Microstructural analysis of silver nanoparticles resulted through bioreduction using *Abelmoschus esculentus* leaf extract

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**Abstract.** Microstructural analysis of silver nanoparticles produced through bioreduction using *Abelmoschus esculentus* leaf extract was carried out. Biosynthetic reactions produce silver nanoparticles by mixing Ag⁺ and *Abelmoschus esculentus* leaf extracts. The formation of nanoparticles characterized by changes in the solution from yellow to brown. Silver nanoparticles were analyzed using XRD, and the analysis results show that the average size of silver nanoparticle crystals is 41.9 nm with strain and stress values of 7.5 x 10⁻⁵ and 0.4908 MPa, respectively. The calculation results show that silver nanoparticles produced have energy density and dislocation density crystals 3.72 J/m² and 5.9 x 10²² m⁻². Based on the prediction of the mechanism carried out the bioreduction process occurs through the use of quercetin-4′-O-methyl-3-O-β-D-glucopyranoside compounds with the orientation of the crystal are FCC and BCC.

1. **Introduction**

Silver nanoparticles have been developed in many fields of medicine, therapy (antimicrobial, antidiabetic, anticancer, antiparasitic, and antioxidant), bio-molecular diagnostics, medicine, textile factories and dental implant raw materials and in the field of water treatment [1–3]. Many researchers are interested in developing silver nanoparticles because these material properties are less toxic, low volatility and high thermal stability. In general silver nanoparticles can be produced through metal reduction methods from their salt forms, pyrolysis methods, sonochemical methods, and microemulsion methods [4–6]. The current production of silver nanoparticles requires high costs, requires specific instruments and is not environmentally friendly. Currently, the synthesis of silver nanoparticles by bioreduction method is an attempt to reduce the shortcomings of conventional synthesis methods.

Green synthesis of silver nanoparticles through bioreduction of silver ions has been carried out using various plants such as using *Urtica dioica* Linn. leaf aqueous extract[4], *Tamarix gallica* leaf extract [7] and *Padina Pavonia algae* [8]. The production of silver nanoparticles through the bioreduction method has a unique challenge because the character of the nanoparticles produced mostly determined by the composition of the chemical compounds contained in the bioreductor. Uniquely the properties provided by silver nanoparticles initiate researchers to understand the various characteristics possessed by these nanomaterials. In addition to nano-sized material, the character of
this material will be slightly different from micro or macro size material. Microstructural analysis of nanomaterial has carried out, namely the study of MgO nanoparticles produced by the hydrothermal process followed by the annealing process [9]. Microstructural analysis was performed on silver nanoparticles doped on cotton enriched with amino groups [10]. Through microstructural analysis of silver nanoparticles, a description of the characteristics of the nanoparticles produced and the effects of changes in material properties will be obtained after being in nano size [9-11]. The character possessed by silver nanoparticles is very closely related to the ability of silver nanoparticles for specific applications [9-11].

Due to the characteristics of silver nanoparticles produced through bioreduction processes using plants are highly dependent on bioreductors, it is very important to characterize these materials structurally. The results of the analysis will provide vital information to determine the process of nanomaterial formation and the factors that determine the application ability of this material. Based on literature studies to date there have been no reports that have examined bioreduction silver nanoparticles using okra microstructure extract. Based on literature studies and the importance of the microstructural properties possessed by nanoparticles, the study has examined the microstructural analysis of silver nanoparticles resulting from bioreduction using *Abelmoschus esculentus* leaf extract.

2. Experimental

2.1 Materials

The materials to be used are *Abelmoschus esculentus* leaves, distilled water, bi-destilled water, Whatman paper No.42, silver nitrate powder (AgNO₃).
2.2 Biosynthesis of silver nanoparticles
The biosynthesis of silver nanoparticles was carried out by mixing Ag$^+$ solution and *Abelmoschus esculentus* leaf extract. A total of 10 mL *Abelmoschus esculentus* leaf extract mixed into a solution of 40 mL Ag$^+$ then stirred for 2 hours. The formation of nanoparticles was characterized by discoloration of the solution from yellow to brown. Furthermore, the formed silver nanoparticle colloid solution was dried with a Spray Dryer (Buchi 190) to obtain samples in powder form. The formed powder was tested with X-ray diffraction (X-RD) at an angle of 20: 20° - 70°. Then a microstructural analysis is carried out.

3. RESULTS AND DISCUSSION

3.1 Synthesis of silver nanoparticles
The biosynthesis process of silver nanoparticles using *Abelmoschus esculentus* leaves extract can be observed through changes in the color of the solution used. The color change shows that the bioreduction process of Ag$^+$ into AgNPs occurs at certain times. The color of the AgNO$_3$ solution is initially bright, changing after adding the extract turns dark yellow. After the incubation process was carried out, the color changes into brown. Variations in color from dark yellow to brown are characteristic of the formation of silver nanoparticles [2,7] as shown in Fig. 1.

3.2 Analysis using XRD
The results of the analysis using XRD in Fig. 2 show the crystalline properties and crystal patterns possessed by the formed silver nanoparticles. XRD analysis carried out by scanning at an angle of 20: 20°-70° indicates a diffractogram at 20: 39.53; 44.06; 57.50 and 64.42 are typical of silver nanoparticles when referring to JCPDS No: 04-0783. Based on the diaphragm nanoparticles, a study of the average crystal size, particle size distribution, crystal strain, crystal stress, crystal density energy, dislocation density and texture coefficient were conducted.

**Figure 2** Diffractogram of silver nanoparticles resulting from bioreduction using *Abelmoschus esculentus* leaves extract
3.3 The average crystal size of silver nanoparticles

Based on the analysis using XRD in Figure 2, it is known that the interplanar distance of each silver nanoparticle diffraction is: 2.277 Å (39.53°); 2.053 Å (44.06°); and 1.444 Å (64.42°). Referring to the angle at 2θ for the characteristic of silver nanoparticles, the average crystal size can be determined using the Debye-Scherrer formula [6] equation 1:

\[ D = \frac{K \times 0.1541 \text{ nm}}{\beta \times \cos \theta} \]  

Where \( D \) is the average size of silver nanoparticle crystals, \( K \) is the Debye-Scherrer constant, \( \beta \) is FWHM (Full Width at Half Maximum) of each peak, 0.1541 nm as light as X-ray radiation wavelength, \( \theta \) is the diffraction angle.

| 2θ (°) | FWHM (°) | hkl | \( D \) (nm) | \( d \)-spacing (nm) | Parameter of lattice (a) (nm) |
|--------|----------|-----|-------------|-----------------|-----------------------------|
| 39.53  | 0.155    | 1 1 1 | 48.44       | 0.227           | 0.39                        |
| 44.06  | 0.181    | 2 0 0 | 40.86       | 0.206           | 0.41                        |
| 64.42  | 0.197    | 3 1 1 | 34.29       | 0.144           | 0.48                        |
| Average|          |      | 41.19       |                 |                             |

The calculation results using Equation 1 then obtained the average crystal size of the silver nanoparticles produced was 41.19 nm. The size of the silver nanoparticles obtained was greater than that of the nanoparticles obtained using a bio-reductor of Urtica dioica Linn leaf extract [4]. While the calculation results of the lattice parameters of silver nanoparticles produced in Table 1 show the average lattice parameter of 0.427 nm. The lattice parameters obtained close to the silver nanoparticle lattice parameter based on JCPDS no. 04-0783 (a = 0.407 nm) [2].

3.4 Determination of particle size distribution

Crystal size distribution is an illustration of the distribution of the size of the silver nanoparticle crystals that have produced. Using a model approach which assumes that all particles formed are dispersed logarithmic-normal [12]. Based on the model, particle distribution formulated with the function in equation 2.

\[ SD(x) = \frac{1}{\sqrt{2\pi} \sigma_{np} x} \exp \left\{ -\frac{[\ln(p/m_{np})]^2}{2\sigma^2} \right\} \]  

Where \( SD \) is a function of particle size distribution, \( p \) is the size of each nanoparticle, \( \sigma_{np} \) is a variant of the size of nanoparticles and \( m_{np} \) is the median of the size of the nanoparticles. The calculation results using the function in equation 2 resulting graph of particle size distribution in Fig. 3.
Fig. 3 shows that the silver nanoparticles produced are mostly measuring less than 40 nm with a variance of 33.55 and a median of 41.38. The size of the silver nanoparticles produced tends to be small indicating that the stability of the nanoparticles formed is quite high, scattered and not agglomerated [10].

3.5 Strain calculation of silver nanoparticle crystals

Crystal strains are quantities that show changes in crystal size or structure caused by changes in temperature, ring shape, and van der Waals bonds, torque. The crystal strain will significantly influence the character and nature of the crystal such as the ability to catalyze. The increase in crystal strains determined by extrinsic factors such as the induction process, which not evenly distributed on all interface lattices or nanoparticle supports [13,14]. Based on the assumption that the crystals have formed are uniform in all directions, the determination of crystal strains can be done using uniform deformation models. In addition, all crystal environments are isotropic, and the existing nanoparticles do not depend on each other in all directions [9]. The models used equation 3:

\[
\beta \cos \theta = \nabla + 4 \varepsilon \sin \theta\\
\]

Where \( \beta \) is FWHM nanoparticles, \( \theta \) is the resulting diffraction angle, \( \nabla = \frac{k \lambda 0.1541}{\theta} \) and \( \varepsilon \) is the nanoparticle strain. Calculations are carried out using equation 4.

\[
\beta = \left[ (\beta^2)_{sample} - (\beta^2)_{stand} \right]^{1/2}
\]

Using equation 3, we can obtain a graph of the relationship of \( 4 \sin \theta \) versus \( \beta \cos \theta \) as shown in Fig. 4.
Based on Fig. 4, the slope value is 0.0003 and intercept obtained at 0.0031. By using the method from a plot of $4 \sin \theta$ versus $\beta \cos \theta$, the strain value ($\varepsilon$) can be obtained from silver nanoparticles of $7.5 \times 10^{-5}$. The silver nanoparticle strain obtained is lower than that of silver nanoparticles produced using *Ocimum sanctum* leaf extract as bio-reductor is 0.3688 [15]. These results indicate that the silver nanoparticles produced using *Abelmoschus esculentus* leaves as bioreductors have a crystal structure that is less subject to change. The result caused the repulsion of Van der Waal between atoms in the crystal structure is not too large and the lack of aversion that occurs due to the rearrangement of bonds between molecules in nanoparticle crystals [13,14,16].

### 3.6 Stress calculation of silver nanoparticle crystals

Stress and crystal strains are two interconnected events in a glass. The value of stress can indicate the difference in the ability of crystals to respond to external pressure caused by crystal imperfections. The modification of the Williamson-Hall equation a uniform stress deformation model (USDM) which is an equation that can be used to determine the stress of a nanoparticle crystal. The application of the USDM equation uses the assumption that crystallographically tested have a uniform shape and the Hook Law used has linearity between strain-stress ($\sigma = \varepsilon Y$) [11,17]. Based on these assumptions, an equation can be obtained to determine the value of crystal stress such as equation 4.

$$\beta \cos \theta = \nabla + \frac{4\varepsilon \sin \theta}{Y}$$

Where $\sigma$ is the stress of the crystal value, $Y$ is the modulus of elasticity of silver (82.5 GPa at 293 K) [11,17], $\beta$ is FWHM. Based on equation 5 then plotted between $4\sin(\theta)/Y$ versus $\beta \cos \theta$ shown in Fig. 5.
The linear regression of plot $4\sin(\theta) / Y$ versus $\beta \cos\theta$ obtained by intercept is $2.38 \times 10^{-5}$. The stress value for silver nanoparticles obtained from bioreduction was obtained using *Abelmoschus esculentus* leaves of 0.4908 MPa. The stress of the silver nanoparticles shows that the amount of internal strength that can be used to respond to external force is 0.4908 MPa. Thus, if the pressure exceeds the stress value, the silver nanoparticle structure will change [11,17].

3.7 Energy density of crystal

The energy density of crystal is the result of calculating energy obtained using the assumption that the material is homogeneous, and the condition is isotropic. The application of the uniform deformation energy density model (UDEDM) equation can be applied to silver nanoparticle crystals. Based on the UDEDM model, equation 5 can be expanded to equation 6 to determine the energy density relationship of the silver nanoparticle crystals obtained.

$$\beta \cos \theta = \nabla + \left(4\sin\theta \left(\frac{2u_{ed}}{Y}\right)^{1/2}\right)$$

Plots of $4\sin\theta \left(\frac{2u_{ed}}{Y}\right)^{1/2}$ versus $\beta \cos \theta$ is the development of the equation and the results of the fittings shown in Fig. 6.
Based on the regression results from Figure 6, the slope value of $2.38 \times 10^{-5}$ obtained. It can be determined the value of the energy density of the silver nanoparticle crystals is 3.72 J/m$^2$. This result approaches the silver density energy value in bulk form which is equal to 1.065 - 1.54 J/m$^2$ and the calculated value using the Kelvin equation is 7.2 J/m$^2$. The crystal density energy obtained is smaller than in its bulk form. This condition can be understood that the silver nanoparticles produced through this process are not pure [18,19].

### 3.8 Dislocation Density

Dislocation is one method for analyzing crystal defects or the arrangement of irregularities that make up crystalline structures. The presence of crystal dislocations will affect various material properties themselves. The dislocation value of material will increase along with the occurrence of the deformation process. In general, the process of dislocation formation can occur through the process of grain boundary initiation and lattice and surface interface, nucleation in a homogeneous, sedimentary, or dispersed phase. In a material composed of crystals, changes in the density of the dislocation of a crystal will be blocked due to another crystal dislocation. Crystals with significant density dislocations will increase the hardness of the crystal. Silver nanoparticle crystals with large dislocation values turned out to have a higher level of hardness compared to the others [4,20]. In addition, crystals with FCC (face center cubic) geometric structure will experience an increase in dislocation along with a reduction in the size of the crystal particles they have. However, it will increase with increasing strain value [20].

Dislocation of silver particles based on analysis using X-rays is $15 \pm 2 \times 10^{14}$ m$^{-2}$ with a stress value of 0.275 GPa [21]. Hardness will increase with decreasing the size of individual crystal grains which is around 20 nm. To determine the dislocation density ($\delta$) of silver nanoparticles can be used in equation 7 [22].

$$\delta = \frac{1}{D^2} \tag{7}$$
Where $\delta$ is the density of the location, $D$ is the average size of the silver nanoparticle crystals. Based on the results of calculation, the dislocation density of the silver nanoparticles obtained from the bioreduction process using the aqueous extract is $5.9 \times 10^{22}$ m$^{-2}$. These results indicate that the dislocation density value of silver nanoparticles produced is higher than that of pure nanoparticles. The density dislocation from the silver produced gives a clue that these silver nanoparticles have a reasonably large impurity compared to pure silver nanoparticles. This condition can be understood because silver nanoparticles are formed through a capping process by reducing organic compounds [2].

3.9 Texture coefficient of silver nanoparticles

The tendency of a crystal to be in the orientation of a particular field known through the determination of structural orientation. The crystal orientation in polycrystalline samples will give an idea of the regularity of the crystals contained in the sample, as well as changes in the electronic properties of the polycrystalline sample [23]. The results of the relative crystal shape orientation for each peak test result with X-RD referring to the relative crystal orientation shown in Table 2.

Table 2. Analysis of the orientation of the structure of silver nanoparticles

| No. | 2$\theta$ | h k l | $S (h^2+k^2+l^2)$ | Silver nanoparticles orientation |
|-----|-----------|-------|------------------|---------------------------------|
| 1   | 39.53     | 1 1 1 | 3                | FCC                             |
| 2   | 44.06     | 2 0 0 | 4                | BCC, FCC                        |
| 3   | 64.42     | 3 1 0 | 10               | BCC                             |

Refer to the silver JCPDS data with two forms of crystal orientation FCC (face center cubic) and BCC (body center cubic). Based on Table 2 shows that the peak at an angle of 2$\theta$: 39.53 tends to choose the orientation of the FCC crystal. The silver FCC structure means that the silver atom will be located at each angle besides that there will also be atoms that occupy all the faces of the cube. For peaks with an angle of 2$\theta$: 44.06, they tend to be BCC and FCC oriented. This condition shows that crystals at the peak can be a transition from the FCC and BCC. The peak at the angle 2$\theta$: 64.42 is estimated to have a BCC crystal orientation. The BCC crystal orientation has 8 atoms at each corner of the cube (cubic unit cell) and one atom at the center of the cube [24].

3.10 Mechanism of the bioreduction reaction

Based on test analysis of the types of alkaloids contained in okra leaves known, namely quercetin-4"-O-methyl-3-O-β-D-glucopyranoside. By using the elucidation results of these compounds, it can be predicted the mechanism of the formation of silver nanoparticles through bio-reduction using Abelmoschus esculentus leaves extract as shown in Fig. 7. Until now there is no exact mechanism for the reaction of the formation of silver nanoparticles using plant, bacterial and fungal extracts. According to some researchers who stated that the -OH group possessed by flavonoids is responsible for reducing silver ions into silver nanoparticles [25]. The content of quercetin-4"-O-methyl-3-O-β-D-glucopyranoside found in the leaves of Abelmoschus esculentus is a compound which is a bio-reductor. This compound is a derivative of quercetin which has a high cell potential and is excellent acting as a bio-reductor.
In addition, there are tautomeric keto-enol which can occur in flavonoids will accelerate the release of silver from the capping process by flavonoid compounds. In general, the formation of silver nanoparticles occurs through stages: Ag⁺ reduction process, followed by cluster formation and then the growth of silver nanoparticles [26,27]. Fig. 7 shows that the process of forming silver nanoparticles begins with the solvation process of AgNO₃ using distillate water to form Ag⁺ and NO₃. Abelmoschus esculentus leaf extract containing quercetin-4‴-O-methyl-3-O-β-D-glucopyranoside has an -OH group which reacts with Ag⁺ by releasing H⁺. After forming a silver bond with quercetin-4‴-O-methyl-3-O-β-D-glucopyranoside, through the reaction of keto-enol changes in bound silver metal will be released as Ag⁰ in the form of silver nanoparticles.

**Figure 7** Prediction of the mechanism of synthesis of silver nanoparticles
4. Conclusions
Based on the results of the analysis carried out, the microstructural parameters of silver nanoparticles obtained as a result of bioreduction using Abelmoschus esculentus. The average size of silver nanoparticle crystals is 41.9 nm with strain and stress values $7.5 \times 10^{-5}$ and 0.4908 MPa, respectively. In addition, the calculation results show that silver nanoparticles produced have energy density and dislocation density crystals $3.72 \text{ J/m}^2$ and $5.9 \times 10^{22} \text{ m}^{-2}$. Based on the prediction of the mechanism carried out the bioreduction process occurs through the use of quercetin-4”-O-methyl-3-O-β-D-glucopyranoside compounds with the orientation of the FCC and BCC crystals.

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