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Anticancer Activities Of Toxic Extract Of *Xestospongia testudinaria* sponge From Sanur Beach, Bali, Indonesia.

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ABSTRACT

Xestospogiatestudinaria spongehas potentially activity as anticancer agent. According to the toxicity assay, X. testudinaria sponge methanol extract was more toxic towards Artemiasalina larvae than that of n-hexane. This study aims to determine the anticancer activity of toxic extracts of X. testudinaria sponge collected from Sanur beach, Bali, Indonesia. Extraction of metabolites was conducted by room temperature using methanol. The extract was assayed its anticancer activity against HeLa cell line. The X. testudinariamethanol extract showed anticancer activity aganitsHeLa cell with IC_{50} value of 1.327 ppm.It was very active categories. These finding suggest that methanol extract of X. testudinariacould be used as natural anticancer towards HeLa cell, and also as a preventive therapy against diseases such as servical cancer.
Keywords: Anticancer, Xestospongiatestudinaria, HeLa cell

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INTRODUCTION

Recently, cancer is still an important public health problem, both in Indonesia and in other countries because of the high mortality rate due to cancer. Cervical cancer is the second most common cancer among women in the world and the third largest cause of cancer deaths in women. It is estimated that in every year more than 270,000 deaths are caused by cervical cancer and more than 85% occur in developing countries [1]. Modern cancer treatments are still done today through surgery, radiation and chemotherapy [2]. The use of synthetic drugs largely not only kills cancer cells, but also kills normal cells [3]. This treatment also requires a high cost. Therefore, the researchers began to look for drugs that come from natural sources including natural marine biological materials. Sponges are one of the natural products of the ocean that produce new compounds with unique structures and have pharmacological activity [4]. Nearly 5,000 compounds have been successfully isolated from sponges with various activities such as antimicrobial, antifungal, antiviral and anticancer [5].

Various studies have been conducted to isolate the compounds of the type sponges Xestospongiatestudinaria (X. testudinaria). Quinn and Tucker [6] isolated brominated bis-acetylenic acetic acid. Furthermore Quinn and Tucker [7] also isolated two new compounds of brominated acetylenic acids. Pham et al. [8]isolated brominatedacetylenic fatty acid and two esterified sterol. Jiang et al. [9] isolated four brominated aliphatic hydrocarbon and sterol. Lee et al. [10] isolated Marinobacterxestospongiaesp Nov. Sun et al. [11] isolated the new compound bisabolanesesquiterpenoid. Nguyen et al. [12] reported isolating anti-fouling compound 26,27-cyclosterol. Toxicity of X. testudinaria has been reported by Zhou et al. [13]. According to their report, the five compounds contained in X. testudinaria(Sapinofuranone; Xestospongic acid; 24-hydroperoxy-24-vinyl-cholesterol; Saringosterol; and 29-hydroperoxystigmasta-5,24-dien-3β-ol) were toxic towards larvae of A. salina with LC_{50} values varies between 0.56 and 6.99 µM. Utamiet al. [14]reported that sponges Jaspis sp. associated with marine bacteria could produce anticancer compounds, flavonoid, alkaloid, and triterpenoid. The extract could inhibit Hela cells with the range IC_{50} values of 242.54 to 267.03 µg/mL.

Prescreening test of a substance potentially having anticancer activity is by toxicity test using Bhrine Shrimp Lethality Test (BSLT) method [15]. A substance has the potential to have anticancer activity if it has toxicity towards A. salina with LC_{50} less than 1000 ppm, so that the test of that can be continued with anticancer tests. Swantara and Rita [16] reported that X.testudinaria sponge methanol extract was toxic to Artemiasalinalarvae with LC_{50} of 31.62 ppm, while that of n-hexane extract showed less toxic because of its LC_{50} value of 177 ppm. The anticancer activity of sponge X. testudinaria has not yet been reported. Hence, the aims of this study to determine the activity of sponge X. testudinaria methanol extract against Hella cell line.

MATERIALS AND METHODS

Materials

X. testudinariasponges were collected at Sanur beach, Bali, Indonsia, on April 25th to Mei 5th, 2017. Methanol, n-hexane, ethyl acetate, and butanol were purchased in Merck, Germany. Brine shrimp Artemiasalina Eggs was purchased in American Technology. SelHeLa Line was purchased in Primate Study Center, Bogor Agriculture University.

Sample Preparation and Extraction

Wet sponge X. testudinaria were cleaned with fresh water, then sliced into small size. Furthermore, they dried in a place that is not subject to direct sunlight for approximately 10 days. After drying, the sample was smoothed using a blander and sieved to a smoothness of 100 mesh. Dry powder samples of 250 grams were extracted using methanol by maceration method. Every 24 hours the extract was filtered and the residue was added with a new solvent. Substitution of this solvent was done up to 3 times. The extracts were then vaporized using a rotary vacuum evaporator until a crude methanol extract was obtained.

Anticancer Assay

The methanol extracts were then assayed for its anticancer activity against HeLa cells. Cervical cancer cells (HeLa) were cultured on RPMI (Roswell Park Memorial Institute) 1640 media, calculated the initial

number of cells under a microscope. Then the cells were harvested with the addition of trypsin. The cells were then centrifuged to form two layers (sediment and supernatant). The supernatant was removed and the precipitate was pelletized and a complete 1 mL medium was added and then counted the number of cells using a hemocytometer. After the cells were sufficient, the cells were grown on micro well plate 96 wells. Each well contained 2x104 cells in 100 µL. Cells were incubated for 1-2 hours so that the cells were attached. After that, extract of the test material was added with various concentrations (1000; 500; 250; 125; 62.5; 31.25; 15.62; 7.81; 3.91; 1.95; 0.97; 0.48; 0.24; 0.12 and 0.06 µg/mL) at each well of 100 µL. So the total volume of each well was 200 µL, then they were incubated for 24 hours at 37° C. After 24 hours, cells were observed under the microscope. MTT (3- (4,5-dimethyltiazole-2-yl) -2,5-diphenyltetrazolium bromide (5µg / 1mL) was added at each well, then incubated for 4 hours. Furthermore, stop solution SDS (sodium dodecyl sulfate) 10% in 0.01 N HCl was added into each well and incubated again one night. The absorbance was read using an ELISA (Enzyme-Linked Immunosorbent Assay) reader at a wavelength of 550 nm.

RESULTS

Sample preparation and Extraction

The wet sponge samples of 7,634 grams were cleaned, sliced, and dried. It produced dry sponge sample of 893 grams. All of the dried samples were mashed and sieved with a fineness level of 100 mesh producing 784 grams of fine sample. About 250 gram sample was extracted with methanol resulting in 757 mg of methanol extract.

Anticancer activity

Anticancer activity against HeLa cells was determined by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) method. The principle of the MTT test was the occurrence of a yellow change mechanism of the Tetrazolium salt (3- (4, 5-dimethyltiazole-2-yl) -2, 5-diphenyltetrazoliumbromide) reduced to the purple Formosan crystals in the mitochondria of living cells. MTT was absorbed into living cells and broken down by the reduction reaction by the reductase enzyme in the mitochondrial respiration chain into a soluble formazan in a purple 10% SDS (sodium dodecyl sulfate) [17].

The absorbance value of formazan formed was measured by micro plate reader at 595 nm wavelength with triple treatment. Data obtained from cytotoxicity test with MTT were absorbance or optical density (OD), and then the mean value of OD was converted to% inhibition. Observation results of absorbance and calculation of% inhibition of HeLa cells after being given toxic extract (sponge X. testudinaria methanol extract) was shown in Table 1.

Based on the data of Table 1 above, a relationship between sample concentrations and % inhibition to calculate IC_{50} can be made (Table 2).Based on the Table 2, it can be made a graph correlation between the sample concentration and the % inhibition for the determination of IC_{50} values was presented in Figure 1.

Table 1: Inhibition of toxic extracts (methanol extract)

Concentration (ppm)	Optical Density			Average	Inhibition (%)
	1	2	3		
100	0.037	0.035	0.035	0.035	82.32
50	0.044	0.042	0.037	0.041	79.29
25	0.052	0.045	0.036	0.044	77.77
12.5	0.062	0.056	0.046	0.054	72.72
6.25	0.071	0.063	0.057	0.063	68.18
3.125	0.078	0.066	0.071	0.071	64.14
1.56	0.094	0.097	0.088	0.093	53.03
0.78	0.102	0.1	0.1	0.100	49.49
0.39	0.135	0.125	0.133	0.131	33.83

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0.195	0.145	0.142	0.150	0.145	26.76
Cell control	0.195	0.197	0.202	0.198	0.00

Table 2: Correlation between sample concentration and inhibition

Concentration , x (ppm)	Inhibition, y (%)
100	82.32[a]
50	79.29[a]
25	77.77[a]
12.5	72.72[b]
6.25	68.18[bc]
3.125	64.14[b]
1.56	53.03[d]
0.78	49.49[d]
0.39	33.83[e]
0.195	26.76[f]
0	0.00[g]

* Values followed by the same letters in the same column are not significantly different according to the Duncan's Multiple Range Test at P<5%.

From the Figure 1, the equation of the graph was y = 47.4729 + 8.9399 in (x), with coefficient determination (R^2) of 0.9393. The IC_{50} can be calculated based on the equation.

50 = 47.4729 + 8.9399 ln (x); ln (x) = (50-47.4729)/8.9399; ln (x) = 0.283; x = 1.327 ppm. Thus the IC_{50} of the toxic extract was 1.327 ppm. Anticancer activity of 1.327 ppm was categorized into very active anticancer [18].

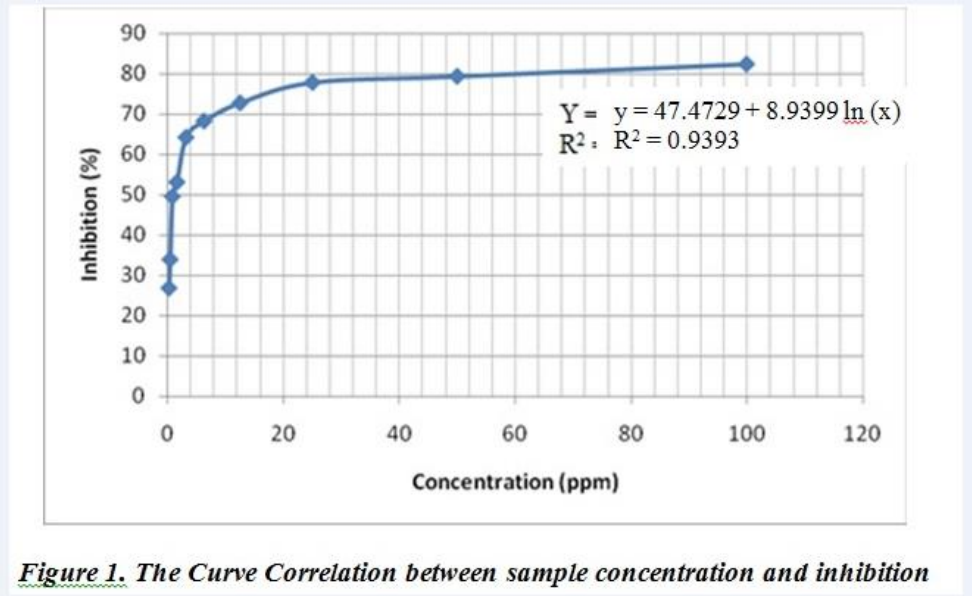

Figure 1. The Curve Correlation between sample concentration and inhibition

DISCUSSION

Based on the Table 2, it can be seen that the concentration of extracts was positively correlated with an inhibition towards HeLa cell growth. The inhibition at the concentration of 0.195 ppm was significantly different from the control. While at concentrations of 0.78 and 1.56 ppm, the inhibition was not significantly different as well as that at concentrations of 25; 50; and 100 ppm. So it can be said that the concentration of 25 ppm was the optimum concentration in inhibiting HeLa cell growth.

Based on the anticancer prescreening test (toxicity test) conducted by Swantara and Rita [16], it showed that the methanol extract of sponge X. testudinaria was toxic towards A. salina larvae with LC_{50} of 31.62 ppm. Thus, the methanol extract was potentially active as anticancer agents. The results of this study were in line with the research conducted by Zhou et al. [13]. He reported that five compounds in the X. testudinaria sponge, namely Sapinofuranone; Xestospongic acid; 24-hydroperoxy-24-vinylcholesterol; Saringosterol; and 29-hydroperoxystigmasta-5,24-dien-3β-ol were toxic to A. salina larvae with LC_{50} values varying between 0.56 to 6.99 µM.

The anticancer activity of X. testudinaria extract against HeLa cell in this study was much more active (IC_{50} = 1.327 ppm) compared to Jaspis sp. extract that was associated with marine bacteria (IC_{50} = 242.54-267.03 µg / mL) [14].

CONCLUSION

Based on the study, the anticancer activities of methanol extract of X. testudinaria sponge was positively correlated with an inhibition towards HeLa cell growth. It was very active categories with IC_{50} value of1.327 ppm.

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Conflict of Interest: We declare that they have no conflict of interest.

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