

## ANTIBACTERIAL ACTIVITY OF BASIL LEAVES EXTRACT TOWARDS BACTERIA *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS* IN DEODORANT SPRAY

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### ABSTRACT

Body odor is a significant problem and is often encountered in some individuals characterized by excessive odor. A deodorant spray made from basil leaf (*Ocimum Basilicum* L.) extract is a product used to treat body odor caused by *Staphylococcus Aureus* and *Staphylococcus epidermidis* bacteria. The purpose of this study is to assess the efficacy of deodorant spray and basil leaf extract on bacterial activity, as well as the optimal dose of deodorant spray for lowering the activity of these bacteria. Method the research was conducted experimentally, testing antibacterial activity using agar diffusion. The results of the deodorant spray preparation test showed that the preparation was homogeneous, organoleptically brown in color, had a distinctive smell, and had a liquid texture; the pH test results met the requirements for deodorant spray 4.5-6.5. The average diameter of the inhibition zones produced from each preparation were *Staphylococcus epidermidis* bacteria, positive control 7 mm, negative control 10.1 mm; formula 5% 12.3 mm; formula 10% 13.3 mm; formula 15% 12.7 mm maximum concentration in inhibiting bacteria is formula 10% by 13.3 mm, while the bacteria *Staphylococcus Aureus* positive control 13 mm, negative control 11.2 mm, 5% formula 13.1 mm; formula 10% 13.4 mm; 15% formula 13.9 mm maximum concentration in inhibiting bacteria is 15% formula 13.9 mm. Based on the inhibitory zone created by 10% basil leaf ethanol extract, *Staphylococcus epidermidis* bacteria had a 13.3 mm clean zone and *Staphylococcus aureus* bacteria had a 26.5 mm clean zone.

**Keywords:** Deodorant spray; *Ocimum basilicum*; *Staphylococcus Epidermidis*; *Staphylococcus Aureus*

## 1. INTRODUCTION

Excessive sweating for a person can cause problems, one of which is body odor (Oktaviana et al., 2019). Body odor (*bromhidrosis*) is one of the most common problems in the postpubertal population, which does characterized by excessive odor and unpleasant odor coming from the skin and is more often the result of secretions from apocrine glands than eccrine glands (Ramdani et al., 2020). One of the apocrine glands in the armpit (*axilla*) area contains some proteins and sugars that bacteria can break down to produce an ammonia-like odor (Setiawan & Suling, 2018). Bacteria that can cause body odor include *Staphylococcus Aureus*, *Staphylococcus Epidermis*, *Corynebacterium Acne* (diphtheroids), *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. *Staphylococcus* can convert certain amino acids into short-chain volatile fatty acids with a strong odor, namely isovaleric acid, which contributes to an underarm odor (Lailiyah et al., 2019).

The use of cosmetic preparation to eliminate body odor commonly used is deodorant, which is usually to control excessive sweating and armpit odor glands. One of the deodorants used in the form of a spray, the deodorant spray has advantages when compared to other forms of deodorant, namely, the deodorant spray delivery system does not involve contact between the deodorant and the user's skin, so it has high hygiene (Veranita et al., 2021).

Basil leaf (*Ocimum basilicum* L.) is one of the plants used in Indonesia as an ingredient in fresh vegetables and as a complementary vegetable. Basil plants in medicine can be used as antioxidants, antibacterials, drugs for skin infections, eliminate body odour, and help treat acne (Afianti & Murrukmihadi, 2015). The main content of basil leaves is an essential oil that can inhibit the growth of harmful bacteria such as *Escherichia coli*, *Staphylococcus Aureus*, *Staphylococcus epidermidis*, and *Staphylococcus Typhimurium* (Al Abbasy et al., 2015; Gürkan & Adiloğlu, 2021). Basil also contains active compounds such as alkaloids, saponins, flavonoids, triterpenoids, steroids, tannins, and phenols. Some of these chemical compounds can inhibit the growth of *Escherichia Coli*, *Staphylococcus Aureus*, and *Klebsiella Pneumonia* bacteria such as alkaloids, essential oils, and phenols. This inhibition is referred to as bacteriostatic or bactericidal (Angelina et al., 2015).

Judging from the potential content of Basil Leaves (*Ocimum basilicum* L.), which is very good at inhibiting the growth of some bacteria, who made basil leaf extract in gel preparations to inhibit bacterial growth. In their research stated that essential oil from basil leaves could inhibit the growth of several bacteria including bacteria that cause body odor, so researchers are interested in examining the antibacterial activity of basil leaves. Ethanol extract against bacteria that cause body odor (*Staphylococcus Aureus* and *Staphylococcus Epidermidis*) and to see the inhibitory power provided by Basil Leaf Extract, in this case, making deodorant spray formula preparations which are still rarely studied against bacteria that cause body odor.

## 2. METHODS

This research includes manufacturing deodorant spray preparations using basil leaf extract with concentrations of 5%, 10%, and 15%. Evaluation of the physical quality of preparations such as homogeneity tests, organoleptic tests, pH tests, and tests for minimum inhibitory levels. The tools and materials used in this study were a 100 ml beaker glass, pH meter, electric scale, blender, petri dish, spray bottle, closed container or largemouth bottle, rotatory evaporator, glass stirrer, measuring cup, incubator, wire loop, water bath. The manufacture of deodorant sprays includes basil leaf extract, aluminium chlorohydrate, propylene glycol, equates, deodorant perfumes on the market, aqua dest, Nutrient agar, cultures of *Staphylococcus Aureus* and *Staphylococcus epidermidis*, and Hockey sticks.

### 2.1. Simplicia Making

Basil plants that have fresh leaves are purchased at the Sukaramai market in Medan. Wet sorting is done on the basil leaves and then dried by spreading them on paper and placing it in a room that is well-ventilated and protected from direct sunlight. Dry sorting was done on the basil leaves; the basil leaves were dry and not rotten and did not contain any fungus.

### 2.2. Basil Leaf Extract process (*Ocimum Basilicum* L.)

The dried basil leaves were mashed using a blender. Weighed 500 grams of basil leaf powder. Soaked 3.75 liters of 96% ethanol in ethanol, left for five days, then filtered. Soaked again with 1.25 liters of 96% ethanol, left for two days. Then filtered again. Put in a rotatory evaporator for 2 hours to get the thick extract (Ariani et al., 2020; Iskandar & Mustarichie, 2019).

### 2.3. Deodorant Spray Preparation Formula

Basil leaf extract deodorant spray formula was made with three concentrations of 5%, 10%, and 15% (Table 1).

**Table 1.** Formula Deodorant Spray Basil Leaf Extract

Components	Concentration (%)				
	Positive Control	Negative Control	Formula 1	Formula 2	Formula 3
Basil Leaf Extract	-	-	5	10	15
Aluminum chlorohydrate	-	20	20	20	20
Propylene glycol	-	5	5	5	5
Aquades	-	Add 100 ml	Add 100 ml	Add 100 ml	Add 100 ml
Perfume	-	q.s	q.s	q.s	q.s

Where:

Positive Control: Posh deodorant 10%

Negative Control: Without using basil leaf extract

Formula 1: Deodorant spray preparation containing 5% ethanol extract of basil leaves

Formula 2: Deodorant spray preparation containing 10% ethanol extract of basil leaves

Formula 3: Deodorant spray preparation containing 15% ethanol extract of basil leaves

## 2.4. Evaluation of Preparation Characteristics

### 2.4.1. Organoleptic Test

Organoleptic tests on deodorant preparations were carried out by observing the preparation's shape, color and odour (Ginting et al., 2021).

### 2.4.2. Homogeneity Testing

The homogeneity test was carried out by applying a small amount of deodorant to the glass object and then observing whether there were visible coarse particles (Ginting et al., 2021).

### 2.4.3. pH test

The pH of the preparation is determined using a pH meter. The pH meter is dipped directly into the deodorant preparation until the number is constant. The number listed on the pH meter is the pH value of the preparation. The pH of the skin is 4.5 – 6.8 (Tuslinah et al., 2021).

### 2.4.4. Minimum Inhibitory Test (MIC)

Determines the ability of an antibacterial or fungal substance in solution, the concentration of an antibacterial or fungal substance against body fluids and tissues, and the sensitivity of a bacterium or fungus to the exposure concentrations. The MIC test is carried out in-vitro. The diffusion method can determine the sensitivity of bacteria or fungi to antibacterials or fungi (Mulyadi et al., 2017).

## 2.5. Testing the Deodorant Spray's Inhibition Against Bacterial Activity

### 2.5.1. Bacterial Inoculation Test

Based on (Gerung et al., 2021), the method of making the media was to take Nutrient agar (NA) as much as 0.46 g dissolved in 20 ml of distilled water using an Erlenmeyer, then homogenized with a stirring rod over a water bath until it boiled. A total of 5 ml was poured into a sterile test tube and covered with aluminium foil. The media was sterilized in an autoclave at 121 °C for 15 minutes, then left at room temperature for ± 30 minutes until the media solidified at a slope of 30 °C. An oblique agar medium was used for bacterial inoculum.

### 2.5.2. Preparation of Nutrient Agar Media (NA)

The manufacture of NA media was carried out by weighing 7.25 grams of NA. NA and aquades in an Erlenmeyer flask were heated using a hotplate for ±10 minutes until the NA dissolved. Then, add 250 ml of distilled water into an Erlenmeyer flask. The homogenized media was sterilized in an autoclave for 15 minutes at 121 °C (Kindangen et al., 2018). After that, wait for the media to cool slightly at around 40-45 °C. The cooled NA media will then be poured into a 20 mL petri dish. The NA media poured into a petri dish is allowed to solidify (Fachriyah et al., 2020).

### 2.5.3. Antibacterial Activity Test

Antibacterial activity of spray deodorant preparations of basil leaf extract using the agar diffusion method with the filter paper disc technique that has been sterilized in an autoclave, put 1 ml of the bacterial test suspension, test 20 cc of liquid Nutrient Agar which has been sterilized into an autoclave, homogenize and allowed to harden. Take the disc paper using tweezers which previously exceeded the Bunsen fire. Dip each paper disc into the extract whose concentration has been determined, namely 5%, 10%, 15%, positive control, and negative control. Put the Nutrient Agar medium in a petri dish and lightly press it. Then the Petri disc is turned over and wrapped with parchment paper, then incubated at 37 °C for 18-24 hours. After that, the zone of inhibition around the disc was measured using a caliper and marked with a clear zone around the disc (Abu & Tandah, 2015).

### 2.5.4. Data Analysis

The data analysis method used was descriptive quantitative analysis which showed the size of the inhibition zone for the growth of *Staphylococcus Aureus* and *Staphylococcus Epidermidis* bacteria around the paper discs. Quantitative analysis was used to process the data generated from the test of the extract's effectiveness as an antibacterial. The data obtained will be analyzed by statistical analysis of variance (ANOVA) with the SPSS 20 program.

## 3. RESULTS AND DISCUSSION

### 3.1. Physical Properties of Deodorant Spray Extract

The formulation of the formulated deodorant spray extract was evaluated organoleptically or from its sensory characteristics, including color, shape, and texture. Figure 1 depicts the organoleptic properties of the gel base and the gel containing 5%, 10%, and 15% deodorant spray extract. The one with the highest concentration (15%) produces the darkest color. All spray preparations are good and do not feel sticky on the skin.



Figure 1. (a) 5% Spray Extract, (b) 10% Spray Extract and (c) 15% Spray Extract

Organoleptic examination, pH, and homogeneity of the preparations showed that the preparation of deodorant spray F0 – F3 could be seen in Table 2 has a liquid texture, varied colors, and a distinctive smell at a concentration of 5% basil leaf extract, produces a brick red color with a similar texture. Liquid has a characteristic odor; at a concentration of 10%, it produces a light brown color with a liquid texture and a characteristic odor. At a concentration of 15%, it produces a dark brown color with a liquid texture and a distinctive smell. For pH, the negative control without extract got a pH of 4.4 and did not meet the pH requirements on the skin, while formulas 1,2 and 3 got a pH of 4.5, 4.6 4.9. Underarm skin has a pH that tends to be more acidic. Maybe due to the high intake of acidic foods where daily consumption reaches 80-95%, pH measurement using a digital pH meter is very sensitive to changes in temperature at the time of measurement. This shows that the greater the concentration of the extract, the higher the pH results on the skin because the basil leaf extract contains a lot of alkaline alkaloids (Zahara, 2018). The homogeneity

test of the preparations starting from F0-F3 showed homogeneous preparations characterized by the absence of coarse grains when the preparation was smeared on a glass object. Homogeneous preparations will give good results because the medicinal ingredients are evenly dispersed in the essential ingredients, so each part of the preparation contains the same amount of medicinal ingredients. If the drug ingredients are not evenly dispersed in the base material, the drug will not achieve the desired therapeutic effect (Sulastri et al., 2016).

**Table 2.** Organoleptic Test Results Data Preparations

Formula deodorant	Organoleptic	Ph Average	Homogeneity
F0	White	4.4	Homogenous
F1	Brick Red	4.5	Homogenous
F2	Light Brown	4.6	Homogenous
F3	Dark Brown	4.9	Homogenous

Where:

F0: Blank (Without Basil Leaf Extract)

F1: Basil Leaf Extract 5%

F2: Basil Leaf Extract 10%

F3: Basil Leaf Extract 15%

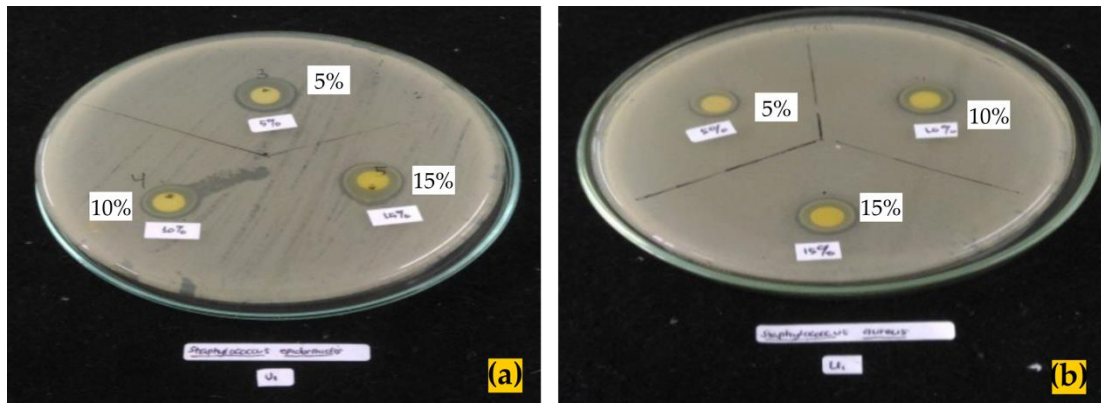
### 3.2. Bacterial Inhibitory Test of *Staphylococcus epidermidis* and *Staphylococcus Aureus*

The inhibition zone indicated the presence of bacterial activity that was inhibited by the concentration of ethanol extract of basil leaves in deodorant spray preparations, in the form of an inhibition zone whose area looked clearer.

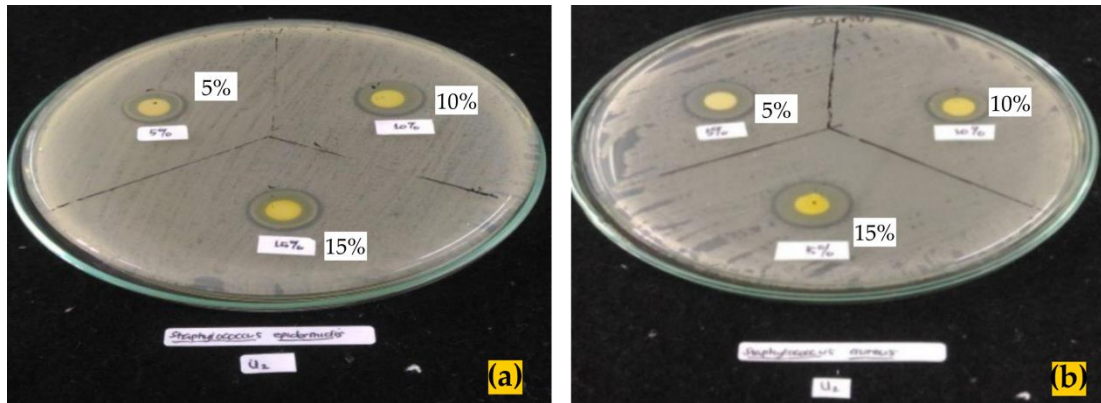
#### 3.2.1. Results of Inhibition Zone Test of *Staphylococcus epidermidis* and *Staphylococcus Aureus*

The difference in the diameter of the inhibition zone shown in [Figure 2](#), [Figure 3](#), and [Figure 4](#) showed that the basil leaf extract effectively inhibited the growth of *Staphylococcus Aureus* and *Staphylococcus Epidermis*. This can be seen in the difference in the inhibition zones produced in Petri dishes in each experiment. [Table 3](#) shows that the average inhibition zones of *Staphylococcus epidermidis* and *Staphylococcus Aureus* in positive controls were 13 mm and 7 mm, negative controls were 10.1 mm and 11.2 mm, 5% basil leaves extract deodorant spray was 12.3 mm and 13.1 mm, 10% are 13.3 mm and 13.4 mm, and 15% are 12.7 mm and 13.8 mm, respectively. [Table 3](#) shows that in *Staphylococcus epidermidis*, the largest inhibition zone occurred at a concentration of 10% with an inhibitory power of 13.3 mm; in *Staphylococcus Aureus*, the largest inhibition zone occurred at a concentration of 15% with an inhibitory power of 13.9 mm. The ANOVA table tests the significance and concludes after the data is proven homogeneous. Does the average data have a significant difference, or is it the same as looking at the F table? In *Staphylococcus epidermidis* bacteria obtained F count < F table  $0.983 < 3.48$ , it can be concluded that the data are not significantly different between the 5 test groups (Ho is accepted, which means it is not significant). For *Staphylococcus Aureus* bacteria, the F count < F table is  $1.029 < 3.48$ . It can be concluded that the data does not show a significant difference between the 5 test groups (Ho is accepted, but the meaning is not significant). Inhibition test of basil leaf extract (*Ocimum Basilicum* L.) against *Staphylococcus epidermidis* and *Staphylococcus Aureus* showed that the basil leaf extract had an inhibitory effect on the growth of *Staphylococcus epidermidis* and *Staphylococcus Aureus*. This is evidenced by the diameter of the clear zone around the disc containing basil leaf extract. The content of secondary metabolites is an important factor through its mechanism against bacteria. The results of the phytochemical screening test showed that basil leaves (*Ocimum Basilicum* L.) were positive for chemical compounds, namely phenols, tannins, flavonoids, terpenoids, saponins, alkaloid (Hidayati & Bahar, 2018; Kumalasari & Andiarna, 2020).

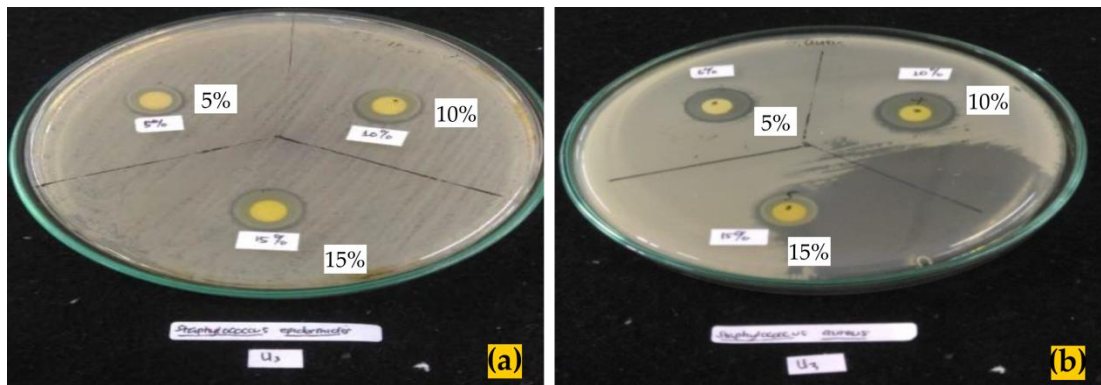




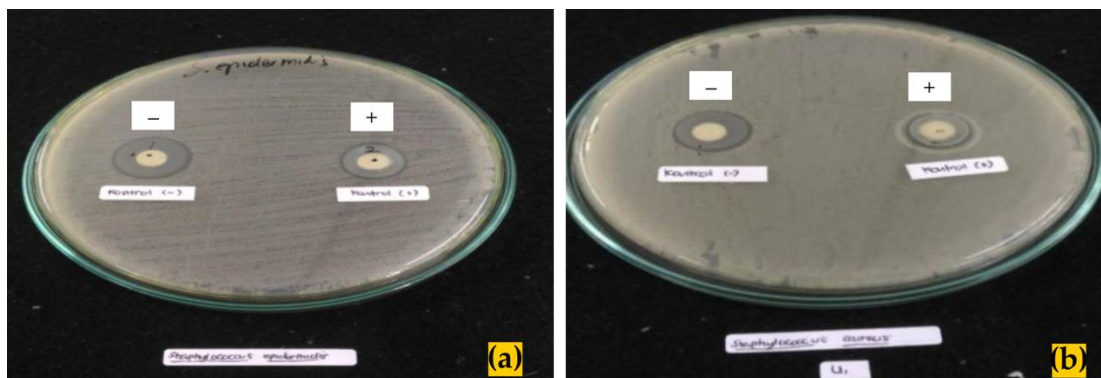
**Figure 2.** First Experiment Inhibition Zone of 5%, 10%, 15% Basil Leaves Extract, (a) *Staphylococcus epidermidis* and (b) *Staphylococcus Aureus*



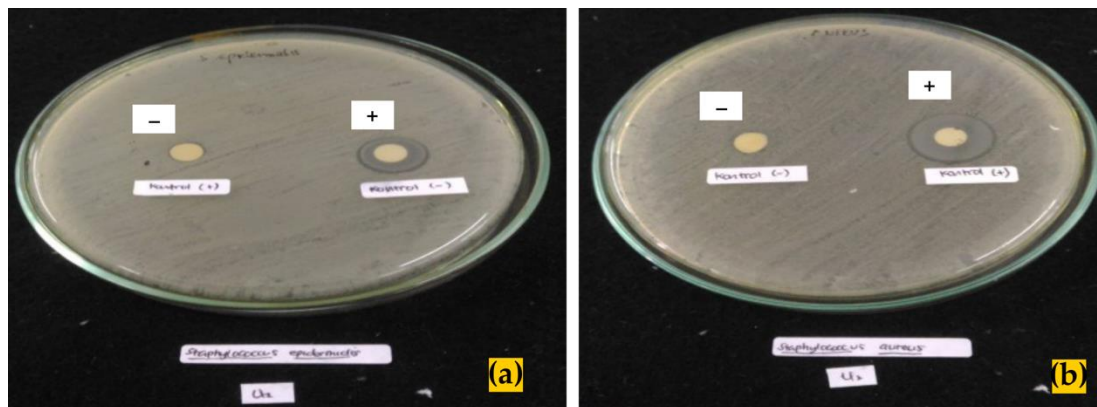
**Figure 3.** Second Experiment Inhibition Zone of 5%, 10%, 15% Basil Leaves Extract, (a) *Staphylococcus epidermidis* and (b) *Staphylococcus Aureus*



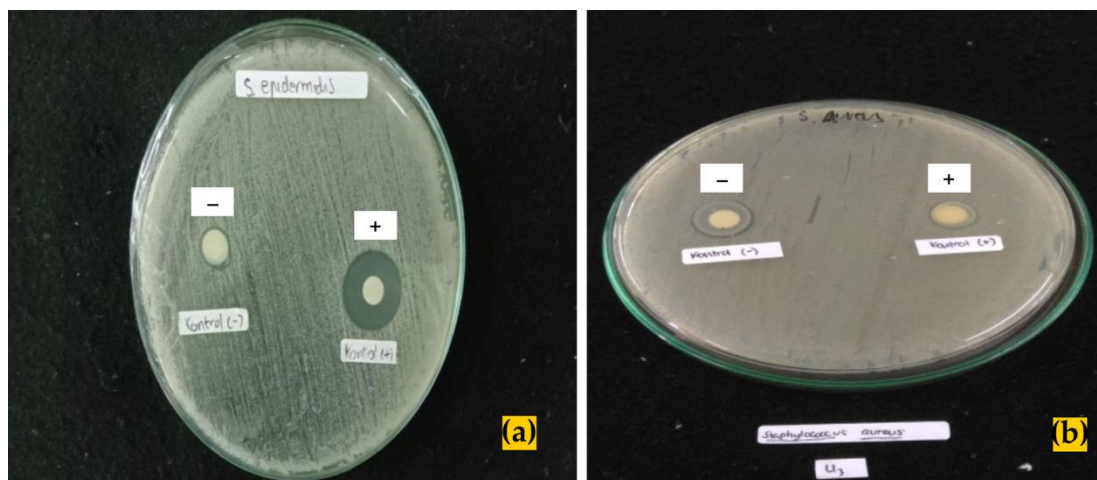
**Figure 4.** Third Experiment Inhibition Zone of 5%, 10%, 15% Basil Leaves Extract, (a) *Staphylococcus epidermidis* and (b) *Staphylococcus Aureus*



**Figure 5.** First Experiment Inhibition Zone of Positive (+) and Negative (-) Control, (a) *Staphylococcus epidermidis* and (b) *Staphylococcus Aureus*



**Figure 6.** Second Experiment Inhibition Zone of Positive (+) and Negative (-) Control, (a) *Staphylococcus epidermidis* and (b) *Staphylococcus Aureus*



**Figure 7.** Third Experiment Inhibition Zone of Positive (+) and Negative (-) Control, (a) *Staphylococcus epidermidis* and (b) *Staphylococcus Aureus*

**Table 3.** Results of Inhibitory Test of *Staphylococcus Aureus* and Bacteria Using Deodorant Spray Preparation of Basil Leaf Ethanol Extract (*Ocimum Basilicum L.*)

Bacteria	Treatment	Clear Zone Diameter (mm)			Average Clear Zone Diameter (mm)	Antimicrobial Zone Index
		U1	U2	U3		
<i>Staphylococcus epidermidis</i>	Positive control	6.5	12.8	1.7	7	0.16
	Negative control	13.1	16.4	0.9	10.13	0.68
	Formula I 5%	13.5	12.7	10.7	12.3	1.05
	Formula II 10%	13.2	13.8	12.9	13.3	1.21
	Formula III 15%	11.5	13.4	13.1	12.66	1.11
<i>Staphylococcus Aureus</i>	Positive control	11.8	17.3	9.9	13	1.16
	Negative control	14.3	6.6	12.9	11.26	0.86
	Formula I 5%	10.6	14.4	14.5	13.16	1.18
	Formula II 10%	11.7	12.3	16.4	13.4	1.23
	Formula III 15%	11.8	14.9	15.2	13.9	3.41

Where:

- Positive control: Posh brand deodorant
- Negative control: Without using basil leaf extract
- Formula I: Basil leaf extract 5%
- Formula II: Basil leaf extract 10%
- Formula III: Basil leaf extract 15%

#### 4. CONCLUSION

Deodorant spray preparations of ethanol extract from basil leaves (*Ocimum Basilicum L.*) with concentrations of 5%, 10%, and 15% can inhibit the growth of *Staphylococcus epidermidis* and *Staphylococcus Aureus* bacteria with a concentration of 10% to obtain a clear zone area of

13.3 mm in *Staphylococcus epidermidis* and *Staphylococcus epidermidis* bacteria. 15% got a clear zone of 13.9 mm in *Staphylococcus Aureus* bacteria. The antibacterial efficacy of the ethanol extract of basil leaves against gram-negative bacteria requires more study.

## 5. ACKNOWLEDGMENT

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## 6. CONFLICT OF INTEREST

All authors declare no conflict of interest.

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