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Antibacterial Activity Test of Basil Leaves (*Ocimum basilicum* L.) Against *Staphylococcus aureus* Using the Diffusion Agar Method

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ABSTRAK

Infeksi yang disebabkan oleh *Staphylococcus aureus* merupakan salah satu penyebab utama morbiditas dan mortalitas di seluruh dunia, terutama dengan meningkatnya resistensi terhadap antibiotik. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri daun kemangi (*Ocimum basilicum* L.) terhadap *S. aureus* menggunakan metode difusi agar. Penelitian Eksperimen laboratorium, Rancangan Acak lengkap: perlakuan konsentrasi ekstrak (100µL, 200µL, 300µL, 400µL), Kontrol positif (antibiotic sintesis@amoxilin) dan kontol negative (aquades) 3 kali pengulangan. Data dianalisis Statistik non parametrik karena data tidak menyebar normal dan homogenitas, Anova *Kruskal Wallis* untuk melihat perbedaan perlakuan. Hasil penelitian menunjukkan bahwa ekstrak daun kemangi memiliki aktivitas antibakteri yang signifikan, dengan diameter zona hambat maksimum ekstrak menunjukkan aktivitas penghambatan terhadap pertumbuhan bakteri uji S. aureus pada semua konsentrasi. Diameter zona hambat tertinggi pada kelompok perlakuan pada konsentrasi 400µL dengan rata-rata diameter 9,28 ± 0,36 mm. Temuan ini menunjukkan potensi daun kemangi sebagai sumber antibakteri alami yang dapat digunakan dalam pengembangan terapi alternatif untuk infeksi S. *aureus*.

Kata kunci: *Ocimum basilicum*, *S. aureus*, *antibakteri*, *difusi agar*, *zona hambat*

ABSTRACT

Infections caused by Staphylococcus aureus are among the leading causes of morbidity and mortality worldwide, particularly due to the increasing resistance to antibiotics. This study aims to evaluate the antibacterial activity of basil leaves (Ocimum basilicum L.) against S. aureus using the agar diffusion method. This laboratory experimental study used a completely randomized design: extract concentration treatments (100µL, 200µL, 300µL, 400µL), a positive control (synthetic antibiotic @amoxicillin), and a negative control (distilled water), each with three replications. The data were analyzed using non-parametric statistics, as the data were not normally distributed and lacked homogeneity. The Kruskal-Wallis ANOVA was used to determine differences between treatments. The results showed that basil leaf extract exhibited significant antibacterial activity, with all concentrations inhibiting the growth of S. aureus. The highest inhibition zone was observed at the 400µL concentration, with an average diameter of 9.28 ± 0.36 mm. These findings suggest the potential of basil leaves as a natural antibacterial source for developing alternative therapies against S. aureus infections.

Keywords*: Ocimum basilicum, S. aureus, antibacterial, agar diffusion, inhibition zone*

1. INTRODUCTION

Pathogenic bacterial infections remain one of the main challenges in healthcare, especially with the rising cases of antibiotic resistance. Antimicrobial resistance has become a global threat that hampers the effectiveness of bacterial infection treatments, thereby demanding the search for more effective and sustainable alternative solutions[1].

This resistance is facilitated by the bacteria's ability to form biofilms and undergo genetic mutations[2]. Infections caused by *Staphylococcus aureus* have become

increasingly difficult to treat due to the emergence of strains resistant to multiple antibiotics, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA), which further complicate treatment strategies[3,4]. *S. aureus* is one of 15 priority pathogenic families with a high burden[4].

This situation has driven the growth of research into alternative antimicrobial agents that are more effective, safer, and pose a lower risk of resistance than synthetic antibiotics. *S. aureus* is a common cause of infections, responsible for various health problems including skin infections, pneumonia, and serious systemic opportunistic infections[3].

Basil (*Ocimum basilicum*), one of the widely used herbal plants in traditional medicine, is known to contain various active compounds such as flavonoids, essential oils (linalool, eugenol), tannins, and saponins, which have potential antimicrobial effects against several pathogenic bacteria[5].

Although many studies have explored the antibacterial activity of basil leaves, several gaps remain to be addressed. For instance, the concentrations and formulations used in existing studies are often limited. Further research is needed to explore a broader range of extract concentrations. Moreover, the findings of this study are expected to provide a scientific basis for the development of safer, more effective natural-based treatment alternatives, potentially addressing the growing issue of antibiotic resistance and paving the way for further research on the clinical application of basil leaf extract[6].

2. RESEARCH METHODS

This study is a descriptive quantitative laboratory experiment. The experimental design used a Completely Randomized Design consisting of six treatments: four basil extract concentrations (100µL, 200µL, 300µL, 400µL), a positive control (amoxicillin), and a negative control (distilled water), each replicated three times using sterile and controlled Petri dishes. Antibacterial activity testing was carried out using the agar disk diffusion method[7]. Inhibition zones around the disks, marked by clear or cloudy areas, were measured to indicate antibacterial activity of basil extract and compared to the synthetic standard.

Materials and Equipment

Materials:

Staphylococcus aureus isolate, Nutrient Broth (NB), Nutrient Agar (NA), distilled water, 70% and 96% alcohol, Amoxicillin 500 mg (as positive control), physiological NaCl (0.9%), basil leaves (*Ocimum basilicum* L.)

Equipment:

Autoclave, Laminar Air Flow (LAF), test tube rack, micropipette, screw micrometer (to measure inhibition zones), hot plate, 6 mm paper disks, incubator, digital balance, Bunsen burner, Whatman No.1 filter paper, tweezers, inoculation loop.

Research Procedures

1. Sample Collection and Preparation
   Fresh, healthy basil leaves (*Ocimum basilicum* L.), free from damage and chemical contaminants, were harvested, collected, and sorted. Only the leaf part was used, as it is the main photosynthetic organ and considered the key source of bioactive compounds[8]. The leaves were washed with running water to remove dirt and dust, followed by rinsing with sterile 0.9% NaCl solution to eliminate microbial contaminants. Leaves were air-dried and then oven-dried at 50°C for 24 hours to obtain durable simplicia. Dried leaves were ground and sieved using a 40-mesh screen[9,10].
2. Basil Extract Preparation
   Extraction was performed using the maceration method. A total of 500 grams of dried basil leaves were extracted with 96% ethanol for 72 hours. The extract was filtered through Whatman No.1 filter paper. The filtrate was then concentrated using a rotary vacuum evaporator and stored in dark bottles at -20ºC until further use[11]. Solvent choice and concentration affect extraction time[12], while temperature and duration influence the solubility and diffusion of compounds[13]. However, excessive temperature may degrade sensitive compounds, so a balance is required[14].
3. Preparation of Basil Extract Concentrations
   Basil extract was diluted with sterile distilled water to create the concentrations of 100µL, 200µL, 300µL, and 400µL. Each concentration was prepared under sterile conditions, stored in sterile test tubes or glass bottles at 4°C until use.

4. Bacterial Suspension Preparation
   Tool Sterilization: All equipment was wrapped in aluminum foil and autoclaved at 121°C for 15 minutes[7,15].
   Media Preparation: 5.6g of Nutrient Agar was dissolved in 200 mL distilled water in an Erlenmeyer flask. The solution was homogenized using a stirrer on a hot plate until boiling, then sterilized in an autoclave at 121°C for 15–20 minutes[16]. Media was cooled to ±45–50°C and used for the test and seed media[12,17].
   Bacterial Cultivation: Dormant *S. aureus* was reactivated by streaking onto sterile NA media under LAF conditions to avoid contamination, then incubated for 24–48 hours. For testing, a suspension of *S. aureus* was prepared in sterile physiological NaCl using a sterile inoculating loop[18] and incubated at 37°C for 3–4 hours. The turbidity was adjusted to McFarland 0.5 standard (≈1.5 × 10^8 CFU/mL), and CFU/mL was confirmed to ensure bacterial concentration[19,20].
   Amoxicillin Solution Preparation (500 µg/mL): One capsule (500 mg) of amoxicillin was dissolved in 1,000 mL of sterile distilled water and mixed thoroughly to obtain a stock solution[20].
5. Testing Procedure
   100µL of *S. aureus* isolate was pipetted into sterilized NA media at ±45–50°C, homogenized, and poured into three Petri dishes using aseptic technique. After solidification, each plate was divided into six sections labeled for each treatment: 100µL, 200µL, 300µL, 400µL basil extract, amoxicillin (positive control), and distilled water (negative control). The testing was done under LAF using aseptic technique[21]. Plates were incubated in an inverted position to minimize condensation at 37°C, an optimal temperature for mesophilic bacteria such as *S. aureus*[22].
6. Measurement of Inhibition Zone Diameter
   After 24 hours of incubation, the inhibition zones (clear areas around the disks) were measured using a ruler or caliper. The effectiveness of antibacterial activity was determined by measuring the inhibition zone with a micrometer screw gauge. The diameter was calculated using the formula[21,23,24]:

$$\mathbf{DzH} = \frac{\mathbf{Sd1} + \mathbf{Sd2} + \mathbf{Sv} + \mathbf{Sh}}{4}$$

   Description:
   DzH = Diameter of Inhibition Zone (mm)
   Sd1 = Left diagonal side
   Sd2 = Right diagonal side
   Sv = Vertical side
   Sh = Horizontal side

Data Analysis

The inhibition zone data were tabulated and averaged. Statistical analysis was performed using SPSS. If the data were normally distributed and homogeneous, parametric tests (ANOVA and Post Hoc) were applied. Otherwise, non-parametric tests (Kruskal-Wallis) were used to determine differences between treatments[25,26].

3. RESULTS AND DISCUSSION

The resulting extract was dark green in color, had a distinctive aroma, and a bitter taste, containing secondary metabolite compounds similar to the simplicia powder. The results of the antibacterial activity test were based on the formation of an inhibition zone around the paper disks, which could be observed on each petri dish after 24 hours of incubation (Figure 1). Measurements were carried out using a digital micrometer screw gauge, based on the vertical, diagonal, and horizontal axis points for each treatment. The measurement data were tabulated and averaged for all petri dishes (replications U1, U2, U3) per treatment (Table 1).

Table 1. Inhibition Zone Diameter of Basil Leaf Extract Against the Growth of *Staphylococcus aureus*

No	Concentration (µL)	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.
1	100	8.05	8.75	8	8.27	0.42
2	200	9	8.5	8.5	8.67	0.29
3	300	7.5	10	8.8	8.77	1.25
4	400	8.75	10	9.1	9.28	0.64
5	Positive Control	29	30	30	29.67	0.58
6	Negative Control	0	0	0	0	0

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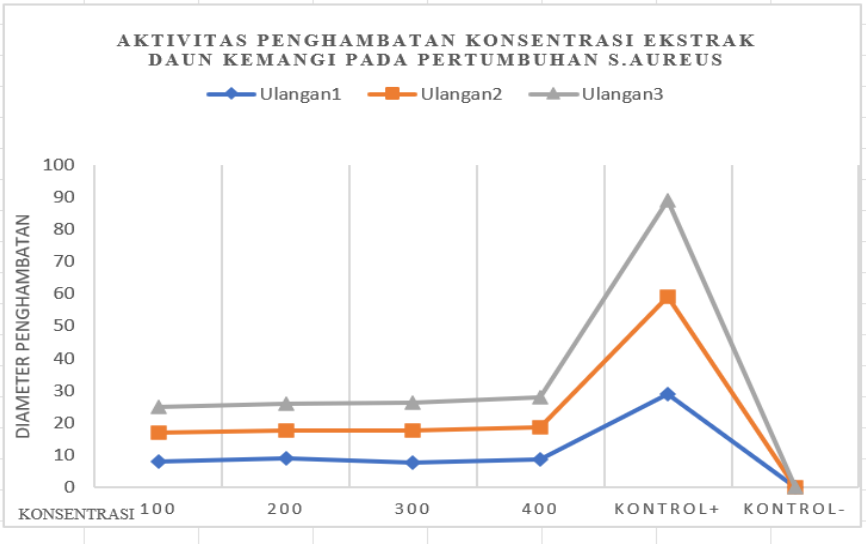

Figure 1. Inhibitory Activity of Basil Leaf (*Ocimum basilicum L.*) Extract on *Staphylococcus aureus*

The results of the antibacterial activity test of the extract against the test bacteria are shown in Table 1 and Figure 1. Based on the obtained data, the extract demonstrated inhibitory activity against *S. aureus* at all tested concentrations (100µL, 200µL, 300µL, 400µL). The highest inhibition zone was observed at the 400µL concentration with an average diameter of 9.28 ± 0.36 mm. The average inhibition zone diameters for each concentration fall into the category of moderate inhibition (5–10 mm), according to the Clinical and Laboratory Standards Institute (CLSI).

The visualization of basil extract's antibacterial activity against *S. aureus* growth on each petri dish is shown in Figure 2.

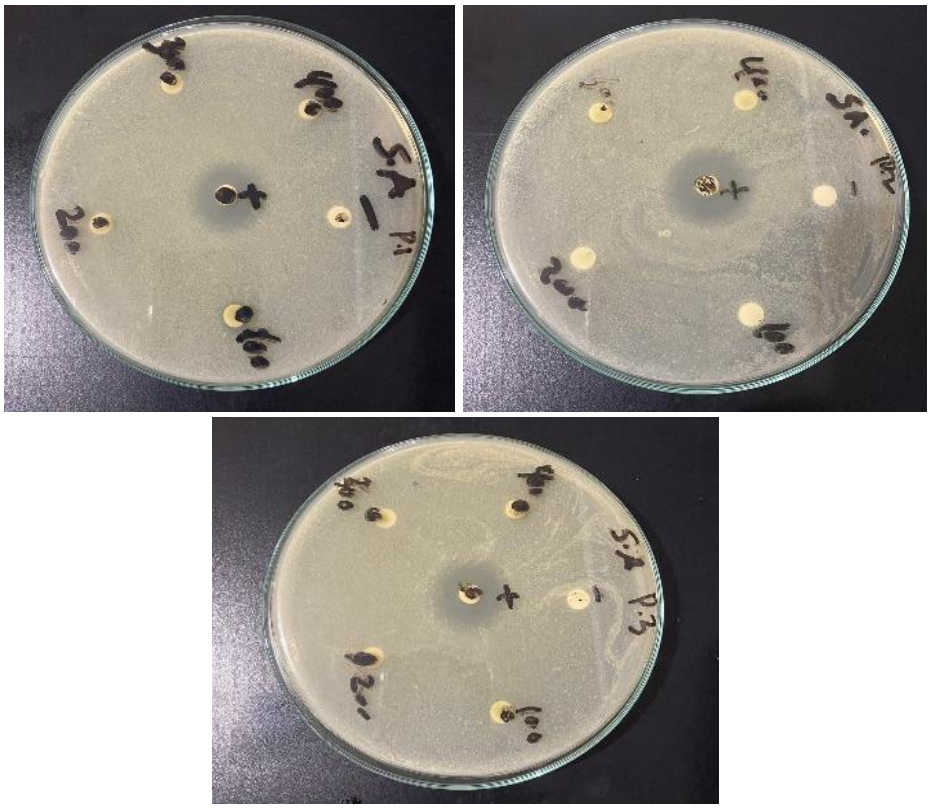

Figure 2. Inhibition of *Staphylococcus aureus* Growth by Basil Leaf Extract

The bacterial growth inhibition by the extract may be due to the presence of antibacterial compounds in basil. This is attributed to the content of various secondary metabolites in basil leaf extract, such as phenolic compounds, flavonoids, carbohydrates, glycosides, and tannins, which contribute to its effectiveness against common pathogens. This plant exhibits a broad spectrum of antimicrobial activity. Its diverse chemical composition supports its traditional medicinal use and potential for drug development, especially antimicrobial agents.

The positive control showed significantly higher inhibition activity with an average diameter of 29.67 ± 0.33 mm, while the negative control showed no inhibition zone. Amoxicillin was used as a comparison because it is a semi-synthetic penicillin compound with bactericidal antibacterial activity and is a broad-spectrum antibiotic. Amoxicillin works by inhibiting the biosynthesis of the bacterial cell wall's mucopeptide during multiplication.

Normality and homogeneity tests of the data from concentrations 100µL, 200µL, 300µL, and 400µL showed that the data were not normally distributed. Therefore, a non-parametric test (Kruskal-Wallis) was used for these treatment groups. The Kruskal-Wallis test for the treatment groups (100µL, 200µL, 300µL, 400µL) yielded H statistic = 3.6148 and p-value = 0.3062, indicating no significant difference among the groups ($p > 0.05$). However, when the positive control (amoxicillin) and negative control (distilled water) were included, there was a significant difference between the groups (H statistic = 13.5858, p-value = 0.0185), indicating a significant difference ($p < 0.05$).

Statistical analysis using the Kruskal-Wallis test on all groups showed a significant difference ($p = 0.015$). However, when the analysis was limited to the treatment groups only (100µL, 200µL, 300µL, 400µL), no significant difference was found ($p = 0.193$). This suggests that increasing the extract concentration from 100µL to 400µL did not result in a statistically significant increase in antibacterial activity.

The therapeutic efficacy of plants is associated with their richness in bioactive compounds, which can exert various pharmacological effects on the human body. These bioactive compounds, also known as phytochemicals, include a wide range of chemical classes such as alkaloids, flavonoids, terpenoids, phenolic acids, and glycosides. Many of these phytochemicals have strong antimicrobial activity, making plants a valuable resource for combating bacterial, viral, and fungal infections. Some sources report that compounds such as alkaloids, flavonoids, and saponins found in chayote leaf extract contribute to its antibacterial activity.

Basil contains a variety of bioactive compounds that contribute to its pharmacological properties, including antibacterial, anti-inflammatory, and antioxidant effects. Essential oils such as linalool, eugenol, methyl chavicol, cineole, and camphor are known to have strong antimicrobial activity against various pathogenic bacteria. Tannins and saponins contribute to the antibacterial and immunomodulatory properties of basil leaves.

Staphylococcus aureus is a Gram-positive bacterium commonly found in the nose, mouth, throat, and skin. It can cause respiratory infections. The cell wall of Gram-positive bacteria consists of 40–80% peptidoglycan by dry weight. The antibacterial effects are due to compounds in basil such as flavonoids, saponins, and tannins. Flavonoids play a role in antibacterial, anti-inflammatory, and antifungal activity. Their mechanism includes inhibiting the development of microorganisms. The bioactive compounds in basil extract act as antibacterial agents; flavonoids disrupt cell membrane permeability, causing leakage in bacterial cell walls. Eugenol (a phenol derivative found in ethanolic basil extract) damages the cell membrane. Phenolic binding to bacterial cell walls disrupts transporter membrane permeability, ultimately killing the bacteria. Tannins act as antibacterial agents by forming hydrogen bonds with proteins, causing protein denaturation and interfering with bacterial metabolism. Alkaloids are nitrogen-containing natural metabolites with rich biological potential due to variations in their atomic structure.

In conclusion, basil leaves have the potential to inhibit bacterial growth. The findings of this study are expected to be useful and serve as a reference for future research.

4. CONCLUSION

Based on the results obtained, it can be concluded that basil leaves exhibit antibacterial activity against the test bacterium *Staphylococcus aureus*. However, their effectiveness is still significantly lower

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compared to the standard antibiotic used as the positive control. Increasing the extract concentration up to 400µL did not result in a significant increase in activity, indicating the need for further research to enhance the extract's effectiveness or explore alternative extraction methods.

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