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Research Article

GREEN-SYNTHESIZED AZADIRACHTA INDICA–MEDIATED AGNPS WITH PHYSICOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY AGAINST DENTAL PSEUDOMONAS AERUGINOSA

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ABSTRACT:

Background:
The growing prevalence of antimicrobial resistance (AMR) in opportunistic bacteria, especially *Pseudomonas aeruginosa*, poses a serious challenge in both dental and broader clinical environments. **Objectives:** In response to this issue, green nanotechnology—particularly plant-mediated silver nanoparticles (AgNPs)—has gained attention as an eco-friendly and biocompatible antimicrobial strategy. This study aimed to synthesize silver nanoparticles using *Azadirachta indica* (neem) leaf extract and to assess their antibacterial efficacy against dental isolates of *P. aeruginosa*. **Methodology:** Silver nanoparticles were fabricated through a modified reduction approach in which *A. indica* leaf extract served simultaneously as a reducing and capping agent. The synthesized nanoparticles were extensively characterized using UV–visible spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and dynamic light scattering (DLS) to determine their structural, morphological, and physicochemical properties. Antibacterial activity was evaluated by agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays at concentrations of 10, 20, and 30 µg/mL. A 0.2% chlorhexidine solution was employed as a positive control. **Results:** XRD patterns confirmed the crystalline structure of the AgNPs, with an estimated average crystallite size of 16.07 nm. FTIR analysis identified bio functional groups from neem extract involved in nanoparticle stabilization, while SEM imaging revealed predominantly hexagonal-shaped particles. UV–visible spectroscopy showed a distinct surface plasmon resonance peak at 238 nm, and DLS analysis indicated a hydrodynamic diameter of 144.22 nm. The neem-mediated AgNPs exhibited dose-dependent antibacterial activity against *P. aeruginosa*, with mean inhibition zones of 12 ± 1.13 mm, 14 ± 2.05 mm, and 17 ± 1.15 mm at 10, 20, and 30 µg/mL, respectively. Notably, the antibacterial effect at 30 µg/mL was comparable to that of chlorhexidine (18 ± 1.46 mm). **Conclusion:** This study demonstrates that *A. indica*–derived silver nanoparticles possess significant antibacterial potential against *P. aeruginosa*. Their strong activity, coupled with a green and sustainable synthesis approach, suggests that these nanoparticles could serve as a promising alternative to conventional antimicrobials however, further in vivo studies and comprehensive toxicity evaluations are necessary before clinical or dental applications can be considered.

Keywords: Acacia honey, *Klebsiella* species, *Klebsiella Pneumoniae*, Urinary tract infections (UTIs), Multidrug resistance (MDR), Antibacterial activity

1 | INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a major global public-health threat, significantly compromising the effectiveness of conventional antibiotics and antiseptic agents.[1] *Pseudomonas aeruginosa* is a highly adaptable opportunistic pathogen characterized by intrinsic resistance mechanisms, robust biofilm formation, and a high

incidence of dental, wound-related, and hospital-acquired infections.[2] Its involvement in periodontal disease, post-surgical complications, and delayed wound healing further exacerbates clinical management challenges, largely due to its resilient biological profile.[3] Silver nanoparticles (AgNPs) have gained considerable attention as next-generation antimicrobial agents owing to their broad-spectrum activity, multimodal mechanisms of action, and comparatively lower propensity for inducing resistance.[4] The antibacterial activity of AgNPs is mediated through multiple pathways, including disruption of bacterial cell membranes, generation of reactive oxygen species (ROS), protein denaturation, and inhibition of DNA replication.[5] However, conventional chemical synthesis methods for AgNPs are often associated with cytotoxic effects, environmental hazards, and the use of unsafe reducing agents, limiting their biomedical applicability.[6] Consequently, green synthesis approaches utilizing plant-based extracts as natural reducing and stabilizing agents have emerged as safer and more sustainable alternatives.[7]

Azadirachta indica (neem) is a well-established medicinal plant recognized for its antimicrobial, antioxidant, and wound-healing properties.[8] Neem leaves are rich in bioactive phytochemicals such as flavonoids, terpenoids, polyphenols, and alkaloids, which play a crucial role in the reduction of metal ions and stabilization of nanoparticles.[9] The integration of neem-derived phytochemicals into AgNPs synthesis not only enhances nanoparticle stability but may also confer synergistic antibacterial effects, thereby increasing their therapeutic potential in biomedical applications.[10] Although several studies have reported the biological synthesis of AgNPs using neem extracts, most investigations have been limited to laboratory reference strains, with minimal focus on clinically relevant isolates—particularly *P. aeruginosa* obtained from dental infections.[11] Moreover, comprehensive physicochemical characterization encompassing nanoparticle size, morphology, crystallinity, and surface chemistry, alongside detailed antibacterial evaluation, remains insufficiently addressed.[12] In addition, the lack of comparative assessments with standard dental antiseptics such as chlorhexidine restricts the clinical and translational relevance of previous findings.[13]

Another critical limitation in existing literature is the absence of systematic dose-dependent antibacterial investigations and standardized concentration benchmarks, which are essential for future formulation and therapeutic development.[14] Furthermore, limited attention has been given to the potential application of neem-mediated AgNPs in dental and oral healthcare, where biocompatibility and minimal toxicity are of paramount importance.[15] The present study was therefore designed to synthesize neem leaf extract-modified AgNPs using a modified chemical reduction method and to systematically evaluate their physicochemical characteristics employing advanced analytical techniques. The antibacterial efficacy of the synthesized AgNPs was assessed against *P. aeruginosa* isolated from dental patients and compared with chlorhexidine, a widely used clinical antiseptic. By addressing existing research gaps, this study establishes a scientific framework for future investigations, including cytotoxicity assessment, antibiofilm activity, in vivo validation, and the development of neem-based AgNP formulations for dental and broader biomedical applications.

2 | MATERIAL AND METHODS

Fresh leaves of *Azadirachta indica* were collected from the campus of Gomal University, Dera Ismail Khan, Pakistan. Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), nutrient agar, nutrient broth, Mueller–Hinton agar, antibiotic assay media, methanol/ethanol, and DPPH reagent were procured from certified scientific suppliers in Dera Ismail Khan. Ascorbic acid was used as a reference antioxidant. All reagents and chemicals employed in the study were of analytical grade.[16]

2.1 | Preparation of *A. indica* Leaf Extract

Freshly collected *A. indica* leaves were thoroughly washed with distilled water to remove dust and surface contaminants, followed by shade drying at room temperature. The dried leaves were finely ground and stored in airtight containers. For aqueous extraction, 10 g of leaf powder was boiled in 100 mL of distilled water for 15 minutes. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was stored at 4 °C for subsequent use as a natural reducing and stabilizing agent.[17]

2.2 | Preparation of Silver Nitrate and Sodium Hydroxide Solutions

A 0.02 M silver nitrate solution was prepared by dissolving 3.336 g of $AgNO_3$ in 1000 mL of distilled water.

Similarly, a 0.02 M sodium hydroxide solution was prepared by dissolving 0.8 g of NaOH in 1000 mL of distilled water.[18]

2.3 | Chemical Reduction and Embedding Process

Silver nanoparticles were synthesized using a modified chemical reduction approach. Briefly, 1 g of $AgNO_3$ was dissolved in 20 mL of distilled water and sonicated for 3 minutes. Separately, 0.5 g of albumin was dissolved in 20 mL of distilled water and sonicated under identical conditions. The two solutions were combined dropwise under continuous magnetic stirring, followed by the addition of 30 mL of distilled water. Subsequently, 10 mL of freshly prepared *A. indica* leaf extract was added slowly to the reaction mixture while stirring continuously. The reaction was allowed to proceed at room temperature for 30 minutes, during which phytochemicals such as flavonoids, terpenoids, and polyphenols facilitated nanoparticle reduction and stabilization.[19]

2.4 | pH Adjustment and Completion of Reduction

To ensure complete deprotonation of Ag^+ ions, 0.02 M NaOH was added dropwise to the reaction mixture until the pH reached 10, as monitored using a digital pH meter. Continuous stirring was maintained for 2 hours, during which a dark brown color developed, indicating successful nanoparticle formation.[20]

2.5 | Purification and Drying of Nanoparticles

The reaction mixture was centrifuged at 3000 rpm for 20 minutes. The resulting pellet was washed three times with distilled water to eliminate unreacted components and residual organic matter. The purified nanoparticles were dried in a hot-air oven at 150 °C until complete moisture removal, yielding a stable dark powder of neem-embedded, albumin-stabilized silver nanoparticles.[21]

2.6 | Characterization of Silver Nanoparticles

The synthesized AgNPs were characterized using multiple analytical techniques. X-ray diffraction (XRD) was employed to confirm crystalline structure and estimate crystallite size. Fourier-transform infrared spectroscopy (FTIR) was used to identify functional groups involved in reduction and stabilization. Surface morphology and particle shape were examined using scanning electron microscopy (SEM). UV–visible spectroscopy confirmed nanoparticle formation through surface plasmon resonance, while dynamic light scattering (DLS) was used to determine hydrodynamic size and size distribution.[22]

2.7 | Bacterial Sampling

Clinical isolates of *Pseudomonas aeruginosa* were obtained from dental patients at Misbha International Hospital, Dera Ismail Khan, Pakistan, following standard ethical guidelines and conventional microbiological procedures.

2.8 | Evaluation of Antibacterial Activity

The antibacterial activity of *A. indica*-mediated AgNPs was assessed using the agar well diffusion method. Bacterial suspensions were adjusted to the 0.5 McFarland standard and uniformly spread onto Mueller–Hinton agar plates. Wells of 6 mm diameter were punched into the agar, and AgNP suspensions at concentrations of 10, 20, and 30 µg/mL were introduced. A 0.2% chlorhexidine solution served as the positive control. Plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters.[23]

2.9 | Determination of MIC and MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined using the broth microdilution method. MIC was defined as the lowest concentration of AgNPs that inhibited visible bacterial growth. For MBC determination, aliquots from non-turbid MIC wells were sub-cultured onto nutrient agar plates and incubated to confirm bactericidal activity.[23]

2.10 | Statistical Analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical comparisons among groups were carried out using one-way analysis of variance (ANOVA), with statistical significance set at $p < 0.05$. Data analysis was conducted using SPSS software (version 22.0).

3 | RESULTS AND DISCUSSION

3.1 | XRD Analysis

XRD was carried out to ensure that AgNPs are crystalline are shown in Fig. 1 (a). The diffraction peaks at 2th degrees of 31.78, 34.48, 47.53, 56.65, 58.54, 62.91 and 68.03 were identified with characteristic lattice planes indicating a large level of crystallinity and purity. The mean crystallite size was determined as per Debye-Scherrer equation, and was found 16.07 nm that revealed the successful synthesis of AgNPs.

3.2 | FTIR Analysis

FTIR further revealed the successful synthesis of AgNPs as shown in Fig. 1 (b)**.** FTIR spectroscopes showed that there were absorption peaks at 3291, 2351, 1640, 1533, 1114, 1043, and 702 cm -1, which represented the existence of hydroxyl, alkene, aromatic, and C-H functional groups. These functional groups are based on neem phytochemicals and albumin which proved their association in reducing and stabilizing NPs.

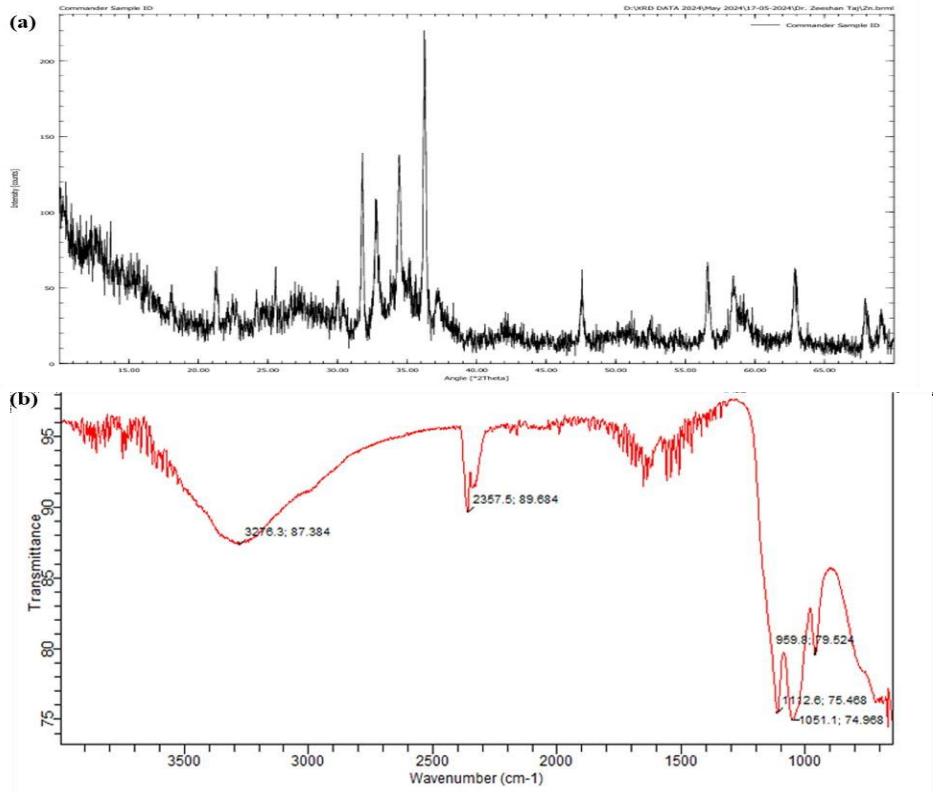

Fig. 1: (a) XRD Spectrum of AgNPs, (b) FTIR Analysis of AgNPs.

3.3 | SEM Analysis

The morphological and structural properties of the AgNPs are investigated using SEM, as shown in Fig. 2 (a). The SEM micrographs revealed that the surface morphology of the AgNPs was homogeneous, with a hexagonal and crystalline structure. The size of the observed particle was comparable to XRD results and once again confirmed the success of the synthesis of AgNPs.

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3.4 | UV–Visible Spectroscopy

UV-Vis spectroscopy is a technique for determining the quantity of light absorbed and scattered by a specific substance, and used to confirm the physicochemical characteristics of AgNPs, as illustrated in Fig. 2 (b). The UV Vis spectra showed a distinct absorption peak at 238 nm, which was the surface plasmon resonance of silver nanoparticles, showing the existence of NPs.

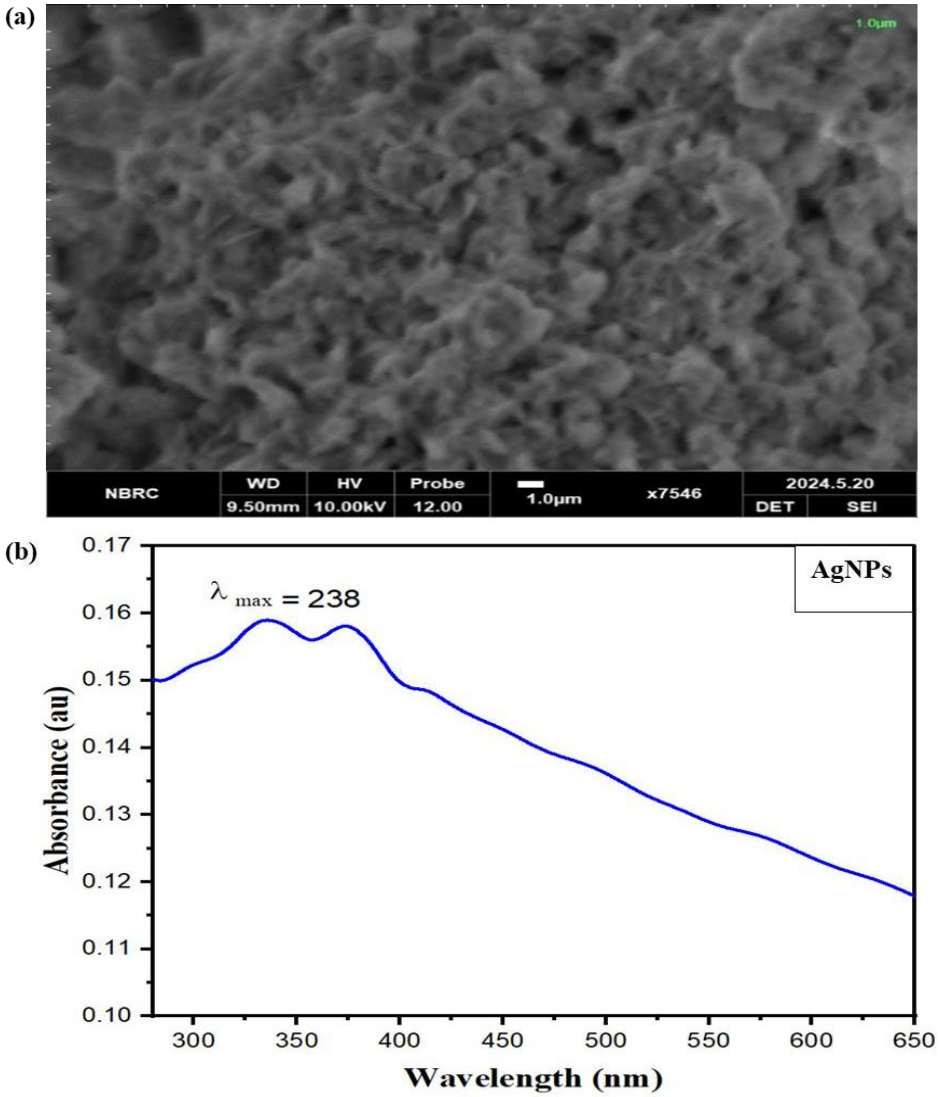

Fig.2: (a) SEM Analysis of AgNPs, (b) UV–vis Absorption Analysis of AgNPs, CuNPs, and ZnNPs.

3.5 | Dynamic Light Scattering

A hydrodynamic diameter of 144.22 nm in the DLS analysis indicated a little aggregation in the aqueous suspension, but the suspension retained good colloidal stability. The diffusion coefficient of AgNPs was 1.4 µm 2 /s and transmittance was 59.7, whereas CuNPs had 0.8 µm 2 /s and transmittance was 60.9. The existing paper demonstrated that AgNPs were very stable in terms of physicochemical properties, preventing aggregations and, therefore, enhancing physical stability and applicability in biomedical practice.

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3.6 | Antibacterial Activity Against *P. aeruginosa*

3.6.1 | Dose-Dependent Antibacterial Effect

The antibacterial efficacy of the test treatment was evaluated using the agar well diffusion method by measuring the zone of inhibition at different concentrations (10, 20, and 30 µg/mL) and compared with the standard antimicrobial agent chlorhexidine (0.2%). A clear dose-dependent increase in antibacterial activity was observed. The zone of inhibition increased from 12.0 ± 1.13 mm at 10 µg/mL to 14.0 ± 2.05 mm at 20 µg/mL, reaching a maximum of 17.0 ± 1.15 mm at 30 µg/mL. The highest concentration exhibited antibacterial activity comparable to chlorhexidine (18.0 ± 1.46 mm), indicating strong inhibitory potential. These findings demonstrate that increasing concentration significantly enhances antimicrobial efficacy, and at higher doses, the treatment shows activity nearly equivalent to the standard control as shown in Tab.1 & Fig. 3 (b). At 30 µg/mL, Neem-AgNPs demonstrated antibacterial activity comparable to 0.2% chlorhexidine, indicating strong antimicrobial potential.

Table 1 Neem-Embedded AgNPs Dose-Dependent Antibacterial Activity

Neem-Embedded AgNPs Dose-Dependent Antibacterial Activity	
Treatment	**Zone of Inhibition (mm)**
10 µg/mL	12 ± 1.13
20 µg/mL	14 ± 2.05
30 µg/mL	17 ± 1.15
Chlorhexidine (0.2%)	18 ± 1.46

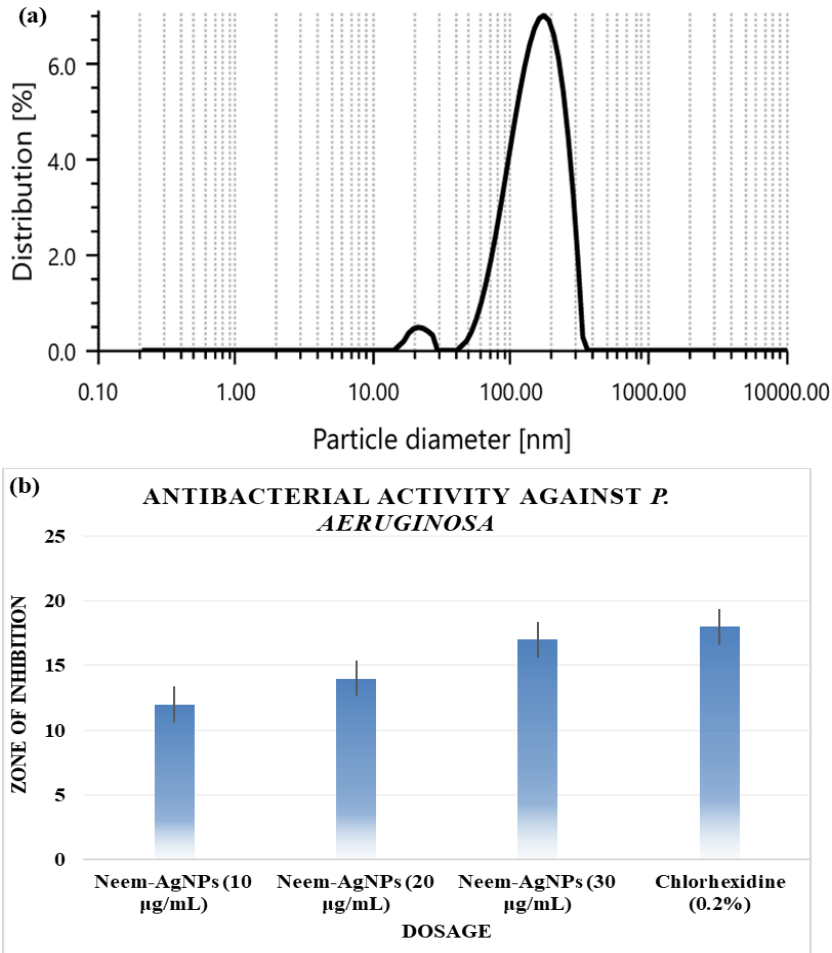

Fig.3: (a) Zeta Sizer of AgNPs, (b) *A. Indica*- AgNPs Antibacterial Effect Against *P. Aeruginosa* Isolated from Dental Patients.

4 | DISCUSSION

The present investigation confirmed that silver nanoparticles can be efficiently synthesized using *Azadirachta indica* leaf extract and that these biogenic AgNPs exhibit pronounced antibacterial activity against *Pseudomonas aeruginosa* isolated from the oral cavity of dental patients. These findings are consistent with growing evidence indicating that biologically fabricated AgNPs represent a sustainable, effective, and biocompatible alternative to chemically synthesized nanoparticles.[24] X-ray diffraction analysis verified the crystalline nature of the synthesized AgNPs, with an average crystallite size of 16.07 nm. Nanoparticles within this size range are known to possess enhanced antibacterial activity due to their large surface-area-to-volume ratio, which facilitates increased interaction with bacterial cell membranes.[25] Comparable crystallite dimensions have been reported for neem-mediated AgNPs in previous studies, where smaller particle sizes were associated with improved antimicrobial efficacy.[26] Furthermore, the presence of well-defined purity peaks in the XRD spectrum confirms the successful synthesis and high purity of the nanoparticles.

FTIR analysis revealed the presence of hydroxyl, aromatic, and alkene C–H functional groups, which are attributed to neem-derived phytochemicals and albumin involved in nanoparticle synthesis. These functional moieties play a crucial role in reducing Ag^{+} ions and stabilizing the nanoparticles, thereby preventing aggregation.[27] The involvement of flavonoids and polyphenols in nanoparticle stabilization has been extensively documented and is known to enhance both colloidal stability and antibacterial performance.[28] The FTIR findings of this study align well with earlier reports on neem-functionalized AgNPs, confirming effective surface functionalization by plant biomolecules. SEM micrographs demonstrated that the synthesized nanoparticles exhibited relatively uniform morphology, with predominantly hexagonal shapes. This observation is consistent with the crystalline characteristics observed in XRD analysis. Particle morphology is a key determinant of antibacterial efficiency, as sharp-edged and well-defined nanostructures are more effective in disrupting bacterial membranes.[29] The hexagonal morphology observed in this study corresponds with previous reports on both chemically and biologically synthesized AgNPs that display enhanced antimicrobial activity.

UV–visible spectroscopy further confirmed AgNP formation by displaying a characteristic surface plasmon resonance (SPR) peak at 238 nm. Although AgNPs typically exhibit SPR peaks in the range of 400–450 nm, blue-shifted peaks at lower wavelengths have been reported for nanoparticles of smaller size or those capped with strong stabilizing agents.[25] The observed shift may be attributed to the combined stabilizing effects of neem phytochemicals and albumin, which influence the optical properties of the nanoparticles. Dynamic light scattering analysis revealed a hydrodynamic diameter of 144.22 nm, which was larger than the crystallite size determined by XRD. This discrepancy is expected, as DLS measures the hydrodynamic size of nanoparticles along with associated biomolecules and solvent layers.[30] Despite the larger hydrodynamic size, the AgNPs exhibited good dispersion and stability, characteristics that are essential for biomedical and dental applications.

Antibacterial evaluation demonstrated a clear concentration-dependent inhibitory effect of neem-mediated AgNPs against *P. aeruginosa*. At a concentration of 30 µg/mL, the nanoparticles produced an inhibition zone (17 ± 1.15 mm) comparable to that of 0.2% chlorhexidine (18 ± 1.46 mm), which is widely regarded as the gold-standard antiseptic in dentistry. Similar antibacterial efficacy of neem-derived AgNPs against multidrug-resistant *P. aeruginosa* and other Gram-negative pathogens has also been reported previously.[31] The pronounced antibacterial activity observed in this study may be attributed to the synergistic interaction between silver ions and neem phytochemicals. This synergy can lead to membrane disruption, reactive oxygen species generation, and interference with bacterial enzymatic and genetic functions.[32] Importantly, the use of plant extracts in nanoparticle synthesis may also reduce the cytotoxic effects commonly associated with chemically produced AgNPs. This study addresses a notable research gap by directly evaluating neem-mediated AgNPs against clinically relevant dental isolates of *P. aeruginosa*. Nevertheless, further investigations focusing on cytotoxicity, antibiofilm properties, and in vivo safety are necessary before clinical translation can be considered.

5 | CONCLUSION

This study demonstrates that *Azadirachta indica*–mediated silver nanoparticles exhibit significant dose-dependent antibacterial activity against *Pseudomonas aeruginosa* isolated from dental patients. Notably, the antibacterial effect observed at a concentration of 30 µg/mL was comparable to that of the commonly used dental antiseptic chlorhexidine (0.2%). The enhanced antimicrobial performance is attributed to the synergistic action of silver ions and bioactive neem

phytochemicals. These findings suggest that neem-embedded AgNPs represent a promising, eco-friendly, and biocompatible alternative to conventional antimicrobial agents. However, comprehensive assessments of long-term safety, cytotoxicity, and optimized delivery strategies are essential to fully realize their therapeutic potential in dental practice.

6 | LIMITATIONS

Despite the promising findings, this study has several limitations that should be acknowledged. First, the antibacterial evaluation was performed **exclusively under in vitro conditions**, which may not fully replicate the complex biological environment of the oral cavity. Factors such as saliva, host immune responses, biofilm formation, and interactions with oral microbiota could influence the effectiveness of *Azadirachta indica*–mediated AgNPs in vivo.

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Informed consent: Not Applicable
Ethical Approval: Not Applicable
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