

# Study on Anticancer Activity of Extracts of Sponges Collected from Biak Water, Indonesia

**A.Trianto<sup>1,2</sup>, A. Ridhlo<sup>2</sup>, D.W. Triningsih<sup>2</sup>, and J. Tanaka<sup>3</sup>**

<sup>1</sup> Natural Product Laboratory, Centre of Research and Services (Laboratorium Terpadu) Diponegoro University,

<sup>2</sup> Department Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Indonesia

<sup>3</sup> Department Chemistry, Biology, and Marine Science, University of The Ryukyus, 1-Senbaru, Nishihara, Okinawa, Japan.

E-mail:

agustrianto.undip@gmail.com

**Abstract.** Indonesia is center of biodiversity where marine sponges are abundant. a source of bioactive compounds with various pharmaceutical properties such as anticancer, antifungal, antibacterial, antioxidants, anti-inflammatory, and anti-malarial. In a continuation of a search for biologically active molecules from marine organisms we investigated the potency of marine sponges as anticancer. A total of 106 sponge specimens were collected between 3-40 m depths by SCUBA diving in Biak waters during August 2005. The specimens were extracted with methanol to provided crude extracts. The methanolic extracts were tested against NBT-T2 cell line. The assay result showed that 8.5 %, 29.2 % and 46.2 % of the extract have activity against the cell line at 0.1, 1.0 and 10.0 µg/mL. While, a 16.0 % of the extract did not showed activity against the cell line.

Keywords : sponge, anti-cancer, NBT-T2, screening, extract

## 1. Introduction

Sponges are the most primitive of multicellular invertebrate that distribute widespread in the marine ecosystem. Sponges are sessile animal with commonly have soft body that easy to be predated by various animal such as fishes, turtles, sea urchin, nudibranches, and flat worms [1][2]. Sponges also compete for space either with other sessile organisms or fouling organisms. For their survival sponges produce various classes of secondary metabolites such as alkaloid, steroid, glycosides, macrolides, and terpenoid [3].

Many researches showed that the secondary metabolites from sponges have pharmacological properties as anticancer, antibacterial, antifungal, antioxidant and other activities [4][5][6][7]. Cancer cells develop the resistance against the drug basically in two method i.e. by develop the membrane cell and development pumping system or known as mdr type 1 and mdr type 2. On the mdr type 1 the cell membrane will selectively pass the substrate that enter to cell. Once the cell recognize the anticancer drug the membrane will refuse it [8].

Marine organisms have proven to be an exceptionally valuable resource for bioactive natural products discovery efforts, particularly in the area of potential anticancer compounds. Metabolites isolated from marine samples often possess unique structural features and incorporate new or unusual assemblages of functional groups. They have demonstrated a broad range of potent biological



activities, and there are currently at least 11 marine-derived compounds under clinical development in the U.S. and Europe [9]. In addition, there are currently two approved marine-derived drugs, the cone snail peptide Prialt for chronic nerve pain and the ascidian alkaloid Yondelis for soft tissue sarcoma treatment [10].

Indonesia is located in tropical area, blessed with a huge marine biodiversity. A number of bioactive compounds derived from Indonesian marine organisms were reported i.e. manadoperoxides originally isolated from a sponge *Plakortis* cfr. *simplex* possessed antimalarial activity [11]. A review on marine natural products in the period of 2001-2010 revealed that Indonesia is the second country that contribute on the finding of the new compounds after Japan [12]. This report indicate that Indonesia provide a good source for the drug development.

In our continuing search on bioactive compounds we collected sponges sample from Biak water. In this paper we described the bioactivity of the methanolic extracts from the marine sponges against NBT-T2 cells.

## **2. Materials and methods**

### **2.1 Animal Material**

The sponges were collected at 10-35 m depths with SCUBA in Biak, Papua-Indonesia on August 2008. The specimens were kept frozen until extraction [13].

### **2.2 Extraction and Isolation**

After cutting into small pieces, the sponges specimens were soaked with methanol (laboratory grade) for 24 h. The extraction process was repeated for three times. Combined methanol extracts were concentrated by a rotary evaporator under vacuum to gave crude materials [13].

### **2.3 Bioassay for Screening process**

NBT-T2, a cell line derived from chemically induced rat bladder carcinoma cells [13] was purchased from RIKEN and used for cytotoxicity screening and MTT assay. The sponge extracts were tested at concentrations of 0.1, 1 and 10 µg/mL in triplicates. The NBT-T2 cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) in the presence of antibiotic-antimycotic (Sigma Aldrich, US), fetal bovine serum (Biowest, Germany), MEM non essential amino acid (Gibco, US) on 24 or 48 well plate (Falcon, US). After adding an aliquot of extracts, cells were incubated for 24 h under 5% CO<sub>2</sub> at 36°C. Then, the cells were observed under a microscope to evaluate cytotoxicity of the extracts [14].

## **3. Result and Discussion**

Marine sponges produce secondary metabolite that can be utilized as prolific source of anticancer agents [4]. A cytotoxic screening of 106 marine sponges methanolic extracts were conducted by using MTT assay method against NBT-T2 cells. The result is displayed on Figure 1, about 84% extract was cytotoxic at a series of concentration levels, the rest 16% extract did not show citotoxicity. Among the active extracts, 46% showed moderate activity at 10.0 µg/mL, 29% showed strong activity at 1.0 µg/mL and 9% showed potent activity at 0.1 µg/mL on the cytotoxixity testing. These results suggested that marine sponges offer a promising anticancer candidates.

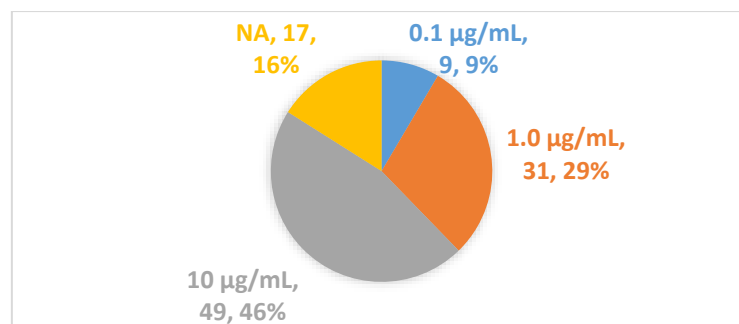


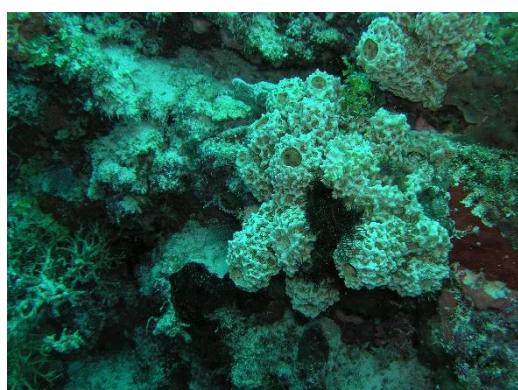
Figure 1. The percentage of the toxicity test result of the Biak collection sponges extracts against the NBT-T2 cells



*Aaptos* sp.



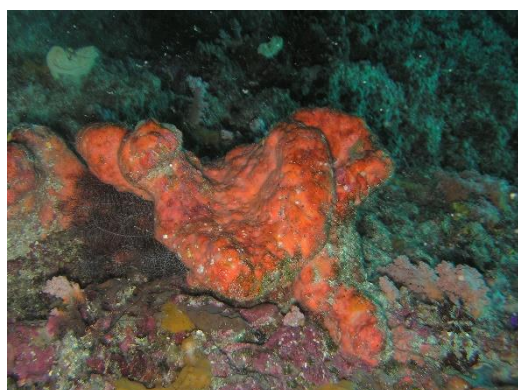
*Strongylophora* sp.



*Lyosina* sp.



*Phylospongia* sp.



*Agelas* sp.



*Dysidea* sp.

Figure 2. Few examples of the sponges collected from Biak water.

Biak Water is located in Coral Triangle, an area housing of an abundant marine organisms. In this study, eighteen sponge genera were revealed from about 22 identified sponge specimens. Figure 2 showed some identified sponges that collected from Biak water during this study. *Dysidea* is one of the genus sponge that can be found in this area. Many studies reported that the genus of *Dysidea* is rich of bioactive compounds.

In our previous paper, we reported two new and four known bioactive compounds isolated from the sponge *Dysidea* sp originally from Biak Water (Figure 3.). Debromoantazirine showed moderate cytotoxicity against NBT-T2 cells with  $IC_{50}$  4.7  $\mu$ g/mL, while, the dyzidazirine was reported cytotoxic to be L1210 cells and inhibited *Pseudomonas aeruginosa*, *Candida albicans*, and *Saccaromyces cereviceae* [16]. Furthermore, frondosin, neoavarol and antazirine were reported to be cytotoxic to PKC [17], inhibitors of IL-8 R $\alpha$  and IL-8 R $\beta$  receptors [18], and piscicidal [19] respectively.

Among the genera, *Theonella* extract showed significant cytotoxic activity which active in the lowest concentration 0.1  $\mu$ g/mL. Marine organisms are recognized as a rich source of compounds that can inhibit HIV infection or modulate specific molecular targets associated with viral entry or replication. Within this group of antiviral marine metabolites is a family of cyclic sponge peptides with potent anti-HIV properties, Papuamides A-D were obtained from Papua New Guinea *Theonella mirabilis* and *T. swinhoei* samples [20]. Theopederins A-E were antitumor originally isolated from *Theonella* sp. against P388 murine leukemia cells [21]. Moreover, member of genus *Theonella* are recognized to have sterols that exhibited anticancer activities [22].

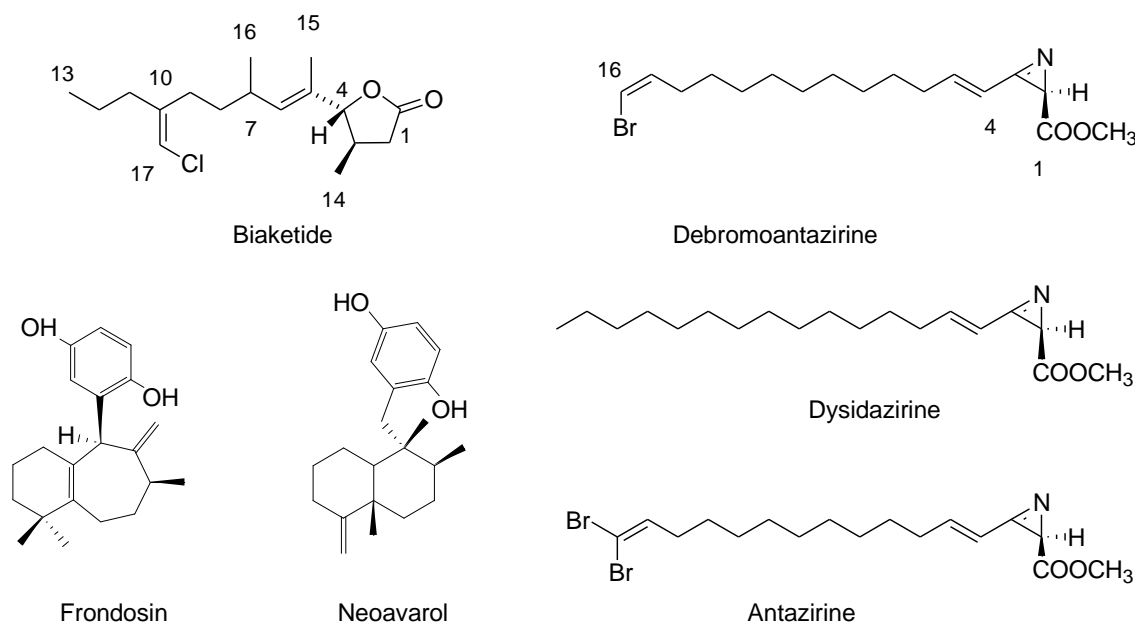


Figure 3. Some bioactive compounds isolated from the sponge *Dysidea* sp. collected from Biak, Papua [15]

#### 4. Conclusion

In conclusion, the preliminary screening on cytotoxic assay of some marine sponges suggested that marine ecosystem is still providing a desirable drug mine. The sponge *Dysidea* sp is one of the promising candidate as drug source.

## 5. Acknowledgement

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