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

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

Green Synthesis and Stability Testing of Gold Nanoparticles Using a Bioreducer from Red Binahong Leaf Extract (*Anredera cordifolia* (Ten.) Steenis)

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Received: May 11, 2026. Accepted: July 17, 2026. Published: October 1, 2026

Abstract: Gold nanoparticles are widely used in various fields due to their unique properties. However, the application of gold nanoparticles is greatly influenced by their stability. One factor affecting nanoparticle stability is the use of bioreducers. The synthesis of gold nanoparticles still largely relies on chemicals that can negatively impact the environment, making more environmentally friendly alternatives necessary. One method that can be used is green synthesis, which utilizes natural materials containing active compounds such as polyphenols to reduce Au^{3+} to Au^0. This study aims to synthesize AuNPs using a green synthesis method with red binahong leaf extract (Anredera cordifolia (Ten.) Steenis) as the bioreductant and 20 ppm $HAuCl_4$ as the precursor, as well as to evaluate the effect of varying bioreductant volumes (0.25;0.75;1.25 and 2 mL) on the characteristics and stability of the AuNPs during 42 days of storage. Stability observations over 42 days provide information on the long-term stability of AuNPs, which has been reported only to a limited extent in previous studies. Characterization was performed using a UV-Vis spectrophotometer to determine the maximum wavelength and the resulting absorbance values. Particle size was calculated using the Brus cluster equation based on the maximum wavelength values. The results showed the formation of gold nanoparticles, as indicated by a color change in the solution to purple and the appearance of absorption peaks in the 539–576.5 nm range. An increase in bioreductant volume led to higher absorbance, indicating greater nanoparticle formation. The resulting particle size was approximately 21.57–23.39 nm. During storage, absorbance decreased, and the absorption peak shifted, indicating aggregation and changes in particle-size distribution. Among the various bioreductant volumes tested, the optimal volume for stability was 0.75 mL. Based on the research results, the volume of the bioreductant affects the characteristics and stability of the resulting nanoparticles.

Keywords: Gold Nanoparticles; Green Synthesis; Red Binahong Leaf Extract; Stability of Gold Nanoparticles.

Introduction

Nanotechnology is one of the major scientific achievements of the 21st century. This field encompasses the synthesis, manipulation, and application of materials smaller than 100 nm. One of the primary focuses in nanotechnology development is nanoparticles. Nanoparticles play a crucial role in various fields, including the environment, agriculture, food, biotechnology, and biomedicine. Additionally, one of the most actively developed application areas is as a drug-delivery system for specific cells or tissues within the body. Nanoparticles are designed to possess specific surface properties so they can selectively target diseased cells while avoiding healthy ones [1]. Nanoparticles offer several advantages, such as biocompatibility, anti-inflammatory and antibacterial activity, bioavailability, tumor targeting, and good bioabsorption. Metal nanoparticles are one of the most extensively studied types of nanoparticles due to their high stability and wide range of functions across various applications [2]. Among various types of metals, gold possesses unique properties, namely resistance to corrosion and oxidation, ease of synthesis, ease of detection, and low toxicity [3].

Generally, gold nanoparticles can be synthesized through two approaches: top-down and bottom-up [4]. The top-down approach involves reducing larger particles to nanoparticles. The bottom-up approach, on the other hand, forms nanoparticles from smaller particles. The top-down approach involves physical methods, while the bottom-up approach involves chemical and biological methods. Physical and chemical methods still have limitations, one of which is the use of toxic chemicals that can adversely affect the environment. Therefore, researchers have developed environmentally friendly, non-toxic, biologically based nanoparticle synthesis methods. This method is known as green synthesis [5]. Green synthesis of nanoparticles is a method that utilizes environmentally friendly materials such as bacteria, fungi, and plants. Compared to bacteria and fungi, plant extracts offer a simpler and more user-friendly alternative for nanoparticle synthesis [6]. The fundamental principle of green synthesis is redox reactions. In gold nanoparticle synthesis, this method utilizes natural extracts capable of reducing Au^{3+} to Au^0 [7].

Various plants have been successfully used as bioreducers in the green synthesis of gold nanoparticles; however, the characteristics of the resulting nanoparticles vary with plant species and synthesis conditions. [8] reported that gold nanoparticles synthesized using bilimbi fruit extract exhibited an SPR band at 534-536 nm with an average particle size of 7.98 nm. Nanoparticles synthesized using *Celastrus hindsii* leaf extract remained colloidally stable for up to 30 days of storage [9]. Likewise, [10] reported that gold nanoparticles synthesized using *Moringa oleifera* leaf extract were monitored for 30 days, and nanoparticles

How to Cite:
I. B. Mauidhah and T. Taufikurohmah, "Green synthesis and stability testing of gold nanoparticles using a bioreducer from red binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis)," *Jurnal Pijar MIPA*, vol. 21, no. 5, pp. 899–908, Oct, 2026. https://doi.org/10.29303/jpm.v21i5.12087

prepared from mature leaves exhibited the highest stability, as indicated by minimal changes in the position of the SPR band during storage. These differences in the optical characteristics and stability of the nanoparticles indicate that different plant species and phytochemical compositions influence the reduction and stabilization processes of the nanoparticles. However, most of these studies have focused on successful synthesis and initial characterization, while research on the effect of variations in bioreductant volume on the long-term stability of gold nanoparticles remains limited.

Gold nanoparticles show promise as a highly effective antioxidant material [11]. Antioxidant activity is greatly influenced by nanoparticle stability. One sign of instability is aggregation [12], which is the process of particle merging due to interparticle forces that cause the formation of increasingly larger clusters [13]. Aggregation leads to a decrease in the colloidal stability of nanoparticles and can result in precipitation [14]. Therefore, stability testing is a key parameter in the synthesis of gold nanoparticles, as it relates to their physical characteristics and potential applications. Several studies have reported that nanoparticle stability is influenced by various synthesis parameters, including the amount or volume of the bioreductant. Variations in the bioreductant volume may affect UV-Vis spectral characteristics, such as broadening of the Surface Plasmon Resonance (SPR) band and decreased peak sharpness, indicating nanoparticle aggregation. Therefore, optimizing the volume of the bioreductant is a key factor in obtaining gold nanoparticles with optimal characteristics and stability.

One natural material that can be used for gold nanoparticle synthesis is red binahong leaves. Binahong leaves contain various secondary metabolites such as flavonoids, tannins, and saponins [15]. Flavonoids possess functional groups that act as bioreducers of Au^{3+} ions while also stabilizing gold nanoparticles [5]. Additionally, binahong leaves exhibit biological activities, including anti-inflammatory and antibacterial properties. The saponin content can enhance collagen production and tissue regeneration, which aids in the wound healing process [16], [17]. Meanwhile, flavonoids can reduce inflammation by decreasing the production of inflammatory mediators such as prostaglandins and cytokines, thereby accelerating the wound healing process and reducing pain [18]. Therefore, the synthesis of gold nanoparticles using red binahong leaves is expected to maximize the plant's potential, as nanoparticles possess anti-inflammatory activity and serve as an effective drug delivery system, thereby optimizing the delivery of active compounds to receptors in wound tissue.

The synthesis of gold nanoparticles using red binahong leaf extract as a bioreductant has been reported in previous studies [19]. However, those studies used $HAuCl_4$ precursors at relatively high concentrations (0.2–1.0 mM), and nanoparticle stability was evaluated over short storage periods, yielding limited information on long-term stability. On the other hand, studies on the effects of variations in bioreductant volume on the characteristics and stability of gold nanoparticles have also been rarely reported. Given these circumstances, this study was conducted to evaluate the effect of varying volumes of red binahong leaf extract as a bioreductant on the characteristics and stability of gold nanoparticles synthesized using a 20 ppm $HAuCl_4$ precursor. Characterization was performed using a UV–Vis spectrophotometer to determine the maximum wavelength and absorbance values. Particle size estimates were calculated using the Brus cluster equation based on the maximum wavelength values, while nanoparticle stability was evaluated by visual observation and by changes in maximum wavelength and absorbance over 42 days of storage. This study is expected to provide information on the effect of variations in the volume of the bioreductant on the characteristics and long-term stability of gold nanoparticles synthesized via the green synthesis method using a low-concentration $HAuCl_4$ precursor.

Research Methods

This study was conducted as an exploratory, descriptive, and experimental laboratory investigation to evaluate the effects of varying volumes of red binahong leaf extract (0.25, 0.75, 1.25, and 2 mL) on gold nanoparticle formation and colloidal stability. The formation characteristics of AuNPs were evaluated through UV–Vis spectrophotometric analysis and cluster size estimation using the Brus equation after synthesis. Furthermore, colloidal stability was monitored over a 42-day storage period by monitoring changes in the surface plasmon resonance (SPR) wavelength and absorbance. The obtained data were analyzed descriptively to identify trends in nanoparticle formation and stability characteristics under different bioreductant volume treatments. Each treatment was conducted using a single experimental unit under controlled synthesis conditions. The synthesized AuNP colloidal solutions were periodically sampled during the storage period for UV–Vis analysis. The selected extract volumes were determined based on preliminary optimization to evaluate the influence of bioreductant availability on Au^{3+} reduction, nanoparticle formation, and colloidal stability.

Materials and Instruments

The equipment used in this study included a blender, an analytical balance, a 250 mL Erlenmeyer flask, a 100 mL beaker, a hot plate, a magnetic stirrer, filter paper, a micropipette, microtubes, a 50 mL measuring cylinder, a 200 mL volumetric flask, and a UV-Vis spectrophotometer (Shimadzu UV 1800). Meanwhile, the materials used in this study were red binahong leaves, distilled water, and a 1000 ppm $HAuCl_4$ stock solution.

Preparation of a 1000 ppm $HAuCl_4$ Stock Solution

The 1000 ppm $HAuCl_4$ stock solution was prepared according to the method of Amiruddin & Taufikurrohmah [20] by weighing 1 gram of gold, dissolving it in aqua regia with an $HCl:HNO_3$ ratio of 3:1 (8 mL), and heating until the gold was completely dissolved. The solution was then cooled to room temperature. After cooling, the solution was diluted in a 1000 mL volumetric flask using aquabidest up to the mark and shaken until homogeneous.

Extraction of Red Binahong Leaves

Red binahong leaves were extracted by infusion. The cleaned binahong leaves were ground using a blender. Next,

a 10-gram sample is weighed and placed into a 250-mL Erlenmeyer flask containing 100 mL of distilled water. The ratio of the sample to distilled water is 1:10. The mixture is then heated on a hot plate at 90 °C for 15 minutes, with occasional stirring [21]. After that, the sample solution was allowed to cool to room temperature and filtered through filter paper to separate the filtrate from the residue, yielding the red binahong leaf extract filtrate. The resulting filtrate was then analyzed using a UV-Vis spectrophotometer to determine the maximum wavelength.

Synthesis of Gold Nanoparticles

A 20 ppm $HAuCl_4$ solution was prepared by diluting a stock solution. Next, the 20 ppm $HAuCl_4$ solution was tested using a UV-Vis spectrophotometer to determine the maximum wavelength. A 50 mL aliquot of the resulting 20 ppm $HAuCl_4$ solution was transferred to a 100 mL beaker, and red binahong leaf extract was added to each solution in varying volumes of 0.25 mL, 0.75 mL, 1.25 mL, and 2 mL. Next, the solutions were heated using a magnetic stirrer at 500 rpm and 90 °C until a color change to red-purple occurred. The solutions were then cooled to room temperature [22].

Characterization Using a UV-Vis Spectrophotometer

Gold nanoparticles synthesized using varying volumes of bioreducer were characterized using a UV-Vis spectrophotometer at wavelengths of 500–600 nm. The maximum wavelength and absorbance values were then determined.

Stability Testing of Gold Nanoparticles

Stability testing was conducted to determine changes in the optical properties of gold nanoparticles during storage. Samples of gold nanoparticles synthesized with varying volumes of bioreducers were stored in tightly sealed containers and placed in a refrigerator. Observations were made over 42 days, and characterization was performed using a UV-Vis spectrophotometer to determine shifts in the maximum wavelength as an indicator of nanoparticle stability.

Calculation of Gold Nanoparticle Size

The size of gold nanoparticles was calculated quantitatively using the cluster diameter formula (Brus Cluster) based on the characterization results. The cluster diameter formula is shown in the following equation [23].

$$\frac{1240,6}{\lambda} = 1,3 + \frac{14,84}{R^2}\left(\frac{1}{me^2} + \frac{1}{mh^2}\right) - \frac{2,6}{6,5R} \quad \text{..... (1)}$$

Notes:
me^2 dan mh^2 = 0,25
λ = wavelength (nm)
R = cluster diameter (nm)

Results and Discussion

Preparation and determination of the wavelength of the $HAuCl_4$ solution

The $HAuCl_4$ solution used as a precursor is obtained by dissolving gold in aqua regia. The resulting solution is yellow. During the preparation of the $HAuCl_4$ solution, Cl^- ions from HCl form complexes, and an oxidation reaction occurs with HNO_3, resulting in the formation of Au^{3+} ions. This reaction produces byproducts, H_2 and NO_2, so heating is required to remove them. The reaction is shown as follows.

$$Au(s) + HNO_3(aq) + 4HCl \rightarrow HAuCl_4(aq) + NO_2(g) + H_2(g) + H_2O \quad \text{(l)}$$

After the gold dissolved, the solution was diluted with aquabidest in a 1000 mL volumetric flask to obtain a yellow $HAuCl_4$ stock solution at 1000 ppm. aquabidest was used to prevent the presence of other ions that could cause contamination in the solution. Next, the stock solution was further diluted to 20 ppm for use in the synthesis of gold nanoparticles. This concentration was chosen because it produces relatively stable nanoparticles, as reported in the study by [24].

The formation of gold nanoparticles can be observed through changes in the maximum wavelength. Therefore, measurements were taken using a UV-Vis spectrophotometer to observe the difference in the wavelength of $HAuCl_4$ before and after the addition of the bioreductant. The measurement results showed a maximum wavelength of 301 nm. The measurement results are shown in Figure 1.

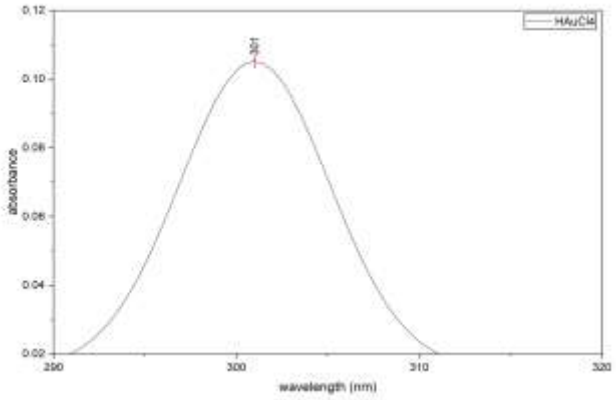


Figure 1. Wavelength of the $HAuCl_4$ solution

Extraction and Determination of the Maximum Wavelength of Red Binahong Leaves

Preparation begins by selecting fresh red binahong leaves free of holes. Next, the leaves are washed to remove dust and dirt, then air-dried until no water remains on their surface. The extraction was performed using the infusion method. The infusion method is an extraction technique that uses water as the solvent and heat to optimally extract polar active compounds. This method was chosen because it is relatively simple, requires little time, and is cost-effective. In this method, distilled water is used as the solvent in accordance with the “like dissolves like” principle, meaning

that compounds dissolve more readily in a solvent of similar polarity. Additionally, distilled water is non-toxic and more biocompatible, making it safe for biomedical applications.

The prepared red binahong leaves were then ground using a blender, weighed to 10 grams, and placed in a 250 mL Erlenmeyer flask. Next, 100 mL of distilled water was added to achieve a sample-to-solvent ratio of 1:10. The mixture was heated to 90°C for 15 minutes. These temperature and time settings are intended to optimize the extraction process while preventing damage to bioactive compounds caused by excessive heating. After the extraction, the solution was filtered through filter paper to separate the filtrate from the residue. The resulting filtrate was yellowish-green in color and was subsequently used for the synthesis of gold nanoparticles.

The filtrate obtained was then analyzed using a UV-Vis spectrophotometer to determine the differences in the wavelength of the red binahong leaf extract before and after reaction with $HAuCl_4$. The characterization results showed an absorption peak at 341 nm. This data was used to observe the difference in the wavelength of the red binahong leaf extract before and after reaction with $HAuCl_4$.

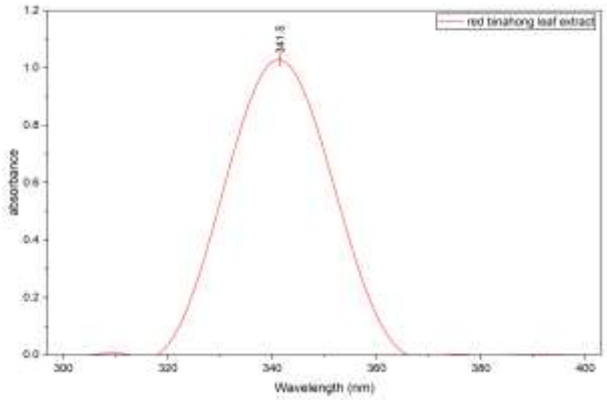
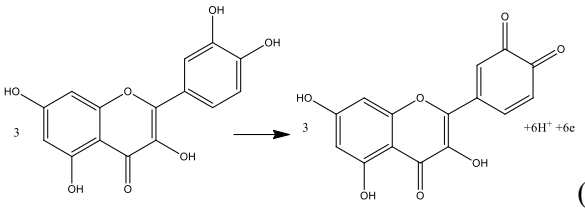


Figure 2. wavelength of red binahong leaf extract

Synthesis and Characterization of Gold Nanoparticles Using Different Volumes of Bioreducers

The synthesis of nanoparticles in this study utilized a bioreductant, namely red binahong leaves. This is because red binahong leaves are reported to contain various polyphenolic compounds with (-OH) groups that can donate free electrons. Additionally, the (-OH) groups also act as stabilizing agents that can prevent nanoparticle aggregation. During the reduction process, the (-OH) groups in the polyphenolic compounds release 2 electrons and 2 protons, which are then used to reduce Au^{3+} ions to Au^0 [25]. The reaction is shown below.



(2)

$$2Au^{3+} + 6e \longrightarrow 2Au^0 \quad (3)$$

The Au^0 atoms that form undergo gold nanoparticle formation, which consists of three stages: activation, growth, and termination. In the activation stage, gold ions (Au^{3+}) from the $HAuCl_4$ precursor solution are reduced to neutral gold atoms (Au^0). This process produces neutral gold atoms that act as the nuclei of the nanoparticles. The growth stage is characterized by an increase in cluster size through the nucleation process. The termination stage occurs when the gold nanoparticles are stabilized by polyphenol compounds. The (-OH) groups interact with the nanoparticle surface and form a protective layer that prevents aggregation, thereby keeping the nanoparticles stable in solution [26]. The reaction mechanism is shown in Figure 3.

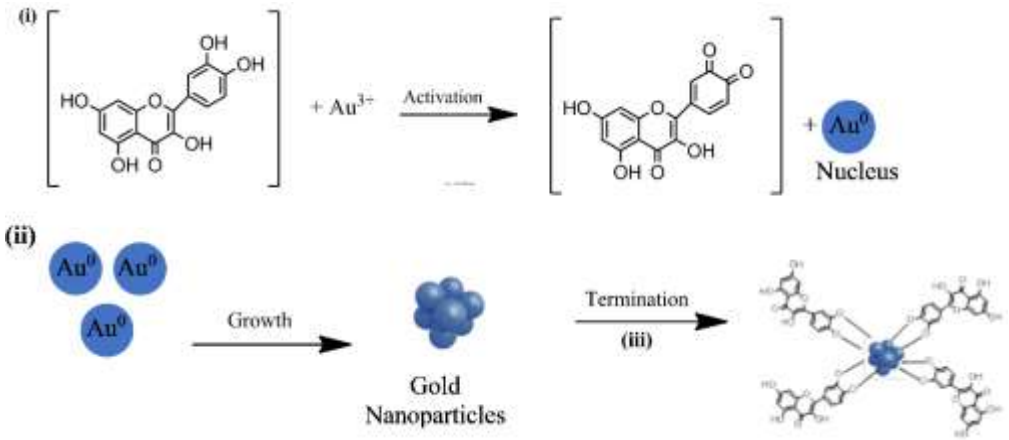


Figure 3. Mechanisms of Nanoparticle Formation and Stability of Gold by Polyphenolic Compounds in Plants. (i) activation process. (ii) growth process. (iii) termination process

Gold nanoparticles were synthesized by adding varying volumes of bioreducer (0.25, 0.75, 1.25, and 2 mL) to 50 mL of a 20 ppm $HAuCl_4$ solution in separate 100 mL beakers. The solution mixture was then heated in a magnetic stirrer at 90 °C at 500 rpm. The use of 90 °C was intended to increase the reaction rate of Au^{3+} reduction to Au^0 and accelerate the nucleation and particle growth processes [27]. Additionally, stirring is necessary during the synthesis process to prevent aggregation [28]. The formation of gold nanoparticles is indicated by a color change in the solution from yellow to red-purple. This signifies the occurrence of Surface Plasmon Resonance (SPR). The oscillation or back-and-forth movement of electrons is called a plasmon. Meanwhile, SPR is the synchronous oscillation of free electrons on the surface of gold nanoparticles when they interact with light. Electron oscillations cause charge separation in the metal, resulting in a dipole aligned with the light's electric field. SPR occurs when the frequency of electron motion matches that of the incident light. This frequency lies within the visible light spectrum, and gold nanoparticles absorb green light. This causes the gold nanoparticle solution to appear red [29]. The results of gold nanoparticle synthesis using varying volumes of red binahong leaf extract are shown in Figure 5.

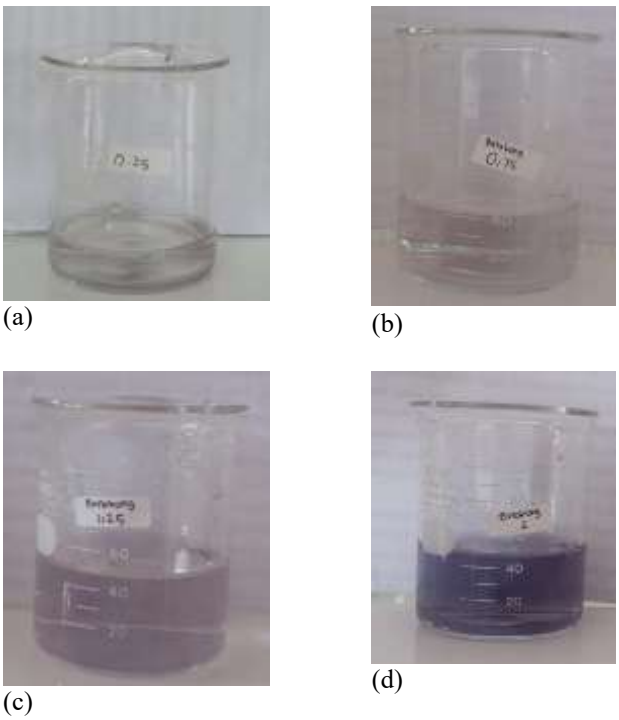


Figure 5. Results of gold nanoparticle synthesis using different volumes of bioreducer: (a) 0.25 mL, (b) 0.75 mL, (c) 1.25 mL, (d) 2 mL

As shown in Figure 5, as the volume of the bioreductant added increases, the solution becomes darker, indicating an increase in the number of gold nanoparticles formed. To confirm this, characterization was performed using a UV-Vis spectrophotometer to determine the wavelength and absorbance. The characterization results are shown in Table 1.

Table 1. Results of characterization using a UV-Vis spectrophotometer

Variations in the volume of the bioreductant	Wavelength (nm)	Absorbance
0.25 mL	539	0.0129
0.75 mL	560.5	0.0136
1.25 mL	561	0.0188
2 mL	576.5	0.0855

The measurement results in Table 1 indicate that as the volume of the bioreductant increases, the obtained absorbance values also increase. An increase in absorbance indicates that more gold nanoparticles are formed because more bioreductant is available to reduce Au^{3+} ions to Au^0. At the lowest bioreductant volume (0.25 mL), the resulting absorbance is relatively low, indicating that the number of nanoparticles formed remains small. This is because the amount of bioreductant is insufficient to fully reduce Au^{3+} ions. Conversely, at the highest bioreductant volume (2 mL), the absorbance increases, indicating that more nanoparticles are formed.

The maximum absorption peak obtained in Table 1 was then used to determine the size of the gold nanoparticle clusters. The cluster size was calculated using the Brus cluster formula, which relates the maximum wavelength to particle size. The results of the cluster size calculation are shown in Table 3

Table 3. Cluster size calculation results

Variations in the volume of the bioreductant (mL)	Cluster Diameter (nm)
0.25	21.57
0.75	22.58
1.25	22.60
2	23.39

Based on the results in Table 3, nanoparticles were produced in the size range 21.57-23.39 nm. These sizes are comparable to those reported in study [30], which found an average size of 22.18 ± 2 nm using an extract of *Benincasa hispida.* However, they are larger than those in study [31], which obtained a size of approximately 13.20 nm using an extract of *Combretum erythrophyllum*, and smaller than those in study [32], which reported a size of 85 nm using an extract of *Crataegus oxyacantha*. These differences in nanoparticle size are likely influenced by the type of plant extract, phytochemical composition, precursor concentration, bioreductant volume, and synthesis conditions such as pH and temperature. Based on the calculations, cluster size increases with bioreductant volume. At a volume of 0.25 mL, the resulting cluster size is the smallest among the volume variations. This is because the number of particles formed is small, so interactions between particles are still minimal and the likelihood of aggregation is lower. As the number of nanoparticles increases, the spacing between particles decreases, leading to more frequent collisions between particles [28]. This causes the particles to aggregate and form larger clusters, as seen in the 2 mL bioreductant volume, which yields the largest cluster size among the bioreductant volumes. As cluster size increases, the maximum wavelength shifts toward the red

due to the SPR phenomenon. This is because as cluster size increases, the distance between charges on the particles also widens, thereby weakening the restoring force. Consequently, the oscillation frequency decreases, causing a shift in wavelength toward longer wavelengths [29].

Based on the research results, an increase in bioreductant volume increased the absorbance, accompanied by a shift in λmax toward longer wavelengths (red shift). This indicates that variations in bioreductant volume affect the optical properties of the resulting gold nanoparticles. These results are consistent with the study by [33], which reported that an increase in the concentration of *Erythroxylum coca* leaf extract during the green synthesis of gold nanoparticles led to an increase in absorbance intensity as well as a red shift in the Surface Plasmon Resonance (SPR) band. The study explains that an increase in extract concentration raises the content of phytochemical compounds, such as phenolic compounds and flavonoids, which act as reducing agents and stabilizers, thereby making nanoparticle formation more effective. In addition to increasing the number of nanoparticles formed, an increase in extract concentration also affects the nanoparticles' optical properties, as indicated by a red shift in the SPR band.

Stability Testing of Gold Nanoparticles

The stability of the gold nanoparticles was assessed over a 6-week storage period by visual inspection and by measuring the maximum wavelength and absorbance using a UV-Vis spectrophotometer over the 200–800 nm range. These observations were conducted to determine changes in the characteristics of the gold nanoparticles during storage. The results of the color observations of the gold nanoparticles are shown in Figure 6, while the results of the λ max and absorbance measurements are presented in Table 2.



Figure 6. Results of Visual Observations of Gold Nanoparticles. (a) Day3. (b) Day 5. (c) Day 7. (d) Day 14. (e) Day 21. (f) Day 28. (g) Day 35. (h) Day 42

Table 2. Gold nanoparticle stability test data

Day	V1 (0.25 mL)		V2 (0.75 mL)		V3 (1.25 mL)		V4 (2 mL)	
	λ max (nm)	Abs	λ max (nm)	Abs	λ max (nm)	Abs	λ max (nm)	abs
3	312.5 [a]	0.778	574	0.039	584	0.153	580	0.118
5	- [b]	- [b]	574	0.052	309	0.035	308.5	0.035
7	- [b]	- [b]	571	0.044	309	0.035	302	0.030
14	- [b]	- [b]	571	0.031	310.5	0.020	306	0.034
21	- [b]	- [b]	564	0.028	311.5	0.018	301	0.028
28	- [b]	- [b]	561	0.022	311	0.013	300	0.021
35	- [b]	- [b]	561	0.017	304,5	0.010	300	0.021
42	- [b]	- [b]	554.5	0.012	304,5	0.008	300	0.022

[a] On day 3, the 0.25 mL bioreductant solution became clear, and no absorption peak was detected in the 500–600 nm range; the observed absorption shifted to around 300 nm, approaching the absorption region of the $HAuCl_4$ precursor, indicating that the gold nanoparticles had redissolved back into Au ions. [b] Measurements for the 0.25 mL bioreductant volume were discontinued after day 3 (see note [a]).

Based on visual observation, the color of the gold nanoparticle colloid at a bioreductant volume of 0.25 mL changed from purple to clear on the third day of storage and showed no sign of precipitate formation through the 42nd day. The loss of color in the solution without the formation of a precipitate indicates that this change was not caused by particle aggregation but is likely related to the redissolution of the gold nanoparticles into their ionic form. This is supported by the UV-Vis analysis results in Table 2, which show an increase in the intensity of the absorption peak in the UV region adjacent to that of the $HAuCl_4$ precursor. Although there was a shift in wavelength from 301 nm to 312.5 nm, the increase in the absorption peak in that region indicates the presence of gold species in ionic form in the solution. The wavelength shift is believed to be influenced by changes in the gold-ion environment arising from interactions with organic compounds still present in the extract. These results are consistent with the study by [34], which reported that dissolved oxygen and residual chloride ions in the solution can affect the dissolution process of gold nanoparticles. In that study, the gold nanoparticle solution, initially red, faded to clear, accompanied by a renewed increase in the intensity of the characteristic absorption peak of the gold ion species in the UV region. Therefore, the color change from purple to clear observed in this study is believed to occur through a similar mechanism, namely the redissolution of gold nanoparticles into ionic species due to the influence of dissolved oxygen and chloride ions still present in the solution.

At a volume of 0.75 mL, the solution underwent color changes, particularly on days 3 and 5; the color of the solution became more intense compared to the first day of synthesis, and the absorbance values continued to increase, indicating that the amount of gold nanoparticles was increasing. This indicates that the nanoparticle formation process is still ongoing. However, from day 7 through day 42, the solution's color gradually faded, accompanied by a decrease in absorbance values, indicating that the gold nanoparticles were becoming less stable.

The 1.25 mL and 2 mL volumes exhibited the same stability. On day 3, the solution's color was darker, and the absorbance values also increased compared to the first day of synthesis. This indicates that the formation process was still ongoing. However, by day 5, the solution's color had turned colorless, indicating that the colloidal system had broken down or become unstable. Based on the data in Table 2, no absorption peaks were detected in the 500–600 nm range from day 5 through day 42 of storage. The disappearance of these Surface Plasmon Resonance (SPR) peaks indicates that the gold nanoparticles have become unstable. This is also evidenced by the formation of precipitates, the number of which increased over time during storage. This indicates that the loss of color in these two variations is caused by particle aggregation and precipitation, rather than by redissolution into an ionic form as in the 0.25 mL variation.

The extract concentration is one of the parameters that control the rate of reduction, particle growth, and nanoparticle stability [35]. Increasing the amount of extract enhances the availability of phytochemical compounds that act as reducing agents and stabilizers. However, beyond the optimum concentration, further addition of the extract can trigger a secondary reduction process on the surface of the already-formed nanoparticle cores and increase biomolecular interactions on the particle surface. These conditions cause particle growth to become less controlled, making the nanoparticles more prone to aggregation. In this study, this process was indicated by the complete disappearance of the SPR band, accompanied by the formation of a precipitate. This suggests that aggregation had progressed to the point of forming a precipitate, so that most of the nanoparticles were no longer in the colloidal phase and could not be detected via UV–Vis measurements.

The findings of this study are consistent with the results reported [36], who synthesized gold nanoparticles using ethanol extract of white guava leaves with bioreductant volumes of 0.25, 0.75, 1.25, and 2.00 mL in 50 mL of a 25 ppm $HAuCl_4$ solution. That study showed that a bioreductant volume of 0.75 mL produced the highest absorbance and the best stability among the variations. This finding suggests that there is an optimal bioreductant volume capable of providing a balanced amount of phytochemicals to reduce Au^{3+} ions while stabilizing the nanoparticle surface. However, the study evaluated stability only up to day 14 and did not report redissolution at low bioreductant volumes. In contrast, this study monitored stability for up to 42 days and revealed two distinct mechanisms of stability decline: the redissolution of nanoparticles at low bioreductant volumes and aggregation that progresses to precipitate formation at high bioreductant volumes. Thus, this study provides a more comprehensive understanding of how variations in bioreductant volume affect the mechanisms underlying the decline in the stability of gold nanoparticles during long-term storage.

Based on the observation results, a bioreductant volume of 0.75 mL was optimal for synthesising gold nanoparticles. This is indicated by the stability of the maximum wavelength in the 500–600 nm range during the

42-day observation period. Based on the data in Table 2, the graph shown in Figure 7 was obtained.

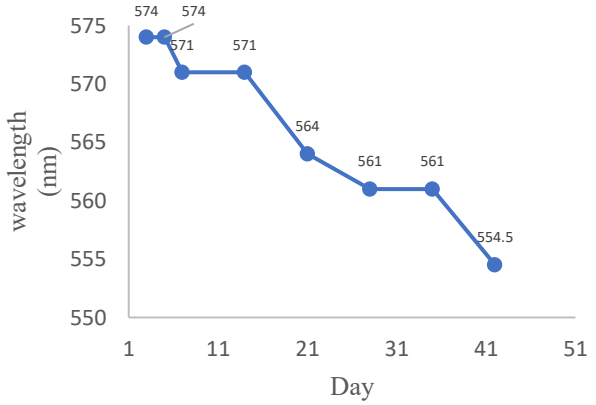


Figure 7. Stability Curve for 0.75 mL of Bioreductant

Based on the graph above, the maximum wavelength shifts toward the blue as storage time increases. This shift indicates a change in the size distribution of the gold nanoparticles. This is related to the decreasing stability of the nanoparticle colloid during storage. The stability of the gold nanoparticles is influenced by bioactive compounds in the red binahong leaf extract, which act as capping agents. These compounds coat the nanoparticles' surfaces, thereby preventing direct interactions between particles and keeping them stably dispersed in solution. As storage time increases, the effectiveness of the capping agent may decrease due to changes in system conditions, thereby increasing interparticle attractive forces. This leads to nanoparticles colliding with one another and aggregating to form larger aggregates. The occurrence of aggregation is one of the main indicators of a decline in the stability of a colloidal system. Larger aggregates cause particles to settle more easily at the bottom of the storage container, thereby reducing the number of nanoparticles still dispersed in the solution [37]. This is indicated by the solution's color becoming increasingly faint as the number of particles contributing to the Surface Plasmon Resonance (SPR) phenomenon decreases [38]. Furthermore, the settling of large aggregates alters the size distribution of the nanoparticles still suspended in the solution. This is indicated by a blue shift in the maximum wavelength, suggesting that the nanoparticles still dispersed in the solution are relatively small.

To support this statement, the size of the gold nanoparticle clusters was calculated using the Brus cluster equation. The results of the cluster size calculations for the optimal extract volume during storage are presented in Table 3.

Based on Table 4.4, the size of the detected gold nanoparticle clusters tends to decrease as storage time increases. These results are consistent with the UV-Vis data, which show a blue shift in wavelength. The measured decrease in cluster size does not necessarily indicate that each nanoparticle has shrunk; rather, it suggests that larger clusters have aggregated and precipitated, rendering them undetectable in the measurements. Thus, the cluster size calculations based on the Brus Cluster equation support the UV-Vis characterization results, which indicate a change in particle size distribution due to a decrease in nanoparticle stability during storage.

Table 3. Changes in the Size of Gold Nanoparticle Clusters at the Optimum Extract Volume During Storage Based on the Brus Cluster Equation

Day	Wavelength (nm)	Cluster Diameter (nm)
3	574	23.25
5	574	23.25
7	571	23.10
14	571	23.10
21	564	22.75
28	561	22.58
35	561	22.58
42	554.5	22.30

Conclusion

Variations in the volume of the red binahong leaf extract bioreductant affect the characteristics of gold nanoparticles. Based on the characterization results using a UV-Vis spectrophotometer, an increase in the volume of the bioreductant led to an increase in the number of gold nanoparticles formed, as indicated by an increase in absorbance in the 500–600 nm wavelength range. Additionally, as the volume of the bioreductant increased, the resulting clusters grew larger, causing a red shift in wavelength. Variations in the volume of the red binahong leaf extract bioreductant affect the stability of gold nanoparticles during storage for up to 6 weeks, with the most stable extract volume being 0.75 mL, at which the maximum wavelength remains within the 500–600 nm range. Nevertheless, the absorbance values gradually decrease over the storage period. These findings demonstrate the potential of red binahong leaf extract as a natural bioreductant and stabilizing agent in sustainable AuNP synthesis.

The use of plant-based reducing agents provides an environmentally friendly approach for nanomaterial development and may support future applications of AuNPs in biomedical fields, including nanoparticle-based drug delivery systems. However, further investigations are required to validate these potential applications. Future studies should focus on optimizing synthesis parameters, including precursor concentration, extract-to-precursor ratio, reaction conditions, and storage factors. In addition, advanced characterization techniques such as transmission electron microscopy (TEM), particle size distribution analysis, zeta potential measurement, and X-ray diffraction (XRD) are recommended to provide more comprehensive information regarding nanoparticle morphology, size distribution, surface stability, and crystallinity.

Author's Contribution

I.B. Mau'idhah: participated in the design and planning of the experiments, the collection and processing of data used in this study, the conduct of the experiments, data analysis, and the writing, editing, and revision of the manuscript. T. Taufikurohmah: contributed to overseeing the research project and provided input to improve the research's overall quality.

Acknowledgements

The author would like to formally express gratitude to the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Surabaya State University, for providing the facilities and essential resources necessary to

successfully complete this research. In addition, special thanks are extended to Ms. Titik Taufikurohmah for her guidance, as well as her excellent input and advice during the preparation of this paper.

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