Physical-chemical and antibacterial properties of green-synthesized silver nanoparticles mediated by leaf extract of *Syzygium aromaticum* L

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**Abstract.** Silver nanoparticles have played an important role in many applications due to their unique properties, and green synthesis of the particles has been a popular choice as opposed to chemical and physical methods. In this study, the physical, chemical, and antibacterial properties of green synthesized silver nanoparticles mediated by leaf extract of *Syzygium aromaticum* L were examined. Initially, the formation of silver nanoparticles was indicated by the yellowish-brown color of the mixture of the extract and silver nitrate solution, followed by the UV-VIS spectrum showing the surface plasmon resonance at 415 nm. The samples then were analyzed using TEM showing the particle diameters varying from 2.9 nm to 33.6 nm with the mean diameter of 20.5 ± 4.9 nm. The FTIR spectrum showed the presence of chemical bonding of organic materials on the particles indicating the involvement of the extract as reducing and capping agents in the formation of the particles. The antibacterial activity on Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* were examined using disc diffusion and spectrophotometric methods, and the results show that the silver nanoparticles inhibit the growth of *S. aureus* and *E. coli*. Results from the disc diffusion method show that the particles inhibit the growth of *S. aureus* and *E. coli* equally, while from the spectrophotometric method, the particles inhibit the growth of *E. coli* faster than they inhibit the growth of *S. aureus*.

1. Introduction

Silver nanoparticles (AgNPs) have been the focus of many studies in recent times. This is related to the fact that AgNPs have been used in many applications. For example, they have been used as an antibacterial agent in cotton fabric [1], in drinking water treatment [2], in products of cosmetics [3], in medical devices [4], and in dental applications [5].

Green synthesis of AgNPs has been proved to be one of the favorite alternatives for producing AgNPs among several methods available, and one of the best is by using plant extracts. This method is environmentally friendly, cost-effective, and less toxic. Extracts of different parts of the plants, such as leaves [6], fruits [7], stem [8], seeds [9], barks [10], and flowers [11] have been used to synthesize AgNPs and to study their antibacterial properties.

*Syzygium aromaticum* L is a plant widely cultivated in Indonesia, and Maluku Island is one of the grown areas. The leaf extract of this plant has been a focus for few studies [12, 13, 14], not as many as the focus on its flower bud. This present study aims to use leaf extract of *Syzygium aromaticum* L to...
synthesize AgNPs, and to characterize the physical, chemical, and antibacterial properties of the particles.

2. Materials and Methods
Leaves of *Syzygium aromaticum* L were collected from a local garden in Ambon, Indonesia. The plant was identified using an identification book and was confirmed by an expert at Pattimura University. Filter papers used for the extraction were Whatman Filter Paper No.1 and silver nitrate (AgNO3) used was the one for analysis (EMSURE, ACS-ISO-Reag-Ph Eur).

2.1. Synthesis of the AgNPs
Preparation of leaf extract followed the protocol described in the previous study [8]. After washing *Syzygium aromaticum* leaves with tap water and followed by distilled water, 20 grams of the leaves were cut into small pieces and dropped into 200 ml distilled water. The mixture was then heated for 20 minutes. The extract was obtained by filtering the mixture through a Whatman filter paper No.1. For the preparation of AgNPs, 1 mM AgNO3 solution was mixed with the leaf extract with a volume ratio of 2:1 at room temperature.

2.2. Physical and chemical characterization of the AgNPs
UV-VIS spectroscopy was used to identify the wavelength, at which the surface plasmon resonance takes place. FTIR spectroscopy was used to identify the chemical bonds, thus functional groups on the AgNPs. TEM was used to characterize the shape and size distribution of the AgNPs.

2.2.1. UV-VIS spectroscopy
The instrument used was UV-VIS spectrophotometer UV-1700 PharmaSpec Shimadzu owned by Departemen Kimia Pattimura University. In the measurements, 3.5 ml of nanoparticle suspension was filled into a 10x10 mm optical path cuvette, and 3.5 ml of the extract was used as a standard. The measurements were taken with wavelengths varying from 300 nm to 700 nm.

2.2.2. FTIR spectroscopy
The instrument used was FTIR spectrophotometer 8201PC Shimadzu, owned by Departemen Kimia Gadjah Mada University. Before the measurement, the sample was centrifuged with 12,000 rpm for 20 minutes and the pellet was taken for the measurements. For the measurements, 2 mg pellet was mixed with 200 mg KBr, and the measurements were taken with wavenumbers varying from 4000 cm⁻¹ to 400 cm⁻¹.

2.2.3. Transmission electron microscopy
The instrument used was TEM JEOL JEM 1400 owned by Departemen Kimia Gadjah Mada University. For TEM measurements, a small drop of nanoparticle suspension was put onto a Cu-substrated grid and was left to dry at room temperature.

2.3. Antibacterial assay
Disc diffusion and spectrophotometric methods were used in the antibacterial assay of the AgNPs against both Gram-positive *S. aureus* and Gram-negative *E. coli*.

2.3.1. Bacterial culture preparation
A loop of each bacterial culture was suspended in the nutrient broth (500 ml) and each suspension was put in a shaker at room temperature overnight. Then, each of the overnight cultures was diluted with distilled water until it reached an inoculum size of about 1.5 x 10⁸ CFU/ml (OD 620 = 0.1).

2.3.2. Disc diffusion method
About 200 µl of the diluted culture for each bacterial suspension was spread on the surface of a nutrient agar plate using a spreader. Sterile paper discs infused with the AgNP solution (20µl/disc) were left in
a laminar airflow to dry for about 30 minutes before placing the discs into the agar surface (3 discs /plate, for 3 replicates). Before that, the AgNP solution was washed with distilled water by centrifugation (12,000 rpm) two times. The plates were then incubated at 37ºC in an incubator for 24 hours before the diameter of the inhibition zones was measured.

2.3.3. Spectrophotometric method

About 500 µl of the diluted culture for each bacterial suspension, 5 ml of AgNPs, and 5 ml of nutrient broth were mixed in a bottle and incubated at room temperature. For a control, 5 ml of sterile distilled water was used to replace AgNPs. The OD values at 620 nm were noted after the incubation time of 0, 2, 4, 6, 8, 12, 16, 20, and 24 hours. The experiment was done in three replicates. To compare the OD 620 between the control (bacteria without the AgNPs) and bacteria with the AgNPs, a t-test with a significant level of 0.05 was used. For OD-620 measurements, a colorimeter (Smart 2 LaMotte) was used. For control (pure bacterial sample), distilled water was used as a standard, while for bacterial sample+nanoparticle suspension, nanoparticle suspension was used as a standard.

3. Results and Discussions

3.1. Physical and chemical properties of the AgNPs

AgNPs were formed a few minutes after the leaf extract of Syzygium aromaticum L was mixed with silver nitrate solution. The formation of the AgNPs was indicated by the change of the color of the mixture becoming yellowish-brown (figure 1.a). This specific color results from the oscillation of electron clouds on the surface of the silver nanoparticles called a plasmon. When light impinges on the particles, the oscillation of the electric field causes an oscillation of the electrons on the surface of the silver nanoparticles, which induces the displacement of the electron cloud from its normal position. The displacement of the negatively charged electron cloud is then restored electrically by positively charged nuclei causing the oscillation of the electron cloud. The electron cloud oscillates with its unique frequency associated with this specific color.

The unique frequency of Syzygium aromaticum AgNPs represented by the wavelength is shown as a peak in the UV-VIS spectrum of the particles, 415 nm (figure 1.b). When light with different wavelengths than 415 nm is applied on the particles, there is a scattering dominating signal coming out from the particles, while at 415 nm, there is an absorption signal, where the resonance takes place, thus the term surface plasmon resonance.

Figure 1.c shows the FTIR spectrum of Syzygium aromaticum AgNPs. The broadband peaked at 3402 cm\(^{-1}\) was contributed from H-bonded O-H stretch, a peak at 1365 cm\(^{-1}\) from in-plane O-H bend, and a peak at 1041 cm\(^{-1}\) from asymmetric C-C-O stretch. These three peaks are indicative of a primary alcohol compound [15]. Peaks at 1705 and cm\(^{-1}\) and 1620 were contributed by C=O carbonyl groups, which might be conjugated: conjugated-carboxylic acid [16]. This functional group is also supported by peak at 1219 contributed from C-O stretch. Another possibility: the peak at 1620 is indicative of amide I. Peaks at 2924 cm\(^{-1}\) was contributed from C-H stretch. The results of the FTIR spectrum indicate the presence of the organic material, thus extract on the AgNPs, and suggest the contribution of the extract in the formation of the nanoparticles (reducing agent) as well as in the stabilization of the particles (capping agent).
Figure 1. a. The sample of AgNPs green synthesized using leaf extract of *Syzygium aromaticum* L, b. The UV-VIS spectrum of the AgNPs, and c. The FTIR spectrum of the AgNPs.

3.2. The Particle size distribution of the AgNPs

Figures 2.a and b show the AgNPs observed under TEM at two different magnifications. The figures indicate that the particles are mostly spherical. The diameters of the particles analyzed from 100 randomly chosen particles varied from 2.9 nm to 33.6 nm with the mean diameters found to be 20.5±4.9 nm. The particle size distribution is shown in figure 2.c.

Figure 2. a. and b. TEM photographs of the AgNPs in different magnifications, c. The particle size distribution of the AgNPs.
3.3. Antibacterial properties of the AgNPs

Figure 3 shows inhibition zones on the NA surfaces containing *E. coli* (a) and *S. aureus* (b), 24 hours after application of the AgNPs. The mean diameter of the zones of 3 replicates for *E. coli* is $13.1\pm1.0$ mm, while for *S. aureus* $13.1\pm0.1$ mm. The results of statistical analysis (t-test, $\rho>0.05$) suggests no significant difference between the two, which implies that the AgNPs inhibit the growth of *S. aureus* as good as they inhibit the growth of *E. coli*.

Figure 3. Inhibition zones on the NA surface containing *E. coli* (a), and on the NA surface containing *S. aureus* (b) as a result of applying the AgNPs.

Figure 4.a shows OD 620 of *E. coli* and *E. coli* with AgNPs. The figure indicates that the growth of *E. coli* was inhibited by the presence of the AgNPs, which is shown by a significant difference (t-test, $\rho<0.05$) in the growth of *E. coli* between samples of *E. coli* and of *E. coli* with the AgNPs, 4 hours after introducing the AgNPs. Figure 4.b shows OD 620 of *S. aureus* and *S. aureus* with AgNPs. This also indicates the inhibition of the growth of *S. aureus* by the AgNPs shown by a significant difference (t-test, $\rho<0.05$) in the growth of *S. aureus* between samples of *S. aureus* and of *S. aureus* with the AgNPs, 8 hours after introducing the AgNPs. These results imply that AgNPs inhibit the growth of *E. coli*, faster than they inhibit the growth of *S. aureus* (4 hours vs 8 hours).

Figure 4. a. OD 620 of *E. coli* and *E. coli* with AgNPs, and b. OD 620 of *S. aureus* and *S. aureus* with AgNPs.
The results of antibacterial assessment from both disc diffusion and spectrophotometric methods conclude that AgNPs green-synthesized using Syzygium aromaticum inhibit the growth of both E. coli and S. aureus. The ability of the AgNPs to inhibit the growth of both E. coli and S. aureus is consistent with the results of previous studies using AgNPs green-synthesized from other plant extracts [6-11]. This ability is related to some properties of AgNPs. First, the small size of the particles (mean diameter of 20.5±4.9 nm for Syzygium aromaticum AgNPs) makes them easy to adhere to the surface of the cell membrane, which is the first step to be able to disrupt the cell. Second, AgNPs can release Ag⁺ ions [17] which can interact electrostatically with the negatively charged membrane of the cell. These ions can penetrate the membrane interior and change the DNA structure of bacteria, which eventually causes the death of the cell. Third, AgNPs can stimulate the formation of reactive oxygen species that may lead to oxidative stress in the cell [18].

4. Conclusion

These studies show that leaf extract of Syzygium aromaticum L can be used as a reducing agent to synthesize AgNPs. The AgNPs produced were yellowish-brown in color indicative of electron cloud oscillation on the surface of the particle, known as a plasmon. Results from UV-VIS measurements specify that the surface plasmon resonance takes place at the wavelength of 415 nm, and the results from FTIR measurements show the presence of the organic materials on the particles, suggesting the involvement of the extract in the particle formation (a reducing agent), and in the particle stabilization (a capping agent). The AgNPs produced are mostly spherical, and the diameter varied from 2.9 nm to 33.6 nm with the mean diameters of 20.5±4.9 nm. The antibacterial assessment using the disc diffusion method shows that AgNPs synthesized using Syzygium aromaticum inhibit the growth of both E. coli and S. aureus equally, while the assessment using spectrophotometric method shows that the AgNPs inhibit the growth of E. coli, faster than they inhibit the growth of S. aureus.

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