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# Green Synthesis of Silver Nanoparticles using Kayu tulak Leaf (*Schefflera Elliptica* Harms) Infusion as a Bio-reductant and Its Antibacterial Activity

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Abstract

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# Article Info

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#### Article history:

Received: 18<sup>th</sup> February 2022 Revised: 18<sup>th</sup> May 2022 Accepted: 11<sup>th</sup> July 2022 Online: 31<sup>st</sup> July 2022 Keywords: Silver nanoparticles; Kayu tulak leaf; bio-reductant; *Escherichia coli; Staphylococcus aureus*  Metal nanoparticles and exploration of green synthesis can be applied to lung tissue therapy, cancer, and even vaccines. Additionally, due to the rise in microbial resistance or the demand for novel antibiotics, the use of NPs as an antibacterial agent has expanded. Meanwhile, using methods to produce metal nanoparticles based on the abundance of biodiversity as a green-reducing agent will be safer. In the present study, Kayu tulak leaves extract served as the greenreducing agent. The synthesized silver nanoparticles were characterized using a UV-Vis spectrophotometer, PSA (Particle Size Analyzer), and TEM (Transmission Electron Microscope). The results showed that the silver nanoparticles synthesized using Kayu tulak leaf extract at concentrations of AgNO<sub>3</sub> 1 and 2 mM had absorbance peaks at 436.5 nm and 467 nm, respectively. The average particle size distributions of the two silver nanoparticles were 88.2 and 16.9 nm, respectively. The characterization of silver nanoparticles using TEM obtained a mixture of spherical, hexagonal, and triangular shapes. The silver nanoparticles synthesized using Kayu tulak as a bio-reductant exhibited antibacterial activity. At AgNO<sub>3</sub> solutions of 1 and 2 mM, silver nanoparticles showed antibacterial activity against Escherichia coli with inhibition zone diameters of  $6.39 \pm 0.30$  mm and 8.28 ± 0.19 mm (moderate inhibition), while against Staphylococcus aureus were  $4.30 \pm 0.24$  mm (weak inhibition) and  $6.39 \pm 0.27$  mm (moderate inhibition).

## 1. Introduction

As an essential aspect of nanotechnology, nanoparticles (NPs) (less than 100 nm in one dimension) has been developed for all kinds of application, particularly in nanomedicine fields. In nanomedicine fields, NPs are usually utilized to transport medicine. Recently, NPs have been applicated in therapy targeted only on a specific part, such as lung tissue, cancer therapy, and vaccination [1]. In addition, the use of NPs continues to increase in microbial applications due to the inevitable potential of NPs, which serve as resistance to microbes and meet the need for new antibiotics for the time being [2, 3, 4]. The number of infections and outbreaks associated with bacteria resistant to multidrug-resistant drugs (MDR) is increasing, threatening public health. Among other metal nanoparticles, silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) have been widely discussed elsewhere for their potential medicinal properties [5]. AgNPs have antibacterial activity and excellent antiviral and antifungal activity [6]. Metal nanoparticles are usually synthesized using chemical and physical methods. The ongoing problems with using current methods have led to the exploration of environmentally friendly and green synthesis to produce metal nanoparticles based on existing biodiversity [7]. This biodiversity is used as an eco-friendly green-reducing agent.

This study used the Kayu tulak leaf (*Schefflera elliptica* (Blume) Harms) as a green-reducing agent. This plant belongs to the Araliaceae family, which has the potential to be a medicinal plant. A paste made from a mixture of *S. elliptica* leaves, *Curcuma longa* rhizoma, *Musa paradisiaca* fruit, and honey can treat the broken fracture, whereas



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the oil extracted from the seeds can cure skin diseases [8]. Its bark is traditionally used for treating rheumatism and as a tonic [9]. The description of *S. elliptica* that Wiart [10] has done is as follows: Woody creeping stems or wide-sized shrubs with scattered headers. Phytochemical studies in plants in the genus Schefflera have revealed the presence of triterpenoids, triterpenoid glycosides, saponins, tannins, flavonoids, phenols, and steroids [11]. Among these compounds that can provide antibacterial effects are triterpenoids, saponins, tannins, and phenols. According to research that has been done, the Kayu tulak leaf has excellent potential as an antibacterial agent [12].

According to Maryati *et al.* [13], infectious diseases are still a significant problem in Indonesia. Various medicinal approaches to treating infectious diseases caused by bacteria are still ineffective, which motivates researchers to develop new and more potent medicines. *Escherichia coli* and *Staphylococcus aureus* are the most pathogenic bacteria that attack humans. *Staphylococcus aureus* is a gram-positive bacteria that lives as a saprophyte in the membrane channels of the human body, the skin surface, sweat glands, and the intestinal tract. At the same time, *Escherichia coli* is a gram-negative bacteria found in the healthy human colon [13].

From the explanation above, this research was performed to determine the antibacterial effects of silver nanoparticles synthesized using Kayu tulak (*Schefflera elliptica*) leaves extract as a bio-reductant. It is envisaged that the results of this study will provide information regarding the antibacterial activity of silver nanoparticles synthesized using Kayu tulak as a bio-reductant.

# 2. Materials and Methods

The analysis of this research is exploratorydescriptive and quasi-experimental. Exploratorydescriptive describes the structure of silver nanoparticles synthesized using Kayu tulak (*Schefflera elliptica*) leaves extract as a bio-reductant, which is formed by performing several characterizations. Quasiexperimental by comparing the antibacterial effects of silver nanoparticles synthesized using Kayu tulak leaf extract (*Schefflera elliptica*) as bio-reductant to controls.

#### 2.1. Tools and Materials

Distilled water, silver nitrate (AgNO<sub>3</sub>) (Merck), extract of Kayu tulak leaves, *Staphylococcus aureus* strain ATCC 25923, *Escherichia coli* strain ATCC 25922, NaCl 0.85%, ethanol 96% (Merck), Mueller Hinton Agar (MHA), paper disks, chloramphenicol-contained paper disks, ovens, hotplates, UV-Vis Spectrophotometer, Transmission Electron Microscope (TEM), Particle Size Analyzer (PSA), rotary evaporator, and water bath.

#### 2.2. Preparation of Kayu tulak leaves

Kayu tulak leaves were cleaned and then dried at room temperature. The dried leaves were cut into small sizes and crushed with a blender. Kayu tulak leaves powder was stored in a clean container and protected from light to avoid deterioration.

#### 2.3. Production of Kayu tulak Leaf Infusion

A total of 20 g of Kayu tulak leaf powder was boiled in 100 mL of distilled water. The mixture was then cooled and filtered to separate the filtrate and its residues. The filtrate was then stored in a clean, sealed container for further use as a bio-reductant in synthesizing silver nanoparticles.

#### 2.4. Green Synthesis of Silver Nanoparticles

A total of 20 mL infusion of Kayu tulak leaf was mixed into 40 mL of  $AgNO_3$  solution of 1 and 2 mM. The mixture was stirred with a magnetic stirrer for 1 hour at room temperature. A change in the color of the solution to brownish-yellow indicated that AgNP had formed. The filtrate was analyzed using a UV-Visible spectrophotometer to identify the formation of silver nanoparticles. The solution was then examined using PSA to determine the size and distribution of the silver nanoparticles and TEM analysis to observe the morphological shape of the silver nanoparticles.

#### 2.5. Production of Medium Mueller Hinton Agar

A total of 36 g of MHA powder was suspended in 1 L of distilled water in an Erlenmeyer and boiled until completely dissolved. The suspension solution was sterilized in an autoclave at 121°C for 5 minutes. The slightly cooled liquid MHA was poured aseptically into sterile plates or tubes and stored.

#### 2.6. Production of Staphylococcus aureus Suspension

*Staphylococcus aureus* was diluted with NaCl, and the turbidity was visually compared until it was the same as the McFarland standard (108 CFU/mL).

#### 2.7. Production of Escherichia coli Suspension

*Escherichia coli* was diluted with NaCl, and the turbidity was visually compared until it was the same as the McFarland standard (108 CFU/mL).

#### 2.8. Antibacterial Activity Test

The antibacterial activity of synthesized silver nanoparticles was qualitatively tested according to the modified Wendri *et al.* [14]. The bacteria used were *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activity test was performed by wetting the sterile paper disk with a nanoparticle solution, then placed in a petri dish containing test bacteria grown on NA media and incubating it for 24 hours at 37°C. The inhibitory effect of silver nanoparticles was known by measuring the width of the clear zone around the disk paper. In addition, control was carried out on the bioreductant extract of Kayu tulak leaf.

# 3. Results and Discussion

# 3.1. Color Change Results in the Synthesis Process of Silver Nanoparticles using Kayu tulak leaf extract as bio-reductant

This study has successfully produced silver nanoparticles synthesized using Kayu Tulak (*Schefflera elliptica*) leaves. At AgNO<sub>3</sub> solutions of 1 and 2 mM, the color changed from clear to golden yellow after 2 minutes and became brownish after 1 hour. The brownish color darkened with time, indicating that the silver nanoparticle solution was utterly formed, as shown in Figure 1. This is in line with research conducted by Wendri *et al.* [14] [15, 16, 17, 18].



**Figure 1.** (a) AgNO<sub>3</sub> solution (1 mM) and synthesized silver nanoparticles at AgNO<sub>3</sub> solution (1 mM) (b) AgNO<sub>3</sub> solution (2 mM) and synthesized silver nanoparticles at AgNO<sub>3</sub> solution (2 mM)

# 3.2. UV-Visible Spectrophotometer

The results of the characterization using UV-Vis spectrophotometer of synthesized silver nanoparticles at concentrations of 1 and 2 mM AgNO<sub>3</sub> can be seen in Figures 2 and 3. In Figure 2, the peak at a wavelength of 436.5 nm was obtained at 1 mM AgNO<sub>3</sub> and 467 nm at 2 mM AgNO<sub>3</sub> (Figure 3). The wavelength is a typical Surface Plasmon Resonance (SPR) absorption characteristic for silver nanoparticles [19, 20, 21, 22, 23, 24].



Figure 2. Spectrum absorption of synthesized silver nanoparticles (1 mM AgNO<sub>3</sub>)



Figure 3. Spectrum absorption of synthesized silver nanoparticles (2 mM AgNO<sub>3</sub>)

# 3.3. Size Distribution of Silver Nanoparticles using PSA (Particle Size Analyzer)

The particle measurement method by PSA is considered more accurate in determining the particle size distribution. The results of the size distribution of silver nanoparticles are shown in Figures 4 and 5. The particle size data obtained are in the form of three distributions, including intensity, number, and volume so that they can describe the overall condition of the sample [25]. Figures 4 and 5 show the size distribution with average values of 88.2 nm and 16.9 nm.



**Figure 4.** Histogram of the size distribution of synthesized silver nanoparticles (1 mM AgNO<sub>3</sub>)



**Figure 5.** Histogram of the size distribution of synthesized silver nanoparticles (2 mM AgNO<sub>3</sub>)

# 3.4. Morphology of Silver Nanoparticles with TEM (Transmission Electron Microscopy)

The results of the TEM analysis in this study are shown in Figures 6 and 7. The TEM pictures show silver nanoparticles in various shapes, including spherical, hexagonal, and triangular [26]. It is identical to the silver nanoparticles synthesized from cannonball leaves [27]. Different compounds are present in Kayu tulak leaf extracts, such as polysaccharides, polyphenols, and proteins responsible for producing nanoparticles in various forms.



Figure 6. Morphology of synthesized silver nanoparticles (1 mM AgNO<sub>3</sub>)



Figure 7. Morphology of synthesized silver nanoparticles (2 mM AgNO<sub>3</sub>)

# 3.5. Antibacterial activity test of synthesized silver nanoparticles with variations in AgNO<sub>3</sub> solution concentration

The disc diffusion method was employed in this study to evaluate the antibacterial activity of synthesized silver nanoparticles 1 and 2 mM AgNO<sub>3</sub> solutions. The inhibition zone of *Escherichia coli* and *Staphylococcus aureus* can be seen in Figures 8 and 9. The calculation results for the antibacterial activity test of synthesized silver nanoparticles can be seen in Tables 1 and 2, which show the inhibition zone of *Escherichia coli* and *Staphylococcus aureus*.



Figure 8. Antibacterial activity test of synthesized silver nanoparticles in 1 and 2 mM AgNO<sub>3</sub> solution against Escherichia coli



Figure 9. Antibacterial activity test of synthesized silver nanoparticles in 1 and 2 mM AgNO<sub>3</sub> solution against Staphylococcus aureus

According to Susanto and Ruga [28], the inhibition zone formed > 20 mm is considered very strong inhibition; 11-20 mm is declared strong inhibition; 6-10mm is declared moderate inhibition, and < 5 is regarded as weak inhibition. Each variant of this research data was carried out with standard deviation calculations to determine the accuracy of measurements in the data obtained. Therefore, the positive control had a very strong antibacterial activity against *Escherichia coli* with an inhibition zone diameter of 25.47  $\pm$  0.19 mm. Meanwhile, the synthesized silver nanoparticles at concentrations of 1 and 2 mM AgNO<sub>3</sub> solution had inhibition zone diameters of 6.39  $\pm$  0.30 mm and 8.28  $\pm$  0.19 mm, where both concentrations are categorized as having moderate inhibition (Table 1).

Table 1. Results of inhibition zone diameter (mm) of synthesized silver nanoparticles in different concentrations of AgNO<sub>3</sub> solution against *Escherichia coli* 

Concentration	1	Replicat	tion (mr	Average	Antibacterial	
	R1	R2	R3	R4	± SD (mm)	properties category
Control (+)	25.20	25.53	25.63	25.50	25.47 ± 0.19	Very strong
Control (-)	0.00	0.00	0.00	0.00	0.00 ± 0.00	No activity
Silver nanoparticle (1 mM)	6.07	6.26	6.43	6.78	6.39 ± 0.30	Moderate
Silver nanoparticle (2 mM)	8.15	8.25	8.56	8.16	8.28 ± 0.19	Moderate

The positive control had very strong antibacterial activity against Staphylococcus aureus with an inhibition zone of 27.22 ± 0.08 mm. Synthesized silver nanoparticles at concentrations of 1 and 2 mM AgNO<sub>3</sub> solution showed inhibition zone diameters of 4.30 ± 0.24 mm (weak inhibition) and  $6.39 \pm 0.27$  mm (moderate inhibition), respectively (Table 2). Based on the results of this study, synthesized silver nanoparticles showed antibacterial properties against Escherichia coli and Staphylococcus aureus. The synthesized silver nanoparticles can generally act as an antibacterial agent because of their ability to interact with bacterial membranes, thus damaging the bacterial membrane and killing bacteria. Silver nanoparticles first attack the surface of the bacterial membrane, then penetrate the bacteria, and eventually alter the permeability of the bacterial membrane. Such mechanisms can damage the membrane. The antibacterial properties of the silver nanoparticle membrane depend on the size, shape, and surface that determine success in damaging the bacterial membrane. The protecting ligand layer on small-sized silver nanoparticles can interact better with bacteria [29].

### Table 2. Results of inhibition zone diameter (mm) of synthesized silver nanoparticles in different concentrations of AgNO<sub>3</sub> solution against *Staphylococcus aureus*

Concentration <sup>-</sup>	1	Replicati	on (mm)	Average ±	Antibacterial	
	R1	R2	R3	R4	SD (mm)	category
Control (+)	27.18	27.23	27.15	27.33	27.22 ± 0.08	Very strong
Control (-)	0.00	0.00	0.00	0.00	0.00 ± 0.00	No activity
Silver Nanoparticles (1 mM)	4.63	4.05	4.25	4.27	4.30 ± 0.24	Weak
Silver Nanoparticles (2 mM)	6.02	6.64	6.53	6.38	6.39 ± 0.27	Medium

#### 4. Conclusion

Research on synthesizing the silver nanoparticles using Kayu tulak leaf extract as bio-reductant has been successful, and characterization was performed using UV-Visible spectrophotometer, PSA, and TEM. The synthesized silver nanoparticles at 1 and 2 mM AgNO3 solution exhibited different inhibition zone against *Escherichia coli* and *Staphylococcus aureus*. Synthesized silver nanoparticles with AgNO<sub>3</sub> solution of 1 and 2 mM showed moderate inhibition against *Escherichia coli* with inhibition zone diameters of  $6.39 \pm 0.30$  mm and  $8.28 \pm$ 0.19 mm, whereas against *Staphylococcus aureus* were 4.30  $\pm$  0.24 mm (weak inhibition) and 6.39  $\pm$  0.27 mm (moderate inhibition).

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