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Antimicrobial and Antioxidant Activities of Ethanol Extracts from Red Guava Leaves (Psidium Guajava L.) Against Staphylococcus Aureus, Escherichia Coli, and Candida Albicans Using the Disk Diffusion Method

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ABSTRACT
Red guava leaves (Psidium guajava L.), a member of the Myrtaceae family, contain secondary metabolites such as flavonoids, alkaloids, and tannins that exhibit potential antibacterial, antifungal, and antioxidant activities. This experimental study employed the maceration method using 96% ethanol as the extraction solvent. Antimicrobial activity was evaluated using the disk diffusion method against Staphylococcus aureus, Escherichia coli, and Candida albicans at concentrations of 20%, 40%, and 60%. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay at concentrations of 10, 20, 40, and 80 µg/mL, with vitamin C used as a comparator. The results showed that the ethanol extract inhibited the growth of all tested microorganisms, with each treatment repeated three times. For S. aureus, the largest inhibition zone was observed at the 60% concentration (16.75 mm on average), whereas the smallest inhibition zone was observed at the 20% concentration (7.53 mm). For E. coli, the largest inhibition zone was also observed at the 60% concentration (8.44 mm on average), while the smallest was recorded at the 20% concentration (6.38 mm). For C. albicans, the largest inhibition zone occurred at the 60% concentration (10.46 mm on average), whereas the smallest was observed at the 20% concentration (8.81 mm). Regarding antioxidant activity, an increase in extract concentration was associated with decreased absorbance and increased free-radical scavenging activity. The ethanol extract exhibited an IC_{50} value of 13.40 µg/mL, indicating very strong antioxidant activity, compared with vitamin C, which showed an IC_{50} value of 2.36 µg/mL.

INTRODUCTION

Indonesia has rich cultural diversity, which is reflected in various aspects of community life, including traditional clothing, traditional houses, arts, and cultural products related to health. One form of health-related cultural practice in Indonesia is the use of traditional plants, which can be obtained from various natural resources, particularly medicinal plants (Nunggut, Y 2020).

Indonesian communities use guava leaves (Psidium guajava L.) as traditional remedies for treating diarrhea, loose stools, oral hygiene problems as a mouthwash, toothache, vomiting caused by cholera, antispasmodic conditions, and local treatments for rheumatism, inflammation, fever, pain, bacterial infections, and fungal infections (Saputro & Purwanto, 2022).

Infections caused by pathogenic microorganisms such as Staphylococcus aureus, Escherichia coli, and Candida albicans remain significant health problems frequently

encountered in communities. Staphylococcus aureus is a Gram-positive bacterium that is a major cause of skin infections, abscesses, and postsurgical wounds. The prevalence of S. aureus infections in Indonesia has been reported to account for approximately 20–30% of skin and soft tissue infection cases (Nunggut, Y 2020).

Escherichia coli is a Gram-negative bacterium and one of the primary causes of diarrhea. The prevalence of diarrhea cases in North Sumatra Province reached 4.7%. Data from Statistics Indonesia (BPS) in 2022 recorded 205,155 cases, while in 2023, diarrhea remained among the ten most common diseases reported in hospitals and healthcare facilities across provinces (Silalahi et al., 2024). East Java Province had the second-highest incidence of diarrhea, with 151,463 cases and a prevalence of 7.6%. Meanwhile, Surabaya City recorded approximately 75,463 cases, accounting for nearly 50% of the total diarrhea cases in East Java (Nunggut, Y 2020).

Pathogenic fungal infections remain a global health concern, with an estimated mortality rate of approximately 1.35 million cases annually. Species from the genera Candida, Aspergillus, and Cryptococcus are among the leading causes of invasive fungal infections, with Candida being the most common causative agent, particularly in developing countries (Buaya et al., 2025). Candida albicans is a globally distributed fungus that can infect individuals of various ages and sexes. Infections caused by fungi of the genus Candida, particularly C. albicans, are among the most frequently reported fungal infections. Based on research data from a hospital in North Sumatra during 2017–2019, there were 3,297 cases of contagious skin diseases among 12,686 registered patients (Yosi et al., 2025). Globally, Candida infections remain a major concern, with C. albicans accounting for a substantial proportion of cases. These infections may affect various body parts, including the hair, skin, nails, and mucous membranes of the mouth and throat (Fadillah et al., 2024).

A study conducted by Manda found that methanol extract of guava leaves (Psidium guajava L.) inhibited the growth of various bacterial species, including both Gram-positive and Gram-negative bacteria such as Staphylococcus aureus, Salmonella spp., Klebsiella pneumoniae, and Escherichia coli. Guava leaves (P. guajava L.) contain various secondary metabolites, including phenolic acids, flavonoids, terpenoids, glycosides, tannins, and saponins. These secondary metabolites play important roles as antibacterial agents. Tannins cause bacterial cell membrane contraction, enzyme inactivation, and cell wall damage. Flavonoids disrupt bacterial cell integrity and degrade proteins, thereby inhibiting bacterial growth. Terpenoids possess bioactive properties that may contribute to bacterial growth inhibition, while saponins exhibit antibacterial activity, particularly against Gram-positive bacteria (Hall et al., 2023).

A study conducted by Ni Nyoman Ayu Prastiwi on the phytochemical content of guava leaf extract (Psidium guajava L.) as an antibacterial agent found that guava leaves contain various phytochemical compounds with antibacterial and antifungal activities, including phenolic acids, flavonoids, terpenoids, glycosides, tannins, and saponins. These compounds have demonstrated effectiveness in inhibiting the growth of both Gram-positive and Gram-negative bacteria. The antibacterial activity of the extract was evaluated using the disk diffusion and well diffusion methods and confirmed through the formation of inhibition zones, indicating the potential of guava leaf extract as an antibacterial agent. The study demonstrated that guava

leaf extract (P. guajava L.) effectively inhibited the growth of bacteria and fungi (Tenaya & Astuti, 2024).

Based on previous studies, several research gaps support the implementation of this study. Previous research has generally focused on white guava leaves (Psidium guajava L.) without differentiating varieties, although red guava leaves contain higher levels of anthocyanins and phenolic compounds, which may contribute to stronger biological activities. In addition, many studies have evaluated only one or two microorganisms separately or focused solely on antimicrobial activity without simultaneously examining antioxidant activity, resulting in limited understanding of the multifunctional potential of guava leaves. This study provides novelty by using 96% ethanol as an extraction solvent to obtain bioactive compounds from red guava leaves collected from specific locations in North Sumatra. Furthermore, this study simultaneously evaluates antimicrobial activity against three microorganisms, namely the Gram-positive bacterium Staphylococcus aureus, the Gram-negative bacterium Escherichia coli, and the fungus Candida albicans, as well as antioxidant activity using the DPPH assay at various concentrations. Therefore, this study provides a more comprehensive understanding of the relationship between solvent type, phytochemical composition, extract concentration, and resulting biological activities.

Based on the studies described above, it can be concluded that guava leaf extract (Psidium guajava L.) has potential as an antimicrobial agent. Therefore, this study was conducted with the title "Antimicrobial and Antioxidant Activity of Ethanol Extract of Red Guava Leaves (Psidium guajava L.) Against Staphylococcus aureus, Escherichia coli, and Candida albicans Using the Disk Diffusion Method." Based on the background described above, the research problem addressed in this study was: how effective is the antimicrobial activity of ethanol extract of red guava leaves (P. guajava L.) against Staphylococcus aureus, Escherichia coli, and Candida albicans using the disk diffusion method, and how does the extract demonstrate antioxidant activity?

This research aimed to determine the antimicrobial and antioxidant activities of ethanol extract of red guava leaves (Psidium guajava L.) against Staphylococcus aureus, Escherichia coli, and Candida albicans using the disk diffusion method, as well as to examine the relationship between solvent type, phytochemical composition, and antimicrobial effectiveness. This research is expected to provide theoretical, practical, and methodological benefits. Theoretically, this study contributes to phytochemical and pharmacological knowledge regarding the potential of guava leaves as antimicrobial and antioxidant agents and provides a basis for the development of phytopharmaceutical products. Practically, this research provides scientific information for communities regarding potential alternative treatments for bacterial and fungal infections, serves as a reference for the herbal industry, and supports government initiatives related to the utilization of biological resources in Indonesia. Methodologically, this study provides a standardized protocol for evaluating the antimicrobial and antioxidant activities of plant extracts that can be replicated in future research.

METHOD

Type and Design of Study

This study employed an experimental laboratory research design using a post-test-only control group approach. The test microorganisms, Staphylococcus aureus, Escherichia coli, and

Candida albicans, were treated with ethanol extract of red guava leaves at various concentrations. The antimicrobial activity was evaluated based on the diameter of the inhibition zones formed around the paper discs using the disk diffusion method.

The experimental design consisted of a treatment group containing red guava leaf ethanol extract at different concentrations and two control groups: a positive control using a standard antimicrobial agent and a negative control using the extraction solvent. The results of each treatment were analyzed to determine the antimicrobial activity of the extract against the tested microorganisms. The research design used in this study is illustrated as follows:

Location and Time of Study

1. **Study Location**
   The study was conducted at the Integrated Basic Science Laboratory of Universitas Prima Indonesia (UNPRI).
2. **Study Period**
   This study was carried out from April 20 to May 27 2026

Research Instruments

1. **Research Equipment**
   The equipment used in this study consisted of a rotary evaporator set, beaker glass, measuring cylinder, Erlenmeyer flask, laboratory blender, test-tube rack, test tubes, funnel, wooden tongs, dropper pipette, analytical balance, filter paper, incubator, petri dishes, sterile swabs, calipers, and dark-colored glass bottles with caps.
2. **Research Materials**
   The materials used in this study were red guava leaves (Psidium guajava L), pure cultures of Staphylococcus aureus, Escherichia coli, and Candida albicans, ethanol, methanol, distilled water, Mueller-Hinton Agar (MHA), Sabouraud Dextrose Agar (SDA), 70% alcohol, NaCl solution, cotton, parafilm, Bunsen burner, acetic acid, acetic anhydride, ethyl acetate, chloroform, micropipette, aluminum foil, UV-Vis spectrophotometer, Erlenmeyer flask, methanol, n-butanol, n-hexane, 1% HCl, 1% $FeCl_3$, concentrated H_2SO_4, 2N HCl, 1% $(CH_3COO)_2$, Mayer's reagent, Bouchardat's reagent, and Dragendorff's reagent.

Research Procedure

Red guava leaves (*Psidium guajava* L.) were collected and prepared as simplicia by wet-sorting, washing, chopping, and air-drying for 14 days, followed by dry-sorting, grinding, and sieving to obtain a fine powder (Maulida, 2020). The ethanol extract was prepared by macerating 500 g of simplicia powder with 96% ethanol at a 1:10 ratio for 3×24 hours with periodic stirring, then filtering and concentrating using a rotary evaporator at 50°C and 70 rpm until a viscous extract was obtained. For antioxidant testing, DPPH solution (0.1 mM) was prepared by dissolving 4 mg DPPH in 100 mL methanol, while test solutions were prepared at concentrations of 10, 20, 40, and 80 µg/mL from a 100 ppm stock; vitamin C as comparator was prepared at concentrations of 2, 4, 6, and 8 µg/mL. Antioxidant activity was measured by mixing 1 mL of DPPH with 1 mL of each test solution, incubating for 30 minutes in darkness, measuring absorbance at 517 nm using a UV-Vis spectrophotometer, and calculating percent inhibition and IC_{50} values through linear regression.

Phytochemical screening was conducted to identify alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids using standard reagents: Mayer's and Dragendorff's reagents

for alkaloids (white/yellow or red precipitate formation), NaOH for flavonoids (yellow precipitate), $FeCl_3$ for tannins (blackish-green color), hot water for saponins (foam formation), chloroform with concentrated H_2SO_4 for steroids (red/brown ring), and acetic anhydride with concentrated H_2SO_4 for terpenoids (purplish-red precipitate) (Maulida, 2020). Extract concentration variations (20%, 40%, and 60%) were prepared from a 200% stock solution diluted with DMSO. Bacterial and fungal media were prepared using Nutrient Agar (28 g/L, autoclaved at 121°C for 15 minutes) and Potato Dextrose Agar (39 g/L with 100 mg penicillin, autoclaved), respectively (Elzuhria A et al., 2023). The McFarland I standard (0.05 mL 1% $BaCl_2$ in 9.95 mL 1% H_2SO_4) was prepared for turbidity adjustment. Microbial cultures were reactivated by streaking on slant agar and incubating at 37°C for 24 hours (bacteria) or 48 hours (fungi). Test suspensions were prepared by transferring cultures into sterile 0.9% NaCl, diluting to achieve McFarland I turbidity (≈3×10^8 CFU/mL), and used within 15 minutes for disc diffusion testing by applying the inoculum to agar surfaces using sterile swabs.

Data Analysis

All data in this study were analyzed using IBM SPSS software. Inhibition-zone-diameter data were analyzed descriptively (mean ± SD). The Shapiro–Wilk normality test and Levene's homogeneity test were performed to determine whether the assumptions for ANOVA were met. Differences in antimicrobial activity were analyzed using two-way ANOVA with extract type and concentration as factors, followed by Tukey's HSD post-hoc test where significant differences were found. Where the ANOVA assumptions were not met, the analysis was replaced with the nonparametric Kruskal–Wallis test and Dunn's post-hoc test. The processed data are presented as mean ± SD bar charts and result-analysis tables.

RESULTS AND DISCUSSION

Research Results

Based on the results of the antimicrobial and antioxidant activity testing of the ethanol extract of red guava leaves (Psidium guajava L) against Staphylococcus aureus, Escherichia coli, and Candida albicans using the disc diffusion method, the results can be seen in the following description:

Extraction Results

The yield of red guava leaf (Psidium guajava L) extract obtained using the maceration method with 96% ethanol solvent was 40 grams.

Phytochemical Test Results

The results of the phytochemical test of the red guava leaf (Psidium guajava L) extract are as follows:

Table 1. Phytochemical Test Results

Phytochemical Test	Reagent	Observation	Result
Alkaloid	– Extract + Mayer's reagent – Extract + Dragendorff's reagent	– White precipitate formed – Yellow-orange precipitate formed	+ / +
Flavonoid	– Extract + Mg powder + amyl alcohol	– Brown precipitate formed	+
Tannin	– Extract + $FeCl_3$	– Blackish-green color formed	+
Saponin	– Extract + hot water / shaken vertically for 10 minutes	– No foam formed after 10 minutes	–
Triterpenoid/Steroid	– Extract + Lieberman–Burchard reagent	– No green color formed	–

Source: Primary data, 2025

Notes:

+ : Contains the secondary metabolite compound

– : Does not contain the secondary metabolite compound

Observation of Inhibition Zone Diameter

After 24 hours of observation, the inhibition-zone diameter of Staphylococcus aureus bacterial growth is presented in the table below:

Based on the results of the antibacterial and antifungal activity testing of the guava-leaf extract at various concentrations (20%, 40%, and 60%), positive control, and negative control, inhibition-zone-diameter parameter data were obtained. Descriptive results of the measurements are presented as mean and standard deviation (SD), as shown in Table 10 below.

Table 2. Inhibition Zone Diameter of Staphylococcus aureus, Escherichia coli, and Candida albicans

Inhibition Zone Diameter – Staphylococcus aureus				
Concentration	**Replicate I**	**Replicate II**	**Replicate III**	**Mean ± SD (mm)**
20%	8.21	6.31	8.06	7.53 ± 1.06
40%	8.81	9.47	9.32	9.20 ± 0.35
60%	17.63	16.19	16.44	16.75 ± 0.77
Positive Control	28.97	28.49	29.82	29.09 ± 0.67
Negative Control	0	0	0	0
Inhibition Zone Diameter – Escherichia coli				
Concentration	**Replicate I**	**Replicate II**	**Replicate III**	**Mean ± SD (mm)**
20%	6.66	6.40	6.08	6.38 ± 0.29
40%	7.44	7.76	6.14	7.11 ± 0.86
60%	8.59	8.98	7.76	8.44 ± 0.62
Positive Control	24.39	23.60	25.60	24.53 ± 0.01
Negative Control	0	0	0	0
Inhibition Zone Diameter – Candida albicans				
Concentration	**Replicate I**	**Replicate II**	**Replicate III**	**Mean ± SD (mm)**
20%	8.96	9.09	8.37	8.81 ± 0.38
40%	9.43	9.72	10.36	9.84 ± 0.48
60%	10.14	10.38	10.86	10.46 ± 0.37
Positive Control	42.28	34.45	39.19	38.64 ± 3.94
Negative Control	0	0	0	0

Source: Primary data, 2025

Based on the results of the antibacterial and antifungal activity testing of the guava-leaf extract at various concentrations (20%, 40%, and 60%), inhibition-zone-diameter parameter data were obtained. The highest average inhibition-zone diameter of Staphylococcus aureus with the guava-leaf extract occurred at a concentration of 60%, with an average of 16.75 mm, while the lowest average occurred at a concentration of 20%, with an average of 7.53 mm.

The highest inhibition-zone diameter of Escherichia coli with the guava-leaf extract occurred at a concentration of 60%, with an average of 8.44 mm, while the lowest occurred at a concentration of 20%, with an average of 6.38 mm. Likewise, the highest inhibition-zone diameter of the fungus Candida albicans with the guava-leaf extract occurred at a concentration of 60%, with an average of 10.46 mm, while the lowest occurred at a concentration of 20%, with an average of 8.81 mm.

Analysis of Bacterial Inhibition Zone Diameter Data

The prerequisite analyses used in this study consisted of two basic tests: a normality test of the data distribution and a homogeneity test of population variance. The normality test was carried out to determine whether the inhibition-zone-diameter data for each treatment group were normally distributed. Given that the sample size in each treatment group was relatively small, the most appropriate and sensitive normality-test method was the Shapiro–Wilk test. Subsequently, a homogeneity-of-variance test was carried out to examine whether the variance among treatment groups was uniform (homogeneous) or constant, using Levene's Test. Both prerequisite tests were analyzed at a significance level of 0.05.

Prerequisite Analysis Tests

Normality Test

The decision criteria in the Shapiro–Wilk normality test are based on the significance value (asymptotic significance / p-value). If the significance value obtained is greater than 0.05 ($p > 0.05$), the hypothesis is accepted, meaning the data are normally distributed. Conversely, if the significance value is less than 0.05 ($p < 0.05$), the data are declared not normally distributed. The results of the normality test on the inhibition-zone-diameter data for the tested microorganisms are as follows.

Table 3. Shapiro–Wilk Normality Test of Inhibition Zone Diameter Data for Staphylococcus aureus and Escherichia coli

Microorganism / Group	Concentration	Normality Test Result (p)
Staphylococcus aureus	20%	0.136
Staphylococcus aureus	40%	0.417
Staphylococcus aureus	60%	0.312
Staphylococcus aureus	Positive Control	0.696
Staphylococcus aureus	Negative Control	–
Staphylococcus aureus	20%	0.886
Staphylococcus aureus	40%	0.358
Staphylococcus aureus	60%	0.608
Staphylococcus aureus	Positive Control	0.770
Staphylococcus aureus	Negative Control	–
Escherichia coli	20%	0.325
Escherichia coli	40%	0.591
Escherichia coli	60%	0.637
Escherichia coli	Positive Control	0.769
Escherichia coli	Negative Control	–

Source: Primary data, 2025

Note: the microorganism/group labels in this table are reproduced exactly as they appear in the original manuscript, including an apparent duplicated "Staphylococcus aureus" block; the source document does not provide a corrected label for this second block.

Based on the results of the analysis in Table 3, it is known that all extract-treatment groups (concentrations of 20%, 40%, and 60%) as well as the positive control for the tested microbes had significance values greater than 0.05 ($p > 0.05$). This shows that the inhibition-zone-diameter data for the microbes concerned satisfy the assumption of normal data distribution. Next, a homogeneity-of-variance test was carried out using Levene's test.

Homogeneity Test

After the normality test was carried out, a homogeneity-of-variance test was performed. This was done to determine whether the variance of the data from each group had the same (homogeneous) distribution or not. The homogeneity test was carried out using Levene's test at

a significance level of 0.05. The results of the homogeneity test on the inhibition-zone-diameter data of the microorganisms are as follows.

Table 4. Levene's Homogeneity Test of Inhibition Zone Diameter Data for Staphylococcus aureus, Escherichia coli, and Candida albicans

Microorganism	Homogeneity Test Result (p)
Staphylococcus aureus	0.026
Escherichia coli	0.074
Candida albicans	0.017

Source: Primary data, 2025

Based on the data in Table 4, the results of Levene's homogeneity test show variation in the significance values for the three tested microbes. The decision criterion in this test states that if $p > 0.05$, the data variance between groups is declared homogeneous; conversely, if $p < 0.05$, the data variance is declared not homogeneous.

For the Escherichia coli data group, a significance value of 0.074 was obtained, indicating that the data variance between treatment groups was homogeneous. Because the Escherichia coli data had already been shown to be normally distributed in the normality test and had homogeneous variance, hypothesis testing for this bacterium met the criteria for analysis using the parametric statistical test, namely one-way ANOVA.

Meanwhile, for the Staphylococcus aureus and Candida albicans data groups, significance values of 0.026 and 0.017 were obtained, respectively, meaning $p < 0.05$, so it can be concluded that the data variance in both microbial groups was not homogeneous. Because the assumption of homogeneity of variance was not met, hypothesis testing for Staphylococcus aureus and Candida albicans was methodologically switched to the nonparametric statistical test, namely the Kruskal–Wallis test.

Antibacterial Activity Test against Staphylococcus aureus

Based on the results of the prerequisite tests carried out previously, it is known that the inhibition-zone-diameter data for Staphylococcus aureus did not meet the assumption of homogeneity of variance. Therefore, hypothesis testing to determine the effect of administering red guava leaf extract on inhibition-zone diameter was carried out using the nonparametric Kruskal–Wallis test.

The results of the Kruskal–Wallis test showed a significance value of 0.009 ($p < 0.05$). This value indicates that there was a significant difference among the treatment groups with respect to the inhibition-zone diameter of Staphylococcus aureus, showing that the guava-leaf extract as well as the positive control elicited different responses in the growth of Staphylococcus aureus. To determine which pairs of groups caused this difference, a post-hoc test using Dunn's test was carried out.

Table 5. Dunn's Test Results for Inhibition Zone Diameter of Staphylococcus aureus

Comparison of Treatment Groups	Dunn's Test Result (p)
Negative Control (K–) vs. Concentration 20%	1.000
Negative Control (K–) vs. Concentration 40%	0.991
Negative Control (K–) vs. Concentration 60%	0.134
Negative Control (K–) vs. Positive Control (K+)	0.010
Concentration 20% vs. Concentration 40%	1.000
Concentration 20% vs. Concentration 60%	0.991
Concentration 20% vs. Positive Control (K+)	0.134
Concentration 40% vs. Concentration 60%	1.000
Concentration 40% vs. Positive Control (K+)	0.991

Comparison of Treatment Groups	Dunn's Test Result (p)
Concentration 60% vs. Positive Control (K+)	1.000

Source: Primary data, 2025

The results of Dunn's test in Table 5 show that the comparison between the Negative Control (K–) and Positive Control (K+) produced a significance value of 0.010, indicating a significant difference in inhibitory power between these two groups. Meanwhile, all comparisons involving the extract groups – whether between the Negative Control and the extract concentrations, or among the various extract concentrations (20%, 40%, and 60%) – produced significance values greater than 0.05. These results show that there was statistically no significant difference among the extract groups, nor between the extract groups and the negative control.

Nevertheless, the overall statistical results show a tendency toward increasing inhibitory power with increasing extract concentration. To provide a clearer picture of this pattern, the average inhibition-zone diameter for each treatment group is presented in the figure below.

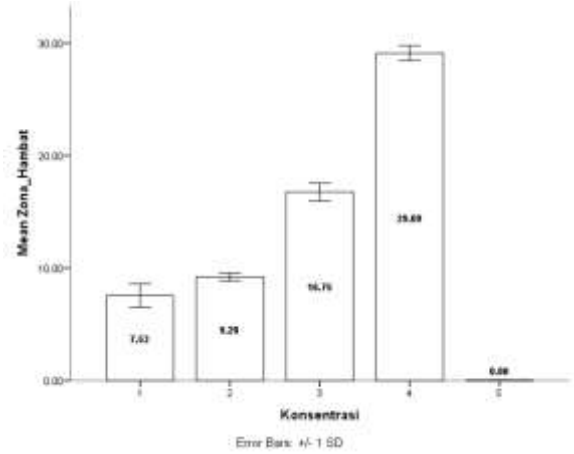

Figure 1. Average Inhibition Zone Diameter of Staphylococcus aureus

Source: Primary data, 2025

In Figure 1, a visual trend of increasing average inhibition-zone diameter can be seen with increasing concentration of the red guava leaf extract. The average inhibition-zone diameters were 7.53 mm at a concentration of 20%, 9.20 mm at 40%, and 16.75 mm at 60%. This shows that increasing the extract concentration tends to be followed by an increase in the ability to inhibit the growth of Staphylococcus aureus.

Nevertheless, the results of Dunn's test show that the differences among the three extract concentrations were not statistically significant. Thus, the increase in average inhibition-zone diameter seen in the chart cannot yet be used as a basis for stating that increasing extract concentration produces a meaningfully different increase in antibacterial effectiveness. These results indicate that the three extract concentrations still showed relatively similar effectiveness in inhibiting the growth of Staphylococcus aureus.

In addition, the Positive Control (K+) group produced the largest average inhibition-zone diameter, at 29.09 mm, while the Negative Control (K–) showed no inhibition zone at all. This condition shows that the positive control had far stronger antibacterial activity than the red guava leaf extract, while the solvent used in the negative control had no inhibitory effect on bacterial growth. The relatively short error bars for each treatment group also indicate that the measurement results between replicates were fairly consistent, so the resulting averages adequately represent the response of each treatment.

Antibacterial Activity Test against Escherichia coli

Based on the results of the prerequisite analysis described earlier, it is known that the distribution of inhibition-zone-diameter data for Escherichia coli was normally distributed and met the assumption of homogeneity of variance. Therefore, hypothesis testing to determine whether there was a significant difference in the effect of administering guava-leaf extract was analyzed using the parametric statistical approach through Analysis of Variance (one-way ANOVA).

The results of the one-way ANOVA test showed a significance value of 0.000 ($p < 0.05$), meaning there was a significant difference in effect among the five treatment groups on the inhibition-zone diameter of Escherichia coli. A post-hoc test was therefore carried out using Tukey's HSD (Honestly Significant Difference) test. This test aimed to identify which groups had significant differences in inhibitory effectiveness.

Table 6. Tukey's HSD Test Results for Inhibition Zone Diameter of Escherichia coli

Comparison of Treatment Groups	Tukey's HSD Test Result (p)
Negative Control (K–) vs. Concentration 20%	0.000
Negative Control (K–) vs. Concentration 40%	0.000
Negative Control (K–) vs. Concentration 60%	0.000
Negative Control (K–) vs. Positive Control (K+)	0.000
Concentration 20% vs. Concentration 40%	0.671
Concentration 20% vs. Concentration 60%	0.023
Concentration 20% vs. Positive Control (K+)	0.000
Concentration 40% vs. Concentration 60%	0.181
Concentration 40% vs. Positive Control (K+)	0.000
Concentration 60% vs. Positive Control (K+)	0.000

Source: Primary data, 2025

The results of the Tukey's HSD post-hoc analysis in Table 6 show that the various extract concentrations (20%, 40%, and 60%) had a significant difference in inhibitory power compared with the Negative Control group ($p < 0.05$). This proves that the guava-leaf extract at all three concentration levels had effective antibacterial activity in inhibiting the growth of Escherichia coli.

In the comparison among extract-concentration groups, the increase from 20% to 40% did not produce a significant difference in inhibition-zone diameter ($p > 0.05$), and likewise for the increase from 40% to 60% ($p > 0.05$). However, a direct comparison between the lowest concentration (20%) and the highest concentration (60%) showed a statistically meaningful difference in effectiveness ($p < 0.05$). This indicates that a statistically significant increase in inhibitory power can be achieved when the interval of increase in extract concentration is sufficiently large. To provide a more comprehensive picture of the inhibition pattern produced by each treatment, the average inhibition-zone diameter of Escherichia coli is presented in the figure below.

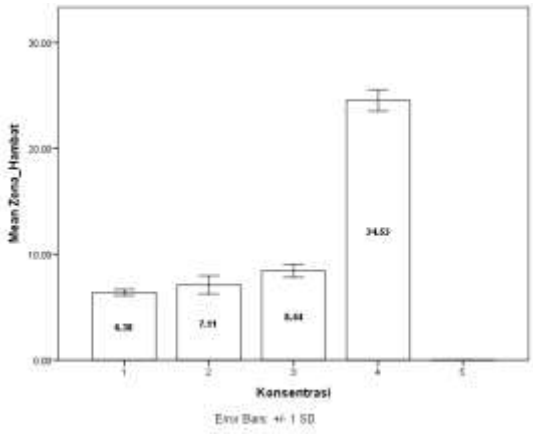

Figure 2. Average Inhibition Zone Diameter of Escherichia coli

Source: Primary data, 2025

Figure 2 visually shows a tendency toward an increasing average inhibition-zone diameter with increasing concentration of the red guava leaf extract. The 20% extract concentration group produced an average inhibition-zone diameter of 6.38 mm, increasing to 7.11 mm at 40%, and reaching 8.44 mm at 60%. This pattern shows that increasing extract concentration tends to be followed by an increase in the extract's ability to inhibit the growth of Escherichia coli.

This visualization is consistent with the results of Tukey's HSD test, which showed that all extract groups differed significantly from the Negative Control (K–) ($p < 0.05$). This indicates that the red guava leaf extract at all tested concentrations was able to exert antibacterial activity against Escherichia coli. However, the increase in inhibitory power between extract concentrations was not entirely statistically significant. The comparisons between concentrations 20% and 40%, and between 40% and 60%, produced significance values greater than 0.05, so the increase in average inhibition-zone diameter across those concentration ranges cannot yet be declared meaningfully different.

In contrast, the comparison between concentrations 20% and 60% showed a significant difference ($p < 0.05$). This result shows that increasing the extract concentration from the lowest to the highest level produced a fairly substantial increase in inhibitory power against the growth of Escherichia coli. Thus, although the gradual changes in inhibitory power did not show significant differences, the overall cumulative increase in extract concentration had a significant effect on the resulting antibacterial activity.

In addition, the Positive Control (K+) group produced the highest average inhibition-zone diameter, at 24.53 mm. This value was far greater than all extract groups and was statistically significantly different from each of the tested extract concentrations. This result shows that the positive control still had stronger antibacterial effectiveness than the red guava leaf extract at the concentrations used in this study. Conversely, the Negative Control (K–) produced no inhibition zone at all, showing that the solvent used had no antibacterial activity against the test bacterium.

The relatively short error bars for each treatment group indicate that the variation in measurement results between replicates tended to be small, indicating that the responses obtained in each treatment group were fairly consistent. Overall, the visualization in the figure shows a tendency toward increasing antibacterial activity with increasing concentration of red

guava leaf extract, although this increase did not always produce a significant difference between consecutive concentration levels.

Antifungal Activity Test against Candida albicans

Based on the results of the prerequisite analysis described earlier, it is known that the distribution of inhibition-zone-diameter data for the fungus Candida albicans did not meet the assumption of homogeneity of variance. Therefore, hypothesis testing to determine whether there was a significant difference in the effect of administering guava-leaf extract was analyzed using the nonparametric statistical approach through the Kruskal–Wallis test.

The results of the Kruskal–Wallis analysis for Candida albicans showed a significance value (Asymp. Sig.) of 0.010. Because the significance value obtained was less than 0.05 ($p < 0.05$), this means there was a significant difference in effect among the five treatment groups on the inhibition-zone diameter of Candida albicans. A post-hoc test using Dunn's test was therefore carried out to identify which pairs of treatment groups had significant differences in inhibitory effectiveness.

Table 7. Dunn's Test Results for Inhibition Zone Diameter of Candida albicans

Comparison of Treatment Groups	Dunn's Test Result (p)
Negative Control (K–) vs. Concentration 20%	1
Negative Control (K–) vs. Concentration 40%	0.817
Negative Control (K–) vs. Concentration 60%	0.172
Negative Control (K–) vs. Positive Control (K+)	0.01
Concentration 20% vs. Concentration 40%	1
Concentration 20% vs. Concentration 60%	1
Concentration 20% vs. Positive Control (K+)	0.134
Concentration 40% vs. Concentration 60%	1
Concentration 40% vs. Positive Control (K+)	1
Concentration 60% vs. Positive Control (K+)	1

Source: Primary data, 2025

Based on the results of Dunn's post-hoc analysis in Table 7, the comparison of effectiveness among groups shows that a significant difference occurred only between the Negative Control (K–) and Positive Control (K+) groups, with a significance value of $p < 0.05$.

Meanwhile, none of the guava-leaf extract concentration variations (20%, 40%, and 60%) showed a significant difference in inhibitory power compared with the Negative Control group ($p > 0.05$). This finding indicates that administering guava-leaf extract at the three concentration levels produced an inhibition profile relatively comparable to that of the base solvent. In other words, the extract at the tested concentration range had not yet shown significant antibacterial/antifungal activity against Candida albicans. A similar condition was also observed among the extract concentration groups themselves, where increasing the dose was not accompanied by a meaningful difference in effectiveness. To provide a clearer picture of the inhibition pattern in each treatment group, the average inhibition-zone diameter of Candida albicans is presented in the figure below.

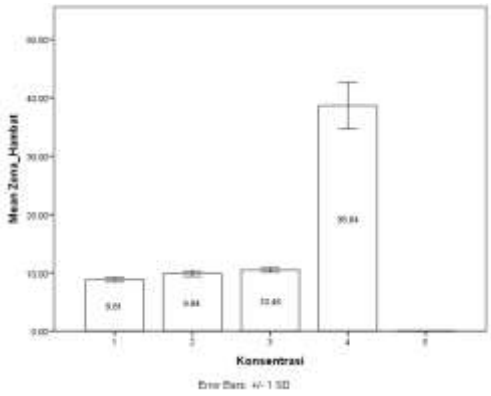

Figure 3. Average Inhibition Zone Diameter of Candida albicans

Source: Primary data, 2025

Figure 3 visually shows a tendency toward an increasing average inhibition-zone diameter with increasing concentration of the red guava leaf extract. The resulting average inhibition-zone diameters were 8.81 mm at a concentration of 20%, increasing to 9.84 mm at 40%, and reaching 10.46 mm at 60%. This pattern shows that increasing extract concentration tends to be followed by an increase in the ability to inhibit the growth of Candida albicans.

Nevertheless, the results of Dunn's test in Table 15 show that this increase in average inhibition-zone diameter was not accompanied by a statistically significant difference. All comparisons among the extract-concentration groups produced significance values greater than 0.05, so there is insufficient evidence to state that increasing the extract concentration from 20% to 60% produced a meaningful difference in antifungal effectiveness. In other words, the three tested extract concentrations still showed a relatively comparable level of inhibitory power against the growth of Candida albicans.

In addition, the comparison between each extract group and the Negative Control (K–) also showed no significant difference ($p > 0.05$). This result indicates that although an inhibition zone formed in the extract groups, the resulting antifungal effectiveness was not yet strong enough to show a meaningful difference compared with the negative control group. This finding shows that the antifungal activity of the red guava leaf extract against Candida albicans, within the concentration range used in this study, remains limited.

On the other hand, the Positive Control (K+) group produced the highest average inhibition-zone diameter, at 38.64 mm. This value was far greater than all extract groups and the negative control. The results of Dunn's test showed that a significant difference was found only between the Negative Control (K–) and Positive Control (K+) ($p < 0.05$). This finding shows that the antifungal agent in the positive control had a far greater inhibitory ability against the growth of Candida albicans than the red guava leaf extract used in this study.

The relatively short error bars for each treatment group indicate that the variation in measurement results between replicates tended to be small, indicating that the observation results obtained were fairly consistent for each treatment group. Overall, the visualization in the figure shows a tendency toward increasing inhibition-zone diameter with increasing concentration of red guava leaf extract. However, based on the statistical analysis, this increase has not yet shown a significant difference, so the antifungal effectiveness of the extract against Candida albicans at the concentrations tested cannot yet be significantly differentiated.

Antioxidant and Vitamin C Test Results

The antioxidant testing of the red guava leaf extract (Psidium guajava L) and of vitamin C produced the following results:

Table 8. DPPH Antioxidant Test Results

Concentration (µg/mL)	Replicate 1	Replicate 2	Mean Absorbance	% Inhibition
10	0.256	0.257	0.26	0.26
20	0.186	0.184	0.19	0.19
40	0.142	0.145	0.14	0.14
80	0.101	0.108	0.10	0.10

Source: Primary data, 2025

Based on the results of the DPPH antioxidant activity test, changes in absorbance value were observed at each concentration variation of the guava-leaf extract. The absorbance value decreased with increasing extract concentration used. At a concentration of 10 µg/mL, an average absorbance of 0.26 was obtained; at 20 µg/mL, an average absorbance of 0.19; at 40 µg/mL, an average absorbance of 0.14; and at 80 µg/mL, an average absorbance of 0.10.

The decrease in absorbance value shows that the higher the concentration of guava-leaf extract, the greater the ability of the antioxidant compounds to scavenge the DPPH free radical. This is reflected in the increase in percent-inhibition values, as more DPPH free radicals were reduced due to the donation of hydrogen atoms or electrons from the antioxidant compounds in the extract.

The antioxidant activity of the guava-leaf extract was determined based on the IC_{50} value, i.e., the sample concentration required to inhibit 50% of the DPPH free radical. Based on the analysis, the IC_{50} value of the guava-leaf extract was 13.40 µg/mL. This value shows that the guava-leaf extract has antioxidant activity classified as very strong, since it has an IC_{50} of less than 50 µg/mL.

The antioxidant capacity of the guava-leaf extract is thought to originate from secondary metabolite compounds such as flavonoids and phenolic compounds, which have the ability to scavenge free radicals. The hydroxyl group in phenolic compounds plays a role in donating hydrogen atoms to the DPPH radical, forming a more stable compound.

Based on these results, the guava-leaf extract has potential as a source of natural antioxidants. An IC_{50} value of 13.40 µg/mL shows that the extract has a good ability to scavenge the DPPH free radical, and its activity can be compared with the positive control, vitamin C, to determine its level of effectiveness.

After the percent-inhibition value of the sample was obtained, the regression equation of sample concentration versus percent inhibition was determined and calculated using Microsoft Excel 2007. The equation was obtained from a graph relating the independent variable, i.e., solution concentration (x), and the dependent variable, i.e., percent inhibition or %RSA (y). The graph and linear regression equation $Y = ax + b$ for the ethanol extract of red guava leaves can be seen in the chart below.

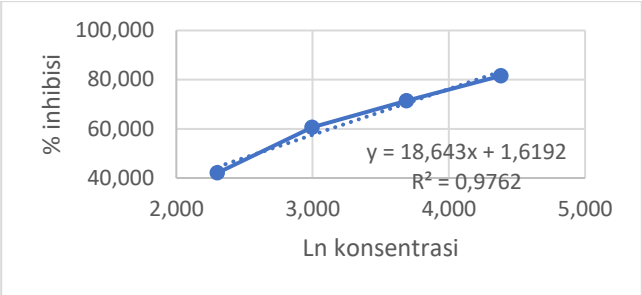

Figure 4. Linear Regression Equation of the Red Guava Leaf Extract in Ethanol Solvent

Source: Primary data, 2025

The antioxidant test of vitamin C on the red guava leaf extract (Psidium guajava L) produced the following results:

Table 9. Vitamin C Test Results

Concentration (µg/mL)	Replicate 1	Replicate 2	Mean Absorbance	% Inhibition
2	0.246	0.248	0.25	44.55
4	0.176	0.177	0.18	62.86
6	0.124	0.125	0.12	76.36
8	0.067	0.065	0.07	91.56

Source: Primary data, 2025

Based on the test results, vitamin C showed an increase in antioxidant activity with increasing concentration. At a concentration of 2 µg/mL, a percent-inhibition value of 44.55% was obtained; at 4 µg/mL, 62.86%; at 6 µg/mL, 76.36%; and at the highest concentration of 8 µg/mL, 91.56%.

The increase in percent-inhibition value shows that the higher the concentration of vitamin C used, the greater its ability to inhibit the DPPH free radical. This is because the amount of available antioxidant compound increases, so its ability to donate electrons or hydrogen atoms to stabilize free radicals also increases.

Based on these results, vitamin C has very strong antioxidant activity. An IC_{50} value of 2.36 µg/mL shows that vitamin C has a good ability to scavenge the DPPH free radical.

After the percent-inhibition value of the sample was obtained, the regression equation of sample concentration versus percent inhibition was determined and calculated using Microsoft Excel 2007. The equation was obtained from a graph relating the independent variable, i.e., solution concentration (x), and the dependent variable, i.e., percent inhibition or %RSA (y). The graph and linear regression equation Y = ax + b for vitamin C can be seen in the chart below.

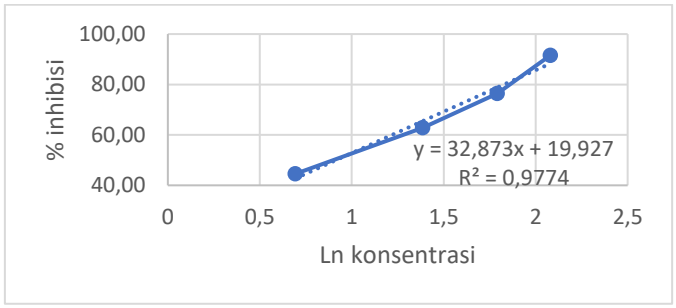

Figure 19. Linear Regression Equation of Vitamin C as the Positive Control

Source: Primary data, 2025

In this study, the antioxidant activity of the ethanol extract of red guava leaves (Psidium guajava L) at concentrations of 10, 20, 40, and 80 µg/mL yielded the regression equation Y = 18.643x + 1.6192, from which an IC_{50} value of 13.40 µg/mL was calculated. The antioxidant

activity of vitamin C at concentrations of 2, 4, 6, and 8 µg/mL yielded the regression equation Y = 32.873x + 19.927, from which an IC_{50} value of 2.36 µg/mL was calculated.

Table 10. Summary of Results

No.	Test Solution	IC_{50} Value	Classification
1	Red guava leaf extract in ethanol solvent	13.40 µg/mL	Very strong antioxidant
2	Vitamin C	2.36 µg/mL	Very strong antioxidant

Source: Primary data, 2025

This study was an in vitro laboratory experiment on the antimicrobial and antioxidant activity of the ethanol extract of red guava leaves (Psidium guajava L) against Staphylococcus aureus, Escherichia coli, and Candida albicans using the disc diffusion method, in which the extract was prepared using the maceration technique with 96% ethanol.

Extraction was carried out using the maceration procedure with 96% ethanol as the solvent. Over three days, the red guava leaf powder (Psidium guajava L) was mixed with 96% ethanol and left to stand for 3×24 hours while being stirred periodically. The resulting extract was then filtered using filter paper. Once the maceration filtrate had been obtained, it was concentrated in a rotary evaporator, in which the extract and solvent were separated by evaporation at a temperature of 50ºC and a speed of 70 rpm, until a viscous extract was obtained.

The extract obtained was then subjected to phytochemical screening to identify the secondary metabolite compounds contained in the red guava leaves (Psidium guajava L). The test results showed that the red guava leaves (Psidium guajava L) contained alkaloid, flavonoid, and tannin compounds. Phytochemical screening of red guava leaves (Psidium guajava L) has also been carried out previously; a study by Ignatius Rinanto Cipto Dwi Saputro and Agus Purwanto (2022) showed that red guava leaf extract (Psidium guajava L) contains alkaloid, flavonoid, and tannin compounds. This finding is also supported by a study by Niken et al. (2022), which showed that the extract yielded alkaloids, flavonoids, and tannins.

The extract obtained was then tested for its antimicrobial effect against the bacteria Staphylococcus aureus and Escherichia coli, and the fungus Candida albicans. The largest inhibitory effect on Staphylococcus aureus occurred at a concentration of 60%, producing an inhibition zone of 16.75 mm, while the positive control produced an inhibition zone of 29.09 mm. This condition shows that the positive control had far stronger antibacterial activity than the red guava leaf extract. For Escherichia coli, the largest inhibition zone occurred at a concentration of 60%, at 8.44 mm, while the positive control produced an average inhibition-zone diameter of 24.53 mm. This result shows that the positive control still had stronger antibacterial activity than the red guava leaf extract. For the fungus Candida albicans, the largest inhibition zone occurred at a concentration of 60%, at 10.46 mm, while the positive control produced the highest average inhibition zone, at 38.64 mm; this result shows that the antifungal agent in the positive control had a far greater antifungal capability than the guava-leaf extract.

The antioxidant test results of the red guava leaf extract showed very strong antioxidant activity based on the DPPH method, with an IC_{50} value of 13.40 µg/mL. This activity shows that the red guava leaf extract has potential as a source of natural antioxidants. In the antioxidant test using the DPPH method, vitamin C as the positive control showed very strong antioxidant activity, with an IC_{50} value of 2.36 µg/mL.

CONCLUSION

The ethanol extract of red guava leaves (Psidium guajava L.) was found to contain alkaloids, flavonoids, and tannins, but no saponins or triterpenoids/steroids. The extract exhibited antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Candida albicans, with inhibition zones increasing at higher extract concentrations (20%, 40%, and 60%). The largest inhibition zones observed at the 60% concentration were 16.75 mm for S. aureus, 8.44 mm for E. coli, and 10.46 mm for C. albicans; however, these values remained considerably lower than those of the positive control (29.09 mm, 24.53 mm, and 38.64 mm, respectively). Statistical analysis showed that the antibacterial activity of the extract against E. coli was significantly different at all tested concentrations compared with the negative control (Tukey's HSD test, $p < 0.05$), with a significant increase in effectiveness from the 20% to 60% concentration ($p = 0.023$). However, the activity against S. aureus and C. albicans showed no significant differences among concentrations or compared with the negative control (Dunn's test, $p > 0.05$), indicating limited effectiveness within the tested concentration range. The extract also demonstrated strong antioxidant activity based on the DPPH assay, with an IC_{50} value of 13.40 µg/mL, approaching the antioxidant activity of vitamin C (IC_{50} = 2.36 µg/mL). These findings suggest that red guava leaves have potential as a natural antioxidant source; however, their antimicrobial potential requires further optimization, such as the use of higher concentrations or combinations with other bioactive compounds, to achieve efficacy comparable to the positive control.

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