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Green synthesis and limited antibacterial activity of silver nanoparticles mediated by neem leaf extract: a characterization-based assessment

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Highlights:

- Synthesis of Silver Nanoparticles.
- Eco-friendly synthesis of AgNPs using neem extract.
- Formation confirmed by UV–Vis, FTIR, and SEM.
- Neem acts as natural reducer and stabilizer.

Abstract: Green synthesis of silver nanoparticles (AgNPs) has become an ecologically safe substitute for traditional chemical methods; nonetheless, responsible use requires rigorous assessment of physicochemical characteristics and biological performance. In this work, *Azadirachta indica* (neem) leaf extract was used in ambient aqueous circumstances to create silver nanoparticles. UV-visible spectroscopy revealed a distinctive surface plasmon resonance peak at about 420 nm, confirming the creation of AgNPs. Neem phytochemicals, including phenolic compounds, flavonoids, proteins, and polysaccharides, have dual roles as surface-stabilizing ligands and reducing agents, according to Fourier Transform Infrared (FTIR) spectroscopy. Incomplete colloidal stabilization was indicated by irregular to quasi-spherical particle morphologies with noticeable aggregation and size heterogeneity, as revealed by scanning electron microscopy (SEM). The ASTM E2149–01 standard technique was used to assess the antibacterial activity of the produced AgNPs against Bacillus subtilis and Escherichia coli. For both bacterial strains, the nanoparticles' poor antibacterial effectiveness resulted in inhibition zones of around 6 mm, which were much smaller than those of the positive control. Particle aggregation, excessive organic surface capping, and low effective nanoparticle concentration all contribute to the poor antibacterial activity by lowering the bioavailability of silver ions. Overall, our study shows that neem-mediated green synthesis of AgNPs is feasible and offers mechanistic insights into their generation and stability.

However, before biomedical applications can be realistically considered, the biological data emphasize the necessity for more optimization, quantitative phytochemical analysis, aggregation control, stability assessment, and thorough biocompatibility evaluation.

Keywords: green synthesis; silver nanoparticles; *Azadirachta indica*

1. Introduction

Nanotechnology is a new scientific discipline that has emerged as a transformative field in contemporary science providing new solutions in various fields such as medicine, agriculture, electronics, catalysis and the removal of environmental pollutants [1]. It is worth mentioning that of the many nanomaterials studied so far, silver nanoparticles (AgNPs) have received exceptional interest because of their unusual physicochemical characteristics like having a high surface-area-to-volume ratio, optical tunability, electrical conductivity, and excellent antimicrobial activity [2,3]. These properties have made AgNPs promising systems to be used in various applications such as biomedical devices and drug delivery systems as well as in water purification and antimicrobial coating.

Although these methods have been widely used, traditional physical and chemical synthesis methods of silver nanoparticles usually require hazardous reducing agents, consume a lot of energy, and yield toxic by-products, which pose severe environmental and health problems [4]. To address these issues, green synthesis methods have become more and more popular as sustainable strategies. These are practices that focus on the exploitation of biological resources including plant extracts, microorganisms, and biopolymers to enable the formation of nanoparticles in mild conditions with little environmental effects [5].

Especially plant-mediated synthesis is an attractive approach, which combines the old botanical concepts with the modern nanoscience. The reduction, capping, and stabilization of the plant extracts occur at the same time thus making the synthesis process simpler and eliminate the need for additional chemical reagents [6]. Among several other species of plants which have been studied, *Azadirachta indica* (neem) has become a most promising biogenic source because of the high phytochemical content and established medicinal activity of the plant [7]. Neem leaves have a variety of bioactive constituents, such as flavonoids, terpenoids, phenolics, alkaloids and proteins, that can reduce silver ions (Ag^{+}) to metallic silver (Ag^{0}) nanoparticles and also give natural surface stabilization [8]. The green synthesis of AgNPs using *Azadirachta indica* extracts has been described in a number of research, although many of these studies are still lacking in terms of systematic synthesis control, repeatability, and standardised evaluation of nanoparticle attributes [9,10]. Additionally, very few investigations have combined thorough physicochemical and biological characterization with globally recognised criteria. By combining multi-technique characterization with ASTM-standardized processes, the current study stands out in this regard and ensures better reproducibility, reliability, and comparability of the data produced.

This study is novel in that (i) it is under ambient conditions, (ii) it is controlled, (iii) it is under aqueous conditions, (iv) it employs a controlling method of green synthesis of silver nanoparticles, (v) the systematic and comprehensive characterization framework that integrates both ASTM standards and UV Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM); and (vi) it involves an assessment of antibacterial performance using standardized methods. This contrasts with earlier reports that focus primarily on nanoparticle formation rather than critical evaluation of synthesis–property–biology relationships.

This study will thus aim to explore the viability and efficiency of a sustainable green synthesis pathway of AgNPs with the *Azadirachta indica* leaf extract. The objectives of the study include the explanation of the chemical reaction of size reduction to the formation of nanoparticles, optical and morphological properties of the produced AgNPs, and their ability to kill bacteria through the use of gram-positive and gram-negative bacteria as representative organisms. Through the integration of both environmentally-friendly synthesis and systematic and thorough characterization, this work will aim to make relevant contributions to the further development of sustainable nanotechnology and the rational design of green-synthesized nanomaterials to be applied in the future.

2. Methods

2.1. Materials

In Dhaka, Bangladesh fresh leaves of *Azadirachta indica* (neem) were collected from the surrounding area. The reason why the plant material was chosen is because it has a well-known phytochemical composition which consists of flavonoid, terpenoid, phenolics and saponins which are known to play a role in reduction of metal ions and stabilization of nanoparticles. Silver precursor ($AgNO_3$, purity $\geq$ 99%) was purchased in the form of silver nitrate at Merck (Germany) and then not purified any further. All the experimental procedures were done in double-distilled water to reduce ionic and particulate contamination. Glassware and all other laboratory equipment were extensively washed using detergent then distilled water, then ethanol and dried before being put into use.

2.2. Preparation of Azadirachta indica leaf extract

Neem leaves were first rinsed with running tap water to wipe out dust and other surface contaminants and then rinsed with distilled water. The shade-dried leaves were dried at ambient temperature (5–7 days) so as to maintain thermolabile bioactive compounds. The mechanized grinder was then sterilized and used to grind the dried leaves into a fine powder.

In the preparation of the aqueous leaf extract, 15 g of neem leaf powder was put into 200 mL of distilled water in a 500 mL borosilicate glass beaker. The blend was cooked at around 80 °C in 30 minutes with frequent stirring in an attempt to extract the aqueous phytochemicals. The solution was then allowed to cool to room temperature after heating and then filtered using Whatman No. 1 filter paper in order to eliminate solids that were remaining. The ensuing filtrate, which was greenish-brown in color, was kept in amber bottles at 4 °C and was used within 48 hours of preparation to maintain chemical stability.

2.3. Green synthesis of silver nanoparticles

The bioreduction of silver nanoparticles also made use of neem leaf extracts. By dissolving the proper quantity of silver nitrate in H_2O double distilled water, a 0.01 M $AgNO_3$ solution was produced. A 200 mL glass beaker was filled with 45 mL of $AgNO_3$ and magnetically swirled at room temperature (about 25 °C) during the synthesis as shown in Figure 1. Then, while continually stirring, 5 mL of prepared neem extract was added dropwise.

Surface plasmon resonance (SPR) caused the reaction mixture's color to gradually shift from pale yellow to dark brown, signifying the reduction of Ag^+ ions to metallic silver (Ag^0) nanoparticles. To enable full reduction and stability, the mixture was incubated for 24 hours at room temperature under static conditions.

To separate the produced nanoparticles, the colloidal solution was centrifuged for 15 minutes at 8000 rpm after incubation. To get rid of extra phytochemicals and unreacted precursors, the final pellet was rinsed three times with distilled water. The purified nanoparticles were kept in a desiccator for further characterization after being dried for six to eight hours at 60 °C in a hot air oven.

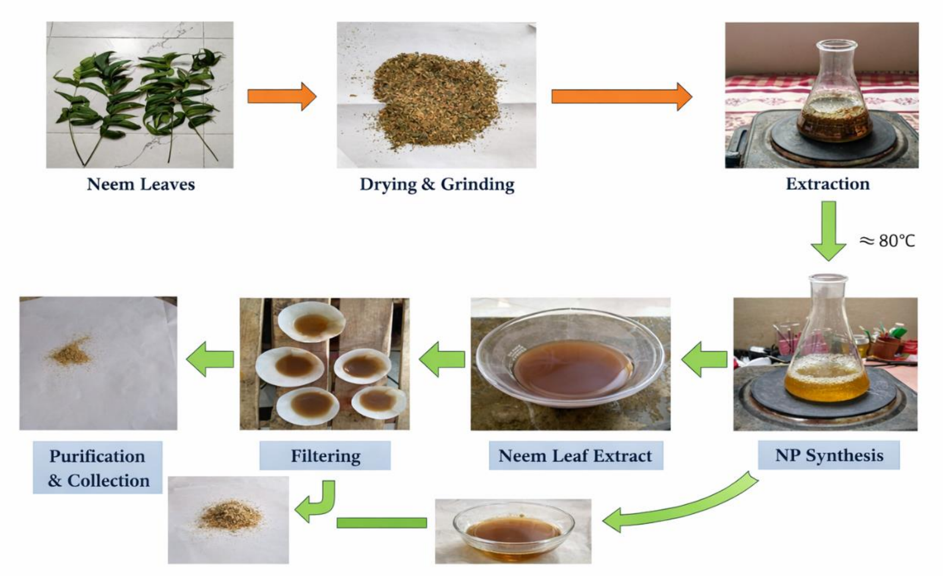

Figure 1. Schematic representation of the green synthesis of AgNPs using *Azadirachta indica* leaf extract.

2.4. UV-visible spectroscopy

UV-visible spectroscopy was used to examine the optical characteristics and formation kinetics of the produced AgNPs. A Shimadzu UV-1800 spectrophotometer was used to evaluate 10 mL aliquots of the reaction mixture that were collected at predefined intervals. A wavelength range of 300–700 nm was used to obtain absorption spectra. Additionally, the effects of temperature (10–60 °C) and reaction time (5–90 minutes) on the creation of nanoparticles were investigated. AgNP synthesis was verified by the emergence of a distinctive SPR absorption band.

2.5. Fourier transform infrared spectroscopy

The functional groups involved in the reduction and stability of silver nanoparticles were identified using FTIR spectroscopy. To create translucent pellets, dried AgNP powder was coarsely crushed and combined with spectroscopic-grade potassium bromide (KBr). A PerkinElmer FTIR spectrometer was used to capture FTIR spectra at room temperature in the 500–4000 cm^{-1} range. The spectra were examined to ascertain how neem phytochemicals' hydroxyl, carbonyl, amine, and ether functional groups contributed to the creation of nanoparticles.

2.6. Scanning electron microscopy

Scanning electron microscopy was used to analyze the produced AgNPs' surface morphology, particle size, and shape. To create a homogenous suspension, a little quantity of dried AgNP powder was mixed with acetone. After being placed on a spotless glass substrate, a drop of this solution was allowed to air dry. Before imaging, a small coating of gold was sputter-coated onto the sample to improve electrical conductivity.

A JEOL JSM-6490LV equipment running at an accelerating voltage of 20 kV was used to perform SEM analysis. The SEM equipment was utilized to validate the elemental composition and presence of silver inside the produced nanoparticles.

2.7. Antibacterial activity (ASTM E2149–01)

The ASTM E2149–01 standard dynamic contact technique was used to assess the antibacterial activity of the produced AgNPs. Gram-negative Escherichia coli and gram-positive Bacillus subtilis were selected as model bacterial strains. Nutrient broth was used to cultivate bacterial cultures, which were then adjusted to a concentration of around 10^6 CFU/mL.

Sterile swabs were used to evenly inoculate Mueller-Hinton agar plates. A sterile cork borer was used to construct wells with a diameter of 6 mm, and each well was filled with 100 µL of AgNP solution. Zones of inhibition were measured in millimeters following a 24-hour incubation period at 37 °C. As a positive control for comparison, a common antibacterial agent was employed.

3. Results

3.1. Analysis of UV-visible spectroscopy

UV–visible spectroscopy was used to establish that *Azadirachta indica* leaf extract promoted the synthesis of AgNPs. The creation of metallic silver nanoparticles is consistent with the reaction mixture's distinctive SPR absorption band, which was centered at about 420 nm. The appearance of this SPR peak shows that the phytochemicals in the neem extract are facilitating the reduction of Ag^+ ions to Ag^0.

The creation of nanoparticles with a rather homogeneous size distribution is suggested by the comparatively small SPR band. However, given the biological nature of the capping agents, UV-Vis spectroscopy by itself cannot rule out the existence of aggregated particles. Conclusions on size evolution or reaction kinetics are limited since no systematic red- or blue-shift study as a function of synthesis parameters was carried out.

3.2. Reduction and capping mechanisms: FTIR analysis

The functional groups involved in the reduction and stabilization of AgNPs were identified using FTIR spectroscopy. The spectra exhibited a large absorption band about 3430 cm^{-1}, corresponding to O–H stretching vibrations of phenolic and flavonoid chemicals. These groups are well known as essential electron donors in plant-mediated nanoparticle production.

C = O stretching vibrations were identified as a unique band about 1630 cm^{-1}, indicating the oxidation of hydroxyl-containing biomolecules during the reduction of Ag^+ ions. This suggests a reduction process where Ag^+ is reduced to Ag^0 and phenolic chemicals are oxidized to quinone-like structures. Further peaks at 1070 cm^{-1} (C–O–C vibrations) and 1380 cm^{-1} (C–N stretching) show that proteins, amino acids, and polysaccharides are involved in nanoparticle capping.

Although the qualitative existence of these functional groups is confirmed by FTIR, no quantitative phytochemical investigation was carried out. As a result, it was unable to ascertain the proportional contributions of proteins, carbs, or flavonoids to the reduction and stabilization processes.

3.3. Morphological analysis by SEM

The surface morphology, particle shape, and aggregation behavior of the produced AgNPs were investigated using SEM at various magnifications (Figure 2). Instead of precisely distinct nanoparticles, the sample is made up of unevenly shaped, mostly angular to quasi-spherical particulate aggregates, according to the SEM micrographs. The particles appear as heterogeneous clusters with rough and textured surfaces, suggesting the presence of organic capping layers produced from *Azadirachta indica* phytochemicals.

The micrographs exhibit significant particle agglomeration at lower magnifications (100×–200×), creating micrometer-scale clusters made up of smaller nanoscale and submicron elements. Strong interparticle interactions mediated by biomolecular capping agents, such as proteins and polyphenols, which encourage adhesion through hydrogen bonding and van der Waals forces, are probably what cause this aggregation. A frequent characteristic of green-synthesized nanoparticles is poor colloidal stabilization, which is indicated by the existence of these aggregates.

Individual particle characteristics contained inside bigger aggregates can be seen in higher-magnification pictures (500×) as shown in Figure 2. Although surface textures and granular characteristics can be used to infer discrete nanoparticles, overlapping structures and the lack of total particle separation make accurate particle size measurement difficult. The constituent particles appear to span a wide size range from the nanometer to submicron scale based on visual estimation from the supplied scale bars, suggesting polydispersity in the synthesized sample.

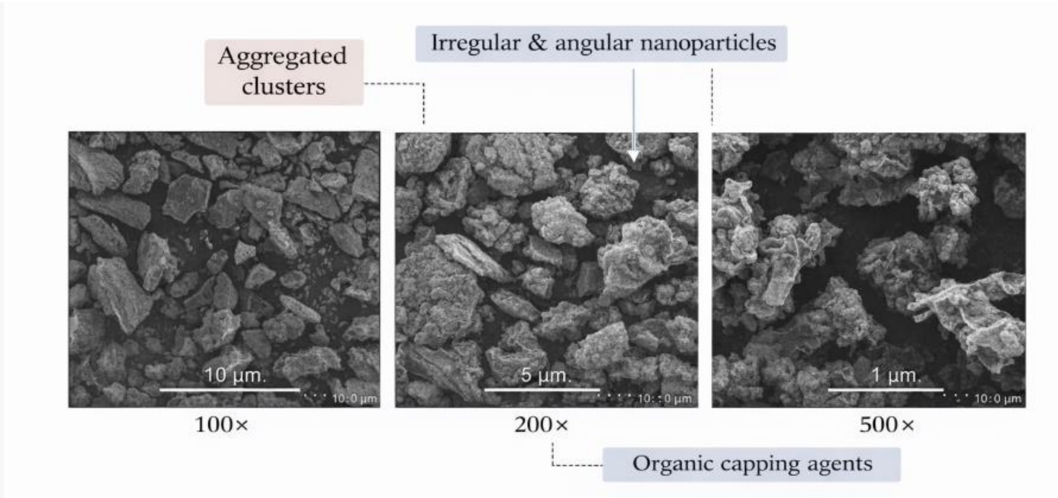

Figure 2. SEM micrographs showing the surface morphology and aggregation behavior of green-synthesized AgNPs mediated by *Azadirachta indica* leaf extract.

Strong organic surface functionalization and efficient reduction of silver ions are suggested by the observed surface roughness and uneven shape. However, the strong aggregation shown at all magnifications would drastically lower the nanoparticles' effective surface area, which could have a detrimental influence on functional characteristics like antibacterial activity. The current morphological evaluation is limited by the absence of quantitative particle size distribution analysis and aggregation measures.

Overall, the SEM data demonstrate the difficulties associated with aggregation and size heterogeneity while also confirming the effective creation of silver-containing particulate forms using green synthesis. To increase particle homogeneity and functional performance, our results highlight the need for additional synthesis condition adjustment, including extract concentration, reaction pH, and post-synthesis dispersion treatments.

3.4. Evaluation of antibacterial activity

Using the ASTM E2149–01 methodology, the antibacterial activity of the produced AgNPs was assessed against Bacillus subtilis and Escherichia coli. For both bacterial strains, the AgNPs generated inhibitory zones of around 6 mm. The positive control, on the other hand, showed much greater antibacterial effectiveness with inhibition zones of 25–26 mm. These findings show that the produced AgNPs performed poorly against bacteria under the investigated circumstances. The restricted inhibitory zones imply that the nanoparticles were either present in insufficient concentrations, showed decreased bioavailability as a result of aggregation, or released silver ions at a pace insufficient to efficiently damage bacterial membranes.

4. Discussion

The current work shows that *Azadirachta indica* leaf extract may be used to create AgNPs in a way that is safe for the environment. The successful formation of nanoparticles with distinct optical and morphological characteristics is confirmed by the physicochemical characterization; however, the biological performance, especially the antibacterial efficacy, reveals significant limitations that must be carefully taken into account before claims of biomedical relevance can be made. This discussion critically assesses the observed antibacterial activity and combines the characterization data with molecular insights into nanoparticle production.

Using UV–visible spectroscopy, the successful synthesis of AgNPs was first verified by the emergence of a distinctive SPR peak with a center of about 420 nm. This SPR band is a well-known indicator of the production of Ag^0 nanoparticles and results from the collective oscillation of conduction band electrons in metallic silver nanoparticles upon contact with incoming light. Limited large-scale aggregation in the colloidal stage and adequately regulated particle production are suggested by the comparatively small SPR peak and the lack of secondary absorption bands at higher wavelengths. These data imply that biomolecules included in the neem extract have an efficient stabilizing function during synthesis.

Important information about the chemical processes behind the stability of the resultant nanoparticles and the decrease of Ag^+ ions was revealed by FTIR spectroscopy. The large O–H stretching band seen at around 3430 cm^{-1} is indicative of hydroxyl groups found in flavonoids and phenolic chemicals, which are prevalent in Azadirachta indica. As shown by the generalized reaction, these phytochemicals are recognized electron donors and probably aid in the reduction of Ag^+ to Ag^0 by oxidizing hydroxyl groups to carbonyl moieties.

$$Ag^+ + Ar\text{–}OH \rightarrow Ag^0 + Ar{=}O + H^+$$

This oxidation process is supported by the appearance of a clear C=O stretching band about 1630 cm^{-1}. The presence of proteinaceous or amino-containing substances is indicated by additional FTIR bands that correspond to C–N stretching (~1380 cm^{-1}) and amide-related vibrations. Through lone-pair electron interactions, these biomolecules may adsorb onto the surface of nanoparticles, creating an organic capping layer that prevents unchecked particle development. The presence of polysaccharides or glycosidic chemicals that contribute to steric stabilization is further suggested by vibrational characteristics at 1070 cm^{-1}. Overall, the FTIR data indicate neem phytochemicals' dual function as capping ligands and reducing agents. Nevertheless, the absence of quantitative phytochemical research restricts the capacity to determine which molecular species dominate the reduction and stabilization processes.

The produced AgNPs mostly display irregular to quasi-spherical morphologies throughout a wide nanoscale to submicron size range, according to SEM examination. Significant aggregation was visible at various magnifications, even though certain areas showed somewhat scattered particles. Green-synthesised nanoparticles frequently exhibit this kind of aggregation, which is probably caused by thick organic capping layers and intermolecular hydrogen bonds between surface-bound phytochemicals. Aggregation significantly lowers effective surface area and restricts the availability of silver ions, both of which are essential for biological interactions, even if this shape could be suitable for some physicochemical applications. The lack of quantitative particle size distribution analysis and aggregation metrics further constrains precise morphology–function correlations.

Weak antibacterial activity under the experimental circumstances was shown by the antibacterial assay, which showed inhibition zones of around 6 mm against both Bacillus subtilis and Escherichia coli. These inhibition zones clearly show limited antimicrobial activity since they are much smaller than those generated by the positive control (25–26 mm) and just slightly larger than the well diameter. This result might be caused by a number of things. First, it's possible that the assay's effective concentration of AgNPs was too low to produce a potent bactericidal reaction. Second, although the thick capping layer formed from phytochemicals is advantageous for the stability of nanoparticles, it may prevent direct interaction between silver surfaces and bacterial membranes and inhibit the release of Ag^+ ions, two crucial mechanisms of antibacterial activity. Third, particle aggregation probably limited the interaction of nanoparticles with microbial cells and decreased their bioavailability. When combined, these elements offer a molecular explanation for the observed poor antibacterial efficacy.

These results highlight a crucial difference between functional biological effectiveness and good green synthesis. Although the sustainable synthesis of chemically stable silver nanoparticles is made possible by *Azadirachta indica* extract, the antibacterial efficacy is largely dependent on the concentration of nanoparticles, surface chemistry, dispersion state, and ion-release behavior. Accordingly, the present antimicrobial data should be taken as a preliminary assessment rather than final proof of biological application. Additionally, this study did not do any biocompatibility or toxicity assessments, which is a crucial omission considering the known dose-dependent cytotoxicity of silver nanoparticles.

Furthermore, neither thorough control tests nor systematic optimization of synthesis factors including extract concentration, $AgNO_3$ concentration, pH, and temperature were included in the study. Despite its significance for practical deployment and commercial translation, long-term colloidal stability and storage-induced aggregation were also not assessed.

Overall, our work demonstrates that *Azadirachta indica*-mediated green synthesis is a feasible method for creating silver nanoparticles with certain physicochemical properties. However, extensive synthesis condition optimization, quantitative phytochemical profiling, aggregation control, stability assessment, thorough antimicrobial benchmarking, and stringent biocompatibility evaluation will be necessary to translate these materials into biomedical or antimicrobial applications. Addressing these factors will be vital for fully realizing the functional potential of green-synthesized AgNPs.

5. Conclusion

This work demonstrates that *Azadirachta indica* leaf extract may effectively mediate the sustainable and eco-friendly production of silver nanoparticles. FTIR research showed that neem-derived phytochemicals serve as both capping ligands and reducing agents, whereas physicochemical evaluation confirmed the synthesis of AgNPs with a distinctive surface plasmon resonance. The considerable effect of organic surface functionalization inherent in plant-mediated synthesis was reflected in the irregular to quasi-spherical morphologies shown by SEM analysis, together with significant aggregation and size variation. When compared to the positive control, the produced AgNPs showed modest inhibitory zones, indicating poor antibacterial action against Escherichia coli and Bacillus subtilis despite effective nanoparticle production. The significance of nanoparticle concentration, surface chemistry, aggregation state, and silver ion release behavior on biological performance is highlighted by this restricted efficacy. Accordingly, the antibacterial findings should be viewed as preliminary and inadequate to justify rapid biological use.

The study makes a clear contrast between the achievement of functional biological efficacy and the viability of green synthesis. Future research should concentrate on methodical synthesis parameter optimization, quantitative phytochemical constituent profiling, aggregation control, long-term stability assessment, thorough antimicrobial benchmarking, and exacting biocompatibility and toxicity evaluations in order to improve the practical applicability of neem-mediated AgNPs. Transforming green-synthesized silver nanoparticles from proof-of-concept materials into dependable functional nanomaterials will require addressing these factors.

Data availability statement

No supplementary or additional data were generated in this study.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Conceptualization, Md. Shakil Chowdhury; methodology, Md. Shakil Chowdhury; supervision, Md. Shakil Chowdhury and Md. Sahadat Hossain; project administration, Md. Shakil Chowdhury; funding acquisition, Md. Shakil Chowdhury; investigation, Md. Zobair Al Mahmud, Md. Thohid Rayhan, Md. Younus and Md. Roman Babu; resources, Md. Younus and Md. Roman Babu; data curation, Md. Zobair Al Mahmud and Md. Abdul Hannan Sarker; formal analysis, Md. Zobair Al Mahmud, Md. Abdul Hannan Sarker and Md. Thohid Rayhan; validation, Md. Abdul Hannan Sarker, Md. Roman Babu and Md. Sahadat Hossain; visualization, Md. Zobair Al Mahmud, Md. Abdul Hannan Sarker and Md. Younus; writing—original draft preparation, Md. Zobair Al Mahmud; writing—review and editing, Md. Sahadat Hossain. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Malik S, Muhammad K, Waheed Y. Nanotechnology: a revolution in modern industry. *Molecules* 2023, 28(2):661.
[2] Duman H, Eker F, Akdaşçi E, Witkowska AM, Bechelany M, *et al.* Silver nanoparticles: a comprehensive review of synthesis methods and chemical and physical properties. *Nanomaterials* 2024, 14(18):1527.
[3] Fatehbasharzad P, Fatehbasharzad P, Sillanpää M, Shamsi Z. Investigation of bioimpacts of metallic and metallic oxide nanostructured materials: size, shape, chemical composition, and surface functionality: a review. *Part. Part. Syst. Char.* 2021, 38(10):2100112.
[4] Rodríguez-Félix F, Graciano-Verdugo AZ, Moreno-Vásquez MJ, Lagarda-Díaz I, Barreras-Urbina CG, *et al.* Trends in sustainable green synthesis of silver nanoparticles using agri-food waste extracts and their applications in health. *J. Nanomater.* 2022, 2022(1):8874003.
[5] Rai M, Yadav A, Ingle A, dos Santos CA. An introduction to biopolymer-based nanofilms, their applications, and limitations. In *Biopolymer-Based Nano Films*. Amsterdam: Elsevier, 2021. pp. 3–17.
[6] Owaid MN, Rabeea MA, Aziz AA, Jameel MS, Dheyab MA. Mycogenic fabrication of silver nanoparticles using Picoa, Pezizales, characterization and their antifungal activity. *Environ. Nanotechnol. Monit. Manage.* 2022, 17:100612.
[7] Sarkar S, Singh RP, Bhattacharya G. Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *3 Biotech* 2021, 11(4):178.
[8] Ullah S, Khalid R, Rehman MF, Irfan MI, Abbas A, *et al.* Biosynthesis of phyto-functionalized silver nanoparticles using olive fruit extract and evaluation of their antibacterial and antioxidant properties. *Front. Chem.* 2023, 11:1202252.

[9] Ahmed M, Marrez DA, Mohamed Abdelmoeen N, Abdelmoneem Mahmoud E, Ali MAS, *et al.* Studying the antioxidant and the antimicrobial activities of leaf successive extracts compared to the green-chemically synthesized silver nanoparticles and the crude aqueous extract from *Azadirachta indica*. *Processes* 2023, 11(6):1644.

[10] Jameel MS, Aziz AA, Dheyab MA, Khaniabadi PM, Kareem AA, *et al.* Mycosynthesis of ultrasonically-assisted uniform cubic silver nanoparticles by isolated phenols from Agaricus bisporus and its antibacterial activity. *Surf. Interfaces* 2022, 29:101774.