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Silver Nanoparticles Synthesized from Kenitu (Chrysophyllum caintio L.) Leaf Extract and Its Potential as Antibacterial Agents

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ABSTRACT

Currently, nanoparticles are one of the promising technologies and have been applied in various sectors. Silver nanoparticles is one of implementation in nanoparticles and have been applied in the industrial and medical. Green synthesis of silver nanoparticles is still in development. This method is carried out using plant extracts as bioreductors, because secondary metabolite compounds in extracts can reduce Ag^{+} ions into nanoparticle form. This study was conducted with the aim of determining the potential of *Chrysophyllum caintio* leaf extract as a bioreductor based on the UV-Vis sepctrophotometric instruments and to determine the potential of synthesized silver nanoparticles as antibacterial agents against *Salmonella typhimurium* through the disc diffusion method. The synthesis was carried out by volume ratio between kenitu leaf extract and $AgNO_3$, 1:1, 1:2, 1:3, and 1:4. The result stated that the four variations have absorption in 422-465 nm. Where silver nanoparticles are formed can be known from the formation of peaks at a maximum wavelength of 400-500 nm. In a ratio of 1:2, a peak was formed at a wavelength of 453 nm with an absorbance of 0.758. Antibacterial activity in synthesized silver nanoparticles produced an inhibitory zone of 17.2 mm so it is classified as strong category.

Keyword: Silver nanoparticles, green synthesis, antibacterial,

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INTRODUCTION

Nanotechnology is one of advanced technology with changing the particle's size to nanometer scale as its purpose. (Fauza *et al.*, 2021). The development of nanotechnology research is still on going with one of those developments is silver nanoparticles. Silver nanoparticles is one of study that caught scientist's attention because its wide potential in several area, such as in health sector (Harso, 2017). Silver nanoparticles have been used as drug delivery agents because its capability to minimizing irritation that could happen in human cell (Sarode *et al.*, 2024).

Generally, silver nanoparticles could be synthesized by two methods. The two method is Top Down and Bottom Up (Khan *et al*., 2019). In Bottom-Up method, there is one technique called green synthesis, which is using plant extract as reducing agent so it's environment friendly (Wulandari & Safaat, 2021). One of plant extract that could be used as reducing agent is kenitu leaf extract, because it contains a lot of secondary metabolite compound, such as flavonoid, tannin, and quinone (Roni *et al*., 2019). And also, there are gallic acid, unsolid acid, and quercetin in kenitu leaf extract (Rahayu, 2020). Those secondary metabolite compound makes kenitu leaf extract used as antioxidant Fernandez-Panchon *et al*., 2008, antidiabetic (Doan *et al*., 2018), antiinflammation (Meira *et al*., 2014), anticancer (Li *et al*., 2015), and anti-hypertensive substance (Mao *et al*., 2015).

Synthesized silver nanoparticles known for having a significant effect in inhibits bacterial growth (Rafique *et al*., 2017). With this terms, synthesized silver nanoparticles from kenitu leaf extract will be tested for its antibacterial activity against *Salmonella typhimurium*.

METHODS

Materials and Equipment

The material used in this study is kenitu leaf, $AgNO_3$, demineralized water, aquades, NaCl 0,9%, KBr, BaCl 1%, H2SO4 1%, filter paper, *ciprofloxacin*, Muller Hilton Agar (MHA) (MerCk), and bacterial suspense of *S. typhimurium*. The tools used throughout the study is spectrophotometry UV-Vis 1800, magnetic stirrer, volumetric flask 100 mL, erlenmeyer, beaker glass, petri dish, glass funnel, reaction tube, stirring rod, vernier caliper and knife.

Kenitu (Chrysophyllum cainito) Leaf Extraction

The process of extracting kenitu leaves begins with the wet sorting stage of ±1 kg of kenitu leaves from contaminants using aquifers. After wet sorting, kenitu leaves enter the drying process by airing at room temperature until dry. Next, the dried kenitu leaves are cut into small sizes and mashed using a smasher so kenitu leaf powder is produced. Next, kenitu leaf powder used in the extraction process by dissolving it with 50 mL of demineralized water and then heating it at a temperature of 50ºC for 30 minutes. After that, the mixture was filtered using Whatman filter paper no. 1 with the aim of separating the filtrate and residue, which is the filtrate is used as reducing agents in the synthesis of silver nanoparticles.

Preparation of $AgNO_3$ 0,01 M Solution

A total of 0.17 g of AgNO3 powder was put into a 100 mL measuring flask and then an aquades is added until reach the limit mark on the measuring flask. Next, the mixture is homogenized using a magnetic stirrer (Yanti *et al*., 2021).

Synthesis of Silver Nanoparticles Using Green Synthesis Method

Kenitu leaf extract is poured into a beaker glass that has contained 0.01 M $AgNO_3$ solution that have been made before. The volume ratio between the extract and the $AgNO_3$ solution is 1:1 (10 mL:10 mL), 1:2 (10 mL:20 mL), 1:3 (10 mL:30 mL), dan 1:4 (10 mL:40 mL) (Rafiqi & Suyatno 2022). After that, the mixture is stirred with a magnetic stirrer at a temperature of 50ºC for

30 minutes. The color change formed in the synthesized nanoparticles was observed. Next, the synthesized silver nanoparticles were characterized using the Shimadzu 1800 UV-Vis spectrophotometry instrument by setting the wavelength of 300-700 nm (Widatalla *et al*., 2022).

Antibacterial Activity

The antibacterial activity test was carried out based on the disc diffusion method. This method recuire the diameter of the clear zone that formed around the disc paper in order to know substance's capability against bacteria. Antibacterial activity testing was carried out on *S. typhimurium* bacteria. The test began with taking the bacterial suspension using an ose wire and then inoculated on Muller Hinton Agar (MHA) media. Next, the suspension of bacteria is distributed to the media's surface using a cotton swab and left to dry. The disc paper was immersed in solution that used in this research, which is synthesized silver nanoparticles, kenitu leaf extract, negative control (aquades), and positive control (*ciprofloxacin*). Next, the disc paper is placed on media that has been overgrown with bacteria in a petri dish. Each petri dish that contains a disc paper is put into an incubator with a temperature set at 37^{o}C for a period of 24 hours. After that, the process is continued with the measurement of the clear zone formed around the paper disc (Pehino *et al*., 2021). Based on the diameter of zone of inhibition formed, the ability of a substance to inhibit the bacteria's growth is divided into 4 categories, that category is weak (<5 mm), medium (5-10 mm), strong (10-20 mm), and very strong (>20 mm) (Santoso *et al*., 2020).

RESULTS AND DISCUSSION

Results of Synthesized Silver Nanoparticles Using Spectrophotometry UV-Vis

Silver nanoparticles (AgNPs) have been synthesized through the green synthesis method including bio reductors and precursors. The bioreceptors used in this study is kenitu leaf extract. Meanwhile, the precursor used was AgNO3 0.01 M. Synthesis was carried out by a comparison between bio reductors and precursors, which is 1:1 (10 mL:10 mL), 1:2 (10 mL:20 mL), 1:3 (10 mL:30 mL), dan 1:4 (10 mL:40 mL). Then, the mixture is stirred using a magnetic stirrer. One of the parameters used to find out that Ag^+ ions have been reduced to Ag^0 is through a color change from yellow to brown (Widatalla *et al*., 2022).

The next step is qualitative test of AgNPs synthesized through UV-Vis spectrophotometer instruments, which was carried out with the aim of determining the maximum wavelength absorption and strengthening the results of visual observations, which is color changes that occur in AgNPs. The four variations of AgNPs were measured through a UV-Vis spectrophotometer performed in the wavelength range of 300-700 nm. The maximum wavelength absorption spectrum of the measurement results is shown in Figure 1.

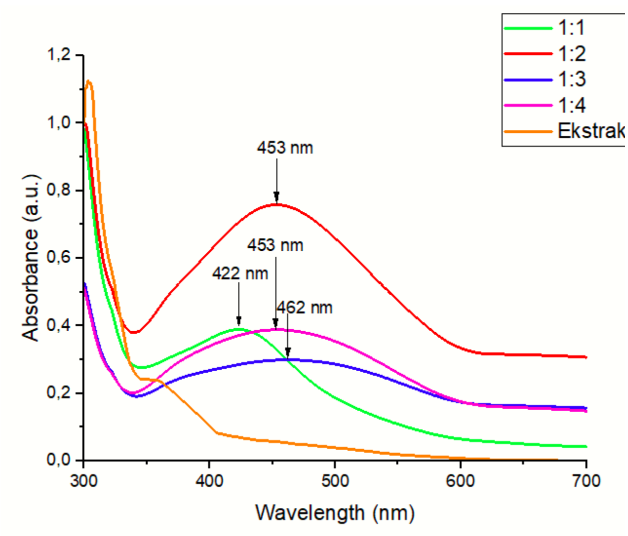

Figure 1. UV-Vis spectrum of AgNPs

The results of the UV-Vis spectrophotometer instrument test showed that of the four volume variations of AgNO3 solution to kenitu leaf extract had a peak absorption at various wavelengths, with peaks at wavelengths ranging from 422-462 nm. In addition, the absorbance values of the four volume variations are also different. The formation of silver nanoparticles can be characterized by the formation of peaks at wavelengths of 400-500 nm (Manosalva *et al.*, 2019). Based on reported research by Nalawati, Suyatma, & Wardhana (2021), silver nanoparticles with a maximum wavelength absorption of 410 nm. Similar results were also reported by Widatala et al. (2022), where silver nanoparticle testing resulted in peak absorption at wavelengths at 405 nm. The results of the UV-Vis spectrophotometry test are shown in Table 1.

Based on the measurements that have been taken, the peak of wavelength absorption is obtained at 453 nm with an absorbance value of 0.758 as the optimal volume comparison in the synthesis of silver nanoparticles.

Table 1. Result in spectrophotometry UV-Vis on synthesized AgNPs

Ratio	Wavelength (nm)	Absorbance
1:1	422	0,389
1:2	453	0,758
1:3	462	0,386
1:4	453	0,391

Results of Antibacterial Activity of Synthesized Silver Nanoparticles

Next, AgNPs synthesized with an optimum ratio of 1:2 was tested for their antibacterial activity against *S. typhimurium* using the disc diffusion method. This test was carried out with the aim of determining the ability of the synthesized AgNPs to inhibit the growth of S. typhimurium bacteria. In this stage, tests were also carried out on kenitu leaf extract, negative control (aquades), and positive control (*ciprofloxacin*). The ability of a substance to inhibit the growth of bacteria can be determined from the formation of a clear zone (inhibition zone) around the disc paper. The results of the antibacterial activity test of the fourth solution are seen in Figure 2.

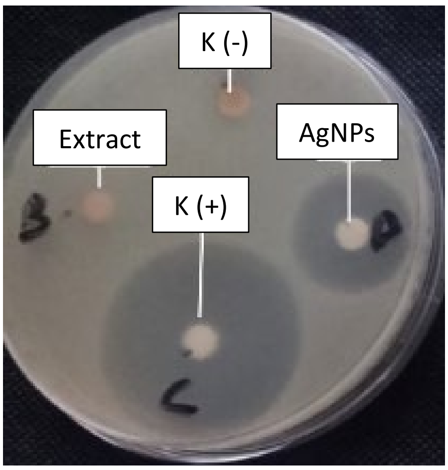

Figure 2. Antibacterial Testing Results

Figure 2 shows the clear zone that forms around the disc paper. Next, the vertical and horizontal diameters of the clear zone are measured using a caliper. The inhibition zone that formed are listed in Table 2.

Table 2. Inhibition Zone Diameter in Antibacterial Testing

Solution	Inhibition zone (mm)	Category
Synthesized AgNPs	17,2	Strong
Kenitu leaf extract	0,00	Weak
Ciprofloxacin (positive control)	34,07	Very strong
Akuades (negative control)	0,00	Weak

Based on Table 2, it can be seen that the synthesized AgNPs test solution has a higher diameter of inhibitory zone than kenitu leaf extract against *S. typhimurium* bacteria. Thus, it can be stated that the synthesized AgNPs have stronger antibacterial activity then kenitu leaf extract. This is because the size of AgNPs's particle is smaller and has a large surface area, so it can interfere with the growth of bacteria. The occurrence of AgNPs in inhibiting bacterial growth is when the diffusion process occurs. AgNPs enter the bacteria through the cell wall and cell membrane of the bacteria and subsequently result in penetration in the bacterial cell. The phosphorus and sulfur content found in bacterial deoxyribonucleic acid (DNA) will interact with AgNPs. The interaction between those two will affecting in decreasement in function of bacterial cells. Thus, DNA can lose its ability to replicate and transcription, leads to the death of bacteria cells (Syarief *et al*., 2015).

CONCLUSIONS

The synthesis of silver nanoparticles has been successfully carried out through the green synthesis method with water extract of kenitu leaves (Chrysophyllum caintio L.) as bio reductor. Secondary metabolite compounds such as the flavonoid group contained in kenitu leaves can reduce Ag^+ ions to Ag^0 in the form of nanoparticles. The results of the study proved that silver nanoparticles had been formed in the ratio between kenitu leaf extract and $AgNO_3$ precursors, which was 1:2. The synthesized silver nanoparticles have a maximum wavelength of 453 nm with an absorbance of 0.758. The magnitude of the absorbance value leads to an increase in the number of silver nanoparticles produced. Silver nanoparticles have antibacterial activity against *S. typhimurium* bacteria with an inhibitory diameter of 17.2 mm, so they are included in the strong category.

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