Biological synthesis and characterization of silver nanoparticles synthesized from *Pometia pinnata* and *Diospyros discolor* Fruits

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**Abstract.** The use of biological agents for nanoparticle biosynthesis is an alternative to the eco-friendly green synthetic method. In this study, fruit extracts from *Pometia pinnata* (Matoa) and *Diospyros discolor* (Bisbul) were used as reducing agents to produce silver nanoparticles (AgNPs). The ratio of silver precursors to water extracts from the fruit and the reaction time was observed to determine optimum reaction conditions. The characterizations were carried out using a UV-Vis spectrophotometer, Transmission Electron Microscope (TEM), and Particle Size Analyzer (PSA) to analyze the size and morphology of the AgNPs. With the increase in the volume ratio of extracts and silver nitrate, the absorption peak intensity tended to increase—these results were shown by the color of the colloid. Based on the comparison of these two extracts, the peak absorbance of the synthesis using *D. discolor* fruit extract was higher than *P. pinnata*. The result showed *D. discolor* fruit extracts had faster reaction times for AgNPs synthesis. The average size of silver nanoparticles from *D. discolor* was 32 nm and from *P. pinnata* was 51 nm. The use of *D. discolor* fruit extract tends to produce smaller AgNPs. This method can be developed for further application for antimicrobial nanoparticles and sensors.

**Keywords:** *Pometia pinnata*, *Diospyros discolor*, silver nanoparticles, biosynthesis

1. Introduction

Phytochemical biodiversity compounds from plants are open to vast potential for their possible utilization and exploration. The role of phytochemical compounds is also very diverse among plants with many functions such as protection for the plants from pests, diseases, and environmental stress [1]. Some even have benefits for human health, such as medicine [2, 3]. *Diospyros discolor* and *Pometia pinnata* are plants that can be found in the tropics. *Diospyros discolor* fruit is known to have high tannin compounds [2] and is also known to have saponin and alkaloid compounds [4]. The fruit has been used for medicine against diarrhea, and the infusion from the fruit can be used for stomatitis medication [5]. The fruit also contains an antioxidant compound to scavenge free radicals [6]. As for the fruit of *P. pinnata*, it is known to be rich in polyphenol compounds and is a known antioxidant [7, 8].
Nowadays, phytochemical compounds play a role in nanotechnology applications. The synthesis of nanoparticles by utilizing phytochemical compounds is interesting to explore [9, 10]. Phytochemical compounds act as reducing agents in the synthesis of AgNPs. The synthesis process using these biological agents affects sizes, shapes, and chemical compositions, which lead to specific functions for applications in the future. This method tends to be eco-friendly, cost-effective, easily ramped up for large-scale synthesizing, and there is no need for high temperature, energy, pressure, and toxic chemicals [11, 12]. Silver nanoparticles can be applied in biomedical sciences for high sensitivity biomolecular detection and diagnostics, antimicrobial agents and therapeutics, and biosensors and bioimaging [13-15]. Among all of the metal nanoparticles, AgNPs have good electrical conductivity, chemical stability, catalytic, and antibacterial properties [14]. Phytochemical compounds in the phenol and flavonoid groups are known to have a role in the reduction process of Ag$^+$ ions to Ag$^0$ [11, 16]. These compounds are known to be contained in *D. discolor* and *P. pinnata* fruit.

Several studies have shown the effectiveness of plant extracts for synthesis of AgNPs using specific plant parts such as leaves [17], roots [15], and stem barks [18]. Therefore, *D. discolor* and *P. pinnata* fruit act as silver ion reducing agents. This study explores the potential of *D. discolor* and *P. pinnata* extracts as reduction agents in AgNPs synthesis. Based on their chemical compounds, they have the requirement that acts as a reductant, yet no previous studies have been conducted using these plant parts. The variation between the plant extract and silver precursors will affect the reaction speed, size, and distribution of AgNPs [10].

2. Materials and Methods

2.1. Plant preparation and extraction

The fruits of *Diospyros discolor* and *Pometia pinnata* plants (figure 1) were taken from FMIPA UI plant collection. The fruit's flesh was washed to remove all the dirt then peeled. Next, the peeled part of the fruits was dried in an oven with a temperature of 40°C. After drying, the fruits were ground into powder and filtered through T32 mesh. The powder of each fruit was weighed to make an aqueous extract with a concentration of 2% (w/v). The powder was boiled in double distillate water for 15 minutes. Then, the aqueous extracts were filtered through Whatman filter paper No.1. These filtrates can be used for the biosynthesis process and stored in the refrigerator at 4°C for a maximum of 2 weeks.

![Figure 1. Diospyros discolor fruits (A) and Pometia pinnata fruits (B).](image1)

2.2. Silver nanoparticle biosynthesis and characterization

Biosynthesis was carried out using AgNO$_3$ [Merck] as the silver precursor with *D. discolor* and *P. pinnata* fruit extracts as the sources of reductant. In this experiment, the ratio of the aqueous extract to 1 mM AgNO$_3$ was varied: 1:20, 1:10, 1:5, and 1:2. Furthermore, each solution was observed visually for any color change and characterized by the UV-Vis spectrophotometer [Thermo Genesys 10S] at 15 minutes, 1 hour, and 24 hours. Characterization was observed with the TEM [TEM FEI Tecnai G2 Supertwin] and PSA [Malvern Zetasizer Nano series Nano-ZS] to determine the size and shape of
3. Results and discussion

Figure 2 shows the synthesis results of the AgNPs with the ratio variations using the fruit extracts *D. discolor* (figure 2A) and *P. pinnata* (figure 2B). The solution using the *D. discolor* extract at the 1:20 ratio had a yellowish color while at a ratio of 1:2 it had a darker brown color. This result is also supported by the absorbance spectrum, which shows AgNPs from a higher ratio of fruit extract showed a sharper and strong absorption band at a wavelength of 430 nm, with a range between 340-600 nm. This result showed the trend to increase with the increasing of ratio until it reaches the higher absorbance value. Meanwhile, the AgNPs synthesis results using fruit extract from *P. pinnata* on the ratio 1:2 with showed absorbance value below 2, while at a ratio of 1:20 below 1.5 and the solution color showed no difference. It was also shown from the peak in the range 350-500 nm and all the solution having absorbance values below 1.5. Therefore, it caused the solution color looked similar in all extract and precursor ratios. The brown color formed is the unique characteristic of AgNPs spectrum Surface Plasmon Resonance (SPR). The formation of these colors indicates the formation of AgNPs and the colloid solution from AgNPs. The research conducted by Masum *et al.* [19], using the fruit section also obtained the color of the solution changing from yellow to brown. The solution color formed looks more concentrated as the ratio between the extract and the precursor of silver increases.

Based on the absorption spectrum observation with the in the increasing of time, both solution with ratio of extracts and precursors 1:2 the color of the solution turned to yellow within 15 minutes. The absorption spectrum indicates that the reaction to reduce precursors in both extracts begin to occur (figure 3). After one hour of synthesis using *D. discolor* there was a very rapid change in color from yellow to brown, getting thicker and tend to be constant at 24 hours. That result shows that the reaction was slowing down due to the lack of availability of reducing agents and silver ions. Meanwhile, when the reaction used extracts from *P. pinnata* after 1 hour, the solution color was still yellow, then it starts to turn brown in 4 hours and becomes thicker in 24 hours. At some point, the
reaction may continue, due to the availability of the reductant and precursors. This result showed that *D. discolor* on that ratio extract still contain reductant to reduce Ag⁺ ions faster than *P. pinnata* fruit. The *P. pinnata* fruit extract tends to have a slower reduction rate. AgNO₃ itself slowly reduced in water, but very low [10]. The speed of this reaction shows the strength of the reducing agent contained from each fruit extracts. Besides, the peak that increases by the time shows the number of nanoparticles formed, the higher the absorbance value can indicate more nanoparticles formed.

**Table 1.** Polydispersity index (PDI) and Zeta potential from *Pometia pinnata* and *Diospyros discolor* fruit with 1:2 ratio between the extract and silver precursor.

| Fruits     | PDI  | Zeta Potential (mV) |
|------------|------|---------------------|
| Matoa      | 0.284| -16.7               |
| Bisbul     | 0.450| -21.4               |

**Figure 3.** Comparison of absorption spectra at λ430 nm on different reaction time from (a) *Diospyros discolor* and (b) *Pometia pinnata* fruit extract to silver precursor from 15 minute to 24 hours of reaction time with extract and precursors (v/v) ratio on 1:2; 1:5; 1:10; 1:20.

Table 1 shows the results of the AgNPs characteristic from PSA, including zeta potential value and polydispersity index. The results showed that the potential zeta value of the synthesis using *P. pinnata* extract lower (-15.7 mV) than using *D. discolor* extract (-21.4 mV). The result shows that the AgNPs formation using *P. pinnata* extract tend to be relatively stable in the range +10-20 mV, which supports dispersion and stability [20]. Based on the result of polydispersity index, AgNPs synthesized with *P. pinnata* tends to moderately dispersed and AgNPs synthesized with *D. discolor* tends to be highly polydispersity. The PSA results confirmed by TEM, where the AgNPs from *P. pinnata* tend to distribute and AgNPs from *D. discolor* tend to form clusters. The TEM results with 59,000x magnification showed that AgNPs synthesized with *D. discolor* fruit extract has the size average at 32 nm. Meanwhile, AgNPs from *P. pinnata* tends to be larger, with an average size of 51 nm (figure 4). The AgNPs formed in all treatments has the spherical shape.
Figure 4. TEM image of silver nanoparticles from *Diospyros discolor* fruit with AgNO$_3$ (1:2 (v/v)), Magnification 59.000x (Scalebar = 50 nm)(A) and their size distribution (B); TEM result of silver nanoparticles from *Pometia pinnata* fruit with AgNO$_3$ (1:2 (v/v)) Magnification 59.000x (Scalebar = 50 nm) (C) and their size distribution (D) tend to disperse and bigger in size.

Biosynthesis mechanism is based on a redox reaction, in which metal ions are reduced to stable the nanoparticles due to the compounds contain in the extract [21]. During the synthesis process plant extracts can have two roles, as reducing agents and as stabilizers to prevent aggregation so that the dispersion and size of nanoparticles can be maintained. Based on information from previous research, *D. discolor* and *P. pinnata* fruit extracts are known to contain groups of alkaloids, flavonoids, tannins, phenolics, and terpenoids compounds (table 2). Compounds such as terpenoids [22] and flavonoids [16] are the potential compounds that play a role as a reducing agent in the synthesis of AgNPs. This synthesis process is a bottom-up process, which can induce crystal growth, where atoms, ions, or molecules are assembled [11].

| Compounds       | *Diospyros discolor* | References       | *Pometia pinnata* | References       |
|-----------------|----------------------|------------------|-------------------|------------------|
| Alkaloid        | +                    |                  | +                 |                  |
| Flavonoid       | +                    | Ragasa et.al.    |                  | -                |
| Tannin          | +                    | 2009; Hung et.al. | +                 | Irawan et.al.    |
| Phenolic        | +                    | 2016.            | +                 | 2017.            |
| Saponin         | +                    |                  | +                 |                  |
| Terpenoid       | +                    |                  | +                 |                  |
There are still not much full reports have been explaining the mechanism for each species of flavonoid or terpenoid role in reduction and stabilization of AgNPs. The mechanism of nanoparticle formation consists of mainly three stages: reduction of ions, clustering, and further nanoparticle growth. The features of each step depend upon the nature of the reducing agent and its concentration. Based on table 2, the phytochemical screening by Irawan et al. [8], did not detect any flavonoid content containing from P. pinnata fruit extract. This condition might cause a slow reduction to happen while using P. pinnata extract than D. discolor extract. The P. pinnata did not contain flavonoids or it have a very small number. Therefore, its possible other compounds can act as the reducing agents. The possible reaction occurs is not a single reaction, and there is a possibility of any synergistic reaction which can involve several compounds as reductant from the extract.

Conclusion
Silver nanoparticles synthesis using D. discolor fruit extract have a faster reduction time than using P. pinnata extract, based on the comparison of AgNPs absorption peak. Diospyros discolor tend to have a higher peak than P. pinnata between 24 hours of reaction and tend to be constant. Meanwhile, P. pinnata has a slow reaction time, and the reaction yet still happened after 24 hours. The PSA and TEM results showed AgNPs from P. pinnata had smaller Polydispersity Index (PI), higher Z-average, and larger AgNPs size than AgNPs from D. discolor. Both extracts can be used for AgNPs synthesis and modify with a further process to get their best AgNPs characteristic based on size, shape, stability, and distribution.

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