

The Difference of Antibacterial Power between Cocoa Peel (Theobroma Cacao L.) Extract 6% Compared to Chlorhexidine Digluconate 2% Against Streptococcus mutans (In vitro)

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Research Report

The Difference of Antibacterial Power between Cocoa Peel (*Theobroma Cacao L.*) Extract 6% Compared to *Chlorhexidine Digluconate* 2% Against *Streptococcus mutans* (In vitro)

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ABSTRACT

Background: Before restoration, it is necessary to clean the cavity from the smear layer and residual bacteria such as *Streptococcus mutans* using a 'gold standard' cavity cleanser, namely 2% Chlorhexidine digluconate (CHX), however CHX 2% has a disadvantage of having a toxic effect on fibroblasts, osteoblasts, myoblasts, odontoblast-like cells, Chinese hamster ovary cells, and buccal epithelial cells. The shortcomings of the 2% CHX triggered researchers to look for alternative cavity cleansers that are more biocompatible, namely cocoa peel extract because it contains of antibacterial compounds including alkaloids, flavonoids, tannins, saponins, and terpenoids with a non-toxic 6% concentration.

Purpose: To analyze the difference of antibacterial activity between cocoa peel extract with a concentration of 6% compared to chlorhexidine digluconate 2% against *Streptococcus mutans*. **Methods:** This research was an in vitro laboratory experimental study with the posttest only control group design which included two treatment groups, namely 6% cocoa peel extract and 2% CHX. This research was conducted using the inhibition zone diffusion method against *S. mutans* to see the antibacterial power of each sample. **Results:** There was a significant difference ($p < 0.05$) in the mean diameter of the inhibition zone between 6% cacao peel extract, namely 11.5406 mm and CHX 2%, namely 13.2156 mm. **Conclusion:** Chlorhexidine digluconate 2% has a greater antibacterial power than 6% cocoa peel extract (*Theobroma cacao L.*) against *Streptococcus mutans*.

Keywords: Cocoa peel extract; Chlorhexidine digluconate; *Streptococcus mutans*; Antibacterial; Inhibition zone.

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INTRODUCTION

Caries is a chronic process due to an ecological imbalance between dental minerals and biofilms that can cause demineralization¹. The occurrence of dental caries can be caused by three factors, namely the host, bacteria, and diet². According to WHO 2017, dental caries is a non-communicable disease which is a global public health problem³. The prevalence of dental caries globally in permanent teeth has reached 2.3 billion people and in primary teeth has reached 560 million children, ranking first in the 2015 Global Burden of Disease Study. The severity of dental caries can be measured through the DMF-T value, which stands for Decay Missing Filled-Teeth. According to Riskesdas 2013, the prevalence of the DMF-T index in Indonesia is 4.6 (high) and 15 provinces have the prevalence of the DMF-T index above the national prevalence.

Streptococcus mutans is a gram-positive bacteria which is one of the primary etiologies of caries. There is a study that states *S. mutans* is the most common bacteria in a caries

lesion where when the caries lesion was isolated there were 45.6% *S. mutans*, 41.2% *Lactobacillus* spp, and 13.2% *S. aureus*⁴. The fermentation of carbohydrates from the *Streptococcus mutans* bacteria colony produces lactic acid which can dissolve hydroxyapatite crystals in teeth^{2,5}.

Tooth affected by caries, the lesion must be removed by preparation, but after the preparation there are still smear layers and residual bacteria that remain attached to the cavity wall, therefore to eliminate bacteria optimally it is necessary to prepare the tooth cavity before restoration using a cavity cleanser⁶. In the field of dentistry, the cavity cleanser is a material to remove debris and bacteria in the cavity wall⁷. There are various kinds of cavity cleansers that can be used by dentists, but the most frequently used in clinical and dental research is Chlorhexidine digluconate 2%⁶.

Chlorhexidine digluconate (CHX) is the "gold standard" cavity cleanser that is most often used because of its broad spectrum properties, which is antibacterial ability against gram-positive and gram-negative bacteria⁸. The interaction between the positive charge on the CHX molecule and

the negative charge on the bacterial wall allows the CHX molecule to penetrate and reduce the resistance of the bacterial cell wall. This results in intracellular discharge and bacterial death due to lysis^{6,9}.

According to several studies, CHX (2.0%) has disadvantages, namely cytotoxic effects on fibroblasts, osteoblasts, myoblasts, and odontoblast-like cells^{10,11}. Other cytotoxicity studies have shown that CHX can lead to increased free radical release followed by Chinese hamster ovary cell death from a concentration of 1-5 x 10⁻⁴%¹². In addition, there are studies that report genotoxicity to buccal epithelial cells due to long-term exposure to 0.2% mouthwash¹³. Therefore, it is necessary to do research on non-toxic alternative cavity cleansers

Indonesia is an agricultural country that produces various kinds of natural ingredients, one of which is cocoa (*Theobroma cacao L.*). Most of the cocoa used is the result of fermentation from cocoa beans, while cocoa shells are categorized as food industry waste. Research states that data on cocoa peel account for 60% of the weight of cocoa pods, which is 3 times heavier than cocoa beans^{14,15}. Cocoa peel contains flavonoids, alkaloids, saponins, tannins and terpenoids which are antibacterial components by inhibiting pathogenic bacteria¹⁶.

Flavonoids are polyphenolic compounds that can interact with bacterial cell membrane proteins by adsorption on the hydrophilic layer on the cell membrane, that can cause bacterial lysis¹⁷. Alkaloids contain nitrogen and alkaline atoms which can cause coagulation of bacterial cell proteins¹⁷. Tannins are compounds that can inhibit bacterial growth because they can bind to teichoic acid, which is the acid owned by peptidoglycan in gram-positive bacteria cell wall⁹. Triterpenoids can bind to transmembrane proteins in the bacterial wall so that they interfere with ion transport causing imbalance of ions in the cell wall and causing cell necrosis and apoptosis⁹. Saponins can cause leakage in the bacterial cell wall by hydrolyzing the cell wall⁹.

Based on the facts mentioned above, it is necessary to do research that proves the antibacterial power between the cocoa peel extract and chlorhexidine digluconate 2% against caries-causing bacteria, namely *Streptococcus mutans*. According to a research by Fitriani et al in 2019, cocoa bark extract has a minimum concentration that can have a cytotoxicity effect on BHK-21 (Baby hamster kidney-21) fibroblast cells¹⁸, which is 6.25%, therefore this research needs to be carried out at a concentration that is more biocompatible, namely the concentration of 6%.

MATERIALS AND METHODS

This type of research is a laboratory experimental in vitro with the post test only control group design. The sample used was a stock of *Streptococcus mutans* bacteria obtained from the Research Center of the Faculty of Dental Medicine, Airlangga University.

The peeled skin of the cocoa pods (1 kg) was washed and cut into 1-2 mm pieces then wind-dried at room temperature

for 3 days. dried in an oven at 40°C for 6 hours. The cocoa peel are then crushed into a powder in a blender. 40 grams of cocoa peel powder is then put into a maceration extractor and macerated by soaking 400 ml of 70% ethanol at room temperature in a ratio of 1: 1.5 (powder: solvent). Stirred with a shaker at a speed of 120 rpm for 24 hours.

The maceration results were filtered from a solution with Whatman filter paper No. 40 so that the pure concentrated extract is obtained. The solvent (ethanol) in maceration is evaporated with a rotary vacuum evaporator to make it free from ethanol solvents and to produce a pure extract of cocoa peel. 2 ml concentrated extract is taken and then diluted to a concentration of 6%.

Streptococcus mutans culture was taken from a stock using sterile osse. *Streptococcus mutans* was implanted in a tube containing Brain Heart Infusion Broth (BHIB) and incubated for 24 hours at 37°C. The cultures were diluted to reach a standard of 0.5 McFarland or the equivalent of 1.5 x 10⁸ CFU / mli. Sixteen petri dishes containing Media Hinton Agar (MHA) were prepared and the streptococcus mutans bacteria that had been implanted in BHIB were taken and smeared on the MHA surface using a sterile cotton swab.

Drop 10 µl 6% cocoa peel extract and Chlorhexidine digluconate 2% on a paper disc with a micropipette then place the paper disc on the surface of the agar medium with tweezers. All petri dishes were incubated in an incubator for 24 hours at 37°C. Observation and measurement of the inhibition zone were done using a caliper (mm).

RESULTS

At the beginning of the research, an extract was made and phytochemical tests were carried out on the cocoa peel extract at the Industrial Research and Consultation Center (BPKI), Surabaya (Table 1).

This study aims to determine the antibacterial power of 6% cacao peel extract (*Theobroma cacao L.*) and Chlorhexidine Digluconate 2% against *Streptococcus mutans* bacteria by looking at the diameter of the inhibition zone using the diffusion method. This study consisted of 2 treatment groups including cocoa peel extract 6% and Chlorhexidine Digluconate 2% (Figure 1). Performed 16 repetitions for each treatment sample. From these results, it can be seen that the Chlorhexidine Digluconate 2% sample group showed greater inhibition zone results than the cacao peel extract 6% (Figure 2).

Normality test is performed using the Kolmogorov-Smirnov Test to determine if the data obtained is normally

Table 1. Phytochemical test results of the cocoa peel extract

Contents	Percentage
Tannins	4.15%
Flavonoids	3.05%
Saponins	4.08%
Terpenoids	2.11%
Alkaloids	5.02%

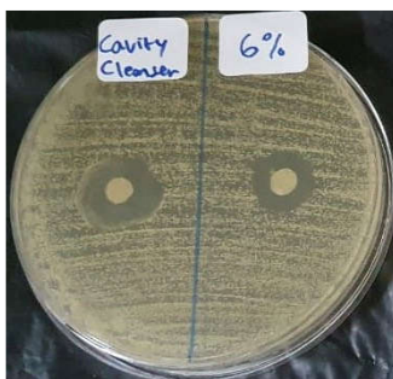


Figure 1. The results of differences in the inhibition zone of cocoa peel extract 6% and CHX 2% (cavity cleanser) against *Streptococcus mutans*.

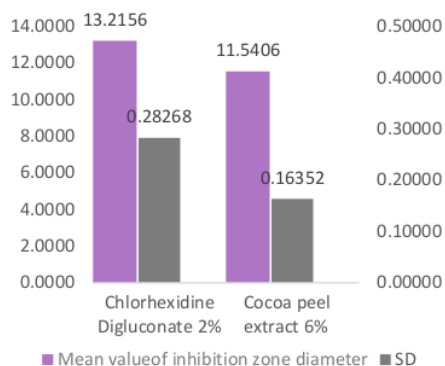


Figure 2. Mean value and standard deviation of inhibition zone diameter cocoa peel extract 6% and CHX 2%.

Table 2. Kolmogrov-Smirnov test results

Treatment Group	p
Cocoa peel extract 6%	0.057
Chlorhexidine Digluconate 2%	0.173

distributed or not. The results of the Kolmogrov-Smirnov Test shows that the data in the two sample groups obtained the p value > 0.05 . From the results of the normality test it can be concluded that the data is normally distributed.

Independent t-test was conducted to determine whether there was a significant difference between the inhibition zone diameter of the two treatment groups. Based on the results of the independent t-test (Table 3), the p value < 0.05 was obtained, it can be seen that there is a significant difference in the mean diameter of the inhibition zone between the two treatments

DISCUSSION

Obtained from the results of this study showed that there was a difference in antibacterial activity between cocoa peel extract 6% and Chlorhexidine digluconate 2% where can be seen from the differences in the inhibition zone diameter. From the results of the independent t-test in this study, there was a significant difference between cocoa peel extract 6% and CHX 2%. The difference in antibacterial power can be influenced by several factors including¹⁹: 1) The type of bacteria being inhibited, 2) The material/extract used, 3) The content of the antibacterial compound of a material, and 4) The concentration of the substance/ extract

The bacteria tested in this study were *Streptococcus mutans*. *S. mutans* is a bacteria that can develop in a facultative anaerobic state. *S. mutans* is a gram-positive bacteria, so the cell wall morphology of *S. mutans* includes glycolax, capsules, peptidoglycan, and plasma membrane, and the inside of *S. mutans* contains cytoplasm²⁰. The wall

Table 3. Independent t-test results

Treatment Group	p
Cocoa peel extract 6%	0.001
Chlorhexidine digluconate 2%	

of gram-positive bacteria has a simpler cell wall and cell membrane structure than gram negative bacteria. The simple cell wall structure makes it easier for antibacterial active compounds to penetrate the cell wall²¹. This can make it easier for these active compounds to find targets to work on in carrying out the antibacterial mechanism²¹.

In this study, two ingredients were carried out, namely cocoa peel extract 6% and Chlorhexidine digluconate 2%, both of which have different antibacterial mechanisms. The antibacterial mechanism of the cocoa peel extract is obtained from the ingredients owned by the cocoa peel, so a phytochemical test has been carried out which states that the cocoa peel extract contains several compounds, including alkaloids (5.02%), flavonoids (3.05%), and tannins. (4.15%), saponins (4.08%), and terpenoids (2.11%). The content of the cocoa peel extract is an antibacterial compound. The mechanism of the antimicrobial compounds in the cocoa peel is by inhibiting bacterial cell wall synthesis, changing the permeability of the bacterial cytoplasmic membrane, and inhibiting enzyme activity in bacterial cells¹⁷

In the content contained in the skin of the cocoa pods, especially compounds, the flavonoid content of 3.05% is the active ingredient of the cocoa peel. Flavonoids have antibacterial properties, namely catechins and anthocyanins, where catechins can denature bacterial proteins with their bactericidal nature, while anthocyanins can digest genetic material in bacteria²². This proves that the cocoa peel extract has antibacterial properties.

The 5.02% alkaloid compound is the largest content in the forastero type of cocoa peel extract. Alkaloids are organic compounds that have nitrogen and alkaline atoms, causing coagulation in bacterial cell proteins. Protein coagulation

5 will disrupt the peptidoglycan components (components of the bacterial cell wall) so that disruption of peptidoglycan can result in inhibition of growth and bacterial cell death^{17,23}. The 4.15% tannin compound contained in the extract of the cocoa pod is an inhibitor of bacterial growth because it can bind to teichoic acid and metal ion complexes. Teichoic acid is an acid that is in peptidoglycan in gram-positive bacteria, whereas metal ion (iron) complex is the need for bacteria in an aerobic state to reduce DNA ribonucleotide precursors. The way tannins bind to these two things can disrupt bacterial function^{9,24}.

7 The 4.08% saponin compound functions to disrupt the permeability of the bacterial cell membrane causing the release of cellular components in bacteria such as nucleotides, proteins, and nucleic acids by hydrolyzing the bacterial cell walls. This can cause leakage in the bacterial cell wall, causing lysis of bacteria^{9,25}. The 2.11% triterpenoid compound is the smallest content in the cocoa peel. The content of terpenoids serves to bind transmembrane proteins to the bacterial walls and bind to fats and carbohydrates. This can interfere with ion transport and cell wall permeability so that it can cause cell necrosis and apoptosis^{9,26}.

On the antibacterial properties of Chlorhexidine digluconate (CHX) The main target of the antibacterial action mechanism on CHX is the integrity of the cytoplasmic membrane (consisting of a phospholipid bilayer and protein) of a bacteria and the function of membrane-bound enzymes. The interaction of the positive charge of CHX molecule and the negative charge of the phosphate particles on the bacterial cell wall causes a progressive decrease in the fluidity of the outer phospholipid layer with the creation of hydrophilic domains in the bilayer^{9,27}. These changes affect the osmoregulation and metabolic activity of the cytoplasmic membrane and enzymes so that the CHX molecule can penetrate the bacterial body. CHX has different antibacterial mechanisms at high and low concentrations, with low concentrations as used in this study (2%), CHX is bacteriostatic by damaging the cell wall and attacking the bacterial cytoplasmic membrane^{9,27}.

From this study, it was obtained that the mean diameter (Figure 2) of the inhibition zone of cocoa peel extract 6% had a diameter of 11.5406 mm with a standard deviation of 0.16352, while chlorhexidine digluconate 2% had a diameter of 13.2156 mm with a standard deviation of 0.28268. These results state that the mean diameter of the inhibition zone of cocoa peel extract 6% is not higher than Chlorhexidine digluconate 2%. This can be due to the choice of concentration in the cocoa peel extract that is used in this study. There is research which states that the concentration of antibacterial substances has a close relationship in inhibiting bacterial growth where the higher the extract concentration, the higher the antibacterial power²⁸. The high antibacterial power causes the bacteria's resistance to lower against the antibacterial compounds contained in the extract. Due to research stating that the minimum concentration of cocoa peel extract, which is 6.25%, has a cytotoxic effect on BHK-21 (Baby hamster kidney-21) fibroblast cells¹⁸, preliminary research before this study has been done on

the antibacterial effectiveness of cocoa peel extract with a non-toxic concentration, namely below 6.25%. In the results of the preliminary research, it was stated that the 6% concentration had the highest antibacterial effect, so that this concentration was carried out in this study. Although the diameter of the inhibition zone produced by cacao peel extract 6% is lower than CHX 2%, there are studies which state that the inhibition zone with a diameter of ≤ 5 mm is classified as weak, 6-10 mm is classified as moderate, 11-20 mm is classified as strong²⁹, because of that statement, the inhibitory power of 6% cacao pod husk extract is still quite strong. In this statement, it can be concluded that the cocoa peel extract 6% is still considered strong as an alternative ingredient for herbal cavity cleanser.

There are several factors that affect the results of this study due to limitations of the study, factors that can affect the levels of active compounds in the extract include genetic factors, environmental factors, and maturity level factors of the cocoa plant. Genetic factors include things related to the inherited traits of the mother plant, such as chemical composition³⁰. Environmental factors include plant external factors that can affect the active compounds of the cocoa plant, such as temperature, sunlight, and also the growing area of cocoa. The maturity level factor is the older/ripe the cocoa plant is, the higher the content of tannin compounds³⁰.

CONCLUSION

2 In this study it can be concluded that there is a difference in antibacterial activity between the cocoa peel extract (Theobroma cacao L.) 6% and Chlorhexidine digluconate 2% against *Streptococcus mutans* bacteria. Chlorhexidine digluconate 2% has greater antibacterial power than cocoa peel extract (Theobroma cacao L.) 6% against *Streptococcus mutans* bacteria

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