

The Effectiveness of Lime Leaf Extract (*Citrus aurantifolia*) in Inhibiting the Growth of Staphylococcus aureus Bacteria

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Abstract

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Herbal plants, such as lime (*Citrus aurantifolia*, *S*), have been widely used by Indonesians as traditional medicine. Limes contain beneficial compounds such as citric acid, amino acids, and essential oils. The number of samples in the study was 25, where each group consisted of 5 treatment groups. The main sample of this study was a certain strain of Staphylococcus aureus bacteria which was tested using all fresh parts of lime leaves (*Citrus aurantifolia*). The extract concentrations used consisted of 10%, 50%, and 90%. the positive control group (CP+) showed a significant difference compared to the negative control group (CN-) and the T1 treatment group ($p=0.001$), but no significant difference was observed with T2 ($p=0.068$) or T3 ($p=0.924$). The CN- group differed significantly from all other groups ($p=0.001$). The T1 group also demonstrated significant differences from all groups except CP+. Meanwhile, no significant differences were found between T2 and T3 ($p=0.067$), nor between these two groups and the positive control group ($p=0.068$ and $p=0.924$, respectively). A 90% concentration of lime leaf extract had the highest antibacterial effect against Staphylococcus aureus, followed by 50% and 10% concentrations. Further studies are needed to determine the most effective and efficient concentration

Keywords: *Citrus aurantifolia*, lime leaves, Staphylococcus aureus, antibacterial, inhibition zone.

Introduction

Herbal plants, such as lime (*Citrus aurantifolia*, *S*), have been widely used by Indonesians as traditional medicine. Limes contain beneficial compounds such as citric acid, amino acids, and essential oils (Lauma et al., 2015). Limes (*Citrus aurantifolia*) are used as a flavor enhancer and in traditional medicine. Their extracts help relieve fever, coughs,

influenza, and treat acne. Their antimicrobial properties (alkaloids, flavonoids, tannins, phenols, and saponins) can inhibit bacteria such as *E. coli*, *S. aureus*, and *S. mutans* (Rizka et al., 2025). Fresh lime juice contains citric acid (6.15%), lactic acid (0.09%), and a small amount of tartaric acid. Its antibacterial activity comes from these organic acids, particularly citric acid. Its mechanism of action involves lowering the pH below the microbial growth limit and inhibiting metabolism by acid molecules (Srilistiany et al., 2021).

Treatment of infection *Staphylococcus aureus* Penicillin G or its derivatives are beginning to face resistance challenges, especially in severe infections. Therefore, the antibacterial effects of medicinal plants such as lime were tested. The results showed that lime leaf essential oil inhibited the growth of *S. aureus* at concentrations of 20%, 40%, and 80%, as well as *E. coli* at concentrations of 40% and 80% (Razak et al., 2013).

As part of the movement back to nature and reduce the negative impacts of synthetic drugs, lime can be an alternative treatment for infections *Staphylococcus aureus*. While this bacteria was once a common cause of wound and post-operative infections in hospitals, cases are no longer as frequent as they were before the advent of antibiotics (Mengga et al., 2021).

There is still a gap in studies regarding lime (*Citrus aurantifolia*) as an antibacterial against antibiotic-resistant *Staphylococcus aureus*. Existing research is limited to conventional tests, while MRSA resistance is also influenced by biofilms and agr system dysfunction. Studies on the mechanisms of biofilm inhibition and the molecular purpose of lime are still very limited. Therefore, this study aims to analyze the antibacterial and antibiofilm activity of lime extract against MRSA and its effect on the expression of resistance- and biofilm-related virulence regulatory genes.

Material and Methods

This study used a laboratory experimental design and a posttest only control group design, with a quantitative experimental research approach. Ethical clearance was obtained from Wijaya Kusuma University on July 3, 2025 (93/SLE/FK/UWKS/2025). The number of samples in the study was 25, where each group consisted of 5 treatment groups. The main sample of this study was a certain strain of *Staphylococcus aureus* bacteria which was tested using all fresh parts of lime leaves (*Citrus aurantifolia*). The extract concentrations used consisted of 10%, 50%, and 90%. In addition, the test bacteria *Staphylococcus aureus* strain ATCC 25923 was also used which was grown on Nutrient Agar media. For the inhibition test,

the disk diffusion method was used with sterile disc paper dripped with extract, then placed on the surface of the media that had been inoculated with bacteria. Ampicillin was used as a positive control, while sterile distilled water was used as a negative control. The entire process was carried out under sterile conditions in the microbiology laboratory of the Faculty of Medicine, Wijaya Kusuma University, Surabaya.

Results and Discussion

Results

Table 1. Inhibitor Zone Diameter

Group	Inhibition zone measurement results (mm)				
	PC (+)	NC (-)	T1 (10%)	T2 (50%)	T3 (90%)
1	6.08	0	2.56	5.02	6.07
2	5.54	0	2.04	5.02	5.56
3	6.04	0	2.54	3.53	7.03
4	5.56	0	3.03	4.05	5.06
5	5.57	0	2.05	5.04	6.52
Average	5.76	0	2.44	4.53	6.05

Note: Positive control (+) using ampicillin, Negative control (-) using sterile distilled water, treatment 1 10% concentration (T1), treatment 2 50% concentration (T2), treatments 3 90% concentration (T3) of lime leaf extract

Table 1 shows that the average diameter of the inhibition zone is in group C1 or the positive control group with the administration of ampicillin antibiotic (KP1) of 5.76 mm, where the inhibitory power of the positive control is smaller compared to the 90% treatment control (T3) and the lowest average value of the inhibition zone diameter is in the negative control group given sterile distilled water, which is 0.00 mm, which can be interpreted that the growth of *Staphylococcus aureus* is not inhibited. The diameter of the inhibition zone has been formed in lime leaf extract with a concentration of 10% (T1) which is 2.44 mm and in the treatment group with lime leaf extract, the highest inhibition zone diameter is at a concentration of 90% (T3) with an average inhibition power of 6.05 mm.

Table 2. Post Hoc Test

Group	PC (+)	NC (-)	T1 (10%)	T2 (50%)	T3 (90%)
CP (+)	-	0.001*	0.001*	0.068	0.924
CN (-)	0.001*	-	0.001*	0.001*	0.001*
T1 (10%)	0.001*	0.001*	-	0.006*	0.001*
T2 (50%)	0.068	0.001*	0.006*	-	0.067
T3(90%)	0.924	0.001*	0.001*	0.067	-

Positive control (+) using ampicillin, Negative control (-) using sterile distilled water, treatment 1 10% concentration (T1), treatment 2 50% concentration (T2), treatments 3 90% concentration (T3) of lime leaf extract

Note: *Significant

Based on the Post Hoc test results presented in Table 2, the positive control group (CP+) showed significant differences compared to the negative control group (CN-) and the

T1 treatment group ($p=0.001$), but no significant differences were observed with the T2 ($p=0.068$) and T3 ($p=0.924$) groups. Meanwhile, the negative control group (CN-) was significantly different from all other groups ($p=0.001$). The T1 treatment group also demonstrated significant differences with all groups except the positive control group (CP+). Furthermore, the T2 and T3 groups did not show any significant differences between each other ($p=0.067$) nor with the positive control group ($p=0.068$ and $p=0.924$, respectively)

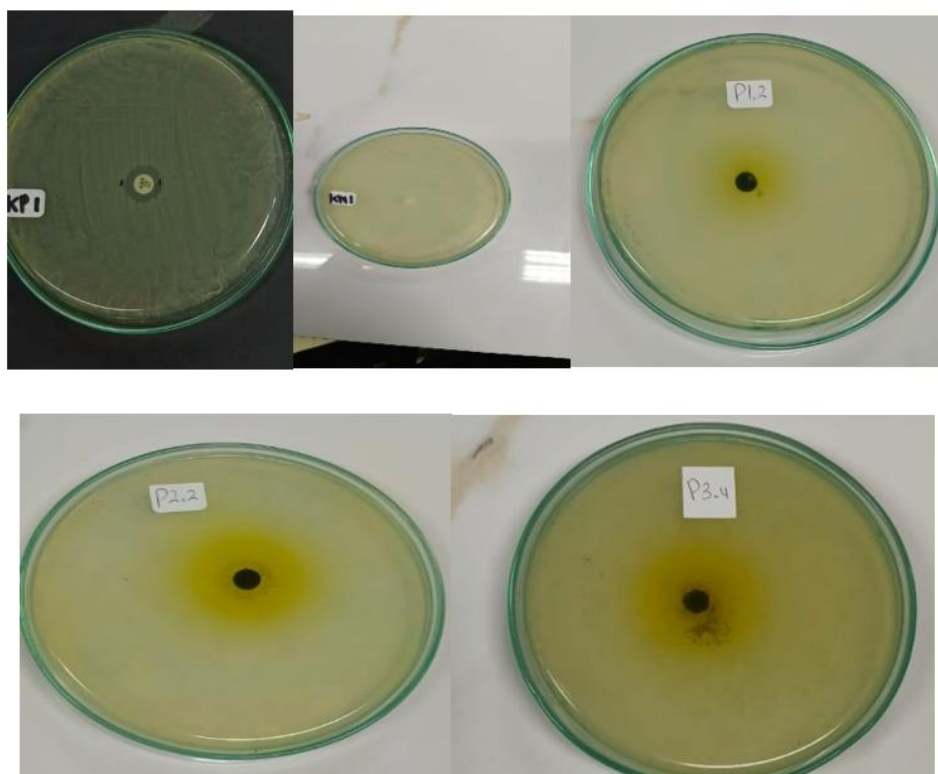


Figure 1. Research results of the control group and treatment with lime leaf extract (*Citrus aurantifolia*)

Discussion

The results of the study showed that lime leaf extract (*Citrus aurantifolia*) has the ability to inhibit the growth of *Staphylococcus aureus*. This is indicated by the formation of an inhibition zone around the disc containing the extract. The average diameter of the inhibition zone increased with increasing extract concentration, with a 90% concentration producing the highest inhibition zone of 6.05 mm, even exceeding the inhibition of ampicillin as a positive control, which averaged 5.76 mm (Kurumisawa et al., 2021).

The antibacterial effect of lime leaf extract (*Citrus aurantifolia*) is primarily derived from its bioactive compounds such as flavonoids (including *quercetin*, *naringin*, and *hesperidin*), tannins, saponins, and essential oils (especially *limonene*, *citral*, and *linalool*).

Flavonoids work by inhibiting bacterial cell wall synthesis by suppressing transpeptidase enzymes and disrupting cell membrane integrity, which causes leakage of intracellular components and inhibits energy metabolism. Tannins can inactivate important enzymes in the bacterial cell wall, inhibit ion transport, and disrupt biofilm formation by inhibiting quorum sensing. Saponins act as biosurfactants that reduce the surface tension of bacterial cell membranes, thereby increasing membrane permeability and triggering cell lysis. Meanwhile, essential oils (*limonene* and *citral*) can inhibit enzymes involved in peptidoglycan cell wall synthesis and disrupt the bacterial aerobic respiration pathway by inhibiting ATPase enzymes. The synergistic combination of these compounds causes disruption of cell wall structure, inhibition of cell division, and effective death of *Staphylococcus aureus* cells (Go' rniak et al., 2019; Hsouna et al., 2017; Xie et al., 2015).

The main secondary metabolite compounds in lime leaf extract (*Citrus aurantifolia*) that play a role in inhibitory activity against *Staphylococcus aureus* include flavonoids (*quercetin*, *naringin*), tannins, saponins, and essential oils (*limonene*, *citral*). Compared with betel leaf extract (*Piper betle*) which contains flavonoids and tannins but is superior due to its high phenol and kavicol content, lime leaf extract shows moderate activity but remains effective at lower concentrations. Meanwhile, garlic extract (*Allium sativum*) with allicin compounds has a thiol enzyme inhibitory mechanism, but the advantage of lime leaf extract lies in the synergistic effect of its multiple active components that work together through various pathways, thereby reducing the risk of bacterial resistance (Hsouna et al., 2017; Lubis & Wahyuni, 2020; Vaou et al., 2022).

Lime leaves (*Citrus aurantifolia*) contain bioactive compounds (flavonoids, alkaloids, saponins, tannins, essential oils) that have antibacterial properties against *Staphylococcus aureus*. Their mechanism of action involves cell membrane damage, protein denaturation, and disruption of bacterial metabolism. Research shows that lime leaf extract, especially at high concentrations, can significantly inhibit the growth of *S. aureus* both indicated by the inhibition zone (diffusion) and a decrease in the number of colonies (dilution) (Najiwa et al., 2022; Sari & Asri, 2022)

Statistical tests showed a significant difference between the treatment and control groups ($p < 0.05$), particularly between the negative control (distilled water) and the group with lime leaf extract. However, a comparison between the high concentration extract group (90%) and the positive control showed no significant difference ($p = 0.924$), indicating that

the effectiveness of the extract at high doses approached that of standard antibiotics (King et al., 2017). This confirms that antibiotics have excellent antibacterial effectiveness, and that distilled water does not inhibit bacterial growth (Shingala, 2015).

The active compounds in lime leaves, such as flavonoids, tannins, saponins, and essential oils, are believed to play a major role in this antibacterial effect. Flavonoids damage bacterial cell membranes, while tannins and saponins damage the protein structure and membranes of bacterial cells, leading to cell lysis. The synergistic effect between these compounds makes lime leaf extract (*Citrus aurantifolia*) effective in inhibiting bacterial growth (Mulangsri et al., 2019).

The method used in this study, disk diffusion, was chosen because it is simple, efficient, and capable of providing fairly representative results regarding the antibacterial activity of a compound. This method also allows direct visualization through the resulting inhibition zone. The use of ampicillin as a positive control serves as a benchmark for effectiveness, given its mechanism of inhibiting cell wall synthesis in Gram-positive bacteria such as *Staphylococcus aureus* (Kurumisawa et al., 2021).

The antibacterial effectiveness of lime (*Citrus aurantifolia*) extract is also determined by the type of solvent, the plant part used, and the extraction method. Ethanol extracts are reported to be superior to water extracts due to their ability to dissolve greater amounts of active compounds. Various studies have also shown that extracts derived from lime roots and peels have antibacterial activity against *Staphylococcus aureus*, through a mechanism that increases bacterial cell membrane permeability. Furthermore, lime essential oil which contains limonene and citral also plays a role in antibacterial activity by damaging the lipid structure of bacterial membranes (Julaeha et al., 2021; Najiwa et al., 2022).

The combination of lime extract with other herbs, such as betel leaf, has been shown to have a stronger synergistic effect in inhibiting *Staphylococcus aureus* than either herb alone. At certain concentrations, its effectiveness is even comparable to chemical antiseptics such as alcohol. Thus, *Citrus aurantifolia* extract has the potential to be a safe and effective natural antibacterial, particularly in the face of increasing antibiotic resistance (Gloria et al., 2021).

Overall, the results of this study indicate that lime leaf extract has significant potential as a natural antibacterial against *Staphylococcus aureus*, especially at high concentrations. While it has not yet fully replaced conventional antibiotics, this extract has the potential to be

further developed as an alternative or complementary treatment for infections, particularly amidst increasing cases of antibiotic resistance (Parama et al., 2019).

Conclusion

Research shows that lime leaf extract (*Citrus aurantifolia*) inhibits growth *Staphylococcus aureus* at concentrations of 10%, 50%, and 90%, as evidenced by the formation of inhibition zones. A concentration of 90% provided the highest antibacterial effect (the largest inhibition zone), although a concentration of 10% was also capable of producing inhibition. However, further research is needed to determine the optimal concentration that is most effective and efficient.

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