RESEARCH ARTICLE

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Aaptamine-rich Fraction from the Indonesian Marine Sponge, *Aaptos suberitoides*, Exhibits a Cytotoxic Effect on DLD-1 Colorectal Cancer Cells

Mutia Hardhiyuna[1], Ucu Yanu Arbi[2], Zuraida Zuraida[1], Peni Ahmadi[1]*

Abstract

Objective: This study aimed to investigate the cytotoxicity effect of the ethyl acetate extract of *Aaptos suberitoides* on colorectal cancer cells (DLD-1) and murine fibroblast cells (NIH-3T3). **Methods:** *A. suberitoides* was collected from Putus Island, Bunaken National Park, North Sulawesi, Indonesia, and was processed with maceration and ethyl acetate extraction. The sponge extract was characterized based on Thin Layer Chromatography (TLC) and then identified by using LCMS/MS analysis. DLD-1 and NIH-3T3 cells were treated with the ethyl acetate extract and then followed by 3- [4, 5-dimethylthiazol-2-yl] -2.5 diphenyl tetrazolium bromide (MTT) assay to assess their cytotoxicity effect. **Results:** LCMS/MS analysis showed that the most abundant compounds in this extract were identified as aaptamine **(1)**. Furthermore, this study revealed that the active ethyl acetate fraction of *A. suberitoides* has cytotoxic effects in colorectal cancer DLD-1 cells with an IC_{50} value of 9.597 µg/mL, higher than NIH-3T3 cells with an IC_{50} value of 12.23 µg/mL Thus, the active ethyl acetate fraction of *A. suberitoides* is considered more toxic to cancer cells than normal cells. **Conclusion:** This study provides the first evidence to support the role of the ethyl acetate extract of *A. suberitoides* sponge extracts to be developed as a colorectal anticancer agent.

Keywords: aaptamine- marine sponge- colorectal cancer cells- cytotoxic

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Introduction

Colon cancer, known as colorectal adenocarcinoma (CRC), is a type of cancer with the third most frequent cases in both men and women worldwide [1] and ranks the fourth cause of death [2]. CRC arises from glands in the epithelial cells of the large intestine followed by a series of mutations, replication, and increasing survival. These hyperproliferative cells trigger benign adenomas, then develop into carcinomas and metastasize for decades [3]. The pathology of CRC is very complex and varied because they were triggered by various factors related to diet, lifestyle, and also related to genetic factors [4]. These risk factors cause genes to change or mutations where cells grow out of control related to oncogenes and tumor suppressor genes [5]. Several therapeutic interventions including surgery, chemo- and radiotherapy have improved the survival of patients with CRC [6]. However, the current failure of cancer treatment that causes mortality of CRC is primarily due to metastasis and tumor relapse or recurrence after therapy [7, 8]. The main challenge now is to discover agents and strategies that target cancer and tumor relapse at their apparent source [8].

Many studies have been carried out to find cancer chemotherapeutic agents derived from natural materials. Marine organisms including sponges, sponge-associated microbes, actinomycetes, and soft corals have been extensively explored as anticancer agents. The effects of marine-sourced bioactive compounds on apoptotic pathways and signaling pathways in cancer therapeutic targets show interesting activity which could be used as an initial step in designing and developing cancer therapeutic agent [9]. One of the most promising marine biota is the sea sponge, as a prolific source of secondary metabolites, some sponges exhibit interesting chemopreventive and tumor chemotherapeutic properties [10]. Sponges have the highest percentage (35.53%) compared to other marine biotas in their commercial use in the pharmaceutical sector [11]. Sponges contain active compounds with greater activity than land plants. Secondary metabolite extracts from sponges contain bioactive compounds that act as cytotoxic and antitumors [12].

Various species of sponges have the potential bioactivity to be developed as anti-colon cancer, one of them is *A. suberitoides*, an abundant marine sponge living in the West Pacific Ocean, particularly in Indonesia, Singapore, and

*[1]Research Center for Vaccine and Drugs, Research Organization for Health, National Research and Innovation Agency (BRIN), Jl. Raya Bogor Km.46, Cibinong, 16911, Indonesia. [2]Research Center for Oceanography, National Research and Innovation Agency (BRIN), Jl. Pasir Putih Raya No.1, North Jakarta, 14430, Indonesia. *For Correspondence: peni.ahmadi@brin.go.id*

Vietnam [13]. *A. suberitoides* has been studied for years. The ethanol extract of *A. suberitoides* has been shown potential anti-cancer activity in different cancer entities, including acting as antiproliferation, proapoptosis, and as proteasome inhibitor [10, 14-18]. Aaptamines (8,9-dimethoxy-1H-benzo[de][1,6] naphthyridine) are a group of bioactive benzo [de][1, 6]- naphthyridine alkaloids, initially isolated from marine sponges mostly belonging to the genus Aaptos [15]. Aaptamine has several bioactive properties such as cytotoxicity, anti-cancer, and anti-inflammation [18-21]. Aaptamine has been proven to have anti-cancer properties in human tumor cell lines such as monocytic leukemia (THP-1), cervical carcinoma (HeLa), colon cancer (SNU-C4), melanoma (SK-MEL-28) and breast cancer (MDA-MB-231) [15].

Although various chemotherapeutic drugs are available for CRC; however, the systemic toxicity to normal cells limits their therapeutic efficacy, which leads to harmful side effects on healthy tissues. Therefore, the development of new anticancer agents with fewer toxic side effects is strongly needed [22] that have better pharmacotoxicological profiles, to be used single or together with other conventional chemotherapy. Furthermore, it is crucial to explore the bioactive fractionation of marine sponges as new colon anticancer drug candidates that are commonly less toxic than traditional chemotherapeutic agents, more effective, cheaper, and have better availability [23], despite the resistance of the cancer cells to anticancer drugs remain the challenge. Hence, it is required to search for anti-cancer compounds from natural ingredients that are specific for certain cancers and inhibit specific proteins that cause cancer cells to continue proliferating without control [24]. Recently, no studies have investigated the cell viability of the ethyl acetate extract of *A. suberitoides* specifically on DLD-1 colorectal cancer cells. Hence, the current study aimed to investigate the cytotoxic activity of DLD-1 cells and NIH-3T3 cells. Subsequently, this research would provide scientific validation of marine sponge natural products as a colorectal anticancer therapeutic agent.

Materials and Methods

Materials

In this experiment, commercially available reagents were employed without additional purification. Analytical-grade solvents were used in this work.

Equipment and Material

In this study the following materials were used, Dulbecco's Modified Eagle Medium (DMEM, Gibco, Invitrogen, USA), Fetal Bovine Serum (FBS; Gibco) and Penicillin/ Streptomycin (Gibco), dimethylsulfoxide (DMSO; Sigma-Aldrich, St. Louis, Missouri, USA) 100%, trypsin-EDTA (Sigma-Aldrich), 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich), trypan blue (Sigma-Aldrich).

Animal Material

The marine sponge was collected from Putus Island, Bitung, North Sulawesi, Indonesia (N 01.52244o E 125.27718o) at 10 - 15 meters depth in September 2022. The specimen was later coded as SSL-013 and kept frozen at the Genomics Laboratory, National Research and Innovation Agency (BRIN), Cibinong, Bogor prior to extraction.

Sponge Identification

The sponge was later identified as *Aaptos suberitoides* by Tri Aryono Hadi, a Marine Sponge Taxonomist at the Research Center for Oceanography, National Research and Innovation Agency (BRIN), Indonesia.

Extraction of Marine Sponge

The marine sponge extraction method refers to [25]. The specimen (146.30 g, wet) was thawed, chopped, and macerated using methanol (MeOH) until submerged overnight before being filtered. The procedure was repeated three times. All the filtrates were combined and concentrated using a rotary evaporator until thick extracts were obtained. The watery crude was then partitioned between ethyl acetate and water three times. The combined organic layer was evaporated under vacuum conditions to give an EtOAc extract of 595.37 mg (SSL-013-1). The EtOAc extract was then used for further analysis.

Thin Layer Chromatography (TLC) based characterization

TLC analyses were performed on normal-phased, silica. The combination mixture of dichloromethane (DCM): methanol (MeOH) with ratio (7:3) was used as the eluents. The plates were dyed using serum sulfate, Dragendorff's reagent, and ninhydrin.

Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LCMS/MS)

The EtOAc extract (0.5 mg) of *Aaptos suberitoides*, coded SSL-013-1, was analyzed using the Waters ACQUITY UPLC® H-Class System (Beverly, MA, USA), which included an ACQUITY UPLC® HSS C18 column (with specifications of 11.8 µm, 2.1 Å, and approximately 100 mm) and was coupled with the Xevo G2-S QTOF Mass Spectrometer. The eluent used MeOH:H_2O with a combination mixture 9:1 with a flow rate of 1 mL/min. The system utilized electrospray ionization (ESI) in a positive ion mode for the mass spectrometry. The resulting data consisted of chromatograms (LC) and spectra (MS). Elemental compositions were determined using the Waters MassLynx software (v4.1). Additionally, the compound type was compared to the PubChem database.

Cells Culture and Conditions

The study was conducted on human colorectal cancer cells (DLD-1) and murine fibroblast cells (NIH-3T3), which were obtained from American Type Culture Collection (ATCC, USA). Cell lines were cultured using DMEM medium (Gibco, Germany) with supplementation of 10% fetal bovine serum (FBS; Gibco, Germany) and 1% Penicillin/Streptomycin (Gibco, Germany) in a cell culture incubator at 37°C and 5% CO_2 content.

MTT Assay

The MTT assay method refers to [26] . Colorectal

cancer cells (DLD-1) and murine fibroblast cells (NIH-3T3) were harvested at 70 - 80% confluency and centrifuged at 1200 rpm/25 °C for 3 min. The cells were counted under a microscope using a haemocytometer and were then seeded into 96 well plates at 1×10^5 cells per mL. Incubation was set to 5% CO_2 at 37 °C for 24 h to allow for cell attachment. After that, the unattached cells were removed carefully. Cells were then treated with 100 µL of series dilution of ethyl acetate fraction of SSL-013-1 marine sponge at concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL, and doxorubicin as a positive control at concentrations of 16, 8, 4, 2, and 1 mg/mL, further incubated for 72 h. MTT reagent (Sigma-Aldrich, Germany) (5 mg/mL) was then added into each well and incubated at 5% CO_2, 37 ◦C for 4 h. SDS solution (Sigma-Aldrich, Germany) was added and further incubated in a dark environment for 15 min at 25 ◦C to stop the reaction. The absorbance was read on the spectrophotometer at 570 nm wavelength. All experiments were performed in triplicate. Cell viability was determined using the formula below:

Cell Viability = (Abs Treated cells / Abs Untreated cells) × 100%

Statistical Analysis

The MTT test results were analyzed using Graphpad Prism 9.0.0 to determine the IC_{50} of ethyl acetate fraction of SSL-013-1 marine sponge using the four parametric logistic regression methods.

Results

The sponge, identified as *Aaptos suberitoides*. and collected from Putus Island, Bitung, North Sulawesi, Indonesia in 2022, as macerated in MeOH and partitioned between EtOAc and H_2O three times. The combined organic layers were concentrated under vacuum conditions to yield 595.37 mg and coded as SSL-013-1.

The SSL-013-1 was assayed against colon cancer, DLD-1, at a final concentration of 100 ppm and showed a 100.87% inhibition. This result indicated that the SSl-013-1 contained potential bioactive compounds as anticancer agents. Therefore, SSL-013-1 was chosen for further analysis.

TLC-Based Characterization of the Structures in SSL-013-1

TLC analyses were performed to characterize the basic moieties in bioactive compounds contained in the SSL-013-1. The TLC analyses were dyed using cerium sulfate indicating the total organic compound contained, Dragendorff's reagent indicating tertiary amine moiety, and ninhydrin indicating primary and secondary amines. The TLC results showed that SSL-013-1 was positive on Dragendorff's and ninhydrin reagents. It indicated that the major compounds in SSL-013-1 contained tertiary amine and primary and or secondary amine moieties.

Table 1. IC_{50} Values of SSL-013-1 against DLD-1 and NIH-3T3 Cells

No	Cell line	Sample	IC_{50} (mg/mL)
1	DLD-1	Aaptamine-rich extract (SSL-013-1)	9.597
		Doxorubicin	2.662
2	NIH-3T3	Aaptamine-rich extract (SSL-013-1)	12.23
		Doxorubicin	2.12

LCMS/MS-Based Metabolite Profiling of SSL-013-1

Sponge with genus Aaptos, such as *Aaptos suberitoides*, has been investigated for years. One of the alkaloids extracted from this type of sponge is aaptamine, showing major potential anticancer activity in different cancer cells acting as antiproliferative, proapoptosis, and proteasome inhibitors [10, 14, 15]. Previous studies of the Indonesian marine sponge *A. suberitoides* isolated aaptamine **(1),** isoaaptamine **(2)**, and demethylaaptamine **(3)** (Figure 1) were collected from North Sulawesi, Indonesia. As inhibitors of the proteasome, the three compounds showed cytotoxic activities against HeLa cells [19].

In this study, the bioactive compounds contained in the SSL-013-1 were analyzed by using LCMS/MS (Figure 2). The data exhibited the major peak at a retention time (Rt) of 4.62 min. The major peak represented the major compound contained in SSL-013-1 and presumed as the bioactive compound that was responsible for the anticancer activity. The major peak was then analyzed by using MassLynx and showing m/z value at 229.0985 [M+H]+ with chemical formula C13H12N2O2. The possible structures for these profiles are aaptamine **(1)** and isoaaptamine **(2)**. Further analyses were performed to determine the corresponding compound in SSL-013-1. Hence, the mass fragmentation analysis was conducted as described in Figure 3.

The main peak was observed at m/z 229.0985 and the fragmented peaks were detected at m/z 196.0645 and 168.0695. The fragmentation characteristic indicated the loss of a methoxy to give m/z 196.0645 and followed by another cleavage of a methoxy moiety to give m/z 168.0695 (Figure 3). It suggested that the compound contains 2 methoxy moieties in its structure. Hence, these fragmentation types lead to the determination of aaptamine

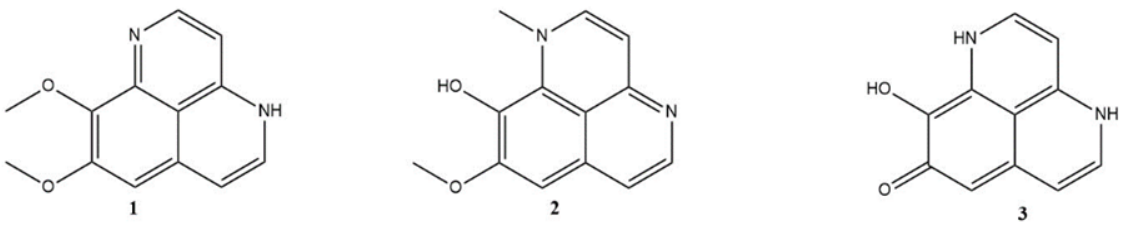

Figure 1. The Structure of aaptamine **(1)**, isoaaptamine **(2)**, and demethylaaptamine **(3).**

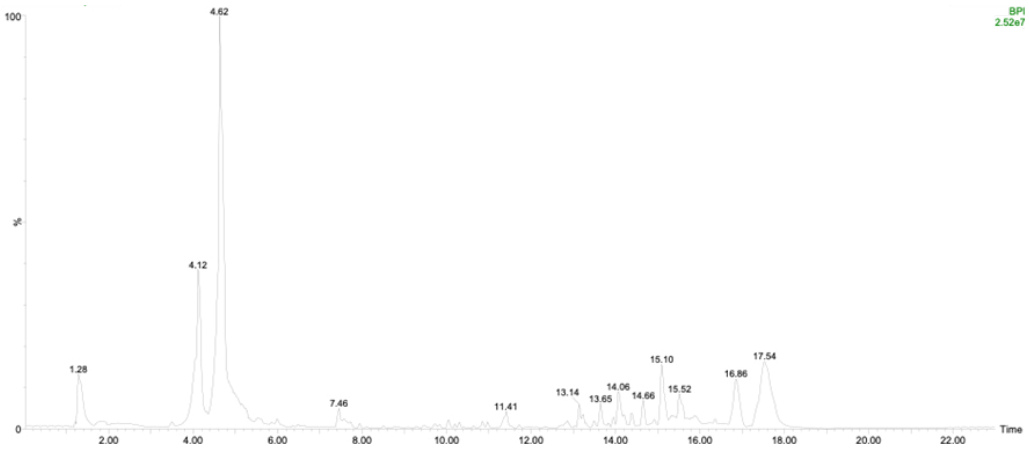

Figure 2. The LCMS/MS Chromatogram of SSL-013-1.

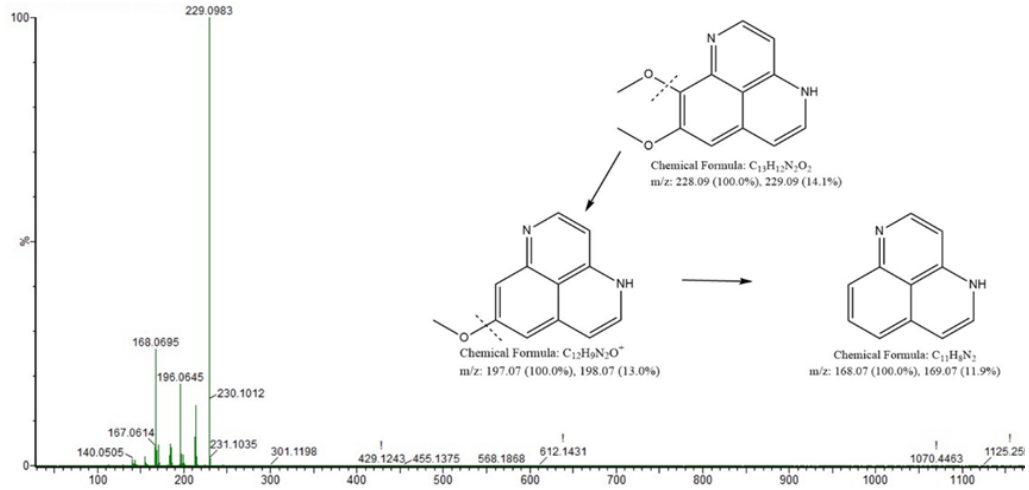

Figure 3. The Mass Fragmentation Peaks at the Corresponding Peak at Rt 4.62 of SSL-013-1.

(1) as the compound responsible for the anticancer activity in SSL-013-1. While isoaaptamine **(2)** has only one methoxy moiety in its structure. This finding was in agreement with TLC analysis results that the compound in SSL-013-1 contained tertiary and secondary amine moieties.

Doxorubicin as positive control induces DLD-1 and NIH-3T3 cell death

The effect of doxorubicin on the viability of the DLD-1 colorectal cancer cells and NIH-3T3 murine fibroblast cells was measured by MTT assay (Figure 4). The cytotoxic assay of doxorubicin showed a dose-dependent inhibitory profile in DLD-1 and NIH-3T3 cells. These data

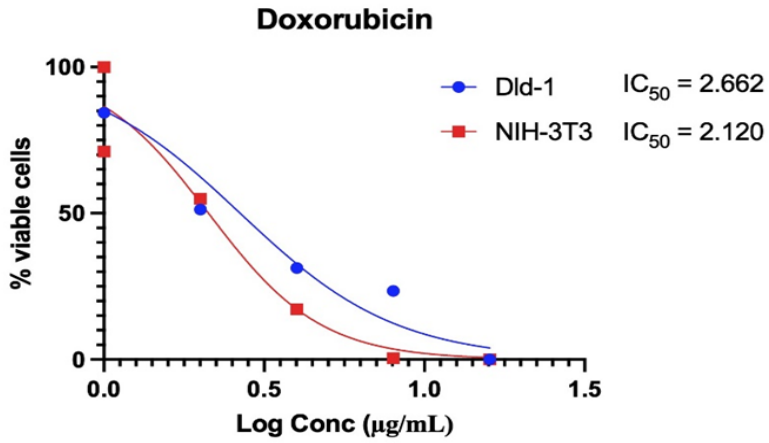

Figure 4. The Cytotoxicity Profile of Doxorubicin on DLD-1 and NIH-3T3 Cells. The viability of doxorubicin-treated cells were measured using an MTT assay.

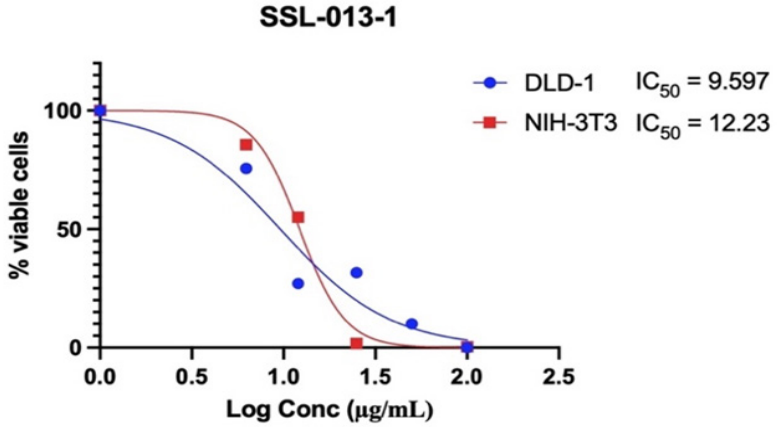

Figure 5. The Cytotoxicity Profile of SSL-013-1 on DLD-1 and NIH-3T3 Cells. The viability of SSL-013-1-treated cells were measured using an MTT assay

indicate that the SSL-013-1 is toxic to DLD-1 colorectal cancer cells with a lethal concentration (IC_{50}) of 2.662 µg/mL on DLD-1 cells (Table 1). Meanwhile, the IC_{50} value of doxorubicin on NIH-3T3 cells was slightly higher at 2.120 µg/mL. Thus, doxorubicin is considered toxic to cancer cells and normal cells.

Ethyl acetate extract (SSL-013-1) of A. suberitoides induces DLD-1 and NIH-3T3 cell death

The effect of SSL-013-1 on the viability of the DLD-1 colorectal cancer cells and NIH-3T3 murine fibroblast cells was measured by MTT assay (Figure 4). The cytotoxic assay of the SSL-013-1 showed a dose-dependent inhibitory profile in DLD-1 and NIH-3T3 cells. These data indicate that the SSL-013-1 is toxic to DLD-1 colorectal cancer cells with a lethal concentration (IC_{50}) of 9.597 µg/mL on DLD-1 cells (Table 1). Meanwhile, the IC_{50} value of SSL-013-1 on NIH-3T3 cells was 12.23 µg/mL. Thus, SSL-013-1 is considered more toxic to cancer cells than normal cells (Figure 5).

Discussion

Marine sponges are a prolific source of secondary metabolites, several of which showed tumor chemopreventive and chemotherapeutic properties. Several marine sponge extracts showed anticancer effects. Aaptamines are alkaloids extracted from different species of Aaptos sp. showing important potential anticancer activity. Aaptamines are also capable of triggering more than one pathway, such as apoptosis, inhibiting cell proliferation, and acting as cytoprotective elements against conventional chemotherapeutic agents. This highlights their multi-target ability to restrain the biological complexity of cancer [10].

A previous study also demonstrated that *A. suberitoides* contains three particular compounds (Aaptamine, Demethyl(oxy)aaptamine, and Isoaaptamine) which exhibit their ability to trigger apoptosis toward human leukemia monocytic cell line (THP-1) [15]. Moreover, another study demonstrated that *A. suberitoides* with major compounds aaptamine exhibit antiproliferative activity targeting the modulators of the cell-cycle progression toward human osteosarcoma cell line (MG63) [27].

The cytotoxicity of marine sponge extract against CRC has been studied in HCT-116 cells [28]. Based on this finding, the ethanolic extract of Stylissa carteri has cytotoxic activity particularly against HCT-116 colorectal cancer cell lines with an IC_{50} value of 5 µg/mL. This marine sponge cytotoxicity activity, however, has not been studied specifically on colorectal cancer cell lines DLD-1 cell. DLD-1 cell is one of the CRC models that has been studied for cytotoxicity activity assay and pro-apoptotic genes target [29].

Investigation of anticancer activity also showed that aaptamine **(1)** and its derivatives demonstrate anticancer effects against five human tumor cell lines (THP-1, HeLa, SNU-C4, SK-MEL-28, and MDA-MB-231 human cancer cell lines) and induced apoptosis in THP-1 human leukemia cells [15]. In another study, the ethanolic extract of *A. suberitoides* on trastuzumab-resistant HER2+ breast cancer cell line demonstrates cytotoxicity, anti-proliferation, and anti-migration effect as well as inhibition effect on three-dimensional spheroid growth [17].

The ethanolic extract of *A. suberitoides* from Tinjil island, Banten, Indonesia has been investigated on trastuzumab-resistant HER2+ breast cancer cell line for their cytotoxicity using MTT assay with IC_{50} was 12ppm [17]. Furthermore, the methanolic extract of *A. suberitoides* also has demonstrated more antiproliferation impact on breast cancer cells than normal cells, indicating oxidative stress-dependent preferential antiproliferation effects on breast cancer cells but not for normal cells [18]. However, to date, there is limited study regarding the ethyl acetate extract study of *A. suberitoides*. Hence, the present study examined the cytotoxic effects of ethyl acetate extract of *A. suberitoides* between CRC and normal cells. Furthermore, it has also opened an opportunity for ethyl acetate extract of *A. suberitoides* to be a novel therapeutic candidate for DLD-1 colorectal cancer cells. Thus, this was the first study in DLD-1 colorectal cancer cells to evaluate the cytotoxic activity of ethyl acetate active fraction of *A. suberitoides*. Our results showed that the ethyl acetate active fraction of

A. suberitoides collected from Putus Island, Bitung, North Sulawesi, Indonesia suppressed cell viability in DLD-1 colorectal cancer cells (Figure 2). The IC_{50} value of the active fraction (SSL-013-1) was 9.597 µg/mL indicating that it has good cytotoxic activity. The American National Cancer Institute (NCI) guidelines set the limit of activity for crude extracts to 50% inhibition (IC_{50}) of the proliferation of less than 30 ppm in 72 hours incubation. Moreover, a crude extract with IC_{50} below 100 µg/mL has the potential to be developed as an anticancer agent [30]. However, further analysis is necessary to identify the exact active compound underlying this cytotoxicity activity.

Based on the findings, ethyl acetate extract (SSL-013-1) of *A. suberitoides* collected from Putus Island, Bunaken National Park, North Sulawesi, Indonesia, suppressed cell viability of DLD-1 colorectal cancer cells and considered more toxic to DLD-1 colorectal cancer cells than murine fibroblast cells (NIH-3T3) as control/normal cells. Later, the bioactive compound responsible for the activity was determined as aaptamine [1]. The IC_{50} of aaptamine-rich extract was evaluated against DLD-1 cells with a value of 9.597 µg/mL and categorized as a potent anticancer agent. Therefore, these data prove that the ethyl acetate extract of *A. suberitoides* could be a potential candidate for the source of novel drugs for colorectal cancer cell therapy. To that end, further in-vivo studies are necessary before recommending the clinical use of aaptamine.

Author Contribution Statement

P. A.; supervision: P. A.; formal analysis: P. A. and M.H..; investigation: P. A. and M. H.; writing original draft: P. A. and M. H.; writing-review and editing: P. A., M. H., U. Y. A., and Z. All authors agreed to the final version of this manuscript.

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General

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Data Availability

Research Center for Vaccine and Drugs, Research Organization for Health, National Research and Innovation Agency (BRIN) – Indonesia.

Conflict of Interest

The authors declare no conflict of interest.

References

1. García-Pinel B, Porras-Alcalá C, Cabeza L, Ortiz R, Prados J, Melguizo C, et al. Bengamide analogues show a potent antitumor activity against colon cancer cells: A preliminary study. Mar Drugs. 2020;18(5). https://doi.org/10.3390/md18050240.
2. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-64. https://doi.org/10.3322/caac.21601.
3. Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Prz Gastroenterol. 2019;14(2):89-103. https://doi.org/10.5114/pg.2018.81072.
4. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther. 2020;5(1):22. https://doi.org/10.1038/s41392-020-0116-z.
5. Markowitz SD, Dawson DM, Willis J, Willson JK. Focus on colon cancer. Cancer Cell. 2002;1(3):233-6. https://doi.org/10.1016/s1535-6108(02)00053-3.
6. Gheytanchi E, Naseri M, Karimi-Busheri F, Atyabi F, Mirsharif ES, Bozorgmehr M, et al. Morphological and molecular characteristics of spheroid formation in ht-29 and caco-2 colorectal cancer cell lines. Cancer Cell Int. 2021;21(1):204. https://doi.org/10.1186/s12935-021-01898-9.
7. Neo JH, Ager EI, Angus PW, Zhu J, Herath CB, Christophi C. Changes in the renin angiotensin system during the development of colorectal cancer liver metastases. BMC Cancer. 2010;10:134. https://doi.org/10.1186/1471-2407-10-134.
8. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: Challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov. 2009;8(10):806-23. https://doi.org/10.1038/nrd2137.
9. Altmann KH. Drugs from the oceans: Marine natural products as leads for drug discovery. Chimia (Aarau). 2017;71(10):646-52. https://doi.org/10.2533/chimia.2017.646.
10. Calcabrini C, Catanzaro E, Bishayee A, Turrini E, Fimognari C. Marine sponge natural products with anticancer potential: An updated review. Mar Drugs. 2017;15(10). https://doi.org/10.3390/md15100310.
11. Gerwick WH, Moore BS. Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. Chem Biol. 2012;19(1):85-98. https://doi.org/10.1016/j.chembiol.2011.12.014.
12. Khalifa SAM, Elias N, Farag MA, Chen L, Saeed A, Hegazy MF, et al. Marine natural products: A source of novel anticancer drugs. Mar Drugs. 2019;17(9). https://doi.org/10.3390/md17090491.
13. Halanych K, Siddall M, Smith V, Penny D. Invertebrate zoology: A functional evolutionary approach. Syst Biol. 2004;53:662-4. https://doi.org/10.1080/10635150490472977.
14. Tsukamoto S, Yamanokuchi R, Yoshitomi M, Sato K, Ikeda T, Rotinsulu H, et al. Aaptamine, an alkaloid from the sponge aaptos suberitoides, functions as a proteasome inhibitor. Bioorg Med Chem Lett. 2010;20(11):3341-3. https://doi.org/10.1016/j.bmcl.2010.04.029.
15. Dyshlovoy SA, Fedorov SN, Shubina LK, Kuzmich AS, Bokemeyer C, Keller-von Amsberg G, et al. Aaptamines from the marine sponge aaptos sp. Display anticancer activities in human cancer cell lines and modulate ap-1-,

nf-κb-, and p53-dependent transcriptional activity in mouse jb6 cl41 cells. Biomed Res Int. 2014;2014:469309. https://doi.org/10.1155/2014/469309.
16. Sa'adah N, Nurhayati A, Shovitri M. The anticancer activity of the marine sponge aaptos suberitoides to protein profile of fibrosarcoma mice (mus musculus). IPTEK J Technol Sci. 2017;27. https://doi.org/10.12962/j20882033.v27i3.1183.
17. Bashari MH, Arsydinilhuda FZ, Ilhamsyah RS, Nugrahani AD, Nurdin RA, Kartika A, et al. The ethanol extract of marine sponge aaptos suberitoides suppress cell viability, cell proliferation and cell migration in her2-positive breast cancer cell line. Asian Pac J Cancer Prev. 2021;22(S1):25-32. https://doi.org/10.31557/apjcp.2021.22.S1.25.
18. Shiau JP, Lee MY, Tang JY, Huang H, Lin ZY, Su JH, et al. Marine sponge aaptos suberitoides extract improves antiproliferation and apoptosis of breast cancer cells without cytotoxicity to normal cells in vitro. Pharmaceuticals (Basel). 2022;15(12). https://doi.org/10.3390/ph15121575.
19. kumar S, Govindan R, Manoharan N. Marine sponge alkaloid aaptamine enhances the anti-bacterial and anti-cancer activity against esbl producing gram negative bacteria and hepg 2 human liver carcinoma cells. Biocatal Agric Biotechnol. 2019;17. https://doi.org/10.1016/j.bcab.2019.01.007.
20. Newman DJ, Cragg GM. Marine natural products and related compounds in clinical and advanced preclinical trials. J Nat Prod. 2004;67(8):1216-38. https://doi.org/10.1021/np040031y.
21. Putra M. The alkaloids from indonesian marine sponges. Oceanography: Open Access. 2014;02. https://doi.org/10.4172/2332-2632.1000125.
22. Wongkaewkhiaw S, Wongrakpanich A, Krobthong S, Saengsawang W, Chairoungdua A, Boonmuen N. Induction of apoptosis in human colorectal cancer cells by nanovesicles from fingerroot (boesenbergia rotunda (l.) mansf.). PLoS One. 2022;17(4):e0266044. https://doi.org/10.1371/journal.pone.0266044.
23. Amin AR, Kucuk O, Khuri FR, Shin DM. Perspectives for cancer prevention with natural compounds. J Clin Oncol. 2009;27(16):2712-25. https://doi.org/10.1200/jco.2008.20.6235.
24. Shaukat A, Levin TR. Current and future colorectal cancer screening strategies. Nat Rev Gastroenterol Hepatol. 2022;19(8):521-31. https://doi.org/10.1038/s41575-022-00612-y.
25. Shingaki M, Wauke T, Ahmadi P, Tanaka J. Four cytotoxic spongian diterpenes from the sponge dysidea cf. Arenaria. Chem Pharm Bull (Tokyo). 2016;64(3):272-5. https://doi.org/10.1248/cpb.c15-00726.
26. Murwanti R, Rahmadani A, Ritmaleni R, Hermawan A, Sudarmanto A. Curcumin analogs induce apoptosis and g2/m arrest in 4t1 murine triple-negative breast cancer cells. Indones J Pharm. 2020;31:11. https://doi.org/10.14499/indonesianjpharm31iss1pp11.
27. Aoki S, Kong D, Suna H, Sowa Y, Sakai T, Setiawan A, et al. Aaptamine, a spongean alkaloid, activates p21 promoter in a p53-independent manner. Biochem Biophys Res Commun. 2006;342(1):101-6. https://doi.org/10.1016/j.bbrc.2006.01.119.
28. Dwiwina R, Sabrina S, Pratiwi Y, Halleyana P, Rahman A, Bashari M. Cytotoxic and anti-proliferative effects of ethanol extract of marine sponge stylissa carteri on colon cancer hct-116 cell line. Indones J Cancer Chemoprevention. 2023;14:33. https://doi.org/10.14499/indonesianjcanchemoprev14iss1pp33-38.
29. Gavrilas LI, Cruceriu D, Ionescu C, Miere D, Balacescu O. Pro-apoptotic genes as new targets for single and combinatorial treatments with resveratrol and curcumin in colorectal cancer. Food Funct. 2019;10(6):3717-26. https://doi.org/10.1039/c9fo01014a.
30. Ueda JY, Tezuka Y, Banskota AH, Le Tran Q, Tran QK, Harimaya Y, et al. Antiproliferative activity of vietnamese medicinal plants. Biol Pharm Bull. 2002;25(6):753-60. https://doi.org/10.1248/bpb.25.753.