Synthesis And Characterization Of Silver Nanoparticles Using Purple Eggplant Peel Extract (Solanum Melongena L.) As A Bioreductor And Testing Its Activity As Antioxidant

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Abstract. Nanoparticles are solid colloidal particles with a diameter of 1-1000 nm. Silver nanoparticles (AgNPs) are one of the most attractive nanotechnology products. The synthesis method using nanoparticles in a green synthesis is environmentally friendly. Purple eggplant (Solanum melongena L.) is a vegetable that contains high antioxidants. The research objectives were to determine the antioxidant activity of silver nanoparticles using purple eggplant peel extract, to determine the purple eggplant skin extract used for the synthesis of silver nanoparticles containing flavonoid compounds and to determine the characterization of silver nanoparticles using purple eggplant peel extract. This research is an experimental study that went through a series of stages, namely sample preparation, extraction, flavonoid screening, synthesis of silver nanoparticles from purple eggplant peel extract, UV-Vis Spectrophotometry, FTIR and PSA tests to determine the formation of silver nanoparticles, and antioxidant activity tests. The results showed that the optimum concentration and time was at a concentration of 2 mM at a wavelength of 447 nm with an optimum time of 5 days. The PSA results showed that the purple eggplant skin silver nanoparticles belonged to a homogeneous and stable nanoparticle size. The IR spectrum after bioreduction using purple eggplant peel extract experienced a shift in the wave number of the –OH group. The IC_{50} value of quercetin is very strong, at 4.723 ppm, while the IC_{50} value of silver nanoparticles is moderate, at 118.536 ppm. Purple eggplant peel extract contains flavonoids, and purple eggplant peel extract nanoparticles have good nanoparticle characterization and moderate antioxidant activity.

Keywords: [Silver Nanoparticles, Purple Eggplant (Solanum melongena L.), Bioreductor, Antioxidant]

INTRODUCTION

Nanoparticles are solid colloidal particles with a diameter of 1-1000 nm (Kurniasari & Atun, 2017). Silver nanoparticles (AgNPs) are one of the most interesting nanotechnology products because they have unique properties such as good chemical conductivity and stability in antimicrobial, antiviral, anti-inflammatory, antioxidant, and anticancer activities (Siakavella et al., 2020). Purple eggplant (Solanum melongena L.) is a vegetable that contains high antioxidants. The basin compound is an anthocyanin that is concentrated in purple eggplant skin (Gallo et al., 2014).

Research on the development of synthesis and characterization of silver nanoparticles using purple eggplant skin extract (Solanum melongena L.) as a bioreductant and testing its activity as an antioxidant by varying the concentration of $AgNO_3$ solution 0.5, 1, 1.5 and 2 mM due to the concentration of $AgNO_3$ in The synthesis of silver nanoparticles affects the size of the particles produced (Ristian et al., 2014). Variations in sample concentration are 100, 150, 200, 250 and 300 ppm. This concentration was determined because of research that has been carried out (Taba et al., 2019); the antioxidant activity of bay leaf extract and silver nanoparticles shows that the greater the concentration (ppm) used, the greater the antioxidant activity, but concentrations of 10, 20, 40, 80, 160 ppm produces bay leaf extract antioxidant activity of 109,63 ppm (medium antioxidant power) and silver nanoparticle antioxidant activity of 582,65 ppm (very weak antioxidant power).

METHODS

This research method is quantitative experimental using purple eggplant skin extract (Solanum melongena L.). Tools: Knife, baking sheet, woven bamboo, test tube, evaporating cup, dropper pipette, Bunsen lamp, simplicial blender (Getra IC-10B), analytical scale (Great scale BS-600), glass funnel (HERMA), moisture balance (Ohaus), alcohol thermometer, beaker (HERMA), measuring flask (HERMA), measuring cup (HERMA), 40 mesh sieve, brown bottle, glass bottle, brown vial, water bath, Erlenmeyer (PYREX), UV-Vis spectrophotometer (BIOBASE BK -D560 spectrophotometer), hot plate magnetic stirrer (I8-ONE), PSA (VASCO DLS), FTIR (Shimadzu IRTracer-100). Ingredients: Purple

eggplant skin, distilled water, 70% ethanol, ethanol p.a, $AgNO_3$ (Merck), quercetin, DPPH, flannel cloth, tissue, label paper, aluminum foil, cling wrap, magnesium powder, concentrated hydrochloric acid and 10% NaOH.

Research procedure

Determination of Purple Eggplant Plants (Solanum melongena L.)

Determine the plants first to ensure that the plants used in the research are the plants in question so that errors in collecting materials can be avoided.

Raw Material Preparation

Purple eggplant (Solanum melongena L.) was wet sorted, and purple eggplants were selected in good condition, fresh, shiny purple, and not rotten. The purple eggplant was washed under running water, the skin was removed, weighed 2500 grams and dried in indirect sunlight, namely covered with a black cloth. The dried purple eggplant skin Simplicia was then ground using a blender and sieved with a 40-mesh sieve (Tandi et al., 2017).

Water Content Test

Determination of water content is carried out using a moisture balance. The determination of water content was replicated three times. The water content of Simplicia is no more than 10% (Ministry of Health of the Republic of Indonesia, 2009).

Preparation of Purple Eggplant Skin Ethanol Extract

Macerated extraction of purple eggplant skin modifies the research of Handayani & Nurcahyanti (2015). 200 g of purple eggplant skin powder was extracted using the maceration method using 800 mL of 70% ethanol solvent with a ratio of 1:4, and the sample was soaked for 24 hours, stirring at room temperature. The maceration results are filtered using a flannel cloth to separate the filtrate and residue (filtrate 1). Remaceration was carried out on the residue in a ratio of 1:4 (in 800 mL of 70% ethanol) and filtered using a flannel cloth to obtain the second filtrate. The residue is soaked again in 70% ethanol in a ratio of 1:2 (in 400 mL of 70% ethanol) and filtered to obtain a filtrate on the third day. The filtrate obtained was collected and evaporated at a temperature of less than 50 ^oC using a water bath until a thick extract was obtained, then the yield of the extract was calculated.

Phytochemical Screening Test (Identification of flavonoids by tube method)

Qualitative analysis of flavonoid compounds in purple eggplant skin extract was carried out by weighing 0.1 gram of thick extract, adding 10 mL of hot water and boiling for 5 minutes. The solution is filtered, and the filtrate is used for testing.

1. Shinoda Test
   1 mL of filtrate was put into a test tube, 0.5 grams of Mg powder and 1 mL of concentrated HCl were added. A positive test for flavonoids produces a yellow or orange color (Harborne, 1987).
2. Bate-Smith test
   1 mL of filtrate was put into a test tube, 1 mL of concentrated HCl was added and then heated. A positive test for flavonoids produces a red or orange color (Achmad, 1987).
3. 10% NaOH Test
   Test 10% NaOH by adding two drops of sample and then adding 2-4 drops of 10% NaOH solution. A positive reaction to flavonoids produces a yellow to brownish-yellow color (Asih, 2009).

Preparation of $AgNO_3$ Solution

The solution was prepared based on the working method of Nisa et al. (2020), namely that the $AgNO_3$ solution is made by dissolving 0.8495 grams of $AgNO_3$ powder in distilled water to a volume of 1000 mL. The silver nitrate solution is shaken and can be used directly. The silver solution was diluted to obtain (0.5, 1, 1.5 and 2 mM) $AgNO_3$ solutions.

Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles was carried out by referring to Taba et al. (2019). 40 mL of (0.5,

1, 1.5 and 2 mM) $AgNO_3$ solutions were pipetted, and each solution was put into a 250 mL Erlenmeyer, then 2 mL of purple eggplant skin extract was added to the Erlenmeyer. The mixture was stirred with a hot plate magnetic stirrer for 15 minutes at a temperature of 50 ºC, then analyzed using UV-Vis spectrophotometry at a wavelength range of 200-700 nm to analyze wavelengths that fall within the absorption range of the silver nanoparticle solution, namely 395-515 nm (Masakke et al., 2015). The characteristics of the solution in the form of color and UV-Vis absorption spectrum after mixing at 1, 2 and 5 days were carried out to obtain the optimum time, and PSA and FTIR characterization were carried out (Taba et al., 2019).

Positive Control Antioxidant (Quercetin)

1. Preparation of Comparator Solution (Quercetin)

A stock solution of 1000 ppm was prepared by weighing 25 mg of quercetin, then dissolving it with 25 mL of ethanol p.a. so that a standard solution of 1000 ppm quercetin is obtained. The 1000 ppm quercetin stock solution was then diluted to 100 ppm. A 100 ppm quercetin solution was made in varying concentrations (2, 4, 6, 8 and 10 ppm) by diluting using 10 mL ethanol p.a (Primadevi & Nafi, 2020).

2. Measurement of the Antioxidant Power of Quercetin Comparative Samples

The test was carried out by pipetting quercetin solutions of various concentrations (2, 4, 6, 8 and 10 ppm). 2 mL of 0.4 mM DPPH standard solution was added to the quercetin standard solution until the limit mark on the 10 mL volumetric flask, then left for the operating time and the absorbance was read at the maximum wavelength obtained (Brand-Williams et al., 1995).

This research method is quantitative experimental using purple eggplant skin extract (Solanum melongena L.). Tools: Knife, baking sheet, woven bamboo, test tube, evaporating cup, dropper pipette, Bunsen lamp, simplicia blender (Getra IC-10B), analytical scale (Great scale BS-600), glass funnel (HERMA), moisture balance (Ohaus), alcohol thermometer, beaker (HERMA), measuring flask (HERMA), measuring cup (HERMA), 40 mesh sieve, brown bottle, glass bottle, brown vial, water bath, Erlenmeyer (PYREX), UV-Vis spectrophotometer (BIOBASE BK -D560 spectrophotometer), hot plate magnetic stirrer (I8-ONE), PSA (VASCO DLS), FTIR (Shimadzu IRTracer-100). Ingredients: Purple eggplant skin, distilled water, 70% ethanol, ethanol p.a, $AgNO_3$ (Merck), quercetin, DPPH, flannel cloth, tissue, label paper, aluminum foil, cling wrap, magnesium powder, concentrated hydrochloric acid and 10% NaOH.

RESULTS AND DISCUSSION

Determination Results of Purple Eggplant Plants

The determination results showed that the plants used were indeed purple eggplant plants (Solanum melongena L.).

Raw Material Preparation Results

The purple eggplant skin is dried in the sun and covered with a black cloth. This is done so that the simplicia is not directly exposed to UV light and the active compounds in the simplicia are not damaged. Sieving aims to facilitate the filtering process and homogeneity; particle size influences the uniformity of the extraction stage as well as the smooth flow and speed of diffusion of substances into the solvent (Maulida & Guntarti, 2015). The results of making purple eggplant skin powder are shown in Table 1.

Table 1. Results of making purple eggplant skin powder

Wet Purple Eggplant Skin (gram)	Dried Simplisia (gram)	Powder (gram)
2500	228	218

Water Content Test Results

Water content is an important parameter that determines the durability of a food product and the activity of microorganisms during storage. Analysis of the water content of purple eggplant skin powder using the moisture balance method at a temperature of 105 ºC and carrying out three replications obtained results of the water content of 6.55%, 6.04% and 6.16% with an average % water content of 6.25 ± 0.21. This shows that the percentage of water content in purple eggplant skin powder meets the

quality requirements, namely no more than 10% (Ministry of Health of the Republic of Indonesia, 2009).

Results of Preparation of Purple Eggplant Skin Ethanol Extract

The results of the extraction are determined from the yield, with the aim of knowing the comparison between simplicia and extract and the amount of extract from simplicia at a certain weight (Ministry of Health of the Republic of Indonesia, 2000). The yield obtained was 6.5%. The yield of purple eggplant skin did not meet the requirements because it was less than 17.9% (Ministry of Health of the Republic of Indonesia, 2006).

Phytochemical Screening Results

The results of phytochemical screening on purple eggplant skin (Solanum melongena L.) were positive for containing flavonoid secondary metabolite compounds, as presented in Table 2.

Table 2. Results of phytochemical screening of purple eggplant skin

Metabolite Compounds	Color Changes That Occur	Test result
Flavonoids	Gray	Negative (Shinoda)
	Pink	Positive (Bate-Smith)
	Yellow	Positive (NaOH 10%)

The Shinoda method of flavonoid identification test showed negative results.

The Bate-Smith test produces a pink color. The formation of a red color indicates the presence of anthocyanidin-type flavonoid compounds (Rahayu et al., 2015). In the flavonoid identification test using the 10% NaOH method, the color changed to yellow after 10% NaOH was dropped. This proves that purple eggplant skin extract contains flavonoid compounds (Kusnadi & Devi, 2017).

Synthesis Results of Silver Nanoparticles

The formation of silver (Ag) nanoparticles is characterized by a change in the color of the $AgNO_3$ solution. The solution changed from clear yellow to brownish yellow after stirring and heating. The purpose of stirring is to speed up the reaction rate, thereby increasing the number of collisions between particles (Wahyudin & Sari. 2004), while the purpose of heating is to control the rate of formation of the size of silver nanoparticles (AgNPs) (Yanti et al., 2021). In this study, the longer the reaction time, the darker the color of the solution. This is in accordance with the literature stated by Shankar et al. (2003) that as the reaction time increases, the solution becomes darker and the color of the silver nanoparticle solution tends to be yellow to brownish, the brown color will continue to increase with the length of the reaction time.

UV-Vis Spectrophotometric Characterization

Determination of the optimum concentration of AgNO3 measured with a wavelength in the range of 200-700 nm was carried out with the aim of knowing the correct concentration ratio of AgNO3 in order to help the process of forming silver nanoparticles (AgNPs). Silver nanoparticles are said to be formed if the peak wavelength is in the absorption range of the silver nanoparticle solution, namely 395-515 nm (Masakke et al., 2015). The result of the four variations in the concentration of the AgNO3 solution, which has a wavelength of 395-515 nm, is a concentration of 2 mM on the fifth day, namely 447 nm with an absorbance of 0.524 (Figure 1). According to Ristian et al. (2014), the concentration of AgNO3 in the synthesis of silver nanoparticles affects the size of the resulting particles. The optimum concentration of reducing agents shows the highest intensity. (Chuchita et al., 2018).

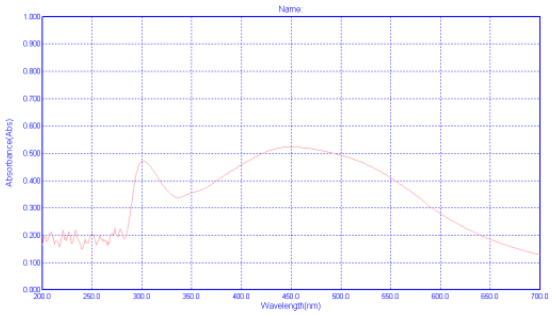


Figure 1. Concentration of 2 mM fifth day

FTIR Characterization Results

FTIR characterization between purple eggplant skin extract, silver nanoparticles from purple eggplant skin extract and silver solution was carried out with the aim of determining the functional groups that act as bioreductors. The FTIR characterization results are presented in Figure 2 and Table 3.

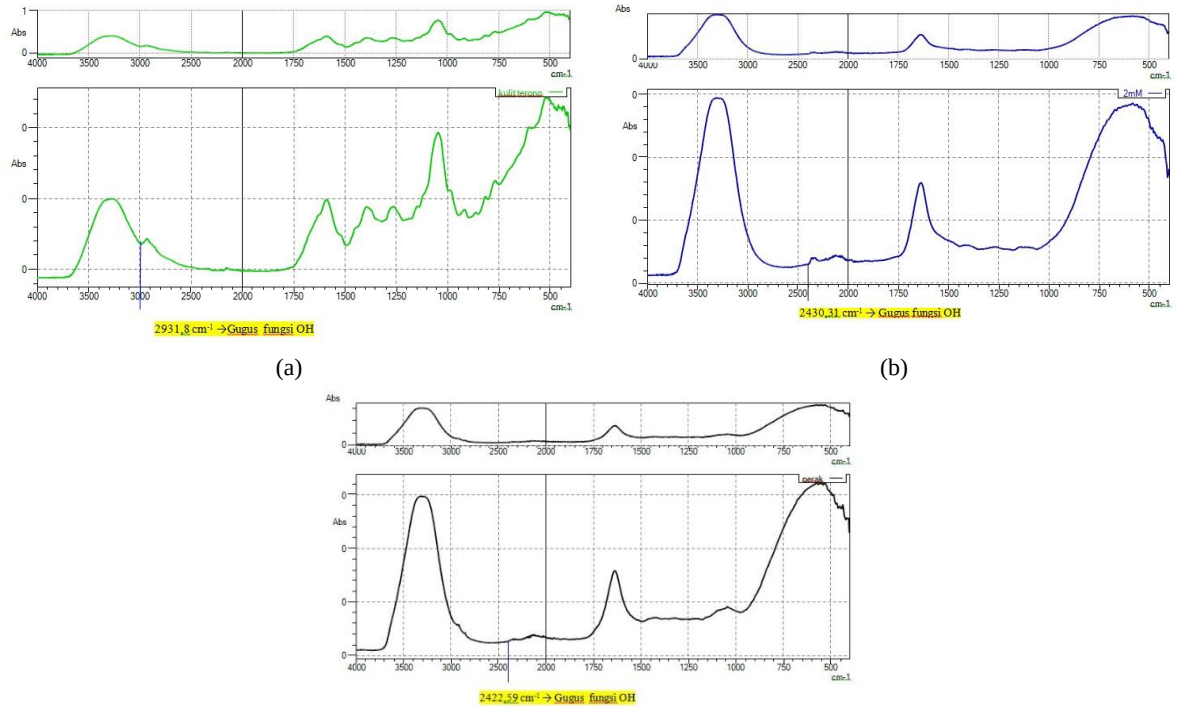


Figure 2. FTIR characterization results (a) purple eggplant skin extract, (b) silver nanoparticles purple eggplant skin extract, and (c) silver nanoparticle solution

Table 3. FTIR Characterization Results

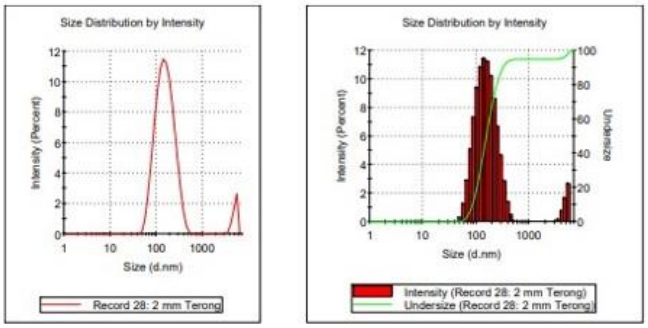
Treatment	Wave number (cm^{-1})	Functional groups	Intensity
Purple eggplant skin extract	2931,8 cm^{-1}	–OH	0,087
Silver nanoparticles Purple eggplant skin extract	2430,31 cm^{-1}	–OH	0,03
Silver nanoparticle solution	2422,59 cm^{-1}	–OH	0,026

The occurrence of a shift in the wave number and a change in the intensity of a functional group indicates an interaction between secondary metabolite compounds from purple eggplant skin extract and

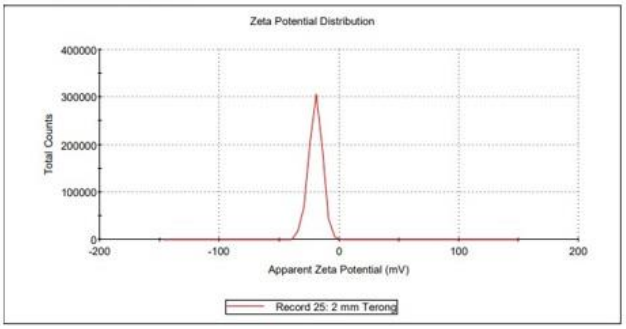
Ag+ ions, which indicates the formation of silver nanoparticles. The decrease in the intensity of the –OH group in the extract is a result of the use of the –OH group to reduce Ag^+ ions to Ag^0 (Trinanda et al., 2019). The presence of flavonoid compounds in purple eggplant skin extract causes a reduction reaction of Ag^+ ions to Ag^0.

PSA Characterization Results

Characterization of silver nanoparticles using a Particle Size Analyzer (PSA) was carried out to determine particle size, polydispersity index (particle size distribution) and zeta potential (Wirasti et al., 2021). The results of PSA characterization can be seen in Figure 3 and Table 4.



(a)



(b)

Figure 3. (a) Size distribution by intensity (b) zeta potential distribution

Table 4. PSA Characterization Results

Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
156,7	0,329	-19,7

The PSA results show that the size of the nanoparticles synthesized with the 2 mM $AgNO_3$ variation is 156.7 nm, so the purple eggplant peel extract nanoparticles in this study comply with the nanoparticle size requirements. The size distribution of the sample concluded that the sample had a good level of uniformity (polydispersity index value 0.329) (Avadi et al., 2009). The zeta potential of purple eggplant peel extract nanoparticles is -19.7 mV. This value meets the criteria for good zeta potential because it is in the zeta potential value range of ±30 mV (Akhtar et al., 2012).

Results of Determination of Antioxidant Activity

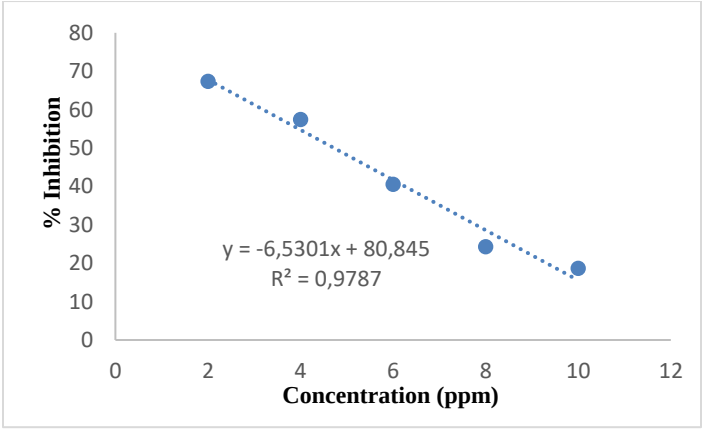
Determination of antioxidant activity using the immersion method (DPPH). The IC_{50} value expresses the antioxidant activity value.

Table 5. IC_{50} Value of Quercetin

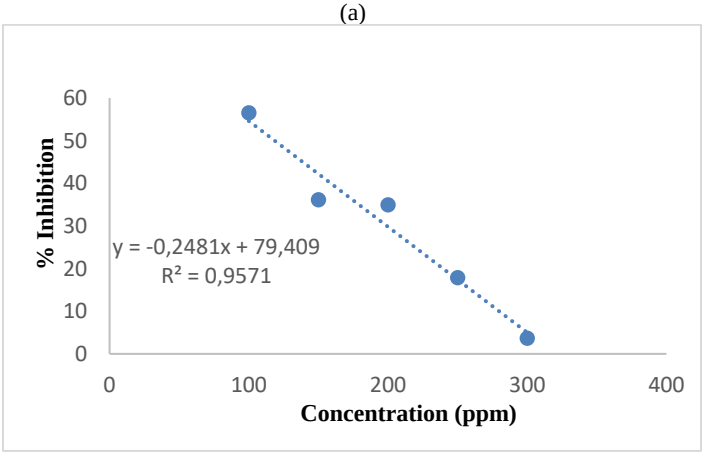
Concentration (ppm)	Average Absorbance	% Inhibition	Persamaan Regresi	IC_{50} (ppm)	Kategori
2	0,243	67,382			
4	0,317	57,449	y = -6,5301x + 80,845 r = 0,989	4,723	Sangat Kuat
6	0,443	40,5369			
8	0,564	24,2953			
10	0,606	18,6577			

Table 6. IC_{50} Value of silver nanoparticles from purple eggplant skin extract

Concentration (ppm)	Average Absorbance	% Inhibition	Persamaan Regresi	IC_{50} (ppm)	Kategori
100	0,324	56,510			
150	0,476	36,107	y = -0,2481x + 79,409 r = 0,9571	118,536	Sedang
200	0,485	34,899			
250	0,612	17,852			
300	0,718	3,624			



(a)



(b)

Figure 4. Inhibition curve of (a) quercetin and (b) silver nanoparticles from purple eggplant skin extract

The equivalent concentration parameter that provides 50% antioxidant activity by scavenging DPPH radicals is the Inhibition Concentration (IC_{50}) value. The IC_{50} value is obtained from a linear regression equation, which states the relationship between concentration and percent inhibition. The smaller the IC_{50} value, the greater the antioxidant activity. From the results above, the IC_{50} value of the quercetin comparison was obtained at 4,723 ppm in the very strong antioxidant category. The results of the antioxidant activity test of silver nanoparticles from purple eggplant skin extract with an IC_{50} value of 118.536 ppm in the medium category (Molyneux, 2004).

CONCLUSION

Purple eggplant skin extract (Solanum melongena L.) used for the synthesis of silver nanoparticles contains secondary metabolite compounds (flavonoids). Purple eggplant skin extract has good silver nanoparticle characteristics (The peak wavelength obtained is 447 nm, included in the absorption range of silver nanoparticle solutions, namely 395-515 nm. PSA results show that the size of the synthesized nanoparticles with variations in $AgNO_3$ 2 mM is 156.7 nm, so the purple eggplant peel extract

nanoparticles in this study comply with the nanoparticle size requirements and the IR spectrum results after bioreduction using purple eggplant peel extract experienced a shift in the wave number of the -OH group). Purple eggplant skin extract nanoparticles have antioxidant activity with an IC_{50} value of 118.536 ppm in the medium category.

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