DENTINO
JURNAL KEDOKTERAN GIGI
Vol IV. No 2. September 2019

COMPARISON OF INHIBITORY ACTIVITY OF KELAKAI LEAVES EXTRACT WITH *Ciprofloxacin* AGAINST *Aggregatibacter actinomycetemcomitans* ATCC® 6514™

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ABSTRACT

Background: *Aggressive periodontitis is an inflammatory disease that exhibit rapid destruction of periodontal tissue and alveolar bone. The disease is caused by several factors, one of them is Aggregatibacter actinomycetemcomitans. The treatment to reduce the growth of Aggregatibacter actinomycetemcomitans is by giving spesific antibiotic like ciprofloxacin. Frequent and inappropriate use of antibiotics can cause side effects, so it needs alternative medicine that have antibacterial activity like kelakai leaves. Ethanol extract of kelakai leaves contains antibacterial compounds, such are flavonoid, tannin, steroid and alkaloid.* ***Objective:*** *The aim of this study was to compare the inhibitory activity of kelakai leaves extract (Stenochlaena palustris) with ciprofloxacin against Aggregatibacter actinomycetemcomitans.* ***Method:*** *This study was true experimental design with post-test only design. The inhibitory activity test was performed using diffusion method in 5 treatment groups and 4 repetitions. The treatment groups were kelakai leaf extract with 25%, 50%, 75%, 100% concentrations and 5µg ciprofloxacin. Inhibitory activity was measured by calculating the diameter of the clear zone (mm) that was formed on MHA.* ***Results:*** *The highest inhibition zone of kelakai leaf extract which found at concentration of 100% was 14.45 mm and the inhibition zone of ciprofloxacin was 26.28 mm. One way Anova test result (P = 0,000) and post hoc LSD test proved that there were significant differences of inhibition zones in each treatment group.* ***Conclusion:*** *Ethanol extract of kelakai leaves with concentration of 25%, 50%, 75% and 100% have inhibitory activity against Aggregatibacter actinomycetemcomitans but it is not as strong as ciprofloxacin.*

Keywords: *Aggregatibacter actinomycetemcomitans, ciprofloxacin, ethanol extract of kelakai leaves, inhibitory activity.*

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INTRODUCTION

Oral health is one of the important things that supports the whole body health. If the health of the oral cavity decreases, the systemic health and daily activities will also disrupt.[1] One of several problems in the oral cavity is periodontal disease, such as aggressive periodontitis. The average incidence of aggressive periodontitis in developed countries is around 0.1% and in developing countries, it is around 5%, while the average prevalence in Indonesia is 8%.[2]

Aggressive periodontitis can be caused by several factors, one of them is the *Aggregatibacter actinomycetemcomitans*. This bacterium presents in approximately 90% of aggressive periodontitis case.[3] *Aggregatibacter actinomycetemcomitans* is a gram negative cocobacillus, around 0.4-0.5x1.0-1.5 µm in size.[4,5] Clinical features of aggressive periodontitis are rapid inflammation of periodontal tissue, alveolar bone destruction and loss of connective tissue attachment at the permanent first molars and incisors or all of the permanent teeth.[6]

One of the treatment for aggressive periodontitis is by giving a spesific antibiotic such as ciprofloxacin. Frequent and inappropriate use of antibiotics can cause side effects, such are hypersensitivity, toxic reaction, and resistance in bacteria. Based on that statement, it is necessary to take alternative medicine from nature that does not cause side effect but have the same antibacterial activity with ciprofloxacin, like kelakai leaves.[5,7]

Ethanol extract of kelakai leaves contains antibacterial compounds such are flavonoid, tannin, steroid and alkaloid. Based on Erwin et al reasearch (2016), ethanol extract of kelakai leaves at concentration of 10%, 15%, 20%, 25% dan 30% have inhibitory activity against *Escherichia coli*.[8] Until today, there has been no research about the inhibitory activity of kelakai leaves extract against bacteria in the oral cavity, *Aggregatibacter actinomycetemcomitans* yet, so it is necessary to investigate the comparison of inhibitory activity of kelakai leaves extracts with ciprofloxacin against *Aggregatibacter actinomycetemcomitans* in vitro.

MATERIAL AND METHODS

The study was conducted at the Laboratory Research Center, Faculty of Dentistry, Universitas Airlangga, Surabaya. This research began with the preparation of research permits and ethical clearance issued by the Ethics Committee of the Faculty of Dentistry, Universitas Lambung Mangkurat No. 098/KEPKG-FKGULM/EC/XII/ 2018. The research was a pure research experimental (true experimental) design with post-test only design. Samples of there study were the *kelakai* leaves extract with concentrations of 25%, 50%, 75%, 100% and ciprofloxacin. Repetitions of each treatment group in this research were obtained using the Federer formula. That were 4 times and the total samples were 20 samples.

The extraction of kelakai leaves ethanol extract

The *kelakai* leaves, used in this study, were taken from the Anjir of Barito Kuala District, South Kalimantan Province of 2 kg with the criteria of young to young adult leaves. Young leaves were red, while young adult leaves were greenish red. The *kelakai* leaves was washed and cut into small pieces, around ±3 cm in size. It was dried at room temperature which is 40^{o}C for 4 hours and mashed into powder with a blender. The maceration process was carried out by mixing one liter of ethanol 96% solution in 200g of simplicia powder for 1x24 hours while it was stirred manually or with a shaker. The processed powder was kept for 24 hours until it was settled. Then, the extract was filtered with flannel cloth and filter paper and was evaporated using a rotary evaporator (Heidolph) at a low pressure of 40-50^{o}C for 4-6 hours then concentrated on the waterbath (SMIC®) to obtain a 100% concentrated solvent. The next step was ethanol-free trial by adding potassium dichromate ($K_2Cr_2O_7$). If there was no color change, then the kelakai leaves extract stated as free-ethanol.

Ethanol extract of *kelakai* leaves with a concentration of 100% was diluted to various concentrations of 75%, 50% and 25%. The diluent that used was aquades. Dilution of *kelakai* ethanol extract was carried out by the formula:

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

Note:
V_1 = Volume of stock solution needed to make the new solution
N_1 = Concentration of stock solution
V_2 = Final volume of new solution
N_2 = Final concentration of new solution

Manufacturing the suspension of *Aggregatibacter actinomycetemcomitans* bacteria on BHIB media

The bacterium that used in this study was originated from the culture of *Aggregatibacter actinomycetemcomitans* ATCC® 6514™, which was available at the Laboratory Research Center of the Faculty of Dentistry, Universitas Airlangga, Surabaya. Some of the colonies from *Aggregatibacter actinomycetemcomitans* were planted on Brain Heart Infusion Broth media and incubated for 1x24 hours at 37^{o}C. The next step was to homogenize the suspension using vortex for 30 seconds and adjusted the turbidity according to the 0.5 Mc Farland standard (1.5x10^{8} CFU/ml).

The inhibition test of *kelakai* leaf extract and ciprofloxacin against *Aggregatibacter actinomycetemcomitans*

The inhibition test was done by inoculating the standardized *Aggregatibacter actinomycetemcomitans* bacteria with Mc

Farland solution ($1.5x10^8$ CFU/ml) on Mueller Hinton media using sticky cotton. The *kelakai* leaf extract was dropped with a concentration of 25%, 50%, 75%, 100%, also on the paper disk of 10 µL. A paper disk containing *kelakai* leaves extract and disc oxoid ciprofloxacin of 5 µg were placed on MHA media. The next step was incubating MHA media for 24 hours at 37°C then reading the results of the size of the bacterial growth inhibition zone using a caliper

RESULT

Based on the research, inhibitory zone of *kelakai* leaves extract at concentration of 25%, 50%, 75% 100% and ciprofloxacin against *Aggregatibacter actinomycetemcomitans* in all groups, as pictures and tables below.

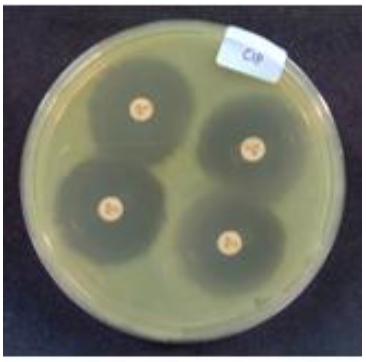

Figure 1. The inhibition zone formed around the disk containing ciprofloxacin in each repetition

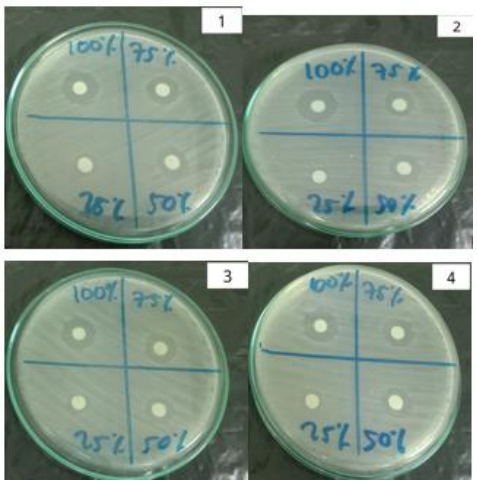

Figure 2. The inhibition zone formed around the disk containing *kelakai* leaf extract at concentration 25%, 50%, 75% and 100% in each repetition

Table 1. Diameter of Inhibitory zone of kelakai leaves extract and c*iprofloxacin* at each repetition (in mm)

No.	EDK 25%	EDK 50%	EDK 75%	EDK 100%	CIP
1.	8,80	10,40	12,50	14,15	26,07
2.	8,35	10,20	12,35	14,80	26,09
3.	8,80	10,50	12,45	14,20	26,28
4.	8,50	10,90	12,85	14,65	26,89

Note:
EDK25% : Ekstrak Daun Kelakai 25%
EDK50% : Ekstrak Daun Kelakai 50%
EDK75% : Ekstrak Daun Kelakai 75%
EDK100% : Ekstrak Daun Kelakai 100%
CIP : *Ciprofloxacin*

Table 2. Mean value and standard deviation of inhibitory zone from kelakai leaves extract and c*iprofloxacin*

	Mean ± SD
EDK25%	8.61 ± 0.22
EDK50%	10.5 ± 0,29
EDK75%	12.53 ± 0,21
EDK100%	14.45 ± 0,32
CIP	26.28 ± 0,28

The collected data of the average of inhibitory zones were tabulated, followed by normality test using *Saphiro-Wilk* and homogeneity test using *Levene's test*. The result of normality test for kelakai leaves extract at 25% concentration (p = 0,22), kelakai leaves extract at 50% concentration (p = 0,73), kelakai leaves extract at 75% concentration (p = 0,31), kelakai leaves extract at 100% concentration (p = 0,20) and ciprofloxacin (p = 0,20) showed p > 0,05, so the data was normally distributed. The data were tested with homogeneity test and obtained for inhibitory zone of kelakai leaves extract and ciprofloxacin is p = 0,76 (p > 0.05) which means the data distribution is homogeneous. Normally distributed and homogeneous data were then managed with *One-way ANOVA* parametric analysis with 95% confidence level.

Table 3. Result Test of *One Way Anova* for inhibitory zone of kelakai leaves extract and ciprofloxacin

	Group	P Value
Inhibitory Zone	EDK25%	0,000
	EDK50%	
	EDK75%	
	EDK100%	
	CIP	

One-way ANOVA test result on table 3 showed that p = 0.000 (p < 0,05), which indicated that there were significant differences between treatment groups. Then, *Post-hoc LSD* was done to know which of the group that showed any difference.

Table 4. Result test of *Post Hoc LSD* for inhibitory zone of kelakai leaves extract and ciprofloxacin

	EDK 25%	EDK 50%	EDK 75%	EDK 100%	CIP
EDK 25%		0,000*	0,000*	0,000*	0,000*
EDK 50%	0,000*		0,000*	0,000*	0,000*
EDK 75%	0,000*	0,000*		0,000*	0,000*
EDK 100%	0,000*	0,000*	0,000*		0,000*
CIP	0,000*	0,000*	0,000*	0,000*	

Note:
*: There is a significant difference (p < 0,05)

From table 4, it can be seen that each of kelakai leaves extract group and ciprofloxacin showed significant difference.

DISCUSSION

The results of the inhibitory zone measurement in table 1 and table 2 showed that *kelakai* leaf extract have inhibitory zones, which gradually increase according to its concentrations. The highest inhibition zone was found in the ethanol extract of *kelakai* leaves with 100% concentration. This was because the higher the concentration of an extract, the more active substances will be contained in it.[10] The size of the inhibitory zone indicates that the inhibitory power of a compound is weak, medium, strong or very strong. Based on the David & Stout classification, < 5 mm inhibition zone is classified as weak, the 5-10 mm inhibition zone is immediate, the 10-20 mm inhibition zone is classified as strong and the > 20 mm inhibition zone is classified as very strong.

Based on this classification, the average inhibition zone of *kelakai* leaf extract with 25% concentration is 8.61 mm was indicated that it is classified as moderate. The average inhibition zone in 50% *kelakai* leaf extract was 10.5 mm, 75% was 12.53 mm and 100% was 14.45 mm, meant as relatively strong. The zone of ciprofloxacin inhibition was 28.26 mm which indicated the inhibition was very strong. This showed that the ciprofloxacin inhibition zone is stronger than the *kelakai* leaf extract at the highest concentration. This might be caused by the difference in the antibacterial mechanism between the ethanol extract of *kelakai* leaves and ciprofloxacin while inhibiting the growth of *Aggregatibacter actinomycetemcomitans*.

The antibacterial mechanism of a compound can affect the inhibitory ability when inhibiting or killing bacteria.[12] The property of *kelakai* leaf extract to inhibit bacteria is influenced by antibacterial content named quercetin flavonoid, alkaloid, tannin and steroid.[13,14]

The research by Chear et al. (2016) mentioned that *kelakai* leaf extract has antibacterial property, named quercetin with a percentage of about 50%.[13] Quercetin is capable of being antibacterial, because it can increase the permeability of cell membrane and form complex compound, that can cause cell membrane become lysis. Quercetin also has bacteriostatic ability because it can inhibit the formation of D-alanine ligation enzyme, so that the bacterial growth is decreased.[15]

The mechanism of the alkaloid as an antibacterial is affected by the presence of OH groups and the nitrogen content. The OH group is able to penetrate the lipopolysaccharide layer on the cell membrane of gram negative bacteria. It can cause depolarization of the cell membrane, protein denaturation and escalation of membrane permeability. The nitrogen content in alkaloid compound can react with amino acid in bacterial cells. This reaction changes the balance of the bacterial's DNA chain, resulting in death of bacterial cells.[16,17,18]

The mechanism of the tannin as an antibacterial is caused by the presence of various chemical structures of tannin, which are

capable of forming hydrogen bond along with the protein in bacterial cell membranes. The bond causes denaturation of protein, so that the cell membrane becomes damage. This damage will reduce metabolism in bacteria and inactivate the bacterial enzyme system. Tannin is also able to form a bond with Ca^{2+} ion in gram negative bacteria. It affects the permeability of cell membranes, which results in the inhibition of important substances absorption for bacterial growth.[19,20]

The steroid interacts with bacterial cell phospholipid membrane, that are permeable to lipophilic compound. The interaction between steroid and phospholipid membrane can cause decrease in the membrane integrity and change in the morphology of the cell membrane, then the cell becomes brittle and lysis.[21]

The action of ciprofloxacin is by inhibiting the replication and transcription of bacterial DNA through the prevention of the gyrase DNA enzyme. Ciprofloxacin can also stop the initiation of replication, reduce the amount of replication and inhibit bacterial mDNA transcription.[22,23] The research that conducted by Hangas et al. (2018) also showed that ciprofloxacin affected cell growth and differentiation, and reduced almost all enzyme activities in bacteria.[24] It can be concluded that the ethanol extract of *kelakai* leaves with 25%, 50%, 75% and 100% concentration was able to inhibit the growth of *Aggregatibacter actinomycetemcomitans* bacteria, but it was not as strong as the inhibitory activity of ciprofloxacin.

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