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INHIBITORY TEST OF FLAVONOID PROPOLIS KELULUT EXTRACTS (*G. thorasica*) ON *Porphyromonas gingivalis* AS AN ETIOLOGIC FACTOR OF CHRONIC PERIODONTITIS

Ursula Valentina[1)*] , Beta Widya Oktiani[2)*] , Fransiska Uli Arta Panjaitan[3)*]
[1)]Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin - Indonesia
[2)]Department of Periodontics, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin - Indonesia
[3)]Department of Periodontics, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin - Indonesia

ABSTRACT

Background*: Natural ingredients have been used recently for its ability in curing various diseases and reducing the risk of antibiotic resistance. One of these natural ingredients is propolis. Bee propolis Geniotrigona thorasica from South Kalimantan has a strong antibacterial activity with active compounds such as flavonoids. Flavonoids can damage cell membranes and can fight the expansion of β-lactamase enzyme produced by gram-negative bacteria. Porphyromonas gingivalis is a gram-negative bacterium that causes chronic periodontitis with a prevalence of 80%.* ***Objective****: This study was to find out the inhibitory test of flavonoid propolis kelulut extract at the concentration of 0.1%; 0.3% and 0.5% on Porphyromonas gingivalis bacteria.* ***Method****: This type of research used a pure experimental method with post-test only and control group design. One-way ANOVA statistical test was performed with follow-up test of Post Hoc Dunnet's T3. Treatment was given to 4 groups with 5 repetitions. Observations were carried out using digital calipers in all groups after incubation for 24 hours with a temperature of 37°C.* ***Results****: One Way Anova and Post Hoc Dunnet's T3 showed significant differences in the diameter flavonoid propolis extract inhibitory zone against Porphyromonas gingivalis. The average value of Flavonoid extract inhibitory zone after 24 hours observation with a concentration of 0.5%; 0.3%; 0.1% and sterile distilled water were 25.24 mm; 18.04 mm; 13.58 mm and there was no inhibitory zone observed in sterile distilled water group.* ***Conclusion****: Extract of flavonoid propolis kelulut at the concentration of 0.1%; 0.3% and 0.5% can inhibit the growth of Porphyromonas gingivalis as an etiologic factor of chronic periodontitis.*

Keywords*: antibacterial activity, flavonoids, Porphyrmonas gingivalis, propolis*

Correspondence: Ursula Valentina, Faculty of Dentistry, Universitas Lambung Mangkurat, Jl Veteran No. 128B, Banjarmasin, South Kalimantan, email: ursulavalentina17@gmail.com

INTRODUCTION

The use of natural materials is lately increased to cure various diseases and reduce the risk of resistance that causes bacteria, parasites or viruses to be insensitive towards commonly used drugs. One of the natural ingredients that can be used is propolis. [1,2]

Propolis is usually associated with sticky bee glue which serves to protect the nest from adverse weather conditions by covering the inside of the walls, cavities and crevices. It is preserved in honeycomb preventing the infection of microorganism.[3] *Geniotrigona thorasica* (kelulut) bee is a type of stingless bee found in South Kalimantan with the lowest propolis toxicity due to the presence of non-toxic vegetative resins content.[4] Propolis has a strong antibacterial activity and has the ability to inhibit bacterial RNA polymeration.[5] Propolis contains compounds that have been identified as a derivate of flavonoids. Previous research obtained results that flavonoids propolis *Trigona sp*. extract from South Sulawesi with

0.1% and 0.5% flavonoid concentrations can inhibit *S. mutans* growth after incubation for 24 hours. Flavonoids are important chemical compounds found in propolis and have antibacterial properties.[6] Antibacterial activity of flavonoids can counteract the expansion of β-lactamase, a group of enzymes that can be produced by gram-negative bacteria.[7]

One type of gram-negative bacteria that can cause inflammation is *Porphyromonas gingivalis*. This bacterium is involved as an etiology in periodontal disease mechanism. *P. gingivalis* bacteria produce a variety of virulence factors that can penetrate the gingiva causing direct or indirect tissue damage and are found in 85.7% of subgingival plaque samples from patients with chronic periodontitis, a common form of periodontitis with a prevalence of around 80%.[8,9] This study aims to determine the inhibitory test of flavonoid propolis kelulut extract (*Geniotrigona thorasica*) against *P. gingivalis* which is the main etiology of chronic periodontitis.

METHODS AND MATERIALS

This study began with an ethical feasibility test which obtained research permit published by the Faculty of Dentistry, Lambung Mangkurat University No.112/KEPKG-FKGULM/EC/I/2019. True experimental laboratory study was conducted in this study by utilizing post-test only with control group design using four treatments with five repetitions based on the large sample formula of unpaired numerical analytical research. The sample in the study was propolis kelulut (*G. thorasica*) originating from Barabai, South Kalimantan.

The extraction process of flavonoid compounds began by weighing 1.5 kg of propolis, then put into a glass jar and soaked with 96% ethanol as a solvent for 5 days. Each immersion required 1.5 liters solvent which replaced every 24 hours and filtered with filter paper to separate filtrate and pulp into Erlenmeyer flask so that ±2.5 liters filtrate was obtained. The filtrate was subsequently evaporated using rotary evaporator at the speed of 180 rpm under 70^{o}C temperature so that the ethanol might evaporate and extracts with high viscosity could be attained (± 100 g).

The viscous extract obtained from the maceration process was continued with fractionation. Viscous propolis extract was dissolved with sterile distilled water. Extract was then poured into separating funnel and mixed with n-hexane solvent with equal volume as sterile distilled water. The mixture was then shook using separating funnel that two non-homogenous layer would be formed. After the sediment was formed, sterile distilled water solution was eliminated and ethyl acetate was added in separating funnel to be shaken and forming two separated layers. Ethyl acetate layers containing flavonoids were obtained and then the ethyl acetate layer was evaporated to obtain a thick flavonoid fraction.

The next step was to determine some concentrations of flavonoid propolis extract from G. thorasica 0.1%; 0.3% and 0.5%. For diluting propolis extract, 10% parental liquor was made by weighing 500 mg flavonoid extract with 5 ml 96% ethanol and mixed homogenously. The concentration of the flavonoid extract obtained was inserted into different tubes, after which the paper disk was immersed in each concentration for 2 hours. After that, the media for Porphyromonas gingivalis bacteria samples which had been bred on nutrient media to be inoculated into 1 ml of Brain Heart Infusion using a inoculating loop. After that, the culture of *Porphyromonas gingivalis* bacteria was added to sterile test tube until the turbidity was equal to 0.5 McFarland standard.

Porphyromonas gingivalis bacteria which had been standardized with Mc Farland 0.5 are applied with sterile cotton sticks on *Mueller Hinton Agar* (MHA) media. Furthermore, the paper disk that had been soaked was attached to the MHA media containing bacteria then MHA media was inserted into the desiccator and incubated in anaerobic state for 1x24 hours at 37°C. Diameter measurement from each zone of bacterial growth inhibition after 24 hours of incubation period was performed using digital calipers.

RESULT

The average value of inhibitory zone measurement in each treatment using flavonoid propolis kelulut (G.thorasica) extract on the growth of *Porphyromonas gingivalis* bacteria in the test media obtained the following results.

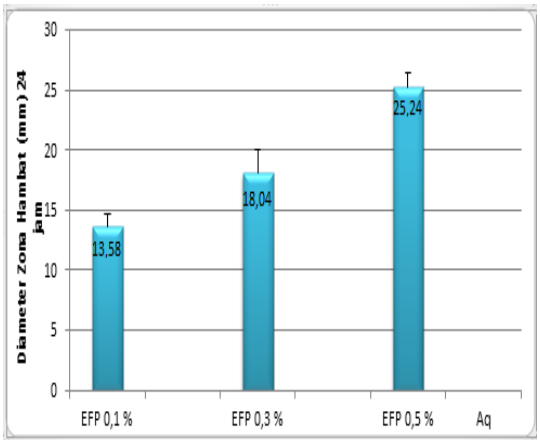

Figure 1. Bar diagram demonstrating the average inhibitory zone in each flavonoid propolis extract treatment group.

In Figure 1, it can be observed that the higher the extract concentration, the wider inhibitory zone diameter will be. The results showed that flavonoid propolis extract which was observed after 24 hours at a concentration of 0.5% had a wider inhibition zone of 25.24 mm while 0.3% concentration obtained 18.04 mm inhibitory zone. The smallest inhibitory zone was observed at the concentration of 0.1% in a diameter of 13.58 mm. No inhibition zone was formed in negative control treated with sterile distilled water.

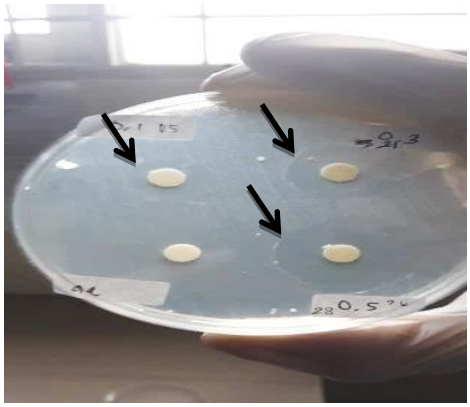

Figure 2. The inhibition zone formed around paper disk.

The testing results for bacterial inhibitory zone were analyzed statistically using SPSS 21. The normality of data distribution was evaluated using *Saphiro-Wilk* test and the result presented p = 0.591 in 0.1% propolis treatment group, p = 0.997 in 0.3% propolis extract treatment group and p = 0.881 in 0.5% extract treatment group. The data shows p>0.05 which means the data is normally distributed, followed by a test of variant homogeneity with Levene 's test which obtained p-value = 0.028 (p<0.05) after 24-hour observation which means that the data variance was non-homogenous.

Normally distributed data was further analyzed using One way ANOVA parametric test which resulted in p value equal to 0.000 (p<0.05). This means that there were significant differences in the inhibitory zone diameter of flavonoid propolis extract. When the data was normally distributed and homogenous, the analysis can be continued with the following test in the form of *Post Hoc Dunnett's T3* to find out which groups have significant differences. The results of the *Post Hoc Dunnett's T3* test can be seen in Table 2.

Table 2. The result of Post Hoc Dunnet's T3 analysis regarding Flavonoid Propolis extract treatment.

	Nilai p 24 jam			
	EFP 0,1%	EFP 0,3%	EFP 0,5%	Aq
EFP 0,1%	-	-	-	0,000*
EFP 0,3%	0,023*	-	-	0,000*
EFP 0,5%	0,000*	0,002*	-	0,000*
Aq	-	-	-	-

* : There are significant differences (p <0,05).

The results of *Post Hoc Dunnett's T3* test showed that there was a significant difference (p <0,05) in the inhibition zone diameter of *Porphyromonas gingivalis* bacteria as the result of 0.1% flavonoids propolis extract; 0.3% and 0.5% after 24 hours of incubation period.

DISCUSSION

This study was conducted to determine the inhibitory activity of flavonoid propolis kelulut (*G. thorasica*) extract on the growth of *Porphyromonas gingivalis* bacteria causing chronic periodontitis. The results showed that after observing the inhibitory zone at 24 hours with 0.1%; 0.3% and 0.5% concentration of flavonoid propolis extract (*G. thorasica*) originating from Barabai, South Kalimantan, it can be obtained that the extract has inhibitory ability against the growth of Porphyromonas gingivalis bacteria.

Porphyromonas gingivalis is the main gram-negative bacterium that causes chronic periodontitis.[10] The cell walls of gram-negative bacteria are surrounded by two membrane structures namely outer membrane consisting of lipopolysaccharide (LPS), phospholipid, lipoprotein and inner membrane which is composed of a double phospholipid membrane

structure.[11] Antibacterial activity of hydrophilic flavonoid compounds can easily penetrate LPS in gram-negative bacteria which causes reduced fluidity in the inner and outer regions of cell membranes.[12,13] Flavonoids also contain many hydroxyl groups that are polar in nature so that it will more easily penetrate the peptidoglycan layer resulting in membrane damage cells. Peptidoglycan itself is a component of gram-negative and gram-positive bacteria cell walls that protect cells from damage.[14,15,16] Flavonoids act as bactericidal and bacteriostatic by destroying the cytoplasmic membrane of the cell. The bacterial cytoplasm membrane itself has a pivotal role in energy conversion and nutrient transport regulation. If the transportation of this nutrients is disrupted then food cannot be produced into energy which will cause the breakdown of the cell.[17,18,19]

Measurements of the inhibition zone after 24 hours showed that 0.5% flavonoid propolis extract obtained 25.24 mm for the average inhibition zone and classified in very strong category (>= 20 mm). It is followed by 0.3% flavonoid propolis extract with 18.04 mm inhibitory diameter which classified in strong category (15-19 mm). Flavonoid propolis extract with the concentration of 0.1% was considered in medium category (8-14 mm) with 13.58 mm inhibitory diameter. Negative control using sterile distilled water demonstrated no inhibitory zone diameter which revealed that antibacterial activity is not affected by solvent factor.[20,21]

The result of flavonoid propolis kelulut extract study showed that there were significant differences in the average diameter of inhibitory zones in each treatment group, namely in the group of flavonoid extract concentrations of 0.5% compared to the concentrations of 0.3%, 0.1% and sterile distilled water, the concentration of 0.3% flavonoid extract compared to 0.1% concentration and sterile distilled water, and 0.1% flavonoid propolis extract group compared to the sterile distilled water control group which was observed after 24 hours incubation. The higher the given concentration, the greater the inhibitory zone around the paper disk will be formed. This is in accordance with the statement of Kavitha et al. (2012) and Roslizawaty et al. (2013) that the higher the concentration of the substance, the higher the number of active compounds in inhibiting bacterial enzymes activity and the greater the killing activity against bacteria.[22,23]

The flavonoid group contains phenol compounds. In high concentration, this compound can stimulate coagulation process within cellular proteins. During division stage, this activity can cause depletion in phospholipid layer around the cell so it can easily penetrate and damage the nucleus of bacterial cells. Conversely, low concentrations of phenol can merely damage cell membranes and cause leakage of the cells.[24,25]

Indoor sterilization should be reconsidered because it can induce contamination and can affect culture purity in the media. This may generate the entry of other undesirable microbes that interfere with the results expected in the study. Based on the research that has been done, it can be concluded that the extract of flavonoid propolis kelulut (*G. thorasica*) at the concentration of 0.1%; 0.3% and 0.5% can inhibit the growth of *Porphyromonas gingivalis* bacteria, with the broadest inhibition zone found at the concentration of 0.5% categorized in strong category.

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