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Research Article

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Synthesis of Silver Nanoparticles Mediated by Stem Extract of *Anredera cordifolia* and Study of their Antimicrobial Properties

Synodalia C. Wattimena[1], Philipus J. Patty[2]*

[1]Departemen Biologi and [2]Departemen Fisika, Fakultas MIPA - Universitas Pattimura, Jl. Ir. M. Putuhena Poka Ambon 97233 Indonesia

Abstract In this study, we synthesize silver nanoparticles using *Anredera cordifolia* stem extract as a reducing agent. We evaluate the antimicrobial properties of these nanoparticles against gram negative bacterial strain (*Escherichia coli*) and gram positive bacterial strain (*Staphylococcus aereus*). It took less than 10 minutes after mixing stem extract with silver nitrate solution for silver nanoparticles to form, which is indicated by the change of the colour of the sample to yellowish brown, and its absorption to the visible light. The particles continue to form and complete the formation in one day. We found from UV-VIS spectra that 424 nm is the wavelength where the Plasmon resonance takes place. The FTIR spectra suggest that the silver nanoparticles formed contained alcohols and phenolic compounds as well as protein which are responsible for their formation and stabilization. Based on the inhibition zones, we found that the silver nanoparticles show antibacterial activity against both *E. coli* and *S. aereus*, while stem extracts alone do not show antibacterial activity against *E. coli* and *S. aereus*. In addition, antibacterial activity of the silver nanoparticles against both *E. coli* and *S. aereus* depends on the silver nitrate concentration. Analysis of variance indicates that the diameter of inhibition zone of 3 mM silver nitrate nanoparticles is significantly larger than that of 1 mM and 2 mM silver nitrate nanoparticles. We found no significant difference in diameter of inhibition zones between *E. coli* and *S. aereus*, which indicates a broad-spectrum antibacterial activity of the *Anredera cordifolia* stem extract mediated nanoparticles.

Keywords Silver Nanoparticles, Antimicrobial activity, Plasmon resonance, Stem extracts

Introduction

Silver nanoparticles (NPs) have been the focus of many researchers due to their broad range applications. Silver NPs have been used in electronics [1], in biosensors [2,3], in medical devices, where they are used as wound dressing [4], to coat catheters [5], and as additive in bone cements [6], in water purification [7], as biolabels [8], and in solar energy absorption system [9]. The use of silver NPs in many applications is due to their antimicrobial, electronic, and optical properties.

Silver NPs can be produced by chemical, physical, and biological methods. However, since there is an issue of toxicity in chemical method and requirement of a large amount of energy in physical method, biological method, which is cost effective and environmentally friendly, becomes more popular. In the biological method: plants [10] and microorganism, such as algae [11], bacteria [12], yeast [13], fungi [13], and plants [14] are used to produce silver NPs. Using plant extracts, however, is advantageous compared to using microorganism, where there is an extra work in maintaining cell cultures.

Synthesis of silver NPs using plant extracts and study of its antimicrobial properties have been done by many researchers. For examples, the researchers used extracts of leafs [15,16], flowers [17,18], fruits [19,20], peels

[21,22], barks [23,24], and stem [25]. These are just recent studies mentioned from large number of studies in the last 10 years. Although, all parts of the plant used for synthesizing silver NPs, the extract of stem is the least.

In this study, we use stem extract of *Anredera cordifolia* to synthesize silver NPs, and evaluate their antimicrobial properties. We observe the initiation and continuation of the formation of silver NPs, and their stabilization. We use different concentrations of silver nitrate to examine the ability of silver NPs to inhibit the growth of gram positive and gram negative bacterial strains.

Materials and Methods

Materials

Stem of *Anredera cordifolia* were collected from local garden in Ambon Indonesia. $AgNO_3$ was used to prepare silver nitrate solution and, Whatman No.1 filter papers were used to prepare the stem extracts.

Synthesis of Silver Nanoparticles

Silver NPs were prepared by mixing leaf extract and solution of silver nitrate. For stem extract preparation, stems of *Anredera cordifolia* were collected and washed under tap water followed by distilled water. The dried stems then were cut into pieces, and 20 g of the stems were put into a beaker containing 200 ml distilled water, continued by heating the mixture to the boiling temperature (around 20 minutes). After cooling, the mixture is filtered through a whatman filter paper No.1. Then, 100 ml of 1 mM solution of silver nitrate was prepared. To synthesize silver NP, required volume of 1mM silver nitrate solution was mixed with stem extract with ratio of silver nitrate solution to stem extract of 2:1.

Characterizations of Optical Properties of Silver NPs

Characterization of optical properties of silver NPs include characterization of Plasmon resonance using UV-VIS spectroscopy and colorimeter, colour characterization using camera, and characterization of their functional groups using Fourier Transform Infra Red (FTIR) spectroscopy. For these purposes, UV-1700 PharmaSpec, Shimattsu Spectrophotometer was used to characterize the wavelength of Plasmon resonance, where the wavelength varies from 300 nm to 700 nm. Still to characterize the Plasmon resonance, Colorimeter Smart 2 LaMotte was used, where there are 4 discrete wavelengths: 430 nm, 520 nm, 470 nm, and 620 nm. To record the colour of the sample as a consequence of Plasmon oscillation, Samsung smart phone was used. To characterize the functional groups of silver NPs, we used FTIR spectrometer MB3000.

The antibacterial assay

Disc diffusion method was used to observe the antibacterial activity of the silver NPs synthesized using *Anredera cordifolia* stems against Escherichia *coli* and *Staphylococcus aereus*. Fresh overnight cultures were adjusted to OD 620 nm 0f 0.1 to give an inoculum size of about 1, 5 x 10^8 cfu/ml. A 200 µL of each bacterial suspension was spread on the surface of a nutrient agar plate. Sterile paper discs of 6 mm were impregnated with 20 µl of each silver nanoparticle solution. The discs were allowed to dry and then placed on the surface of the inoculated agar plates (4 discs/plate). The plates were incubated at 37 °C for 24 hours. Zone of inhibitions around the discs were measured after the incubation time.

Results and Discussion

Formation of Silver Nanoparticles

The formation of silver NPs is indicated by the Plasmon oscillation resulting in the colour change of the sample and its response to the visible and UV light. Silver NPs were formed after mixing stem extract and silver nitrate solution. The formation of the Silver NPs, therefore, is indicated by the change of the mixture from colourless to yellowish brown. Figure 1 shows the change of colour of the mixture in the first 60 minutes to show the initial formation of silver NPs, in 7 hours to show the continuation of the formation, and in 7 days to observe the stability of the silver NPs. The figure shows that the colour of the mixture became yellowish brown in less than 10 minutes, indicating the formation of silver NPs. The colour of the mixture became browner in several hours and after one day, it turned into dark brown. The change from bright to dark brown indicates that more silver NPs were formed.

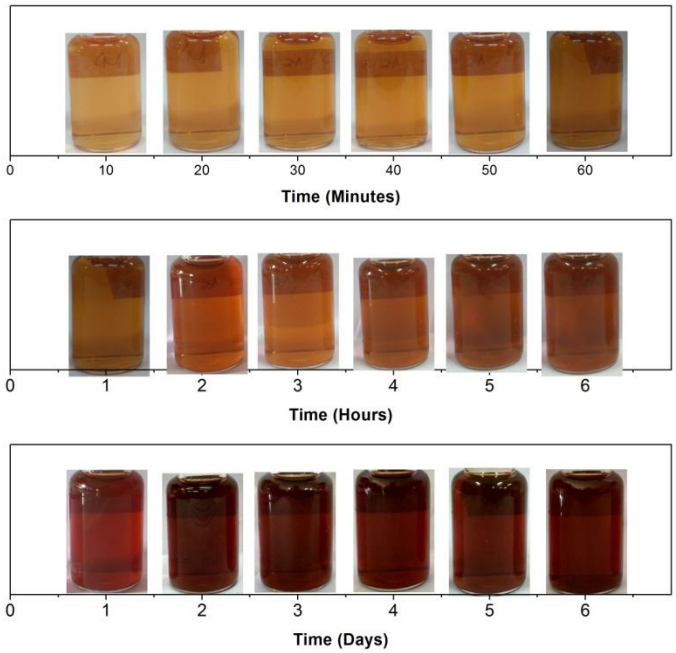

Figure 1: The colour of the sample after mixing stem extract and silver nitrate solution in 60 minutes, 6 hours, and 7 days

At the same time when the colour of the mixture was captured, the absorbance of silver NPs was recorded using colorimeter. Figure 2 shows the absorbance of the silver NPs measured after mixing the stem extract and silver nitrate solution. This is a quantitative data of the colour change in Figure 1. The absorbance was measured at four different wavelengths, and the highest intensity is at the wavelength of 430 nm, suggesting that 430 nm is closer to the wavelength of Plasmon resonance. The absorbance increases exponentially, reaches the maximum point after one day, and fluctuates around that value up to 7 days. The increase in absorbance is related to the increase in the number of silver NPs formed as stem extract mediating reduction of silver ions in the solution. The increase in intensity in one day, which relates to the change of colour, relates to the increase in the number of silver NPs formed. Hence, the formation of the silver NPs completes after one day, which can be interpreted from constant intensity and no change in colour.

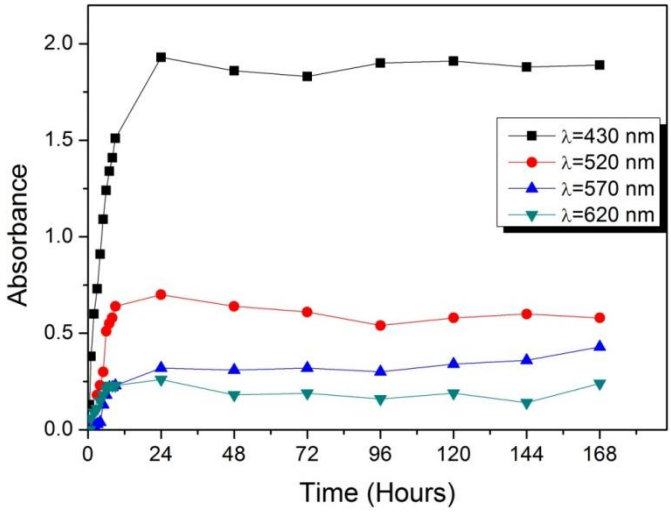

Figure 2: Absorbance of the NPs samples as a function of time as a response to light with wavelengths of 430 nm, 520 nm, 570 nm, and 620 nm

The formation of silver NPs after mixing stem extract and silver nitrate solution must be due to the content of the extract. It has been suggested that phytochemical plant extract are responsible for reduction of silver ion becoming silver NPs [26]. These phytochemicals include terpenoids, flavonoids, ketones, aldehydes, amides, and carboxylic acids.

UV VIS and FTIR Spectra

Figure 3 shows UV-Vis spectra of Silver NPs synthesized using stem extract of *Anredera cordifolia*. The sample was scanned from 700 nm to 300 nm. The maximum absorption is in the range of 400 nm to 450 nm with the peak at the wavelength of 424 nm. The peak at the wavelength of 424 nm indicates that 424 nm is the wavelength for surface Plasmon resonance for this particular sample of silver NPs. Higher absorbance of the sample at 430 nm compared to other wavelengths in Figure 2 is due to its closer value to the wavelength of Plasmon resonance of 424 nm.

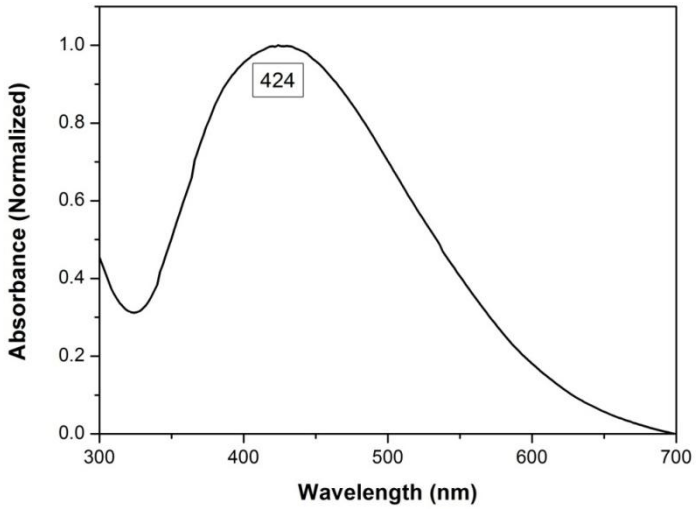

Figure 3: UV-Vis spectra of silver NPs synthesized using stem extract of Anredera cordifolia. The sample was scanned from 700 nm to 300 nm. The peak of absorbance is at the wavelength of 424 nm.

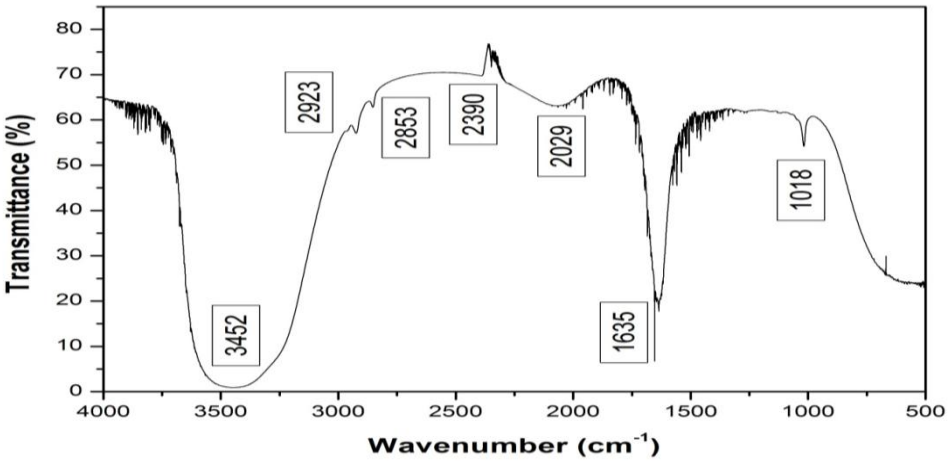

Figure 4: FTIR spectra of silver NPs synthesized using stem extract of Anredera cordifolia

Figure 4 shows FTIR spectra of silver NPs mediated by stem extract of *Anredera cordifolia*. There is a broadband peaked at 3452 cm^{-1} and an intense peak at 1635 cm^{-1}. In addition to these two peaks, several minor peaks are also indicated, including 1018 cm^{-1}, 2029 cm^{-1}, 2390 cm^{-1}, 2853 cm^{-1}, 2923 cm^{-1}. The following interpretation of the spectrum was based on the practical approach by John Coates [27]. The broadband peaked at 3452 cm^{-1} associates with an O-H stretching vibration, indicating the presence of hydroxyl groups in silver NPs: OH stretching in

alcohols and phenolic compounds. Intense peak at 1635 cm^{-1} associates with a C=O stretching vibration, indicating the presence of carbonyl groups in nanoparticles: a carbonyl stretch in the amide linkages of the proteins. The carbonyl group has been observed to have a strong binding ability to silver, which is believed to cover layer of silver NPs, thus act as a capping agent to prevent aggregation, and the proteins is believe act as reducing and stabilizing agents [28].

Antibacterial Activities

Antibacterial activities of silver nanoparticles mediated by *Anredera cordifolia* stem extract against gram negative bacterial strain (*E. coli*) and gram positive bacterial strain (*S. aereus*) were examined at different concentrations of silver nitrate. Figure 5 shows an example of inhibition zones for silver NPs formed by 1mM (Figures 1A and 2A) and 3 mM (Figures 1B and 2B) silver nitrate against *E. coli* and *S. aerueus*. The diameter of inhibition zones around each disc with silver nitrate concentration of 1 mM, 2mM, and 3 mM is summarized in Figure 6.

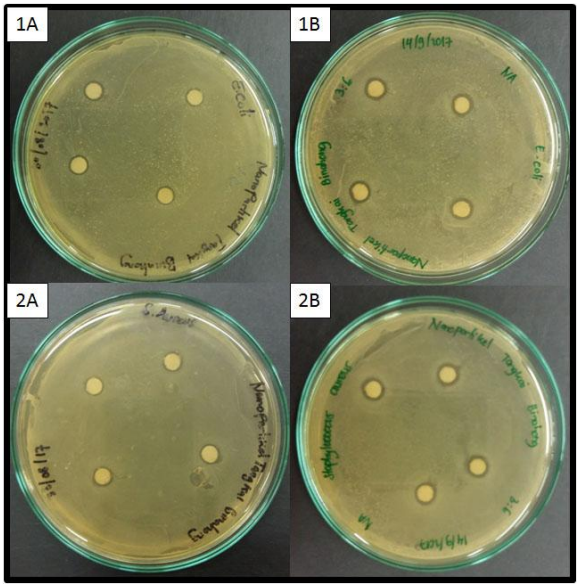

Figure 5: Inhibition zones for E. coli (1A and 1B) and S. coccus (2A and 2B). At 1B concentrations of silver nitrate is three times of those at 1A. Likewise, at 2B concentrations of silver nitrate is three times of those at 2A

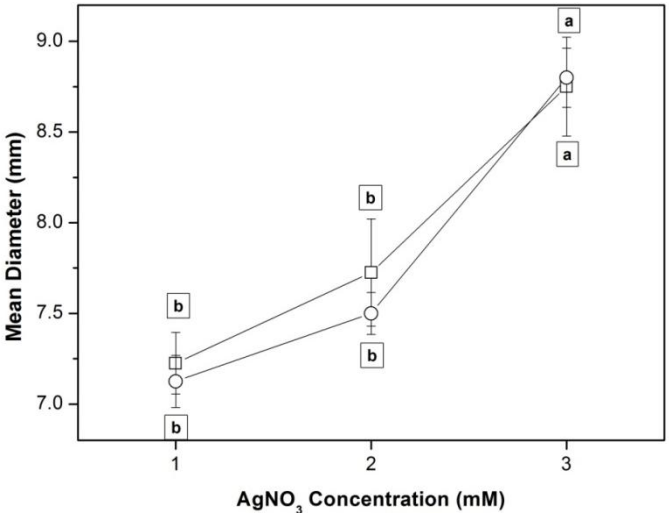

Figure 6: Mean diameter of inhibition zone against E. coli and S.coccus as a function of silver nitrate concentration. The same letters on the graph indicate that there is no significant difference, while different letters indicate that there is a significant difference.

Based on the zones of inhibition produced, the silver NPs show antibacterial activity against both *E. coli* and *S. aereus*. On the other hand, stem extracts alone do not show antibacterial activity against *E. coli* and *S. aereus* (Data not shown). Antibacterial activity of the silver NPs against both *E. coli* and *S. aereus* depends on the silver nitrate concentration (Fig. 6). Based on analysis of variance, we found that the diameter of inhibition zones with silver nitrate concentration of 3 mM for both *E. coli* and *S. aereus* are significantly larger than that of 1 mM and 2mM. The ability of silver NPs to cause disruption of bacterial membranes leading to protein leakage from the membranes has been reported and has been related to their antimicrobial activity [29]. Free radicals generated by silver NPs also have been related to bacterial membrane disruption [29]. Silver NPs synthesized by many plant extracts such as *Alternanthera dentate*, *Acorus calamus*, *Coleus aromaticus* and *Boerhaavia diffusa* have been found to have antibacterial activity against gram positive bacterial strains and gram negative bacterial strains [30,31, 32,33]. They also show antifungal activity [34].

The antimicrobial property of silver nanoparticles is due to the large surface areas of silver nanoparticles that enable them to have appropriate contact with cell membranes of microbes. The analysis of variance shows that the diameters of inhibition zones of *E. coli* and *S. aereus* are not significantly different (Fig. 6). This result shows that the silver nanoparticles synthesized by *Anredera cordifolia* stems have the same antibacterial activity on gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*, although gram-positive bacteria have thicker cell wall than gram-negative bacteria because they have more peptidoglycan on their cell wall than gram negative bacteria. This indicates a broad-spectrum antibacterial activity of the nanoparticles.

Conclusion

Formation of silver nanoparticles took place in around 10 minutes after mixing stem extract of *Anredera cordifolia* and silver nitrate solution. The number of silver nanoparticles continually increased, and completed after one day. Results from UV-VIS spectra indicates that 424 nm is the wavelength of surface Plasmon resonance on the silver nanoparticles. The FTIR spectra suggest that the silver nanoparticles formed contained alcohols and phenolic compounds as well as protein which are responsible for their formation and stabilization. We found that the silver nanoparticles show antibacterial activity against both *E. coli* and *S. aereus*. In addition, antibacterial activity of the silver nanoparticles against both *E. coli* and *S. aereus* depends on the silver nitrate concentration.

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