Journal of Ayurveda and Integrative Medicine 10 (2019) 88–93

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Original Research Article (Experimental)

Study on physical properties of Ayurvedic nanocrystalline *Tamra Bhasma* by employing modern scientific tools

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ARTICLE INFO

Article history:
Received 5 June 2017
Accepted 25 June 2017
Available online 15 December 2017

Keywords:
Ayurvedic *Tamra Bhasma*
Nanomedicine
Physical properties
Magnetism

ABSTRACT

Background: *Tamra Bhasma* is derived from metallic copper that is recommended for different ailments of liver and spleen, dropsy, abdominal pain, heart disease, colitis, tumors, anemia, loss of appetite, tuberculosis, as well as eye problems.
Objectives: The knowledge of crystallite size and active ingredients in *Bhasma* materials is limited restricting its use as nanomedicine in the modern era. Also, the 2015 Nobel prize in medicine has motivated many researchers towards traditional medicines. Therefore, the different chemical and physical properties of prepared *Tamra Bhasma* has been studied by modern experimental tools (XRD, VSM, SEM, FTIR and PL spectrometer) and the preliminary testing of *Tamra Bhasma* nanoparticles was examined on bacteria.
Materials and methods: *Bhasma* is prepared by metals and minerals using three step procedures e.g. *Shodhana, Bhavana* and *Marana*. In the present work, for the preparation of *Tamra Bhasma*, pulverized copper wire was used and prepared by the principle of *Puta* (incineration) in an Electrical Muffle Furnace (EMF).
Results: X-ray diffraction analysis and scanning electron microscopy results revealed that the crystallite size of *Bhasma* powder was less than 100 nm and nanocrystallites of aglomerated size in micrometer. Magnetometer measurement supports its medicinal value. Photoluminescence (PL) properties of nanocrystalline *Bhasma* powder was investigated in UV-NIR region and shows luminescence in visible region. The antimicrobial study of *Tamra Bhasma* shows effectiveness on bacteria and, may be useful to control the bacterial infection disease.
Conclusion: Scientific data obtained using modern scientific tools and evidence would support in utilizing the ancient Indian wisdom of Ayurveda for the development of newer drugs as a modern nanomedicine and in other possible technological applications.

1. Introduction

As mentioned in India's *Vedic lore*, the whole system of preventive and curative health is "Ayurveda" *i.e science of life*. It is the traditional and natural system of healing and is accepted as the oldest scientific medicinal system of Indian subcontinent since ancient period, which utilizes herbs, metal, minerals and formulations in therapeutics [1–3]. It is a science of life or a way of living with rhythm of nature i.e connecting the physical, mental and spiritual connectivity of the human body. According to this system '*Bhasmas*' are metal or metal oxide based drugs [4,5]. Metal/metal oxide in the field of medicine got the status during *"Samhita"* period in its sense. A complex and elaborate procedure for the preparation of *bhasma* was described by *Nagarjun* around 800 AD in *Rasashastra*. According to Ayurvedic metallurgy, *bhasma* is product of herbo-metallic process and contains both metallic and herbal ingredients They are basically calcined form of metals/minerals treated with herbs. This process is strictly followed till date [2,3]. *Bhasmas* are believed to be ancient part of India as nanomedicine

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Peer review under responsibility of Transdisciplinary University, Bangalore.

http://dx.doi.org/10.1016/j.jaim.2017.06.012

and are more efficacious than any other healing system. This is because their nanosize (10^{-9} m) particles being insoluble can absorb and enter into the blood stream and, are more biocompatible as compared to any chemically produced entity due to its size/volume ratio. The size of nanoparticles lies between 1 and 100 nm at least in one dimension [6]. *Bhasmas* as compared to their herbal drug counterparts are stable over a longer period of time, require lower dose, are easy to store and sustainable availability. Currently, there is an urgent need for the practitioners of modern and traditional systems to standardize the synthesis procedure, rigorous scientific analysis for the quality, safety of these metal-based *bhasmas*. One of the widely used metal oxide based Ayurvedic drug is *Tamra Bhasma*. *Tamra Bhasma* is derived from metallic copper and is recommended for different ailments of liver and spleen, dropsy, abdominal pain, heart disease, colitis, tumors, anemia, loss of appetite, tuberculosis, as well as eye problems. The ancient Egyptian medical texts mention the use of copper for the purpose of sterilizing chest wound and evidences of first use of copper can be found in these papyri. Greek also applied the dry powder form of copper oxide and copper sulfate on wounds. Inorganic ways of copper synthesis were found to be effective in treating eczema, impetigo, tubercular infections etc. [7,8]. Copper is present in all tissues of animals and promotes hemoglobin synthesis, connecting tissue metabolism and growth of bone [9].

In this work, our objective is to explore the physical properties of *Tamra Bhasma* by modern scientific techniques and gather scientific evidence to corroborate the authenticity of such *bhasma* as a modern nanomedicine, which is the ancient Indian wisdom. Hence, the present article may help to design a drug from nanocrystalline *Tamra Bhasma* in future.

2. Materials and methods

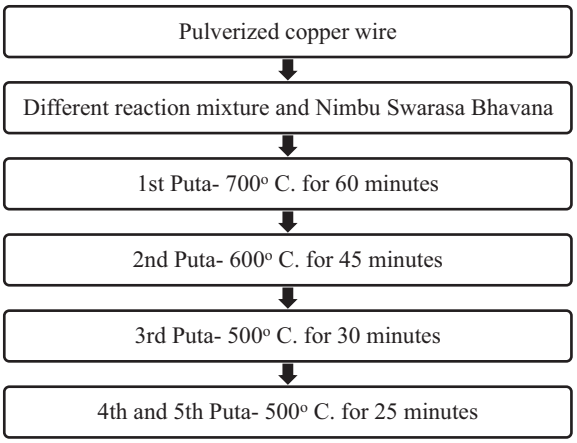
2.1. Pharmaceutical procedure of making Bhasma

According to *Rasatarangin*, edited by Pandit Kasinath Shastri (Motilal Banarshidas publication, Delhi).

वशिुद्धशुल्बस्य दलानि नम्बिूद्रवम पषि्टेश्र्वर गन्धलेपात्।
वशिाष्य धर्मे प्रटतानि नूनं वारत्रयेणोह मृतं प्रयान्तति॥ २५॥

Vishudhshulbsy Dalani Nimbudrawam Pisheshwar Gandhlepat
।Vishashay Dharme Pratitani Nunan Wartrayenoh Mritim Prayanti॥25॥

Bhasma is prepared by metals and minerals using three step procedures e.g *Shodhana*, *Bhavana and Marana* [10,11]. In the present work, for the preparation of *Tamra Bhasma*, pulverized copper wire was used and prepared by the principle of *Puta* (incineration) in an Electrical Muffle Furnace (EMF). *Samaguna Kajjali* equal to the amount of pure *Tamra* was mixed with wet trituration with lemon juice (*Nimbu Swarasa Bhavana*) till a paste-like consistency appeared and was smeared on *Tamra patra*. After drying in shade, it was put in *Sharava* (earthen saucer), which was covered by another *Sharava* and junction was sealed by multifold of *multani mitti* with smeared clothes. It was subjected for *Puta* in EMF. On the next day, after self-cooling *Sharava Samputa* was separated and, the material was collected and triturated for another *Puta*. The same process was repeated and total five *Puta* were given to complete the preparation of *Bhasma*. Following temperature pattern was followed: *1*st *Puta* – 700 °C for 60 min, *2*nd *Puta* – 600 °C for 45 min, 3rd *Puta* – 500 °C for 30 min, *4*th and *5*th *Puta* – 500 °C for 25 min. The flow chart 1 is given below and materials used are mentioned in Table 1.



Flow chart 1. Preparation of *Tamra Bhasma*.

Table 1
Materials used for the preparation of *bhasma*.

Materials	Purpose
Til Taila and Takra (butter milk)	*Samanya Shodhana (Purification)*
Gomutra and Kanji	*Vishesha Shodhana (Potentiation)*
Gandhak (Sulfur)	*Marana (Calcination)*
Nimbhu Swarasa (lemon juice)	*Bhavana (Levigation)*

3. Results

3.1. Crystal and micro-structural measurement

The crystallographic phase analysis of prepared *Tamra Bhasma* material was carried out by using Rigaku TTRX-III X-ray diffractometer, Japan with Cu-Kα radiation source (λ = 1.5418 Å) and is shown in Fig.1. The average crystallite size found to be ~88 nm, which was calculated by employing Scherrer's formula ($d=K\lambda/\beta \cos\theta$, where K is a constant that has value 0.9, β is full width half minimum (FWHM), θ is Bragg's angle and λ is the wavelength of Cu-Kα radiation.

Measure peaks in XRD spectrum can be indexed to different oxides and salt of Cu with different oxidation state, shown in Table 2 and depicts search of ICDD database. The chemical formulae are also enlisted in Table 2. Similar results have been reported by other research groups [12].

Grain size and morphological observation of prepared *bhasma* materials, shown in Fig. 2 has been carried out by the SEM

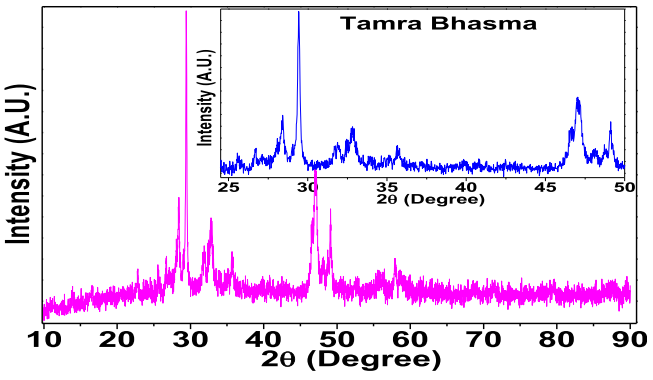


Fig. 1. XRD pattern of *Tamra Bhasma*.

Table 2
The ICDD data base card numbers with chemical formula and corresponding compounds are present in *Tamara Bhasma* (XRD peak position analysis).

No.	2θ (deg)	Chemical formula	ICDD card number
1	26.65 (7)	$Fe_2 O_3$, Zn S	01-074-6271,01-074-5014
2	28.39 (3)	$Cu_4 O_3$, Zn S	01-083-1665,01-074-5014
3	29.427 (6)	Na_2 S O_3, Zn S	01-075-2067,01-074-5014
4	31.84 (5)	$Cu_4 O_3$, Zn S, K O_2	01-083-1665,01-074-5014,01-077-0137
5	32.84 (4)	Cu O, $Fe_2 O_3$, $Na_2 SO_3$, Zn S	03-065-2309,01-074-6271,01-075-2067,01-074-5014
6	35.64 (5)	$Cu_4 O_3$, Cu O, $Fe_2 O_3$, Zn S	01-083-1665,03-065-2309,01-074-6271,01-074-5014
7	46.69 (4)	Cu O	03-065-2309
8	46.98 (2)	$Cu_4 O_3$, $Fe_2 O_3$, Zn S	01-083-1665,01-074-6271,01-074-5014
9	47.209 (14)	K O_2	01-077-0137
10	47.43 (3)	Zn S	01-074-5014
11	48.13 (7)	Mg O, Zn S	01-075-9567,01-074-5014
12	48.74 (7)	$Cu_4 O_3$, $Fe_2 O_3$, Zn S	01-083-1665,01-074-6271,01-074-5014
13	49.116 (12)	Cu O, Na_2 S O_3	03-065-2309,01-075-2067
14	58.6 (3)	$Cu_4 O_3$, Cu O, $Fe_2 O_3$, Na_2 S, Zn S	01-083-1665,03-065-2309,01-074-6271,01-075-2067,01-074-5014
15	79.6 (3)	$Cu_4 O_3$, Cu O, $Fe_2 O_3$, Na_2 S O_3	01-083-1665,03-065-2309,01-074-6271,01-075-2067

technique using Evo 18 research and Zeiss tungsten electron source equipment. *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 applied 10.00 kv electron beam and the distance between sample and electron source was 4.5 mm of magnification of 50 kx. SEM micrographs reveal that, the size of particles is in micrometer (~ 30 μm) of agglomeration of nanocrystallites.

3.2. FTIR Measurement

FTIR measurement of *Tamra Bhasma* sample was prepared by the KBr palate method. In this method, obtained sample was properly grind and mixed with potassium bromide (KBr) at approximately 1:20 mass ratio with the help of mortar and pestle. The mixture was then pressed to make thin pellets by palatalizer and applied pressure up to 6.5 tons. Spectra were observed with Perkin-Elmer Model (Frontier, Thermo fisher) at room temperature. The FTIR absorption bands of crystalline solid are usually assigned to the vibrations of ions in the crystal lattice. The FTIR finger prints are shown in Fig.3. In case of *Tamra Bhasma*, the single carbon and chlorine (C-Cl) bond with stretching vibration were in the range of 80-700 cm^{-1}. The carbon oxygen stretching bond (C-O-C) vibration were found at 1200 cm^{-1} and a number of inorganic groups such as sulfate, phosphate and carbonate were also observed at wave number below 1200 cm^{-1}. The wave number and functional group details are shown in Table 3.

3.3. Magnetic measurement

Magnetic parameters were measured using the vibrating sample magnetometer (7410, LakeShore,USA) at room temperature. The M-H hysteresis loop is shown in Fig. 4. The magnetization, of this material was found to be 18 emu/g for *Tamra Bhasma*. Thus, *Tamra Bhasma* nanomaterials show magnetic properties as well.

3.4. Luminescence Measurement

Color in Ayurvedic methodology indicates the formation of specific compounds. Room temperature photoluminescence (PL) spectra of *Tamra Bhasma* were measured using the fluorescence spectrometer (LS-55, PerkinElmer, U.K.). PL spectra taken in the wavelength range 200-900 nm and excited by a photon of wavelength of 200 nm and 225 nm are shown in Fig. 5.1 and 5.2 respectively. A characteristic luminescence spectrum is exhibited with 18 peaks with different intensities in UV, visible-NIR region as shown in Fig. 5.1 through excitation of 200 nm laser source and 10 peaks using 225 nm excitation source.

3.5. Antimicrobial test

Preliminary testing of the *Tamra Bhasma* nanoparticles was conducted on gram negative (*P. aeruginosa, K. pneumoniae*) and gram positive (*S. aureus*) bacteria and results are shown in Fig.6. The determination of antimicrobial property of *Tamra Bhasma* was

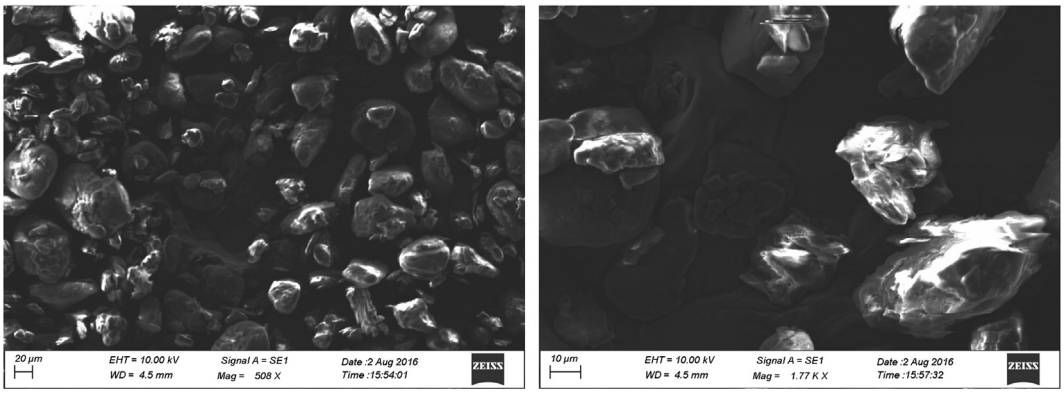


Fig. 2. SEM micrograph of *Tamra Bhasma* nanoparticles.

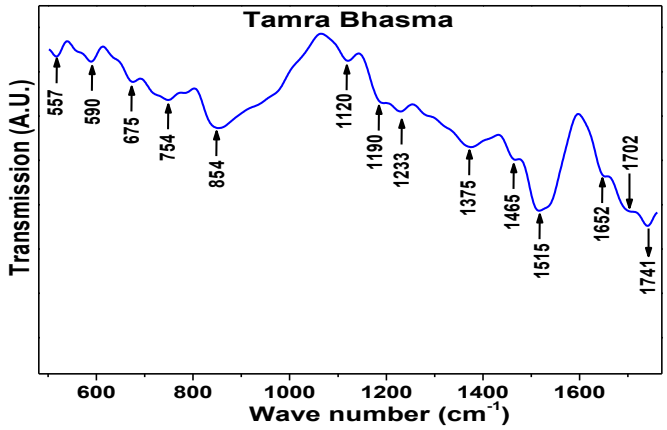


Fig. 3. FTIR spectrum of *Tamra Bhasma.*

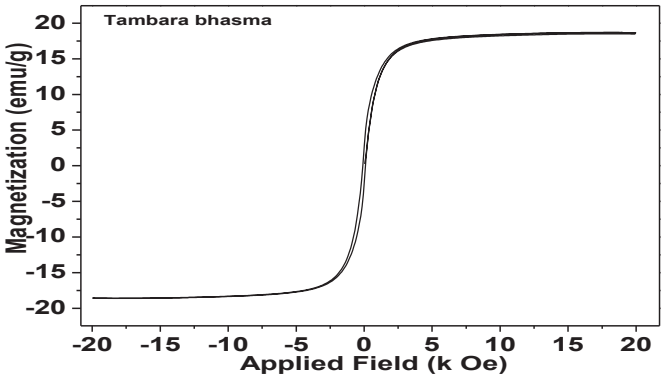


Fig. 4. M–H hysteresis loop of *Tamra Bhasma.*

observed by Kirby–Bauer disc diffusion method. In this method, a standard suspension of bacteria to be tested was inoculated on the surface of Mueller-Hinton Agar (MHA) plates. 40μL of *Tamra Bhasma* acetonic solution having concentration of 25mg/ml was used. Zone of inhibition are shown in Table 4.

4. Discussion

Based on the X-ray diffraction (XRD) results shown in Fig. 1, prepared Ayurvedic *Tamra Bhasma* which was nanocrystalline in nature had size less than 100 nm; however, the Scanning Electron Microscope (SEM) micrograph, shown in Fig. 2, reveals the formation of micrometer samples due to agloromation of nanocrystallites. Also *Tamra Bhasma* is in polycrystalline form. In a single particle there may exist several crystallographic grain boundaries. This may be due to heat treatment for multiple times (*Puta*) and mixing with different herbs like lemon, aloe vera etc. The difference between the crystallite size obtained from XRD and particle size obtained from SEM has been observed by other researchers and our result is comparable with those results [13–15]. Measurement of peaks of different intensities in XRD can be indexed to different oxides and salt of copper with different oxidation state, shown in Table 2 and depicts search peaks of ICDD database. The chemical formulae are also enlisted in Table 2. Thus, this study reveals that prepared *bhasma* has nanocrystalline materials of average size less than 100 nm.

The FTIR results, shown in Fig. 3, support the different ingredients present in the *Tamra Bhasma* nanoparticles [16]. The single carbon and chlorine (C–Cl) bond with stretching vibration were in the range of 80–700 cm^{-1}. The carbon oxygen stretching bond (C–O–C) vibration were found at 1200 cm^{-1} and a number of inorganic groups such as sulfate, phosphate and carbonate were also observed at wave number below 1200 cm^{-1}. The wave number and functional group details are shown in Table 3. Thus, XRD data shown in Table 2 support the FTIR results, shown in Table 3.

The magnetization (M–H) loop, shown in Fig. 4 suggests the super-paramagnetic nature of *Tamra Bhasma* nanoparticles. Also, the magnetization saturates at low field which is a signature of soft magnetic nanomaterials. Hence, these materials can also be used for soft magnetic applications. This super-paramagnetic property also favors any medicinal values of traditional medicine [17,18]. Mostly, Cu is an anti-ferromagnetic material at room temperature. But the high magnetic moments and hysteresis loop suggest the presence of magnetic phase oxide and other elements, which are present during the preparation. Also, XRD shows (Table 2) the presence of iron oxide (Fe_2O_3).This justifies the appearance of magnetic hysteresis loop. This *bhasma* can be used to treat the diseases, where magnetic *bhasmas* are required [19].

The luminescence in the present material may be due to presence of different chemicals as enlisted in Table 2 from XRD analysis. It needs a theoretical analysis and support from other experimental results such as, X-ray Photoelectron Spectroscopy (XPS), Electron Spin Resonance (ESR) etc. Hence, it is our future research problem to establish the PL spectra of '*Tamra Bhasma*'. This observation of magnetic and luminescence investigation reveals that, the '*Tamra Bhasma*' not only can be used as a very good medicine particularly for diseases treatment but can also be employed as magnetic material for other technological applications. The advantage is that, the production is natural which will be environment friendly.

The antimicrobial study, shown in Fig. 6 reveals that *Tamra Bhasma* is effective on both gram positive as well as gram negative bacteria. As a result, a zone of inhibition of 30 mm in *P. aeruginosa,* 22 mm in *K. pneumoniae* and 15 mm in *S. aureus* was observed. Such bacterial studies on *Tamra Bhasma* nanoparticles were also reported by few research groups [8,20–23]. Generally antimicrobial mechanism is found to be due to some possible reasons e.g. changes in the bacterial cell membrane preventing the uptake of an antimicrobial, production of enzyme which inactivates antibiotics, modification of target so that it no longer interacts with the antimicrobial agent, alteration of ribosomes - plasmid mediated methylation of the 30s ribosome block attachment of the drug to

Table 4
Zone of inhibition of *Tamra Bhasma* against bacteria.

S.N.	Name of bacteria	Zone of inhibition
1.	*P. aeruginosa*	30 mm
2.	*K. pneumoniae*	22 mm
3.	*S. aureus*	15 mm

Table 3
FTIR absorption wave number and corresponding functional groups.

Wavenumber (cm^{-1})	517	590	675	749	854	1120	1190	1233	1375	1465	1652	1702	1741
Functional Group	Alkanes	C–Cl	C–H	C–H Stretching	C–H	C–O Ether	C–O Ester	C–O–C	C–H	C=C	C=O	C=C	C=O

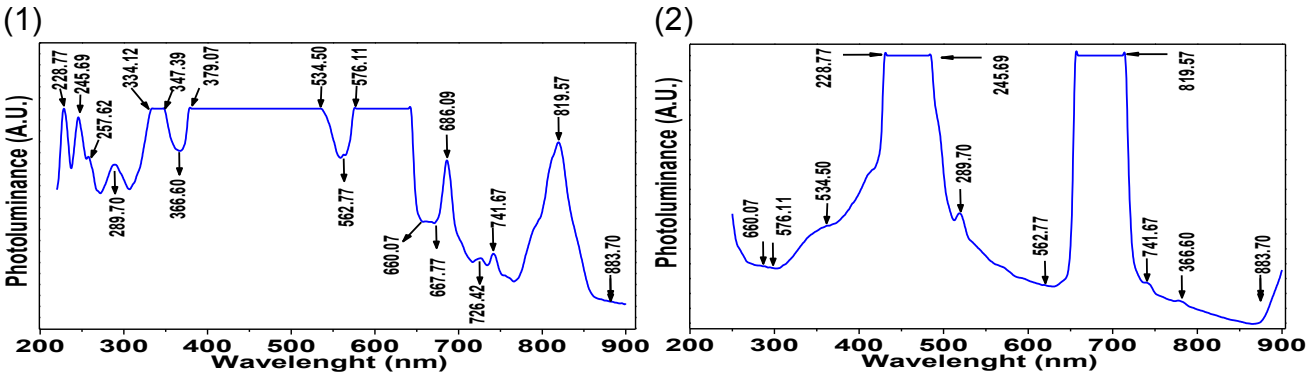
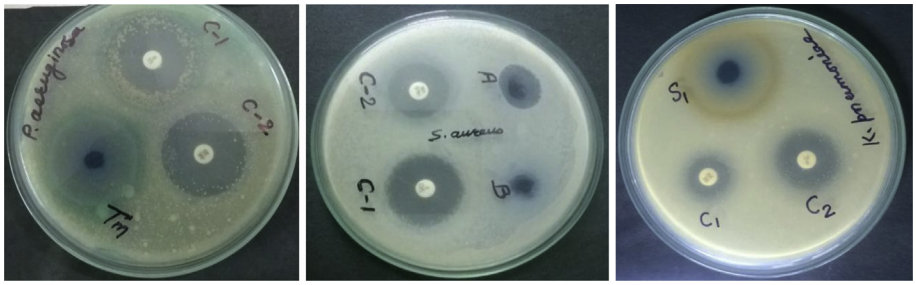


Fig. 5. (1) PL at 200 nm Excitation. (2) PL at 225 nm Excitation source.



(A) **(B)** **(C)**

Fig. 6. Antimicrobial test of *Tamra bhasma* on *P. aeruginosa*, *S aureus* and *K. pneumoniae* (A,B,C,).

the ribosome. Therefore mentioned mechanisms may be involved in *Tamra Bhasma* nanomaterials antimicrobial properties and further study is needed at this stage. The present study reveals that *Tamra Bhasma* nanomaterials can be used in bacterial infection diseases for both gram positive and gram negative. Ayurvedic *bhasma* shows nanometric size with optical behavior and treated as a nanomedicine are in agreement with findings reported by other research groups [24–29].

5. Conclusion

Based on the XRD result, it can be concluded that Ayurvedic *Tamra Bhasma* in nanocrystalline nature has size less than 100 nm; however, the SEM micrograph reveals the formation of micrometer samples due to agloromation of nanocrystallites. FTIR results reveal different crystalline oxides and salts of Cu are present in this Ayurvedic *bhasma*. The luminescence properties of *Tamra* are found to be in visible range that reveals color. VSM measurement reveals super-paramagnetic nature of the *bhasma* that supports its medicinal value. The antimicrobial study of *Tamra Bhasma* shows effectiveness on both gram positive and gram negative bacteria and, may be useful in controlling bacterial infections. The observations of magnetic behaviour and luminescence in visible region results reveals that, the '*Tamra Bhasma*' not only can be used as a very good medicine particularly for disease treatment but can also be employed as magnetic materials for other technological applications. The advantage is that, the production is natural which will be environment friendly. These results may help to establish a relation among modern science and technology, modern medicine and ancient medicine. In the present study, scientific data and evidence would support in utilizing the ancient Indian wisdom of Ayurveda for the development of newer drugs as a modern nanomedicine.

Sources of funding

Aryabhatta Knowledge University Patna, Bihar, India and Department of Science and Technology, Govt. of Bihar, India.

Conflict of interest

None

Acknowledgements

Authors are thankful to Dr. R. K. Kotnala, CSIR-NPL, Delhi for his motivation in this work and entered the VSM technique for magnetic measurement, Dr. Jitendra Kumar, Dept. of Biotechnology, Patna University for antimicrobial study help and Dr. Prabhat Kr Dwedi of Govt. Ayurvedic College, Patna for fruitful discussion and support.

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