Green-route synthesis and characterization of the silver nanoparticles resulted by bio-reduction process

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Abstract. The synthesis of silver nanoparticles by using the leaves extract of Abelmoschus esculentus as a bio-reductor agent have been conducted. Leaf extract produced by using the boiling process of the leaves in boiling water. Ag⁺ ions as a source of silver metal formed by dissolving AgNO₃ into distilled water. A solution containing Ag⁺ ions was added to the leaf extract of Abelmoschus esculentus, followed by an incubation process accompanied by stirring at medium speed. Silver nanoparticles formed were characterised by UV-VIS, FT-IR, PSA and SEM. Based on the results known that the highest nanoparticles formed during the incubation period of 6 days with the size of silver nanoparticles mostly less than 100 nm. SEM results show that silver nanoparticles have a non-uniform cubic shape morphology. Band gap energy for each incubation period 1; 2; 3; 6 and 7 days are 2.263 eV; 2.228 eV; 2.227 eV; 2.096 eV; and 2.227 eV respectively.

1. Introduction
Various synthesis methods (physical and chemical methods) to produce metal nanoparticles have been carried out by researchers to produce nanoparticles with a variety of characters and functions. The primary considerations for selecting nanoparticle synthesis methods are production costs, the potential for development, toxicity, and impact on the environment [1–6]. The development of a synthesis method with a green synthesis model is a promising opportunity for the production of nanoparticles such as silver nanoparticles. One of the most widely used green synthesis methods for silver nanoparticles is bioreduction using plant extracts, algae, enzymes and bacteria [7–9].

The synthesis pathway using the bioreduction method continues to be developed because the various application can be produced from these silver nanoparticles such as for anti-oxidants, antimicrobials, antifungals and as active ingredients in chemical sensors [10–13]. Silver nanoparticles also play a significant role as inhibitors that deactivate the enzymes are needed for the life of a single cell, a virus, and various fungi to provide oxygen supply to the metabolic system. The performance of silver nanoparticles does not interfere with or cause side effects or damage to other enzymes in the human body and other parts of the chemical system found in the human body. Silver nanoparticles only damage certain parts that interfere with the performance system of the human body [14,15]. Recently, bioreduction of silver nanoparticles using plant extracts has been explored by the researchers, apart from being very diverse in function of plant extracts, there are also many plant species that have not been studied.
Silver ion bio-reduction by using *Muntingia calabura* leaf extract has been carried out by previous researchers. The results showed that the size of the silver nanoparticles produced was 54.50 nm. Silver nanoparticles products can be used as an active chemical sensor to detect blood sugar levels [10]. Bioreductors from *Azadirachta indica* leaf extract have been able to produce silver nanoparticles with nanoparticle size of 34 nm. These silver nanoparticles can be applied as antibacterial [17].

In general, the ability of bioreduction from plant extracts depends on the reducing compounds contained in the extract, especially alkaloids, flavonoids and derivatives. One of the plants that contain the reducing compounds is *Abelmoschus esculentus*. The *Abelmoschus esculentus* plant is a plant that contains compounds of quercetin-3-O-gentiobio.pyranoside, quercetin-3-O-[β-D-xylo-(1→2)]-β-D-glucopyranoside, quercetin-4"-O-methyl-3-0β-D-gluco.pyranoside [18]. The presence of the flavonoid derivative which has the properties of reducing is a great potential of this plant to be used as a bio-reductor. The utilisation of *Abelmoschus esculentus* as a bio-reductor material to produce silver nanoparticles is the latest research because there is no published literature or research. Based on the background, this study carried out the synthesis and characterisation of silver nanoparticles using bioreducers of *Abelmoschus esculentus* leaf extract.

2. Experimental

2.1 Materials and Instruments

The instruments to be used are analytical scales (Acculab), UV-Vis (Shimadzu UV-2600), *Scanning Electron Microscopy* (SEM) JSM-651OLA JEOL, *Particles Size Analyser* (PSA) Vasco DLS, *Magnetic Stirrer* (VWR Scientific), *Spray Dray* (Buchi 190). The materials used are *Abelmoschus esculentus* leaves, distilled water, bi-distilled water, silver nitrate powder (AgNO₃), filter paper, Whatman paper No. 1.

2.2 Preparation of the *Abelmoschus esculentus* leaf extract

*Abelmoschus esculentus* plants were obtained from Moncongloe Bulu Village, Moncongloe District, Maros Regency, South Sulawesi. The part of the plant used is fresh leaves. The leaves are taken and washed thoroughly with distilled water and drained. The leaves are cut into small pieces and weighed 10 grams, then boiled with 100 mL of distilled water in 500 mL Erlenmeyer. Blowing is carried out for 5 minutes, then cooled. After reaching room temperature, poured boiling water and filtered using Whatman no. 1. The extract ready to be used directly for the biosynthesis of silver nanoparticles.

2.3 Synthesis of silver nanoparticles with *Abelmoschus esculentus* leaf extract

The silver ions reduced to produce the silver nanoparticles initiated by dissolving AgNO₃ into in bi-distilled water. Then, The Ag⁺ ion mixed in extracts that produced from *Abelmoschus esculentus* leaves. A solution containing 40 mL of Ag⁺ ion was added to the extract of *Abelmoschus esculentus* leaves as much as 2 mL of boiled water. The mixture is incubated and stirred at medium speed. The incubation period and stirring were varied, namely 1, 2, 3, 6 and 7 days. The colour changes of each mixture were observed for each incubation period, and UV-Vis was carried out. The band gap energy of silver nanoparticles was determined based on UV-vis results for each incubation period. Characterization of changes in functional groups that occur during the process of forming silver nanoparticles was observed using FT-IR. Morphological tests using SEM of colloidal silver nanoparticles were dried to form a powder.
3. RESULTS AND DISCUSSION

3.1 Bio-reduction of Ag⁺ into silver nanoparticles

Bio-reduction process of Ag⁺ by using the leaf extract of *Abelmoschus esculentus* was carried out, through the addition of liquid extract of the leaves of this plant into a glass containing silver-ionized by water. The colour of silver nitrate solution is bright after being added to the leaf extract of *Abelmoschus esculentus* changed into a homogeneous yellow color. After the incubation process was carried out with stirring, the color change from the curl became brownish yellow and finally browned (Figure 1). The phenomenon of color changing of the solution at the duration of incubation period occurs due to the excitation of Surface Plasmon Resonance (SPR) on the surface of the nanoparticles. The color changing of the solution into brown color indicates that the silver nanoparticles have been formed. The similar color changing also obtained by previous researchers [13,19].

![Figure 1 Biosynthesis process of silver nanoparticles](image)

3.2 Analysis of the SPR phenomenon through UV-Vis

In this study, the effect of incubation time on changes in SPR and nanoparticles was observed. Silver nanoparticles synthesized at various incubation times were analyzed by UV spectra to observe changes in SPR, changes in the size of silver nanoparticles (Figure 2). The change in the coloration of the yellowish solution to brown and the absorption of UV-Vis light in the range 446-448 nm is characteristic of SPR excitation and the formation of silver nanoparticles as reported by previous researchers [20–22]. The value of absorbance intensity which has increased from the incubation time of the first day to the sixth day shows that in the solution there is an increase in the number of silver nanoparticles formed [23,24,16]. When incubation time increasing until the seventh day there is a decrease in absorbance intensity. This phenomenon shows that the process of bio-reduction of Ag⁺ into silver nanoparticles in the solution has decreased due to the reduced number of reducing compounds in the solution. The highest UV-Vis absorption intensity was observed after the sixth day of the incubation period began, this result showed that the process of forming silver nanoparticles had been carried out correctly in the reactant mixture at the sixth day incubation time.
Figure 2 shows a shifting of the peak wavelength of silver nanoparticles produced towards higher wavelengths or bathochromic shifts (redshift) during the incubation period of the first day to the third day. The occurrence of the bathochromic shift from the first day to the third day shows that along with the addition of the incubation period there has been an increase in the average diameter size of the silver nanoparticles produced. However, the incubation period of the sixth day until the seventh day was found to have changed the type of shift from the bathochromic shift to the hypochromic shift (blue shift). The presence of a hypochromic shift indicates that the average diameter size of the silver nanoparticles produced decreases with increasing incubation period [21,22].

3.3 Determination of the band gap of silver nanoparticles

Band gap energy is the difference in energy values between HOMO (highest occupied molecular orbital) and LUMO (Lowest unoccupied molecular orbital). The band gap energy also shows the difference in energy possessed by the valence band with the conduction band. The nanomaterial that will be applied as an electronic base material that functions as an introduction to electron flow is largely determined by the band gap energy possessed by the material [25]. Various methods can be used to determine the band gap energy of one of the ingredients using the UV-Vis method. Through spectra produced from UV-Vis, we can know the electronic transition of electrons in a material. The maximum λ value generated from UV-Vis spectra is closely related to the band gap energy possessed by nanoparticles. In addition, the maximum λ value is also a symptom that arises because of the interaction of electrons in accordance with quantum theory.

In general, the band gap energy of silver nanoparticles can be determined using the Tauc equation [25–27]. Based on the Tauc equation, the band gap energy is determined by the following equation below.

\[ n = \frac{Q(hv - E_{gap})^2}{hv} \]

where, \( n \) is the absorptivity coefficient, \( Q \) is the proportionality constant, \( h \) is the Plank constant, \( \nu \) is the photon frequency. The results of the calculation of band gap energy for each band from the UV-Vis test with the incubation period of the first day until the third day shown in Figure 3. The
band/wavelength band of silver nanoparticles for consecutive first, second, and third day incubation period 2.226 eV (447 nm), 2.228 eV (446 nm), and 2.227 eV (444.5 nm).

These results indicating that the energy band gap of silver nanoparticles decreases with increasing particle size on average nanoparticle molecules produced. This phenomenon is due to quantum mechanics if the size of the material is greater (especially the material on the nanoscale), the number of orbitals that overlap or the level of energy possessed increases. This condition causes the distance between the valence band and conduction to get closer [25,26]

The band gap energy for each band produced by the UV-vis test with an incubation period of 6 days and 7 days is shown in Figure 4. The band/wavelength band of silver nanoparticles for the sixth and seventh day incubation periods respectively is 2.096 eV (449 nm) and 2.227 eV (449.5 nm). These data indicate that the band gap energy of silver nanoparticles increases with decreasing average particle size of the nanoparticle molecules produced, characterized by a shift in SPR to a higher wavelength. The fact is due to the smaller size of the nanoparticles resulting in an overlapping number of orbitals or decreasing levels of energy. This condition causes the distance between the valence band and conduction to be wider [27,26]
3.4. Functional group analysis using FT-IR

The results of the test analysts using FT-IR on silver nanoparticles and *Abelmoschus esculentus* leaf extract are shown in Figure 1. The broad peak at 3446.79 cm\(^{-1}\) and 667.32 cm\(^{-1}\) indicating the presence of stretching vibrations originating from OH and alcohol deformation out of the plane. The appearance of a weak band at wave number 2958.60 cm\(^{-1}\) comes from the vibration stretching of the \(-\text{CH}_2\) group. The ribbon with weak intensity at 2904.29 cm\(^{-1}\) and 2746.63 cm\(^{-1}\) originated from the vibration stretching of the aldehyde group (-CHO). Stretching vibration that occurs in the \(-\text{CH}_2\)-group is indicated by the appearance of the band at 2852.72 cm\(^{-1}\).

![Figure 4](image)

**Figure 4** Band gap energy of silver nanoparticles for an incubation period (a) of sixth days (b) seventh days

![Figure 5](image)

**Figure 5** FT-IR (a) extract of *Abelmoschus esculentus* leaves, and (b) silver nanoparticles

The band at 2398.94 cm\(^{-1}\) originates from the stretching vibration of the acetaldehyde group while the C-C stretching radiation from the R-CH = \text{CH}_2 bond appears at 1805.37 cm\(^{-1}\). Stretching vibration (C = C) of aliphatic compounds, stretching vibrations C = C which is in cyclic compounds and stretching vibrations of C-N from successive groups of amines appear at wave numbers 1651.07 cm\(^{-1}\); 1541.12 cm\(^{-1}\); 1063.39 cm\(^{-1}\). The tape at 1394.53 cm\(^{-1}\) showed the occurrence of C-H deformation from –\text{CH}_3 and \text{CH}_2 deformation from long-chain carboxylates appearing at 1240.23 cm\(^{-1}\). Based on the analysis shows the number of groups that can function as bioreductors (undergoing oxidation) which are owned by the extract of *Abelmoschus esculentus*. 
The results of previous studies showed that the functional groups responsible for the bioreduction process using plant extracts were –CN, -CHO, and –OH [27-30]. Figure 51 (b) shows the presence of a slight FT-IR band breakdown of the leaf extract of *Abelmoschus esculentus* after forming silver nanoparticles. In addition, there was also a reduction in bandwidth intensity at 3442.94 cm\(^{-1}\); 1643.35 cm\(^{-1}\); 1047.35 cm\(^{-1}\); 609.81 cm\(^{-1}\) and the band loss at 2746.63 cm\(^{-1}\). The band at 3442.94 cm\(^{-1}\) and 609.81 cm\(^{-1}\) came from alcohol groups. The band 1047.35 cm\(^{-1}\) originated from the C-N group of protein compounds and the band 2746.63 cm\(^{-1}\) was a characteristic of the aldehyde compound. The reduced intensity and loss of the band at the wave number after the formation of gold nanoparticles shows that these groups are responsible for the bioreduction process and capping silver ions. The results of FT-IR analysis are similar to those obtained by previous researchers [15,28].

3.5 Analysis of particle distribution with Particle Surface Analyzer (PSA)

The size distribution of silver nanoparticles in the form of the solution produced was tested using a PSA to determine the distribution of nanoparticles and the size distribution of nanoparticles formed in the aqueous phase. The results of the nano-size distribution of silver particles using a PSA are shown in Figure. 6.

![Figure 6](image_url)

**Figure 6** Number of distribution of silver nanoparticles

The size of silver nanoparticles obtained by the average obtained was 24.9 ± 6.9 nm. The result showed that the synthesized silver nanoparticles meet nanoscale particle sizes (<100 nm). The PSA test results showed the polydispersity index of the nanoparticles produced. The polydispersity index gives a homogeneous picture of the size distribution of scattered nanoparticles in the solution phase. The polydispersity index value is calculated by dividing the average weight of the nanoparticles by the average amount of weight of the nanoparticles. The higher the polydispersity index value, the more the size of the nanoparticles is not uniform. The ideal nanoparticle polydispersity index value is less than 0.5. The significant difference in the size of the nanoparticles (smaller and larger) will affect the
characteristics of nanoparticles. Larger size nanoparticles tend to experience precipitation. Silver nanoparticles produced from bioreduction using *Abelmoschus esculentus* leaf extract had a polydispersity index of 0.381. These results indicate that the size of the nanoparticles produced is more homogeneous because it has a polydispersity index value of less than 0.5 [12,29].

3.6 **Morphological analysis of silver nanoparticles with SEM**

Silver nanoparticles analyzed using SEM aims to determine the morphology of the nanoparticles produced. The atomic composition of the nanoparticle constituents was carried out using SEM-EDX. The SEM test results were tested using SEM 1000 x magnification, and voltage acceleration which was reduced by 20 keV, the silver nanoparticles produced in this study are shown in Figure 7.

![Figure 7 Image of SEM silver nanoparticles](image)

![Figure 8 Image of SEM-EDX silver nanoparticles](image)
Based on these test results it appears that silver nanoparticles formed have scattered cubic morphology among compounds that capping Ag. One of the silver nanoparticles assemblies formed is quite clearly shown in Figure 8, which is in the red circle. Grouped silver nanoparticles showed that the formed nanoparticles had agglomerated during the incubation and drying process. The results of the SEM-EDX silver nanoparticles test produced were carried out using voltage acceleration from 0 keV to 21 keV (Figure 8). The peak that appears at 0.1 keV and 3 keV shows that the content of silver nanoparticles in solid samples from bioreduction results using a large extract of *Abelmoschus esculentus* leaves which is indicated by a red arrow.

### 4. Conclusions
Silver nanoparticles produced through the bioreduction process using *Abelmoschus esculentus* leaf extract were successfully carried out. The UV-Vis test showed that the optimum incubation time to produce silver nanoparticles was on the sixth day. The characteristics of the silver nanoparticles produced have an average particle size of 24.9±6.9 nm and tend to be homogeneous. SEM results show that the resulting nanoparticles are cubic in size and shape that are uneven. Based on the results of the calculation illustrate that the resulting nanoparticles have a band gap value that can be used as a semiconductor or a pretty good electron delivery.

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