Bioprospection of *Diospyros discolor* Willd. fruit for the biosynthesis of silver nanoparticles

I Nolia¹,², W Handayani¹,², K Secario², D Djuhana²,³ and C Imawan²,³

¹Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
²Bionanotechnology, Department of Physics, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
³Department of Physics, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Corresponding author’s email: windri.h@sci.ui.ac.id

Abstract. Leaves from *Diospyros discolor* Willd. plants can act as a reducing agent in the biosynthesis of silver nanoparticles. This raises the question of whether other parts of the plant can also be used for this purpose. The present study aimed to explore the prospect of *D. discolor* fruit for the synthesis of silver nanoparticles. The fruit and seeds of the plant were extracted and used for the biosynthesis process, and the resulting silver nanoparticles were characterized using UV–visible light spectrophotometry, Transmission electron microscopy (TEM), and particle size analysis (PSA). During biosynthesis, aqueous extracts from the fruit flesh or seeds were reacted with 1 mM silver nitrate at various ratios. A ratio of 1:2 (v/v) resulted in the highest absorbance values (indicating the greatest production of nanoparticles), and the absorbance at all ratios increased with time. The results were similar for the fruit flesh and seed extracts, with absorbance values observed in the same peak areas for each treatment. However, the use of *D. discolor* fruit flesh resulted in faster reactions during the initial 24 h.

Keywords: Biosynthesis, *Diospyros discolor*, fruit, silver nanoparticles

1. Introduction

Biosynthesis provides an alternative method for the environment-friendly synthesis of nanoparticles, using natural materials as a reducing agent in the synthesis process [1, 2]. Often, the natural products used in nanoparticle biosynthesis are derived from plants and microorganisms [3, 4]. The use of plants has numerous advantages, including allowing cheaper reducing agents to be produced and using less toxic compounds to benefit the environment [1, 5]. Several studies have been conducted on the biosynthesis of nanoparticles using different parts of the plants as reducing agents [2-7]. This open the possibility for further bioprospection in other application of plant phytochemistry beside their medicinal use. Most studies have used leaf extracts, with only a few using the fruit and seeds to make reducing agents for biosynthesis. Compounds with reducing properties are expected to play a role as reducing agents for silver ions.

*Diospyros discolor* Willd. is a plant from the Ebenaceae family; it grows to 7–32 m in height, and its fruit has whitish, slightly dry, sweet, aromatic flesh [8]. Plants from the same genus have been shown to contain secondary metabolite compounds [9], and the fruit of *D. discolor* contains high
concentrations of tannins, saponins, and alkaloids [10]. Leaves from *D. discolor* have previously been used as a reducing agent in the biosynthesis of silver nanoparticles. The present study aimed to investigate whether the fruits and seeds of *D. discolor* could also be used to form a reducing agent to synthesize silver nanoparticles and to examine the absorbance characteristics of the biosynthesized silver nanoparticles.

2. Materials and method

2.1. Plant preparation and extraction

Fruit and seed materials were taken from a *D. discolor* tree at the Faculty of Mathematics and Natural Sciences, Universitas Indonesia. The fruit was washed, and its flesh and seeds were separated. Each part was dried in an oven at 40 °C, after which each part was milled separately to produce a powder. The powders from the fruit flesh and seeds were each weighed and then mixed with distilled water to make 2 % (w/v) aqueous extracts. These extracts were then boiled for 15 min and allowed to cool to room temperature. They were then filtered through Whatman No. 1 filter paper for further use in the biosynthesis process.

2.2. Biosynthesis of silver nanoparticles and characterization

The biosynthesis of silver nanoparticles was carried out using a silver nitrate (AgNO₃) precursor purchased from Merck, which was mixed with the aqueous extracts at extract:AgNO₃ (v/v) ratios of 1:20, 1:10, 1:5 and 1:2. The color of each mixture was observed at 15 min, 1 h, 24 h, 48 h, 1 week, 3 weeks and 5 weeks by taking photographs and characterizing the mixture’s spectrum absorbance using a UV-Vis Genesys 10S spectrophotometer (Thermo Scientific) at wavelengths of 200–800 nm.

The resulting silver nanoparticles’ morphology and stability were characterized using a transmission electron microscopy (TEM; FEI Tecnai G2 SuperTwin) and a particle size analyzer (Malvern Zetasizer Nano series) from Nanotechnology Laboratory, Balit Pasca Panen, Cimanggu, Bogor. The TEM was used to characterize the shape and size of the silver nanoparticles and the particle size analyzer to determine the size distribution of the nanoparticles.

3. Results and discussion

Figure 1 shows the absorbance spectra at 24 h for the various concentration ratios of fruit and seed aqueous extracts with AgNO₃. The color of the mixture changed from light yellow to darker brown as the ratio of reducing agent to silver precursor increased, indicating the formation of silver nanoparticles. This was confirmed by UV–visible light spectrophotometry, which showed the absorbance peak at 350–500 nm, which is the spectrum of silver nanoparticles. The silver nanoparticle absorbance curves and colors of the mixtures changed similarly for the fruit and seed extracts, with the absorbance increasing as the concentration ratios increased, indicating that higher concentrations of both types of extract resulted in the formation of greater numbers of silver nanoparticles. This was the result of a greater number of reactions with the AgNO₃.

The highest absorbance was at 425 nm. Figure 2 shows the increase in absorbance at this wavelength from 15 min to 5 weeks for the various concentrations of each extract that showed almost the same condition of absorbance after 5 weeks. At 15 min, the absorbance had increased slightly, and the mixtures were yellowish (figure 3). With the fruit extract, the absorbance then increased rapidly during the first 24 h. The biosynthetic reaction was slower with the seed extract, with lower absorbance values at each time point. After 24 h, the reaction slowed, with little change for the higher concentrations (1:2 and 1:5 for the fruit extract and 1:2 for the seed extract). At lower concentrations (1:10 and 1:20) the reaction continued after 48 h, although at a slower rate. Over time, the mixtures became darker in color (figure 3), indicating the formation of nanoparticles in increasing numbers.
The particle size analysis produced polydispersity index (PDI) values for the silver nanoparticles in the seed and fruit mixtures at ratios of 1:2, 1:5, 1:10 and 1:20 (table 1). The PDIs showed that the nanoparticles resulting from the seed extract and AgNO₃ mixtures were moderately disperse at all four concentrations, but the nanoparticles from the fruit extract mixtures were highly polydisperse at ratios

![Absorbance spectra and mixture colors for various ratios of aqueous extracts of D. discolour, (a) fruit, (b) seeds to silver nitrate at 24 h.](image)

**Figure 1.** Absorbance spectra and mixture colors for the various ratios of aqueous extracts of *D. discolour*, (a) fruit, (b) seeds to silver nitrate at 24 h.

![Absorbance at 425 nm from 15 min to 5 weeks for various ratios of aqueous extracts of D. discolour, AgNPs-fruits (red line) and AgNPs-seeds (black line) to silver nitrate.](image)

**Figure 2.** Absorbance at 425 nm from 15 min to 5 weeks for the various ratios of aqueous extracts of *D. discolour*, AgNPs-fruits (red line) and AgNPs-seeds (black line) to silver nitrate.

![Comparison of 1:10 (v/v) ratio mixtures from aqueous extracts of D. discolour fruit and seed with silver nitrate, showing the changes in color from 15 min to 48 hours.](image)

**Figure 3.** The comparison of 1:10 (v/v) ratio mixtures from aqueous extracts of *D. discolour* fruit and seed with silver nitrate, showing the changes in color from 15 min to 48 hours.
Table 1. Polydispersity indexes for the silver nanoparticles biosynthesized using aqueous extracts of seeds and fruit flesh of *D. discolor* at various ratios to silver nitrate.

| Sample       | Ratio | Polydispersity Index | Distribution      |
|--------------|-------|----------------------|-------------------|
| AgNPs-seeds  | 1:2   | 0.339                | Moderately disperse |
|              | 1:5   | 0.349                | Moderately disperse |
|              | 1:10  | 0.306                | Moderately disperse |
|              | 1:20  | 0.244                | Moderately disperse |
| AgNPs-fruit  | 1:2   | 0.450                | Highly polydisperse |
|              | 1:5   | 0.480                | Highly polydisperse |
|              | 1:10  | 0.381                | Moderately disperse |
|              | 1:20  | 0.253                | Moderately disperse |

Figure 4. Transmission electron microscopy images of silver nanoparticles from *D. discolor* Willd. (a) seeds, and (b) fruit flesh AgNO₃ (1:2 (v/v)), 195,000× magnification.

of 1:2 and 1:5. These results indicate that the silver nanoparticles varied in size to a moderate or high degree and were not monodisperse.

TEM was used to assess the morphological characterization and distribution of silver nanoparticles in samples from the mixtures at 1:2 ratio. The results revealed differences in the size and distribution of silver nanoparticles between the biosynthesis using the fruit flesh and that using the seeds. With the seed extract, the resulting silver nanoparticles tended to be smaller and more dispersed than those from the fruit extract, which were larger and tended to be more clustered, indicating agglomeration (figure 4). All the nanoparticles were roughly spherical.

The zeta potential values resulting from the particle size analysis are shown in table 2. The zeta potentials for samples of the seed extracts mixtures at 1:2 and 1:10 ratios and of fruit extract mixture at a 1:10 ratio showed the silver nanoparticles to be relatively stable, whereas the zeta potential value for the fruit extract mixture at a ratio of 1:2 showed the nanoparticles to be moderately stable, and therefore, more stable than the other three mixtures. The magnitude of the electrostatic repulsion or attraction between particles affects their dispersion and suspension. In general, it is better to use more stable particles with high dispersity. These findings provide an insight about the parts of the plant and the characteristics of the resulting silver nanoparticles product that will provide better options for future applications.
Table 2. Zeta potential values from the particle size analysis for the biosynthesis of silver nanoparticles using aqueous extracts of D. discolor fruit flesh and seeds.

| Biosynthesis using          | Ratio | Zeta potential (mV) | Stability     |
|-----------------------------|-------|---------------------|---------------|
| Seed extract and AgNO₃      | 1:2   | -15.3               | Relatively stable |
|                             | 1:10  | -17.6               | Relatively stable |
| Fruit extract and AgNO₃     | 1:2   | -21.4               | Moderately stable |
|                             | 1:10  | -17.3               | Relatively stable |

4. Conclusion

Silver nanoparticles can be synthesized by using aqueous extracts from the fruit flesh or seeds of D. discolor. A ratio of aqueous extract to AgNO₃ of 1:2 (v/v) resulted in the highest absorbance peak, with a tendency to produce nanoparticles of greater stability after reacting for 24 h. There was little difference in the shape of silver nanoparticles produced using the aqueous extracts from flesh or seeds, but those with the fruit extract were larger. Also, the use of aqueous extract from the fruit flesh resulted in a faster reaction over the initial 24 h.

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