Research Article

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Antioxidant, Antimicrobial, and Potential Biomedical Applications of Green Synthesized Silver Nanoparticles Using *Aloe barbadensis* Root

Sivaranjani Varadharajan[1] and Parthiban Pandian[2*]
[1]Department of Biotechnology, Periyar Maniammai Institute of Science & Technology, Vallam, Thanjavur, TN, India
[2]Centre for Environmental Engineering, Ponnaiyah Ramajayam Institute of Science & Technology, Thanjavur, TN, India
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*dr.parthi2009@gmail.com

ABSTRACT

The present study focuses on the green synthesis and physicochemical characterization of silver nanoparticles (AgNPs) using *Aloe barbadensis* root extract. The green synthesized AgNPs were characterized using X-ray diffraction (XRD), UV-visible spectrophotometry, Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and scanning electron microscopy (SEM). The characteristic UV absorption peak at 328 nm confirmed the successful synthesis of AgNPs. The antioxidant activity of AgNPs was assessed through DPPH and hydrogen peroxide scavenging assays, with maximum free radical scavenging activities of 75.5 % and 73%, respectively, at 500 µg/mL. Antimicrobial activity of the AgNPs was demonstrated against both Gram-positive and Gram-negative bacteria, with a maximum zone of inhibition of 22 mm. Additionally, the synthesized AgNPs displayed potential biomedical applications. These findings highlight the potential of *Aloe barbadensis* root-mediated AgNPs for therapeutic, antimicrobial, and antioxidative applications, underscoring their relevance in sustainable nanotechnology and biomedicine.

Keywords: *Aloe barbadensis* root extract; Antioxidant activity; Antimicrobial properties; Biogenic synthesis; Green nanotechnology; Silver nanoparticles

1. INTRODUCTION

Silver nanoparticles (AgNPs) have drawn considerable attention recently due to their unique properties and potential applications in diverse fields, including medicine, electronics, and environmental science. AgNPs can be synthesized using various methods such as chemical reduction, physical techniques, and biological approaches. Among these, green synthesis using plant extracts has emerged as a promising alternative, owing to its eco-friendliness and cost-effectiveness (Roy *et al*. 2019). *Aloe barbadensis*, commonly known as aloe vera, is one such plant extensively studied for its phytochemical constituents and antioxidant properties. The bioactive compounds in aloe vera, including flavonoids, phenolics, and vitamins, play a crucial role in reducing metal ions into nanoparticles (Khan *et al*. 2022). The plant species aloe vera belongs to the order Asparagales within the Liliaceae family and the Plantae kingdom. It is a perennial plant characterized by its pointed leaves forming a rosette at the stem (Minjares and Femenia, 2019). Its highwater content and solid material composition include over 75 active compounds such as vitamins, minerals, enzymes, and complex polysaccharides (Khan *et al*. 2022). As a xerophyte, aloe vera thrives in arid regions with minimal water availability. It is a stemless or short-stemmed plant with elongated leaves capable of storing significant amounts of water. Aloe vera exhibits antiproliferative and immuno-stimulatory activities (Chakraborty *et al*. 2016).

Aloe vera is reported to strengthen the immune system, reduce oxidative damage, enhance memory, and act as an antidepressant based on recent findings. However, herbal medications with antiplatelet effects, such as aloe vera, are recommended to be discontinued before surgeries to prevent complications. Recognized for its medicinal value, aloe vera has gained popularity as a source of functional components for the development of health-oriented foods and beverages (Fu *et al*. 2021). It can lower triglycerides, reduce cholesterol, prevent kidney stones, and has shown antiviral properties. Zinc, a vital mineral found in aloe vera, has demonstrated effectiveness in inhibiting SARS-CoV-2 replication by preventing RNA polymerase activity during cell culture studies.

Aloe vera possesses numerous therapeutic attributes, including anti-inflammatory, immune-modulating, hepatoprotective, antiulcer, anticancer, antioxidant, antimicrobial, antidiabetic, and antiallergic properties. Phytochemicals are a promising source of bioactive molecules, offering efficacy and safety for treating chronic diseases. Despite advancements in cancer therapy, cancer remains one of the leading causes of global mortality, second only to cardiovascular diseases (Zhang *et al.* 2016). Aloe vera gel has been shown to reduce body weight, body fat mass, and insulin resistance in obese patients with prediabetes or early-stage diabetes. Preliminary studies suggest that aloe vera may benefit HIV patients by boosting CD4 counts. Furthermore, a randomized double-blind clinical trial revealed that aloe vera leaf gel significantly lowered total cholesterol and LDL levels in hyperlipidemic type 2 diabetes patients.

Aloe vera roots have also been a focus of research. Anthraquinones isolated from aloe roots have been characterized using advanced techniques like ^{1}H NMR, ^{13}C NMR, and LC-HRMS. These studies have enabled the identification and derivatization of new compounds with antiviral properties (Lateef *et al*. 2015; Radha and Laxmipriya, 2015). Similarly, *in vitro* assays such as CPE reduction and plaque inhibition tests have highlighted the potential of these derivatives (Hu *et al*. 2022).

Methods for synthesis of nanoparticles continue to evolve, with biogenic synthesis gaining prominence due to its simplicity and environmental compatibility. For instance, AgNPs can be synthesized by adding plant extracts to silver nitrate solutions, where phytochemicals act as reducing and stabilizing agents (Kavitha *et al.* 2020; Nalimu, 2021; Sagar *et al*. 2023). Key compounds include flavonoids, glycosides, and polyphenols, which prevent nanoparticle aggregation and enhance stability (Oladipo *et al*. 2017; Alharbi *et al.* 2022).

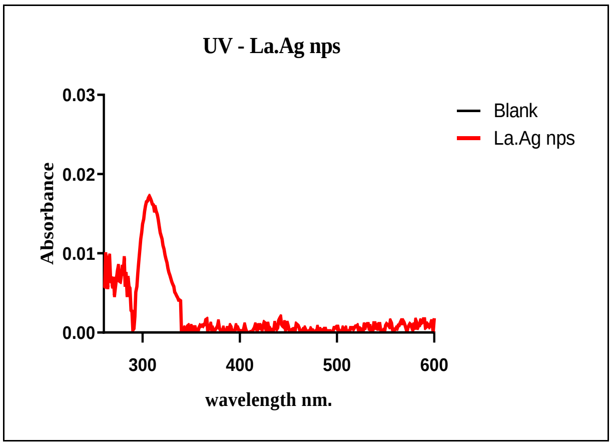

Fig. 1: UV–visible spectrum of AgNPs synthesized from aloe vera root extract

The use of green synthesis methods for producing AgNPs offers several benefits over traditional approaches. These methods are efficient, cost-effective, and environmentally benign (Kumar *et al*. 2020). Additionally, plant-based synthesis avoids hazardous by-products, aligning with sustainability goals. AgNPs, classified as noble metal nanoparticles due to their resistance to corrosion and oxidation, have unique physicochemical and biological properties that have fueled interest in their applications (Alabdallah and Hasan, 2021). Researchers are increasingly exploring green technologies as viable solutions to overcome the challenges posed by conventional synthesis methods (Castillo *et al*. 2020; Selvakumar *et al*. 2024).

To date, research on the synthesis and characterization of silver nanoparticles using *Aloe barbadensis* roots, along with their antioxidant analysis, remains underexplored. This study aims to address that gap, contributing valuable insights into the development of eco-friendly nanomaterials with biomedical and environmental applications.

2. EXPERIMENTAL SECTION

2.1 Materials

Fresh Aloe vera roots (*Aloe barbadensis*) were sourced locally for the study. Silver nitrate ($AgNO_3$) was obtained from Sigma-Aldrich Pvt. Ltd., while DPPH (2,2-Diphenyl-1-Picrylhydrazyl), H_2O_2, ascorbic acid, Phosphate Buffer Solution (PBS), were supplied by HiMedia Pvt. Ltd.

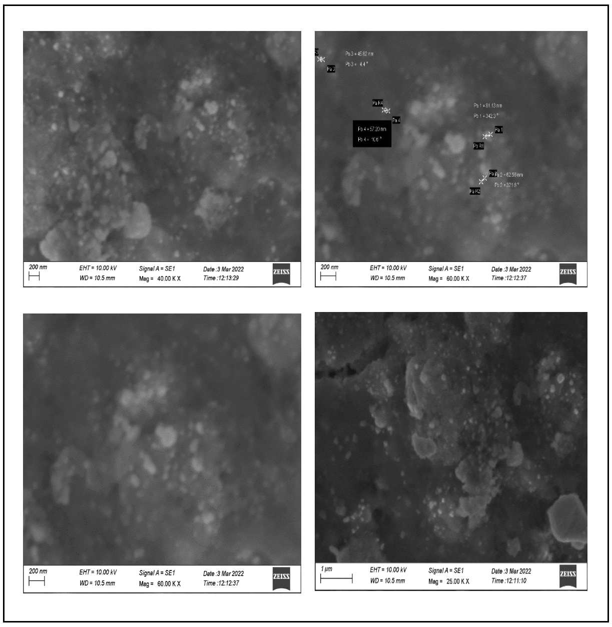

Fig. 2: SEM image of AgNPs

2.2 Biogenic Synthesis of AgNPs

The synthesis of silver nanoparticles was performed using silver nitrate and *Aloe barbadensis* root extract. A 0.1 M silver nitrate solution was prepared by dissolving 1 g of silver nitrate in 100 mL of double-distilled water. The solution was mixed with the aloe vera extract in ratios of 5:5, 6:4, 7:3, 8:2, and 9:1. The 5:5 ratio was selected for bulk preparation due to higher nanoparticle yield. The reaction mixture was stirred at 800 rpm, heated below 100°C for 1 hour, and maintained in darkness to prevent degradation. The mixture turned brown, indicating nanoparticle formation. The suspension was centrifuged at 15,000 rpm for 15 minutes, and the pellet was filtered using Whatman filter paper. The nanoparticles were washed with deionized water, dried, and stored for further characterization.

2.3 Physicochemical Characterization

The formation of silver nanoparticles was visually confirmed by observing the distinct colour change in the reaction mixture. Further confirmation was provided by UV-visible spectroscopy, scanning the absorbance spectrum between 300–800 nm to detect characteristic peaks. XRD analysis was employed to evaluate the crystalline structure of the nanoparticles, and FTIR analysis identified functional groups on their surface within the spectral range of 4000–500 cm^{-1}. The shape, morphology, and size distribution were analysed using SEM, while DLS assessed nanoparticle dispersibility and stability.

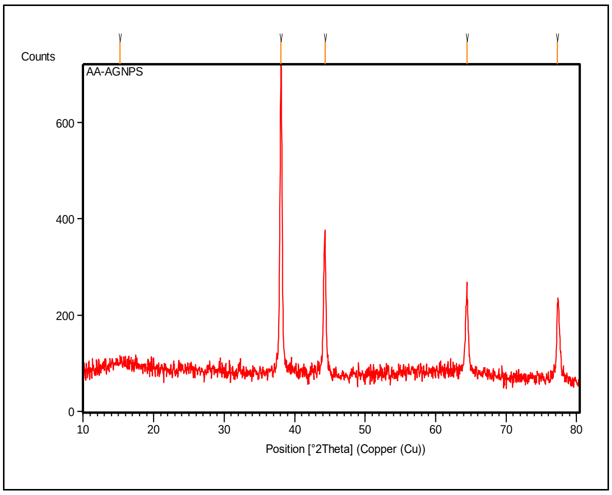

Fig. 3: XRD spectrum of AgNPs

2.4 Antioxidant Activity Assays

The antioxidant potential of biogenic AgNPs was evaluated using both DPPH and H_2O_2 scavenging assays. For the DPPH assay, various concentrations of AgNPs (10–500 µg/mL) were mixed with 3 mL of 0.1 mM DPPH solution. The mixture was incubated in the dark at room temperature for 30 minutes, and the decolorization from purple to yellow was measured at 517 nm. For the H_2O_2assay, AgNPs were added to 3 mL of 20 mM hydrogen peroxide solution in phosphate buffer (pH 7.4) and incubated for 15 minutes. The absorbance was recorded at 230 nm to determine the scavenging activity. The scavenging efficiency for both assays was calculated using the equation:

$$\text{Scavenging Activity (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} x100$$

2.5 Antimicrobial Activity of Biogenic AgNPs

The antimicrobial activity of biogenic AgNPs was evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) clinical strains using the standard well diffusion method. Overnight log cultures of the strains were uniformly spread onto sterile nutrient agar plates. Wells were created in the agar using a sterile gel puncture tool and loaded with 0.3 M AgNP solution, sterile distilled water (negative control), and a standard antibiotic (positive control). After 24 hours of incubation at 37°C, the diameter of the inhibition zones around each well was measured to determine antimicrobial efficacy.

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Characterization of Biogenic AgNPs

3.1.1 UV-vis Spectrometry

The formation of AgNPs was preliminarily confirmed using UV–vis spectroscopy. The absorption spectrum of AgNPs was recorded in the range 700–300 nm. The surface plasmon resonance (SPR) band observed at 328 nm confirmed the synthesis of AgNPs, as shown in Fig. 1. This characteristic peak indicated the reduction of silver ions (Ag^+) to metallic silver nanoparticles (Ag^0) in the reaction mixture. The UV–vis analysis validated the successful formation of AgNPs through the distinct SPR signal.

3.1.2 Scanning Electron Microscopy (SEM)

The size, morphology, and uniformity of biogenic AgNPs were analyzed using SEM. Fig. 2 displays SEM images of the synthesized nanoparticles at a scale of 500 nm. The images reveal that while some nanoparticles appear agglomerated, others are visible as distinct grains. The analysis indicates an average particle size of approximately 45.82 nm for the AgNPs, confirming their nanoscale dimensions determined from SEM images using ImageJ software. Multiple nanoparticles were measured, and their average size was calculated by analyzing well-defined particles observed in the SEM micrographs, offering valuable insights into their structural characteristics.

3.1.3 XRD Analysis

The crystalline nature of biogenic AgNPs was confirmed through XRD analysis. The XRD spectrum revealed four distinct diffraction peaks at 2θ values of 38°, 45°, 65°, and 78°, indicated the miller indices 111, 200, 220 and 311 respectively. These sharp peaks indicate the crystalline structure of the AgNPs. The data, as shown in Fig. 3, confirm the successful synthesis of crystalline silver nanoparticles.

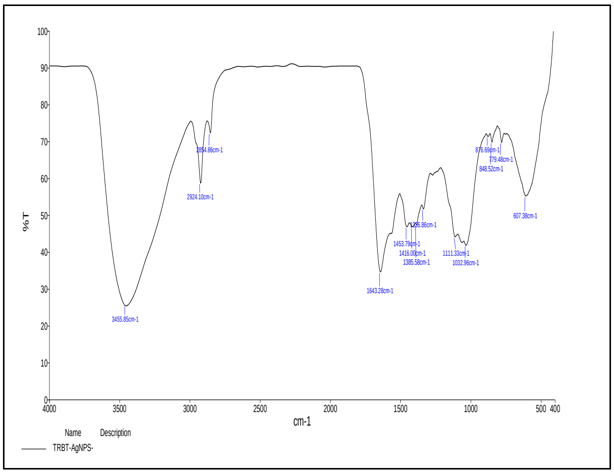

Fig. 4: FTIR spectra of AgNPs

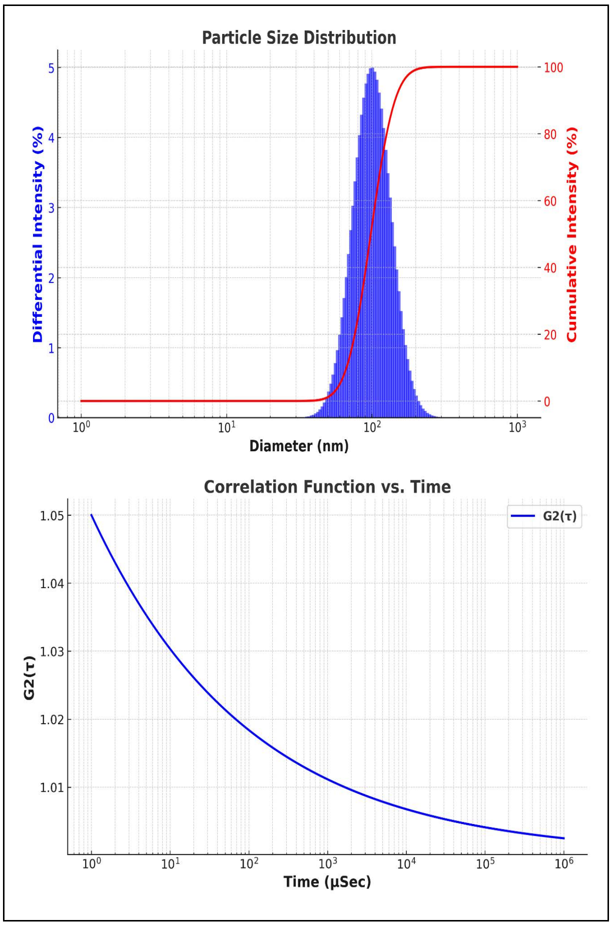

Fig. 5: DLS of AgNPs

3.1.4 FTIR Analysis

The FTIR analysis of biogenic AgNPs, as shown in Fig. 4, revealed several significant absorption peaks. The spectrum revealed a prominent peak at 3455.85 cm^{-1}, corresponding to N-H/C-H/O-H stretching vibrations of amines and amides. A peak at 2924.10 cm^{-1} indicated C-H bonds, while 2854.88 cm^{-1} represented carboxylic acid bonds. The peak at 1453.79 cm^{-1} corresponds to C-O/C-H bonds, and a peak at 1416.00 cm^{-1} indicated alkanes. These functional groups confirm their role in reducing and stabilizing the nanoparticles.

3.1.5 Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) was employed to determine the particle size distribution of the synthesized AgNPs. The results showed that the nanoparticles were widely distributed and highly dispersed within the mixture, with an average diameter of 172.1 nm and a standard deviation of 127.5 nm suggesting a broader distribution with some larger particles contributing to the overall average. Fig. 5 highlights the nanoparticle size and dispersion characteristics in the solution.

3.2 Antioxidant Activity

The antioxidant potential of biogenic AgNPs was evaluated using DPPH and H_2O_2 scavenging assays, with results indicating robust free radical neutralization capabilities. Various concentrations (10, 50, 100, 250, and 500 µg/mL) of AgNPs were tested alongside ascorbic acid as the standard. The results demonstrated a dose-dependent increase in free radical scavenging activity. In the DPPH assay, the AgNPs effectively scavenged radicals, as evidenced by a color change from violet to yellow. The scavenging activity increased in a dose-dependent manner, from 19.74% at 10 µg/mL to 75.5% at 500 µg/mL (Fig. 6). These findings align with recent studies highlighting the antioxidant efficacy of green-synthesized nanoparticles (Bhowmik *et al.* 2016; Khan *et al.* 2022; Alharbi *et al.* 2022). Lower concentrations displayed proportionally reduced inhibition percentages, signifying effective but concentration-dependent activity.

The H_2O_2 scavenging ability of AgNPs was similarly tested at the same concentrations. At the highest concentration (500 µg/mL), the scavenging effect was 73%, slightly lower than ascorbic acid (84%) (Fig. 6). The scavenging effect decreased with lower AgNP concentrations, indicating a similar trend of dose dependency. These results highlight the significant antioxidant activity of AgNPs, aligning with recent findings on their reactive oxygen species (ROS) scavenging properties.

The biogenic AgNPs demonstrated a promising antioxidative potential in both DPPH and H_2O_2 assays. Their dose-dependent behavior aligns with other studies, confirming the efficiency of green-synthesized nanoparticles in mitigating oxidative stress. The observed antioxidant activities can be attributed to the reduction potential of AgNPs, which facilitates free radical neutralization. Moreover, the comparable activity of AgNPs with standard ascorbic acid underscores their potential for therapeutic applications, including in oxidative stress management and related disorders. Further investigations are warranted to elucidate the underlying mechanisms of their antioxidative properties.

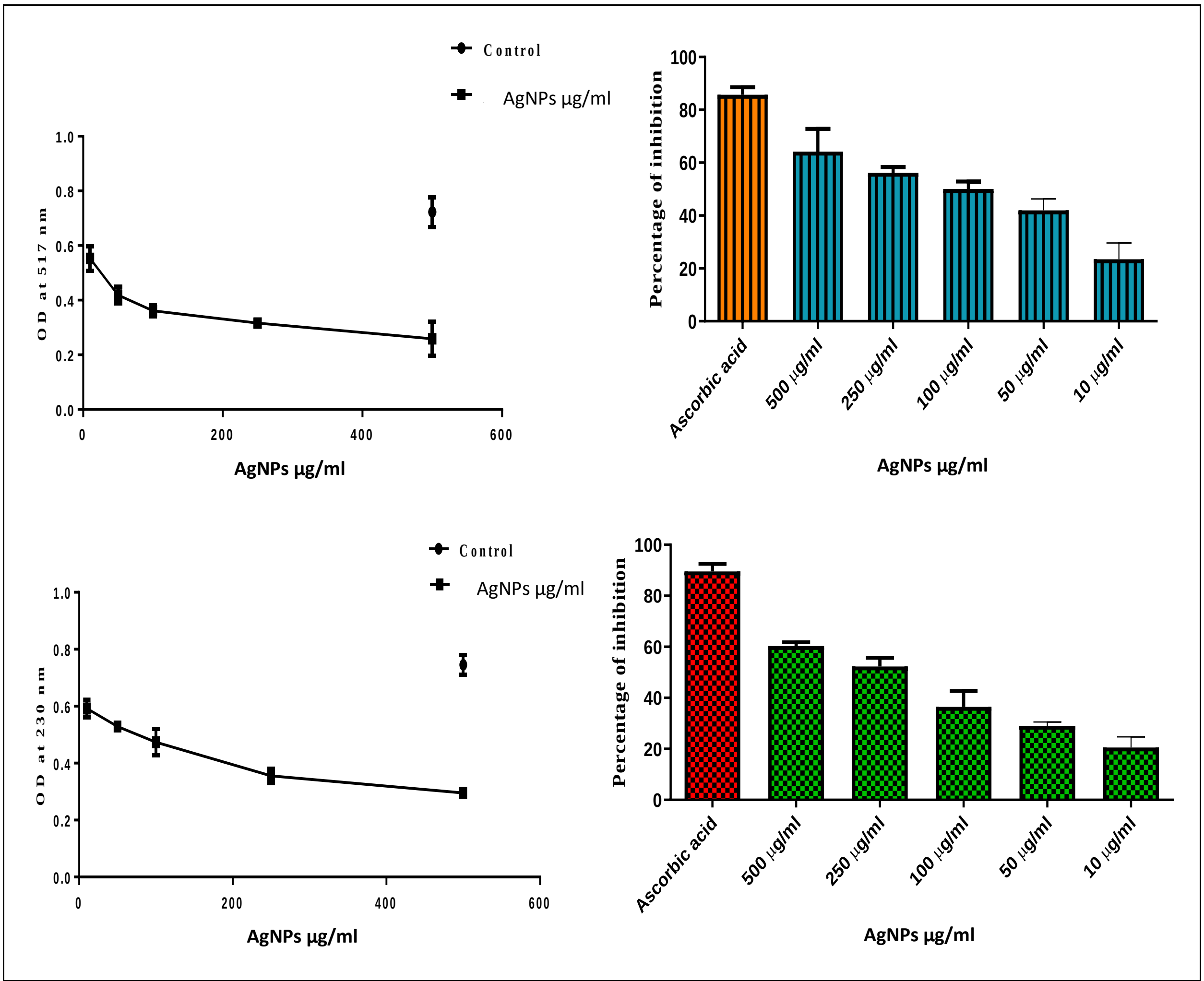

Fig. 6: Antioxidant results of AgNPs

When compared to similar studies, the antioxidant performance of these AgNPs exceeds that reported for chemically synthesized nanoparticles (Roy *et al*. 2019; Kumar *et al.* 2020; Ghatage *et al.* 2023). These results underscore the potential of biogenic AgNPs for biomedical applications where oxidative stress mitigation is critical.

3.3 Antimicrobial Activity of Biogenic AgNPs

The antimicrobial efficacy of biogenic AgNPs was assessed against both Gram-positive and Gram-negative bacteria to evaluate their broad-spectrum antibacterial properties. Specifically, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were selected for this study. The zones of inhibition observed around the wells containing AgNPs are depicted in Fig. 7. The biogenic AgNPs exhibited significant antibacterial activity, with zones of inhibition measuring 16 mm for *Staphylococcus aureus* and 22 mm for *Escherichia coli*, as detailed in Table 1. Gram positive microorganisms demonstrated least susceptibility to AgNPs with a zone of inhibition of 16 mm.

Notably, all tested microbial strains showed a significant increase in the zone of inhibition when compared to the reference drug, azithromycin, as illustrated in Fig. 7. The Gram-negative bacterium *Escherichia coli* exhibited the largest zone of inhibition, measuring 22 mm in diameter. These findings align with recent studies that have reported the potent antibacterial activity of biogenic AgNPs against both Gram-positive and Gram-negative bacteria (Raj *et al.* 2021; Yan *et al.* 2018).

The observed variations in inhibition zones can be attributed to differences in the peptidoglycan composition of bacterial cell walls. Gram-positive bacteria possess a thicker peptidoglycan layer, which may impede the penetration of AgNPs compared to the thinner peptidoglycan layer in Gram-negative bacteria. The relatively small size of AgNPs facilitates their entry into bacterial cells, where they can interact with intracellular components, leading to cell death. Additionally, silver ions (Ag^+) released from the nanoparticles are known to interact with thiol groups in cellular enzymes, disrupting essential cellular functions and contributing to the antimicrobial effect (Borges *et al.* 2019; Rai *et al*. 2009; Durán *et al*. 2011). These results underscore the potential of biogenic AgNPs as effective antimicrobial agents against a broad spectrum of bacterial pathogens.

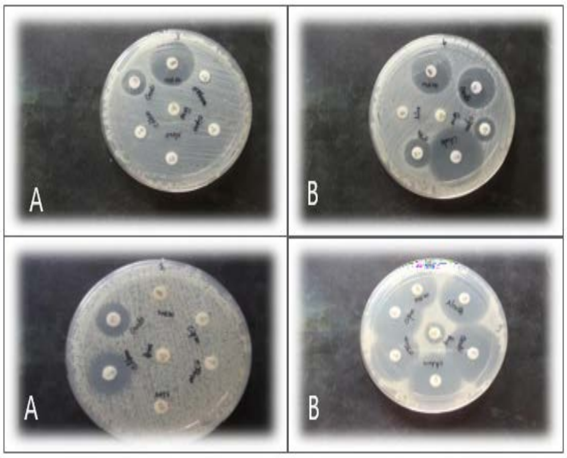

Fig. 7: Well diffusion method; zone of inhibition of biogenic AgNPs against (a) *Staphylococcus aureus* and (b) *Escherichia coli*

4. CONCLUSIONS

The green synthesis of AgNPs using *Aloe barbadensis* root extract demonstrated a cost-effective, eco-friendly, and efficient method for nanoparticle production. The synthesized AgNPs exhibited promising antioxidant, antimicrobial, and potential biomedical applications. Characterization using UV-Vis spectroscopy revealed a maximum absorption peak at 328 nm, confirming the formation of nanoparticles. XRD analysis established their crystalline nature, while FTIR, DLS, and SEM provided further structural and morphological insights.

The AgNPs showed notable antioxidant activity, highlighting their potential in mitigating oxidative stress. Antimicrobial studies confirmed broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria.

These findings underline the significant biomedical and environmental applications of biogenic AgNPs synthesized using *Aloe barbadensis* root extract. The environmentally friendly and scalable synthesis approach opens avenues for large-scale production and utilization in pharmaceuticals, biomedicine, and other industries. Future research could focus on optimizing synthesis conditions and exploring additional therapeutic and industrial applications of these nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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