Inhibitory of PANC-1 produced by Indonesian’s Marine Sponge *Endectyon delaubenfelsi* Adapted to Nutrient Starvation

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Abstract. The bioactivity of Indonesian Marine Sponge *Endectyon delaubenfelsi* has been conducted on July 2018. The active metabolite based on bioassay guided-separation with several steps of chromatography. The FTIR spectrum shows that the active metabolite has N-H amine functional groups at 3434.6 cm⁻¹ C-H methyl was detected at 3090.46 cm⁻¹, and the fingerprint region at 1637.27 cm⁻¹ as C-N imine. Based on the FTIR data shows indicate that the active compound as alkaloid. Metabolite active has activity IC₅₀ = 0.2 ug / mL indicates that the active compound from of *Endectyon delaubenfelsi* has potential activity as the new model to the drug discovery against PANC-1 cell.

1. Introduction.
Cancer is a disease caused by abnormal development at various levels of uncontrolled growth. Mortality and morbidity rates caused by men and women reach 216,000 people/year [1]. Cancer therapy can be done in various ways, such as conventional surgery to chemotherapy, radiation, hormones, and monoclonal antibodies. However, chemotherapy has some disadvantages and side effects that are dangerous because it causes damage to the surrounding tissue and other organs as well as expensive and long treatment times [2,3].

Various ways of drug models have been found as a substitute for chemotherapy such as Alkeran, Anzatax, Blenamax, and antimycin but the use of these drugs is only effective against cancer cells that are nutritionally deficient [4,5].

The latest biological studies of cancer cells state that nutritionally deficient cancer cells have abnormal development activities that are faster in the cell growth phase, so this condition has attracted the attention of looking for a drug model for cancer cells that are nutritionally deficient [6,7].
Marine resources sourced from Sabang island is still limited, so it becomes an opportunity for us to exploit with the aim to know the potential of marine natural resources in Sabang Island.

2. Experimental Section

2.1 Materials

NMR spectrum using JEOL ECA-500 (¹H: 500 MHz, ¹³C: 125 MHz). ESI-TOF-MS analysis using Q-Tof Ultima (Waters Co., MA, U.S.A). IR spectrum analysis using JASCO FT / IR-5300. UV spectrum analysis using UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Active component separation using Silica gel open chromatography column BW-200 (Fuji Sylisia, Aichi, Japan), Cosmosil ODS (75C18-OPN, Nacalai Tesque, Kyoto, Japan), and Cosmosil 5C18-MS-II (10 mm id × 250mm, Nacalai Tesque). Further analysis using TLC Silica gel 60F254 chromatography (Merck Chemical, Darmstadt, Germany) and HPLC (UV detector: L-4000H). Bioradic spectroscopy, biorad plates, 96-well plastic plates, CO₂ incubators are used for preparation of toxicity tests. Bioanalysis used Kanamycin 50 µg / mL, medium trypsin Mc-Coys, trypsin blue, panc-1 human cells, general glucose media, glucose deficiency media, Phosphate buffer saline (PBS), methanol, ethanol, ethyl acetate, Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), n-Hexane and aquadest.

2.2 Biomaterial

Indonesian marine sponge *Endectyon delaubenfelsi* is collected from Anoi Itam, Sabang in 2018. The sponge is cut into small pieces and air-dried. Then analysis and analysis of toxicity was carried out at the Natural Products Laboratory for Drug Findings, the Graduate School of Pharmaceutical Sciences, Osaka University.

2.3 Cell Culture and Toxicity Test

PANC-1 was cultivated in Dulbecco’s modified Eagle’s medium (DMEM) using a supplement of 10% fetal bovine serum (FBS) with an inactive high temperature regulator and kanamycin (50 µg / mL) in humidity below 5% CO₂ at 37°C [8,9]. Under conditions of glucose deficiency, PANC-1 was cultivated on Glucose Deficient Medium [Basal Medium (25mM N- (2-hydroxyethyl) piperazine-N’-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) supplement with 6.4g / L NaCl, 700mg / L, NaHCO₃, 400mg / L, KCl, 265mg / L CaCl₂2H₂O, 200mg / L MgSO₄.7H₂O, 125mg / L, NaH₂PO₄, 0.1mg / L Fe (NO₃) .9H₂O, 15mg / L Phenolred, 10mL / L vitamin solution (X100) (GIBCO, Carlshad, CA, USA), 200mmol / L L-glutamine solution (GIBCO), 50g / L kanamycin) containing 10% FBS dialysis. Glucose medium with 10% FBS and 2.0 g / L glucose (25mM) is also used for bioassays in order to compare cell activity in conditions of glucose deficiency [10,11,12].

PANC-1 cells were monitored for 24 hours using DMEM with 10% FBS. Medium Regular Glucose or Fat Glucose is used in the media to be replaced so that it can adjust glucose deficiency. After 12 hours of incubation, the sample has been dissolved and incubated in a humid atmosphere of 5% CO₂ at 37°C. Colorimetric Reagents WTS-8 detects cell proliferation. The growth inhibitor curve is interpolated to determine the IC₅₀ value. The results of selectivity on anti-proliferation activity (S.I) on the basis of differences in IC₅₀ values in general glucose media and glucose Medium Medium [13,14].

3. Extraction and Isolation

The ethyl acetate fraction (9.6 g) was fractionated using an open chromatography column and second fraction produced shows activity [(12.45 g) IC₅₀ = 50 µg / mL]. The second Fraction was fractionated using High Performance Liquid Chromatography (HPLC-Cosmosil 5C-18 MS II), compared with chloroform (CHCl₃), compared to Methanol: water with low phase, resulting the fourth fraction has activity [(1.26 g) IC₅₀ = 20 µg / mL]. Fraction three extract was fractionated again using HPLC (Cosmosil 5C-18 MS II) with
MeCN: H₂O compared to TFA 0.1% resulting the three fraction [(0.63 g) IC₅₀ = 0.2 ug / mL] shows the lowest viability against PANC-1 cell. The final extraction and isolation of yellow amorphous compounds.

4. Result and Discussion

4.1 Identification of Structure

Endectyon delaubenfelsi [(0.63 g) IC₅₀ = 0.2 ug / mL] was isolated as a yellow amorphous compounds. Based on the FTIR spectrum shows that the active metabolite has N-H amine functional groups at 3434.6 cm⁻¹, C-H methyl was detected at 3090.46 cm⁻¹, and the fingerprint region at 1637.27 cm⁻¹ as C-N imine (Figure 1). Interpretation of the data indicates that the active metabolite compound know as an alkaloid [15,16,17].

![Figure 1. FTIR Spectrum of Active Compound](image)

The bioactivity results show cytotoxic against PANC-1 cell. The lowest viability from active compound indicates that the compound has potential activity compared to antimycin.

5. Conclusion

Metabolite active from Endectyon delaubenfelsi have cytotoxic activity against PANC-1 cell with IC₅₀ = 0.2 μg/mL value very close to MIC₅₀ value against commercial drug antimycin = 0.1 μg/mL.

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