DENTIN
JURNAL KEDOKTERAN GIGI
Vol VII. No 2. AGUSTUS 2023

ANTIBACTERIAL EFFECTIVENESS OF WATER HYACINTH (*Eichhornia crassipes*) LEAF EXTRACT ON THE GROWTH OF *Porphyromonas gingivalis*

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ABSTRACT
Background*: The most common periodontal disease is periodontitis which is frequently found in the form of chronic periodontitis. Such disease is caused by the accumulation of the bacterium Porphyromonas gingivalis where supporting therapy such as chlorhexidine gluconate mouthwash 0.2% can be given. However, its use in the long term can have a negative effect on the oral cavity. There is a natural plant, namely water hyacinth (Eichhornia crassipes) which contains compounds that can be used as antibacterial, such as: tannins, phenols, flavonoids, alkaloids, saponins, terpenoids, and steroids. As a result of this, the water hyacinth plant can be used as an alternative herbal medicine.* ***Purpose****: To analyze the antibacterial effectiveness of water hyacinth leaf extract (Eichhornia crassipes) against the growth of* Porphyromonas gingivalis *bacteria.* ***Methods****: This study used True Experimental with posttest only with control group design. Antibacterial test was performed using liquid dilution method to determine MIC and solid dilution to determine MBC.* ***Results****: Based on the results and data analysis, it was found that the water hyacinth leaf extract had MIC at 1,56% and MBC at a concentration of 50%. Conclusion: Leaf extract of water hyacinth (Eichhornia crassipes) can inhibit and have antibacterial properties against the growth of* Porphyromonas gingivalis *bacteria.*

Keywords: *Chronic periodontitis, Leaf extract of Water Hyacinth, Porphyromonas gingivalis*
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INTRODUCTION

Periodontitis is a chronic infectious disease caused by microorganisms. The most common periodontitis disease is chronic periodontitis. Chronic periodontitis is an infectious disease of the gingiva that can cause damage to the periodontal tissue due to the accumulation of plaque and bacteria. The most common bacteria found in patients with chronic periodontitis is Porphyromonas gingivalis. Porphyromonas gingivalis is a type of anaerobic gram-negative bacteria commonly found in subgingival plaque. Bacteria in subgingival plaque can cause periodontal tissue damage due to bacteria penetrating into the gingival sulcus and causing the sulcus to deepen.[1]

Treatment for chronic periodontitis can be done by scaling root planning (SRP) along with drug therapy. Other chemical therapy can be given with mouthwash such as 0.2% chlorhexidine gluconate which is the gold standard of periodontal disease treatment. Chlorhexidine gluconate 0.2% is considered effective for reducing the formation of bacterial biofilms in periodontal pockets, such as Porphyromonas gingivalis bacteria which is the dominant bacterium in the incidence of chronic periodontitis.[2] bacteria, discoloration of tooth enamel, and often xerostomia or dryness of the oral mucosa. The number of negative impacts caused by 0.2% chlorhexidine gluconate, so it is necessary to use herbal ingredients as an alternative to mouthwash made from natural ingredients.[3]

Water hyacinth (*Eichhornia crassipes*) is considered to have a negative impact such as spreading over a large area and can cover the water surface and dead water hyacinth can also settle to the bottom of the water and cause siltation. As a result of this, the water hyacinth plant can be used as an alternative herbal medicine. In a study by Kiristos *et al.* (2018) stated that water hyacinth leaf

extract contains secondary metabolites that act as antibacterials such as tannins, flavonoids, alkaloids, saponins, terpenoids, and steroids. The amount of compound content in water hyacinth has been done before.[4] Research by Kumar *et al.* (2014) stated that the levels of phenolic compounds in water hyacinth leaves were known to be 10.63±0.13mg PGE/g sample.[5] Research by Mohamed *et al.* (2019) stated that the flavonoid content was 4.20±0.30µg CAE/mg extract and the tannin content was 3.13±0.25µg TAE/mg extract. Phenol compounds act as antibacterial by denaturing proteins and damaging the composition of cell membranes. Flavonoid compounds act as antibacterial by inhibiting nucleic acid synthesis, energy metabolism, and membrane function. Tannin compounds are antibacterial because of their high toxicity to cell permeability.[6] The amount of alkaloid content in water hyacinth has also been previously carried out by Lata and Dubey (2010) with 0.66 ± 0.630% in rhizomes and as much as 0.36 ± 0.077% in shoots. These alkaloid compounds can act as antibacterial by destroying the peptidoglycan component in the bacterial cell wall.[7]

A study by Joshi and Kaur (2013) revealed that water hyacinth leaves have antibacterial activity by inhibiting the growth of pathogenic microorganisms, namely *Escherichia coli*, *Staphylococcus epidermidis*, and *Bacillus subtilis* at concentrations of 25 g/ml, 50 g/ml, 75 g/ml. , and 100 g/ml in the extract using ethanol, methanol or aquadest.[8] Research from afidati *et al.* (2019) regarding water hyacinth leaf extract has also been carried out previously using *Aggregatibacter actinomycetemcomitans* bacteria with concentrations of 0.78%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50% and 100% . In this study it was said that water hyacinth extract contains compounds that function as antibacterial, namely flavonoids, terpenoids, phenols, and alkaloids. At a concentration of 6.25%-100% the results were obtained as MBC (Minimum Bactericidal Concentration) and at a concentration of 1.56%-3.125% as MIC (Minimum Inhibitor Concentration), while at a concentration of 0.78% it was seen that bacterial growth was the same as the control. (not given water hyacinth leaf extract).[9] Based on this, the purpose of this study was to analyze the antibacterial effectiveness of water hyacinth (Eichhornia crassipes) leaf extract against the growth of Porphyromonas gingivalis bacteria (in vitro study with dilution method) with concentrations of 1.56%, 3.125%, 6.25%, 12 ,5%, 25%, and 50%.

METHOD

Early Preparation

Licensing was done with Ethical clearance No. 033/KEPKG-FKGULM/EC/III/2021 from the Faculty of Dentistry, Lambung Mangkurat University. Before conducting the research, the tools to be used should be washed first and then sterilized using an autoclave for 5 minutes at a temperature of 121ºC.[1]

Research Tools and Materials

The tools needed in making water hyacinth leaf extract and antibacterial test were analytical balance, erlenmeyer, measuring cup, petri dish, test tube, sterile loop, Spectrophotometer UV-Vis, pipette, micropipette, colony counter, rotary evaporator, mask, handscoon, cotton sterile, tube rack, yellow tip, blue tip, incubator, hot plate, blender, water bath, and autoclave.

The materials needed in this study were water hyacinth leaves, Porphyromonas gingivalis bacteria, 0.2% chlorhexidine gluconate as a positive control, aquadest as a negative control, liquid dilution media in the form of Brain Heart Infusion Broth (BHIB) and Nutrient Agar (NA) as a dilution medium. solid. Other materials needed in the research were 70% ethanol for the maceration method, acetic acid, sulfuric acid, and potassium dichromate for an ethanol-free test.

Water Hyacinth Plant Determination Test

The plant determination test was carried out to avoid errors in the materials to be used for research. The selected water hyacinth plants had leaf sizes with a diameter of 12-18 cm. Water hyacinth plants were obtained from the banks of the Martapura River, South Kalimantan. The water hyacinth plant determination test carried out at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

Making Water Hyacinth Leaf Extract

The production of water hyacinth leaf extract was carried out using the maceration method. The first step of washing as much as 1 kg of water hyacinth leaves and cutting them into small pieces and then drying using an oven at a temperature of 50ºC. After that, the dried water hyacinth leaves were crushed using a blender to obtain water hyacinth leaf powder. Before use, the powder was stored in a tightly closed container. After that, the water hyacinth leaf powder was soaked into 70% ethanol solvent used per day. The water hyacinth powder was soaked for 3 days. The results of the immersion were filtered using filter paper so that the filtrate became clear 3 times. The results of three times the clear filtrate from water hyacinth leaf

extract (*Eichhornia crassipes*) were then combined into one, then evaporated using a rotary evaporator until the filtrate was reduced. The filtrate was then released from the solvent by heating over a water bath until the concentration of the filtrate thickens and produces a thick extract.[9]

Ethanol Free Test

Ethanol free test was carried out to ensure pure extract without any ethanol contamination. This is because ethanol has antibacterial properties so that if the extract still contains ethanol, it can cause false positive results in the sample. Ethanol-free testing was done by adding acetic acid and sulfuric acid to water hyacinth leaf extract and then heating. The extract can be said to be free of ethanol. Another way was done by adding drops of sulfuric acid and potassium dichromate to the extract and then see the color change. If there is no color change, it can be said that the extract is free from ethanol.[10]

Bacteria Test Preparation

Porphyromonas gingivalis was cultured on BHIB media into sterile test tubes until the turbidity was equivalent to the standard Mc Farland 0.5 (1.5 x 10^8).[11]

Antibacterial Test

Testing the antibacterial effectiveness of water hyacinth leaf extract (*Eichhornia crassipes*) can be done by solid and liquid dilution methods. The first step was to make a mother liquor of water hyacinth leaf extract. The extract was then diluted using aquadest and made into several concentrations, namely 1.56%, 3.125%, 6.25%, 12.5%, 25%, and 50% using the dilution formula V1 x C1 = V2 x C2. Then put the diluted extract into a vacuum tube using a sterile micropipette as much as 1 ml per tube and also add 1 ml of standardized bacterial suspension using a Mc Farland turbidity 0.5 ($1.5x10^8$). In another tube, 2 ml of 0.2% chlorhexidine gluconate and 2 ml of distilled water were added to different tubes. The vacuum tubes were closed using sterile cotton and homogenize using a vortex mixer. At the time before and after incubation for 24 hours, the vacuum tubes were measured using a Spectrophotometer UV-Vis for their absorbance which had previously been adjusted to the maximum wavelength.[11]

In the MIC assessment, namely the Optical Density (OD) value or the difference from the absorbance value is lower or there is a decrease compared to the delta OD in the negative control group, it is considered that there is still bacterial growth in the media and there is no MIC. After the MIC assessment was carried out, it was continued with testing to determine MBC by taking the concentration that had previously shown the presence of MIC and then put it in a petri dish containing sterile NA media and then incubated for 24 hours at 37ºC. After that, the bacterial colonies were counted using a colony counter. If the result of the total number of bacterial colonies is 0 CFU/µL or the absence of bacterial growth, the smallest concentration with the value of the colony is MBC.[11]

RESULT

The results of the measurement of the difference in the absorbance value or MIC and the number of bacterial colonies can be seen in tables 1 and 2 below:

Table 1 Measurement of MIC of Water Hyacinth Leaf Extract on *Porphyromonas gingivalis* Bacterial Growth Bakteri

Treatment	Amount	*Mean*			Standard Deviation	Information
		Before Incubation	After Incubation	Difference (Absorbance)		
1,56%	7	0,503	0,227	-0,276	0,012	Turun
3,125%	7	0,830	0,420	-0,410	0,015	Turun
6,25%	7	1,177	0,911	-0,265	0,036	Turun
12,5%	7	1,811	1,236	-0,575	0,060	Turun
25%	7	2,000	1,633	-0,367	0,035	Turun
50%	7	2,000	1,946	-0,053	0,020	Turun
C (+)	7	1,433	0,475	-0,958	0,018	Turun
C (-)	7	0,357	1,427	1,070	0,044	Naik

The table above shows that the average difference in absorbance values or MIC in the treatment group of water hyacinth leaf extract concentrations of 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, and positive control showed a decrease. The mean difference in absorbance values or inhibition of the growth of Porphyromonas gingivalis bacteria, while the negative control group had an increased mean value from before incubation which indicated an increase in the mean absorbance value so that the results obtained that the MIC of water hyacinth leaf extract on the growth of Porphyromonas gingivalis bacteria was a concentration of 1 ,56%.

Table 2 MBC Data of Water Hyacinth Leaf Extract on Porphyromonas gingivalis Bacterial Growth Bakteri

Treatment	Amount	Number of Colonies (CFU/µL)	
		Mean	Standard Deviation
1,56%	7	2101,714	295,818
3,125%	7	1701,571	157,193
6,25%	7	1608,857	182,770
12,5%	7	1215,714	157,676
25%	7	891,714	188,284
50%	7	0,000	0,000
C (+)	7	0,000	0,000
C (-)	7	2303	468,225

The table above shows the MBC of water hyacinth leaf extract based on the mean number of colonies after 24 hours of incubation. In the water hyacinth leaf extract group of 1.56%, 3.125%, 6.25%, 12.5%, and 25%, it was known that there was still growth of Porphyromonas gingvalis bacterial colonies, while the water hyacinth leaf extract group with a concentration of 50% showed no growth of Porphyromonas gingvalis bacterial colonies, so that the results obtained that MBC from water hyacinth leaf extract against the growth of Porphyromonas gingvalis bacteria was at a concentration of 50%.

The data that has been obtained from the results of the study was carried out statistical analysis using statistical software version 26.0. The data were then tested for normality using the Kolmogorov-Smirnov test. This method was used because the samples used were more than 50 samples and carried out a homogeneity test using Levene's test. MIC data is normally distributed, then parametric analysis is carried out using the One Way Anova hypothesis test and the resulting data has different variances, then followed by the Post-Hoc Games Howel test. The MBC data is not normally distributed or the variance is not the same, then a non-parametric test will be carried out using Kruskal-Wallis and the resulting data has a significant difference, then proceed with the Mann Whitney Post-hoc test. The results of the Post Hoc Test can be seen in tables 3 and 4 below:

Table 3 Post Hoc Games Howel MIC Test Results Water Hyacinth Extract

	Water Hyacinth Leaf Extract Concentration						Control	
Treatment	**1,56%**	**3,125%**	**6,25%**	**12,5%**	**25%**	**50%**	**(+)**	**(-)**
1,56%	-	0,000*	0,985	0,000*	0,004*	0,000*	0,000*	0,000*
3,125%		-	0,000*	0,003*	0,175	0,000*	0,000*	0,000*
6,25%			-	0,000*	0,000*	0,000*	0,000*	0,000*
12,5%				-	0,000*	0,000*	0,000*	0,000*
25%					-	0,000*	0,000*	0,000*
50%						-	0,000*	0,000*
Control +							-	0,000*
Control -								-

Table 4 Post Hoc Test Results Mann Whitney MBC Water Hyacinth Leaf Extract.

	Water Hyacinth Leaf Extract Concentration						Control	
Treatment	**1,56%**	**3,125%**	**6,25%**	**12,5%**	**25%**	**50%**	**(+)**	**(-)**
1,56%	-	0,006*	0,002*	0,002*	0,002*	0,001*	0,001*	0,277
3,125%		-	0,406	0,002*	0,002*	0,001*	0,001*	0,004*
6,25%			-	0,009*	0,002*	0,001*	0,001*	0,002*
12,5%				-	0,013*	0,001*	0,001*	0,002*
25%					-	0,001*	0,001*	0,002*
50%						-	1,000	0,001*
Control +							-	0,001*
Control -								-

DISCUSSION

Based on the results of the research above, it can be seen that the hypothesis is proven because there is an antibacterial effectiveness of water hyacinth (*Eichhornia crassipes*) leaf extract against the growth of Porphyromonas gingivalis bacteria. In accordance with the data seen from the MIC value at a concentration of 1.56% and MBC at a concentration of 50%, it was indicated that there was no growth of Porphyromonas gingivalis bacterial colonies after incubation for 24 hours. This shows that water hyacinth leaf extract is able to inhibit and kill the growth of gram-negative bacteria, namely Porphyromonas gingivalis, as well as research conducted by Joshi and Kaur (2013) which also used gram-negative bacteria, namely *Escherichia coli*.[8]

The antibacterial effect of water hyacinth leaf extract on the growth of Porphyromonas gingivalis bacteria can occur due to the content of secondary metabolic compounds that can work with certain mechanisms. Water hyacinth leaf extract contains secondary metabolic compounds such as tannins, saponins, steroids, terpenoids, alkaloids, flavonoids, and phenols. Tannins have a toxic effect on cell permeability. The mechanism of tannins can shrink the bacterial cell wall and cause the permeability of the cell to be disrupted by targeting the peptidoglycan wall of the bacterial cell, as a result the cell cannot carry out live activities and make growth stunted or even die.[12]

Saponins have antibacterial activity by lowering cell surface tension, resulting in increased permeability or leakage from cells and resulting in the release of intracellular compounds and disrupting cell membrane stability resulting in bacterial lysis. Steroids can damage the plasma membrane and cause the cytoplasm to leak out of the cell. This happens because steroid molecules have non-polar (hydrophobic) and polar (hydrophilic) groups so that they have a surfactant effect that can dissolve the phospholipid components of the plasma membrane.[13]

Terpenoids have a toxic effect on the walls of bacteria, both gram-positive and gram-negative bacteria. This happens because of the interaction with proteins on the cell membrane and intracellular components, causing disruption of the membrane structure of gram-negative bacteria. Alkaloids are able to damage the peptidoglycan component of the bacterial cell wall. These conditions can cause the structure of the cell wall layer to be unable to fully form and result in the death of bacterial cells.[9]

Flavonoids function to inhibit nucleic acid synthesis, bacterial energy metabolism, cell membrane function. Flavonoids also have functions as antioxidants, anticancer, anti-inflammatory, antiallergic, antiviral, and antibacterial. Phenol serves to inhibit the growth of bacteria by denaturing proteins and damaging the membrane structure. The active phenol can penetrate into the bacterial cytoplasmic membrane either by active or

passive diffusion. At the cytoplasmic level, phenol will cause damage to the mitochondrial membrane, endoplasmic reticulum, and nucleus. Phenol can also damage the organelle components of the cytoplasm.[9]

Secondary metabolic content in water hyacinth leaf extract, namely tannins, saponins, steroids, terpenoids, alkaloids, flavonoids, and phenols has the same mechanism of action as 0.2% chlorhexidine gluconate, which can cause rupture of bacterial cell walls, reduce adhesion of epithelial cells, and result in the death or lysis of bacterial cells.[9,11,13]

Positive control in the form of chlorhexidine gluconate is an antimicrobial agent. Chlorhexidine gluconate is commonly used in the form of mouthwash, gel, varnish, toothpaste, spray, and sugar-free gum. Chlorhexidine gluconate can react in the cytoplasmic membrane. As a result of this reaction will occur dicationic at a pH above 3.5. It can prevent plaque from accumulating, so chlorhexidine gluconate is an anti-plaque agent. Chlorhexidine gluconate is a cationic biguanide that can be bacteriostatic in low amounts and will be bactericidal in higher concentrations.[3] Chlorhexidine gluconate with a concentration of 0.2% is an antiseptic that is often used or the gold standard for mouthwash in the treatment of periodontitis today, because it has antibacterial properties that can also serve to reduce the buildup of bacteria or plaque. Chlorhexidine gluconate 0.2% can also inhibit the virulence factors of Porphyromonas gingivalis by reducing the adhesion of epithelial cells, damaging cell walls, and causing bacterial cell death.[14]

The negative control group in the form of distilled water showed that there was still bacterial colony growth after treatment and could not inhibit or stop bacterial growth. This is in line with the research of Khotimah *et al.* (2017) which states that distilled water or condensed water is distilled water that is free from impurities so that it is pure in the laboratory which is usually used as a solvent and cleaning tools.[15] The use of aquadest as a negative control is also in line with research conducted by Muljono *et al.* (2016) and who state that its use as a negative control is essential to rule out the possibility of antimicrobial effects.[16]

Based on statistical tests on the difference in absorbance values and the number of bacterial colonies of Porphyromonas gingivalis in the concentration groups of 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, positive control, and negative control, it was found that almost all concentrations have a significant difference or there is a significant difference with each other. In the statistical test of the difference in the absorbance value of the 1.56% concentration group, there was no significant difference to the 6.25% concentration, and the 3.125% concentration group to the 25% concentration. In the statistical test of the number of Porphyromonas gingivalis bacteria colonies there were no significant differences in several groups, namely the concentration of 1.56% against the negative control group, the concentration of 3.125% against the 6.25% concentration, and the concentration of 50% against the positive control group. There was no significant difference or significant difference because the results of the decrease in absorbance values between the groups were not much different. This is in line with research conducted by Sinarsih *et al.* (2016), which is due to the unstable antibacterial performance at a certain concentration, indicated by the absence of greater inhibition (not significantly different) when a concentration is increased. The instability is caused by the secondary metabolite compound at a certain concentration increasing which has a limited ability in bioactivity, so it does not provide a significant increase in response or is not significantly different.[17]

Based on research conducted by Kumara *et al.* (2019) states 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 will be stronger. It is also known that the mean difference in the absorbance value of the 6.25% concentration is lower than that of the 1.56% concentration. This situation can occur in the presence of noise on the Spectrophotometer UV-Vis which is used to read the absorbance value of 6.25% concentration.[18] Noise is an image quality that is disturbed by images and pixels caused by digital data deviations received by the image data receiver and interferes with image quality. This also happened in the research of Khairiah *et al.* (2020) that there is noise when the treatment group reads 60 mg/ml so that the difference in absorbance values is lower than the concentration of 40 mg/ml. In the concentration group of 1.56%, 3.125%, 6.25%, 12.5%, and 25%, the mean number of bacterial colonies was higher than the 50% concentration group and positive control, but lower than the mean bacterial colony of the control group. negative. The 50% concentration group and the positive control group had the same mean bacterial colony value of 0.000 CFU/µL so it could be said that they had the equivalent ability to stop the growth of the bacterial colony, namely Porphyromonas gingivalis, but had different abilities to inhibit bacterial growth because they contained different amounts of metabolic compounds.[11] This is in line with the research of Ariyana *et al.* (2021) namely the higher the concentration of an extract used, the higher the

content of antibacterial active substances in the extract.[19]

The positive and negative control groups can determine the absorbance value using a wavelength of 480 nm or using visible light, while in the treatment group with concentrations of 1.56%, 3.125%, 6.25%, 12.5%, 25%, and 50% absorbance can be assessed using a wavelength of 740 nm or near infrared. This can be supported by research by Kafle (2020) which states that the control group has a lower level of concentration or turbidity so it can be penetrated by light, while the treatment group with several concentrations is cloudy and very concentrated so it is not easily penetrated by light.[20]

It can be concluded that water hyacinth (*Eichhornia crassipes*) leaf extract is able to inhibit and has antibacterial properties against the growth of Porphyromonas gingivalis bacteria.

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