Characterization of green silver nanoparticles of *Graptophyllum pictum* leaf extract: from the localized surface plasmon resonance to the antimicrobial activity

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**Abstract.** This study aims to synthesize silver nanoparticles using leaf extract of *Graptophyllum pictum* and to characterize their properties, starting from localized surface plasmon resonance, functional groups and the particle size distribution, to their antibacterial activity. The measurement of the wavelength of localized surface plasmon resonance was conducted using UV-VIS spectroscopy, while FTIR spectroscopy was used to identify the chemical bonds of organic compounds in the particle. The particle size distribution was analyzed using TEM. The spectrophotometric method was used to assess the antimicrobial properties of the particles against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. For this purpose, OD-620 of the bacterial sample was compared to OD-620 of the bacterial sample mixed with the silver nanoparticles, where the data was taken in 24 hours. The wavelength of localized surface plasmon resonance was found to be 455 nm, while FTIR spectrum showed the chemical bonds of organic compounds, denoting the presence of the extract on the particle. The particles were mostly spherical with diameters varying from 5.4 nm to 50.6 nm and the mean diameter was found to be 21.5±9.9 nm. The results from the antimicrobial assessment show that *Graptophyllum pictum* silver nanoparticles inhibit the growth of both *S. aureus* and *E. coli*, where during 24 hours of observation time, the particles affected *E. coli*, faster than the particles affected *S. aureus*.

1. **Introduction**

Silver nanoparticles (AgNPs) have been used in many applications due to their unique properties, i.e., optical, electronic, magnetic, and chemical properties, which are different from the bulk material. AgNPs have been used as biosensor [1] due to the optical properties, as conductive coatings [2] due to electronic properties, and as an agent in drug delivery [3] due to the magnetic properties. AgNPs also have been used in many applications due to their antibacterial properties. For example, AgNPs have been used in products for wound healing [4], medical devices [5], textiles [6], and many other products.

AgNPs can be synthesized using chemical, physical, and biological methods [7]. Chemically AgNPs can be synthesized using silver precursor and reducing as well as stabilizing agents, which involve chemical solvents. Physically, AgNPs can be synthesized by evaporation condensation and laser ablation. The methods require a great deal of energy. Biologically, AgNPs can be synthesized using the same procedure as the chemical method but using biology materials as reducing and stabilizing agents.
The materials can be microorganisms or plant extracts. Although each method has its advantages and disadvantages, the biological method becomes popular recently since it is environmentally friendly, cost-effective, and there is no issue of toxicity like in the chemical method or a great deal of energy requirement like in the physical method. Moreover, the biological method using plant extract as a reducing agent becomes a popular choice for its simplicity compared to using microorganisms.

Many researchers have studied the physical and chemical properties as well as antibacterial and other properties of AgNPs synthesized using plant extracts as reducing and stabilizing agents. For instance, the leaf extract of *Cucumis prophetarum* [8], fruit extract of *Phyllanthus emblica* [9], flower extract of *Fritillaria* [10], and stem extract of *Garcinia mangostana* [11] have been used to synthesize AgNPs and to study antibacterial as well as other properties.

This study aims to synthesize AgNPs using leaf extract of *Graptophyllum pictum* and to characterize their properties, starting from localized surface plasmon resonance (LSPR), functional groups and the particle size distribution, to their antibacterial activity. *Graptophyllum pictum* plant originated from New Guinea is one of the traditional medicinal plants grown in Indonesia. Phytochemical analysis of the aerial parts of this plant showed the presence of glycosides, alkaloids, saponins, flavonoids, and tannins in the ethanolic extract [12].

### 2. Materials and methods

#### 2.1. Preparation of leaf extract and AgNPs

For the preparation of the AgNPs, the protocol from the previous study was used [13]. Leaves of *Graptophyllum pictum*, collected from a local garden in Ambon, Indonesia, were washed with tap water, followed by distilled water, and were cut into small pieces. 20 grams of the leaves were then dropped into 200 ml distilled water, and the mixture was heated for 20 minutes. The mixture was left to cool down and was filtered through Whatman Filter Paper No.1 to obtain the extract. For the preparation of AgNPs, an aqueous solution of silver nitrate salt (EMSURE, ACS-ISO-Reag-Ph Eur) of 0.1 mM was prepared. The silver nitrate solution was then mixed with the extract at the volume ratio of 9:1 at room temperature.

#### 2.2. Physical characterization of the AgNPs

**2.2.1. Characterization of LSPR wavelength.** LSPR wavelength was determined using UV-VIS spectroscopy. For this purpose, UV-VIS spectrophotometer UV-1700 PharmaSpec Shimadzu owned by Departemen Kimia Pattimura University, was used. An optical path cuvette of 10x10 mm was used to put 3.5 ml nanoparticle suspension, where 3.5 ml extract was used as a standard. The wavelength used in the measurements was varied from 300 nm to 700 nm.

**2.2.2. Characterization of functional groups.** FTIR spectroscopy was used to characterize chemical bonds, thus functional groups of the extract on AgNPs. For this purpose, FTIR spectrophotometer 8201PC Shimadzu, owned by Departemen Kimia Gadjah Mada University, was used. Sample preparation was started by centrifuging nanoparticle suspension with 12,000 rpm for 20 minutes to get the pellet, and followed by mixing the pellet with 200 mg KBr. For the measurements, the wavenumber was varied from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\).

**2.2.3. Characterization of the particle size distribution of AgNPS.** Transmission electron microscopy was used to characterize the particle size distribution of AgNPs. For this purpose, TEM JEOL JEM 1400 owned by Departemen Kimia Gadjah Mada University, was used. In the measurement, 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

2.3.1. Bacterial culture preparation. A sterile loop was used to scrape off each bacterium from the agar surface of its culture and then the loop was shaken in 500 ml of nutrient broth. The broth was put in a shaker for 12 hours before it was diluted to $1.5 \times 10^8$ CFU/ml (OD 620 = 0.1) of bacterial concentration.

2.3.2. Spectrophotometric method. In spectrophotometric measurements of the antibacterial assay, OD 620 of bacterial sample mixed with AgNPs, and pure bacterial sample (control) was compared [14]. A mixture of the diluted culture of each bacterium (500 µl), nutrient broth (5 ml), and AgNPs (5 ml) was prepared in a glass bottle. For the control, in another bottle, a mixture of the diluted culture of each bacterium (500 µl), nutrient broth (5 ml), and sterile distilled water (5 ml) was prepared. The experiment was conducted in three replications at room temperature. After certain incubation times (0, 2, 4, 6, 8, 12, 16, 20, and 24 hours) the OD 620 nm were recorded. A statistical test, the t-test ($\alpha=0.05$), was used for the OD value comparison between the control and AgNP samples.

3. Result and discussion

3.1. The wavelength of LSPR of the AgNPs

Figure 1 shows a sample of Graptophyllum pictum AgNPs formed after mixing the extract and silver nitrate solution (a), and one hour later (b). The change of colour from transparent light yellow to yellowish-brown shows the process of AgNP formation. When the extract was mixed with silver nitrate solution, the silver ions $\text{Ag}^+$ from silver nitrate salt was reduced by the extract to atom Ag. The nucleation of the atoms into a small cluster then takes place, followed by growing into nanoparticles [15]. Biomolecules such as glycosides, alkaloids, saponins, flavonoids, and tannins are found in the plant extract [12] and these biomolecules are likely involved in the reduction of the $\text{Ag}^+$ ions. The yellowish-brown colour shown in Figure 1.b is an indication of the formation of the AgNPs. The colour is a consequence of the oscillation of the conduction electrons occurred when light impinges on the particles, which are smaller than the wavelength of light. The frequency of oscillation is unique and depends on the size, shape, and dielectric properties of the particles [16]. When the frequency of incident light matches the frequency of electron oscillation, the localized surface plasmon resonance (LSPR) takes place. Figure 1.c shows the spectrum of UV-VIS, indicating the maximum absorption at 455 nm, denoting the wavelength of LSPR. This wavelength is associated with the colour of the AgNPs shown in Figure 1.b.

![Figure 1. Graptophyllum pictum AgNPs after mixing the extract and silver nitrate solution (a), one hour later (b) and UV-VIS spectrum of Graptophyllum pictum AgNPs (c)](image)

3.2. FTIR spectrum of the AgNPs

Figure 2 shows the FTIR spectrum of the AgNPs. The peak at $3425 \text{ cm}^{-1}$ is likely contributed by O-H and N-H stretches. If it was only contributed by an O-H stretch, the band should look broader, and the slightly sharper peak was contributed by an N-H stretch. Peaks at 2924 and 2854 are signals of CH2
asymmetric and symmetric stretches, respectively, while at 1627 is of C=O conjugated. Peaks (weak) at 1542 and 1527 are the signal of N-H bends, peaks at 1381, and 825 indicate O-H and C-H bend, respectively, and peak at 1041 indicates C-C-O symmetric stretch.

![FTIR spectrum](image)

**Figure 2.** FTIR spectrum of *Graptophyllum pictum* AgNPs. The dash-line circle denotes the presence of CO$_2$ artifact peaks [17]

The presence of O-H stretch, in-plane O-H bends, and asymmetric C-C-O stretch is likely an indication of alcohol (primary) compound class [18]. Likewise, the presence of C=O and N-H stretches and N-H bend is likely an indication of amide II class [19]. Finally, the presence of CH$_2$ asymmetric and symmetric stretches indicates alkane class, while C-H bend at 825 is indicative of an aromatic ring. The results of the chemical bonds of organic material on the AgNPs suggest the presence of the extract on the particles. This indicates that the extract acted as reducing and capping/stabilizing agents. For instance, it has been shown that O-H groups played an important role in reducing Ag$^+$ ions to Ag [20]

3.3. *Particle size distribution of the AgNPs*

The result of the size characterization of the AgNPs using TEM is shown in Figure 3 with three different magnifications. The particles are mostly spherical. The analysis of 100 randomly chosen particles from TEM pictures reveals that the diameter of the particles ranges from 5.4 nm to 50.6 nm. The mean diameter determined is 21.5±9.9 nm. The particle distribution analyzed from 100 randomly chosen particles is shown in Figure 4. Although the distribution does not look Gaussian, it only has one peak, where particles with diameters from 9 nm to 27 nm dominate in the distribution. This one peak distribution is consistent with one peak of the UV-VIS spectrum shown in Figure 1.c. A previous study shows a correlation between the particle size distribution of silver nanoparticles and their associated UV-VIS spectrum [21].
The range of diameters found was typical for green AgNPs, varying from several nm to less than 100 nm. For example, the mean diameter of AgNPs using *L. acapulcensis* extract was found to be 5 nm [22], using Berry extract 21.5 nm [23], and using *Arbutus unedo* extract 58 nm [24]. The variation of the size of AgNPs has an impact on their application as an antibacterial agent. Studies have shown that smaller silver nanoparticles have greater antibacterial activity than larger particles [25].

**Figure 3.** *Graptophyllum pictum* AgNPs observed under TEM with three different magnifications

Antimicrobial activity of the AgNPs was observed against *E. coli* and *S. aureus* using a spectrophotometric method. Figure 5.a shows OD 620 of samples of *E. coli* and of *E. coli* mixed with the AgNPs observed 24 hours after applying AgNPs on the bacterial sample. OD 620 of the *E. coli* sample increased with time during the observation, while OD 620 of *E. coli* mixed with the AgNPs leveled off. Figure 5.b shows OD 620 of samples of *S. aureus* and of *S. aureus* mixed with the AgNPs observed 24 hours after applying AgNPs on the bacterial sample. Similar to OD 620 of *E. coli*, OD 620 of *S. aureus* increased with time, while *S. aureus* mixed with the AgNPs leveled off.

Data of OD 620 from samples of *E. coli* and of *E. coli* mixed with the AgNPs for each measurement time was analysed statistically using t-test, and it was found that there is a significant difference between the two at 4 hour of measurement time and later (t-test, $p<0.05$). This suggests that the presence of the AgNPs inhibits the growth of *E. coli* effectively 4 hours after application of the AgNPs. Likewise, data of OD 620 from samples of *S. aureus* and of *S. aureus* mixed with the AgNPs for each measurement time was analysed statistically using t-test, and it was found that there is a significant difference between the two at 8 hour of measurement time and later (t-test, $p<0.05$). This suggests that the presence of the

**Figure 4.** Particle size distribution of 100 randomly chosen *Graptophyllum pictum* AgNPs observed under TEM. The numbers in the axes are medians of intervals.

3.4. Antimicrobial activity of the AgNPs

Antimicrobial activity of the AgNPs was observed against *E. coli* and *S. aureus* using a spectrophotometric method. Figure 5.a shows OD 620 of samples of *E. coli* and of *E. coli* mixed with the AgNPs observed 24 hours after applying AgNPs on the bacterial sample. OD 620 of the *E. coli* sample increased with time during the observation, while OD 620 of *E. coli* mixed with the AgNPs leveled off. Figure 5.b shows OD 620 of samples of *S. aureus* and of *S. aureus* mixed with the AgNPs observed 24 hours after applying AgNPs on the bacterial sample. Similar to OD 620 of *E. coli*, OD 620 of *S. aureus* increased with time, while *S. aureus* mixed with the AgNPs leveled off.

Data of OD 620 from samples of *E. coli* and of *E. coli* mixed with the AgNPs for each measurement time was analysed statistically using t-test, and it was found that there is a significant difference between the two at 4 hour of measurement time and later (t-test, $p<0.05$). This suggests that the presence of the AgNPs inhibits the growth of *E. coli* effectively 4 hours after application of the AgNPs. Likewise, data of OD 620 from samples of *S. aureus* and of *S. aureus* mixed with the AgNPs for each measurement time was analysed statistically using t-test, and it was found that there is a significant difference between the two at 8 hour of measurement time and later (t-test, $p<0.05$). This suggests that the presence of the
AgNPs inhibits the growth of *S. aureus* effectively 8 hours after application of the AgNPs. Results of OD 620 measurements on *E. coli* and *S. aureus* imply that the AgNPs inhibit the growth of *E. coli* faster than they inhibit the growth of *S. aureus*. In a previous study using green AgNPs from seed extract of *Sauropus androgynous* [26], the AgNPs were also found to inhibit the growth of both *E. coli* and *S. aureus*, but with an effective time of 16 hours for both *E. coli* and *S. aureus*.

There are some possible mechanisms to explain the ability of AgNPs to inhibit bacterial growth. Physically, the nanosize of the particles is a benefit for AgNPs to interact with the cell wall and even to penetrate the cell wall to change the membrane structure, thus denaturation of the membrane, which eventually causes the cell lysis. Alternatively, AgNPs release Ag\(^+\) ions [27], which electrostatically interact with the negatively charged cytoplasmic membrane. The ions can penetrate into the cell interior and deactivate respiratory enzymes, stimulating the formation of reactive oxygen species [28]. This can cause both the destruction of the cell membrane and the change of DNA structure.

**Figure 5.** OD 620 of sample of *E. coli* and *E. coli* mixed with AgNPs (a) and OD 620 of sample of *S. aureus* and *S. aureus* mixed with AgNPs (b). The same letters indicate no significant difference, while the different letters indicate a significant difference.

### 4. Conclusion

In this study, the properties of silver nanoparticles synthesized using leaf extract of *Graptophyllum pictum* were characterized, starting from localized surface plasmon resonance, functional groups, and the particle size distribution to their antibacterial activity. The wavelength of localized surface plasmon resonance was found to be 455 nm, while the FTIR spectrum showed the chemical bonds of organic compounds, denoting the presence of the extract on the particle. The particles were mostly spherical with diameters varying from 5.4 nm to 50.6 nm, mean diameter of 21.5±9.9 nm. The results from the antimicrobial assessment show that *Graptophyllum pictum* silver nanoparticles inhibit the growth of both *S. aureus* and *E. coli*, where during 24 hours of observation time, the particles affected *E. coli*, faster than the particles affected *S. aureus*.

This study shows that leaf extract of *Graptophyllum pictum* can be used as a reducing agent to synthesize silver nanoparticles. The extract was involved in the formation of the particles and also in stabilizing the particles. The wavelength of localized surface plasmon resonance of the synthesized nanoparticles was found to be 455 nm, while the particles were mostly spherical with diameters varying...
from 5.4 nm to 50.6 nm, and the mean diameter was found to be 21.5±9.9 nm. The results from the antimicrobial assessment show that *Graptophyllum pictum* silver nanoparticles inhibit the growth of both *S. aureus* and *E. coli*, where during 24 hours of observation time, the particles affected *E. coli*, faster than the particles affected *S. aureus*.

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