakrikas* out of it, while in later *Putas*, as the material gradually became soft, less time was required for trituration. After three *Putas*, an equal quantity of *Gandhaka* was added instead of *Kajjali* till the nineteenth *Puta*. After each *Puta*, chakrikas were formed easily probably due to a reduction in particle size. Specific change was also observed in the physical appearance and weight of the Chakrikas as shown in **Tables 5** and **6**. After nineteen *Putas*, a good quality TB was formed, as it passed all the *Bhasma Parikshas i.e*; *Nishachandra*, *Rekhapurna*, *Sukshma*, *Varitara*, *Unama*, *Avaami*, *Amla Pariksha*, *Apunarbhava* as shown in **Fig. 7**. Stepwise assessment of *Bhasma Pariksha* was shown in **Table 7**. *Amla Pariksha* is specific for the metals/ minerals containing copper as an ingredient.

TABLE 5: WEIGHT OF TB AFTER EACH CYCLE OF CALCINATIONS

Puta number	Wt. of cow dungs (in kg)	Media *Nimbu swarasa* (in ml)	Wt. of *Kajjali* or *Gandhaka* (in g)	Initial wt. of the product (in g)	Final wt. of the product (in g)	loss/ gain	% loss / gain
1st	7.5	120	150 *Kajjali*	150	160	10 gain	6.66%
2nd	7.5	120	150 *Kajjali*	160	158	2 loss	1.25%
3rd	7.5	110	150 *Kajjali*	158	156	2 loss	1.26
4th	7.5	110	150 *Gandhaka*	156	153	3 loss	1.92
5th	7.5	100	150 *Gandhaka*	153	150	3 gain	1.96
6th	7.5	100	150 *Gandhaka*	150	148	2 loss	1.33
7th	05	100	150 *Gandhaka*	148	150	2 gain	1.35
8th	05	90	150 *Gandhaka*	150	153	3gain	2.00
9th	05	90	150 *Gandhaka*	153	150	3loss	1.96
10th	05	90	150 *Gandhaka*	150	154	4gain	2.66
11th	05	90	150 *Gandhaka*	154	151	3loss	1.94
12th	05	80	150 *Gandhaka*	151	152	1gain	0.66
13th	2.5	80	150 *Gandhaka*	152	148	4loss	2.63

14th	2.5	85	150 *Gandhaka*	148	146	2loss	1.35
15th	2.5	85	150 *Gandhaka*	146	149	3gain	2.05
16th	2.5	80	150 *Gandhaka*	149	145	4loss	2.68
17th	1.5	80	150 *Gandhaka*	145	148	3gain	2.06
18th	1.5	70	150 *Gandhaka*	148	148	--	No change
19th	1.5	70	150 *Gandhaka*	148	148	--	No change

TABLE 6: OBSERVATIONS AT EACH STEP OF CALCINATION OF TB.

***Puta* number**	**Colour change**	**Other observations**
1st	Black, mild yellow	Pellets were hard and shiny, the upper surface was gritty
2nd	Half chakrikas were dull red and grey, yellow	Pellets were hard and shiny, the surface was smooth now.
3rd	Half *chakrikas* were dull red and grey, yellow	The hard, shiny, periphery of a few *chakrikas* was an orange and dull red.
4th	Black and dull red	Hard, the shine was less compared to previous *Puta*, the upper surface of C*hakrikas* was dull black and the lower surface was a dull red.
5th	Black and dull red	Hard, the shine was less comparatively previous *Puta*, the upper surface of *Chakrikas* was dull black and the lower surface was dull red
6th	Black and maroon	Hardness and shine were less comparatively previous *Puta*, Patchy appearance of maroon colour over the *Chakrikas*,
7th	Black, red mix	Fewer *Chakrikas* were red, the only centre part of few *Chakrikas* was orange in colour, hardness and shine comparatively to previous *Puta*
8th	Black, dark maroon	Most of the *Chakrikas* are black, and only a lower portion of a few chakrikas was red, hardness and shine comparatively previous *Puta*
9th	Black, dark maroon	Most of the *Chakrikas* are black, the only lower portion of few *Chakrikas* was red, hardness and shine comparatively previous *Puta*
10th	Black, maroon, orange	Most *Chakrikas* are maroon and red in colour, hardness and shine comparatively previous *Puta*
11th	Black, maroon	Black with few *Chakrikas* was maroon in colour, hard comparative to previous *Puta*, no shine
12th	Black, maroon.	Black with few *Chakrikas* are maroon in colour, soft, no shine
13th	Black, mild maroon	Black with few *Chakrikas* was maroon in colour, more soft compared to previous *Puta*, no shine, rough in texture,
14th	Black, mild maroon	Black with few *Chakrikas* was maroon in colour, soft, with no shine
15th	Black	All *Chakrikas* were black, softer than the previous one, with no shine,
16th	Black	All *Chakrikas* were black, softer than the previous one, with no shine,
17th	Black	All *Chakrikas* were black, softer than the previous one, with no shine,
18th	Black	All *Chakrikas* were black, softer than the previous one, with no shine,
19th	Black	All *Chakrikas* were completely black, very soft, and fine, no shine was present

TABLE 7: THE STATUS OF *BHASMA PARIKSHA* AFTER EACH CYCLE OF CALCINATION OF TB.

***Puta* number**	***Nishchandra***	***Rekhapurna***	***Varitara***	***Sukshma***	***Unama***	***Avami***	***Apunarbhava***	***Amla pariksha***
1st	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2nd	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3rd	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
7th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
10th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
11th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12th	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
13th	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
14th	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
15th	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve

16th	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve
17th	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve
18th	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve
19th	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

FIG. 7: *BHASMA PARIKSHA* FOR "TB".

Finally prepared TB underwent the *Amritikarana* procedure to remove any remaining leftover flaws, the quantity of TB taken for *Amritikarana* was 130 g and the final product obtained was 148 g. In the process of *Amritikarana*, three hours of trituration were required for the formation of a bolus. After *Putapaka*, *Surana* was found burnt. Bolus inside was black and breakable by some pressure. After trituration, it converted to a black, smooth, and fine powder. All *the Bhasma Parikshas* were positive and the colour of the bolus was blackish. Weight variations after each *Puta* were shown in Table [8]. The results of various physiochemical parameters colour, taste, texture, loss on drying, ash value, acid-insoluble ash, and water-soluble ash are shown in **Table 9.**

TABLE 8: WEIGHT AFTER *AMRITIKARANA* OF TB.

No. of *Puta*	Initial Wt. of *Tamra bhasma* (in gms)	Final Wt. of *Bhasma* (gms)	Loss/ gain (gms)	% loss / gain
1	130	148	18 gain	13.84

TABLE 9: PHYSICO-CHEMICAL ANALYSIS OF TB.

Sr. no.	Tests	Results
1.	Colour	*Krishna Varna*
2.	Taste	Tasteless
3.	Texture	Smooth
4.	Loss on drying (%)	0.18
5.	Total Ash value (%)	97.69
6.	Acid Insoluble Ash (%)	1.79
7.	Water-soluble Ash (%)	0.80

The XRD pattern of "TB" was shown in **Fig. 8**. Diffraction peaks at 2θ = 29.218, 31.748, 32.847, 47.929, 52.646 and 59.280◦ are identical to those reported for the standard cupric sulphide [25]. The compound identified is covellite, having the same XRD pattern as the sample drug "TB". The XRD pattern confirms the presence of CuS (Hexagonal) as the major crystalline phase in the sample. The other researchers also proved the similar chemical composition of TB prepared by the aforesaid method [26].

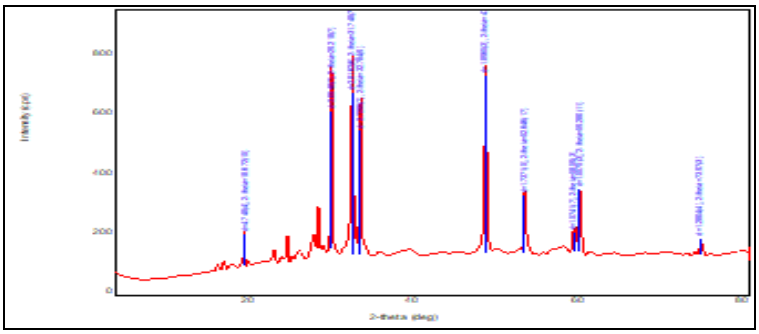


FIG. 8: SHOWING XRD PATTERN OF "TB".

SEM images of TB are shown in **Fig. 9**. The photographs show spongy, relatively compact microcrystalline aggregates with loss of grain boundaries. The average size of the cluster observed is in the range of 10– 50 µm, due to the agglomeration of the crystals, which are also covered by the small crystallites. The agglomeration occurs because these particles have a high surface area to volume ratio, so they tend to get agglomerated and neutralize this high surface energy [27]. It has been reported that the preparation method also influences the particles' morphology, particularly the range and duration of temperature provided to the material during calcination [28].

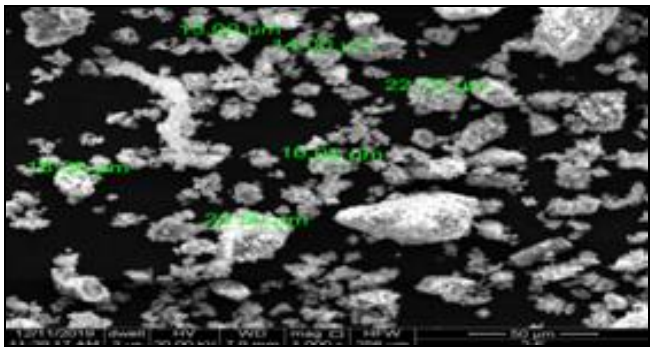


FIG. 9: SEM IMAGE OF "TB" AT 1000 X MAGNIFICATION

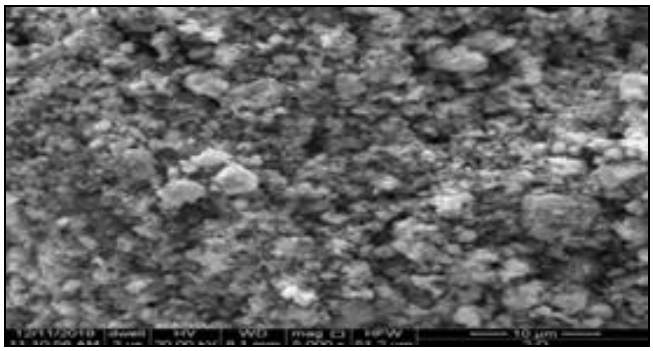


FIG. 10: SEM IMAGE OF "TB" 5000X MAGNIFICATION

EDXA analysis showed the percentage of Cu was 61.2 % by weight, and the percentage of S was 36.3 % by weight in the sample of TB. Apart from that, certain trace elements are also present, *i.e.*, Si 1.2% by weight and Fe 1.3% by weight in the given sample.

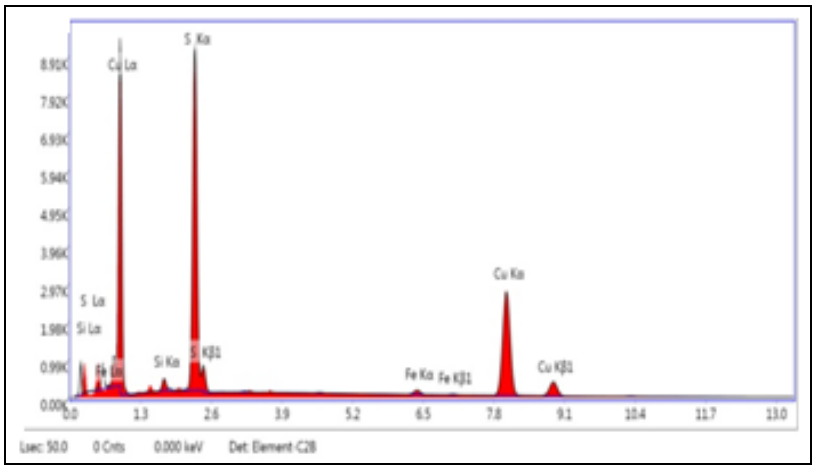


FIG. 11: SHOWING THE EDXA OF "TB".

TABLE 10: THE ELEMENTAL COMPOSITION OF "TB".

Element	Weight %	Atomic %	Error %
Si K	1.2	2.0	14.2
S K	36.3	52.3	4.9
Fe K	1.3	1.1	17.8
Cu K	61.2	44.5	2.4

DISCUSSION AND CONCLUSION: The preparation of Ayurvedic *Bhasmas* is a very intricate process that requires pharmaceutical processing, as mentioned in the classical Ayurvedic texts as well the expertise in this field. The main challenge faced by herbometallic/ herbomineral formulation is safety issues. Consequently, the processing of the *Bhasma* should be given prime importance, starting from authentication of the raw drugs until the formation of the desired product. In the present study, TB was prepared following all the steps – *Shodhana, Marana* and *Amritikarana*. During *Samanya Shodhana*, Cu turnings were heated and quenched into various liquid media having an alteration in their pH. The use of the acidic and alkaline solution as cooling media in the quenching process affects the hardness of the metal [3]. Furthermore in *Vishesha Shodhana* too, Cu turnings were heated in cow's urine, heating the metal will displace atoms from their position and

the displaced atoms form a new structure. This process was known as allotropic phase transformation. It alters the hardness, strength and ductility of the metal [29], increases brittleness, reduces the tensile strength and reduces the particle size of Cu turnings, which will aid the further processing of *Bhasma*.

Marana of *Shodhita Tamra* was done by triturating it with *Samaguna Kajjali* and *Nimbu Swarasa* and subjected to the traditional *Puta* system. *Nimbu Swarasa* is the best chelating agent [30] to remove metallic impurities and thus helps enhance TB's therapeutic efficacy. This method was selected because the *Parada Marita Bhasma* was considered to be the best as per the reference of classical texts of RasaShastra [31]. While after the third *Puta* only *Gandhaka* was added because, as per the classical reference, in the context of the general *Maraka dravyas* for *Dhatus* (substances antagonistic to specific metals), *Gandhaka* was considered as *Maraka dravya* for *Tamra* [32]. TB containing *Gandhaka* formed as copper (II) sulphide, as per the previously published research work, this form was considered therapeutically best, was non-toxic and thus highly effective. In the process of *Amritikarana* also, *Gandhaka* was added as *Maraka dravya*. Henceforth, all these points favour the addition of it in the later cycles of calcinations. Instead of EMF, traditional *Puta* was chosen for the preparation of TB to understand the concept of *Puta*, factors affecting the type of *Puta* such as weight of the material, number or weight of cow dung cakes to be used, atmospheric conditions, duration of heating and self-cooling. In the classical textbooks of RasaShastra, the classification of *Puta* relied on two criteria: the dimension of the pit and the number of cow dung cakes. Also, Rasatarangini mentioned that the type of *Puta* to be given should provide a sufficient amount of heat as required by the material [33]. It means that other aspects should be looked upon apart from these two basic parameters. The basic heat transfer equation can understand the relation between the energy required and mass.

According to the equation of Heat Transfer:

$$Q = ms\Delta T$$

Where Q = amount of heat supplied, m = mass of the substance: **s** = specific heat capacity (it is constant as the material is the same during the process), T= change in temperature.

Here, Q (amount of heat supplied) is directly proportional to m (the mass of the substance).

The amount of heat required by the material is directly proportional to the quantity of that particular material, and here cow dung cakes were used as a source of heat.

Along with this, the atmospheric conditions will also affect the process. In a hot climate, self-cooling will occur late, and vice versa in cold weather. Hence fuel requirement varies depending upon the climate. The quality of cow dung cakes used also affect the process of *Puta.* In the previous research, it was given that the cow dung cakes containing more cow dung and less saw husk had more calorific value than one having more husk[34]. The quality of cow dung cakes will also affect the *Puta* system.

It was observed during the study that cow dung cakes weighing 7.5, 5.0, 2.5 and 1.5 kg were used depending upon the physical characteristics of *Chakrikas* after every *Puta*. As the weight of cow dung cakes was reduced, *Bhasma* became soft, lusterless, and blackish. It was also observed during the study that by increasing the number of cow dung cakes, the colour of the pellets turned red, which indicated copper (I) oxide formation and was undesirable for consumption. The colour of TB should be *Krishna varna* (blackish) as mentioned in classical textbooks of Rasashastra. Previous research also showed that the colour of TB was blackish. It indicates the compound formed is either CuS or CuO [3, 26]. The sulphide form of copper is non-toxic and therapeutically effective. The XRD analysis of the present study also revealed that the compound formed is CuS.

The number of cow dung cakes to be given for the next *Puta* was decided by observing the product of the previous *Puta*. The maximum temperature range was approx 500-650°C for the four sequential weight variations adopted as given in the material and method section. The important difference was the duration of that particular range of temperature. It was inferred from **Table 1** that increased weight directly influences the duration of the temperature, hence depending upon the amount of material and

nature of the material (metals or minerals) such as its hardness, melting point, boiling point, specific gravity and density, the weight of cow dung cakes should be selected. The most reliable criteria for the *Bhasma* formation were the completion tests (*Bhasma Siddhi Lakshanas*). *Bhasma* passing all these criteria could be used therapeutically.

After the nineteenth *Puta*, TB passed all the *Bhasma Siddhi Lakshanas* with 100%, *Rekhapurnatva*, 100% *Varitaratva*, *Nishchandratva*, *Unama*, *Avaami*, *Amla Pariksha*. Positive *Amla pariksha* indicates the absence of free copper or copper sulphate. The colour of *Bhasma* was *Krishna varna* (blackish) which was due to the formation of copper sulphide (black) as evidenced in XRD and EDXA analysis. In the process of *Amritikarana*, 13.18% gain in weight was observed, it might be due to the toting up of some organic (calcium oxalate crystal) content from the *Amorphophallus campanulatus* (*Surankanda*) [35] and also some amount of sulphur leftover that was evidenced by the **Table 10** of SEM analysis. Organic matter present in the tuber could act as a source of carbon. The carbon reduction process could reduce any unstable metallic compound (especially oxides) to the metallic state, which will further react with sulphur and form copper (I) sulphide [36]. Therefore, this process further crafted TB to be more stable chemically as well as therapeutically. By the practical observation from the present study, it can be suggested that for the preparation of 150 g of TB, in the first 3 *Putas* 7.5 kg cow dung cakes should be given and later on, in each *Puta* 500 g will be reduced till the attainment of completion tests (*Bhasma Siddhi Lakshanas*).

Several physical and chemical parameters have been described for ascertaining the purity of TB. Physical parameters include lustre, colour, fineness, floatability, *etc*., while chemical parameters include a test of TB for irreversibility to a metallic state, floatability test, *etc*. In this study, after a quality check done by classical parameters, TB was subjected to different physicochemical characterization studies using modern analytical tools. Our results showed negligible moisture content (0.18% loss on drying), total ash value (97.69%), water-soluble ash (0.80%) and acid-insoluble acid (1.79%) **Table 9**. The results are comparable to the reported value [26]. A lesser percentage of loss of drying indicates minimum chances of microbial contamination due to moisture content, hence more stability of TB. The total ash value is useful in determining the purity of *Bhasma* and signifies the presence of more inorganic contents in the *Bhasmas* [37]. As TB was having a composition of Cu and S and only traces of Si and Fe as evidenced in EDXA analysis, it also favoured the same. The lower the acid-insoluble ash higher will be bioavailability of the drug [38]. XRD analysis shows that 29.218, 31.748, 32.847, 47.929, 52.646 and 59.280◦ peaks present in "TB" indicate the formation of CuS (covellite). The SEM images of TB revealed its non-homogenous character; particles were small, having a size ranging between 10-50µm and most of the particles were agglomerated, which might be because these particles have a high surface area to volume ratio so they tend to get agglomerated and neutralize this high surface energy [39]. EDXA analysis showed Cu -61.2% by weight, S- 36.5% by weight, Si-1.2% by weight, and Fe- 1.3% by weight. As copper was the prime constituent of the process, its percentage was more, sulphur was added half of it so it is also in major amount. Other elements, such as iron and silica, are present in small amounts because lemon juice contained Fe as its chemical constituent and was used for the trituration before each cycle of Marana [40]. The presence of silica might be due to the earthen pots and mortar pestle used in the incineration steps [41].

ACKNOWLEDGEMENTS: The authors are thankful to Dr. N. V. Chalapathi Rao, Professor, Department of Geology, Banaras Hindu University, for providing lab facilities for SEM and EDXA analysis and also to the technical staff of the Department of Chemical Engineering, Indian Institute of Technology, Banaras Hindu University for providing lab facilities for XRD analysis.

CONFLICT OF INTEREST: None

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How to cite this article:
Gulati S, Joshi N and Jayakumar R: Pharmaceutical processing and analytical characterization of metal-based ayurvedic drug "*Tamra Bhasma*". Int J Pharm Sci & Res 2023; 14(4):1940-53. doi: 10.13040/IJPSR.0975-8232.14(4).1940-53.

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