ANTIBACTERIAL ACTIVITY OF THE ANTISEPTIC DETERGENT MAHOGANY SEED EXTRACT (SWIETENIA MAHOGANY L.)

Widiastuti Agustina E. S., Mela Yuliyanti, Vinsensius Maunia S. H., Halida Anwar A. F.

ABSTRACT

The antibacterial activity of detergents is needed to inhibit the growth of bacteria found on clothes. Mahogany seeds (Swietenia mahogany L.) are medicinal plants that contain saponins and have antibacterial activity, thus mahogany seed extract can be developed as a foaming agent as well as an antibacterial agent in the manufacture of antiseptic detergents. This research aimed to determine the antibacterial activity of antiseptic detergent from mahogany seed extract. Detergent was made by varying the ratio of Linear alkylbenzene Sulfonate (LAS) as LAS : mahogany seed extract (g/g) = 1: 0 (control), 1 : 1 (formula 1), 1 : 2 (formula 2) and 0 : 1 (formula 3). Antibacterial activity test was carried out by using the disc diffusion method. Detergent with a ratio of LAS : mahogany seed extract = 1 : 2 (formula 2) has conformity with the national standard of Indonesia for detergents. Formula 2 provides antibacterial activity against Staphylococcus aureus ATTC with an inhibition zone diameter of 12.43 mm (strong). Saponins from mahogany seed extract can inhibit the growth of gram (+) bacteria such as Staphylococcus aureus ATTC by reducing the surface tension of the bacterial cell walls and damaging membrane permeability resulting in denaturation of membrane proteins so that the cell membrane will be damaged and lysis.

Keywords: Mahogany seed extract, saponin, antiseptic detergent, antibacterial activity.

INTRODUCTION

Bacteria are the cause of various diseases suffered by Indonesians. One of the media for transmitting bacteria is through clothing. The most common bacteria on clothing is Staphylococcus aureus [1 - 3]. Bacteria on the skin can transfer from clothing to the skin, causing acne, itching, and skin irritation [4]. Thus a detergent is needed to remove the dirt on clothes and is antibacterial at the same time.

The use of synthetic cleaning agents (known as detergents) is increasingly common in modern society. Detergents have been used by the community for more than 100 years, but the effect of various detergent ingredients on the environment is still very pronounced [5]. Antiseptic detergent is one of the many detergents developed. Antiseptic detergents contain a special composition that acts as an antibacterial agent. A commonly used antibacterial agent is triclosan (TCS), which is a broad-spectrum antimicrobial agent [6, 7]. TCS is biodegradable, photo-unstable and continues to break down after being released into the aquatic environment. TCS is degraded in aerobic soil for 18 days whereas in water it takes 11 days [6]. The use of TCS can be replaced with organic materials which have faster degradation power. Besides, organic compounds can be a source of nutrition for soil or water.

Mahogany trees (Swietenia mahogany L.) grow in many parts of Indonesia. The seeds of mahogany contain chemicals such as flavonoids, saponins, tannins, cardiac glycosides, essential oils, alkaloids, and anthraquinones [8]. Saponins in mahogany seeds have an important role in inhibiting microbes. Also, mahogany seed extract contains antibacterial flavonoids so that it functions as a disinfectant and bacteriostatic in inhibiting cellular metabolic activity. Also, saponins can produce foam.
when reacted with water [9] and can be used as a foaming agent. Therefore mahogany seed extract can be used as an antibacterial agent.

In this study, mahogany seed extract was used to develop an antiseptic detergent. This study was conducted to determine the antibacterial activity of an antiseptic detergent developed using mahogany seed extract as a foaming agent as well as an antibacterial agent.

**EXPERIMENTAL**

**Materials**

The main material in this research is mahogany seeds. The solvent used for extraction is ethanol. The materials used for detergents are distilled water, coconut oil, KOH 40 %, carboxymethyl cellulose (CMC), linear alkylbenzene sulfonate (LAS), butylated hydroxytoluene (BHT), stearic acid, and fragrances. The materials for antibacterial analysis are Mueller Hinton Agar (MHA), chloramphenicol, distilled water and *Staphylococcus aureus*.

**Sample preparations**

250 grams of mahogany seed powder was macerated with 500 ml of absolute ethanol for 3x24 hours at room temperature. The filtrate was evaporated using a rotary evaporator at 50°C to get 31.125 grams of concentrated orange mahogany seed extract. The formulation and process of making liquid detergents refer to previous studies [12].

**Making Antiseptic Detergents**

The detergents are made in 3 formulations, each containing 1.5 g (formula 1), 2 g (formula 2), and 3 g (formula 3) mahogany seed extract, while the control

<table>
<thead>
<tr>
<th>Table 1. Liquid detergent formulations.</th>
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<tbody>
<tr>
<td><strong>Material</strong></td>
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<tr>
<td>Mahogany seeds extract (g)</td>
</tr>
<tr>
<td>Coconut oil (mL)</td>
</tr>
<tr>
<td>KOH 40 % (mL)</td>
</tr>
<tr>
<td>CMC (g)</td>
</tr>
<tr>
<td>LAS (g)</td>
</tr>
<tr>
<td>Stearic acid (g)</td>
</tr>
<tr>
<td>BHT (g)</td>
</tr>
<tr>
<td>Perfume (mL)</td>
</tr>
<tr>
<td>Aquades (mL)</td>
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</table>

*BHT - Butylated hydroxytoluene; CMC - Carboxymethyl cellulose; LAS - Linear alkylbenzene sulfonate.*
detergent does not contain mahogany seed extract. All formulas contain 15 ml of coconut oil and 1.5 g CMC but different quantity of LAS. After the LAS dissolved, 3.6 g of stearic acid, 0.5 g of BHT, 2 ml of perfume, and 65 mL of distilled water were added.

The test for the quality of liquid detergent according to the Indonesian National Standard (SNI-06-4075-1996) includes tests for pH, specific gravity, and free alkali. Other detergent quality tests were also carried out, including organoleptic, foam stability, and detergency power. An organoleptic test is done by looking at the physical appearance of liquid detergent which includes color and odor. A density test is done by checking the density of liquid detergent in a water bath system using a pycnometer as a reference for calculating specific gravity. The free alkali test is carried out by checking the free alkaline content of the liquid detergent through a distillation process.

Antibacterial Activity Test

Antibacterial activity was analyzed by the disc diffusion method. Sterile sticky cotton swabs dipped in a bacterial suspension have been compared to the Mc.Farland experienced turbidity, and wait for it to soak into cotton, then rubbed it on Mueller Hinton Agar (MHA) medium until blended. Blank paper discs with a diameter of 6 mm which have been sterilized in an oven at 121°C for 15 minutes are then taken out and placed on the surface of the agar medium. As a negative control, distilled water was used which was dripped using a pipette microliter while chloramphenicol was used as a positive control. 20 microliters of formula 1 were dropped on blank disc paper, as well as of formula 2, formula 3, and control, respectively, then incubated for 24 hours at 37°C and measured the bacterial inhibition zone formed [11].

RESULTS AND DISCUSSION

The extraction was done by using the maceration method which is a simple method and tool, not damage the compounds that cannot stand the heat. The solvent used for the extraction is ethanol because it is selective for dissolving polyphenols, flavonoids, alkaloids, tannins, and essential oils.

Identification of saponins

The presence of saponins in mahogany seed extract is characterized by the formation of a stable foam with 1 cm high for 1 minute and Lieberman Burchard reagents show a brownish red ring on the surface that indicates triterpenoid saponins are present in the extract [10]. In triterpenoid testing, analysis is based on the ability to form a color with concentrated H₂SO₄ in acetic acid solvents.

Test of detergency is used to assess the effectiveness of foam in cleaning dirt on the fabric. The pH of the control solution to formula 3 has increased from 9.2 to 12.5. This increase in pH is due to the difference in the addition of mahogany seed extract and LAS to each formula. The more mahogany seed extract is added, the detergents are more alkaline. This is due to the presence of OH⁻ functional groups in saponins. The presence of OH⁻ ions affects the detergency power value [11]. The OH⁻ ions will bind nonpolar (positively charged) groups of fats/fatty acids so that more fat will be separated from the fabric. Therefore, the more additions to the extract, the pH from the liquid will be increased so that the greater detergency is produced.

The formula 1 and formula 2 showed good detergency with almost the same value. If connected with foam stability, formula 2 had the optimum detergency because it has a foam stability value of 70 % based
The more stable foam is formed, the faster is the surfactant in removing dirt because the contact with dirt will be better. Based on previous research, formula 2 has values for pH of 11.1, the specific gravity of 1.1 g/mL, free alkaline of 0.10 % and detergency > 90 % [12]. Based on the result, the quality tests of formula 2 showed that its pH, specific gravity, and free alkaline values have met the standard of Indonesian National Standards for liquid detergent so the mahogany seed extract can be used as a foaming agent to clean dirty clothes.

**The antibacterial activity**

Chloramphenicol was used as a positive control to compare whether mahogany seed extract had a better antibacterial effect than standard antibiotics, meanwhile, untreated distilled water was used as a negative control. After being incubated for 24 hours at 37°C, a clear zone was formed around the paper disk on the petri dish, indicating the inhibition zone for the growth of *Staphylococcus aureus* ATCC bacteria was formed due to the influence of saponins. The difference in the inhibition of growth of *Staphylococcus aureus* ATCC can be caused by differences in the antibacterial ability of the test compounds. The result of antibacterial activity test is illustrated on Fig. 3.

This study showed that the mahogany seed extract added to the three formulas could inhibit the growth of *Staphylococcus aureus* ATCC with different effectiveness. This can be seen by the formation of a clear zone indicating that there is a zone free from bacteria. The larger the diameter of the formed inhibition zone, the wider it proves the strength of bioactive compounds in inhibiting bacterial growth [13]. The difference in the mass of LAS and extract is a factor that also affects the size of the formed inhibition zone.

The diameter of the inhibition zone measured between 10-15 mm on average, this shows that the liquid detergent sample has antibacterial properties from the mahogany seed extract. From the results in Table 2, it can be seen that formula 2 has an antibacterial activity against *Staphylococcus aureus* ATCC with an inhibition zone diameter of 12.43 mm (strong category). The inhibitory activity of formula 2 is not much different from the control detergent. Although the use of mahogany seed extract in formula 2 is only half the mass of LAS, the results given are quite good. The quality test of antiseptic liquid detergent according to SNI has shown that formula 2 detergent has the best quality, so it can be concluded that formula 2 is the best antiseptic liquid for detergent preparation. The inhibitory activity of liquid detergent against *Staphylococcus aureus* ATCC was influenced by the concentration of mahogany seed extract given.

A decrease in the diameter of the inhibition zone in formula 3 can occur because the extract is not able to diffuse well. The concentration of the extract that is too thick makes it difficult for the extract to fully diffuse.
Formula 3 uses 3 grams of mahogany seed extract without the addition of LAS, so that a saturation can occur and cause the active compounds contained in the extract to not dissolve completely and there is no increase in the diameter of the bacterial inhibition zone. The quality of formula 3 as a detergent does not provide good acetylide according to SNI standards such as its pH value which is too alkaline. Mahogany seed extract contains saponins and flavonoids which have antibacterial activity [14]. Saponins from the extract at concentrations of 25 and 50 mg/ml can inhibit the growth of *Staphylococcus aureus* so that it has antibacterial properties. Gram (+) bacteria and fungi. Gram (-) bacteria and fungi are more resistant to antibiotics [15]. This ineffectiveness is because saponins are not able to penetrate the cell membrane of gram (-) bacteria such as *Escherichia coli* [9]. It can be concluded that saponins are more effective in inhibiting the growth of *Staphylococcus aureus* [9] while flavonoids are more active in inhibiting the growth of *Escherichia coli*. *Staphylococcus aureus* as gram-positive bacteria only has a single layer on the cell wall with a lot of peptidoglycans, little lipids, and polysaccharides (teichoic acid). This causes antibacterial compounds to enter the cell more easily and find the target to work. Damage to bacterial cells will cause inhibition of biosynthesis of specific enzymes needed in a metabolic reaction [16].

Saponins can act as antibacterial because their surface-active are similar to detergents, consequently, saponins will reduce the surface tension of bacterial cell walls and damage membrane permeability causing denaturation of membrane proteins so that cell membranes will be damaged and lysis. Damage to cell membranes greatly interferes with the survival of bacteria. Saponin diffuses through the outer membrane and the susceptible cell wall and then binds to the cytoplasmic membrane to disrupt and reduce the stability of the cell membrane. This causes the cytoplasm to leak out of the cell which results in cell death [9].

### CONCLUSIONS

The results of the research showed that all of the detergent formulas with different compositions of extracts and LAS have antibacterial activity against *Staphylococcus aureus* ATCC. The optimum conditions for liquid detergent preparation were obtained in formula 2. It gives detergency > 90 % and antibacterial activity against *Staphylococcus aureus* ATCC with an inhibition zone diameter of 12.43 mm (strong category). Formula 2 with a LAS : extract ratio of 1 : 2 (g/g) is the best formula that the addition of mahogany seed extract can function as a foaming agent as well as an antibacterial agent in the detergent well. Saponin from extract can inhibit the growth of gram (+) bacteria such as *Staphylococcus aureus* by reducing the surface tension of bacterial cell walls and damage membrane permeability causing denaturation of membrane proteins so that cell membranes will be damaged and lysis.

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**Table 2. Inhibitory zone diameter of *Staphylococcus aureus* ATCC on detergents.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Diameter of inhibition zone (mm)</th>
<th>Average diameter of inhibition zone (mm)</th>
<th>Inhibitory power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replication 1</td>
<td>Replication 2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.75</td>
<td>14.84</td>
<td>14.80</td>
</tr>
<tr>
<td>Formula 1</td>
<td>11.87</td>
<td>11.98</td>
<td>11.93</td>
</tr>
<tr>
<td>Formula 2</td>
<td>12.67</td>
<td>12.19</td>
<td>12.43</td>
</tr>
<tr>
<td>Formula 3</td>
<td>10.39</td>
<td>10.31</td>
<td>10.35</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30.05</td>
<td>29.72</td>
<td>29.89</td>
</tr>
<tr>
<td>Sterile aqua</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Information: a) weak, inhibitory zone of 5 mm or less; b) medium, 5-10 mm inhibition zone; c) strong inhibition zone 10-20 mm; and d) very strong, a zone of inhibition of 20 mm or more [13].
REFERENCES