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Antiproliferative and pro-apoptotic effects on cancer cell line HCT116 using marine sponge *Tedania* species collected from Kalangahan, Lugait, Misamis Oriental, Philippines

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ABSTRACT

Cancer is a major cause of mortality worldwide, and marine sponges have emerged as potential sources of anticancer agents. In this study, we investigated the antiproliferative and pro-apoptotic effects of a marine sponge extract from *Tedania* sp. against the human colorectal carcinoma cell line (HCT116) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Caspase-Glo® 3/7 assays. The extract was prepared using a polar solvent mixture of ethanol:water, and was subjected to reversed-phase flash chromatography consisiting of water(W), acetonitrile(ACN), and ethyl acetate(EA) gradient eluent, yielding 16 Fraction. Treatment of HCT116 cells with Fractions 1-16 showed that only Fraction 16, consisting of 100% EA, exhibited significant ($p<0.05$) cytotoxic activity with the lowest viability of 90.9% compared to the negative control. Furthermore, Fraction 9, consisting of 20:80 W and ACN, showed a significantly ($p<0.05$) highest fold change (FC) value of 2.89 indicating an almost threefold increase in caspase-3/7 activity. These results demonstrate that the *Tedania* sp. extract has promising antiproliferative and pro-apoptotic effects against HCT116 cell lines, indicating its potential as an anticancer agent.

Keywords: *Tedania* sp., Antiproliferative, Pro-apoptotic, Viability, Caspase-glo 3/7, HCT116 cell line.

INTRODUCTION

Cancer is a worldwide disease caused by abnormal cell growth and can affect people of all ages. It is caused by a combination of environmental factors, genetics, and lifestyle habits like unhealthy food and tobacco use. Cancer cells can invade and spread, disrupting cell communication and gene function [1]. Specifically, colorectal cancer (CRC) is the third most prevalent malignant disease and the second leading cause of cancer-related mortality. It is estimated that there were 1.9 million cases of CRC and 0.9 million deaths worldwide in 2020 [2]. Thus, the discovery of novel anticancer compounds that may help to entirely prevent, slow down, or reverse by the administration of one or more naturally occurring and/or synthetic agents is desired [3].

Natural products hold great promise in the fight against cancer since they are often less toxic than traditional chemotherapy drugs and they have the potential to be effective, affordable, and readily available [4]. Although natural products that are used for serious illness often come from land-based sources, 70% of the earth is covered by the ocean with abundant marine life. With this, medical science has turned to exploring the marine world for the last 60 years, unraveling its vast potential for medical treatment [5].

In the marine world, marine sponges are valuable resources for discovering new cancer drugs because they contain a variety of

secondary metabolites with unique molecular configurations [6]. These compounds are used as a defense mechanism in response to the aggressive and demanding surroundings where the sponges live [7]. The genus *Tedania* (family Tedaniidae, order Poecilosclerida) is a marine sponge that comprises of 83 species and can be found worldwide [8]. The secondary metabolites produced by marine sponges, including *Tedania*, have been found to have antiviral, antioxidant, antiobesity, antihypertensive, antidiabetic, anticancer, and antiproliferative capabilities, among other biological actions. These compounds have been shown to modulate cellular and molecular pathways, including growth inhibition, induction of apoptosis and autophagy, antiangiogenic effects, and antimigration, making them promising candidates for drug discovery and development [9]. However, despite the abundance of marine biodiversity in the Philippines, only a few marine sponges have been studied for their potential as a source of bioactive secondary metabolites, particularly anticancer compounds [10].

Hence, this study aims to examine the anticancer properties, specifically, the antiproliferative and pro-apoptotic effect of polar extract *Tedania* sp. against HCT116 cell lines.

MATERIALS AND METHODS

Materials

The marine sponge *Tedania* sp. was collected from Kalangahan, Lugait, Misamis Oriental, located off the coast of Iligan Bay. Its identification based on morphological characteristics was conducted by Marine Biologist Prof. Angelo Responte from Mindanao State University - Iligan Institute of Technology, Iligan City, Philippines.

The HCT116 cell line was obtained from the American Type Culture Collection. McCoy's 5A media and Fetal Bovine Serum (FBS) were purchased from Gibco. The 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Celltiter96® Nonradioactive Cell Proliferation Assay) and Caspase-Glo® 3/7 Assay kits were obtained from Promega Corporation. Sodium butyrate, digitonin, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich.

Preparation of *Tedania* sp. Crude Extract

The Tuklas Lunas Development Center (TLDC) at Mindanao State University-Iligan Institute of Technology (MSU-IIT), established and funded by the Philippine Council for Health Research and Development (PCHRD) of the Department of Science and Technology (DOST) supplied a crude sample of the marine sponge *Tedania* sp., which was then processed, freeze-dried and weighed. The freeze-dried sample was chopped and steeped in a 1:1 ethyl acetate-methanol mixture for 3 days to obtain the nonpolar extract. The sponge residues were then soaked in a 1:1 ethanol-water mixture for 3 days to obtain the polar extract. Then, extracted samples were concentrated *in vacuo* and/or freeze-dried to obtain a final weight. This study utilized a fraction of a sample previously obtained in a prior study by TLDC, with a total weight of 3.9 g of *Tedania* sp. polar extract.

Fractionation of Polar Crude Extract of *Tedania* sp.

Flash chromatography (BUCHI Pure C-815 Flash) was used for the purification and fractionation of the sponge *Tedania* sp. polar extract using reversed-phase chromatography. A total of 1.1 g of dried *Tedania* sp. extract and 9.8 g of Wakogel® were dissolved in a 1.25 mL of 1:1 distilled water-acetonitrile mixture. This was then subjected to fractionation with a water/acetonitrile/ethyl acetate gradient eluent, yielding 16 major Fractions (F1 to F16). The bioactivity of each Fraction was then evaluated against the HCT116 cell line using MTT and Caspase-Glo® 3/7 assays.

Preparation of Sample and Positive Control

A 10,000-µg/mL stock solution was created by combining 1 mg of a sample with 100 µL of dimethyl sulfoxide (DMSO) in a microcentrifuge tube. From this stock solution, a 1000-µg/mL solution was made by mixing 25 µL of the stock solution with 225 µL of cell culture medium. A 300-µg/mL solution was created from the 1000-µg/mL solution by adding 75 µL of the 1000-µg/mL solution to 175 µL of cell culture medium. 10 µL of the 300-µg/mL solution was then placed into the wells, resulting in a final concentration of 30-µg/mL in the assay.

Cell Culture

The HCT116 cells were cultured in McCoy's media which was supplemented with 10% fetal bovine serum (FBS). Following that, the cells were placed in an incubator and maintained at a temperature of 37°C in an atmosphere containing 5% CO_2.

MTT Assay

The CellTiter96® Non-radioactive Cell Proliferation Assay, which is a modified version of the MTT assay method, was used to evaluate the cytotoxic activity of the extracts against the HCT116 cell lines. A 90 µL cell suspension containing 20,000 cells was placed in a 96-well microplate except for the blank wells and incubated overnight at 37 °C in a 5% CO_2 atmosphere. The next day, 10 µL of 300-µg/mL test samples and positive control (digitonin) were added to their corresponding wells to yield a 30 ug/mL final assay concentration. A 0.3% vehicle DMSO-treated negative control was used, along with a blank control that contained 0.3% vehicle DMSO and the cell culture medium without any cells. The 96-well plate was then incubated overnight at 37 °C under a 5% CO_2 atmosphere. Then, a 15 µL dye solution was added to all wells and incubated for 4 hours at 37 °C under the same conditions. Finally, a solubilization solution/stop mix was added to all wells (100 µL) and incubated for 1 hour at room temperature in the dark. The absorbance was then measured at 570 nm wavelength using a Perkin Elmer Victor X3

multimode spectrophotometer, and the cytotoxicity of cells was calculated using the formula (1).

$$Cell\ Viability, \% = \frac{Corrected\ Absorbance\ of\ Sample}{Corrected\ Absorbance\ of\ Negative\ Control} x100 \quad (1)$$

Caspase-Glo® 3/7 Assay

A commercially available kit (Promega G8091 Caspase-Glo® 3/7 Assay) was used to identify the pro-apoptotic property of *Tedania* sp. extract. The HCT116 cell lines were seeded in a 96-well white-opaque plate with 10,000 cells per well. Following a six-hour incubation at 37 ºC and 5% CO_2, 10 µL of 300-µg/mL test samples and positive control (sodium butyrate) were added to their corresponding wells to achieve a final concentration of 30 ug/mL. A 0.3% vehicle DMSO-treated negative control was used, along with a blank control that contained 0.3% vehicle DMSO and the cell culture medium without any cells. The cells were incubated for 20 hours under the same conditions. Afterward, Caspase-Glo® 3/7 reagent with a volume of 10 µL was added to each well, and the plate was covered with foil and shaken for 2 hours. The luminescence was quantified using a luminometer (Perkin Elmer Victor X3 multimode spectrophotometer).

The pro-apoptotic activity of the samples was expressed as the mean of the corrected relative light units (RLU) for Caspase-Glo® 3/7 activity. The pro-apoptotic activity was evaluated by calculating the fold change (FC) relative to the untreated cells, with higher RLU values indicating higher Caspase-Glo® 3/7 activity as seen in formula (2).

$$Fold\ Change\ (FC) = \frac{RLU\ test\ sample\ or\ positive\ control}{RLU\ negative\ control} \quad (2)$$

Statistical Analysis

All experiments were performed in triplicate and repeated at least three times. The results are presented as the mean, with the standard deviation (SD) represented by error bars. Statistical significance between the negative control (DMSO) and test fractions against HCT116 cell lines was determined by *T*-test at a 95% confidence level.

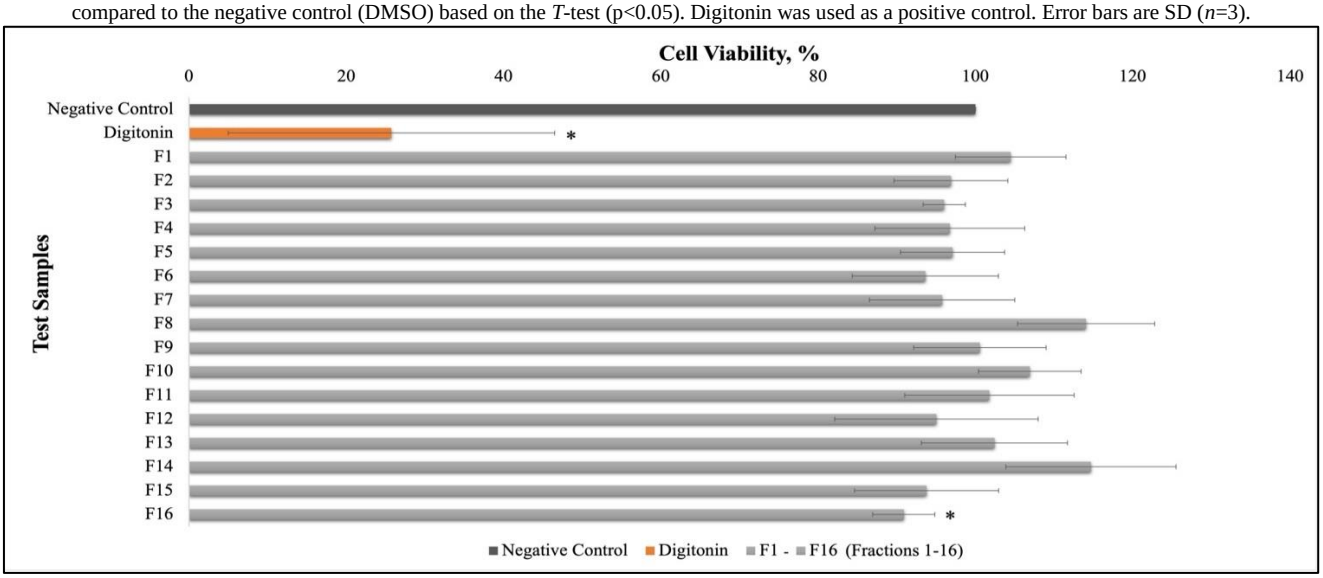
RESULTS AND DISCUSSION

The MTT assay showed that Fraction 16, obtained using a gradient eluent consisting of 100% EA from the *Tedania* sp. extract, exhibited cytotoxic activity against HCT116 cell lines, as indicated in Figure 1. This fraction demonstrated a significant decrease ($p<0.05$) in cell viability, reaching 90.9% compared to the negative control. Although the viability of cells treated with Fraction 16 was significantly lower, the cytotoxicity index was still considered low since the percent viability fell within the range of 90.00-100% [11].

The presence of cancer cells in the Fractions, apart from Fraction 16, with viability below 100% as shown in Figure 1, indicates a possible inhibitory effect on cell growth. This effect may lead to a slowdown in metastasis and an antiproliferative pathway [12]. However, this effect was not statistically significance ($p>0.05$), indicating the need for further investigation.

Previous research has also identified potent bioactive compounds from *Tedania* sp. that have demonstrated strong antimicrobial activity and potential as an antitumor agent. Furthermore, a novel cyclic peptide with significant bioactivity was discovered from a microorganism associated with *Tedania* sp. [13]. Specifically, tedanolide, a compound isolated from *Tedania ignis* (a species from the genus Tedania found in the Caribbean), exhibited high cytotoxicity with a median effective dose ED_{50} value of 2.5×10^{-4} µg/mL against KB cells (human carcinoma of the nasopharynx) [14]. These findings suggest that *Tedania* sp. extract can be further studied to determine its bioactive compounds that is responsible for cytotoxicity.

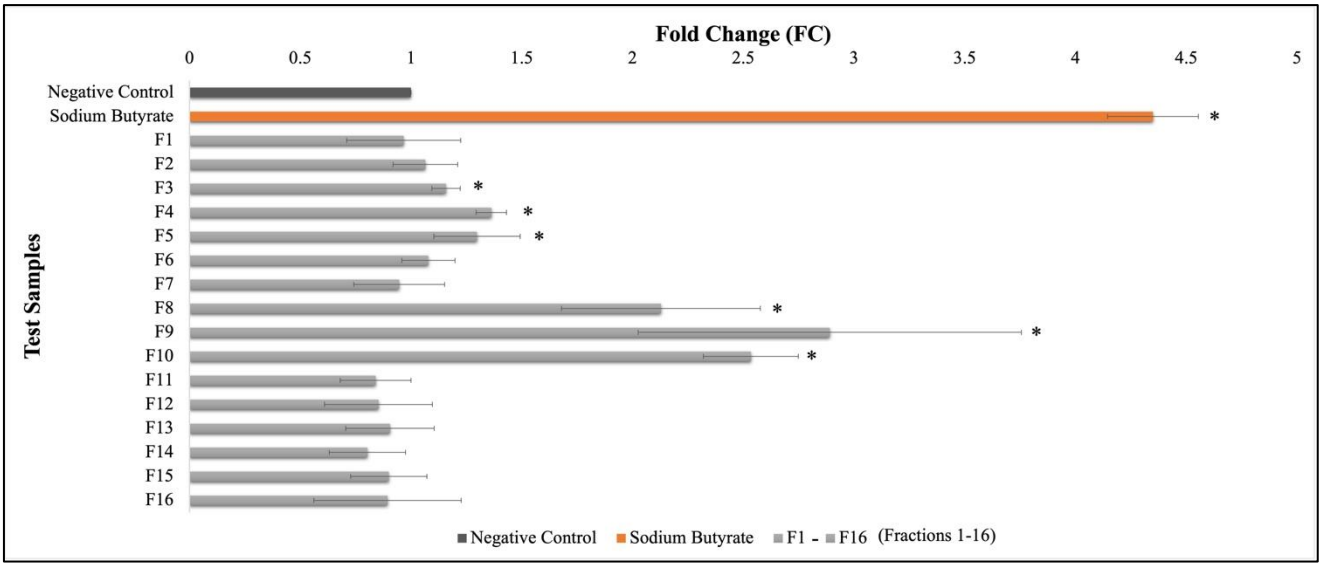
Figure 1: The percent viability of HCT116 cell lines treated with 30-ppm *Tedania* sp. extract. The asterisk (*) indicates significantly lower percent cell viability compared to the negative control (DMSO) based on the *T*-test ($p<0.05$). Digitonin was used as a positive control. Error bars are SD ($n=3$).



On the other hand, in the Caspase-Glo® 3/7 assay illustrated in Figure 2, only specific Fractions exhibited significant results. Among the 16 Fractions tested, Fractions 3, 4, and 5, consisting of a gradient eluent of 80:20 W and ACN, as well as Fractions 8 (60:40 W and ACN), 9 (20:80 W and ACN), and 10 (100% ACN), demonstrated significantly higher ($p<0.05$) fold change (FC) signals compared to the negative control. This increased FC signal indicates higher luminescence relative to the negative control. Notably, among the six significant Fractions, Fraction 9 had the highest FC value of 2.89 as shown in Figure 2, suggesting its potential proapoptotic activity through the activation of caspase-3/7 with an almost threefold increase compared to the negative control [9]. This result is consistent with a previous study on the *Tedania* sp. extract, which showed its ability to induce cell apoptosis in breast cancer cells [15]. Furthermore, the remaining Fractions that had higher FC values than the negative control in Figure 2 may induce apoptosis, but the results were not statistically significant ($p>0.05$).

Regardless of the specific trigger that initiates cell death, caspase-3, and caspase-7 are commonly activated during the process of apoptosis. These two enzymes are commonly believed to work together to carry out the destruction phase of apoptosis by cutting a wide range of protein substrates [16]. Thus, the induction of caspase-3/7 activity is proportional to the activation of apoptosis [9]. The result of the pro-apoptotic effect of *Tedania* sp. extract against HCT116 cell lines can be further studied by identifying the specific bioactive compound responsible for the apoptosis.

Figure 2: The pro-apoptotic activity of the test samples from *Tedania* sp. extract expressed in Fold Change (FC). The asterisk (*) indicates significantly higher FC than the negative control (DMSO) based on the *T*-test ($p<0.05$). Sodium butyrate (NaBut) was used as a positive control. Error bars are SD ($n=3$)



CONCLUSION

In summary, the polar ethanol:water extract of *Tedania* sp. demonstrated potential antiproliferative and pro-apoptotic effects against HCT116 cell lines. Interestingly, although the extract did not demonstrate significant activity compared to the positive control, further research can be conducted to assess the anticancer properties of the *Tedania* sp. extract and explore its potential as a promising chemo preventive agent targeting other hallmarks of cancer. Confirmatory techniques, structure determination, and target identification methods can also be performed to determine its mechanism of action and identify its cellular targets.

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Conflict of interest:

The authors declare that there are no conflicts of interest exist.

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