Antibacterial Differences Effect between Purple Leaves (Graptophyllum Pictum (L) Griff.) 70% And 96% Ethanol Extract Against Aggregatibacter Actinomycetemcomittans Bacteria

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Abstract

Since the main causative agent for local aggressive periodontitis was the Aggregatibacter actinomycetemcomitans bacteria (A. actinomycetemcomittans). Purple leaves (Graptophyllum pictum (L) Griff.), which contain antibacterial compounds such as flavonoids, tannins, saponins, steroids, and alkaloids, are chosen as one of the natural medicinal ingredients. However, such antibacterial compounds that contain 70% ethanol and 96% ethanol are different. This study proves the differences between the antibacterial effect of 70% and 96% of ethanol extract of purple leaves against the A. actinomycetemcomitans bacteria. This study, purple leaf Simplicia, was extracted with 70% and 96% ethanol using the maceration method. The antibacterial power test has carried out using the serial dilution method to determine MIC and MBC. The diffusion method on the Luria Bertani media utilized to measure the inhibition zone has formed. It has revealed that there was no significant difference between the number of growths of bacterial colonies on MIC and MBC for 70% and 96% ethanol extract of purple leaves. MIC and MBC in 70% and 96% ethanol extract of purple leaves were 3.125% and 6.25%. Unlike the serial dilution test, the diffusion test obtained a significant difference with p=0.019 (p=<0.05). In ethanol, 96% of the inhibition zone's diameter is 13.13 mm, and in 70% ethanol, is 16.5 mm. In short, 70% and 96% ethanol extract of purple leaves has the same antibacterial effect on A. actinomycetemcomittans bacteria. However, the extra antibacterial activity of 70% ethanol in bacteria is more than the 96% ethanol extract of purple leaves.

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Introduction

Aggressive periodontitis is the second most of the periodontal diseases in the world after teeth caries[1]. In Indonesia, periodontal disease has a prevalence of 96.58% in all age groups. It is often in the form of gingivitis and periodontitis[2]. Moreover, the prevalence of the number of patients with gingivitis was 55.25%. Those with periodontitis were 44.75%[3,4]. The pathogenesis of aggressive periodontitis is influenced by the host and bacteria interactions.

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It has dominated by *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)* bacteria with a frequency of around 90% compared to chronic periodontitis, which is only 21%[5,6]. A. actinomycetemcomitans are gram-negative bacteria that become the main cause of localized aggressive periodontitis[7].

Nowadays, periodontitis treatment is still using antibiotics to eliminate bacteria[8–10]. Several cases found that the use of antibiotics in periodontitis is not suitable. It causes antibiotic resistance. Therefore, there is much research that is has been developed to discover antibacterial and supportive therapy using herbal plants[11,12].

Plant-derived medicines, also known as herbal medicines, can be used as alternatives to chemical drugs due to their lower potential to cause harmful effects[13–15]. One of the herbal

plants that tend to have antibacterial contents is Purple leaves (*Graptophyllum pictum* (L) Griff.)[16]. Purple leaves have alkaloid, flavonoid, steroid, saponin, and tannin that act as antibacterial. Compounds contained in it can be drawn through the extraction process and strongly influenced by various solvents. For example, the flavonoid is easily dissolving in polar solvents such as ethanol, butanes, and acetone[17,18]. Tannin also cannot be dissolved in a non-polar solvent, as either but tannin can easily dissolve in water, acetone, and alcohol[19].

The extraction process is the separation specific compound or active substance from a solid mixture or liquid using certain solvents. Solvents are used to attract active components of the medicinal-herb sample mixture. The bioactive components in the mixture will move into the solvent with intensive contact[20,21].

Some different solvents can be used in the extraction process: water, ethanol, methanol, acetonitrile, diethyl ether, and acetone[22]. Ethanol was chosen as a solvent in the natural medicines' extraction process because it had high solubility, most of the secondary metabolites were insoluble, non-toxic, and inert, so they did not interfere with other components. The use of ethanol as a solvent could prevent the growth of fungi and bacteria in the extract, therefore minimizing the occurrence of contamination in the extract. The low ethanol boiling point facilitated the evaporation process in the extraction process using less heat[23–25].

Several previous research that has conducted on antibacterial inhibitory testing by comparing several solvents with various ethanol concentrations. Recent research by Sagita *et al* (2017) stated that 96% of ethanol extract could extract the wet samples because of its lots of water, proved by extracted tannin and flavonoid substances in *Pyyrosia piloselloides (L)* herbs with 96% ethanol[26]. Other research conducted by Mubarak *et al* (2018) showed that bioactive components extraction was better in 70% ethanol than in 96% ethanol. In order to that, 70% of ethanol is more polar than 96%. Therefore, it gave the best antibacterial inhibition in bligo fruit (*Benincasa hispida Thunb*) extract against *Salmonella typhimurium*[27].

No previous research has investigated the antibacterial differences test between 70% and 96% ethanol extract of purple leaves against *A. actinomycetemcomittans* bacteria. Therefore, this study has conducted to prove antibacterial differences between 70% and 96% ethanol extract of purple leaves against *A. actinomycetemcomittans* bacteria. This research could provide the readers with more information about the best ethanol concentration as a solvent for purple leaves extract, which provides antibacterial potential against *A. actinomycetemcomittans* bacteria.

Materials and methods

Ethical clearance

This study has given the ethical clearance approval from the Committee of Dental Medicine, Universitas Airlangga, Indonesia (No. 246/HRECC.FODM/IX/2018).

Extract Processing

Purple leaves (*Grapthophyllum pictum*) has collected from the local market Surabaya-Indonesia. 300gr of purple leaves powder macerated with 3,6 L ethanol 70% and 96%, which have been redistilled with an ultrasonic method for 2 minutes and repeated three times then filtered. The extract is separated from the residue. The residue has re-macerated with 4,8 L of ethanol 70% and 96% with the same methods. The collected extracted then concentrated with a rotavapor vacuum until thick extract obtained. The thick extract was then evaporated in a 40°C oven.

Bacterial Test Preparation

The *A. actinomycetemcomitans* ATCC 43718 bacteria were inoculated into BHIB media and incubated for 24 hours at 37°C temperature, anaerobic. The turbidity of *A. actinomycetemcomitans* suspension was adjusted to 0,5 McFarland standard.

Inhibition Rate Test, Dilution Method

Eppendorf was given a 1-11 number. Serial dilution was done in *Eppendorf* 1-8. *Eppendorf* 1 contained 1 ml of 70% ethanol extract and 96% ethanol extract of purple leaves in 100% concentration. In each *Eppendorf* 2-8 is filled with 0,5 ml BHIB media. Serial dilution was done by aspirating 0.5 ml 70% ethanol extract and 96% ethanol extract of purple leaves in *Eppendorf* 1, then dispensed into *Eppendorf* 2, then continued to aspirate 0,5 ml from *Eppendorf* 2 then dispensed into Eppendorf 3 and so on until *Eppendorf* 8. The last aspiration was taken 0,5 ml from *Eppendorf* 8 and thrown away so that the *Eppendorf* sequence is obtained with the

following concentration of 100%, 50%. 25%, 12,5%, 6,25%, 3,125%, 1,56%, and 0,78%. Then each *Eppendorf* 1-8 was added 0,01 ml *A. actinomycetemcomitans* bacteria culture.

Eppendorf 9 controlled positive contained 0.5 BHIB media and 0,01 ml *A. actinomycetemcomitans* bacteria culture. *Eppendorf* 10 controlled negative contained 0,5 BHIB media. *Eppendorf* 11 was control of extract sterility contained 5 ml of 70% and 96% ethanol extract of purple leaves. The eleventh *Eppendorf* tube was then incubated in anaerobic for 24 hours at 37°C temperature. Bacterial growth was determined by replanting in Luria *Bertani media* in each *Eppendorf* **(Figure 1).**

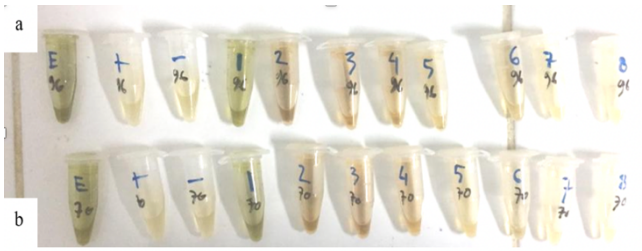

Figure1. *Serial dilution* **(a)** 96% ethanol extract and **(b)** 70% ethanol extract of purple leaves against *A. actinomycetemcomittans* in each concentration.

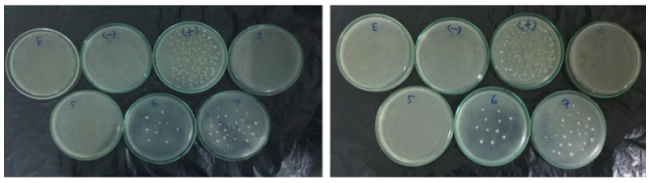

Figure 2. Replanted results of purple leaves 96% ethanol extract and 70% ethanol extract Luria Bertani media.

The replanting results determined the MIC and MBC 70% ethanol extract and 96% ethanol extract of purple leaves on each of *A. actinomycetemcomitans* bacteria. Then, colonies are counted in *Luria Bertani* media to reassure MIC and MBS, comparing the inhibitory strength between 70% ethanol extract and 96% ethanol extract of purple leaves against *A actinomycetemcomittans* bacteria (**Figure 2**). 0.01 ml of serial dilution on *Eppendorf* MIC, MBC, one concentration below MIC, one concentration above MBC, also control was planted in *Luria Bertani* media with spreading method then incubated anaerobically in 37°C temperature for 24 hours.

Inhibition Rate Test, Diffusion Method

After determining the minimum inhibitory concentration and minimum bactericidal concentration in the dilution test, then continued with diffusion test to find the ability of 70% and 96% ethanol extract of purple leaves in MIC and MBC. *A. actinomycetemcomitans* bacterial suspension was spread in the petri dish contained *Luria Bertani* media. 5 mm disc paper dropped by 10µl 70% and 96% ethanol extract of purple leaves with 3,125% concentration (MIC) and 6,25% concentration (MBC) then placed in above the media surface. Plates are incubated upside-down for 2 x 24 hours in 37°C temperature, anaerobically. Then the calipers were used to measure the Minimum Inhibitory concentration.

Results

Extraction Yield

The results of thick extract maceration of 96% ethanol were 44,89 grams, and 70% ethanol was 66,28 grams (Table 1).

Table 1. Extraction Yield Results

Solvent	Simplicia	Thick Extract	Rendement
Ethanol 96%	300 gr	44,89 gr	14,9%
Ethanol 70%	300 gr	66,28 gr	22,09%

Table 1. The results of thick extract maceration.

Inhibition Rate Test, Dilution Method

The results of the inhibition rate test and serial dilution method found that in 3,125% concentration was the MIC and 6,25% concentration was the MBC of purple leaves extract against *A. actinomycetemcomittans* **(Figure 1, 3 and Table 2).**

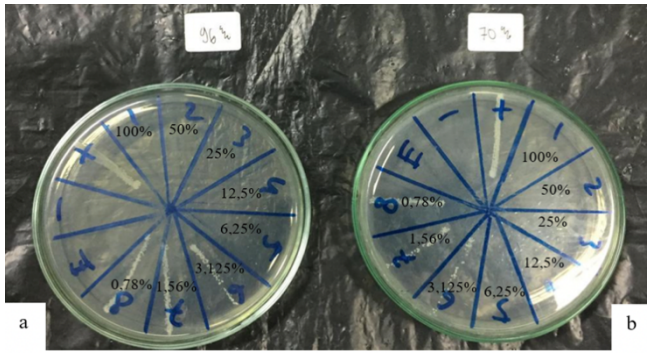

Figure 3. Replanting (streak) results in each concentration of 96% ethanol extract **(a)** and 70% ethanol extract **(b)** of purple leaves against *A. actinomycetemcomittans* bacteria.

Solvent	Treatment	Extract concentration							Control		
		100 %	50 %	25 %	12,5 %	6,25 %	3,125%	1,56 %	Extract	Positive	Negative
96% Ethanol	Bacterial Growth Existences	-	-	-	-	-	+	+	-	+	-
	Mean Growth	0	0	0	0	0	1040	2680	0	14660	0
70% Ethanol	Bacterial Growth Existences	-	-	-	-	-	+	+	-	+	-
	Mean growth	0	0	0	0	0	1000	2720	0	15020	0

Table 2. *A. actinomycetemcomittans* bacterial colonies calculation result on *Luria Bertani* media in CFU/ml in each concentration of 70% and 96% ethanol extract of purple leaves.

The results of the inhibition rate test and diffusion method found that there was no inhibitory zone in 3,125% concentration. Meanwhile, in 6,25% concentration found that 70% ethanol extract had more significant inhibitory zone activity with a mean of 16,5 mm than 96% ethanol extract with a mean of 13,1 mm (Figure 4, Table 3).

Figure 4. Diffusion test result of 70% ethanol extract and 96% ethanol extract of purple leaves with 3,125% and 6,25% concentration against *A. actinomycetemcomittans* bacteria.

70% Ethanol Extract		96% Ethanol Extract	
3,125% Concentration (mm)	6,25% Concentration (mm)	3,125% Concentration (mm)	6,25% Concentration (mm)
0	16,5	0	13,1

Table 3. Inhibition zone diameter between 70% and 96% ethanol extract with 3,125% and 6,25% concentration of Purple leaves against *A. actinomycetemcomittans* bacteria.

Discussion

This research aims to discover an antibacterial rate of 70% and 96% ethanol extract of purple leaves against *A. actinomycetemcomittans* bacteria. A large amount of yield produced shown the total of substances extracted and suspected as the bioactive compounds. The results showed that 96% ethanol extract of purple leaves outcome was fewer than 70% ethanol extract yield. The extract yield caused this had concentrated gel form, which was thick and sticky.

The difference in yield between 70% and 96% ethanol extract of purple leaves could benefit the manufacturer because the exact Simplicia amount of the 70% ethanol extract could produce more extract than 96% ethanol extract. 70% ethanol extract is a mixture of two solvents, namely; ethanol and water, with 70% ethanol (v/v) that effectively producing the optimal amount of bioactive compounds. The advantages of the water contained in 70% ethanol are; reusable, minimalizing ethanol toxicity, and reducing the production cost in the industrial field.

In this research, purple leaves extract obtained with 70% and 96% ethanol solvent, which has the same MIC and MBC; 3,125% and 6,25% (no significant difference). After MIC and MBC were determined, it has continued with the inhibition rate test with the diffusion test. Inhibitory zone diameter showed bacteria sensitivity of the antibacterial compound. The formed zone diameter, determined by the concentration of bioactive compounds in the extract, identified that compounds have diffused to the entire medium. Thus, the wider the inhibitory zone diameter, the more potent and sensitive it will be[28].

The results showed a significant difference in inhibitory zone growth between 70% ethanol extract and 96% ethanol extract of purple leaves in 6,25% concentration (MBC). In those concentrations, 70% ethanol extract has a larger inhibitory zone with a mean of 16,5 mm rather than 96% ethanol extract with a mean of 13,1 mm. It concluded that both ethanol extracts have the same minimum bactericidal concentration. Furthermore, 70% of ethanol extract has a more significant bactericidal effect than 96% ethanol extract.

There was a significant difference in *A.*

actinomycetemcomittans bacterial growth in ethanol extract with 6,25% concentration (MBC), which suspected came from dissolved bioactive compounds. Based on the extraction principle, the extraction of a component has based on its polarity. Research conducted by Irwan (2011) stated that 70% of ethanol could attract both polar and non-polar compounds such as alkaloid, saponin, tannin, steroid, and flavonoid. On the contrary, 96% ethanol solvent could extract compounds group of alkaloids, flavonoid, steroid, and tannin[29].

The non-exist content in 96% ethanol extract and 70% ethanol extract of purple leaves are saponins [29]. Saponins' solubility causes this in water and ethanol [30]. Saponins mechanism as an antibacterial agent is by reducing the surface tension of the bacterial cell wall and damaging the membrane's permeability because its active compound is similar to a detergent. Saponins diffused through its fragile outer membrane and cell wall the binding cytoplasm to disturb and reduce membrane cell stability. Thus, causes cytoplasm leaked out from the cell and cell death [31]. The cell membrane damage causes inhibition of the activity and biosynthesis of specific enzymes needed in metabolic reactions, and this condition ultimately causes the death of bacteria cells.

Overall, this discussion summarized that there is a difference in antibacterial activity in the diffusion test caused by semi-polar to polar properties of 70% ethanol extract. Moreover, it has expected to dissolve compound with ideal antibacterial activity, both polar and semi-polar such as polyphenols, flavonoid, and saponins contrast with 96% ethanol extract [32].

It lessens the antibacterial compound extracted in 96% ethanol extract. Compound extraction is more capable of providing more significant inhibition.

Conclusions

The main conclusion that there are no differences in antibacterial rate between 70% and 96% ethanol extract of purple leaves against *A. actinomycetemcomitans* bacteria. An antibacterial test found that 3,125% concentration extract as MIC and 6,25% concentration extract as MBC in 70% and 96% ethanol extract of purple leaves by the dilution method. Meanwhile, 70% ethanol extract of purple leaves has a greater bactericidal rate when viewed from the inhibitory zone formed.

Declaration of Interest

The authors report no conflict of interest.

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