Antibacterial effect of cinnamon essential oil (*Cinnamon cassia*) in different concentration towards *Streptococcus sanguis*

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ABSTRACT

Introduction: *Streptococcus sanguis* is a type of normal bacteria found in the oral cavity, particularly in plaque on tooth surfaces and can also be found in saliva, buccal mucosa, tongue and gingival sulcus. At present, the usage of herbal remedy for reducing plaque in oral cavity has been implemented widely and one of the herbal remedy used is cinnamon essential oil. The purpose of this study is to prove that there are differences in the antibacterial activity of cinnamon essential oil with different concentrations against *Streptococcus sanguis*. **Methods:** The study was performed using laboratory experiments, in which the sensitivity of the *Streptococcus sanguis* bacteria from the saliva of 10 KPBI batch 2006 dental students were tested *in vitro* against cinnamon essential oil by using Kirby-Bauer method. **Results:** The study shows that the cinnamon essential oil has different antibacterial abilities in inhibiting the growth of *Streptococcus sanguis* in different concentrations. This is due to the element cinnamaldehyde and eugenol that they posses as their main constituent. There is a significant difference between the antibacterial effects of *Cinnamon cassia* essential oil in different concentrations towards *Streptococcus sanguis*. **Conclusion:** Cinnamaldehyde and eugenol has been proved to have antibacterial effect towards wide variety of bacteria including *Streptococcus sanguis*.

Keywords: Antibacterial effect, cinnamon essential oil, *Streptococcus sanguis*.

INTRODUCTION

There are lots of bacteria of the oral cavity, most of them are opportunistic. The bacteria which can be found in the oral cavity are *Streptococcus* species, *Staphylococcus* species, *Actinomyces* species and many more. All of these bacteria cause oral disease such as caries, periodontal disease and many more infections of the oral cavity.[1]

One of the main bacteria that cause plaque formation in the oral cavity are *Streptococcus sanguis*, known as a facultative anaerobe bacteria which belongs to the *Streptococcus viridans* group, a Gram positive bacteria, coccus in shape and have a chain formation. This specific type of bacteria is included in normal oral microflora where it is mainly found on gingival sulcus, coronal plaque, dorsum of tongue, buccal mucosa and saliva. [2,3]

Streptococcus sanguis are known as the main bacteria which causes the formation of supragingival plaque with the ability to form extracellular polysaccharides in the form of

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dextran which adheres completely to the tooth structure.[1] Once the bacteria adhere to the tooth surface it creates an interbacterial matrix which gives ideal condition for other bacteria to adhere to the tooth surface. Besides this *Streptococcus sanguis* also have the ability to form an acidic metabolite paraaminobenzoat which serves as nutrient for *Streptococcus mutans* growth, hence leading towards caries formation.[4]

Plaque formation is a major dental health problem, a lot of step to overcome it have been done but mostly using drugs and mouthwash that consist substance that have side effects on oral health. Hence, researcher finds way to implant the usage of herbs in dental health to prevent oral diseases and one of them is cinnamon.

Cinnamon has been proved to possess antibacterial effect on bacteria that causes typhoid and posses chemopreventive, antispasmodic, anti-ulcer, antifungal, antibacterial, antipyretic and also stimulates immune system.[5,6,7] It also said that almost a huge population of Indonesia uses cinnamon as medicine for ulcers where they boil cinnamon and drink the boiled water. [8] Cinnamon also has been proved to work against E coli.[9] In the year 2005 it is also proven that essential oil from cinnamon works as antibacterial agent towards food borne bacteria and fungal.[10] The cinnamaldehyde in cinnamon also been proved to inhibits fungal growth such as *Aspergillus niger* and *Candida albicans*.[11]

Other research proved that cinnamon also has an antibacterial effect towards *Streptococcus sanguis* in the form of aqueous which is higher than normal mouth rinse solution.[12] In contrast between cinnamon oil and cinnamon in aqueous preparation, cinnamon oil has been proved to inhibit the growth of *Streptococcus sanguis* more than cinnamon in aqueous solution.[10] All the previous research makes it certain that cinnamon essential oil has antibacterial effect and this raises the thought of whether the antibacterial effect of cinnamon essential oil works on *Streptococcus sanguis*. The aim of this research is to prove that there are differences in the antibacterial activity of *Cinnamon cassia* essential oil with different concentration towards *Streptococcus sanguis*.

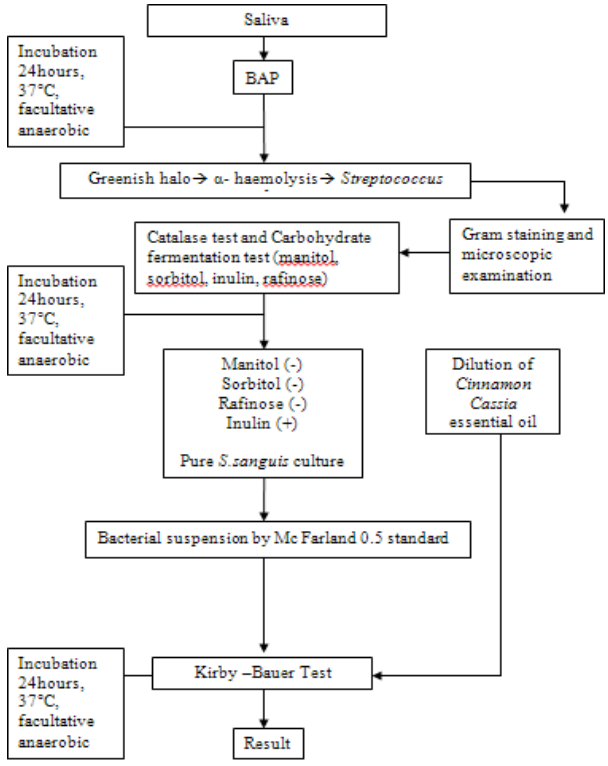


Figure 1. Research framework

METHODS

Type of research is laboratory experiment techniques to find out whether cinnamon oil has antimicrobial effect towards *Streptococcus sanguis* using diffuse agar method. Population is *Streptococcus sanguis* in the oral cavity of KPBI 2006 batch dental students. The sample is *Streptococcus sanguis* taken from saliva of 10 KPBI dental students.

In this research the materials used are Cinnamon Essential Oil, S*treptococcus sanguis* bacteria isolated from saliva, gram staining reagents, hydrogen peroxide 3% (H_2O_2), Carbohydrate Sugar (Raffinose and Inullin, Sorbitol, Manitol), and Polyethyleneglycol (PEG) with laboratory apparatus for bacterial culture.

RESULTS

The results of microscopic examination of samples taken from saliva with Gram staining showed cocci-shaped Gram-positive bacteria with the formation of the chain (Figure 2).

After microscopic examination, the suspected colonies were cultured on blood agar plates and incubated in a candle jar to create a facultative anaerobes environment at 37° Celsius for 24 hours. After 24 hours, the blood agar plate shows a greenish halo zone around the colony suggesting a partial haemodigestion activity has occurred. The result of such reactions is a characteristic that indicates that these bacteria belong to Streptococcus α-haemolyticus group that could reduce the hemoglobin (Hb) into methemoglobin (metHb).

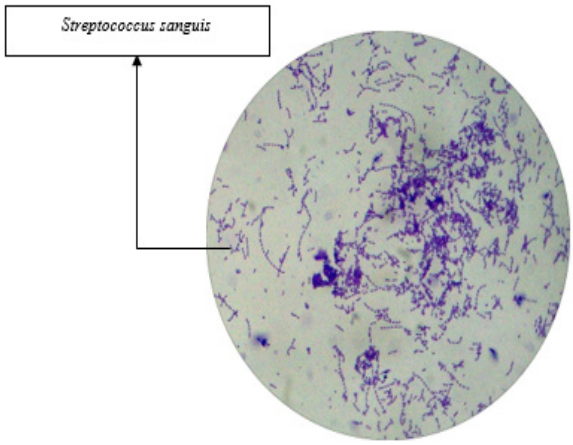
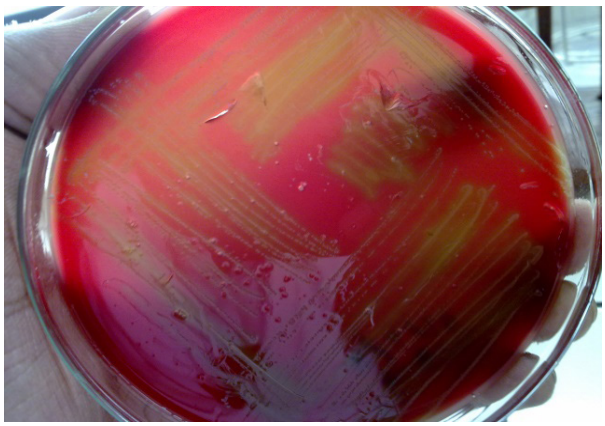


Figure 2. Microscopic view of *Streptococcus sanguis*

The result of this culture was further identified using the catalase test and fermentation tests. Catalase test using 3% H_2O_2 solution showed negative results, as there were no formations of air bubbles. This indicates that the bacteria in the culture are not a Staphylococcus but Streptococcus bacteria group. In the carbohydrate fermentation test of Inulin, Raffinose, Manitol and Sorbitol, after incubated in facultative anaerobes in 37° C for 24 hours, the results showed only Inulin change colour from red to yellow, which means positive result while others did not change colour (Figure 4). From this fermentation test, it can be confirmed that the bacteria in the culture are *Streptococcus sanguis*.[3,13]



1 - Inulin; 2 - Raffinose; 3 - Manitol; 4 - Sorbitol

Figure 3. Greenish halo zones

Figure 4. Carbohydrate fermentation test

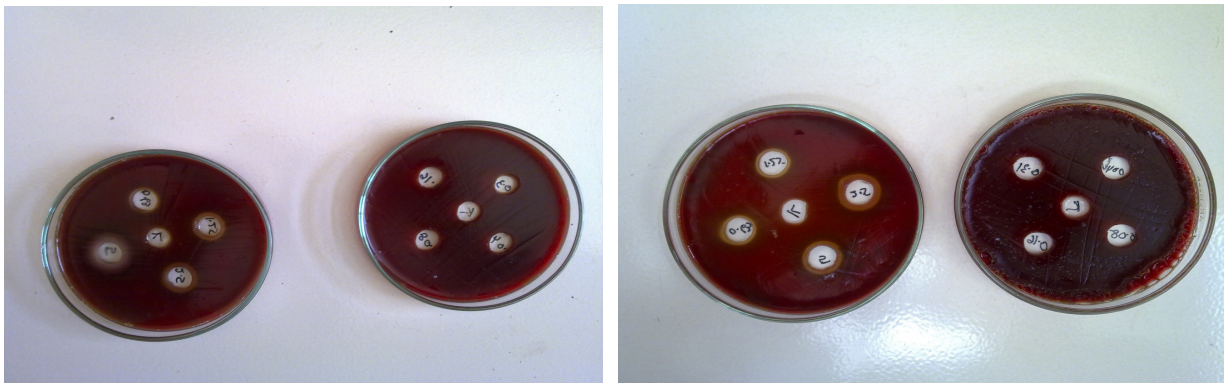


Figure 5. Bacterial growth inhibition zone of *Cinnamon cassia* essential oil towards *Streptococcus sanguis*

The assessment of the antimicrobial activity of cinnamon essential oil based on agar diffusion method according to Kirby-Bauer against *Streptococcus sanguis* after incubated in facultative anaerobic environment at 37°C for 24 hours, showed bacteria free zone around the hole which are filled with cinnamon essential oil of various concentrations as figure 5.

Results of measurement of inhibitory zone of cinnamon essential oil against *Streptococcus sanguis* from ten samples tested are shown in Table 1 below

Based on the results shown in table 1, it is proven that there are differences in inhibition zones at each concentration. Average inhibition area formed on 5 % concentration of cinnamon essential oil is 8.85mm, 2.5% is 6.2mm, 1.25% is 4.35mm, and 0.63% is 2.45mm. While, 0.31% of cinnamon essential oil is 0.65mm, 0.16% is 0.15mm, 0.08% is 0.05mm and lastly 0.04% shows averagely no inhibition zone. From this we can assume that 5% concentration of cinnamon essential oil has the biggest zone of inhibition with 8.85mm in average while 0.04% concentration has the lowest inhibition zone with 0 mm, suggesting that the cinnamon essential oil at 0.04% concentration does not have any antibacterial activity.

According to Sudjana (1995), the results of calculations with 95% confidence level (α = 0.05) showed significant results because the calculated F value is greater than F table as shown in Table 4.2. Since the test statistic (F_{value}) is much larger

Table 1. The result of the inhibition zone measurement of cinnamon essential oil towards *Streptococcus sanguis*

Sample	Repetition	Inhibition Zone (mm)								C
		5%	2,5%	1,25%	0,63%	0,31%	0,16%	0,08%	0,04%	
A	1	12	7	9	3	1	0	0	0	0
	2	7	12	10	3	2	2	0	0	0
B	1	4	2	3	0	0	0	0	0	0
	2	7	4	1	0	0	0	0	0	0
C	1	10	7	4	3	0	0	0	0	0
	2	13	5	5	4	1	1	1	0	0
D	1	6	5	3	2	0	0	0	0	0
	2	5	6	4	4	0	0	0	0	0
E	1	9	6	2	1	1	0	0	0	0
	2	11	5	4	3	0	0	0	0	0
F	1	7	5	4	4	1	0	0	0	0
	2	9	7	3	2	1	0	0	0	0
G	1	8	6	3	2	0	0	0	0	0
	2	7	4	3	1	0	0	0	0	0
H	1	12	9	5	4	2	0	0	0	0
	2	10	8	6	4	2	0	0	0	0
I	1	13	9	6	4	1	0	0	0	0
	2	11	7	6	3	1	0	0	0	0
J	1	7	5	3	1	0	0	0	0	0
	2	9	5	3	1	0	0	0	0	0
Average		8.85	6.2	4.35	2.45	0.65	0.15	0.05	0	0

Tabel 2. Varians analysis

Source of Variation	SS	DF	MS	F	F table
Treatment	179	2064.91	212.20	6.53	3.23
Error 1	8	1697.61	32.48	70.15	
Error 2	9	292.30	0.46		
	162	75.00			

than the critical value (F_{table}), the null hypotheses were rejected therefore accepting that there are significant differences in antibacterial effect of cinnamon essential oil with the concentration of 5%, 2.5%, 1.25%, 0.63%, 0.31%, 0.15%, 0.08% and 0.04% towards *Streptococcus sanguis*.

Based on data in Tables 1 and 2, it can be it can be concluded that the cinnamon essential oil with a concentration of 5%, 2.5%, 1.25%, 0.63%, 0.31%, 0.16% and 0.08% have antibacterial capacity, as they inhibit the growth of *Streptococcus sanguis* and form a inhibition zone around the hole on blood agar, filled with cinnamon essential oil.

DISCUSSION

Table 1 shows the results of the experiment where there are slight variation of inhibition zone diameter among samples which are due to human error and limitation of Kirby Bauer Sensitivity Test where it depends on the ability and rate of diffusion of the antibacterial agent into the medium and its interaction with test organism, the number of organism inoculated, the growth rate of the organism and the degree of susceptibility of the organism to the antibacteria.[13]

To determine whether a substance posses antibacterial effect and the degree of sensitivity of the bacteria towards the substance, according to Stokes[14], generally when the inhibition zone is greater than 3mm, the bacteria is sensitive towards the test material, if the inhibition zone is 2-3mm, the bacteria are moderately sensitive and if the zone of inhibition are less than 2mm it means that the bacteria are resistant towards the test material.[12] While according to Case and Johnson[15] generally the bacteria are categorized sensitive only when the inhibition zone diameter were 16mm and above and if the inhibition zones are less than 10mm it is categorized as resistant.[15]

There are differences between both references above is due to evolution process of the resistance of a particular bacteria.[16] Bacteria may manifest resistance to antibacterial drugs through a variety of mechanisms. Some species of bacteria are innately resistant to ≥ 1 class of antimicrobial agents. In such cases, all strains of that bacterial species are likewise resistant to all the members of those antibacterial classes. Of greater concern are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent.[17]

Microorganisms have developed seven major mechanisms to evade the bactericidal or bacteriostatic actions of antimicrobial agents such as, enzymatic inactivation, modification/ protection of the target (receptor) site, limiting access to the target site (altering cell wall or membrane permeability), active drug efflux from the cell, failure to activate the antibiotic within the cell, use of alternate growth requirements, and overproduction of target sites.[16,17]

Most microorganisms have developed ways to alter their cell wall or membrane permeability either by deleting outer membrane pores or by closing these membrane channels. This mechanism usually confers only low level resistance but when coupled with other more efficient resistance mechanisms can add significantly to the defenses of the microorganism. Multidrug antibiotic efflux pumps have been adapted by microorganisms from their original purpose (to expel waste products or toxins) to a very efficient means of antibiotic resistance.[18] More than 50 such efflux systems (multidrug efflux pumps, cytoplasmic membrane efflux proteins) have been described operating in many microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, Enterococci, Staphylococci, and Streptococci.[19]

In this research according to Table 1, *Streptococcus sanguis* were resistant towards Cinnamon essential oil and after ANOVA statistical test were performed on the data (Ttable 1), the result specified in table 2 were obtained. At 95% confidence level ($\alpha = 0.05$) showed significant results because the calculated F value is greater than F table as shown in Table 2, therefore H_0 were rejected. From these results we can conclude there is antibacterial effect of cinnamon essential oil to inhibit the growth of *Streptococcus sanguis* and there are also differences in the antibacterial efficacy of the cinnamon essential oil in different concentration in inhibiting the growth of *Streptococcus sanguis*.

Cinnamon cassia essential oil has the ability to inhibit the growth of *Streptococcus sanguis* because they contain the element cinnamaldehyde and eugenol as their main constituent.

Cinnamaldehyde and eugenol has been proved to possess antibacterial effect towards wide variety of bacteria including *Streptococcus sanguis*.[10]

Cinnamon oil had the most potent bactericidal properties compared to 20 other essential oils against different important pathogens.[20] Since its identification as the major antimicrobial substance of cassia[21], cinnamaldehyde has been reported to be an effective inhibitor of the growth of various micro-organisms including yeasts, bacteria and moulds as well as toxin production by micro-organisms.[21,18] It completely inhibits the growth of a number of food-borne bacteria such as Staphylococcus, Streptococcus, Micrococcus, Bacillus and Enterobacter spp. in a liquid media Masuda *et al*.[22] Against these five strains, cinnamaldehyde showed the strongest antibacterial activity among 15 essential oil components including citral, geranial, eugenol and menthol.[22] Application of cinnamaldehyde revealed potent antimicrobial effects against *Clostridium perfringenes*, *Bacteroides fragilus* and *Bifidobacterium bifidus*.[23] It also exhibits insecticidal and fumigant activities against *Mechoris ursulus*.[18] Application of the compounds to alfalfa seeds and sprouts were attempted for the purpose of inactivation of Salmonella sp.

Cinnamaldehyde, a major antimicrobial compound found in cassia, has been well known to possess strong inhibitory effects against various micro-organisms as well as against toxin production.[21] However, its mechanism to kill the microbial cells is yet to be identified.

CONCLUSION

There is differences between the antibacterial effects of *Cinnamon cassia* essential oil in different concentrations towards *Streptococcus sanguis*. Cinnamaldehyde and eugenol has been proved to have antibacterial effect towards wide variety of bacteria including *Streptococcus sanguis*.

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