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Evaluation of The Antibacterial Activity of Tallow-Based Soap with the Addition of Tea Tree and Peppermint Extracts

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ABSTRACT

Tallow is a by-product of the meat processing industry. Tallow is commonly used as a raw material in soap making. This studi aims to evaluate the antibacterial activity of tallow-based soap with varying amounts of tea tree and peppermint extracts. This study used a completely randomized design with the addition of tea tree and peppermint extracts 0% (P1), addition 2% (P2), addition 5% (P3), addition 8% (P4). The parameters of this study were water content, pH, free fatty acids, and antibacterial activity tests against *Escherichia coli*, *Staphylococcus aureus*, and *Propionibacterium acnes*. The results showed that tea tree and peppermint extracts had no significant effect on the water content, pH and free fatty acid values. Meanwhile, the results of antibacterial activity showed significantly different results ($P<0.05$) for *E. coli* and *S. aureus* but were not significant for *P. acnes*. Adding tea tree and peppermint extracts to tallow-based soap can enhance its antibacterial properties without affecting the physical quality of the soap.

Keywords: antibacterial, peppermint, solid soap, tallow, tea tree

INTRODUCTION

Soap can be used for daily personal hygiene and skin protection. According to the National Socioeconomic Survey by the Central Bureau of Statistics (BPS) in 2017, the percentage of the Indonesian population using soap is very high, at 99.8%, making soap a primary necessity. Bath soap, as one of the skin cleansing materials, contains potassium compounds with fatty acids from vegetable or animal fat and comes in solid, soft, or liquid forms, foamy, with or without additional substances such as fragrances and other ingredients that do not harm health or cause skin irritation (Rita *et al.* 2018). Soap protects the skin from pathogenic bacteria (Baehaki *et al*. 2019). Pathogenic bacteria are one of the causes of skin infections. These bacteria can invade the human body via the respiratory, skin, digestive, and urinary systems. Bacteria, viruses, protozoa, and other microorganisms can cause skin infections. Akib *et al*. (2019) also stated that The most frequently encountered pathogenic bacteria that cause human infections are *Staphylococcus aureus* and *Escherichia coli*. Using solid soap is a method to safeguard the skin against bacterial infections and mitigate the risk of infectious skin diseases. Synthetic antibacterial agents can prevent infections but may cause side effects such as irritation. It has encouraged the shift to natural preparations (Rosdiyawati, 2014).

Soap can be made from animal fat. Tallow is a triglyceride primarily composed of saturated fats (acids), with tristearin as its most significant component. Tallow is an oil obtained from the extraction of bovine abdominal fat. It is solid at room temperature and melts at 64 °C. The main components of tallow are oleic acid (40-45%), stearic acid (14-19%), palmitic acid (24-37%), myristic acid (2-8%), linoleic acid (3-4%), and lauric acid (0.2%) (Kowalska *et al*. 2015). Tallow as a raw material for solid soap can be enhanced by adding natural ingredients such as tea tree and peppermint extracts to increase the soap's benefits.

Tea tree (*Melaleuca alternifolia*) is a plant native to Australia, resembling the tea plant (Camelia spp.), and is used as an antiseptic against bacteria, fungi, and viruses. According to research by Folorunso Olufemi *et al*. (2020), the essential oil from tea trees can inhibit the growth of pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, and *Salmonella paratyphi*. Peppermint (Mentha piperita) contains essential oil with menthol (50-60%) as its main component. Peppermint leaves are aromatic herbs with antiseptic and antibacterial properties. The primary constituents found in peppermint leaves include essential oil (0-5.4%), consisting

predominantly of menthol (30-55%) and menthone (14-32%). Due to the menthol oil content, Peppermint leaves have a fragrant aroma and a refreshing cool taste (Setiawan *et al*. 2018). These two ingredients can enhance soap's antibacterial action to kill skin bacteria. Therefore, this study aims to evaluate the antibacterial activity of tallow soap by adding tea tree and peppermint extracts at different concentrations.

MATERIALS AND METHODS

Materials and Tools

The materials used in this study include beef fat (tallow), tea tree oil (Jaya Abadi Chemical) and peppermint extract (Jaya Abadi Chemical), NaOH crystals, distilled water, canola oil, coconut oil, soap colorant, as per the formulation in Table 1. The materials for soap quality testing include distilled water, 0.1 N KOH solution, 0.1 N HCl standard solution, phenolphthalein indicator, and ethanol. The materials for antibacterial testing include distilled water as the inoculum for gram-negative bacteria, amoxicillin for gram-positive bacteria, nutrient agar (NA) suspension solution, physiological NaCl solution, McFarland 0.5 solution, and Muller Hinton Agar (MHA) suspension solution.

The equipment used in the soap-making process for this research includes a digital scale, stove, thermometer, stainless steel pot, blender, containers, sieve, and silicone molds. The equipment used for soap testing includes a pH meter, magnetic stirrer, volumetric flask, beaker, analytical balance, oven, desiccator, burette, water bath, microtubes, Petri dishes, spreader, loop, and paper discs.

Table 1. Formulation of tallow-based soap enriched with tea tree and peppermint extracts

Ingredients	Treatments			
	P1 (0%)	P2 (2%)	P3 (5%)	P4 (8%)
Tea Tree (g)	0	2	5	8
Peppermint (g)	0	2	5	8
Tallow (g)	50	50	50	50
Canola Oil (g)	25	25	25	25
Coconut Oil (g)	25	25	25	25
NaOH (g)	13	13	13	13
Aquadest (g)	39	39	39	39
Coloring agent (g)	3	3	3	3

Note: P1 = 0% addition of tea tree and peppermint extracts, P2 = 2% addition of tea tree and peppermint extracts, P3 = 5% addition of tea tree and peppermint extracts, P4 = 8% addition of tea tree and peppermint extracts. The treatments was repeated three times

Procedure

Tallow Production

Before making soap, the initial step is rendering beef fat to produce tallow. This is done by cooking the fat on a stove over medium to low heat. One kilogram of beef fat is cleaned of impurities, blood, and non-fat parts. The cleaned fat is then blended until smooth. During cooking, 10 grams of crystal salt is added for every 1 kilogram of beef fat. The rendering process is complete when the beef fat fully melts into oil. Once finished, the rendered oil is poured into a container and can be used within 2-3 days. The tallow will harden again, turning white.

Solid Soap Making (Rita *et al*. 2018)

The soap-making process begins by mixing the fat fraction, which includes stearic acid (tallow), VCO oil, and canola oil, with alkali (30% NaOH) at 35 ˚C. During the addition of NaOH, the mixture will become hard and sticky, indicating the formation of soap stock. Then, additional ingredients such as tea tree and peppermint extracts obtained from the market (Jaya Abadi Chemical) and soap colorant are added to the soap stock. The soap-making materials are prepared in advance, and two processes are involved. The first process is preparing the lye solution, which involves mixing NaOH and distilled water. During the addition of NaOH, the mixture will become hard and sticky, indicating the formation of soap stock. Then, additional ingredients such as tea tree, peppermint extracts, and soap colorant are added to the soap stock. Prepare the tallow, canola oil, and coconut oil in the second process. First, heat the tallow, and once it has melted, immediately mix in the other oils. When the temperature of the NaOH solution and oils reach 35-40 °C, the

two liquids can be combined into one container. After that, the soap is left for an aging time of 30 days until it is ready for analysis.

Soap Quality Analysis

Water Content Test (SNI 3532 2016)

Weigh 1 gram of the sample in a dish, then place it in an oven at 105 °C for 8 hours. Afterwards, weigh the water content.

pH Test (SNI 3532 2016)

Place 1 gram of the sample into a 1000 mL volumetric flask and add distilled water. Calibrate the pH meter using standard buffer solutions with pH 4 and 7, then immerse the electrode into the test sample solution while stirring. Record the pH result displayed on the pH meter.

Free Fatty Acid Test (SNI 3532 2016)

Place a 10-gram soap sample, weigh it, and mix it with 30 ml of neutral ethanol and a boiling stone. The mixture is heated in a water bath and then cooled. Next, 3 drops of phenolphthalein are added. If the solution does not turn red, it indicates the presence of free fatty acids, and the sample is titrated with NaOH.

Antibacterial Analysis (Dhiman *et al.*, 2011)

Preparation of Test Bacteria

The bacteria used in this test include *Propionibacterium acnes*, *Escherichia coli*, and *Staphylococcus aureus* at a concentration of 1.5×10^6 CFU/mL. Each bacteria was subcultured using the streak method. A bacterial stock was taken with an inoculation loop and streaked onto nutrient agar (NA) in petri dishes. The Petri dishes were incubated at 37 °C for 24 hours in an inverted position. After incubation, bacteria subcultured for 24 hours were taken with an inoculation loop, transferred into 9 mL of 0.85% physiological saline solution, and then homogenized until no precipitate was present. The turbidity of the bacterial suspension was measured using a UV-VIS spectrophotometer at a wavelength of 620 nm to achieve an optical density (OD) corresponding to the McFarland 0.5 standard, ensuring a bacterial count of 1.5×10^8 CFU/mL. The suspension with the same wavelength was diluted once for a bacterial count of 1.5×10^6 CFU/mL.

Antibacterial Testing

A bacterial suspension of 0.1 mL was taken using a micropipette and spread onto Petri dishes containing solid Mueller Hinton agar medium. Nine soap samples were treated with P1, P2, P3, and P4. Samples of Tea Tree, Peppermint, and TTPP (Tea tree + Peppermint) extracts, as well as negative control (sterile distilled water) and positive control (amoxicillin), were placed onto paper disks and positioned on the surface of the Mueller Hinton agar using forceps. Petri dishes were covered with filter paper and incubated at 37 °C for 24 hours. After incubation, the diameter of the clear zones around the paper disks was observed, and the inhibition zones were measured and recorded using a caliper.

Data Analysis

The design used in this study is a Completely Randomized Design (CRD) with the addition of Tea tree and peppermint extracts at different concentrations, namely: 1) Without the addition of extract oil (control); 2) Addition of 2%; 3) Addition of 5%; 4) Addition of 8%. The addition of oil treatments was repeated three times.

According to Steel and Torrie (1997), the statistical model used is as follows.

$$Y_{ij} = \mu + \alpha i + \varepsilon_{ij}$$

Note:

Y_{ij} = Research Response at the level of adding tea tree and peppermint extract to - i (0%, 2%, 5%, and 8%) at replication - j (1, 2, and 3)

μ = Response

α_i = The effect of the level of addition of tea tree and peppermint to - i (0%, 2%, 5%, and 8%) on research response

ε_{ij} = The effect of error in adding tea tree and peppermint extract to - i (0%, 2%, 5%, and 8%) on replication - j (1, 2, and 3).

The data were analyzed using analysis of variance (ANOVA) with a 95% confidence interval to determine the effects of the treatments, and if the treatments had a significant or highly significant effect, Tukey's test was performed.

RESULTS AND DISCUSSION

Soap Quality Testing

Testing the quality standards for solid bath soap aims to determine the quality, which refers to SNI 3532 (2016). The soap quality test consists of water content, pH and free fatty acids, as seen in Table 2.

Table 2. Results of quality test analysis of tallow soap enriched with tea tree and peppermint extracts

Treatments	Water content (%)	pH	Free fatty acid (%)
P1	18.60±1.23	9.11±0.148	0.32
P2	17.88±3.00	9.01±0.171	0.27
P3	20.03±2.15	9.213±0.21	0.24
P4	22.08±3.68	9.17±0.172	0.25
SNI	Max 25	6.0-11.0	Max 2.5

Note: P1 = addition of 0% tea tree and peppermint extract, P2 = addition of 2% tea tree and peppermint extract, P3 = addition of 5% tea tree and peppermint extract, P4 = addition of 8% tea tree and peppermint extract. SNI standards for pH 6-11.

Water Content

The research results showed that the treatment with the addition of tea tree and peppermint extracts had no significant effect on the water content of the soap. The water content obtained ranged from 18.60-22.08%. This value still fulfilled the SNI standard of a maximum of 25%. This can be caused by using tallow, which has the property of immediately solidifying if left at room temperature. During storage, the elevated water content in soap leads to a reaction where excess water reacts with unsaponified fats, forming free fatty acids and glycerol. This process is known as soap hydrolysis (Idoko *et al*., 2018). Soap with a very high water content will experience weight loss more quickly. The amount of water added to the soap will also affect its solubility. The more water added to the soap, the easier it will shrink when used (Mumpuni and Sasongko, 2017). Meanwhile, soap with a small water content will increase the shelf life of the soap product (Habib *et al*., 2016).

pH

The results of the pH value analysis showed no different effect on the treatment. The pH value of soap ranges from 9.01-9.21. This value still fulfilled SNI standards, namely 6-11. The saponification process influences soap's high and low pH during soap making. The high pH value of soap results from the hydrolysis reaction in the saponification process. The solution was to add excess fat or oil. However, adding fat or oil will reduce the hardness of the soap (Habib *et al*., 2016). In general, soap is alkaline in water solutions because soap is a salt of a weak acid (fatty acid) and a base. Newly-made soap still has a high free alkali content, so it needs an aging time of 30 days to reach optimal condition. The purpose of aging soap is to allow for chemical rearrangement and stabilization within the soap. During the aging period, the soap undergoes a reaction known as further saponification, where any excess alkali continues to react with the remaining fatty acids in the soap. This process helps reduce the level of free alkali in the soap, enhancing its hardness and durability and improving its final quality in terms of softness and smoothness. Therefore, aging enables the soap to achieve optimal quality before use.

Free Fatty Acids

The results of measuring free fatty acids showed no significantly different results. The free fatty acids produced ranged from 0.24-0.32%. The maximum free fatty acid content in solid transparent soap based on SNI was 2.5%. The soap tends to smell rancid if the free fatty acid content is high. Free fatty acids that were too high could interfere with soap's effectiveness in cleaning dirt. High levels of free fatty acids could reduce the binding power of soap to dirt, oil, grease or sweat. Free fatty acids had no dirt binding because they were polar, unlike fatty oils, or dirt could bind with free fatty acids.

Antibacterial Properties

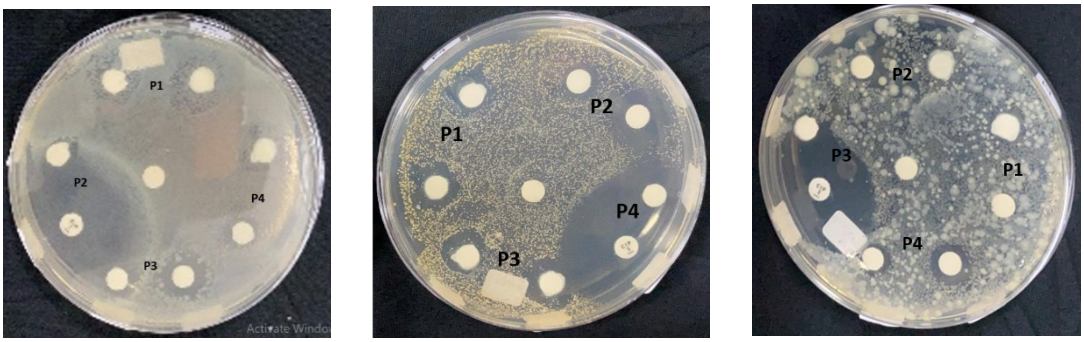
The antibacterial properties of soap were tested using *Escherichia coli, Staphylococcus aureus*, and *Propionibacterium acnes*. The test results for the antibacterial properties of soap can be seen in Table 3 and Figure 1.

Antimicrobial activity can be determined based on the inhibition zone around the paper disc (Dhiman *et al.* 2011). The antibacterial test results against *E. coli* showed a significant effect on performance. The addition of 5% tea tree and peppermint extracts showed the highest inhibitory value, and the S. aureus significantly affected the addition of 8% tea tree and peppermint extracts. Meanwhile, for *P. acnes*, the addition of tea tree and peppermint extracts had no significant effect on antibacterial activity. Based on research results, the inhibitory ability of tallow soap with adding tea tree and peppermint extracts on Gram-positive bacteria was more significant than on Gram-negative. It is due to differences in the structure of the cell walls of Gram-positive and Gram-negative bacteria.

Table 3. Results of inhibition zone analysis of antibacterial solid soap enriched with tea tree and peppermint extracts

Treatments	Inhibition zone diameter (mm)		
	E. coli	*S. aureus*	*P. acnes*
P1	5.50±0.05b	8.25±3.18b	5.25±1.06
P2	6.00±0.08ab	3.25±0.35c	6.00±2.83
P3	8.75±1.06a	8.25±1.06b	8.00±4.24
P4	5.00±0.71b	9.50±0.00a	7.75±4.60

Note: P1 = addition of 0% tea tree and peppermint extract, P2 = addition of 2% tea tree and peppermint extract, P3 = addition of 5% tea tree and peppermint extract, P4 = addition of 8% tea tree and peppermint extract. Different letters in the same column indicate significant differences (P<0.05).



A B C

Figure 1. Inhibition zone in A). *E coli*, B). *S. aureus*, C). *P. acnes*

Gram-positive bacteria only have a single plasma membrane surrounded by a thick cell wall in the form of a peptidoglycan layer (Marwati *et al*. 2012). Gram-negative bacteria have a double membrane system, so the peptidoglycan layer is located between the two double membranes (Beveridge 1999). The antimicrobial mechanism for inhibiting microorganisms operates through several methods, including (i) damaging the cell walls of microorganisms during formation or after the cells have formed, (ii) disrupting cell membrane permeability, which inhibits cell growth or kills the cells, (iii) denaturing proteins and nucleic acids, which are vital components that can damage cells, (iv) inhibiting enzyme activity, resulting in disrupted cell metabolism and potentially cell death, and (v) inhibiting the synthesis of nucleic acids and proteins, which have essential functions in the cell; impeded synthesis of DNA, RNA, and proteins results in cell damage (Moulia *et al*. 2018). The growth of bacteria that cause infections and diseases must be inhibited with antibacterial agents. Antibacterials are substances that can inhibit the growth of bacteria and kill pathogenic bacteria (Paju *et al*., 2013).

The antimicrobial compounds contained in tea tree oil are terpinen-4-ol and α-terpineol (Carson et al., 2006). Meanwhile, the antibacterial compounds found in peppermint are limonene, 1,8-cineole, menthol, and menthone (Sharafi et al., 2010; Dorman & Deans, 2000). The inhibition zone produced in this study was included in the medium category, namely around 5-10 mm. The antibacterial inhibition test, according to Pal *et al*. (2009), is categorized based on the diameter of the inhibition zone formed; namely, an inhibition zone diameter of 5 mm or more minor is classified as weak, 5-10 mm as moderate, 10-20 mm as vital, and 20 mm or more as extreme resistance.

CONCLUSION

Tallow-based solid soap enriched with tea tree and peppermint extracts fulfilled the SNI. This research soap also had antibacterial activity against *E. coli* and *S. aureus* at additional 5 and 8% concentrations with a medium category inhibition zone. The menthol, menthone, limonene, and 1,8-cineole in peppermint and the terpinene-4-ol and α-terpineol in tea tree extract contribute to a broad-spectrum antibacterial effect. This combination can help create a soap that not only cleanses effectively but also helps reduce bacterial presence on the skin, potentially improving skin health and hygiene.

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