

Synthesis of Nano Silver From *Melastoma malabathricum* Leaf Extracts Modified PVA and its Antibacterial Activity Test

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ABSTRACT

Synthesis of silver nanoparticles from the extract of senduduk leaf (*Melastoma malabathricum*) with modified polyvinyl alcohol (PVA) has been carried out. The purpose of this study was to determine the effect of the addition of PVA on the characteristics of silver nanoparticles formed using the extract of sensaat leaf (*Melastoma malabathricum*). Variations in the concentration of PVA used were 0%; 0.75%; 1.5%; and 3%. Synthesis was carried out in a ratio of 1:10:1 (Extract: AgNO₃: PVA) for 2 hours. The characteristics of the synthesis of nanoparticles using a UV-Vis spectrophotometer resulted in a consecutive wavelength of 414 nm; 435 nm; 438 nm; and 420 nm. To determine the particle size using Particle Size Analyzer (PSA) analysis at 0% PVA and 3% PVA with sizes obtained 61.9 nm and 216.2 nm. The average particle size using X-Ray Diffraction (XRD) analysis showed the results in the form of crystals and using the Debye-Scherrer equation to obtain the average particle size of PVA 0% 22.97 nm and PVA 3% 10.15 nm. Antibacterial activity test on silver nanoparticles used disc diffusion method, which showed that silver nanoparticles had moderate to strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria.

Keywords: *Bioreductor, senduduk leaf extract, silver nanoparticles, and polyvinyl alcohol*

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1. INTRODUCTION

The development of studies and research in the field of nanotechnology is developing very rapidly, innovations to create nano products continue to be developed by researchers that are useful among the public. One of the material developments of nano products is silver nanoparticles (nanosilers). Silver nanoparticles are materials that have antimicrobial properties that function to inhibit antibacterial growth so that they can be used in various applications, one of which is in the field of health (plasters and socks) (Xiu dkk.,2012). The synthesis of environmentally friendly nanoparticles can use the biosynthesis method with plant extracts (Shittu and Ihelbunna, 2017). In this method, the procedures used in the synthesis process are not many, but have environmentally friendly properties, easy to find at a more economical cost so that this method is more attractive compared to other methods (Jerrard, 2007)

According to Oćwieja et al (2017) the synthesis of plant extract nanoparticles acts as a substitute for reductors that are very reactive so that they have the potential to damage the environment. Plant extracts that contain phenolic compounds such as flavonoids, saponins and tannins can be used as a silver metal (Ag) reducer. Therefore, the synthesis of plant extracts can be used as an alternative in the production of silver nanoparticles.

Bangka Belitung Islands is one of the regions that has a variety of endemic plants that have the potential to be developed. One of them is the somber plant (*Melastoma malabathricum*). Somber leaves are often used by the surrounding community as traditional medicine to overcome wounds, diarrhea medicine and so on (Dewi, 2019). Phytochemical studies in the melastomaceae family contain phenolic content such as flavonoids, saponins and tannins. With this phenolic content, somber leaves show that somber leaves are able to be used as bioreductors in the synthesis of silver nanoparticles and there is activity as an antibacterial (Danladi et al., 2015).

Most of the chemical methods used to synthesize Ag nanoparticles involve the use of chemicals that are toxic in nature so as to potentially cause environmental damage. In addition to

reductors, the synthesis of nanoparticles uses particle stabilizing agents (capping agents) which function to prevent clumping from forming on particles (agglomeration). Generally, such chemical stabilizers are synthetic polymers such as polyvinyl alcohol (PVA) (Navaladian *et al.*, 2007).

This study used green synthesis to synthesize nanoparticles using PVA modified solute leaf extract (*Melastoma malabathricum*) and tested its activity as an antibacterial. By utilizing the local potential from Bangka Belitung to become a functional material and applied in various health products. Synthesis of silver nanoparticles was carried out by reasuring the extract of leaf somber with a 1 mM (1:10) solution of silver nitrate (AgNO_3) for 2 hours. The nanoparticles from the synthesis are characterized by UV-Vis, PSA and XRD analysis.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were Senduduk Leaf Extract (*Melastoma malabathricum*) obtained from delas village, AgNO_3 (Merck), Akuades, Polyvinyl Alcohol (PVA) Technical, Nutrient Agar (NA) technical, Disc Paper (Merck), Amoxilin and dimethyl sulfoxide (DMSO) Pa (Merck).

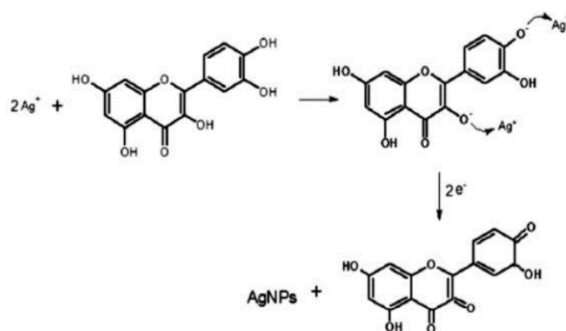
2.2. Methods

A total of 35 grams of Senduduk leaves were added with 350 ml of aqueous with a volume ratio of 1:10 and then heated at 70 °C for 15 minutes. After heating, the extract is allowed to stand first and then centrifuged and the filtrate is taken. The filtrate that has been obtained is stored in a sealed container so that it is not contaminated and used as a bioreductor. Soggy leaf extract is added in AgNO_3 solution with a concentration of 1 mM, next 0% PVA is added; 0,75% ; 1.5% and 3%. The volume ratio used is 1:10:1 (Extract : AgNO_3 : PVA). After the addition of PVA, the solution of distirrer for 2 hours, while observing the discoloration that occurs. The obtained Silver nanoparticles were centrifuged to separate the precipitate from the filtrate. Characterization of silver nanoparticles was performed by UV-Vis analysis to observe the maximum wavelengths of silver nanoparticles. The maximum wavelengths that are in the formation range of silver nanoparticles range from 400-450 nm (Solomon *et al*, 2007). Then the precipitate is characterized by XRD analysis and the filtrate is characterized by PSA analysis to determine particle size. Furthermore, the antibacterial activity test of silver nanoparticles was carried out on how to make a series of dilutions of test compounds (colloidal solution of silver nanoparticles from synthesis) with variations of 25%, 50%, 75% and 100%. The inhibitory power test of control using Amoxilin (positive control) and dimethyl sulfoxide (DMSO) (negetive control) was carried out on the test compound media by wetting sterile paper discs with a dilution silver nanoparticle solution, then placed in a petri dish that had contained escherichia coli and Staphylococcus aureus test bacteria grown on Nutrient Agar (NA) media. The inhibitory power of the test material is known by measuring the clear zone around the disc paper (Wahyudi dkk, 2011).

3. RESULTS AND DISCUSSION

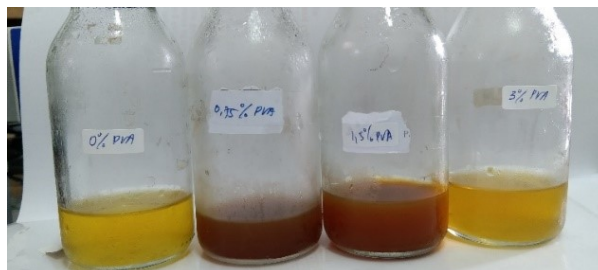
3.1. Synthesis Of Silver Nanoparticles

The method used in the synthesis of silver nanoparticles using plant extracts is green synthesis. The sorpent leaf extract used acts as a bireductor that can reduce Ag^+ silver ions to Ag^0 (Fabiani *et al*, 2018). The reaction mechanism that occurs is:



Gambar 2. Mechanism of The Reaction of Ag^+ to Ag^0 (Jain dan Mehata, 2017).

At the synthesis of silver nanoparticles undergo a process of agglomeration or clumping. To prevent the occurrence of agglomeration and to obtain a stable particle size can be done with the addition of polyvinyl alcohol stabilizing agents (PVA). Synthesis was carried out by adding extracts of leaves of stir and AgNO₃ 1 mM and PVA in a ratio of 1:10:1, the addition of PVA with a concentration variation of 0.75%, 1.5%, 3% and without the addition of PVA (0%). Aims to determine the effect of variations in PVA concentrations on the size of silver nanoparticles. Based on the results of the synthesis of silver nanoparticles, the color of the solution changes to brownish-yellow which indicates the formation of silver nanoparticles (Bakir, 2011).



Gambar 3 Results of Synthesis Without PVA (0%) and Addition of PVA (0,75%; 1,5% dan 3%)

3.2. Characteristics of Silver Nanoparticles

a. Spektrofotometer UV-Vis

one of the analyses to identify the formation of silver nanoparticles through wavelength uptake. From the results of previous research analysis shows that the formation of silver nanoparticles is characterized by a maximum wavelength absorption area in the range of 400-450 nm. The range is a typical value of silver nanoparticles (Ag₀) (Solomon et al, 2007). The results of the UV-Vis analysis of this study are as follows.

Table 1. Silver Nanoparticle Analysis with Spektrofotometer UV-Vis

Concentration AgNO ₃ (mM)	Concentration PVA (%)	Wevelength Maks (nm)
1 mM	0	414
	0,75	435
	1,5	438
	3	420

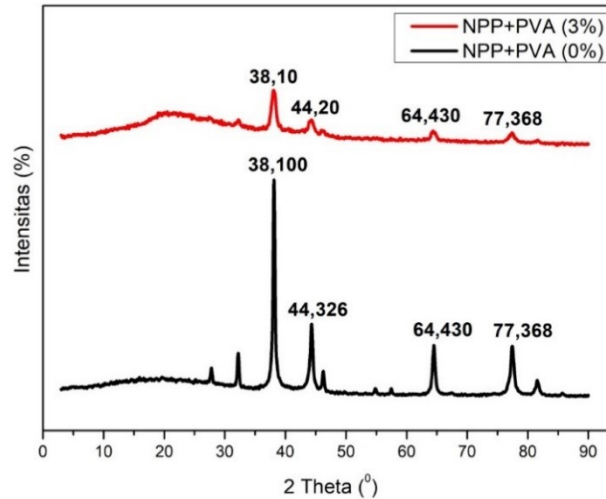
Based on the results of UV-Vis analysis, it shows that the wavelength absorption area of silver nanoparticles without the addition of PVA has a lower wavelength when compared to the addition of PVA. At a PVA concentration of 0.75% it has a wavelength absorption of 435 nm, while a PVA concentration of 1.5% indicates a wavelength absorption area of 438 nm. However, at a PVA concentration of 3% wavelength absorption area of 420 nm, this shows that the greater the wavelength, the more the size of the silver nanoparticles formed. (Bakir, 2011).

b. Particle Size Analyzer (PSA)

Particle Size Analyzer (PSA) is one of the analyzes used to determine the particle size in silver nanoparticles. Where particles are dispersed into a liquid medium so that agglomeration does not occur. Generally the particle size of silver nanoparticles ranges from 1-100 nm (Lu, 2008). Based on the results of the analysis of 0% silver nanoparticles, The resulting particle size of 61.9 nm is included in the nanoscale category with a polydispersity index value of 0.345, while the silver PVA nanoparticles (3%), the particle size produced is 216.2 nm with a polydispersity index (PI) value of 0.566. The results of the PSA analysis are different from the UV-Vis results where the particle size produced should be able to maintain the size of the nanoparticles, this is because in the PSA measurements particles are dispersed into the medium, where at the time of measurement the chances of small particles fusing are very large, so that the size obtained is greater than the size of the nanoparticles due to agglomeration.

c. X-Ray Diffraction (XRD)

Silver nanoparticles can be characterized using X-Ray Diffraction (XRD) analysis with the aim of knowing the crystallinity degree information of the silver nanoparticles. Through the pattern of diffraction and intensity formed XRD can also determine the particle size based on the Debye-Scherrer equation. The diffractogram of the silver nanoparticle can be seen in the following figure:



Gambar 4 Difaktogram NPP 0% (Bottom) and NPP PVA 3% (Top)

The XRD analysis diffractogram of silver nanoparticles without PVA showed that there was a peak in the 2θ diffraction pattern of 38.10 $^{\circ}$; 44.33 $^{\circ}$; 64.43 $^{\circ}$; 77.36 $^{\circ}$ and full width at half maximum (FWHM) value of 0.33; 0.47; 0.34 and 0.49. Meanwhile, in the silver nanoparticle diffractogram, the addition of 3% PVA shows that there are peaks in the 2θ diffraction pattern, namely 38.1 $^{\circ}$, 44.2 $^{\circ}$, 64.43 $^{\circ}$, 77.36 $^{\circ}$ and FWHM values of 0.86, 0.97, 0.9 and 1.04. Based on the results of XRD analysis of silver nanoparticles without PVA and with the addition of PVA in accordance with international centre for diffraction data (ICDD) No-01-071-4613 regarding silver diffraction pattern 2θ 38.09 $^{\circ}$; 44.27 $^{\circ}$; 64.41 $^{\circ}$; 77.35 $^{\circ}$. To find out the average particle size can use the Debye-Scherrer equation.

$$D = \frac{K \times \lambda}{\beta \times \cos\theta}$$

D = Average particle size

λ = Wevelenght Cu-K α (1, 5406 \AA)

θ = Bragg corner (rad)

K = Shape factor (0,9)

β = FWHM (rad)

Table 2 XRD Data Analysis Results of Silver Nanoparticles

Sample	2 θ (deg)	FWHM (rad)	D (nm)	Particle size (D) average (m)
Ag/PVA 0%	38,10	0,33	25,24	22,97 m x 10 ⁻⁹
	44,33	0,47	18,25	
	64,43	0,34	27,62	
	77,36	0,49	20,77	
Ag/PVA 3%	38,1	0,86	7,52	10,15 m x 10 ⁻⁹
	44,2	0,97	9,77	
	64,26	0,9	12,88	
	77,46	1,04	10,42	

Based on **Table 2** the average particle size of the silver PVA nanoparticles (0%) was obtained at 22.97 nm while the average particle size of the 3% PVA silver nanoparticles was obtained at 10.15 nm. The results of XRD data analysis show the conformity of diffraction patterns with Fabiani's research (2018). So that the results of the analysis show the formation of silver nanoparticles. It is based on the intensity values obtained that the XRD analysis at PVA is 0% higher compared to PVA 3%. So that the relationship between crystallinity and intensity is directly proportional, where the higher 2 θ (x-ray / crystallinity) eats the higher the intensity.

3.3. Antibacterial test

Antibacterial activity test to determine the inhibition zone in silver nanoparticles (0%) and silver nanoparticles with a PVA of 3%. Antibacterial tests were carried out using the disc diffusion method against *S. aureus* and *E. coli* bacteria. Based on the dilution concentration of silver nanoparticles, namely 25%, 50%, 75% and 100%, the antibacterial test results on disc paper produced a clear zone. Amoxilin is used as a positive control because it can block the formation of antibacterial cell membranes that cause death in bacteria (Sandika *et al*, 2017), while dimethyl sulfoxide (DMSO) negative control as a solvent reference that has no effect on antibacterial test results (Utomo *et al*, 2018). The inhibition zone of antibacterial test can be seen as follows:

Table 3 Diameter inhibition zone antibacterial test

Sample	Concentration	Diameter inhibition zone (mm)			
		<i>S. aureus</i>	Inhibition category	<i>E. coli</i>	Inhibition category
NPP/PVA 0%	25%	8,66	Medium	8,00	Medium
	50%	9,83	Medium	10,00	Medium
	75%	10,33	Strong	10,16	Strong
	100%	11,33	Strong	10,33	Strong
NPP+PVA 3%	25%	8,33	Medium	8,00	Medium
	50%	9,33	Medium	8,16	Medium
	75%	10,00	Medium	8,66	Medium
	100%	10,66	Strong	9,16	Medium
Control +	-	21,24	Very Strong	20,53	Very Strong
Control -	-	-	-	-	-

According to Table 3 silver nanoparticles have the ability to inhibit bacterial growth. This is because *S. aureus* is a gram-positive bacterium by having only a more accessible (simpler) peptidoglycan coating for permeation by silver solutions. While *E. coli* is a gram-negative bacterium that has a thin layer of lipopolysaccharides as an effective permeability barrier (Connel *et al*, 2013). So it can be concluded that gram-positive bacteria are easier to damage cell walls than gram-negative bacteria (Helmiyati, 2010).

Based on the category of inhibitory strength, the antibacterial test results obtained showed moderate to strong. According to Lestari et al (2016) the inhibitory power of bacteria is classified into 4, namely valley inhibition power (<5 mm), medium (5-10 mm), strong (10-20 mm) and very strong (> 20 mm). The inhibitory power of bacteria is classified into 4 categories, namely weak inhibitory power (less than 5 mm), medium inhibitory power (5-10 mm), strong inhibitory power (10-20 mm), very strong inhibitory power (more than 20 mm). The inhibition zone produced on the 0% PVA silver nanoparticles showed good activity compared to the 3% PVA silver nanoparticles. Because based on Particle Size Analyzer (PSA) data which shows the size of silver nanoparticles PVA 0% 61.9 nm and PVA 3% 216.2 nm. The addition of PVA which aims to maintain particle size is clumping due to the prevention of nanoparticle stability. Based on these results, PVA silver nanoparticles are 0% smaller so that the antibacterial activity produced is stronger (Sotiriou and Pratsinis, 2010).

4. CONCLUSION

Based on the results of the research that has been obtained, it can be concluded that based on the results of the UV-Vis spectrophotometer, the optimum wavelength absorption was obtained by 420 nm at the addition of 3% PVA, the PSA analysis resulted in a particle size of 61.9 nm at the addition of 0% PVA and included in the nanoscale (1-100 nm). While the particle size is 216.2 nm in the addition of 3% PVA, XRD analysis average particle size of 22.97 nm at 0% PVA and average particle size of 10.15 nm at 3% PVA addition. And antibacterial tests on silver nanoparticles of PVA modified stir leaf extract showed that 0% PVA silver nanoparticles have strong antibacterial activity whereas on the addition of PVA 3% have moderate antibacterial activity against staphylococcus aureus bacteria and Escherichia coli.

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