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THE INHIBITORY ACTIVITY OF KELAKAI LEAF EXTRACT AGAINST THE GROWTH OF *Porphyromonas gingivalis* ATCC® 33277™

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ABSTRACT
Background: Chronic periodontitis is an infectious disease that causes damage on periodontal ligament and alveolar bone. The severity of periodontitis is caused by several types of bacterial species which one of them is *Porphyromonas gingivalis* bacteria with a prevalence of 85% in oral cavity. The extract of kelakai leaf contained antibacterial in the form of flavonoid, alkaloid, tannin, and steroid. Flavonoid consists of some chemical compounds which is one of them is *quercetin*. The level of *quercetin* in kelakai leaf is 503.56 mgQE/g. From some secondary metabolites, kelakai leaf has inhibitory power toward gram negative bacterial, *Porphyromonas gingivalis*. **Objective:** This research was intended to know the activity of inhibitory power of kelakai leaf toward *Porphyromonas gingivalis* bacteria. **Method:** This research was an experimental research consisted of 5 experimental groups that were group of kelakai leaf extract on the concentrations of 100 mh/ml, 75 mg/ml, 50mg/ml, and 25 mg/ml and the control group (0.2% chlorhexidine). Each treatment was done in 4 repetitions. The test of inhibitory power used diffusion method by measuring the inhibitory zone around the growth of *Porphyromonas gingivalis* on *Mueller Hinton Agar* media. The data were analyzed by using One Way Anova 95% and then continued with LSD. **Results:** Based on the LSD test, it was known that the extract of Kelakai leaf had inhibitor power activity toward *Porphyromonas gingivalis*. The highest inhibitory zone was on the concentration of 100 mg/ml with inhibitory zone of 14.61 mm. **Conclusion:** The extract of kelakai leaf had inhibitory power activity toward *Porphyromonas gingivalis* bacteria in vitro.

Keywords: 0.2% chlorhexidine, Diffusion method, Inhibitory power, *Stenochlaena palustris* extract, *Porphyromonas gingivalis.*

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INTRODUCTION

Periodontal disease is an inflammation occured on the support tissue of teeth. Chronic periodontal disease based on the result of *World Health Organitation* (WHO) was found in 15-20% adults aged 35-44 years old.[1] Periodontal disease in Indonesia is in second ranked, most after dental caries with a prevalence of almost 90%.[2] Periodontal disease included gingivitis and periodontitis.

Periodontitis causes damage to the alveolar bone and periodontal ligament. The severity of periodontitis is caused by the presence of pathogenic bacteria on the dental plaque and resulting in the formation of deep pockets and tooth shaking.[3] Bacteria play an important role in the process of periodontitis. *Porphyromonas gingivalis* is a gram negative species with a prevalence of 85% in the oral cavity of patients with periodontitis.[4]

Porphyromonas gingivalis is rod-shaped and black pigmented as a predominant bacterium against periodontitis. *Porphyromonas gingivalis* releases a chemical compound in the form of lipopolysaccharide (LPS) which is a powerful stimulant for host responses. These stimulants can increase excessive levels of *Polymorphonuclear* (PMN) and *Reactive oxygen species* (ROS), thus triggering tissue destruction in the gingiva, periodontal ligaments and alveolar bone.[5] Periodontitis can be prevented by the use of mouthwash.[6] One type of mouthwash that is often used in periodontal disease is 0.2%

chlorhexidine, which has antibacterial and antiplaque properties. Chlorhexidine is a broad-spectrum antibacterial agent, which has antifungal and antibacterial effect in the oral cavity. However, excessive side effects in the use of 0.2% chlorhexidine. It can cause burning sensation, discoloration of the teeth and uncomfortable.[7]

The use of chemical based mouthwash can provide side effects that are not good for the oral cavity. It is necessary to use herbal mouthwash as an alternative to traditional medicine which has minimum side effects.[8] One of the plants in Kalimantan which contains antibacterial, antioxidant and antifungal is *kelakai* (*Stenochlaena palustris*).[9] Kelakai plants have several compounds, one of which is quercetin The qurcetin level in kelakai leaf is 503.56 mgQE/ g.[10] Quercetin has the ability to reduce bacterial production by inhibiting DNA gyrase, thus preventing the occurrence of *supercoiled* DNA needed by bacteria for the transcription and replication of bacteria.[11.12]

The results of a research conducted by Roanisca (2018) stated that kelakai extract inhibited the growth of gram negative *E. coli* bacteria in concentrations of 10 mg/ml, 50 mg/ml, 25 mg/ml, and 100 mg/ml. Kelakai extract at a concentration of 12.5 mg/ml was able to inhibit *E. coli* with an inhibition of 5.28 mm.[13] Inhibitory activity was measured based on the amount of inhibitory zone formed around the paper disk, which had been planted with test bacteria in mm units. There was no information about the inhibitory activity of kelakai leaf extract with the concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml against the *Porphyromonas gingivalis* bacteria. Based on the review above, it was necessary to conduct research on the inhibitory activity of leaf extract with the concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml toward *Porphyromonas gingivalis* in vitro.

MATERIAL AND METHOD

This research was carried out in the Microbiology Laboratory of FMIPA Lambung Mangkurat University, Industrial Consultation Research Center, and FKG Microbiology Research Center Laboratory of Airlangga University. This research was begun with managing permits and ethical clearance issued by the Ethics Committee of the Faculty of Dentistry, Lambung Mangkurat University No.093/KEPKG-FKGULM/EC/XII/2018.

This research used a pure experimental method with post-test only with control group design. The treatment consisted of the preparation of kelakai leaf extract with concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml. The control treatment was 0.2% chlorhexidine. Each treatment was repeated four times. This research was conducted at the Microbiology Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya.

Kelakai leaf extract was made by using the maceration method, carried out by taking greenish-red color kelakai leaf as much as 2 kilo grams, clean washed with running water and then drained. The leaves were cut into small pieces with a size of ± 3 cm and dried using an oven at 40^{o}C for 4 hours. Kelakai leaf was mashed up with a blender, then sifted to fine powder or simplicia obtained ± 200 grams. Simplicia was macerated by adding 1 liter of 96% ethanol as a whole, soaking it all for 24 hours, and changing the solvent 3 times. Kelakai extract was filtered using flannel cloth and evaporated with a rotary evaporator at a temperature of 40-50 C for ± 4-6 hours until the liquid extract was obtained which experienced a shrinkage of 1/10 of its part, then transferred to the waterbath and were obtained blackish-red characteristics and obtained thick extract weighing as much as 150 ml of kelakai leaf extract.

Thick kelakai leaf extract was made with several concentrations, which were 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml using the formula V1 x C1 = V2 x C2. To make the required concentrations, 5 test tubes were prepare. Tube 1 for a concentration of 100 mg/ml containing 10 ml of kelakai leaf extract. Tube 2 for a concentration of 75 mg/ml containing 7.5 ml of a solution of kelakai leaf extract and 2.5 ml of distilled water. Tube 3 for a concentration of 50 mg/ml containing 5 ml of a solution of kelakai leaf extract and 5 ml of distilled water. Tube 4 for a concentration of 25 mg/ml containing 2.5 ml of a solution of kelakai leaf extract and 7.5 ml of distilled water. Tube 5 contained 10 ml of 0.2% chlorhexidine as a control group.

Bacterial culture was prepared by taking several colonies of *Porphyromonas gingivalis* that had been stored on the agar media with sterile ose, then planted on BHI media and incubated for 1x24 hours at 37^{o}C. The next step was homogenized the suspension by using the vortex for 30 seconds and was adjusted its turbidity according to the 0.5 Mac Farland standard which had the similar number of bacteria as ($1.5x10^{8}$ cfu/ml). If the turbidity was still not the same, then the media was diluted again with BHI media or added the bacteria to obtain the similar turbidity.

The inhibitory test of kelakai leaf extract was carried out by taking a suspension of bacteria that had been standardized with 0.5 Mac Farland which had a number of bacteria equivalent to ($1.5x10^{8}$ cfu/ml) applied with sterile cotton sticks on Mueller Hinton Agar (MHA) media. The paper disk was dropped by 10 microliters of kelakai leaf extract with concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 0.2% chlorhexidine as the control group. The paper disk that had been dropped was put on the

MHA media containing bacteria, then MHA was inserted into the Incubator and incubated in anaerobic state at 37^{o}C for 1x24 hours. The reading of the results of the inhibitory power was obtained by using a *caliper*.

RESEARCH RESULTS

Each treatment was tested by diffusion, using the paper disk and was conducted for 4 time repetitions. The measurement results of inhibitory zone of each treatment on the *Porphyromonas gingivalis* can be seen in the Table 1.

Concentration Group of Kelakai Leaf Extract	**Mean Diameter of Inhibitory Zone (mm)** ***Mean* ± Standard Deviation**
100 mg/ml	14.61 ± 0.11
75 mg/ml	11.88 ± 0.08
50 mg/ml	9.51 ± 0.21
25 mg/ml	7.21 ± 0.10
0,2% Chlorhexidine	18.11 ± 0.08

Table 1. Inhibitory Zone of Kelakai Leaf Extract and 0.2% Chlorhexidine on the Growth of *Porphyromonas gingivalis.*

The table 1 shows the data obtained from the research. Table 1 revealed the variation of inhibitory zone in the several treatments. The results of this research was known that kelakai leaf extract with a concentration of 100 mg/ml had a greater value of inhibitory zone than the concentrations of 75 mg/ml, 50 mg/ml and 25 mg/ml, but it had a smaller mean value of inhibitory power than 0.2% chlorhexidine.

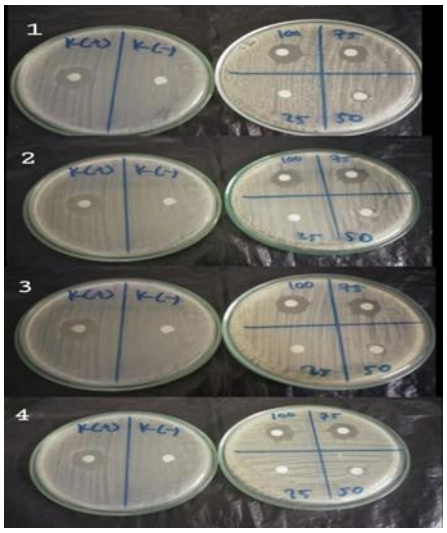

Figure 1. Measurement results of inhibitory zone in the repetitions 1, 2, 3 and 4.

Each treatment was carried out by the Shapiro-Wilk normality test because the number of samples was less than 50 (n <50). The results of the normality test, covered the significance value was above (p> 0.05), the results of the test of kelakai leaf extract with a concentration of 100 mg/ml was 0.798, the concentration of 75 mg/ml was 0.850, the concentration of 50 mg/ml was 0.310, the concentration of 25 mg/ml was 0.572 and the significance value of 0.2% chlorhexidine as the control group was 0.850. The data analysis was continued with Levene's test to find out the variance or homogeneity of the groups. The homogeneity test results showed the sig value=0.332 (p> 50) which meant as homogeneous data. The requirements of parametric test were the data must be normally distributed and homogeneous, so that the data were continued with the One-Way ANOVA parametric test with a 95% confidence level.

The results of the statistical analysis with One-Way ANOVA test of the 5 treatments showed the Sig value=0,000 (p <0.05) which meant that there were significant differences in the several treatment groups. This indicated that kelakai leaf extract inhibited the growth of *Porphyromonas gingivalis* in vitro. In the results of the LSD test, it can be seen that the inhibitory zone of kelakai leaf extract with concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 0.2% chlorhexidine were statistically difference.

DISCUSSION

The results of the research on the inhibitory activity of Kelakai leaf extract (*Stenochlaena palustris*) toward the growth of *Porphyromonas*

gingivalis showed that kelakai leaf extract with concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml inhibited *Porphyromonas gingivalis* bacteria through diffusion.

The resulted inhibitory zone was characterized by the formation of a clear zone that was brighter than the surrounding area. The formation of this clear zone was because Kelakai leaf extract contained secondary metabolites which inhibited the growth of *Porphyromonas gingivalis* bacteria. Numbers from the diameter of the inhibitory zone formed indicated the antibacterial strength of the extract used.[14] The clear zone consisted of several groups found by David and Stout (1971) in Lingga (2016), >20 mm clear zone formed was considered to have very strong inhibitory activity, 10-20 mm clear zone formed was stated to have a strong inhibitory activity, 5-10 mm clear zone formed was stated to have moderate inhibitory activity and <5 mm clear zone formed was said to have a weak inhibitory activity.[15]

Based on this classification, Kelakai leaf extract which included as strong group (100 mg/ml, 75 mg/ml) and medium group (50 mg/ml, 25 mg/ml) in inhibiting the growth of *Porphyromonas gingivalis* bacteria. Kelakai extract at a concentration of 100 mg/ml was the highest concentration that was able to inhibit the growth of *Porphyromonas gingivalis* compared to concentrations of 75 mg/ml, 50 mg/ml, and 25 mg/ml.

The inhibitory zone found in kelakai leaf inhibited the growth of *Porphyromonas gingivalis*. This is also in accordance with a research conducted by Roanisca (2018), the results showed that kelakai leaf extract with the content of flavonoid secondary metabolites at a concentration of 100 mg/ml was able to inhibit *E. coli* bacteria by damaging the structure of cell membranes that caused intracellular compounds to emerge from the bacteria. Fitriana's research (2018) of onion dayak leaf extract with the content of flavonoid was able to play a role in damaging the cell wall of *Porphyromonas gingivalis* bacteria. Flavonoid belongs to the hydrophilic group, which can easily penetrate the cell membrane of *Porphyromonas gingivalis* bacteria such as lipopolysaccharide.[16,17]

The kelakai leaf contained several ingredients, named tannin, flavonoid, Alkaloid, steroid. Tanin had antibacterial properties. Each compound had a diverse mechanism of action to inhibit bacterial work.[18] The mechanism of action of flavonoid as antibacterial is by inhibiting the function of energy metabolism from bacteria. When inhibiting the metabolic function of bacteria, flavonoid secreted extracellular proteins which later caused damage to the cell membrane of *Porphyromonas gingivalis* bacteria, followed by the release of the bacterial intracellular compounds.[19] Flavonoid had several chemical compounds, one of which is *quercetin*.[20] The level of *quercetin* in the kelakai leaf is 503.56 mgQE/g.[10] *Quercetin* had the ability to reduce bacteria as bacteriostatic, because of its capacity to inhibit ligation of D-Ala-D-Ala in bacterial cells.[11,20]

According to Cushnie (2017), the content of alkaloid was also believed to have an antibacterial effect by inhibiting DNA synthesis mechanisms. Alkaloid was heterocyclic nitrogen compounds that contained at least one nitrogen atom and was alkaline. The alkaline group experienced a reaction with the acid compound in the DNA which was the main constituent of the cell nucleus. The disruption of DNA synthesis caused bacteria to experience doubling difficulties. This caused a decrease in the number of bacteria.[21.22]

The antibacterial mechanism of tannin is by interfering with the transport of proteins found in the cell layer and activating enzymes. This compound also had target in cell wall, polypeptides. It is one of the most important substances in the formation of enzymes, so that, in forming, cell walls became imperfect. The presence of osmotic and physical pressure cause bacterial cells to become lysis and die. Another effect of tannin is also able to make bacterial cells unable to form by inhibiting topoisomerase DNA.[23]

The other content is steroid, antibacterial content that interferes with lipid membranes, which later resulted in leakage of liposomes. Steroid is also known to reduce cell membrane integrity and cell morphology due to the steroid properties of lipophilic compounds, which result in lysis and fragile cells.[24]

0.2% chlorhexidine as the control group showed different results in the concentration group, which had a higher antibacterial power compared to kelakai leaf extract with concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml. According to Abouassi et al (2014), 0.2% chlorhexidine inhibited antimicrobial activity. The action mechanism of 0.2% chlorhexidine is very effective in inhibiting the growth of gram-negative and gram-positive bacteria. This is influenced by the concentration used. The strength of the 0.2% chlorhexidine attachment to bacterial cell membranes is caused by the presence of a positive charge (cation) and most of the bacterial molecules are negatively charged (anions). 0.2% chlorhexidine causes changes in the permeability of cell membranes, which later secreted cell cytoplasm from bacteria.[25] This research concluded that kelakai leaf extract at concentrations of 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml had the ability to inhibit *Porphyromonas gingivalis*, but it is not equivalent to the antibacterial effect of 0.2% chlorhexidine.

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