Exploration of the potential of *Holothuria atra* bioactive compounds based on their habitat characteristics

[1]Bambang Sulardiono, [2]Sutrisno Anggoro, [3]Renni Yuniati

[1] Laboratory of Hydro-biology, Aquatic Resources Department, Fisheries and Marine Sciences Faculty, Diponegoro University, Tembalang Semarang, Indonesia; [2] Laboratory of Aquatic Resource Management, Aquatic Resource Department, Fisheries and Marine Sciences Faculty, Diponegoro University, Tembalang Semarang, Indonesia; [3] Department of Dermatology and Venereology, Faculty of Medicine, Diponegoro University, Tembalang Semarang, Indonesia. Corresponding author: R. Yuniati, renniyuniati@yahoo.com

Abstract. An extreme environment produces an increase in the secretion rate of bioactive compounds of sea cucumbers. The research aims to explore the bioactive compounds of *Holothuria atra* from different habitats in Karimunjawa National Park, Indonesia. A total of 15 individual samples of sea cucumber *H. atra* were collected from the waters of Menjangan Besar, and 20 individuals from Alang-Alang waters. Sample extraction was carried out by maceration. Crude extract was obtained using an evaporator at 40°C. The extract yield was obtained by comparing the weight of the crude extract with the extract that has been evaporated. The analysis of steroid compounds, flavonoids, alkaloids, saponins, and phenols was conducted. Both sample lots from the Menjangan Besar and Alang-Alang waters contained the aforementioned compounds. However, the concentration of phenol, flavonoid, and alkaloid compounds in Menjangan Besar waters are higher than those from Alang-Alang waters, while the saponin compound content is relatively lower. The results of the analysis of variance at 95% confidence level showed that the content of flavonoid, alkaloid, and saponin compounds in the 2 observation locations were not significantly different, but the contents of phenol compounds were significantly different. The discovery of the potential of phenol and flavonoid compounds of *H. atra* sea cucumber, especially from Menjangan Besar waters as a cultivation zone, provides an alternative development of pharmaceutical products and health supplements to support conservation-based management plans.
Key Words: Karimunjawa National Park, maceration, sea cucumber.

Introduction. Overexploitation of commercial sea cucumbers in the waters of the Karimunjawa National Park are causing a significant population decline, thus threatening the sustainability of its use. Sea cucumbers have an important role in increasing diversity and preventing the process of eutrophication in their habitat (Hou et al 2017), and this is the effect of the sea cucumber bioturbation process. Therefore, alternative management of sea cucumbers based on conservation, integrated with the cultivation system is needed.

Most of the Karimunjawa region is part of the Karimunjawa National Park, which is divided into various zones, including the Menjangan Besar cultivation zone and the rehabilitation zone in the west coast of Karimunjawa Island (Alang-Alang waters) (Sulisyati et al 2018). These regions are suitable to be used as sea cucumber habitats (Sulardiono et al 2017). There are 18 species of sea cucumbers that are captured and traded in Karimunjawa (Purwati et al 2010). These species are categorized in 3 groups, according to their commercial value: high, medium and low. The sea cucumber *Holothuria atra* (Jaeger 1833) (Echinoderm: Holothuria) belongs to the *Holothuria* genus and is classified under the non-commercial sea cucumbers, living as a benthic invertebrate, and appearing abundantly in the waters of the Karimunjawa National Park.

Sea cucumbers have the potential to produce bioactive compounds that can be used as raw materials in medicine (Albuntana et al 2011). This is because sea cucumbers contain various bioactive compounds including peptides, phenols, polysaccharides, glycosides, triterpenes and several others. There is a relationship between the environment and the rate of secretion of sea cucumber bioactive compounds. In extreme environments, sea cucumbers tend to secrete more bioactive compounds. This relates to the function of bioactive compounds as protection against interference from pathogenic bacteria on their bodies as a result of these extreme environmental impacts. Verpoorte & Alfermann (2000) state that bioactive compounds are a form of secondary metabolic compounds that function to defend organisms from adverse environmental conditions, and to protect themselves physiologically by increasing the rate of secretion of these bioactive compounds. Referring to the statement, Menjangan Besar waters, as a tourism and cultivation zone, tends to have a poor environmental quality and increased environmental degradation due to the impact of human activities. The more extreme the environment is, the more increased the secretion of bioactive compounds released from the body of sea cucumbers will be. The hypothesis proposed is that sea cucumbers in the waters of Menjangan Besar tend to produce higher bioactive compounds compared to the sea cucumbers in Alang-Alang, which is assumed to have a better environmental quality, as a rehabilitation zone.

This study aims to explore *H. atra* bioactive compounds from different habitats in Karimunjawa National Park, Indonesia. The results of the study can be used as information for conservation-based resource management in Karimunjawa National Park, Indonesia.

Material and Method

Materials used. The tools used in the study include: macerator, incubator, distillators, micropipettes with 50, 100, and 100 μL volume, micropipette tips, knife, oven, Erlenmeyer flask, analytic scales, separating funnel, measuring cup, autoclave, tweezers, Bunsen (spirit) burners, test tubes, magnetic stirrers, drop pipettes, stirring rods, refrigerator, and tweezers. The samples analyzed in this study are *H. atra* sea cucumbers harvested from both study regions. The chemicals used to analyze the contents are Aquadest, 96% ethanol, 10% ammonia (Merck), chloroform (Merck), 2N hydrochloric acid (Merck), Mayer reagent, magnesium powder, iron reagent, chloride reagent, 1% gelatin solution, ether (Merck), vanillin sulfate reagent and 1N sodium hydroxide (Merck).

Research location. *H. atra* sea cucumber samples were collected from the waters of the Karimunjawa National Park Region, Central Java Province, Indonesia, in July 2018. The samples were collected from two places with different environmental characteristics, namely from the waters of Menjangan Besar (station A), which is a tourism and cultivation zone and from Alang-Alang waters (station B), which is a rehabilitation zone. A map detailing the research locations is presented in Figure 1.

Sampling sea cucumbers. 15 individual *H. atra* sea cucumbers from the waters of Menjangan Besar and 20 individual *H. atra* sea cucumbers from the Alang-Alang waters were collected. The sea cucumbers were cleaned, dissected and part of the body wall was collected. The body wall sample was inserted in a cool box and transported to the Fisheries Product Laboratory, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, for further analysis.

Extraction and analysis. The extraction was carried out by a modified maceration process after Roihanah et al (2012). 1 kg of samples from both observation stations were sliced into small pieces, and stored in a 300 mL ethanol 96% solution with the ratio of samples to solvents of 1:4 (b/v) for 72 hours. The samples were filtered with Whatman filter paper. The separation was done with an evaporator at 40°C, so that crude extracts could be obtained. The results of weighing the crude extract were compared with the extract that has been evaporated, so that the extract yield could be obtained.

Analysis of steroid compounds was conducted using the technique defined by Rasyid (2012), which involved taking 1 g of sea cucumber extract and dissolving it in 0.5 mL chloroform, in a test tube. 0.5 mL of anhydrous acetic acid was added to the dissolved extract, then 1-2 mL of concentrated H_2SO_4 was slowly dropped in the tube and was left to dry. The appearance of a blue or purple color indicates the presence of steroid compounds.

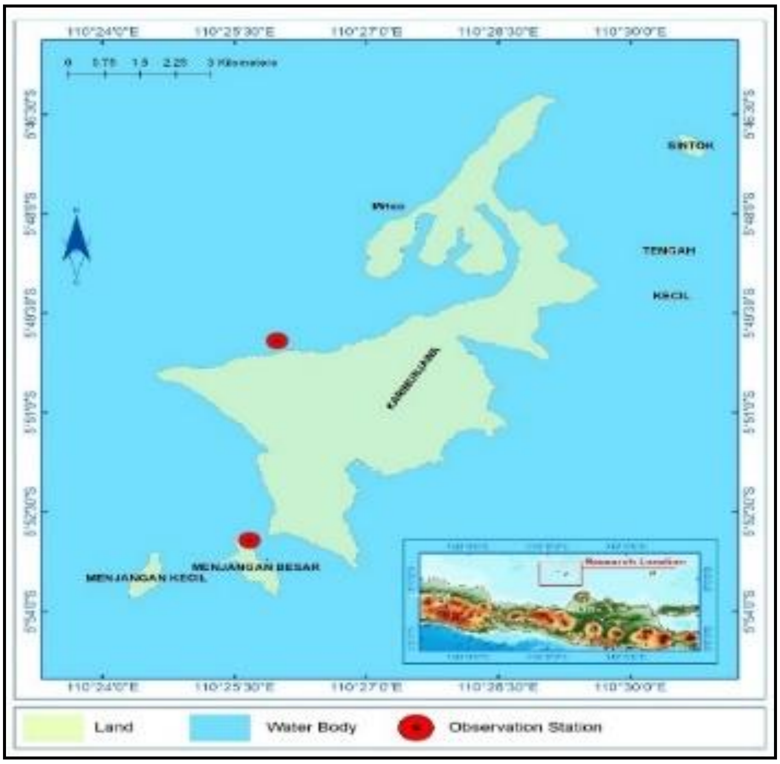

Figure 1. The research location at Karimunjawa National Park, Indonesia.

Analysis of flavonoids was conducted using the technique defined by Suryanto (2008). 5 g of sea cucumber extract were dissolved in 100 mL of distilled water, and 25 mL of 10% acetate acid in ethanol was added to the mixture. The sample was grinded and the solution was separated from the filtrate by using filter paper. 1 mL of filtrate was taken, and 2 mL of 5% $AlCl_3$ solution was added to the filtrate. 10 mL of Aquadest was added. The absorbance of the final solution was determined using a spectrophotometer with a wavelength of 420 µm. The results were calculated using the Quercetin standard curve.

Analysis of alkaloids was conducted using the technique defined by Harborne (1987). 5 g of sea cucumber ethanol extract were dissolved in 100 mL of distilled water. 25 mL of 10% acetic acid in ethanol was added to the solution, and the solution was stirred until a smooth consistency was obtained. The solution was incubated for 2 hours and then filtered, to obtain alkaloid filtrate deposits, so that the filtrate could be obtained. The filtrate was evaporated. NH_4OH was added to the filtrate until a white precipitate was formed as an alkaloid. The filter paper was weighed for further use. The precipitate was filtered and washed using 1% ammonium hydroxide solution. Filter paper containing sludge was dried in an oven at 60°C for 30 minutes. After cooling, the precipitate was weighed to obtain a constant weight. The alkaloid yield was determined from the weight presentation of the alkaloids obtained from the initial weighing sample. Testing was repeated 3 times.

Analysis of saponins was carried out using the technique defined by Harborne (1987). 5 g of sea cucumber extract were dissolved in 100 mL of distilled water into the test tube. 25 mL of ethanol 75% were added and then stirred until a homogeneous mix resulted and the mix was left for 30 minutes, until the suspensions settled. The solution in the top layer was collected with a pipette and placed in a container, then dried with the oven to a constant. Saponin levels can be obtained by calculating the difference in constant weight (final weight).

Analysis of phenols was done using the technique defined by Harborne (1987). 5 g of sea cucumber extract were dissolved in 100 mL of distilled water in a test tube. The filter paper was used to separate the solution from the filtrate. 1 mL of filtrate was collected. 0.5 mL of Follin type and 1 mL Na_2CO_3 saturated solution were added to the filtrate and the mix was left to rest for 10 minutes. Distilled water was added until the volume of the solution reached 10 mL. The mixture was homogenized with a vortex. The absorbance reading was carried out using a spectrophotometer with a wavelength of 730 μm. The results were calculated by using the standard curve for phenol measurement.

Data were processed using SPSS for Windows version 17.0 (IBM Corp, USA). To verify the difference in the value of the bioactive compound content observed between stations, statistical tests were used with variance analysis methods at a 95% confidence level.

Results and Discussion

Potential of H. atra bioactive compounds. Various types of bioactive compounds, including steroids, flavonoids, alkaloids, phenols, and saponin compounds were found in sea cucumbers from both study sites. Extracts presented a yield of 2.37% in the Menjangan Besar habitat and 4.38% in the Alang-Alang habitat, with a pH value of 4.79±0.04 and 4.89±0.03, respectively. The results of phytochemical screening tests on each class of bioactive compounds can be seen in Table 1.

Table 1
Bioactive compounds of *Holothuria atra* from the Menjangan Besar and Alang-Alang habitats

Test compound	*Average content of bioactive compounds ± SD*	
	Menjangan Besar habitat	Alang-Alang habitat
Steroids	+	+
Flavonoids (ppm)	58.5±7.90	48.8±2.80
Alkaloids (%)	7.39±1.28	6.98±1.22
Saponins (%)	3.05±1.02	5.65±1.30
Phenols (ppm)	1277±67.0	614±37.0
pH	4.79±0.04	4.89±0.03
Extract yield (%)	2.37	4.38

Note: + - compound was found.

The potential of H. atra bioactive compounds based on their habitat characteristics. There were no significantly different results between the bioactive compounds of sea cucumbers from Menjangan Besar and Alang-Alang waters, except for phenol compounds with a P value of 0.0001 (<0.05) (Table 2).

Table 2
Analysis of variance test results for the content of *Holothuria atra* bioactive compounds from the Menjangan Besar and Alang-Alang habitat

Bioactive compound	*P value*	*95% confidence level (α=0.05)*
Flavonoids (ppm)	0.1179	0.05
Alkaloids (%)	0.7099	0.05
phenols (ppm)	0.0001*	0.05
Saponins (%)	0.0840	0.05

Note: * - shows a significant difference.

The over-exploitation of seawaters causes highly commercial sea cucumbers to experience a significant population decline, which renders the loss of some sea cucumber species. This is demonstrated by the results of previous research conducted by Sulardiono (2016), which explains that the commercial sea cucumber *H. scabra* found in Karimunjawa waters had the lowest biomass from the species identified, which implies the status of overexploitation. Globally, the decline of *H. scabra* populations is observed by a CITES (2006) study, which stated that *H. scabra* species are overexploited. Therefore, alternative conservation based resource management is needed.

One of the alternatives that can be used to preserve the current state of the sea cucumber populations is using alternative non-commercial sea cucumbers as pharmaceutical and health industry materials. This method allows unlocking the hidden potential possessed by several species of sea cucumbers that have never been used as a medical product ingredient, and it can reduce the exploitation of the other species of sea cucumbers in the sea. Therefore, we need data and information about bioactive compounds in these non-commercial sea cucumbers.

Many organisms from the sea provide various extraction metabolites (Shakouri et al 2017; Gallimore 2017) as source material for the development of therapeutic drugs (Sohair 2018). Sea cucumbers are a potential source of high value compounds, such as triterpenes glycosides, carotenoids, bioactive peptides, vitamins, minerals, fatty acids, collagen, gelatin, chondroitin sulfate, and amino acids (Pangestuti & Arifin 2018), which can be utilized for medicinal purposes. As in other sea cucumber species categorized in the Aspidochirotida order, *H. atra* sea cucumber, which is currently a non-commercial sea cucumber, contains bioactive compounds as well as secondary metabolites, which can be used as a source of medicinal ingredients (Farjami et al 2013). The structure, physicochemical properties and pharmacological effects of bioactive compounds that have anticoagulant, antithrombotic, antioxidant, anticancer, anti-infection and other important activities have been described by Khotimchenko (2018). Through biotechnology, these marine resources can be utilized as ingredients or can be modified to produce several medicines with various benefits to support the development of pharmaceutical and health products.

H. atra belongs to the genus *Holothuria* and the order of Aspidochirotidae (Rowe 1969). In Karimunjawa, this species has not been utilized optimally and has a low commercial value, because it is considered to have a low beneficial value. Although *H. atra* extract does not present a high content in beneficial compounds, if its potential is enhanced with technological support and community participation, it will provide opportunities for local communities to increase its economic value, and reduce sea cucumber fishing activities, so that conservation and economic goals can be integrated and made sustainable.

The results showed that phenolic bioactive compounds and flavonoid extracts of *H. atra* sea cucumber are relatively high compared to other bioactive compounds, especially for the samples collected from Menjangan habitat, which show 1.277±67 ppm phenolic compound and 58.5±7.9 ppm flavonoids. The samples from Alang-Alang had 614±37 ppm phenolic compounds and 48.8±2.8 ppm flavonoids. Dhinakaran & Lipton (2014) confirm that *H. atra* has various compounds, including flavonoids, phenols, saponins, and alkaloids. According to Putram et al (2017), *H. atra* sea cucumber crude extract contains a class of flavonoids compounds known to have antihypertensive activities. Moreover, according to Tungmunnithum et al (2018), the phenolic and flavonoid compounds of *H. atra* possess antioxidant properties and other important functions with beneficial effects to human health, like antibacterial, anti-coagulation, anti-fungal, anti-inflammatory, anti-malaria, and anti-virus properties (Bhuyan & Basu 2017), and immune properties, cardio-protective properties, and skin protection from ultra violet radiation.

The phenolic compounds of *H. atra* discovered in this study were in accordance with the results of a previous study (Esmat et al 2012), which stated that *H. atra* sea cucumber contains relatively high levels of phenol compounds derived from phytoplankton and macro-algae degradation, as the main food of sea cucumbers. According to Mfilinge & Tsuchiya (2016), sea cucumbers consume algae (diatoms and green macroalgae) and detritus, these substances being a potential source of phenol compounds. There is an increase in domestic waste originating from the activity zone of cultivation in the waters of Menjangan Besar. This facilitates phytoplankton blooming, which contributes to a significant increase in bioactive compounds. According to Sulardiono et al (2018), the results of the chlorophyll-a (phytoplankton) measurement in Menjangan Besar waters include them in the oligotrophic category. Based on this result, it can be explained that the content of phenol compounds in *H. atra* is higher in the Menjangan Besar waters (cultivation zone), than that of *H. atra* from Alang-Alang waters (rehabilitation zone).

Sea cucumbers in unfavorable environmental conditions tend to adapt by increasing structural and functional secretion of bioactive compounds (Rizzo & Lo Giudice 2018). Marine organisms produce a variety of bioactive compounds because of the diversity of biological resources that are found in harsh environments (Mondol, et al 2017), including a decrease in the quality of their habitat. The implication of this condition supposedly causes the phenolic and flavonoid bioactive compounds in *H. atra* from Menjangan Besar waters to be higher than those from Alang-Alang waters. The Menjangan Besar waters are designated as a cultivation zone, which allows the area to be used as a development zone of *H. atra* for the pharmaceutical and health fields through technology enhancement and community participation.

The interesting thing in this research is that there are significant differences in phenolic MOM compounds between Menjangan Besar habitat and Alang-Alang habitat. Based on these facts, the existence of sea cucumber phenolic compounds is closely related to the environment of sea cucumber growth. The presence of phenols in the body of sea cucumbers is influenced by the level of ocean acidity and the dynamics of sedimentary organic matter. According to Min et al (2015), an increase in CO_2, temperature, nitrogen deposition, and drought can increase the production of phenolic compounds, which leads to changes in the dynamics of sedimentary organic matter. This happens because phenolic compounds are used to control the rate of decomposition of sedimentary organic matter, stabilizing organic carbon in the ecosystem. The increase in ocean acidity can affect the structure and function of phenol chemical compounds. As stated by Jin et al (2015), ocean acidity increases the phytoplankton phenolic production by 46-212%, as a growing source of phenol below the CO_2 concentrations in the surrounding environment.

Based on the results of the study, the potential for bioactive compounds in the waters of Menjangan Besar as a cultivation zone can be developed by cultivating *H. atra*, and changing its classification from a non-commercial into a commercial sea cucumber for local communities. According to Lawrence et al (2009) and Mayer et al (2013), sea cucumbers have the potential to be developed as medicinal products and healthy food. Based on this data, the development of new products from bioactive compounds has become an important aspect in innovations in new drug products.

Conclusions. The discovery of the potential of phenol compounds and flavonoids of *H. atra*, specifically from Karimunjawa waters, provides an alternative development of pharmaceutical products and health supplements to support conservation based management plans, such as integration between conservation and economic aspects. This needs to be done because conservation is able to reduce sea cucumber harvesting efforts and might increase the population of sea cucumbers, while the economic aspect can be achieved through increasing biotechnology and local community participation, which provides economic value. The results of this study indicate that the Menjangan Besar waters, as a cultivation zone, can be used as a development area for sea cucumbers.

Acknowledgements. We would like to thank the Dean of the Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, for the year 2018 Grant funding support for this research.

References

Albuntana A, Yasman, Wisnu W., 2011 [Toxicity test of four Holipuriidea sea cucumber extracts from Penjaliran Timur Island, Kepulauan Seribu, Jakarta using brine shrimp lethality test (BSLT)]. Jurnal Ilmu dan Teknologi Kelautan Tropis 3(1):65-66. [In Indonesian].

Bhuyan D. J., Basu A., 2017 Phenolic compounds: potential health benefits and toxicity. In: Utilisation of bioactive compounds from agricultural and food production waste. Vuong Q. V. (ed), CRC Press, Boca Raton, pp. 27-59.
Dhinakaran D. I., Lipton A. P., 2014 Bioactive compounds from *Holothuria atra* of Indian ocean. SpringerPlus 14(3):673, 10 p.
Esmat A. Y., Said M. M., Soliman A. A., El-Masry K. S. H., Badiea E. A., 2012 Bioactive compounds, antioxidant potential, and hepatoprotective activity of sea cucumber (*Holothuria atra*) against thioacetamide intoxication in rats. Nutrition 29(1):258-267.
Farjami B., Nematollahi M. A., Moradi Y., Irajian G. R., Nazemi M., Ardebili A., Pournajaf A., 2013 Antibacterial activity of the sea cucumber *Holothuria leucospilota*. International Journal of Molecular and Clinical Microbiology 1:225-230.
Gallimore W., 2017 Marine metabolites: oceans of opportunity. In: Pharmacognosy Fundamentals, Applications and Strategies. Badal S., Delgoda L. (eds), Academic Press, Cambridge, pp. 377-400.
Harborne J. B., 1987 [Phytochemical method: guide to modern ways to analyze plants]. Penerbit ITB, Bandung, 354 p. [In Indonesian].
Hou Y. R., Sun Y. J., Gao Q. F., Dong S. L., Wen B., Pan Z., Liu C., 2017 Bioturbation by sea cucumbers *Apostichopus japonicus* affects sediment phosphorus forms and sorption characteristics. Aquaculture Environmental Interactions 10:201-211.
Jin P., Wang T., Liu N., Dupont S., Beardall J., Boyd P. W., Riebesell U., Gao K., 2015 Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels. Nature Communications 6:8714, 6 p.
Khotimchenko Y., 2018 Pharmacological potential of sea cucumbers. International Journal of Molecular Sciences 19(5):1342, 42 p.
Lawrence A. J., Afifi R., Ahmed M., Khalifa S., Paget T., 2009 Bioactivity as an options value of sea cucumbers in the Egyptian Red Sea. Conservation Biology 24(1):217-225.
Mayer A. M. S., Rodriguez A. D., Taglialatela-Scafati O., Fusetani N., 2013 Marine pharmacology in 2009-2011: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. Marine Drugs 11(7):2510-2573.
Mfilinge P. L., Tsuchiya M., 2016 Changes in sediment fatty acid composition during passage through the gut of deposit feeding holothurians: *Holothuria atra* (Jaeger, 1883) and *Holothuria leucospilota* (Brandt, 1835). Journal of Lipids 2016:4579794, 9 p.
Min K., Freeman C., Kang H., Choi S. U., 2015 The regulation by phenolic compounds of soil organic matter dynamics under a changing environment. BioMed Research International 2015:825098, 11 p.
Mondol M. A. M., Shin H. J., Rahman M. A., Islam M. T., 2017 Sea cucumber glycosides: chemical structures, producing species and important biological properties. Marine Drugs 15(10):317, 35 p.
Pangestuti R., Arifin Z., 2018 Medicinal and health benefit effects of functional sea cucumbers. Journal of Traditional and Complementary Medicine 8(3):341-351.
Purwati P., Hartati R., Widianingsih, 2010 Eighteen sea cucumber species fished in Karimunjawa Island, Java Sea. Marine Research in Indonesia 35(2):23-30.
Putram N. M., Setyaningsih I., Kustiariyah T., Nursid M., 2017 [Anticancer activity from active fraction of sea cucumber]. JPHPI 20(1):53-62. [In Indonesian].
Rasyid A., 2012 [Identification of secondary metabolite compounds and antibacterial activity test and antioxidant of *Stichopus hermanii* methanol extract]. Jurnal Ilmu dan Teknologi Kelautan Tropis 4(2):360-368. [In Indonesian].
Rizzo C., Lo Giudice A., 2018 Marine invertebrates: underexplored sources of bacteria producing biologically active molecules. Diversity 10(3):52, 36 p.
Roihanah S., Sukoso, Andayani S., 2012 [Antibacterial activity of *Holothuria* sp. sea cucumber extract against *Vibrio harveyi* bacteria *in vitro*]. Journal of Experimental Life Science 2(1), 5 p. [In Indonesian].

Rowe F. W. E., 1969 Review of the family Holothuriidae (Holothurioidea:Aspidochirotida). Bulletin of the British Museum (Natural History) Zoology 18(4):119-170.
Shakouri A., Shoushizadeh M. R., Nematpour F., 2017 Antimicrobial activity of sea cucumber (*Stichopus variegatus*) body wall extract in Chabahar Bay, Oman Sea. Jundishapur Journal of Natural Pharmaceutical Products 12(1):e32422, 5 p.
Sohair R. F., 2018 *Holothuria arenicola* as promising material for therapeutic drugs. SOJ Pharmacy & Pharmaceutical Sciences 5(2):1-6.
Sulardiono B., 2016 [The potential of sea cucumbers (Holothurians) use in Karimunjawa waters of Jepara district, Central Java province]. Bulletin Oseanografi Marina 5(1):64-72. [In Indonesian].
Sulardiono B., A'in C., Muskananfola M. R., 2018 Profiles of water quality at Menjangan Besar Island, Karimunjawa, Central Java Province, Indonesia. Biodiversitas 19(6):2308-2315.
Sulardiono B., Pujiono W. P., Haerudin, 2017 [The level of suitability of the aquatic environment sea cucumber habitat (Echinodermata: Holothuria) in Karimunjawa]. Saintek Perikanan 12(2):93-97. [In Indonesian].
Sulisyati R., Prihatinningsih P., Mulyadi, 2018 [Zoning review of Karimunjawa National Park as an effort to compromise natural resource management]. Proceedings of the National Geomatics 2018: Use and Development of Geospatial Information Products Support National Competitiveness, pp. 713-724. [In Indonesian].
Suryanto E., 2008 [Antioxidant activity of flavonoid extract from andaliman fruit (*Zanthoxylum acanthopodium* DC) in carp (*Cyprinus carpio* L). Journal MIPA Unsrat 2(1):50-55. [In Indonesian].
Tungmunnithum D., Thongboonyou A., Pholboon A., Yangsabai A., 2018 Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel) 5(3):93, 16 p.
Verpoorte R., Alfermann A. W., 2000 Metabolic engineering of plant secondary metabolism. Springer Science & Business Media, Dordrecht, 286 p.
***CITES, 2006 Sea cucumber. Convention on international trade in endangered species of wild fauna and flora. Twenty-second meeting of the Animal Committee, Lima, Peru, 17-13 July.

Received: 07 December 2019. Accepted: 01 March 2020. Published online: 30 June 2020.
Authors:
Bambang Sulardiono, Laboratory of Hidro-biology, Aquatic Resources Department, Fisheries and Marine Sciences Faculty, Diponegoro University, Prof. Soedarto, SH Street, 50275 Tembalang Semarang, Central Java, Indonesia, e-mail: bambangsulardiono@gmail.com
Sutrisno Anggoro, Laboratory of Aquatic Resource Management, Aquatic Resource Department, Fisheries and Marine Sciences Faculty, Diponegoro University, Prof. Soedarto, SH Street, 50275 Tembalang Semarang, Central Java, Indonesia, e-mail: sutrisnoanggoro52@gmail.com
Renni Yuniati, Department of Dermatology and Venereology, Faculty of Medicine, Diponegoro University, Prof. Soedarto, SH Street, 50275 Tembalang Semarang, Central Java, Indonesia, e-mail: renniyuniati@yahoo.com

How to cite this article:
Sulardiono B., Anggoro S., Yuniati R., 2020 Exploration of the potential of *Holothuria atra* bioactive compounds based on their habitat characteristics. AACL Bioflux 13(3):1715-1722.