Antibacterial Activity of Water Hyacinth (Eichhornia Crassipes) Leaf Extract Against Bacterial Plaque from Gingivitis Patients

Agung Krismariono[1]*, Ernie Maduratna Setiawatie[1], Rita Yuana Rachmawati[2], Yosua Adi Setiawan[2], Hafidzah Nareswari Padmarini[2], Nurul Annisa Apriliyanti[2]

1. Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Abstract

Water hyacinth (Eichhornia crassipes) is one of the free-floating aquatic perennials that are still rarely utilized. Water hyacinth leaf extract contains alkaloid compounds, flavonoids, phenols, glutathione, terpenoids, and saponins, which have potent antibacterial activity. This study aimed to determine the antibacterial activity of Water hyacinth leaf extract against bacterial plaque from gingivitis patients. This study used the maceration technique to prepare Water hyacinth leaf extract with 70% ethanol. A dilution technique was used to form eight concentration extracts: 100%, 50%, 25%, 12.5%, 6.125%, 3.125%, 1.56%, and 0.78%.

The antibacterial effect of the extract was studied by counting the number of growth colonies that grew on the Mueller-Hinton Agar (MHA) medium. Post-hoc HSD Test statistical analysis of the antibacterial potency of Water hyacinth leaf extract on colony bacterial plaque growth in patients with gingivitis showed a significant result (p = 0.00) with inhibition activity of Water hyacinth leaf extract starting at 3.125%. Water hyacinth leaf extract was potential effective to inhibit bacterial plaque growth in patients with gingivitis from the concentration of 3.125%.

Experimental article (J Int Dent Med Res 2022; 15(3): 966-971)

Keywords: Water Hyacinth, Antibacterial, Bacterial Plaque.

Received date: 11 January 2022 **Accept date:** 25 May 2022

Introduction

Periodontal disease is one of two main dental and oral diseases that affect human populations worldwide with high prevalence rates. In the world, periodontal disease is the most common disease in permanent teeth, affecting about 20-50% of the global population. One of the periodontal diseases is gingivitis, characterized by inflammation in periodontal tissue without periodontal attachment loss. Plaque accumulation is one of the main etiologies causing gingivitis.[1,2]

Plaque is a biofilm composed of various microbial species encased in a matrix of extracellular polymer substances such as polysaccharides, proteins, and nucleic acids, and consists of organic materials such as glycoproteins, lipids, and DNA. Microbiota on subgingival plaques that have a role in the occurrence of gingivitis consists of a comparison of approximately gram-positive bacteria (56%) and gram-negative (44%) with facultative species (59%) and anaerobes (41%). The predominant gram-positive bacterium that triggers gingivitis is *Streptococcus spp*, one of which is *Streptococcus sanguinis*, which forms and initiates the formation of plaque, and there are also microorganisms frequently found in subgingival plaque of patients with chronic periodontitis like *Porphyromonas gingivalis* and *Treponema denticola*.[3,4]

Pathogenesis of bacteria increases in proportion to the formation of biofilm which acts as an energy source, aggregation, and barrier to bacterial protection. The interaction between toxins produced by microorganisms in plaque that attach to the surface of the tooth and surrounding tissue and host inflammatory cells results in an inflammatory response and initiates an immune response through cytokines. If left untreated, this condition will cause severity with the accumulation and increase of collagenase enzymes that play a role in the destruction of

Corresponding author:
Agung Krismariono,
Department of Periodontology, Faculty of Dental Medicine,
Universitas Airlangga, Jl. Mayjen. Prof. Dr. Moestopo 47
Surabaya 60132, Indonesia.
E-mail: agung-k@fkg.unair.ac.id

soft tissue supporting the teeth. It will increase the progression of the disease into periodontitis and even cause tooth loss and other systemic diseases.[5–7] So we needed substances that can be used to reduce and eliminate the formation of plaque accumulation to prevent the incidence and progression of periodontal disease.

Based on the phytochemical analysis of Water hyacinth (*Eichhornia crassipes*) leaf extract containing alkaloids, flavonoids, phenols, glutathione, terpenoids, and saponins. Water hyacinth leaf extract is known to show broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, and fungi depending on pH, concentration, and duration of action.[8,9] Research shows that the content of flavonoids, terpenoids, and saponins act as antibacterial against the activity of the glycosyltransferase (GTF) enzyme that plays a role in the formation of biofilms, by changing the permeability of cell membrane.[10,11] So indirectly, this mechanism is expected to inhibit the attachment of bacteria to host cells.

There has not been a specific study to discuss the effect of Water hyacinth leaf extract on bacterial activity on plaques of gingivitis sufferers. In addition, the population control of Water hyacinth plants has only been carried out manually so that it increases the amount of unused waste. It is necessary to use water hyacinth in an effort to utilize water hyacinth as a waste product into pharmaceutical potential through pharmacological extraction and fraction methods. Based on this background, the authors would like to conduct further research on the inhibitory power of Water hyacinth leaf extract on bacterial growth in plaques of gingivitis sufferers.

Materials and methods

The study was approved by the Ethics Committee of the Dental Medicine, Universitas Airlangga. This type of research is a laboratory experiment with the design of the post-test only control group design. This research was conducted at the Research Center Faculty of Dental Medicine, Universitas Airlangga, and Laboratory of Materia Medika, Batu, East Java, Indonesia.

The tools and materials used are racks and test tubes, brander spirits, petri dishes, measuring cups, incubators, micropipette, rotary evaporators, osse, spreaders, analytical scales, Water hyacinth leaf extract, gingivitis patient plaque bacterial suspension, Brain Heart Infusion Broth Media (BHIB), Mueller Hinton Agar (MHA), Mc Farland standard solution 0.5 (1.5 × 108 CFU / Ml).

Hyacinth Leaf Extract Preparation

Water hyacinth plants are washed using running water until clean, then thinly cut using a stainless steel knife and separated from the leaves and stem of the plant. After being cut, the leaves are dried using an oven at 50 °C. The dried results are blended and sifted to form a fine powder. The powder from the water hyacinth plant was weighed at 50 g and then macerated with 100 ml of 70% ethanol in the 500 ml Erlenmeyer. The maceration process is carried out for 24 hours with boiling using an orbital shaker. A 70% ethanol filtrate is separated from the residue using a Buchner funnel which has been coated with filter paper with the help of a vacuum pump. The filtrate is stored at room temperature. The residue obtained is macerated for 24 hours, up to 3 times replication to get a clear filtrate. The 70% ethanol extract was evaporated at 60 °C using a rotary evaporator and continued with evaporation in the water bath until a thick ethanol extract was obtained.

Dental Plaque Sampling

Plaques are taken directly from the cervical portion of gingivitis patients in 20-30 year-olds with sterile excavator. Plaque that has been taken was bred in the BHIB media. Incubating BHIB media containing plaque for 1 × 24 hours at 37 °C. The turbidity BHIB media was compared with the standard 0.5 Mc Farland (1.5 × 108 CFU / Ml).

Inhibition Test of Water Hyacinth Leaf Extract on Plaque Bacterial Growth

The inhibitory test in this study used serial liquid dilution method to obtain 8 extract concentrations of 100%, 50%, 25%, 12.5%, 6.125%, 3.125%, 1.56%, and 0.78%. Then each extract concentration was mixed with 1 ml of a suspension of bacteria and incubated for 24 hours at 37°C. The suspension was then planted in Mueller-Hinton Agar (MHA) media and incubated at 37 °C for 24 hours. Observation of the results was done by counting

the number of colonies grown on Mueller-Hinton Agar (MHA) media manually. The obtained data were statistically tested, including a normality test with the Shapiro-Wilk test, a homogeneity test with the Levene test, intergroup testing with one-way ANOVA, and post-hoc Tukey HSD.

Results

Plaque bacterial colonies that grow on MHA media by adding water hyacinth extract in certain variant concentrations can be seen in Figure 1. The results showed that there was a decrease in the number of plaque bacterial colonies that grew on the media along with the increase in extract concentration. A Comparison of the number of bacterial colonies grown on Mueller Hinton Agar (MHA) media after administration of water hyacinth extract at various concentrations can be seen in Table 1, Figure 2, and 3.

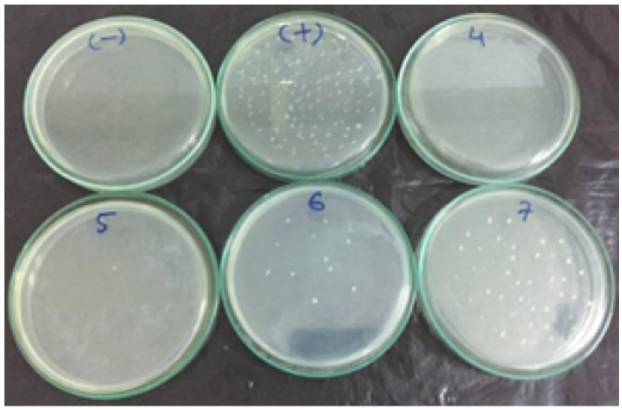

Figure 1. Water hyacinth (Eichhornia crassipes).

Replication	Concentration							Control +	Control -
	100%	50%	25%	12.5%	6.25%	3.125%	1.56%		
1	0	0	0	0	0	11	42	145	0
2	0	0	0	0	0	12	44	141	0
3	0	0	0	0	0	13	40	139	0

Table 1. Bacterial colonies growth on MHA (Mueller Hinton Agar).

Statistical Analysis

Analysis of research data was carried out by calculating the results of the research data in the form of a number of statistical colony growths. Data analysis was only performed on three treatment groups, namely the positive group (culture of plaque bacteria with gingivitis patients), water hyacinth extract with a concentration of 1.56%, and water hyacinth extract with a concentration of 3.125%. Other groups were not included in the statistical calculations because there was only 0 in the group.

Figure 2. Bacterial colonies growth on MHA (Mueller Hinton Agar): (-) negative control, (+) positive control, (4) concentration of 12.5%, (5) concentration of 6.25%, (6) concentration of 3.125%, (7) concentration of 1.56%.

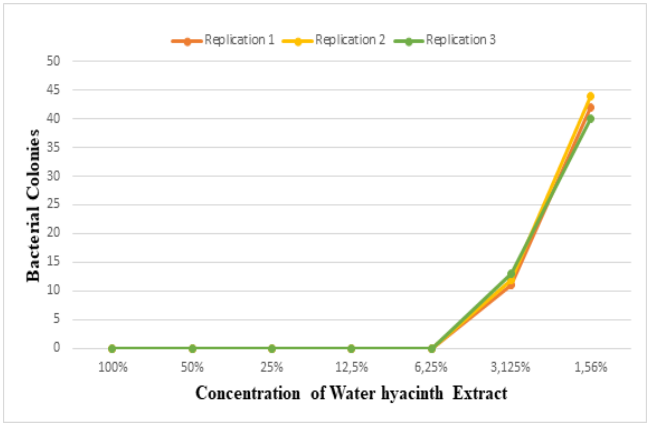

Figure 3. Growth chart of plaque bacterial colonies at each concentration of Water hyacinth leaf extract.

In this study, normality data analysis was used in the Shapiro Wilk Test. The results of the normality test show the value of p-value= 1.0 for the positive control group and extract with a concentration of 6.25% and p-value= 0.64 for extract with a concentration of 3.125% which indicates all three have significant values (p-value > 0.05), it can be concluded that the data for each group tested were normally distributed.

Testing for homogeneity using Levene's Test, the results showed p-values > 0.05 in the three test groups; it can be concluded that the variation in data is homogeneous. Significant tests were performed with the One-Way ANOVA Test to see the significance of differences between groups of variables. The results of the test showed the value of p = 0 in the three test groups, so it can be concluded that the hypothesis is accepted and the data between groups has a significant difference (p < 0.05). Then a further test with post-hoc Tukey HSD showed significant results, so it could be concluded that the data between treatment groups had a significant difference.

In the positive control group, the average number of bacterial colonies was 141.67 CFU/ml assuming the number of colonies lived 100%. In group 4 (extract 12.5%) the percentage of living bacterial colonies was 0% so it can be concluded that bacterial colonies die 100%. This also applies to group 5 (extract 6.25%). However, in group 6 (extract 3.125%) it was found that the average number of living colonies was 12 CFU/ml or 8.5% of living colonies with the conclusion that it could kill 91.5% of plaque bacterial colonies in gingivitis patients. In group 7 (extract of 1.56%) it was found that the average number of live colonies was 42 CFU/ml or as large as 29.6 live colonies with the conclusion that it could kill 70.4% of the plaque bacteria in gingivitis patients. Based on the analysis of the data tested through statistical processing in this study, it can be concluded that the inhibitory power of water hyacinth extract against plaque bacteria in patients with gingivitis at a concentration of 3.125% was more effective than the concentration of 1.56%.

Discussion

Observations made in the negative control group did not show any growth in bacterial colonies. This is due to the negative control group, where no bacteria is embedded. Therefore, it can be ascertained that in this study, there was no contamination in the BHIB (Brain Heart Infusion Broth) transfer medium.

In accordance with the theory, group 6 (concentration of 3.125%) was the minimum inhibitory concentration (MIC) of water hyacinth extract against plaque bacteria in gingivitis patients, because it succeeded in killing 91.5% (> 90%) of plaque bacterial colonies in gingivitis patients. The minimum bactericidal concentration (MBC) was in group 5 (concentration of 6.25%) because it was the smallest concentration that succeeded in killing 99.9% of plaque bacterial colonies in gingivitis patients. However, this study has not been able to determine the minimum inhibitory and bactericidal concentration of water hyacinth extract on the growth of plaque bacteria in gingivitis patients because the study was carried out at a ratio of large concentration ranges.

Water hyacinth leaf extract can inhibit bacteria (bacteriostatic) and kill bacteria (bacteriocides) which are influenced by the presence of chemical compounds contained therein, alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glutathione, and other metabolites such as cardiac glycosides, phlobatannin, quinine, and anthraquinone are active compounds contained in Water hyacinth leaf extract.[8,9] Based on phytochemical tests showed that the results of Water hyacinth leaf extract showed positive results in testing the content of flavonoids, tannins, saponins, and phenols, but showed negative results in the alkaloid content test.

Flavonoids are one of the compounds contained in water hyacinth extract that has antibacterial potential. The mechanism of action of flavonoids in inhibiting bacterial growth by causing damage to bacterial cell wall permeability and inhibiting bacterial motility. Flavonoids have bacteriostatic properties and at higher concentrations can kill broad-spectrum bacteria, both gram-positive and gram-negative bacteria.[12] Flavonoids have the ability to inhibit bacteria directly through lipid bilayers and disrupt bacterial cell defense functions. Through adsorption, flavonoid compounds bind to the hydrophilic part of the cell membrane, which then interacts with bacterial cell membrane proteins. Flavonoid compounds then enter the cell membrane, resulting in precipitation of cell proteins, disrupting the permeability of cell membranes so that the cell membrane becomes lysis. Decreased surface tension and increased permeability of cell membranes result in intracellular leakage and the release of cell components needed by bacteria to metabolize and maintain normal life functions, such as mitochondria, lysosomes, ribosomes, golgi

bodies, and other important intracellular components. This results in biosynthetic reactions of specific enzymes needed in inhibited metabolic reactions. The role of flavonoids as antibacterial is through inhibition of DNA-RNA nucleic acid synthesis and inhibition of energy metabolism.[13–15]

Saponins are triterpene and sterol glycosides that are widely found in plants. Saponin has a bitter taste, foams, and hemolytic activity toward red blood cells. Saponins work as antimicrobials by inhibiting growth and killing microbes through interactions with sterol.[16-18] The main effect of saponin on bacteria is the release of proteins and enzymes from within the cell. Hence, saponins work to disrupt the permeability of bacterial cell membranes. A hydrophobic tip will bind to proteins in cell membranes through polar group bonds, while non-polar saponin groups will bind to cell membrane fat. This situation results in damage to cell membranes and the release of important components in bacterial cells such as proteins, nucleic acids, and nucleotides.[19] Indirectly, this mechanism will inhibit bacterial attachment to the host cell.

The content of tannin compounds in the ethanol extract of Water hyacinth leaf has antibacterial action associated with its ability to disrupt early colonization by deactivating bacterial adhesion, inhibiting the action of enzymes, and inhibiting protein transport in cell sheath.[20,21] The mechanism of action of tannin as an antibacterial material includes the destruction of bacterial cell membranes due to tannin toxicity and the formation of bonds of metal ion complexes from tannins, which play a role in tannin toxicity.[22,23] Bacteria that grow in aerobic conditions require iron for various functions, including the reduction of ribonucleotide DNA precursors. The bond between tannins and iron will cause the disruption of various bacterial functions. *Streptococcus sanguinis* is an early colonizer that aids in the adhesion of succeeding bacteria and plays an important role in biofilm development.[24] *Streptococcus sanguinis* is a facultative anaerobic bacteria, so it can still live in aerobic conditions. If living in aerobic conditions, the bacteria will be disrupted by the presence of tannin compounds, as the study was carried out in aerobic conditions.

Conclusions

Based on the results of research that has been done, it can be concluded that the Water hyacinth (*Eichhornia crassipes*) leaf extract can inhibit bacterial growth in plaques of patients with gingivitis with a minimum concentration of extract of 3.125%.

Acknowledgements

The authors would like to thank the Research Center of the Faculty of Dental Medicine, Universitas Airlangga, and Laboratory of Materia Medika, Batu, East Java, Indonesia for the support in this study.

Declaration of Interest

The authors report no conflict of interest.

References

1. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. Sci World J. 2020;2020:1-8.
2. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. J Clin Periodontol. 2017;44(5):456-462.
3. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza's clinical periodontology. 13th ed. Philadelphia: Elsevier Saunders; 2018:112-132.
4. Sunarto H, Soeroso Y, Dwiyanti S, Anggraeni ED, Bachtiar B, Radi B, Sulijsya B, Kemal Y. Quantitative measurement of Porphyromonas gingivalis and Treponema denticola levels on dental plaque and its relationship with periodontal status and coronary heart disease. J Int Dent Med Res. 2016;9(3):144-150.
5. Marre ATO, Domingues RMCP, Lobo LA. Adhesion of anaerobic periodontal pathogens to extracellular matrix proteins. Braz J Microbiol. 2020;51(4):1483-1491.
6. Bamadi M. Gingivitis and the environmental health: An overview. Middle East J Sci Res. 2013;18(3):283-287.
7. Bartold PM, Van Dyke TE. Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontology 2000. 2013;62(1):203-217.
8. Haggag WM, Abou El Ella SM, Abouziena HF. Phytochemical analysis, antifungal, antibacterial activities and application of Eichhornia crassipes against some plant pathogens. Planta Daninha. 2017; v35(e017159560:1-11.
9. Mala A, Tulika T, Mala A. Pharmaceutical potential of aquatic plant Pistia stratiotes (L.) and Eichhornia crassipes. J Plant Sci Spec Issue Med Plants. 2015;3(1):10-18.
10. Zhang Q, Ma Q, Wang Y, Wu H, Zou J. Molecular mechanisms of inhibiting glucosyltransferases for biofilm formation in Streptococcus mutans. Int J Oral Sci. 2021;13(1):1-8.
11. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Front Microbiol. 2019;10(911):1-12.
12. Biharee A, Sharma A, Kumar A, Jaitak V. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. Fitoterapia. 2020;146(104720):1-17.
13. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A,

Mukhtar S, Zakaria ZA. Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. Molecules. 2022;27(4):1-43.
14. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Curr Med Chem. 2015;22(1):132-149.
15. Ahmad A, Kaleem M, Ahmed Z, Shafiq H. Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections-A review. Food Res Int. 2015;77(2):221-235.
16. Moses T, Papadopoulou KK, Osbourn A. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. Crit Rev Biochem Mol Biol. 2014;49(6):439-462.
17. Faizal A, Geelen D. Saponins and their role in biological processes in plants. Phytochem Rev. 2013;12(4):877-893.
18. Kazan K, Gardiner DM. Targeting pathogen sterols: Defence and counterdefence? PLoS Pathog. 2017;13(5):1-7.
19. Dong S, Yang X, Zhao L, Zhang F, Hou Z, Xue P. Antibacterial activity and mechanism of action saponins from Chenopodium quinoa Willd. husks against foodborne pathogenic bacteria. Ind Crops Prod. 2020;149(112350):1-13.
20. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials-A minireview. Materials. 2020;13(3224):1-10.
21. Narsy S, Elgamily H, El-Refai I, Mehanna N. The clinical efficacy of a probiotic miswak oral spray in patients with gingivitis. J Inter Dent Med Res. 2018;11(2): 433-440.
22. Mailoa MN, Mahendradatta M, Laga A, Djide N. Antimicrobial activities of tannins extract from Guava leaves (Psidium guajava L) on pathogens microbial. International Journal of Scientific and Technological Research. 2014;3(1):236-241.
23. Štumpf S, Hostnik G, Primožič M, Leitgeb M, Salminen JP, Bren U. The effect of growth medium strength on minimum inhibitory concentrations of tannins and tannin extracts against E. coli. Molecules. 2020;25(2947):1-13.
24. Zhu B, Macleod LC, Kitten T, Xu P. Streptococcus sanguinis biofilm formation & interaction with oral pathogens. Future Microbiol. 2018;13(8):915-932.