

Volume 17, Number 1
June, 2018

ISSN: 1816-1820 (Print version)
1816-1839 (Online version)

The Dhaka University Journal of
**Pharmaceutical
Sciences**



A Publication of the Faculty of Pharmacy
University of Dhaka, Dhaka-1000, Bangladesh

A PEER REVIEWED JOURNAL
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Dhaka University Journal of Pharmaceutical Sciences
ISSN 1816-1820 eISSN 1816-1839



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Published: 2020-06-30

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Dhaka University Journal of Pharmaceutical Sciences
ISSN 1816-1820 eISSN 1816-1839

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

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(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 IC₅₀ 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 IC₅₀ 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

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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 Na₂SO₄ 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 × 10³ cells/wells) were transferred to 96 well plate and incubated for 24 hrs in 37°C, 5% CO₂ (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 at room 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 IC₅₀ values were determined by Probit analysis using SPSS software. Experiments were done in triplicate.

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

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 cervical 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 IC₅₀ value of 18.1 µg/ml. The other two extracts, *D. debeauforti* and *H. amboinensis* displayed similar potency with IC₅₀ 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 IC₅₀ 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 IC₅₀ value of 68.6 µg/ml.

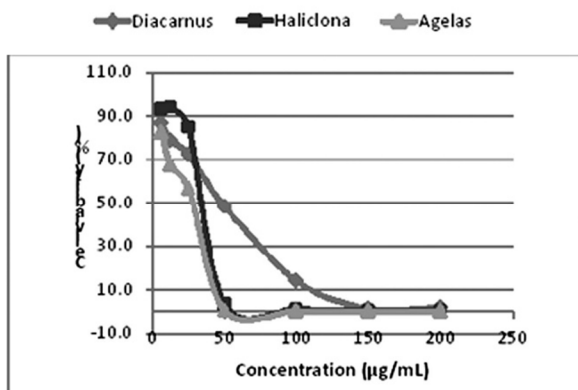


Figure 1. Effect of marine sponges extracts on cell viability in T47D cells.

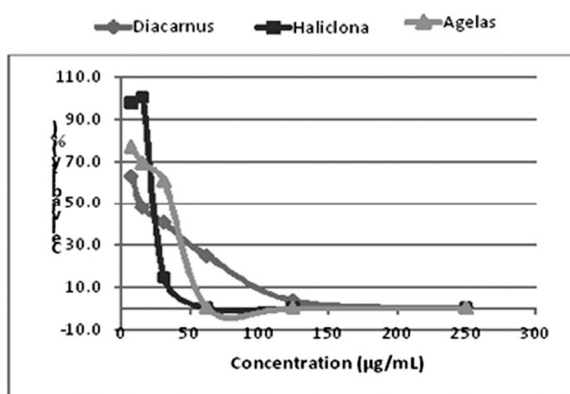


Figure 2. Effect of marine sponges extracts on cell viability in HeLa cell lines.

Table 1. IC₅₀ values of marine sponge extracts against T47D and HeLa cell lines.

Samples	IC ₅₀ values	
	T47D cells	HeLa cells
<i>D. debeauforti</i>	32.7 ± 1.5	15.4 ± 3.3
<i>H. amboinensis</i>	29.0 ± 1.5	19.4 ± 2.3
<i>A. cavernosa</i>	18.1 ± 4.3	68.6 ± 1.6

Data are represented as mean ± SD (standard deviation); Experiments were done in triplicate; IC₅₀: 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 epimuquibilin B and diacarpoxide F isolated from Indonesian *Diacarnus megaspinorhabdosa* which exhibited cytotoxic activity against HeLa cell with EC₅₀ of 1.00 and 0.60 µg/ml, respectively.¹⁰ Several metabolites isolated from *Agelas* spp. have shown anticancer property, such as bromopyrrole alkaloid agelasine B from Okinawan *Agelas* sp. which exerted cytotoxic

activity against murine lymphoma (L1210) cell at IC₅₀ 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 IC₅₀ 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.

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Dhaka University Journal of Pharmaceutical Sciences

Scopus coverage years: from 2007 to 2018

(coverage discontinued in Scopus)

Publisher: University of Dhaka

ISSN: 1816-1820 E-ISSN: 1816-1839

Subject area: Pharmacology, Toxicology and Pharmaceutics: Pharmaceutical Science Medicine: Pharmacology (medical)

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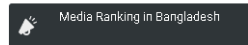
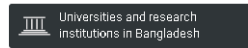
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PUBLICATION TYPE

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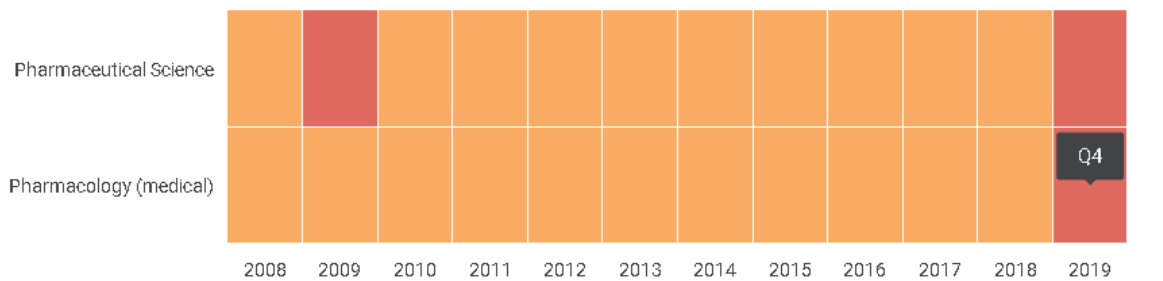
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