

COMPARATIVE EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SOFT CORALS COLLECTED FROM THE WATER OF PANGGANG ISLAND, KEPULAUAN SERIBU

Iriani Setyaningsih, Tati Nurhayati, Roni Nugraha* and Indra Gunawan

Department of Aquatic Product Technology, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia.

Received: 3 May 2012; Revised: 15 May 2012; Accepted: 27 May 2012; Available online: 5 June 2012

ABSTRACT

The need for new antibiotic compounds to overcome the resistance of pathogenic bacteria on existing antibiotics is increasing at this time. Soft corals are a rich source of bioactive compounds, have biological activity such as antifungal, cytotoxic, antimicrobial, anti-inflammatory, etc. This study evaluated antibacterial activity of soft corals collected from the waters of Panggang Island, Kepulauan Seribu. Five collected soft corals, namely *Sinularia* sp., *Sarcophyton* sp., *Xenia* sp., *Dendronephthya* sp., and *Nephthea* sp. were extracted with the methanol, ethyl acetate and hexane. Antibacterial activity of soft corals was determined by disc diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, and *Vibrio harveyi*. The result showed that extract of soft corals exhibited antibacterial activity on one or more tested bacteria. Soft coral *Sinularia* sp. produced the highest inhibitory zone, while *Xenia* sp and *Sarcophyton* sp. produced the weakest antibacterial activity. Methanol extracts had the highest antibacterial activity on all tested soft corals, with maximum activity against *Vibrio harveyi*. The results of this study indicate that the methanol extract of the soft coral *Sinularia* sp. has potential as a source of antibacterial compounds.

Keywords: Soft corals; antibacterial; disc diffusion; methanol extract; inhibition zone.

INTRODUCTION

The need for new antibiotic compounds to overcome the resistance of pathogenic bacteria on existing antibiotics is increasing at this time. Various types of pathogenic bacteria resistance against antibiotics increase a serious threat to the healing of infectious diseases, especially in patients who are undergoing immunity treatment. Research on the discovery of new drugs from marine organisms are currently being developed, such as from a soft coral¹, sponge², gorgonian³, actinomycetes⁴ and other marine organisms since it was introduced for the first times in 1960 with conference entitled "Biochemistry and Pharmacology Derived from Marine Organisms" organized by the New York Academy of Sciences.

Soft corals (Cnidaria: Octocorallia: Alcyonacea: Alcyoniina) are animals that live in low tidal areas to depths of 200 meters, which is the main component of coral reef ecosystems. Soft corals are distributed mainly in the Indo-Pacific region, with centers of diversity in Indonesia, Malaysia and the Philippines.⁵ The ability of soft corals to adapt is influenced by the presence of various secondary metabolites components under high concentration produced by the body.⁶ Soft corals are a rich source of bioactive compounds such as prostaglandins, cembrene diterpenoids, sesquiterpenoids and sterols.⁷ Several active compounds have biological activity such as cytotoxic^{8,9}, antitumor compounds¹⁰, anticancer¹¹ and antibacterial¹²,

some are in preclinical and clinical trials¹³. Soft coral contain various terpenes compounds and not like a sponge, these compounds can be synthesized de novo. However, the content of secondary metabolites as bioactive compounds and their ability not equally shared by all types of soft corals. Formation of chemical compounds in the body of soft coral is affected by predation, space competition, reproduction and environmental stress.¹⁴ This study aimed to identify potential soft coral collected from waters of Panggang Island, as a source of antibacterial compound.

MATERIAL AND METHODS

Collection and characterization

Five samples of soft corals were collected by Scuba Diving from waters of Panggang Island at a depth of 2-14 meters. Samples were transported to laboratory of Aquatic Product Technology Department, then characterized and identified on morphologic similarity based on Manuputty, 2002.¹⁵

Consecutive extraction of antibacterial compound of different polarity

Antibacterial component from soft coral were extracted by gradual extraction method based on Nurhayati *et al*, 2010¹⁶ using three solvents with different polarity, i.e methanol; ethyl acetate and hexane. The soft corals (100 g) was crushed and soaked in 200 mL of methanol for 24 hours at room temperature. The mixture was then filtered through Whatman no 1 filter paper and the residue were extracted with ethyl acetate and hexane respectively in the same way. All of three solutions were evaporated and dried under vacuum (below 40 °C), to yield the methanol,

*Corresponding Author:

Roni Nugraha
Lecturer, Department of Aquatic Product Technology,
Faculty of Fisheries and Marine Science, Bogor Agricultural University,
Bogor, Indonesia.
Contact no: +62 81316909654; Email: nugraha.thp@gmail.com

ethyl acetate, and hexane extracts, respectively.

Antibacterial activity assay

Antibacterial activity assays were performed with the agar diffusion method (Kirby-Bauer). Lyophilised extracts were impregnated on to sterile filter paper disc and placed on to the Mueller-Hinton agars which were previously swabbed with *E. coli*, *P. aeruginosa*, *A. hydrophila*, and *V. harveyi*. Chloramphenicol was used as positive control. All the plates were incubated overnight at 37 °C under static conditions. Antibacterial activities were observed from inhibition zone formed around the paper disc.

RESULTS AND DISCUSSION

Collection and characterization

Panggang Island waters are still pretty good location, this is due to the closure of coral reefs in the category of moderate to good (from 34.72 to 62.68%) with a diversity index ranged from 0.2 to 2.81%. Soft corals that were collected are shown in figure 1. The soft corals were identified as *Sinularia* sp., *Sarcophyton* sp., *Xenia* sp., *Dendronephthya* sp. and *Nephthea* sp based on the shape and morphology.

Antibacterial Activity Assay

Table 1 show results of antibacterial activity assay from soft coral extract against four bacterial cultures at a

Table 1. Antibacterial activity of soft corals

Soft coral	Solvent	Yield (g)	inhibition zone for (mm)			
			<i>E. Coli</i>	<i>P. Aeruginosa</i>	<i>v. harveyi</i>	<i>a. Hydrophilia</i>
<i>Sinularia</i> sp	Methanol	1,2	2	5	6	1,5
	Ethyl acetate	1,1	2	NZ	6	4
	hexane	0,24	1,5	NZ	4	2
<i>Nephthea</i>	Methanol	0,64	NZ	1,5	NZ	1,5
	Ethyl acetate	1,6	NZ	1,5	NZ	1,5
	hexane	0,695	NZ	1	NZ	NZ
<i>Dendronephthya</i>	Methanol	0,55	1,5	NZ	NZ	NZ
	Ethyl acetate	1,01	1	NZ	NZ	NZ
	hexane	0,15	1	NZ	NZ	NZ
<i>Xenia</i> sp	Methanol	1,7	1	NZ	NZ	NZ
	Ethyl acetate	0,68	0,5	NZ	NZ	NZ
	hexane	0,285	1	NZ	NZ	NZ
<i>Sarcophyton</i> sp	Methanol	1,71	1	NZ	NZ	NZ
	Ethyl acetate	0,845	0,5	NZ	NZ	NZ
	hexane	0,28	1	NZ	NZ	NZ
Chloramphenicol (Control)			11	10	13	10

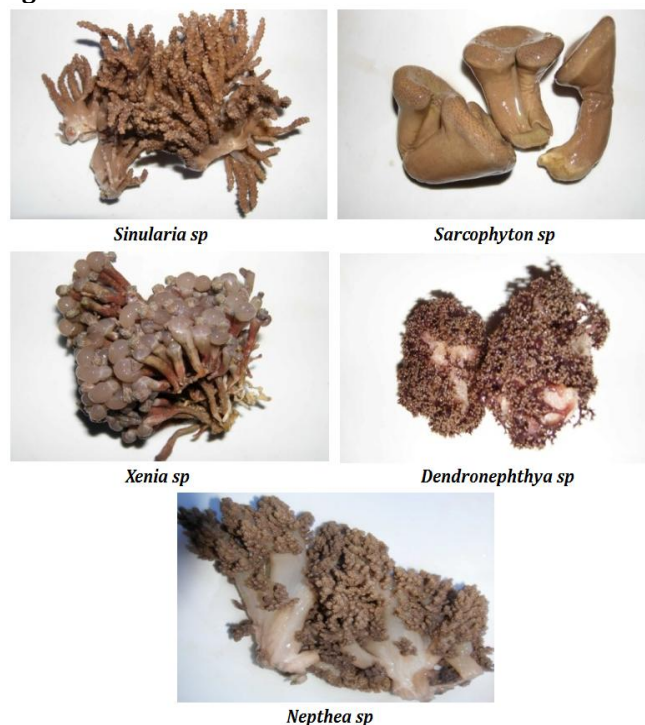
NZ: No inhibition zone detected

Here, we report the in-vitro result of antibacterial assays of five soft corals in methanol, ethyl acetate and hexane on selected bacterial cultures (Table 1). Extracts of soft corals have different antibacterial activity against all bacterial cultures. Extract of soft coral *Sinularia* sp has the most effective inhibitory activity compared to other soft corals which inhibited all bacterial tests, while *Nephthea*, *Xenia* sp and *Sarcophyton* sp have low antibacterial activity which only inhibited *E. coli* culture. Similar results were also obtained by Devi *et al*¹⁷, who found that the genus *Sinularia* produce antibacterial compounds that are stronger than with other soft coral genus. The antibacterial compound of *Sinularia* sp has strong activity against *V. harveyi*. *Sinularia* is one type of soft coral that has been studied extensively as a producer of bioactive compounds. Khalesi *et al*¹⁸ reported that from 30-year period there is more than 70 species of *Sinularia* which have been tested for their chemical compounds and more than 60% of the soft coral *Sinularia* contain terpenoid compounds.

The results also showed that methanol extracts had higher

concentration of 300 µg/disc.

Figure 1. Soft corals collected



antibacterial activity than other solvents. Methanol extracts of the five types of soft corals could inhibit one or more bacterial cultures with inhibition zones larger than the other solvent. Similar result were obtained by Darah *et al*¹⁹ that found methanol extracts of soft corals *Haliclona* sp. have antibacterial activity to some types of gram-positive and gram negative. The use of different types of solvents may result in different activities due to differences in the polarity of each solvent.²⁰ Antimicrobial activity of the methanolic extract appeared to be more effective than ethyl acetate and hexane extracts, since methanol could extract a wide variety of active components compared to than ethyl acetate and hexane.

Antibacterial activity in this study was determined using disc diffusion method. This method has the ability to rapidly identify active metabolites contained in the extract and can be used as an initial screening of antibacterial activity. However, this method greatly depends on the rate of diffusion of chemical compounds in order to extract that has a small diffusion rate, despite having a potentially active compounds, will produce a small test activity.²¹

Thus, some soft corals that do not produce resistance zone, does not indicate the absence of antibacterial compounds.

CONCLUSION

This study showed that some of soft corals have a potential as a source of antibacterial compound. Soft coral

REFERENCES

1. Sulistiyani Nugraheni S A, Radjasa O K, Sabdono A, Khoeri M M; Antibacterial Activities of Bacterial Symbionts of Soft Coral *Sinularia* Sp. Against Tuberculosis Bacteria. *Journal of Coastal Development*. 2010; 14(1):45-50.
2. Dhinakaran D I, Manohari V, Atchya B, Tamilselvi K, Lipton A P; Antifungal and Cytotoxic Activities of Some Marine Sponges Collected from the South East Coast of India. *World Journal of Fish and Marine Sciences*. 2012; 4(2):155-158.
3. Koh L L, Tan T K, Chou L M, Goh N K C; Antifungal properties of Singapore gorgonians: a preliminary study. *Journal of Experimental Marine Biology and Ecology*. 2002; 273:121-130.
4. Athoor R S R, Janardhan A; Evaluation of anti-microbial and anti-cancer activity of secondary metabolites from marine actinomycetes. *Journal of Pharmacy Research*. 2012; 5(1):391-393.
5. Fabricius K, De'ath; Biodiversity of the Great Barrier Reef: Large-Scale Patterns and Turbidity-Related Local Loss of Soft Coral Taxa. In: Wolanski E, eds. *Oceanographic Processes of Coral Reefs: Physical and Biological Links in the Great Barrier Reef*. CRC Press, Boca Raton. 2001; 127.
6. Sammarco P W, Coll J C; Chemical adaptations in the Octocorallia: evolutionary consideration. *Mar Ecol Prog Ser*. 1992; 88:93-104.
7. Changyun W, Haiyan L, Changlun S, Yanan W, Liang L, Huashi G; Chemical defensive substances of soft corals and gorgonians. *Acta Ecologica Sinica*. 2008; 28(5):2320-2328.
8. Suh J H, Lin F Y, Huang H C, Dai C F, Wu Y C, Hu W P, Hsu C H, Sheu J H; Novel steroids from the soft coral *Nephthea chabrolii*. *Tetrahedron*. 2006; 63:703-707.
9. Duh C Y, Lu I W, Wang S K, Dai C F; New cytotoxic steroids from the soft coral *Clavularia viridis*. *Steroids*. 2007; 72:573-579.
10. Watanabe K, Iwashima M, Iguchi K; New bioactive marine steroids from the Okinawan soft coral *Clavularia viridis*. *Steroids*. 1996; 61:439-446.
11. Ahmed A, Dai C F, Kuo Y H, Shen J H; 1 α , 3 β , 5 β -trihydroxy-24-methylene-cholestan-6-one a novel steroid from a soft coral *Sinularia gibberosa*. *Steroids*. 2003; 68:377-381.
12. Shnit-Orland M, Kushmaro A; Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiol Ecol*. 2009; 67:371-380.
13. Newman D J, Cragg G M; Marine natural products and related compounds in clinical and advanced preclinical trials. *J Nat Prod*. 2004; 67(8):1216-38.
14. Geffen Y, Ron E Z, Rosenberg E; Regulation of release of antibacterials from stressed scleractinian corals. *FEMS Microbiol Lett*. 2009; 295:103-109.
15. Manuputty A E W. *Karang lunak (Soft Coral) Perairan Indonesia*. Puslitbang Oseanologi-LIPI, Jakarta, 2002.
16. Nurhayati T, Fikri D, Desniar; Aktivitas Inhibitor Protease dari Ekstrak Karang Lunak, Asal Perairan Pulau Panggang Kepulauan Seribu. *Indonesian Journal of Marine Sciences*. 2010; 15(2):59-65.
17. Devi P, Wahidulla S, Kamat T, D'Souza L; Screening marine organism for antimicrobial activity against clinical pathogens. *Indian Journal of Geo-Marine Science*. 2011; 40(3):338-346.
18. Khalesi M K, Beeftink R K, Wijffels R N; The soft coral *Sinularia flexibilis*: potential for drug development, In: Leewis RJ, Janse M, eds. *Advances in Coral Husbandry in Public Aquariums*. Burgers' Zoo, Arnhem, the Netherlands. 2008; 47-60.
19. Darah I, Lim C L, Nurul A Z, Noor A S, Shaida F S; Effects of methanolic extract of a soft sponge, *Haliclona* sp. on bacterial cells: structural degeneration study. *International Journal of Comprehensive Pharmacy*. 2011; 7(03):1-6.
20. Tian F, Li B, Ji B, Yang J, Zhang G, Chen Y, Luo Y; Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*: The polarity affects the bioactivities. *Food Chemistry*. 2009; 113:173-179.
21. Kelman D, Kashman Y, Hill R T, Rosenberg E, Loya Y; Chemical warfare in the sea: The search for antibiotics from Red Sea corals and sponges. *Pure Appl Chem*. 2009; 81(6):1113-1121.