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**COMPARISON OF KELAKAI AND KATUK EXTRACT COMBINATION TO
 0.2% CHLORHEXIDINE GLUCONATE AGAINST *Porphyromonas gingivalis***

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ABSTRACT

Background: Periodontal disease is the second most frequent oral disease in Indonesia, which includes periodontitis. *Porphyromonas gingivalis* is the predominant bacteria in chronic periodontitis. This disease is usually treated with 0.2% chlorhexidine gluconate mouthwash. However, 0.2% chlorhexidine gluconate has several adverse effects, such as tooth discoloration, irritation of the oral mucosa, and changes in taste sensation. Therefore, an herbal-based mouthwash is needed. A combination of kelakai leaf and katuk leaf extract contains antibacterial substances such as flavonoid, tannin, steroid, alkaloid, and saponin, which can be used as an alternative therapy for chronic periodontitis. **Purpose:** To compare the inhibitory zone of kelakai and katuk extract combination with 0.2 Chlorhexidine gluconate against *Porphyromonas gingivalis*. **Method:** True experimental design with 13 treatment groups and 3 repetitions for each group. The treatment groups include combinations of kelakai leaf extract with 25%, 50%, 75%, 100% concentrations and katuk leaf extract with 20%, 40%, 80% concentrations and 0.2% chlorhexidine gluconate, which were tested on *Porphyromonas gingivalis* with a total of 39 samples. The inhibitory zone is measured with caliper after incubated for 48 hours. **Results:** One-Way ANOVA revealed significant differences between all treatment groups and 0.2% chlorhexidine gluconate. Consequently, the Bonferroni post-hoc test was performed, which showed that the antibacterial activity of 75% kelakai leaf and 80% katuk leaf extract did not differ significantly from the activity of 0.2% chlorhexidine gluconate. **Conclusion:** The combination of 75% kelakai leaf and 80% katuk leaf extract has an equal antibacterial activity to 0.2% chlorhexidine gluconate against *Porphyromonas gingivalis* statistically.

Keywords : 0.2 % Chlorhexidine gluconate, chronic periodontitis, combination of kelakai and katuk leaf extract, inhibitory activity, *Porphyromonas gingivalis*

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Introduction

Periodontitis causes damage to the periodontal tissues and generates bone resorption¹. *Porphyromonas gingivalis* is considered as the main bacteria that cause periodontitis and was identified in 85.75% samples of patients with chronic periodontitis^{2,3}. It excreted various virulence factors, which degrades host defensive molecules and causes damage to the periodontal tissues and failure to regenerate, then lipopolysaccharides (LPS) will induce inflammatory process and stimulates osteoclasts to resorb bone, which leads to chronic periodontitis⁴. The gold standard therapy for chronic periodontitis

is 0.2% chlorhexidine gluconate. However, this agent has several adverse effects, such as tooth discoloration, irritation of the oral mucosa, and changes in taste sensation⁵. The community has started to shift from synthetic drugs to herbal medicine with minimal adverse effects. Herbal mouthwash can be derived from plants that are well-known by the people within the area⁶. Kalimantan has many typical wetland plants that are utilized as medicines, including kelakai and katuk.

Kelakai leaves are often consumed by the people to counter anemia, fever, diarrhea, and skin diseases. Kelakai leaves contain flavonoid, tannin, steroid, and alkaloid, which act as antibacterial

agents⁷. Flavonoid is predominant in kelakai leaf, which consisted of 166.1779 mgQE/g^{8,9}. Other than kelakai, katuk leaf is also renowned to increase breast milk production and to ease fever. Katuk leaves contain secondary metabolites, including saponin, tannin, flavonoid, steroid, and alkaloid as antibacterial and anti-inflammatory substances¹⁰. Flavonoid is also predominant in katuk leaf, with a total of 148.94 mgQE/g¹¹.

A study investigating kelakai leaf extract with 25%, 50%, 75%, and 100% concentrations against *Aggregatibacter actinomycetemcomitans* showed that the minimum inhibitory concentration (MIC) was at 25% concentration with an inhibitory diameter of 8.61 mm. Kelakai extract with 100% concentration has 14.45 mm inhibition zone. Kelakai extract still showed a lower diameter compared to positive control¹². A study researching the effect of katuk leaf extract with 5%, 10%, 20%, 40%, and 80% concentrations on *Escherichia coli* showed that the MIC was at 20% concentration. However, 80% katuk leaf extract alone could not compete with the inhibition zone of the positive control¹³.

Single preparations of kelakai leaf and katuk leaf had insufficient antibacterial activity compared to the positive control, thus a combination of both ingredients can be made. The combination is expected to give better results compared to single preparation, known as the synergistic effect. This effect is the result of interaction between secondary metabolites in plants, which contributes to better results compared to single preparation¹⁴.

Based on the background above, the author tested and compared the antibacterial activity of kelakai and katuk leaves combination extract with 0.2% *chlorhexidine gluconate* against *Porphyromonas gingivalis* using 25%, 50%, 75%, 100% kelakai extract and 20%, 40%, and 80% katuk extract. The parameter observed was the inhibition zone from all treatment on *Porphyromonas gingivalis* in mm.

MATERIALS AND METHOD

This study was conducted in Microbiology Laboratory of Natural Science Faculty, Lambung Mangkurat University, Industrial Consultation Research Center, and Microbiology Laboratory of Faculty of Dentistry Research Centre Airlangga University. The study was begun by obtaining permission and ethical clearance from the Ethical Committee of Faculty of Dentistry, Lambung Mangkurat University No. 026/KEPKG-FKGULM/EC/I/2020. The method used in this study was true experimental laboratory with post test only control group design using 13 treatment groups and 3 times repetition. The treatment groups consisted of combinations of kelakai leaf extract

with 25%, 50%, 75%, 100% concentrations and katuk leaf extract with 20%, 40%, 80% concentrations and 0.2% *chlorhexidine gluconate*, which were tested on *Porphyromonas gingivalis* with 39 total samples.

Kelakai and katuk leaves extract were produced using the maceration method in 96% ethanol. Four kilograms of leaves were used, which were cleaned and dried at room temperature, then put into the oven at 40°C for 4 hours. The leaves were subsequently made into powder, immersed with 96% ethanol for 24 hours while stirred occasionally. The extracts were filtered with a filter paper, evaporated, and heated in a waterbath to eliminate the solvent. A total of 15 grams pure kelakai extract and 14.8 grams katuk extract were obtained. Ethanol testing was conducted by adding a few drops of potassium dichromate. The extract was free from ethanol if there were no color changes. The concentration obtained was in accordance with the formula as follows:

$$V_1 \times N_1 = V_2 \times N_2$$

V_1 = baseline volume

N_1 = baseline concentration

V_2 = final volume

N_2 = final concentration

The combination of kelakai extract with 25%, 50%, 75%, 100%, and katuk with 20%, 40%, 80% concentrations, and 0.2% *chlorhexidine gluconate* were tested on *Porphyromonas gingivalis* ATCC®33277™ obtained from the Research Centre Microbiology Laboratory, Airlangga University. Pure isolates of *Porphyromonas gingivalis* were inoculated in a BHIB medium, then incubated for 2x24 hours at 37°C according to the 0.5 McFarland standard. The bacteria were smeared on MHA media and 0.01 ml of extract combination and positive control (0.2% *chlorhexidine gluconate*) were added to the media and incubated for 2x24 hours at 37°C. The resulting inhibition zone was measured with a caliper.

RESULTS

Table 1. The average inhibition zone diameter of kelakai and katuk leaf extract combination in comparison to 0.2% *Chlorhexidine gluconate* against *Porphyromonas gingivalis*.

Group	Mean±SD
EDKE 25% + EDKA 20%	7.25±0.05
EDKE 25% + EDKA 40%	11.46±0.07
EDKE 25% + EDKA 80%	12.20±0.05
EDKE 50% + EDKA 20%	8.15±0.05
EDKE 50% + EDKA 40%	13.38±0.07
EDKE 50% + EDKA 80%	15.60±0.05
EDKE 75% + EDKA 20%	9.15±0.05
EDKE 75% + EDKA 40%	14.13±0.07
EDKE 75% + EDKA 80%	16.51±0.10
EDKE 100% + EDKA 20%	10.11±0.08
EDKE 100% + EDKA 40%	14.45±0.05
EDKE 100% + EDKA 80%	16.85±0.05
CHX 0.2%	16.32±0.10

Description :

EDKE : Kelakai Leaf Extract

EDKA : Katuk Leaf Extract

CHX 0.2% : 0.2% *Chlorhexidine gluconate*

There were variety in inhibition zone between the combination of 25%, 50%, 75%, 100% kelakai leaf extract and 20%, 40%, and 80% katuk leaf extract in comparison to 0.2% *chlorhexidine gluconate* on the growth of *Porphyromonas gingivalis* that was served with 3 times repetition. The combination of 25% kelakai and 20% katuk extract already showed an inhibition zone, which means that antibacterial activity was evident even at the lowest concentration. The table also indicated that the largest mean diameter was found in 100% kelakai and 80% katuk extract with 16.85 mm.

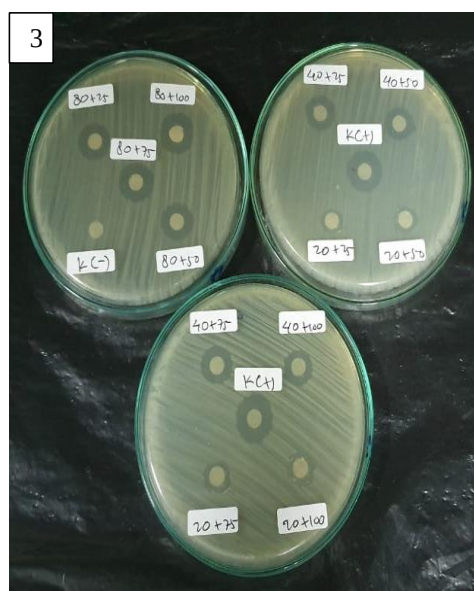
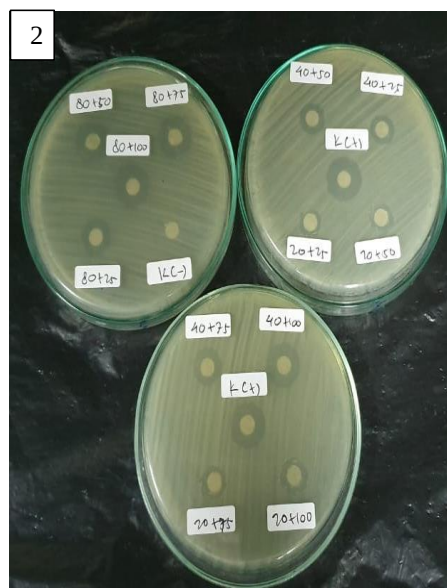


Figure 1. Inhibition zone of kelakai leaf and katuk leaf extract combination in comparison with 0.2% *Chlorhexidine gluconate* against *Porphyromonas gingivalis*.

The data were tested for normality using *Shapiro-Wilk* test and all groups were revealed to be normal, with $p > 0.05$. Data homogeneity was identified using *Levene's test*, resulted in $p > 0.05$. Normal and homogenous data were then tested with one-way ANOVA, which produced a significant difference between each group and 0.2% *chlorhexidine gluconate* p value = 0.000. *Bonferroni post-hoc* test was performed consequently, as seen in table 2.

Tabel 2. The result of *Bonferroni Post Hoc* of kelakai leaf and katuk leaf extract combination in comparison to 0.2% *Chlorhexidine gluconate* against *Porphyromonas gingivalis*.

Group	1	2	3	4	5	6	7	8	9	10	11	12	13
(1) EDKE 25% + EDKA 20%		0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(2) EDKE 25% + EDKA 40%			0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(3) EDKE 25% + EDKA 80%				0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(4) EDKE 50% + EDKA 20%					0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(5) EDKE 50% + EDKA 40%						0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(6) EDKE 50% + EDKA 80%							0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(7) EDKE 75% + EDKA 20%								0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
(8) EDKE 75% + EDKA 40%									0.000*	0.000*	0.001*	0.000*	0.000*
(9) EDKE 75% + EDKA 80%										0.000*	0.000*	0.009	0.122
(10) EDKE 100% + EDKA 20%											0.000*	0.000*	0.000*
(11) EDKE 100% + EDKA 40%												0.000*	0.000*
(12) EDKE 100% + EDKA 80%													0.000*
(13) CHX 0.2%													

* significantly different ($p < 0,05$)

The result of post-hoc test showed that all combinations of kelakai and katuk extract had significant differences with 0.2% *chlorhexidine gluconate*, except the combination of 75% kelakai and 80% katuk with $p = 0.122$ ($p > 0.05$).

DISCUSSION

Antibacterial comparison of kelakai and katuk extract combination to 0.2% *chlorhexidine gluconate* against *Porphyromonas gingivalis* showed that the combination of 25%, 50%, 75%, and 100% kelakai extract and 20%, 40%, and 80% katuk extract could produce inhibition zones on *Porphyromonas gingivalis*. The inhibition zone surrounding the treatment disk indicated that both plant extracts had antibacterial active substances, thus could reduce the growth of *Porphyromonas gingivalis*. The David-Stout method to measure antibacterial potency was determined from the diameter produced by antibacterial substances. Inhibition zone with less than 5 mm diameter was considered weak, inhibition zone with 5-10 mm diameter was considered moderate, inhibition zone with 10-20 mm diameter was considered strong, and more than 20 mm was considered very strong. Based on this method, the combination of 25% kelakai and 20% katuk extract, which was the lowest concentration in this study, showed 7.25 mm inhibition zone diameter and classified in moderate category. The results of this study showed moderate to strong inhibition zone categories (mean inhibition zones from 7.25 mm – 16.85 mm).

The addition of katuk leaf concentration enhance the diameter in agar media. The highest concentration of kelakai and katuk extract combination (100% and 80% respectively) had the highest mean of inhibition zone compared to other concentrations, and this concentration (100% and 80%) is higher than the positive control of 0.2% *chlorhexidine gluconate*. The combination of 25%, 50%, 75%, and 100% kelakai leaf with 40%, and 80% katuk leaf showed larger inhibition zone diameter compared to single kelakai leaf extract¹². The rise in the extract concentration may increase the content of active antibacterial substances, thus intensifying the ability to inhibit bacteria¹⁵. Other than that, the synergistic effect of both contents enhanced the antibacterial activity, thus extending the inhibition zone diameter¹⁴.

A combination of 75% kelakai leaf and 80% katuk leaf extract produced an inhibition zone that is statistically equal when compared to 0.2% *chlorhexidine gluconate*, as shown in *Bonferroni post-hoc* test. Therefore, the hypothesis of this study was accepted. This was possible due to the synergistic effect, whereas the effect of combination extract was higher than its single counterpart¹⁶. Antibacterial substances in kelakai and katuk leaf which can inhibit the growth of gram-negative bacteria were secondary metabolite components, such as flavonoid, alkaloid, steroid, and tannin, while katuk leaf had an additional secondary metabolite component of saponin. These substances also improve the ability to inhibit *Porphyromonas gingivalis* from both plants because saponin had better lipophilic property,

which attaches to the bacterial cell membrane, thus affecting physiological activity of the cells¹⁷.

A study on Tin leaf extract which has flavonoid as its secondary metabolite said that flavonoid could inhibit the use of oxygen by bacteria so that the metabolism process will be affected. Moreover, flavonoid could disrupt cell walls function of *Porphyromonas gingivalis* and lead to cell death¹⁸.

The working mechanism of alkaloid on *Porphyromonas gingivalis* is by disrupting the outer membrane of gram-negative bacteria, whereas the attachment to the peptidoglycan walls will inhibit the construction of cell walls that will inhibit the growth of bacteria. Alkaloid also affects virulence gene; thus, bacteria could not express their virulence factors and may reduce the formation of biofilm by bacteria¹⁹.

Steroid works by breaking down lipid layers which resulted in damaged liposomes. Steroid is known to interact with phospholipid membrane. The permeable property of steroid on lipophilic substances reduced membrane integrity, and leads to cell lysis due to a surfactant effect which can dissolve phospholipid components in the cell plasma membrane^{20,21}.

Tannin, as one of the components in kelakai and katuk, also possess antibacterial activity on *Porphyromonas gingivalis*. Tannin works on cell wall polypeptides, leading to imperfect cell wall formation. This will cause lysis of bacterial cells due to osmotic pressure or physical pressure, that leads to cell death²².

Chlorhexidine gluconate at 0.2% concentration as positive control showed the inhibition zone diameter at 16.32 mm. It was effective to inhibit the growth and kill gram-negative bacteria by disrupting bacterial cell permeability, causing cytoplasm to come out from the cells. Organelles with low molecular weight will penetrate cell membranes, causing cell death²³. Tannin, alkaloid, and steroid contents in kelakai leaf and katuk leaf extract had a similar antibacterial mechanism to 0.2% *chlorhexidine gluconate* and a substantial amount of flavonoid were responsible for equal inhibition zone to 0.2% *chlorhexidine gluconate*. One of the factors affecting the results of this study is the environmental factor, including nutrition, temperature, pH, and moisture. The combination of kelakai and katuk leaf extract has the potential for herbal mouthwash because it contains antibacterial substances that can inhibit *Porphyromonas gingivalis*. This study concludes that the combination of 75% kelakai leaf and 80% katuk leaf extract has an antibacterial activity that is statistically equal to 0.2% *chlorhexidine gluconate* against *Porphyromonas gingivalis*.

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