ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 27 (8) (2024): 381-387

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Green Synthesis of Gold Nanoparticles Using *Basella alba* Leaf Extract and Their Antioxidant Activity

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https://doi.org/10.14710/jksa.27.8.381-387

Article Info

Article history:

Received: 28th June 2024
Revised: 14th August 2024
Accepted: 20th August 2024
Online: 31st August 2024

Keywords:
green synthesis; gold nanoparticles; *Basella alba* leaf extract; characterization; antioxidant

Abstract

In this research, gold nanoparticles were synthesized using the green synthesis method with the bioreduction extract of *Basella alba* leaf infusion. This research aims to characterize gold nanoparticles synthesized using UV-Vis spectroscopy and TEM and assess their antioxidant activity, which is quantified by the IC_{50} value. The UV-Vis spectrophotometric characterization of the gold nanoparticles revealed an absorption peak at a maximum wavelength of 543 nm. TEM characterization indicated a particle diameter of 9.463 ± 2.273 nm. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction method with concentration variations of 2.5, 5, 10, and 20 ppm, resulting in reduction percentages of 40.6%, 44.3%, 57.4%, and 77.9%, respectively, and an IC_{50} value of 7.05 µg/mL, indicating very strong antioxidant activity. Based on these findings, it can be concluded that *Basella alba* leaf infusion extract is effective as a biological reducing agent in synthesizing gold nanoparticles, and the resulting nanoparticles show significant potential as antioxidants for reducing free radicals.

1. Introduction

The field of nanoparticle technology has advanced rapidly in recent years. Nanotechnology focuses on the study of materials science and engineering at the nanometer scale, ranging from 1 to 100 nm [1]. Nanomaterials exhibit superior chemical and physical properties compared to their bulk materials. The use of metal nanoparticles has garnered significant attention due to their catalytic, electrical, magnetic, therapeutic, and optical properties, offering a wide range of applications in biomedicine, catalysis, healthcare, and cosmetics [1, 2, 3].

Gold nanoparticles (AuNPs) have attracted considerable attention among all metal nanoparticles due to their unique characteristics, including high biocompatibility, low toxicity, and the ability to be synthesized through simple methods [4]. Gold nanoparticles are an inorganic metal material in the form of a colloidal solution that undergoes a particle size reduction process to become nanoscale with a size ranging from 5-400 nm [5]. Synthesis of metal nanoparticles can be carried out using top-down and bottom-up methods. In the bottom-up synthesis process, reducing agents can be categorized into synthetic and biological, with the latter derived from natural sources [6]. While both top-down and bottom-up methods can produce high-quality nanoparticles, they have certain drawbacks, including high demands on time, energy, and cost and the potential generation of hazardous waste that can harm the environment [7]. Consequently, green synthesis has emerged as a more environmentally friendly alternative for nanoparticle production.

Green synthesis involves using an environmentally friendly chemical-based or bottom-up approach by utilizing natural materials such as plants or microorganisms as reducing agents. In this study, gold nanoparticles were synthesized using a bottom-up approach with *Basella alba* leaf extract as a biological reducing agent due to its active compounds that can act as effective reducing agents. This plant is also known as Malabar spinach, Indian spinach, Ceylon spinach, climber spinach, and vine spinach [8]. *Basella alba* is a plant that contains several nutrients and various types of vitamins

as well as active phenolic compounds, flavonoids, alkaloids, saponins, tannins, carotenoids, and terpenoids [9]. Specifically, *Basella alba* contains the flavonoid kaempferol at a concentration of 1.4 mg/100 g [10]. The antioxidant content in plant extracts such as flavonoids, phenolics, saponins, and tannins act as bioreductors, enabling the reduction of Au^{3+} ions (auric) to gold nanoparticles (Au^0). These compounds also function as stabilizing agents (capping agents), helping to stabilize the metal atoms as they form into nanoparticles [2].

Gold nanoparticles are synthetic antioxidants that do not exhibit carcinogenic effects [11]. Antioxidant compounds prevent and inhibit the formation of free radicals or Reactive Oxygen Species (ROS) in the body by donating electrons to free radicals, thereby pairing the unpaired electrons and halting cellular damage. The presence of free radicals in the body can lead to degenerative diseases such as cancer, stroke, hypertension, coronary heart disease, and premature aging [12]. In an effort to overcome this degenerative disease, antioxidants such as gold nanoparticles can be utilized due to their small size and neutral charge, which enhance the mobility of active compounds. Additionally, gold nanoparticles exhibit robust and long-lasting antioxidant activity, making them highly effective in reducing free radicals [11].

In this research, AuNPs were synthesized using a bottom-up approach through green synthesis. The materials used in the synthesis included $HAuCl_4$ solution as a precursor and *Basella alba* leaf extract as a bioreductant. The study also aimed to characterize the synthesized gold nanoparticles using UV-Vis spectroscopy and Transmission Electron Microscopy (TEM), as well as to evaluate their antioxidant activity through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction method.

2. Experimental

2.1. Materials and Instruments

The materials used in this research were the leaves of the *Basella alba* plant obtained from the village of Kalijaten, Sepanjang, Sidoarjo, East Java. Other materials included double-distilled waters (Otsuka), 1000 ppm $HAuCl_4$ stock solution (Chemistry Department, State University of Surabaya), filter paper, aluminum foil, technical 98% ethanol solution, and DPPH powder (TCI Japan). The instruments used in this research were a UV- Vis Spectrophotometer (Shimadzu UV-1800) and a TEM (JEOL JEM-1400).

2.2. Extraction of *Basella alba* Leaf Extract

In this study, fresh *Basella alba* leaves were selected, ensuring they were free from yellow spots, white spots, or holes. The leaves were washed to remove dirt, dust, and other plant debris and dried using tissue or a clean cloth to eliminate any remaining moisture. A 25-gram of the dried leaves was ground using a chopper or blender. The finely ground leaves were placed into a 250 mL Erlenmeyer flask and combined with 100 mL of double-distilled water. The mixture was heated in a water bath at 90°C for 15 minutes, with occasional stirring [13]. After heating, the mixture was filtered through filter paper to obtain a clear, light green extract, which was used as a bioreductant to synthesize gold nanoparticles.

2.3. Synthesis Process of AuNPs

2.3.1. $HAuCl_4$ Solution Preparation

The materials for synthesizing the gold nanoparticles included an $HAuCl_4$ solution as the precursor and *Basella alba* leaf extract as the bioreductant. The $HAuCl_4$ solution was prepared by dissolving 1 gram of gold metal in aqua regia, which is a mixture of 6 mL of hydrochloric acid (HCl) and 2 mL of nitric acid (HNO_3) in a 3:1 ratio. This solution was then diluted with double-distilled water in a 1000 mL measuring flask to the mark, resulting in a 1000 ppm $HAuCl_4$ solution [11].

2.3.2. Synthesis of AuNPs with *Basella alba* Leaf Extract

AuNPs were synthesized with an $HAuCl_4$ concentration of 20 ppm. This was achieved by measuring 2 mL of a 1000 ppm $HAuCl_4$ solution using a measuring cup, transferring it to a 100 mL measuring flask, and then filling the flask with double-distilled water up to the mark, followed by thorough mixing. The homogenized solution was then transferred to a 250 mL beaker, and 2 mL of *Basella alba* leaf extract was added. The mixture was heated to a temperature of 80-90°C while stirring with a magnetic stirrer at 200 rpm until the color changed to burgundy [14, 15, 16].

2.4. Characterization of AuNPs Using UV-Vis Spectrophotometer

Measurements were carried out on the $HAuCl_4$ solution and 20 ppm AuNPs from the synthesis with the aim of determining the maximum wavelength shift of the $HAuCl_4$ solution so that it is known that gold nanoparticles have been formed. The 20 ppm $HAuCl_4$ solution was put into a cuvette and analyzed at a maximum wavelength of 200–800 nm. The colloidal AuNPs 20 ppm from the synthesis were put into a cuvette and analyzed at a maximum wavelength of 400–800 nm [14].

2.5. Characterization of AuNPs Using TEM

Characterization of the nanoparticles using TEM was conducted by placing a drop of colloidal AuNPs onto a copper grid, which was then dried under a vacuum. After that, the grid was placed on a specimen holder and analyzed with the TEM instrument to determine the shape and size of the gold nanoparticles [6].

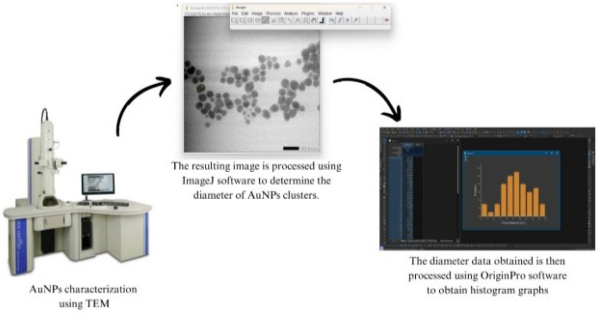

Figure 1. Characterization of AuNPs Using TEM

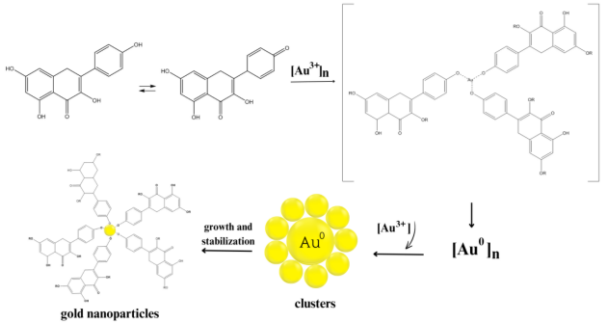

Figure 2. Reaction of kaempferol compounds with Au [17]

2.6. Antioxidant Activity Test for AuNPs

A 0.003% DPPH solution was prepared by dissolving 3 mg of DPPH powder in 100 mL of 98% ethanol. The solution, which turned deep purple, was left in a dark place for 30 minutes. Subsequently, the DPPH solution was measured using a UV-Vis spectrophotometer at a wavelength range of 400–600 nm. This resulted in determining the maximum λ for DPPH, which was then used to measure the sample's absorption [11].

The synthesized AuNPs at a concentration of 20 ppm were diluted to obtain concentrations of 10, 5, and 2.5 ppm. To prepare the 10 ppm AuNPs, 50 mL of the 20 ppm AuNPs were measured and transferred into a 100 mL volumetric flask. Double-distilled water was added to the calibration mark, and the mixture was shaken until homogeneous. The same procedure was followed for the 10 ppm and 5 ppm AuNPs to obtain 5 ppm and 2.5 ppm AuNPs, respectively.

In the next step, 2 mL of each AuNPs sample (2.5, 5, 10, and 20 ppm) was measured and transferred into reaction tubes lined with aluminum foil. Each tube was then filled with 2 mL of a 0.003% DPPH solution, homogenized, and left in a dark room for 30 minutes. Following this, the sample was placed in a cuvette, and its absorption value was measured using a UV-Vis spectrophotometer at the maximum wavelength of DPPH [11].

3. Results and Discussion

3.1. Synthesis Process of AuNPs

During the dissolving process, a redox reaction occurs in which a neutral Au atom (Au^0) is oxidized to a trivalent Au ion (Au^{3+}), forming a tetrachloroaurate(III) anion [11]. The chemical reaction is represented by Equation (1).

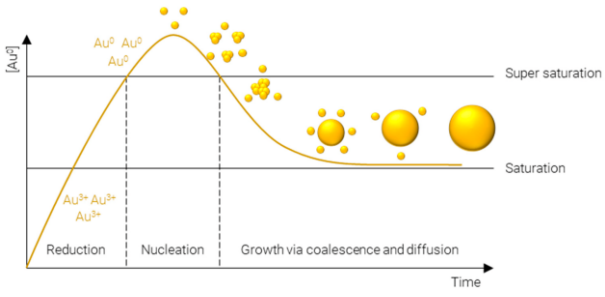

Figure 3. LaMer model of gold nanoparticle formation [18]

$$Au_{(s)} + HNO_{3\,(aq)} + 6HCl_{(aq)} \rightarrow HAuCl_{4\,(aq)} + NO_{2\,(g)} + 2H_{2\,(g)} + 3Cl_{2\,(g)} + H_2O_{(l)} \quad (1)$$

The AuNPs synthesis process was carried out at a concentration of 20 ppm. A concentration of 20 ppm was chosen for this process because the AuNPs are stable at this concentration. This is based on research by 'Aini and Taufikurohmah [19], who synthesized AuNPs using sodium citrate as a reducing agent at a concentration of 20 ppm. Their study reported a reduction percentage of 52.32% and optimal stability at this concentration, while AuNPs synthesized at higher concentrations of 25 ppm and 30 ppm were more prone to aggregation. Therefore, this research was conducted at a concentration of 20 ppm to synthesize AuNPs.

The synthesis was conducted at 80–90°C, with the temperature carefully maintained below 90°C throughout the heating process [15]. A study by Suliasih *et al.* [16] supports this, stating that the synthesis process optimally reduced gold ions at 80–90°C. However, if the heating was too intense or prolonged, the AuNPs might agglomerate due to increased particle consistency, leading to the formation of larger clusters. This aggregation resulted in a suspension with a purple-brown color [15, 16].

The antioxidant content of kaempferol in *Basella alba* leaf extract can act as a bioreductant and stabilizer capable of reducing Au^{3+} ions to Au^0 atoms. Figure 2 describes the process of forming gold nanoparticles through the oxidation-reduction reaction of the Au^{3+} ion. The active compounds in plant extracts possess a hydroxyl cluster with a free electron pair, which they can use to reduce Au^{3+} ions. The hydroxy cluster in the flavonoid compound kaempferol in the sample has the ability to reduce the Au^{3+} ion by releasing its hydrogen atom, transforming it into a ketone cluster [6]. When Au^0 accumulates to a certain point, it naturally merges into clusters that gradually grow to nanometer size. Bioreductants also serve as stabilizing agents, preventing aggregation by electrostatically interacting with the positive partial charge of the gold nanoparticles and the negative partial charge of phenolic compounds [17, 20].

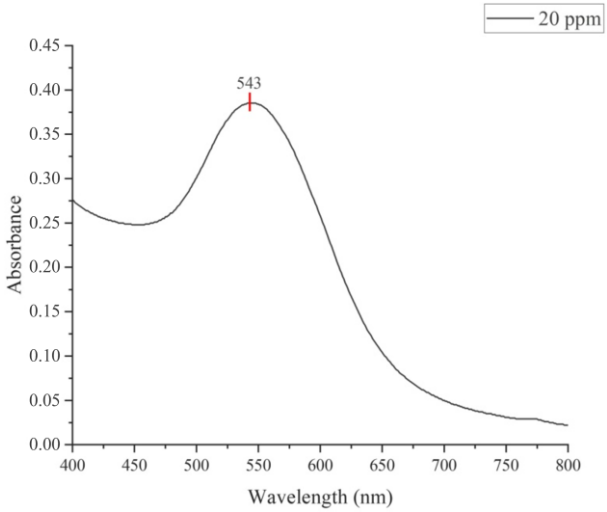

Figure 4. Results of characterization analysis of AuNPs using a UV-Vis spectrophotometer

Figure 5. Color changes that occur during the AuNPs synthesis process

During the formation of gold nanoparticles, a disproportionation reaction may occur. This type of redox reaction involves the same substance acting as both the oxidant and the reductant, where part of the substance is oxidized, and another part is reduced. $HAuCl_4$, a weak acidic solution, can establish an equilibrium system during the formation of gold nanoparticles. Due to their similar charges, Au^{3+} ions repel each other in their ionic form. When a reducing agent converts Au^{3+} ions to Au^0, it neutralizes the atomic charge of gold, initiating a nucleation process where Au atoms interact to form nanometer-sized clusters through intermetallic bonds. As these clusters grow, they undergo stabilization, leading to the formation of AuNPs. A color change in the resulting solution indicates the successful formation of AuNPs [18, 20].

3.2. Characterization of AuNPs Using UV-Vis Spectrophotometry

The synthesis of AuNPs using a bioreductant from *Basella alba* leaf extract was characterized using a UV-Vis spectrophotometer to confirm the formation of AuNPs and determine their maximum wavelength. The formation of gold nanoparticles is typically indicated by a shift in the maximum wavelength observed in the $HAuCl_4$ solution [14]. For this research, the maximum wavelength of a 20 ppm $HAuCl_4$ solution was 309 nm. After synthesis with the bioreductant, the UV-Vis spectrophotometric analysis of the AuNPs, performed over the wavelength range of 400-600 nm, showed a maximum absorption peak at 543 nm. This shift from 309 nm to 543 nm, as illustrated in Figure 4, confirms the successful formation of AuNPs. The observed wavelength range of 500-600 nm is consistent with the typical range for AuNPs [6, 14].

This is supported by the research of Sodeinde *et al.* [21], which synthesized AuNPs using *Basella alba* leaf extract and produced a maximum wavelength in the range of 530–560 nm. The maximum absorption peak observed in this study aligns with the typical wavelength range of AuNPs, which is approximately 500–600 nm [14, 21, 22]. The shift in wavelength in AuNPs occurs due to the collective oscillation of conduction electrons, resulting in resonant excitation, which produces photons. Based on the Surface Plasmon Resonance (SPR) theory, the maximum wavelength is related to the size of the nanoparticle, where the larger the size of the nanoparticle, the higher the wavelength produced. This is because the excitation energy decreases with increasing distance electrons travel to excite the state basis [14].

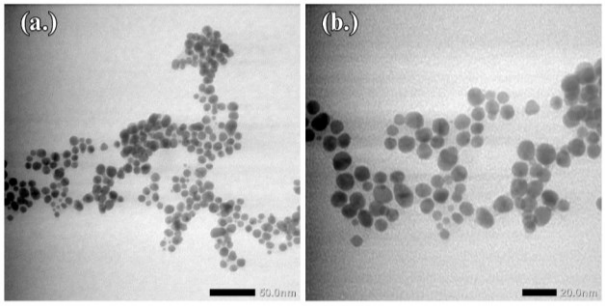

Figure 6. Results of characterization of gold nanoparticles with a magnification of (a) 80,000 and (b) 150,000

The formation of AuNPs can be qualitatively indicated by a change in the color of the solution mixture from colorless to burgundy [14]. This color change during the synthesis process signifies the growth of gold clusters. Initially, a colorless solution indicates that gold atoms have not yet interacted significantly. As the number of gold clusters increases, the color gradually shifts to purplish. When the clusters grow to nanometer size, the color transitions to burgundy [3].

3.3. Characterization of AuNPs Using TEM

In Figure 6b, magnified at 150,000×, the diameter of the AuNPs clusters can be observed using ImageJ. The size distribution of the AuNPs clusters was analyzed by processing the ImageJ data with OriginPro software. According to the graph in Figure 7, the most common cluster size is 9-10 nm in diameter, with 11 particles in this size range. The average diameter of the AuNPs clusters is 9.463 ± 2.273 nm. This indicates that AuNPs were successfully synthesized using a bioreductant from *Basella alba* leaf extract, resulting in nanoparticles with diameters ranging from 1 to 100 nm.

3.4. Antioxidant Activity Testing of AuNPs

The working principle of testing antioxidant activity with DPPH is capturing electrons from DPPH free radicals through hydrogen atoms from antioxidant compounds, which is indicated by a color change in the sample from dark purple to pale yellow [20]. This method uses a UV- Vis spectrophotometer to measure the free radical scavenging activity value expressed in percent scavenging (IC_{50}). In this study, the wavelength of the 0.003% DPPH solution was measured using a UV-Vis spectrophotometer, producing a λ_{max} of 517 nm and an absorbance value of 0.600. Antioxidant activity testing on AuNPs samples at each concentration was measured at the λ_{max} of 517 nm. The absorbance value was used to calculate the percent reduction of free radicals.

Table 1. Absorbance measurements of gold nanoparticles and DPPH at a wavelength of 517 nm

Concentration of gold nanoparticles	Average ± Standard deviation
2.5 ppm + DPPH	0.408 ± 0.003
5 ppm + DPPH	0.432 ± 0.013
10 ppm + DPPH	0.447 ± 0.025
20 ppm + DPPH	0.482 ± 0.012

Table 2. Percent reduction of free radicals calculated from absorbance measurements

AuNPs concentration	DPPH	Absorbance of AuNPs + DPPH (A)	Absorbance of AuNPs at λ 517 nm (B)	A-B	Percent inhibition
2.5 ppm	0.600	0.408	0.051	0.357	40.6%
5 ppm		0.432	0.098	0.334	44.3%
10 ppm		0.447	0.191	0.256	57.4%
20 ppm		0.482	0.349	0.133	77.9%

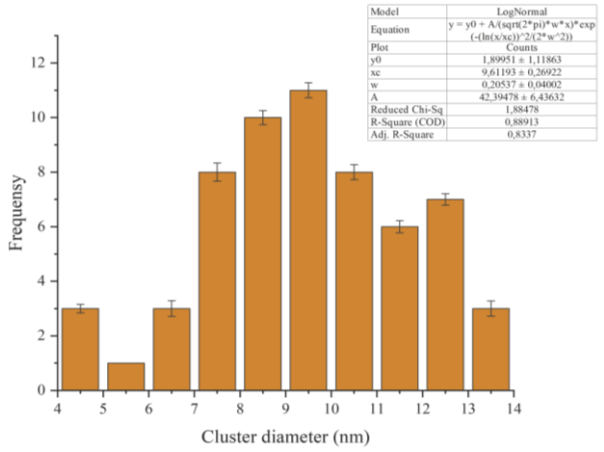

Figure 7. Results of characterization of AuNPs using Origin Pro

Antioxidant activity testing on AuNPs was performed by reacting samples at 2.5, 5, 10, and 20 ppm (Figure 8) with DPPH in a 1:1 ratio. This variation in concentration was intended to assess the percentage of free radical scavenging activity. The absorbance values for each concentration of AuNPs, after reaction with DPPH, were measured using a UV-Vis spectrophotometer, and the results are presented in Table 1.

Based on Table 1, the absorbance values for each concentration of the sample can be used to determine the percent reduction of free radicals. The percent reduction is calculated by subtracting the absorbance value of the AuNPs-DPPH mixture from the absorbance value of AuNPs without DPPH, measured at a wavelength of 517 nm. The presence of AuNPs affects the absorbance readings of the DPPH solution, as the absorption of AuNPs overlaps with that of DPPH. Therefore, the absorbance values are calculated using Equation (2).

$$\% \text{ Attenuation} = \frac{(absorbance\ of\ DPPH - (A-B))}{absorbance\ of\ DPPH} \times 100\% \quad (2)$$

Where, A is the absorbance of gold nanoparticles + DPPH and B is the absorbance of AuNPs at λ 517 nm.

Based on Table 2, it can be observed that the absorbance value of the sample (A-B) decreases as the concentration of AuNPs increases. This reduction is attributed to the DPPH being neutralized by the antioxidant compounds in the sample. As the concentration of AuNPs rises, the absorbance decreases, indicating an increase in the percentage of scavenged DPPH radicals. This suggests that higher concentrations of AuNPs are more effective at suppressing or stabilizing free radicals [11]. Among the tested concentrations, AuNPs at 20 ppm demonstrate the highest free radical scavenging activity, with a resistance value of 77.9%.

Figure 8. Results of gold nanoparticles diluted to various concentrations

The antioxidant activity of a sample is determined by calculating the inhibition concentration value (IC_{50}), which can be derived from the percent attenuation data presented in Table 2. To obtain the IC_{50} value, a curve is plotted with the test sample concentration on the x-axis and the percentage of attenuation on the y-axis. A linear regression equation is then fitted to this data, resulting in the equation y = 2.1739x + 34.67 with an R^2 value of 0.9967, as shown in Figure 9.

The IC_{50} value represents the concentration of a sample required to inhibit or reduce free radical activity by 50%. A lower IC_{50} value indicates better antioxidant activity, as it means that a smaller concentration of the sample is needed to achieve a 50% reduction in DPPH free radicals [18]. The IC_{50} value is derived from the linear regression equation and can be calculated using Equation (3) [22].

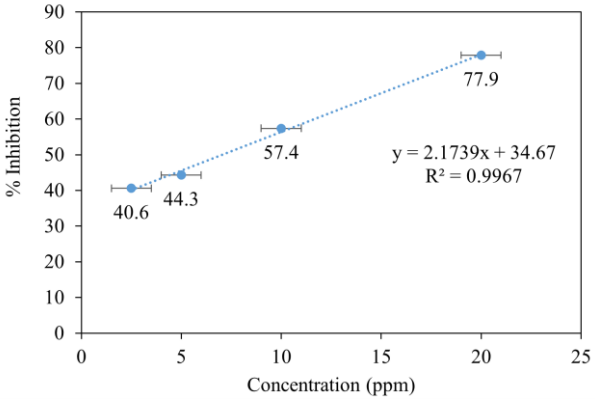

Figure 9. Graph of percent attenuation of AuNPs at various concentrations

$$IC_{50} = \frac{(50-b)}{a} \quad (3)$$

The IC_{50} value obtained from synthesizing AuNPs using a bioreductant extract of *Basella alba* leaf, calculated using Equation (3), is 7.05 µg/mL. This IC_{50} value indicates that the AuNPs produced exhibit very strong antioxidant activity [22]. The linear regression equation yielded an R^2 value of 0.9967, which is close to 1, suggesting a strong correlation between the sample concentration and the percentage of free radical scavenging, thereby allowing for an accurate assessment [21].

4. Conclusion

The green synthesis of AuNPs using a bioreductant from *Basella alba* leaf extract produced a burgundy-colored colloid. Characterization with a UV-Vis spectrophotometer revealed a maximum wavelength of 543 nm, while TEM showed an average cluster diameter of 9.463 ± 2.273 nm. Antioxidant activity testing at concentrations of 2.5, 5, 10, and 20 ppm using the DPPH method resulted in percentages of 40.6%, 44.3%, 57.4%, and 77.9%, respectively. The IC_{50} value was 7.05 µg/mL, indicating very strong antioxidant activity. The *Basella alba* leaf extract has shown potential as a reducing agent in synthesizing AuNPs. These AuNPs could be further explored for health, biomedicine, cosmetics, and environmental applications.

Acknowledgment

Surabaya State University, FMIPA chemistry lecturers, and all parties who cannot be mentioned one by one participated in helping directly or indirectly in completing this journal.

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