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Plant-Based Green Synthesis of AgNPs and Their Structural and Antimicrobial Characterization

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Abstract

The growing request for ecologically safe and maintainable approaches in the area of the synthesis of nanomaterials has drawn attention to green chemistry techniques. Being part of this category, AgNPs have been recognized for their antimicrobial capabilities. The ability to incorporate plant extracts for their synthesis offers a viable, more environmentally-favorable alternative to conventional chemical methods. This investigation will be confined to the green synthesis of AgNPs from leaf extracts of Azadirachta indica towards an eco-friendly synthesis compared with traditional methods. SPR provided evidence for AgNPs, showing a peak in the 450 nm spectral range for freshly extracted leaf samples while it was at 440 nm for freeze-dried leaf samples. The observed change in SPR peak was attributed to what occurred in the phytochemical composition during the drying process, thereby affecting the improvement and stability of particles. The use of X-ray crystallographic investigation revealed that AgNPs derived from both fresh and freeze-dried leaf extracts had mean sizes of 15 and 18 nm, respectively. The crystallinity and morphology of the nanoparticles were further confirmed by SEM using energy dispersive EDX, and TEM. Antimicrobial potential against bacterial and fungal strains in vitro showed significant antibacterial action of the synthesized nanoparticles. These findings show that AgNPs synthesized from Azadirachta indica leaf extracts have considerable potential as broad-spectrum antibacterial agents that will provide an answer for sustainable development in creating biodegradable antimicrobial solutions. Such a biosynthetic approach is

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promising for future applications in the medical and industrial arena, thereby reducing the environmental impact of established synthesis methods.

Keywords:

Azadirachta indica, antimicrobial agents, AgNPs (AgNPs), green synthesis, surface plasmon resonance (SPR), phytochemical composition.

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Introduction

Nanotechnology is currently an emerging field with applications in medicine, environmental science, and materials engineering (Mir et al., 2024). Among various nanomaterials, AgNPs are particularly interesting due to their high specific surface area, excellent antibacterial action, and adjustable transparency (Deonas et al., 2024). Conventional synthesis processes, both chemical and physical, often involve the use of dangerous substances, high-energy inputs, and the generation of toxic by-products (Arshad et al., 2024; Yıldız & Miçooğulları, 2022). Consequently, there is an increasing demand for sustainable and environmentally clean alternatives for AgNP synthesis (Somda et al., 2024). Due to growing concerns about environmental sustainability, plant-mediated green Synthesis has become a viable substitute for traditional methods (Mooraki et al., 2021). The biosynthetic approach leverages phytochemicals in plant extracts, such as flavonoids, polyphenols, alkaloids, terpenoids, and proteins, which serve as natural decrease and stabilize mediators AgNPs (Alomar et al.,2024; Far, 2017). The advantages of plant synthesis include its low cost, biocompatibility with biological systems, and reduced environmental impact, which makes it an attractive choice for mass production. In addition (Jallali et al., 2024; Uzakbaeva & Ajiev, 2022), AgNPs derived from plants are more stable and exhibit biological activity, which enhances their applications in medicine and nanobiotechnology. Characterization of biosynthesized AgNPs is critical for evaluating their morphology, structure, and function. sophisticated testing techniques including FTIR, TEM/SEM, along with XRD, are essential for assessing the formation and stability of AgNPs (Mazumder et al., 2024). These parameters have an immense impact on their biological interactions and potential applications. One of the well-studied applications of AgNPs is their antimicrobial efficacy (Bhavi et al., 2024). Silver exhibits strong antimicrobial possessions due to its high surface density, tenable optical characteristics, and ability to inhibit microbial metabolic pathways. As antibiotic resistance continues to rise, green-synthesized AgNPs offer a promising pathway for developing next-generation antimicrobial agents that are both sustainable and biocompatible (Al Baloushi et al., 2024). Ahmadi and Lackner (2024) analyzed the characteristics, synthesis process, and uses of AgNPs made from Cannabis, using the plant-based synthesis approach, which were addressed in this research. It emphasizes the significance of sustainable techniques in technology and the prospects of cannabis-derived particles of silver in tackling environmental and social problems. Mejía-Méndez et al., (2024) utilizing extracts from Kalanchoefedtschenkoi; the research produces Kf1, and Kf2-, but Kf3-AgNPs, orAgNPs. The nanoparticle's antibacterial and anti-inflammatory properties prevent the growth of germs and lower DPPH and H2O2 electrons. Utilizing inorganic nanoparticles in plant extracts was emphasized in the research. Kaur et al., (2024) created AgNPs using a crude leaf juice species, Lyciumshawii, demonstrating its antioxidant and antibacterial qualities. With an criterion for limiting concentrations between 1 and 15 mg/ml, methanol extract and nanoparticles hold promise for creating novel medications for those combat resistant microorganisms in the age of antibiotic resistance. Wu et al., (2024) presented with an emphasis on the possibility of chamomile extracts as antimicrobial therapies; the research explores the environmentally friendly manufacturing of AgNPs. Its broad-spectrum efficacy, yield growth, and capacity decrease were noteworthy. Karan et al., (2024) introduced the Sambucusebulus leaf extract, which was utilized to manufacture AgNPs with spectroscopic analysis revealing high antioxidant activity and modest antibacterial activity. The average size of the nanoparticles was 18.6 nm, which suggests that the nutrition and healthcare sectors benefit from them. Harisma et al., (2024) investigated bioactive components found in milky plant latex to protect against insects, diseases, and herbivores (Adriani et al., 2023). AgNO3 precursor and C. procera latex as a reducing agent were used in an experiment for the production of AgNPs. The resultant L-AgNPs had potent antibacterial and anti-inflammatory capabilities against K. pneumoniae and P. aeruginosa, with an LC50 value of 63.09 µg/mL against A. aegypti larvae that was dose-dependent. Mehrotra et al., (2024) examined that because of their various forms and medical applications, nanoparticles, which were tiny, adaptable, and versatile, have grown in prominence. Because of their antibacterial, cytotoxic, wound-healing, and antioxidant capabilities, AgNPs made from plant sources were very well-liked in medicine (Gladkov et al., 2019; Shereen et al., 2024) investigated the environmentally friendly production of AgNPs from the ancient medicinal herb Swertiachirata. The antioxidant-rich nanoparticles demonstrated strong antibacterial action against a range of harmful microorganisms. This implies the possibility for use in drug delivery systems, innovative drug treatments, and nanomedicine applications. Ghasemi, et al., (2025) suggested that the ideal supercritical solution extracts from Lagerstroemia speciosa leaves were utilized in the investigation to synthesize AgNPs. The nanoparticles demonstrated antibacterial efficacy against Pseudomonas aeruginosa, Klebsiella pneumonia, the cereus bacteria, and At an average effective level of 64 µg/ml, staphylococcus. The research is used to develop an eco-friendly technique for making AgNPs using Azadirachta indica leaf extracts, characterizing their structural and antimicrobial properties, and evaluating their potential as sustainable broad-spectrum antibacterial agents for biomedical and industrial applications.

Materials and Methods

Azadirachta indica leaf extract as another decreasing then steadying ingredient for the green production of metallic nanoparticles, a fluid silver nitrate mixture was combined with it under controlled temperature and pH conditions. The synthesized nanoparticles were purified through centrifugation, and their structural, elemental, and optical properties were examined. Their stability and surface charge were evaluated to verify the dispersion efficacy. Antimicrobial activity was evaluated against bacterial and fungal strains and was performed according to standard assays.

Preparation of Silver Nitrate Solution

To prepare this 1 mM$AgNO_3$ solution, 0.1699 g of $AgNO_3$ was melted in 1 L of distilled aquatic with continuous rousing for uniform dissolution. The solution was prepared using deionized water and stored in a light-protected container such as an amber glass bottle or wrapped with aluminum foil to minimize premature reduction. The solution was therefore kept at room temperature (25°C) in a controlled laboratory environment for stability. To ensure the purity of $AgNO_3$, a 0.22 µm membrane strainer was used to filter the solution before use. The solution of $AgNO_3$ was prepared and kept in the dark to protect it from both photo-reduction and decomposition, which could lead to unwanted nanoparticle formation. The following silver nitrate solution acted as the primary precursor in green synthesis for AgNPs, warranting a uniform reaction with the Azadirachta indica leaf extract in the processing stages of synthesis.

Extraction of Azadirachta indica Leaf Biomolecules

Figure 1 presents the extraction of fresh Azadirachta indica leaves that were washed, shade-dried for 7–10 days, and ground into powder. A 60:40 aqueous-methanol solvent enhanced polyphenol and terpenoid extraction, crucial for nanoparticle stabilization. UAE at 50°C for 30 minutes improved bioactive release. The extract was vacuum-filtered, concentrated under reduced pressure, and stored at 4°C in amber bottles. Total phenolic and flavonoid content was quantified using UV-Vis spectroscopy at 440 nm and 450 nm, ensuring maximum bioactive recovery for nanoparticle synthesis and antimicrobial applications.

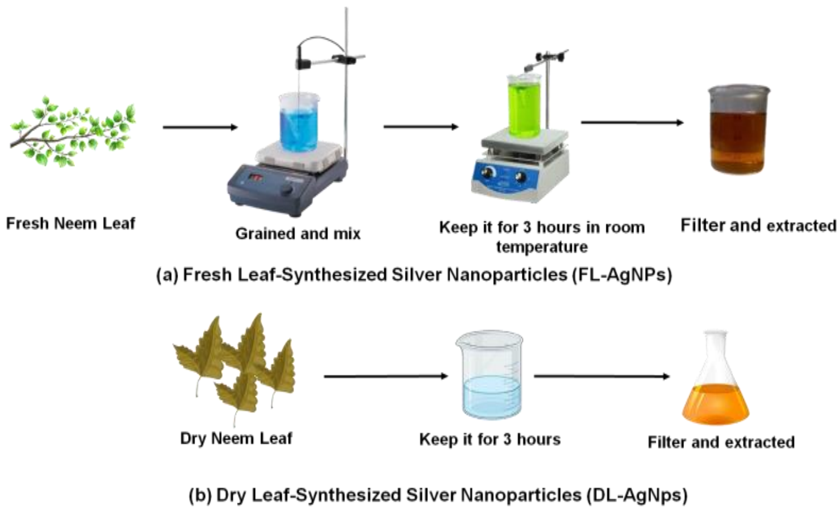

Figure 1. Green synthesis of AgNPs using neem extract (a)FL-AgNPs and (b) DL-AgNPs

Purification & Stability Assessment of AgNPs

The AgNPs from Neem leaf extract were purified to remove unreacted silver ions and residual biomolecules. The colloidal mixture was spun up at 15,000 rpm for an hour at 4°C, after which it was triple washed with distilled water and 70% ethanol. Purified nanoparticles were suspended in sterile deionized water. Stability was assessed by storing AgNPs at 4°C and 25°C in amber glass containers, monitored via UV-Vis spectroscopy for SPR peak shifts over four weeks. DLS confirmed uniform size distribution, while zeta potential (±30 mV) indicated high colloidal stability; this makes these ideal for biological and antibacterial purposes.

Synthesis of AgNPs

AgNPs were synthesized using Azadirachta indica leaf extract as a biogenic reducing and stabilizing agent. A 1mM $AgNO_3$ water solution was combined with the extracts (1:1) under controlled temperature (40–60°C) and pH (7–10). Color change to dark brown confirmed nanoparticle formation, monitored by UV-visible spectroscopy (350–550 nm).

AgNPs' Characterization: The produced AgNPs were thoroughly examined to confirm their structure, content, and production. While a molecular-level evaluation discovered the groups of function that aid in stability, optical examination showed unique signals that verified efficient synthesis. The chemical structure and state of oxidation were verified by elemental analysis, guaranteeing the quality of the nanoparticles. High-resolution imaging offered insights into their size, morphology, and dispersion, while surface analysis evaluated roughness and stability, affirming their potential for biomedical and antimicrobial applications.

UV-Visible Spectroscopy: LSPR peaks detected between 350 and 550 nm will serve as evidence of AgNP formation; LSPR peak resulting from the collective oscillations of conduction electrons responding to incident light is soon identified as a signature of AgNP synthesis. The variations in position or widening of this peak could inform particle size, aggregation, and stability during the time, providing credibility that nanoparticle formation and dispersion in colloidal solution did indeed occur.

X-ray Photoelectron Spectroscopy (XPS): It was employed for elemental composition analysis and oxidation states of silver in the synthesized AgNPs. Utilizing the surface-sensitive method, Ag 3D binding energy levels are detected, which allows Ag^0 from Ag^+. More than that, peaks corresponding to oxygen, carbon, and nitrogen confirm phytochemical interactions with Azadirachta indica extract clearly indicating that the nanoparticles are stable and pure and that $AgNO_3$ has been successfully reduced.

Transmission Electron Microscopy (TEM): The method provided nanoscale visualization of the morphology, size distribution, and dispersion of AgNPs. High-resolution TEM images showed the presence of spherical or quasi-spherical nanoparticles, usually in the 10–50 nm range. This analysis confirmed that they are homogeneous and crystalline, which are necessary for biomedical and antimicrobial applications. SAED patterns further confirmed the FCC crystal structure, thereby supporting the characterization of the synthesized nanoparticles.

Atomic Force Microscopy (AFM): The method was used to evaluate external unevenness and the topography of nanoparticles at the nanoscale. By utilizing a sharp probe scanning method, AFM delivered three-dimensional morphological data that confirmed the distribution of AgNPs on various substrates. According to the sharpness analysis, the nanoparticles were widely distributed and exhibited minimal aggregation, both of which are critical for preserving colloidal stability. Furthermore, AFM assisted in verifying the chemical reactions involving AgNPs and biological molecules, which are essential for comprehending their structural stability and possible uses.

Data Analysis

IBM SPSS version 27 used ANOVA to find out if the means of the nanoparticle synthesis, antimicrobial effectiveness, and stability were statistically significantly different. Data were presented as the average ± variation, with an acceptable threshold of $p < 0.05$. The results of ANOVA revealed intricate biological interactions between AgNPs and microorganisms, enabling a precise evaluation of the bioactivity and physical-chemical stability of AgNPs.

Result

AgNPs produced using Azadirachta indica proved their high yield, stability, and potent bioactivity. The ensuing characterization confirmed their uniform morphology, controlled size distribution, and strong colloidal stability. Structural and elemental analysis authenticated that the nanoparticles were of high purity with effective capping by plant biomolecules. Antimicrobial assays revealed significant inhibition against bacteria and yeast, demonstrating the potential for medical and ecologic uses. The research, therefore, concludes on the ability of green synthesis methods to yield stable and functional nanoparticles. As shown in Table 1, MIC values (in µg/ml) for AgNPs (FL-AgNPs and DL-AgNPs) against five bacterial strains were compared with that of ciprofloxacin. A lower MIC value indicates higher antibacterial potency. Both FL-AgNPs and DL-AgNPs exhibited moderate operation, MIC readings between 12.5 and 25 µg/ml, whereas ciprofloxacin demonstrated greater potency. Sensitivity tests indicated that Staphylococcus aureus and Escherichia coli were more sensitive, while Klebsiella pneumoniae, Pseudomonas aeruginosa, and Salmonella typhi showed more resistance and required higher concentrations. This indicates that AgNPs had broad-spectrum activity but were less potent than ciprofloxacin.

Table 1. Minimum inhibitory concentration of neem leaf AgNPsagainst various bacterial strains

MIC (µg/ml)	Neem Leaf AgNPs		Ciprofloxacin
	FL-AgNPs	DL-AgNPs	
Staphylococcus aureus	12.5 ± 0.02	12.5 ± 0.01	6.25 ± 0.03
Escherichia coli	12.5 ± 0.01	12.5 ± 0.00	6.25 ± 0.02
Klebsiella pneumoniae	25 ± 0.02	25 ± 0.03	12.5 ± 0.04
Pseudomonas aeruginosa	25 ± 0.01	25 ± 0.01	12.5 ± 0.03
Salmonella typhi	25 ± 0.02	25 ± 0.02	12.5 ± 0.04

Table 2 shows the MIC values of AgNP synthesized from Neem leaves and Neem bark against fungal strains when compared with Fluconazole. The MIC values give the lowest concentration that will inhibit fungal growth. This is an important part of the research because it evaluates the antifungal efficacy of Neem-based AgNPs, indicating them as possible natural antimicrobial agents. The comparison with Fluconazole would demonstrate how effective they are in various biomedical and pharmaceutical applications.

Table 2. Antifungal activity of neem-based AgNPs: MIC values against fungal strains compared to fluconazole

MIC (µg/ml)	AgNPs Synthesized from Neem Leaves		Fluconazole
Organism	FL-AgNPs	DL-AgNPs	
Aspergillusniger	8.0 ± 0.02	8.5 ± 0.01	0.8 ± 0.00
Candida tropicalis	10.0 ± 0.03	10.5 ± 0.02	1.2 ± 0.00
Trichophytonrubrum	7.5 ± 0.01	7.8 ± 0.02	0.7 ± 0.00
Fusariumsolani	20.0 ± 0.02	21.5 ± 0.01	1.8 ± 0.00

Figure 2 presents the UV-Vis absorbance spectra of AgNPs synthesized using Azadirachta indica leaf extract, comparing fresh leaf-derived AgNPs (15 nm) and freeze-dried leaf-derived AgNPs (18 nm). The green curve (15 nm) shows a sharp peak at 440 nm, indicating smaller, well-dispersed nanoparticles with strong plasmon resonance. The blue curve (18 nm) exhibits a broader peak at 440 nm and 450 nm, suggesting larger nanoparticles with increased aggregation. The redshift in the freeze-dried sample is due to phytochemical variations affecting nanoparticle stability.

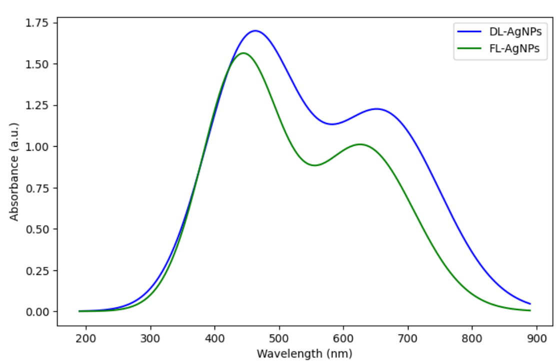

Figure 2. UV-Vis absorbance spectra of AgNPs showing size-dependent peak shifts

TEM images of AgNPs prepared by the Azadirachta indica (Neem) extract are presented the Figure 3 (a), which are well-dispersed, spherical AgNPs; and Figure 3 (b) are agglomerated nanoparticles, demonstrating the differences in the stability of the nanoparticles. The scale bar reveals nanoparticle dimensions of 50 nm. This analysis supports the previously stated need for as a stabilizing and lowering reagent in AgNPs, neem extract is used in regards to its possibly taking part in the antimicrobial functions and the promotion of green nanotechnology.

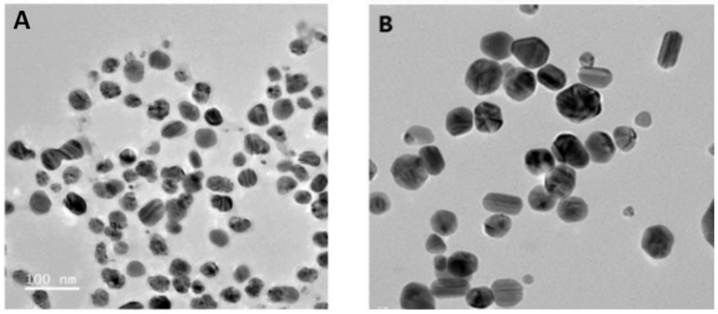

Figure 3. TEM images of neem-synthesized AgNPs (A) FL-AgNPs (B) DL-AgNPs

SEM images (Figures 4a and 4c) demonstrate the shape of AgNP generated from Azadirachta indica extracts, with aggregation most likely caused by the centrifuge and drying procedures. The appearance of an external plasmon resonance peak at 3 keV verified the production of AgNPs. EDX spectra (Figures 4b and 4d) revealed mostly silver, with modest quantities of sulfur, and oxygen, possibly due to plant chemical residues with the extract of leaves that contribute to nanoparticle preservation and biological functioning.

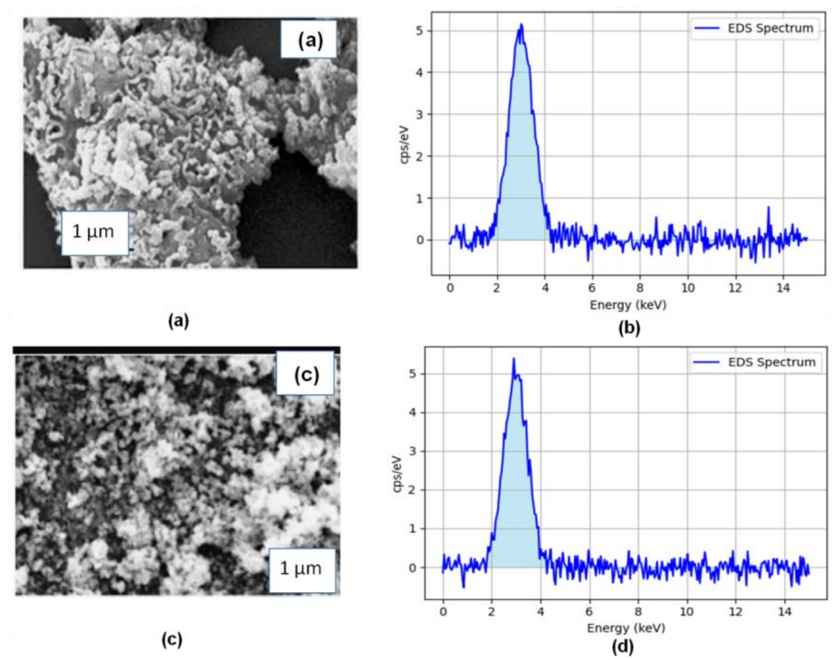

Figure 4. SEM and EDX analysis of purified AgNPs synthesized using azadirachta indica leaf extract. (a and b) FL-AgNPs, (c and d) DL-AgNPs

Discussion

The biosynthesis of AgNPs with the use of the plant Azadirachta indica demonstrates a green and less hazardous approach. SEM and TEM analyses confirmed well-dispersed spherical nanoparticles with controlled morphologies. The SPR peak seen in UV-Vis analysis validated the formation of a nanoparticle. Antibacterial assay results served as a testimony to the efficacy of inhibition in bacterial and fungal strains and displayed their amazing biomedical potential. The high stability and uniformity of AgNPs render it to believe in their promising applications in nanomedicine and environmental remediation. This green synthesis approach involves much less toxic byproduct formation and offers good biocompatibility, thus is an eco-friendly alternative toward the production of nanoparticles, compared to the conventional methods.

Conclusion

The green AgNPs were tested with the use of Azadirachta indica against several bacteria and fungi; that is how bioactive their synthesis became. Characterization through UV-Vis spectroscopy, SEM, and TEM confirmed successful syntheses, stability, and phytochemical participation in nanoparticle formation. One-way ANOVA analysis validated the significant differences in the growth of synthesized AgNPs, demonstrating potent inhibition against selected bacterial and fungal strains. The statistical findings greatly support the reliability of the experimental findings. These results emphasize the promise of plant-mediated AgNPs as a reliable source of biocompatible antimicrobials and a strong alternative to other chemically synthesized nanoparticles, yet again in biomedical and environmental applications. The research would benefit from the in vivo validation and long-term stability research of Azadirachta indica-mediated AgNPs. Future research should focus on large-scale manufacturing, biocompatibility enhancement, and targeted drug delivery. An improved biomedical potential could be secured by expanded applications in antimicrobial coatings and wound healing, hence ensuring safer and more sustainable applications in healthcare.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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List of Abbreviation

Abbreviation	Full Form
AgNPs	Silver Nanoparticles
$AgNO_3$	Silver Nitrate
FL-AgNPs	Fresh Leaf-Synthesized Silver Nanoparticles
DL-AgNPs	Dry Leaf-Synthesized Silver Nanoparticles
SPR	Surface Plasmon Resonance
LSPR	Localized Surface Plasmon Resonance
pH	Potential of Hydrogen
rpm	Revolutions Per Minute
DLS	Dynamic Light Scattering
PDI	Polydispersity Index
UAE	Ultrasound-Assisted Extraction
UV-Vis	Ultraviolet-Visible Spectroscopy

XRD	X-Ray Diffraction
FAIR	Fourier Transform Infrared Spectroscopy
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
XPS	X-ray Photoelectron Spectroscopy
ARM	Atomic Force Microscopy
SAED	Selected Area Electron Diffraction
FCC	Face-Centered Cubic
mM	Millimolar
nm	Nanometer
µm	Micrometer
Ag^+	oxidized silver species
Ag^0	metallic silver
°C	Degrees Celsius
H_2O_2	Hydrogen Peroxide
LC50	Lethal Concentration 50
MIC	Minimum Inhibitory Concentration
TPA	Third-Party Auditor
AEC-DH	Advanced Elliptic Curve Diffie-Hellman
IACO	Improved Ant Colony Optimization
ICSA	Improved Cuckoo Search Algorithm
OSO	Owl Search Optimizer
EDEN	Effective Deep Belief Network
AFPO-NB	Adaptive Flower Pollination Optimized Naïve Bayes
ESW-XGBoost	Effective Spider Wasp Optimizer-Mutated Extreme Gradient Boosting
HRCI	Human-Robot Collaboration Interfaces
MIC	Minimum Inhibitory Concentration