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The Role of pH in Controlling Size and Distribution of Silver Nanoparticles using Biosynthesis from *Diospyros discolor Willd.* (Ebenaceae)

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Abstract. The silver nanoparticles generated by biosynthesis have a quite diverse result, both in size and shape. Structures of silver nanoparticles can be controlled by modifying the parameters of the biosynthesis such as the ratio between the precursors and reducing agents, as well as pH of the solution. In this study, the pH of *Diospyros discolor* (Bisbul) leaves aqueous extract was varied to 4, 7, 9, and 11. The extract then was added to 1 mM $AgNO_3$ precursor (1:2; v/v ratio). The result of the silver nanoparticles characterized using spectrophotometer UV-Vis to find if there was any absorbance peak formed between 400 nm to 500 nm. TEM characterization was used to determine the size and shape of silver nanoparticles, and PSA was used to see their size distribution and stability. The higher pH tends to produce smaller silver nanoparticles rapidly. The synthesis parameters that were varied in this research have affected the size, size distribution patterns, and stability of silver nanoparticles.

Keywords. Biosynthesis, *diospyros discolor,* pH, and silver nanoparticles.

1. Introduction

Silver nanoparticles (Ag NPs) have a unique characteristic and have the ability to efficiently absorb and scatter light because of the surface plasmon resonance (SPR) [1]. Ag NPs are also known to have applications in various fields such as sensors [2], biomedical and health [3]. Based on their unique characteristic, scientists develop some different methods to synthesize silver nanoparticles, which are chemical, physical and biological methods [4, 5]. Biological methods for nanoparticles synthesis can be done by using single cell microorganism such as bacteria or fungi through intracellular processes [6]. However, the intracellular biosynthesis requires a long reaction time. In contrast, the synthesis of silver nanoparticles using plant extract as an extracellular extruded agent requires shorter reaction time than the intracellular biosynthesis.

Diospyros discolor Willd. (bisbul) is known for containing triterpenes [7], alkaloids, flavonoids, and tannins [8]. The specific compound from those group can be used as reducing agents [9]. Silver nanoparticles characteristics such as size, shape, dispersion, and stability can be influenced by temperature, the ratio of precursor concentrations with reducing agents, and pH during the biosynthesis [10]. The degree of acidity (pH) can affect the size of the nanoparticles. The higher the

pH value, the nanoparticles formed tend to be smaller and can even reach the size of 5 nm [11]. Therefore, it is expected that the adjustment of pH in this study during the biosynthesis using *D. discolor* leaves aqueous extract can affect the size, shape, and stability of Ag NPs.

2. Materials and methods

2.1. Plant collection and extract preparation

Leaves aqueous extract of *D. discolor* was prepared using raw materials collected from Universitas Indonesia garden collection. The raw materials were firstly washed to remove the dirt. After that, the samples were dried in the oven at a temperature of 40 °C until it had constant dry weight, then it was ground into powder. Two grams of leaves powder were boiled for (10 to 15) min with 100 mL of bidistilled water (2 % w/v). Then, the water extract filtered with Whatman filter paper No 1. As the experiment variable, pH of the extract was adjusted to 4, 7, 9, 11, using 1 M NaOH and 0.1 M HCl. One sample was prepared for control without pH adjustment. LAQUA Horiba PH1100 was used to measure the pH solution. A solution of 1 mM $AgNO_3$ (Merck) was developed from $AgNO_3$ powder.

2.2. Biosynthesis and characterization

To perform the biosynthesis, 30 mL of leaves aqueous extract with various pH, respectively were mixed with 60 mL AgNO3 solution (1:2; v/v ratio) at room temperature. The silver nanoparticles formed were characterized using spectrophotometer (GENESYS 10SUV-VIS) to confirm the absorbance peak from the Ag NPs in between 200 nm and 800 nm. Beside the spectroscopy result, the change of the solution's color between 24 h was also observed.

The Ag NPs resulted from biosynthesis was evaluated by observing their size and shape morphology. To observe the size and shape we used transmission electron microscope (TEM). Meanwhile, the Particle Size Analyzer (PSA) was used for particle size and zeta potential determination. This experiment was using TEM FEI Tecnai G2 Supertwin and PSA (Malvern Zetasizer) from Balai Besar Pascapanen, Departement Pertanian, Bogor and TEM JEOL JEM 1400 from Department of Chemistry, FMIPA UGM, Yogyakarta.

3. Results and dıscussıon

3.1. Absorption spectrum analyses

Biosynthesis of Ag NPs with pH variation was performed by mixing $AgNO_3$ and aqueous extract of the *D. discolor* leaves. The color of Ag NPs colloid was examined after 24 h of reaction. The results showed that each of Ag NPs colloid changed its color during the reaction after 24 h (Figure 1). Biosynthesis using leaves extract at pH 4 resulted in a yellowish solution. Meanwhile, the Ag NPs without any pH adjustment the color tended to be brownish and got darker in pH 7, pH 9 and pH 11. The colloid color becomes darker as the pH value increases. Color variations in colloidal silver nanoparticles are associated with surface plasmon resonance and optical characteristic of silver nanoparticles [12]. The interaction between silver nanoparticles with light will result in a combined oscillation of electrons on the surface of nanoparticles when excited by light at certain wavelengths [13]. The reflectance light makes colloidal silver nanoparticles look yellow to brown [14]. Therefore, the changes of colloid's color indicate the formation of silver nanoparticles.

Based on UV-Vis spectrophotometer analyses, Ag NPs-control samples using aqueous extract leaves showed a specific peak around the 400 nm wavelength. The peak indicates the presence of silver nanoparticles [15]. During the 24-hour incubation period, there is a reaction between *D. discolor* leaves aqueous extract and $AgNO_3$. The reaction occurs when silver ions (Ag^+) derived from $AgNO_3$ reduces to neutral silver (Ag^0) [9]. The metallic silver (Ag^0) subsequently undergoes a nucleation phase until silver nanoparticles formed which are showed by the existence of spectrum absorbance between 350 nm to 500 nm (Figure 2).

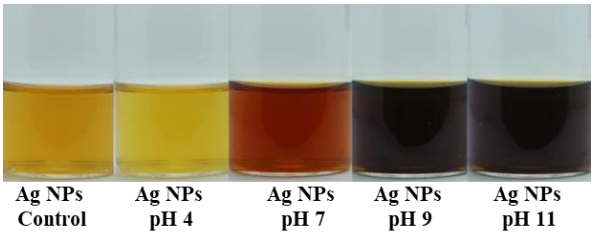

Figure 1. Ag NPs colloid color from various pH value of the aqueous extract of *D. discolor* leaves using biosynthesis with silver precursor after 24 h reaction time.

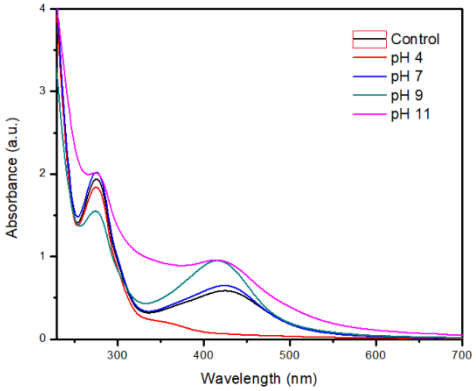

Figure 2. Spectrum UV-Vis result from silver nanoparticles synthesized using *Diospyros discolor* leaves extract pH after 24 h.

The UV-Vis spectrum also shows a difference in absorbance values that qualitatively indicates the number of silver nanoparticles [16]. Ag NPs at pH 4 showed no significant peak at 24 h reaction; it was assumed that the Ag NPs not yet formed during that time. The Ag NPs samples with pH 7, pH 9, and pH 11 show the higher significant peak. The absorbance values tended to be larger along with the higher pH values. Biosynthesis results from control sample tended to be higher than at pH 4, the pH value of aqueous extract at sample control was 6.5. The peak on the Ag NPs sample pH control and pH 7 have the same pattern. According to the absorbance spectra, the formation of Ag NPs tends to be faster in the alkaline condition.

The λ_{max} from spectrum absorbance of Ag NPs also showed varied value, where the sample control showed λ_{max} at 425 nm, pH 7 at 424 nm, pH 9 at 416 nm and pH 11 at 414 nm. This phenomenon suggests that those Ag NPs tend to have a smaller size at lower λ_{max}. The absorbance value in the UV-Vis spectrum qualitatively shows the number of silver nanoparticles formed, as well as the specific wavelength range, indicates the size of the resulting Ag NPs [17]. The size of nanoparticles tends to be smaller when the peak shifted to smaller wavelength range. The degree of acidity (pH) given an effect on binding of silver ions and biomolecules that have an impact on the silver ions reduction process and nucleation growth into silver nanoparticles [17].

3.2. Silver nanoparticles morphology, distribution, and stability

The distribution and shape of the Ag NPs have been confirmed using TEM results. The results showed that Ag NPs formed in spherical shapes and varied in sizes (Figure 3). For the size of Ag NPs based on images of the TEM results sized changed from 5 nm to 80 nm. Ag NPs-control tends to have more significant dimensions, ranging from 20 nm to 70 nm. The biosynthesis results using leaves extract at pH (4 to 7) showed that the Ag NPs exhibits a size ranging 50 nm (Table 1). Meanwhile, in pH 9 and pH 11, the dimensions of Ag NPs reach up to (15 to 30) nm. The result showed that pH could control the size of Ag NPs. In Ag NPs-pH 11, Ag NPs formed in small sizes (23 ± 8) nm. The TEM picture

showed that after the Ag NPs created, some of Ag NPs seem to aggregate. The aggregation may be originated from the attraction between tiny particles. Furthermore, it could also cause due to the rapid reducing of Ag^+ where the capping agent can no longer cap the growing nanoparticles. Therefore, it can be expected that in the alkaline condition the nanoparticles have smaller size and poor balance between nucleation and growth processes could be the cause of this result [18]. Meanwhile, the result of Particles Size Analyzer (PSA) showed Polydispersity Index (PDI) and zeta potential (Table 1). The PDI value of Ag NPs tends to be higher along with increasing of pH. Ag NPs-control and Ag NPs-pH 4 have PDI values 0.364 and 0.412 indicating a moderate disperse level. Meanwhile, the PDI value of Ag NPs at pH (7 to 11), subsequently 0.480, 0.556, and 0.739 are within highly polydisperse range.

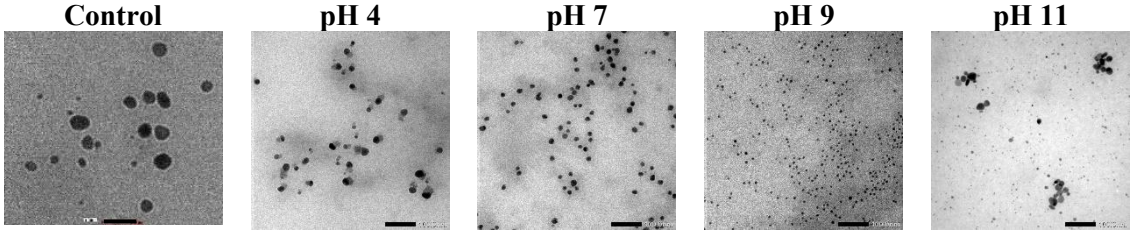

Figure 3. TEM result of silver nanoparticles morphology and distribution from biosynthesis using an aqueous extract of *Diospyros discolor* leaves (scale bar showed 100 nm scale).

Table 1. Polydispersity index (PDI), sizes and zeta potential values from Ag NPs from biosynthesis using an aqueous extract of *Diospyros discolor* leaves.

Sample	PDI	Sizes (nm)	Zeta Potential (mV)
Control	0.364	45 ± 24	–14.3
pH 4	0.412	54 ± 20	–22.7
pH 7	0.480	35 ± 12	–25.1
pH 9	0.556	21 ± 5	–28.1
pH 11	0.739	23 ± 8	–30.0

The stability level of Ag NPs during biosynthesis was indicated by its zeta potential (Table 1). It is shown that the zeta potential is higher as pH increase. Ag NPs-control has the smallest zeta potential of 14.3 mV, which indicates that the nanoparticles are less stable. The samples synthesized at pH 7 to pH 11 are in the range of moderate stable of (20 to 30) mV. The addition of NaOH to the *D. discolor* aqueous extract was found to influence the reduction rate of silver ions. The balance between the nucleation process and the growth of nanoparticles affects the shape of the nanoparticles [18]. Also, under very alkaline conditions, phenol compounds contained in *D. discolor* leaves extracts might be deprotonated, which cause the functional group on the mixture becoming unstable and unable to prevent the aggregation of silver nanoparticles formed [19].

4. Conclusıons

Silver nanoparticles resulted from biosynthesis using an aqueous extract of *D. discolor* leaves after treatment with various pH modification in solution showed different characteristic in size, distribution, and stability. In this experiment, alkaline condition tended to control the size and distribution of the Ag NPs. The size of Ag NPs at pH below 7, at pH 7, and at pH 9 and pH 11 are in the range of 80 nm, under 50 nm, under 35 nm respectively. Some aggregation might happen due to lack of capping agent that can prevent attraction between the Ag NPs in colloid solution. In this experiment, pH shows potential as a dependent parameter in biosynthesis to get the desired Ag NPs in size and stability.

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