Denta: Jurnal Kedokteran Gigi

Accredited No. 79/E/KPT/2023
p-ISSN: 1907-5987 e-ISSN: 2615-1790

RESEARCH ARTICLE

Cinnamomum zeylanicum Essential Oil as an Antibacterial Agent and Dental Caries Prevention

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Received : 04 Dec 2025
Revision : 04 Feb 2026
Accepted : 06 Feb 2026
Published : 18 Feb 2026

ABSTRACT

Background: *The prevalence of dental caries in Indonesia reaches 88.8%. Chlorhexidine, although effective, has side effects such as tooth discoloration. Cinnamomum zeylanicum is rich in cinnamaldehyde and eugenol, with antibacterial activity that makes it a natural alternative.* ***Objective:*** *To assess the effectiveness of Cinnamomum zeylanicum essential oil against Streptococcus mutans inoculated in Wistar rats (Rattus norvegicus) in preventing dental caries.* ***Method:*** *This study used 20 male Wistar rats, divided into five groups: negative control, positive control, and three treatment groups with Cinnamomum zeylanicum essential oil concentrations of 6.25%, 10%, and 12.5%. Streptococcus mutans (S. mutans) was inoculated orally in 0.2 ml using a pipette for three consecutive days. During the study, rats were fed a diet mixed with a sucrose solution (600 mg of sucrose dissolved in 1 liter of Aquadest) at a 1:500 ml-to-pellet ratio to induce caries. After 20 days of treatment, data were collected using a bacterial inhibitory zone assay by disk diffusion and salivary pH measurement. Data analysis was performed using one-way analysis of variance (ANOVA), Tamhane's post-hoc test, and the Wilcoxon signed-rank test.* ***Results:*** *One-way ANOVA test showed no significant differences in the inhibitory zone (p>0.05). Post-hoc tests showed that all groups were not significant (p>0.05). The Wilcoxon signed-rank test showed significant differences in salivary pH before and after treatment (p<0.05).* ***Conclusion:*** *Cinnamomum zeylanicum essential oil has potential as an antibacterial agent and natural alternative in preventing dental caries against S. mutans, and is able to change the salivary pH to alkaline.*

Keywords: *Antibacterial agent, cinnamomum zeylanicum, dental caries prevention, essential oils*

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Available at: http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/41
DOI: https://doi.org/10.30649/denta.v20i1.2

INTRODUCTION

Based on data from the 2018 National Basic Health Research (Riskesdas), the prevalence of dental caries in the Indonesian population was 88.8%, with the highest prevalence among people aged 15–24. The national Decayed, Missing, and Filled Teeth (DMF-T) index was reported to be 7.1, while the community Oral Hygiene Index–Simplified (OHI-S) was 1.4 (Riskesdas, 2020). According to World Health Organization (WHO) criteria, a DMF-T index greater than 6.6 indicates severe dental caries, whereas an OHI-S score of 1.4 indicates fair oral hygiene. However, the national oral health targets set for 2020 aimed to achieve a DMF-T index of less than 1 and an OHI-S index below 1.2, indicating a substantial gap between current conditions and desired outcomes.[1,2]

Dental caries is a multifactorial disease that affects the hard tissues of the teeth. It is primarily caused by bacterial activity, particularly *Streptococcus* species, with *Streptococcus mutans (S. mutans)* recognized as the main etiological agent in plaque formation and caries progression. Caries is a pathological process characterized by the progressive demineralization and destruction of enamel, dentin, and cementum, resulting from acid production by microorganisms during carbohydrate metabolism, ultimately causing structural damage to tooth tissues.[3,4] In recent decades, preventive approaches in oral health care have been increasingly emphasized over curative treatment. Preventive strategies play a critical role in reducing the incidence of dental caries, especially given that excessive consumption of sweet and sticky foods, inadequate motivation, improper toothbrushing techniques, and infrequent dental visits (ideally recommended every six months) remain major contributing factors.[5,6]

Chlorhexidine is widely used in dentistry as an anti-caries mouthwash due to its broad-spectrum antimicrobial activity. At a concentration of 0.2%, chlorhexidine is effective in inhibiting plaque formation and reducing *S. mutans* colonies. In clinical dental practice, chlorhexidine is also used as a cavity cleanser at an optimal concentration of 2%. At this concentration, chlorhexidine has been shown to effectively reduce *Enterococcus faecalis* within dentinal tubules and root canals when applied for approximately two minutes during endodontic treatment. Despite its effectiveness, prolonged use of chlorhexidine is associated with undesirable side effects, including tooth staining and discoloration.[7-10]

Indonesia is well known for its abundant production of essential oils, which have gained increasing attention, particularly as natural and alternative therapeutic agents. One such essential oil is derived from cinnamon (*Cinnamomum* spp.). Approximately 300 species of cinnamon exist worldwide, with at least 12 species found in Indonesia, including *Cinnamomum zeylanicum*.[11-14] The essential oil of *Cinnamomum zeylanicum* contains several bioactive compounds, such as cinnamaldehyde, linalool, caryophyllene, and eugenol. Cinnamaldehyde constitutes approximately 60–70% of the essential oil extracted from cinnamon bark and exhibits strong antibacterial and antifungal properties. Linalool, a terpenoid compound with a pleasant aroma, contributes to fragrance intensity, while caryophyllene provides a warming and spicy sensation when consumed.[13-15] Given its widespread use and antibacterial potential, cinnamon essential oil is a promising natural alternative for cavity cleaning. Essential oils were selected in this context due to their ability to inhibit plaque growth associated with dental caries and their potential to protect tooth structures from caries-related damage.[16-19]

Eugenol, a major constituent of cinnamon essential oil, exhibits strong antimicrobial activity and functions as an effective antiseptic agent. Eugenol has been reported to eliminate various bacterial species, including antibiotic-resistant strains, such as *S. mutans*. Its mechanism of action involves

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DOI: https://doi.org/10.30649/denta.v20i1.2

penetration of the cytoplasmic membrane and disruption of bacterial cell wall permeability. Additionally, the hydrophobic nature of eugenol facilitates its passage through lipopolysaccharides, leading to structural alterations in bacterial cell walls, particularly in Gram-negative bacteria. Several studies have demonstrated that eugenol possesses anti-caries properties, consistent with its antimicrobial and antiseptic effects, which inhibit *S. mutans* activity and plaque formation on tooth surfaces.[20-22]

Furthermore, the novelty of this study lies in using an in vivo experimental model to evaluate the antibacterial and anticaries potential of *Cinnamomum zeylanicum* essential oil. Most previous studies investigating the antimicrobial effects of essential oils against cariogenic bacteria have predominantly employed in vitro methods, which, although valuable, do not fully represent the complex biological conditions of the oral environment. Using male *Wistar* rats as an in vivo model, this study provides a comprehensive assessment of the antibacterial efficacy of *Cinnamomum zeylanicum* essential oil under physiological conditions, including bacterial colonization, host response, and oral ecological dynamics. Therefore, the findings of this study are expected to contribute a novel, and clinically relevant evidence supporting the potential application of *Cinnamomum zeylanicum* essential oil as a natural alternative for dental caries prevention.

MATERIALS AND METHODS

This study was a true experimental laboratory study using a post-test-only control-group design. The instruments used in this study included rat cages (five cages, each containing four rats), a digital scale, rat feeding and drinking containers, sucrose solution bottles, micropipettes, 1 ml syringes, microtubes, Petri dishes, gloves, masks, and laboratory coats. The materials used included *Cinnamomum zeylanicum* essential oil, *S. mutans* bacteria, a mixed diet containing sucrose, pilocarpine, 96% ethanol, ketamine, and xylazine.

This research was conducted at the Experimental Animal Laboratory of the Faculty of Dentistry, Universitas Hang Tuah, and the Oral Biology Laboratory of the Faculty of Dentistry, Universitas Hang Tuah. This study received ethical approval from the Ethics Committee of the Faculty of Dentistry, Universitas Hang Tuah, Surabaya, with ethical clearance certificate number S.Ket/120/KEPKFKGUHT/XI/2024.

Preparation of Experimental Animals

This study used male *Rattus norvegicus* weighing 180–200 grams, with a total of 20 rats. The animals were housed in cages measuring 37 cm × 30 cm and 50 cm × 37 cm, with four rats per cage. *Rattus norvegicus* were divided into five groups. The negative control group (K−) received no treatment. The positive control group (K+) received chlorhexidine treatment. The first treatment group (P1) received a mixture of *Cinnamomum zeylanicum* essential oil in drinking water at 6.25%. The second treatment group (P2) received a mixture of *Cinnamomum zeylanicum* essential oil in drinking water at a concentration of 10%, and the third treatment group (P3) received a mixture of *Cinnamomum zeylanicum* essential oil in drinking water at a concentration of 12.5%. All groups were subjected to bacterial induction with *S. mutans* and fed a sucrose-containing diet. *Rattus norvegicus* were acclimated to the new environment for seven days. The rats were fed 15–25 g/day in small containers, while drinking water was provided in bottles with a drinking tube, filled with boiled water. This treatment was administered for 20 days.[23]

Preparation of *Cinnamomum zeylanicum* Essential Oil

The preparation of *Cinnamomum zeylanicum* essential oil in this study involved drying the cinnamon bark for 48 hours without direct exposure to sunlight. The dried bark was then blended until finely ground and placed into

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Accredited No. 79/E/KPT/2023
p-ISSN: 1907-5987 e-ISSN: 2615-1790

a Soxhlet apparatus containing 700 ml of 96% ethanol as the solvent. The highest concentration (12.5%) was prepared first by diluting an appropriate volume of *Cinnamomum zeylanicum* essential oil with 96% ethanol to obtain the desired final volume. Subsequently, 10% and 6.25% concentrations were prepared by serially diluting the higher concentration with the same solvent. The mixture was heated to the boiling point of 78.3°C. The extraction process lasted for 10 hours. After extraction, the extract was mixed with propylene glycol and flavored with menthol.[13-15]

Induction of *S. mutans* in Experimental Animals

S. mutans were administered orally to the experimental animals at a volume of 0.2 ml using a pipette, and the procedure was repeated continuously for three days.[23]

Administration of Sucrose-Containing Diet

The experimental animals were fed a cariogenic diet containing sucrose as the cariogenic agent. A total of 600 mg of sucrose was dissolved in 1 liter of distilled water. Subsequently, 1 kg of pellet feed was mixed with the sucrose solution at a ratio of 1 kg pellets to 500 ml of sucrose solution and mixed thoroughly. The moisture content of the pellets was then removed using microwaves.[23]

Collection of Saliva Samples

Saliva was collected from the experimental animals' oral cavities using a micropipette, specifically from the sublingual region. A volume of 50 microliters of saliva was collected from each rat. Before saliva collection, a pilocarpine injection was prepared by mixing 15 ml of distilled water with 5 ml of pilocarpine, then administered subcutaneously at a dose of 0.75 ml per 100 g of body weight.[24]

Salivary pH Measurement

Each saliva sample was immediately tested by two experienced examiners. Salivary pH was first measured using pH paper. pH-indicating paper (Whatman® indicator paper) was used in the current study. Droplets of randomly selected saliva were dropped onto a disinfected stainless-steel plate with a micropipette. An examiner placed a piece of pH paper on the droplet for one second, and the color change was immediately compared to the manufacturer's color-coded chart. The other examiner duplicated this procedure. No communication between the examiners was allowed during all tests. Salivary pH was measured before and after treatment on the 20th day in *Rattus norvegicus*.[24]

Antibacterial Inhibitory Activity

This method is an antibacterial testing procedure in which a substance is placed on agar media and inoculated with the test microorganism. After incubation, the inhibition zone around the disc was measured to determine the effectiveness of the tested substance. The antibacterial activity test in this study used the disk diffusion method and included the following groups: without extract (K-), chlorhexidine (K+), and extract concentrations of 6.25% (P1), 10% (P2), and 12.5% (P3). Measurements were taken after 24 hours of incubation. *S. mutans* colonies were counted in colony-forming units per milliliter (CFU/ml) using a colony counter. [25,26]

Data analysis

The data were analyzed using the Shapiro–Wilk test for normality and Levene's test for homoscedasticity in IBM SPSS Statistics (version 28; SPSS, Chicago, Illinois, United States). Since the data were normally distributed and showed homoscedasticity ($p>0.05$), one-way ANOVA and post hoc Tamhane multiple comparisons were performed to detect substantial differences across concentrations of *Cinnamomum zeylanicum* essential oil. pH of saliva before and after treatment was evaluated using the Wilcoxon signed-rank test. A statistically significant difference was set at

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p<0.05. Descriptive statistics were used to assess the mean, standard deviation, and 95% confidence interval for the antibacterial activity test and salivary pH before and after treatment.

RESULTS

Table 1. Mean, standard deviation, and 95% confidence interval of antibacterial activity test.

Group	n	Mean ± SD (CFU/ml)	95% CI
K-	4	1.570.00 ± 587.95	(634.43, 2505.56)
K+	4	1.450.68 ± 15.04	(1200.79, 1700.56)
P1	4	1.387.88 ± 361.34	(812.91, 1962.85)
P2	4	1.328.22 ± 320.44	(818.32, 1838.12)
P3	4	1.271.70 ± 352.49	(753.77, 1789.63)

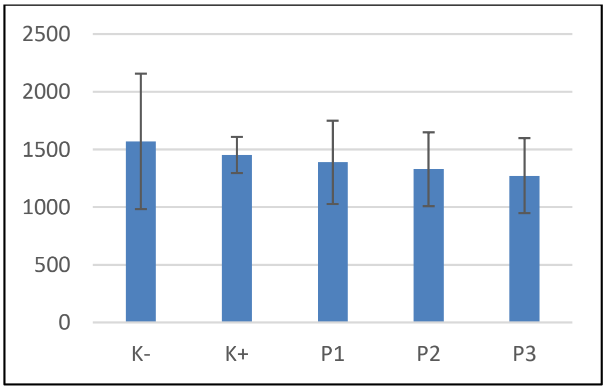

Figure 1. Graph showing the mean and standard deviation of the antibacterial activity test

Table 2. Mean, standard deviation, and 95% confidence interval of salivary pH before and after treatment.

Group	n	Before treatment	After treatment
K-	4	7.50 ± 0.57	6.85 ± 0.19
95% CI		(6.58, 8.41)	(6.54, 7.15)
K+	4	6.65 ± 0.25	7.25 ± 0.50
95% CI		(6.24, 7.05)	(6.45, 8.04)
P1	4	6.95 ± 0.10	7.75 ± 0.50
95% CI		(6.79, 7.10)	(6.95, 8.54)
P2	4	7.00 ± 0.16	7.75 ± 0.50
95% CI		(6.74, 7.25)	(6.95, 8.54)
P3	4	6.75 ± 0.19	7.25 ± 0.50
95% CI		(6.44, 7.05)	(6.45, 8.04)

Based on Table 1 and Figure 1, the highest antibacterial inhibitory activity was observed in the positive control group (K+), with a value of 1.450.68. In contrast, the lowest antibacterial inhibitory activity was found in the P3 group, with a value of 1.271.70. Salivary pH was higher after treatment than before in all experimental groups (Table 2). Data analysis using a One-Way ANOVA showed no significant differences in the inhibitory zone (P=0.823). Further analysis using the Wilcoxon signed-rank test showed significant differences in salivary pH before and after treatment (P=0.001), with higher pH in the K+, P1, P2, and P3 after-treatment groups. Based on the results of Tamhane's post hoc test, all experimental groups showed p-values greater than 0.05 (p>0.05), indicating that there were no statistically significant differences in the antibacterial activity against *S. mutans* among the treatment groups (Table 3).

Table 3. Post Hoc Tamhane test results

Research Group	K-	K+	P1	P2	P3
K-		1.000	1.000	0.999	0.996
K+			1.000	0.999	0.991
P1				1.000	1.000
P2					1.000
P3					

DISCUSSION

Dental caries is one of the most common oral health problems worldwide. The primary etiological agent of dental caries is *S. mutans*, which plays a major role in biofilm formation and enamel demineralization. In recent years, preventive strategies for dental caries using natural materials with antibacterial properties have gained increasing attention. One such natural material is *Cinnamomum zeylanicum* essential oil, which contains bioactive compounds such as cinnamaldehyde, eugenol, and linalool, all of which have demonstrated antibacterial activity against *S. mutans* .[27,28]

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This study aimed to evaluate whether *Cinnamomum zeylanicum* essential oil could be used as an antibacterial agent against *S. mutans* to reduce dental caries. The study used male *Wistar* rats aged 8–10 weeks, weighing 180–200 g, and in healthy physical condition, as indicated by clear eyes, active movement, and normal feces. The rats were inoculated with *S. mutans*, fed a cariogenic diet containing sucrose, and administered *Cinnamomum zeylanicum* essential oil in their drinking water starting on day three, followed by antibacterial testing using the disk diffusion method and saliva pH measurement.

Descriptive analysis showed that the group inoculated with *S. mutans* without any treatment (K−) had an antibacterial inhibition mean of 1.570.00 ± 587.95, which was higher than that of the groups receiving *Cinnamomum zeylanicum* essential oil in their drinking water. A lower inhibition value indicates greater suppression of bacterial growth or activity, suggesting that *S. mutans* was more effectively inhibited, thereby reducing its ability to proliferate or form dental plaque. In other words, the lower the antibacterial inhibition value, the more effective the substance is at reducing the risk of caries. Without antibacterial inhibition, bacterial growth occurs more readily, increasing the risk of dental caries because *S. mutans* plays a critical role in plaque formation.[29] The groups treated with cinnamon essential oil at concentrations of 6.25% (P1) and 10% (P2) also demonstrated better antibacterial inhibition than the untreated group. However, their effectiveness was lower than that observed in the 12.5% concentration group (P3; $p<0.05$). These findings indicate that cinnamon essential oil exhibits antibacterial activity even at low concentrations, with greater effectiveness at higher concentrations, suggesting a concentration-dependent effect.[30]

The antibacterial activity of cinnamon essential oil is attributed to its active compounds, including cinnamaldehyde, eugenol, and linalool. Cinnamaldehyde, the major component, disrupts bacterial cell membrane integrity, inhibits protein synthesis, and interferes with bacterial metabolism, thereby suppressing bacterial growth. It has also been reported to reduce biofilm biomass and metabolic activity of *S. mutans* at sub-MIC concentrations, increase cell surface hydrophobicity, reduce bacterial aggregation, inhibit acid production, and enhance acid tolerance. Eugenol and linalool further contribute to the antibacterial efficacy of cinnamon essential oil.[28]

In the absence of intervention, *Streptococcus mutans* grows freely under favorable environmental conditions, particularly in carbohydrate-rich environments such as sucrose. Although the treated groups showed antibacterial inhibition, the differences were not statistically significant compared with K−, except for P3, which showed better results descriptively but not statistically.[31] Cinnamon essential oil at concentrations of 6.25% and 10% had antibacterial effects comparable to 0.2% chlorhexidine. The antibacterial effects of P1 and P2 were attributed to cinnamaldehyde and eugenol, although at lower concentrations than in P3. Previous studies have shown that the effectiveness of chlorhexidine may be limited by its concentration, while cinnamon essential oil at 6.25% and 10% can produce comparable antibacterial effects.[32] Descriptively, P2 exhibited greater antibacterial inhibition than other groups. The reduced effectiveness in P1 may be due to lower concentrations of active compounds that were insufficient to achieve optimal antibacterial effects. Previous studies have reported a linear increase in the antibacterial activity of essential oils with increasing concentration.[33] Chlorhexidine (0.2 %) primarily exerts a bacteriostatic effect by disrupting bacterial cell membranes and causing leakage of intracellular components. In contrast, 12.5% cinnamon essential oil contains higher concentrations of cinnamaldehyde and eugenol, which effectively disrupt bacterial cell membranes, interfere with enzymatic function, and increase cellular leakage. These findings

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Accredited No. 79/E/KPT/2023
p-ISSN: 1907-5987 e-ISSN: 2615-1790

confirm that higher concentrations of cinnamon essential oil are more effective at inhibiting *S. mutans*, thereby helping prevent dental caries.[34-36] Cinnamaldehyde, a hydrophobic compound, disrupts bacterial lipid membranes more effectively at higher concentrations. Additionally, higher concentrations of eugenol increased cell membrane permeability, resulting in greater leakage of bacterial cellular components.[37]

These findings are consistent with studies by Waty (2022), which reported that cinnamon essential oil at 12.5% showed a descriptively significant antibacterial effect against *S. mutans* compared with lower concentrations. Similar conclusions were reported by Nugraha, Astuti, and Tunggadewi (2021), who demonstrated that higher concentrations of cinnamon essential oil resulted in stronger antibacterial effects.[14] Eugenol is generally considered safe for oral consumption at doses of 5–10 mL, without dilution. However, prolonged use or high doses without dilution may exhibit toxic effects, particularly on vital tissues, and excessive oral exposure may lead to periodontal tissue damage due to diffusion from the cavity. Therefore, cinnamon essential oil should be used within safe limits and in diluted form. Overall, *Cinnamomum zeylanicum* essential oil shows strong potential as a natural antibacterial agent for caries prevention.[31,38,39]

The increase in salivary pH observed in the treatment groups (K+, P1, P2, and P3) may be closely associated with the bioactive compounds present in *Cinnamomum zeylanicum* essential oil, particularly eugenol and cinnamaldehyde. These compounds exhibit strong antimicrobial activity against *Streptococcus mutans* by disrupting bacterial cell membranes, altering cell wall permeability, and inhibiting bacterial metabolic processes responsible for acid production. By suppressing *S. mutans* acidogenic activity, the production of organic acids is reduced, thereby preventing a decline in salivary pH. An alkaline oral environment plays a critical role in caries prevention, as elevated pH decreases enamel solubility and inhibits the demineralization of hydroxyapatite crystals. The reduced acidogenic potential of *S. mutans* under the influence of cinnamon essential oil limits the diffusion of hydrogen ions into the enamel and promotes remineralization. Therefore, the ability of *Cinnamomum zeylanicum* essential oil to modulate salivary pH through its antimicrobial constituents provides a plausible biological mechanism for its protective effect against enamel demineralization and progression of dental caries.[40-42]

Cinnamomum zeylanicum essential oil has potential as a natural antibacterial agent for preventing dental caries, inhibiting the growth of oral bacteria in *Rattus norvegicus* infected with *Streptococcus mutans*, and altering salivary pH to an alkaline state.

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Denta: Jurnal Kedokteran Gigi, Februari 2026; Vol.20 No.1; Hal 7-15
Available at: http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/41
DOI: https://doi.org/10.30649/denta.v20i1.2

Accredited No. 79/E/KPT/2023
p-ISSN: 1907-5987 e-ISSN: 2615-1790

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