JNCS
Journal of Nepal Chemical Society
June/July 2022, Vol. 43, No. 1, 5-16
ISSN: 2091-0304 (print)
DOI: https://doi.org/10.3126/jncs.v43i1.47031
RESEARCH ARTICLE

Synthesis, Characterization, Biological Study of Synthesized *Lauha Bhasma*

Rajesh Paudel[1], Gopinand Lal Karn[1], Girja Mani Aryal[2], Jyoti Giri[3], Rameshwar Adhikari[1,2], Motee Lal Sharma[1*]

[1]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

[2]Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kirtipur, Kathmandu

[3]Department of Chemistry, Trichandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal

[*]*Corresponding author:* mlsharma.chem@gmail.com

Submitted: **16 June 2022,** Revised **27 June 2022,** Accepted **29 June 2022**

Abstract:

Bhasmas are prepared from metallic and herbal ingredients and are also referred to as herbo-metallic preparations. *Lauha bhasma* (LB) is one of the iron-based herbo-metallic preparations used in *Ayurvedic* medicine for treating various ailments due to iron deficiency. The preparation of LB involves normal purification (*samanya sodhana*), special purification (*vishesha sodhana*) followed by drying under sunlight (*bhanupaka*), heating in a frying pan (*sthalipaka*), and calcination (*putapaka*) with *Triphala kwatha* as a medium under the temperature of 650 °C in an electric muffle furnace (EMF) and maintained for 1 hour. LB is subjected to different physicochemical analysis and modern analytical methods using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Spectroscopy (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX). Prepared LB is also undertaken to antibacterial study and is compared with market samples.The results suggest that organoleptic characters, preliminary test and physicochemical result of LB suggest that these steps were necessary to obtain a good quality of *bhasma* and also make it acceptable during the *Bhasmikarna* process. From physicochemical analysis data, it was observed the negligible moisture content (0.42 %; loss on drying), total ash value (17.3 %), acid insoluble ash value (7.6 %). It was observed that LB was prepared in 21 *puta* where the average crystalline size was found to be 57.23 nm from XRD spectra. SEM analysis shows the fine coarse structure with uniform particle size of LB. EDX graph shows the presence of Fe (75.43 %) as a major element. FTIR spectra suggest the presence of different organic moieties which enhance the therapeutic action due to which *bhasma* shows significant antibacterial properties.

Keywords: *Lauha bhasma*, EMF, FTIR, XRD, SEM, EDX.

Introduction

Ayurveda is the traditional system of health science which was developed around 3000 B.C [1]. Its name means, "science of life" or "life knowledge"; as it is the combination of two Sanskrit words *ayush* (life) and *veda* (science or knowledge).

From the twentieth century, Ayurveda became politically, conceptually, and commercially dominated by modern biomedicine, resulting in Modern Ayurveda and Global Ayurveda. Modern Ayurveda is geographically located in the Indian subcontinent and tends towards secularization through minimization of the magic and mythic aspects of Ayurveda [2]. Practitioners of Ayurveda in south Asian countries Nepal, India and Sri Lanka follow the Sanskrit texts. However, they do differ in some aspects, particularly in the herbs used [3]. The *ayurvedic* method of holistic

health care emphasizes balancing the body, mind, and spirit to treat and prevent disease. This 5000 years old practice focuses on harmonizing the body with nature through diet, herbal remedies, *yoga* and meditation, exercise, lifestyle, and body cleansing [4].

According to *ayurveda*, the normal homeostasis of the human body is governed by three *doshas*, namely *vata* (for transport), *pitta* (for metabolism), and *kapha* (for storage). When the three *doshas* are in equilibrium, biological systems exhibit normal functions in the body, and any distortion or disturbance, or imbalance between these *doshas* may lead to a diseased condition [5], [6]. When *dosha* becomes imbalanced, the natural flow of "*prana*" (life force energy) becomes disrupted which causes a build-up of toxic waste in the body, mind, and spirit, which creates disease[7]. From the *Ayurvedic* philosophy, the entire cosmos is interplay of the energies of the five basic elements, namely ether, air, fire, water, and earth. The *vata* (air and ether), *pitta* (fire and water), and *kapha* (water and earth) are combinations of the five elements that manifest as patterns in all creation[8].

Biomedicine, in contrast, is founded on the reductionist approach to health and disease, and attempts, first and foremost, to eliminate pathology. Rheumatoid arthritisis the prototype of a severely painful chronic disease that affects multiple joints, causing swelling and crippling deformities in most patients. Pain relief for painful, swollen joints is produced by local application of plant extracts (for example, *Semecarpus anacardium* or marking nut), which produce chemical cauterization and superficial burn-like reactions. Similar cauterization may be achieved by applying heated probes of gold, copper, or iron known as *Agnikarma*. *Bhasma is first time introduced in Rasashastra. It is a specialized branch of Ayurveda dealing mainly with the materials which are known as "Rasa dravyaas"*[9]. *The branch of Rasashastra involves procedures such as; Sodhana, Marana, and Puta. Rasashastra is a pharmaceutical branch of Nepalese and Indian system of medicine which mainly deals with the metals, minerals, animal origin product, toxic herbs and their use in therapeutics.The drugs used in Rasashastra have enormous advantages towards the maintenance of normal physical and mental status.*

Bhasma is a unique ayurvedic metallic/minerals preparation, treated with herbal juice or decoction and exposed for Ayurveda, which is known in the Indian subcontinent since 7th century A.D and is widely recommended for the treatment of a variety of chronic ailments [10], [11].*Bhasma is a Sanskrit word that means "ash". It comes from the root "bha" meaning "delusion" and "sma" meaning "ever". According to Hindu mythology and Yoga, bhasma is sacred ash. In the traditional Himalayan region medical system of Ayurveda, bhasma is a type of medicinal power made through calcinations of stones, gems, minerals or metals. There are wide ranges of bhasmas used to treat many types of ailments* [12]. *Bhasma means an ash obtained through incineration in which the starting material undergoes an elaborate process of purification and this process is followed by the reaction phase, which involves incorporation of some other minerals and herbal extract* [13], [14]. *Bhasmas are the nanomedicine which is the relevance of nanotechnology in the area of healthcare, diagnosis of disease, cure and prevention of disease* [15]. Iron (Fe) is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid synthesis, and electron transport [16], [17]. The incinerated Fe preparations of *Ayurveda* are known as *Lauha bhasma* . It is a herbo-metallic product that has several therapeutic applications [18], [19].

Materials and Methods

Materials

Iron turning was bought from Nike Chemical India, Product code: E-009408. Apart from that, Sesame oil and *Triphala churna*was purchased from the Pharmacy ahead of *Nardevi Ayurved* Hospital, Kathmandu. Cow urine and butter milk was collected from the local cow farm present in Kirtipur, Kathmandu. A decoction of horse gram and rice gruel was prepared in Research Centre for Applied Science and Technology (RECAST) Laboratory, Tribhuvan

University, Kirtipur, Kathmandu. For the heating sample, EMF was used from RECAST Laboratory.

Methods

Synthesis of LB was done by following the standard protocol present in *Rasashastra* of *Ayurveda t*ext [8]. Preparation of LB includes *Samanya sodhana* (normal purification), *Vishesha sodhana* (special purification), and *Marana* (the process of conversion of metal in nanoparticles).

The normal purification process was done in five steps which included Quenching in *Sesame* oil, Quenching in Butter Milk, Quenching in Cow Urine, Quenching in Rice Gruel, and Quenching in a decoction of Horse Gram [20], [21]. Special purification process was done in single step by Quenching in decoction in *Triphala churna* (*Triphala kawath*)[22].*Marana* was done in three steps which include *Bhanupaka* (exposure to sunlight), *Sthalipaka* (frying in the iron pan), and *Putapaka* (calcination) [23]. *Lauha bhasma* was subjected to various organoleptic and physicochemical analysis such as color, taste, texture, loss on drying,ash value, acid insoluble ash, and water-soluble ash [24]. Analytical instruments such as FTIR, XRD, SEM and EDX wereemployed to determine the organic moieties, particle size, surface morphology and elemental composition respectively.

Normal Purification (*Samanya shodhana*)

In *Samanya shodhana* process, 700 g of raw material (Fe turning)was heated in EMF till red hot condition (900 ^{o}C) and immersed in 700 mL of each medium viz. *Tila taila* (sesame oil), *Takra* (buttermilk), *Gomutra* (cow's urine), *Kanji* (rice gruel), and*Kulattha kwatha* (decoction of horse gram) and kept for self-cooling(approximately 1 h) at room temperature [25]. This quenching process was repeated for seven times consecutively in *Tila taila* followed by seven times consecutively in *Takra*, *Gomutra*, *Kanji*, and *Kulattha kwatha* by using fresh media every time [26]. After completion of the process, material was filtered by Fe mesh anddried under sunlight. The material obtained at this stage is called *Samanya shodhita Lauha*.

Special Purification (*Vishesha shodhana*)

In this purification step, quenching was done in *Triphala kwatha*. It was prepared by taking coarse powders of three myrobalans, taken without seed: Haritaki (*Terminalia chebula*), Bibhitaki (*Terminalia bellirica*), and Amalaki (*Phyllanthusemblica*) in equal quantity (each 1 kg) and boiled in 24 L of watertill reduction to 1/4th of the original volume of water to obtain *Triphala kwatha*. Using this, repeated quenching process of *Samanya shodhita* lauha was done. This purification step was repeated seven times using freshly prepared *Triphala kwatha* [22]. The lauha churna (coarse powder of Fe turning) obtained at this stage is called *Vishesha shodhitaLauha*.

Exposure to sunlight (*Bhanupaka*)

Triphala kwatha was prepared by heating equal quantity of *Triphala*to *Vishesha shodhita Lauha bhasma*with two parts ofwater andreduced to 1/4th of original volume. This *Triphala kwatha* wasadded to *Lauha* obtained after *Vishesha shodhana* and allowed to dryunder sunlight [27]. It took a maximum of 3 days for complete drying of *Triphala kwatha*. This process was repeated seven times to yield intermediate after *Bhanupaka*.

Heating in frying pan (*Sthalipaka*)

In this step, *Triphala kwatha* was prepared by taking *Triphala*3 times of *Lauha* obtained after *bhanupaka* and 16 times of water was added to it. The whole material was boiled in a stainless-steel container to reduce the volume to 1/8th of the original volume ofwater. *Lauha* obtained after *bhanupaka* was washed with hot waterand placed in a sthali (Fe pan), to which above freshly prepared *Triphala kwatha* was added and intense heating was given for complete evaporation of water contents of *Triphala kwatha*. On complete drying of the material, again *Triphala kwatha* was addedand subjected to heat. This process required 4.5 hours for complete drying of *Triphala kwatha*. The whole process was repeated seven times to yield intermediate after *Sthalipaka*[20].

Calcination (*Putapaka*)

The process of *puta* (calcination) refers to controlled heating of herbo-mineral preparations and allowing the preparation to cool to room temperature. In this

process, freshly prepared *Triphala kwatha* was mixed with *Lauha* obtained after *Sthalipaka*in mechanized *Khalva yantra* and trituration was done with afrequency of 60 times/min. The paste formed during this trituration was made into *Cakrikas* (pellets) and dried under sunlight. After complete drying of *Cakrikas*, it was taken in an earthen vessel (*Sarava*) and covered with another inverted earthen vessel. The space between the two earthen vessels was covered with clay smeared cloth; this specific process is known as *Sarava samputikarana*(sealed earthen pot). After this, it was subjected to *puta* inhorizontal EMF and temperature was allowed to gradually rise to650 ^{o}C in 2 h and maintained for 1 h. The furnace was then switched off and allowed for self-cooling. The next day, pellets werecollected from *sarava* and again triturated with *Triphala kwatha*.Same process of *puta* was repeated for 20 times to obtain *Lauha bhasma* of desired quality.

Antimicrobial activity:

The antimicrobial efficacy of synthesized *bhasma* was assayed by the standard Kirby Bauer disk diffusion method against the bacterial pathogens. The bacterial suspension was swabbed on the Muller Hinton Agar by using the cotton swab. The agar was impregnated and well was made. The sample was dissolved in Dimethyl Sulfoxide (DMSO) and its antibacterial activity was studied by agar well diffusion method. The disk were gently incubated at 37°C for 24 hour. After the incubation period the susceptibility of the test organisms was determined by measuring the inhibition zone given and result was evaluated.

Results and Discussion

Preliminary Test

The results of various physiochemical parameters color, taste, texture, ash value, acid insoluble ash, water soluble ash, particle size and elemental composition are recorded.

Modern instrumental analysis (characterization)

FTIR

LB was also prepared with different organic sources. Therefore, different organic fragments such as C-H stretching at 1465 cm^{-1}, O-H group at 1391 cm^{-1}, C=C at 967 cm^{-1}, C-O group at 1050 cm^{-1} and Fe-O bonding at 567 cm^{-1} are found for all three spectrum of LB, C_LB-2 and C_LB-3 respectively as shown in Figure 1. This clearly showed that synthesized LB is completely in agreement with commercial LB C_ LB-2 and C_LB-3 [14].

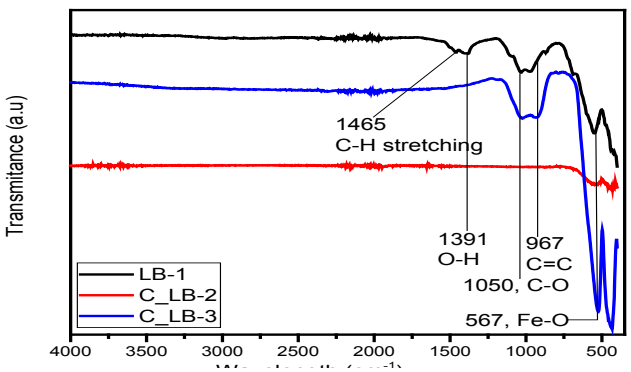

Figure 1: The plot showing FTIR spectrum of synthesized LB (LB-1) and two commercial LB, (C_LB-2 and C_LB-3).

XRD

Similarly, XRD diffraction shown in Figure 2 is for LB, C_LB-2 and C_LB-3 and are similar in all aspect. The *Lauha bhasma* identification was done by using standard JCPDS data (card no.39-1346) for "d" spacing. The result shows that ferric oxide (Fe_2O_3, cubic) is the major crystalline phase present in the LB [28].

The diffraction pattern of final product of LB sample shows major peaks at 2θ angles 30.43°, 35.89°, 43.78°, 54.10°, 54.75°, 63.41° and 74.54° corresponding to reflection from (2 2 0), (3 1 0), (3 1 1), (4 0 0), (4 2 1), (4 3 0), (5 1 1) and (4 4 0) respectively. It indicates the cubic system with lattice parameters. Formation of Fe_2O_3 indicates that we have obtained the desired results. Fe^{2+} ions produced were used as medicine for the treatment of anemia.

The XRD of raw iron turning shows crystalline peaks of iron metal whereas that of C-LB show cubic crystalline Fe_2O_3 peaks in its very effective ferric form as shown in Figure 2b and c. The absence of any crystalline iron metal peaks and presence of nanosize particles is confirmed. It is observed that iron which was present in raw metal has been converted into Fe_2O_3 nanoparticles after proper incineration process.

Table 1: List of the *Bhasma* samples prepared in laboratory and physical properties.

S. N.	Name of Bhasma/Company name	Sample Designation	Colour	State
1	*Lauha Bhasma*	LB	Reddish Brown	Amorphous Powder
2	Commercial *Lauha Bhasma*/Singh Darbar Baidhya Khaana	C_ LB-2	Reddish Brown	Amorphous Powder
3	Commercial *Lauha Bhasma*/Shree Dhootapapeshwar LTD. Batch No. P180100034 Manufacture Date 01/2018	C_LB-3	Black	Amorphous Powder

LB= Lauha Bhasma (Synthesized)
C_LB-2= Lauha Bhasma (Commercial)
C_LB-3= Lauha Bhasma (Commercial)

Table 2: Description on observations performed on the synthesized and commercial *Bhasma* samples.

Different Tests/Analysis	Name of Bhasma Parameters	LB	C_LB-2	C_LB-3
Organoleptic	Touch	Smooth Fine powder	Smooth Fine powder	Smooth Fine powder
	Colour	Brownish red	Brownish red	Brownish red
	Odour	Odourless	Odourless	Odourless
Preliminary test	Luster	Dull	Dull	Dull
	Fineness (+/-)	+ve	+ve	+ve
	Buoyancy (+/-)	-ve	-ve	-ve
	Fume (+/-)	-ve	-ve	-ve
Physiochemical analysis	LOD (%)	0.42	-	-
	TAV	17.3	-	-
	AIAV (%)	7.6	-	-

LOD= Loss on drying, **TAV=** Total ash value. **AIAV=** Acid insoluble ash value

Particle size analysis of LB was calculated by using Scherrer formula, the average particle size of synthesized LB was found to be D = 57.23 nm.

XRD study was carried out to identify the major compound present in the LB. Crystallographic information obtained by XRD is very useful to predict action of the product. The sample of *Lauha bhasma* (LB) identification was done by matching "d" spacing with standard JCPDS data (card no.39-1346). The result shows that ferric oxide (Fe_2O_3, cubic) is the major crystalline phase present in the final product of *Lauha bhasma.*

The diffraction pattern of final product of LB sample shows major peaks at angles 30.43°, 35.89°, 43.78°, 54.10°, 54.75°, 63.41° and 74.54° corresponding to reflection from (2 2 0), (3 1 0), (3 1 1), (4 0 0), (4 2 1), (4 3 0), (5 1 1) and (4 4 0) respectively. It indicates the cubic system with lattice parameters. Formation of Fe_2O_3 indicates that we have obtained the desired results. Fe^{2+} ions produced were used as medicine for the treatment of anemia.

The XRD of raw iron turning shows crystalline peaks of iron metal whereas that of *Lauha Bhasma* sample shows cubic crystalline Fe_2O_3 peaks in its very effective Fe^{3+} state. The absence of any crystalline iron metal peaks and presence of nanosize particles is observed. It is clear that iron which was present in raw metal has been converted into Fe_2O_3 nanoparticles after proper incineration process. Particle size analysis of LB was calculated by using Scherrer formula, the average particle size of synthesized LB was found to be D = 57.23 nm.

Scherrer's Equation

$$D = \frac{K\lambda}{\beta \cos\theta}$$

D= Nanocrystallite size

λ= wavelength

β= full width at half maxima (in radian)

θ= Angle (in degrees)

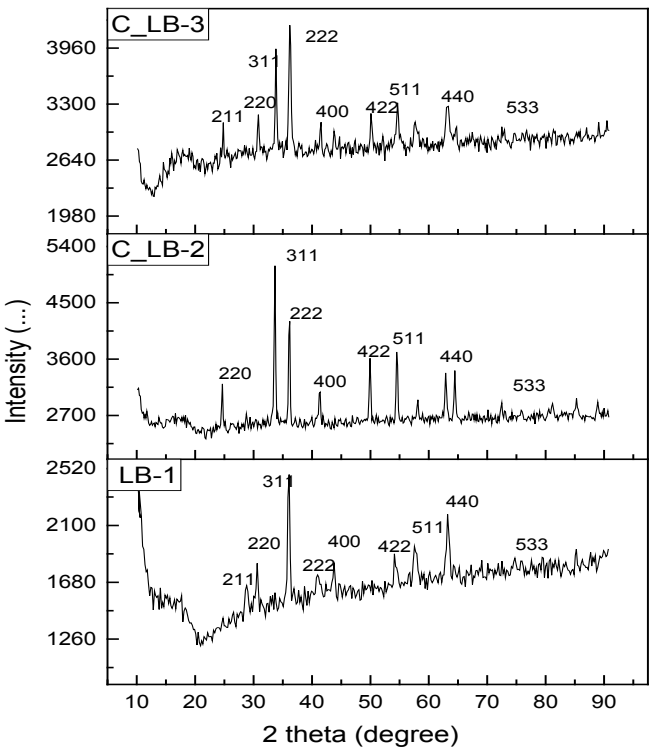

Figure 2: The plot showing X-ray diffraction pattern of synthesized LB (LB-1) and two commercial LB (C-LB-1 and C-LB-3)

SEM

The C_LB from different companies studied in this work also have various agglomerated sizes [29]. C_LB-2 has random agglomeration sizes in comparison to C_LB-3. C_LB-3 are fine coarse structure with uniform particle size. The Figure 3 (a) shows the surface morphology of C_LB-2, the random agglomeration size of the *Bhasma* particles is due to the moisture absorption [30]. Similarly,also due to the different organic constituents during intoxication process [31].

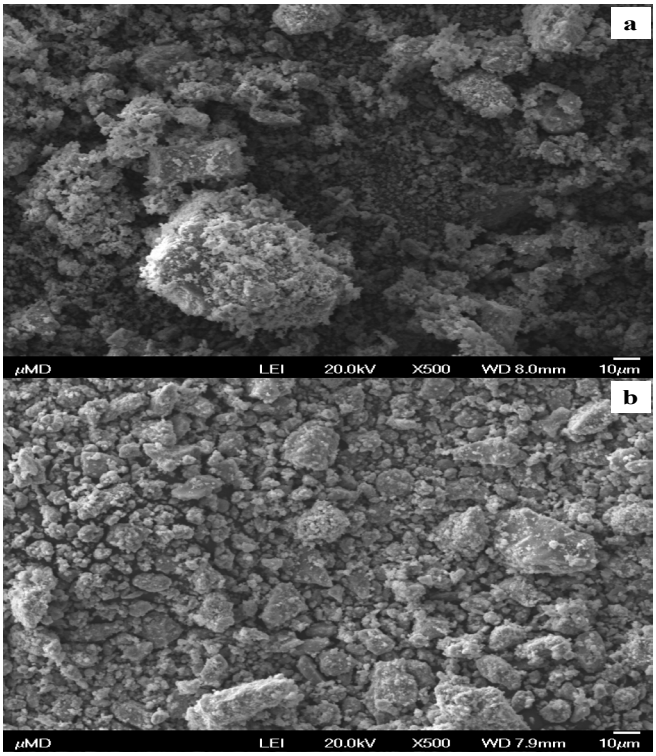

Figure 3: Micrographs showing surface morphology of commercial *Lauha bhasmas* (a) C_LB-1 and (b) C_LB-2

EDX

EDX gives semi quantitative analysis of elements. Elemental compositions of LB are recorded and presented in the bar graph Figure 4. Major elements observed in *Lauha bhasma* are Fe, K, Cl and Ca. Mn, Cu, Cr and Br [32].

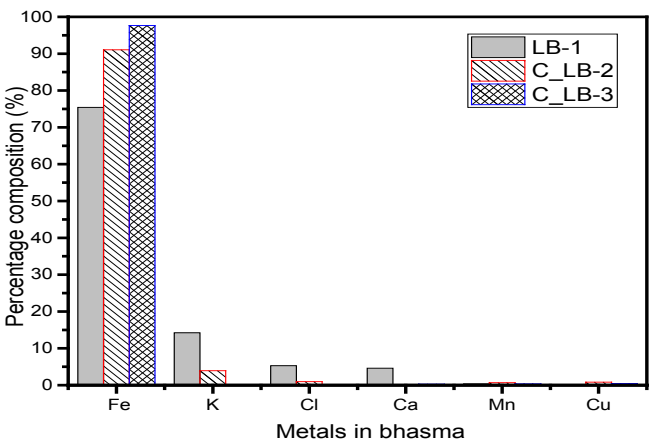

Figure 4: Plot showing bar graphs of metal content in the *Lauha bhasma* from different origin

Biological Test

Bhasmas are used for many antibacterial effects from very long ago and studied by clear zone method in agar plate. The inhibition zone formed on the plate by

the action of material kept in the agar hole indicates the antibacterial activity. Higher the zone of inhibition higher would be the activity. The LB synthesized are slightly antibacterial for *Escherichia coli* but not for *Staphylococcus aureus* middle row compares C_LB-1 & C_LB-2 and last row compares antibiotic tetracycline with DMSO.

Therapeutic effect of LB was evaluated against the bacterial strains *E. coli*, and *S. aureus* for LB in media Dimethyl Sulfoxide (DMSO). Iron nanoparticles have good antibacterial properties. The zone of inhibition with different bacteria *Escherichia coli* and *Staphylococcus aureus* are presented in Table 3 for LB, C_LB-2 and C_LB-3 with respect to the antibiotic Tetracycline 10 mg/disc.

Table 3: Antimicrobial activity shown by *Lauha bhasma* with microbes

S.No.	*Lauha Bhasma* Test drugs	Dose (mg/mL)	Zone diameter (mm)	
			Escherichia coli	*Staphylococcus aureus*
1	LB	30	3	~ 1
		15	~ 1	~ 1
2	C_LB-2	30	1	~ 1
		15	~ 1	~ 1
3	C_LB-3	30	1	~ 1
		15	~ 1	~ 1
4	Tetracycline	10 µg/disc	9	12
5	Ampicillin	10 µg/disc	-	-

Conclusion

In conclusion, synthesis of LB was done by following the standard *ayurvedic* text "*Rasashastra",* which involves normal purification, special purification, and calcination through which desired quality of LB was obtained. The synthesized LB was analyzed by different classical parameter and modern analytical techniques for standardization. Physico-chemical analysis of LB involves loss on drying (0.42 %), total ash value (17.3 %) and acid insoluble ash value (7.6 %). FTIR spectra show the presence of valuable organic moieties in LB which probably improve the medical properties. EDX data help to know the higher composition in LB was iron (75.43 %) while other elements were present in traces amount. XRD value shows the synthesized LB have an average particle diameter was 57.23 nm which is nanoscale range; as iron nanoparticles have a wide medical application to treat various diseases. The antimicrobial study helps to know a wider application of iron nanoparticles in therapeutic action to cure different iron deficiency diseases.

Conflicts of Interest

None

Competing Interest Statement

None

Ethics Approval and Consent to Participate

Not applicable

Additional information

No additional information is available for this paper.

Acknowledgment

The authors are highly thankful to the University Grants Commission (UGC), Nepal for financial support (Award No. FRG-73/74-S&T-17) for this research. Authors are also thankful to Nepal Academy of Science and Technology (NAST), Dr. Sven Henning, Prasamsha Pant, Bidit Lamsal and Tika Ram Bhandari for various supports to conduct this research work.

References

[1] S. Pal, "The Ayurvedic Bhasma: The Ancient Science of Nanomedicine," *Recent Pat. Nanomedicine*, vol. 5, no. 1, pp. 12–18, Jun. 2015, doi: 10.2174/1877912305666150417233945.

[2] S. Raj, K. Sivashanmugam, and K. M. Gothandam, "Ayurveda -A glance," *Res. Plant Biol.*, vol. 1, pp. 1–14, Jan. 2011, doi: 231157965.

[3] R. Jayasundar, "Ayurveda: a distinctive approach to health and disease," *Curr. Sci.*, vol. 98, no. 7, p. 8, 2010.

[4] A. Hankey, "Ayurvedic physiology and etiology: Ayurvedo Amritanaam. The doshas and their functioning in terms of contemporary biology and physical chemistry," *J. Altern. Complement. Med. N. Y. N*, vol. 7, no. 5, pp. 567–574, Oct. 2001, doi: 10.1089/10755530152639792.

[5] Amit S. Deshmukh, Renu Rathi, and Bharat Rathi, "Concept of Rasayana: Unique Ayurvedic Approach in Preventing Infectious Diseases with Special Emphasis on COVID-19," *Int. J. Res. Pharm. Sci.*, vol. 11, no. SPL1, pp. 938–941, Sep. 2020, doi: 10.26452/ijrps.v11iSPL1.3216.

[6] T. K. Biswas, "Drug Discovery From Ayurveda," in *Natural Products and Drug Discovery*, Elsevier, 2018, pp. 3–28. doi: 10.1016/B978-0-08-102081-4.00001-0.

[7] G. Hussain, "Concept of mardana: a review," *J. Drug Deliv. Ther.*, vol. 8, pp. 25–27, Oct. 2018, doi: 10.22270/jddt.v8i5-s.1929.

[8] S. Savrikar and B. Ravishankar, "Introduction to 'Rasashaastra'- The Iatrochemistry of Ayurveda," *Afr. J. Tradit. Complement. Altern. Med.*, vol. 8, no. 5S, Jul. 2011, doi: 10.4314/ajtcam.v8i5S.1.

[9] D. V. N. Hirudkar and D. R. M. Ganvir, "General Principles, Approaches and Therapeutic Utility of Rasashastra: An Ayurveda Review," *J. Drug Deliv.*, p. 3.

[10] D. Pal, C. K. Sahu, and A. Haldar, "Bhasma : The ancient Indian nanomedicine," *J. Adv. Pharm. Technol. Res.*, vol. 5, no. 1, pp. 4–12, 2014, doi: 10.4103/2231-4040.126980.

[11] K. Patwardhan, "The history of the discovery of blood circulation: unrecognized contributions of Ayurveda masters," *Adv. Physiol. Educ.*, vol. 36, no. 2, pp. 77–82, Jun. 2012, doi: 10.1152/advan.00123.2011.

[12] K. A and G. Ak, "A clinical study on Pandu Roga, iron deficiency anemia, with Trikatrayadi Lauha suspension in children.," *J. Ayurveda Integr. Med.*, vol. 3, no. 4, pp. 215–222, Oct. 2012, doi: 10.4103/0975-9476.104446.

[13] N. OD, "Critical Review: Bhasma Kalpana," *J. Homeopathy Ayurvedic Med.*, vol. 03, no. 04, 2014, doi: 10.4172/2167-1206.1000168.

[14] J. Liu, F. Zhang, V. Ravikanth, O. A. Olajide, C. Li, and L.-X. Wei, "Chemical Compositions of Metals in Bhasmas and Tibetan Zuotai Are a Major Determinant of Their Therapeutic Effects and Toxicity," *Evid. Based Complement. Alternat. Med.*, vol. 2019, pp. 1–13, Jan. 2019, doi: 10.1155/2019/1697804.

[15] S. Farooq, Z. Mehmood, F. A. Qais, M. S. Khan, and I. Ahmad, "Nanoparticles in Ayurvedic Medicine," in *New Look to Phytomedicine*, Elsevier, 2019, pp. 581–596. doi: 10.1016/B978-0-12-814619-4.00023-9.

[16] S. R. Lynch, "Interaction of Iron with Other Nutrients," *Nutr. Rev.*, vol. 55, no. 4, pp. 102–110, Apr. 2009, doi: 10.1111/j.1753-4887.1997.tb06461.x.

[17] R. S. Eisenstein, "Iron regulatory proteins and the molecular control of mammalian iron metabolism," *Annu. Rev. Nutr.*, vol. 20, pp. 627–662, 2000, doi: 10.1146/annurev.nutr.20.1.627.

[18] N. Singh and K. R. C. Reddy, "Pharmaceutical study of Lauha Bhasma," *AYU Int. Q. J. Res. Ayurveda*, vol. 31, no. 3, p. 387, Jul. 2010, doi: 10.4103/0974-8520.77157.

[19] A.-S. Zhang and C. A. Enns, "Molecular mechanisms of normal iron homeostasis," *Hematol. Am. Soc. Hematol. Educ. Program*, pp. 207–214, 2009, doi: 10.1182/asheducation-2009.1.207.

[20] T. R. Singh, L. N. Gupta, and N. Kumar, "Standard manufacturing procedure of Teekshna lauha bhasma," *J. Ayurveda Integr. Med.*, vol. 7, no. 2, pp. 100–108, Apr. 2016, doi: 10.1016/j.jaim.2015.08.003.

[21] B. Krishnamachary *et al.*, "Scientific validation of the different purification steps involved in the preparation of an Indian Ayurvedic medicine, Lauha bhasma," *J. Ethnopharmacol.*, vol. 142, no. 1, pp. 98–104, Jun. 2012, doi: 10.1016/j.jep.2012.04.021.

[22] V. K. L. Gupta and P. Bj, "Standard manufacturing procedure of lauha bhasma using triphala media and by employing electric muffle furnace heating," vol. 1, no. 3, p. 8, 2012.

[23] N. Joshi, M. K. Dash, and L. K. Dwivedi, "Critical review and concept of marana with special reference to lauha," *Int. J. Res. Ayurveda Pharm.*, vol. 6, no. 3, pp. 404–412, Jun. 2015, doi: 10.7897/2277-4343.06379.

[24] T. Pavani, K. V. Rao, C. S. Chakra, and Y. T. Prabhu, "Comparative study of ancient and modern procedures-Synthesis of Bhasma," p. 6, doi: 270475038.

[25] N. Gebreselassie, R. Abrahamsen, F. Beyene, F. A. Abera, and J. Narvhus, "Chemical composition of naturally fermented buttermilk," *Int. J. Dairy Technol.*, vol. 69, Feb. 2016, doi: 10.1111/1471-0307.12236.

[26] G. K. Randhawa and R. Sharma, "Chemotherapeutic potential of cow urine: A review," *J. Intercult. Ethnopharmacol.*, vol. 4, no. 2, pp. 180–186, Mar. 2015, doi: 10.5455/jice.2015022210032.

[27] B. Krishnamachary *et al.*, "Bhanupaka: A Green Process in the Preparation of an Indian Ayurvedic Medicine, Lauha Bhasma," *J. Chem.*, vol. 2013, pp. 1–8, 2013, doi: 10.1155/2013/951951.

[28] S. C. Bhargava, K. R. C. Reddy, and G. V. S. Sastry, "Identifications studies of Lauha Bhasma by X-ray diffraction and X-ray fluorescence," *Ayu*, vol. 33, no. 1, pp. 143–145, 2012, doi: 10.4103/0974-8520.100332.

[29] B. T. Punchihewa, M. A. B. Prashantha, P. I. Godakumbura, and S. K. M. K. Herapathdeniya, "The chemical role of natural substances used in Lauha Bhasma preparation," *J. Ayurveda Integr. Med.*, vol. 13, no. 1, p. 100412, Jan. 2022, doi: 10.1016/j.jaim.2021.02.003.

[30] N. Singh and K. R. C. Reddy, "Particle size estimation and elemental analysis of Lauha bhasma," *Int J Res Ayu Pharm*, vol. 2, pp. 30–35, Feb. 2011, doi: 285713566.

[31] P. Tambur, C. Chakra, and K. Rao, "A Green approach for the synthesis of nano-sized iron oxide, by Indian ayurvedic modified bhasmikaran method," Mar. 2013, doi: 270453965.

[32] W. A. Yusmaniar, Y. Adi, Taryana, and R. Muzaki, "Synthesis and Characterization of Composite UPR/Fe_3O_4 for Its Use as Electromagnetic Wave Absorber," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 196, p. 012033, May 2017, doi: 10.1088/1757-899X/196/1/012033.

[33] H. K. Farshchi, M. Azizi, M. R. Jaafari, S. H. Nemati, and A. Fotovat, "Green synthesis of iron nanoparticles by Rosemary extract and cytotoxicity effect evaluation on cancer cell lines," *Biocatal. Agric. Biotechnol.*, vol. 16, pp. 54–62, Oct. 2018, doi: 10.1016/j.bcab.2018.07.017.

[34] N. Joshi, M. Dash, L. Dwivedi, and G. Khilnani, "Toxicity study of Lauha Bhasma (calcined iron) in albino rats," *Anc. Sci. Life*, vol. 35, no. 3, p. 159, 2016, doi: 10.4103/0257-7941.179870.

Appendix

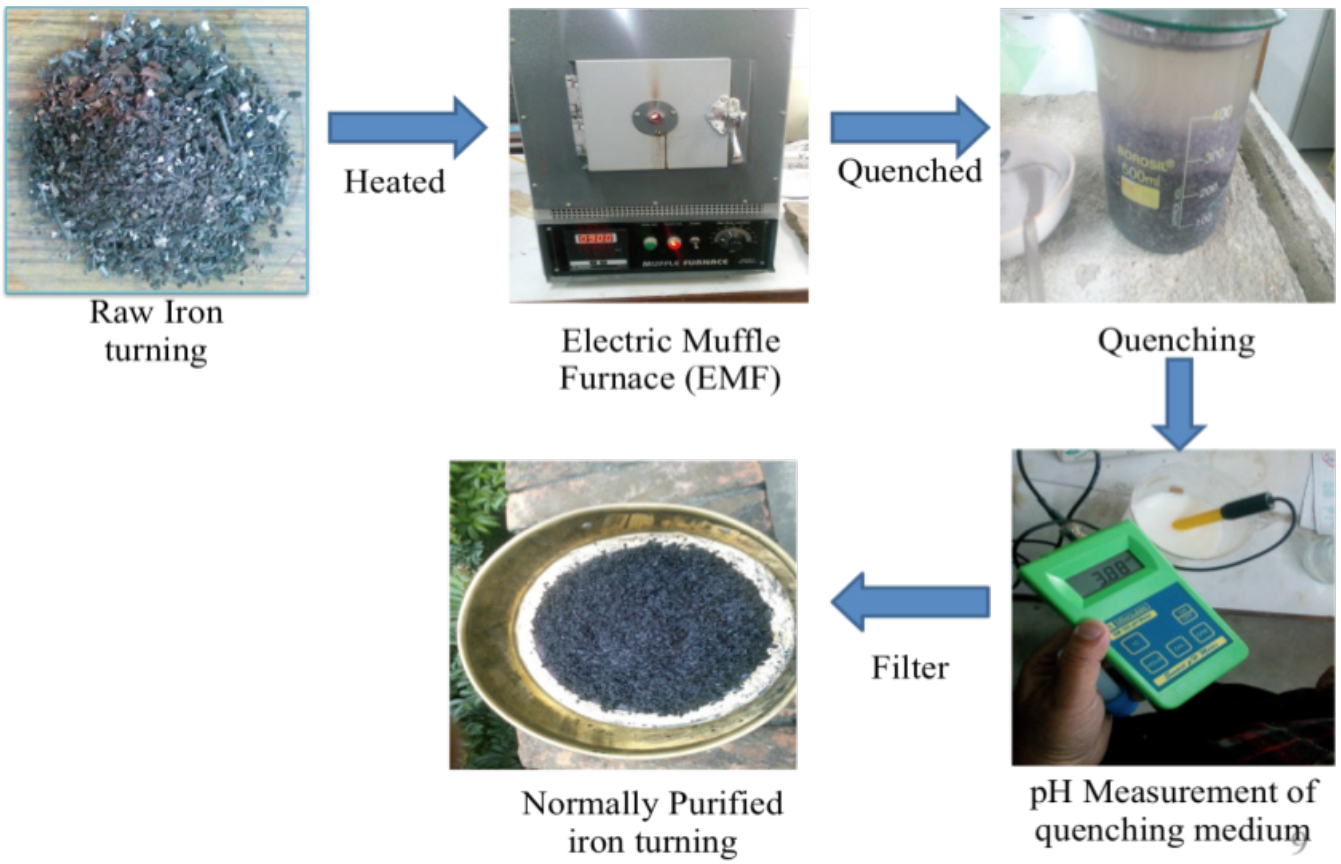

Figure 5: Diagrammatic scheme of normal purification step during *Lauha Bhasma* preparation

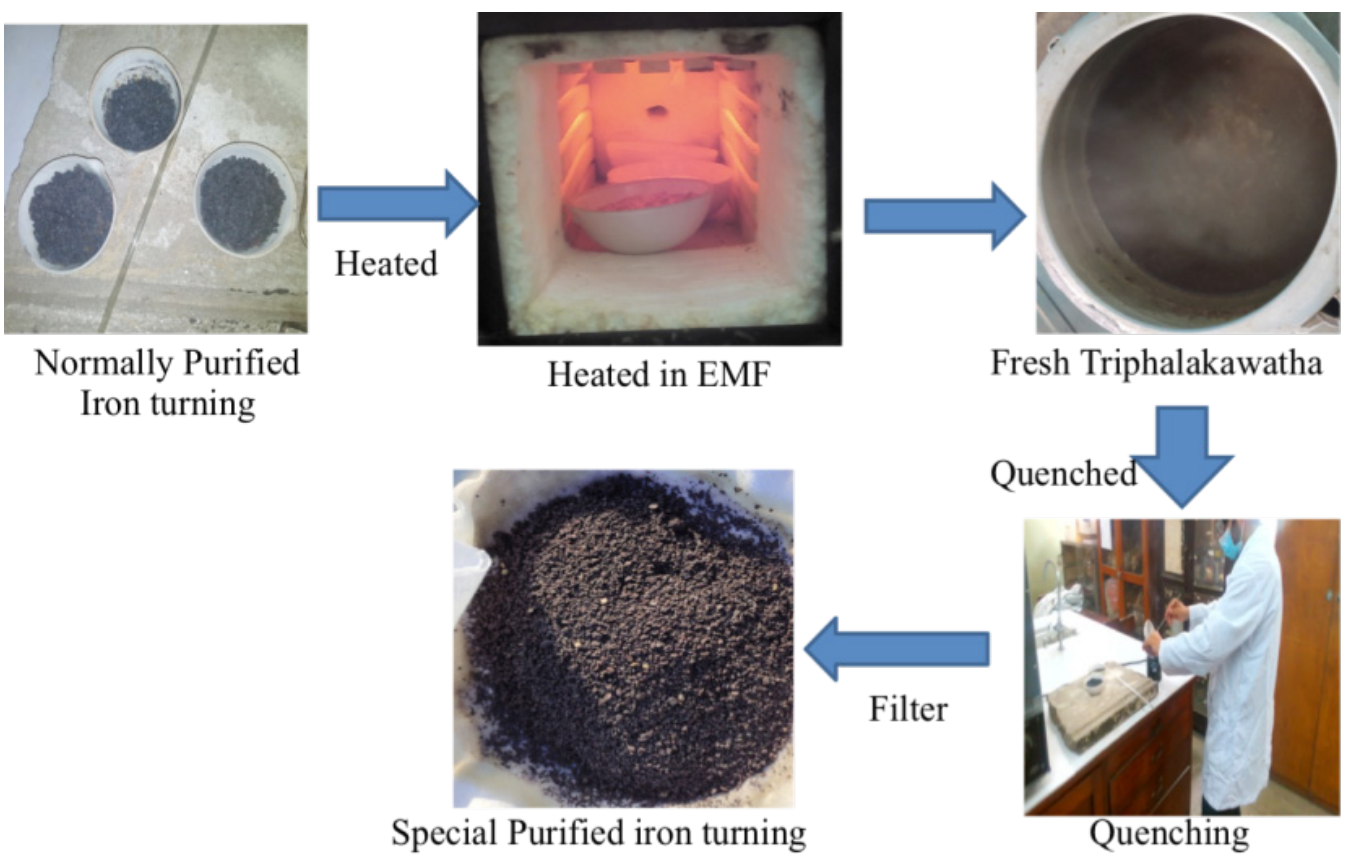

Figure 6: Diagrammatic scheme of special purification step during *Lauha bhasma* preparation

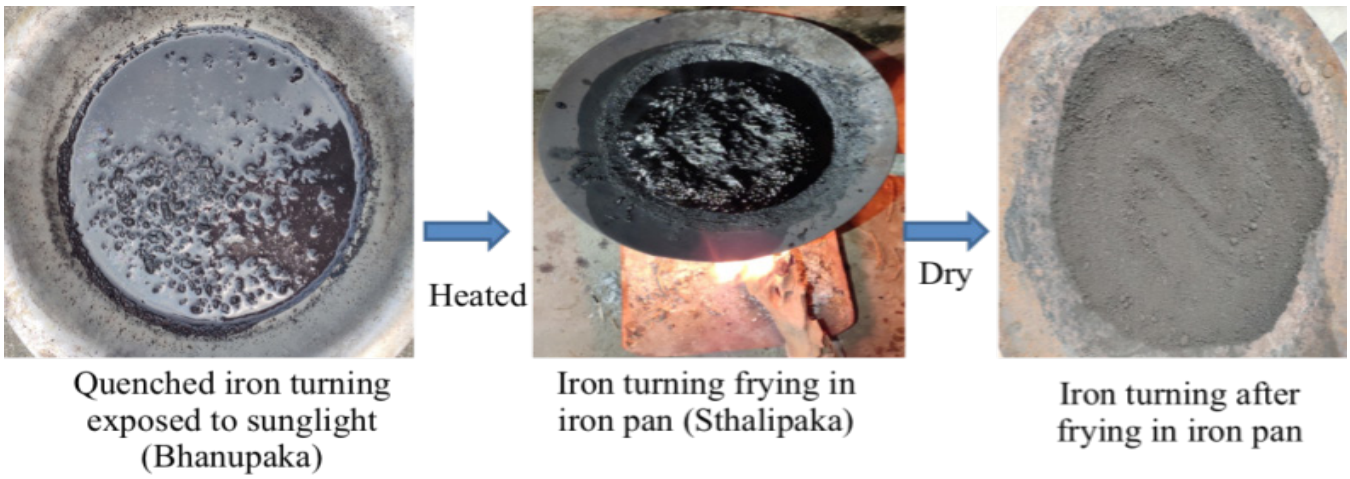

Figure 7: Diagrammatic scheme of *Bhanupaka* and *Sthalipaka* step during *Lauha Bhasma* preparation

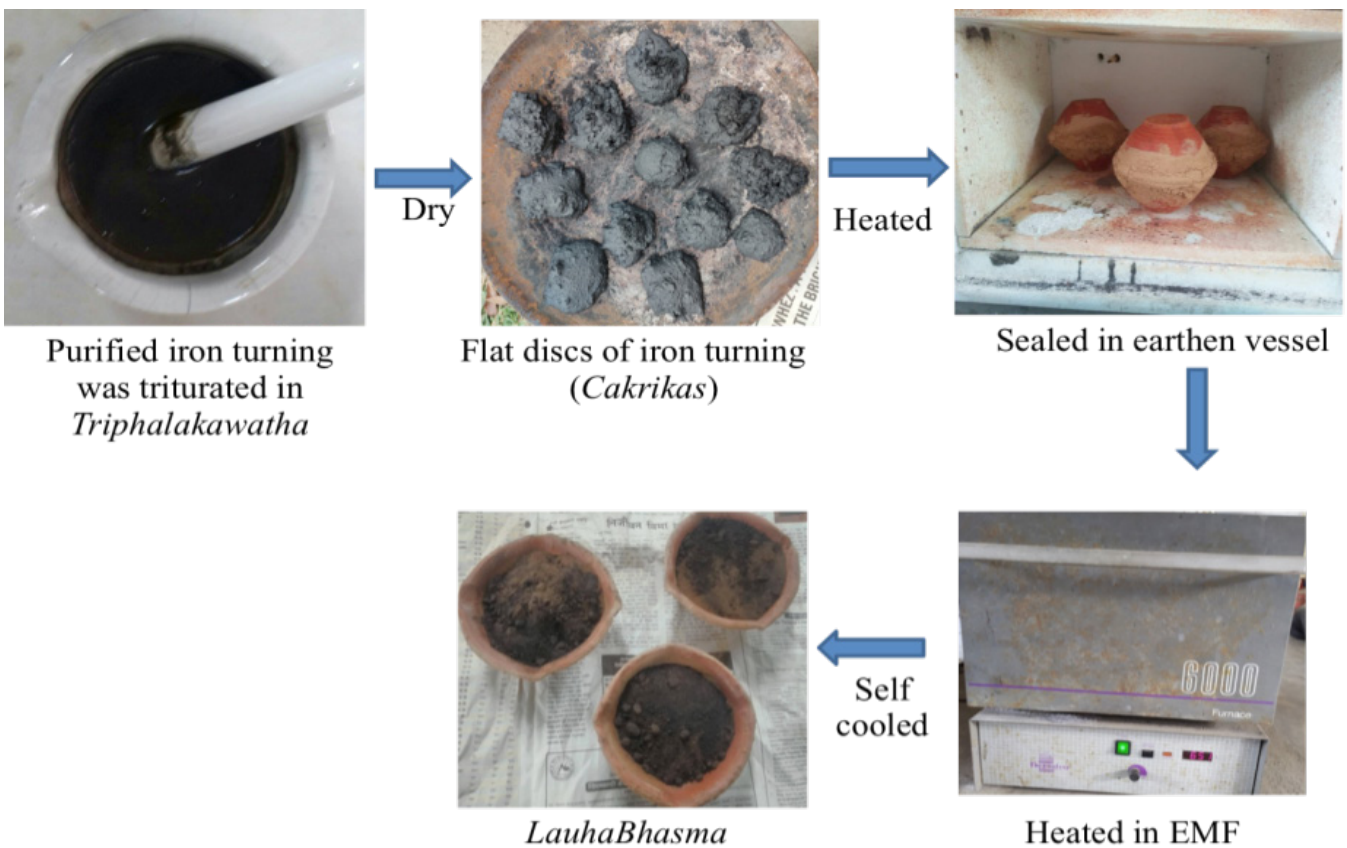

Figure 8: Diagrammatic scheme of calcination (*Marana*) step during *Lauha Bhasma* preparation

Figure 9: Antimicrobial study of *LB* in a) *E. coli* and b) *S. aureus*

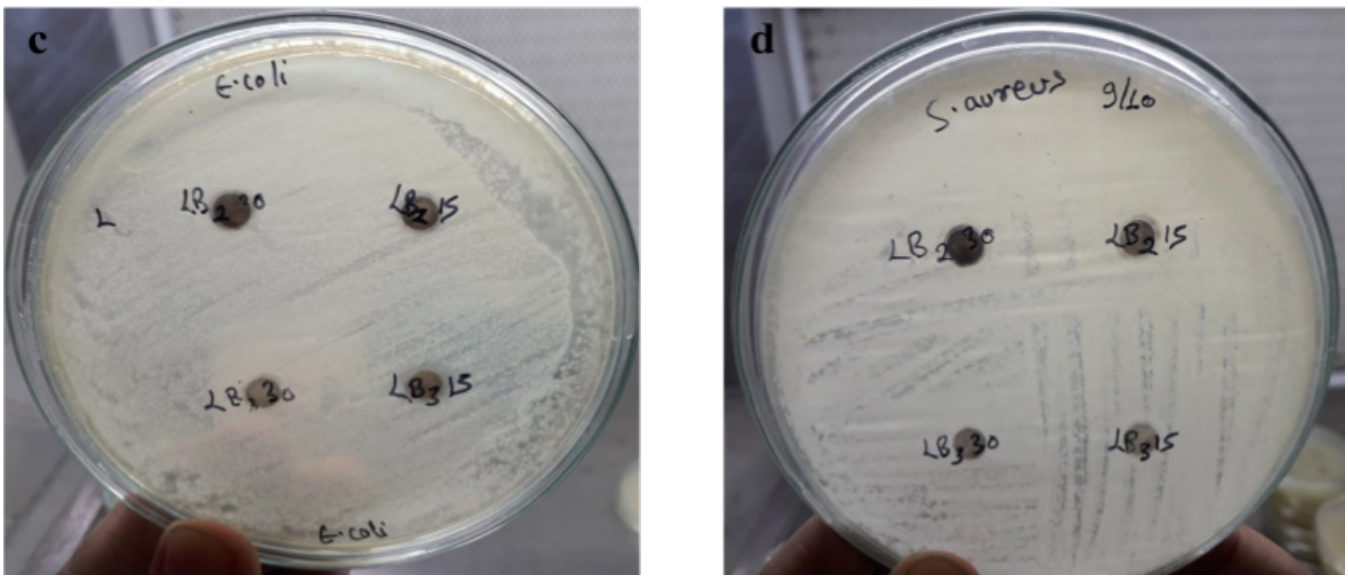

Figure 10: Antimicrobial study of C_*LB-2* and C_*LB-3* in c) *E. coli* and d) *S. aureus*

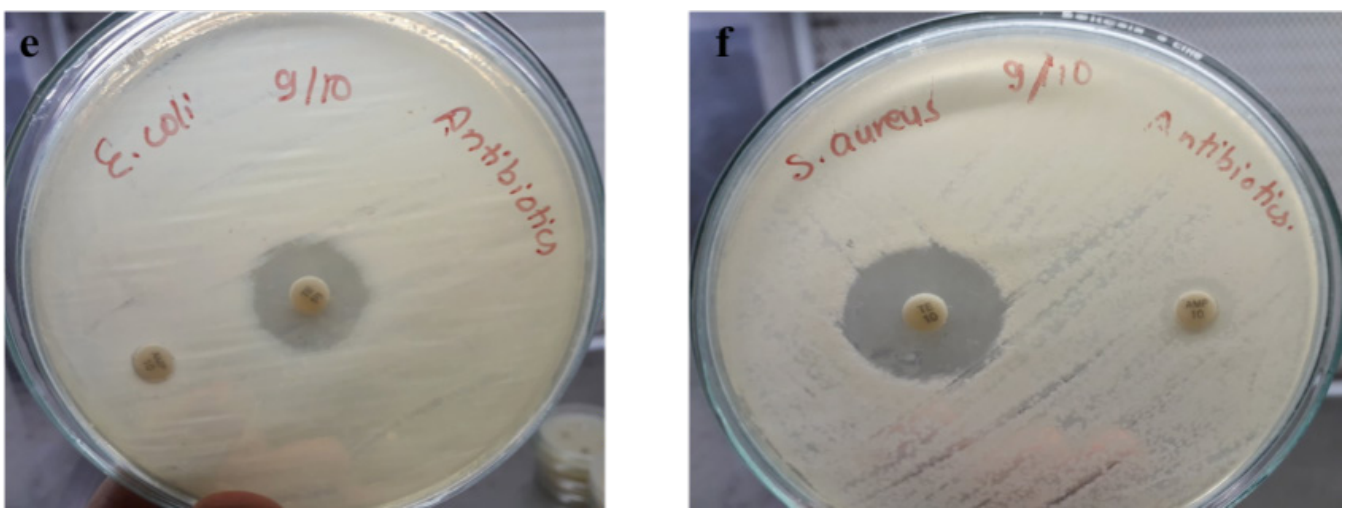

Figure 11: Antimicrobial study of standard antibiotics in e) *E. coli* and f) *S. aureus*