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Active Compounds Ethyl Acetate Extract of *Stylotella* Sp. Sponges from Selayar Island Against MCF-7 Breast Cancer Cells

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***Abstract**: Stylotella sp. sponge is a type of marine invertebrate animals that widely distributed in Indonesian marine areas. One of the spreading areas of the Stylotella sp. sponge is in the Selayar Island, South Sulawesi, Indonesia. Sponges have potential as a drug because contained secondary metabolites. The aim study was identified the types of secondary metabolite compounds and determined the bioactivity of Stylotella sp. Metabolite compound was used against MCF-7 breast cancer cells. The extraction method used process of obtaining isolates because its easy and economical. Testing the level of purity of isolates was carried out with three eluent systems which showed a single stain result. FTIR characterization result showed that pure isolates contained O-H, C-N, and C-H functional groups, alkaloids compound. The activity of MCF-7 cells used the colorimetric method that showed IC_{50} value of 14987.50 µg/mL, can be inhibited expansion of MCF7.*

***Keywords**: Stylotella sp, alkaloid, IC_{50}, MCF-& cells*

INTRODUCTION

The potential of marine biodiversity can be utilized in various industrial fields including food, dyes, cosmetics, and health (Handayani et al., 2010). There have been many studies on marine biota both in classifying, isolating, characterizing and testing the activity of natural compounds derived from the sea (Faisal et al., 2008). Very high molecular diversity in marine biota is caused by changes in extreme temperatures, salinity, pressure, and the spread of viruses and pathogens (Herdhiansyah et al., 2015). Marine organisms, especially invertebrates, contain the most chemical compounds compared to other marine plants (Handayani et al., 2010). As a marine biota that has no spine in the spine ranks first as a source of chemical compounds that have bioactivity, known as bioactive compounds (Murniasih, 2003). It has been reported in the last few decades that 50% of bioactive compounds have been found originating from marine invertebrates, especially from the phylum of Porifera (Asaf et al., 2001). Chemical compounds that have been successfully isolated from sponges are alkaloids, terpenoids, polyketides, acetogenin and others (Murniasih, 2003). Extracts of secondary metabolites from sponges have bioactivity such as cytotoxic, antitumor, antileukimia, antiviral, antibacterial, antifungal, immunomodulatory, and anti-inflammatory properties (Suparno, 2017).

Sponges have diversity and around 7000 species have been published (Handayani et al., 2012). (Wewengkang et al., 2014) stated that 830 types of sponges were scattered in Indonesian sea areas. Outside the territory of Indonesia many discoveries of new compounds from the genus *Stylotella* sponge which have potential as medicine. Some of these include the discovery of a cycloheptapeptide compound called stylostatin 1 having cytotoxic bioactivity, this compound was isolated from a sea sponge type of *Stylotella* aurantium originating from the waters of Papua New Guinea (Pettit et al., 1992).

Stylotella sponges are known to have varied natural compounds such as Axinellin C, Wainunumida and Styloguanidines. It has been mentioned that research on the study of biological activity originating from the *Stylotella* aurantium sponge is rarely reported (Ko et al., 2017). Therefore this study was conducted which aims to determine the types of secondary metabolite compounds contained in the ethyl acetate extract sponge *Stylotella* sp. and to know the bioactivity of secondary metabolite compounds found in ethyl acetate extract sponge *Stylotella* sp. against MCF-7 breast cancer cells.

RESEARCH METHODS

Materials and Tools

Sponge *Stylotella* sp, sulfuric acid (H_2SO_4) pa merck, sulfuric acid (H_2SO_4) 10%, acetone (C_3H_6O), iron (III) chloride ($FeCl_3$) 5%, cisplatin (cis-$PtCl_2(NH_3)_2$), dimethyl sulfoxide (C_3H_6O), iron (III) chloride ($FeCl_3$) 5%, cisplatin (cis-$PtCl_2(NH_3)_2$), dimethyl sulfoxide (C_3H_6O) DMSO), ethanol 96% (C_2H_5OH), ethyl acetate ($C_4H_8O_2$) Bratachem, Bratachem fetal serum (FBS), chloroform ($CHCl_3$), microtube, sodium hydroxide (NaOH) 10%, n-Hexane (C_6H_{14}) Bratachem, FBS), chloroform ($CHCl_3$), microtube, sodium hydroxide (NaOH) 10%, n-Hexane (C_6H_{14}) Bratachem, phosphate-buffered saline (PBS), PrestoBlue cell viability reagents, Roswell park memorial medium (RPMI), MCF-7 breast cancer cells, trypan blue, trypsin-EDTA, tubes, T-flask, and 96 well plates.

Prestige-21 Shimadzu Fourier Transform Infra-Red (FTIR), Biosafety Cabinet (BSC), inverted microscope, multimode reader, centrifuge, CO_2 incubator, Kirin oven, UV lamps 254-336 nm, rotary evaporator Hanshin Scientific co. Model Hs. 2000NS, analytical balance, vacuum liquid chromatography column (KKCV), gravity chromatography column (KKG), vacuum pump, microplate, adapter, hotplate, condenser, steel heat, 110 ° C thermometer, and Beaker glassware.

Procedures

Samples by Selayar Island with a depth of about 3-10 m because the growth and community of coral reefs and sponges were optimum at these depths (Suharyanto et al., 2008). Then the sample is cleaned and stored in the refrigerator. Subsequently, the taxonomy was dried and identified in the Faculty of Fisheries and Maritime Affairs, Hasanuddin University. The next process is formed into small sizes.

500 grams sample was immersed using an ethyl acetate solvent for 24 hours which was repeated up to 3 times. The maserate obtained is then concentrated using a rotary evaporator until a thick extract is obtained. Then performed vacuum liquid column chromatography (KKCV) with various solvent ratios sorted by polarity starting from non-polar to polar. The fraction obtained in the KKCV process was tested by TLC, the fraction which had crystal markings combined and continued on purification by gravity column chromatography with a solvent ratio sorted by polarity. The continued fractionation process through gravity column chromatography obtained pure fractions which proced to the purity test, identification and anticancer test.

Pure isolates showed one stain by testing three eluent systems namely chloroform: ethyl acetate (9: 1), chloroform: acetone (9: 1) and n-hexane: acetone (8: 2). The pure isolates obtained were identified by a qualitative test and continued with characterization using the Fourier Transform Infra-Red (FTIR) spectrophotometer. Anticancer testing uses the colorimetric method with positive control of cisplatin.

RESULTS AND DISCUSSION

Stylotella sp. from the Selayar Islands macerated using ethyl acetate solvent because its semipolar in nature. Maceration for 24 hours was three times in a row. Thick an extract obtained 36.4 grams and dark brown colour. Thick extracts were tested for their secondary metabolite content through phytochemical screening. The test results showed *Stylotella* sp. positive alkaloids.

Fractionation

Fractionation using column of vacum liquid chromatography (KKCV) produced 17 fractions combined into 11 fractions based on color, polarity and TLC test results showing the same Rf value. The results of the combined fraction showed yellowish-white crystal weighing 0.0161 grams.

Identification

The purity test is carried out to determine the purity level of an isolate by elution using several eluent mixtures that have different polarity levels. The eluent polarity affecting the Rf value of a substance is shown in Figure 1.

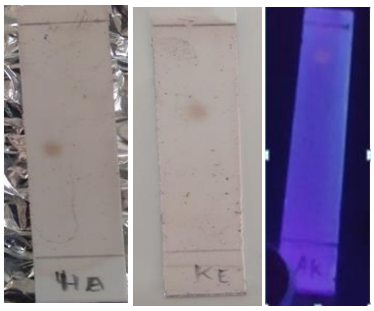

Figure 1. TLC results for three eluent (a) acetone eluent: n-Hexane (8: 2) eluent (b) eluent chloroform: ethyl acetate (9: 1), (c) chloroform eluent: acetone (9: 1)

The stains showed in the three eluents system test purification process showed a single stain with Rf value of 0.45; 0.60 and 0.82. The crystals have been thought to be pure alkaloid compounds. This is also proved by the results of phytochemical screening and functional group tests. In the Wagner test, K^+ metal ions will form covalent coordinate bonds with nitrogen in the alkaloids to form a precipitating alkaloid complex (Setyowati et al., 2014), such as the following reaction:

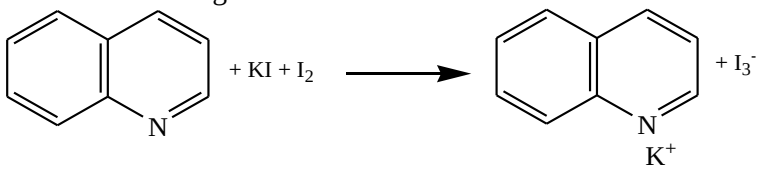

Figure 2. Reaction of Alkaloid with Wagner's reagent.

Characterization with FTIR spectrophotometer

Identification of the functional group isolates of ethyl acetate extract Sponge *Stylotella sp*. using Fourier Transform Infra-Red (FTIR) spectrophotometer. The test results show some typical absorption for some functional groups. The uptake at 3425.83 cm^{-1} shows the widening absorption as a stretch vibration of the O-H (hydroxyl) group. Absorption at 2930.06-2855.41 cm^{-1} indicating strain vibrations towards aliphatic C-H (alkane) groups. Sharp and strong absorption at 1062.84 cm^{-1} gives an indication of the presence of C-N (nitrile) groups so that based on the test results it can be concluded as an alkaloid compound.

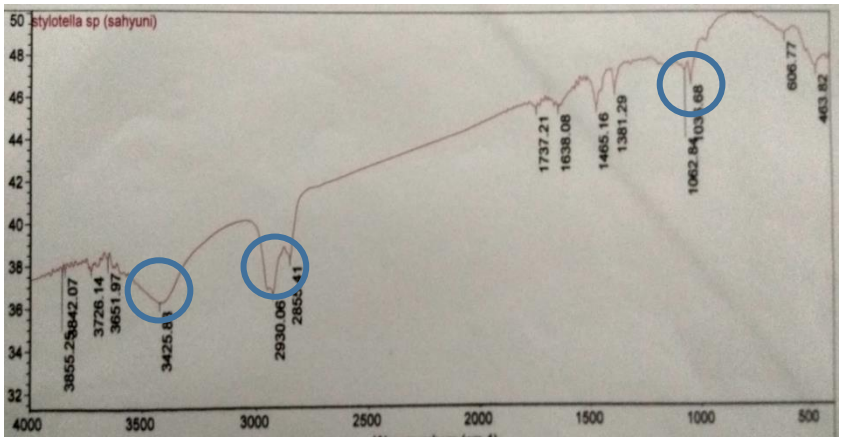

Figure 3. The spectrum of FTIR Isolate of ethyl acetate extract was Sponge *Stylotella* sp.

Anticancer Test on MCF7 Breast Cancer Cells

Cytotoxic activity testing is carried out to determine the IC_{50} value of a sample and its effect on cell activity and cell multiplication. Cytotoxic testing is carried out in several methods, this test uses the colorimetric method or color changes due to oxidation-reduction reactions, where resazurin as a blue indicator is reduced to pink resorufin, color changes indicate cell activity.

Cells which were still actively dividing carry out metabolic activities, thus producing enzymes derived from mitochondrial cell organelles causing the reduction in resazurin such as dihydrolipoamine dehydrogenase (Matsumoto et al., 1990). Resazurin used is presto blue reagent added to cells and the final result is absorbance measurement using a multimode reader. This test uses Dimethyl Sulfoxide solution (DMSO) as a negative control and as a sample solvent because it can dissolve well in a variety of organic solvents, which are polar or nonpolar in nature so as to increase the solubility of the sample. The cisplatin as a positive control which is a pure anticancer agent and is usually used as a comparison.

Tests using complete Roswell Park Memorial Institute Medium (RPMI) liquid culture media (containing 10% Fetal Bovine Serum (FBS) and 50 µL / 50mL antibiotics). According to Freshney (2010), FBS functions as a nutrient for cell survival because it contains a source of lipids, minerals, and hormones. The work solution used is PrestoBlue ™ Cell Viability Reagent.

The Isolate Test was made into eight concentration variants to see the relationship between the concentration patterns and cell activity. Each concentration in dilution can represent each category of toxic concentration parameters, concentrations of 1000.00 µg / mL and 500.00 µg / mL for the less toxic category, concentrations of 250.00 µg / mL, 125.00 µg / mL and 62, 50 µg / mL for the moderate toxic category. As for the very toxic category that is at a concentration of 31.25 µg / mL, 15.63 µg / mL and 7.81 µg / mL.

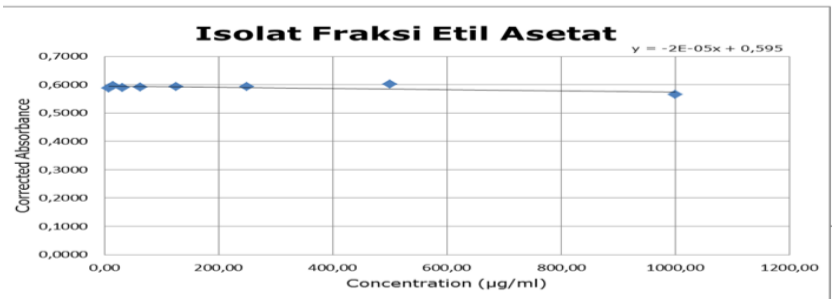

Figure 4. Curve Test Results of Ethyl Acetate Fraction

Results IC_{50} ethyl acetate extract sponge *Stylotella sp*. of 14987.50 µg/mL which means it is not toxic. Cytotoxic activity is categorized into three depending on the IC_{50} value, namely IC_{50} <100 µg/mL including potential cytotoxics, 100 µg/mL <IC_{50} <1000 µg/mL including moderate cytotoxic and IC_{50}> 1000 µg/mL means that it does not have cytotoxic potential to inhibit cancer cells (Prayong et al., 2020).

The breast cancer cells used are MCF-7 cells (Michigan Cancer Foundation-7) derived from the breast tissue of a 69-year-old woman with blood type O, with Rh-positive, in the form of cells attached and can be grown in the media, MCF-7 cells has been reported to have characteristics that are resistant to chemotherapy agents (CCRC, 2008). The cis-Diammineplatinum (II) dichloride (Cisplatin) compound is usually used in small concentrations as a standard in cytotoxic testing as in antiproliferation studies of T47D breast cancer cells with the use of cisplatin as a positive control in a concentration of 0.43 µg/mL (Subeki & Muhartono, 2015) and is used as a positive control with various concentrations (1-10 µg/mL) in research as a lung cancer chemotherapy agent on A549 cancer cells (Ihsan, et al., 2013). Ethyl acetate extract isolates Sponge *Stylotella* sp. non-toxic to MCF-7 cells because IC_{50} values are not included in the toxic category, however, the addition of sample concentrations gives the effect of differences in appearance between cells sampled in concentrations of 1000 µg/mL and in concentrations of 7.81 µg/mL. While the cells given Cisplatin showed signs of reducing the number of cells.

Reduction in the number of cells can be an indication of apoptosis, according to the CCRC (2008), cells undergoing apoptosis can be observed using an electron microscope through the morphological features shown. These characteristics include cells being rounded because the structure of proteins that make up the cytoskeleton is digested by specific peptidase enzymes that have been activated in the cell. The chromatin begins to degrade and condensate, the chromatin undergoes further condensation, becoming more solid. At this stage the membrane surrounding the cell nucleus still appears intact, the environment in the cell nucleus appears to be interrupted and the DNA within it is fragmented, the cell nucleus breaks apart releasing various forms of chromatin or nucleosome units due to DNA degradation.

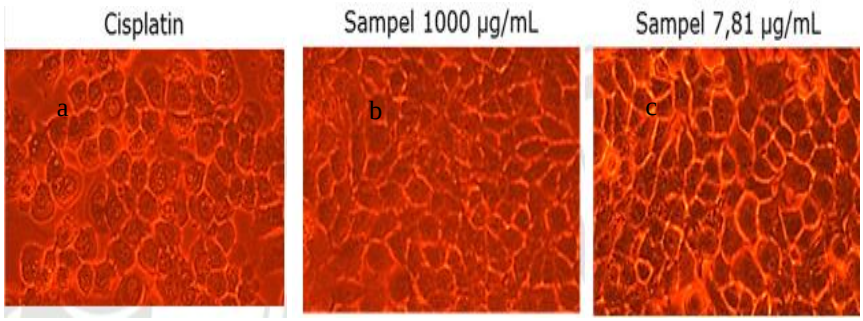

Figure 5. MCF-7 Cancer Cell Test Results

Apoptosis shows cell death that begins with the formation of indentations in the cell membrane and fragmented DNA (Goodlett et al., 2005). Compounds are toxic if they show antiproliferation effects, cell cycle inhibition, angiogenesis inhibition, direct cell destruction (necrosis) and induction of apoptosis (Ren et al., 2003)

Alkaloids can be anticancer agents as antitubulin, alkaloids can bind microtubule proteins in the formation of spindles, thereby inhibiting the cell division cycle, precisely at the metaphase stage (Thurston, 2007). Microtubules are polymers of tubulin whose existence is important in cell division. Cells that cannot divide can experience apoptosis.

CONCLUSIONS

Extract ethyl acetate of sponges *Stylotella sp*. contained alkaloids compound. Results of FTIR showed functional group of alkaloids such as O-H (hydroxyl) group, C-H (alkane) groups, C-N (nitrile) groups. IC_{50} values of ethyl acetate extract isolates from *Stylotella* sp. is 14978.50 µg/mL, can be inhibited expansion of MCF7.

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