(Antibacterial Ability of Cacao Peel Waste Extract (Theobroma cacao L.) against Streptococcus sanguinis in Dental Root Canal)

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Abstract

Background: *Cocoa pod peel waste extract is a natural ingredient with active compounds. As much as 78% of the total production of cocoa pods is in the form of waste with processing directed at fertilizers and alternative energy sources. Streptococcus sanguinis in chronic and acute root canals has a percentage of 10% and has the ability to penetrate to a depth of 792µm in root canals. 2.5% NaOCl plays an important role in root canal treatment mainly as an irrigation material.* ***Purpose:*** *to determine the inhibitory concentration of cacao pod waste extract (Theobroma cacao L.) which is close to the inhibitory power of 2.5% NaOCl against Streptococcus sanguinis.* ***Methods:*** *The type of research is experimental laboratory in vitro with post test-only control group design using 4 sample groups, namely E6,25, E3,125, E1,56, and K. Inhibition test was measured in the clear area around the disc on the culture medium. Data were analyzed using non-parametric test (Kruskal-walis test) and different test (Mann-Whitney test).* ***Results:*** *the average diameter of the inhibition zone E1.56 was 0±0, E3.125 was 14.125±0.444, E6.25 was 17.225±0.909, and K was 25.680±0.329. Based on the Mann-Whitney test there were significant differences between different groups.* ***Conclusion:*** *cacao pod peel waste extract with a concentration of 3.125% had the lowest mean diameter of inhibition, while the concentration of 6.25% had an average diameter of inhibition close to 2.5% NaOCl.*

Keywords: *Cacao peel waste extract, NaOCl, Streptococcus sanguinis*

Abstrak

Latar Belakang: Ekstrak limbah kulit buah kakao merupakan bahan alami dengan kandungan senyawa aktif. Sebanyak 78% dari total produksi buah kakao berupa limbah dengan pengolahan yang diarahkan pada pupuk dan sumber energi alternatif. *Streptococcus sanguinis* pada saluran akar kronis maupun akut memiliki presentase sebesar 10% dan memiliki kemampuan berpenetrasi hingga kedalaman 792µm dalam saluran akar. NaOCl 2,5% berperan penting dalam perawatan saluran akar utamanya sebagai bahan irigasi. **Tujuan**: menganalisis konsentrasi daya hambat ekstrak limbah kulit buah kakao (*Theobroma cacao L*.) terhadap *Streptococcus sanguinis*. **Metode:** Jenis penelitian berupa eksperimental laboratoris secara *in vitro* dengan rancangan *post test-only control group design*

menggunakan 4 kelompok sampel yaitu E6,25, E3,125, E1,56, dan K. Uji daya hambat diukur pada daerah jernih di sekitar cakram pada media biakan. Data dianalisis menggunakan uji non-parametrik (Uji *Kruskal-walis*) dan uji beda (Uji *Mann-whitney*). **Hasil:** rata-rata diameter zona hambat E1,56 adalah 0±0, E3,125 adalah 14,125±0,444, E6,25 adalah 17,225±0,909**,** dan K adalah 25,680± 0,329. Berdasarkan uji *Mann-Whitney* terdapat perbedaan yang signifikan antar kelompok berbeda. **Kesimpulan:** ekstrak limbah kulit buah kakao konsentrasi 3,125% memiliki rerata diameter daya hambat paling rendah sedangkan konsentrasi 6,25% memiliki rerata diameter daya hambat mendekati NaOCl 2,5%.

Kata Kunci: Ekstrak limbah kulit buah kakao, NaOCl, *Streptococcus sanguinis*

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INTRODUCTION

Cocoa is the mainstay commodity of plantations, the third largest contributor to foreign exchange, the plantation sub-sector after rubber and palm oil.[1] As much as 22% of the cocoa pods in the form of seeds are used to be processed into chocolate products and the rest is in the form of skins and placenta of cocoa pods.[2] Increased production of cocoa pods has an impact on an increase in the amount of waste that is currently used only as fertilizer and alternative energy sources such as biochar briquettes. Cocoa pod peel waste is known to have benefits as analgesic, antifungal, anti-inflammatory, anti-wrinkle, antioxidant, and antibacterial. 25% has greater antibacterial power than the antibacterial power of 2.5% NaOCl concentration.[3,4]

Streptococcus sanguinis is a commensal bacteria that is widespread in the oral cavity such as on the surface of the teeth, the surface of the oral mucosa, and in human saliva with the ability as a pioneer bacterium for plaque formation, causing dental caries, periodontal disease, and failure of root canal treatment. Root canals are important to prevent more bacteria from entering and re-infection.[6] Irrigation is one of the factors that also plays a role in root canal treatment.[7]

NaOCl is a source of root irrigation that is currently often used. NaOCl has advantages in the form of broad-spectrum antibacterial power, is able to dissolve vital and non-vital pulp tissue, can break down proteins into amino acids, is able to dissolve organic compounds, spores, viruses, lubricants, and is easy to obtain at an economical price. It is capable of irritating the vital periradicular, less able to remove the smear layer, toxic, uncomfortable feeling, corrosive to instruments, destructive when irrigation reaches the periradicular tissue, causing extensive pain, bleeding, and swelling.[4] Based on this background , researchers are interested in conducting research related to the concentration of the inhibitory power of cocoa pod peel extract (Theobroma cacao L.) which is close to 2.5% NaOCL against Streptococcus sanguinis.

RESEARCH METHODS

This type of research is an experimental laboratory with a post test only control group design. The research was conducted at the Research Center of the Faculty of Dentistry, Airlangga University, Plant Laboratory, Department of Agricultural Production, State Polytechnic of Jember, and the Bioscience Laboratory of the Dental and Oral Hospital, University of Jember.

The waste skin of 1 kg cocoa pods was washed and cut transversely to a thickness of 1-2 mm.[4] The pieces of waste skin were dried on a plastic mat and covered with black cloth in the open air for 7 days, after drying, they were blended to a fine powder.[10,11] The powder was then macerated as much as 100 grams with 500 mL of 70% ethanol in an Erlenmeyer tube for 3 days with stirring every 6 hours and the solution was changed every 24 hours. The solutions on days 1,2, and 3 were combined and filtered using Whatman paper no. 41 and obtained maserate. The macerate was then concentrated using a rotary vacuum evaporator to obtain a pure extract of cocoa pod waste with a concentration of 100% dark brown and thick.[13]

The Streptococcus sanguinis suspension which will be treated has been tested for bacterial identification using Gram stain with the results of purple, cocci, chain-shaped, and uncontaminated. The suspension was grown on BHI-B media and diluted using standard Mc. Farland 0.5 (1.5 x 108 CFU/ml).[14]

This study used the disc diffusion method, namely placing discs on BHI-A that had been inoculated with Streptococcus sanguinis. Each petridish is labeled at the bottom. Furthermore, cocoa pod peel waste extract 6.25%, 3.125%, 1.56% and NaOCl 2.5% was dripped as much as 10µl on each disc and then placed on the surface of the BHI-A media using sterile tweezers. The petridish was closed and put upside down in an anaerobic incubator for 48 hours at 37°C. After incubation, the clear area or transparent zone around the disc was measured using a caliper, the data were tabulated and analyzed.

RESULTS

The results showed the average inhibition zone of cocoa pod waste extract against Streptococcus sanguinis as shown in Table 1.

Table 1. Average diameter of inhibition zone of cocoa pod peel waste extract against Streptococcus sanguinis (mm).

Pengulangan	**E1 (1,56%)**	**E2 (3,125%)**	**E3 (6,25%)**	**K+ (NaOCl 2,5%)**
n	6	6	6	6

Std. Deviasi	0	0,44	0,90	0,32
Mean	0	14,12	17,22	25,68

Data were analyzed using the Kruskal-Wallis non-parametric test showing significant differences between treatment groups. Furthermore, the Mann-Whitney test was carried out showing a significant difference between the treatment groups.

DISCUSSION

Cocoa pod peel waste extract is known to have active compounds in the form of tannins, alkaloids, terpenoids, saponins, and flavonoids. . The significant difference between cocoa pod peel waste extract and 2.5% NaOCl lies in the antibacterial activity of NaOCl. Hypochlorous acid which is the substance of the hypochlorite solution will combine with the amino groups of proteins to form chloramines. The reaction between chlorine and amino groups (NH) forms chloramines which can interfere with cell metabolism, inhibit bacterial enzymes, and cause oxidation of SH groups (sulphydryl groups) of bacterial enzymes.

The results of this study are different from the research conducted by Nugroho[4], which stated that the concentration of 6.25% had a greater inhibitory power than 2.5% NaOCl, while in this study the concentration of 6.25% had a lower inhibitory power than that of NaOCl. 2.5% NaOCl. The difference in the results in this study is assumed to be due to first, the difference in the maceration method. According to Pratyaksa[10] and Ananta[17], temperature, particle size, and maceration time affect the total phenolic extract of cocoa pod waste. Second, the difference in the location for taking cocoa pod waste. According to Pappa[18], differences in planting locations affect the active compound content of a plant. Other factors that influence the results of this study include genetic factors and the maturity level of cocoa plants. Genetic factors include things related to traits that are inherited from the parent plant such as the composition of the plant. Plants derived from different strains of the same variety are very likely to produce different amounts of active compounds.[19]

The results of this study are the same as those conducted by Nurrezeki, who stated that there was an inhibition zone at a concentration of 3.125% and no zone of inhibition at a concentration of 1.56%. Based on the results of the research that has been carried out, it can be concluded that the inhibitory power of cocoa pod waste extract (Theobroma cacao L.) has a lower average diameter of inhibition than the diameter of 2.5% NaOCl.

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