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Antibacterial Activity of Limau Kuit (*Citrus hystrix* DC.) Leaf Essential Oil Against Methicillin-Resistant *Staphylococcus aureus* and Multidrug-Resistant *Pseudomonas aeruginosa*

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Abstract: Antibiotic resistance in pathogenic bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Multi-drug-resistant *Pseudomonas aeruginosa* (MDR *P. aeruginosa*), has encouraged the development of alternative antibacterial agents from natural sources. This study aimed to analyse the antibacterial Activity of Limau Kuit (*Citrus hystrix* DC.) leaf essential oil against MRSA and MDR *P. aeruginosa*. The study used a true experimental design with a posttest-only control group design. The essential oil was obtained through steam-water distillation of Limau Kuit leaves and then tested at concentrations of 50%, 60%, 70%, 80%, 90%, and 100% using the well diffusion method. Positive controls included ciprofloxacin and ceftazidime, while dimethyl sulfoxide served as the negative control. Each treatment was repeated four times. Data were analysed using Welch ANOVA and Games-Howell tests for MRSA and Kruskal-Wallis and Mann-Whitney tests for MDR *P. aeruginosa*. The essential oil yield was 0.60% (v/w). All concentrations showed antibacterial activity against both test bacteria. The largest inhibition zone diameter against MRSA was obtained at 90% (22.23 ± 1.75 mm), while against MDR *P. aeruginosa* at 80% (13.98 ± 1.48 mm). Statistical analysis showed a significant difference between concentrations in MRSA ($p < 0.001$) and MDR *P. aeruginosa* ($p = 0.003$). Antibacterial activity against MRSA is higher than against MDR *P. aeruginosa*, which is thought to be related to differences in cell wall structure and resistance mechanisms of the two bacteria. Lime leaf essential oil has potential as a natural antibacterial against antibiotic-resistant bacteria. However, further research is needed to determine active compound content, mechanism of action, and the minimum inhibitory concentration and minimum bactericidal concentration.

Keywords: Antibacterial; *Citrus hystrix* DC**.**; essential oil; methicillin-resistant *Staphylococcus aureus*; multidrug-resistant *Pseudomonas aeruginosa*.

INTRODUCTION

Infectious diseases remain a major global health problem and are caused by various pathogenic microorganisms, such as bacteria, viruses, fungi, protozoa, and worms[1]. The burden of infectious diseases remains very high, as reported by the Global Research on Antimicrobial Resistance (GRAM) study, which found that in 2019 there were 13.7 million deaths from infectious diseases, with 7.7 million of these deaths caused by 33 pathogenic bacteria, including over one million deaths caused by *Staphylococcus*

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aureus[2]. In Indonesia, bacterial infections also remain a major cause of various infections, particularly in chronic wounds in patients with diabetes mellitus. Research by Zuliana et al. (2023) reported that of 40 diabetic ulcer samples at Sidoarjo Hospital, 22.5% were caused by *Staphylococcus aureus* and 7.5% by *Pseudomonas aeruginosa*[3].

Staphylococcus aureus is a Gram-positive, coccus-shaped, facultative aerobic bacterium that can cause infections ranging from mild to severe systemic disease[4]. Meanwhile, *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped, aerobic bacterium that often causes opportunistic infections in individuals with weakened immune systems, such as urinary tract infections, pneumonia, burn infections, chronic diabetic wounds, and infections in AIDS patients[5]. Both bacteria are generally treated with antibiotics. However, inappropriate, irrational, or excessive antibiotic use has accelerated the emergence of antimicrobial resistance[4,6].

One form of resistance of concern is Methicillin-Resistant *Staphylococcus aureus* (MRSA), a strain of *S. aureus* resistant to β-lactam antibiotics, including penicillin, cephalosporins, carbapenems, and monobactams[7]. Based on a global meta-analysis, MRSA prevalence reached 14.69%[8], while in Indonesia it was reported to reach 52%[9]. In addition, Multidrug-Resistant *Pseudomonas aeruginosa* (MDR *P. aeruginosa*) is a strain resistant to three or more classes of antibiotics, such as β-lactams, penicillins, aminoglycosides, and fluoroquinolones. This resistance occurs through various mechanisms, including the production of β-lactamase enzymes (AmpC and ESBL), changes in membrane porins, increased efflux pumps, and biofilm formation[10]. In Indonesia, Estiningsih et al. (2016) reported that MDR bacteria in NICU patients at Dr Soeradji Tirtonegoro General Hospital were dominated by Pseudomonas sp. strains, accounting for 26.9%[11]. The increasing incidence of antibiotic resistance has prompted the need to develop new, more effective, and safer antibacterial sources derived from natural sources[12].

Natural materials, particularly plants, produce various secondary metabolites in the form of bioactive compounds with antibacterial Activity and the potential to serve as an alternative to overcome antibiotic resistance. In addition to their good biological Activity, natural antibacterials are also relatively safer because they have fewer side effects than synthetic antibiotics[13]. One group of plants widely used as a source of antibacterial compounds is the Citrus genus. Citrus is a plant with high diversity and widespread distribution in Indonesia, including kaffir lime (*Citrus hystrix* DC.). In South Kalimantan, *Citrus hystrix* DC. is locally known as limau kuit and is widely utilised by the local community. Limau kuit is particularly associated with Sungai Tuan Village, Astambul District, Banjar Regency, which serves as a production and marketing area for this local citrus plant[14]. Although *C. hystrix* is widely known internationally as kaffir lime, this study uses the term limau kuit to refer specifically to the locally sourced plant material investigated.

The leaves of limau kuit are commonly utilised and can be processed to obtain essential oil[14]. In the present study, the botanical identity of the plant material was confirmed as *Citrus hystrix* DC through botanical identification before essential oil extraction.

Essential oils are secondary plant metabolites in the form of volatile aromatic liquids and contain various bioactive compounds, such as terpenes, alcohols, aldehydes, ketones, and phenols, which are known to have antibacterial Activity[15]. The essential oil

of lime leaves contains the main compounds in the form of γ-terpinene, prehnitene, (+)-4-karena, β-cis-osimene, and β-pinene, which are included in the monoterpene or terpenoid group[14]. Terpenoid compounds are known to work as antibacterials through the mechanism of damaging bacterial cell membranes, increasing

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Various studies have shown that essential oils from various plants have antibacterial Activity against pathogenic bacteria. Mohideen et al. (2022) reported that the essential oil of kaffir lime (*Citrus hystrix*) peel at a concentration of 40% produced an inhibition zone diameter of 19.3 mm against *Staphylococcus aureus* using the disc diffusion method[17]. Research by Ropiqa et al. (2023) showed that the essential oil of Pontianak orange (*Citrus nobilis Lour*. var. microcarpa) peel at a concentration of 50% produced an inhibition zone diameter of 10.27 mm against *Staphylococcus aureus*[18]. Furthermore, Kindangen et al. (2018) reported that kalamansi orange (*Citrus microcarpa Bunge*.) peel essential oil at 100% produced an inhibition zone diameter of 11.16 mm against *Staphylococcus aureus* using the well diffusion method[19]. For MDR *Pseudomonas aeruginosa*, mint leaf essential oil (*Mentha piperita* L.) had an MIC of 4% and an MBC of 8%[20]. Furthermore, grapefruit peel essential oil (Citrus maxima) at 80% and 100% concentrations produced inhibition zone diameters of 6.45 mm and 7.15 mm, respectively[21], while cinnamon essential oil (*Cinnamomum verum*) at 100% concentration produced an average inhibition zone diameter of 24.72 mm against 72 MDR *Pseudomonas aeruginosa* isolates[22].

Although various studies have reported the antibacterial Activity of essential oils from several plant species, information on the effectiveness of essential oil from lime leaves (*Citrus hystrix* DC.) against antibiotic-resistant pathogenic bacteria, particularly MRSA and MDR *Pseudomonas aeruginosa*, remains very limited. However, the dominant monoterpene content in lime leaves essential oil shows promising potential as a natural antibacterial source. Therefore, this study aims to analyse the antibacterial Activity of lime leaves essential oil (*Citrus hystrix* DC.) against the growth of antibiotic-resistant bacteria by testing various concentrations using the well diffusion method.

MATERIALS AND METHODS

This is a true experimental study with a posttest-only control group design to evaluate the antibacterial efficacy of lime leaf essential oil (*Citrus hystrix* DC.) on the growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and MDR *P. aeruginosa*. The study was conducted from October 2025 to January 2026 in the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, for essential oil extraction, and in the Bacteriology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Banjarmasin, for antibacterial activity testing.

The plant material used in this study was limau kuit, a locally recognised *Citrus* plant obtained from Astambul District, Banjar Regency, South Kalimantan, at an altitude of approximately 10–15 m above sea level. The leaves were selected based on the following criteria: fresh, free from pest infestation, and free from visible holes or physical damage. Botanical identification was conducted at the Basic Laboratory, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, and the plant specimen was identified as *Citrus hystrix* DC. The local name limau kuit is retained throughout this manuscript to refer specifically to the plant material investigated in this study.

Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the Microbiology Laboratory of Ulin Hospital, Banjarmasin. The *S. aureus* isolate was recovered from a wound swab collected on August 27, 2025, and identified using the VITEK system with a 95% probability of identification. The isolate was classified as methicillin-resistant *S. aureus* (MRSA) based on a positive cefoxitin screen and oxacillin resistance (MIC ≥4 µg/mL), with the laboratory report indicating a positive MRSA phenotype.

The *P. aeruginosa* isolate was recovered from a wound specimen collected on November 29, 2024, and identified using the VITEK system with a 90% probability of identification. Antimicrobial susceptibility testing showed resistance to piperacillin/tazobactam (MIC ≥128 µg/mL), cefepime (MIC 32 µg/mL), aztreonam (MIC ≥64 µg/mL), meropenem (MIC ≥16 µg/mL), amikacin (MIC ≥64 µg/mL), and ciprofloxacin. In contrast, ceftazidime showed an intermediate interpretation (MIC 16 µg/mL). Based on non-susceptibility to antimicrobial agents from at least three antimicrobial classes, the isolate was classified as multidrug-resistant (MDR) *P. aeruginosa*. This study has received ethical approval from the Ethics Commission of Poltekkes Kemenkes Banjarmasin under number 732/KEPK-PKB/2025 and 734/KEPK-PKB/2025.

Essential oils were obtained through steam-water distillation. A total of 1,500 g of lime leaves were cleaned, wilted for one week at room temperature, and then cut into approximately 0.5 cm pieces. The samples were distilled for 6 hours, maintaining the condenser temperature at 40–60°C. The distillate was separated using a separating funnel, and the essential oil was purified using anhydrous sodium sulfate (1–3 g/L), stored in dark-colored vials, and wrapped in aluminium foil until use. The essential oil yield was calculated as the ratio of the volume of oil obtained to the initial weight of the leaf sample.

Antibacterial Activity was evaluated using the well diffusion method on Mueller–Hinton Agar (MHA) with an agar thickness of 4 mm. For each bacterial isolate, 180 µL of the 0.5 McFarland suspension was evenly inoculated onto the MHA surface using a sterile cotton swab and allowed to stand for 5 minutes. Eight wells (6 mm diameter) were then prepared, with a minimum distance of 15 mm between wells. The wells consisted of six essential oil concentrations (50%, 60%, 70%, 80%, 90%, and 100%), one positive control, and one negative control. Each well was filled with the corresponding test solution. Ciprofloxacin at 5 µg was used as the positive control for MRSA, while ceftazidime was used as the positive control for MDR *P. aeruginosa*. The negative control consisted of pure DMSO. The plates were allowed to stand for 10 minutes to permit pre-diffusion of the test solutions into the agar and were subsequently incubated at 37°C for 18 hours. Each treatment was performed in four replicates. The inhibition zone diameter was

measured with a digital calliper and expressed in millimetres (mm). Measurements were taken across the entire clear zone, including the 6-mm well diameter.

Data were analysed using IBM SPSS Statistics. MRSA data were analysed using a Welch ANOVA followed by a Games–Howell post hoc test, while MDR *Pseudomonas aeruginosa* data were analysed using a Kruskal–Wallis test followed by a Mann–Whitney test. A p-value <0.05 was set as the limit of statistical significance.

RESULTS AND DISCUSSION

The limau kuit leaves (*Citrus hystrix* DC.) used in this study were obtained from Astambul District, Banjar Regency, South Kalimantan. Samples were selected based on the criteria of fresh leaves that were pest-free and free of holes. Botanical identification (determination) was conducted at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, and confirmed that the species used was *Citrus hystrix* DC.

A total of 1,500 g of fresh limau kuit leaves decreased to 835 g after wilting for one week at room temperature. The wilted leaves were then subjected to steam-water distillation, yielding 9.0 mL of essential oil (0.60%, v/w). The resulting essential oil was light yellow in colour with a distinctive limau kuit aroma.

Table 1. Inhibition Zone Diameter of *Citrus hystrix* DC. Leaf Essential Oil Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Essential Oil Concentration (%)	Inhibition Zone (mm) Repetition				Mean (mm)
	I	II	III	IV	
50	14.3	13.7	13.6	13.9	13.88
60	17.7	18.2	17.6	18.2	17.93
70	19.3	19.4	19.4	19.1	19.30
80	19.8	19.6	19.9	19.7	19.75
90	22.6	21.7	21.8	22.8	22.23
100	15.7	17.8	14.5	15.5	15.88
Positive control	25.6	25.8	25.5	26.8	25.93
Negative control	0	0	0	0	0

Table 2. Inhibition Zone Diameter of *Citrus hystrix* DC. Leaf Essential Oil Against Multidrug-Resistant *Pseudomonas aeruginosa*

Essential Oil Concentration (%)	Inhibition Zone (mm) Repetition				Mean (mm)
	I	II	III	IV	
50	10.0	11.0	10.0	11.6	10.65
60	13.0	12.8	13.0	13.0	12.95
70	14.0	14.0	13.5	13.3	13.70
80	14.3	14.3	13.8	13.5	13.98
90	13.8	14.1	13.6	14.0	13.88
100	13.4	13.8	12.0	11.8	12.75
Positive control	24.0	25.0	25.0	24.6	24.65
Negative control	0	0	0	0	0

The antibacterial activity of lime leaf essential oil (*Citrus hystrix* DC.) against MRSA and MDR *P. aeruginosa* is presented in Tables 1 and 2. For both test bacteria, all essential oil concentrations produced inhibition zones, while the negative control (DMSO) showed no inhibition zone formation. In contrast, the positive control produced the largest inhibition zone diameter for both bacteria.

According to Table 1, the average inhibition zone diameter against MRSA increased with increasing essential oil concentration from 50% to 90% (13.88 mm to 22.23 mm), then decreased to 15.88 mm at 100% concentration. The largest inhibition zone diameter was obtained at 90% concentration, while the lowest was obtained at 50%. Meanwhile, Table 2 shows that the average inhibition zone diameter against MDR *P. aeruginosa* ranged from 10.65–13.98 mm. The inhibition zone diameter increased from 50% to 80% concentration, then decreased slightly at 90% and 100% concentrations. The largest inhibition zone diameter was obtained at 80% concentration (13.98 mm), while the smallest was obtained at 50% concentration (10.65 mm).

In general, lime leaf essential oil exhibited antibacterial activity against both test bacteria. However, the inhibition zone diameter formed for MRSA was larger than for MDR *P. aeruginosa* at all concentrations, indicating that lime leaf essential oil has higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria.

Figure 1 illustrates the inhibition zones produced by *Citrus hystrix* DC. leaf essential oil against (A) methicillin-resistant *Staphylococcus aureus* (MRSA) and (B) multidrug-resistant *Pseudomonas aeruginosa*. Clear inhibition zones were observed around the essential oil treatments, whereas no inhibition zone was observed around the negative control (DMSO). The positive control showed the largest inhibition zone for both bacterial isolates. For MRSA, the inhibition zone increased with increasing essential oil concentration up to 90%, which produced the largest inhibition zone, followed by a decrease at 100% concentration.

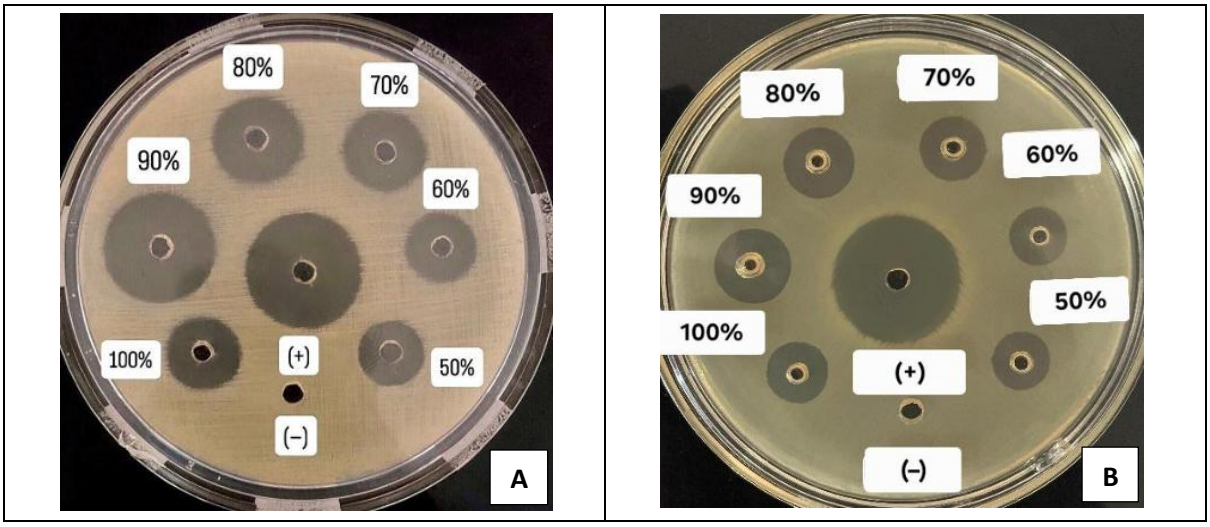

Figure 1. Inhibition zones of *Citrus hystrix* DC. leaf essential oil against (A) methicillin-resistant *Staphylococcus aureus* (MRSA) and (B) multidrug-resistant *Pseudomonas aeruginosa*.

The Shapiro–Wilk normality test showed that the inhibition zone diameter data were normally distributed (p = 0.138). However, the homogeneity test indicated that the variance between groups was not homogeneous (p = 0.018). Therefore, the analysis of differences between groups continued using Welch ANOVA, which is appropriate for normally distributed data but does not meet the assumption of homogeneity of variance.

The Welch ANOVA results showed a significant difference in the inhibition zone diameter of lime leaf essential oil against the growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among the various concentrations tested (p < 0.001) (Table 3).

Further analysis using the Games–Howell post hoc test (Table 4) showed that most concentration pairs differed significantly in inhibition zone diameter (p < 0.05). No significant differences were found only between the concentrations of 50% and 100% (p = 0.237), 60% and 100% (p = 0.237), and 70% and 100% (p = 0.068). These results indicate that increasing essential oil concentration does not always increase antibacterial activity, as shown by the smaller inhibition zone diameter at 100% than at 90%. Conversely, the 90% concentration produced significantly higher antibacterial activity than most other concentrations.

Table 3. Statistical Analysis of the Inhibition Zone of *Citrus hystrix* DC. Leaf Essential Oil Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Statistical Test	*p*-value	Interpretation
Shapiro–Wilk normality test	0.138	Normally distributed
Homogeneity of variance test	0.018	Variances were not homogeneous
Welch ANOVA	<0.001	Significant difference among treatment groups

Table 4. Games–Howell Post Hoc Analysis of the Inhibition Zone Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Comparison	*p*-value	Interpretation
50% vs 60%	<0.001	Significant
50% vs 70%	<0.001	Significant
50% vs 80%	<0.001	Significant
50% vs 90%	<0.001	Significant
50% vs 100%	0.237	Not significant
60% vs 70%	0.007	Significant
60% vs 80%	0.003	Significant
60% vs 90%	0.001	Significant
60% vs 100%	0.237	Not significant
70% vs 80%	0.024	Significant
70% vs 90%	0.008	Significant
70% vs 100%	0.068	Not significant
80% vs 90%	0.013	Significant
80% vs 100%	0.049	Significant
90% vs 100%	0.005	Significant

The normality test indicated that the inhibition zone diameter data were not normally distributed ($p < 0.001$), while the homogeneity test indicated that the variance between groups was not homogeneous ($p < 0.001$). Therefore, we used the Kruskal–Wallis test to compare groups, as the data did not meet the assumptions of normality and homogeneity.

The Kruskal–Wallis test results showed a significant difference in the inhibition zone diameter of lime leaf essential oil on the growth of Multidrug-Resistant *Pseudomonas aeruginosa* (MDR *P. aeruginosa*) at various concentrations tested ($p = 0.003$) (Table 5).

Further analysis using the Mann–Whitney test (Table 6) showed significant differences primarily between the 50% and 60% concentrations and the higher concentrations. All comparisons between 50% and 60–100% concentrations and between 60% and 70–90% concentrations showed significant differences ($p < 0.05$). Conversely, there was no significant difference between 60% and 100% concentrations ($p = 1.000$), or between 70%, 80%, 90%, and 100% concentrations ($p > 0.05$). These results indicate that increasing the concentration of essential oils above 70% did not significantly increase antibacterial Activity against MDR *P. aeruginosa*, so the antibacterial effect tended to reach its optimum condition within that concentration range.

Table 5. Statistical Analysis of the Inhibition Zone of *Citrus hystrix* DC. Leaf Essential Oil Against Multidrug-Resistant *Pseudomonas aeruginosa*

Statistical Test	*p*-value	Interpretation
Normality test	<0.001	Not normally distributed
Homogeneity of variance test	<0.001	Variances were not homogeneous
Kruskal–Wallis test	0.003	Significant difference among treatment groups

Table 6. Mann–Whitney Post Hoc Analysis of the Inhibition Zone Against Multidrug-Resistant *Pseudomonas aeruginosa*

Comparison	*p*-value	Interpretation
50% vs 60%	0.017	Significant
50% vs 70%	0.019	Significant
50% vs 80%	0.019	Significant
50% vs 90%	0.020	Significant
50% vs 100%	0.020	Significant
60% vs 70%	0.017	Significant
60% vs 80%	0.017	Significant
60% vs 90%	0.018	Significant
60% vs 100%	1.000	Not significant
70% vs 80%	0.304	Not significant
70% vs 90%	0.375	Not significant
70% vs 100%	0.146	Not significant
80% vs 90%	0.661	Not significant
80% vs 100%	0.058	Not significant
90% vs 100%	0.059	Not significant

This study shows that lime leaf essential oil (*Citrus hystrix* DC.) has antibacterial Activity against two antibiotic-resistant bacteria, namely methicillin-resistant

Staphylococcus aureus (MRSA) and MDR *Pseudomonas aeruginosa*. The essential oil yield obtained in this study was 0.60% (v/w), which is higher than the yield obtained in the study by Panjaitan and Irwan (2023), which was 0.59% (v/w)[14]. Variations in essential oil yield can be influenced by various factors, including soil conditions and altitude, leaf age, maturity at harvest[23], climate conditions and harvest season[24], plant geographic origin, rainfall intensity, soil organic carbon, soil pH, nitrogen, phosphorus, and magnesium levels[25], and technical factors such as sample preparation, material size, distillation time, and sample conditions during the extraction process[26].

Statistical analysis showed that lime leaf essential oil significantly affected the growth of both bacteria. For MRSA, the data were normally distributed but not homogeneous. Therefore, analysis using Welch ANOVA showed a significant difference between treatment groups ($p<0.001$), followed by a Games–Howell test. These results align with Brożyna et al. (2025)27, who used Welch ANOVA on data with non-homogeneous variance and obtained significant results. For MDR *P. aeruginosa*, the data were not normally distributed or homogeneous, so they were analysed using the Kruskal–Wallis test, which also showed a significant difference between groups ($p=0.003$), followed by the Mann–Whitney test. Overall, the statistical results confirmed that variations in essential oil concentration affected antibacterial Activity against both bacteria.

Increasing essential oil concentration increased antibacterial Activity until it reached an optimum concentration, followed by a decrease at higher concentrations. For MRSA, the inhibition zone diameter increased from 50% concentration to a peak at 90% concentration (22.23 mm), while for MDR *P. aeruginosa*, it increased up to 80% concentration (13.98 mm). These results support Chamidah et al. (2017), who stated that increasing concentration increases the amount of antibacterial compounds available to diffuse into the medium and interact with bacterial cells[27]. However, the response of MDR *P. aeruginosa* was not consistently proportional to the increase in essential oil concentration, as the inhibition zone decreased slightly at 90% and 100%. This pattern is consistent with Brożyna et al. (2025), who reported substantial variability in *P. aeruginosa* susceptibility to essential oils, with MIC values differing by up to 1,000-fold among strains[28]. These findings suggest that *P. aeruginosa*'s antibacterial response to essential oils may not depend solely on concentration but may also be influenced by bacterial characteristics.

The decrease in Activity at the highest concentration is likely related to the physical properties of the essential oil. Reducing the proportion of DMSO makes the essential oil more concentrated, increasing its viscosity and potentially reducing the diffusion of active compounds into the agar medium. Kruk et al. (2022) explained that increasing viscosity can inhibit molecular diffusion[29], while Yunilawati et al. (2021) stated that the hydrophobic nature of essential oils can limit their distribution in water-based media[30]. Hulankova (2024)23 also explained that DMSO facilitates the homogeneous dispersion of essential oils, thereby supporting the diffusion of active compounds into the agar medium[23]. Furthermore, Angane et al. (2022) reported that increased viscosity at high concentrations can reduce the diffusion area and consequently decrease the inhibition zone diameter[24].

Differences in the Activity of essential oils against the two bacteria are closely related to the characteristics of their cell walls and resistance mechanisms. MRSA, as a

Gram-positive bacterium, has a simpler cell wall without an outer membrane, allowing lipophilic compounds in essential oils to reach the cytoplasmic membrane more easily. Conversely, MDR *P. aeruginosa*, a Gram-negative bacterium, has an outer membrane containing lipopolysaccharide (LPS), which limits the penetration of hydrophobic compounds. This explains why the inhibition zone diameter in MRSA is consistently larger than in MDR *P. aeruginosa*[31,32].

In addition to cell wall structure, the two bacteria also differ in resistance mechanisms. MRSA resistance is primarily due to expression of the mecA gene, which produces the PBP2a protein and causes β-lactam antibiotics to lose affinity for their targets. However, this mechanism does not protect the bacteria against compounds that damage the cell membrane. Therefore, monoterpenes in essential oils can still exert antibacterial effects against MRSA[24,33]. In contrast, MDR *P. aeruginosa* has more complex resistance mechanisms, including biofilm formation that inhibits antimicrobial penetration[34] and efflux pump activity that actively releases antimicrobial compounds from within the cell[10]. This combination of mechanisms likely explains the lower Activity of essential oils against MDR *P. aeruginosa* compared to MRSA.

The antibacterial Activity of lime leaf essential oil is thought to originate from a combination of various active compounds. Based on research by Afifah (2025), the main components of essential oils include γ-terpinene, β-cymene, 3-carene, α-thujene, and o-cymene, which belong to the monoterpene hydrocarbon group[35]. These compounds work by increasing membrane permeability, disrupting enzyme function, and causing leakage of intracellular components[36]. Furthermore, essential oils also contain thymol, a phenolic monoterpene with stronger antibacterial Activity through membrane depolarisation, increased permeability, and inhibition of biofilm formation[24,37]. Although monoterpene hydrocarbons individually have lower Activity than phenolic compounds[38], the combination of all essential oil components can produce a synergistic effect, enhancing antibacterial Activity[39].

This study has several limitations. First, antibacterial Activity was evaluated only using the well diffusion method, which did not allow determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Second, the chemical composition of essential oils was not analyzedanalyzed directly in the research samples, but was inferred from GC–MS results from previous studies at the same sampling site. Third, this study used only one MRSA isolate and one MDR *P. aeruginosa* isolate, so it could not evaluate variation in response between clinical isolates. Furthermore, the study was limited to in vitro testing, so in vivo conclusions cannot be drawn about the effectiveness and safety of essential oils.

The results indicate that lime leaf essential oil has potential as a natural antibacterial agent against antibiotic-resistant bacteria, particularly MRSA. However, its Activity against MDR *P. aeruginosa* is still lower than that of standard antibiotics. These findings provide a scientific basis for developing lime leaf essential oil as a candidate for adjunctive therapy or a source of new antibacterial compounds. Further research should identify the active components directly using GC–MS, determine MIC and MBC values, evaluate antibiofilm activity, test for synergistic effects with antibiotics, and conduct in vivo testing to confirm its therapeutic potential.

CONCLUSION

Lime leaf essential oil (*Citrus hystrix* DC.) has antibacterial Activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Pseudomonas aeruginosa*. The highest Activity against MRSA was achieved at a concentration of 90%, while against MDR *P. aeruginosa* it was 80%. The essential oil's Activity against MRSA was higher than against MDR *P. aeruginosa*, possibly due to differences in cell wall structure and resistance mechanisms between the two bacteria. These findings suggest that lime leaf essential oil has potential as a natural antibacterial source for developing therapies against antibiotic-resistant bacteria.

CONFLICT OF INTEREST

In this study there is no conflict of interest

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