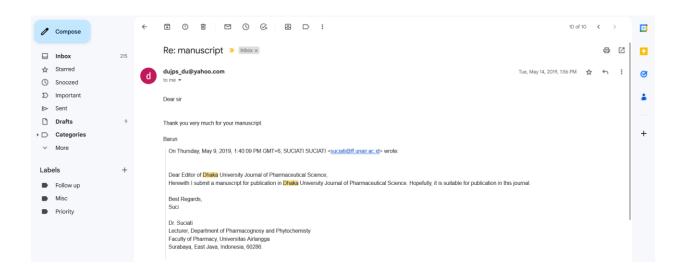
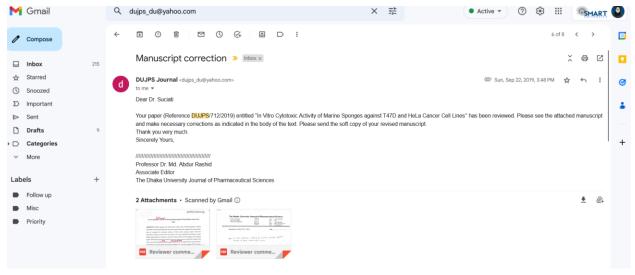
1. Proses Submit



2. Proses Review



In Vitro Cytotoxic Activity of Marine Sponges against T47D and HeLa Cancer Cell Lines

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 MTT method. The results showed that the three sponges gave anticancer activity against both cancer cell lines. The lowest IC₅₀ of 18.2 μg/mL was given by extract of *A. cavernosa* against T47D cell line, while in the screening against HeLa cancer cell line, the extract of *D. debeauforti* gave the highest potency with IC₅₀ of 15.7 μg/mL. Our results suggested that marine sponges *D. debeauforti*, *H. amboinensis* and *A. cavernosa* can be candidates for development of anticancer agents.

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

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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 World Wide. Diverse metabolites have been reported from marine sponges and many of these compounds showed pronounced bioactivity including anticancer.

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 10.3. million people each year unless they take appropriate action to combat this illness. In recent years, investigation of marine sponges as an anticancer agent has produced a considerable number of drug candidates. Example of marine sponge-derived anticancer agents including, we 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 FDA and marketed in the USA. 455

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. 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 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 around 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 freezed 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 were kept in ethanol 70% at Faculty of Pharmacy, Universitas Airlangga under accession number 17-5-14-6, 17-5-14-9 and 17-5-14-14.

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In vitro cytotoxicity assay. A modification of the method described by Freshny (2010) was used. T47D and HeLa cells were cultured in RPMI 1640 medium containing foetal bovine serum 10% and penicillin-streptomycin 1% (v/v). Cells (5 x 10³ cells/wells) were transferred to 96 well plate and incubated for 24 h in 37°C, 5% CO2 (70-80% confluent). Cells were then treated with serial dilution of sponge extracts made in DMSO. After 24 h incubation, MTT (3-(4,5-dimethylthiazol-2-yl)2-5-diphenyl tetrazolium bromide) of mg/ml were added to each wells, followed by incubation for 4 h. A solution of sodium dodecylsulfate (10%) in 0-11 N HCl was added to dissolve formazan crystal. Cells were further incubated overnight in room temperature and protected from light. Reaction mixtures were homogenize by shaking for 0.5 min before measurement of absorbance using ELISA reader at λ 595 nm. Percentage of cell viability was calculated by using the equation below and the IC₅₀ values were determined by Probit analysis using SPSS software. Experiments were done in triplicate.

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

RESULTS AND DISCUSSION

Cytotoxic test against cancer cell lines is one of the most widely used assays to preliminary screen natural product extracts to be developed as an anticancer drug. 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, ethyl acetate extracts of three marine sponges (Figure 1), namely Diagrams debeauforti, Halicloria amboinensis and Agelas cavernosa, were prepared and subjected to anticancer screening against human breast carcinoma cell T47D and human

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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 compounds and many of them demonstrated anticancer activity. Fixample include epimuqubilin B and diacarperoxide F isolated from Indonesian *Diacarnus megaspinorhabdosa* which exhibited cytotoxic activity against HeLa cell with EC50 of 1.00 and 0.60 μg/mL, respectively. 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 IC50 of 3.1 μg/mL, Agelasine B obtained from *Agelas clathrodes* also showed anticancer activity by inducing apoptosis in human breast cancer cell MCF7. The ethyl acetate extract of marine sponge *Haliclona exigua* demonstrated several activities including anticancer against Hep2 and MCF7, with IC50 of approximately 31 μg/mL. The methanolic extract of *Haliclona* sp. have shown both dose and time-dependent cytotoxicity against nonsmall cell lung cancer (A549), the extract demonstrated significant inhibition of cell proliferation and viability.

CONCLUSION

The results of the present study provided evidence for potential anticancer activities of marine sponges Diagrams debeauforti. Halielona amboinensis and Agelas cavernosa and provide supportive data for future development of their usage in cancer therapy. Further study is needed, such as bioassay-guided isolation in order to determine the active metabolites.

discovery

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ACKNOWLEDGEMENT

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

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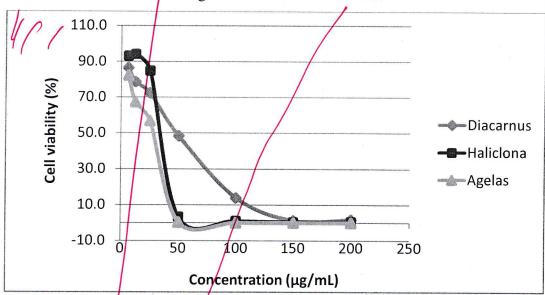


Figure 1. Effect of marine sponges extracts on cell viability in T47D cells

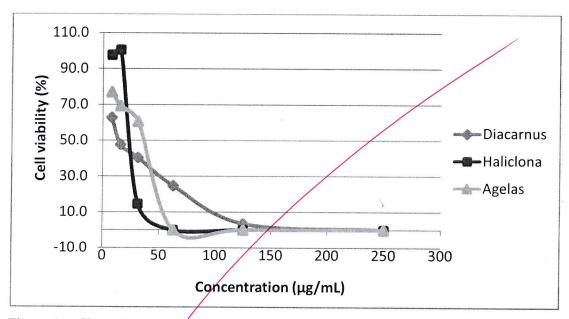


Figure 2. Effect of marine sponges extracts on cell viability in HeLa cells

Reviewer 2

The Dhaka University Journal of Pharmaceutical Sciences

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Manuscript No.: DUJPS/ ヲバ	2 /2019	Date
Title: In vitoro cyto	otoxic activi eLa Comessi (ty of marine sponges against cell lines.
Report of the Reviewer:		
Is the contribution new and substantial?	Yes □ No □	Is the literature citation satisfactory? Yes $\hfill\square$ No $\hfill\square$
Is the experimental design adequate?	Yes □ No □	Is the paper sufficiently concise? Yes□ No □
Are the statistics adequate, if any?	Yes □ No □ NA □	Is the linguistic quality satisfactory? Yes □ No □
Are the formulae correct?	Yes □ No □ NA □	Are all the tables and illustrations necessary?
		Yes No NA NA
Is/are the structure(s) properly characterized?	Yes □ No □ NA □	If not delete Table(s) □ □ Illustrations □ □
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Table 1. IC_{50} values of marine sponges extracts against T47D and HeLa Cells

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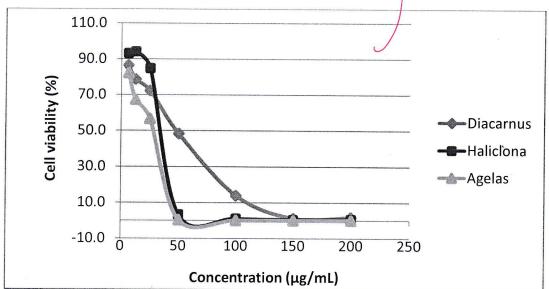


Figure 1. Effect of marine sponges extracts on cell viability in T47D cells

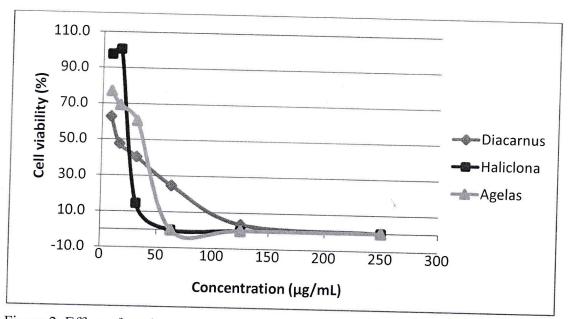


Figure 2. Effect of marine sponges extracts on cell viability in HeLa cells

Tanggapan Terhadap Komentar Reviewers

Response to the reviewer comments

Manuscript Title: In Vitro Cytotoxic Activity of Marine Sponges against T47D and HeLa

Cancer Cell Lines

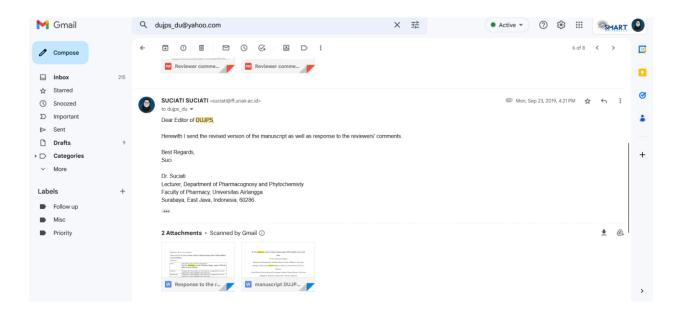
Reviewer 1.

Title	The title has been revised as susggested to In Vitro Anticancer Activity of Marine Sponges against T47D and HeLa Cancer Cell Lines
Abstract	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
Introduction	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
Material and Method	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
Results and Discussion	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
Conclusion	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
References	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
Table 1	Changes have been made in several sentences as suggested by reviewer indicated by yellow highight in the manuscript
Figure 1 and 2	Author prefer to keep figures 1-2 since it is needed to explain the results of the study.

Reviewer 2.

Abstract	Changes have been made in several sentences as suggested by reviewer indicated by green highlight in the manuscript
Introduction	Changes have been made in several sentences as suggested by reviewer indicated by green highlight in the manuscript
Material and Method	Explanation how the samples were prepared has been added.
Results and Discussion	 Author prefer to keep paragraph 1 in the manuscript since it explains why the method used in this study Changes have been made in several sentences as suggested by reviewer indicated by green highlight in the manuscript
Table 1	Number of replicates have been added "Experiments were done in triplicate."

Figure 1 and 2 The concentration of DMSO used in this study is less than 1%, and based on our study this concentration did not kill the cells.



3. Artikel Diterbitkan

