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Research Article

INHIBITION ACTIVITY OF WATER HYACINTH LEAF EXTRACT (*EICHHORNIA CRASSIPES*) AGAINST *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*

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ABSTRACT

Objective: Water hyacinth (*Eichhornia crassipes*) contains bioactive compounds that have antibacterial properties. The antibacterial agent is required in the treatment of periodontitis. The aim of this study was to determine the inhibition activity of water hyacinth leaf extract against *Aggregatibacter actinomycetemcomitans* that was the major cause of aggressive periodontitis.

Methods: This research divided into two groups (treatment and control group). The treatment group was conducted using a serial dilution of water hyacinth leaf extract at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78% in brain heart infusion broth media with *A. actinomycetemcomitans* suspension. The control group was prepared without water hyacinth leaf extract. All media were incubated at 37°C for 24 h. Inhibition activity was measured by calculates the number of bacterial colonies on Mueller-Hinton agar media. The data were statistical analysis using one-way ANOVA.

Results: There were significant differences between the treatment group (concentrations of 100% to 1.56%) and the control group ($p < 0.05$). Water hyacinth leaf extract at concentrations of 100% to 6.25% showed that there was no growth of *A. actinomycetemcomitans*.

Conclusion: The results suggest that water hyacinth leaf extract has inhibition activity against *A. actinomycetemcomitans* at a minimal concentration of 1.56%.

Keywords: Water hyacinth, antibacterial, *Aggregatibacter actinomycetemcomitans*.

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INTRODUCTION

Periodontal disease is one of the oral problems which have a high prevalence in the community. Periodontal disease is the most common disease in the human oral cavity besides caries. Periodontal disease is a chronic disease that caused periodontal pathogenic bacteria characterized by inflammation and periodontal tissue breakdown. Aggressive periodontitis is one of the periodontal diseases with rapid tooth-supporting tissue destruction [1,2]. Aggressive periodontitis typically attacks patients under 35 years old. A specific clinical sign of aggressive periodontitis is less plaque accumulation but the rapid loss of alveolar bone. Aggressive periodontitis is the leading cause of tooth mobile and tooth loss. Aggressive periodontitis caused by *Aggregatibacter actinomycetemcomitans* is one of the bacteria with high virulence factor [3].

A. actinomycetemcomitans is non-motile Gram-negative anaerobic coccobacillus bacteria which is dominantly found in aggressive periodontitis patients. These bacteria colonize in the oral cavity as normal flora. It becomes potentially pathogenic when present in large numbers in the gingival sulcus. These bacteria invade periodontal tissue and disrupt the body's immune defense system [4]. *A. actinomycetemcomitans* has the ability to express and release various virulence factors, one of them is leukotoxin. It grows within a biofilm that is composed of complex periopathogens [5]. This condition constitutes of infection process that is required a potential agent to inhibit the growth of *A. actinomycetemcomitans*.

Water hyacinth (*Eichhornia crassipes*) is one of the plants which have a potential therapeutic effect. This plant can be used as an antimicrobial, antioxidant, antitumor, and accelerate wound healing. Water hyacinth has many ingredients including phenols, alkaloids, flavonoids, terpenoids, glycosides, tannins, anthraquinones, quinones, carbohydrates, proteins, and amino acids [6,7]. Water hyacinth is a free-floating herbaceous plant, spread throughout the world, including Indonesia's rivers.

Plant identification is conducted by UPT Materia Medica, Batu, East Java, Indonesia. The taxonomy of water hyacinth (*E. crassipes*) (Fig. 1) is in the kingdom (Plantae); subkingdom (Viridiplantae); infrakingdom (Streptophyta); superdivision (Embryophyta); division (Tracheophyta); subdivision (Spermatophyta); class (Magnoliopsida); subclass (Lilianae); order (Commelinales); family (Pontederiaceae); genus (*Pontederiaceae*); and species (*Pontederiaceae*).

Considering the above-mentioned properties, water hyacinth became candidate herbal medicine to inhibit the activity of *A. actinomycetemcomitans* growth. Later, it can be used for therapy of aggressive periodontitis. However, this assumption has not been proved. Inhibitory activity of water hyacinth for *A. actinomycetemcomitans* growth has not been studied. In this first study, we examine the inhibition activity of water hyacinth leaf extract against *A. actinomycetemcomitans*.

METHODS

Extraction

The water hyacinth leaf ethanol extract used in this study was processed at UPT Materia Medica, Batu, East Java, Indonesia. Water hyacinth leaf was washed using running water until clean and thinly cut. Then, the leaf is dried using an oven at 50°C. The drying results are blended and sifted to form a fine powder. The powder of water hyacinth leaf was weighed 50 g, then macerated with 100 ml of 70% ethanol in the 500 ml Erlenmeyer. The maceration process is carried out for 24 h with boiling using an orbital shaker. About 70% ethanol filtrate

Fig. 1: Water hyacinth (*Eichhornia crassipes*)

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 h up to 3 times replication to get a clear filtrate. About 70% ethanol extract was evaporated at 60°C using a rotary evaporator and continued with evaporation on the water bath until a thick ethanol extract was obtained.

Inhibition activity test

This study was an experimental laboratory with post-test only control group design. Bacterial sampling using *A. actinomycetemcomitans* ATCC 6514. Bacterial testing was conducted at Research Center, Faculty of Dental Medicine, Airlangga University, Surabaya. The experiment was performed in triplicates (n = 3).

Inhibition test using serial tube dilution technique method as previously described by Haggag *et al*. [8]. Serial dilution from 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78% was used. First, we prepare nine test tubes. Tube no. 1 containing 100% (w/v) water hyacinth leaf extract solution as much as 10 ml. Tube no. 2–9 containing 5 ml brain heart infusion broth. About Five ml solution of tube no. 1 was added into the tube no. 2 and stirred. After that, 5 ml liquid from tube no. 2 was added into the tube no. 3 and stirred. Then, 5 ml liquid from tube no. 3 was added into the tube no. 4 and stirred again. These procedures were repeated until tube no. 8. Then, 5 ml of liquid from tube no. 8 is removed so that the liquid volume in each tube was the same. Tube no. 9 controlled tube, without additions of water hyacinth leaf extract.

Each tube was added with 1 ml of *A. actinomycetemcomitans* suspension 1×10^6 colony-forming unit (CFU). All the tubes were incubated at 37°C for 24 h. After 24 h, each tube was taken 1 osse and planted on the entire surface of Mueller-Hinton agar (MHA) media by streak method. The procedure of plating bacteria on MHA media is replicated 3 times to get internal validity. All the MHA media were incubated for 24 h at 37°C. The growth of *A. actinomycetemcomitans* on MHA media was identified by count the number of this bacteria colony that expressed as CFU.

RESULTS

Water hyacinth leaf extract at concentrations of 100%, 50%, 25%, 12.5%, and 6.25% showed that there was no growth of *A. actinomycetemcomitans* after incubated 24 h. Growth of this bacteria on MHA media is seen in the concentration of 3.125%, 1.56%, 0.78%, and 0% (control group without water hyacinth leaf extract) (Table 1).

Test of normal distribution using the Shapiro–Wilk test indicated the data in a normal distribution ($p > 0.05$). Test of homogeneity using Levene's test demonstrates that the variance data were homogeneous ($p > 0.05$). Analyzing variance data using one-way ANOVA showed that significant difference between groups ($p < 0.05$). Furthermore, the results of *post hoc* test with least significant difference comparing each group showed start from the concentration of 100% to 6.25% significantly different with a concentration of 3.125%, 1.56%, and 0.78% as well as control group ($p < 0.05$). Comparison between control group and concentration at 0.78% explained no significant difference ($p > 0.05$) (Table 2).

DISCUSSION

The previous study stated that natural product from various plants such as *Mentha pulegium*, *Citrus aurantium*, and *Cymbopogon citratus* has antibacterial ability against *A. actinomycetemcomitans*. These Gram-negative bacteria release high virulence factor that leads to periodontal tissue breakdown and tooth loose [9]. Hence, the growth of these bacteria must be inhibited. Many plants have been used as antibacterial. One of the plants that have antibacterial properties is water hyacinth.

The present study demonstrated that water hyacinth leaf extract exhibited totally inhibitory effect against the growth of *A. actinomycetemcomitans* in various concentrations that are 100% to 6.25%. Water hyacinth leaf extract at a concentration of 3.125% to 1.56% represents partially inhibitory effect, whereas a concentration of 0.78% similar to control. The ability of water hyacinth leaf extract to inhibit *A. actinomycetemcomitans* growth depends on the concentration of extract.

The growth and proliferation of *A. actinomycetemcomitans* on media are influenced by various factors and environmental conditions including temperature, pH, osmotic pressure, and oxygen and chemicals present in the culture media [10]. In this study, all the factors used in the experiment, but only the bioactive compounds in the water hyacinth leaf extract were the most appropriate decisive in the inhibitory growth of *A. actinomycetemcomitans*.

Gram-negative bacteria, such as *A. actinomycetemcomitans*, have two physiologically distinct membranes, called an inner membrane and an outer membrane. These membranes separated by a narrow space, namely periplasm. It is essential to structure for bacterial survival. The bacterial membrane has a role as a permeability barrier, allowing cells to resist toward adverse effect from its environment. Bacterial membranes are protein anchorage points for various proteins related to the synthesis of substantial molecules and energy metabolism for bacterial growth [11, 12].

In this study, water hyacinth leaf extract showed bacteriostatic and bactericidal activities. Water hyacinth leaf extract shows its nature as bactericide with increased concentration, the higher the concentration, the more *A. actinomycetemcomitans* are killed. Its bacteriostatic and bactericidal activities may be influenced by the presence of chemical compounds contained therein. Based on phytochemical tests and scientific research stated that extracts of water hyacinth leaf contain several chemicals. Water hyacinth leaf extract contains bioactive compounds such as phenols, terpenoids, flavonoids, and alkaloids [8, 13, 14].

Phenols have potential antibacterial properties. Phenols inhibit bacterial growth by denaturing protein and disrupting bacterial membrane integrity. Structure of membrane proteins is stabilized by hydrogen bonds. Phenols can interfere with the stability of the hydrogen bonds. The hydrogen-bonded complex between phenol and protein provokes the protein structure of the plasma membrane was damage. Destruction of the plasma membrane causes imbalance of intracellular interaction between macromolecules and ions that result in cell disruption and lysis [15, 16].

Active phenol can penetrate cytoplasmic membrane bacteria in both active and passive diffusion. In cytoplasmic level, phenols cause damage membranes of mitochondria, endoplasmic reticulum, and nucleus. Phenols also damage the components of cytoplasmic organelle such

Table 1: The number of colonies of *Aggregatibacter actinomycetemcomitans* treated with water hyacinth leaf extract at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, and 0% (n=3)

Parameter	Concentration (%)								
	100	50	25	12.5	6.25	3.125	1.56	0.78	0
Number of colony (CFU)	0±0	0±0	0±0	0±0	0±0	11±1	25±2	123±2	125±2
One-way ANOVA	p=0.00								

Results are expressed as mean±SD; n=3. SD: Standard deviation, CFU: Colony-forming unit

Table 2: p value of *post hoc* test (least significant difference) between each group

Concentration (%)	100	50	25	12.5	6.25	3.125	1.56	0.78	0
100	-	-	-	-	-	*	*	*	*
50	-	-	-	-	-	*	*	*	*
25	-	-	-	-	-	*	*	*	*
12.5	-	-	-	-	-	*	*	*	*
6.25	-	-	-	-	-	*	*	*	*
3.125	*	*	*	*	*	-	*	*	*
1.56	*	*	*	*	*	*	-	*	*
0.78	*	*	*	*	*	*	*	-	NS
0	*	*	*	*	*	*	*	NS	-

*Significant difference, NS: Not significant

as enzymes. In the nucleus level, phenols degrade DNA by interfering nucleic acids synthesis [15, 17].

Another bioactive compound from water hyacinth leaf extract is terpenoids. Terpenoids have toxic effect on bacterial cell wall, both Gram-positive and Gram-negative. It can interact with proteins in the cell membrane and intracellular components, lead to disruption on the membrane structure of Gram-negative bacteria. This results in degradation of cytoplasmic membrane both in functional and structural. Damaging the cytoplasmic membrane causes cytoplasm coagulation and increased membrane permeability, leading to leakage of vital intracellular substance and reducing ATP synthesis [18, 19]. This process was confirmed as the most likely cause of cell death.

Flavonoids are also found in water hyacinth leaf extract. Flavonoids have antibacterial properties that affect bacterial growth through various mechanisms. Antibacterial activity of flavonoids generated by alteration membrane permeability as a result of porin disruption, inhibition of cytoplasmic membrane function, inhibition of nucleic acid synthesis, inhibition of energy metabolism, and inhibition of biofilm formation. Antibacterial activity of flavonoids depends on its structure, such as hydroxyl groups on special sites are favorable for the activity, substitutions on the aromatic rings in flavonoids structure increase antibacterial properties [20, 21].

Water hyacinth leaf extract also contains alkaloids. Alkaloids have the ability as a bactericide for Gram-positive and Gram-negative bacteria. Alkaloids can damage peptidoglycan components in the bacterial cell wall. This condition leads to the structure of the cell wall layer is not fully formed and causes bacterial cell death. Alkaloids also can inhibit bacterial growth by inhibiting nucleic acid synthesis [22].

The compounds contained in water hyacinth leaf extract that has mentioned above are the possibility cause of the emergence of the inhibition growth of *A. actinomycetemcomitans* in this study. In general, this inhibitory effect of antibacterial natural compounds begins from injury to the outer membrane of the bacterial cell wall. This condition causes increased the permeability of the bacterial cell membrane that leads to cytoplasmic and DNA synthesis disturbance and finally bacterial cell death.

CONCLUSION

The results suggest that water hyacinth leaf extract has inhibition activity against *A. actinomycetemcomitans* at a minimal concentration of 1.56%.

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AUTHORS' CONTRIBUTIONS

The first author (Yasinta Izzah Afidati) performed laboratory test and prepared the manuscript. The second author (Dr. Irma Josevina Savitri) supervised the laboratory test and corrected the manuscript. Corresponding author (Dr. Agung Krismariono) initiated the idea of this research and corrected the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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