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Journal of Multidisciplinary Applied Natural Science Vol. 6 No. 1 (2026) https://doi.org/10.47352/jmans.2774-3047.333
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Research Article

Antibacterial Activities of Silver Nanoparticles Prepared using Extract of *Drymoglossum piloselloides*

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Received : December 29, 2024	Revised : August 13, 2025	Accepted : November 7, 2025	Online : December 26, 2025

Abstract
This study aims to synthesize and evaluate the antibacterial activity of silver nanoparticles (AgNPs) produced from the ethanolic extract of *Drymoglossum piloselloides*, highlighting its unique phytochemical composition rich in flavonoids, phenolics, and steroids that are expected to enhance nanoparticle stability and antibacterial performance. The methodology employed includes the synthesis of AgNPs through the reduction of leaf extract, followed by comprehensive characterization using UV-Vis spectroscopy, XRD, FTIR, TEM, and particle size analysis. The results indicate that AgNPs exhibit a spherical morphology with varying average sizes, with AgNPs 30 demonstrating the smallest size of 20.33 nm. The antibacterial activity of AgNPs was tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*, producing inhibition zones up to 9.26 ± 1.02 mm against *S. aureus*. The proposed antibacterial mechanism involves the generation of reactive oxygen species and disruption of bacterial membranes, leading to oxidative stress and cell lysis. In conclusion, AgNPs synthesized from *D. piloselloides* show considerable potential as effective antibacterial agents, thereby opening opportunities for further applications in nanomedicine and infection control.

Keywords: antibacterial activity, biosynthesis, *Drymoglossum piloselloides*, silver nanoparticles

1. INTRODUCTION

Silver nanoparticles (AgNPs) are materials with unique properties, particularly in atomic-scale interactions, offering significant potential in various fields such as medicine, catalysis, sensors, electronics, and cosmetics [1]-[5]. Notably, AgNPs exhibit antibacterial activity, catalytic ability, high sensitivity, and good conductivity, making them effective in combating bacterial infections and addressing antibiotic resistance. Thus, AgNPs represent innovative materials that could revolutionize multiple sectors and provide solutions to global challenges. Recent trends in nanoparticle synthesis emphasize the use of natural materials, which present several advantages, including biodegradability and lower production costs compared to synthetic chemicals [2][6][7]. The complex chemical composition of natural materials facilitates the production of nanoparticles with unique and functional characteristics. The synergy between bioactive compounds in these materials contributes to the formation of stable, uniform, and well-distributed nanoparticles [8]-[10]. Moreover, synthesizing nanomaterials through green chemistry approaches is environmentally friendly, contrasting with traditional biological methods that often involve complex and costly cell cultures. Utilizing plants for biosynthesis offers abundant and accessible raw materials, with their secondary metabolites acting as natural reducing and stabilizing agents.

One compelling example is the biosynthesis of nanoparticles using *Drymoglossum piloselloides* (locally known as dragon scales), a sustainable approach with potential applications in antibacterial fields [1][11]. This unique epiphytic fern is rich in polyphenolic compounds, essential oils, steroids, flavonoids, sugars, and tannins, providing sustainable alternatives for medicinal materials and chemical synthesis [12]. Research has shown that various plant species, including *D. piloselloides*, can synthesize AgNPs with varying sizes and promising antimicrobial activity against both Gram-negative and Gram-positive microorganisms. While many plant species have been used for synthesis of AgNPs, *D. piloselloides* have rarely been explored despite its rich content of phenolics, flavonoids, and steroids that could enhance nanoparticle stability

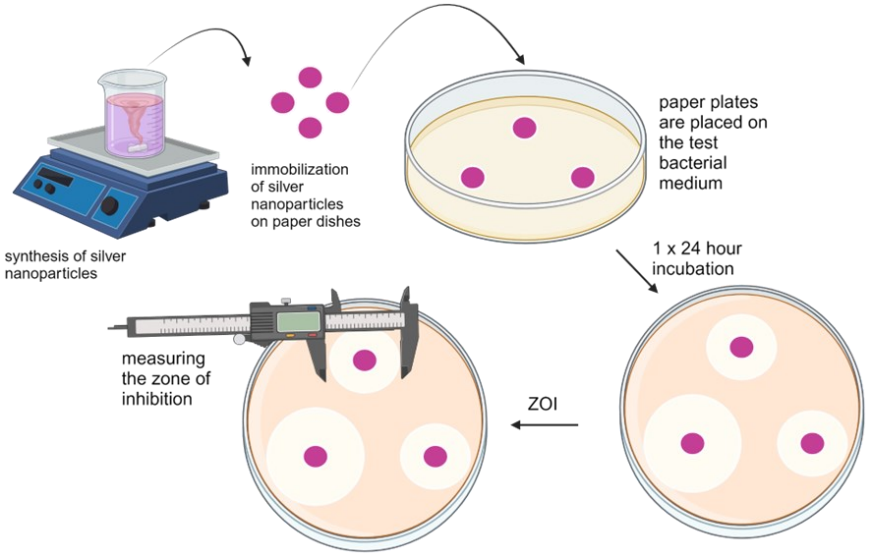

Figure 1. Schematic method from antibacterial activity assay of silver nanoparticles.

and antibacterial activity. This knowledge gap highlights the novelty of the present study, which aims to investigate the potential of *D. piloselloides* in producing stable and bioactive AgNPs. The plant samples were collected from peatland areas in Palangkaraya City and Pulang Pisau District, Central Kalimantan, Indonesia, and their identity was confirmed morphologically based on descriptions from published botanical references [11]-[13]. This study focuses on *D. piloselloides* due to its potential in synthesizing environmentally friendly nanoparticles. The extraction process using ethanol effectively isolates active phytochemical compounds from the plant, which can form polydisperse nanoparticles with significant nanocatalytic properties. By employing *D. piloselloides* extract, this research aims to explore the synthesis of AgNPs that not only exhibit high antimicrobial activity but also align with the principles of green chemistry, minimizing hazardous chemicals and toxic waste. This study seeks to provide innovative and ecological solutions for antimicrobial applications, promoting sustainability and energy efficiency.

2. MATERIALS AND METHODS

2.1. Materials

The leaves of the epiphytic plant *D. piloselloides* used in this study were collected from peatland areas in Palangkaraya City and Pulang Pisau District, Central Kalimantan, Indonesia. Laboratory chemicals used for the synthesis of AgNPs included silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), ethanol (C_2H_5OH), Nutrient Agar (NA), and Nutrient Broth (NB). All chemicals were obtained from Merck Sigma-Aldrich Reagent Pte, Singapore, and were used without additional purification steps, ensuring their suitability for the synthesis process.

2.2. Methods

2.2.1. Preparation of Ethanolic Extract of D. piloselloides

The ethanolic extract of *D. piloselloides* was prepared by dissolving 1 g of dried leaves in 40 mL of ethanol at 60 °C for 40 min. This specific temperature was selected to optimize the solubility of the bioactive compounds while minimizing the risk of thermal degradation. Following the extraction period, the solution was allowed to cool naturally to room temperature. The mixture was then filtered through Whatman filter paper, and the resulting filtrate was collected. This filtrate, enriched with phytochemicals, served as the foundational stock for the subsequent synthesis of AgNPs. To ensure its stability, the filtrate was stored in a cool, dark environment until further use.

2.2.2. Phytochemical Analysis

The phytochemical analysis of the ethanolic extract of *D. piloselloides* was conducted using a combination of standard qualitative tests and High-

Performance Liquid Chromatography (HPLC). Firstly, the Shinoda test for flavonoids involved adding a few drops of concentrated HCl to a small amount of the extract, followed by Mg turnings; the appearance of a pink or red color indicated the presence of flavonoids. Subsequently, for steroids, the Liebermann-Burchard test was conducted by adding a few drops of acetic anhydride to the extract, followed by concentrated H_2SO_4, which resulted in a blue or green color at the interface, thereby confirming the presence of steroids. In addition, the Dragendorff's test for alkaloids was performed by adding a few drops of Dragendorff's reagent to the extract, where the formation of an orange or reddish precipitate confirmed the presence of alkaloids. Furthermore, the hydrochloric acid test for saponins involved mixing a small amount of the extract with HCl, and the formation of stable froth indicated the presence of saponins. Lastly, the $FeCl_3$ test for phenolics was conducted by adding a few drops of $FeCl_3$ solution to the extract, and the development of a blue, green, or purple color indicated the presence of phenolic compounds. HPLC characterization of the ethanolic extract was performed using a mobile phase consisting of a mixture of acetonitrile (MeCN) and 1% phosphoric acid (36:64 v/v).

2.2.3. Synthesis of AgNPs

AgNPs were synthesized utilizing a synthesis approach. In this procedure, 1 mL of ethanolic extract of *D. piloselloides* was added to 18 mL of $AgNO_3$ solution and subjected to sonication for 5 min. Following this, varying volumes (10, 20, and 30 µL) of 0.4 M $NaBH_4$ solution were introduced, and the mixture was sonicated for an additional 10 min to facilitate the complete formation of AgNPs. The resulting AgNPs were characterized using a Shimadzu UV-1700 spectrophotometer (Shimadzu Co. Ltd., Kyoto, Japan) to ascertain the peak of surface plasmon resonance (SPR). Subsequently, the AgNPs exhibiting the most pronounced SPR were further analyzed using JEOL JEM-1400 transmission electron microscopy (TEM) operating at 120 kV to evaluate their morphology and size. Additionally, a particle size analyzer (PSA)

Table 1. Phytochemicals analysis of *D. piloselloides* extracts.

Chemical Constituents	Testing Methods	Results
Flavonoids	Shinoda test	Present
Steroids	Lieberman Burchard	Present
Alkaloids	Dragendroff's test	Absent
Saponins	Hydrogen chloride	Present
Phenolics	Ferric chloride test	Present

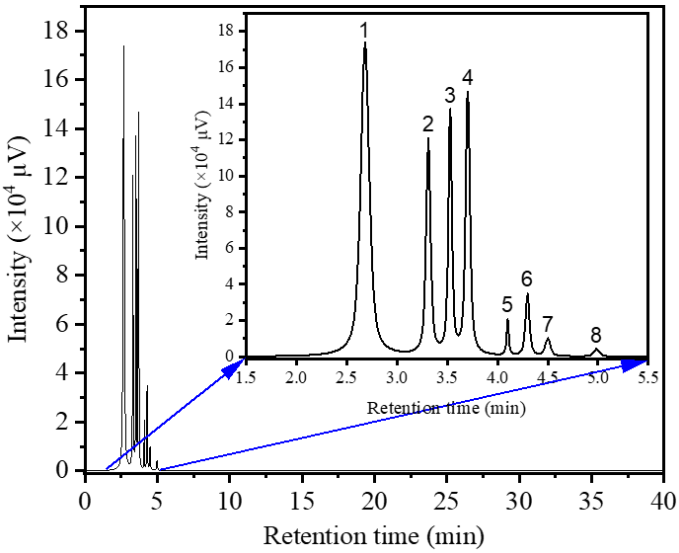

Figure 2. HPLC chromatogram of *Drymoglossum piloselloides* extract.

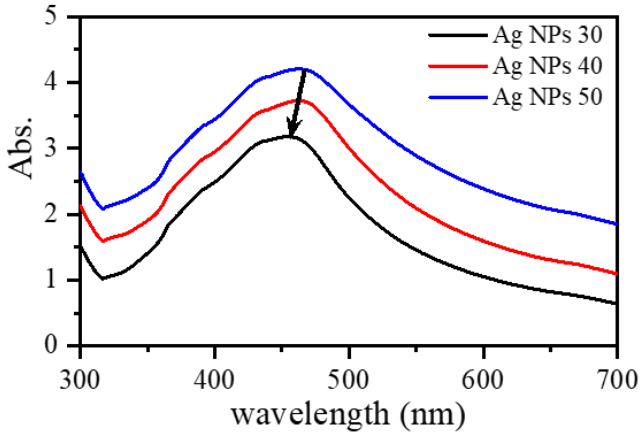

Figure 3. UV-Vis spectra of AgNPs synthesized from *Drymoglossum piloselloides* extract.

MALVERN was employed to determine the size distribution of the synthesized nanoparticles, providing valuable insights into their uniformity and stability. Characterization of the synthesized nanoparticles was also conducted using Fourier transform infrared spectroscopy (FTIR) BRUKER to identify the functional groups present within the nanoparticles.

2.2.4. Antibacterial Activity

The antibacterial activity of AgNPs was evaluated against both Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram -negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), as well as the fungus *Candida albicans*, all of which were obtained from the microbiology laboratory of Airlangga University. For the antibacterial testing, bacterial cultures were cultivated on NA for 24 h. A single bacterial colony was then selected and transferred to NB using a sterilized loop. The culture was subsequently shaken at 100 rpm at 37 ° C overnight to achieve a normalized population. The optical density of the bacterial suspension was adjusted to the standard 0.5 for the MacFarland scale by adding sterilized NB, ensuring consistency for the antibacterial activity tests.

The antibacterial activity of AgNPs was assessed using the Kirby-Bauer disc diffusion method. A 1 mL aliquot of the test bacteria was inoculated onto the surface of NA media. Sterile paper discs, previously soaked in AgNPs, were then placed on the inoculated agar surface using sterile forceps. The antibacterial assay used 5 µg ciprofloxacin disks (Oxoid) as positive controls. AgNPs were tested at three concentrations (AgNPs 30, 40, and 50), prepared through serial dilutions to ensure reproducibility. The plates were incubated for 24 h to allow for the observation of the zones of inhibition. The effectiveness of the AgNPs in inhibiting the growth of the test bacteria was determined by measuring the diameter of the zones of inhibition surrounding the discs (see Figure 1).

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Screening of D. piloselloides Extracts

The phytochemical analysis of the ethanolic extract of *D. piloselloides* was conducted using standard qualitative tests (Table 1), which successfully identified the presence of several bioactive compounds, including flavonoids, steroids, saponins, and phenolics [13][14]. These compounds are recognized for their diverse therapeutic properties, such as antioxidant, anti-inflammatory, and antimicrobial activities, which may contribute to the potential health benefits associated with this plant. The Shinoda test confirmed the presence of flavonoids, while the Liebermann-Burchard test indicated the existence of steroids. Additionally, the hydrochloric acid test revealed the presence of saponins, and the $FeCl_3$ test confirmed the presence of phenolic compounds. However, the analysis did not detect alkaloids, suggesting that *D. piloselloides* may exert its biological activities primarily through the other identified phytochemicals. The absence of alkaloids is noteworthy, as these compounds are often linked to various pharmacological effects.

The results of the HPLC analysis of the ethanolic extract of *Drymoglossum piloselloides* are presented in Figure 2. The HPLC analysis revealed the presence of polyphenolic compounds, which were detected based on their retention times and corresponding peak areas. According to the principle of "like dissolves like," polar solvents effectively dissolve polar compounds, while non-polar solvents dissolve non-polar compounds. The HPLC data indicated a retention time of 2.680 min with an area percentage of 44.13%, suggesting the presence of water as the primary polar solvent. Subsequent retention times at 3.310, 3.527, and 3.703 min showed peaks with area percentages of 15.447%, 14.208%, and 18.686%, respectively, which are likely attributed to various polyphenolic compounds, consistent with the characteristics of polar solvents dissolving polar substances. Additionally, other peaks observed in the chromatogram are presumed to originate from secondary metabolite compounds with non-polar properties. These findings align with the earlier phytochemical analysis, which identified the presence of phenolics among other bioactive compounds in the extract.

The phytochemical analysis of *D. piloselloides* revealed the presence of several bioactive compounds, including flavonoids, steroids, saponins, and phenolics, which are known for their reducing and stabilizing properties. These compounds play a crucial role in the synthesis of AgNPs [1][15][16]. The presence of flavonoids and phenolics, in particular, is significant, as they can effectively reduce Ag^{+} ions to form AgNPs while simultaneously stabilizing the nanoparticles to prevent agglomeration. The results from the HPLC analysis further support this potential, as the identified polyphenolic compounds are likely to contribute to the reduction process during nanoparticle synthesis.

3.2. *Characterization of AgNPs Synthesized from D. piloselloides*

The synthesis of AgNPs from the ethanolic extract of *D. piloselloides* was marked by a distinct color change from colorless to a vibrant red-violet hue, indicating the successful formation of nanoparticles [1][17][18]. The UV-Vis spectrum of the synthesized AgNPs exhibited a strong and broad peak at 453 nm, with the highest absorbance value of 3.222 observed for the AgNPs 40 sample an optical feature characteristic of silver nanoparticles and absent in the spectrum of the *D. piloselloides* extract. The observed red shift from 408 to 453 nm, along with the broad SPR peak, indicates the formation of anisotropic structures and an increase in particle size due to surface plasmon coupling among aggregated nanoparticles (Figure 3). This phenomenon suggests that the bioactive compounds present in the *D. piloselloides* extract such as polyphenols, flavonoids, and proteins function not only as reducing agents but also as natural capping agents, providing surface stabilization, controlling nanoparticle growth, and preventing agglomeration [19]-[21].

To accurately assess the atomic positions within the lattice structure, the crystalline phase and structure of the synthesized AgNPs from the

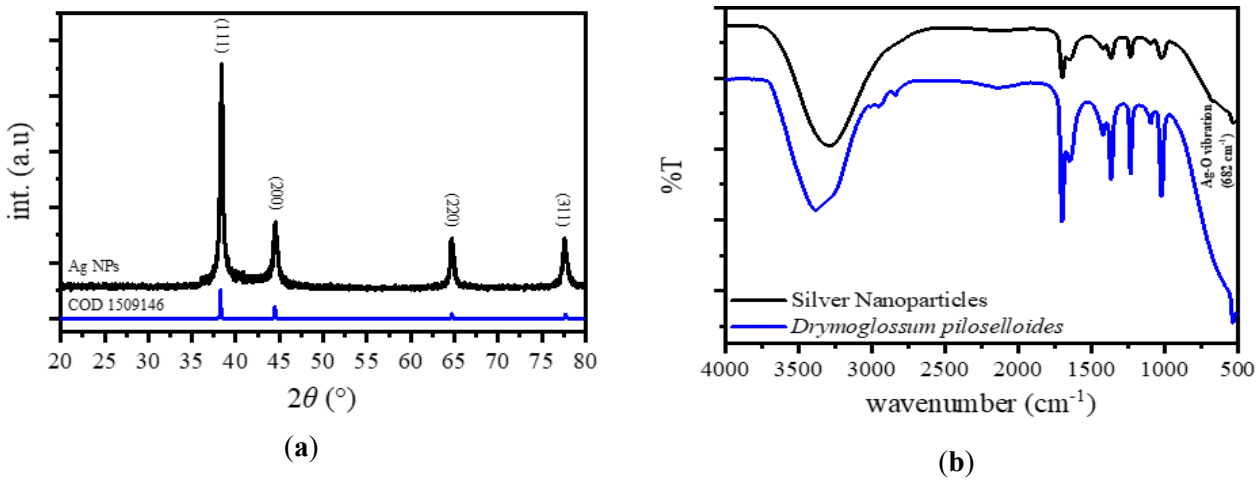

Figure 4. (a) XRD and (b) FTIR profiles of the synthesized AgNPs.

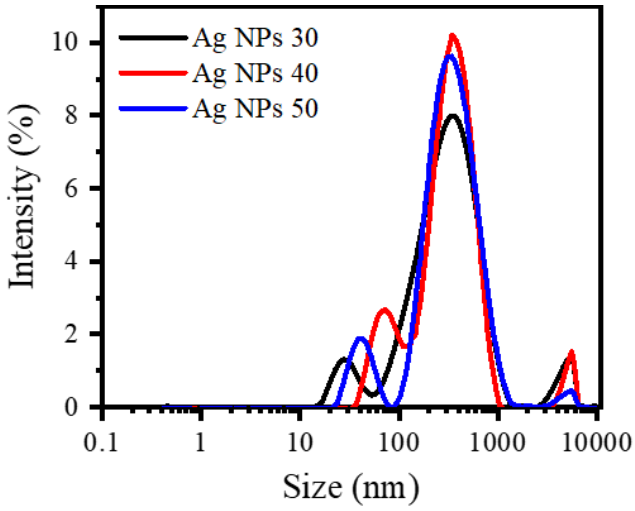

Figure 5. Particles size distribution of AgNPs.

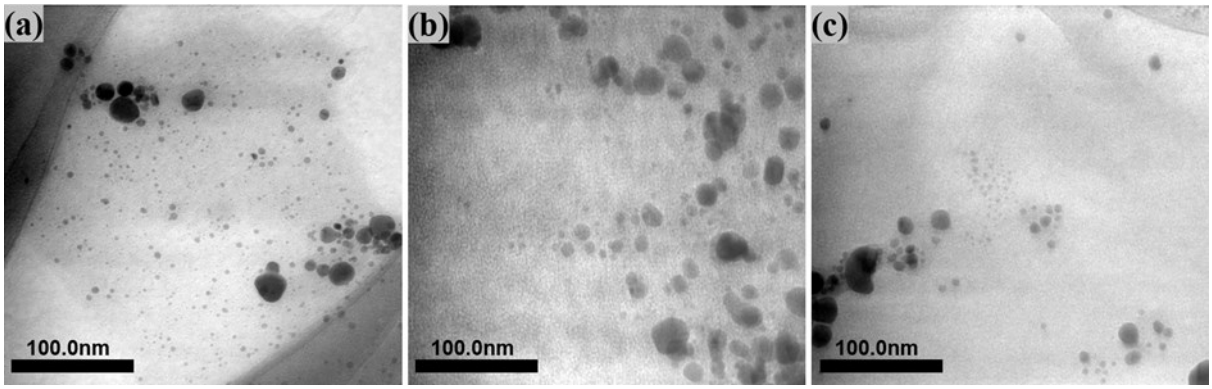

Figure 6. TEM of (a) AgNPs 30, (b) AgNPs 40, and (c) AgNPs 50.

ethanolic extract of *D. piloselloides* were investigated using XRD and FTIR examinations. The XRD patterns of the AgNPs are presented in Figure 4(a). The XRD results were compared with the Crystallography Open Database (COD) number 1509146, confirming the successful formation of zero-valent silver. This finding indicates that the synthesized AgNPs possess a crystalline structure characteristic of metallic silver. To further investigate the interaction between the *D. piloselloides* extract and silver, FTIR analysis was performed. The FTIR results, shown in Figure 4(b), reveal a low absorption peak at 682 cm^{-1}, which aligns with findings reported in several previous studies. This peak suggests the presence of functional groups in the extract that may play a role in stabilizing the nanoparticles and facilitating their synthesis.

The characterization of AgNPs synthesized from the ethanolic extract of *D. piloselloides* was conducted using TEM and PSA. The PSA results indicated that AgNPs at concentrations of 30, 40, and 50 exhibited predominantly spherical morphologies, suggesting a uniform particle shape across the samples, as shown in Figure 5. Notably, AgNPs 30 demonstrated the smallest average particle size, measuring 20.33 nm. The most significant peak for this sample was observed at 28.36 nm, with a full width at half maximum (FWHM) of 19.23, indicating a relatively narrow size distribution. In contrast, AgNPs 40 exhibited a minimum particle size of 42.35 nm, with the first peak also recorded at 42.35 nm and a FWHM of 73.82, reflecting a broader size distribution compared to AgNPs 30. Furthermore, AgNPs 50 displayed a minimum size of 26.77 nm, with the first peak at 39.82 nm and a FWHM of 32.47. Importantly, all AgNPs demonstrated a secondary peak at larger diameters, specifically 322.43 nm for AgNPs 30, 356.80 nm for AgNPs 40, and 335.18 nm for AgNPs 50. TEM micrographs of synthesized AgNPs are depicted in Figure 6.

3.3. Antibacterial Activity of AgNPs

The antibacterial activity of AgNPs synthesized from the ethanolic extract of *D. piloselloides* was evaluated against four types of bacteria, namely *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, while using ciprofloxacin (CIP) as a positive control. The effectiveness of each sample in inhibiting bacterial growth was assessed by measuring the inhibition zones. The results indicated that AgNPs exhibited significant antibacterial activity, with the mechanism of action illustrated in Figure 7, which proposes several chemical and physical pathways involved in the antibacterial effects of AgNPs.

Specifically, AgNPs generate reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which lead to oxidative stress in bacterial cells [22]-[24]. This oxidative stress, in turn, damages cellular components, including proteins, lipids, and nucleic acids, ultimately resulting in cell lysis. Furthermore, the interaction of AgNPs with bacterial cell surfaces enhances membrane permeability, thereby causing the leakage of ions and molecules. Moreover, the synthesized AgNPs, with an average size of approximately 12 nm, possess a high surface area that facilitates direct contact with bacteria, allowing for easier penetration into cellular structures and enhancing their destructive effects [15][25][26]. The bioactive compounds present in the *D. piloselloides* extract, such as phenolics and flavonoids, act as reducing and stabilizing agents, which potentially enhance the antibacterial effects by promoting ROS formation and disrupting bacterial cells.

Overall, the combination of ROS production and direct interaction with DNA and proteins demonstrates the ability of AgNPs to penetrate cell membranes and interiors, making them effective antibacterial agents, particularly against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*. While AgNPs show promising antibacterial activity, it is noteworthy that ciprofloxacin remains more

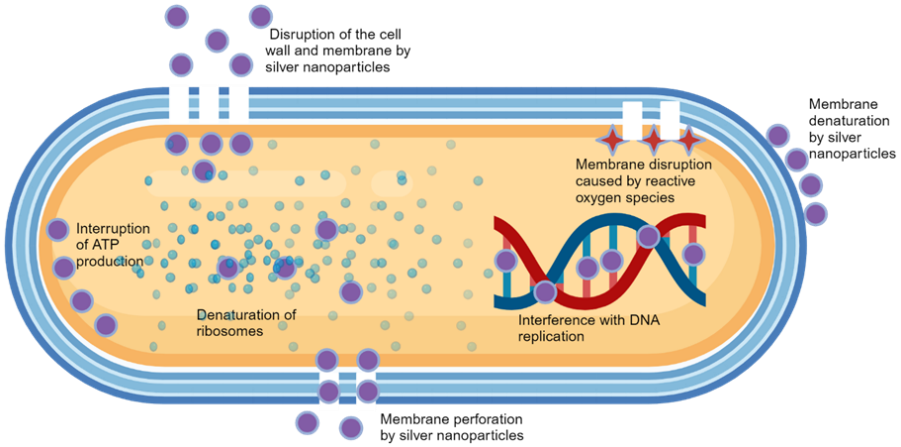

Figure 7. Antibacterial mechanism activity of AgNPs.

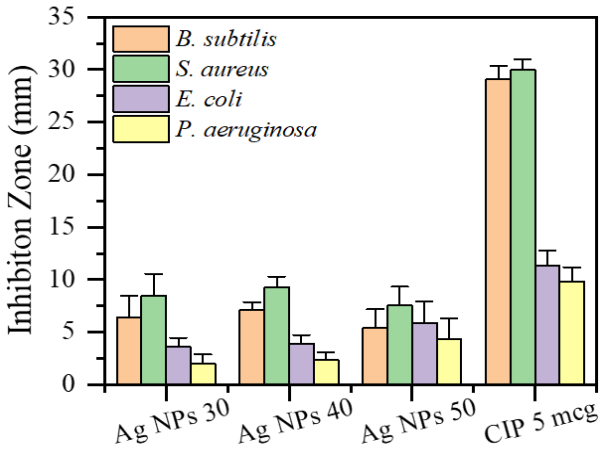

Figure 8. Antibacterial activity of AgNPs.

effective due to its specific molecular mechanisms against bacteria, as it is a well-established antibiotic with broad-spectrum action. In addition, the antibacterial activity of AgNPs increased with higher concentrations, as shown in Figure 8. The synthesized AgNPs from *D. piloselloides* effectively inhibited the growth of Gram-positive bacteria compared to Gram-negative bacteria. The inhibition zones for AgNPs at different concentrations indicated that AgNPs 30 produced inhibition zones of 6.36 ± 2.09 mm for *B. subtilis*, 8.48 ± 2.09 mm for *S. aureus*, 3.58 ± 0.87 mm for *E. coli*, and 2.01 ± 0.87 mm for *P. aeruginosa*. Meanwhile, AgNPs 40 gave the inhibition zones were 7.14 ± 0.72 mm for *B. subtilis*, 9.26 ± 1.02 mm for *S. aureus*, 3.91 ± 0.82 mm for *E. coli*, and 2.34 ± 0.72 mm for *P. aeruginosa*. These results indicate that AgNPs synthesized from *D. piloselloides* have significant potential as antibacterial agents, particularly against Gram-positive bacteria, due to their structural characteristics and the absence of an outer membrane that protects Gram-negative bacteria. The thick peptidoglycan layer in Gram-positive bacteria makes them more susceptible to direct interactions with AgNPs, leading to cell wall damage and ROS formation, while the additional outer membrane in Gram-negative bacteria hinders AgNPs penetration, thereby reducing their effectiveness.

4. CONCLUSIONS

This study demonstrated the synthesis of AgNPs using the ethanolic extract of *D. piloselloides*, which exhibited a predominantly spherical morphology with variable particle sizes, as confirmed by TEM and PSA analyses. The synthesized AgNPs showed notable antibacterial activity against both Gram-positive and Gram-negative bacteria, with particularly strong efficacy observed against *B. subtilis* and *S. aureus*. The antibacterial mechanism was attributed to the generation of ROS and interactions with bacterial cell membranes, leading to oxidative stress and subsequent cellular damage. The bioactive compounds present in the extract may have further enhanced the antibacterial potential of the nanoparticles. Nevertheless, possible limitations, including nanoparticle aggregation, cytotoxic effects on mammalian cells, and instability under physiological conditions, should be addressed in future studies. A comprehensive evaluation of these factors will be essential to ensure the safe and effective utilization of *D. piloselloides* derived AgNPs in nanomedicine and other fields.

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Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

We extend our heartfelt appreciation to DRTPM Kementrian Pendidikan, Kebudayaan, Riset, dan Teknologi Republik Indonesia for their funding support through the Fundamental Research Grant in 2024 under contract number 0992/UN24.13/AL.04/2024. Their invaluable assistance greatly contributed to the successful completion of this research endeavor.

DECLARATION OF GENERATIVE AI

Not applicable.

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