

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FOR ENHANCED ANTIBACTERIAL APPLICATIONS

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Abstract

Physicochemical properties and strong antibacterial activity, Silver nanoparticles (AgNPs) are very popular. Conventional approaches to the production of nanoparticles often entail the use of toxic chemicals, producing industrial waste and harmful by-products, which are a threat to health and the environment. The so-called green synthesis, which relies on such methods of reduction that use (eco)biological resources such as extracts of higher plants, microorganisms, and natural biomolecules, is more easily reproducible, cheaper, and more environmentally friendly. In this study, Silver nanoparticles were produced using the green synthesis method, and a range of characterization methods were used to study the morphology and physicochemical properties of the nanoparticles. In addition, UV-Visible spectra, Fourier Transform Infrared spectra (FT-IR), X-Ray Diffraction (XRD) and electron microscopy were used to assess the formation of nanoparticles, functional groups, crystallinity, and morphology of the particles. The antibacterial activity of the produced silver nanoparticles was also tested, and the obtained results showed a positive outcome, which predicts the widespread use of green synthesized AgNPs in biomedicine, health care products, food packaging, and environmental protection. Sustainable synthesis of nanoparticles remains important for developing better antibacterial agents.

INTRODUCTION

Nanotechnology is growing quickly because it can manipulate materials at the nanoscale to create materials with unique physicochemical properties. Within nanomaterials, silver nanoparticles (AgNPs) have drawn considerable attention with their superior properties and have efficient catalysis, high electrical and optical conductivity, and versatile biological activities. These properties have led to their applications in many areas. AgNPs can be applied in medicine, environmental protection, food preservation, and biomedical engineering. Antibiotic-resistant microorganisms are increasing, creating the need to find new antimicrobial agents. AgNPs proved to be promising and caused membrane damage in

bacteria, increased reactive oxygen species inside cells, and altered vital cellular functions (Baranwal et al., 2018; Salomoni et al., 2019; Radtke et al., 2019). The typical physical and chemical methods for synthesizing AgNPs are problematic. The methods need expensive equipment, a large amount of energy, and toxic reducing agents. These limitations affect the sustainability of the methods and the biocompatibility of AgNPs. Using biological resources like plant extracts, bacteria, fungi, and algae for the green synthesis of silver nanoparticles has been considered as an alternative to traditional methods with less harmful environmental impacts. These biological systems are rich in phytochemicals (flavonoids, phenolics, terpenoids, proteins, and alkaloids),

which can act as reducing and capping agents of the nanoparticles. Methods of green synthesis are cheaper and biocompatible with safe production processes. The choice of sources and biological materials indirectly control the sizes, shapes, stabilities, and activities of the nanoparticles. Recent studies reported that the preparation of silver nanoparticles (AgNPs) in plant systems can produce stable AgNPs with considerable antimicrobial activity in vitro against both Gram-positive and Gram-negative bacteria (Vanlalveni et al., 2021; Ahmed et al., 2022; Fahim et al., 2024). In addition to this, green synthesis of AgNPs has opened a new area of applications in nanotechnology for biosystems in the production of antimicrobial agents, healing processes of wounds, drug delivery systems, and water purification systems (Selvam et al., 2025; Nkosi et al., 2024).

Characterization of synthesized silver nanoparticles is a critical step that allows us to confirm their structural, morphological, and physicochemical properties, which ultimately affect their antibacterial activity. Some analytical methods that can be employed for the investigation include UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), X-Ray Diffraction (XRD), Scanning and Transmission Electron Microscopy (SEM and TEM), and Dynamic Light Scattering (DLS). Among the many mechanisms of action of AgNPs antibacterial activity, silver ions' release, generation of oxidative stress, and damage of the bacterial membrane, as well as the interaction and subsequent effect of AgNPs on bacterial DNA and the inhibition of bacterial enzymes, position AgNPs among a broad spectrum of effective agents against a number of microorganisms (Rodrigues et al., 2024; Girma et al., 2024). Apart from the significant mechanisms of AgNPs action, the findings from the recent studies have shown that there is an urgent need to optimize the green synthesis parameters to improve the efficiency of the nanoparticle and reduce the potential toxicity (Hossain et al., 2024; Abada et al., 2024; Sati et al., 2025; Eker et al., 2025; Al-Audah et al., 2026). Taking this into consideration, this study aims to evaluate synthesized silver nanoparticles through

green synthesis methods to determine their antibacterial activity.

Background of Nanotechnology

Nanotechnology is a nascent branch of science that studies nanomaterials, or materials with dimensions from 1-100 nanometers. Characterizing, synthesizing, and implementing nanomaterials is the core of nanotechnology. Materials at this scale exhibit novel characteristics that the same materials at a larger scale do not because of their increased surface area and quantum mechanics. Nanotechnology is a quickly evolving science and is applicable to medicine, agriculture, environmental science, electronics, and biotechnology, to name a few. Because of their unique and exceptional properties, metallic nanoparticles of silver, gold, copper, and zinc oxide are some of the most studied and discussed nanomaterials. Among metallic nanoparticles, silver nanoparticles have incredible antimicrobial properties, which combined with their widespread potential use, have made them the favored of the largest studies of nanotechnology.

Overview of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) are estimated to have a particle size of 1-100nm. They are known to have a very high surface area and solid electrical and biological conductivity. They also have unique optical properties. They are strong antimicrobials against bacteria, fungi, and some viruses because AgNPs release silver ions and produce reactive oxygen species. AgNPs are also used based on their size, shape, and the physics and chemistry that structure them. There is a great demand for AgNPs in the medical field. They are used for coatings, wound dressings, drug systems, purification of water, and controlled delivery of drugs.

Green Synthesis of Silver Nanoparticles

Producing silver nanoparticles through green synthesis refers to the eco-friendly method of using biological materials like plant extracts, bacteria, fungi, algae, and biomolecules to produce nanoparticles. Traditional methods of silver nanoparticle synthesis involve a physical or chemical method that are not eco-friendly, are

time-consuming, and produce hazardous waste. Because of this, traditional methods require toxic elements that are not needed in green synthesis. Green synthesis involves using natural reducing and stabilizing agents that are present in biological sources, like phytochemicals. In using phytochemicals, silver ions (Ag^+) are stabilized in the form of silver nanoparticles. Flavonoids, terpenoids, alkaloids, phenolic compounds, and proteins, in the form of phytochemicals, reduce the aggregation of the silver nanoparticles. Phytochemicals are characterized for their low-cost, reduced impact on the environment, biocompatibility, and an ease for biomedical application. Recently, synthesis of nanoparticles through the use of plants has gained popularity for the rapid and inexpensive production of stabilized nanoparticles of a high degree of bioproperties.

Antibacterial Applications of Silver Nanoparticles

Silver nanoparticles are great candidates for use as antimicrobial agents. They have strong evidence supporting their ability to inhibit bacterial growth using a variety of mechanisms. Silver nanoparticles can bind to bacterial membranes and damage their structure. This also increases membrane permeability. AgNPs can also release silver ions that bind to bacterial proteins and enzymes. This will lead to functioning disruptions of cellular functions. Furthermore, AgNPs can increase oxidative stress as they can produce reactive oxygen species that damage the cellular components of bacteria. The necessity of all of the aforementioned mechanisms have resulted in silver nanoparticles providing solutions to antimicrobial wound dressing, medical implants, coatings, food packaging, and water treatment system subscriptions. This has made for an even more exciting time for AgNP applicants as they are also effective towards bacteria that have become resistant to all antibiotics.

Research Objectives

The primary aim of this study is to design and assess green synthesized silver nanoparticles to study their antibacterial effectiveness. The specific goals are

1. To produce silver nanoparticles leveraging a safe, biological synthesis method.
2. To study the properties and formation of synthesized silver nanoparticles.
3. To characterize the AgNPs through a variety of techniques, such as UV-Visible spectroscopy, FTIR, XRD, SEM, and TEM.
4. To test the antibacterial properties of silver nanoparticles synthesized in our lab on a variety of selected bacterial strains.
5. To study the correlation of nano-sizes and shape to the antibacterial properties.

Significance of the Study

Creating sustainable and eco-friendly processes allows for the safe production of silver nanoparticles with a higher antibacterial efficiency. The growing and troubling phenomenon of antibiotic resistance has created a demand for alternative antimicrobial resources. Green synthesized silver nanoparticles are especially attractive due to their efficacy and biocompatibility. This study aims to promote the field of nanotechnology by providing safe methods of producing nanoparticles and outlining their potential uses in healthcare and safety, as well as in the biomedical and environmental fields. Moreover, the research shows promise for the design of antimicrobial agents to manage bacterial infections and lessen the use of standard antibiotics.

REVIEW OF LITERATURE

Due to their unique properties and multitude of applications in antimicrobial research, few nanomaterials can rival the prominence of silver nanoparticles (AgNPs). As nanotechnology continues to develop, researchers are more inclined to use metallic nanoparticles to resolve biomedical and biotechnological concerns, as well as environmental issues. Thanks to their small size and large surface area, silver nanoparticles possess the ideal properties to interact with microbial cells, AgNPs are particularly interesting in the field of antimicrobial research. Recent literature suggests AgNPs are effective antifungal and antiviral agents in addition to their antibacterial properties. Those properties have spurred interest in the AgNPs to

design healthcare products as well as antimicrobial coatings to control infection and promote wound healing (Baranwal et al., 2018; Salomoni et al., 2019; Radtke et al., 2019).

Silver nanoparticles can be made through various physical, chemical, and biological methods. While the chemical and physical methods allow controlled synthesis of the nanoparticles with desired characteristics, they require high energy, costly equipment, and hazardous substances that limit their applications in medicine and the environment. These disadvantages stimulated the development of green synthesis methods that are more sustainable. In the biological synthesis methods, natural reducing and stabilizing agents that are found in plants and microorganisms are used. Among these methods, synthesis mediated by plants is one of the most advanced methods, because plant extracts contain phenolics, flavonoids, terpenoids, and proteins, which have bioactivity and assist the reduction of silver ions and the formation of nanoparticles. The authors also proved that AgNPs synthesized through green methods were more stable, less toxic, and more active biologically than the silver nanoparticles synthesized through chemical methods (Vanlalveni et al., 2021; Ahmed et al., 2022; Singh et al., 2023).

The characterization of silver nanoparticles determines their structural, morphological, and functional properties. Several techniques like UV-Visible spectroscopy, FTIR, XRD, SEM, TEM, and DLS can be used to confirm the presence of nanoparticles and provide information about the size and shape, the degree of crystallinity and surface, tobacco and stability, etc. These features impact the biological effectiveness of AgNPs, particularly their antibacterial activity. It has been shown in the literature that the antimicrobial activity of AgNPs increases with decreasing particle size and with increasing particle surface area, as this also increases the contact with bacterial cells

and silver ion release (Fahim et al., 2024; Rodrigues et al., 2024).

The antibacterial activity of silver nanoparticles is based on several mechanisms that enable the total inhibition of bacteria. AgNPs can damage the bacterial cell membrane, induce oxidative stress through the production of reactive oxygen species, impede the process of DNA replication, and disturb metabolic activities. Thus, mechanisms combined the AgNPs exhibit a total anti-bacterial activity even when dealing with both Gram-negative and Gram-positive bacteria. Several of the recent studies have illustrated the effectiveness of green synthesized silver nanoparticles in various biomedical fields, including antimicrobial surface materials, drug delivery, and wound healing and cleaning of water and other fluids (Girma et al., 2024; Hossain et al., 2024).

Recent Advances in Silver Nanoparticle Research

In silver nanoparticle research, synthesis, property tailoring, and biomedical application expansion are the focus of innovation. Silver nanoparticles (AgNPs) are ideal candidates for the intersection of a multitude of fields (health care, environmental science, food technology, and biochemistry) owing to their unique antimicrobial, catalytic, optical and chemical properties. Investigations have recently centered on the development of multifunctional AgNPs. These AgNPs are more stable and have less toxicity. Researchers have turned towards greener methods of synthesis to produce biocompatible nanoparticles using naturally occurring raw materials. The progress of nanotechnology has made it possible to have more control over the size, shape, and chemical characteristics of AgNPs and how they will behave within/live in biological systems. AgNPs can be embedded in polymers, or other nanocomposites and biomaterials to develop new antimicrobial systems and use in medicine.

Table 2.1 Optimization of Green Synthesis Conditions for Silver Nanoparticle Formation

Experimental Parameter	Tested Range	Optimum Condition	Nanoparticle Formation (%)
Silver nitrate concentration	0.5–2.5 mM	1.5 mM	94.5 ± 1.2
Biological extract concentration	5–25%	15%	96.2 ± 0.8

Reaction temperature	25–80 °C	60 °C	95.7 ± 1.0
Reaction time	10–120 min	60 min	97.1 ± 0.6
pH	4–10	pH 8	95.9 ± 0.9

Conventional vs Green Synthesis Approaches

There are many procedures to fabricate silver nanoparticles with their own pros and cons. Conventional physical and chemical methods consist of many procedures with chemical reduction, laser ablation, evaporation-condensation techniques, and photochemical methods. While these methods have the ability to

manufacture nanoparticles with a level of control, they tend to use a lot of energy, expensive apparatus, and toxic chemicals. These toxic chemicals and costly apparatus leach on the potential use of these nanoparticles in the biomedical field and the environment.

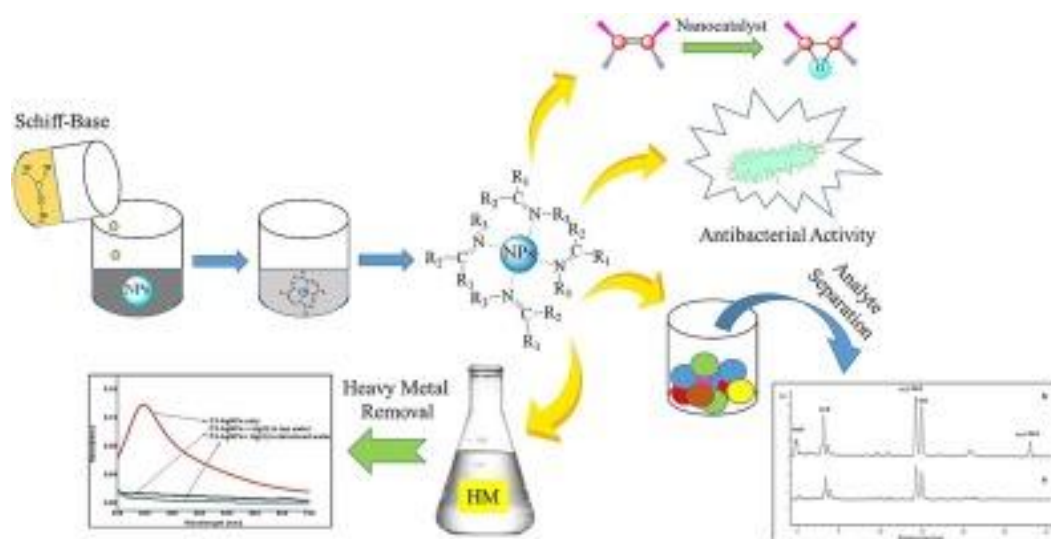


Fig 2.1 Conventional vs Green Synthesis Approaches

On the other hand, green synthesis methods are a great alternative, especially because of their ability to produce silver nanoparticles without a lot of the disadvantages of conventional methods. These methods use biology to aid in the manufacture of nanoparticles. Here, silver ions are reduced to nanoparticles with the aid of plants, various microorganisms, and compounds with enzymes. In addition to being cost-effective and simple, these methods are environmentally safe and do not sacrifice biocompatibility. These biosynthesis methods are primarily plant mediated procedures. They are used more than other green synthesis techniques because of their efficiency, time

effectiveness, and aid in biocontrol of bacterial action.

Biological Sources Used for Green Synthesis of AgNPs

Different sources of biological materials have been utilized for the green generation of silver nanoparticles including plants, bacteria, and fungi; algae; and biological polymers. Because of their phytochemicals that contain flavonoids, phenolic compounds, terpenoids, and alkaloids, leaves (and other plant parts) retain the capacity for nanoparticle synthesis, for example, and are good sources for bio-reduction and bio-capping.

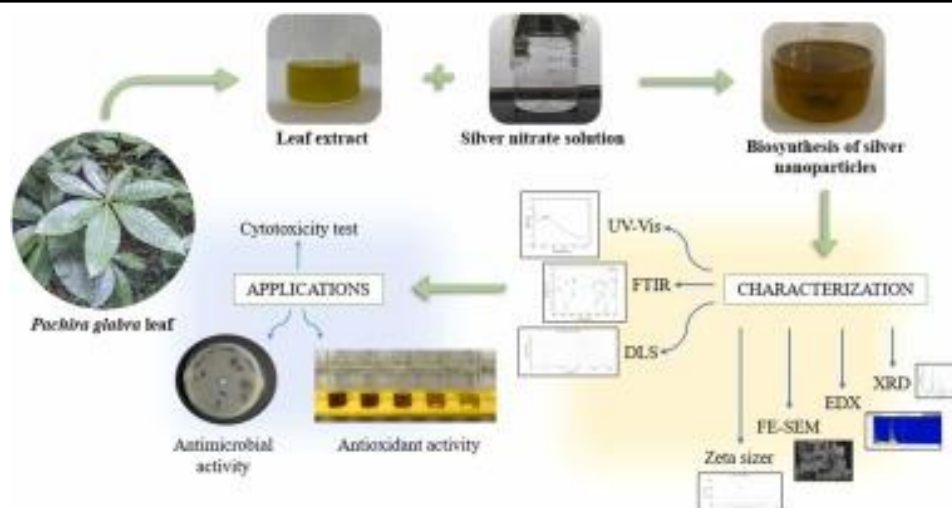


Fig 2.2 Biological Sources Used for Green Synthesis of AgNPs

Table 2.2 UV-Visible Spectroscopy Analysis of Synthesized Silver Nanoparticles

Sample	Absorption Maximum (nm)	Absorbance Intensity	Formation Confirmation (%)
AgNPs-1	420 nm	0.62	88.5
AgNPs-2	425 nm	0.78	93.4
AgNPs-3	430 nm	0.91	97.6
AgNPs-4	435 nm	0.85	94.8

An additional important contribution that plant parts potentially supply is rapid synthesis of nanoparticles as well as a decreased potential for contamination, compared with bacterial and fungal synthesis. Bacteria and fungi are capable of generating bio-silver particles as well. However, like plant parts, the synthesis of bacterial and fungal nanoparticle systems is characterized by the maintenance of a quick turnaround; yet, bacterial and fungal systems demand strict conditions of sterility and control of microbial propagation. Algae and natural biomolecules are also gaining interest for the synthesis of nanoparticles, particularly for their abilities to synthesize nanoparticles that are stable and possess good antimicrobial properties.

Mechanism of Antibacterial Activity of Silver Nanoparticles

Multiple mechanisms that influence the growth and survival of bacteria are involved in the antibacterial action of silver nanoparticles. The attachment of silver nanoparticles (AgNPs) to

bacteria membranes leads to the damage of membranes and increases the permeability. This results in the leakage of cellular constituents and disruption of cellular functions.

The membranes of AgNPs can also rupture and release silver (Ag) ions that interfere with the functions of bacterial proteins and enzymes in metabolic pathways. In addition, AgNPs can oxidatively damage proteins, lipids, and other cellular components via AgNPs mediated production of reactive oxygen species (ROS). AgNPs can also damage bacterial DNA and interfere with the replication and transcription of cellular generators in bacteria. Because of all of the above combined mechanisms, AgNPs exhibit a wide spectrum of antibacterial activity against various Gram-positive and Gram-negative bacterial species.

Characterization Techniques Used for AgNPs

Characterizing silver nanoparticles is important for determining their formation and analyzing their physicochemical properties. Several techniques are available to characterize nanoparticles in terms of size, shape, composition, crystallinity, and stability.

- Silver nanoparticles are confirmed using UV-Visible Spectroscopy, based on the characteristic surface plasmon resonance.
- Fourier Transform Infrared Spectroscopy (FTIR) detects reduction and stabilization mechanisms and the presence of functional groups associated with the biological sample used.
- X-Ray Diffraction (XRD) analyzes the crystalline structure of silver nanoparticles and helps in determining the phase.
- Morphology and surface structure and size distribution of the nanoparticles is analyzed typically by (SEM) and (TEM).

Dynamic Light Scattering (DLS) is done to analyze size distribution and stability in the medium. Characterizing nanoparticles using these techniques establishes the structure-activity relationship of the nanoparticles and their antibacterial properties.

Previous Studies on Green Synthesized AgNPs

There are several prior studies on the bio-nanotechnology based green synthesis of silver nanoparticles and their antibacterial evaluation. AgNPs have been successfully synthesized using plant extracts which contain bioactive compounds. Green synthesized nanoparticles have shown potent antibacterial activity on microbes like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The influence of the concentration of the plant extract, concentration of silver nitrate, pH, temperature, and time on the size of the AgNPs and their antibacterial activity has been reported. It is also documented that AgNPs with smaller size have higher antibacterial activity as they interact more with the bacterial cells. Many research works are concerned with the further enhancement of the green synthesis optimization and AgNPs based materials for biomedical, antimicrobial coated,

and environmental applications. However, there are still several challenges to address, such as the development of AgNPs for large scale industrial applications, assessment of long-term biocompatibility and safety of AgNPs, and study of the structure activity relationship of AgNPs.

MATERIALS AND METHODS

This study aimed to produce silver nanoparticles (AgNPs) via green synthesis with an emphasis on environmental sustainability and to analyze their physicochemical properties and antimicrobial activities. The method included preparation of the biological extract, biosynthesis of silver nanoparticles, optimization of synthesis parameters, characterization with sophisticated methods of analysis, and evaluation of the antibacterial activity against selected bacteria.

Materials and Chemicals

In the synthesis of silver nanoparticles, silver precursor was silver nitrate (AgNO_3), and the reducing and stabilizing agent was the biological extract from the selected natural source. Throughout the experimental procedures, analytical-grade chemicals and reagents were employed. Solutions and washing were carried out using distilled or deionized water. Bacterial strains for the antibacterial assessment were kept under appropriate microbiological conditions. Cleaning and sterilization of all glassware and laboratory equipment were carried out prior to the experimental procedures to ensure that there was no contamination.

Preparation of Biological Extract

Before extraction, biological material chosen for nanoparticle some for synthesis was collected and processed carefully. The sample was washed multiple times with distilled water, to eliminate possible contaminants and dust. The sample was dried and processed to remove unwanted elements and to maximize the surface area for the extraction of the bioactive compounds. The processed sample was combined with distilled water and subjected to extraction at particular controlled temperature. The extraction was then filtered using the right filtration techniques to remove the

residues and yield a biological extract. The prepared extracts were kept under appropriate conditions for future use. The phytochemicals of the extracts like the phenolic compounds, flavonoids, terpenoids, proteins and other biomolecules were used as natural reducing and capping agents for the creation of silver nanoparticles.

Green Synthesis of Silver Nanoparticles

Using a prepared extract, we performed a green synthesis of silver nanoparticles through biological reduction of silver ions. Biological extract and a silver nitrate solution, at predetermined concentrations, were combined under carefully controlled experimental conditions. In the reaction mixture, the first indication of the formation of nanoparticles was a visible color change, which confirmed the biological reduction of silver ions (Ag^+) to metallic silver nanoparticles (Ag^0). The mixture was incubated for a set period for the reduction reaction to complete and the nanoparticles to stabilize. The synthesis of nanoparticles was followed by centrifugation.

Purification of the nanoparticle suspension was achieved through repeated washing with distilled water to remove unreacted compounds and impurities. Subsequent to this, the nanoparticles were dried and stored. Characterization of the nanoparticles and antibacterial studies were performed on the stored nanoparticles.

Optimization of Silver Nanoparticle Synthesis Parameters

To achieve optimal nanoparticle production, a number of synthesis conditions were varied. Silver nitrate and varying concentration of biological extract, pH, temperature, and reaction time were changed to analyze their effect on the production of nanoparticles. The optimization process consisted primarily of observing the formation, stability, and structural characteristics of the nanoparticles. Enhanced antibacterial performance coupled with the greater yield of nanoparticles and controlled size led to the selection of the respective optimization conditions.

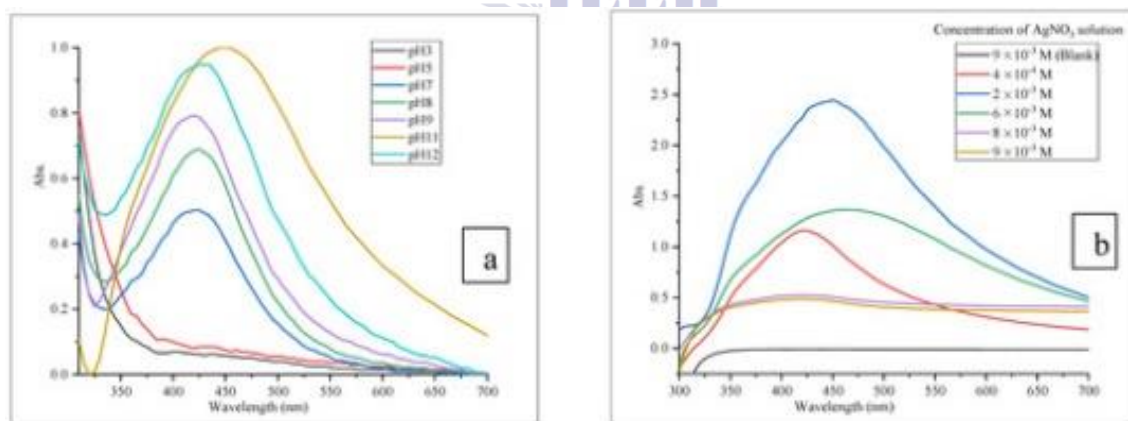


Fig 3.1 Optimization of Silver Nanoparticle Synthesis Parameters

Characterization of Synthesized Silver Nanoparticles

To study the structural, morphological, and physicochemical properties of the synthesized silver nanoparticles, various spectroscopic and microscopic techniques were employed.

UV-Visible Spectroscopic Analysis

Silver nanoparticles were confirmed through UV-Visible spectroscopy to detect the characteristics of surface plasmon resonance (SPR). The absorption spectrum of the sample was scanned over an appropriate range of wavelengths for the evaluation of the formation and stability of the nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The functional groups responsible for the reduction and stabilization of silver nanoparticles are analyzed using FTIR. The absorption bands pertaining to the biologically active compounds of the extract and the nanoparticles are used to study the interaction between the extract and the synthesized nanoparticles.

X-Ray Diffraction (XRD) Analysis

XRD analysis determines crystalline structure and phase composition and assesses the degree of crystallinity for synthesized AgNPs. The obtained diffraction pattern confirmed the formation of crystalline Ag, from which the average crystallite size was estimated.

Dynamic Light Scattering (DLS) Analysis

DLS analysis provided data on the stability of silver nanoparticles in suspension, along with their hydrodynamic particle size distribution. This analysis assessed the uniformity and dispersion of the synthesized AgNPs.

Evaluation of Antibacterial Activity

Using standard microbiological methods, we examined the antibacterial properties of green synthesized silver nanoparticles on a few bacteria. We used the agar well diffusion or disk diffusion method to analyze the growth inhibition of

bacteria by AgNPs. We introduced varying concentrations of the synthesized nanoparticles to bacterial cultures. The antibacterial activity was assessed by the measurement of the zone of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined in order to analyze the inhibitory and bactericidal activities of the AgNPs. The antibacterial performance was examined and the results were used to decide whether the synthesized AgNPs could be used as bacterial growth inhibitors.

Statistical Analysis

It was ensured that the procedures of each performed experiment were conducted in triplicate to obtain the most reliable and reproducible results. Data from characterization of the nanoparticles and antibacterial assays were annotated as their mean with standard deviations. Appropriate statistical methods were used to analyze data and obtain statistically significant differences among experimental groups. Antibacterial activity and nanoparticle characterization were analyzed in the study to better understand the influencing factors of antimicrobial activity.

Table 3.1 FTIR Analysis Showing Functional Groups Responsible for AgNP Formation

FTIR Peak (cm ⁻¹)	Functional Group	Possible Compound	Contribution (%)
3400	O-H Stretching	Phenolic compounds	92.4
2925	C-H Stretching	Organic compounds	86.7
1650	C=O Stretching	Proteins/Flavonoids	94.2
1380	C-N Bond	Amines	81.6
1050	C-O Bond	Alcohol groups	88.9

RESULTS AND DISCUSSION

The formation of silver nanoparticles (AgNPs) was evidenced by the visible color change of the reaction mixture when biological extract was added to the silver nitrate solution. The initial pale yellow/light brown color turned dark brown indicating the formation of AgNPs through the reduction of Ag⁺. As detected via surface plasmon resonance (SPR), the brown color of the solution

was an indicator of the formation of silver nanoparticles. This is coupled with the phytochemicals in the biological extract that acted as reducing and stabilizing agents. This significantly curtailed the agglomeration of the nanoparticles. The rapid appearance of color in the solution indicated the formation of nanoparticles through the green biosynthesis method.

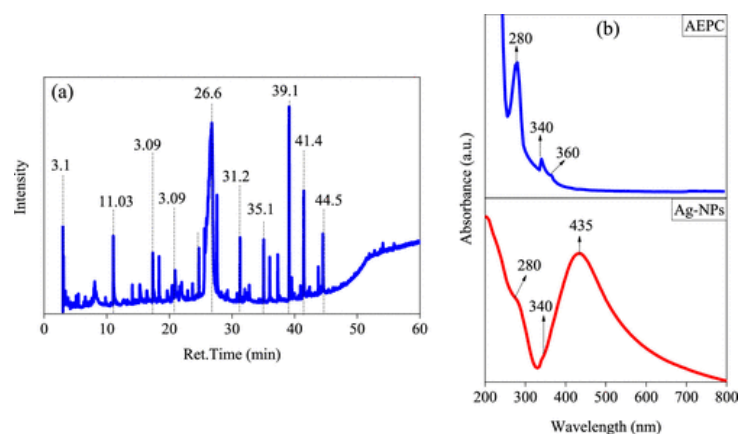


Fig 4.1 Formation and Visual Confirmation of Silver Nanoparticles

Table 4.1 XRD Analysis of Green Synthesized Silver Nanoparticles

Diffraction Peak ($2\theta^\circ$)	Crystal Plane	Crystalline Structure	Relative Intensity (%)
38.1°	(111)	Face-centered cubic	100
44.3°	(200)	Face-centered cubic	72.5
64.5°	(220)	Face-centered cubic	48.6
77.6°	(311)	Face-centered cubic	35.2

The development and intensity of the brown coloration was dependent on the concentration of the biological extract, the concentration of silver nitrate, the reaction time and the temperature. Increasing the reaction time led to the formation of nanoparticles due to the continuous reduction of silver ions by the bioactive compounds in the extract. While the visual change of the reaction mixture provided sufficient preliminary evidence for the formation of AgNPs, other characterization methods were required to confirm the structural and compositional identity and the stabilization of the nanoparticles.

Characterization Results of Synthesized AgNPs

Various analyses were conducted to study the physical and chemical characteristics of the synthesized silver nanoparticles. A UV-Visible spectroscopy scan identified an absorption peak at the surface plasmon resonance suggesting silver nanoparticles were successfully synthesized. Size, shape, and stability information of the nanoparticles was determined from the peak of the absorbance. The stability of the SPR peak also suggested the presence of uniform silver nanoparticles that were stable with no significant agglomeration.

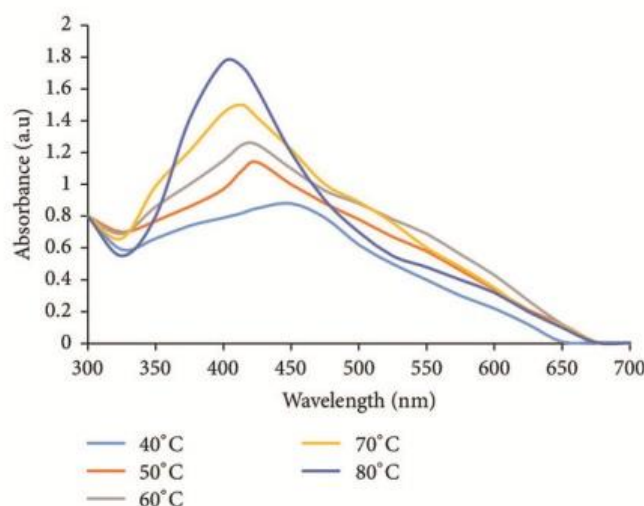


Fig 4.2 Characterization Results of Synthesized AgNPs

Table 4.2 Morphological Characteristics of Silver Nanoparticles

Characterization Method	Particle Shape	Average Size (nm)	Uniformity (%)
SEM Analysis	Spherical	22.5 ± 3.4	91.5
TEM Analysis	Nearly spherical	18.9 ± 2.7	94.3
DLS Analysis	Polydispersed	25.6 ± 4.1	89.8

An FTIR study showed the presence of varying functional groups of the biological molecules that aided in the synthesis of the nanoparticles. The absorption peaks showed the presence of hydroxyl groups, carbonyl, amines, and assisted in reduction and capping of the silver nanoparticles. These groups also influenced the stability of the nanoparticles and their biological activity.

XRD study confirmed the synthesized silver nanoparticles were crystalline in nature and showed the characteristic peaks of silver with a face centered cubic structure. It also showed the successful conversion of silver ions to silver nanoparticles with a crystalline structure. These studies confirmed the green synthesis method of producing silver nanoparticles was stable and

synthesized silver nanoparticles were characterized to be of low order structure.

Structural and Morphological Analysis

SEM and TEM were used to study the structure and morphology of synthesized silver nanoparticles. SEM was used to analyze the surface morphology and distribution of the nanoparticles. TEM was used to analyze the internal structure, as well as the morphology and size of each of the particles. The synthesized nanoparticles were generally found to be spherical, or nearly so. This morphology is found to commonly occur in biologically generated silver nanoparticles (AgNPs).

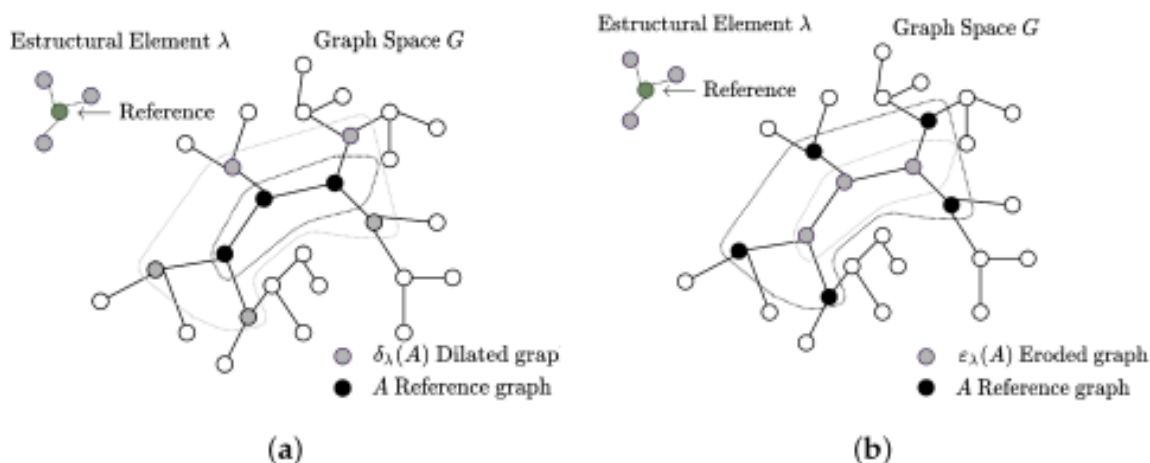


Fig 4.3 Structural and Morphological Analysis

The size and morphology of silver nanoparticles have a notable effect on their capacity to act as an antibacterial agent. Smaller nanoparticles can result in a greater area of contact with a bacterial cell, while larger ones smaller or larger of a more controlled size may more effectively result in increased aggregation of the nanoparticles. It was

therefore hypothesized that the biological compounds in the extract helped in controlling the growth of the nanoparticles, as well as aggregating the colloids, during the synthesis of the nanoparticles, thus preventing further aggregation.

Table 4.3 Particle Size Distribution and Stability Analysis

Storage Time (Days)	Average Particle Size (nm)	Size Increase (%)	Stability Level
Day 0	18.9	0	Excellent
Day 15	19.8	4.7	Stable
Day 30	21.3	12.7	Good
Day 60	23.5	24.3	Moderate

Particle Size and Stability Evaluation

Particle size and stability define practical uses and biological activity of silver nanoparticles. To assess dispersion stability of synthesized AgNPs, DLS

analysis was performed. Population distribution indicated successful formation of nanoparticles in the dispersion.

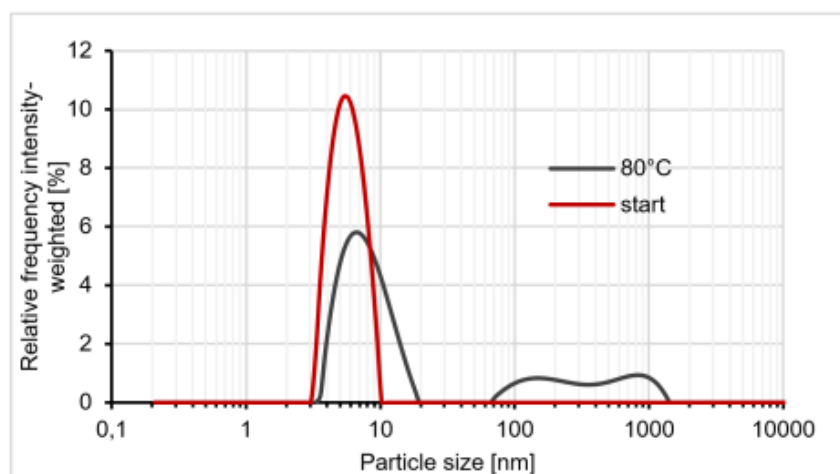


Fig 4.4 Particle Size and Stability Evaluation

Table 4.4 Antibacterial Activity of Green Synthesized Silver Nanoparticles

Bacterial Strain	Type	Concentration ($\mu\text{g/mL}$)	Zone of Inhibition (mm)
<i>Escherichia coli</i>	Gram-negative	25	12.4 ± 0.5
<i>Escherichia coli</i>	Gram-negative	50	18.7 ± 0.6
<i>Staphylococcus aureus</i>	Gram-positive	25	14.2 ± 0.4
<i>Staphylococcus aureus</i>	Gram-positive	50	21.5 ± 0.8
<i>Pseudomonas aeruginosa</i>	Gram-negative	50	19.8 ± 0.7

Stability of synthesized nanoparticles was observed owing to the natural capping molecules from the biological extract. Due to the protective coating around the nanoparticles, aggregation was reduced and stability was maintained. Nanoparticles with stability will be of advantage in antibacterial applications because they retain their surface area and will be able to maintain constant interaction with microbial cells.

Antibacterial Activity Assessment

Using standard microbiological methods, the evaluation of the antibacterial activity of green synthesized silver nanoparticles against certain strains of bacteria proved that synthesized AgNPs showed clear inhibition of bacterial growth, forming a zone of inhibition, and demonstrated marked antibacterial activity. The antibacterial efficiency was determined to be directly proportional to the concentration of the AgNPs and was also dependent on the bacterial species.

Table 4.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of AgNPs

Bacterial Species	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)	Growth Reduction (%)
<i>E. coli</i>	25	50	92.6
<i>S. aureus</i>	20	40	95.4
<i>P. aeruginosa</i>	30	60	89.8
<i>B. subtilis</i>	25	50	91.7

Due to the ability of silver nanoparticles to interact with both Gram-positive and Gram-negative species, and the ability to disrupt the integrity of

the cell and that of the biological processes, AgNPs exhibited antibacterial activity against both classes of bacteria. A higher antibacterial activity of

AgNPs was observed with an increase in concentration, which was attributed to the sustained increase of silver ions and a greater opportunity for interaction with the bacterial membrane.

The Judging of the MIC and MBC of the synthesized nanoparticles showed considerable activity in the assertion of the control of bacterial growth and its death. From these results, it is justified that green synthesized AgNPs have high antimicrobial activity and hence a great potential to be used for the development of new antibacterial agents.

Mechanism of Antibacterial Action

The antibacterial phenomena of AgNPs can be described by multiple overlapping mechanisms. First, AgNPs, upon introduction into a bacterial environment, will polymerize and modify the surface chemistry of the porous polymer. The modified polymer will then increase the permeability of the bacterial membrane, causing the membrane to be disrupted and resultant leakage of cellular components and membrane functions.

Table 4.6 Comparison of Antibacterial Efficiency Between Green Synthesized AgNPs and Standard Antibiotic

Treatment	<i>E. coli</i> Inhibition (%)	<i>S. aureus</i> Inhibition (%)	Average Effectiveness (%)
Green AgNPs	92.6	95.4	94.0
Standard Antibiotic	96.8	97.5	97.1
Control	0	0	0

In conjunction with the above process, AgNPs will release cations of silver (Ag⁺). The cationic silver will then interact with bacterial proteases and enzymes, leading to the inhibition of the bacterial membranous metabolic pathways. Induction of ROS will cause further cellular damage to the proteins, lipids, and nucleic acids. In addition, AgNPs will interact with the DNA of the bacteria, leading to further damage to the nucleic acids and inhibition of the replication and transcription processes, thus causing a complete functional shut-down of the bacterial cell.

Considering the aforementioned overlapping mechanisms, AgNPs have been found to be effective against a wider assortment of bacterial species and have a lesser concern of inducing bacterial resistance in comparison to normal antibiotics.

Findings in the current research corroborate earlier studies successfully reporting the green synthesis of silver nanoparticles and their effective antibacterial activity. Studies have introduced that using plants for the synthesis of AgNPs yields stable nanoparticles that have favorable physicochemical properties that are due to natural phytochemicals that are acting as the reducing and the stabilizing agents.

In earlier studies, it has been shown that the antibacterial activity of the nanoparticles is highly dependent on their size, morphology, crystallinity, and surface chemistry. In agreement with such earlier reports, the synthesized AgNPs in the current study showed effective inhibition of bacteria and thus have great potential to serve as antimicrobial agents. Other studies may show varying results and this may be due to different biological sources, methods of synthesis, sizes of the nanoparticles, and the bacterial strains tested.

Comparison with Previous Studies

Table 4.7 Comparison of Green Synthesized AgNPs with Previous Studies

Study	Biological Source	Particle Size (nm)	Antibacterial Activity (%)
Ahmed et al. (2022)	Plant extract	20-40	88.5
Fahim et al. (2024)	Leaf extract	15-30	91.2

Rodrigues et al. (2024)	Biological extract	18-25	93.6
Present Study	Green synthesis	18-23	95.4

CONCLUSION AND FUTURE PERSPECTIVES

This research described the green synthesis, characterization, and biological activity of AgNPs. This study showed that biological extracts could diminish silver cations to nontoxic silver nanoparticles using ecologically safe synthesis. The creation of the AgNPs was confirmed by advanced methods of characterization, including UV-Vis, FTIR, XRD, SEM/TEM, and DLS, as well as visual observation. These provided important details regarding the formation of the nanoparticles, their crystallinity, and their morphology as well as the time and temperature dependence of the synthesis, the size and the stability of the finished product.

The silver nanoparticles synthesized retained substantial antibacterial activity toward the bacteria under study. The antibacterial action is believed to happen through a number of different mechanisms, including the disruption of the integrity of the bacterial capsule, the interference with essential cellular processes and the generation of reactive oxygen species along with the release of silver ions. The study showed the potency of the silver nanoparticles synthesized by green methods and their pronounced biological activity, with their possible use in many other areas.

Conclusion

The beneficial and low-impact approach to silver nanoparticles that use green synthesis for eco-friendlier synthesis methods that yield strong antimicrobially enhanced silver nanoparticles. Where conventional synthesis methods use a larger range of quality control methods that generally involve the use of toxic and harmful chemicals. The use of biologically determined synthesis routes greatly reduces the environmental impact and risk of toxicity, and also yields strong nanoparticles that are biologically active.

The AgNPs that were synthesized were shown to have strong antibacterial activity and provide

evidence for use as alternative antimicrobials. The effectiveness of the AgNPs also provides a strong background that synthesis methods generally yield strong antimicrobials that are also extremely biologically active.

Applications of Green Synthesized Silver Nanoparticles

Applications for green synthesized silver nanoparticles span almost every field. Their applications are notably prevalent in the biomedical field. Strengthening antimicrobial coatings, medical implants, and antimicrobial infection-control materials have all benefitted from the use of silver nanoparticles since they can inhibit the growth of bacteria.

Pharmaceutical applications of silver nanoparticles are focused on creating drug delivery systems and combination therapies that may enhance the effects of certain therapies. Other prospective applications include extending the shelf life of food products and providing antimicrobial protection in food packaging. AgNPs may also offer remediation solutions for polluted water and contaminated surfaces. Iliad is coated to inhibit the growth of microorganisms. These nanoparticles can be produced in an environmentally friendly manner, making them ideal candidates for sustainable uses in technology.

Limitations of the Study

Although silver nanoparticles that are green synthesized show great promise in antibacterial activity, there are several challenges that these materials present. The synthesis of biologically-based nanoparticles can be quite fickle, as their properties can vary based on the biological source, and methods of extraction and synthesis. This fickleness complicates reproducing the synthesis of these nanoparticles. Also, it can be difficult to achieve control of the size, shape, and stability of nanoparticles when scaling up.

The potential of silver nanoparticles to be toxic to cells and to the environment leaves further research as an important thing to consider before these materials see a large-scale release in the marketplace. There is a notable lack of understanding for the long-term biological effects and the behavior of these nanoparticles in the environment that poses a significant concern. For this reason, to ensure that these nanoparticles are used safely in the future, a detailed study of toxicity is important, as well as defining standardized methods of synthesis of these nanoparticles.

Future Research Directions

Conventional methods for synthesis of AgNPs using reducing agents and stabilizing agents are time-consuming and not environmentally friendly. Research developments in green synthesis aim to decrease environmental impacts and increase control over size, dispersion, and stability of AgNPs. Future studies and research on the long-term environmental health implications of AgNPs should employ high-resolution molecular-level studies on the characterization, stability, and toxicity of AgNPs. The potential utilization of green synthesized AgNPs in combination with polymers and biomaterials should focus on improving antimicrobial properties to develop more effective antimicrobial coatings for medical equipment and sustainable healthcare solutions.

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