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ANTIBACTERIAL ACTIVITY OF CINNAMON BARK EXTRACT (CINNAMOMUM BURMANNII) AGAINST *STAPHYLOCOCCUS AUREUS* (ATCC) BACTERIA

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Abstract

Staphylococcus aureus is one of bacteria causing dental caries. More efforts to control bacterial growth in oral cavity including using natural resources like cinnamon bark *(Cinnamomum burmanni)*. Cinnamon bark contains bioactive compounds such as flavonoids, saponins, and phenols, which are recognized have antibacterial activity. This research was an experimental study with a post-test only control group design. Cinnamon bark extract was obtained using maceration method with 80% ethanol and tested using disc diffusion method at concentrations of 6.25%, 12.5%, and 25%. Chlorhexidine was used as a positive control, and DMSO (dimethyl sulfoxide) as a negative control. Data were analyzed using ANOVA and Post Hoc tests. Results showed that average diameter of the inhibition zone of cinnamon bark extract against *Staphylococcus aureus* was in strong category at concentrations of 6.25% (11.93 mm), 12.5% (13.33 mm), and 25% (17.1 mm). Shapiro-Wilk test showed that data were normally distributed, and ANOVA indicated a significant difference ($p < 0.05$). Post-hoc Tukey test showed that cinnamon bark significantly inhibited *Staphylococcus aureus* ($p < 0.05$) at a 25% concentration. Conclusion : cinnamon bark extract demonstrate potential antibacterial activity in vitro against *Staphylococcus aureus*.

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Introduction

Dental caries occur due to plaque accumulation on the teeth, which contains bacteria that interact with food and oral conditions, leading to the destruction of tooth structure (Satrio et al., 2023). According to the 2023 Indonesian Health Survey (SKI) prevalence of dental caries among children aged 5–9 years was 49.9%, and among those aged 10–14 years was 37.2%. Meanwhile, the percentage of children who practiced proper tooth brushing behavior was only 4.6% for the 5–9 age group and 5.3% for the 10–14 age group. This condition indicates that proper tooth brushing behavior remains very low, highlighting the need for education on oral and dental health.

Dental plaque is a soft, smooth, whitish-yellow layer formed from colonies of bacteria that adhere to the surfaces of teeth with poor oral hygiene (Rosalia & Rahmawati, 2023). The formed plaque accumulates microorganisms within its sticky layer, creating an acidic environment that comes into contact with the teeth, leading to enamel demineralization and the formation of dental caries (Hamsar & Ramadhan, 2019).

Dental plaque contains more than 500 types of bacteria. (Waty et al., 2023). Various microorganisms associated with dental caries include Gram-positive cocci such as *Streptococcus mutans, S. mitis, S. salivarius, S. intermedius, Staphylococcus aureus, Atopobium* spp., *Peptostreptococcus* spp., and *Enterococcus faecalis*; Gram-positive rods such as *Lactobacillus fermentum, L. acidophilus, Actinomyces odontolyticus, A. naeslundii, A. viscosus, A. israelii, Bifidobacterium dentium*, and *Propionibacterium* spp.; Gram-negative cocci such as *Veillonella parvula* and *Neisseria* spp.; Gram-negative rods such as *Bacteroides denticola, B. melaninogenicus, Fusobacterium necrophorum, F. mortiferum, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter freundii, Pseudomonas fluorescens, Haemophilus* spp., *Prevotella* spp., and *Leptotrichia* spp.; as well as fungi such as *Candida albicans, C. tropicalis*, and *C. glabrata*. (Satrio et al., 2023).

Bacteria that cause dental caries are part of the normal oral flora and generally exist as commensal microorganisms as well as opportunistic pathogens. These bacteria exhibit pathogenicity under specific environmental conditions. Certain conditions, such as high carbohydrate intake and reduced salivary flow, promote the local dominance of *Streptococcus mutans*, often accompanied by *Lactobacillus* spp., leading to enamel demineralization and cavity formation. *Streptococcus mutans* is considered the most significant bacterium involved in the development of dental caries. This bacterium forms dental plaque together with other cariogenic bacteria, including *Staphylococcus aureus*, which is also classified as a Gram-positive coccus. (Satrio et al., 2023).

Plaque control can be performed through mechanical, chemical, or natural methods to maintain oral health. The most common mechanical techniques used for daily plaque control include tooth brushing and flossing. Chemical methods for plaque control generally involve the use of antiseptic mouth rinses (Karyadi & Roza, 2021). Several commercially available mouthwashes contain varying concentrations of alcohol (ethanol). Alcohol (ethanol) functions to maintain the stability of active ingredients; however, it may also cause a burning sensation in the mouth, pain, tooth discoloration, and even increase the risk of oral cancer (Oktanauli et al., 2017).

Ingredients commonly used in mouthwash products to inhibit microbial growth include chlorhexidine, fluoride, and povidone-iodine (Sinaredi et al., 2014). Chlorhexidine is considered an effective oral solution for reducing plaque accumulation, inhibiting its development, and preventing periodontal diseases. Its effectiveness is attributed to its bactericidal and bacteriostatic properties against various types of bacteria, including those found in dental plaque (Hamsar & Ramadhan, 2019). Herbal mouthwashes have been increasingly developed due to their antibacterial properties. According to a study conducted by Waty et al., (2018), a cinnamon bark mouthwash at a concentration of 6.25% using 80% ethanol was effective in reducing dental plaque accumulation.

Cinnamon bark is a herbal ingredient believed to possess antibacterial compounds such as flavonoids, saponins, and cinnamaldehyde, which are capable of inhibiting bacterial growth in the oral cavity. As a traditional medicinal spice, cinnamon bark is easily accessible and affordable; however, its utilization remains suboptimal. Both the bark and leaves of cinnamon contain essential oils, saponins, and flavonoids, which have long been used as medicinal plants beneficial for treating various diseases (Waty et al., 2018).

A study conducted by Rosalia & Rahmawati, (2023) reported that mouthwash formulated with moringa leaf extract demonstrated antibacterial effectiveness against *S. aureus*, with an inhibition zone diameter of 11.3 mm, which falls into the strong category. In line with this finding, Umayah et al. (2024) developed a mouthwash utilizing cinnamon extract at concentrations of 10%, 12.5%, and 15%, and confirmed its ability to inhibit the growth of *Streptococcus mutans*, with inhibition zones measuring 7.33 mm, 8.50 mm, and 9.33 mm, respectively.

Since most previous studies have used different concentrations of extracts, the author is interested in investigating the antibacterial effectiveness of cinnamon bark extract against *Staphylococcus aureus* (ATCC) at various concentration levels. This study aims to determine the antibacterial activity of cinnamon bark (*Cinnamomum burmannii*) extract against *Staphylococcus aureus* (ATCC).

Method

This study employed a true experimental design using the post test only control group design, which involves conducting observations or measurements after treatment and comparing the results with a control group. The antibacterial activity of cinnamon bark (*Cinnamomum burmannii*) extract against *Staphylococcus aureus* (ATCC) was evaluated using the maceration method. The research was conducted at the Phytochemistry Laboratory and Microbiology Laboratory Faculty of Pharmacy, Universitas Sumatera Utara from March to ay 2025. The study population consisted of *Staphylococcus aureus* obtained from bacterial culture stocks at the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, and cinnamon bark from Pusat Pasar, Medan.

The study was carried out through several stages, beginning with the preparation of cinnamon extract. Dried cinnamon bark was ground using a spice grinder and then sieved to obtain fine cinnamon powder. A total of 500 grams of cinnamon powder was placed into a 5-liter glass jar and submerged completely in 80% ethanol as the solvent for the maceration process. The jar was tightly covered with aluminum foil and left to stand for 24 hours, with stirring performed every 30 minutes during the first 6 hours. The jar was kept away from direct sunlight throughout the maceration process. Afterward, the mixture was filtered using Whatman filter paper to separate the residue from the filtrate. The residue was

re-extracted with 80% ethanol for an additional 48 hours. The collected filtrate was then concentrated using a vacuum evaporator and a water bath at 60°C to remove the remaining solvent. Upon completion of this process, a pure cinnamon extract was obtained. The cinnamon extract solution was then prepared at concentrations of 6.25%, 12.5%, and 25%. A 6.25% solution contained 0.0625 ml of cinnamon extract and 0.9375 ml of DMSO; a 12.5% solution contained 0.125 ml of cinnamon extract and 0.875 ml of DMSO; and a 25% solution contained 0.25 ml of cinnamon extract and 0.75 ml of DMSO.

The antibacterial activity using Kirby Bauer disc diffusion method. The study consisted of three groups such as cinnamon bark extract at 6.25%, 12.5%, and 25% concentrations in group I, negative control (DMSO) in group II, positive control (chlorhexidine) in group III. Each test was performed in triplicate. Sterile blank discs were impregnated with the respective extract concentrations, chlorhexidine (positive control) or DMSO (negative control) and allowed to dry. Each Petri dish contained three discs with the extract concentrations and two discs with the control agents. Plates were incubated at 37°C for 24 hours, after which inhibition zones were measured in millimeters. The data was analyzed statistically using One Way ANOVA and Post Hoc (Tukey) test.

Result

This study utilized cinnamon bark (*Cinnamomum burmannii*) as the sample to evaluate its antibacterial activity against *Staphylococcus aureus*. The ethanol extract was obtained through maceration of 1 kg of cinnamon bark simplicia using 80% ethanol as the solvent, resulting in 7 liters of brown filtrate. After evaporation using a rotary evaporator, a thick reddish-brown extract weighing 108.25 grams was obtained, which was subsequently used for antibacterial testing. Phytochemical screening was also conducted to identify the secondary metabolites present in the cinnamon extract, as shown in the table below.

Table 1. Phytochemical Test Results of cinnamon bark (*Cinnamomum burmannii*) Ethanol Extract

Phytochemical Result	Screening Result (+/-)
Flavonoid	+
Saponin	+
Terpenoid	-
Phenol	+

According to table 1 that cinnamon bark extract positively contains metabolite compunds such as flavonoid, saponin, Terpenoid, and phenol.

Table 2. Average Diameter of Inhibition Zone cinnamon bark (*Cinnamomum burmannii*) Extract against Staphylococcus aureus Bacteria

Inhibition Zone (mm)	Cinnamon bark Extract Activity Test Results			
	Repitition 1	Repitition 2	Repitition 3	Ave
6.25% Concentration	11.1	12.7	12.0	11.93
12.5% Concentration	13.2	13.1	13.7	13.33
25% Concentration	15.1	20.1	16.1	17.1
Positive Control	10.7	11.7	10.7	10.8
Negative Control	0	0	0	0

Table 2 indicates that the inhibition zone diameter of cinnamon bark extract against *Staphylococcus aureus* was categorized as strong at concentrations of 6.25% (11.93 mm), 12.5% (13.33 mm), and 25% (17.1 mm). For comparison, chlorhexidine, which served as the positive control, showed an average inhibition zone of 10.8 mm, classified as moderate. Meanwhile, the negative control using DMSO (Dimethyl Sulfoxide) showed no inhibition zone (0 mm).

Table 3. Analyze The Inhibition Zone Diameter Using ANOVA

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	.064	3	.021	16.263	.001
Within Groups	.011	8	.001		
Total	.075	11			

Based on the table 3, a significant difference was observed among the test groups in inhibiting the growth of *S. aureus*, with a p-value of 0.001 ($p < 0.05$).

Table 4. Comparison Test of Inhibition Zone Diameters With Post Hoc (Tukey)

(I) Kelompok Uji	(J) Kelompok Uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Kelompok Positif	Kelompok EEKM 6,25%	-.04272	.02963	.511	-.1376	.0522
	Kelompok EEKM 12,5%	-.09147	.02963	.059	-.1864	.0034
	Kelompok EEKM 25%	-.19628*	.02963	.001	-.2912	-.1014
Kelompok EEKM 6,25%	Kelompok Positif	.04272	.02963	.511	-.0522	.1376
	Kelompok EEKM 12,5%	-.04875	.02963	.408	-.1436	.0461
	Kelompok EEKM 25%	-.15356*	.02963	.004	-.2484	-.0587
Kelompok EEKM 12,5%	Kelompok Positif	.09147	.02963	.059	-.0034	.1864
	Kelompok EEKM 6,25%	.04875	.02963	.408	-.0461	.1436
	Kelompok EEKM 25%	-.10481*	.02963	.031	-.1997	-.0099
Kelompok EEKM 25%	Kelompok Positif	.19628*	.02963	.001	.1014	.2912
	Kelompok EEKM 6,25%	.15356*	.02963	.004	.0587	.2484
	Kelompok EEKM 12,5%	.10481*	.02963	.031	.0099	.1997

The Post Hoc (Tukey) test show, the 25% Ethanol Extract of Cinnamon Bark group showed a significant difference in inhibiting bacterial growth ($p < 0.05$) compared with the positive control group ($p = 0.001$), the 6.25% EECB group ($p = 0.004$), and the 12.5% EECB group ($p = 0.031$). In contrast, the 6.25% and 12.5% EECB groups showed no significant difference compared with the positive control. This finding indicates that even at a concentration of 6.25%, the EECB already possesses antibacterial potential comparable to that of chlorhexidine. Increasing the concentration of EECB up to 25% resulted in greater antibacterial activity than the positive control (chlorhexidine).

Discussion

The results of the phytochemical screening of cinnamon bark (*Cinnamomum burmannii*) extract, as shown in Table 4.1, indicated the presence of secondary metabolites such as flavonoids, saponins, and phenols. This finding is supported by the study of Waty et al., (2023), which reported that cinnamon bark contains alkaloids, saponins, flavonoids, and glycosides. Similarly, the research conducted by Febrianti & Sumarya, (2023) stated that cinnamon bark extract contains antibacterial compounds, including flavonoids, tannins, saponins, and alkaloids. This is in line with the findings of Putri et al., (2023), who explained that flavonoids act as antimicrobial agents by interacting with proteins located on the bacterial cell surface as well as within the cells, thereby damaging the cell membrane and inhibiting bacterial activity.

The diameter of the inhibition zone is categorized based on the strength of its antibacterial activity. According to Savitri & Harris., (2018), inhibition zones measuring <5 mm are classified as weak, 5–10 mm as moderate, 10–20 mm as strong, and >20 mm as very strong. The antibacterial activity of the ethanolic extract of cinnamon bark (*Cinnamomum burmannii*) against *Staphylococcus aureus* shown in Table 4.2 indicates that the mean diameter of the inhibition zones falls within the strong category at all tested concentrations, namely 6.25% (11.93 mm), 12.5% (13.33 mm), and 25% (17.1 mm). As a comparison, chlorhexidine, which was used as a positive control, exhibited an inhibition zone of 10.8 mm, categorized as moderate. Meanwhile, the negative control using DMSO (Dimethyl Sulfoxide) showed no inhibition zone (0 mm), indicating the absence of antibacterial activity from the solvent.

A study conducted by Intan et al., (2021) reported that the average inhibition zone diameters of cinnamon extract against *Staphylococcus aureus* were 3.7 mm at 30% concentration, 4.8 mm at 50%, 5.7 mm at 65%, 9.7 mm at 70%, and 12.7 mm at 75%. The inhibition zones were classified as weak at concentrations of 30%, 50%, and 65%, moderate at 70%, and strong at 75%. Therefore, the cinnamon extract was most effective in inhibiting *S. aureus* at a concentration of 75%, producing an inhibition zone of 12.7 mm. This finding is consistent with the study by Waty S., (2022), which reported that toothpaste containing cinnamon bark extract (*Cinnamomum burmannii*) demonstrated mean inhibition zones against *Streptococcus mutans* (ATCC) of 10.0 mm at 6.25% concentration and 15.72 mm at 12.5% concentration.

The statistical analysis of the antibacterial activity of cinnamon bark extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* at concentrations of 6.25%, 12.5%, and 25% began with the Shapiro-Wilk normality test. The results of the normality test showed a significance value of 0.0176 ($p > 0.05$). A significance value greater than 0.05 indicates that the data are normally distributed. Therefore, the data meet the assumption of normality and can be further analyzed using a parametric statistical test, namely ANOVA, followed by a Post Hoc Tukey test.

Based on the results of the ANOVA analysis of the mean inhibition zone diameter of cinnamon bark extract (*Cinnamomum burmannii*) against *Staphylococcus aureus* (Table 3), a significance value (p-value) of 0.001 was obtained with an F-value of 16.263. Since the p-value is less than 0.05, it can be concluded that there is a statistically significant difference among the treatment groups. This indicates that the variation in extract concentration truly affects its antibacterial capability in inhibiting the growth of

S. aureus. In other words, the ethanol extract of cinnamon bark has been proven to exhibit antibacterial activity, with its effectiveness increasing along with higher concentrations. To further determine which groups showed significant differences, a Post Hoc Tukey test was conducted.

Furthermore, the Post Hoc Tukey analysis (Table 4.4) showed that the 25% concentration group of Ethanol Extract of Cinnamon Bark (EECB) exhibited a significant difference in inhibiting bacterial growth ($p < 0.05$) compared with the positive control ($p = 0.001$), EECB 6.25% ($p = 0.004$), and EECB 12.5% ($p = 0.031$). Meanwhile, the EECB 6.25% and 12.5% groups showed no significant difference compared with the positive control (chlorhexidine). This indicates that the antibacterial potential of EECB begins to be comparable to chlorhexidine at a concentration of 6.25% in inhibiting *Staphylococcus aureus* growth. Increasing the concentration up to 25% demonstrated even greater inhibitory effects than the positive control.

The study conducted by Waris & Ambarwanto., (2024) reported that the ethanol extract of cinnamon bark (*Cinnamomum burmannii*) at concentrations of 2%, 4%, and 8% was able to inhibit the growth of *Staphylococcus aureus*. The mean inhibition zone diameters obtained were 10.43 ± 0.85 mm, 12.53 ± 0.85 mm, and 14.27 ± 0.8 mm, respectively. Statistical analysis using the ANOVA test showed a significant difference with a p-value < 0.05, indicating a notable variation among the concentration groups in inhibiting bacterial growth. These findings are consistent with the study conducted by Waty, S., (2022), which demonstrated a significant difference ($p < 0.05$) in the ANOVA analysis of toothpaste formulations containing cinnamon bark extract against *Streptococcus mutans*.

The post hoc test conducted by oleh Putu Yetty Nugraha et al., (2021) revealed a significant difference among the concentrations of 20%, 40%, 80%, 100%, the positive control, and the negative control, with a p-value < 0.05, indicating statistically significant differences between the groups. In this study, the Minimum Inhibitory Concentration (MIC) was determined to be 40%, while the Minimum Bactericidal Concentration (MBC) was 80%. Furthermore, ANOVA analysis was performed to assess the variation among treatment groups, yielding a significance value ($p < 0.05$), which confirmed the presence of significant differences.

Conclusions and Suggestions

The cinnamon bark extract (*Cinnamomum burmannii*) inhibited the growth of *Staphylococcus aureus* at concentrations of 6.25% (11.93 mm), 12.5% (13.33 mm), and 25% (17.1 mm), all of which were categorized as strong antibacterial activity, with a statistically significant difference ($p < 0.05$) observed at the 25% concentration ($p = 0.001$). This antibacterial activity assay can be further extended to other bacterial species, both Gram-positive and Gram-negative, to determine the broad-spectrum antibacterial potential of the extract.

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