Majalah Farmasi Indonesia, 14(1), 238-243, 2003

BIOACTIVITY SCREENING OF SPONGES COLLECTED FROM BUNAKEN, MENADO BY *Brine Shrimp Lethality Test* AGAINST *Artemia salina* Leach

SKRINING BIOAKTIVITAS SPONS YANG DIKOLEKSI DARI BUNAKEN, MENADO DENGAN METODE *Brine Shrimp Lethality Test* TERHADAP *Artemia salina* Leach

Puji Astuti*, Gemini Alam dan Subagus Wahyuono***
* Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University
**Department of Pharmacy, Fac. of Math and Nat. Science, University of Hasanuddin, Makassar

ABSTRACT

Indonesia is one of the countries rich of natural resources. Sponges are marine invertebrates widely found in Indonesia and known to have various bioactive compounds with unique chemical structures.

This study was aimed to screen potentially bioactive extracts of sponges collected from Bunaken and identify on their toxicity level against larva *Artemia salina* Leach.

Samples were macerated using acetone followed by partition using chloroform and methanol. The extracts were prepared at the concentration of 100, 250, 500 and 1000 μg/ml and their toxicity was tested using Brine shrimp Lethality Test. LC_{50} (μg/ml) of the extracts was calculated using probit analysis.

From the 4 extracts tested, all of them showed toxicity to larva *Artemia*. Chloroform extract of MD-02 was found to be the most toxic with the LC_{50} of 48.15 μg/ml and considered as a potential candidate for new drug research. Further characterization is still needed for the development in the future. This active sponge was then identified as *Petrosia sp*.

Key words: sponges, Bunaken, bioactivity, *Artemia salina* Leach

ABSTRAK

Indonesia adalah salah satu negara yang kaya akan sumber alam. Spons adalah invertebrata laut yang banyak ditemukan di perairan laut Indonesia dan telah diketahui banyak mengandung senyawa bioaktif dengan struktur yang unik.

Penelitian ini bertujuan untuk melakukan skrining bioactivitas ekstrak spons yang dikoleksi dari taman laut Bunaken dan melaporkan toksisitasnya terhadap larva *Artemia salina* Leach.

Sampel dimaserasi dengan aseton yang dilanjutkan dengan partisi menggunakan kloroform dan metanol. Ekstrak dibuat dengan konsentrasi 100, 250, 500 and 1000 μg/ml dan diuji dengan *Brine Shrimp Lethality Test*. LC_{50} (μg/ml) dari ekstrak uji dihitung dengan analisis probit.

Dari 4 sampel yang diuji, semua ektrak menunjukkan toksisitas terhadap larva *Artemia*. Ekstrak kloroform MD-02 ditemukan sebagai ektrak yang paling aktif (LC_{50} = 48,15 μg/ml) dan dipertimbangkan berpotensial untuk pengembangan obat baru. Karakterisasi lebih lanjut masih diperlukan untuk usaha pengembangan lebih jauh. Sampel aktif ini lebih lanjut diidentifikasi sebagai *Petrosia sp.*

Kata Kunci: spons, Bunaken, toksisitas, *Artemia salina* Leach

INTRODUCTION

Most of the currently available medications for cancer are unable to eradicate the disease or have yield severe side effects (Green *et al*., 1982; Herzig *et al*., 1987). The lack of any current chemotherapy augmented the necessity to search for new and better anti cancer drug leads. Despite the introduction of new

drugs for the treatment of cancer, the overall survival of patients suffering from this malignancy is far from satisfactory.

Research has been conducted to search anticancer with renewed vigor. Natural products are the major source of lead compounds for drugs against cancer. Sponges are known as rich sources of compounds with unique chemical structures and pronounced chemical activities, which suggests potential value as lead structures for the development of new pharmaceuticals. Three isomalabaricane triterpenes have been isolated from marine sponge *Rhabdastrella globostellata* and these compounds were found to be toxic to human colon tumor (Tasdemir *et al*., 2002). Manzamines, other examples, isolated from *Xestopongia ashmorica* were toxic to a mouse lymphoma cell line (Edrada *et al*., 1996).

In this paper we screen potential bioactivesponges extracts collected from Bunaken island and report on their toxicity against larva *Artemia salina* Leach.

METHODOLOGY

Materials

The main source of sponges were collected from Bunaken Bay. Identification for the species of the active marine sponge was conducted in the Faculty of Biology, Gadjah Mada University. Based on its morphology, tissue and the specula, the most active sample (MD-02) was identified as *Petrosia sp*.. All the solvents used in this study are qualified as pro-analysis (E. Merck), otherwise stated. These include n-hexane, ether, acetone, chloroform, ethanol, methanol, ethyl acetate. TLC plates, silica gel GF 254. Spot detectors Dragendorf. Brine Shrimp Lethality Test (BST): Brine shrimp eggs from local fish shop, sea water, yeast.

Methods

Extraction

The extracts are obtained by maceration using acetone followed by partition using chloroform and methanol. The scheme for extraction processes can be seen in figure 1. The solvent was evaporated using rotavapor.

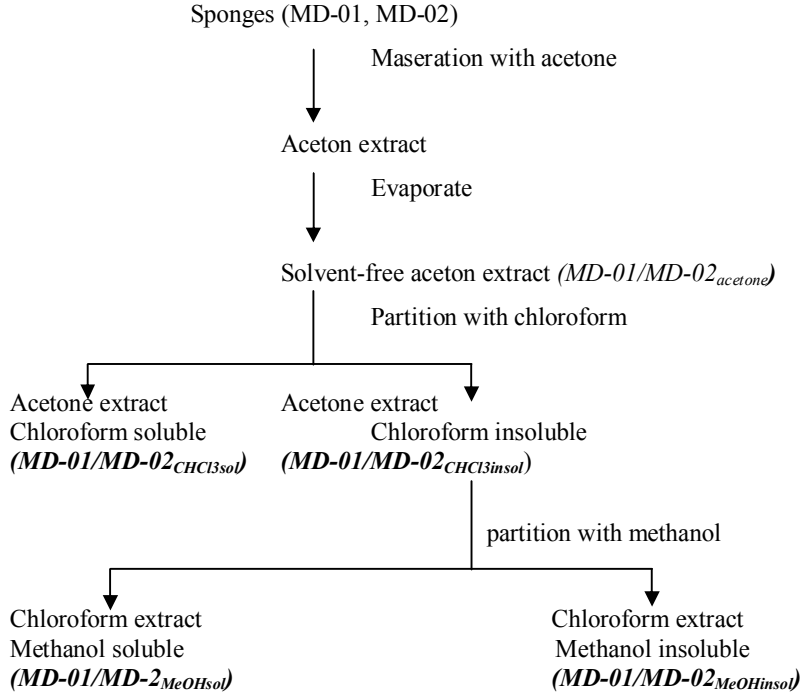


Figure 1. Scheme of sponge extraction methods.

Sample preparation

Samples were prepared by dissolving 50 mg of extract in 5 ml of chloroform: methanol (1:1). Appropriate amount of solution (50, 100, 250 and 500 µl) was transferred into the vials to get final concentration of 100, 250, 500 and 1000 µg/ml, respectively. Negative control was made by adding the solvent without extract into the vials at the same volume as the samples. The solvent was evaporated and the extracts were ready for bioassay. Five replicates were prepared for each concentration level.

Brine Shrimp Lethality Test (BST)

BST was conducted according to Meyer *et al* (1982). Brine shrimp eggs were hatched in dark divided rectangular dishes containing seawater commercially purchased from pet shop. Wholes were made between the two areas inside the dish allowing the larva (nauplii) to move from the dark area to the light one. After 48 hours the larva were collected and were ready for bioassay. Ten shrimp were transferred into vials containing evaporated tested extracts at certain concentration. Seawater was added into the vials to make 5 ml. A drop of yeast suspension (3 mg in ml seawater) was added to each vial. The number of viable larva was determined after 24 hours and the percent of death was determined.

Analysis

Percent deaths was calculated and LC_{50} of the samples was determined by probit analysis.

RESULTS AND DISCUSSION

Bioactivity study of the sponge extracts was conducted using *Brine Shrimp Lethality Test* (BST) employing 48 hours larva *Artemia salina* Leach. This method was reported to be a simple, fast and reproducible bioassay which is able to utilize a large number of this organism necessary for statistical validation. This method requires no special equipment and a relatively small amount of sample and can be useful for screening bioactive compounds (McLaughlin *et al*., 1991a; 1991b; 1993). Toxicity study using this method is reported to be comparable to cell culture so that it can be associated with anti cancer activities (Meyer *et al*., 1982; McLaughlin *et al*., 1993, Carballo *et al.,* 2002). In addition, the ability to kill larva *Artemia* can be used as test for prescreen pesticidal natural products and other toxicity-related assay.

Two samples collected from Bunaken Bay were extracted using acetone employing maceration technique. This solvent was chosen with consideration that it was able to extract non-polar as well as polar compounds with minimal sea salt contamination. Following maceration with acetone, partition was conducted using chloroform followed by methanol. To avoid cross contamination with the solvent used for extract solubilization, in the Brine Shrimp Lethality Test negative control was made by treating the solvent without extract the same as the tested samples. Results of the toxicity study can be seen in table 1.

As can be seen in table 1, from the 4 tested extracts all of them showed toxicity and were considered active. At the concentration of 1000 µg/ml, these extracts were able to kill larva *Artemia* with percent death of 98%, 100%, 92% and 96 for MD-01_{CHCl3}, MD-01_{MeOH}, MD-02_{CHCl3} and MD-02_{MeOH} extracts, respectively. According to Meyer *et al* (1982), an extract was considered active if it is able to kill 50% larva *Artemia* at the concentration $\leq$ 1000 µg/ml. In this study all extracts of MD-01 and MD-02 displayed toxicity in the brine shrimp bioassay and MD-02_{CHCl3} was found to be the most toxic with LC_{50} of 48.15 µg/ml (table 2). Based on the TLC profiles (figure 2) this extract contained alkaloids and other compounds which were necessary to be determined for characterization in the future.

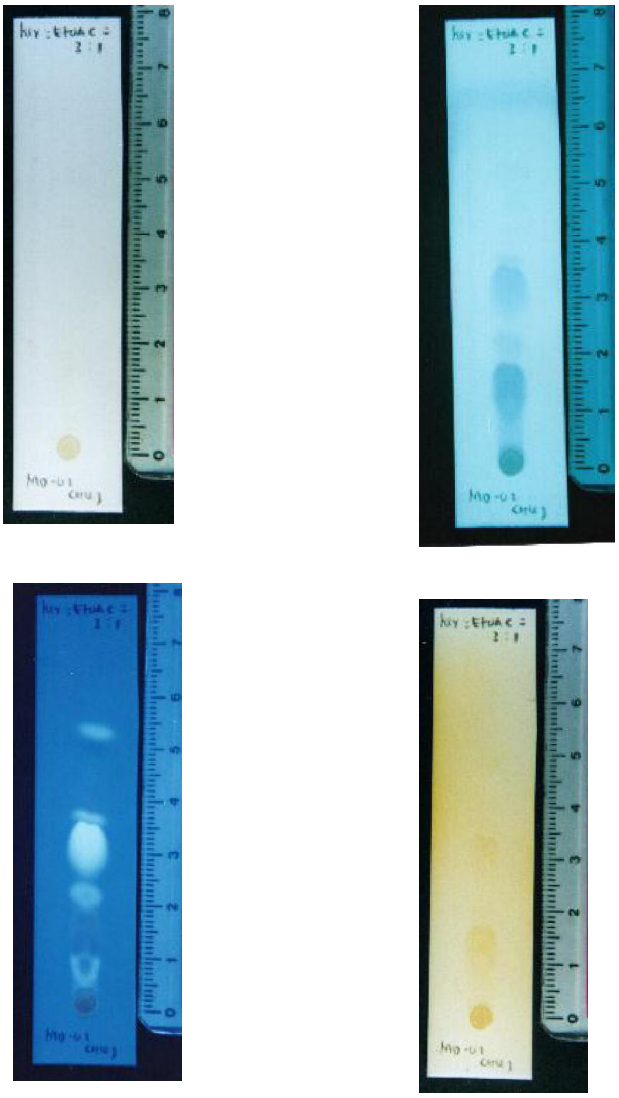
Table 1. Percent death of *larva Artemia salina* Leach by the administration of sponge extracts collected from Bunaken Bay (n=5).

Samples	Concentration (μg/ml)	Percent death (%)
MD-01 (CHCl3)	1000	98
	500	96
	250	64
	100	48
MD-01 (MeOH)	1000	100
	500	78
	250	20
	100	14
MD-02 (CHCl3)	1000	*92*
	500	88
	250	80
	100	62
MD-02 (MeOH)	1000	96
	500	46
	250	8
	100	6
Negative (-) control	500 μl	100
	250 μl	100
	100 μl	100
	50 μl	100

Table 2. LC_{50} (μg/ml) of sponges extracts collected from Bunaken Bay.

Samples	LC_{50} (μg/ml)
MD-01 (CHCl3)	119.77
MD-01 (MeOH)	255.6
MD-02 (CHCl3)	48.15
MD-02 (MeOH)	445.7

Screening for bioactive compounds is needed for the exploration of anticancer drug leads. As marine sponges have not been widely explored and this invertebrate are known to be rich of various bioactive compounds, the results of this study will give valuable information for the development of new pharmaceuticals. Example of similar studies were reported by Edrada *et al* (1996) and Tasdemir *et al* (2002) who found a number of compounds having high cytotoxicity level against tumor cells (Edrada, *et al*., 1996; Tasdemir *et al*., 2002). Considering the toxicity level of $MD\text{-}02_{CHCl3}$, this extract can be considered as potential bioactive source for new drug research.



(b)

Figure 2. TLC profiles of chloroform extract of MD-02. (a) visual, (b) detection using UV_{254} lamp (c) detection using UV_{366} lamp (d) detection using Dragendorff spot detector

CONCLUSION

$MD\text{-}02_{CHCl3}$ was found to be the most active extract against larva *Artemia salina* Leach and be considered as a potential candidate for new drug research. Fractionation, isolation and structure elucidation are still needed for determination of the active compounds.

ACKNOWLEDGEMENT

This work was partly supported by DIK/S Faculty of Pharmacy, Gadjah Mada University, 2002.

REFERENCES

Carballo, J., Hernandez-Inda, Z.L., Perez, P., Garcia-Gravalos, M.D., 2002, A Comparison Between Two Brine Shrimp Assays to Detect *in vitro* Cytotoxicity in Marine Natural Products, *BMC Biotechnol.*, Sep 23 : 2(1) : 17

Edrada, R.U., Proksch, P., Wray, V., Witte, L., Muller, W.E.G., Van Soest, R.W.M., 1996, Four New Bioactive Manzamine-Type Alkaloids from the Philippine Marine Sponge Xestospongia ashmorica, *J.Nat.Prod.*, 59, 1056-1060.

Green D., Tew, K.D., Hisamatsu, T., Schein, P.S., 1982, Correlation of Nitrosourea Murine Bone Marrow Toxicity With Deoxyribonucleic Acid Alkylation and Chromatin Binding Sites. *Biochem. Pharmacol.*, 31, 1671-1679.

Herzig R.H., Hines, J.D., Herzig, G.P., Wolf, S.N., Cassilesh, P.A., Lazamus, H.M., Adelstein, D.J., Brown, R.A., Loccia, P.F., Standjord, 1987, Cerebellar Toxicity with High-dose Cytosine Arabinoside *J. Clin. Oncol.*, Jun 1, 927-932.

McLaughlin, J.L., Chang, C.J., Smith, D.L., 1991a, S., 1987,. Bench topbioassay for the discovery of bioactive natural products: An update. In: Atta-ur-Rahman, ed, *Studies in Natural Products Chemistry*, Amsterdam, Elsevier, 9, 388-409.

McLaughlin, J.L., 1991b, Crown-gall tumours in potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation, in: Hostettmann, K., ed, *Methods in Plant Biochemistry*, London, Academic Press, 6, 1-31.

McLaughlin, J.L., Chang, C.J., Smith, D.L., 1993, Simple bench top bioassays (brine shrimp dan potato discs) for the discovery fo plant antitunour compounds: Review of recent progress, In: Kinghorn, A.D., and Balandrin, M.F., eds., *Human Medical Agents from Plants*, Washington, DC., ACS Symposium Series 534, American Chemical Society, 112-134.

Meyer, Ferrigni, Putnam, Jacobsen, Nichols, Mc. Laughlin, 1982, *Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents, Plant Medica*, Vol 45.

Tasdemir D., Mangalindan, G.C., Concepcion, G.P., Verbitski, S.M., Rabindran, S., Miranda, M., Greenstein, M., Hooper, J.N., Harper, M.K., Ireland, C.M., 2002, Bioactive isomalabaricane triterpenes from the marine sponge *Rhabdastrella globostellata*, *J. Nat. Prod.*, 65 (2), 210-214.