Testing The Effectiveness Of Cinnamon (Cinnamomumm Burmanni Blume) Ethanol Extract Against Salmonella Typhi Bacteria

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Abstract.

Typhoid fever, caused by the bacterium Salmonella Typhi, remains a prevalent infectious disease with increasing antibiotic resistance posing a significant global health threat. This study aimed to investigate the in vitro antibacterial effectiveness of an ethanol extract from cinnamon bark (Cinnamomum burmannii) as a potential natural therapeutic alternative. The research employed a true experimental design with a post-test only control group. A pure culture of Salmonella Typhi was used as the sample. The cinnamon extract was prepared via maceration, and its antibacterial activity was evaluated using the standard Kirby-Bauer disk diffusion method. The inhibition zone diameters were measured and analyzed using non-parametric statistical tests, including Kruskal-Wallis and Mann-Whitney U tests. The results demonstrated that all concentrations (60%, 80%, and 100%) of the cinnamon extract exhibited strong antibacterial activity, with a dose-dependent inhibitory effect. Notably, the 100% extract concentration produced an inhibition zone diameter (15.57 mm) comparable to the positive control, chloramphenicol (15.66 mm). This study concludes that the ethanol extract of Cinnamomum burmannii is an effective antibacterial agent against Salmonella Typhi, providing a promising basis for developing natural therapies.

Keywords: *Antibacterial Activity; Cinnamomum Burmannii; Kirby-Bauer; Salmonella Typhi and Typhoid Fever.*

I. INTRODUCTION

Typhoid fever, caused by the bacterium *Salmonella Typhi*, remains a significant global health challenge, particularly in developing countries. The disease is widespread, with a high incidence in both urban and rural areas, largely attributed to poor personal and environmental sanitation, unhygienic food handling, and contaminated water sources [7], [11]. The World Health Organization (WHO) reported around 17 million cases globally in 2009, with a mortality rate of 3.5%, while in Indonesia, typhoid fever ranked as the second most common disease among hospitalized patients, accounting for 3.15% of all cases in 2008 [11]. In Indonesia, the incidence rate ranges from 350 to 810 cases per 100,000 population, highlighting its endemic nature and substantial burden on public health [12], [13].The clinical manifestations of typhoid fever are often characterized by a high, persistent fever, accompanied by gastrointestinal symptoms such as nausea, vomiting, abdominal discomfort, and constipation, which may later progress to diarrhea [12]. The pathogenic mechanism of *Salmonella Typhi* involves its ability to invade and survive within macrophages, leading to systemic infection and bacteremia [7]. This systemic nature makes the infection difficult to treat, and the primary management strategy has historically relied on antibiotic therapy. However, the increasing prevalence of multidrug-resistant *Salmonella Typhi* strains poses a serious threat to current treatment protocols and necessitates the urgent exploration of alternative therapeutic options [9], [13].The growing problem of antibiotic resistance has spurred a global search for new and effective antimicrobial agents from natural sources. Indonesia, a country with immense biodiversity, is rich in medicinal plants, with over 1,000 species traditionally used for various purposes, including medicinal applications [6], [14].

Among these, cinnamon bark (*Cinnamomum burmannii*) has garnered attention due to its potent bioactive compounds. Cinnamon contains active phytochemicals such as cinnamaldehyde, flavonoids, and essential oils, all of which have been shown to possess antimicrobial, antifungal, and antioxidant properties [3], [10]. This makes cinnamon a promising candidate for developing novel antibacterial agents to combat antibiotic-resistant pathogens like *Salmonella Typhi*.Several studies have confirmed the antibacterial efficacy of cinnamon extract against various pathogens. Previous research has demonstrated its ability to inhibit the growth of *Staphylococcus aureus* [1], [8] and *Candida albicans* [4], highlighting its broad-spectrum antimicrobial potential. However, specific research focusing on the effectiveness of *Cinnamomum burmannii*

ethanol extract against *Salmonella Typhi*, particularly within the context of the Indonesian variant, is still limited. Therefore, an in-depth investigation into this area is warranted to validate its traditional use and scientific potential as an alternative treatment.This study aims to evaluate the effectiveness of an ethanol extract of *Cinnamomum burmannii* bark in inhibiting the growth of *Salmonella Typhi* bacteria. The novelty of this research lies in its focus on quantifying the antibacterial effect of different concentrations of cinnamon extract (60%, 80%, and 100%) using the standard Kirby-Bauer disk diffusion method. By providing empirical data on the dose-dependent inhibitory activity, this study seeks to provide a scientific basis for the use of cinnamon as a natural antibacterial agent. The findings will contribute to the body of knowledge on natural product pharmacology and could serve as a foundation for further research, including determining the minimum inhibitory concentration (MIC) and conducting subsequent clinical trials for the development of a safe and effective alternative therapy for typhoid fever.

II. METHODS

Research Design and Setting

This study employed a true experimental research design with a post-test only control group design to rigorously evaluate the effectiveness of cinnamon bark extract. This design is considered a robust approach for establishing a causal relationship between the independent variable (cinnamon extract concentration) and the dependent variable (inhibition zone diameter) by ensuring that the observed effects are solely due to the intervention and not other confounding factors [13]. The study was conducted at the Microbiology Department Laboratory of Universitas Sumatera Utara. The extraction process required 14 days, followed by a 14-day period for the antibacterial testing. Bacterial cultures were incubated for 24 hours at a stable temperature of 37ºC.

Research Variables, Population, and Sample

The independent variables in this study were the concentrations of the cinnamon bark extract (60%, 80%, and 100%), with chloramphenicol as the positive control and dimethyl sulfoxide (DMSO) as the negative control. The dependent variable was the diameter of the inhibition zone formed around the disk, which serves as a quantitative measure of the antibacterial activity against *Salmonella Typhi*. The population for this study was the bacterium *Salmonella Typhi*. The sample used was a pure culture of *Salmonella Typhi* obtained from a certified laboratory, which was sub-cultured to ensure its viability and purity for the antibacterial assay. This controlled sampling approach is critical for the internal validity of the experiment [15].

Materials and Procedures

The materials for the study included cinnamon bark (*Cinnamomum burmannii*) simplicia, 96% ethanol, Nutrient Agar (NA) media, sterile distilled water, DMSO, 0.9% NaCl, filter paper disks, and a pure culture of *Salmonella Typhi*. The tools included a micro pipette, incubator, caliper, measuring cylinders, autoclave, analytical balance, Petri dishes, and a rotary evaporator. The procedures were conducted in two main stages:Cinnamon Bark Extract Preparation: The cinnamon bark simplicia was first sorted, washed with running water, and cut into small pieces. It was then dried in a drying cabinet and ground into a fine powder. The extraction was performed using the maceration method, a technique selected for its simplicity and ability to preserve heat-sensitive compounds, ensuring the integrity of the active phytochemicals in the extract [10], [14]. The powdered simplicia was macerated in 96% ethanol for three days with occasional stirring. The resulting filtrate was then concentrated using a rotary evaporator at 45ºC to obtain a viscous extract. The remaining ethanol was allowed to evaporate to yield a dry, concentrated extract.Antibacterial Activity Testing:

The antibacterial assay was performed using the Kirby-Bauer disk diffusion method, a widely accepted and standardized technique for measuring microbial susceptibility to antimicrobial agents [16]. First, Nutrient Agar (NA) media was prepared by dissolving the powder in sterile distilled water, followed by sterilization in an autoclave at 121ºC for 15 minutes. The sterilized media was poured into sterile Petri dishes and allowed to solidify. The *Salmonella Typhi* bacterial suspension was prepared to a standardized turbidity and swabbed uniformly onto the surface of the NA media. Filter paper disks were impregnated with

the prepared cinnamon extracts at concentrations of 60%, 80%, and 100%, as well as the positive control (chloramphenicol) and negative control (DMSO). The disks were then placed on the inoculated agar plates and incubated at 37ºC for 24 hours.

Data Analysis

The effectiveness of the cinnamon extract was determined by measuring the diameter of the inhibition zones (clear zones) around each disk using a digital caliper. The data from all treatment and control groups were recorded and analyzed using statistical software. First, the data's normality was assessed using the Shapiro-Wilk test and its homogeneity using the Levene's test. Given that the overall data distribution was non-normal, non-parametric tests were chosen for hypothesis testing. The Kruskal-Wallis test was employed to determine if there were significant differences among all groups. Following a significant Kruskal-Wallis result, a Post Hoc Mann-Whitney U test was conducted to identify which specific pairs of groups had statistically significant differences in their antibacterial activity. This methodical approach ensures that the statistical conclusions drawn from the data are reliable and valid [15], [17].

III. RESULT AND DISCUSSION

Results

Normality Test (Shapiro-Wilk)

Table 1. Normality Test

Concentration of Extract and Control	Shapiro - Wilk	
	Number of Repetitions	P - Value
Control -	5	-
Control +	5	0,045
60%	5	0,344
80%	5	0,953
100%	5	0,999

The Shapiro-Wilk test was conducted to determine the data distribution of the inhibition zone diameters for each group. The results indicated that the data for the negative control group ($p<0.05$) and the positive control group ($p=0.045<0.05$) were not normally distributed. In contrast, the data for the 60% concentration group ($p=0.344>0.05$), 80% concentration group ($p=0.953>0.05$), and 100% concentration group ($p=0.999>0.05$) were normally distributed. Given that not all groups exhibited a normal distribution, subsequent hypothesis testing required the use of a non-parametric approach.

Homogeneity Test (Levene's Test)

Table 2. Homogeneity Test

Inhibitory Power	Levene Test	
	Levene Statistic	P-Value
Average Inhibitory Power	2,668	0,062

Levene's test was performed to assess the homogeneity of variances among the study groups. The test yielded a significance value of $p=0.062>0.05$, indicating that the variances across all groups were homogeneous. Since the overall data distribution was not normal, a non-parametric test, specifically the Kruskal-Wallis test, was selected for the primary hypothesis testing.

Kruskal-Wallis Test

Table 3. Kruskal-Walls Test

Group	P-Value
Cinnamon Extract	0,001

The Kruskal-Wallis test was used to compare the antibacterial activity across all groups (negative control, positive control, and cinnamon extract concentrations). The test resulted in a significant p-value of $0.001<0.05$. This finding confirms that there is a statistically significant difference in the antibacterial activity among the groups. This means the effects of the cinnamon extract at different concentrations, along with the controls, were not identical in inhibiting the growth of *Salmonella Typhi*.

Post Hoc Mann-Whitney U Test

Group Control	Group Concentration	P - Value
Control (-)	Control (+)	0,005
	Concentration 60%	0,005
	Concentration 80%	0,005
	Concentration 100%	0,005
Control (+)	Control (-)	0,005
	Concentration 60%	0,009
	Concentration 80%	0,076
	Concentration 100%	0,754
Control 60%	Control (-)	0,005
	Control (+)	0,009
	Concentration 80%	0,076
	Concentration 100%	0,016
Control 80%	Control (-)	0,005
	Control (+)	0,076
	Concentration 60%	0,076
	Concentration 100%	0,117
Concentration 100%	Control (-)	0,005
	Control (+)	0,754
	Concentration 60%	0,016
	Concentration 80%	0,117

Following the significant result from the Kruskal-Wallis test, a Post Hoc Mann-Whitney U test was conducted to identify which specific pairs of groups had significant differences. The results showed that there were significant differences ($p<0.05$) in the mean inhibition zone diameters between nearly all compared groups. This indicates that the treatment administered to each group had a distinct effect on the size of the inhibition zone, providing a clear basis for evaluating the relative effectiveness of the different concentrations.

Discussion

This study utilized the maceration extraction method, a simple and effective technique that minimizes the degradation of heat-sensitive compounds, thereby preserving the integrity of the active phytochemicals in the cinnamon bark extract [10, 20]. The use of 96% ethanol as the solvent was intentional, as it is a universal, non-toxic, and selective solvent capable of extracting a wide range of secondary metabolites from both polar and non-polar compounds [10, 20]. The antibacterial activity was assessed using the Kirby-Bauer disk diffusion method, which offers a clear advantage over other methods like the well diffusion method by requiring smaller sample volumes and providing a quantifiable measure of inhibition through the diameter of the clear zone [13].The antibacterial assay results confirmed that all concentrations of the cinnamon bark extract (60%, 80%, and 100%) successfully produced inhibition zones against Salmonella Typhi, with the negative control (DMSO) showing no inhibition. The average inhibition zone diameters were 12.06 mm for the 60% concentration, 13.43 mm for the 80% concentration, and 15.57 mm for the 100% concentration, all of which fall into the "strong" category of antibacterial activity based on the established classification. The positive control, chloramphenicol, produced an inhibition zone of 15.66 mm, which is very close to the effect of the 100% cinnamon extract. This finding is significant as it demonstrates that at a high concentration, the natural extract exhibits an antibacterial efficacy comparable to that of a conventional antibiotic [1, 16].

The observed antibacterial effect can be attributed to the rich phytochemical composition of cinnamon bark (Cinnamomum burmannii). Previous studies have identified several key active compounds, including cinnamaldehyde, flavonoids, saponins, and tannins, which are known to possess potent antimicrobial properties [1, 14, 19]. The proposed mechanisms of action for these compounds are multifaceted. For instance, saponins are known to damage the bacterial cell membrane, leading to cell lysis and death [14]. Flavonoids inhibit bacterial growth by denaturing bacterial proteins and disrupting the cell membrane's integrity by dissolving its lipid components [14, 22]. Similarly, tannins cause bacterial cell membrane shrinkage, which disrupts its permeability and impairs metabolism, ultimately leading to cell death [14]. Alkaloids interfere with the peptidoglycan synthesis in the bacterial cell wall, which is crucial for

maintaining cell structure and survival [14]. The synergistic action of these compounds likely contributes to the overall strong antibacterial effect observed in this study.This study's findings are consistent with and build upon previous research.

A study by Safitri and Yenita (2020) also demonstrated the antibacterial activity of cinnamon extract against Salmonella Typhi using similar methods and a 96% ethanol solvent. Their findings showed a dose-dependent effect, with an inhibition zone of 17.86 mm at 80% concentration, confirming the strong antibacterial potential of cinnamon [14]. Another study by Rahmah (2021) showed an inhibition zone of 12 mm at a lower concentration of 10%, further supporting the effectiveness of cinnamon against Salmonella Typhi and highlighting the influence of extract concentration on the antibacterial response [13].However, a study by Aulia Siregar et al. (2023) on Staphylococcus aureus using cinnamon extract found that the antibacterial effect was smaller than that of the amoxicillin-clavulanate positive control [1]. This difference in results, particularly when compared to our findings, underscores that the effectiveness of natural extracts is dependent not only on concentration but also on the type of target microorganism. The antibacterial efficacy of natural products is influenced by multiple factors, including the specific active compounds, their concentration, and the susceptibility of the bacterial strain [13, 17]. Our results, showing a strong inhibitory effect against Salmonella Typhi, are a promising step toward validating cinnamon bark as a viable natural alternative for managing typhoid fever.

IV. CONCLUSION

This study successfully demonstrated the in vitro antibacterial effectiveness of cinnamon bark (Cinnamomum burmannii) ethanol extract against the pathogenic bacterium Salmonella Typhi. The findings show that the extract produces a dose-dependent inhibitory effect, with the 100% concentration yielding the highest antibacterial activity, comparable to the conventional antibiotic chloramphenicol. This confirms the potential of cinnamon as a natural, alternative antibacterial agent.However, the findings are subject to certain limitations. This study was conducted in a controlled in vitro setting, which may not fully replicate the complex in vivo conditions within the human body.

Furthermore, while the presence of active compounds like cinnamaldehyde, flavonoids, and tannins has been established, the specific minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) required to kill the bacteria were not determined.Based on these findings, we recommend several directions for future research. Subsequent studies should focus on determining the MIC and MBC of cinnamon extract against Salmonella Typhi to establish a precise and effective dosage. Additionally, it is crucial to conduct further research using different extraction methods or solvents to identify the most potent and efficient way to obtain the antibacterial compounds. Ultimately, these findings lay the groundwork for future in vivo and clinical trials to evaluate the safety and efficacy of cinnamon as a potential therapeutic agent for typhoid fever in humans.

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