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GREEN SYNTHESIS OF SILVER NANOPARTICLES VIA *Cratoxylum glaucum* LEAF EXTRACT LOADED POLYVINYL ALCOHOL AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

In this study, green synthesis of silver nanoparticles via *Cratoxylum glaucum* leaf extracts loaded with polyvinyl alcohol and its bacterial activity were tested. Nanosilver from 'Pucuk Idat' leaves has a non-uniform size and tends to agglomerate, making the nanoparticle size unstable and challenging to apply further. Therefore, so that nanoparticles do not aggregate, efforts are made to add stabilizers. Polyvinyl alcohol (PVA) is a polymer that can be used as a stabilizer because it can prevent unwanted aggregation and oxidation processes. UV-Vis analysis results show that PVA as a stabilizer can avoid an increase in wavelength shift. Based on the results of this XRD analysis, it can be concluded that in this research sample, the silver nanoparticle is formed with a cubic crystal system, and it can be observed that the smallest average particle size is in the 0.75% Ag/PVA sample of 10.32 nm. Furthermore, based on the antibacterial test against E. coli and S. aureus, it can be explained that the 0.75% PVA-modified nanosilver sample showed weak to medium antibacterial activity.
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Keywords: *antibacterial, particle size, polyvinyl alcohol, silver nanoparticles, stabilizer.*

INTRODUCTION

The development of research and studies of nanotechnology is currently growing very rapidly. One of the exciting developments of nanomaterials to study is silver nanoparticles. A silver nanoparticle is a material that has antimicrobial properties. It can be used in various applications such as wound dressings [1] and cotton fibres [2], which function to inhibit bacterial growth, antiseptic sprays and antimicrobial coatings for medical devices which sterilize air and surfaces [3]. Silver nanoparticle synthesis continues to be directed at economical and environmentally friendly synthesis. The use of plant media is an innovation in producing silver nanoparticles. Indonesia is rich in tropical plants, and this is an opportunity for researchers to continue to develop silver nanoparticles based on local Indonesian plants, especially in Bangka Belitung.

Several methods of synthesizing silver nanoparticles have been used, including chemical methods [4], electrochemistry [5], radiation [6], photochemical methods [7], and Langmuir Blodgett [8]. However, most chemical methods used to synthesize these Ag nanoparticles involve toxic chemicals that pose a risk to the environment. Therefore, several researchers have used green synthesis to synthesize nanoparticles using microorganisms [9], nata de coco [10], and plants [11].

The Bangka Belitung Islands have various potential endemic plants to be developed [12]. One of the interesting plants to study is the 'Pucuk Idat' *(Cratoxylum glaucum)*. The people in Bangka Belitung know Pucuk Idat very well because it is often used for food seasoning. In addition, pucuk Idat leaves contain many antioxidant compounds such as polyphenols, flavonoids and tannins [13]. Furthermore, the phenolic content in the leaves of Pucuk Idat has been proven to be used as a bioreduction in synthesising silver nanoparticles [14]. Some plants that have been used to synthesize silver nanoparticles are *Tristaniopsis merguensis* [15], *Dilenia indica* [16], *Jatropha curcas* [17], and tiger grass (*Lantana camara L.)* [18].

In a previous study, silver nanoparticles from *Cratoxylum glaucum* leaf extracts had a non-uniform size. As a result, they tended to agglomerate so that the nanoparticle size became unstable and challenging to apply further [14]. Therefore, to prevent nanoparticle aggregation, efforts were made to add stabilizers [19]. Polyvinyl alcohol (PVA) is a polymer that can be used as a stabilizer because it can prevent aggregation and oxidation processes [20].

In this study, the green synthesis of silver nanoparticles via *Cratoxylum glaucum* leaf extracts loaded with polyvinyl alcohol and its bacterial activity were tested. Antibacterial activity tests were carried out on Escherichia coli and Staphylococcus aureus, bacteria that can cause human digestive infections. Silver ions interacting with cells will prevent protein synthesis, decrease membrane permeability, and ultimately cause cell death in both bacteria. Green silver nanoparticles are expected to be an environmentally friendly material that is economical and effective as an antibacterial [21].

METHODS

Extraction of *Cratoxylum glaucum* Leaves

Pucuk Idat samples were obtained in Sempan Village, Bangka Regency. First, the leaves of Pucuk Idat are dried by aerating for 3-5 days. Then, the dried Pucuk Idat were mashed using a blender and weighed 10 grams. Next, add a solution of 25 mL of distilled water: 25 mL of ethanol, mix in a shaker for 30 minutes, then filter to obtain Pucuk Idat leaf extract.

Green synthesis of silver nanoparticles via *Cratoxylum glaucum* leaf extracts loaded with polyvinyl alcohol

The silver nanoparticle synthesis was carried out by adopting and referring to the method developed by Kudle et al. [22] and Wahyudi et al. [23]. The synthesis of silver nanoparticles was carried out by the reduction method by reacting 3 mL of a bioreductor of Pucuk Idat leaf extract and 20 mL of $AgNO_3$ 2mM solution and a variation of 12 mL PVA of 0%; 0.75%; 1% ; 3% and 5%. The process of adding $AgNO_3$ solution to the bioreductor solution of Pucuk Idat leaf extract, which had been added with PVA, was carried out gradually drop by drop through the burette while continuously stirring using a magnetic stirrer for 2 hours at room temperature. The visual indicator for the formation of silver nanoparticles is a change in the color of the solution from yellowish to brown. Silver nanoparticles was characterized using UV-Vis and XRD. To determine the average silver nanoparticles particle size using the Debye-Scherrer equation (1) [24].

$$D = \frac{K\lambda}{\beta \cos \theta} \qquad (1)$$

Description :
D = average particle size
λ = wavelength of Cu-Kα (1,5406 Å)
θ = bragg's angle (rad)
K = *shape factor* (0,9)
β = FWHM (rad)

Antibacterial activity

The antibacterial activity of silver nanoparticles was tested according to the AATCC 147-1998 and AATCC 100-1999 standards. Antibacterial activity testing was carried out by making a series of dilutions of the test compound (synthesized colloidal silver nanoparticles parent solution) with variations of 25%, 50%, 75% and 100%. Control was carried out on the test compound media. The inhibition test was carried out by wetting sterile paper discs with a diluted nano silver solution and then placing them in a petri dish containing Escherichia coli and Bacillus subtilis test bacteria grown on NA media. The inhibition of the test material is known by measuring the clear zone around the paper disc. At the same time, microbial activity is determined by calculating the percentage reduction in bacterial culture [23]

RESULTS AND DISCUSSION

Cratoxylum glaucum leaf extract was extracted using ethanol as a solvent. The ethanol solvent effectively extracts flavonoid compounds as bioreductors on silver particles [25]. *Cratoxylum glaucum* leaf extract contains polyphenols, flavonoids and tannins [13]. In several studies, these compounds can produce or form nanoparticles from a metal [26]. Phenolic compounds have hydroxyl groups that can bind metals. The ability to chelate metals from phenolic compounds is due to the high nucleophilic character of aromatic rings [27]. One of the green synthesis methods that can minimize the use of hazardous and environmentally unfriendly inorganic materials can be done using plant extracts [28]. In synthesising silver nanoparticles, reducing silver ions in solution generally produces colloidal silver with a nanometers diameter. Over time, silver nanoparticles can aggregate so that the surface area decreases and causes the particle size to get more significant due to the instability of the particle size. Therefore, a stabilizing material is needed to maintain the stability of the nanoparticles. Materials used as stabilizers include ligands and polymers [29]. In this study, the synthesis of silver nanoparticles using Polyvinyl Alcohol (PVA) as a stabilizer is known to be an effective stabilizer in synthesising silver nanoparticles [20].

Silver nanoparticles modification was carried out with variations in PVA concentrations of 0%, 0.75%, 1%, 3% and 5%, and this aims to determine the effect of the addition of PVA on the silver nanoparticles size. A change in the color of the solution to brown marks indications for the formation of silver nanoparticles. The longer it turns black, the color of the solution gets darker with time. The reduction process of Ag^+ to Ag^0 due to the bonding of the -OH functional group derived from phenolic compounds [21] in the *Cratoxylum glaucum* leaf extract. The prediction of silver nanoparticle formation from the reduction reaction by the polyphenolic compounds contained in the *Cratoxylum glaucum* leaf extract can be seen Figure 1, show the reduction reaction between the polyphenolic compounds and Ag^+ ions. In the early stages, the polyphenolic compounds in the solution change from the R-OH group to the R-O group, which is ready to react. Furthermore, polyphenols bind Ag^+ and form RO-Ag groups. At this stage, a reaction occurs in which the bound Ag^+ ion undergoes a polyphenol chain termination reaction and then releases to form Ag° [30].

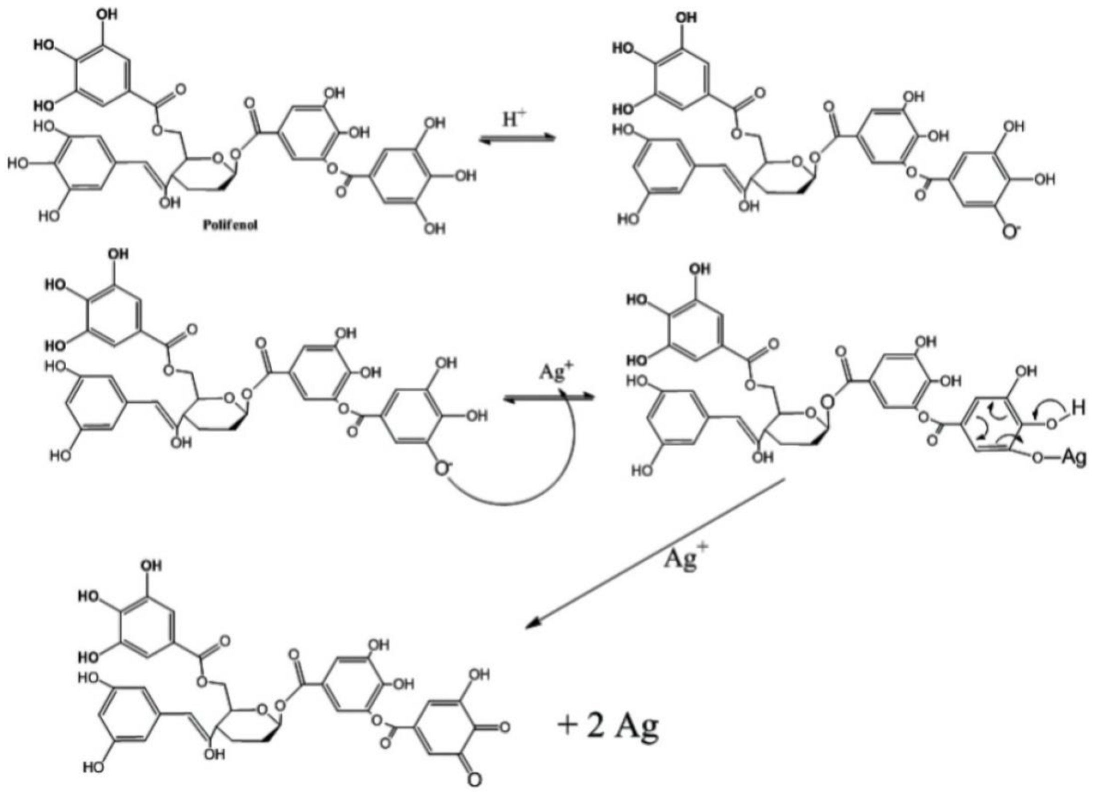

Figure 1. Prediction reactions in the synthesis of silver nanoparticles by polyphenols [31]

Table 1. Data of UV-Vis Analysis

$AgNO_3$ (mM)	PVA (%)	λ Max (nm)	*Range Silver nanoparticles*
2	0	451	395-438 nm [32]
	1	370.5	
	3	449	
	5	392.5	
50	0	405	
	0,75	360	
	3	370	

The stability of the synthesized silver nanoparticles colloid is known through measurements using a spectrophotometer. The stability of the colloidal silver nanoparticles solution is known from the change in the absorption peak. Suppose there is a shift in the absorption peak to a larger wavelength. In that case, it indicates that the stability of the silver nanoparticles colloid is still low due to an agglomeration event [23]. The results of the UV-Vis analysis are presented in Table 1.

Based on Table 1 shows that the addition of PVA affects the stability of silver nanoparticles. $AgNO_3$ 2mM shows a large maximum wavelength shift from 1% to 3% and a shift to a smaller wavelength at 5% PVA concentration. It indicates that 5% PVA produces relatively stable silver nanoparticles compared to 3% PVA. The larger the wavelength, the larger the size of silver nanoparticles due to the instability of the nanoparticle size. The results of UV-Vis analysis from the use of PVA at $AgNO_3$ 2 mM did not show a wavelength in the wavelength range of silver nanoparticle formation, so another effort was made, namely by increasing the concentration of $AgNO_3$. Increasing the concentration of $AgNO_3$ can increase the yield of silver nanoparticles produced [33]. The concentration of $AgNO_3$ used is 50 mM. This concentration is used because it is the optimum concentration in silver nanoparticle synthesis using *Cratoxylum glaucum* leaf extract [14]. In Table 1, it can be observed that at 0% PVA, the maximum wavelength produced is in the range of silver nanoparticle formation, but when PVA is added, the wavelength absorption shifts to be smaller. It explains that PVA as a stabilizer can prevent an increase in the wavelength shift and decrease the wavelength.

XRD Analysis

The Debye-Scherrer equation characterisation using XRD was carried out to obtain information about crystal degree and orientation and to determine crystal size. The PVA-modified silver nanoparticles XRD diffractogram is presented in **Figure 2**.

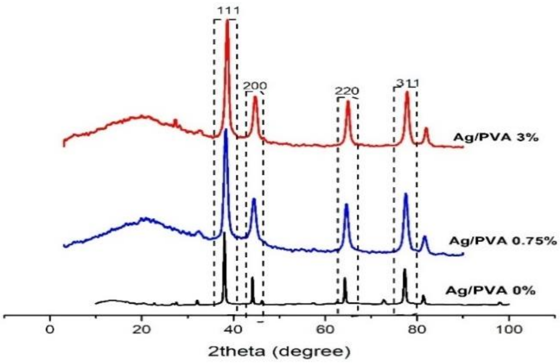

Figure 2. Diffractogram of silver nanoparticles/PVA

Silver nanoparticles diffractogram shows a diffraction pattern that corresponds to the silver diffraction pattern based on ICDD data (International Center For Diffraction Data) No. 01-071-4613 are at 2θ 38.09°, 44.27°, 64.41° and 77.35° with Index of Miller (111), (200), (220), and (311). Therefore, based on the results of this XRD analysis, it can be concluded that the sample in this study formed silver nanoparticles with a cubic crystal system.

Table 2. Size analysis of silver nanoparticles

Sample	2θ (deg)	θ (rad)	FWHM (rad)	D (nm)	Average Particle size (nm)
Ag/PVA 0%	37.99	0.3315	0.0054	27.36	35.60
	44.11	0.3849	0.0031	47.84	
	64.24	0.5606	0.0040	40.71	
	77.26	0.6742	0.0067	26.49	
Ag/PVA 0.75%	38.309	0.3343	0.0150	9.79	10.32
	44.45	0.3879	0.0180	8.33	
	64.54	0.5632	0.0143	11.45	
	77.5	0.6763	0.0152	11.70	
Ag/PVA 3%	38.40	0.3351	0.0155	9.47	11.22
	44.73	0.3903	0.0159	9.44	
	64.92	0.5665	0.0127	12.89	
	77.80	0.6789	0.0136	13.08	

Based on Table 2, the smallest average particle size in the Ag/PVA sample of 0.7% indicates that the resulting particle size is smaller at lower PVA concentrations. PVA in small concentrations, which can preserve and stabilise a small number of silver nanoparticles, can effectively produce small-sized silver nanoparticles [34].

Antibacterial activity

A qualitative antibacterial activity test was carried out by measuring the growth inhibition zone of the test bacteria. The tested silver nanoparticles sample was 0.75% Ag/PVA silver nanoparticles because it had the smallest average particle size. An antibacterial activity test was carried out on *Escherichia coli* and *Staphylococcus aureus* bacteria. The antibacterial activity test using the disc diffusion method was carried out by placing a paper disc that had been soaked in a solution on a solid medium that had been inoculated with the test bacteria. The results of the antibacterial activity test can be observed in Figure 3.

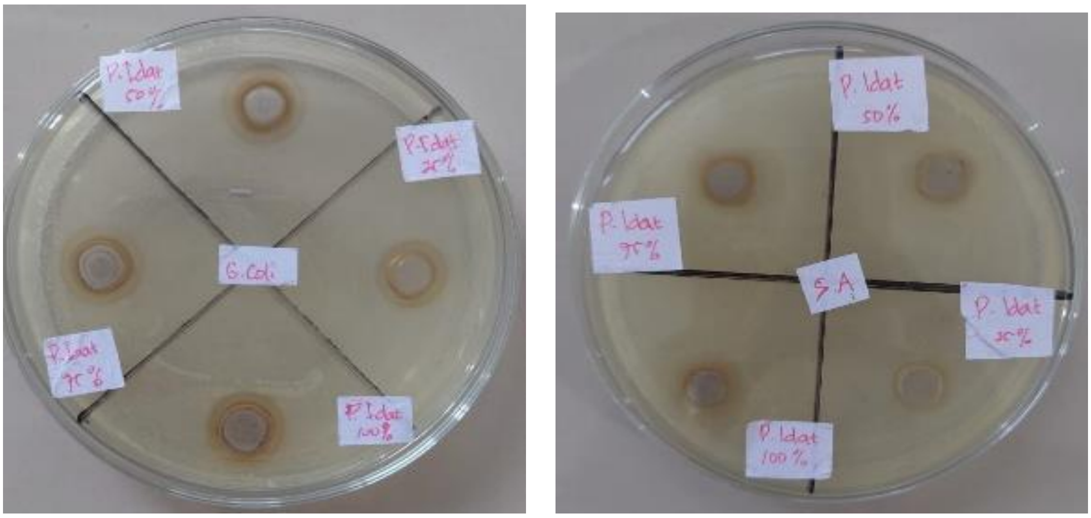

Figure 3. Antibacterial activity of silver nanoparticles against (a) *Escherichia coli* and (b) *Staphylococcus aureus*

Table 3. Inhibition zone diameter

Ag/PVA 0,75%	Inhibition zone diameter (mm)		Category	
	E. coli	*S. aureus*	*E. coli*	*S. aureus*
25%	4,20	3,03	Weak	Weak
50%	4,78	3,25	Weak	Weak
75%	5,50	3,63	Medium	Weak
100%	5,70	4,63	Medium	Weak

Figure 3 shows that the activity of inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria was indicated by the formation of an inhibition zone on MHA media. The size of the inhibition zone means the silver nanoparticle's inhibitory power [23]. The results of the inhibition zone measurements are presented in Table 3.

Based on Table 3, it can be explained that the 0.75% PVA-modified silver nanoparticles sample showed weak to moderate antibacterial activity. The antibacterial inhibitory zone activities were grouped into four categories, namely: weak activity (<5mm), medium (5-10 mm), strong (>10-20 mm), and very strong (>20-30 mm). The antibacterial inhibitory activity was expressed based on the clear zone produced around the paper disc. The diameter of the zone of inhibition of bacterial growth is measured in mm [35]. When viewed from the size of the inhibition zone, the antibacterial activity against *E.coli* was greater than *S.aureus* because Escherichia coli is a class of Gram-negative bacteria where the cell wall of Gram-negative bacteria consists of one or more thin layers of peptidoglycan and an outer membrane. The peptidoglycan layer, while *Staphylococcus aureus*, is a group of Gram-positive bacteria whose cell walls consist of several layers of peptidoglycan that form a thick and rigid structure and contain a cell wall substance called teichoic acid [36]. Because it has only a tiny layer of peptidoglycan and does not contain teichoic acid, the cell walls of Gram-negative bacteria are more susceptible to physical treatment, such as the administration of antibiotics or other antibacterial agents [37]

CONCLUSION

The formation of silver nanoparticles occurs through green synthesis, where the reduction reaction of Ag^+ to Ag^0 occurs by polyphenolic compounds in the *Cratoxylum glaucum* leaf extract. The effect of adding PVA on the synthesis of nanosilver from *Cratoxylum glaucum* leaf extract provides good stability in particle size. Adding 0.75% PVA gives the smallest nanosilver particle size of 10.32 nm based on Debye Scherrer's calculations from XRD data analysis. Antibacterial activity resulting from nanosilver extract of Pucuk Idat leaves showed weak to moderate antibacterial activity against *E.coli* and *S.aureus* bacteria. The development of this research will continue to be carried out in the future related to optimising the synthesis method and application of silver nanoparticles.

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