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GREEN SYNTHESIS AND ANTIBACTERIAL EVALUATION OF SILVER NANOPARTICLES USING NEEM (AZADIRACHTA INDICA) LEAF EXTRACT

Syeda Farheen ANDERABI[a], Jahanzeb SHEIKH[b,c], Sania TANVIR[c], Rehana KOUSER[a], Iftikhar Ahmed CHANNA[f], Tanzeela IKRAM[a], Sidra Abid SYED[c], Ali Dad CHANDIO[f], Tan Tian SWEE[b], Jose-Javier SERRANO OLMEDO[d], Rashid KHAN[e], Muhammad FAIZAN[f], Madeeha SADIA[a, b, *]

ABSTRACT. The synthesis of silver nanoparticles (AgNPs) using traditional physical and chemical methods often involves toxic reagents, high energy consumption, and poor biocompatibility, making them unsuitable for many biomedical applications. Moreover, existing green synthesis approaches frequently lack control over nanoparticle size, shape, and stability, limiting their reproducibility and scalability. To address these limitations, this study employed a green synthesis route using Azadirachta indica (Neem) leaf extract as a natural reducing and stabilizing agent. Silver nitrate ($AgNO_3$) solutions of varying concentrations (1 mM, 5 mM, and 10 mM) were reacted with the Neem extract under ambient conditions. UV–Visible spectroscopy confirmed the formation of AgNPs with a characteristic surface plasmon resonance peak at 402 nm. Scanning Electron Microscopy (SEM) showed that the 5 mM $AgNO_3$

[a] *Department of Biomedical Engineering, NED University of Engineering & Technology, Karachi, Pakistan.*
[b] *Department of Biomedical Engineering and Health Sciences, Universiti Teknologi Malaysia, Johor Bahru 81310, Johor, Malaysia.*
[c] *Department of Biomedical Engineering, Sir Syed University of Engineering and Technology, Karachi, Pakistan.*
[d] *Centre for Biomedical Technology Madrid, Universidad Politécnica de Madrid, Madrid, Spain.*
[e] *Mechanical Engineering Department, College of Engineering, Al Imam Muhammad Ibn Saud Islamic University, Riyadh, Saudi Arabia.*
[f] *Department of Metallurgy, NED University of Engineering & Technology, Karachi, Pakistan.*
[*] *Corresponding author: madeehaoz@neduet.edu.pk*

concentration produced the most desirable morphology, uniformly spherical nanoparticles with an average size of 98 nm. Energy Dispersive X-ray (EDX) analysis further confirmed the presence of pure elemental silver with no silver compounds. Moreover, antibacterial testing, conducted against Total Coliform bacteria and Propionibacterium acnes, revealed that the synthesized AgNPs, particularly in powder form, effectively inhibited bacterial growth over extended incubation periods. In conclusion, this study demonstrates that Neem-mediated synthesis is a viable, sustainable, and efficient approach for producing biologically active silver nanoparticles.

Keywords: *Silver nanoparticles; Green synthesis; Azadirachta indica (Neem); Antibacterial activity; SEM characterization; Plant-based nanomaterials.*

INTRODUCTION

Nanotechnology has revolutionized material science by enabling the manipulation of matter at the atomic and molecular scale, typically within 1–100 nm [1]. Among the diverse applications, the synthesis of metallic nanoparticles, particularly silver nanoparticles (AgNPs), has garnered significant attention due to their exceptional antimicrobial, anti-inflammatory, and optical properties [2]. Silver, a noble metal known historically for its antimicrobial efficacy [3], becomes remarkably more reactive at the nanoscale due to an increased surface area-to-volume ratio [4].

Traditionally, AgNPs have been synthesized using physical and chemical methods [5], such as evaporation-condensation [6] and chemical reduction using agents like sodium borohydride and citrate [7]. While these methods are efficient and scalable, they often involve high energy consumption [8], toxic reagents [9], and hazardous by-products [10], limiting their suitability for biomedical applications. For example, tube furnace-based evaporation methods consume significant energy and produce environmental heat pollution [11]. Similarly, chemical synthesis can lead to nanoparticle agglomeration [12] due to insufficient capping agents, and the residual chemicals may remain on particle surfaces, posing cytotoxic risks [13].

To overcome these drawbacks, green synthesis using biological systems such as bacteria [14], fungi [15], and plant extracts [15 – 17] has emerged as a safer and more sustainable approach [18]. Microbial synthesis, though effective, often requires complex culturing conditions and longer synthesis times [19]. Fungal methods, while yielding higher nanoparticle quantities, involve risk of contamination [20] and require precise control over pH and temperature [21]. Furthermore, both microbial and fungal synthesis approaches may involve challenges in downstream processing and scale- up.

Among plant-based green synthesis methods, the use of Azadirachta indica (Neem) has shown great promise [22, 23]. Neem is a medicinally valuable plant known for its antibacterial, antifungal, and anti-inflammatory properties [24]. Moreover, Research has established that limonoid compounds extracted from neem seeds exhibit minimum inhibitory concentrations (MICs) ranging from 32 µg/ml to 128 µg/ml against *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus epidermidis* (*S. epidermidis*), an opportunistic skin pathogen [25]. Its extract contains various phytochemicals such as flavonoids, terpenoids, and phenolics, which serve as both reducing and stabilizing agents during nanoparticle formation. For instance, Zanjage [26] and Ghazali [27] demonstrated the rapid biosynthesis of AgNPs using aqueous Neem extracts. However, the limitations included poor reproducibility, lack of control over particle size, and agglomeration due to unstable phytochemical profiles in extracts. Moreover, the influence of synthesis parameters such as extract concentration, reaction time, temperature, and pH were not rigorously optimized in many prior studies.

In terms of characterization, studies employing UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) analysis have confirmed the successful formation and elemental composition of Neem-derived AgNPs [28, 29]. However, gaps remain in detailed structure-property correlation, and many works lack thorough antibacterial efficacy validation against clinically relevant strains. Therefore, in this research, we addressed the limitations of conventional AgNP synthesis methods by developing a green, eco-friendly approach using Neem leaf extract. This method eliminated the need for toxic chemicals and high energy input. The study involved optimizing critical synthesis parameters to achieve controlled particle size and minimize agglomeration. Additionally, detailed characterization using UV-Vis spectroscopy, SEM, and EDX was conducted to verify the successful synthesis and purity of the silver nanoparticles. Antibacterial activity was further evaluated against *Total Coliform* and *Propionibacterium acnes* to confirm their potential for biomedical applications.

RESULTS AND DISCUSSION

Visual Confirmation of Silver Nanoparticle Formation

The initial visual indicator of silver nanoparticle (AgNP) formation was the change in color of the Neem extract and silver nitrate solution mixture. Upon mixing, the solution exhibited a rapid transition from pale yellow to dark brown within five minutes, which is characteristic of silver nanoparticle synthesis due to surface plasmon resonance (SPR). The color intensity increased with incubation time, confirming nanoparticle growth [32].

The SPR peak at 402 nm observed in this study aligns with previous reports on Neem-mediated AgNP synthesis, where similar peaks (400–420 nm) have been linked to spherical particle morphology and stable colloidal dispersion [33, 34]. However, unlike some earlier studies that reported broader peaks due to polydispersity, the sharp peak at 5 mM $AgNO_3$ suggests a relatively narrow size distribution, indicative of improved synthesis control in our method [35].

UV–Visible Spectroscopy

UV–Visible (UV-Vis) spectroscopy was employed to confirm the formation of silver nanoparticles and to study their optical properties at three different silver nitrate concentrations: 1 mM, 5 mM, and 10 mM (Figure 3.1). All samples exhibited a characteristic surface plasmon resonance (SPR) peak at 402 nm, which is indicative of the presence of silver nanoparticles and corresponds to their spherical morphology.

At 1 mM concentration (Figure 1a), a sharp absorption peak was observed at 402 nm with an absorbance value of 1.6, indicating successful synthesis but with moderate nanoparticle yield. Increasing the concentration to 5 mM resulted in a more intense absorption peak at the same wavelength (Figure 1b), with a maximum absorbance of 1.8, suggesting a higher nanoparticle concentration and improved synthesis efficiency. However, at 10 mM, the absorbance decreased to 1.4, even though the SPR peak remained at 402 nm (Figure 1c). This decline may be attributed to agglomeration or particle over-saturation, which can occur when the silver ion concentration exceeds the optimal threshold for stable nanoparticle formation.

The results demonstrate a clear correlation between silver ion concentration and nanoparticle formation efficiency. Specifically, absorbance increased from 1 mM to 5 mM, reflecting a greater number of nanoparticles, but declined at 10 mM, likely due to particle instability and clustering. Moreover, since smaller nanoparticles typically exhibit higher absorbance due to greater surface area-to-volume ratio, the findings also support the conclusion that 5 mM $AgNO_3$ yields smaller, more stable nanoparticles compared to the other concentrations. Based on the combination of peak intensity and stability, 5 mM was identified as the optimal concentration, and samples from this condition were used for further characterization.

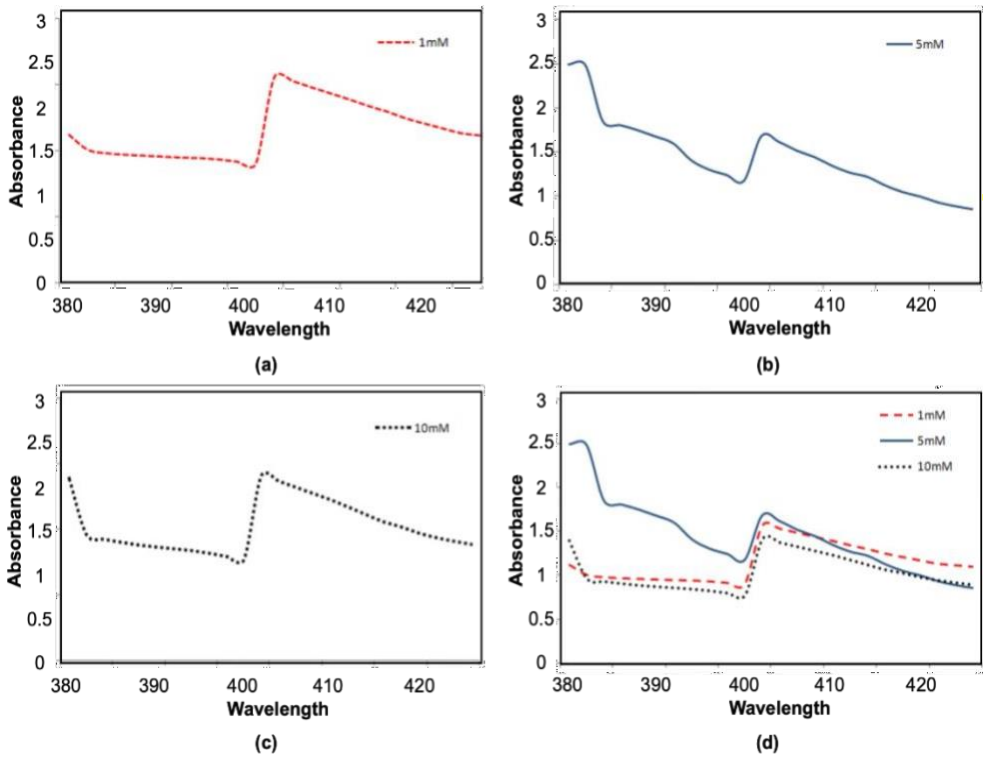

Figure 1. The UV-VIS spectroscopy spectrum of: (a) 1mM, (b) 5mM, (c) 10mM

Moreover, SEM analysis was carried out to investigate the morphology and size of silver nanoparticles synthesized using different concentrations of silver nitrate (1 mM, 5 mM, and 10 mM). At 1 mM, the nanoparticles predominantly exhibited a spherical shape, though some rod- like structures were also observed Figure 2. The presence of rod-shaped particles, while uncommon in green synthesis, suggests minor deviation in nucleation or growth dynamics under lower precursor concentration. The particle sizes ranged from 109.2 nm (Figure 2a) to 289.8 nm (Figure 2b), with an average diameter of approximately 325.1 nm. The irregularity in shape and relatively large size at this concentration indicates suboptimal synthesis conditions.

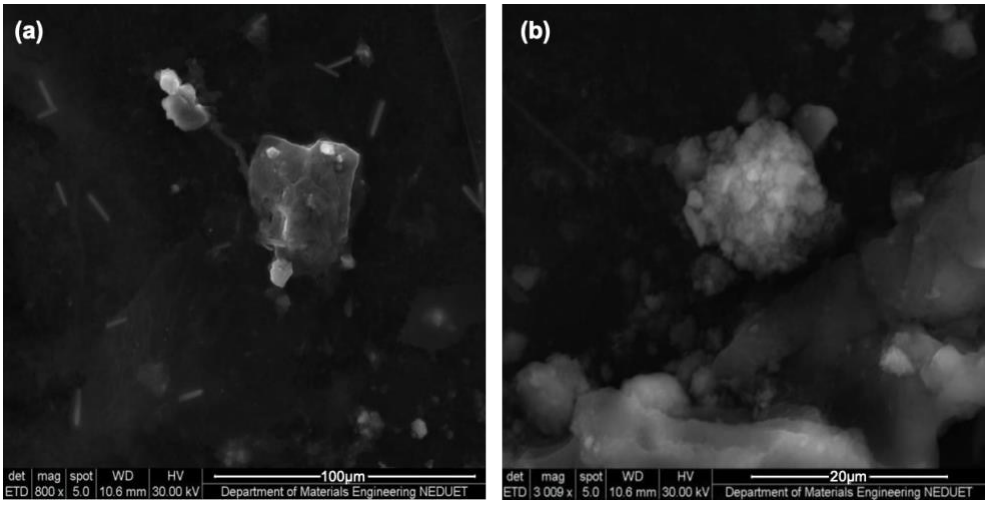

Figure 2. SEM analysis of silver nanoparticles synthesized using different concentrations of silver nitrate, (a–b) SEM images for 1 mM $AgNO_3$ showing primarily spherical nanoparticles with some rod-shaped structures at magnifications of 800X and 3009X, respectively.

The 5 mM sample produced the most desirable results, with SEM images (Figures 3a and 3b) showing uniformly distributed, spherical nanoparticles. The particles had diameters of 96 nm and 100 nm, with an average size of 98 nm. Some clustering was observed, likely due to the presence of biomolecules from the Neem extract acting as natural capping agents. Additional SEM images (Figures 3c and Figure 3d) confirmed the consistency of spherical morphology and narrow size distribution, reinforcing that 5 mM $AgNO_3$ is the optimal concentration for producing stable, monodispersed silver nanoparticles using green synthesis. Overall, the SEM results demonstrate that 5 mM silver nitrate yields the best combination of controlled shape, smaller particle size, and uniform morphology, making it the most suitable condition for further applications.

Earlier studies from demonstrated the impact of bacterial morphology on various disinfection efficacies [61-62], which aligns with the present investigation into morphology-dependent antibacterial responses to AgNPs. The SEM micrographs revealed that only the 5 mM $AgNO_3$ concentration yielded predominantly spherical nanoparticles (~98 nm), a morphology often associated with enhanced antibacterial efficacy due to higher surface energy [36, 37]. The absence of dendritic structures at this concentration contrasts with the

morphological deviations observed in other plant-mediated syntheses, where excess precursor concentration (>10 mM) accelerates nucleation and disrupts uniform growth [38, 39].

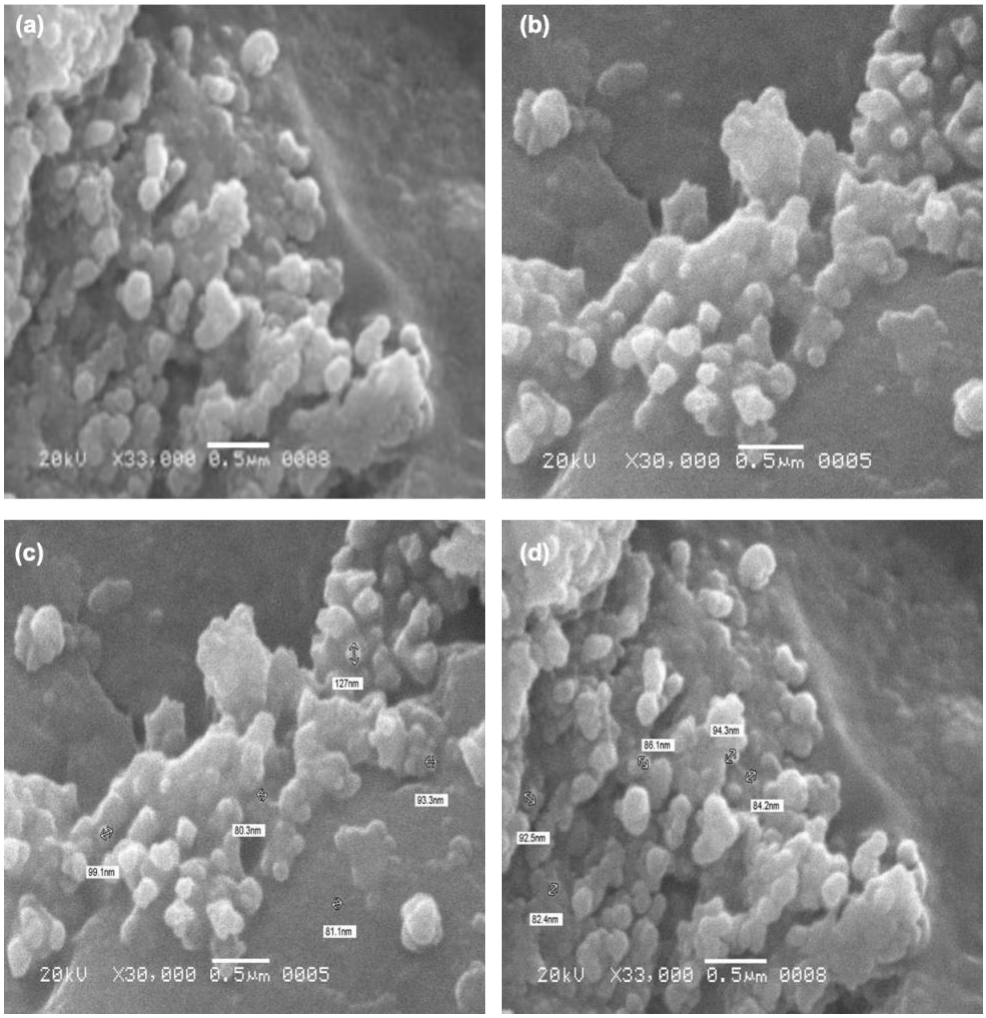

Figure 3. SEM analysis of silver nanoparticles synthesized using different concentrations of silver nitrate, SEM images for 5 mM $AgNO_3$ illustrating uniformly spherical nanoparticles with minimal agglomeration at higher magnifications (a) 33000X, (b) 30000X, (c) 30000X and (d) 33000X, with average particle sizes of 96.1 nm and 87.9 nm (a-d), respectively.

In the case of 10 mM, the nanoparticles exhibited dendritic and rod-shaped morphologies, as shown in Figure 4a and Figure 4b. These complex structures are typically associated with physical or chemical synthesis methods and are rarely observed in green synthesis. The particle diameters recorded were 195.3 nm and 146.4 nm, respectively, resulting in an average size of around 174 nm. The formation of such unusual structures at higher precursor concentrations may be due to rapid nucleation and uncontrolled particle growth.

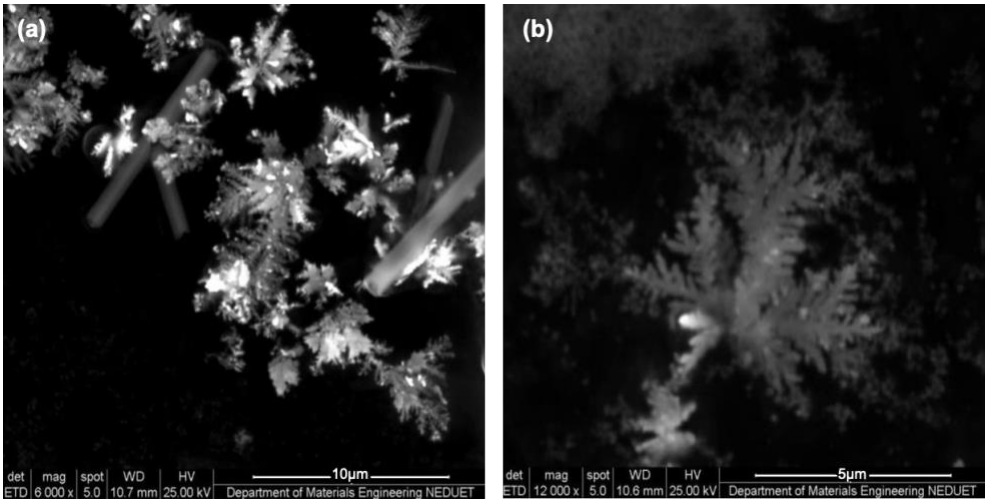

Figure 4. SEM images for 10 mM $AgNO_3$ displaying dendritic and rod-shaped nanoparticles at (a) 6000X and (b) 12000X magnification, indicating morphological changes at higher precursor concentration

Energy Dispersive X-ray (EDX) Analysis

EDX spectroscopy confirmed the elemental composition of the synthesized nanoparticles (Figure 5). A strong characteristic peak for silver was observed at 2.98 keV, affirming the successful reduction of silver ions to elemental silver. The analysis showed the presence of 100% pure silver with no significant contamination from other elements, validating the efficacy of the green synthesis method.

The EDX spectra confirming 100% elemental silver purity are noteworthy, as many plant-based syntheses report minor impurities from plant metabolites or environmental contaminants [40, 41]. This high purity underscores the efficiency of Neem phytochemicals as both reducing and capping agents, minimizing

unwanted secondary phases [42, 43]. The EDS spectrum (Figure 5) confirms the presence of Ag through its characteristic peak, while minor peaks of C, O, Na, S, and Cl originate from residual plant phytochemicals due to analysis over the full BSE image. This representative spectrum is considered adequate for elemental confirmation at this proof-of- concept stage, with spot EDS planned in future studies for refined compositional profiling.

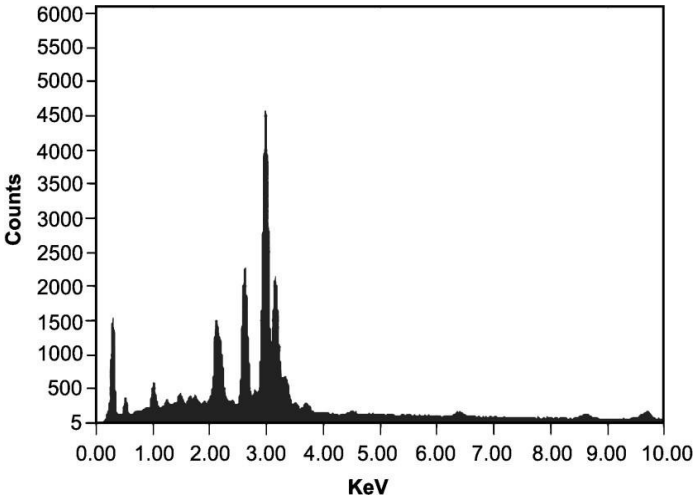

Figure 5. Energy-dispersive X-ray spectroscopy (EDS) spectrum of the synthesized Ag nanoparticles, showing a prominent Ag peak confirming their successful formation. Additional peaks corresponding to C, O, Na, S, and Cl are attributed to residual phytochemicals from the plant extract, as the spectrum was acquired over the entire BSE image.

Antibacterial Assay Results

By selecting one environmental bacterium (*T. coliform*) and one skin-associated opportunistic pathogen (*P. acnes*), the study assessed the broad-spectrum antibacterial potential of Neem- synthesized silver nanoparticles for skin wound care. This combination simulates real-world wound contamination scenarios: Environmental exposure (dirt, water, surfaces) tested with *T. Coliform* [44]. Endogenous skin bacteria turning pathogenic tested with *P. acnes* [45]. Testing against this bacterium assesses whether the antibacterial properties of the plant leaf extract attributed to its phytochemical compounds along with silver nanoparticles can synergistically prevent or treat infections that develop directly in skin and wound tissues [47, 48].

The results showed that silver nanoparticles, particularly in powdered form [49], effectively inhibited both bacteria, meaning they could help protect wounds from contamination and infection during the healing process. Neem extract alone also showed antibacterial activity, but the nanoparticles were more effective and longer lasting in their protection [23, 50].

Powdered synthesized silver nanoparticles (AgNPs) and neem (Azadirachta indica) based formulations typically outperform simple AgNP solutions against both Gram-positive and Gram-negative bacteria because they (i) maintain higher colloidal/surface stability, (ii) provide sustained and localized Ag^+ release at the cell–material interface, and (iii) add polyphenolic/terpenoid phytochemicals from neem that yield true multi-target synergy and reduce aggregation [51, 52].

AgNP solutions are prone to aggregation and oxidative changes in typical media (ionic strength, proteins), which reduces bioactive surface area and bactericidal performance; repeated bacterial exposure can even drive NP aggregation as a resistance strategy. Dry (powdered) AgNPs or solid-state/biocomposite formats preserve particle dispersion until contact with moisture, limiting pre-exposure aggregation and enabling higher effective surface contact on cells [51, 53, 54].

Therefore, powdered AgNPs has better stability. They are more consistent to cell-wall contact and ROS/protein–thiol interactions, especially on surfaces and in gels/agar, than the same particles stored and aged in liquid [54].

Bactericidal action correlates with controlled Ag^+ dissolution at the microbe material interface. Powdered/solid matrices (including coatings, composites, solid-state, green-synthesized powders) typically meter Ag^+ over time, maintaining inhibitory levels without rapid bulk dilution or scavenging that occurs in free colloids. Several recent studies on solid carriers/coatings show tunable, long-lasting Ag^+ release with superior inhibition zones compared with freely dispersed AgNPs [52, 55, 56]

Hence powdered synthesized AgNPs Sustained release helps maintain activity across growth phases in both Gram-positive and Gram-negative bacteria while limiting one-shot spikes that are neutralized by broth constituents [57].

Neem leaves/bark provide flavonoids, terpenoids (e.g., nimbin), tannins and quercetin-like polyphenols that (a) reduce and cap AgNPs (smaller, more stable particles), (b) perturb membranes/enzymes/DNA directly, and (c) inhibit biofilm and efflux, thereby potentiating silver. Green-synthesized or neem-infused AgNPs repeatedly show broader and stronger activity than either AgNPs or neem alone [18, 58]

Silver's multi-site mechanism (membrane disruption, metabolic/protein thiol binding, ROS, DNA damage) is not wall-type specific; neem's phytochemicals add outer-membrane permeabilization and quorum/biofilm interference helpful for Gram-negatives with lipopolysacchride barriers and for Gram-positives with thick peptidoglycan. Contemporary reviews confirm broad-spectrum efficacy and enhanced performance when AgNPs are combined with bio-actives [59].

Table 1 show that synthesized powdered (dry) silver nanoparticles, diluted silver nanoparticles, and Neem extract all showing bacterial inhibition properties. But powdered silver nanoparticles show better bacterial inhibition growth.

Table 1. Comparative antibacterial activity of synthesized powdered (dry) silver nanoparticles, diluted silver nanoparticles, and Neem extract.

Bacterial Assays	Time (Hrs)	Time Days	Powdered Silver Nanoparticles	Silver Nanoparticles solution + distilled water	Neem extract
T. cloriform (Gram-Negative)	0	0	-	-	-
	24	1	-	-	-
	48	2	-	-	-
	96	4	+++	+	+++
P. acne (Gram-Positive)	0	0	-	-	-
	24	1	-	-	-
	48	2	-	-	-
	96	4	-	-	-
	168	7	+++	+	++

"+++" = Clear inhibition zone observed (High antibacterial activity present)
"++" = Inhibition zone observed (Medium antibacterial activity present)
"+" = Inhibition zone observed (Minimum antibacterial activity present)
"–" = inhibition zone observed (no antibacterial activity)

Against *Total Coliform* Bacteria

The antibacterial activity of the synthesized silver nanoparticles against Total Coliform bacteria was assessed using the disc diffusion method (Figure 6). Observations were recorded at 24, 48, and 96 hours of incubation. At 24 and 48 hours, all three discs, containing silver nanoparticle powder (Disc 1), silver nanoparticle solution (Disc 2), and Neem extract (Disc 3), showed limited or no visible bacterial growth around the treated areas. By 96 hours, Disc 1 (silver nanoparticle powder) exhibited complete inhibition of bacterial growth, with a clear and well-defined zone of inhibition. Disc 2 (silver nanoparticle solution) showed slight bacterial regrowth, indicating moderate antibacterial activity. Disc 3 (Neem extract) also exhibited no bacterial

growth, suggesting that the plant extract retained antibacterial potential, though likely milder than nanoparticle treatments. These results confirm that silver nanoparticles, particularly in powdered form, have a strong inhibitory effect on Total Coliform bacteria, maintaining clear zones of inhibition over a 96-hour period.

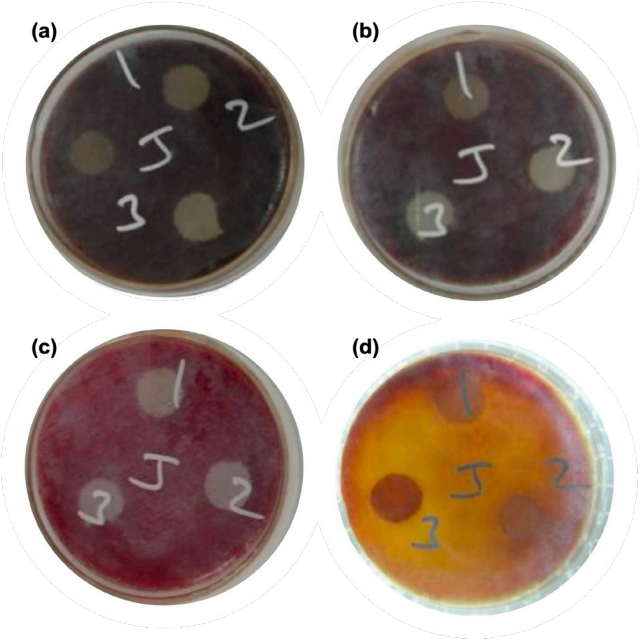

Figure 6. Antibacterial activity of silver nanoparticles and Neem extract against Total Coliform bacteria observed at different incubation times. (a) At 0 minutes showing initial placement of discs: 1 (silver nanoparticle powder), 2 (silver nanoparticle solution), 3 (Neem extract). (b) At 24 hours, showing early signs of bacterial inhibition. (c) At 48 hours, indicating progressive bacterial suppression around the treated discs. (d) At 96 hours, with clear zones of inhibition, particularly around Disc 1 (AgNP powder), confirming strong antibacterial efficacy.

Against Propionibacterium acnes

The antibacterial effect against *P. acnes* was evaluated over a similar incubation period (Figure 7), and up to 7 days, due to the slow-growing nature of this bacterium. At 24 and 48 hours, initial signs of inhibition were observed, but no significant difference between treatments was apparent. By 96 hours and especially at 7 days, Disc 1 (silver nanoparticle powder) and Disc 2 (silver nanoparticle solution) both demonstrated complete inhibition of

bacterial growth, as evidenced by clear zones around the discs. Disc 3 (Neem extract) also showed no bacterial growth after 7 days, although its inhibitory effect appeared delayed compared to the nanoparticle treatments. These findings indicate that both forms of synthesized silver nanoparticles exhibit effective antibacterial activity against *P. acnes*, with the powdered form showing slightly superior performance. Neem extracts also showed potential antimicrobial properties, though its activity may be slower or less potent.

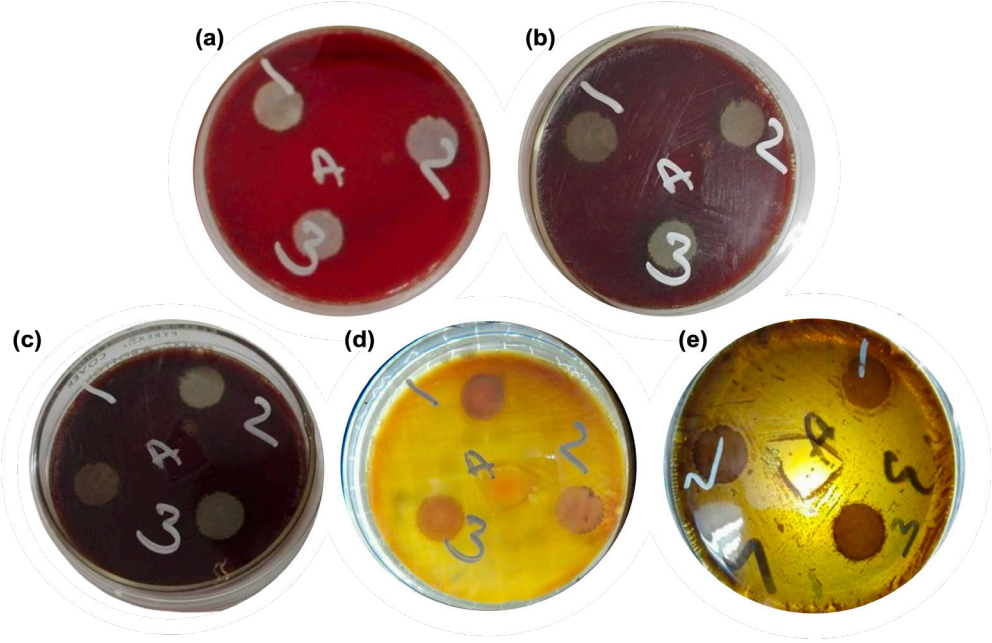

Figure 7. Antibacterial activity of silver nanoparticles and Neem extract against Propionibacterium acnes observed over time. (a) At 0 minutes showing placement of discs 1 (silver nanoparticle powder), 2 (silver nanoparticle solution), 3 (Neem extract), (b) At 24 hours with initial bacterial inhibition beginning to appear, (c) At 48 hours showing increased suppression around nanoparticle discs, (d) At 96 hours with clearer zones of inhibition, especially around Disc 1 and Disc 2, (e) After 7 days, exhibiting complete bacterial growth inhibition around all discs, confirming strong antimicrobial efficacy, particularly of the powdered silver nanoparticles.

The superior antibacterial performance of AgNP powder against *Total Coliform* and *P. acnes* can be attributed to its higher surface contact and potential for sustained ion release compared to colloidal forms, as supported by previous work demonstrating enhanced bacterial membrane disruption

with increased nanoparticle–cell wall interactions [3, 9, 32]. Furthermore, the slower yet observable inhibition by Neem extract alone reaffirms its intrinsic antimicrobial activity, likely driven by limonoids and flavonoids [60, 50], which may also synergistically enhance the bioactivity of the synthesized AgNPs. While earlier Neem-based AgNP syntheses have faced challenges in reproducibility and particle agglomeration due to fluctuating phytochemical profiles [27], our optimization of precursor concentration and reaction conditions resulted in monodispersed particles with stable morphology. This improvement addresses a key limitation highlighted in recent reviews, where the need for process standardization is emphasized for clinical translation of phytofabricated nanoparticles [23, 45].

These findings hold promise for developing biocompatible, non-toxic antimicrobial agents for wound healing and dermatological applications, areas where both AgNPs and Neem phytochemicals have demonstrated complementary bioactivities [3, 29]. The ability to produce high-purity, optimally sized particles through an eco-friendly route further strengthens the case for scaling up this approach for biomedical device coatings and environmental disinfection systems [5, 28].

CONCLUSIONS

The present study successfully demonstrated the green synthesis of silver nanoparticles using Azadirachta indica (Neem) leaf extract as a natural reducing and stabilizing agent. This eco-friendly and cost-effective method proved to be efficient in terms of reaction time, stability, and reproducibility, offering a cleaner and safer alternative to conventional chemical and physical synthesis techniques. The formation of silver nanoparticles was confirmed through UV–Visible spectroscopy, which showed a consistent SPR peak at 402 nm. SEM analysis revealed that the morphology and size of the nanoparticles were highly dependent on the silver nitrate concentration, with the most desirable spherical and uniformly distributed nanoparticles observed at 5 mM concentration, averaging 98 nm in diameter. EDX analysis further validated the presence of pure elemental silver with no detectable silver compounds, confirming complete reduction by the phytochemicals in the Neem extract. Antibacterial testing showed that the synthesized silver nanoparticles exhibited strong inhibitory effects against both Total Coliform bacteria and *P. acnes*, particularly in powdered form. Neem extract alone also demonstrated antimicrobial potential, though the nanoparticles were significantly more effective in inhibiting bacterial growth over extended incubation periods. Overall, the study confirms that Neem leaf extract is a powerful and sustainable agent

for the green synthesis of silver nanoparticles with excellent antibacterial properties. These findings highlight the potential of this method for biomedical and environmental applications where non- toxic, biocompatible nanomaterials are essential.

MATERIALS AND METHOD

Preparation of Neem Leaf Extract

Fresh Azadirachta indica (Neem) leaves were collected (Figure 8a), washed thoroughly with distilled water to eliminate any surface impurities, and then air-dried under sunlight. A total of 25 grams of the dried leaves were chopped into smaller pieces and boiled in 100 mL of distilled water for 15 minutes [30] (Figure 8b). The boiling process resulted in a yellow-colored solution, indicating the extraction of bioactive compounds [31]. After cooling to room temperature, the mixture was filtered using standard filter paper. The resulting filtrate, rich in phytochemicals, was stored at 6 °C and used as a reducing and stabilizing agent in the synthesis of silver nanoparticles

Green Synthesis of Silver Nanoparticles

Silver Nitrate Solution was prepared of 1mM, 5mM, and 10mM concentrations where 1mM Silver Nitrate solution contains 0.1699g of AgNO3 in 1000ml of distilled water. These solutions were each mixed with the Neem extract in a 1:6 ratio (extract to silver nitrate solution). Upon mixing, a rapid color change from pale yellow to dark brown was observed within five minutes (Figure 8c), indicating the reduction of silver ions and the formation of silver nanoparticles. To ensure complete synthesis and prevent photoactivation, the mixtures were incubated in the dark at room temperature for 24 hours.

Collection and Drying of Nanoparticles

After incubation for 24 hours, the silver nanoparticles were collected by centrifugation at 4000 rpm for 15 minutes. The supernatant was discarded, and the solid pellet was dried in a laboratory oven set at 50 °C. The supernatant was discarded and the solid mass was kept in an oven to dry, at 50 degree Celsius. The resulting dried product was a black powder, which was carefully collected using spatula and stored in airtight containers for further characterization and testing (Figure 8d).

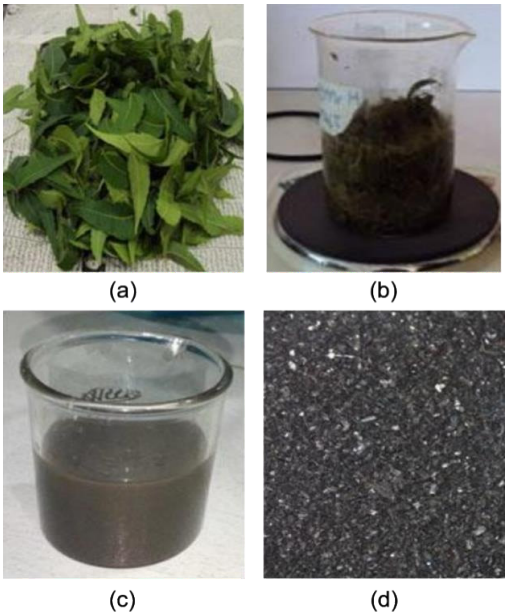

Figure 8. Visual representation of the green synthesis process of silver nanoparticles using Azadirachta indica (Neem) leaf extract, including (a) Collection phase, (b) Boiling phase, (c) Visualization of color change, (d) Stereo optical microscope image of black colored powder at 30X

Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were characterized using three analytical techniques. UV–Visible spectroscopy (UV-Vis) was used to confirm nanoparticle formation by detecting the surface plasmon resonance (SPR) peak, typically centered around 402 nm. The UV–Vis absorption spectra were recorded using a Spectrum Lab 22 PC spectrophotometer operating in the range of 340–1000 nm with a spectral band width 6nm. Scanning Electron Microscopy (SEM) was employed to examine the morphology, size, and distribution of the nanoparticles, confirming their predominantly spherical shape with average diameters around 98 nm. The morphological analysis of the nanoparticles was performed using a JSM-6380A JEOL (JEOL Ltd., Japan) operated at an accelerating voltage of 5–15 kV under high-vacuum mode**.** In addition, Energy Dispersive X-ray (EDX) analysis verified the elemental composition of the samples, showing a distinct silver peak, which confirmed the presence and purity of the nanoparticles without contamination from other metals or compounds. Elemental composition was determined using JEOL Japan model number EX-54175JMU attached to the SEM, operating at 15 kV.

Antibacterial Testing

The antibacterial activity of the synthesized silver nanoparticles was evaluated using the disc diffusion method on blood agar medium. Two bacterial strains were selected for testing: *T. Coliform* and *P. acnes*. To prepare the blood agar, nutrient agar powder was dissolved in distilled water and heated until completely dissolved. The mixture was then autoclaved for 15 minutes. Once cooled to approximately 50 °C, 5% warm sheep blood was added to the medium, which was gently mixed to avoid air bubble formation. The prepared blood agar was poured into sterile petri dishes and allowed to solidify under aseptic conditions. For the antibacterial assay, each solidified agar plate was uniformly inoculated with one of the bacterial strains using sterile cotton swabs. Three types of discs were prepared for testing: one impregnated with silver nanoparticle powder, another with silver nanoparticle solution, and a third with Neem leaf extract. These were labeled as 1 (AgNP powder), 2 (AgNP solution), and 3 (Neem extract), respectively. The discs were gently pressed onto the agar surface to ensure firm contact. All plates were incubated at 37 °C under aerobic conditions, and bacterial growth inhibition was monitored at regular intervals. In this study, the antibacterial activity was assessed using the disc diffusion method. The evaluation was qualitative and based on the extent of clear inhibition zones observed around the discs impregnated with AgNP powder, AgNP solution, and Neem extract. To provide a comparative interpretation of the results, the inhibition zones were categorized into four levels: “+++” indicating high antibacterial activity with a prominent and clear zone; “++” indicating medium activity with a moderately visible zone; “+” indicating minimum activity with a faint inhibition zone; and “–” indicating no observable antibacterial activity. This scale was adopted in place of direct zone measurements (in mm) because the primary aim was comparative evaluation of treatment effectiveness rather than precise quantification. The formation of clear zones around the discs indicated antibacterial activity, allowing for comparative evaluation of the effectiveness of each treatment.

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