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Minimum inhibitory [concentration of cocoa pod husk extract on Enterococcus faecalis](#) extracellular polymeric substance [biofilm thickness](#) ABSTRACT Background: Root canal treatment is a treatment sequence for the infected pulp to eliminate these etiological factors of pulp necrosis and periapical lesion. Enterococcus faecalis (E. faecalis) is an organism that [commonly found in high percentage of root canal failure because its ability to form biofilm](#). Degradation of extracellular polymeric substance (EPS) by oxidizing agent such as sodium hypochlorite is the first step to remove biofilm. However sodium hypochlorite [toxicity is the main concern, so the safest alternative irrigants needed](#). The development of herbal uses, especially in the fields of medicine and dentistry, fruit and plants are widely used. Food crops are known to be rich in bioactive compounds, especially polyphenols, which have properties as antioxidants and antimicrobials. Cocoa pod husk extract can be an alternative choice. Purpose: The study aimed to determine the minimum inhibitory [concentration of cocoa pod husk extract on E. faecalis EPS biofilm thickness](#). Methods: [Four groups sample of E. faecalis cultured biofilm; group one is E. faecalis without cocoa pods husk as positive control, group two for E. faecalis with 1.56 % cocoa pod husk extract, group 3 for E. faecalis with 3.125% cocoa pod husk extract and group 4 for E. faecalis with 6.25% cocoa pod husk extract. All groups will be measured biofilm thickness with confocal laser scanning microscopy and then statistical analysis is taken](#) by post hoc test and Tukey HSD. Results: [The average value of EPS biofilm thickness, group 1: 9500 nm, group 2: 8125 nm, group 3: 8000 nm, and group 4: 6375 nm. Post Hoc Tukey HSD test showed a significant difference between group 1 and group 4](#). While in [group 1 and group 2 compared to the group 1](#), there were no significant differences with [the values of each p = 0.340 and p = 0.267 \(p>0.05\)](#). Conclusion: 6,25% cocoa pod husk extract reduce E. faecalis EPS biofilm thickness. Keywords: cocoa pod husk extract; endodontic; Enterococcus faecalis; extracellular polymeric substance biofilm INTRODUCTION Pathology of pulp tissue and periapical tissue directly or indirectly related to microorganisms. The microbes can be removed and minimized by root canal treatment. [The success of root canal treatment is influenced by](#) several factors that are interrelated with one another.¹ These factors include proper diagnosis, aseptic action, knowledge of dental anatomy, chemical-mechanical

preparation, three-dimensional obturation and use of root canal dressing. All of these factors are based on one point, namely root canal decontamination.¹ The effectiveness of root canal preparation can be increased by the use of irrigation solutions such as [sodium hypochlorite \(NaOCl\)](#), [chlorhexidine](#), [ethylenediaminetetraacetic acid \(EDTA\)](#). NaOCl is the golden standard for root canal irrigation solutions, because until now, no other solution has been similar effectiveness with NaOCl, however the disadvantage of NaOCl is its cytotoxic activity which can cause acute injury if it reaches the periapical region. If NaOCl is in contact with tissue, it rapidly oxidizes the surrounding living tissue and triggers rapid hemolysis, inhibits the neutrophil migration, damages endothelial and fibroblast cells.² The higher concentration of NaOCl the higher anti-bacterial effect and tissue dissolution, but the higher toxicity.³ In addition, in vitro cell culture, very low concentration of NaOCl (>0.01%) caused death of human fibroblast cell.⁴ [Root canal treatment](#) can [fail due to the](#) absence [of](#) a good coronal seal, microleakage, failure in chemical-mechanical preparation, and poor quality root canal filling so that there are microorganisms that still survive or reinfection also occurs. Some microorganisms are associated with [failure of root canal treatment](#), one of [the](#) most common microorganisms is *Enterococcus faecalis* (*E. faecalis*).⁵ This is due to the ability to survive *E. faecalis* in environmental conditions that are low in nutrition and its ability to form biofilms so that 1000 times more resistant to phagocytic cells, antibodies and antimicrobials compared to organisms that are unable to make biofilm.^{1,6} Biofilms are defined as [multicellular microbial](#) communities [characterized by cells that](#) attach strongly [to](#) the [surface and](#) produce matrix extracellular polymeric substance (EPS).⁷ EPS [consists of bacterial proteins,](#) [nucleic acids,](#) polysaccharides [and](#) fats. Microbes that form biofilms are thought to be the cause of 80% of infections.⁸ The development of herbal uses, especially in the fields of medicine and dentistry, fruit and plants are widely used. Food crops are known to be rich in bioactive compounds, especially polyphenols, which have properties as antioxidants and antimicrobials. One of the food plants that are rich in antioxidants and has an antimicrobial effect is cocoa.⁹⁻¹² The antioxidant and antimicrobial properties of cocoa can be found in the [cocoa pod husk](#). The [cocoa pod husk](#) contains unsaturated fatty acids and epitakin polymers which have antibacterial and antiglucocyl transferase activity, whereas the coco pods consist mainly [of polysaccharides \(cellulose and hemicellulose\),](#) lignin [and a small portion of phenolic compounds,](#) tannin, [purine alkaloids and cocoa butter](#).¹⁰ The minimum concentration that can inhibit *E. faecalis* biofilm formation is equal to 3.125%.¹³ To the best of our knowledge, there have been no [studies to date](#) evaluating [the](#) effect [of cocoa](#) pod husk [on](#) the thickness of *E. faecalis* EPS biofilm. Thus, [the purpose of this study](#) was [to](#) determine [the](#) minimum inhibitory concentration [of](#) cocoa pod husk extract on *E. faecalis* EPS biofilm thickness.

MATERIALS AND METHODS

The ingredients used in this study were Forastero type cocoa fruit (*Theobroma cacao* L.) extract with concentrations of 1.56%, 3.125% and 6.25%. The cocoa pods used for Forastero type is obtained from the Coffee Research Center and Cocoa Jember, the cocoa pods taken is the one that has been cooked with the yellowing mark when picked. [Before processing, the](#) pods [that has been picked is](#) left [for](#) about 5 [days](#) to facilitate [the](#) release of [seeds](#) from the cocoa husk. The cocoa pods husk in fresh form is separated by seeds or the entire contents of the pods.¹³ The extraction process of cocoa pods husk is done by maceration. The cocoa pods husk used in this study is 6 kg fresh then cut and aerated. When half-dried pods husk is then put into an oven with a temperature of 50° C. After drying, 1 kg of cocoa pods husk is obtained. Cocoa pods (1 kg) was milled then macerated with 70% ethanol for 24 hours then filtered. After filtering cocoa pods, filtrate and dregs were obtained. The dregs is then soaked again after it is filtered again. The process of maceration and filtration occurs repeatedly until a clear filtrate is obtained. After obtaining a clear filtrate, ethanol evaporation was carried out by [using a rotary evaporator](#) with [a temperature of 50° C to obtain](#) cocoa pods husk [extract](#) with a thick texture. During the solvation process, 5 liters of ethanol are needed. Cocoa pods husk after extraction has a weight of 134 grams. This study used 32 samples divided into 4 treatment groups, namely group 1 is *E. faecalis* without giving cocoa pods husk extract (control group), group 2 is *E. faecalis* bacterial culture with 1.56% cocoa pods husk extract, group 3 is *E. faecalis* bacterial culture with 3.125% cocoa pods husk extract and group 4 is *E. faecalis* bacterial culture with 6.25% cocoa pods husk extract. Stock of *E. faecalis* bacteria according to standard Mc. Farland 0.5 or 1.5 x 10⁸ CFU/ml was diluted with dilution

method to reach a density of 106 CFU/ml, then culture on TSB media in a microtiter plate flat bottom 24 well then incubated for 3 x 24 hours at 35° C.13,14 Cocoa pods husk extract was applied to each titer with concentrations of 1.56%, 3.125%, and 6.25% after the biofilm formation process. Then incubated again at 35° C for 24 hours. Then the contents of each microtiter plate were aspirated and washed 4 times with [0.2 ml of phosphate- buffered saline \(pH 7. 3\)](#) using [a](#) pipette to remove planktonic bacteria and then dried. Biofilms attached to the microtiter plate were stained with 1 ml Alexa Dextran (Thermo Fisher Scientific, Singapore) under dark conditions for 30 minutes and finally rinsed with aquadest to remove dyestuffs. After the staining procedure, the appropriate specimens were immediately examined with a confocal laser scanning microscope (CLSM) under 400X magnification (Olympus, Tokyo, Japan). The preliminary research has been carried out to get the minimum concentration using the calculation of bacterial density in biofilms as measured by the bacterial optical density (OD) unit using ELISA reader. The [difference between the treatment group and the control group](#) were determined with [Post Hoc Test \(p = 0.05\). Tukey HSD](#) was used to test the significance of the differences between treatment groups. RESULTS The figure 1 shows the intensity values on EPS biofilms which are reviewed through 3D slices. The brighter color of the graph shows the remaining EPS biofilms with dyes that are still attached to this EPS and the higher intensity value. From the picture shows that there is a difference of intensity value in the EPS biofilm of [the control group \(Group 1\) and the treatment group \(Group 2, Group 3 and Group 4\)](#). The 6.25% cocoa pod husk has the lowest intensity and the control group has the highest intensity. [Post Hoc Tukey HSD test \(Table 2\) showed a significant difference between Group 1 and Group 4](#). While in [Group 1 and Group 2](#) compared to the [Group 1](#), there were no significant differences with [the values of each p=0. 340 and p=0. 267 \(p>0.05\)](#). DISCUSSION This study aims to obtain the concentration of inhibitory formation E. faecalis EPS biofilm due to exposure to the extract of cocoa (Theobroma cacao) which is expected to be used as an alternative material for root canal irrigation. This study used cocoa pod husk extract with a concentration of 1.56%, 3.125% and 6.25%. [Post Hoc Tukey HSD test showed a significant difference between the control group and the concentration of 6.25%](#). While cocoa pods husk extract with a concentration of 1.56% and a concentration of 3.125%, [there was no significant difference of the formation of E. faecalis EPS biofilm](#). It can be shown that the amount of concentration in cocoa pod husk extract affects the [inhibition of the E. faecalis EPS biofilm formation](#), which obtained [the minimum inhibitory concentration is 6.25%](#). This is due to the fact that there is an alkaloid, flavonoid, tannin and saponin content in the husk of cocoa pods husk that has antibacterial properties. 14 The mechanism of tannin inhibition against the formation of EPS biofilms is by binding and precipitating proteins on EPS. In addition, tannins are also able to bind to carbohydrates, where the greater the molecular weight the stronger the interaction with tannin. Tannin is also a chelating agent because it is able to form bonds with iron ions which will result in breaking of the EPS matrix bond.25 Mechanism of saponin as antibiofilm by decreasing bacterial extracellular DNA component, so that it will result in decreased biofilm formation. Bioactive fractions that are rich in saponins can also inhibit the formation of biofilms by preventing the initial cell-surface attachment of bacteria.26 From the description above, it can be concluded that the presence of [compounds found in the extract of cocoa pod husk](#) can inhibit [the formation of E. faecalis EPS biofilms](#). The extract of 6.25% cocoa pod husk is a concentration that can reduce the thickness of E. faecalis EPS biofilm. REFERENCES 1. Hargreaves KM, Berman LH. Cohen's Pathways of The Pulp. 11th Edit. Elsevier Health Science. USA. 2016; p. 621-5 2. Guivarc'h M, Ordioni U, Ahmed HM, Cohen S, Catherine JH, Bukiet F. Sodium hypochlorite accident: a systematic review. Journal of endodontics. 2017 Jan 1;43(1):16-24. 3. Spencer HR, Ike V, Brennan PA. the use of sodium hypochlorite in endodontics—potential complications and their management. British dental journal. 2007 May;202(9):555. 4. Zhu WC, Gyamfi J, Niu LN, Schoeffel GJ, Liu SY, Santarcangelo F, Khan S, Tay KC, Pashley DH, Tay FR. Anatomy of sodium hypochlorite accidents involving facial ecchymosis—a review. Journal of dentistry. 2013 Nov 1;41(11):935-48. 5. Murad CF, Sassone LM, Faveri M, Hirata Jr R, Figueiredo L, Feres M. Microbial diversity in persistent root canal infections investigated by checkerboard DNA-DNA hybridization. Journal of endodontics. 2014 Jul 1;40(7):899-906. 6. Jhajharia K, Parolia A, Shetty KV, Mehta LK. Biofilm in endodontics: a review. Journal of International Society of Preventive & Community

Dentistry. 2015 Jan;5(1):1. 7. Mohammadi Z, Palazzi F, Giardino L, Shalavi S. Microbial biofilms in endodontic infections: an update review. *Biomedical journal*. 2013;36(2):59-70. 8. Kreth J, Herzberg MC. Molecular principles of adhesion and biofilm formation. In *The Root Canal Biofilm*. Springer, Berlin, Heidelberg. 2015 (pp. 23-53). 9. Matsumoto, M., Tsuji, M., Okuda, J., Sasaki, H., Nakano, K., Osawa, K., Simura, S. & Ooshima, T. Inhibitory effects of cocoa bean husk extract on plaque formation in vitro and in vivo. *European journal of oral sciences*. 112(3) 2016: 249-252. 10. Chung, BY., Cho, JY., Lee, SS., Nishiyama, Y., & Matsumoto, Y. The relationship between lignin and morphological characteristics of the tracheary elements from cocoa (*Theobroma cacao* L.) hulls. *Journal of Plant Biology*.51(2) 2016: 139-144. 11. Mulyatni Sri A., Budiani A. & Taniwiryono D. Aktivitas antibakteri ekstrak kulit buah kakao(*Theobroma cacao* L.) terhadap *Escherichia coli*, *Bacillus subtilis*, dan *Staphylococcus aureus*. *Menara Perkebunan*.80(2) 2012: 77-84 12. Sartini, M., & Alam, G. Ekstraksi Komponen Bioaktif Dari Limbah Kulit Buah Kakao Dan Pengaruhnya Terhadap Aktivitas Antioksidan Dan Antimikroba. *Journal of Traditional Medicine* 14 2017: 47-54. 13. Yuanita T, Putri DV, Rukmo M, Zubaidah N, Agustin D. Antibiofilm Power of Cocoa Bean Pod Husk Extract (*Theobroma cacao*) Against *Enterococcus Faecalis* Bacteria (In Vitro). *International Medical Device and Technology Conference 2017*:129-131 14. Rachmawaty R, Mu'nisa AMNA, Hasri H. Analisis Fitokimia Ekstrak Kulit Buah Kakao (*Theobroma cacao* L.) Sebagai Kandidat Antimikroba. In *Seminar Nasional Lembaga Penelitian UNM*. 2(1) 2017: 667-670 15. Garg N, Garg A. *Textbook of endodontics*. *Boydell & Brewer Ltd*. 2014: p. 56-57 16. Matthew, S., & Boopathy, T. *Enterococcus faecalis – An Endodontic Challenge*. *The journal of Indian Academy of Dental Specialist* Vol. 1(4) 2016: p. 33-7 17. Siqueira Jr JF, Rôças IN, Ricucci D, Hülsmann M. Causes and management of post-treatment apical periodontitis. *British dental journal*. 2014 Mar;216(6):305. 18. Lim SY, Teh CS, Thong KL. Biofilm-related diseases and omics: global transcriptional profiling of *Enterococcus faecium* reveals different gene expression patterns in the biofilm and planktonic cells. *Omics: a journal of integrative biology*. 2017 Oct 1;21(10):592-602. 19. Van Tyne D, Martin MJ, Gilmore MS. Structure, function, and biology of the *Enterococcus faecalis* cytolysin. *Toxins*. 2013 May;5(5):895-911. 20. Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. *Critical Reviews in Oral Biology & Medicine*. 2004 Sep;15(5):308-20. 21. Flemming, H. C. EPS—then and now. *Microorganisms*. 2016: 41-59

a b c d Figure 1. Fluorescence color intensity chart and EPS thickness. (a) Group 1 (b) Group 2 (c) Group 3 (d) Group 4. Table 1. Mean and standard deviation of *E. faecalis* EPS biofilm thickness

Group	N	Mean(nm)	SD (nm)
Group 1	8	9500.00	1195.23
Group 2	8	8125.00	1727.89
Group 3	8	8000.00	2000.00
Group 4	8	6375.00	1408.00

Note: N = number of samples; Mean = average; SD = standard deviation

Table 2. Difference test between treatment groups (Tukey HSD test)

Group 1	Group 2	Group 3	Group 4
0.340	0.267	0.03*	0.999
0.156	0.206	0.206	0.206

Note: * there is a significant difference (p<0.05)

1 2
3 4 5 6 7 8