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Comparative Antibacterial Activity of Citrus Peel Infusions from Sweet Orange, Lime, and Pomelo with or without Ultrasonication against *Staphylococcus aureus*

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Abstract

Infectious diseases still become a health burden in various country, one of them is Indonesia. *Staphylococcus aureus* infection can affect either part of body. Some previous studies, which were looked for alternative antibacterial source, have investigated antibacterial activity from some herbs, including various type of Citrus peels. This study aimed to compare antibacterial activity from some citrus peel infusions with and without ultrasonication against *Staphylococcus aureus* bacteria. This experimental study used Post-Test Only Control Group design. All type of orange peel was extracted using two comparative methods: conventional infusion (without ultrasonication) and ultrasound-assisted infusion (with ultrasonication). After that, these samples underwent phytochemicals screening and antibacterial assay using disc diffusion (Kirby-Bauer) methods. Phytochemical screening revealed that all citrus peel infusions contained flavonoids and phenolic compounds. Among these ultrasonicated infusions, lime (*Citrus aurantifolia*) exhibited the greatest phytochemical diversity, containing flavonoids, tannins, phenolic compounds, and alkaloids, whereas pomelo (*Citrus maxima*) contained only flavonoids and phenolic compounds. All ultrasonicated citrus peel infusions showed a weak antibacterial activity against *Staphylococcus aureus* (P-Value: 0.124). All ultrasonicated citrus peel infusions exhibited potential antibacterial activity, although further optimization of extraction methods is necessary to improve their antibacterial effects.

Keywords: Sweet Orange; Lime; Pomelo; *Staphylococcus aureus*; Disc Diffusion.

INTRODUCTION

Infectious diseases remain a significant health burden in Indonesia, particularly due to its tropical climate, which supports the growth and transmission of various pathogenic microorganisms. (Bramantono et al., 2021) One of the important infectious agents is *Staphylococcus aureus*, which can cause a wide spectrum of health problems, ranging from mild skin infections to severe conditions such as sepsis and pneumonia. (Thean et al., 2021) *Staphylococcus aureus* is a Gram-positive bacterium belonging to the group of pyogenic cocci. Antibiotics remain the primary therapeutic approach for staphylococcal infections; however, inadequate or inappropriate treatment may allow the infection to disseminate into the systemic circulation. Furthermore, irrational antibiotic use has contributed to the emergence of resistant bacterial strains. (Ghassani et al., 2022; Niken et al., 2023)

Recently, Methicillin-Resistant Staphylococcus Aureus (MRSA) infections have become an increasing global health concern and one of the most significant public health problems worldwide. It has been reported that approximately 94,000 MRSA infection cases occur annually in the United States, resulting in approximately 18,650 associated deaths. The increasing prevalence of MRSA is strongly associated with the irrational use of antibiotics in staphylococcal infections. (Garoy et al., 2019; Mcguinness et al., 2017) Although antibiotics have long been the primary treatment option, the emergence of bacterial resistance to synthetic antimicrobial agents poses substantial challenges in modern medicine. Therefore, there is an urgent need to explore natural resources as alternative antibacterial agents that are safer, more economical, and more accessible to the community. (Jieputra et al., 2024; Nurhidayanti, 2022; Pudiarifanti & Farizal, 2022)

Indonesia is rich in various herbal plants that have long been utilized in traditional medicine and community practices. One of the most commonly used herbal groups is the citrus species, including sweet orange, lime, pomelo, and other related fruits. Each citrus species possesses distinct characteristics and bioactive compounds that contribute to their pharmacological potential. Numerous studies have investigated the phytochemical contents and biological activities of citrus plants. Although citrus fruits are widely consumed and utilized in household and food industries, their peels are commonly discarded as waste products. In fact, citrus peels contain various bioactive compounds with potential medicinal benefits, making them promising natural resources for further pharmacological studies. (Furqan, 2025)

Several studies have been performed to investigate the pharmacological potential of citrus plants, particularly their peels. Pomelo peel, for instance, has been reported to contain various phytochemicals, including flavonoids, phenols, alkaloids, terpenoids, steroids, saponins, and tannins, which contribute to its antioxidant and antibacterial activities. (Angelia et al., 2022) Kartini *et al.* (2024) further reported that pomelo peel extract exhibited antibacterial activity at concentrations ranging from 8% to 15% against bacterial pathogens. (Kartini et al., 2024)

Another citrus species, lime (*Citrus aurantifolia*), has long been used in traditional medicine due to its antibacterial, anti-inflammatory, and antipyretic properties. Angelia *et al.* (2022) also demonstrated that lime extract was able to inhibit the growth of *Escherichia coli*, *Streptococcus haemolyticus*, and *Staphylococcus aureus*. Similarly, Najiya *et al.* (2022) reported that lime root extract showed antibacterial activity against *Staphylococcus aureus* at a concentration of 50%. (Angelia et al., 2022; Najiya, 2022)

In addition, antibacterial activity has also been reported in sweet orange peel (*Citrus sinensis*). Yumiko et al. (2024) found that both infusion and decoction of sweet orange peel exhibited weak antibacterial activity against *Enterobacteriaceae* isolated from a water depot. Another study by Niken and Arman (2023) also demonstrated that sweet orange peel extract showed antibacterial activity against *Staphylococcus aureus*. (Niken et al., 2023)

The increasing prevalence of antibiotic resistance in *Staphylococcus aureus* highlights the urgent need for alternative natural antibacterial agents. (Putri et al., 2024) Considering the antibacterial potential of citrus peels and their flavonoid content, these agricultural waste products may serve as valuable natural antibacterial sources. While most previous studies employed extraction methods, infusion offers a simpler, faster, and more economical preparation technique. Therefore, this study aims to evaluate the inhibitory potential of lime peel, pomelo peel, and sweet orange peel infusions against *Staphylococcus aureus* in vitro.

MATERIALS AND METHODS

Study area

This experimental study used disc diffusion test or (Kirby-Bauer) assay with a Post-Test Only Control Group design. This study was performed in Laboratorium Terpadu, Universitas Prima Indonesia.

Materials

This study used some materials including some citrus peels (sweet orange, lime, and pomelo), isolate of *Staphylococcus aureus* ATCC6538p, distilled water, Mueller Hinton Agar (MHA) media, Nutrient Agar Media, distilled water, methanol solution, normal saline, 70% alcohol, acetone alcohol, sodium hydroxide, hydrochloric acid, ferric chloride, Mayer reagent, Dragendroff reagent, Chloroform, anhydrous acetate acid, and sulfur acid.

Procedure

Identification of Citrus species

This study collected three different citrus types, which commonly found in local market. The collected citrus samples were identified at Herbarium Medanense, Universitas Sumatera Utara. (Gulo et al., 2021; Suhartomi et al., 2020)

Preparation of Citrus Peels Simplicial Powder

The citrus samples were rinsed under running water, drained, and sliced. The peels were then separated and dried using a food dehydrator at 70 °C for 24 hours. The dried citrus peels were subsequently ground into powder and packed into some tea bags. Each tea bag contained a gram of simplicia powder derived from a single citrus peel species. (Husni et al., 2025; Imen et al., 2024; Yumiko et al., 2024)

Citrus peel Infusions Process

The infusion process was carried out by heating one tea bag containing citrus peel powder in 25 mL of distilled water at 90 °C for 15 minutes with periodic stirring. The infusion was performed under two distinct conditions: with and without ultrasonication. In the non-ultrasonicated group, the mixture was heated at 90 °C for 15 minutes with periodic manual stirring to facilitate extraction. Conversely, the ultrasonicated group underwent the same temperature and time parameters while being subjected to ultrasonic waves to enhance diffusion of phytochemicals into the solvent. (Woitassek et al., 2022) Both infusions were then separately collected for further analysis. (Husni et al., 2025; Yumiko et al., 2024)

Phytochemical screening
Phytochemical screening of all infusions was performed according to the method described by Yumiko *et al.* (2024) to identify flavonoids, tannins, alkaloids, and phenolic compounds (Yumiko et al., 2024)

Preparation of Staphylococcus aureus Isolate
The antibacterial assay was performed using *Staphylococcus aureus* ATCC 6538P, that has been cultured at Laboratorium Terpadu, Universitas Prima Indonesia. The bacterial isolate was subcultured on nutrient agar (NA) using the four-quadrant streak technique and incubated for 24 hours. A single bacterial colony was then inoculated into normal saline and adjusted to a turbidity equivalent to the 0.5 McFarland standard. Furthermore, bacterial concentration also confirmed by UV-Vis Spectrophotometer at a wavelength of 600 nm. (Husni et al., 2025; Mutia et al., 2021; Yumiko et al., 2024)

Antibacterial Assay
The antibacterial activity assay was performed using the Kirby–Bauer disc diffusion method with triple replications. Mueller–Hinton Agar (MHA) was aseptically poured into sterile Petri dishes and allowed to solidify. The agar surface was subsequently inoculated by evenly swabbing a standardized suspension of *Staphylococcus aureus* ATCC6538P. Sterile paper discs impregnated with various citrus peel infusions were then placed onto the surface of the inoculated MHA plates. Similar procedures were applied to both the standard and control groups. Chloramphenicol discs were used as the standard, while discs impregnated with distilled water served as the control. All plates were incubated at 37 °C for 18–24 h. Following incubation, the diameter of the inhibition zones surrounding each disc was measured using a vernier caliper and expressed in millimeters (mm) (Husni et al., 2025; Mutia et al., 2021; Yumiko et al., 2024)

Data Analysis
All data were analyzed using IBM SPSS Statistics 26. Phytochemical screening results and inhibition zone diameters underwent a descriptive statistical analysis. Data distribution of inhibition zone was subsequently analysed by Shapiro-Wilk test. If data distribution was normal, then it was analyzed using one-way analysis of variance (One-Way ANOVA) and expressed as Mean ± Standard Deviation (SD). Elseif data distribution was not normal, then it was analysed using the Kruskal-Wallis test and presented as Median (Minimum–Maximum).

RESULTS AND DISCUSSION

This study utilized several citrus species, namely sweet orange, lime, and pomelo. Identification of sample was performed by Herbarium Medanense and confirmed the scientific names of the samples as *Citrus sinensis*, *Citrus aurantifolia*, and *Citrus maxima*, respectively. Subsequently, infusions prepared from the peels of each citrus species underwent phytochemical screening, which were described in Table 1.

Table 1. Phytochemicals Screening of All Ultrasonicated Citrus Peel Infusions.

Phytochemicals	Methods	Sweet orange (*Citrus sinensis*)	Lime (*Citrus aurentifolia*)	Pomelo (*Citrus maxima*)
Flavonoid	NaOH(aq)+ HCl(aq)	Positive	Positive	Positive
Tanin	FeCl33%	Positive	Positive	Negative
Alkaloid	Mayer	Negative	Positive	Negative
	Dragendorff	Negative	Positive	Negative
Saponin	Distilled Water+ HCl(aq)	Negative	Negative	Negative
Steroid Triterpenoid	Lieberman-Burchard	Negative	Negative	Negative
Phenol	FeCl3 5%	Positive	Positive	Positive

Table 1 demonstrates that lime (*Citrus aurantiifolia*) contained a greater variety of phytochemical compounds compared to the other citrus species, including flavonoids, tannins, phenolic compounds, and alkaloids. This was followed by sweet orange (*Citrus sinensis*), which contained flavonoids, tannins, and phenolic compounds. In contrast, pomelo (*Citrus maxima*) contained only flavonoids and phenolic compounds. Furthermore, antibacterial activity assays were performed on ultrasonicated citrus peel infusions and the inhibition zones formed around the discs on the Mueller–Hinton agar (MHA) plates are shown in Figure 1.

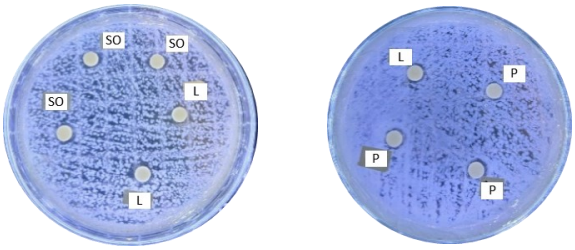

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Figure 1. Inhibition zones formed by various citrus peel infusions against Staphylococcus aureus on Mueller–Hinton agar (MHA) plates using the Kirby–Bauer disc diffusion method. SO: Sweet Orange; L: Lime; P: Pomelo.

Figure 1 showed that all ultrasonicated citrus peel infusions, which exhibited visible inhibition zones against *Staphylococcus aureus*. In contract to all non-ultrasonicated citrus peel infusions did not show any antibacterial activity against *Staphylococcus aureus*. Furthermore, the diameter of formed inhibition zone from all ultrasonicated citrus peel infusions were described in Table 2.

Table 2. Comparison of Antibacterial Activity of Ultrasonicated Citrus Peel Infusions against *Staphylococcus aureus.*

Type of Ultrasonicated Citrus Peel Infusions	Diameter of Inhibition Zone (mm)			Mean	SD	P-Value
	I	II	III			
Sweet Orange (Citrus sinensis)	8.82	8.39	8.56	8.59	0.22	
Lime (Citrus aurantifolia)	7.60	9.48	9.21	8.76	1.01	0.124
Pomelo (Citrus maxima)	6.88	7.92	7.72	7.51	0.55	

*P-Value was obtained from One-Way ANOVA

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Table 2 revealed that the antibacterial activity of the ultrasonicated citrus peel infusions. Lime (*Citrus aurantifolia*) exhibited the highest mean inhibition zone (8.76 ± 1.01 mm), followed by sweet orange (8.59 ± 0.22 mm) and pomelo (7.51 ± 0.55 mm). Despite these variations, one-way ANOVA analysis revealed no statistically significant differences in antibacterial potential among the three groups (P = 0.124).

Discussion

The present study demonstrated that no significant differences were observed in the antibacterial activity of all citrus peel infusions against *Staphylococcus aureus*. The inhibition zone diameters from each citrus peel infusions ranged from 7.51 to 8.76 mm, which were categorized as weak antibacterial activity based on Greenwood Classification.

The antibacterial activity observed in this study may be associated with the phytochemical constituents present in the citrus peel infusions. Lime (*Citrus aurantiifolia*) exhibited the greatest variety of phytochemicals, including alkaloids, which were exclusively found in lime. Alkaloids have been reported to exert antibacterial activity by interfering with the peptidoglycan layer of the bacterial cell wall, ultimately leading to bacterial cell death (Park et al., 2022)

All citrus peel samples in this study contained flavonoids and phenolic compounds, which inhibit bacterial growth by disrupting membrane integrity and inducing protein denaturation. (Liu et al., 2025) While both *Citrus sinensis* and *Citrus aurantiifolia* contain tannins that coagulate bacterial protoplasm, (Olchowik-Grabarek et al., 2022) their specific flavonoid profiles differ. *Citrus sinensis* peel is characterized by polymethoxyflavones—such as hesperidin, narirutin, naringin, naringenin, and tangeretin—whereas *Citrus maxima* peel is heavily dominated by naringin, which constitutes over 90% of its flavonoid fraction, alongside neohesperidin and limonene. (Karo et al., 2020) Despite reports that these specific phytochemicals and limonene-rich essential oils exert robust activity against *Staphylococcus aureus* (Chen et al., 2018), the inhibition zones observed in this study remained weak across all samples (Shehata et al., 2021) (Oikeh et al., 2020) This lower efficacy does not negate the antibacterial potential of these peels; rather, it underscores the limitations of the simple infusion method used, which likely failed to yield the high bioactive concentrations achieved in optimized extractions.

These findings suggest that all three citrus peels possess potential as natural antibacterial agents against *Staphylococcus aureus*. The presence of bioactive compounds in these infusions further indicates that citrus peel waste retains considerable antibacterial potential, even when prepared using a simple infusion method. (Pedreira et al., 2021)

This study demonstrated that all tested citrus peel infusions exhibited relatively similar antibacterial activity, which was categorized as weak antibacterial activity. Despite the limited inhibitory effect observed, the findings suggest that citrus peel waste still possesses potential as a natural antibacterial source derived from agricultural by-products. However, the ultrasonication potentially enhanced the antibacterial activity from all tested citrus peel. Previous studies have reported that ultrasonication may enhance the antibacterial activity of plant-derived extracts by improving the extraction efficiency of bioactive compounds. Ultrasonic-assisted extraction (UAE) utilizes ultrasound waves to disrupt plant cell walls and enhance solvent penetration, accelerating the release of intracellular phytochemicals like flavonoids and phenolics. Consequently, Sandhu *et al.* (2021) and Oliveira *et al.* (2022) demonstrated that UAE significantly increases phenolic extraction from citrus processing waste and *Citrus latifolia* by-products compared to conventional methods. Beyond improving yield, sonication directly enhances antibacterial efficacy; the mechanical shear forces can reduce particle size and increase the surface area-to-volume ratio of bioactive compounds, facilitating deeper penetration into bacterial membranes. Due to this reason, all ultrasonicated citrus peel infusions in this study exhibited visible inhibition zones against *Staphylococcus aureus*, whereas non-ultrasonicated infusions failed to show any antibacterial activity. (Sandhu et al., 2021) (Oliveira et al., 2022)

The weak antibacterial activity observed in this study may be attributed to the simple extraction method employed, namely infusion extraction, which may not have produced sufficient concentrations of bioactive compounds to achieve strong bacteriostatic or bactericidal effects. Therefore, further studies are required to optimize the extraction process and enhance the antibacterial potential of citrus peels, particularly lime (*Citrus aurantiifolia*), as a promising natural antibacterial agent.

CONCLUSIONS

In conclusion, all tested citrus peel infusions demonstrated weak antibacterial activity against *Staphylococcus aureus* ATCC 6538P. This activity may be attributed to the presence of bioactive phytochemicals, particularly phenolic-derived compounds and alkaloids detected in lime peel infusion.

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Competing Interests: The authors declare that there are no competing interests.

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