

Antibacterial activity of cocoa bean husk extract on the growth of *Streptococcus alpha*

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ABSTRACT

Introduction: *Streptococcus alpha* is the most common bacteria found in dental plaques of children and roled as pioneer bacteria in plaque formation. One of the natural agents which has antibacterial activity is cocoa bean husk (*Theobroma cacao*, L.). This study aimed to analyze antibacterial activity of cocoa bean husks extract concentration 12.5%, 25%, 37.5% and 50% on the growth of *S. alpha*. **Methods:** This study was a laboratory experiment conducted in March, 2021 at Microbiology Laboratory, Faculty of Medicine Universitas Negeri Surakarta. The bacteria was isolated from the buccal tooth surface plaque of caries-free-9-years-old girl, then incubated on the blood agar medium to grow the *S. alpha*. The extract of cocoa bean husk concentration 12.5%, 25%, 37.5%, 50% and 0,2% Chlorhexidine Gluconate (CHX) as positive control were used to identify the antibacterial activity by measure the diameter of inhibition zone growth of *S. alpha* in the blood agar medium. The data was analyzed using one-way Anova. **Results:** Significant differences were found among the treatment groups ($p < 0.05$). The highest mean of the inhibitory zone was the 50% concentration of cocoa bean husk extract group ($17.07 \text{ mm} \pm 1.01$), but lower than CHX. The lowest inhibitory zone was the 12.5% ($10.12 \pm 0.05 \text{ mm}$). **Conclusion:** The cocoa bean husk extract has antibacterial activity on the growth of *Streptococcus alpha*, but it is lower than Chlorhexidine Gluconate.

Keywords: cocoa bean husk extract; antibacterial activity; *S. alpha*'s growth; child's dental plaque

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INTRODUCTION

Dental plaque is the main cause of dental caries and periodontal disease. Formation of dental plaque is preceded by acquired pellicle, then colonized by pioneer bacteria, such as *Streptococcus*. *Streptococcus alpha* is the most common bacteria

found in early plaques formation. The acidogenic effect of *S.alpha* was shown their ability to ferment sucrose fructose, and glucose, and causes pH declined.¹

The cocoa beans have active ingredients such as flavonoid, polyphenol, terpenoid/ steroid, and alkaloid, which has antibacterial activity. To

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produce a chocolate product, the cocoa beans have to be fermented, dried then roasted² and this process produces a large amount of cocoa bean husk as a waste.³ The cocoa bean husk has the same active ingredients with the beans of cocoa.^{4,5,6} It has capacity to damage the membrane cell of bacteria and inhibit its growth,⁷ so it can be considered as chemical dental plaque control, such as mouthwash or gargle. Currently, Chlorhexidine (CHX) is the standard mouthwash as an effective agent for inhibiting plaque growth.^{8,9,10}

The producer claimed it has ability to ionically adhere to teeth and mucosal surfaces in high concentration for hours. However, the use of CHX has side effects such as unpleasant sensation and stains if used for a long period.^{11,12} This study used to explore the antibacterial activity of cocoa bean husk and compare it to CHX. In the preliminary study, the minimal inhibitory of cocoa bean husk extract on the growth of *S.alpha* was 12.5%. The aim of this study was to analyze the antibacterial activity of 12.5%, 25%, 37.5% and 50% cocoa bean husk extract on the growth of *S.alpha* isolated from child's dental plaque and compared to 0.2% CHX.

METHODS

This experimental laboratory study was conducted on a nine-year-old girl who was caries-free, in Microbiology Laboratory, Faculty of Medicine Universitas Negeri Sebelas Maret. The cocoa bean husk was wasted from the chocolate processing. Where, the cocoa beans were peeling off from the husk after fermentation and roasted. It comes from a chocolate factory at Nglanggeran village, district of Pathuk, Gunung Kidul, Yogyakarta. Ethanol extract of cocoa bean husk was made from 1 kilogram of the husk added 5 grams of cellulose then soaked in 4,75 liters of aquades at 50°C for 4 hours. The 96% ethanol was added up to 50% and distilled for 1 hour, after filtration and the ethanol evaporated, it will produce 120 mg thick gel of cocoa bean husk extract. Then the concentrations of cocoa bean husk's extract 12.5%, 25%, 37.5%, and 50% were made by adding the aquadest.

Plaques on the buccal tooth surface were swabbed with cotton swab sterile, smeared to blood agar medium and incubated at 37°C for 24 hours. The greenish zone on the blood agar

medium was identified as the colony of *S.alpha*, then added with aquadest to obtain as 0,5 McFarland. The bacterial suspension was spread evenly with a spreader on the Mueller Hinton Agar medium. Six wells with 6 mm diameter were made by puncher in each plate and filled with the cocoa bean husk extract concentration 12,5%, 25%, 37,5%, 50%, 0,2% CHX (positive control) and aquadest (negative control), then incubated at 37°C for 24 hours. The inhibitory growth zone of *S.alpha* on the plate was measured by a caliper at 3 different diameters. In this study, replication was carried out 6 times. Normality and homogeneity of data were tested using the Shapiro Wilk and Levene test. The one-way Anova test was used to determine each treatment. The research was previously obtained Ethical Clearance No. 00560/KKEP/FKG-UGM/EC/2020.

RESULTS

The mean and standard deviation of *S.alpha*'s inhibition growth zone diameter by 12.5%, 25%, 37.5%, 50% cocoa bean husk extract, 0.2% CHX and the probability of the value of one-way Anova test were presented in Table 1 and Figure 1. The distribution of data was normal and homogeneous in all treatment groups, so it used parametric statistics for data analysis. The one-way ANOVA analysis shows that there are significant differences ($p < 0,05$) of the mean diameter of *S.alpha* inhibition growth zone among the treatment groups. The highest inhibition growth zone *S.alpha* was shown by the 50% concentration and the inhibition decrease by the decrease of concentration, however it is still lower compared to 0.2% CHX namely 17.07 ± 1.01 mm and 24.59 ± 0.23 mm.

Table 1. Mean and standard deviation of diameter inhibition zone on the growth *S.alpha* by cocoa bean husk extract and CHX, as well as the values probability of one-way Anova

Groups (%)	n	$\bar{x} \pm SD$ (mm)	F	p value
50	6	$17,07 \pm 1,01$	2074.57	0.00
37.5	6	$13,40 \pm 0,19$		
25	6	$11,45 \pm 0,19$		
12.5	6	$10,12 \pm 0,05$		
0.2% CHX (+ control)	6	$24,59 \pm 0,23$		

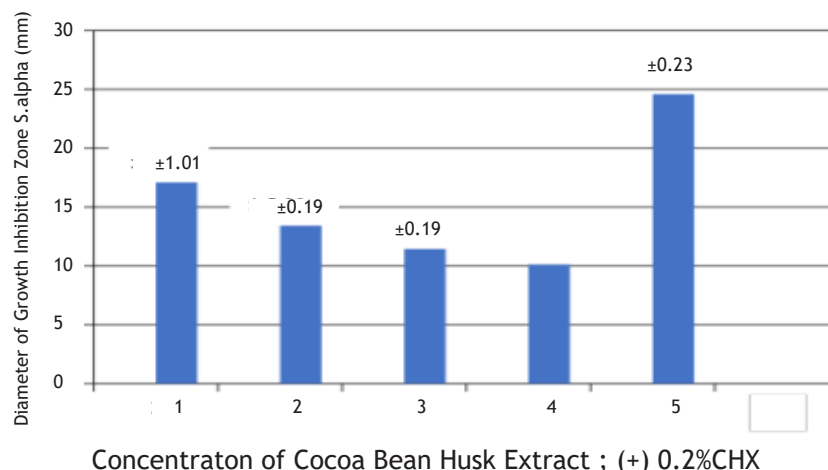


Figure 1. Diameter of inhibition growth zone S.alpha by cocoa bean husk extract and CHX

The result of the one-way Anova analysis showed $p < 0.05$, so it can be assumed that the concentration of cocoa bean husk extract influences S.alpha's growth. Comparative analysis

of the treatment groups by the post hoc analysis (Table 2) showed significant differences ($p < 0.05$) between treatment groups.

Table 2. Post Hoc test of inhibition zone on the growth S.alpha by 12.5%, 25%, 37.5%, 50% of cocoa bean husk extract, and 0.2% CHX

	Group	Mean differences	p
50%	37.5%	3.67*	0.00
	25%	5.62*	0.00
	12.5%	6.95*	0.00
	0.2% CHX	-7.52*	0.00
37,5%	25%	1.95*	0.00
	12.5%	3.28*	0.00
	0.2% CHX	-11.19*	0.00
25%	12.5%	1.33*	0.00
	0.2% CHX	-13.14*	0.00
12,5%	0.2% CHX	-14.47*	0.00

(*) Significant mean differences ($p < 0.05$)

DISCUSSION

Our preliminary study with chromatography was known that the cocoa bean husk contained flavonoid 0.52%. The minimum concentration of cocoa bean husk which had potency in growth inhibition of S.alpha was 12.5%, with diameter zone 10.78 mm. In this study we used ethanol extract of cocoa bean husk with concentration 12.5%, 25%, 37.5% and 50%. Since we planned to use this extract as mouthwash, we compared with Chlorhexidine Gluconate 0.2% as positive control.

These results in Figure 1 showed that the extract of cocoa bean husk had capacity as an

antibacterial agent to S.alpha at concentration 12.5%, 25%, 37.5% and 50%. The diameter inhibitory growth zone S.alpha of 50% cocoa bean husk extract was 17,07 mm, greater than 37,5% cocoa bean husk extract (13,40 mm), 25% cocoa bean husk extract (11,45 mm), and 12,5% cocoa bean husk extract (10,12 mm). According to Maleki, et al, higher concentration of the extract, higher antibacterial capacity¹⁴ this was proven in this study which showed that the cocoa bean husk extract 50% has greater diameter growth zone S.alpha than the 12,5%, 25%, and 37,5% cocoa bean husk extract. Cockerill said that inhibition growth zone diameter 5 mm or less was categorized as a

weak antibacterial agent, 5-10 mm as moderate, 10-20 mm as strong, and 20 mm or more was the strongest one.¹⁵

This study shown 12.5% of cocoa bean husk has diameter 10,12 mm so 12.5% considered has strong antibacterial potency. The strain of *Streptococcus alpha* contains many bacterial species such as *S.mutans*, *S.sanguis*, *S.mitis*, *S.salivarius*, *S.anginosus*, *S.bovis*, etc. Study of Matsumoto showed that cocoa bean husk inhibits the attachment of *S.mutans* on the hidroksiapatit and reduces the number of *S.mutans* in plaques.¹⁶ While study of Babu et.al showed the children's mouthwash from cocoa bean husk was as effective as CHX in inhibiting *S.mutans*.¹⁷

The diameter inhibitory growth zone *S.alpha* of CHX in Figure 1 has recorded 24.59 mm, showed that it was greater than the 50% cocoa bean husk extract. The antibacterial capacity of 50% cocoa bean husk extract compared to 0.2% CHX is around 70%, because this study used a strain of *S.alpha*, which was not specific in one species.

CHX is more effective in inhibiting *S.mutans* and *Porphyromonas gingivalis*.¹⁸ The use of CHX in the long term causes side effects such as stains and unpleasant taste, so the purpose of this study was to make mouthwash which has minimal side effects and is appropriate to children. However, the 50% cocoa bean husk extract was very concentrated and tasted bitter, so it should be added with other ingredients. Alternatively, it may be considered as a topical application to inhibit the attachment of bacteria on the tooth surface.

CONCLUSIONS

The cocoa bean husk extract has antibacterial activity on the growth of *Streptococcus alpha*, but it is lower than Chlorhexidine Gluconate.

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