BIOSYNTHESIS AND CHARACTERIZATION GOLDNANOPARTICLES USING EXTRACTS OF *SARANG SEMUT (Myrmecodia pendans)*

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Abstrak. Nanopartikel emas berhasil disintesis dengan metode sederhana dan relatif aman dengan menggunakan kaemferol dan quersetin yang terkandung dalam ekstrak air sarang semut sebagai agen pereduksi, Pembentukan nanopartikel diidentifikasi oleh perubahan warna (kuning ke merah) terjadi dalam larutan asam kloroaurat setelah ditambahkan ekstrak air dari tanaman sarang semut. Spektrum UV-Visible menunjukkan keberhasilan pembentukan AuNPs dengan panjang gelombang maksimal 521,5 nm yang stabil selama 4 hari, dibuktikan dengan Ananlisis PSA yang mengkonfirmasikan kehadiran partikel dengan ukuran 53,2 nm. Ananlis XRD menampilkan puncak difragtokram yang merupakan nanopartikel emas. Hasil SEM memperlihatkan permukaan nanopartikel emas berbentuk bulat dan batang. Dengan demikian, ditemukan bahwa kinerja pereduksi dan penstabil dapat disediakan oleh ekstrak sarang semut. Oleh karena itu, penggunaan reduktor dan agen penstabil sintetis tidak diperlukan untuk sintesis partikel nano. Penelitian ini menunjukkan bahwa zat pereduksi dan penstabil kinerja tinggi dapat diisolasi secara biosintesis yang hijau secara kimia dan dapat menggantikan metode berbahaya yang ada saat ini.

Kata kunci: biosintesis, nanopartikel emas, sarang semut.

Abstract. Gold nanoparticles have been synthesized with simple and relatively safe method by using kaemferol and quercetin containing aqueous extract of Myrmecodia pendans (sarang semut plant). Under this method, it was able to obtain AuNPs and measured the size. The formation of the nanoparticles were identified by the color change (yellow to red) occurred in the chloroauric acid solution after additional aquoeos extract of sarang semut plant. The UV-Visible spectra and particle size analyzer indicated the successful formation of AuNPs with wavelength of 521.5 nm which was stable for 4 days, evidenced by the presence of particles with a size 53.2 nm. XRD analysis showed the difractogram peak of gold nanopartcle. The result of SEM analysis indicated gold nanopartcle surface with spherical and rod-like shapes. Thus, it is found that the reduction and capping performances can be provided by the extract. Therefore, the use of synthetic reducing and stabilizing agents was not necessary for nanoparticles synthesis. This work exemplifies that high performance reducing and stabilizing agent could be isolated biosynthetically that is chemically green and may replace the harmful method present nowadays.

Keywords: biosynthesis, gold nanoparticles, sarang semut.

INTRODUCTION

The synthesis of nanoparticle utilizing plant extract allow for controlling the shape and size of the synthesized materials potential for several applications. The plant extract which is categorized as secondary metabolic compounds can be acted as reductor and stabilizing agent in synthesizing nanoparticles. The characteristics and properties of the plant extract is become pivotal factor in influencing the morphology of the synthesized nanoparticles (Mukunthan dan Balaji, 2012).

One of plant that potential to be utilized for an active stabilizing agent is Myrmecodia pendan which well-known as 'umbi sarang semut (Indonesia)". The metabolic compounds that be found in Sarang semut plants are Falvonoid, phenolic acid, thanine, polyphenol, tocopherol, terpenoid, ketone, aldehyde, protein, amino acid, vitamine, alkaloid, tanine, phenolate, saponine, and polysaccharide and also minerals ((Engida, et. al. (2013); Nath (2013) and Kurniawati and Santuri (2016)). Those organic compounds are known to have ability as metal-ion reductot agent in metal nanoparticle synthesis (Philip, et. al , 2010). Thus, Sarang semut plants extract is potential as bio-reductor for biocomaptibel gold nanoparticle synthesis in different fields such as medicine, catalytic, cosmetic and food.

MATERIAL AND METHOD

Instruments

The instruments were employed in this research were, dropping pipettes, volumetric pipette, beaker-glass, Erlenmeyer flask, volumetric flass, analytical balance, stirring rod, spatula, petri-dish, magnetik stirrer, spray bootle, oven, blender, gold electrode, scissor, electrical freezer, sample bootle, centrifuce (TOMY mx-305, freese-drier Alpha 1-2 LD, voltammetric cyclic, Automated Analyzed Clinical Chemistry Pentra C-200, and also in characterization step, UV-Vis spectrometer (Shimadzu UV-2600), Scanning Electron Microscopy (SEM) dan Energy Dispersive X-Ray Spectrometer (EDS) MA10-14-37 ZEI ss EVO, X-Ray Diffraction (XRD) Shimadzu 7000 and Particle Size Analyzer (PSA) VASCO DLS were substantially employed.

Materials

Materials used in this research including glucose anhydride, "sarang semut" plant, HAuCl4 (Antam), HCl 12 N (Merck), HNO_3 12 N (Merck), PAA (Sigma Aldrich), NaOH (Merck), Kaemferol (Alibaba, Cina), quercetin (Sigma Aldrich), methanol (Merck), aquabides, Whatmann paper No. 42, tissue, dan aluminium foil.

Methods

1. **Preparation of a standard glucose solution**

Standard glucose 10 mM solution was prepared by dissolving 1,8 g of glucose anhydride into 1000 ml of aquabides. Subsequently, the dissolution was performed to obtain glucose with concentration in the range of 1 mM – 10 mM.

2. Preparation of kaempferol 0,1M and Quercetin 0,1M

a. Kaemferol 0,1M

Kaempherol 0.1 M solution was prepared by dissolving 0.286 g of pure kaemperol into 10 mL of methanol, stirred till

dissolved. Finally, this solution was used in synthesizing of the gold nanoparticle.

b. Quersetin 0,1M

Quercetin 0.1 M solution was prepared by dissolving 0.302 g of pure quercetin into 10 mL of methanol, stirred till dissolved. Finally, this solution was used in synthesizing of the gold nanoparticle.

3. Preparation of Sarang semut extracts

The plants which used in this biosynthesis experiment was Myrmecodia pendan (Sarang semut plants). This plant was received from Merauke regency, Papua province.

a. **Preparing of Sarang semut**

Sarang semut plants obtained from deep-forest was previously cleaned. The leaves was cut-off using a knife. The outer skin was also cut-off by knife. It further divided into four parts and cut again into pieces. Those pieces were dried unde undirect sun-light. Finally the dried powder was obtained after milling treatment by particular milling machine

b. Extraction of Sarang semut

Sarang semut powder was prepared as much as 10 g. Subsequently, it was extracted aquabides (1 g : 10 mL) at 30 ^{0}C for 15 min. The supernatant was filtered using whatman paper and further used as for biosynthesis process.

4. Preparation of $HAuCl_4$

1 g of gold metal was dissolved into 8 mL aquaregia while heating. The heating treatment was performed till the gold metal was perfectly dissolved and both nitrite and hydrogen gaseous were evaporated. The resultant pure gold solution was then diluted into volumetric flask 1000 mL with distilled water. Aquaegia was prepared by mixing 6 mL of HCl 12 N and 2 mL of HNO_3 12 N solution (3:1, v/v).

5. Synthesis of gold nanoparticles

a. **Biosynthesisof Sarang semut extracts**

The biosynthesis of gold nanoparticle was performed by mixing 30 ppm $HAuCl_4$ solution and Sarang semut extract. Typically, as much as 15 mL of Sarang semut extract was mixed with 30 mL of $HAuCl_4$ 30 ppm and stirred for 2h. the formation of nanoparticle was observed by the change of colour from yellow to red.

b. **Synthesis with kaemferol**

The biosynthesis gold nanoparticle was performed by mixing 30 mL $HAuCl_4$ 30 ppm solution and 3 drops of kaempherol 0.1 M and stirred for 2h. The formation of nanoparticle was observed by the change of colour from yellow to purple.

c. Synthesis with quercetin

The biosynthesis gold nanoparticle was performed by mixing 30 mL $HAuCl_4$ 30 ppm solution and 3 drops of quercetin 0.1 M and stirred for 2h. The formation of gold nanoparticle was observed by the change of colour from yellow to purple.

6. Characterization the gold nanoparticles

a. Using UV-Visspectroscopy

The obtained gold nanoparticle solution was characterized by using UV-Vis spectroscopy after 0 day, 1 day, 2 days, 3

days to observe the stability of the formed gold nanoparticles. The formation of gold nanoparticle characteristic intense peack on several adsorptions observed under UV-Vis spectroscopy analysis. Spcifically, Au nanoparticle possessed high absortion in the range of 500 – 550 nm, originated from surface plasmon resonance (SPR) effect occurred on metal nanoparticle (Noruzi, 2015).

b. Particel Size Analyzer (PSA)

The obtained gold nanoparticles solution was characterized using a PSA to see the particle size distribution of the gold nanoparticles produced.

c. X-RAY Diffraction (XRD), *Scanning Electron Microscopy* (SEM), and *Energy Dispersive X-Ray Spectrometer* (EDS).

The obtained gold nanoparticles solutionwere centrifuged for 30 minutes under 1000rpm, solid gold nanoparticles produced after 24 hours dried with freeze dryer were then characterized using XRD to confirm the purity of the gold nanoparticles produced (Emery, 2016). The crystal size was determined using the Scherrer formula as follows:

$$D = \frac{K\lambda}{\beta COS(\theta)} \qquad (1)$$

K = is a constant whose magnitude depends on the shape crystal factor (0.98), crystal latice (hkl) diffraction,

λ = wavelength Cu (1.54A),

θ = diffraction angle,

β = Full width at half maximum (FWHM) or Integral Breadth of the peak (Satoshi and Nick, 2013).

Meanwhile, the SEM was employed to explore the morphology of the obtained gold nanoparticles.

RESULT AND DISCUSSION

1. Biosynthesis of gold nanoparticles

The change of color of the gold solution after the additional Sarang semut extract from yellow to red wine (Vijayaraghavan, 2017) indicates the occurrence of reduction reaction of gold which transformed Au^{3+}) to Au^0 confirminf the successful formation gold nanoparticles (Figure 1). This phenomenon has been reported by several several reported work in the synthesis of gold nanoparticles, including Fachrurrazie and Wibowo (2017) whose obtained a gold nanoparticle of red-wine color using of sodium citrate and Ahmad, et al. (2016) by using coconut leaf oil extract (Elaise guineensis).

Figure 1. Gold Nanoparticles

a. Characterization of gold nanoparticles

The size, morphology and the shape of the obtained gold nanoparticles were characterized by UV-Vis spectroscopy, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectrometer (EDS) and Particle Size Analyzer (PSA).

b. Characterization of golf nanoparticles by UV-Visible.

The UV-Vis spectroscopy showed spectra at wavelength of 523.5 - 521.5 nm after additional Sarang semut extract into $HAuCl_4$ solution(Figure 2). PAL and Kryschi (2015) reported that the UV-Vis absortion of gold nanoparticles of occurs at a wavelength of 520-580nm. Thus, UV-Vis spectroscopy analysis evidenced the formation of gold nanoparticle and more importantly, the absorption absorbance was increased during the prolong reaction time in the respective wavelength (Noruzi, 2015).

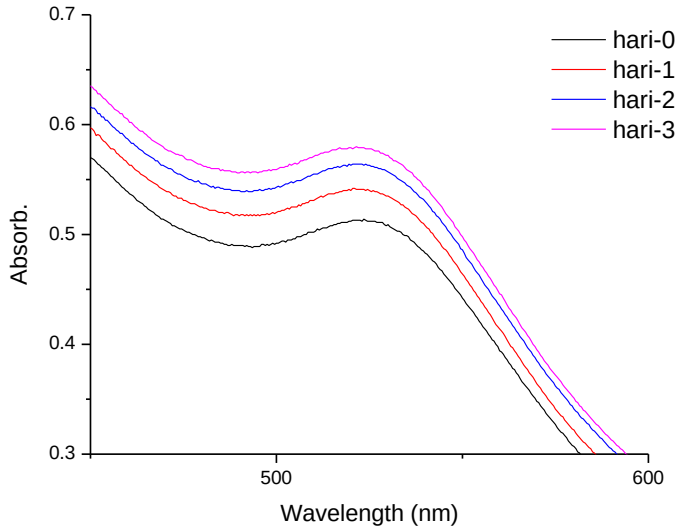


Figure 2. Spectrum of UV-Vis the Gold Nanoparticles

Figure 2 shows no significant changes on the maximum absorption (523.5, 520.5, 521.5 and 521) during consecutive time which inferred that obtained gold nanoparticles of gold relatively stable for 4 days observation and the amount of nanoparticles formed was increased as the absorbance was also enhanced from 0.514 to 0.580.

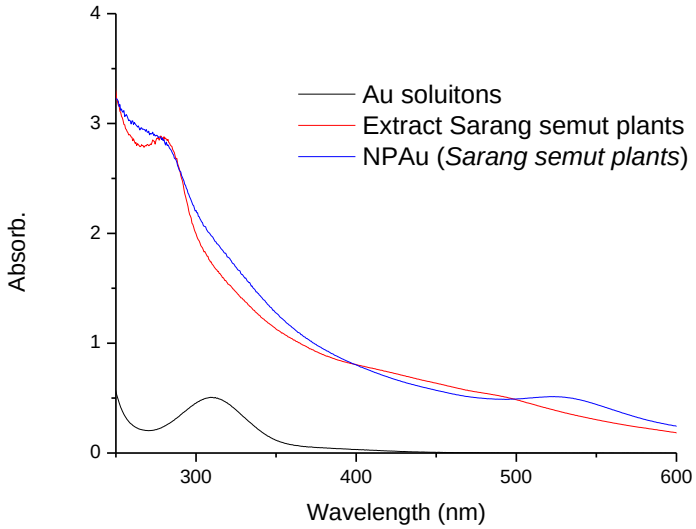


Figure 3. Spectrum UV-Vis Au, *Myrmecodia Pendan extracts,* and NPAu

Figure 3 shows the UV-Vis spectra for $HAuCl_4$ solution, Sarang semut extract and Sarang semut extract added HAuCl4 solution for. 309nm, 230.5nm and 523.5nm, respectively. The difference on the maximum absorption wavelength indicates a specific character of a compound or particles. The spectra of HAuCl4 solution after added Sarang semut extract confirming the formation of gold nanoparticle, in which the maximum absorption was righ-shifted and broader in the area of gold nanoparticle absorption (500-550 nm) (Noruzi, 2015). This is in accordance with the previous work reported by Nagaraj, et al. (2012) that he found the maximum absorption of gold nanoparticleswas at ~ 550 nm.

Gold nanoparticles was formed because of the ability of flavonoid compounds (Zhou, 2010) from Sarang semut extracts, included kaemferol and quersetin. Kaemferol and quersetin have the ability to reduced gold ions ($Au3^+$) to Au^0 (Basavegowda et al., 2014). Figure 4 shows the UV-Vis spectrum of gold nenoparticles obtained used kaemferol and quersetin. After being reduced to Au^0, the Au atom charge becomes neutral so that Au electron atoms to interacted with each other through metal bonds formed a nano-sized cluster.

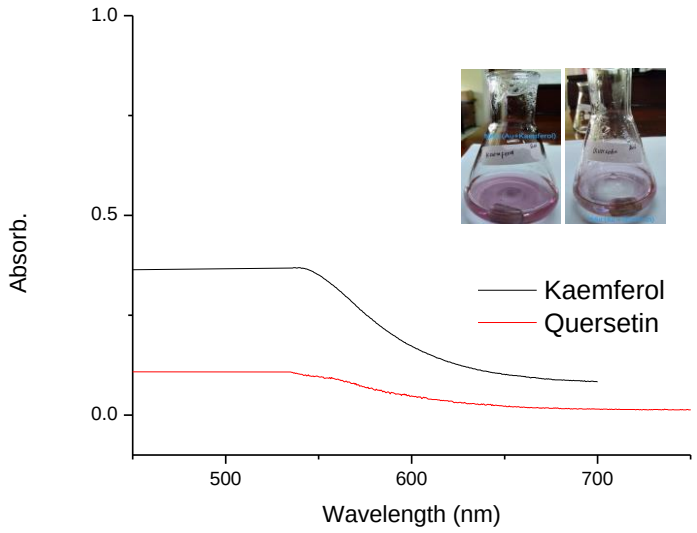
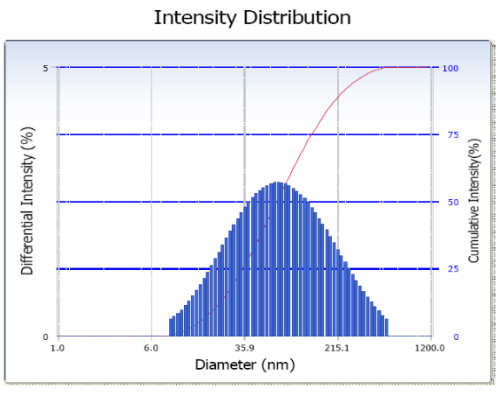


Figure 4. UV-Vis Spectrum of gold nanoparticles from kaemferol and quersetin

The maximum absorption of gold nanoparticles used kaemferol and quercetin standard are 536.5 and 565.5 nm which was regions of gold nanoparticle absorption. Thus the Sarang semut extract has the ability as a reducing agent needed for the synthesis of nanoparticles. This is in accordance with the previous work reported by Ahmed and Ikram (2015) that plant extracts can act both as reducing and stabilizing agents in the synthesis of nanoparticles.

c. Characterization of Gold Nanoparticles Colloid by*Particle size Analyzer* (PSA)

Particle Size Analyzer (PSA) shows the size of the gold nanoparticles obtained is 53.2 nm, this is in accordance with the theory, and the material is classified as a nanoparticle if it has a size of 1-100nm. Figure 5 illustrates the overall condition of the sample based on data on the intensity, volume and quantity distribution. The size distribution of nanoparticles ranges from 55.2 ± 8.6 nm.



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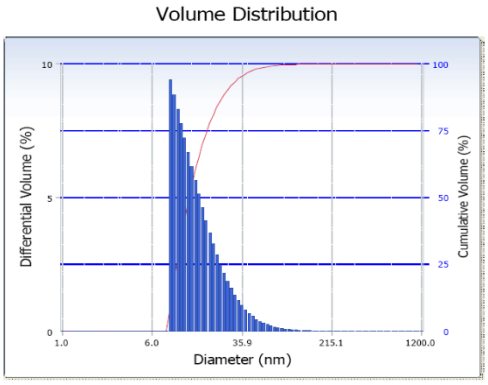

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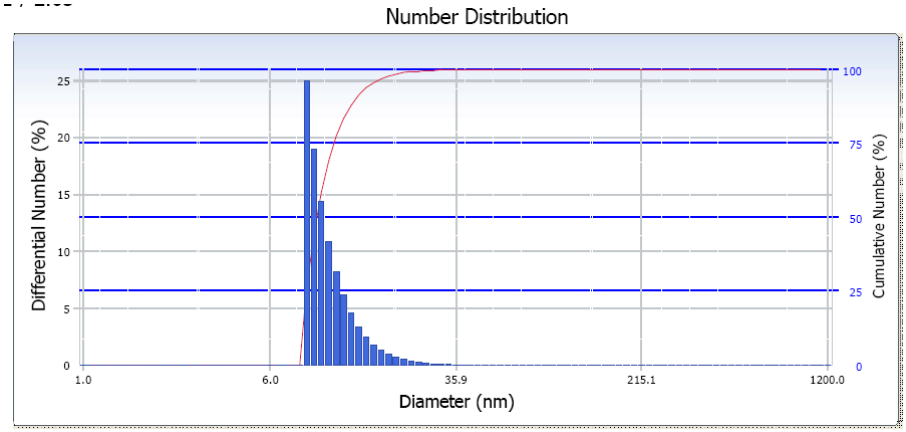

c

Figure5. Results of measurements of gold nanoparticles PSA; a. size distribution based on intensity, b. size distribution based on volume, c. size distribution by number.

The resulted of size proved that the metabolic compounds from Sarang semut extract have the ability to reduce Au^{3+}ions, electron transfer occured so that gold nanoparticles was formed.

d. Characterization of gold nanoparticles by X-RAY Diffraction (XRD)

XRD analysis showed four different peaks at 37,890, 44,030, 64,400 and 77,460 with Miller index (111), (200), (202) and (311) . The Miller index is a crystal lattice field (hkl) which is the crystal system of a material. The crystal system of the gold nanoparticles obtained was the cubic system (Davey, 1925) with crystal structure is face centered cubic (FCC) (Amanda dan Larry, 2006).

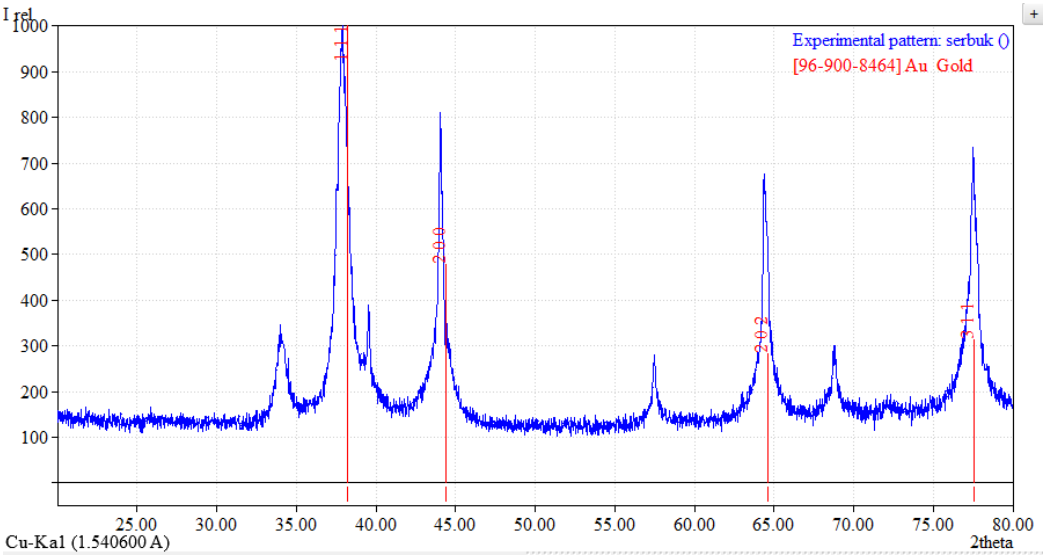

Figure 6. Diffractogram ofgold nanoparticles

The diffractogram data (Figure 6) shows the peak besides the typical gold peak, this shows that the gold nanoparticles produced are not 100% pure gold nanoparticles or still contained other compounds. Varied peak widths indicated the size of the gold nanoparticles crystals obtained varies, the size of the crystal is estimated using the equation 1 Scherrer formula (Satoshi and Nick, 2013).

Table 2. Diffractogram data of gold nanoparticles

No.	2-theta	d(A)	Miller index	Size (nm)
1	37,89	2,37	111	10,63
2	44,03	2,05	200	14,80
3	64,40	1,44	202	17,83
4	77,46	1,23	311	12,89

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The size distribution of gold naoparticles were successfully synthesized the varying crystalline sizes of 10.63 - 17.83 nm (Table 2). The size of the crystals obtained on the nano scale proved that bioactive molecules from the Sarang semut extracts was good reducers in synthesizing gold nanoparticles.

e. Characterization of gold nanoparticles by *Scanning Electron Microscopy* (SEM) and*Energy Dispersive X-Ray Spectrometer* (EDS).

The results of SEM analysis have shown uneven shape of gold nanoparticles. The morphology of gold nanoparticles obtained was in the shape of spherical and rods (Figure 7 a and b). This is in accordance with the previous work reported by of Ahmed and Ikram (2015) that gold nanoparticles have varying structures. Figure 19 shows that some parts was in the formed of nano, while others are still in the form of lumps, the shape of the average carbon particle shows an imperfect spherical shapes and there is little clumping of some particles contained in the test sample.

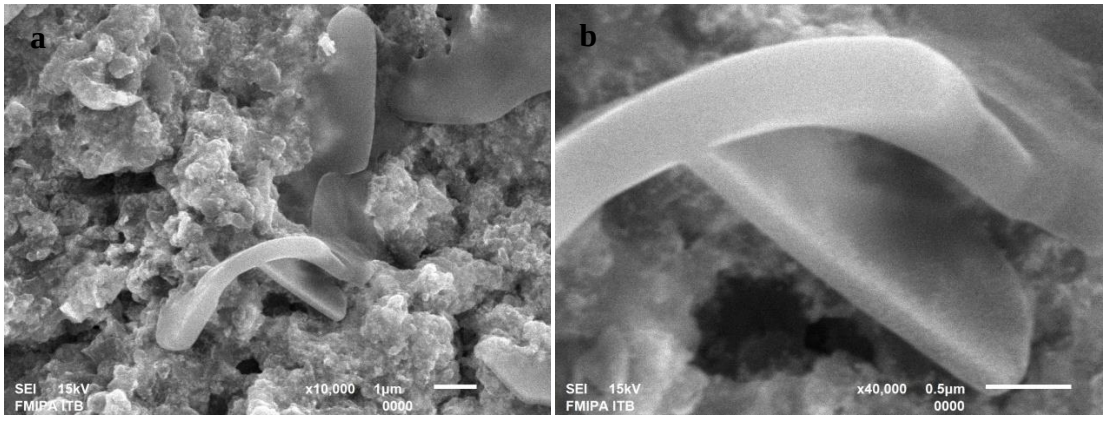
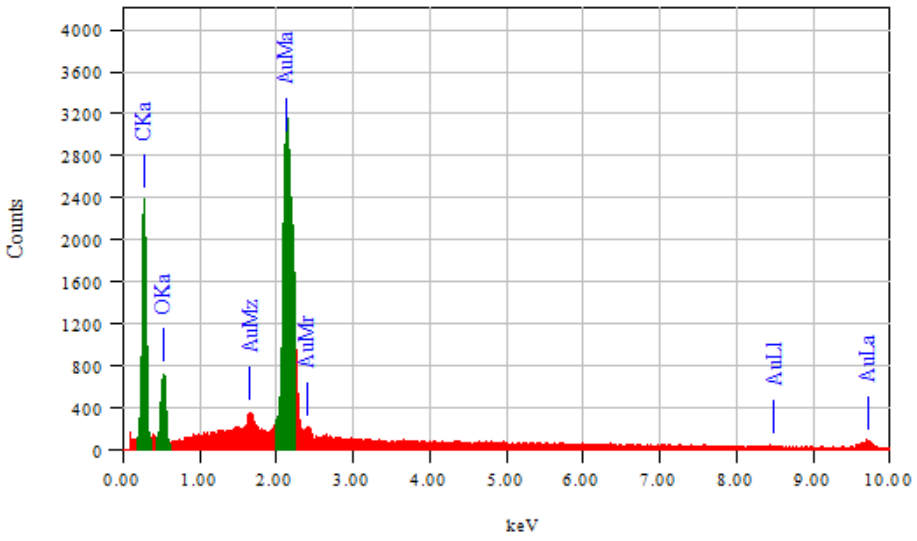


Figure 7. Scanning of Electron Microscopy (SEM) the gold nanoparticles, (a) 10.000, (b) 40.000



Element	(keV)	Mass%	Error%	Atom%	Compound	Mass%	Cation	K
C K	0.277	57.12	0.18	83.79				48.7903
O K	0.525	12.23	0.38	13.47				5.2921
Au M	2.121	30.65	0.38	2.74				45.9176
Total		100.00		100.00				

Figure 8. EDS Spectrum of Gold Nanoparticles

The Energy Dispersive X-Ray Spectrometer (EDS) analysis shows the composition of the sample where the mass percent of gold obtained is 30.36%. The presence of carbon and oxygen elements shows that extracellular organic groups was adsorbed on the surface of metal nanoparticles (Raghunandan, 2010) (Figure 8). The most dominant sample composition was carbon element where 57.12%, this shows that the gold nanoparticles formed was not 100% pure.

CONCLUSION

Sarang Semut extracts were successfully used as a reducing solution for gold to synthesize gold nanoparticles. The resulting gold nanoparticles were 53.2 nm in size, varying crystalline size of 10.63 - 17.83 nm with a cubic-shaped crystal system, the morphology of the gold nanoparticles obtained was spherical, square and stem and the mass percent of gold obtained was 30.36 %.

REFERENCES

Ahmad, T., Irfan, M., Azmi, M. B., Bhattacharjee, S. 2016. Effect of Reaction Time on Green Synthesis of Gold Nanoparticles by Using Aqueous Extract of *Elaise Guineensis* (Oil palm leaves).*Procedia Engineering*. 148: 467 – 472

Ahmed, S. dan Ikram, S. 2015. Synthesis of Gold Nanoparticles using Plant Extract.An Overview. 1(1):5

Amanda S. Barnard dan Larry A. Curtiss. 2006. Predicting the shape and structure of face-centered cubic gold nanocrystals smaller than 3 nm. *ChemPhysChem*. 7, 1544 – 1553.

Basavegowda, N., Idhayadhulla, A., Lee, Y.R. 2014.Phyto-Synthesis of Gold

Nanoparticles Using Fruit Extract of Hovenia Dulcis and Their Biological Activities.*Ind. Crops Prod*. (52): 745–751.

Davey, W. P. 1925. Precision Measurements Of The Lattice Constants Of Twelve Common Metals. Physical Review. 25: 753-761

Engida, A. M., Kasim, N. S., Tsigie, Y. A., Ismadji, S., Huynh, L. H., Yi-Hsu, J., 2013. Extraction, Identification and Quantitative HPLC Analysis of Flavonoids from Sarang Semut (Myrmecodia pendan).*Industrial Crops and Product*, 41: 392-396.

Fachrurrazie, T. A.I dan Wibowo, W., 2017.Preparation of Glucose Sensors using Gold Nanoparticles Modified Diamond Electrode. *Material Science and Engineering.*

Kurniawati, E., Sianturi, Y.C. 2016.Manfaat Sarang Semut (*Myrmecodiapendans)* sebagai Terapi Antidiabetes.*Majority*.**5**(3).

Mukunthan, K. dan Balaji, S. 2012. Cashew Apple Juice (Anacardium Occidentale L.) Speeds Up The 778 Synthesis of Silver Nanoparticles.*Int J. Green Nanotechnol*.**4**(2).

Nagaraj, B., Malakar, B., Divya, T., Krishnamurthy, N., Liny, P., Dinesh, R. 2012. Environmental Benign Synthesis of Gold Nanoparticles From The Flower Extracts of Plumeria Alba Linn,(Frangipani) and Evaluation of Their Biological Activities. *Int. J. Drug Dev Res*. **4**(1):144-150.

Nath, D. dan Banerjee, P. 2013. Green Nanotechnology - A New Hope For Medical Biology, Environ. *Toxicol.Pharmacol*. 36: 997-1014.

Noruzi, M. 2015. Biosynthesis of gold nanoparticles using plant extracts. *Bioprocess Biosyst Eng*. 38:1–14

Pal, N.B., Kryschi, C,. 2015. A Facile One-Pot Synthesis of Blue and Red Luminescent Thiol Stabilized Gold Nanoclusters: A Thorough Optical And Microscopy Study. *Phys. Chem*. 17, 21423-21431.

Philip, D. 2010. Low-dimensional Systems and Nanostructures. *Physica E*. **42**(5): 1417.

Raghunandan D, Bedre MD, Basavaraja S, Sawle B, Manjunath SY. 2010. Rapid Biosynthesis of Irregular Shaped Gold Nanoparticles from Macerated Aqueous Extracellular Dried Clove Buds (*Syzygium Aromaticum*) Solution. *Colloids Surfaces B Biointerfaces.* 79: 235-240.

Satoshi Horikoshi and Nick Serpone. 2013. Microwaves in Nanoparticle Synthesis, First Edition. Wiley-VCH Verlag GmbH & Co. KGaA.

Vijayaraghavan, K., dan Ashokkumar, T. 2017. Plant-Mediated Biosynthesis of Metallic Nanoparticles: A Review of Literature, Factors Affecting Synthesis, Characterization Techniques and Applications. *J. Env. Chem. Eng*. 5:4866.

Zhou, Y., Lin, W., Huang, J., Wang, W., Y., Lin, L., Li, Q. and Du, M. 2010. Biosynthesis of Gold Nanoparticles by Foliar Broths: Roles of Biocompounds and Other Attributes of the Extracts. *Nanoscale Res. Lett.***5**(8) :1351.