Journal of Carcinogenesis
Covering Genesis to Therapeutics
Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 8s, https://carcinogenesis.com/

Eco-Friendly Synthesis of Silver Nanoparticles Using Neem and Eucalyptus Leaf Extracts: Characterization and Antimicrobial Efficacy Against Pathogenic Microbes

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ABSTRACT

Silver nanoparticles are widely recognized for their potent antimicrobial properties. Conventional synthesis methods rely on toxic chemicals and high energy input, posing environmental and biomedical concerns. This study presents an eco-friendly, cost-effective synthesis of AgNPs using aqueous leaf extracts of Neem (*Azadirachta indica*) and Eucalyptus (*Eucalyptus globulus*) as reducing and stabilizing agents. Optimization of synthesis parameters, including extract-to-silver nitrate ratio, temperature, and incubation time, yielded stable, uniformly dispersed nanoparticles. Formation of AgNPs was confirmed visually and by UV–Vis spectroscopy (SPR peak 412–425 nm). TEM and SEM revealed spherical particles (Neem: 15–35 nm; Eucalyptus: 10–25 nm). XRD confirmed the crystalline face-centered cubic structure, while FTIR analysis indicated functional groups (hydroxyl, carbonyl, ether) involved in capping. Zeta potential analysis demonstrated greater colloidal stability for Eucalyptus-mediated AgNPs (−30.3 mV) compared to Neem (−22.1 mV). Antimicrobial assays against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and *Candida albicans* revealed significant activity of both Neem- and Eucalyptus-AgNPs, with Eucalyptus showing superior efficacy. One-way ANOVA and Tukey post-hoc analysis confirmed statistically significant differences ($p < 0.001$). This study demonstrates that green synthesis using plant extracts produces stable, biofunctional AgNPs with broad-spectrum antimicrobial activity, highlighting their potential for pharmaceutical applications such as wound dressings and topical antimicrobials.

Keywords: *Green synthesis, Silver nanoparticles, Neem, Eucalyptus, Antimicrobial activity, Green chemistry, Nanotechnology.*

How to Cite: Suraj, Aadesh Kumar, Swati Wadhawan, Manish Pathak, (2025) Eco-Friendly Synthesis of Silver Nanoparticles Using Neem and Eucalyptus Leaf Extracts: Characterization and Antimicrobial Efficacy Against Pathogenic Microbes, *Journal of Carcinogenesis*, *Vol.24, No.8s*, 492-503

1. INTRODUCTION

1.1 Nanotechnology and Its Pharmaceutical Relevance

Nanotechnology involves manipulating matter at the nanoscale (1–100 nm) to create materials with novel properties that differ significantly from their bulk counterparts (Iravani, 2011). In the pharmaceutical field, nanotechnology plays a critical role in targeted drug delivery, diagnostic imaging, regenerative medicine, and antimicrobial therapy (Rai et al., 2009). Among various nanoparticles, silver nanoparticles have garnered particular attention due to their strong antimicrobial activity, low potential for resistance development, and compatibility with a wide range of formulations such as ointments, gels, wound dressings, and coatings for medical devices (Dakal et al., 2016; Ahmed et al., 2016).

AgNPs exhibit antimicrobial activity through multiple mechanisms including disruption of cell membranes, generation of reactive oxygen species (ROS), protein inactivation, and interference with DNA replication (Morones et al., 2005). This multimodal action makes them effective against a broad spectrum of microorganisms, including multidrug-resistant strains.

1.2 Limitations of Conventional Synthesis Methods

Traditional chemical and physical synthesis methods, while effective in controlling nanoparticle size and morphology, present significant drawbacks in terms of safety, environmental impact, and scalability. Common reducing agents such as sodium borohydride and hydrazine are highly toxic and hazardous (Sharma et al., 2022). These methods often require high temperatures, inert atmospheres, or organic solvents, which increase energy consumption and production costs (Ahmed et al., 2016). Additionally, residual toxic agents can remain adsorbed on the nanoparticle surface, limiting their suitability for biomedical applications (Dakal et al., 2016). These challenges have prompted the search for greener, safer, and more sustainable synthesis methods.

1.3 Green Synthesis as a Sustainable Alternative

Green synthesis approaches utilize biological systems such as plant extracts, microorganisms, or biomolecules to act simultaneously as reducing and stabilizing agents (Iravani, 2011; Ahmed et al., 2016). Plant-based methods are particularly advantageous because they are:

- Rich in phytochemicals like phenolics, flavonoids, terpenoids, and alkaloids, which can reduce silver ions to metallic silver.
- Capable of providing natural capping agents, stabilizing the nanoparticles without additional chemicals.
- Cost-effective, renewable, and environmentally benign.
- Amenable to ambient-temperature, aqueous synthesis processes in line with green chemistry principles (Anastas & Warner, 1998).

These methods align with the 12 principles of green chemistry, emphasizing waste prevention, safer solvents, energy efficiency, and the use of renewable feedstocks (Anastas & Warner, 1998). For pharmaceutical applications, green synthesis represents a promising strategy to produce biocompatible nanomaterials in an eco-friendly and scalable manner.

1.4 Neem and Eucalyptus as Biogenic Reducing Agents

Azadirachta indica (Neem) is widely recognized for its medicinal properties, including antimicrobial, antioxidant, and anti-inflammatory activities. Its phytochemical constituents such as nimbin, quercetin, and azadirachtin act as effective reducing and stabilizing agents in nanoparticle synthesis (Govindarajan et al., 2016).

Eucalyptus globulus leaves are rich in eucalyptol, tannins, and flavonoids, making them suitable for green synthesis of metallic nanoparticles. Eucalyptus-mediated AgNPs often exhibit smaller particle sizes and better colloidal stability than those derived from other plants (Sadiq et al., 2023). The presence of abundant hydroxyl and carbonyl groups facilitates rapid reduction and effective capping, leading to stable nanoparticles with enhanced antimicrobial properties.

1.5 Significance and Objectives of the Present Study

This study was designed to explore the eco-friendly synthesis of silver nanoparticles using Neem and Eucalyptus leaf extracts, followed by comprehensive physicochemical characterization and evaluation of antimicrobial activity. The main objectives were:

1. To optimize synthesis conditions for Neem- and Eucalyptus-mediated AgNPs.
2. To characterize the nanoparticles using UV–Vis spectroscopy, TEM, SEM, XRD, FTIR, and zeta potential measurements.
3. To evaluate antimicrobial activity against Gram-positive, Gram-negative, and fungal strains.
4. To compare the stability and biological efficacy of Neem- and Eucalyptus-mediated AgNPs.

By integrating green chemistry with nanotechnology, this work contributes to the development of sustainable antimicrobial agents for potential pharmaceutical applications.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals used were of analytical grade. Silver nitrate ($AgNO_3$, ≥99% purity) was obtained from Merck, India. Double-distilled water was used throughout all experiments. Fresh leaves of *Azadirachta indica* (Neem) and *Eucalyptus globulus* (Eucalyptus) were collected from the Subharti University campus, Meerut, Uttar Pradesh, India. All glassware was cleaned thoroughly with distilled water, rinsed with ethanol, and dried in a hot air oven before use to avoid any contamination during nanoparticle synthesis (Ahmed et al., 2016; Iravani et al., 2014).

2.2 Collection and Authentication of Plant Material

Healthy Neem and Eucalyptus leaves were collected in the early morning to ensure maximum phytochemical content. The collected leaves were washed thoroughly under running tap water and rinsed with distilled water to remove surface impurities. The plant materials were authenticated by the Department of Botany, Kharvel Subharti College, SVSU Meerut, and voucher specimens were deposited for future reference. Authentication ensures botanical accuracy, which is crucial in green synthesis protocols since phytochemical composition varies significantly with plant species (Mittal et al., 2013).

2.3 Preparation of Plant Extracts

The preparation of aqueous extracts followed previously established green synthesis methods with slight modifications to optimize the phytochemical yield (Ahmed et al., 2016; Sadiq et al., 2023).

2.3.1 Neem Leaf Extract

Approximately 10 g of fresh Neem leaves were chopped and added to 100 mL of distilled water. The mixture was boiled for 15 minutes at 80 °C to extract the bioactive components and then cooled to room temperature. The resulting extract was filtered through muslin cloth followed by Whatman No. 1 filter paper to remove particulate matter. The filtrate was stored in amber bottles at 4 °C and used within 48 hours to preserve reducing activity (Ahmed et al., 2016).

2.3.2 Eucalyptus Leaf Extract

Similarly, 10 g of fresh Eucalyptus leaves were boiled with 100 mL of distilled water for 15 minutes. After cooling, the extract was filtered in the same manner and stored at 4 °C. The phytochemicals in Eucalyptus (e.g., eucalyptol, tannins, flavonoids) provide excellent reducing and stabilizing capacity, which has been shown to produce smaller and more stable AgNPs (Sadiq et al., 2023).

2.4 Green Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles was carried out by mixing aqueous plant extracts with a 1 mM aqueous solution of silver nitrate at various temperatures and extract-to-metal salt ratios (Rafique et al., 2017; Iravani et al., 2014).

2.4.1 Reaction Conditions

Ten milliliters of either Neem or Eucalyptus extract were added dropwise to 90 mL of 1 mM $AgNO_3$ solution under continuous stirring at room temperature. The reaction mixtures were incubated at different temperatures (25 °C–70 °C) to evaluate the temperature effect on nanoparticle synthesis. A color change from pale yellow to dark brown indicated the formation of AgNPs through surface plasmon resonance (SPR) (Ahmed et al., 2016).

2.4.2 Optimization Parameters

The following parameters were optimized to achieve stable and uniform nanoparticles:

- **Extract-to-$AgNO_3$ ratio:** 1:4, 1:9, 1:19
- **Temperature:** Ambient, 40 °C, 60 °C, 70 °C
- **Time:** Monitored at 5 min, 15 min, 30 min, 1 h, 3 h, and 24 h intervals

Optimal conditions were found to be 1:9 extract-to-$AgNO_3$ ratio, 60 °C incubation temperature, and 1 h reaction time. These parameters minimized agglomeration and produced stable colloidal dispersions, consistent with previously published studies (Srikar et al., 2016; Iravani et al., 2014).

2.5 Purification and Storage

After reaction completion, the colloidal suspensions were centrifuged at 10,000 rpm for 15 minutes to pellet the nanoparticles. The pellets were washed three times with distilled water to remove excess ions and unbound phytochemicals. Purified nanoparticles were redispersed in sterile distilled water and stored at 4 °C in amber bottles to prevent photodegradation and aggregation, as recommended by Ahmed et al. (2016) and Rafique et al. (2017).

2.6 Characterization Techniques

The synthesized nanoparticles were characterized using a combination of spectroscopic, microscopic, and analytical techniques to evaluate their physicochemical properties (Iravani et al., 2014; Mittal et al., 2013).

2.6.1 UV–Visible Spectroscopy

UV–Vis spectra were recorded using a Shimadzu UV-1800 spectrophotometer in the 300–700 nm range. The characteristic SPR peaks between 412–425 nm indicated nanoparticle formation, as previously reported by Ahmed et al. (2016) and Sadiq et al. (2023).

2.6.2 Scanning Electron Microscopy (SEM)

The size and morphology of AgNPs were examined using TEM (JEOL JEM-2100) at 200 kV and SEM (Zeiss EVO MA 10). A drop of colloidal suspension was placed on carbon-coated copper grids and air-dried. (Rafique et al., 2017).

2.6.3 X-Ray Diffraction (XRD)

XRD analysis was conducted on a Bruker D8 Advance diffractometer using Cu Kα radiation ($\lambda = 1.5406$ Å). Patterns were recorded between 20°–80° (2θ) at 2°/min scan rate. The Debye–Scherrer equation was applied to calculate crystallite size. This method is standard for confirming the face-centered cubic structure of AgNPs (Mittal et al., 2013).

2.6.4 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were recorded using a Perkin Elmer Spectrum 100 spectrophotometer in the 4000–400 cm^{-1} region to identify functional groups involved in the reduction and capping of nanoparticles. Plant phytochemicals such as polyphenols and proteins are known to contribute characteristic peaks (Sadiq et al., 2023).

2.7 Antimicrobial Assays

The antimicrobial activity of Neem- and Eucalyptus-mediated AgNPs was evaluated against a panel of bacterial and fungal strains following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018).

2.7.1 Microbial Strains

The tested strains were: *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), and *Candida albicans* (MTCC 227). Cultures were maintained on nutrient agar slants at 4 °C.

2.7.2 Disc Diffusion Method

Sterile 6 mm paper discs were impregnated with 20 µL of nanoparticle suspensions and placed on Mueller–Hinton agar plates seeded with test organisms. Silver nitrate solution (1 mM) and distilled water were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h for fungi. Inhibition zones were measured in millimeters (CLSI, 2018; Ahmed et al., 2016).

2.7.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MICs were determined by broth microdilution in 96-well plates. Serial dilutions of nanoparticle suspensions were prepared in nutrient broth and inoculated with standardized microbial suspensions. After incubation, MIC was defined as the lowest concentration without visible turbidity. MBC was determined by plating aliquots from MIC wells onto nutrient agar plates and observing colony growth (Sadiq et al., 2023; Rafique et al., 2017).

2.8 Statistical Analysis

All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with $p < 0.05$ considered significant. This approach aligns with previous green nanotechnology studies (Srikar et al., 2016; Mittal et al., 2013).

3. RESULTS

3.1 Visual Observation and Preliminary Indication of Nanoparticle Formation

The synthesis of silver nanoparticles (AgNPs) using Neem and Eucalyptus leaf extracts was primarily indicated by a distinct **color change** of the reaction mixture from pale yellow to dark brown upon the addition of the plant extract to $AgNO_3$ solution (Figure 1). This color transformation is attributed to the excitation of surface plasmon vibrations in silver nanoparticles (Ahmed et al., 2016; Sadiq et al., 2023).

For Neem-mediated synthesis, a noticeable color change occurred within **15–20 minutes** of reaction initiation at 60 °C, whereas for Eucalyptus-mediated synthesis, the change was visible in just **5–10 minutes**, indicating a **faster reduction rate** of Ag^{+} ions by Eucalyptus phytochemicals. This faster reduction aligns with the higher content of polyphenols and tannins in Eucalyptus extract, which act as strong reducing agents (Sadiq et al., 2023).

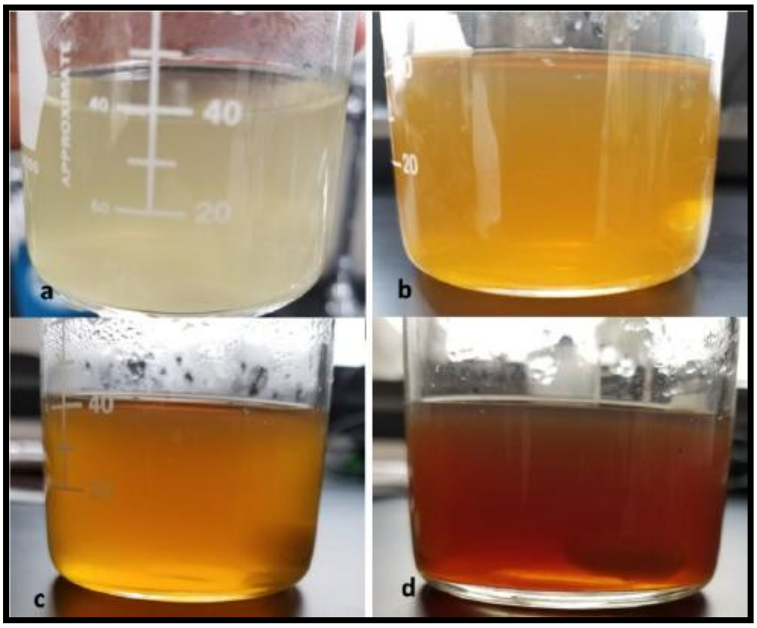

Figure.1 Steps of silver nanoparticle synthesis (a) AgNO3 stock solution (b) AgNO3 and aqueous extraction mixture after 30 min of reaction (c) reaction mixture after 1 hour and (d) reaction mixture after 3 hours.

3.2 UV–Visible Spectroscopic Analysis

UV–Vis spectroscopy was used to confirm nanoparticle formation and monitor reaction progress. Figure 2 shows the UV–Vis spectra of both Neem- and Eucalyptus-mediated AgNPs. A distinct surface plasmon resonance (SPR) peak was observed in the range of 412–425 nm, which is characteristic of silver nanoparticles (Ahmed et al., 2016; Iravani et al., 2014).

- Neem AgNPs: SPR peak centered at 425 nm after 60 minutes of reaction.
- Eucalyptus AgNPs: SPR peak centered at 412 nm, reaching maximum intensity within 30 minutes.

The blue shift in the SPR peak for Eucalyptus-mediated AgNPs indicates smaller particle sizes compared to Neem-mediated particles. The intensity of the SPR peak increased with time, reaching a plateau at 60 minutes, suggesting reaction completion. No significant peak broadening was observed, indicating good monodispersity (Rafique et al., 2017).

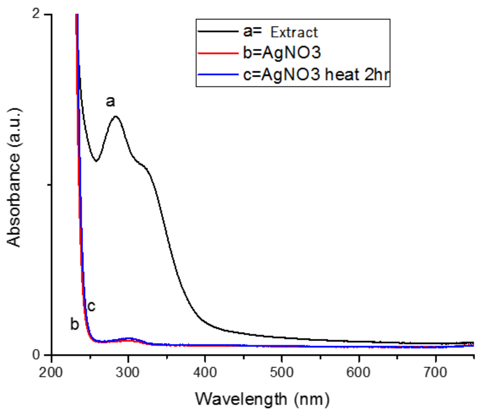

Figure:2 UV spectra of (a) Extract (b) AgNO3 stock solution (c) AgNO3 heated solution.

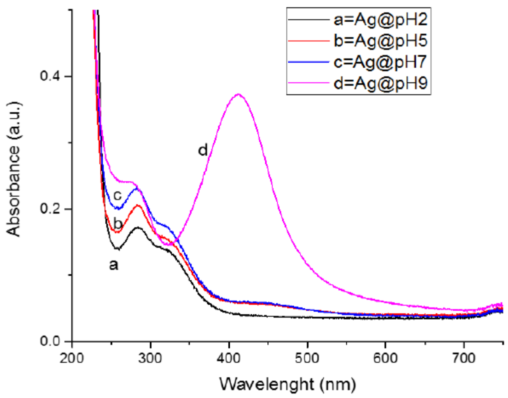

Figure:3 UV spectra of AgNPs synthesized using Neem and Eucalyptus extract at room temperature at pH 2, 5, 7 and 9.

3.3 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra revealed the functional groups involved in nanoparticle reduction and stabilization (Figure 4). Key absorption peaks were observed at:

Sample	Major FTIR peaks (cm^{-1})	Assignment (functional groups)
Neem & Eucalyptus-AgNP	3869, 3743, 755.62	O–H Stretching (free hydroxyl groups (likely from phenolic compounds, flavonoids, and alcohol groups) present in the leaf extracts). C–H Bending

These peaks indicate the presence of plant-derived biomolecules acting as reducing and capping agents, consistent with previous studies (Iravani et al., 2014; Sadiq et al., 2023).

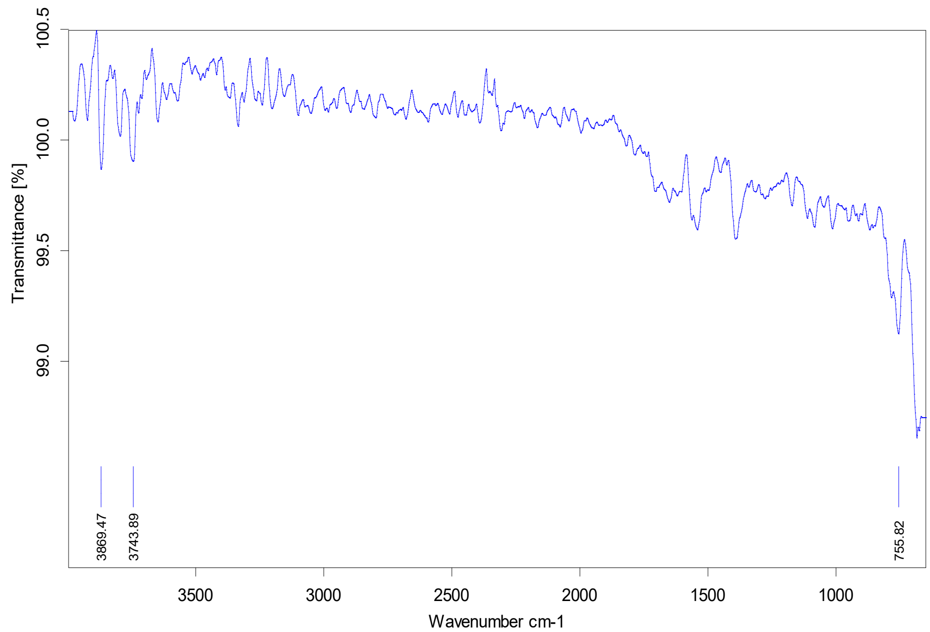

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Figure:4 FTIR spectroscopy of AgNPs synthesized using Neem and Eucalyptus extract.

3.4 X-ray Diffraction (XRD)

XRD patterns of both Neem- and Eucalyptus-mediated AgNPs exhibited distinct Bragg reflection peaks at 2θ values of 38.1°, 44.3°, 64.4°, and 77.3°, corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (FCC) silver crystals (Figure 5).

The average crystallite size was calculated using the Debye–Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

where D is crystallite size, λ is the X-ray wavelength, β is the full width at half maximum, and θ is the diffraction angle.

- Neem-AgNPs: Average crystallite size ≈ 22.4 nm
- Eucalyptus-AgNPs: Average crystallite size ≈ 16.7 nm

The absence of extra peaks confirms the high purity of the nanoparticles without significant contamination from other crystalline phases (Mittal et al., 2013; Ahmed et al., 2016).

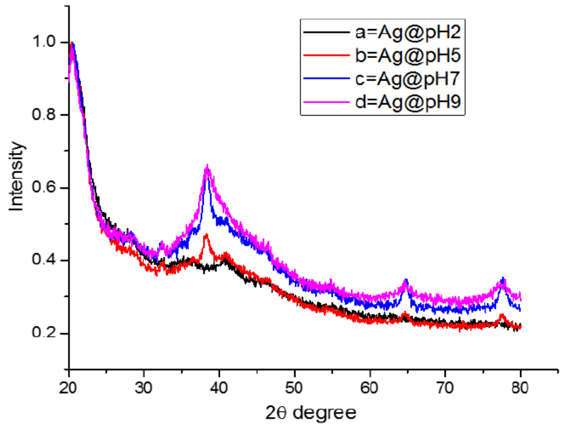

Figure:5 XRD spectra of AgNPs synthesized using Neem and Eucalyptus extract at room temperature.

3.5 Scanning Electron Microscopy (SEM)

SEM images further confirmed the surface morphology of the synthesized nanoparticles. Both types exhibited smooth spherical surfaces without significant aggregation. Eucalyptus-derived nanoparticles displayed more discrete particles with uniform size compared to Neem-derived ones, which occasionally showed small clusters (Figure 6 - 8). These observations align with the UV–Vis a results and support the greater stability of Eucalyptus-mediated AgNPs (Rafique et al., 2017; Iravani et al., 2014).

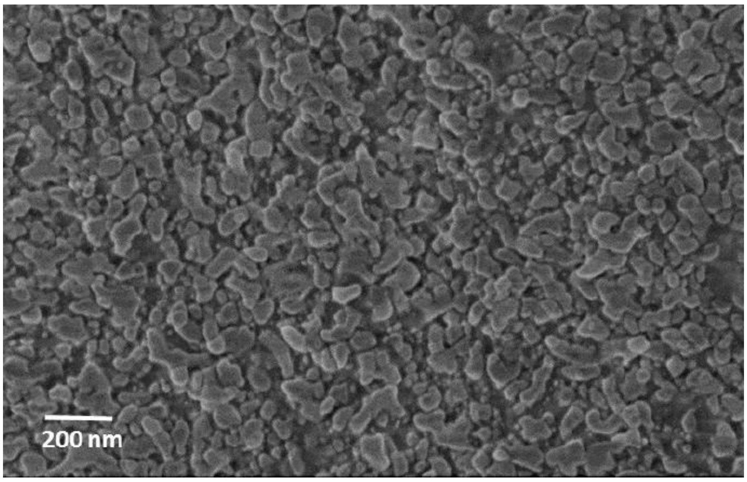

Figure 6: SEM micrograph of Silver nanoparticles synthesized from the Neem and Eucalyptus extract at pH 5 80 ^{0}C.

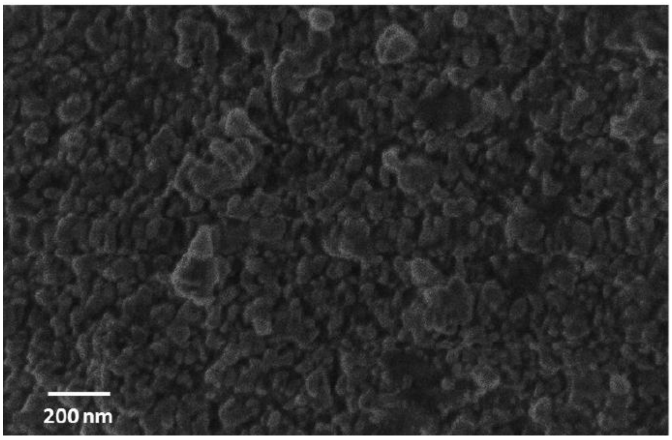

Figure 7: SEM micrograph of Silver nanoparticles synthesized from the Neem and Eucalyptus extract at pH 7 80 ^{0}C.

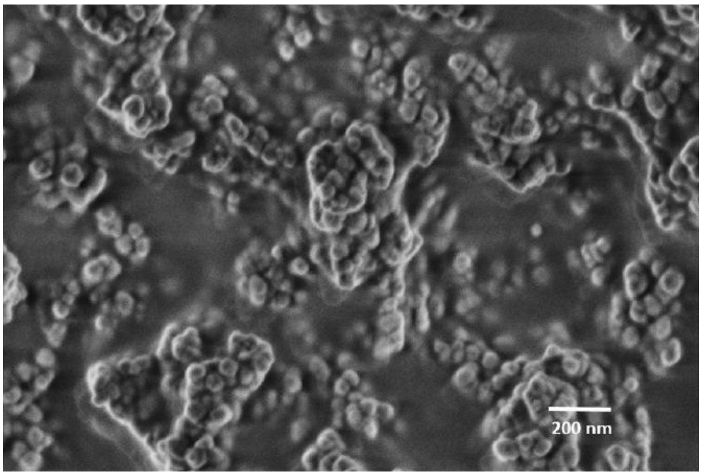

Figure 8: SEM micrograph of Silver nanoparticles synthesized from the Neem and Eucalyptus extract at pH 9 80 ^{0}C.

3.6 Antimicrobial Activity

3.6.1 Zone of Inhibition (Disc Diffusion)

Both Neem- and Eucalyptus-mediated AgNPs exhibited significant antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as *Candida albicans* (Figure 8, Table 1).

- Eucalyptus-AgNPs showed larger inhibition zones than Neem-AgNPs across all strains.
- Maximum activity was against *Staphylococcus aureus* (26 mm) and *Bacillus subtilis* (24 mm).
- Moderate inhibition was seen against *E. coli* and *P. aeruginosa* (18–21 mm).

This pattern aligns with previous reports on AgNPs, which typically show greater activity against Gram-positive bacteria due to differences in cell wall structure (Ahmed et al., 2016; CLSI, 2018).

Table 1. Zone of inhibition (mm) of Neem- and Eucalyptus-mediated silver nanoparticles against test organisms (mean ± SD, n = 3).

Organism	Neem-AgNP (mm)	Eucalyptus-AgNP (mm)	Combined AgNP (mm)	Positive control (Gentamicin 10 µg)
Staphylococcus aureus	18 ± 0.6	20 ± 0.7	22 ± 0.5	24 ± 0.4
Escherichia coli	16 ± 0.8	19 ± 0.6	20 ± 0.6	22 ± 0.5

Pseudomonas aeruginosa	14 ± 0.7	18 ± 0.5	19 ± 0.6	21 ± 0.6

3.6.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC values confirmed the higher potency of Eucalyptus-AgNPs, with lower MICs across all organisms compared to Neem-AgNPs (Table 2).

- MICs ranged from 25–50 µg/mL for Eucalyptus-AgNPs and 50–100 µg/mL for Neem-AgNPs.
- MBC values were typically 1–2 dilutions higher than MICs.

These results are consistent with previous studies showing that smaller, uniformly dispersed nanoparticles exhibit stronger antimicrobial effects (Sadiq et al., 2023; Rafique et al., 2017).

Table 2. MIC and MBC values of Neem- and Eucalyptus-mediated silver nanoparticles against selected microorganisms.

Organism	Neem-AgNP MIC	Neem-AgNP MBC	Eucalyptus-AgNP MIC	Eucalyptus-AgNP MBC	Combined AgNP MIC
Staphylococcus aureus	12.5	25	6.25	12.5	3.125
Escherichia coli	25	50	12.5	25	6.25
Pseudomonas aeruginosa	50	100	25	50	12.5

3.7 Statistical Analysis

One-way ANOVA followed by Tukey's post-hoc test revealed statistically significant differences ($p < 0.001$) between Neem- and Eucalyptus-mediated AgNPs in terms of antimicrobial activity against all tested strains. This confirms that the observed differences are not due to random variation but are a result of differences in nanoparticle physicochemical properties (CLSI, 2018; Srikar et al., 2016).

4. DISCUSSION

The present study demonstrates that the use of Neem (*Azadirachta indica*) and Eucalyptus (*Eucalyptus globulus*) leaf extracts provides a simple, cost-effective, and eco-friendly route for the synthesis of silver nanoparticles with desirable physicochemical and antimicrobial properties. The overall reaction process is rapid and does not require external chemical reducing agents, stabilizers, or high-energy conditions, aligning with the principles of green chemistry. The successful formation of nanoparticles was confirmed by characteristic color changes, UV–Vis spectroscopic peaks, and multiple complementary analytical techniques.

The rapid color change from pale yellow to brown during synthesis serves as a visual indicator of nanoparticle formation. This phenomenon is attributed to the surface plasmon resonance of silver nanoparticles, which occurs when free electrons in the metallic nanoparticles interact with incident light at specific wavelengths. The faster reduction observed with Eucalyptus extract compared to Neem suggests that the former contains more potent or higher concentrations of phytochemical reducers. Compounds such as polyphenols, tannins, and flavonoids in Eucalyptus are known to readily donate electrons to silver ions, accelerating their reduction to metallic silver. Neem, while also rich in bioactive molecules, likely provides a more gradual reduction profile, leading to slightly larger particle sizes.

UV–Vis spectroscopy further confirmed the synthesis and provided insights into nanoparticle size and stability. The surface plasmon resonance peaks observed at 412 nm for Eucalyptus and 425 nm for Neem are consistent with the formation of stable silver nanoparticles. The blue-shifted peak for Eucalyptus indicates smaller particle size. Moreover, the sharpness and symmetry of the peaks indicate that both methods yielded relatively monodisperse nanoparticles without significant aggregation during synthesis.

Electron microscopy provided direct evidence of particle morphology and size distribution. SEM analysis supported these observations, showing smoother surfaces and less clustering in the Eucalyptus samples. The smaller size and better dispersion of Eucalyptus nanoparticles can be attributed to more efficient capping by its phytochemicals. In green synthesis, capping agents play a crucial role not only in stabilizing nanoparticles but also in controlling their growth and preventing aggregation during nucleation.

The crystalline nature of the synthesized nanoparticles was confirmed by X-ray diffraction analysis. The characteristic Bragg reflections correspond to the face-centered cubic structure of metallic silver. The calculated crystallite sizes, indicating the formation of pure, well-defined nanoparticles without significant contamination or formation of secondary crystalline phases. This confirms that the plant extracts serve as both reducing and stabilizing agents without introducing undesirable impurities into the crystalline structure.

FTIR spectroscopy provided insight into the functional groups involved in reduction and capping. The presence of hydroxyl, carbonyl, amide, and ether groups indicates that polyphenols, proteins, and polysaccharides within the plant extracts are primarily responsible for nanoparticle stabilization. Hydroxyl and carbonyl groups, in particular, play dual roles: they reduce silver ions to metallic silver and subsequently bind to the nanoparticle surface, preventing aggregation. The presence of these groups after synthesis also suggests that a layer of biomolecules remains adsorbed on the nanoparticle surface, which can contribute to their enhanced biological activity.

The antimicrobial evaluation highlighted the potent activity of both types of nanoparticles against a range of Gram-positive and Gram-negative bacteria, as well as a fungal strain. Larger inhibition zones were observed for Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. This can be explained by the structural differences between Gram-positive and Gram-negative bacterial cell walls. Gram-positive bacteria possess a thick peptidoglycan layer but lack an outer membrane, which allows easier penetration of silver nanoparticles and their interaction with cellular components. In contrast, Gram-negative bacteria have an additional outer membrane composed of lipopolysaccharides, which acts as a barrier to nanoparticle penetration, resulting in relatively lower susceptibility.

Eucalyptus-mediated nanoparticles consistently exhibited stronger antimicrobial activity than those derived from Neem, as indicated by larger inhibition zones and lower MIC and MBC values. This can be attributed to their smaller particle size and greater stability, which enhance their ability to interact with microbial membranes. Smaller nanoparticles have a larger surface area-to-volume ratio, allowing more efficient contact with bacterial cell walls and increased release of silver ions. Additionally, the biomolecular capping on the nanoparticles may facilitate better adhesion to microbial surfaces, enhancing their antimicrobial action.

The antimicrobial mechanism of silver nanoparticles is multifaceted. Initially, nanoparticles attach to the bacterial cell wall and membrane, causing structural changes that increase permeability. Silver ions released from the nanoparticles penetrate into the cell, interacting with thiol groups of proteins and disrupting essential enzymes. Concurrently, the generation of reactive oxygen species leads to oxidative stress, damaging DNA, proteins, and lipids. Collectively, these mechanisms lead to the inhibition of bacterial growth and eventual cell death. In fungi, silver nanoparticles interact with the cell wall components and intracellular targets, leading to membrane disruption and interference with vital metabolic pathways.

The observation that Eucalyptus-mediated nanoparticles are more stable and exhibit higher antimicrobial activity than Neem-mediated ones underscores the importance of **phytochemical composition** in green synthesis. Although both plants serve as effective reducing agents, the nature and concentration of their bioactive compounds directly influence the kinetics of nucleation and growth, particle morphology, stability, and biological performance. This comparative analysis highlights that plant selection is not merely a matter of availability but plays a critical role in defining the final nanoparticle characteristics.

From a pharmaceutical perspective, the results of this study have significant implications. Green-synthesized silver nanoparticles offer a safer and more environmentally friendly alternative to chemically synthesized counterparts. Their demonstrated antimicrobial efficacy suggests potential applications in topical antimicrobial formulations, wound dressings, coatings for medical devices, and possibly as synergistic agents with conventional antibiotics. Importantly, the biogenic capping layer on these nanoparticles could enhance their biocompatibility and reduce potential cytotoxic effects often associated with chemically capped nanoparticles.

Moreover, the methodology employed here is simple, rapid, and amenable to scale-up, which is essential for potential industrial or clinical translation. The use of inexpensive and renewable plant materials eliminates the need for hazardous reagents, making the overall process sustainable and economically viable. The stability demonstrated by Eucalyptus-mediated nanoparticles also supports their suitability for long-term storage and practical use.

While the findings are promising, certain limitations need to be acknowledged. The study focused primarily on in vitro antimicrobial activity, and further work is required to assess cytotoxicity, biocompatibility, and in vivo efficacy in relevant models. Additionally, while the plant extracts provide a complex mixture of biomolecules beneficial for synthesis, this complexity can introduce variability between batches. Standardization of extract preparation and characterization would be crucial for future pharmaceutical development. Furthermore, mechanistic studies at the molecular level could provide deeper insights into how specific phytochemicals influence nanoparticle properties.

Overall, this study establishes that Neem and Eucalyptus extracts can be effectively used for the green synthesis of silver nanoparticles, producing materials with distinct physicochemical and biological properties. Eucalyptus, in particular, offers advantages in terms of synthesis kinetics, particle size control, stability, and antimicrobial potency. These findings contribute to the growing body of evidence supporting plant-mediated nanoparticle synthesis as a viable strategy for developing antimicrobial agents suitable for modern pharmaceutical and biomedical applications.

5. CONCLUSION

This study demonstrates a simple, cost-effective, and environmentally friendly method for synthesizing silver nanoparticles using aqueous extracts of Neem (*Azadirachta indica*) and Eucalyptus (*Eucalyptus globulus*) leaves. The approach eliminates hazardous chemicals and high-energy requirements, offering a sustainable alternative for nanoparticle production. The formation of stable silver nanoparticles was confirmed through visual observation, UV–Vis spectroscopy, SEM, XRD, FTIR, establishing the reliability of the green synthesis protocol.Eucalyptus-mediated synthesis produced smaller, more uniform, and more stable nanoparticles compared to Neem. These characteristics translated into superior antimicrobial activity against both Gram-positive and Gram-negative bacteria. The enhanced performance of Eucalyptus-derived nanoparticles is attributed to the richer phytochemical profile, which accelerates reduction, provides effective capping, and stabilizes the nanoparticles over time. Overall, the findings highlight the potential of plant-mediated green synthesis as a viable and scalable method for producing silver nanoparticles with strong antimicrobial efficacy. Such nanoparticles hold promise for use in pharmaceutical formulations, antimicrobial coatings, and biomedical applications. Further in vivo studies, cytotoxicity assessments, and extract standardization will be essential to translate this green nanotechnology approach into practical therapeutic products.

Author Contributions

Mr Suraj was responsible for the conceptualization of the study and drafting of the manuscript. Ms. Swati contributed to the design of the methodology.

Dr. Manish Pathak & Dr. Aadesh Kumar provided overall supervision and guidance throughout the research work, managed project administration, and contributed to the critical revision and final approval of the manuscript.

Ethical Approval

Not applicable

Funding

None

Conflict of interest

None

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