Synthesis of silver nanoparticles using bioreductors from clove leaf extract (Syzygium aromaticum) and test of its antibacterial activity

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Abstract. Synthesis of silver nanoparticles using clove leaf extract (Syzygium aromaticum) bioreductor and its antibacterial activity has been studied. Silver nanoparticles were synthesized using the green synthesis method with clove leaf extract (Syzygium aromaticum) as a bioreductor. Nanoparticles were analyzed and characterized by a spectrophotometer by UV-Vis, FTIR, SEM, XRD, and antibacterial activity. Antibacterial activity testing was carried out against Pseudomonas Aeruginosa and Bacillus Subtilis using the disc paper method. Silver nanoparticles were successfully synthesized by a reduction method using clove leaf extract (Syzygium aromaticum). The resulting nanoparticles have varying wavelengths in the range 420-435 nm and have non-uniform shapes with the resulting particle crystal size diameter based on the Debye-Scherer formula is 36.57 nm. FT-IR spectrum comparison of clove leaf extract and silver nanoparticles shows the contribution of the OH functional group in reducing silver ions which is indicated by its presence decrease in absorbance intensity in the area of wave number 3400 cm⁻¹ after the formation of nanoparticles silver. Antibacterial activity of silver nanoparticles against Pseudomonas Aeruginosa and Bacillus Subtilis which showed that the inhibition zone diameter was 11 mm and 9 mm.

1. Introduction

Nanoparticles are particles that have a size of 1-100 nm. Nanoparticles can be applied in the health sector as antibacterial agents. Silver has long been known to have antimicrobial properties [1]. In general, the synthesis of silver nanoparticles is carried out using top-down (physics) and bottom-up (chemistry) methods. However, this method uses excessive chemicals, pollutes the environment due to the presence of hazardous by-products and contamination from chemical precursors, and is expensive [2]. Another alternative nanoparticle synthesis can be done using green synthesis methods that are environmentally friendly with the help of natural materials derived from organisms (animals, plants, and microorganisms) both land and sea [3].

The principle of nanoparticle biosynthesis is to utilize plants and microorganisms as reducing agents [4]. A plant that has a high antioxidant content is clove (Syzygium aromaticum) leaves which can be used as a bioreductor. Cloves are one of the most abundant natural ingredients and are considered to have antibacterial properties [5]. The purpose of this study was to synthesize silver nanoparticles using clove leaf extract (Syzygium aromaticum) as a bioreductor and to test its antibacterial activity against Pseudomonas aeruginosa and Bacillus subtilis.
2. Materials and methods

2.1. Materials
The materials used are clove leaves (Syzygium aromaticum), aquades, Aquabides, AgNO₃, chloromphenicol, bacterial cultures of Pseudomonas aeruginosa and Bacillus subtilis, disc paper, nutrient broth (NB), nutrient agar (NA), plastic-wrap, Whatman No.42 filter paper, and aluminum foil.

2.2. Methods

2.2.1. Clove leaf extract preparation. The clove leaves are washed with distilled water to remove impurities and then dried. The leaves are cut into small pieces into clove powder. The powder is weighed as much as 5 g then put into a 200 mL beaker and 100 mL of aquabides are added and then heated to boiling then cooled to room temperature. The cooking water is filtered using Whatman filter paper no. 42. The cooking water was analyzed by FTIR [6].

2.2.2. Nanoparticle synthesis. Silver nanoparticles were synthesized using the green synthesis method. A total of 40 mL of AgNO₃ solution was put into erlenmeyer then added 1 mL of clove leaf extract and stirred using a magnetic stirrer for 30 minutes. The characterization of the solution in the form of color, UV-Vis absorption spectrum at 1 day, 2 days, 3 days, 4 days, and 7 days. Then dried in a freeze dryer to be characterized UV-Vis, XRD, SEM, and test for antibacterial activity.

2.2.3. Antibacterial testing. Inhibition testing of silver nanoparticles against the growth of the bacteria Pseudomonas aeruginosa and Bacillus Subtilis was carried out using the disc paper method. The antibacterial activity test was carried out by observing the inhibition zone by immersing paper discs in colloidal silver nanoparticles and then attaching them to the surface of agar nutrients that had been grown by the tested bacteria. The agar nutrients that had been affixed with disc paper were incubated for 24 hours at 37°C. Positive tests for bacteria were carried out by wetting the discs with chloromphenicol while the negative tests were carried out using distilled water. These positive and negative tests were carried out as controls. Inhibition can be identified by the presence of a clear area around the disc paper [7].

3. Results and discussion

3.1. Characterization of silver nanoparticles with a UV-Vis Spectrophotometer
UV-Vis characterization was carried out by measuring the maximum wavelength of a solution of clove leaf extract, 2 mM of AgNO₃, and a solution of silver nanoparticles in the wavelength range of 200-700 nm.

| Sample                  | Wavelength (nm) | Absorb  |
|-------------------------|-----------------|---------|
| Clove leaf extract      | 413             | 10      |
| AgNO₃ 2 mM              | 285.5           | 0.175   |
| Silver nanoparticle     |                 |         |
| Day 1                   | 421.5           | 1.717   |
| Day 2                   | 423             | 1.796   |
| Day 3                   | 422             | 1.899   |
| Day 4                   | 424.5           | 1.955   |
| Day 7                   | 424.5           | 2.112   |

Clove leaf extract absorbs energy at a maximum wavelength of 413 nm and 2 mM of AgNO₃ solution at a wavelength of 285.5 nm. The absorbance peak at a wavelength of 424.5 nm was observed after
mixing the two. This shows that there has been a reduction process between clove leaf extract and AgNO₃ solution which forms a solution of silver nanoparticles. Colloidal solutions of silver nanoparticles give absorption peaks at a wavelength of around 400-500 nm which shows the typical plasmon surface absorption peaks of silver nanoparticles [8]. Analysis of the UV-Vis spectrum measurements of silver nanoparticles periodically was carried out to see the growth and stability of the silver nanoparticles that were synthesized with several time variations (Figure 1).

![Figure 1. UV-Vis absorption spectrum of clove leaf extract with 2 mM AgNO₃](image)

The absorbance value shows a trend towards the number of nanoparticles produced. The increase in absorbance value from time to time can indicate the reaction speed of the formation of silver nanoparticles and during the observation time, there is a shift in the position of $\lambda_{\text{maks}}$. According to Bakir, the number of nanoparticles formed qualitatively can be determined based on the absorbance value obtained from the analysis using a UV-Vis spectrophotometer [9].

3.2. Nanoparticle characterization using FTIR

Analysis using FTIR was carried out to determine functional groups that play a role in the process of reducing Ag⁺ ions to Ag⁰ contained in clove leaf extract and the shift in wave numbers that occurs when silver nanoparticles have been formed. The FTIR spectrum of clove leaf extract and silver nanoparticles (Figure 2) shows that there are different functional groups.

The absorption band of clove leaf extract before the addition of AgNO₃ shows the absorption that is unique to several functional groups, including the absorption of the -OH group in the 3379 cm⁻¹ showing the vibrational stretching of the O-H (free) bonds or phenolic compounds. The Csp³-H group on the 2937 cm⁻¹ absorption band. The vibration of the aromatic C = C bond shows the presence of absorption at a wavelength of 1635 cm⁻¹ and the presence of a hydroxyl group with the appearance of absorption at 1039 cm⁻¹ originating from the C-O group. The shift in wave numbers occurred after reacting to the clove leaf extract with AgNO₃. The -OH group absorption shifted from the wave number 3379 cm⁻¹ to 3446 cm⁻¹, the Csp³-H group absorption shifted from 2937 cm⁻¹ to 2924 cm⁻¹ and the other group absorption also experienced a shift in the wave number from 1635 cm⁻¹ to 1610 cm⁻¹ originating from the C = C aromatic group and the CO group absorption shifted from 1039 cm⁻¹ to 1031 cm⁻¹. The wave number 1382 cm⁻¹ indicates the C-O stretching vibration. Wave number 2424 cm⁻¹ indicates a stretching vibration of C≡C.
Based on the results of the wave number shift data, it shows that there is an interaction between functional groups and silver nanoparticles due to the oxidation process due to the reduction of silver nanoparticles. The –OH group of compounds such as tannins, flavonoids, saponins, and alkaloids in clove leaf extract is responsible for the reduction of silver ions [10].

### Table 2. FTIR absorption data of clove leaf extract before adding AgNO₃ and after adding AgNO₃

| Functional groups | Clove leaf extract before addition of AgNO₃ | Clove leaf extract after addition of AgNO₃AgNO₃ |
|-------------------|------------------------------------------|----------------------------------------------|
| O-H               | 3379                                     | 3446                                         |
| C-H               | 2937                                     | 2924                                         |
| C=C               | 1635                                     | 1610                                         |
| C-O               | 1039                                     | 1031                                         |

3.3. **Silver nanoparticle morphology by SEM**

SEM analysis aims to determine the morphology of silver nanoparticles synthesized from clove leaf extract. Analysis using SEM can also provide particle size distribution information.

The results of observations using SEM can be seen that the nanoparticles formed have random shapes and some are spherical with varying sizes due to the effects of aggregation. SEM was measured by magnification of the silver nanoparticle sample image on a scale of 5 μm and 10 μm and a voltage acceleration of 15 kV, as shown in Figure 3. It is consistent with the research of Nurafni, et al. that the resulting silver nanoparticles have a non-uniform structure. They are spherical and have sizes that tend to vary due to the aggregation of nanoparticles [11].

3.4. **Characterization with XRD**

XRD characterization was carried out to determine the crystal characteristics of the silver nanoparticles that had been synthesized. The XRD results of the silver nanoparticles are shown in Figure 4.
Figure 3. Results of silver nanoparticle sample analysis using SEM on (a) 5 μm scale and (b) 10 μm

Figure 4. XRD diffractogram of silver nanoparticles with clove leaf extract bioreductors

In Figure 4, the peaks formed are generally Ag peaks. The silver nanoparticle diffractogram shows the peaks at the diffraction angle (2θ) which conforms to the standard Ag diffraction data published in the Joint Committee of Powder Diffraction Standards data (JCPDS No. 990094). This is indicated by the 2θ values of silver nanoparticles at 37.86, 44.10, 64.44 and 77.62, respectively, with the Miller index of each diffraction peak (111), (200), (220) and (311).

The diffractogram shows that there are peaks other than the typical peaks of silver nanoparticles, this indicates that the silver nanoparticles produced are not yet pure or still contain impurity particles. The diffractogram data also provides information on the grain size of the nanoparticles. The approximate size of the nanoparticles can be calculated from the Debye-Scherer equation [12]. Analysis of nanoparticle crystal size using the Debye-Scherer equation is shown in the table. The nanoparticle crystal size that was successfully synthesized had an average particle size of 36.57 nm.

Table 3. XRD diaphragmatic data of silver nanoparticles

| 2θ  | FWHM | Miller | Miller | Size (nm) |
|-----|------|--------|--------|-----------|
| 37.86 | 0.19 | 111    |        | 43.31     |
| 44.10 | 0.21 | 200    |        | 42.01     |
| 64.44 | 0.27 | 220    |        | 33.80     |
| 77.62 | 0.38 | 311    |        | 27.17     |

Average particle size 36.57
3.5. Antibacterial test

Antibacterial activity test was carried out using the paper disc diffusion method or paper disc diffusion test against Gram-negative pathogenic bacteria (*Pseudomonas aeruginosa*) and Gram-positive bacteria (*Bacillus Subtilis*). The test process is using paper discs that have been immersed in silver nanoparticles and then attached to a bacterial growth medium and incubated for 24 hours. Inhibitory activity against bacterial growth if the inhibition zone value $<5$ mm is categorized as weak, $5-10$ mm is categorized as moderate, $11-20$ mm is categorized as strong and $>20$ mm is categorized as very strong [13].

Table 4. Results of inhibition zone measurements on antibacterial activity testing

| Test sample                  | Obstacles zone (mm) | Average (mm) |
|------------------------------|---------------------|--------------|
|                              | Repetition I | Repetition II |              |
| *Pseudomonas aeruginosa*     |             |              |              |
| Control (+)                  | 24          | 26           | 25           |
| Control (-)                  | 0           | 0            | 0            |
| Clove Leaf Extract 5%        | 7           | 8            | 7.5          |
| Clove Leaf Extract 2%        | 0           | 0            | 0            |
| AgNO$_3$ 2 mM               | 8           | 9            | 8.5          |
| Silver nanoparticle 2 mM     | 10          | 12           | 11           |
| *Bacillus Subtilis*          |             |              |              |
| Control (+)                  | 22          | 24           | 23           |
| Control (-)                  | 0           | 0            | 0            |
| Clove Leaf Extract 5%        | 8           | 7            | 7.5          |
| Clove Leaf Extract 2%        | 0           | 0            | 0            |
| AgNO$_3$ 2 mM               | 7           | 8            | 7.5          |
| Silver nanoparticle 2 mM     | 8           | 10           | 9            |

Table 4 shows that silver nanoparticles can inhibit bacterial growth. This can be seen from the width of the clear zone on the media that has been planted with bacteria. The wider the clear zone, the stronger the inhibitory power of these compounds against bacterial growth. Silver nanoparticles showed an inhibition zone diameter of 11 mm in *Pseudomonas aeruginosa* bacteria was stronger than the bacterium *Bacillus subtilis* which showed an inhibition zone diameter of 9 mm. The results of these observations indicated that silver nanoparticles were in the strong category against *Pseudomonas aeruginosa* bacteria while *Bacillus subtilis* was in the moderate category.

4. Conclusions

Silver nanoparticles were successfully synthesized by a reduction method using clove leaf extract (*Syzygium aromaticum*). The resulting nanoparticles have varying wavelengths in the range 420-435 nm and have non-uniform shapes with the resulting particle crystal size diameter based on the Debye-Scherer formula is 36.57 nm. Antibacterial activity of silver nanoparticles against *Pseudomonas aeruginosa* and *Bacillus Subtilis* which showed that the inhibition zone diameter was 11 mm and 9 mm.

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