In vitro Anticancer Activity of Marine Sponges Against T47D and HeLa Cell Lines

Suciati[1,2] and Lusiana Arifianti[1]

[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga
Nanizar Zaman Joenoes Building, Jl. Mulyorejo, Surabaya 60115, East Java, Indonesia
[2]Natural Product Medicine Research Development, Institute of Tropical Diseases, Universitas Airlangga
Jl. Mulyorejo, Surabaya 60115, East Java, Indonesia

(Received: December 23, 2018; Accepted: May 10, 2020; Published (web): June 28, 2020)

ABSTRACT: Marine sponges have been known as the source of natural products. Various metabolites with potent bioactivities have been reported from this organism. The current study aims to investigate the anticancer potency of three marine sponges namely *Diacarnus debeauforti*, *Haliclona amboinensis* and *Agelas cavernosa* collected from Barrang Lompo Island, South Sulawesi, Indonesia. The ethyl acetate extracts of the sponges were screened against T47D breast cancer cells and HeLa cervical cancer cells by using the MTT method. The results showed that these sponges demonstrated anticancer activity against both cancer cell lines. The lowest IC_{50} of 18.2 µg/ml was given by the extract of *A. cavernosa* against T47D cell line, while in the screening against HeLa cancer cell line, the extract of *D. debeauforti* revealed the highest potency with IC_{50} of 15.7 µg/ml. Our results suggested that the marine sponges namely *D. debeauforti*, *H. amboinensis* and *A. cavernosa* can be good candidates for the development of anticancer agents.

Key words: Marine sponges, Anticancer, T47D, HeLa, MTT

INTRODUCTION

The oceans, which cover 70% of the Earth's surface, is a unique environment consisting of extreme variation in pressure, salinity, and temperature which have been the habitat of various organisms, including sponges. Amongst marine resources, sponges have been the focus of study for many years. So far more than 15,000 species have been discovered worldwide. Diverse metabolites have been reported from marine sponges and many of these compounds showed pronounced bioactivity including anticancer property.[1,2]

Cancer is still a major health problem, which causes a large number of deaths around the world. According to WHO, it is estimated that in 2020, cancer would kill almost 10.3 million people unless they take appropriate action to combat this illness.[3] In recent years, investigation of marine sponges as anticancer agents has produced a considerable number of drug candidates. Example of marine sponge-derived anticancer agents includes, cytarabine (Ara-C), a derivative from the Caribbean sponge *Tethyacrypta* and eribulin mesylate, a derivative developed from *Halichondria okadai*. These two anticancer agents have been approved by the FDA and marketed in the United States.[4,5]

In our ongoing research, we investigated the bioactivity of marine sponges before classifying and isolating their bioactive metabolites. In the previous study, we have reported the antiviral activity of several marine sponges collected from Barrang Lompo, South Sulawesi, Indonesia.[6] This current study aims to investigate the anticancer activity of three marine sponges, namely *Diacarnus debeauforti*, *Haliclona amboinensis* and *Agelas cavernosa* collected from the same location. The samples were

Correspondence to: Suciati
E-mail: suciati@ff.unair.ac.id
Phone: +62-81-231-779-793;

Dhaka Univ. J. Pharm. Sci. **19**(1): 25-28, 2020 (June)

DOI: https://doi.org/10.3329/dujps.v19i1.47815

screened against human breast cancer cell line (T47D) and cervical cancer cell line (HeLa) using MTT method.

MATERIALS AND METHODS

Sponge collection. Sponges were collected using SCUBA at a depth of 8-10 m from the Barrang Lompo Island, Makassar, South Sulawesi on May 17, 2014. Samples were kept in a plastic pack in ice boxes immediately after collection and transported to Surabaya. Sponge specimens were then frozen at -20°C until analysis. Identification of sponges was conducted in Ecology Laboratory, Department of Biology, Faculty of Mathematics and Sciences, Institut Teknologi Sepuluh November. Voucher specimens have been kept in ethanol (70%) at the Faculty of Pharmacy, Universitas Airlangga under accession number 17-5-14-6, 17-5-14-9 and 17-5-14-14.

Extraction. Small scale extraction of the sponges was carried out. Fresh sponges (wet weight 13-60 g) were sliced into small pieces and filled in a glass container. Samples were extracted in a mixture of dichloromethane-methanol (1:1, 100 ml) by using ultrasonic vibration for 3 × 5 mins each extraction. The solvent was removed by filtration. The residue was then re-extracted using the same procedure twice. The collected filtrate was evaporated under reduced pressure to give an aqueous residue, which was then partitioned with ethyl acetate (3 × 50 ml). The organic layer was removed, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give brown viscous extracts.

***In vitro* cytotoxicity assay.** A modification of the method described by Freshny (2010) was used.[7] T47D and HeLa cells were cultured in RPMI 1640 medium containing fetal bovine serum 10% and penicillin-streptomycin 1% (v/v). Cells (5×10^3 cells/wells) were transferred to 96 well plate and incubated for 24 hrs in 37°C, 5% CO_2 (70-80% confluent). Sponge extracts were dissolved in DMSO and further diluted with media to make a series of concentrations. The final concentration of DMSO in the test solution was not more than 1%. Cells were then treated with a serial dilution of sponge extracts. After 24 h of incubation, MTT [3-(4,5-dimethylthiazol-2-yl)2-5-diphenyl tetrazolium bromide] 0.5 mg/ml was added to each wells, followed by incubation for 4 h. A solution of sodium dodecylsulfate (10%) in 0.1 N HCl was added to dissolve formazan crystals. Cells were further incubated overnight atroom temperature and protected from light. Reaction mixtures were homogenized by shaking for 0.5 min before measurement of absorbance using ELISA reader at λ 595 nm. Percentage of cell viability was calculated from triplicate observations by using the equation below and the IC_{50} values were determined by Probit analysis using SPSS software. Experiments were done in triplicate.

$$\%\,Viability = \left(\frac{absorbance\ of sample - absorbance\ of control\ media}{absorbance\ of\ control\ cell - absorbance\ of control\ media}\right) x\ 100\%$$

RESULTS AND DISCUSSION

Cytotoxic test against cancer cell lines is one of the most widely used assay to preliminary screen natural product extracts to be developed as anticancer drugs. In this study, the MTT method was chosen because it offers many advantages as it is highly reliable, simple, applicable to a wide range of cells and can be performed in microtitre plates. The assay was based on the colorimetric reaction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide with enzyme dehydrogenase inside living cells to form a colored formazan dye, which corresponded to the number of viable cells.[8]

In this study, the ethyl acetate extracts of three marine sponges, namely *D. debeauforti*, *H. amboinensis* and *A.cavernosa*, were prepared and subjected to anticancer screening against human breast carcinoma cell (T47D) and human cervica; carcinoma cell (HeLa). The results presented in figures 1-2 and table 1 demonstrated that all three

extracts had inhibitory effect against cancer cell growth. In the assay against T47D cells, the highest inhibitory activity was given by *A. cavernosa* with an IC_{50} value of 18.1 µg/ml. The other two extracts, *D. debeauforti* and *H. amboinensis* displayed similar potency with IC_{50} values of 32.7 and 29.0 µg/ml, respectively. The extract of *D. debeauforti* exhibited the strongest cytotoxic activity against HeLa cell with an IC_{50} value of 15.4 µg/ml. In this assay *H. amboinensis* also demonstrated a strong anticancer activity, however, extract of *A. cavernosa* only showed moderate cytotoxicity against HeLa as reflected from IC_{50} value of 68.6 µg/ml.

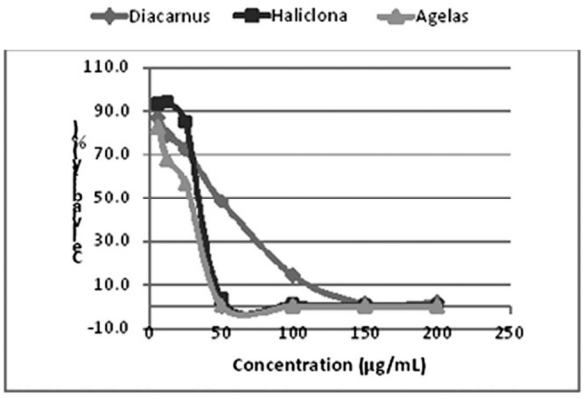

Figure 1. Effect of marine sponges extracts on cell viability in T47D cells.

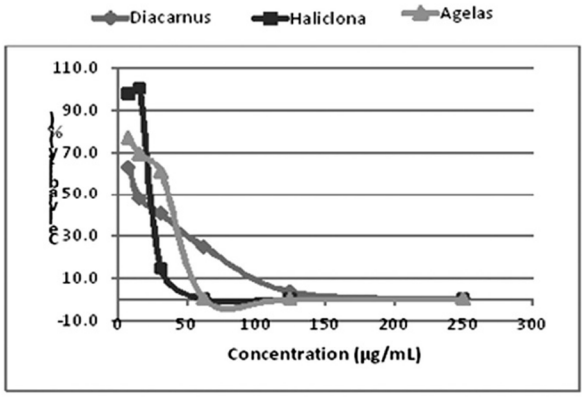

Figure 2. Effect of marine sponges extracts on cell viability in HeLa cell lines.

Table 1. IC_{50} values of marine sponge extracts against T47D and HeLa cell lines.

Samples	IC_{50} values	
	T47D cells	HeLa cells
D. debeauforti	32.7 ± 1.5	15.4 ± 3.3
H. amboinensis	29.0 ± 1.5	19.4 ± 2.3
A. cavernosa	18.1 ± 4.3	68.6 ± 1.6

Data are represented as mean ± SD (standard deviation); Experiments were done in triplicate; IC_{50}: Concentration of extracts causing 50% inhibition of the cells.

Marine sponges from the genus *Diacarnus*, *Haliclona* and *Agelas* have been reported for their activity against various cancer cells. Sponges from the genus *Diacarnus* have been known to contain unique norsesterterpene cyclic peroxide derivatives and many of them demonstrated anticancer activity.[9-12] The examples include epimuqubilin B and diacarperoxide F isolated from Indonesian *Diacarnus megaspinorhabdosa* which exhibited cytotoxic activity against HeLa cell with EC_{50} of 1.00 and 0.60 µg/ml, respectively.[10] Several metabolites isolated from *Agelas* spp. have shown anticancer property, such as bromopyrolle alkaloid agelasine B from Okinawan *Agelas* sp. which exerted cytotoxic activity against murine lymphoma (L1210) cell at IC_{50} of 3.1 µg/ml.[3,13] Agelasine B obtained from *A. clathrodes* also showed anticancer activity by inducing apoptosis in human breast cancer cell MCF7.[14] The ethyl acetate extract of marine sponge *Haliclona exigua* exhibited several activities including anticancer property against Hep2 and MCF7 with IC_{50} of approximately 31 µg/mL.[15] The methanolic extract of *Haliclona* sp. showed both dose and time-dependent cytotoxicity against non-small lung cancer cell (A549), where the extract demonstrated significant inhibition of cell proliferation and viability.[16]

CONCLUSION

The results of the present study revealed evidence for potential anticancer activities of marine sponges namely *D. debeauforti*, *H. amboinensis* and *A. cavernosa* and provided supportive data for future discovery of anticancer lead compounds. Further study, such as bioassay-guided isolation is required in order to determine the active metabolites.

ACKNOWLEDGEMENT

Authors acknowledge the Faculty of Pharmacy, Universitas Airlangga for BOPTN research grant.

REFERENCES

1. Ye, J., Zhou, F., Al-Kareef, A.M.Q. and Wang, H. 2015. Anticancer agents from marine sponges. *J. Asian Nat. Prod. Res.* **17**, 1-23.
2. Blunt, J.W., Carroll, A.R., Copp, B.R., Davis, R.A., Keyzers, R.A. and Prinsep, M.R. 2018. Marine natural products. *Nat. Prod. Rep.* **35**, 8-53.
3. Schumacher, M., Kelkel, M., Dicato, M. and Diederich, M. 2010. A survey of marine natural compounds and their derivatives with anti-cancer activity reported in 2010. *Molecules.* **16**, 5629-5646.
4. Gerwick, W.H. and Moore, B.S. 2012. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. *Chem. Biol.* **19**, 85-98
5. Calcabrini, C., Catanzaro, E., Bishayee, A., Turrini, E. and Fimognari, C. 2017. Marine sponge natural products with anticancer potential: an updated review. *Mar. Drugs*. **15**, 310-343.
6. Suciati, Fauzi, A., Adianti, M. and Hafid, A.F. 2018. Potency of a marine sponge from the genus *Stylissa* as anti hepatitis C agent. *Malays. Appl. Biol.* **47**, 121-127
7. Fresney, R.I. 2010. *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications*. John Wiley and Sons, New Jersey, pp. 365-377.
8. McCauley, J., Zivanovic, A. and Skropeta, D. 2013. Bioassays for anticancer activities. *Methods Mol. Biol.* **1055**, 191-205.
9. Dai, J., Liu, Y., Zhou, Y.D. and Nagle, D.G. 2007. Hypoxia-selective antitumor agents: norsesterterpene peroxides from the marine sponge *Diacarnus levii* preferentially suppress the growth of tumor cells under hypoxic conditions. *J. Nat. Prod*. **70**, 130-133.
10. Ibrahim, S.R.M., Ebel, R., Wray, V., Müller, W.E.G., Edrada-Ebel, R. and Proksch, P. 2008. Diacarperoxide, norterpene cyclic peroxide from the sponge *Diacarnus megaspinorhabdosa*. *J. Nat. Prod*. **71**, 1358-1364.
11. Ibrahim, S.R.M. 2012. Diacarperoxide S, new norterpene cyclic peroxide from the sponge *Diacarnus megaspinorhabdosa*. *Nat. Prod. Commun*. **7**, 9-12
12. Ibrahim, S.R.M., Al Haidari, R.A. and Mohamed, G.A. 2014. Megaspinoxide A: new norterpene cyclic peroxide from the sponge *Diacarnus megaspinorhabdosa*. *Nat. Prod. J.* **4**, 38-42.
13. Ishida, K., Ishibashi, M., Shigemori, H., Sasaki, T. and Kobayashi, J. 1992. Agelasine G, a new antileukemic alkaloid from the okinawan marine sponge *Agelas* sp. *Chem. Pharm. Bull.* **40**, 766-767.
14. Pimentel, A.A., Felibertt, P., Sojo, F., Colman, L., Mayora, A., Silva, M.L., Rojas, H., Dipolo, R., Suarez, A.I., Compagnone, R.S., Arvelo, F., Galindo-Castro, I., De Sanctis, J.B., Chirino, P. and Benaim, G. 2012. The marine sponge toxin agelasine B increases the intracellular $Ca^{(2+)}$ concentration and induces apoptosis in human breast cancer cells (MCF-7). *Cancer Chemother. Pharmacol*. **69**, 71-83.
15. Bhimba, V., Beulah, M.C. and Vinod, V. 2013. Efficacy of bioactive compounds extracted from marine sponge *Haliclona exigua*. *Asian J. Pharm. Clin. Res*. **6**, 343-345.
16. Bae, W., Lim, H.K., Kim, K.M., Cho, H., Lee, S.Y., Jeong, C.S., Lee, H.S and Jung, J. Apoptosis-inducing activity of marine sponge *Haliclona* sp. extracts collected from Kosrae in non-small cell lung cancer A549 cells. *Evid. Based Complement. Alternat. Med*. Article ID 717959.