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Research Report

The antibacterial activity of red betel (*Piper crocatum)* leaf extract toward *Staphylococcus aureus*

Ahmad Alfan Surya Dilaga Zulkarnain[1], Ida Bagus Narmada[2], I Gusti Aju Wahju Ardani[2]
[1]Undergraduate Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Orthodontic, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: Red betel (*Piper crocatum*) is a plant that grows a lot in the tropical area, especially Indonesia. The use of red betel leaves for medicine is due to the fact that betel leaves contain a lot of essential oils. **Purpose:** To examine the antibacterial activity of red betel leaf extract against *Staphylococcus aureus* by investigate at the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. **Methods:** This study used an in vitro laboratory experiment as its research method. The post-test only control group design experiment was used in this study, in which the experimental and control groups were not chosen at random and each of the two groups was compared. **Results:** Red betel leaves that had been extracted at a concentration of 100% were diluted into several concentrations, namely 50%, 25%, 12.5%, 6.25%, and 3.75%. The results of this dilution obtained the minimum inhibitory concentration (MIC) value of red betel leaf extract against *Staphylococcus aureus* bacteria at a concentration of 6.25%, while the minimum bactericidal concentration (MBC) value was obtained at a concentration of 12.5%. **Conclusion:** The MBC value in this study was in red betel leaf extract with a concentration of 12.5%, and the MIC value in this study was in red betel leaf extract with a concentration of 6.25%.

Keywords: Red betel leaf extract; *Staphylococcus aureus*; antibacterial; Minimum Inhibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC)

Correspondence: Ida Bagus Narmada, Department of Orthodontic, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo no. 47 Surabaya, 60132, Indonesia. Email: ida-b-n@fkg.unair.ac.id

INTRODUCTION

Plaque accumulation can be prevented by carrying out plaque control efforts, which can be mechanical or chemical. Mechanical plaque control requires the addition of chemical plaque control agents, one of the chemical plaque control methods is mouthwash.[1,2] The use of mouthwash is effective in preventing the accumulation of dental plaque if it is used as a complement to the mechanical control of dental plaque. Some mouthwashes have bacteriostatic and bacteriocidal properties, so they have a stronger cleaning power against the formation of dental plaque. The use of natural mouthwash has advantages because the therapeutic effect of natural ingredients is constructive and the side effects are very small, so natural ingredients are relatively safe compared to chemicals.[3,4]

Red betel (*Piper crocatum*) is a plant that grows a lot in the tropical area, especially in Indonesia. The use of red betel leaves for medicine is due to the fact that betel leaves contain a lot of essential oils.[2] Essential oils are volatile oils that give off a distinctive aroma. Ingredients in red betel, such as flavonoids, alkaloids, polyphenolics, tannins, and essential oils, are known to have antibacterial properties by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cells.[5,6] In red betel essential oil, there is also a kavikol compound, which is a derivative of phenol and has a bacterial killing power five times greater than phenol.[7–9]

Utilization of red betel as a natural antibacterial agent has the advantage that these compounds are safer than synthetic materials. The use of synthetic materials raises concerns about side effects that are detrimental to health.[8,10] Based on the compound content of red betel, namely polyphenolic compounds, this plant can also have potential as an antibacterial. Several groups of antibacterial agents exist, namely phenols, alcohols, halogens, heavy metals, and detergents.[11,12] The aim of this study is to examine the antibacterial activity of red betel leaf extract against *Staphylococcus aureus* by examine at the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

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MATERIALS AND METHODS

This study used an in vitro laboratory experiment as its research method, which was approved by the health ethics committee of the Faculty of Dental Medicine, Universitas Airlangga. The post-test only control group design experiment was used in this study, in which the experimental and control groups were not chosen at random and each of the two groups was compared.

The sample in this study was a *S. aureus* culture obtained from the gingival plaque of patients with fixed orthodontic appliances, which was then cultured in Brain Heart Infusion Broth (BHIB) medium at 37°C for 24 hours. The microdilution method was used to conduct MIC and MBC tests. Red betel extract preparations were serially diluted in BHIB medium with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Then, a negative control with media and *S. aureus* bacterial suspension, and a positive control with media, bacterial suspension, and 2% NaF. The previously prepared McFarland 0.5 *S. aureus* bacterial suspension was placed in a test tube that also contained the treatment and control groups. The samples were then incubated in an incubator at 37°C for 48 hours.

The clarity of the suspension indicates the lowest concentration of red betel extract suspension that produces an inhibitory (bacteriostatic) effect on *S. aureus* bacteria as the MIC. Then, using the spreading technique, 0.1 ml of each clear tube was planted on TSA agar and incubated at 37°C for 48 hours. The absence of bacterial colonies growing on agar media indicates the lowest concentration of red betel extract that produces a bactericidal effect on *S. aureus* bacteria, which is recorded as MBC. The test was performed approximately three times.[13]

Several tests were used to process the data, including the data normality test, the data homogeneity test, and the significance test between treatment groups. The Shapiro-Wilk test should be used to determine data normality. After performing the normality test, use the Levene test to determine data homogeneity. If the test data is normally distributed, perform a parametric test in the form of a one-way ANOVA test. If the data is not normally distributed or homogeneous, it must be followed by a non-parametric test such as the Mann-Whitney test. Statistical data was calculated using IBM Statistical Package for Social Science (SPSS) (Illinois, Chicago, USA) software version 27.

RESULTS

Red betel leaves that had been extracted at a concentration of 100% were diluted into several concentrations, namely 50%, 25%, 12.5%, 6.25%, and 3.75%. The results of this dilution obtained the MIC value of red betel leaf extract against *S. aureus* at a concentration of 6.25%, while the MBC value was obtained at a concentration of 12.5% (Table 1).

The research data was then used to calculate the average number of colonies using the Total Plate Count (TPC) method from four replicates for each sample group. The average yield of the positive control group (K(+)) was 0. The negative control group (K(-)) 182 colonies. Concentrations of 100%, 50%, 25%, and 12.5% are 0. Concentrations of 6.25% are 11.75 colonies. Concentration of 3.125% for 37 colonies. The average value of the number of colonies is then made a multilevel diagram to compare and visualize the data (Figure 1).

This test was conducted to determine significant differences in variables. The following are the results of the Mann Whitney test which can be seen in the following Table 2.

Analysis of the data from the post-hoc test using the Mann-Whitney method, namely, if the significance value is less than 0.05 ($p<0.05$), then there is a significant difference between the two groups of variables. In the positive control group, the concentrations are 100%, 50%, 25%, and 12.5%. There is no significant difference between them because the data shows a significance value greater than 0.05. In the negative control, there is a significant difference from the positive control: 100% concentration; 50%; 25%; 12.5%; 6.25%; and 3.125% because the data shows a significance value of 0.014. Other significant results were obtained at concentrations of 6.25% and 3.125% to each other, which showed a significance value of 0.021, and to the positive control, concentrations of 100%, 50%, 25%, and 12.5%, with a significance value of 0.0.14 ($p<0.05$). Thus, a concentration of 12.5% is expressed as MBC, and a concentration of 6.25% can be expressed as MIC against *S. aureus.*

Table 1. Total plate count (TPC) test results

Treatment Group	Replication 1	Replication 2	Replication 3	Replication 4
K (+)	0	0	0	0
K (-)	186	175	179	188
100%	0	0	0	0
50%	0	0	0	0
25%	0	0	0	0
12.5%	0	0	0	0
6.25%	10	12	14	11
3.125%	42	37	34	35

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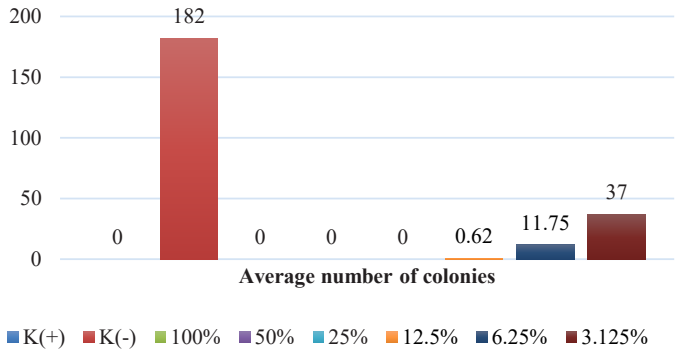

Figure 1. Comparison of the average number of *S. aureus* colony after treated with Red betel extract.

Table 2. Multiple comparison test results with Mann Whitney

Significance	Control +	Control -	100%	50%	25%	12.5%	6.25%
Control +							
Control -	0.014*						
100%	1	0.014*					
50%	1	0.014*	1				
25%	1	0.014*	1	1			
12.5%	1	0.014*	1	1	1		
6.25%	0.014*	0.021*	0.014*	0.014*	0.014*	0.014*	
3.125%	0.014*	0.021*	0.014*	0.014*	0.014*	0.014*	0.021*

* Significant different at $p<0.05$

DISCUSSION

The most appropriate method for laboratory experimental research on *S. aureus* carried out in vitro is the microdilution method. This study aims to determine the value of MBC and MIC) and to see the effectiveness between concentrations to eradicate and inhibit the growth of *S. aureus* from the compounds contained in red betel leaf extract. This study used red betel leaf extract, which was diluted serially to obtain concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. The positive control (K (+)) was also used in this study, namely 2% NaF solution as the gold standard value. Positive control, 2% K(+) NaF was proven to be an anti-cariogenic agent. Research conducted by Nadhira et al. (2020 shows that 2% NaF can inhibit the growth of *S. aureus* in vitro. 2% NaF contains fluorine, which can directly inhibit bacterial growth through inhibition of enzymes involved in glycolysis.[14,15]

Red betel leaf extract that has been diluted serially will produce a concentration at which, in the next stage, red betel leaf extract is dripped as much as 100–200 l into the Staphylococcus aureus bacterial suspension and BHIB media and into the control group. Then incubated at 37°C for ± 24 hours. The results of the research conducted showed that several test tubes turned clearer, which in turn led to these tubes being considered MIC. More accurate MIC and MBC values can be seen by inoculating on Triptic Soy Agar (TSA) media with the spreading technique, which involves incubation for 48 hours.[16] The results of the inoculation will show the growth of *S. aureus* colonies in each concentration. The concentration levels of 100%, 50%, 25%, and 12.5% showed no growth of S. aureus, which indicated that these four concentrations were optimal concentrations for killing *S. aureus* or could be called bactericidal. The theory obtained explains that the MBC value of an antimicrobial can kill ≥ 99.9% of microbes. The results of this study indicate that the lowest concentration capable of killing 100% of *S. aureus* is a concentration of 12.5%, which results in a concentration of 12.5% being considered the MBC value.

The IC_{90} value as a form of anticipation of the MIC value, which must be determined individually for the microbes in the patient. Based on the cumulative epidemiological analysis, the MIC_{90} value was obtained. MIC_{90} is expressed as the inhibitory concentration of microbial agents with a value of ≥ 90% of the microbial isolates that can be inhibited by their growth. From the results of the TPC test obtained, there was visible growth of bacterial colonies at concentrations of 6.25% and 3.125%. A concentration of 6.25% indicated that there were isolates that grew in each replication, namely 10, 12, 14, and 11. The isolates that grew at these concentrations were then compared with isolates in the negative control of each replication. Red betel leaf extract with a concentration of 6.25% is proven to inhibit the growth of Staphylococcus aureus with a percentage ratio of 91%–93%. The percentage comparison value is included in the MIC_{90} range because this value is ≥ 90%. Meanwhile, at a concentration of 3.125%, a comparison percentage of 77%–81% is obtained, so the percentage comparison value is not included in the MIC_{90} range because the value is ≤ 90%.[17]

The antibacterial effectiveness of red betel leaf extract is also supported by a significant difference between the

MBC concentration (12.5%) and the MIC_{90} concentration (6.25%). This was indicated by a concentration of 12.5%, which was assessed as MBC, which was more effective in killing *S. aureus* compared to a concentration of 6.25%, which was assessed as MIC. A comparison between a concentration of 6.25% and a concentration of 3.125% showed that a concentration of 6.25%, which was assessed as the MIC, was more effective at inhibiting the growth of *S. aureus* than the lower concentration. Concentrations that were not considered MBC and MIC were also evaluated for effectiveness, which resulted in no significant difference between these concentrations.

CONCLUSION

Red betel (*Piper crocatum*) leaf extract contains antibacterial activating compounds against *S. aureus*. Antibacterial activity can be seen through the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. The MBC value in this study was in red betel leaf extract with a concentration of 12.5%, and the MIC value in this study was in red betel leaf extract with a concentration of 6.25%.

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