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# Green Synthesis Of Silver Nanoparticles And Evaluation Of Their Antibacterial Activity Against Selected Pathogens

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**ABSTRACT**

Green synthesis of silver nanoparticles (AgNPs) using plant extracts has emerged as an eco-friendly alternative to traditional methods , offering benefits like safety, sustainability, and enhanced bioactivity . This study focuses on the synthesis of AgNPs using leaf extracts from *Adathoda vasica*, *Plectranthus amboinicus*, *Pimenta dioica* and evaluates their antibacterial properties against selected pathogens. The plant-mediated synthesis relies on phytochemicals—such as flavonoids, terpenoids, and phenolic compounds—which act as both reducing and stabilizing agents, facilitating the conversion of silver ions ($Ag^{+}$) to elemental silver ($Ag^{0}$) and providing stability to the nanoparticles. The formation of AgNPs confirmed by a characteristic color change and spectrophotometric analysis, showing absorption peaks between 350–500 nm, indicative of surface plasmon resonance. The synthesized nanoparticles were purified and tested for antibacterial efficacy using the agar well diffusion method against pathogens, including *Escherichia coli*, *Salmonella spp.*, *Pseudomonas spp.*, and *Staphylococcus spp.* Results showed that AgNPs exhibit broad-spectrum antibacterial activity, especially AgNPs synthesized from *Adathoda vasica*. These findings reveal the potential of green-synthesized AgNPs in medical and environmental applications as an alternative to conventional antibiotics, especially in combating multidrug-resistant bacteria.

***Keywords:*** *Green synthesis, Silver nanoparticles , Agar well diffusion*

## 1. INTRODUCTION

In recent years, eco-friendly approaches for synthesizing nanomaterials have gained significant attention, especially in green nanotechnology. Silver nanoparticles (AgNPs) have emerged as promising options for diverse biomedical applications due to their potent antimicrobial properties (Ahmed *et al*., 2016). Traditionally, AgNPs have been synthesized through chemical and physical methods that often involve hazardous substances, high energy consumption, and toxic byproducts. These conventional approaches raise environmental and health concerns, necessitating the development of safer and more sustainable synthesis methods (Franci *et al*., 2015).

Green synthesis techniques have become popular as an alternate strategy, using natural resources such as enzymes, microorganisms, and plant extracts as stabilising and reducing agents in the synthesis of nanoparticles. (Bhatt & Negi, 2012). This approach not only minimizes the use of toxic chemicals but also exploits bioactive compounds naturally found in plants, which can enhance the functionality of the nanoparticles. The potential of plant-mediated synthesis lies in the phytochemicals present in various plant species, such as flavonoids, terpenoids, alkaloids, and phenolic compounds, which play a dual role as reducing and capping agents in nanoparticle synthesis (Rai *et al*., 2015).

A major advantage of using plant extracts for synthesizing AgNPs is the simplicity and cost-effectiveness of the method, which makes it feasible for large-scale production. In addition, the biocompatibility of plant-synthesized nanoparticles reduces the risk of toxicity, enhancing their suitability for various medical and environmental applications (Subbaraj *et al*.,2023). Recent studies have explored a variety of plants with medicinal properties as potential sources for synthesizing AgNPs. The leaves of *Adathoda vasica* (Malabar nut), *Plectranthus amboinicus* (Panikoorka), and *Pimenta dioica* (Allspice) have been used in the synthesis of silver nanoparticles, taking advantage of their phytochemical composition to enhance the antimicrobial efficacy of the nanoparticles produced (Roy *et al*., 2019).

The process of synthesizing AgNPs using plant extracts typically involves mixing the extract with a silver nitrate solution. Upon reaction, a color change indicates the reduction of silver ions ($Ag^{+}$) to elemental silver ($Ag^{0}$), marking the formation of nanoparticles. This color change occurs due to surface plasmon resonance, a phenomenon wherein free electrons in the nanoparticles oscillate in response to light (Gong *et al*., 2014). The size, shape, and stability of these nanoparticles depend on various factors, including the concentration of the silver precursor, the pH of the reaction medium, temperature, and incubation time.

Spectrophotometric analysis, commonly used to characterize synthesized AgNPs, shows characteristic absorbance peaks in the range of 350–500 nm, confirming the presence of silver nanoparticles (Medda *et al*., 2015). These spectral characteristics are essential for determining the efficacy of the green synthesis process. Techniques such as centrifugation and sonication are often employed to purify and stabilize the nanoparticles, ensuring they are free from impurities and uniformly dispersed (Rai *et al*., 2018).

The antibacterial properties of AgNPs have been widely documented, with studies showing significant inhibitory effects against various pathogens, including both Gram-positive and Gram-negative bacteria (Gurunathan *et al*., 2014). The mechanism of antibacterial activity is attributed to the ability of AgNPs to interact with bacterial cell walls, disrupt membrane integrity, induce oxidative stress through reactive oxygen species (ROS) generation, and interfere with essential cellular processes, ultimately leading to cell death (Yilmaz *et al*., 2019). These nanoparticles exhibit broad spectrum antibacterial activity, which makes them particularly valuable in combating multidrug-resistant bacteria—a growing concern in public health (Rai *et al*., 2018).

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

Fresh leaves of three medicinal plants—*Adathoda vasica* (Malabar nut), *Plectranthus amboinicus* (Panikoorka), and *Pimenta dioica* (Allspice)—were collected to synthesize silver nanoparticles. The leaves were thoroughly washed, dried, and finely ground using a mortar and pestle to obtain a consistent paste.

### 2.2 Preparation of Leaf Extracts

To prepare the plant extracts, 1 gram of the finely ground leaf material from each plant (*A. vasica*, *P. amboinicus*, and *P. dioica*) was weighed and added to 50 mL of distilled water. These mixtures were agitated using a rotary shaker at room temperature to maximize the extraction of bioactive compounds. Following extraction, the mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were stored at 4°C for further use.

### 2.3 Preparation of Silver Nitrate Solution

A 10 mM solution of silver nitrate ($AgNO_3$) was prepared by dissolving 0.17 grams of silver nitrate crystals in 100 mL of deionized water. The solution was stirred until all crystals were completely dissolved, and then stored in a dark environment to prevent photodegradation.

### 2.4 Synthesis of Silver Nanoparticles

To synthesize silver nanoparticles, 500 µL of each plant extract was added to 10 mL of the prepared 10 mM silver nitrate solution. The mixtures were then incubated in a water bath at 60°C for 24 hours. The formation of silver nanoparticles was indicated by a color change in the solution, signifying the reduction of silver ions ($Ag^+$) to metallic silver ($Ag^0$).

### 2.5 Spectrophotometric Analysis of Silver Nanoparticles

The synthesized silver nanoparticles were analyzed using a UV-visible spectrophotometer (Shimadzu UV-1800) to confirm nanoparticle formation. Deionized water was used as a blank, and the absorbance spectrum was recorded in the range of 350–500 nm to detect the characteristic surface plasmon resonance peak of silver nanoparticles.

### 2.6 Separation and Purification of Silver Nanoparticles

The silver nanoparticles were purified by centrifugation at 15,000 rpm for 15 minutes. The resulting supernatant was discarded, and the nanoparticle pellet was resuspended in deionized water. This washing step was repeated three times to remove any unbound substances. Finally, the purified nanoparticles were resuspended in 1 mL of deionized water and sonicated to ensure dispersion.

### 2.7 Antibacterial Activity Testing: Well Diffusion Method

The antibacterial activity of the synthesized silver nanoparticles tested using the well diffusion method on Mueller-Hinton agar (MHA). Selected bacterial (*E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella spp.*) lawn cultures were prepared by evenly swabbing the agar surface with bacterial suspensions. Wells of 4 mm diameter were made using a sterile borer, and 50 µL of the silver nanoparticle suspension was added to each well. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured in millimeters.

## 3. RESULTS AND DISCUSSION

In this study, leaf extracts of *Plectranthus amboinicus, Adathoda vasica,* and *Pimenta dioica* were used in the green synthesis of silver nanoparticles (AgNPs). The formation of nanoparticle was indicated by a color change and confirmed through spectrophotometric analysis, which is similar to the findings of recent studies that highlight the role of plant phytochemicals as reducing agents in nanoparticle synthesis (Ahmed *et al*., 2016; Roy *et al*., 2019). The choice of these specific plant species is particularly relevant, as they contain various bioactive compounds such as alkaloids, flavonoids, and phenolic compounds, which aid in the reduction and stabilization of AgNPs, thus enhancing their antimicrobial activity (Rai *et al*., 2015; Prabhu *et al*.2012).

Spectrophotometric data from this study, showing characteristic peaks of AgNPs in the 350-500 nm range, correspond with previous studies confirming the successful synthesis of silver nanoparticles via green methods (Franci *et al*., 2015). These findings are consistent with the surface plasmon resonance properties of AgNPs synthesized using other plant extracts, where peak wavelengths often fall within a similar range. Previous studies using *Plectranthus amboinicus* have also observed efficient synthesis and stability of AgNPs, supporting this study's observation of effective nanoparticle formation under controlled conditions (Subbaraj *et al*., 2023).

The antibacterial assessment of the synthesized AgNPs revealed significant inhibitory effects against *Staphylococcus* spp. *Salmonella* spp., *Pseudomonas* spp., demonstrating the broad-spectrum antibacterial efficacy of these nanoparticles. (Table 1). Earlier studies reported that AgNPs can disrupt bacterial cell walls, create oxidative stress through reactive oxygen species (ROS) generation, and inhibit essential cellular processes (Gurunathan *et al*., 2014; Yilmaz *et al*., 2019). AgNPs synthesized using *Plectranthus amboinicus* showed potent antibacterial effects, which may be due to the high content of polyphenols in the plant, compounds known to enhance ROS production and thereby strengthen the bactericidal activity of AgNPs (Bhatt *et al*. 2012).

| Organism | Extract | | |
|---|---|---|---|
| | *Pimenta diocia* | *Plectranthus ambonicus* | *Adathoda vasica* |
| *Staphylococcus sp*. | 15 mm | 15mm | 17mm |
| *E. coli* | nil | nil | nil |
| *Pseudomonas spp.* | nil | 15 mm | nil |
| *Salmonella sp.* | nil | 9 mm | 11 mm |

**Table :1 Antimicrobial activity of nanoparticles from plant extracts**

The agar well diffusion method employed in this study showed clear zones of inhibition, indicating that the synthesized nanoparticles exhibited effective antimicrobial activity (Figure 1,2). The results showed similarities with findings from previous studies that have successfully used the same method to assess the antimicrobial properties of green-synthesized AgNPs against Gram-positive and Gram-negative bacteria (Medda *et al*., 2015). The efficacy of AgNPs against these pathogens underscores their potential as a powerful antimicrobial agent, particularly given the rising concern over multidrug resistant bacteria (Gong *et al*., 2014; Rai *et al*., 2018).

**Figure 1: Antibacterial activity of AgNps formed by *Adathoda vasica (A)* (17mm), *Pimenta dioica (S)* (15mm), *Plectranthus amboinicus (P)* (15mm) against *Staphylococcus spp.***

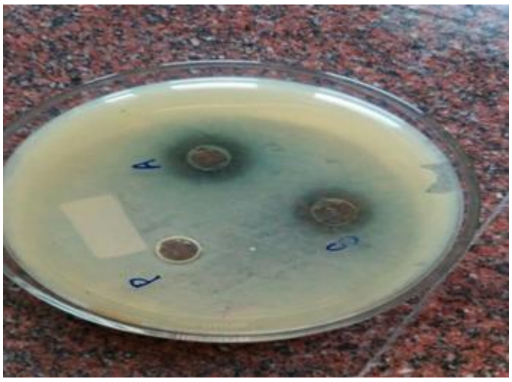


**Figure 2: Antibacterial activity of AgNps formed by *Adathoda vasica(A)* (11mm) and Plectranthus *amboinicus (S)* (9mm) against *Salmonella sp***

## 4. CONCLUSION

This study supports the utility of green-synthesized silver nanoparticles as an alternative to conventional antibiotics. By considering the effectiveness, low toxicity, and environmental sustainability, AgNPs hold promise for applications in biomedical fields, including wound dressings, coatings for medical devices, and food preservation. Future research could explore the potential of these AgNPs in synergy with traditional antibiotics to counteract bacterial resistance. Optimizing the synthesis parameters and assessing the long-term stability of AgNPs could further enhance their effectiveness for practical applications.

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