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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SOAP ETHANOL EXTRACT OF GAHARU LEAVES (*GYRINOPS VERSTEEGII*)

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ABSTRACT
Gyrinops versteegii leaves can be used as an alternative to prevent skin infections caused by microbes by being formulated into solid soap. This study aims to determine the best formulation of agarwood leaf extract solid soap as antibacterial and antifungal and its compliance with Indonesian National Standards (SNI). Soap formulation was carried out with 5 different mass variations of agarwood leaf extract, namely 0, 1, 3, 5, and 7 grams. The antibacterial activity test against *Staphylococcus aureus* bacteria and the antifungal activity test against *Candida albicans* fungus were carried out by the diffusion well method, while the soap quality test was carried out in accordance with the Indonesian National Standard (SNI) which includes acidity (pH) test, foam stability, water content, total fat, insoluble ingredients in ethanol, free alkali or free fatty acids, unsaponifiable fat, and chloride content. The results obtained by all solid soap formulations of agarwood leaf extract meet the SNI quality standards. The best solid soap formula as antibacterial is in formula IV with the addition of 5 grams of agarwood leaf extract producing an inhibition of 11.08 mm while the best solid soap formula as antifungal is in formula III with the addition of 3 grams of agarwood leaf extract producing an inhibition of 12.5 mm.

KEYWORDS: *Candida albicans, Gyrinops versteegii leaves, soap, Staphylococcus aureus.*

INTRODUCTION

Skin disease is one of the health problems that is often found in Indonesia. The 2020 Indonesian Health Profile states that Indonesia has a high prevalence rate of skin diseases, namely 0.49 (49%) cases per 10,000 population and new skin disease cases of 4.2 cases per 100,000 population (Ministry of Health of the Republic of Indonesia, 2021). The types of skin diseases experienced by the public are generally infectious diseases which can be caused by microbes such as bacteria (Junairiah, 2013). One of the pathogenic bacteria that causes infectious diseases on the skin is *Staphylococcus* in the type *Staphylococcus aureus* (Tivani and Sari, 2021). Infection caused by *Staphylococcus aureus* begins with the entry of this bacteria into the skin through a wound scratch. Infection will be characterized by tissue damage accompanied by purulent abscesses such as boils or acne (Haryati et al., 2015).

Too rapid growth of the *Candida albicans* fungus can cause an infection in the vagina called candidiasis vaginitis. This infection often occurs due to the use of water such as toilets that contain *Candida sp. Candida albicans* often causes vaginitis in women with the main symptom being fluor albus which is often accompanied by itching (Nelwan, 2014).

The use of antiseptic soap is one option used by the public to treat infectious diseases. However, the use of antiseptic soap on the market can have a negative impact due to the addition of synthetic active substances, such as triclocarban and triclosan. Continuous use of triclocarban and triclosan on the skin can cause resistance to bacteria, allergies and disruption of the hormone system for brain growth and reproduction.

Triclocarban and triclosan can also trigger the creation of superbugs, namely bacteria that have undergone many changes (cell mutations), making these bacteria difficult to kill (Pratiwi and Endang., 2020).

The global scale states that every year there are 700,000 cases of death due to Antimicrobial Resistance (AMR) and it is estimated that this will increase to 10 million people by 2050 (O'Neill, 2016). Based on these problems, it is necessary to develop antibacterial and antifungal preparations derived from natural ingredients, one of which is derived from plants. The use of plants as antibacterials and antifungals is related to the secondary metabolite groups produced by plants. One of the plants

that has this potential is gaharu (*Gyrinops versteegii*).

Agarwood (*Gyrinops versteegii*) is an Indonesian forest plant that has great potential as a medicinal ingredient. People in various regions have used parts of the agarwood-producing plant, such as the roots, stems, leaves, skin and fruit. The use of plant extracts which contain secondary metabolites is believed to inhibit microbial growth activity which is very helpfulin healing and preventing infectious diseases. Based on research by Hendra (2015), it was found that agarwood leaf extract with chloroform solvent hadantibacterial activity with an inhibition zone of 10.83 mm against *S. aureus* bacteria and 9.92 mm against *E. coli* bacteria at a concentration of 300 mg/ml. Other research explains that the ethanol extract of agarwood leaves contains secondary metabolites including flavonoids, tannins, saponins and alkaloids which can inhibit the activity of *Staphylococcus aureus* bacteria at concentrations of 300mg/mL, 350mg/mL, 400mg/mL and 450mg/mL respectively 15.33 mm, 16.33 mm, 20.50 mm, and 21.17 mm (Abdul and Dzun, 2019). Putra's research results (2023) also reported that the ethanol extract of agarwood leaves showed an inhibitory power of 10.61 mm at a concentration of 30% against the fungus *Candida albicans*. Secondary metabolite compounds have different inhibitory mechanisms against microbial growth. The mechanism of action of flavonoids is that the hydroxyl group (-OH) contained in flavonoids disrupts the cell wall components of bacterial/fungal cells so that the wall layer does not form completely and causes the death of the bacterial/fungal cells (Kurama et al., 2020). The mechanism of action of tannins is by inactivating bacterial/fungal cell adhesion and inactivating enzymes, as well as disrupting protein transport in the inner layers of cells. Tannins damage cell wall polypeptides so that the formation of bacterial/fungal cell walls becomes less than perfect, this causes bacterial/fungal cells to lyse (Kurama et al., 2020). The mechanism of action of alkaloid compounds as antibacterial or antifungal is bydisrupting the cell wall components of bacterial/fungal cells so that the cell wall layer does not form completely and causes cell death. Apart from that, the alkaloid component is known to be a DNA interchelator and inhibits the topoisomerase enzyme in bacterial/fungal cells (Ningsih et al., 2016). The mechanism of action of saponin as an antibacterial or antifungal is by denaturing proteins, because the surface active substance of saponin is similar to detergent, saponin can be used as an antibacterial or antifungal where the surface tension of the bacterial/fungal cell walls will be reduced and the permeability of the bacterial/fungal membrane is damaged. Saponin will then diffuse through the cytoplasmic membrane so that the stability of the membrane will be disturbed, causing the cytoplasm to leakand leave the cell, resulting in cell death (Sudarmi et al., 2017).

Plant extract dosage forms can generally be used to treat and prevent infections. Extract preparations for treatment are usually in the form of cream or ointment, while for prevention they are usually in the form of antiseptic soap, either solid or liquid. In this research, agarwood leaf extract will be formulated into a natural antiseptic soap to prevent skin infections caused by bacteria and fungi. Soap contains surfactant compounds which work by lowering the surface tension of water, so that the process of pulling dirt on the skin becomes easier. Dirt in the form of fat, sweat or dust particles that stick to the surface of the skin will bind to hydrophobic groups and the reaction results of bacteria or fungi in the dirt with the added active ingredients are automatically attracted when rinsed with water. This is what causes water to attract dirt much more easily, because its surface tension decreases (Fessenden and Fessenden, 1992).

Based on the background above, the author is interested in conducting research with the title "Antibacterial and Antifungal Activity of Solid Soap Ethanol Extract of Agarwood Leaves (*Gyrinops versteegii*)". In this research, the mass formulation of agarwood leaf extract added was different in the hope of obtaining the best solid soap as antibacterial and antifungal as well as soap quality that complies with the soap quality requirements permitted by the Indonesian National Standard (SNI).

MATERIALS AND METHODS

Research Materials and Equipment

The material used in this research was agarwood leaves (*Gyrinops versteegii*) which had been inoculated and obtained from Klaci Village, Marga District, Tabanan Regency. Plant determination was carried out at LIPI-UPT Bali Branch, namely at the Baturiti "Eka Karya" Botanical Garden, Tabanan.

The chemicals used are distilled water, ethanol, HCl, coconut oil, olive oil, stearic acid, NaOH, glycerin, cocamid-DEA, NaCl, methyl orange, n-hexane, magnesium nitrate, anhydrous sodium sulfate, phenolphthalein, $AgNO_3$, K_2CrO_4 KOH, *Nutrient Agar* (NA), and *Sabouraud Dextrose Agar* (SDA).

The bacteria used were *Staphylococcus aureus* bacteria while the fungi used were *Candida albicans* fungi obtained from the Microbiology Laboratory of the Biology Study Program, Faculty of Mathematics and Natural Sciences.

The equipment used in this research includes a set of glassware, drying machine (oven), aluminum foil, blender, 200 mesh sieve, maceration container, gauze, vacuum rotary evaporator, stir bar, hand blender, spatula, soap mold, pH meter, set of titration tools, ruler, stopwatch, porcelain cup, disposable petri dish, analytical balance, desiccator, separating funnel, filter paper, boiling stone, water bath, set of reflux tools, Buchner filter, autoclave, tube needle, bunsen, laminar air flow (LAF), tweezers, plastic wrap, hot plate, cork borer, sterile cotton swab, incubator, dropper and

micropipette and blue tip.

Method

Sample preparation

A total of 5 kg of agarwood leaf samples that had been collected were wet sorted by washing with running water. The clean aloe leaves are then dried using a drying machine at a temperature of 50°C. The dried simplicia is sorted dry, then ground using a blender until it becomes powder. The resulting powder was sieved using a 200 mesh sieve, until a fine powder wasobtained. The results were put into a closed glass container.

Extraction of agarwood leaves (*Gyrinops versteegii*)

The extraction process is carried out using themaceration method. A total of 500 g of gaharu leaf simplicia powder (*Gyrinops versteegii*) was put into a maceration container, then macerated with 3 liters of ethanol solvent for 2x24 hours. The results of the maceration are then filtered using filter paper to obtainagarwood leaf extract. The liquid extract was thenconcentrated using a vacuum rotary evaporator at a temperature of 40°C with a rotation speed of 80 rpm to obtain a thick extract of gaharu leaves and the extract yieldwas determined using the following formula:

$$\% \text{ Yield} = \times 100\% \times \frac{b^2}{b1}$$

Information

b_1= initial simplicia weight (g)

b_2= final extract weight (g).

Soap making

The soap making process begins by mixing coconut oil and olive oil first in a container (the volume of each according to the formulation). The oil mixture is then mixed with 9 g of stearic acid which has been melted by heating (70°C) until it melts. The stearic acid and oil have been homogenized, then 30% NaOH solution is added.

When NaOH is added, the dough will become hard and sticky, indicating the formation of soap stock. Stirring is done until it is homogeneous, then glycerin is added to make the mixing process easier. The solution has been homogenized, then cocamid-DEA, NaCl, distilled water and agarwood leaf extract are added. The soap is poured into molds and left for ± 24 hours at room temperature. Thesoap is removed from the mold and then stored. The aging process is carried out for 3-4 weeks before the soap can be used and analyzed.

The soap formula is designed in 5 different formulas to find out the best formulation. The best formulation will be used as the main ingredient in antibacterial and antifungal soap. Five soap formula designs were made with different massesof agarwood leaf extract used. The mass of agarwood leaf extract for formulas I, II, III, IV, and V is 0; 1; 3; 5; and 7 g. The soap formula is presented in Table 1.

Table 1: Solid soap formulation from agarwood leaf extract (*Gyrinops versteegii*).

Material	Unit	Formulas				
		I	II	III	IV	V
Agarwood leaf extract (*Gyrinops versteegii*)	g	0	1	3	5	7
Coconut oil	g	65	65	65	65	65
Olive oil	g	25	25	25	25	25
NaOH 30%	mL	54	54	54	54	54
Stearic acid	g	9	9	9	9	9
Aquades	g	10	10	10	10	10
Cocamid-DEA	g	25	25	25	25	25
Glycerin	g	7	7	7	7	7
NaCl	g	0.2	0.2	0.2	0.2	0.2
Total Mass	g	195.2	196.2	198.2	200.2	202.2

Source: Widyasanti and Hasna, 2016 (with modifications)Information

Formula I: Solid soap preparation without adding agarwood leaf extract

Formula II: Solid soap preparation with 1 g agarwood leaf extract

Formula III: Solid soap preparation with 3 g agarwood leaf extract

Formula IV: Solid soap preparation with 5 g agarwood leaf extract

Formula V: Solid soap preparation with 7 g agarwood leaf extract.

Soap Quality Test

The tests carried out in this research were pH test, foam stability test, water content test, total fat test, ethanol insoluble material test, free alkali or free fatty acid test, unsaponifiable fat test and chloride content test.

Preparation of test media and sterilization

Media for the antibacterial test was made by weighing 5.75 g of *Nutrient Agar* (NA), then dissolving it in 250 mL of distilled water using an Erlenmeyer flask. Meanwhile, the antifungal test was made by weighing 3.37 g of *Sabouraud Dextrose Agar* (SDA), then dissolving it in 250 mL of distilled water using an Erlenmeyer. The media is heated using a hot plate until

it boils and stirred to ensure the media is perfectly homogeneous. The homogenizedmedia and blue tip tools were sterilized in anautoclave at 121˚C for 60 minutes. Meanwhile, the tube needle and tweezers equipment is sterilized by heating it over a Bunsen flame. The media that has been sterilized is then cooled for a few minutes and then placed in a disposable petri dish to a uniform depth of ± 0.5 cm and left to solidify.

Antibacterial activity test

Testing of the antibacterial activity of agarwood leaf soap was carried out using the agar diffusion method (diffusion well). The *S. aureus* bacterial suspension was inoculated into each petri dish using the streak plate method with a sterile cotton swab and left for 10- 15 minutes. Next, a diffusion well is made using acork borer which is heated first and pressed against themedia to form a diffusion well. Samples of solid soap formula IV with concentrations of 50, 25, and 10% were pipetted at 20 µL each and then placed into marked diffusion wells, then incubated at 37°C for 2x24 hours. The test was carried out with 3 repetitions. The clear zone that appears after incubation in the area around the well is a bacterial inhibition zone.

Antifungal activity test

Testing of the antifungal activity of agarwood leafsoap was carried out using the agar diffusion method (diffusion well). The *C. albicans* fungus suspension was inoculated into each petri dish using the streak plate method with a sterile cotton swab and left for 10- 15 minutes. Next, a diffusion well is made using acork borer which is heated first and pressed against themedia to form a diffusion well. Samples of solid soap formula IV with concentrations of 50, 25 and 10% were pipetted at 20 µL each and then placed into marked diffusion wells, then incubated at 37°C for 2x24 hours. The test was carried out with 3 repetitions. The clear zone that appears after incubation in the area around the well is a fungalinhibition zone.

RESULTS AND DISCUSSION

Simple preparation

A total of 5 kg of fresh samples of gaharu leaves were wet sorted with the aim of separating the leaves from dirt in the form of soil, stems and leaf petioles. The clean agarwood leaves are then dried to reduce the water content contained in them, thereby preventing the sample from being damaged by microbes. A sample of 1 kg of dried aloe leaves was then ground with a blender to produce agarwood leaf powder. The purpose of this refinement is to increase the surface area of the sample and facilitate contact between the sample and the solvent during the maceration process.

Extraction of agarwood leaves

The maceration results obtained were 56 g of concentrated green aloe leaf extract with a yield of 11.2%. The green color results in the concentratedextract of gaharu leaves is because the ethanol solvent can also extract natural pigments from gaharu leaves in the form of chlorophyll. The yield results in the research that has been carried out can be declared good because the value is more than 10% as in the research of Ilyas et al. (2023) which states that the yield is said to be good if the value is more than 10%.

Soap making

The soap obtained from the results of this research has different weights and colors according to the formula that has been made. One soap formulation recipe produces 4 molds for each formula. The results of the solid soap formulation in this study are presented in Table 1 as follows.

Table 1: Results of solid soap formulation.

No.	Formulas	Weight ofsoap (g)	Color of soap
1.	F I	27,72	White
2.	F II	27,90	Light green
3.	F III	27,55	Light green
4.	F IV	28,80	Deep green
5.	F V	29,21	Deep green

Table 1 shows that the soap obtained produces different colors. This color difference is caused by the mass of agarwood leaf extract added to the soap formula, where themore extract added, the darker the green color of the soap. The results of solid soap obtained physically in this study are supported by research on making solid soap from mangosteen peel extract reported by Aminudin et al. (2019), namely that the more extract added, the darker the brown color of the soap produced.

Soap quality test results

Quality testing of solid bath soap preparations is carried out to determine whether the soap is made in accordance with SNI or permitted soap standards. The tests carried out in this research were pH test, foam stability test, water content test, total fat test, ethanol insoluble material test, free alkali or free fatty acid test, unsaponifiable fat test andchloride content test.

Acidity test results (pH)

The results of the acidity (pH) test for solid soap in thisstudy are presented in table 2 as follows.

Table 2: Acidity (pH) test results for solid soap.

Formulas	Acidity (pH)
I	10.44
II	10.35
III	10.42
IV	10.30
V	10.23

Table 2 shows the results of the pH value of agarwood leaf extract solid soap respectively from formula I-V, namely 10.44; 10.35; 10.42; 10.30; 10.23. The results of the acidity (pH) test of solid soap from agarwood leaf extract formula I-V showed that the solid soap produced was in accordance with the pH requirements for

permitted soap preparations, namely in the range of 9 - 11 (SNI, 2016). The pH value of the solid soap produced is alkaline,this is because most of the components of the soap base such as NaOH have an alkaline pH (Sulastri and Yayan, (2016). The pH value of soap is very important for repairing and improving the quality of the skin and isvery influential in minimizing irritation to the skin.

Foam stability test results

The results of the solid soap foam stability test in this study are presented in Table 3.

Table 3: Results of foam stability tests on solid soap.

Formulas	Foam stability (%)
I	67.87
II	69.46
III	68.56
IV	63.86
V	61.27

Table 5: Total fat test results in solid soap.

Formulas	Total fat (%)
I	65.26
II	68.60
III	70.00
IV	72.46
V	73.46

Table 3 shows that the stability value of solid soap foam for agarwood leaf extract from formula I-V is 67.87; 69.46; 68.56; 63.86; and 61.27%. The results of testing the foam stability of the agarwood leaf extract solid soap formula I-V showed that the stability of the foam produced met SNI (2016) standards, namely in Table 5 shows the test results for the total solid soap fat of agarwood leaf extract respectively from formula I-V, namely 65.26; 68.60; 70.00; 72.46; and 73.46%. The total fat test results from solid soap from agarwood leaf extract formula I-V show that the solid soap produced is in accordance with the total fat quality standard according to SNI (2016), namely a minimum of 65%. The total fat produced is the amount of all the fat in the soap that has undergone a saponification reaction with alkali (Ningrum etal., 2021). The quality of soap will be better when the total fat in the soap increases, because fat is non-polar so it is the range of 60-70%. The foam in soap plays an important role in the process of cleaning dirt attached to the skin, where the characteristics of the foam are influenced by the surfactant ingredients and composition contained in the soap (Hutapea, 2019). The use of VCO oil as a soap raw material can provide excellent foam results due to the high content of lauric acid and myristic acid (Widyasanti et al, 2017). The saponin content in agarwood leaf extract can also increase the production and stability of foam in solid soap. Santoso et al.'s research. (2022) stated that agarwood plant leaf extract contains secondary metabolite compounds, namely saponins, amountingto 2.47-4.00%.

Water content test results

The results of the solid soap water content test in this study are presented in Table 4.

Table 4: Water content test results in solid soap.

Formulas	Water content (%)
I	14.71
II	14.76
III	14.44
IV	14.32
V	14.72

Table 4 shows the test results for the water content of agarwood leaf extract solid soap respectively from formula I-V, namely 14.71; 14.76; 14.44; 14.32; and 14.72%. The water content test results of formula I-V agarwood leaf extract solid soap show that after going through an aging process for 30 days, the water content has met SNI (2016) standards, namely a maximum of 15%. Too high a water content in soap causes the soap to shrink more quickly when used and also causes the soap to smell rancid. On the other hand, if the water content is too low, it will cause the soap to be harder, making it uncomfortable to use. Testing water contentis important because water affects the quality and storage duration of soap, as well as affecting the solubility of soap when used (Widyasanti et al., 2016).

Total fat test results

The total fat test results in this study are presented in difficult to dissolve in water and low fat content will have an effect in regulating the consistency of soap so that the less fat content a soap has, the easier it will be to break down when used because it is easy to use. absorb water (Marpaung et al., 2019).

Test results: the material is insoluble in ethanol

The test results for materials insoluble in ethanol in thisstudy are presented in Table 6.

Table 6: Test results for substances insoluble in ethanolin solid soap.

Formulas	Substances insoluble in ethanol (%)
I	0.40
II	0.26
III	0.40
IV	0.40
V	0.40

Table 6 shows the test results for ethanol insoluble substances in agarwood leaf extract solid soap respectively from formula I-V, namely 0.40; 0.26; 0.40; 0.40; and 0.40%. The test results for ingredients insoluble in ethanol from solid soap from agarwood leaf extract formula I-V show that the solid soap preparation produced complies with quality standards according to SNI (2016), namely good solid soap contains ingredients insoluble in ethanol ata maximum of 5%. Soap with high amounts of insoluble ingredients in ethanol can cause skin health problems and disrupt the appearance of the

soap (Zainal, 2020).

Test results for the amount of free alkali or free fatty acids

The test results for the amount of free alkali from solid soap from agarwood leaf extract formula I-V showed that the solid soap produced did not contain free alkali, where when the phenolphthalein indicator was added the solution did not change color to pink, meaning that there was no free alkali but there were free fatty acids present on soap. The free fatty acid test results are presented in Table 7.

Table 7: Free fatty acid test results in solid soap.

Formulas	Free fatty acids (%)
I	0.59
II	0.80
III	0.97
IV	1.18
V	1.36

Table 7 shows the results of the free fatty acid test on agarwood leaf extract solid soap respectively from formula I-V, namely 0.59; 0.80; 0.97; 1.18; and 1.36%. The results of the free fatty acid test from solid soap with agarwood leaf extract formula I-V obtained in this study have met the SNI (2016) solid soap quality standards, namely a maximum of 2.5% and increase with the addition of the mass of agarwood leaf extract, where more extract is added the higher the free fatty acids produced. This is because gaharu leaves contain fatty acids as antioxidants such as palmiic acid (Rizkyani, et al. 2023). The results of this study are also in accordance with the tests of Sukeksi et al. (2018) which states that a NaOH concentration of 30% is the best concentration to produce optimal soap (free of alkali) so that the soap produced does not cause irritation to the skin.

Unsaponifiable fat test results

The results of the unsaponifiable fat test in this study are presented in Table 8.

Table 8: Test results for unsaponifiable fat in solid soap.

Formulas	Unsaponifiable fat (%)
I	0.0037
II	0.0036
III	0.0033
IV	0.0031
V	0.0029

Table 8 shows the results of the unsaponifiable fat test in agarwood leaf extract solid soap respectively from formula I-V, namely 0.0037; 0.0036; 0.0033; 0.0031; and 0.0029%. This shows that almost all the fat in the I-V soap formulation has been saponified by alkali and is in accordance with the quality standards according to SNI (2016), namely a maximum of 0.5%. The unsaponifiable fraction relates to substances contained in unsaponified oils or fats in the form of sterols and hydrocarbons. Testing the amount of unsaponifiable fat is important because if the amount is high in the soap, it can reduce the soap's ability to clean oil and dirt (Agustini and Agustina, 2017).

Chloride level test results

The results of the chloride level test in this study are presented in Table 9.

Table 9: Test results for chloride levels in solid soap.

Formulas	Chloride content (%)
I	0.52
II	0.35
III	0.46
IV	0.40
V	0.35

Table 9 shows the results of the chloride content test in agarwood leaf extract solid soap respectively from formula I-V, namely 0.52%; 0.35%; 0.46%; 0.40%; 0.35%. The results of the chloride content test from solid soap from agarwood leaf extract formula I-V show that the solid soap produced is in accordance with the quality standards according to SNI (2016), namely a maximum of 1%. The presence of chloride in this study was caused by the addition of NaCl which was used when making soap, however the chloride levels obtained were not high because if the chloride levels in the soap were high it could be dangerous for skin health because it is corrosive and causes skin irritation (Ningrum et al, 2021).

Antibacterial test results

The results of the antibacterial test were marked by the formation of an obstacle zone (clear area) around the well, which in this study showed that gaharu leaf extract had antibacterial activity against *S. aureus* bacteria after being formulated in a soap preparation. The results of the antibacterial activity test for solid soap, agarwood leaf extract formula I-V against *S. aureus* bacteria are presented in Table 10 as follows.

Table 10: Results of antibacterial tests on solid soap.

Formulas	Resistance (mm)		
	50%	25%	10%
I	7.83	6.83	4.58
II	9.16	7.58	5.83
III	10.08	8.45	6.91
IV	11.08	8.75	7.00
V	7.16	6.16	5.58

Table 10 shows that agarwood leaf extract solid soap inhibits the growth of *S. aureus* bacteria with an inhibitory power range of 4.58-7.00 mm at a concentration of 10%; 6.16-8.75 mm at a concentration of 25%, and 7.16-11.08 mm at a concentration of 50%. The resulting antibacterial activity showed an increase in bacterial inhibition with the addition of agarwood leaf extract, but in formula V soap there was a decrease in inhibition. The increase in inhibitory power of 42% in formulation IV was caused by the addition of agarwood

leaf extract which acts as an antibacterial compound, so that formulation IV is the optimum formulation in inhibiting bacteria. The results of this research are supported by Bayu's research (2021) which reported that ethyl acetate extract of gaharu leaves has high levels of total flavonoids, namely 186.976 mg QE/g and is able to inhibit the growth of S. aureus with a resulting inhibitory zone diameter of 19 mm at a concentration of 20%. The decrease in the antibacterial inhibitory power of formula V soap was caused by exceeding the maximum amount of added extract. Adding too much extract can change the interaction between the active compounds in the soap and the *S. aureus* bacteria, thereby reducing its inhibitory activity.

In this study, formula I soap (positive control) without the addition of agarwood leaf extract also showed results in inhibiting the growth of *S. aureus* bacteria. According to Hapsari (2015) the strength of the inhibition zone of 20 mm or more is categorized as very strong, the inhibition zone of 10-20 mm is categorized as strong, 5-10 mm is categorized as medium and the resistance area of 5 mm or less is categorized as weak so that the results of the inhibition power of formula I soap are included in the category currently. This is caused by the influence of additional ingredients that are antiseptic and antimicrobial such as VCO in the composition of solid soap with agarwood leaf extract. The research results of Yeyen et al. (2020) stated that VCO was able to minimally inhibit the growth of *S. aureus* bacteria with a turbidimetric test occurring at a concentration of 100%. VCO has antibacterial capabilities based on the content of saturated fatty acids including Medium Chain Fatty Acid (MCFA) and Medium Chain Tryglycerides (MCT). MCFA in the form of lauric acid has antiviral, antibacterial, antiprotozoal properties and is currently being developed as an antiviral for HIV (Human Immunideficiency Virus) (Yeyen et al., 2020).

Antifungal test results

The results of the antifungal test were marked by the formation of an obstacle zone (clear area) around the well, which in this study showed that agarwood leaf extract had antifungal activity against the fungus *C. albicans* after being formulated in a soap preparation. The results of the antifungal activity test of solid soap agarwood leaf extract formula I-V against the fungus *C. albicans* are presented in Table 11 as follows.

Table 11: Antifungal test results on solid soap.

Formulas	Resistance (mm)		
	50%	25%	10%
I	7.91	3.3	1.75
II	10	3.58	2.08
III	12.5	4.58	2.75
IV	6.3	4.08	1.75
V	6.16	2.08	1.00

Table 11 shows that agarwood leaf extract solid soap inhibits the growth of the fungus *C. albicans* with an inhibitory power range of 1.00-2.75 mm at a concentration of 10%; 2.08-4.58 mm at a concentration of 25%, and 6.16-12.5 mm at a concentration of 50%. The resulting antifungal activity showed an increase in fungal inhibition with the addition of agarwood leaf extract, but in soap formulas IV and V there was a decrease in inhibition. The increase in inhibitory power of 58% in formulation III was caused by the addition of agarwood leaf extract which acts as an antifungal compound, so that formulation III is the optimum formulation in inhibiting fungi. The results of this research are supported by research by Putra (2023) who reported that analysis of the phytochemical test of the ethanol extract of gaharu leaves showed the presence of flavonoid, alkaloid, saponin, tannin and steroid compounds and the results of the antifungal activity test against the fungus *C. albicans* showed an inhibitory power of 10 .61 mm at 30% concentration.

The decrease in antifungal inhibitory power in soap formulas IV and V was caused by exceeding the maximum amount of added extract. Adding too much extract can change the interaction between the active compounds in the soap and the *C. albicans* fungus, thereby reducing its inhibitory activity.

In this study, soap formula I (positive control) without the addition of agarwood leaf extract also showed inhibition of the growth of the fungus *C. albicans*. According to Hapsari (2015) the strength of the inhibition zone of 20 mm or more is categorized as very strong, the inhibition zone of 10-20 mm is categorized as strong, 5-10 mm is categorized as medium and the resistance area of 5 mm or less is categorized as weak so that the results of the inhibition power of formula I soap are included in the category currently. This is caused by the influence of additional ingredients that are antiseptic and antimicrobial such as VCO in the composition of solid soap with agarwood leaf extract. The research results of Meyok and Jasman (2023) stated that VCO has very strong antifungal activity which is caused by the active substances from coconut oil which diffuse into the fungal growth medium. The active substances are caprylic acid (C8), capric acid (C10), and lauric acid (C12) which work by damaging the fungal membrane.

Selection of the best agarwood leaf extract solid soap

The results of the research we conducted showed that the quality test of formula I-V agarwood leaf extract solid soap met SNI in all parameters, so the selection of

the best formula was carried out by comparing the results of the antibacterial and antifungal tests for each soap formulation. Agarwood leaf extract solid soap formula IV has the highest antibacterial activity test value on *Staphylococcusaureus* bacteria and formula III agarwood leaf extract solid soap has the highest antifungal activity test value on *Candida albicans* fungus. The difference in the results of the activity of agarwood leaf extract solid soap against bacteria and fungi can be caused by differences in the morphology of the bacteria and fungi. Bacterial cells have a cell wall morphology composed of thick peptidoglycan, while fungal cell wall morphology is composed of chitinand does not have peptidoglycan (Sukmiwati et al., 2018). A recapitulation of the results of the analysis of solid soap from agarwood leaf extract can be seen in Table 12 as follows.

Table 12: Recapitulation of results of analysis of agarwood leaf extract solid soap.

Parameter	FI	F II	F III	F IV	FV	SNI
Acidity (pH)	10.44	10.35	10.42	10.30	10.23	Range9-11
Foam stability (%)	67.87	69.46	68.56	63.86	61.27	Range60-70
Water content (%)	14.71	14.76	14.44	14.32	14.72	Max 15.0
Total fat (%)	65.26	68.60	70.00	72.46	73.46	Min 65.0
Substances insoluble in ethanol (%)	0.40	0.26	0.40	0.40	0.40	Max 5.0
Free alkali (%)	-	-	-	-	-	Max 0.1
Free fatty acids (%)	0.59	0.80	0.97	1.18	1.36	Max 2.5
Unsaponifiable fat (%)	0.0037	0.0036	0.0033	0.0031	0.0029	Max 0.5
Chloride content (%)	0.52	0.35	0.46	0.40	0.35	Max 1.0
Antibacterial activity (50%)	7.83	9.16	10.08	11.08	7.16	-
Antifungal activity (50%)	7.91	10	12.5	6.3	6.16	-

CONCLUSION

The best formulation of solid soap with agarwood leaf extract as an antibacterial that causes skin infections is formula IV with the addition of 5 grams of agarwood leaf extract. The best formulation of solid soap with agarwood leaf extract as an antifungal that causes skin infections is formula III with the addition of 3 gramsof agarwood leaf extract. The agarwood leaf extract solid soap produced in this research meets the SNI quality standard requirements.

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