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The Potential of Daruju Leaf Extract (*Acanthus Ilicifolius*) as a Bioreductor in the Synthesis of Silver Nanoparticles and Antibacterial Activity Test

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ABSTRACT: Silver nanoparticles had been synthesized through the bioreduction process using daruju (*Acanthus ilicifolius*) leaf extract as a bioreductant. The variation of the formula used is a mixture of daruju leaf extract with a 0.01 M $AgNO_3$ solution with volume ratios of 2:1, 1:1, 1:2, and 1:3. The research results show that UV-Visible spectrophotometry confirmed that a volume ratio of 1:1 is the optimum formula with an absorption value of 2.346 at the maximum wavelength 440 nm. Synthesis of silver nanoparticles with daruju leaf extract has a strong inhibition zone about 10.42 mm. Proving that the synthesis of silver nanoparticles with extract daruju leaf can be used as an antibacterial agent against *E.coli* bacteria.

KEYWORDS: Bioreductor, Daruju Leaf extract, E.Coli, Silver nanoparticle, Synthesis nanoparticle.

I. INTRODUCTION

Silver nanoparticles are one of the nanotechnology products that have become the object of research by researchers recently because they have better interactions with microorganisms so that their antimicrobial properties are effective compared to other nanoparticles [1]. Silver nanoparticles have a very small size, ranging from 1–100 nm [2]. Now silver nanoparticles have been developed and are widely used in the medical world and as a substitute for antibiotics [3].

Silver nanoparticles can be made using plant extracts as a bioreductant, known as the green synthesis method. Green synthesis is relatively more environmentally friendly, cheap, simple, and reduces the use of hazardous waste. Green synthesis using plant extracts has received attention from researchers to be developed because plant extracts contain secondary metabolite compounds such as flavonoids, tannins, alkaloids and polyphenols which function as reducing agents [4].

Natural ingredient that can be used as a bioreductor is daruju leaves (*Acanthus ilicifolius*). Daruju leaves are widely used to treat diarrhea, fever, malaria and coughs [5]. Based on the results of phytochemical tests, daruju leaf extract contains flavonoid and polyphenol compounds which have the potential to act as bioreductors [6]. Daruju leaf extract has the potential as a bioreductor for the synthesis of silver nanoparticles if reacted with $AgNO_3$ solution as a precursor. Because of their effective antimicrobial properties, silver nanoparticles synthesized using daruju leaf extract can be used as an anti-bacterial agent.

Indonesia has a fairly high number of diseases caused by *E.coli* bacteria. *E.coli* is a gram-negative bacterium, usually colonizing the digestive tract. However, *E.coli* includes both commensal bacteria and pathogenic clones. Commensal *E. coli* rarely causes disease, except in hosts with weakened immune systems. Meanwhile, pathogenic *E.coli* causes various major outbreaks such as diarrhea [7].

Efforts to overcome bacterial disease problems often use chemical-based antibiotics. However, giving antibiotics continuously and with increasing doses will cause new problems, namely bacterial resistance to antibiotics. Therefore, an alternative is needed to overcome the problem of bacterial diseases using silver nanoparticles which can be used as an antibacterial agent, especially for the pathogenic bacteria *E.coli* [8].

Based on the background that has been explained, this research is the first step to evaluate the potential of daruju leaves (*Acanthus ilicifolius*) as a source of secondary metabolite compounds that have the potential to act as bioreductors in the synthesis of silver nanoparticles and test their activity as an antibacterial for *E. coli.*

II. MATERIAL AND METHODS

2.1 Tools

In this research, equipment such as Shimadzu UV-Vis spectrophotometry was used 1800, caliper (Mitutoyo 505-646-50), vortex mixer (kataoka Mixer TM-100), incubator (Eyela WFO 600ND), autoclave (Tomy SX 500), magnetic stirrer (Heidolph

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MR Hei-Standard), analytical balance (Adventurer Ohaus), beaker (Pyrex), volume pipette, measuring flask, test tube (Iwaki), spatula, glass funnel (Pyrex), enlemeyer 300 mL, bunsen, tweezers, petri dish, mortar.

2.2 Material

Also materials such as daruju leaves, $AgNO_3$ 0.01 M, Whatman No.1 filter paper, aquabides, disc paper, Mueller Hinton Agar media, aluminum foil, plastic wrap, ciprofloxacin, cotton buds, *Eschericia coli* bacterial suspension, magnesium powder, concentrated HCl, $FeCl_3$ 10%, reagents (Dragendorf, Meyer, Werner), glacial acetic acid.

2.3 Extraction of Daruju Leaves (Acanthus Ilicifolius)

Daruju leaf extract was obtained by dissolving 10 g of daruju leaf powder in 100 mL of distilled water. The mixture was stirred using a magnetic stirrer at a temperature of 50° C with a speed of 1000 rpm for 30 minutes. Next, the extract was filtered using Whatman No.1 paper to separate the filtrate and residue. The filtered filtrate is used as a bioreductant stock solution [9].

2.4 Daruju Leaf Extract Phytochemical Test (Flavonoid Test)

5 mL of daruju leaf extract was added with 0.05 g of Mg powder and 1 mL of concentrated HCl, then shaken. A positive test is indicated by the formation of a red, yellow, or orange color [10].

2.5 Daruju Leaf Extract Phytochemical Test (Tannin and Polyphenol Test)

1 mL of daruju leaf extract was added with 10 drops of 10% $FeCl_3$. Extracts are positive for containing tannins if they produce a blackish green or blackish blue color [10].

2.6 Daruju Leaf Extract Phytochemical Test (Alkaloid Test)

2 mL of daruju leaf extract was put into 3 different test tubes. Then 1 mL each of Dragendorf, Meyer, and Werner Wagner reagents was added. The formation of white, red-orange and brown precipitates with Dragendorf, Meyer and Wagner reagents respectively indicates that the sample contains alkaloids [9].

2.7 Daruju Leaf Extract Phytochemical Test (Steroid and Triterpenoid Test)

1 mL of daruju leaf extract was added with 10 drops of glacial acetic acid and 2 drops of concentrated H_2SO_4. The solution was shaken gently and left for several minutes. A steroid test is positive if it produces a blue or green color, while triterpenoids produce a red or purple color [11].

2.8 Daruju Leaf Extract Phytochemical Test (Saponin Test)

1 mL of daruju leaf extract is added to 10 mL of water while shaking for 1 minute, then 2 drops of 1 N HCl are added. If the foam formed remains stable for approximately 7 minutes, then the extract is positive for containing saponin [10].

2.9 Optimization of the comparison of the volume of silver nitrate solution and the volume of Daruju leaf extract in the synthesis of silver nanoparticles

The process of synthesizing silver nanoparticles begins with making an $AgNO_3$ precursor solution by weighing 0.17 grams of the $AgNO_3$ compound, putting it in a 100 mL volumetric flask, then adding distilled water to the mark to form an $AgNO_3$ solution with a concentration of 0.01 M. The mixture is stirred using a magnetic stirrer. at a speed of 1000 rpm and for 30 minutes with the aim that the solution can be dissolved homogeneously. Next, the daruju leaf extract and the 0.01 M $AgNO_3$ solution were mixed with a ratio of 2:1, 1:1, 1:2, and 1:3. Next, the mixture of daruju leaf extract and $AgNO_3$ solution was stirred for 30 minutes using a magnetic stirrer at a temperature of 50° C with a speed of 1000 rpm. The formation of silver nanoparticles was marked by a change in color to brownish yellow. The synthesized silver nanoparticles had their UV-Vis spectrum measured at a wavelength of 400-500 nm. Based on the absorbance value at the maximum wavelength, the optimum mixture is determined, namely the one that shows the highest absorbance value [12].

2.10 Making Agar Media

3.8 grams of Muller Hinton Agar (MHA) powder was diluted in an Erlenmeyer with 100 mL of distilled water, then homogenized, then the mixture was covered with aluminum foil and sterilized by autoclaving at 121° C for 15 minutes. The media has been sterilized, cooled and then poured into each petri dish. Next, wait for ± 30 minutes until the media solidifies. Solid media

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is stored in the refrigerator. Muller Hinton Agar (MHA) media will be used for bacterial inoculation and antibacterial activity tests [13].

2.11 Bacterial Rejuvenation E.coli Test

Rejuvenation of test bacteria is carried out by taking 1 dose of test bacteria and streaked on MHA media, then incubated at 37° C for 24 hours [14].

2.12 Preparation of E.coli Test Bacterial Suspension

A suspension of *E.coli* test colonies is made by taking one loop of colonies from the media solid MHA into a test tube containing 5 mL of physiological NaCl. Turbidity in the test colony suspension standardized to a standard of 0.5 Mc. Farland (approximately 1.5 x 108 CFU/mL) [15].

2.13 Antibacterial Activity Testing

Antibacterial activity testing was carried out using the disc diffusion method. Suspension the test bacteria were inoculated on Muller Hinton Agar (MHA) media 1 dose, then Flatten with a sterile cotton bud and let sit until dry (± 5 minutes). Disc paper soaked in silver nanoparticles, daruju leaf extract, and positive control (ciprofloxacin) and negative control (aquabides) were carried out ± 15 minutes later placed on the surface of the MHA media aseptically using tweezers. Each Petri dishes were incubated for 24 hours at 37° C. Next, observe the clear zone or inhibition zone around the paper disc. The diameter of the inhibition zone is measured in millimeters (mm) using a caliper, which then determines the category of inhibition zone this is based on the strength of its antibacterial power based on the classification that has been made established [16].

III. RESULT AND DISCUSSION

In the process of identifying secondary metabolite compounds from daruju leaf extract, positive results were found for several groups of secondary metabolite compounds. Positive results indicate the presence of flavonoids with the formation of an orange color. Daruju leaf extract also showed positive results for containing tannins and polyphenols because it produced a blackish green color in the sample [10]. Meanwhile, for the alkaloid content, daruju leaf extract in the Dagendorf reagent showed positive results, namely showing the formation of an orange precipitate. In Wagner's reagent a brown precipitate also forms. However, for Mayer's reagent, a white precipitate was formed. Thus, daruju leaf extract contains alkaloid compounds [9]. The content of saponin compounds in the extract was shown by the appearance of stable foam for 7 minutes after treatment [10]. However, negative results were shown by the steroid and triterpenoid test, because the sample did not show a color change to blue or green for steroids and red or purple for triterpenoids [11]. Based on the results of the phytochemical test, daruju leaf extract contains secondary metabolites, namely flavonoids, polyphenols, tannins, alkaloids and saponins. In detail the results of the phytochemical tests are presented in **Table 1**.

Table 1. Phytochemical test result daruju leaf extract

No	Phytochemical test	Test Result	Analysis	Figure
1	Flavonoid	Orange	Positive	
2	Tannin and Polyphenol	Blackish Green	Positive	

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3	Saponin	Foam formed	Positive	
4	Steroid and Triterpenoid	Yellow	Negative	
5	Alkaloid (Mayer's Reagent)	White precipitate	Positive	
6	Alkaloid (Wagner's Reagent)	Brown precipitate	Positive	
7	Alkaloid (Dragendorf's Reagent)	Orange precipitate	Positive	

These results indicate the presence of secondary metabolites that have the potential to act as bioreductors in the silver nanoparticle synthesis process. Flavonoids and polyphenols are secondary metabolites that play an important role in the Ag metal ion reduction process [6][12][17]. The silver nanoparticle synthesis process involves mixing daruju leaf extract with silver nitrate solution in various ratios (2:1; 1:1; 1:2; 1:3), and the mixture is then stirred using a magnetic stirrer. During this process, a color change will occur which is an indication of the formation of silver nanoparticles, namely the process of reducing the metal Ag^{+} to Ag^{0} which comes from the reductant, namely daruju leaf extract [18].

Figure 1: Synthesis of Silver Nanoparticles using Dauju leaf extract (1:1)

The color changes that occurred were observed using a UV-Vis spectrophotometer, this was done to strengthen the results of visual observations and to determine the maximum wavelength and absorbance. In this research, silver nanoparticles were synthesized in various ratios (2:1; 1:1; 1:2; 1:3). Then, the synthesis results were measured in the wavelength range 300-600 nm using the UV-Vis spectrum. The UV-Vis spectrum results of silver nanoparticles produced from daruju leaf extract are shown in **Figure 2**.

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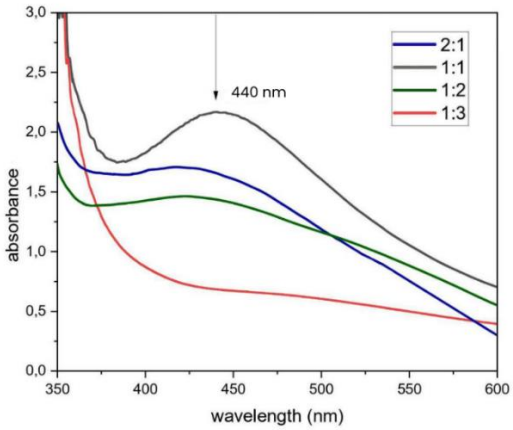

Figure 2: UV-Vis spectrum of silver nanoparticles synthesized using daruju leaf bioreductor

The results of UV-Vis spectroscopy show that the four comparisons have different peaks. In a 1:1 ratio, Daruju leaf extract with $AgNO_3$ precursor, has the highest absorbance value, namely 2,346 nm, so the maximum absorption wavelength of the UV-Vis spectrum is 440 nm. At this wavelength, the most optimal formation of silver nanoparticles occurs [12][17]. The UV-Vis absorption wavelength between 400 – 500 nm is a specific wavelength for Ag^0, so the change in Ag^+ to Ag^0 can be easily observed as a control for the formation of silver nanoparticles. The results of peak wavelength readings using UV-Vis Spectroscopy are presented in **Table 2**.

Table 2. UV-Vis spectroscopy test results

Ratio	Wavelength (nm)	Absorbance
2 : 1	435	1,760
1 : 1	440	2,346
1 : 2	425	1,450
1 : 3	415	0,790

In this research, silver nanoparticles synthesized using a bioreductant from daruju leaf extract were tested for their antibacterial activity against *E.coli* bacteria. *E.coli* is a gram-negative bacterium which is a common pathogen in the digestive tract. The antibacterial activity test was carried out using the disc diffusion method. The principle of this method is that the material used as an antibacterial is soaked in a disc and then the disc is placed on an agar seed medium that has been inoculated with the bacteria to be tested. After that, incubate for 18-24 hours at a temperature of 37°C. Next, the clear zone formed around the disc paper was measured. The larger the clear zone formed, the more effective it is used as an antibacterial [19][12]. The results of the antibacterial test of the synthesized silver nanoparticles are presented in **Figure 3**.

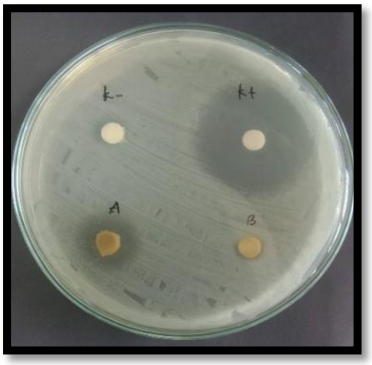

Figure 3: Results of the actibacterial test of silver nanoparticles synthesized using daruju leaf bioreductor

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Based on the results of the actibacterial test, the results showed that disk A filled with 100 ppm of synthesized AgNPs produced an inhibition zone of 10.42 mm (strong category), disk B filled with 100 ppm of daruju leaf extract produced an inhibition zone of 2.84 mm (weak category), K- disks filled with aquabides showed no zone of inhibition, and K+ disks filled with ciprofloxacin showed a very strong zone of inhibition (25 mm). Thus, the synthesis of silver nanoparticles produced using daruju leaf bioreductors has a relatively strong inhibition zone (10.42 mm) and its activity is stronger than daruju leaf extract (2.84 nm).

Table 3. Classification of bacterial growth inhibitory power [19].

Inhibition Zone Diameter	Growth Inhibitory Power
>20 mm	Very Strong
10-20 mm	Strong
5-10 mm	Medium
<5 mm	Weak

IV. CONCLUSION

Synthesis of silver nanoparticles was carried out using daruju leaf extract as a bioreductant. Daruju leaf extract contains secondary metabolites, namely flavonoids, polyphenols, tannins, alkaloids and saponins. The flavonoid and polyphenol compounds contained in daruju leaves have an important role in reducing Ag metal ions. The results showed that a 1:1 ratio between daruju leaf extract and silver nitrate solution precursor, Synthesis of silver nanoparticles was carried out using daruju leaf extract as a bioreductant. Daruju leaf extract contains secondary metabolites, namely flavonoids, polyphenols, tannins, alkaloids and saponins. The flavonoid and polyphenol compounds contained in daruju leaves have an important role in reducing Ag metal ions. The research results showed that a 1:1 ratio between daruju leaf extract and silver nitrate solution precursor was the optimum ratio because it had the highest absorbance value (2.346). The synthesis of silver nanoparticles using daruju leaf extract has a strong inhibition zone (10.42 mm) compared to daruju leaf extract which has an inhibition zone of 2.84 mm. Proving that the synthesis of silver nanoparticles with duan daruju extract can be used as an antibacterial agent against *E.coli* bacteria.

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