

CHARACTERIZATION OF GOLD NANOPARTICLES FROM CLOVE FLOWER WATER EXTRACT AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

CHARACTERIZATION OF GOLD NANOPARTICLES FROM CLOVE FLOWER WATER EXTRACT AND ITS ANTIOXIDANT ACTIVITY. Green chemistry is a safe and cost-effective method for producing metal nanoparticles. The objective of this study is to synthesize, characterize and tested the antioxidant activity of gold nanoparticles (AuNPs). In this research gold nanoparticles were synthesized using HAuCl_4 as the gold precursor and water extract of *Syzygium aromaticum* (L.) flowers 0.5% as the bioreductor. Characterization of the gold nanoparticles was carried out using UV-Vis spectrophotometer to measure the maximum wavelength, zeta analyzer to determine the particle size, and SEM EDS to investigate the morphology and content of the gold nanoparticles. The antioxidant activity was determined by measuring its free radical scavenging activity using the DPPH method. The research found the AuNPs has a maximum wavelength at 533 nm, and particle size of 73.52 nm. Furthermore, the AuNPs show free radical scavenging activities of up to 70%.

Keywords: Green Synthesis, Gold Nanoparticles, Antioxidant, *Syzygium Aromaticum* (L.)

ABSTRAK

KARAKTERISASI NANOPARTIKEL EMAS DARI EKSTRAK AIR BUNGA CENGKEH DAN AKTIVITAS ANTIOKSIDANNYA. Sintesis hijau adalah metode yang aman dan hemat biaya untuk memproduksi nanopartikel logam. Penelitian ini bertujuan untuk mensintesis, mengkarakterisasi nanopartikel emas (NPAu) dan menguji aktivitas antioksidan dari NPAu. Dalam penelitian ini nanopartikel emas disintesis menggunakan HAuCl_4 sebagai prekursor emas dan ekstrak air bunga *Syzygium aromaticum* (L.) 0,5% sebagai bioreduktor. Karakterisasi nanopartikel emas dilakukan menggunakan spektrofotometer UV-Vis untuk mengukur panjang gelombang maksimum NPAu, zeta analyzer untuk mengetahui ukuran partikel, dan SEM EDS untuk mengetahui morfologi dan kandungan nanopartikel emas. Aktivitas antioksidan ditentukan dengan mengukur aktivitas peredaman radikal bebas menggunakan metode DPPH. Hasil penelitian menemukan NPAu memiliki panjang gelombang maksimum 533 nm, dan ukuran partikel 73,52 nm. Selanjutnya, NPAu menunjukkan aktivitas peredaman radikal bebas hingga 70%.

Kata kunci: Sintesis Hijau, Nanopartikel Emas, Antioksidan, *Syzygium Aromaticum* (L.)

INTRODUCTION

Nanoparticles are particles with diameters from 1 to 100 nm. These particles are commonly used in the industries, household, and healthcare products. The main challenge during the nanoparticle formation process relates to creating a size, shape and monodispersity that fits the nanoparticle criteria; and therefore improvements in the process of forming nanoparticles are required consistently [1]. Nanoparticles can be prepared using two methods, namely “bottom-up” (chemical method) and “top-down” (physical method) [2,3]. Both chemical and physical methods have succeeded in producing good nanoparticles, but there are drawbacks in chemical and physical methods so that the use of plant or herbal extracts in the fabrication of nanoparticles has emerged as an alternative approach over the last few decades. This nanoparticle biosynthetic method is environmentally friendly and easy to perform [4], cost-effective, and simple. The biosynthesis involves reduction of metal ions by plant secondary metabolites which are obtained by extracting the plant parts in green solvent, ie water. The secondary metabolites such as proteins, reducing sugars, flavonoids, terpenoids, and alkaloids act as reducing agents and capping agents to stabilize the particle size [5-12]. The extracts used to synthesize AuNPs were cloves (*Syzygium aromaticum* (L.) Merr. & L.M.Perry). Cloves (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) contains eugenol which is the main compound (72-90%). Eugenol is a secondary metabolite that can be used as a bioreductor and capping agent in synthesizing AuNPs [13]. Clove flowers are showed in Figure 1.



Figure 1. *Syzygium aromaticum* (L.) Merr. & L.M.Perry

The AuNPs has attracted attention in recent years because of their widespread use. The AuNPs have a larger surface area, higher dispersion due to their very small size, stable and biocompatible. The AuNPs have tremendous benefits in medicine, for treatment of diabetes mellitus, cancer, cardiovascular disease, tuberculosis, and in controlling antibiotic resistance [14-19]. Apart from the above benefits, AuNPs also have the

ability to act as antioxidants [20], which, when used as dietary supplements it will have helped people fight the disorders like cardiovascular disease, aging, and cancer [21].

The antioxidant properties of the nanoparticles are important applications in therapeutic and biomedicine field [22-25]. There have been no reports of the antioxidant properties of AuNPs synthesized from *Syzygium aromaticum* (L.). We investigated the characterization of these AuNPs and their potential as antioxidants.

EXPERIMENTAL METHOD

Materials and Instruments

Materials used in this experiment: *Syzygium aromaticum* (L.) Merr. & L.M.Perry flowers were obtained from the Negara Bali, Indonesia, and properly identified by the Indonesian Institute of Sciences, Bedugul, Bali, Indonesia. The HAuCl_4 solution used as a precursor in this study was prepared by dissolving 99.99% of pure Au metal (PT Antam) in aqua regia solution. 1,1-diphenyl-2-picrylhydrazyl (DPPH) for the antioxidant test was obtained from Nitra Kimia. Demineralised water (Aqua DM) was obtained from PT Brataco

The equipment used in this study were glassware (Pyrex), blender, hot plate (IKA C-MAG HS 7), analytical balance (Acis), filter paper, 50 mesh sieve, UV-Vis spectrophotometer (Genesys 10S UV-Vis) to measure the maximum wavelength of AuNPs, magnetic stirrer, zetasizer (Malvern) to determine the particle size, SEM-EDS (JSM-6510LA) to investigate the morphology and content of the gold nanoparticles.

Method and Procedure

Five grams dried of *Syzygium aromaticum* (L.) Merr. & L.M.Perry flowers were ground and blended with 100 mL of Aqua DM, then heated for 15 minutes at 60 °C. The mixture was filtered with Whatman 42 filter paper. An extract concentration of 0.5% was prepared by diluting 100 mL of the filtrate in 1 L using the Aqua DM.

The synthesis of gold nanoparticles was carried out by mixing 0.5 mM HAuCl_4 and 0.5 % *Syzygium aromaticum* (L.) Merr. & L.M.Perry flowers water extract with a ratio of 1:10. Then the solution is heated at 40 °C and 80 °C. As a visual indicator for the formation of

AuNPs, the colloid changed color from clear yellow to cherry red. The AuNPs formed were characterized by some methods. First, the wavelength of gold nanoparticles was measured using a UV-Vis spectrophotometer (Genesys 10S UV-Vis) in the range of 400-800 nm. The morphology and composition of the AuNPs was determined by scanning electron microscopy energy dispersive spectroscopy (SEM-EDS) (JEOL JSM-6510LA). The particle sizes were measured using dynamic light scattering technology (DLS) with a zetasizer (Malvern, UK).

The antioxidant activity of the AuNPs was studied by their free radical scavenging ability of DPPH [26]. Various concentrations colloid of AuNPs (10, 20, 30, 40, 50, 60, and 70 mg/mL) were mixed with DPPH and incubated for 30 minutes. After 30 minutes, the absorbance of the solution was measured using a UV-Vis spectrophotometer at 517 nm. For the blank used Aqua DM and DPPH without sample.

Antioxidant testing use synthesis temperature of 80 p C, but the ratio between clove flower water extract and HAuCl₄ solution varies based on the concentration to be made. From measurements using a UV-Vis spectrophotometer, all colloid concentrations must be showed a maximum wavelength between 500-600 nm, but the zetasizer test was not carried out for these 7 concentrations, so that the particle size of each concentration is not known with certainty. The formation of AuNPs was only suspected based on spectrophotometer UV-Vis measurements. The percentage of scavenging activity is calculated using equation (1).

$$\text{Percent Scavenging Activity (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

The maximum wavelength of AuNPs

AuNPs were synthesized using different synthesis temperatures. The purpose of this variation is to determine whether temperature has an effect in reducing gold nanoparticles. Measurements are carried out at wavelength range of 400-800 nm on days 1, 2, 3 of the synthesis so that the measurements were carried out 3 times.

The AuNPs formed under the two varied temperatures show the same maximum wavelength of 533 nm. This proves that gold nanoparticles have been

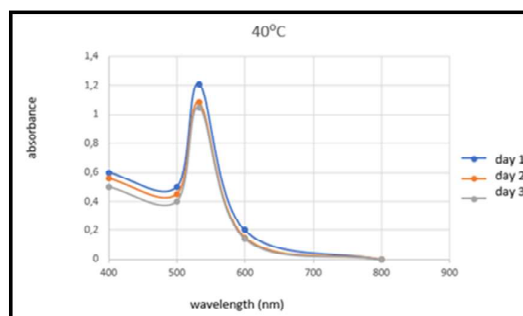


Figure 2. The absorbance of gold nanoparticles synthesized at 40 °C

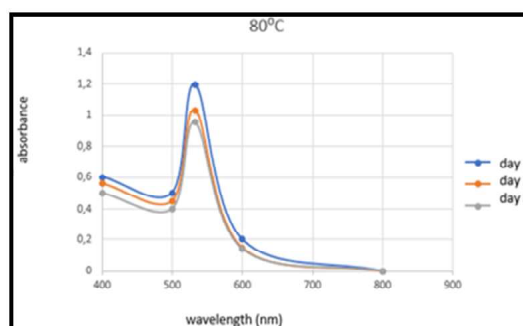


Figure 3. The absorbance of gold nanoparticles synthesized at 80 °C

formed since they have maximum absorbances at a wavelength between 500-600 nm [27]. Surface plasmon resonance peak analyzed using the UV-Vis spectrum showed a peak at a wavelength of 533 nm (Figures 2 and 3). The flower of *Syzygium aromaticum* (L.) Merr. & L.M.Perry succeeded in synthesizing AuNPs where the maximum wavelength of these AuNPs is 533 nm with a color change to cherry red. AuNP exhibits surface plasmon resonance due to oscillation of free electrons in the presence of a specific wavelength of light. In AuNP, a change in color was observed from yellow to cherry red and in some cases light to purple, depending on the shape and size of the nanoparticles. Phenomena of surface plasmon resonance on gold nanoparticles occur in visible light (500-600 nm wavelength) [28-30]. In Figure 4, it can be seen that the secondary metabolite components of plant extracts are responsible for reducing metal salts which will later lead to the growth and stability of gold nanoparticles [31-33].

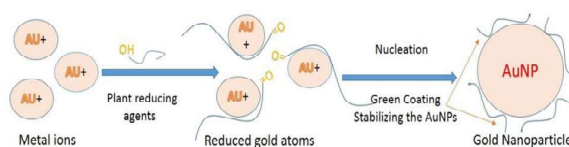


Figure 4. Biosynthesis of AuNPs with plant extract

After a statistical test was carried out on the effect of synthesis temperature on the formation of gold nanoparticles (Table 1), it was concluded that

sig (2-tailed) > 0.05 so that the different synthesis temperatures did not give a difference in results for the gold nanoparticle wavelength analysis. It can be concluded that the synthesis temperatures of 40 °C and 80 °C both produce gold nanoparticles which have a maximum wavelength of 533 nm.

Table 1. Results of statistical tests on the effect of synthesis temperature on the maximum wavelength of AuNPs

		Independent Samples Test				
		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
Absorbansi	Equal variances assumed	.589	.486	.630	4	.563
	Equal variances not assumed			.630	3.493	.568

Particle Size Analysis

Samples of AuNPs synthesized under both temperatures were analyzed by zetasizer. Table 2 shows the results of the analysis.

Table 2. Results of Particle Size Analyzer

	AuNPs (40 °C)	AuNPs (80 °C)
Particle size (nm)	109.83	73.52
Polydispersity	0.439	0.431
Index		

Table 2 shows that the particle sizes vary with temperature. The higher of reaction temperature, the size gold nanoparticles formed get smaller. Synthesis nanoparticles at higher temperatures produce particles with the average size smaller where the smallest AuNPs size was obtained through 73.52 nm. This is because the higher temperature (50 °C - 100 °C), most of the gold ions are formed turns into a gold nanoparticle nucleus (nuclei) and inhibits further reduction reaction processes on the surface of the pre-formed nuclei [34]. The smallest AuNPs size obtained was 73.52 nm with polydispersity index value 0.431. Polydispersity index shows the uniformity of the particle sizes. The smaller polydispersity index value, state that the more homogeneous the particle size [35]. The polydispersity index value greater than 0.5 indicates heterogeneity size of AuNPs. The results of the PSA showed that the nanoparticles obtained were in accordance with the literature. The particle size distribution can be seen in Figure 5 and 6. In Figure 5, there are 2 peaks. This is because the particle size is not evenly distributed where the PDI is 0.439. There are 2 peaks below 100 nm and above 100 nm, but the size of AuNPs above 100 nm is more dominant.

After a statistical test was carried out on the ef-

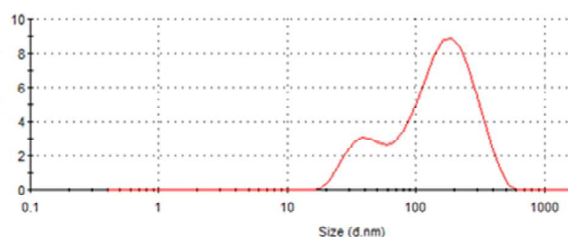


Figure 5. Particle size distribution of AuNPs in 40 °C

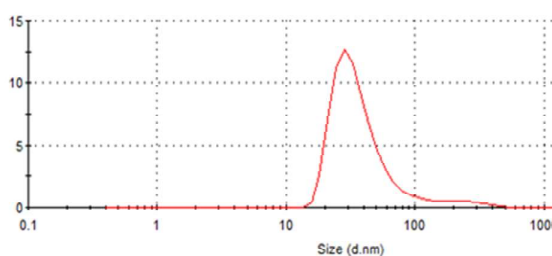


Figure 6. Particle size distribution of AuNPs in 80 °C

fect of synthesis temperature on the formation of gold nanoparticles (Table 3), it was concluded that sig (2-tailed) < 0.05 so that the different synthesis temperatures did give difference results for the gold nanoparticle sizes. It can be concluded that the synthesis temperature of 40 °C and 80 °C resulted in different sizes of gold nanoparticles where at 40 °C the size of gold nanoparticles obtained was 109.83 nm while at 80 °C the size of gold nanoparticles obtained was 73.52 nm.

Table 3. The results of statistical tests of the effect of synthesis temperature on the particle size of AuNPs

		Independent Samples Test				
		Levene's Test for Equality of Variances				
		F	Sig.	t	df	Sig. (2-tailed)
Ukuran Partikel	Equal variances assumed	.012	.918	14.590	4	.000
	Equal variances not assumed			14.590	3.997	.000

Scanning Electron Microscopy Energy Dispersive X-Ray Spectroscopy

Figure 7 and 8 shows a SEM image of AuNPs at the nanometric scale and clearly shows that the synthesized AuNP is spherical. SEM images of AuNPs formed after cessation of the reaction between *Syzygium aromaticum* (L.) Merr. & L.M.Perry and HAuCl₄ solution. From Figure 7 and 8, it is evident that the AuNPs are spherical with a diameter about 73.52 nm.

Result obtained from the EDS provides a clear indication of nanoparticle elements. The strong signal of the gold atom confirmed that the gold nanoparticles contained pure gold (Figure 9). Typical optical absorption is between 2-4 KeV confirms metal nanoparticles due to surface plasmon resonance [36]. Other element

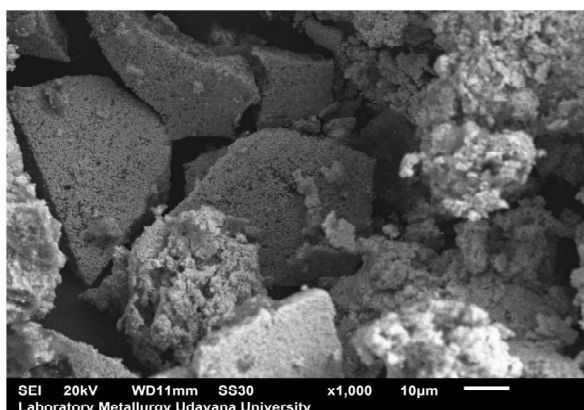


Figure 7. SEM image of AuNPs with magnification of 1.000x

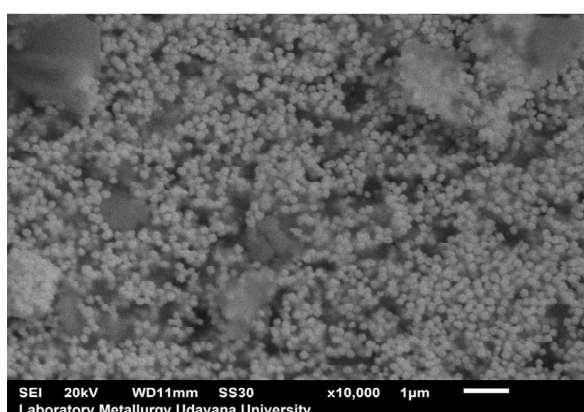


Figure 8. SEM image of AuNPs with magnification of 10.000x

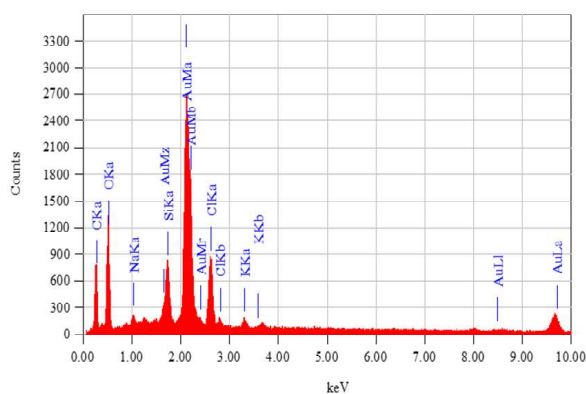


Figure 9. The composition of AuNPs determined by EDS

signals on the EDS spectrum is derived from plant secondary metabolites which function as stabilizing agents.

Antioxidant Activity of AuNPs

DPPH can change color from purple to yellow due to reduction by hydrogen or electrons [37]. In the DPPH free radical reduction test, compounds that can reduce DPPH are considered as antioxidants. In the

antioxidant analysis, it is shown that the AuNPs are able to react with free radicals because they have antioxidant activity. Figure 11 represents the percentages of DPPH scavenging activities. Samples can be seen in Figure 10.

Gold nanoparticles showed the ability to react with free radicals at several concentrations. The percentage of free radical reduction by AuNPs is good, above 30% and up to 70%. The antioxidant activity of gold nanoparticles highly dependent on the surface area. The smaller the particle size, the larger the surface area, and thus the higher the antioxidant activity [38]. Gold nanoparticles are smaller, have a larger total surface area, are more efficient in the antioxidant activity test. Free radical scavenging reaction mechanism from nanoparticles gold is gold nanoparticles giving electrons or hydrogen to free radicals thus free radicals become stable [39].

From Figure 11, it is apparent that the free radical scavenging ability increase from 10 to 30 µg/mL AuNPs, and then tend to decrease with increasing concentrations up to 70 µg/mL. This is probably because the formation of the nanoparticles is incomplete. Formation imperfections can occur because the stirring is not stable, ratio between extract and HAuCl₄, either the duration or the speed. Moreover, perhaps in the higher concentrations the particles aggregate more, hence decreases the surface area. This is because the higher concentration, the more gold particles formed, resulting in more collisions [40]. Based on the results of



Figure 10. Samples with various concentrations

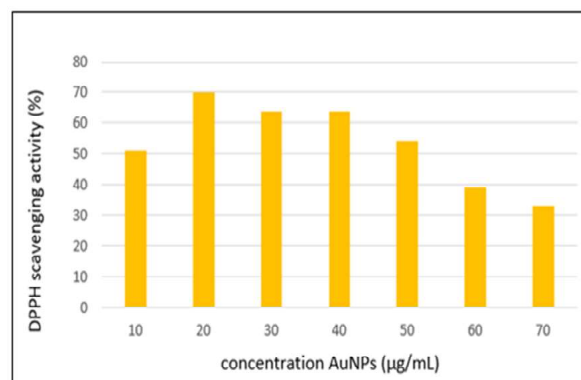


Figure 11. Antioxidant activity of AuNPs

Figure 11, the IC₅₀ of NPAu is 26.28 ppm where NPAu is classified as a very strong antioxidant.

CONCLUSION

This study finds that the *Syzygium aromaticum* (L.) flowers water extract has been successfully used to synthesize gold nanoparticles with a maximum wavelength of 533 nm, have a spherical crystal morphology with the main component of gold. The particle size obtained by the synthesis at 80 p C is 73.52 nm. The gold nanoparticles show antioxidant activity with free radical scavenging up to 70% and IC₅₀ is 26.28 ppm.

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