Antioxydant activity of the silver nanoparticles (AgNPs) synthesized using Nephrolepisradicans extract as bioreductor

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Abstract. The silver nanoparticle is a silver metal in nanosize which is currently widely applied in various fields, such as optics, electronics, biology, catalysts, health, food, and the environment. Silver nanoparticles can be synthesized by top-down and bottom-up methods. Bottom-up method can be done by reacting silver nitrate solution with plant extracts or known as green synthesis. This research is aimed to synthesis the silver nanoparticles using the methanolic extracts of *Nephrolepisradicans* ferns as bioreductor and testing their antioxidant activity. The characterization of silver nanoparticles synthesized was carried out using the UV and FTIR spectrophotometer. The antioxidant activity test was carried out using the DPPH method. The results showed that the silver nanoparticles synthesized had an average diameter of 19,194 nm. Based on the IR spectrum, the functional group that played an important role in the process of synthesis of silver nanoparticles were hydroxyl (OH), carbonyl (C = O) and ether (C-O). The silver nanoparticles had antioxidant activity in the medium category with IC_{50} of 106.442 ppm. Thus, the silver nanoparticles synthesized had the potential to be developed as antioxidants agent.

1. Introduction

Silver nanoparticles are a silver metal in nano size. Silver nanoparticles are widely studied because they have wide applications in everyday life, such as optics, electronics, biology, catalysts, health, food, and the environment [1]. To synthesize nanoparticles can be done by two methods, namely top-down and bottom-up. The bottom-up method has advantages over the top-down method because it is easy to manipulate the synthesized nanoparticles. Bottom up method can be done through the reduction reaction, namely the reduction reaction of silver ion (Ag^+) to silver atom (Ag^0) in the form of silver nanoparticles. To realize the reduction reaction, it takes a reducing agent [2].

Based on the composition there are two types of reducing agents, namely synthetic reductor and bioreductor (reducing agents derived from natural materials). One example of material that can be used as a bioreductor is a plant extract. It contains various antioxidant compounds such as flavonoids, phenolics, tannins, etc., so that it can be used as a bioreductor and capping agent that can reduce silver ion to silver nanoparticles [3]. Therefore, bioreductors from plant extracts can be an alternative choice as bioreductors.

The fern *Nephrolepisradicans*are one of the ferns that become Indonesia's biological natural wealth. This plant had been widely used for vegetables, worm medicine and stomach cancer [4]. Previous research has shown that methanol extract of *N. radicans* fern contains phenolic compounds, flavonoids,

alkaloids. A flavonoid compound that has potential as an antioxidant has been successfully separated from the plant. Therefore, the methanol extract of the *N. radicans* fern has the potential to be used as a bioreductor for the synthesis of silver nanoparticles [4,5,6].

In this paper we will report the results of the synthesis of silver nanoparticles using bioreductor extracts of the *N. radicans* fern, nanoparticles characterization, as well as the results of antioxidant activity testing of silver nanoparticles using the DPPH method.

2. Methods

2.1. Material

The dried powder of *Nephrolepisradicans*'s aerial parts, methanol, aquabidest, $AgNO_3$, DPPH (Sigma), ascorbic acid (Sigma), whatman filter paper no. 42.

2.2. Instruments

Spectrophotometer UV-Vis (Shimadzu UV-1800), magnetic stirrer (DLAB MS7-H550), rotary vacuum evaporator (BuchiLabortechnik B-491), oven (Heraeus ST-5042), analitical balance (Advanturer Ohaus), vacuum pump (Gast DOA-P-504-BN), waterbath (Memmert), freeze dryer (Martin Christ Alpha 1-2), volumetric flask, beaker glass, Buchner funnel, test tube, volumetric pipette, spatula, maceration vessel.

2.3. Research procedures

2.3.1 Collection and preparation of sample

Samples of the *N. radicans*were collected from the Kletak forest, Nongkojajar, Pasuruan, East Java. Before further investigation, the sample was identified at LIPI Kebun Raya Purwodadi. Furthermore, the sample is cleaned of attached dirt, then dried at room temperature. The dried sample was grinded into a fine powder that was ready for extraction.

2.3.2. Extraction

Three kg of dried powder of *N. radicans* were macerated with methanol for 3 x 24 hours. Then filtered using a funnel Buchner. The methanol extract obtained was evaporated in vacuo using rotary vacuum evaporator, resulting the concentrated extract. It was dried in freeze dryer for 8 hours, resulted the dark green solid [7,8].

2.3.3. Preparation of methanol extract solution

A total of 2 g of dried methanol extract of *N. radicans* was dissolved with 80 mL aquabidest, then filtered with whatman filter paper No. 42. The obtained methanol extract solution is stored in refrigator [9].

2.3.4. Preparation of silver nitrate solution

The silver nitrat powder as much as 0.085 g was dissolved with aquabidest in a 250 mL volumetric flask to produce an silver nitrate solution with a concentration of 2mM [9].

2.3.5. Optimization of the ratio of the amount of extract solution with silver nitrate solution in synthesis of silver nanoparticles using the methanolic extract of N. radicans as bioreductor

The methanolic extract solution of *N. radicans* was added with 2 mM $AgNO_3$ solution in 4 beakers each with a volume ratio of 1: 1, 1: 2, 1: 3, and 1: 4. The mixture is stirred using amagnetic stirrer for 15 minutes. Measure the UV-Vis spectrum to determine the maximum wavelength and absorbance in the range 300 - 700 nm. Determine the optimum ratio of volume of methanol extract to silver nitrate solution. The optimum mixture is centrifuged and then separated between the filtrate and pellet. The pellets were dried using a frezze dryer to obtain silver nanoparticle dry powder. Then the silver nanoparticles were characterized by FTIR and tested for their antioxidant activity [9].

2.3.6. Antioxidant activity assay of silver nanoparticles synthesized

A total of 5 mg of silver nanoparticles powder synthesized was dissolved with methanol to a volume of 10 mL to produce a mother liquor with a concentration of 500 ppm. From the 500 ppm mother solution was taken 0.1;0.2; 0.4; 0.8; 1.6 mL is put in a separate test tube so that the solution will be produced with various concentrations of 10, 20, 40, 80, 160 ppm. To each test tube 1 mL of DPPH 0.4 mM solution was added. Methanol was added to a volume of 5 mL into each test tube. The mixture was incubated for 30 minutes at room temperature in a dark room. The absorbance of the solution was measured at a maximum wavelength of 514 nm. Using the same procedure, measurement of the antioxidant activity of ascorbic acid was carried out as a positive control. The experimental procedure was carried out in triplo. Based on the absorbance data obtained, the antioxidant activity was determined which was stated at the IC-50 value [9].

3. Results and discussion

3.1. Extraction of the N. radicans's aerial part

The aerial part of *N. radicans* (3 kg) was macerated using methanol as solven for 24 hours. Evaporation of the solvent in vacuo, followed by drying at freeze drier, yielded the dark green solid (15 gr).

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3.2. Optimation composition of $AgNO_3$solution in synthesis silver nanoparticles using the methanolic extract of N. radicans as bioreductor

In this research, the synthesis of silver nanoparticles was carried out by reacting the extract of methanol N radicans with 2 mM silver nitrate solution using 4 volume ratio variations, namely 1: 1, 1; 2, 1: 3, and 1: 4. Each nanosilver produced was analyzed by its UV-Vis spectrum at wavelengths between 300 - 700 nm. The results of the UV-Vis spectrum measurements are presented at Figure 1 and Table 1.

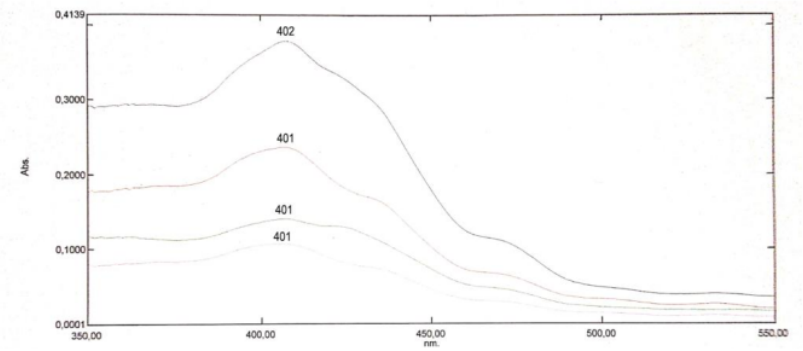

Figure 1. The UV-Vis spectra of silver nanoparticles

Table 1. The maximum wavelength and absorbance of silver nanoparticles synthesized

No.	Ratio of methanol extract and silver nitrat solution	The maximum wave lengh(nm)	Absorbance
1	1 : 1	402	0.3795
2	1: 2	401	0.2644
3	1 : 3	401	0.1395
4	1 : 4	401	0.1087

All reaction mixtures with various ratios showed the maximum absorption wavelength between 400 – 500 nm, namely 401 and 402 nm. Thus the silver nanoparticles had been formed in all reaction

mixtures because the wavelength value of silver nanoparticles are generally found 400-500 nm [10]. Based on the data Table 1 can be stated that the optimum composition for the synthesis of silver nanparticles using bioductors extract of *N. radicans* fern plant is 1: 1. In the composition the silver nanoparticles produced have a maximum absorption wave length and maximum absorbance.

3.3. Size of the silver nanoparticles synthesized using the methanol extract of N. radicans as bioreductor

Teoritically, based on the UV-Vis spectra data, the particle size of silver nanoparticles synthesized was predicted using the following Brust equation [11]:

$$E_g = E_g(\infty) + \frac{14.84}{R^2}\left(\frac{1}{m_e^2} + \frac{1}{m_h^2}\right) - \frac{2{,}6}{kR}$$

Note: $E_g(\infty)$ = 1.3 eV, m_e and m_h= 0.25, k = 6.5, h (Planck constant) = 4.1356 x 10^{-6} eV, and c (light speed) = 3x10^8 m/s. The calculation results of silver nanoparticles were presented at Table 2.

Table 2. The size of silver nanoparticles synthesized

No.	Ratio of methanol extract and silver nitrat solution	The maximum wave lengh (nm)	Size of silver nanoparticles prediction (nm)
1	1 : 1	402	16,1943
2	1: 2	401	16,1597
3	1 : 3	401	16,1597
4	1 : 4	401	16,1597

The silver nanoparticles resulted from the reaction mixtures had particle size between 16.1597 nm and 16.1943 nm. The silver nanoparticles synthesized using bioreductor had various particle size from 5 to 500 nm. The diversity of silver nanoparticle sizes was influenced by the ability of each secondary metabolites contained the extract of plant to act as a capping agent. The better the performance of secondary metabolites as a capping agent, the more stable the size of the silver nanoparticles produced [12].

3.4. Characterizationof silver nanaparticlesusing the FTIR spectroscopy

The IRspectraof *N. radicans*metanol extract and silver nanoparticle were shown at Fig 2 and 3, respectively. The IR spectrum of metanoli extract of *N. radicans* were diffrent with it's of the silver nanoparticles. The IR spectrum of methanol extract showed the existence of hydroxyl group with the sharp peak at 3284.48 cm^{-1} and C-H alkyl group at 2924.37 cm^{-1} with low intensity. Absorption band at 1605.66 cm^{-1}caused by C=C streching showed the existence of phenolic compound in this extract. At the IR spectrum of silver nanoparticles still showed the presence of hydroxyl group at 3348.65 cm^{-1} but with low intensity. While the peak absorption of C-H alkyl at 321.43 cm^{-1}showed the high intensity and a sharp peak arised at 1709.15 cm^{-1} caused bay carbonyl group. The decreasing in the intensity of the hydroxyl group from the methanol extract and the increasing in the intensity of the carbonyl group in nanoparticles indicated that there had been a reduction of silver ions by the hydroxyl group of phenolic compound to produce a carbonyl compound (quinone) [9,13].

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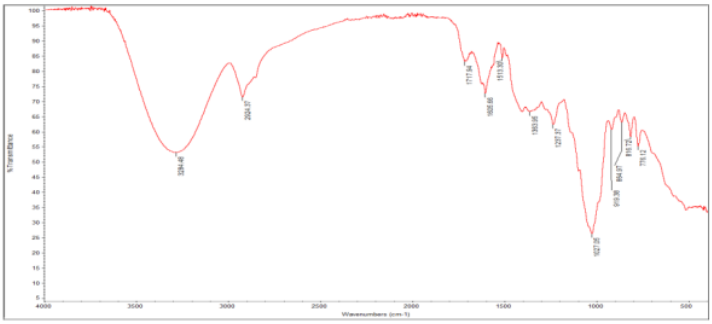

Figure 2. The IR spectrum of *N radicans* methanol extract

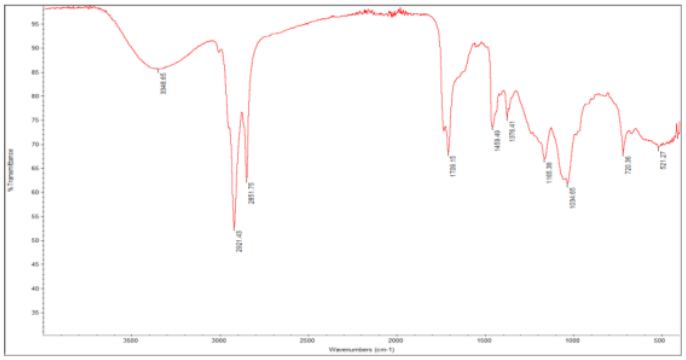

Figure 3. The IR spectrum of silver nanoparticles

3.5. Antioxidant activity assay of silver nanoparticles

The antioxidant activity of the silver nanoparticles synthesized was determined using the DPPH method. In this assay, the silver nanoparticles with various concentrations of 10, 20, 40, 80, and 160 ppm were reacted with DPPH 0.4 mM. The results of antioxidant activity assay were presented at Table 3.

Table 3. Results of antioxidant activity assay

Concentration of nanosilver (ppm)	Average of absorbance value	Average of absorbance reduction percentage (%)
Control	0.911	0
10	0.788	13.56
20	0.652	28.43
40	0.578	36.61
80	0.473	48.13
160	0.354	61.20

The antioxidant activity of silver nanoparticles can be determined using linear regression analysis relationship between the DPPH absorbance reduction percentage with concentration of silver nanoparticles. Based on the results of the analysis, it could be stated that silver nanoparticles synthesized had antioxidant activity in the medium category with the IC-50 of 106.422 ppm [14]. Thusit has the potential to be used as an antioxidant agent. The antioxidant activity of the silver nanoparticles is caused by the ability of the silver atom to donate one electron to a DPPH molecule so it change to produce a stable molecule. Besides that, a hydrogen atom donor from a phenolic hydroxyl group from a phenolic compound contained in the methanol extract acts as a capping agent to silver nanoparticles [15,16].

4. Conclusion

The silver nanoparticles coud be synthezed using metahnol extract of *N. radicans* as bioreductor. The silver nanoparticles havethe maximum wave length of 402 nm and particle size between 16.1597 - 16.1943 nm. It contained the hydroxyl, C-H alkyl, carbonyl and C-O groups. The silver nanopartcles had antioxidant activity in medium category with IC-50 of 106.422 ppm.

Acknowledgements

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