



MINIMUM INHIBITORY CONCENTRATION OF PURPLE LEAF EXTRACT (*GRAPTOPHYLLUM PICTUM* L. GRIFF) AGAINST *LACTOBACILLUS ACIDOPHILUS* ATCC 4356

KONSENTRASI HAMBAT MINIMUM EKSTRAK DAUN UNGU (*GRAPTOPHYLLUM PICTUM* L. GRIFF) TERHADAP PERTUMBUHAN *LACTOBACILLUS ACIDOPHILUS* ATCC 4356

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ABSTRACT

Background: Dental caries is an oral cavity disease that affects most Indonesians. *Lactobacillus acidophilus* (*L. acidophilus*) is one of the bacteria that causes dental caries. Control of bacteria in the form of antibacterial agents is needed to suppress the growth of *L. acidophilus*. Purple leaves (*Graptophyllum pictum* L. Griff) are a medicinal plant with antibacterial compounds, namely flavonoids, alkaloids, saponins, and tannins. **Purpose:** Determine the Minimum Inhibitory Concentration (MIC) of purple leaves extract on the growth of *L. acidophilus*. **Method:** The sample consisted of seven groups, including positive control (chlorhexidine 0.2%), negative control (BHI-B), and purple leaves extract with concentrations of 25%, 12.5%, 6.25%, 3.12%, and 1.56%. The antibacterial activity of purple leaves extract was carried out quantitatively using a spectrophotometer with a wavelength of 600 nm. After that, it was incubated at 37°C for 48 hours, followed by absorbance measurement. The absorbance results were then analyzed using the Paired T-Test (before and after incubation). **Result:** Purple leaves extract concentrations of 6.25%, 12.5%, and 25% had an inhibitory effect on *L. acidophilus*. **Conclusion:** Minimum Inhibitory Concentration (MIC) of purple leaves extract on the growth of *L. acidophilus* was 6.25%

ABSTRAK

Latar belakang: Sampai saat ini karies gigi merupakan penyakit di rongga mulut yang paling banyak diderita masyarakat Indonesia. *Lactobacillus acidophilus* (*L. acidophilus*) merupakan salah satu bakteri penyebab karies gigi. Pengendalian bakteri berupa bahan antibakteri sangat diperlukan untuk menekan pertumbuhan *L. acidophilus*. Daun ungu (*Graptophyllum pictum* L. Griff) merupakan tanaman obat yang mengandung senyawa antibakteri yaitu flavonoid, alkaloid, saponin, dan tanin. **Tujuan:** Mengetahui Konsentrasi Hambat Minimum (KHM) dari ekstrak daun ungu terhadap pertumbuhan *L. acidophilus*. **Metode:** Sampel terdiri tujuh kelompok meliputi kontrol positif (chlorhexidine 0,2%), kontrol negatif (BHI-B), dan ekstrak daun ungu dengan konsentrasi 25%, 12,5%, 6,25%, 3,12%, dan 1,56%. Aktivitas antibakteri ekstrak daun ungu dilakukan secara kuantitatif menggunakan spektrofotometer dengan panjang gelombang 600 nm. Setelah itu, diinkubasi pada suhu 37°C selama 48 jam, dilanjutkan pengukuran absorbansi. Hasil absorbansi kemudian dianalisis menggunakan *Paired T-Test* (sebelum dan sesudah inkubasi). **Hasil:** Ekstrak daun ungu konsentrasi 6.25%, 12.5% dan 25% memiliki daya hambat terhadap *L. acidophilus*. **Kesimpulan:** Konsentrasi Hambat Minimumnya (KHM) ekstrak daun ungu terhadap pertumbuhan *L. acidophilus* adalah 6,25%.

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INTRODUCTION

Dental caries is the most common problem affects in the world's population, including Indonesians, with a prevalence of 88.8% (Riskasdas, 2018). Dental caries is a disease caused by many factors, one of which is the *Lactobacillus acidophilus* (*L. acidophilus*) (Hakim, 2018). *L. acidophilus* is a facultative, anaerobic, rod-shaped, Gram-positive bacterium found in saliva samples from caries sufferers and on the tongue's surface (Ahirwar *et al.*, 2019). *L. acidophilus* produces lactic acid after the fermentation of carbohydrates and can survive in low pH environment. The repeated cycle of acid formation causes the pH in the oral cavity to decrease and will facilitate the growth of acidogenic bacteria, including *L. acidophilus*. A repeated decrease in pH for a specific time also results in demineralization of the tooth surface (Viranda Sutanti *et al.*, 2021).

Control of bacteria in the form of antibacterial agents is needed to suppress the growth of *L. acidophilus*. The antibacterial material that is commonly used is chlorhexidine. Research by Evans *et al.* (2015) said chlorhexidine gluconate 0.2% could inhibit the growth of *Streptococcus mutans*, *Streptococcus sanguinis*, and *L. acidophilus*. However, chlorhexidine has side effects such as xerostomia, hypogeusia, parotid gland swelling, and oral paresthesia (Tartaglia *et al.*, 2019). Another problem that arises from the use of chlorhexidine is *Antimicrobial Resistance* (AMR), where bacteria become resistant, which means the antibacterial becomes less effective (Brookes *et al.*, 2020). Based on this, the use of new natural materials with minimal side effects and high antibacterial activity.

The use of plants for treatment has been widely used because they have low side effects (Sumayyah, 2018). One of the medicinal plants that can be used is the purple leaves plant (*Graptophyllum pictum* L. Griff). Purple leaves are 1 of 66 medicinal plant commodities stipulated through the Decree of the Minister of Agriculture Number 104/KPTS/HK.140/M/2/2020. The part that is often used is the leaves. Purple leaves are shrubs, single leaf shape, dark purple, short stems located facing each other and crossed, wavy edges with tapered leaf tips (Wibowo *et al.*, 2020; Sya'haya and lyos, 2016).

Phytochemical analysis of purple leaves revealed the presence of flavonoids, steroids, glycosides, tannins, saponins, chlorophyll, nontoxic alkaloids, and anthocyanins (Makkiyah *et al.*, 2021). Research on the antibacterial activity of purple leaves extract against *L. acidophilus* has been carried out using the liquid dilution method to obtain a *Minimum Inhibitory Concentration* (MIC) of 6.25% (Juniarti *et al.*, 2021). However, determining MIC by dilution is difficult for dark concentrated extracts (Sari *et al.*, 2015). Purple leaves extract has a deep purple-black color due to the high content of flavonoids (Juniarti *et al.*, 2021). Therefore, research is needed to determine MIC with other methods.

Bacterial test measurements were carried out by visually looking at the turbidity of the test medium and quantitatively using a spectrophotometer. The advantages of the obtained spectrophotometric test results are pretty accurate and quantitative, and the numbers read directly are recorded by the detector (Seniati *et al.*, 2019). The use of spectrophotometry to measure the antibacterial inhibition of purple leaves extract has yet to be researched. This research aims to strengthen previous studies and to obtain MIC of purple leaves extract on the growth of *L. acidophilus* with different bacterial test methods.

MATERIAL AND METHOD

The current study is laboratory experimental research with a *pre test-post test* control group research design. The study was conducted in the Bioscience Laboratory Jember Dental and Oral Hospital (RSGM) University of Jember. *L. acidophilus* obtained from the Biomedical Laboratory of the Faculty of Dentistry, University of Jember with ATCC 4356 strain. Purple leaves (*Graptophyllum pictum* L. Griff) were taken from the plantation of the Indonesian Medicines Education Forum (WETO), University of Jember, then macerated with 1500 ml of 70% ethanol for three days. The dilution of the extract was done by serial dilution method (serial dilution) with a ratio of 1 : 2 (w/v) and obtained concentrations of 25%, 12.5%, 6.25%, 3.12% and 1.56%. Suspension preparation by taking *L. acidophilus*, which had been cultured, was taken using an ose wire and placed in a test tube containing 4 ml of BHI-B media. The test tube was closed and incubated at 37°C for 48 hours (Balram Bhargava, 2019). The turbidity of the bacterial suspension was according to the standard 0.5 McFarland.

This research included seven groups of positive control (chlorhexidine 0.2%), negative control (BHI-B), and purple leaf extract with concentrations of 25%, 12.5%, 6.25%, 3.12%, and 1.56%. All groups were given 0.1 ml of *L. acidophilus* suspension. All groups were taken 1 ml and put into the eppendorf to measure the absorbance value. Measurements using a spectrophotometer with a wavelength of 600 nm. After that, they were incubated at 37°C for 48 hours. The test tube that had been incubated was observed for turbidity visually and continued with a measurement with a spectrophotometer.

Visual observation was conducted by looking at the turbidity or precipitate in the test solution. There is no antibacterial activity if there is turbidity or sediment (Kusumaningsih *et al.*, 2021). The group whose turbidity level is the same as or close to the negative control is labeled (-), which means there is no antibacterial activity. Determination of MIC, namely the lowest concentration that has inhibited the growth of bacteria in the tube, is characterized by the absence of turbidity or sediment (Balram Bhargava, 2019). The values of the

observations using a spectrophotometer before and after incubation are compared. If the final absorbance value (after incubation) increases or is greater than the initial absorbance value (before incubation), it can be concluded that bacterial growth is still occurring. Suppose there is no change in the absorbance value between the start and end, or the final absorbance value is smaller than the initial absorbance value. In that case, it can be concluded that the growth of bacteria is inhibited. MIC is determined by the smallest concentration in a tube that has experienced a decrease in absorbance (Astutiningsih *et al.*, 2014; Wuon *et al.*, 2018). The absorbance results were then analyzed using analyzed using *Paired Sample T-Test* with SPSS application which aims to determine the average difference between the two paired samples (before and after incubation).

RESULT

The results of the purple leaves extract treatment (Figure 1) which had been given *L. acidophilus* suspension and incubated for 48 hours. In Figure 1, can be seen that tube K(+): positive control, K(-): negative control; A: 25% concentration of purple leaves extract; B: 12.5% concentration of purple leaves extract; C: 6.25% concentration of purple leaves extract; D: purple leaves

extract concentration of 3.12%; and E: purple leaves extract concentration of 1.56%.

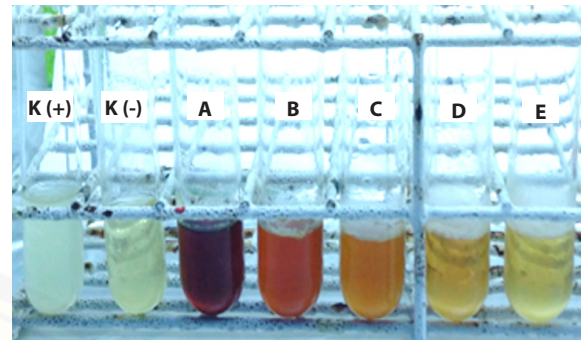


Figure 1. Visual observation results of purple leaves extract with *L. acidophilus* suspension after incubation

The results of visual observations were carried out by five different observers with five repetitions (Table 1). Minimum inhibition of bacterial growth by visual observation occurs at a concentration of 3.12%. After observing visually, it is followed by measuring the absorbance value using a spectrophotometer -wavelength before and after incubation at 600 nm. The wavelength of 600 nm is light that bacteria can absorb (McBirney *et al.*, 2016). The results of quantitative observations, namely the average absorbance value of each group before and after using the spectrophotometer, can be seen in Table 2.

Table 1. Visual observation results of purple leaves extract with *L. acidophilus* suspension after incubation

Group	Result					Desc.
	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5	
K (+)	+	+	+	+	+	+
K (-)	-	-	-	-	-	-
25%	TT	TT	TT	TT	TT	TT
12.5%	TT	TT	TT	TT	TT	TT
6.25%	+	+	+	+	+	+
3.12%	+	-	-	+	+	+
1.56%	-	-	-	-	-	-

Note: (+) inhibition of bacterial growth; (-) no inhibition of bacterial growth, TT was not observed.

Table 2. Results of measuring the difference in the average absorbance before and after incubation of purple leaves extract

Group	Results			Desc.
	Average		ΔOD	
	Before incubation	After incubation		
K (+)	0.2224	0.2016	- 0.0208	Down
K (-)	0.0676	0.6162	0.5486	Up
25%	0.5914	0.4828	- 0.1086	Down
12.5%	0.4650	0.3572	- 0.1078	Down
6.25%	0.3512	0.2508	- 0.1004	Down
3.12%	0.1216	0.4828	0.3612	Up
1.56%	0.0852	0.4662	0.3810	Up

Note: (down) inhibition of bacterial growth; (up) no inhibition of bacterial growth

At a concentration of 6.25%, it can be seen that the absorbance value before and after incubation decreased, so this concentration was determined as the MIC of purple leaves extract on growth *L. acidophilus*. The absorbance values that have been obtained are grouped and analyzed using the *Paired Sample T-Test* (Table 3).

Table 3. The results of the *Paired Sample T-Test*

Group	N	SD	Sig. (2-tailed)
K (+)	6	0.020	0.085
K (-)	5	0.098	0.001*
25%	5	0.109	0.088
12.5%	5	0.093	0.062
6.25%	5	0.038	0.743
3.12%	5	0.012	0.001*
1.56%	5	0.048	0.000*

Note: * there was a significant difference before and after incubation

There was a significant difference in the significance value of the purple leaves extract group at concentrations of 3.12%, 1.56%, and K(-). This difference was caused by a significant increase in *L. acidophilus* growth after incubation because purple leaves extract could not inhibit *L. acidophilus* growth. There was no significant difference in the purple leaves extract concentration of 25%, 12.5%, and 6.25% before and after incubation. The average results before and after incubation were relatively the same due to the antibacterial activity of purple leaves extract.

DISCUSSION

This research is a laboratory experimental study that aims to determine the *Minimum Inhibitory Concentration* (MIC) of purple leaves extract (*Graptophyllum pictum* L. Griff) on the growth of *L. acidophilus*. The antibacterial test method of this study was based on visual observations and quantitative measurements using spectrophotometry. Minimum inhibition of bacterial growth by visual observation occurs at a concentration of 3.12%. MIC was determined visually by comparing the test solution tube with the control group tube. MIC determination is done by looking at the turbidity of the solution in the tube, not by looking at the color density of the solution in the tube. The reading of the MIC results is the lowest concentration that inhibits bacterial growth marked by no turbidity or sediment (Kusumaningsih and Febiyanto, 2015). The results of measuring the absorbance of the purple leaves extract at concentrations of 25%, 12.5%, and 6.25% decreased the absorbance value after incubation. An increased absorbance value indicates bacterial cell growth, while a constant and/or reduced absorbance value after incubation indicates inhibition of bacterial growth.

MIC is determined by the smallest concentration, which has inhibited bacterial growth marked by a decrease in absorbance (Astutiningsih *et al.*, 2014; Wuon *et al.*, 2018). The smallest concentration in the tube that inhibited the growth of bacteria in this study was 6.25%.

There are differences in the results of MIC between visual observations and quantitative observations using a spectrophotometer. Visual observation depends on the observer's subjectivity, lighting, and room conditions when making observations, which can lead to possible errors. While observations using a spectrophotometer, the results obtained are quite accurate because the numbers that are read are recorded directly by the detector and are quantitative in nature. Based on this, purple leaves extract concentration of 6.25% was concluded as MIC from *L. acidophilus*.

The MIC results in this study are in line with previous studies. Juniarti *et al.* (2021) found that purple leaf extract using 96% ethanol has antibacterial activity against *L. acidophilus* with a minimum inhibitory concentration of 6.25%. This research used the dilution method and then streaked on tryptone yeast cystine media to determine MIC. This research uses a different solvent, using 70% ethanol. There was no difference in the MIC results obtained. Kusumaningsih *et al.* (2021) found no difference in the MIC value between 70% and 96% ethanol solvent purple leaves extract against *Aggregatibacter actinomycetemcomitans*. However, the diameter of the inhibition zone of 70% ethanol was 3.37 mm larger than that of 96% ethanol.

The inhibition of *L. acidophilus* growth is thought to be due to the antibacterial activity of purple leaves. The antibacterial activity of purple leaves is obtained from secondary metabolites of purple leaves extract. Phytochemical analysis of 70% ethanol extract of purple leaves contains alkaloids, saponins, tannins, and flavonoids. Quantitative analysis of chemical content, namely 2.76% total saponins, 2.66% total flavonoids, 0.13% tannins (Makkiyah *et al.*, 2021). The antibacterial activity of purple leaves extract in the presence of these secondary metabolites will provide a synergistic and mutually reinforcing effect because both flavonoids, alkaloids, tannins, and saponins all have antibacterial activity (Kurniawati *et al.*, 2020; Dyasti *et al.*, 2021).

It was known that the active compounds contained in purple leaves extract were flavonoids, alkaloids, steroids, saponins, and tannins. These active compounds have different mechanisms as antibacterials. Flavonoids, as antibacterial, cause the denaturation of proteins found in cell walls so that they can damage the composition and change the mechanism of cell wall permeability (Juniarti *et al.*, 2021). Disruption of cell wall permeability results in damage to the plasma membrane so that the processes of osmoregulation, respiration, and transportation are disrupted. The mechanism of saponin as an antibacterial is damaging membrane permeability, causing the cytoplasm to leave the cell and cell death. This condition ultimately

causes bacterial cell death (Anandhi *et al.*, 2014). Alkaloid compounds can damage the peptidoglycan component of *L. acidophilus* so that the cell wall is not formed and causes bacterial death (Juniarti *et al.*, 2021; Kurniawati *et al.*, 2020). Alkaloids inhibit bacterial growth by inhibiting nucleic acid synthesis, thereby inhibiting the bacterial replication process (Ryan *et al.*, 2018). Tannins work by freezing the protoplasm, precipitating proteins, and binding to proteins to inhibit cell wall formation (Juniarti *et al.*, 2021). Tannins can bind to proteins mainly through hydrophobic interactions and hydrogen bonds. This disrupts *L. acidophilus* metabolism and decreases lactic acid production (Kaczmarek, 2020).

The limitation of this research, spectrophotometric measurements are not selective in differentiating samples from contaminants or other particles that can absorb light at the same wavelength. In addition, too concentrated extracts can affect light absorption by bacterial cells (Permata *et al.*, 2016). Therefore, further research is needed using *High-Performance Liquid Chromatography* (HPLC). HPLC is a tool with a separation method in pharmaceutical analysis that can separate specific compounds and measure the amount of these compounds in solution.

CONCLUSION

The *Minimum Inhibitory Concentration* (MIC) of purple leaves extract (*Graptophyllum pictum* L. Griff) against *L. acidophilus* is 6.25%.

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