

The role of aqueous leaf extract of *Tinospora crispa* as reducing and capping agents for synthesis of gold nanoparticles

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Abstract. Environmentally friendly method for green synthesis of Au nanoparticles (AuNP) using aqueous leaf extract of *Tinospora crispa* (TLE) was reported. TLE has the ability for reducing and capping AuNP. Identification of active compounds in aqueous leaf extract was obtained by phytochemical analysis and Fourier transform infrared spectroscopy (FTIR). The AuNP-TLE growth was characterized using UV-Vis spectrophotometer. The particle size and the distribution of AuNP were confirmed by particle size analyzer (PSA). AuNP-TLE formation was optimized by varying the extract concentration and time of the synthesis process. UV-Vis absorption spectrum of optimum AuNP formation displayed by the surface plasmon resonance at maximum wavelength of λ_{\max} 536 nm. The PSA result showed that AuNP has size distribution of 80.60 nm and stable up to 21 days. TEM images showed that the size of the AuNP is \pm 25 nm.

Keywords: Au nanoparticle, *Tinospora crispa*, green synthesis, capping agent

1. Introduction

Au nanoparticle (AuNP) is (one) of the most stable metal nanoparticles which used in various fields such as medicine, cosmetics, water treatment and catalysis [1-4]. AuNP is known to be selective in various catalytic reactions [5-6].

Synthesis of AuNP has been successfully carried out by the chemical reduction method. It was obtained by using reducing agents such as sodium borohydride, sodium citrate, hydrazine hydrate, dimethylformamide, tollens reagents, and polyethylene glycol [7]. However, these methods used toxic chemical compounds, so the compound can be adsorbed on the surface of AuNP, and caused a toxic side effect [8]. Along with the development of science and technology, AuNP can be obtained by green synthesis methods using environmentally friendly raw materials. This method also has advantages such as lower cost, easily produced in large scale, lower energy, temperature, and pressure requirement, when compared with the physical and chemical methods. Many raw materials are used for reducing and capping agent in AuNP synthesis, such as microorganisms, either bacteria or fungi [9], biopolymer like alginate [10-11], or plant extract [12]. Green synthesis of AuNP has been developed by using a various of plants, namely Guava, Pomelo, Moringa, noni, *Nepenthes khasiana*, hibiscus flower, Ginger, *Solanum nigrum*, *Gymnema sylvestre*, and *Plumeria alba* [13-19].

Indonesian plants can be used as reducing agents in AuNP synthesis. One of them is brotowali leaf (*Tinospora crispa*) as flavonoids source of *glucosylflavones* type as *α -glucosidase* inhibitor [20].



Traditionally, brotowali leaf has been used for the treatment of fever, diabetes, skin infections and antimalarial [21].

Tinospora crispa contains metabolites compounds such as phenols, polyols, amines, flavonoids, tannins, saponins, gallic acid, protein, and reducing sugar which have the ability as a reducing agent [21]. It also has an antioxidant activity [22] that acts as a substitute for the chemical reducing agent in synthesis of AuNP. There is no report of AuNP synthesis from *Tinospora crispa* leaf. The present study describes the synthesis of AuNP by using the aqueous extract of *Tinospora crispa*.

2. Materials and methods

2.1. Material

Gold metal 99.99% was obtained from PT Antam, Indonesia, and dissolved into aqua regia. Stock solution of HAuCl₄ was prepared in 100 mL deionized water. Precursor solutions of the desired molar concentrations were prepared by a proper dilution of the stock solution. *Tinospora crispa* leaves were collected from Balitro in Bogor, Indonesia.

2.2. Preparation of *Tinospora crispa* leaves extract

Tinospora crispa leaves were thoroughly washed with distilled water. They were dried by placing them outdoor for a week. The dried *Tinospora crispa* leaves were grinded to get the fine powder. The 50 g leaf powder was soaked in 250 mL methanol for a week while regularly stirred every day. The result of soaking was separated by filtration. The filtered *Tinospora crispa* leaves was extracted by hexane, ethyl acetate, and water gradually to get the aqueous leaf extract of *Tinospora crispa* (TLE).

2.3. Preliminary phytochemical screening

Each extract was phytochemically tested for the presence of flavonoids, tannins, phenolic, saponosides, terpenoids, steroids, and alkaloids.

2.4. Synthesis of gold nanoparticles

Synthesis of AuNP was done at room temperature by adding 1ml of aqueous leaf extract of *Tinospora crispa* in 9 mL of 0.1 mM HAuCl₄ solution. AuNP formation was detected by observing colloids color changed from yellow to light red.

2.5. Characterization of AuNP

Shimadzu 2600 UV-Visible spectrophotometer was used to get absorption spectra of AuNP. Average particles diameter and particle size distribution were measured with Particle Size Analyzer MalvernZEN 1600. Identification of functional groups of biocompounds in *Tinospora crispa* leaves extract before and after the reaction were done using FTIR spectrophotometer (Perkin-Elmer). The Transmission Electron Microscopy JEM 1400 was used to determine the morphology and particle size of the nanoparticles.

2.6. Concentration effect on aqueous AuNP synthesis and its stability

AuNP stability was observed at various concentration of aqueous leaf extract of *Tinospora crispa* (0.20-0.40%). The Effect of concentration on AuNP synthesis was investigated by monitoring the UV-Vis spectrophotometer.

3. Results and discussion

The preliminary phytochemical was performed to investigate the secondary metabolite presence of flavonoids, tannins, phenolic, steroids, saponosides, terpenoids, alkaloid in the *Tinospora crispa* leaves extracts. The methanol fraction of *Tinospora crispa* leaves extract showed a positive result of all secondary metabolite. Meanwhile, the aqueous fraction showed the presence of flavonoids and phenolic compounds.

UV-Vis absorption spectra of AuNP-TLE observed at various different times are displayed below in figure 1a. There was no significant reduction of HAuCl₄ occurred at 25 min of the reaction which was

proved from SPR peak formation. A color change was observed from yellow to pink after 90min reaction at room temperature, confirmed the AuNP formation.

Figure 1b shows the various concentrations of AuNP formation and observation of UV-Vis spectra with high absorbance value of 0.329, the smallest λ_{\max} and the sharpest peak shape at 0.25% concentration indicated the optimum concentration of AuNP synthesis.

AuNP has a tendency to agglomerate at the certain time and conditions. Figure 1c shows the stability of AuNP for 21 days, observed from the UV-Vis absorption. At the first day of the synthesis, AuNP-TLE 0.25% has λ_{\max} of 536 nm and absorbance value of 0.329. After 21 days, λ_{\max} of AuNP shifted to 532 nm. This shifting shows that AuNP is stable for 21 days. AuNP stability is affected by the active compounds in TLE which act as a reducing and capping agent of AuNP. The absorbance values continued to increase up to the 21st days. It decreased after 21 days due to the increasing of AuNP formation. However, after day 21 the decrease was due to the AuNP agglomeration.

TEM characterization was performed to determine the morphology, shape and size of AuNP. Figure 2a and 2b shows the TEM images with a magnification of 40000 and 150000 times. The results showed that the morphology of AuNP-TLE has a sphere shape with a diameter ± 25 nm. To confirm the AuNP, the Miller indices were adjusted with data Join Committee on Powder Diffraction Standards of

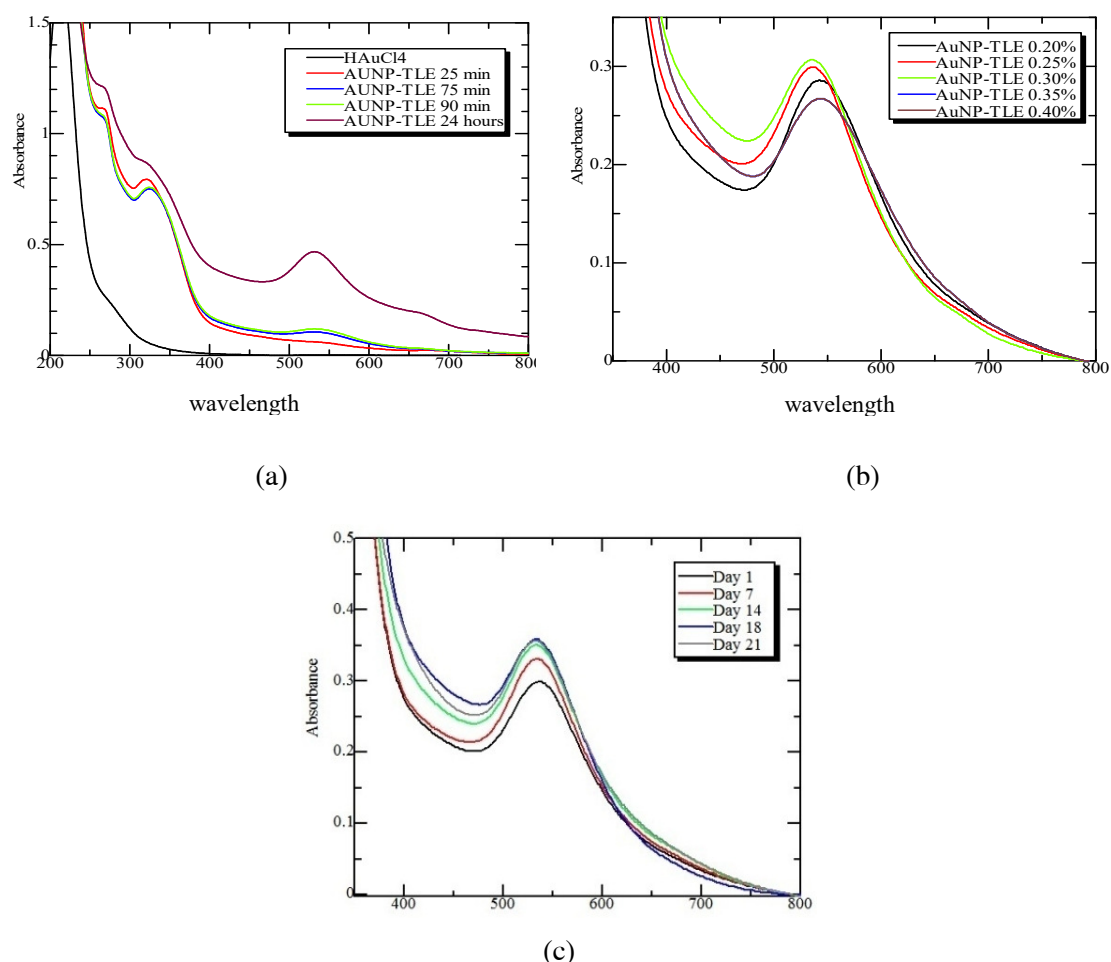


Figure 1. UV-Vis absorption spectra of AuNP-TLE (a) against time reaction (b) at various concentration (c) stability observation in 21 days.

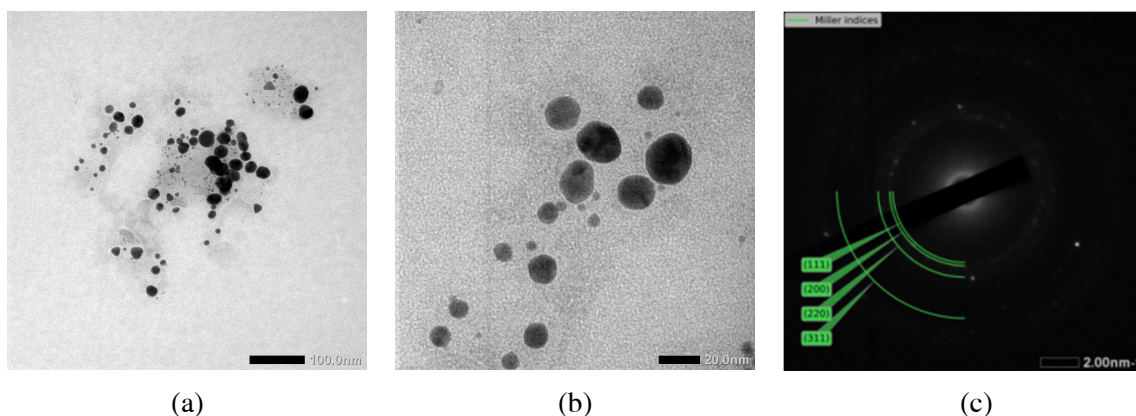


Figure 2. (a) and (b) TEM images of AuNP-TLE, (c) SAED images of AuNP-TLE diffraction pattern.

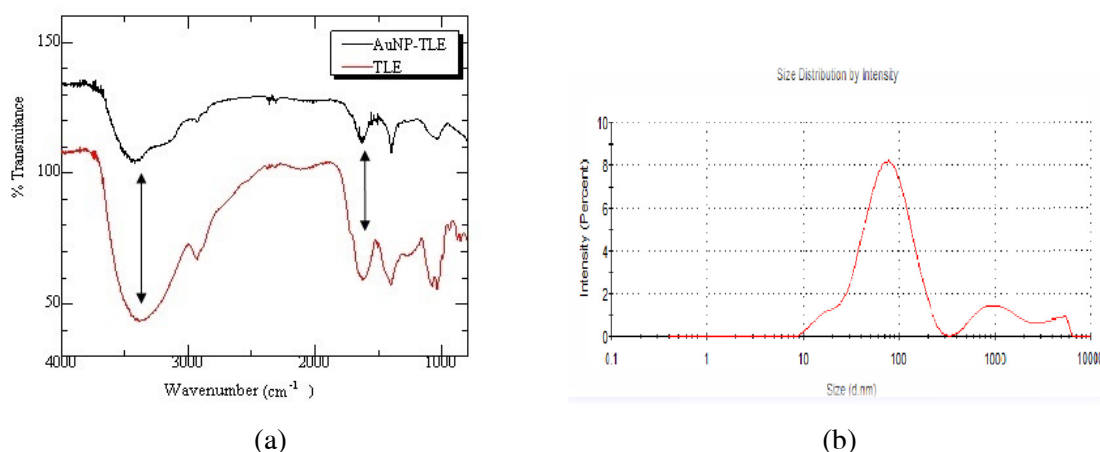


Figure 3. (a) FTIR spectra for AuNP-TLE and TLE, (b) PSA profile: size distribution of AuNP-TLE.

Au (JCPDSNo. 04-0748) by SAED analysis. The results are shown in figure 2c. The miller indices are (111), (200), (220) and (311) indicated the synthesized AuNP.

FTIR characterization was conducted to determine the interaction between the functional groups of TLE in the AuNP synthesis as shown in figure 3a. FTIR spectra show the wavenumber shifting of TLE before and after AuNP formation. The stretch of -OH group shifted from 3086 to 3138 cm⁻¹. C = O group shifted from 1766 to 1706 cm⁻¹. This shift was due to the interaction of functional group (-OH and -C=O) in TLE compounds with AuNP.

The significant shifts of 3086 and 1766 cm⁻¹ was supposed to be the role of -OH group (phenol, alcohol) and -C=O (carboxylic acid and its derivative) as the reducing and capping agent of AuNP. The phenolic, carboxylic, nitrogen compounds, vitamins, and reducing sugars have been reported to play the role in reduction of HAuCl₄ and capping of AuNP [22].

PSA Characterization was conducted to determine the particle size and size distribution of AuNP as shown in figure 3b. PSA spectrum peak of AuNP is 80.60 nm. The controlled size of AuNP proves the TLE act as a good reducing agent in the AuNP synthesis.

4. Conclusions

AuNP was obtained using aqueous *Tinospora crispa* leaves extract by green synthesis method. Effect of TLE concentration as HAuCl₄ reducing agent in the AuNP formation was investigated. The variations

of TLE concentration affected the wavelength, absorbance, and the spectrum shape of AuNP. The AuNP was observed to be stable up to 21 days and the AuNP formation was optimum at λ_{\max} 536 nm. The size distribution of AuNP-TLE was confirmed at 80.60 nm using PSA. Based on FTIR characterization, the phenolic and carboxylic compounds acted as reducing and capping agents in the AuNP synthesis. TEM images showed the AuNP size of ± 25 nm.

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