JPPIPA 10(12) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Green Synthesis of Silver Nanoparticles Using Gempur Batu Leaf Extract (*Ruellia napifera*) as Antibacterial, Antibiofilm, and Antioxidant

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Received: May 08, 2024
Revised: October 06, 2024
Accepted: December 25, 2024
Published: December 31, 2024

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DOI: 10.29303/jppipa.v10i12.9356

Abstract: Silver Nanoparticles basically focus 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 human living conditions and health. The method of 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 of a simple method, environmentally friendly, non-polluting, antitoxic, and cost-effective. The purpose of this study was to determine the antibacterial and antioxidant activities of AgNPs. In the spectrophotometer absorption spectrum appears at a wavelength of 450 nm. FTIR measurements were used to determine the presence of bioactive molecules that may be responsible for the stabilization that acts as a capping agent. The absorption spikes at 3256, 1552, 1048, and 934 cm^{-1} were determined for gempur batu leaf extract, while silver nanoparticles showed absorption spikes at 3369, 1576, 1080, and 822 cm^{-1}. The results of XRD analysis of AgNPs showed that they had been successfully synthesized, which can be seen from the formation of narrow peaks indicating the crystalline nature of the formed nanoparticles. The results of TEM analysis of AgNPs in this study are a mixture of spherical, hexagonal, and triangular shapes of silver nanoparticles. The antibacterial activity test of silver nanoparticles with gempur batu leaf extract with variations in $AgNO_3$ solution concentration has been successful, which is indicated by the formation of inhibition zones for *Eschericia coli* and *Staphylococcus aureus* bacteria. The results of antioxidant activity in AgNPss show an increasing percentage of inhibition along with increasing concentration of AgNPss increasing from 1 to 15 ppm and ascorbic acid increasing from 1 to 5 ppm. Antibiofilm activity in AgNPs has a good ability to inhibit the formation of biofilm layers in *Staphylococcus aureus* and *Escherichia coli* bacteria by having a biofilm inhibition percentage of more than 50%.

Keywords: Antibacterial; Antibiofilm; Antioxidant; Gempur batu leaves; Green synthesis; Silver nanoparticles

Introduction

Recently, nanotechnology has attracted the attention of researchers due to its wide application in medicine, agriculture, environment, and food (Pandit et al., 2022). This technology basically focuses on the synthesis of nano-sized particles produced through chemical, physical, and biological processes, which

How to Cite:
Aryasa, I. W. T., Artini, N. P. R., & Setiawan, P. Y. B. (2024). Green Synthesis of Silver Nanoparticles Using Gempur Batu Leaf Extract (Ruellia napifera) as Antibacterial, Antibiofilm, and Antioxidant. *Jurnal Penelitian Pendidikan IPA*, *10*(12), 10556–10567. https://doi.org/10.29303/jppipa.v10i12.9356

contribute significantly to the control of plant and animal diseases and have shown great promise in improving the quality of human living conditions and health (Nguyen et al., 2022). The size of these nanoparticles ranges from 1 to 100 nm. However, green synthesis of nanoparticles has become more important than conventional 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 et al., 2022). Green synthesis has the advantages of being simple, environmentally friendly, non-polluting, antitoxic, and cost-effective. Metal nanoparticles from 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 et al., 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. These stabilization substances are very important to change biological processes and environmental perception (Javed et al., 2020). Currently, silver nanoparticles (AgNPs) have shown their potential as alternative antibacterial agents in many studies (Das et al., 2020; Sibbald et al., 2007; Liu et al., 2010). The resulting AgNPs showed antiseptic activity against several bacterial species, including multidrug-resistant bacteria such as methicillin-resistant bacteria, and were 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 free radical-induced damage (Nguyen et al., 2022; Zulfiqar et al., 2022; Nagaich et al., 2016; Ahmed et al., 2019). Some of the biological uses of AgNPss include antioxidant, antibacterial, anti-inflammatory, wound healing, anticancer, antiproliferative, antifungal, antiviral, antibiofilm and antidiabetic (Hussain et al, 2020; Rajeswari et al., 2022; Selem et al., 2022; Kumar et al., 2023). Due to these unique properties, silver nanoparticles (AgNPs) are widely used for drug delivery agents, therapeutic devices, sensing and diagnostics and 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. However, 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 (Sings et al., 2016; Aryasa et al., 2022; Aryasa et al., 2023), antiplatelet (Krishnaraj, 2013), anticancer (Castro-aceituno et al., 2016), antibiofilm (Ispiryan et al., 2024; Ounjaijean et al., 2024) 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 AgNPs 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), *Acacia farnesiana* (Mohammed et al., 2018), *Blumea balsamifera* (Aryasa et al., 2023) and *Schefflera Elliptica Harms* (Aryasa et al., 2022).

One of the medicinal plants that can be used in the formation of silver nanoparticles is the gempur batu plant (*Ruellia napifera*). This plant grows abundantly in forests, fields, and slightly damp soil in lowlands up to an altitude of 500 meters above sea level. The gempur batu plant has spear-shaped leaves and roots, the leaves are rather rough and the flowers are small and white. The gempur batu plant contains flavonoids, saponins and polyphenols. Related literature studies show that flavonoids have direct or indirect wound healing effects, such as soy isoflavones on rat burns (Zhang et al., 2013) and the inhibitory effects of xanthine oxidase and corylin on human fibroblast cells in a wound healing vitro model (Pang et al., 2017). The efficacy of the gempur batu plant is as a natural antioxidant, antimicrobial, and helps protein metabolism and PPAR alpha gene expression in diabetics, treats gallstones, treats kidney stones and external medicine for diarrhea (Anggraini et al., 2018; Wahyuni et al., 2023). Thus, in this study, we evaluated the effectiveness of silver nanoparticle synthesis from gempur batu (*Ruellia napifera*) leaf extract as antibacterial and antibiofilm on bacteria (Gram positive and negative) and as an antioxidant.

Method

Materials

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

Preparation of Gempur Batu Leaves

The collected gempur batu leaves are cleaned and then dried by airing them at room temperature. The dried leaves are cut into small pieces and then ground with a blender. The gempur batu leaf powder is stored in a clean container and protected from light to prevent damage and quality degradation. The expected output is in the form of smooth gempur batu leaves.

Gempur Batu Leaf Extract

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

Green Synthesis of Silver Nanoparticles

A total of 0.5 mL of gempur batu leaf extract was mixed into 49.5 mL of $AgNO_3$ solution with concentrations of 1, 2, and 3 mM for each. The mixture was left to stand until it changed color at room temperature. The color change of the solution that occurred was brownish yellow which indicated that silver nanoparticles had formed. The filtrate obtained was then analyzed using a UV/Vis Spectrophotometer (Thermo Scientific AQ 8100 Spectrophotometer UV-Vis type) to identify the formation of silver nanoparticles. For the silver nanoparticle solution obtained, a freeze drying process was carried out (Freeze dry Buchi Lyovapot L200) to be used for FTIR, XRD and TEM characterization.

Characterization of the Formed Silver Nanoparticles

Silver nanoparticle solution was characterized by several analytical methods such as UV-Vis Spectrophotometry was performed 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 performed 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 (type XRD PANalytical AERIS) with an angular width 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 silver nanoparticles was tested qualitatively by the disc diffusion method. The bacteria used were Eschericia coli and Staphylococcus aureus. The inhibition test was carried out by wetting sterile paper discs with AgNPs solution, then placed on a petri dish containing test bacteria grown on NA media and incubated for 24 hours at 37°C. The inhibition of the test material was determined by measuring the width of the clear zone around the paper disc.

Biofilm Formation Inhibition Activity Test

The biofilm inhibition activity test was carried out using a 96-well polystyrene round bottom microplate with BHI media. A total of 70 µL of sample in the media was added to each well, then 70 µL of bacterial suspension in the media equivalent to 1.5 x 108 CFU/mL was added to the well containing the sample, incubation was carried out at a temperature of ± 37 ^{o}C for 72 hours. After incubation, the contents of the wells were discarded and the plate was washed with running water and then dried for 15 minutes by inverting the microplate at room temperature. A total of 200 µL of 1% crystal violet solution was added to each well with a staining time of 15 minutes. The contents of the wells were discarded and the wells were rinsed again with running water. The microplate was dried by inverting it at room temperature for one hour. Then 200 µL of 96% ethanol solution was added to each well on the plate and the optical density was read at λ 540 nm. Each test was replicated 10 times.

$$\% \text{ Biofilm Inhibition} = \frac{\text{DO Negative Control} - \text{DO Sample}}{\text{DO Negative Control}} \times 100\% \quad (1)$$

With DO = Optical density

Biofilm inhibition was categorized on a scale of 0 to 100%. Values below 0% were recorded as 0% biofilm inhibition, values falling between 0 and 50% indicated weak anti-biofilm activity, and values exceeding 50% indicated effective biofilm inhibition. Any value exceeding 100% was reported as 100% biofilm inhibition (Akinboye et al., 2024).

Determination of Antioxidant Activity Using the DPPH Method

DPPH radical scavenging activity was carried out in the following manner: as much as 50 µL of test samples with various concentrations (Concentrations that provide IC50 values, namely the fraction concentration that provides a percentage of radical scavenging activity of 50% compared to the control through a linear regression line equation), added with 1.0 mL of 0.4 mM DPPH, and 3.950 mL of ethanol. The mixture was then vortexed and left for 30 minutes. The solution was then measured for absorbance at a wavelength of 517 nm against the blank (consisting of 50 µL of extract and 4.950 mL of ethanol). The absorbance of the control was also measured consisting of 1.0 mL of DPPH and 4.0 mL of ethanol. Vitamin C was used as a comparison, both of which are known to be antioxidants.

$$\text{Percentage of radical scavenging} = \left(\text{Ao} - \frac{\text{A1}}{\text{Ao}}\right) \times 100\% \quad (2)$$

Note: Ao is the absorbance of the control (does not contain the test sample) and A1 is the absorbance in the presence of the test sample or reference compound.

Result and Discussion

Results of Color Changes in the Silver Nanoparticle Synthesis Process with Gempur Batu Leaf Extract

In this study, gempur batu leaf extract was mixed with silver nitrate ($AgNO_3$) solution with concentrations of 1, 2, and 3 mM and 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 gempur batu leaf extract, then for a moment the solution began to form silver nanoparticles which were indicated by a color change according to Figure 1. The first color change was observed at 10 minutes, this indicates that the synthesis of nanoparticles using gempur batu leaves has been formed. This is in accordance with the study of silver nanoparticles using leaves in previous studies (Willian et al., 2020; Aryasa et al., 2023). 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 constant, the color changes from clear, light yellow to brown and dark brown due to surface plasmon resonance, as shown in Figure 1.

UV-Vis Spectrophotometer Test Results of Silver Nanoparticles

The formation of AgNPss was confirmed by the analysis of UV-Vis absorption spectrum between 300–800 nm which was marked by the emergence of Surface Plasmon Resonance (SPR) peaks as shown in Figure 2. The AgNPs solution formed was taken 3 mL and its absorption spectrum was measured at room temperature. The baseline in this measurement was determined using distilled water as a solvent. The silver reduction process from Ag^+ ions to Ag^0 became the basis for the formation of SPR peaks in the spectrum analysis. As 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 emergence of the UV-Vis spectrum peak. The broad absorption peak appeared at a maximum λ of 450 nm. These results are also supported by the research of Isaac et al. (2013) where the synthesis of silver nanoparticles using Averrhoa bilimbi Linn. fruit extract displayed an SPR peak at 450 nm (Isaac et al., 2013). Furthermore, Salari et al. (2019) found the SPR peak on AgNPss in the range of 425–460 nm using Prosopis farcta fruit extract (Salari et al., 2019). The growth of silver nanoparticles can also be qualitatively observed from the shift of the SPR peak of silver nanoparticles. The position of the plasmon peak depends on the size and shape of the particles (Suárez-cerda et al., 2015).

Figure 1. Formed silver nanoparticle solution

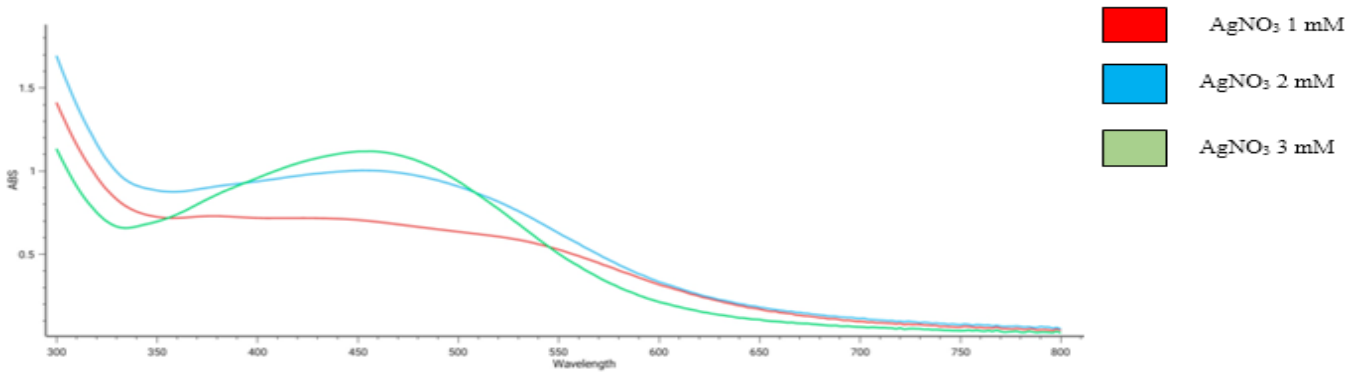
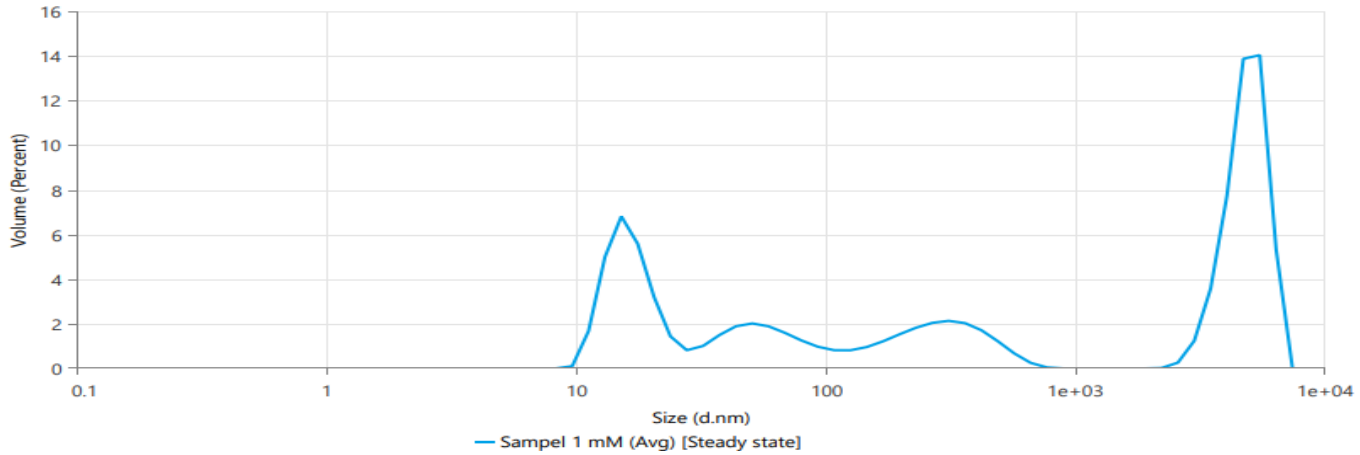


Figure 2. Absorption spectrum of silver nanoparticles with gempur batu leaf extract bioreductor with variations in $AgNO_3$ solution concentration (1, 2, and 3 mM)

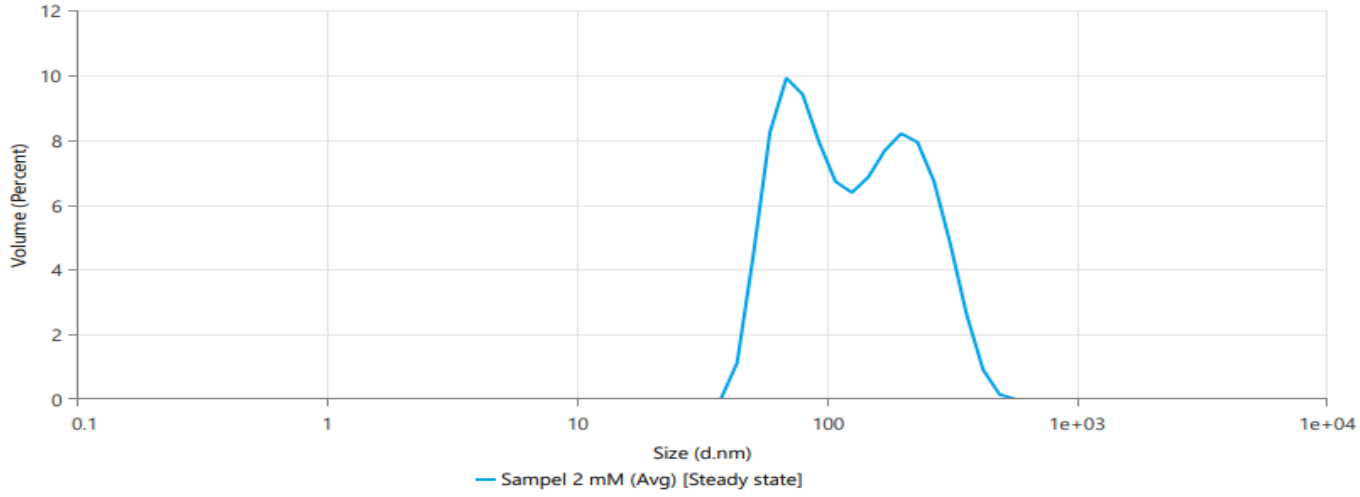
Silver Nanoparticle Size Distribution with PSA (Particle Size Analyzer)

The particle measurement method with PSA is considered more accurate in determining the 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 are in the form of three distributions, namely intensity, number and volume, so that they can describe the overall

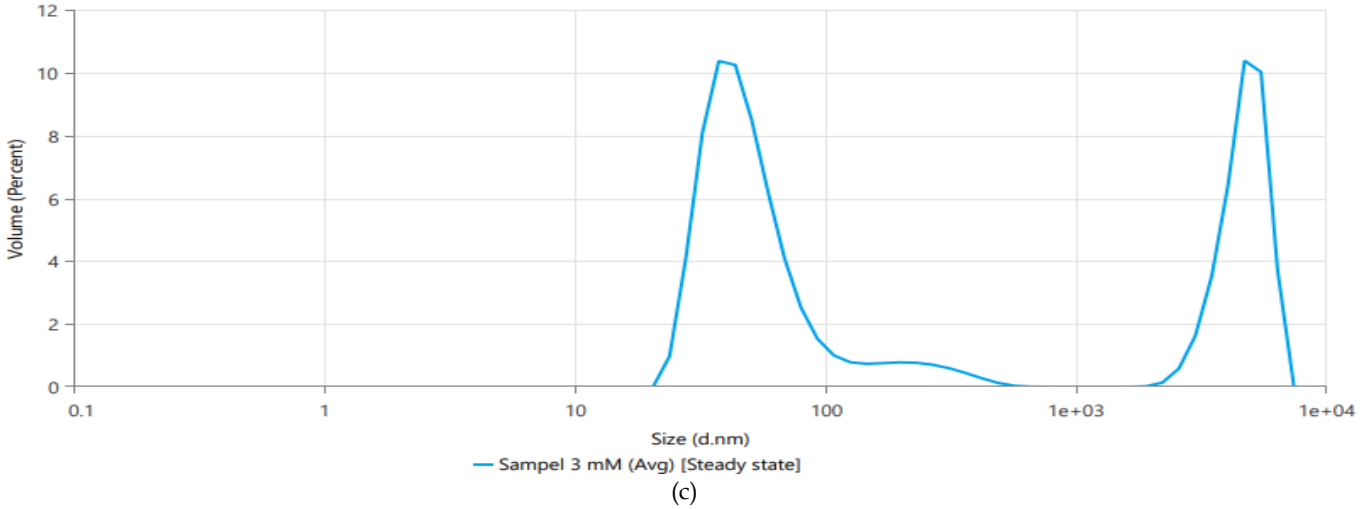
condition of the sample (Nikmatin et al., 2011). Figure 3 shows the particle size distribution of silver nanoparticles with gempur batu leaf extract bioreductor with variations in $AgNO_3$ solution concentration (1, 2, and 3 mM) which obtained average values of 148.8, 126.5, and 89.78 nm accompanied by all polydispersity indices with a value of 0.27, which means that the particles in the silver nanoparticle sample are categorized as highly heterogeneous nanoparticles (PDI below 0.4), this value is also in accordance with the results of research conducted by Ghasemi et al. (2024).



(a)



(b)



(c)

Figure 3. Size distribution graph of Silver Nanoparticles with gempur batu leaf extract with concentration variations of (a) $AgNO_3$ solution (1 mM), (b) $AgNO_3$ solution (2 mM) and (c) $AgNO_3$ solution (3 mM)

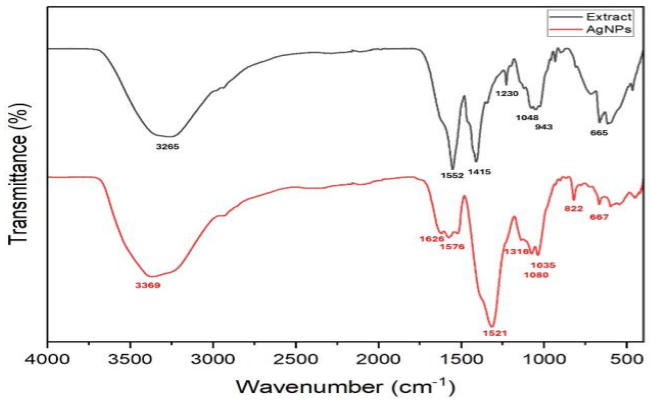


Figure 4. FTIR Spectrum of gempur batu leaf extract (*Ruellia napifera*) (A) and silver nanoparticles synthesized from gempur batu leaf extract (B)

FTIR Test Results of Silver Nanoparticles and Gempur Batu Leaf Extract Solution

FT-IR analysis was conducted to compare functional groups contained in water extracts from gempur batu (*Ruellia napifera*) plants with silver nanoparticles. In addition, it can also be used to determine the functional groups involved in reducing Ag+ ions to Ago. The following is a comparison of the FTIR spectrum profile of water extracts from gempur batu (*Ruellia napifera*) with silver nanoparticles (Figure 4).

Water extract from *Ruellia napifera* shows broad absorption with strong intensity at wave number 3256 cm-1 indicating the presence of stretching vibration of O-H bonds originating from alcohol or phenol compounds. This is reinforced by absorption with strong intensity at wave number 1048 cm^{-1} which is the stretching vibration of C-O bonds of Alcohol, wave number 1552 cm^{-1} indicating the presence of stretching vibration of N-O bonds originating from aromatic nitro compounds and absorption at wave number 934 cm^{-1} indicating the presence of C=C bonds of aromatic rings (Table 1). Changes in absorption intensity in the wave number region 3419 cm^{-1} (O-H bond vibration), 1552 cm^{-1} (N-O bond vibration) and 1048 cm^{-1} (C-O bond vibration) can provide clues to functional groups that play a role in the formation of silver nanoparticles as capping ligands.

Table 1. Functional Group of AgNPs

Functional Group	Wave number (cm^{-1})	
	Water extract of Gempur Batu leaves	Silver nanoparticles (AgNPss)
O-H stretching	3256	3369
C=C stretching	-	1626
N-O stretching	1552	1576
		1521
C-N stretching	1230	1316
C-H bending	1415	-
C-O stretching	1048	1080
		1035
C=C bending	934	822

XRD Test Results on Silver Nanoparticles

Figure 5 shows the XRD results of the synthesized AgNPss. The narrow peaks indicate the crystalline nature of the formed nanoparticles. Four peaks that appear continuously correspond to the detected 2θ values, namely 38.23, 44.44, 64.82, and 77.85° which represent the face-centered cubic (FCC) silver lattice with Miller indices (111), (200), (220), and (331), respectively. From the comparison of the intensity of the peak (111) compared to other diffraction peaks, it can be concluded that the plane (111) is the main orientation in the silver crystal structure of the synthesized AgNPss.

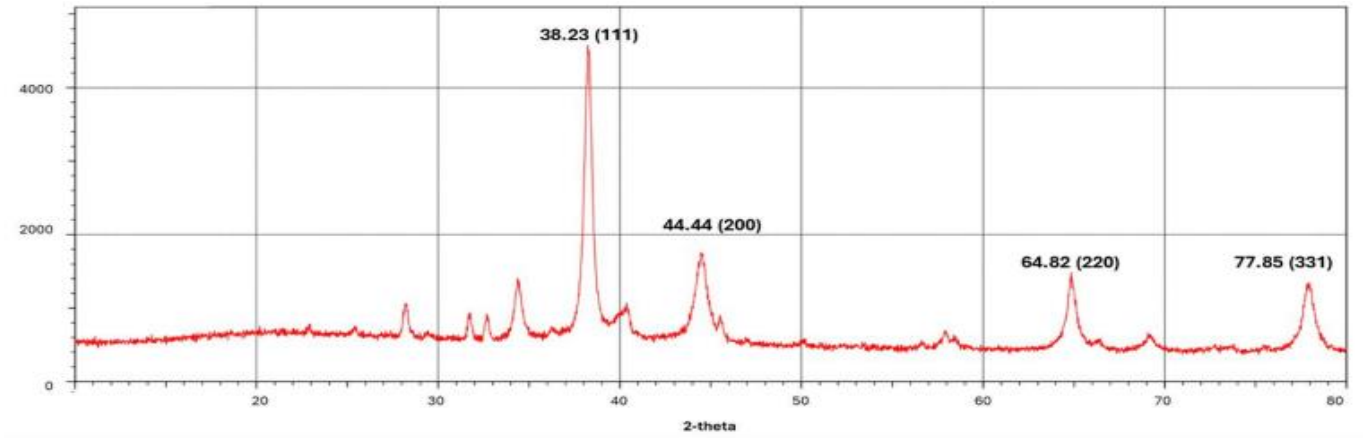


Figure 5. XRD pattern of silver nanoparticles synthesized with gempur batu leaf extract

Morphology of Silver Nanoparticles by TEM (Transmission Electron Microscopy)

The results of TEM analysis in this study are shown in Figure 6. The TEM image is a mixture of spherical, hexagonal, and triangular shapes of silver nanoparticles (Bhuvaneswari et al., 2017). This is identical to silver nanoparticles synthesized from cannonball leaves,

sembung leaves and tulak leaves (Preetha et al., 2013; Aryasa et al., 2023; Aryasa et al., 2022). Different compounds are present in gempur batu leaf extract such as polysaccharides, polyphenols, and proteins which are responsible for producing nanoparticles in various shapes.

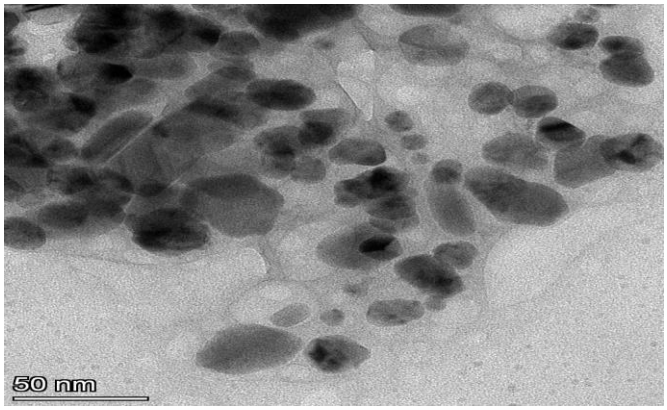


Figure 6. TEM morphology of silver nanoparticles with gempur batu leaf extract

Antibacterial Activity Test of Silver Nanoparticles with Gempur Batu Leaf Extract with Variations in $AgNO_3$ Solution Concentration

This study used the disc diffusion method to test the antibacterial activity of Silver Nanoparticles (AgNPs) with gempur batu leaf extract with variations in $AgNO_3$ solution concentrations of 1, 2, and 3 mM. This method was used with the aim of determining the inhibitory power caused by silver nanoparticles with gempur batu leaf extract with variations in $AgNO_3$ solution concentrations of 1, 2, and 3 mM on bacterial growth.

The results of the inhibition zone on *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 gempur batu leaf extract can be seen in Tables 2 and 3 which show that there is an inhibition zone on the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. Silver nanoparticles with gempur batu leaf extract with variations in $AgNO_3$ solution concentrations with concentrations of 1, 2, and 3 mM, when associated with the category of inhibition zone diameter stated by Sudrajat et al. (2012) where the inhibition zone formed > 20 mm is considered to have very strong inhibition, 11-20 mm is stated as strong inhibition, 6-10 mm is stated as moderate inhibition and < 5 nm is stated as weak inhibition. Each variant of this research data is calculated using standard deviation to determine the accuracy and precision of the measurements on the data obtained. Silver nanoparticles with gempur batu leaf extract with variations in $AgNO_3$ solution concentration of 1, 2, and 3 mM have an inhibition zone diameter of 9.4 ± 0.3 mm, 10.0 ± 0.3 and 10.0 ± 0.4 mm on *Escherichia coli* bacteria where the three concentrations are categorized as having moderate inhibition power.

In silver nanoparticles (AgNPs) with gempur batu leaf extract with variations in $AgNO_3$ solution concentration of 2 and 3 mM, the inhibition zone diameter is 8.5 ± 0.2 mm and 8.4 ± 0.3 mm, which is categorized as having moderate inhibition, but in silver nanoparticles (AgNPss) with gempur batu leaf extract with $AgNO_3$ solution concentration of 1 mM did not show an inhibition zone against *Staphylococcus aureus* bacteria. Based on the results of this study, silver nanoparticles with gempur batu leaf extract show antibacterial properties against *Staphylococcus aureus* bacteria.

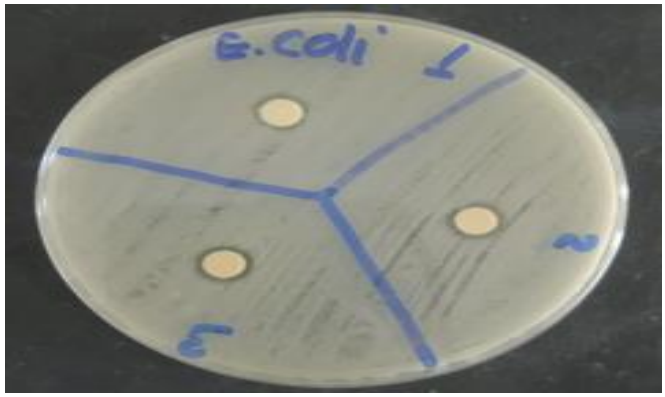


Figure 7. Antibacterial activity of silver nanoparticles with gempur batu leaf extract against bacteria in *Escherichia coli* (a) $AgNO_3$ 1 mM, (b) $AgNO_3$ 2 mM and (c) $AgNO_3$ 3 mM

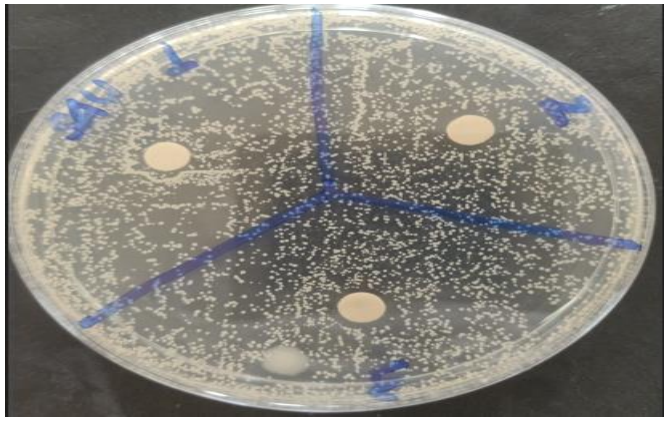


Figure 8. Antibacterial activity of silver nanoparticles with gempur batu leaf extract against bacteria in *Staphylococcus aureus* (a) $AgNO_3$ 1 mM, (b) $AgNO_3$ 2 mM and (c) $AgNO_3$ 3 mM

In general, the mechanism of silver nanoparticles can function as 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 the silver nanoparticle membrane depend on the size, shape, and surface that determine the success in damaging the bacterial membrane. Small-sized silver nanoparticles can interact better with the protective lignin layer in bacteria (Zheng et al., 2018).

Table 2. Results of the Inhibition Zone Diameter (mm) of Silver Nanoparticles with Gempur Batu Leaf Extract Bioreductor with Variations in $AgNO_3$ Solution Concentration Against *Escherichia coli* Bacteria

Test Solution	Average Diameter of Inhibition Zone (mm)				Average (mm)	SD (mm)	Mean ± SD (mm)
	I	II	III	IV			
Control (+)	15.0	15.4	14.6	14.4	14.9	0.4	14.9 ± 0.4
Control (-)	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
Silver Nanoparticles 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0 ± 0.0
Silver Nanoparticles 2	8.3	8.5	8.7	8.6	8.5	0.2	8.5 ± 0.2
Silver Nanoparticles 3	8.2	8.4	8.8	8.2	8.4	0.3	8.4 ± 0.3

Table 3. Results of the Inhibition Zone Diameter (mm) of Silver Nanoparticles with Gempur Batu Leaf Extract Bioreductor with Variations in $AgNO_3$ Solution Concentration Against *Staphylococcus aureus* Bacteria

Test Solution	Average Diameter of Inhibition Zone (mm)				Average (mm)	SD (mm)	Mean ± SD (mm)
	I	II	III	IV			
Control (+)	41.8	43.5	43.8	42.6	42.9	0.9	42.9 ± 0.6
Control (-)	0.00	0.00	0.00	0.00	0.0	0.0	0.0 ± 0.0
Silver Nanoparticles 1	7.7	7.1	7.4	7.5	7.4	0.3	7.4 ± 0.3
Silver Nanoparticles 2	8.8	8.2	7.8	8.3	8.3	0.4	8.3 ± 0.4
Silver Nanoparticles 3	9.0	8.6	8.6	8.4	8.7	0.3	8.7 ± 0.3

Antioxidant Activity of Silver Nanoparticles and Ascorbic Acid

Antioxidants are substances that counteract free radicals and protect against degenerative diseases, such as cancer, Parkinson's disease, Alzheimer's disease, and atherosclerosis, which are caused by oxidative stress (excess 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 showed that AgNPs have antioxidant properties at various concentrations (1–15 ppm) as determined using the DPPH assay. The results showed that the inhibition percentage increased as the concentration of AgNPss and ascorbic acid increased from 1 to 5 ppm (Table 4). Higher concentrations of AgNPss and ascorbic acid were used in the DPPH experiment to measure antioxidant activity. The higher polyphenol content of leaf extracts restricted to AgNPss may be the reason for the increased antioxidant capacity (Moorthy et al., 2022). By effectively reducing reactive oxygen species, AgNPss showed a broad spectrum of antioxidant activity in this study (Berridge et al., 2005).

Inhibitory Activity of Biofilm Formation Using Silver Nanoparticles with Gempur Batu Extract

To evaluate the antibiofilm activity of the extracts with three sub-minimum inhibitory concentrations, this study focused on *Staphylococcus aureus* and *Escherichia coli* bacteria, since these bacteria have the best biofilm formation ability in their respective species. The results of the investigation are presented in Table 5, where the percentage of biofilm inhibition is documented. In our analysis, silver nanoparticles with varying concentrations of $AgNO_3$ solution (1, 2, and 3 mM) with any gempur batu leaf extract showing an inhibition percentage exceeding 50% were considered to have substantial anti-biofilm activity. In contrast, extracts with inhibition percentages ranging from 0 to 50% were categorized as having limited or poor anti-biofilm activity. It is important to note that the values equal to or greater than 100%, were recorded as 100%, indicating complete inhibition of biofilm formation, while values less than or equal to 0% were considered to have no effect. The observed anti-biofilm activity was consistent with the criteria set by Sandasi et al. (2008). The findings in this study revealed the biofilm inhibitory activity of silver nanoparticles with varying concentrations of $AgNO_3$ solution (1, 2, and 3 mM) with gempur batu leaf extract against *Staphylococcus aureus* and *Escherichia coli* bacteria (Table 5). All silver nanoparticles with varying concentrations of $AgNO_3$ solution (1, 2, and 3 mM) with gempur batu leaf extract exhibited biofilm inhibition values of more than 50% against *Staphylococcus aureus* and *Escherichia coli* bacteria, these results are in accordance with research conducted by Akinboye et al. (2024).

Table 4. Percentage Inhibition Values of Silver Nanoparticles and Ascorbic Acid

Sample	Concentration (ppm)	% Inhibition
Silver Nanoparticles 1 (1 mM $AgNO_3$)	1	13.3
	2	72.4
	3	143.4
	4	247.0
	5	265.4
Silver Nanoparticles 2 (1 mM $AgNO_3$)	2	77.0
	4	106.9
	6	207.0
	8	275.1
	10	391.6
Silver Nanoparticles 3 (1 mM $AgNO_3$)	3	201.6
	6	297.7
	9	397.7
	12	473.7
	15	513.9
Ascorbic Acid	1	17.54
	2	21.84
	3	27.27
	4	35.34
	5	41.57

Table 5. Antibiofilm Activity of Silver Nanoparticles with Gempur Batu Leaf Extract

Name of Bacteria	Nanoparticle Samples	Biofilm Inhibition Percentage (%)
Staphylococcus aureus ATCC 25923	Silver Nanoparticles 1	70.92
	Silver Nanoparticles 2	87.73
	Silver Nanoparticles 3	93.29
Escherichia coli ATCC 25922	Silver Nanoparticles 1	63.86
	Silver Nanoparticles 2	73.35
	Silver Nanoparticles 3	83.31

Conclusion

Gempur batu leaf extract has been effectively used to synthesize AgNPs, and the benefits of gempur batu leaves have been proven as a natural ingredient that acts as a silver bioreduction agent and capping agent. Characterization of synthesized nanoparticles using UV-visible, FTIR, TEM, PSA and XRD studies have shown the results of the formation of AgNPs and reinforced with functional groups of gempur batu leaf extract with silver nanoparticle sizes with gempur batu leaf extract bioreductors with variations in $AgNO_3$ solution concentrations (1, 2, and 3 mM) obtained average values of 148.8, 126.5, and 89.78 nm, with spherical, hexagonal, and triangular shapes of silver nanoparticles. AgNPs show quite good antibacterial activity and have antioxidant activity as indicated by the percentage of inhibition increasing with increasing concentration of AgNPs and ascorbic acid increasing from 1 to 15 ppm. Antibiofilm activity of AgNPs has good ability to inhibit the formation of biofilm layer in Staphylococcus aureus and Escherichia coli bacteria as seen from the percentage of silver nanoparticle antibiofilm. Therefore AgNPs are very important for biomedicine as antibacterial and antioxidant in the pharmaceutical sector.

Acknowledgments

The researchers would like to thank the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia because this research has been fully funded through the Beginner Lecturer Research Grant scheme from the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia in 2024. The authors also express their appreciation to all parties who have helped during this research.

Author Contributions

I.W.T.A.: Conceptualization, methodology, investigation, writing—original draft preparation; data curation, and software. N.P.R.A: validation, formal analysis. P.Y.B.S: writing—review and editing, visualization, supervision. All authors have read and agreed to the published version of the manuscript.

Funding

This research has been fully funded through the Beginner Lecturer Research Grant scheme from the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia in 2024.

Conflicts of Interest

All author declares that there is no conflict of interest.

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