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Biosynthesis of Gold Nanoparticles Mediated by Andaliman Fruit Water Extract and Its Application as Antioxidants

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Abstract

Plant extract-mediated green synthesis of gold nanoparticles (AuNPs) is currently gaining significant interest in the field of nanotechnology. In this study, AuNPs were synthesized using an aqueous extract of Andaliman fruit (*Zanthoxylum acanthopodium* DC.). The formation of AuNPs was confirmed by observing the color change of the solution from clear to cherry red. The reaction parameters, namely the extract concentration and the ratio of the mixture of the extract with $HAuCl_4$ solution, were optimized for the AuNPs biosynthesis. The gold nanoparticles were characterized using a UV-Vis spectrophotometer, SEM-EDS, and particle size analyzer. The characterization suggested that AuNPs had a maximum wavelength ranging of 540–559 nm, with spherical crystals morphology where the highest component was gold at 36.01% and the size below 100 nm on average. The antioxidant activity of the synthesized AuNPs was determined using the DPPH method. It showed that the highest free radical scavenging activity was 83%, given by 20 ppm AuNPs.

1. Introduction

Metal nanoparticles, such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs), have received much attention recently because of their catalytic, electrical, magnetic, and optical properties exhibited when compared to the respective bulk metal state [1]. Nanoparticles are dispersed particulates with 1–100 nm [2]. Currently, many researchers are competing to synthesize gold nanoparticles using environmentally friendly methods such as green synthesis. Gold nanoparticles have the potential as a catalyst, anticancer, antibacterial, biosensor, and antioxidant. The antioxidant is a term that is quite popular among nutritionists and other health professionals. In recent years, the term has been used more and more frequently and began to seize the public's attention, especially people with health concerns [3]. Various studies on active compounds with antioxidant activity have been carried out, one of which is gold nanoparticles (AuNPs). Gold nanoparticles are one of the nanoscience products which has many potential uses. The Surface Plasmon Resonance (SPR) character of AuNPs is the reason why nanomaterials are essential to investigate and engineer because of their application in health research [4].

Gold nanoparticles can be synthesized by physical and chemical methods [5]. However, physical and chemical methods have drawbacks, such as time-consuming, labor-intensive, costly, and not environmentally friendly. The new approach is thus needed to overcome the shortcomings of the physical and chemical methods. The new method uses to synthesize a metal into nanoparticle size is reduction using plant extracts, known as phytosynthesis. Many plant extracts have been reported to be effective as bioreductor to form and stabilize nanoparticles [6]. Plants contain secondary metabolites such as flavonoids, alkaloids, tannins, and saponins with functional groups that are strongly suspected of taking parts involved in the bioreduction of metallic ions to NPs and their stabilization [7].

The advantages of using plants to synthesize gold nanoparticles are a straightforward, cost-effective method, utilizing the available natural resources and non-toxic so that it is suitable for application in the pharmaceutical and biomedical fields [8]. However, there

has been no scientific report on Andaliman fruit water extract (*Zanthoxylum acanthopodium* DC.) which is used to synthesize gold nanoparticles. This plant is an endemic plant of Sumatra, Indonesia, especially the Batak area. Andaliman fruit contains terpenoid compounds, polyphenols, quinones, essential oils, flavonoids, alkaloids, pyranoguinoline alkaloids, quaternary isoquinoline alkaloids, and aporphyrine alkaloids [9]. In several studies, compounds mentioned above are capable of forming nanoparticles of metal [10].

This research aims to synthesize gold nanoparticles using Andaliman fruit water extract as a bioreductant. These gold nanoparticles will be tested for their ability as a free radical scavenger as a preliminary study in synthesizing an antioxidant. In this research, the characterization of the synthesized gold nanoparticles was carried out using several instruments, such as UV-Vis spectrophotometer, scanning electron microscopy energy dispersive X-Ray spectroscopy (SEM-EDX), and particle size analyzer (PSA). UV-Vis spectrophotometer monitored the initial formation of the gold nanoparticles at 500–600 nm. This absorption is due to the surface plasmon resonance, also known as SPR, which is the resonance of excited electrons in the conduction band around the surface of the nanoparticles. The electrons' vibration adjusts the shape and size of the nanoparticles. Therefore, metal nanoparticles display characteristic optical absorption spectra in the UV-Vis region [11]. Scanning electron microscopy energy dispersive X-Ray spectroscopy (SEM-EDX) is used to identify the topography, morphology, and constituent elements of the gold nanoparticles. The particle size analyzer (PSA) is used to determine the size and zeta potential.

The method used for the antioxidant testing was the DPPH method which is based on the ability of the antioxidants to inhibit free radicals by donating a hydrogen atom [12]. Smaller-sized nanoparticles with a larger surface area are much more efficient in the antioxidant activity test [13].

2. Methodology

This research was designed into four stages: (i) extraction of Andaliman fruit water extract, (ii) preparation of 0.5 mM $HAuCl_4$ solution as a precursor for the formation of gold nanoparticles, (iii) synthesis and characterization of the formed gold nanoparticles, consisting of the optimization of Andaliman fruit water extract concentration and the ratio of Andaliman fruit water extract with $HAuCl_4$ solution, and the characterization of the gold nanoparticles using UV-Vis spectrophotometer, SEM-EDX and particle size analyzer, (iv) the gold nanoparticles application as an antioxidant using the DPPH method.

2.1. Equipment and Materials

The equipment used in this study were a blender, analytical balance, magnetic stirrer, UV Vis spectrophotometer (Genesys 10S UV-Vis), particle size analyzer (Zetaziser nano ZSP Malvern), scanning electron microscopy energy dispersive X-Ray spectroscopy (JSM-6510LA JEOL), volume pipette, glassware (Pyrex), centrifugation (Gemmy PLC 05). The materials used in this study were 99.9% pure gold (Antam), HNO3 69% (Brataco), HCl 37% (Merck), distilled water (Brataco), demineralized water (Brataco), Andaliman fruit, ethanol (Onemed), DPPH (NitraKimia).

2.2. Preparation of Andaliman Fruit Water Extract

Andaliman fruit was dried and mashed until Andaliman fruit powder was obtained. Twenty grams of the powder was heated with 100 mL of demineralized water in a 250 mL beaker for 15 minutes at 60°C, then allowed to cool. After that, the suspension was filtered using filter paper. Then Andaliman fruit water extract was made into two concentrations, 3% and 5%.

2.3. Preparation of $HAuCl_4$ Solution

One mM $HAuCl_4$ solution was prepared by dissolving 0.09848 grams of pure gold with 4 mL of aqua regia (HCl: HNO_3 (3:1)) under heating, then the volume was brought up to 500 mL with demineralized water. This $HAuCl_4$ solution was then diluted to a concentration of 0.5 mM. The solution was characterized by a UV-Vis spectrophotometer at 200–800 nm.

2.4. Synthesis and Characterization of AuNPs

The synthesis of gold nanoparticles was carried out by mixing 0.5 mM $HAuCl_4$ solution with Andaliman fruit water extract with a ratio of 1:1 and 2:1 (v/v). The extract concentrations used were 3% and 5%. The mixture was heated at 80°C for 20 minutes. The biosynthesis process of the AuNPs is illustrated in Figure 1. As an indicator for the formation of the gold nanoparticles, the color of the solution changed to cherry red. After indicated to form, the gold nanoparticles were characterized using UV-Vis spectrophotometer, SEM-EDX, and PSA to ensure that the synthesis of gold nanoparticles had been successfully carried out.

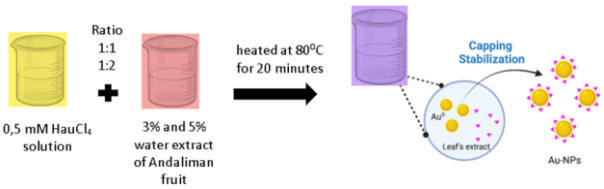

Figure 1. Illustration of the biosynthesis of AuNPs

2.5. The Determination of the Antioxidant Activity of AuNPs

The antioxidant activity was determined based on the differences between the absorbances of DPPH with and without AuNPs addition. The 40 ppm DPPH solution was prepared by adding 2 mg DPPH powder into a 50 mL volumetric flask, followed by a few mL 96% ethanol up to the mark and shaken. The absorbance of the solution was measured at its maximum wavelength. Furthermore, for the antioxidant activity test, 2 mL of 40 ppm DPPH solution was added into 2 mL of colloidal gold nanoparticles (with varying concentrations of 10, 20, 30, 40, 50, 60, and 70 ppm). The mixture was shaken vigorously left for 60 minutes in a dark room, and the absorbance was measured at the maximum wavelength of the DPPH. Data processing was done using Excel software.

3. Results and Discussion

3.1. Preparation of Andaliman Fruit Water Extract

Sample preparation was the first step in a study that determine the success of the results. The fruit Simplicia samples were determined at LIPI Bedugul Bali as Andaliman fruit (*Zanthoxylum acanthopodium* DC.). The Andaliman fruits were dried outdoors but not in direct sunlight for 3–4 days and removed moisture content from the samples. This was carried out to prevent microorganisms such as fungi from growing on the sample, which might affect the analysis results. In the extraction process, a temperature of 60℃ was used to ensure the active ingredients in the extract were not damaged by high heating temperatures (Figure 2).

Figure 2. The process of making Andaliman fruit water extract

The wavelength of Andaliman fruit water extract in Figure 3, where the wavelength is below 350 nm.

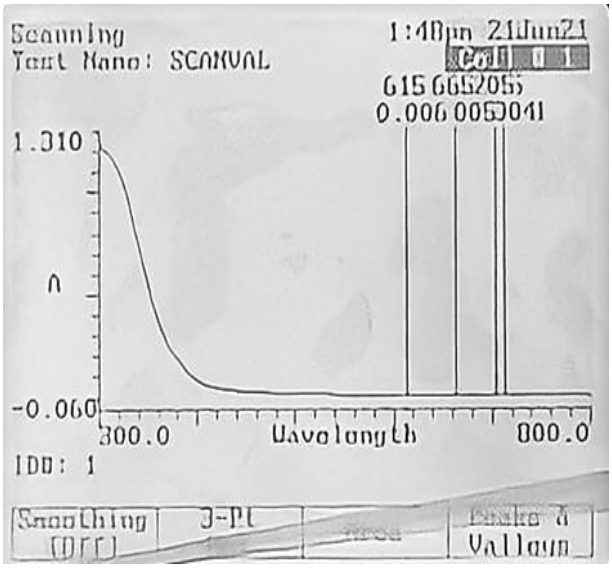

Figure 3. The wavelength of Andaliman fruit water extract

3.2. Preparation of $HAuCl_4$ Solution

$HAuCl_4$ solution was prepared from 99.99% gold plates. The solution concentration was modified from 1 mM $HAuCl_4$ to 0.5 mM to minimize the gold and solvents. The solvent used was aqua regia ($HCl:HNO_3$), it dissolved the gold quickly because aqua regia is a strong acid capable of dissolving metals [14]. When gold (Au) and aqua regia react, oxidation-reduction occurred, producing $HAuCl_4$, NO, and H_2. A heating step is required to obtain only $HAuCl_4$. Heating using a hot plate aimed to accelerate the reaction process and evaporate the by-products, NO and H_2 [15]. The heating process was conducted at 120℃ until the solution was orange-red in color. All these processes were carried out in a fume hood to prevent the toxic gasses from spreading throughout the room.

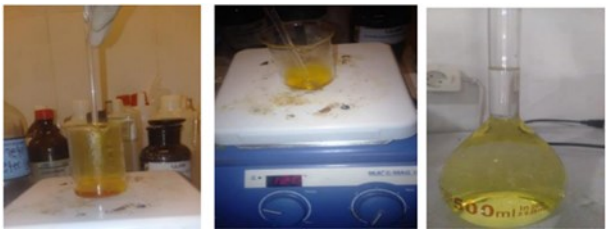

Figure 4. The preparation process of the $HAuCL_4$ solution

The maximum wavelength of the 0.5 mM $HAuCl_4$ (the starting material for the formation of gold nanoparticles) was measured in the wavelength range of 200–800 nm using UV-Vis spectrophotometer. The analysis showed the maximum wavelength of 315 nm with an absorbance of 3.915. The spectra can be seen in Figure 5. The result is similar to the result reported by Hidayat [16], where $HAuCl_4$ wavelength was 319 nm with an absorbance of 2.429. The strong absorption peak of $HAuCl_4$ is formed due to the charge transfer interactions between the chloro ligand and the metal [17].

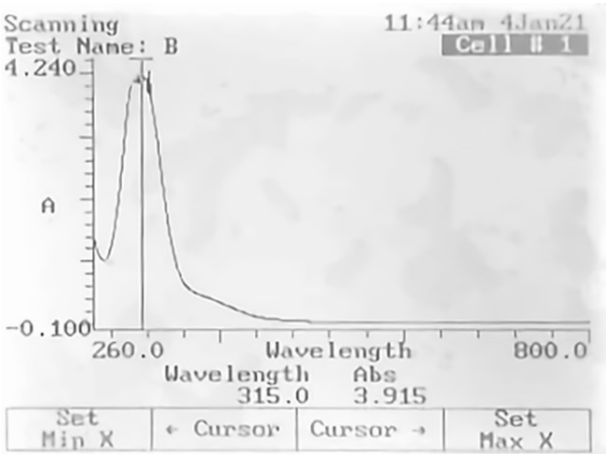

Figure 5. The UV-Vis spectra of 0.5 mM $HAuCl_4$

3.3. Synthesis and Characterization of AuNPs

3.3.1. Synthesis of AuNPs

The synthesis of the gold nanoparticles was carried out by mixing 0.5 mM $HAuCl_4$ with fruit water extract of Andaliman (*Zanthoxylum acanthopodium* DC.). The concentrations of the extract were 3% and 5%. A comparison of the mixture ratios between extracts to 0.5 mM $HAuCl_4$ solution was also carried out for 1:1 and 1:2 ratios. These variations were made to obtain the optimum formulation for the formation of the gold nanoparticles. As a visual indicator for the formation of gold nanoparticles, the color of the solution changed to cherry red [18]. The changes in the color of the solution in this research are shown in Figure 6. This suggested that the synthesis has successfully produced the expected AuNPs.

There was a shift in the maximum wavelength of the precursor (3.15 nm) to 500–600 nm that according to Hidayat [16], it can be stated that the formation of gold nanoparticles has occurred after mixing with Andaliman fruit water extract (as bioreductant).

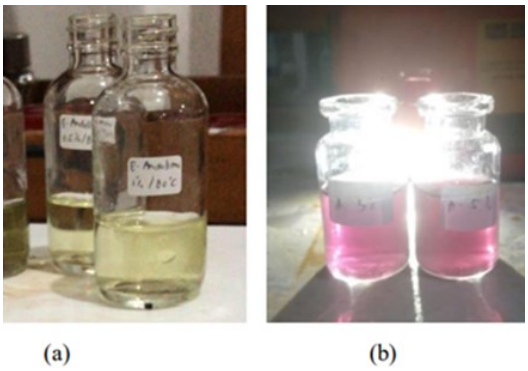

Figure 6. (a) Before forming AuNPs (b) after forming AuNPs

These wavelengths arise from the collective oscillations of the conduction electrons; therefore, resonance excitation occurs, producing photons [19]. Based on SPR (surface plasmon resonance) theory, the maximum wavelengths inversely corelate with the nanoparticle size because the excitation energy is getting smaller with increasing particle size. Since the distance traveled by the electron to be excited from the ground state to the excitation state is getting smaller [16]. The formation of gold nanoparticles occured starting with Au polymer, which then formed Au core [20].

3.3.2. Characterization of AuNPs using UV-Vis Spectrophotometer

UV-Vis spectrophotometer was the first technique to identify AuNPs through the maximum wavelength and absorbance formed.

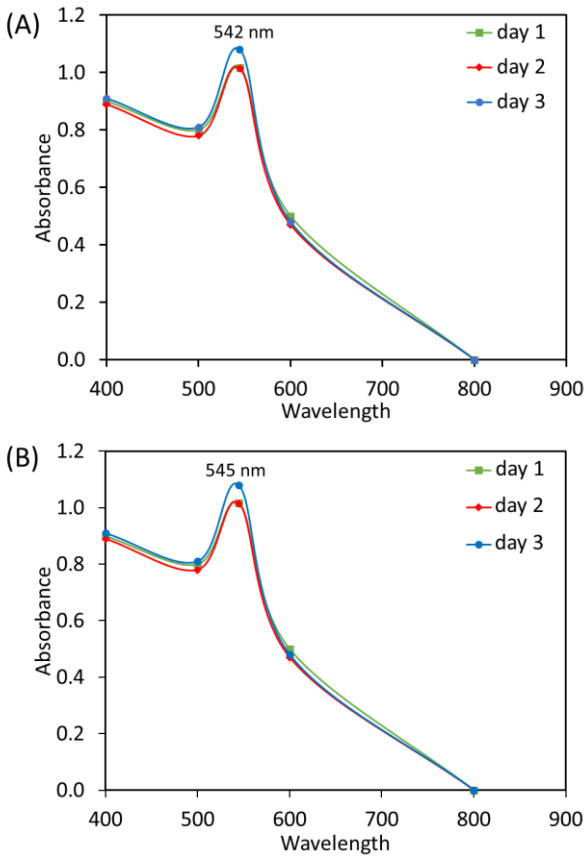

Figure 7. The spectra of AuNPs were prepared using 3% extract. (A) Ratio 1:1, (B) Ratio 1:2

From the UV-Vis spectrophotometer results, the AuNPs from 3% extract with ratios of 1:1 and 1:2 have varying wavelengths between 542 and 545 nm. The 5% extracts have a maximum wavelength between 540 and 559 nm (Figure 7 and 8).

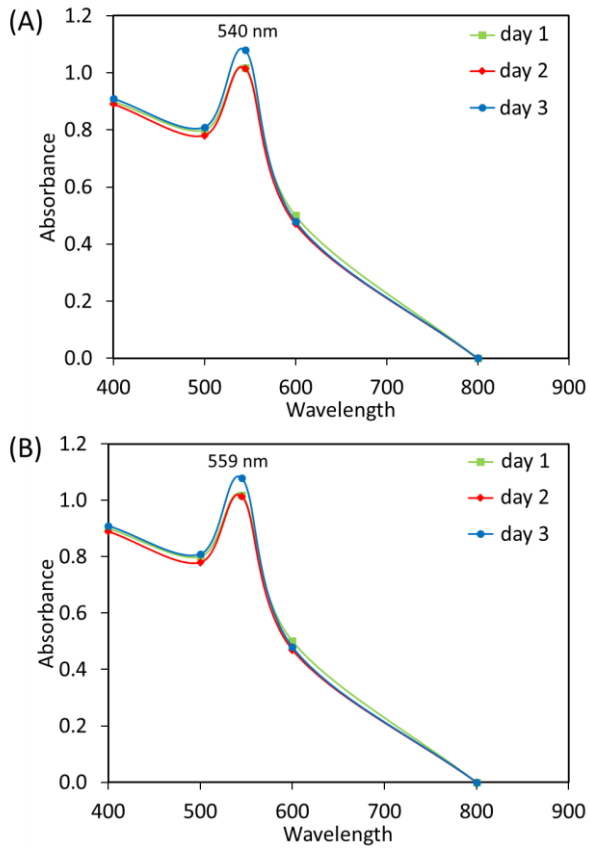

Figure 8. The spectra of AuNPs prepared using 5% extract (A) Ratio 1:1, (B) Ratio 1:2

Compared with the maximum wavelength of the $HAuCl_4$ solution, which is 315 nm, the AuNPs wavelength appears to have a wavelength shift identified as the formation of AuNPs. The different wavelengths between the AuNPs prepared by the varied concentrations and ratios indicated that the sizes of the AuNPs obtained by those variations also vary (Table 1.). Formation of red-purple due to the excitation of the surface plasmon nanoparticles. When in their ionic form, the formation process of gold nanoparticles, $AuCl_4^-$ would repel each other due to the effect of similar charges. In contrast, after being reduced to Au^0 by bioreductor, the atomic charge of Au becomes neutral; therefore, it allows the Au atoms to interact with each other through bonds between metals to form nano-sized clusters [15].

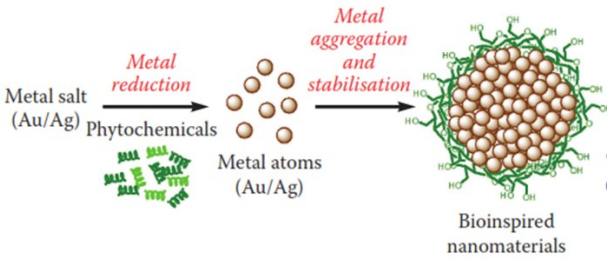

Figure 9. Illustration of the formation of metal nanoparticles

3.3.3. Characterization of AuNPs using Particle Size Analyzer

The particle size analyzer technique was carried out to determine the particle size of the AuNPs that have been synthesized. The AuNPs prepared by all variations were sampled and analyzed. The results are shown in Table 1.

Table 1. Result of PSA

Concentration (%)	Ratio of variation	Size of AuNPs (nm)	PdI
3	1:1	41.76 ± 0.5	0.747
	1:2	49.70 ± 2.37	0.570
5	1:1	73.56 ± 22.66	0.346
	1:2	151.93 ± 11.51	0.619

The PSA results show that the AuNPs prepared by three variations fulfil the 1–100 nm nanoparticle size criteria. The smallest particle size is the sample prepared with a ratio of 1:1 from the extract concentration of 3%. However, the value of PdI is exceptionally high, indicating that the sample obtained is not homogeneous; therefore, further analysis is required to analyze the parameters of the synthesis of AuNPs. On the other hand, the AuNPs prepared by 1:1 ratio from 5% extract is the most homogenous sample, although the size is much larger, still categorized as nanoparticles (Table 1, Figure 10).

3.3.4. Characterization of AuNPs using SEM-EDX

Characterization using SEM aimed to show the morphology of the AuNPs. Samples were analyzed in powder forms. The method used to obtain AuNPs powder is by centrifugation process at a speed of 10,000 rpm for 30 minutes. The centrifugation results were collected in a porcelain dish and dried to obtain gold nanoparticles powder. The drying was carried out in an oven at a temperature of 50°C for 4 hours. The yield of the gold nanoparticles powder obtained in this study was 15 mg.

The first step was to identify the morphological shape of the gold nanoparticles with the SEM process. The gold nanoparticles powder was placed in a cell and fed into the instrument for SEM analysis. The magnification used was 500x, 1,000x and 3,000x and 10,000x. Spherical crystal-shaped particles are found at a magnification of 10,000x (Figure 11).

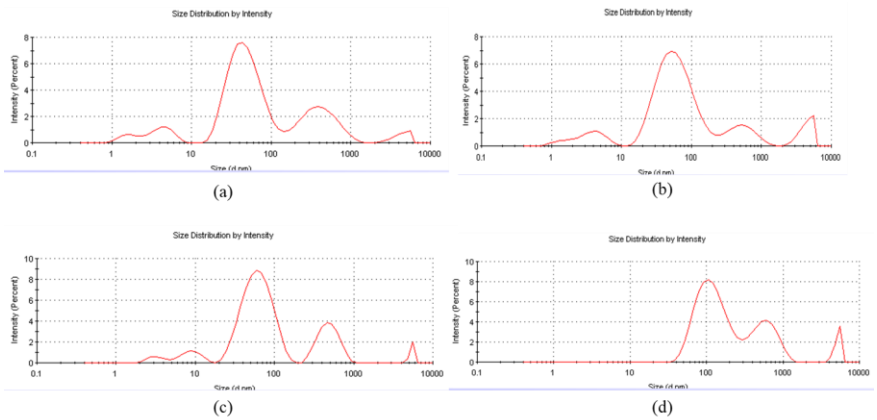

Figure 10. The he size distribution of the sample. (a) extract 3% ratio 1:1; (b) extract 3% ratio 1:2; (c) extract 5% ratio 1:1; (d) extract 5% ratio 1:2

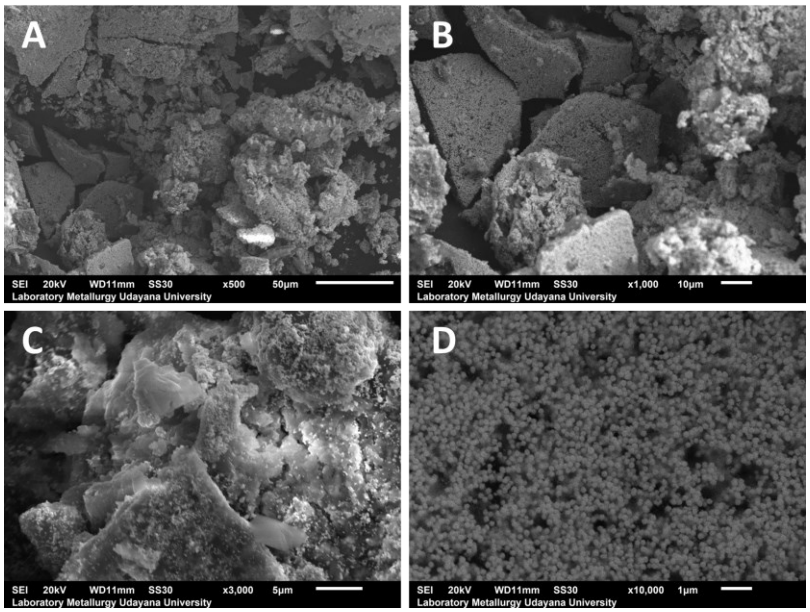

Figure 11. The morphology of the AuNPs, (a) 500x magnification; (b) 1000x magnification; (c) 3000x magnification and (d) 10000x magnification

The EDX analysis was carried out to obtain the content of the gold nanoparticles. Characterization of gold nanoparticles with EDX gives the highest content yield on gold nanoparticles is 36.01% gold (Figure 12).

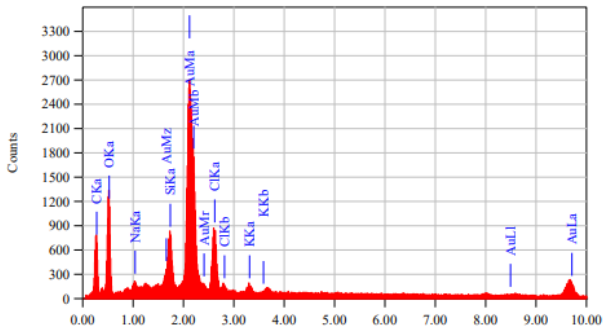

Figure 12. The composition elements in the AuNPs by EDX

3.4. The Antioxidant Activity of the AuNPs

Antioxidants are substances that can slow down or prevent the oxidation process caused by free radicals. The method used in this study is the DPPH method. DPPH is a free radical which is stable at room temperature. The principle of this method is to measure the occurrence of color fading (violet to yellow) of DPPH radicals due to the presence of antioxidant compounds that can neutralize free radical molecules [21]. The DPPH method is based on the ability of the antioxidants to inhibit free radicals by donating hydrogen atoms. Based on the spectrophotometer results at the maximum wavelength of the DPPH at 517 nm, the percentage of the free radical scavengers obtained is shown in Table 2.

Table 2. Free Radical Scavenging of the AuNPs

Concentration of AuNPs (ppm)	Absorbance	% Inhibition
10	0.227	42%
20	0.065	83%
30	0.168	57%
40	0.168	57%
50	0.212	46%
60	0.215	45%
70	0.27	31%

Based on the data, it can be seen the free radical scavenging activity of the AuNPs. The highest activity shown was 83%, given by the AuNPs at a concentration of 20 ppm. Theoretically, the greater the concentration of the AuNPs, the greater the percentage of free radical scavenging. But the results show that if the concentration is greater than 20 ppm, DPPH radical scavenging activity decreases. This can be caused by incomplete particle formation due to unstable stirring control, both time and speed.

The greater concentration of gold, the more gold particles were formed to reduce DPPH free radicals. However, the reduction will decrease at a specific concentration because colloidal AuNPs will increase in size clusters. The increase in the size of the AuNPs cluster is due to the more concentrated $HAuCl_4$ solution used. The more gold particles produced, the more collisions between the particles. This will increase the chance of the particles combining with each other to form larger aggregates. The mechanism for the scavenger is that the Au atom will stabilize the N atom in the DPPH by mutual bonds. The Au atom will donate its lone pair of electrons to the N atom to form Au-N coordination covalent bonds. In the presence of covalent bonds, the coordination between Au-N can reduce DPPH free radicals because the N atom has been stabilized by the Au atom [22].

4. Conclusion

The optimum concentration of Andaliman fruit water extract (*Zanthoxylum acanthopodium* DC.) required to produce gold nanoparticles in this study was 3%. The optimum ratio to obtain the smallest gold nanoparticles was 1:1. Characterization of the gold nanoparticles with UV-Vis spectrophotometer showed maximum wavelength that varies between 540–559 nm. PSA results revealed the obtained gold nanoparticles with the smallest size of 41.75 nm. The results of SEM-EDX suggested the morphology of spherical crystal AuNPs with the highest gold content of 36.01%. The maximum free radical scavenging activity was at 20 ppm AuNPs, with an inhibition percentage of 83%.

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