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From Traditional medicine to cutting-edge science: Abhrak and Tamra Bhasma's dual Antimicrobial and Anticancer abilities

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Abstract

Antibiotic resistance and cancer are two of the major public healthcare challenges the world is facing today. As the number of deaths caused by these diseases continues to rise, there is a growing need for new, naturally derived drugs that can target these problems effectively. This study explores the potential use of the Ayurvedic compound bhasma as an antibacterial and anticancer agent. The bhasma samples were characterized using various techniques, including FTIR, SEM, and EDAX. Abhrak and Tamra bhasma was tested against five isolates each of *E. faecalis*, *K. pneumoniae*, and *P. aeruginosa* for antibacterial activity at three different concentrations (2.5mg, 5mg, 10mg), as well as against MCF7/Breast cancer cell lines for anticancer activity. The results suggest that Tamra bhasma is effective in inhibiting the growth of all isolates of *E. faecalis*, while Abhrak bhasma is effective against *K. pneumoniae* and *P. aeruginosa* isolates. Tamra bhasma also shows greater potential in controlling the growth of cancerous cells compared to Abhrak bhasma. The study concludes that bhasma has the potential to be a promising therapeutic agent for treating cancer and bacterial infections and could revolutionize modern healthcare.

Key words : Bhasma, Antibacterial, Anticancer, FTIR, SEM with EDAX.

The global public healthcare system is currently facing two major serious healthcare issues that will result in a high rate of morbidity and mortality. The first is the rise of infections due to pathogens that are resistant to multiple drugs (MDR), and the other is cancer. Drug resistance has contributed to toxicities and the ineffectiveness of currently available medications,

representing a threat to the health of the world. Bacteria undergo mutational changes and modulate gene expression to evolve resistance against current antibiotics as a result of nature's "survival of the fittest" principle. Both Gram-positive and Gram-negative organisms exhibit drug resistance due to plasmids obtained by gene transfer. A report from the Centre for Disease Control (CDC) suggests that in the year 2019, about 1.27 million people died due to antimicrobial resistance[33].

Cancer stands as one of the deadliest diseases, responsible for every second death globally. Recent reports indicated that among every five patients, one dies due to cancer[12]. The gene mutation is a prime cause of cancer and also the carcinogenic agents to which we are exposed daily intentionally or unintentionally. These factors bring about changes in cell functioning and alter the cell metabolism influencing the rapid cell growth and multiplication of cells causing tumours at different sites of the body. The American Cancer Society predicted about 1,918,030 new cancer cases and 609,360 deaths due to cancer in the United States in the year 2022. In 2030, the number of people dying due to cancer will be around 23.6 million if this current trend continues[25].

The research and development of new anticancer and antibacterial agents is the need of an hour. People are suffering drastically due to these disorders and the number of deaths is increasing day by day. Unless and until new targeted, active, efficient drug molecules will not develop the battle against these disorders will be the same. In the current study, an attempt was made to tackle these problems and put forward an Ayurvedic compound bhasma that can act as both an antibacterial and anticancer agent effectively.

The history of medicine reflects humanity's struggle against emerging diseases and the evolution of treatments. Ayurvedic drugs, although effective, remain unchanged over time as they target the intrinsic causes of human health[21]. *Rasashastra*, a branch of Ayurveda, focuses on processing natural medicines to eradicate the root causes of diseases. Bhasma, such as Abhrak and Tamra, are herbal, metallic, and mineral compounds prepared through Ayurvedic methods and are effective in treating various ailments and infections[8]. There is a need to screen more drugs to find an innovative drug therapy for cancer and drug-resistant pathogens. This was one of the reasons for screening Abhrak and Tamra bhasma for antibacterial and anticancer activity.

The aim and objective of the current study were to study the *in vitro* antibacterial activity of Abhrak and Tamra bhasma against human pathogens in a dose-dependent manner. And also, to put forward Abhrak and Tamra bhasma as an anticancer agent to inhibit cell growth of MCF7 cancer cell line (*in vitro*).

Procurement of bhasma samples :

The ancient secrets of therapeutic healing, in the form of metal, minerals, and herbs are confined in bhasma. Bhasma such as Abhrak bhasma which is mica-based and Tamra bhasma which contains copper as a primary element were procured from the Patanjali store.

Physicochemical characterization of bhasma samples :

Different analytical techniques were

employed to analyze the bhasma samples, allowing the determination of their organic constituents, particle size, and elemental composition. The Fourier transform infrared spectrophotometry (FTIR) analysis was conducted using the FTIR model: SHIMADZU-8400. The FTIR spectrum of bhasma was recorded in the range of 4000 to 500 cm^{-1} by the KBr pellet method. Scanning Electron Microscope (SEM) Model- ZEISS equipped with Energy-dispersive X-ray spectroscopy (EDAX) was used to determine the particle size and elemental composition of bhasma.

Isolation of bacteria:

The human pathogenic bacteria were isolated from clinical samples on UTI agar plates (HiMedia- M135R). Five isolates each of *E. faecalis*, *K. pneumoniae,* and *P. aeruginosa* were taken as test organisms for the research study.

Antibacterial activity of bhasma:

Preparation of stock solutions- 0.1 g/ml concentration of stock solutions of bhasma was prepared in respective solvents. To prepare the suspensions, 1 gram of Abhrak bhasma was added to 10 ml of sterile distilled water, and 1 gram of Tamra bhasma was added to sterile Dimethyl sulfoxide (DMSO). The suspensions were mixed using a vortex machine to distribute the particles uniformly in the solvents.

Antibacterial activity :

The antibacterial potential of bhasma was tested against several bacterial species using Kirby Bauer well diffusion technique[4]. A 0.5 MacFarland turbidity standard bacterial broth was spread on sterile Muller Hinton Agar plates (MHA) (HiMedia- 173) with the help of sterile cotton swabs. Four wells were punctured on the solidified MHA agar plates using a well borer (8 mm diameter). Bhasma samples of 2.5 mg (25 µl), 5 mg (50 µl), and 10 mg (100 µl) concentrations along with a control (distilled water/DMSO) of 100 µl were added to the wells in each plate. The plates were incubated at 37°C for 24 hours. The zone of inhibition was measured by repeating the sets in triplicates for each of the five isolates of *E. faecalis, K. pneumoniae,* and *P. aeruginosa*. The mean zone of inhibition and standard deviation for each bacterial isolate was calculated.

MTT Assay of bhasma :

The cytotoxic effect of the bhasma was tested on MCF7 breast cancer cell lines (*in vitro*). The MCF7 cell line was procured from NCCS Pune and the cells were maintained in DMEM medium (HiMedia). Serial two-fold dilutions of 6.25 to 100 µg/ml of bhasma were prepared in respective solvents and tested. Bhasma test solutions of varying concentrations (100 µl each) were added to a partial monolayer of cells in a 96-well microtiter plate. Then the plates were incubated at 37°C for 24 hours with 5% CO_2 incubator. After this the test solutions were discarded and the 100 µl MTT reagent was added to each well followed by incubation for 4 hours in a CO_2 incubator. After incubation, the supernatant was removed and 100 µl DMSO was added to solubilize the formed formazan. The absorbance at 570 nm was measured using a microplate reader (ROBONIK-Readwell Touch Elisa Plate Analyzer). The percentage (%) of viable cells was calculated using the following formula.

$$\% \text{ Viability} = \frac{\text{Sample Absorbance}}{\text{Control absorbance}} \text{ x } 100$$

The IC_{50} quantifies a compound's efficacy in inhibiting biological processes. IC_{50} values for cytotoxicity assessments were determined using a sigmoidal dose-response curve and nonlinear regression analysis.

Fourier transform infrared spectrophotometry (FTIR) :

The functional groups that are present in the bhasma were identified by FTIR characterization study. The FTIR spectrum and peak of Abhrak bhasma are showcased in Figure 1. Meanwhile, the banding pattern and peak values of Tamra bhasma are shown in Figure 2. In the FTIR spectrum, various vibrational peaks with different intensities were observed. The banding patterns of Abhrak bhasma exhibited two sharp peaks at 3807.48 cm^{-1} and 3568.31 cm^{-1}, which indicate the presence of hydroxy group (-OH) in the compound. The peaks at 603.72 cm^{-1} and 435.91 cm^{-1} indicate the alkyl halide (-CH_2-X) stretching pattern in the test compound. Moreover, the peaks at 2002.11 cm^{-1} and 2206.57 cm^{-1} represent the presence of aromatic compounds (C_4r+2H_2r+4) and aliphatic amines (-CN), respectively, in the Abhrak bhasma. The peaks at 420.48 cm^{-1}, 480.28 cm^{-1}, and 680.87 cm^{-1} indicate the presence of acid chloride, titanium oxide, and nickel oxide in the test compound. A broad peak at 972.12 cm^{-1} indicates that barium cation is present in traces in Abhrak bhasma.

The IR Spectrum of Tamra bhasma showcased about 40 peaks with varying intensities. There are 15 prominent vibrational peaks at frequencies 3078.39 cm^{-1}, 3159.40 cm^{-1}, 3375.43 cm^{-1}, 3441.01 cm^{-1}, 3518.16 cm^{-1}, 3574.10 cm^{-1}, 3612.67 cm^{-1}, 3645.46 cm^{-1}, 3664.75 cm^{-1}, 3687.90 cm^{-1}, 3726.47 cm^{-1}, 3826.77 cm^{-1}, 3840.27 cm^{-1}, 3932.86 cm^{-1}, 3946.36 cm^{-1}, and 3990.72 cm^{-1} which indicate the presence of -OH (hydroxyl) stretching bonds in Tamra bhasma. The peaks at frequencies 3118.90 cm^{-1}, 3041.74 cm^{-1}, 2958.80 cm^{-1}, 2544.11 cm^{-1}, and 588.29 cm^{-1} represent the presence of organic hydrocarbons alkane group (-CH) group. Four sharp peaks were observed at frequencies 405.05 cm^{-1}, 414.70 cm^{-1}, 445.56 cm^{-1}, and 489.92 cm^{-1}, indicating the presence of copper oxide particles. The intense vibrational peak obtained at 756.10 cm^{-1} symbolizes the presence of alkenes (CH_2) bending. The peaks at frequencies 518.85 cm^{-1}, 1381.03 cm^{-1}, 1967.39 cm^{-1}, and 2098.55 cm^{-1} indicate the presence of disulfide (S-S), aromatic amine groups, ammonium nitrate, and allenes functional groups, respectively.

Scanning Electron Microscope with Energy-dispersive X-ray spectroscopy :

Scanning electron microscopy was used to determine the particle size and shape of particles in the bhasma. The average particle size of Abhrak bhasma was found to be 316 ± 84.74 nm. The Abhrak bhasma had uneven particle size distribution and a multilayered crystalline structure with an oblong to spherical shape was observed (Figure 3). In the case of Tamra bhasma, the average particle size was 306 ± 99.35 nm, and a large number of agglomerated clumps were observed in the

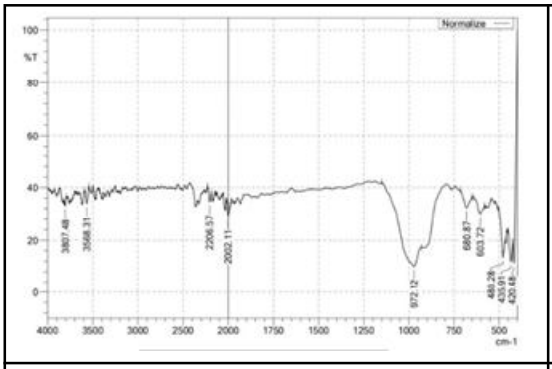


Figure 1- FTIR spectrum of Abhrak bhasma

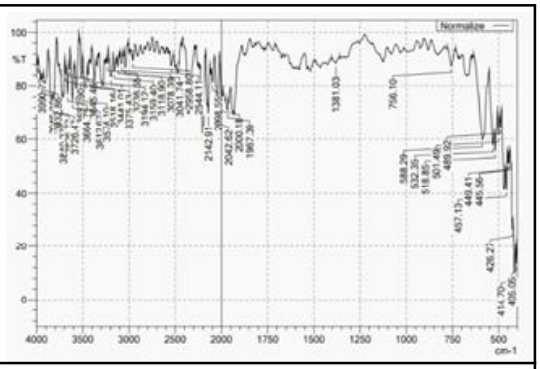


Figure 2- FTIR spectrum of Tamra bhasma

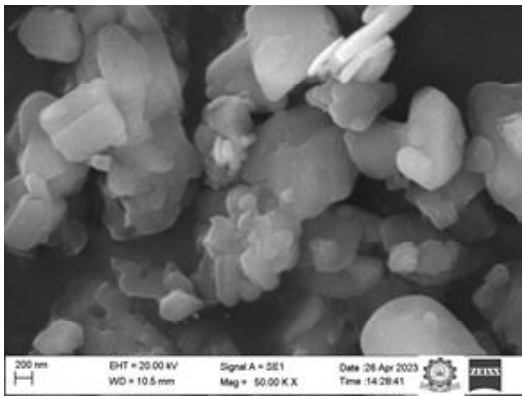


Figure 3- SEM image of Abhrak bhasma

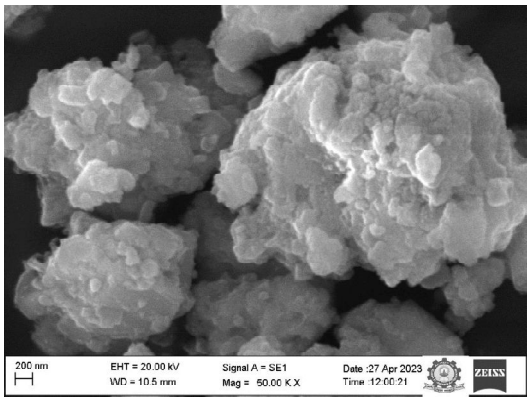


Figure 4- SEM image of Tamra bhasma

microscopic image (Figure 4). The elemental compositions of the bhasma were determined by EDAX, which was attached with SEM. The EDAX analysis revealed that Abhrak bhasma contained elements such as oxygen (40.5%), aluminum (7.4%), magnesium (5%), silicon (13.8%), potassium (12.2%), calcium (1.1%), and iron (17.7%) in moderate amounts. It also contained elements such as chromium (1%), titanium (1.3%), and carbon (1.0%) in trace amounts (Figure 5).

In the case of Tamra bhasma, the elements copper (54.0%) and oxygen (26.3%) were found in abundant amounts, while elements such as silicon (1.0%), chromium (1.3%), potassium (1%), chlorine (0.7%), iron (5.0%), and aluminum (2.4%) were found in trace amounts (Figure 6).

Antibacterial activity of bhasma :

The antibacterial efficacy of bhasma samples at three different concentrations was tested using the well diffusion technique. The results showcased that the bacterial isolates

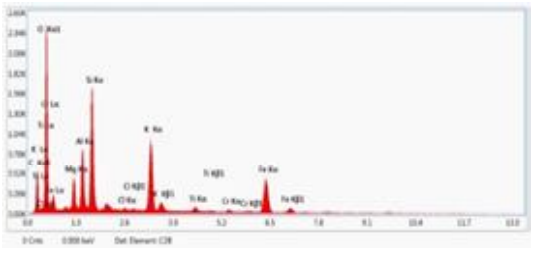


Figure 5- Abhrak bhasma EDAX

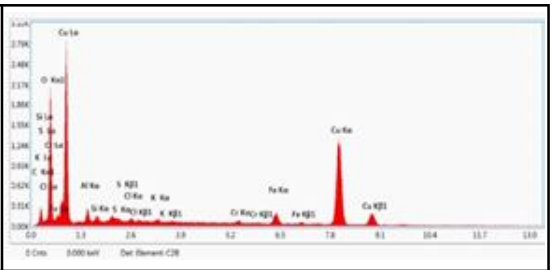
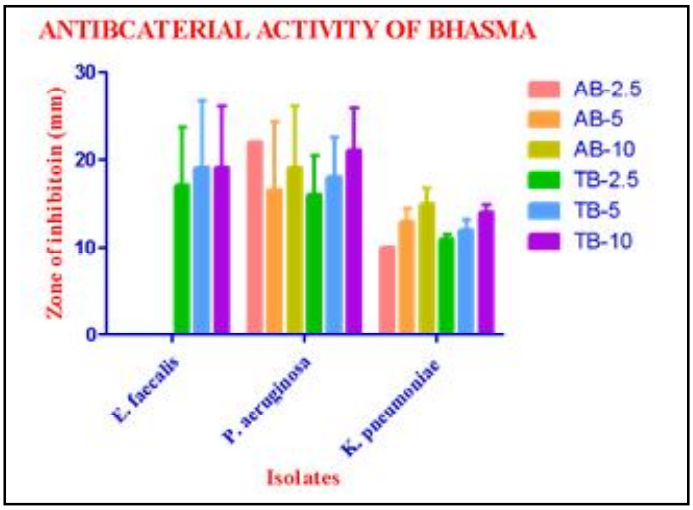


Figure 6- Tamra bhasma EDAX

had different degrees of sensitivity to the bhasma samples, with a range of zones of inhibition. *E. faecalis*, a Gram-positive isolate, was found to be completely resistant to Abhrak bhasma at all concentrations, while showing remarkable sensitivity to Tamra bhasma at all concentrations, with a zone of inhibition values ranging from 19.8 ± 7.72 mm (5 mg) to 19.8 ± 7.04 mm (10 mg).

In contrast, a moderate range of growth inhibition was noted in *P. aeruginosa* and bhasma. The diameter of the zone of inhibition increased with increasing concentrations, and the maximum zone of inhibition was observed at 10 mg (19 ± 7.07 mm) for *P. aeruginosa* isolates and 15 ± 1.73 mm (10 mg) for *K. pneumoniae* isolates.

For Tamra bhasma, a moderate range of antibacterial activity was seen for *K. pneumoniae* isolates, while the maximum zone of inhibition noted for growth inhibition of Tamra bhasma against *P. aeruginosa* was 21.2 ± 4.81 mm at 10 mg concentration (Graph 1).

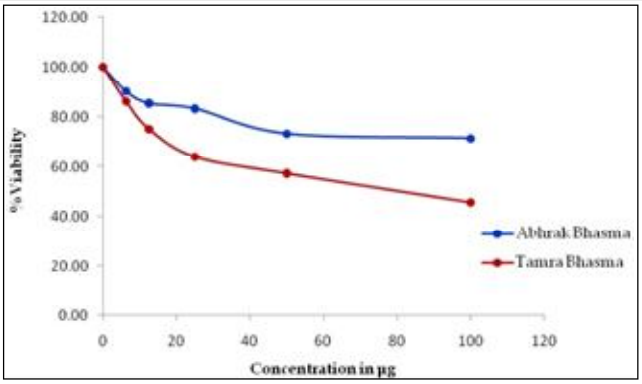


Graph-1 - Graphical representation of the antibacterial activity of Bhasma

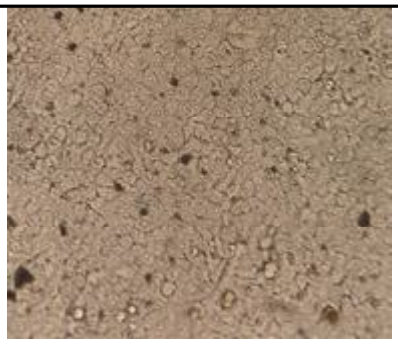
MTT Assay of bhasma :

Abhrakand Tamra bhasma are compounds made up of metallic, mineral, and herbal elements that have medicinal properties. They can prevent cancer cells from multiplying and spreading by affecting cellular metabolism. To determine the effectiveness of Abhrak and Tamra bhasma as an Ayurvedic anticancer agent with a particle size in the nanometer range, they were tested on MCF7 (Breast Cancer) cell lines. The IC50 value was calculated, which is the concentration at which the bhasma can inhibit the growth of cancer cells by 50%.

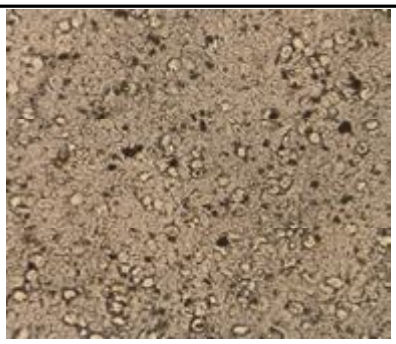
The results of the study revealed that Abhrak bhasma exhibited an average cell growth inhibition of 1.287 ± 0.006, 1.210 ± 0.005, 1.189 ±0.007, 1.046± 0.004, 1.021 ± 0.005 at concentrations of 6.25, 12.50, 25.00, 50.00, 100.00 µg/ml respectively. Tamra bhasma, on the other hand, exhibited an average cell growth inhibition of 1.231 ± 0.004, 1.073 ± 0.005, 0.914 ± 0.003, 0.818 ± 0.004, 0.653± 0.005 at concentrations of 6.25, 12.50, 25.00, 50.00, 100.00 µg/ml respectively. The IC_{50} values of Abhrak bhasma and Tamra bhasma were >100 µg and 74.99 µg, respectively (Graph 2).



Graph 2- Graphical Representation of Effect of Bhasma on Cell Viability of MCF7 cancer cell lines

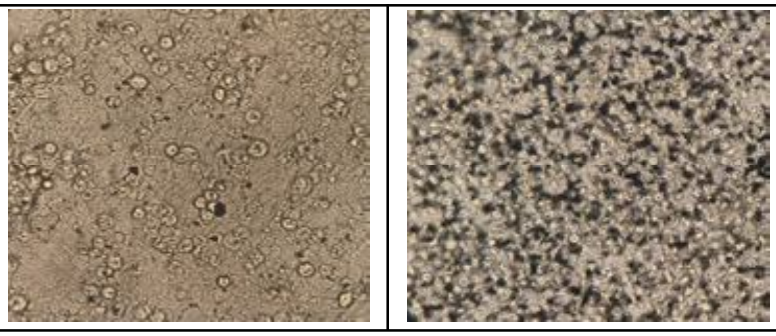


6.25 µg/ml



100.00 µg/ml

Figure 7a- MTT Assay of Abhrak bhasma on MCF7 cell lines



6.25 µg/ml	100.00 µg/ml

Figure 7b- MTT Assay of Tamra bhasma on MCF7 cell lines

Based on the analysis, it was found that Tamra bhasma is more effective than Abhrak bhasma in preventing the growth of cancer cells. The study also showed the disintegration of cancer cells for increasing concentrations of bhasma, which can be seen in Figures 7a and 7b.

FTIR uses infrared light to determine a sample's chemical composition, identifying functional groups and bonds to assess purity, properties, and reactivity. The findings of the current study suggest that Abhrak bhasma have presence of several functional groups such as alcohol, alkyl halides, aromatic compounds, aliphatic amines, and acid chlorides, as identified through FTIR analysis. A similar study by Wele *et al.,*[34] reported the presence of -OH groups in the peak range of 3700-2700 cm^{-1}, as well as free alcohol groups in the vibrational peak range of 3700-3600 cm^{-1} in Abhrak bhasma. They also detected carbonate groups, nitro groups, acid anhydrides, benzene derivatives, silicate groups, and alkyl bromide functional groups at 2350-2340 cm^{-1}, 1550-1500 cm^{-1}, 1045-980 cm^{-1}, 900-400 cm^{-1},750-470 cm^{-1}, and 685-500 cm^{-1}, respectively[34]. The research conducted by Kantak *et al.,* in 2020 provided insights into the characterization of Abhrak bhasma, synthesized through two different methods. The study revealed the presence of a hydroxyl group in the peak range of 2700-3700 cm^{-1}, which the researchers attributed to the bhasma's hydrophilic nature. Additionally, the study reported the presence of Mg_2Al-OH stretching at 3654 cm^{-1} and $MgFe_3^+$-OH stretching at 3594 cm^{-1} and 3502 cm^{-1}, respectively. A sharp peak at 1000-1250 cm^{-1} was observed due to Si-O stretching. The study also reported the presence of Mg_2-OH and alkyl halides at 712-681 cm^{-1} and 500-690 cm^{-1}, respectively. These findings are relevant to our current study as we have also observed similar vibrational peak intensities corresponding to hydroxyl groups, alkyl halides, and aromatic compounds[17]. The findings of Singh *et al.,*[27] provide strong evidence in support of the current study. The researchers identified functional group bonds like Ca-O, C-H, C-O, C-O-C, Fe_2O_3, MgO, and Si-O-Si at specific vibrational peaks of 1516 cm^{-1}, 1369 cm^{-1}, 752 cm^{-1}, 1220 cm^{-1}, 516 cm^{-1}, 891 cm^{-1}, and 423 cm^{-1} respectively, during their characterization

of Abhrak bhasma[27]. The presence of Mg_2Al-OH and Mg_2-OH groups in our Abhrak bhasma samples showcase its purity as these functional groups play a vital role in composition and activity of Abhrak bhasma.

The FTIR analysis of Tamra bhasma in current study revealed the presence of -OH, CuO, -CH, $-C_2H_4$, and S-S functional groups. These results align with the findings of a previous study by Wadekar *et al.,* back in 2005, which characterized Tamra bhasma and reported the existence of copper oxide particles in it. Interestingly, their analysis further detected peak intensities between 468-488 cm^{-1}, which adds to the evidence supporting the presence of copper oxide[32]. Chaudhari *et al.,*[9] conducted FTIR analysis of Tamra Bhasma at different stages of synthesis, and reported the presence of alkane groups (1380 cm^{-1}, 2862.54 cm^{-1}, 22921.99 cm^{-1}, 748.67 cm^{-1}, 694.67 cm^{-1}, 748.67 cm^{-1}) and alkenes (1634.56 cm^{-1}, 1634.56 cm^{-1} 1454.37 cm^{-1}). These findings are strikingly similar to our own FTIR analysis, further affirming the authenticity of our results[9]. The current study suggests that Tamra bhasma contains copper oxide particles, indicating the presence of copper in various forms. This has great potential in treating clinical ailments.

Scanning Electron Microscopy (SEM) is an analytical technique that is combined with Energy Dispersive X-ray Analysis (EDAX) to characterize materials at the micro- and nanoscale levels. This technique is comprehensive and helpful in determining the size, shape, structure, and elemental composition of a compound. Many researchers have investigated bhasma in various aspects like production, its activity, and characterization, and the majority of them suggested bhasma as a nanomedicine[10,16,18,23,31]. However, this contradicts the findings of the current study. The particle size of bhasma was not in the range of nanoparticles as the literature suggests[1,7,8,19,22,24]. The recorded particle size was in the range of 300 to 400 nm, which is on the borderline of the nanoparticle dimension range. In the present study scanning electron microscopy examination revealed that Abhrak bhasma possesses an average particle size of 316 ± 84.74 nm, with particles exhibiting an irregular shape and structure. Our findings are consistent with those of Balkrishna *et al.,* who reported a diameter size of 100 nm to ~1 μm for Abhrak bhasma[3]. The study of Wele *et al.,*[34], have reported a particle size of 28-88 nm which contradicts our findings[34]. In a research study by , Kantak *et al.,*[17] they reported a unique and complex multilayered structure of Abhrak bhasma. Additionally, the study revealed that the particles are relatively small, measuring in at around 200 nm [17]. Our results are consistent with the study conducted by Singh and colleagues in 2018, which found that the average size of Abhrak bhasma particles falls within the range of 200 nanometres[27]. The varying particle size of the bhasma may be due to the different methods followed by each manufacturer in *Bhasmikarana* process. The current study's EDAX analysis found that Abhrak bhasma contains essential elements like Oxygen, Aluminium, Magnesium, Silicon, Iron, and more. This study's findings align with Balkrishna *et al., 's* research in 2021, where they also identified all these vital elements in Abhrak bhasma[3]. The presence of Si, Ca, Fe, and Al elements in Abhrak bhasma has been extensively reported by numerous researchers, highlighting the significance of these minerals

in their composition. These minerals are known to play a vital role in several physiological processes, underscoring the importance of Abhrak bhasma as a potential therapeutic agent[15,17,34]. The results of the present study indicate that Tamra bhasma contains a significant number of essential elements such as oxygen, copper, and aluminum. The use of EDAX analysis has enabled us to identify these elements with great precision. These findings are in line with those reported by Wadekar *et al.,* in 2005, who also observed the presence of copper oxide through a stoichiometry study along with EDAX analysis[32]. Tamra bhasma is a traditional Ayurvedic medicine that has been used for centuries. It is a copper-based formulation that is believed to have therapeutic properties. Recent studies have shed light on the elemental composition of Tamra bhasma, revealing that it contains a significant amount of oxygen and copper. These elements are thought to play a crucial role in the medicinal properties of Tamra bhasma[2,4,26].

The first step in establishing the efficacy of an antibacterial agent for human or animal use is to test its antibacterial activity *In vitro*. In the present study, Abhrak and Tamra bhasma showcased potential antibacterial properties when tested *invitro*. Previous research by Singh *et al.,*[26] also showcased similar results with Gram-positive *S. aureus* species with a range of zones of inhibition up to 15 mm [26]. The study by Wijenayake *et al.,*[35] reported that Gram-positive isolates of *S. aureus* to be resistant to Abhrak bhasma which contradicts our findings[35]. Tambekar and Dahikar conducted a research study in 2010, where they evaluated the effectiveness of Tamra bhasma, an antibacterial agent, against enteric pathogens. They tested it in three different solvents and concluded that it could be an effective way to combat bacterial infections[29]. In comparison to the current research, a study conducted by Begum and Chavadi in 2017 found that Abhrak bhasma did not exhibit any efficacy against Gram-negative isolates of *P. aeruginosa* at a concentration of 200 mcg [5]. Gopinath and Shivashankar's critical review in 2016 suggests that Abhrak bhasma, a substance with trace minerals, has antiviral and antibacterial properties that improve the immune system[13]. Singh *et al.,* 2017 conclusively demonstrated that Tamra Bhasma is a potent antibacterial agent that can effectively inhibit the growth of *K. pneumoniae* and *P. aeruginosa*[26].

The *in vitro* anticancer activity is a type of testing that is carried out in a laboratory to assess the potential of a compound to inhibit or destroy cancer cells. This testing helps researchers identify promising candidates that may be further studied and developed as anticancer agents outside of a living organism. The present study has reported that Tamra and Abhrak bhasma possess potent anticancer activity against breast cancer cell lines. In a similar study by Tamhankar and Gharote[30], Abhrak bhasma was found to be effective against three different cell lines, with a concentration of about 80 µg, LC_{50} value was obtained[30]. In a recent study conducted by Naikare *et al.,* (2022), Abhrak bhasma was reported as a promising cytotoxic compound with potential anti-cancer properties. The study found that at a concentration of 168.60 µg/ml (IC_{50} value), Abhrak bhasma exhibited significant cytotoxicity against Adenocarcinoma Human Alveolar Basal Epithelial Cells

(A549) cell lines[20]. In 2020, Bera *et al.,* reported Vanga Bhasma's dual nature as an antibacterial and anticancer agent which is similar to our study[6]. It is also worth noting that other types of bhasma, such as Swarna bhasma, Yashsdha bhasma, Shankha bhasma, and Rajat bhasma, may also have potential as anticancer drugs[11,28]. This exciting discovery could pave the way for new and innovative treatments that could help millions of people worldwide.

The current era poses significant threats to both cancer treatment and combating antibiotic-resistant bacteria. Furthermore, chemotherapy's adverse effects make it imperative to find more effective therapeutic solutions. Our research indicates that bhasma presents a viable option for a combined therapeutic approach, addressing the pressing demands of modern healthcare. Through our in-depth investigations, we have found that bhasma exhibits promising potential as a therapeutic agent against a range of ailments, including cancer and various bacterial infections such as those in the urinary tract, skin, and after surgery. In summary, our research suggests that bhasma is a promising dual-action therapeutic agent that can effectively combat pathogenic bacteria and breast cancer cells. We believe that our findings could revolutionize modern healthcare and bring much-needed relief to patients suffering from these illnesses.

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