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Toona sinensis Mediated Green Synthesis of Silver Nanoparticles

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Abstract:
Background*: Plant-mediated silver nanoparticles have gained much attention in the past decade because of its features, such as being eco-friendly, effective cost, rapid and simple. Silver nanoparticles can be made by reducing silver ions using secondary metabolites which have reducing properties.*
Materials and Methods*: In this study, Surian leaf extract (Toona sinensis) was used as a bioreductor in the formation of silver nanoparticles. Surian leaf extract was made by adding 5 g of Surian leaf powder into 50 mL distilled water, boiled at 90 ^{o}C for 15 min, and then filtered. Silver nanoparticles were made by mixing 0.2 mL of this Surian leaf extract with 0.5 mL of silver nitrate 0.1 M and the volume was adjusted to 50 mL with distilled water, then stirred with varying the stirring times, 15 min; 30 min; 1; 2; 3; 4; 5; 6 hours. To observed the effect of variations in extract concentration on the formation of nanoparticles, 0.05; 0.1; 0.15; 0.2; 0.25; 0.3; 0.35 mL of Surian leaf extract was mixed with 0.5 mL of silver nitrate 0.1 M, the volume was adjusted to 50 mL with distilled water. The mixture was stirred for 4 hours and the absorbance was measured by UV-Vis spectrophotometer at reaction time 4; 24; 48; 168; 336; 672 hours. To evaluate the formation of silver nanoparticles the color changes from pale yellow to brownish yellow and brown were observed, then the absorbance of samples was measured by UV-Vis spectrophotometer at wavelength 300-800 nm at specified times.*
Results*: UV-Vis spectroscopy analysis showed Surface Plasmon Resonance (SPR) band of silver nanoparticles range from 400-440 nm. Absorbance value at the stirring time 15 min; 30 min; 1; 2; 3; 4; 5; 6 hours respectively were 0.111; 0.144; 0.172; 0.204; 0.264; 0.295; 0.304 and 0.312, the average absorbance value in the formation of silver nanoparticles with extract concentration 0.05; 0.1; 0.15; 0.2; 0.25; 0.3; 0.35 mL respectively were 0.153; 0.276; 0.382; 0.455; 0.544; 0.573; 0.607, and the average absorbance value of silver nanoparticles after reaction time 4; 24; 48; 168; 336; 672 hours respectively were 0.254; 0.376; 0.433; 0.486; 0.508; and 0.505.*
Conclusion*: Toona sinensis leaf extract has potential as a silver ion bioreductor to form silver nanoparticles. Formation of optimal and stable silver nanoparticles from biosynthesis was using 0.25 mL Surian leaf extract with stirring time 4 hours and determined after reaction time 672 hours.*
Key Word*: nanotechnology; silver nanoparticle; Toona sinensis; eco-friendly.*

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I. Introduction

Nanotechnology is an important field that relates to design, synthesis, and manipulation of the structures and sizes of particles around 1-100 nm (1). Nanotechnology attracts many scientists throughout the world and has become one of the research fields that it is widely developed. The unique properties of nanoparticles that differ from large-sized materials have become their advantages. It includes optical, magnetic, electronic properties, as well as smaller shapes and sizes thus providing superior bioactivities (2). In recent years, the use of nanomaterials for biomedical and pharmaceutical applications has gained significant appeal. A large percentage of nanomaterials is used in a variety of biomedical applications, such as for drug delivery, wound dressing, and other medical purposes (3).

Among all metal nanoparticles, silver has received much attention to be studied because the synthesis process is not difficult and can be applied in various fields of science and technology. In the medical field, silver nanoparticles have antibacterial, anticancer, and anti-inflammatory activity. It is also widely applied as diagnostic agents, drug delivery, and antimicrobial material in health products and cosmetics (4).

Silver has long been known to have antibacterial properties. Silver has been used for the treatment of burns, dental care, catheters, and infection control, in the form of silver metal, silver nitrate, and silver sulfadiazine (5). Silver nanoparticles have lower toxic effects than silver in ionic form (6). Silver nanoparticles have a large surface area to volume ratio, thus increasing its effectiveness. Therefore, silver nanoparticles are more effective to kill resistant bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (7,8).

Synthesis of silver nanoparticles can be done through several methods, one of the easiest is the chemical reduction method (9). However, this method generally uses synthetic chemicals that are harmful for medical applications and have a negative impact on the environment. Therefore, a more eco-friendly method has gained more attention nowadays, called 'green synthesis' method (10). In this method, plants and microorganisms could act as reducing and stabilizing agents in the synthesis of silver nanoparticles, thereby reducing the disposal of substances harmful to the environment.Generally plants that used as reducing agents are contain secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids and others (11).

One of the plants that contain many secondary metabolites is surian. Surian (*Toona sinensis)* is a species of the Meliaceae family which is widely distributed in the Asian region, especially Indonesia. Traditionally, Surian plants are widely used to treat diseases such as infections, diarrhea, diabetes, etc. From the results of previous studies noted that Surian leaves contain phenolics (gallic acid, methyl gallate) and flavonoids compounds (quercetin, quercetrin, kaempferol, rutin) (12). Its usefulness as a drug is related to the content of its secondary metabolites which can act as natural antioxidants (13). It is expected that this Surian leaf extract content can play a role in reducing silver ions to silver nanoparticles. This study will synthesize silver nanoparticles using surian (*Toona sinensis*) leaf extract by evaluating variations of extract concentration and stirring time for the formation of silver nanoparticles.

II. Material And Methods

Materials

Fresh Surian leaves (*Toona sinensis)* were collected from Bukittinggi, West Sumatera, Indonesia. Silver nitrate ($AgNO_3$) as precursor (Merck, Germany), acetonitrile, phosphoric acid, and double distilled water grade for HPLC, gallic acid (Sigma), distilled water, aluminum foil, and Whatman No.1filter paper.

Preparation of plant extract

The plant of *Toona sinensis* was identified by Anda Herbarium, Andalas University, Padang, West Sumatra, Indonesia. *Toona sinensis* leaves were washed several times to remove any impurities and dried at room temperature for 14 days. The dried leaves were ground and sieved to get uniform size range. The fine powder was stored in an air-tight container and dark place. The aqueous extract of *Toona sinensis* leaf was made by adding 5 g powder to 100 mL Erlenmeyer flask with 50 mL distilled water and were boiled at 90^{o}C for 15 min. The aqueous extract was cooled to room temperature and filtered through Whatman No.1filter paper, then stored in a refrigerator at 4 ^{o}C for further use.

The absorption spectrum of aqueous extract was determined using the UV-Vis spectroscopy (Shimadzu UV-1700) in a wavelength range of 200-800 nm.

Phytochemical screening of *Toona sinensis*leafextract

Test for Phenolic

2 mL of the extract was put into a test tube, a solution of iron (III) chloride was added and a change in the color of the solution was observed. If a blue or black purple solution is formed, it indicates the presence of phenolic compounds.

Test for Flavonoid

2 mL of extract was put into a test tube, then it was added concentrated hydrochloric acid and a few grains of magnesium powder. The formation of orange to red indicates the presence of flavonoid compounds.

Test for Alkaloid

2 mL of extract was put into a test tube, added 2 mL of HCl and 1 mL of Mayer reagents. If the solution becomes turbid or the formation of white precipitate, it indicates the presence of alkaloid compounds.

Test for Saponin

2 mL of extract was put into a test tube and shaken. Then a few drops of concentrated hydrochloric acid were added. If foam forms and does not disappear after the addition of concentrated hydrochloric acid, it indicates the solution contains saponin compounds.

Test for Triterpenoid and Saponin

Triterpenoid and steroid tests were carried out by dripping the extract on a drop plate, then adding concentrated sulfuric acid and acetic anhydride. If red or purple color is formed, indicates the sample contains triterpenoid compounds and if a green or blue green ring is formed, indicates the sample contains steroid compounds.

Determination of gallic acid content in *Toona sinensis* leaf extract using HPLC

Determination of gallic acid levels in *Toona sinensis* leaf extract was carried out using the HPLC method. About 20 µL of gallic acid series standard solution were injected into the HPLC(Agilent 1260) with Diode Array Detector (DAD), column C_{18} stationary phase, mobile phase containing 50% acetonitrile and 0.01% H_3PO_4,

running with a flow rate of 0, 7 mL/min. Measurements were taken at wavelengths of 260, 272 and 310 nm. The sample of *Toona sinensis* leaf extract was analyzed using the same condition.

Synthesis of silver nanoparticle

Silver nanoparticles were synthesized by mixing aqueous solution of silver nitrate 1 mM and 0.2 mL *Toona sinensis* leaf extract while stirred at a constant rate at a room temperature. Absorbance measurements were measured using a UV-Vis spectrophotometry the wavelength range 300-700 nm.

Effect of stirring time

About 0.5 mL of $AgNO_3$ 0.1 M is mixed with 0.2 mL of *Toona sinensis* leaf extract in 100 mL Erlenmeyer flask and distilled water was added until the solution has a final volume of 50 mL. The reaction of the formation of silver nanoparticles was allowed to take place while stirring at room temperature using a magnetic stirrer at a speed of 150 rpm. 2 mL samples were taken at 15 min; 30 min; 1; 2; 3; 4; 5; and 6 hours to determine the absorbance spectrum using a UV-Vis spectrophotometer in the wavelength range 300-700 nm.

Effect of Toona sinensis leaf extract concentration and reaction time to AgNP

About 0.5 mL of $AgNO_3$ 0.1 M is mixed with each 0.05; 0.1; 0.15; 0.2; 0.25; 0.3 and 0.35 mL of *Toona sinensis* leaf extract in 100 mL Erlenmeyer flask and distilled water was added until the solution has a final volume of 50 mL. The reaction of the formation of silver nanoparticles was allowed to take place while stirring at room temperature using a magnetic stirrer at a speed of 150 rpm for 4 h. 2 mL samples were taken to determine the absorbance spectrum using a UV-Vis spectrophotometer in the wavelength range 300-700 nm. Absorbance measurements were continued 24; 48; 168; 336 and 672 hours after the reaction took place.

III. Result

Toona sinensis leaves that have been made into powder form were extracted using water solvent at ratio of 1:10 w/v. Extraction using water solvent produces a dark brown extract as shown in Figure 1c. The extract was then further used as bioreductor in the formation of silver nanoparticles (AgNPs). The results of maximum absorption measurements of *Toona sinensis* leaf extract using UV-VIS spectrophotometer were obtained at a wavelength of 271 nm as shown in Figure 2.

Figure 1. (a). *Toona sinensis* leaves, (b).*Toona sinensis*leaves powder, (c).Water extract of *Toona sinensis* leaves

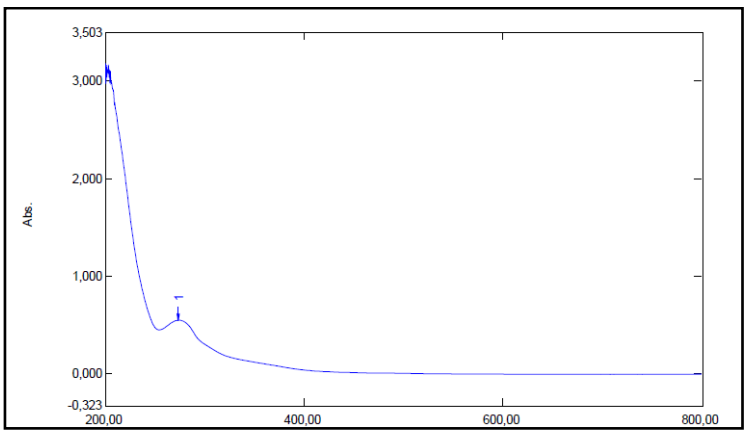


Figure 2. Maximum absorption of *Toona sinensis* leaf extract

Phytochemical screening of *Toona sinensis* leaf extract is shown in Table 1. The results indicate the presence of secondary metabolites compounds such as phenolic, flavonoids, saponins, triterpenoids. Phenolic and flavonoid compounds are groups of compounds that act as primary antioxidants and play an important role in the silver ion reducing properties of extracts.

Gallic acid is one of the main components contained in *Toona sinensis* leaves (12). This compound is a group of phenolic compounds that can potentially be used as bioreductors in the formation of silver nanoparticles. Gallic acid content in *Toona sinensis* leaf extract was determined by the HPLC method. Measurement of standard solution series at a wavelength of 272 nm showed linear regression with equation y = 121.6x-8.3262, R^2= 0.9993 and correlation coefficient (r) = 0.9996. Gallic acid content in *Toona sinensis* leaf extract was obtained at 56.571mg/L.

Table 1. Phytochemical screening result from water extract of *Toona sinensis*

NO	Secondary Metabolite Compound	Reagent	Observation	Result
1	Phenolic	$FeCl_3$	A blue or black purple solution is formed	+
2	Flavonoid	Shinode test	An orange-red solution is formed	+
3	Alkaloid	Mayer	A white precipitation is not formed	-
4	Saponin	H_2O/ HCl concentrate	Formed foam does not disappear by addition concentrated chloric acid	+
5	Triterpenoid	Liebermann-Burchard	Red or purple ring is formed	+
6	Steroid	Liebermann-Burchard	Green/Green-blue ring is not formed	-

Biosynthesis of silver nanoparticles was done by reducing silver ions using *Toona sinensis* leaf extract as a bioreductor. Silver nanoparticles were formed in yellow to brown colloids. Figure 3 shows the absorption spectrum of $AgNO_3$, *Toona sinensis* leaf extract and silver nanoparticles mediated by *Toona sinensis* leaf extract. Silver nanoparticles that were formed had maximum absorption in the wavelength range of 400-440 nm.

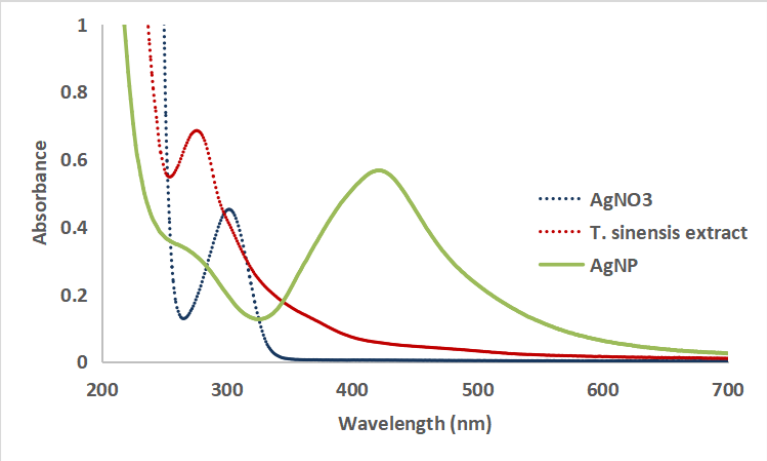


Figure 3. UV-Vis absorption spectrum of silver nitrate, *Toona sinensis* leaf extract, and AgNPs that was produced

The formation of silver nanoparticles mediated by *Toona sinensis* leaf extract was affected by several factors, such as the stirring time as shown in Figure 4. The UV-Vis absorbance value of silver nanoparticles increased with the increasing of stirring time.

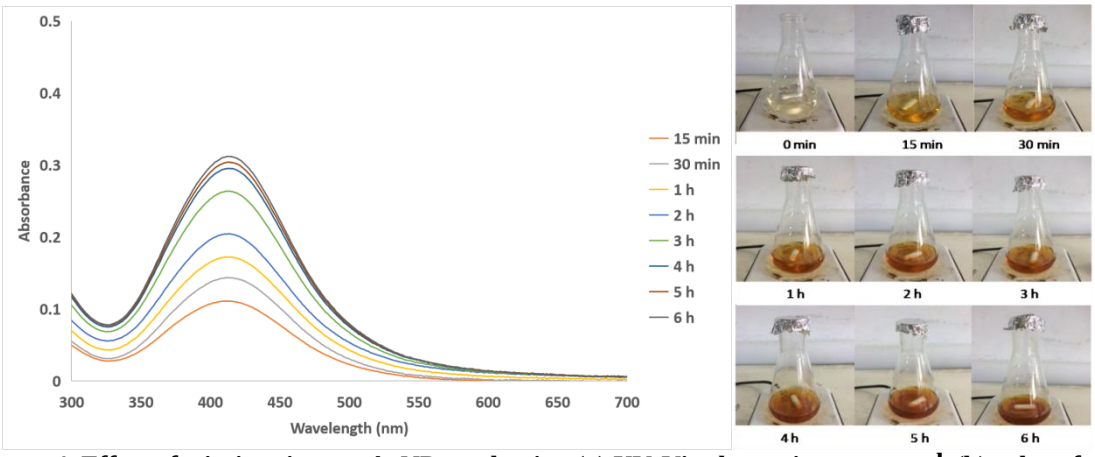


Figure 4. Effect of stirring time on AgNP production (a) UV-Vis absorption spectrum (b) color of the solution

In addition, the formation of silver nanoparticles mediated in *Toona sinensis* leaf extract was also affected by the concentration of the extract added. Increasing the concentration of *Toona sinensis* leaf extract could increase the absorbance value of silver nanoparticles as shown in Figure 5. Besides, the increase in reaction time of reactant also affected the absorbance value and wavelength of the silver nanoparticles formed as listed in Table 2 and Figure 6.

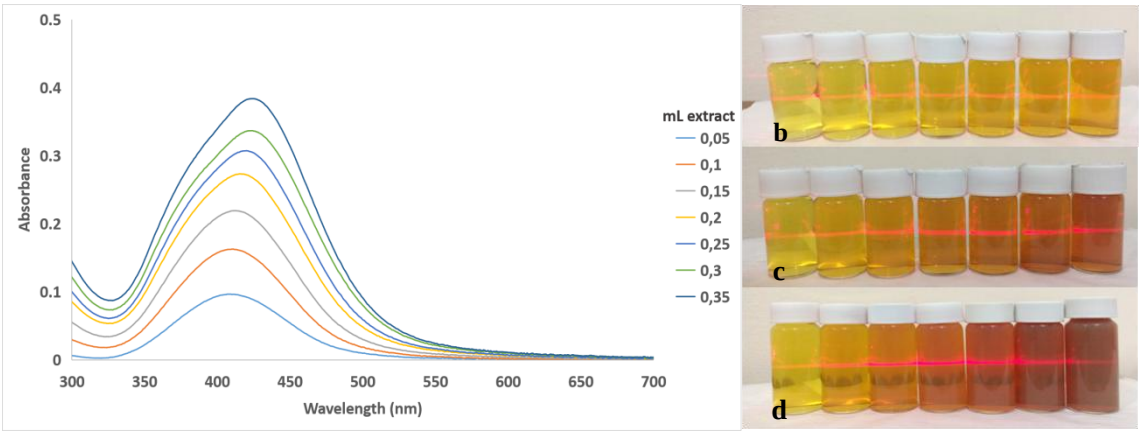
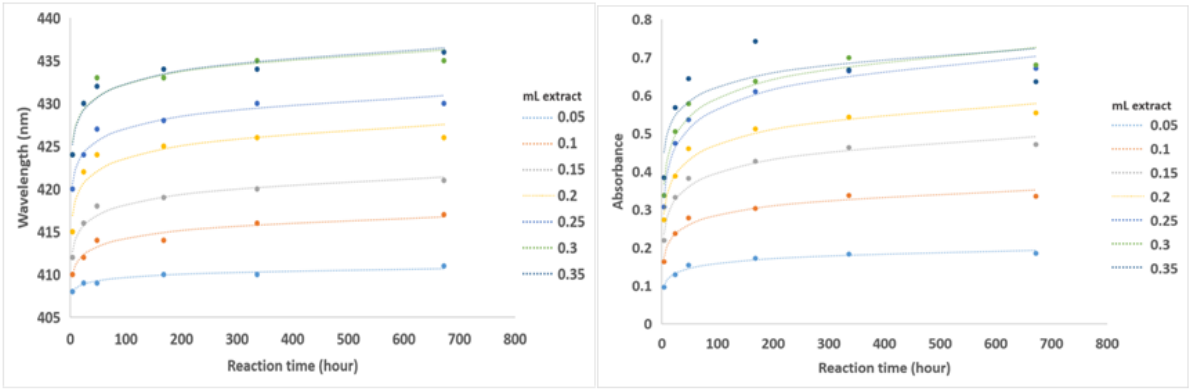


Figure 5. Effect of *T. sinensis* extract concentration on AgNP production (a) UV-Vis absorption spectrum after 4 hours time reaction; laser beam radiation of silver nanoparticles after time reaction (b) 4 hours, (c) 168 hours, and (d) 336 hours

Table 2. Absorbance and wavelength of *Toona sinensis* mediated silver nanoparticles with variation of extract concentration and reaction time

Reaction time (hour)	Wavelength (nm)							Absorbance (a.u)						
	0.05 mL	0.1 mL	0.15 mL	0.2 mL	0.25 mL	0.3 mL	0.35 mL	0.05 mL	0.1 mL	0.15 mL	0.2 mL	0.25 mL	0.3 mL	0.35 mL
4	408	410	412	415	420	424	424	0.096	0.163	0.219	0.273	0.307	0.337	0.384
24	409	412	416	422	424	430	430	0.129	0.237	0.332	0.388	0.474	0.505	0.568
48	409	414	418	424	427	433	432	0.154	0.278	0.382	0.46	0.536	0.578	0.644
168	410	414	419	425	428	433	434	0.172	0.303	0.427	0.512	0.61	0.637	0.742
336	410	416	420	426	430	435	434	0.183	0.337	0.463	0.543	0.664	0.699	0.667
672	411	417	421	426	430	435	436	0.185	0.335	0.471	0.554	0.671	0.68	0.636



a **b**

Figure 6. (a) Wavelength and (b) absorbance of AgNPs in variated *Toona sinensis* leaves extract concentration

IV. Discussion

Aqueous extract of *Toona sinensis* leaf that was used in this study contains secondary metabolite compounds, such as phenolic, flavonoids, saponins and triterpenoids. Yang *et al*., was reported that *Toona sinensis* leaf aqueous extract contains gallic acid, methyl gallate, ethyl gallate, kaempferol, kaempferol-3-O-b-D-glucocide, quercetine, quercitrine, quercetine-3-O-b-D-glucocide and rutin. Based on the study the major constituent in *Toona sinensis* leaf extract is gallic acid. Gallic acid is a phenolic compound that can play a role as bioreductor in the formation of silver nanoparticles (12).

The formation of silver nanoparticles from reduction of silver ion by *Toona sinensis* leaf extract was visually recognized by colour changing from pale yellow to brownish yellow and brown. This was supported by UV-Vis spectrophotometer analysis in Figure 3. The absorption peaks of silver nitrate and *Toona sinensis* leaf extract were shifted to the wavelength at range between 400-440 nm which gives the confirmation for the AgNPs formation. Previous studies about green synthesis of silver nanoparticles have been reported. Singh et al, reported absorption band of silver nanoparticle using *Tinospora cordifolia* at 420-425 nm (14), and Soshinikova *et al.*, reported that colloidal spherical AgNPs absorb light in approximately 400-450 nm (15).

Stirring time during the process of forming silver nanoparticles affected the absorbance value. As the stirring time increased, the absorbance value also increased as shown in Figure 4. The absorbance value increased until stirring time for 4 hours, while stirring at 5 and 6 hours did not show a significant difference in absorbance value.

The increasing concentration of *Toona sinensis* leaf extract as bioreductor would increase the absorbance value of silver nanoparticles, as shown in Figure 5a. Similar study was reported by Ahmed *et al.*, that the increasing concentration of extract *Azadirachta indica* will increase the absorbance of AgNPs (16). The absorbance value of silver nanoparticles is also affected by the reaction time, the longer the reaction occurs, the absorbance value would be increased. However the addition of 0.3 mL and 0.35 mL *Toona sinensis* leaf extract during the reaction time of 336 hours and 672 hours did not give a significant increase (Table 2). In the formation of silver nanoparticles, the reaction occurs in a stoichiometric manner. A number of silver ions will react with biomelocules contain in extract. So the addition of excess bioreductor compound will not increase the formation of silver nanoparticles.

Colloidal silver nanoparticles that formed were also conducted by laser beam radiation as the colloid dispersion could scatter the laser light. A light was scattered by the particle in colloid and resulting in a light line. The higher concentration of silver nanoparticles formed, the intense light could be observed. As shown in Figure 5b, the intensity of light observed in reaction time 336 > 168 > 4 hours.

The effect of *Toona sinensis* leaf extract concentration and the reaction time can also be observed from the color of the silver nanoparticles formed as shown in Figure 5d. It shows that the greater the concentration of extract used, resulting in a more intense color. Similar observations were also reported on *Musa paradisiaca* peel extract (17) and *Cinnamomum zeylanicum* bark extract (18).

In Figure 6it can be seen that at the reaction time of 336 hours, the increase in absorbance was not significant. Meanwhile the maximum absorption wavelength shifts towards a larger wavelength as the concentration of *Toona sinensis* leaf extract increases. Based on the Mie theory, the increasing wavelength (red-shifted) indicates the increasing of particle size (19).

Biosynthesis of silver nanoparticles using plant extracts as a reducing agent can be conducted if plant extracts contain secondary metabolites which contain hydroxyl group. Gallic acid has hydroxyl groups which can reduce silver ions to form silver nanoparticles and the hydroxyl groups itself is oxidized to ketones as shown in Figure 7.

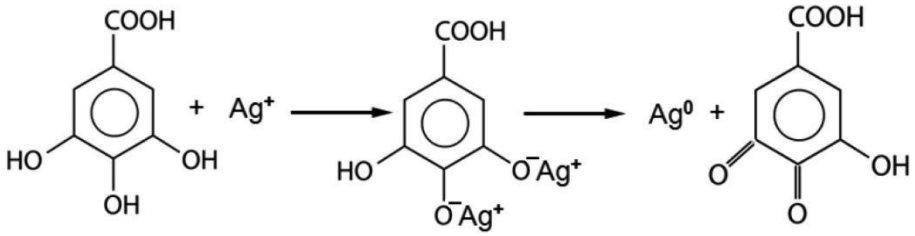


Figure 7. Mechanism of silver nanoparticle formation from gallic acid in Ref (20)

Beside be able to act as a bioreductor, gallic acid can also act as a stabilizer on silver nanoparticles. Based on Pacioni *et al*., gallic acid can stabilize the silver nanoparticles that are formed, so that the nanoparticles are stable (21). The role of gallic acid as a stabilizing agent in silver nanoparticles is illustrated in Figure 8.

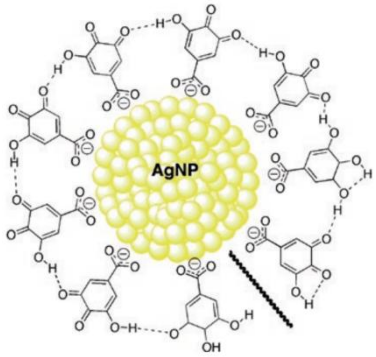


Figure 8. Illustration of the stabilized capped silver nanoparticle using gallic acid proposed in Ref (21)

This research has only reached the formation of silver nanoparticles. Study on the characterization of AgNPs, such as TEM, FTIR, XRD, DLS analysis needs to be continued.

V. Conclusion

Green synthesis of silver nanoparticles using one-pot method mediated by *Toona sinensis* leaf extract was reported in this study. This method provides a cost effective, eco-friendly, and efficient way to form AgNPs. UV-Vis spectrophotometry analysis of AgNPs shows maximum absorption in the wavelength range between 400-440 nm. The gallic acid present in extract may play role in reducing silver ion to silver nanoparticles.

Acknowledgment

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