ANTIBACTERIAL ACTIVITY OF KELAKAI AND KATUK LEAVES EXTRACT COMBINATION IN COMPARISON TO 1% Povidone iodine AGAINST Aggregatibacter actinomycetemcomitans

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ABSTRACT

Background: Aggregatibacter actinomycetemcomitans is one of the causes of aggressive periodontitis. Povidone iodine mouthwash at 1% concentration can be used to reduce the growth of these bacteria, but long-term use may lead to side effects that initiate the need for an alternative herbal mouthwash. Kelakai leaves contain antibacterial substances such as flavonoid, tannin, alkaloid and steroid, while katuk leaves contain flavonoid, tannin, alkaloid and saponin. Combination of these two extracts can be used to increase the effectiveness produced. Objective: To prove that the antibacterial activity of kelakai and katuk leaves extract has the same effectiveness as 1% Povidone iodine against Aggregatibacter actinomycetemcomitans. Methods: True experimental method with post-test only and control group design was applied in this study. The antibacterial activity test was performed using diffusion method in 13 treatment groups with triple repetition. The total samples used were 39 samples. The treatment groups were the combination of kelakai leaves extract with 25%, 50%, 75%, 100% concentration and katuk leaves extract with 20%, 40%, 80% concentration, and 1% Povidone iodine. The parameter measured was the inhibitory zone (mm). Results: Combination of 100% kelakai leaves and 80% katuk leaves extract has the highest inhibitory zone of 17.33 mm. Post Hoc Bonferroni test results showed that the combination of 75% kelakai leaves and 80% katuk leaves extract were the only group that had no significant difference to 1% Povidone iodine. Conclusion: Combination of 75% kelakai leaves extract and 80% katuk leaves extract is statistically equivalent to 1% Povidone iodine against Aggregatibacter actinomycetemcomitans.

Keywords: Aggregatibacter actinomycetemcomitans, inhibitory zone, katuk leaves extract, kelakai leaves extract, 1% Povidone iodine.

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INTRODUCTION

Aggressive periodontitis is a type of periodontitis characterized with prompt bone destruction and loss of attachment with minimal plaque involvement. One of the causes of aggressive periodontitis is Aggregatibacter actinomycetemcomitans bacteria.¹ This bacteria is an opportunistic pathogen and a part of normal flora colonizing on the surface of oral mucosa, teeth and oropharyng.² Aggregatibacter actinomycetemcomitans plays a role in the development of aggressive periodontitis as the consequence of its virulence which promotes colonization and inflammation, generates tissue destruction and inhibits host tissue repair.³

Treatments of aggressive periodontitis can be done with local therapy such as the use of mouthwash.⁴ Povidone iodine with 1% concentration is a broad spectrum antiseptic mouthwash that is proven to be effective in resolving problems in the oral cavity due to its ability to attack pathogenic periodontal bacteria at low concentration.⁵ However, long term use of
1% Povidone iodine cause some side effects such as sensitivity, local erythema, pain, mucosal erosion and impairment of thyroid gland functions.©

Herbal mouthwash derived from plants that contain antibacterial properties can be used since the components exert less significant side effects. Kelakai (Sienochlaena palustris) is a typical swamp plant which grows in South Kalimantan. The leaves is commonly used as herbal medicine by the local community because it contains antibacterial substances such as flavonoid, tannin, alkaloid and steroid.© The predominant metabolite found in kelakai leaves is flavonoid with a total of 166.17 mgQE/g.©

Katuk (Sauropus androgynous) is a type of plant that grows in tropical area. The leaves have been commonly used as herbal medicine for its antibacterial potency presented by the bioactive substances including flavonoid, tannin, alkaloid and saponin.© Flavonoid is the main active substance in katuk leaves with a total of 148.94 mgQE/g.©

According to a study by Setyorini et al. (2019), kelakai leaves extract with 25%, 50%, 75% and 100% concentration can inhibit the growth of Aggregatibacter actinomycetemcomitans but still not as effective as 1% Povidone iodine against gram negative bacteria as reported in a study by Montevacchi et al. (2013). Combination of kelakai and katuk leaves extract can be used to achieve higher antibacterial activity. There has not been any study regarding the antibacterial activity of kelakai and katuk leaves extract combination against Aggregatibacter actinomycetemcomitans up to this day, therefore it is necessary to investigate the antibacterial activity of combination of kelakai and katuk leaves extract compared with 1% Povidone iodine against Aggregatibacter actinomycetemcomitans in vitro.

**MATERIALS AND METHODS**

This study was conducted in Research Center Laboratory, Faculty of Dentistry, Airlangga University, Surabaya. This study has obtained research and ethical approval from the Ethical Committee of Faculty of Dentistry, Universitas Lambung Mangkurat No. 015/KEPKG-FKGULM/EC/1/2020. This study employed a true experimental laboratory method with post-test only and control group design using 13 treatment groups. The number of repetitions for each group was 3 times which calculated using the Federer formula. The sample size used was 39 samples. Samples used were the combination of kelakai leaves extract with 25%, 50%, 75%, 100% concentration and katuk leaves extract with 20%, 40%, 80% concentration and also 1% Povidone iodine.

**Extraction of kelakai and katuk leaves**

Kelakai leaves for this study were obtained from Anjir, Barito Kuala District, while katuk leaves were obtained from Gambut sub-district, Banjar district, South Kalimantan. Kelakai leaves and katuk leaves were each taken in a total of 4 kg with criteria of young leaves. The leaves were then cleaned and cut into small pieces. The leaves were dried at room temperature and put in an oven with a temperature of 40°C for 4 hours, then blended until it formed a simplicial powder. The method used was maceration, that is mixing 96% ethanol solution with simplicial powder while stirring the mixture using a shaker for 1x24 hours. The immersion was carried out for 24 hours until the mixture turned into sedimentation. The extract was filtered and the solvent was evaporated with rotary evaporator at low pressure with a temperature of 50-60°C for 4-6 hours, then the extract was heated on a waterbath until it generates 15 g of pure kelakai extract and 14.8 g of pure katuk extract. Ethanol free test was carried out by adding potassium dichromate (K₂CrO₄).

Ethanol extract of kelakai leaves with a concentration of 100% was diluted with aquades to obtain 25%, 50% and 75% concentration, while katuk leaves extract was diluted into 20%, 40% and 80% concentration. The concentration obtained was calculated using the following formula:

\[ V_1 \times M_1 = V_2 \times M_2 \]

Note:
- \( V_1 \) = Volume of stock solution required to make the new solution
- \( M_1 \) = Concentration of stock solution
- \( V_2 \) = Final volume of new solution
- \( M_2 \) = Final concentration of new solution

**Antibacterial activity test of combination of kelakai and katuk leaves extracts against Aggregatibacter actinomycetemcomitans**

The bacteria used in the study was an isolate of Aggregatibacter actinomycetemcomitans ATCC® 43718™ obtained from Research Center Laboratory, Faculty of Dentistry, Airlangga University, Subaraya. Bacterial colony of Aggregatibacter actinomycetemcomitans were inoculated in Brain Heart Infusion Broth media and incubated for 2x24 hours in a temperature of 37°C. Bacteria suspension turbidity was then homogenized using Mc Farland standard of 0.5 (1.5 x 10⁶ CFU/ml) and smeared on Mueller Hinton Agar media with a sterile cotton swab.

Combination of kelakai leaves extract with 25%, 50%, 75%, 100% concentration and katuk
leaves extract with 20%, 40%, 80% concentrations as many as 0.01 ml each were dripped on a paper disk with a micropipette. The paper disk were placed on MHA media using tweezers and incubated in a temperature of 37°C for 24 hours. The inhibitory zone of bacterial growth was measured using caliper.

RESULTS
The results for inhibitory zone measurement of kelakai extract at 25%, 50%, 75%, and 100% concentration combined with katuk leaves extract at 20%, 40%, and 80% concentration whilst compared to 1% Povidone iodine against Aggregatibacter actinomycetemcomitans on all treatment groups is presented on the following tables and picture.

Table 1. The Average Diameter (mm) for Inhibition Zone of Kelakai and Katuk Leaves Extract Combination in Comparison to 1% Povidone iodine against Aggregatibacter actinomycetemcomitans.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>EDKE 25% + EDKA 20%</td>
<td>7.30 ± 0.22</td>
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<tr>
<td>EDKE 25% + EDKA 40%</td>
<td>12.18 ± 0.07</td>
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<tr>
<td>EDKE 25% + EDKA 80%</td>
<td>14.02 ± 0.10</td>
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<td>EDKE 50% + EDKA 20%</td>
<td>8.16 ± 0.07</td>
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<td>EDKE 50% + EDKA 40%</td>
<td>13.31 ± 0.10</td>
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<tr>
<td>EDKE 50% + EDKA 80%</td>
<td>14.43 ± 0.21</td>
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<td>EDKE 75% + EDKA 20%</td>
<td>9.13 ± 0.12</td>
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<td>EDKE 75% + EDKA 40%</td>
<td>13.55 ± 0.05</td>
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<tr>
<td>EDKE 75% + EDKA 80%</td>
<td>16.58 ± 0.76</td>
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<td>EDKE 100% + EDKA 20%</td>
<td>10.11 ± 0.07</td>
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<tr>
<td>EDKE 100% + EDKA 40%</td>
<td>14.32 ± 0.10</td>
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<tr>
<td>EDKE 100% + EDKA 80%</td>
<td>17.33 ± 0.76</td>
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<tr>
<td>PVP-I 1%</td>
<td>16.88 ± 0.07</td>
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Note: EDKE : Kelakai Leaves Extract  
EDKA : Katuk Leaves Extract  
PVP-I 1% : 1% Povidone iodine

According to the data above, it is known that a combination of 100% kelakai leaves extract and 80% katuk leaves extract has the highest mean inhibitory zone, that is 17.33 mm. The inhibitory zone for 100% kelakai leaves extract combination with 80% katuk leaves extract exceeded 1% Povidone iodine which has a mean inhibitory zone of 16.88 mm. The treatment group which also has a similar mean inhibitory zone with 1% Povidone iodine is combination of kelakai leaves extract with 75% concentration and katuk leaves extract with 80% concentration with a mean inhibitory zone of 16.58 mm.

Data distribution in each treatment groups was analyzed for the normality using Saphiro-wilk test and homogeneity using Levene’s test. Normality test on all treatment groups showed a p value>0.05, therefore all data were distributed normally.

Homogeneity test result showed that combination of kelakai leaves extract and katuk leaves extract compared to 1% Povidone iodine obtained a p value = 0.061 (p>0.05), which means that the variant between groups were homogenous.

Normally-distributed and homogenous data were then analyzed using parametric analysis of One Way Anova with 95% confidence interval. The result for One Way Anova test showed a p value=0.000 (p<0.05), which indicates that there is a significant difference between each treatment groups. The data were then analyzed using Post Hoc Bonferroni to determine the significant difference in each treatment groups.
Table 2. The Result of Post Hoc Bonferroni for Antibacterial Activity of Kelakai and Katuk Leaves Extract Combination in Comparison to 1% Povidone iodine against Aggregatibacter actinomycetemcomitans.

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<th>Treatment</th>
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<td>(1) EDKE 25% + EDKA 20%</td>
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<td>(3) EDKE 25% + EDKA 80%</td>
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<td>(4) EDKE 50% + EDKA 20%</td>
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<td>(5) EDKE 50% + EDKA 40%</td>
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<td>(6) EDKE 50% + EDKA 80%</td>
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<td>(7) EDKE 75% + EDKA 20%</td>
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<td>(8) EDKE 75% + EDKA 40%</td>
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<td>(9) EDKE 75% + EDKA 80%</td>
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<td>(10) EDKE 100% + EDKA 20%</td>
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<td>(11) EDKE 100% + EDKA 40%</td>
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<td>(12) EDKE 100% + EDKA 80%</td>
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<td>(13) 1% Povidone iodine</td>
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Note: *: Significant differences (p<0.05)

The result for Post Hoc Bonferroni test on Table 2 showed that combination of kelakai leaves extract with 75% concentration and katuk leaves extract with 80% concentration is the only group that had no significant difference in their antibacterial activity when compared to 1% Povidone iodine. Combination of 75% kelakai leaves and 80% katuk leaves extract showed a p value=0.317 (p>0.05). Based on that result, it is known that the combination of kelakai leaves extract at 75% concentration and katuk leaves extract at 80% concentration is statistically equivalent with 1% Povidone iodine against Aggregatibacter actinomycetemcomitans.

DISCUSSION

The results for antibacterial activity of kelakai and katuk leaves extract combination when compared to 1% Povidone iodine against Aggregatibacter actinomycetemcomitans showed that combination of kelakai leaves extract at 25%, 50%, 75%, 100% concentration and katuk leaves extract at 20%, 40%, 80% concentration could inhibit the growth of Aggregatibacter actinomycetemcomitans. This study showed that the combination of kelakai leaves extract and katuk leaves extract has an antibacterial activity which efficacy is gradually intensified following the increase of
extract concentration. Suriawati et al. (2018) mentioned that a higher concentration of extract combination will produce a stronger antimicrobial activity. Combination of 100% kelakai leaves extract and 80% katuk leaves extract has the highest mean inhibitory zone compared to other concentration. This result is also higher than the average inhibitory diameter of 1% Povidone iodine.

Aforementioned outcome has been expressed by Qomar et al. (2018) that the higher the concentration used, the more active substances are contained. Rising the antibacterial substances concentration may enhance the penetration of antimicrobial substances into the microbial cells and destroy the metabolism system and cause cell death. David-Stout method presented that the antibacterial efficacy can be observed by measuring the inhibition zone diameter. Based on this classification, ≥20 mm inhibition zone is considered as very strong, 10-20 mm as strong, 5-10 mm as moderate and ≤5 mm as weak. According to this method, the antibacterial efficacy of kelakai and katuk leaves extract combination against Aggregatibacter actinomycetemcomitans is categorized as strong in all concentrations, except for the combination of 25% kelakai leaves extract and 20% katuk leaves extract, the combination of 50% kelakai leaves extract and 20% katuk leaves extract, and the combination of 75% kelakai leaves extract and 20% katuk leaves extract which was categorized as moderate.

Combination of kelakai and katuk leaves extract against Aggregatibacter actinomycetemcomitans in this study produced a larger inhibitory zone than single extract of kelakai leaves from a study by Setyorini et al. (2019). This result corresponds to a statement by Padalia et al. (2016), that combining plants may be performed to increase antibacterial efficacy. Combination of the two extracts resulted in the significant increase of inhibiton zone emerging from the active substances contained within two extracts that synergistically work to disrupt the functions of bacteria.

The inhibitory zone that is statistically equivalent between the combination of 75% kelakai leaves extract and 80% katuk leaves extract when compared to 1% Povidone iodine against Aggregatibacter actinomycetemcomitans is generated by the synergistic effect of the active substances which affect the antibacterial potency. Other factor that may also contribute is the similar mechanism of action between the active substances of kelakai and katuk leaves extract with 1% Povidone iodine.

Secondary metabolite substances contained in kelakai leaves are flavonoid, tannin, alkaloid, and steroid, while the secondary metabolites substances contained in katuk leaves are flavonoid, tannin, alkaloid, and saponin. Syamsul et al. (2019) and Bunawan et al. (2015) reported that the dominant antibacterial ingredient of kelakai and katuk leaves is flavonoid. The mechanism of flavonoid from kelakai leaves and katuk leaves as an antibacterial product is by inhibiting nucleic acid synthesis, cell membrane function and bacterial energy metabolism. Flavonoid inhibits nucleic acid synthesis by piling nucleic acid bases with B-ring from the flavonoid compound which inhibits DNA and RNA synthesis. When inhibiting the cell membrane functions, flavonoid binds in a complex binding with extracellular protein hence the cell membrane integrity is compromised. Inhibitory mechanism of energy production by flavonoid is carried out by inhibiting oxygen utilization by bacteria, therefore the bacterial molecules cannot develop into a complex molecule.

Tannin, an antibacterial substance from kelakai leaves and katuk leaves, works by lysing cells both osmotically and physically. Tannin disrupt the synthesis of peptidoglycan which causes incomplete development of cell membrane. This substance also suppress DNA replication of Aggregatibacter actinomycetemcomitans. Reverse transcriptase and DNA topoisomerase will be restrained so that the bacteria cell will not develop. Tannin inactivates cell adhesion of microbes and interrupts protein transport in the blood sets.

The role of alkaloid contained in kelakai and katuk leaves against Aggregatibacter actinomycetemcomitans is by disrupting the integrity of bacterial cells peptidoglycan, therefore the cell wall will not be developed entirely. Alkaloid is also able to change the amino acid in the DNA chain and cause changes in genetic balance, therefore the bacterial DNA is disintegrated and the bacteria became inactive.

The other secondary metabolite of kelakai leaves is steroid. The antibacterial effect of this substance is related to the lipid membrane and its sensitivity to steroid which cause bacterial liposomal leakage. Steroid interacts with the phospholipid membrane of gram negative bacteria, causing cell lysis due to the decrease of membrane integrity and changes in the cell membrane morphology.

The antibacterial mechanism of saponin from katuk leaves is by interrupting the stability and development of cell membrane and cell walls of Aggregatibacter actinomycetemcomitans. This substance can also cause protein and enzymes leakage by reducing tension of the cell wall. Saponin will diffuse through the cytoplasmic membrane, causing
cytoplasmic leakage out of the cells until cell death.\(^5\)

This study used 1% Povidone iodine as the positive control which has bacteriostatic and bactericidal properties, so it can reduce the microorganism growth in the oral cavity. Povidone iodine with 1% concentration is an iodine complex that work as a broad spectrum antiseptic capable of destructing microbial DNA and proteins.\(^5\) The iodine element will be released gradually after direct contact with tissues. The release of iodine will kill cells by ionicization of lipids and oxidation of cytoplasmic and membrane substances.\(^6\) This will result in interrupt metabolism of bacterial enzymes which in turn disrupts bacterial multiplication therefore cause the bacteria to become weak.\(^3\)

Combination of kelakai leaves and katuk leaves extract is investigated as an alternative to synthetic mouthwash such as 1% Povidone iodine in reducing Aggregatibacter actinomycetemcomitans, the dominant bacteria in aggressive periodontitis. The conclusion of this study is that the combination of 75% kelakai leaves extract and 80% katuk leaves extract is statistically equivalent with 1% Povidone iodine against Aggregatibacter actinomycetemcomitans.

REFERENCES
3. Afrina, Santi C, Risa YM. Konsentrasi Hambat dan 80% katuk leaves extract as an alternative to synthetic mouthwash such as 1% Povidone iodine in reducing Aggregatibacter actinomycetemcomitans, the dominant bacteria in aggressive periodontitis. The conclusion of this study is that the combination of 75% kelakai leaves extract and 80% katuk leaves extract is statistically equivalent with 1% Povidone iodine against Aggregatibacter actinomycetemcomitans.

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