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Evaluation of the Antimicrobial Efficacy of Red Ginger Extract (*Zingiber Officinale Rosc. Var Rubrum*) Against *Staphylococcus aureus* Using the Kirby-Bauer Disc Diffusion Method

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ABSTRACT

The increasing prevalence of antibiotic-resistant bacteria, particularly Staphylococcus aureus, necessitates the exploration of alternative treatments. This study investigates the antibacterial efficacy of red ginger extract (*Zingiber officinale Rosc. var. Rubrum*) against *Staphylococcus aureus* using the Kirby-Bauer disc diffusion method. Fresh red ginger was extracted using 96% ethanol, and varying concentrations (50%, 75%, and 100%) were tested for their ability to inhibit bacterial growth. The results indicated that at 50% and 75% concentrations, the inhibition zones ranged from 6.2 mm to 9.5 mm, categorizing them as resistant. In contrast, the 100% concentration yielded inhibition zones between 14.2 mm and 16.9 mm, classified as intermediate. These findings suggest that red ginger extract possesses significant antibacterial properties, particularly at higher concentrations, although it remains less effective than standard antibiotics like chloramphenicol. The study highlights the potential of red ginger extract as a natural alternative for treating *Staphylococcus aureus* infections, emphasizing the need for further research on dosage variations, solvent types, and toxicity assessments to optimize its therapeutic application. Overall, red ginger extract could serve as a promising candidate in the fight against antibiotic-resistant bacterial infections.

Keywords: Red ginger, *Staphylococcus aureus*, Antibacterial activity, Antibiotic resistance, Bioactive compounds

INTRODUCTION

Plants play a vital role in human life, including their use in traditional medicine. Traditional herbs are predominantly derived from plants such as roots, wood, leaves, flowers, bark, or seeds. To ensure their scientific validity, traditional medicine requires research in toxicology, pharmacology, and the identification and isolation of active chemical compounds (Fitriah et al., 2017). Antibacterial agents, which are natural chemical compounds, can inhibit or kill bacteria even in small concentrations. Testing for minimum inhibitory concentration aims to enhance the effectiveness of these compounds while preventing bacterial resistance caused by excessive and continuous administration, which can lead to resistant bacterial strains. Antibacterial activity depends on several factors, including extract concentration, the content of antibacterial compounds, diffusion ability, and the type of bacteria inhibited (Kuspradini et al., 2016; Marselia et al., 2015; Menon & Satria, 2017). Traditional medicine, a cultural heritage, must be continuously preserved and developed, with Indonesia's rich biodiversity offering various spice plants such as ginger (Muabas, 2018). Ginger (*Zingiber officinale*), originating from South Asia, has been used globally, from food flavoring in ancient China to herbal remedies for ailments like vertigo and nausea in Greece. In England, King Henry VIII recommended ginger during the plague, and Queen Elizabeth I utilized it to enhance sexual arousal (Redi Aryanta, 2019). Indonesia ranks third globally in ginger production, with Central Java alone producing 30 tons annually (Azizah et al., 2019). Among the commonly cultivated ginger types elephant ginger (*Zingiber officinale var. Roscoe*), red ginger (*Zingiber officinale var. Rubrum*), and emprit ginger (*Zingiber officinale var. Amarum*) red ginger stands out due to its high essential oil and gingerol content, making it highly effective for treating various diseases (Muabas, 2018; Redi Aryanta, 2019; Setiawan & Selmitri, 2022). Its processed products, such as oleoresin, contain key bioactive compounds like gingerol, shogaol, and zingerone, which provide therapeutic benefits (Arifianto et al., 2019; Sulistyaningsih et al., 2023). Empirically, ginger is used for managing swelling, rheumatism, muscle pain, osteoarthritis, diabetes-related complications, LDL cholesterol reduction, and even cancer prevention (Redi Aryanta, 2019; Yuniati et al., 2019).

The growing prevalence of antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), has heightened the urgency for alternative treatments (Abhisubesh Vijayakumar et al., 2023; Tania et al., 2023). *Staphylococcus aureus*, a versatile pathogen, is linked to infections ranging from minor skin issues to life-threatening conditions like sepsis. Red ginger, with its bioactive compounds including gingerol and shogaol, has demonstrated significant antibacterial properties against *Staphylococcus aureus* in recent studies, suggesting its potential as a natural antimicrobial agent (Prasetyo & Vifta, 2022; Sandrasari et al., 2023; Srikandi et al., 2020). The mechanism involves disrupting bacterial membranes and inhibiting bacterial growth, supported by its antioxidant properties, which may enhance the immune response (Abhisubesh Vijayakumar et al., 2023; Mbouche et al., 2022). Given these attributes, red ginger could serve as a valuable alternative to conventional antibiotics in combating resistant strains of *Staphylococcus aureus* (Luhurningtyas et al., 2021; Prasetyo & Vifta, 2022).

This study explores the effectiveness of red ginger extract against *Staphylococcus aureus*, aiming to contribute to the understanding of its role in addressing antibiotic resistance. With the increasing challenge of resistant bacterial strains, identifying effective natural alternatives like red ginger is crucial for advancing therapeutic options and improving public health outcomes (Abhisubesh Vijayakumar et al., 2023; Mbouche et al., 2022; Prasetyo & Vifta, 2022).

METHODS

The Kirby-Bauer disc diffusion method was chosen for this study due to its reliability, simplicity, and widespread use in evaluating the antimicrobial activity of plant extracts (Agnesia et al., 2023; Bayot & Bragg, 2024; Hudzicki, 2016; Liberty, 2024). This method involves impregnating filter paper discs with the test substance (red ginger extract at varying concentrations) and placing them on an agar plate inoculated with *Staphylococcus aureus*. The extract diffuses into the agar, creating a concentration gradient, and the resulting inhibition zones around the discs are measured to determine antibacterial efficacy. This method is particularly suitable for comparing the effectiveness of different concentrations of red ginger extract against a standardized bacterial strain, providing clear and quantifiable results. The study subjects included fresh red ginger (*Zingiber officinale Rosc. var. Rubrum*) of approximately 8–10 months of age, as this stage is optimal for the highest concentration of bioactive compounds such as gingerols and shogaols, which are known for their antimicrobial properties. The ginger was sourced from the Makassar market to ensure consistency and local relevance. The sample size was determined using Federer's formula, which is commonly applied in experimental designs to ensure statistical robustness and minimize variability. Five groups were established to test different concentrations of red ginger extract (50%, 75%, and 100%), along with positive (*chloramphenicol*) and negative (distilled water) controls. Each group contained five samples with five repetitions, totaling 25 samples. This design was chosen to provide sufficient data for statistical analysis, ensuring the reliability and reproducibility of the results while accounting for potential experimental variability.

Inclusion criteria for the study included fresh red ginger with a distinct red color and Mueller-Hinton agar medium overgrown with *Staphylococcus aureus*, while exclusion criteria included damaged media, such as broken or melted agar. Red ginger extract was prepared by macerating 500 g of fresh red ginger in 5 liters of 96% ethanol for five consecutive 24-hour periods with periodic stirring, followed by filtration and concentration using a rotary evaporator. Extract concentrations of 50%, 75%, and 100% were prepared using a dilution formula. A bacterial suspension was prepared by growing a pure culture of *Staphylococcus aureus* in a 0.9% NaCl solution until the turbidity matched the McFarland standard 0.5 (equivalent to 1.5×10^8 CFU/mL). Control solutions included *chloramphenicol* antibiotic discs and distilled water.

The antibacterial activity was tested by spreading a bacterial suspension onto Mueller-Hinton Agar plates, followed by placing paper discs soaked in the red ginger extract solutions onto the plates. After incubation at 37°C for 24 hours, the inhibition zones were measured using a ruler. The diameter of the inhibition zone was classified based on CLSI standards, with results being analyzed using descriptive statistics. Ethical clearance was obtained from the Ethics Committee of the Faculty of Medicine with the number (520/A.1/KEP-UMI/X/2024), it is get from Muslim Indonesia University, to ensure adherence to ethical guidelines for experimental research.

RESULTS AND DISCUSSION

Table 1.

Results of inhibition zones formed at various concentrations of red ginger extract (*zingiber officinale Rosc. Vas Rubrum*)

Concentration	Zone of Inhibition of *Staphylococcus aureus*	Interpretation of Growth Inhibition Response
50%	6.2 mm 6.4 mm 6.6 mm 6.8 mm 6.9 mm	Resistant
75%	6.5 mm 6.8 mm 7.3 mm 8.6 mm 9.5 mm	Resistant
100%	14.2 mm 14.7 mm 15.2 mm 15.9 mm 16.9 mm	Intermediate

Source: Primary Data Processed, 2024

Table 1 presents the results of the inhibition zones formed by various concentrations of red ginger extract (*Zingiber officinale Rosc. Vas Rubrum*) against *Staphylococcus aureus*, highlighting the antibacterial efficacy of the extract at different concentrations (Jieputra et al., 2024; Lukita et al., 2021). At a concentration of 50%, the recorded inhibition zones ranged from 6.2 mm to 6.9 mm, indicating a resistant response to bacterial growth, as the zones were relatively small and insufficient to effectively inhibit the bacteria. Similarly, at a 75% concentration, the inhibition zones varied from 6.5 mm to 9.5 mm, which also fell within the resistant category, suggesting that this concentration was still inadequate for significant antibacterial action. In contrast, at a 100% concentration, the inhibition zones exhibited a marked increase, ranging from 14.2 mm to 16.9 mm, which categorized the response as intermediate. This substantial increase in inhibition zone size at the highest concentration indicates that red ginger extract possesses enhanced antibacterial properties at elevated concentrations, although it does not reach the level of sensitivity typically observed with standard antibiotics.

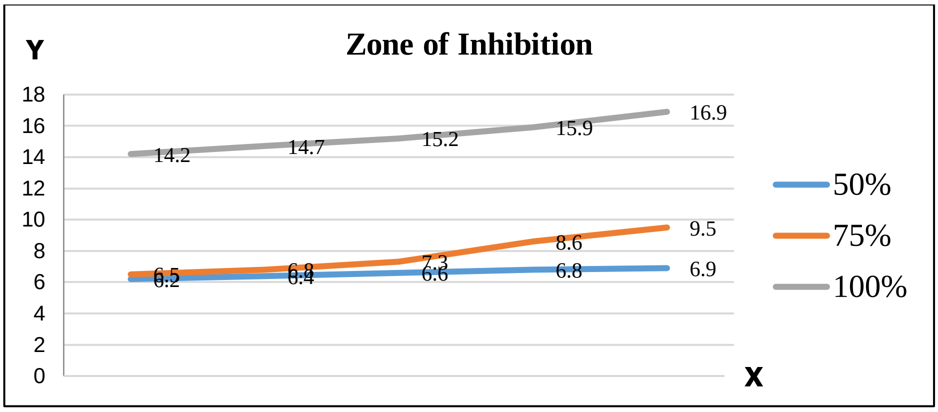

Figure 1. Zone of Inhibition

The line graph illustrates the relationship between different concentrations of a substance (50%, 75%, and 100%) and their corresponding "Zone of Inhibition," which is measured in millimeters (mm) and represented on the y-axis, ranging from 0 to 18. The x-axis represents the progression of time or experimental conditions, though it is unlabeled in this graph. Overall, all three lines exhibit an increasing trend across the x-axis, with the 100% concentration consistently yielding the highest values, indicating the largest Zone of Inhibition. The 75% line closely follows the 100% line, while the 50% line displays the lowest values among the three. This pattern suggests that higher concentrations of the tested substance are more effective at inhibiting bacterial growth, supporting the notion of a concentration-dependent effect. The y-axis quantifies the effectiveness of the substance, while the x-axis likely represents sequential measurements or time intervals, demonstrating how the Zone of Inhibition changes with varying concentrations over the course of the experiment.

DISCUSSION

The results showed that red ginger extract (*Zingiber officinale Rosc. Vas Rubrum*) has antibacterial ability against *Staphylococcus aureus* which is concentration-dependent. At 50% and 75% concentration, the antibacterial activity was classified as low (resistant), while at 100% concentration, the activity increased to intermediate category. This suggests that the active compounds in red ginger, such as gingerol, shogaol, and zingerone, are able to inhibit bacterial growth, although their effectiveness cannot match standard antibiotics such as *chloramphenicol*, which produces an inhibition zone of 39.6 mm. Comparison with positive and negative controls also confirmed that the observed antibacterial activity came from the red ginger extract, not from the solvent or media used.

This is in line with the research which reported the antibacterial activity of red ginger with the largest inhibition zone at 100% concentration (13.17 mm) and smaller inhibition zone at lower concentration (10.21 mm for 25%). The study also confirmed that the inhibition of red ginger increased with concentration, although the effectiveness was still lower than positive controls, such as ciprofloxacin (21.75 mm) (Isramilda et al., 2024; Juariah et al., 2023; Widiiastuti et al., 2024). At 100% concentration, the inhibition zone produced by this study (14.2-16.9 mm) was greater than the results which reported an inhibition zone of 12.54 ± 0.76 mm. Inhibition of bacterial growth by red ginger occurred at all tested extract concentrations (20-100%), with the smallest inhibition zone at 20% concentration (10.17 ± 2.26 mm). These findings suggest that bioactive compounds such as gingerol, shogaol, and zingeron, which were identified through phytochemical analysis conducted in this research, play a major role in the antibacterial activity of red ginger extract. The phytochemical analysis involved qualitative and quantitative methods to detect and measure the presence of these compounds, confirming their contribution to the observed inhibition zones. The larger inhibition zones at higher concentrations (e.g., 14.2–16.9 mm at 100%) align with the increased presence of these bioactive compounds, which are known for their antimicrobial properties. This supports the concentration-dependent antibacterial effect observed in the study, as higher concentrations of red ginger extract contained greater amounts of these active compounds, leading to more significant bacterial growth inhibition (Widiastuti & Pramestuti, 2018).

The difference in inhibition zone results between this study may be influenced by the concentration of extracts used, the testing method (wells vs discs), or laboratory conditions such as culture media and incubation techniques. Nevertheless, both studies support the conclusion that red ginger extract effectively inhibits the growth of *Staphylococcus aureus* at various concentrations, although the activity is still below positive controls, such as ciprofloxacin or *chloramphenicol* antibiotics (Widiastuti & Pramestuti, 2018). In another study found similar results, although in terms of concentration the research was still stronger, the diameter of the inhibition zone of red ginger extract at a concentration of 40% reached 12.10 ± 0.20 mm and was categorized as strong. In fact, at concentrations as low as 20%, an inhibition zone of 10.03 ± 0.56 mm was seen, also classified as strong. This shows that even though the concentration of the extract was lower than this study (50-100%), red ginger still showed significant antibacterial activity (Ruth Mayana Rumanti et al., 2021).

Ginger extract (*Zingiber officinale*) has antibacterial ability against *Staphylococcus aureus* with an inhibition zone that increases with increasing extract concentration. At 80% concentration, the inhibition zone reached 11.0 mm, and at 100% concentration, the inhibition zone increased to 12.3 mm. However, at lower concentrations (20-60 mg/ml), no significant zone of inhibition was formed. This supports the finding that extract concentration plays an important role in enhancing antibacterial activity (Zainal et al., 2022). The results of this study indicate that red ginger extract (*Zingiber officinale var. rubrum*) has antibacterial activity against *Staphylococcus aureus*, which is influenced by the type of bioactive compounds, concentration, and solvent used. The antimicrobial activity of red ginger mainly comes from monoterpenoid compounds such as 1,8-cineol, linalool, and geraniol, which work by damaging the bacterial cell wall and disrupting its cell function (Dewi & Wahyunitisari, 2018).

The results of research reported that raw ginger extract (CGE) was unable to inhibit the growth of *Staphylococcus aureus* at concentrations of 5% to 60%, but began to show inhibition at 70% concentration, with total inhibition at 100% concentration. These results suggest that to achieve optimal antibacterial effectiveness, the concentration of ginger extract needs to be high enough. This finding is in line with the results of this study which showed an increase in the zone of inhibition as the concentration of ginger extract increased, although there were differences in the concentration required to achieve maximal inhibition (Rahman et al., 2020). Ginger extract showed consistent antibacterial activity against *Staphylococcus aureus*. In this study, the higher the concentration of ginger extract, the larger the zone of inhibition formed, which is in line with the results of previous studies showing the effectiveness of ginger in inhibiting bacterial growth at various concentrations. Although there were differences in the MIC (Minimal Inhibitory Concentration) values, which were higher in this study, this could be due to variations in the ginger species, its biological conditions, or the extraction method used.

The results showed that *Staphylococcus aureus* bacteria showed susceptibility to several antibiotics with varying zones of inhibition, namely Nitrofurantion (14 mm), Vancomycin (15 mm), Levofloxacin (22 mm), and Oxacillin (16 mm). Interestingly, the zones of inhibition produced by antibiotics such as Vancomycin and Nitrofurantion were relatively similar in size to the results obtained in our study using red ginger extract, especially at higher extract concentrations, such as at 100% concentration which produced zones of inhibition around 16-17 mm. This suggests that although red ginger extract is not a synthetic antibiotic, its potential in inhibiting the growth of S. aureus can be comparable to some standard antibiotics, albeit with different mechanisms of action (Mahdi, 2022).

This study shows that red ginger extract has potential as an effective antibacterial agent against *Staphylococcus aureus*, with zones of inhibition comparable to some standard antibiotics such as Vancomycin and Nitrofurantion at certain concentrations. These findings provide important implications in the development of alternative medicinal plant-based therapies, particularly for the treatment of antibiotic-resistant bacterial infections. Red ginger extract could be a candidate for further development as a safer and more accessible natural medicine, given its potential that is almost comparable to conventional antibiotics. This study also opens up opportunities to dig deeper into the mechanism of action of ginger extract in inhibiting bacterial growth as well as potential combinations with other antibiotics to increase therapeutic effectiveness.

Some limitations in this study need to be noted. First, the concentration of ginger extract used in this study is limited to a certain level, so it is not certain whether higher or lower concentrations can provide more optimal results. Secondly, this study only tested the antibacterial effect against *Staphylococcus aureus* without considering the effect on other bacteria or potential toxicity on human cells. The test methods used, such as pitting diffusion, may have limitations in representing the effectiveness of ginger extracts under more complex biological conditions. Differences in the chemical composition of the ginger used (e.g., in terms of age, place of growth, or extraction method) may also affect the results, so further research is needed to optimize the most effective extraction procedure and concentration.

CONCLUSIONS

Red ginger extract at 100% concentration showed better effectiveness compared to lower concentrations, as evidenced by the inhibition zones ranging from 14.2 mm to 16.9 mm, which fall into the intermediate category. In contrast, the 50% and 75% concentrations produced significantly smaller inhibition zones, ranging from 6.2 mm to 9.5 mm, indicating that these concentrations are ineffective and categorized as resistant to Staphylococcus aureus. This data highlights that while the 100% concentration demonstrates a notable potential for inhibiting bacterial growth, there remains room for improvement in the effectiveness of lower concentrations, which did not achieve significant inhibition. Therefore, the results of this study can be used as a reference for institutions to develop red ginger extract-based therapy as an alternative treatment for Staphylococcus aureus infection. The public is also advised to understand the potential of red ginger extract as a natural treatment while still consulting with medical personnel to ensure the safety and effectiveness of its use. In addition, researchers are expected to continue the study with variations in dosage and different types of solvents, as well as testing the potential toxicity of red ginger extract to gain a more comprehensive understanding of the benefits and risks of its use.

SUGGESTION

Based on the findings of this study, several recommendations can be made to further explore the potential of red ginger extract as an alternative treatment for *Staphylococcus aureus* infections. Future research should test a wider range of concentrations, especially between 75% and 100%, to identify the optimal dosage for more effective results. Additionally, exploring different solvents, such as methanol or water, could enhance the extract's antibacterial activity. Testing against a broader range of pathogens, particularly antibiotic-resistant strains, would provide a clearer picture of its wider applicability. It is also crucial to conduct toxicity assessments to ensure the safety of red ginger extract for medicinal use, and clinical trials should be considered to evaluate its real-world effectiveness and safety, particularly in combination with conventional antibiotics. By addressing these suggestions, further studies could refine the therapeutic potential of red ginger extract, providing a promising natural alternative for treating bacterial infections.

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