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Comparison of antibacterial activities of lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*

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Abstract

Lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices have the potential to be used as natural alternative therapies for infections caused by Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. The aim of this research was to compare antibacterial activities between lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria. This was a laboratory experimental study. In vitro testing was carried out using the diffusion method. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* were cultured on Mueller Hinton Agar (MHA) media. The diameter of the inhibition zones produced on the culture media after being treated with multiple concentrations of lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices were measured. All concentrations of lime juice and lemon juice (25%, 50%, 75%, and 100%) produced the diameter of the inhibition zones in the culture media of the three bacteria, with the greatest diameter seen at the concentration of 100%. Lime juice and lemon juice affects the diameter of the three bacteria's inhibition zones, and there is a significant difference between the two, especially at a concentration of 100% on the growth of Escherichia coli. Against *Staphylococcus aureus*, lime juice has an antibacterial effectiveness range of 23-12mm. Meanwhile, lemon juice has a range of 21-12 mm for the same bacteria and concentration. For *Pseudomonas aeruginosa*, the antibacterial effectiveness range of lime juice is 21-13 mm and lemon juice is 20-11 mm. Lime juice and lemon juice have antibacterial effectiveness ranges of 18-10 mm and 16-9 mm respectively against *Escherichia coli*. The antibacterial effectiveness value of lime juice against the three bacteria tested was always slightly higher than lemon juice.

Keywords: Antibacterial; Lime (*Citrus aurantifolia)*; Lemon (*Citrus limon)*; Juice

1. Introduction

Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Escherichia coli* are three of the top five bacteria responsible of infectious diseases that cause half of these deaths [1]. These bacteria are well recognized as important human pathogens. Staphylococcus aureus is capable of causing a wide range of infectious diseases, including skin and soft tissue infections, endocarditis, osteomyelitis, bacteremia, and life-threatening pneumonia [2]. Bloodstream infections caused by Escherichia coli represent one of the most common sources of infection [3], and this bacterium is estimated to cause millions of cases and hundreds of thousands of deaths in developing countries due to infectious diarrheal diseases, particularly among young children [4]. Pseudomonas aeruginosa accounts for approximately 10–20% of nosocomial infections and is considered a hallmark of opportunistic hospital-acquired infections, often causing severe and life-threatening conditions in immunocompromised patients [5].

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Previous study shows that there has been resistance in Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa to the use of antibiotics. Therefore, other alternatives are needed to overcome this problem [6], [7], [8]

Natural products is one of the potential alternatives that can help to overcome this problem. Several herbal plants contain citric acid and flavonoids that have been reported to inhibit bacterial growth [9]. These compounds are widely distributed in plants of the genus Citrus, including oranges, lemons, and grapes [10].

Lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices are known to have antibacterial compounds, these compounds include citric acid and flavonoids [11]. Although limes and lemons have many similarities in terms of content and almost the same properties, they show differences in antibacterial activity [12], [13], [14], [15]

Based on this background, research was conducted with the aims to compare antibacterial activities between lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* bacteria.

2. Material and methods

2.1. Study Design and Setting

This study was conducted in the Microbiology Laboratory, Faculty of Medicine, Airlangga University, from June to August 2024. This study used a true experimental in-vitro laboratory design with a post-test only control group design to evaluate the antibacterial activity of lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juice against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. This study had received ethical clearance from the Ethics Committee for Health Research, Faculty of Medicine, Universitas Airlangga, Surabaya.

2.2. Collection of test pathogenic microorganisms

Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli bacterial isolates were obtained from the Microbiology Lab Faculty of Medicine, Airlangga University.

2.3. Collection of plant materials

Limes from the *Citrus aurantifolia* species are taken from fresh lime plantations in the Mojokerto area. Lemons from the *Citrus limon* species were taken from the Plant Research Institute Oranges and Subtropical Fruit (BSIP Jestro) Batu city, Malang.

2.4. Preparation of extracts

Limes and lemons are washed using clean water. Lime and lemon are cut using a knife. After being cut, they are squeezed using an orange squeezer. The squeezed product is collected in a container and given an appropriate name using a marker. Lime and lemon juice was obtained in each container with a concentration of 100% without any additional solution.

2.5. Checking for contaminants from research materials

Checking for contaminants is done by taking lime juice or lemon juice with a hose wire that has been heated using a spirit bus and then rubbing it on Mueller-Hinton Agar (MHA) media. After that, the media was put into an incubator machine and incubated at 37℃for 24 hours

2.6. Re-filtering of research materials

Re-filtering research materials for lime juice or lemon juice that still have contaminants is by using a micro syringe membrane filter. Filtering is done by taking research material that is still contaminant using a syringe, then directing it to a micro syringe membrane filter and collecting it in a new container.

2.7. Making lime and lemon juice concentration

The concentration of lime and lemon juice to be made are 25%, 50%, 75%, and 100%. To obtain this concentration, dilution is carried out using aquades (distilled water) as a solvent. Determining the concentration of lime and lemon using the dilution formula M1 x V1 = M2 x V2.

2.8. Making bacterial suspensions

Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli bacterial suspensions are made by embedding 1-2 bacteria in Nutrient Broth (NB) liquid media and then combining them with the help of a vortex. After that, the turbidity was measured until it reached the McFarland standard of 0.5.

2.9. Culture of bacterial suspension

Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli isolates were each spread onto Mueller Hinton Agar (MHA) media using a cotton swab that had been dipped in the bacterial suspension.

2.10. Antibacterial test of limes and lemons

Bacterial cultures of Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli were taken which had dried on MHA solid media. Holes or wells are made using a cork borer with a diameter of ±7.5mm. Add lime and lemon juice with concentrations ranging from 25%, 50%, 75%, to 100% into MHA media containing bacteria using a micro pipette and yellow tip. For lime juice there were 18 trials as well as lemon juice. So the total media for lime juice and lemon juice alone was 36 trials. Incubate the media at 37°C for 24 hours.

2.11. Positive and negative control

The positive control used for each bacteria is different. Erythromycin as a positive control for Staphylococcus auerus, Ciprofloxacin for Pseudomonas aeruginosa, and Gentamicin for Escherichia coli bacteria. All positive controls were in the form of antibiotic discs. Meanwhile, the negative control used for this research was aquades.

2.12. Inhibition zone measurement

The inhibition zone appears clearer than the surrounding area and is not overgrown with bacteria. The diameter of the inhibition zone is circular around the well and the distance is measured using millimeters (mm). Measurement of the diameter of the inhibition zone is carried out visually by reading the numbers that appear on the digital caliper.

2.13. Data Analysis

The data obtained from this study were analyzed using a normality test at a 95% confidence level. Data categorization was performed using Microsoft Excel 2019. Normality testing was conducted using the Shapiro–Wilk test. The Kruskal–Wallis test was applied to analyze the effectiveness of the treatments. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 27 (International Business Machines Corporation, IBM), with a significance level set at $p < 0.05$.

3. Results and discussion

The antibacterial effects of limes (*Citrus aurantifolia*) and lemons (*Citrus limon*) against *Staphylococcus aureus* are presented in Tables 1 and 2.

Table 1 Antimicrobial activity of limes (*Citrus aurantifolia*) against *Staphylococcus aureus*

No.	Concentration	Mean of inhibition zone ± SD (mm)
1.	25% [a]	11.69 ± 5.28
2.	50% [b]	17.69 ± 5.28
3.	75% [c]	21.30 ± 5.28
4.	100% [c]	23.22 ± 5.28

Note: Differences in notation (a, b, or c) indicate significant differences. Tested by ANOVA test ($p \leq 0.05$).

Table 2 Antimicrobial activity of lemons (*Citrus limon*) against *Staphylococcus aureus*

No.	Concentration	Mean of inhibition zone ± SD (mm)
1.	25% [a]	11.70 ± 4.02
2.	50% [b]	16.19 ± 4.02
3.	75% [c]	18.78 ± 4.02
4.	100% [c]	21.07 ± 4.02

Note: Differences in notation (a, b, or c) indicate significant differences. Tested by ANOVA test ($p \leq 0.05$).

Lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) are natural materials with potential use as alternative treatments for bacterial infections[9]. Based on the results of this study, both lime and lemon demonstrated effective antibacterial activity against *Staphylococcus aureus* at concentrations of 25%, 50%, 75%, and 100% (Table 1 and 2, Figure 1A and 1B). These findings are consistent with previous studies reporting that lime and lemon extracts or juices are capable of inhibiting the growth of *Staphylococcus aureus*, with inhibition zones increasing proportionally to higher extract or juice concentrations. This antibacterial activity is associated with the presence of bioactive compounds such as citric acid, flavonoids, tannins, and essential oils, which are known to disrupt the cell wall of gram-positive bacteria such as *Staphylococcus aureus* [16], [17], [18]

The antibacterial effects of limes (*Citrus aurantifolia*) and lemons (*Citrus limon*) against *Pseudomonas aeruginosa* are presented in Tables 3 and 4.

Table 3 Antimicrobial activity of limes (*Citrus aurantifolia*) against *Pseudomonas aeruginosa*

No.	Concentration	Mean of inhibition zone ± SD (mm)
1.	25% [a]	13.08 ± 3.79
2.	50% [a]	15.44 ± 3.79
3.	75% [b]	18.48 ± 3.79
4.	100% [c]	21.49 ± 3.79

Note: Differences in notation (a, b, or c) indicate significant differences. Tested by ANOVA test ($p \leq 0.05$).

Table 4 Antimicrobial activity of lemons (*Citrus limon)* against *Pseudomonas aeruginosa*

No.	Concentration	Mean of inhibition zone ± SD (mm)
1.	25% [a]	11.14 ± 3.71
2.	50% [b]	16.78 ± 3.71
3.	75% [c]	19.00 ± 3.71
4.	100% [d]	20.34 ± 3.71

Note: Differences in notation (a, b, c, or d) indicate significant differences. Tested by ANOVA test ($p \leq 0.05$).

Lime and lemon at concentrations of 25%, 50%, 75%, and 100% also exhibited antibacterial effects against *Pseudomonas aeruginosa* (Table 3 and 4, Figure 1C and 1D). This result is supported by previous studies indicating that lemon juice, particularly in the form of ethanolic extracts, shows significant antibacterial activity against *Pseudomonas aeruginosa*. The observed inhibition zones and minimum inhibitory concentration (MIC) values suggest increased bacterial sensitivity with rising extract concentrations. This activity is thought to be related to the strong acidic nature of lemon juice and the presence of flavonoids, which may interfere with the integrity of the gram-negative bacterial cell membrane [19]

The antibacterial effects of limes (*Citrus aurantifolia*) and lemons (*Citrus limon*) against *Escherichia coli* are presented in Tables 5 and 6.

Table 5 Antimicrobial activity of limes (*Citrus aurantifolia*) against *Escherichia coli*

No.	Concentration	Mean of inhibition zone ± SD (mm)
1.	25% [a]	9.94 ± 3.55
2.	50% [b]	14.35 ± 3.55
3.	75% [c]	16.53 ± 3.55
4.	100% [d]	18.65 ± 3.55

Note: Differences in notation (a, b, c, or d) indicate significant differences. Tested by ANOVA test ($p \leq 0.05$).

Table 6 Antimicrobial activity of lemons (*Citrus limon*) against *Escherichia coli*

No.	Concentration	Mean of inhibition zone ± SD (mm)
1.	25% [a]	9.09 ± 2.92
2.	50% [b]	12.48 ± 2.92
3.	75% [c]	14.55 ± 2.92
4.	100% [c]	15.93 ± 2.92

Note: Differences in notation (a, b, or c) indicate significant differences. Tested by ANOVA test ($p \leq 0.05$).

The effectiveness test of lime and lemon against *Escherichia coli* demonstrated that both citrus fruits at concentrations of 25%, 50%, 75%, and 100% contain antibacterial substances capable of inhibiting bacterial growth (Table 5 and 6, Figure 1E and 1F). These findings are in accordance with previous studies reporting that lemon and lime juices possess antibacterial activity against *Escherichia coli*. This effect is presumed to be associated with their acidic properties and the presence of bioactive compounds such as flavonoids and citric acid, which contribute to the inhibition of bacterial growth [18], [19], [20]

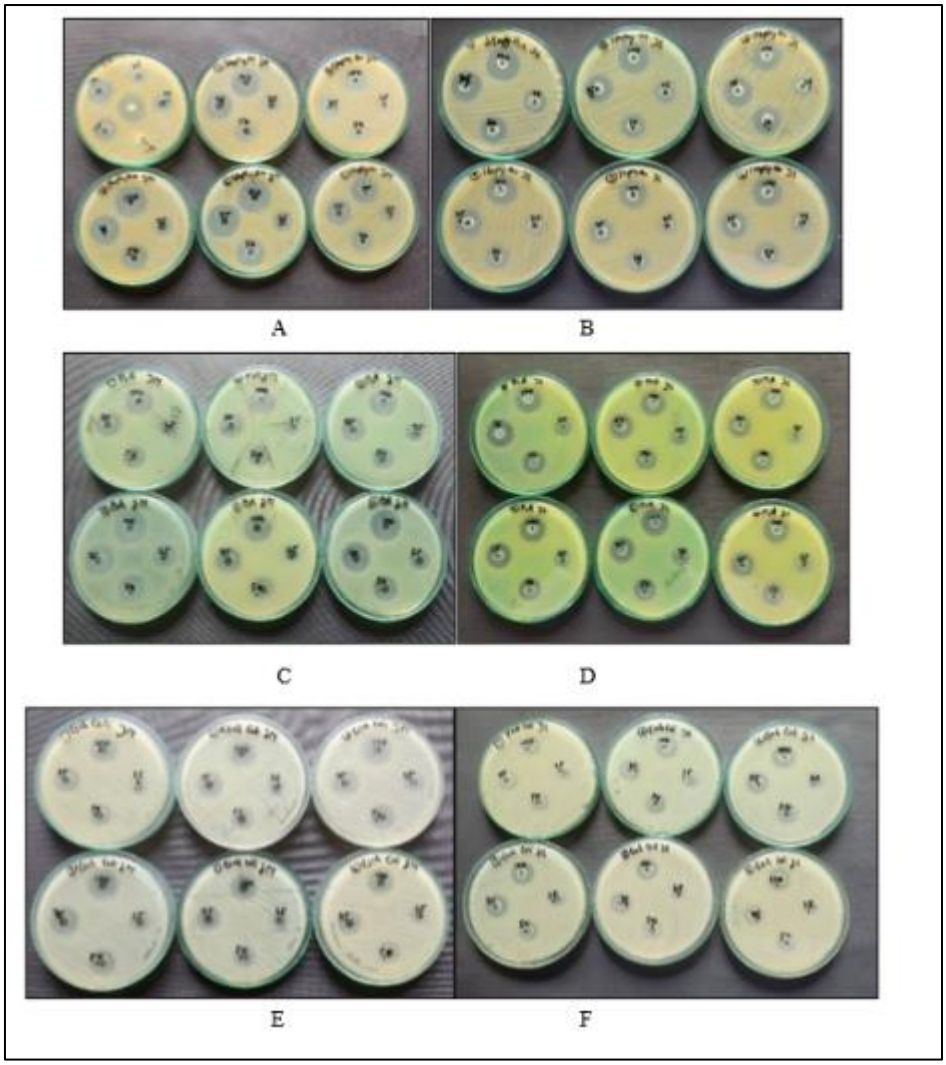

Figure 1 Inhibition zone of limes (*Citrus aurantifolia)* and lemons (*Citrus limon*) juice against three bacteria. A and B *Staphylococcus aureus* bacteria. C and D *Pseudomonas aeruginosa* bacteria. E and F *Escherichia coli* bacteria.

Table 7 Comparation of antibacterial activity between limes (*Citrus aurantifolia)* and lemons (*Citrus limon*)

Bacteria	**Various concentrations of each lime and lemon**	***Citrus aurantifolia*** **(Mean Inhibitory Zone)**	***Citrus limon*** **(Mean Inhibitory Zone)**
Staphylococcus aureus	25%	11.69 ± 5.28	11.70 ± 4.02
	50%	17.69 ± 5.28	16.19 ± 4.02
	75%	21.30 ± 5.28	18.78 ± 4.02
	100%	23.22 ± 5.28	21.07 ± 4.02
Pseudomonas aeruginosa	25%	13.08 ± 3.79	11.14 ± 3.71
	50%	15.44 ± 3.79	16.78 ± 3.71
	75%	18.48 ± 3.79	19.00 ± 3.71
	100%	21.49 ± 3.79	20.34 ± 3.71

Escherichia coli	25%	9.94 ± 3.55	9.09 ± 2.92
	50%	14.35 ± 3.55	12.48 ± 2.92
	75%	16.53 ± 3.55	14.55 ± 2.92
	100%	18.65 ± 3.55	15.93 ± 2.92

*p-value significance at <0.05

In this study, it was found that the antibacterial effectiveness value of lime juice against the three bacteria tested was always slightly higher than lemon juice. It contradicts a study where it was found that lemon juice generally exhibited greater inhibitory effects than lime juice against *Staphylococcus aureus* and *Escherichia coli* [21]. Another study supported that lemon juice had a significantly higher inhibitory effect (P<0.05) on *Escherichia coli* O157:H7 than lime juice. Both juices achieved a 5- log reduction of stationary phase cells after 72 hours at room temperature, but the inactivation was consistently more rapid in lemon juice[22]. These results partially support the idea of citrus potency but emphasize that lemon's effectiveness is not universal across all isolates or fruit stages[21].

Lime and lemon juices has an antibacterial potency because the citric acid and flavonoids contained in it have antibacterial capabilities. The citric acid content makes the fruit acidity (pH). An acidic pH can inhibit bacterial growth and cause the internal pH of bacterial cells to decrease [16]. Flavonoids can inhibit the process of protein and nucleic acid synthesis in bacteria by destroying nucleic acids and denaturing proteins, thereby causing disruption of the bacterial protein and nucleic acid synthesis process as well as total cell damage [23].

It was shown that there were phytochemical differences between lime juice and lemon juice where ketose (fructose) and reducing sugars were found in large quantities only in lemon juice, both of these components were not found at all in lime. The saponin and terpenoid content of lemon juice was also detected to have a higher quantity than in lime. While other contents such as carbohydrates, flavonoids and cardiac glycosides in both have the same quantity [11]. This is different from other studies where cardiac glycosides content was only found in lime juice. In this study, lime juice was also detected as not containing tannins. Meanwhile, saponin content was not detected in either of them [24]. The diversity of lime juice content according is thought to be due to differences in species and geographical location of citrus fruits[25]. This statement is again supported by other studies which state that differences in the volatile profile of citrus fruits can occur due to differences in cultivars, environmental conditions, geographical origin, season, fruit ripeness, and citrus fruit extraction methods [26]

In Table 7, significant differences in effectiveness between lime and lemon juice were only found when both were tested against the growth of *Escherichia coli* bacteria at a concentration of 100%. Furthermore, in several other data from this study, many similarities were found in the effectiveness of inhibiting bacteria especially between concentrations of 75% and 100%. This finding is supported by a study conducted by Saleh in 2016, which reported that antibacterial activity did not show a significant difference among concentration variations in terms of the inhibition zone diameters against *Escherichia coli* and *Staphylococcus aureus*. However, that study employed a different material from the present research, namely the *n*-butanol fraction of libo stem bark. This phenomenon was presumably due to the narrow range of concentrations used, resulting in antibacterial activity that did not exhibit a statistically significant difference [27]. This shows that increasing the concentration of a test material does not always increase its antibacterial properties. Further studies are required with more specific subspecies of citrus with a more definite strain of bacteria.

4. Conclusion

Lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices have antibacterial substances that are effective against the growth of Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli bacteria. The antibacterial effectiveness value of lime juice against the three bacteria tested was always slightly higher than lemon juice. There is a difference in effectiveness between lime (*Citrus aurantifolia*) and lemon (*Citrus limon*) juices at a concentration of 100% against the growth of Escherichia coli bacteria.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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