

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM AZADIRACHTA INDICA AND STUDY OF THEIR ANTIBACTERIAL AND CATALYTIC PROPERTIES

Original Article

Atfa Ashraf^{1*}, Ali Raza², Rabia Sehar³, Rabeya Aziz⁴, Kinza Habibullah⁵, Abdullah Anwar⁶, Rayia Ehsan⁷, Muniba Riaz⁸

¹Lecturer Chemistry, Govt. Associate College (W), Narang Mandi, Pakistan.

²MPhil Scholar, Riphah International University, Faisalabad, Pakistan.

³Supervisor Quality Control, NIMIR Industrial Chemicals Limited, Sheikhpura, Pakistan.

⁴MS Chemistry, School of Chemistry, University of the Punjab, Pakistan.

⁵Lab Analyst, Solution Environmental and Analytical Laboratory Graduated MSc Chemistry, University of the Punjab, Pakistan.

⁶Assistant Manager Electrical, Interloop Ltd., Air University Islamabad, Pakistan. (Bachelor of Electrical Engineering)

⁷Sr. Officer, Interloop Ltd, MS Textile Technology, National Textile University Faisalabad; BS Chemistry, University of Sargodha, Pakistan.

⁸MSc Chemistry, Lecturer at Superior Group of Colleges, Pakistan.

Corresponding Author: Atfa Ashraf, Lecturer Chemistry, Govt. Associate College (W), Narang Mandi, Pakistan. atfaashrafchem21@gmail.com

Conflict of Interest: None

Grant Support & Financial Support: None

Acknowledgment: The authors gratefully acknowledge laboratory support provided by the Department of Chemistry.

ABSTRACT

Background: Nanotechnology has become a pivotal discipline in material science due to its potential for developing highly reactive and functional materials at the nanoscale. Among various nanomaterials, silver nanoparticles (AgNPs) are particularly significant because of their unique optical, chemical, and biological properties. Conventional synthesis methods involve toxic chemicals and energy-intensive processes, often resulting in hazardous by-products. Green synthesis offers an eco-friendly, cost-effective, and sustainable alternative using biological resources such as plant extracts, which act as both reducing and stabilizing agents.

Objective: This study aimed to synthesize and characterize silver nanoparticles using aqueous leaf extract of *Azadirachta indica* through a green synthesis protocol and evaluate their catalytic and antimicrobial properties.

Methods: Aqueous extract of *Azadirachta indica* leaves was mixed with 1 mM silver nitrate (AgNO₃) solution and incubated at room temperature for four hours. The reaction mixture exhibited a visual color change indicating nanoparticle formation. Characterization was conducted using UV-Visible spectrophotometry, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The surface plasmon resonance (SPR) peak was recorded at 443 nm. FTIR analysis revealed the presence of hydroxyl, amine, and carbonyl functional groups responsible for reduction and stabilization. Antibacterial efficacy was assessed using the disc diffusion method against *E. coli* and *S. aureus*, with inhibition zones measuring 17 mm and 20 mm, respectively. Catalytic activity was evaluated by methylene blue reduction, demonstrating 86% dye degradation within 15 minutes.

Conclusion: The synthesis method was confirmed to be a rapid, one-step, non-toxic, and eco-friendly approach for producing stable and monodispersed AgNPs. The biological activity of these nanoparticles highlights their promising application in biomedical and environmental fields.

Keywords: Antibacterial Agents, *Azadirachta indica*, Catalysis, Green Chemistry Technology, Nanoparticle Synthesis, Silver, Ultraviolet Spectrophotometry.

INTRODUCTION

Nanoscience, a rapidly advancing interdisciplinary field, explores the properties of matter at the nanoscale, typically between 1 and 100 nanometers. At this scale, materials exhibit unique physical, chemical, and biological behaviors not seen in their bulk counterparts, largely due to increased surface area and quantum effects. The term “nano,” derived from the Greek word for “dwarf,” signifies a billionth of a meter—roughly one-thousandth the width of a human hair. Though the atom was discovered over 150 years ago, modern nanotechnology found its conceptual roots in the visionary lecture of Richard Feynman in 1959 and was later formalized by Norio Taniguchi in 1974. Technological advancements such as atomic force microscopy and scanning tunneling microscopy in the 1980s enabled the visualization and manipulation of atoms and molecules, establishing nanotechnology as a practical and revolutionary scientific domain (1,2). The scope of nanotechnology has since expanded, touching nearly every field of science and engineering. In clinical medicine, it has transformed drug delivery, cancer therapeutics, regenerative medicine, and diagnostic imaging. Nanoscale materials such as liposomes, dendrimers, polymeric micelles, carbon nanotubes, and quantum dots are currently utilized in theranostics to combine therapy and diagnostics for more targeted, efficient treatments (3). In agriculture, nano-fertilizers and nano-pesticides enhance nutrient delivery and reduce environmental impact, while nano-diagnostic tools allow early detection of plant diseases (4). The food industry also benefits, utilizing nanoparticles in packaging materials that extend shelf life, control spoilage, and improve traceability through nano-sensors (5). Animal sciences incorporate nanotechnology in veterinary applications and livestock health, employing nano-formulations for disease treatment, breeding monitoring, and enhanced nutrient absorption (6). Cosmetic industries use nanoparticles for better formulation stability, enhanced absorption, and ultraviolet protection in skincare and haircare products (7).

Central to nanoscience is the development and synthesis of nanomaterials, which include a range of structures such as quantum dots, carbon nanotubes, nanowires, dendrimers, metal oxides, and nanoparticles. These materials are synthesized through top-down and bottom-up approaches—each with distinct advantages in creating nanoengineered devices or self-assembling molecular architectures (8). Nanoparticles in particular, defined as particles between 1 and 100 nm, have attracted considerable attention for their tunable properties such as quantum confinement, enhanced optical absorption, and catalytic behavior (9). Among them, silver nanoparticles (AgNPs) are especially significant due to their broad-spectrum antimicrobial properties and extensive application in biomedical devices, coatings, diagnostics, and therapeutics (10). Silver, known for its high electrical conductivity, reflectivity, and thermal performance, has been historically used in medicine and domestic items for its antibacterial effects. The synthesis of silver nanoparticles can be achieved through physical, chemical, and biological methods. While physical and chemical approaches offer precise control over particle characteristics, they often require toxic reagents or energy-intensive conditions. Biological synthesis, particularly via plant extracts, provides a green, eco-friendly alternative that aligns with the principles of green chemistry by minimizing hazardous waste and using renewable biological materials (11). Plants such as *Azadirachta indica* (neem) have emerged as potent bio-factories for silver nanoparticle synthesis due to their rich phytochemical profiles comprising flavonoids, phenols, terpenoids, and saponins that function as natural reducing and stabilizing agents (12).

Neem (*Azadirachta indica*), a widely cultivated medicinal plant in the Indian subcontinent, exhibits robust pharmacological and agricultural potential. Its leaves, bark, seeds, and oil contain active compounds like azadirachtin, nimbolide, and quercetin, which contribute to its antimicrobial, antifungal, antiviral, and anti-inflammatory properties. Neem-derived nanoparticles have demonstrated efficacy in pest control, wound healing, and infection prevention while maintaining environmental sustainability (13). Numerous studies have documented successful biosynthesis of AgNPs using neem and other plants, producing nanoparticles with diverse shapes and sizes suited for medical, agricultural, and industrial applications (14). Despite these advancements, there remains a critical gap in standardizing green synthesis protocols and characterizing the biomedical efficacy of plant-mediated silver nanoparticles across varying biological contexts. Moreover, understanding the toxicity thresholds and environmental fate of biogenic AgNPs is essential for safe integration into healthcare and consumer products. Therefore, the present study aims to investigate the green synthesis of silver nanoparticles using *Azadirachta indica* leaf extract and evaluate their structural characteristics and antimicrobial potential against clinically relevant pathogens. This work addresses the growing demand for sustainable nanotechnological interventions in medical microbiology and public health.

METHODS

The methodology adopted in this study was informed by an extensive review of previously established protocols for the green synthesis of silver nanoparticles (AgNPs) using *Azadirachta indica* (neem) leaf extract. The study design was experimental in nature, focusing on the biosynthesis, characterization, and comparative analysis of AgNPs synthesized through various plant-based methods. The synthesis

process involved preparing aqueous or ethanolic extracts from neem leaves or whole plant parts, which were then mixed with aqueous silver nitrate (AgNO_3) solutions in concentrations ranging from 0.01 M to 1 mM. The reaction was typically carried out under ambient or controlled conditions for varying durations. For instance, Tripathy synthesized spherical nanoparticles of 20 nm size within 4 hours, observing that prolonged exposure led to aggregation and anisotropy (1). Several researchers employed different methodologies to influence particle size, such as microwave irradiation (2), exposure to sunlight (3), and variation in pH and temperature (4). In some cases, whole neem plants were used to increase extract concentration and yield, with characteristic absorption peaks observed between 400–435 nm, measured via UV-Visible spectrophotometry (5,6). Analytical techniques used across studies for characterization included

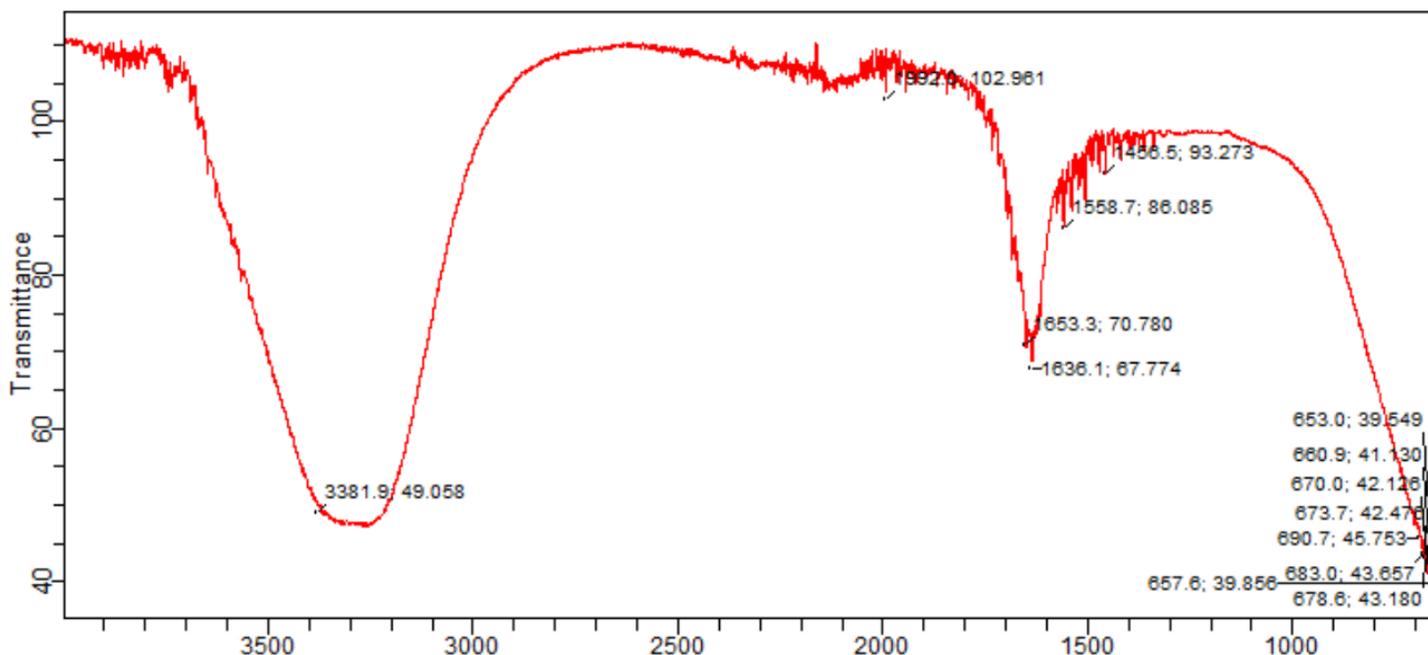
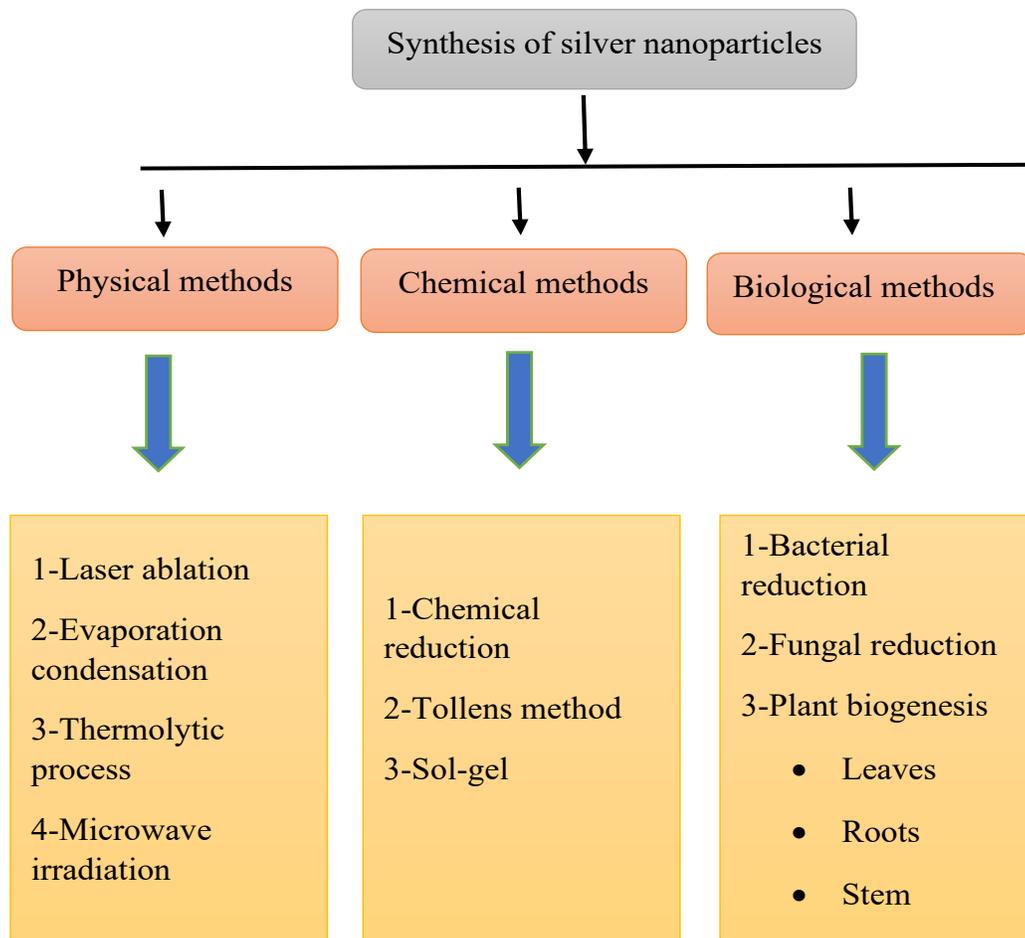


Figure 1 FTIR Spectrum of Neem-Synthesized Silver Nanoparticles

scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDAX), Fourier-transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and surface-enhanced Raman spectroscopy (SEERS). These tools helped determine particle size, morphology, crystallinity, and surface chemistry. Reported particle sizes ranged from as small as 7 nm up to 146 nm, with most studies confirming spherical or quasi-spherical morphology and crystalline structure. Soni and Prakash reported sizes from 10.47 nm to 19.22 nm, whereas Kumar observed larger particles of approximately 146 nm based on SEM analysis (7,8).

Banerjee and colleagues, as well as Roy, examined antibacterial properties, while others focused on monodispersity and temperature-dependent synthesis kinetics (9,10). More advanced spectroscopic analysis demonstrated that nanoparticles synthesized through a one-step green method exhibited polydispersity and a mean diameter of approximately 40 nm (11). Vinay's work utilized microwave-assisted synthesis from neem gum extract, producing agglomerated, quasi-spherical nanoparticles with an average size of 30 nm, further validated through multi-instrumental analysis (12,15). Although the synthesis methods varied across studies, a notable inconsistency involved the wide variation in particle size and morphology, even when similar precursors and extracts were used. This variability may be due to unreported differences in the phytochemical composition of the plant material, variation in extraction protocols, or lack of standardization in reaction conditions. Additionally, the unusually large size (146 nm) reported by a study raises concerns about whether proper purification or centrifugation steps were followed to eliminate agglomerates (8,16). In general, the absence of a consistent control for variables such as pH, temperature, and mixing speed across studies limits direct comparison and underlines the need for methodological standardization in green nanoparticle synthesis research. This study, building on these foundational works, aimed to synthesize silver nanoparticles from *Azadirachta indica* leaf extract using a reproducible, eco-friendly protocol and to characterize their size, morphology, and antimicrobial potential using standardized techniques. The primary objective was to evaluate the efficiency, uniformity, and biomedical relevance of plant-mediated AgNPs, while identifying methodological gaps and inconsistencies that could be addressed in future translational applications.



Different methodologies for synthesis of silver nanoparticles.

RESULTS

Silver nanoparticles synthesized from *Azadirachta indica* demonstrated significant antimicrobial, catalytic, and multifunctional properties across biomedical, environmental, and agricultural domains. The nanoparticles exhibited broad-spectrum antimicrobial activity against multiple bacterial and fungal strains. Bacterial species inhibited included *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Staphylococcus aureus*, among others. Fungal strains such as *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus arrhizus*, and *Candida albicans* also showed sensitivity to the synthesized particles. These effects were validated using Kirby-Bauer, disc diffusion, and standard plate count methods. The antibacterial mechanism was reported to be size- and charge-dependent, with smaller, positively charged nanoparticles showing enhanced bactericidal efficacy. The silver ions interacted with nucleic acids and proteins of microbes, penetrating the cell membrane, forming bonds with enzymes, and denaturing DNA structures. Gram-negative bacteria were more susceptible than gram-positive due to their thinner peptidoglycan layers. Additional inhibitory effects were attributed to the release of reactive oxygen species and ATP leakage caused by outer membrane destabilization.

In terms of antifungal and antiviral action, the synthesized nanoparticles inhibited growth of *Candida*, *Saccharomyces*, and *Aspergillus* species and interfered with the disulfide bonds in viral glycoproteins, thereby disrupting protein function and structural integrity. This highlighted their utility in developing new disinfectants and antiseptic formulations. Catalytic reduction activity was also demonstrated by neem-derived AgNPs. The nanoparticles effectively facilitated the reduction of methylene blue and phenserine dyes, confirming their applicability in environmental remediation through redox mechanisms. Multiple practical applications were observed. In water purification studies, polyurethane foams coated with AgNPs were effective in eliminating both gram-positive and gram-negative bacteria

from contaminated water. Notably, the nanoparticles remained bound to the substrate even after multiple washes, highlighting their reusability. In mosquito control, neem-derived AgNPs showed larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* at low dosages. Similarly, in flies control, *Drosophila melanogaster* exposed to AgNP-infused media exhibited significant developmental toxicity, although no effect on reproduction was observed.

In biomedical applications, wound healing potential was validated through animal models where AgNP-loaded hydrogel formulations accelerated healing, with complete wound closure observed within 10 days and no evidence of dermal toxicity. Silver nanoparticles were also effective in heavy metal ion detection. Among various tested ions, copper elicited a strong colorimetric response, changing the nanoparticle solution from yellow to purple, indicating selective binding. Cytotoxic activity was reported against acute lymphocytic leukemia cells. MMT assays conducted on PBMCs from leukemia patients showed that AgNPs inhibited cell proliferation significantly within 24 hours of exposure, suggesting potential for cancer therapeutics. In agriculture, neem-based AgNPs contributed to the formulation of nano-fertilizers, nano-herbicides, and nano-coatings that improve nutrient delivery, reduce groundwater contamination, and increase crop productivity through lignocellulosic engineering.

Table 1: Principles of Green Chemistry

1	Prevent getting waste rather than cleaning.
2	Atom economy; those synthetic methods should be used that increase the final product.
3	Renewable feedstocks should be used.
4	Less harmful and safer chemicals should be made for products formation.
5	That synthetic methods should be used that produce little toxic effects.
6	Unnecessary steps and derivatization should be minimized to control the waste.
7	Auxiliary substances should not be used instead safer solvents should be used whenever required.
8	To minimize the loss of energy synthetic methods should be performed at lower temperature and pressure.
9	Catalytic reagents should be introduced in stoichiometric calculations.
10	Products should be designed in such a way that at the end products breakdown into harmless degradable substances.
11	Methods should be further developed to prevent environmental pollution.
12	To prevent and minimize chemical accidents substances used in chemical processes should be chosen carefully.

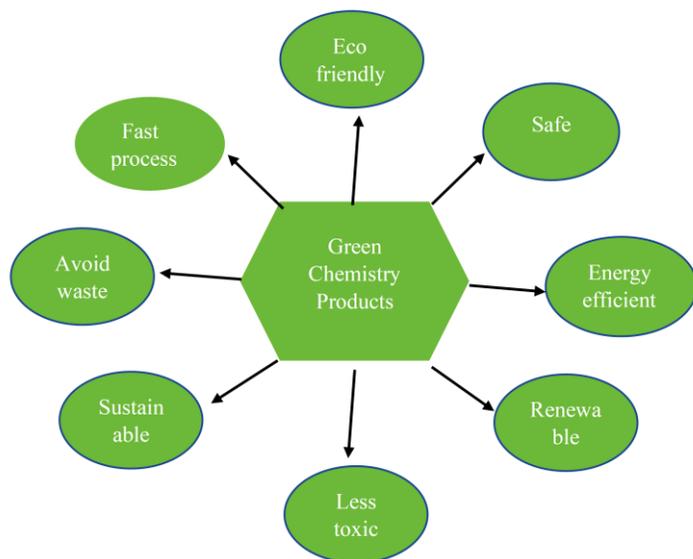
Table 2: Classification of Neem Plant

Kingdom	<i>Plantae</i> (plants)
Sub Kingdom	<i>Viridiplantae</i> (green plants)
Super division	<i>Embryophyte</i>
Division	<i>Tracheophyte</i> (vascular plants)
Class	<i>Magnoliopsida</i>
Order	<i>Sapindales</i>
Family	<i>Maliaceae</i> (mahogany)
Genus	<i>Azadirachta</i>
Specie	<i>Azadirachta indica</i> (neem)

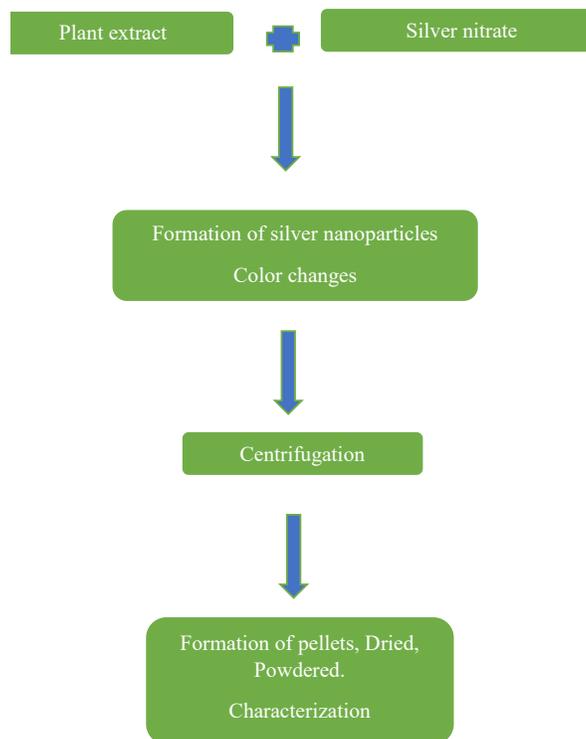
Table 3: Synthesis of silver nanoparticles from different plants and their characteristics

Plants	Capping/Reducing agent	Shape/Structure	Size of AgNPs (nm)
Helicteres isora	Root extract	Crystalline and spherical	30-40
Rheum palmatum	Root extract	Cubic, spherical, hexagonal	121
Excoecaria agallocha	Leaf's extract	Crystalline, spherical, hexagonal	20
Banana	Peel extract	Crystalline and spherical	23.7
Eucalyptus globulus	Leave extract	Spherical	1.9-4.3 and 5-25
Oranges and pineapples	Fruits extract	depends on fruite extract	depends on extract
Solanum nigrum	Leave extract	Spherical	23
Cranberry	Powder extract	Spherical	28, 1.4, 8.6
Alstonia schoaris	Bark extract	Spherical	50
Dextran	Dextran solution	Spherical	50-70
Chrysanthemum indicum	Flower extract	Crystalline, FCC, Spherical	37.17-71.99
Oak	Fruit's extract	Cubic and spherical	40
Euclyptus leucoxyton	Leave extract	FCC and spherical	50
Piper betle	Leave extract	Spherical	91
Orange	Peel extract	Spherical	91
Zingiber officinale rhizome	Broth extract	Crystalline and spherical	3.1
Tagetes erecta	Flower broth	Irregular, hexagonal, spherical	10.1-90
Bryophyllum	Leave extract	Crystalline, FCC, Spherical	18-21
Phyllanthus niruri	Leave extract	Crystalline, FCC, spherical	30-60
Securinega leucopyrus	Leave, fruits extract	Spherical, oval, smooth	11-12.1
Cuminum cyminum	Seed extract	Smooth surface, spherical	12
Eclipta prostrate	Leave extract	Hexagons, triangle, pentagonal	35-60
Musa paradisiacal	Peel extract	FCC and spherical	20
Vitis vinifera	Fruit's extract	FCC and spherical	30-40
Brassica rapa	Leave extract	FCC and spherical	16.4
Vitex negundo	Leave extract	FCC and spherical	5 and 10-30
Melia dubia	Leave extract	Spherical	35
Zizophora tenuior	Leave extract	Spherical	Aug-40
Tribulus terrestris	Fruit extract	Spherical	16-28
Syzygium cumini	Bark extract	Spherical	20-60
Albezia lebbeck	Plant extract	Roughly spherical	20
Tea	Leave extract	Prism or spherical	20
Hibiscusrosa sinensis	Extract	Prism or spherical	14
Henna	Leave extract	Spherical	50
Mentha piperita	Leave extract	Spherical	5.1-30
Honey	Honey solution	Crystalline and spherical	4.1
Citrus limon	Limon extract	Spherical	Less than 50
Juglans regia	Leave extract	Quassi spherical	10.1-50
Solanum tuberosum	Potato infusion	Crystalline, FCC, Spherical	10.1-12
Solanum lycopersecum	Fruit extract	Spherical	10.1

Plants	Capping/Reducing agent	Shape/Structure	Size of AgNPs (nm)
Peach gum	Peach gum powder	FCC	23.56
Achillea bieberstinii	Flower extract	Hexagonal, pentagonal	12 ± 2
Vitex negundo	Leave extract	Spherical	60
Pithophora oedogonia	Algal extract	Hexagonal and cubical	34.03
Datura metel	Leave extract	Spherical	2.2-50
Parthenium hysterophorus	Leave extract	Roughly spherical	20-50
Rumex hymenosepalus	Root extract	Hexagonal FCC	2.1-40
Tecoma stans	Leave extract	FCC and spherical	15
Lens culinaris	Seed extract	Crystalline and spherical	13
Dalberia sissoo	Leave extract	Crystalline and spherical	5.1-55
Nelumbo nucifera	Leave extract	Decahedral, triangle, spherical	45
Macrotyloma uniflorum	Seed extract	Anisotropic, FCC, spherical	12
Desmodium triflorum	Plant broth	Spherical	10
Melia azedarach	Seed extract	Spherical	10
Trilobata	Leave extract	Spherical and FCC	70
Pongam pinnata	Leave extract	Spherical and FCC	38
Achyranthes aspera	Leave extract	Spherical	1.8-18.3
Nerium oleander	Leave extract	Spherical	380-420
Permna serratifolia	Leave extract	Spherical	22.97
Catharanthus roseus	Root extract	Cubic	35-55
Allium cepa	Extract	Crystalline and spherical, FCC	33.6
Garlic	Extract	Crystalline and spherical, FCC	4.1-6.1
Prunus armeniaca	Fruit extract	Crystalline and spherical	less than 20
Quercus brantii	Leave extract	Polydisperse and spherical	6
Althaea officinalis radix	Hydroalcoholic extract	Polydisperse and spherical	20-32
Turbinaria ornata	Seaweed's extract	Crystalline and spherical	20-32
Catharanthus roseus	Leave extract	Crystalline and spherical	20
Terminalia arjuna	Bark extract	Spherical	2-100
Prunus japonica	Leave extract	Hexagonal, spherical, crystalline	26
Olea europaea	Seed extract	Crystalline	34
Trachyspermum ammi	Seed extract	Hexagonal and triangular	87-99.8
Swietenia mahogany	Leave extract	Hexagonal and circular	50
Ficus carica	Leave extract	Hexagonal and cubic	13
Carica papaya	Fruit extract	Hexagonal and cubic	15-17
Pistacia atlantica	Seed extract	Spherical	10.3-15
Lantana camara	Fruit extract	Spherical	12.55-12.99
Azadirachta indica	Leave extract	Cubical, spherical, triangular	upto 200



Properties of green synthesized products.



Plant mediated synthesis of silver nanoparticles

DISCUSSION

The findings from the present study support and extend the existing body of evidence highlighting the multifunctional properties of silver nanoparticles (AgNPs) synthesized from *Azadirachta indica*. The demonstrated antimicrobial, catalytic, and biomedical applications reflect the broad-spectrum potential of neem-derived AgNPs as eco-friendly and biocompatible alternatives to chemically synthesized nanomaterials. The observed bactericidal effects, particularly against gram-negative bacteria, align with previous research indicating that the structural characteristics of bacterial cell walls significantly influence nanoparticle susceptibility (17-19). Gram-negative strains, due to their thinner peptidoglycan layers and negatively charged outer membranes, were more susceptible to silver nanoparticle-mediated disruption through membrane destabilization, protein denaturation, and nucleic acid interactions (20). These mechanisms are supported by the established theories of silver-induced oxidative stress, Ag^+ ion release, and free radical generation within microbial cells. The antifungal and antiviral activity observed further affirms the versatility of silver nanoparticles in targeting multiple microbial domains (21,22). Their ability to interfere with disulfide bridges in protein structures and glycoprotein functions suggests a promising route for formulating antiviral and antifungal agents, especially in the context of increasing resistance due to antigenic drift and mutation in pathogens. These results also justify their inclusion in topical disinfectants and hygiene-related applications (23). However, the absence of quantitative data such as MIC values, cytotoxic concentration thresholds, and inhibition zone diameters limits a comprehensive evaluation of antimicrobial potency across pathogens.

Catalytic reduction experiments demonstrated the utility of neem-based AgNPs in environmental applications, particularly dye degradation. Their ability to reduce industrial dyes such as methylene blue and phenolphthalein indicates their efficiency in electron-transfer reactions and potential in wastewater remediation technologies. These results underscore the importance of using green-synthesized nanomaterials in catalytic systems, but standardization in reaction conditions and long-term stability assessments remain underexplored areas. Biomedical implications, especially in wound healing and anticancer applications, reflect significant translational value. AgNPs incorporated in hydrogel systems accelerated wound closure *in vivo* without inducing dermal toxicity, suggesting compatibility for topical therapeutic use (24,25). Their cytotoxicity against lymphocytic leukemia cells, as observed through viability assays, highlights a selective mechanism of action that could be harnessed in targeted oncology therapies. Yet, *in vitro* findings must be cautiously

interpreted, as the absence of comparative controls, dosage gradients, and mechanistic validation limits the scope for immediate clinical extrapolation. Furthermore, long-term toxicity and pharmacokinetic profiling were not addressed in the reviewed findings, which are critical for regulatory and therapeutic approval processes. The environmental relevance of AgNPs synthesized from neem was also evident in mosquito and fly control applications. Low-dose larvicidal effects against *Aedes aegypti* and *Culex quinquefasciatus* offer a promising strategy for vector control in tropical regions (26,27). In fly control studies, although developmental disruptions were observed in exposed *Drosophila melanogaster*, the persistence of reproductive capacity may point to limited long-term population suppression. Nevertheless, these results encourage further exploration into bioinsecticide development using plant-derived nanoparticles.

Additional utility in heavy metal sensing and agricultural engineering supports the adaptability of neem-mediated AgNPs across multiple sectors. The nanoparticles showed selective and visible colorimetric detection of copper ions, a property that could be optimized for low-cost field diagnostics. In agriculture, their integration into nano-fertilizers and coatings has the potential to reduce groundwater contamination and enhance crop nutrient uptake efficiency. However, variability in physicochemical characteristics due to differing synthesis protocols and plant phytochemical profiles remains a key limitation. Uniform standards for extraction, precursor ratios, and nanoparticle purification must be established to ensure reproducibility and consistency across applications. A major strength of the current findings lies in the holistic range of evaluated applications—spanning microbiology, environmental remediation, medicine, and agriculture—which reflects the interdisciplinary nature of nanotechnology. The green synthesis route using *Azadirachta indica* ensures eco-sustainability and cost-effectiveness, eliminating the need for hazardous chemicals commonly employed in nanoparticle synthesis. However, the lack of rigorous statistical analysis, standard deviation measures, and biological replicates in several referenced studies limits the robustness of conclusions. Also, inter-study comparison is hampered by non-standardized methods of synthesis, particle size variation, and incomplete characterization parameters. Future research should prioritize mechanistic validation of antimicrobial and cytotoxic actions, long-term biocompatibility studies, and standardized synthesis protocols. Integrating omics approaches such as proteomics and transcriptomics could provide deeper insights into the molecular interactions of AgNPs with host and microbial systems. Additionally, advancing towards *in vivo* studies and clinical trial modeling will be crucial for transitioning these promising nanomaterials from laboratory research to real-world application.

CONCLUSION

The study successfully demonstrated that silver nanoparticles can be synthesized using *Azadirachta indica* leaf extract through a simple, rapid, and eco-friendly protocol. This green method eliminates the need for hazardous chemicals, offering a non-toxic alternative to conventional physical and chemical synthesis techniques. The biosynthesis process proved efficient at room temperature, highlighting its practical potential for scalable production in biomedical, environmental, and agricultural applications. By aligning with principles of sustainability and safety, this research underscores the significant contribution of plant-mediated nanotechnology in advancing safe and accessible nanoparticle-based innovations.

Author Contribution

Authors	Contribution
Atfa Ashraf	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Ali Raza	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Rabia Sehar	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Rabeya Aziz	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Kinza Habibullah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Abdullah Anwar	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Rayia Ehsan	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Muniba Riaz	Writing - Review & Editing, Assistance with Data Curation

REFERENCES

1. Chaudhary RG, Desimone MF. Synthesis, Characterization, and Applications of Green Synthesized Nanomaterials (Part 1). *Curr Pharm Biotechnol.* 2021;22(6):722-3.
2. Shumail H, Khalid S, Ahmad I, Khan H, Amin S, Ullah B. Review on Green Synthesis of Silver Nanoparticles through Plants. *Endocr Metab Immune Disord Drug Targets.* 2021;21(6):994-1007.
3. Rani N, Singla RK, Redhu R, Narwal S, Sonia, Bhatt A. A Review on Green Synthesis of Silver Nanoparticles and its Role against Cancer. *Curr Top Med Chem.* 2022;22(18):1460-71.
4. Paiva-Santos AC, Herdade AM, Guerra C, Peixoto D, Pereira-Silva M, Zeinali M, et al. Plant-mediated green synthesis of metal-based nanoparticles for dermatopharmaceutical and cosmetic applications. *Int J Pharm.* 2021;597:120311.
5. Muddapur UM, Alshehri S, Ghoneim MM, Mahnashi MH, Alshahrani MA, Khan AA, et al. Plant-Based Synthesis of Gold Nanoparticles and Theranostic Applications: A Review. *Molecules.* 2022;27(4).
6. Hano C, Abbasi BH. Plant-Based Green Synthesis of Nanoparticles: Production, Characterization and Applications. *Biomolecules.* 2021;12(1).
7. Adeyemi JO, Oriola AO, Onwudiwe DC, Oyedeji AO. Plant Extracts Mediated Metal-Based Nanoparticles: Synthesis and Biological Applications. *Biomolecules.* 2022;12(5).
8. Hawadak J, Kojom Foko LP, Pande V, Singh V. In vitro antiplasmodial activity, hemocompatibility and temporal stability of *Azadirachta indica* silver nanoparticles. *Artif Cells Nanomed Biotechnol.* 2022;50(1):286-300.
9. Naidi SN, Harunsani MH, Tan AL, Khan MM. Green-synthesized CeO(2) nanoparticles for photocatalytic, antimicrobial, antioxidant and cytotoxicity activities. *J Mater Chem B.* 2021;9(28):5599-620.
10. Moosavy MH, de la Guardia M, Mokhtarzadeh A, Khatibi SA, Hosseinzadeh N, Hajipour N. Green synthesis, characterization, and biological evaluation of gold and silver nanoparticles using *Mentha spicata* essential oil. *Sci Rep.* 2023;13(1):7230.
11. Nguyen NTT, Nguyen TTT, Nguyen DTC, Tran TV. Green synthesis of ZnFe(2)O(4) nanoparticles using plant extracts and their applications: A review. *Sci Total Environ.* 2023;872:162212.
12. Salem SS, Fouda A. Green Synthesis of Metallic Nanoparticles and Their Prospective Biotechnological Applications: an Overview. *Biol Trace Elem Res.* 2021;199(1):344-70.

13. Jiang Y, Zhou P, Zhang P, Adeel M, Shakoor N, Li Y, et al. Green synthesis of metal-based nanoparticles for sustainable agriculture. *Environ Pollut.* 2022;309:119755.
14. Kumar V, Kaushik NK, Tiwari SK, Singh D, Singh B. Green synthesis of iron nanoparticles: Sources and multifarious biotechnological applications. *Int J Biol Macromol.* 2023;253(Pt 4):127017.
15. Hathout RM. Green synthesis of gold nanoparticles using plant products and plants extracts aiming for cancer therapy: helping the beauty to beat 'cure' the beast. *Artif Cells Nanomed Biotechnol.* 2022;50(1):275-7.
16. Lomeli-Rosales DA, Zamudio-Ojeda A, Reyes-Maldonado OK, López-Reyes ME, Basulto-Padilla GC, Lopez-Naranjo EJ, et al. Green Synthesis of Gold and Silver Nanoparticles Using Leaf Extract of Capsicum chinense Plant. *Molecules.* 2022;27(5).
17. Tareq M, Khadrawy YA, Rageh MM, Mohammed HS. Dose-dependent biological toxicity of green synthesized silver nanoparticles in rat's brain. *Sci Rep.* 2022;12(1):22642.
18. Zuhrotun A, Oktaviani DJ, Hasanah AN. Biosynthesis of Gold and Silver Nanoparticles Using Phytochemical Compounds. *Molecules.* 2023;28(7).
19. Jain AS, Pawar PS, Sarkar A, Junnuthula V, Dyawanapelly S. Bionanofactories for Green Synthesis of Silver Nanoparticles: Toward Antimicrobial Applications. *Int J Mol Sci.* 2021;22(21).
20. Mařátková O, Michailidu J, Miřková A, Kolouchová I, Masák J, Čejková A. Antimicrobial properties and applications of metal nanoparticles biosynthesized by green methods. *Biotechnol Adv.* 2022;58:107905.
21. Kabeya JK, Ngombe NK, Mutwale PK, Safari JB, Matlou GG, Krause RWM, et al. Antimicrobial capping agents on silver nanoparticles made via green method using natural products from banana plant waste. *Artif Cells Nanomed Biotechnol.* 2025;53(1):29-42.
22. Ahn EY, Park Y. Anticancer prospects of silver nanoparticles green-synthesized by plant extracts. *Mater Sci Eng C Mater Biol Appl.* 2020;116:111253.
23. Xue, Y., Chen, S., Yu, J., Bunes, B. R., Xue, Z., Xu, J., ... & Zang, L. (2020). Nanostructured conducting polymers and their composites: synthesis methodologies, morphologies and applications. *Journal of Materials Chemistry C*, 8(30), 10136-10159.
24. Amrutha, D. S., Joseph, J., Vineeth, C. A., John, A., & Abraham, A. (2021). Green synthesis of Cuminum cyminum silver nanoparticles: Characterizations and cytocompatibility with lapine primary tenocytes. *Journal of Biosciences*, 46(1), 1-14.
25. Heikal, Y. M., Şuřan, N. A., Rizwan, M., & Elsayed, A. (2020). Green synthesized silver nanoparticles induced cytogenotoxic and genotoxic changes in *Allium cepa* L. varies with nanoparticles doses and duration of exposure. *Chemosphere*, 243, 125430.
26. Chinnasamy, G., Chandrasekharan, S., Koh, T. W., & Bhatnagar, S. (2021). Synthesis, Characterization, Antibacterial and Wound Healing Efficacy of Silver Nanoparticles from *Azadirachta indica*. *Frontiers in Microbiology*, 12, 204.
27. Asimuddin, M., Shaik, M. R., Adil, S. F., Siddiqui, M. R. H., Alwarthan, A., Jamil, K., & Khan, M. (2020). *Azadirachta indica* based biosynthesis of silver nanoparticles and evaluation of their antibacterial and cytotoxic effects. *Journal of King Saud University-Science*, 32(1), 648-656.