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ANTIBACTERIAL EFFECTIVENESS OF RAMBAI (*Sonneratia Caseolaris*) LEAVES EXTRACT AGAINST *STREPTOCOCCUS MUTANS*

Aniqah Nor Rahmatika[1)], Didit Aspriyanto[2)], Muhammad Yanuar Ichrom Nahzi[3)]
[1)] Faculty of Dentistry, Universitas Lambung Mangkurat, Banjarmasin
[2)] Department of Oral Biology, Faculty of Dentistry, Universitas Lambung Mangkurat, Banjarmasin
[3)] Department of Conservative Dentistry, Faculty of Dentistry, Universitas Lambung Mangkurat, Banjarmasin

ABSTRACT
Background: *The 2018 Riskesdas data stated that dental caries prevalence in Indonesia was 88.8%. Dental caries is a multifactorial disease characterized by hard tissue damage to the enamel, dentin, and cementum of the teeth. One of the causes of caries is the presence of a microorganism called Streptococcus mutans. Rambai leaf extract (Sonneratia caseolaris) is a typical plant of South Kalimantan with content flavonoids, tripernoids, tannins, phenols, steroids that have antibacterial properties against the growth of Streptococcus mutans which can prevent caries.* ***Purpose****: To determine the difference in the effectiveness of the antibacterial extract of rambai leaves (Sonneratia caseolaris) against bacteria Streptococcus mutans in vitro.* ***Methods****: This research was a true experimental with a post-test-only design and a control group, using six treatment groups, namely 20%, 40%, 60%, 80%, positive control (povidone iodine 1%), and negative control (aquadest) respectively. Each treatment got 3 repetitions. The antibacterial test method used in this study was the liquid dilution method to determine the Minimun Inhibitory Concentration (MIC) by using Spectrophotometer and solid dilution to determine the Minimum Bactericidal Concentration (MBC) by using tools Colony Counter.* ***Results:*** *Based on the results and data analysis, it can be seen that the rambai leaf extract has a MIC of 20% and a MBC of 60%.* ***Conclusion:*** *Rambai leaf extract (*Sonneratia caseolaris*) can inhibit and have antibacterial activity against the growth of Streptococcus mutans.*

Keywords: *Antibacterial, Rambai leaf extract, Streptococcus mutans*
Correspondence**:** Aniqah Nor Rahmatika; Faculty of Dentistry, Universitas Lambung Mangkurat, Jl. Veteran No. 128B, Banjarmasin, South Kalimantan, email: tikaka65@gmail.com

INTRODUCTION

The 2018 Riskesdas data stated that caries in Indonesia was 88.8% and in South Kalimantan Province it was found that the proportion of the population who experienced dental caries at the age of 3 years was 46.90% with the highest caries percentage owned by Barito Kuala Regency of 59.67%. Barito Kuala has a pH level of 3.65 which plays an important role in the process of tooth decay.[1,2]

Dental caries is a condition where the hard tissue of the teeth is damaged, which includes enamel, dentin, and cementum. Caries have quite a lot of causal factors, but the main causes of caries are microorganisms (plaque), substrate, host (tooth), and time. One of the microorganisms that play a role in the occurrence of caries is *Streptococcus Mutans* bacteria. These bacteria have glucosyltransferase enzymes that act as virulence factors in caries by synthesizing glucose into glucans by *Streptococcus mutans* colonies. The glucans formed will adhere to the tooth surface. The amount of glucan can trigger demineralization of enamel due to a decrease in the pH value of the biofilm. Unmineralized enamel after demineralization results in dental caries. The antiseptic commonly used in plaque control to prevent caries is povidone iodine mouthwash.[3,4,5]

Povidone iodine is an antibacterial that can penetrate cell membranes. Povidone iodine 1% can kill bacterial cells because of its cytotoxic properties. The synthesis of glucosyltransferase (GTF) and fructosyltransferase (FTF) which are virulence factors in *Streptococcus mutans* can be inhibited by povidone iodine. However, the use of 1% povidone iodine for a long time will cause sensitivity, local erythema, pain, mucosal erosion, and impaired thyroid function. Therefore, it is necessary to use antiseptics from natural ingredients with minimal side effects.[6,7]

Indonesia has abundant biodiversity, especially in South Kalimantan, which can be used as herbal alternatives. One of these natural resources is rambai leaf (*Sonneratia caseolaris*). This plant has been used as a traditional medicine with anti-inflammatory and antibacterial properties. The results of the phytochemical screening show that rambai leaf (*Sonnoratia caseolaris*) contains flavonoid compounds, tripenoids, tannins, phenols, steroids.[8]

Based on this, it is necessary to analyze the differences in the antibacterial effectiveness of rambai leaf extract (*Sonneratia caseolaris*) at concentrations of 20%, 40%, 60%, 80% against the growth of *Streptococcus mutans* bacteria as the main cause of caries.

RESEARCH METHODS

This research was conducted after obtaining ethical approval from the Ethics Commission of the Faculty of Dentistry ULM on No. 045/ KEPKG-FKGULM/EC/III/2021. This research was true experimental with posttest only design with control group and 6 treatments which include rambai leaf extract at concentrations of 20%, 40%, 60%, 80%, distilled water as a negative control, and a positive control of 1% povidone iodine. The MIC test used the liquid dilution method with a UV-Vis spectrophotometer to determine the absorbance value and the MBC with solid dilution used a colony counter to calculate the number of colonies on the agar medium.

Determination of Rambai Plants

Rambai leaves were found on the banks of the Barito river, Anjir Serapat Village, Anjir Muara District, Barito Kuala Regency, South Kalimantan Province. The determination test of rambai leaves was conducted at Faculty of Mathematics and Natural Sciences Laboratory, Universitas Lambung Mangkurat and the results were obtained in the form of determination with the local name of Rambai plant or the Latin name of the plant is *Sonneratia caseolaris*.

Rambai Leaves Extract

Rambai leaves extract was prepared using the maceration method, with the first step being to wash 1 kg of rambai leaves and dried in an oven at 40ºC for 4 hours. After dried, the rambai leaves were crushed sufficiently using a blender so that 100 gr of rambai leaves simplicia powder was obtained. Rambai leaves powder was soaked in 96% ethanol solvent for 3x24 hours and replaced every day. The results of the immersion were filtered with filter paper 3 times until the filtrate was clear. The extract was then released from the solvent by heating at a temperature of 40ºC over a water bath and examined using the addition of acetic acid and concentrated sulfuric acid. Rambai leaves extract which did not show any ester odor from ethanol, meant that the extract has been positively free from ethanol solvent.

The filtrate of rambai leaves extract (*Sonneratia caseolaris*) which was free from ethanol solvent was then combined and evaporated with a Rotary Evaporator until it thickened and became a thick extract of 45.26 gr. The thick extract was then diluted with aquadest using the formula V1.C1 = V2.C2 to obtain a concentration of 20%, 40%, 60%, 80%.

Sample Making

The cultured bacterial isolate was then taken as much as 1 ml and mixed in each vacuum tube. The vacuum tube was covered with sterile cotton and homogenized with a vortex mixer. Tubes containing bacteria, control (+) and control (-) were incubated at 37ºC for 24 hours. Then the control absorbance value was measured using a UV-Vis Spectrophotometer which had been adjusted to the maximum wavelength to determine the MIC value. Then, to determine the MBC value, it is calculated through the solid dilution method on NA agar media with a colony counter.

Bacterial Inhibitory Test with Liquid Dilution (Minimum Inhibitory Concentration)

The MIC value was obtained by comparing the difference in the absorbance value of the extract group to the difference of the negative control group. If the difference in the absorbance value of the extract group was significantly lower than the negative control group, then the concentration had an inhibitory power on bacterial growth. The control solution and extract that had been mixed with *Streptococcus Mutans* bacteria were measured using a UV-Vis Spectrophotometer to determine the absorbance value before incubation, then the solution was incubated at 37ºC for 24 hours. Next, the solution was measured again to get the absorbance value after incubation, then calculated the difference in absorbance values.

Bacterial Inhibition Test with Liquid Dilution (Minimum Bactericidal Concentration)

MBC value was calculated by adding sterile nutrient agar in petri dishes at all concentrations that had been incubated with bacteria. The petri dishes were subsequently incubated for a period of 24 hours at 37°C. After incubation, the number of bacteria was calculated by utilizing the colony counter. The killing power and the smallest concentration would be obtained if the number of bacterial colonies indicated a value of zero.

RESULTS

The results of the MIC research were obtained by measuring with a Uv-Vis Spectrophotometer (500 nm) to obtain the difference in absorbance values and the MBC obtained through a colony counter to measure the number of bacteria. The results of the study can be observed in the following table:

Table 1. Mean and Standard Deviation (SD) of the Difference between Absorbance Value and Colony Number

		Difference in Absorbance Value	Colony Number
		Mean ± SD	Mean ± SD
20%	6	-1.81925 ± 0.006396	1347.75 ± 301.244
40%	6	-1.541 ± 0.009486	0.75 ± 1.5
60%	6	-1.29175 ± 0.0075	0 ± 0
80%	6	-0.833 ± 0.02070	0 ± 0
K (+)	6	-0.06225 ± 0.00585	0 ± 0
K (-)	6	1.339 ± 0.00697	971.25 ± 345.048

The table above shows the difference absorbance value before and after incubaton. In the rambai leaf extract group with concentrations of 20%, 40%, 60%, 80% and the positive control, it showed a decrease in the mean value of the difference in absorbance values or inhibition of *Streptococcus mutans*, while the negative control group had an increased mean value from before incubation which showed no inhibition of the growth of *Streptococcus mutans* so that the MIC of rambai leaf extract against the growth of *Streptococcus mutans* was obtained at a concentration of 20%.

Figure 1. Result of Absorbance Value of All Treatment Groups to determine the MIC against *Streptococcus mutans*

The results of colonies number at concentrations of 20% and 40% show that the growth of *Streptococcus mutans* was still detected, the growth of *Streptococcus mutans* bacteria colonies was still detected, while the concentrations that could kill bacteria were found in the 60% and 80% concentration groups. The mean value at these concentrations was amounted to 0 CFU/µL and had the same mean value with the positive control. Regarding to that matter, a concentration of 60% was indicated as a minimal concentration that may inhibit and kill bacterial growth.

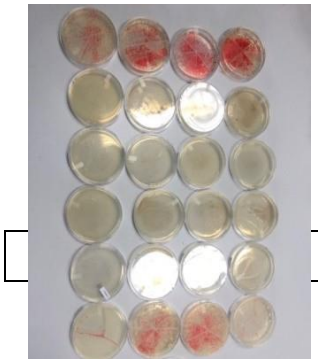

Figure 2: Results of Colony Number of All Treatment Groups against *Streptococcus Mutans* bacteria.

The data were then tested for normality with Saphiro Wilk test and it showed that the data were not normally distributed. The data was then tested for homogeneity with Levene's test and the results were not homogeneous, so it was continued with the Kruskal Wallis test. The results of the Kruskal Wallis test with a value of $p < 0.05$ showed that the data had a significant difference or there was an effect of treatment on *Streptococcus mutans*.

The results of the Post Hoc Mann Whitney test in the treatment group of absorbance value showed that there was a significant difference, because it had a significance value of $p<0.05$. The results of the Post Hoc Mann Whitney test for the number of colonies showed that there was a significant difference in the rambai leaf extract because it had a significance value of $p<0.05$..

DISCUSSION

The results showed that there is antibacterial effectiveness in rambai leaf extract (*Sonneratia caseolaris*) against *Streptococcus mutans* bacteria with concentrations of 20%, 40%, 60%, 80%, povidone iodine (positive control) and aquadest (negative control). This can be seen in the value of the Minimum Inhibitory Level (MIC) at a concentration of 20% which shows the smallest concentration in inhibiting bacterial growth with a decrease in absorbance of 1.81925 and a Minimum Bactericidal Concentration (MBC) which is found at a concentration of 60% which indicates that for 24 hours, *Streptococcus mutans* did not experience growth after incubation. This shows that the growth of gram-positive bacteria, namely *Streptococcus mutans*, and negative bacteria, namely *Escherichia coli* can be inhibited and killed by rambai leaf extract.[9]

The results above occur because rambai leaves have several phytochemical compounds that are antibacterial, anti-inflammatory, antimicrobial, antifungal and antioxidant. Rambai leaf extract has secondary metabolites, namely flavonoids, terpenoids, tannins, phenols, steroids. Flavonoid and phenolic compounds in rambai leaves are the most dominant compounds in inhibiting *Steptococcus mutans*. These flavonoids will form a complex of compounds with extracellular proteins so that they will interfere with cell membrane function and metabolism of *Streptococcus mutans*. Rambai leaf phenol works in inhibiting *Streptoccus mutans* by inactivating bacterial cell enzymes, thereby damaging the bacterial cell system and causing lysis.[10]

The mechanism of terpenoids in rambai leaves is to reduce membrane permeability which causes bacterial cell lysis.[11] Tannin compounds in rambai leaves work by inhibiting extracellular enzymes and inactivating bacterial cells.[12,13] While the mechanism of steroid in rambai leaves causes a decrease in membrane integrity so that the membrane will be brittle and lysed.[14]

The positive control in this study was 1% povidoine iodine. Povidone iodine is able to deactivate bacterial enzymes that will cause damage to the function and structure of bacterial cells. Povidone iodine is also able to inhibit the glucosyltransferase (GTF) and fructosyltransferase (FTF) enzymes in *Streptococcus mutans*.[15] Another control group, namely the negative control in the form of aquadest, was unable to inhibit or interfere with the growth of bacteria. This is because aquadest is pure distilled water.[16]

In this study, it can be seen that the secondary metabolite content in rambai leaf extract, namely tannins, has a similar mechanism with 1% povidone iodine, which is capable of inactivating enzymes, especially glucosyltransferase and frutosyltransferase enzymes in *Streptococcus mutans*.[6]

A significant difference means that they have different abilities in inhibiting the growth of bacterial colonies. Based on the statistical tests, concentrations of 20% and 40% have a lower mean difference in absorbance values compared to concentrations of 60%, 80%, and positive control. It was proven that the solutions with concentrations of 20% and 40% had a weaker inhibitory ability than concentrations of 60%, 80% and positive control, but had a higher antibacterial effect than the negative control. The concentration of 80% in rambai leaves had an antibacterial effect that surpassed the positive control. This study is in accordance with Mufti's research (2017) which stated that increasing the concentration of the extract used will affect the increase in the content of antibacterial compounds contained in it, so that the inhibition obtained is stronger.[17]

The results of the MBC statistical test showed the mean value of the number of bacterial colonies growth in solid media. Concentrations of 20% and 40% had a mean value of colony count of 971 CFU/µL and 1 CFU/µL. The concentration of 20% had the highest mean value of colonies number among all treatment groups, except for the negative control. This shows that the concentrations of 20% and 40% have no killing ability, while at the concentrations of 60%, 80% and positive control, there is no bacterial growth or the number of colonies is 0 CFU/µL.

Based on this value, it can be concluded that the 80% concentration and positive control are equivalent or have the ability to kill bacteria and it may be inferred that the minimum inhibitory concentration of rambai leaves extract against *Streptococcus mutans* was at a concentration of 20% and the minimum kill concentration was at 60%.

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