UJI AKTIVITAS ANTIBAKTERI MINYAK ATSIRI BUNGA LILI (*Lilium auratum*) TERHADAP *Propionibacterium acnes*

The Antibacterial Activity Test of Lily Flower Essential Oil (*Lilium auratum*) against *Propionibacterium acnes*

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Abstrak

Propionibacterium acnes merupakan bakteri utama yang terlibat dalam patogenesis *acne vulgaris* atau jerawat. Peningkatan resistensi *Propionibacterium acnes* terhadap antibiotik seperti klindamisin mendorong pencarian alternatif obat antibakteri dari bahan alam yang lebih aman dan murah. Salah satu tanaman lili dikenal sebagai salah satu yang terbaik di dunia karena keindahan dan keragaman jenisnya serta memiliki potensi sebagai antibakteri. Minyak atsiri bunga lili mengandung senyawa benzaldehid, linalol, simen, borneol, dan osimen yang dapat merusak dinding sel bakteri. Penelitian ini bertujuan mengevaluasi potensi minyak atsiri bunga lili dalam menghambat pertumbuhan bakteri *Propionibacterium acnes* dengan berbagai konsentrasi (12 µL, 25 µL, 50 µL) menggunakan metode sumuran. Hasil penelitian menunjukan bahwa pengukuran zona hambat minyak atsiri bunga lili (*Lilium auratum*) terhadap bakteri *Propionibacterium acnes* menunjukkan hasil sebagai berikut: pada konsentrasi 12 µl, rata-rata zona hambat sebesar 14,54 ± 0,51 mm (aktivitas kuat); pada konsentrasi 25 µl, rata-rata zona hambat sebesar 15,93 ± 0,24 mm (aktivitas kuat); dan pada konsentrasi 50 µl, rata-rata zona hambat sebesar 20,17 ± 0,66 mm (aktivitas sangat kuat). Dengan itu, penelitian ini dapat disimpulkan bahwa minyak atsiri bunga lili dapat menghambat pertumbuhan bakteri *Propionibacterium acnes* secara *in vitro*.

Kata Kunci:
Antibakteri, Bunga lili, Minyak atsiri, *Propionibacterium acnes*

Keywords:
Antibacterial, Lily Flower, Essensial oil, Propionobacterium acnes

Abstract

Propionibacterium acnes is the main bacterium involved in the pathogenesis of acne vulgaris. The increasing resistance of Propionibacterium acnes to antibiotics such as clindamycin drives the search for alternative antibacterial drugs from safer and cheaper natural sources. One of the lilies is known as one of the best in the world due to its beauty and variety and has potential as an antibacterial agent. Lily essential oil contains compounds such as benzaldehyde, linalool, cymene, borneol, and ocimene, which can damage bacterial cell walls. This study aims to evaluate the potential of lily essential oil in inhibiting the growth of Propionibacterium acnes bacteria at various concentrations (12 µL, 25 µL, 50 µL) using the well diffusion method. The results showed that the measurement of the inhibition zone of lily (Lilium auratum) essential oil against Propionibacterium acnes bacteria was as follows: at a concentration of 12 µL, the average inhibition zone was 14.54 ± 0.51 mm (strong activity); at a concentration of 25 µL, the average inhibition zone was 15.93 ± 0.24 mm (strong activity); and at a concentration of 50 µL, the average inhibition zone was 20.17 ± 0.66 mm (powerful activity). Therefore, this study concludes that lily essential oil can inhibit the growth of Propionibacterium acnes bacteria in vitro.

INTODUCTION

Propionibacterium acnes is the main bacterium involved in the pathogenesis of acne vulgaris or acne. Acne vulgaris, commonly known as acne, is one of the most common skin problems experienced by people worldwide, especially among teenagers and young adults. This condition affects the pilosebaceous unit, which is a skin structure consisting of hair follicles and sebaceous glands that produce sebum, or skin oil.

When sebum production increases or there is a blockage in the hair follicles, it can trigger the formation of comedones, which are the initial stages of acne development (Saragih et al., 2016).

The antibiotics clindamycin, erythromycin, tetracycline, and doxycycline can be beneficial in reducing the growth of *Propionibacterium acnes*, which causes acne. In addition to antibiotics, retinoids and acetic acid are also capable of reducing the amount of *Propionibacterium acnes*. However, their long-term use is not recommended and overuse of these medications can exacerbate acne and lead to antibiotic resistance (Dermawan et al., 2015). The occurrence of antibiotic resistance is a condition where microorganisms require higher concentrations of antibiotics to inhibit their growth, compared to microorganisms that are still sensitive (Yenny, 2018).

The increasing resistance of *Propionibacterium acnes* to antibiotics such as clindamycin has driven the search for alternative antibacterial drugs from natural sources that are safer and more affordable. Antibiotic resistance poses a threat to the treatment of infectious diseases worldwide, making it necessary to develop alternative trerapies from natural materials, such as plants, which have antibacterial properties (Poejiani et al., 2021). Several studies in Indonesia indicate that various types of medicinal plants, including lily plants, have potential antibacterial agents.

The research conducted by Febrianti et.al (2021), reporting that Lily flower essential oil effectively inhibits *Salmonella typhi* bacteria with inhibition zones at concentrations of 10% (13.83 mm), 30% (13.25 mm), 50% (15.05 mm), and 70% (15.26 mm), it falls into the strong category. Other research conducted by Aryzki & Febrianti (2023), reporting that Lily flower essential oil effectively inhibits Pseudomonas aeruginosa bacteria with inhibition zones as follows: 3.025 mm (low activity) at 12 µl, 8.675 mm (moderate activity) at 25 µl, and 13.018 mm (strong activity) at 50 µl. Components of the essential oil, such as benzaldehyde, linalool, thymol, borneol, and osthmane, can damage bacterial cell walls and inhibit their growth. Therefore, the purpose of this research is to determine the potential of lily flower essential oil in inhibiting the growth of *Propionibacterium acnes*.

MATERIALS AND METHODS

Sample

The sample used in this research is lily flowers essential oil (*Lilium auratum*) obtained from PT. Lansida, Yogyakarta.

Tools and Materials

Tools used in this research include: measuring glassware, erlenmeyer flask, tweezers, needle holder, weighing scale, gloves, microscope, incubator, petri dish, laminar air flow, marker, scissors, Bunsen burner, slide, cover slip, aluminum foil, mask, test tube, caliper, autoclave, and tissue.

Materials used in this research include: essential oil from lily flowers, ethanol, *Propionibacterium acnes* bacteria, Nutrient Agar media, and Clindamycin.

Sterilization of Instruments

Heat Instruments and materials that are heat-sensitive will be sterilized using an autoclave at 121°C for 15 minutes, while heat-resistant instruments will undergo sterilization in an oven at 180°C for 1 hour. Microbiological testing will be conducted meticulously and under sterile conditions in a Laminar Air Flow (LAF) cabinet to ensure cleanliness and the accuracy of results (Febrianti & Ariani, 2020).

Bacterial Cultivation

Propionibacterium acnes bacteria used in this study were obtained from the Microbiology Laboratory of STIKES ISFI Banjarmasin. To cultivate pure bacterial cultures, these bacteria were inoculated onto Nutrient Agar solid media. This process was carried out using aseptic techniques, where a sterile needle containing *Propionibacterium acnes* bacteria was used to streak the Nutrient Agar solid media (Febrianti et al., 2021).

Media Preparation

The process of media preparation begins with weighing 2.4 grams of Nutrient Agar (NA) and adding 120 ml of distilled water. This mixture is then heated while stirring until all the Nutrient Agar (NA) powder dissolves completely and reaches a pH of 6.8. After that, the dissolved media is sterilized using an autoclave at 121°C for 15 minutes to ensure the elimination of any possible microorganisms (Febrianti & Ariani, 2020).

Antibacterial Activity Test

The antibacterial activity testing in this study was conducted using a modified Kirby-Bauer method with the well diffusion technique. Initially, 20 ml of Nutrient Agar (NA) was poured into each Petri dish. Once the agar solidified, 200 µl of bacterial suspension was evenly spread onto the agar surface. Subsequently, small wells were created in the solid agar using a sterile tool after bacterial inoculation. The number and positions of the wells were adjusted according to the predetermined experimental design. Each well was filled with various concentrations of lily flower essential oil (LFEO): 12 µL, 25 µL, and 50 µL, to test their antibacterial effects. The inoculated Nutrient Agar (NA) plates were then incubated at 37°C for 24 hours. After the incubation period, clear zones formed around the wells, indicating inhibition zones of bacterial growth, were observed. To ensure the accuracy of the results, each test was conducted in triplicate (Korompis et al., 2017).

Measurement of Inhibition Zones

The measurement of inhibition zones formed around wells is conducted by measuring the vertical and horizontal diameters in millimeters (mm) using a caliper (Warbung et al., 2013).

RESULTS AND DISCUSSION

The Content of Lily Flower Essensial Oil (LFEO)

The analysis by PT. Lansida Yogyakarta shows that the lily flower essential oil (LFEO) contains 12 major compounds, namely benzene, *1,4-diethoxy-2-methyltridecane, tetradecane, pentadecane, 7-hexadecene, hexadecane, 8-heptadecene, heptadecane, 9,17-octadecadienal, 1,6,10-dodecatriene, 7,11-dimethyl-3-methylene, and heneicosane*. Several of these compounds function as antibacterial agents, making lily flower essential oil (LFEO) potentially useful in natural medicine, thereby aiding in addressing various health issues related to bacteria and viruses (Febrianti et al., 2021).

The Results of Sterilization of Tools and Materials

The tools to be used in the antibacterial activity test process must be sterilized first to prevent contamination with other microorganisms that could affect the test results. One of the sterilization methods used is autoclaving, a device that uses high-pressure steam. The sterilization process with an autoclave is effective because high-pressure steam can penetrate microbial cells, causing protein denaturation. This denaturation results in damage to the structure and function of proteins within microbial cells, rendering the microbes unable to survive. Therefore, the autoclave ensures that all microorganisms on sterilized tools are killed, maintaining cleanliness and accuracy in the antibacterial activity test (Rizal et al., 2016).

The use of an oven in the sterilization process involves the application of significant high heat. This high temperature causes dehydration of bacterial cells and denaturation of proteins, thereby efficiently inhibiting bacterial biological functions and metabolic activities (Utami et al., 2016). Tools such as scalpels and cork borers are prepared for use with sterilization procedures using the Flaming method. This process involves soaking the tools in 70% alcohol solution for 5 minutes, followed by direct exposure to the flame from a Bunsen burner. The Flaming method was chosen because these tools cannot withstand direct heating, which could cause damage. This sterilization method is necessary to ensure the cleanliness and safety of the

tools in the experimental process (Febrianti et al., 2021).

The Results of Antibacterial Activity of Lily Flower Essensial Oil (LFEO)

This study employed the well method, where each well had a diameter of 6 mm. Within a single media plate, there were four wells of varying volumes: 12 µL, 25 µL, and 50 µL respectively. The media utilized in this research was Nutrient Agar (NA). The choice of NA was based on its complexity and high nutritional content, which are essential for bacterial growth. Additionally, the economic advantages of NA were also considered in selecting this medium (Febrianti et al., 2019).

This research utilizes sterile distilled water (aquadest steril) as a universal solvent for preparing high concentrations and equating solvent levels in experiments. Sterile distilled water is water that has undergone intensive distillation processes, ensuring it is free from minerals and other impurities. This purity makes it highly effective and precise as a solvent in various scientific and technical applications. Therefore, sterile distilled water can assist in achieving the necessary concentrations and solvent equilibrations in chemical and biological processes, thereby ensuring better and more accurate results (Aryzki & Febrianti, 2023).

In this study, the addition of concentrations of lily flower essential oil (LFEO) and distilled water (aquadest) was carried out by first mixing the essential oil with aquadest into the wells. In this procedure, the lily essential oil used was from Lilium auratum. Testing was conducted with three replicates to ensure result reliability. Petri dishes treated were then incubated for 24 hours at 37°C and measured using a digital caliper to determine the diameter of inhibition zones. The results of the inhibition zone diameters for lily flower essential oil (LFEO) with replicates can be seen in **Table 1**.

Table 1. The results of antibacterial activity of lily flower essential oil (LFEO) against *Propionibacterium acnes*

Treatment	Replication (mm)			Mean (mm) ± SD
	1	2	3	
LFEO 12 µL	15,12	14,18	14,32	14,54 ± 0,51
LFEO 25 µL	16,19	15,72	15,89	15,93 ± 0,24
LFEO 50 µL	18,29	18,34	18,95	18,53 ± 0,37
Clindamycin	20,83	19,51	20,19	20,17 ± 0,66

The measurement results of the inhibition zones of lily essential oil against *Propionibacterium acnes* bacteria were conducted using concentrations of 12 µl, 25 µl, and 50 µl, as shown in **Figure 1**. The inhibition zone measurement results of the antibacterial activity of lily essential oil are divided into four levels: weak, moderate, strong, and very strong. Bacterial activity is considered weak if the inhibition zone diameter is <5 mm, moderate between 5-10 mm, strong between 10-20 mm, and very strong if >20 mm (Emelda et al., 2021).

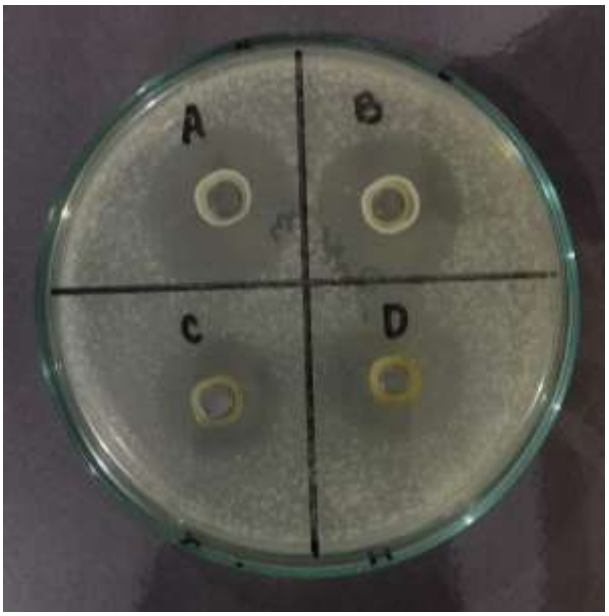

Figure 1. The results of the inhibitory zones of lily flower essential oil (LFEO) against *Propionibacterium acnes* Bacteria. A: Clindamycin, B: LFEO 50 µL, C: LFEO 25 µL, D: LFEO 12 µL.

The measurement results of the inhibition zone of lily essential oil showed that at a concentration of 12 µl, the average inhibition zone obtained was 14.54 ± 0.51 mm, which can be categorized as strong activity. At a concentration of 25 µl, the average inhibition zone obtained was 15.93 ± 0.24 mm, also categorized as strong activity. Meanwhile, at a concentration of 50 µl, the average inhibition zone obtained was 20.17 ± 0.66 mm, which can be categorized as very strong activity.

The difference in inhibition zones at various volumes is influenced by factors such as volume, diffusion method, type of bacteria, type of antimicrobial material, and the content of active compounds like heneicosane. According to Vanitha et al (2020), plants containing heneicosane compounds show very good antimicrobial activity. Lily essential oil also contains compounds such as benzaldehyde, linalool, cymene, borneol, and ocimene, which can inhibit the growth of bacteria (Hui-xiu et al., 2013).

This research uses clindamycin as a positive control. Clindamycin is a broad-spectrum antibiotic that can reduce the growth of both Gram-positive and Gram-negative bacteria. It is effective against aerobic Gram-positive bacteria, anaerobic Gram-negative bacilli, and *methicillin-resistant Staphylococcus* bacteria (Wati et al., 2022). Clindamycin is the only antibiotic that can reduce bacterial adhesion to epithelial cells on the mucosal surface by suppressing the expression of virulence factors (Luchian et al., 2021).

The mechanism of action of clindamycin exerts its bacteriostatic effect by inhibiting bacterial protein synthesis through binding to the RNA, specifically the 50S subunit of the bacterial ribosome. Clindamycin halts the growth and spread of bacterial infections. Additionally, clindamycin can enhance the process of bacterial elimination that is bound or coated with antibodies by macrophages and the process of phagocytosis through the disruption of bacterial protein synthesis, leading to the alteration of the cell wall surface and decreasing bacterial adhesion to host cells (Luchian et al., 2021).

The antibacterial activity of essential oil of lily flowers against P. *acnes* bacteria shows a significant increase with rising concentration. According to Gonelimali et al (2018), the concentration of antibacterial substances affects the content of active compounds that function as antibacterials. The higher the concentration of the antibacterial substance, the greater the number of antibacterial compounds, and thus, the stronger the antibacterial inhibitory effect.

In this study, lily plants can be used as antioxidants and antimicrobials because the components of essential oil compounds can react with the components of the bacterial cell wall, leading to inhibition and even damage to the bacterial cell wall (Hui-xiu et al., 2013). According to Izzari (2007), there are several factors affecting antibacterial inhibition, namely diffusion rate, molecular size, and stability. Differences in the diameter of the inhibition zone are influenced by the properties of the medium, the number of organisms inoculated, bacterial growth rate, concentration of chemicals, and incubation conditions.

CONCLUSION

This research concludes that the essential oil of lily flowers (*Lilium auratum*) at a concentration of 12 µl exhibited an average inhibition zone of 14.54 ± 0.51 mm (strong activity); at a concentration of 25 µl, the average inhibition zone was 15.93 ± 0.24 mm (strong activity); and at a concentration of 50 µl, the average inhibition zone was 20.17 ± 0.66 mm (very strong activity). Therefore, it can be concluded that the essential oil of lily flowers inhibits the growth of *Propionibacterium acnes*.

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