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Green Synthesis of Silver Nanoparticles Using *Basilicum polystachyon* Leaf Extract and Their Antibacterial Activity Against *Staphylococcus aureus*

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ABSTRACT

AgNPs are nanomaterials with significant potential as antibacterial agents and can be synthesized in an environmentally friendly manner using plant extracts. This study aims to synthesize AgNPs using *B. polystachyon* leaf extract as a bioreductant and evaluate their antibacterial activity against *S. aureus*. The synthesis was performed by mixing *B. polystachyon* leaf extract with a 0.01 M $AgNO_3$ at volume ratios of 1:1, 1:2, 1:3, and 1:4, followed by pH optimization in the range of pH 8–11. The synthesized AgNPs were characterized using UV–Vis spectrophotometry, FTIR spectroscopy, PSA, and XRD. Antibacterial activity was evaluated using the disk diffusion method. The results showed that optimal synthesis conditions were achieved at a volume ratio of 1:4 and a pH of 11. The UV–Vis spectrum of the synthesized AgNPs exhibited a maximum absorption peak at 412 nm. FTIR analysis revealed the presence of O–H, aromatic C=C, and C–O bonds. The average particle size was 58.79 nm (PDI = 0.29). The XRD pattern confirmed the formation of crystalline silver. The AgNPs inhibited the growth of *S. aureus*, with an inhibition zone diameter of 26.51 mm (very strong). Therefore, the synthesized AgNPs have the potential to be developed as antibacterial agents.

Keywords: Antibacterial activity, *B. polystachyon* leaf extract, green synthesis, silver nanoparticles

1. Introduction

Nanotechnology is a rapidly advancing field of science that focuses on the engineering of materials at the nanoscale (1–100 nm), which exhibit distinct physical and chemical properties compared to their bulk counterparts due to the increased surface area-to-volume ratio and quantum effects [1][2][3]. One of the most extensively developed products of nanotechnology is nanoparticles, owing to their high surface area and enhanced reactivity, which enable their potential application across various fields, including biomedicine [4][5].

Silver nanoparticles (AgNPs) are one of the most extensively studied metal nanoparticles due to their broad biological activities, including antibacterial, antifungal, antioxidant, and anticancer properties [6][7]. The antibacterial activity of AgNPs operates through multiple mechanisms, including disruption of the cell membrane, enzyme inactivation, interference with DNA replication, and the generation of reactive oxygen species (ROS) [8]. This multitarget mechanism means that AgNPs have a lower potential to induce resistance compared to conventional antibiotics [9].

AgNPs can be synthesized using physical, chemical, and biological methods. However, physical and chemical methods generally require extreme conditions and hazardous chemicals that have the potential to pollute the environment. [10][11]. Therefore, the green synthesis approach offers a more environmentally friendly alternative by utilizing plant extracts as bioreducers and stabilizers. [12]. Phytochemical compounds, particularly phenolic groups such as flavonoids, alkaloids, and tannins, play a crucial role in reducing Ag^+ ions to Ag^0 and stabilizing the resulting nanoparticles [13][14].

One plant with potential as a bioreductant is *Basilicum polystachyon* (L.) Moench leaf of the Lamiaceae family. *B. polystachyon* is a traditional

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medicinal plant commonly found in various regions of Indonesia. It has been traditionally used to treat fever and infant shock, heal wounds, alleviate joint inflammation, manage diarrhea, and prevent insect bites [15]. Based on phytochemical screening, this plant is known to contain phenolic compounds, flavonoids, and alkaloids with strong antioxidant activity [15][16]. The ethanol extract of *B. polystachyon* leaves has been reported to exhibit very high antioxidant activity, with an IC_{50} value of 7.25 mg/L, indicating a strong electron-donating capacity [15]. These secondary metabolites suggest a significant potential as reducing and stabilizing agents in the synthesis of silver nanoparticles. AgNPs have been successfully synthesized using bioreductants derived from various plant extracts, such as *Erythrina lithosperma* [17], *Mesona palustris* [18], *Cordyline fruticosa* [19], and *Camellia sinensis* [20]. However, the use of *B. polystachyon* extract for the synthesis of AgNPs has not yet been reported, highlighting the novelty of this study in exploring a natural bioreductant.

Evaluation of the antibacterial activity of AgNPs is essential for evaluating their potential applications. In this study, the Gram-positive bacterium *Staphylococcus aureus* was used, which is a pathogen responsible for various infections such as pneumonia, skin infections, and wounds. [21]. This bacterium was selected for its clinical relevance, given the high incidence of infections reported globally and nationally. According to data from the World Health Organization, pneumonia is the leading cause of death among children under five years of age worldwide, with a mortality rate of 740,180 in 2019 [22]. Meanwhile, the Indonesian Ministry of Health reports approximately 800,000 cases of pneumonia in children annually in Indonesia, making it one of the diseases with the highest number of cases among toddlers [23]. Tests of the antibacterial activity of AgNPs synthesized using *B. polystachyon* extract as a bioreductant against *S. aureus* have also not been previously reported.

Based on this background, this study aims to synthesize silver nanoparticles via a green synthesis approach using *B. polystachyon* leaf extract as a bioreductant, to characterize the synthesized nanoparticles, and to evaluate their antibacterial activity against *S. aureus*

2. Methods

2.1. Material

B. polystachyon leaves, $AgNO_3$ (Merck), NaOH (Merck), Mueller-Hinton Agar (Merck), NaCl (Merck), *S. aureus* (ATCC 25923), Whatman filter paper (No. 42), paper discs, ciprofloxacin, and demineralized water (Otsuka).

2.2. Experiment

2.2.1. Preparation of *B. polystachyon* leaf extract

B. polystachyon leaves were collected from Sumbersuko Village, Malang Regency, East Java. A total of 3 kg of fresh leaves were washed under running water and then air-dried at room temperature for 7 days. The dried leaves were ground using a blender to obtain a fine powder suitable for extraction [17].

Extraction was carried out using the infusion method, in which 20 g of *B. polystachyon* leaf powder was extracted with 200 mL of distilled water at 50 °C under continuous stirring using a magnetic stirrer for 60 minutes. The mixture was then filtered using Whatman No. 42 filter paper to obtain the *B. polystachyon* leaf extract [24].

2.2.2. Preparation of $AgNO_3$

A total of 0.17 g of $AgNO_3$ was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water. The solution was then homogenized using a magnetic stirrer to obtain a 0.01 M $AgNO_3$ solution [25].

2.2.3. Green synthesis of AgNPs

AgNPs were synthesized by mixing *B. polystachyon* leaf extract with a 0.01 M $AgNO_3$ solution at volume ratios of 1:1, 1:2, 1:3, and 1:4 to a total volume of 60 mL. The mixture was heated at 50 °C and stirred using a magnetic stirrer at 1500 rpm for 60 minutes. Subsequently, each mixture was subjected to pH optimization in the range of pH 8, 9, 10, and 11 by the addition of 0.1 M NaOH solution. The mixtures were further stirred at 1500 rpm for 90 minutes [26].

The mixtures were then analyzed using a UV–Vis spectrophotometer over a wavelength range of 200–800 nm to determine the optimum conditions, indicated by the highest absorbance value [27]. The AgNPs colloidal obtained under optimum conditions were subsequently centrifuged at 5000 rpm for 30 minutes. The precipitate was separated from the supernatant by decantation and dried using a freeze dryer to obtain AgNPs powder [17].

2.2.4. Characterization of AgNPs

The synthesized silver nanoparticles were characterized using a UV–Vis Spectrophotometer (Shimadzu UV-Vis 1800), an FTIR Spectrophotometer (Nicolet iS10), a Particle Size Analyzer (Biobase BK-802N DLS Nano), and X-ray diffraction (Rigaku MiniFlex 600). The UV–Vis Spectrophotometer was used to determine the maximum absorption wavelength corresponding to the surface plasmon resonance (SPR). The functional groups present in the synthesized AgNPs were identified using FTIR Spectroscopy, while the average particle size and polydispersity index were determined using the Particle Size Analyzer (PSA). The crystalline structure of the AgNPs was characterized using X-ray diffraction (XRD) to confirm the formation of silver nanoparticles [17].

2.2.5. Antibacterial activity test

The antibacterial activity of the synthesized AgNPs was evaluated using the disk diffusion method against the Gram-positive bacterium *S. aureus*. The bacterial suspension was adjusted to the 0.5 McFarland standard, inoculated onto solid Mueller–Hinton Agar (MHA), and incubated for 24 hours [28]. Sterile paper discs impregnated with the AgNPs solution were placed on the surface of the inoculated agar and incubated at 37 °C for 24 hours [29]. After incubation, the diameter of the inhibition zones formed around the discs was measured using a caliper as an indicator of antibacterial activity [30]. The same procedure was also applied to *B. polystachyon* leaf extract, ciprofloxacin as a positive control, and distilled water as a negative control.

3. Results and Discussion

3.1. Results of synthesized AgNPs using UV-Vis Spectrophotometry

In this study, AgNPs were synthesized via a green synthesis approach using *B. polystachyon* leaf extract as a bioreductant and a 0.01 M $AgNO_3$ solution as the precursor. The synthesis was carried out by varying the volume ratio of extract to $AgNO_3$ solution (1:1, 1:2, 1:3, and 1:4). The results of UV–Vis spectrophotometric analysis of the mixtures with different volume ratios of extract to $AgNO_3$ are presented in Table 1 and Figure 1.

Table 1. The UV-Vis maximum wavelength and absorbance of extract-$AgNO_3$ mixtures at variation of volume ratio (composition)

Composition	λ max (nm)
1:1	313
1:2	316
1:3	316
1:4	314

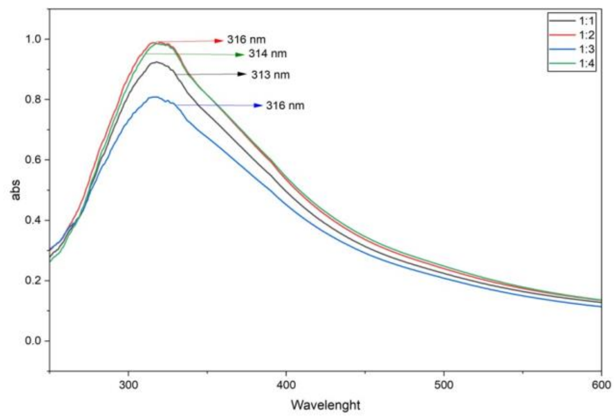

Figure 1. UV-Vis spectra of extract-$AgNO_3$ mixtures at variation of volume ratio

Based on Table 1 and Figure 1, all four mixture compositions exhibited maximum UV–Vis absorption wavelengths below 400 nm. Therefore, AgNP formation had not yet occurred in these compositions, as AgNPs typically show a characteristic surface plasmon resonance (SPR) absorption band in the range of 400–500 nm [31].

To accelerate the formation of AgNPs in all four mixture compositions, pH optimization was performed within the range of pH 8–11. The UV–Vis spectral results are presented in Table 2.

Table 2. The absorbance of synthesized silver nanoparticles

Composition	Absorbance value at pH			
	8	**9**	**10**	**11**
1:1	0.807	0.818	0.669	0.720
1:2	0.579	0.413	0.588	0.795
1:3	0.432	0.703	0.786	1.144
1:4	0.579	0.818	0.921	1.261

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Based on Table 2, the differences in absorbance values observed for each variation indicate that both the composition ratio and pH significantly influence the quantity and stability of the nanoparticles formed. Higher absorbance values correspond to a greater amount of nanoparticles and improved stability [32].

Of all the variations tested, the 1:4 volume ratio at a pH of 8–11 showed a tendency toward higher absorbance values compared to other volume ratios, and was therefore determined to be the optimal composition for the synthesis of AgNPs. This indicates that increasing the amount of $AgNO_3$ precursor enhances the reduction of Ag^+ ions to Ag^0 more effectively.

Table 3. The maximum wavelength and absorbance of the synthesized AgNPs at optimum composition (1:4) with variation of pH values

pH	λ max (nm)	Absorbance
8	417	0.579
9	415	0.818
10	336	0.921
11	412	1.261

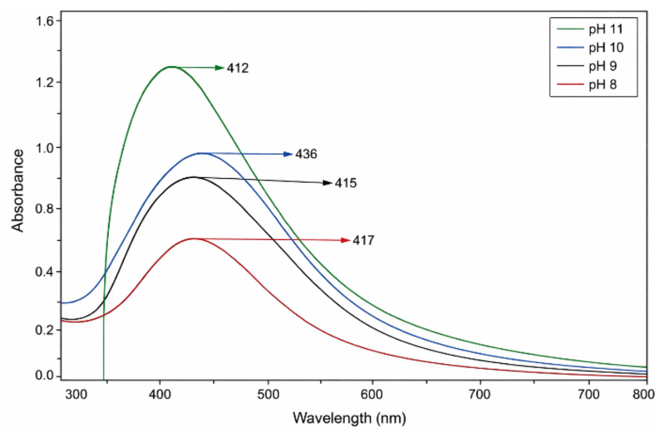

Figure 2. The UV-Vis spectra of AgNPs at variation of pH values

Table 3 and Figure 2 show the UV-Vis spectrum of AgNPs formed at a volume ratio of 1:4 with pH values ranging from 8 to 11. It can be seen that pH variations affect the maximum absorption wavelength and absorbance of the synthesized AgNPs. AgNPs were formed in all compositions and pH variations because the maximum absorption wavelength of $AgNO_3$ no longer appears in the region below 350 nm, and the absorption peak of AgNPs appears between 400 and 450 nm [33]. The highest absorbance value (1.261) was obtained at the 1:4 ratio and pH 11, indicating the most optimal condition for AgNPs synthesis using *B. polystachyon* leaf extract as a bioreductant. The formation of AgNPs is favored under alkaline conditions because, at higher pH, phenolic OH groups from secondary metabolites in *B. polystachyon* are more readily ionized to form phenoxide ions, thereby enhancing their ability to reduce Ag^+ ions to Ag^0 (AgNPs) [19].

3.2. Characterization of AgNPs with Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectral analysis was performed to identify the functional groups of secondary metabolites present in *B. polystachyon* leaf extract that are involved in the reduction of Ag^+ ions to Ag^0, as well as their role as capping and stabilizing agents for silver nanoparticles (AgNPs). The FTIR spectra of *B. polystachyon* leaf extract and the synthesized AgNPs are presented in Figure 3.

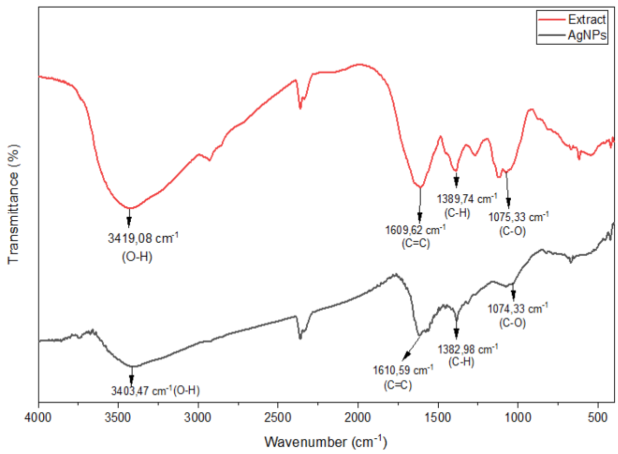

Figure 3. The FTIR spectra of *B. polystachyon* leaf extract and AgNPs

Table 4. Comparison of wavenumber shifts between *B.polystachyon* extract and AgNPs

Functional group	Wave number (cm^{-1})	
	Extract	**AgNPs**
O-H	3419.08	3403.47
C=C	1609.62	1610.59
C-H	1389.74	1382.98
C-O	1075.33	1074.33

The FTIR spectrum of *B.polystachyon* leaf extract indicates the presence of O–H, aromatic C=C, and C–O (alcohol) functional groups at 3419.08,

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1609.62, and 1075.33 cm^{-1}, respectively. In contrast, the FTIR spectrum of the synthesized AgNPs exhibits absorption peaks at 3403.47, 1610.59, and 1074.33 cm^{-1}. The decreased intensity of the O–H and C–O (alcohol) absorption bands in the AgNPs compared to the extract suggests that phenolic O–H groups in the *B. polystachyon* leaf extract were involved in the reduction of silver ions to silver nanoparticles [19][34]. The FTIR spectrum of AgNPs shows a shift in wavenumber compared to the extract. The peaks of the O–H, aromatic C=C, and alcohol C–O groups shifted from 3419.08 cm^{-1} to 3403.47 cm^{-1}, 1609.62 cm^{-1} to 1610.59 cm^{-1}, and 1075.33 cm^{-1} to 1074.33 cm^{-1}, respectively. These shifts indicate that an interaction has occurred between the phenolic OH groups of the secondary metabolites remaining in the extract and the AgNPs, thereby forming a capping layer that stabilizes the synthesized AgNPs [34].

3.3. Characterization of AgNPs with Particle Size Analyzer (PSA)

Based on the PSA measurement results in Figure 3, it was found that the AgNPs have an average particle size of 58.79 nm. Thus, the synthesized AgNPs are classified as nanoparticles because their particle size falls within the range of 1–100 nm [1][2]. The obtained Polydispersity Index (PDI) value of 0.29 indicates a homogeneous particle size distribution and a high degree of size uniformity, as the PDI value is less than 0.3 [35].

These results are consistent with previous findings that produced silver nanoparticles with an average size of 78.2 nm using *Artocarpus integer* (Thunb.) Merr. leaf extract [36].

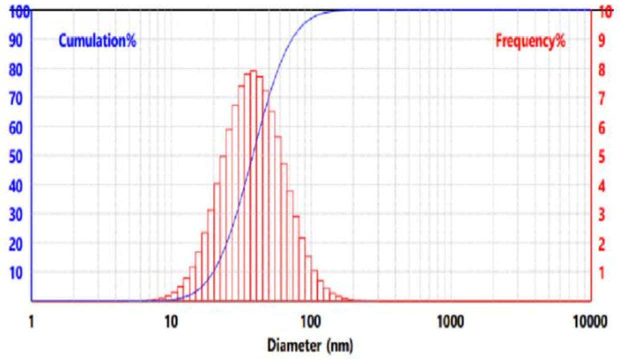

Figure 4. Characterization results of AgNPs using the PSA instrument

3.4. Characterization of AgNPs with X-Ray Diffraction

The XRD analysis results of the synthesized silver nanoparticles are presented in Figure 4. The XRD diffractogram exhibits characteristic diffraction peaks at 2θ values of 38.18°, 44.36°, 64.46°, and 77.36°, which are indicative of metallic silver. These peaks are consistent with the ICDD database (No. 03-065-2871), with standard 2θ values at 38.1°, 44.3°, 64.4°, and 77.4° [17]. The observed peaks can be indexed to the crystallographic planes (111), (200), (220), and (311), corresponding to the face-centered cubic (FCC) crystal structure of silver [26]. This confirms that the synthesized AgNPs possess a well-defined crystalline structure.

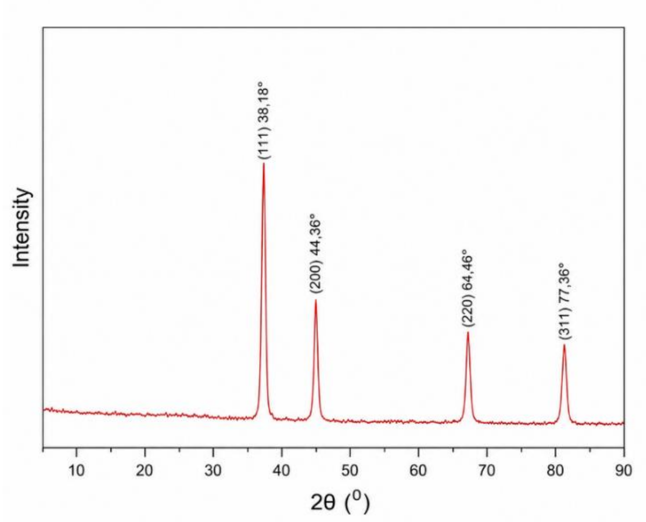

Figure 5. Diffractogram of AgNPs

The sharpness of the diffraction peaks indicates a high degree of crystallinity, while the absence of significant additional peaks suggests that the synthesized nanoparticles possess good phase purity without major impurities. The crystallite size of the AgNPs was calculated using the Debye–Scherrer equation and was found to be 49.98 nm. These results demonstrate that green synthesis using *B. polystachyon* leaf extract is capable of producing silver nanoparticles with a well-defined crystalline structure. The particle size of AgNPs obtained using the D-Scherrer equation (49.98 nm) is smaller than that measured by PSA (58.79 nm) because XRD measures crystallite size, whereas PSA measures the hydrodynamic particle size [37].

Similar findings have been reported in the synthesis of silver nanoparticles using *Rhizophora stylosa* extract, which exhibited diffraction peaks at 2θ values of 38.29° (111), 44.50° (200), 64.76° (220), and 77.80° (311), with crystallite sizes calculated using the Scherrer equation ranging from 38 to 77 nm [38].

Based on the results of this study, the synthesized AgNPs were well characterized using UV-Vis spectroscopy, FTIR, PSA, and XRD. However, the addition of Scanning Electron Microscopy (SEM) data

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would further complement the characterization results, as it can provide information on the surface morphology of the synthesized AgNPs [17].

3.5. Antibacterial Activity test against *S. aureus*

AgNPs synthesized using a bioreductant derived from *B. polystachyon* leaf extract were tested for their antibacterial activity against *S. aureus* (ATCC 25923) using the disk diffusion method. This test aimed to determine the ability of AgNPs to inhibit bacterial growth. At this stage, testing was also conducted on *B. polystachyon* leaf extract, a negative control (distilled water), and a positive control (ciprofloxacin) for comparison. Each test was performed in triplicate. The disk diffusion method is a standard method for evaluating antibacterial activity based on the formation of inhibition zones around the disks [28].

The ability of a substance to inhibit bacterial growth is indicated by the presence of a clear zone surrounding the paper disc. The results showed that AgNPs, *B. polystachyon* leaf extract, and the positive control produced inhibition zones, whereas the negative control exhibited no antibacterial activity. The antibacterial activity results of the four test samples are presented in Figure 6.

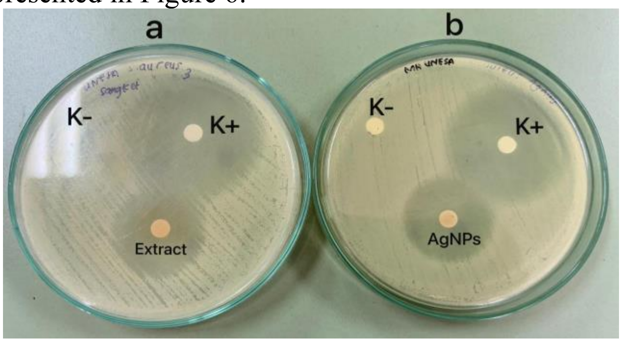

Figure 6. Antibacterial activity test of (a) *B. polystachyon* leaf extract, (b) AgNPs against *S. aureus*

The diameter of the inhibition zones was subsequently measured using a caliper, and the results are presented in Table 5.

Based on the data in Table 5, the AgNPs exhibited greater antibacterial activity against *S. aureus* compared to the *B. polystachyon* leaf extract (26.51 mm vs 10.17 mm). This indicates that nanoparticle formation enhances antibacterial activity relative to the crude extract. However, the antibacterial activity of AgNPs was still lower than that of the positive control (ciprofloxacin), although both are categorized as having very strong activity (26.84 mm vs 33.97 mm).

To evaluate the significance of differences in antibacterial activity among the synthesized AgNPs, *B. polystachyon* leaf extract, ciprofloxacin, and aquabidest, statistical analysis was performed. Based on the Shapiro–Wilk normality test, all datasets were normally distributed because all p-values were greater than 0.05. Furthermore, Levene's test indicated homogeneity of variances among the groups (p-value > 0.05). The One-Way ANOVA analysis revealed a significant difference among the treatment groups because p-value < 0.05 (0.001). Subsequent Bonferroni post hoc analysis demonstrated that the antibacterial activity of AgNPs differed significantly from that of *B. polystachyon* leaf extract and ciprofloxacin as the positive control, as indicated by p-values < 0.05 (p = 0.001 for each comparison). Thus, the synthesized AgNPs exhibited significantly higher antibacterial activity than *B. polystachyon* leaf extract [39].

Table 5. Inhibition zone diameter measurements from the antibacterial activity test

Sample	Inhibition zone (mm)			Avarage
	1	2	3	
Synthesized AgNPs	25.56	28.04	25.94	26.51 ± 1.48
***B.polystachyon* leaf extract**	8.86	10.16	11.50	10.17 ± 0.47
Ciprofloxacin (positive control)	39.84	39.58	40.50	39.97 ± 1.32
Aquabidest (negative control)	0.00	0.00	0.00	0.00

This clear difference indicates that the antibacterial activity was not solely attributed to the bioactive compounds present in *B. polystachyon* leaf extract but was also enhanced by the formation of silver nanoparticles. Nanoparticle size plays an important role in antibacterial activity, as smaller particles provide a larger surface area for interaction with bacterial cells, thereby enhancing their antibacterial efficacy [40][41].

The antibacterial activity of AgNPs is associated with their small particle size and large surface area, which enhance interactions with bacterial cells [4]. The inhibitory mechanisms involve disruption of the cell wall and membrane, interference with enzymatic functions, and the generation of reactive oxygen species (ROS) that damage cellular components [8]. In addition, AgNPs can interact with bacterial DNA, inhibiting replication and transcription processes, ultimately leading to cell death [8]. The antibacterial mechanism of silver nanoparticles is illustrated in Figure 7.

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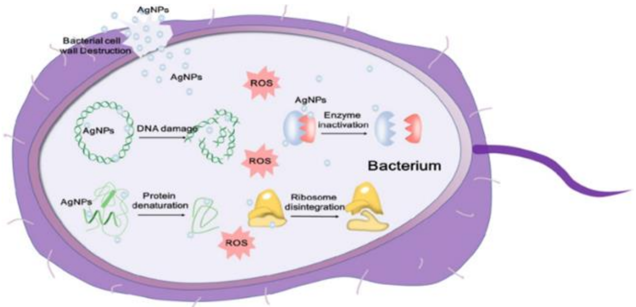

Figure 7. Antibacterial mechanism of silver nanoparticles [42]

The antibacterial mechanism of silver nanoparticles (AgNPs) begins with the attachment of particles ranging in size from 1 to 100 nm to the cell wall, which alters membrane permeability, followed by the release of Ag+ ions into the cell, thereby inhibiting metabolism and DNA replication [43]. AgNPs can penetrate the membrane and induce the formation of reactive oxygen species (ROS), causing oxidative stress and damage to vital cellular components [44]. Additionally, the interaction of silver ions with thiol groups (-SH) on proteins and enzymes leads to protein denaturation and disruption of cellular respiration in both Gram-positive and Gram-negative bacteria [45]. Overall, the effectiveness of AgNPs as potential antimicrobial agents is determined by a combination of membrane damage, oxidative stress, and enzymatic inhibition through chemical interactions with bacterial biomolecules [46].

However, the disk diffusion method is still a preliminary test for antibacterial activity. Therefore, further testing using the dilution method is highly necessary to better confirm the antibacterial potential of the synthesized AgNPs. Through this method, the minimum inhibitory concentration (MIC) value can be determined, and the actual efficacy of the synthesized AgNPs can be validated [47].

4. Conclusion

The synthesis of silver nanoparticles (AgNPs) using a bioreductant derived from *B. polystachyon* leaf extract was successfully optimized at a volume ratio of 1:4 and a pH of 11. The UV-Vis spectrum of the synthesized AgNPs showed a maximum absorption peak at 412 nm. The synthesized AgNPs contained OH, aromatic C=C, and C-O alcohol functional groups. PSA analysis indicates that the synthesized AgNPs have an average particle size of 58.79 nm with a polydispersity index of 0.29. The XRD diffraction pattern confirmed that AgNPs had formed and possessed a crystalline structure consistent with silver metal atoms. The synthesized AgNPs exhibited very strong antibacterial activity against *S. aureus*, with an inhibition zone diameter of 26.51 mm, making them a promising candidate for development as an antibacterial agent.

CRediT Authorship Contribution Statement

DLA: Conceptualization, Methodology, Investigation, Writing – Original Draft. SS: Supervision, Project administration, Validation, Writing – Review & Editing. RYA: Methodology, Validation, Resources. WS: Methodology, Validation, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available upon reasonable request.

Acknowledgment

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