INHIBITION OF *STREPTOCOCCUS MUTANT* GROWTH BY PURPLE LEAF EXTRACT (*Graptophyllum pictum*)

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Abstract

Tooth decay is an infectious disease of microbial origin. Considering the increasing prevalence of antibiotic resistance due to their overuse and also their side effects, medicinal plants are now considered for use against bacterial infections. This study aimed to assess the effects of *Graptophyllum pictum* extract on inhibition of *Streptococcus mutans*. In this experimental study, serial dilutions of the extract were prepared in 13 test tubes for each bacterium. Standard amounts of bacterial suspension were added; 100λ of each tube was cultured on prepared solid agar plates and incubated at 37°C for 24 hours. Serial dilutions of the extract were prepared in another 20 tubes and 100λ of each tube was added to blood agar culture medium while being prepared. The mixture was transferred to the plates. The bacteria were inoculated on plates and incubated as described. The minimum inhibitory concentration (MIC) was 0.04 mg/mL for *S. mutans*. The minimum bactericidal concentration (MBC) was 0.09 mg for *S. mutans*. *Conclusion. Graptophyllum pictum* extract has significant antibacterial activity against *S. mutans* cariogenic microorganisms.

Keyword: inhibition, *Streptococcus Mutan, Graptophyllum pictum.*

INTRODUCTION

Tooth decay is an infectious, progressive disease disrupting the normal molecular interactions between the tooth surface and microbial biofilm. If not treated early, tooth decay can result in tooth cavity and subsequent dentin loss and pulp injury.[1,2] At this point, tooth decay and loss of mineral content (calcium and phosphorous) are irreversible and the lost tooth structure can only be restored with restorative dental materials.[3] In Indonesia prevalence of tooth decay (caries) reach 90,05%. *Streptococcus mutans* belong to the viridans streptococci group, which are the most commonly found members of the normal flora of the oral cavity. These bacteria synthesize large polysaccharides such as dextran and levan from sucrose and play an important role in development of dental caries. Moreover, following an injury or trauma to the mucosa, they may enter the blood stream and cause endocarditis of the heart valves.[3,4]

Graptophyllum pictum plant, commonly known as purple leaf. Purple leaves (*Graptophyllum pictum*) is a natural resource that is widely found around the yard and has many benefits for the health of the human body. Purple leaves contain nutritious substances that are tannins, flavonoids, anthocyanins, leukoantocyanins, and flavonols . Given the complex and diverse content of purple leaves, has well recognized antibacterial , antifungal, antiviral, and anticancer properties.[5-9]

Considering the previous reports regarding the antimicrobial activities of ethnic medicinal plants in Indonesia and the increasing prevalence of antibiotic resistance due to their overuse as well as their side effects, researchers have become increasingly interested in medicinal plants to find new sources of antibacterial remedies.[10]

Formulation of purple leaf extract in case of confirming its antibacterial efficacy can be used as a substitute for chemical antimicrobial agents. This study aimed to assess the antibacterial effect extract on *S. mutans*. The null hypothesis was that *Graptophyllum pictum* extract would have no antibacterial effect on *S. mutans*

RESEARCH METHODS

Amount of fresh purple leaves (25 gram) were washed with running water until clean and dried. Put purple leaves into the pressing that has been coated with sterile gauze. Then the purple leaves were collected in a beaker glass. Testing of concentrations of purple leaf extract on growth of

Streptococcus mutans in this research was done by using direct contact method A total of 0.1 ml of bacterial suspension was grown in a 0.5 BHIB liquid medium in a tube and added as much as 0.5 ml of purple leaf extract with different concentrations of 100%, 50%, 25%, 12.5%, 6.25 %, 3.12%, 1.56%, 0.78%, 0.39, 0.19%, 0.09%, 0.04%, and 0.02% respectively. Then incubate at 370C for 24 hours. After incubation, do subculture on nutrient media so that where each bacterial culture on BHIB media is taken as much as 0.1 ml suspension inoculum from purple leaf extract then flattened by means of swab on nutrient agar media. Incubate at 37 ° C for 24 hours, then observe the number of growing colonies. Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined by counting the number of colonies grown on rogosa media to manually declared with a colony forming unit (CFU) and compared with positive controls and negative controls. The calculation is repeated three times by three different observers. The data of this study were analyzed by one way ANOVA test to find out the inhibitory power of Streptococcus mutans bacteria after administration of extract.

RESULTS

Based on observations of streptococcus mutans after administration of purple leaf extract (*Graptophyllum pictum*) with the series thinning method, the results obtained as listed in here.

Table 1. Observation Results Of Viscosity In The Test Tube Visually

Concentration of Extract Purple Leaf (%)	**Result**
Control (+)	+
Tube 1 (100%)	-
Tube 2 (50 %)	-
Tube 3 (25%)	-
Tube 4 (12,5%)	-
Tube 5 (6,25%)	-
Tube 6 (3,125%)	-
Tube 7 (1,56%)	-
Tube 8 (0,78%)	-
Tube 9 (0,39%)	-
Tube 10 (0,19%)	-
Control (-)	-

Table 2. Observation Results Of Viscosity On The Test Tube Visually After Continued Dilution

Concentration of Extract (%)	Sample						
	1	2	3	4	5	6	7
Control +	+	+	+	+	+	+	+
Tube 9 (0,39%)	-	-	-	-	-	-	-
Tube 10 (0,19%)	-	-	-	-	-	-	-
Tube 11 (0,09%)	-	-	-	-	-	-	-
Tube 12 (0,04%)	-	-	-	-	-	-	-
Tube 13 (0,02%)	+	+	+	+	+	+	+
Control -	-	-	-	-	-	-	-

Information:
+ = There is turbidity and or sediment in the test tube
- = No turbidity and or sediment in the test tube

To determine the minimum inhibitory concentration violet leaf extract on the growth of Streptococcus mutans then further dilution series start at 0.39% concentration as much as 4 times until it reaches a concentration of 0.02%. In Table 2 it appears that at a concentration of 0.04% violet leaf extract was able to inhibit bacterial growth of Streptococcus mutans, which is on show in the absence of turbidites stacked or precipitate at the reaction tube.

To check for turbidity contained in the test tube derived from the bacterium Streptococcus mutans then planted on media nutrient agar on the limit bacterial growth, ie a concentration of 1 levels below the MIC, MIC, and one level above the MIC so that it can be proved that the turbidity contained in the tube Derived from the bacterium Streptococcus mutans, not derived from the influence of the extract. In this research, cultivation on nutrient agar media at 5 final concentration in the test is the concentration of 0.39, 0.19%, 0.09%, 0.04% and 0.02%. The results showed plantings at a concentration of 0.04% violet leaf extract was able to inhibit bacterial growth of Streptococcus mutans is more than 90%, so that a concentration of 0.04% is the minimum inhibitory concentration (MIC) of the purple leaf extract (Graptophyllum pictum) on the growth of Streptococcus mutans.

Table 3. Average And Standard Deviation Of Streptococcus Mutans Colony.

Concentration of Extract (%)	N	Average CFU	SD
Control +	7	276,143	3,388
Media 11 (0,09%)	7	0	0
Media 12 (0,04%)	7	8,429	1,272
Media 13 (0,02%)	7	74,714	2,138

N = Sample size
CFU = Colony Forming Unit
SD = Standard deviation

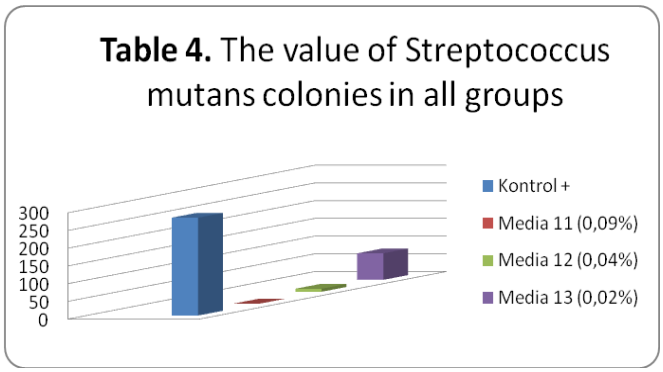

Table 5. The Value Of P Test Results Kolmogorov Smirnov Test And Levene's Test Number Of Streptococcus Mutans Colonies In All Groups

Groups	Kolmogorov Smirnov Test	Levene's Test
Control +	p= 0,986	p= 0,081
Media 12 (0,04%)	p= 0,699	
Media 13 (0,02%)	p= 0,934	

TABLE 6. THE P VALUE OF ONEWAY ANOVA TEST RESULTS TEST THE NUMBER OF COLONIES OF STREPTOCOCCUS MUTANS BETWEEN GROUPS

Groups	Control +	Media 12 (0,04%)	Media 13 (0,02%)
Control +		0,000	0,000
Media 13 (0,02%)			0,000

In table 5 above we can know that the results of data distribution test by using statistical test Kolmogorov Smirnov Test all groups have probability value greater than 0.05 (p> 0.05). This indicates that the group has a normal data distribution. While for homogeneity test of variance by using Levene's Test statistic test have value p = 0,899 (p> 0,05). This shows that the three groups have homogeneous variance. Because the data obtained homogeneous and consists of more than 2 groups of samples so to know the differences of each group tested significance using the one-way ANOVA test. The results of the test differences in the number of colonies of Streptococcus mutans between groups can be seen in table 6 below.

In table 3 it can be seen that at 0,09% concentration have not found any bacterial growth. In the positive control group (+) had an average number of colonies of Streptococcus mutans ie 276,143 ± 3,388 CFU. In table 6 it can be seen that the results of different test between groups of colony *Streptococcus mutans* measurement have p value <0.05. This suggests that there are significant differences in the value of colony of *Streptococcus mutans* between groups

DISCUSSION

This research aims to determine the inhibition and bactericidal of purple leaf extract (*Graptophyllum pictum*) to *Streptococcus mutant*. The minimal inhibitory concentration was observed visually and with a cross-media turbidity test plantings, while the minimum bactericidal concentration is done by counting the number of bacterial colonies. Turbidity or sediment appears on dilution tube showed growth of Streptococcus mutants as purple leaf extract ingredients are not able to

inhibit the growth of bacteria at these concentrations. Invisibility of turbidites stacked or sediment on the tube with a concentration of 0.04% is determined as the minimum inhibitory concentration (MIC) of violet leaf extract on the growth of Streptococcus mutants.

The examination of turbidity obtained on the test tube is derived from the growth of bacteria hence done planting test on nutrient agar media. Based on the observation results obtained that at 0.09% concentration has not found any bacterial growth in nutrient agar. At concentration 0,04% which is the minimum inhibitory concentration is still found the existence of bacterial colony but the amount of less than 10%.

The minimal bactericidal concentration (MBC) of purple leaf extract against the mutant Streptococcus bacteria was tested by counting the number of colonies. The result showed no mutant Streptococcus bacteria at concentration 0,09%. The result of the calculation of colony Streptococcus mutants showed that the higher concentration of purple leaf extract, the number of successful mutant Streptococcus colonies grew decreasing. The results of the calculation on Oneway Annova statistics showed that the different test results between the colonies of the mutant streptococcus colony had a p value <0.05 which means that there was a significant difference from the number of mutant Streptococcus colonies between the test groups.

In purple leaf content there are tannins and flavonoids that play a major role in inhibiting the growth of mutant Streptococcus.[12] Tannin has an antibacterial effect that can inhibit the formation of glucosyltranferase by mutant Streptococcus so that sucrose can not be converted into glucose as a source of mutant Streptococcus energy.[12]

The antibacterial mechanism of flavoid compounds can be through various means such as inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function through the formation of complexes against extracellular proteins, and inhibition of metabolism.[13] Flavonoid compounds contained in purple leaf extracts can cause disruption in the formation of nucleic acids, the main ingredient of DNA and RNA forming the genetic core of bacterial cells. Disorders in the formation of nuclei of bacterial genetic cells can cause apoptosis in bacterial cells. This cell death occurs due to inhibition of phase S (synthetic phase) during the process of DNA or RNA formation so that the genetic core and the regulator of the life of bacterial cells are not formed and slowly experiencing death.[14]

Flavonoids are antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes. Damage to cell membranes can cause the surface tension of the bacterial cell membrane to decrease in order to increase the permeability of bacterial cell membranes. This leads to leakage of molecules and ions that can cause cellular damage or death. [15] Intracellular leakage of bacteria causes the discharge of cell components such as nucleus, mitochondria, lysosomes, ribososm, and others. The cell organelle functions to run the life of bacterial cells and maintain normal function of bacterial life, when disturbed the bacterial cell will be damaged and bacteria lytic. [16,17]

CONCLUSION

This study has investigated the effect of crude extract Purple Leaf (*Graptophyllum pictum*), against virulence properties of *S. mutans*. It reflects a prospective role of *Graptophyllum pictum* as a potential therapeutic agent against virulence traits of *S. mutans*. Hence, it can be a promising prophylactic therapeutic agent for dental caries.

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