

The UV-VIS Spectrum Analysis From Silver Nanoparticles Synthesized Using *Diospyros maritima* Blume. Leaves Extract

Tiara Egga Agustina¹, *Windri Handayani¹, Cuk 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. Email: windri.h@sci.ui.ac.id

ABSTRACT

Research on the biological synthesis of silver nanoparticles (AgNPs) using plant extracts as reducing agents is growing rapidly because they are eco-friendly and cost-effective. *Diospyros maritima* Blume. is one of the plant species that had potential in the biosynthesis of AgNPs. In this study, biosynthesis of AgNPs was carried out by varying volume ratio of the extract and AgNO₃ (5.0: 1; 2.0: 1; 0.5: 1; 0.2: 1; 0.1: 1) in reaction time (25 minutes, 2, 3, 24, 48, and 96 hours). The phytochemical screening results showed leaves extract of *D. maritima* contained phenols, flavonoids, saponins, and alkaloids, which may act as a reducing agent of Ag⁺ to Ag⁰. The biosynthetic solution's color was changed from brown to dark brown or black with the increasing reaction time. The UV-Vis spectra confirmed the formation of silver nanoparticles. The UV-Vis spectrum results showed the highest absorption peaks at 400 nm in solutions with a volume ratio of 0.5 :1, which showed the localized surface plasmon resonance (LSPR) characteristics of silver nanoparticles. From the UV-Vis spectrum can be analyzed that there are more than one peak of absorption at 350–500 nm in solutions with a volume ratio of 2.0:1. We carried out deconvolution of UV-Vis spectrum to separate each absorption peak. The formation of more than one absorption peak in the UV-Vis spectrum indicates a certain shape and silver nanoparticles size.

Keywords: Biosynthesis, *Diospyros maritima* Blume., phytochemicals, silver nanoparticles, volume ratio

1. INTRODUCTION

Diospyros maritima Blume. is a plant from the Southeast Asia region [1]. *Diospyros maritima* in Central Sulawesi Province is called Biluat [2]. The leaves of these *Diospyros* species generally contain a group of alkaloids, phenol, flavonoid, and terpenoid compounds [3,4,5,6,7]. The use of *D. maritima* for AgNPs biosynthesis is unknown. However, the leaves of *D. maritima* are known to contain a group of chemical compounds, such as phenolic, flavonoids, terpenoids, and saponins which are known to have the potential for the biosynthesis of AgNPs [8,9].

Silver nanoparticles are silver particles with a size 1–100 nm [10]. Silver nanoparticles can be applied as antibacterial agents, biosensors, composite fibers,

cosmetic products, medicines, coatings for medical equipment [11]. One of the factors influencing the reaction rate, size, and shape of AgNPs resulted from synthesis by biological methods is the volume ratio of Ag⁺ ions and extracts [12]. Characterization of AgNPs can be done by observing the color change of the solution resulting from reacting the extract with AgNO₃. The change in the color of the solution to dark brown or blackish brown can indicate the reduction of Ag⁺ ions by the extract [13]. The characterization of AgNPs can also be done by observing the absorption peak of the SynthesizedAgNPs using aqueous extract of *Tithonia diversifolia* leaves with a volume ratio parameter of *T. diversifolia* extract and AgNO₃ 0.001 M (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) [12]. The results showed that the optimum absorption peak was found

in a solution with a volume ratio of 1:9 appeared at a wavelength of 430 nm. Meanwhile, the results of AgNPs biosynthesis research using water extract of *Cinnamomum tamala* leaves by [16] which also uses the parameters of the volume ratio extracts and AgNO₃ 0.01 M (1:1, 1:2, 1:3, 1:4, 1:5 and 2:1) showed the highest absorption peak in the solution with the ratio volume 1:1 at a wavelength of 429 nm. Based on those studies, there was a difference in the absorption peak from the UV-Vis spectra, which was influenced by the parameters of the extract volume ratio and AgNO₃. Therefore, AgNPs biosynthesis using the plant extract can be affected by variations in the volume ratio of the extract and AgNO₃.

Therefore, this study carried out on AgNPs biosynthesis using *D. maritima* leaves extract by varying the volume of the extract and AgNO₃ to see how that affects the AgNPs biosynthesis. The UV-Vis spectrum's absorption peak was analyzed to determine the reaction rate, size, shape, and stability of the AgNPs.

2. MATERIAL AND METHODS

2.1. *Diospyros maritima* Leaves Powder

Diospyros maritima collected from Faculty of Engineering, Universitas Indonesia. The leaves of *D. maritima* washed under running water and rinsed with distilled water. The leaves are then left at room temperature to dry. The dried leaves are then oven-dried at 40 °C until the leaves mass is constant. After the leaves mass is constant, the leaves then crushed using a blender to become a powder. The leaves powder is then filtered using a sieve.

2.2. *Diospyros maritima* Leaves Extract

The leaves powder (2 g) was added with 100 ml of temperature of 100 °C for 15 minutes. Furthermore, the solution is filtered using Whatmann paper No. 1 [4].

2.3. Phytochemical Qualitative Test of *Diospyros maritima* Leaves Extract

2.3.1. Alkaloid test

Extract (1 ml) was dropped with 2–3 ml of Wagner's reagent. The formation of chocolate deposits indicates that the positive extract solution contains alkaloid compounds [17].

2.3.2. Phenolic test

Two drops of 5% FeCl₃ solution were added to 1 ml of extract. Extracts containing phenolic compounds will produce green, purple, blue or black colors [18].

2.3.3. Flavonoid test

Magnesium powder and 2 ml of concentrated HCl are dropped into 1 ml of extract. Extracts containing flavonoid compounds will produce an orange, red or yellow color change [19].

2.3.4. Terpenoid test

Extract (1 ml) was added with 4 drops each of chloroform, acetic acid anhydride, and concentrated sulfuric acid. Extract color changes into dark green indicates a positive solution containing terpenoid compounds [20].

2.3.5. Saponin test

Extract (1 ml) was added to 1 ml of aquabides and then shaken. If after being shaken there is foam, this indicates the extract positively contains saponin compounds [17].

2.4. Silver Nanoparticle Biosynthesis

The leaves extract of *D. maritima* was reacted with 1 mM AgNO₃ solution to form various variations in the volume ratio of the extract and AgNO₃ (v/v) (0.1: 1, 0.2: 1, 0.5: 1, 2.0: 1, and 5.0: 1) with a final volume of 10 ml. Then the solutions were incubated in the dark room up to the time specified for the characterization of AgNPs (25 minutes, 2, 3, 24, 48, and 96 hours).

2.5. Characterization of Silver Nanoparticles

Characterization of the AgNPs formed was carried out visually and spectroscopically using a UV-Vis spectrophotometer. Color changes were observed at reaction times of 0 minutes, 25 minutes, 2, 3, 24, 48, and 96 hours. The absorption spectrum using a spectrophotometer was observed at a wavelength of 200–800 nm and was carried out at a reaction time of 25 minutes, 2, 3, 24, 48, and 96 hours. Before we measured the solution's absorption spectrum, the solution diluted by taking 0.5 ml and then adding bidestilated water to the final volume of 5 ml.

The data obtained are presented in the form of pictures, graphs, and tables. The data generated from the measurement of the absorption spectrum of AgNPs biosynthesis were processed using 64-bit Origin 2018 software.

3. RESULT AND DISCUSSION

3.1. Phytochemical Qualitative Test

Based on the qualitative phytochemical test, it shows that the leaves extract of *D. maritima* contains groups of chemical compounds alkaloids, phenols, flavonoids, and saponins can be seen in Table 1. The qualitative phytochemical test in this study used the colorimetric method, which is a method to determine the presence of compounds with color change indicators after a reagent is given [21]. Certain flavonoid compounds are known to have hydroxyl groups (-OH). The group is negatively charged so that it can attach to the Ag⁺ surface and reduce the ion [22]. It is also known that the -OH group in flavonoids is also involved in the reduction of the Ag⁺ ion [23].

Table 1. Phytochemical qualitative test results of leaves Extract of *D. maritima*

Phytochemicals	Presence
Alkaloid	+
Phenols	+
Flavonoids	+
Terpenoids	-
Saponins	+

In addition, phenolic compounds function as antioxidants so that they can act as reducing agents in neutralizing free radicals and reducing metal ions [24]. Tannins, terpenoids, ketones, aldehydes, amides, and carboxylates can also donate electrons to reduce Ag⁺ ions to Ag⁰ [25]. Therefore, the saponins, phenols, flavonoids, and alkaloids in the extract of *D. maritima* leaves in this study are strongly suspected to play a role in the biosynthesis of AgNPs.

3.2. UV-Vis Absorption Spectrum

At 25 minutes and 2 hours, no absorption peak was formed in all solution. However, an absorption peak began to form at a reaction time of 3 hours in solutions with a ratio of 0.1: 1 and 0.2: 1 at a wavelength of 400–500 nm. At 24 hours, absorption peaks of 0.5:1 and 2.0: 1 began to form (Figure 1a). The highest absorption peak starting from the reaction time of 24 hours is a solution with a volume

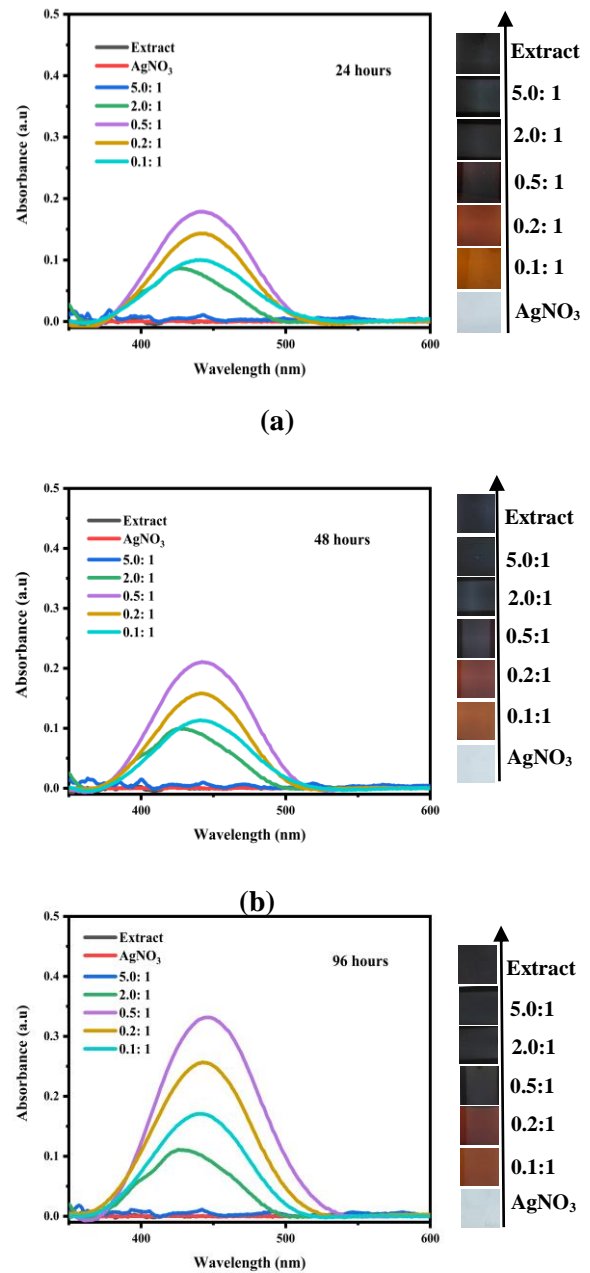


Figure 1. The UV-Vis^(c) Spectrum Graphs and Changes in Color of The Solution with Different Volume Ratios at Reaction Time (a) 24 hours, (b) 48 hours, (c) 96 hours

ratio of 0.5: 1, then 0.2: 1, 0.1: 1, and 2.0: 1. The absorption peak gets higher with increasing reaction time. The appearance of an absorption peak at a wavelength of 400–500 nm is an indication that AgNPs have formed [26,27]. The higher absorption intensity in Figure 1. shows that the number of AgNPs formed in the solution increases with increasing reaction time [26,27]. The absorption peak

formed in Figure 1. also looks wider, indicating that there is aggregation. Silver nanoparticles can be stable if there is strong Coulomb repulsion between particles [28].

A solution with a volume ratio of 2.0: 1 form the lowest absorption peak compared to 0.1: 1, 0.2: 1, and 2.0: 1. This may occur because the volume of the extract is very concentrated, causing the extract to reduce Ag⁺ particles very quickly so that accelerate the formation of AgNPs and cause aggregation. The occurrence of aggregation in a solution with a ratio of 2.0: 1 is indicated by the formation of more than one absorption peak that overlap each other [28]. Changes in color gradations in the entire solution become darker brown over time, indicating that the formation of AgNPs continues to occur, which is confirmed by increasing the maximum absorption value over time in all solutions (Figure 2). The maximum absorption value from ratio of 0.1: 1 increased from 3 hours to 96 hours, from 0.01 to 0.17. Meanwhile, the solution's maximum absorption value with a ratio of 0.2: 1 at 3 hours was 0.018 and increased to 0.256 at 96 hours. The solution's maximum absorption value with a ratio of 0.5: 1 increased from 24 hours to 96 hours, that is 0.178 to 0.331. The solution with a ratio of 2.0: 1 had a maximum absorption value of 0.073 at 24 hours and 0.098 at 96 hours (Figure 2.). The maximum absorption value with a 5.0: 1 ratio tend to remain because the solution does not form an absorption peak for up to 96 hours, as shown in Figure 1.

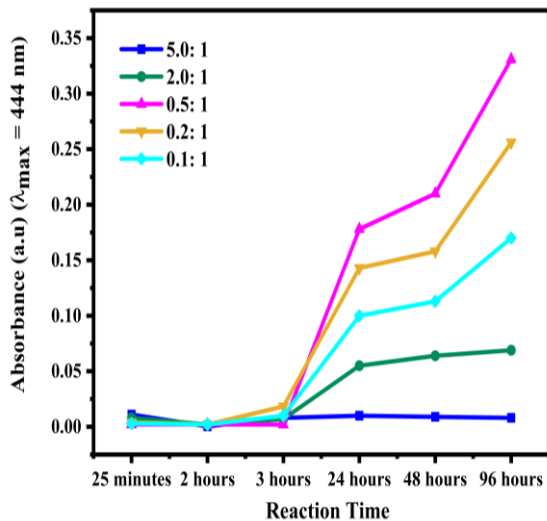


Figure 2. Graph Increase the Maximum Absorption Value (λ_{max}= 444 nm) at 25 Minutes, 2, 3, 24, 48 and 96 Hours

3.3. FWHM (Full Width at Half Maximum) analysis

The FWHM value from the solution with a ratio of 0.5: 1 was the highest after a reaction time of more than 3 hours. Those results followed by solutions with a ratio of 0.2: 1, 0.1: 1, and 2.0: 1 (Figure 3.). The FWHM value of the solution with a ratio of 0.1: 1 increased from 69.2 to 76.84 nm at 3 to 96 hours. The FWHM value of the solution with a ratio of 0.2: 1 increased from 57.09 to 77.33 nm at 3 to 96 hours. The FWHM value of the solution with a ratio of 0.5: 1 at 24 to 96 hours increased, from 75.9 nm to 82.49 nm. The solution with a ratio of 2.0: 1 had an increased FWHM value from 24 to 96 hours, namely 44.09 to 45.33 nm.

All solutions had an increase in the FWHM value. The increase in the value of FWHM indicates that there is an increase in the polydisperse rate of AgNPs formed [32]. The increase in the size and polydisperse of AgNPs may occur due to variations in the nucleation process and the nanoparticles' growth rate during synthesis [33]. Based on Figure 3, it indicates that the polydisperse level increases in all solutions up to 96 hours reaction time.

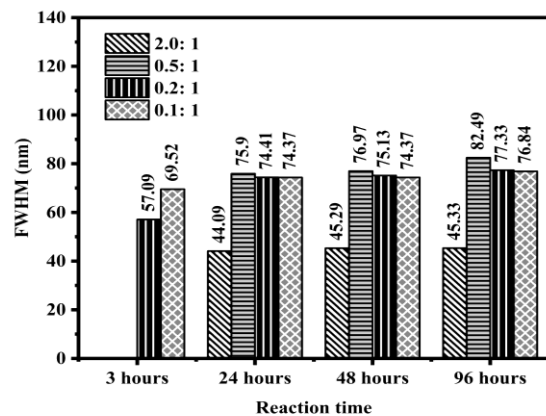


Figure 3. Graph of FWHM Value of Solution with Ratio 0.1: 1, 0.2: 1, 0.5: 1, and 2.0: 1

The size of AgNPs analyzed the value of the maximum wavelength and FWHM values. The size of the AgNPs refers to the study of Solomon et al. (2007). Based on the prediction of the size of the AgNPs in Table 2., the AgNPs formed from biosynthesis using *D. maritima* leaf extract were in the range of 35–80 nm. Solutions with a ratio of 2.0: 1 predicted to have an AgNPs size of 35-50 when viewed from the smallest λ_{max} value compared to solutions with a ratio of 0.1: 1, 0.2: 1, and 0.5: 1 [27,31,34]. The range of size solution with a ratio of 2.0: 1 is also smaller than the solution with a ratio of 0.1: 1, 0.2: 1 and 0.5: 1 because it has the smallest FWHM [32,34].

Table 2. Estimated size AgNPs results biosynthesis using leaf extract *D*.

Reaction time	Volume ratio	λ_{max}^a (nm)	FWHM ^a (nm)	AgNPs size ^b (nm)
3 hours	0.1: 1	441	69.52	60–80
	0.2: 1	441	57.09	60–80
24 hours	0.1: 1	438	74.37	60–80
	0.2: 1	440	74.41	60–80
	0.5: 1	441	75.9	60–80
	2.0: 1	427	44.09	35–50
48 hours	0.1: 1	441	74.37	60–80
	0.2: 1	441	75.13	60–80
	0.5: 1	442	76.97	60–80
	2.0: 1	427	45.29	35–50
96 hours	0.1: 1	443	76.84	60–80
	0.2: 1	440	77.33	60–80
	0.5: 1	444	82.49	60–80
	2.0: 1	426	45.33	35–50

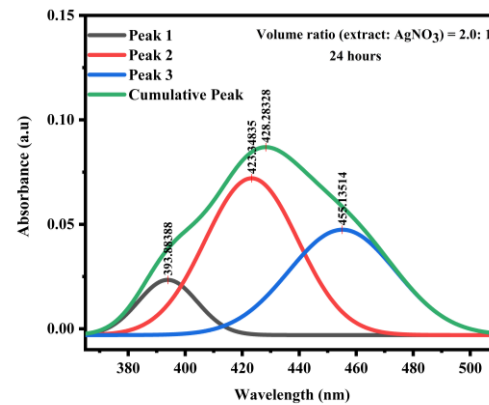
Note: ^a: experiment data, ^b: reference

3.4. UV-Vis spectrum deconvolution analysis

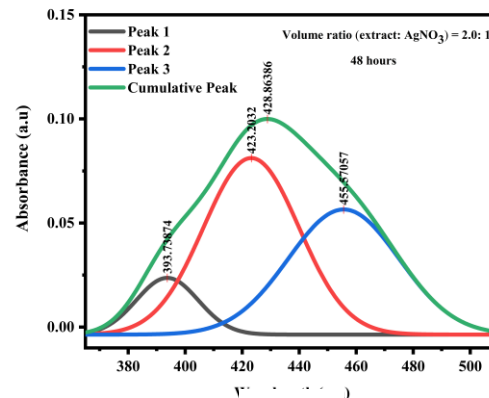
The absorption peaks formed sometimes appear more than one and appear to overlap. These overlapping peaks can be detected and analyzed by deconvolution through multiple peak fit with the Origin 2018 64-bit software [35]. The deconvoluted absorption peak analysis was carried out because it was suspected that each of the overlapping peaks had a unique nanoparticle size. The deconvolution absorption peak will also determine the size distribution of the nanoparticles formed [36].

Absorption peak in each reaction time (24, 48, and 96 hours) there are three peaks (Figure 4 (a–c)). Peak 1 is the smallest peak at a wavelength of about 393 nm, while peak 2 is at a wavelength of about 423 nm. Peak 3 is at a wavelength of about 455 nm. Deconvolution of the UV-Vis absorption spectrum of 2.0: 1 solution was carried out to see the absorption peaks that overlapped separated.

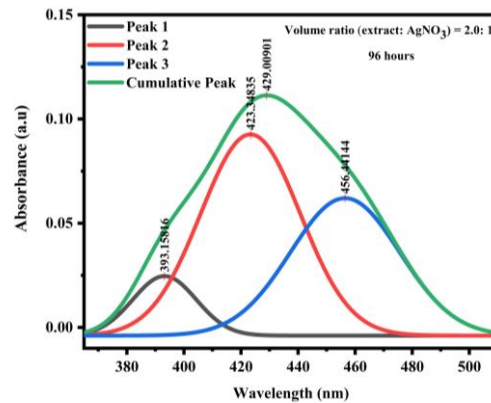
The formation of more than one peak can occur because of the different levels of aggregation in AgNPs [28] and the morphological transitions of AgNPs [37]. Desai et al. (2012) stated that the presence of more than one peak could arise due to the presence of non-spherical particles in AgNPs agglomerates. The shape of the AgNPs or their transition in this study cannot be ascertained but can be estimated based on the absorption peak formed after deconvolution.



(a)



(b)



(c)

Figure 4. Deconvolution Results Spectrophotometer Spectra Solution with Volume Ratio 2.0: 1 at Time Reaction (a) 24 hours, (b) 48 hours, and (c) 96 Hours

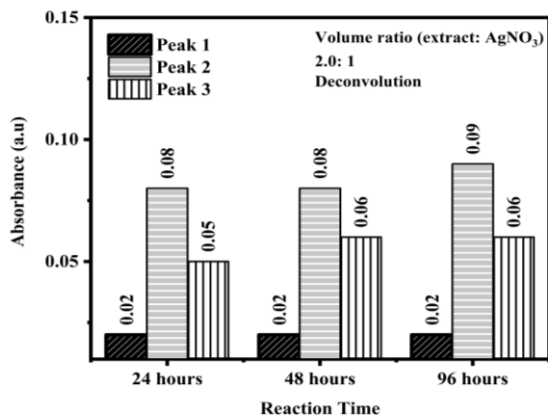


Figure 5. Graph of the maximum absorption deconvolution value of solution with a ratio of 2.0: 1 at 24, 48 and 96 hours

The absorption peaks 1, 2, and 3 are symmetrical so that it can be estimated that the form of AgNPs in a solution with a ratio of 2.0: 1 is spherical [22,28,38]. The formation of more than one peak in 2.0: 1 solution was due to variations in the size of the AgNP formed.

The maximum wavelength (λ_{max}) used to determine the maximum absorption deconvolution value of 2.0:1 solution is the maximum absorption wavelength at the reaction time of 24 hours. The maximum absorption value ($\lambda_{max} = 393.88$ nm) peak 1 did not change from the reaction time of 24 to 96 hours, which was 0.02. The maximum absorption value ($\lambda_{max} = 423.35$ nm) of peak 2 tends to increase from the reaction time of 24 hours to 96 hours, which is 0.08 to 0.09. The maximum absorption value ($\lambda_{max} = 455.14$ nm) of peak 3 increased when the reaction time was 24 to 96 hours, from 0.05 to 0.06 (Figure 5). According to Figure 5, peak 1 does not show an increase in the number of AgNPs up to 96 hours of reaction. Peaks 2 and 3 show an increase in the number of AgNPs at 96 hours [27].

Peak 1 shows a smaller λ_{max} value and show a decrease λ_{max} value during the reaction time of 24 to 96 hours, from 393.88 to 393.16 nm. The peak 2 λ_{max} value at 24 and 96 hours is constant (423.35 nm). Peak 3 shows a shift in the value of λ_{max} to be greater than 24 to 96 hours, from 455.14 nm to 456.44 nm. Based on Figure 6. it can be said that peak 1 has the smallest size of AgNPs because it is at the smallest λ_{max} , while the size of AgNPs at peak 3 is the largest because it is at the largest λ_{max} [27,31]. Peaks 1 and 2 tend to be the same size over time, whereas peak 3 has an increase in the size of the AgNPs at 96 hours (Figure 6).

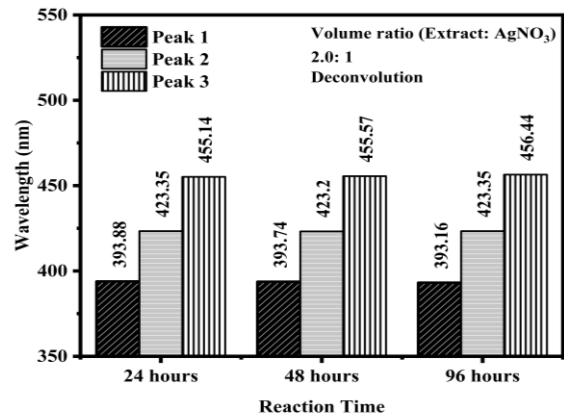


Figure 6. Graph of Maximum Wavelength (λ_{max}) Deconvolution Solution with Ratio 2.0: 1 at 24, 48, and 96 Hours

The FWHM value from peak 1 at 24 to 96 hours increased from 24.36 nm to 26.19 nm. The peak 2 FWHM value also continued to increase, that is 38.29 nm at 24 hours to 41.07 nm at 96 hours. The peak 3 FWHM value at 24 hours was 44.35 nm, increasing to 45.1 nm at 96 hours (Figure 7). Based on the FWHM value, almost all AgNPs at those peaks showed an increase in polydispersity, except for peak 3 which tended to be the same at 48 to 96 hours [33].

Based on the estimated size of AgNPs in Table 3., AgNPs range in size from 10–50 nm [34]. Peak 1 is thought to have an AgNPs size of about 10–14 nm at 24 to 96 hours. Peaks 2 and 3 are estimated to have AgNPs in size of about 35–50 nm. This is in accordance with the values of λ_{max} and FWHM in Figure 6. and Figure 7., where peak 1 is at the smallest wavelength and the FWHM value is the smallest compared to peaks 2 and 3.

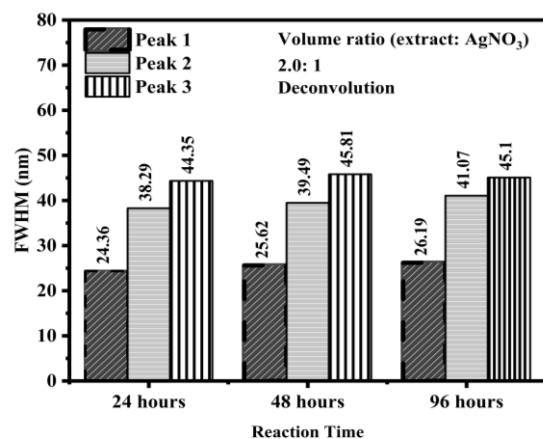


Figure 7. Graph of FWHM Deconvolution Value of Solution with a ratio of 2.0: 1 at 24, 48, and 96 hours

Table 3. The Estimated Size AgNPs Results Biosynthesis solution with a ratio of 2.0: 1 Deconvolution

Reaction Time	Peak	λ_{\max} ^a (nm)	FWHM ^a (nm)	AgNPs Size ^b (nm)
24 hours	Peak 1	393.88	24.36	10–14
	Peak 2	423.35	38.29	35–50
	Peak 3	455.14	44.35	35–50
48 hours	Peak 1	393.74	25.62	10–14
	Peak 2	423.2	39.49	35–50
	Peak 3	455.57	45.81	35–50
96 hours	Peak 1	393.16	26.19	10–14
	Peak 2	423.35	41.07	35–50
	Peak 3	456.44	45.1	35–50

Note: ^a: experiment data, ^b: reference

4. CONCLUSION

The leaves extract of *D. maritima* in this study contains phenolic, flavonoids, alkaloids, and saponins compounds which are predicted to play a role in the biosynthesis of AgNPs. *D. maritima* leaves extract with the potential for AgNPs biosynthesis were the solution with volume ratio of 0.1:1, 0.2:1, 0.5:1 and 2.0:1. The volume ratio of *D. maritima* leaves extract and 1 mM AgNO₃ affect AgNPs biosynthesis time. AgNPs biosynthesis time was analysed from the absorption peak formed. The higher the extract ratio the faster AgNPs formed. It is indicated by a higher absorption peak between 400–500 nm. However, if the extract ratio too high it caused the absorption peaks to overlap with AgNPs or even no nanoparticles formed.

AUTHORS' CONTRIBUTIONS

T. E. Agustina developed the experiment, performed the data analytic and manuscript. Both C. Imawan and W. Handayani contributed to develop the research design, analysis, and supervised the project and manuscript.

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