

Formulation and Effectiveness Test of Handmade Soap Essential Oil of Basil Leaves (*Ocimum basilicum* L.) Against *Staphylococcus epidermidis* Bacteria

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Abstract

Introduction: Basil essential oil is one of the ingredients that evaporates easily when used directly, so it's formulated in the form of handmade soap so that it's comfortable to use. if *Staphylococcus epidermidis* balance is disturbed can be one of the triggers for acne and cause body odor. **Aims:** This research aims to formulate and evaluate handmade soap from the essential oil of basil leaves (*Ocimum basilicum* L.) of the Basil brand from PT Nusaroma Essential Indonesia, and test it's effectiveness in inhibiting *S.epidermidis*. **Method:** Evaluation carried out include organoleptic test, pH, foam stability, water content and free alkali/free fatty acid content test. This research uses laboratory experimental methods, where preparations are tested against *S.epidermidis* using well diffusion with essential oil concentrations 0%, 1%, 2%, and 3%. **Result:** Handmade soap preparations of basil leaf essential oil of 0% it shows the smallest inhibition zone, namely 6,1 mm and of 3% it shows the largest zone of inhibition is 7,9 mm. From the results obtained, there is a large influence of increasing the concentration of basil essential oil on its effectiveness in inhibiting the growth of *S.epidermidis* bacteria. Data analysis used the Anova test $p < 0,05$, which means there is a significant influence between the concentration of basil leaf essential oil in handmade soap preparations. **Conclusion:** The essential oil of basil leaves can be formulated into a handmade soap dosage form, and is able to inhibit the growth of *S.epidermidis* with largest average inhibition zone at a concentration of 3%, namely 7,9 mm.

Keywords : Bessential Oil, Handmade Soap, *Staphylococcus epidermidis*

Abstrak

Pendahuluan: Minyak esensial kemangi merupakan salah satu bahan yang mudah menguap bila digunakan langsung, sehingga diformulasikan dalam bentuk sabun buatan tangan agar nyaman digunakan. Jika keseimbangan *Staphylococcus epidermidis* terganggu, dapat menjadi salah satu pemicu jerawat dan menyebabkan bau badan. **Tujuan:** Penelitian ini bertujuan untuk memformulasikan dan mengevaluasi sabun buatan tangan dari minyak esensial daun kemangi (*Ocimum basilicum* L.) merek Kemangi dari PT Nusaroma Essential Indonesia, dan menguji efektivitasnya dalam menghambat *S.epidermidis*. **Metode:** Evaluasi yang dilakukan meliputi uji organoleptik, pH, stabilitas busa, kadar air, dan kadar alkali bebas/asam lemak bebas. Penelitian ini menggunakan metode eksperimen laboratorium, di mana preparat diuji terhadap *S.epidermidis* menggunakan sumur difusi dengan konsentrasi minyak esensial 0%, 1%, 2%, dan 3%. **Hasil:** Preparat sabun buatan tangan minyak esensial daun kemangi 0% menunjukkan zona inhibisi terkecil, yaitu 6,1 mm dan 3% menunjukkan zona inhibisi terbesar yaitu 7,9 mm. Dari hasil yang diperoleh, terdapat pengaruh besar peningkatan konsentrasi minyak esensial kemangi terhadap efektivitasnya dalam menghambat pertumbuhan

bakteri *S.epidermidis*. Analisis data menggunakan uji Anova $p<0,05$, yang berarti terdapat pengaruh signifikan antara konsentrasi minyak esensial daun kemangi dalam sediaan sabun buatan tangan. **Kesimpulan:** Minyak esensial daun kemangi dapat diformulasikan menjadi bentuk sediaan sabun buatan tangan, dan mampu menghambat pertumbuhan *S.epidermidis* dengan zona inhibisi rata-rata terbesar pada konsentrasi 3%, yaitu 7,9 mm.

Kata kunci: Bessential Oil, Sabun Buatan Tangan, *Staphylococcus epidermidis*

I. INTRODUCTION

Skin is the largest human organ located on the surface of the body. Therefore, the skin has a great risk of being exposed to adverse influences from the environment and tends to contain microorganisms that are temporary.⁷ If there is an abnormality in the form of an incomplete skin barrier on the skin, for example due to microtrauma, it will allow the emergence of skin diseases, one of which is skin infection. One of the main causes is *Staphylococcus epidermidis* bacteria.

The chemical content of basil leaf essential oil (*Ocimum basilicum* L.), namely linalool, eugenol, methyl eugenol, and flavonoids have a major role in antibacterial activity.³ Essential oil of basil leaves (*Ocimum basilicum* L.) is volatile when used directly, so a dosage form is needed for ease of application and convenience when used.⁴

Dirty skin if not cleaned will cause the skin to be easily infected with bacteria. The use of antibacterial soap is used as a solution because it is believed to have the ability to clean the skin and can treat and prevent diseases caused by bacteria.⁹

II. METHODS

A. PREPARATION OF ESSENTIAL OIL OF BASIL LEAVES (*Ocimum basilicum* L.)

The essential oil of basil leaves (*Ocimum basilicum* L.) used was purchased in one of the commercial markets, with the trademark Basil from PT Nusaroma Essential Indonesia which has a certificate of analysis.

B. FORMULATION OF BASIL LEAF ESSENTIAL OIL HANDMADE SOAP

TABLE 1. HANDMADE SOAP FORMULATION

Ingredients	Unit	F0	F1	F2	F3	Usability
essential oil of basil leaves (<i>Ocimum basilicum</i> L.)	g	0%	1%	2%	3%	Active Substance
Colorant	g	1	1	1	1	Coloring
Base	Unit	F0	F1	F2	F3	Usability
VCO	g	95	95	95	95	oil
Olive oil	g	225	225	225	225	oil
NaOH	g	45	45	45	45	Alkaline
Aquadest	g	110	110	110	110	Solvent
Volume	g	ad	ad	ad	ad	
Total		475	475	475	475	

C. WORK PROCEDURE FOR MAKING HANDMADE SOAP

The procedure for making handmade soap begins with mixing the oil part, namely VCO and olive oil with alkali, namely NaOH which has been dissolved in distilled water at room temperature. When the addition of NaOH is done slowly little by little, the consistency will then turn thick and sticky which indicates the formation of soap stock. Then add the essential oil of basil leaves. Stir until homogeneous then divide into several parts in different containers add fragrance oil and color variations. Stir again until the consistency becomes thicker, the mixing and stirring process is carried out using a hand blender. Pour into the handmade soap mold and shape as interesting as possible, then let stand for 24 hours. Cut the handmade soap to the desired size using a soap cutter. Let stand again for 3 weeks (aging process), then evaluate the physical stability and activity test against the growth inhibition of *Staphylococcus epidermidis* bacteria.²

D. EVALUATION OF HANDMADE SOAP

Organoleptic test

Testing is done by visually observing the physical appearance of handmade soap preparations which include shape, color, and smell.

Acidity test (pH)

Measurement of the pH of a solution is carried out using a pH meter (Orion Star A211) by dipping it into a handmade soap preparation solution. Before pH measurement, calibration is carried out on the pH meter first using a pH 4 buffer solution, pH 7 buffer, and pH 10 buffer. The number listed on the pH meter indicates the acidity scale of the solution.⁵

Foam stability test

A sample of 1 gram of the preparation is dissolved in 10 ml of distilled water, then shaken by inverting the test tube. Calculate the height of the foam produced, then let stand for 5 minutes and measure the foam height again. Foam stability is calculated using the formula:

$$\% \text{ lost foam} = \frac{\text{initial foam height} - \text{final foam height}}{\text{initial foam height}} \times 100\%$$

$$\% \text{ foam stability} = 100\% - \% \text{ lost foam}$$

Moisture content test

Petri dish is put into the oven at 105 for 30 minutes then weighed on an analytical balance and record the results. Weigh 5 grams of handmade soap sample into a Petri dish. Heat the sample in the oven at 105 for 1 hour and then let it cool to room temperature, weigh it using an analytical balance and record the results. Measurement of weight deficiency after drying at 105, using the formula:¹⁰

$$\text{moisture content} = \frac{b_0 - b_1}{b_0} \times 100\%$$

Description: b_0 = weight of test and petri dish before heating (g)
 b_1 = weight of test and petri dish after heating (g)

Free alkali/free fatty acid test

The test is done by heating 5 grams of sample in 200 ml of ethanol. When the solution is almost boiling, enter 0.5 ml of 1% phenoflatein indicator. If the nature of the

solution is acidic, then no color appears so that it continues with titration using KOH standard solution until a pink color appears. If the solution is alkaline, a red color will appear so continue with HCl standard solution until the red color disappears, record the test results.¹⁰

The formula :

$$\text{free alkali} = \frac{40 \times V \times N}{b} \times 100\%$$

Description : N = normality of HCl used
 V = volume of HCl used (ml)
 b = sample weight (mg)
40 = equivalent weight of

NaOH

$$\text{free fatty acid} = \frac{282 \times V \times N}{b} \times 100\%$$

Description : N = normality of NaOH used
 V = volume of NaOH used (ml)
 b = sample weight (mg)
282 = equivalent weight of oleic acid ($C_{18}H_{34}O_2$)

E. STAPHYLOCOCCUS EPIDERMIDIS ATCC 12228 ANTIBACTERIAL ACTIVITY TEST

Antibacterial activity testing of essential oils

Take a suspension of test bacteria then streak into MHA media bent in a zigzag manner from top to bottom until evenly distributed using a sterile cotton stick. Each paper disc was placed on the agar media and then pipette essential oil as much as 20 μ l with a concentration of 0%, 1%, 2%, and 3%, essential oil was diluted using DMSO. Negative control used DMSO and positive control tetracycline. Then all Petri dishes that had been treated were put in an incubator with a temperature of 37°C and incubated for 24 hours. This test was carried out three times (triplo). The results of the inhibition zone formed were measured using a caliper, observed and recorded.

Antibacterial activity testing of handmade soap preparations

Take a suspension of test bacteria then streak into MHA media bent in a zigzag manner from top to bottom until evenly distributed using a sterile cotton stick. Make a well with a diameter of 6 mm using a cork borer tool, then the handmade soap preparation solution is pipetted as much as 50 µl with formulas

F0, F1, F2, F3 and tetracycline positive control. Then all Petri dishes that have been treated are placed in an incubator with a temperature of 37°C and incubated for 24 hours. This test was carried out three times (triplo). The results of the inhibition zone formed were measured using a caliper, observed and recorded.

III. RESULTS AND DISCUSSION

A. ACTIVE INGREDIENT IDENTIFICATION RESULTS

TABLE 2. DENSITY OF ESSENTIAL OIL OF BASIL LEAVES (*OCIMUM BASILICUM* L.)

Sample	Organoleptic			density
	Color	fragrance	consistency	
Basil leaf essential oil (<i>Ocimum basilicum</i> L.) brand from PT Nusaroma Essential Indonesia	yellow	Typical of basil leaves	liquid	0,92 g/ml

The density standard of basil essential oil (*Ocimum basilicum* L.) according to the Essential Oil Association (EOA) is in the range of 0.952-0.973 g/ml.¹

B. EVALUATION RESULTS OF BASIL LEAF ESSENTIAL OIL HANDMADE SOAP PREPARATION

Organoleptic test results

TABLE 3. ORGANOLEPTIC TEST RESULTS OF HANDMADE SOAP

No.	Sample	Organoleptic Test		
		Form	Color	Smell
1.	F0	Solid	pink, blue	odorless
2.	F1	Solid	pink, blue	Weak characteristic odor
3.	F2	Solid	pink, blue (more faded colors)	Weak characteristic odor
4.	F3	Solid	pink, blue (more faded colors)	Strong characteristic odor

The results of color observations, F0 and F1 have a deep color, F2 and F3 have a faded color which is influenced by the addition of greater concentrations of essential oils so that the resulting color is increasingly faded. The results of aroma observations, F0 has no aroma, F1 and F2 have a weak characteristic aroma of essential oils, and F3 has a strong characteristic aroma of essential oils. The difference in aroma in the preparation is influenced by differences in the concentration of essential oil added to each preparation, the higher the concentration of essential oil in the preparation, the stronger the distinctive aroma produced. From the observations, it was found that the four dosage formulations were organoleptically

stable and good, which was characterized by the formation of an evenly distributed solid consistency in the preparation, the absence of bleaching on the surface of the preparation due to excessive administration of alkali, and an even formation which indicated that there was no air trapped during the preparation making process.

pH test results

The degree of acidity (pH) is a very important parameter in a product in order to ensure that the preparation does not cause irritation to the skin. The pH of soap preparations that meet skin criteria is in the range of 8-11.¹²

TABLE 4. RESULTS OF HANDMADE SOAP pH TEST

Observations	F0	F1	F2	F3	Category
1.	9,74	10,27	10,24	9,13	Eligible
2.	10,22	10,29	9,22	10,05	Eligible
3.	10,00	9,19	9,26	9,15	Eligible
Mean $\pm$ SD	9,97 $\pm$ 0,2	9,92 $\pm$ 0,6	9,57 $\pm$ 0,6	9,44 $\pm$ 0,5	

The four formulas have a pH that meets the criteria with an interval of 8-11.¹² pH measurement results show that the preparation meets the requirements for the use of body soap.

Foam stability test results

The criteria for good foam stability is if within 5 minutes a range of foam stability is obtained in the range of 60-70%.¹² The following table shows the results of the foam stability test of basil leaf essential oil handmade soap:

TABLE 5. HANDMADE SOAP FOAM STABILITY TEST RESULTS

No.	Sample	Foam Stability	Category
1.	F0	67,6%	Eligible
2.	F1	70,0%	Eligible
3.	F2	67,5%	Eligible
4.	F3	69,0%	Eligible

The four formulas have good foam stability according to the criteria in the range of 60-70%.¹²

Moisture content test results

The Indonesian National Standard (SNI) sets the maximum level for testing water content at 15%. The following table shows the results of the water content test of basil leaf essential oil (*Ocimum basilicum* L.) handmade soap:

TABLE 6. HANDMADE SOAP MOISTURE CONTENT TEST RESULTS

No.	Sample	Moisture Contents	Category
1.	F0	7,0%	Eligible
2.	F1	11,8%	Eligible
3.	F2	10,8%	Eligible
4.	F3	10,3%	Eligible

The level of hardness and durability of soap can be influenced by the content of water content contained in the soap, where the

higher the water content in the soap, the level of hardness or durability of the soap can decrease, causing the soap to shrink easily.⁸

The four formulas meet the criteria with good water content according to SNI 2016.

Free fatty acid test results

The Indonesian National Standard (SNI) sets the maximum level for testing free fatty acid levels at 2.5%. The following table shows the test results of free fatty acid content of handmade soap essential oil of basil leaves (*Ocimum basilicum* L.):

TABLE 7. TEST RESULTS OF HANDMADE SOAP FREE FATTY ACID CONTENT

No.	Sample	Free Fatty Acid Contents	Category
1.	F0	1,07%	Eligible
2.	F1	0,85%	Eligible
3.	F2	0,90%	Eligible
4.	F3	0,71%	Eligible

The four formulas meet the criteria with maximum fatty acid levels that meet the 2016 SNI criteria. This means that the four handmade soap formulas do not cause irritation and have unwanted free fatty acid levels in the cleaning process but still meet the SNI criteria.

C. *STAPHYLOCOCCUS EPIDERMIDIS* ATCC 12228 ANTIBACTERIAL ACTIVITY TEST RESULTS

Observation of antibacterial tests on the growth of *Staphylococcus epidermidis* bacteria from basil leaf essential oil with concentrations of 1%, 2%, 3%, positive control of tetracycline antibiotics, and negative control of DMSO. Observation of antibacterial tests on the growth of *Staphylococcus epidermidis* bacteria from handmade soap of basil leaf essential oil

from F0, F1, F2, F3, and positive control of Dettol soap. Determination of the inhibition category is based on the criteria for the inhibition area, namely ≥ 20 mm is a very strong category, 11-19 mm is a strong category, 6-10 mm is a medium category.

and ≤ 5 mm is a weak category.¹³ The following is a table of antibacterial test results from basil leaf essential oil and handmade soap preparations:

TABLE 8. ESSENTIAL OIL ANTIBACTERIAL TEST RESULTS

No.	Sample	zone of inhibition (mm)			Mean of zone of inhibition (mm) $\pm$ SD	Category
		P1	P2	P3		
1.	0%	0	0	0	$0 \pm 0,00$	Weak
2.	1%	3	3,5	4	$3,5 \pm 0,50$	Weak
3.	2%	4	4,5	3,5	$4 \pm 0,50$	Weak
4.	3%	5	4,5	3,5	$4,3 \pm 0,76$	Weak
5.	K+	10,5	12	10,5	$11 \pm 0,87$	Strong

TABLE 9. ANTIBACTERIAL TEST RESULTS OF HANDMADE SOAP

No.	Sample	Zone of Inhibition (mm)			Mean of zone of inhibition (mm) $\pm$ SD	Category
		P1	P2	P3		
1.	F0	5,75	5,75	7	$6,1 \pm 0,72$	Medium
2.	F1	6	5	9,5	$6,8 \pm 2,36$	Medium
3.	F2	7	6,25	9,5	$7,4 \pm 1,70$	Medium
4.	F3	7	6,25	10,5	$7,9 \pm 2,27$	Medium
5.	K+	2	2	3	$2,3 \pm 0,58$	Weak

Based on the results of measuring the diameter of the inhibition zone, it shows that basil leaf essential oil and basil leaf essential oil handmade soap preparations have weak to moderate inhibition against *Staphylococcus epidermidis* bacteria. The concentration of essential oil of basil leaves (*Ocimum basilicum* L.) Basil brand from PT Nusaroma Essential Indonesia at a concentration of 1%, 2%, and 3% has a weak inhibition category. Meanwhile, after being formulated into a handmade soap dosage form, it has moderate category inhibition in each formulation. Determination of the inhibition category is based on the criteria for the inhibition area, namely ≥ 20 mm is a very strong category, 11-19 mm is a strong category, 6-10 mm is a medium category. and ≤ 5 mm is a weak category.¹³ Weak or less effective inhibition is produced because essential oils are more potent against gram-negative bacteria than gram-positive bacteria. This happens in relation to the permeability of the bacterial

cell wall which is influenced by the thickness of the peptidoglycan layer in the bacterial cell. Gram-positive bacteria have a peptidoglycan layer of 30 layers and a compact cell wall arrangement so that the permeability is low, causing essential oils to have difficulty penetrating the cell membrane.³ In addition, this may be caused by essential oils in the soap that evaporate during the aging period (6 weeks), thus reducing the antibacterial activity of the preparation.⁴

The diameter of the inhibition zone in basil leaf essential oil was the largest at a concentration of 3% at 4.3 mm and the smallest at a concentration of 1% at 3.5 mm. This shows that the higher the concentration of basil leaf essential oil, the more effective it is in inhibiting the growth of *Staphylococcus epidermidis* bacteria. The diameter of the inhibition zone of the basil leaf essential oil handmade soap preparation was the largest in F3 with a concentration of 3% essential oil and the smallest was in F0 with a concentration of 0% essential oil. This shows that the greater the concentration of basil leaf essential oil in the formulation proves the more effective the preparation is in inhibiting the growth of *Staphylococcus epidermidis* bacteria. The inhibition produced by antibacterial ingredients is higher when the concentration is high. However, it is better to know the effective

concentration of an antibacterial substance to test the antibacterial activity of the dilution method, so as to obtain the minimum inhibitory concentration of basil leaf essential oil with the Basil brand from PT Nusaroma Essential Indonesia.

IV. SUMMARY

1. Essential oil of basil leaves (*Ocimum basilicum* L.) of Basil brand from PT Nusaroma Essential Indonesia can be formulated into a handmade soap dosage form.
2. Basil leaf essential oil handmade soap from PT Nusaroma Essential Indonesia effectively inhibits the growth of *Staphylococcus epidermidis* bacteria. It was found that basil essential oil handmade soap can inhibit bacterial growth in F0, F1, F2, and F3. The most effective formulation that has the largest inhibition zone diameter against the growth of *Staphylococcus epidermidis* bacteria is the formulation with 3% essential oil concentration with an average inhibition zone diameter of 7.9 mm.

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