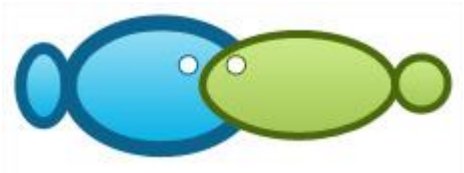

Ecological study and preliminary culture of the sponge *Candidaspongia* a source of anticancer molecules

[1,2]Agus Trianto, [1,2]Ambariyanto, [3]Sarjito, [1]Rini Pramesti, [1]Nirwani Soenarjo, [1]Retno Hartati, [1]Nur Taufiq-Spj, [1]Hadi Endrawati, [1]Destio, [4]Isai Yusidarta

[1] Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java Indonesia; [2] Marine Biopharmaceutical Research and Development Center, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java Indonesia; [3] Department of Fisheries, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java Indonesia; [4] Natural Resources Conservation Agency (BBKSDA), St. Perintis Kemerdekaan Kelapa Lima, Kupang, Indonesia.
Corresponding author: A. Trianto, agustrianto.undip@gmail.com

Abstract. Sponge *Candidaspongia* sp. is a source of candidaspongiolide, a very potent anticancer macrolide that is active against various cell lines at nanogram level. However, low abundance of the sponge in nature and structurally complex of candidaspongiolide have become the major obstacles for the drug development. This study aims to assess the feasibility of the production of the anticancer compounds, the candidaspongiolides, using sponge culture. The study was conducted in Kupang, Nusa Tenggara Timur, Indonesia. The sponge abundance was observed using modified Line Intercept Transect method at 12 m and 20 m. Some sponge colonies were cut and preserved for culture and chemical analyses. Then, the recovery rate of the sponge was observed after 60 days. Sponge culture was carried out at 6 m, 12 m and 25 m depth during 60 days. Inventory of the *Candidaspongia* sp. showed that the sponge density at around 12 m depth is lower than those in 25 m depth. All of the sponges were survive after the cut and fully recovered in 60 days. The length and width increments of the basal part were 0.25-2.1 cm/month and 0.5-1.75 cm/month, respectively. The sponges cultured at 12 m and 25 m depth have higher survival and growth rates than those at 6 m depth. Descriptively, the sponge cultured in deeper water have higher ethyl acetate extracts content than the sponge cultured at the shallower water. Sponge mariculture is a possible method to supply candidaspongiolide for further studies.
Key Words: Ethyl acetate, *Candidaspongia*, anticancer, mariculture**,** natural stock.

Introduction. Sponges have been proven as productive sources of various compounds classes with pharmacological potency as antibacterial, antifungal, anthelmintic, antimalarial, antiviral, anti-inflammatory, anticoagulant, antioxidant and antitumor (Trianto et al 2014; Balansa et al 2017). *Candidaspongia* sp. is a rare marine sponge that produces a potent anticancer compound called candidaspongiolide, a unique 18-membered macrolide. Candidaspongiolides have also been reported as exhibited remarkable cytotoxicity in NCI (National Cancer Institute) 60-cells-panel with GI_{50} of 14 ng mL-1. Originally, the compound was isolated from the sponge collected from Australian and Papua New Guinean waters (Meragelman et al 2007). Candidaspongiolide and its derivatives also exhibited activity against melanoma (UACC-257, LOXIMVI, and M14), breast (MCF7) and lung cancer (NCI-H460) cell lines (Whitson et al 2011).

In 2011, we identified two new derivatives of the candidaspongiolide along with the known one isolated from the sponge collected in Indonesia. The compounds exhibited potent cytotoxicity with IC_{50} 37.0, 4.7 and 19.0 ng mL^{-1}, against NBT-T2 cells (Trianto et al 2011). However, low abundance of the sponge in nature and structural complexity of candidaspogiolide have become the major obstacles for the drug development. This study

aims to assess the feasibility of the production of the anticancer compounds, the candidaspongiolides, using sponge culture. Mayer et al (2010) noted that among thousands of bioactive compounds isolated from marine organisms, those were only a few compounds entered a clinical trial. Material supply is the main problem besides the bioactivity and the pharmacological profile.

There are several methods that are commonly used to supply the bioactive compounds including chemical synthesis, mariculture, closed system culture, and fermentation (Mendola 2003). The most preferred method to produce a drug is chemical synthesis because of its efficiency, economically, and robust. However, considering that candidaspongiolide has several stereocenters, total synthesis would be impractical due to the longer pathway. Stereochemistry is a key to activity in biological systems (Butler 2004). The best synthesis method of one of the related compounds, tedanolide, has been achieved in 31 steps and gave only 0.31% of overall yield from the starting material (Smith & Lee 2007). Therefore, big-scale production of candidaspongiolide or its analogs via chemical synthesis may not be an economical method due to the high price of some starting materials. Tedanolide, isolated from the Caribbean marine sponge *Tedania ignis,* has been reported to exhibit strong cytotoxicity at pico to the nanomolar level (Schmitz et al 1984).

Sponges have been cultured for mass production of bath sponge and providing bioactive substances (Milanese et al 2003). Sipkema et al (2005) showed that culture of sponges *Lissodendoryx* sp. and *Dysidea avara* was able to produce anticancer compounds as halichondrin B and avarol, respectively. Muller et al (2000) successfully produced avarol from *Dysidea avara* via a cell culture that is known as primmorphs. To the best of our knowledge, this study is the first effort to culture the sponge *Candidaspongia* sp.

Material and Method

Candidaspongia inventory. The survey was conducted by Line Intercept Transect (LIT) method with a slight modification (Cleary et al 2005). The sponges were observed along transects (6 x 100 m) placed in 12 and 20 m depth with observation area around 3 m on both sides.

Sponge collection. The sponge colonies for explant and extract were collected by hand during the survey. The upper part of the sponge colonies was cut to let the basal part (about 5 cm height) re-growth for future stock (Mendola 2003).

Sponge culture. The sponge was cultured *in situ* in Kupang Bay, East Nusa Tenggara, Indonesia. Before the culture, the sponge colonies were tied at 12 m depth in a net for acclimation. After four days, the sponge colonies were cut into 3 cm x 5 cm (width x length). 15 fragments were explanted in three different nets placed at 6, 12, and 25 m depths (five fragments per net). The sponge growth rate was measured in the end culture period for 60 days. All the procedures applied were based on the methods proposed by DeCaralt et al (2003), Mendola (2003), and Osinga et al (2003).

Monitoring of the recovery and growth rates of the sponges. The basal parts of the sponge colonies were observed by SCUBA diving method to evaluate the recovery and the growth rates after 60 days from cutting. The height, width, and the number of new branches were recorded. The ruler was used for measuring the height and width increment.

Extraction of the sponges. The harvested sponges were cut into small pieces and extracted with methanol for 24 hours with triplicates. The extract was filtered with filter paper and concentrated with a rotary evaporator under vacuum. Then, the extract was subjected to the separatory funnel using ethyl acetate and water to provide the organic and water fractions (Trianto et al 2011).

Data analysis. The width increment of the sponges was analyzed by ANOVA test, and followed by Tukey HSD test to indicate the factors having significant effect. Ethyl acetate (EA) extract contents data was analyzed with Mann-Whitney test, to indicate whether the

treatments had a significant effect or not. Mann-Whitney test is a non-parametric test that has been chosen due to the data were not distributed normally and homogenously. Statistical analyses were performed using SPSS ver. 16 software.

Results

Candidaspongia inventory and collection. The sponge inventory was conducted using a modified Line Intercept Transect Method at 12 and 20 m depths. The average sponge densities were 0.3 and 1.43 colonies transect at 12 m and 20 m respectively, as shown in Tables 1 and 2.

Table 1

The sponges of *Candidaspongia* sp. colonies observed at 12 m depth

Station	*Colony(s) number*	*Average height (cm)*	*Average width (cm)*
I	1	12.10	8.30
II	0	-	-
III	0	-	-
IV	0	-	-
V	0	-	-
VI	0	-	-
VII	1	8.20	10.40
Total colonies	2	2	2
Average	0.3	10.15	9.35

Table 2

The sponges of *Candidaspongia* sp. colonies observed at 20 m depth

Station	*Colony(s) number*	*Average height (cm)*	*Average width (cm)*
I	2	18.75	10.70
II	2	15.20	9.00
III	2	14.20	7.30
IV	0	-	-
V	1	19.00	13.20
VI	1	10.00	5.10
VII	2	10.20	7.00
Total colonies	10		
Average	1.43	14.56	8.72

However, four small colonies of the sponges were observed at 10 m depth under the port out of the line transects. Light intensity probably plays an important role in the larval settlement. However, the hypothesis still needs to be proven with further study.

Monitoring of sponge colonies survival and growth rates after the cut. The sponges of *Candidaspongia* sp. colonies were observed by SCUBA diving method to evaluate the recovery rate and the growth after the cut. The basal parts of the sponge were survive 100% and grown well (see Table 3). The average height and width increments were 2.33 cm and 2.53 cm, respectively.

Table 3

The basal parts of sponge *Candidaspongia* sp. grown after 60 days from cut at around 20 m depth

Colony no.	*Growth*		*Number of new lobes*
	Δ Height (cm)	*Δ Width (cm)*	
1	0.5	1	5
2	3.5	3.6	0
3	0.5	4.2	4
4	3.5	2.9	0
5	3.0	1	2
6	3.0	2.5	2
Average	2.33	2.53	2.17

Sponge culture. The survival and growth rates of the sponge explanted at 6, 12 and 25 m depths were observed *in situ* by SCUBA diving. The sponge cultured at 6 m has lower survival and growth rates, while the sponge cultured at 12 and 25 m depths have higher survival and growth rates as shown in Table 4.

Table 4

The average growth and survival cultured sponges after 60 days

Depth	*Growth*			*Survival rate (%)*
	ΔL (cm)	*ΔW (cm)*	ΔA *(cm²)*	
6 m	-0.75	-1.50	- 1.125	40
12 m	1.40	1.50	2.100	80
25 m	1.50	2.25	3.375	80

ANOVA test indicated that depth has a significant effect on the sponge colonies growth (P=0.008). The further test, Tukey HSD test showed that the sponge growth rate at 60 days for 6 m was different with a sponge growth rate at 12 and 25 m, but there are no differences of the growth rates between 12 and 25 m (see Table 5).

Table 5

The Tukey test of the growth cultured sponges expressed by the area increment for 60 days

Test	*Depth*	*N*	*Subset*	
			1	*2*
Tukey HSD[a,b]	6	4	-78.325	
	12	4		120.825
	25	4		139.975
	Significance		1.000	0.931

Means for groups in homogeneous subsets are displayed based on observed means.
The error term is Mean Square (Error) = 5626.500.
a. Uses Harmonic Mean Sample Size = 4.000.
b. Alpha = 0.05.

(a) **(b)** **(c)**

Figure 1. The sponge *Candidaspongia* sp. explanted at 6 m depth showed loss of biomass (a), while the explants at 12 m (b), and 25 m (c) grew well.

Chemical analysis. The sponges were extracted with methanol pro analysis in laboratory of Biotechnology, department of Marine Sciences, Diponegoro University. Then, the crude extracts were separated into ethyl acetate (EA) and water fraction using a test tube. The result is shown in Table 6.

Table 6

Ethyl acetate and water extracts from cultured and natural sponges of *Candidaspongia* sp.

No.	*Sponge code*	*Sponge wet weight (g)*	*Ethyl acetate extract*		*Water extract*	
			Weight (mg)	*Content (%)*	*Weight (mg)*	*Content (%)*
1	C-12	14.97	0.14	0.93	0.26	1.63
2	C-25	16.56	0.20	1.24	0.30	1.82
3	N-12	26.70	0.05	0.17	0.61	2.30
4	N-20	20.48	0.09	0.46	0.20	0.96

Note: C-12: Cultured sponge at 12 m, C-25: Cultured sponge at 25 m, N-12: Natural sponge cultured at 12 m, N-20: Natural sponge cultured at 20 m.

The Mann-Whitney U test indicated that the ethyl acetate extract concentration in natural and cultured is no significantly difference (see Table 7a). However, water depth gives significant effect to the EA extract concentration both in natural and cultured sponges (see Table 7b and c).

Table 7

The Mann-Whitney U test of ethyl acetate extracts of the *Candidaspongia* sp.
a. Cultured vs natural sponge. b. The Shallow water vs deep water natural sponge extracts. c. The Shallow water vs deep water culture sponge extracts

a	EA extract
Mann-Whitney U	*5,000*
Wilcoxon W	*15,000*
Z	*-,866*
Asymp. Sig. (2-tailed)	*,386*
Exact Sig. [2(1-tailed Sig.)]*	*,486[b]*

a. Grouping variable: Depth
b. Not corrected for ties.

b	EA extract
Mann-Whitney U	*.000*
Wilcoxon W	*3.000*
Z	*-1.549*
Asymp. Sig. (2-tailed)	*.121*
Exact Sig. [2(1-tailed Sig.)]*	*.333[a]*

a. Not corrected for ties.
b. Grouping Variable: Depth

c	EA extract
Mann-Whitney U	*.000*
Wilcoxon W	*3.000*
Z	*-1.549*
Asymp. Sig. (2-tailed)	*.121*
Exact Sig. [2(1-tailed Sig.)]*	*.333[a]*

a. Not corrected for ties.
b. Grouping Variable: Depth

Discussion. Sponge *Candidaspongia* sp. is known as a source of bioactive compounds with unique structure and high potent as anticancer drug candidate called candidaspongiolide (Meragelman et al 2007; Trianto et al 2011; Whitson et al 2011). However, the scarcity of the sponges in nature and structural complexity of the candidaspongiolide have been hampering the development of the compounds into a commercial drug. Synthesis, the most preferred method by pharmaceutical companies, is an unsuitable method for mass production of the candidaspongiolides due to compounds containing many stereo-centers. Tadpecth et al (2017) showed the synthesis of a macrolide greensporone C using 16 steps with overall yield 3%. Synthesis of the (-)-hortonone C has also provided a yield as low as 1 % with 11 steps (Niroula et al 2017). Light has a great effect on the metabolism rate for the sponge-associated microorganisms since spicule can be used as light transduction for the microorganisms live inside the colony (Brümmer et al 2016). In turn, the metabolites will give an impact to the host.

To develop a mass production method, we conduct initial research for the sponge culture in Kupang water including the sponge inventory, recovery rate after cutting, and mariculture. Based on our observation, the sponge density in nature is quite low. The average sponge density is 1.7 colonies per 100 m line transect length or 17 colonies per km at around 20 m depth. Even, the sponge density at around 15 m depth is as lower as 0.3 colonies per 100 m transect length or 3 colonies per km length. However, a group of small sponge colonies could be found at 10 m below the port, a protected area either from strong current or light intensity. The situation leads to the assumption that strong current and light intensity may be the limiting factors for sponge growth. Larval swimming behavior is highly affected by light and temperature. The stronger light intensity reduces the swimming periods of the larval of sponge *Hymeniacidon perlevis* (Xue et al 2009).

Current is an important factor for the sponge growth because current brings the nutrient and oxygen, and at the same time flush the CO_2 and metabolisms products away. However, the strong current may damage the sponge colony. The sponge has photo sensory that are sensitive to the certain light wave (Muller et al 2006). The sponge grows on hard substratum such as dead coral.

All of the sponge colonies survived after the cut, and they have fully recovered after 60 days. However, the growth rates of the basal parts were varied among the colonies. The highest length and wide increments were 4.2 cm and 3.5 cm in 60 days, respectively. The fastest grew on the basal part with uncut lamellae, for example, the growing direction on sponge no. 2 was down the side where the hanging lamella was uncut. The lowest length and wide increments was 0.5 cm and 1.0 cm in 60 days, respectively. However, the sponges that were fully cut developed a growing strategy by increasing the number of lamellae instead of increase the colony size. Even though the *Candidaspogia* sp. maintains its colony basic shape as lamella, but it may become a complex branching lamellar colony with various thickness. The complexity of growth form has correlated with water depth and the protected level of the natural habitat the sponge. The sponge able to survive after cut, however, the smaller size will reduce the survival rate (Duckworth and Wolff, 2007). Naturally, sponge mortality mostly caused by sedimentation and diseases, even though, sponge has predators but they do not cause mortality on the sponge (Wulff 2006a; Bell et al 2017). Wulff reported that erect sponge has 70% survival rate post damage by hurricane. Sponge has also change the morphology for adaptation to the ecological condition (Wulff 2006b).

Even though the *Candidaspogia* sp. maintains its colony basic shape as lamella, but it may become a complex branching lamellar colony with various thickness. The complexity of growth form has correlated with water depth and the protected level of the natural habitat the sponge.

The sponges were able to survive after cut, however, the smaller size will reduce the survival rate (Duckworth & Wolff 2007). Naturally, sponge mortality was mostly caused by sedimentation and diseases, even though, sponges have predators but they do not cause mortality on the sponge (Wulff 2006a; Bell et al 2017). Wulff (2006a) reported that erect sponge has 70% survival rate post damage by hurricane. Sponge has also change the morphology for adaptation to the ecological condition (Wulff 2006b).

Mariculture is a feasible method for mass production of the sponge; even though, the further study still needed to standardize and to improve the method. The sponges explanted at 12 m and 25 m depth have an 80% survival rate. However, the sponges explanted at 6 m depth had only a 40% survival rate). The explant with four side incision could not survive due to loss of the mass and died or untied and driven away by the current. The explants grown in 6 m depth indicated the loss of mass because the sponge dead or lost (see Figure 1). Louden et al (2007) reported the *in situ* culture of sponges *Rhopaloeides odorabile,* and *Coscinoderma* sp. have survival rates of 65% and 90% for 78 days. The sponge has also dead up to 3% naturally, and up to 7% caused by accidentally fishing (Butler et al 2017). The total growth of *R. odorabile* (146.0±40.3%) and *Coscinoderma* sp. (195.9±39.8%) was not significantly different over the 21 month experimental period but was highly variable between explants from the same individual.

Descriptively, the sponge explanted in deeper water has a higher average growth rate than those explanted at the shallower water. The average growth rate of sponges explanted at 25 m, 12 m, and 6 m depth were 3.375 cm^2, 2.1 cm^2, and -1.125 cm^2, respectively. Size of the sponge explanted at 6 m decreased due to loss of biomass. However, further analyses with ANOVA showed a significant effect between the culture depth. There are many factors related to a depth that may affect *Candidaspongia* sp. live and growth such as pressure, light intensity, nutrient, current, and sedimentation rate. We observed four small colonies of the sponge Candidaspongia that grow at 10 m depth below the harbor, a small and protected area either from current or direct sunlight. Based on our observation, there are only few *Candidaspongia* sp. that grow in open area, and among them usually, grow under the hard coral or crevices. Further environmental study is needed to reveal the key factor of the sponge growth rate.

Our previous research showed that the anticancer compounds the candidaspongiolide and its analogs were obtained from organic fraction (Trianto et al 2011). So, in this study, we pay more attention to the ethyl acetate content in the sponge. The EA extract content in the transplanted and the natural sponges were not significantly different (Mann-Whitney test, U=6.00). However, water depth affects not only the growth rate and survival rate but also the chemical content. The EA extracts of the natural and explanted sponges were affected by the depth. The extracts content in transplanted sponges were higher in the deeper water (Mann-Whitney test, U=0.00), on the other hand, the extract contents in natural sponges were higher in the lower water (Mann-Whitney test, U=0.00). Naturally, sponges produce bioactive compounds that support their survival, including from bacterial infection. The marine sponge reported produces the antibacterial compounds to protect the colony (Yu et al 2017).

The development of large-scale production of the candidaspongiolide via sponge culture is still in a preliminary study that needs many strategies to overcome the problem regarding the environmental factors and the number of explants. However, the difficulties are in proportion to the potency of the sponge as a source of the anticancer drug candidate. Sponge culture is a promising method to overcome the bottleneck in drug development and to avoid the over-exploitation of wild population (Pérez-López et al 2014).

Conclusions. The sponge *Candidaspongia* sp. density in deeper water is higher than in the lower water. All of the sponges were survive after the cut, and they have fully recovered in 60 days. The growth rate of the explants were 10-60% and 73-203% at 12 m and 25 m respectively. The explants in 6 m have a negative growth rate.

The sponges explanted at 12 and 25 m depth have higher survival and growth rates than the sponge explanted at 6 m depth. The sponge explanted in deeper water has higher EA extract than those explanted at the shallower water.

Acknowledgements. We thank Yusup, S.Kel of The Nature Conservation Kupang for assisting in field work. We also indebted BKKPN-Kupang for providing Diving Equipment. This work and publication partially supported by BBKSDA Kupang and Faculty of Fisheries and Marine Science Grant 2018.

References

Butler M. S., 2004 The role of natural product chemistry in drug discovery. Journal of Natural Product 67:2141-2153.

Brümmer F., Pfannkuchen M., Baltz A., Hauser T., Thiel V. 2008 Light inside sponges. Journal Experimental Marine Biology and Ecology 367(2):61-64

Butler M. S., Behringer D. C., Valentine M. M., 2017 Commercial sponge fishery impacts on the population dynamics of sponges in the Florida Keys, FL (USA). Fisheries Research 190:113-121.

Balansa W., Trianto A., de Voogd N. J., and Tanaka J., 2017 A new cytotoxic polyacetylenic alcohol from a sponge *Callyspongia* sp. Natural Product Communications 12(12):1009-1011.

Cleary D. F. R., Becking L. E., de Voogd, N. J., Renema W., de Beer M., van Soest R. W. M., Hoeksema B. W., 2005 Variation in the diversity and composition of benthic taxa as a function of distance offshore, depth and exposure in the Spermonde Archipelago, Indonesia. Estuarine, Coastal and Shelf Science 65:557-570.

De Caralt S., Agell G., Uriz M. J., 2003 Long-term culture of sponge explants: conditions enhancing survival and growth, and assessment of bioactivity. Biomolecular Engineering 20:339-347.

Duckworth A. R. and Wolff C., 2007 Bath sponge aquaculture in Torres Strait, Australia: Effect of explant size, farming method and the environment on culture success. Aquaculture 271:188-195.

Mayer A. M. S., Rodríguez A. D., Berlinck R. G. S., Hamann M. T., 2009 Marine pharmacology in 2005–6: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities, affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action. Biochimica et Biophysica Acta 1790:283-308.

Mendola M., 2003 Aquaculture of three phyla of marine invertebrates to yield bioactive metabolites: process developments and economics. Biomolecular Engineering 20:441-458.

Meragelman T. L., Willis R. H., Woldemichael G. M., Heaton A., Murphy, P. T., Snader K. M., Newman D. J., van Soest R. W. M., Boyd M. R., Cardellina II J. H., McKee T. C., 2007 Candidaspongiolides, distinctive analogues of tedanolide from sponges of the genus *Candidaspongia*. Journal of Natural Product 70:1133-1138.

Milanese M., Chelossi E., Manconi R., Sara A., Sidri M., Pronzato R., 2003 The marine sponge *Chondrilla nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. Biomolecular Engineering 20:363-368.

Muller W. E. G., Wendt K., Geppert C., Wiens M., Reiber A., Schroder H.C., 2006 Novel photoreception system in sponges? Unique transmission properties of the stalk spicules from the hexactinellid *Hyalonema sieboldi*. Biosensors and Bioelectronics 21:1149-1155.

Müller W. E. G., Böhm M., Batel R., De Rosa S., Tommonaro G., Müller I. M., Schröder H. C., 2000 Application of cell culture for the production of bioactive compounds from sponges: synthesis of avarol by primmorphs from *Dysidea avara*. Journal of Natural Product 63:1077-1081.

Niroula D., Hallada l. P, Rogelj S., Tello-Aburto R. 2017 A total synthesis of (-)-hortonone C. Tetrahedron 73:359-364.

Osinga R., Belarbi E. H., Grima E. M., Tramper J., Wijffels R. H., 2003 Progress towards a controlled culture of the marine sponge *Pseudosuberites andrewsi* in a bioreactor. Journal of Biotechnology 100:141-146.

Pérez-López P., Ternon E., González-García S., Genta-Jouve G., Feijoo G., Thomas O. P., Moreira M. T. 2014. Environmental solutions for the sustainable production of bioactive natural products from the marine sponge *Crambe crambe*. Science of the Total Environment 475:71-82.

Schmitz F. J., Gunasekera S. P., Yalamanchili G., Hossain M. B., van der Helm D., 1984 Tedanolide: a potent cytotoxic macrolide from the Caribbean sponge *Tedania ignis.* Journal of American Chemical Society 106:7251-7252.
Sipkema D., Osinga R., Schatton W., Mendola D., Tramper J., Wijffels R. H., 2005 Large-Scale Production of Pharmaceuticals by Marine Sponges: Sea, Cell, or Synthesis? Biotechnology and Bioengeering 90:201-222.
Smith A. B., Lee D., 2007 Total Synthesis of (+)-Tedanolide. Journal of American Chemical Society 129:10957-10962.
Tadpetch K., Jeanmard L., Rukachaisirikul V. 2017 Total synthesis of greensporone C. Tetrahedron Letters 58:3453-3456.
Trianto A., Hermawan I., Suzuka T., Tanaka J., 2011 Two new cytotoxic candidaspongiolides from an Indonesian sponge. ISRN Pharmaceutics, article ID 852619, 6 pages, doi:10.5402/2011/852619.
Trianto A., de Voogd N.J., Tanaka J., 2014 Two new compounds from an Indonesian sponge *Dysidea* sp. Journal of Asian Natural Product Research 16(2):163-168.
Xue L, Zhang X., Zhang W., 2009 Larval release and settlement of the marine sponge *Hymeniacidon perlevis* (*Porifera*, *Demospongiae*) under controlled laboratory conditions. Aquaculture 132:290-139.
Whitson E. L., Pluchino K. M., Hall M. D., McMahon J. B., McKee T. C., 2011 New candidaspongiolides, tedanolide analogues that selectively inhibit melanoma cell Growth. Organic Letter 13:3518-3521.
Wulff J. L., 2006[a] Rapid diversity and abundance decline in a Caribbean coral reef sponge community. Biological Conservation 127:167-176.
Wulff J. L., 2006[b] Resistance vs recovery: morphological strategies of coral reef sponges. Functional Ecology 20:699-708.
Yu H. B., Gu B. B., Wang S. P., Cheng C. W., Yang F., Lin H. W., 2017 New diterpenoids from the marine sponge *Dactylospongia elegans*. Tetrahedron 73:6657-6661.

Received: 18 May 2018. Accepted: 21 May 2019. Published online: 5 January 2020.
Authors:
Agus Trianto, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia. Marine Biopharmaceutical Research and Development Center, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: agustrianto.undip@gmail.com
Ambariyanto, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia. Marine Biopharmaceutical Research and Development Center, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: ambariyanto.undip@gmail.com
Sarjito, Department of Fisheries, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: sarjito_msdp@yahoo.com
Rini Pramesti, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: rinipramesti63@gmail.com
Nirwani Soenarjo, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: nirwanisoenardjo@gmail.com
Retno Hartati, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: retnohartati.undip@yahoo.com
Nur Taufiq-Spj, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: taufiqspj_1999@yahoo.com
Hadi Endrawati, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: hadiendrawati@yahoo.co.id
Destio, Department of Marine Sciences, Faculty of Fisheries and Marine Sciences, Diponegoro University, St. Prof. Soedarto, SH., Tembalang, Semarang, Central Java, Indonesia, e-mail: desdestiotio@gmail.com
Isai Yusidarta, The Natural Resources Conservation Agency (BBKSDA), St. Perintis Kemerdekaan Kelapa Lima, Kupang, Indonesia, e-mail: isaiyusidarta@yahoo.com

How to cite this article:
Trianto A., Ambariyanto, Sarjito, Pramesti R., Soenarjo N., Hartati R., Taufiq-Spj N., Endrawati H., Destio, Yusidarta I., 2020 Ecological study and preliminary culture of the sponge *Candidaspongia* a source of anticancer molecules. AACL Bioflux 13(1):36-45.