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Potential Of Red Ginger (Zingiber Officinale Roscoe Var. Rubrum) Extract To Inhibit The Growth Of Staphylococcus Aureus In Vitro

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Abstract
*Antimicrobial resistance is a global health issue, including in Indonesia. In 2019, antimicrobial resistance was associated with approximately 4.95 million deaths worldwide. In Indonesia, 133,800 deaths were linked to antimicrobial resistance, with 34,500 deaths directly attributable to it. Red ginger (*Zingiber officinale* Roscoe var. *rubrum*) contains various bioactive compounds—such as phenolics, alkaloids, flavonoids, cardiac glycosides, glycosides, saponins, steroids, terpenoids, and tannins—that possess potential anti-inflammatory, antioxidant, and antibacterial properties. This study aimed to investigate the potential of red ginger extract to inhibit the growth of *Staphylococcus aureus* *in vitro*. Methods: This study employed a true experimental design. Red ginger extract was obtained via maceration using 96% ethanol. Antibacterial activity was assessed using the disc diffusion method at extract concentrations of 25%, 50%, 75%, and 100%. Results: Red ginger extract demonstrated an inhibitory effect on the growth of *Staphylococcus aureus*. Concentrations of 50%, 75%, and 100% showed significant differences ($p<0.05$). The mean diameters of the inhibition zones at concentrations of 25%, 50%, 75%, and 100% were 8.69 mm, 9.87 mm, 9.30 mm, and 13.82 mm, respectively. The One-Way ANOVA test yielded a p-value of <0.05, indicating that the concentration of red ginger extract influences the inhibition of *Staphylococcus aureus* growth. Conclusion: Red ginger extract exhibits antibacterial activity against *Staphylococcus aureus*, with a concentration of 100% being the most effective for inhibiting bacterial growth.*

Keywords: Red ginger; Staphylococcus Aureus; Zingiber officinale Roscoe Var. Rubrum

INTRODUCTION

Staphylococcus aureus ranks fourth among pathogens that cause deaths due to antimicrobial resistance. Research in Malaysia reports that approximately 40% of adults are colonized by *Staphylococcus aureus* in their oral cavity. A meta-analysis in Nigeria indicates a high prevalence of *Staphylococcus aureus* resistance to various classes of antimicrobials. Furthermore, a systematic study in Indonesia—encompassing 2,182 search results and 102 publications—analyzed 19,517 bacterial isolates; it found that 22.2% of *Staphylococcus aureus* isolates from hospitals and 11.1% of those from the community exhibited resistance to methicillin.

Antimicrobial resistance is a global health issue, including in Indonesia. In 2019, antimicrobial resistance was associated with approximately 4.95 million deaths worldwide. That same year, 133,800 deaths in Indonesia were linked to antimicrobial resistance, with 34,500 of these deaths directly attributable to it.

Red ginger (*Zingiber officinale* Roscoe var. *rubrum*) contains various bioactive compounds with health benefits, including phenolics, alkaloids, flavonoids, cardiac glycosides, glycosides, saponins, coumarins, quinones, betacyanins, steroids, terpenoids, and tannins. These compounds are known to possess anti-inflammatory, antioxidant, immunomodulatory, and antibacterial properties.

Based on this background, this study aims to investigate the potential of red ginger (*Zingiber officinale* Roscoe var. *rubrum*) extract at various concentrations to inhibit the growth of *Staphylococcus aureus* *in vitro*.

RESEARCH METHODS

Research Design

This study employs a true experimental design, specifically a posttest-only control group design. The research involved administering treatments—consisting of red ginger (*Zingiber officinale* Roscoe var. *rubrum*) extract at various concentrations—to observe their effects on the growth of *Staphylococcus aureus* bacteria. *Staphylococcus aureus* isolates were obtained from the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences at the University of Muhammadiyah North Sumatra. Antibacterial activity was assessed using the disc diffusion method, with double-distilled water (*aquabidest*) serving as the negative control and cefoxitin as the positive control. The concentrations of red ginger extract tested were 25%, 50%, 75%, and 100%.

Number of Replications

The study consisted of six treatment groups: red ginger extract at concentrations of 25%, 50%, 75%, and 100%, a positive control (cefoxitin), and a negative control (double-distilled water). Each group underwent five replications, resulting in a total of 30 samples. The number of replications was determined using Federer's formula.

(n-1) (t-1) ≥ 15
(n-1) (t-1) ≥ 15
(n-1) (6-1) ≥ 15
(n-1) (5) ≥ 15
(5n-5) ≥ 15
(5n) ≥ 20
n ≥ 4

n: Number of samples
t: Number of groups

RESULTS AND DISCUSSION

Data from the measurement of the inhibition zone diameters were analyzed using statistical software. Normality and homogeneity tests were conducted to determine the distribution and homogeneity of the data. Data that were normally distributed and homogeneous were subsequently analyzed using a One-Way ANOVA, followed by a Tukey Honest Significant Difference (HSD) post hoc test to identify differences in the mean inhibition zone diameters among the treatment groups. A p-value of <0.05 was considered statistically significant.

Research Results

This study was conducted at the Microbiology and Biochemistry Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, to investigate the inhibitory activity of red ginger (*Zingiber officinale* Roscoe var. *rubrum*) extract against the growth of *Staphylococcus aureus*. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones at extract concentrations of 25%, 50%, 75%, and 100%, and compared against a positive control (cefoxitin) and a negative control (distilled water).

Table 1. Measurement Results of the Inhibition Zone Diameter of Red Ginger Extract against Staphylococcus aureus

Diameter of Inhibition Zone						
P	**Extract Concentration**				**K (+)**	**K (-)**
	25%	**50%**	**75%**	**100%**		
P 1	8,75	8	9,3	15,95	15,65	0
P 2	14,35	14,55	0	11,8	23,85	0
P 3	10,16	10,25	9,8	12,05	11,2	0
P 4	0	9,1	14,3	16,65	19,95	0
P 5	10,2	7,45	13,1	12,65	30,4	0
Mean	8,69	9,87	9,3	13,82	20,21	

The table above shows that the average diameter of the inhibition zone for red ginger (*Zingiber officinale* Roscoe var. *rubrum*) extract at concentrations of 25%, 50%, 75%, and 100% are 8.693 mm, 9.87 mm, 9.3 mm, and 13.82 mm, respectively.

Table 2. Results of Normality and Homogeneity Tests

Group	**Shapiro-Wilk Normality Test**	**Homogeneity Test**
Ekstrak 25%	0,278	0,078 or > 0,05
Ekstrak 50%	0,249	
Ekstrak 75%	0,231	
Ekstrak 100%	0,119	
Cefoxitin	0,985	

This study presents a normality test with a sig. value >0.05, indicating that the treatment data are normally distributed; subsequently, a One-Way ANOVA was performed.

Table 3. One-Way ANOVA Test Results

Group	***Mean ± SD***	***P***
Cefoxitin (K+)	20,21 ± 7,40	0,000
Aquadest (K-)	0,00 ± 0,00	
Ekstrak 25%	8,69 ± 5,29	
Ekstrak 50%	9,87 ± 2,82	
Ekstrak 75%	9,30 ± 5,61	
Ekstrak 100%	13,82 ± 2,29	

The One-Way ANOVA test yielded a p-value of <0.05, indicating that the administration of red ginger (Zingiber officinale Roscoe var. rubrum) extract has an effect on inhibiting the growth of the bacterium Staphylococcus aureus.

Subsequently, a post-hoc test—specifically the Tukey Honest Significant Difference (HSD) test—was conducted to determine whether there were significant differences ($p<0.05$) in the mean bacterial growth across the various treatment groups.

Table 4. Results of the Tukey Honest Significant Difference (HSD) Post-Hoc Test

Treatment Group	n	p-value	Information
(K-) vs Extract 25%	5	0.063	Not significantly different
(K-) vs Extract 50%	5	0,026	Significantly different
(K-) vs Extract 75%	5	0,042	Significantly different
(K-) vs Extract 100%	5	0,001	Significantly different
(K+) vs Extract 100%	5	0,278	Not significantly different

The results of the Tukey Honest Significant Difference (HSD) post-hoc test indicate that only the 25% concentration of red ginger extract did not differ significantly from the negative control (p=0.063). Conversely, red ginger extracts at concentrations of 50%, 75%, and 100% showed significant differences compared to the negative control ($p<0.05$). Meanwhile, there was no significant difference between the 100% concentration of red ginger extract and the positive control (cefoxitin) (p=0.278).

Discussion

This study demonstrates that the administration of red ginger (*Zingiber officinale* var. *rubrum*) extract inhibits the growth of *Staphylococcus aureus*. This is evidenced by the formation of inhibition zones in each treatment group, with mean diameters varying according to the concentration of the extract applied. These results indicate a trend toward increased antibacterial activity as the extract concentration rises.

The antibacterial activity of the red ginger extract is likely linked to the presence of secondary metabolites identified through phytochemical analysis, namely flavonoids, alkaloids, phenolics, and tannins. Alkaloids are known to interfere with bacterial cell wall synthesis, leading to cell membrane damage caused by pressure differentials between the intracellular and extracellular compartments. Flavonoids contribute to increased bacterial cell membrane permeability, resulting in cell lysis, while phenolic compounds have been reported to inhibit the enzyme 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase, which is essential for bacterial folic acid synthesis. Furthermore, tannins exert antibacterial effects through mechanisms such as cell membrane disruption, protein binding, enzyme inactivation, and the inhibition of bacterial toxin production. It is hypothesized that this combination of mechanisms contributes to the antibacterial activity of red ginger extract against *Staphylococcus aureus*.

The study results show that the mean inhibition zone diameters for red ginger extract at concentrations of 25%, 50%, 75%, and 100% were 8.69 mm, 9.87 mm, 9.30 mm, and 13.82 mm, respectively. In general, an increase in extract concentration corresponds to greater inhibition of *Staphylococcus aureus* growth, with the largest inhibition zone diameter observed at a concentration of 100%. These findings are consistent with research by Bonita Yunpay, which reported that the mean diameter of the inhibition zone increased alongside the concentration of red ginger extract; this supports the conclusion that a higher extract concentration is associated with greater antibacterial activity.

The results of the Tukey test analysis indicate that red ginger extract at concentrations of 50%, 75%, and 100% shows a significant difference compared to the negative control, whereas the 25% concentration does not show a significant difference (p=0.063). Although the 25% concentration produced an inhibition zone, its antibacterial activity did not differ statistically from that of the negative control. In contrast, concentrations of 50%, 75%, and 100% demonstrated superior antibacterial activity compared to the negative control. These

findings suggest that increasing the extract concentration contributes to an enhanced ability to inhibit the growth of *Staphylococcus aureus*.

The results of this study are consistent with research by Siti Zamiyatul Azkiyah, who reported that red ginger extract influences the growth of *Staphylococcus aureus*, although not all concentrations showed a significant difference. In that study, concentrations of 5%, 10%, and 80% showed significant differences, while concentrations of 20% and 40% did not differ significantly. This similar pattern indicates that the antibacterial activity of red ginger extract is influenced by the concentration used, although the antibacterial response at each concentration does not necessarily increase in a linear fashion.

In the present study, the 100% concentration produced the largest inhibition zone diameter compared to the other concentrations. This result differs from research by Shavila Lukita, who reported that a 75% concentration was the most effective for inhibiting the growth of *Staphylococcus aureus*. Discrepancies in results between studies may be influenced by various factors, such as the extraction method, the type of solvent used, the content of active compounds, the characteristics of the bacterial isolates, and differences in laboratory testing conditions.

A comparison between the 100% concentration red ginger extract and the positive control (cefoxitin) yielded a p-value of 0.278 ($p > 0.05$), indicating no statistically significant difference in the diameter of the inhibition zone between the two groups. These results suggest that, under the conditions of this study, the 100% concentration red ginger extract exhibits an inhibitory effect that is statistically comparable to that of the positive control. However, this does not imply that the effectiveness of the red ginger extract is equivalent to that of cefoxitin, as the study was not designed to test for equivalence in efficacy. Therefore, further research—including *in vivo* studies as well as the determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)—is required to evaluate the effectiveness and safety of red ginger extract as a potential antibacterial agent.

The selection of extract concentrations for this study was based on research by Purba, which reported that red ginger extracts at concentrations of 5%, 10%, 15%, 20%, and 25% failed to provide an optimal inhibitory effect against *Staphylococcus aureus*.[26] In light of these findings, the present study employed higher concentrations—25%, 50%, 75%, and 100%—to assess whether antibacterial activity could be enhanced. The results demonstrate that higher concentrations yield a greater inhibitory effect, particularly at 50%, 75%, and 100%, supporting the hypothesis that increasing the concentration of the extract contributes to enhanced antibacterial activity of red ginger extract against *Staphylococcus aureus*.

CONCLUSION

Red ginger (Zingiber officinale var. rubrum) extract exhibits antibacterial activity against Staphylococcus aureus, as evidenced by the formation of inhibition zones at all tested concentrations. The study results indicate that red ginger extract at concentrations of 50%, 75%, and 100% provides a significantly different inhibitory effect compared to the negative control, whereas a concentration of 25% shows no significant difference. The 100% concentration produces the largest inhibition zone diameter and shows no statistically significant difference compared to the positive control (cefoxitin).

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