THE INHIBITORY POWER OF BROWN MACROALGAE (*Turbinaria ornata*) EXTRACT TO THE GROWTH OF PATHOGENIC BACTERIA IN TILAPIA (*Oreochromis* niloticus)

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Abstract

Turbinaria ornata is a potential source of phatogenic antibacterial in tilapia. This study aims to determine the inhibitory power of *Turbinaria ornata* macroalgae extract on the growth of disease causing pathogenic bacteria in tilapia. This study included experimental research with Completely Randomized Design (CDR), consisting of 6 treatments and 3 replications to produce 18 experimental units. The experiment consisted of the control - (distilled water), control + (ampicillin), extract concentration (20%, 40%, 60%, 80%). *Turbinaria ornata* extract can inhibit growth of phatogenic bacteria at K +, extract concentration (20%, 40%, 60%, 80%). Positive rounds bacteria, the diameter of the inhibition power is 20 mm, 31,67 mm, 34,3 mm, 36,67 mm, 38,3 mm. Positive short stems bacteria, the diameter of the inhibition power is 50 mm, 33,3 mm, 38,3 mm, 42 mm, 45,3 mm. Negative short stems bacteria, the diameter of the inhibition power is 25 mm, 25,3 mm, 29,3 mm, 32,3 mm, 34,67 mm. It can be concluded that, *Turbinaria ornata* extract has inhibitory activity against positive arounds bacteria, positive short stems, and negative short stems in tilapia. The extract concentration of 80% showed the most effective results in suppressing bacterial growth positive rounds, positive short stems, and negative short stems were in tilapia.

Keywords: *Turbinaria ornata*, bacterial inhibition, tilapia pathogenic bacteria.

I. Introduction

Fish is an important source of animal protein for the human body, with a protein content of around 15-24 % of its body weight. Protein levels of some types of freshwater fish vary from eel fish by 14.0%, tilapia fish by 16.0%, to tilapia by 17.5% of their body weight (Ramlah *et al* ., 2016; Khairuman *et al.*, 2011; Ghufran *et al*., 2011; Setianto, 2012). With the high levels of fish protein, fish meat is needed for human life to support their health.

Some types of freshwater fish cultivated by the community include tilapia, and eels. Of the several types of freshwater, the most cultivated by the community is tilapia. This is because the tilapia has a distinctive flavor meat and selling prices are affordable by the community. As an illustration, the price of tilapia in the local market ranges from Rp. 20,000-40,000/kg. In addition, tilapia has the ability to grow well in intensive cultivation systems (Winasis, 2015; Ghufran *et al.*, 2013) . Thus, tilapia cultivation is very economically profitable, and can increase the income of farmers or the community in general.

The main obstacle in tilapia cultivation is disease. Disorders of disease can cause a decrease in production, a decrease in water quality and total death. The disease can be caused by pathogens such as parasites, viruses, fungi and bacteria. Among these pathogens, bacteria are known to be one of the dominant disease pathogens that kill tilapia. As an illustration, bacteria commonly attacks tilapia such as *Aeromonas* sp. and *Streptococcus* sp. which causes tilapia to have wounds on the surface of the body, bleeding in the gills, scales and caudal fins is released, and if done surgically you will see swelling and damage to the liver and kidneys. *Aeromonas hydrophila* can result in death reaching 100% (Ashari *et al.*, 2014; Muslikha *et al*., 2016). Besides being able to turn off fish, bacterial diseases can cause fish appetite to decrease, resulting in the inhibition of fish growth, as well as a decrease in the quality of infected fish meat (Wiyanto, 2010).

Based on the description above, the problem of diseases in tilapia is important to be taken seriously. Disease prevention can be done by way of prevention and treatment. Prevention of disease in fish is usually done by creating a sterile environment and feeding the good nutritional value. Treatment that is done when fish are infected with pathogenic bacteria, usually given antibiotics containing chemicals. However, the use of

chemicals can pollute the environment and can cause a resistance to pathogenic bacteria (Wiyanto, 2010) . In addition, chemicals can accumulate in the body of tilapia, so that it will affect the health of humans who consume tilapia. Another factor is high public awareness of health, eventually people are more likely to choose sources of organic animal protein.

Based on this fact, efforts are needed to find out the types of natural ingredients that can inhibit the growth of these bacteria. Based on the study of macroalgae literature reported to contain antibacterial. Pakidi *et al* (2016) reported makroa brown Iga *(Phaeophyceae)* contain secondary metabolites such as steroid, alkaloida, fenol, and triterpenoids all known as antibacterial, antiviral and antifungal.

Based on the facts above, then in order to suppress the growth of tilapia fish, it is necessary to know the species of brown macroalgae extract which can inhibit the growth of disease-causing bacteria in tilapia. Therefore, it is necessary to do a research entitled the inhibition of brown macroalgae extract (*Turbinaria ornata*) on the growth of pathogenic bacteria of tilapia (*Oreochromis niloticus*). This study aims to obtain information about brown macroalgae (*Turbinaria ornata*) which effectively suppresses the growth of pathogenic bacteria in tilapia. The results of this study are expected to contribute to the increase in tilapia production which ultimately leads to increased community income.

2. Method

This research was conducted in May-August 201 8 . The research took place at Central Laboratory of Leading Bioscience and Biotechnology (PUBB) Faculty of Mathematics and Natural Sciences, University of Mataram .

3. Results and Discussion

3 .1. Characterization of Tilapia Pathogen Bacteria

The results ar k akterisasi Gram pathogenic bacteria in tilapia can be seen in Figure 1. Meanwhile, the results of the morphological characterization of pathogenic bacteria in tilapia can be seen in Table 1 .

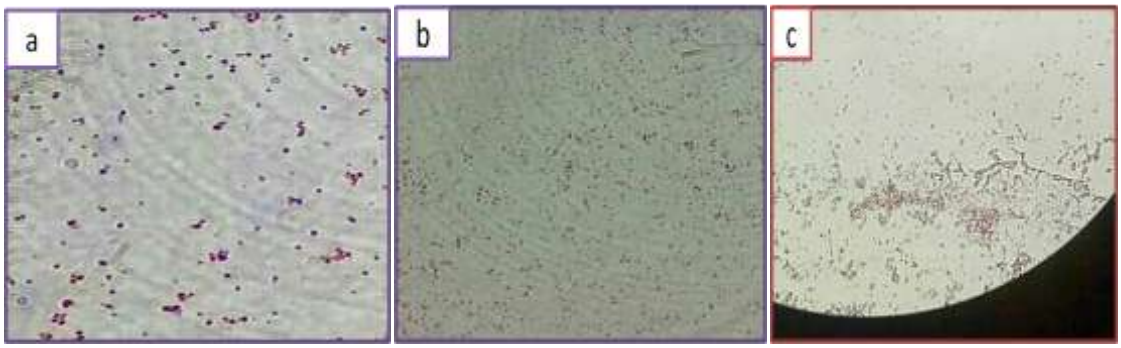

Figure 1 . Characterization of grams of pathogenic bacteria in tilapia. a). gram positive/ purple bacteria, b). gram positive/purple, c). gram negative/red bacteria.

Table 1 . K arakterisasi mor fologi form of bacterial pathogens in tilapia.

Isolate Code	Form
Bacteria a	Round
Bacteria b	Short stem
Bacteria c	Short stem

Characterization of pathogenic bacteria in tilapia can be done microscopically. Morphological characterization of observed bacteria such as shape, round, stem and spiral. Besides morphology, bacterial characterization can also be observed from gram staining, purple indicates gram positive and red indicates gram negative. Based on the data above, the characterization of pathogenic bacteria in tilapia was obtained by three types of bacteria, positive round, positive short stem, and negative short stem. These pathogenic bacteria, suspected pathogenic bacteria in tilapia that have been successfully identified include *Streptococcus* sp., *S taphylococcus* sp., *Basillus* sp., *Aeromonas* sp., *Pseudomonas* sp .(Lubis *et al.*, 2014; Manurung *et al.*, 2017).

P endugaan other based on a sample of tilapia used look sick. In the sample tilapia there are red patches on the abdomen, protruding and suppurating eyes, and darker body colors on the back and body of skinny tilapia. Sampling is in accordance with the statement of Napitupulu *et al* (2016) which states that, examining the clinical symptoms of fish attacked by bacteria can be observed on the outside of the body of the fish such as fish lacking mucus, rough body, the presence of wounds and abnormal body shape. Ashari *et al.* (2014) stated that, with a slightly prominent red spot on the abdomen and eyes, the fish was indicated to have *Motile Aeromonad Septicemia* (MAS) or red spot disease. This disease is caused by the bacterium *Aeromonas hydrophila* . Muslikha *et al* (2016) added that statement, *Aeromonas hydrophila* is a gram negative

bacterium which can cause death to reach 80-100%. Some gram-negative bacteria do not release toxic fluids, but make endotoxin is released when the cell dies or breaks. Endotoxin is lipopolysaccharide on bacterial cell walls. Bacteria too produce extracellular enzymes that can attack healthy fish. According to Kurniawan (2012), in addition to being able to kill fish, al bacterial diseases can cause fish appetite to decrease, resulting in the inhibition of fish growth, as well as a decrease in the quality of infected fish meat.

4.2. Inhibitory Power of *Turbinaria ornata* Extract Against Pathogenic Bacteria

4.2.1. Positive Round Bacteria

In terms of the inhibitory power of *Turbinaria ornata* extract against bacteria round the positive position shown in Figure 2. While the percentage increase in bacterial growth inhibition power is positive for the Control + shown in Figure 3 .

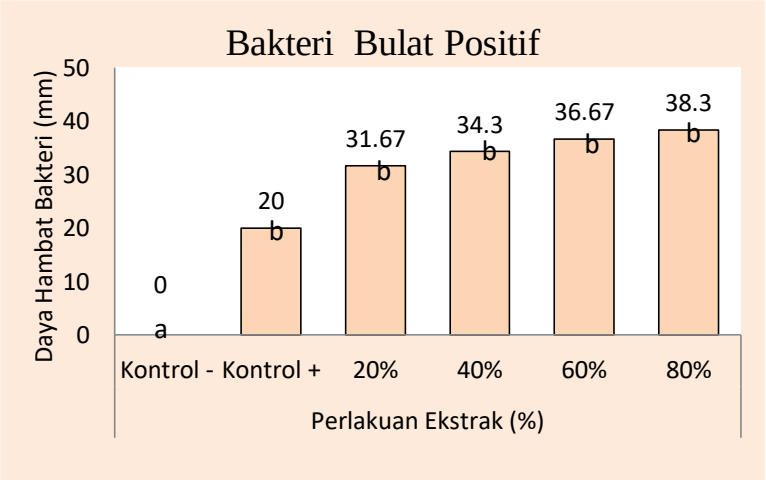

Figure 2 . Diameter inhibitory extract *Turbinaria ornata* against positive round bacteria. Different letters in the diagram show significant results (P<0,05). Treatment Controls - (distilled water), Control + (ampicillin), K onsentrasi extract 20%, K onsentrasi extract 40%, K onsentrasi extract 60%, and 80% K onsentrasi extract.

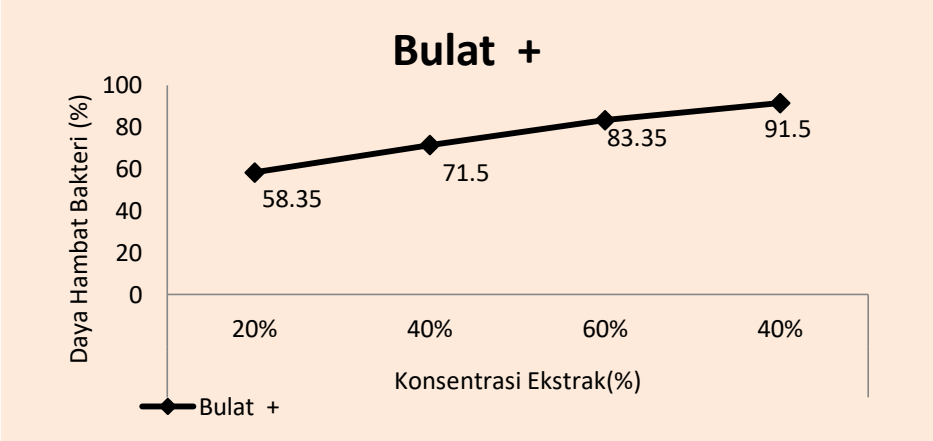

Figure 3 . Effect of *Turbinaria o rnata* extract , percentage increase in positive round bacterial growth inhibition on Control (ampicillin). K ounce entrasi extract 20%, 40% extract concentration, the extract concentration of 60%, and 80% extract concentration.

Statistical analysis *One-Way Annova* trials have been conducted, showed inhibition of extracts *Turbinaria ornata* has a significant effect (P<0.05) on positive round bacteria. Effect of *Turbinaria ornata* extract, inhibitory diameter formed on positive round bacteria ranged from 0 to 38 , 3 mm. And the percentage increase in the inhibitory growth of a positive bacterial growth against a roller contributor + above 50%. Based on the results of *Duncan's* advanced test analysis, the treatment at 80% extract concentration showed the best results with the highest inhibitory value of 38.3 mm and significantly different for all treatments. Meanwhile, the lowest inhibitory value is shown in the Control treatment - (0 mm) and significantly different for all treatments.

Inhibitory activity of *Turbinaria ornata* extract against positive round bacteria is characterized by the formation of a clear zone on the agar surface around the well. The clear zone indicates that no bacteria is growing. It is indicated that extract of *Turbinaria ornata* has antibacterial compounds. Makroalgae *Turbinaria Ornata* has secondary metabolites, alkaloids, flavonoids and triterpenoids which have antibacterial activity (Diachanty *et al*., 2017). Alkaloid compounds can inhibit the growth of gram positive and gram negative bacteria, this compound causes cell lysis and changes in bacterial morphology. Flavonoid is antibacterial by damaging the cell wall components so that the cell wall layer is not intact and causes cell death. And triterpenoid has a working mechanism of antibacterial reacts with Porin (transmembrane protein) on the

outer membrane of the bacterial cell wall, forming a strong bond polymers resulting in the destruction of Porin. Damage to the porin which is the entrance and exit of the compound will reduce the permeability of the bacterial cell wall which results in bacterial cells lacking nutrients, so that bacterial growth is blocked or dead (Karou, 2006; Dewi, 2010; Ganiswarna, 1995).

The diameter of the positive bacterial growth inhibitory power in tilapia has an average clear zone diameter above 20 mm except negative controls. This indicates that active inhibitory itas macroalgae extract against bacteria b caterpillar relatively strong positive. This statement is supported by the opinion of Greenwood (1995), stating that the inhibitory power has a diameter of more than 20 mm, the inhibitory activity is very strong, while the diameter is less than 20 mm, the inhibitory activity is classified as moderate.

The inhibitory power of *Turbinaria ornata* extract against round bacteria is positively affected by concentration. With high concentration, the inhibition power is more effective in suppressing bacterial growth, it is proven that 80% concentration shows the most effective results in suppressing the growth of positive round bacteria in tilapia and is significantly different with other concentrations. These results indicate that the higher the concentration, the more the amount of active substance dissolved in the extract of *Turbinaria ornata,* so that the higher the ability *Turbinaria ornata* extract in inhibiting the growth of positive round bacteria in tilapia. This is confirmed by the statement of Putri *et al* (2017), stating that extract concentration affects speed diffusion of active substances. The high concentration of extract causes the diffusion process to accelerate, as a result the greater the antibacterial power and the wider the diameter of the inhibition zone formed.

The inhibitory power can also be influenced by the ability of positive round bacteria response to resist *Turbinaria ornata* extract compounds. This is supported by the statement of Adisasmito *et al* (2006) stating that, gram positive has a high level of sensitivity to antibacterial. Gram positive has a simple cell wall consisting of thin layers of fat 1-4%, 90% peptidoglycan, and teikoic acid. So that the gram-positive cell wall is more easily damaged by the antibacterial compound extract of *Turbinaria ornata* .

4.2.2. Positive Short Stem Bacteria

Values diameter inhibitory extract *Turbinaria ornata* against short positive stems bacteria seen in Figure 4. And the percentage increase in bacterial growth inhibitory power round the positive position of Control + can be seen in Figure 5 .

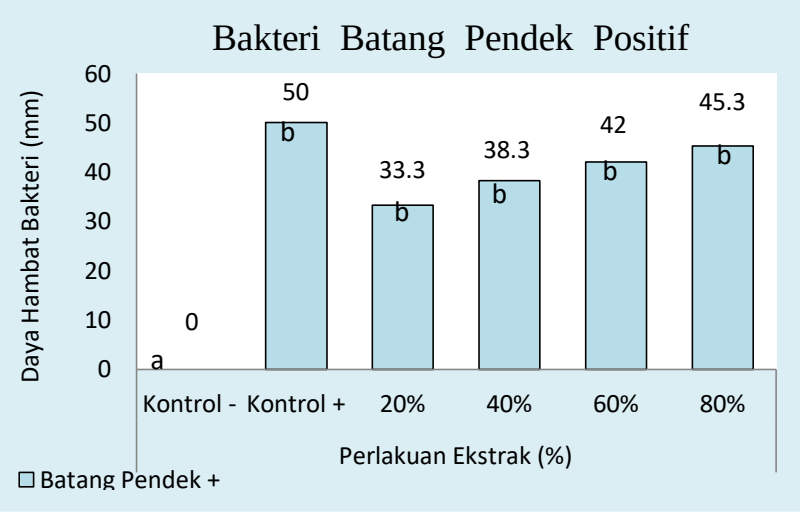

Figure 4 . Inhibitory diameter of *Turbinaria* extract *ornata* against positive short stem bacteria . Different letters in the diagram show significant values (P<0,05). Treatment of control concentration (distilled water), control + (ampicillin), extract concentration of 20% , extract concentration of 40%, extract concentration of 60% , extract concentration of 80%.

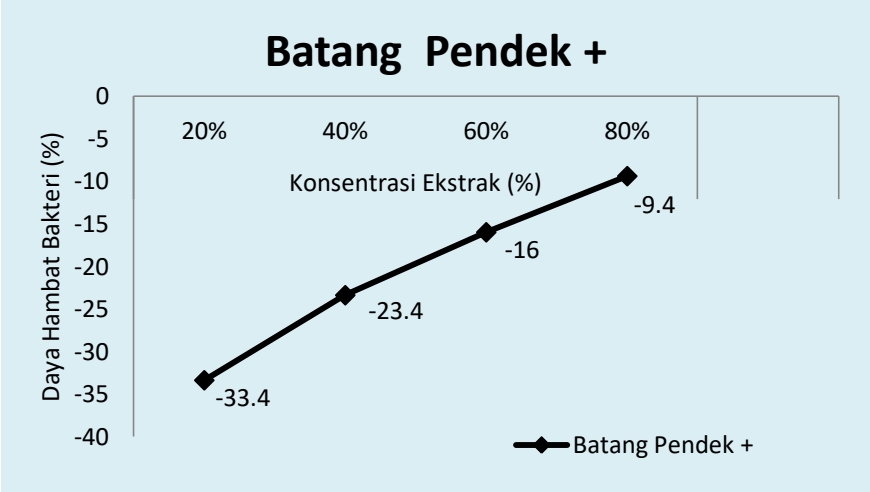

Figure 5 . The effect of *Turbinaria o rnata* extract , the percentage increase in inhibitory growth of positive short stem bacteria on Control + (ampicillin). The extract 20%, 40% extract concentration, K onsentrasi extract 60%, and 80% extract concentration.

Statistical analysis *One-Way Annova* trials have been conducted, showed inhibitory effect *ornata Turbinaria* extract significantly (P <0, 05) against the positive short rod bacteria. Inhibitory diameter of *Turbinaria* extract *ornata* against positive short rod bacteria ranging from 0-50 mm. Based on the data above, the Control + treatment showed the best results with the highest inhibitory value of 50 mm and significantly different from all treatments. However, the percentage increase in inhibitory power growth of positive short stem bacteria is below 1%. In the *Duncan* test, *the* treatment at 80% extract concentration showed the best results with the highest inhibitory value of 45.3 mm and significantly different for all treatments. Meanwhile, the lowest inhibitory value is shown in the Control treatment - (0 mm) and significantly different for all treatments.

Clear zones formed on the agar surface around the well indicate a bacterial inhibition in the treatment except for the negative control. The highest inhibition in the treatment of bacterial b Control + indicates positive short Atang have the ability to respond more sensitive to antibiotics. This is supported by the statement Adisasmito *et al* (2006) stating that, gram positive has a lower resistance level to antibiotics compared to gram negative. Mechanism of action of antibiotics by inhibiting bacterial cell wall synthesis, cell wall biosynthesis consequently become obstructed and bacterial cells into lysis. While the advanced test *Duncan*, treating the extract concentration of 80% showed the best results if suppress bacterial growth efekt b Atang positive short.

A positive short-rod bacterial inhibitory activity formed on the agar medium, has an average treatment diameter value ranging from 33.3-50 mm except for the negative control. This indicates that the inhibition of *Turbinaria ornata* extract against positive short stem bacteria has a relatively strong inhibitory power. This statement is supported by Greenwood (1995) stating that, the inhibitory power which has a diameter of more than 20 mm, the inhibitory activity is classified as very strong.

4.2.3. Negative Short Stem Bacteria

The inhibitory diameter formed in negative short rod bacteria is shown in Figure 6, and the percentage increase in inhibition of the growth of negative short rod bacteria against roller + contruction is shown in Figure 7 .

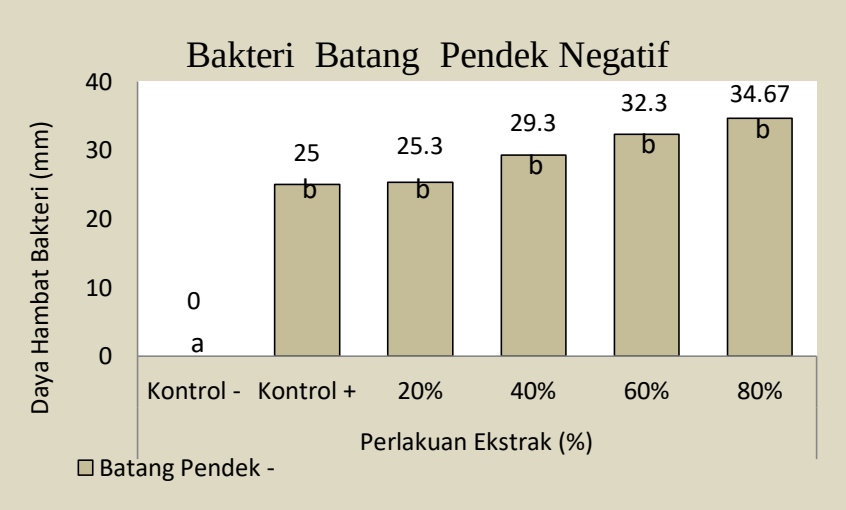

Figure 6. Diameter inhibitory extract *Turbinaria ornata* short rod bacteria to bacteria n egatif. Different numbers in the diagram show significant results (P<0,05). Treatment of Control concentration - (distilled water without administration of extract and ampicillin), Control + (ampicillin), Extract concentration of 20%, Extract concentration of 40%, Extract concentration of 60%, Extract concentration of 80%.

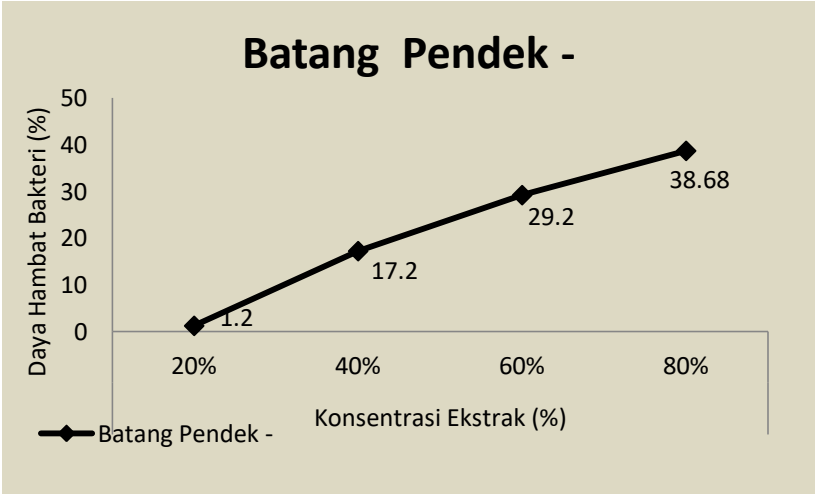

Figure 7. The effect of *Turbinaria o rnata* extract, the percentage increase in inhibition of the growth of negative short stem bacteria against + (ampicillin) Control. 20% extract concentration, 40% extract concentration, 60% extract concentration, and 80% extract concentration.

Statistical analysis *One-Way Annova* trials have been conducted, showed inhibition of extracts *Turbinaria Ornata* has a significant effect (P<0,05) on negative short stem bacteria. The effect of *Turbinaria ornata*, inhibitory diameter on negative

short rod bacteria ranged from 0-34, 67 mm. And the percentage of the increase in the inhibition of short stem bacteria is negative against the Control + range of 1-3 %. Based on the results of the *Duncan* test, the treatment at 80% extract concentration showed the best results with the highest inhibitory value of 34.67 mm and significantly different for all treatments. Meanwhile, the lowest inhibitory value is shown in the Control - treatment (0 mm) and significantly different for all treatments.

Inhibitory activity of *Turbinaria* extract *Ornata* against negative short stem bacteria is characterized by the formation of a clear zone on the agar surface around the well. The clear zone indicates that no bacteria is growing. It is indicated that extract of *Turbinaria ornata* has antibacterial compounds. Makroalgae *Turbinaria ornata* has secondary metabolites, alkaloids, flavonoids and triterpenoids which have antibacterial activity (Diachanty *et al.*, 2017). Secondary metabolite compounds have almost the same inhibitory mechanism that causes damage to the bacterial cell wall, so that the bacterial metabolism becomes disrupted and bacterial growth becomes blocked or dies (Karou, 2006; Dewi, 2010; Ganiswarna, 1995).

The diameter of the inhibitory power of negative short stem bacteria growth in tilapia has an average clear zone diameter above 25 mm except negative controls. This indicates that the inhibitory activity of macroalgae extracts against pathogenic bacteria is classified as very strong. This statement is supported by the opinion of Greenwood (1995), stating that the inhibitory power has a diameter of more than 20 mm, the inhibitory activity is very strong, the inhibitory power has a diameter ranging from 10-20 mm, the inhibitory activity is classified as medium and diameter the inhibition power is less than 5 mm, the inhibitory power activity is classified as very weak.

The inhibitory power of *Turbinaria ornata* extract against negative short stem bacteria is effected by concentration. This statement is reinforced by Katzung (2001) stating that, concentration effects the increase in the effectiveness of the drug against bacteria . The higher the concentration, the more effective the inhibition of suppressing bacterial growth, it is evident that the concentration of 80% shows the most effective results of suppressing the growth of negative short stem bacteria in tilapia . In addition to concentration, the inhibitory power can be influenced by the negative response ability of the negative rod bacteria in resisting the antibacterial compound extract of *Turbinaria ornata*. Gram negative has a low sensitivity to antibacterial. Pelezar and

Chan in Lingga *et al* (2016) stated that gram negative bacteria have multilayered cell walls consisting of peptidoglycan layers and fat layers 11-12% thick. And the other layer is the outer membrane layer which is composed of lipids, proteins and polysaccharides. Thus, gram negative bacteria have a higher resistance to environmental changes that are caused by the antibacterial compound extract of *Turbinaria ornata* .

5 . Conclusion

Based on the results of the research that has been done it can be concluded that the extract *Turbinaria Ornata* has bacterial inhibitory activity against positive round bacteria, positive short stems and negative short stems in tilapia . The inhibitory activity of *Turbinaria ornata* extract was characterized by the formation of a clear zone on the agar surface around the well. Treatment at 80% extract concentration showed the most effective results in suppressing the growth of pathogenic bacteria in tilapia.

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