The Antibiofilm Activity of Extract Propolis Against Biofilm Enterococcus Faecalis as Herbal Medicine Potential in Root Canal Treatment

by Dian Agustin Wahjuningrum

The Antibiofilm Activity of Extract Propolis Against Biofilm Enterococcus Faecalis as Herbal Medicine Potential in Root Canal Treatment

Dr. Dian Agustin Wahjuningrum

Ari Subijanto

ABSTRACT

Background

Endodontic root canal treatment is a treatment that can be performed in the dental pulp necrosis. Failure in endodontic root canal treatment can still occur, although it has been done in accordance with procedures. One cause of failure of root canal treatment is bacterial resistance to conservative treatment. Some microorganisms in pulp necrosis were able to form biofilm to enhance pathogen virulence. This happens because the necrotic pulp tissue is an opportunistic environment for development of microorganisms due to organic residues or nutrients, which serve as a substrate or microorganism culture. One of these microorganisms is Enterococcus faecalis. Medicament needed to eliminate microorganisms in the root canal pulp necrosis, especially in the form of bacterial biofilms. The problem faced by this time almost all the materials used in dentistry is a chemical and it has side effects, it is necessary for natural ingredients from nature that has antibacterial or antibiofilm. Antibiofilm or antibacterial agent can be found in propolis. Propolis contains tt-farnesol and apegenin that have mechanisms for inhibiting growth and development of bacterial biofilm.

Purpose

The aim of this study was to know the antibiofilm effects of propolis extracts by determining its minimum inhibitory concentration to Enterococcus faecalis biofilm.

Methods

This study is an in-vitro experimental research laboratory. Propolis extract used is 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 the minimum inhibitory concentration of propolis extracts to Enterococcus faecalis biofilm

Results

Minimum concentrations of propolis extract can inhibit the growth and development of Enterococcus faecalis biofilm is 5,75%.

Conclusion

Influence of propolis extract in inhibiting the formation of biofilm produced by Enterococcus faecalis, compared with no propolis extract. Propolis extract concentration by 5,75% is Minimum Inhibitory Concentration to Enterococcus faecalis biofilm in vitro.

INTRODUCTION

Pulp necrosis is death of part or all of the pulp tissue, usually caused by inflammation or traumatic injury. Pulp necrosis is also a process of bacterial infection. The treatment plan can be made in the treatment of pulp necrosis is a root canal treatment. Root canal treatment aims to restore the state of the sore tooth to be accepted by the biological surrounding tissues. This means that the tooth can function and no signs of other pathology.[1,2]

Root canal treatment failure can still occur despite conducted in accordance with procedures. Root canal treatment failure can be caused of bacteria resistant to conservative treatment. Allegedly some microorganisms in endodontic infections able to form biofilms as a significant mechanism in avoiding the host defense system and increase the virulence of the pathogen.[3,4]

Biofilms can be defined as microbial populations containing organic or inorganic substrate, which is coated by extracellular microbial products, which form the matrix inter microbial. In biofilms, microorganisms showed higher resistance, both on antimicrobial agents and host defense mechanisms when compared in the form of planktonic cells. Biofilm formation is a complex process involving the formation of attachment and immobilization, cell to cell interactions, forming micro colonies, confluent biofilm formation, and biofilm formation of three-dimensional structures. Bacteria in biofilms have different properties to the planktonic form. Biofilm production is regulated by quorum sensing system in several pathogenic bacteria. Quorum sensing is a regulatory system of bacterial gene expression in response to population density of microorganisms obtained through the production of extracellular signaling molecules called auto inducers.[3,5]

Biofilm formation on root canal may be started after the first invasion of the pulp chamber by oral planktonic microorganism. Necrotic pulp tissue into a favorable environment for the proliferation of microorganisms due to the presence of

organic residues or nutrients, which serves as a substrate medium or culture microorganisms. In the study of microbial biofilm communities found some root canal one is *E. faecalis*.[6,7]

E. faecalis is a microorganism that despite persistent small amounts of necrosis in the root canal, but it plays a role in the occurrence of persistent periradicular lesions after root canal treatment. *E. faecalis* can survive in adverse conditions, such as root canal instrumentation and obturation was done with only a few nutrients are available. Model of growth through the formation of biofilms.[8]

There are three basic stages in the definitive root canal treatment has known as the "Triad of Endodontic", which consists of the biomechanical preparation, irrigation and disinfection, and obturation. Eliminating the remaining pulp tissue in dentin and eliminate microorganisms contained in the root canal is dominant during root canal treatment. Irrigation and biomechanical preparation can't eliminate the entire microorganisms in the root canal. Thus, the use of root canal sterilization is required for root canal treatment. Problems encountered in the field of dentistry today is almost all materials used in dental treatment is a chemical and has side effects.[9,10,11]

Studies in Indonesia today is mostly done to find substitute materials chemicals using basic ingredients of traditional plant or materials which may be obtained from the natural environment in Indonesia. Indonesia has known to have various types of local bees. Honeybees produce some products that have utility both for itself and for the human. One of their products is bee glue or propolis. Bee glue or propolis has known to have antibacterial activity that can be used for both pulp therapy in primary teeth and permanent.[11,12]

Propolis is a sticky resin that comes from a tree trunk or bark, collected and processed by bees salivary fluid secretion. Each type of bee has a specific resin existing resources in their respective areas so that the composition of propolis varies. The main components of propolis are flavonoids and phenolic acids, including caffeic acid phenylesthylester (CAPE) that its content reaches 50% of the entire composition. Based on the results of the study, in the commercial propolis and extract contained the same active compound, which contains flavonoids, phenolic, hidroquinon, tannins, volatile oils, steroids, saponins and reducing sugars. In the propolis extract also contained tt-farnesol (terpenoid) and apigenin.[13,14,15]

The purpose of this study was to determine the minimum inhibitory concentration of propolis extract against bacterial biofilms of *E. faecalis*, which later can be considered the use of propolis extracts as an ingredient for the success of the sterilization of the root canal treatment to inhibit bacterial biofilm formation of *E. faecalis*.

METHODS AND MATERIALS

This research is an experimental research laboratory in-vitro with post-test only control group design. Samples of this research are propolis extracts which diluted in various concentrations using distilled water diluent. The materials used are oese, brander spirits, incubators, anaerobic jar, test tube, test tube rack, petri dish, pipettes, microscopes, 96-well flat-bottomed plastic tissue culture plate, ELISA reader, Tryptone Soy Broth (TSB) medium, the stock of *E. faecalis* bacteria, a solution of phosphate-buffered saline (pH 7,3), propolis extract, sterile distilled water, crystal violet 0,2 ml of 2%, and isopropanol.

Propolis extract obtained from Balai Penelitian dan Konsultasi Industri (BPKI) Surabaya which extracted by maceration method (number of laboratory test results 03573/KI/VI-2012). Furthermore, the method of thinning of the series to get a wide range of concentrations, ie 11,45%, 5,75%, 2,86%, 1,43%, 0,715%, 0,38%, 0,19%, and 0,10%. E. faecalis bacteria were used obtained from Balai Besar Laboratorium Kesehatan (BBLK) Surabaya.

Biofilm formation and determination of minimum inhibitory concentration: Culture *E. faecalis* in Trypticase Soy Broth (TSB) diluted to 1:100 in TSB glu overnight. Then 0,1 ml of *E. faecalis* at the concentration of 1x 106 bacteria/ml loaded on 96-well flat-bottomed plastic tissue culture plate and 0,2 ml of *E. faecalis* loaded in 96-well flat-bottomed plastic tissue culture plate as a positive control. Furthermore, microtitter was incubated for 24 hours at the temperature of 37°C. After 24 hours, propolis extract was applied into each microtitter in 11,45%, 5,75%, 2,86%, 1,43%, 0,715%, 0,38%, 0,19%, and 0,10% and then incubated for 24 hours at a temperature of 37°C and the contents of each microtitter plate aspirated and washed 3 times with 0,2 ml of phosphate-buffered saline (pH 7,3) by using a pipette. Biofilm microorganisms which attached to the well were painted with crystal violet. Flushing is done by using distilled water and dried. To analyze biofilm formation, added 0,2 ml of isopropanol in each well then measured by Optical Density (OD) at 570 nm using an ELISA reader.

The data was obtained from the quantitative data in the form of readings on spectophotometric Optical Density (OD) of each well plates which treated differently that propolis extract with a concentration of 11,45%, 5,75%, 2,86%, 1,43%, 0,715%, 0,38%, 0,19%, 0,10%, and the positive control well without application of propolis extract. After that, the data were analyzed using one-way ANOVA test.

RESULTS

Researcher did the study using a bacterial biofilm-forming *E. faecalis* and subsequently given propolis extract concentration of 11,45%, 5,75%, 2,86%, 1,43%, 0,715%, 0,38%, 0,19%, 0,10%, and 0% as the positive control treatment given to the microtitter plate.

The results showed that the propolis extract is able to inhibit *E. faecalis* biofilm formation. In this study, concentration of the extract was ranging from 0,10% to inhibit biofilm growth (Table 1). From the analysis, it is known that at the concentration of 5,75% of propolis extract, contained biofilm bacteria less than 10%, so the concentration of 5,75% is the minimum inhibitory concentration of propolis extract against E. faecalis biofilm.

DISCUSSION

Biofilm formation on root canal may be started after the first invasion of the pulp chamber by oral planktonic microorganisms. Necrotic pulp tissue become a favorable environment for the proliferation of microorganisms due to the presence of organic

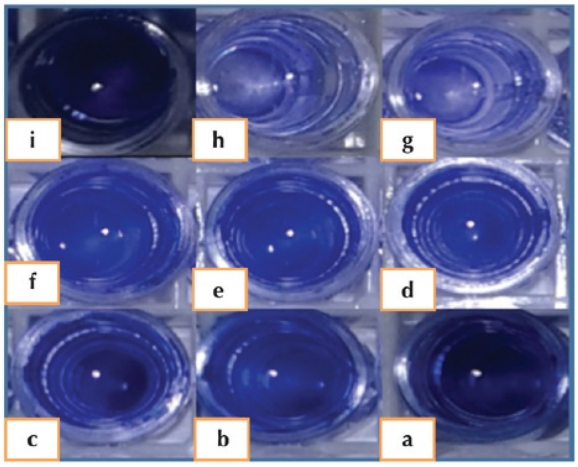


Fig 1. The results of staining with Crystal Violet on *E. faecalis* Biofilm Bacteria Application of Different Concentrations with Propolis Extract
a. Control
b. Propolis extract at the concentration of 11,45%
c. Propolis extract at the concentration of 5,75%
d. Propolis extract at the concentration of 2,86%
e. Propolis extract at the concentration of 1,43%
f. Propolis extract at the concentration of 0,715%
g. Propolis extract at the concentration of 0,38%
h. Propolis extract at the concentration of 0,19%
i. Propolis extract at the concentration of 0,10%

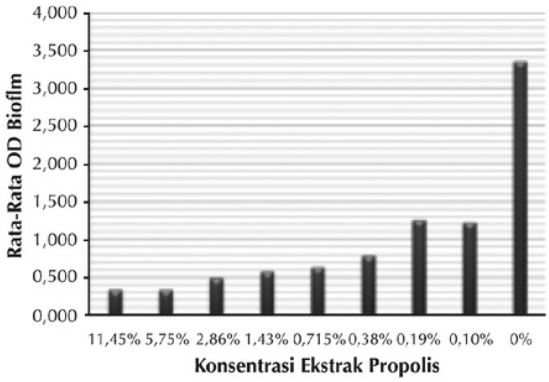
Table 1. Measurement Result of ELISA reader OD *E. faecalis* Biofilm

Extract concentration	n	Mean OD	OD
0%	5	3,344	100%
0,10%	5	1,2264	36,67%
0,19%	5	1,2536	37,48%
0,38%	5	0,7802	23.33%
0,715%	5	0,6292	18,81%
1,43%	5	0,5706	17,06%
2,86%	5	0,4898	14,65%
5,75%	5	0,3304	9,88%
11,45%	5	0,3360	10,04%

residues or nutrients, which serves as a substrate or culture medium mikroorganism.[6]

Research in the field of health to propolis have been carried out, both in vitro and in vivo. Fokt et al 2010, the study showed propolis has multiple biological and pharmacological activity, propolis in addition to act as antibacterial against Gram positive and Gram negative, propolis also has anti-inflammatory, anti-fungal, antiviral, anti-oxidant, anti -protozoal, anti-tumor, hepatoprotective, anti-ulcer, cardio-protective, neuroprotective, radioprotective, immunomodulatory, lowering blood pressure and cholesterol levels of the body. Propolis also promote the regeneration of tissue, bone, and cartilage. Propolis can also stop

Fig 2. Graphs Measurement Results OD ELISA Reader Biofilm



the formation of bacterial biofilms.[16,17,18,19]

Tt-Farnesol and apigenin content in propolis have instrumental in stopping the formation of biofilm microorganisms. This is consistent with the study of Koo et al., 2003, which found that the content of propolis, namely apigenin (4',5,7-trihydroxyl-flavone) and tt-farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol), it causes a decrease in the amount of polysaccharides in the biofilm microorganisms without interfering with the survival of the bacteria. Because apigenin and tt-farnesol have bacteriostatic capability. This means being able to overcome the infection without killing the microorganisms of the oral cavity of normal and not cause resistance of bacteria.[15]

Apigenin and tt-farnesol affects one of the polysaccharides in the biofilm, which is alkali-soluble glucan, so that will inhibit biofilm formation. Alkali-soluble glucan is a function of extracellular glucan to adherence to the cell surface. Insoluble glucans synthesized from sucrose by GTFs (glucosyltransferases) also play an important role in the attachment and colonization of microorganisms. Apigenin and tt-farnesol have a significant impact on the further development and biofilm accumulation by influencing the synthesis of polysaccharides in the biofilm.[15]

Apigenin and tt-farnesol have different mechanisms to reduce the synthesis of glucan. The main targets for apigenin is GTF enzymes (glucosyl transferase). Inhibition of glucan synthesis by tt-farnesol have an effect on the cell membrane, rather than enzymatic activity, because tt-farnesol is poor for GTFs inhibitor. The chemical structure and lipophilic nature of tt-farnesol support for membrane damage, which is caused by changes in permeability and fluidity of cell membranes. Agents are capable of causing damage to the cell membrane not only reduces the metabolism of bacteria, but it also affects the synthesis of glucan by microorganism.[15]

The results showed that the extract of propolis is able to inhibit the formation of biofilms by E. faecalis bacteria. At extract concentrations ranging from 0,10% can inhibit biofilm growth (Table 1). From the analysis, it is known that the concentration of propolis extract contained 5,75% of biofilm bacteria with a percentage of less than 10% or at this concentration of propolis extract could inhibit biofilm by 90%, resulting in a concentration

of 5,75% is the minimum inhibitory concentration of the extract of propolis against *E. faecalis* biofilm bacteria. This is because propolis extract contains tt-farnesol/terpenoid and apigenin that can cause disorders of biofilm membrane and cause a decrease in the amount of polysaccharides in biofilms that occur subsequent release of cellular content of biofilms. It is characterized by elevated concentrations of proteins and polysaccharides outside cells.[15,19]

It can be concluded that propolis extract plays an important role in the inhibition of biofilm formation produced by the *E. faecalis* bacteria, compared with no extract of propolis and propolis extract the minimum inhibitory concentration against *E. faecalis* biofilm bacteria in vitro of 5,75%.

REFERENCES

1. Abbott P V, Yu C. A Clinical Classification of The Status of The Pulp And The Root Canal System. Australian Dental Journal Supplement;52:(1 Suppl):2007. S17-S31

2. Wintarsih O, Partosoedarmo M, dan Santoso P. A Comparative Study of Apical Leakage on Irrigation Using and Without EDTA. 2009. Vol. 58(2), p 14-19

3. Distel J W, Hatton J F, Gillespie M J. Biofilm Formation in Medicated Root Canals. Journal of Endodontics, 2002. Vol. 28(10)

4. Siqueira J F. Aetiology of Root Canal Treatment Failure: Why Well-Treated Teeth Can Fail. International Endodontic Journal, 2001. 34, 1–10

5. Mohamed J A, Huang D B. Biofilm Formation by Enterococci. Journal of Medical Microbiology. 2007. 1581–1588

6. Usha H L, Kaiwar A, Deepak M. Biofilm In Endodontics: New Understanding To An Old Problem. IJCD. 2010, 1(3)

7. Luis E C de P. Development of a Multispecies Biofilm Community by Four Root Canal Bacteria. JOE, 2012. 38(3):318-23.

8. Pujar M, Patil C, Kadam A. *Comparison of antimicrobial efficacy of Triphala, (GTP) Green tea polyphenols and 3% of sodium hypochlorite on Enterococcus faecalis biofilms formed on tooth substrate: in vitro.* JIOH. 2011. Vol 3(2).p. 23.

9. Shahani M N, Subba Reddy V V. *Comparison of Antimicrobial Substantivity of Root Canal Irrigants in Instrumented Root Canals up to 72 h: An in vitro study.* Journal of the Indian Society of Pedodontics & Preventive Dentistry; 2011, Vol. 29(1),

10. Kustarci A, Altunbas D, Akpinar K E. Comparative Study of Apically Extruded Debris Using One Manual and Two Rotary InstrumentationTechniques For Endodontic Retreatment. Journal of Dental Sciences 7. 2012.

11. Abidin T. *Inovasi Perawatan Konservasi Gigi Melalui Teknologi Tissue Engineering*. Universitas Sumatera Utara Medan. 2007

12. Ahuja V and Ahuja A. Apitherapy - *A sweet approach to dental diseases. Part II: Propolis*. Journal of Academy of Advanced Dental Research, 2011.Vol 2, p. 3, 6

13. Riyanti E, Hadidjah D, Iswari A P.. *Pemakaian Propolis Sebagai Antibakteri Pada Pasta Gigi.* Fakultas Kedokteran Gigi Universitas Padjadjaran, 2010. p.3

14. Agustrina G.. *Potensi Propolis Lebah Madu Apis Mellifera Spp Sebagai Bahan Antibakteri.* Departemen Biokimia Fakultas Matematika Dan Ilmu Pengetahuan Alam Institut Pertanian Bogor, p. 1-2, 5-7. 2011

15. Koo H, Pearson S K, Scott-Anne K, Abranches J, Cury J A, Rosalen P L, Park Y K, Marquis R E, Bowen W H. *Effects of apigenin and tt-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats.* Oral Microbiology Immunology: . 2003. Vol. 17, p. 339

16. Fokt *et al.* How does propolis prevent hive protection? The antimicrobial properties of propolis. Current research, technology and education topics in Applied Micro-biology and Microbial Biotechnology. 2010

17. Abishek *et al.* Propolis and its potential uses in oral health. Int Jou Med Sci. 2010. 2(7):210

18. Lotfy M. Biological Activity of Bee Propolis in Health and Disease. Asian Pasific Jou of Cancer. 2006

19. Gomes F, Leite B, Teixeira P, Oliveira R.. Strategies to control *Staphylococcus epidermidis* biofilms. Science against microbial pathogens: communicating current research and technological advances. 2011

The Antibiofilm Activity of Extract Propolis Against Biofilm Enterococcus Faecalis as Herbal Medicine Potential in Root Canal Treatment

ORIGINALITY REPORT

19%	14%	13%	9%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	znaturforsch.com Internet Source	1%
2	L.R. Rodrigues. "Interference in adhesion of bacteria and yeasts isolated from explanted voice prostheses to silicone rubber by rhamnolipid biosurfactants", Journal of Applied Microbiology, 3/2006 Publication	1%
3	www.jpbms.info Internet Source	1%
4	doaj.org Internet Source	1%
5	Submitted to Royal Australasian College of Dental Surgeons Student Paper	1%
6	Üstün, Y, BC Çanakçi, AN Dinçer, O Er, and S Düzgün. "Evaluation of apically extruded debris associated with several Ni-Ti systems",	1%

	International Endodontic Journal, 2014. Publication	
7	Submitted to King Saud University Student Paper	1%
8	www.publicscienceframework.org Internet Source	1%
9	www.devosendo.nl Internet Source	1%
10	www.sciencepublishinggroup.com Internet Source	1%
11	www.ukm.my Internet Source	1%
12	Morohoshi, T.. "Identification of quorum-sensing signal molecules and the LuxRI homologs in fish pathogen Edwardsiella tarda", Journal of Bioscience and Bioengineering, 2004 Publication	1%
13	unair.ac.id Internet Source	1%
14	Submitted to UC, San Diego Student Paper	1%
15	www.portaldeperiodicos.unisul.br Internet Source	<1%
16	tel.archives-ouvertes.fr Internet Source	<1%

17	Şimşek, Neslihan, Kerem Engin Akpinar, and Zeynep Sümer. "Evaluation of Bacterial Microleakage of Root Canals Irrigated with Different Irrigation Solutions and KTP Laser System", Photomedicine and Laser Surgery, 2012. Publication	<1%
18	tryl2012.blogspot.in Internet Source	<1%
19	repository.usu.ac.id Internet Source	<1%
20	jdr.sagepub.com Internet Source	<1%
21	Submitted to Universidad San Francisco de Quito Student Paper	<1%
22	journal.ugm.ac.id Internet Source	<1%
23	www.j3.jstage.jst.go.jp Internet Source	<1%
24	Submitted to University of Wales Institute, Cardiff Student Paper	<1%
25	www.unic.br Internet Source	<1%

26	mdpi.com Internet Source	<1%
27	docslide.us Internet Source	<1%
28	etheses.whiterose.ac.uk Internet Source	<1%
29	geneontology.org Internet Source	<1%
30	orca.cf.ac.uk Internet Source	<1%
31	hub.hku.hk Internet Source	<1%
32	Arunajatesan Subbiya, Kesavaram Padmavathy, Krishnan Mahalakshmi. " Evaluation of the Antibacterial Activity of three Guttapercha Solvents against ", The International Journal of Artificial Organs, 2018 Publication	<1%
33	pt.scribd.com Internet Source	<1%
34	irjponline.com Internet Source	<1%
35	Peter Murray. "A Concise Guide to Endodontic Procedures", Springer Nature America, Inc,	<1%

2015
Publication

36 Jose F. Siqueira. "Reaction of periradicular tissues to root canal treatment: benefits and drawbacks", Endodontic Topics, 3/2005 <1%
Publication

Exclude quotes	On	Exclude matches	< 5 words
Exclude bibliography	Off		

The Antibiofilm Activity of Extract Propolis Against Biofilm Enterococcus Faecalis as Herbal Medicine Potential in Root Canal Treatment

GRADEMARK REPORT

FINAL GRADE

/100

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4