Effectiveness of the binahong leaf extract (*Anredera cordifolia*) in devoting bacterial growth *Vibrio cholerae* in vitro

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Abstract. Parameters that determine biological water quality are microbial parameters of pollutants, pathogens and toxin producers. Many microbes often mix with water, especially in shallow groundwater. One of the antioxidant-producing plants is the Binahong plant which has a high enough antioxidant level which inhibits bacterial growth. The purpose of this study was to determine whether binahong leaf extract can inhibit the growth of *Vibrio cholerae* bacteria. This study was a laboratory experimental study using a completely randomized design (CRD) with five treatments. The treatment was repeated 3 times with various concentrations of 30%, 40%, 50%, and 100%, and chlorine was used as a positive contract. The material used in this study was binahong leaf extract macerated with ethanol solvent, a thick extract was produced which was then tested for antibacterial activity on *Vibrio cholerae* bacteria. The parameters observed were the diameter of the inhibition zone of the growth of *Vibrio cholerae* bacteria. The result is known that binahong leaf extract has the ability to inhibit the growth of *Vibrio cholerae* bacteria seen by the presence of inhibitory zones formed. The most effective concentration to inhibit the growth of *Vibrio cholerae* bacteria was at a concentration of 100% at 6.10 mm at 24 hours of observation.

1. Introduction

Parameters that determine biological water quality are microbial parameters of pollutants, pathogens and toxin producers. Many microbes often light up with water, especially in shallow groundwater. The most dangerous microbes are microbes derived from feces, namely Coli bacteria. Microbes originating from polluted water can cause health problems for humans (Viessman, 2015). Types and numbers of microorganisms in waters have a relationship with waste pollution in a waters. Dense city and village wastewater not only increases the growth of coliform bacteria but also increases the number of pathogenic bacteria such as *Salmonella*, *Shigella* and *Vibrio cholerae*. Coliform group bacteria, Escherichia coli and Streptococcus faecalis are microorganisms which are generally found in large amounts of domestic waste. Coliform, fecal coli and *Salmonella* are some bacteria which are indicators of the quality of a waters. There are three groups of indicators of bacterial pollution of coastal recreational waters, namely faecal coliform, faecal *Streptococcus* and pathogens (Metcalf & Eddy, 2013).

Water disinfection is one of the end of processing clean water before it is distributed to community customers. One of the disinfection techniques used, the

most common, cheap and dominant method used for processing clean water is chlorination. This method is used to remove non-beneficial bacteria or pathogens in the water. Sodium hypochlorite, chlorhexidine and hydrogen peroxide are disinfectants that are widely used and have the effectiveness of disinfection in pathogenic microorganisms. Sodium hypochlorite and chlorhexidine have a broad spectrum, work fast and have low toxicity which is safe to use for disinfection of printed materials. The use of sodium hypochlorite as a disinfectant is effective at concentrations of 0.5% and chlorhexidine as an effective disinfectant at a concentration of 0.2%. The use of hydrogen peroxide is effective at a concentration of 3% and is active in gram-negative and gram-positive microorganisms. One alternative that can be used in the prevention of pollutants by hydrogen peroxide and protection for living organisms is by giving antioxidants as compounds that can reduce the level of reactivity of free radicals caused by pollutants. One of the antioxidant-producing plants is Binahong which has a high antioxidant level of 9.614% flavonoids (Noss & Olivieri, 2015).

2. Materials and Methods

2.1. Materials

The tools used in measuring biological parameters in the analysis of identification of bacteria include autoclaves, analytic balance, microscopes, incubators, thermometers, aquariums, petri dishes, test tubes, erlenmeyer, beakers, ovens, measuring cups, pipettes, straight and round ose , knives, bottles, separating funnels, magnetic stirrers, pH meters, vortex, hot plates, bunsen, colony counters, ovens, refrigerators, digital cameras, and writing instruments, ropes, tissues, cotton, gloves, masks, paper, paper label, aluminum foil. The materials used in this study are binahong leaves obtained from the Aceh Tamiang area, as much as 5 kg, 96% ethanol used as a solvent in maceration processes, pure culture of pathogenic bacteria, aquades, NA (Oxoid) media and media NB (Merck).

2.2. Binahong Leaf Extract

Making binahong leaf extract is done by maceration method using 96% ethanol. The maceration process is carried out 3 times. The results of maceration are then evaporated using a rotary vacuum evaporator. The concentration of binahong leaf extract used was 30%, 40%, 50%, and 100% with sterile aquadest solvents (Darsana et al, 2012).

2.3. Antibacterial Test

Antibacterial activity test was carried out by making a well (6 mm diameter) on *Nutrient Agar* media which was mixed with 2 drops of test bacteria per media. Each media is made 3 wells, so in each binahong leaf extract wells are included with different concentrations, namely 30%, 40%, 50% and 100%. (Wisudaningrum, 2008). Data were obtained by measuring the diameter of the inhibitory zone (clear zone) formed in *Vibrio cholerae* culture media in millimeters.

2.4. Data analysis

Data obtained from measurements of inhibition zone diameters were analyzed using Anova with the following criteria:

Table 1. Classification of antibacterial resistance zones

Diameter of the inhibition zone	Growth inhibition response

< 3	Weak
3 – 6	Middling
> 6	Strong

3. Results and Discussion

Analysis of the potential of binahong leaves is done by making a well (6 mm diameter) on *Nutrient Agar* media which has been mixed with 2 drops of test bacteria per media. Each media is made 3 wells and then in each binahong leaf extract wells are included with different concentrations of 30%, 40%, 50% and 100%. Furthermore, the measurement of the inhibition zone is done by using a calipers after 24 hours. The response from the binahong leaf potential test results on the inhibition of the growth of *Vibrio cholerae* bacteria is presented in Tabel 2.

Tabel 1.2. The results of measurements of inhibition area diameter (DDH) test the potential of binahong leaves on the growth of *Vibrio cholerae* bacteria in vitro

Handling	Inhibited Area Diameter (mm)			
	30%	40%	50%	100%
1	1, 11	1,,30	3,,01	6,09
2	1,09	2,,20	3,,03	6,11
3	1,13	2,,22	3.06	6.11
Average	1,,11	1,,90	3,,03	6,10

Based on Tabel 2, it was found that the application of binahong leaves as a bacterial growth inhibition at a concentration of 100% increased the average inhibitory power of bacterial growth compared with concentrations of 30%, 40,% and 50%. The higher the binahong extract extract concentration was applied to NA media which had been rubbed with *Vibrio cholerae* bacteria, the greater the

inhibitory power of bacterial growth was produced. This is because binahong leaves have benefits and pharmacological effects where, if consumed, binahong has high antioxidant and antiviral properties. Binahong leaves contain compounds of flavonoids, alkaloids, saponins and polyphenols (Noss & Olivieri, 2015). In the research of Silvana Rhargaok, et al. (2015) binahong leaf extract was obtained by extracting materials namely binahong leaves extracted by maceration extraction using 96% ethanol. The method used is the agar plate diffusion method (*Kirby-Bauer*) which is a direct sensitivity test method. *Muller-Hinton's* order (MHA) provided five petri dishes. According to this study, binahong leaves are effective as antimicrobial bacteria, because binahong leaves contain flavonoids, saponins, and tannins which can interfere with the growth of *Vibrio cholerae* bacteria.

Giving different extracts on the leaves of binahong showed different results. These results showed that the leaves of binahong (*Anredera cordifolia*) at all concentrations had varying antibacterial activity. Binahong leaf extract (*Anredera cordifolia*) at all concentrations has antibacterial activity which is classified as weak, moderate and strong. Antibacterial inhibitory zone classification starts from the weak, medium and strong category, which is <3 mm, so the inhibitory response of bacterial growth is said to be weak, in the diameter of the inhibitory zone 3-6 the antibacterial ability is classified in the medium category. In the inhibitory zone diameter> 6, the growth inhibition response is categorized as strong antibacterial.

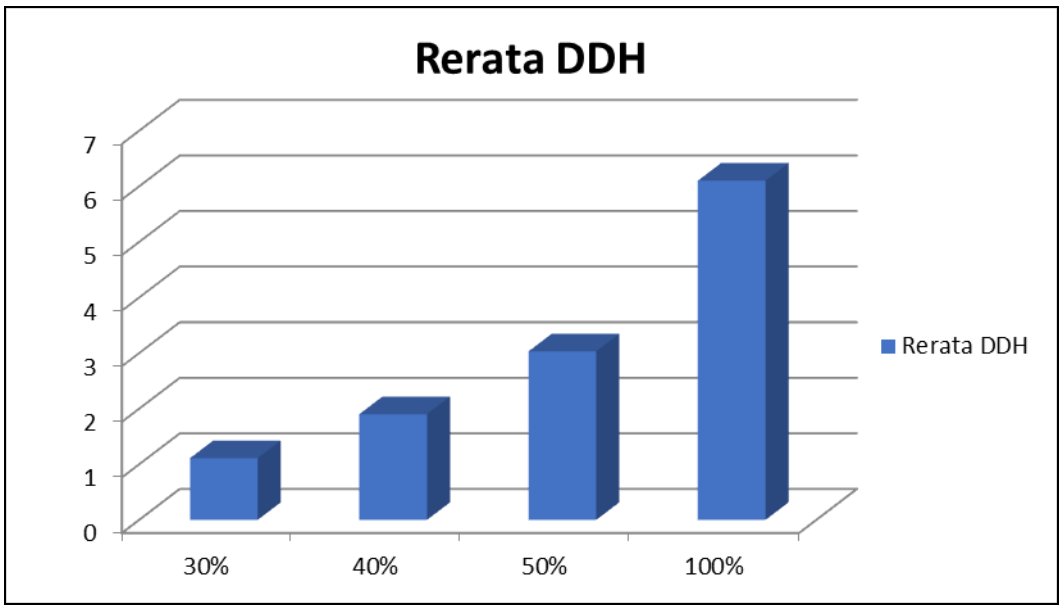

Figure 1. The measurement of inhibition area diameter (DDH) tested the potential of binahong leaves in the growth of Vibrio cholerae bacteria in vitro.

Based on Figure 1 shows that the effectiveness test of binahong leaf extract in inhibiting the growth of *Vibrio cholerae* bacteria in In vitro shows an increase in the average inhibition area. The lowest average treatment at a concentration of

30% with a value of 1.11 mm. While the highest average at a concentration of 100% with a value of 6.10 mm.

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