Available online at http://www.journalijdr.com

IJDR

International Journal of DEVELOPMENT RESEARCH

ISSN: 2230-9926

International Journal of Development Research
Vol. 4, Issue, 11, pp. 2398-2403, November, 2014

Full Length Research Article

THE ANALYSIS OF THE ANTIBACTERIAL POTENCY OF THE CALLYSPONGIA EXTRACT AND UTILIZATION POSSIBLITY AS BIOPROTECTOR OF BIOLOGICAL CONTROL OF THE ICE-ICE DISEASE ON SEAWEED

[1*]Moses Kopong Tokan, [2]Gufran Darma Dirawan and [3]Mbing Maria Imakulata

[1,3]Faculty of Teacher Training and Education of Undana
[2]Post Graduated of Makassar State University

ARTICLE INFO

Article History:
Received 11th August, 2014
Received in revised form
19th September, 2014
Accepted 19th October, 2014
Published online 18th November, 2014

Key words:
Antibacterial,
Callyspongia,
ice-ice.

ABSTRACT

The purpose of this study was to determine the antibacterial potency of *Callyspongia* and utilization possibility as a companion in the biological control of ice-ice disease. This study is a factorial experiment consists of 3 factors, namely the kind of bacterial isolates from seaweed suffering of ice-ice disease, kind of solvent and the kind of Callyspongia. Each factor consists of three levels and replicated three times. Callyspongia antibacterial potency testing performed by agar diffusion method. Data in form the inhibition zone diameter. Data were analyzed by multiple ANOVA through SPSS. The results showed that the kind of solvent, kind of bacterial isolates and the kind of *Callyspongia*, either individually or jointly showed significantly different effects on inhibition zone diameter. Antibacterial of ethanol extract showed the most potent inhibitory effects compared with the methanol extract and diethyl ether extract. While the influence of the type *Callyspongia*, *Callyspongia biru* showed strongest inhibition compared with *Callyspongia subarmigera* and *Callyspongia monilata*. The results also showed that the three isolates were categorized as susceptible to antibacterial use of Callyspongia antibacterial. Based on these results, *Callyspongia* can be used as a companion on seaweed farming as a biological control agent of ice-ice disease.

INTRODUCTION

The main constraint of seaweed cultivation is the ice-ice disease that attacks the seaweed before the turn of the season. The existence of the disease negatively impact to the seaweed production decline resulting in a decline in the income of farmers. Ice-ice disease is the cause of the failure of seaweed farming. Until recently, the disease is yet to be overcome (Tokan, 2009). The main inhibition to control this disease is the presence of an aquatic environment as the growth of seaweed. Therefore the water environment as a place to live, then control this disease becomes very difficult to do. There is no barrier between the bulkhead cultivation location to the another location, so there is no insulation barrier between the rope tied with rope belt to another, and therefore when the invasion of ice-ice disease, the cultivation of seaweed on the location or tie rope others will be susceptible to this disease.

Corresponding author: Moses Kopong Tokan
Faculty of Teacher Training and Education of Undana

This is because water is a medium spread of disease. In connection with the constraints above, it is necessary to apply appropriate techniques of disease control in the aquatic environment. Controlling the use of pesticides altogether unfavorable views of both ecological and economic aspects. Pesticides will be easily rinsed and carried away by the flow of water away from the location of culture so that the duration of pesticide exposure to bacteria becoming shorter. Short duration of exposure would not be enough to kill disease-causing microbial populations. Another limiting factor in the determination of the ideal pesticide concentration to be able to kill all pathogenic microbial populations. When applied, the concentration of the most concentrated pesticides though will soon undergo dilution by a huge mass of sea water. Therefore we need large amounts of pesticides with a frequency applications more frequently thereby increasing the duration of pesticide exposure to disease-causing microbes. This has a huge impact on the economic aspects. Of ecological aspects, chemical disease control approaches give negatively impact to the aquatic ecosystems as a whole. Pesticides not only kill disease-causing microbes but also kill non target organisms

are in the vicinity of cultures and far away from the location of cultivation due to water swept away. The negative impact is greatest when pesticides containing heavy metals entering the food chain and accumulate in plankton in aquatic ecosystems. Bioaccumulation and biomagnification are very likely to occur at each trophic level that will eventually harm humans. Ice-ice disease control with other techniques, such as eradication and quarantine also not possible on the marine environment. Techniques of ice-ice disease control is more suitable to be applied is through the cultivation of cultivars seaweed *Eucheuma cottonii* that are resistant to ice-ice disease and biological control through polyculture cultivation. Ice-ice disease control through polyculture cultivation by utilizing seaweed has been done Tokan and Awang (2009) and the results showed that 11 % of the total planted clumps with polyculture technique in November, infected ice-ice disease after third week. The same was done by Tokan, *et al*. (2010) in the waters of Kojadoi of Sikka regency East Nusa Tenggara.

In the monoculture techniques, ice-ice disease invasion had occurred after the first week on nearly all clumps. Although polyculture is quite successful but the main drawback is the growth of seaweed slower than the monoculture techniques. This is caused by the competition between seaweed in fighting of the nutrients. Another workable alternative is biological control by utilizing porifera Callyspongia. Callyspongia are animals, so if it used as a companion organisms on seaweed farming would be profitable. This is due to nutrient competition is not going to happen. Even here there will be mutual needs between one another. *Callyspongia* provide CO_2 for photosynthesis needs of seaweed and seaweed instead provide O_2 for respiration of Callyspongia. The fact is that some kind of Callyspongia producing secondary metabolites with biological activity. Several bioactive compounds exhibit antimitotic properties, cytotoxic, antibacterial, and antifungal. Blunt and Munro (1999) and van Soest and Braekman (1999) suggested that more than 3500 different compounds have been isolated from about 475 species. Faulkner (2000) suggested that sponges are a rich source of secondary metabolites are unique and diverse. Several such compounds have potential pharmacological activity as an anti-tumor, anti-fungal and anti-bacterial.

Kelman, *et al*. (2001) explains that the compounds isolated from the sponge is active against human pathogenic bacteria and marine bacteria. The research results from Newbold, *et al*. (1999) showed that extracts of 33 species of sponges showed antibacterial activity diversity where 48 % of all species of sponges can inhibit at least one bacterial isolates. Goa (2004) showed that *Callyspongia fibrosa* and seaweed *Stoecheospermum margilatum* can totally inactivate fungi. Imakulata (2007) explained that in Kupang Bay waters there are a number species of Callyspongia showed antimicrobial potency against bacteria and *Callyspongia biru* showed the strongest inhibitory effects than others. Tokan and Lodo (2008)[11] showed that *Callyspongia biru*, *C. subarmigera* and *Callyspongia* sp showed antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus*. Using *Callyspongia* as companion organisms in control ice-ice disease in seaweed, it is important that research carry out is testing extract antibacterial potency of *Callyspongia* against bacterial isolates derived from seaweed ice-ice disease. If ekskrak antibacterial of Callyspongia able to inhibit or kill bacterial isolates from seaweed suffering ice-ice diseasee, then Callyspongia can be used as a companion organisms on seaweed cultivation to control this disease. During the growth period, *Callyspongia* always exposed to pathogenic bacteria, thus to maintain itself, *Callyspongia* release of antibacterial compounds into the environment to control the invasion of the disease. Thus, if *Callyspongia* used as a companion, protection mechanism itself can be used to protect seaweed *Eucheuma cottoni* of ice-ice disease invasion.

MATERIALS AND METHODS

Location and Time of Research

Callyspongia collection conducted in waters Tablolong West Kupang, Kupang regency. Naturally, many Tablolong intertidal area covered by different species of sponges including Callyspongia and is the center of seaweed production in East Nusa Tenggara. This study to assess the ability of bioactive antibacterial compounds against bacterial isolates and tests carried out in the Laboratory of Biology and Chemistry University of Nusa Cendana Kupang NTT. The study lasted for 6 months, June-November 2012.

Experiment Design

This study is a factorial experiment and designed by Completely Randomized Design, which species Callyspongia as the first factor consists of three levels, namely *Callyspongia monilata* , *C. subarmigera*, and *C. biru*, and the second factor is the type of bacterial isolates consists of three levels and the third factors is solvent consists of three levels, namely ethanol, methanol and diethyl eter. Each treatment combination was replicated three times. Thus the total treatment unit in this study are 81 units.

Equipment and Materials Research

The tools used in the laboratory is a stereo microscope with camera, incubators, laminar air flow, micropipette (1000 µl and 200 µl), measuring pipettes, autoclave, glass objects and glass cover, petri dish, inoculation loops, UV sterilizer, analytical balance, sterile knife, pH meter, test tube, erlenmeyer 1000 ml, measuring cup (10 ml, 25 ml and 100 ml), sensi-disc dispensers, durham tube, funnel, mortar, container maceration, vacuum evaporator, colony counter, spirit lamp, and a water bath. Materials used in this study were dried explan of *Callyspongia monilata*, *C. subarmigera*, and *C. biru*, three isolates of seaweed ice-ice disease, ethanol 70 % and 95 %, pure cultures of *Staphylococcus aureus* and *Escherichia coli*, NA medium, 30 µg tetracycline, methanol, ethanol, diethyl ether, aquadest, medium Mueller Hinton Agar, Whatman 3 MM paper, phosphate buffer, medium phenol red sucrose broth, medium phenol red lactose broth, medium phenol red dextrose broth, SIM agar, broth MR-VP, Trypticase soy agar, reagents Kovac 's, indicators methyl red, reagents Barrit 's, hydrogen peroxide 3%, reagent Tetramethyl-p-phenylenediamine didhydrochoride, sterile cotton, oil immersion, and water-iodine solution.

Collection and Data Analysis

The data collected in this study it is characteristic of colonies and cells as well as inhibition zone diameter of antibacterial

compounds of Callyspongia. Isolation and characterization of microorganisms from seaweed suffering ice-ice disease. Microorganisms extraction, suspension dilution, cultivation, colony counting, colony and cell morphology characterization based on Tokan (2006), Cappucino and Sherman (1983). Preparation of antimicrobial extract base on Tuney, *et al*. (2007) and determination of resistance of isolates based on Cappuccino and Sherman (1983) and Prescott *et al*., (1990). Data of inhibition zone diameter were analyzed by two way ANOVA at 5% significance level using SPSS for Window version 16 and to know the differences between the treatment carried out by Tukey HSD 5%.

Interpretation of Analisys Result

After data analysis, the interpretation of the results of the analysis carried out as follows: If the value F calculated (combination treatment Callyspongia * solvent * isolates) is greater than the F table, it was concluded that there was an interaction between type of Callyspongia, the type of solvent and type of isolates to inhibition antibacterial.

RESULTS AND DISCUSSION

Isolation of Bactaerial

Isolation result of bacterial in seaweed suffering of ice-ice disease with colony characteristics as listed in table 1. Based on Table 1 it can be argued that all three isolates of seaweed suffering of ice-ice disease showed the different morphology colony characteristic from one colony to another colony.

Table 1. Colonies Characteristic on medium cultivation

No	Characteristic	Colony		
		1	2	3
1	Shape	Round	Round	Amuboid
2	Edge	flat	Flat	Wavy
3	Elevation	arise	Arise	Flat arise
4	Inner Structure	Smooth	Smooth	Coarse grained
5	Color	Yellow	White	Yellow
6	Zize	0.1 cm	0.3 cm	0.5 cm
7	Number	$0.4x\ 10^4$	$1\ x\ 10^4$	$2\ x\ 10^4$

Morphology and Biochemical Characteristics of Bacteria

Cell characteristics of the three isolates as shown in table 2. Based on tables 1 and 2, it was concluded that isolate 1 *is Acinetobacter* sp, isolates II is *Pseudomonas* type A and isolate III is *Pseudomonas* type B. Pathogenicity test of the microbiology laboratory of P2O - LIPI of 8 species of bacteria shows that there are 5 bacteria that can cause the ice ice diseases. The fith of these bacteria are *Pseudomodas nigricaciens*, *Pseudomonas fluorescens*, *Vibrio granii*, *Bacillus cereus* and *Vibrio agarliquefaciens* with the highest pathogenicity is *Vibrio agarliquefaciens*. The results research of Santoso and Nugraha, (2008) showed that the bacteria can be isolated from seaweed with ice-ice symptoms include *Pseudomonas* spp., *Pseudoalteromonas gracilis*, and *Vibrio* spp. Agarase (arginase) of bacteria is one of the virulence factors that contribute to ice-ice infection. The results research of Aris (2011) showed that the bacterial species isolated from the seaweed *Kappaphycus alvarezii* suffering ice-ice disease in the management of seaweed farming in the island waters Roast are *Vibrio alginolyticus, Pseudomonas cepacia*, *Flavobacterium meningosepticum*, *Pseudomonas diminuta* and *Plesiomonas shigelloides.*

Table 2. Cell characteristics and gram staining results from each isolate

Characteristics	Bacteria Isolate		
	I	*II*	*III*
1. Morfologi:			
- Shape	Rod	Rod	Rod
- Arrangemnent pattern	Strepto	Strepto	Strepto
- Zize	0.2-0.3 x 0.7-0.8 ↘m	0.5-0.8 x 1.3-1.4 ↘m	0.3-0.5 x 0.9-1.1 ↘m
2. Grams	Negative	Negative	Negative
Color	Pink	Pink	Pink
3. Biochemycal:			
- Glucose	+ (A)	+ (A)	+ (A)
- Lactose	-	-	-
- Sucrose	-	-	-
- Catalase	+	+	+
- Oxsidase	-	+	+
- Methyl Red	-	-	-
- V-P	-	-	-
- Indole	-	-	-
4. Respiration:			
- Aerobic	+	+	+
- Anaerobic	-	-	-

Test of Inhibition power of Antibacterial Callyspongia to Isolate From Seaweed of Diseased Ice-Ice

Antibacterial activity of Callyspongia was evaluated based on inhibition zone diameter (IZD). To determine the antibacterial potency, it needs to be extracted the antibacterial using organic solvents. There are 3 organic solvents used in this study, namely methanol, ethanol and diethyl ether. The variance analysis results indicate that the interaction between antibacterial Callyspongia, solvents and isolates against IZD.

Table 3. Summary Anova of interaction effect of Callyspongia, solvents and isolates

Dependent Variable: DZH					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	263.556[a]	26	10.137	18.661	.000
Intercept	22400.111	1	22400.111	4.124E4	.000
Pelart	16.667	2	8.333	15.341	.000
Callysp	68.963	2	34.481	63.477	.000
Baktri	30.519	2	15.259	28.091	.000
Pelart * Callysp	18.593	4	4.648	8.557	.000
Pelart * Baktri	5.481	4	1.370	2.523	.051
Callysp * Baktri	9.407	4	2.352	4.330	.004
Pelart * Callysp * Baktri	113.926	8	14.241	26.216	.000
Error	29.333	54	.543		
Total	22693.000	81			
Corrected Total	292.889	80			

a. R Squared = ,900 (Adjusted R Squared = ,852)

The interaction F value with df numerator 8 and db denominator 54 is 26,216 with a probability 0,000 ≤ α 005. The third factors were tested, namely Callyspongia antibacterial, solvents and isolates independently or jointly establish a clear difference to IZD. The factors of Callyspongia antibakeri, solvents, isolates, the interaction of Callyspongia antibacterial with the solvent, the interaction of antibacterial Callyspongia with the isolates and the interaction of solvent with isolates have a probability 0,000 ≤ α 0.05. Result of analysis listed in table 3. The interaction occurs with probalilitas 0.000 < α 0.05 and then do further analysis with Tukey HSD 5% to see homogeneity subset of each factor tested. Homogeneity subset of Callyspongia antibacterial can be seen in Table 4.

Table 4. Homogeneity subsets based on Tukey HSD antibacterial Callyspongia

Callysp	N	Subset		
		1	2	3
C.monila	27	15.5185		
C.subar	27		16.5926	
C.biru	27			17.7778
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = 543.

Based on Table 4, it was concluded that the *Callyspongia biru* antibacterial extracts has a stronger inhibitory or significantly different from *C. subarmigera*, and *C. monilata*. Analysis of bacterial isolates subset homogeneity can be seen in table 5. Based on the table 5 above, it was concluded that the isolates I (Acinetobacter) more sensitive or have a weaker defense system compared with the other two isolates (*Psedudomonas* type A and *Pseudomonas* type B).

Table 5. Homogeneity subset of bacteria based on Tukey

Tukey HSD				
Baktri	N	Subset		
		1	2	3
Isolat2	27	15.8148		
Isolat3	27		16.7778	
Isolat1	27			17.2963
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = ,543.

Results of pathogenicity tests conducted by LIPI LON showed that *Pseudomonas* and *Vibrio* are more virulent than others derived from seaweed suffering of ice-ice disease. The results of a subset analysis of the homogeneity of the type of solvent can be seen in table 6.

Tabel 6. Homogeneity subset subset solvent based on Tukey

Tukey HSD				
Pelart	N	Subset		
		1	2	3
Diter	27	16.0741		
Metanol	27		16.6296	
Etanol	27			17.1852
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = ,543.

Table 6 above showed that ethanol produces antibacterial inhibition significantly different with the two other solvents. Ethanol attracts more antibacterial, both in terms of the number and types resulting in greater inhibition compared with other solvents. Profile plot interaction between Callyspongia antibacterial*bacterial* Solvent based on estimates of marginal mean inhibition zone diameter can be seen in Figure 1. Figure 1 above shows that antibacterial *Callyspongia biru* methanol extract showed the most potent inhibition against 1 isolates with inhibition zone diameter of 21 mm and the smallest is the solvent diethyl ether with IZD of 15.33 mm.

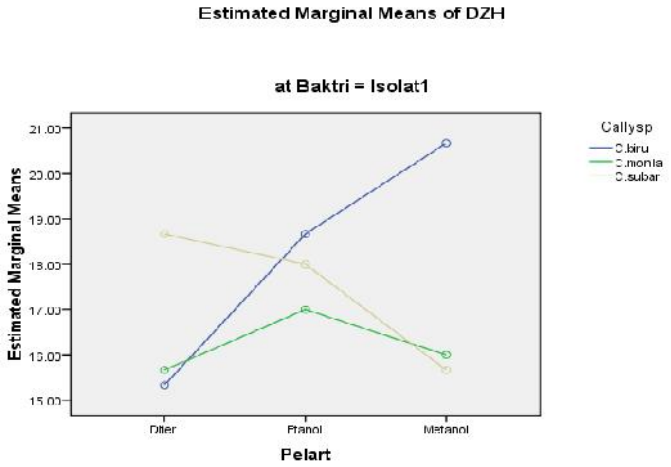

Figure 1. The average diameter of inhibition zone of Callyspongia antibacterial extracts from each solvent to Isolate I

Figure 2 above shows that antibacterial of Callyspongia subarmigera methanol extract showed the most potent inhibitory effects on the isolates 2 and the weakest in diethyl ether solvent. Figure 3 above shows that the methanol Extract, antibacterial of *C. biru* has the most potent inhibitory effects against isolates III and the weakest in *C. monilata* with methanol extract. In this study it was found that at the same concentration, antimicrobial from the selected species of Callyspongia showed the different inhibition. This indicates that the type and amount of antimicrobials contained in Callyspongia is varied. Because it vary in type and amount, then the mechanism of action in inhibiting the growth of microorganisms also vary. For example, Prescott, *et al*. (1990) explains that penicillin, ampicillin plays a role in inhibiting

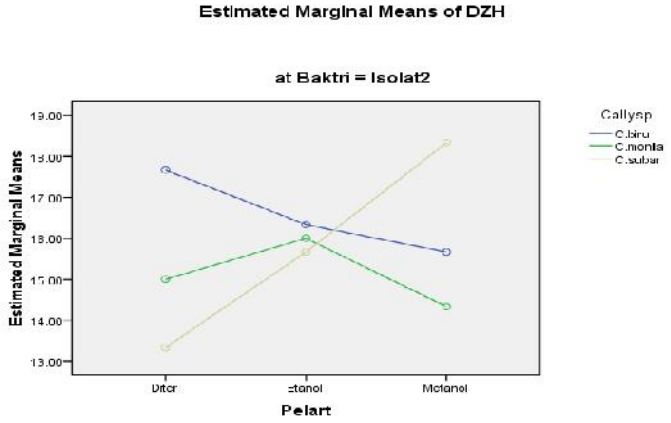

Figure 2. The average diameter of inhibition zone Callyspongia antibacterial extracts from each solvent to Isolate II

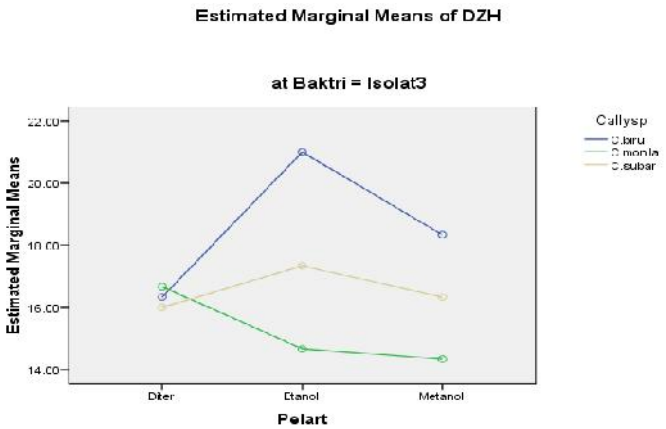

Figure 3. The average diameter of inhibition zone Callyspongia antibacterial extracts from each solvent to Isolate III

cell wall synthesis; streptomycin and cloramphenycol inhibit protein synthesis as well as polymicyn B and sulfonamides damaging bacterial cell walls. In addition, the inhibition of antimicrobials also varies according to the nature. The main effect may sidal (to kill) or static (inhibits growth) with broad or narrow spectrum. Broad-spectrum antimicrobial capable of killing or inhibiting microorganisms, both gram positive and gram negative, whereas a narrow spectrum only kill of gram-positive or negative bacteria only, clamydia alone or rickets alone. For example Prescott, *et al*. (1990) explains that the main effect of ampicillin is sidal with broad spectrum, which kills bacteria gram positive and some gram negative bacteria. Meanwhile, the main effect of bacitracin is sidal with a narrow spectrum, only kill gram-positive bacteria. In table 3 and 4 above shows that Callyspongia extract antibacterial can inhibit bacterial isolates from Eucheuma cottonii suffering ice-ice disease. This indicates that Callyspongia can be used as a companion or protector or bioprotector to protect seaweed from invasion ice-ice disease. Results of this study also proves that Callyspongia able to produces secondary metabolites in the form of antibacterial to protect themselves from disease invasion.

Isolation and identification of bioactive of sponge for bactericidal obtained some species of sponge that is able to inhibit the growth of several types of bacteria that cause disease in fish commodities, such as *Auletta* sp, *Halichondria* sp and *Callyspongia* sp containing compounds such as sterols, peptides and phenolic acids (Ahmad *et al*., 1995, Muliani, *et al*., 1996, and Suryati, *et al*. 1996). Suryati, *et.al* (1999) explains that, in addition to beneficial in the field of pharmacy and medicine in humans and animals, bioactive sponge can also be used to combat pests and diseases in fishery commodities amongst others against bacteria, fungi, viruses and biofouling often a problem in coastal aquaculture. According to Nakao, *et al*. (2003) that *Callyspongia truncata* (Class Demospongiae) containing polyacetylene compounds can be inactivated glucosidase. *Callyspongia* sp obtained from the Red Sea reported there were 6 new polyacetylene compounds that aikupikanynes A-F, but it is not known pharmacological activity (Alam, *et al*., 2003). Research that has been conducted by Yulianty, *et al*. (2011) to 14 sponge extracts collected from Barrang Lompo Island. All extracts of spons are active against the bacteria *S. aureus*, *S. typhii* and *E. coli* and the yeast *C. albicans* at the concentration of 1000 mg/ml. Results of this experiment indicate that the crude extract of 5 species of Callyspongia can inhibit the growth of bacterial isolates from seaweed *Eucheuma cottonii* suffering ice-ice disease and bacterial test *E. coli* and *S. aureus*. Besides the factors mentioned above, other factors that determine antimicrobial inhibitory effects, namely: (1) the population size of microorganisms. Antimicrobials will work more effectively on a smaller population compared with larger populations of microorganisms. In this study, the authors do dilution up to 1 million times, however, the initial population before dilution not necessarily the same as the result depends on the ability to grow in culture media. (2) The composition of the population.

Antimicrobial inhibition varies according to the nature of microorganisms, whether bacteria organisms (gram positive or gram negative), fungi or rickets. Results of this study showed that all of the bacteria found were gram negative, however, resistance to antimiroba not necessarily the same. (3) The concentration of antimicrobial. Concentration will result in a more concentrated IZD larger than the more dilute concentrations. If viewed from the type isolate, the *Acinetobacter* is relatively more vulnerable compared to other isolates. Vulnerability isolates can be seen from the inhibition zone diameter are wider compaired with other isolates. Results of this study indicate that the antibacterial compounds contained in the *Callyspongia biru* extract gives negative impact far greater compaired with other *Callyspongia*. Antibacterial mechanisms to bacteria can through inhibition of synthesis of proteins, synthesis of cell walls or other mechanisms. Through this inhibition mechanism, the growth of bacterial cells around sensi-disc dispencer inhibited so it formed a clear zone around sensi-disc dispencer without colonies growth.

Callyspongia Utilization Opportunities as a Bioprotector againts Ice-ice Disease on Seaweed

The results showed that all three species of Callyspongia has the potential to inhibit the growth of bacteria that cause ice-ice disease. It can be seen from the diameter of the inhibition zone formed when applied to the three types of disease-causing bacteria cause ice-ice disease and bacterial test (*E. col*i and *S. aureus*). Based on its potential as a source of antibacterial, so ability Callyspongia then can be used to control the ice-ice disease in seaweed. Callyspongia can be used as bioprotektor to protect seaweed from invasion ice-ice disease. In the natural habitat, Callyspongia always exposed by different types of microorganisms. To defend itself, *Callyspongia* form and release the antibacterial to the environment to protect themselves from microorganisms invasion. Self-protection mechanism of Callyspongia can be used to protect seaweed by planting seaweed side by side with Callyspongia. Bottom method and off bottom method most likely be used to adopt a self-defense mechanism of Callyspongia. In the farming location that naturally overgrown by Callyspongia and suitability of aspects suitable for seaweed cultivation, the seeds of seaweed can be sown in this location. This Cultivation method has weaknesses especially level production is lower and interference from grazer much higher. This drawback can be overcome by select the location that has a high brightness level so that the penetration of light is able to reach the seaweed. Encourage the presence of light to perform photosynthesis of seaweed so it does not interfere with normal

production levels. Disturbance of the seaweed grazer can be addressed by using net cages to prevent the entry of rabbitfish, sea urchins and sea turtles.

Conclusion

Based on the above results and discussion, it was concluded some of the following:

1. Three types of *Callyspongia* selected, bacterial isolates from seaweed and type of solvent, either individually or jointly significantly different effect on the inhibition zone diameter of antibacterial applications.
2. *Callyspongia biru* showed the most potent antibacterial activity compared with *Callyspongia subarmigera* and *Callyspongia monilata*
3. All three isolates of bacteria susceptible to antibacterial from *Callyspongia* with the highest vulnerability to bacterial isolates *Acinetobacter*.
4. Ethanol extracts showed inhibition zone diameter of greatest diameter compared with methanol extract and extract of diethyl ether.
5. Based on these results it can be used as an organism Callyspongia companion on seaweed cultivation to control ice-ice disease biologically.

Acknowledgment

Authors would like to thank the head of the laboratory and technicians of Biology and chemistry laboratory from Faculty of teacher training and education science of Nusa Cendana university and my family who have always supported me.

REFERENCES

Tokan, M.K. 2009. Morphology Characteistics of Microorganisme of the Seaweed (*Eucheuma spinosum)* Suffering of The Ice-Ice Disease. *Journal Biotropical Sains*. No. 2.Vol. 3: 18 – 25.

Tokan, M.K, dan Awang, Y. 2009. Controling of the Ice-Ice Disease Using The Macroalgae. PGRI University : Kupang

Tokan, M.K., Bhuja P dan Pani, Emil. 2010. The Control Strategy of The Ice-Ice Disesase of the Seaweed Trought Polyculture dan New Cultivar Intrducing .*UNWIRA* on Sikka District. Research Report: Kupang

Munro, John W. Bluntand Eric J. Dumdei. Sarah J.H. Hickford, Rachel E. Lill, Shangxiao Li, Christopher N. Battershill, Alan R. Duckworth 1999. The discovery and development of marine compounds with pharmaceutical potential. *Journal of Biotechnology* 70 1999, 15–25

Kelman, D., Yoel Kashman, Eugene Rosenberg, Micha Ilan, Ilan Ifrach, Yossi Loya. 2001. Antimicrobial activity of the reef sponge *Amphimedon viridis* from the Red Sea: evidence for selective toxicity. *Aquatic Microbial Ecology* Vol. 24: 9–16.

Soest, R.W.M. van, 1989. The Indonesian sponge fauna: A status report.— *Neth. J. Sea Res*. 23 (2): 223-230.

Newbold, Rochelle W., Paul R. Jensen, William Fenical, Joseph R. Pawlikl. 1999. Antimicrobial activity of Caribbean sponge extracts. *Aquatic Microbial Ecology* Vol. 19: 279-284

Imakulata, M.M. 2007. Biodiversity adn Antimicroba Contain of Porifera Callyspongia at Pasir Panjang Costal Zone of Kupang Gulf. Report Research Undana: Kupang.

Tokan, M.K. dan Lodo, 2008. He Effect of Callyspongia extract toward *Escherichia coli dan Staphylococcus aureus*. University of Nusa Cendana: Kupang

Tokan, M.K. 2006. The Characteristics of Microorganisms on Seaweed *(Eucheuma spinosum)* Suffering Ice-Ice Disease and Resintance toward *Antimicroba of Some Algae*. Thesis., Post Graduated Of Hassanuddin University: Makassar

Cappuccino, J.G. dan Sherman, N. 1983. *Microbiology:* A Laboratory Manual. Addison-Wesley Publishing Company, Reading Massachusetts.

Tüney, I., Çadirci, B.H., Ūnal, D., and Sutakar, A. 2006. Antimicrobial Activities of Extracts of Marine Algae from the Coast of Urla (Izmir, Turkey). *Turk J. Biol*. 30:171-175

Prescott, L.M., John P. Harley, and Donald Klein. 1990. *Microbiology*, WmC. Brown Publisher. Dubuque.

Aris, M. 2011. Identification, Pathogenicity of Bacteria and 16S-rRNA gene Emanfaatan For Ice-ice Disease Detection In Seaweed Cultivation (*Kappaphycus alvarezii*). Thesis. Not published. Bogor: Institute of Agriculture Graduate School Bogor.

Suryati, Emma, Andi Parenrengi, Rosmiati, Dan Asda Laining. 1999. Screening and Analysis Sponge Effective As Antibiofouling. In Pond And Keramba cage Marina Chimica Acta, Special Edition. October*: 16 – 20*

Nakao, Y., Uehara, T., Matsunaga, S., Fusetani, N., van Soest, R.W., Matunaga, S. 2003. Callysponginic Acid, A Polyacetylenic Acid which Inhibits Alpha Glucosidase, from The Marine Sponge Callyspongia truncata. *Journal of Natural Product*. 66(1). 156.

Alam, G., Adnan, A., Makhmud, A.I., Djide M.N., 2003, TLC Analysis- Bioautografi Antibacterial Compounds Methanol Extracts Callyspongia Sponge sp. Traditional Medicine magazine

Adriani, Alfian Noor, Abdul Rauf Patong, Maming., 2012. Analysis of Cu Metal Levels In Bacterial Symbiont OnSpons Callyspongia sp. *Marina Chimica Acta.* Vol 13 No 2. Hal.1-6.

Voogd, N.J. de. 2004. Callyspongia (Euplacella) biru spec. nov. (Porifera: Demospongiae: Haplosclerida) from Indonesia. Zool. Med. Leiden 78 (28), 31.xii. 2004: 477-483.

Yulianty, R. Herlina Rante, Gemini Alam Dan Akbar Tahir. 2011. Screening and Analysis of Antimicrobial Compounds Klt-Bioautografi Originally Some Extracts Sponges Sea waters Lompo Barrang Island, South Sulawesi. *Majalah Obat Tradisional, 16(2), 88 – 94.*

****