Bioreduction properties of *Pometia pinnata* J. R. Forst. & G. Forst (*Sapindaceae*) for silver nanoparticles synthesis

A P Pridyantari¹, A S Ningrum¹, W Handayani¹ and C Imawan²

¹Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia
²Department 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. Plant extracts have been recognized as a substitute for chemical reducing agents in the synthesis of nanoparticles. Plants with antioxidants, including phenols and flavonoids, are expected to be reducing agents. Preliminary research has shown that *Pometia pinnata* (Matoa) stem bark aqueous extract can be used for silver nanoparticle (AgNP) biosynthesis. However, the compounds that serve as reducing agents in this process are still unknown. In this study, we studied antioxidant strength using DPPH radical reduction method. AgNP biosynthesis was performed by mixing powdered Matoa stem bark aqueous extract (2 %) with 1 mM AgNO₃ (1:2). Further, pH of the Matoa stem bark aqueous extract was varied (pH 4, 7, 9 and 11), and one sample without pH adjustment was used as the control. Additionally, we synthesized AgNPs using the standard antioxidants gallic acid and rutin trihydrate. Our results showed that with increasing pH, changes in the color of solutions and escalation of UV–Vis spectrum absorbance were observed. The sizes and shapes of the AgNPs were further characterized using TEM and PSA, which revealed spherical and short rod-shaped particles. Our findings about the strength of the antioxidant activity of the Matoa stem bark aqueous extract under different pH conditions provide relevant information on the processes that can affect silver nanoparticle biosynthesis.

Keywords: Biosynthesis, *Pometia pinnata*, silver nanoparticles, stem bark

1. Introduction

Nanoparticle biosynthesis, a synthesis method, uses natural compounds, including microorganism or plant extracts, as reducing agents [1]. Among these, nanoparticles synthesized using plant extracts are produced faster and are more stable than those synthesized using microorganism extracts. Furthermore, plant extracts can be used to produce nanoparticles at a larger scale. Currently, nanoparticle biosynthesis using plant extracts as reducing agents is in demand because of its ease of production, low cost, and limited effects on the environment [2, 3]. Silver nanoparticles (AgNPs) are known for their multiple applications in medical and pharmaceutical products. To date, various plant sources have been successfully used as reducing agents in AgNP biosynthesis. These sources include banana peel extract [4], stem bark extracts of *Dalbergia rostrata* [5] and *Zizyphus xylopyrus* [6], and *Lantana camara* flowers [7].

According to Lee et al. [8], antioxidant compounds in plants play the role of reducing agents in nanoparticle biosynthesis. However, the mechanism of photosynthesis is still under exploration.
We hypothesize that plants rich in antioxidant compounds have the potential to serve as reducing agents. Examples of such compounds include flavonoids [9], phenolic acids, terpenoids, polyphenols, polysaccharides, alkaloids, proteins, and reducing sugars [10]. In addition to the reducing compounds, there are several parameters (such as temperature, reactant ratio and pH [11]) in the biosynthesis process that can affect the size, shape, and stability of nanoparticles.

Pometia pinnata (Matua) stem bark is known for possessing various types of chemical compounds, including tannins, saponins, flavonoids, terpenoids, alkaloids, and glycosides [12]. The stem bark has been used in previous research for AgNPs biosynthesis. In this study, we used varying pH conditions of the environment to study the role of pH on the size, shape, and stability of AgNPs and examined the antioxidant activities of Matoa stem bark aqueous extracts as a reducing agent.

2. Materials and method

2.1. Plant preparation, extraction and nanoparticle biosynthesis

Matoa stem bark aqueous extracts were prepared using the stem bark was collected from the area surrounding the Universitas Indonesia and was washed to remove dirt. The samples were dried in an oven (Memmert UN55) at 40 °C until dried to a constant dry weight and were ground. Further, 2 g of the stem bark powder was subsequently boiled for 10–15 min with 100 mL bidistilled water (2 % w/v). Then, the aqueous extract was filtered with Whatman filter paper no 1. Next, the pH of the extracts was adjusted to 4, 7, 9 and 11 using 1 M NaOH and 0.1 M HCl. One sample was prepared as the control without pH adjustment. The pH of the solutions was measured with a pH meter (LAQUA Horiba PH1100). A solution of 1 mM AgNO3 (Dhucefa) was prepared from AgNO3 powder. Next, to perform nanoparticle biosynthesis, 30 mL Matoa stem bark aqueous extracts with various pH values were mixed with 60 mL AgNO3 solution (1:2 v/v ratio) at 24 °C. The AgNPs formed were characterized using a spectrophotometer (GENESYS 10S UV-VIS).

2.2. Spectroscopic study, morphology, and zeta potential determination of the AgNPs

The AgNPs were evaluated by observing several characteristics: color of the solution, spectroscopy absorbance between 200 and 800 nm (Genesys 10S UV), size, and shape. To observe their morphologies, we used the transmission electron microscope (TEM) FEI Tecnai G2 Supertwin at Pasca Panen, Deptan, Bogor, and TEM JEOL JEM 1400 at the Department of Chemistry, FMIPA UGM, Yogyakarta, Indonesia. Meanwhile, particle size, distribution, and zeta potential were evaluated using a particle size analyzer (PSA; Malvern Zetasizer) to determine the dispersity and stability of the nanoparticles.

2.3. Evaluation of antioxidant potential for AgNP biosynthesis

Radical scavenging activity of the Matoa stem bark aqueous extracts and biosynthesized AgNPs were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Antioxidant activity was evaluated by measuring the scavenging activity of the samples using DPPH as the free radical. The method used in this experiment is based on that described by Kumar et al. (2014) [7]. The samples used in the test were Matoa stem bark aqueous extract (2 %), gallic acid (1 mM), and rutin (1 mM). Each sample (0.2 mL) was added to 1.8 mL distilled water and 2 mL DPPH (0.2 mM) in methanol; vigorously vortexed; and allowed to stand at room temperature for 30 min in the dark. The absorbance of the mixtures was spectrophotometrically measured at 517 nm, and the free radical scavenging activity was calculated using the following formula: Scavenging effect (%) = (1 – [sample absorbance / control absorbance]) × 100 [13].

2.4. Silver nanoparticle biosynthesis using standard antioxidant

Nanoparticle biosynthesis using antioxidant compounds was performed using gallic acid (Merck) and rutin trihydrate (Sigma–Aldrich). Accordingly, 30 mL aqueous solution of rutin trihydrate or gallic acid
(1 mM) was added with 60 mL AgNO₃ aqueous solution (1 mM) to reduce the concentration of Ag⁺ ions. The mixed solution was incubated for 24 hours at room temperature in the dark. The formation of AgNPs was confirmed by change in the color of the solution, which darkened after the 24 hour incubation. The absorbance of the solution was also measured using a spectrophotometer.

3. Results and discussion

3.1. AgNP biosynthesis using Matoa stem bark aqueous extract

AgNP biosynthesis resulting from different pH conditions using Matoa stem bark aqueous extracts produced different colored solutions (figure 1a). Colloid from biosynthesis products tended to be brownish. With an increase in pH, the color of the product became darker. Starting from pH 4, the color of the solution tended to be tawny; however, at pH 7, the solution turned brown. At pH 9 and 11, the solution turned dark brown (almost black). This color change indicated the formation of AgNPs, which was supported by the spectroscopy results. The AgNPs showed an absorbance spectrum between 350 and 500 nm (figure 1b). The darker the color, the higher the absorbance tended to be. This color change indicated that at higher pH, Ag⁺ ion reduction rapidly occurred [11]. The color of the solution varied due to the excitation of the surface plasmon resonance of metal nanoparticles [12]. The darkness of the solution can be a general indication of the concentration of AgNPs [14].

3.2. Characteristic of the nanoparticles: morphology and distribution

pH of a solution is known to be an important factor controlling nanoparticle biosynthesis [11]. This study showed that spherical nanoparticles were formed under all pH conditions (figure 2). However, at pH 11, short rod-shaped nanoparticles were also formed. Based on TEM images of the spherical nanoparticles, the size of the nanoparticles varied between 10 and 50 nm diameters. The short rod-shaped nanoparticles had a length between 20 and 60 nm and width between 10 and 20 nm.

The solution with higher pH tended to have relatively small particles. PSA results showed that the polydispersity index (PDI) of the control and pH 4, 7, 9 and 11 solutions were 0.09, 0.827, 0.723, 0.509 and 0.370, respectively (figure 3), indicating that the AgNPs formed under the control condition (unmodified pH) had a highly monodispersed form of distribution. The AgNPs at pH 4, 7 and 9 also

![Figure 1](image_url)
displayed the same property, whereas those formed at pH 11 had moderately dispersed distribution. The polydispersity was caused by agglomeration or aggregation of the AgNPs. The zeta potential value of the nanoparticles had a range of 13–16 mV, indicating that the AgNPs were relatively stable (figure 3). Therefore, the TEM result shows that pH affects the size and shape of nanoparticle. Meanwhile, the PSA result also showed that the nanoparticles formed under all pH conditions were relatively stable.

3.3. Antioxidant activity analysis
Antioxidant activity analysis was conducted to explore the role of phytochemical compound during nanoparticle biosynthesis. Assumedly, compounds with higher antioxidant activity have higher reducing activities during nanoparticle biosynthesis. Therefore, this analysis was used to compare the biosynthesis abilities between plant extracts and compounds known for their antioxidant activities, which included rutin and gallic acid.

Antioxidant compounds in plant extracts play some roles in reduction–oxidation reactions [11]. The percentage of scavenging activity of the Matoa stem bark aqueous extract was 80.43 % (table 1). This result was compared with the activities of rutin and gallic acid, and the percentage of scavenging activity of rutin and gallic acid was 97.09 % and 97 %, respectively (table 1). When 1 mM rutin and gallic acid were used for nanoparticle biosynthesis, the results revealed that the rutin trihydrate–AgNO₃ mixture had the ability to form AgNPs, which was indicated by the yellow solution produced (figure 4a) and the presence of peaks in the absorbance spectrum between 350 and 400 nm (figure 4b).

![Figure 2. Transmission electron microscopy image of silver nanoparticle biosynthesis for the control and pH 4, 7, 9 and 11 solutions. The arrows indicate the short rod-shaped nanoparticles at pH 11. Scale bar = 20 nm.](image)

![Figure 3. Polydispersity index and zeta potential value based on particles size analyzer results.](image)
Table 1. DPPH scavenging activity from *Pometia pinnata* stem bark aqueous extract and standard antioxidants.

| No. | Sample                                | Percentage of scavenging activity (%) |
|-----|---------------------------------------|---------------------------------------|
| 1   | *P. pinnata* stem bark aqueous extract (2 %) | 80.43                                 |
| 2   | Rutin (1 mM)                          | 97.09                                 |
| 3   | Gallic acid (1 mM)                    | 96.00                                 |

Figure 4. Silver nanoparticle biosynthesis using *Pometia pinnata* stem bark aqueous extract compared with that using standard antioxidants. (a) Colors of the solution (b) Absorbance spectrum results.

Conversely, the absorbance spectrum of the gallic acid–AgNO₃ mixture showed no absorbance peak between 300 and 500 nm, and the color of the solution remained relatively clear. This result indicates that not all antioxidant compounds can act as reducing agents in AgNP biosynthesis; however, the experimental conditions and the concentrations of the compound used also contributed to this result. There are many compounds in plants that are expected to have strong reducing abilities, but their mechanism is yet to be discovered. Therefore, further research is warranted to investigate and explore the role of certain secondary metabolites in AgNPs biosynthesis and their strengths as reducing agents.

4. Conclusion
This study indicates that pH has a role in AgNP biosynthesis using Matoa stem bark aqueous extract. The pH of a solution affects the shape and PDI of AgNP colloids. Based on the zeta potential values, the AgNPs under all pH conditions were relatively stable. The DPPH test showed that the percentage of scavenging activity of the Matoa stem bark aqueous extract was lower than that of the standard antioxidants at 1mM concentration. However, the extract used in this experiment had a better ability to form AgNPs. These results show that there are certain compounds and activities in the Matoa stem bark aqueous extract that act as reducing agents in the process of AgNPs biosynthesis.

Acknowledgments
This research supported by Hibah PITTA 2016 from Universitas Indonesia.
References

[1] Prasad R 2014 J. Nanopart. 2014 963961
[2] Ahmed S, Ahmad M, Swami B L and Ikram S 2016 J. Adv. Res. 7 17-28
[3] Iravani S 2011 Green Chem. 13 2638-50
[4] Ibrahim H M M 2015 J. Radiat. Res. Appl. Sci. 8 265-75
[5] Muniyappan N and Nagarajan N S 2014 Int. J. Green Nanotechnol. 2 1-9
[6] Maria B S, Devadiga A, Kodialbail V S and Saidutta M B 2015 Appl. Nanosci. 5 755-62
[7] Kumar B, Smita K and Cumbal L 2016 J. Sol-Gel Sci. Technol. 78 285-92
[8] Lee J, Park E Y and Lee J 2014 Bioprocess Biosyst. Eng. 37 983-9
[9] Ghasemzadeh A and Ghasemzadeh N 2011 J. Med. Plants Res. 5 6697-703
[10] Makarov V V et al. 2014 Acta Naturae 6 35-44
[11] Iravani S, Korbekandiand H and Zolfaghari B 2015 Phytosynthesis of nanoparticles
    Nanotechnology and Plant Sciences Nanoparticles and Their Impact on Plants ed Siddiqui M
    H et al. (New York: Springer International Publishing) pp 203-58
[12] Elya B et al. 2015 Pak. J. Biol. Sci. 18 279-84
[13] Ray A and Gupta S D 2013 Ind. Crops Prod. 51 130-7
[14] Mallikarjuna K et al. 2011 Dig. J. Nanomater. Biostruct. 6 181-6