



Antibacterial Activity of Tulsi Ethanolic Extract (*Ocimum tenuiflorum* L.) against *Staphylococcus aureus*

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Abstract: Infectious disease is a type of disease caused by invasion by microbiological agents, such as bacteria. One type of bacteria is *Staphylococcus aureus* which is a Gram-positive bacteria. The definitive therapy for bacterial infections is the administration of antibiotics. However, overuse of antibiotics leads to bacterial resistance. One of the efforts to overcome this is with alternative antibiotics made from herbs, such as Tulsi (*Ocimum tenuiflorum* L.). This study aims to determine the antibacterial activity of Tulsi ethanolic extract against *S. aureus*. This research was conducted at the Analysis Laboratory of the Faculty of Agriculture, Warmadewa University from October to November 2024. The research design used a true experimental method with a posttest only control group design model, namely by testing the antibacterial activity of Tulsi ethanolic extract with concentrations of 25%, 50%, and 75% against *S. aureus*. In the antibacterial test results, the diameter of the inhibition zone formed at concentrations of 25%, 50%, and 75% was 5.25 mm, 5.90 mm, and 7.22 mm, respectively. Based on the One Way ANOVA test results, the P value <0.001 was obtained, which means there is a significant difference between the treatment groups. Based on this study, it can be concluded that Tulsi ethanolic extract has moderate antibacterial activity.

Keywords: Antibacterial; *Ocimum tenuiflorum* L.; *Staphylococcus aureus*

Introduction

Infectious diseases are illnesses caused by the invasion of microbiological agents, such as bacteria. The human body naturally hosts normal organisms in certain organs, such as the intestines or mouth. However, this condition can change when there is an invasion by highly pathogenic bacteria, followed by the proliferation of these bacteria, leading to disease (Kulla & Herrani, 2022). In 2019, infectious diseases accounted for 52.9% of deaths in Africa and nearly 25% of deaths in the Eastern Mediterranean and Southeast Asia (WHO, 2022). That same year, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* contributed to 30.9% of 7.7 million infection-related deaths globally. *Staphylococcus aureus* alone was the leading bacterial cause of death in

135 countries, associated with 1,105,000 deaths in 2019 (Linz et al., 2023).

Staphylococcus aureus has a grape-like cluster shape and appears golden in color (Widiastuti & Pramestuti, 2018). This bacterium is often found colonizing the nasal cavity and nasal mucosa of humans, with about 30% of the healthy adult population being invaded by *S. aureus*. Although *S. aureus* is a normal flora coexisting with humans, it has the potential to cause adverse effects, ranging from skin infections to severe conditions such as bloodstream infections, endocarditis, osteomyelitis, and necrotizing infections (Jenul & Horswill, 2019).

Definitive therapy for bacterial infections is the administration of antibiotics (Huemer et al., 2020). For *S. aureus* infections, commonly used antibiotics include penicillin and methicillin (Guo et al., 2020). However, previous studies have reported an increase in antibiotic

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resistance cases in *S. aureus*, attributed to years of antibiotic use without proper responsibility (Breijyeh et al., 2020). Therefore, alternative antibiotics are needed to replace conventional antibiotics in eradicating *S. aureus*.

Tulsi, also known as *Ocimum tenuiflorum*, is a plant in the Lamiaceae family. In Ayurvedic teachings, this plant is referred to as the "holy basil" or "queen of herbs" due to its ability to treat various diseases (Sintya et al., 2023). Previous studies have identified Tulsi's antibacterial properties, attributed to its content of terpenoids, phenolics, flavonoids, glycosides, and phenylpropanoids (Permatananda et al., 2025). According to a study conducted by Yamani et al. (2016), Tulsi essential oil at concentrations of 2.25% and 4.5% could inhibit the growth of *S. aureus*, including Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Yamani et al., 2016). Thus, researchers are interested in analyzing the phytochemical content of Tulsi extract and its ability to inhibit the growth of *S. aureus*.

Method

The study was conducted at the Analytical Laboratory of the Faculty of Agriculture, Warmadewa University, Denpasar, Bali. This research was carried out from October to November 2024. The study design used a true experimental research method with a posttest-only control group design model. Antibacterial activity testing of Tulsi ethanolic extract against *S. aureus* ATCC 25923 was performed using the disk diffusion method to measure the diameter of the inhibition zone (Zone of Inhibition, ZOI) (Dharsono et al., 2022). The population

in this study was *S. aureus* ATCC 25923. The sample used was *S. aureus* ATCC 25923 cultured in the Analytical Laboratory of the Faculty of Agriculture, Warmadewa University. The bacteria were grown on Luria Bertani medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 20 g/L bacto agar).

The independent variable in this study was the ethanolic extract of Tulsi (*Ocimum tenuiflorum* L.), while the dependent variable was the diameter of the inhibition zone (ZOI). Data analysis was performed using Microsoft Excel 2019. Descriptive analysis involved observing the antibacterial activity of Tulsi ethanolic extract (Hamidah et al., 2019). The formation of an inhibition zone indicated a positive result. The data obtained in this test included the mean diameter of the inhibition zone and standard deviation. The data were processed using JAMOMI software and analyzed using One-Way Analysis of Variance (ANOVA) to examine the differences in the inhibition zones of *S. aureus* ATCC 25923 growth.

Result and Discussion

This study was conducted after obtaining an ethical clearance letter issued by the Ethics Committee of the Faculty of Medicine and Health Sciences, Warmadewa University, with the reference number 512/Unwar/FKIK/EC-KEPK/X/2024.

The research aimed to determine the antibacterial activity of Tulsi (*Ocimum tenuiflorum* L.) ethanolic extract against *S. aureus* ATCC 25923. The inhibition zones for each treatment group are presented in Table 1.

Table 1. Inhibition Zone of Bacterial Growth

Experiment No.	Tulsi Ethanolic Extract 25%	Tulsi Ethanolic Extract 50%	Tulsi Ethanolic Extract 75%	Negative Control (Aquadest)
1	5.28	6.07	6.87	0
2	5.25	6.36	7.49	0
3	5.13	6.33	7.96	0
4	5.32	5.64	6.83	0
5	5.27	5.51	7.11	0
6	5.23	5.49	7.06	0
Mean	5.25	5.90	7.22	0
SD	0.065	0.403	0.432	0.000

Table 1 shows that the 25% Tulsi ethanolic extract produced an inhibition zone of 5.25±0.065 mm, the 50% Tulsi ethanolic extract produced 5.9±0.403 mm, and the 75% Tulsi ethanolic extract produced 7.22±0.432 mm. Meanwhile, the negative control showed no inhibition zone.

This study employed a One-Way ANOVA test for data analysis. Certain prerequisites must be met before conducting a One-Way ANOVA, including a normality test to determine whether the data distribution is

normal. The Shapiro-Wilk test was chosen as the normality test because the sample size was less than 50. Data is considered normally distributed if the significance value is >0.05. The results of the normality test are presented in Table 2.

Table 2 shows that the 25% extract has p = 0.648, the 50% extract has p = 0.136, and the 75% extract has p = 0.260. The negative control was not included in this data analysis because the result was static, i.e., 0. All

treatment groups are considered to have normal data because the significance values $p > 0.05$.

Table 2. Normality Test

Group (Inhibition Zone)	Statistic	Saphiro Wilk df	Sig
Tulsi Ethanolic Extract 25%	0.909	6	0.648
Tulsi Ethanolic Extract 50%	0.842	6	0.136
Tulsi Ethanolic Extract 75%	0.878	6	0.260

Since the data follows a normal distribution, a One-Way ANOVA test was conducted, with the results shown in Table 3. In Table 3, a p-value of <0.001 was obtained, indicating a significant difference between the treatment groups.

Table 3 One Way Anova

Variable	F	P Value
Inhibition Zone Diameter	682	<0.001

The results of the antibacterial activity test of Tulsi ethanolic extract showed a difference in the inhibition zone diameter for each concentration. The study found that the 75% concentration extract had the largest average inhibition zone diameter of 7.22 ± 0.432 mm, followed by the 50% and 25% extracts, with inhibition zones of 5.9 ± 0.403 mm and 5.25 ± 0.065 mm, respectively. All three extract concentrations exhibited inhibition zone diameters ranging from 5-10 mm, categorizing them as having moderate antibacterial activity (Morales, 2003).

Several factors influence antibacterial activity, including extract concentration, the type of bacteria tested, and the content of secondary metabolites. An increase in the concentration of antibacterial compounds correlates with enhanced ability to penetrate microbial cells, ultimately damaging the cell's metabolic systems and inducing apoptosis. In other words, bacterial growth inversely correlates with increasing extract concentration (Ballo et al., 2021). Angelina (2015) also mentioned that higher extract concentrations increase its inhibitory effect on bacterial growth, which is known as the dose-related pharmacological effect.

The antibacterial activity of Tulsi ethanolic extract is likely due to the secondary metabolites it contains (Mallikarjun et al., 2016; Permatananda et al., 2024; Yamani et al., 2016). A qualitative phytochemical test by Borah & Biswas (2018) reported that Tulsi ethanolic extract contains phenolic compounds, tannins, flavonoids, saponins, terpenoids, and alkaloids. These compounds have been shown to possess antibacterial activity by disrupting bacterial cell membranes,

inhibiting essential enzymes, or interfering with the metabolic functions of bacteria. Phenols, for example, can halt bacterial cell metabolism through protein denaturation (Silhavy et al., 2010). They can also damage bacterial cell membranes by dissolving lipids in the membrane, causing leakage of ions and essential molecules, leading to cell death (Ghosh et al., 2022; Nourbakhsh et al., 2022). At low concentrations, phenols act as bacteriostatic agents, while at higher concentrations, they become bactericidal, killing bacteria by disrupting membranes and essential proteins (Marfuah et al., 2018).

Flavonoids damage bacterial cytoplasmic membranes, leading to the release of intracellular compounds by forming complexes with extracellular proteins (Ariani et al., 2020). Alkaloids disrupt the formation of bacterial cell walls, causing apoptosis due to the interference with peptidoglycan components. Tannins disrupt bacterial metabolism by denaturing proteins. This occurs because tannins work by forming polysaccharide complexes in bacterial cell walls. Saponins cause damage to the membrane, leading to the release of components from bacterial cells due to the instability of the bacterial cell. Terpenoids inhibit bacterial protein synthesis by blocking processes in the protein synthesis pathway (Marfuah et al., 2018).

This study successfully found the potential for Tulsi ethanolic extract to inhibit *S. aureus* bacteria, with increased antibacterial activity observed as the concentration of the extract increased. However, variations in the level of antibacterial activity were noted (Angelina, 2015; Ariani et al., 2020). In this study, Tulsi ethanolic extract concentrations ranging from 25% to 75% showed moderate antibacterial activity. In contrast, the study by Angelina (2015) on the antibacterial activity of ethanolic basil leaf extract (*Ocimum sanctum* L.) against *Escherichia coli* and *Staphylococcus aureus* at concentrations between 20% and 100% found strong antibacterial activity. Similarly, the study by Ariani et al. (2020) on the antibacterial activity of ethanolic basil leaf extract (*Ocimum sanctum* L.) against *Staphylococcus aureus* in vitro showed weak to strong antibacterial activity at similar concentrations. Several factors may explain the differences observed, including the variability of Tulsi's secondary metabolites based on geographic conditions, climate, soil type, and cultivation methods. Additionally, Tulsi has various cultivars, such as the Rama and Krishna varieties, which may have different active compound contents. Differences in antibacterial activity testing methods, such as pH, temperature during testing, bacterial strain, and culture medium, may also affect research outcomes (Nirmalasari, 2024; Suputra, 2024).

Limitations of the Study

The main limitation of this study is the potential composition of phytochemicals in the extract, which heavily depends on the extraction method, extract concentration, as well as the environmental and geographical conditions where Tulsi was obtained (Borah & Biswas, 2018; Priya & Peddha, 2023). This variation may lead to differences in the content of active compounds, which directly affect the antibacterial potential of the extract. Furthermore, the use of the disk diffusion method, which is influenced by the ability of active compounds to diffuse in the agar medium, may limit the accuracy of measuring antibacterial activity. Measurement bias may also occur in this study, as the inhibition zone was manually measured using a ruler or caliper, which is prone to subjective error.

Conclusion

Based on the research results, Tulsi ethanolic extract at concentrations of 25%, 50%, and 75% produced inhibition zone diameters on *S. aureus* ATCC 25923 of 5.25 mm, 5.90 mm, and 7.22 mm, respectively. These inhibition zone diameters, when classified, fall into the moderate antibacterial activity category. When compared to the control (aquades), the Tulsi ethanolic extract showed a significant difference in antibacterial activity (p-value <0.05).

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Author Contributions

All author contributed in conceptualization and performed this research. All author had part in drafting the article. Permatananda finished and submitted the article.

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Conflicts of Interest

No conflicts of interest.

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