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BIOSYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES AND THEIR INTERACTION STUDY WITH METFORMIN

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ABSTRACT

BIOSYNTHESIS AND CHARACTERIZATION OF GOLD NANOPARTICLES AND THEIR INTERACTION STUDY WITH METFORMIN. Synthesis of gold nanoparticles successfully carried using Imperata cylindrica L leaf extract. In this study, the approach used through green synthesis method is a reaction betwen of the $HAuCl_4$ solution (concentration variation as 3; 5; 7 x 10^{-4} M) with Imperata cylindrica L leaf extract. Results of UV-Vis showed of gold nanoparticles has a maximum wavelength at 530 nm with absorbance value of 1.4. Results of FTIR shows a shift the absorption peak at wavenumber of 3392 cm^{-1} to 3404 cm^{-1}. PSA and PZC showed the distribution of gold nanoparticles was 48.84 nm with a charge of 20.5 mV. Gold nanoparticles has a spherical shape and an average particle size of 20 nm which can be seen from the results of the characterization using TEM. XRD showed crystalize size average of gold nanoparticles as 20.47 nm. The interaction between of gold nanoparticles with metformin can be seen in the absorbance decrease of 0.38 at a wavelength of 531 nm and the results of PSA shows an average particle size of AuNPs@metformin is 122 nm. From the characterization data can be concluded the gold nanoparticles were successfully synthesized using natural bioreductors by utilizing secondary metabolites from Imperata cylindrica L leaf extract.

Keywords: Biosynthesis, Gold Nanoparticles, *Imperata cylindrica L*, Metformin

ABSTRAK

BIOSINTESIS DAN KARAKTERISASI NANOPARTIKEL EMAS DAN STUDI INTERAKSI DENGAN METFORMIN. Sintesis nanopartikel emas berhasil dilakukan menggunakan ekstrak daun ilalang (*Imperata cylindrica L*). Metode yang digunakan pada penelitian ini yaitu pendekatan melalui *green synthesis* yaitu mereaksikan larutan $HAuCl_4$ (variasi konsentrasi 3; 5; 7 x 10^{-4} M) dengan ekstrak daun ilalang. Hasil UV-Vis menunjukkan koloid nanopartikel emas memiliki serapan panjang gelombang maksimum pada 530 nm dengan nilai absorbansi 1.4. Hasil FTIR menunjukkan adanya pergeseran puncak serapan pada bilangan gelombang 3392 cm^{-1} menjadi 3404 cm^{-1}. Hal ini mengindikasikan adanya interaksi gugus –OH pada ekstrak daun ilalang terhadap ion Au^{3+}. Hasil PSA dan PZC menunjukkan distribusi ukuran nanopartikel emas sebesar 48.84 nm dengan muatan sebesar 20.5 mV. Nanopartikel emas memiliki bentuk bulat dan rata-rata ukuran partikel 20 nm yang terlihat dari hasil karakterisasi menggunakan TEM. Hasil XRD memperlihatkan kristal nanopartikel emas dengan rata-rata ukuran kristal sebesar 20.47 nm. Adanya interaksi antara nanopartikel emas dengan metformin terlihat pada penurunan nilai absorbansi nanopartikel emas sebesar 0.38 pada panjang gelombang 531 nm dan hasil PSA menunjukkan ukuran rata-rata partikel AuNPs@metformin sebesar 122 nm. Dari data karakterisasi dapat disimpulkan bahwa nanopartikel emas berhasil disintesis menggunakan bioreduktor alami dengan memanfaatkan metabolit sekunder dari ekstrak daun ilalang.

Kata kunci: Biosintesis, Nanopartikel Emas, *Imperata cylindrica L*, Metformin

INTRODUCTION

Gold nanoparticles are one of the metal that are many of still being studied, because has many uniqueness. Gold nanoparticles has particle size shapes from 1-100 nm and high particle stability. Precursors used in the synthesis of gold nanoparticles typically use tetrachloroauric (III) acid ($HAuCl_4$) as a source of Au^{3+} ions. Visually, the formation of gold nanoparticles is characterized by changes in the color of $HAuCl_4$ solution. In the process of reducing Au^{3+} ions to Au^0 involving reductants such as natrium tetrahidridoborat ($NaBH_4$), citric acid and sodium citrate [1]. This reducing agent is very dangerous and not environmentally friendly. Therefore, it is necessary to replace natural reductants through the green synthesis approach. Extracts from leaves, flowers, roots, and fruits on plants can be used as natural reducing agents in the synthesis of gold nanoparticles.

Tinospora crispa leaf extract [2], *Pistacia integerrima gall* extract [3], *Salix alba* extract [4], *Polyscias fruticosa* leaf extract [5], *Citrus maxima* aqueous extracts [6], cornelian cherry (*Cornus mas*) fruits extract [7], *Nerium oleander* leves extract [8], aqueous *garlic (Allium sativum L)* extract [9], successfully used as a reducing agent in the synthesis of gold nanoparticles. Visually, the color changes occur in colloidal gold nanoparticles such as: purple-blue and ruby-red [3,4], yellow, pink, blue and purple-grey [10], light yellow to violet [11], and yellow to red purple [12]. The difference in color produced in colloidal gold nanoparticles proves varying particle size. Gold nanoparticles has localized surface plasmon resonance (LSPR) properties that can vary according to the shape and size of the particles produced. The shape of the nanosphere, nanoshells and nanorod in particles of gold has different optical properties [13].

Colloid of gold nanoparticles can function as a drug delivery system that can interaction with ubiquicidin (29-41) [14], amoxicillin coated-AuNPs [15], vancomycin capped-AuNPs [16], ampicillin, streptomycin, and kanamycin conjugated-AuNPs [17], cefaclor and aminoglycoside-conjugated AuNPs [18,19]. In this study, colloid of gold nanoparticles will be interacted with the metformin drug.

Metformin is a drug used for diabetes mellitus of type 2. Giving the drug metformin will cause side effects if it is not on target. Side effects such as tremors, hypoglycemia, vomiting and nausea [20]. In this research, extraction of leaves of *Imperata cylindrica L* using water solvent will be carried out. Water fraction of *Imperata cylindrica L* leaf will be used as a medium and natural reductant agent in the synthesis of gold nanoparticles. Colloid of gold nanoparticles that are formed are expected to be able to interact with metformin drug which are seen on decreasing theabsorbance value on the spectrophotometer UV-Vis.

EXPERIMENTAL METHOD

Materials and Instruments

Sample of *Imperata cylindrica L* leaf were taken from Depok Area (West Java, Indonesia). Metformin was purchased from Merck (Darmstad, Germany). Gold metal 99.9% from PT Antam (Jakarta, Indonesia) and HNO_3 and HCl purchased from Sigma-Aldrich (Missouri, United States).

Characterization of gold nanoparticles using Shimadzu UV-Vis Spectrophotometer 2600 (Kyoto, Japan) with wavelength from 200-800 nm set and equiped with wolfram lamp as a light source. Interactions of functional group are analyzed using Shimadzu Fourier Transform Infrared (FTIR) Prestige 21 Spectroscopy with wavenumbers from 4000-400 cm^{-1} set and using nernst lamps (Kyoto, Japan). Particle size distribution seen using Particle Size Analyzer (PSA) and Potential Zeta Charge (PZC) Malvern ZEN 1600 with dynamic light scattering system (Malvern, United Kingdom). Morphological shapes of gold nanoparticles were seen using Transmission Electron Microscopy (TEM) JEM 1400, using 350 keV of electron beam energy (Nagoya, Japan). Crystal of gold nanoparticles in analyzed using Shimadzu X-ray Diffraction (XRD) 610 with Cobalt us a source of electron (Kyoto, Japan).

Method and Procedure

About 5 g of *Imperata cylindrica L* leaf was added 50 mL aquadest and stirred for 4 hours at 60 °C temperature. The results of *Imperata cylindrica L* leaf extract, are calculated percent yield and solution stock concentration. $HAuCl_4$ solution was synthesized by dissolving 99.9% gold metal with aqua regia (HNO_3:HCl) solvent with ratio of 1:3.

Synthesis of gold nanoparticles using various concentration of $HAuCl_4$ (3; 5; 7 x 10^{-4} M) respectively. About 10 mL $HAuCl_4$ was added 1 mL *Imperata cylndrica L* leaf extract (v/v). The reaction takes place at room temperature for 24 hours. Test of interaction was carried out by reacting 5 mL colloid of gold nanoparticles (7x10^{-4} M) with 2 mL of metformin solution (4x10^{-4} M).

RESULT AND DISCUSSION

The process of extraction of *Imperata cylindrica L* leaf lasts for four hours and produces a brown solution. After calculation, the results of *Imperata cylindrica L* leaf extract has a 0.5% yield and a stock concentration of the solution of 5%. Synthesis of $HAuCl_4$ solution of gold metal 99.9% produces a yellow color. Figure 1 shows the results of *Imperata cylindrica L* leaf extract the water fraction and the $HAuCl_4$ solution. $HAuCl_4$ discoloration is seen after the addition of *Imperata cylindrica L* leaf extract. The colloid of gold nanoparticles with various

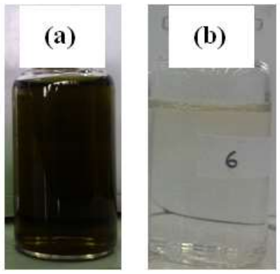

Figure 1. (a). Imperata cylindrica L Leaf Extract and (b). $HAuCl_4$ Solution

concentrations of $HAuCl_4$ produces a red-purple color. Figure 2 shows the color of the synthesis of gold nanoparticles using *Imperata cylindrica L* leaf extract.

Figure 2. Colloid of Gold Nanoparticles with Various Concentration of $HAuCl_4$ (a). 3x10^{-4} M, (b). 5x10^{-4} M, and (c). 7x10^{-4} M

The higher the concentration of $HAuCl_4$ used, the colloidal color of gold nanoparticles produced is increasingly red-purple. This indicates that many nanoparticles are formed [21]. The reduction reaction that occurs is seen in equation 1.

$$Au^{3+} + e^{-} \longrightarrow Au^{0} \quad \text{..........} \quad (1)$$

The source of electrons used to reduce Au^{3+} ions is predicted to come from phenol groups found in the flavonoid compound of *Imperata cylindrica L* leaf extract [12]. The colloid of gold nanoparticles has surface plasmon resonance (SPR) properties that can be seen on the UV-Vis spectrum. Figure 3 shows a spectrum of

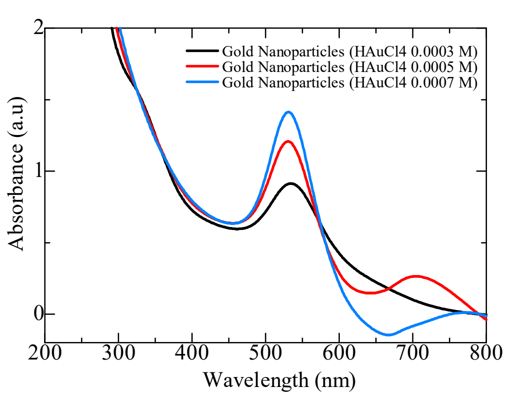

Figure 3. UV-Vis spectrum of gold nanoparticles colloid with various concentration of $HAuCl_4$.

gold nanoparticles with various concentrations of $HAuCl_4$ used. The wavelength of 530-532 nm is used to see the maximum absorption peak (λ_{max}) of gold nanoparticles formed. Phenomena of surface plasmon resonance on gold nanoparticles occur in visible light (530-532 nm wavelength).

The higher concentration of $HAuCl_4$ used, the greater of absorbance value of gold nanoparticles produced. The higher absorbance value of gold nanoparticles, the better of colloid stability, and the tendency of gold particles to form agglomeration is getting smaller [22]. Data on wavelength and absorbance values of gold nanoparticles are shown in Table 1.

Table 1. Data of Wavelength and Absorbance Values of Gold Nanoparticles with Various Concentration of $HAuCl_4$

Concentration $HAuCl_4$	λ_{max} Gold Nanoparticles	Absorbance Value
3x10^{-4} M	532 nm	0.9
5x10^{-4} M	530 nm	1.2
7x10^{-4} M	530 nm	1.4

Apart from act as a reducing agent, the function group such as C-H, C-N, C=C in *Imperata cylindrica L* leaf extract also functions as a capping agent for gold nanoparticles [23]. Figure 4 shows the functional groups found in secondary metabolites on *Imperata cylindrica L* leaf extract and the spectrum of gold nanoparticle colloids.

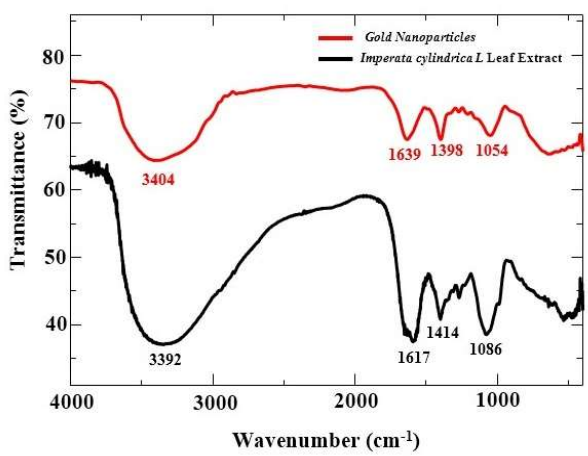

Figure 4. FTIR Spectrum of Imperata cylindrica L leaf Extract and results of biosynthesis of gold nanoparticles.

FTIR analysis functions to determine the functional groups found in *Imperata cylindrica L* leaf extract. The functional groups in secondary metabolites of *Imperata cylindrica L* leaf extract act as Au^{3+} ion reduction agents and capping agents for gold nanoparticles [23]. There is a shift in the absorption peak at the wavenumber from 3392 cm^{-1} to 3404 cm^{-1} (-OH), 1617 cm^{-1} to 1639 cm^{-1} (N-H), 1414 cm^{-1} to 1398 cm^{-1} (C-N), and 1086 cm^{-1} to 1054 cm^{-1} (C=C) [24]. This functional group indicates the presence of a compound as such flavonoids, alkaloids, terpenoids, and protein [25].

PSA and PZC results show the particle size distribution of gold nanoparticles. The peak of one has a size of 9.7 nm and a peak of two has a size of 87.98 nm. The average size of gold nanoparticles was 48.84 nm with a potential zeta charge is 20.5 mV. Figure 5a shows the graph of PSA and PZC of gold nanoparticles (5b).

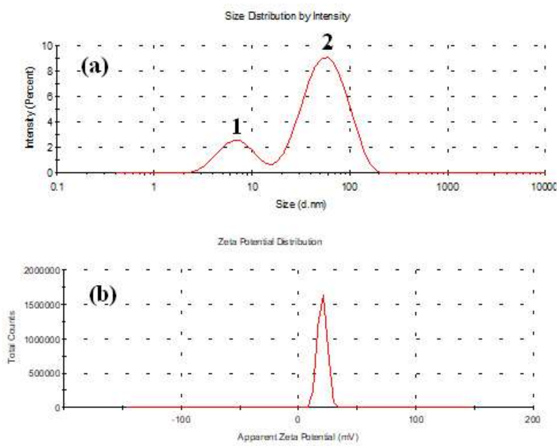

Figure 5. Spectrum Image of Gold Nanoparticles (a). PSA, and (b). PZC.

Results of the monodisperse nature of gold nanoparticles (PDI=0.578) and the value of PZC is corresponding to TEM images, gold nanoparticles have good stability.

Figure 6 showed of TEM images of gold nanoparticles. Gold nanoparticles have spherical shapes with particle size is 20 nm. The results of TEM showed the good homogeneity of gold nanoparticles. The density of gold particles was seen to be higher than the capping agent of *Imperata cylindrica L* leaf extract which was marked with strong black color on TEM.

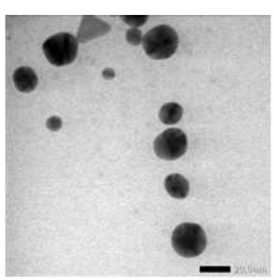

Figure 6. TEM of Gold Nanoparticles

There is a difference in the size of gold nanoparticles from the results of measurement using PSA and TEM. Results of PSA, the size of gold nanoparticles (87.98 nm) is greater than the results of TEM (20 nm). The measurement using PSA, there are several functional groups of *Imperata cylindrica L* leaf extract which acts as a capping agent for colloid of gold nanoparticles to be measured. Characterization using TEM, only shows of gold nanoparticle metals that measured, because the density of gold is greater than the functional groups in the extract.

The crystal structure of gold nanoparticles is analyzed as seen in XRD (fig. 7). Four Bragg reflection peaks are distinctly exhibited at 38.24^0, 44.49^0, 64.78^0, and 77.85^0 were indexed with the planes (111), (200), (220), and (311) of the face-centered cubic (fcc) structure of metallic gold (JCPDS no. 65-2870). These results are in corresponding with previous studies [26]. After the calculated using the Debye-Scherrer's, the average size of gold nanoparticles was obtained is 20.47 nm.

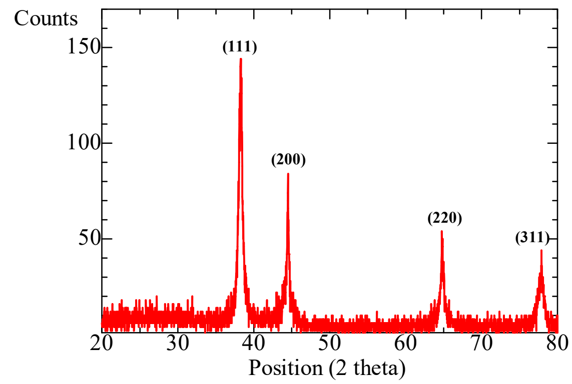

Figure 7. XRD Spectrum of as Biosynthesis Gold Nanoparticles.

Interactions of metformin with gold nanoparticles will increase the particle size of gold nanoparticles. Figure 8 showed a shift in wavelength from 530 nm (a) to 531 nm (b). The absorbance value of gold nanoparticles also decreased from 1.4 to 0.38. The presence of a new peak at wavelength 233 nm with an absorbance value of 0.54 is a typical peak of the metformin drug [27].

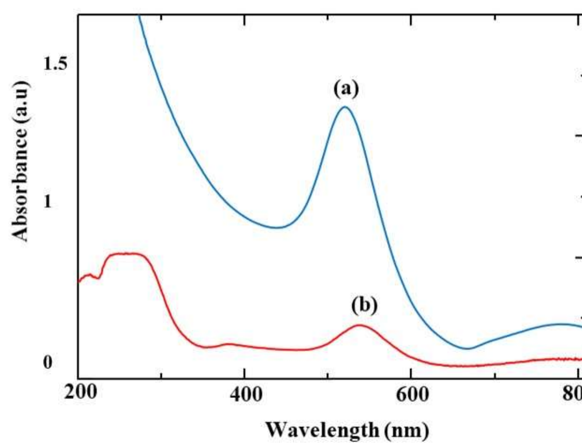

Figure 8. UV-Vis Spectrum of (a). Gold Nanoparticles and (b). Gold Nanoparticles@Metformin.

The interaction of gold nanoparticles with the metformin drug resulted in average particle size of 122 nm (fig. 9). Gold nanoparticles@metformin has a PDI value = 0.588 with three peaks of different sizes. The presence of active groups contained in metformin such as $-NH_2$, $-CH_3$, and -NH can interact on the surface of gold nanoparticles, causing the gold particle size to be larger as seen from the PSA analysis [20].

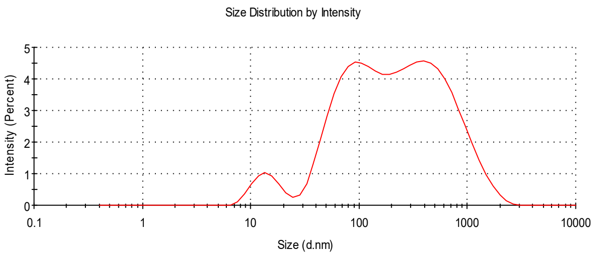

Figure 9. PSA Spectrum Image of Gold Nanoparticles @Metformin

CONCLUSION

Secondary metabolites contained *Imperata cylindrica L* leaf extract is very influential on the success of the synthesis of gold nanoparticles. UV-Vis showed the characteristic of gold nano particles with $HAuCl_4$ $7x10^{-4}$ M solution has the highest absorbance value of 1.4 with a wavelength of 530 nm. FTIR showed the sharp bands at 3392 cm^{-1} corresponding to –OH, –N–H, –C–N, –C=C– functional group for *Imperata cylindrica L* leaf extract. PSA and PZC values of 48.84 nm and 20.5 mV. The morphology of gold nanoparticles has sphere shapes with particle size is 20 nm. The crystal size of gold nanoparticles is 20.47 nm with face-centered cubic shapes. The interaction between metformin with gold nanoparticles is seen at wavelength shifts from 530 to 531 nm and PSA shows an average particle size of 122 nm.

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