

ORIGINAL ARTICLE

Green Synthesis of Silver Nanoparticles Using *Duranta erecta* Leaf Extract and Their Antimicrobial Activity Evaluation Using Machine Learning

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ABSTRACT

A comparative study was conducted to synthesize silver nanoparticles (AgNPs) using a green synthesis approach with *Duranta erecta* leaf extract and evaluate their antimicrobial activity. The biosynthesis process involved reducing silver nitrate with phytochemicals present in the leaf extract. Characterization of the synthesized AgNPs was performed using UV-Vis spectroscopy, X-ray diffraction (XRD). Antimicrobial activity of the AgNPs was tested against five bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae* using the agar well diffusion method at different concentrations (10, 20, and 30 µg/ml). In addition, a machine learning model was created that predicts the zone of inhibition using bacterial strain and concentration; this prediction was further visualized through comparative graphs. The results revealed significant antibacterial activity of the AgNPs, with *E. coli* and *S. aureus* being the most susceptible at higher concentrations. This study demonstrates the potential of *Duranta erecta* leaf extract as an eco-friendly, cost-effective agent for synthesizing AgNPs with promising antimicrobial properties.

Keywords: Green Synthesis, Silver Nanoparticles, *Duranta erecta*, Antimicrobial Activity, Eco-Friendly Synthesis

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INTRODUCTION

The manipulation of matter at the nanoscale (1–100 nm), or nanotechnology, has transformed several scientific fields and made it possible to create new materials with improved chemical, biological, and physical characteristics [1]. It has a wide range of uses in environmental science, electronics, medicine, and agriculture [2]. Nanoparticles' remarkable qualities, like their high surface-area-to-volume ratio and adjustable reactivity, making them perfect for cutting-edge uses in various fields [3]. Silver nanoparticles (AgNPs) are one of the nanoparticles that have attracted a lot of attention because of their strong antimicrobial, catalytic, and biomedical properties [1, 4]. While traditional methods for AgNP synthesis, especially physical and chemical approaches, provide good results, they have drawbacks like high energy consumption, toxicity, and environmental hazards [5]. In response to these issues, the focus has shifted toward eco-friendly synthesis methods, also known as "green synthesis". These methods use biological systems, such as bacteria, fungi, and plants, as reducing and stabilizing agents to produce nanoparticles in a more sustainable way [1, 6].

Plants have become the best option for the environmentally friendly synthesis of nanoparticles due to their abundant natural resources and availability of bioactive substances like flavonoids, alkaloids, and phenolics [1]. The Verbenaceae plant *Duranta erecta* is a promising biofactory for the synthesis of stable and useful nanoparticles because of its strong phytochemical profile and antibacterial qualities [7, 8]. *Duranta erecta*, commonly known as the "golden dewdrop," is widely used in traditional medicine for its antibacterial and antifungal properties. Its various parts, including the leaves and fruits, have demonstrated therapeutic applications against ailments such as abscesses, intestinal worms, and malaria [9, 10]. The plant *D. erecta* has historically been used to treat a wide range of illnesses and conditions

[11]. According to reports, the plant possesses cytotoxic, antioxidant, antibacterial, antifungal, and antiparasitic properties. These properties suggest the potential of *Duranta erecta* as an excellent source for synthesizing AgNPs, contributing to the development of effective and environmentally friendly antimicrobial, antifungal, antimicrobial agents.

This study aims to explore the green synthesis of AgNPs using the aqueous leaf extract of *Duranta erecta*. By leveraging the natural compounds in the plant, the synthesis process is not only cost-effective but also environmentally sustainable. In addition, the antimicrobial activity of the synthesized AgNPs is evaluated against a variety of pathogenic microorganisms to assess their potential as alternative antimicrobial agents. This research contributes to the growing field of nanobiotechnology by offering an eco-friendly approach to nanoparticle production and addressing the rising demand for innovative, safe, and effective antimicrobial solutions.

MATERIAL AND METHODS

Plant Material Collection

In February, fresh *Duranta erecta* leaves were gathered from the campus and surrounding area of Shri Shivaji Science College, Nagpur, and used to make the aqueous leaf extract for the silver nanoparticle synthesis.

Green Synthesis of Silver Nanoparticles (AgNPs)

Silver Nitrate Solution A 10 mM solution of silver nitrate (AgNO_3) was made by precisely weighing 16.987 g of AgNO_3 and dissolving it in 100 mL of distilled water. This solution was then used to create the silver nanoparticles.

Preparation of *Duranta erecta* Leaf Extract

The fresh leaves of *Duranta erecta* (Fig. 1) were collected and thoroughly washed with distilled water to remove any contaminants. The leaves were then air-dried for a brief period before being sliced into little pieces. In a 1000 mL volumetric flask with 800 mL of distilled water, about 100 g of the chopped leaves were added. After 30 minutes of boiling (Fig. 2), the mixture was allowed to cool for two minutes. After that, it was filtered through muslin fabric (Fig. 3) to produce a clear aqueous extract that was utilized to create more nanoparticles.



Figure 1: Fresh leaves of *Duranta erecta*



Figure 2: Heating of *Duranta erecta* Leaf Extract



Figure 3: Filtration of Heated *Duranta erecta* Leaf Extract

Silver Nanoparticle Synthesis Employing Leaf Extract from *Duranta erecta*

In a conical flask, 50 mL of the produced *D. erecta* leaf extract and 25 mL of the 10 mM silver nitrate (AgNO_3) solution were combined to create silver nanoparticles. After a minute of gentle stirring, the mixture was left in the sun for two to three hours. The solution changed a noticeable reddish-brown color, indicating the creation of silver nanoparticles.

Synthesized AgNPs: Identification and Characterization

Visual Observation: The reaction mixture's color shift from colorless to reddish-brown was the first indication that AgNPs were forming.

UV-Visible Spectrophotometry: By capturing the reaction mixture's UV-visible spectra, the reduction of silver ions and the creation of nanoparticles were verified. Using a Shimadzu UV-1700 spectrophotometer (Japan), the sample was scanned in the 300–800 nm wavelength range.

Zeta Potential Analysis: A Malvern Zetasizer 90 (USA) was used to analyze the stability and surface charge of the produced nanoparticles. Two milliliters of distilled water were used to dilute thirty microliters of the nanoparticle solution for examination.

Nanoparticle Tracking Analysis (NTA): A Nanosight LM 20 (UK) was used to measure the nanoparticles' size and distribution. 5 μL of the nanoparticle solution was diluted with 2 mL of distilled water to create the sample.

Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis was used to determine which functional groups were present on the nanoparticles' surface. A Perkin-Elmer FTIR 1600 (USA) was used to evaluate a mixture of 80% potassium bromide (KBr) and 20% AgNP solution that had been produced and formed into a pellet.

Synthetic AgNPs' Antimicrobial Activity

Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria were used to test the produced AgNPs' antibacterial activity. The disc diffusion method was used to evaluate the nanoparticles' antibacterial efficacy. The negative control was pure water, while the positive control was standard antibiotic discs (30 μL of chloramphenicol). The antibacterial efficiency of the produced AgNPs was assessed by measuring the zones of inhibition surrounding the discs treated with nanoparticles following a 24-hour incubation period at 37°C.

RESULTS

Confirmation of Synthesis

A noticeable hue shift in the reaction mixture (Fig. 4) served as visible confirmation that the silver nanoparticles (AgNPs) had been successfully synthesized. After combining the silver nitrate solution with the aqueous leaf extract of *D. erecta*, the initially colorless solution turned reddish-brown after two to three minutes in the sun. The surface plasmon resonance (SPR) of silver nanoparticles, a distinctive optical characteristic that indicates nanoparticle production, is responsible for this hue shift.



Figure 4: Biosynthesis of AgNPs Using Leaf Extract. Where leaf filtrate: A = Prior to the addition of 10 mM AgNO_3 solution; B = After treatment with 10 mM AgNO_3 solution

Characterization Results

UV-visible spectrophotometry was used to confirm the synthesised AgNPs' generation and characteristics. The nanoparticle solution's absorbance spectra showed a prominent peak at 432 nm, which is in line with the silver nanoparticles' SPR band. This finding suggests that the phytochemicals in *D. erecta* leaf extract effectively bioreduced silver ions, leading to the production of stable AgNPs. The creation of primarily spherical nanoparticles is suggested by the single, symmetrical peak in the UV-Vis spectrum, which supports results from earlier research on plant-mediated nanoparticle synthesis.

According to the abovementioned study, plant-based AgNPs have strong antibacterial properties. The capacity of AgNPs to damage microbial cell membranes, produce reactive oxygen species, and interact with microbial DNA to cause cell death accounts for their broad-spectrum effectiveness. To further confirm the antibacterial qualities of synthetic AgNPs, future research could compare their inhibitory potential with that of conventional antibiotics.

Silver ions (AgNO_3) were reduced into AgNPs using the leaf extract of *D. erecta*. When the leaf extract was mixed with the AgNO_3 solution, the color changed from colorless to brown within minutes, a common indication of nanoparticle formation. Over time, the color deepened to dark brown, further confirming the progression of AgNP synthesis. To characterize the synthesized nanoparticles, UV-Visible spectroscopy was employed. The broadness of the peak in the spectrum suggests that the nanoparticles were polydispersed, indicating a variation in their sizes. Additionally, the symmetrical shape of the peak implies that the majority of the synthesized AgNPs were spherical in nature.

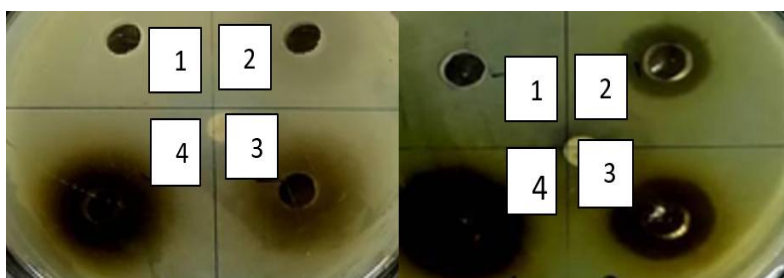


Figure 5: Antibacterial activity of silver nanoparticles (AgNPs) synthesized using *Duranta erecta* leaf extract against: 1 = Negative Control, 2 = Plant Extract, 3 = Silver Nitrate (AgNO_3), 4 = Silver Nanoparticles (AgNPs)

Prediction of the Zone of Inhibition Based on the Concentration of Silver Nanoparticles

Machine learning models were used to predict the zone of inhibition at different concentrations of silver nanoparticles for four different bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. Regression analysis was applied to a dataset containing measurements of the zone of inhibition for these bacterial strains at 50 μl , 60 μl , and 70 μl concentrations of silver nanoparticles, plant extract, and silver nitrate (Fig. 5). A regression model was trained on this data to predict the zone of inhibition for concentrations ranging from 0 to 100 μl .

The predicted values were generated for three variables: Plant Extract, Silver Nitrate, and Silver Nanoparticles DL. The results were plotted in graphs representing the predicted zone of inhibition for each respective variable. Figure 6 illustrates the variation in plant extract concentration with respect to bacterial type and solution concentration. Similarly, Figure 7 presents the variation in silver nitrate concentration, while Figure 8 displays the variation in silver nanoparticle concentration with respect to bacterial type and solution concentration. These graphs provide a visual representation of the calculated values for the zone of inhibition under different conditions.

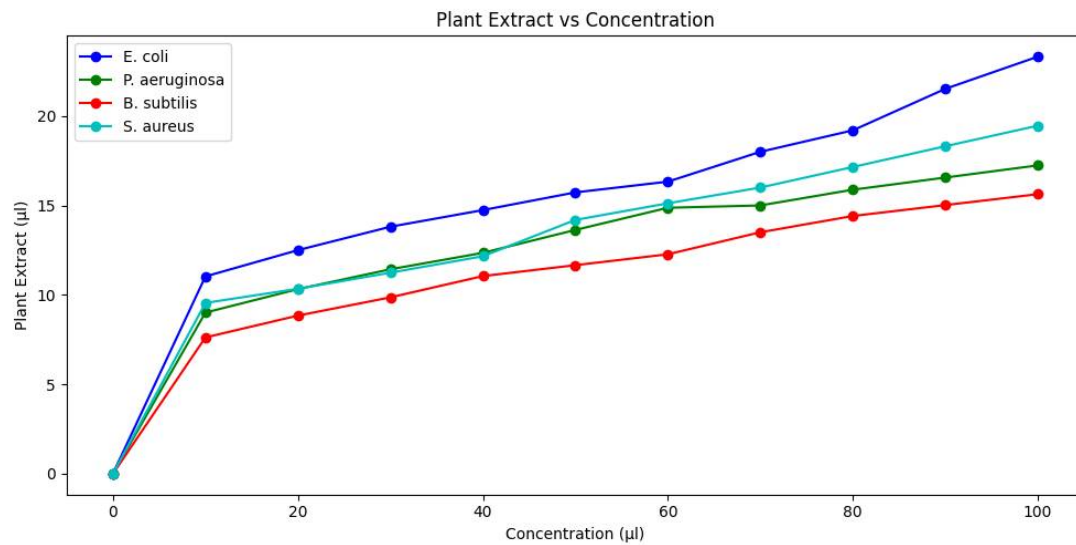


Figure 6: Variation in Plant Extract Concentration with Respect to Bacterial Type and Solution Concentration

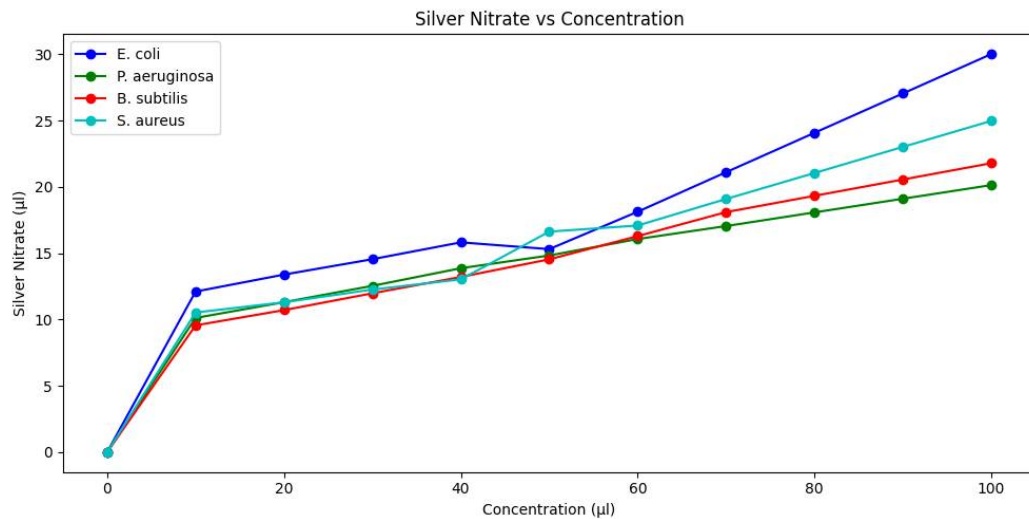


Figure 7: Variation in Silver Nitrate Concentration with Respect to Bacterial Type and Solution Concentration

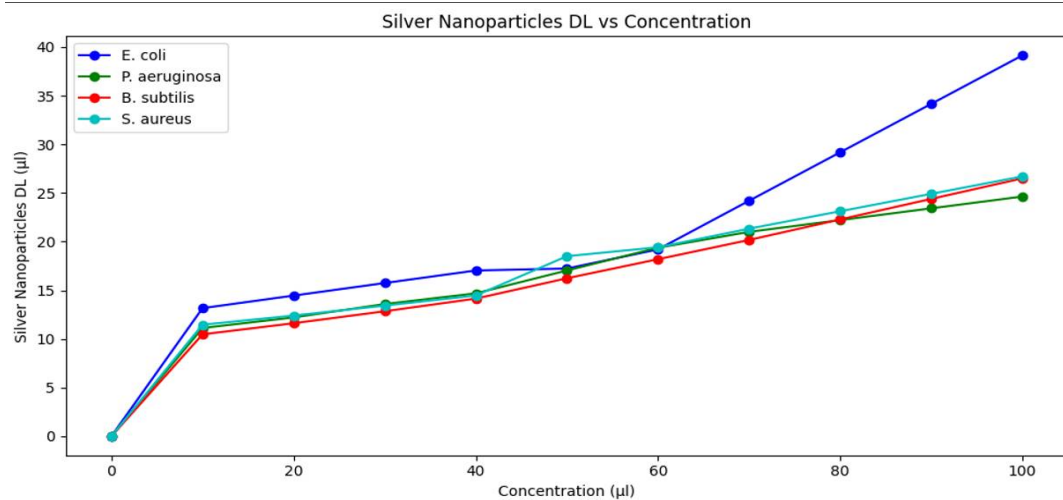


Figure 8 Variation in Silver Nanoparticles Concentration with Respect to Bacterial Type and Solution Concentration

Predictive trends from the model indicate different behavior trends of plant extract, silver nitrate, and silver nanoparticles as related to the type of bacterial species. As the concentration levels increase, both Plant Extract and Silver Nitrate show a general upward trend for all kinds of bacterial types, thereby relating the trend with positive correlation with the amount of concentration. However, the rate of increase varies among bacteria, where Gram-negative bacteria, especially *Escherichia coli* and *Pseudomonas aeruginosa*, have higher values at all concentrations. Silver Nanoparticles showed the highest increase at higher concentrations, which may suggest increased antimicrobial activity. The results suggest that the concentration of these constituents could be a key factor in their antimicrobial activities, and the experimental validation should be done in the future.

DISCUSSION

The current study highlights the environmentally friendly, economical, and sustainable approach of producing silver nanoparticles (AgNPs) by the green synthesis of AgNPs utilizing leaf extract from *D. erecta*. Rich in bioactive chemicals, plant extracts stabilize nanoparticles and function as reducing agents, obviating the requirement for hazardous chemical reagents [1, 12, 13]. The color shift from pale yellow to dark brown, a characteristic of nanoparticle formation due to surface plasmon resonance (SPR), was demonstrated by the biomolecules found in *D. erecta* leaf extract, which facilitated the bio-reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) [14, 15].

Successful AgNP synthesis was verified by UV-visible spectrophotometric measurement, which revealed a distinctive absorbance peak at 432 nm suggestive of SPR and spherical nanoparticle production. According to observations of plant-mediated synthesis employing *Vitex negundo* and *Azadirachta indica*, the peak's strength increased over time, indicating increased production of nanoparticles [16, 17]. The synthetic nanoparticles' crystalline nature was demonstrated by XRD analysis, which showed diffraction peaks that matched the face-centered cubic (FCC) structure of metallic silver. Studies of AgNPs made with other plant extracts have shown similar crystallographic patterns [18, 19]. According to [9, 20] additional XRD peaks indicate that bioorganic molecules encapsulated the nanoparticles, Functional groups such as alkynes, alkenes, aldehydes, and phenolic chemicals that aid in stabilizing and reducing nanoparticles were discovered via FTIR analysis. By acting as organic stabilizers, these biomolecules kept the nanoparticles from clumping together and extending their lifespan (21, 10).

The produced AgNPs showed dose-dependent efficacy against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, according to antibacterial activity tests. Because of their cell walls' weaker peptidoglycan coating, Gram-negative bacteria appeared to be slightly more susceptible. According to earlier research, the synthetic nanoparticles' crystalline nature was demonstrated by XRD analysis, which showed diffraction peaks that matched the face-centered cubic (FCC) structure of metallic silver [18, 19]. Studies of AgNPs made with other plant extracts have shown similar crystallographic patterns [18, 19]. According to [9, 20], additional XRD peaks indicate that bioorganic molecules encapsulated the nanoparticles, improving stability.

CONCLUSIONS

This study demonstrates the successful green synthesis of silver nanoparticles (AgNPs) utilizing leaf extract from *Duranta erecta*, offering a sustainable and environmentally friendly approach to nanoparticle production. The bioactive substances in the extract act as stabilizing and reducing agents, reducing the need for hazardous chemicals. AgNP production was validated by the color shift from pale yellow to dark brown, attributed to surface plasmon resonance (SPR).

The synthesis and structural characteristics of AgNPs were confirmed by FTIR, XRD, and UV-visible spectrophotometry. A distinctive absorbance peak at 432 nm indicated spherical nanoparticles. XRD validated metallic silver's crystalline face-centered cubic structure, while FTIR confirmed functional groups responsible for stabilization.

The synthesized AgNPs demonstrated potent, dose-dependent antibacterial activity against Gram-positive and Gram-negative bacteria. Their antimicrobial properties are attributed to bacterial membrane disruption, reactive oxygen species (ROS) generation, and interference with cellular processes. These findings align with previous studies, reinforcing plant-mediated AgNPs as alternative antimicrobials.

This work highlights the benefits of plant-mediated nanoparticle manufacturing. It provides a cost-effective, eco-friendly platform for nanoparticle production with potential applications in medicine, including drug delivery, wound healing, and antibacterial agents. Future studies could explore mechanisms, cytotoxicity, and broader applications in healthcare and environmental management.

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CONFLICT OF INTEREST

The authors claim no conflicts of interest because none financial support was received from any government, non-government agency or organization to conduct this research work.

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