

The Effect of *Callyspongia* biru Antibacterial Extract Concentration towards the Growth of Bacterial Isolates of *Kappaphycus Alvarezii* Seaweed with Ice-ice Disease

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Submission date: 26-Sep-2021 08:17PM (UTC+0700)

Submission ID: 1657665476

File name: Feliksitas_Masing_edited.pdf (843.09K)

Word count: 5824

Character count: 31226



The Effect of Callyspongia biru Antibacterial Extract Concentration towards the Growth of Bacterial Isolates of Kappaphycus Alvarezii Seaweed with Ice-ice Disease

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Article Info

Article History

Received: 28 August 2021

Revised: 18 September 2021

Published: 25 August 2021

Keywords

Callyspongia biru,
 antibacterial,
 Kappaphycus alvarezii,
 ice-ice disease.

Abstract

This research was conducted at the Biology Education Laboratory, Faculty of Teacher Training and Educational Sciences, Nusa Cendana University, with the aim of knowing the effect of the concentration of the antibacterial extract of Callyspongia biru on the growth of bacteria that caused Ice-ice disease. The method used in this study was an experimental method, with a Completely Randomized Design (CRD) consisting of 4 treatments (concentration of Callyspongia biru extract of 10%, 20%, 30% and 40%) with 3 times repetition. Therefore, the total treatment were 12 units. The parameter that has been measured in this study was the diameter of the inhibition zone. The inhibition zone diameter data was then analyzed by using one-way ANOVA with the Tukay method. If Fcount is greater than Ftable then it would be tested further using Honest Reality Difference test at 5% significant level. The result showed that the different concentrations of Callyspongia biru extract gave significantly different effects on the growth of bacterial isolates from seaweed Kappaphycus alvarezii with Ice-ice disease. At a concentration of 40%, Callyspongia biru extract showed the strongest average inhibition of 27.67 mm.

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Citations: Masing, F. A. & Tokan, M. K. (2021). The Effect of Callyspongia biru Antibacterial Extract Concentration towards the Growth of Bacterial Isolates of Kappaphycus Alvarezii Seaweed with Ice-ice Disease. *Science Education and Application Journal*, 3 (2), 102-112.

INTRODUCTION

The abundance of marine life in our country makes it rich in marine products and certainly become one of the exporting countries of marine products in the world. East Nusa Tenggara province stores regional income assets from marine products such as seaweed. Kupang Regency is one of the seaweed cultivation areas in East Nusa Tenggara Province (National Water Conservation Area Agency, NTT province, 2020).

The coastal and marine areas of Kupang Regency have rich natural resource potential such as mangrove forests, sea grass beds, seaweed, various types of coral reefs, fisheries and other marine biota (Temu et al, 1999 in Kamlasi, 2011). The coastal area of the West Kupang District, one of the sub-districts in Kupang Regency, has the potential of water resources for business development in the field of fisheries (cultivating and catching). Seaweed cultivation is one of the potential being developed currently. Seaweed is one of the marine resources that has benefits for the food, pharmaceutical, and other industries because it produces agar, carrageenan and alginate. Seaweed also contains carbohydrates, protein and a little fat as well as sodium and potassium salt compounds. Utilization of coastal and marine biological resources such as seaweed and others has long been carried out by seaweed farming communities in West Kupang District. So far, people have been using seaweed to be limited to nature and few are cultivating it. The type of seaweed that is cultivated and utilized in this

area is the *Eucheuma* seaweed (Kamlasi, 2011). *Eucheuma* seaweed dominates Indonesia's production and exports. Indonesia is even the number two the supplier of *Kappaphycus alvarezii* seaweed in the world after the Philippines (Kordi, 2011).

The main obstacle of seaweed cultivation is the presence of ice-ice disease that attacks seaweed before the change of seasons. The disease impacts the decline in seaweed production resulted in a decrease of farmers' income. This ice-ice disease is the cause of failure of seaweed cultivation (Tokan, et al. 2012). The development of pathogenic bacteria activity in the seaweed thallus resulted in the appearance of white spots on the thallus and gradually became porous and finally the thallus was broken. Symptoms of ice-ice disease are generally characterized by bleaching at the base of the thallus, middle and tip of the young thallus, which begins with a change in color of the thallus to clear white or transparent. In general, the spread of ice-ice disease occurs vertically by thallus seeds and horizontally through the mediation of water (DKP, 2004 in Aris, 2011). Until now, this disease has not been overcome. The main difficulty in controlling this disease is the existence of a marine environment as a place for seaweed growth. Controlling this disease becomes too difficult because of the water environment. There is no barrier between one cultivation location and another, as well as there is no barrier between one rope and another. Thus, when an ice-ice disease invades, other cultivation locations or ropes will be susceptible to attacked. This is because water is a medium for the spread of disease (Tokan et al, 2012). Another alternative that can be applied is biological control by using *Callyspongia* sponges. *Callyspongia* is an animal, so if it is used as a companion organism in seaweed cultivation, it will be more profitable. This is because the competition for nutrients will not occur. Moreover, there will be mutual fulfillment of each other's needs. *Callyspongia* provides CO₂ to meet the photosynthetic needs of seaweed, and on the other hand seaweed provides O₂ for *Callyspongia*'s respiration needs. The fact shows that some types of *Callyspongia* produce secondary metabolites with biological activity (Tokan et al, 2012).

Secondary metabolites of this sponge have been reported to have anticancer, antimicrobial and anti parasitic activity (Amir and Budiyo, 1996). Immaculata (2007) in Tokan et al (2012) suggested that in the waters of Kupang Bay there were a number of *Callyspongia* species showing antimicrobial potential against test bacteria and *Callyspongia* biru showed the strongest inhibition compared to others. Voogd et al. (2006) in Fariska (2010) stated that *Callyspongia* biru has a higher bio activity than the sponges *Amphimedon paraviridis*, *Niphates olemda*, and *Aaptos suberi-toides*. Because of its ability to produce bio actives including antibacterial, research on controlling ice-ice disease in seaweed has also used *Callyspongia* as a companion and the results are quite encouraging because it can reduce the attack of ice-ice disease (Tokan and Oheli, 2010 in Tokan, 2011). The purpose of this research was to determine the effect of the concentration of *Callyspongia* biru extract on the growth of bacterial isolates from seaweed *Kappaphycus alvarezii* with ice-ice disease and to determine the concentration that produced the strongest inhibition on the growth of bacterial isolates from seaweed *Kappaphycus alvarezii* with ice-ice disease. This research is useful as information material for agencies engaged in marine and fisheries as well as seaweed farming communities so that they can use *Callyspongia* biru as a bioprotector for seaweed, as well as for researchers who are interested in conducting further research.

METHODS

The *Callyspongia* biru was taken from Timor Beach and seaweed samples were gotten from Tablolong Beach, Kupang, East Nusa Tenggara.

1. Tools and Materials

- a. Tools. The tools used in this research are incubator, Laminar Air Flow, micropipette (1000 μ l and 200 μ l), measuring pipette, autoclave, hot plate, petri dish, inoculation loop, analytical balance, test tube, erlenmeyer 1000 ml, measuring cup (200 ml),

Sensidisc dispenser, funnel, mortar, maceration container, vacuum evaporator, spirit lamp and water bath.

- b. Material. The materials used in this study were dry explants of *Callyspongia* biru, bacterial isolates from seaweed *Kappaphycus alvarezii* with ice-ice disease, modified NA medium, MHA medium, aquades, 30 g tetracycline and gasoline petrol.

2. Research Design

This study is an experimental study, using a Completely Randomized Design (CRD) consisting of 4 treatments (concentration) with 3 replications. There are 12 experimental units total.

3. Work Procedure

- a. Room preparation. The room of inoculation was cleaned and sprayed with alcohol to sterilize other microorganisms.
- b. Preparation of tools and materials. The tools and materials used in this research were prepared in the laboratory. Pure cultures of isolated bacterial from seaweed with ice-ice disease were obtained from the rejuvenation results in the P. Biology laboratory.
- c. Manufacture of modified NA medium (Seawater NA). Seawater NA medium is a growth medium for bacterial isolates from seaweed with ice-ice disease. This medium is made by adding 150 mL of meat extract then adding 300 mL of sea water and then heating it on a hotplate. Then dissolve the agar and add seawater until the volume reaches 500 ml. Then it was removed and poured into 10 mL petri dishes each and allowed to cool.
- d. Isolation of bacteria from seaweed *Kappaphycus alvarezii* with ice-ice disease. Bacterial isolates from seaweed with ice-ice disease are readily available at the Biology Laboratory of FKIP UNDANA, from student research. This bacterial isolate consisted of *Vibrio* sp and *Pseudomonas* sp.
- e. Preparation of *Callyspongia* biru extract. The extracts of the *Callyspongia* biru were made by using the maceration method while the solvent used was petroleum gasoline. The dried extract of the *Callyspongia* biru type was cut into small pieces/mashed and weighed as much as 300 grams, then were put in a maceration container and added 750 ml of gasoline gasoline solvent, let stand for 5 days while stirring occasionally. Furthermore, it is filtered and the extract obtained is accommodated and concentrated until it became thick with a vacuum evaporator at a temperature of 80.1°C according to the boiling point of gasoline gasoline. Petrol gasoline is a non-polar solvent (Toka, 2012). Turbone (1983) in Tokan (2012) explains that the principle of solubility is that polar solvents will dissolve polar compounds, semi-polar solvents will dissolve semi-polar compounds and non-polar solvents will dissolve non-polar compounds. So the extract obtained is an extract containing non-polar compounds.
- f. Rejuvenation of test bacteria *Staphylococcus aureus* and *Escherichia coli* were used as a comparison of resistance with isolates obtained from ice-ice diseased seaweed. These test bacteria were inoculated in inclined NA medium and incubated at 37°C (optimum temperature for growth of both types of test bacteria) for 24 hours.
- g. Preparation of MHA medium 500 ml of aquadest was heated on a hot plate. Then 20 grams of MHA powder was added. After that stirred and heated until thick. The finished MHA medium is removed and ready to be used. This MHA medium need to be sterilized before being used. The MHA medium was put in an Erlenmeyer flask, covered with aluminum foil, then sterilized using an autoclave with a pressure of \pm 1.5-2 atm and at a temperature of 121°C for 35 minutes.
- h. Preparation of extract concentration *Callyspongia* biru. Based on the results of research by Suryati and Mulyani (2002) regarding the effectiveness of the use of bio active sponges for bacterial control, it is known that the sponge extract of the

Callyspongia sp species is able to kill bacteria maximally at a concentration of 20%. Therefore, in this study the concentration of 20% will be determined as the middle concentration which will be increased twice up and twice down, calculated by multiples of 10. So that the concentration is 40%, 30%, 20% and 10% while 0% was used as a negative control. How to make Callyspongia biru extract, namely: a) 40% = 4.0 ml of extract diluted with sterile distilled water until the volume reaches 10 ml b) 30% = 3.0 ml of extract diluted with sterile distilled water until the volume reaches 10 ml. c) 20% = 2.0 ml of extract diluted with sterile distilled water until the volume reaches 10 ml. d) 10% = 1.0 ml of extract diluted with sterile distilled water until the volume reaches 10 ml. e) 0% = 0 ml of extract diluted with sterile distilled water until the volume reaches 10 ml. This concentration is also negative.

- i. Preparation of Comparison Solution Tetracycline was weighed as much as 0.00030 grams and dissolved in sterile distilled water until the volume reached 10 ml, so that a concentration of 30 g/ml was obtained.
- j. Testing the antibacterial inhibition of Callyspongia biru extract against bacterial isolates from seaweed and test bacteria. 10 ml of MHA medium was poured aseptically into a petri dish and allowed to freeze as a base layer. A total of 5 ml of MHA medium which was slightly cooled at 45 - 48 C, after being thoroughly mixed with the test bacteria, was poured over the bottom layer of the medium and spread evenly using a sterile speeder. Furthermore, the sensidisk or cover is placed on the surface of the medium and filled with 0.2 ml of the comparison solution and the test solution. For 1 medium consisted of 6 pens which were divided into 1 positive control containing tetracycline, 1 negative control (0% extract concentration) and 3 pens with different concentrations of the extract of the bioactive compound Callyspongia biru then incubated at 37 C. for 24 hours. Furthermore, the diameter of the inhibition zone or the distance that is not overgrown with bacteria is measured with a caliper. Determination of the inhibition of bacterial isolates based on Cappuccino and Sherman (1983) and Prescott et al. (1990) and according to Davis and Stout (1997).

4. Data Collection

The data needed in this study is the diameter of the inhibitory zone of the antibacterial compound of Callyspongia biru from each treatment concentration.

5. Data Analysis and Interpretation

Data on the zone of inhibition of antibacterial were analyzed by one-way ANOVA at a significance level of 5% with the SPSS for Windows version 14 program. To see the differences between treatments, further analysis was carried out with 5% BNJ. If the calculated F value (treatment with different concentrations) is greater than F table, it can be concluded that the difference in concentration has a significantly different effect on the inhibition of bacterial isolates from seaweed *Kappaphycus alvarezii* with ice-ice disease.

RESULTS AND DISCUSSION

1. *Callyspongia biru* Antibacterial Extract

In this study, 300 grams of Callyspongia birupowder was weighed and macerated with 750 ml of gasoline as solvent. This maceration process occurred for 5 days while stirring occasionally, then evaporated and obtained 21 ml of black filtrate. Then 10 ml of the filtrate was taken to make concentrations of 10%, 20%, 30% and 40% by dilution.

2. Tested Bacteria

The tested bacteria were taken from the seaweed *Kappaphycus alvarezii* suffered ice-ice, which consisted of *Vibrio* sp and *Pseudomonas* sp.. The bacteria were taken at a dilution rate of 10⁻⁴. Prior to the research, these bacteria were rejuvenated for 24 hours

in an incubator at 30°C. There are also other bacteria used as comparisons in this study, namely *S. aureus* as a representative of gram-positive bacteria and *E.coli* as a representative of gram-negative bacteria. These two bacteria were also rejuvenated for 24 hours in an incubator. As a negative control, sterile distilled water was used.

3. Inhibition Zone Diameter Data

Callyspongia biru extract can inhibit the growth of bacteria that cause ice-ice disease as well as comparison bacteria, namely *S. aureus* and *E. coli* as seen from the clear zone that appears after incubation for 24 hours. The following table shows the result of laboratory test on the effect of the concentration of *Callyspongia biru* extract towards the bacteria that cause ice-ice disease in *Kappaphycus alvarezii* seaweed.

Table 1. Inhibitory Zone Diameter of *Callyspongia biru* extract towards bacteria causing ice-ice disease in seaweed *Kappaphycus alvarezii* E.cottonii

Treatment (%)	Inhibition Zone Diameter (mm)			Amount	Average
	1	2	3		
K ₁	17	19	18	18	18
K ₂	20	21	20	20	20,33
K ₃	23	25	22	22	23,33
K ₄	27	30	26	26	27,67
K ⁺	18	20	22	22	20
K ⁻	-	-	-	-	-

Note table 1:

K1 = 10% concentration

K2 = 20% concentration

K3 = 30% concentration

K4 = 40% concentration K+ (positive control): Tetracycline K- (negative control): sterile Aquades

Table 1 above shows the average value of the diameter of the different inhibition zones at each treatment concentration. The average value of the smallest inhibition zone diameter was at a concentration of 10%, i.e 18, while the largest was at a concentration of 40%, i.e 27.67. The difference in the effect of the concentration of *Callyspongia biru* extract on the growth of ice-ice bacteria isolates can also be seen more clearly in Figure 1 below.

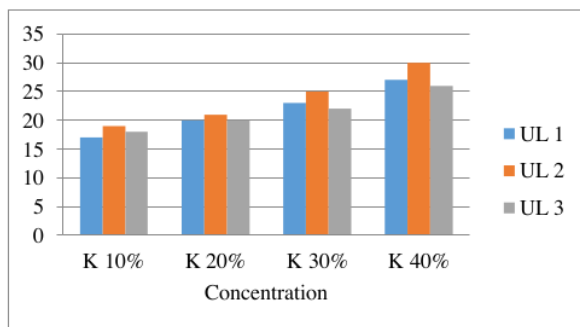


Figure 1. Graph of the average difference on the effect of the concentration of *Callyspongia biru* extract towards the growth of ice- ice bacteria.

From Figure 1 above, it is clear that the average diameter of the inhibition zone for each treatment is different. The higher the concentration, the greater the inhibition. The

concentration that has the strongest inhibition is the concentration of 40%. Further, data on the effect of concentration of *Callyspongia* biru extract on *S. aureus* and *E. coli* bacteria can be seen as follows:

1. Bacteria *S. aureus*

Data on the diameter of the inhibition zone of the antibacterial extract of *Callyspongia* biru against *S. aureus* bacteria are presented in table 2 below.

Table 2. Inhibitory zone diameter of *Callyspongia biru* extract against *S. aureus* bacteria.

Treatment (%)	Inhibition Zone Diameter (mm)			Amount	Average
	1	2	3		
K ₁	14	13	14	41	13,67
K ₂	16	15	16	47	15,67
K ₃	17	15	16	48	16
K ₄	20	18	17	55	18,33
K ⁺	16	15	16	47	15,67
K ⁻	-	-	-	-	-

Note table 2:

K1 = 10% concentration

K2 = 20% concentration

K3 = 30% concentration

K4 = 40% concentration

K+ (positive control): Tetracycline

K- (negative control): sterile Aquades

Table 2 above also shows the effect of the concentration of *Callyspongia biru* extract on *S. aureus* bacteria in each treatment. The smallest average is at a concentration of 10% which is 13.67 mm and the largest average is at a concentration of 40% with a value of 18.33 mm.

2. *Escherichia coli*

The effect of the concentration of the antibacterial extract of *Callyspongia* biru on the growth of the comparison bacteria *E. coli* can be seen in table 3 below.

Table 3. Inhibitory zone diameter of *C. biru* extract towards *E. coli* bacteria.

Treatment (%)	Inhibition Zone Diameter (mm)			Amount	Average
	1	2	3		
K ₁	16	16	16	48	16
K ₂	16	17	17	50	16,67
K ₃	17	17	18	52	17,33
K ₄	18	18	20	56	18,67
K ⁺	15	14	16	45	15
K ⁻	-	-	-	-	-

Table 3:

K1 = 10% concentration

K2 = 20% concentration

K3 = 30% concentration

K4 = 40% concentration

K+ (positive control): Tetracycline

K- (negative control): sterile Aquades

Table 3 also shows the difference in the average of each treatment with the smallest average at a concentration of 10% with a value of 16 mm and the largest average is at a concentration of 40%, which is 18.67 mm. From the three tables presented above, it can be stated that the diameter of the inhibition zone increased as the concentration of the given *Callyspongia biru* extract increased. In this study, tetracycline was used as a comparison solution. 0.0003 gram of tetracycline was weighed and then dissolved with water to reach 10 ml. Yellow tetracycline solution, which was used as a positive control. The following is data on the effect of tetracycline on bacterial isolates from seaweed that was attacked by ice-ice disease, *S. aureus* and *E. coli* bacteria.

Table 4. Effect of tetracycline on isolated bacterial, *S. aureus* and *E. coli*.

Bacteria	Inhibition Zone Diameter (mm)			Amount	Average
	1	2	3		
Seaweed isolate (<i>Vibrio sp</i> and <i>Pseudomonas sp</i>)	18	20	22	60	20
<i>S. aureus</i>	16	15	16	47	15,67
<i>E. coli</i>	15	14	16	45	15

Table 4 above shows the diameter of the inhibition zone in the 3 types of bacteria tested due to the effect of tetracycline. It is also seen that there is a difference in the average diameter of the inhibition zone. Bacterial isolates from seaweed diseased with ice-ice had the largest average of 20 mm, and then followed by 15.67 mm *S. aureus* bacteria and 15 mm *E. coli* bacteria.

The data obtained in table 1, table 2 and table 3 above illustrate different results. The *Callyspongia biru* extract was more active on isolated bacterial from ice-ice diseased seaweed than on *S. aureus* and *E. coli* bacteria. The data in table 1 reveals an analysis of variance (one way ANOVA). The complete summary of the analysis results can be seen in table 5.

Table 5. Summary of ANOVA the effect of concentration (%) of *Callyspongia biru* extract on the growth of bacteria causing ice-ice.

Source of diversity	Free Degrees	Number of Squares	Middle Square	F. Count	Sign.	F. Table 5%
Treatment	3	156,67	52,22	26,11*	.000	4,07
Error	8	16,00	2,00			
Total	11	172,67				

Description: * : Very real effect

The results of the ANOVA in table 5 above show that the value of F. Count = 26.11 > F. Table 5% = 4.07 (db for the numerator of 3 and db for the denominator of 8). Because F. Calculate > F. Table, this research hypothesis is accepted. Thus, it can be concluded that the concentration treatment has a very significant effect on the growth of ice-ice bacteria. To find out the treatment or concentration that gave a very real effect, it was continued with the Tukey's Honest Significant Difference Analysis (HSD) at a significance level of 5%. The results of the significant difference test can be seen in table 6.

Table 6. The results of the Honestly Significant Difference (HSD) test in the diameter of the ice bacteria growth inhibition zone.

Tukey HSD				
concentration	N	Subset for alpha = 0.05		
10	3	1	2	3
20	3	18.00	20.33	
30	3	20.33	23.33	
40	3		23	27.67
Sig.		.257	.117	1.000

Means for groups in homogeneous subsets are displayed.

The results of the Honest Significant Difference test in table 6 above show the average value of the 10% concentration treatment is in subset 1. The average value of the 20% concentration treatment is in subsets 1 and 2. The average value of the 30% concentration treatment is in subset 2. The result reveals that these three concentrations are not significantly different. While the average value of the 40% concentration treatment is in subset 3 which shows a very significant difference with other treatments. From the results obtained, it can be concluded that the higher the concentration of *Callyspongia biru* extract, the great the antibacterial power. In the *Callyspongia biru* extract, the concentration that showed the strongest/maximum inhibition on the growth of ice-ice bacteria was at the concentration of 40%. *Callyspongia biru* that is still wet is aerated to dry for 7 days. After drying, it is mashed and taken as much as 300 grams to be macerated for 5 days with 750 ml of gasoline solvent for then be evaporated. Petrol gasoline is a non-polar solvent that dissolves non-polar compounds based on the solubility principle. Thus, the extracts obtained from evaporation containing non-polar compounds. The filtrate obtained was 21 ml, black in color and had a sharp aroma. Then 10 ml of the filtrate was taken to make concentrations of 10%, 20%, 30% and 40% dilution.

Callyspongia biru extract was proven to inhibit the growth of bacteria from the ice-ice diseased *Kappaphycus alvarezii* seaweed as seen from the clear zone produced around the area of bacterial growth. In addition, this extract was also active on the compared bacteria, namely *S.aureus* and *E.coli* which also seen from the presence of a clear zone produced. According to De Voogd et al. (2007), *Callyspongia biru* extract contains amphitoxin bioactive compounds A and B. Amphitoxin is a promising compound because it is active in various test organisms and various biological tests (Wikanta, et al. 2011). This amphitoxin which is also anti-microbial has a mechanism to kill microbial growth, including damaging the microbial cell wall, changing the permeability of the cytoplasmic membrane which causes the release of nutrients from the microbial cell, inhibiting the work of enzymes in microbial cells and inhibiting the synthesis of nucleic acids and proteins, and releasing free sulfhydryl groups (Jawetz et al, 1996 in Wibawaty, 2012). In determining the antibacterial activity of *Callyspongia biru* extract, agar diffusion method was used. Determination of the inhibition zone diameter category according to Cappuccino and Sherman (1983) in Tokan et al, (2012) using standard tetracycline antibiotics, namely the resistant category 14 mm, the medium category 15-18 mm and the susceptible category 19 mm. The tetracyclines (positive control) in this study had an average diameter of 15-20 mm of moderate to susceptible inhibition zones. The effect of tetracycline that has been tested shows that bacterial isolates from seaweed with ice-ice disease have an average inhibition zone diameter of 20 mm, including the susceptible category. Then, the average *S. aureus* bacteria was 15.67 mm which was included in the medium category and the average *E. coli* bacteria was 15 mm which also included the medium category.

In addition, according to Davis and Stout (1971) in Pantun (2011) Keo (2013), if the diameter of the inhibition area is 5 mm or less, the inhibitory activity is categorized as weak, 6-10 mm is categorized as moderate, 11-17 mm is categorized as weak, and 20 mm or more is considered as very strong. The average diameter of the inhibition zone on the growth of ice-ice bacteria for 10% concentration was 18 mm, categorized as strong. The concentration of 20% has an average diameter of 20.33 mm, categorized as very strong. For a concentration of 30%, the average diameter of the inhibition zone was 23.33 mm, which was classified as very strong. Furthermore, the average 40% concentration of 27.67 mm is also classified as very strong category. In the comparison bacteria *S. aureus*, the overall diameter of the inhibition zone ranged from 13-18 mm, which means that it belongs to the strong category. For comparison bacteria, *E.coli* ranging from 16-18 mm is also included in the strong category. This means that *C.25* extract is not only active in inhibiting the growth of ice-ice bacteria isolates but also can inhibit the growth of *S. aureus* and *E. coli* bacteria. From the results above, it has been proven that *Callyspongia biru* extract besides being able to inhibit the growth of gram-positive bacteria isolates from seaweed, can also inhibit the growth of gram-negative bacteria represented by *E.coli* bacteria. This proves that *Callyspongia biru* extract has a broad spectrum of inhibition in inhibiting bacterial growth. The calculated F value from the data on the diameter of the ice-ice bacterial inhibition zone is greater than the F. table (F. Count = 26.11 > F.Table 5% = 4.07). This means that the hypothesis of this study is accepted, that different concentrations of *Callyspongia biru* extract have different effects on the growth of bacterial isolates from *Kappaphycus alvarezii* seaweed with ice - ice disease. Further test results with the Honest Significant Difference Test (HSD) shows, when associated with the four concentrations (10%, 20%, 30% and 40%), then the 40% concentration had a very significantly different effect with other concentrations. (is in subset 3). In addition to depending on concentration, according to Tokan, et al (2012), the activity of an antimicrobial is also influenced by the following factors: (1) population size. A large population takes a longer time to kill than a small population, (2) Population composition. (3) The effectiveness of the agent varies, depends on the nature of the organism, the concentration or intensity of the antimicrobial agent, (4) the duration of exposure. The longer a population is exposed to an antimicrobial agent, the more members of that population are killed, (5) Temperature. An increase in temperature sometimes increases the activity of a chemical substance. Often, lower concentrations of chemicals can be used at higher temperatures, (6) Local environment. Controlled populations are not isolated, but are surrounded by environmental factors that may help or protect them from destruction. The *Callyspongia biru* extract in this study only reached the level of inhibiting bacterial growth but have not touch the level of bacteria killing. From the test results of the effect of the concentration of *Callyspongia biru* extract on the growth of bacterial isolates from seaweed *Kappaphycus alvarezii* with ice-ice disease, it is found that the higher the concentration, the greater the diameter of the inhibition zone, which means the antibacterial inhibition is stronger. Or in other words, the concentration is directly proportional to the inhibitory power or the diameter of the resulting inhibition zone

CONCLUSION

From the results of this study it can be concluded that:

1. *Callyspongia biru* extract with different concentrations has different effects in inhibiting the growth of bacteria that causes ice-ice disease in *Kappaphycus alvarezii* seaweed.
2. From the four concentrations used (10%, 20%, 30% and 40%), the one with the strongest inhibition was at a concentration of 40%. Thus it is inferred that the higher the concentration the stronger the inhibition.

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