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Synthesis of Silver Nanoparticle Using Bioreductor Method from Cempedak (*Artocarpus integrifolius* L. f) Bark Extract

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Abstract

Synthesis of silver nanoparticle using bioreductor method from cempedak (*Artocarpus integrifolius* L. f) bark extract has been conducted. Steps in this research performed with synthesis of silver nanoparticle using AgNO_3 solution with variation of concentration reacted with bioreductor in form of Cempedak (*Artocarpus integrifolius* L. f) bark extract, then characterized with Spectrophotometer UV-Vis, TEM (Transmission Electron Microscope) and PSA (Particle Size Analyzer). Results of maximum wave length measurements with Spectrophotometer UV-Vis shows the most stable silver nanoparticle is with AgNO_3 0,004 M concentration. TEM analysis results shows silver nanoparticle morphology as rounded and varies in size and shows average particle size in nanoparticle using bioreductor is 78,66 nm. PSA result analysis obtained particle size distribution in nanoparticle using bioreductor from 50-170 nm shows that nanoparticles has formed.

Keywords: Silver Nanoparticle, bioreductor, cempedak (*Artocarpus integrifolius* L. f), stability

Introduction

Nanotechnology is an aspect of biology, physics, chemistry and engineering which is interesting and also important today and in the future. One kind of nanotechnology greatly developed today is nanoparticle. Nanoparticle that mostly used is metal nanoparticle due to its wide application. One mostly used metals is silver (Ag). Among available methods, the most effective method to produce silver nanoparticle is reduction method. This method has fast, convenient, low-cost also utilizing low temperature (Khaydaroy *et al.*, 2009). In reduction method, bioreductor can be used.

Reduction method done in this silver nanoparticle synthesis by utilizing bioreductor. Bioreductor used in silver nanoparticle synthesis is betel leaf extract (*Piper betle* linn), where in said betel leaf extract contains secondary metabolite which can reduce silver in silver nanoparticle synthesis (Purnamasari, 2015). In this research utilizes cempedak bark extract (*Artocarpus integrifolius* L. f). Cempedak bark chosen due to expectation in containing secondary metabolite which is not only found in leaf, branch, and root but also diffused in barks.

Research Methodology

Materials and Methods

Materials

Material in this research including AgNO_3 solution, aquadest, methanol 99,9%, cempedak bark (*Artocarpus integrifolius* L. f).

Research Procedure

Cempedak bark sample preparation

Cempedak bark taken from cempedak tree in Handil, Muara Jawa, Kutai Kartanegara. Cempedak bark dried with room temperature, then cut into small pieces with size of 0,5-1 cm.

Sample Extraction

Extraction method of cempedak bark extract bioreductor in the research referring to Lestari *et al.*, (2016) using maceration and evaporation. Cempedak bark powder as much as 1 kg macerated with methanol for 3 x 24 hours. Later, obtained methanol extract concentrated with evaporator until thick methanol extract obtained.

Alkaloid Test

Crude extract of cempedak bark put in test tube, then added 2 drops of Dragendorff reagent (mixture of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ in nitric acid and KI solution). Positive



result of alkaloid test marked by formed sediments with orange to brownish red color (Sitorus, 2010).

Steroid and Triterpenoid Test

Crude extract of cempedak bark put in test tube, then added 3 drops of Liebermann-Burchard reagen (mixture of acetic acid anhydrate and $H_2SO_{4(p)}$). Positive result of steroid and triterpenoid test marked by color shift of sample into red or purple (Harborne, 1987).

Flavonoid Test

Crude extract of cempedak bark put in test tube, then dissolved with corresponding solvent. Added 2 mg of Mg powder and 3 drops of $HCl_{(p)}$. Positive result of flavonoid tes marked by color shift of sample into red, yellow, or orange (Harborne, 1987).

Phenolic Test

Crude extract of cempedak bark put in test tube, then dissolved with corresponding solvent. $FeCl_3$ 1% solution added. Positive result of phenolic test marked with color shift of sample into red, green, black, blue, or strong purple (Harborne, 1987).

Saponins Test

Crude extract of cempedak bark put in test tube, then dissolved with 2 mL aquadest and shaken until foams formed. Added 2-3 drops of $HCl_{(p)}$ solution. Positive result of saponins test marked by formed foam with 1-3 cm height that lasts ± 15 minutes (Harborne, 1987).

Synthesis of silver nanoparticle with bioreductor from extract of cempedak bark (*Artocarpus integrifolius* L. f)

Extract of cempedak bark taken as much as 5 mL and mixed in $AgNO_3$ as much as 45 mL with concentration vary from $1,5 \times 10^{-3}$, 2×10^{-3} , 3×10^{-3} and 4×10^{-3} M. solution synthetized into *microwave* with $60^\circ C$ temperature until the color shift occur into brownish yellow indicating nanoparticle formed. (Kudle *et al.*, 2014).

Stability analysis of nanoparticle with spectrophotometer UV-Vis.

Characterization of silver nanoparticle formed carried out with spectrophotometer UV-Vis instrument. This characterization aim to find out formed silver nanoparticle and its stability.

Analysis with Transmission Electron Microscope (TEM)

Characterization of silver nanoparticle with TEM (Transmission Electron Microscope) aim to understand the morphology of silver nanoparticle synthesis result showed in picture.

Analysis with PSA (Particle Size Analyzer)

Characterization with PSA (Particle Size Analyzer) on silver nanoparticle sample aim to determine the size of nanoparticle.

Results and Discussion

Synthesis of silver nanoparticle with bioreductor

Synthesis of silver nanoparticle with bioreductor carried out with utilizing cempedak bark extract, where extraction of dried cempedak bark extract as much as 1 kg with maceration method using organic solution which is methanol. Extraction process with organic solution done to pull out secondary metabolite compound contained in cempedak bark. Result obtained in form of methanol extract of 202,2 g cempedak bark with 20,22 % yield percentage result. After methanol extract of cempedak bark obtained, phytochemicals test carried out qualitatively. Result from phytochemicals test of methanol extract of cempedak bark showed in table 1.

Table 1. Result of phytochemical test of ethanol extract of cempedak bark

No.	Secondary Metabolite	Test Result
1.	Alkaloid	-
2.	Flavonoid	+
3.	Phenolic	+
4.	Quinone	-
5.	Steroid	-
6.	Triterpenoid	+
7.	Saponin	-

Annotation:

(+) = Positive containing secondary metabolite compound.

(-) = Negative containing secondary metabolite compound.

According to phytochemical test, positive test result obtained in secondary metabolite compound flavonoid, phenolic and triterpenoid. Therefore, cempedak bark extract can be utilized as bioreductor in synthesis of silver nanoparticle with bioreductor. Based on research done by Purnamasari (2015), it is discovered that every plants contain various kinds of secondary metabolite which has groups of phenolic

compound. Phenolic compound has high nucleophilic, hence able to reduce metal.

Synthesis process of silver nanoparticle with bioreductor carried out by means of micro wave from *microwave* with temperature of 60°. In this synthesis process of silver nanoparticle with bioreductor, cempedak bark extract will reduce silver ion in AgNO_3 from Ag^+ into Ag^0 , hence formed a solution with yellow brownish color as shown in Figure 1, marking the forming of nanoparticle.



Figure 1. Synthesis result of silver nanoparticle with bioreductor (cempedak bark extract) with concentration level of AgNO_3 0,0015; 0,002; 0,003; 0,004 M.

Synthesis result of silver nanoparticle with bioreductor (cempedak bark extract) is in shape of solution with yellow brownish color, where the higher the level of concentration of AgNO_3 used, the thicker the color of the solution, whereas nanoparticle obtained with lower concentration level of AgNO_3 produce lighter color solution. It is proven in result test of spectrophotometer UV-Vis, where in synthesis result of silver nanoparticle with bioreductor, maximum wave length measurement applied in each silver nanoparticle with variation of concentration levels and a chart obtained showed Figure 2.

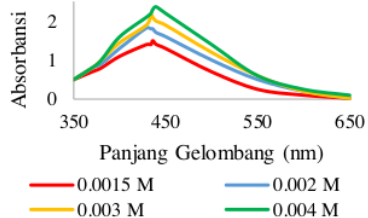
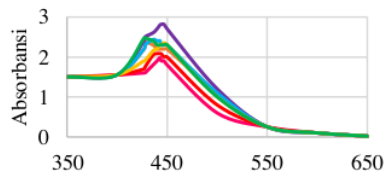
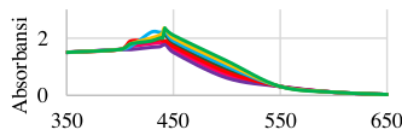


Figure 2 Result of maximum wavelength measurement on silver nanoparticle with bioreductor.

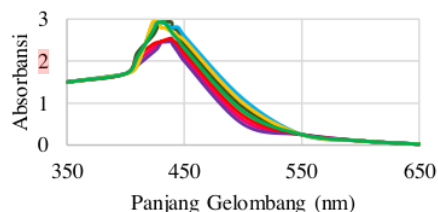
Analysis of Silver nanoparticle stability with Spectrophotometer UV-Vis



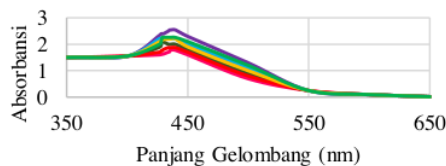
(a) Panjang Gelombang (nm)
 Hari 1 Hari 2 Hari 3
 Hari 4 Hari 5 Hari 6
 Hari 7



(b) Panjang Gelombang (nm)
 Hari 1 Hari 2 Hari 3
 Hari 4 Hari 5 Hari 6
 Hari 7



(c) Panjang Gelombang (nm)
 Hari 1 Hari 2 Hari 3 Hari 4
 Hari 5 Hari 6 Hari 7



(d) Panjang Gelombang (nm)
 Hari 1 Hari 2 Hari 3 Hari 4
 Hari 5 Hari 6 Hari 7

Figure 3 Observation result of silver Nanoparticle with bioreductor stability for 7 days (a) AgNO_3 0,0015 M, (b) AgNO_3 0,002 M, (c) AgNO_3 0,003 M and (d) AgNO_3 0,004 M.

Stability data of silver nanoparticle with bioreductor can be viewed with wave length measurement method with Spectrophotometer *UV-Vis*, where in range of 400-450 nm denote the unique properties of silver nanoparticle formed (Solomon *et al.*, 2007). Nanoparticle formed measured its maximum wavelength for 7 days to perceive the stability of the nanoparticle. Maximum wave length measurement result on silver nanoparticle with bioreductor done for 7 days can be viewed from spectrophotometer *UV-Vis* measurement result shown in Figure 3.

Measurement result of silver nanoparticle with bioreductor stability for 7 days using spectrophotometer *UV-Vis* showed nanoparticles in each concentration stable. Research result shows wave length shifting in silver nanoparticle with AgNO_3 0,0015 M concentration approximately 428-447; in 0,002 M approximately 429-443; in 0,003 M approximately 423-440 and in 0,004 M approximately 428-440 nm. Based on said wave length measurement result, silver nanoparticle with AgNO_3 0,004 M concentration level is more stable than the silver nanoparticle with AgNO_3 0,0015 M, 0,002 M and 0,003 M concentration level due to silver nanoparticle with AgNO_3 0,004 M concentration level has the smallest deviation in wave length shifting than other AgNO_3 concentration. As for maximum wave length measurement for 7 days result on silver nanoparticle with bioreductor using various concentration level of AgNO_3 can be viewed in Figure 4.

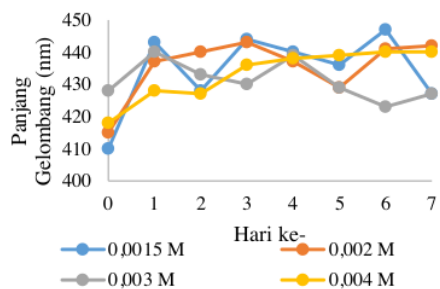


Figure 4 Result of maximum wavelength measurement on silver nanoparticle with bioreductor for 7 days.

Based on result of maximum wave length measurement for 7 days, shows that wave length shifting occur in nanoparticle with various AgNO_3 concentration level. This maximum wave length position shifting showed that changes occur in size of nanoparticle from each concentration. From obtained data, for silver nanoparticle with bioreductor has stability on silver nanoparticle with AgNO_3 0,004 M due to insignificant wave length shifting in deviation

than in other concentration levels of AgNO_3 . According to Ridwan *et al.*, (2019), storing length also affecting silver nanoparticle absorbance due to silver nanoparticle forming in process, where reduction process of Ag^+ into Ag^0 still occur.

TEM (Transmission Electron Microscope) analysis

Based on result test of silver nanoparticle with bioreductor stability using spectrophotometer *UV-Vis*, silver nanoparticle with AgNO_3 0,004 M concentration level characterized using TEM (Transmission Electron Microscope). The aim of characterization with TEM is to understand the morphology and average particle size based on diameter measurement using ImageJ software, also to ensure that silver nanoparticle formed by diffraction picture made. Characterization result of silver nanoparticle with bioreductor using TEM can be viewed in Figure 5.

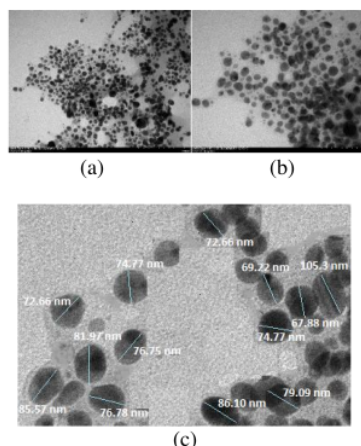


Figure 5. Transmission Electron Microscope (TEM) characterization result on silver nanoparticle with bioreductor (a) 200 nm scale (b) 100 nm scale (c) Size of silver nanoparticle with bioreductor.

Based on characterization result of silver nanoparticle with bioreductor using TEM, morphology of silver nanoparticle obtained in round shape with various particle sizes. Particle size measurement with ImageJ software, smallest particle size obtained is 67,88 nm and largest particle is 105,3 nm. Average size of particle is 78,88 nm, where in characterization with TEM the scale used is 200 and 100 nm.

PSA (Particle Size Analysis)

Silver nanoparticle with bioreductor also characterized with PSA (Particle Size Analyzer). Aim of PSA characterization is to determine the size of particle and

uniformity of particle analyzed. PSA (Particle Size Analyzer) characterization result of silver nanoparticle with bioreductor as shown in Figure 6.

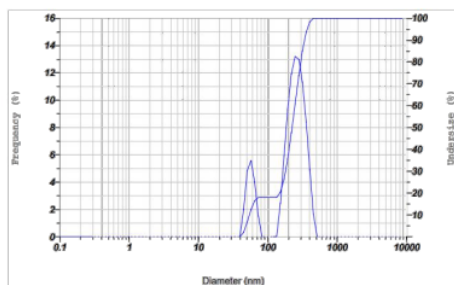


Figure 6 PSA (Particle Size Analyzer) characterization result of silver nanoparticle with bioreductor

Based on PSA characterization result of silver nanoparticle with bioreductor, size distribution of nanoparticle in range of 100 to 170 nm. Particle size obtained generally from 40 nm to 100 nm. From said data, average size of particle discovered suitable with the research done by Prasetiowati *et al.*, (2018), where particle size obtained is 112,8 nm.

Conclusions

Silver nanoparticles produced by bioreductor shows stability for 7 days on AgNO_3 with 0,004 M concentration level. Morphology of nanoparticles are round in shape and average size of nanoparticle with bioreductor is 78,88 nm. While according to PSA data, distribution of particle size on nanoparticle with bioreductor in range of 50-170 nm.

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