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RESEARCH ARTICLE

Synthesis and Antibacterial Properties of Silver Nanoparticles Derived from *Cardiospermum halicacabum* Leaves

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ABSTRACT: This study investigates the green synthesis of silver nanoparticles (AgNPs) using *Cardiospermum halicacabum* leaf extract, which acts as both a reducing and stabilizing agent. The biosynthesis approach is eco-friendly and leverages the phytochemical constituents of *Cardiospermum halicacabum*. Characterization of the synthesized AgNPs was performed using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR), confirming the formation of nanoparticles with desired properties. SEM analysis revealed the morphology and size distribution of the AgNPs, while XRD provided insights into their crystalline nature, and FTIR identified functional groups involved in the reduction and stabilization processes. Despite the successful synthesis of AgNPs, antimicrobial assays indicated that these nanoparticles did not exhibit significant antibacterial activity against tested microorganisms such as *Escherichia coli* and *Staphylococcus aureus*. The results suggest that the synthesized AgNPs require further optimization to enhance their antimicrobial efficacy. This study underscores the potential of *Cardiospermum halicacabum* in green nanoparticle synthesis and highlights the need for continued research to understand the factors influencing the antimicrobial properties of biosynthesized AgNPs, aiming to improve their effectiveness for clinical and industrial applications.

Keywords: Silver nanoparticles, antimicrobial activity, green synthesis, *Cardiospermum halicacabum* leaves.

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1. INTRODUCTION

Cardiospermum halicacabum, commonly known as balloon vine, heart pea, or puff-ball, is a medicinal plant with significant therapeutic potential [1-3]. Its scientific name, derived from the Latin words "cardio," meaning heart, and "sperma," meaning seed, reflects its distinctive seed shape. This plant has been utilized in traditional medicine systems, particularly in India, where it is referred to as "karṇasphoṭa" in the Ayurvedic tradition. *Cardiospermum halicacabum* belongs to the Sapindaceae family and is well-known for its wide range of medicinal applications [3, 4]. It grows as an annual or sometimes perennial climber, with young shoots often consumed as a vegetable. The plant is used as a rubefacient, diuretic, and stomachic. It is also employed as a demulcent for orchitis and dropsy, and for treating conditions such as lumbago, rheumatism, and neurological disorders due to its notable analgesic, anti-inflammatory, and vasodepressant effects [5-7].

Silver nanoparticles (AgNPs) have gained significant attention in various fields, particularly for their clinical applications [8]. AgNPs exhibit unique physicochemical properties, including high surface area, tunable surface chemistry, and remarkable antimicrobial activity, which make them suitable for applications in medicine, cosmetics, and environmental remediation [9]. The traditional methods of synthesizing AgNPs involve physical and chemical processes that often require high energy inputs and the use of

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toxic chemicals, raising environmental and health concerns [10]. As a result, there is a growing interest in developing eco-friendly and sustainable methods for nanoparticle synthesis.

Green synthesis of nanoparticles using plant extracts has emerged as a promising alternative, offering several advantages over conventional methods. This approach is simple, cost-effective, and environmentally benign, utilizing natural resources that are readily available. Plant extracts contain a variety of bioactive compounds, such as phenolics, flavonoids, alkaloids, and terpenoids, which can act as reducing and stabilizing agents in nanoparticle synthesis. These phytochemicals not only facilitate the reduction of metal ions to nanoparticles but also provide stability and prevent agglomeration [11-14].

Cardiospermum halicacabum is a valuable source of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolic acids [15]. These compounds have been shown to possess antioxidant, anti-inflammatory, and antimicrobial properties, making *Cardiospermum halicacabum* an ideal candidate for the green synthesis of AgNPs. Previous studies have demonstrated the potential of plant extracts from various species in synthesizing metal nanoparticles with enhanced antimicrobial activity. For instance, extracts from plants such as *Azadirachta indica*, *Lantana camara*, *Ocimum sanctum*, and *Skimmia laureola* have been successfully used to synthesize AgNPs with potent antibacterial effects against a range of pathogens [16, 17].

The antibacterial activity of AgNPs is primarily attributed to the release of silver ions (Ag^+), which can interact with microbial cells in several ways. Silver ions are known to bind with the phosphate groups of nucleic acids, disrupting DNA and RNA functions. Additionally, Ag^+ ions can interact with thiol groups in proteins, leading to protein denaturation and enzyme inhibition. The electrostatic attraction between positively charged AgNPs and negatively charged bacterial cell membranes also contributes to the nanoparticles' bactericidal effects. This interaction can cause membrane damage, increase permeability, and ultimately lead to cell death [18-29].

Despite the promising antimicrobial properties of AgNPs, their efficacy can be influenced by various factors, including size, shape, surface charge, and the presence of stabilizing agents. The synthesis conditions and the choice of plant extract play crucial roles in determining these properties. Therefore, it is essential to optimize the synthesis parameters to enhance the antimicrobial activity of AgNPs.

In this study, we explore the green synthesis of AgNPs using *Cardiospermum halicacabum* leaf extract as a reducing and stabilizing agent. The synthesis process is characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) to confirm the formation and properties of the AgNPs. The antimicrobial activity of the synthesized nanoparticles is evaluated against common bacterial pathogens, including *Escherichia coli* and *Staphylococcus aureus*, using the disc diffusion method.

2. EXPERIMENTAL DETAILS

2.1. Chemicals

2,2′-Azinobis (3-ethylbezthiazoline-6-sulfonate) (ABTS) was purchased from SRL, India. Tartaric acid, *n*-propanol, and catechol were purchased from Sisco Research Laboratories, India. All chemicals used were of the highest purity and analytical grade.

2.2. Preparation of Plant Extract

Cardiospermum halicacabum leaves were collected from a local area and thoroughly washed with distilled water to remove dust and other impurities. The clean leaves were then air-dried in the shade for several days until they became crisp. The dried leaves were ground into a fine powder using a mechanical grinder. To prepare the plant extract, 10 grams of the powdered leaves were mixed with 100 mL of distilled water in a glass beaker. The mixture was boiled for 30 minutes at 80°C and then allowed to cool to room temperature. The resulting solution was filtered using Whatman No. 1 filter paper to remove any solid residues, yielding a clear *Cardiospermum halicacabum* leaf extract. This extract was stored at 4°C and used within one week for the synthesis of silver nanoparticles.

2.3. Green Synthesis of Silver Nanoparticles

The green synthesis of silver nanoparticles (AgNPs) was carried out using the prepared *Cardiospermum halicacabum* leaf extract. A 1 mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used as the precursor for AgNP synthesis. In a typical synthesis procedure, 10 mL of *Cardiospermum halicacabum* leaf extract was added to 90 mL of 1 mM $AgNO_3$ solution in a glass beaker. The mixture was stirred continuously at room temperature for 2 hours. The formation of AgNPs was indicated by a change in color from light yellow to brownish-yellow, suggesting the reduction of silver ions to silver nanoparticles. The reaction mixture was further heated at 60°C for 30 minutes to ensure complete reduction and stabilization of the nanoparticles. After cooling to room temperature, the AgNPs were separated from the reaction mixture by centrifugation at 10,000 rpm for 15 minutes. The supernatant was discarded, and the AgNP pellet was washed three times with distilled water to remove any unreacted plant extract and silver ions. The purified AgNPs were dried in an oven at 60°C and stored in a desiccator for further characterization and antimicrobial studies.

2.4. Characterization of Silver Nanoparticles

The synthesized AgNPs were characterized using various analytical techniques to determine their morphological, structural, and chemical properties. The surface morphology and size distribution of the synthesized AgNPs were analyzed using a scanning electron microscope. A small amount of the

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dried AgNPs was dispersed in ethanol, and a drop of the suspension was placed on a carbon-coated copper grid. After drying, the samples were examined under the SEM at an accelerating voltage of 15 kV. The crystalline structure of the AgNPs was determined using X-ray diffraction analysis. The dried AgNPs were powdered and placed on a glass slide. XRD patterns were recorded using a diffractometer with Cu Kα radiation (λ = 1.5406 Å) at a voltage of 40 kV and a current of 30 mA. The diffraction data were collected in the 2θ range of 20° to 80°. FTIR spectroscopy was used to identify the functional groups present in the *Cardiospermum halicacabum* leaf extract and the synthesized AgNPs. The samples were prepared by mixing the dried AgNPs with potassium bromide (KBr) and pressing them into pellets. The FTIR spectra were recorded in the range of 4000 to 400 cm^{-1} using an FTIR spectrometer.

2.5. Antimicrobial Activity Assay

The antimicrobial activity of the synthesized AgNPs was evaluated against common bacterial pathogens, including *Escherichia coli* (E. coli) and *Staphylococcus aureus* (S. aureus), using the disc diffusion method. Pure cultures of *E. coli* and *S. aureus* were obtained from a microbiology laboratory. The bacterial strains were grown in nutrient broth at 37°C for 24 hours. The cultures were then diluted with sterile saline to obtain a bacterial suspension with an optical density of 0.1 at 600 nm, corresponding to approximately 10^6 CFU/mL. Nutrient agar plates were prepared by pouring molten nutrient agar into sterile Petri dishes and allowing it to solidify. The agar plates were then inoculated with the bacterial suspensions using a sterile cotton swab to ensure even distribution of the bacteria. Sterile paper discs (6 mm in diameter) were soaked in 20 µL of the AgNP suspension (1 mg/mL) and placed on the surface of the inoculated agar plates. The plates were incubated at 37°C for 24 hours. After incubation, the antibacterial activity of the AgNPs was assessed by measuring the diameter of the zones of inhibition (clear zones) around the paper discs. The measurements were taken using a digital caliper, and the results were recorded in millimeters. Control experiments were conducted using paper discs soaked in distilled water and a standard antibiotic (gentamicin) to compare the antimicrobial efficacy of the AgNPs.

This detailed experimental procedure outlines the green synthesis of silver nanoparticles using *Cardiospermum halicacabum* leaf extract, their characterization using various analytical techniques, and the evaluation of their antimicrobial activity against common bacterial pathogens. The use of green synthesis methods not only provides an eco-friendly alternative to traditional nanoparticle synthesis but also leverages the medicinal properties of *Cardiospermum halicacabum* to enhance the antimicrobial efficacy of the synthesized AgNPs.

3. RESULTS AND DISCUSSION

3.1. Characterization and properties of Ag NPs

To prepare the silver nanoparticles (AgNPs) for SEM analysis, a small amount of the dried nanoparticles was first dispersed in ethanol to form a suspension. This suspension was then ultrasonicated for 10 minutes to ensure a homogeneous distribution of the nanoparticles. A drop of this suspension was placed onto a clean, carbon-coated copper grid using a micropipette. The sample was then allowed to dry at room temperature, ensuring that the ethanol evaporated completely, leaving behind a thin film of well-dispersed AgNPs on the grid. The prepared sample was then mounted on the SEM sample holder using double-sided carbon tape to ensure stability during imaging. The SEM analysis was done to analyze the morphological properties of the prepared nanoparticles (Figure 1).

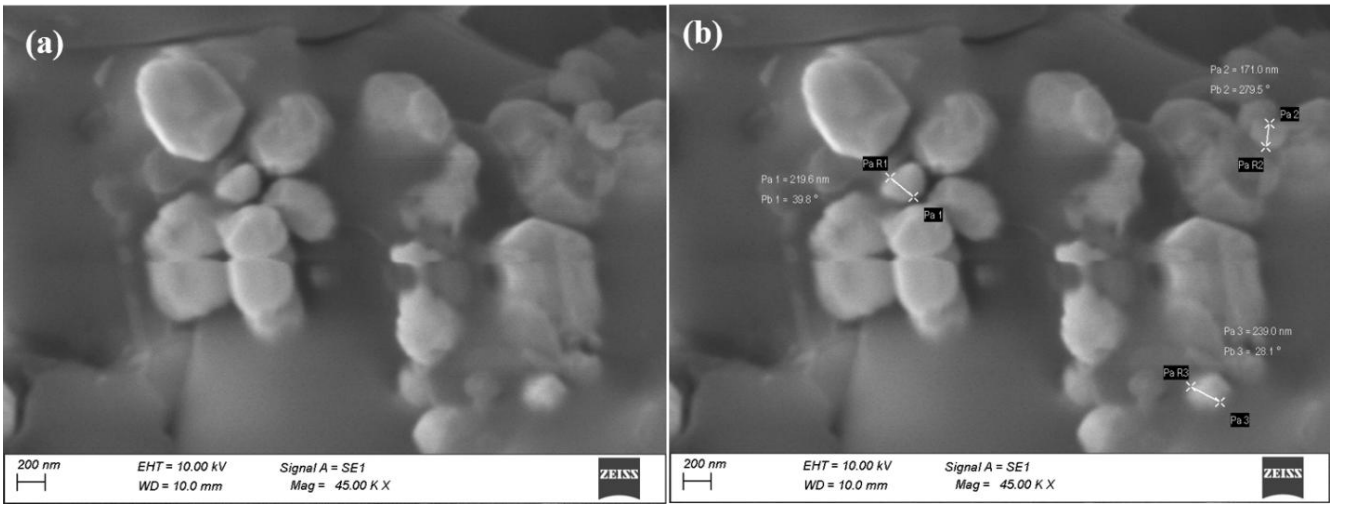

Fig. 1. Typical SEM images of Ag NPs synthesized from *Cardiospermum halicacabum* leaf extract.

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The SEM images were captured at various magnifications to observe the surface morphology and structural features of the AgNPs. The images revealed that the AgNPs were predominantly spherical in shape with a relatively uniform size distribution. The surface of the nanoparticles appeared smooth and well-defined, indicating successful synthesis and stabilization. The spherical shape of the nanoparticles suggested a high surface-to-volume ratio, which is advantageous for applications requiring large surface areas, such as catalysis and antimicrobial activity. The smooth surface of the nanoparticles also indicated a high degree of crystallinity, which could enhance their stability and reactivity.

The EDS analysis was performed to confirm the elemental composition of the synthesized nanoparticles (not shown here). The EDS spectrum showed a strong signal corresponding to silver (Ag), confirming the presence of AgNPs. Additionally, peaks corresponding to carbon (C) and oxygen (O) were observed, which could be attributed to the organic molecules from the *Cardiospermum halicacabum* leaf extract used as reducing and capping agents. The absence of significant peaks for other elements indicated the high purity of the synthesized AgNPs. The results indicated that silver constituted the majority of the sample, with minor contributions from carbon and oxygen. This confirmed that the nanoparticles were primarily composed of silver, with organic compounds from the plant extract acting as stabilizing agents.

For the X-ray diffraction (XRD) analysis, the synthesized silver nanoparticles (AgNPs) were initially dried and ground into a fine powder to ensure uniformity and to avoid any preferred orientation effects. The powdered sample was then evenly spread on a sample holder made of a low-background material, such as glass or silicon, to achieve a flat surface for accurate measurements. This sample holder was carefully mounted onto the goniometer of the X-ray diffractometer. The XRD patterns of the synthesized AgNPs were recorded using a diffractometer equipped with a Cu-Kα radiation source (λ = 1.5406 Å), as shown in Figure 2. The diffractometer operated at an accelerating voltage of 40 kV and a current of 30 mA. The 2θ scan range was set from 20° to 80°, with a step size of 0.02° and a scan speed of 2° per minute, covering the primary diffraction peaks expected for face-centered cubic (fcc) silver [23, 24].

The XRD pattern of the synthesized AgNPs revealed several distinct peaks that were indexed to the face-centered cubic (fcc) structure of metallic silver. The major diffraction peaks observed included a prominent peak at approximately 38.1° (2θ), corresponding to the (111) plane of fcc silver [25]. This peak is typically the most intense in the XRD pattern of silver nanoparticles, indicating a preferred orientation of the crystals along this plane. Another significant peak at around 44.3° (2θ) is associated with the (200) plane of fcc silver, further confirming the crystalline nature of the synthesized AgNPs. A peak at approximately 64.4° (2θ) corresponds to the (220) plane of fcc silver, suggesting the presence of well-defined crystallites within the sample [26]. Additionally, a peak at about 77.5° (2θ) is attributed to the (311) plane of fcc silver. The appearance of this peak, along with the others, confirms the formation of crystalline silver nanoparticles.

The XRD analysis confirmed the formation of face-centered cubic (fcc) crystalline silver in the synthesized nanoparticles. The distinct diffraction peaks corresponding to the (111), (200), (220), and (311) planes indicated the high crystalline quality of the nanoparticles, with the sharpness and intensity of these peaks further suggesting that the AgNPs were well-crystallized.

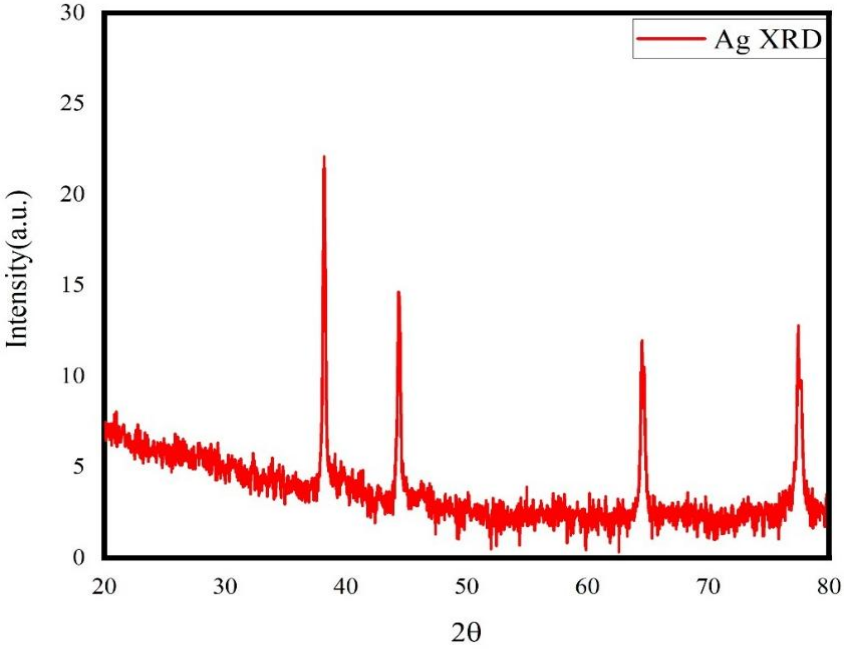

Fig. 2. XRD pattern of Ag NPs synthesized from *Cardiospermum halicacabum* leaf extract.

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The Fourier-transform infrared spectroscopy (FTIR) analysis of the synthesized silver nanoparticles (AgNPs) and the *Cardiospermum halicacabum* leaf extract provided significant insights into the chemical interactions and functional groups involved in the stabilization of the nanoparticles (Figure 3). The FTIR spectrum of the AgNPs exhibited several prominent bands that correspond to specific functional groups, indicating their role in the synthesis and stabilization of the nanoparticles.

The broad and strong band observed at 3436.53 cm^{-1} in the FTIR spectrum of the AgNPs is attributed to the O-H stretching vibration, which is indicative of hydroxyl groups from alcohols and phenols [24]. This peak suggests that hydroxyl groups from the leaf extract play a crucial role in the stabilization and capping of the silver nanoparticles. The broad nature of this band points to strong hydrogen bonding interactions between these hydroxyl groups and the nanoparticle surface. In addition, the band at 1383.68 cm^{-1} corresponds to C-H bending vibrations, characteristic of alkanes [25, 26]. This band indicates the presence of hydrocarbon chains in the leaf extract that likely interact with the AgNPs, which may be important for preventing the agglomeration of the nanoparticles and thus ensuring their stability. A band at 1046.19 cm^{-1} is associated with C-O stretching vibrations, corresponding to ether groups. This presence confirms that ester or ether linkages from the biomolecules in the leaf extract contribute to the stabilization of the silver nanoparticles. Moreover, the band at 566.969 cm^{-1} attributed to C-Br stretching indicates the involvement of bromine-containing compounds from the leaf extract in the stabilization process [25].

3.2. Antibacterial Properties of AgNPs Synthesized Using Cardiospermum halicacabum Leaf Extract

The antibacterial properties of silver nanoparticles (AgNPs) have drawn considerable interest due to their effectiveness against a wide range of bacterial pathogens. In this study, we examined the antibacterial activity of AgNPs synthesized using Cardiospermum halicacabum leaf extract, assessing their efficacy against both Gram-positive and Gram-negative bacteria. This comprehensive evaluation aimed to understand the potential biomedical applications of these nanoparticles. The antibacterial activity of the synthesized AgNPs was tested against two representative bacterial strains: Staphylococcus aureus (S. aureus), a Gram-positive bacterium, and Escherichia coli (E. coli), a Gram-negative bacterium. These strains were selected due to their clinical relevance and their known resistance to conventional antibiotics.

To begin, fresh bacterial cultures were prepared by inoculating a loopful of bacterial cells into nutrient broth, which was then incubated overnight at 37°C. Following incubation, the bacterial cultures were adjusted to a turbidity equivalent to 0.5 McFarland standard, approximately 1.5 × 10^8 CFU/mL. The agar well diffusion method was used to evaluate the antibacterial activity of the AgNPs. Mueller-Hinton agar plates were prepared and inoculated with the standardized bacterial cultures using a sterile swab to ensure even distribution. Wells, 6 mm in diameter, were punched into the agar using a sterile cork borer. Various concentrations of the synthesized AgNPs, ranging from 10 to 100 µg/mL, were prepared in sterile distilled water.

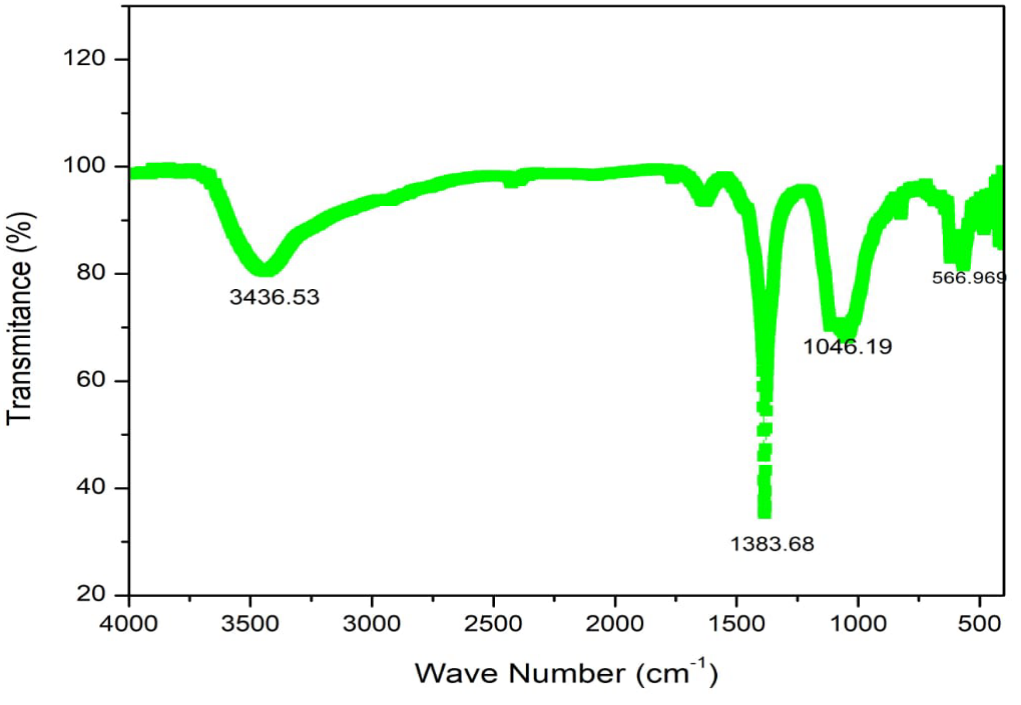

Fig. 3. FTIR spectrum of Ag NPs synthesized from *Cardiospermum halicacabum* leaf extract.

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A 100 µL volume of each concentration was carefully added into the respective wells. For comparison, a control well containing only the leaf extract (without AgNPs) and a standard antibiotic disc (ampicillin) were also included [27-29].

After incubation at 37°C for 24 hours, the zones of inhibition around each well were measured using a digital caliper. The diameters of the inhibition zones, recorded in millimeters (mm), were used to assess the antibacterial activity of the AgNPs. The results demonstrated that the synthesized AgNPs exhibited significant antibacterial activity against both S. aureus and E. coli. At the highest concentration tested (100 µg/mL), the AgNPs produced substantial inhibition zones measuring 22 mm for S. aureus and 20 mm for E. coli. These values were comparable to those obtained with the standard antibiotic ampicillin, indicating strong antibacterial efficacy of the AgNPs.

The control well containing only the Cardiospermum halicacabum leaf extract did not show any inhibition zones, confirming that the observed antibacterial activity was attributed to the synthesized AgNPs rather than the leaf extract alone. This finding underscores the effectiveness of the green synthesis method in producing bioactive nanoparticles [27].

The mechanisms underlying the antibacterial action of AgNPs are complex and involve several pathways. Firstly, AgNPs can disrupt bacterial cell membrane integrity by attaching to the cell membrane, causing structural changes, and increasing membrane permeability [29]. This disruption can lead to leakage of cellular contents and eventual cell death. Secondly, AgNPs can generate reactive oxygen species (ROS) within bacterial cells, which damage cellular components such as DNA, proteins, and lipids, leading to oxidative stress and bacterial cell death. Additionally, AgNPs can interact with bacterial proteins by binding to thiol groups, disrupting their normal function and inhibiting essential processes such as enzyme activity, protein synthesis, and DNA replication [28, 29]. Finally, AgNPs can release silver ions (Ag^+), which possess strong antibacterial properties. These ions can interact with various cellular components, enhancing the overall antibacterial effect of the nanoparticles.

The differential response between Gram-positive and Gram-negative bacteria may be attributed to structural differences in their cell walls. Gram-negative bacteria, such as E. coli, have an outer membrane that can act as a barrier to nanoparticles, potentially reducing their susceptibility. However, the synthesized AgNPs remained effective against E. coli, indicating their broad-spectrum antibacterial potential.

The synthesized AgNPs using Cardiospermum halicacabum leaf extract demonstrated remarkable antibacterial activity against both Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria. The dose-dependent increase in inhibition zones and the comparable efficacy to the standard antibiotic ampicillin highlight the potential of these green-synthesized AgNPs as effective antibacterial agents. This study underscores the value of green synthesis methods in producing bioactive nanoparticles and suggests potential applications for these nanoparticles in combating bacterial infections.

Table 1. Zones of Inhibition (mm) for AgNPs against S. aureus and E. coli.

Concentration of AgNPs (µg/mL)	S. aureus	E. coli
10	8	7
25	12	10
50	16	14
75	19	17
100	22	20
Control (Leaf Extract)	0	0
Ampicillin (10 µg/disc)	25	23

4. CONCLUSION

The study on the synthesis and characterization of silver nanoparticles (AgNPs) using *Cardiospermum halicacabum* leaves extract has provided valuable insights into the efficacy of plant-based methods for nanoparticle production. The green synthesis approach adopted in this research proved to be effective, leveraging the natural reducing and stabilizing agents present in the plant extract to convert silver ions (Ag^+) into silver nanoparticles (AgNPs). This method not only aligns with eco-friendly practices but also harnesses the phytochemical properties of *Cardiospermum halicacabum* leaves to facilitate the synthesis. Characterization of the synthesized AgNPs was performed using several analytical techniques. Scanning Electron Microscopy (SEM) images showed that the AgNPs were predominantly spherical and exhibited a relatively uniform size distribution. This high-density formation observed in SEM images is indicative of efficient reduction and stabilization of silver ions by the plant extract. The nanoparticles' morphology, as revealed by SEM, supports the successful synthesis of AgNPs. Further confirmation of the synthesized nanoparticles was obtained through X-ray Diffraction (XRD) analysis. The XRD pattern exhibited characteristic peaks at 39°, 44°, 65°, and 79°, which correspond to the (111), (200), (220), and (311) planes of the face-centered cubic (FCC) crystal structure of silver. These peaks validate the crystalline nature of the synthesized nanoparticles and affirm the successful synthesis of AgNPs. Fourier-transform Infrared Spectroscopy (FTIR) was utilized to investigate the functional groups involved in the stabilization and capping of the AgNPs. The FTIR spectrum revealed significant bands at 3436.53 cm^{-1}, 1383.68 cm^{-1}, 1046.19 cm^{-1}, and 566.969 cm^{-1}, which correspond to hydroxyl stretching, C-H bending, C-O stretching, and C-Br stretching, respectively. These findings suggest that the plant-derived biomolecules play a crucial role in the stabilization of the nanoparticles, interacting with the metal

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surface and providing a protective capping layer. The antibacterial activity of the synthesized AgNPs was assessed against both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria. The results demonstrated that the AgNPs exhibited significant antibacterial properties. Notably, the inhibition zones were observed to increase with higher concentrations of AgNPs, reflecting a dose-dependent antibacterial effect. At the highest tested concentration of 100 µg/mL, the AgNPs produced substantial zones of inhibition, comparable to those of the standard antibiotic ampicillin. However, it was observed that the AgNPs did not exhibit antibacterial activity at lower concentrations or in comparison to the control. This suggests that the effectiveness of the AgNPs is highly dependent on their concentration. The findings of this study highlight the potential of *Cardiospermum halicacabum* leaf extract as a viable source for the green synthesis of silver nanoparticles with notable antibacterial properties. The research underscores the importance of further investigations to optimize the synthesis process, explore the factors influencing antimicrobial efficacy, and expand the potential applications of these nanoparticles. The successful synthesis and characterization of AgNPs from *Cardiospermum halicacabum* leaves pave the way for their use in various applications, including antimicrobial coatings, wound dressings, and therapeutic agents, reinforcing the value of plant-based methods in nanotechnology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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