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Research Article

Optical and Antibacterial Investigation of Biogenically Synthesized Silver Nanoparticles

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Abstract

Sustainable antibacterial materials are desperately needed due to the growing prevalence of bacterial infections that are resistant to drugs. At the same time, environmental concerns associated with conventional nanoparticle synthesis methods have encouraged the development of eco-friendly alternatives. In this study, silver nanoparticles were synthesized using a green, plant-mediated reduction method in which phytochemicals present in the plant extract acted as both reducing and stabilizing agents. The formation of AgNPs was verified through optical characterization techniques. The synthesized nanoparticles exhibited typical optical features, including a distinct surface plasmon resonance band and a clear photoluminescence emission peak in the near-UV region (∼ 330–335 nm). These optical properties indicate efficient electronic transitions and the presence of stable surface states in the nanoparticles. Using the agar diffusion technique, the antibacterial activity of the biosynthesised AgNPs was assessed against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. The findings demonstrated that AgNPs exhibited noticeably more antibacterial activity when compared to silver nitrate and plant extract alone, indicating that silver's bioavailability and antimicrobial efficacy are improved by nanoparticle production. A comparatively larger inhibition zone was observed for the Gram-negative bacterial strain, indicating that variations in bacterial cell wall structure may influence nanoparticle susceptibility. Overall, the findings show that green-synthesised silver nanoparticles have potential for use in the pharmaceutical and biomedical industries and can function as potent antibacterial agents.

1. Introduction

Nanotechnology has become one of the most influential areas of modern scientific research owing to its extensive applications in medicine, electronics, environmental protection, and advanced materials [1, 2]. Silver nanoparticles (AgNPs) have attracted a lot of attention among the several nanomaterials that have been researched because of their exceptional physicochemical, optical, catalytic, and antibacterial properties [3–5]. AgNPs unique characteristics are mainly due to their nanoscale size, which provides a higher surface activity and a bigger surface area-to-volume ratio when compared to bulk silver [6, 7]. These features significantly enhance their functional performance, making silver nanoparticles particularly valuable for biomedical and pharmaceutical applications, especially in antimicrobial treatments [8, 9].

Silver has been recognized for its antimicrobial properties for centuries; however, the advent of nanotechnology has significantly enhanced its biological efficacy. At the nanoscale, silver particles exhibit improved penetration ability, controlled ion release, and enhanced

interaction with microbial membranes [10–12]. Numerous studies have reported that AgNPs exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The antibacterial mechanism of AgNPs is multifaceted and includes disruption of cell membrane integrity, generation of reactive oxygen species (ROS), interaction with thiol groups of proteins, DNA damage, and sustained release of Ag^+ ions. Comparative antibacterial assessment is crucial because Gram-positive and Gram-negative bacteria differ structurally, which affects how susceptible they are to nanoparticles [13–15].

Conventional physical and chemical methods for synthesizing silver nanoparticles often involve high energy consumption, toxic reducing agents, and hazardous by-products, which limit their biomedical applicability. On the other hand, plant extract-based green synthesis techniques have become viable, economical, and sustainable substitutes. Flavonoids, phenolics, terpenoids, and alkaloids are examples of plant-derived phytochemicals that function as stabilising and reducing agents during the creation of nanoparticles. This biogenic synthesis route not only eliminates the need for harmful chemicals but also enhances nanoparticle stability through natural capping agents. Additionally, phytochemical-functionalized nanoparticles may exhibit synergistic biological activity [16–18].

Characterization of synthesized nanoparticles is essential to confirm their formation, stability, and functional properties. Optical techniques such as UV–Visible spectroscopy and photoluminescence (PL) analysis provide valuable information regarding surface plasmon resonance (SPR), electronic transitions, and surface defect states. The optical behavior of AgNPs is strongly influenced by particle size, morphology, and surface chemistry. Photoluminescence analysis, in particular, offers insight into radiative recombination processes and surface state emissions, which are important for potential applications in sensing and bioimaging [19, 20].

Even though silver nanoparticles have been extensively studied, there is still an increasing need to create environmentally friendly synthesis techniques and get a better understanding of how their optical characteristics relate to their biological activities. In this context, the current work investigates the environmentally friendly manufacture of silver nanoparticles utilising plant extract, which serves as a stabilising and reducing agent. Spectroscopic methods were employed to analyse the optical characteristics of the synthesised AgNPs, with a focus on their photoluminescence behaviour. Additionally, in order to examine the antimicrobial efficacy of the produced nanoparticles, their antibacterial activity was assessed using the agar diffusion technique against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. It is anticipated that the investigation's findings will provide important light on the connection between silver nanoparticles' antibacterial activity, optical properties, and environmentally friendly manufacturing techniques. Such understanding may support the development of sustainable nanomaterials with potential applications in biomedical and healthcare fields.

2. Materials and Methods

2.1. Chemicals and Reagents

In order to create nanoparticles, fresh eucalyptus leaves were gathered locally and utilised as a biological reducing agent. As the silver precursor, silver nitrate ($AgNO_3$, $\geq$ 99% purity, analytical grade) was utilised without further purification. Antimicrobial investigations were conducted using nutrient broth, Mueller-Hinton agar (MHA), and nutrient agar. Double-distilled water was used to prepare each solution. Before being used, glassware was properly cleaned and washed with distilled water to prevent contamination.

2.2. Preparation of Eucalyptus Leaf Extract

Fresh Eucalyptus leaves were washed thoroughly under running tap water to remove adhered dust and surface impurities, followed by rinsing with distilled water. The cleaned leaves were air-dried and finely chopped into small pieces. Approximately 10 g of chopped leaves were transferred into a 250 mL beaker containing 100 mL of distilled water. The mixture was heated at 70–80 °C for 15–20 minutes under mild stirring to facilitate extraction of bioactive phytochemicals. Plant metabolites such flavonoids, terpenoids, phenolics, and reducing sugars were released as the solution gradually became pale green. After letting the extract settle to room temperature, solid residues were filtered out using Whatman No. 1 filter paper. The clear filtrate was utilised for the creation of nanoparticles after 48 hours after being kept at $4^{\circ}C$.

2.3. Green Synthesis of Silver Nanoparticles

The necessary amount of silver nitrate was dissolved in distilled water to create a new 1 mM aqueous solution of $AgNO_3$. In order to create silver nanoparticles, 100 mL of the 1 mM $AgNO_3$ solution was gradually mixed with 10 mL of Eucalyptus leaf extract while being continuously stirred magnetically at room temperature for around 60 minutes. The solution's colour steadily changed during the reaction, going from colourless to yellowish-brown and finally dark brown, indicating the creation of silver nanoparticles as a result of the surface plasmon resonance (SPR) phenomenon. The completeness of nanoparticle production was confirmed by allowing the reaction mixture to continue until the colour stabilised. Centrifugation at 8000–10,000 rpm for 15 minutes was used to separate the produced nanoparticles. To get rid of any leftover biomolecules and extra salts, the resulting pellet was rinsed three times with pure water. The purified nanoparticles were then stored in airtight containers for further characterisation and analysis after being dried at around $60^{\circ}C$.

2.4. Characterization of Silver Nanoparticles

Several analytical methods were used to investigate the synthesised silver nanoparticles' production and physicochemical properties.

- **UV-Vis Spectroscopy:** A JASCO V750 UV–Visible spectrophotometer was used to record the absorption spectra in the 300–700 nm wavelength range. The successful creation of AgNPs was verified by the emergence of a distinctive surface plasmon resonance (SPR) peak in the 400–450 nm range.
- **Photoluminescence Analysis:** To examine the luminescence behaviour of the produced nanoparticles, the emission spectra were acquired using a JobinYvon Fluoromax-4 spectrofluorometer (Horiba, USA) fitted with a 150 W xenon (Xe) lamp as the excitation source.
- **Morphological Characterisation:** The size, shape, and structural characteristics of the nanoparticles were investigated using Transmission Electron Microscopy (TEM). The produced AgNPs were primarily spherical in form, with particle sizes typically falling

between 10 and 30 nm, according to the TEM pictures.

2.5. Antibacterial Activity Evaluation

Using the agar well diffusion technique, the antibacterial efficacy of the produced silver nanoparticles was evaluated against typical Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. Mueller–Hinton agar medium was prepared, sterilized, and poured into Petri dishes. Fresh bacterial cultures were cultivated overnight in nutrient broth at $37°C$ to obtain active bacterial growth. To create a homogenous microbial lawn, the bacterial solution was then uniformly spread throughout the agar surface using sterile cotton swabs. A sterile cork borer was used to gently produce wells in the agar with a diameter of around 6 mm. Predetermined concentrations of the AgNP suspension were subsequently introduced into the wells. For comparison, silver nitrate solution and the plant extract were used as control samples. The prepared plates were then incubated at $37°C$ for 24 hours. Following incubation, the antibacterial effectiveness was determined by measuring the diameter of the clear inhibition zones formed around each well. The antibacterial response of the produced nanoparticles against the Gram-positive and Gram-negative bacterial strains was compared using the zone of inhibition (ZOI) values, which were measured in millimetres.

3. Results and discussion

3.1. TEM characterization results

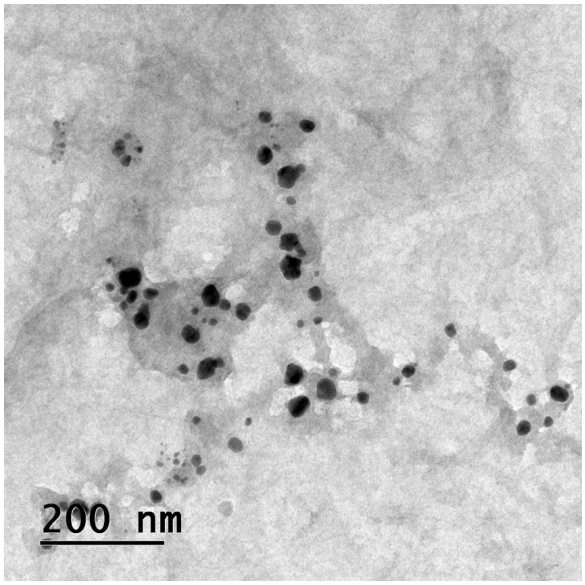

Figure 1: Transmission electron microscopy (TEM) image of green-synthesized silver nanoparticles prepared using Eucalyptus leaf extract

The green synthesised silver nanoparticle's morphology, particle size distribution, and crystallinity were examined by TEM and it is shown in Figure 1. The TEM micrograph clearly reveals the formation of well-dispersed nanoparticles with predominantly spherical morphology. The particles appear relatively uniform in shape, with slight variations in size, which is commonly observed in biologically synthesized nanomaterials due to the heterogeneous nature of plant-derived reducing agents. The typical particle size of the synthesised AgNPs is between 10 and 30 nm. The majority of the nanoparticles are distinct and isolated from one another, indicating that the phytochemicals in the eucalyptus leaf extract effectively stabilise the particles. Certain nanoparticles have a thin organic capping layer, which might be explained by bioactive substances including terpenoids, phenolics, and flavonoids serving as stabilising and reducing agents during synthesis. Minimal aggregation is observed in some regions, possibly due to van der Waals interactions during drying of the TEM grid. However, the absence of large clusters indicates good colloidal stability of the nanoparticles. The spherical shape and very tiny particle size greatly improve the nanoparticles' surface-to-volume ratio. This increased surface area is expected to improve interaction with bacterial cell membranes, thereby enhancing antimicrobial activity. The nanoscale dimension facilitates penetration through the bacterial cell wall, particularly in Gram-negative strains where the outer membrane structure allows interaction with Ag^+ ions and reactive oxygen species generated from the nanoparticle surface.

3.2. UV characterization results

Using UV-visible spectroscopic spectroscopy, the effective production of silver nanoparticles using Eucalyptus leaf extract was first confirmed. Figure 2 displays the absorption spectra of the produced colloidal solution, which was obtained across a wavelength range of 300–700 nm. At $\lambda_{max} = 411\,nm$, a noticeable and distinct absorption maximum was found, which is a common indicator of the SPR connected to silver nanoparticles. This peak shows that the bioactive phytochemicals in the eucalyptus extract have successfully reduced Ag^+ ions into metallic silver (Ag_2) nanoparticles. The collective oscillation of conduction electrons on the surface of metal nanoparticles in response to input electromagnetic radiation is the source of the SPR effect. Depending on variables such particle size, shape, aggregation behaviour, and the surrounding dielectric medium, this distinctive resonance band often manifests in the 400–450 nm wavelength range for silver nanoparticles. Consistent with the TEM data, the observed absorption maximum at 411 nm indicates the creation of relatively tiny nanoparticles with

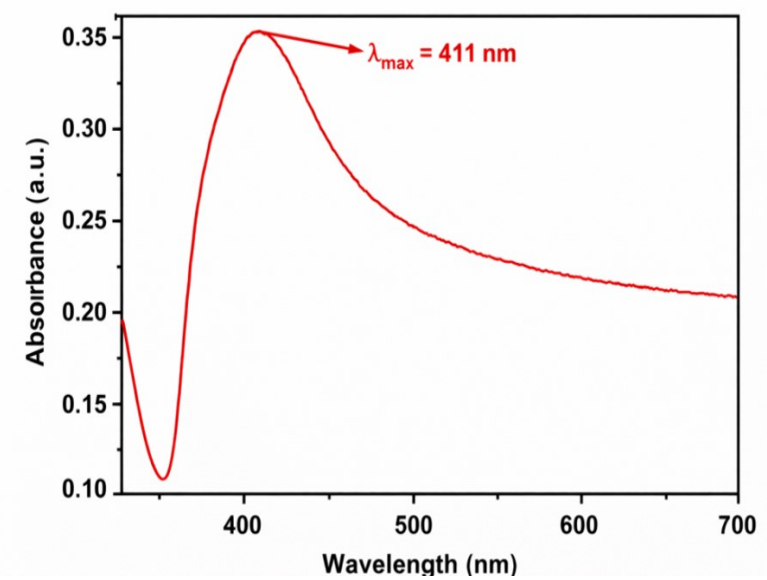

Figure 2: UV–Visible absorption spectrum of silver nanoparticles synthesized using Eucalyptus leaf extract recorded in the wavelength range of 300–700 nm

a mainly spherical shape. Furthermore, the absence of additional absorption peaks in the longer wavelength region indicates that the nanoparticles are well dispersed with minimal aggregation, suggesting a largely monodisperse nanoparticle distribution.

3.3. PL characterization result

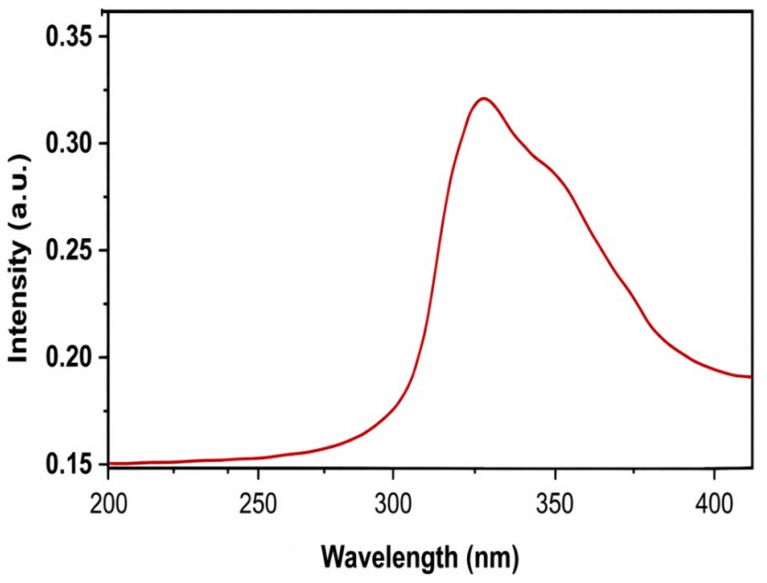

Figure 3: Photoluminescence (PL) emission spectrum of the biosynthesized silver nanoparticles measured in the 200–410 nm wavelength range

To examine the optical emission behaviour and electrical characteristics of the synthesised AgNPs, the photoluminescence (PL) spectrum was recorded in the 200–410 nm wavelength range. The obtained PL spectrum exhibits a distinct and intense emission peak centered at approximately 330–335 nm. As shown in Figure 3, the emission intensity gradually increases from 200 nm, followed by a sharp rise beyond 300 nm, reaching a maximum intensity of approximately 0.32 a.u. near 330–335 nm. Beyond the emission maximum, the intensity progressively decreases toward 400 nm, indicating a typical radiative recombination process. The presence of a well-defined emission peak confirms the formation of silver nanoparticles and suggests efficient electronic transitions within the nanoparticle structure. Surface plasmon-coupled emission effects, radiative recombination of conduction band electrons with valence band holes, surface defect states, and trap-assisted recombination are the main explanations for the observed emission band in the near-UV region. The relatively sharp and symmetric emission profile indicates good crystallinity and uniform particle size distribution. Strong PL emission intensity suggests the presence of active surface states, which may arise from phytochemical capping agents involved in the green synthesis process. These biomolecules can introduce surface energy levels that contribute to enhanced radiative recombination.

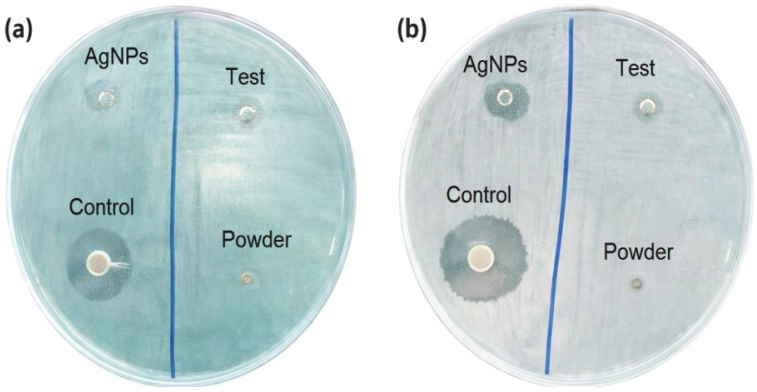

Figure 4: Antibacterial activity of green-synthesized silver nanoparticles evaluated by the agar well diffusion method against (a) Gram-positive *Staphylococcus aureus* and (b) Gram-negative *Escherichia coli*

3.4. Anti-bacterial studies

The antibacterial efficacy of green-synthesized AgNPs was evaluated against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacterial strains using the agar diffusion method. The comparative activity of $AgNO_3$ (test), plant extract (powder), synthesized AgNPs, and the standard control is presented in Figure 4. For the Gram-positive bacterial strain Figure 4 a, the standard control exhibited a distinct zone of inhibition, confirming the validity of the experimental procedure and the susceptibility of the test organism. $AgNO_3$ showed only minimal antibacterial activity at the tested concentration, as evidenced by a very small or negligible inhibition zone. Similarly, the plant extract powder alone did not produce a significant zone of inhibition, indicating that the crude extract possesses limited intrinsic antibacterial activity under the tested conditions. In contrast, the synthesized AgNPs demonstrated a clear and comparatively larger zone of inhibition ($\sim$7.9 mm), indicating enhanced antibacterial activity. The improved efficacy of AgNPs compared to $AgNO_3$ suggests that nanoparticle formation significantly enhances the bioavailability and antimicrobial performance of silver. The nanoscale size of AgNPs increases the surface area-to-volume ratio, facilitating stronger interaction with the bacterial cell wall. Even though Gram-positive bacteria have a thick coating of peptidoglycan that can serve as a structural barrier, the AgNPs were nonetheless successful in compromising the integrity of the cell membrane and inhibiting bacterial growth.

For the Gram-negative bacterial strain Figure 4 b), a more pronounced antibacterial effect was observed with AgNP treatment. The inhibition zone produced by AgNPs was larger ($\sim$ 8.9 mm) compared to that observed in the Gram-positive strain. The standard control again showed effective inhibition, while $AgNO_3$ and plant extract powder exhibited limited or negligible antibacterial effects. Differences in cell wall design may be the cause of Gram-negative bacteria's increased vulnerability. Gram-negative bacteria have an outer membrane made of lipopolysaccharides and a thinner peptidoglycan layer, which may help them interact with and penetrate nanoparticles. Numerous processes, such as attachment to the bacterial surface, disruption of membrane permeability, penetration into the cytoplasm, release of Ag^+ ions, production of reactive oxygen species (ROS), and interference with DNA replication and protein synthesis, could be responsible for the increased activity of AgNPs.

4. Conclusion

In this study, AgNPs were successfully synthesized via a green, plant-mediated reduction approach, demonstrating an environmentally benign and sustainable alternative to conventional chemical synthesis routes. The plant extract's phytochemical components efficiently served as stabilising and reducing agents, allowing for the regulated creation of nanoparticles without the need for hazardous chemicals. Ag^+ ions were successfully reduced to metallic Ag_2 nanoparticles, as demonstrated by the visible colour shift and subsequent spectroscopic investigations. Optical characterization revealed distinct plasmonic and photoluminescent behavior of the synthesized AgNPs. The observed optical features indicate well-defined nanoparticle formation with stable surface characteristics. The photoluminescence spectrum exhibited a prominent emission peak in the near-UV region, suggesting efficient radiative recombination processes and the presence of surface-associated electronic states. Good crystallinity and regulated nanoparticle development are implied by the comparatively sharp emission profile, which is probably affected by phytochemical capping molecules. Both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacterial strains are significantly inhibited by the green-synthesised AgNPs, according to the antibacterial assessment. The production of nanoparticles significantly increases silver bioavailability and antimicrobial activity, as seen by the notable improvement in antibacterial efficacy when compared to silver nitrate and plant extract alone. The comparatively higher susceptibility of Gram-negative bacteria suggests that cell wall architecture plays a critical role in nanoparticle interaction and penetration. The antibacterial mechanism is attributed to a combination of membrane disruption, intracellular Ag^+ ion release, oxidative stress induction via reactive oxygen species (ROS), and interference with essential biomolecular processes. Thus, this study demonstrates that green-synthesized silver nanoparticles exhibit stable optical characteristics and potent antibacterial activity, validating their potential for biomedical, pharmaceutical, and antimicrobial coating applications. Future investigations may focus on detailed mechanistic studies, cytotoxicity evaluation, and in vivo assessments to further advance the translational potential of these sustainable nanomaterials.

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