IJIRR

International Journal of Information Research and Review
Vol. 03, Issue, 12, pp. 3304-3308, December, 2016

Research Article

IMPACT ON INCREASING ANTHROPOGENIC PRESSURE TO THE VARIABILITY OF CEMBRANOID COMPOUNDS DERIVED OF SOFTCORAL *SINULARIASP* FROM MANADO THE WATERS AND THEIR ANTITUMOR ACTIVITY

[1,*]Nickson J. Kawung, [1]Remy E.P. Mangindaan, [1]Rizald M. Rompas, [2]Ekowati Chasanah, [2]Hedi I. Januar, [2]Dewi Fajarningsih and [3]Adolfina Sumangando

[1]Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado North Sulawesi
[2]Indonesian Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, Jl. KS Tubun Petamburan VI, Jakarta 10260
[3]Christian Indonesia Tomohon University

ARTICLE INFO

Article History:

Received 18th September, 2016
Received in revised form
22nd October, 2016
Accepted 24th November, 2016
Published online December, 30th 2016

Keywords:

Anthropogenic,
Soft Corals,
Cembranoid,
Toxicity,
Antitumor.

ABSTRACT

The aim of this research was to understand the impact on increasing anthropogenic pressure to the variability of Cembranoid Compounds derived of Coral*Sinularia* sp derived from Manado the waters and their cytotoxic activity against HeLa tumor.The coverage of corals was conducted at 4-6 mdepth, with 30 m using line intercept transects (LIT). Analysis of the structure of coral reef communities is based on underwater photography by category of sponges, hard corals, soft corals, algae, rubble and sand, using software CPCe 3.6 (Kohler & Gill, 2006). In situ analysis of of phosphate, nitrate, nitrite, ammonia, dissolved oxygen, and pH variable using portable devices (HACH DR-890 and HACH HQ40d). Ethanolicextract was subsequent from inorganic materials using reverse phase flash chromatography, the stationary phase in the form of C18 (Phenomenex C18) as well as the mobile phase of methanol and dichloromethane (1: 1). The isolation of bioactive compounds is done by using Preparative HPLC (Shimadzu HPLC column using a Shimadzu Preparative C18 measures 250 × 21 mm). Cytotoxic test performed on HeLa cancer cell types by using the method of 3- (4,5-dimethylthiazol-2yl) -2,5-diphenyltetrazolium bromide (MTT assay). Results of water quality in Malalayang; 0:55 mg/l of phosphate, 0.035 mg/l of nitrate, 0.008 mg/l nitrite, 0.0044 mg/l ammonia, 7.94 mg/lDO and a pH of 8.12, while Bunaken; 0.1 mg/l phosphate, 0.03 mg/l nitrate, 0.05 mg/lnitrite, 0.004 mg/l ammonia, 7.97 mg/lDO and a pH of 8.22. individual characteristic compositions in Malayalang; 0-10 % of hard corals, sponges 3.12 to 17.26%, algae 2.14 to 4.16%, and sand rubble 0-20.42% 0-51.12% while Bunaken; 22.24-44.12% of hard corals, sponges 0-27.18%, algae 1.12 to 2.14%, and sand rubble 0-33.24% 0-23.42%. Antitumor activity (100mg/l) on cell mortality in Malalayang; 7864-38936% and Bunaken 11861-81281%. While types of cembranoid compounds in Malalayang 0.14% Sinularide, 0.08 % Dehydrosinularide, 0:09%Flexilarin-B, 12.10% Episinularidide acetate while in Bunaken 0.15%, 0.08%, 0.16% 12.23% respectively.

INTRODUCTION

Increasing anthropogenic pressure on waters causing coral reefs cover shift patterns resulted in a decrease of hard corals and the possibility of increasing domination of macroalgae, soft corals, corallimorpharia or sponge. Soft corals are sessile organisms that are not able to move causing the production of secondary metabolites serves as a defense mechanisms in maintaining competition in benthic zone and resistance ability in degradation of water quality.

****Corresponding author: Nickson J. Kawung,***
Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado North Sulawesi

The research of Chanyun *et al*. (2008) and Sotka *et al*. (2009) soft corals in the ability to dominate space and resistance to changes in water quality is a part in producing bioactive compounds.Soft coral resiliency is the ability to live in the water column under anthropogenicpressure. Moreover, the invasion of soft corals due to its ability in producing compounds allelophatic cembranoid compound, which is also has pharmacological potency. There are very small information related to the research of the production cembranoid compounds in soft corals in regards to the space competition in benthic organisms due to the anthropogenic pressure and does not comprehensively discuss the type and quantity of allelophaticcompound insoft coral in their adaptation to the

benthic competition. Manado water is the area with the highest marine biodiversity, including soft corals. However, the high anthropogenic pressure can cause a shift bioprospecting pharmacological compounds within these organisms. Eco-physiological responsesallelophaticbioactive compound production of soft corals, *Sinularia* sp is important to explain the impact of anthropogenic pressures to the production of cembranoid compounds in soft corals*Sinularia*sp.in Manado waters especially in Bunaken and Malalayang.Because of the background mentioned above we would like to know the impact of anthropogenic pressure in Manado waters (Malalayang and Bunaken) tocharacteristic of cembranoid compounds to their antitumor activity. The novelty of the results of this study are characteristics cembranoid compound produced from soft coral *Sinularia* sp, as a result of anthropogenic pressures and their potential in the pharmacological field.

RESEARCH METHODOLOGY

Analysis of coral cover is done in a depth of 4-6 m, with 30 m using line intercept transects (LIT) of 3 replications parallel at each point of the study. Data were collected using underwater photos on each meter on either side of the transect line with the guide pipe 0.5 x 0.5 m square. Analysis of the structure of coral reef communities is based underwater photos by category sponges, hard corals, soft corals, algae, rubble and sand, using software CPCe 3.6 (Kohler & Gill, 2006). Samples of water parameters were taken at the same depth. Variable water in the form of phosphate, nitrate, nitrite, ammonia, dissolved oxygen, and pH, was selected as a variable water level mementukan anthropogenic impacts. All variables in the analysis directly in the field using portable devices (HACH DR-890 and HACH HQ40d).

In the laboratory, the samples in Extraction repeated 3 times. Furthermore, the entire maserat (3 L) filtered and dried using an evaporator (Buchi 250 Rotavapor) and Concentrator (Savant concentrator). The dry extract is then in the filtration of inorganic materials using reverse phase flash chromatography, the stationary phase in the form of C18 (Phenomenex C18) as well as the mobile phase of methanol and dichloromethane (1: 1). Solvents from the filtrate is dried again using evaporator and concentrator, and then diluted with 4 mL of methanol. The isolation of bioactive compounds is done by using Preparative HPLC (Shimadzu HPLC column using a Shimadzu Preparative C18 measures 250 × 21 mm). Mobile phase used is elution gradient of 20% acetonitrile / water to 100% acetonitrile for 60 minutes with a flow rate of 15 mL / min. Fractionation of the sample is done automatically using the fraction collector every 30 seconds. The results of each subsequent fraction in a qualitative analysis of the tumor cells using the method of 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolinon bromide (Zachary, 2003). In this test using HeLa cancer cell types. The cells were cultured in RPMI complete media () containing 10% fetal bovine serum in a CO2 incubator at 37 ° C using the test MTT {3- (4,5-dimethylthiazol-20 yl) 2-5-diphenyltetrazolium bromide}. For the whole test cytotoxic, where the number of cells used were 10,000 cells / well, this method following the instructions of Freshney (2005) and Ebada *et al*. (2008) ang modified by Nursid M. (2013). Measurement of absorbance of each well with microplate reader made at a wavelength of 570 and 690 nm. Persetanse cell death is calculated based on the formula [(xA.xD) - (xB.xC) / (xA-xD)] x 100%. Analysis of coral cover is done in a depth of 4-6 m, with 30 m using line intercept transects (LIT) of 3 replications parallel at each point of the study. Data were collected using underwater photos on each meter on either side of the transect line with the guide pipe 0.5 x 0.5 m square. Analysis of the coral reef structure communities is based underwater photos by category of sponges, hard corals, soft corals, algae, rubble and sand using software CPCe 3.6 (Kohler & Gill, 2006).The parameters of water Samples were taken at the same depth. Variable water in the form of phosphate, nitrate, nitrite, ammonia, dissolved oxygen, and pH, was selected as a variable water level that determine the anthropogenic impacts. All variables in the analysis directly in the field using portable devices (HACH DR-890 and HACH HQ40d).

In this test we useHeLa cancer cell types (cervical cancer), breast cancer cells (MCF-7). The cells were cultured in complete media RPMI containing fetal bovine serum 10% in the incubator CO_2 at 37 ° C by using MTT test {3- (4,5-dimethylthiazol-20 yl) 2-5-diphenyltetrazolium bromide}. For the whole cytotoxic test, the number of cells 10,000 cells / wellwere used, this method following the instructions of Freshney (2005) and Ebada et.al (2008) as modified by Nursid M. (2009). Measurement of absorbance of each well with microplate reader made at a wavelength of 570 and 690 nm. Percentage of cell death was calculated based on the formula [(xA.xD) - (xB.xC) / (xA-xD)] x 100%.

RESULTS

The results of water quality measurements in Manado Waters specifically in Malalayang and Manado Waters shown in

In Figure 1 above we could seen the chemical content of water as an indicator of water quality in Malalayang and Manado Waters and contrastive as content of phosphate, nitrate, nitrite, ammonia and ammonia is higher in Malalayang compared to Bunaken, whereas pH in Bunaken still more base. Analysis of competition space place live soft coral *Sinularia* sp from the waters of Bunaken and Manado's Malalayang in particular and its effects on the activity of the toxicity of the extract coarse*Sinularia* sp. shown in Table 1 below. Table 1 above shows antitumor activity from the soft coral*Sinularia*sp. Theywere grouped in three categories, which islow, moderate and high. and Figure 2 shows that the competition from the soft coral *Sinularia* sp. affects the antitumor aktivity, where the higher the space competition is, antitumor activity of the extract of soft coral *Sinularia* sp. is getting higher too, this indicates that to sustain the life of the soft coral *Sinularia* sp., compound metabolites produced is getting bigger. Malalayang and Bunaken waters area give different characteristics against a competition space where Malalayang in domination by the sand while in Bunaken is dominated by hard coral soft coral while the distinction hardly amount to both locations compared to sponges. Analysis of thecytotoxicitythat is plotted against the competition of benthic samples from space showed that benthic space competition became the main factors that lead to increased cytotoxic activity of the sample (Figure 3) In the space competition where was low, with the high soft coral cover itself and the abiotic component in the form of rubble or sand, then this can lead to low production of cytotoxic compounds from the particular biota.

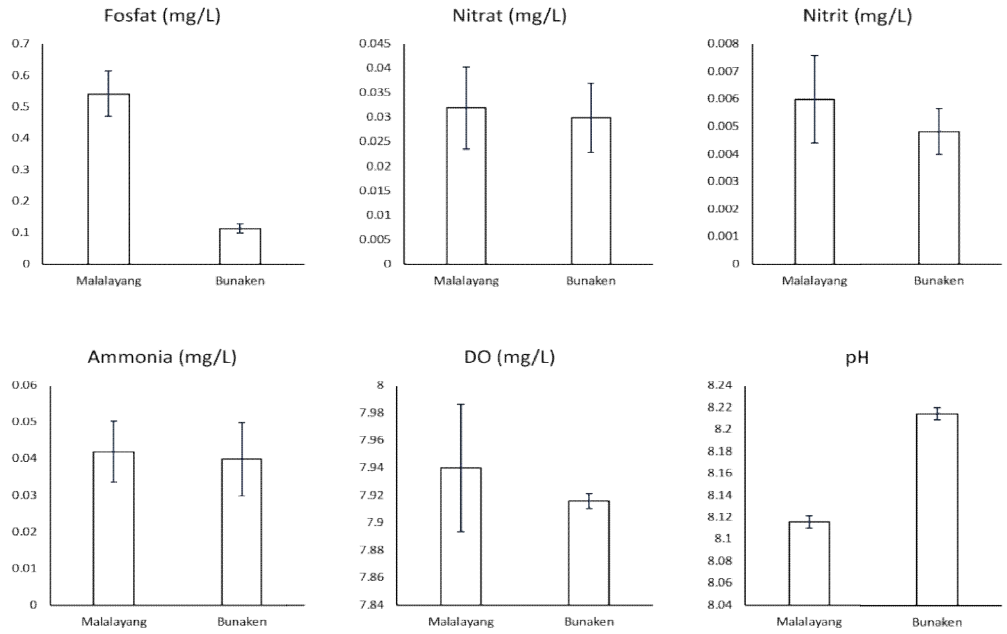

Table 1. Composition of the individual Characteristics and antitumor activity

Sinularia sp.	Karakteristik Kompetisi Individu (%)						Antitumor (100mg/l)	
	Sample	Biotic			Abiotic		HeLa	Bioactivity Group
		Hard corals	Sponnge	Alga	Rubbie	Sand		
Malalayang	56.56	0	0	3.22	0	40.22	18.701	Low
	97.76	0	0	2.24	0	0	12.788	Low
	31.4	10.22	3.12	2.34	0	53.12	38.936	Moderate
	38.96	0	7.44	3.14	0	50.14	10.285	Low
	82.44	0	0	3.46	0	13.42	15.575	Low
	18.04	0	17.26	4.14	3.22	57.32	36.670	Moderate
	76.4	0	0	3.18	20.42	0	7.864.	Low
Bunaken	20.52	44.12	0	2.12	33.24	0	81.281	High
	97.86	0	0	2.14	0	0	17.309	Low
	91.74	0	0	1.12	7.14	0	11.861	Low
	44.98	20.24	0	1.22	17.44	16.12	73.907	High
	68.12	23.24	0	1.34	7.12	0	53.884	High
	48.18	0	27.18	1.22	0	23.42	57.388	High
	37.82	23.48	0	1.42	23.12	14.16	69.627	High

On the contrary space competition with hard corals and sponges will cause increased production of compounds from the bioactivity soft coral (Figure 3).

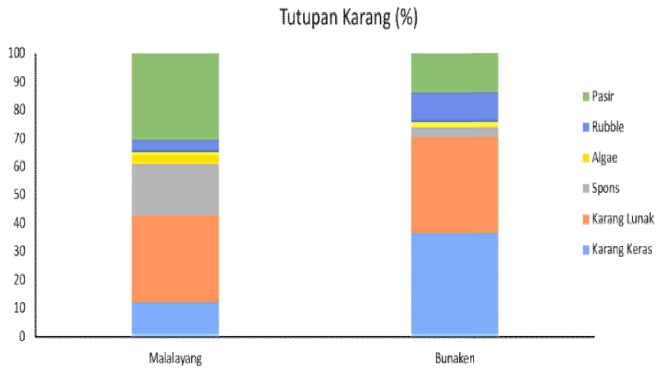

Figure 2. The composition of coral cover in Malalayang and Bunaken

Characterisitics of the cembranoid compounds between two locations namely Malalayang and Bunaken plotted in the diagram below (Figure 4). Overall the cembranoid compounds commonly found in soft coral *Sinularia* sp living in two locations, but the composition of each of the different compounds, from Figure 4 above the production of cembranoid compound in Bunaken is higher than in Malalayang waters, this is due to higher coverage of hard corals where the soft coral growth generally attach to them. Anthropogenic pressure gives the cembranoid compound production is shifting in the soft coral.

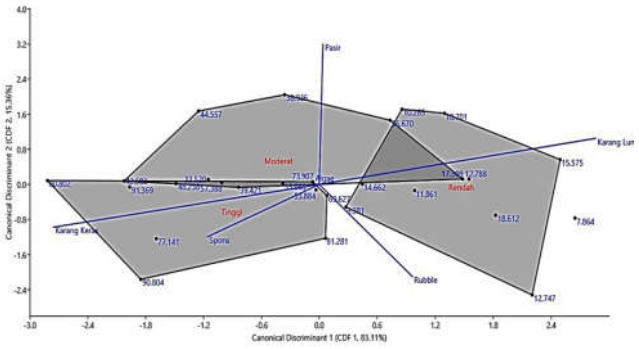

Figure 3. Antitumor activity based on the composition of coral cover in Malalayang and Bunaken

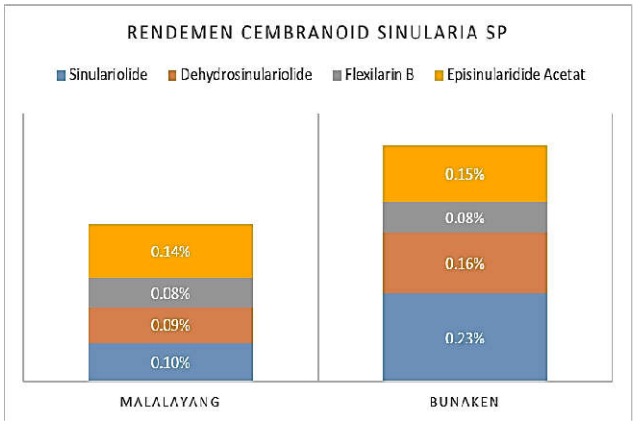

Figure 4. The characteristics of the soft coral *Sinularia* sp. cembranoid yield in Malalayang and Bunaken

DISCUSSION

Malalayang Waters has decreased water quality compared to the waters of Bunaken. High chemical content of water such as phosphate, nitrate, nitrite and ammonia in Malalayang is caused due to the presence of waste discarded laundry or weathering rocks. Furthermore the low pH due to high organic impurities, which causes the aerobic decompose by bacteria, producing carbon dioxide. The decline in water quality influence on coverage of the hard corals that invaded a non-coral biota builder. Analysis result of hard coral coverage in Bunaken is higher than in Malalayang Waters. Anthropogenic Pressure may cause a shift in the pattern of coral cover, a living space of life hard corals, which have a high vulnerability to changes in water quality, will be diokupasi by other biota, which has higher durability, such as soft corals, makroalga, corallimorpharia, or sponge (Norström *et al*. 2009). Lasut *et al*. (2005) stated Manado obtained anthropogenic pressure in the form of discarded waste water of high terrestrial, for example of the Bailang, Maasing and Tondano. The impact of this can be seen by the existence of the condition of the coral cover declined from 1990 to 2000 (Sills & Tioho, 2009).

The analysis of antitumor activity result of soft coral*Sinularia*sp. extract, from two different locations and Bunaken Malalayang, where there is the highest on the antitumor toxicity extract soft coral *Sinularia*sp origin of Bunaken. This difference is due to the characteristics of the individual compositions in Bunaken more dominated by hard corals, coral and sponges causing rubbie software producing content of bioactive compound which is toxic to beused in space and nutrient competitions. The diversity of soft coral is obtained between the two sites showed. The high level indicated with toxicity mortality of tumor cells HeLa 81,281%, i.e. the same compound cembranoid yield of characterisitics of the two sites showed a difference, highest cembranoid yield in Bunaken Island. There are four (4) successful cembranoid compounds are characterized from the soft coral *Sinularia*sp which is Sinulariolide i.e. Dehydirosinulariolide, antitumor, Flexilarin-B and Episinulariodide acetat where the structure was compared to2014 marinlit database (Blunt and Blunt 2014). The largest Flexilarin cembranoid-B yield with 0.23% originated from the Bunaken island. On the origin of the soft coral species of biota aquatic Manado, has found a variety of new compounds, from the soft coral Sarcophyton *Sinularia*sp., *Lobophytum*sp., and *Xenia* sp., such as Sinulasulfoxida, sinulasulfonat, polihidroksida, sterol sinulariocyd, Chloroscabrolide, sarcofuranocembrenolide (type A and B), Decaryiol, fatty acid, Lobozoanthamine, and Xenimanadins (Fattorusso *et al*. 2008a; 2008b; 2009; 2012; Wang 2009; Kapojos *et al*. 2010; Son *et al*. 2012a; 2012b; 2012c). This type of compound cembranoid found in this research is different from what is found by the researchers. This difference is due to the conditions change of the aquatic environment caused due to anthropogenic pressure. Quantitatively, the study of bioactive compounds in soft coral has discovered the existence of a relation of bioactivity andbioactive compounds production in soft coral with the condition of the aquatic habitat quality (January *et al*. 2011; 2012; 2015a; 2016a).

Conclussion

Due to the anthropogenicpressure in Malalayang waters has decreased water quality compared to the waters of Bunaken. Phosphate is higher due to the presence of waste discarded laundry or rocks weathering. The lower pH cause by the high organic impurities, which causes the aerobic decomposition by bacteria, producing carbon dioxide and decrease the pH of the soft coral *Sinularia* sp. is one type of soft coral which became the major component of the invaded areas of coral reefs. Anthropogenic pressure in Manado (Malalayang and Bunaken) gives the difference of compoundcharacteristicas well as the effect on the cembranoid antitumor toxicity. The antitumour activity of the soft coral *Sinularia* sp. extract taken from Bunaken Island higher than from Malalayang.

REFERENCES

Changyun, W., Haiyan, L., Changlun, S., Yanan, W., Liang, L., Huashi, G. 2008. Chemical defensive substances of soft corals and gorgonians. *Acta Ecologica Sinica.* 28(5):2320-28.

Januar HI, Marraskuranto E, Patantis G, Chasanah E. 2012. LCMS metabolomic analysis of environmental stressors impacts to the metabolites diversity in Nephthea sp. Chronicles of Young Scientist Journal, 3(1):57-62.

Januar, H.I. 2016b. North-South BioDiscovery Research Collaboration of Indonesian Sponge and Soft Coral : A Bibliographic Analysis of Publications Over the Last Two Decades J Scientometric Res. 5(1):43-8.

Januar, H.I., Chasanah, E., M Tapiolas, D., A Motti, C., H Liptrot, C. and D Wright, A., 2015a. Influence of anthropogenic pressures on the bioactivity potential of sponges and soft corals in the coral reef environment. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 10(2), pp.51-59.

Januar, H.I., Hendrarto, B., Chasanah, E., Wright, A. 2011. Nephthea sp.: correlation between natural products production and pressure from local environmental stressors. Marine Science Research & Development, S8:001.

Januar, H.I., Hendrarto, B., Chasanah, E., Wright, A.D. 2011. Nephthea sp.: Correlation between natural products production and pressure from local environmental stressors. *J Mar Sci Res Dev.,* S8(001):1-6

Januar, H.I., Zamani, N.P., Soedarma, D. and Chasanah, E., 2016a. Changes in soft coral *Sarcophyton* sp. abundance and cytotoxicity at volcanic CO2 seeps in Indonesia.AIMS Environmental Science, 3(2): 239-248.

Januar, HI, Pratitis A, and Bramandito A. (2015b). Will the Increasing of Anthropogenic Pressures Reduce the Biopotential Value of Sponges? Scientifica. Article ID 734385: 7pp.

Kapojos, M.M., Lee, J.S., Oda, T., Nakazawa, T., Takahashi, O., Ukai, K., Mangindaan, R.E., Rotinsulu, H., Wewengkang, D.S., Tsukamoto, S. and Kobayashi, H., 2010. Two unprecedented cembrene-type terpenes from an indonesian soft coral sarcophyton sp. Tetrahedron, 66(3), pp.641-645.

Kohler, K.E., Gill, SM. 2006. Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. Computers & Geosciences. 32(9): 1259–1269.

Kusen, J.D., & Tioho, H. 2009. The present status of coral reef condition in Bunaken National Park and Manado Bay, North Sulawesi, Indonesia. Galaxea, Journal of Coral Reef Studies, 11(2), pp.219-222.

Lasut, M.T., Jensen, K.R. and Shivakoti, G., 2008. Analysis of constraints and potentials for wastewater management in the coastal city of Manado, North Sulawesi, Indonesia. Journal of environmental management, 88(4), pp.1141-1150.

Lasut, M.T., Jensen, K.R., Arai, T. and Miyazaki, N., 2005. An assessment of water quality along the rivers loading to the Manado Bay, North Sulawesi, Indonesia. Coastal Marine Science 29(2): 124-132.

Norström, A.V., Nyström, M., Lokrantz, J., Folke, C. 2009. Alternative states on coral reefs: beyond coral–macroalgal phase shifts. Marine Ecology Progress Series. 376: 295–306.

Nursid, M., N. Dewi Fajarningsih, Th. Wikanta. 2009. Isolation of Cytotoxic Compound from *Nephthea* sp. Soft Coral. Jurnal of Biotechnology Research in Tropical Rdegion, Vol. 2, No. 1, Apr. 2009 *(Special Edition)* ISSN: 1979-9756**.** *Research Center for Marine and Fisheries Product Processing and Biotechnology, Agency of Marine and Fisheries Research, Jalan KS. Tubun Petamburan VI Jakarta*

Putra, M. Y., Ianaro, A., Panza, E., Bavestrello, G., Cerrano, C., Fattorusso, E., & Taglialatela-Scafati, O. 2012a. Sinulasulfoxide and sinulasulfone, sulfur-containing alkaloids from the Indonesian soft coral *Sinularia* sp. Tetrahedron Letters, 53(30), 3937-3939.

Putra, M. Y., Ianaro, A., Panza, E., Bavestrello, G., Cerrano, C., Fattorusso, E., Taglialatela-Scafati, O. 2012c. Sinularioside, a triacetylated glycolipid from the Indonesian soft coral *Sinularia* sp., is an inhibitor of NO release. Bioorganic & medicinal chemistry letters, 22(8), 2723-2725.

Putra, M.Y., Bavestrello, G., Cerrano, C., Renga, B., D'Amore, C., Fiorucci, S., Fattorusso, E. and Taglialatela-Scafati, O. 2012b. Polyhydroxylated sterols from the Indonesian soft coral *Sinularia* sp. and their effect on farnesoid X-activated receptor. Steroids, 77(5), pp.433-440.

Sotka, E., Forbey, J., Horn, M., Poore, A., Raubenheimer, D. The emerging role of pharmacology in understanding consumer-prey interactions in marine and freshwater systems. Integr Comp Biol. 2009.49:291-313. 5.

Wang, W., Lee, J.S., Nakazawa, T., Ukai, K., Mangindaan, R.E., Wewengkang, D.S., Rotinsulu, H., Kobayashi, H., Tsukamoto, S. and Namikoshi, M. 2009. (25S)-Cholesten-26-oic acid derivatives from an Indonesian soft coral Minabea sp. Steroids, 74(9), pp.758-760.

Zachary I. Determination of cell number. In: Cell proliferation and apoptosis, D. Hughes and H. Mehmet (Eds), Bios Scientific Publishers, 2003; 13-35

****