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INHIBITORY TEST ANTIMICROBIAL OF SEAWEED EXTRACT FROM *Padina* sp. AGAINST THE GROWTH OF *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli* AND *Salmonella thypimirium*

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ABSTRACT

The content of secondary metabolites of seaweed as an antibacterial, antiviral, antifungal and sitotastik have great potential as a source of cure various diseases. *Padina* sp is one of brown algae which is potential to be a natural antibacterial agent. This research aims to determine the inhibitory potency of *Padina* sp. extract on the growth of *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella thypimirium* bacterial. The extraction *Padina* sp using methanol as the solvent while the treated samples dilution of 0, 5, 10 and 50. Then measuring the inhibition by paper disc methods. Result showed that the diameter methanol extract of seaweed *Padina* sp. against bacterial the growth inhibition of *E. coli* each 26.5 mm with the very strong barriers response and 17.5 mm were classified as strong; on the growth of *Salmonella thypimirium* bacteria by 19 mm with a response barriers that were moderate but not have inhibitory effect on the growth of *Vibrio parahaemolyticus* and *Staphylococcus aureus* bacterial.

Keywords: *Antimicrobial*; *Inhibitory test; Padina* sp.

1. INTRODUCTION

Various attempts have been made to prevent the attacks of human pathogenic bacteria, such as Escherichia coli, *Staphylococcus aureus, Bacillus subtilis*, *Candida maltose*, *Vibrio parahaemolyticus* and *Salmonella thypimirium* (Renhoran, 2012). According to Maduriana and Sudira (2009), an attempt to prevent attacks carried pathogenic bacteria such as by use of synthetic antibiotic that is bacteriostatic or bactericidal among others. The use of chemical drugs are effective in the short term, but use for the long-term can cause undesirable side effects, it can even lead to drug

resistance so that the use of drugs of natural origin can be used as an alternative cure of the diseases.

One of Indonesia's marine resources that have potential as a source of natural compounds that are useful are macroalgae or seaweed. In addition to the content of the primary economic value, according to Hutasoit *et al*., (2013), research on the content of secondary metabolites of seaweed show that this plant has the potential as a producer of metabolites bioactive (bioactive substance) that have the potential to be developed as an antibacterial, antiviral, antifungal and cytostatic. The nature of secondary metabolites in seaweed is a means of self defense marine organisms that turned out to have great potential as a source of cure various diseases (Winston, 1988; Cragg *et al*., 1997 *in* Andi Reskika, 2011).

Seaweed is divided into three major groups based on their chemical composition, namely green algae (*Chlorophyta*), red algae (*Rhodophyta*) and brown algae (*Phaeophyta*). Brown algae has many genera, such as *Padina* sp, *Sargassum* sp and *Turbinaria* sp that can be used for a variety of benefits. Brown seaweed species as *Padina* sp. contains alkaloids used to antimicrobials also contains fucoidan and phenolic components.

Choudhury *et al*. (2005) *in* Renhoran (2012), stated that in South Africa 56 methanol extract of seaweed that comes from the class *Chlorophyta* (green algae), *Phaeophyta* (brown algae) and *Rhodophyta* (red algae). Of the three classes of seaweed, brown seaweed has the highest antibacterial activity.

According to Davis and Stout (1971) *in* Renhoran (2012), antimicrobial activity is determined by measuring the diameter of the resistance is the clear region formed around the disc paper. Strength Conditions of Antibacterial Compounds are shown in Table 1 below.

Table 1. Strength Conditions of Antibacterial Compounds.

Diameter Clear Zone	Obstacle Response
> 20 mm	Very Strong
10-20 mm	Strong
< 10 mm	Intermediate
≤ 5 mm	Low

Chemical content of brown seaweed can be obtained by solvent extraction. Extraction using a solvent such as ethanol, methanol, ethyl acetate, hexane and water capable of separating compounds that are important in a material. Methanol is a universal solvent that can dissolve the analytic are polar and nonpolar. Methanol can be interested alkaloids, steroids, saponins and flavonoids. According to research Wiyanto (2010), methanol is more effective than ethanol when used as a solvent to extract the antibacterial component of seaweed. Meanwhile, according to research conducted Suryanto and Wehantouw (2009) *in* Andi (2015), indicates that methanol is able to attract more number of secondary metabolites of phenolic compounds, flavonoids, and tannins than ethanol. So that the antibacterial component seaweed extract used methanol solvent.

Based on this, the study will be measured inhibitory power of seaweed *Padina* sp. using methanol to the growth of *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella thypimirium*. This study was conducted to explore and characterize bioactive compounds of the type of brown algae in the waters of Ambon. From the results of this study are expected to be obtained inhibition information isolate the methanol extract of seaweed *Padina* sp. on the growth of the bacteria *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella thypimirium* that can be used as new agent of anti-bacterial.

2. RESEARCH METHOD

The research was conducted at the Laboratory of Fishery Processing Products Faculty of Fisheries, Pattimura University, Ambon. The stages of the implementation of this study are as follows: (1) preparation of tools and materials include: sterilization, manufacture of bacterial growth media, rejuvenation of bacteria, preparation of test bacterial suspension, the manufacturing of test bacteria inoculum; (2) extracting active substances brown alga *Padina* sp. using methanol; (3) measuring the pH levels of each dilution; and (4) performing antibacterial activity of methanol extract of seaweed *Padina* sp. according of dilution level as 0, 5, 10 and 50 times, respectively.

The tools used on this study are as follows: petridish, tweezers sterile micropipette 100 mL, paper discs, incubators, sliding term, laminar, bunsen lamps, autoclaves, ovens, pH meter. While the materials used are: Salmonella Shigella Agar (SSA), Tiocyanate Bile

Sucrose Agar (TCBSA), Eosin Methylene Blue Agar (EMBA), Tryptone Soya Broth (TSB), Vogel Johnson Agar (VJA), *Vibrio parahaemolyticus* bacteria isolates, *Staphylococcus aureus* bacteria isolates, *Escherichia coli* bacteria isolates, *Salmonella thypimirium* bacteria isolates, methanol extract of *Padina* sp. seaweed, 70% alcohol and aquadest.

The methods used in this research that uses methods of direct visual observation and testing of the inhibition of the antimicrobial extract of seaweed *Padina* sp. seaweed by paper disc method. Observations were conducted a careful review of whether or not the zone of inhibition (clear zone without microorganisms) are formed around the paper disc in the form of a diameter of clear zone by using calipers then interpreted zone of inhibition of bacterial growth which refers to the classification table of sensitivity clinic of microbes (Supandi and Gantini, 2009).

The procedure of this study are follows: (1) Sterilize tools and materials to be used for analysis. (2) Brown seaweed *Padina* sp. obtained from the waters of Latuhalat Village, Ambon Island. Extracted with methanol by maceration for 24 hours and then filtered. The filtrate obtained was dried with a vacuum dryer until all the solvent evaporates. Concentrated extract obtained is collected to be tested. (3) The methanol extract of seaweed *Padina* sp. diluted by 5 times, 10 times and 50 times then measured pH. Dilution aims to produce some concentration to be used from extracts of *Padina* sp. which can inhibit the growth of bacteria. (4) Take each 10% pure bacterial isolates was 24 hours with a micropipette, pour into a petri dish. (5) Pour the appropriate media to isolate the growth media as much as 10-15 mL, homogenized and allow to solid. (6) Pipette 100 mL of methanol extract of seaweed *Padina* sp. then used as drops on a paper disk. After that placed the paper disc on agar media each contained the growth of each suspension of *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella thypimirium*. Incubated in an incubator for 24 hours at temperature of 35^0C. (7) The reading of the results by measuring the diameter of inhibition zone formed around the paper disc by using calipers with millimeter or paper. (8) Record the pH of each dilution and the diameter of the clear zone of each dilution were formed.

3. RESULTS AND DISCUSSION

3.1. pH Test

Results of testing the pH of the methanol extract of seaweed *Padina* sp each dilution are shown in Table 2 below.

Table 2. The pH of the methanol extract of *Padina* sp. seaweed each dilution pH test.

0x dilution	5x dilution	10x dilution	50x dilution
5.1	5.2	5.2	5.3

Based on the results of measurements of the pH of the methanol extract is known that the higher the dilution turns the methanol extract of seaweed *Padina* sp. remain at acidic pH ranges are between 5.1 and 5.3. This is in accordance with Greenwood (1992) *in* Pratama (2005), that one of the factors that could affect the zone of inhibition and should be controlled is the pH value of the medium because of some antibacterial works well in acidic and some alkaline conditions.

3.2. Inhibitory Test of Methanol Seaweed Extract *Padina* sp.

The observation and testing diameter of clear zone suspensions methanol extract of *Padina* sp. seaweed each dilution of each type of bacteria can be seen in Table 3 below.

Table 3. Results of testing diameter of clear zone suspensions methanol extract of *Padina* sp. seaweed at each dilution of each type of bacteria.

Bacteria Isolates	Clear zone diameter (mm)			
	0x dilution	5x dilution	10x dilution	50x dilution
Vibrio parahaemolyticus	0	0	0	0
Escherichia coli	26,5	1,75	0	0
Salmonella thypimirium	19	0	0	0
Staphylococcus aureus	0	0	0	0

Table 3 shows that the clear zone formed after an incubation period of 1 x 24 hours at room temperature with a concentration of 0x, 5x, 10x and 50x. The antibacterial activity of seaweed extract is indicated by a clear zone around the well, which indicates that the bacteria cannot grow in the regions that diffuse the active ingredient from seaweed. The measurement results of the antibacterial testing of isolates methanol extract of seaweed

Padina sp. against *E. coli* bacteria appear where treatment without dilution and the 5 times dilution produces the diameter of the inhibition of 26.5 mm and 17.5 mm which is categorized the response as very strong and strong, respectively (as shown as Table 3.)

On 10 and 50 times dilution or at a low concentration is not formed a clear zone that has a weak inhibition on the growth of *E. coli* bacteria. This suggests that the greater the concentration of extract *Padina* sp. seaweed the inhibition zone is formed greater. This is caused by the higher concentration of the active substances contained causes the inhibition of bacteria growth is getting bigger. This is in line with research conducted by Andi (2015), the width of the zone diameter indicates the growing strength of bioactive compounds that inhibit bacterial growth. It also indicates that the antibacterial active compounds in *Padina* sp. seaweed soluble in polar solvents (methanol) and showed the greatest antibacterial activity against *E. coli* bacteria.

The results showed that the extent or area diameter barrier formed from extracts of seaweed *Padina* sp. on EMBA (Eosin Methylene Blue Agar) medium in the growth of *E. coli* bacteria can be seen in Figure 1 below. Clear zone area around the filter paper show wide barriers resulting from extract *Padina* sp. seaweed.

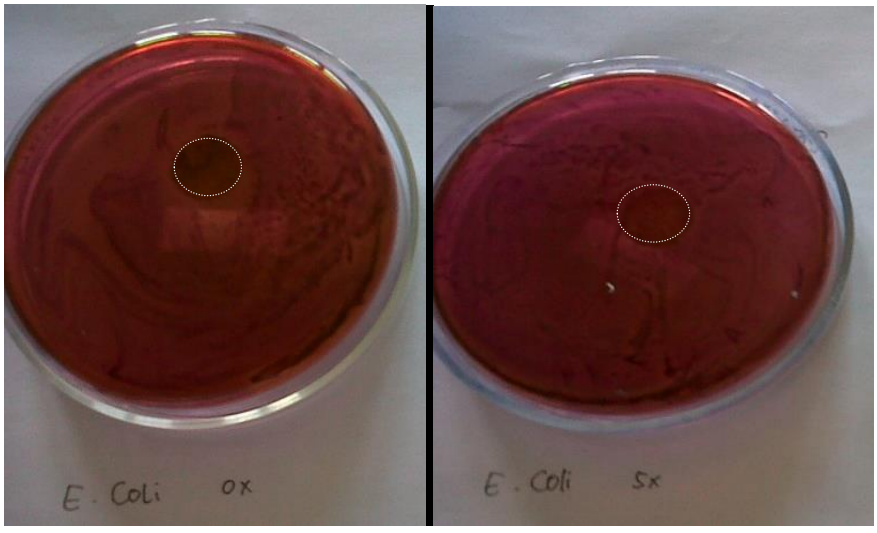

Figure 1. Diameter barriers of extract *Padina* sp seaweed on EMBA media for the growth of *E. coli* bacteria without dilution and the 5 times dilution.

The methanol extract of seaweed *Padina* sp. can affect the growth of *E. coli* because this extract can inhibit those bacterial growth. Inhibition ability of the methanol extract of *Padina* sp. seaweed against *E. coli* bacteria showed that the extract can be used as an

active compounds for the *E. coli* bacteria. Inhibitory activity of the methanol extract of seaweed *Padina* sp. On SSA (Salmonella Shigella Agar) medium in inhibiting the growth of *Salmonella thypimirium* bacteria can be seen in Figure 2 below.

Figure 2. The diameter of the methanol extract of seaweed barriers *Padina* sp. on SSA (Salmonella Shigella Agar) medium inhibited the growth of *Salmonella thypimirium* bacteria.

Methanol extract of *Padina* sp. seaweed without dilution resulted in the inhibition diameter of 19 mm were classified as moderate. While the dilution 5 times and 50 times not formed a clear zone, so it is not inhibit the growth of *Salmonella thypimirium* bacteria. Then, the methanol extract of *Padina* sp. seaweed can affect the growth of *Salmonella thypimirium* bacteria because it can inhibit the growth of *Salmonella thypimirium* bacteria. Inhibit the ability of the methanol extract of seaweed *Padina* sp against *Salmonella thypimirium* bacteria showed that the methanol extract of seaweed *Padina* sp. can be used as an active compound for the *Salmonella thypimirium* bacteria.

The methanol extract of *Padina* sp. seaweed has not been able to inhibit the growth of bacteria either *Vibrio parahaemolyticus* bacteria on selective medium for Tiocyanate Bile Sucrose (TCBS) and *Staphylococcus aureus* bacteria on selective medium for Vogel Johnson Agar (VJA) because they do not form clear zones on both medium.

All of inhibitory tests were conducted demonstrates that the inhibition zones included in the category of lethal on the methanol extract of the bacterium *Escherichia coli* and *Salmonella thypimirium*.

4. CONCLUSION

The methanol extract of *Padina* sp. seaweed has the ability to very strong inhibitory effects on the growth of *Escherichia coli* bacteria and a strong inhibitory ability against the growth of *Salmonella thypimirium* bacteria but has no inhibitory effect on the growth of *Vibrio parahaemolyticus* and *Staphylococcus aureus* bacteria

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