

Antibacterial Activity Test of Sunkist Orange (*Citrus sinensis*) Peel Ethanol Extract against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* Using the Disc Diffusion Method

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ABSTRAK

Penyakit infeksi yang disebabkan oleh *Escherichia coli*, *Staphylococcus aureus*, dan *Pseudomonas aeruginosa* masih menjadi beban kesehatan masyarakat yang significant, sementara meningkatnya resistensi antibiotik mendorong pencarian alternatif antibakteri alami. Kulit jeruk Sunkist (*Citrus sinensis*), limbah agroindustri yang melimpah, mengandung senyawa bioaktif seperti flavonoid, limonene, tanin, dan minyak atsiri yang berpotensi sebagai antibakteri. Penelitian ini bertujuan menilai aktivitas antibakteri ekstrak etanol kulit jeruk Sunkist terhadap ketiga bakteri tersebut menggunakan metode difusi cakram. Penelitian eksperimental laboratorium murni ini menggunakan Rancangan Acak Lengkap dengan pendekatan post-test only control group design. Ekstrak diperoleh melalui maserasi

dengan etanol 96% dan diuji pada konsentrasi 25%, 50%, 75%, dan 100%, dengan Ciprofloxacin sebagai kontrol positif dan akuades sebagai kontrol negatif, masing-masing dengan tiga kali pengulangan. Diameter zona hambat dianalisis secara deskriptif dan inferensial menggunakan uji Shapiro-Wilk, Levene's Test, Kruskal-Wallis, dan post hoc Mann-Whitney U. Hasil menunjukkan ekstrak mampu membentuk zona hambat pada seluruh konsentrasi, dengan pola peningkatan yang bergantung pada dosis. Rata-rata zona hambat tertinggi terlihat pada *E. coli* (17,28 mm pada konsentrasi 100%), diikuti *P. aeruginosa* (10,73 mm) dan *S. aureus* (9,92 mm). Uji Kruskal-Wallis menunjukkan perbedaan bermakna antar kelompok konsentrasi pada ketiga bakteri ($p < 0,05$), yang mengonfirmasi adanya hubungan dosis-respons, meskipun seluruh konsentrasi ekstrak masih jauh lebih lemah dibandingkan kontrol positif. Temuan ini mendukung potensi kulit jeruk Sunkist sebagai agen antibakteri alami, meskipun diperlukan penelitian lanjutan berupa pemurnian senyawa dan uji fitokimia kuantitatif.

ABSTRACT

Infections caused by *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* remain a major public health burden, and the growing prevalence of antibiotic resistance has driven the search for natural antibacterial alternatives. Sunkist orange (*Citrus sinensis*) peel, an abundant agro-industrial waste, contains bioactive compounds such as flavonoids, limonene, tannins, and essential oils with reported antibacterial potential. This study aimed to evaluate the antibacterial activity of ethanol extract of Sunkist orange peel against the three bacteria using the disc diffusion method. A true experimental laboratory design with a completely randomized post-test only control group design was applied. The extract was obtained through maceration with 96% ethanol and tested at concentrations of 25%, 50%, 75%, and 100%, with Ciprofloxacin as positive control and distilled water as negative control, each in triplicate. Inhibition zone diameters were analyzed descriptively and inferentially using Shapiro-Wilk, Levene's Test, Kruskal-Wallis, and Mann-Whitney U post hoc tests. Results showed that the extract produced inhibition zones at all concentrations, increasing in a dose-dependent manner. The highest mean inhibition was observed against *E. coli* (17.28 mm at 100%), followed by *P. aeruginosa* (10.73 mm) and *S. aureus* (9.92 mm). Kruskal-Wallis tests revealed significant differences among concentration groups for all three bacteria ($p < 0.05$), confirming a genuine concentration-dependent effect, although all extract concentrations remained significantly weaker than the positive control. These findings support the potential of Sunkist orange peel as a natural antibacterial agent, though further purification and phytochemical quantification are recommended.

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INTRODUCTION

Infectious diseases remain one of the leading causes of morbidity and mortality in developing countries, including Indonesia. The three bacteria most frequently involved in clinical infections are *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, which are widely associated with urinary tract infections, skin and soft tissue infections, respiratory tract infections, and even sepsis. Hamers et al. (2024) stated that *E. coli* is dominant in urinary tract infections, *S. aureus* is common in skin infections, while *P. aeruginosa* is often the cause of nosocomial infections that are difficult to treat, especially in immunocompromised patients. The infection burden of these three bacteria in Indonesia is high, accompanied by continually rising antibiotic resistance.

Irrational antibiotic use, whether excessive, at incorrect doses, or without clear indication, has led to the emergence of drug-resistant bacteria, making infections harder and costlier to treat and putting patients at risk of fatal outcomes. The World Health Organization (2024) classifies antimicrobial resistance as one of the ten greatest global health threats, with projections of up to 10 million deaths per year by 2050. In Indonesia, the prevalence of resistance among the three test bacteria continues to rise, with many

strains resistant to first- and second-line antibiotics (Weezenbeek et al., 2024), making the search for natural antibacterial agents highly important.

These conditions have encouraged the exploration of safer and more sustainable alternatives, one of which is the use of plant bioactive compounds. Herbal plants and organic waste such as fruit peels are known to contain secondary metabolites, namely flavonoids, phenols, alkaloids, and essential oils, that can inhibit pathogenic bacteria. Musyaffa et al. (2023) stated that Indonesian herbal plants have the potential to be developed as natural antibacterials to replace synthetic antibiotics, while Qaralleh et al. (2021) showed that the ethanol extract of *Psidium guajava* has moderate to strong antibacterial effects against all three test bacteria and is also able to inhibit biofilms. These findings reinforce the potential of fruit peels, including orange peel, as an environmentally friendly source of natural antibacterials.

Sunkist orange (*Citrus sinensis*) peel is an organic waste rich in phytochemical compounds such as flavonoids, limonene, tannins, and essential oils with antibacterial activity against both Gram-positive and Gram-negative bacteria. Bouyahya et al. (2023) reported that sweet orange peel essential oil inhibits the growth of *S. aureus*, *E. coli*, and *P. aeruginosa* through its limonene and linalool components, which damage bacterial cell membranes. Saxena et al. (2021) also affirmed that the ethanolic extract of *C. sinensis* peel contains flavonoids and tannins that act as antibacterial and antioxidant agents. Its utilization is also valuable from environmental and economic perspectives, considering that the volume of orange peel waste continues to grow with the expansion of the juice and processed food industries.

Ethanol 96% was chosen as the solvent because it can dissolve both polar and nonpolar compounds, making it effective for extracting flavonoids, alkaloids, phenols, and essential oils. Sugiharto et al. (2022) showed that extracting orange peel with ethanol yields a high extract recovery and a complete phytochemical composition, in addition to being safe, non-toxic, and volatile, so the extract can be purified without solvent contamination. The selection of the three test bacteria is important to describe the full spectrum of antibacterial activity: *S. aureus* represents Gram-positive bacteria with a thick peptidoglycan wall, while *E. coli* and *P. aeruginosa* represent Gram-negative bacteria whose lipopolysaccharide layer makes them more resistant. Eladl et al. (2025) also tested an antimicrobial peptide extract against these three bacteria to assess its broad effectiveness, an approach that is important in the development of new natural antibacterial agents.

The disc diffusion method was chosen because it is simple, economical, and valid for comparing antibacterial effectiveness among treatments. Its principle is to measure the inhibition zone around a disc containing the test compound on a medium inoculated with bacteria, where the zone diameter reflects antibacterial potency. Usamo et al. (2024) demonstrated that this method is effective for identifying the preliminary antibacterial potential of a plant extract and yields quantitative data that can be used for further development. Although research on fruit peel waste as an antibacterial has been widely conducted, studies testing Sunkist orange peel extract against *E. coli*, *S. aureus*, and *P. aeruginosa* simultaneously remain limited, particularly in Indonesia, because most studies focus on only one or two bacteria or use other types of oranges.

Based on the above, this study aims to: (1) assess the antibacterial activity of Sunkist orange peel ethanol extract against *E. coli*, *S. aureus*, and *P. aeruginosa* using the disc diffusion method; (2) analyze differences in antibacterial effectiveness across extract concentrations; (3) compare the effectiveness of the extract with a positive control in the form of a standard antibiotic; and (4) measure the inhibition zone diameter as an indicator of antibacterial inhibitory strength. This study is expected to enrich the scientific literature, support the development of natural antibacterials, and reduce organic waste in an environmentally friendly way.

RESEARCH METHODS

Research Design

This study was a true experimental laboratory study conducted *in vitro* using a Completely Randomized Design (CRD) with a post-test only control group design. The study aimed to test the antibacterial activity of Sunkist orange peel ethanol extract against *E. coli*, *P. aeruginosa*, and *S. aureus* using the disc diffusion method. There were six treatment groups for each test bacterium: extract at concentrations of 25%, 50%, 75%, and 100%, a positive control of the antibiotic Ciprofloxacin, and a negative control of distilled water, with three replicates (triplicate) in each group to ensure data reliability.

The study was carried out at the integrated laboratory of Universitas Prima Indonesia, Medan, from February to March 2026.

Population and Samples

The study population was specimens of pathogenic bacteria that cause infections in humans and can be tested *in vitro* using the disc diffusion method. The test bacterial samples consisted of *E. coli* and *P. aeruginosa* (Gram-negative) and *S. aureus* (Gram-positive),

which were chosen because they are clinical pathogens causing both nosocomial and community infections with significant levels of antibiotic resistance. The extract test samples were Sunkist orange peel ethanol extract at four concentrations (25%, 50%, 75%, 100%), with three replicates for each combination of extract and bacterium. The inclusion criteria were test bacteria in the form of pure, actively growing strains, and extracts prepared according to standard phytochemical procedures; the exclusion criteria were contaminated bacteria or degraded extracts.

Research Variables

The independent variable was the concentration of Sunkist orange peel ethanol extract (25%, 50%, 75%, 100%). The dependent variable was antibacterial activity, measured by inhibition zone diameter (mm) for each test bacterium. The controlled variables included the type of medium (Nutrient Agar), incubation temperature and duration, inoculum density (0.5 McFarland standard), disc volume and size, the use of positive and negative controls, and the sterilization techniques for tools and materials.

Research Procedure

Sunkist orange peels were collected, washed, cut into small pieces, dried, ground, and sieved into simplicia powder. Extraction was performed by maceration using 96% ethanol, filtered through Whatman No. 1 filter paper, then concentrated using a rotary evaporator to obtain a viscous extract. The viscous extract was then diluted to four concentrations (25%, 50%, 75%, 100%) using sterile distilled water and homogenized with a vortex mixer. All equipment was sterilized in an autoclave, and bacterial inoculation was carried out inside a Laminar Air Flow (LAF) cabinet to maintain aseptic conditions.

Nutrient Agar (NA) medium was prepared and sterilized, then poured into sterile Petri dishes. Test bacterial suspensions were prepared in 0.85% physiological NaCl solution and adjusted to the turbidity of the 0.5 McFarland standard. The bacterial suspension was spread evenly over the medium surface using a sterile cotton swab. Sterile paper discs soaked in each extract concentration, the positive control (Ciprofloxacin), and the negative control (distilled water) were placed on the medium surface at spacing arranged so that inhibition zones between discs would not overlap. The dishes were then incubated at 37°C for 18–24 hours. After incubation, inhibition zone diameters were measured using a caliper or a millimeter ruler, and results were averaged from the three replicates.

Data Analysis

Analysis was performed using SPSS statistical software. Descriptive analysis included the mean, standard deviation (SD), median, and minimum and maximum values of the

inhibition zone diameter in each concentration group. Inferential analysis began with the Shapiro-Wilk normality test (chosen because of the small sample size, $n = 3$ per group) and Levene's Test for homogeneity of variance. If the data were normally distributed and homogeneous, differences were tested with One Way ANOVA followed by Tukey HSD post hoc; however, if the assumptions were not met, the non-parametric Kruskal-Wallis test followed by Mann-Whitney U post hoc was used to identify which pairs of concentration groups differed significantly. Differences were considered significant when the significance value was < 0.05 .

Research Ethics

The test bacteria were handled using aseptic procedures in accordance with laboratory biosafety standards. Data were reported objectively without fabrication or manipulation. Chemical and bacterial waste was disposed of according to hazardous and toxic waste (B3) management procedures, including autoclave sterilization before disposal. All scientific references were credited to their original sources using the APA citation style. This study did not use human or animal subjects and therefore did not require ethical clearance, but it still upheld the principle of not harming the environment and microorganisms.

RESULTS AND DISCUSSION

Overview of Inhibition Zone Measurement Results

This study was conducted on three types of bacteria, namely *E. coli*, *S. aureus*, and *P. aeruginosa*, with six treatment groups (extract concentrations of 25%, 50%, 75%, 100%, positive control, and negative control), each repeated three times. The mean inhibition zone diameters from the three replicates are presented in Table 1.

Table 1. Mean Inhibition Zone Diameter (mm) of Sunkist Orange Peel Ethanol Extract against Three Test Bacteria

Bacteria	Control (-)	Control (+)	25%	50%	75%	100%
<i>E. coli</i>	-	36,25	9,66	9,94	12,90	17,28
<i>P. aeruginosa</i>	-	28,62	7,76	8,54	9,55	10,73
<i>S. aureus</i>	-	29,32	7,33	8,30	9,09	9,92

Note: The negative control (distilled water) showed no inhibition zone against any of the three bacteria.

In general, Sunkist orange peel ethanol extract was able to form inhibition zones against all three test bacteria at all concentrations. Inhibition zone diameters tended to increase as the extract concentration rose from 25% to 100%. Among the three bacteria, *E. coli* showed the highest mean inhibition zone diameter at every concentration, followed by *P. aeruginosa* and *S. aureus*. The positive control, a standard antibiotic, produced inhibition zone diameters far larger than all extract concentrations.

Descriptive Statistics of Inhibition Zone Diameter

Descriptive statistics for the three test bacteria are presented in Table 2, covering N, mean, standard deviation (SD), median, minimum, and maximum for each concentration group.

Table 2. Descriptive Statistics of Inhibition Zone Diameter in Three Test Bacteria

Bacteria	Group	N	Mean (mm)	SD	Median	Min	Max
<i>E. coli</i>	25%	3	9,66	0,44	9,90	9,15	9,92
<i>E. coli</i>	50%	3	9,90	0,05	9,91	9,84	9,94
<i>E. coli</i>	75%	3	12,90	1,72	11,96	11,86	14,89
<i>E. coli</i>	100%	3	17,28	2,38	15,96	15,85	20,02
<i>E. coli</i>	Control (+)	3	36,25	0,68	35,93	35,79	37,03
<i>P. aeruginosa</i>	25%	3	7,76	0,73	7,47	7,22	8,59
<i>P. aeruginosa</i>	50%	3	8,54	0,24	8,55	8,30	8,78
<i>P. aeruginosa</i>	75%	3	9,55	1,18	9,35	8,49	10,82
<i>P. aeruginosa</i>	100%	3	10,73	0,23	10,82	10,47	10,91
<i>P. aeruginosa</i>	Control (+)	3	28,62	2,70	30,09	25,50	30,27
<i>S. aureus</i>	25%	3	7,33	0,43	7,27	6,94	7,79
<i>S. aureus</i>	50%	3	8,30	0,99	8,52	7,22	9,17
<i>S. aureus</i>	75%	3	9,09	0,69	9,48	8,29	9,49
<i>S. aureus</i>	100%	3	9,92	1,43	9,73	8,59	11,44
<i>S. aureus</i>	Control (+)	3	29,32	0,52	29,61	28,72	29,62

A consistent pattern was seen across all three bacteria: the mean inhibition zone diameter increased as the extract concentration rose. In *E. coli*, the mean inhibition zone increased from 9.66 mm (25%) to 17.28 mm (100%); in *P. aeruginosa* from 7.76 mm to 10.73 mm; and in *S. aureus* from 7.33 mm to 9.92 mm. Positive control values remained far higher than all extract concentrations in all three bacteria. The standard deviation in most groups

was small, indicating that data across replicates were relatively uniform, although the 100% *E. coli* group showed a slightly larger data spread (SD = 2.38).

Normality and Homogeneity of Variance Tests

Before determining the type of difference test, the Shapiro-Wilk normality test and Levene's Test for homogeneity of variance were performed. The results of both tests are summarized in Table 3.

Table 3. Results of Normality Test (Shapiro-Wilk) and Homogeneity of Variance Test (Levene's Test)

Bacteria	Groups with Sig. > 0.05 (Normal)	Levene Statistic	df1	df2	Sig. Levene
<i>E. coli</i>	No group is normal (all Sig. = 0.00)	7,96	4	10	0,004
<i>P. aeruginosa</i>	Only the 75% group (Sig. = 0.32)	7,55	4	10	0,005
<i>S. aureus</i>	25% (0.07), 50% (0.27), 100% (0.39) groups	1,41	4	10	0,300

Based on Table 3, most groups in all three test bacteria had normality significance values below 0.05, meaning the data were not normally distributed. The homogeneity test results showed that the data variances for *E. coli* and *P. aeruginosa* were not homogeneous (Sig. = 0.004 and 0.005), whereas for *S. aureus* they were homogeneous (Sig. = 0.300). Because the majority of data groups did not meet the assumptions of normality and homogeneity, the requirements for the parametric One Way ANOVA were not fully met, so the difference test analysis proceeded with the non-parametric Kruskal-Wallis test.

Kruskal-Wallis Test

The Kruskal-Wallis test was used to determine whether there were significant differences in inhibition zone diameter among concentration groups for each test bacterium. The results are presented in Table 4.

Table 4. Kruskal-Wallis Test Results for Inhibition Zone Diameter

Bacteria	Chi-Square	df	Asymp. Sig.
<i>E. coli</i>	12,90	4	0,012
<i>P. aeruginosa</i>	11,50	4	0,022

Bacteria	Chi-Square	df	Asymp. Sig.
<i>S. aureus</i>	11,33	4	0,023

The significance values for all three test bacteria were below 0.05, namely 0.012 for *E. coli*, 0.022 for *P. aeruginosa*, and 0.023 for *S. aureus*. This indicates a statistically significant difference in inhibition zone diameter among concentration groups for each test bacterium, so the alternative hypothesis (H1) is accepted and the null hypothesis (H0) is rejected.

Mann-Whitney U Post Hoc Test

Because the Kruskal-Wallis test only shows an overall significant difference, the Mann-Whitney U follow-up test was performed to compare each pair of concentration groups. A summary of the results for the ten group pairs across the three bacteria is presented in Table 5.

Table 5. Summary of Mann-Whitney U Post Hoc Test Results (Significance Notes)

Group Pair	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
25% vs 50%	Not significant (0,513)	Not significant (0,275)	Not significant (0,275)
25% vs 75%	Significant (0,050)	Not significant (0,127)	Significant (0,050)
25% vs 100%	Significant (0,050)	Significant (0,050)	Significant (0,050)
25% vs Kontrol (+)	Significant (0,050)	Significant (0,050)	Significant (0,050)
50% vs 75%	Significant (0,050)	Not significant (0,275)	Not significant (0,275)
50% vs 100%	Significant (0,050)	Significant (0,050)	Not significant (0,127)
50% vs Kontrol (+)	Significant (0,050)	Significant (0,050)	Significant (0,050)
75% vs 100%	Significant (0,050)	Not significant (0,184)	Significant (0,050)
75% vs Kontrol (+)	Significant (0,050)	Significant (0,050)	Significant (0,050)
100% vs Kontrol (+)	Significant (0,050)	Significant (0,050)	Significant (0,050)

In *E. coli*, only the 25% vs 50% pair showed no significant difference; all other pairs differed significantly, including against the positive control. In *P. aeruginosa*, adjacent concentration pairs (25% vs 50%, 25% vs 75%, 50% vs 75%, 75% vs 100%) were not significant, and significant differences only emerged across wider concentration ranges. In *S. aureus*, a similar pattern occurred for the 25% vs 50%, 50% vs 75%, and 50% vs 100% pairs.

Discussion

The results show that Sunkist orange peel ethanol extract has antibacterial activity against all three test bacteria, as evidenced by the formation of inhibition zones at all tested concentrations. Based on the classification of Davis and Stout (1971), which divides inhibitory responses into weak (< 5 mm), moderate (5–10 mm), strong (>10–20 mm), and

very strong (>20 mm) categories, antibacterial activity against *E. coli* was in the moderate category at concentrations of 25% and 50%, rising to the strong category at 75% and 100%. In *P. aeruginosa*, inhibition was moderate at 25–75% and only reached the strong category at 100%. In *S. aureus*, all concentrations produced inhibition in the moderate category. The positive control produced inhibition zones in the very strong category for all three bacteria.

Antibacterial activity and active compound content. The antibacterial ability of the extract is inseparable from the secondary metabolites in orange peel, such as flavonoids, tannins, saponins, and limonene-containing essential oils. The use of 96% ethanol as the solvent supports the extraction of these compounds because it can effectively dissolve both polar and non-polar compounds (Sugiharto et al., 2022). Each compound works through a different but complementary mechanism: flavonoids disrupt bacterial cell membrane permeability and, in Gram-negative bacteria, also inhibit the DNA gyrase enzyme (Yan et al., 2024); tannins precipitate cell wall proteins and inactivate microbial enzymes; saponins increase membrane permeability, causing leakage of intracellular components (Jubair et al., 2021); while limonene damages the integrity of bacterial cell membranes and triggers leakage of intracellular material leading to cell death (Gupta et al., 2021). This combination of mechanisms explains the formation of inhibition zones in all three test bacteria.

Effect of concentration on inhibitory strength. The main finding of this study is the increase in inhibition zone diameter as extract concentration rose in all three test bacteria, indicating a dose-dependent relationship: the higher the concentration, the more active compounds diffuse and interact with bacterial cells. This finding is consistent with Amilia et al. (2021) on calamondin orange peel extract against *S. aureus*, which showed increasing inhibition zones with rising concentration, as well as Oikeh et al. (2020), who found that the antibacterial activity of *C. sinensis* peel extract was directly proportional to its total phenolic, flavonoid, and tannin content. Nevertheless, the Mann-Whitney U post hoc results show that not every increase in concentration produced a statistically significant difference, particularly for adjacent concentration pairs in *P. aeruginosa* and *S. aureus*, indicating that a larger concentration difference is needed for the increase in inhibitory strength to be truly evident.

Differences in sensitivity among bacteria. *E. coli* showed the highest inhibition, followed by *P. aeruginosa*, and the lowest in *S. aureus*, an interesting pattern because *S. aureus*, a Gram-positive bacterium, was actually more resistant than the two Gram-negative bacteria. In theory, Gram-positive bacteria are generally considered more susceptible because their cell wall consists only of peptidoglycan with no outer membrane (Yan et al.,

2024), yet this result, which runs contrary to the general theory, is in line with several previous studies. The high sensitivity of *E. coli* is presumed to be related to the mechanism of limonene, which specifically damages the integrity of the *E. coli* cell membrane, causing leakage of proteins, lipids, nucleic acids, and ATP, and disrupting DNA transcription (Gupta et al., 2021). The low inhibition against *S. aureus* is presumed to be related to its thick peptidoglycan layer and teichoic acid content, which resist the penetration of certain antibacterial compounds. Meanwhile, *P. aeruginosa*, although Gram-negative like *E. coli*, showed lower inhibition due to its intrinsic resistance: very low outer membrane permeability, active efflux pump systems, and the production of antibiotic-degrading enzymes (Pang et al., 2019).

Comparison with the positive control. All extract concentrations, including 100%, produced inhibition zones that differed significantly from, and were far smaller than, the positive control. This is reasonable given that the extract used was still a crude extract containing a mixture of various compounds, so the concentration of pure active compounds was relatively lower than that of the antibiotic, which is a single compound with a specific target of action. Nevertheless, this does not diminish the potential of Sunkist orange peel as a source of natural antibacterials, considering that the extract had not undergone further purification or fractionation.

Implications and limitations. This study demonstrates that Sunkist orange peel waste, which is often discarded, has the potential to be developed as a natural antibacterial source, particularly in facing the challenge of antibiotic resistance, while also strengthening the value of organic waste utilization through an environmentally friendly and economically valuable applied science approach. The 100% concentration gave the best inhibitory activity against all three bacteria, with the highest sensitivity in *E. coli*. However, this study has limitations: the extract used was still a crude extract, so the single most dominant active compound is not yet known, and measurement of the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC), and quantitative phytochemical screening were not performed.

CONCLUSION AND RECOMMENDATIONS

Based on the results, it can be concluded that ethanol extract of Sunkist orange (*Citrus sinensis*) peel exhibits antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* at concentrations of 25%, 50%, 75%, and 100%. The antibacterial activity increased with increasing extract concentration, with the 100%

concentration showing the highest inhibitory activity against all three test bacteria. Among the tested bacteria, *E. coli* showed the greatest sensitivity to the extract, followed by *P. aeruginosa* and *S. aureus*. Nevertheless, the antibacterial activity of the extract was still lower than that of the standard antibiotic used as the positive control. These findings indicate that Sunkist orange peel has potential as a natural antibacterial source with added environmental and economic value.

Further research is recommended to identify and characterize the active antibacterial compounds through fractionation and purification, as well as to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Quantitative phytochemical analysis and testing against a broader range of pathogenic bacteria are also recommended to strengthen the evidence for the development of Sunkist orange peel extract as a potential natural antibacterial agent.

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