Squalen Bulletin of Marine & Fisheries Postharvest & Biotechnology, 8 (2), 2013, 87-94

Antibacterial Activity of Sea Cucumbers Harvested from Karimunjawa

Aktivitas Antibakteri Teripang yang dipanen dari Karimunjawa

Delianis Pringgenies*

Department of Marine Sciences, Faculty of Fishery and Marine Sciences, Universitas Diponegoro, Semarang
*Correspondence Author: pringennies@yahoo.com

Article history:
Received: 4 March 2013, revised: 10 July 2013, accepted: 10 July 2013

ABSTRACT

This research was conducted to discover bioactive compounds in sea cucumbers collected from Karimunjawa as anti-microbial agents against several pathogenic bacteria. Five sea cucumbers species used in this study were Teripang Gamat (*Stichopus variegatus*), Teripang Nanas (*Stichopus chloronotus*), Teripang Getah (*Bohadschia mamorata*), Teripang Emas (*Stichopus herrmanni*) and Teripang Babi (*Bohadschia argus*). Sea cucumbers extract were subjected to a series of anti-microbial tests using *Staphylococcus aureus*, *Escherichia coli*, *Vibrio anguila*, *Vibrio voinivica*, *Bacillus subtilis*, and *Pseudomonas* sp. Results showed that in the preliminary study, tissue extract of *Bohadschia mamorata* and *Bohadschia argus* were able to suppress the growth of all tested bacteria. Further study used these two sea cucumber extracts, and the results show that *Bohadschia mamorata* extract at 10 mg/ml showed the largest antibacterial (2.18 mm) towards the pathogenic bacterium *Pseudomonas* sp., while the smallest antibacterial zone (0.63 mm) was against the growth of *Bacillus subtilis*. At the concentration level of 20 mg/ml *Bohadschia argus* extract showed the highest antibacterial zone of 3.68 mm against *Staphylococcus aureus* and the lowest inhibition zone (1.75 mm) against *Pseudomonas* sp.

Keywords: sea cucumber, anti-bacterial, pathogenic bacteria

ABSTRAK

Penelitian ini bertujuan untuk mengetahui senyawa bioaktif antibakteri terbaik pada sampel teripang yang dikoleksi dari perairan Karimunjawa terhadap beberapa jenis bakteri pathogen. Ada lima jenis teripang yang digunakan, yaitu teripang Gamat (*Stichopus variegatus*), teripang Nanas (*Stichopus chloronotus*), teripang Getah (*Bohadschia mamorata*), teripang Emas (*Stichopus herrmanni*) dan teripang Babi (*Bohadschia argus*). Uji antibakteri dilakukan terhadap bakteri uji *Staphylococcus aureus*, *Escherichia coli*, *Vibrio anguila*, *Vibrio voinivica*, *Bacillus subtilis*, *Pseudomonas* sp. Penelitian pendahuluan dilakukan dengan menguji jaringan/daging teripang secara langsung terhadap pertumbuhan bakteri patogen penguji. Dari hasil penelitian pendahuluan terbaik, teripang dalam bentuk ekstrak kasar diuji. Hasil penelitian menunjukkan bahwa jaringan daging teripang getah *Bohadschia mamorata* dan teripang babi *Bohadschia argus* memperlihatkan kemampuan untuk menghambat pertumbuhan semua bakteri uji, yaitu *Staphylococcus aureus*, *Escherichia coli*, *Vibrio anguila*, *Vibrio voinivica*, *Bacillus subtilis*, *Pseudomonas sp*. Hasil uji lanjutan menunjukkan bahwa ekstrak teripang getah *Bohadschia mamorata* pada konsentrasi 10 mg/ml memiliki sifat antibakteri dengan diameter penghambatan terbesar (2,18 mm) terhadap bakteri pathogen *Pseudomonas* sp dan diameter zona hambatan terkecil (0,63 mm) terhadap bakteri pathogen *B. substilis.* Ekstrak kasar teripang babi *Bohadschia argus* pada konsentrasi 20 mg/ml menghasilkan diameter zona hambatan tertinggi (3,68 mm) terhadap bakteri pathogen *S. aureus* dan diameter zona hambatan terendah (1,75 mm) terhadap bakteri patogen *Pseudomonas* sp.

Kata Kunci: teripang, anti bakteri, bakteri patogen

Permalink/DOI: http://dx.doi.org/10.15578/squalen.v8i2.90

1. Introduction

Marine organisms have proven to be rich sources of interesting organic molecules. A great number of compounds with diverse structural features and sound biological activities have been reported in many studies. Among marine organisms, the compounds isolated from sea cucumbers are gaining more due to their bioactivities. Sea cucumbers are soft-bodied worm-like echinoderms which belong to the class Holothuroidea.

Pathogenic bacteria induced infections are some of the most prevalent health issues in Indonesia. Improper treatment of these health problems by unrestrained use of antibiotics has created new issues

of new antibiotic resistant. The developed resistance of bacteria against antibiotics is a prevalent issue in both communities and medical institutions throughout the world (Bax et al., 2001; Goldmann and Huskins, 1997; Tenover and Hughes, 1996; Cohen, 1994) which requires serious attention in the management of patients suffering from infection. The main cause of this problem is attributed to the overused of antibiotics by prescribers or patients (Essential Drugs Monitor, 2000). Reserved antibiotics which supposed to be administered to patients with a terrible case of infection are used on a regular basis. These results the shortage of antibiotics reserves to manage patients with infectious disease. On the other hand, research and development of new antibiotics require a massive amount of time and resources. Therefore, possibility of the return of pre-antibiotics era is imminent.

Sea cucumber is a species of marine life rich in chemical substances with promising pharmacological properties (Parulekar & Shrvoiker, 1991). Sea cucumber-derived bioactive compound, known as holotoxin, is a very potential anti-bacterial agent (Sudiro et al.,1993; Jawahar et al., 2002). The magnitude of inhibition of pure saponin was same as unsaponiable fraction of *Holothuria atra*. The results revealed the presence of antimicrobial steroidal sapogenins in the unsaponiable fraction of *H. atra.* Previous studies revealed the presence of antitumor, antifertility, ichthyotoxic, antiviral, immunomodulation, anti bacterial and anti fungal activities in Holothuria species. A more detailed research on the potential of bioactive compound in sea cucumber as an anti-bacterial agent is urgently needed.

The aims of this study were to obtain potent bioactive compounds derived from sea cucumbers from Karimunjawa, Indonesia.

2. Material and Methods

2.1. Sample Collection

Sea cucumber samples (size> 15 cm) were collected from the islands of Karimunjawa. Sample were cleaned from the dirt and soaked with fresh water for one night to remove salt and parasites that were attached to the body and then dried in a drying cabinet cucumbers in temperature < 40 °C. The samples were cut into small pieces for preliminary tests.

2.2. Preliminary Test

The preliminary test was conducted to discover whether samples of sea cucumber tissues possess biological activity when subjected to tested bacteria. The test used diffusion method (Conception *et al*, 1994), in which several Petri dishes were filled with 20 ml of Zobell 2216E agar medium. The prepared media was then inoculated with tested bacteria. As much as 1 gram of tissue pieces was put on the surface each of media. The prepared media was then incubated under 30 °C for 24 h. Positive result was obtained when a inhibition zone around the tissue sample was formed as a proof of biological activity of the secondary metabolites in the respective sea cucumber.

2.3. Sea Cucumber Extract

Sea cucumber extract were prepared according to Montano and Glorioso (1994). Each of the collected sea cucumber samples was cleaned and cut into 3-10 cm. The samples were then soaked in n-hexane solution at 1:5 ratios. The soaked sample was left under room temperature for 24 h and then filtered using filtering paper. The extract from the prepared samples was obtained by means of homogenization with hexane (non-polar) and 10% methanol in chloroform (polar) using a blender. Separation of filtrate from solution was accomplished by using rotary evaporator. The filtrate obtained was crude extract ready for further.

2.4. Isolation of Clinically Pathogenic Bacteria

Various clinical specimens (blood, urine, feces, etc) was obtained from Clinical Microbiology Laboratory, Karyadi Hospital/Faculty of Medicine UNDIP, Semarang. Bacteria obtained from the specimens were cultured using Nutrient Agar, Mac Conkey and Blood agar media. After 18-24 h of incubation, each bacteria colony on the media was screened based on their appearance. Advanced testing was conducted by means of API strip with GNB for Gram negative bacteria and API staph for *Staphylococcus*. Pure isolates were stored in Trypticase Soy Agar medium for further analysis.

2.5. Antibacterial Activity Test

Antibacterial test were carried out by the method of disk diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS). Muller Hinton (MH) media were prepared in a plate. The bacteria suspension was made in sterile saline to match the turbidity standard of 0.5 Mac Farland. Sea cucumber extract were spreaded on MH medium plate with a sterile cotton stick. Subsequent antibiotic disks was placed on the surface of the agar, and the plates were incubated at 37 ° C for 18-24 h. Inhibition zone formed in each antibiotic disc were measured with caliper (mm). Standard NCCLS was then used to interpret the results in the category susceptible/ sensitive, intermediate and resistant. Control strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 were used.

Table 1. Result of preliminary (qualitative) study

Species	*S. aureus*	*E. coli*	*Pseudomonas* sp	*V. voinivica*	*V. anguila*	*B. substilis*
S.variegatus	-	+	+	+	-	
S. chloronatus	+	+	+	+	-	-
S. herrmanni	+	+	+	+	-	-
B. argus	+	+	+	+	+	+
B. mamorata	+	+	+	+	+	+

3. Results and Discussion

3.1. Preliminary Antibacterial Test of Sea Cucumber Tissues

After being separated from the viscera, the tissue of sea cucumber was used in the preliminary study. Preliminary test showed that tissues from *Bohadschia mamorata* and *Bohadschia argus* have the ability to suppress the growth of all tested bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Vibrio anguila*, *Vibrio voinivica*, *Bacillus subtilis*, *Pseudomonas sp*). Tissue of *Stichopus variegatus* was able to suppress the growth of *Escherichia coli, Vibrio voinivica,* and *Pseudomonas sp* while tissue sample from *Stichopus chloronatus* showed the ability to hamper the growth of *Escherichia coli, Pseudomonas* sp, *Vibrio voinivica* and *Staphylococcus aureus.* Table 1 gives a resume of the preliminary test results.

Further quantitative experiment used *Bohadschia mamorata* and 'pigy-sea cucumber' *Bohadschia argus* which were relatively easy to be collected at Karimunjawa islands during the west monsoon.

3.2. Antibacterial Test of Bohadschia mamorata *Extract Against Pathogenic Bacteria*

Result of the antibacterial test of *Bohadschia mamorata* extracts are shown in Figure 1, which revealed that crude extract of *Bohadschia mamorata* posses positive antibacterial activity. Increasing exctract concentration treatment showed an increasing antibacterial activity up to a certain concentration, then decreasing. Antibacterial test of *Bohadschia mamorata* at concentration of 10 mg/ml had resulted on the formation of the largest diameter of the antibacterial activity zone (2.18 mm) against pathogenic bacteria of *Pseudomonas* sp. and the smallest antibacterial activity zone (0.63 mm) against pathogenic bacteria *B. substilis* (Figure 1). Burgess et al. (1999) and Radjasa et al. (2007) reported that a secondary metabolites had an antibacterial activity if it has the anti-bacterial diameter activity zone larger than 1 mm from the size of standard paper disc (8 mm).The antibacterial activity of the crude exctract in several concentrations after 3-days incubation periode were shown in Figure 2, and tend to decrease after 2-days incubation.

The antibacterial activity decreased and was followed by the re-growths of the bacteria. Hence, it may be assumed that *Bohadschia mamorata* act as a bacteriostatic, meaning that the biocative compound had a temporary antibacterial capability. So that with the absence of the bioactive compound, the pathogenic bacteria will grow again. Surprisingly at concentration treatment of 10 mg/ml with tested bacteria *Pseudomonas* sp. had the bactericidal capability, i.e showing antibacterial capability even in the absence of the bioactive compound, and the pathogenic bacteria could not grow again.

In order to understand the antibacterial capability of the bioactive compound from *Bohadschia argus,* a comparison test with an antibiotic would be needed. An antibiotic compound was known as a bactericidal or a bacteriostatic mechanism. In this study an Amoxylin antibiotic was used as control. Chemical stucture of Amoxylin was $C_{16}H_{19}N_3O_5S$ atau *(2S, 5R, 6R)-6-[(R)-2-amino-2-(4-hydroxyphenyl)* (Styrer, 1988).

Based on the result as in Figure 4, the antibacterial activity of the sea cucumber extract was better than the Amoxylin antibiotic for all bacterial groups especially in days 3 for *E. coli*.

3.3. Antibacterial Test of Bohadschia Argus *Extract Against Pathogenic Bacteria*

Figure 5 shows the result of antibacterial activity of *Bohadschia argus* crude extract, indicating that increasing concentration of the exctract would be increasing of the antibacterial activity up to a certain concentration, then start to decreasing. At concentration of 20 mg/ml of *Bohadschia argus* extract, the antibacterial activity showed the biggest inhibition against *S. aureus* forming 11.68 mm diameter zone and the smallest inhibition was against *Pseudomonas* sp (9.75 mm). However, the

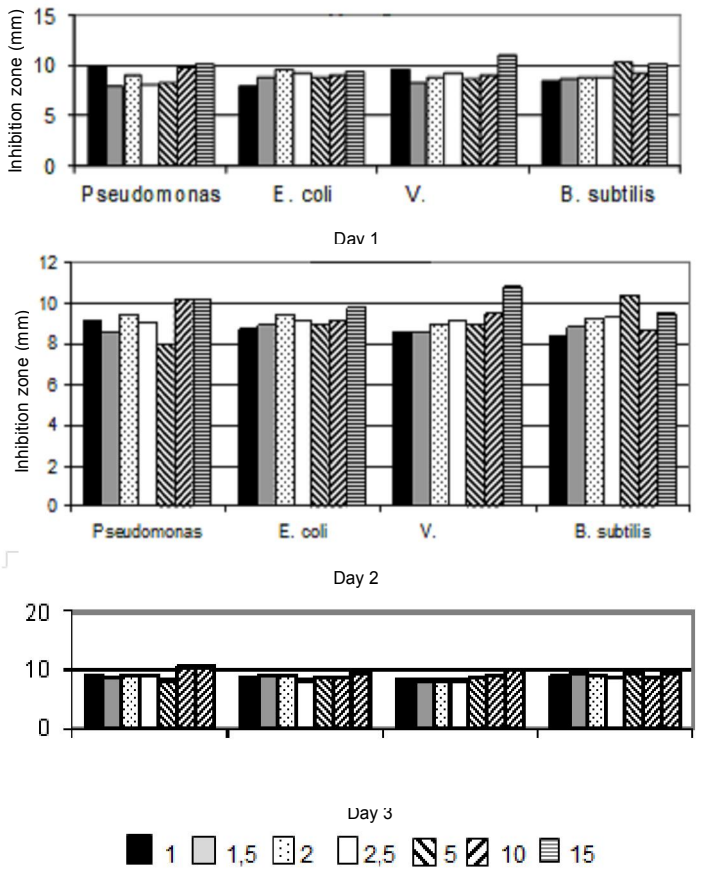

Figure 1. Results of the quantitative test of *Bohadschia mamorata* extract against pathogenic bacteria *Pseudomonas* sp, *E. coli, V. voinivica* and *B. subtilis*

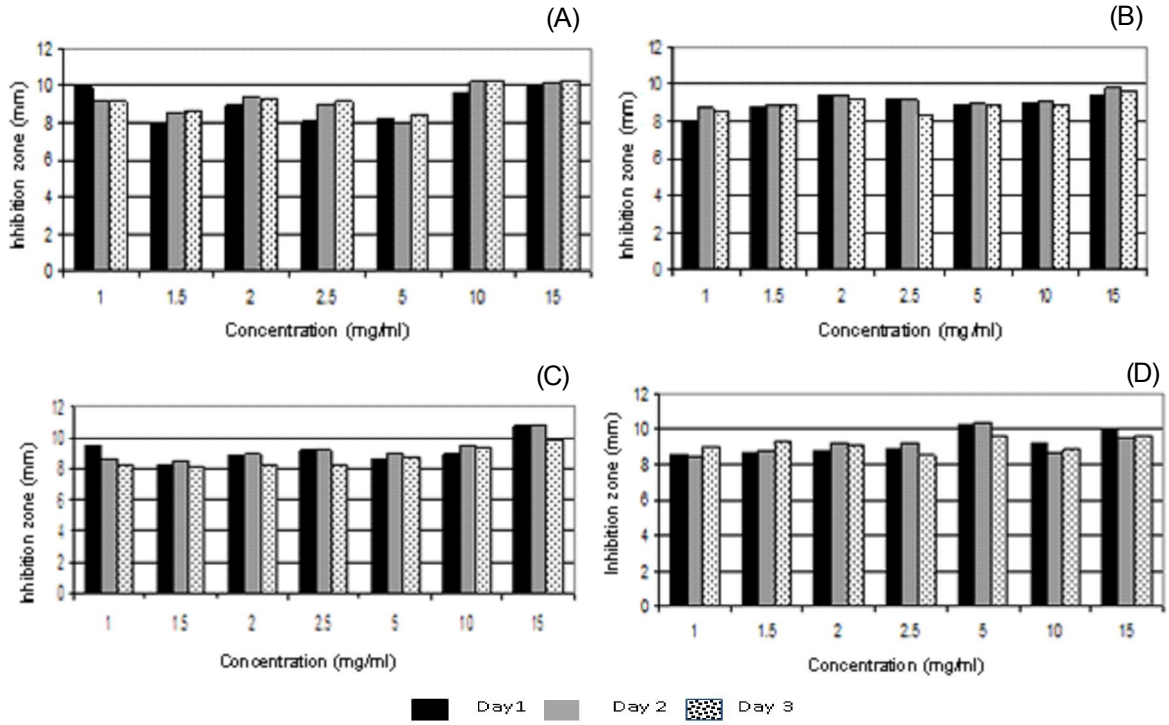

Figure 2. Results of the quantitative test of *Bohadschia argus* extract against *Pseudomonas* sp (A), *E.coli* (B), *V.voinivica* (C) and *B. subtilis* (D).

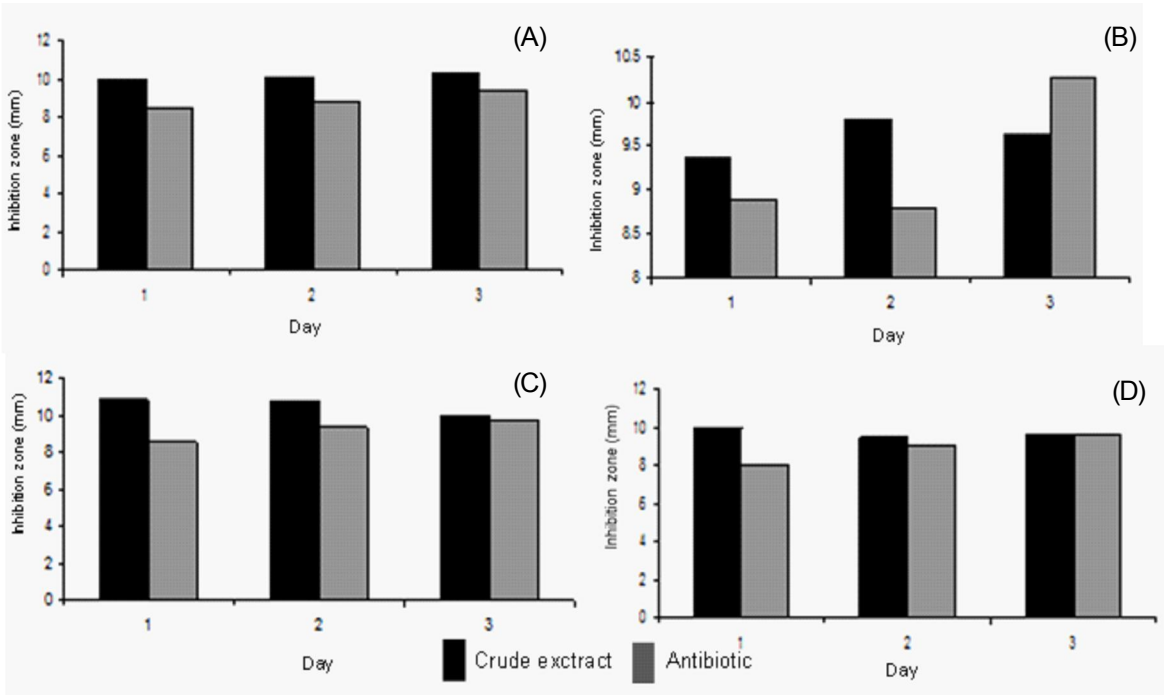

Figure 3. Antibacterial test of *Bohadschia marmorata* exctract against *Pseudomonas* sp. (A), *E. coli* (B), V. voinivica (C) and *B. substilis* (D)

antibacterial capability of the crude extract tend to decrease after 2-days incubation as shown in Figure 4.

Diameter of the antibacterial activity will decrease and followed by the re-growths of bacteria at the transparent zone of the antibacterial activity zone as happened with *Bohadschia mamorata* extract which is assumed that the extract act as a bacteriostatic, meaning that the bioactive compound had a temporary antibacterial capability. So that with the absence of the bioactive compound, the pathogenic bacteria will grow again. However, extract treatment of 30 mg/ml had shown the bactericidal capability against *Pseudomonas* sp.

Figure 6 indicates that the antibacterial extract of the sea cucumber *Bohadschia argus* was better than Amoxylin antibiotic especially for *S.aureus and Pseudomonas*. The extract forms smaller inhibition zone compared to the control antibiotics Amoxylin during the three days incubation period when tested against *Escherichia coli.*

Infectious diseases caused by pathogenic bacteria become one health problem in Indonesia. Treatment of the disease by judicious use of antibiotics has led to a new problem with the development of strains resistant to antibiotics. Bacteria resistance to antibiotics is a worldwide problem either in the hospital or in the community (Bax *et al*, 2001) which requires seriousness in the management of patients with infectious diseases (World Health Organization. 2001). This is because the use of antibiotics / antimicrobials that are not rational / wise by prescribers (prescribing doctors) or the patients themselves. Based on the facts above, then it should be pursued efforts to obtain new sources of antibiotics that are capable of handling-bacteria pathogen.

Pelczar & Chan (1988) explain that the concentration at which antibiotics is administered is inversely proportional with the time needed to kill the disease causing bacteria. In other words, more dose used of antibiotics administered, less time is required to kill the bacteria. Purbowatiningrum and Mulyani (2006) suspect that bacterial activity is increasing up to certain concentration of antibiotics administration, after which maximum resistance is reached and its activity tend to be constant. This phenomenon is caused by the maximum limit at which the antibiotics resistance capability reaches. Therefore, sea cucumber extract may be proven to be a powerful antibiotic agent. In contrary, Mokhlesi et al. (2012) reported that, not all sea cucumbers could serve as an anti-bacterial agents. Numerous chemical and pharmacological studies carried out on several species of sea cucumbers, indicated that these invertebrate contain triterpene glycoside with antifungal, anti bacterial and cytotoxic properties. Other species of sea cucumber have been examined for antibacterial activities but the results were different.

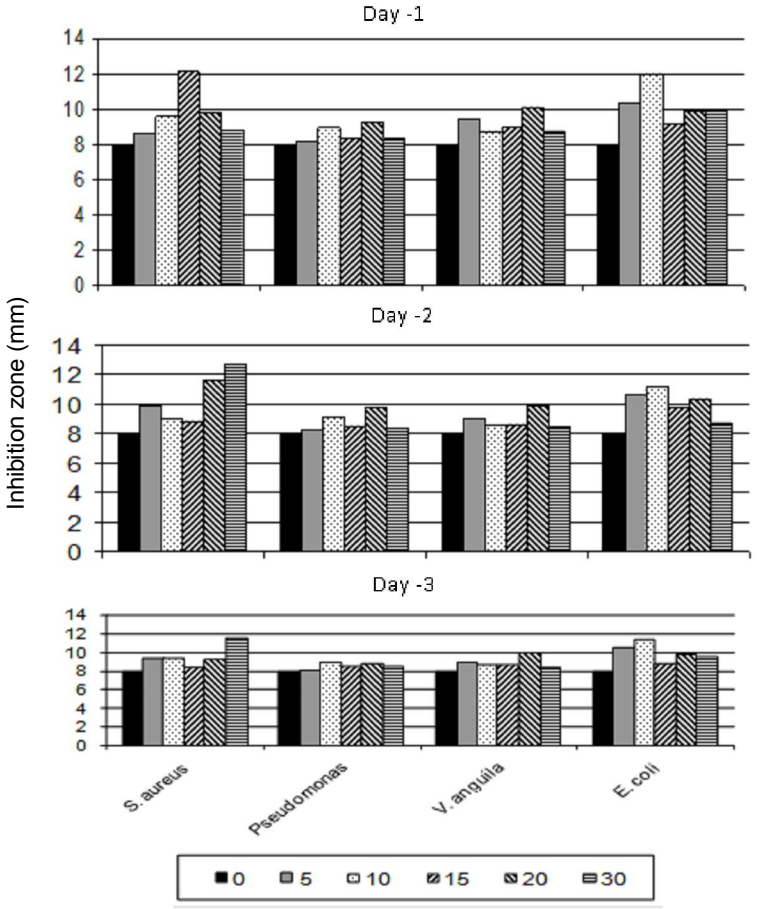

Figure 4. Results of the quantitative test of *Bohadschia argus* extract against *Pseudomonas* sp, *E.coli, V.anguila* and *B. subtilis.*

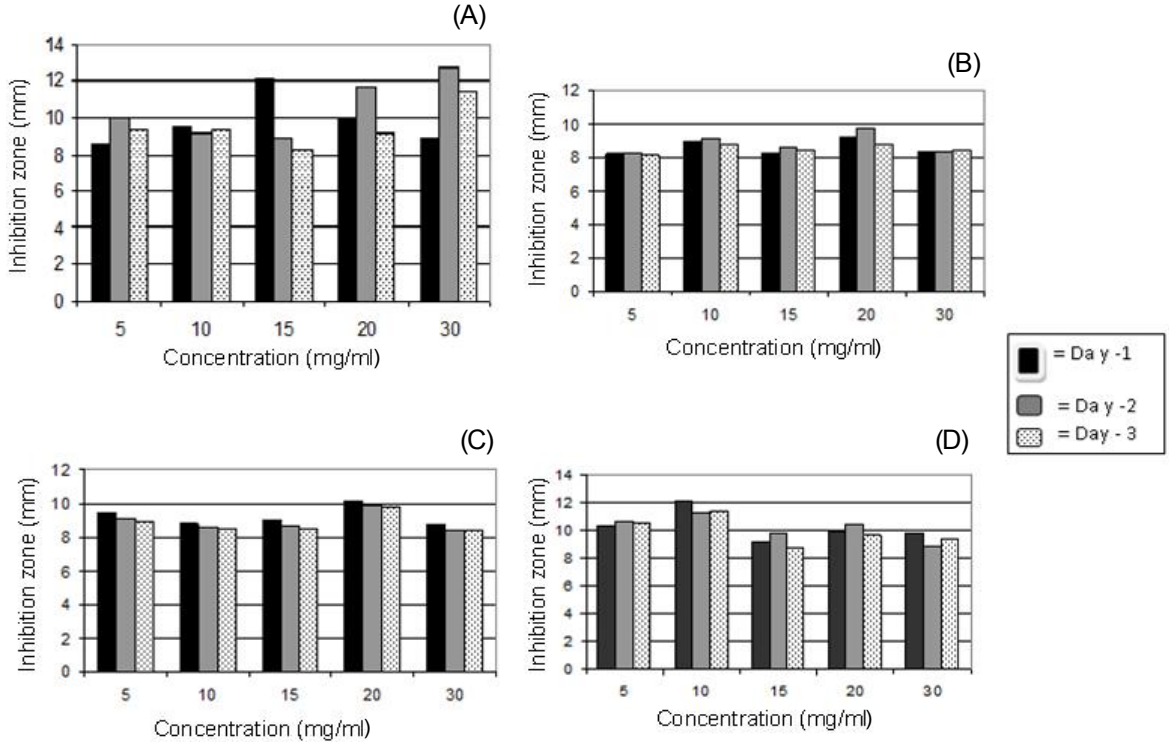

Figure 5. Results of antibacterial activity against pathogenic bacteria during 3 days incubation period

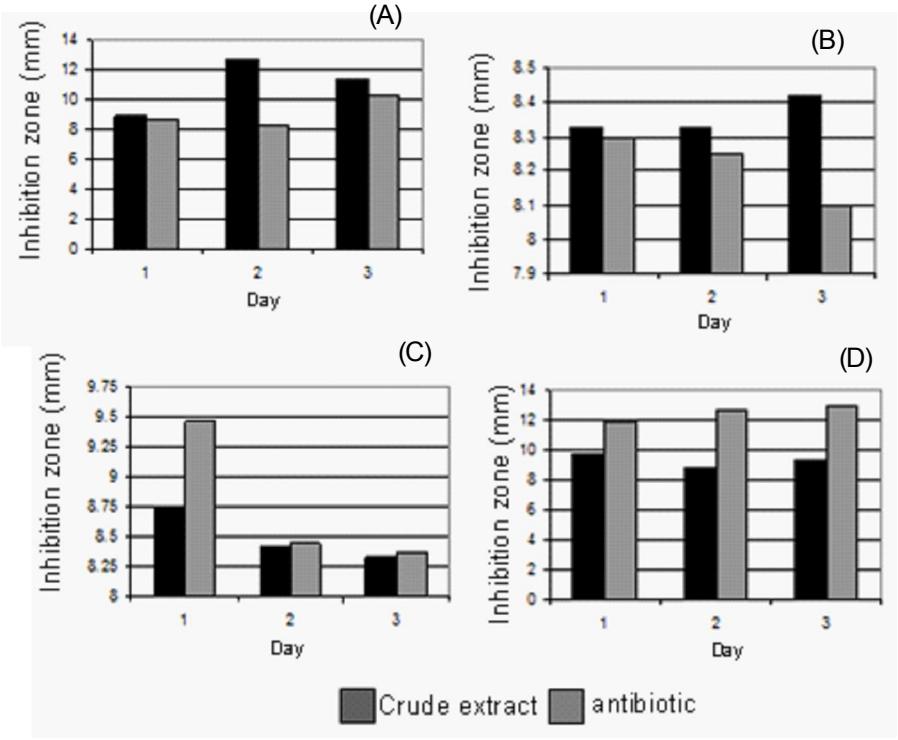

Figure 6. Sensitivity test of the *Bohadschia argus* crude extract for the growth of *S.aureus* (A), *Pseudomonas* (B), *V anguilla* (C) *E.coli* (D)

Kuznetsova et al. (1982) reported the evaluation of *H. atra*, *H. scabra* and *Bohadschia argus* against seven species of bacteria and found that lipid and methanolic extracts have no inhibitory activity, while a phosphate buffered saline extract showed inhibitory activity. Villasin and Pomory (2000) showed that the extract of *Parastichopus parvimensis* did not inhibit bacteria compared to tetracyline and Ampicillin. Interestingly, Gowda et al. (2000) showed that *H. scabra* have strong broad spectrum antibacterial activity against both Gram positive and Gram negative because of T-antigen binding lectin with anti bacteria. Farouk et al. (2007) reported new bacterial species isolated from Malaysian sea cucumbers with optimized secreted antibacterial activity.

4. Conclusion

Of five sea cucumbers species used in this study, i.e Teripang Gamat (*Stichopus variegatus*), Teripang Nanas (*Stichopus chloronotus*), Teripang Getah (*Bohadschia mamorata*), Teripang Emas (*Stichopus herrmanni*) and Teripang Babi (*Bohadschia argus*), preliminary study showed that only *Bohadschia mamorata* and *Bohadschia argus* were qualitatively having antibacterial properties against all tested bacteria.

Bohadschia mamorata extract at 10 mg/ml showed the largest antibacterial (2.18 mm) activity against *Pseudomonas* sp. and the smallest antibacterial zone (0.63 mm) againts *Bacillus subtilis*.

Bohadschia argus extract at the concentration level of 20 mg/ml showed highest antibacterial activity (3.68 mm) against *Staphylococcus aureus* and the smallest inhibition activity (1.75 mm) against *Pseudomonas sp*.

References

Bax, R., Bywater, R., Cornaglia, G., Goossens, H., Hunter, P., Isham, V., & Jones, R. (2001). Surveilellance of antimicrobiol resistance-ehat, how and whither. *Clin. Microbiol. Infect.* 7, 316-325.

Burgess, J. G., E. M. Jordan, M. Bregu, & A. Mearns-spragg. 1999. Microbial antagonism: a neglected avenue of natural products research. *J. Biotechnology*. 70, 27-32.

Cohen. (1994) . Tests for detecting herpes simplex virus and varicella-zoster virus infections. Department of Dermatology, University of Texas-Houston Medical School. *PubMed. Jan*; 12(1), 51-68.

Conception, G. P., Caraan, G. B., & Lazaro, J. E. (1994). Biological Assays for Screening of Marine Samples. In: *Workbook Second Natural Products Workshop: Strategies in the Quest for Novel Bioactive Compounds from the Sea.* Marine Science Institute

and Institute of Chemistry, University of Philippines. Deliman, Quezon City, Philippines.

Essential Drugs Monitor. (2000). World Health Organization. No. 28/29. p. 1.

Farouk, A. A., Ghouse, F. A. H., & Ridzwan, B. H. (2007). New bacterial species isolated from Malaysian sea cucumbers with optimized secreted antibacterial activity. *Am. J. Biochem. Biotechnol.* 3, 60- 65.

Gowda, N. M., Goswami, U., & Khan, M. I. (2008). T. Antigen binding lectin with antibacterial activity from marine invertebrate, sea cucumber (*Holothuria scabra*); Possible involvement in differential recognition of bacterial. *J. Invertebrate Pathol.* 99, 141-145.

Goldmann, D. A. & Huskins, W. C. (1997). Control of nosocomial antimicrobialresitant bacteria: A strategy priority for hospitals worldwide. *Clin. Infec. Dis.* 24, 139-145.

Jawahar, Abraham, T., Nagarajan, J., & Shanmugan, S.A. (2002). Antimicrobial substances of potential biomedical importance from Holothurian sp. *Indian Journal of Marine Science.* 31(2). June 2001. 161 - 164

Kuznetsova, T. A., Anisimov, M. M., Popov, A. M., Baranova, S. I., & Afiyatullov, S. H. (1982). A comparative study in vitro of physiological activity of triterpene glycosides of marine invertebrates of echinoderm type. *Comp. Physiol.* 73, 41-43

Montano, N. E. & Glorioso, B. A. (1994). Extraction and isolation of Marine Natural products. In: *Work book second natural workshop: Strategies in the quest for Novel Bioactive Compound from the sea.* Marine Science institute and Institute of Chemistry. University of the Philippines. Diliman, Quezon city. Philippines

Mokhlesi, A., Soodabeh, Saeidnia, Ahmad, R. G. I., Ahmad R. S., Ali, N., Farzanah, F., Reza, K., & Nasrin Es'haghi. (2012). Biological activities of the sea cucumber holothuria leucospilota. *Asian Journal of Animal and Veterinary Advanced.* 7(3), 243-249.

Parulekar, A.H. & Sirvoiker, P. (1990). Bioactive Compound from Marine Organisms, Bioactivity and Chemical Ecology of Some Intertidal Animals. An Indo-United States Symposium. A.A. Balkema. Rotterdam. 29-36.

Pelczar, M.J. & Chan, E. C. S. (1988). Dasar-dasar Mikrobiologi Jilid 1, UI Press, Jakarta.

Purbowatiningrum & Mulyani (2006). Uji Aktivitas Antibakteri Rimpang Temu Putih (*Curcuma Mangga* Vali) (Laporan Penelitian). Fakultas Matematika dan Ilmu Pengetahuan Alam. Universitas Diponegoro. Semarang. Laporan Kegiatan. 47 p.

Stryer, L. (1988). Biochemistry, 3rd ed. W. H. Freeman & Co., New York, N.Y.

Sudiro, I. (1998). Marine Conservation and Natural Products Utilization Prospects in the Field of Health and Cosmetics *in* Soemadihardjo *et al*., (Ed.). Proceedings of the Seminar on Marine Biotechnology LIPI Indonesia I. Jakarta.

Tenover, F. C. & Hughes, J. M. (1996a). The challenges of emerging infectious diseases. Development and spread of methicillin resistant bacterial pathogens, *J. Am. Med. Assoc*. 275: 300-304.

Villasin, J. and Pomory, C.M. (2006). Antibacterial activity of extracts from the body wall of *Parastichopus parvimensis* (Echinodermata: Holothuroidea): Fish Shell fish. Immurol- 10: 465-467.

WHO. (2001). Antimicrobial resistant in Neisseria gonorihoeae. Departement of Communicable Disease Surveillance and Response. p. 65