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# Antibacterial Irrigation Solution Cocoa Bean Cuticle Extract (*Theobroma cacao L*) Against *Streptococcus viridans*

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## ABSTRACT

**Background:** Pulp necrosis is the demise of the pulp due to persistent infection in the pulp area, such as *Streptococcus viridans*. Irrigation steps during biomechanical preparation and sterilization play a very important role. The irrigation material often used is 2.5% NaOCl because it has a broad antibacterial spectrum but is irritating and has an unpleasant aroma. The cuticle of cocoa beans (*Theobroma cacao L.*) might be an alternative root canal irrigation material.
**The Objective:** To determine the antibacterial properties of irrigation solution of cocoa bean cuticle extract against *Streptococcus viridans*.
**Materials and Methods:** This study was laboratory experimental with a post-test-only control group design by calculating the number of bacterial colonies. Consisting of eight research groups, namely cocoa bean cuticle extract with concentrations of 25% (P1), 12.5% (P2), 6.25% (P3), 3.12% (P4), 1.56% (P6), 0.78% (P6), NaOCl 2.5% (K+), and sterile distilled water. 0.5 ml of each group was taken and then mixed with 0.5 suspension of *Streptococcus viridans*, diluted $10^{-4}$. Next, the extract and bacterial suspension mixture $10^{-4}$ were incubated for 24 hours, then 0.1 ml was inoculated on BHIA media and incubated. After that, the colony counter manually counted the number of colonies.
**Results:** The result showed a significance of $p<0.5$, followed by the Mann-Whitney test, which showed significant differences except in group P1 (25%) with K+ (NaOCl 2.5%).
**Conclusion:** Cocoa bean cuticle extract irrigation solutions with concentrations of 25%, 12.5%, 6.25%, 3.12%, 1.56% and 0.78% presented antibacterial power against *Streptococcus viridans*.

***Keywords:*** *Streptococcus viridans*, Antibacterial, Cocoa bean epidermis extract, Irrigation solution.

## INTRODUCTION

Pulp necrosis or pulp tissue demise is an irreversible condition characterized by damage to the pulp tissue. The initial occurrence of pulp necrosis results from damage to the protective layers of the pulp (cementum, enamel, and dentin). Deterioration to the protective layer of the pulp allows microorganisms to resettle to the pulp through the dentin tubules, resulting in aggravation of the pulp tissue.[1] The most ordinary microorganisms encountered in pulp necrosis are *Streptococcus viridans* 75%.[2] *Streptococcus viridans* is a gram-positive anaerobic bacterium of the Streptococcus alpha-hemolytic which is pathogenic in the blood and provokes subacute bacterial endocarditis in humans.[3,4,5]
Root canal treatment (endodontic treatment) aims to eliminate microorganism infections in the root canal.[6] Moreover, this treatment maintains teeth function and periodontal tissue health, lasting as long as possible in the oral cavity. The Endodontic Triad

consists of biomechanical preparation actions (shaping and cleaning), sterilization, and obturation.[2] The irrigation stage during biomechanical preparation and sterilization plays a vital role because it kills microorganisms, dissolves the smear layer, removes debris and acts as a lubricant.[7]

The most frequently used root canal irrigation agent is Sodium Hypochlorite (NaOCl) 2.5% because it is antimicrobial with a broad spectrum, dissolving vital, necrotic pulp tissue and organic/inorganic compounds. Moreover, it is economical and easy to obtain. However, on the other hand, it stimulates irritation if pushed into the periapical tissue, comes into contact with the oral mucosa, and delivers an unpleasant aroma. Alternative irrigation materials made from natural ingredients are needed to overcome these weaknesses.[8,9]

Jember Regency is the second-highest producer of cocoa (*Theobroma cacao L.*) in East Java after Banyuwangi.[10] The cocoa bean is the part used in the chocolate-making process. Of course, waste is produced in the production process, including the cocoa bean shell, especially the epidermis. Cocoa bean epidermis waste has alternative potential as a root canal irrigation material because it contains antibacterial compounds.[11,12]

The results of research by Yumas[13] regarding the antibacterial activation of cocoa bean cuticle extract with concentrations of 0.25%, 0.5%, 1%, 1.5% and 2% were proven to inhibit *Streptococcus mutans* bacteria. The highest inhibitory power was at concentrations of 1.5% and 2%, with an inhibitory zone diameter of 11 mm - 11.8 mm and an observation time of 2 days. In another study, the antibacterial test of cocoa beans at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12% and 1.56% against *Streptococcus mutans*, it was shown that the effective concentration was at a concentration of 12.5%.

Antibacterial compounds in cocoa bean husk include polyphenols, flavonoids, and tannins.[13] Polyphenols are active compounds that function as antibacterial agents because they damage cell walls and precipitate bacterial cell proteins.[14] Flavonoids function as antibacterial agents because they can damage cell walls and precipitate proteins. Bacterial cells, while the tannins in cocoa bean husk waste extract, present the potential to be antimicrobial because they can disrupt bacterial protein transport, inhibit enzyme work, and deactivate bacterial adhesion.[15,16]

## MATERIALS & METHODS

The type of research carried out was experimental laboratory post-test only control group design, by counting the number of *Streptococcus viridans* colonies that grew on BHI-A media. The cocoa bean husk waste used was for a stereo (lindak) type obtained from PTPN XII's Banjarsari Plantation, Jember.

Cocoa bean cuticle extract, namely 1 kg of dried cocoa bean epidermis, was ground into powder using a blender, and the powder was placed in the oven at 500C for two days. Cocoa bean epidermis powder was sifted and then weighed 200g. Cocoa husk simplicial was extracted by maceration using a 96% ethanol solution to obtain the filtrate. The filtrate was evaporated at low pressure and a temperature of $50^0$C.[13] Cocoa bean epidermis extract with a concentration of 100% was diluted in stages to P1 (25%); P2 (12.5%); P3 (6.25%); P4 (3.12%); P5 (1.56%); and P6 (0.78%). There were two treatment groups, namely the cocoa bean cuticle extract group and the positive control (NaOCl 2.5%).

A suspension of *Streptococcus viridans* was made by mixing one dose of pure *Streptococcus viridans* into 2 ml of BHI-B solution in an incubator at 37ºC for 24 hours. Next, the *Streptococcus viridans* suspension was vibrated using a thermocline and the turbidity level was measured using a densitometer and compared with the standard Mc solution. Farland 0.5. Then, the suspension was diluted $10^{-4}$ with sterile distilled water.

Mix 0.5ml of the extract groups of various concentrations and the control group into the Eppendorf containing 0.5 ml of $10^{-4}$ bacterial suspension, then incubate at 37°C for 24 hours. Inoculation uses the streak plate method by dripping 0.1ml of the mixture into BHI-A media, spreading it using a cotton swab and incubating. After 24 hours, the number of colonies was counted. Data analysis resulting from counting the number of colonies used the Kruskal-Wallis and Mann-Whitney statistical tests.

## RESULT

The results of antibacterial research on the irrigation solution of cocoa bean husk extract (*Theobroma cacao L.*) against the bacteria *Streptococcus viridans* showed colony growth, as seen in Table 1 and Figure 1.

**Table 1. The number of *Streptococcus viridans* colony after treatments (CFU)**

| Groups | Number of Bacteria Colony | P value |
|---|---|---|
| P1 | 0.00 ± 0.00 | 0.000* |
| P2 | 2.00 ± 1.83 | |
| P3 | 28.00 ± 6.58 | |
| P4 | 67.25 ± 5.56 | |
| P5 | 100.5 ± 7.23 | |
| P6 | 134.5 ± 4.65 | |
| CP | 0.00 ± 0.00 | |

Data presented mean and standard of deviation; Data was analyzed by Kruskal Wallis test ($p<0.05$); *, significant difference ($p<0.05$); P1, 25% cocoa bean cuticle extract group; P2, 12.5% cocoa bean cuticle extract group; P3, 6.25% cocoa bean cuticle extract group; P4, 3.12% cocoa bean cuticle extract group; P5, 1.56% cocoa bean cuticle extract group; P6, 0.78% cocoa bean cuticle extract group; CP, positive control (NaOCl)

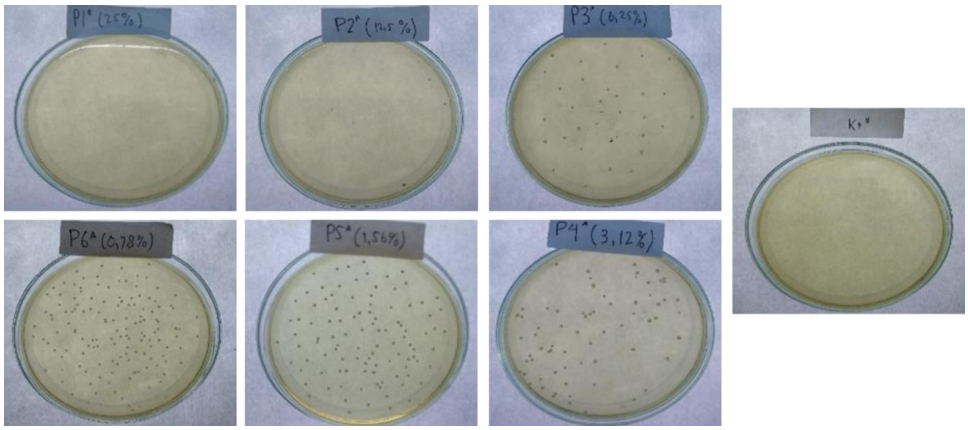

**Figure 1.** *Streptococcus viridans* colonies of groups in BHI-A media. Colonies were obtained at 24 h post incubation. P1, 25% cocoa bean cuticle extract group; P2, 12.5% cocoa bean cuticle extract group; P3, 6.25% cocoa bean cuticle extract group; P4, 3.12% cocoa bean cuticle extract group; P5, 1.56% cocoa bean cuticle extract group; P6, 0.78% cocoa bean cuticle extract group; CP, positive control (NaOCl)

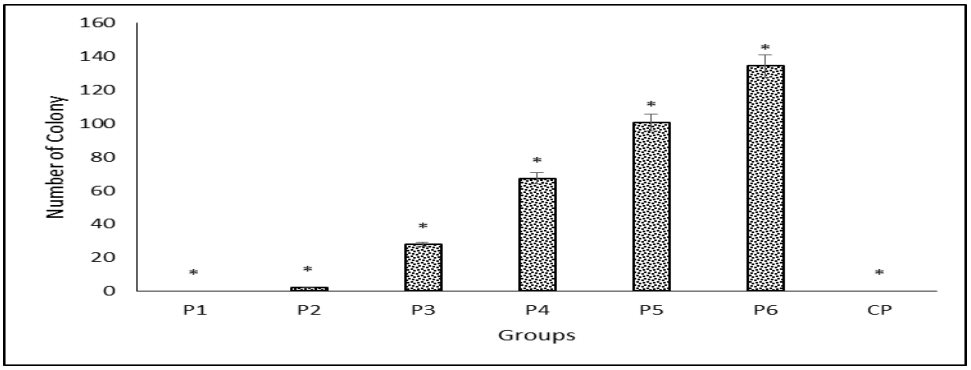


**Figure 2. Data presented mean and standard of error; Data was analyzed by multiple comparison test ($p<0.05$); *, significant difference ($p<0.05$)**

Counting bacterial colonies using solid media aims to measure the density of living bacteria. This study revealed that cocoa bean cuticle extract group reduced the growth of *Streptococcus viridans* bacterial colonies. Cocoa bean cuticle extract group with a concentration of 25% presented the same ability as the positive control (NaOCl 2.5%) in reducing the growth of *Streptococcus viridans* bacterial colonies (mean ± SD = 0.00 ± 0.00) (table 1 and Figure 1). Meanwhile, concentrations below 25% were still able to grow bacterial colonies. However, there was a significant increase in the number of *Streptococcus viridans* colonies following a decrease in the concentration of cocoa epidermis extract (figure 2).

## DISCUSSION

The cocoa bean cuticle extract group with a concentration of 25% presented the same ability as the positive control (NaOCl 2.5%). A concentration of 25% might present the ability to reduce the number of *Streptococcus viridans* colonies. The active ingredient content at a concentration of 25% is relatively high, reducing the growth of *Streptococcus viridans*. The results also indicated that the greater the extract concentration, the smaller the number of colonies. Moreover, this study indicated that each concentration presented different antibacterial compounds. The difference in the number of compounds between concentrations is caused by dilution using distilled water; the higher the dilution, the fewer antibacterial compounds in a solution. In addition, crude extracts of medicinal plants which contain many combinations of active compounds may produce antimicrobials that are more effective than antibiotics/antiseptics. In addition, several studies show that plant antimicrobials often do not cause resistance. However, it remains to be understood whether these antimicrobials will experience the same antimicrobial resistance as existing antibiotics.[17]

Plants and bacteria presented "genetic instability" in that they can respond to environmental stress by rearranging their genotypes. The chemical complexity and high level of bioactive components become therapeutic power, although identifying the most potent active compounds as therapeutic targets is challenging.[17] The ability of cocoa bean epidermis waste extract to inhibit the growth of *Streptococcus viridans* is due to the content of active substances such as flavonoids, tannins and polyphenols, which have antibacterial properties.[13,18] Polyphenols in cocoa bean epidermis waste extract are active compounds that function as antibacterial agents because they damage cell walls and precipitate bacterial cell proteins. Flavonoids function as antibacterial agents because they can damage cell walls and precipitate bacterial cell proteins. At the same time, tannins in cocoa bean epidermis waste extract have the potential to act as antimicrobials because they can disrupt bacterial protein transport, inhibit enzyme work, and deactivate bacterial adhesion.[19]

According to Sumawinata[20], *Streptococcus viridans* presented more irritating properties than Streptococcus mutans. The irritating nature of *Streptococcus viridans* is the leading cause of pulp necrosis. If left untreated, *Streptococcus viridans* can infiltrate the blood circulation and cause subacute bacterial endocarditis in humans. Apart from that, according to Bertha Ayi[3], the virulence of *Streptococcus viridans* is higher than that of Streptococcus mutants because *Streptococcus viridans* is a combination of several alpha-hemolytic gram-positive bacteria, while Streptococcus mutants only consist of one type of bacteria. *Streptococcus viridans* consist of several types of bacteria, such as Streptococcus mitis, Streptococcus salivarius, Streptococcus mutans and Streptococcus sanguinis.

## CONCLUSION

Briefly, cocoa bean cuticle waste extract (*Theobroma cacao L.*) at concentrations of 25%, 12.5%, 6.25%, 3.12%, 1.56% and 0.78% presented antibacterial power on growth *Streptococcus viridans*. Moreover, Cocoa bean cuticle waste extract (*Theobroma cacao L.*) at a concentration of 25% represented antibacterial power equivalent to 2.5% NaOCl against the growth of *Streptococcus viridans*. However, this study presented limitations, so further research about the toxicity, safety, and ability to remove the smear layer of cocoa bean epidermis waste extract as a root canal irrigation material. Moreover, it needed to study the antibacterial power of cocoa bean epidermis waste extract (*Theobroma cacao L.*) on other microflora in the oral cavity, especially in the root canal.

### *Declaration by Authors*

**Ethical Approval:** Approved

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**Conflict of Interest:** The authors declare no conflict of interest.

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