Daya Hambat Ekstrak Limbah Kulit Buah Kakao (Theobroma cacao L.) terhadap E. faecalis Saluran Akar

(Inhibition Ability of Cacao Pod Peel Waste's Extract (Theobroma Cacao L.) against Dental Root Canal's E. faecalis)

Dhea Kartika Suradi[1], Dwi Warna Aju Fatmawati[2], Raditya Nugroho[3]

[1]Fakultas Kedokteran Gigi Universitas Jember, Jember, Indonesia

[2]Bagian Konservasi, Fakultas Kedokteran Gigi, Universitas Jember, Indonesia

[3]Bagian Konservasi, Fakultas Kedokteran Gigi, Universitas Jember, Indonesia

Abstract

Background: *Cacao peels are the largest part of cacao fruit (67-76%) ad it is the largest waste in chocolate production whose processing is only made as animal feed, compost, paper, and soap. 2.5% NaOCl plays a role in root canal treatment mainly as irrigation material. E. faecalis is a gram-positive bacterium that causes primary (4-40%) and secondary (24-77%) root canal infection.* ***Purpose:*** *to analyze the inhibition ability of cacao pod peel waste's extract towards E. faecalis' growth.* ***Methods:*** *This type of research is an in vitro experimental laboratory with a post-test only control group design using four sample groups, namely E6.25, E3.125, E1.56 and K(+). The Inhibition test was carried out by measuring the diameter of the clear part around the disc with a digital caliper. The data were then analyzed using a non-parametric test (Kruskal-walis test) and a differential test (Mann-Whitney test).* ***Results:*** *the average diameter of the inhibition zone K(+) was 27.10 mm, E6.25 was 17.72 mm, E3.125 was 14.36 mm, while E1.56 was 0.* ***Conclusion:*** *sample groups E6.25 and E3.125 had inhibition against E. faecalis, while E1.56 had no inhibitory activity.*

Keywords: *E. faecalis, cacao pod peel waste's extract, NaOCl*

Abstract

Latar Belakang: Kulit buah kakao merupakan bagian terbesar dari buah kakao (67-76%) dan limbah terbesar dalam produksi coklat yang pengolahannya hanya dibuat sebagai pakan ternak, pupuk kompos, kertas, dan sabun. Ekstrak limbah kulit buah kakao memiliki senyawa-senyawa antibakteri seperti alkanoid, flavonoid, tanin, terpenoid, dan saponin. NaOCl 2,5% ikut berperan dalam perawatan saluran akar utamanya sebagai bahan irigasi. *Enterococcus faecalis* merupakan bakteri gram positif penyebab infeksi saluran akar primer (4-40%) dan sekunder (24-77%). **Tujuan:** menganalisis daya hambat ekstrak limbah kulit buah kakao dalam menghambat *Enterococcus faecalis*. **Metode:** Jenis penelitian ini adalah eksperimental laboratoris secara in vitro dengan *post-test only control group design* menggunakan empat

kelompok sampel yaitu E6,25, E3,125, E1,56 dan K(+). Uji daya hambat dilakukan dengan cara mengukur diameter bagian yang jernih di sekitar cakram dengan jangka sorong digital. Data kemudian dianalisis menggunakan uji non-parametrik (Uji *Kruskal-walis*) dan Uji beda (Uji *Mann-whitney*) **Hasil:** rerata diameter zona hambat K(+) adalah 27,10 mm, E6,25 adalah 17,72 mm, E3,125 adalah 14,36 mm, sedangkan E1,56 adalah 0. **Kesimpulan:** kelompok sampel E6.25 dan E3.125 memiliki daya hambat terhadap *Enterococcus faecalis* sedangkan pada E1.56 tidak ditemukan daya hambat, dan kelompok sampel yang mendekati rerata diameter daya hambat NaOCl 2,5% yaitu kelompok sampel E6.25.

Kata Kunci: *Enterococcus faecalis*, ekstrak limbah kulit buah kakao, NaOCl

***Correspondence*:** Dhea Kartika Suradi, Kedokteran Gigi Universitas Jember, Jl. Kalimantan, Rumah Sakit Gigi dan Mulut Universitas Jember, Jember, Jawa Timur 68121. Email: dheakartika000@gmail.com

INTRODUCTION

Root canal treatment is one of the endodontic treatments to maintain teeth that have experienced pulpal or periapical infection.[9,10] *E. faecalis* is one of the dominant facultative anaerobic gram-positive bacteria in pulp or periapical infections.[4,5] Thisbacteria has the ability to survive in conditions with low nutrient availability, bind to dentin, invade the dentinal tubules, alter host response, inhibit lymphocyte action, compete with other bacteria, form biofilms, and are resistant to calcium hydroxide administration.[1] *E. faecalis* is the cause of primary root canal infection (4-40%) and secondary root canal infection (24-77%).[18] *E. faecalis* remaining in the root canal will significantly reduce the success rate after root canal treatment.

2,5% NaOCl is a strong base with a pH of more than 11.[10] 2,5% NaOCl is one of the frequently used irrigation solutions, which not only has an antibacterial effect but also has the ability to dissolve organic solutions in root canals.[5] However, 2,5% NaOCl has an unpleasant taste, is unable to dissolve inorganic components of the smear layer, cytotoxicity and caustic effects on healthy periradicular tissues on inadvertent extrusion during the irrigating procedure.[4]

Cacao peels are the largest part of cacao pods (67-76%) which will be the largest waste in chocolate production.[2,14] Based on phytochemical tests, cacao peels contain several antibacterial compounds, namely alkaloids, flavonoids, tannins, terpenoids, and saponins which potential as antibacterial.[9] Based on research by Nugroho, et al (2019), cacao pod peel waste's extract concentration of 6.25% (mean: 19.20 mm) had greater antibacterial power than 2.5% NaOCl (mean: 17.28 mm) against *Streptococcus sanguinis*. In Yuanita et al. (2019)'s study using a confocal laser scanning microscope (CLSM), cacao pod peel waste's extract (*Theobroma cacao L.*) with a concentration of 6.25% had the power to reduce the biofilm thickness of *E. faecalis*. This is shown through the extracellular polymer matrix of the *E. faecalis* biofilm viewed through 3D sectional images. In *E. faecalis* given a concentration of 6.25% cacao pod peel waste's extract, the 3D cut image is faded, showing a reduced level of *E. faecalis* biofilm extracellular polymer matrix. The purpose of this study was to analyze the inhibitory effect of cacao pod peel waste's extract (*Theobroma cacao L.*) against *E. faecalis* tooth root canal.

RESEARCH METHODS

This type of research is an experimental laboratory in vitro with a post-test only control group design. Using 4 treatment groups consisting of 6.25% (E6.25) cacao pod peel waste's extract, 3.125% (E3.125) cacao pod peel waste's extract, 1.56% (E1.56) cacao pod peel waste's extract, and 2.5% NaOCl (K(+)), repeated 6 times.

Making extract of cacao pod peel waste's extract is by weighing 1 kg of cacao pod's peels that has been cut to a thickness of 1-2 mm, placed on a plastic mat then covered with black cloth, and dried in the sun for seven days. After drying, mashed with a blender until it becomes a fine powder, filtered using a 20 mesh sieve. Maceration process, by weighing 100 grams of cacao peels powder soaked in 500mL of 70% ethanol. Immersion was carried out by remaceration for 3 days in an Erlenmeyer tube covered with black plastic so that it was protected from light, at room temperature (20-25℃), and stirred 2 times a day with an interval of 6 hours. The soaking results were filtered using Whatmann filter paper number 41 produces maserate and pulp. Then the macerate was evaporated using a Rotary Vacuum Evaporator at a temperature of 40℃ to obtain a thick brown extract and then diluted to 6.25%, 3.125%, and 1.56%.

Inoculate *E. faecalis* on BHI-A in laminar flow. Dropping 10µl of disc paper of cacao pod peel waste's extract with concentrations of 6.25% (E6.25), 3.125% (E3.125), 1.56% (E1.56), and 2.5% NaOCl (K(+)) using a micropipette. Place the paper disc on the surface of the BHI-A using sterile tweezers. The petridish was closed then put into a desiccator in an inverted position and incubated in an incubator for 48 hours at 37℃.

Measuring the diameter of the inhibition zone in the form of a clear area around the paper disc using a digital caliper. The results of the measurements were averaged and then analyzed with the statistical test of Kruskall Wallis and Mann Whitney.

RESULT

The results of the research on the inhibitory power of cacao pod peel waste's extract (*Theobroma cacao L.*) and 2.5% NaOCl against *E. faecalis* are presented in Figure 1.

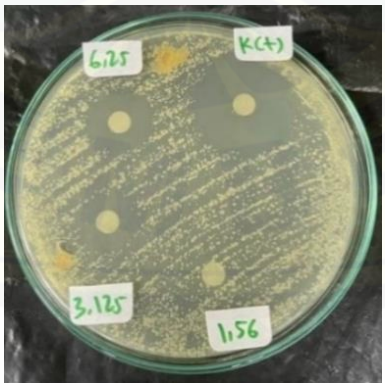

Figure 1. The results of the inhibition zone of cacao pod peel waste's extract 6.25%, 3.125%, 1.56%, and 2.5% Naocl (K(+)) against *E. faecalis.*

The average diameter of the growth inhibition zone of *E. Faecalis* obtained is as shown in Figure 2 and Table 1.

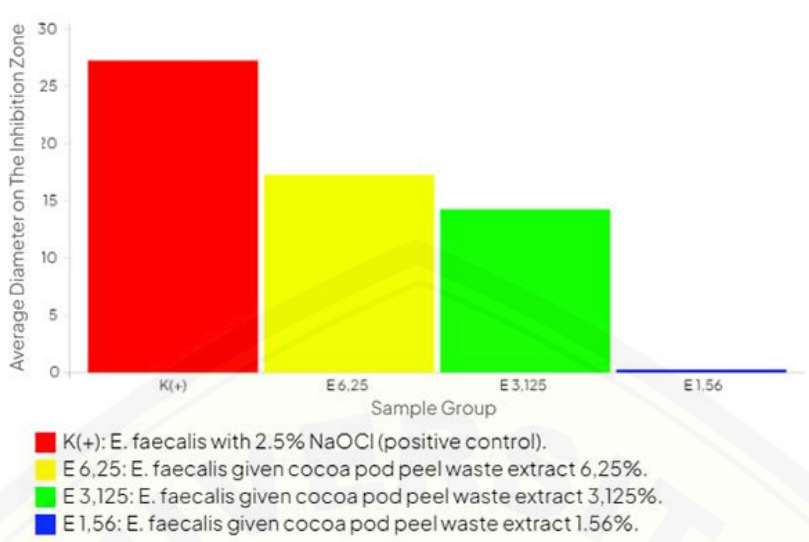

Figure 2. Bar chart of the average diameter on the inhibition zone of *E. faecalis*

Table 1. The average results of the calculation of the diameter of the inhibition zone of *E. faecalis*

	K(+)	E 6,25	E 3,125	E 1,56
Average	27,10 mm	17,72 mm	14,36 mm	0

DISCUSSION

The results of this study showed that there was an inhibition against *E. faecalis* in the sample group of 2.5% NaOCl and cacao pod peel waste's extract with concentrations of 6.25% and 3.125%, respectively. 2.5% NaOCl showed the largest average diameter of the inhibition zone against *E. faecalis* was 27.10 mm. The inhibitory power of the 2.5% NaOCl group was due to its ability to make saponification reactions that would produce soap and glycerol. Soap reduces the surface tension, making it easier to remove debris from the root canal wall. If the hypochlorous acid (HOCl−) and hypochlorite ion (OCl−) formed from the reaction come into contact with organic tissue, it will release chlorine which can inhibit bacterial enzymes, damage DNA synthesis, and hydrolyze amino acids, thereby damaging bacterial metabolism.

The inhibition zone formed around the disc paper which was given a concentration of 6.25% and 3.125% cacao pod peel waste's extract was thought to be due to the presence of active compounds that act as antibacterials including alkanoids, flavonoids, tannins, terpenoids, and saponins.[9] Alkaloids can inhibit DNA synthesis and interfere with peptidoglycan in bacterial cells which causes the cell wall layer to not form intact, causing cell death, disrupting cell metabolism by inhibiting ATP formation, and inhibiting the efflux pump. [8, 13, 17] Flavonoids can inhibit DNA-RNA synthesis, inhibition of bacteria toxins, inhibition of quorum sensing, inhibition of cell envelope formation, inhibition of efflux pump, inhibition of peptidoglycan synthesis, inhibition of cell respiration so that ATP is not formed.[3] Saponins can reduce surface tension causing increased permeability of cell membranes so that bacterial cells lyse and die.[11] Tannins can inhibit enzymes activity, interfere with protein transport so that cell metabolism

stops, and targets polypeptides on the cell wall so that the formation of the cell wall becomes less than perfect causing bacterial cells to lyse and die.[8,11] Terpenoids will interact with biomembranes and proteins on the cell membrane which will increase the instability and membrane permeability thus causing leakage on bacterisa cells characterized by the presence of cell apoptosis.[16] The absence of inhibition zone in *E. faecalis* given cacao pod peel waste's extract concentration of 1.56% was assumed because it did not have an active compound that was described above.

The results of this study are different from the results of research by Nugroho et al (2019) which showed that the concentration of cacao pod peel waste's extract at 6.25% (mean: 19.20 mm) had a greater inhibition zone than 2.5% NaOCl (mean: 17.28). mm). In this study, cacao pod peel waste's extract with a concentration of 6.25% had an average inhibition zone diameter below 2.5% NaOCl. This is assumed to be due to the different extraction methods. In the study of Nugroho et al (2019), the immersion was carried out at room temperature in a shaker at a speed of 120 rpm continuously for 24 hours while in this study it was carried out only in an Erlenmeyer tube and stirred 2 times a day with an interval of 6 hours.

Based on the results of the research that has been carried out, it can be concluded that the cacao pod peel waste's extract at concentrations of 6.25% and 3.125% had inhibitory power against *E. faecalis* while at a concentration of 1.56% no inhibition was found.

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