Anticancer potency of black sea cucumber (*Holothuria atra*) from Mentawai Islands, Indonesia

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ABSTRACT

Introduction: The source of bioactive compounds believed to have strong anticancer potency is derived from sea cucumber. Black sea cucumber (*Holothuria atra*) is a dominant species in Mentawai Islands, West Sumatera, Indonesia. Key factor compound that acts as anticancer in sea cucumber extract is tritepenoid also known as Frondoside A. The purpose of this study was to determine the effectiveness of the active compound taken from black sea cucumber as anticancer. **Methods:** Methods used was experimental laboratory. Anticancer activity of black sea cucumber was determined by using the MTT assay cytotoxic test to measure the IC_{50}. Cytotoxic test was conducted with Supris clone-1 (SP-C1) derived from cloned lymphadenopathy of patients with oral squamous cell carcinoma on lingual part. **Results:** It was found that the compounds contained in black sea cucumber has strong anticancer activity (IC50 = 5.25 µL/mL) against SP-C1. **Conclusion:** Black sea cucumber extract has strong cytotoxic effects against cancer cells SP-C1, therefore black sea cucumber extract potentially developed as a new source of cancer medicine.

Keywords: Black sea cucumber (*Holothuria atra*), Anticancer, Supri's Clone 1 (SP-CI)

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INTRODUCTION

Cancer is one of the leading cause of death worldwide. Data taken from Ministry of Health Republic of Indonesia mentioned that about 10 million deaths was caused by cancer. More than 60% of new cases and about 70% of deaths caused of cancer worldwide occurs annually in Africa, Asia, Central and South America. It is estimated that cancer cases will increase by 0.5 million cases annually (WHO).[1]

Cancer is not a mild disease. The first step in cancer treatment is detection of cancer cell malignancy. This detection can be done by biopsy examination to make quick and precise treatment. The next step usually done is the conventional way treatment, such as chemotherapy, radiotherapy, surgery, or the combinations of those therapies.

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But as widely known, conventional cancer therapies are giving adverse side effects, with the healing rate that is not commensurate with the side effects. Chemotherapy, for example, having side effects such as decreased in blood cell count, multiple infections, anemia, nosebleed, alopecia, skin dryness, nausea, dehydration, hypotension, constipation, diarrhea, and nervous system disorders. Whilst radiotherapy having side effects such as nausea, white decreased in leukocytes count, multiple infections/inflammations, sunburn like effect, fatigue, mouth and throat pain, severe diarrhea, and alopecia (BPOM).[2]

The alternative cancer treatment with minimum side effects is natural treatment. Natural treatment uses natural materials derived from clinically tested extracts from animals or plants. This kind of treatment able to detoxify blood tissue and stimulate the immune system to jointly fight the cancer cells. The use of natural compounds in this type of cancer treatment appear from several approaches, based on the concept from the research that cancer was reversible; concept of treatment by inhibiting cancer growth by eliminating carcinogens and environments that support the genetic mutation of cancer cell growth; concept of cancer cells aging by inhibiting the growth of cancer cells in order to eradicates the opportunity to develop, then the cancer cells will aging and death; concept of strengthening other healthy cells around the cancer cell to form a cell defense that can withstand the development of cancer cells.

Sea cucumber is classified in Echinodermata phylum Holothuridea class.[3] Most species of sea cucumbers are having high economic value as a food source with also some promising biological activities.[4] Demand for sea cucumbers in global market for food and pharmaceutical use increases significantly[5] and has been recognized as a traditional treatment in Chinese and Malaysian literatures for hypertension, asthma, rheumatism, wounds and burns, and impotence.[6] The benefits from sea cucumbers as medicinal compounds can be attributed to the presence of several bioactive compounds, most notably triterpen glycoside.[4]

This study described the pharmacology of black sea cucumber extract by fractionating hexane, ethyl acetate, and butanol to measured the cytotoxic activity as anticancer. The black sea cucumbers were collected from Mentawai Island and Supris clone-1 (SP-C1) derived from cloned lymphadenopathy of patients with oral squamous cell carcinoma on lingual part as anticancer test sample, and cytotoxic activity was tested by using Microculture Tetrazolium (MTT) Assay test.[7]

METHODS

The research was done under experimental laboratory methods. Samples of black sea cucumber were taken in Smasin Waters, Sikabaluan Districts, Mentawai Islands Regency, West Sumatra, at depth of ± 15 meters. Black sea cucumber was then cleaned and weighed as much as 3 kg, and soaked with 96% ethanol solution. Taxonomically, the classification of black sea cucumber has been identified in the Animal Ecology Laboratory Universitas Andalas West Sumatra.

Samples were then cut into small pieces and put into a bottle and soaked with 96% ethanol solution for three days and stirred daily. Soaking process then repeated with new ethanol solution until got clear bath result. The result of ethanol extracts obtained was then distilled by rotary (Rotavapor R-210) on the temperature of ± 40°C to got the viscous extract then stored in temperature 20°C.

As a next step, the ethanol extract was fractionated by dissolving in aquadest and partitioned in separating funnel by using hexane solvent in order to obtained hexane fraction and aquadest fraction. The aquadest fraction then re-partitioned by using ethyl acetate solvent resulted in an ethyl acetate fraction and aquades fraction. The aquadest fraction was then partitioned again by using butanol solvent resulted in butanol fraction and aquadest fraction. The use of partition methods with various solvents with different polarity level which showed certain bioactive compound for anticancer. The results from 3 partitions was then evaporated by using rotary evaporator.

The culture of Supri's Clone-1 Cells (SP-C1) was done in Dulbecco's Modified Eagle's Medium (DMEM) contained 10% Fetal Bovine Serum (FBS). Previously trephination of cell was done by using 0,05% of trypsin-EDTA of 0,53 mM, then added growth medium until became cell suspension. After that the cells were calculated by using hemocytometer, then planted with cell density of

Table 1. Viability percentage of SP-C1 tested with extracts and fractions of black sea cucumber

Sample 100 µg / mL	Cell viability percentage		
	Repetition I	Repetition II	Repetition III
Ethanol extracts	12.00	7.00	17.50
Hexane fraction	108.50	118.00	117.50
Ethyl acetate fraction	98.50	95.00	114.00
Butanol fraction	99.50	105.00	93.50

Table 2. Cell absorbance average measured by using ELISA Reader on the wavelength of 550 nm

Black sea cucumber (extracts and fractions) 100 µg / mL	SP-C1 culture	
	Absorbance average*	Viability average
Ethanol extracts	12.00	12.167
N-Hexane fraction	108.50	114.667
Ethyl acetate fraction	98.50	102.500
Butanol fraction	99.50	99.500

25000 cells/mL, then incubated, and the growth medium was changed every 2 days when the medium color showed pH change. Cells then viewed under a microscope, when the confluent rate reached 80-90%. Then added 1 ml of complete homogenized medium and counted treatment ready cells.

Cytotoxic test was done using MTT assay test. On the 96-well plates added as much as 100 µl of cell suspension with cell density of $2x10^4$/20.000 cell/well, then let stand for 1-2 hours. After that, added 100 µl of the extracts and the results from fractionation of hexane, ethyl acetate, and butanol of black sea cucumber. Then incubated in CO^2 incubator for 24 hours, with CO^2 level of 5%, the temperature was 37^oC, and humidity level of 98%). After 24 hours, observed under microscope, and suspensions patched on tissue paper was then discarded. After, added 100 µl of MTT (5 mg of MTT+1 ml of Phosphate Buffered Saline (PBS)+9 ml of complete Roswell Park Memorial Institute (RPMI) medium/growth) on each well and incubated over night. Then read the results on Enzyme-Linked Immunosorbent Assay (ELISA) Reader with the wavelength of 550 nm and the data analyzed by using viability formula.

RESULTS

The cytotoxic activity test on condensed ethanol extract and the three fractions of black sea cucumber resulted on the calculation on the value of viability percentage as shown in Table 1.

Cytotoxic test of ethanol extracts and fractionations of black sea cucumber were done with 3 treatments. In sample concentration of 100 µg/mL, the highest viability percentage was on the administration of hexane fraction of 118.00% at the 2nd repetition, and the lowest viability percentage was on the administration ethanol extracts of 7% at the 2nd repetition. The value of IC_{50} was obtained by doing probit analysis on viability percentage of SP-C1 cells, resulted in IC_{50} value was 12.167, means that the ethanol extracts of black sea cucumber has a strong cytotoxic effect ($12.167<IC_{50}<100$) against SP-C1 cancer cells. The increasing concentration of black sea cucumber extracts resulted in decreasing viability percentage of SP-C1 cells, as shown in Table 2.

DISCUSSION

The reason for the search for medicine that made from marine animals comes from the fact that marine animals have adapted to all sorts of marine environments and are always reproducing to keep the survival of their life.[8] The development of safe and effective anticancer drugs has revolutionized the cancer treatment in the past 70 years.[9] The increasing of human disease resistance towards conventional medicine, as in this case conventional chemotherapy treatment, has required to a discovery for new anticancer substances from other sources including natural sources from marine sources.[10]

The result from this research was showed that significant anticancer activity was seen in black sea cucumber extracts and no anticancer activity in hexane, ethyl acetate and butanol fractions, as shown in Tables 1 and 2. Sea cucumbers are known to contain several anticancer compounds with antiproliferative agents.[2] Antiproliferative and anticancer functions of sea cucumbers are derived from triterpen glycosides that considered as effective antioxidants to protect from oxidative stress and degenerative diseases.[11]

The results of MTT test showed that black sea cucumber ethanol extracts had IC_{50} value of 12.167%, whilst the hexane fraction was 114.667%, the ethyl acetate fraction was 102.500%, and butanol fraction was 99.50%. According to The American National Cancer Institute, an extracts was classified of having cytotoxic activity when the value of IC50<20 µg/ml.[12] Thus, the black sea cucumber extract can be classified as anticancer substance, because has cytotoxic activity against SP-C1 cancer cells. The value of 12.167% was the average result number from three repetitions.

Components of compounds contained in sea cucumbers can mutually weaken, strengthen, improved or changed altogether. In addition, the quality and quantity of existing substances in sea cucumbers was determined by environmental factors, living places such as climate, water and sea cucumber conditions at the time of capture.[13]

CONCLUSION

From the results of the study can be concluded that the extracts of black sea cucumber has cytotoxic effect towards SP-C1 cancer cells, whilst the hexane, ethyl acetate and butanol fractions have no cytotoxic effect, therefore black sea cucumber extract is a potential new source of cancer medicine.

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