

Green Synthesis and Characterization of Silver Nanoparticles using Aloe vera Plant Extract and Evaluation of their Antibacterial Activity

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ABSTRACT

This study demonstrated the successful green synthesis of silver nanoparticles (AgNPs) using Aloe vera leaf extract as a reducing and stabilizing agent. The proposed synthesis method represents an environmentally friendly and sustainable alternative to conventional chemical approaches. The formation of AgNPs was initially indicated by a visible color change from light yellow to yellowish-brown, providing preliminary evidence of nanoparticle formation. UV-Vis spectroscopy further confirmed the synthesis of AgNPs through the appearance of a characteristic surface plasmon resonance (SPR) band within the typical spectral range of silver nanoparticles, indicating the effective reduction of Ag⁺ ions to metallic Ag⁰. The colloidal stability of the synthesized AgNPs in the aqueous medium was further evaluated using zeta potential measurements. Scanning electron microscopy (SEM) revealed a predominantly agglomerated morphology, with the nanoparticles occurring as dense and irregular clusters at the nanoscale. Energy-dispersive X-ray spectroscopy (EDX) confirmed the elemental composition of the synthesized material, with silver constituting the predominant detected element. Furthermore, X-ray diffraction (XRD) analysis exhibited four characteristic diffraction peaks corresponding to the face-centered cubic (FCC) crystalline structure of metallic silver. The synthesized AgNPs showed an inhibitory effect against both *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The antibacterial effect was more pronounced against *S. aureus* at all tested concentrations. Bacterial enumeration further revealed a clear reduction in bacterial load in the Aloe vera-derived AgNP-treated group compared with the control group, resulting in a 1.02-log reduction, corresponding to a 90.5% decrease in bacterial count.

Keywords: Silver nanoparticles (AgNPs), Green synthesis, Aloe vera, Antibacterial activity, Surface plasmon resonance, Nanomaterials.

1. INTRODUCTION

Nanotechnology has emerged as a major scientific advancement owing to its broad applicability and significant impact across a wide range of scientific and technological

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disciplines (Al-Musawy et al., 2025). A fundamental aspect of nanotechnology is the synthesis and utilization of nanoparticles (NPs), which exhibit physicochemical characteristics that differ markedly from those of their corresponding bulk materials. These distinctive properties arise primarily from the ability to engineer materials at the atomic and molecular scales, thereby modifying their structural, optical, electronic, catalytic, and biological characteristics (Nauroze et al., 2025; Lee and Moon, 2020).

Among the various nanomaterials, silver nanoparticles (AgNPs) have attracted considerable scientific interest because of their remarkable optical, electronic, catalytic, and biological properties. These characteristics have enabled their extensive application in diverse fields, including biomedicine, agriculture, industry, and environmental remediation (Srikar et al., 2016; Singh and Kaur, 2019). The physicochemical properties of nanomaterials are strongly influenced by nanoparticle size, morphology, shape, and surface characteristics, all of which play a critical role in determining their functional performance (Qamer et al., 2021). In particular, AgNPs exhibit a pronounced surface plasmon resonance (SPR) phenomenon, resulting from the collective oscillation of conduction electrons at the nanoparticle surface. This characteristic produces strong optical absorption in the visible region and can be readily detected using ultraviolet–visible (UV–Vis) spectroscopy (Li et al., 2023).

Various conventional techniques have been employed for the synthesis of AgNPs, including chemical, photochemical, and electrochemical reduction, thermal evaporation, sol–gel processing, microwave-assisted synthesis, and ultrasonication (Zhang et al., 2019; Maharjan et al., 2020; León-Valencia et al., 2023). Despite their effectiveness, many conventional synthesis routes rely on chemical reducing and stabilizing agents that may be toxic, expensive, and environmentally hazardous. These limitations have stimulated increasing interest in the development of safer, more sustainable, and environmentally compatible approaches for nanoparticle synthesis. In this context, plant-mediated synthesis has emerged as a promising alternative, as demonstrated by (Baran et al., 2021; Firdhouse and Lalitha, 2013). The use of plant extracts can minimize the reliance on hazardous chemicals while providing a relatively simple, rapid, cost-effective, and energy-efficient route for nanoparticle production.

Green synthesis, also referred to as biological or eco-friendly synthesis, represents an environmentally sustainable approach to nanoparticle fabrication that exploits biological resources, particularly plant extracts and microorganisms, as sources of reducing, capping, and stabilizing agents. The effectiveness of plant-mediated synthesis is largely attributed to the diverse array of bioactive phytochemicals naturally present in plant tissues. These compounds can participate in the reduction of metal ions and subsequently contribute to the stabilization and surface functionalization of the resulting nanoparticles (Shahzadi et al., 2025; Rai and Avila-Quezada, 2024). Consequently, plant-based synthesis has gained considerable attention as a sustainable alternative to conventional physicochemical methods.

Aloe vera is a widely distributed plant found predominantly in tropical and subtropical regions and is well known for its diverse phytochemical composition and established medicinal value (Alara and Alara, 2019). Aloe vera leaf gel consists predominantly of water, accounting for approximately 99% of its composition, while the remaining fraction contains water-soluble polysaccharides and other organic constituents, including glucomannan (Hamman, 2008). In addition, Aloe vera gel contains a broad spectrum of biologically active compounds, including emodin, polysaccharides, anthraquinones,



flavonoids, polyphenols, phenolic acids, amino acids, enzymes, and vitamins (**Sharm and Kaur, 2026**). Several of these phytochemicals, particularly polysaccharides and polyphenolic compounds, possess functional groups capable of reducing metal ions and stabilizing the resulting nanoparticles. Therefore, Aloe vera extract represents a promising biological resource for the green synthesis of metal nanoparticles, including AgNPs (**Bhatt et al., 2025**).

The unique physicochemical properties of AgNPs have resulted in their widespread application in biological, biomedical, pharmaceutical, and other technological fields. Their nanoscale dimensions facilitate interactions with biological membranes and cellular components, enabling their internalization into living cells through mechanisms such as endocytosis (**Yusuf, 2018; Abbas et al., 2024; Das and Paul, 2025; Khalid, 2025**). Accordingly, the development of efficient and environmentally sustainable methods for AgNP synthesis is of considerable importance, particularly approaches that utilize renewable biological resources and reduce the environmental and health risks associated with conventional chemical synthesis.

Silver nanoparticles (AgNPs) have attracted considerable attention as promising antimicrobial and biomedical agents because of their broad-spectrum biological activities, including antibacterial, antimicrobial, and anticancer effects (**Asif et al., 2022; Sofi et al., 2022**). The increasing prevalence of antibiotic-resistant pathogens, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Escherichia coli*, has intensified the search for alternative antimicrobial agents with effective and sustainable therapeutic potential (**Gajic et al., 2025; Ehmud et al., 2025**). In this context, the development of biologically synthesized AgNPs represents a promising strategy for addressing the growing challenge of antimicrobial resistance.

The present study aims to synthesize silver nanoparticles (AgNPs) using Aloe vera leaf extract as a natural reducing and stabilizing agent and to characterize the synthesized nanoparticles using ultraviolet–visible (UV–Vis) spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, energy-dispersive X-ray (EDX) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD). Furthermore, the antibacterial activity of the synthesized AgNPs against *Escherichia coli* and *Staphylococcus aureus* will be evaluated using the disc diffusion method. The study is intended to assess the potential of Aloe vera-mediated synthesis as an environmentally friendly approach for producing AgNPs with effective antibacterial properties.

2. EXPERIMENTAL WORK

2.1. Preparation of Aloe vera leaf extracts

Silver nanoparticles (AgNPs) were synthesized using the leaves of Aloe vera, a medicinal plant that is readily available locally and represents a low-cost biological resource. Fresh Aloe vera leaves were collected locally, thoroughly washed with tap water, and subsequently rinsed several times with distilled water to remove adhering dirt and impurities. The cleaned leaves were dried at room temperature for 15–30 min and then cut into small pieces. Approximately 100 g of the prepared leaf material was transferred into a 250 mL beaker containing 100 mL of distilled water. The mixture was heated to 80 °C and maintained at this temperature for 20 min to facilitate the extraction of bioactive phytochemical constituents. After heating, the mixture was allowed to cool to room temperature and subsequently centrifuged at 12,000 rpm for 15 min. The resulting supernatant was collected and filtered through Whatman No. 1 filter paper to obtain a clear Aloe vera leaf extract. The freshly



prepared extract was stored at 4–8 °C until further use. The extract was subsequently employed as a natural reducing and stabilizing agent for the green synthesis of AgNPs, following the approach reported by (Nivethaa and Sidhdharth, 2021).

2.2 Synthesis of Silver Nanoparticles (AgNPs)

An aqueous silver nitrate (AgNO_3) solution was prepared at a concentration of 0.001 M and used as the silver precursor for nanoparticle synthesis. Subsequently, 10 mL of the prepared Aloe vera leaf extract was added dropwise to 90 mL of the AgNO_3 solution under continuous magnetic stirring at 600 rpm and 60 °C for 2 h. The final concentration of AgNO_3 in the reaction mixture was 0.0009 M. During the reaction, the color of the solution gradually changed from yellow to dark brown, indicating the reduction of Ag^+ ions to elemental silver (Ag^0) and the subsequent formation of silver nanoparticles (AgNPs). Following the synthesis reaction, the pH of the reaction mixture was adjusted to 7.0 using 0.1 M sodium hydroxide (NaOH) solution. The pH adjustment was performed to promote nanoparticle stability and minimize particle agglomeration. The reaction mixture was subsequently incubated in the dark for 24 h to facilitate the completion of the reduction process.

The resulting nanoparticles were collected and washed three times with deionized water and twice with ethanol. Each washing step was performed by centrifugation at 6000 rpm for 10 min to remove unreacted silver ions, residual plant-derived compounds, and other impurities. The purified nanoparticles were subsequently dispersed by ultrasonication for 20 min at room temperature using a probe sonicator operating at 40 kHz and 100 W. This step was performed to minimize particle aggregation and improve the uniformity of the nanoparticle suspension. The resulting AgNP suspension was then used for subsequent physicochemical characterization.

2.3 Study of the Prepared Nanomaterial Properties

An integrated set of analytical techniques was employed to confirm the formation and characterize the physicochemical properties of the synthesized silver nanoparticles (AgNPs) (Kareem et al., 2023). The formation of AgNPs was initially indicated by a visible change in the color of the reaction mixture from pale yellow to yellowish-brown, which was subsequently verified through instrumental characterization. The optical properties of the synthesized AgNPs were investigated using ultraviolet–visible (UV–Vis) spectroscopy. Prior to analysis, the nanoparticle suspension was appropriately diluted with deionized water, and its absorption spectrum was recorded using a Shimadzu UV-1650 PC UV–Vis spectrophotometer (Shimadzu Corporation, Japan). The surface functional groups and chemical interactions associated with the synthesized nanoparticles were examined using Fourier-transform infrared (FTIR) spectroscopy with a Shimadzu IRTracer-100 spectrometer over the spectral range of 400–4000 cm^{-1} . The surface charge characteristics of the synthesized AgNPs were evaluated using electrophoretic light scattering (ELS). The zeta potential was determined based on the electrophoretic mobility of the nanoparticles, with the Smoluchowski model applied as an appropriate approximation for aqueous dispersions. The morphological characteristics and elemental composition of the synthesized nanoparticles were investigated using scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDX). The crystalline structure and phase composition of the synthesized AgNPs were analyzed using an X-ray diffractometer (Shimadzu XRD-600) equipped with Cu $\text{K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). The XRD measurements



were conducted over a 2θ range of 10° – 80° . The average crystallite size of the synthesized AgNPs was estimated from the XRD data using the Scherrer equation (**Barman et al., 2020**).

$$D = K\lambda / \beta \cos\theta \quad (1)$$

Where D is the average crystallite size

K is the Scherrer constant (≈ 0.94),

λ is the X-ray wavelength (0.154 nm for Cu K α radiation),

β is the peak width at half maximum intensity (FWHM),

and θ is the Bragg angle.

2.4 Comparative Antibacterial Activity Assay

The antibacterial activity of the prepared AgNPs was evaluated using the agar well diffusion method against *Escherichia coli*. Four treatments were tested: AgNPs alone (1A), Aloe vera extract alone (ALO), Amoxicillin as a standard antibiotic (Am), and a combination of AgNPs with Amoxicillin (mix). Sterile distilled water was used as a negative control.

2.5 Assessment of Antibacterial Activity

The antibacterial activity of the synthesized AgNPs was evaluated using the agar well diffusion method against two bacterial strains, *Escherichia coli* and *Staphylococcus aureus*. Bacterial suspensions were prepared and adjusted according to the McFarland turbidity standard to obtain a standardized bacterial inoculum. Subsequently, 0.1 mL of each bacterial suspension was uniformly spread over the surface of sterile agar plates using a sterile cotton swab. After inoculation, a sterile 6 mm diameter well was aseptically prepared in the center of each agar plate. Subsequently, 0.1 mL (100 μ L) of AgNP suspension at concentrations of 1, 10, and 100 μ g/mL was separately introduced into the wells. The inoculated plates were incubated at 37 $^\circ$ C for 24 h. Following incubation, the antibacterial activity was determined by measuring the diameter of the clear inhibition zones surrounding the wells. The antibacterial activity of the synthesized AgNPs was evaluated based on the observed inhibition zones (**Kumar et al., 2025**). In addition, the ability of the synthesized AgNPs to inhibit bacterial growth in contaminated water was investigated to assess their potential application in the treatment of biologically polluted water.

2.6 AgNPs-loaded Active Carbon

Activated carbon impregnated with silver nanoparticles (AgNPs) was prepared by mixing 30 g of carbon with 100 mL of an aqueous suspension of these particles at a concentration of 25 ppm. The mixture was maintained for one hour to facilitate adsorption and the immobilization of the silver nanoparticles onto the activated carbon surface. Subsequently, the mixture was incubated for 24 hours to enhance the interaction and bonding of the nanoparticles to the carbon matrix. The nanoparticle-loaded carbon material was then washed three times with 100 mL of sterile, deionized water to remove unadsorbed or loosely bound particles. After washing, the material was allowed to drain and dried in an oven at 75 $^\circ$ C (**Karthik and Radha, 2016**). Finally, the AgNP-loaded activated carbon was evaluated for its potential use in treating contaminated water, particularly in reducing biological contamination and inhibiting bacterial growth.



2.7 Measuring the Efficiency of Bacteria Removal

The antibacterial activity of silver nanoparticles (AgNPs) was evaluated using the pour-plate technique. For each water sample, 1 mL of untreated contaminated water served as the control, while 1 mL of silver-treated water served as the test sample. Each 1 mL sample was transferred to a sterile Petri dish, and serial decimal dilutions were prepared using sterile distilled water to obtain plates with a countable number of colonies. Subsequently, 18–20 mL of molten Nutrient Agar, cooled to approximately 45–50°C, was added to the dishes. The contents were thoroughly mixed to ensure even distribution of the water sample throughout the agar medium. After the agar solidified, the plates were incubated at 37°C for 18–24 hours. After incubation, the viable bacterial colonies were enumerated to assess bacterial growth in the untreated and AgNP-treated water samples. The antibacterial effect of the AgNP treatment was evaluated by comparing the number of viable colonies obtained from the treated samples with those observed in the untreated control. Plate containing 30–300 colonies were selected for enumeration, whenever application, and the results were expressed as colony-forming units per milliliter (CFU/ml).

3. RESULTS AND DISCUSSION

3.1 Formation of Silver Nanoparticles (AgNPs)

A pronounced visual change in the reaction mixture was observed during the green synthesis of AgNPs using Aloe vera leaf extract. As illustrated in **Fig. 1**, the initial reaction mixture exhibited a light-yellow coloration, which progressively intensified and changed to dark brown after 24 h. This characteristic color transition provides a preliminary qualitative indication of the reduction of Ag^+ ions to elemental silver (Ag^0) and the subsequent formation of AgNPs in the colloidal suspension. The progressive increase in color intensity was particularly evident during continuous magnetic stirring at 60 °C for approximately 2 h, indicating the progression of the reduction process. The pH of the reaction mixture was subsequently adjusted to 7.0 using 0.1 M sodium hydroxide (NaOH), after which a more pronounced development of the characteristic brown coloration was observed. This observation suggests that near-neutral conditions may favor the reduction of Ag^+ ions. The enhanced reduction under these conditions may be associated with changes in the ionization state and chemical reactivity of phytochemical constituents present in the Aloe vera extract, which may act as reducing and stabilizing agents during nanoparticle formation. Following the initial reaction period, the reaction mixture was maintained in the dark for 24 h to facilitate the further reduction of residual silver ions and allow the nanoparticle formation process to proceed. Protection from light may also minimize potential photochemical reactions that could influence the physicochemical properties of the synthesized nanoparticles. Overall, the observed color transformation served as a simple qualitative indicator of the progress of the green synthesis process. Nevertheless, visual observation alone is insufficient to conclusively establish nanoparticle formation. Therefore, complementary instrumental techniques, including UV–Vis spectroscopy, FTIR spectroscopy, XRD, SEM, and EDX, were employed to confirm the formation and characterize the physicochemical properties of the synthesized AgNPs.



Figure 1. Aloe vera plant extract before and after the addition of silver nitrate and the formation of silver particles.

3.2 UV-VIS Spectral Analysis

The UV-Vis absorption spectrum of the synthesized silver nanoparticles (AgNPs) exhibited a distinct and well-defined absorption maximum at 409 nm, as shown in **Fig. 2**. The observed absorption band lies within the characteristic spectral region associated with AgNPs and provides strong preliminary evidence of their formation. This band is attributed to the localized surface plasmon resonance (LSPR) of the nanoparticles, which originates from the collective oscillation of conduction electrons at the nanoparticle surface upon interaction with incident electromagnetic radiation (**Mubarak and Al-Haddad, 2019**). The presence of a well-defined LSPR band at 409 nm is consistent with the successful formation of AgNPs through the green synthesis route employing Aloe vera leaf extract. The spectral position, intensity, and width of the LSPR band can be affected by several physicochemical parameters, including nanoparticle size, shape, morphology, degree of aggregation, and the dielectric properties of the surrounding medium. Therefore, the observed absorption maximum at 409 nm not only supports the formation of AgNPs but also provides an important optical characteristic that can be interpreted in conjunction with the structural, morphological, and elemental information obtained from FTIR, XRD, SEM, and EDX analyses.

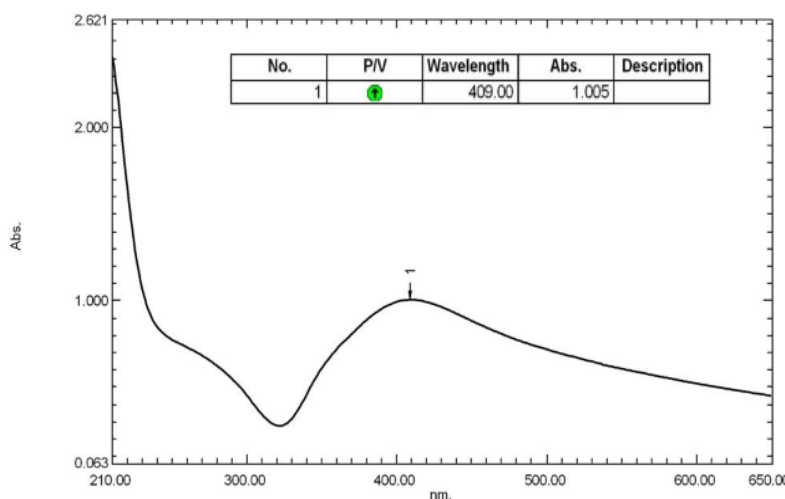


Figure 2. UV-visible spectrum of the prepared silver nanoparticles.

The UV-Vis absorption spectrum of the synthesized AgNPs exhibited a distinct absorption maximum at 409 nm, as shown in **Fig. 2**. This absorption band falls within the characteristic spectral region of approximately 400–450 nm commonly associated with the surface

plasmon resonance (SPR) of silver nanoparticles (**Liknaw et al., 2025**). The presence of this well-defined absorption band provides strong evidence for the formation of AgNPs and is consistent with the reduction of silver ions (Ag^+) to elemental silver (Ag^0) during the synthesis process. The observed optical response may be associated with the phytochemical constituents present in the Aloe vera leaf extract, including acemannan, polyphenolic compounds, and flavonoids, which may participate in the reduction of Ag^+ ions and contribute to the stabilization of the newly formed nanoparticles.

3.3 FTIR Spectrum

The FTIR spectrum of the synthesized AgNPs is presented in **Fig. 3**. The observed absorption bands were assigned to characteristic functional groups, including hydroxyl (O–H), carbonyl (C=O), amide, C–O, and C–N groups, indicating the presence of various bioactive phytochemical constituents associated with the Aloe vera extract. These functional groups may play important roles in the reduction of Ag^+ ions and the subsequent stabilization of the synthesized nanoparticles. In particular, hydroxyl- and carbonyl-containing compounds may contribute to the reduction process, whereas proteins and other nitrogen-containing biomolecules may interact with the nanoparticle surface and act as capping or stabilizing agents, thereby limiting particle aggregation. These observations support the proposed role of plant-derived phytochemicals as both reducing and stabilizing agents during the green synthesis of AgNPs (**Thomas et al., 2024**). Furthermore, shifts in the positions and/or intensities of the characteristic FTIR absorption bands observed for the AgNPs compared with those of the Aloe vera leaf extract suggest that hydroxyl, carbonyl, and amide-containing functional groups may be involved in the reduction of Ag^+ ions and the subsequent stabilization and surface functionalization of the synthesized nanoparticles. Such spectral changes provide evidence of interactions between the phytochemical constituents of the plant extract and the AgNP surface, indicating their potential contribution to nanoparticle formation and stability.

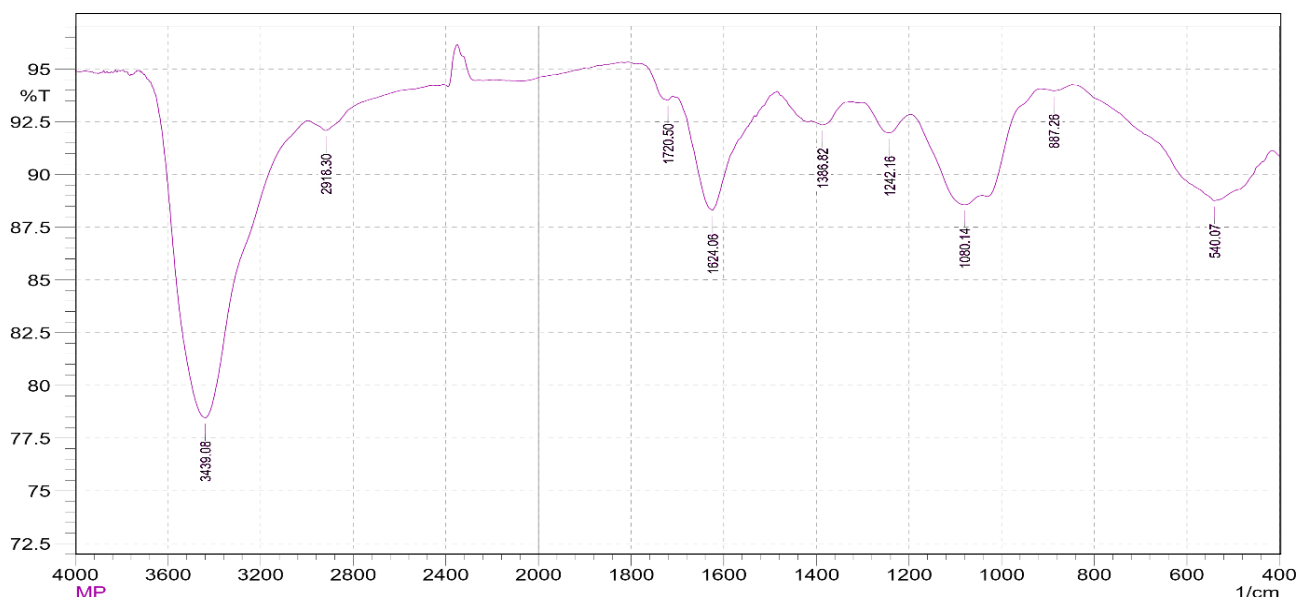


Figure 3. The FTIR spectrum of bioactive chemicals extracted from Aloe vera plant.

**Table 1.** The FTIR spectra of various compounds that are involved in AgNPs synthesis

Wavenumber cm ⁻¹	Functional group detection	Interpretation
3439	-OH	Electron donor (Reducing agent)
2918	C-H	This indicates the presence of an organic compound in the Aloe vera extract, which contributes to the formation of a coating layer around the nanoparticles, thus helping to partially stabilize them.
1720	C=O	It participates in oxidation-reduction reactions and helps reduce silver ions.
1624	-CONH-	This group plays an important role as a stabilizing agent, binding to the surface of the silver nanoparticles and forming a coating layer that helps stabilize them and reduce clumping.
1242-1386	C-O and C-N	It helps reduce and stabilize particles.
1080	C-O (polysaccharides)	They play an important role as reducing and stabilizing agents in the process of forming silver nanoparticles.
887-540	Metallic vibrations	This indicates the vibration of atoms in metallic bonds, and these bonds work to form silver nanoparticles (AgNPs).

Table 1 presents the FTIR spectral characteristics of the synthesized AgNPs. A prominent absorption band was observed at 3439 cm⁻¹, which can be attributed to O–H stretching vibrations associated with hydroxyl-containing compounds, particularly polysaccharides present in Aloe vera. Among these polysaccharides, acemannan is an important bioactive constituent that may contribute to the reduction of Ag⁺ ions and the stabilization of the resulting AgNPs. The absorption bands observed at 2918 and 1720 cm⁻¹ can be assigned to C–H stretching and C=O stretching vibrations, respectively. These functional groups may participate in the reduction of silver ions and the subsequent stabilization of the nanoparticle surface. The absorption bands at 1624 and 1242 cm⁻¹ may be associated with amide and C–O-containing functional groups originating from proteins, polysaccharides, and other phytochemical constituents adsorbed onto the nanoparticle surface. These interactions suggest that plant-derived biomolecules may act as capping agents, contributing to the stabilization of the synthesized AgNPs. In addition, a low-frequency band observed at 540 cm⁻¹ may be associated with Ag–O-related vibrations, suggesting possible interactions between silver species and oxygen-containing functional groups derived from Aloe vera phytochemicals (**Liknaw et al., 2025**). Overall, the FTIR results indicate the presence of several oxygen- and nitrogen-containing functional groups associated with Aloe vera phytochemicals on the surface of the synthesized AgNPs. The observed spectral features support the proposed involvement of these biomolecules in both the reduction of Ag⁺ ions and the stabilization of the resulting nanoparticles.

3.4 Zeta Potential Analysis

The surface charge characteristics of the synthesized AgNPs were evaluated by zeta potential analysis, as shown in **Fig. 4**. The nanoparticles exhibited a zeta potential of –37.33 mV, indicating a relatively high negative surface charge and suggesting good electrostatic stability of the colloidal suspension (**Jain and Das, 2024**). The negative surface charge may be attributed to the adsorption of ionizable phytochemical constituents from the Aloe vera

leaf extract onto the nanoparticle surface. Flavonoids, polyphenolic compounds, polysaccharides, and other oxygen-containing biomolecules may contribute to the surface charge and act as capping or stabilizing agents. The relatively high magnitude of the negative zeta potential promotes electrostatic repulsion between adjacent nanoparticles, thereby reducing the likelihood of particle–particle aggregation and contributing to the stability of the colloidal dispersion. Thus, the measured zeta potential suggests that the phytochemical constituents of Aloe vera may provide an effective stabilizing layer around the synthesized AgNPs. The electrophoretic mobility of the synthesized nanoparticles was measured as $-2.92 \text{ } (\mu\text{m/s})/(\text{V/cm})$. The negative electrophoretic mobility is consistent with the negative zeta potential and indicates that the nanoparticles possess a negatively charged surface. Under an applied electric field, negatively charged particles migrate toward the positively charged electrode. The measured electrophoretic mobility is related to the zeta potential through the appropriate electrokinetic model, such as the Smoluchowski approximation for suitable aqueous dispersions. The combined negative values of the zeta potential and electrophoretic mobility support the presence of a negatively charged and electrostatically stabilized colloidal system. In conjunction with the FTIR findings, these results provide evidence that biomolecules derived from Aloe vera are associated with the nanoparticle surface and may contribute to the stabilization of the synthesized AgNPs.

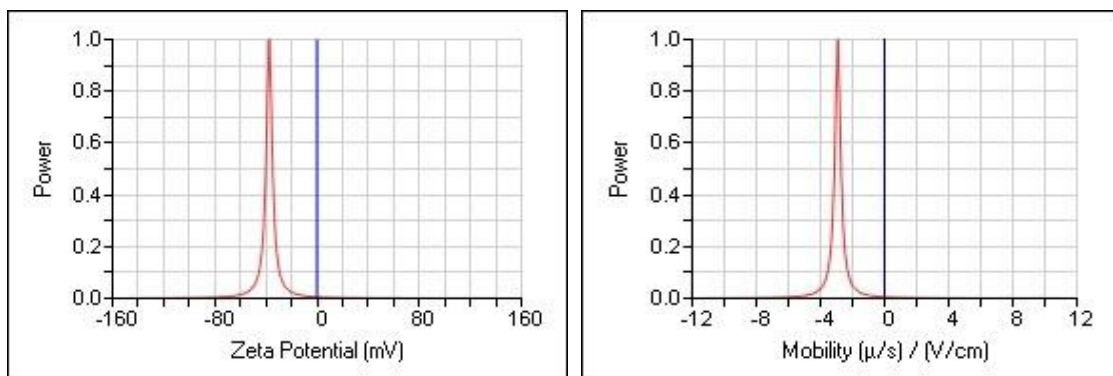


Figure 4. Analysis of the zeta potential for the prepared silver nanoparticles.

3.5 SEM-EDX ANALYSIS

The elemental composition of the synthesized AgNPs was analyzed using energy-dispersive X-ray spectroscopy (EDX), and the corresponding spectrum and elemental weight percentages are presented in **Fig. 5**. The EDX analysis revealed that silver was the predominant element in the sample, accounting for approximately 90 wt%, while smaller contributions from oxygen and chlorine were also detected. The strong silver signal provides substantial evidence for the presence of silver in the synthesized material and is consistent with the successful formation of AgNPs. The detected oxygen may be associated with oxygen-containing phytochemical residues originating from the Aloe vera leaf extract, surface oxidation, or adsorbed species on the nanoparticle surface. The presence of chlorine may be related to residual chloride-containing compounds from the synthesis procedure or, potentially, to the formation of silver chloride (AgCl). However, the EDX result alone is insufficient to conclusively establish the presence of AgCl, and further structural analysis would be required to distinguish between these possible sources. Overall, the predominance of silver in the EDX spectrum, together with the relatively low contributions of other detected elements, indicates that silver constitutes the major elemental component of the

synthesized nanomaterial. These findings, when considered alongside the UV–Vis, FTIR, XRD, and morphological results, further support the successful synthesis of AgNPs using Aloe vera leaf extract.

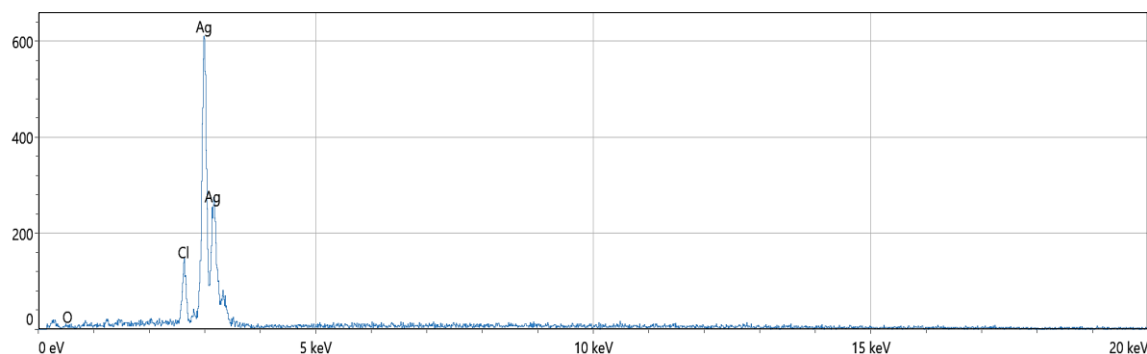


Figure 5. EDX spectrum of the prepared silver nanoparticles.

The SEM micrograph presented in **Fig. 6** reveals that the synthesized AgNPs exhibit a predominantly agglomerated morphology, characterized by dense and irregular clusters at the nanoscale. The observed aggregation may be attributed to interparticle interactions and the high surface energy of nanoparticles, which promote particle–particle attraction. In addition, the drying and sample-preparation procedures required for SEM analysis may have contributed to the formation of the observed aggregates. Such agglomeration is commonly observed in nanoparticle systems and may also be influenced by the surface chemistry and capping effects of phytochemical constituents derived from Aloe vera. The SEM observations provide valuable information regarding the surface morphology and aggregation behavior of the synthesized AgNPs and complement the zeta potential results. Although the nanoparticles exhibited a relatively high negative zeta potential, suggesting electrostatic stabilization in aqueous suspension, some degree of aggregation may occur during sample drying and preparation for SEM analysis. Therefore, the agglomerated structures observed in the SEM micrograph should not necessarily be interpreted as representative of the dispersion state of the nanoparticles in their original aqueous suspension.

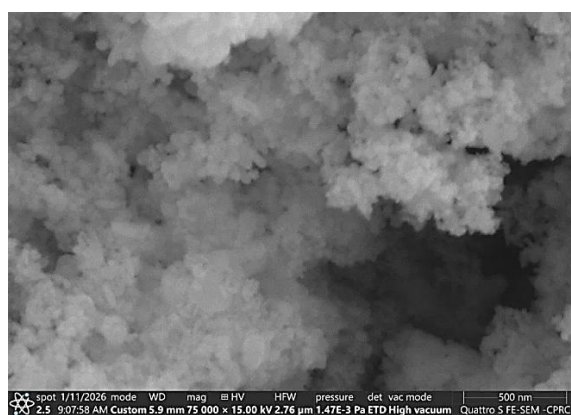


Figure 6. SEM for silver nanoparticles.

3.6 XRD Analysis

The crystalline structure and phase composition of the synthesized AgNPs were investigated using X-ray diffraction (XRD). As shown in **Fig. 7**, the XRD pattern exhibited four distinct and well-defined Bragg diffraction peaks at 2θ values of 38.2461° , 44.3° , 64.5787° , and 77.5138° .

These reflections can be indexed to the (111), (200), (220), and (311) crystallographic planes, respectively, and are characteristic of the face-centered cubic (FCC) crystal structure of metallic silver. The presence of these characteristic diffraction reflections provides strong evidence for the formation of crystalline AgNPs. The crystallite size of the synthesized AgNPs was estimated from the full width at half maximum (FWHM) of the diffraction peaks using the Scherrer equation. The corresponding diffraction peak positions, intensities, FWHM values, and calculated crystallite sizes are presented in **Table 2 (Sharma et al., 2022)**. The estimated crystallite sizes ranged from approximately 15 to 18 nm, with an average of 16.5 nm, indicating that the synthesized material possessed a nanoscale crystalline structure. The observed diffraction pattern and crystallite-size range are consistent with the formation of crystalline silver nanoparticles through the green synthesis route. The phytochemical constituents present in the Aloe vera leaf extract may contribute to the reduction of Ag^+ ions to metallic Ag^0 and subsequently act as capping and stabilizing agents during nanoparticle formation. These biomolecular interactions may influence the nucleation and growth processes and, consequently, the crystallite size and structural characteristics of the synthesized AgNPs.

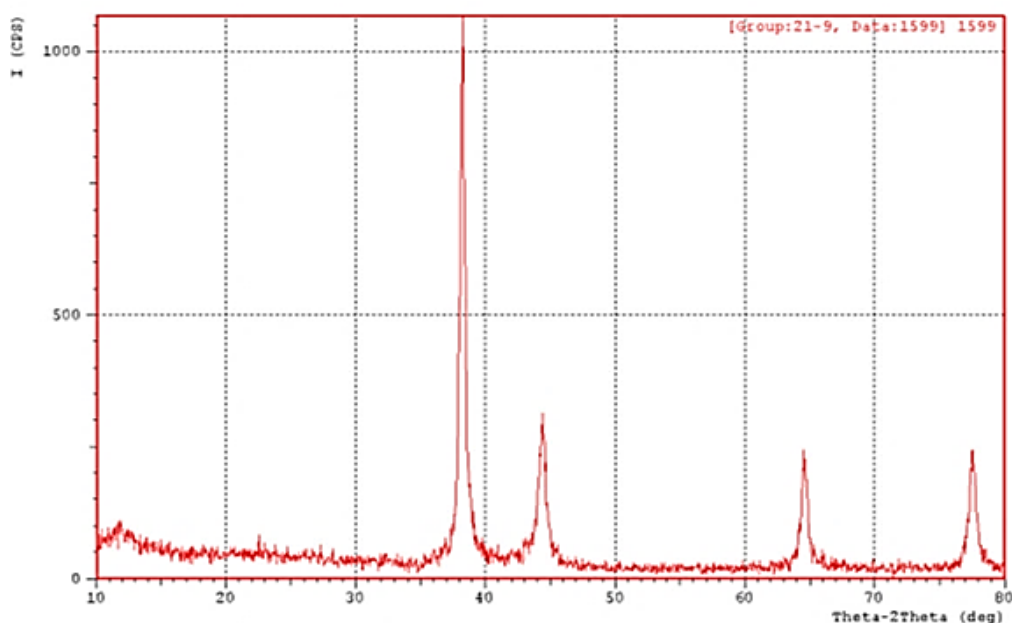


Figure 7. X-ray diffraction spectrum of the prepared silver nanoparticles.

Table 2. Spectral data of XRD analysis for AgNPs

(hkl)	D-spacing($\text{\AA}$)	Intensity	($^\circ$)2 θ	FWHM ($^\circ$)	Crystallized D(nm)
(111)	2.351	642	38.25	0.45	~18
(200)	2.039	188	44.40	0.48	~17
(220)	1.444	146	64.50	0.552	~16
(311)	1.230	146	77.55	0.55	~15

3.7 AgNPs Antibacterial Activity

Fig. 8 shows that four substances were tested and that AgNPs exhibited the highest antibacterial efficacy when compared with Aloe vera extract alone, being either comparable to or higher than that of amoxicillin.

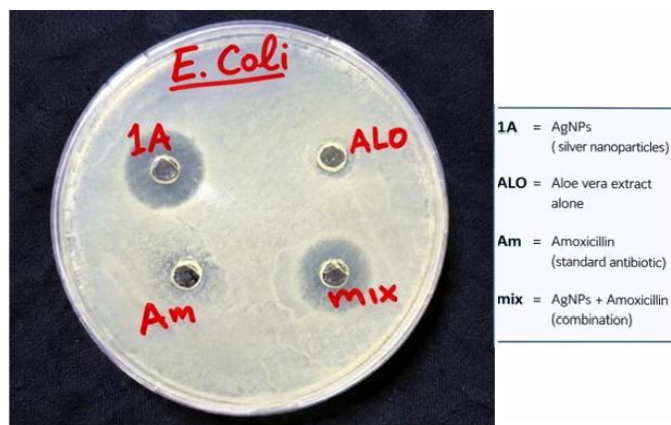


Figure 7. Antibacterial activity of AgNPs, Aloe vera extract, amoxicillin, and their combination (AGNPs + amoxicillin) against *E. coli* using the agar well diffusion method.

The antibacterial activity of the synthesized silver nanoparticles (AgNPs) was evaluated using the agar well diffusion method against *Staphylococcus aureus*, a Gram-positive bacterium, and *Escherichia coli*, a Gram-negative bacterium. Three different concentrations of the AgNP suspension were tested, namely 100 $\mu\text{g/ml}$ (A1), 10 $\mu\text{g/ml}$ (A2), and 1 $\mu\text{g/ml}$ (A3). The antibacterial activity was assessed based on the formation and diameter of the inhibition zones surrounding the wells. The corresponding inhibition zones observed for both bacterial strains at the different AgNP volumes are presented in **Fig. 8**.

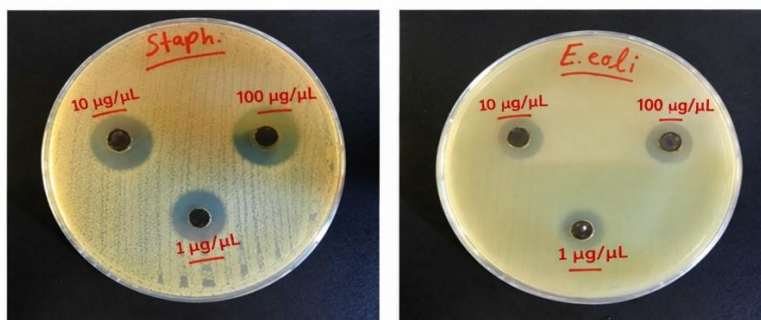


Figure 8. Inhibition zones resulting from the effect of silver nanoparticles prepared from Aloe vera extract at different concentrations on bacteria *S. aureus* (left) and *E. coli* (right) using the disc diffusion method in nutrient agar medium

Table 3. Diameters of inhibition halos (mm) of silver nanoparticles against the tested bacteria at different concentrations

Sample	AgNPs Conc. $\mu\text{g/ml}$	Size(mm) <i>S. aureus</i>	Size(mm) <i>E. coli</i>
A1	100	14	10
A2	10	12	10
A3	1	9	8

Table 3 shows a clear increase in the antibacterial activity of the synthesized AgNPs with increasing concentration against both bacterial strains. The highest concentration tested, A1 (100 μL), produced the largest inhibition zones, measuring 14 mm against *Staphylococcus aureus* and 10 mm against *Escherichia coli*. At A2 (10 μL), inhibition zones of 12 and 10 mm were observed for *S. aureus* and *E. coli*, respectively, whereas the lowest concentration, A3

(1 μL), produced inhibition zones of 9 and 8 mm. These findings indicate that the synthesized AgNPs retained measurable antibacterial activity even at the lowest concentration tested. The greater inhibition at higher concentrations may be associated with the increased amount of AgNPs available to interact with bacterial cells, thereby enhancing their antibacterial effect (**Salayová et al., 2021**). A comparison of the two bacterial species showed that *S. aureus* was generally more susceptible to the synthesized AgNPs than *E. coli* under the experimental conditions used. This difference may be related to structural differences between Gram-positive and Gram-negative bacterial cell envelopes. Gram-positive bacteria possess a relatively thick peptidoglycan layer.

In contrast, Gram-negative bacteria have a more complex cell envelope with a thin peptidoglycan layer and an outer membrane containing lipopolysaccharides (LPS). These structural differences can influence the interaction, adsorption, and penetration of AgNPs and released Ag^+ ions at the bacterial surface. However, the precise susceptibility of each bacterial species depends on several factors, including nanoparticle size, surface charge, aggregation state, bacterial strain, and experimental conditions.

3.8 Efficiency of Bacterial Removal

The antibacterial performance of the synthesized AgNPs was further evaluated by comparing viable bacterial counts in untreated contaminated water with those obtained following AgNP treatment. As shown in **Fig. 9**, a pronounced reduction in bacterial colony numbers was observed after treatment. In the first experiment, approximately 2,000 colonies were observed in the untreated control.

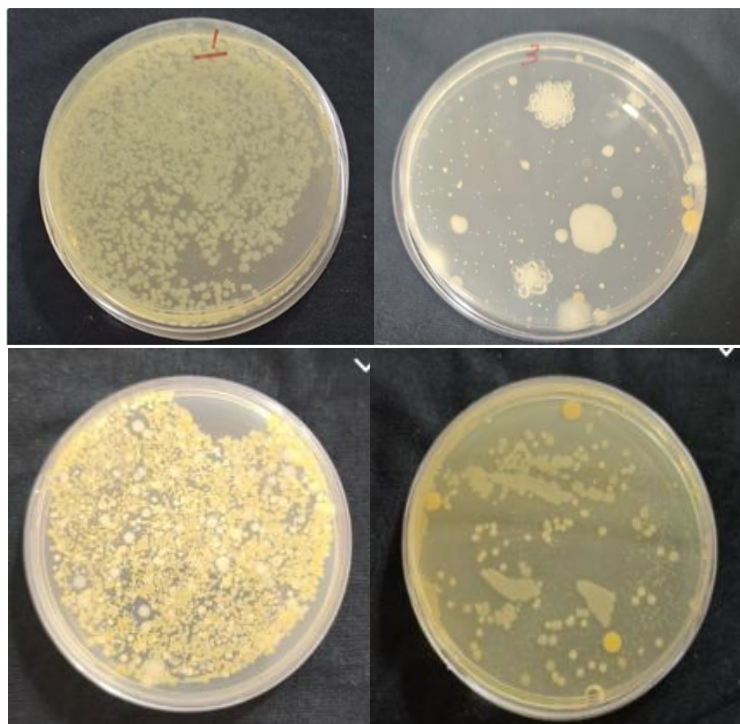


Figure 9. Bacterial colony growth in untreated control and AgNP-treated water samples on nutrient agar after incubation at 37 °C for 24 h. Control samples are shown on the left and AgNP-treated samples on the right; the upper and lower panels represent the first and second experiments, respectively.



Whereas approximately 180 colonies were detected in the AgNP-treated sample. In the second experiment, the untreated control contained more than 2,000 colonies, compared with approximately 200 colonies in the treated sample. The observed reduction in viable bacterial colonies demonstrates the effectiveness of the synthesized AgNPs in suppressing bacterial growth in contaminated water. Based on the reported colony counts, the calculated log reductions were approximately 1.04 and 1.00 for the first and second experiments, respectively, with an average log reduction of approximately 1.02. This corresponds to an approximate bacterial reduction of 90.5% under the experimental conditions employed. The relatively close bacterial counts obtained in the two treatment experiments (approximately 180 and 200 colonies) indicate good consistency between the experimental measurements. The observed antibacterial activity of the synthesized AgNPs may be attributed to several complementary mechanisms. AgNPs can interact with bacterial cell envelopes and, through the release of Ag^+ ions, interfere with membrane integrity and cellular functions. These interactions may increase membrane permeability, promote leakage of intracellular components, and ultimately compromise bacterial viability. In addition, AgNPs and released silver ions may contribute to the generation of reactive oxygen species (ROS), which can induce oxidative damage to essential cellular components, including proteins, lipids, and nucleic acids. Such combined effects can disrupt vital metabolic processes and ultimately lead to bacterial cell death (Godakhindi et al., 2025).

4. CONCLUSIONS

Silver nanoparticles (AgNPs) were successfully synthesized using a green synthesis approach based on *Aloe vera* leaf extract. The combined results obtained from UV-Vis spectroscopy, FTIR, XRD, EDX, SEM, and zeta potential analysis provided complementary evidence for the formation, crystalline structure, elemental composition, surface functionalization, morphology, and colloidal stability of the synthesized AgNPs. The characteristic UV-Vis absorption band at 409 nm, the crystalline diffraction pattern corresponding to the face-centered cubic structure of metallic silver, and the predominant silver signal observed by EDX collectively supported the successful synthesis of AgNPs. The synthesized AgNPs exhibited measurable antibacterial activity against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, as demonstrated by the formation of inhibition zones in the agar diffusion assay. Furthermore, treatment of contaminated water with the synthesized AgNPs resulted in an average log reduction of approximately 1.02, corresponding to an estimated bacterial reduction of approximately 90.5% under the experimental conditions employed. These findings demonstrate the potential of *Aloe vera*-mediated AgNPs as an effective antimicrobial material, particularly for applications involving the reduction of bacterial contamination in aqueous systems. The findings also highlight the potential of *Aloe vera* leaf extract as a readily available and environmentally favorable source of reducing and stabilizing phytochemicals for AgNP synthesis. However, further optimization is required to improve nanoparticle dispersion, reduce aggregation, and establish the relationship between synthesis parameters and nanoparticle size and morphology. Future studies should investigate different extract concentrations and reaction conditions, together with more extensive antimicrobial and toxicity assessments, to determine the suitability of *Aloe vera*-derived AgNPs for potential biomedical, environmental, and food-related applications.



Credit Authorship Contribution Statement

Salma S. Alani: Writing –original draft, Project administration, Formal analysis. Mustafa H. Al-Furaiji: Writing –review & editing, Validation. Azhar Q. Getan: Project administration, Investigation. Suhaila K. Ali: Methodology, Validation. Safauldeen Adnan: Data curation, Conceptualization. Rouhollah Y. Farsani: Writing – review & editing.

Declaration of Competing Interests

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.

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التخليق الأخضر لجسيمات الفضة النانوية باستخدام مستخلصات نباتية وتطبيقها في مجال معالجة المياه كمضاد للجراثيم

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الخلاصة

أظهرت هذه الدراسة نجاح التخليق الأخضر لجسيمات الفضة النانوية (AgNPs) باستخدام مستخلص أوراق الألوفيرا (Aloe vera) بوصفه عاملاً مختزلاً ومثبتاً. وتمثل طريقة التخليق المقترحة نهجاً مستداماً وصديقاً للبيئة، يمكن أن يشكل بديلاً مناسباً للطرائق الكيميائية التقليدية. وقد تم الاستدلال مبدئياً على تكوّن جسيمات الفضة النانوية من خلال التغير اللوني الواضح من الأصفر الفاتح إلى الأصفر المائل إلى البني، مما قدّم مؤشراً أولياً على تكوّن الجسيمات لنانوية. وأكدت مطيافية الأشعة فوق البنفسجية-المرئية (UV-Vis) عملية التخليق بصورة إضافية من خلال ظهور حزمة مميزة للرنين البلازموني السطحي (Surface Plasmon Resonance, SPR) ضمن النطاق الطيفي المعتاد لجسيمات الفضة النانوية، مما يدل على الاختزال الفعال لأيونات الفضة (Ag^+) إلى الفضة المعدنية (Ag^0). وأوضحت مطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR) مشاركة مجموعات وظيفية مرتبطة بالمركبات الفينولية والفلافونويدية الموجودة في مستخلص Aloe vera، مما يشير إلى دورها في اختزال أيونات الفضة، وتثبيت الجسيمات النانوية المتكونة، وتكوين طبقة سطحية واقية حولها. كما تم تقييم الاستقرار الغروي لجسيمات الفضة النانوية المحضّرة في الوسط المائي باستخدام قياسات جهد زيتا (Zeta Potential) وأظهر المجهر الإلكتروني الماسح (SEM) مورفولوجيا يغلب عليها التكتل، حيث ظهرت الجسيمات النانوية على هيئة تجمعات كثيفة وغير منتظمة على المقياس النانوي. وأكد تحليل مطيافية الأشعة السينية المشتتة للطاقة (EDX) التركيب العنصري للمادة المحضّرة، إذ كانت الفضة العنصر الغالب ضمن العناصر المكتشفة. كذلك أظهر تحليل حيود الأشعة السينية (XRD) أربع قمم حيود مميزة تتوافق مع التركيب البلوري المكعب متمركز الوجوه (Face-Centered Cubic) FCC للفضة المعدنية. أظهرت جزيئات الفضة النانوية (AgNPs) المحضّرة تأثيراً مشبّطاً تجاه كل من Staphylococcus aureus (بكتيريا موجبة الغرام) و Escherichia coli (بكتيريا سالبة الغرام). وكانت الفعالية المضادة للبكتيريا أكثر وضوحاً تجاه S. aureus عند جميع التراكيز المختبرة. وأظهر التعداد البكتيري كذلك انخفاضاً واضحاً في الحمل البكتيري في المجموعة المعاملة بجسيمات الفضة النانوية المحضّرة باستخدام Aloe vera مقارنةً بالمجموعة الضابطة، حيث بلغ الانخفاض 1.02 لوغاريتم، وهو ما يعادل انخفاضاً بنسبة 90.5% في العدد البكتيري.

الكلمات المفتاحية: جسيمات الفضة النانوية (AgNPs)، التخليق الأخضر، Aloe vera، النشاط المضاد للبكتيريا، الرنين البلازموني السطحي، المواد النانوية.