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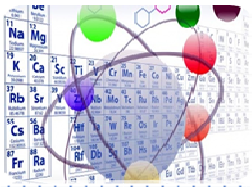

Antibacterial Activity Test of Ag Nanoparticle Soap Turi Leaf Extract (*Sesbania grandiflora*) Against *Staphylococcus aureus* Bacteria

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ABSTRACT

Silver metal nanoparticles have biocidal properties as a non-toxic inorganic antibacterial agent. Flavonoid compounds from the Turi Leaf (Sesbania grandiflora) plant extract as a natural bio reductant. In the research, UV-Vis spectrophotometric testing parameters and antibacterial soap preparations were carried out. Synthesis and Characteristics of Silver Nanoparticles were carried out, with soap preparations made into 3 formulas with different variations in Agno3 concentration, namely 1 mM, 2 mM, and 3 mM and antibacterial activity tested using well diffusion. The results of the absorption spectrum of Ag nanoparticles show the highest peak at a value of 280-290 nm. In the antibacterial activity test, the greater the $AgNO_3$ concentration in the Ag nanoparticle soap, the greater the inhibition zone created by Staphylococcus aureus bacteria. The optimum concentration in soap preparations is 3 mM producing a wavelength of 300 nm with an absorbance value of 1.105. In terms of antibacterial activity, the most effective way to inhibit the growth of Staphylococcus aureus bacteria is formulation III, with an inhibitory power of 7.69 cm^2.

Keywords: Antibacterial, Nanoparticles, *Staphylococcus aureus*.

1. INTRODUCTION

Infectious diseases are health problems that drive high mortality rates. The infection has an acute nature and easily attacks all layers of the body, especially on the skin caused by the polluted surrounding environment. Infections on the skin, both mild and moderate, are often caused by bacteria, one of which is the bacterium Staphylococcus aureus. Staphylococcus aureus is one type of bacteria that has a role as the body's normal microflora so it is very easy to attack the human body.[1] A commonly used treatment is the use of antibiotics. However, improper use of antibiotics can lead to antibiotic resistance. Therefore, in order to break the chain of bacterial growth, it is necessary to have antibacterial agents as the first step in preventing

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outermost skin infections, namely by using soap. The soap made is formulated with silver nanoparticles and Turi Leaf content as a bio reducing agent that is able to have antibacterial properties.

Soap is one of the most commonly used forms of cleaning preparations. The purpose of cleaning is to help remove dirt or oil from the surface, especially bacteria. Soap is considered to have more clean power when compared to solid soap, liquid soap has economic value and is more hygienic because it is not touched directly by hand.[2] Soap preparations sold in the market still contain many chemical compounds that are not good for the body, so they can irritate the skin. With that made soap with basic ingredients from nature which is certainly safe for the human body.

Turi plant (*Sesbania grandiflora*) is most popular especially the leaves. Based on research.[3] states that all Turi plant samples containing saponin compounds with the highest percentage are leaves. According to Mariando, et al in a study stated that liquid soap preparations using Turi leaves contain saponins, flavonoids, and tannins that can kill microorganisms.

Soap making in this study with the help of nanoparticles to obtain antibacterial agents. Nanoparticles are nanotechnology that has attracted many researchers[4], one of which is silver nanoparticles. Silver metal nanoparticles have biocidal material properties as non-toxic inorganic antibacterial agents. The potential possessed by nanoparticles as antibacterial agents is due to properties that can be applied especially in the medical field and most likely can interact strongly with microorganisms, including bacteria.[5] In research[6] produced silver nanoparticles of 3.9 Ev and 3.88 Ev and had an inhibitory power in bacteria of 5.52 mm and 6.65 mm. Therefore, in this study, it aims to synthesize Ag with the help of bio reducing natural ingredients Turi Leaves (*Sesbania grandiflora*) and formulated in soap preparations, then test antibacterial activity with *Staphylococcus aureus* bacteria.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The tools used are analytical scales, magnetic stirrers, beaker glass, measuring cups, stirring rods, test tubes, petri dishes, mortars and stampers, drip pipettes, micropipettes and tips, baths, Erlenmeyer flasks, cotton, Whatman filter paper, boor prop, calipers, ose wire, UV-Vis *spectrophotometers*, incubators, autoclaves. As well as the materials used, namely Turi leaves (*Sesbania grandiflora*), $AgNO_3$, aquadest, SLS (Sodium Laury Sulfate), Na_2SO_3, STTP, Citric Acid, Foam Booster, *Oleum Rosae*, Nutrient Agar (NA), Brain Heart Infusion (BHI), Mueller Hinton Agar (MHA), *Staphylococcus aureus*.

2.2. Research Procedure

2.2.1. Making Turi Leaf Infusion

Done by weighing the powder of Turi Leaf simplisia (*Sesbania grandiflora*) as much as 10 grams in a 10% infusion. Pour in beaker glass and add with aquadest as much as 100 ml. Boil in a pot filled with water, for 30 minutes at 80°C and occasionally while stirring. Wait for the temperature to drop and then strain it using Whatman filter paper.

2.2.2. Flavonoid Compound Content Test

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A total of 2 ml of extract infusion of Turi leaves (*Sesbania grandiflora*) was inserted into a test tube. Add 1 ml of 95% ethanol and 2 ml of HCl 2N, then add 10 drops of concentrated HCl. If there is a red, yellow, orange, purple or blue color change, it can indicate that the extract contains flavonoid group compounds.

2.2.3. Synthesis and Characteristics of Silver Nanoparticles

As much as 1 ml of extract was mixed into 15 ml of $AgNO_3$ solution with variations in concentration of 1 mM, 2 mM, 3 mM. Then synthesized using a magnetic stirrer for 1 hour until the color change in the solution. If the color of the solution changes to brownish-yellow, it can be said that silver nanoparticles are formed.

2.2.4. UV-Vis Spectrophotometry Measurement

Turn on the UV-Vis Spectrophotometry Instrument, let it sit for 30-60 minutes. Prepare a solution of aquadest blanks and sample solutions with concentration variations of 1 mM, 2 mM, 3 mM. Insert in each cuvette according to the boundary mark. Perform a scan set wavelength of 200-600 nm, with an interval value of 5. Record the absorbance value at each wavelength setting.

2.2.5. Nanoparticle Soap Formulation

Table 1. Silver Nanoparticle Soap Preparation Modification Formula (*modified*)

Materials	Unit	Material function	Formula		
			I	II	III
Silver Nanoparticles + Turi Leaf Extract	mM	Antibacterial active ingredients	1	2	3
SLS	gram	Surfaktan	9	9	9
Na_2SO_3	gram	Soap formers	6	6	6
STTP	gram	Chelate	3	3	3
Citrit acid	gram	pH neutralizer	0,15	0,15	0,15
Essensial oil	drip	Fragrance	q.s	q.s	q.s
Foam booster	gram	Foam generator	1,4	1,4	1,4
Aquadest	mL	Solvent	Ad 100	Ad 100	Ad 100

2.2.6. Liquid Soap Making

The first step in the soap-making process is to put SLS and foam reinforcement into a mortar, grinding slowly until well mixed. Then add Na_2SO_3 little by little. Dissolve STTP with enough aquadest, put little by little into the mortar and then mix again. Dissolve citric acid with aquadest to taste, put in a mortar mix until homogeneous. Incorporate a solution of silver particles according to the variation in concentration in each formula. Add with essential oil (*oleum rosae*) to taste. Then let the soap preparation stand for ± 24 hours, the soap will turn out to be denser and shaped like a clear gel.

2.2.7. Antibacterial Activity Testing

Antibacterial testing with the well method is carried out aseptically through a sterilization process. First inoculate the parent bacteria into the NA medium, by scraping using a sterile ose needle onto the surface of the inclined media. The second inoculates from inclined NA media to liquid BHI media. Third, inoculating

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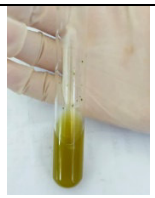
Staphylococcus aureus bacteria from BHI media to MHA media using sterile cotton by scratching it on the surface zigzag. Make a well hole with boor supports. After that, enter a sample of 50 µL in a well consisting of variations in the concentration of Ag nanoparticles, negative control, namely aquadest and positive control, namely 50 µg amoxicillin antibiotic. Then incubate for ±48 hours, observe until a clear zone is formed.

3. RESULTS AND DISCUSSION

Nanoparticles can be referred to as nanospheres or Nano capsules are dispersions of solids that have a size of 10-100 nm. Nanoparticle synthesis is carried out by biological methods or Green synthesis that utilizes plant extracts, namely Turi Leaf extract (*Sesbania grandiflora*) containing terpenoid compounds and flavonoids that play a role in the ion reduction process as a bio reducing agent, the final result is obtained brownish-yellow silver nanoparticles. Then the synthesis of silver nanoparticles (Ag) is used as an antibacterial agent of *Staphylococcus aureus* in soap preparations.

3.1. Flavonoid Compound Content Test

Table 2. Test results of flavonoid compound content

Test	Test result	Information
Flavonoids	+	

As a bioreducing agent to help the process of Ag nanoparticles in plant extracts, flavonoid compounds are needed. So it is necessary to test the content of flavonoid compounds. In table 2 samples of Turi Leaf extract (*Sesbania grandiflora*) showed positive results containing flavonoid compounds. The purpose of adding 95% ethanol, because flavonoids are easily soluble in ethanol. Then added HCl 2N with its polar properties can help the process of distribution of flavonoid compounds optimally. Finally, the addition of concentrated HCl can help hydrolyze flavonoid compounds into aglicon. So that the final result shows a yellow liquid.[7]

3.2 Synthesis and Characteristics of Silver Nanoparticles

The process of making nanoparticle synthesis from $AgNO_3$ solutions of 1 mM, 2 mM, and 3 mM with the help of Turi Leaf extract (*Sesbania grandiflora*) as a bio reducing agent. Both materials are mixed through a process using a magnetic stirrer. Stirring is carried out constantly for 1 hour at a speed of 900 rpm at room temperature. The purpose of this stirring process is to accelerate the reaction rate so as to cause more collisions between particles and the resulting particle size is smaller and stronger. [8] The use of silver metal in the process of oxidation and release of Ag+ ions into the environment can have antibacterial properties. So that the synthesis of Ag nanoparticles has antimicrobial activity. Visually, in the final result, there is a

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brownish-yellow discoloration and the formation of deposits. So this is appropriate, according to research[9] . Nanoparticles are formed visually, characterized by a change in the color of the solution to yellow to brown.

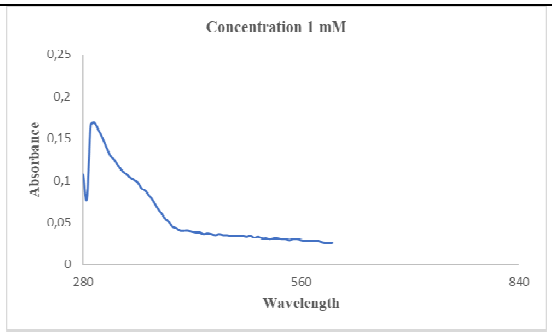

Figure 1. Absorbance spectrum of 1mM Ag nanoparticles

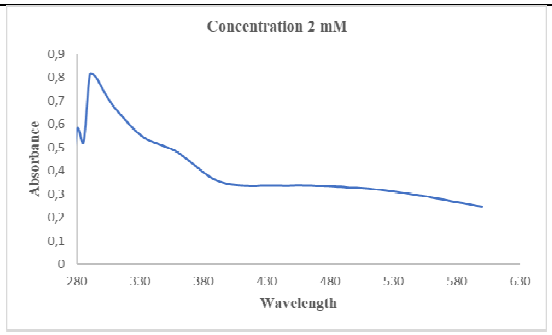

Figure 2. Absorbance spectrum of 2mM Ag nanoparticles

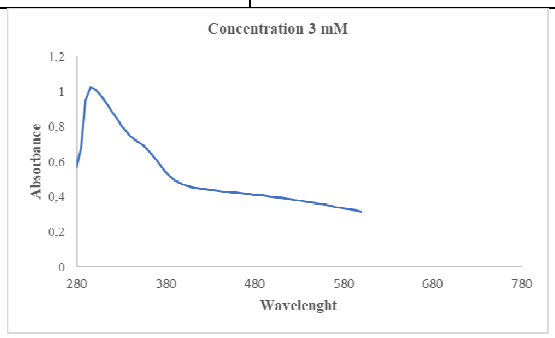

Figure 3. Absorbance spectrum of 3mM Ag nanoparticles

Measurements were made using UV-Vis Spectrophotometry tools to analyze the formation of Ag nanoparticles based on the resulting wavelengths obtained from each nanoparticle concentration. Determination of Wavelength at values of 200nm - 600nm with interval values of 5. Based on the results of the absorption spectrum of Ag nanoparticles of Turi leaf extract (*Sesbania grandiflora*). At each concentration, namely 1 mM, 2 mM, and 3 mM, the highest peak produced is at a value of 280-290 nm. From figure 1. a concentration of 1mM yields a wavelength of 295 with an absorption value of 0.169, from table 2. A concentration of 2 yields a wavelength with an absorption value of 0.814. From figure 3. A concentration of 3 yields a wavelength of 300 with an absorbance value of 1.105. So that the results show the optimum concentration variation in soap preparations is a concentration of 3 mM. From research[10] states that the greater the concentration of silver nitrate, the greater the indication of its formation. And the higher the absorption value, the higher the concentration of nanoparticles in the solution. Based on research [11] optical properties studied using Perkin Elmer UV-Vis spectrophotometry (model no. Lambda 35) with a range length of 190 nm-1100 nm. From the results of his research showed with an absorption edge of 210 nm-240 nm.

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3.3 Turi Leaf Extract Ag Nanoparticle Soap

Soap making refers to[12] using Ag + Turi Leaf Extract nanoparticles (*Sesbania grandiflora*) as active substances containing antimicrobials, which are formulated into soap form. The first ingredient is the mixing of SLS (Sodium Laury Sulfate) which functions as a surfactant with clean power capabilities. Then Foam Booster as a foam generator while the addition of Na_2SO_3 as a soap former along with SLS. Next is STTP (Sodium Tripoli Phosphate) used for chelating soap preparations. In helping the process of dissolving oil and water to dissolve, citric acid is used. Solvents by using aquadest and the addition of essential oils to additional ingredients as fragrances for soap preparations. The final result of the soap preparation after being allowed to stand (aging process) for ± 24 hours shows a homogeneous and transparent soap preparation.

3.4 Antibacterial Activity Testing

Table 3. Area of inhibition area of soap nanoparticle Ag Turi leaf extract (*Sesbania grandiflora*)

Replication	Inhibition area (cm^2)				
	FI	FII	FIII	(+)	(-)
1	6,88	4,78	7,73	6,5	0
2	5,53	2,01	8,67	5,18	0
3	6,22	2,08	6,69	3,27	0
Average	6,21	2,95	7,69	4,98	0

Based on the results of this antibacterial activity test referring to[13], Ag nanoparticle soap Turi leaf extract (*Sesbania grandiflora*) shows that each soap formula has inhibitory power against *Staphylococcus aureus* bacteria. The inhibitory zone produced from each formula is different, because it contains different $AgNO_3$. From the average value of the area of the inhibitory area in formula I with a concentration of $AgNO_3$ 1 mM is 6.21 cm^2, formula II with a concentration of $AgNO_3$ 2 mM is 2.95 cm^2, while formula III with a concentration of $AgNO_3$ 3 mM is 7.69 cm^2. So it can be concluded that the greater the concentration of $AgNO_3$ of each formula in the Ag nanoparticle soap of Turi leaf extract (*Sesbania grandiflora*), the greater the inhibitory zone caused against *Staphylococcus aureus* bacteria. This is based on the synthesis of Ag nanoparticles from the reduction process of reduced silver metal ions to form nanoparticles with the help of bio reducing natural materials that contain flavonoid compounds so that they can form inhibitory zones as antibacterials. Meanwhile, the results of the positive control using a broad-spectrum type antibiotic, namely amoxicillin 50 µg with an average inhibitory area of 4.98 cm^2 and the negative control using aquadest did not show an inhibitory area.

4. CONCLUSION

As a role in Green Synthesis Ag Nanoparticles with the help of bio reducing agents Turi leaf extract (*Sesbania grandiflora*) in the compound content test showed positive results containing flavonoid compounds. Based on UV-Vis spectrophotometric measurements of the three concentrations, the most optimal concentration in soap preparations is a concentration of 3 mM resulting in a wavelength of 300 nm

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with an absorbance value of 1.105. In testing the antibacterial activity that most effectively inhibits the growth of *Staphylococcus aureus* bacteria is formulas III with an inhibitory power of 7.69 cm^2.

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