

Green Synthesis Gold Nanoparticles using Bioreductant Red Shoot Leaf Extract (*Syzygium myrtifolium* Walp.) and Activity as Antioxidant

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Abstract: Gold nanoparticles (AuNPs) are small-sized materials important in various commercial and industrial applications. Several methods have been developed to synthesise AuNPs. This study was conducted by synthesizing AuNPs using HAuCl₄ as a precursor and red shoot leaf extract as a bioreductor. The concentrations of AuNPs used were 20, 10, 5, and 2.5 ppm. Characterization of gold nanoparticles at a concentration of 20 ppm was carried out using a UV-Vis spectrophotometer to measure the maximum wavelength and Transmission Electron Microscopy (TEM) to determine particle size. Antioxidant activity at 20, 10, 5, and 2.5 ppm was determined by measuring free radical capture activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The results showed that gold nanoparticles had a maximum wavelength of 530 nm with a ruby red color and an average particle size of 14.907 nm. The synthesized AuNPs showed high antioxidant activity: 99.6% (20 ppm), 98.9% (10 ppm), 96.7% (5 ppm), and 91.0% (2.5 ppm), indicating that higher concentrations of AuNPs resulted in more significant free radical scavenging. This study successfully synthesized gold nanoparticles using red shoot leaf extract as an environmentally friendly bioreduction. The resulting nanoparticles have a nanometer particle size with very high antioxidant activity, especially at a concentration of 20 ppm. The method used in this study offers a more environmentally friendly alternative for synthesizing gold nanoparticles, which previously often used hazardous chemicals. The use of red shoot leaf extract as a bioreductor has not been widely reported in the literature, thus providing a new contribution to green nanotechnology. Further research is recommended to explore the potential applications of gold nanoparticles synthesized by this method in medical and other industrial fields. In addition, additional studies are needed to optimize the synthesis conditions and more in-depth characterization of the stability and toxicity of gold nanoparticles in practical applications.

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

Introduction

Nanotechnology has revolutionized various industrial applications and has seen exponential growth over the past five decades [1]. This field, encompassing the design, development, and implementation of components sized between 1-100 nm [2], holds significant promise across numerous sectors such as environmental science, agriculture, food technology, biotechnology, biomedicine, and pharmaceuticals [3]. Among the various metallic nanoparticles, gold nanoparticles (AuNPs) have garnered substantial attention due to their ease of synthesis [4] and multifaceted benefits in medical applications, including the treatment of diabetes mellitus, cancer, cardiovascular diseases, tuberculosis, and the mitigation of antibiotic resistance [5]. Moreover, AuNPs exhibit robust and enduring antioxidant properties, making them effective agents against free radicals and Reactive Oxygen Species (ROS), which are implicated in various degenerative diseases [6].

Degenerative diseases caused by oxidative stress and free radicals pose a major global health challenge, with projected increases in mortality rates, as the World Health Organization (WHO) indicates that deaths caused by degenerative diseases will continue to increase. Overall, it is estimated that 52 million people will die each year by 2030, an increase of 14 million people from 38 million people this year. More than two-thirds (70%) of the world's population

will die from degenerative diseases. According to the 2018 Riskesdas data, the rate of degenerative diseases in Indonesia reached 65.7%. Overcoming this health problem requires the development of effective antioxidant therapy [6].

Gold nanoparticles are usually synthesized through chemical reactions. Two methods can be used for this: top-down, known as the physical approach, and bottom-up, known as the chemical approach [7]. Although effective, these methods often involve high costs, labour-intensive processes, and potential environmental and biological hazards [8]. In recent years, green synthesis has emerged as a sustainable alternative, utilizing biological entities such as plants for environmentally friendly production of nanoparticles [9].

Red shoot (*Syzygium myrtifolium* Walp.) was identified as a promising bioreductant for the green synthesis of gold nanoparticles. The plant is rich in secondary metabolites, including triterpenoids, steroids, saponins, flavonoids, phenolics, and anthocyanins, which facilitate the reduction of Au⁺ ions to Au⁰ and also act as capping agents to stabilize the nanoparticles [10]. Although red shoots are new in nanoparticle synthesis, previous studies have demonstrated the potential of plants containing similar secondary metabolites [11].

This research explores the green synthesis of gold nanoparticles using red shoot extract using an infusion method for extraction, which is economical, simple and

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minimizes thermal degradation of the active compound [12]. The synthesized nanoparticles will be characterized using UV-Vis spectroscopy and Transmission Electron Microscopy (TEM) to determine their optical properties and size distribution. Additionally, the antioxidant activity of the nanoparticles will be assessed through their interactions with the synthetic free radical DPPH, providing insight into their potential efficacy as therapeutic antioxidants. This research aims to increase the usefulness of red shoots in nanoparticle synthesis and contribute valuable information regarding their antioxidant properties.

Research Methods

Materials

The materials used in this research were the leaves of the red shoot plant (*Syzygium myrtifolium* Walp.), aquadest, 1000 ppm H₂AuCl₄ stock solution, filter paper, 96% ethanol solution, and 1,1-diphenyl-2-picrylhydryl (DPPH) powder.

Extraction of red shoot leaves

Extraction of red shoot leaves was carried out using the infusion method [13]. The red shoot leaves that have been prepared are ground using a blender/copper and put into a 250 mL Erlenmeyer flask with 100 mL of distilled water added (the ratio of sample: distilled water is 3:4). The extraction process was carried out by heating an Erlenmeyer flask containing red shoot leaves using a water bath for 15 minutes, stirring occasionally [14], [15]. Filter using filter paper separates the filtrate and residue to obtain the red shoot leaf extract filtrate. The extraction process for red shoot leaves is shown in Figure 1a.

Synthesis of Gold Nanoparticles with Red Shoot Leaf Extract as a bioreductant

The 1000 ppm H₂AuCl₄ solution was measured in 2 mL and put into a 100 mL volumetric flask. Distilled water as a colorless solution was added to the mark and shaken until homogeneous. The homogenized solution was put into a 250 mL beaker, and 2 mL of red shoot leaf extract was added. Homogenize with a magnetic stirrer at a speed of 300 rpm until the color changes to ruby red [16]. The synthesis of gold nanoparticles is shown in Figure 1b.

Characterization

Gold nanoparticles (20 ppm) were characterized using a UV-Vis spectrophotometer in the range of 200-800 nm to see the maximum wavelength [17], and Transmission Electron Microscopy (TEM) to determine the size of the gold nanoparticles [18].

Antioxidant Activity Test of Gold Nanomaterials Using DPPH

The antioxidant activity test was carried out using a UV-Vis spectrophotometer. The gold nanoparticle solution (20 ppm) was diluted to 10, 5, and 2.5 ppm. Each sample (2 mL) was mixed with 2 mL of 0.003% DPPH solution [19], homogenized, and left for 30 minutes in the dark [20].

Absorbance was measured at λ max DPPH to calculate the percent scavenging of free radicals [21].

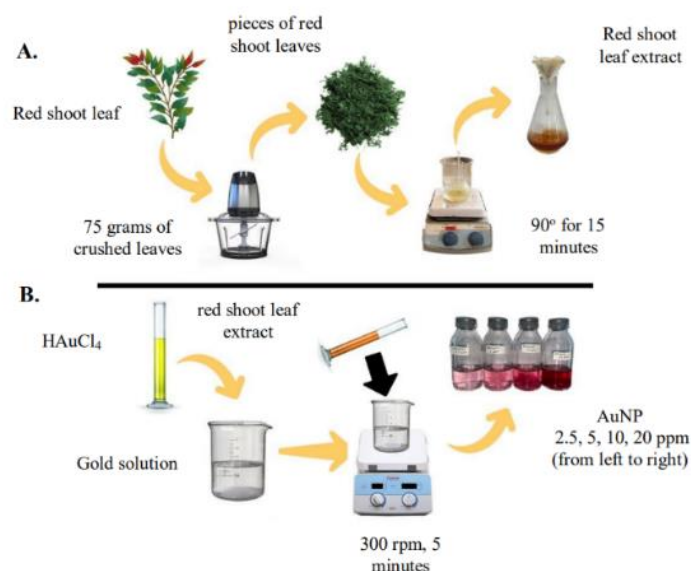


Figure 1. (a) Extraction of red shoot leaf, (b) Synthesis of AuNP using red shoot leaf extract

Results and Discussion

Extraction of red shoot leaves

Red shoot leaves are extracted using the infusion method. Rich in active compounds such as triterpenoids, steroids, saponins, flavonoids, and phenolics [22]. This method was chosen because it is faster and reduces the risk of damage to active compounds due to excessive heating [23]. In addition, the infusion method is more cost-effective and easy to apply to complex plant parts such as leaves and bark [12].

The extraction process includes cleaning fresh red shoot leaves, grinding, and mixing with distilled water as a polar solvent [24]. This mixture was heated at 90°C for 15 minutes to ensure efficient extraction without damaging the active compounds [25]. After heating, the mixture is filtered to separate the filtrate and residue to obtain red shoot leaf extract, which is then used as a bioreduction agent to synthesise AuNPs.

Synthesis of gold nanoparticles

This research synthesized gold nanoparticles using a bottom-up synthesis method that utilized 1000 ppm H₂AuCl₄ stock solution, red shoot leaf extract, and distilled water [5]. The H₂AuCl₄ solution is produced from 1 gram of gold dissolved in aqua regia and diluted to a 1000 ppm solution. Gold dissolution is done by heating to evaporate the gas from the by-product [12]. Red shoot leaf extract rich in flavonoid compounds is used as a reducing agent, converting gold ions (Au³⁺) into gold nanoparticles (Au⁰) [22]. This process is explained in detail in Figure 2a, showing the oxidation-reduction reaction of Au³⁺ ions with flavonoid compounds in red shoot leaf extract. The synthesis process was carried out at room temperature because the secondary metabolite compounds in red shoot leaves are active at this temperature, encouraging the formation of gold nanoparticles.

The reaction mechanism for forming gold nanoparticles using flavonoid compounds is also explained through reduction, oxidation, and disproportionation reactions, which trigger the formation of nanometer-sized gold nanoparticles [26]. The growth process of gold nanoparticles consists of three phases: activation, growth, and termination. The activation phase involves the reduction of gold ions to Au⁰, while the growth phase allows small nanoparticles to combine into larger particles spontaneously. The termination phase produces gold nanoparticles with the

desired size and structure. This is illustrated in Figure 2b due to the synthesis of gold nanoparticles with a concentration of 20 ppm [26].

Overall, the gold nanoparticle synthesis process using red shoot leaf extract as a reducing agent clearly shows gold nanoparticles' reaction mechanism and growth and potential applications in various scientific fields such as biomedicine and catalysis.

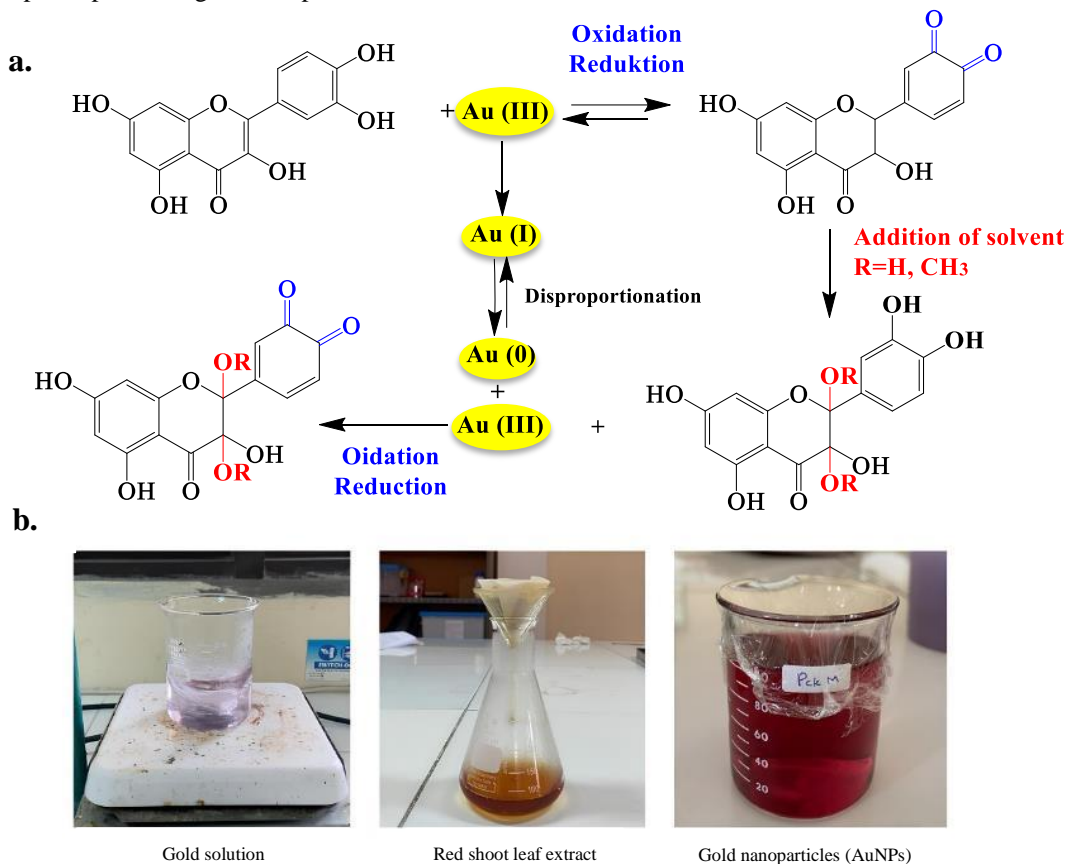


Figure 2. (a) The reaction mechanism for the formation of gold nanoparticles uses flavonoid compounds (b) Gold solution, Red shoot leaf extract, Gold nanoparticles

Characterization of Nanoparticles

The results of the characterization of gold nanoparticles at a concentration of 20 ppm using a Shimadzu 1800 UV-Vis Spectrophotometer show a maximum absorption wavelength at 530 nm with an absorbance of 0.244, as seen in Figure 3. The change in wavelength from 309 nm (the initial wavelength of HAuCl₄) to 530 nm indicates the formation of gold nanoparticles, which is an indicator of successful synthesis [27]. Other research by [28], [17] also supports this finding by showing a similar wavelength shift in the 500-600 nm range when the formation of gold nanoparticles occurs.

Apart from the shift in wavelength, the color change from pale yellow to ruby red is also an indicator of successful synthesis [29]. The increasingly intense color at higher concentrations indicates an increase in the density of gold nanoparticles, which can be explained by approaches based on cluster diameter and cluster concentration or density [30]. This can be seen in Figure 4, which illustrates the effect of cluster density on colloid color, where the color density is

more caused by the concentration or cluster density at a concentration of 20 ppm.

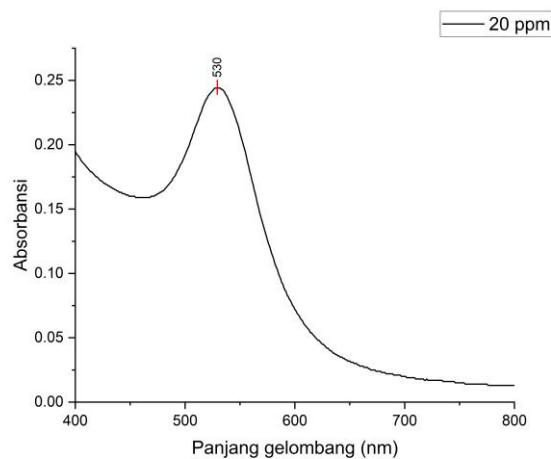


Figure 3. Gold nanoparticle absorption graph

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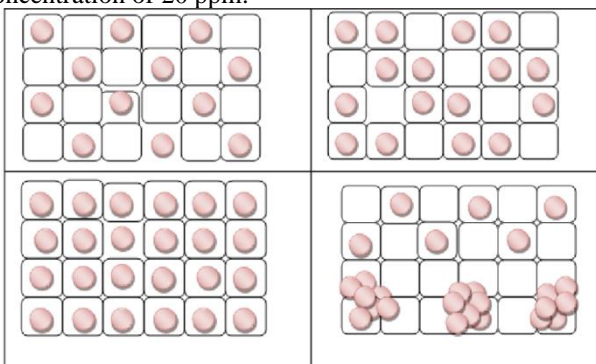


Figure 4. Illustration of the effect of cluster density on colloid color [31]

Characterization using TEM (Transmission Electron Microscopy) on a gold nanoparticle sample with a concentration of 20 ppm (Figure 5) shows that the smallest particle size is 5.89 nm, and the largest is 19,319 nm, with an average particle size of 14,907 nm. The particle size distribution was also analyzed via Origin Lab software, showing particle size variations between 8-22 nm, as shown in Figure 6.

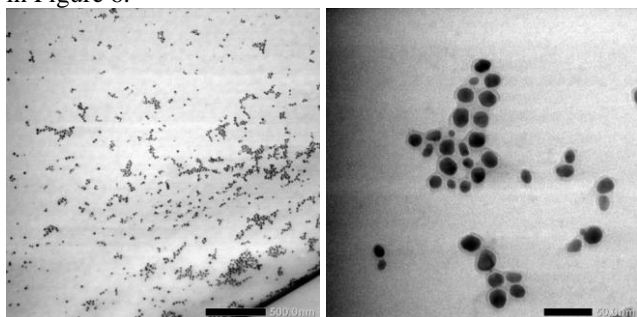


Figure 5. Characterization of 20 ppm gold nanoparticles using TEM (a) magnification 10000 (b) magnification 80000

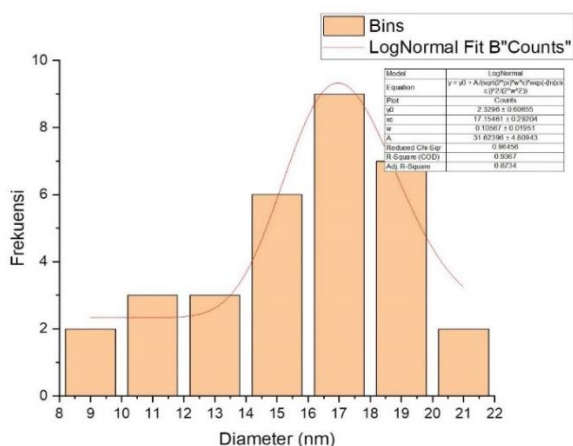


Figure 6. The results at 80,000 magnification were characterized using Image J software

Thus, characterization using a UV-Vis Spectrophotometer and TEM provides comprehensive information about the physical and optical properties of the synthesized gold nanoparticles at a concentration of 20 ppm.

Antioxidant Activity

In testing antioxidant activity, gold nanoparticles were diluted with distilled water to 20 ppm in a 100 mL volumetric flask, resulting in gold nanoparticle concentrations of 10, 5, and 2.5 ppm, as shown in Figure 7. This concentration variation is used to determine the Optimal antioxidant activity of gold nanoparticles synthesized using red shoot leaf extract bioreduction.



Figure 7. The results of the synthesis of gold nanoparticles at a concentration of 2.5; 5; 10; and 20 ppm

The synthesis results with concentrations of 20, 10, 5, and 2.5 ppm were tested for antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction method. DPPH is an artificial free radical used to test the effectiveness of antioxidant compounds [32]. The principle of reducing free radicals is that when antioxidants interact with free radicals, the color of the solution will change from dark purple to bright yellow [33]. The results of measuring the absorbance of samples at a DPPH wavelength of 517 nm are used to calculate the percent reduction of free radicals.

The test was carried out by preparing 2 mL of sample and 2 mL of DPPH solution, then placing it in a test tube covered with aluminium foil in a 1:1 ratio. The solution mixture was shaken until homogeneous and incubated for 30 minutes for interaction between DPPH and gold nanoparticles. The absorbance measurement results show the percent reduction of DPPH by gold nanoparticles, as recorded in Table 1.

Further analysis showed that the highest antioxidant activity was at a concentration of 20 ppm with a reduction percentage of 99.1%. This shows that red shoot leaf extract has the potential to be a gold nanoparticle bioreductant with high antioxidant activity. The relationship between sample concentration and percent attenuation shows a consistent increase, where the higher the concentration, the higher the percent attenuation of DPPH [24]. Things that can influence the percent attenuation value as a benchmark for determining the level of strength of antioxidant activity include measuring the test material, solubility of the test material, pipetting the sample and DPPH for incubation. The better the researcher's way of conducting this research, the better the R₂ value obtained from each linear regression, namely the line relationship between sample concentration and % inhibition, where a good R₂ value is an R₂ value that is almost 1. This shows a correlation between sample concentration and % inhibition [34].

Based on Figure 8, the linear equation $y = 3.8231x + 88.947$ is obtained with an R^2 regression of 0.8206, so the average IC_{50} value obtained is 0.0000699 $\mu\text{g/mL}$, which indicates it has very strong antioxidant activity. The IC_{50} value indicates antioxidant power that inhibits free

radicals by 50%. The results of the antioxidant activity test show that gold nanoparticles synthesized using red shoot leaf extract have high antioxidant activity, showing potential as an effective gold nanoparticle bioreductant.

Table 1. Data on the percent reduction of DPPH radicals by gold nanoparticles

Gold nanoparticle concentration (ppm)	DPPH Absorbance	Absorbance of gold nanoparticles + DPPH (A)	The absorbance of gold nanoparticles (B)	A-B	% Inhibition
2,5		0.083	0.046	0.037	91.0%
5	0.409	0.099	0.086	0.013	96.7%
10		0.137	0.132	0.005	98.9%
20		0.233	0.229	0.004	99.1%

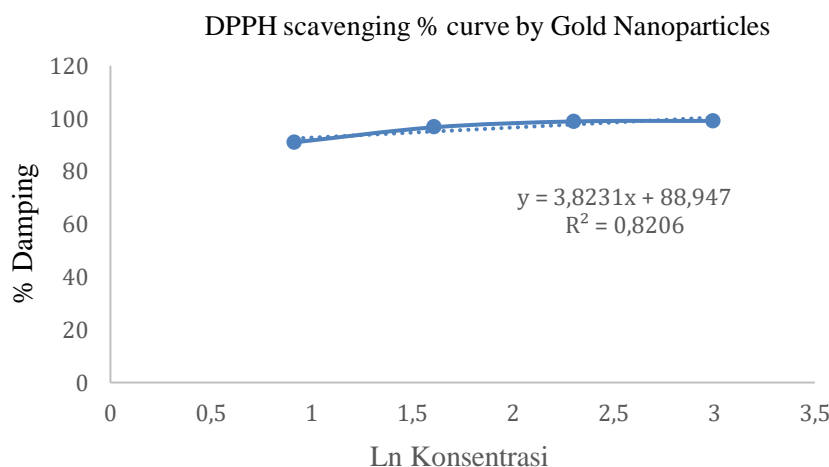


Figure 8. Percent DPPH reduction curve by gold nanoparticles

Conclusion

The results of green synthesis of gold nanoparticles using a bioreductant from red shoot leaf extract (*Syzygium myrtifolium* Walp.) are ruby red, with a maximum wavelength of 535 nm and an average particle size of 14,907 nm. The results of the antioxidant activity test show that gold nanoparticles synthesized using the bioreduction extract of red shoot leaves (*Syzygium myrtifolium* Walp.) have high antioxidant activity, as seen from the results of the percent reduction, namely 99.1% (20 ppm), 98.9% (10 ppm), 96.7 % (5 ppm), and 91.0 (2.5 ppm) this shows that the relationship between the sample concentration and the percent scavenging of free radicals increases from low concentration to high concentration, where the higher the concentration, the higher the percent scavenging of free radicals.

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