CYTOTOXIC ACTIVITY OF ASCIDIAN *Eudistoma* sp. FROM MANTEHAGE ISLAND MANADO

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ABSTRAK

Koloni Ascidian genus Eudistoma telah dikoleksi dari perairan Pulau Mantehage, Sulawesi Utara Indonesia. Ekstrak kasar metanolik dari *Eudistoma* sp. yang diuji sitotoksisitasnya terhadap koloni hamster China (V79) memperlihatkan aktivitas sitotoksik yang tinggi pada pertumbuhan koloni hamster (V79) dimana *Eudistoma* sp. menunjukkan daya hambat yang kuat (99,5% inhibisi pada 50 µg/mL), uji sitotoksisitas ekstrak kasar *Eudistoma* sp. terhadap sel kanker HCT-15 (kanker usus) dan sel Jurkat (leukemia) memperlihatkan bahwa organism ini menghambat pertumbuhan HCT-15, dan pada sel Jurkat (leukeumia) menunjukkan penghambatan aktivitas yang lemah. Nilai konsentrasi penghambatan (IC_{50}) terhadap HCT-15 adalah 81.2% pada 50 µg/Ml dan terhadap sel Jurkat adalah 35.4% pada 50 µg/mL. Uji bioesei antimikrobial tidak menunjukkan adanya aktivitas terhadap pertumbuhan bakteri (*Staphylococcus aureus*, *Escherichia coli*, *Candidaalbicans* dan *Mucorhiemalis*). Dari hasil penelitian ini, dapat disimpulkan bahwa Ascidian *Eudistoma* sp. memiliki sifat aktivitas sitotoksik yang tinggi dan sebagai antikanker sehingga dapat direkomendasikan untuk diteliti lebih lanjut sebagai bahan obat di bidang farmakologi.

Kata Kunci: Ascidian, *Eudistoma* sp., sitotoksik, antikanker

INTRODUCTION

The ascidian include a wide variety of invertebrates that are classified within the Phylum Chordata based on the presence of a larval notochord during their early development. They are about 3% of the 45,000 or so species of chordates and contain about 3,000 species, usually divided into Ascidiacea (Aplousoobranchia, Phlebobranchia, and Stolidobranchia), Thaliacea (Pyrosomida, Doliolida and Salpida), Appendicularia (Larvacea), and Sorberacea classes. The great majority are benthic sac-like filter feeders (Ascidiacea and Sorberacea); they live on the ocean floor and filter water by a variety of mechanisms to extract fine planktonic food particles. Those within the two much smaller classes (Thaliacea and Appendicularia) have abandoned the benthic existence in favor of a holoplanktonic lifestyle, that is they live in the pelagic zone as adults (Collin *et al.*, 1995; Fattorusso *et al.*, 2012)

Over the past decades, ascidians have been shown to be a prolific source of natural products with promising biomedical potentials (Blunt *et al*., 2012). Ascidians

are sources of structurally interesting and biologically active natural products. Most of these products fall within the area of cancer therapy, and several ascidian-derived compounds have entered into preclinical and clinical trials as antitumor agents (Fattorusso *et al*., 2012; Blunt *et al*., 2012; Faulkner, 2002). For instance, trabectedin, a tetrahydroisoquinoline alkaloid obtained from *Ecteinascidia turbinata*, was recently approved for the treatment of soft tissue sarcoma, with the commercial name Yondelis (Newman and Cragg, 2014) and Eribulin (Halichondrin B) for breat cancer obtained from ascidian *Halichondria okadai*, with the commercial name Halaven (Newman and Cragg, 2014). Tunicates belonging to the genus *Eudistoma* have been the sources of numerous novel and bioactive secondary metabolites. (Montenegro *et al.*, 2012, Weihong *et al.*, 2008; Jimenez *et al.* 2008)

The aims of this work to search for novel and useful metabolites from marine ascidian, *Eudistoma* sp. has been collected from Bunaken National Park, especially Mantehage Island at North Sulawesi, Indonesia. Extraction, isolation and cytotoxic assay of this species also were done at Laboratory of Marine Natural Product, Tohoku Pharmaceutical University Japan.

METHODS

Ascidian

The colonial ascidian was collected by scuba diving at Mantehage Island, North Sulawesi, Indonesia in September 2010 and identified as *Eudistoma sp.*
The voucher specimen is deposited at Tohoku Pharmaceutical University as 10-09-12=1-7.

Extraction and Isolation

The ascidian (187 g, wet weight) was cut into small pieces and soaked in EtOH on a boat immediately after collection. The organism was further extracted three times with EtOH to give the crude extract (1365.13 mg).

Materials

Fetal bovine serum (FBS) and other culture materials were purchased from Invitrogen (Carlsbad, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and organic solvents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Antimicrobial Assay

The growth inhibitory activity was examined by the paper disc method using agar medium (B1) against *Mucor hiemalis* IAM 6088 (fungus), *Candidaalbicans* IFM 4954 (yeast), *Staphyloccoccus aureus* IAM 12544T (Gram-positive bacterium), and *Escherichia coli* IAM 12119T (Gram-negative bacterium) as test microorganisms.

Influence on the Colony Formation of Chinese Hamster V79 Cells

Chinese hamster V79 cells were grown as a monolayer culture in Eagle's MEM with 10% heat-inactivated FBS. The relative plating efficiencies 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 mL) was added to the culture medium, cells were further cultured for four days. The numbers of colonies in the sample plates were counted and compared with those in control cultures.

Cytotoxicity Assay Against HCT-15 and Jurkat Cells

HCT-15 and Jurkat cells were obtained from the Center for Biomedical Research, Institute of Development, Aging, and Cancer, Tohoku University (Miyagi, Japan). The cell lines were cultured in RPMI-1640 medium. The medium was supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Exponentially growing cells, cultured in a humidified chamber at 37 ^{o}C containing 5.0% CO_2, were used for the experiments. Cytotoxic activity was evaluated using the colorimetric MTT assay (Mosmann, 1983). HCT-15 (1.0 x 10^4 cells in 100 µL) or Jurkat cells (2.0 x 10^4 cells in 100 µL) were added to each well of a 96-well plastic plate. Each sample (1.0 µL in MeOH) was added to each well to make the final concentration from 0 to 39-47 µM, and the cells were incubated for 48 h at 37 ^{o}C. MTT (10 µL of 5.5 mg/mL stock solution) and a cell lysate solution (90 µL, 40% *N*,*N*-dimethylformamide, 20% sodium dodecyl sulfate, 2.0% CH_3COOH and 0.03% HC1) were added to each well, and the plate was shaken thoroughly by agitation at room temperature for overnight. The optical density of each well was measured at 570 nm using an MTP-500 micro plate reader.

Result and Discussion

The colonial ascidian genus *Eudistoma* (Figure 1) was collected at

Mantehage Island, North Sulawesi, Indonesia and extracted with ethanol to give the crude extract (1365.13mg).

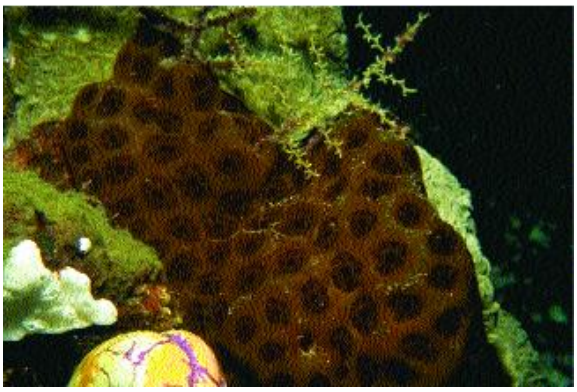

Figure 1. *Eudistoma* sp. (Colin *et al.*, 1995)

Table 1. Effect of ascidian *Eudistoma* (crude extract) against V79, Jurkat, and HCT-15 cells

	Cytotoxicity Assay		
Organism (Ascidian)	**V79**	**HCT-15**	**Jurkat**
	EC_{50} (µg/mL)	**IC_{50} (µg/mL)**	
***Eudistoma* sp.**	**99.5%**	**81.2%**	**35.4%**

The crude extracted (Table 1) were tested for their influence on the colony formation of Chinese hamster V79 cells and the colonian ascidian *Eudistoma* inhibited the colony formation of V79 cells (99.5% inhibition at 50 µg/mL) in the screening bioassay. The effect of crude extract *Eudistoma* showing the highest inhibition (99.5%) against the colony formation of Chinese hamster V79 cells at 50 µg/mL. Antimicrobial assay showed no activity against bacteria (*Staphylococcus aureus, Escherichia coli, Candidaalbicans, Mucor hiemalis*). In addition, cytotoxicity the crude extract of *Eudistoma* against HCT-15 and Jurkat cells was evaluated after 48 h by the MTT method. The 50% inhibitory concentration (IC_{50}) values of crude extract against HCT-15 and Jurkat cells were 81.2% inhibition at 50 µg/mL and 35.4% inhibition at 50 µg/mL, respectively. The inhibitory activity crude extract against HCT-15 was potentd in Jurkat Cells (leukeumia) showed weak inhibitory activity. The relationship is comparable with the results of inhibition effects of crude extracton the colony formation of Chinese hamster V79 cells and HCT-15. Since the influence on V79 cells reflects the direct action of compounds on the cells, this assay system is used to select active compounds for the bioassay of inflammatory cytokine production (Wang *et al.*, 2007). This result indicate that

ascidian *Eudistoma* sp. has remarkable anticancer and cytotoxic activity. The observed result strongly suggests that the ascidian extracts can be used as anticancer agents. Other findings on endemic ascidian *Eudistomavannamei* which collected from Northeast Brazilrevealed as the most promising of anticancer agents on HCT-8 (Montenegro *et al.*, 2012). Weihong *et al.* (2008) reported genus *Eudistoma* sp. collected near Tong-Yeong City, South Sea, Korea, have obtained 7 new compounds; Eudistomins Y1−Y7 were evaluated for their antibacterial activity, and eudistomin Y6 exhibited moderate antibacterial activity against Gram-positive bacteria *Staphylococcus epidermis* and *Bacillus subtilis* without cytotoxicity in the MTT assay at 100 μM.

CONCLUSIONS

Ascidian *Eudistoma* sp. from Mantehage Island, North Sulawesi has most promosing compound and remarkable as anticancer and cytotoxic activity, and bioassay test of the crude extract of *Eudistoma* sp. against HCT-15 was potent inhibitor and has high influence against colony formation of Chinese hamster V79 cells.

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