



Antibacterial Activity of Green Synthesized Silver Nanoparticles from Aloe Vera Plant

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Abstract— This study offers an environmentally friendly and cost-effective method for synthesizing silver nanoparticles (AgNPs) using *Aloe vera* leaf extract, without the use of harmful chemicals. The phytochemicals, such as polysaccharides and flavonoids, acted as reducing and stabilizing agents to convert silver ions (Ag^+) to metallic silver (Ag^0). Best synthesis conditions were found by mixing 90 mL of 1 mM AgNO_3 with 10 mL of 10% *Aloe vera* extract at pH 8, followed by a 48-hour incubation. The mixture color changed from light yellow to dark brown, indicating AgNPs formation, confirmed by UV-Vis spectroscopy with a peak at 411 nm. Characterization via Dynamic Light Scattering (DLS), Zeta potential, and Fourier Transform-Infrared Spectroscopy (FTIR) showed that AgNPs had an average size of 147.6 nm, a polydispersity index of 0.387, and a zeta potential of -40.3mV , indicating high stability. Antibacterial efficacy was evaluated using the disc diffusion assay against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) at 2, 5, and 10 $\mu\text{g}/\mu\text{L}$ concentrations. The test showed dose-dependent inhibitory zone diameters of 7.4-10.2 mm against *S. aureus* and 8-9.1 mm against *E. coli*. These results highlight the potential of green synthesized AgNPs in antibacterial activity against resistant tested strains. Future studies are recommended to evaluate long-term stability and biocompatibility.

Keywords—green synthesis; silver nanoparticles; *Aloe vera*; antibacterial activity.

I. INTRODUCTION

Antibiotic resistance is considered one of the major public health challenges. As bacteria are becoming increasingly resistant to traditional antibiotics, the World Health Organization (WHO) listed multiple priority strains in 2017 for which new, safe, and effective therapeutic agents are urgently needed. *Staphylococcus aureus* (*S. aureus*) is one of the high-priority antibiotic resistant bacteria [1]. *Escherichia coli* (*E. coli*) is not in the list; however, recent studies reported its growing resistance [2].

Nanotechnology is an emerging approach that has proven its efficacy in many biomedical applications, including antibacterial resistance. Nanomaterials are one of the products of nanotechnology research, where nano-sized particles ranging between 10 to 100 nm are used increasingly due to their characteristic physicochemical features [3]. Nanoparticles are known for their large surface area, ability to interact with bacteria membranes, and subsequently cytotoxic and antibacterial potential [4]. Metal nanoparticles are a promising

subtype of nanomaterials where metals such as silver, gold, and copper are used to synthesize nanoparticles with characteristic optical, catalytic, and antimicrobial properties [5].

Silver nanoparticles (AgNPs) are a type of metal NPs that have been extensively employed as an antibiotic agent in many health conditions such oral infections and wound healing. While AgNPs are well-documented to inhibit bacterial growth, the underlying mechanisms are not yet fully understood. Some studies report that AgNPs can penetrate bacterial wall and cause DNA damage and increase reactive oxygen species (ROS) inside the bacteria, leading to cell death [6-7].

The physicochemical properties, especially the size and shape of AgNPs plays a crucial role in dictating their bactericidal activity. These features mainly rely on the synthesis approach. There are two synthesis methods, chemical synthesis and biosynthesis. The latter involves producing NPs from various living organisms such as bacteria, fungi and plant extracts [8].

Green synthesis is an eco-friendly, sustainable, cost-effective, and non-toxic approach of producing nanoparticles. It refers to the use of plant extracts as reducing and stabilizing agents for converting silver ions (Ag^+) to metallic silver (Ag^0). These agents are the phytochemical components of plants such as flavonoids, phenolics, alkaloids, and proteins. Green synthesis is reported to produce NPs with higher surface area to volume ratio compared to other methods, leading to better bioactivity [8-9].

Aloe vera plant (AV) belongs to the *Liliacea* family. It is a xerophyte, arborescent, and succulent plant known for its medicinal properties, traditionally used for wound healing and burns. It contains various bioactive compounds, including polysaccharides, glycoproteins, and anthraquinones, which contribute to its therapeutic effects. These compounds possess antimicrobial properties, making *Aloe vera* an ideal candidate for the green synthesis of silver nanoparticles in the context of antibiotic resistance [9-10].

The present study aims to develop a green and sustainable method for synthesizing silver nanoparticles (AgNPs) using *Aloe vera* plant extract. This biosynthesis approach harnesses the natural reducing and stabilizing agents present in *Aloe vera* to produce environmentally friendly nanoparticles. The synthesized AgNPs are then thoroughly characterized to assess their physicochemical properties. Additionally, the paper aims to evaluate the antibacterial efficacy of the AgNPs against both

gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacterial strains using the disc diffusion method. This research explores the potential application of green-synthesized AgNPs as an effective antibacterial agent, particularly in addressing the growing challenge of antibiotic resistance.

II. METHODS

A. Synthesis and Characterization of AgNPs from *Aloe vera* plant extract.

AgNPs were synthesized based on previously published protocol [11], with some modifications. First, 10 g of washed *Aloe vera* (AV) leaves were chopped into small pieces in a mortar and pestle, then transferred into a glass beaker containing 100 mL of distilled water. The solution was heated for 30 min at 60°C with continuous stirring until it turned light yellow. After cooling, the extract was purified using filter paper. The resulting plant extract solution was stored at 4°C for future use. Silver nitrates (AgNO_3) solution was prepared at 1mM concentration by mixing 0.017g of AgNO_3 powder with 100 mL of distilled water. For AgNPs synthesis, 90 mL of AgNO_3 solution was mixed with 10 mL of 10% AV extract and few drops of 1M NaOH to reach pH 8. The resulting 100 mL of NPs were left in the magnetic stirrer at 300 rpm for 1 hour and then incubated at 40°C for 48h in dark. After incubation period, the AgNPs were centrifuged at 4100 rpm for 30 min. The supernatant was discarded and residual pellet was resuspended with distilled water and put in a petri dish and dried in the oven at 40°C. The collected AgNPs powder was stored at 4°C for further applications. The experimental framework for AgNPs synthesis is schematically presented in Fig. 1.

The biosynthesized silver nanoparticles from *Aloe vera* plant were characterized using the following methods: UV-Vis spectroscopy to understand the optical properties of AgNPs within the wavelength range of 200-900 nm, Dynamic Light Scattering (DLS) to measure the hydrodynamic diameter of the particles, Zeta potential to assess the surface charge and stability of nanoparticles, and Fourier transmission-infrared spectroscopy (FTIR) to analyze the functional groups resulting from the reduction and stabilization of AgNPs. DLS and Zeta measurements were conducted three times. Data are presented as mean $\pm$ standard error.

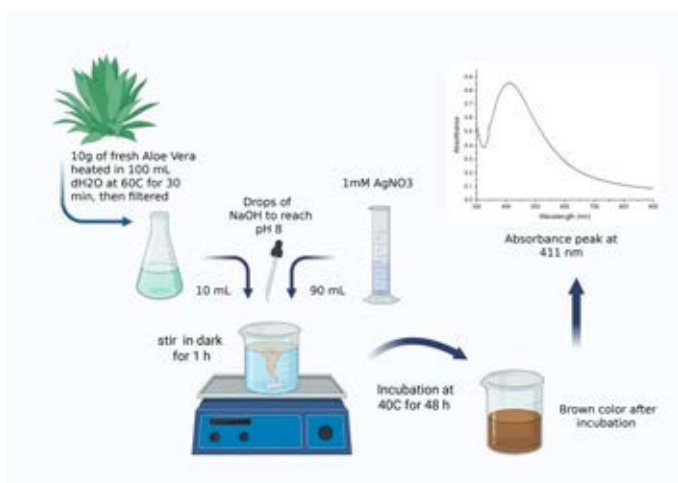


Fig. 1. The experimental framework of AgNPs synthesis

B. Antibacterial Activity of silver nanoparticles

The antibacterial activity of green synthesized AgNPs was assessed using agar disc diffusion assay [12]. One gram-negative (*E. coli* ATCC-8739) and one gram-positive (*S. aureus* ATCC-6538) bacterial strains were used.

38 grams of Mueller-Hinton agar were weighed, dissolved in 1 liter of distilled water, sterilized, then cooled and poured into petri dishes. Three AgNPs concentrations were prepared (2, 5, 10 $\mu\text{g}/\mu\text{L}$), and 6 mm discs were added into the NPs dilutions (discs immersed together into a NPs solution of 1 ml and left to absorb it) and allowed at 4°C for absorption. Bacteria were incubated overnight in the broth liquid medium. After incubation, the young bacteria were diluted and 100 μL of the diluted sample was taken and spread throughout the petri dish. The AgNPs-absorbed discs and Tetracycline discs (30 μg) for a positive control were placed on top of the bacteria and incubated overnight at 37°C. The inhibition zones were determined by measuring the diameters of the zones around the discs. Each AgNPs concentration was tested three times. Data are expressed as mean $\pm$ standard error and a paired two-tailed Student's t-test was employed for P-value. Each concentration was compared to the antibiotic control group (Tetracycline).

III. RESULTS AND DISCUSSION

Silver nanoparticles were successfully synthesized using *Aloe vera* (AV) leaves extract. The solution color changed from light yellow to dark brown (Fig. 2a, 2b), indicating the conversion of silver ions (Ag^+) to silver metal. This change is justified by the presence of chemical reducing agents in the AV extract [13].

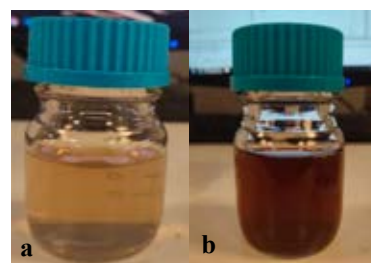


Fig. 2. Color change of AgNPs. a. Initial mixture. b. after 48h incubation.

In addition to the color testing, UV-Vis spectroscopy was used to confirm the formation of silver nanoparticles. The absorbance peak between 400 and 450 nm is an indicator of AgNPs formation [14]. In our context, a peak was observed at 411 nm (Fig. 3).

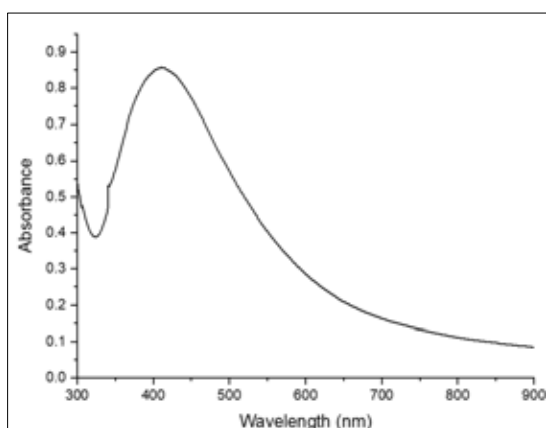


Fig. 3. Absorption spectra of AgNPs.

The FTIR results highlight the chemical composition of the dried AgNPs synthesized from *Aloe vera* (Fig. 4). From the observed peaks, the bio-compounds in the *Aloe vera* were essential for good nanoparticles as reducing, capping, and stabilizing agents. Table I gives more details about the present functional groups. Based on published literature, the wavenumbers were associated to their corresponding active groups [11,13].

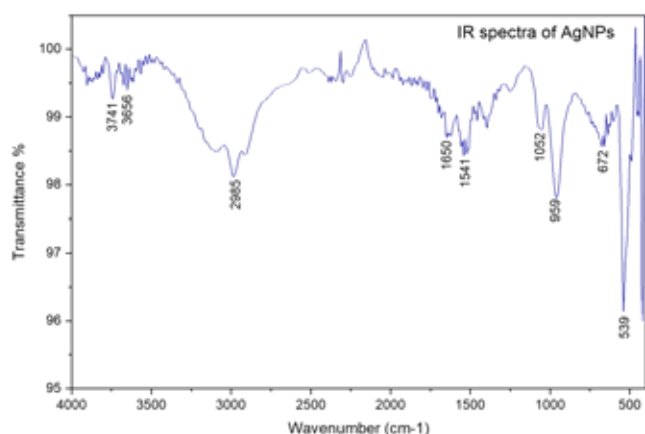


Fig. 4. FTIR spectra of AgNPs synthesized from *Aloe vera*.

Table I. Active compounds involved in AgNPs stabilization [11,13].

Wavenumber (cm ⁻¹)	Functional group	Comment
3656 and 3741	O-H stretching	Hydroxyl groups in polysaccharides or phenols
2985	C-H stretching	Aliphatic groups
1650	C=O stretching	carbonyl group aldehydes and aliphatic amides
1541	C = O and C-N stretching	nitro compounds
1052	C-O stretching	alcohols
959	C = C bending	Polysaccharides or aromatic compounds
672	C-H bending	Aromatic compounds
539	Ag-O or Ag-N bond stretching	Metal-ligand bond

Dynamic Light Scattering (DLS) and zeta potential measurements were employed to assess the size distribution and

surface charge stability of AgNPs (Table II). The average diameter was found to be 147.6 nm, with a polydispersity index (PDI) of 0.387. This indicates the moderate size distribution homogeneity and the fairly uniform nanoparticles suitable for biological applications. Previous studies reported that AgNPs with moderate size and low-to-moderate PDI demonstrate acceptable antimicrobial/antibacterial activities [15,16].

Zeta potential showed a surface charge of -40.3mV , suggesting strong electrostatic repulsion between particles. This high negative value indicates good colloidal stability and low aggregation potential, which is essential for prolonged suspension and effective interaction with bacterial membranes [15].

Table II. Size, Polydispersity Index, and Zeta Potential of AgNPs (n=3)

Measurement	Hydrodynamic diameter (DLS) (Mean $\pm$ SD)	Polydispersity index (PDI)	Surface charge (Zeta potential) (Mean $\pm$ SD)
AgNPs	147.6 ± 3.08 nm	0.387	-40.3 ± 0.66 mV

The antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* plant was evaluated using the disc diffusion assay against *S. aureus* and *E. coli* at three concentrations (2 μg , 5 μg , and 10 μg). Results from images (Fig. 5) were quantified and illustrated in the bar graph (Fig. 6).

The inhibition zone diameters clearly indicate that AgNPs exhibited dose-dependent antibacterial effects against both tested strains. At 2 μg , AgNPs showed moderate inhibition zones of 7.4 ± 0.67 mm for *S. aureus* and 8 ± 0.25 mm for *E. coli*. When the concentration increased to 10 μg , the diameters extended to 10.2 ± 0.76 mm and 9.1 ± 0.17 mm, respectively.

This observed antibacterial effect of AgNPs is explained by their ability to attach to bacterial membrane proteins, triggering permeability and entrance of nanoparticles inside the cell. Subsequently, enzyme deactivation, protein denaturation, and DNA damage occur, leading to bacteria death. Another mechanism is the release of silver ions (Ag^+) from the nanoparticles. These ions can disrupt the uptake and release of essential physiological ions required for cellular function [13,17]. In Fig. 7, the possible antibacterial effect of AgNPs is schematically illustrated.

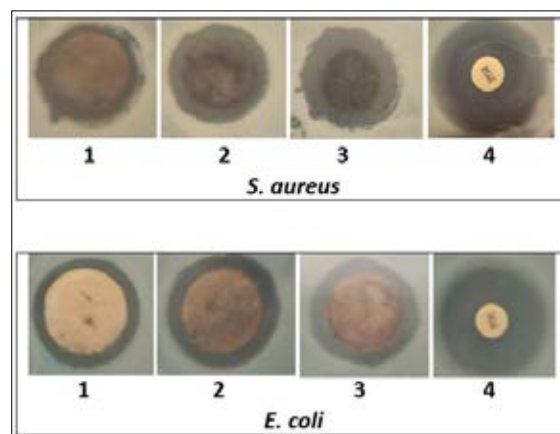


Fig. 5. Antibacterial activities of different concentrations of AgNPs. 1 (2 μg), 2 (5 μg), 3 (10 μg), 4 (Tetracycline 30 μg).

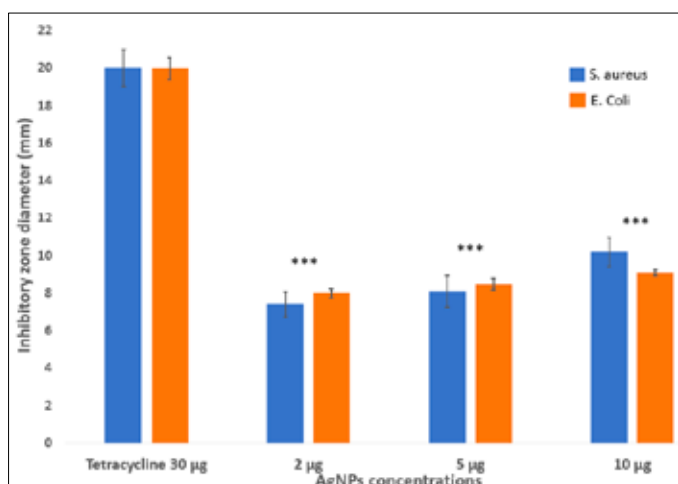


Fig. 6. Inhibitory zone diameter of AgNPs synthesized from *Aloe vera* against *S. aureus* and *E. coli*. (n=3) (***)p < 0.001.

Table III. Inhibitory zone diameter of AgNPs (n=3) (***)p < 0.001)

Micro-organism	Inhibition zone diameter in millimeters (Mean ± SD)			
	Positive control	AgNPs (µg/µL)		
	Tetracycline 30 µg	2	5	10
<i>S. aureus</i>	20 ± 1	7.4 ± 0.67***	8.1 ± 0.85***	10.2 ± 0.76***
<i>E. coli</i>	20 ± 0.58	8 ± 0.25***	8.5 ± 0.32***	9.1 ± 0.17***

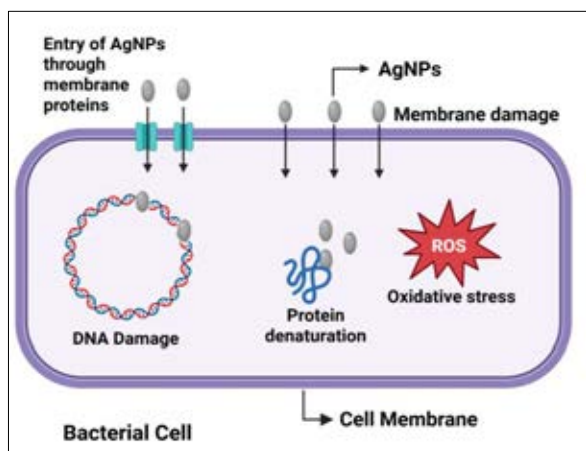


Fig. 7. Schematic illustration of possible antibacterial mechanisms of AgNPs, created by the author based on concepts described in the literature [13, 17]. Created by BioRender.

IV. CONCLUSION

In the present study, a simple, eco-friendly, and inexpensive green synthesis method was proven to be effective in producing biologically active silver nanoparticles using *Aloe vera* plant extract. The successful formation of AgNPs was confirmed by the brown color and the characteristic absorbance peak at 411 nm in UV-Vis spectroscopy. DLS, Zeta potential, and FTIR analyses verified the nanoparticles' size (147.6 nm), stability (-40.3 mV), and functional groups composition, respectively. The biosynthesized AgNPs exhibited dose-dependent antibacterial activity against both gram positive (*S. aureus*) and gram negative (*E. coli*) bacterial strains. This study highlights the

biomedical potential of AgNPs against antibiotic-resistant microorganisms. Further studies are needed to check nanoparticles long-term stability and biocompatibility.

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