

Antibacterial Efficacy of 5% Ethanolic Extract of Propolis (EEP) Solution against *Enterococcus faecalis* (Laboratory Experiment)

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Abstract

2% chlorhexidine is proven to be effective against *Enterococcus faecalis* (*E. faecalis*), however chlorhexidine is known to be toxic against several particular cells. Propolis is a non-toxic natural resinous substance. Furthermore, 5% Ethanolic Extract of Propolis (EEP) solution is proven to be effective against *E. faecalis*. The aim of this study is to investigate the comparison of the antibacterial efficacy of 5% EEP solution and 2% chlorhexidine solution against *E. faecalis*.

24 tubes were divided into 4 groups. On group 1 and 2, the tubes were filled with 1 ml of BHI suspension inoculated by *E. faecalis* and 1 ml of the tested solutions. On group 3 and 4, the tubes were filled with 2 ml of BHI suspension, with and without *E. faecalis*, as control groups. All tubes were then incubated at 37°C for 24 hours. The value of optical density (OD) was measured using Elisa reader. The data were analyzed statistically using non-parametric Kruskal-Wallis test and Mann-Whitney test.

5% EEP solution has the same antibacterial efficacy as 2% chlorhexidine against *E. Faecalis*.

Antibacterial efficacy of 5% EEP solution is statistically equal to that of 2% chlorhexidine solution in eliminating *E. faecalis*

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Introduction

The purpose of root canal treatment is to remove all the pulp tissue, bacteria and endotoxin from the root canal system. The main cause of endodontic treatment failure is due to lagging facultative anaerobic bacteria that survive in the root canal. Facultative anaerobic gram-positive bacteria such as *Enterococcus faecalis* (*E. faecalis*) are the most predominant bacteria found in root canal treatment failure. Chlorhexidin gluconate is a broad spectrum anti bacterial but cytotoxic against fibroblast, leukocyte, PMN, macrophage, and erythrocyte.¹ *E. faecalis* bacteria can survive after chemomechanical preparation and administration of medicaments in root canal.^{2,3,4} *E. faecalis* bacteria is resistant to antibacterial medicament, able to survive without

nutrition in a long period of time, able to adapt to environmental changes at high pH, and are protected by biofilm.⁵ The increase of potential side effects and safety concerns of conventional medicaments have led to recent popularity of herbal alternative medicaments.

The herbal products are known for its high antimicrobial activity, biocompatibility, anti inflammatory, and anti oxidant properties. Propolis as a herbal product which is a natural resin material produced by honeybees. Propolis has antioxidant properties, antibacterial, antifungal, antiviral, anti-inflammatory, anti-tumor, and has the ability to modulate the immune process. Propolis in dentistry has been used in the field of cariology, oral surgery, periodontics, and endodontics. In the field of endodontic, propolis can be used as root canal medicaments and irrigation solutions.^{6,7} Propolis is a natural ingredient that is safe and non-toxic.⁸ Propolis is generally extracted to facilitate subsequent processing into antibacterial medicament.

Alcohol (ethanol) is the most common type of solvent.⁹ Previous study evaluated the effects of ethanol extract of propolis against 94 types of anaerobic bacteria in vitro. The results showed

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that the antibacterial efficacy of ethanol extract of propolis is more effective against anaerobic gram-positive bacteria compared to anaerobic gram-negative bacteria.^{10,11} In this study we will compare the capabilities of antibacterial solution of Ethanol Extract Propolis (EEP) 5% and 2% chlorhexidine solution against gram-positive facultative anaerobic bacteria *E. faecalis*.

Materials and methods

Twenty four tubes were divided into 4 groups. On group 1 and 2, the tubes were filled with 1 ml of BHI suspension inoculated by *E. faecalis* and 1ml of the tested solutions. On group 3 and 4, the tubes were filled with 2 ml of BHI suspension, with and without *E. faecalis*, as control groups. All tubes were then incubated at 37°C for 24 hours. After 24 hours the reaction tubes were removed from the incubator and turbidity seen in each tube to measure bacterial growth. Every 200 mL test tube was taken, put in a microplate, and then inserted into the ELISA reader and calculated optical density (OD) with a wavelength of 490 nm.

The data obtained in numerical form will then be analyzed statistically with SPSS using non parametric statistical tests Kruskal-Wallis and Mann-Whitney with significance level ($p = 0.005$).

Results

Table 1 shows that the average value of OD group BHI (negative control) is the lowest at 0.00000, while the highest value seen in the group BHI cultured with bacteria *E. faecalis* (positive control) which is 0.21000. OD mean value of 2% chlorhexidine group (0.02400) and group EEP 5% (0.03194) lower than the average value OD positive control group (0.21000). However, 5% EEP group OD (0.03194) has an average value higher than mean value at 2% chlorhexidine group OD (0.02400). The results shows that the group EEP 5% more turbid than the 2% chlorhexidine group. This indicates that, based on the average value OD, antibacterial capability EEP solution of 5% in inhibiting the growth of bacteria *E. faecalis* is lower than 2% chlorhexidine solution.

Furthermore, the data were statistically tested. In the test of homogeneity of variance, the data above has a normal distribution but does not

meet the assumption of homogeneity of variance, since the value of $p=0.004$ (<0.005). Then Kruskal-Wallis test to determine whether there were significant differences between the treatment group OD values, gained significance $p=0.000$. It can be concluded that there is a significant difference in the growth of *E. faecalis* bacteria between 4 groups, because the p -value less than 0.01. Mann-Whitney analysis used to find out which group has the difference.

Table 2 shows significant differences between OD value growth of the *E. faecalis* negative control group compared to all groups. You can also see a significant difference between the value of the growth of the bacteria *E. faecalis* OD positive control group compared to an antibacterial solution both groups ($p = 0.002$). This indicates that 2% chlorhexidine solution and a solution of 5% EEP has the ability antibacterial against *E. faecalis*. In Table 2 also shows that the OD value of *E. faecalis* growth between 2% chlorhexidine group and 5% EEP groups did not show statistically significant difference, with $p = 0.699$. This suggests that, based on the value of significance, antibacterial ability EEP 5% solution in inhibiting the growth of bacteria *E. faecalis* is similar with 2% chlorhexidine solution.

Groups	n	Minimum	Maximum	Mean	SD
BHI (negative control)	6	0,00000	0,00000	0,00000	0,00000
BHI + <i>E.f</i> (Positive control)	6	0,20167	0,22133	0,21000	0,00670
BHI + <i>E.f</i> + CHX 2%	6	0,01400	0,04033	0,02400	0,00973
BHI + <i>E.f</i> + EEP 5%	6	0,00233	0,06267	0,03194	0,02216
Total	24	0,00000	0,22133	0,06649	0,08629

Table 1. The mean growth of *E. faecalis* bacteria in BHI media after mixed antibacterial solution represented by the value OD.

(*E.f*: *E. Faecalis*, *CHX*: Clorheksidine, *EEP*: Etanol Extracts of Propolis)

Groups	BHI + <i>E.f</i> (positive control)	BHI + <i>E.f</i> + CHX 2%	BHI + <i>E.f</i> + EEP 5%
BHI (negative control)	0,002*	0,002*	0,002*
BHI + <i>E.f</i> (positive control)		0,002*	0,002*
BHI + <i>E.f</i> + CHX 2%			0,699

Table 2. The predictive value of the growth of the bacteria *E. faecalis* in the study group.

(*Ket.** limit of significance $p= 0.012$ using the Mann-Whitney test analysis. *E.f*: *E. Faecalis*, *CHX*: Clorheksidine, *EEP*: Ethanol extracts/ of propolis)

Discussion

The results showed that a solution of 5% EEP has antibacterial capabilities comparable with 2% chlorhexidine solution against *E. faecalis* ($p = 0.699$). Table 1 and Table 2. Table 1 shows that the average OD value of 2% chlorhexidine group (0.02400) and group EEP 5% (0.03194) lower than the average OD value of the positive control group (0.21000). Table 2 also shows that there are significant differences between OD value growth of the *E. faecalis* positive control group with an antibacterial solution in both groups ($p = 0.002$). This indicates that 2% chlorhexidine solution and a solution of 5% EEP has the antibacterial ability against *E. faecalis*.

In Table 1 shows also that 5% EEP group has a higher value of OD (0.03194) compared with the average OD value of 2% chlorhexidine group (0.02400). The results showed that 5% EEP group had higher levels of turbidity compared with 2% chlorhexidine group. This indicates that, when seen by the average OD value, the antibacterial ability of EEP 5% solution in inhibiting the growth of *E. faecalis* is lower than the 2% chlorhexidine solution. But in Table 2 shows that the OD value of *E. faecalis* growth between 2% chlorhexidine group and 5% EEP group was not statistically significantly different, with $p = 0.699$. It shows that, when viewed by the value of significance, then the antibacterial ability EEP 5% solution in inhibiting the growth of *E. faecalis* can be said to be comparable with 2% chlorhexidine solution. The results showed similar antibacterial ability can be caused by the similarity of antibacterial properties owned by 2% chlorhexidine solution and a solution of 5% EEP. High concentrations of chlorhexidine (chlorhexidine 2%) has bactericidal properties, as it can lead to precipitation and coagulation of cytoplasm bacteria that lead to cell death.^{3,12}

The basic principle of the mechanism of action of chlorhexidine is the release of positively charged molecules (cations) which in turn will interact with negatively charged phosphate compound (anion) contained in the cell walls of bacteria. Chlorhexidine and the bond between the bacterial cell wall will change the osmotic balance of the bacterial cell and cause a decline in the integrity of the bacterial cell wall. This will increase the permeability of the bacterial cell wall, thus allowing the chlorhexidine molecule to be adsorbed into the bacterial cells and cause

precipitation and coagulation cytoplasm bacteria that lead to cell death.¹² EEP also known to have bactericidal properties even in a low concentration.^{9,13} It means that the EEP 5% also has a strong bactericidal properties.

Many studies revealed that propolis (EEP) is equal to or more effective against *E. Faecalis* or oral pathogens than some other antibacterial properties such as chlorhexidine, calcium hydroxide, tri antibiotic mixture or herbal medications. Previous research evaluated the disinfection of dentinal tubules using Propolis, *Azadirachta indica* (alcoholic and aqueous extracts), 2% Chlorhexidine gel and calcium hydroxide against *Candida albicans* biofilm formed on tooth substrate. The study conclude that Propolis and alcoholic extract of *Azadirachta Indica* performed equally well as that of 2% Chlorhexidine and thus can be probable alternatives to Chlorhexidine.¹⁴ Kayaoglu also studied about the antimicrobial of Propolis compared to Chlorhexidine and Calcium hydroxide against *E. faecalis*. The study conclude the antimicrobial activity of the Propolis sample was between Calcium hydroxide and Chlorhexidine. Propolis was antimicrobial effective, however their activity did not exceed Chlorhexidine¹⁵. Other study about Propolis were conducted by Madhubala et al . This study evaluated and compared the antimicrobial activity of Calcium hydroxide, Tri Antibiotic Mixture (TAM) and an ethanol extract of Propolis (EEP) as intracanal medicaments on *E. Faecalis* in infected root canal. The result is that Propolis is more effective than triantibiotic mixture against *E. Faecalis* at a 2- day time period, and both are equally effective at 7-days. Calcium hydroxide showed gradual increase in antibacterial activity with maximum of 59. 4% on day 7.¹⁶

Saha S et al investigated and compared the effectiveness of Propolis, metronidazole with Chlorhexidine gel, *Curcuma longa* and Calcium hydroxide for elimination of *E. faecalis* bacteria in extracted teeth samples. The result of the study is Propolis produce a better antimicrobial efficacy followed by Chlorhexidine metronidazole combination, *Curcuma longa* and Calcium hydroxide. Propolis showed better antimicrobial properties against *E. faecalis* than other medicaments.¹⁷ The study of A. Eralp Akca et al revealed that Propolis was more effective in inhibiting Gram-positive bacteria than the Gram-negative bacteria in their planktonic state and it

was suggested that ethanolic extract of Propolis (EEP) could be as effective as chlorhexidine on oral microorganisms in their biofilm state. It concluded that the administration of Propolis at appropriate concentration might be effective on oral microorganisms. Propolis may serve as a natural alternative in reliable antimicrobial mouth rinse in order to avoid the side effect of chlorhexidine.¹⁸ The above studies revealed that Propolis (EEP) has a strong antimicrobial property against *E. faecalis* and other oral pathogen microorganisms and thus can be probable alternatives to other conventional medicaments.

Mechanism of action of the active ingredient EEP conducted by flavonoids, aromatic components (acid kafeat), and phenol component.^{4,8,19} Active ingredients EEP will inhibit the growth of bacteria by preventing cell division through inhibition of bacterial DNA replication. In addition, the active ingredients will also destroy the cytoplasm, cytoplasmic membrane and cell wall of bacteria, so that bacterial protein synthesis is inhibited and cause partial bakteriolysis.^{4,20} This study showed that chlorhexidine 2% and 5% EEP has the same antibacterial properties, which is bactericidal. The similarity of the antibacterial properties of this possibly could be the cause of obtaining the results in Table 2 above, which indicates that there is no difference between the antibacterial ability of 5% EEP solution compared with 2% chlorhexidine solution against *E. faecalis*.

In this study used the *E. faecalis* because *E. faecalis* is persistent, difficult to remove and often found in cases of repeated root canal treatment.^{5,21} *E. faecalis* bacteria is resistant to antibacterial properties, being able to adapt to environmental changes drastically, has a small size so that it can enter and live in the dentinal tubules, can survive without nutrition for a long time, protected by the biofilm, and resistant to high pH (due to *E. faecalis* ability to maintain pH homeostasis).⁵ The bacteria *E. faecalis* used in this study is a pure strain of *E. faecalis* ATCC 29 212 from a portion of Microbiology Faculty of Medicine. Ethanolic extracts of propolis (EEP) 5% used in this study was prepared by following the method of making EEP 5% ie by dissolving chunks of raw propolis in 95% ethanol solution with a ratio of 1: 5 and stirred for 15 minutes, then soaked for 5 days. Furthermore, filtering to obtain filtrate, then the filtrate is evaporated to obtain a thick EEP. EEP condensed dissolved in

dimethyl sulfoxide (DMSO) to achieve a concentration of 5%.⁴ Alcohol (ethanol) is a type of solvent chosen for extracting propolis as it can dissolve the entire active substances in propolis, easily available and relatively cheap. The study was conducted one day after manufacturing EEP 5%, so that the storage time of EEP 5% in this study is the first day.

The weakness of this study is the way bacterial count done indirectly by measuring turbidity test materials using the tool ELISA reader, so that when the nature or antibacterial solution color became dark or cloudy, it can affect the OD value to become higher. This can lead to bias, because the antibacterial ability of the solution will be seen to be lower. If counting living bacteria, it can be known with certainty the level of antibacterial ability of a material. In addition, this study is a preliminary research in the form of direct contact between antibacterial solution tested with *E. faecalis*, so it does not describe the real situation of the root canal in the oral cavity. Therefore, further research needs to be done using the natural teeth as the bacterial culture media.

Conclusions

The ability of antibacterial solution Ethanol Extract Propolis (EEP) 5% is statistically similar to the ability of the antibacterial chlorhexidine solution 2% in inhibiting the growth of *Enterococcus faecalis* (*E. faecalis*), Ethanol Extract Propolis (EEP) 5% are natural materials that can be used as alternative irrigation in eliminating *E. faecalis*.

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Declaration of Interest

The authors report no conflict of interest.

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