

Original Research Article

Evaluation of Medicinal Properties of *Azadirachta indica* Extracts and Their Biosynthesized Silver Nanoparticles

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Abstract

Purpose: Harnessing the therapeutic wealth of medicinal plants through nanotechnology has emerged as a powerful frontier in drug discovery. This study explores the medicinal properties of *Azadirachta indica* (Neem) leaf extracts and their biosynthesized silver nanoparticles, bridging traditional knowledge with modern biomedical innovation.

Methods: Aqueous and ethanolic leaf extracts of *A. indica* were prepared and analyzed for proximate composition, mineral content, and key phytochemicals using standard procedures. Antioxidant activity was assessed by DPPH, nitric oxide, FRAP, and TBARS assays. Silver nanoparticles were synthesized using the aqueous extract and monitored by UV-Visible and Fourier-transform infrared (FTIR) spectroscopy. Antibacterial activity of the extracts and biosynthesized AgNPs was evaluated using the disk diffusion method.

Results: Proximate analysis indicated that *A. indica* leaves are nutritionally dense, with high protein (25.35%) and carbohydrate (33.32%) content, and moderate levels of crude fat (10.20%) and fiber (9.74%). Mineral profiling revealed appreciable amounts of essential elements such as magnesium, calcium, potassium, iron, and zinc. Phytochemical evaluation showed that the extracts contained flavonoids, steroids, saponins, and phenolic constituents. Antioxidant screening demonstrated efficient scavenging of DPPH and nitric oxide radicals, along with notable ferric reducing antioxidant power. The aqueous extract displayed measurable antibacterial activity, whereas the biosynthesized silver nanoparticles produced markedly stronger antibacterial effects. FTIR analysis confirmed the involvement of hydroxyl, carbonyl, and amine functional groups in AgNP formation.

Conclusion: These findings show that *A. indica* possesses rich bioactive constituents and effectively mediates AgNP synthesis, producing nanoparticles with enhanced antibacterial activity.

Keywords: Antibacterial activity; Silver nanoparticles (AgNPs); Antioxidant activity; Natural products; *Azadirachta indica*

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INTRODUCTION

Medicinal plants have long been recognized as valuable reservoirs of bioactive compounds with significant therapeutic potential. Among these, *Azadirachta indica* A. Juss. (neem), a tree native to the Indian subcontinent and widely cultivated in Africa, has attracted considerable attention due to its broad spectrum of pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities.¹ Its leaves, bark, seeds, and flowers contain abundant bioactive compounds such as flavonoids, alkaloids, tannins, terpenoids, and phenolic constituents, which act synergistically to account for its broad medicinal effectiveness.² Notably, nimbin, nimbidin, and nimbidol are regarded as the principal bioactive constituents responsible for the therapeutic properties of neem leaves.³

Experimental evidence supports these findings. Ola⁴ demonstrated that neem leaf extract effectively countered oxidative stress and protected hepatic tissue from toxin-induced damage. Similarly, Alzohairy⁵ reported hepatoprotective effects of neem leaves against drug-induced liver injury, attributing the activity to their potent free-radical-scavenging capacity.

In addition to hepatoprotection, several studies have documented the antibacterial potential of neem leaves. Effiong⁶ showed significant inhibitory activity of neem leaf extract against pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*. Supporting these results, another study⁷ reported strong antibacterial activity against oral pathogens, including *Streptococcus mutans* and *Porphyromonas gingivalis*. Neem leaves have also been traditionally employed for their antidiabetic effects. In an experimental study, researchers⁸ observed significant reductions in blood glucose levels and improved insulin sensitivity in diabetic rats following treatment with neem leaf extract. Furthermore, another investigation⁹ highlighted the effectiveness of neem leaves against drug-resistant bacterial strains, reinforcing their relevance as natural alternatives to synthetic antibiotics.

More recently, the integration of nanotechnology and phytochemical research has gained increasing attention as an effective strategy for improving the biological performance of plant-based compounds.¹⁰ Among various nanomaterials, biosynthesized metallic nanoparticles—especially silver nanoparticles (AgNPs)—are of particular interest due to their distinctive physicochemical characteristics, including large surface-area-to-volume ratios and enhanced interaction with

biological membranes. These features significantly strengthen the antimicrobial, antioxidant, and therapeutic activities of plant extracts.^{11,12} Furthermore, the fabrication of nanoparticles through plant-mediated routes provides an environmentally benign, economically viable, and sustainable substitute for traditional chemical synthesis methods while minimizing the use of toxic reagents¹³. Although the medicinal properties of *Azadirachta indica* and the biomedical potential of silver nanoparticles have been widely documented, relatively few studies have investigated the combined evaluation of neem leaf extracts and their biosynthesized AgNPs. Understanding this interaction may provide valuable insights into developing natural, plant-based antimicrobial agents and enhancing therapeutic efficacy. This study therefore investigates the medicinal properties of *A. indica* leaf extracts and their biosynthesized silver nanoparticles, with particular emphasis on their antibacterial activities.

MATERIALS AND METHODS

Fresh leaves of *Azadirachta indica* were collected from Ekiti State University (EKSU), Ado-Ekiti, Nigeria, on 02/02/2023 (GPS coordinates: 7.7181° N, 5.2793° E). The collected plant material was verified at the Herbarium of the Department of Plant Science, Faculty of Science, Ekiti State University, Ado-Ekiti, Nigeria, where it was deposited as a reference specimen. Taxonomic identification was performed and confirmed by Mr. Omotayo, Chief Technologist, and assigned voucher number UHAE 2023019. The collected leaves were washed thoroughly, air-dried in the open laboratory, crushed, and subsequently ground into fine powder using a Marlex Excella laboratory blender.

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and were used without further purification.

Preparation of plant extract

Twenty grams of each plant leaf sample were weighed and blended in 100 mL of distilled water and filtered to obtain the aqueous solution which was used for the determination of the various parameters

Phytochemical Screening

The aqueous extract of *Azadirachta indica* leaves was screened for the presence of secondary metabolites using standard phytochemical tests as

described by Harborne (1973) and Trease and Evans (1996). The extract was qualitatively tested for saponins, tannins, flavonoids, steroids, and phenols.

Antioxidant assay

The antioxidant activities of the *Azadirachta indica* extracts were evaluated using established colorimetric assays with slight modifications. DPPH radical scavenging activity was assessed following Brand-Williams et al.¹⁴ using extract concentrations of 100 and 200 µg/mL, with absorbance measured at 516 nm. Nitric oxide radical scavenging activity was determined according to the method of Green et al.¹⁶ with absorbance recorded at 520 nm. Ferric reducing antioxidant power (FRAP) was evaluated following the procedure of Benzie and Strain¹⁷, and absorbance was measured at 593 nm. Lipid peroxidation inhibition was assessed using the TBARS method of Ohkawa et al.¹⁵ with absorbance measured at 532 nm. Total phenolic content was determined using the Folin–Ciocalteu method of Singleton et al.¹⁸ and expressed as gallic acid equivalents with absorbance at 765 nm. Total flavonoid content was evaluated using the aluminum chloride colorimetric method of Zhishen et al.¹⁹ with absorbance measured at 510 nm.

Proximate analysis of *Azadirachta indica*

Proximate composition of the dried powdered leaf samples was determined using standard procedures recommended by the Association of Official Analytical Chemists.²⁰ Moisture, ash, crude fiber, crude protein, and crude fat contents were quantified using established analytical protocols, while total carbohydrate content was obtained by difference. This was calculated by subtracting the combined percentages of moisture, ash, fat, protein, and fibre from 100 using the expression: % Total carbohydrate = 100 – (% Moisture + % Ash + % Fat + % Protein + % Fibre). Nitrogen content of the samples was determined using the Kjeldahl method, and crude protein was estimated by multiplying the nitrogen value by a conversion factor of 6.25.

Mineral composition of *Azadirachta indica*

Mineral content was quantified from the ash obtained during proximate analysis after acid digestion with dilute hydrochloric acid. The resulting solution was analyzed for elemental composition using an Atomic Absorption Spectrophotometer (Buck Scientific, East Norwalk, CT, USA) and a Flame Photometer (FP 202 PG).

Green synthesis

A total of 52.92 g of the crushed leaf sample was weighed into a beaker and transferred to a 1000 mL quick-fit round-bottom flask where 500 mL of distilled water was added and heated in a mantle for 1 hour for proper extraction. At the end of the process, the substance was removed and poured into a Buckner funnel (lined with filter paper) for the first round of filtration. A second time filtration was done to obtain a clean filtrate (cotton wool was used at this point). The filtrate obtained was kept in the fridge at 40C for the nanoparticle synthesis.

Preparation of Metal Ion Solutions

Silver Solution (0.1 M): 17 g of silver nitrate salt was weighed into a clean beaker and then dissolved with deionized water and made up to the mark in a 1000 mL standard volumetric flask. The solution was labeled and kept.

Characterization of the synthesized silver nanoparticles (AgNPs)

The formation of silver nanoparticles in the reaction mixture was monitored using UV-visible spectrophotometry. Spectral measurements were obtained with a double-beam UV-visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) over a wavelength range of 300–800 nm at a resolution of 1 nm, with distilled water serving as the reference blank. Functional groups involved in the reduction and stabilization processes were examined using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of both the plant extract and the synthesized AgNPs were recorded with a Shimadzu FTIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan) operated in diffuse reflectance mode at a resolution of 4 cm⁻¹. The spectra were recorded over a wavenumber range of 4000–400 cm⁻¹ using KBr pellet techniques. Prior to analysis, dried extract and AgNP samples were finely ground with spectroscopic-grade KBr and compressed into pellets. Background correction was performed before scanning, and all spectra were obtained as the average of multiple scans to improve signal quality.

Antimicrobial analysis

Antibacterial and antifungal analyses were carried out on the synthesized nanoparticle and plant extract 50 mg of the synthesized samples were weighed and dissolved in 2 ml of distilled water separately and heated for a few minutes to allow for proper dissolution. The extract was poured into a sterile petri-dish, and an antimicrobial disk was

inserted for proper pre-diffusion into the synthesized sample.

Disk Diffusion Method

The disk diffusion plate method was used for the antibacterial analysis. Sterile nutrient agar (14 g/L) was prepared, poured into sterile plates, and allowed to gel. Cultures of the bacterial organisms were taken from stock and inoculated onto the surface of the agar plates. Sterile paper disks pre-diffused with the *Azadirachta indica* extract and the synthesized silver nanoparticles (AgNPs), respectively, were placed on the inoculated agar plates. The plates were left on the bench to allow proper diffusion of the samples into the agar. The plates were incubated at 37 °C for 24 hours. After

incubation, the plates were observed, and it was clear that some samples showed clear zones of inhibition, whereas others showed none.

Statistical Analysis

All experimental data were analyzed using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA, 2007). Results were expressed using appropriate descriptive statistical measures.

RESULT AND DISCUSSION

The proximate composition of *Azadirachta indica* leaf presented in Table 1.0 reveals its substantial nutritional and biochemical significance.

Table 1: Proximate Composition of *Azadirachta indica*

Parameters	Values (%)
Moisture content	13.17 ± 0.02
Ash	8.22 ± 0.01
Crude fat	10.20 ± 0.00
Crude fiber	9.74 ± 0.00
Crude protein	25.35 ± 0.01
Carbohydrate (CHO)	33.32 ± 0.02

The moisture content (13.17 ± 0.02%) indicates a relatively low water level, suggesting good shelf stability and reduced susceptibility to microbial spoilage during storage, consistent with previous reports on the storage quality of medicinal plants.^{21,22} The ash content (8.22 ± 0.01%) reflects a rich presence of mineral elements such as calcium, potassium, magnesium, and trace elements that play vital roles in physiological and therapeutic functions.²³ The crude fat content (10.20 ± 0.00%) demonstrates the presence of lipid-soluble bioactives—such as terpenoids and steroids—known to contribute to neem's anti-inflammatory and antioxidant properties.²⁴ The crude fiber value (9.74 ± 0.00%) indicates that the leaf contains a considerable amount of indigestible polysaccharides, which aid digestion, support detoxification, and improve bowel movement.²⁵ The high crude protein content (25.35 ± 0.01%) highlights the nutritional potential of *A. indica* leaves, as proteins and amino acids contribute to cellular repair and the biosynthesis of secondary

metabolites responsible for its pharmacological actions.²⁶ The carbohydrate content (33.32 ± 0.02%) constitutes the major fraction of the leaf's dry matter, serving as an energy source and as a precursor for the synthesis of glycosides and polyphenolic compounds.²⁷

The mineral composition of *Azadirachta indica* leaf (Table 2.0) reveals a nutritionally valuable profile containing both macro- and micro-elements essential for metabolic and physiological functions. Phosphorus (43.7 ppm) and potassium (2170 ppm) were predominant among the major minerals, reflecting the leaf's role in cellular metabolism, energy transfer, and maintenance of osmotic balance.²⁸ Calcium (2140 ppm) and magnesium (1000 ppm) were also present in appreciable quantities, supporting previous findings that plant-derived calcium and magnesium are vital for bone formation, neuromuscular coordination, and enzymatic catalysis.²⁹

Table 2: Mineral Composition of *Azadirachta indica*

Mineral	Concentration (ppm)
Phosphorus (P)	43.7
Potassium (K)	2170
Sodium (Na)	2260
Calcium (Ca)	2140
Magnesium (Mg)	1000
Cobalt (Co)	0.04
Copper (Cu)	0.10
Chromium (Cr)	0.10
Iron (Fe)	0.61
Manganese (Mn)	0.08
Lead (Pb)	0.01
Zinc (Zn)	1.00

Among the trace elements, iron (0.61 ppm) and zinc (1.00 ppm) were relatively abundant compared with other micro-minerals, suggesting the plant's potential to aid hemoglobin synthesis, immune modulation, and antioxidant defense.³⁰ The low levels of heavy metals such as lead (0.01 ppm) and cobalt (0.04 ppm) indicate minimal

environmental contamination and affirm the leaf's safety for medicinal and nutraceutical use.²³ Overall, the mineral distribution pattern underscores the dual nutritional and therapeutic relevance of *A. indica*, complementing its established phytochemical and pharmacological properties.

Table 3: Phytochemical screening of *Azadirachta indica*

Phytochemical	Observation
Saponins	+
Phenols	+
Tannins	-
Flavonoids	+
Steroids	+

- = Not present, + = Present,

The phytochemical screening of *Azadirachta indica* leaf (Table 3.0) revealed the presence of saponins, phenols, flavonoids, and steroids, while tannins were absent. The high saponin content (++) indicates the plant's potential for anti-inflammatory and immune-boosting effects, consistent with previous reports.³¹ The presence of phenols and flavonoids suggests strong antioxidant properties, as these compounds are known for their

free radical-scavenging activity.³² Steroids, also detected, may contribute to the plant's antimicrobial and anti-inflammatory actions.²⁶ Overall, the qualitative phytochemical profile supports the established therapeutic uses of *A. indica* and provides a biochemical basis for its bioactivity in the biosynthesis of silver nanoparticles and related medicinal applications.

Table 4: Antioxidant composition of ethanol and aqueous extracts of *Azadirachta indica*

Conc(g/mL)	Ethanol		Aqueous	
	Flavonoids (mg AAE/g)	Phenolics (mg GAE/g)	Flavonoids (mg AAE/g)	Phenolics (mg GAE/g)
100	21.04 ± 0.05	8.65 ± 0.21	36.04 ± 0.05	23.65 ± 0.21
200	39.79 ± 0.26	18.68 ± 0.04	54.79 ± 0.26	33.68 ± 0.04

The antioxidant composition of *Azadirachta indica* leaf extracts (Table 4.0) shows that both ethanol and aqueous extracts possess notable flavonoid and phenolic contents, which increased with concentration from 100 to 200 µg/mL. The aqueous

extract exhibited comparatively higher values (flavonoids = 54.79 ± 0.26 mg AAE/g;

phenolics = 33.68 ± 0.04 mg GAE/g) than the ethanolic extract (flavonoids = 39.79 ± 0.26 mg AAE/g; phenolics = 18.68 ± 0.04 mg GAE/g),

indicating greater solubility of these compounds in polar solvents. This observation agrees with

previous findings that aqueous and methanolic extracts of *A. indica* yield higher concentrations of phenolic and flavonoid compounds responsible for antioxidant activity.³³ The high phenolic content directly correlates with

free-radical-scavenging potential, while flavonoids contribute to lipid-peroxidation inhibition and metal-chelating properties.³⁴ Overall, the results confirm that *A. indica* leaf extracts are rich in natural antioxidants, supporting their therapeutic relevance in preventing oxidative stress-related disorders.³⁵

Table 5: Antioxidant potentials of aqueous extracts of *Azadirachta indica*

Conc(g/mL)	Aqueous Antioxidant			
	DPPH	FRAP	NO	TBAR
100	54.30±0.28	57.51±0.16	51.50±1.56	22.24±0.44
200	64.60±0.28	66.74±1.15	64.42±0.08	38.35±0.01

Table 6: Antioxidant potentials of ethanol extracts of *Azadirachta indica*

Conc(g/mL)	Ethanol Antioxidant			
	DPPH	FRAP	NO	TBAR
100	75.04±1.46	98.87±0.04	95.08±0.13	37.39±0.21
200	54.79±0.26	57.99±0.16	52.13±1.56	57.42±0.04

The antioxidant assays (Tables 5.0 and 6.0) show that both aqueous and ethanolic extracts of *Azadirachta indica* possess substantial free-radical scavenging and reducing abilities. Antioxidant activity increased with concentration in the aqueous extract, as indicated by higher DPPH, FRAP, NO, and TBARS values at 200 µg/mL compared to 100 µg/mL. This dose-dependent behavior highlights the efficiency of water as a polar solvent for extracting hydrophilic antioxidant compounds such as phenolic acids and flavonoids.³⁶ The ethanolic extract also demonstrated considerable antioxidant activity, with the highest DPPH, FRAP, and NO scavenging observed at 100 µg/mL, indicating a

possible saturation or degradation effect at higher concentrations. Its comparatively strong TBARS inhibition at 200 µg/mL suggests the presence of lipophilic constituents capable of preventing lipid peroxidation.³⁷ The variation in performance between solvents can be attributed to differences in polarity, which influence the solubility and extraction efficiency of phytochemicals.³⁸ Overall, both extracts exhibited strong and consistent antioxidant potential, corroborating earlier findings that *A. indica* leaves are rich in polyphenolic antioxidants that contribute to free-radical neutralization and oxidative-stress mitigation.

Table 7: Antimicrobial activity of aqueous extract and silver nanoparticle of *Azadirachta indica* on some selected bacteria

Sample	Conc(mg/ml)	<i>E. coli</i> (mm)	<i>S. typhi</i> (mm)	<i>S. aureus</i> (mm)	<i>P. aeruginosa</i> (mm)
Aqueous extract	100	6.3	1.0	4.1	1.6
	50	5.5	ND	2.4	1.3
Silver Nanoparticles (AgNP)	100	11.6	3.2	7.3	2.4
	50	10.2	2.0	5.8	2.0

ND: Not Detected

The antimicrobial results (Table 7.0) clearly demonstrate that biosynthesized silver nanoparticles (AgNPs) of *Azadirachta indica* exhibit stronger antibacterial activity than the crude aqueous extract across all tested organisms.

At 100 mg/mL, the AgNPs produced inhibition zones of 11.6 mm against *E. coli*, 7.3 mm against *S. aureus*, 3.2 mm against *S. typhi*, and 2.4 mm against *P. aeruginosa*, whereas the corresponding

aqueous extract produced lower inhibition zones of 6.3, 4.1, 1.0, and 1.6 mm, respectively. This enhanced potency is likely related to the nanoscale dimensions and increased surface area of AgNPs, which promote stronger interactions with microbial cell membranes.³⁹ Among the highest test organisms, *E. coli* showed the highest susceptibility, followed by *S. aureus*, *S. typhi*, and

P. aeruginosa. This trend is partially consistent with known differences in bacterial cell wall structures, where membrane composition and permeability influence nanoparticle interaction.⁴⁰ The lower inhibition observed for *P. aeruginosa* may result from its efflux pump systems and biofilm-forming capacity, which reduce AgNP uptake.⁴¹

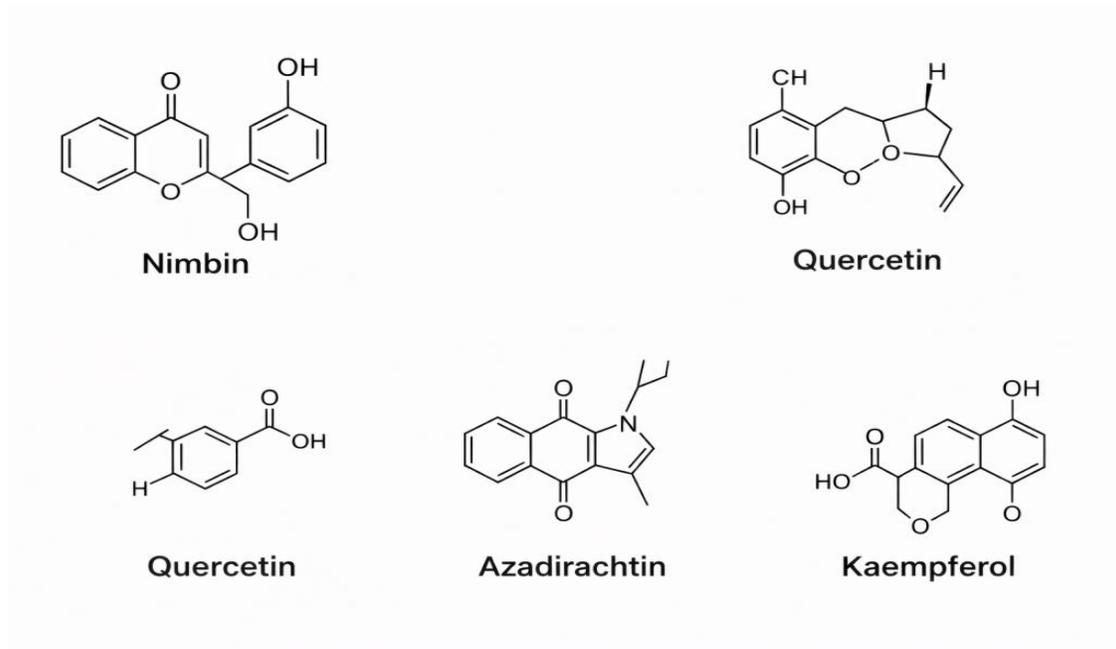


Figure 1: Chemical Compounds Found in the Leaf of *Azadirachta Indica*

The phytochemicals in the neem extract—such as nimbin, azadirachtin, and quercetin (Fig. 1)—likely contribute to enhanced bactericidal activity through oxidative stress induction, protein denaturation, and DNA damage pathways when combined with silver ions.⁴² Furthermore, the higher inhibition zones at 100 mg/mL compared to 50 mg/mL for both the extract and AgNPs confirm a clear concentration-dependent

antibacterial effect, supporting the role of increased nanoparticle density in accelerating reactive oxygen species (ROS) generation and microbial cell death.⁴³ Overall, the findings confirm that *A. indica*-derived AgNPs are more effective than the crude extract in inhibiting both Gram-positive and Gram-negative bacteria, validating their potential as eco-friendly antimicrobial agents.

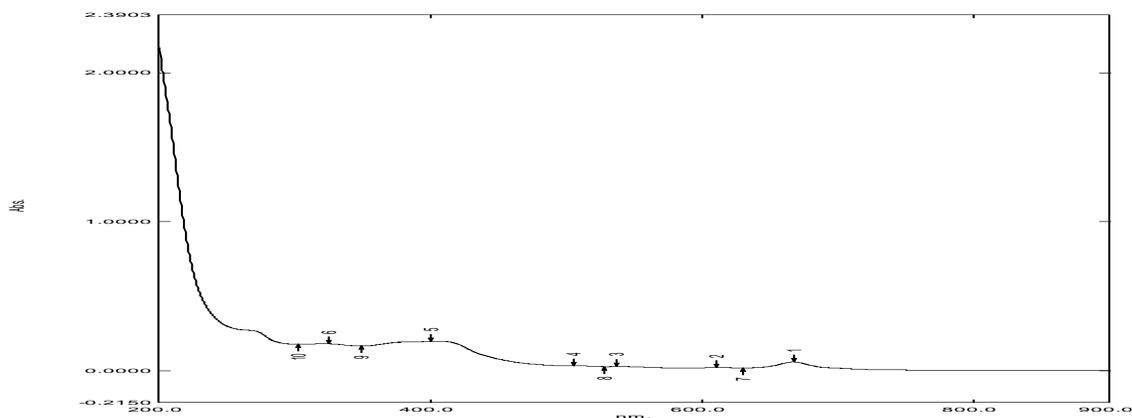


Figure 2a: UV–Visible wave scanning spectrum of *Azadirachta indica* leaf ethanolic extract showing multiple absorption peaks at 301.5–400.0 nm with notable maxima at 324.0 nm (Abs. 0.1810) and 301.5 nm (Abs. 0.1772), consistent with electronic transitions of phenolic and flavonoid phytoconstituents involved in redox activity.

The UV-Visible wave scanning of *Azadirachta indica* ethanolic extract (Fig. 2.0a) revealed multiple absorption peaks between 300 and 400 nm, attributed to $\pi \rightarrow \pi^*$ transitions in phenolic and flavonoid compounds such as quercetin and nimbin. These bioactive constituents are known to act as natural reducing and capping agents in nanoparticle synthesis. The silver nanoparticle spectrum (Fig. 2.0b) showed sharp peaks at 255.5

and 272 nm, confirming the successful formation of AgNPs. The shift from the extract's absorption bands to lower wavelengths reflects the conversion of Ag^+ ions into stable metallic nanoparticles mediated by phytochemicals.⁴⁴

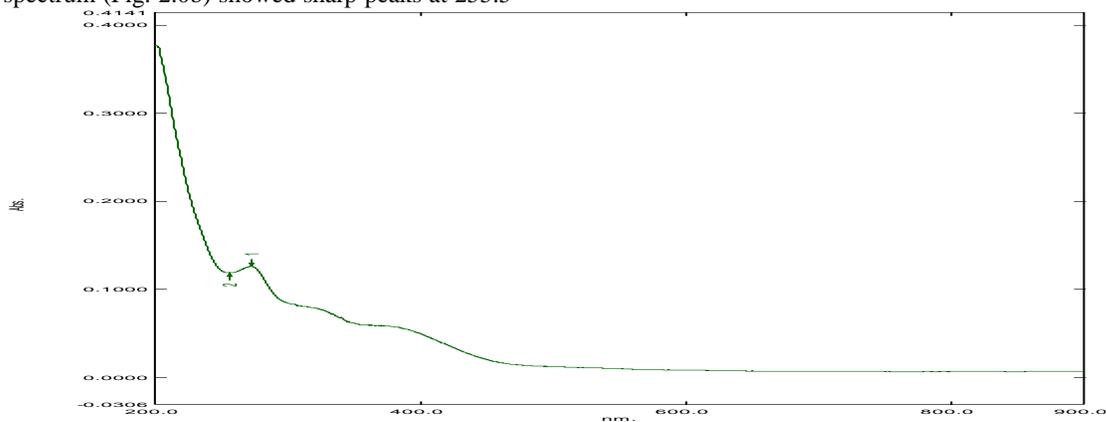


Figure 2b: UV–Visible wave scanning spectrum of silver nanoparticles synthesized using *Azadirachta indica* leaf extract, exhibiting distinct absorption peaks at 255.5 nm (Abs. 0.1188) and 272.0 nm (Abs. 0.1263), confirming the reduction of Ag^+ ions and formation of phytochemically stabilized silver nanoparticles.

This spectral evidence therefore supports the biochemical pathway linking the antioxidant constituents of *A. indica* to its enhanced antibacterial efficacy when combined with silver ions.

The FTIR spectrum of the *Azadirachta indica* ethanolic extract (Fig. 3.0a) displayed prominent absorption bands at 2950.3, 2921.6, 1454.6, 1375.7, and 1242.5 cm^{-1} , corresponding to C–H, C=C, O–H, and C–O vibrations of organic

functional groups. These peaks confirm the presence of phenolic, flavonoid, and amine compounds, consistent with the plant's known phytochemical profile.^{45,46} The band near 818.1 cm^{-1} represents aromatic C–H bending, while the absorption at 522.9 cm^{-1} may indicate metal–oxygen interactions already present due to the mineral content in the extract.⁴⁷

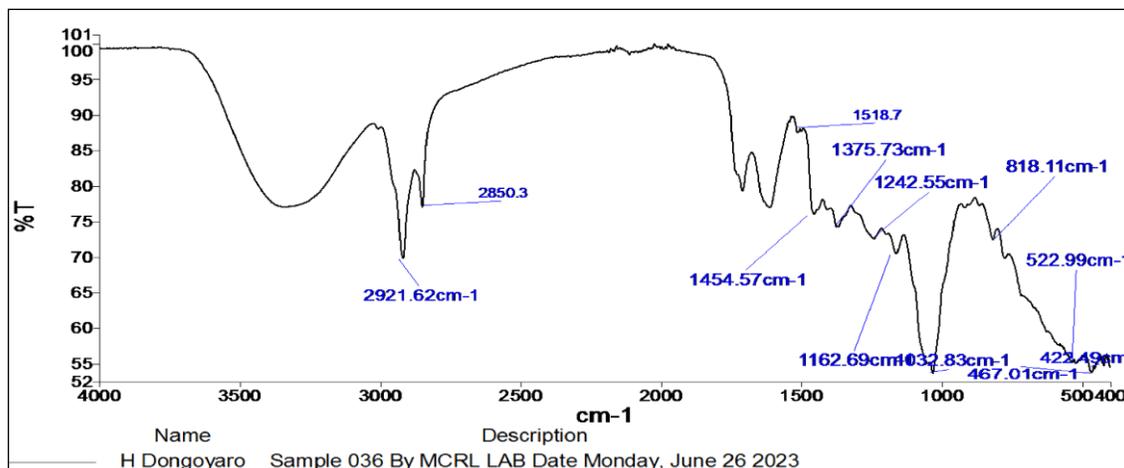


Figure 3a: FTIR spectrum of the ethanolic extract of *Azadirachta indica* leaf showing characteristic absorption bands at approximately 2921.62 cm^{-1} (aliphatic C–H stretching), 2850.3 cm^{-1} , 1454.57 cm^{-1} (C–H bending), 1375.73 cm^{-1} , 1242.55 cm^{-1} (C–O stretching), 1162.69 cm^{-1} , 1032.83 cm^{-1} , and fingerprint region peaks at 818.11, 522.99, and 467.01 cm^{-1} , indicative of diverse phytochemical functional groups present in the extract.

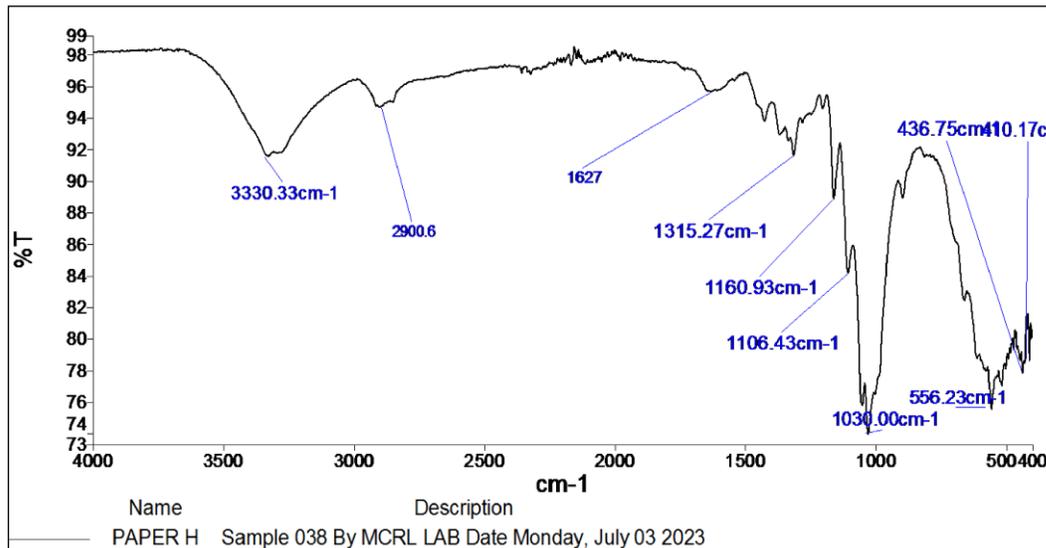


Figure 3b: FTIR spectrum of *Azadirachta indica*-mediated silver nanoparticles showing absorption bands at approximately 3330.33 cm^{-1} (O–H/N–H stretching), 2900.6 cm^{-1} (C–H stretching), 1627 cm^{-1} (amide I or C=C stretching), 1315.27 cm^{-1} , 1160.93–1106.43 cm^{-1} (C–O stretching), and distinct low-frequency bands at 556.23, 436.75, and 410.17 cm^{-1} , indicating interaction of silver with phytochemical functional groups involved in nanoparticle stabilization.

.After silver nanoparticle biosynthesis, the FTIR spectrum (Fig. 3.0b) exhibited notable band shifts and intensity reductions. The broadband at 3330.3 cm^{-1} indicates O–H stretching of hydroxyl groups involved in hydrogen bonding, while new peaks at 1627 and 1315 cm^{-1} (C=O and C–N vibrations) confirm the participation of carbonyl and amine groups in Ag^+ reduction and nanoparticle capping.⁴⁸ The appearance of new low-frequency bands at 556.2 and 436.7 cm^{-1} corresponds to Ag–O and Ag–N vibrations, directly confirming the formation of AgNPs.⁴⁹ These spectral changes collectively demonstrate that biomolecules in the

nem extract—particularly phenolics, flavonoids, and amines—played a dual role as reducing and stabilizing agents, converting silver ions into metallic nanoparticles and forming a protective organic coating around them. This capping layer enhances nanoparticle stability and contributes to the enhanced antibacterial activity observed in Table 7.0.⁴⁸

CONCLUSION

The ethanolic and aqueous extracts of *Azadirachta indica* exhibited substantial phytochemical

profiles and notable antioxidant and antibacterial activities. UV–Vis and FTIR analyses verified the green synthesis of silver nanoparticles and confirmed the role of phenolic and flavonoid compounds as natural reductants and stabilizers. The *A. indica*-mediated AgNPs demonstrated significantly greater antibacterial efficacy than the crude extracts, indicating that nanoparticle formation enhances the plant's intrinsic bioactivity. Collectively, these results support the use of *A. indica* as a sustainable precursor for green synthesis of silver nanoparticles and highlight the promise of plant-derived AgNPs as eco-friendly antimicrobial agents

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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