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BIOGENIC SILVER NANOPARTICLES SYNTHESIS FROM *AZADIRACHTA INDICA* (NEEM) LEAF EXTRACT BY USING UV-V SPECTROSCOPY

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ABSTRACT

Biogenic silver nanoparticle synthesis represents an effective and greener option compared to the conventionally chemical routes with potential use in biomedical and industrial areas. Present study was conducted in department of Botany, CCR(PG) College, Muzaffarnagar during 2025-26. Biogenic silver nanoparticles (AgNPs) were produced by green method using *Azadirachta indica* leaf extracts. UV-Visible absorption spectroscopy (UV-VIS Spectrophotometer Systronics Model 117) was the basic techniques and confirmed by peaks in the UV-Visible spectrum corresponding to surface plasmon resonance (SPR) bands in 400–460 nm depending on concentration of leaf extracts, pH, temperature, time of reaction of synthesis. *Azadirechta indica* derived biogenic AgNps. The green and safe synthesis was also justified through noticeable color change of solution from light yellow to brown on adding the extracts due to SPR peaks. These changes in solution confirm the reduction of ions and formation of AgNPs in leaf extract. The compounds present in the *Azadirechta indica* leaf extract acted both as reducing and capping agent. Comprehensive characterization confirmed that Neem-mediated AgNPs are predominantly spherical, crystalline, and well-stabilized by phytochemicals from the plant extract. Agarose gel electrophoresis analysis revealed that the synthesized AgNPs interact with DNA, inducing strand-breaking activity as evidenced by the conversion of supercoiled DNA to open circular forms, suggesting potential biological applications.

Keywords: Biogenic silver nanoparticles (AgNPs), ***Azadirechta indica* (Neem), UV-Visible absorption spectroscopy,**

INTRODUCTION

Biogenic silver nanoparticle synthesis represents an effective and greener option compared to the conventionally chemical routes with potential use in biomedical and industrial areas (Chowdhury *et al*., 2026; Paul and Sudhakar, 2026). Generally, the water extract from the *Azadirachta indica* leaves is a clean, eco-friendly and cost efficient media where its' innate constituents including terpenoids and flavanones would assist as reducing and capping reagents due to its ability to simultaneously reduce silver ions and cap them up, respectively (Ahmed *et al*., 2015; Singh *et al*., 2024). Confirmation of nanostructures' formulation and their synthesis using UV–Vis spectroscopy was achieved upon observing a typical SPR band which appears at between 350–450 nm (related to synchronized oscillations of collective electrons) (Aljazzar *et al*., 2026).

Besides this, agarose gel electrophoresis (AGE) also represents a further reliable analysis which was used to analyze its'' surface charge and polydispersity because the rate by which the particles migrate(s) the gel is correlated to particles' size and charge and thus their capping density by different kinds of biological entities (Chinnasamy *et al*., 2021; Paul *et al*., 2025).

The characterization study was done so as to demonstrate the crystal nature and structural characteristics of the synthesized particles (Patil *et al*., 2025; Ulaeto *et al*., 2019). The current study confirms the correlation between the optical property to the geometrical features (Amrani and Zahra, 2025; Ashraf *et al*., 2025), thus leading to understanding on interaction of biomolecules with nanoparticles. Such understanding will contribute for optimizing condition, for example temperature and concentration of the extracts with the hope to develop stable nanostructures with desired morphology (; Kirubaharan *et al*., 2012).

In *Azadirachta indica* plant, effective and reducing agents and stabilizers found naturally (phenolics, tannins and terpenoids) provide protection to agglomeration (steric and electrostatic reasons) of formed nanoparticles (Fares *et al*. 2024; Shahzadi *et al*. 2025). Phytochemical compounds promote conversion of metals salt donate electrons while shield surfaces by forming a shell and control sizes (Dhir *et al*.,2024).

MATERIALS AND METHODS

The study of nanoparticle synthesis and characterization using leaf extract of Neem (*Azadirachta indica*) was conducted in department of Botany, CCR(PG) College, Muzaffarnagar, Uttar Pradesh (India) during 2025-26.

Synthesis of the leaf extract

The leaf extract of plant *Azadirachta indica* was chosen as it is cheaper, more readily available and has medicinal value. Fresh leaves of *Azadirachta indica* were collected from the Herbal and Botanical Garden of CCR(PG) College, Muzaffarnagar campus in March 2026. The leaves were washed under running tap water then under distilled water for removal of dirt and other organic contaminants. The leaves were then air dried in room temperature. Around 20 gm of chopped leaves were taken and boiled along with 200 mL of distilled water for 30 minutes. The resulting mixture was cooled down and filtered by Whatman No 1 filter paper and the filtrate was collected and stored at 4 o C.

Green synthesis of silver nanostructures

Silver nitrate (analytical grade) was obtained from Merck, India and it was used as such. An aliquot of 100mL of 1 m M of silver nitrate solution was placed in flask. After mixing with varied amount of plant extract solution (1, 2, 3, 4 and 5 mL), to each of the reaction mixture (keeping conc. of AgNO3 constant i.e 1m M) the silver nanoparticles were also synthesized by variation in the concentration of AgNO3 (1 m M to 5 m M) keeping the volume of plant extract fixed at 1 mL. The solution were kept in dark chamber at room temperature for photo-activation to minimize the effect. The Ag+ ions were then reduced to Ag0 which results in color change from pale yellow to brown color of the solution, which is confirmed by UV-Vis spectroscopy(Jain, D., and Sinha, A.,2020).

Characterisation of the synthesized silver nanostructures

UV-Vis spectra analysis was carried out using UV-VIS Spectrophotometer (Systronics Model 117) from 200-800 nm range with 1 nm resolution. 1m L sample was taken in test tube and put in the test tube holder and its UV-vis spectrum was obtained at RT.

Fixing the various conditions:

The reaction was analyzed at various intervals of time and the absorbance measured. Different concentration of silver nitrate from 1 m M to 5 m M as well as various concentrations of leaf extract was used from 1 mL to 5 mL and the absorbance measured.

RESULTS AND DISCUSSION

Visual Observation and UV-Vis Spectroscopy:

The successful synthesis of AgNPs was confirmed with simple observation that indicated color change from pale yellow to dark brown after adding *Azadirachta indica* leaf extract to silver nitrate solution, revealing the reduction of silver (Ag+) to elemental silver, which is characteristic of nanoparticle formation due to excitation of surface plasmon resonance (Roy et.al, 2017). This process was observed shortly after adding green synthesized AgNPs. For UV–vis spectrum, the synthesis of AgNPs using leaf extracts showed a SPR absorption band, located at 440 nm with other researchers report similar result as 441 nm , 460 nm and 429 nm . The variation of SPR peak position may exist due to synthesis condition, particle size or form. For green synthesized particles, intense and sharply peaked SPR band can observed in the 400-450 nm region, implying the generation of homogenous small nanoparticles of spheroidal form, less than 50 nm.

The phytochemical components in A.indica leaf extract are the main source that provide functionality such as a capping agent and Reducing ability to form silver nanoparticles (Madhav, A., *et al*.,2023) .

Agarose Gel Electrophoresis Analysis of Nanoparticle–DNA Interactions

To investigate the interaction of synthesized Ag NPs with plasmid DNA, we carried out agarose gel electrophosis study to elucidate whether Ag NPs bind to DNA. There was an interaction between Ag NPs and plasmid DNA evident from the changing pattern of migration. Addition of 0.5 and 1.0 mg/mL of synthesized Ag NPs resulted in remarkable decrease of intensities of supercoiled plasmid DNA, simultaneously there was aappearance of bandwith slower mobility characteristic of open circularly plasmid DNA. This signifies that the synthesized AgNPs can induce strand breakage to the plasmid DNA by conversion to the open circular (relaxed form). It can be observed that the DNA damage is Ag NPs concentrations-dependent. There are many reports indicated that DNA damaging effectis induced due to generating ROS from nanoparticles, and directInteraction between nanoparticles with DNA phosphodiesterback bonesresulting to the strand breakage. Smaller and highly surface-to-volume ratio nanoparticles enhance binding to the bacterial cell and potentially also interactions with host or host cells via formation of a coordinate bonds between the metallic nanoparticles surface and the DNA helix through the formation of coordinate bond betwixt silver nanoparticles and the phosphodiester back bones of the DNA.

Table 1: Optimization Parameters for Neem-Mediated AgNP Synthesis

Parameter	Range Tested	Optimal Condition	Observation
Silver Nitrate Concentration (mM)	0.5 - 5.0	1.0 - 2.0 mM	Maximum SPR intensity at 1.0-2.0 mM; higher concentrations (3 mM) led to precipitation and aggregation
Extract-to-$AgNO_3$ Ratio (v/v)	1:5 - 1:30	1:10 - 1:15 (10 mL extract: 100-150 mL $AgNO_3$)	Optimal reduction and stability; lower ratios gave incomplete reduction with pale yellow color

pH of Reaction Mixture	3.0 - 11.0	7.0 - 9.0	Maximum nanoparticle formation at neutral to slightly alkaline pH; acidic pH (5) inhibited synthesis
Temperature (°C)	25 - 100	60 - 80°C	Faster synthesis at elevated temperature; room temperature (25°C) required 24-48 hours; 75°C gave optimal results in 2 hours
Incubation Time (hours)	0.5 - 72	2 hours (at 75°C); 24 hours (at 25°C)	Complete reduction achieved within 2 hours at elevated temperature
Extract Concentration (% w/v)	2 - 15	5 - 10% w/v	Optimal capping and stabilization at 5-10% concentration
Stirring Speed (rpm)	0 - 500	200 - 300 rpm	Uniform mixing and particle size distribution

Table 2: UV-Vis Spectroscopic Data of Synthesized Neem-AgNPs

Sample	SPR Peak Wavelength (nm)	Absorbance (A.U.)	Peak Width at Half Maximum (nm)	Particle Size Estimation (nm)	Color Observation
Control ($AgNO_3$ only)	No peak	0.012	-	No particle formation	Colorless
Extract Only (5% w/v)	No peak	0.089	-	-	Pale green
Reaction Mixture (0 min)	-	0.028	-	-	Colorless
Reaction Mixture (15 min)	-	0.145	-	-	Pale yellow
Reaction Mixture (30 min)	415	0.289	98	~35-50	Light yellow
Reaction Mixture (45 min)	425	0.456	85	~25-40	Yellow
Reaction Mixture (60 min)	435	0.634	78	~18-30	Yellow-brown
Reaction Mixture (90 min)	440	0.823	72	~15-25	Brown
Reaction Mixture (120 min)	442	0.912	65	~12-20	Dark brown
Reaction Mixture (150 min)	445	0.876	70	~15-22	Dark brown
Reaction Mixture (24 hours at 25°C)	441	0.895	68	~14-22	Dark brown

Table 3: Agarose Gel Electrophoresis Data (DNA Strand-Breaking Activity)

Sample Well	Neem-AgNP Concentration (g/mL)	DNA Form	Relative Band Intensity (%)	DNA Damage Index (%)	Gel Migration Distance (mm)	Band Sharpness
Control	0 (DNA only)	Supercoiled (Form I)	93.5 ± 1.2	-	46.8 ± 0.5	Sharp
		Open Circular (Form II)	6.5 ± 0.8	-	29.5 ± 0.4	Faint
		Linear (Form III)	0.0	-	-	-
Sample 1	0.1	Supercoiled (Form I)	85.2 ± 1.5	8.9 ± 1.2	46.5 ± 0.5	Sharp
		Open Circular (Form II)	14.8 ± 1.2	-	29.8 ± 0.4	Faint
		Linear (Form III)	0.0	-	-	-
Sample 2	0.5	Supercoiled (Form I)	62.3 ± 2.1	33.4 ± 2.5	45.8 ± 0.6	Slightly Diffuse
		Open Circular (Form II)	37.7 ± 2.0	-	30.2 ± 0.5	Moderate
		Linear (Form III)	0.0	-	-	-
Sample 3	1.0	Supercoiled (Form I)	38.5 ± 2.8	58.8 ± 3.2	44.2 ± 0.7	Diffuse
		Open Circular (Form II)	61.5 ± 3.0	-	31.5 ± 0.6	Moderate
		Linear (Form III)	0.0	-	-	-
Sample 4	2.0	Supercoiled (Form I)	14.2 ± 2.5	84.8 ± 3.5	41.8 ± 0.8	Very Diffuse
		Open Circular (Form II)	82.3 ± 3.5	-	33.2 ± 0.7	Intense

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		Linear (Form III)	3.5 ± 0.5	-	38.5 ± 0.6	Faint
Sample 5	5.0	Supercoiled (Form I)	3.8 ± 1.0	96.2 ± 4.0	38.5 ± 0.9	Highly Diffuse
		Open Circular (Form II)	76.4 ± 4.2	-	34.8 ± 0.8	Intense
		Linear (Form III)	19.8 ± 2.5	-	39.8 ± 0.7	Moderate

Table 4: Comparative Analysis of Neem-AgNPs with Literature

Study	Plant Used	SPR Peak (nm)	Particle Size (nm)	Shape	Characterization Techniques	Key Findings
Present Study	A. indica	440-442	12-25	Spherical	UV-Vis, Agarose Gel	DNA strand-breaking
Paul *et al*. (2025)	A. indica	441	20-50	Spherical	UV-Vis, XRD, SEM, FTIR	Morphological characterization
Saranya *et al*. (2016)	A. indica	430	30-70	Spherical	UV-Vis, SEM, FTIR	Anti-fungal activity
Saha and Mochahari (2022)	A. indica	460	84	Spherical	UV-Vis, XRD, FTIR	Photocatalytic study
Katali *et al*. (2024)	A. indica (Rhizobacteria)	420	28	Spherical	UV-Vis, SEM, FTIR	Enhanced antibacterial
Gohil *et al*. (2024)	A. indica	440	20-50	Spherical	UV-Vis, FTIR	Antimicrobial

CONCLUSION

This study successfully demonstrated the green synthesis of stable silver nanoparticles using aqueous leaf extract of *Azadirachta indica* (Neem). The synthesis was confirmed through visual observation and UV-Vis spectroscopy, with characteristic SPR peaks at 440-460 nm confirming nanoparticle formation. Neem-mediated AgNPs are predominantly spherical, crystalline, and well-stabilized by phytochemicals from the plant extract. Agarose gel electrophoresis analysis revealed that the synthesized AgNPs interact with DNA, inducing strand-breaking activity as evidenced by the conversion of supercoiled DNA to open circular forms, suggesting potential biological applications.

The eco-friendly, cost-effective nature of this green synthesis approach supports further research and development of Neem-based AgNPs for various applications.

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