FORMATION KINETICS OF GREEN SILVER NANOPARTICLES USING *GNETUM GNEMON* L. LEAF AND FRUIT EXTRACT AS BIOREDUCTORS

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ABSTRACT

This research was carried out with the aim of determining the formation rate of green silver nanoparticles using Gnetum gnemon L. leaves and fruit as a reducing agent. In the synthesis of silver nanoparticles, each bioreductant extract is mixed with a silver nitrate solution. From color observations, the silver nanoparticles using Gnetum gnemon L. leaves and fruit showed a color change from clear to yellowish-brown. The results of measurements using a colorimeter at a wavelength of 430 nm showed an increase in the absorbance value after 1 hour of mixing for the AgNPs using Gnetum gnemon L. leaves and 1.33 hours for the AgNPs using Gnetum gnemon L. fruit. The color change and increase in absorbance values of the two samples began to stabilize after 5 hours of mixing until measurements on the 5th day. The rate of formation of silver nanoparticles using Gnetum gnemon L. leaves is 7×10^{-6} s^{-1} and the rate of formation of AgNPs using Gnetum gnemon L. fruit is 3.8×10^{-4} s^{-1}. Measurements using UV-Vis showed that LSPR silver nanoparticles with Gnetum gnemon L. leaf as bioreductor at a wavelength of 439 nm and nanoparticles with Gnetum gnemon L. fruit as bioreductor at a wavelength of 405 nm.

Keywords: *Silver nanoparticles; Gnetum Gnemon L; Bioreductor; Kinetic of formation; UV-Vis*

INTRODUCTION

One of the products of nanotechnology developments is nanoparticles, particles with diameters varying from 1 to 100 nm (Shariat and Ali A., 2016). Nanoparticle research is developing rapidly because nanoparticles can be widely applied in the fields of environment, electronics, materials, optics, biomedicine, and others (Khan et al., 2019). Nanoparticles which are very intensive studied recently are metal nanoparticles, such as gold, silver, iron, zinc, and metal oxides (Yılmaz et al., 2023).

Silver nanoparticles (AgNPs) have become the focus of many researches because of their unique properties, including: chemical stability, good conductivity, and anti-bacterial and anti-fungal properties, as well as their application in many products, including: shampoo, soap, cosmetics, toothpaste, and drugs (Liao et al., 2019). There are various methods used for the synthesis of AgNPs, including: evaporative condensation (Handoko et al., 2018), electrical irradiation (Güzel and Erdal, 2018), gamma irradiation (Perkasa et al., 2022), laser irradiation (Qazi et al., 2018), lithography (Güzel and Erdal, 2018), electrochemical techniques (Rahmah and Kurniawan, 2017), chemical reduction (Siddiqui et al., 2023), thermal decomposition (Nakano et al., 2016), radiation (Rheima et al., 2019), microwaves (Özkar and Finke, 2017), and biological methods that use leaves, fruit, seeds, bark, and roots as reducing agents (Vanlalveni et al., 2021). From all the methods mentioned, the biological methods is the favorite method, because they are environmentally friendly, cheap, can be produced on a large scale, do not require high temperatures, pressure and energy, and do not contain dangerous chemicals (Xu et al., 2020). Silver nanoparticles produced using biological methods are called green silver nanoparticles. One of the popular biological methods is by using plant extract. Many researchers have used extract like *Ficus variegata* Leaf (Wattimena et al., 2022), *Musa balbisiana* Peel (Rengga et al., 2018), *Inula viscosa* (Okka et al., 2023), *Graptophyllum pictum* leaf (Wattimena et al., 2021), and *Sauropus androgynus* (Hutagalung et al., 2018) as a reducing agen.

Previously, there had been a lot of researches on the green AgNPs studying the physical, chemical, antioxidant, anticancer, antifungal and antibacterial properties (Wattimena, et al., 2021; Rengga et al., 2018; Okka et al., 2023). However, not many studies focused on the rate of formation of green AgNPs, which is one of the important parameters to control the process of forming AgNPs (Kumar et al., 2022). Although formation rate of green AgNPs have been determined by some researchers, the measurement time is in the range of 100-200 minutes after mixing the plant extract and silver nitrate solution

(Wattimena et al., 2021; Hutagalung et al., 2018; Wattimena et al., 2021). In fact, AgNPs still continue to form after 1 day (Wattimena et al., 2021). Therefore, data on the formation of AgNPs over several days is needed to determine the overall rate of silver nanoparticle formation carried out in this study. Recent studies (Wattimena et al., 2022) determined the formation rate of green AgNPs up to several day measurement time, where the exponential model used involved 5 parameters.

In these studies, we use extracts of leave and fruit *Gnetum gnemon* L. to produce green AgNPs and to determine the formation rate of the particles. For the formation rate determination, we used an empirically exponential formula involving only 3 parameters, with time of measurement up to several days. With these studies we can compare to the results of the previous studies using empirically exponential formula involving 5 parameters.

METHOD

Synthesis of AgNPs with Gnetum Gnemon L. Leaves and Fruit as Bioreductors

The steps taken to produce AgNPs using *Gnetum gnemon* L. leaf and fruit follow previous studies (Wattimena et al., 2022; Kumar et al., 2022). First, the *Gnetum Gnemon* L. leaves and fruit were cleaned with tap water and sterilized with distilled water which is then left to dry. Next, the dried *Gnetum gnemon* L. leaves and fruit were cut in pieces. Then 20 grams of *Gnetum gnemon* L. leaf and fruit were put separately into a beaker containing 200 ml of distilled water and was heated until boiling and then cooled to room temperature. The cool water from the *Gnetum Gnemon* L. leaves and fruit was then filtered separately using a whatmann filter paper No.1 assisted by a vacuum pump. Finally, *Gnetum gnemon* L. leaf and fruit extracts were mixed with 1 mM $AgNO_3$ solution in a glass bottle separately in a ratio of 1:20 for leaves and 1:3 for *Gnetum Gnemon* L. fruit. Determination of the ratio between $AgNO_3$ solution and *Gnetum gnemon* L. leaf and fruit extract is based on the results of varying the ratio to get the appropriate ratio.

Determination of the Rate of Formation of AgNPs

To determine the rate of formation of AgNPs using *Gnetum gnemon* L. leaves and fruit, the absorbance of the samples was measured using a LaMotte Smart 2 Colorimeter at wavelengths of 430, 520, 570 and 620 nm. Measurements were carried out after mixing the extract and $AgNO_3$ solution for up to 5 days. The results of the sample absorbance as a function of time were plotted. The data was then fit using the model (Wattimena and Patty, 2023)

$$A = A_0(1 - A_1 e^{-k_1 t})$$

where $A_0, A_1,$ are fit parameters and k_1 is the formation rate constants, thus the parameter has been reduced from 5 to 3. The fitting model uses non-linear least squere fit data, where iteration is carried out with initial parameter values until it reaches convergent final parameter values. The degree of suitability of the fit results is difined by

$$\chi^2$$

On top of that, color changes were also recorded. The change from clear to yellow-brown indicates that the formation of AgNPs. Therefore, to support the results of the absorbance test, the sample is also photographed after each absorbance test is carried out so that color changes and their relations with the absorbance value can be observed.

Physical Characterization of AgNPs

The LSPR wavelength values of AgNPs were determined using UV-Vis spectrophotometry. Characterization was carried out using UV-VIS (UV-1700 PharmaSpec Shimadzu) in the chemistry laboratory of the Faculty of Mathematics and Natural Sciences, Pattimura University. A total of 3.5 ml of AgNPs was put into a 10x10 mm optical cuvette and scanned at a wavelength of 300-700 nm.

RESULT AND DISCUSSION

Kinetics of the Formation of AgNPs

Kinetics formation of AgNPs were indicated by the color changes and the absorbance of the sample. Figures 1 and 2 shows the color changes of AGNPs 6 days after mixing silver nitrate solution with leave extraxt of *Gnetum gnemon* L. and fruit extract of *Gnetum gnemon* L., respectively. In each of the sample, it is obvious that the color of the sample starts from transparent to yellowish-brown. However, the results show that formation of AgNPs is faster for leaf extract than for fruit extract. When the silver nitrate solution is mixed with the extract, the nucleation process occurs due to the Ag^+ ion being reduced to Ag^0 which is caused by secondary metabolites contained in *Gnetum gnemon* L. leaf and fruit extracts such as steroids, alkaloids and flavonoids resulting in the formation of AgNPs (Wattimena et al., 2022).

In addition to color changes, the absorbance of the sample was also measured after

the extract and precursor were mixed. Absorbance measurements were carried out at wavelengths of 520, 570, and 620 nm using a colorimeter. The measurement results (Figure 3) show that the absorbance intensity at a wavelength of 430 nm is higher than the absorbance intensity at other wavelengths. The higher absorbance intensity at a wavelength of 430 nm indicates the formation of AgNPs in accordance with the wavelength range of silver bio-nanoparticles which varies between 400 nm to 500 nm (Wattimena and Patty, 2023).

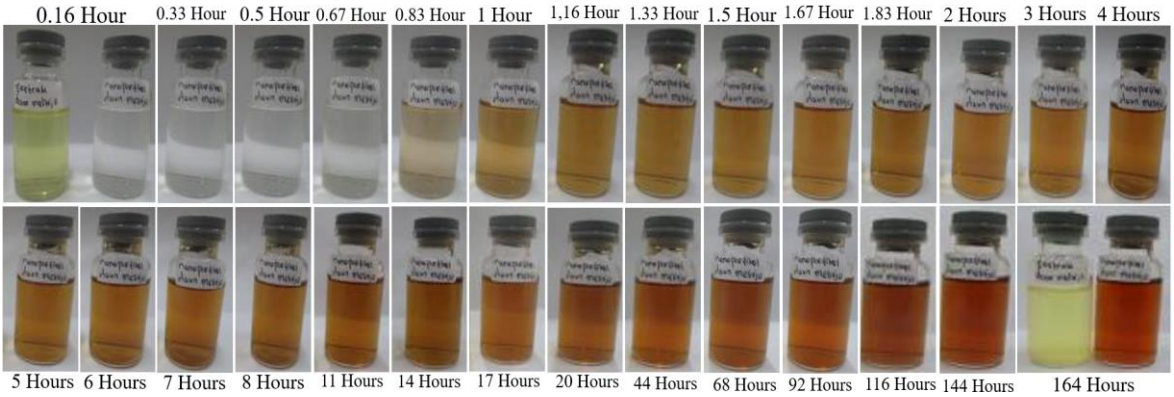

Figure 1. Color changes of AgNPs of *Gnetum gnemon* L. leaves

Figure 2. Color changes of AgNPs of *Gnetum gnemon* L. fruit

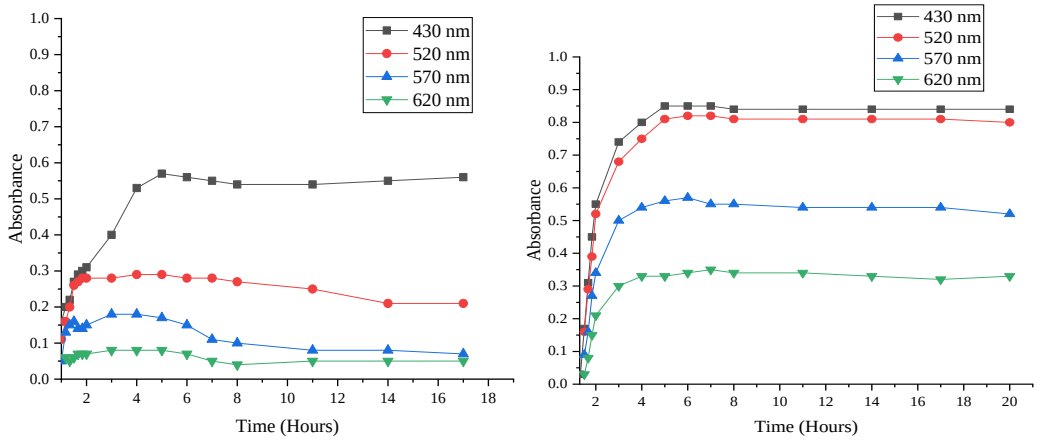

Figure 3. Absorbance of AgNPs using *Gnetum gnemon* L. leaf (left) and *Gnetum gnemon* L. fruit (right)

The color of the sample observation was yellowish-brown which is the typical color of silver bio-nanoparticles. The color of direct observation is different from the color at the LSPR wavelength which is in the 400's nm (bluish). This is because the observed color is the result of scattering, while the absorbance measurement results are the result of the absorbed color, so there is a difference between the observed color and the color resulting from the absorbance measurement.

For determining the growth rate of AgNPs, the discussion is continued only on the results of

absorbance measurements at a wavelength of 430 nm, which is the wavelength range of AgNPs. The results of measuring the absorbance of the two samples showed that AgNPs began to form from 1 hour after mixing the silver nitrate solution with *Gnetum gnemon* L. leaf and fruit extracts which was characterized by the absorption of light at a wavelength of 430 nm. This result is in line with the time required for the sample color change to occur which can be observed in Figure 3. The absorbance intensity increases with time, indicating that AgNPs continue to form. The formation of AgNPs increased exponentially as seen in the curve in Figure 3. Both samples had a significant increase in absorbance at 5 hours after mixing and then the increase in absorbance intensity slowed down after 5 hours. This shows that the formation of AgNPs significantly occurs 1-5 hours after mixing.

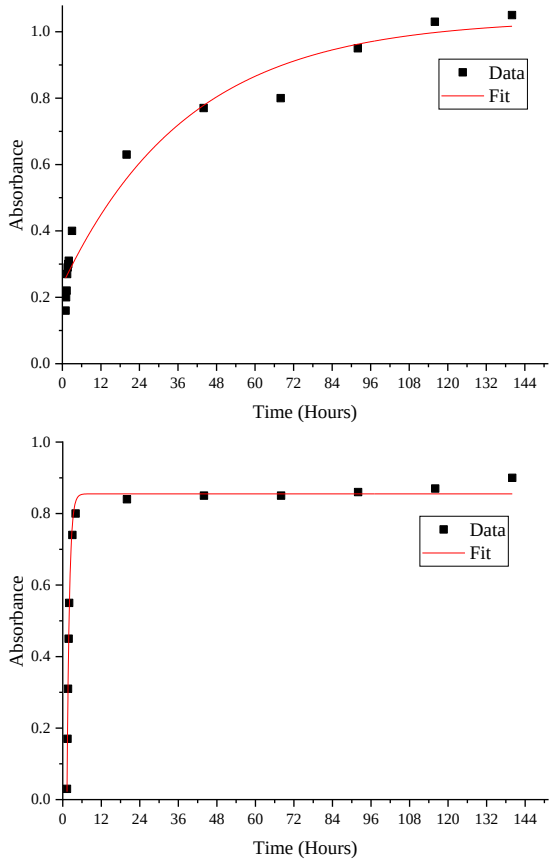

Figure 4. Absorbance of AgNPs using *Gnetum Gnemon* L. leaf (top) and *Gnetum Gnemon* L. fruit (bottom) at a wavelength of 430 nm as a function of time for 5 days.

The growth rate of AgNPs can be difined using the model in equation 1 to fit the data. The fitting results using the exponential model to get parameters A_0, A_1, and k at the smallest value of χ^2. The parameters of the fitting results are shown in table 1 with χ^2 values of 4.11×10^{-3} for AgNPs using *Gnetum gnemon* L. leaf and 6.47×10^{-4} for AgNPs using *Gnetum gnemon* L. as indicators of the suitability of the data to the model.

Table 1. Fitting result parameter values

No	Parameters	AgNPs Using *Gnetum Gnemon* L. leaf	AgNPs Using *Gnetum Gnemon* L. Fruit
1	A_0	1.05 ± 0.06	0.85 ± 0.009
2	A_1	0.77 ± 0.03	5.94 ± 0.8
3	k	0.026 ± 0.006 /Hour	1.36 ± 0.09 /Hour

The rate of formation of AgNPs using *Gnetum Gnemon* L. leaves, based on the fitting results, is 0.026/Hour or 7×10^{-6} s^{-1} and the rate of formation of AgNPs using *Gnetum gnemon* L. fruit is 1.36/Hour or 3.8×10^{-4} s^{-1}. The rate of formation of AgNPs using *Gnetum gnemon* L. leaves was slower than the rate of formation of AgNPs from *Gnetum gnemon* L. fruit. The difference in the rate of formation of AgNPs is very clearly visible in the fitting results curve where the increase in absorbance value in the first 5 hours of AgNPs using *Gnetum gnemon* L. fruit is higher than that of AgNPs using *Gnetum gnemon* L. leaves. The goodness of the fit shown by the value of χ^2 implies that the fitting model using 3 parameters in these studies is as good as the model using 5 parameters in previous studies (Wattimena et al., 2022).

Previously, several studies had discussed the rate formation of Green AgNPs. Table 2 shows the data of the rate formation of Green AgNPs in this study and the data of the rate formation of Green AgNPs in several studies.

Base on Table 2 (No 1-3), AgNPs using *Gnetum gnemon* L. leaf, *Ficus variegata* Leaf, and *Gnetum gnemon* L. fruit with same consentration of $AgNO_3$, 1 mM, have different rate formations. AgNPs using *Ficus variegata* Leaf is faster than AgNPs using *Gnetum gnemon* L. fruit and *Gnetum gnemon* L. leaf. The three AgNPs have the same $AgNO_3$ concentration but have different types of reducers and ratios. Previously, researcher had studied the rate formation with the same type of reducer and ratio but with variations in the concentration of the $AgNO_3$ solution (Okka et al., 2023). The results, Table 2 No 5-7, show that *Inula viscosa* with 5 mM $AgNO_3$ is faster than the rate formation of AgNPs using *Inula viscosa* with 20 mM $AgNO_3$ and AgNPs using *Inula viscosa* with 10 mM $AgNO_3$.

Table 2. Rate formation of AgNPs.

No.	Silver Nanoparticels	Rate formation (s^{-1})	Ratio	Molar (mM)
1	*Gnetum gnemon* L. leaf (this study)	$7x10^{-6}$	1:20	1
2	*Gnetum gnemon* L. fruit (this study)	$3.8x10^{-4}$	1:3	1
3	*Ficus variegata* Leaf (Wattimena et al., 2022)	2.1×10^{-4}	1:9	1
4	*Musa balbisiana* Peel (Rengga et al., 2018)	$4.35x10^{-4}$	-	50
5	*Inula viscosa* (Okka et al., 2023)	$8.1x10^{-3}$	3:1	5
6	*Inula viscosa* (Okka et al., 2023)	$3.4x10^{-3}$	3:1	10
7	*Inula viscosa* (Okka et al., 2023)	$3.6x10^{-3}$	3:1	20

Furthermore, the study of rate formatioan of AgNPs using *Musa balbisiana* peel with 50 mM $AgNO_3$ (higher concentration than AgNPs *Inula viscosa*) had slower rate formation than AgNPs using *Inula viscosa*. From the results in Table 2, it cannot be concluded that there is a relationship between the ratio and concentration of the $AgNO_3$ solution on the rate of formation of AgNPs due to limited data studying the rate of formation of AgNPs. However, futher investigation is needed to confirm the effect of the ratio and the concentration of $AgNO_3$ solution and the extract of samples on the rate formation of AgNPs or other factors.

In addition, Characterization using UV-Vis needs to be carried out to strengthen the results of observing color changes and measuring absorbance as a function of time using a colorimeter to confirm the formation of AgNPs.

Physical Characterization of AgNPs

Furthermore, AgNPs were characterized using UV-Vis. AgNPs have as many free electrons on their surface as other metal nanoparticles. These free electrons have a high density which is called plasma. The electron plasma oscillates around positive ions with certain frequencies called plasmons. AgNPs have a unique plasmon so that when light is illuminated with the same frequency, the light will be absorbed by the AgNPs which is called localized surface plasmon resonance (LSPR). UV-VIS has a light source with a wavelength of 300-700 nm. Figure 4 shows the UV-VIS results together with the pictures of AgNPs. Sample pictures are the colour of AgNPs when characterize using UV-Vis.

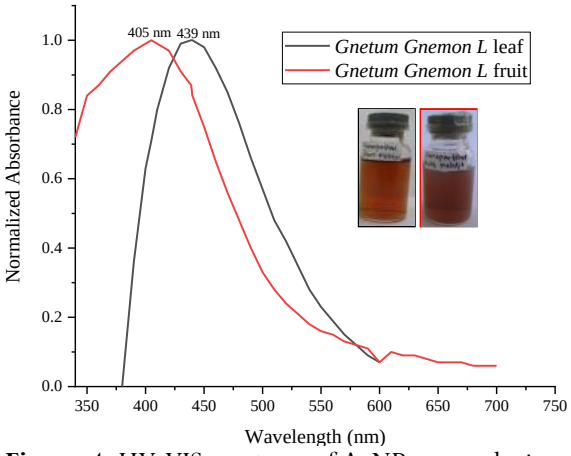

Figure 4. UV-VIS spectrum of AgNPs as a reducing agent for *Gnetum Gnemon* L. leaves and fruit with figures of the samples.

The UV-Vis results show that AgNPs using *Gnetum gnemon* L. leaves has LSPR at a wavelength of 439 nm and AgNPs using *Gnetum gnemon* L. fruit have LSPR at a wavelength of 405 nm. This result is in line with the Colorimeter measurement results. In previously studies, the LSPR wavelengths of AgNPs were ranges between 400-500 nm. The LSPR wavelength of AgNPs using *Ficus variegata* leaf is 415 nm (Wattimena et al., 2022), using *Graptophyllum pictum* is 455 nm (Wattimena et al., 2021), using *Sauropus androgynus* seeds is 428 nm (Hutagalung et al., 2018), and using *Syzygium aromaticum* L is 415 nm (Wattimena et al., 2021). Therefore, this is indicated that both samples produce AgNPs.

The LSPR wavelength can be related to the size of the AgNPs. The particle size affects the number, shape, half-peak width, and LSPR wavelength of AgNPs. UV-VIS can be used to determine the effect of size and shape on the optical properties of AgNPs (Jin et al., 2001). The larger silver particle size causes the absorbance peak to widen and shift towards longer wavelengths which is called "red shifting" while the smaller particle size generally absorbs light at wavelengths approaching 400 nm (Mekuye, 2023). This means that the smaller the LSPR wavelength, the smaller the size of the AgNPs. Therefore, from the UV-VIS results, it can be indicated that the size of the AgNPs using *Gnetum gnemon* L. fruit is smaller than the size of the

AgNPs using *Gnetum gnemon* L. leaf when viewed from the LSPR wavelength.

CONCLUSION

The results of this research show that AgNPs can be synthesized from *Gnetum gnemon* L. leaf and fruit extracts as bioreductors. The formation of AgNPs is confirmed through a color change from clear to yellowish-brown which is an indicator of the formation of AgNPs. Moreover, AgNPs were also confirmed through the LSPR wavelength of AgNPs with *Gnetum gnemon* L. leaves as bioreductor (439 nm) and AgNPs using *Gnetum gnemon* L. fruit as bioreductor (405 nm) which are in the LSPR wavelength range of silver bio-nanoparticles (400-500 nm). The rate of formation of AgNPs using *Gnetum gnemon* L. leaves is 0.026/Hour or 7×10^{-6} s^{-1} and the rate of formation of AgNPs using *Gnetum gnemon* L. fruit is 1.36/Hour or 3.8×10^{-4} s^{-1}.

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