Research Report

Effectiveness of flavonoid from mangosteen pericarp (*Garcinia mangostana L.*) as *Enterococcus faecalis* antibiofilm

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ABSTRACT

Background:*Enterococcus faecalis (E. faecalis) is a microorganism that is commonly found in endodontic failure treatment, this due to several characteristics of E.faecalis which has the capabillity to living in environments with high salt levels, high temperature, and pH broad spectrum. Bacteria in biofilms form is one of the adaptive process that allows bacteria to survive in an environment with low nutrients in the root canals. Bacteria in biofilms form have different characteristics from planktonic form, resistance to phagocytic cells and drugs, which can effect to persistent infection. Mangosteen (Garcinia mangostana) has many benefits, especially on the pericarp of the fruit contains alkaloids, tannins, phenolics, flavonoids, and triterpenoids. Flavonoids are the largest group of phenolic compounds that have a nature effectively inhibit the growth of viruses, bacteria, and fungi.* ***Purpose:****Purpose of this study wasto find out the role of the antibiofilm of the flavonoid in garcinia mangostana pericarp against E. faecalis bacterial biofilm.* ***Methods:****Laboratory experimental in-vitro with post test only group design. The method used is microtitter plate biofilm assay and continued with the readings use Elisa reader at a wavelength of 595 nm.* ***Results:****Flavonoids mangosteen pericarp effective as antibiofilm E.faecalis bacteria at a concentration of 12.5%.* ***Conclusion:****The study showed that flavonoids from mangosteen pericarp has antibiofilm activity against E. faecalis bacterial biofilm.*

Keywords: *Flavonoid Garcinia mangostana; Enterococcus faecalis; biofilm*
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INTRODUCTION

Enterococcus faecalis (*E. faecalis*) is a microorganismcommonly found because of endodontic failure treatment.[1] These bacteria can live in high salinity, high temperature, and broad spectrum pH. *E. faecalis* can attach to host cell releasing protein that enable it to compete with other bacteria, and alter the host immune response. *E. faecalis* can suppress the activity of lymphocytes, which could potentially make a failed endodontic treatment.[2]

Endodontic treatment have three basic principles, know as the "Triad Endodontics" consisting of biomechanical preparation, irrigation, and disinfection, as well as obsturation.[3]Irrigation composition is needed to minimalize biofilm presence. Bacteria in

the form of biofilm is an adaptive process that make bacteria can live through low nutrition environment in the root canals.[4]Bacteria in biofilms have different characteristics from planktonic form, including resistance towards phagocytic cells and drugs, which can lead to persistance infection.[5]

There are various kind of irrigation solution in the root canal treatment, such as sodium hipoklorit (NaOCl), hydrogen peroxide (H_2O_2), chlorhexidine, citric acid, iodine-potasium-iodida, and Ethylenediaminetetraacetic acid (EDTA). One of the irrigation source has antibiofilm power and most frequently used in the root canal treatment is NaOCl.[6] NaOCl active on necrotic tissue, vital tissue, and is a disinfectant with bactericidal power which has been recognized. If NaOCl have contact with the vital soft tissues, it will become cytotoxic.[7]

Currently, there is so many research is done by using natural materials in the field of dentistry. One of it is the use of mangosteen (*Garcinia mangostana L*) which has shown many health benefit in the field, like ulcers drug, hemorrhoids, and wounds. Phytochemical screeming that has been done to fruit skin mangosteen indicates there is a compunds like alkaloid, tanin, fenolik, flavonoid, and triperpenoid. These compound known to have antibacterial properties. Previous research has suggested that the antibacterial activity from mangosteen pericarp is effective for inhibiting gram positive bacteria, such as (*Bacillus subtilis*, *Staphylococcus aureus*,and *Streptococcus faecalis*).[8]

Flavanoid are the largest group of fenol compounds that have a effectively nature for inhibit the growth of viruses, bacteria, and spore.[9] Mechanism of flavonoid as antibacterial is to make a complex compound with extracellular protein and dissolve so it can damage bacteria cell membranes and is followed by releasing intracellular compunds.[10] No research about flavonoid mangosteen pericarp as antibiofilm against biofilm *E. faecalis*. Based on the reason, it is necessarry to make a research about the role of flavonoids mangosteen pericarpas antibiofilms against *E. faecalis* biofilms. This study is to determine the effective concentration offlavonoids mangosteen pericarp (*Garnicia mangostana* L.) as *Enterococcus faecalis* bacterial antibiofilm.

MATERIAL AND METHODS

The type of research that is used is an experimental laboratory research in-vitro with study design post test only control group design. The sample of this study is a single species biofilms *E. faecalis* which cultured from tripticase soy broth media (TSB). The research is conducted at the Laboratory of Tropical Infectious Disease Hospital (RSPTI) Airlangga University in August - October 2014.

Mangosteen pericarp (*Garnicia mangostana* L.) which used as a raw material for the flavonoid mangosteen pericarp obtained from UPT Materia Medica, Batu. Extract is obtained by extracting mangosteen pericarp using ethanol 96% with maseration method. Flavonoids obtained from mangosteenpericarp extraction using acetone benzene solvent. Flavonoid mangosteen pericarp extract has been obtained by conducted thinning series to obtain a variety of concentrations, ie a concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.562%, and 0.781%.

To test the biofilm, microtitter method plate biofilm assay method is selected. Suspension initial test is made equivalent to 0.5 Mc Farland turbidity (Cloudiness of mixture $BaSO_4$ and HCl) diluted to gain a concentration of 10^6 bacterial. *E. faecalis* bacterial suspention were cultured into the microplate and incubated at 37^{o}C. For 6x24 hours. Added 0.1 ml suspension from flavonoid mangosteen pericarp with concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.562%, and 0.781%, respectively to microplate wells.Incubation carried back in the 37^{o}C in the incubator for 24 hours. Microplate is washed with phospate buffered saline (PBS) 3 times to remove planctonic bacteria and dried for further reading. Staining with crystal violet 0.1% about 0.2 ml. The remaining coloring liquid washed with aquadest a few time until there is no color in the rinse water, then dried, and the

solvent is added colors, 100% DMSO 0.1 ml. After that, plate will be shaked for 1 minute, and placed in the microplate reader to be observed the formation of net layers in the sample. Each isolation will be replicate 3 times. There is a measure for optical density (OD) at 595nm using ELISA reader.[11]

RESULT

Antibiofilm flavonoid mangosteen pericarp test result towards bacterial biofilms *E. faecalis* on microtitler plate usingflavonoids mangosteen pericarp concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. The final result converted into biofilm viability percentage with the following formula :

$$\text{Viability} = \frac{\text{OD value of treatment group}}{\text{OD value of control group}} \times 100\%$$

From the data in Table 1, can be seen at 12.5% concetration, contained bacteria biofilm by 16% and capable of damaging biofilm bacterial as much as 84% of bacteria amount that managed to grow in positive control. So it can be concluded that on 12.5% concentration flavonoid mangosteen pericarp effectively destruct*E. Faecalis*bacterial biofilm.

The result of this study is analyzed using SPSS statistical analysis. Test data distribution is done by using Kolmogorov-Smirnov Statistical Test. The test result showed significance with p equal to 0.156 (p>0.05), so the data is concluded as normal distribution. Homogenity of variance data is tested by statistical test using Levene's Test. Test result indicate homogeneous data (p>0.05) with p equal to 0.081.

Parametric test was performed using the test One Way Annova and continued Post-hoc multiple comparasion Test (Tukey Test) with a significance level of 5%. One Way Annova test is used to determine the differences between groups of data, followed with the Tukey Test to see significance of differences between groups OD biofilm research. The result of the test One Way Annova shows the results of significance of 0.000 (p <0.05), which means there are significant differences in flavonoid mangosteen pericarp with different concentrations on the growth of *E. faecalis* biofilm bacteria.

DISCUSSION

Flavonoid is an one of the elements contained in the mangosteen pericarp which has the ability as an anti-bacterial. In this research flavonoid used as antibiofilm bacteria *E. faecalis*. The results showed all concentrations have antibiofilm ability against biofilm bacteria *E. faecalis*. Because the results obtained indicate that all OD values biofilms treated lower than the value of OD biofilm untreated, this agrees with the theory that the flavonoid have an antibiofilmeffect.Effective concentration which can damage the biofilm bacteria *E. faecalis* present in a concentration of 12.5%. At a concentration of 12.5% contained biofilm bacteria with the smallest percentage that is equal to 16%, so it can inferred that the concentration of 12.5% is an effective concentration of flavonoids mangosteen pericarp to destroy bacterial biofilms of *E. faecalis*. In group concentration of 100%, 50%, and 25% indicate average of value an increasingly large, this was due to the concentration of flavonoid extract of mangosteen pericarp which is high enough to have difficulty in reading the OD. ELISA reader is spectrophotometry, so with flavonoidmangosteen pericarp have crimson thickness, then the result material is sufficiently strong to stick to the walls of microtiter plate so that it can affect results of the reading ELISA reader.

The results showed flavonoid mangosteen pericarp could damage bacterial biofilms of *E. faecalis*. It is appropriate with the theory that a material which has the power antibiofilm can destroy biofilms in various ways, such as by penetration of the extracellular matrix, dispersing the cells of the biofilm, or eliminating stability extracelullar polyshacaride substance (EPS) in the biofilm. The release of the cells occurs due to an increase in fluid pressure or degradation of endogenous enzymes, as well as the release of EPS or adhesions protein.[12]

Flavonoid is an the largest group from phenol compounds that have a nature effectively inhibit the growth of viruses, bacteria, and fungi. The ability phenol compounds to create a bacterial enzyme becomes inactive cause glycosyltransferase disrupted enzyme activity, enzyme glycosyltransferase consequently can not produce glucans which serves as a medium for bacterial adhesions.[13] Besides interfere enzymes glycosyltransferase work, flavonoid also interfere with signaling molecules in bacteria (quorum sensing) that serves as a medium of communication between bacteria and contribute in the process of biofilm formation. Inhibition of quorum sensing caused due inhibition synthase enzyme responsible for producing the protein receptors or signaling molecules which modulate the quorum sensing.[12] In positive gram bacteria, signaling molecules which modulate the quorum sensing is a signal peptides.[14]

Flavonoids contribute to change the permeability of cells which work by altering the permeability of the cell membrane bacteria that cause changes in the pump system Na^+ and K^+ in bacterial cells. In normal conditions the pump system Na^+ and K^+, K^+ ion concentration in the cell is greater from in the outside of the cell, whereas the concentration of Na^+ ions outside the cell is greater than in the cell. To maintain these conditions, the Na^+ions and K^+ should always be pumped against a concentration gradient with the energy from ATP hydrolysis. Three Na^+ ions is pumped out and two K^+ ions pumped into the cell.Terpenoids binds to the transmembrane protein that causes damage to the transmembrane, thus triggering the occurrence arrest of sodium ions causes the fluid out into plasma cells and swelling cell which ended the outbreak of the inhibitory bakteri.[15,16] cell signaling molecule in bacteria process, glycosyltransferase enzyme activity, and changes in cell permeability causing biofilm bacteria *E. faecalis* can not thrive.

Result from this research concluded that the of flavonoid extract mangosteen pericarp (*Garcinia mangostana* L.) have antibiofilm power to biofilm bacteria Enterococcus faecalis with an effective concentration of 12.5%.

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