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Original Research Paper

Microbiology

"BIOFABRICATION OF SILVER NANOPARTICLES USING *ARGEMONE MEXICANA L.* FOR ANTIMICROBIAL EFFICACY"

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ABSTRACT The present study reports an eco-friendly and cost-effective green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Argemone mexicana*. The biosynthesized AgNPs were characterized by UV–Visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). UV–Vis analysis confirmed nano particle formation through a characteristic surface plasmon resonance peak at 441 nm. XRD patterns revealed the crystalline nature of AgNPs with cubic and hexagonal phases, while SEM analysis showed poly dispersed nanoparticles with sizes ranging from 20–50 nm. FTIR spectra indicated the involvement of plant-derived phytochemicals as reducing and stabilizing agents. The antimicrobial efficacy of the synthesized AgNPs was evaluated against a broad panel of clinically relevant pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, and *Candida albicans*, using the agar well diffusion method. The nanoparticles exhibited strong, concentration-dependent antimicrobial activity, with enhanced susceptibility observed among Gram-negative bacteria compared to Gram-positive bacteria. Significant antifungal activity against *Candida albicans* was also recorded. The results demonstrate that *Argemone mexicana*–mediated AgNPs possess broad-spectrum antimicrobial potential, highlighting their applicability in biomedical and pharmaceutical formulations. This green synthesis approach provides a sustainable alternative to conventional chemical methods and contributes to the advancement of environmentally benign nanotechnology.

KEYWORDS : Green synthesis, Silver nanoparticles, Argemone Mexicana, Antibacterial activity, Antifungal activity.

INTRODUCTION

The rapid emergence and dissemination of antimicrobial resistance have become a major challenge to modern healthcare systems worldwide. The declining effectiveness of conventional antibiotics has intensified the search for alternative antimicrobial agents capable of combating resistant pathogens. In recent years, nanotechnology has gained considerable attention as a promising approach for developing advanced materials with enhanced biological activity.

Silver nanoparticles (AgNPs) are among the most extensively studied nanomaterials due to their unique physicochemical properties and well-documented antimicrobial potential. The antimicrobial mechanisms of AgNPs involve multiple pathways, including cell membrane disruption, oxidative stress induction, and inhibition of vital cellular processes, which collectively contribute to their effectiveness against a wide range of microorganisms.

Despite their advantages, conventional chemical synthesis methods of AgNPs are often associated with toxic by-products and environmental concerns. Green synthesis strategies have therefore emerged as sustainable and eco-friendly alternatives. Among biological approaches, plant-mediated synthesis is particularly attractive because plant extracts contain diverse secondary metabolites that act as natural reducing and stabilizing agents.

Argemone mexicana is a traditionally used medicinal plant recognized for its antimicrobial, anti-inflammatory, and therapeutic properties. Its phytochemical composition makes it a suitable candidate for nanoparticle synthesis. However, limited information is available regarding the synthesis of silver nanoparticles using A. mexicana and their antimicrobial evaluation against clinically significant bacterial and fungal pathogens.

The present study focuses on the green synthesis of silver nanoparticles using Argemone mexicana leaf extract and their characterization using standard analytical techniques. Furthermore, the antimicrobial activity of the synthesized nanoparticles is investigated against selected pathogenic microorganisms, aiming to explore their potential applications in biomedical and pharmaceutical fields.

MATERIALS AND METHODS

Chemicals And Reagents

Silver nitrate ($AgNO_3$, analytical grade) was procured from Sigma-Aldrich and used without further purification. All solutions were prepared using double-distilled water. Nutrient agar and Sabouraud dextrose agar media were obtained from HiMedia Laboratories, India.

Plant Material Collection And Preparation

Fresh and healthy leaves of *Argemone mexicana* were collected from local fields of Amravati and Akola districts, Maharashtra, India. The collected plant material was thoroughly washed with running tap water followed by double-distilled water to remove adhering dust and impurities. The leaves were shade-dried, chopped into small pieces, and used for extract preparation.

Preparation Of Aqueous Leaf Extract

Approximately 5 g of finely chopped leaves were boiled in 100 mL of double-distilled water for 30 minutes. The mixture was allowed to cool to room temperature and subsequently filtered through Whatman No. 1 filter paper. The clear filtrate was stored at 4 °C and used as a reducing and stabilizing agent for nanoparticle synthesis.

Green Synthesis Of Silver Nanoparticles

A 5 mM aqueous solution of silver nitrate was prepared by dissolving the appropriate amount of $AgNO_3$ in double-distilled water. The leaf extract was mixed with the silver nitrate solution in a 1:1 (v/v) ratio under constant stirring at room temperature (25 ± 2 °C). The reaction mixture was incubated for 24 hours. Formation of silver nanoparticles was preliminarily confirmed by the visible color change from pale yellow to dark brown, indicating the reduction of Ag^+ ions to Ag^0 nanoparticles.

UV–Visible Spectroscopic Analysis

The reduction of silver ions and formation of nanoparticles

were monitored using a UV–Visible spectrophotometer in the wavelength range of 300–700 nm. The characteristic surface plasmon resonance (SPR) peak was recorded to confirm nanoparticle synthesis.

X-Ray Diffraction (XRD) Analysis

The crystalline nature and phase structure of the synthesized silver nanoparticles were analyzed using X-ray diffraction (XRD). Diffraction patterns were recorded using Cu K radiation (λ = 1.5406 Å) over a 2 range of 20–80°. The average crystallite size was calculated using the Debye–Scherrer equation.

Scanning Electron Microscopy (SEM)

The surface morphology and particle size distribution of the synthesized nanoparticles were examined using scanning electron microscopy. A thin film of dried nanoparticle suspension was coated onto a carbon stub and sputter-coated with gold prior to imaging.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed to identify the functional groups responsible for the reduction and stabilization of silver nanoparticles. The spectra were recorded in the range of 400–4000 cm^{-1} using dried nanoparticle samples mixed with potassium bromide (Kbr).

Antimicrobial Activity Assay

Test Microorganisms

The antimicrobial activity of the synthesized silver nanoparticles was evaluated against Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, and Candida albicans. All microbial strains were maintained on appropriate culture media and subcultured prior to experimentation.

Agar Well Diffusion Method

Antimicrobial activity was assessed using the agar well diffusion method. Sterile molten nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi) were poured into Petri dishes and allowed to solidify. Fresh microbial cultures were uniformly spread onto the agar surface using sterile cotton swabs. Wells of 6 mm diameter were aseptically punched into the agar plates, and different concentrations of silver nano particles (10, 20, and 30 ppm) were added into the wells. Plates were incubated at 37 °C for 24 hours for bacterial strains and at 30 °C for 48 hours for fungal strains. Zones of inhibition were measured in millimeters.

RESULTS

Visual Observation and UV–Visible Spectroscopic Analysis

The formation of silver nanoparticles was initially indicated by a distinct color change of the reaction mixture from pale yellow to dark brown within 24 h of incubation, suggesting the reduction of Ag^{+} ions to metallic Ag^{0} nanoparticles. UV–Visible spectroscopic analysis further confirmed nanoparticle synthesis by exhibiting a strong and well-defined surface plasmon resonance (SPR) absorption peak at approximately 441 nm. The presence of this characteristic peak is indicative of the formation of silver nanoparticles and reflects their nanoscale dimensions and stability in the colloidal suspension.

X-Ray Diffraction (XRD) Analysis

XRD analysis was performed to determine the crystalline nature and phase composition of the biosynthesized silver nanoparticles. The diffraction pattern displayed prominent peaks corresponding to the (111), (200), (220), and (311) lattice planes, confirming the crystalline structure of silver. The observed diffraction peaks were consistent with cubic and hexagonal phases of metallic silver, indicating high crystallinity of the nanoparticles. The average crystallite size, calculated using the Debye–Scherrer equation, was found to be in the range of 20–50 nm, corroborating nanoscale particle formation.

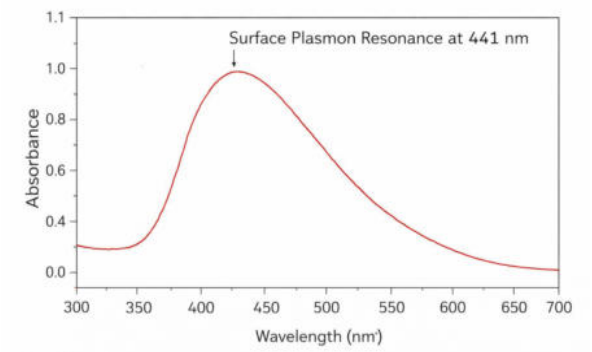


Figure 1. UV-visible spectra of Biosynthesized Silver Nanoparticles

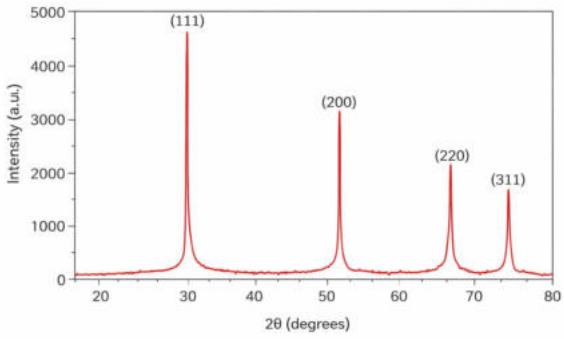


Figure 2. XRD pattern of Biosynthesized Silver Nanoparticles

Scanning Electron Microscopy (SEM) Analysis

SEM micrographs revealed that the synthesized silver nanoparticles were predominantly polydispersed with irregular, cubic, and hexagonal morphologies. Particle size estimation from SEM images indicates that the synthesized silver nanoparticles fall predominantly within the range of 20–50 nm, confirming their nanoscale dimensions. The nanoparticles were uniformly distributed with minimal agglomeration, suggesting effective stabilization by phytochemicals present in the *Argemone mexicana* leaf extract. The observed particle size distribution was in good agreement with XRD findings, confirming successful biosynthesis of nanosized silver particles.

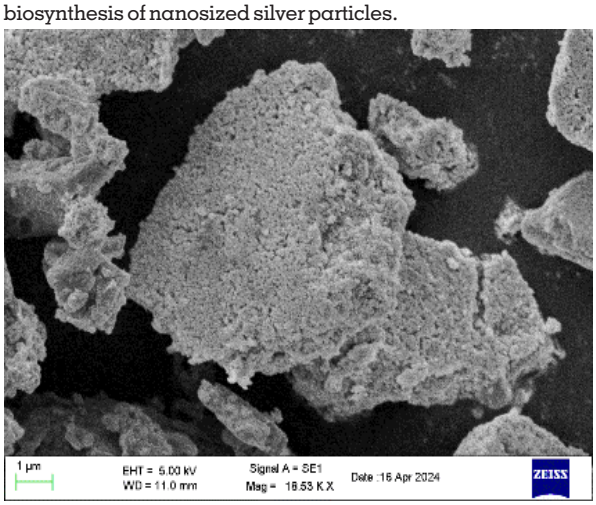


Figure 3. Scanning Electron magnified image of Biosynthesized Silver Nanoparticles

Fourier Transform Infrared (FTIR) Spectroscopic Analysis

FTIR spectroscopy was employed to identify the functional groups involved in the reduction and stabilization of silver

nanoparticles. The FTIR spectrum exhibited characteristic absorption bands corresponding to hydroxyl (–OH), carbonyl (–C=O), amine (–NH), and aromatic groups. These functional groups are attributed to bioactive phytochemicals present in the leaf extract, indicating their involvement as reducing and capping agents during nanoparticle formation. The presence of these functional moieties confirms the role of plant metabolites in nanoparticle stabilization.

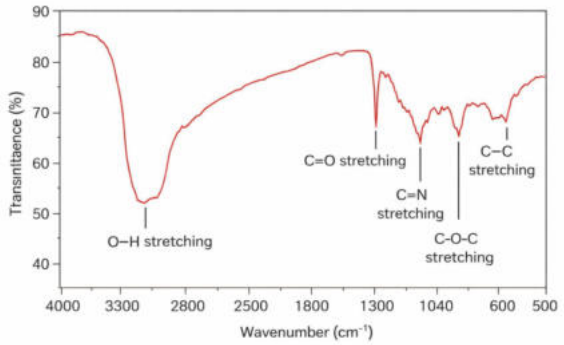


Figure 4. FTIR spectra of Biosynthesized Silver Nanoparticles

Antimicrobial Activity Of Silver Nanoparticles

The antimicrobial activity of the biosynthesized silver nanoparticles was evaluated against a panel of pathogenic microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and fungi, using the agar well diffusion method. The results demonstrated a clear, concentration-dependent antimicrobial effect against all tested microorganisms.

At lower concentrations (10 ppm), moderate inhibitory activity was observed, while significantly enhanced zones of inhibition were recorded at higher concentrations (20 and 30 ppm). Among the tested bacteria, Gram-negative strains exhibited greater sensitivity to the nanoparticles compared to the Gram-positive strain. Notably, *Klebsiella pneumoniae* and *Escherichia coli* showed the largest zones of inhibition at 30 ppm, followed by *Salmonella typhi*. *Staphylococcus aureus* exhibited comparatively lower susceptibility, which may be attributed to its thicker peptidoglycan cell wall.

The synthesized nanoparticles also displayed notable antifungal activity against *Candida albicans*, with inhibition zones increasing proportionally with nanoparticle concentration. The observed antifungal efficacy highlights the broad-spectrum antimicrobial potential of the biosynthesized silver nanoparticles.

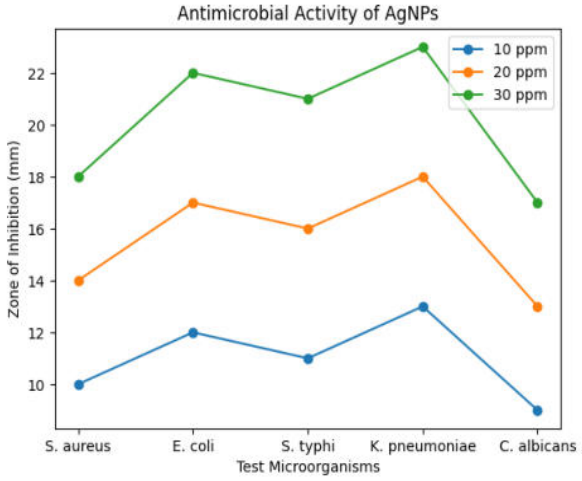


Figure 5. Graph showing Zone of Inhibition (mm) of Biosynthesized Silver Nanoparticles Against Test Microorganisms

Table 1. Zone of Inhibition (mm) of Biosynthesized Silver Nanoparticles Against Test Microorganisms

Microorganism	Zone of inhibition (in mm)		
	10 ppm	20 ppm	30 ppm
Staphylococcus aureus	10	14	18
Escherichia coli	12	17	22
Salmonella typhi	11	16	21
Klebsiella pneumoniae	13	18	23
Candida albicans	9	13	17

DISCUSSION

Green synthesis of silver nanoparticles using plant extracts has been widely reported as an eco-friendly and effective approach. In the present study, Argemone mexicana leaf extract was successfully utilized for the biosynthesis of silver nanoparticles, and the results are consistent with earlier reports on plant-mediated AgNP synthesis.

The appearance of a dark brown color and the UV–Visible absorption peak at approximately 440 nm confirm the formation of silver nanoparticles. Similar surface plasmon resonance (SPR) peaks in the range of 400–450 nm have been reported by Shankar et al., Ahmed et al., and Iravani et al., indicating that the optical behavior observed in this study is characteristic of nanoscale silver particles.

XRD analysis revealed diffraction peaks corresponding to the (111), (200), (220), and (311) planes of face-centered cubic silver. Comparable crystalline patterns have been reported by Philip et al., Bindhu and Umadevi et al., and Rai et al., confirming that green synthesis does not alter the crystalline structure of metallic silver. The crystallite size range of 20–50 nm observed in this study is in agreement with the findings of Elumalai et al. and Veerasamy *et al*..

SEM analysis showed that the synthesized silver nanoparticles were polydispersed with spherical, cubic, and hexagonal morphologies. Similar morphological diversity has been reported by Anandalakshmi *et al*. and Sathishkumar *et al*., who attributed such variation to the influence of plant-derived biomolecules on nanoparticle growth. The limited agglomeration observed is consistent with reports by Dubey et al., suggesting effective stabilization by phytochemicals.

FTIR analysis confirmed the presence of hydroxyl, carbonyl, and amine functional groups, which are commonly associated with plant metabolites involved in nanoparticle reduction and stabilization. Comparable FTIR results have been reported by Prabhu and Poulose *et al*. and Kumar *et al*., supporting the role of phytochemicals in green synthesis.

The biosynthesized silver nanoparticles exhibited strong antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungal pathogens. Similar broad-spectrum antimicrobial effects have been reported by Morones *et al*., Rai *et al*., and Shrivastava *et al*., who suggested that differences in cell wall structure account for higher susceptibility of Gram-negative bacteria. The antifungal activity observed in this study is also consistent with earlier reports by Kim *et al*. and Jo *et al*..

Overall, the present findings are in strong agreement with earlier research, confirming that Argemone mexicana-mediated silver nanoparticles are crystalline, stable, and biologically active, and highlighting their potential for biomedical and antimicrobial applications.

CONCLUSION

The present study successfully demonstrates an eco-friendly and cost-effective green synthesis of silver nanoparticles using Argemone mexicana leaf extract. The formation of silver

nanoparticles was confirmed through UV–Visible spectroscopy by the appearance of a characteristic surface plasmon resonance peak, indicating the successful reduction of silver ions. X-ray diffraction analysis revealed the crystalline nature of the synthesized nanoparticles with a face-centered cubic structure, while scanning electron microscopy confirmed their nanoscale size and diverse morphology, including spherical, cubic, and hexagonal forms.

Overall, this study confirms that Argemone mexicana leaf extract is an efficient biological resource for the green synthesis of stable and crystalline silver nanoparticles. The simplicity, environmental compatibility, and biological efficacy of this method suggest that the synthesized silver nanoparticles hold promise for applications in biomedical, pharmaceutical, and antimicrobial formulations. Further studies focusing on toxicity assessment and in vivo applications are recommended to explore their practical utility.

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