



Research Article

PHARMACEUTICAL AND PHYSICO CHEMICAL ANALYSIS OF *TUTTHA BHASMA*

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ABSTRACT

Rasa sastra is an ancient science dealing with various drugs of mineral and metallic origin *Tuttha* (copper sulphate) is a mineral useful in various clinical conditions externally as well as internally. According to *Rasasastra*, through the process of *Shodhana* (purification) and *Marana* (incineration) the harmful effects of *Tuttha bhasma* (incinerated copper sulphate) is nullified resulting in the formation of a newer compound that is therapeutically more potent. To prepare *Tuttha Bhasma* by adopting standard manufacturing procedure explained in Ayurvedic texts as well as to study its physical and chemical characters using traditional and modern analytical tools. The pharmaceutical processing of *Tuttha bhasma* was carried out in three *Kukkuta puta* (incineration) with a peak temperature of 610^{0C} according to *Rasa Tarangini* reference. Physico chemical analysis, Energy-Dispersive X-ray Fluorescence (EDXRF), X-ray Diffraction (XRD), Particle Size Analysis (PSA) were conducted. The final product shows presence of nano particles which was confirmed by particle analysis. XRD results of *Tuttha bhasma* revealed the presence of copper sulphide (covellite) with hexagonal lattice and sodium sulphate with orthorhombic structure. All of the preparation stages and changes in the properties were documented and validated, and they may now be used as a valuable tool for standardization and quality assurance of *Tuttha bhasma*.

INTRODUCTION

Tuttha is a mineral categorized under "Maharasa" in the text *Rasa Ratna Samucchaya* [1] and under "Upadhatwadi Vignaneeya" in *Rasa Tarangini*. Chemically it is identified as copper sulphate [CuSO₄.5H₂O] [2].

The aim of pharmaceutical study is to produce good quality drug. It mainly depends upon the standard operative procedure followed during the manufacture. *Tuttha bhasma* is a preparation mentioned in *Rasatarangini* [3]. This formulation contains *Tuttha* (copper sulphate), *Gandhaka* (sulphur) and *Tankana* (borax) as mineral drugs and *Bhavana* was done using *Lakucha swarasa* (*Atrocarpus lakoocha*). All the ingredients used in this formulation were taken after proper purification processes.

Tuttha bhasma is indicated in diseases like *Hridroga* (cardiac ailments), *Amlapitta* (dyspepsia), *Kushta, switra* (skin disorders), *Netraroga* (eye diseases)[4]. Efficacy of the mineral mainly depends upon its physical and chemical properties. Despite the fact that different properties of *Tuttha bhasma* are documented in Ayurvedic classics, it is rarely used. This could be due to a lack of understanding of how procedures like *Shodhana* and *Maarana* affect the chemical structure of minerals. This study is an attempt to interpret the physico-chemical changes of *Tuttha bhasma* and to develop a standard manufacturing procedure.

MATERIALS AND METHODS

Tuttha and *Tankana* in crystal form were collected from a local store in Trivandrum. *Gandhaka* in powder form was collected from laboratory supplies near Ayurveda college hospital Trivandrum. All the mineral drugs were identified and authenticated by *Rasa shastra* experts of Govt. Ayurveda College, Trivandrum. *Lakucha* fruit (*Atrocarpus lakoocha*) was collected from Rishikul campus, Uttarakhand Ayurveda University which was identified and authenticated by *Dravya guna* department. The composition of *Tuttha bhasma* is provided in table no 1. The procedure

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includes *Nirmalakarana* of *Tuttha*, *Shodhana* of *Tuttha*, *Gandhaka* and *Tankana* all the above ingredients were mixed together and triturated with *Lakucha swarasa* and subjected to *Kukkuta puta*. For the next *Putta*, equal

quantity of *Shodhita Gandhaka* was added to obtain *Bhasma* and the process is continued till the *Tuttha bhasma* attains *Bhasma sidhi lakshanas*.

Table 1: Ingredients and Proportion for *Tuttha Bhasma*

S.No	Drugs	English Name	Chemical/ Botanical Name	<i>Shodhana</i>	Proportion
1.	<i>Tuttha</i>	Blue vitriol	CuSO ₄ 5H ₂ O	<i>Nimbu swarasa bhavana</i>	1 part
2.	<i>Gandhaka</i>	Brim stone	S	<i>Go ksheera</i>	1 part
3.	<i>Tankana</i>	Borax	Na ₂ B ₄ O ₇ .10H ₂ O	<i>Bharjana</i>	1 part
4.	<i>Lakucha</i>	Monkey jack	<i>Artocarpus lakucha</i>	-	Sufficient quantity

Pre-procedures for the Preparation of *Tuttha bhasma*

Nirmalakarana of *Tuttha* [5]

Nirmalakarana was carried out according to *Rasa Tarangini* reference. *Tuttha* crystals was weighed, powdered and taken in a glass beaker and to this 4 times hot water was added. It was stirred continuously to form a solution. This solution was filtered using a filter paper into another beaker and wait for crystallization. Three batches of *Tuttha* (400gm- One batch) was taken for *Nirmalakarana* process.

Note -5gm of *Nirmalakarutha Tuttha* was kept for EDXRF analysis.

Table 2: Quantity of *Nirmalakarutha Tuttha*

Weight of <i>Tuttha</i>	Weight after <i>Nirmalakarana</i>	Loss in weight
1200gm	1033gm	167gm (13.9%)

Tuttha Shodhana [6]

Powdered *Tuttha* was taken in a *Khalwa yantra*. *Nimbu swarasa* was added and *Bhavana* was done to transform *Tuttha* into powder consistency. *Shodhana* was carried out in three batches and 998gm of *Shoditha Tuttha* was obtained. 30gm was lost due to spilling during *Bhavana*. The quantity analysis is provided in table no 3. Note 5gm *Shoditha Tuttha* was kept for EDXRF analysis

Table 3: Quantity Analysis of *Tuttha* after *Shodhana*

<i>Bhavana</i>	Weight before <i>Bhavana</i>	Weight after <i>Bhavana</i>	Medium for <i>Bhavana</i>
1st	342g	330	<i>Nimbu rasa</i> 370ml
2nd	342g	334	
3rd	344g	334	
Total	1028g	998g	

Gandhaka Shodhana [7]

Gandhaka shodhana was done by *Koormaputa* method explained in *Ayurveda Prakasha*. *Ashoditha Gandhaka churna* was weighed exactly to 500gm. 3 litres of *Gavya ksheera* was filled inside the ghee smeared earthen pot and a double layered clean cloth was tied to the mouth of the pot. All the powdered *Gandhaka* was slowly placed over this cloth and an earthen *Sharava* was placed over the mouth of the pot covering the powdered *Gandhaka* below. The joint between the mouth of the pot and earthen *Sharava* was sealed this apparatus was placed inside a pit and covered with dry coconut husk. This was lighted and change in temperature was noted. After attaining *Swangaseetha* the mud coverings and *Sharava* were removed and milk was drained to obtain purified sulphur globules. It was washed well with warm water to remove milk fat, later dried, weighed and sieved through sieve no 120 and stored. *Shodhana* was carried out in two batches.

Table 4: Quantity Analysis During *Gandhaka Shodhana*

Batches	Quantity taken	After <i>Shodhana</i>	Loss in weight	Time taken	Peak Temperature
1	500gm	475gm	25gm (5%)	4 hrs	423 ^o C
2	500gm	468gm	32gm (6.4%)	4 hrs	492 ^o C

Tankana Shodhana [8]

Bharjana was the method adopted here for *Shodhana* as per *Rasatarangini* reference. *Tankana* crystals was taken in a *Khalwa Yantra* and pounded into fine powder and fried with continuous stirring. *Tankana* started to pop up and stirring was continued till it loses the water of crystallization and the material become puffed. Later it was powdered in *Khalwa Yantra*, weighed and preserved. *Shodhana* was carried out in three batches.

Table 5: Observation and Quantity Analysis- Tankana shodhana

Weight of <i>Tankana</i>	Weight after <i>Shodhana</i>	Loss in weight
2000gm	1127.6gm	872gm (43.6%)

Preparation of Tuttha bhasma [9]

Out of total 998gm of *Shoditha Tuttha* only 250gm was taken to prepare *Bhasma*. Reference from *Rasa Tarangini* was selected for *Maarana* procedure. Equal quantity (250gm) of *Shoditha Tuttha*, *Shoditha Gandhaka* and *Shoditha Tankana* were taken in a large *Khalwa yantra* and mixed well. To this sufficient quantity of *Lakucha swarasa* was added and grinding was continued *Chakrikas* prepared were dried under shade. The colour of *Chakrikas* slightly changed to cyan blue. It took almost 2-3 days for complete drying. They were brittle and easily broken by hand. Weight of each dried *Chakrika* was approximately 3-5gm. All dried *Chakrikas* were placed in a clean *Sharava* and *Sandhi sheelaman* was done and carried out *Kukkuta puta*. The quantity analysis of *Tuttha bhasma* is provided in table no 6.

In *Rasa Tarangini kukkuta puta* was mentioned for *Tuttha bhasma*. 24cm×24cm measuring *Kukkuta puta* was first filled with cow dung cake pieces upto one fourth of its height. Each *Vanopala* was weighed (68gm) and its average weight was calculated. One cow dung cake was divided in to 4 parts to occupy maximum number of *Vanopalas* in *Kukkuta puta* for optimum temperature. Total 40-42 *Vanopalas* were required for one *Putta*. Temperature was noted uniformly throughout the *Putta*. Maximum attainment of temperature was 610° celsius for first *Putta*. After *Swangasheetha*, *Sharava* was carefully taken out from the *Kukkuta puta*. The obtained sample was collected and *Bhasma pariksha* were carried out.

For the second *Putta*, equal quantity of *Shuddha Gandhaka* was added to the obtained *Bhasma* and triturated with *Lakucha swarasa* and was subjected to *Kukkuta Puta*. The colour of *Chakrika* turned to be black after second *Putta* and a total of 3 *Putta* was required to attain all the *Bhasma lakshanas*. Pictures of preparations were provided in figure 1.

Table 6: Quantity analysis of Tuttha bhasma- kukkuta puta

No of <i>Putta</i>	Initial weight	Weight of <i>Gandhaka</i>	Quantity of <i>Lakucha swarasa</i>	Weight of <i>Chakrika</i>		Weight Loss after <i>Putta</i>
				Before <i>Putta</i>	After <i>Putta</i>	
1	750g	250g	490ml	557g	167g	390g (70.01%)
2	334g	167g	227ml	419.5g	139g	280g (66.7%)
3	278g	139g	180ml	393g	166g	227g (57%)

Figure 1: Tuttha Bhasma Preparation*Bhavana lakucha rasa**Chakrika nirmana*In *Kukkuta puta**Chakrikas* after 1st *Putta**Tuttha bhasma* after 3rd *Putta***OBSERVATIONS AND RESULTS****Table 7: Properties of Ashoditha, Nirmalikirutha and Shoditha tuttha**

Features	<i>Ashoditha Tuttha</i>	<i>Nirmalikirutha Tuttha</i>	<i>Shoditha Tuttha</i>
Colour	Dark blue	Light to dark blue	Greenish blue
Touch	Hard, crystalline form	Small crystalline forms, hard	Fine powder, soft
Smell	No specific smell	No specific smell	Smell of lemon juice

Table 8: Observations of Chakrikas after Puta

Parameters	Before Puta	1 st Puta	2 nd Puta	3 rd Puta
Colour	Greenish blue	Greyish black	Black	Black
Consistency	Solid and brittle	Soft, smooth and brittle	Powdery and smooth	Smooth
Appearance	Non shiny	shiny	Non shiny	Non shiny

Table 9: Bhasma pareeksha of Tuttha bhasma

Bhasma characteristics	1 st Puta	2 nd Puta	3 rd Puta
Rekhapurnatwa	+	+	+
Varitaratwa	-	+	+
Niswadu	-	+	+
Dadhi pareeksha	-	-	+

Figure 2: Bhasma pareeksha

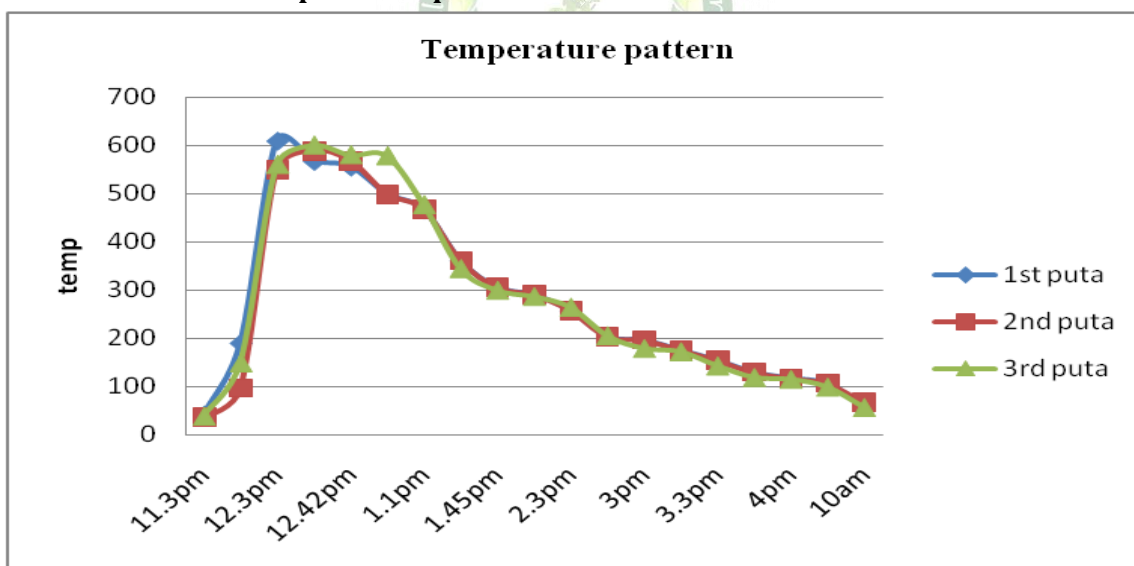


Varitaratwa

Amla Pareeksha

Rekhapurnatwa

Graph 1: Temperature Pattern of Tuttha bhasma



Analytical parameters to determine the quality of Tuttha Bhasma includes

- Organoleptic Characteristics
- Physico-Chemical Analysis
- Instrumental Analysis

Table 10: Organoleptic Features of Tuttha bhasma

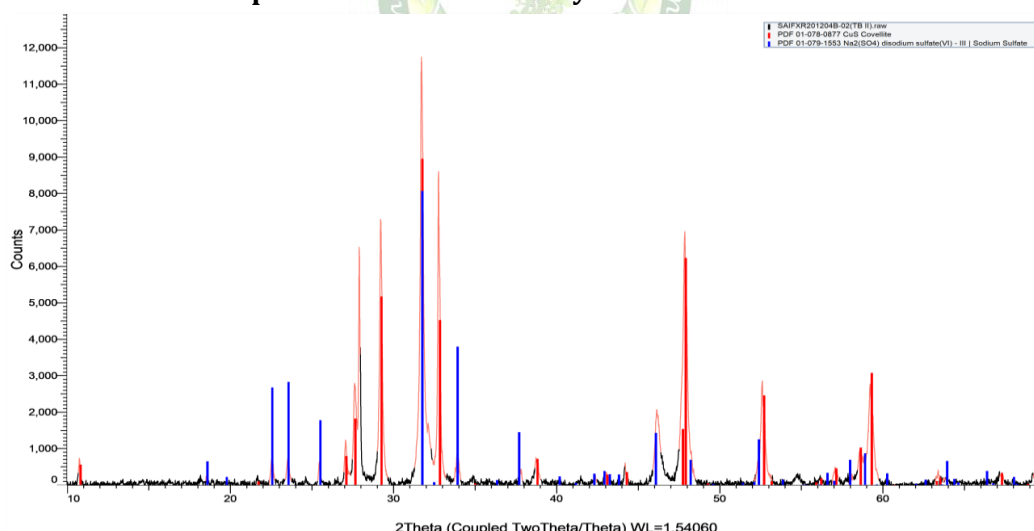
Organoleptic features	Observations
Colour	Deep black
Taste	Tasteless
Touch	Smooth, fine

Table 11: Result of Physicochemical Analysis of Tuttha bhasma

Parameters	<i>Tuttha Bhasma</i>
pH	4.77
Loss on drying	0.0264%
Total ash	58.47%
Acid insoluble ash	0.5%
Water soluble extractive	33.1%
Alcohol soluble extractive	2.6%

Table 12: Result of EDXRF of Ashoditha Tuttha, Nirmalikirutha Tuttha and shoditha Tuttha

<i>Ashoditha Tuttha</i>			<i>Nirmalikirutha Tuttha</i>			<i>Shoditha Tuttha</i>		
Comp.	Conc	Unit	Comp.	Conc	Unit	Comp.	Conc	Unit
Al ₂ O ₃	0.307	%	Al ₂ O ₃	0.227	%	Al ₂ O ₃	0.267	%
P ₂ O ₅	0.977	%	P ₂ O ₅	0.92	%	P ₂ O ₅	0.986	%
SO ₃	34.938	%	SO ₃	36.865	%	SO ₃	35.614	%
CaO	0.202	%	CaO	0.184	%	K ₂ O	864.5	ppm
Fe ₂ O ₃	0.292	%	CuO	61.494	%	CaO	0.252	%
CuO	63.139	%	SrO	56.2	ppm	TiO ₂	0	ppm
As ₂ O ₃	27.7	ppm	CdO	0.116	%	Fe ₂ O ₃	0.248	%
CdO	0.128	%				CuO	62.525	%
Tl ₂ O ₃	30.9	ppm						
PbO	113.7	ppm						

Graph 2: Result of XRD analysis of Tuttha Bhasma**Table 13: Result of Particle Size Analysis of Tuttha bhasma**

PSA after 2 nd Puta	PSA after 3 rd Puta
566.2343718 nm	476.7252034 nm

DISCUSSION

Aim of present study was to standardize the preparation of *Tuttha bhasma*. According to API a genuine raw drug of *Tuttha* should contain not less than 20% copper [10]. CuO was found to be 62.2% from this the percentage of copper was calculated to be 50.439% which was the highest constituent in the raw

sample of *Tuttha* and this sample was selected for the study.

Nirmalikirana process helps to remove mainly physical impurities and is specifically mentioned for some minerals only in *Rasatarangini* (like *Tuttha* and *Tanka*) [11]. According to *Rasatarangini* this

Nirmalikruta Tuttha can use for external application but not for internal use. Hence it can be considered as a pre procedure of *Shodhana*. When half quantity of hot water was added to *Tuttha* as per reference but it was not sufficient to form a solution. After increasing the ratio of water to four times it began to form a solution. It was kept undisturbed for crystallization. Crystallization initiated from second to third day onwards. It almost took 5 to 7 days to form crystals from 100gm *Tuttha*. The duration of *Nirmalakarana* procedure depends on the temperature needed to evaporate the filtrate and also the surface area of the container. Only 13.9% was the loss in the entire *Nirmalakarana* process, it could be due to the impurities present.

During *Tuttha shodhana*, 8 hours of *bhavana* was required to transform *Tuttha* into a powder consistency. However, *Bhavana* did not completely dry the *Tuttha* it might be because of the hygroscopic nature of copper sulphate. The product obtained after *Bhavana* was greenish blue in colour and had a faint smell of lemon. Since *Nimbu swarasa* was used as *Bhavana dravya*, this helps in the addition of useful components to the prepared drug as observed in the elemental composition in EDXRF analysis (table no 12). 30gm (2.9%) was lost due to spilling while doing *Bhavana*.

Kurmaputa method was adopted for *Gandhaka shodhana*, it is a practice that necessitates both talent and labour, *Kurmaputa* method has the advantage of more yield in one go. Here dry coconut husk was used as the fuel.

During *Bharjana* of *Tankana* the chemical compound of borax changed into an amorphous form. At a time *Shodhana* of 100g *Tankana* was carried out for 1hr. *Tankana shodhana* (2000gm) was carried out in 3 batches. 43.6% loss was noted and this may be due to evaporation of moisture content.

While preparing *Tuttha bhasma* when all the ingredients (*Tuttha*, *Tankana*, *Gandhaka*) ground with *Lakucha swarasa*. The colour slightly changed to cyan blue. By the addition of *Tankana* the mixture becomes more sticky. *Shoditha Tankana* was very light in weight but more amounts were present, making the mixture more sticky. The *Kukkuta puta*, which measures 24cm*24cm (1 *Vitasthi*) as per *Bhavaprakasha* reference^[12], was first filled to one-fourth of its height with cow dung cake. Total 40-42 *Vanopalas* was required for one *Putra*. One cow dung cake weighs approximately 65-70gms.

From 1st *Putra* onwards *Rekhapurnatwa* can be seen. For the second *Putra*, equal quantity of *Shuddha Gandhaka* was added to the obtained *Bhasma* and triturated with *Lakucha swarasa* and was subjected to *Kukkuta Putra*, the colour of *Chakrika* turned to be black after second *Putra* and *Varitaratwa* was obtained after

second *Putra*. 70% loss was observed after first *Putra*, then 66.7%, 57% loss after second and third *Putra* respectively, it can be seen that the amount of weight loss has been reduced after each *Putra* this may be because of the temperature pattern, a total of 3 *Putra* was required to attain all the *Bhasma lakshanas*.

The EDXRF data of *Ashodhita Tuttha* reveals the presence of oxides of Aluminium, Phosphorous, sulphur, Calcium, iron and copper oxide (CuO) with highest percentage and also heavy metals like, lead, arsenic in ppm. This may be present in raw *Tuttha* as contaminants. (Table no.12)

EDXRF results on *Nirmalikrutha Tuttha* showed the presence of compounds such as oxides of Aluminium, Phosphorous, Copper, Sulphur, Calcium. Heavy metals like lead and arsenic was absent after *Nirmalakarana*. Source of cadmium could be from the water used for *Nirmalakarana* process. The quantitative analysis shows the presence of CuO as 61.4% which decreased after *Nirmalakarana* and SO₃ as 36.8%. (Table no.12)

Shoditha Tuttha showed complete absence of heavy metals and the presence of oxides of aluminium, phosphorous, copper, sulphur, calcium, potassium and iron. The concentration of CuO and Fe₂O₃ is said to be 62.52 % and 35.6% respectively. (Table no.12)

The colour of *Bhasma* is an indicative of the compounds that are produced in the final product. It is observed that final product contains Copper sulphide as a major compound which is black in colour. This may be the reason for the black colour of *Tuttha bhasma*. No specific taste and odour was noted for the sample.

Bhasma Pariksha mentioned in the classics is highly informative and scientific. They validate physico-chemical characters of the *Bhasma. Tuttha bhasma* were found to be *Rekhapoorna*, they filled the finger creases. This indicates the particle fineness. Also when tested for *Varitaratwa*, *Bhasma* sample floated on stand stiff water, there by passing *Bhasma Pariksha. Amla/Dadhi pareeksha* which is specific for *Tamra bhasma* was also conducted for *Tuttha bhasma* due to the presence of copper. If there is a free copper metal in the *Bhasma* or if the conversion process is incomplete, the *Bhasma* reacts with the *Amla dravya* and give rise to blue colour. *Tuttha bhasma* was un reactive. (figure.2)

Physicochemical analysis is the important characteristics to evaluate the quality, standardisation and safety of Ayurvedic drugs. The physico chemical parameters analyzed in this study were pH, loss on drying, water soluble extract and alcohol soluble extract, total ash, acid insoluble ash.

Loss on drying of a drug gives the percentage of moisture present in a given sample^[13]. If *Bhasma* is hygroscopic, it may absorb moisture. Higher amount of

moisture may deteriorate the shelf life and therapeutic efficacy of the sample and it was found to be 0.0264%.

The ash value mainly represents the inorganic salts present in the drug. The ash value of prepared *Tuttha bhasma* was 68.47%. Acid insoluble ash was carried out to assess the percentage of acid insoluble inorganic content of the sample, indicates the presence of silica and oxalates in drugs, the acid insoluble ash of *Tuttha bhasma* was 0.5%. This might be due to the earthen *Sharava* used for *Putta*.

Extractive values give an idea about solubility in specific solvent when administered clinically. Water soluble extract of *Tuttha bhasma* was 33.1%. XRD analysis of *Tuttha bhasma* showed the presence of sodium sulphate also (29.8% solubility), this might be the reason for its solubility since copper sulphide is insoluble in water and alcohol soluble extractive of *Tuttha bhasma* was 2.6%.

pH is a measure of hydrogen ion concentration of a solution. It is a numeric scale used to specify the acidity or basicity of aqueous solution. pH of *Tuttha bhasma* was 4.77 which was acidic. It is observed that final product contains Copper sulphide as a major compound which has a pH of 5.3 which is acidic as well. This may be the reason for the acidic nature of the *Tuttha bhasma*.

Patterns of final sample of *Tuttha bhasma* suggest the presence of copper sulphide with hexagonal lattice and sodium sulphate with orthorhombic structure which is evident from the major peaks in two theta scale values at 10.746°, 27.628°, 27.903°, 29.220°, 31.721°, 32.721°, 32.766°.

The size of the *Bhasma* particles should be optimal for absorption and penetration into minute capillaries, as well as assimilation within the body. The rate of drug absorption is proportional to the particle size of the drug. The smaller the particle size, the faster the absorption, and thus the faster and more drug activity^[14]. The solubility increases significantly on reducing particle size below 1µm (0.5µm in radius). This is because the reduction of size below 1µm increases solvation pressure, giving rise to an increase on solubility and also causes disruption of solute-solute interaction which eases the solubilization process, which was revealed in a study conducted by Prakash Khadka et al^[15]. In the present study *Tuttha* has been subjected to a variety of processing methods, including *Peshana*, *Bhavana*, and *Marana*, to assist reduction in particle size. By Particle Size Analysis (PSA) it is revealed that the hydrodynamic diameter of particles in *Tuttha Bhasma* after second *Putta* was 566.2 nanometre (0.566µ) and after third *Putta* was 476.7 nanometre (0.476µ) (table no.13). The particle size decreased after each *Putta*, from this we can infer that particle size also depends on the nature of the drug and number of calcination cycles. In this study

Particle size of *Tuttha bhasma* after second and third *Putta* has been studied, may be particle size decreases after another *Putta*. This is an important factor to be considered if future studies were carried out on this drug.

All of these advanced analytical techniques contribute in evaluating the facts stated in Ayurvedic treatises and thus helps in demonstrating their validity.

CONCLUSION

The following conclusions can be drawn from this study.

1. *Tuttha bhasma* is a preparation mentioned in *Rasatrangini*, which includes *Shoditha tuttha*, *Gandhaka* and *Tankana* as mineral drugs and *Lakucha swarasa bhavana*.
2. The method of preparation is less laborious and simple and can be prepared in three *Kukkuta puta*.
3. EDXRF of *ashoditha tuttha*, *Nirmalikirutha tuttha*, *Shoditha tuttha* were conducted to find the elemental composition. Study revealed the presence of major compounds such as copper, sulphur, calcium and iron.
4. No traces of heavy metals were present after *Nirmalikirana* and *Shodhana*.
5. *Tuttha Bhasmas* chemical composition was discovered by XRD analysis. The outcome revealed the Copper sulphide and sodium sulphate as the contents.
6. Particle size analysis shows that the mean diameter of *Tuttha bhasma* after third *Putta* was 476nm.

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