Synthesis of Silver and Gold Nanoparticles through Reduction Method using Bioreductor of Leaf Extract of Ketapang (*Terminalia catappa*)*

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Abstract

Synthesis of silver and gold nano-particles was carried out by the reduction method with leaf extract of Ketapang (*Terminalia catappa*). The biomolecules present in the extract generated the reduction of Ag^{+} and Au^{3+} ions from $AgNO_3$ and $HAuCl_4$, respectively. The growth of nanoparticles was followed by UV-Vis spectrophotometer. The maximum absorption of biosynthesis of AgNP and AuNP are observed in the respective range of 421-431nm and 530-535nm. Those peaks correspond to surface plasmon absorbance of AgNP and AuNP, respectively. Analysis on the functional groups change of the extract by Fourier Transform Infra Red (FTIR) showed the formation of carbonyl- from hydroxyl-groups which suggested the oxidation and reduction processes involved in the formation of both nanoparticles. The average size distributions determined by PSA (Particle Size Analyzer) are 55-71nm and 18-44nm for AgNP and AuNP, respectively. Morphology of the silver nanoparticles was observed by Scanning Electron Microscope (SEM) and the structure of the compounds was characterized using X-ray Diffraction (XRD). The shape of AgNP varied from triangular, cubic and hexagonal polyshaped, while AuNP were spherical. XRD studies showed that the nanoparticles obtained were crystalline gold and silver.

Keywords: silver nanoparticles (AgNP); gold nanoparticles (AuNP); reduction method; Ketapang; material characterization

1. Introduction

Nanoparticles exhibit unusual optical and catalytic properties and are considered as a new physico-chemical dimension between molecules and bulk materials. The surface to volume ratio of nanoparticles is extremely large. Electronic properties of nanoparticles such as light absorption, color, fluorescence, catalytic activity and electrochemistry depend on the size of nanoparticles. Due to these properties of nanoparticles, variable researches for nanoparticles synthesis are extended. A lot of nanoparticles preparation methods were reported. Nanoparticles can be prepared not only by chemical methods, but also by photochemical,

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electrochemical, radiolytic methods, sonolytic methods, and bioreduction using natural products (Zakir, 2006). The last method is usually classified as a new branch of nanotechnology, the so called nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. These methods of synthesis can be divided into intra- and extra-cellular. Biosynthetic methods can employ either microorganism cells or plant extract for nanoparticles production (Duran et al., 2005; Kumar and Yadav, 2009; Vaidyanathan et al., 2009). Formation of metal nanoparticles, such as silver and gold nanoparticles, is an example that will be discussed here.

Gold and silver nanoparticles are presently under intensive study for applications in optoelectronic devices, ultrasensitive chemical and biological sensors and as catalysts. The nanoparticles have been used for their potential applicability in bioremediation of radioactive wastes (Zakir, 2006), sensor technology, opto-electronics recording media and optics (Kumar and Yadav, 2009), and their bioactivity effects (Sotiriouand and Pratsinis, 2010; Marambio-Jones and Hoek, 2010; MubarakAli et al, 2011). Their ecotoxicity to the environment is another reason to study the metal nanoparticles (Fabrega et al., 2011).

Utilization of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. The above synthetic protocol by plant extract or biomass exemplifies the promising application of the green synthesis of metal nanoparticles. Very recently green metal nanoparticles have been synthesized using various natural products like opuntia (Gade et al., 2014), jatropha (*Jatropha curcas*) seed extract (Bar et al, 2009), cinnamon (*Cynnamon zeylanicum*) bark extract and powder (Sathishkumar et al, 2009), leaf extract of *Dalbergia sissoo* (Singh et al., 2012) etc.

Ketapang (Makassarese: *talise*) is found as a specific plant in South Sulawesi Area and has been used for traditional medicine since the ancient time. The plant is a medium sized tree with leaves clustered towards the ends of the branches. The various extracts of leaves and bark of the plant have been reported to be anticancer antioxidant (Masuda et al., 1999), hepatoprotective (Lin et al., 1997), hepatitis (Chen et al., 2000), and aphrodisiac (Ratnasooriya and Dharmasiri, 2000). Fruit of Ketapang contains cyanidin-3-glucoside, corilagin (Topoisomerase I and II) inhibitor (Kashiwada et al., 1993), Xanthin oxidase inhibitor (Hatano et al., 1990), ellagic-acid as anti-HIV (Tan et al., 1991) and flavonoids (Lin et al, 2000). Ketapang is rich in tannins that are reported to be antidiabetic (Teotia and Singh, 1997; Nagappa et al., 2003). Those compounds might be responsible in the bioreduction reaction of silver and gold ions to produce AgNP and AuNP, respectively.

In this paper we demonstrate method for the synthesis of silver and gold nanoparticles by the reduction of respective aqueous silver and chloroaurate ions using leaf extract of Ketapang (Makassarese: talise). The scenario of oxidation and reduction mechanism is proposed in this paper. To the best of our knowledge, it is rarely discussed in the previous papers related to biosynthesized gold and silver nanoparticles (Ankamwar, 2010).

2. Materials and methods

2.1. Materials

The silver nitrate ($AgNO_3$, 99.8%) and gold(III)chloride hydrate, ($HAuCl_4.3H_2O$, 99.999%) were purchased from the Merck Agent (Makassar, Indonesia) and have been used for the synthesis of AgNP and AuNP, respectively. The fresh leaves were taken from the tree of Ketapang (Makassarese: talise) located in Hasanuddin University Campus, Makassar, South Sulawesi.

2.2. Preparation of leaf extract

Synthesis of silver and gold nanoparticles was carried out through the following procedure: 10 gm fresh leaves of Ketapang (Makassarese: talise) were taken and washed thoroughly to remove dust and other impurities. These washed leaves were cut into 2cm x 2cm pieces and immersed into the 50 ml Millipore water and then boiled for 5 min. The extract was filtered and the residual material was thrown away.

2.3. Synthesis of silver and gold nanoparticles

For the bioreduction of Au(III) into the Au(0), a freshly prepared leaf extract (3mL) was added drop wise using a syringe to 50 mL 0.5 mM $HAuCl_4$ solution. Similarly for the bioreduction of Ag(I) into the Ag(0), 1mL of leaf extract was added to 40mL of 1 mM $AgNO_3$ solution. After the addition of leaf extracts both the solutions were kept in the incubator at 37°C.

2.4. UV-Visible spectroscopic analysis

The reduction of both Ag^+ and $AuCl_4^-$ in the aqueous solution was monitored with the regular sampling of the 0.3 ml aliquots. The sample was diluted with 3 ml of the Millipore water and measuring the UV-visible spectra of the diluted sample. Shimadzu UV-2600 spectrophotometer at a resolution of 1 nm was used for the analysis electronic transition in the sample.

2.5. Particle size analysis

Solution of AgNP and AuNP as much as 50μL is pipetted and then put into the sample holder of Particle Size Analyzer (PSA) Vasco DLS. The Vasco DLS uses the thermal motions of particles in suspension (Brownian Motion) to determine their size. Here the sample suspension is irradiated by a laser and the light scattered in a certain direction detected with high time resolution. From the fluctuation of the intensity of the scattered light, the mobility of the particles can be calculated and then again via the Stokes-Einstein formula, their size can be calculated

2.6. X-ray diffraction analysis

Solution of Ag(0) and Au(0), which was obtained from complete reduction of $AgNO_3$ and $HAuCl_4$ solution, respectively, was maintained at -80°C for 5 hours and then lyophilized for 24 hours. This lyophilized powder was further used for the XRD analysis. The XRD analysis was done using an XRD Rigaku MiniFlex diffractometer operating at 40mA current and 45 kV voltages with CuKα radiation to confirm the crystalline form of silver and gold nanoparticles.

2.7. Fourier transform infrared (FTIR) spectroscopic analysis

Sample preparation for FTIR analysis is as follows: 15 ml solution of silver and gold nanoparticles were taken separately and centrifuged at 4000 rpm for 10 min. The resulting suspension was redispersed into 20 ml of sterile water and centrifuged again. The process of centrifugation and redispersion was repeated three times

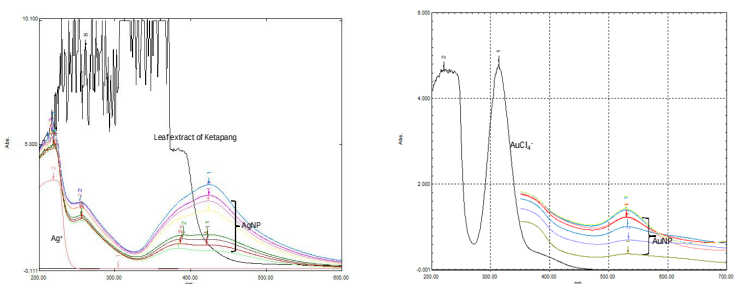
to make the solution free from any biomass which is not present as the capping agent in the solution. The sample is then put into FTIR instrument (Shimadzu IRPrestige-21).

2.8. Scanning electron microscopy (SEM) measurements

Samples for SEM measurement were taken after the complete bioreduction of Ag(I) and Au(III) into Ag(0) and Au(0), respectively. SEM samples of aqueous silver and gold nanoparticles were prepared by taking a small drop and putting it on the carbon-coated copper grid and dried at room temperature. The SEM observations were performed on the instrument Tescan Vega3SB Analytical SEM, operating at accelerating voltage of 100 kV.

3. Results and discussion

The reduction of aqueous solution of Ag^+ and $AuCl_4^-$ can be easily monitored by UV-Vis spectroscopy. Qualitative analysis for the formation of AgNP and AuNP can also be determined due to the specific characteristic of surface plasmon resonance ("absorption") of both nanoparticles. The excitation of surface plasmon vibrations of silver and gold nanoparticles exhibits yellowish-brown and red wine color, respectively. This makes possible to follow the formation of AgNP and AuNP in the aqueous solution (Ankamwar, 2010; Singh et al., 2012). The colour of gold solution in AuNP formation was initially pale yellow and after the addition of boiled leaf extract of Ketapang (*Terminalia catappa* Linn) the colour transformed into red wine colour within 5 minutes. The reduction continued for 4 hours. However, in the case of AgNP formation, the solution was initially colorless and after the addition of boiled leaf extract of Ketapang (*Terminalia catappa* Linn), the colour was changing into light yellow in at least 2 hour. The colour proceeded further into yellow and yellowish-brown, but the complete reduction took more time, i.e 24 hours as compared to that for the complete reduction of AuNP. The most possible reason could be difference in their redox potential (Ag^+/Ag, E^o_{red} = 0.8 volt; Au^{3+}/Au, E^o_{red} = 1.4 volt). The maximum absorption for AuNP was observed in the range 530-535nm (Fig. 1b). In the case of AgNP, the maximum absorption was observed in the range 420-430nm (Fig. 1a). Both of them are characteristic surface plasmon absorption of both nanoparticles (Ankamwar, 2010; Singh et al., 2012).



(a) (b)

Fig. 1. UV-Visible absorption spectra in the synthesis of (a) AgNP and (b) AuNP

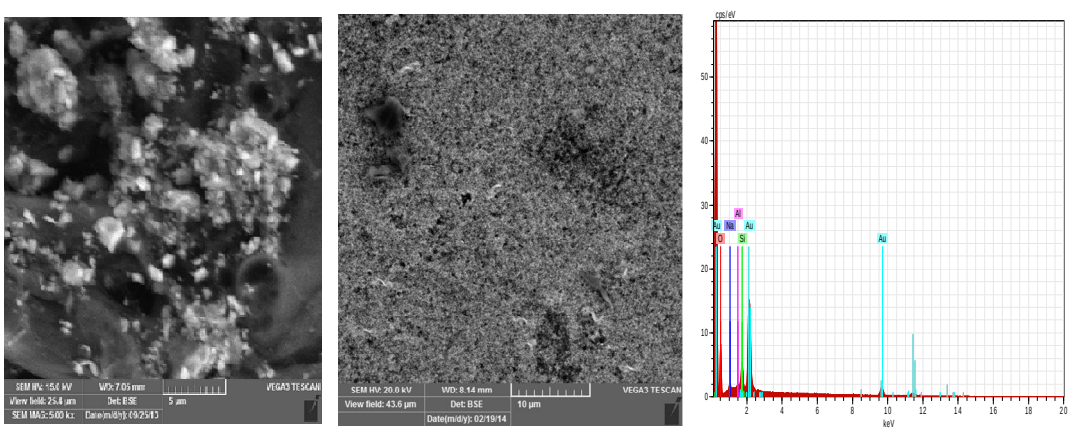
The SEM images of AgNPs show that their morphology were cubic, hexagonal and triangular in nature [Fig. 2a]. The SEM images of AuNPs clearly show regularity in shape, which was spherical in nature [Fig. 2b]. The EDS spectrum of AuNP is shown in Fig. 2c, while AgNP spectrum is not available. It is clearly seen the elemental composition presents in AuNP precipitate. Based on the spectrum, AuNP precipitate consists of Au (79.75%) and O (9.01%), and the remainings are Si, Na and Al (1.44-5.56%). This suggests that biosynthesis reaction produced gold nanoparticles (AuNP). The average diameter of silver nanoparticles observed with PSA was in the range 55-71nm while AuNP was in the range 18-44nm.

FTIR measurements were carried out to identify the possible biomolecules in the leaf extract of Ketapang which is responsible for the reduction of Ag^{+} and $AuCl_4^{-}$ ions in the formation AgNP and AuNP, respectively. Phytochemical test carried out on the leaf extract of Ketapang showed that phenolic compounds are the dominant compound found in the extract. The phenolic compounds might be tannins as it is known that Ketapang is rich in tannins (Teotia and Singh, 1997; Nagappa et al., 2003). Tannin from Ketapang is a potential inhibitor for DNA-topoisomerase II (Kashiwada et al., 1993) and xanthine oxidase (Hatano et al., 1990). Therefore, it is supposed in this paper that tannins also play an important role in the reduction process of Ag^{+} and Au^{+3} ions. Table 1 shows the anaysis results on the FTIR spectra recorded in the synthesis of AgNP and AuNP. The increase in the intensity of –C=O at around 1720-1740cm^{-1} and the decrease in the intensity of –OH at around 3400cm^{-1}indicate the formation of –C=O groups from –OH groups which is also an indication of oxidation of –OH groups into –C=O groups. The respective Ag^{+} or $AuCl_4^{-}$ ions will be reduced concurrently into their metal nanoparticles AgNP or AuNP. The change in the amplitudo of peaks and a small shift was observed in both cases which also may be attributed to the formation of carbonyl groups from hydroxyl groups. Based on that information, we propose the scenario of oxidation and reduction mechanisms in the formation of AgNP and AuNP as follows:

(i) Formation of bonding between Ag^{+} with –OH group from tannins, and

(ii) Reduction of Ag^{+} to Ag or Au^{+3} to Au is occurred concurrently with the oxidation of –OH groups into carbonyl groups (-C=O).

This scenario is described in the scheme of reduction and oxidation mechanism in Figure 3(c).



(a) AgNP (b) AuNP (c) EDS spectra of AuNP

Fig. 2. SEM images of (a) AgNP and (b) AuNP, and (c) EDS spectrum of AuNP

Table 1. Changes in wave number of functional groups of biosynthesized silver and gold nanoparticles observed in FTIR analysis

Wave number (cm^{-1})			Functional groups
Leaf extract of Ketapang	AgNP	AuNP	
3414	3444	3446	O-H
1051	1049	1045	C-O
1707	1739	1720	C=O

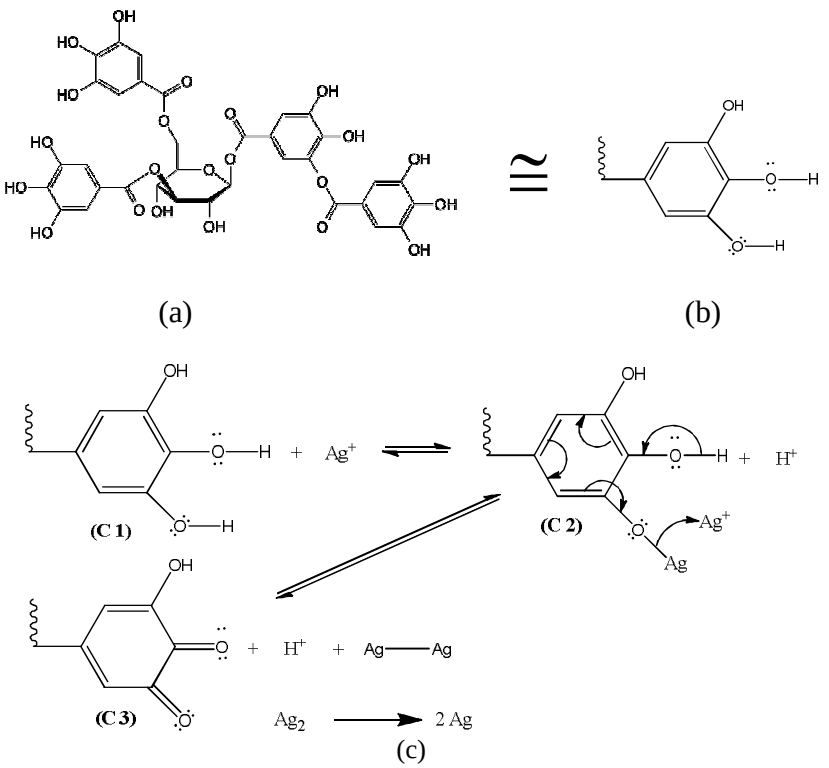


Fig. 3. Molecular structure of (a) tannin, (b) simplified structure of tannin, (c) Possible reaction mechanism of AgNP formation

The reaction stoiciomethry for AgNP formation can be summarized as follows

$$(C\ 1) + 2\ Ag^{+} \rightleftharpoons 2\ Ag + (C\ 3) + 2\ H^{+}$$

It is clearly shown that for the reduction of 2 moles of Ag^{+} ions as one ion system (Ag(I)), one molecule of

tannins is required and 2 protons (H^+) will be released. Interestingly, if we apply it to the formation of AuNP, the reaction stoiciomethry will be as follows

$$3\ (C\ 1) + 2\ Au^{3+} \rightleftharpoons 2\ Au + 3\ (C\ 3) + 6\ H^+$$

So, for the reduction of 2 moles of Au^{3+} ions as three ions system (Au(III)), three molecules of tannins are required and 6 protons (H^+) will be released. This mechanism is consistent with our experimental results where the final pH is 5 and 3 for the AgNP and AuNP synthesis, respectively. Therefore, the more protons (H^+) are released the lower will be the pH. Clarification at the molecular level to this reaction will be carried out in the future. Very recently, Mittal et al. (2014) also clarifiied the formation of –C=O groups from –OH groups in the bioreducing agents of flavonoid families. However, the occurrence of reaction of $NADP^+$ → NADPH should be further clarified (Duran et al., 2005), since plant extract is not a living organism (Gade et al., 2014), including leaf extract of Ketapang.

The XRD analysis was conducted to determine the crystalline nature of biologically synthesized silver and gold nanoparticles on the formation of AgNP and AuNP. Various Bragg's reflections are clearly visible in both silver and gold XRD pattern. In Figure 3a the diffraction pattern is clearly shown with Miller indices (111), (021), (120), (031), (101), (11-2), and (21-1). Diffraction pattern with Miller indices (111) correspond cubic crystal system of AgNP. Crystal system for AgNO3 is ortorhombik occured with Miller indices (021), (120), (031), and (101) while crystal system for AgCN is trigonal (rhombohedral) with Miller indices (11-2) and (21-1). The occurrence of both AgNO3 and AgCN patterns appears from silver ions which were not reduced in the biosynthesis reaction. Bragg's reflection are also seen for XRD pattern of AuNP (Fig. 3b), which are corresponding to the (111) and (200) set of lattice planes. This pattern suggests that synthesized gold nanoparticles are face centered cubic and essentially crystalline in nature. (200) set of lattice plane is observed to be very weak.

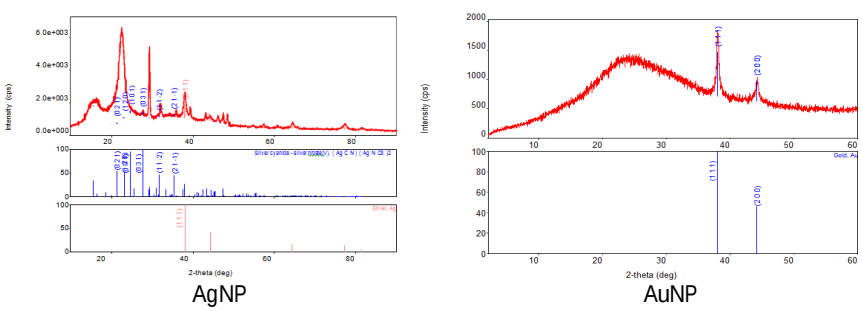


Fig. 4. (a) XRD pattern of biosynthesized (a) AgNP and (b) AuNP

4. Conclusions

Green and rapid synthesis of AgNP and AuNP using the leaf extract of Ketapang (Terminalia catappa Linn) has been performed with possible role of phenolic compounds (tannins) as reducing agent. It is proposed that reduction reaction takes place concurrently with the oxidation of reducing agents, i.e tannins, which was clarified by analysis on the functional groups change of the extract by Fourier Transform Infra Red (FTIR).The current research has shown a new possibility for synthesis of AgNP and AuNP using leaf extract

of Ketapang (Makassarese: talise) as a traditional plant in South Sulawesi Area. The existence of both AgNP and AuNP is veryfied by (i) surface plasmon absorption of AgNP and AuNP, (ii) average size distribution measured by PSA, and (iii) determination of their structure and morphology by XRD and SEM, respectively.

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