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Green Synthesis Silver Nanoparticles Using Sembung (*Blumea balsamifera*) Leaf Extract as an Antibacterial and Antioxidant

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Abstract: Silver Nanoparticles essentially focuses on the synthesis of nano-sized particles produced through chemical, physical and biological processes, which contribute significantly to the control of plant and animal diseases and have shown considerable promise in improving the quality of living conditions and human health. Currently, silver nanoparticles (AgNP) have shown their potential as an alternative antibacterial and antioxidant agent in many studies. The method for making silver nanoparticles, namely Green synthesis, involves the use of plants for the synthesis process of various types of nanoparticles. Green synthesis has the advantages, namely that the method is simple, environmentally friendly, non-polluting, antitoxic and cost-effective. The aim of this research is to determine the antibacterial and antioxidant activity of AgNPs. In this research, the nanoparticle solution was visually determined by the yellow-brown color change. In the spectrophotometer absorption spectrum appears at a wavelength of 425 nm. The results of determining the size distribution of silver nanoparticles using PSA show that the particle size distribution obtained has an average value of 40.5 nm and 59.6 nm for silver nanoparticles for $AgNO_3$ concentrations of 1 mM and 2 mM, respectively. FTIR measurements are used to determine the presence of bioactive molecules that may be responsible for the stabilization that acts as a capping agent. Absorption spiked at 3.280; 2.919; 1.583; 1.379; 1.239 and 1.068 cm^{-1} were determined for sembung leaf extract, while silver nanoparticles showed an absorption spike at 3.368; 2.850; 1.594; 1.369; 1.244 and 1.108 cm^{-1}. The results of XRD analysis of AgNP show that it has been successfully synthesized which can be seen from the formation of a narrow peak indicating the crystalline nature of the nanoparticles formed. The results of TEM analysis of AgNPs in this study are mixed shapes such as spherical, hexagonal and triangular shapes of silver nanoparticles. The antibacterial activity test of silver nanoparticles with sembung leaf extract with varying concentrations of $AgNO_3$ solution was successful as indicated by the formation of an inhibition zone for *Eschericia coli* and *Staphylococcus aureus* bacteria. The results show that the antioxidant activity of AgNPs shows that the percentage of inhibition increases as the concentration of AgNPs increases from 100 to 500 ppm and ascorbic acid increases from 1 to 5 ppm.

Keywords: Antibacterial; Antioxidant; Green synthesis; Sembung leaves; Silver nanoparticles

Introduction

Recently, nanotechnology has attracted the attention of researchers because it can be widely applied in the fields of medicine, agriculture, environment and food (Pandit et al., 2022). This technology essentially focuses on the synthesis of nano-sized particles produced through chemical, physical and biological processes, which contribute significantly to the control of plant and animal diseases and have shown considerable promise in improving the quality of living conditions and human health (Nguyen & Lai, 2022). The sizes of these nanoparticles range from 1 to 100 nm. However, green synthesis of nanoparticles has become

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more important than ordinary chemical and physical methods (Ramamurthy et al., 2013).

Green synthesis involves the use of plants for the synthesis process of various types of nanoparticles (Alhumaydhi, 2022). Green synthesis has the advantages, namely that the method is simple, environmentally friendly, non-polluting, antitoxic and cost-effective. Metal nanoparticles from the green synthesis process are innovative and promising agents for various biological and catalytic activities, such as antibacterial, antiviral, anticancer and others, and without negative side effects (Arya & Chundawat, 2020). In green synthesis, plant extracts are used as important capping agents and stabilizers that control the growth of nanoparticles and prevent them from aggregation or coagulation. This stabilization of substances is essential for changing biological processes and environmental perception (Javed et al., 2020).

Currently, silver nanoparticles (AgNP) have shown their potential as an alternative antibacterial agent in many studies (Das et al., 2020; Sibbald et al., 2007; Liu et al., 2010). The AgNPs that have been produced show antiseptic activity against several bacterial species, including multidrug-resistant bacteria such as methicillin-resistant bacteria, and are safe for mammalian cells at low concentrations (Shahverdi et al., 2007). Various studies have confirmed that silver nanoparticles obtained from green synthesis have stronger antioxidant activity due to the presence of various biomolecules on their surface and can be used as radical scavengers against damage caused by free radicals (Nguyen & Lai, 2022; Zulfiqar et al., 2022; Nagaich et al., 2016; Ahmed et al., 2019). Some of the biological uses of AgNPs include antioxidant, antibacterial, anti-inflammatory, wound healing, anticancer, antiproliferative, antifungal, antiviral, and antidiabetic (Hussain et al., 2020). Due to these unique properties, silver nanoparticles (AgNPs) are widely used for drug delivery agents, therapeutic, sensing and diagnostic devices as well as other applications (Tehri et al., 2022).

Silver has long been known as an antimicrobial agent and AgNPs are non-toxic to eukaryotes such as humans. Nevertheless, AgNPs are highly toxic to prokaryotic cells such as bacteria (Hussain et al., 2016). Researchers are very interested in studying the application of AgNPs in nano-based medicine because of their useful antimicrobial (Singh et al., 2016), antiplatelet (Rathinam & Berchmans, 2013), anticancer (Castro-aceituno et al., 2016) and wound healing properties (Rigo et al., 2013). The high surface area to volume ratio of AgNPs ensures high reactivity in biomedical research (Chaloupka et al., 2010). Various studies have been reported on plant extracts used for AgNP synthesis, such as *Papaver somniferum* (Vijayaraghavan et al., 2012), *Bauhinia variegata L.* (Kumar et al., 2012), *Hevea brasiliensis* (Guidelli et al., 2011), *Aloe vera* (Tippayawat et al., 2016), and *Acacia farnesiana* (Mohammed et al., 2018).

Blumea balsamifera is a medicinal plant that grows widely, especially in Southeast Asian countries such as Malaysia, the Philippines, Vietnam, Thailand and Indonesia. In Indonesia, this plant is often called sembung leaf which shows biological effects on the stomach, anti-neuroinflammatory (Ma et al., 2018), receptor system (Nguyen et al., 2004), anti-cancer (Norikura et al., 2008), antioxidant (Fazilatun et al., 2005), antiobesity and antifungal effects (Ragasa et al., 2005). *Blumea balsamifera* leaves are commonly used as an ingredient in cigarettes and tea. Phytochemical analysis reveals that *Blumea balsamifera* leaves contain a large amount of flavonoids (Nessa et al., 2004; Pang et al., 2014), and related literature studies also show that many flavonoids have direct or indirect wound healing effects, such as soy isoflavones in burns mice (Zhang et al., 2013), the inhibitory effect of xanthine oxidase and corylin on human fibroblast cells in an in vitro model of wound healing (Pang et al., 2017). Flavonoids from *Blumea balsamifera* leaves can be utilized as antioxidant agents and other secondary metabolites may have cytotoxic and antibacterial activity. In this study, we evaluated the effectiveness of the synthesis of silver nanoparticles from *Blumea balsamifera* leaf extract as an antibacterial agent on bacteria (Gram positive and negative) and as an antioxidant.

Method

Materials

The materials used in this research were fine powder of sembung leaves (*Blumea balsamifera*), distilled water, solid $AgNO_3$ p.a (Merck), Whatman filter paper and aluminum foil, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 bacteria.

Sembung Leaf Preparation

Sembung leaves that have been collected are cleaned and then dried by air-drying at room temperature. The dried leaves are cut into small pieces and then ground with a blender. Sembung leaf powder is stored in a clean container and protected from light to avoid damage and loss of quality.

Sembung Leaf Extraction

A total of 20 grams of sembung leaf powder is mixed into 100 mL of distilled water, then boiled until boiling. The mixture is then cooled and filtered to separate the filtrate and residue. The filtrate obtained is then stored in a clean and closed container to be used as a bioreductant in the synthesis of silver nanoparticles.

Green Synthesis Silver Nanoparticles

A total of 0.5 mL of sembung leaf extract was mixed into 49.5 mL of $AgNO_3$ solution with a concentration of 1 mM and 2 mM for each. The mixture was allowed to stand until it changed color at room temperature. The change in color of the solution that occurs is brownish yellow, which indicates that silver nanoparticles have been formed. The filtrate obtained was then subjected to UV/Vis Spectrophotometer analysis (Thermo Scientific AQ 8100 Spectrophotometer UV-Vis type) to identify the formation of silver nanoparticles. The silver nanoparticle solution obtained was subjected to a freeze drying process to be used for FTIR, XRD and TEM characterization.

Characterization of the Silver Nanoparticles Formed

The silver nanoparticle solution was characterized using several analytical methods such as UV-Vis Spectrophotometry which was carried out to determine the maximum wave length (λ) using a UV-Vis spectrophotometer from 250 to 1100 nm at a resolution of 1 nm. Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out using an FTIR spectrometer (Thermoscientific Nicolet iS-10) in total reflection mode and a spectral range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1}. XRD analysis (XRD type PANalytical AERIS) with wide angles ranging from 20 to 80. Transmission electron microscope (TEM) (type Tecnai G2 20S-Twin Function Transmission Electron Microscope) analysis was used to study the shape of silver nanoparticles.

Antibacterial Activity Test

The antibacterial activity of AGNPs (silver nanoparticles) was tested qualitatively by the disk diffusion method. The bacteria used are *Eschericia coli* and *Staphylococcus aureus*. The inhibition test was carried out by wetting a sterile paper disc with AgNP solution, then placing it in a petri dish containing the test bacteria grown on NA media and incubating for 24 hours at a temperature of 37°C. The resistance of the test material is determined by measuring the width of the clear zone around the disc paper.

Determination of Antioxidant activity DPPH Method

DPPH radical scavenging activity is carried out in the following way: 50 µL of test samples with various concentrations (The concentration that gives the IC50 value, namely the concentration of the fraction that gives a percentage of radical scavenging activity of 50% compared to the control via a linear regression line equation), is added by 1.0 mL 0.4 mM DPPH, and 3,950 mL ethanol. The mixture was then vortexed and left for 30 minutes. The absorbance of the solution was then measured at a wavelength of 517 nm against a blank (which consisted of 50 µL of extract and 4,950 mL of ethanol). Also measured was the absorbance of a control consisting of 1.0 mL DPPH and 4.0 mL ethanol. As a comparison, Vitamin C is used, both of which are known to be antioxidants.

$$\text{(\%) of radical capture} = \left(Ao - \frac{A1}{Ao}\right) \times 100\% \quad (1)$$

Description: Ao is the absorbance of the control (not containing the test sample) and A1 is the absorbance in the presence of the test sample or comparison compound.

Results and Discussion

Color Changes in the Synthesis Process of Silver Nanoparticles with Sembung Leaf Extract

In this study, sembung leaf extract was mixed with a solution of silver nitrate ($AgNO_3$) with a concentration of 1 mM and 2 mM was allowed to react with a total synthesis volume of 50 mL. Where 49.5 mL of $AgNO_3$ solution was added with 0.5 mL of sembung leaf extract, then for a moment the solution began to form silver nanoparticles which were marked by a color change according to Figure 1. The first color change was observed at 10 minutes, this indicated synthesis. Nanoparticles using sembung leaves have been formed. This is in accordance with research on silver nanoparticles using leaves in a previous study (Willian et al., 2020). The color change occurs from clear to brownish yellow. The color change indicates the excitation of surface plasmon vibrations in metal nanoparticles (Bakshi et al., 2015). The nanoparticle synthesis process stops when the color of the solution becomes fixed, the color changes from clear, light yellow to brown and dark brown due to surface plasmon resonance, as shown in Figure 1.

(a) (b)

Figure 1. (a) $AgNO^3$ solution (1 mM and 2 mM) and (b) Silver Nanoparticles Solution

Silver Nanoparticle UV-Vis Spectrophometer Test Results

The formation of AgNPs was confirmed by analysis of the UV-Vis absorption spectrum between 300–800 nm which was characterized by the appearance of Surface Plasmon Resonance (SPR) peaks as shown in Figure 2. 3 mL of the AgNP solution formed was taken and the absorption spectrum was measured at room temperature. The baseline for this measurement was determined using distilled water as a solvent. The silver reduction process from Ag+ ions to Ag0 is the basis for the formation of SPR peaks in spectrum analysis. As can be seen in Figure 2, after the synthesis had been running for 60 minutes, the reaction was clearly visible in the formation of silver nanoparticles as seen from the appearance of the UV-Vis spectrum peak. A broad absorption peak appears at a maximum λ of 425 nm. These results are also supported by research by Isaac et al. (2013) where the synthesis of silver nanoparticles used Averrhoa bilimbi Linn fruit extract display an SPR peak at 420 nm. Furthermore, Salari et al. (2019) found SPR peaks in AgNPs in the range 425–438 nm using Prosopis farcta fruit extract. The qualitative growth of silver nanoparticles can also be observed from the shift in the SPR peak of silver nanoparticles. The position of the plasmon peak depends on the size and shape of the particle (Suárez-cerda et al., 2015).

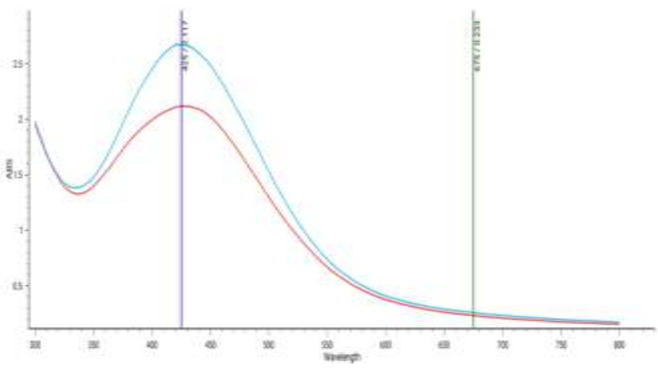
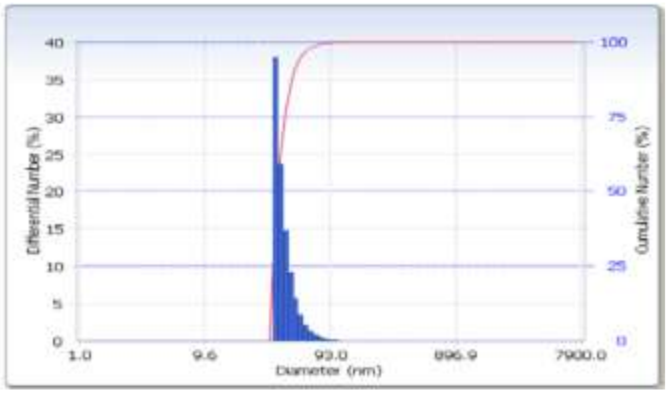


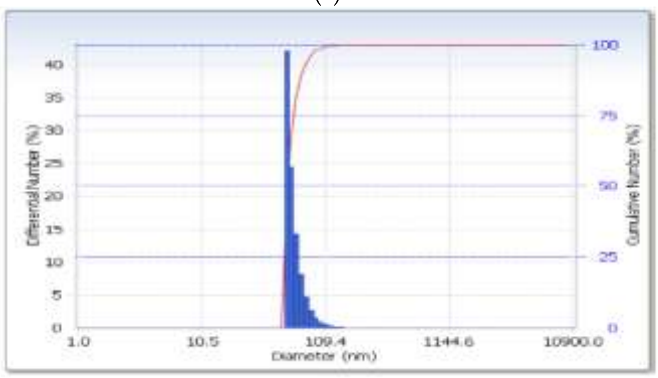
Figure 2. Absorption spectrum of silver nanoparticles with sembung leaf extract bioreductant with varying concentrations of $AgNO_3$ solution (1 mM and 2 mM)

Size Distribution of Silver Nanoparticles with PSA (Particle Size Analyzer)

The PSA particle measurement method is considered more accurate in determining particle size distribution. The results of determining the size distribution of silver nanoparticles using PSA are shown in Figure 3. The particle size data obtained is in the form of three distributions, namely intensity, number and volume, so that it can describe the overall condition of the sample (Nikmatin et al., 2011). Figure 3 shows the particle size distribution obtained with average values of 40.5 nm and 59.6 nm.



(a)



(b)

Figure 3. Size distribution histogram of Silver Nanoparticles with sembung leaf extract with varying concentrations of (a) $AgNO_3$ solution (1 mM) and (b) $AgNO_3$ solution (2 mM)

FTIR Test Results of Silver Nanoparticles and Sembung Leaf Extract Solution

FTIR measurements are used to determine the presence of bioactive molecules that may be responsible for stabilization by acting as capping agents. Absorption spiked at 3,280; 2,919; 1,583; 1,379; 1.239 and 1.068 cm^{-1} were determined for sembung leaf extract, while silver nanoparticles showed an absorption spike at 3,368; 2,850; 1,594; 1,369; 1,244 and 1,108 cm^{-1} shown in Figure 4. The higher peaks at 3,280 and 3,368 cm^{-1} can be caused by the presence of bound hydroxyl groups (O–H) in the alcohol compound in sembung leaf extract. The presence of smaller bands at 2,917 and 2,919 cm^{-1} for sembung leaf extract and AgNP is caused by the stretching of the C–H groups on the alkanes. The peaks at 1,583 cm^{-1} (extract) and 1,594 cm^{-1} (AgNP) correspond to the N–H groups. These bands at 1,379 cm^{-1} (extract) and 1,369 cm^{-1} (AgNPs) correspond to the C–H group. The peaks at 1,239 cm^{-1} (extract) and 1,244 cm^{-1} (AgNPs) as well as 1,068 cm^{-1} (extract) and 1,108 cm^{-1} (AgNPs) were attributed to C–O in the ester stretch found in the leaf extract. The observed spectrum of AgNPs (Figure 4A is similar to that in Figure 4B regarding the location of its functional groups.

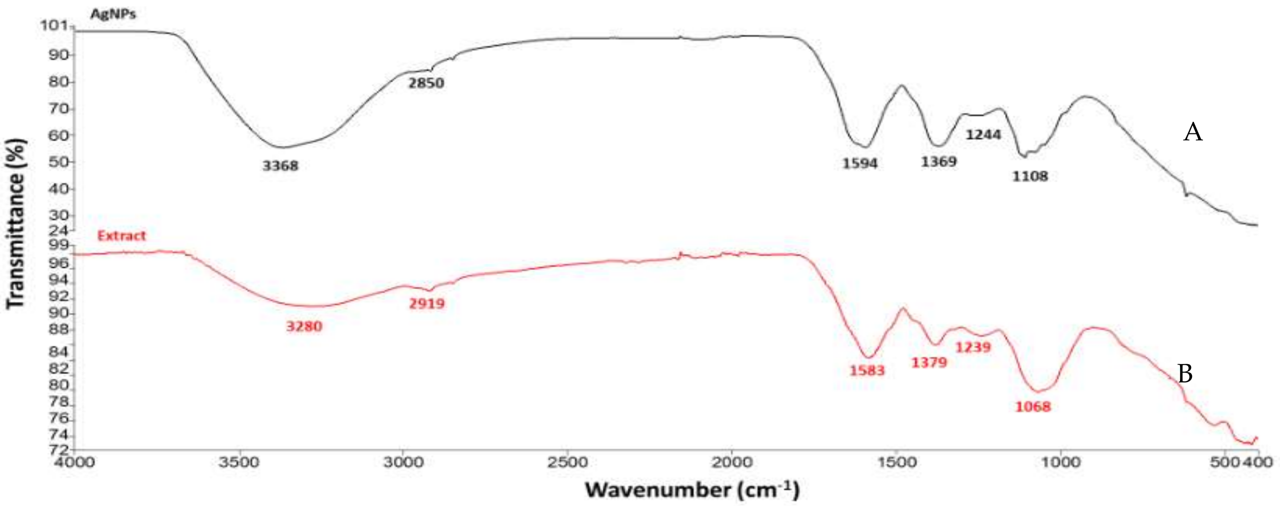


Figure 4. FTIR spectra of sembung leaf extract (A) and Silver nanoparticles synthesized from sembung leaf extract (B)

XRD Test Results on Silver Nanoparticles

Figure 5 shows the XRD results of the synthesized AgNPs. The narrow peak indicates the crystalline nature of the nanoparticles formed. Four continuously appearing peaks corresponding to the detected 2θ values of 38.14, 44.33, 64.47, and 77.42° represent the face-centered cubic (fcc) silver lattice with Miller index (111), (200), (220), and (311), respectively. From the comparison of the intensity of the (111) peak compared to other diffraction peaks, it can be concluded that the (111) plane is the main orientation in the silver crystal structure of the synthesized AgNPs.

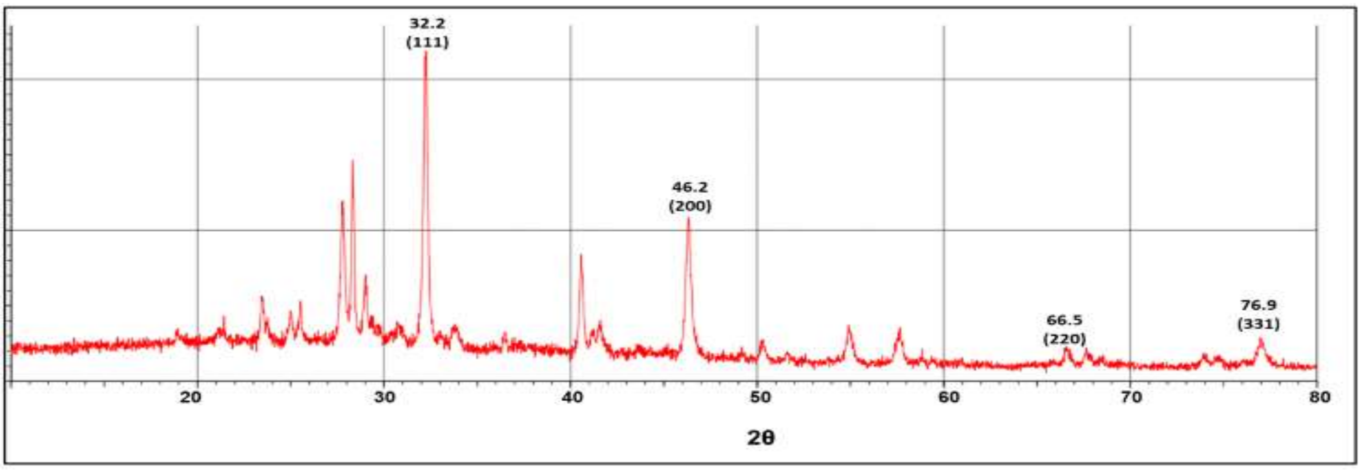


Figure 5. XRD pattern of silver nanoparticles synthesized with Sembung leaf extract

Morphology of Silver Nanoparticles Using TEM (Transmission Electron Microscopy)

The results of TEM analysis in this study are shown in Figure 6. The TEM image is a mixture of shapes such as spherical, hexagonal and triangular shapes of silver nanoparticles (Bhuvaneswari et al., 2017). This is identical to silver nanoparticles synthesized from cannonball leaves (Preetha et al., 2013). Different compounds present in sembung leaf extract such as polysaccharides, polyphenols, and proteins are responsible for producing nanoparticles in various forms.

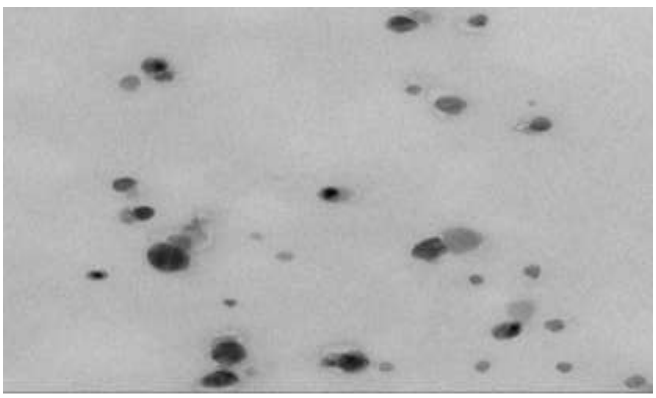


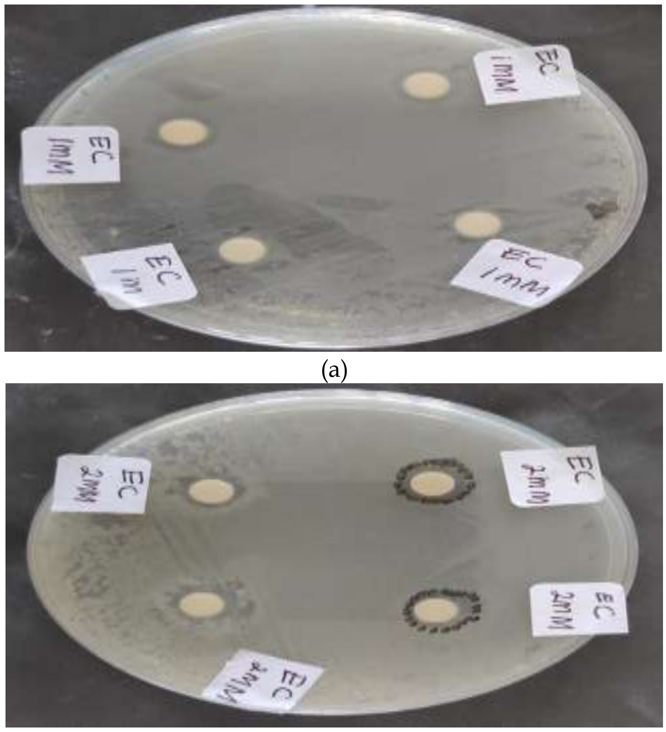
Figure 6. TEM morphology of silver nanoparticles with sembung leaf extract

Test of the Antibacterial Activity of Silver Nanoparticles with Sembung Leaf Extract with varying concentrations of $AgNO_3$ Solution

In this study, the disk diffusion method was used to test the antibacterial activity of Silver Nanoparticles (AgNP) with sembung leaf extract with varying concentrations of 1 mM and 2 mM $AgNO_3$ solutions. This method was used with the aim of determining the inhibitory power exerted by silver nanoparticles with sembung leaf extract with varying concentrations of 1 mM and 2 mM $AgNO_3$ solutions on bacterial growth.

The results of the inhibition zone for *Escherichia coli* and *Staphylococcus aureus* bacteria can be seen in Figures 7 and 8. The calculation results for the antibacterial activity test of silver nanoparticles with sembung leaf extract can be seen in tables 1 and 2 which show that there is an inhibition zone for the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. Silver nanoparticles with sembung leaf extract with varying concentrations of $AgNO_3$ solution with concentrations of 1 mM and 2 mM, if related to the inhibition zone diameter category stated by Susanto et al. (2012) where the inhibition zone formed > 20 mm is considered to have power. very strong inhibition, 11-20 mm is considered a strong inhibition, 6-10 mm is a medium inhibition and < 5 mm is a weak inhibition. For each variant of research data, the standard deviation was calculated to determine the accuracy and precision of measurements in the data obtained. Silver nanoparticles with sembung leaf extract with varying concentrations of 1 mM and 2 mM $AgNO_3$ solution had an inhibitory zone diameter of 9.2 ± 0.2 mm and 10.9 ± 0.4 mm for *Escherichia coli* bacteria where both concentrations were categorized as having medium inhibitory power.

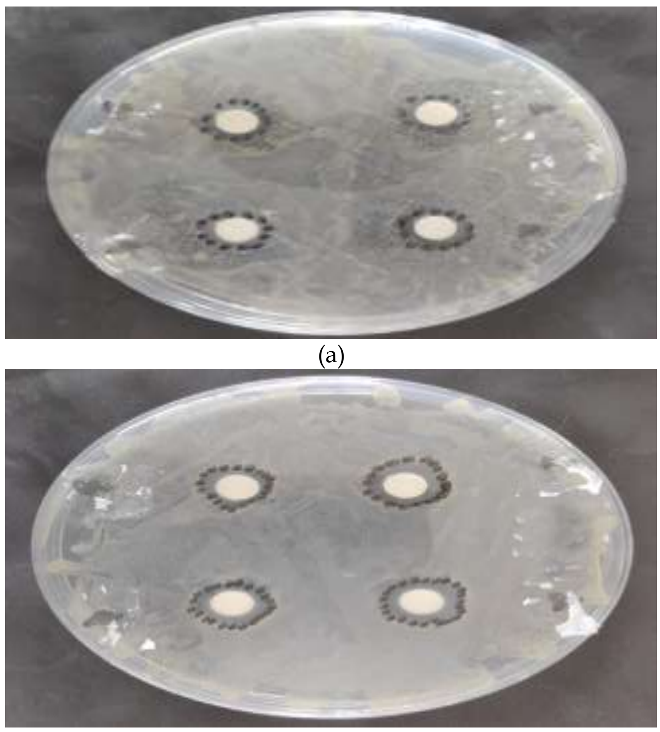
Silver nanoparticles (AgNP) with sembung leaf extract with varying concentrations of 1 mM and 2 mM $AgNO_3$ solution had an inhibitory zone diameter of 9.4 ± 0.3 mm and 10.0 ± 0.3 mm, which was categorized as having moderate inhibitory power. Based on the results of this research, silver nanoparticles with sembung leaf extract showed antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* bacteria. In general, the mechanism of silver nanoparticles can function as an antibacterial because silver nanoparticles can interact with bacterial membranes, causing damage to the bacterial membrane which will then kill the bacteria. Silver nanoparticles first attack the surface of the bacterial membrane, penetrate into the bacteria, and finally change the permeability of the bacterial membrane. This mechanism causes damage to the membrane. The antibacterial properties of silver nanoparticle membranes depend on the size, shape and surface which determine their success in destroying bacterial membranes. Silver nanoparticles with a small size can interact with the protective lignin layer on bacteria better (Zheng et al., 2018).



(a)

(b)

Figure 7. Antibacterial activity of silver nanoparticles with sembung leaf extract against bacteria in *Escherichia coli* (a) $AgNO_3$ 1 mM and (b) $AgNO_3$ 2 mM



(a)

(b)

Figure 8. Antibacterial activity of Silver Nanoparticles with sembung leaf extract against *Staphylococcus aureus* bacteria (a) $AgNO_3$ 1 mM and (b) $AgNO_3$ 2 mM

Table 1. Results of Inhibition Zone Diameter (mm) of Silver Nanoparticles with Sembung Leaf Extract Bioreductor with Varying Concentrations of $AgNO_3$ Solution Against *Escherichia coli* Bacteria

Test Solution	Average Inhibition Zone Diameter (mm)				Average (mm)	SD (mm)	Average ± SD (mm)
	I	II	III	IV			
Control (+)	29.2	28.8	29.4	29.6	29.3	0.3	29.3 ± 0.3
Control (-)	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
Silver nanoparticle 1	9.1	8.9	9.4	9.3	9.2	0.2	9.2 ± 0.2
Silver nanoparticle 2	10.80	10.40	11.00	11.30	10.9	0.4	10.9 ± 0.4

Table 2. Results of Inhibition Zone Diameter (mm) of Silver Nanoparticles with Sembung Leaf Extract Bioreductor with Varying Concentrations of $AgNO_3$ Solution Against *Staphylococcus aureus* Bacteria

Test Solution	Average Inhibition Zone Diameter (mm)				Average (mm)	SD (mm)	Average ± SD (mm)
	I	II	III	IV			
Control (+)	31.7	31.1	30.2	31	31.0	0.6	31.0 ± 0.6
Control (-)	0.00	0.00	0.00	0.00	0.0	0.0	0.0 ± 0.0
Silver nanoparticle 1	9.3	9.4	9.1	9.7	9.4	0.3	9.4 ± 0.3
Silver nanoparticle 2	9.8	9.9	10.4	9.8	10.0	0.3	10.0 ± 0.3

Antioxidant Activity of Silver Nanoparticles and Ascorbic Acid

Table 3. Percent Inhibition Values of Silver Nanoparticles and Ascorbic Acid

Sample	Concentration (ppm)	% Inhibition
Silver nanoparticle 1 (1 mM $AgNO_3$)	100	14.29
	200	32.87
	300	44.93
	400	61.25
	500	73.33
Silver nanoparticle 2 (2 mM $AgNO_3$)	100	23.17
	200	38.93
	300	53.02
	400	63.58
	500	75.67
Ascorbic Acid	1	10.50
	2	27.68
	3	37.10
	4	50.39
	5	61.63

Antioxidants are substances that ward off free radicals and protect against diseases that cause degeneration, such as cancer, Parkinson's disease, Alzheimer's disease, and atherosclerosis, which are caused by oxidative stress (excessive production of free radicals) (Balashanmugam et al., 2015). The role of phenolic compounds, such as phenolic acids and flavonoids, found in medicinal plants is to provide hydrogen atoms to the plant and scavenge free radicals (ROS) (Labulo et al., 2022). Findings from the current study indicate that AgNPs have antioxidant properties over a wide range of concentrations (100–500 ppm) as determined using the DPPH assay. The results showed that the percentage of inhibition increased as the AgNP concentration increased and ascorbic acid increased from 100 to 500 ppm (Table 3). Higher concentrations of AgNPs and ascorbic acid were used in DPPH experiments to measure antioxidant activity. The higher polyphenol content of leaf extracts restricted to AgNPs may be the reason for the increased antioxidant capacity (Moorthy et al., 2022). By effectively reducing reactive oxygen species, AgNPs showed a broad spectrum of antioxidant activity in this study (Berridge et al., 2005).

Conclusion

Sembung leaf extract has been effectively used to synthesize AgNP, and the benefits of sembung leaves have been proven as a natural ingredient that acts as a silver bioreduction agent and limiting agent. Characterization of the synthesized nanoparticles using UV-visible, FTIR, TEM, PSA and with spherical, hexagonal, and triangular shapes of silver nanoparticles. AgNPs showed quite good antibacterial activity and had antioxidant activity as indicated by the percentage of inhibition increasing as the concentration of AgNPs and ascorbic acid increased from 100 to 500 ppm. Therefore, AgNP is very important for biomedicine as an antibacterial and antioxidant in the pharmaceutical sector.

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Author Contributions
I Wayan Tanjung Aryasa conceptualized the research idea, designed of methodology, management, conducted a research

and coordination responsibility; Ni Putu Rahayu Artini analyzed data, conducted an investigation process, conducted literature review and provided critical feedback on the manuscript.

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Conflicts of Interest

The author declared no conflict of interest.

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