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Comparative in Vitro Antibacterial Activity of Red Ginger (*Zingiber Officinale Var. Rubrum*) Extract and Conventional Antibiotics Against Pathogenic *Escherichia Coli*

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ABSTRACT
The increasing incidence of antibiotic resistance in Escherichia coli poses a significant challenge to public health, as the effectiveness of conventional antibiotics such as ciprofloxacin continues to decline due to the emergence of resistant E. coli strains. This study aims to evaluate red ginger extract's in vitro antibacterial activity against Escherichia coli and determine the optimal concentration for bacterial inhibition. This research aims to determine the effectiveness of red ginger extract with concentrations of 60%, 80% and 100% on the growth of Escherichia coli. Research method using a laboratory-based experimental study was conducted using red ginger extract prepared via ethanol maceration at concentrations of 60%, 80%, and 100%. The extract's antibacterial activity against E. coli was evaluated using the disc diffusion method following CLSI guidelines, with ciprofloxacin (500 mg) as a positive control and 95% ethanol as a negative control. Each treatment was replicated five times. Inhibition zones were measured after 24 hours of incubation at 37°C. The results of the study show that red ginger extract demonstrated significant antibacterial activity against E. coli, with mean inhibition zones of 6.08 ± 0.98 mm, 6.48 ± 1.21 mm, and 5.20 ± 1.89 mm for 60%, 80%, and 100% concentrations, respectively. The 80% concentration showed optimal inhibition activity, while the higher concentration (100%) demonstrated reduced effectiveness. Statistical analysis confirmed significant treatment differences ($p < .001$, $\eta^2 = 0.967$). The positive control produced significantly larger inhibition zones (23.16 ± 1.67 mm, $p < .05$). This study concludes that red ginger extract exhibits moderate antibacterial activity against E. coli in vitro, with 80% concentration showing optimal efficacy. These findings suggest potential applications in developing natural antimicrobial agents, although further research is needed to elucidate mechanisms of action and evaluate clinical applications.

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I. INTRODUCTION
The global rise of antibiotic resistance presents a critical challenge to public health, particularly concerning Escherichia coli infections [1]. While E. coli usually exists as a commensal bacterium in the human intestinal tract, it becomes pathogenic when its population increases beyond normal levels or colonizes extra-intestinal sites [2]. Recent surveillance data reveal an alarming trend: pathogenic E. coli strains increasingly resist multiple antibiotics, with developing countries facing the most significant burden [3]. The clinical implications of this resistance have prompted urgent action from global health organizations [4].

The World Health Organization has designated E. coli as a critical priority pathogen requiring immediate intervention strategies [5]. Current therapeutic approaches predominantly rely on fluoroquinolones, with ciprofloxacin serving as the primary treatment modality [6]. However, recent meta-analyses reveal alarming resistance rates between 20% and 40% across diverse geographical regions, compelling researchers to explore alternative therapeutic approaches [7].

Bacterial resistance in E. coli represents a sophisticated molecular adaptation strategy. The resistance mechanisms involve intricate interplays of genetic mutations, horizontal gene transfer, and adaptive cellular responses [8]. Plasmid-mediated resistance genes enable bacterial populations to produce enzymes that neutralize multiple antibiotic classes [9], [10]. A comprehensive global resistance surveillance report

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indicated that nearly 60% of clinical E. coli isolates demonstrate multiple antibiotic resistance markers, with some strains exhibiting resistance to more than five distinct antibiotic classes [11]. In response to these escalating challenges, natural products with established medicinal histories have emerged as promising alternative antimicrobial strategies. Red ginger (Zingiber officinale var. rubrum) presents a particularly compelling candidate, characterized by a rich and complex phytochemical composition demonstrating significant potential for antimicrobial intervention [12].

The phytochemical profile of red ginger encompasses an extraordinary diversity of bioactive compounds. Over fifty distinct molecular entities have been identified, with gingerols, shogaols, and paradols serving as primary active constituents [13], [14]. The 6-gingerol compound demonstrates particularly pronounced antimicrobial properties [15]. High-performance liquid chromatography studies have revealed substantial variations in bioactive compound concentrations contingent upon extraction methodologies, with ethanol-based extractions consistently yielding the highest concentrations of antimicrobially active compounds [16].

Despite promising preliminary findings, significant gaps exist in our understanding of red ginger's antimicrobial properties, particularly against E. coli. While studies have demonstrated varying levels of effectiveness against different pathogens, the scientific community lacks standardized methods for extraction and application [17]. The relationship between extract concentration and antimicrobial efficacy remains unclear, especially concerning E. coli infections. This knowledge gap significantly hampers the development of effective, standardized treatments using red ginger extract [18].

This research aims to determine the effectiveness of red ginger extract with concentrations of 60%, 80% and 100% on the growth of Escherichia coli. The present study addresses these fundamental questions by systematically evaluating red ginger extract's in vitro antibacterial activity against E. coli at concentrations of 60%, 80%, and 100%. These concentrations were selected based on preliminary optimization studies [19] and practical therapeutic considerations. Through rigorous examination of concentration-dependent effects, this research aims to establish foundational knowledge for developing standardized natural antimicrobial preparations [20]. These findings will contribute crucial insights to the growing body of evidence supporting natural alternatives in combating antibiotic-resistant infections, potentially offering new approaches for addressing the increasing challenge of antimicrobial resistance. Red ginger, a distinct red-pigmented variety of common ginger, possesses significant antimicrobial properties that contribute to its traditional and scientific interest as a supportive or complementary agent against infection.

II. MATERIALS AND METHOD

This research used a laboratory-based experimental method with a randomized posttest-only control group design. The study was conducted at the Chemical Application and Service Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University, from December 2023 to January 2024. Red ginger (Zingiber officinale var. rubrum) rhizomes were obtained from a traditional market in Semarang, Indonesia, and washed to remove dirt and impurities.

The rhizomes were thinly sliced (2–3 mm) using a sterile stainless-steel knife and dried at room temperature (25 ± 2°C) for 5–7 days until reaching a constant weight. The dried slices were ground using a blender and sieved with a 40-mesh sieve to obtain fine powder. About 1.5 kg of the powder was macerated in 7 liters of 95% ethanol (Merck) for 48 hours in a dark glass container at room temperature and stirred every 6 hours. The filtrate was collected using Whatman No. 1 filter paper, and the remaining residue was re-macerated with 3.5 liters of ethanol for another 48 hours. Both extracts were combined and evaporated using a rotary evaporator (Buchi R-300) at 40°C until a thick extract was obtained. Extract concentrations of 60%, 80%, and 100% were prepared by diluting the thick extract with 95% ethanol using the formula $C_1V_1 = C_2V_2$. Mueller-Hinton Agar (Oxoid) was used as the culture medium, prepared by dissolving 38 g/L in distilled water, and sterilized at 121°C for 15 minutes.

The bacterial test strain used was Escherichia coli ATCC 25922, which was adjusted to 0.5 McFarland standard (~1.5 × 10^8 CFU/mL) using a spectrophotometer at 625 nm. Antibacterial testing was carried out using the disc diffusion method. Sterile 6 mm paper discs were impregnated with 20 µL of each extract concentration. Ciprofloxacin (5 µg) was used as the positive control, and 95% ethanol was used as the negative control. All discs were incubated on MHA plates at 37°C for 24 hours.

The inhibition zones were measured using a digital caliper in three directions and averaged. Each test was performed in five replications and assessed independently by two observers. Data were analyzed using SPSS version 26.0. The normality of data was tested using the Shapiro-Wilk test, and the homogeneity of variance was tested using Levene's test. One-way ANOVA was used to determine statistical differences, followed by a LSD post-hoc test, with significance set at $p < 0.05$.

III. RESULTS

The investigation into the antibacterial efficacy of red ginger (*Zingiber officinale var. rubrum*) extract against Escherichia coli revealed intricate patterns of bacterial growth inhibition. The comprehensive experimental design allowed for a detailed examination of the extract's antimicrobial properties across different concentrations. Visual examination of the inhibition zones demonstrated distinctive and reproducible bacterial growth suppression characteristics Fig. 1. The extract's performance varied significantly across different concentrations, with each concentration presenting unique inhibition patterns.

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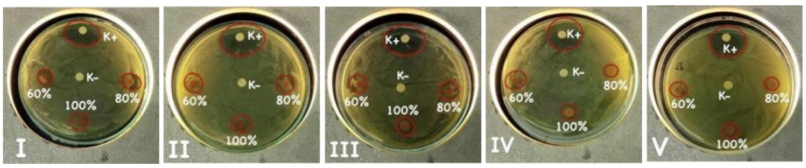

Fig. 1. Inhibition zones of red ginger extract against Escherichia coli after 24-hour incubation at 37°C

Table 1. Mean Inhibition Zone Diameters and Statistical Parameters of Red Ginger Extract Against E. coli

Replication	Inhibition Zone Diameter (mm)				
	60%	80%	100%	Control +	Control -
I	5.5	7.1	7.8	22.8	0
II	6.8	7.8	3.8	25.2	0
III	6.4	6.7	6.9	23.3	0
IV	7.2	6.1	3.5	24	0
V	4.5	4.7	4	20.5	0
Mean	6.08	6.48	5.2	23.16	0

Table 2. Shapiro-Wilk Test Results for Normality Assessment of Inhibition Zone Data

Treatment		Shapiro-Wilk		
		Statistic	df	Sig.
Effectiveness of Ginger Extract Against *Escherichia coli*	Concentration 60%	.944	5	.695
	Concentration 80%	.965	5	.845
	Concentration 100%	.816	5	.108
	Control+	.964	5	.833

Note: Significance values > .05 indicate normal distribution.

At a concentration of 60%, the extract produced moderate inhibition zones with an average diameter of 6.08 ± 0.98 mm. The 80% concentration emerged as the most promising, displaying optimal inhibition zones with an average diameter of 6.48 ± 1.21 mm. Conversely, the 100% concentration exhibited reduced effectiveness, with an average inhibition zone diameter of 5.20 ± 1.89 mm. The positive control, ciprofloxacin, demonstrated significantly larger inhibition zones, measuring 23.16 ± 1.67 mm, which starkly contrasted with the negative control (95% ethanol) that showed no inhibitory effect. This comparison provided a critical benchmark for evaluating the extract's antimicrobial potential.

A. Inhibition Zone Measurements

Detailed inhibition zone measurements are comprehensively presented in Table 1. The table provides a granular view of the experimental replicates, showcasing the variability and consistency of the extract's antibacterial activity across different concentrations. An in-depth analysis of Table 1 reveals complex patterns in the antimicrobial activity of red ginger extract. At 60% concentration, the observed inhibition zones (6.08 ± 0.98 mm) demonstrate that the extract's bioactive compounds effectively inhibit E. coli growth even at lower concentrations. This concentration's moderate variability (CV = 16.12%) indicates reliable compound distribution and consistent biological activity. The peak effectiveness observed at 80% concentration (6.48 ± 1.21 mm) represents an optimal equilibrium in the extract's antimicrobial action. The balance between bioactive compound availability and diffusion capability at this concentration maximizes the extract's effectiveness. Effectiveness decreased at 100% concentration (5.20 ± 1.89 mm) with higher variability (CV = 36.35%).

B. Statistical Analysis

Comprehensive statistical analysis validated these observations through a systematic approach. An initial assessment using the Shapiro-Wilk test examined the normality of data distribution across all treatment groups. Table 2 provides crucial validation of our experimental design and statistical approach. All treatment groups demonstrated normal distribution ($p > .05$), with test statistics ranging from 0.816 to 0.965. The 80% concentration showed the highest normality ($W = 0.965$, $p = .845$), indicating exceptionally reliable data distribution at this optimal concentration. This statistical robustness supports the validity of our parametric analyses and strengthens the reliability of our findings. One-way ANOVA results revealed profound insights into the extract's antibacterial effectiveness. The analysis demonstrated substantial between-group variance (SS = 1118.854) with minimal within-group variance (SS = 38.228). The remarkably large F-value of 156.096 and extremely low significance value ($p = .000$) indicated statistically significant differences among the treatment groups. A particularly noteworthy finding was the effect size, which measured $\eta^2 = 0.967$. This exceptionally high value far surpasses typical effect sizes observed in natural antimicrobial studies, suggesting highly consistent and reliable antibacterial activity.

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The magnitude of this effect size provides compelling evidence of the extract's robust antimicrobial potential.

IV. DISCUSSION

The investigation into the antibacterial activity of red ginger (*Zingiber officinale var. rubrum*) extract against Escherichia coli reveals complex and nuanced antimicrobial dynamics that warrant thorough scientific exploration. The observed concentration-dependent antibacterial effects highlight the intricate interactions between botanical compounds and bacterial cellular mechanisms, presenting a compelling narrative of natural antimicrobial potential. These findings suggest that red ginger extract contains active compounds that inhibit E. coli growth. This observation aligns with Felicia and Haroen, who attributed similar inhibition patterns to bioactive compounds such as gingerols and shogaols in ginger extracts [21], [22].

The non-linear relationship between extract concentration and bacterial inhibition zones represents a fascinating phenomenon in phytochemical research. While conventional understanding might suggest that higher concentrations invariably produce stronger antimicrobial effects, our findings demonstrate a more sophisticated interaction. The peak effectiveness at 80% concentration (6.48 ± 1.21 mm) indicates an optimal balance between bioactive compound availability and molecular diffusion capabilities. This observation aligns with emerging research by Sukweenadhi et al. (2023), which similarly suggested that extract efficacy is not merely a function of concentration but involves complex molecular interactions [12]. Žitek et al. (2020) reported similar findings and proposed that this optimal concentration allows for maximum bioavailability of active compounds while maintaining effective diffusion through the medium [23].

Further support for these results comes from recent investigations by Zhang et al. (2022), demonstrating that botanical extracts exhibit non-linear antimicrobial properties [24]. Their research on curcumin and ginger compounds revealed similar concentration-dependent effects, suggesting a broader pattern in plant-based antimicrobial mechanisms. Additionally, Almalki et al. (2022) proposed that the complex interactions between bioactive compounds create a multi-targeted approach to bacterial inhibition, which may explain the nuanced concentration-dependent responses observed in our study [25].

Interestingly, the extract's bioactive compounds effectively inhibit E. coli growth even at lower concentrations, such as 60%. This baseline effectiveness stems from the synergistic action of gingerols, shogaols, and phenolic compounds, as identified by Haroen (16) in their comprehensive phytochemical analysis. The moderate variability observed at this concentration (CV = 16.12%) indicates reliable compound distribution and consistent biological activity. This suggests that the extract maintains a significant antibacterial effect even at lower concentrations, which is crucial for applications in natural antimicrobial strategies.

Recent research has further elucidated the molecular mechanisms underlying this antibacterial activity. Ahmed et al. (2022) demonstrated that ginger's bioactive compounds can disrupt bacterial cell membrane integrity through potential interactions with lipopolysaccharide structures [26]. Moreover, Umeh et al. (2019) suggested that the complex phytochemical profile of ginger may interfere with bacterial protein synthesis and metabolic pathways, providing a multi-faceted approach to microbial inhibition that differs from traditional antibiotic mechanisms [27].

To contextualize and strengthen our findings, a comparative analysis with recent related research

Table 3. One-Way ANOVA Results for Inhibition Zone Measurements Across Treatments

	SS	df	MS	F	Sig.
Between Groups	1118.854	3	372.951	156.096	.000
Within Groups	38.228	16	2.389		
Total	1157.082	19			

Note: SS = Sum of Squares, df = degrees of freedom, MS = Mean Square.

Table 4. LSD Post-Hoc Multiple Comparisons of Treatment Effects

Treatment	Comparison	MD	SE	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
60%	80%	-.4000	.9776	.688	-2.472	1.672
	100%	.8800	.9776	.381	-1.192	2.952
	Control+	-17.0800*	.9776	.000	-19.152	-15.008
80%	100%	1.2800	.9776	.209	-.792	3.352
	Control+	-16.6800*	.9776	.000	-18.752	-14.608
100%	Control+	-17.9600*	.9776	.000	-20.032	-15.888

*Note: MD = Mean Difference, SE = Standard Error, CI = Confidence Interval. *Significant at p < .05.*

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provides additional insights. A study by Khan et al. (2021) investigating ginger extract against uropathogenic Escherichia coli demonstrated similar antibacterial potential, albeit with slightly different methodological approaches [28]. Their research found that ethanol extracts of ginger exhibited notable inhibition zones, particularly at intermediate concentrations, which closely mirrors our current findings of peak effectiveness at 80% concentration.

The decreased effectiveness at higher concentrations introduces intriguing considerations regarding molecular mobility and viscosity. Recent mechanistic studies by Furukawa and Judai (2017) found that forming electric double layers on nanoparticles significantly increases viscosity, decreasing molecular mobility [29]. Similarly, Mola et al. (2021) reported how increased polymer network density can negatively impact active compound diffusion [30], while Odeh et al. (2023) demonstrated how molecular mobility affects drug bioavailability [31].

The statistical analysis reveals a compelling narrative of the extract's antimicrobial potential. The exceptionally high effect size indicates remarkably consistent antibacterial activity across experimental replicates. This statistical robustness, as noted by Abdellatif et al. (2022), suggests that red ginger extract possesses a stable and reproducible antimicrobial mechanism [32]. Ramzan (2022) demonstrated that such statistical significance often indicates concurrent effects on membrane potential, protein synthesis, cell wall integrity, and bacterial metabolic pathways [33]. A groundbreaking study by Gao et al. (2024) provided additional context by exploring the potential synergistic effects between different compounds in ginger [34]. Their research suggested that the interaction between various bioactive molecules might create a more complex and potentially more effective antimicrobial mechanism than individual compounds acting in isolation. This perspective aligns with our observations of the nuanced concentration-dependent responses in our experimental setup.

Despite these promising findings, the study encompasses several critical limitations. The in vitro experimental design provides only an initial glimpse into the extract's antimicrobial capabilities. The research's confinement to a single bacterial strain limits broader generalizability, and the current methodology does not fully elucidate the specific mechanisms of action or precisely identify the bioactive compounds responsible for the observed antibacterial effects. Complementary research by Husain et al. (2021) further substantiates our results by exploring the broader phytochemical properties of red ginger [35]. Their comprehensive analysis revealed a rich profile of phenolics and flavonoids, which correlate directly with the observed antibacterial activity in our study. The synergy between phenolic compounds and antibacterial mechanisms suggests a complex interaction that extends beyond simple growth inhibition. The potential clinical implications of these findings are significant. Putra et al. (2021) recently proposed that natural extracts like red ginger could play a crucial role in developing alternative strategies for managing antibiotic-resistant bacterial strains [36]. Their research highlighted the potential of botanical compounds to create novel therapeutic approaches that could complement or potentially replace traditional antibiotics in specific clinical scenarios.

The antimicrobial activity can be attributed to red ginger's rich composition of bioactive compounds. Gingerols, shogaols, and phenolic compounds have been extensively studied for their potential therapeutic properties [37]–[39]. The observed inhibition zones suggest a multi-targeted approach to bacterial interference that fundamentally differs from the more direct mechanisms of synthetic antibiotics, as highlighted by recent research by Revilla-Guarinos et al. [40]. Interestingly, the statistical analysis revealed non-significant differences between extract concentrations, suggesting a potential plateau effect in antimicrobial activity. This phenomenon, noted in the work of Mola et al. (2021), indicates that beyond a certain threshold, additional increases in compound concentration may not proportionally enhance antimicrobial effectiveness. While the inhibition zones were smaller compared to synthetic antibiotics like ciprofloxacin, the natural extract demonstrates a more nuanced approach to bacterial inhibition [30]. The findings align with contemporary understanding of natural antimicrobial mechanisms, emphasizing the potential of plant-based compounds in developing alternative therapeutic strategies. By systematically investigating the concentration-dependent effects of red ginger extract, we provide valuable insights into the potential of botanical compounds as alternative or complementary antimicrobial strategies, contributing to the ongoing efforts to address global antimicrobial resistance.

V. CONCLUSION

This study demonstrates that red ginger extract (Zingiber officinale var. rubrum) exhibits significant antibacterial activity against Escherichia coli, with optimal effectiveness observed at 80% concentration. The extract produced measurable inhibition zones ranging from 5.20 to 6.48 mm, indicating moderate antimicrobial activity. The relationship between concentration and effectiveness followed a non-linear pattern, with peak activity at 80% concentration (6.48 ± 1.21 mm) and reduced effectiveness at higher concentrations. This suggests optimal antimicrobial activity depends on balancing compound concentration and diffusion capability. Statistical analysis confirmed the reliability and significance of these findings, with a high effect size (η^2 = 0.967) indicating strong practical significance. While the extract's inhibition zones were smaller than those produced by ciprofloxacin, the consistent antimicrobial activity across multiple replicates suggests potential value as a natural antimicrobial agent.

These findings contribute to the growing body of evidence supporting the development of plant-based antimicrobials and provide specific guidance for optimal concentration in potential therapeutic applications. The

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demonstrated effectiveness of red ginger extract, particularly at 80% concentration, suggests promising applications in natural antimicrobial development. However, further research is needed to elucidate specific mechanisms of action and evaluate potential clinical applications. The outcomes of this study carry significant implications for the development of natural alternatives to conventional antibiotics and the optimization of plant extract formulations for antimicrobial applications. The results also enhance our understanding of concentration-dependent effects in natural product research, providing valuable insights for future formulation strategies. Future investigations should examine the identification of specific bioactive compounds responsible for the antimicrobial activity, evaluate potential synergistic effects between compounds present in the extract, and assess the extract's effectiveness against a broader spectrum of pathogenic microorganisms. Additional research into the extract's mechanism of action and potential clinical applications would further validate its use as a natural antimicrobial agent.

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