A New Approach to the Treatment of Oral Cancer With Cempedak Sea Cucumber (Bohadsch Marmorata) of Mentawai Islands

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ABSTRACT

Oral Squamous cell carcinoma cases tend to increase and account for more than 90% of all oral cancers. Among the standard treatments are surgery, radiotherapy, chemotherapy or combination, however most patients with oral carcinoma, especially tongue carcinoma, are admitted to the hospital at an advanced stage. During the last few decades, there have been no effective systemic therapy for patients with advanced tongue carcinoma, thus the prognosis for patients with advanced tongue carcinoma is still very limited. One of the treatments being developed is the search for natural or herbal ingredients. Sea cucumbers have important bioactive compounds and are believed to have strong anticancer activity. The bioactive compounds that act as anti-cancer in sea cucumbers are triterpene glycosides. The *Bohadschia marmorata* or brown sea cucumber (Indonesian: *Teripang Cempedak*) is one of the dominant species in the West Sumatran. This study aims to determine the anticancer activity of the extract and fractionation of *cempedak* sea cucumber (Bohadschia marmorata) of the Mentawai islands, West Sumatra. This research is a laboratory experimental study to identify the anticancer activity of the extract and fractionation of *cempedak* sea cucumber (Bohadschia marmorata) using the MTT cytotoxic assay test to measure IC50 against Supri's-Clone 1 (SP-C1) tongue cancer cells derived from the results of lymphodenopathy cloning of patients with carcinomacell squamous tongue. The bioactive compounds in the *cempedak* sea cucumber fractions etyl acetate (*Bohadschia marmorata*) indeed have strong anti-cancer activity against SP-C1, IC_{50} value was 18,833, means that the fractions etyl acetate of *cempedak* sea cucumber has a strong cytotoxic effect ($18,833 < IC_{50} < 100$) against SP-C1 cancer cells. *Cempedak* sea cucumber etyl acetate fractions indeed has a strong cytotoxic effect on SP-C1 cancer cells, therefore the *cempedak* sea cucumber etyl acetate fractions has the potential to be developed as a source of new cancer drugs.

Keywords: *Cempedak sea cucumber (Bohadschia marmorata) etyl acetate fractions, anticancer, Supri's Clone 1 (SP-CI)*

1. INTRODUCTION

Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million after heart disease. In Indonesia, the number of death due to cancer is reported to increase every year from 1.4% in 1972 to 4.4% in 1992 [1].

Squamous Cell Carcinoma (SCC) of the oral cavity is a cancer whose incidence is increasing from year to year [2]. The frequency of oral SCC tends to increase and until now it has reached the 6th rank of the top 10 cancers worldwide [3]. More than 90% of all oral cancers are SCC [4]. In general, SCC of the oral cavity affetcs the tongue (ventral and lateral), lips, floor of the mouth and the oropharynx [5]. The tongue is an area of concern to the incidence of oral SCC as SCC of the tongue represents 25% to 50% of the total number of SCC of the oral cavity.

Conventional cancer therapies such as surgery, chemotherapy, radiotherapy and hormonal therapy usually cause many side effects because conventional therapies cannot distinguish between cancer cells and healthy cells: thus, they damage both cell types and cause serious and often debilitate side effects, thereby often forcing the patient to abandon this treatment. Therefore, many cancer patients seek complementary and alternative therapies. Balunas et al (2005) reported that more than 60% of drugs approved for cancer treatment are of natural origins.

One of the natural ingredients that can be used as anti-cancer is sea cucumber, which is a type of marine invertebrate related to the sea urchins and sea

stars, collectively known as the echinoderms that can be developed and used for anticancer. This biopotency has been reported in many publications describing various activities of sea cucumber extract as anti-cancer.7 In the Mentawai islands, it is called Swallou. Among the types of sea cucumbers that are widely available in the Mentawai Islands and very easy to get, include: Brown Sea Cucumber or Bohadschia marmorata (In Indonesia: Teripang Cempedak).

Thus, this study was conducted in order to determine the anticancer activity of the extract and fractionation of *cempedak* sea cucumber (*Bohadschia marmorata*) of Mentawai islands, West Sumatra using the MTT cytotoxic assay to measure IC50 against tongue cancer cells Supri's-Clone 1 (SP-C1) derived from the cloned results of lymphodenopathy in squamous cell carcinoma of the tongue.

2. METHOD

This type of research is experimental research, which is quantitative research with a pure in vitro experimental method because the treatment of the subject was carried out under artificial conditions. The samples used were sea cucumbers taken directly from Mentawai sea cucumber divers. The sea cucumbers were cleaned and cut into small pieces, weighed as much as 3 kg, put in plastic, soaked in 96 ethanol solution, left for 3 x 24 hours and stirred every day.

The solution resulting from the immersion of the sample was later filtered using filter paper, then soaked again three times with a new solution, a process called maceration. Maceration was carried out at room temperature and protected from light. The ethanol extract obtained by maceration was then distilled. Distillation is the process to separate pure extract from the solvents. After obtaining the pure extract, a rotary was carried out with a Rotavapor R- 210 to obtain a thick extract with a temperature of ± 40ºC.

Furthermore, the ethanol extract was dissolved in distilled water and partitioned in a separating funnel using n-hexane as a solvent to obtain the n-n-hexane fraction and equates fraction. The distilled water fraction was re-partitioned using ethyl acetate as a solvent to produce an ethyl acetate fraction and a distilled water fraction. The distilled water fraction was re-partitioned using butanol as a solvent in order to produce the butanol fraction and aquades fraction. The use of various methods of partitioning aims to classify compounds based on their level of polarity. The resulting fractions (n-n-hexane, ethyl acetate and butanol) were evaporated using a rotary evaporator.

Sp-C1 tongue SCC cells were cultured in medium (DMEM) containing 10% FBS. Trypsination of cells was previously carried out using trypsin 0.05% - EDTA0.53 mM, growth medium was later added to make it a cell suspension. The cells were further counted using a hemocytometer and then grown with a cell density of 25,000 cells/mL, the cells were then incubated and the growth medium was changed every two days when the color of the medium showed a change in pH.

Cell harvesting was carried out by observing the cells under a microscope, if they are 80-90% confluent then the medium in the flash discard was discarded, the medium without FBS was washed, put into flash, then shaken to remove the remnants of FBS attached to the cells. The discarded medium was poured and added with trypsin-EDTA 0.25% 1-2 ml into the flask containing the cell culture, to be incubated for 10 minutes. Once the cells were separated, 3 mL of medium was added. 10 µL of cell suspension was put into each cell counting box of the hemocytometer and calculations under a microscope was performed to determine the average number of active cells present to be able to make a suspension of 2,000 cells in each well on a 96-well plate. After the cells were counted and the number was obtained, the cells wereready for treatment.

By using 96 well test plates, 100 µl of suspension each with a density of $2x10^4$/20,000 cells/well was added. Then the sieves were incubated for 24 hours. Each section was designed for 3 replications. 100 µl of extract solution and fractionate the *cempedak* sea cucumber (Bohadschia marmorata) were added. Microplates were incubated in a CO_2 incubator for 24 hours (CO_2 content 5%, temperature 370^0C, humidity 98%). After 24 hour-observation under a microscope, any existing medium was discarded (by turning it upside down) on tissue paper (for adhering cells) in each well to be incubated for 4-6 hours, added with 100 µl of stop solution to each well and then incubated overnight. The MTT reaction was terminated by adding sodium dedosil. The wavelength of 550 nm was read on the ELISA Reader. Finally, the data can be analyzed using the viability formula.

3. RESULTS

Cytotoxic test of extract and fractionation of *Cempedak* sea cucumber (*Bohadschia marmorata*) of Mentawaiislands against Sp-C1 culture using the MTT assay method was carried out 3 times with a concentration of 100 µg/ml. The absorbance values that produce viability values obtained from the test results can be seen in the following table:

Table 1: the value of extract viability and fractionation of *cempedak* sea cucumber (*Bohadschia marmorata*) against Sp-C1 culture

Sample 100µg/ml	Percentage of cell viability		
	I	II	III
Ethanol extract	106.50	98.50	112.50
Hexane fraction	98.00	109.50	115.00
Ethyl acetate fraction	22.00	15.00	19.50
Butanol fraction	100.00	114.50	109.00

Table 1 shows the highest viability value was produced by the butanol fraction of 114.50% in the second iteration. The lowest viability value was produced by the ethyl acetate fraction of 15,00% also in the second iteration. From the viability value, probit analysis was carried out to obtain the IC_{50} value, the resulting IC_{50} value was 18.833 indicating that the ethyl acetate fraction of *cempedak* sea cucumber (*Bohadschia marmorata*) has a strong cytotoxic effect on SP-C1 cancer cells which can be seen from the decrease in the percentage of SPC1 cell viability, as shown in table 2 below:

Table 2. the mean value of absorbance andviability of the extract and fractionation of*cempedak* sea cucumber (*Bohadschia marmorata*) against Sp-C1 culture

Sample 100µg/ml	Culture of Sp-C1	
	Absorbance Mean	Viability Mean
Ethanol extract	0,852	105,833
Hexane fraction	0,855	107,500
Ethyl acetate fraction	0,678	18,833
Butanol fraction	0,856	107,833

4. DISCUSSION

Based on the research that has been carried out, it shows significant anticancer activity produced by the ethyl acetate fraction of *cempedak* sea cucumber (*Bohadschia marmorata*) and no anticancer activity produced by ethanol extract, hexane fractionation and butanol fractionation from *cempedak* sea cucumber (*Bohadschia marmorata*).

The ability of sea cucumbers as anticancer is caused by triterpenoid compounds which are secondary metabolites that are mostly found in marine organisms such as sea cucumbers [7]. Over the past few years, a large number of biologically active triterpenoids have been found to have cytotoxicity against various tumor cells [8]. Anticancer triterpenoids isolated from sea cucumbers are triterpene glycosides (saponins) where these compounds are measured to have the ability to block activation of nuclear factor-*kappaB*, induce apoptosis, inhibit signal transducers, and activate transcription and angiogenesis [9].

The results of the MMT assay showed that the ethyl acetate fraction of *cempedak* sea cucumber (*Bohadschia marmorata*) had an IC_{50} value of 18.833%, while the others were ethanol extract with the value of 105.833%, hexane fraction with the value of 107.5% and butanol fractionwith the value of 107.833%.

According to The American National Cancer Institute, an extract is classified as having cytotoxic activity when the IC_{50} value is < 20 g/ml [10]. Therefore, the ethyl acetate fractionation of *cempedak* sea cucumber (*Bohadschia marmorata*) is classified as an anticancer agent, as it has cytotoxic activity against SP-C1 cancer cellswith value 18,833%.

5. CONCLUSION

Ethyl acetate fractionation of *cempedak* sea cucumber (*Bohadschia marmorata*) has a strong cytotoxic effect on SP-C1 cancer cells, therefore the Ethyl acetate fractionation of cempedak sea cucumber has the potential to be developed as a source of new cancer drugs.

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