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Green Synthesis of Silver Nanoparticles from *Trapa bispinosa* Fruit Extract: Characterization and Promising Antimicrobial Activity Against Multidrug-Resistant Pathogens

Nisha Rani[1,*], Anil Kumar[2], Kaushal Sanghi[3]

Abstract

Silver nanoparticles (Ag NPs) are widely recognized for their applications in medicine, electronics, and environmental remediation. This study presents a new green synthesis method for Ag NPs using Trapa bispinosa fruit extract as a natural reducing agent and stabilizing agent. Characterization of the synthesized Ag NPs through X-ray diffraction (XRD) confirmed a crystalline structure consistent with the face-centered cubic (fcc) arrangement, with peaks at (111), (200), (220), (311), and (222), indicating metallic silver. Fourier Transform Infrared (FTIR) spectroscopy revealed the presence of functional groups, such as O–H and C–N, which play a key role in stabilizing the nanoparticles. Scanning electron microscopy (SEM) and particle size analysis confirmed the formation of spherical nanoparticles with an average size of 82.93 nm, while transmission electron microscopy (TEM) revealed an average particle size of 13.36 nm. Antimicrobial tests demonstrated inhibition zones of 6.9 ± 0.22, 9.7 ± 0.45, and 12.9 ± 0.33 mm at 100, 150, and 200 µg/ml against Staphylococcus aureus, respectively, while Pseudomonas aeruginosa exhibited no inhibition. This study highlights the potential of Trapa bispinosa fruit extract in Ag NPs synthesis, demonstrating its dual role in nanoparticle production and biomedical applications.

Keywords: Ag NPs, *Trapa bispinosa,* XRD, SEM, TEM, FTIR

INTRODUCTION

Nanotechnology is a rapidly evolving field with immense potential in biomedical applications, particularly drug delivery, diagnostics, and therapeutics. The unique features of nanomaterials, such as their high surface-area-to-volume ratio, size-dependent properties, and molecular-level interactions, make them valuable in diverse areas including healthcare, food safety, and environmental sustainability (El-Kady et al., 2023) [1]. Silver nanoparticles (Ag NPs) stand out among these nanomaterials because of their remarkable antimicrobial properties, straightforward synthesis process, and ability to combat multi-drug-resistant bacteria effectively. Additionally, Ag NPs have shown the ability to enhance drug delivery and improve the efficacy of chemotherapeutic agents, positioning them as promising candidates for cancer treatment [2]. Studies have shown that the peels and leaves of *Trapa bispinosa* have medicinal properties and have been traditionally used to treat conditions, such as gastric ulcers and oesophageal cancer. Additionally, they exhibit antibacterial and antitumor activities. These therapeutic effects are attributed to the presence of various biologically active compounds, including phenols and flavonoids, which have been extensively studied. The peels are particularly rich in flavonoids, such

***Author for Correspondence**
Nisha Rani
E-mail: nishaarya424@gmail.com

[1]Assistant Professor, Department of Zoology, Baba Mastnath University, Asthal Bohar, Sector-29, Rohtak, Haryana, India
[2]Associate Professor, Chaudhary Bansi Lal University. Bhiwani, Haryana, India

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as naringenin, dihydromountanol, delphinidin, and delphinidin-3-O-glucoside. These compounds are known for their antioxidant, anti-inflammatory, and anticancer properties, making *Trapa bispinosa* a valuable plant for both pharmaceutical and biomedical applications (Chen et al., 2024) [3]. *Pseudomonas aeruginosa* is a significant opportunistic pathogen that impacts humans, plants, and animals, frequently linked to cases of sporadic clinical mastitis. Conversely, *Staphylococcus aureus* is an opportunistic pathogen and a common contaminant that holds medical significance as it can cause a wide range of infections in humans, from mild skin and wound infections to severe, life-threatening diseases [4].

The rise of multidrug-resistant (MDR) strains of *Pseudomonas aeruginosa* in clinical settings has become a serious concern, emphasizing the critical need for innovative treatment approaches. *S. aureus* is a Gram-positive, facultative anaerobic bacterium that typically resides on the skin and mucous membranes of healthy individuals. It is a leading cause of healthcare-associated infections, responsible for conditions, such as skin and soft tissue infections, pneumonia, endocarditis, and sepsis. A key concern with *S. aureus* is its exceptional capacity to develop antibiotic resistance, *exemplified* by the emergence of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). MRSA *poses* a significant threat due to its widespread presence in healthcare environments and its potential to cause severe infections [5].

In recent years, the use of plant extracts for synthesizing nanoparticles has garnered significant interest as an eco-friendly and cost-effective alternative to traditional physical and chemical methods. Among the different types of nanoparticles, Ag NPs have attracted considerable attention for their unique properties and wide-ranging applications in fields, such as biomedicine, electronics, and agriculture. Ag NPs have shown remarkable antibacterial, antifungal, antiviral, and larvicidal properties, making them highly desirable for a range of applications. In this regard, *Trapa bispinosa*, a medicinal plant commonly found in Asia, has been utilized for the eco-friendly synthesis of Ag NPs. This plant is renowned for its medicinal properties, including its antibacterial, antifungal, and anti-inflammatory effects. The phytosynthesis of Ag NPs using T. bispinosa extract has been shown to yield stable and monodisperse nanoparticles with outstanding antimicrobial properties. This study proposed a straightforward and eco-friendly green synthesis method for Ag NPs utilizing the fruit extract of the medicinal plant *Trapa bispinosa*. The synthesized Ag NPs were thoroughly characterized using multiple analytical techniques, including Fourier Transform Infrared Spectroscopy (FT-IR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). The antibacterial activity of the synthesized Ag NPs was evaluated in vitro against a range of bacterial strains. This approach highlights the potential of *Trapa bispinosa* fruit extract as a natural and sustainable source for the synthesis of Ag NPs with promising antimicrobial properties [6].

MATERIAL AND METHODS

Plant Collection and Extract Preparation

Trapa bispinosa (water chestnut) fruits were collected from the local market in Rohtak, Haryana, India. The fruits were thoroughly washed (2–3 times) with distilled water to remove dust and impurities, then dried in an oven at 70°C and ground into fine powder using an electronic blender (Figure 1). The powdered sample was stored at –20°C for later use. To prepare the extract, 10 g of dry powder was added to 100 ml distilled water in a 250 ml Erlenmeyer flask and heated at 80°C for 30 min with constant stirring (250 rpm). After cooling, the mixture was filtered through a muslin cloth to obtain a clear extract, which was stored at 4°C for further use in silver nanoparticle synthesis [7].

Green Synthesis of Ag NPs

Green synthesis Ag NPs was done by the following method of Prakash et al. (2013) [2] with minor modification, 20 mL $AgNO_3$ was dissolved in deionized water to prepare a 10 mM $AgNO_3$ solution. A total of 60 mL of *Trapa bispinosa* fruit extract was added dropwise to the reaction mixture while stirring at 250 rpm. The mixture was then stirred continuously for 2 hours to facilitate the reduction of

silver ions and the formation of Ag NPs. To purify the nanoparticles, the reaction mixture was centrifuged at 10,000 rpm, and the resulting nanoparticle pellet was thoroughly washed with distilled water and ethanol to remove impurities. The washed nanoparticles were dried overnight at 60°C. To prevent the photoactivation of $AgNO_3$, the reaction was carried out in the dark at room temperature.

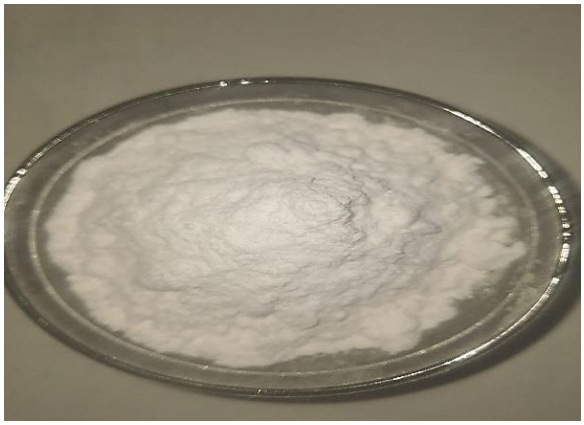


Figure 1. Fine powder of dried plant material.

Characterization of Ag NPs

The characterization of Ag NPs was performed using various techniques to analyze their structural, morphological, and functional properties. XRD (PANalytical X'pert PRO) analysed crystallinity and phase purity. TEM with a (JEOL 2100F) provided high-resolution imaging of the morphology and size distribution of the nanoparticles, while SEM using a (Zeiss EVO40) enabled surface morphology examination. Additionally, Fourier-transform infrared spectroscopy (FTIR) (Varian 7000 FTIR) was used to investigate the optical properties of the synthesized nanoparticles [8].

Antibacterial Activity of Ag NPs

This study includes the resistant bacterial strains *Pseudomonas aeruginosa* (MTCC 1688) (Gram – ve) and *S. aureus* (MTCC 737) (Gram +ve), were procured from IMTECH, Chandigarh [9]. The antibacterial activity of Ag nanoparticles was performed by the agar well diffusion method (Geoprincy et al., 2012) [4]. Nutrient agar plates were prepared by pouring 20 mL of freshly prepared media into sterilized petri dishes. Subsequently, 1 mL of freshly prepared bacterial suspension was evenly spread across the surface of the agar using a sterile swab. Five equidistant wells were made by cutting agar media. Eight wells were filled with different concentrations of NPs (10 µl/ml, 20 µl/ml, 30 µl/ml, 40 µl/ml 50 µl/ml, 100 µl/ml, 150 µl/ml, 200 µl/ml) and one plate with antibiotic kanamycin solution. The plates were allowed to incubate at 37°C for 24 hours. The diameter of ZOI obtained was measured in mm using a plastic ruler "(Hi Antibiotic Zone Scale TM-C, HiMedia Laboratories Pvt. Ltd. India)". All experiments were triplicates and recorded the mean value [10, 11].

STATISTICAL ANALYSIS

For characterization of Ag NPs graphs were analyzed using Origin software (version 23). The antimicrobial activity data were expressed as mean ± standard deviation (SD). Statistical differences between groups were evaluated using one-way ANOVA, performed in GraphPad Prism (version 10). P-values were calculated to indicate significance, with the significance level set at $*p < 0.05$ [12, 13].

RESULT AND DISCUSSION

Green Synthesis of Ag NPs

The synthesis of Ag NPs using *Trapa bispinosa* fruit extract was confirmed through a visible color change in the reaction mixture [14–16]. Upon adding the plant extract to silver nitrate ($AgNO_3$) solution, the initially colorless solution turned yellowish brown consist with the previous studies Desalegn et al. (2021) [17], indicating the reduction of silver ions and successful formation of Ag NPs Figure 2).

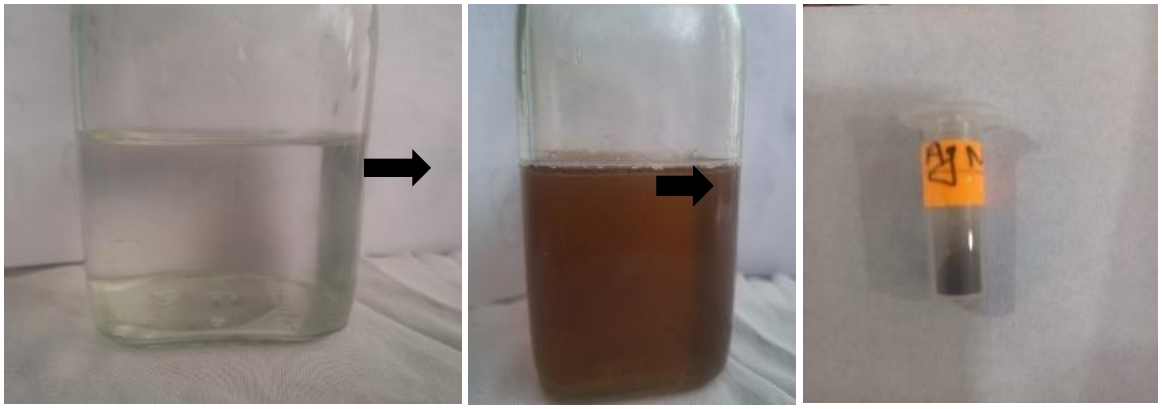


Figure 2. Shown colorless solution turned yellowish brown and Ag NPs in powder form.

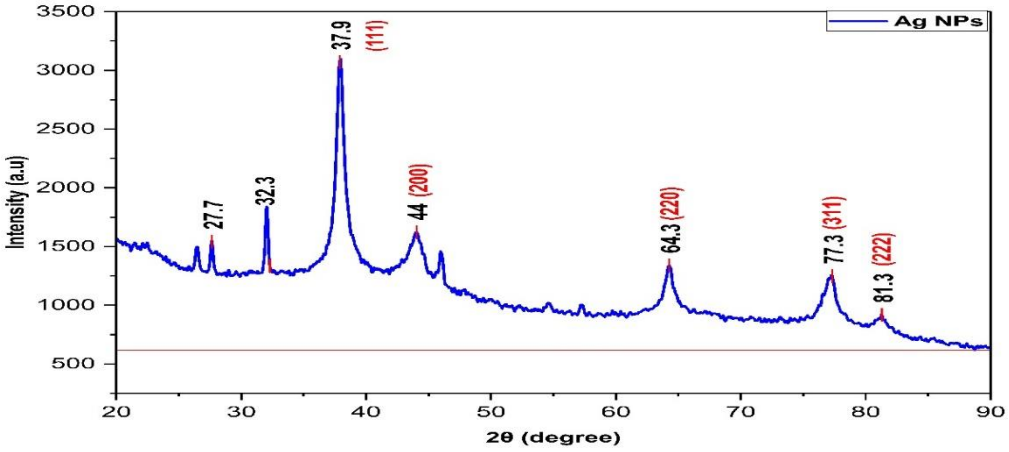
Characterization of Ag NPs

XRD analysis of silver nanoparticles shows distinct diffraction peaks at specific 2θ values, which correspond to the face-centered cubic (fcc) structure of silver (Ag). The indexed peaks validate the crystalline structure and phase purity of the synthesized nanoparticles. The observed peaks match well with the standard reference pattern for silver (Ag) from JCPDS (Joint Committee on Powder Diffraction Standards) card number 04-0783. The presence of peaks at (111), (200), (220), (311), and (222) confirm that the nanoparticles crystallize in an fcc structure, which is the expected crystal structure of metallic silver. The highest intensity peak at 37.9° (111) shows that the nanoparticles have a preferred orientation along this plane. This is typical for silver nanoparticles because the (111) plane is the most stable and densely packed surface in fcc metals consist with the previous studies Yuan et al. (2017), and Desalegn et al. (2021) [14] leading to increased activity in this direction ((Figure 3(A)).

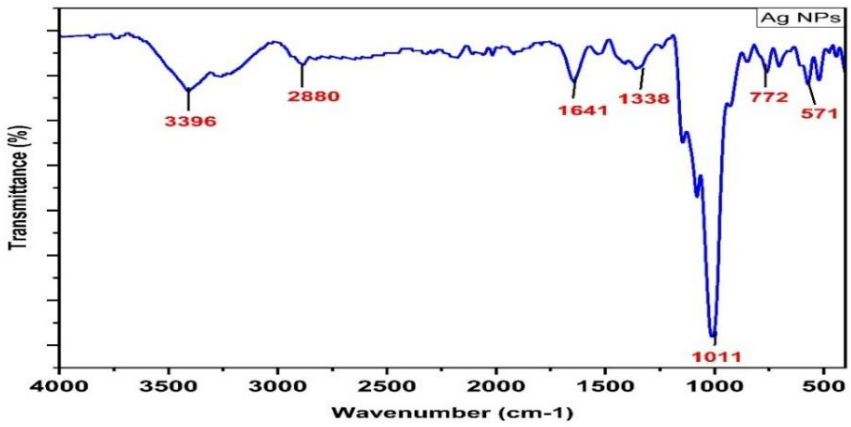
FTIR analysis offers crucial information about the functional groups responsible for the capping and stabilization of silver nanoparticles (Ag NPs). The broad peak at 3396 cm^{-1} corresponds to O–H stretching, indicating the presence of hydroxyl groups from biomolecules, such as phenols or alcohols, which may play a role in the stabilization of the nanoparticles consist with the previous studies [15]. The peak at 2880 cm^{-1} corresponds to C–H stretching, suggesting the presence of alkene or organic residues from reducing agents. The peak at 1641 cm^{-1} is attributed to C=O stretching, which is characteristic of amide I bonds from proteins or carbonyl groups from organic compounds. This suggests the involvement of proteins or other organic molecules in the synthesis process, potentially acting as capping agents. The presence of C–N stretching at 1338 cm^{-1} supports the role of amine groups, which are known to interact with metal surfaces. The peak at 1011 cm^{-1}, which corresponds to C–O stretching, indicates the presence of alcohols, esters, or ethers, which may contribute to the stabilization of the nanoparticles consist with the previous studies Yuan et al. (2017) [14] and Kalishwaralal et al. (2010). The band at 772 cm^{-1} is attributed to C–H bending vibrations, confirming the presence of aromatic structures. Finally, the peak at 571 cm^{-1} is associated with Ag–O or Ag–N bonds nanoparticles consist with the previous studies conducted by Desalegn et al. (2021) [17], indicating possible interactions between silver nanoparticles and organic ligands (Figure 3(B)).

The morphological features and dimensional properties of the synthesized Ag nanoparticles were systematically analyzed using SEM. High-energy electron beam imaging helped to comprehensively evaluate their structural features, including size, shape, orientation, and overall arrangement. The SEM micrographs presented in Figure 3(C) demonstrated a diverse range of nano-sized particles with distinct morphological variations. Notably, the synthesized Ag nanoparticles exhibited well-defined structures with prominent patterns. Various geometric forms, including nearly spherical, cylindrical, triangular, and prismatic shapes, were identified, indicating the heterogeneous nature of the sample. Particle size distribution analysis revealed a wide range, with the average grain size spread between 32 and 148 nm, while the average particle diameter was determined to be 82.93 nm shown in histogram consist with the previous studies conducted by Desalegn et al. (2021) [17].

TEM analysis was conducted to examine the crystalline characteristics and size distribution of the synthesized silver nanoparticles (Ag NPs). TEM images confirmed that the nanoparticles were predominantly spherical in shape, with slight variations in thickness, consistent with the observations from SEM analysis. Histogram analysis showed that the particle size ranged from 3.53 to 23.89 nm, with an average particle size of 13.36 nm consisting with the previous studies Yuan et al. (2017) and Desalegn et al. (2021) (Figure 3(D)) [17].



(a)



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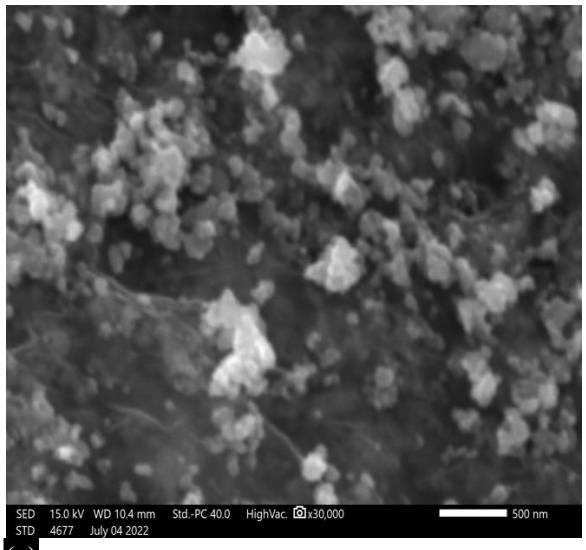

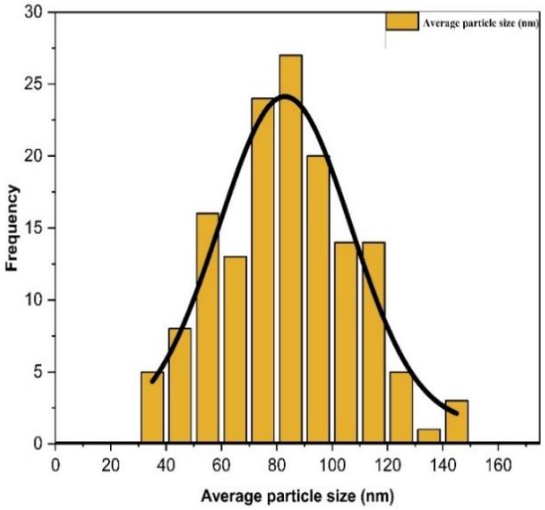

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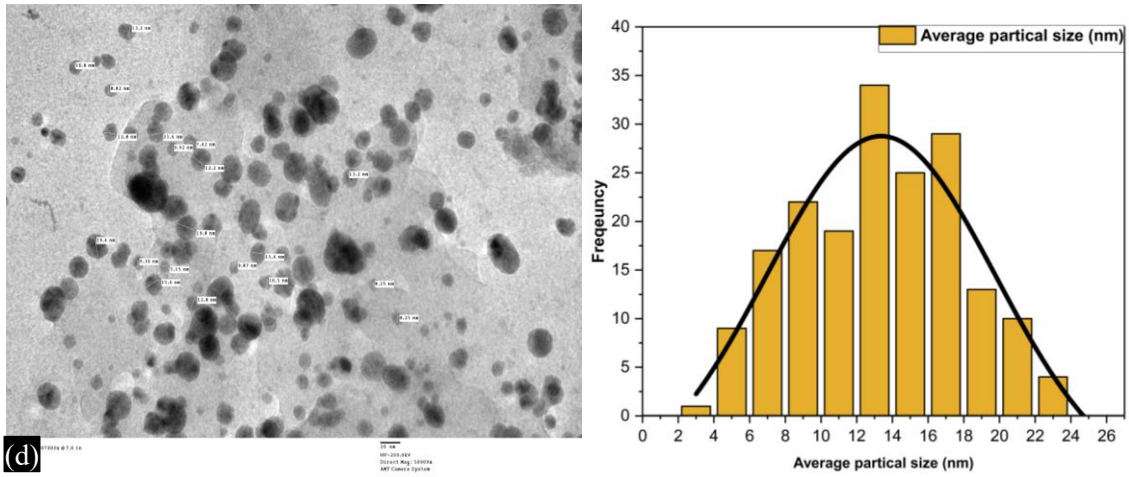

Figure 3. Synthesis and characterization of silver nanoparticles (Ag NPs) from *Trapa bispinosa* fruit extract: (A) XRD spectra of Ag NPs; (B) FTIR spectra of Ag NPs; (C) SEM images of Ag NPs; (D) Additional TEM images of Ag NPs. Several regions were analyzed to determine the particle size, which revealed Ag NPs ranging from 3.53 to 23.89 nm based on TEM measurements.

Antibacterial Activity of Silver Nanoparticles

The antibacterial activity of silver nanoparticles (Ag NPs) against *Pseudomonas aeruginosa* was evaluated at various concentrations ranging from 10 µg to 200 µg. The results showed that none of the tested concentrations displayed any detectable antibacterial activity against *Pseudomonas aeruginosa* which is not similar with the previous studies of Desalegn et al. (2021). This suggests that the synthesized Ag NPs were ineffective in inhibiting the growth of this bacterial strain under the given experimental conditions (Figures 4(A) and (B)).

The antibacterial activity of silver nanoparticles (Ag NPs) against *S. aureus* was evaluated at different concentrations, showing dose-dependent inhibitory effect. At low concentrations (10–40 µg), no antibacterial activity was observed, indicating insufficient nanoparticle concentration to exert bactericidal effect. However, at 100 µg, an inhibition zone of 6.9 ± 0.22 mm was recorded, indicating the onset of antimicrobial activity. As the concentration increased to 150 µg and 200 µg, the inhibition zones expanded to 9.7 ± 0.45 mm and 12.9±0.33 mm, respectively, indicating enhanced bacterial suppression consist with the previous studies [17]. The antimicrobial action of Ag NPs is possibly due to membrane disruption, oxidative stress through reactive oxygen species (ROS) generation, and interference with intracellular biomolecules, ultimately leading to bacterial cell death (Table 1). These findings highlight the potential of Ag NPs as effective antibacterial agents against *S. aureus* at high concentrations and support their potential application in biomedical and antimicrobial therapies, while emphasizing the need for further cytotoxicity and resistance studies (Figures 4(C) and (D)).

- The concentration of Ag nanoparticles in agar well is 10–200 µg/ml did not show any activity against *Pseudomonas aeruginosa* bacterial strain shown in Figures 4(A) and (B).
- The concentration of Ag nanoparticles in agar well is 10–50 µg/ml did not show any activity against *S. aureus* bacterial strain shown in Figure 4(C).
- The concentration of Ag nanoparticles in agar well is 100 µg/ml with 6.9 ± 0.22 mm shown in Figure 4(D).
- The concentration of Ag nanoparticles in agar well is 150 µg/ml with 9.7 ± 0.45 mm shown in Figure 4(D).
- The concentration of Ag nanoparticles in agar well is 200 µg/ml with 12.9 ± 0.33 mm shown in Figure 4(D).

Another possible mechanism for the inhibitory effect of silver nanoparticles (Ag NPs) on bacterial growth could be linked to the interaction between Ag NPs and the cell walls of bacteria. Studies have shown that Gram-positive bacteria tend to bind larger amounts of metal ions, including silver (Ag), compared to Gram-negative bacteria. This phenomenon may be due to the thicker peptidoglycan layer

in the cell walls of Gram-positive bacteria, which may hinder the efficient penetration of Ag NPs. However, in contrast, Gram-negative bacteria have thinner cell walls, making them more susceptible to the action of Ag NPs. This difference in bacterial cell wall structure has been noted in several studies, which typically report that Gram-negative bacteria are more affected by Ag NPs than Gram-positive bacteria. One possible explanation for the reduced susceptibility of Gram-positive bacteria is that their thicker cell walls act as a barrier to the Ag NPs, reducing their ability to penetrate the bacterial cell and exert their antimicrobial effects. Furthermore, it has been suggested that Ag NPs may disrupt the helical structure of DNA molecules within bacterial cells. The interaction between Ag NPs and the bacterial cell could cause the release of silver ions (Ag+), which may bind to DNA and other cellular components, ultimately leading to DNA damage and cell death. In addition, the electrochemical potential across the bacterial cell membrane could decrease when Ag NPs release Ag+ ions upon interaction with the bacterial cell. The disruption of the electrochemical gradient across the cell membrane can compromise its integrity, resulting in the leakage of essential cellular components, loss of cell viability, and ultimately cell death. These interactions collectively contribute to the antimicrobial activity of Ag NPs, although their exact mechanism of action may vary depending on the bacterial strain and environmental conditions.

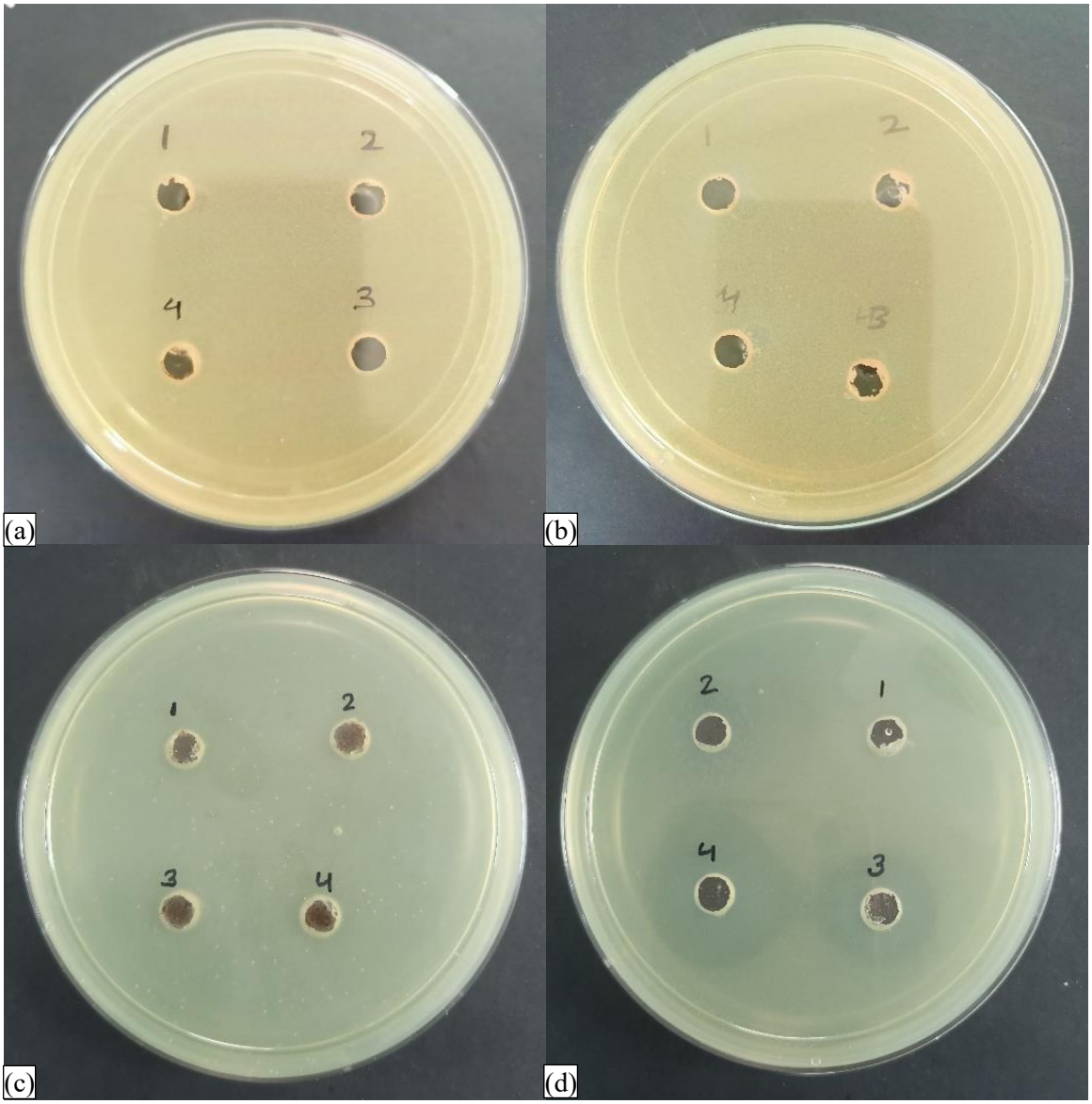

Figure 4. Inhibition assay of *Pseudomonas aeruginosa* (A), (B) and *Staphylococcus aureus* (C), (D) in the presence of different concentrations of Ag NPs synthesized from *Trapa bispinosa*.

Table 1. Growth inhibition assay of *Staphylococcus aureus* in the presence of different concentrations of Ag NPs synthesized from *Trapa bispinosa*.

	Zone of inhibition (mm)				
Bacterial Strain	***Staphylococcus aureus***				**Positive Control (Kanamycin)**
Concentration of Ag nanoparticles	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	50 mg/ml
Mean ± SD	–	6.9 ± 0.22 mm	9.7 ± 0.45 mm	12.9 ± 0.33*** mm	19 ± 3 mm

Note: Value is shown in mean ± SD, with the significance level set at $*p < 0.05$.

CONCLUSIONS

This study successfully demonstrated the green synthesis of silver nanoparticles (Ag NPs) utilizing *Trapa bispinosa* fruit extract as both a reducing and stabilizing agent. The use of a plant extract in the synthesis process not only provides an environmentally friendly approach but also reduces the dependence on toxic chemicals, aligning with the growing demand for sustainable nanomaterial production. XRD analysis confirmed that the synthesized Ag NPs have a crystalline fcc structure with characteristic diffraction peaks at (111), (200), (220), (311), and (222), confirming the formation of metallic silver nanoparticles. FTIR spectroscopy These findings suggest that the bioactive compounds present in *Trapa bispinosa* fruit extract play a key role in both the reduction and stabilization of Ag NPs. The morphology and size of the Ag NPs were analyzed using SEM and TEM, which confirmed that the nanoparticles had a spherical shape. The average particle size was found to be 13.36 nm based on TEM analysis, which indicates that the nanoparticles are within the optimal size range for enhanced antimicrobial activity. Antimicrobial testing demonstrated that the synthesized Ag NPs exhibited potent inhibitory effects against *S. aureus*, with clear inhibition zones observed at concentrations of 100, 150, and 200 µg/ml. However, *Pseudomonas aeruginosa* did not exhibit any significant inhibition, indicating that the Ag NPs synthesized in this study may be more effective against Gram-positive bacteria than Gram-negative strains. The results highlight the potential of Ag NPs as a selective antimicrobial agent, especially in addressing infections caused by multidrug-resistant *S. aureus*. This study contributes to the growing body of research on biologically synthesized nanoparticles and emphasizes the versatility of *Trapa bispinosa* fruit extract as a sustainable and cost-effective resource for nanoparticle production. The findings suggest that Ag NPs synthesized using *Trapa bispinosa* extract may serve as a promising option in the development of new antimicrobial therapies, especially in the face of rising antibiotic resistance. Future studies may explore the detailed mechanisms of action of these nanoparticles, their efficacy in vivo models, and their potential applications in other biomedical and environmental fields.

Conflict of Interest

The authors have declared no conflicts of interest.

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