Production of the nanoparticles using leaf of *Muntingia calabura* L. as bioreductor and potential as a blood sugar nanosensor

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Abstract. Research on the synthesis of gold nanoparticles using *Muntingia calabura* L. leaf extract as a bioreductor and its potential analysis as a blood sugar nanosensor has been done. The synthesis Au nanoparticles are characterized and applied as sensors to detect glucose. The results show that gold nanoparticles have been successfully synthesized with a maximum wavelength of 544 nm. The measurements with PSA show that the average size of the gold nanoparticles is 78.2 nm. The gold nanoparticle-based glucose sensor design has a measurement range of 1-4 mM with a regression is 0.9248, minimum detection limit of the sensor at a concentration of 0.1500 mM and a maximum detection limit is 4.1744 mM with sensitivity is 0.6837 A. mM⁻¹.mm⁻².

1. Introduction

Nanotechnology is the science and engineering in the creation of materials, functional structures, and devices in the nanometer scale. Nanoparticles are part of nanotechnology that is very popular and growing rapidly because it can be widely applied in agriculture, electronics, optical, industrial, health, biomedical, catalyst, textile and environment [1, 2]. The size of a nano-sized particle is about 1-100 nm will have a characteristic slightly different from its larger size [3, 4].

Utilization of plants in the process of synthesis of nanoparticles utilizes organic compounds contained in plants such as enzymes, proteins and carbohydrates. The content of secondary metabolite compounds such as flavonoid compounds, terpenoids and tannins, especially those with high activity as antioxidants may be thought of as bioreductors [3, 5]. The secondary metabolite compounds act as bioreductors to reduce Au³⁺ ions to be Au uncharged (Au⁰) such as quercetin compounds [6] and tannins. One plant with a high antioxidant content and potential as a bioreductor in the synthesis of gold nanoparticles is *Muntingia calabura* L. leaf. *Muntingia calabura* L. leaf extract contains flavonoid compounds, triterpenes, tannins, alkaloids, polyphenols, quinones, monoterpenoid-sesquiterpenoids, steroids-triterpenoids, and saponins [7]. Proteins that play an important role in the biosynthesis of nanoparticles using *Muntingia calabura* L. leaf extract [8], whereas the compounds involved are tannins and phenolic compounds [9].
Generally, nanoparticles tend to be unstable because they have high surface energy and can cause aggregation and thus need to be stabilized. Stabilization plays an important role when nanoparticles will be characterized and applied in a product [10]. One of the polymers that can be used to stabilize gold nanoparticles is the poly acrylic acid (PAA) [11]. The nanoparticles resulted can be applied as glucose detection sensors to monitor blood sugar levels. Diabetes mellitus or diabetes is one of the diseases that arise in a person due to the body has problems in controlling blood sugar levels. Normal human blood contains glucose in a fixed amount or concentration, which is between 70-100 mg/10 mL of blood. However, in people with diabetes mellitus, the amount of blood glucose is greater than 130 mg/100 mL of blood [12].

Recently, research of glucose sensors for blood sugar determination has been widely developed. However, the method used usually involves a highly specialized enzyme for glucose and the activity of the enzyme will decrease significantly over time [1]. Now, many researchers developed non-enzymatic based sensors by modifying the material, using metals [13], metal alloys [14], metal oxides [15, 16] or metal nanoparticles [17]. Based on this background, this study has been done nanoparticle production using Muntingia calabura L as bioreductors. Furthermore, these nanoparticles are used to design sensors to analyze glucose levels as a reference blood sugar levels.

2. Experimental

2.1. Materials
The materials used in this study were leaf of Muntingia calabura L., gold metal, destilled water, poly acrylic acid (PAA), HCl p.a, HNO₃ p.a, NaOH, anhydrous glucose, gold wire, copper wire, electrode gold, Ag/AgCl electrode, Pt electrode, universal pH paper (E-Merck), Whatman filter paper No.42, aluminum foil.

2.2. Preparation of Muntingia calabura L Leaf Extracts
Plants used in this study are Muntingia calabura L leaf. The leaves Muntingia calabura L. plucked then washed with distilled water until clean and dried at room temperature. After drying, the leaves are cut into pieces and weighed as much as 10 grams. Then put in a 250 mL glass of water and boiled with 100 mL of bi-distilled water. Furthermore, boiling water was allowed to boil for 5 minutes. Then, the boiling water cooled to room temperature and filtered using Whatman filter paper No.42.

2.3. Synthesis of Gold Nanoparticles
The synthesis of gold nanoparticles was carried out by mixing 40 mL of a 25 ppm HAuCl₄ solution with 2 mL of 0.1 g/mL Muntingia calabura L. leaf water extract as bioreductor. Furthermore 10 mL of 1% PAA is added to the solution and the mixture is stirred with a magnetic stirrer for 2 hours. Then characterized by UV-Vis, SEM and PSA

2.4. Preparation of Gold Nanoparticles Electrode
The gold electrode is made of gold wire cut with a length of 3 cm as much as two pieces. Then both electrodes are connected with 5 cm copper wire (Cu) by solder using lead wire. Furthermore, both gold electrodes are inserted into a blue tip that serves as the body of the electrode. Both of these electrodes will be used as working electrodes. One of the gold electrodes has been made, modified with gold nanoparticles. The precipitation of gold nanoparticles is done by LBL (layer by layer) technique. The gold electrode was immersed in a 0.2% (pH 10) PAA solution for 30 min, then rinsed with Iabides and immersed into a gold nanoparticle suspension for 15 min, then rinsed again with Iabides. This cycle is repeated three times. These electrodes are called modified gold electrodes. Subsequently both electrodes were immersed into a standard solution of 8 mM glucose for 24 hours.
2.5. Measurement of Glucose Standard Solution
Measurement of standard glucose solution was performed by cyclic voltammetry method. The cell type used is a three-electrode system consisting of unmodified gold electrodes and modified gold electrodes (working electrode), platinum electrode (auxiliary electrode), and Ag/AgCl electrode (comparative electrode) with 0.1 M NaOH solution as electrolyte. Then measured standard solution of glucose 1, 2, 3, 4, 5, 6, 7, and 8 mM using potentiostat at potency -1 to +1 V. Next is calculated detection limit and selectivity of electrode based on data obtained.

3. Results and Discussion

3.1. Preparation of HAuCl₄ solution 1000 ppm
Initial stages in synthesis of gold nanoparticles with *Muntingia calabura* L. leaf as bioreductors are the preparation of 1000 ppm HAuCl₄ solution by dissolving 1 g of gold metal with 8 mL aquaregia. Aquaregia is a mixture of HCl and HNO₃ solution with a 3:1 ratio of 6 mL HCl and 2 mL HNO₃. The equation of the reaction that occurs is:

\[
\text{Au}^{(s)} + \text{HNO}_3^{(aq)} + 6\text{HCl}^{(aq)} \rightarrow \text{HAuCl}_4^{(aq)} + \text{NO}_2^{(g)} + 2\text{H}_2^{(g)} + \text{Cl}_2^{(g)} + \text{H}_2\text{O}^{(l)}
\]  

At the equation of reaction 1 occurs a redox reaction that Au uncharged oxidizes into a trivalent charged Au ion. The reaction process of dissolution of gold metal can take place spontaneously and the reaction produced NO₂ and H₂ gas so that required heating. The process of dissolving gold is left open in acid space as has been done. Heating is needed with the purpose to keep the remnants of acid remaining in the solution can be evaporated completely and odorless. The heating process is stopped when all the gold solids are completely dissolved so only the clear yellow HAuCl₄ and H₂O solution as shown in figure 1(a). The reaction HAuCl₄ solution was then diluted to obtain 1000 ppm HAuCl₄ as shown in figure 1(b). The parent solution of HAuCl₄ 1000 ppm is further used as a precursor solution in the synthesis of gold nanoparticles.

3.2. Synthesize of Gold Nanoparticles
Gold nanoparticles in this research was synthesized by biosynthesis method that utilizing plant extract of *Muntingia calabura* L. leaf as bioreductors. The 0.1 g/mL *Muntingia calabura* L leaf extract was added dropwise to a 25 ppm HAuCl₄ solution accompanied by stirring using a magnetic stirrer to homogenize the mixture to form a nanoparticle. The compounds contained in *Muntingia calabura* L leaf extract play a role reducing Au³⁺ ions to Au⁰. Reduction of Au³⁺ to Au⁰ is necessary because in the form of AuCl₄ ions-rejects to occur due to the effect of similar charges. However, after being reduced, the
charge of Au atoms becomes neutral. It’s allowing Au atoms to interact with each other to form nanosized clusters through metallic bonds.

3.3. Characterization of Gold Nanoparticles
The gold nanoparticles formed can be determined by the color change of the solution at difference of time. A solution color changed from a clear to purple with increasing time as indicates that gold nanoparticles have been formed [18]. The color change of the solution initially turns to gray and the color will be change after increasing time and the stirring process into purple clear.

The interaction between gold atoms occurs very rapidly and continues to grow, sometimes uncontrolled therefore the particle size of gold becomes very large even exceeds the nanometer size. In this condition, a stabilizer is needed to control the growth of the gold cluster. Based on the results polyacrylic acid (PAA) serves to stabilize the size of the gold nanoparticles and prevent agglomeration of particles. Based in this fact in this study used PAA stabilizer. The results show that PAA plays a good role in stabilizing gold nanoparticles. It can be seen in the change color of the solution which remains purple until day 8.

3.4. Characterization of Gold Nanoparticles using UV-Vis Spectrophotometers
The formation and growth of gold nanoparticles can be determined by qualitative analysis based on the specific characteristics of Plasmon Resonance Surface (SPR). SPR is a collection of oscillations of conduction electrons on the surface of the material. The existence of electromagnetic fields generated by light causes the excitation of the conduction band. The light interacting with the AuNPs surface can be localized, manipulated, and strengthened on a nanometer scale through the excitation of a collection of electron oscillations on metal nanoparticles, called Localized Surface Plasmon Resonance (LSPR). The existence of SPR caused a phenomenon in the color change from clear to clear purple which indicated the formation of gold nanoparticles as a result of the SPR phenomenon. Analysis using UV-Vis spectrophotometer showed that there was a change of intensity of UV-Vis wavelength absorption along with increasing time. In the first hour until the 5 hour the wavelength is 545 nm, 542.5 nm, 542 nm, 541 nm and 540 nm respectively with absorbance of 0.241; 0.243; 0.247; 0.255 and 0.258. The intensity of absorbance that increases with time increases the number of nanoparticles while the decreasing absorbance value signifies the formation of larger clusters due to agglomeration of particles [18].

There is a decrease in UV-Vis absorption intensity according to the increasing time. The intensity of absorption on the first day was 540 nm decreased into 534.5 nm on day 22. Likewise absorbance, on the first day was 0.258 decreased into 0.209 on the 22 day. But decreasing of the intensity is still linear from one to eight days. Based on it’s condition can be categorized that the nanoparticles produced tend to be stable until 8 days. The stability of the nanoparticles produced is caused by the addition of a stabilizer PAA at synthesis time. Application of the the PAA in this study caused it has the best affinity power in stabilizing nanoparticles. The resulting gold nanoparticles tend to stabilize up to 8 days due to the influence of the PAA with the ability as a capping agent.

3.5. Characterization of Gold Nanoparticles with PSA
Characterization with Particle Size Analyzer (PSA) aims to determine the diameter and distribution of gold nanoparticles in the sample. The measured particle size is the size of a single particle. The particle size data obtained in the form of three distributions are intensity, number, and volume of distribution, it can be assumed to describe the overall condition of sample while Polydispersity Index (PI) or polydispersity index shows equality of nanoparticle size in liquid medium. The determination result of gold nanoparticle size distribution using PSA obtained the mean size of gold nanoparticle diameter is 78.2 nm with polispersersity index equal to 0.318. The measure shows that the particles that are successfully synthesized.
3.6. **Characterization of Gold Nanoparticles with SEM**

Characterization using SEM instrument have purpose to show the morphology of the gold nanoparticles have formed. The enlargement of the sample images was performed on a scale of 5 μm, 10 μm, 20 μm and 50 μm with HV 20 kV and 7.50 mm Working Distance (WD). In figure 2 the resulting AuNPs have a non-uniform and round shape.

![SEM image of gold nanoparticles](image)

**Figure 2.** SEM image of gold nanoparticles (a) 5 μm scale (b) 10 μm scale

3.7 **Application of Nanoparticle-Based Gold Sensors**

Applications of gold nanoparticles as sensors for measuring glucose levels were performed using the cyclic voltammetry method. Observations were made by comparing the voltammogram of 2 working electrodes used in glucose measurement with a variation of 1 mM-8 mM concentration. The working electrode employed is a non-coated working electrode of gold nanoparticles and a working electrode coated with gold nanoparticles. The result analysis of the two working electrodes to measure glucose level shown in voltamogram figure 3.

Figure 3 (a) shows that the voltammogram oxidation peak of the working electrode not coated with gold nanoparticles is not clearly visible and tends to accumulate. The results of the voltammogram show the irregularity of measured current patterns in various variations of glucose concentration. It’s shows that the working electrode non-coated with gold nanoparticles is less sensitive to glucose. Figure 3 (b) is a voltammogram of a working electrode coated with gold nanoparticles is appearing the rising of current along with increasing glucose concentration. The mechanism of glucose reaction (figure 4) on the electrode surface coated with gold nanoparticles is as follows [19].

Measurable current patterns at regular glucose concentrations indicate that the working electrode with gold nanoparticle coating is sensitive to glucose and may be used in glucose analysis [6]. The result range of the working electrode measurements coated with gold nanoparticles can be seen in figure 5. Measurements of the working electrodes coated with gold nanoparticles are in the range of 1-4 mM measurements. Therefore the linear regression equation $y = 0.3435x - 0.05$ with $R^2 = 0.9248$. Furthermore, to know the capability and performance of electrode coated by gold nanoparticle, determination of limit value of detection and sensitivity.
Figure 3. Voltammogram of working electrode (a) non-coated gold nanoparticles (b) coated with gold nanoparticles

Figure 4. Mechanism of glucose reaction on the surface of the coated electrode gold nanoparticles
3.8 Detection Limit

The detection limit the smallest concentration of analyte that can be measured by instrument. The smaller the concentration that can be detected shows the better characteristics of the sensor. The detection limits of the working electrode coated by the gold nanoparticles are determined by making a tangent to the non-nuclarian linear function as shown in figure 6.

Figure 5. Linear regression curve of concentration vs. current strength

Figure 6. Detection limit of working electrodes coated with gold nanoparticles

Figure 6 shows the intersection points of the two lines then extrapolated to the x-axis to obtain the concentration of the detection limit. The extrapolation between linear nerstian curve and non-null linear curve obtained minimum detection limit of sensor at 0.1500 mM and maximum detection limit 4.1744 mM.
3.9 Sensitivity

Sensitivity is the ratio of signal changes per unit of change in analytical concentration [20]. The sensitivity test is performed to find out the sensitivity of a sensor to the analyte. Based on the equation of line \( y = 0.3435x - 0.05 \) then obtained the result of sensitivity calculation is \( 0.6837 \) A.mM\(^{-1}\).mm\(^2\). The results of the sensor analysis of glucose only have a measurement range at a concentration of 1 - 4 mM. The graph result has high regression value of 0.9248 which indicates that this sensor is quite sensitive to glucose detection.

4. Conclusion

Based on the result of research can be concluded that the leaf *Muntingia calabura* L extract is a potential as an agent of biosynthesis of gold nanoparticles. The gold nanoparticle shows at 544 nm with the incubation time is more than 5 minutes. The XRD characterization shows that the gold nanoparticle crystals have a particle size distribution of 36.93 nm. The SEM results shows the gold nanoparticles have uniform and rounded surface structures. The average size of the gold nanoparticles using PSA is 78.2 nm. Application of the gold nanoparticles as sensors are used in measure of the glucose levels with the measurement range is 1-4 mM with a regression value of 0.9248. The minimum detection limit of the sensor is 0.1500 mM and the maximum detection limit is 4.1744 mM with sensor sensitivity is 0.6837 A.mM\(^{-1}\).mm\(^2\).

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