

Inhibition Activity of Water Hyacinth Leaf Extract (Eichornia Crassipes) to the Growth of Subgingival Plaque Bacteria Colony

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Abstract

Background: Periodontitis is an infectious disease in periodontal tissue and is able to cause a plaque that contains bacteria on the teeth. These bacteria colonize the oral cavity, invade the periodontal tissue, and attack the body's host system. Indonesia has many floras and one of them is called Water hyacinth. This flora is commonly known as waste because its ability to pollute the environment. However, water hyacinth has a useful material including alkaloids, flavonoids, and tannins that can be used as the antibacterial agents. The study aims to determine the effect water hyacinth leaf extract (Eichorniacrassipes) to the growth of subgingival plaque bacteria colony. **Method:** The study was conducted by using serial dilution techniques in the BHIB media with 100%; 50%; 25%; 12,5%; 6,25%; 3,125%; 1,56%; 0,78% concentrations and planting on MHA medium. The analysis method in this study uses the Shapiro-Wilk Test, Levene's Test, and statistical tests with One-Way Anova.

Results: There are significant differences at the growth of sub gingival plaque bacteria. There is no growth of subgingival plaque bacteria in groups of 100%; 50%; 25%; 12.5% and 6.25%. The growth of subgingival plaque bacteria colonies was only seen at 3.125% concentration and increased the number of bacteria colonies at a 1.56%.

Conclusion: Water hyacinth leaf extract was effective to inhibit the growth of sub gingival plaque bacteria colonies.

Keywords: Antibacterial, bacteria colonies, Eichorniacrassipes, sub gingival plaque bacteria.

Introduction

Periodontal disease and dental caries are two diseases that mostly affect the severity of dental and oral diseases. Tooth loss in adults is a result of the severe periodontal abnormalities and is found in 5-15% of the world's population^{1,2}.

Periodontitis is an infectious disease in periodontal tissue with various manifestations, starting from inflammation of the gingiva, formation of periodontal pockets, loss of supporting bone until the occurrence of a loose tooth³. In addition, this disease is also characterized by the inflammation that causes damage to collagen fibers, the gingival matrix, periodontal ligaments, and alveolar bone⁴. Periodontitis is often associated with infections from various bacteria, such as *Porphyromonasgingivalis*, *Tannerella forsythia* and *Aggregatibacteractinomycetemcomitans*. The occurred tissue damage that is the result of complex interactions of these bacteria with the response of the host as well as the inflammatory process⁵.

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During the inflammation process, bacterial products are released and penetrate into the periodontal tissues. Afterward, those bacterial products activate the host immunoinflammatory response by producing cytokines and matrix metalloproteinase which can cause damage to the extracellular matrix of the periodontal tissue. The matrix metalloproteinase is a proteolytic enzyme that causes damage to the extracellular matrix, periodontal ligament, and the bone protein matrix. Thereby, MMP plays an important role in the regulation of periodontal tissue damage⁶.

Dental plaque is the main factor which causes the early periodontal abnormalities and known as gingivitis – a process of inflammation in the gingiva⁷. Plaque formation begins with the formation of dental pellicles on the clean surface of the tooth in minutes. These bacteria can bind to receptors on the dental pellicle. Initial colonization is started by gram-positive facultative bacteria, such as *A. viscosus*, *S. sanguis*. This plaque develops rapidly in the early development of the biofilm and progressively slows down during the biofilm maturation⁸. The next stage is secondary colonization by bacteria such as *Prevotellaintermedia*, *Prevotelleloescheii*, *Capnocytophagaspp*, *Fusobacteriumnucleatum*, and *Porphyromonasgingivalis*. These bacteria have the ability to attach at the different species of plaque microorganisms. If the plaque is not cleaned, it will develop into the subgingival area which causes subgingival plaque formation⁹.

Nowadays, the modern medical world of the molecular field is very advanced, but this does not stop the traditional medicine to exist. This is proved by the many traditional medicines that are widely used by the medical world to treat diseases. Some plants are known for their antibacterial activity¹⁰.

Water hyacinth is a plant that floats on the surface of the water as well as has thick leaves and air-filled stems which make this plant float. This plant is considered disturbing because it can spread in large areas of the water and cover the surface of the water. Thereby, it can reduce the light that penetrate trough the water and result in reduced oxygen in the water. Other affect include silting the lake or other waters due to water hyacinth that dies and settles on the bottom. But the content of the water hyacinth plants is secondary metabolites of alkaloids, flavonoids, tannins, which known as antibacterial, antiviral, and anticancer¹¹. Based on the background, the aims of the study is to analyse the inhibition activity of

water hyacinth leaf extract (*echiorniacrassipes*) to the growth of subgingival plaque bacteria colony.

Material and Method

This type of research is a laboratory experiment with the design of the Post-Test Only control group design¹². This research was conducted in the Research Center Dental Medicine Faculty, Airlangga University and Pharmacy Laboratory, Airlangga University, East Java.

The tools and materials used are test tubes and shelves, buchner funnel, petri dish, glass gourd, incubator, micropipette, osse, spreader, rotary evaporator, scales, auctoclave, water hyacinth leaf extract (*Eichornia Crassipes*), subgingival plaque bacteria stock, *Brain Heart Infusion Broth* (BHIB) media, and *Mueller Hinton Agar* (MHA)media

Making Hyacinth Leaf Extract: Water hyacinth leaves are dried with oven in 50°C until dry. After the drying process, then the grinding one is conducted using a blender to get water hyacinth powder granule, as much as 600 grams of water hyacinth leaves macerated with 2500 ml ethanol 96% solvent. Moreover, stirred with a stirrer for 24 hours and filtered using whatmann paper which is placed on a Buchner funnel and obtained by filtrate. Centrifuged 50 rpm for 24 hours. The sediment obtained is then evaporated using a rotary evaporator for 4 hours. Finally, the conduct and evaporation above the water bath until finished, and then evaporated again until there is no residual ethanol content.

Stage of Inhibition Activity Test for Water Hyacinth Leaf Extract to The Growth of Subgingival Plaque Bacteria

The inhibitory test in this study uses a liquid dilution method with serial dilution as well as some steps. Firstly, we have to prepare 9 test tubes with 1 test tube containing subgingival plaque bacteria stock which has been prepared and labeled. The test tube no. 1 containing 10 ml of water hyacinth leaf extract, eight other test tubes filled with BHIB media as much as 5 ml. After the other eight tubes were filled with BHIB, 5 ml of extract was put in the tube no. 1 then stirred it. Afterward, 5 ml of liquid from the tube no. 1 were taken and put in tube no. 2 and stirred again. The next one, 5 ml of liquid from the tube no. 2 are taken and put in the tube no. 5. Stirred it again, and repeated until the liquid is put in the tube No. 8. In addition, the 5 ml of liquid from tube no. 8

is removed so that the liquid in each tube has the same liquid volume.

After all of the media are prepared, 1 ml of subgingival plaque bacteria stock is inserted into each tube containing a mixture of BHIB and the extract. The remaining tubes containing only 5 ml of BHIB media were negative controls, and the tubes containing subgingival plaque bacteriastock were positive controls. All the treatment above was repeated three times to obtain adequate sample size. The tubes were incubated at 37°C for 24 hours

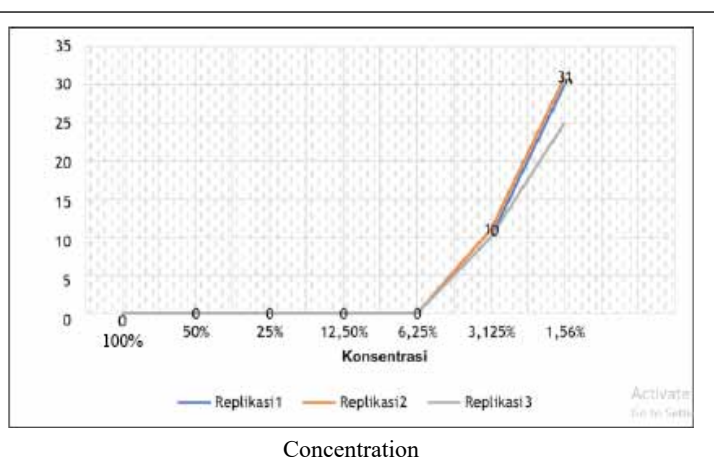
After 24 hours incubated, each tube is taken 1 osse and planted on Mueller Hinton Agar (MHA) media by strike move to check there is bacteria growth or not. Incubation is conducted for 24 hours at 37°C. After the second incubation, we have to take 0.1 ml from the boundary tube between the bacteria growth and the non-positive. The negative control then planted on the MHA media. Enter it into the incubator at 37°C for 24 hours. Calculates the number of bacteria colonies that grow on MHA media after the last incubation and do the data analysis.

Results

The results of this study can be seen from the number of subgingival plaque bacteriacolonies growth.

Table 1. The number of subgingival plaque bacteriacolonies given water hyacinth leaf extract (*Eichhorniacrassipes*) at a concentration of 1,56%-100%

Concentrations	1	2	3
100%	0	0	0
50%	0	0	0
25%	0	0	0
12,5%	0	0	0
6,25%	0	0	0
3,125%	10	12	11
1,56%	31	30	33
Positive controls	149	151	152
Negative controls	0	0	0



Based on the results of the study it can be seen that there is no growth of subgingival plaque bacteria in groups of 100%; 50%; 25%; 12.5% and 6.25%. The growth of subgingival plaque bacteria colonies was only seen at 3.125% concentration and increased the number of bacteria colonies at 1.56%.

The measurement results are tabulated according to each group sample and followed by testing the normal distribution using the Shapiro-Wilk Test with Sig > 0.05. It can be concluded that the data are normally distributed. Afterward, the homogeneity testing using Levene’s Test with Sig. <0.05 can be concluded that the data is homogeneous. Afterward, the statistical tests were conducted using One-Way Anova at the significance level of Sig. <0.05 and significant differences were obtained. Finally, it then continued by performing

multiple comparisons using Post-hoc Tukey Anova which concluded that all groups had significant differences.

Discussion

The results of the analysis showed that the number of colonies at a concentration of 3.125% had a significant difference with the number of colonies at a concentration of 1.56% and positive control. Likewise, the number of colonies in the extract of water hyacinth leaves concentration of 1.56% has a significant difference to the number of colonies in the positive control group. This shows that the extract of water hyacinth leaves at a concentration of 3.125% and 1.56% has a fairly good antibacterial effect. The results of this study also showed a bactericidal effect of 6.25% where subgingival plaque bacterial colonies were completely invisible and the

inhibitory power was best at a concentration of 3.125%, where the subgingival plaque bacteria could grow at minimal number. This is due to the presence of bioactive substances in water hyacinth leaves which have been studied as antibacterial, and the quantity of concentration that contains bioactive substances that play a role in inhibiting bacteria.

Based on scientific research stated that water hyacinth leaf extract contains alkaloids, flavonoids, and tannins which are known to have antibacterial power that can inhibit bacterial growth¹⁰. The alkaloids contained in the hyacinth leaf extract has antibacterial biological activity that can interfere with the constituent components of the bacterial cell that make the cell wall not formed and easily get lysed¹¹. Antibacterial flavonoids has a working mechanism that can interfere bacterial membrane synthesis, and inhibits bacterial metabolism – which can damage bacterial breeding pathways and damage bacterial membrane walls that will be followed by the bacterial death¹³. This compound will also interfere with the energy metabolism in a similar manner to the respiratory system because the energy demand for active absorption of various metabolites and the biosynthesis of macromolecules insufficient so that lead to bacterial death¹⁴. Tannin known to has antibacterial activity related to their ability to form hydrogen bonds which results in protein denaturation in the membrane so that cells experience damage. Membrane damage causes the fulfillment of nutrients needed by bacteria cannot be achieved. This can be disrupted the bacterial metabolism and reduce the bacterial energy¹⁵.

Conclusion

Water hyacinth leaf extract was effective to inhibit the growth of sub gingival plaque bacteria colonies. So, it is expected to preserve the water hyacinth plant and cultivate it.

Ethical Clearance: The research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was conducted in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: There is no conflict of interest for all authors

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