

Physical Characteristic and Antibacterial Activity of Silver Nanoparticles from Green Synthesis Using Ethanol Extracts of *Phaleria macrocarpa* (Scheff.) Boerl Leaves

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ABSTRACT

The Green Synthesis method is widely developed due to its environmentally friendly, cost-effective, and easy application for nanoparticle-scale synthesis. Among all metal nanoparticles, silver nanoparticles are the most utilized products in the field of nanotechnology. Biomolecules contained in plant extracts can reduce the size of silver particles to nano size. This study aims to determine the antibacterial properties and activity of silver nanoparticles synthesized with the ethanol extract of *mahkota dewa* leaves. The formation of silver nanoparticles is monitored with UV-Vis absorption and its change in color. Parameters evaluated are shape, size, particle size distribution, composition, metal residue, and a functional group of nanoparticles, using Scanning Electron Microscopy (SEM), Particle Size Analyzer (PSA), X-Ray Diffraction (XRD), and Fourier Transform InfraRed (FTIR) instrument. The research on UV-Vis color and absorption show black silver color with the wavelength of 450-465nm. The characterization result shows spherical-shaped silver nanoparticle. Furthermore, PDI best value on concentration of 0.125% is $0,221 \pm 0,0482$ with average particle size of $130,300 \pm 12,6858$ nm. The diffraction pattern of silver nanoparticle with XRD test indicates that the nanoparticles contain the silver component. Antibacterial activity test shows that silver nanoparticles have a greater inhibition zone than AgNO₃, and 0.125% ethanol extract of *mahkota dewa* leaves against *Escherichia coli* and *Staphylococcus aureus*. From the results of the study, it can be concluded that the ethanol extract from *mahkota dewa* leaves can be used as a bioreductor agent to produce silver nanoparticles which have greater antimicrobial activity compared to Ag and ethanol extract from *mahkota dewa* leaves.

Key words: Antibacterial, Green Synthesis, Mahkota Dewa, *Phaleria macrocarpa*, Silver Nanoparticle

INTRODUCTION

Diabetes mellitus is a chronic disease in which complication could affect the quality of the patient's life. In addition, WHO has predicted an increase in diabetics from 6.4% (285 million) in 2010 to 7.7% (439 million) in 2030 (Mihardja *et al.*, 2014). *Diabetic foot ulcer* (DFU) is one of the chronic complications in diabetes mellitus caused by the presence of neuropathy and vascular disorders in the legs, and these complications could have a large long-term impact on the patient's morbidity, mortality, and quality of life (Sing *et al.*, 2013). DFU occurs when there is an infection of *polymicrobial*. The most common pathogen that can be seen is gram-positive aerobic cocci, specifically *Staphylococcus aureus*, and β -hemolytic *streptococci*. Resistance or multi-resistance has become a major problem in the health sector, for instance, the resistance of *Staphylococcus aureus* to methicillin and *Candida albicans* to fluconazole. The new introduction to

wound care dressing using silver has become a new breakthrough for wound healing or infection for it is known that silver ions and silver-based compounds are highly toxic to microorganisms (Duran, *et al.*, 2007).

The method of synthesizing nanoparticles with a chemical approach using chemicals mostly has dangerous and toxic risks and is not environmentally friendly. Biological method approach then becomes the choice for synthesizing nanoparticles using plants (*green synthesis*). The choice of plants for biosynthesis is based on the content of reducing substances such as citric acid, ascorbic acid, flavonoids *etc.* which provides an important role in the biosynthesis of silver nanoparticles (Roy & Das, 2015). The use of plants can be developed for the synthesis of nanoparticles on a large scale by controlling the parameters for synthesis according to their size, shape, and dispersion (Nabikhan, *et al.*, 2010). Silver nanoparticles have antibacterial and anti-inflammatory properties that can be used for faster wound healing. Silver nanoparticles can affect cell metabolism and inhibit cell growth when the silver

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nanoparticles are in contact with bacteria, thus preventing the occurrence of protein synthesis which causes a decrease in membrane permeability which ultimately leads to cell death (Hajimirzababa *et al.*, 2012).

Phaleria macrocarpa (Scheff.) Boerl or *mahkota dewa* had been widely used for various kinds of needs including anti-cancer, anti-inflammatory, and anti-bacterial applications. The extract from this plant contains flavonoids, polyphenols, saponins, essential oils, alkaloids, and phalerin (Shodikin, 2010; Andrian *et al.*, 2014). Phalerin from *Mahkota Dewa* extract has the activity of accelerating wound healing (Easmin *et al.*, 2014; Kamal *et al.*, 2015)

From the background, it is considered necessary to do research on silver nanoparticles synthesized with *mahkota dewa* leaves extract (*Phaleria macrocarpa* (Scheff.) Boerl). Silver nanoparticles produced from synthesis are characterized to determine the shape and size of nanoparticles using *Scanning Electron Microscopy* (SEM) instrument, nanoparticle distribution using *Particle Size Analyzer* (PSA), metal content with *X-Ray Diffraction* (XRD), and detection of compound groups formed using *Fourier Transform InfraRed* (FTIR). After characterizing the optimal result from the concentration of ethanol extract of *mahkota dewa* leaves, the antibacterial activity is tested with agar well diffusion method using *Staphylococcus aureus* and *Escherichia coli* bacteria.

METHODOLOGY

Material and Equipment

The materials used in this study include dry simplicia of *mahkota dewa*, AgNO₃ metal salts (Merck, Germany), NaOH (Merck, Germany), *Staphylococcus aureus* bacteria, *Escherichia coli*, *Mueller-Hinton Agar* (MHA) media (Sigma-Aldrich, Germany), Chloramphenicol antibiotics (Sigma-Aldrich, USA), and Gentamicin antibiotics (Sigma-Aldrich, USA).

The equipment used are Branson 8510R-MTH Ultrasonic (Danbury, USA), Malvern Zetasizer Nano ZS (Rhode Island, USA), Hitachi U-2900 UV-Vis Spectrophotometer (Tokyo, Japan), *Scanning Electron Microscope* (SEM) Jeol JSM-6510 (Japan), *Particle Size Analyzer* (PSA), Microtrac Nanotrac Wave II, X-Ray Diffraction (XRD) PAN analytical (United Kingdom), OHAUS Pioneer analytical balance (Shanghai, China), *Centrifuge* Rotofix 32 (Hettich, Germany), *Biofuge Stratos* Thermo Scientific (USA), *Hotplate Stirrer*, FTIR (*Fourier Transform Infra Red*) Jasco R-4200, and laboratory glassware.

Sample Extraction Method

The dried simplicia of *mahkota dewa* leaves (*Phaleria macrocarpa* (Scheff.) Boerl) was mashed using a mortar to become powder and then sieved using a 20-mesh sieve. Ten grams of plant powder were extracted in 100 ml ethanol using *ultrasound-assisted extraction*. Filtering was done using Whatman paper and then the same solvent was added up to 100.0 ml using a measuring flask.

Synthesis of Silver Nanoparticles

The content of plant extract is used with different ratios to obtain optimum concentration. The nanoparticle synthesis was started by mixing plants sample with sterile water until a concentration of 0.125; 0.25; 0.50% b / v was reached. Then, AgNO₃ was added until a solution concentration of 1 mM was reached. Addition of AgNO₃ was carried out slowly along with the stirring treatment. After all the salts dissolved, 0.2 M NaOH was added slowly while stirring accompanied by heating at a temperature of 60°C and 80°C to reduce Ag⁺ to Ag ion. The suspension was stirred constantly.

The collection and purification of nanoparticles were carried out by centrifugation with 2000 rpm for 15 minutes (eliminating unwanted impurities). The next step for collecting yield was centrifugation at 11000 rpm for 15 minutes. Pellets were formed, which were then washed 3 times using sterile water to remove unwanted impurities or components. Washed particles were stored in the desiccator until they became dry and powdery. This yield was stored for further characterization and testing (Kim, 2015).

Characterization of Ethanol Extract Nanoparticles from *Mahkota Dewa* Leaves

The yield of the synthesis of ethanolic extract of *mahkota dewa* leaves silver nanoparticles were characterized by measuring the particle distribution using *Particle Size Analyzer* (PSA), the morphological shape of the nanoparticles was seen using *Scanning Electron Microscopy* (SEM), metals formed was analyzed using *X-ray Diffractometer* (XRD), and the functional groups contained in nanoparticles was observed using FTIR.

Determination of Antibacterial Activity with Agar Well Diffusion Method

The antimicrobial effectiveness of silver nanoparticles was carried out on pathogenic bacteria, such as *Staphylococcus aureus* and *Escheria coli*. The test was carried out using the *Agar Well Diffusion* method conducted on *Muller*

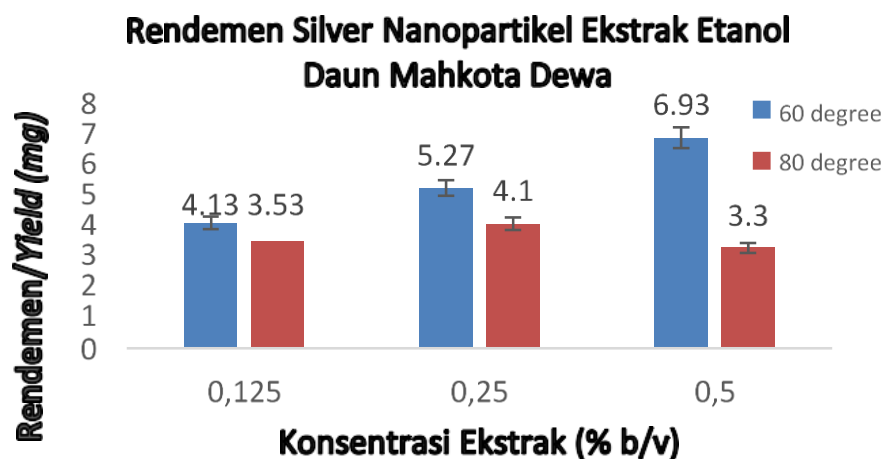


Figure 1. The average histogram of silver nanoparticles yield

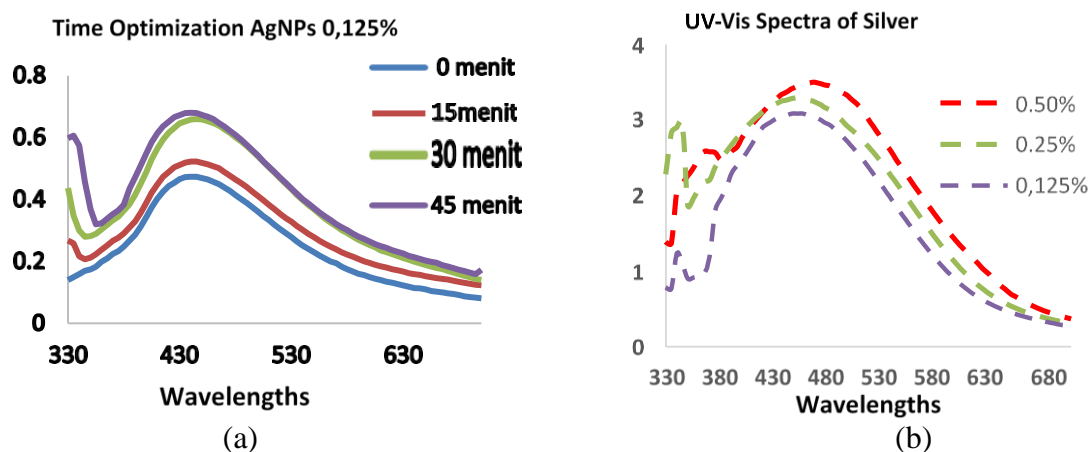


Figure 2. (a) UV-Vis Spectrum Graph. The formation of Silver Nanoparticles using ethanol extract of *mahkota dewa* leaves was 0.125% b/v, at various time ranges. (b) UV-Vis Spectrum Graph. Silver nanoparticles at various concentrations after the addition of NaOH.

Hinton Agar (MHA). As much as 16 ml of melted agar (45 °C) were poured into a petri dish and left to freeze at room temperature. A well hole was made with a diameter of 6 mm using a sterile *cork borer* on the agar that had hardened. The standardized bacterial suspension was applied aseptically according to the standardized concentration of 1 Mc Farland into the petri dish which was taken with a sterile osseous needle and then implanted in the media by scraping. A total of 0.1 ml of silver nanoparticles dissolved in DMSO were placed in the well pit and incubated at 37°C for 24 hours. As a positive control, chloramphenicol 250 ppm and gentamicin 100 ppm were used. Antibacterial activity was measured from the diameter (mm) of the clear zone formed around the well hole (Rojas *et al.*, 2003).

RESULTS AND DISCUSSION

Temperature is one parameter that can affect the formation of silver nanoparticles. The results obtained illustrate that the higher the temperature, the smaller the yield obtained has become. This is because agglomeration occurs at high temperatures and therefore particles formed in large size.

The UV-Vis spectrophotometer was carried out to confirm the readings of the absorbance of silver nanoparticles. In Figure 2, it can be seen that with increasing time, the absorption of the solution increases, namely the emergence of peak wavelengths that are included in the range of absorption of silver nanoparticles, namely 400-500 nm (Udapudi *et al.*, 2012). The scanning result showed that the optimum reaction time of silver nanoparticles biosynthesis was 30 minutes,

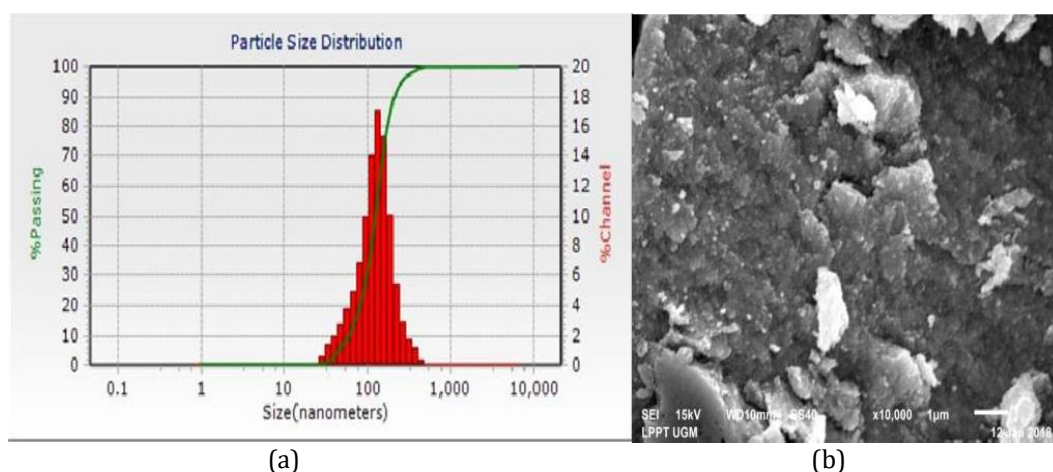


Figure 3. (a) PDA AgNPs Result Graph of Ethanol Extract of *Mahkota Dewa* Leaves 0.125% b/v. (b) SEM result of silver nanoparticles with a concentration of mahkota dewa leaf ethanol extract 0.125% b/v at 10,000x magnification.

Table I. The Nanoparticle Size and the Value of *Polydispersity index* (PDI) Silver Nanoparticles in Various Concentrations

<i>Mahkota dewa</i> leaves ethanolic extract concentration	PDI Value	Measurement (nm)
0,125% b/v	0,221 ± 0,0482	130,300 ± 12,6858
0,25%, b/v	0,322 ± 0,0335	178,467 ± 20.7760
0,50% b/v	0,603± 0,1450	111,200 ± 19,2333

In which in the 45th minute there was no significant increase.

The description of the formation of silver compounds in the synthesis process using extract concentrations of 0.125%, 0.25%, and 0.50% b/v can be seen after the addition of NaOH. The addition of NaOH is performed to increase the reduction rate in the formation of silver nanoparticles. The absorbance graph in (Figure 2) illustrates the comparison between the amount of absorbance of silver particles formed after the addition of NaOH and the different variations in extract concentration. The Scan shows that the greater the concentration of ethanol extract of the *mahkota dewa* leaves were used, the greater the absorbance obtained. The maximum absorbance of each concentration is in the range 400 - 465nm.

The size characterization and size distribution of silver nanoparticles were carried out using the PSA (*Particle Size Analyzer*) instrument. Table I below shows the results of particle measurements in each concentration on the PSA reading. It is known that the particle size values generated from the three concentrations range at 150 nm. At a concentration of 0.125% b/v,

the mean for particle size was 130,300±12,6858, followed by a concentration of 0.25% b/v with an average of 178.467±20.7760, and a concentration of 0.5% b/v having an average of 111,200 ± 19,2333. From this analysis, the value trend of *polydispersity index* (PDI) is directly proportional to the increase in concentration of plant extracts, for instance, the concentration of 0.125%, 0.25%, and 0.5% b/v is 0.221±0.0482, 0.322±0.0335, and 0.603±0.1450. The higher PDI value indicates that the nanoparticle sample has a very wide size distribution (heterogeneous). The increase in PDI value at a concentration of 0.5% b/v shows the instability of silver nanoparticles due to the agglomeration process. The process of agglomeration is influenced by the electrostatic energy on the nanoparticles and also the influence of the *Van der Waals* force where due to the force the pull between electrons occurs so that the distance between one another is getting closer and the nanoparticles merge and form large particles (Sulistawaty, 2015).

The morphology of silver nanoparticles yield was analyzed using *Scanning Electron Microscopy* (SEM) at magnifications of 1000x,

Table II. The position of 2θ Silver Nanoparticles Theta 0.125% b/v

Pos. [2θ .]	Height [cts]	FWHM Left [2θ .]	d-spacing [Å]	Rel. Int. [%]
28,0213	7,09	0,6691	3,18435	14,16
32,3980	21,24	0,2007	2,76347	42,41
38,2396	50,09	0,2007	2,35369	100,00
46,3239	13,54	0,3346	1,96002	27,03
64,4111	14,26	0,5353	1,44652	28,47
77,4183	19,41	0,4684	1,23277	38,76
83,3259	3,91	0,4684	1,15974	7,80

Table III. The Result of Inhibitory Zone Measurement on Silver Nanoparticles Antibacterial Activity Test on *Staphylococcus aureus* and *Escherichia coli*

Sample	The diameter of inhibition zone	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
AgNPs	10,10 \pm 0,35 mm	9,17 \pm 0,45 mm
AgNO ₃	7,37 \pm 0,21 mm	7,87 \pm 0,25 mm
Plant Extract	-	-
Positive Control (+)	18,13 \pm 0,38 mm	18,43 \pm 0,15 mm
Negative Control (-)	-	-

3000x and 10,000x. The result of the observation shows that the morphological shape of the silver nanoparticle yield is spherical.

To determine the presence of silver compounds, the yield of the silver nanoparticles produced was analyzed using XRD. The characteristics testing using XRD were carried out on silver nanoparticles extracted from *mahkota dewa* leaves with a concentration of 0.125% b/v. Diffractogram readings were carried out and compared with the standard for silver from (JCPDS) file No. 4078 (Swanson *et al.*, 1955). The diffractogram profile formed illustrates the same peak at a value of 2θ which shows similarities with the reference XRD pattern from the standard Ag. The diffractogram profile of silver nanoparticles at a concentration of 0.125% b/v shows the same peak at a value of 2θ 38.23, 64.41, and 77.41 (Table II).

The antimicrobial activity test was carried out using the *Well Diffusion* method in gram negative and positive bacteria. The bacteria used were *Escherichia coli* as gram-negative bacteria and *Staphylococcus aureus* as the gram-positive. As a positive control, Gentamicin antibiotic (100 μ g/mL) was used, while for gram-positive bacteria, Chloramphenicol (250 μ g/mL) was used. The negative control used was sterile aquademineralisata. The silver nanoparticle samples tested were results of yield with a plant extract concentration of 0.125 b/v. The result of the measurement of the inhibition zone in testing antibacterial activity (Table III).

The inhibition zone diameter produced by the silver nanoparticles yield is higher than the inhibition diameter produced by AgNO₃ as seen in table.

4.5. From the table, it can also be seen that the negative control and ethanol extract of *mahkota dewa* leaves with a concentration of 0.125% does not have an inhibitory zone, which means it has no antibacterial activity. It occurred because the concentration of ethanol extract used is too small. Similar research conducted by Sodikin *et al.*, (2010) found that the ethanol extract of *mahkota dewa* leaves can only have an antibacterial effect with a concentration of 4x10⁴ μ g/mL, which inhibits the bacterial activity of *Pseudomonas aeruginosa*. The same study was also conducted on the ethanol extract of *mahkota dewa* leaves in which the results show that the extract had weak activity against *Staphylococcus aureus* and *Escherichia coli* bacteria at a concentration of 10 mg/ml (Matsjeh *et al.*, 2015).

CONCLUSION

From this study, we can conclude that from the physical character of silver nanoparticles from *green synthesis* using ethanol extract of *mahkota dewa* leaves, the yield is black. From the results of the physical character test, the results of *Particle Size Analyzer* (PSA) analysis with the average size of nanoparticles at a concentration of 0.125% was 130,300 \pm 12,6858 nm with a value of *Polydispersity Index* (PDI) 0,221 \pm 0,0482. The results of the *Scanning Electron Microscopy* (SEM) analysis show

a spherical shape, and the diffractogram pattern at position 2θ as XRD result illustrates that silver nanoparticles are contained in the yield. The concentration of *mahkota dewa* (*Phaleria macrocarpa* (Scheff.) Boerl) leaves ethanolic extract has an effect on the results of the *green synthesis* of silver nanoparticles. The greater the concentration was used, the higher the PDI value obtained. Silver nanoparticles from *green synthesis* with water extract of *mahkota dewa* leaves at a concentration of 0.125% b/v had antibacterial activity against gram-positive bacteria namely *Staphylococcus aureus* and gram-negative, namely *Escherichia coli*.

ACKNOWLEDGMENT

The researcher expresses gratitude to the Directorate of Research and Community Service and Development of the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the National Competitive Grants.

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