

Antibacterial activity of cinnamon ethanol extract (*cinnamomum burmannii*) and its application as a mouthwash to inhibit *streptococcus* growth

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Abstract. Cinnamon bark has been commonly used as spicy and traditional medicine. It contains several antibacterial compounds such as flavonoids, saponins, and cinnamaldehyde. Several studies have been done to know the antibacterial effect on bacteria such as *Streptococcus* in vitro. This study aimed to examine the antibacterial activity of cinnamon ethanol extract against *Streptococcus* and its application as mouthwash to inhibit the bacteria. The cinnamon bark was macerated followed by extracted in 80% ethanol. Bacterial samples were isolated from dental plaque of patients visiting dental clinic drg. Syahdiana Waty in Medan, North Sumatra. The isolates were identified using Vitek 2 compact. Secondary metabolites were detected using previously described method. Antibacterial assay was done at extract concentration of 6.25%, 12.5%, and 25%. The result showed that alkaloids, flavonoids, saponins, and glycoside were detected in the extract. Nine bacterial species were identified as *Streptococcus mitis*, *S. sanguinis*, *S. salivarius*, *S. pluranimalium*, *S. pneumoniae*, *S. alactolyticus*, *Kocuria rosea*, *Kocuria kristinae*, and *Spingomonas paucimolis*. It showed that the extract of Cinnamon bark significantly inhibited *Streptococcus* growth, and it was effective as mouthwash.

Keywords: antibacterial activity, cinnamon bark, mouthwash, *Streptococcus*

1. Introduction

Cinnamon bark has been used for spicy and traditional medicine. Cinnamon bark and its leaves contain essential oils, saponins and flavonoids that have been widely used for healing various diseases [1]. The main compound of the essential oil is cinamaldehyde (60.72%), eugenol (17.62%) and coumarin (13.39%) which has antibacterial effect [2].

Prevalence of dental and oral diseases in Indonesia tends to increase in which caries is the most one. The dental caries and abnormalities of the dental begin with the formation of dental plaque [3]. The formation of plaque in the form of a thin layer attached to the tooth surface and sometimes also found in



the gums and tongue is caused by food debris used by pathogenic bacteria in the oral cavity [4]. Pathogenic bacteria present in the oral cavity include *Streptococcus mutans*, *S. viridans*, *S. pneumoniae*, *St. epidermidis*, and *St. aureus* [5].

There are various ways to reduce the accumulation of plaque in the oral cavity, among those are brushing teeth regularly, rinsing with antiseptic solution, cleaning interdental with dental floss, cleansing the tongue, chewing gum, and avoiding fermented carbohydrates [6]. The easiest way to remove plaque is by rinsing. Several studies have demonstrated the effectiveness and usefulness of an antiseptic mouthwash containing active compounds such as chlorhexidine and essential oils to prevent the formation of plaque and gingivitis [7].

One of the potential compounds in controlling plaque formation is in cinnamon bark. Puspita *et al.* (2013) showed that cinnamon extract has an effect on the growth of *S. mutans* which is cariogenic bacteria, but there is no information about the effectiveness of using cinnamon extract as a mouthwash to reduce dental plaque [2]. Therefore, in this study cinnamon ethanol extract applied as a mouthwash in inhibiting the growth of these bacteria was conducted.

2. Methods

2.1. Cinnamon bark maceration, extraction, and phytochemical test

Cinnamon bark was macerated followed by extraction in ethanol. A-2 kg of dried cinnamon bark was immersed in 20 L of distilled ethanol. Maserate was evaporated using a rotary evaporator at 60° C. The extract was fed into a water bath to remove remaining moisture. The extract was subjected to phytochemical tests including alkaloids, glycosides, anthraquinone glycosides, saponins, tannins, flavonoids, and steroids/triterpenoids.

2.2. Patients, plaque samples, and bacterial identification

Forty patients from Dental Clinic of Drg. Syahdiana Waty, Medan were selected based of Slovin's equation. Sample inclusion criteria were patients with dental caries of at least one tooth, aged 35-44 years, in good health and did not previously use antibiotic drug, willing to fulfill informed consent and willing not to brush their teeth before taking plaque samples. Samples were taken on buccal portion of 16, 26, 36 and 46 and labial of teeth 11 and 31. Samples were stored in containers and immediately taken to laboratory for bacterial culture. The samples were dissolved in sterilized water and subjected to be vortexed. Vortexed sample was streak on trypticase yeast cysteine and incubated at 37° C overnight. Single colony was identified using Vitek 2 compact.

2.3. Antibacterial assay of cinnamon bark extract and prepared mouthwash

Cinnamon bark extract was prepared in concentration of 6.25%, 12.5%, and 25% using dimethyl sulfoxide as solvent. Bacterial solution was prepared in 0.90% of NaCl and equalised to 0.5 Mc Farland. The bacterial solution was steak on entire surface of Muller Hinton agar using sterilized cotton bud. Paper disc immersed in each cinnamon extract concentration was put on the bacterial lawn in petri dish. The petri dish was incubated at 37° C overnight. Inhibition zone was measured as diameter of clearing zone around the paper disk subtracted to 0.5 cm. The extract with Minimum Inhibitory Concentration (MIC) was used as active compound in prepared mouthwash. pH and viscosity of prepared mouthwash were measured. Application of 10 ml mouthwash was conducted in patients for 30 seconds. The plaque sample

and measuring bacterial cell number were conducted as previously described. Number of bacterial cell was measured as colony growth in total plate count agar.

2.4. Design and data analysis

This study was a pretest-post test one group design. Data was analyzed using Shapiro Wilk, Kolmogorov-Smirnov, Kruskal-Wallis, Wilcoxon and Man Whitney tests.

3. Results and Discussion

3.1. Cinnamon bark maceration, extraction, and phytochemical test

Maceration and extraction of 20 kg of the bark yielded 17.5 L of the brown macerate and 670 gram reddish brown extract, respectively. Phytochemical test showed that cinnamon bark ethanol extract contains secondary metabolite compounds such as alkaloid, saponin, flavonoid, and glycoside groups. Puspita *et al* (2013) showed that cinnamon bark extracts contained transinamaldehyd, polyphenols, flavonoids, saponins, and tannins [2]. Awang *et al* (2013) reported that cinnamon bark essential oil contained cinnamaldehyde which is potential as an antimicrobial compound [8]. Alkaloid is one of the most organic compounds found in nature. It has prominent antimicrobial activity and has been widely used in antimicrobial treatment [9]. Saponin is a compound that has a working mechanism as antibacterial with its lipophilic properties capable of damaging cell membranes [10]. Flavonoid compounds disrupt bacteria by destroying the cytoplasmic membrane and causing leakage of important metabolites that inactivate bacterial enzyme systems [11].

3.2. Isolation of plaque bacteria

There were nine bacterial species found in the plaque samples, in which *Streptococcus* was the most common bacteria. There were six species of *Streptococcus* including *S. mitis*, *S. sanguinis*, *S. salivarius*, *S. alactolyticus*, *S. pneumoniae*, and *S. pluranimalium*. The other three bacterial species were *Kocuria rosea*, *K. kristinae*, and *Sphingomonas paucimolis* (Table 1).

Table 1. Frequency of *Streptococcus* and other bacterial species from dental plaque

No.	Bacteria species	Number of samples	Frequency (%)
1.	<i>S. mitis</i>	12	30
2.	<i>S. sanguinis</i>	8	20
3.	<i>S. salivarius</i>	4	10
4.	<i>S. alactolyticus</i>	3	7.5
5.	<i>S. pneumoniae</i>	1	2.5
6.	<i>S. pluranimalium</i>	3	7.5
7.	<i>K. rosea</i>	2	5
8.	<i>K. kristinae</i>	6	15
9.	<i>S. paucimolis</i>	1	2.5
	Total	40	100

Streptococcus mitis, *S. sanguinis* and *S. salivarius* were the most common bacteria in dental plaque. *Streptococcus mitis* is one of the bacteria of the mutans streptococci group (MSG). This group is known as cariogenic. It also included other species such as *S. mutans* and *S. sobrinus*. This group of bacteria is known as the pioneer bacteria involved in plaque formation and initiates dental caries [12].

Streptococcus sanguinis is one of species of *Viridans Group Streptococci* (VGS) which is most commonly found in dental plaque in the oral cavity. *S. sanguinis* and MSG have a strong association as *oral streptococci* and the most commonly found in dental plaque [12]. *Streptococcus salivarius* together with *S. sanguinis*, *S. mitis*, and *S. gordonii* known as the first colonizer the tooth surface, in contact with salivary glycoproteins on tooth surfaces via specific polymer capsules such as glucan and fructan. Furthermore, these bacteria play an important role in the process of dental caries together with other *Oral Streptococci* [13]. *Streptococcus plurimalium* was a species of VGS that is rarely encountered in dental plaque. Dhotre *et al.* (2016) found three strains of *Streptococcus* that were very rare and unusual in dental plaque subgingival plaque such as *S. plurimalium*, *S. thoralensis* and *S. hyointestinalis* [14].

Bacteria *K. rosea* and *K. kristinae* are a gram-positive bacteria belonging to the family *Micrococcus*. Sabtie *et al* (2015) found 16 species of bacteria in dental supragingival plaque from 50 patients, in which two were *K. rosea* and *K. kristinae* [15]. The normal habitat of the *Kocuria* is on the skin but can also be found in the urinary tract in patients with urinary tract infections. This non-pathogenic bacteria are human normal flora. However, many studies showed that these bacteria might cause many infections of the urinary and gastrointestinal tract [16]. Many diseases in the gastrointestinal tract, urinary, and even other vital organ diseases are believed to be associated with the oral cavity diseases.

3.3. Antibacterial activity

The result of antibacterial test of cinnamon bark ethanol extract showed all concentration of the extract inhibited the bacterial growth (Figure 1.). Cinnamon bark extract of 6.25%, 12.5% and 25% showed inhibitory zone of 6.78, 9, and 11.68 mm, respectively (Table 2).

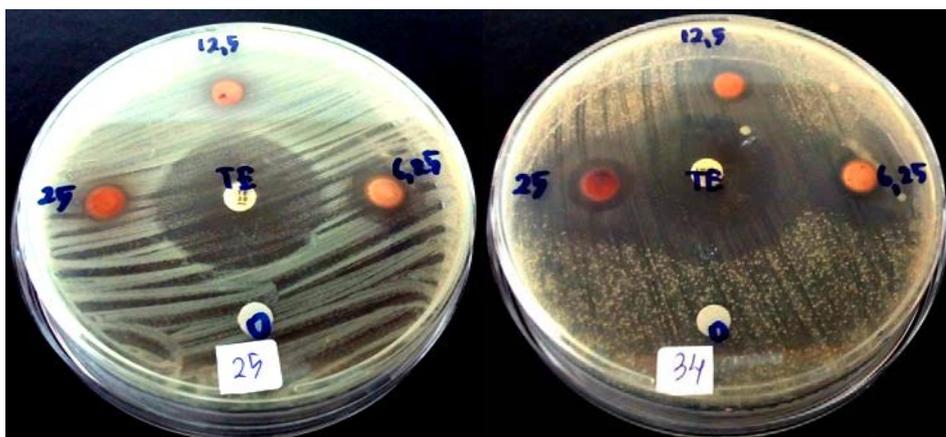


Figure 1. The bacterial inhibitory zone of cinnamon bark ethanol extract to *S. mitis* (25) and to *S. sanguinis* (34)

Table 2. Inhibitory zone of cinnamon ethanol extract to bacteria from dental plaque

Concentration of cinnamon extract	Diameters of inhibitory zone(mm)
6.25%	6.78
12.5%	9
25%	11.68
<i>Tetracyclin</i>	28.3

Data analysis showed that there was a significant difference in bacterial inhibition zone of the three concentrations. Furthermore, it was shown each concentration showed significantly different on inhibitory zone. The similar result showed in Puspita *et al.* (2013) to inhibit the growth of *S. mutans*. Al-Duboni *et al.* (2013) reported that cinnamon bark extract showed to have more antibacterial activity than that of ginger extract on *S. mutans* [17].

3.4. Examination of cinnamon extract mouthwash as antibacterial agent

It was shown that the prepared mouthwash had pH of 6.97 with viscosity of 1.01 cP. The results of antibacterial activity found that population of bacteria were reduced (Figure 2.). It was shown that there was a significant difference to bacterial activity of the prepared mouthwash in the mouth before and after rinsing.

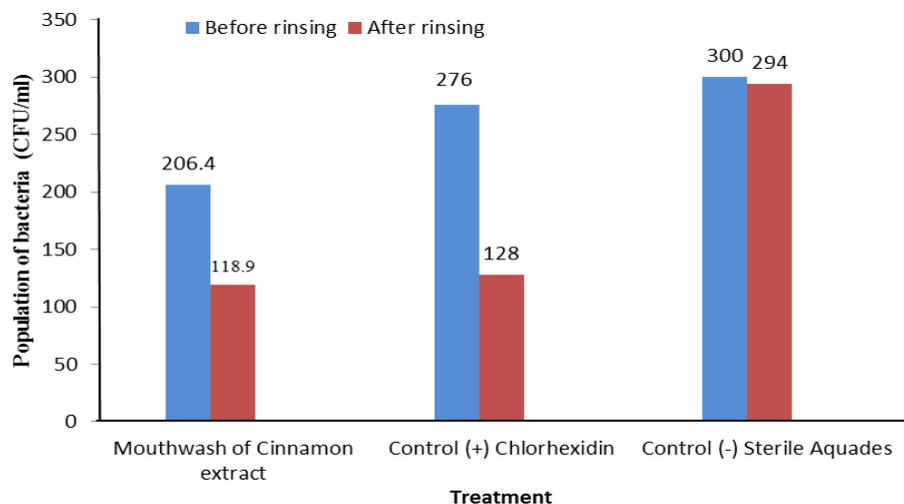


Figure 2. The average population of bacteria before and after rinsing with the mouthwash of Cinnamon extract, Chlorhexidin and sterile aquades.

From this study it was suggested to use this extract as an alternative in controlling pathogenic oral cavity bacteria. Many other plants such as starfruit leaves, gambir leaves, siwak, and lemon were reported to be used in mouthwash against bacteria in dental plaque in vivo. Fajriani and Mahrum (2015) reported

that 40% lemon extract showed a significant effect in reducing bacterial number 30 minutes after rinsing [18].

4. Conclusions

Cinnamon bark ethanol extract contained phytochemical compounds such as alkaloid, flavonoid, saponin, and glikosid. The extract of each concentration showed antibacterial activity in vitro, and as mouthwash as well.

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