Araguspongine: an indole alkaloid as panc-1 cell inhibitory adapted to nutrient starvation from Indonesian’s marine sponge *spongionellapulchella*

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Abstract. Isolation of active metabolite from Indonesian’s Marine Sponge *Spongionellapulchella* has been conducted on July 2018. Active compound isolated based on bioassay-guided separation with several steps of chromatography. The rate of viability shows that IC50 = 0.2 μg / mL against PANC-1 cell. The active compound known as C28H51N2O2 determined by LCMS-ESI with molecular weight [M+H]+447.3951m/z. Based on the FTIR spectrum shows that the active metabolite has functional groups at 3435.56 cm⁻¹ as N-H secondary amines, 2853.39 cm⁻¹ as C-H methyl, 2769.64 cm⁻¹ as C-H methylene and the fingerprint region of amine at 1637.27 cm⁻¹ as C-N imine. The active compound which known as araguspongine, an indole alkaloid shows potential activity against PANC-1 cell.

1. Introduction
The pancreas is a human digestive organ located under the stomach. The pancreas has the potential to get cancer because of smoking, a history of diabetes, alcohol consumption, gastric infection, obesity and there are offspring of pancreatic cancer [1,2].

Pancreatic cancer is difficult to diagnose when at the beginning, it can usually be diagnosed at an advanced stage. The initial symptoms that can be felt from pancreatic cancer are jaundice that has no pain. The cause of the tumor lies in the head of the pancreas and causes interference with the bile duct. Other indications of pancreatic cancer sufferers are itching and yellowing of the skin and eyes [3,4].

The main method of cancer treatment is surgery. Pancreatic cancer is performed curette surgery. But surgery can only be done by 20% of the total patients diagnosed with pancreatic cancer who have been diagnosed early [5]. Whipple's actions can be performed for patients who have tumors located on the head of the pancreas. Chemotherapy is usually given after surgery to minimize the risk of recurrence of the disease. There are a number of marine sponge studies that have the potential as anticancer agents such as *Chondrosiaacorticata, Xestospongia sp., Clathrinacoriacea* [6,7].

Based on the results of the research that has been conducted, we are looking for active compounds that can be used as selective cancer cell inhibitors derived from natural marine substances that are
adapted to nutritional deficiencies by utilizing culture media that lack glucose. Bioactivity was carried out to obtain filtration results from natural marine material isolated from the Indonesian marine sponge *Spongionellapulchella*.

2. Materials and Methods

2.1 Materials

NMR Spectrum analysis was carried out using JEOL ECA-500 (1H: 500 MHz, 13C: 125 MHz). Extraction sample study using the ESI-TOF-MS brand Q-Tof Ultima (Waters Co., MA, U.S.A). The IR spectrum was analyzed using the JASCO FT/IR-5300 tool. The UV spectrum was analyzed using UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). The active component of the sample was separated using an open chromatography column with Silica gel BW-200 (Fuji Silysia, Aichi, Japan), Cosmosil 5C18-MS-II (10 mm id x 250mm, Nacalai Tesque) and Cosmosil ODS (75C18-OPN, Nacalai Tesque, Kyoto, Japan). Subsequent research using a TLC Silica gel 60F254 chromatography (Merck Chemical, Darmstadt, Germany) and HPLC (UV detector: L-4000H). Extraction results were also analyzed with support tools such as bioradic spectroscopy, bioradic plates, and 96-well plastic plates. For the preparation of toxicity tests using a CO2 incubator. Biological analysis using Kanamycin 50 µg / mL solution, medium trypsin Mc-Coys, blue trypan, panc-1 human cells, general glucose media, glucose deficiency media, Phosphate buffer saline (PBS), Fetal Bovine Serum (FBS), ethanol, methanol, Dulbecco's modified Eagle's medium (DMEM), ethyl acetate, n-hexane and aquadest.

2.2 Cell Culture and Toxicity Test

PANC-1 processed in Dulbecco’s modified Eagle’s medium (DMEM) required additional 10% fetal bovine serum (FBS) with high temperature deactivation and kanamycin (50 µg / mL) with moist conditions not more than 5% CO2 and 37 °C. When the situation is negative glucose, PANC-1 is treated in Glucose deficient Medium (Basal Medium (25mM N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES) buffer (Ph 7.4) plus 4.6g / L NaCl, 700mg / L, NaHCO3, 400mg / L, KCl, 265mg / L CaCl2,2H2O, 200mg / L MgSO4,7H2O, 125mg / L, NaH2PO4, 0.1mg / L Fe (NO3) .9H2O, 15mg / L Phenolred, 10MI / 1 vitamin solution (X100) (GIBCO, Carlsbad, CA, USA), 200mmol / L L-glutamine solution (GIBCO), 10% FBS contained in 50mg / L (kanamycin) which has been dialyzed [8,9]. Glucose media with 10% Fetal Bovine Serum (FBS) and 2.0 g / L glucose (25mM) are useful for toxicity tests to determine the difference in cell activity when the condition is negative glucose [10,11,12].

For 24 hours PANC-1 cells were monitored using DMEM with 10% FBS. Medium Glucose Regular or Glucose Fat is used in the media to be replaced so that it can adjust nutritional hunger. After 12 hours of incubation, the sample was dissolved and incubated in moist conditions of 5% CO2 at 37 °C [13,14]. Cell proliferation was detected by the WTS-8 Colorimetric Reagent. The growth curve is determined to determine the IC50 value. The difference in IC50 values was determined based on the results of selectivity on anti-proliferation activity (S.I) in general glucose and glucose medium [15,16].

2. 3 Extraction and Isolation

*Spongionella pulchella* (62.50 g) partitioned using n-Hexane: EtOAc: EtOH (1: 1: 1 v / v) produce n-Hexane fraction (1.60 g), EtOAc fraction (17.62 g), and EtOH fraction (43.28 g). The viability results show that EtOAc fraction has activity against PANC-1 [17.62 g (IC50 = 40 µg / mL)]. Then, EtOAc fraction (17.62 g) fractionated using open column of chromatography (OPN-C18) with EtOAc: EtOH gradient produce 8 fractions. The activity of the fourth fraction [9.43 g (IC50 = 15 µg / mL)] purified using column 5C-18 MS II HPLC with eluent EtOAc: MeOH gradient obtain 7 fractions. The third fraction activity [4.72 g (IC50 = 10 µg / mL)] repurified using column 5C-18 MS II HPLC (EtOAc: MeCN gradient) produce 4 fractions. The activity of the third fraction [0.46 g (IC50 = 0.2 µg / mL)] shows cytotoxic against PANC-1 cell.
3. Results and Discussions

Compound 1 (colorless solid) which has been isolated known to has the molecular formula C_{28}H_{51}N_{2}O_{2} determined by LCMS-ESI with molecular weight [M+H]^+ 447.3951 m/z. Based on the FTIR spectrum shows that the active metabolite has functional groups at 3435.56 cm\(^{-1}\) as N-H secondary amines, 2853.39 cm\(^{-1}\) as C-H methyl, 2769.64 cm\(^{-1}\) as C-H methylene and the fingerprint region of amine at 1637.27 cm\(^{-1}\) as C-N imine. The results show that the active compound as an alkaloid [17,18].

| Position | δ_H | δ_C (t) | Mult. (J), int |
|----------|-----|--------|----------------|
| 2        | 3.65| 75.8   | m, 2H          |
| 3        | 1.72| 28.7   | m, 1H          |
| 4        | 3.08| 53.2   | m, 1H          |
| 6        | 2.79| 54.6   | m, 1H          |
| 7        | 1.76| 23.5   | -              |
| 8        | 1.36| 24.6   | m, 1H          |
| 9        | 2.26| 40.4   | m, 2H          |
| 10       | 4.14| 95.2   | m, 1H          |
| 11       | 1.32| 31.3   | -              |
| 12       | 1.17| 29.2   | m, 1H          |
| 13       | 1.12| 32.6   | m, 1H          |
| 14       | 1.82| 28.3   | m, 1H          |
| 15       | 1.18| 24.5   | m, 1H          |
| 16       | 1.38| 35.9   | m, 1H          |

The \(^1\)H and \(^{13}\)C NMR signals of 1 were confirmed by the analysis of 1H–1H COSY and HMHC spectral data and comparison with the signals of 2 and 3. The 1H–1H COSY spectrum of 1 revealed the carbon sequences of 2–3–4 and 6–7–8–9–10 together with the geminal couplings of H2 to 3, H2 to 4, H2 to 6, H2 to 7, and H2 to 8 [19].

The \(^{13}\)C spectrum (Table 1) showed 14 signals ascribed to three methines and 11 methylenes which suggested that 1 have a C2-symmetric structure. The larger coupling constant shows for H10 (δ 4.14, J = 10.0 Hz) indicated that H9 is axially oriented. The NOESY and ROESY spectra confirm that a larger coupling constant at H2 (3.65, J = 10.8 Hz) is also at axial orientation. Based on the interpretation of data indicate that the active metabolite as indole alkaloid [20].

Figure 1. Structure of active metabolite
The viability against PANC-1 cell in glucose deficiency medium from *Spongionella pulchella* shows potential activity of cell proliferation was detected by WST-8 colorimetric reagents. The IC$_{50}$ value is determined from the linear interpolation of the growth-survival curve. Based on IC$_{50}$ values, observed differences in viability in glucose deficiency medium. The active metabolite has anti-proliferative activity in glucose deficiency medium [21].

![Cytotoxic of Indonesian’s Marine Sponge against PANC-1 cell](image)

**Figure 2.** Cytotoxic of Indonesian’s Marine Sponge against PANC-1 cell

**4. Conclusion**

The active compounds from *Spongionella pulchella* have lower viability against PANC-1 cell with IC$_{50}$ = 0.2 μg/mL. The compound has potential activity to alternative drug models against commercial drug antimycin = 0.1 μg/mL.

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