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Anticancer Pre-screening of Marine Sponge *Chynachyrella* Extract From Spermonde Archipelago, South Sulawesi, Indonesia.

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ABSTRACT

Anticancer pre-screening of marine sponge extract of *Chynachyrella* genus from Spermonde Archipelago of South Sulawesi has been conducted. The sponge *Chynachyrella australiensis* and *Chynachyrella* sp. was immersed in methanol. The crude methanol extract was partitioned by n-hexane and followed by ethyl acetate. Each fraction was evaporated to produce the n-hexane, ethyl acetate, and methanol extracts. N-hexane and ethyl acetate extracts of both sponge are positively contain steroid compounds. Methanol extracts of both sponges contain alkaloids compounds. N-hexane extract of *Chynachyrella* sp., ethyl acetate extract of both sponge and methanol extract of *C. australiensis* showed toxicity against *Artemia salina* nauplii. IC_{50} value of extracts all species sponge is proportional to toxic activity, and ethyl acetate extracts of all sponge species has potential as anticancer.

Keywords: *Cinachyrella*, *Chynachyrella australiensis*, toxicity, antioxidant.

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INTRODUCTION

Sponge is marine organisms as source of metabolites with unique or new structures. The functional group compounds in sponge are not commonly found in terrestrial organisms. These compounds have pharmacological activity that is toxic properties can be used to inhibit or kill cancer cells [1].

Identified 150 species of sponge in the Spermonde Archipelago, Strait of Makassar [2]. One of them is sponge *Cinachyrella* genus of family Tetillidae.

Some compounds components were found in sponge of *Cinachyrella* genus showed pharmacological activity, especially as anti-cancer. (3E)-cholest-4-en-3,6-dion-3-oxime compound of *C. australiensis* sponge from China have cytotoxic properties against hepatitis B virus [3]. Cinachyramine compounds of *Cinachyrella* sp., sponge have cytotoxic properties against HeLa S_3 cells (IC_{50}: 6.8 μg /mL) [4]. Enigmazole A compound of *C. enigmatica* sponge have cytotoxic properties against the NCi 60 cell-line screen antitumor with an average of GI_{50} is 1.7 μM [1]. Sponge *C. apion* contain lectin with antiproliferative potency against tumor cell line [5]. Dichloromethane and ethanol extracts of *C. tarentine* sponge showed antifungal and antibacterial activity [6].

In this research was conducted of secondary metabolites identification, toxicity and the antioxidant activity test of two species marine sponge extract of *Cinachyrella* genus from Spermonde Archipelago South Sulawesi. Toxicity and antioxidants tests of a prescreening (anti-cancer) is determining activity of toxic (toxic effects) and damping ability of free radical extracts of *C. australiensis* and *Cinachyrella* sp. sponge as an indication of active potential compound in extract against cancer cells.

MATERIALS AND METHODS

Marine Sponge *C. australiensis* and *Cinachyrella* sp. sampling

Sponge samples were collected from Spermonde Archipelago South Sulawesi by SCUBA at a depth of 10-25 m in Sep-Oct 2015. A voucher specimen (SVP 01/10/15, Figure 1) and (SVP 02/10/15, Figure 2) was deposited at the Research Center for Oceanography Indonesian Institute of Sciences in North Jakarta.

Figure 1: *Chynachyrella australiensis*

Figure 2: *Chynachyrella* sp.

Extraction of Marine Sponge

Dried sponge samples were immersed in methanol for three cycles and allowing enough time to achieve color fading to get optimal extraction of the sample. Each cycle of extraction was carried out with stirring overnight at room temperature. The methanol extracts were collected and dried by rotary evaporator to obtain the crude extracts. The crude methanol extract was partitioned with n-hexane and followed by ethyl acetate. Each fraction was dried to get of n-hexane, ethyl acetate, and methanol extracts. These extract was tested of the phytochemical screening, toxicity and antioxidant activity.

Brine Shrimp Lethal Assay

The lethality assay modified method [7]. The larvae of *A. salina* were obtained after incubation of eggs in a brine shrimp media and equipped with an aerator was kept under 40 watts lamp for 24 hours. The larvae that hatch were left for 24 hours. After 48 hours, old larvae (nauplii) ready to take toxicity study.

The n-hexane, ethyl acetate, and methanol extracts was tested at 1000, 100, and 10 µg/mL and evaluated by triplicate for each test concentration and control. Then, ten *A. salina* naupliis were transferred to each vial. The volume was then adjusted to 5 mL by artificial sea water.

After 24 hours of incubation, the number of survivors was counted and mortality percentage for each concentration, analyzed the data further probit to determine LC_{50} values of extracts.

Antioxidant Activity of the Extracts of marine Sponge.

The modified method [8] was employed in this study. DPPH solution was prepared in 95% methanol.

The n-hexane, ethyl acetate, and methanol extracts were mixed with 95% methanol to prepare the stock solution (5 mg/5mL). The test samples were prepared from stock solution by dilution with methanol to attain a concentration of 5, 10, 25, 50, and 100 µg/mL respectively (triplicate). The freshly DPPH solution was added into each of these test tubes that contain sponge extracts and after 30 min, the absorbance was taken at 517 nm using a UV-Vis spectrophotometer.

Absorbance and percentage inhibitions for each concentration of test samples is calculated and tabulated. The data was analyzed probit to determine the IC_{50} value of each extracts.

RESULTS AND DISCUSSION

Amount of n-hexane, ethyl acetate and methanol extracts of sponge *Chynachyrella* genus from Spermonde Archipelago are presented in Table 1.

Table 1 showed that the acquisition of n-hexane extracts of both sponge species greatest, then methanol extracts, and the smallest is ethyl acetate extracts.

Table 1: Amount of Extracts Sponges Genus *Chynachyrella*.

Extracts	Species Sponges	
	C. australiensis	*Chynachyrella* sp.
n-hexane	245,00 g	265,64 g
ethyl acetate	33,28 g	28,00 g
methanol	166,00 g	214,87 g

The result showed compounds content in both of sponge species are dominated by non-polar compounds, semi-polar compounds content is very low.

Secondary metabolites content in extracts of both sponge species, toxicity, and antioxidant showed in Table 2.

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Table 2: The content of Secondary Metabolites, Toxicity, and Antioxidant Extract Sponges Genus *Chynachyrella*

Species Sponges	Extracts	Secondary Metabolites						Toxicity (LC_{50} ppm)	Antioxidant (IC_{50} ppm)
		Steroid	Terpenes	Flavonoid ($FeCl_3$)	Alkaloid				
					W	M	D		
C. australiensis	n-hexane	(+)						7265,92	25176,76
	ethyl acetate	(+)						247,57	1076,46
	methanol				(+)	(+)	(+)	624,97	5023,42
Chynachyrella sp.	n-hexane	(+)						742,56	849,37
	ethyl acetate	(+)						338,31	639,73
	methanol				(+)	(+)	(+)	1952,84	1172,19

Annotation

W: Wegner
M: Meyer
D: Dragendorf

An extract with highly toxic is value of LC_{50}<30 ppm, toxic is value of LC_{50}; 30-1000 ppm and not toxic is value of LC50> 1000 ppm [9].

Based on these criteria, four extracts showed toxic activity against larvae of *A. salina* there are n-hexane extract of *Chynachyrella* sp., sponge, ethyl acetate extracts of both species sponge and methanol extract of *C. australiensis* sponge. The toxic activity of fourth extracts is an indication of active potential compound contained in extracts against tumor cells/cancer cells.

LC_{50} value of ethyl acetate extracts both of sponge species is lower than the other extracts. The data showed that the greater toxicity of compounds was found in sponge of *Chynachyrella* genus are semi-polar included in the steroid class. The similarity class of secondary metabolites and toxic activity of ethyl acetate extracts from both species sponge, cannot be used as an indication that the chemical compounds contained in ethyl acetate extracts of both species are exactly the same compounds.

N-hexane extracts of *C. australiensis* and *Chynachyrella* sp. contains steroids compound, but steroids compounds in n-hexane extracts of both sponge species are not the same, it was showed on the toxic activity of both extract. While, the methanol extracts of both species also showed different toxic activity, it showed differences of alkaloids compound content.

The whole extracts of both species sponge genus *Chynachyrella* showed IC_{50}> 200 ppm (antioxidant activity is very weak) [10]. IC_{50} value of each sponge extracts is proportional to the toxic activity of extracts, in this case LC_{50} and IC_{50} value ethyl acetate extracts of both species is lowest compared to other extracts. The result is an indication that the compounds have highest anticancer potential contained in ethyl acetate extracts of both sponge species.

CONCLUSION

Ethyl acetate extracts of both of sponge *C. australiensis* and *Chynachyrella* sp. containing the steroid compound as a anticancer potential.

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