# INHIBITORY ACTIVITIES OF ASCIDIAN *Herdmania momus* ON THE COLONY FORMATION OF CHINESE HAMSTER V79 CELLS, COLLECTED IN MANADO NORTH SULAWESI, INDONESIA

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**ABSTRACT**

The ethanol extract of ascidian *Herdmania momus* collected from Manado North Sulawesi was assayed against V79, the bioassay tested to V79 cells reflects the direct action of the compounds on the cells and also is used to select active compounds for the bioassay of inflammatory cytokine production. The inhibition activity against the colony formation of Chinese hamster V79 cells showed very weak inhibited. The HPLC separation of the ethanol extract yielded two interesting fractions **Fr.2.2.2** and **Fr.2.2.4.** were obtained from marine ascidian *H. momus.*

***Keywords*:** Ascidian *Herdmania momus*, inhibitor, North Sulawesi, V79 Cells

## INTRODUCTION

Ascidians or sea squirt belong to a group of sessile marine filter feeders, hermaphroditic, and many species are tolerant of wide range environmental conditions. Ascidian characterized by though outer tunic and their ability to attach themselves to reefs and shells. Ascidians take many variations in form, which are conveniently divided into colonial and solitary species. The solitary ascidians generally live as isolated individuals, and colonial species are characterized by many small individuals, called zooids and living together in a common tunic.

The solitary ascidian *Herdmania momus* (Subclass Pleurogona, Order Stolidobranchia, Family Pyuridae) is found at tropical Indo-Pacific waters, subtropical and temperate water of South Africa and Australia. Most solitary ascidians develop externally, make them amenable for experimental, however, over the past years there are only three papers about chemical study have been reported.

Indonesia is one of ASEAN countries, have a great marine invertebrates potential for the production of bioactive molecules of pharmaceutical interest. Research of marine natural product from Indonesia will be very useful to find new active secondary metabolites, can be developed as a drug candidate with SNI standart and also become the promising target for ASEAN *Economic Community* (AEC).

Over the past years, ascidians have been shown to be a prolific source of natural products with promising biomedical potential (Blunt, *et al*., 2012). The ascidian has turned out to be a source of chemical structurally interesting and biologically active natural products, and most of these products fall within the area of cancer therapy, and significant number of ascidian-derived compounds have entered into preclinical and clinical trials as antitumor agents (Blunt, *et al*., 2012; Faulkner, 2002).

Li, *et al.* (2012) was reported the most recent study of *H. momus* collected at Jeju Is, Korea they afforded seven new amino derivatives named as herdmanines E–J. The bioactivity of these compounds was tested against (PPAR)-γ and (herdmanines I, K, and J) showed significant PPAR-γ activation in Ac2F rat liver cells, while hermanine J exhibited strong PPAR-γ activation at 1 and 10 μg/mL. The third studied of *H. momus* collected at Dayawan Bay and obtained five compounds; oleic acid, β-sitosterol, p-hydroxybenhyde, ethyl-α-D-glucopyranoside, and thymidine (Cheng, *et al*., 1995).

Therefore, this report was to investigate the influence of the ascidian extract on the colony formation of Chinese hamster V79 cells. This bioassay reflects the direct action of compounds on the cells (Oda, *et al.*, 2007).

As the part of our course studies to search the bioactive compound of ascidian, we were collected *H. momus* from Manado, North Sulawesi, Indonesia on September 2010, and the extract collected at Manado Tua Island was chosen for analysis. The HPLC separation of the ethanol extract led to the isolation of **Fr. 2.2.2** and **Fr. 2.2.4**.

## METHODS

### General

EI-MS was performed by a JMS-MS 700 mass spectrometer (JEOL, Tokyo, Japan). $^{1}H$ and $^{13}C$ NMR spectra were recorded on a JNM-AL-400 NMR spectrometer (JEOL). Preparative HPLC was carried out using L-6200 system (Hitachi Ltd., Tokyo, Japan). All chemicals and organic solvents such as ethanol, methanol, ODS C-18 were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

### Ascidian

The solitary ascidian was collected by scuba diving at the Manado, North Sulawesi, Indonesia in September 2010 and identified as *Herdmania momus* sp. The voucher specimen was deposited at the Tohoku Medical and Pharmaceutical University, Miyagi – Japan.

**Cytotoxicity Assay of V79 cells**

Chinese hamster V79 cells were grown as a monolayer culture in Eagle's MEM with 10% heat-inactivated FBS MEM (Nissui Seiyaku Co., Ltd., Tokyo, Japan). Culture were maintained at 37$^{o}$C in a humidified atmosphere of 5% CO2 and 95% air (Sato, 1992).

**Relative plating efficiency**

The relative plating efficiencies against V79 cells were determined as the ratio of the number of colonies in various concentrations of samples to that in the sample-free control. Two hundred cells were seeded on a 60/15-mm plastic plate with 4 mL culture medium and incubated overnight at 37$^{o}$C. After each sample in DMSO (4 µl) was added to the culture medium, cells were further cultured for four days. The dishes were then fixed with methanol and then stained with 7% Giemsa solution. The numbers of colony (>50 cells/colony) in the sample plates were counted and compared with those in control cultures to assess the effects of the drugs on the plating efficiency (Sato, 1992).

**Extraction and Isolation**

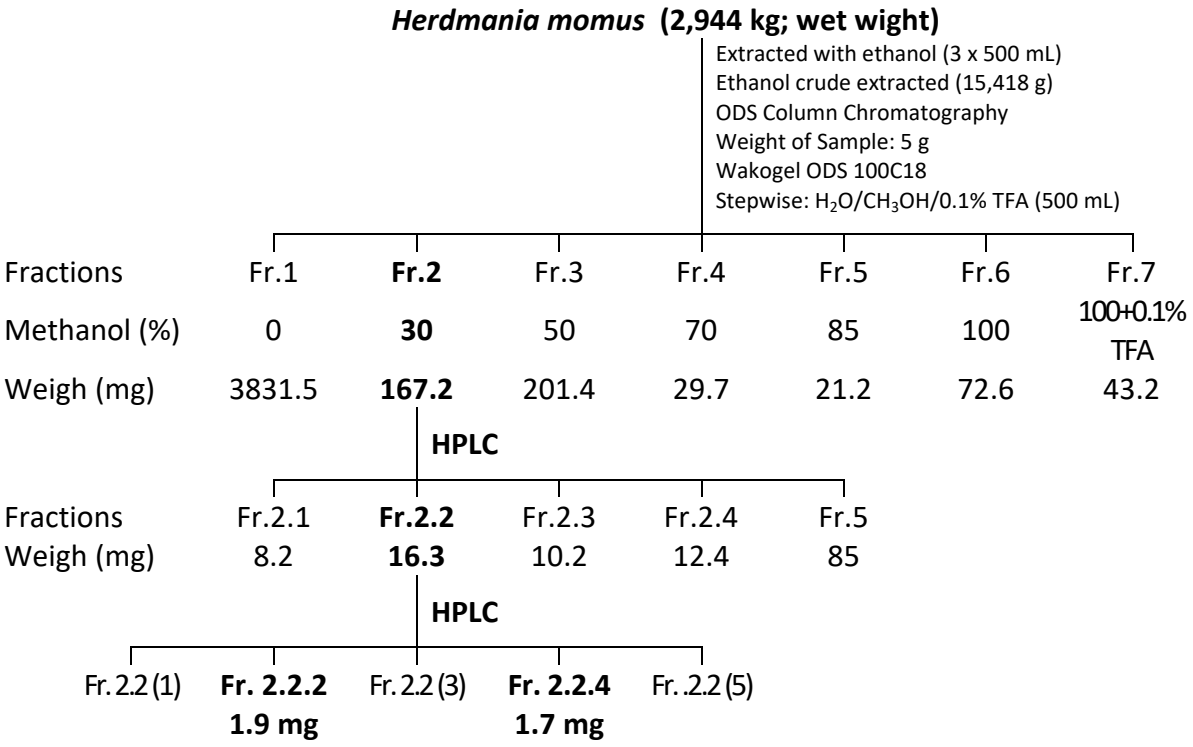


**Figure 1.** Bioassay-guided isolation procedure and HPLC for ascidian *Herdmania momus*

The ascidian (2.944 kg, wet weight) which collected from Manado Tua Island was immediately cut into small pieces and soaked in EtOH on the boat immediately after collection. The organism was further extracted three times with EtOH. The EtOH crude extract (5 mg) was suspended in $H_2O$ and adsorbed on an ODS column (100 g). The ODS

column was eluted stepwise with 0, 30, 50, 70, 85, 100, 100% MeOH in 0.10% TFA aqueous solution into seven fractions (Fr.1 – Fr.7). Fr. 2, eluted with 30% MeOH, was concentrated to yield dark brown - gum (167.2 mg), and 25 mg/mL in MeOH (100 mg) of the fraction was purified by preparative HPLC (column, Pegasil ODS (10 mm x 250 mm); solvent 30% MeOH containing 0.1% TFA; flow rate, 2 mL/min; detection, UV at 220 nm) to give five fractions, then Fr.2.2. again was purified by preparative HPLC (column, Pegasil ODS (10 mm x 250 mm); solvent 20% MeOH containing 0.1% TFA; flowrate, 2 mL/min; detection, UV at 220 nm) to give **Fr.2.2.2** eluted at 37.34 min) as dark brown-gum (1.9 mg) and **Fr. Fr.2.2.4** (eluted at 47.58 min) as dark brown-gum (1.7 mg).

## RESULT AND DISSCUSSION

### Marine ascidian *Herdmania momus*

Marine ascidian *Herdmania momus* collected at Manado North Sulawesi, Indonesia (Figure 2).

**Figure 2.** Ascidian *Herdmanis momus*

### Effect of crude extract to V79 cells

The ethanol extract was assayed against V79, bioassay tested to V79 cells reflects the direct action of the compounds on the cells and is used to select active compounds for the bioassay of inflammatory cytokine production. The crude extract showed 16.1 – 45.4 % of inhibition activity against the colony formation of Chinese hamster V79 cell with concentration at 50 µg/mL (Table 1). The inhibition showed very weak activity to the cells found in *H. momus* collected at Bunaken Island North and the highest collected in Bunaken Island South, 45.4%. All the sample collected from 5 sampling sites showed their inhibition very weak activity to the cells, < 50 % with concentration at 50 µg/mL.

**Table 1.** Cytotoxicity activity (V79) *Herdmanis momus* collected in Manado

| Sample Number/ Code | Weight | | Remarks (Identification) | Cytotoxicity V79 | Sampling Site |
|---|---|---|---|---|---|
| | Organism (g) | Extract (mg) | | | |
| ***Hm* 1** | 5,183 | 333750 | *Herdmania momus* | 45.4 | Bunaken South |
| ***Hm* 2** | 239 | 2437.21 | *Herdmania momus* | 26.6 | Mantehage |
| ***Hm* 3** | 685 | 5910.84 | *Herdmania momus* | 16.1 | Bunaken North |
| ***Hm* 4** | 2,944 | 15418.40 | *Herdmania momus* | 23.4 | Manado Tua South |
| ***Hm* 5** | 347 | 1694.14 | *Herdmania momus* | 29.4 | Manado Tua East |
| V79: inhibitory activity (%) against the colony formation of Chinese hamster V79 cells at 50 mg/mL (90-100%, 60-90%, 50-60%) | | | | | |

**Isolation of Fr.2.2.2 and Fr.2.2.4**

By the repeated HPLC of the marine ascidian *Herdmania* sp. collected at Manado Tua, Is. Indonesia, found two interesting fractions, as **Fr.2.2.2** and **Fr.2.2.4.**

**Fr.2.2.2** (1.9 mg) was obtained as dark brown gum;· The $^{1}$H and $^{13}$C-NMR spectra ($CD_3OD$) at δ 1.12 and 22.76 $^{1}$H-NMR ($CD_3OD$) δ 1.12 (1H), 1.28 (1H), 1.40 – 1.46 (2H), 1.57 – 1.64 (1H), 3.00 (2H), 3.26 (1H), 3.66 (1H); $^{13}$C-NMR ($CD_3OD$) δ 22.76, 26,98, 36.72, 38.88, 39.11, 48.6, 49.66, 161.46; EI mass spectra of **Fr.2.2.2** showed molecular ion peak at *m/z* 265 $[M]^+$. (Figs. 3 - 6)

**Fr.2.2.4** (1.7 mg) was obtained as dark brown gum; $^{1}$H-NMR ($CD_3OD$) δ 0.89 – 0.95 (2H), 1.28 (1H), 1.38 – 1.44 (1H), 1.61 (1H), 2.83 (2H), 2.99 (1H), 3.47 (2H), 3.61 (2H), 3.74 (1H), 7.16 – 7.29 (m, 8H); $^{13}$C-NMR ($CD_3OD$) δ 22.77, 36.27, 36,72, 42.09, 127.46, 129.55, 129.79, 140.14, 161.50. EI mass spectra of **Fr.2.2.4** showed molecular ion peak at *m/z* 299 $[M]^+$. (Figs. 7 – 10).

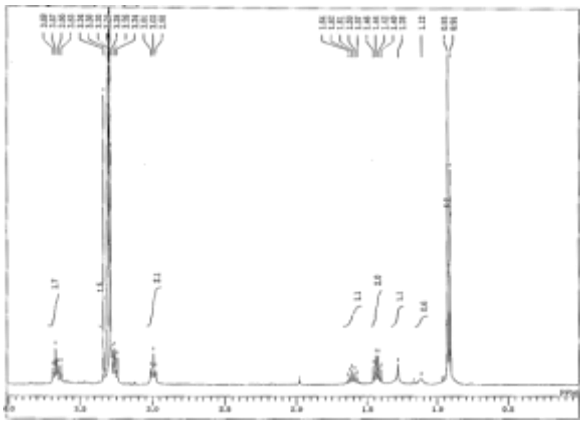

**Figure 3.** $^{1}$H-NMR spectrum of **Fr.2.2.2**

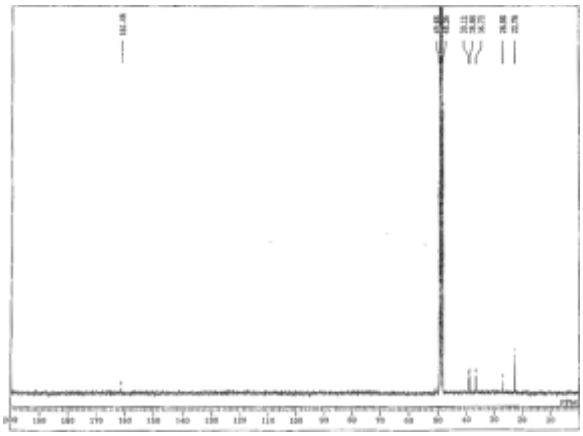

**Figure 4.** $^{13}$C-NMR spectrum of **Fr.2.2.2**

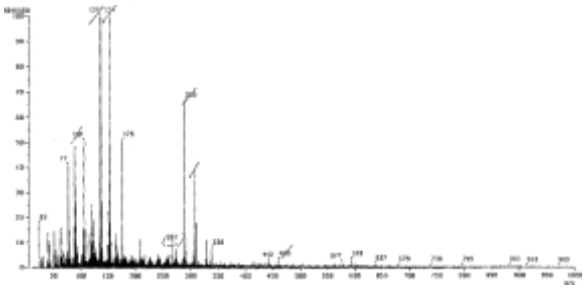

**Figure 5.** FAB-MS spectrum of **Fr.2.2.2**

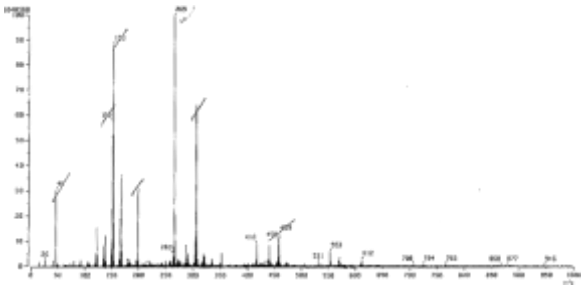

**Figure 6.** FAB-MS spectrum of **Fr.2.2.2**

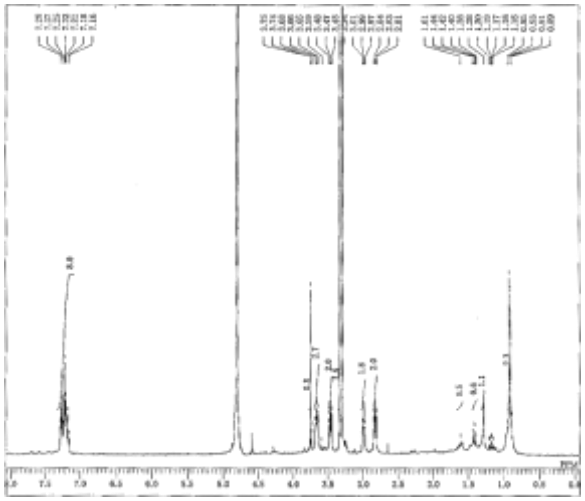

**Figure 7.** $^{1}$H-NMR spectrum of **Fr.2.2.4**

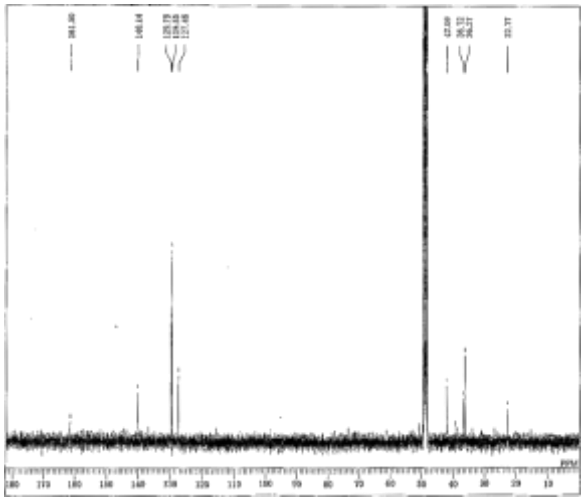

**Figure 8.** $^{13}$C-NMR spectrum of **Fr.2.2.4**

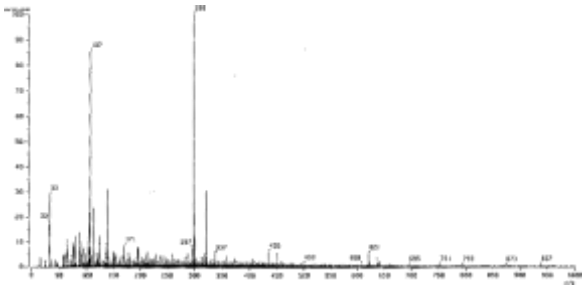

**Figure 9**. FAB-MS spectrum of **Fr.2.2.4**

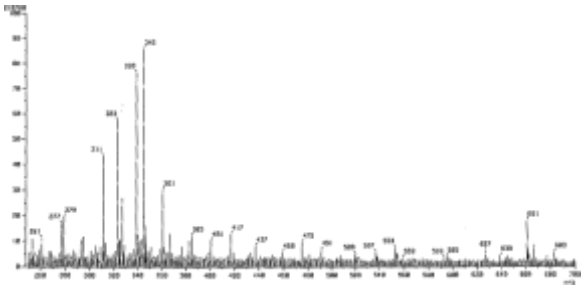

**Figure 10.** FAB-MS spectrum of **Fr.2.2.4**

## CONCLUSION

Two interesting fractions **Fr.2.2.2** and **Fr.2.2.4** were obtained from marine ascidian *Herdmania* sp. collected in Manado Tua, Is. Indonesia. The inhibition activity against the colony formation of Chinese hamster V79 cells showed very weak inhibited. The structure has not been yet to determined because the amounts were not enough to further analysis.

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