

# MINIMUM BACTERICIDAL CONCENTRATION OF EAST JAVA PROPOLIS EXTRACT TO BIOFILM OF *Enterococcus faecalis*

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## ABSTRACT

**Background.** Endodontic root canal treatment is a treatment that can be performed in inflammation pulp or dental pulp necrosis. *Enterococcus faecalis* (*E faecalis*) bacteria identified 89,6% in root canal treatment failure. Sterilization and irrigation of the root canal are the bacteria elimination stages of root canal treatment, but materials that used do not killing *E faecalis* specially the bacterial biofilm, so *E faecalis* can survive to cause periapical disease after root canal treatment. Propolis contain apigenin and tt-farnesol that have mechanisms for inhibiting growth and development of bacterial biofilm. **Purpose.** To measured Minimum Bactericidal Concentration of East Java Propolis Extract to Biofilm of *E faecalis*. **Methods.** This research was laboratory experimental study. Propolis extract used is East Java propolis extracted by maceration method, and dilution into several concentrations using aquadest. Biofilm formation was observed using the microtitter plate method then continued reading of Optical Density (OD) using ELISA reader to determine Minimum Biofilm Eradication Concentration (MBEC) of East Java propolis extract to *E faecalis* biofilm. **Result.** East Java Propolis extract had MBEC at concentration of 11,45% against *E faecalis* biofilm. **Conclusion.** East Java Propolis extract concentration 11,45% is MBEC to *E faecalis* biofilm in-vitro.

**Keywords :** East Java propolis extract, Minimum Biofilm Eradication Concentration, *E faecalis*

## INTRODUCTION

*Enterococcus faecalis* (*E faecalis*) are the most species of bacteria found in root canal treatment failure. Sometimes treatment failure without clinical symptoms so that radiographic picture is the only way to see the presence of a periapical lesion known as Chronic apical periodontitis . Chronic apical periodontitis is an asymptomatic condition that originates in the dental pulp is infected by bacteria ,it was associated with inflammation and periapical tissue destruction (Cohen,2011). Failed root canal treatment can be seen in the presence of periapical radiolucency. The prevalence of periapical radiolucency high at 36 % of the 28,881 treated teeth root canal treatment (Pak et al,2012).

*E faecalis* is a facultative anaerobic Gram-positive cocci that are often found in the root canal that has been treated with a prevalence of 30 % to 90 % of cases . *E faecalis* can survive in root canals that have been treated , including resistance to intracanal medicaments premises the ability to form biofilm , invasion into the dentinal tubules and survive in the long term in nutrient limitation (Fouad ,2009).

Biofilms are microbial populations containing organic or inorganic substrates coated with extracellular microbial products that form the matrix intermikrobial . On microbial biofilms are more resistant to microbial agents than planktonic cells form . Biofilm formation is a complex process that involves attachment and immobilitation , interactions between cells, the formation of microcolonies, confluent biofilm formation after the formation of three-dimensional structure of the biofilm. Biofilm production is regulated by Ouorum sensing system . There is quorum sensing in several pathogenic bacteria to regulate bacterial gene in response to population density of microorganisms obtained through the production of extracellular signaling molecules known as autoinducers (Mohamed and Huang, 2007). Biofilm formation on root canal probably started after the entry of Oral planktonic microorganisms into the root canal. The necrotic pulp tissue would be a favorable environment for the proliferation of microorganisms because of the organic residue that serves as a nutrient medium substrate bacteria (Usha et al, 2010).

Propolis is a sticky resin , colored brown and is thermoplastic . A natural product that is a mixture of beeswax and resin collected from the flowers especially important plant and leaf buds by honey bees (*Apis and Trigona melifera Sp*). Some studies revealed that propolis has anti-oxidant properties, anti-bacterial, anti- fungal, anti-viral and anti-inflammatory (Surendra et al, 2012).

Components of propolis depends on the season and the source where the resin exudate was collected by bees. Bees use propolis to protect the hive against intruders from outside factors such as insects, beetles and mice, as an adhesive gap contained in the honeycomb and bee larvae to protect from disease and bacteria. Komposisi chemistry of propolis, color and aroma according to geographical places. The composition of propolis is also very varied and close relationship with propolis plant species where it originates. The main components of propolis are flavonoids, which is one of the largest class of natural phenols. Inhibition effect on microorganisms propolis depends on the synergy of many components (Ozen et al, 2010) . Anti-microbial power possessed by propolis extracts is influenced by the presence of the active compounds in the extract are flavonoids, polyphenols, terpenoids, galangin, quercetine, myrecetine, robinetin, epighalocatechin, licochalcones AB, caffeic acid, tannins and essential

oils. Each active ingredient has its own mechanism in its activity as an anti- microbial (Fokt et al ,2010) . Propolis also contains terpenoids or tt - farnesol which is a sesquiterpene alcohol found in the essential oils of various plant extracts that can affect the growth of many bacteria (Gomes et al , 2011).

Indonesia is also the world community and especially East Java, now is beginning to prioritize the use of drugs from natural materials (back to nature). The use of herbal medicine began to become the people's choice because in addition to reducing the use of chemicals and synthetic substances that are harmful , low price and can increase the potential use of natural materials that are easy to obtain . In East Java, *Apis mellifera* propolis collected from bee hives located in Lawang area located on the plateau which is dominated by the silk cotton tree (*Ceiba pelandra* L).

Objective in this study was to determine the Minimum Bacterial Concentration of East Java Propolis Extract to biofilms of *Enterococcus faecalis* to determine Minimum Biofilm Eradication Concentration ( MBEC ) .

## **MATERIALS AND METHODS**

Raw propolis Lawang macerated with 70 % ethanol in a sealed container and then shaken using a shaker with a speed of 80 rpm . After 7 days maceration stopped and filtered . Maceration for 24 hours for 7 days repeated again so that total maceration time was 14 days to obtain a stock solution of propolis (Victorio et al , 2009).

*E faecalis* culture in trypticase soy broth (TSB) diluted to 1:100 for 24 hours and then 0.1 ml concentration of  $1 \times 10^6$  *E faecalis* bacteria / ml loaded on a 96 well-plate bottomed plastic tissue culture plate . Propolis extract was applied into each microtiter concentration of 11.45%, 5.75%, 2.86%, 1.43%, 0.715%, 0.38%, 0.19%, 0.10% and 0% as a positive control. Painting done biofilm microorganisms attached to the well using crystal violet. To quantitatively analyze biofilm formation isopropanolol added 0.2 ml of each well then measured Optical Density (OD) at 570 nm using ELISA reader . Data analysis using one-way ANOVA and LSD at  $p < 0,05$ .

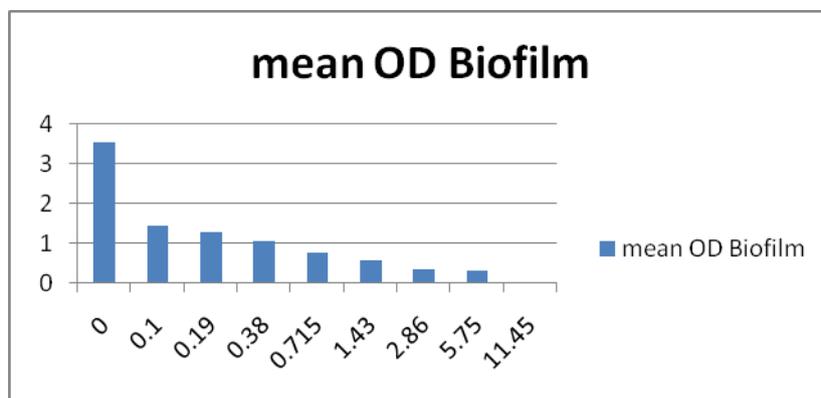
## **RESULTS AND DATA ANALYSIS**

Propolis extract with a concentration of 11.45 % , 5.75% , 2.86% , 1.43% , 0.715% , 0.38% , 0.19% , 0.10% and 0% as a positive control to *E faecalis* biofilm were calculated by ELISA Reader with a wavelength of 570 nm can be seen in Table 1 and Picture 1.

Table 1. Extract Concentration, Mean OD, Percentage OD to *E. faecalis* biofilm

Extract Concentration	n	Mean OD	OD Concentration
0%	5	3,542	100%
0,10%	5	1,458	41,17%
0,19%	5	1,304	36,83%
0,38%	5	1,050	29,65%
0,715%	5	0,759	21,44%
1,43%	5	0,576	16,28%
2,86%	5	0,363	12,32%
5,75%	5	0,313	8,86%
11,45%	5	0	0

Picture 1. Mean OD graph to *E faecalis* biofilm



From Table 1 and Picture 1, it can be seen that there is a concentration of 11,45% biofilm bacteria is zero, it can be concluded that the concentration is 11.45% is Minimum Biofilm Eradication Concentration (MBEC), that is the lowest concentration of propolis extracts can inhibit the growth of test bacteria 100% of the amount of bacteria that managed to grow in a positive control.

One way ANOVA analysis found significant differences between the positive control group 11.45%, 5.75%, 2.86%, 1.43%, 0.715%, 0.38%, 0.19%, 0,10%. ( $p < 0,05$ ).

## DISCUSSION

Biofilm is an aspect of microbial physiology that almost all species of bacteria can form it . Multicellular structure of the biofilm enables the bacteria to perform dormancy and hibernation so that bacteria can survive (Bordi and Bentzmann,2011). *E faecalis* is a pathogenic bacteria that plays a role in most cases of failure of root canal treatment. The prevalence of *E faecalis* in the re-treatment of root canals treatment is 89.6% and the primary infection is 67.5% (Fouad ,2009).

Propolis honey bee *Apis mellifera spp* is antibacterial that can kill all the germs that enter the hive. Propolis is often referred to as Russian penicillin because of intensive research scientist at the Russian worker bees. Propolis is a natural antibiotic called because of its antibacterial ability (Finstrom and Spivak,2010). Propolis can also stop the formation of bacterial biofilms (Gomes et al ,2011).

In this study, the concentrations of propolis extracts used 11.45% , 5.75%, 2.86%, 1.43 % , 0.715%, 0.38%, 0.19% , 0.10% and 0% concentration of the used as a positive control. Subsequently the extract was applied to the plate microtiter previously established biofilm bacteria *E. faecalis*, and the the determination biofilm OD using ELISA Reader.

The results showed that propolis extract at a concentration of 0.10 % can reduce bacterial growth at a concentration of 5.75 % and there is a number of bacterial colonies that live around 10 % compared to the number of colonies on the control and concentration of 11.45 % has no found bacterial growth, so the concentration is referred as the Minimum Biofilm Eradication concentration (MBEC).

East Java propolis extract containing 1,03% tt-farnesol (terpenoids) and the content 5,12% flavonoids (apigenin), tt - farnesol and apigenin content in propolis is useful to destroy biofilms. This is consistent with research Kao et al (2003) who found that the content of propolis is apigenin and tt-farnesol which led to a decrease in the amount of biofilm polysaccharides. tt-farnesol also have hydrophobic properties that are likely to cause disruption of biofilm membrane, which causes the release of cellular biofilm. This will cause the rupture of the cell (Gomes et al,2011).

The results showed that the extract of propolis East Java is able to inhibit biofilm formation of *E faecalis*. This is because the East Java propolis extract contains apigenin and tt-farnesol which may cause the membrane biofilm and cause a decrease in the number of polysaccharide in biofilms to biofilm cell contents regardless. This is evidenced by an increase in the concentration of protein and polysaccharide outside the cell (Kao et al,2003; Gomes et al,2011). **The conclusion** of this study is, 11,45% concentrations of East Java propolis extract eradicate biofilm *E faecalis* bacteria.

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