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Synthesis of Silver Nanoparticles Using Nyamplung Leaf Extract (*Calophyllum Inophyllum* L.) As A Bioreducing Agent with Microwave Irradiation and Evaluation of Its Antibacterial Activity

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Abstract

Silver nanoparticles (AgNPs) were synthesized using a bioreduction method, with nyamplung leaf extract (*Calophyllum inophyllum L.*) serving as a reducing agent for $AgNO_3$, in which Ag^+ was reduced to Ag^0 through microwave irradiation. The formation of AgNPs was indicated by a color change and subsequently analyzed by measuring the absorption wavelength using UV-Vis spectrophotometry, FT-IR, and Particle Size Analyzer (PSA). The AgNPs were successfully synthesized under the optimum conditions of an $AgNO_3$ concentration of 0.0025 M, an optimum $AgNO_3$ solution-to-amplung leaf extract volume ratio of 1:1, and an optimum storage time of 8 days. The average particle size of the synthesized AgNPs was 107.1 nm. The antibacterial activity test showed that the silver nanoparticles synthesized using nyamplung leaf extract exhibited strong antibacterial activity against S. aureus and E. coli, with inhibition zone diameters of 12 mm and 10.3 mm, respectively.

Keywords: Silver nanoparticles, bioreduction, Calophylluminophyllum L., microwave, antibacterial activity

INTRODUCTION

Nanotechnology is one of the fields experiencing rapid development because it plays an important role in the application of science and technology, as well as in many other areas. In general, nanotechnology involves the engineering, production, and application of structures or materials at the nanometer scale, particularly in dimensions ranging from 1 to 100 nm. The rapid development of nanotechnology is driven by the fact that materials produced at the nanoscale can possess multifunctional properties and exhibit superior physical and chemical characteristics compared to their larger-scale counterparts. One of the current areas of extensive nanotechnology research is the synthesis of noble metal nanoparticles, such as silver and gold (Ariyanta, 2014).

Silver has advantages over gold due to its superior optical properties (Mathur et al., 2018). Silver nanoparticles have been extensively studied because their size and shape can significantly influence their optical and magnetic properties, making them effective antibacterial agents. The antibacterial properties of silver nanoparticles are influenced by several factors, including concentration, particle size and shape, and the contact time between the nanoparticles and bacteria. In general, smaller silver nanoparticles exhibit stronger antibacterial effects (Heydaryan, 2026). Their smaller size allows for better contact with bacteria or fungi, thereby increasing the effectiveness of their bactericidal and fungicidal properties (Madivoli et al., 2020).

In general, nanoparticle synthesis can be carried out using two methods: top-down (physical) and bottom-up (chemical) approaches. The physical method involves breaking down larger particles into smaller or nanoscale particles, whereas the chemical method involves the formation of nanoparticles through the combination or assembly of atoms or molecules (Aperti, 2018). Nanoparticle synthesis using the top-down (physical) method is considered less effective and has several drawbacks, including high energy consumption, which can contribute to environmental pollution, relatively high costs, and the production of particles with non-uniform sizes. Therefore, the bottom-up method based on green synthesis is considered a better alternative to the top-down method. Green synthesis is an alternative approach based on the principles of green chemistry. This method is simpler, safer, more environmentally friendly, and more cost-effective because it utilizes natural materials, such as plants and microorganisms, as bioreducing agents (Hudaya et al., 2023).

The use of natural materials, particularly plant extracts, as bioreducing agents in the synthesis of silver nanoparticles is highly advantageous because they are readily available and do not require complex culture maintenance, as is the case with microorganisms, resulting in a shorter synthesis time (Hammood et al., 2026). In addition, plant extracts are non-toxic, require relatively small amounts of chemicals, and contain various metabolites that can act as bioreducing agents in the synthesis of silver nanoparticles.

Plant-derived extracts have been widely employed as bioreducing agents in the green synthesis of silver nanoparticles (AgNPs). These extracts contain various bioactive constituents that are capable of converting Ag^+ ions present in a solution into metallic silver (Ag^0), thereby facilitating the formation of silver nanoparticles. The phytochemicals responsible for this reduction process may include terpenoids, phenolic compounds, flavonoids, tannins, steroids, saponins, and alkaloids (Priya et al., 2016). Nyamplung (*Calophyllum inophyllum* L.) is one plant species that contains several of these bioactive compounds and therefore has potential to serve as a natural reducing agent in the biosynthesis of silver nanoparticles

The nyamplung plant (*Calophyllum inophyllum L.*) is a type of tree that commonly grows in coastal and rocky areas. In Indonesia, this plant is found in almost all regions, including Maluku (Sudrajat et al., 2007). Traditionally, the people of Maluku have used nyamplung as a medicinal plant to treat various conditions, such as rashes, boils, and wounds. Nyamplung can also be used as a source of biodiesel, as its seeds can produce a considerable amount of oil (Kurniaty et al., 2021; Musta et al., 2017; Sutapa et al., 2013), while its leaves can be used as a bioreducing agent. The nyamplung plant contains various bioactive chemical compounds, including secondary metabolites from the flavonoid, saponin, alkaloid, tannin, and steroid groups. These compounds have the potential to function as antibacterial agents as well as reducing agents in the synthesis of silver nanoparticles (Artanti et al., 2020).

Conventional approaches have traditionally been employed for the synthesis of silver nanoparticles; however, these methods may require relatively prolonged reaction times and may provide limited control over the uniformity of the synthesized nanoparticles. Microwave-assisted synthesis has therefore attracted considerable attention as an alternative approach for accelerating nanoparticle formation. Microwave irradiation can promote rapid and relatively uniform heating of the reaction medium, thereby facilitating the reduction of Ag^+ ions and influencing the nucleation and subsequent growth of silver nanoparticles. This approach has been reported to enable shorter reaction times while producing nanoparticles with improved consistency and size uniformity (Rahim et al., 2020).

The application of microwave irradiation in the green synthesis of silver nanoparticles has previously been demonstrated using betel leaf extract as a bioreducing agent. Purnamasari, Harjono, & Wijayati (2016) synthesized silver nanoparticles from betel leaf extract under microwave irradiation and subsequently evaluated their physicochemical characteristics using UV–Vis spectrophotometry, transmission electron microscopy (TEM), and particle size analysis (PSA). TEM analysis revealed predominantly spherical crystalline nanoparticles with a particle size of approximately 11.94 nm, while PSA indicated an average particle size of 37.44 nm. The synthesized nanoparticles exhibited a face-centered cubic (FCC) crystalline structure. Furthermore, the study demonstrated that the antibacterial activity of the resulting silver nanoparticles was influenced by the concentration of $AgNO_3$ used during synthesis, with higher $AgNO_3$ concentrations associated with enhanced antibacterial activity.

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METHODOLOGY

Materials and Instrumentals

The materials employed in this study comprised fresh nyamplung leaves (Calophyllum inophyllum L.) and silver nitrate ($AgNO_3$; Merck) as the primary materials for nanoparticle synthesis. Aquabidest and distilled water were used throughout the experimental procedures. Staphylococcus aureus and Escherichia coli bacterial cultures were used for the antibacterial assay, with Nutrient Agar (NA) and Nutrient Broth (NB) serving as the respective growth media. Amoxicillin was used as the reference antibacterial agent. Additional laboratory supplies included labeling paper, aluminum foil, and Whatman No. 42 filter paper. The synthesized silver nanoparticles were characterized using a UV–Vis spectrophotometer (Shimadzu UV-1800), Fourier-transform infrared (FTIR) spectrophotometer (Shimadzu), and particle size analyzer (PSA).

Methods

Preparation of Nyamplung Leaf Extract (Bioreducing Agent)

The nyamplung leaves were initially washed thoroughly to remove adhering impurities and subsequently dried before being cut into smaller pieces. Approximately 25 g of the prepared leaves was transferred into a 250 mL beaker, followed by the addition of aquabidest to the predetermined volume. The mixture was heated for 30 min to facilitate the extraction of phytochemical constituents and then cooled to room temperature. After cooling, the extract was separated from the plant residue by filtration through Whatman No. 42 filter paper. The resulting filtrate was collected and subsequently employed as a bioreducing agent for the microwave-assisted synthesis of silver nanoparticles (AgNPs).

Synthesis of Silver Nanoparticles with Concentration Optimization

Silver nitrate ($AgNO_3$) solutions with concentrations of 0.001, 0.0015, 0.002, and 0.0025 M were prepared, and 6 mL of each solution was transferred into separate Erlenmeyer flasks. Subsequently, 3 mL of nyamplung leaf extract (*Calophyllum inophyllum* L.) was added to each $AgNO_3$ solution. The resulting mixtures were subjected to microwave irradiation at 30 °C for 10 min using a microwave equipped with magnetic stirring. The formation of silver nanoparticles was preliminarily indicated by a visible color transition from yellow to brown. Following synthesis, the resulting AgNP suspensions were subjected to UV–Vis spectrophotometric analysis over the wavelength range of 400–500 nm to monitor the characteristic optical absorption associated with nanoparticle formation.

Synthesis of Silver Nanoparticles with Volume Ratio Optimization

To determine the optimum extract-to-$AgNO_3$ ratio, different volumes of aqueous nyamplung leaf extract (25, 10, 9, 2, 4, 5, and 6 mL) were transferred into seven separate Erlenmeyer flasks. The optimized $AgNO_3$ solution was subsequently added to each flask in volumes of 25, 20, 27, 16, 24, 40, and 60 mL, respectively, corresponding to extract-to-$AgNO_3$ volume ratios of 1:1, 1:2, 1:3, 1:4, 1:6, 1:8, and 1:10. Each reaction mixture was subjected to microwave irradiation at 30 °C for 10 min under magnetic stirring. The formation of AgNPs was initially indicated by a characteristic color change from yellow to brown. The resulting nanoparticle suspensions were subsequently characterized by UV–Vis spectrophotometry within the wavelength range of 400–500 nm to evaluate their optical absorption characteristics and identify the surface plasmon resonance associated with AgNP formation.

Synthesis of Silver Nanoparticles with Storage Time Optimization

Nyamplung leaf extract (25 mL) was pipetted into an Erlenmeyer flask, followed by the addition of 25 mL of $AgNO_3$ solution at the optimum concentration (0.0025 M), using the optimum volume ratio of 1:1. The mixture was then heated using a modified microwave at 30°C for 10 minutes until its color changed from yellow to brown. Characterization was performed by observing the UV-Vis absorption spectrum in the wavelength range of 400–500 nm at 0, 1, 2, 3, 4, 5, 7, 8, 9, 10, 15, and 20 days after mixing.

Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were initially evaluated by UV–Vis spectrophotometry to assess their characteristic optical absorption. The sample exhibiting the most favorable synthesis conditions was subsequently selected for further characterization using a Particle Size Analyzer (PSA) to determine the particle-size distribution and mean hydrodynamic diameter. Fourier-transform infrared (FTIR) spectroscopy was also performed to identify the functional groups of phytochemical constituents potentially involved in the reduction and stabilization of the synthesized silver nanoparticles.

Antibacterial Activity Test

The antibacterial activity test was conducted according to the procedure using nyamplung leaf extract at the optimum concentration and solvent as a control, and the antibacterial activity was compared with that of samples synthesized using microwave irradiation.

Bacterial Suspension Preparation

Bacterial suspensions of *Escherichia coli* and *Staphylococcus aureus* were prepared using Nutrient Broth (NB) as the growth medium. Briefly, a single loopful of each bacterial colony obtained from solid Nutrient Agar (NA) was aseptically inoculated into an Erlenmeyer flask containing 30 mL of NB. The cultures were subsequently incubated at 37 °C for 24 h to allow bacterial growth. Prior to the antibacterial assay, the resulting suspensions were standardized to a final cell density of approximately 10^6 CFU/mL.

Antibacterial Testing Using the Well Diffusion Method

The antibacterial activity of the synthesized silver nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus*, which had been previously purified and maintained through laboratory subculturing. The assay was performed using a double-layer Nutrient Agar (NA) diffusion method. The lower layer functioned as the nutrient base, whereas the upper layer was prepared by incorporating the bacterial suspension at a cell density of 10^6 CFU/mL into the molten NA medium. After solidification, wells measuring approximately 6–7 mm in diameter were aseptically prepared in the upper agar layer using a sterile well punch. Separate two-layer NA plates were prepared for *E. coli* and *S. aureus*. Subsequently, 40 µL of the synthesized AgNP suspension obtained from nyamplung leaf extract was introduced into three wells on each bacterial plate. Distilled water (40 µL) was used as the negative control, while amoxicillin (40 µL) served as the positive control. The inoculated plates were incubated at 37 °C for 24 h. Following incubation, the diameter of the clear inhibition zones surrounding each well was measured and recorded to assess the antibacterial activity of the synthesized AgNPs.

RESULTS AND DISCUSSION

Nyamplung Leaf Extraction

The nyamplung leaf samples were cleaned and dried to remove impurities. The leaves were then cut into small pieces to increase the surface area, thereby facilitating contact between the sample and solvent and accelerating the extraction process. The nyamplung leaf extract was prepared by dissolving 25 g of chopped nyamplung leaves in 250 mL of aquabidest and then heating the mixture. The mixture was filtered to obtain a cloudy yellow extract, which was used as a bioreducing agent in the synthesis process.

Concentration Optimization

The optimization of $AgNO_3$ concentration in the synthesis of silver nanoparticles was conducted to determine the optimum $AgNO_3$ concentration that produces the best AgNPs. In this study, $AgNO_3$ solutions with concentrations of 0.001, 0.0015, 0.002, and 0.0025 M were used. AgNPs can be readily detected using UV-Vis spectrophotometry based on their characteristic Surface Plasmon Resonance (SPR). SPR is strongly dependent on the size, shape, and dielectric properties of the particles and their surrounding medium; therefore, different types of nanoparticles exhibit different SPR ranges (Wendri et al., 2017). The optimum $AgNO_3$ concentration for the nanoparticle synthesis was determined by examining the absorbance values obtained from the UV-Vis spectrophotometer, as shown in Figure 1.

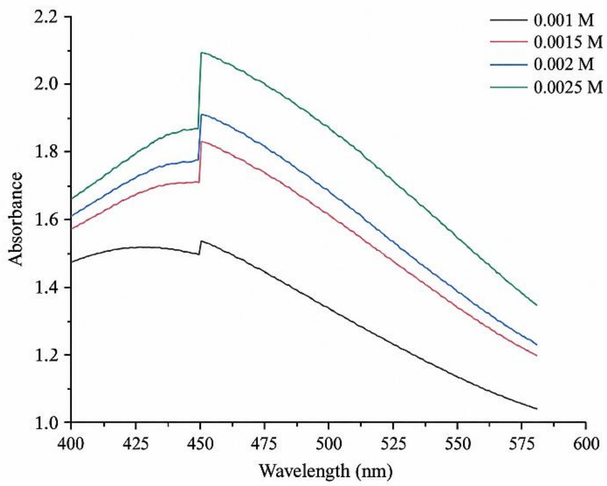

Figure 1. UV-Vis Spectra of AgNPs at Various $AgNO_3$ Concentrations

The UV-Vis absorption measurements at a wavelength range of 400–500 nm for the predetermined concentration variations yielded an absorbance of 2.105, with a maximum absorption wavelength of approximately 430.4 nm. This result is consistent with the characteristic Surface Plasmon Resonance (SPR) of silver nanoparticles, which occurs in the range of 400–450 nm (Velgosova et al., 2022). The results are presented in Table 1.

Table 1. Maximum Absorbance at Various $AgNO_3$ Concentrations

	Maximum Absorbance

Maximum Wavelength (nm)	$AgNO_3$ 0.001 M	$AgNO_3$ 0.0015 M	$AgNO_3$ 0.002 M	$AgNO_3$ 0.0025 M
430.4	1.459	1.793	1.876	2.105
430.6	1.458	1.791	1.877	2.105

The optimum concentration of $AgNO_3$ in the presence of nyamplung leaf extract as a bioreducing agent was determined to be 0.0025 M. At this optimum concentration, the bioreducing compounds present in the extract effectively reduced Ag^+ ions to metallic Ag^0. This finding was indicated by a shift in the surface plasmon resonance (SPR) peak toward longer wavelengths, accompanied by a decrease in absorbance intensity. The shift toward longer wavelengths is characteristic of a red shift, which may indicate changes in the physicochemical properties and particle size of the synthesized silver nanoparticles (Xuan Hoa et al., 2018).

Optimization of Volume Ratio

Optimization of the volume ratio in the synthesis of silver nanoparticles (AgNPs) was conducted to determine the optimum ratio capable of producing nanoparticles with the most desirable characteristics. $AgNO_3$ solution at the previously determined optimum concentration (0.0025 M) was reacted with nyamplung leaf extract using various volume ratios of nyamplung leaf extract to $AgNO_3$ solution, namely 1:1, 1:2, 1:3, 1:4, 1:6, 1:8, and 1:10.

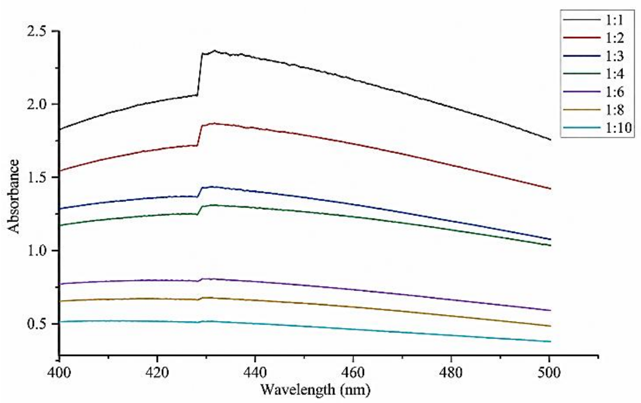

Figure 2. UV–Vis spectra of silver nanoparticles (AgNPs) at various volume ratios

As shown in Figure 2, the 1:1 volume ratio exhibited a more intense yellowish-brown coloration compared with the other volume ratios. This more pronounced coloration indicates a greater formation of silver nanoparticles (AgNPs) at this ratio. A similar finding was reported by Parveen et al., (2025), who stated that the volume of plant extract used should be proportional to the amount of $AgNO_3$ added. The coloration of metal nanoparticles is influenced by their shape and size. This observation is consistent with the findings of Essghaier et al., (2023), who reported that a more intense yellowish-brown coloration corresponds to a greater amount of AgNPs formed. The optimization of the volume ratio between $AgNO_3$ and nyamplung leaf extract in the AgNP synthesis was further evaluated using UV–Vis spectrophotometry.

Based on the UV–Vis absorption measurements conducted over the wavelength range of 400–500 nm for the various volume ratios, the optimum absorbance was 2.374 at a wavelength of 430.8 nm. As shown in Table 2, among all the volume ratios of $AgNO_3$ solution to nyamplung leaf extract, the 1:1 ratio produced the highest absorbance compared with the other ratios. This result indicates that the 1:1 volume ratio was the most favorable condition for the formation of silver nanoparticles (AgNPs).

Table 2. UV–Vis Measurement Results for Different Volume Ratios of Nyamplung Leaf Extract to $AgNO_3$ Solution

Maximum Wavelength (nm)	Maximum Absorbance						
	1:1	1:2	1:3	1:4	1:6	1:8	1:10
430.4	2.374	1.891	1.440	1.131	0.806	0.680	0.498
430.6	2.374	1.892	1.440	1.131	0.805	0.680	0.498

Silver nanoparticles (AgNPs) were successfully synthesized using nyamplung leaf extract as a bioreducing agent. The synthesis was carried out by reacting 25 mL of nyamplung leaf extract with 25 mL of $AgNO_3$ solution at an optimum concentration of 0.0025 M, corresponding to a 1:1 volume ratio, using a modified microwave-assisted method. The formation of AgNPs was visually indicated by a color change in the reaction solution from cloudy yellow to yellowish-brown. A comparison of the nyamplung leaf extract before and after the reaction is presented in Figure 3.

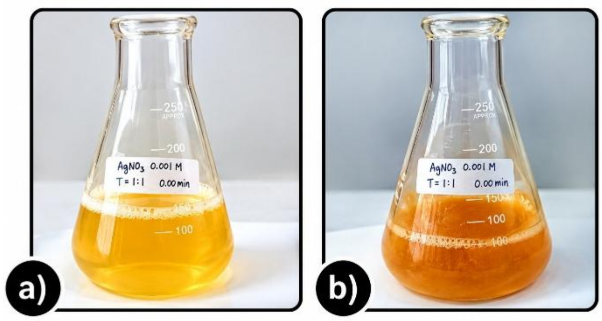

Figure 3. AgNP synthesis: (a) before microwave irradiation and (b) after microwave irradiation

The color change observed during the synthesis of silver nanoparticles (AgNPs) can be attributed to the reduction of Ag^+ ions and the subsequent formation of metallic silver (Ag^0). In their ionic form, Ag^+ ions in $AgNO_3$ repel one another due to their like positive charges. However, upon reduction by biomolecular compounds, such as polyphenols and flavonoids

present in the aqueous nyamplung leaf extract, Ag^+ ions are converted into neutral Ag^0 atoms. The neutral Ag^0 atoms can then approach one another and interact through intermetallic bonding, leading to the formation of nanoscale silver clusters (Essghaier et al., 2023). The general reaction equation for the formation of AgNPs is presented in Figure 4.

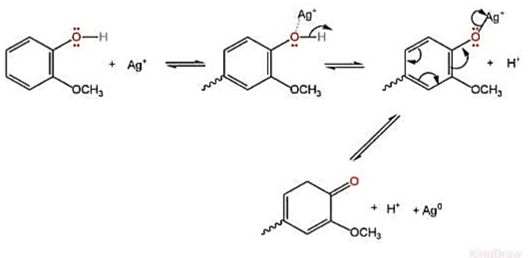

Figure 4. General reaction mechanism for the formation of silver nanoparticles (AgNPs) (Putra & Lestari, 2024)

Storage Time Optimization

Storage time optimization was conducted to evaluate the effect of storage duration on the formation and stability of silver nanoparticles (AgNPs). According to Morales-Sánchez et al. (2011), storage duration during the synthesis process can affect the amount of AgNPs formed. As shown in Appendix 2, differences in the characteristic color of the colloidal AgNPs were observed over the storage period, which can be attributed to surface plasmon resonance (SPR) arising from the silver nanoparticles formed (Sutapa et al., 2013). Based on the observations, the resulting AgNP colloids exhibited various colors, ranging from pale yellow, cloudy yellow, and yellowish-brown to dark brown, depending on the storage duration. From day 0 to day 8, noticeable changes in color were observed, indicating variations in the absorbance values of the samples. However, from day 9 to day 20, no further visible color changes were observed, suggesting that the formation of AgNPs had reached a relatively stable state. This observation is consistent with the UV–Vis measurements presented in Appendix 3, which showed relatively stable absorbance values from day 9 to day 20. The optimum storage time was further determined by monitoring the AgNP samples using UV–Vis spectrophotometry daily from day 0 to day 9. The UV–Vis absorption spectra obtained throughout the observation period are presented in Figure 5.

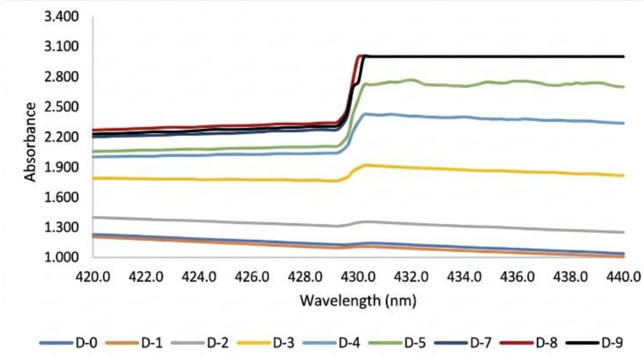

Figure 3. UV–Vis spectra of AgNPs at different storage times (0–9 days)

Based on the UV–Vis absorption measurements obtained over the storage period, as shown in Figure 8, shifts in the maximum absorption wavelength (λmax) and changes in absorbance values were observed. The absorbance increased with increasing storage time. A relatively substantial increase in absorbance was observed from day 0 to day 5, whereas from day 7 to day 9, the increase in absorbance was relatively small from one day to the next. The relatively small increase in absorbance during the synthesis process indicates good stability of the formed nanoparticles. In this study, the optimum storage time was determined to be 8 days. This finding is supported by the UV–Vis spectrum presented in Figure 8, where a decrease in absorbance was observed on day 9. The corresponding absorbance and maximum wavelength values are also presented in Table 3.

Table 3. UV–Vis Measurement Results for the Optimum Storage Time

Maximum Wavelength (nm)	Maximum Absorbance								
	D-0	D-1	D-2	D-3	D-4	D-5	D-7	D-8	D-9
430.4	1.142	1.114	1.349	1.865	2.266	2.431	2.757	2.785	2.745
430.6	1.146	1.118	1.357	1.898	2.355	2.589	3.000	3.000	3.000

The relatively small increase in absorbance during the synthesis process indicates good stability of the nanoparticles formed. In this study, the optimum storage time was determined to be 8 days. This finding is supported by the UV–Vis spectrum presented in Figure 3, which shows a decrease in absorbance on day 9. As presented in Table 3, the decrease in absorbance indicates that the sample solution had begun to reach saturation, potentially due to nanoparticle aggregation. The occurrence of aggregation was further evidenced by the formation of sediment at the bottom of the Erlenmeyer flask, indicating the formation of particles that were no longer within the nanoscale range (Ragunath et al., 2024). Therefore, the optimum formation of AgNPs was achieved on day 8, with an optimum absorption wavelength (λmax) of 430.0 nm.

Characterization of Silver Nanoparticles (AgNPs)

The AgNPs synthesized using aqueous nyamplung leaf extract as a bioreducing agent under the optimum conditions—an $AgNO_3$ concentration of 0.0025 M, an $AgNO_3$-to-extract volume ratio of 1:1, and an optimum storage time of 8 days—were subsequently subjected to further characterization. Particle Size Analysis (PSA) was performed to determine the average particle size of the synthesized AgNPs, while Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups involved in the bioreduction process.

PSA (Particle Size Analyzer)

PSA characterization was conducted to determine the average particle size of the AgNPs synthesized using nyamplung leaf extract as a bioreducing agent through a modified microwave-assisted synthesis method.

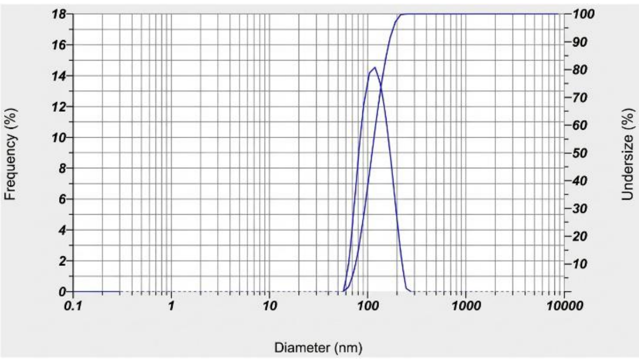

Figure 4. Particle size distribution of AgNPs synthesized using nyamplung leaf extract as a bioreducing agent

As shown in Figure 4, the synthesized AgNPs exhibited an average particle size of 107.1 nm. This value exceeds the commonly accepted nanoscale range of less than 100 nm. The relatively large particle size may be attributed to the occurrence of nanoparticle agglomeration or aggregation, resulting in larger particle clusters and consequently reducing the proportion of particles within the nanoscale range.

Characterization using Particle Size Analysis (PSA) provides information on the average particle size of the nanoparticles; therefore, the smallest and largest particle sizes of the AgNPs present in the sample cannot be directly determined from the PSA results alone. This represents one of the limitations of PSA characterization. The analysis also provides the Polydispersity Index (PDI), which describes the distribution of particle sizes within the sample. According to Nury (2023), a PDI value greater than 1.0 indicates a broad and non-uniform particle size distribution, whereas a PDI value below 1.0 indicates a more homogeneous particle size distribution. In this study, the PDI value obtained was 0.127, which is below 1.0, indicating a relatively narrow and homogeneous particle size distribution. Thus, the synthesized AgNPs exhibited a relatively uniform particle size distribution.

FT-IR (Fourier Transform Infrared)

FTIR characterization of the synthesized AgNPs was performed to compare the functional groups present in the aqueous nyamplung leaf extract before and after the addition of $AgNO_3$ during AgNP formation. In addition to identifying changes in the functional groups, FTIR analysis was used to determine the functional groups potentially involved in the synthesis process, particularly in the bioreduction of Ag^+ ions to metallic Ag^0.

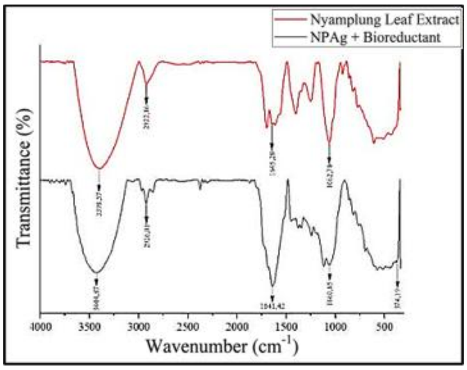

Figure 5. FTIR spectra of nyamplung leaf extract (bioreducing agent) and AgNPs synthesized using the bioreducing agent

Figure 5 presents the FTIR spectra of the aqueous nyamplung leaf extract as the bioreducing agent and the AgNP sample synthesized using the bioreducing agent, recorded over the wavenumber range of 500–4000 cm^{-1}. According to previous research by Panjaitan, Syamsurizal, Maharini, Putra, & Septiana (2025), characteristic signals associated with synthesized AgNPs can be observed in the wavenumber ranges of 3500–3200 cm⁻¹, approximately 2300 cm⁻¹, 2100–2000 cm⁻¹, 1610–1650 cm⁻¹, and 1610–600 cm⁻¹. The FTIR spectrum of the synthesized AgNPs exhibited a broad absorption band at 3444.87 cm⁻¹, while the aqueous nyamplung leaf extract showed an absorption band at 3398.57 cm⁻¹. These bands are characteristic of the O–H stretching vibration of phenolic hydroxyl groups. Furthermore, weak absorption bands were observed at 2926.01 cm⁻¹ and 2922.16 cm⁻¹, which can be attributed to the stretching vibration of alkyl C–H groups. A sharp absorption band at 1641.42 cm⁻¹ was observed in the AgNP spectrum, whereas the nyamplung leaf extract exhibited weaker absorption bands at 1645.28 cm⁻¹ and 1614.42 cm⁻¹. These absorptions can be associated with C=C stretching vibrations. In addition, absorption bands at 1060.85 cm⁻¹ and 1062.78 cm⁻¹ were observed in the FTIR spectra, indicating the presence of C–O groups. A distinct absorption band was also observed in the AgNP spectrum but was absent in the spectrum of the nyamplung leaf extract, namely at 374.19 cm⁻¹. According to Kasim, Taba, Ruslan, & Anto (2020), this absorption can be attributed to the vibrational mode of the Ag–O group. The appearance of this band after $AgNO_3$ addition provides evidence of the interaction between silver and oxygen-containing functional groups during the synthesis process.

Table 4. Interpretation of the FTIR spectra of the nyamplung leaf extract as a bioreducing agent and AgNPs synthesized using the bioreducing agent

Functional Group	Wavenumber (cm-1)	
	Nyamplung Leaf Extract	AgNPs + Bioreducing Agent
−OH	3398,57	3444,87
−C−H	2922,16	2926,01
−C=C− (Aromatic)	1645,28 and 1614,42	1641,42
$-CH_3$	1350,17	1379,10 and 1346,31
−C−O	1062,78	1060,85
Ag−O	-	374,19

The absorption bands of the AgNPs and nyamplung leaf extract observed in the FTIR spectra are presented in Table 4. The changes in the intensity of the absorption peaks indicate the involvement of compounds present in the nyamplung leaf extract in the synthesis of AgNPs. Based on the FTIR characterization results, the observed changes in the spectra suggest the occurrence of Ag^+ ion reduction to metallic Ag^0, with hydroxyl (–OH), C=C, C–O, and methyl ($-CH_3$) groups potentially contributing to the reduction process. However, further characterization is required to identify more specifically the compounds or biomolecules that act as bioreducing agents in the synthesis of AgNPs.

Antibacterial Activity Testing Using the Well Diffusion Method

The antibacterial activity of the AgNPs synthesized using aqueous nyamplung leaf extract under the optimum synthesis conditions was evaluated using the well diffusion method. This method is based on the formation of wells in a prepared agar medium that has been previously inoculated with bacterial cultures, followed by the addition of the test samples into the wells. After 24 hours of incubation, the formation of a clear zone surrounding the wells, referred to as the inhibition zone, indicates the inhibition of microbial growth and, consequently, antibacterial activity. In this study, the antibacterial activity was evaluated against two bacterial strains with different cell wall characteristics: the Gram-positive bacterium Staphylococcus aureus and the Gram-negative bacterium Escherichia coli.

Six nutrient agar plates were prepared for the well diffusion assay and inoculated with the two bacterial strains, namely the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli*. Two test samples were evaluated: the synthesized AgNPs and the aqueous nyamplung leaf extract, with 40 µL of each sample added to the respective wells. Distilled water was used as the negative control, while amoxicillin solution was used as the positive control.

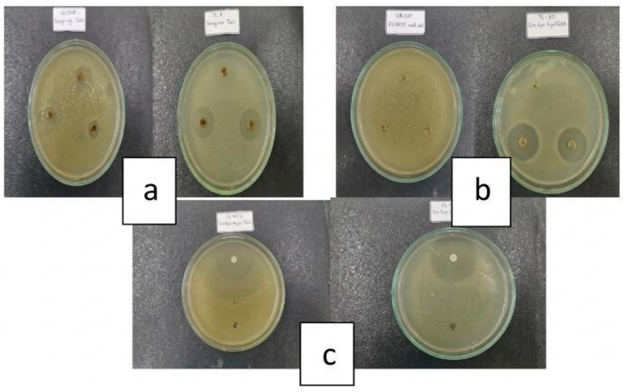

Figure 6. Antibacterial activity against S. aureus and E. coli: (a) AgNPs synthesized using the bioreducing agent, (b) nyamplung leaf extract as the bioreducing agent, and (c) positive (+) and negative (−) controls

The results of the antibacterial activity test after 24 hours of incubation are presented in Figure 6. The AgNP sample exhibited a clear inhibition zone surrounding the well, indicating antibacterial activity, whereas no inhibition zone was observed for the nyamplung leaf extract sample. A larger inhibition zone diameter indicates greater antibacterial activity of the tested sample. Conversely, the absence of an inhibition zone indicates that the sample did not exhibit detectable antibacterial activity under the conditions of the assay. The inhibition zone diameters were measured using a ruler and expressed in millimeters (mm), as presented in Table 5.

Table 5. Inhibition zone diameters of AgNPs synthesized using the bioreducing agent and nyamplung leaf extract against *S. aureus* and *E. coli*

Sample	Inhibition Zone Diameter (mm)	
	Staphylococcus aureus	*Escherichia coli*
NPAg + bioreductor nyamplung leaf extract	12	10.3
Nyamplung leaf extract	0	0
Positive control (+) amoxicillin	40	40
Negative control (-) aquadest	0	0

As shown in Table 5, the synthesized AgNPs demonstrated antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, as indicated by the formation of inhibition zones around the sample wells. The AgNP preparation produced inhibition zones of 12.0 mm against *S. aureus* and 10.3 mm against *E. coli*, indicating a difference in the response of the two bacterial species to the synthesized nanoparticles. The variation in antibacterial activity may be associated with differences in the structural characteristics and physicochemical properties of the bacterial cell envelopes. *S. aureus* is a Gram-positive bacterium characterized by a relatively thick peptidoglycan layer, whereas *E. coli*, a Gram-negative bacterium, possesses a comparatively thin peptidoglycan layer located between the inner cytoplasmic membrane and an additional outer membrane. The outer membrane of Gram-negative bacteria contains lipopolysaccharides and can act as an additional permeability barrier to various antimicrobial agents. Consequently, differences in cell-envelope architecture may affect the interaction of AgNPs with bacterial surfaces, their cellular uptake, and their subsequent antibacterial effects.

The antibacterial activity of AgNPs has been associated with several possible mechanisms, including adsorption onto the bacterial cell surface, disruption of membrane integrity, generation of reactive oxygen species, and interactions with intracellular biomolecules. Therefore, the differences in inhibition zones observed between *S. aureus* and *E. coli* may reflect the combined effects of bacterial cell-envelope structure and the physicochemical characteristics of the synthesized AgNPs. Further characterization of particle size, surface properties, and nanoparticle–cell interactions would be required to elucidate the predominant antibacterial mechanism. This is consistent with the findings of Arya et al. (2024), who reported that antibacterial activity can vary among bacterial species due to differences in cell wall structure and susceptibility to antibacterial agents. The extent of bacterial inhibition is also influenced by the bacteriostatic properties and susceptibility of each bacterial strain to the tested sample.

The inhibition zones produced by the AgNPs synthesized using the bioreducing agent against *S. aureus* and *E. coli* were classified as strong, with inhibition zone diameters of 12.0 and 10.3 mm, respectively. According to Davis and Stout (1971), antibacterial activity based on inhibition zone diameter can be classified into four categories: weak activity (<5 mm), moderate activity (5–10 mm), strong activity (10–20 mm), and very strong activity (>20 mm). Based on these criteria, the positive control (amoxicillin) exhibited very strong antibacterial activity against both bacterial strains, with an inhibition zone diameter of 40 mm. The strong antibacterial activity of the amoxicillin positive control is consistent with its use as a broad-spectrum β-lactam antibiotic against various bacterial infections. In contrast, the negative control (distilled water) did not produce an inhibition zone against either bacterial strain. The absence of inhibition confirms that the solvent itself did not exhibit detectable antibacterial activity under the conditions of the assay and therefore did not interfere with the antibacterial activity observed in the test samples. Similarly, the nyamplung leaf extract did not produce an inhibition zone against either *S. aureus* or *E. coli*. This finding indicates that, under the experimental conditions used, the extract did not exhibit detectable antibacterial activity. Therefore, in this study, the aqueous nyamplung leaf extract primarily served as a bioreducing agent in the synthesis of AgNPs rather than as the direct source of the observed antibacterial activity. However, the absence of an inhibition zone does not necessarily indicate that the extract contains no antimicrobial secondary metabolites, as their activity may depend on factors such as extraction method, concentration, bacterial susceptibility, and assay conditions.

The antibacterial activity of AgNPs against both bacterial strains may be attributed to their strong interactions with bacterial cells. AgNPs can interfere with bacterial growth and cellular functions through several mechanisms, including the release of Ag^+ ions, interaction with cellular biomolecules, and induction of oxidative stress. The released Ag^+ ions can interact with bacterial DNA and interfere with DNA replication, thereby inhibiting bacterial proliferation and potentially leading to cell death. In addition, Ag^+ ions and AgNPs may induce the production of reactive oxygen species (ROS), which can cause oxidative damage to cellular components, including DNA and RNA (Lestari et al., 2022). Gatoo et al. (2014) also reported that the physicochemical properties of nanoparticles can influence their toxicological effects. Silver in its ionic form exhibits strong antibacterial and cytotoxic effects due to its ability to interact with cellular components. These interactions may result in damage to the bacterial cell wall, inhibition of cellular biosynthesis, and disruption of cellular metabolism. Disruption of cellular metabolism may occur through interactions between silver species and intracellular macromolecules, particularly proteins and DNA, ultimately contributing to the inhibition of bacterial growth.

CONCLUSION

Based on the research findings, it can be concluded that the synthesis of silver nanoparticles (AgNPs) using nyamplung leaf extract as a bioreducing agent achieved optimum conditions at an $AgNO_3$ concentration of 0.0025 M, a volume ratio of $AgNO_3$ solution to nyamplung leaf extract of 1:1, and an optimum storage time of 8 days. The resulting AgNPs had an average particle size of 107.1 nm as determined by Particle Size Analysis (PSA), and exhibited strong antibacterial activity against S. aureus and E. coli, with inhibition zone diameters of 12.0 mm and 10.3 mm, respectively.

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