Optimization of Silver Nanoparticles Synthesis using Kawista (Limonia Acidissima Groff.) leaves ethanol extract

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Abstract. Nanotechnology is one of science technology that current interest due to their wide variety of application in fields. Nanoparticle has different characteristic from previous macroscopic characteristic before it is turned to Nano-size. This research aims to get the optimum condition to synthesize silver nanoparticle from ethanol extract of Kawista (Limonia acidissima Groff). Extraction was done by refluxs method. Different variation does the optimization of silver nanoparticle, i.e., with and without incubation, with and without stabilizer, with and without stir, and stirrer time variation. The optimum condition to synthesize silver nanoparticle was conducted based on characterization result from UV-Vis Spectrophotometric method, and particle size from particle size analyzer result. The optimum condition to synthesize silver nanoparticles was done using AgNO₃: 1% ethanol extract = (10 :1), 5 hours of stirred and 15 hours of incubation time. Those silver nanoparticles are 130, 4 nm in particle size and 0,295 in polydispersity index. Per SEM characterization, silver nanoparticles have a rod and ellipse in shape.

1. Introduction
Nanotechnology is one of science technology which is related to modern research technology i.e. design, synthesis, and structure engineering with particle size scale 1-100 nm. In recent years, nanoparticle becomes one of nanotechnology product which is researched because of their new characteristics which is different from their macroscopic form. Their particle size, which is very small in size, influence their physiochemistry and electrical [1,2].

Nowadays, there is so many researches about nanoparticle especially silver nanoparticle. Silver nanoparticle becomes one of famous nanoparticles because of their unique characteristic than gold nanoparticle [3]. Their Nano-size have raised their reactivity. Silver nanoparticle can be synthesized based on chemical, physical and biological methods. Physical method (top-down method), use high temperature, and chemical method (bottom-up method) use anorganic substances and need high pressure application. This explained that physical and chemical methods need more difficult condition to achieve than if we use biological method. Hence, we need another method, which is more economic, and green chemistry. Green synthesis nanoparticle is more likely to chemical method with oxidation reduction reaction as the main reaction. This method uses eco-friendly reagent such as plant extract. Plant extract act as a bioreductor to reduce Ag⁺ to Ag⁰ [4]. Biomolecule component that acts as a bioreductor in plants i.e. ascorbic acid, polisacharide, and flavonoid [1]. One of plant that contains flavonoid is Kawista (Limonia acidissima), especially in their leaves. Phytochemical screening showed that Kawista contains
flavonoid, phenol, terpenoid, tannin, steroid, saponin and glycoside [5]. Flavonoid in Kawista as a reductor in biosynthesis of silver nanoparticle [4]. Hence, kawista leaves extract potent to become a bioreductor in silver nanoparticle.

This research is aimed to synthesize silver nanoparticle from Kawista leaves extract. Silver nanoparticle will be synthesized in various treatment, which is AgNO₃ variation concentration, Kawista leaves extract volume variation, variation of stabilizer, string time variation, pH variation, and incubation time variation.

2. Methods

This research was conducted in Laboratory of Research, Pharmacy Department, Bandung Islamic University. Particle size analysis was conducted in Central Laboratory, Padjadjaran University and Laboratory of Semisolid and Liquid Preparation, School of Pharmacy, Bandung Institute of Technology. Morphology characteristic was analyzed in P3GL Laboratories.

This research use Kawista (Limonia acidissima Groff) leaves crudes drug, aquadest, aquabidest, AgNO₃ (pro analysis), aluminium foil, polyvinyl alcohol (pro analysis), citric acid (pro analysis), ammonia (pro analysis). Hot plate (IKA C-MAG HS 7), refrigerator, crude drug dryer, micropipette (Effendorf Research Plus), magnetic stirrer, pH meter (Mettler Toledo SevenCompact), particle size analyzer (Beckman Coulter Delsa Nano-C), reflux apparatus (Thermo Scientific), Rotary Vacuum Evaporator (Stuart RE300DB), Spectrophotometer UV-Vis (Shimadzu, UV-Mini 140 and UV-1800), stopwatch, analytical balance (Mettler Toledo ME204E), L. acidissima Groff. plant derivatives (leaves) were collected from Subang, Bandung, Indonesia. Kawista leaves were immersed in a distilled water due to remove dust particles, and then allowed to dry. Kawista leaves extract were made by reflux method using ethanol 70% as a solvent. 10 grams of Kawista leaves were added to 100 mL of solvent. Reflux was done for 2 hours. Silver nanoparticle was synthesized per variation of AgNO₃ concentration (i.e. 0,01 M and 0,1 M), variation of stabilizer (i.e. polyvinyl alcohol 1%, citric acid and ammonia 25%), stirring time, pH and incubation time.

Each of those variation of synthesized condition was characterized by spectrophotometric UV-Vis to detect the formation of silver nanoparticles based on their wavelength. The particle size was characterized using particle size analyzer and the morphology was characterized using Scanning Electron Microscope.

3. Results and discussion

In this research, silver nanoparticles were synthesized by green synthesis method using Kawista leaves ethanol extract as a bioreductor. Silver nanoparticles were synthesized according to the reduction oxidation reaction between silver nitrate and bioreductor substances. The chemical reaction mechanism to synthesize silver nanoparticles is first, bioreductor substances, such as flavonoid and polyphenol will reduce Ag⁺ ion to Ag⁰. Second, the collision of Ag⁰ will generate the silver nanoparticle [6].

Silver nanoparticles synthesis was done by various concentration of AgNO₃, various ratio between volume of Kawista extract and AgNO₃, variation of stabilizer, variation of stirrer time, pH and incubation time. Those silver nanoparticles synthesize divided by 6 variation condition. That variation of synthesize condition are done to obtain the most optimum condition to synthesize silver nanoparticle which is meet the requirement of stability and particle size.

First and second condition of silver nanoparticle synthesis were done to determine the effect of incubation period to silver nanoparticles size. Each of condition consisted of AgNO₃ 0.01 M solution. 1% Kawista leaves extract, PVA 1% as a stabilizer, with the same ratio is 2: 1: 2 (v/v/v), differentiation was done only in incubation period, that is no incubation period for first condition, while for second condition incubation was done for 24 hours. According to characterization of particle size using particle size analyzer, silver nanoparticle size of second condition is smaller than first condition. This mean that the incubation period in 45°C influence the redox reaction rate between AgNO₃ and bioreductor substance. This is taking effect in silver nanoparticle size [7]. Based on particle size analyzer characterization, first silver nanoparticles are 626 nm in size, while second nanoparticles are 570 nm.
Both conditions haven’t produced a qualify silver nanoparticles, because of those particle size still haven’t met the requirement (particle size > 100 nm).

Third condition of synthesis was done by adding stabilizer solution, PVA 1%. This condition was expected to influence the silver nanoparticle size. The addition of stabilizer was done after 24 hours of incubation time; hence the stability of silver nanoparticle was improved well. However, the addition of stabilizer solution after 24 hours of incubation period, increase the particle size of silver nanoparticle to 930 nm in size, instead of lowering the particle size. This happened because silver nanoparticles were aggregated each other in period of incubation, so that the polymer chain of PVA can’t influence the stability of silver nanoparticle. Therefore, stabilizer solution is better added in the beginning of synthesis period, hence the silver nanoparticle will bond directly to polymer chain of PVA, and the stability of silver nanoparticle will increase well [8].

Forth and firth condition of silver nanoparticle synthesis, were done to find out the influence of stabilizer type and pH to particle size of silver nanoparticle. Forth condition using citric acid as a stabilizer so that the acidity of the solution is lowered between 4 – 5. While, fifth condition using ammonia as a stabilizer. Ammonia made the silver nanoparticle solution is increased between 9 – 11. Silver nanoparticle with citric acid as a stabilizer, generate brownish green solution, and 452 nm in wavelength. According to Solomon et al., that wavelength showed the possibility of silver nanoparticle size is more than 100 nm in size [9].

The fifth condition of synthesis, also generate brownish green solution with 476 nm in wavelength. According to Majumdar, that wavelength showed the possibility of silver nanoparticle size is more than 100 nm in size [6]. Moreover, there is a precipitation in the solution after having been incubate for 24 hours. This is showed that silver nanoparticle has been aggregated each other after being incubated for 24 hours (Figure 1). This precipitation phenomenon was caused by base condition that affected the reaction between bioreductor substance and AgNO₃. So that, those wavelengths are not the surface plasmin resonance (386-438 nm) [9].

![Figure 1. Silver nanoparticle which undergo the precipitation.](image)

The sixth condition of synthesis were done to find out the influence of AgNO₃ concentration, stirrer duration, component ratio and incubation time, to silver nanoparticle size. Silver nanoparticle synthesis were done by mix AgNO₃ 1 mM solution with Kawista leaves ethanol extract (ratio 10: 1). This mixture was stirred for 5 hours. Silver nanoparticle were spotted as the color of solution will change time after time. First, the mixture of AgNO₃ 1 mM and 1% Kawista leaves extract is yellow in color and then change to light brown after 3.5 hours [Figure 2 (c)]. After 5 hours of string period, the color change to reddish brown [Figure 2 (d)].

Beside the color change, UV-Vis spectrum can be used to identify the characteristic of silver nanoparticle. Per [9], silver nanoparticle has λ_mak around 395 and 438 nm. AgNO₃ 1 mM solution has maximum wavelength at 221 nm, 299 nm, and 537 nm. 1% Kawista leaves extract has maximum wavelength at 386 nm and 668 nm. After they have been mixed, there is new peak at 395–450 nm. This indicate that a new component has been detected, that is silver nanoparticle. After 3.5 hours, there is still no peak at 395–450 nm, but there is a color change. After 4.5 hours, there is peak at 434 nm, which indicate the silver nanoparticle formation. The formation of yellowish-brown color indicates this.
Silver nanoparticle which have been synthesized from sixth condition has a peak at 436 nm, which indicate > 80 nm in particle size according to Solomon [9]. Based on characterization by particle size analyzer, this silver nanoparticle has 130.4 nm in particle size, and 0.295 in polydispersity index (PI). Polydispersity index is particle size distribution unit, especially for silver nanoparticle. According to Ayadi, a good polydispersity index has a range from 0 to 1 [10]. Polydispersity index limit to 0 indicate a homogen dispersion, while polydispersity index higher than 0.5, indicate high heterogenicity dispersion. Thus, silver nanoparticle from sixth condition has met the requirement of particle size and polydispersity index.

Because of their result of characterization has met the requirement, then silver nanoparticle from sixth condition is being chosen for further evaluation up to 15 days of stability. Silver nanoparticle formation from sixth condition, was affected by various factor, including AgNO₃ concentration, type of bioreductor substance, stirring duration, pH and temperature [7]. Appropriate ratio factor between concentration of AgNO₃ and bioreductor substance in Kawista leaves ethanol extract, must be chosen. If there is no enough concentration of bioreductor to react with silver nitrate, then the reduction of Ag⁺ to Ag⁰ can’t be achieved. Thus, the amount of silver nanoparticle core is reduced. If the concentration of bioreductor substances is too low, then the only slight of silver nanoparticle is formed. But, if the concentration of bioreductor substances is too high, the agglomeration of silver nanoparticle is formed then the size of silver nanoparticle is higher than the requirement [7]. In this research, the optimum ratio of silver nitrate and Kawista leaves ethanol extract 1% is 10: 1 (v/v).

Stirring time is one of the factors that affect the formation of silver nanoparticle. Stirring process is important to increase the contact between AgNO₃ and bioreductor substance. The longer the stirring time, then the reaction rate will increase and more Ag⁺ is reduced to Ag⁰. pH is also become another factor that affect the formation of silver nanoparticle. The most optimum pH is around 4 -5. This acidity is important for formation from keto to enol. This transformation contributes in the formation of silver nanoparticle by bioreduction process. If too base, the silver nanoparticles become less stable. This is indicated by the formation of precipitation.

Incubation temperature also plays a rule in formation of silver nanoparticle. It can increase flow rate of the reaction and the formation silver nanoparticle become faster. The effect of temperature to the formation of silver nanoparticle can be seen in Figure 3. The absorbance of silver nanoparticle has raised
significantly after being incubated for 15 hours. This indicated that the amount of silver nanoparticle formed has been increased.

The stability of silver nanoparticle was observed for 15 days. The formation of silver nanoparticle has increased significantly for 24 hours. This is indicated by the raised of absorbance which is proportional to the amount of silver nanoparticle formed. After 24 hours, the formation of silver nanoparticle tends to stable up to 15 days. This is indicated from stable absorbance ± 2A (Figure 3). Fluctuation of maximum wavelength happened in the range 430 – 450 nm (Figure 3). This indicate that the stability of silver nanoparticle fluctuates because of the aggregation between those particles. Therefore, it is important to add the stabilizer compound (other than citric acid and ammonia) with the appropriate type, concentration, and quantity, to avoid the aggregation of those particles.

The color of silver nanoparticle is stable for 15 days. This indicate that the silver nanoparticle tends to stable, and the degree of aggregation tend to low. If the degree of aggregation is high, then color of the solution is become colorless and clear. Ag₀ turn to Ag⁺ again. The result of silver nanoparticle characterization using Scanning Electron Microscope, showed that silver nanoparticles from Kawista leaves ethanol extract have a rod and ellipse in shape (Figure 4).

4. Conclusion
In this research, the synthesis of silver nanoparticle using Kawista leaves ethanol extract has been optimized. The optimum condition and composition to synthesize those silver nanoparticles are ratio of AgNO₃ 1 mM and Kawista leaves ethanol extract 1% = (10: 1) (v/v). The optimum stirring time is 5 hours, and incubation time 15 hours. Those silver nanoparticles are 130, 4 nm in particle size and 0,295 in polydispersity index. Per SEM characterization, silver nanoparticles have a rod and ellipse in shape.
For further research, it is recommended to add another stabilizer (other than Polyvinyl alcohol, citric acid and ammonia), so that the particle size of silver nanoparticle is more fulfilling the requirement, and more stabilize.

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