ORIGINAL ARTICLE

Antibacterial Activity and Phytochemical Analysis of Ethanolic Purple Leaf Extract (*Graptophyllum Pictum* L.griff) on *Lactobacillus Acidophilus*

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ABSTRACT

Introduction: : *Lactobacillus acidophilus* is an anaerobic facultative gram-positive bacterium that contributes to secondary caries. A cavity cleanser was needed as an antibacterial agent to eliminate oral microorganism which contaminated the tooth cavity. *Graptophyllum pictum* L.Griff is one of the herbal medicaments which has the potential to be used as an antibacterial agent as it contains active antibacterial chemical-compound. **Methods:** Qualitative phytochemical analysis of purple leaves extract was determined with several methods. *Lactobacillus acidophilus* ATCC 4356 was suspended into several concentrations of *Graptophyllum pictum* L.Griff extract from the dilution on BHIB medium. **Results:** Purple leaves extract contained some bioactive compounds flavonoid, alkaloid, tannin, triterpenoid/steroid, and saponin. Minimum Inhibitory Concentration (MIC) of the ethanolic purple leaves extract was 6.25% and Minimum Bactericidal Concentration (MBC) of the ethanolic purple leaf extract was 12.5% against *Lactobacillus acidophilus*. **Conclusion:** Ethanolic purple leaves extract (*Graptophyllum pictum* L.Griff) has antibacterial activity against *Lactobacillus acidophilus*.

Keywords: *Lactobacillus acidophilus*, Phytochemicals, Herbal medicine, Antibacterial

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INTRODUCTION

Lactobacillus acidophilus is the most dominant bacteria among other Lactobacillus species; it is a gram-positive bacteria and grows in anaerobic conditions (1). An antibacterial is needed to suppress the growth of *Lactobacillus acidophilus* and prevent secondary caries. The use of antibacterial agents to eliminate pathogenic microorganisms in the tooth cavity and prevent secondary caries can be done through cavity disinfection procedures with the application of a cavity cleanser (2).

Purple leaves (*Graptophyllum pictum* L.Griff) is one of Indonesia's traditional medicinal plants, which has antibacterial properties. It is expected that the use of these plants as herbal medicines can reduce the number of *Lactobacillus acidophilus* bacteria, which is one of the pathogenic bacteria that cause dental caries (3).

MATERIALS AND METHODS

Phytochemical Screening

In the detection of phenols by ferric chloride test, extracts were treated with 3-4 drops of ferric chloride solution. To detect the flavonoid using alkaline reagent test, extracts were treated with few drops of sodium hydroxide solution. In the detection of tannins by gelatin test, a 1% gelatin solution containing sodium chloride was added to the extract. The extract was diluted with distilled water to 20 ml and then shaken in a cylinder for 15 minutes for the froth test to detect saponins. Detection of alkaloids by Mayer's Test was done by dissolving the extract individually in hydrochloric acid and then filtered. Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Detection of terpenoid/steroid was done using Salkowski's test and the extract was treated with chloroform and filtered. The filtrates

were treated with few drops of Concentrated Sulphuric acid, shaken, and then allowed to stand (4).

Antimicrobial Activity of *Lactobacillus acidophilus* Culture Supernatants

The formulation of purple leaves extract with various concentrations was carried out by dilution, where the extract was obtained with concentrations of 25%, 12.5%, 6.25%, 3.125%. 0.05 ml of bacterial suspension that has been standardized with 0.5 McFarland was planted in a tube containing BHIB. The control (+) test tube was filled with 0.05 ml of *Lactobacillus acidophilus* suspension and BHIB media without the addition of purple leaves extract, while the control (-) test tube contained BHIB media without the addition of *Lactobacillus acidophilus* and purple leaves extract to ensure that there would be no bacterial contamination of the media. Each group consisted of 7 samples, then the entire test tubes were incubated in an anaerobic incubator at 37°C for 24 hours. Because dark extracts and turbidity occurred in all tubes, each tube was taken of 1 osse, then etched on Tryptone Yeast Cystine media and incubated anaerobically at 37°C for 24 hours where the presence or absence of bacterial growth was observed. The result of the bacterial growth limit streak was used as the MIC. 0.1 ml of the boundary tube between bacterial growth and non-positive control was taken to be planted on Tryptone Yeast Cystine media using a spreader and incubated at 37°C for 24 hours to cross-check the growth of *Lactobacillus acidophilus* (5).

The calculation was repeated three times by three different observers in which the average amount was taken. The data analysis test used in this study was the Kolmogorov-Smirnov normality test and the non-parametric test using Kruskal-Wallis, followed by a signification test using the HSD Post Hoc Tukey Test.

RESULTS

The phytochemical analysis of purple leaves is shown in Table I, while the average growth rate of *Lactobacillus acidophilus* on nutrient agar can be seen in Table II.

Before an analysis test was carried out between the research groups on the *Lactobacillus acidophilus*, a normality test was carried out in each group using the Kolmogorov-Smirnov test as shown in Table III.

Table I : Phytochemical screening results for purple leaves extract

Active compound	Phytochemical test	Result
Phenol	Ferric Chloride Test	+
Flavonoid	Alkaline Reagent Test	+
Tannin	Gelatin Test	+
Alkaloid	Mayer's Test	+
Saponin	Froth Test	+
Terpenoid	Salkowski test	+
Steroid	Salkowski test	+

Table II : Dilution test results of 96% ethanol extract of purple leaf (*Graptophyllum pictum* L.Griff) with the number of *Lactobacillus acidophilus* from each tube (CFU/ml)

Treatment Groups	N	Concentration				Control Groups	
		25%	12.5%	6.25%	3.125%	Positive	Negative
L.acidophillus	7	-	-	+	+	+	-
Average (CFU/ml)		0	0	12.33±0.30	22.5±0.28	131±0.48	0

Table III : The Kolmogorov-Smirnov test between groups

Groups	6.25%	3.12%	Control (+)
Kolmogorov Smirnov test	p = 0.833	p = 0.828	p = 0.918

From the results of normality test data using the Kolmogorov-Smirnov Test in the control (+) group, the concentration of 6.25% and 3.12% had a p-value > 0.05. This shows that the group has a normal data distribution.

On the results of the Post Hoc Test HSD Test (Tukey HSD Test), Table IV shows a significant difference in the number of *Lactobacillus acidophilus* between the control (+) concentrations of 6.25% and 3.12%.

Table IV : Results of differences in the significance of effectiveness between concentration groups with the Tukey HSD Test

	Concentration 6.25%	Concentration 3.12%	Control (+)
Concentration 6.25%	-	0.000*	0.000*
Concentration 3.12%	0.000*	-	0.000*
Control (+)	0.000*	0.000*	-

DISCUSSION

The average number of colonies growing at a concentration of 3.125% purple leaves extract (*Graptophyllum pictum* L.Griff) was 131 CFU/ml. The bacterial growth was inhibited by 90.6%, causing the concentration of 6.25% purple leaves extract (*Graptophyllum pictum* L.Griff) to be considered as the minimum inhibitory concentration (MIC) of the *Lactobacillus acidophilus*. At a concentration of 12.5%, there was no growth of *Lactobacillus acidophilus*. Thus, at a concentration of 12.5%, according to the requirements of the minimum bactericidal concentration (MBC), the extract can eliminate bacteria by 99.9% of the total average bacteria that managed to grow in control positive.

The active compounds of an extract will bind damage to the cell wall, causing the growth of the bacteria to be inhibited. Alkaloids work as an antibacterial by damaging the component of bacterial cells, preventing the wall layer to be formed intact and causes bacterial cell death. Polyphenols work by reacting with bacterial cell membranes and cause bacterial cell lysis, denaturation of proteins, and inhibit the formation of cytoplasmic proteins, nucleic acids, and ATP-ase bonds in bacterial cell membranes. Tannin works by coagulating bacterial protoplasms, precipitating proteins, and binding to proteins as prevention towards the formation of bacterial cell walls. Active flavonoids as an antibacterial power cause the denaturation of proteins found in cell walls that can damage the composition and change the permeability mechanism of microsomes, lysosomes, and cell walls (6,7). Saponins can interact with bacterial cell walls, causing the walls to lysis because saponins can form a foam (which is similar to detergent) that disrupts the surface tension of cell walls (7). The active compounds work synergistically in inhibiting and eliminating the growth of *Lactobacillus acidophilus*.

CONCLUSION

The purple leaf extract (*Graptophyllum pictum* L.Griff) has antibacterial activity against *Lactobacillus acidophilus*. The minimum inhibitory concentration was 6.25% and the minimum bactericidal concentration was 12.5%.

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