Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

Green Synthesis of Gold Nanoparticles Using Moringa Oleifera Leaf Extract Bioreductor (*Moringa oleifera* L.) and Activity Test as Antioxidant

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Received: July 11, 2024. Accepted: August 19, 2024. Published: September 30, 2024

Abstract: Gold nanoparticles are inorganic metal materials with sizes ranging from 5-400 nm and are in the form of a suspension solution that undergoes a process of reducing particle size to nano form. This study aimed to determine the results of synthesizing gold nanoparticles (AuNPs) at a concentration of 20 ppm using a bottom-up approach with a green synthesis method characterized using a UV-Vis spectrophotometer and TEM and antioxidant activity test using DPPH. In this study, the synthesis of gold nanoparticles (AuNPs) with a concentration of 20 ppm was carried out using a bottom-up approach with the green synthesis method. The parent solution of $HAuCl_4$ was reduced with Moringa oleifera leaf extract (*Moringa oleifera* L.) bioreductor, which produced burgundy-colored gold nanoparticles. The results of the characterization of gold nanoparticles using a UV-Vis spectrophotometer obtained the previous wavelength of $HAuCl_4$ of 309 nm. After being synthesized using moringa leaf extract bioreductor (Moringa oleifera L.), the maximum wavelength was shifted to 542.80 nm, with an absorbance value of 0.213. The characterization results using TEM obtained a diverse cluster size of gold nanoparticles with an average length of 6.635 and still in the nanometer size range with the highest frequency at 4-5 nm. The antioxidant activity test of gold nanoparticles was carried out at a concentration variation of 2.5, 5, 10, and 20 ppm, obtaining the percent of free radical suppression sequentially 69, 77, 76, and 83% with an IC_{50} value <50, which is 0.11 ppm which indicates that the antioxidant activity of gold nanoparticles is extreme. It can be concluded that gold nanoparticles synthesized using Moringa leaf extract have the potential to be a good antioxidant.

Keywords: Antioxidant Activity; Gold Nanoparticles; Green Synthesis; Moringa Leaf Extract.

Introduction

In recent decades, the development of nanotechnology has contributed significantly to many applications of nanoscale technology (nanoparticles) in various fields such as the environment, agriculture, food, biotechnology, biomedicine, drug delivery systems, optics, electronics, and the chemical industry [1]. Nanoparticles are particles that have a diameter between 10-9 m (1 nm) to less than 100 nm (1 µm) and have unique optically active properties compared to macro sizes [2]. Most of the applications of inorganic materials in the form of nanoparticles are materials derived from metals, including Au (gold), Cu (copper), Ti (Titanium), Zn (zinc), and Ag (silver) [2]. The most researched metal is gold (Au) [3].

Gold nanoparticles are inorganic metal materials in a suspension solution that reduces the particle size to nano form with sizes ranging from 5-400 nm [4]. Synthesizing nanoparticles can be done with two approaches: top-down approaches, such as physical methods, and bottom-up ones, such as chemical and biological methods. Physical and chemical methods in this synthesis process have limitations, such as harsh chemicals that pose environmental risks due to harmful solvents or additives, so nanoparticle researchers have turned to biological synthesis for more comprehensive application [5].

The process of biologically synthesizing gold nanoparticles is known as green synthesis. Green synthesis has various advantages, including simplicity, one-step nature, environmental friendliness, cost-effectiveness, biocompatibility, and the need for external stabilizers because biogenic components of plants and microorganisms serve as stabilizers or limiters [5]. One of the plants that can be used as a bioreductor is Moringa leaf extract (Moringa oleifera, L.) because it has active compounds that have potential as reducing agents [6]. The active compounds of Moringa leaves are various types of vitamins (A, C, E, K, B1, B2, B3, B6), flavonoids, alkaloids, saponins, tannins, and terpenoids [7]. The main flavonoid found in Moringa oleifera L. is quercetin. The active compounds in Moringa leaves are readily soluble in water, so an effective way to withdraw these compounds is to use the infusion method. This is by research conducted by Rahayu & Suharti (2021) that moringa leaf extract can be obtained by the infusa method, which is the process of extracting vegetable simplisia with water, which is heated for 10-15 minutes at 90°C.

There are two steps in the biosynthesis process of gold nanoparticles; first, Active groups derived from natural materials such as flavonoids function to reduce the charged Au metal (Au^{1+} (aurous) or Au^{3+} (aurat)) to the size of gold nanoparticles (Au^0) [8]. The second step is growth and stabilization, which results in the formation of AuNPs with a change in the color of the solution to burgundy [8]. The way to know that a particle is successfully synthesized is by characterizing it using a UV-Vis spectrophotometer and Transmission Electron Microscopy (TEM).

How to Cite:
Indriyani, R., & Taufikurohmah, T. (2024). Green Synthesis of Gold Nanoparticles Using Moringa Oleifera Leaf Extract Bioreductor (Moringa oleifera L.) and Activity Test as Antioxidant. *Jurnal Pijar Mipa*, *19*(5), 881–887. https://doi.org/10.29303/jpm.v19i5.7325

Gold nanoparticles have antioxidant activity that is very strong, durable, and effective in reducing free radicals, so the drug's efficacy will increase, and the healing process will also be faster [9]. Percent free radical suppression by gold nanoparticles can be done through an antioxidant activity test with UV-Vis spectrophotometric method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [10]. The process was chosen because it is simple, easy, and uses a small sample quickly. The antioxidant ability will increase as the number of compounds is reduced [10].

Based on the description, the urgency of this research is to find out how to synthesize gold nanoparticles (AuNPs) using the green synthesis method from 20 ppm $HAuCl_4$ using moringa leaf extract (Moringa oleifera L.) as a reductant which offers an environmentally friendly alternative compared to conventional synthesis methods that often involve toxic chemicals. In addition, the antioxidant activity test of gold nanoparticles was carried out with concentration variations of 20, 10, 5, and 2.5 ppm using DPPH to determine the optimum concentration in reducing free radicals and IC_{50} value. This study contributes to the development of new science and technology in nanotechnology and medical applications; namely, it can open up innovations in manufacturing medicines and other health products.

Research Methods

Materials

The materials used in this study were 1000 ppm $HAuCl_4$ solution, Moringa leaves (*Moringa oleifera* L.), distilled water, filter paper, 96% ethanol solution, and DPPH powder**.**

Method

Sample Preparation

The sampling process is carried out in the morning, and fresh green fruit is selected in good condition without any hollows or decay in certain areas. Then, put into a container to be washed to remove dirt, dust, and other plant parts. Next, the moringa leaves that have been cleaned are drained so that the water that is still attached to the fruit is completely gone. Next, the dried moringa leaves were weighed as much as 75 grams.

Extraction of Moringa oleifera Leaf

Moringa leaf extract is obtained from extraction using the infusion method. Prepared moringa leaves; as much as 75 grams were mashed using a blender/cooper. Then, put into a 250 mL Erlenmeyer with 100 mL of distilled water added (sample ratio: distilled water is 3: 4). Next, prepare a water bath to heat distilled water with a 1000 mL beaker on a hot plate. Delicate moringa leaves were heated at 90°C for up to 15 minutes with occasional stirring. Then, it is filtered using filter paper to separate the filtrate and residue so that the brownish-yellow moringa leaf extract is obtained. The process of this method is supported by the research of Rahayu & Suharti (2021), that moringa leaf extract can be obtained by the infusion method, namely the process of extracting vegetable simplisia with water which is heated for 10-15 minutes at 90°C.

Synthesis of Gold Nanoparticles Using Moringa oleifera Leaf Extract Bioreductor

1000 ppm $HAuCl_4$ solution was measured as much as 2 mL using a measuring cup and then put into a 100 mL volumetric flask. Then, it was added to distilled water as a colourless solution until the limit mark and shaken until homogeneous. The solution was put into a 250 mL beaker, adding moringa leaf extract as much as 1 mL, and heated at 90°C using a magnetic stirrer at 500 rpm until there was a change in colour to burgundy. And finally cooled to room temperature [11].

Characterisation of Gold Nanoparticles

The results of green synthesis of 20 ppm gold nanoparticles were characterized using a Shimadzu UV-1800 UV-Vis spectrophotometer instrument by inserting them into a cuvette and then measuring the maximum wavelength value and absorbance using UV-Vis spectrophotometry in the range of 200-800 nm.

And characterized using transmission electron microscopy (TEM) to determine the diameter of the gold nanoparticles formed. The first step is to prepare a TEM grid cleaned with organic solvents such as acetone or ethanol. Then, a small sample of the synthesized gold nanoparticles using a moringa leaf extract bioreductor (Moringa oleifera L.) was dried on the TEM grid. Then, it was observed using TEM at the appropriate magnification to see the structure and size of the gold nanoparticles formed. Furthermore, characterize the gold nanoparticles formed, such as determining the size and shape of the particles.

Antioxidant Activity Test of Gold Nanoparticles Using DPPH

This antioxidant activity test was conducted using a UV-Vis spectrophotometer instrument. The first step in making 0.003% DPPH solution is to weigh DPPH powder in black powder, as much as 3 mg or 0.003 grams, using an analytical balance. Then, put into a measuring flask measuring 100 mL, coated with aluminum foil. Then, it was added with ethanol p.a. solution as a colorless solution until the limit mark and shaken until homogeneous. DPPH solution will change color to dark purple and be left for 30 minutes in a dark place. After that, the DPPH solution was measured using a UV-Vis spectrophotometer at a wavelength of 400-600 nm so that the maximum λ DPPH data obtained will be used to measure the absorbance of the sample [12].

The next step was to dilute the solution of synthesized gold nanoparticles using moringa leaf extract bioreductor with a concentration of 20 ppm to 10; 5; 2.5 ppm. The synthesized solution with a concentration of 20 ppm as a burgundy solution was measured as much as 50 mL and put into a 100 mL volumetric flask. Then, it was added to distilled water until the limit mark and shaken until homogeneous to obtain a solution of synthesized gold nanoparticles with moringa leaf extract bioreductor with a concentration of 10 ppm. A solution of gold nanoparticles synthesized with moringa leaf extract bioreductor

concentrations of 5 ppm and 2.5 ppm was obtained using the same procedure.

Each 2.5, 5, 10, and 20 ppm gold nanoparticle sample was measured as much as 2 mL using a measuring cup and put into a test tube coated with aluminum foil. Then, each test tube containing gold nanoparticle samples was added with 0.003% DPPH solution in as much as 2 mL. Then homogenized and allowed to stand for 30 minutes in a dark room. After that, the sample was put into a cuvette. Then, the absorbance value of the sample was measured using a UV-Vis spectrophotometer instrument at λ max DPPH. The absorbance value was recorded, and the % free radical suppression was calculated [13].

Results and Discussion

This study was conducted to determine how to synthesize gold nanoparticles (AuNPs) with a concentration of 20 ppm through a bottom-up approach with the green synthesis method from $HAuCl_4$ as a precursor and using moringa leaf extract bioreductor (Moringa oleifera L.). The green synthesis results were characterized by a UV-Vis spectrophotometer and Transmission Electron Microscope (TEM) to determine whether gold nanoparticles have been formed, which can be known from the maximum wavelength and cluster size of AuNPs. In addition, the purpose of this study was to determine the results of the antioxidant activity test (IC_{50} value) of gold nanoparticles with concentration variations of 20, 10, 5, and 2.5 ppm using artificial free radicals, namely 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Extraction of Moringa oleifera Leaf

Indonesia has a diverse wealth of biological natural resources, which can be an opportunity for research on using plants as reducing agents in the green synthesis of nanoparticles. The green synthesis process of gold nanoparticles was chosen because it is more environmentally friendly, non-toxic, simple, and requires less time than chemical synthesis [5]. The bioreductor used in this study is Moringa leaf extract (Moringa oleifera, L.) because it has active compounds that have the potential as reducing agents. According to Oktavia & Sutoyo (2021), active compounds in plant extracts such as flavonoids, phenolics, tannins, and others can act as bioreductors and capping agents that can reduce Au^{3+} ions into gold nanoparticles (Au^0). Active compounds in moringa plants are most commonly found in the leaves [6].

Moringa leaves contain flavonoid compounds, namely quercetin, which is the most abundant compared to other types of flavonoids [14]. The main content in moringa leaves that plays a significant role in the reduction process is flavonoids (quercetin) because it has a greater concentration of 384.61 mg/100 g [15]. Quercetin is found in dried MO leaves, at concentrations of 100 mg/100 g, as quercetin-3-O-β-d-glucoside (iso-quercetin or isotrifolin) [16]. From the research of Pakade, Cukrowska, and Chimuka (2013), Moringa leaves had higher concentrations of quercetin (1362.6 mg/kg), so that moringa has high nutritional value and is safe for consumption [17].

Extraction of Moringa leaves in this study was obtained through the infusion method, namely by blending 75 grams of fresh Moringa leaves until smooth and then extracting vegetable simplistic using 100 mL of distilled water by heating 90°C for 10-15 minutes on a hotplate while occasionally stirring [7]. Then, it was filtered using filter paper to obtain yellow moringa leaf extract, as shown in Figure 1. The infusion method is used for soft materials that are resistant to heating using distilled water as a solvent. In addition, the effectiveness of extraction depends on how soluble the compound is in the solvent according to the solubility principle, namely, ***like dissolved,*** meaning that the compounds dissolved in the solvent have the same polarity [18].

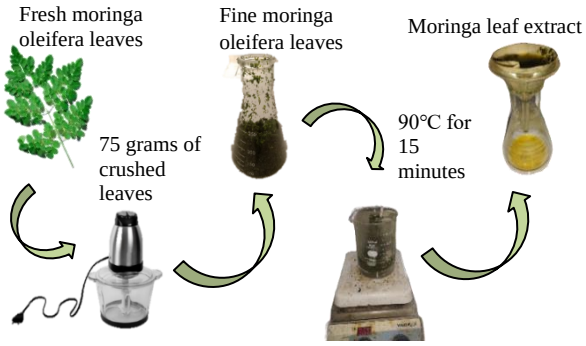

Figure 1. Extraction of Moringa oleifera Leaf

Synthesis of Gold Nanoparticles Using Moringa oleifera Leaf Extract Bioreductor

The synthesis of gold nanoparticles at a concentration of 20 ppm was carried out through a bottom-up approach namely the nanoparticle synthesis process begins with small particles (Au^{3+} from $HAuCl_4$ molecules) formed into large particles, namely gold nanoparticles [19]. The yellow-colored $HAuCl_4$ solution was tested using a UV-Vis spectrophotometer in the 200-800 nm wavelength range. In this study, the maximum wavelength of $HAuCl_4$ was obtained at 309 nm with an absorbance value of 4.000, which was recorded as the initial wavelength of $HAuCl_4$ before the formation of gold nanoparticles (AuNPs).

Synthesis of gold nanoparticles at a concentration of 20 ppm was carried out using a 1000 ppm $HAuCl_4$ solution of 2 mL diluted in a 100 mL volumetric flask using distilled water and then shaken until homogeneous. The solution was put into a 250 mL beaker and added Moringa leaf extract as much as 1 mL and then heated at 90ºC on a magnetic stirrer hotplate at a speed of 500 rpm until the colour changed to burgundy and then cooled at room temperature as shown in Figure 2. The increase in temperature causes collisions between particles to accelerate, causing aggregation and particle size to increase [20].

The color change in the synthesis process shows that the longer the resulting cluster grows, the more significant it is. The process of color change when starting with a solution of gold (Au) has a clear yellow color turns into a colorless solution, which indicates that gold atoms have not interacted with each other, then turns into a dark blue solution, which suggests that slowly the cluster in a certain amount interacts with each other, continued to be dark red. When the cluster reaches nanometer size, the solution changes to a stable burgundy [19], as shown in Figure 3.

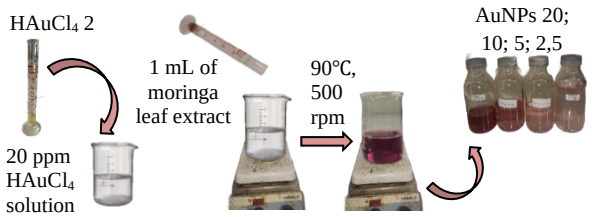

Figure 2. Green synthesis of gold nanoparticles

Figure 3. Gold nanoparticles 20 ppm

This study uses Moringa oleifera leaf extract as a reductant and stabilizer for forming gold nanoparticles (AuNPs). The active group derived from quercetin in moringa leaves reduces the charged Au metal Au^{3+} (aurat) to the size of gold nanoparticles (Au^0). Gold nanoparticles occur due to electron transfer from the reducing agent to Au metal ions [21].

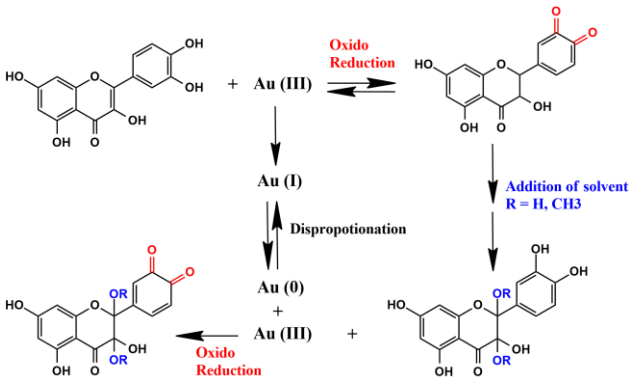

Figure 4. Reaction Mechanism of Gold Nanoparticle Formation with Flavonoid Compound (Quercetin)

Figure 4 explains that what is reduced in the reaction mixture is Au(III) ions, while Au(I) is not proportioned back into Au(III) and Au(0). The newly generated Au(III) ion further oxidizes the additional three ′,4′-dihydroxyquercetin group obtained after the first $2e^-$ oxidation, giving the final reaction product.

Characterisation of Gold Nanoparticles

Characterisation Using UV-Vis Spectrophotometer

The results of green synthesis of gold nanoparticles with a concentration of 20 ppm were then characterized using a Shimadzu 1800 UV-Vis spectrophotometer by reading the absorption at a 200-800 nm wavelength. An indicator of the success of gold nanoparticle formation is looking at the shift in the maximum wavelength of $HAuCl_4$ [22]. This research succeeded in synthesizing gold nanoparticles because the $HAuCl_4$ wavelength previously obtained was 309 nm. After being synthesized using a reductant from Moringa oleifera L. leaf extract, the maximum wavelength shifted to 542.80 nm with an absorbance value of 0.213, as seen in Figure 5. The results of this research are based on the theory that gold nanoparticles have a wavelength ranging from 500-600 nm [23].

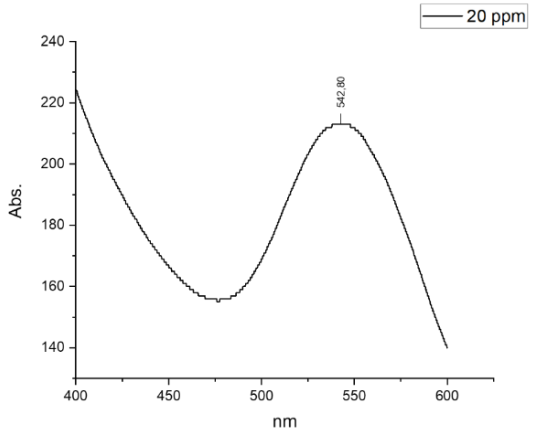

Figure 5. UV-Vis Spectrophotometer Results of 20 ppm Gold Nanoparticles

The SPR (Surface Plasmon Resonance) theory states that the size of the nanoparticle is correlated with the maximum wavelength, meaning that the larger the nanoparticle size, the longer the wavelength is produced. This is because the further the excited electron is from the ground state, the smaller the excitation energy [22].

Characterisation Using Transmission Electron Microscope (TEM)

The 20 ppm gold nanoparticles from green synthesis were characterized using TEM JEOL JEM-1400 at the Chemistry Laboratory, Gajah Mada University, to determine the diameter of gold nanoparticles. TEM has the advantage of clearly observing particles with a size of several nanometres due to its very high resolution (high-resolution TEM) [24]. The cluster size of 20 ppm gold nanoparticles with a scale of 20 nm or magnification of 80000 and 150000 varies significantly, as shown in **Figure 6**.

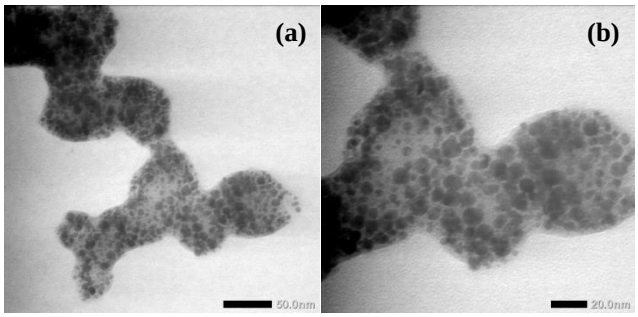

Figure 6. Characterization of 20 ppm Gold Nanoparticles Using TEM (a) Magnification 80000 (b) Magnification 150000

TEM results were analyzed using ImageJ software, and then the data obtained was processed through OriginLab software by creating a histogram to determine the particle size distribution of 20 ppm gold nanoparticles, as shown in Figure 7.

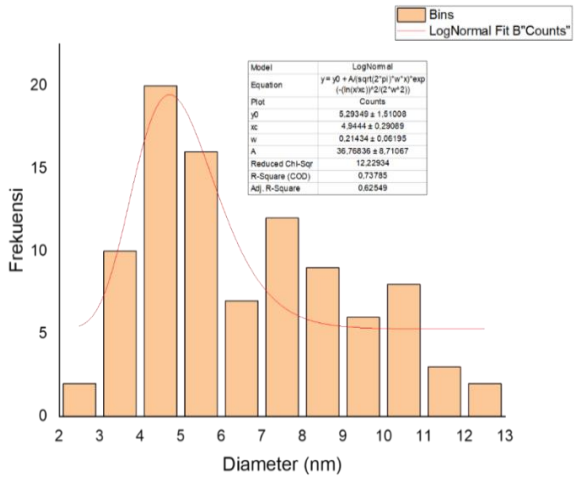

Figure 7. Histogram of Particle Distribution Pattern of Gold Nanoparticles

The histogram shows that the smallest cluster size is 2.542 nm, the most significant cluster size is 12.225 nm, and the average cluster size is 6.635 nm. The lowest frequency is at a size of 2-3 nm, and the highest frequency for gold nanoparticle cluster sizes is at a size of 4-5 nm. This shows that AuNPs have been successfully synthesized using the bioreductor Moringa oleifera L. leaf extract because, according to the theory, nanoparticles usually have a diameter of 1-100 nm, and the smaller the size, the higher the activity [25].

Antioxidant Activity Test of Gold Nanoparticles Using DPPH

This study tested the antioxidant activity of gold nanoparticles using artificial free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) with a concentration variation of 20, 10, 5, and 2.5 ppm to determine the optimum concentration in reducing free radicals as shown in Figure 8.

Figure 8. Gold Nanoparticles 20; 10; 5; and 2.5 ppm

Figure 8 shows a difference in colour intensity due to the closer distance between clusters and the greater diameter of the gold nanoparticle (AuNPs) colloidal cluster, which makes the color more intense as the concentration increases [21]. The process of determining the ability of gold nanoparticles (AuNPs) to reduce free radicals requires three absorbance measurements, namely determining the maximum wavelength (λ_{max}) of DPPH, measuring the absorbance of colloidal gold nanoparticles (AuNPs), and measuring colloidal gold nanoparticles (AuNPs) after adding DPPH [10]. Gold nanoparticles with varying concentrations have absorption values at different wavelengths, as shown in Table 1.

Table 1. Maximum Wavelength Data and Absorbance of Gold Nanoparticles of Various Concentrations

Concentration of Gold Nanoparticles (AuNPs) (ppm)	Maximum wavelength (nm)	Absorbance
2.5	541.80	0.031
5	545.80	0.057
10	541.80	0.112
20	542.80	0.213

This table shows that at each concentration of gold nanoparticles (AuNPs), the maximum wavelength is obtained in the range of 541-545, and the higher concentration of gold nanoparticles produces a more excellent absorption value. This statement proves that more gold nanoparticles (AuNPs) are produced [26].

This study measured DPPH using a UV-Vis spectrophotometer at 400-600 nm to obtain an absorbance value of 1.047 at a maximum wavelength of 517 nm. Antioxidant activity testing using DPPH was chosen because it is fast, cheaper, simple, and only requires a small sample [27]. Gold nanoparticles with various concentrations have different wavelengths and absorbance values, so to determine the percentage of free radical silencing, the absorbance value of gold nanoparticles must be measured at λmax DPPH, which is 517 nm, as in Table 2.

Table 2. Absorbance Value of AuNPs of Various Concentrations at λ 517 nm

Concentration of Gold Nanoparticles (AuNPs) (ppm)	Absorbance at λ 517 nm
2.5	0.029
5	0.050
10	0.101
20	0.193

The absorbance value will decrease when DPPH reacts with free radical scavenging compounds. The mechanism of suppression of DPPH free radicals by AuNPs is shown in Figure 9. The mechanism of suppressing DPPH free radicals by AuNPs by bonding between Au atoms with N atoms so that the N atoms in DPPH will become stable. Free electron pairs (PEB) owned by N atoms are given to Au atoms to form Au-N coordination covalent bonds. This indicates that the Au atom has stabilized the N atom and suggests that the Au atom can reduce DPPH free radicals.

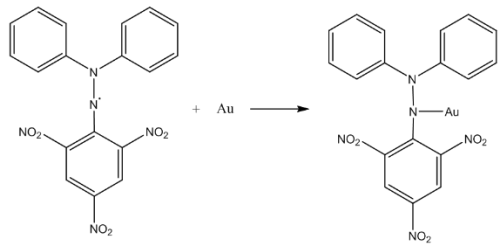

Figure 9. DPPH Inhibition Reaction by AuNPs

Free radical capture activity can be expressed in units of percent (%) of free radical suppression, which is written as follows [27] :

$$\frac{(\text{Initial DPPH absorbance} - \text{DPPH absorbance})}{\text{Initial DPPH absorbance}} x\ 100\%$$

The data obtained from the calculation of the percent of free radical silencing with various concentrations of gold nanoparticles is shown in Table 2. This table shows that the greater the concentration of gold nanoparticles, the greater the percentage of suppression [10].

Table 2. Percent Free Radical Reduction by Gold Nanoparticles of Various Concentrations

Conc. of AuNPs (ppm)	Abs.of AuNPs + DPPH at λ517 nm (A)	Abs.of AuNPs at λ517 nm (B)	A-B	% Inhibition
2.5	0.358	0.029	0.329	69%
5	0.290	0.050	0.240	77%
10	0.352	0.101	0.251	76%
20	0.367	0.193	0.174	83%

A linear regression equation can determine the IC50 value for each concentration of gold nanoparticles, y = ax + b. The equation is obtained from the curve between the concentration of the solution (located on the x-axis) and the % free radical suppression (located on the y-axis), as in Figure 9 [28]. From the equation y = ax + b, the IC_{50} value can be calculated using the following formula [29] : $IC_{50} = \frac{(50-b)}{a}$

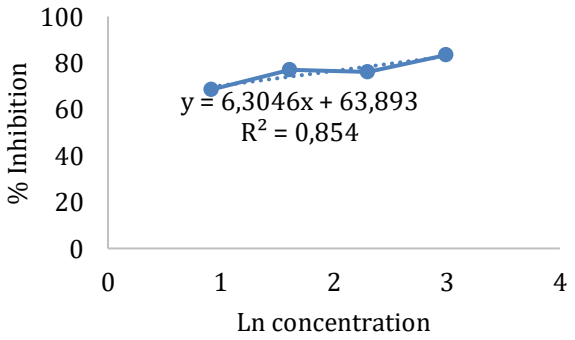

Figure 9. Inhibition curve

In the curve drawing, the regression equation y = 6.3046x + 63.893 was obtained with a value of R^2 = 0.854. The regression curve data shows a good correlation between sample concentration and % inhibition, as indicated by the R2 value (correlation coefficient), which is almost close to +1 (positive value). In this study, the resulting IC_{50} value is 0.11 ppm. Hoshyar et al. reported the one-step synthesis of AuNPs using crocin as a reducing agent. Crocin was obtained from the saffron stigma, which contains many active phytocompounds. The IC_{50} values of crocin-AuNPs were 1.8 mg/mL ± 0.08 and 1.2 mg/mL ± 0.04 after incubating for 24 and 48 h, respectively [30]. In a study conducted by Meliani (2021), gold nanoparticles from the biosynthesis of clove flower water extract (Syzygium aromaticum (L.) Merr. & L.M.Perry) also produced an IC_{50} value in the <50 ppm range, namely 26.28 ppm [31].

So this indicates that gold nanoparticles synthesized using moringa leaf extract (*Moringa oleifera* L.) bioreductor are classified as powerful antioxidants because they are in the range of <50 ppm [32]. From the results of this study, gold nanoparticles are very suitable as antioxidants to counteract free radicals because they have very strong antioxidant activity.

Conclusion

Based on the research results, the following conclusions can be obtained from gold nanoparticles from green synthesis using moringa leaf extract (*Moringa oleifera* L.) bioreductor have a burgundy colour, and the maximum wavelength is 542.80 nm with an absorbance value of 0.213 and an average cluster diameter size of 6.635 nm. Gold nanoparticles' antioxidant activity test results at concentrations of 20, 10, 5, and 2.5 ppm were 69, 77, 76, and 83%, respectively. This proves that the higher the concentration of gold nanoparticles (AuNPs), the greater the percentage of silencing because gold nanoparticles silence the more DPPH. This study obtained the IC50 value of 0.11 ppm, which is classified as a powerful antioxidant because it is in the range of <50 ppm.

Acknowledgements

First, I thank Surabaya State University, especially the Department of Chemistry and Faculty of Mathematics and Natural Sciences, for providing opportunities and support in this research. I would also like to thank the gold nanoparticle team for providing support during this research process.

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