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Evaluation of Antibacterial Activity of Tamra Bhasma-Synthesized Silver Nanoparticles Against *Pseudomonas aeruginosa*

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ABSTRACT

The emergence of multidrug-resistant *Pseudomonas aeruginosa* strains poses a serious challenge to modern medicine. This study investigates the green synthesis of silver nanoparticles (AgNPs) using Tamra Bhasma, a traditional Ayurvedic copper preparation, and evaluates their antibacterial effectiveness against clinical isolates. AgNPs were synthesized by reducing silver nitrate with Tamra Bhasma in the dark. UV-Visible spectroscopy confirmed their formation, while Fourier Transform Infrared Spectroscopy (FTIR) identified functional groups that suggest stabilization by bio-organic compounds. X-ray Diffraction (XRD) analysis revealed a crystalline structure with peaks for silver and copper oxide.Antibacterial testing show cased inhibition zones of 13.5± 2.38 mm (2.5 µg), 14.5± 3.00 mm (5 µg), and 15.25± 2.62 mm (10 µg), indicating a synergistic effect from silver ions and minerals in Tamra Bhasma. These findings highlight the potential of combining traditional Ayurvedic formulations with nanotechnology to develop effective agents against antibiotic-resistant bacteria.

Figures : 03 References : 31 Table : 00

KEY WORDS : Antimicrobial activity, Green synthesis, *Pseudomonas aeruginosa,* Silver nanoparticles, Tamra Bhasma

Introduction

The emergence of multidrug-resistant bacterial strains presents a significant challenge to modern medicine, limiting treatment options for infectious diseases. The rise in antibiotic resistance underscores the urgent need for new antimicrobial agents and strategies to combat resilient pathogens such as *Pseudomonas aeruginosa*[3,16]. This opportunistic pathogen is known for its resistance mechanisms and ability to form biofilms, complicating eradication efforts and making traditional antibiotics less effective, particularly in immunocompromised patients[15].Amid this public health crisis, research is increasingly focused on alternative therapies, including natural products and nanotechnology[18]. Traditional medicinal systems like Ayurveda offer a wealth of naturally derived substances that could serve as promising antimicrobial agents. One such compound is Tamra Bhasma, an incinerated copper-based Ayurvedic formulation known for its substantial antibacterial properties against various strains, including *P. aeruginosa*[25].This renewed interest in Tamra Bhasma has opened avenues for its application as either a direct antimicrobial agent or as part of advanced drug delivery systems[28]. By integrating ancient medicinal wisdom with modern nanotechnology, we could develop innovative antibacterial solutions[26]. This article specifically explores the use of Tamra Bhasma as a reducing agent in the green synthesis of silver nanoparticles, aiming to harness both the antimicrobial properties of silver and the traditional benefits of Tamra Bhasma to combat resistant bacterial strains effectively.

Material and Method

Isolation and Preliminary Identification of *Pseudomonas aeruginosa* isolates-

Sample Collection and Primary Isolation

Clinical specimens presumed to contain *Pseudomonas aeruginosa,* were collected under aseptic conditions and immediately processed for microbiological analysis. HiCrome UTI Agar plates

(HiMedia, M1353) were inoculated using the spread plate technique and incubated at 37 °C for 24 hours. This chromogenic differential medium facilitates the identification and differentiation of uropathogens by producing distinct colony colours based on bacterial enzymes, enabling rapid differentiation between Gram-positive and Gram-negative bacteria.

Selective Isolation of *Pseudomonas aeruginosa* isolates

Pseudomonas sp. were selectively isolated by subculturing colonies with distinctive traits onto *Pseudomonas* Isolation Agar (PIA) (HiMedia, M406) and incubating for 24 to 48 hours at 37°C. This medium inhibits non-*Pseudomonas* bacteria and facilitates the isolation of *P. aeruginosa* by promoting the production of pigments, such as pyocyanin and pyoverdin. Glycerol serves as a carbon source, enhancing growth and pigment production.

Preliminary Identification

Gram Staining: Bacterial smears were heat-fixed, stained, and examined microscopically for theGram reaction.

Motility Testing:A drop of bacterial suspension was placed on a coverslip, inverted over a concave slide, and observed at 40X for cell movement**.**

Biochemical Confirmation

The VITEK®2 automated identification system confirmed the isolates by accurately identifying bacterial species through biochemical profiling.

Collection of Bhasma

Tamra Bhasma, a traditional Ayurvedic formulation made from purified copper, served as a reducing agent in the synthesis of silver nanoparticles. Renowned for its antimicrobial and anti-inflammatory properties, this formulation undergoes multiple stages of incineration and purification to ensure chemical stability and biocompatibility for biomedical applications. The Tamra Bhasma used in this study was purchased from a Patanjali Ayurvedic retail outlet in Chandrapur, Maharashtra. This choice ensured uniformity and adherence to quality control protocols, providing greater reliability for experimental reproducibility compared to local or laboratory-generated alternatives.

Synthesis of Tamra Bhasma-based Silver Nanoparticles.

Tamra Bhasma was used as a reducing agent for synthesizing silver nanoparticles. A 1 mM solution of silver nitrate ($AgNO_3$, AgNOƒ) was prepared using analytical-grade silver nitrate, and a 1 mg/mL suspension of Tamra Bhasma was created by mixing it with DMSO and vigorously vortexing.To prevent light-induced degradation of silver ions, the AgNOƒ solution was mixed with the Tamra Bhasma suspension in a 9:1 ratio and allowed to stand in the dark for 24 hours. The formation of nanoparticles was monitored through colour changes and surface plasmon resonance spectra recorded at 0 and 24 hours.The mixture was then centrifuged at 10,000 rpm for two hours, and the supernatant was discarded [31]. The nanoparticle pellets were washed twice with sterile distilled water, dried at room temperature, and collected for further analysis of antibacterial properties. Surface plasmon resonance was determined using ultraviolet-visible spectroscopy, with absorbance spectra recorded from 300 to 600 nm to confirm nanoparticle formation.

Physiochemical characterization of Tamra Bhasma-based Silver Nanoparticles

Silver nanoparticles were synthesized using a green method and characterized for their optical and structural properties. FTIR spectroscopy identified the bioactive components in Tamra Bhasma, while X-ray diffraction analyzed the crystalline nature of the nanoparticles.

Antibacterial activity of Tamra Bhasma-based Silver Nanoparticles

The antibacterial activity of Tamra Bhasma silver nanoparticles (AgNPs) was tested against five *Pseudomonas aeruginosa* isolates using the well diffusion technique[11]. A stock solution of AgNPs at 1 mg/mL was prepared and agitated for 30 minutes.Bacterial cultures were adjusted to the 0.5 McFarland standard and spread on Mueller-Hinton Agar plates. Three 8 mm wells were bored into each plate, and different volumes of AgNPs (25 µL, 50 µL, and 100 µL) were added.After incubating at 37°C for 24 hours, the zones of inhibition around each well were measured to evaluate antibacterial efficacy.

Result and Discussion

Isolation and Preliminary Identification of *Pseudomonas aeruginosa* isolates-

In this study, we isolated five stains of *P. aeruginosa* from clinical specimens. The initial culture on HiCrome UTI agar produced colourless, non-pigmented colonies, consistent with the earlier works[5] which noted that *P. aeruginosa* often displays pale colonies. Isolates cultured on *Pseudomonas* Isolation Agar formed creamy white colonies. *P. aeruginosa* produces pigments like pyocyanin and pyoverdine, with quantities varying by environmental conditions and media. It was noted that these fluctuations could create creamy or pale colonies[23]. Gram staining confirmed all

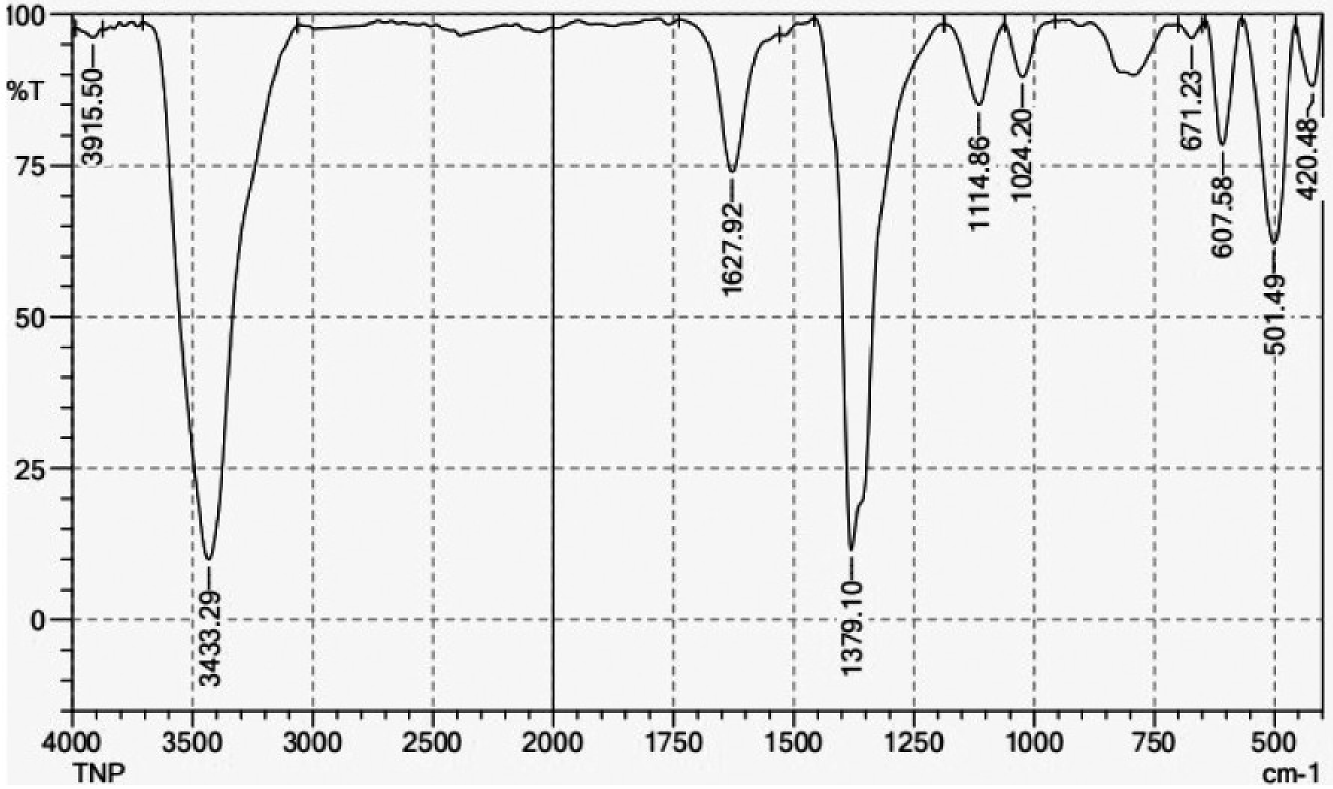

Fig. 1 : Fourier Transform Infrared (FTIR) spectroscopy

five isolates as Gram-negative bacilli with a pink-red rod morphology, strongly aligning with *P. aeruginosa* characterization . The results aligned with findings which reported a uniform Gram-negative rod morphology in *P. aeruginosa* isolates derived from both clinical and environmental sources[1]. Isolates were identified as *P. aeruginosa* using the VITEK 2 automated system, showcasing its effectiveness in clinical microbiology. This supports earlier finding which highlighted the reliability of such systems for identifying non-fermenting Gram-negative bacteria[23].Morphological analysis and biochemical profiling confirm the isolates as *P. aeruginosa*, highlighting the value of various detection methods.

Synthesis of Tamra Bhasma-based Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) was indicated by a colour change from light charcoal-black to dark brown-black after 24 hours, due to surface plasmon resonance. UV-visible spectroscopy showcased absorbance peaks at 340 nm, 390 nm, 400 nm, and 440 nm, confirming the presence of spherical AgNPs and suggesting smaller nanoparticles. This aligns with findings which identified similar indicators of AgNP synthesis[24]. Reported SPR peaks of 400 to 450 nm in silver nanoparticles from plant extracts, indicating polydispersity or shape variation[27]. Other workers found that peaks below 390 nm in AgNPs suspensions indicated smaller nanoparticles or intermediates from eco-friendly production methods[2].

Physiochemical characterization of Tamra Bhasma-based Silver Nanoparticles

Fourier Transform Infrared (FTIR) spectroscopy

FTIR analysis of Tamra Bhasma-synthesised AgNPs showed a peak at 3433.29 cm^{-1}, indicating O–H stretching vibrations from hydroxyl groups in bio-organic compounds[22]. A peak at 3915.50 cm^{-1} might indicate metal-oxide interactions. The absorption at 1627.92 cm^{-1} suggests C=O stretching from proteins or phenolics, while the peak at 1379.10 cm^{-1} indicates C–N stretching or CH_3 bending from biological molecules[9]. Peaks at 1114.86 cm^{-1} and 1024.20 cm^{-1} suggest C–O and C–O–C stretching, indicating ether or ester linkages that stabilize AgNPs. Absorption bands at 671.23, 607.58, 501.49, and 420.48 cm^{-1} are linked to metaloxygen (M–O) vibrations, matching earlier FTIR studies on nanoparticles and Bhasma compounds[7,10], Ag–O and Cu–O bonds confirm the incorporation of silver and copper into nanoparticles, suggesting enhanced antimicrobial properties. Similar FTIR findings in green

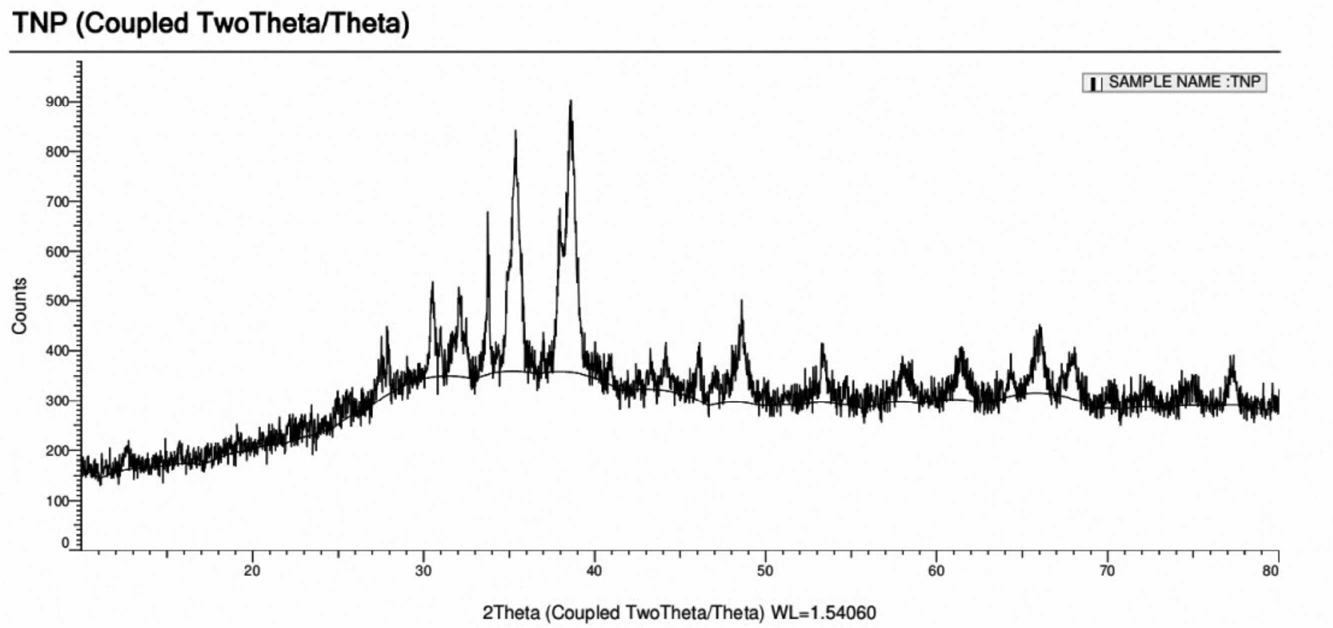

Fig. 2 : X-ray diffraction (XRD) analysis

synthesis of AgNPs from *Vitis vinifera* indicate phenolic and protein-based capping agents[20]. Metallic Rajata Bhasma had fewer stabilizing groups in the FTIR spectrum, leading to reduced effectiveness against *P. aeruginosa*[11](Fig. 1).

X-ray diffraction (XRD) analysis

The XRD analysis of silver nanoparticles synthesized using Tamra Bhasma confirmed the formation of a crystalline nanomaterial. Distinct diffraction peaks were observed at 2θ values of 38.61°, 35.34°, 33.76°, 48.61°, and 53.36°, with the most prominent peak at 38.61° exhibiting a relative intensity of 100%. This peak is associated with the (111) plane of FCC silver, indicating successful synthesis of well-crystallized AgNPs with a preferred growth orientation along the (111) plane. This characteristic is indicative of high surface energy and may contribute to antimicrobial activity [2,4,20] .Additional diffraction patterns at 35.34°, 33.76°, and 30.52° correspond to reported values for CuO and Cu, O phases, suggesting the integration of copper oxide nanocrystallites derived from Tamra Bhasma[10,7] . These peaks aligned with the JCPDS reference patterns for CuO (111, 110) and Cu, O (110) and have been identified in prior research on Ayurvedic metallic Bhasmas and bimetallic Ag–Cu nanoparticles[14,19]. The peak at 48.61° corresponded to the (220) plane of Ag, while peaks at 53.36° and 66.06° might be related to higher-index planes such as (311) of Ag or CuO, further supporting the polycrystalline nature of the sample[6,9].

The presence of multiple sharp and intense peaks throughout the 2è range indicated a well-defined crystalline structure of the nanoparticles, with no evidence of amorphous phases. This pattern was consistent with that of green or biosynthesized AgNPs, where plant-derived or mineral matrices assist in both reduction and structural stabilization[17]. The overlapping peaks of Ag and CuO also imply the potential formation of bimetallic or core-shell structures, akin to those observed in nanoparticle systems derived from *Vitis vinifera* peel and *Phyllanthus emblica*extracts[4,22]. The observed combination of Ag and Cu phases enhanced the appeal of these nanoparticles due to their synergistic antibacterial and catalytic properties[12,13]. Overall, the XRD profile demonstrates that the Tamra Bhasma synthesized AgNPs exhibited high crystallinity, clear phase purity, and a complex composition, positioning them as promising one for medicinal applications and antimicrobial use (Fig. 2).

Antibacterial activity of Tamra Bhasma-based Silver Nanoparticles

The antibacterial effect of AgNPs from Tamra Bhasma on *P. aeruginosa* was tested by well diffusion method. Concentrations of 2.5 µg, 5.0 µg, and 10.0 µg produced average inhibition zones of 13.50 ± 2.38 mm, 14.50± 3.00 mm, and 15.25± 2.62 mm, respectively (Fig. 3).Antibacterial activity increases with AgNPs concentration, as higher concentrations enhanced interaction with bacterial membranes due to increased surface area[2,22]. Tamra Bhasma AgNPs exhibited notable antibacterial activity at the lowest concentration, with an inhibition zone of 13.5 ± 2.38mm. This suggests that minimal doses could disrupt bacterial growth. Earlier workers[22] found similar results with chemically synthesized AgNPs, while another investigator reported

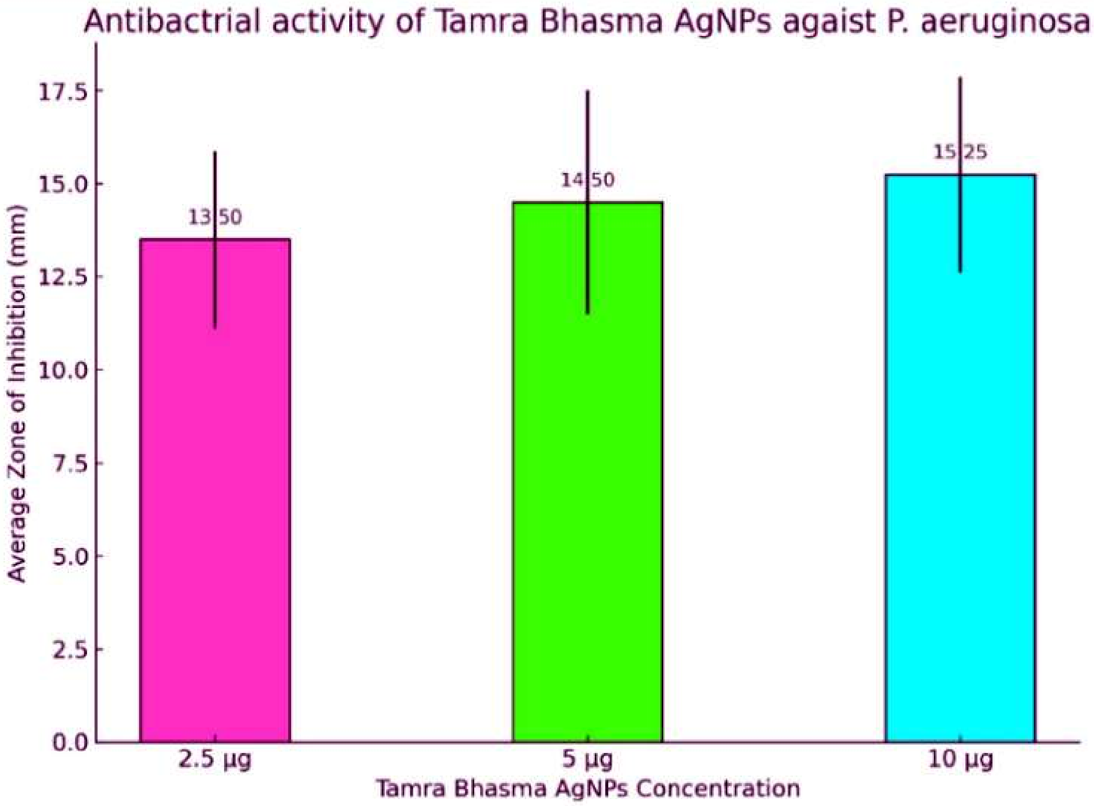

Fig. 3 : Antibacterial activity of Tamra Bhasma-based Silver Nanoparticles

that green-synthesized AgNPs could harm proteins and DNA in*P. aeruginosa* at very low concentrations[8,22]. The inhibition zones increased to 14.5± 3.00 mm and 15.25± 2.62 mm for the 5 µg and 10 µg concentrations, indicating the antibacterial effect may be plateauing. This could be due to limited diffusion of AgNPs in the agar or saturation of bacterial targets. Some investigator noted that exceeding a certain AgNPs dosage could lead to diminishing returns in inhibition diameter[2].

The antimicrobial effect might be boosted by Tamra Bhasma's bioactive components alongside silver. Other report noted that its weak effects improve with metallic ions[30]. Bhasma enhanced nanoparticle activity[21]. The antibacterial effect against *P. aeruginosa* likely results from the interaction between silver ions and Tamra Bhasma, highlighting the potential of Ayurvedic-nanotech combinations.

Conclusion

This study demonstrates the effective use of Tamra Bhasma for the eco-friendly synthesis of silver nanoparticles (AgNPs). FTIR analysis revealed key functional groups, while X-ray diffraction confirmed their crystalline face-centred cubic structure. Antibacterial assays showed that AgNPs exhibited increased antimicrobial activity with higher concentrations, with inhibition zones of 13.5± 2.38 mm (2.5 µg), 14.5 ± 3.00 mm (5 µg), and 15.25± 2.62 mm (10 µg). Overall, Tamra Bhasma provides a sustainable and cost-effective approach to synthesizing AgNPs with notable antibacterial properties.

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