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The effectiveness of red betel leaf (Piper crocatum) extract against periodontal pathogens

Herryawan,[1*] Indah Puti Rahmayani Sabirin[2]

ABSTRACT

Introduction: Red betel (Piper crocatum) has long been known as one of herbs that has antibacterial properties as it contains several useful compounds such as essential oils, flavonoid, alkaloids and tannins. The objective of this study is to learn more about the effectiveness of red betel leaf extract based on in vitro test against periodontal pathogen bacteria such as Aggregatibacter actinomycetemcommitans (Aa) and Porphyromonas gingivalis (Pg).

Method: This study was conducted using diffusion method that has Kirby-Bauer test sensitivity. The experiment was performed eight times on four treatments consisting of 10% DMSO as negative control and 2.5%, 5% and 10% of red betel leaf extract. The sterile paper disc was soaked into sterile water and red betel leaf extract at each concentration before evaporation inside the incubator. After that, the dry paper disc was placed in the Nutrient Agar (NA) that had previously been inoculated and incubated for 24 hours at 37° Celsius. ANOVA test was used to compare inhibition zone in four group intervention.

Result: The result of the study shows that the 2.5%, 5% and 10% concentration of red betel leaf extract respectively could inhibit the growth of bacteria Aa and Pg. The result of the study also confirms that the 10% red betel leaf extract concentration created the largest inhibition zone (10.5786 mm for bacteria Aa and 10.7638 mm for bacteria Pg).

Conclusion: Red betel leaf extract, particularly at 10% concentration is significantly effective in inhibiting the growth of Aa and Pg bacteria.

Keywords: Aggregatibacter actinomycetemcommitans, Red betel leaf extract, Porphyromonas gingivalis.

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[1]Periodontic Laboratory, Dentistry Education, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi
[2]Oral Biology Laboratory, Dentistry Education, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi

[*]Correspondence to:
Herryawan, Periodontic Laboratory, Dentistry Education, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi
herryawan@lecture.unjani.ac.id

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INTRODUCTION

Currently, more people worldwide are beginning to pay more attention to the use of natural, herbal medicine as they believe that herbal medicines are quite useful in treating various illnesses without causing excessive side effects. One of the herbs that are empirically used as a traditional drug in Indonesia is red betel (Piper crocatum), a herb that has long been known to have medicinal properties to treat various diseases including dental and oral diseases like canker sores and gingivitis as it is believed to have strong and effective antibacterial and anti-inflammation agents.[1-3]

There have been many studies conducted to reveal the effectiveness of red betel. In 2011, Fitriyani and her team experimented on white mice to test the anti-inflammatory effect of red betel leaf's methanol extract. The result shows that 25, 50 and 100 mg/kg BB methanol extract of red betel leaf can decrease inflammation respectively by 72.37%, 61% and 81.02%.[3] Another research conducted by Reveny (2011) concluded that 80% of the ethanol extract from the red betel leaf has anti-bacterial effectiveness that can inhibit the growth of Escherichia coli by 2.5%, Staphylococcus aureus by 2.5% and Candida albicans by 10%.[4] Red betel's anti-bacterial and anti-inflammatory agents comes from the flavonoid, alkaloid, tannin, monoterpene and sesquiterpene compounds found in the leaf.[1-6]

Although there have been many studies conducted on the benefits of red betel leaf as an anti-bacterial agent, the number of scientific literature that reveals the effectiveness of the herb in treating periodontal tissue is still limited. The lack of scientific research that focuses its study on eliminating periodontal pathogens bacteria concerns, as bacteria is also the main cause of all periodontal tissue diseases.[7] Therefore, this study aims to highlight the antibacterial effect of red betel leaf extract against periodontal pathogen bacteria, particularly Aggregatibacter actinomycetemcommitans (Aa) and Porphyromonas gingivalis (Pg).

MATERIAL AND METHOD

The research used red betel leaf as the main material. The leaf extraction and its concentration were made in Phytochemistry laboratory at the Faculty of Pharmacy, Jenderal Achmad Yani University, Cimahi. The microorganism used for this research were Aggregatibacter actinomycetemcommitans strain ATCC 29523 and Porphyromonas gingivalis strain ATCC 49417. The anti-bacterial

Table 1 Average data of Aa bacteria inhibition zone measurement

Treatment Group	n	Mean ± SD (mm)	p-value
10% DMSO	8	0.40 ± 0.00	0.001*
2.5% extract	8	9.42 ± 1.17	
5% extract	8	10.00 ± 0.51	
10% extract	8	10.57 ± 0.61	

*significant ($p < 0.05$)

Table 2 Average data of Pg bacteria inhibition zone measurement

Treatment Group	n	Mean ± SD (mm)	p-value
10% DMSO	8	0.50 ± 0.00	0.000*
2.5% extract	8	9.15 ± 0.66	
5% extract	8	9.92 ± 0.55	
10% extract	8	10.76 ± 0.47	

*significant ($p < 0.05$)

effectiveness test was performed in August 2017, at the Microbiology laboratory of the Faculty of Medicine, Jenderal Achmad Yani University, Cimahi in August 2017.

The first step of the research began with hot processed leaf extraction by using decocta. The leaves that were picked for the test were the mature ones (the 3rd, 4th and 5th leaves from the top). The dry simplicia of red betel leaves were extracted with water at 96-98^{0}C for 30 minutes prior filtration. After that, the filtrate was evaporated using a rotary vacuum evaporator at 70^{0}C until it produced thick, pure, brownish and aromatic extract liquid[8]. The extract then was diluted with 10% DMSO (Dimethyl Sulfoxide) based on the expected concentration (2.5%, 5%, and 10%) with composition ratio as follow:

1. Concentration 2.5 %: 0.125 ml red betel leaf extract + 4.875 ml DMSO 10 %
2. Concentration 5 %: 0.25 ml red betel leaf extract + 4.75 ml DMSO 10%
3. Concentration 10 %: 0.5 ml red betel leaf extract + 4.5 ml DMSO 10 %

The test discs were soaked into each concentration for 15 minutes.

In the next step, bacterial suspension was prepared by culturing the Nutrient Agar (NA) media. Four to five colony of cultured Aa and Pg bacteria were taken with a transfer loop and put into a test tube containing 5 ml PBS. It was then incubated at 37°C for two hours to form the turbidity that is equivalent to 1 Mc Farland standard with bacterial concentration 3×10^8 / ml. The number of bacteria used for the sensitivity test is $10^5 - 10^8$ / ml. These bacteria were swabbed to the Nutrient Agar media and after that the test disc was placed in the NA media and re-incubated at 37° C for 24 hours. These experiments were performed in eight experiment groups consisting of four samples (three red betel leaf extract samples with varied concentration and one negative control sample DMSO 10%).[10,11,12] The inhibition zones formed from the cultured bacteria were measured with vernier scale in millimeter. Statistical analysis using ANOVA test to evaluate the differences between bacterial inhibition zone in four group intervention.

RESULT

Aggregatibacter actinomycetemcommitans

Comparison of Aa bacteria inhibition zone measurement in four group intervention can be seen in Table 1.

The largest inhibition zone for Aa bacteria resulted from the 10% concentration of red betel leaf extract zone (10.5786 ± 0.61470), and each inhibition zone of each sample showed significant differences ($p = 0.001$) (Table 1).

Porphyromonas gingivalis

Comparison of Pg bacteria inhibition zone measurement in four group intervention can be seen in Table 2.

The largest inhibition zone for Pg bacteria resulted from the 10% concentration of red betel leaf extract zone (10.7638 ± 0.47901), and each inhibition zone of each sample showed significant differences ($p = 0.000$) (Table 2).

DISCUSSION

The test result of Aa bacteria shows that the three red betel leaf extract concentrations contained anti-bacterial effectiveness with the average inhibition diameter as much as 9.4220 mm for 2.5% concentration, 10.0000 mm for 5% concentration and 10.5786 mm for 10% concentration. Similar result was obtained from the test on Pg bacteria, showing an average inhibition diameter of 9.1563 mm for 2.5% concentration, 9.9225 mm for 5% concentration and 10.7638 mm for 10% concentration. These results prove that the three red betel leaf extract concentrations can be used as an anti-bacterial agent against Aa and Pg bacteria. The inhibition zone diameter of the three extract concentrations to both bacteria has far exceeded 10% DMSO as the negative control, which only produced 0.4000 mm inhibition zone for Aa bacteria and 0.5000 mm inhibition zone for Pg bacteria. The research used Mann-Whitney statistical analysis to find out significant inhibition zone within each treatment

groups. The result showed that the inhibition power in each treatment group was significantly different, p = 0.001 in Aa bacteria and p = 0.000 in Pg bacteria.

The anti-bacterial effectiveness of red betel leaf comes from its essential oil. Emamghoreishi et al. (2005) explained that essential oils contain volatile compounds that have anti-bacterial and anti-inflammatory effects such as monoterpene and sesquiterpene.[13-16] Other researchers also found other beneficial properties of red betel leaf such as alkaloid, flavonoid and tannins.[6-10]

Essential oils that contain activity against some gram positive and gram negative bacteria inhibit bacterial growth by disrupting the forming of plasma membrane or cell wall, preventing the cell from forming perfectly.[10-12] Parwata and Dewi (2008) concluded that essential oils that have active anti-bacterial properties generally contain hydroxyl functional groups (-OH) and carbonyls. Phenol derivatives interact with bacteria cell through absorption process that includes hydrogen bonds, which at high level can cause coagulation protein and membranous cells to lysis.[17]

The effectiveness of anti-inflammatory agent in essential oils come from its volatile compounds such as monoterpenes and sesquiterpene. Silveira, Andrade and de Sousa (2013) in their research concluded that monoterpene compound from plants proved to inhibit the production of proinflammatory cytokines such as TNF α, IL-1β, IL-4, dan IL-5.[14] Another study that was conducted by Chadwick et al. (2013) showed that sesquiterpenes work by inhibiting the response of interleukin, endotoxins, tumor necrosis and bacterial antigens.[15]

Antibacterial activity in alkaloid has been described in several studies. In 2001, Akiyama et al. Prove alkaloids could serve as antibacterial agent and can kill bacteria cells.[18] The working mechanism of alkaloid as antibacterial by interfering with peptidoglycan component of bacteria cell, preventing the cell wall from forming perfectly and thus killing the cell.[19]

Flavonoid is also contained antibacterial properties. It works by forming complex compounds against extracellular protein that disrupts the integrity of bacteria cell membrane.[1,5] Furthermore, flavonoid inhibits an important phase in biosynthesis of prostaglandins, the cyclooxygenase pathway. Flavonoid also inhibits phosphodiesterase, aldoreductase, monoamine oxidase, protein kinase, DNA polymerase and lipoxygenase.[4,19]

According to Akiyama et al. (2001), tannin's antibacterial effect comes from its toxicity, which is damaging for the cell membrane of bacteria.[1,5,18] Ajizah et al. (2004) concluded that tannin is thought to be able to shrink cell wall or membrane and it would interfere with the permeability of the cell, inhibiting its activity and growth, which will eventually lead to cell death.[20] According to Masduki et al. (1996), tannin also has antibacterial property that has similar effect with phenol and it works by precipitating the protein.[21]

CONCLUSION

Red betel leaf extract, particularly at 10% concentration is significantly effective in inhibiting the growth of Aa and Pg bacteria.

CONFLICT OF INTEREST

Author's has no conflict of interest regarding all aspect in this study.

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