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PROLIFERATION INHIBITORY ACTIVITY OF THE ACTIVE FRACTION MARINE SPONGE *Cinachyrella* sp. AGAINST CELL LINE T47D

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ABSTRACT

Marine sponges *Cinachyrella* sp. (Family:Tetillidae) in Kukup beach, Kemadang Village, Tanjungsari District, Gunung Kidul, DIY were producing diversity secondary metabolites such as polyketides, alkaloids, peptide and terpene. The purpose of this study was investigated proliferation inhibitory activity of active fraction *Cinachyrella* sp. against cell line T47D. Sponges samples were collected manually from rocky substrate at depth 0.5 m. The sponges was minced and extracted with 95% ethanol. The ethanol extract was partitioned sequentially with ethyl acetate. The extract ethyl acetate was fractionation with 4 organic solvent, in increasing order of polarity with vacuum liquid chromatography column (VLC) method. Doubling time method was applied to analyse the inhibition proliferative cell line T47D. Resulted showed ethyl acetate extract of *Cinachyrella* sp. were 12 fractions and all tested fraction obtained by thin layer chromatography (TLC). Fractions that have the same value Rf grouped together to obtain 6 fractions. The fraction number 5 exhibited proliferation inhibitory activity to cell line T47D. The Rf value of active fraction number 5 were 0.125; 0.25 and 0.437. The active fraction 5 than isolation by preparative thin-layer chromatography (PTLC) was 5 isolate fractions preparative. The isolate fractions preparative number 5 exhibited proliferation inhibitory activity against cell line T47D. Fraction which determined by cerium sulfate and results was expressed terpene and alkaloid.

Key words: *Cinachyrella* sp., Doubling time method, proliferation inhibitory activity.

INTRODUCTION

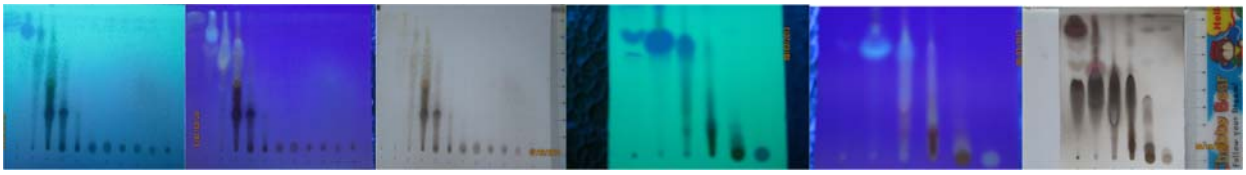
Cancer is the fourth leading cause of death after stroke, hypertension and diabetes (Ullah & Aatif, 2009). In Indonesian patients of cancer also tended to increase (Tjindarbumi & Mangunkusumo, 2002). Data Riskesdas reported that in 2007 there were 100 new cancer patients than 100,000 residents. Many attempts have been made to overcome cancer. In America and Europe is estimated 65% of cancer drugs derived from natural materials commercially (Wei *et al.*, 2007). Derivate of bioactive compound from natural products have specific targets and has no side effects (Iwamaru *et al.*, 2007). Sponges as sessil animals, filter feeders have a strategy physiology, reproduction and defense mechanisms are effective against bacterial infections, fungi, viruses and predators (Brackman & Daloze, 1986), space competition with other organisms (Schupp *et al.*, 2009) marine and defense against pathogens (Muller *et al.*, 2004) by producing secondary metabolites (Bell, 2008). Kukup beach, village Kemadang, Tanjungsari District, Gunung Kidul, Yogyakarta has a diverse marine life. The lectin proteins, which we refer to as the Cinachyrella galectins (CchGs), were identified as the active principles in an aqueous sponge extract that modulated the function of mammalian ionotropic glutamate receptors (Ueda *et al.,* 2013). In the test anticancer ethanol fraction *Cinachyrella* sp. against cancer cells WiDr showed arrest results in G0-G1 phase was 57.31% (Nurhayati *et al.*, 2011). In the cytotoxicity assay HeLa cancer

cells, T47D, WiDr and normal Vero cells obtained results targets cancer cells ethyl acetate fraction sponge *Cinachyrella*sp. is WiDr at 253.548 ug / ml (Nurhayati *et al.*, 2012). Ethyl acetate fraction has several active fractions are not known WiDr cell proliferation inhibition activity.

MATERIALS AND METHODS

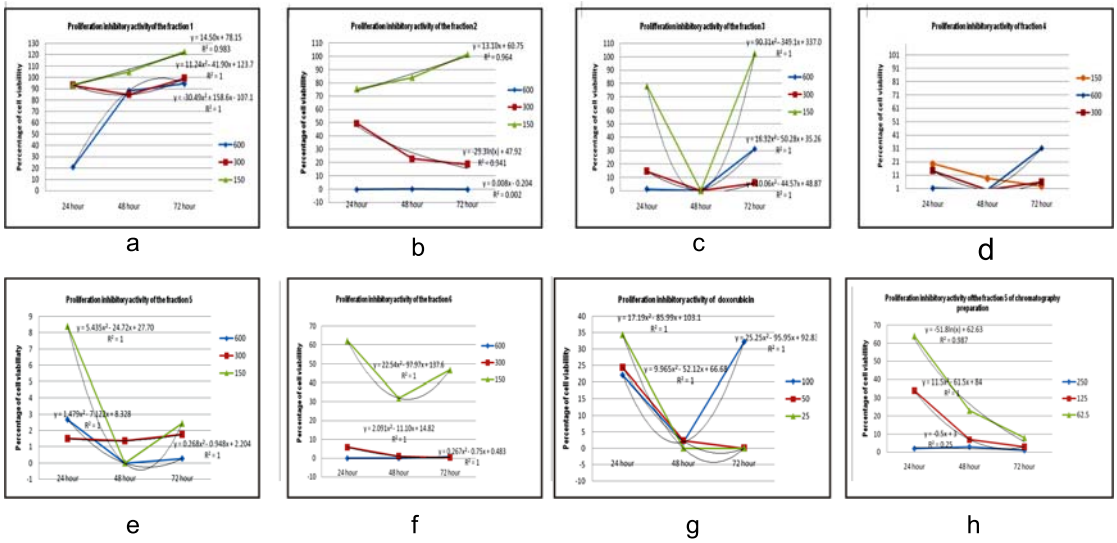
Sponge *Cinachyrella* sp. sampling from Kukup beach, village Kemadang, Tanjungsari District, Gunung Kidul, Yogyakarta at intertidal areas by way of direct collection. Sponge inserted at plastic bag and stored in the icebox temperature of 5 ° C. Sponge mass of macerated with 96% ethanol for 2-3 days. Ethanol extract partitioned with ethyl acetate. The filtrate was fractionated by vacuum liquid chromatography column. The solvent used was n-hexane (100%), n-hexane: ethyl acetate (9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8 ; 1:9), ethyl acetate (100%) and methanol (100%). Fractionation results obtained 12 fractions. Fractions showed the same Rf value combined to obtain 6 fractions. Further proliferation inhibition test with Double-time method. Variations are tested concentrations of 600, 300 and 150 µg/mL for fraction and 25, 50, 100 µg/mL for doxorubicin. The most active fraction was isolated by preparative thin layer chromatography and proliferation inhibition test with a concentration of 62.5; 125; 250 µg/mL.

RESULTS AND DISCUSSION



a b c d e f

Figure 1. Test results of thin layer chromatography (TLC) of 12 fractions results column, UV 254 (a), UV 366 (b), (c) after using cerium sulfate (d). Test results of thin layer chromatography (TLC) of 6 fractions results column, UV 254 (d), UV 366 (e) and after using cerium sulfate (f).



a b c d e f g h

Figure 2. Test results WiDr cell proliferation inhibition of fraction 1 (a), fraction 2 (b), fraction 3 (c), fraction 4 (d), fraction 5 (e), fraction 6 (f), doxorubicin (g) and fraction 5 of preparative chromatography (h).

Doubling time test was conducted to determine the effect of marine sponge *Cinachyrella* sp. fraction of the cancer cells WiDr. Concentrations used were 600, 300 and 150 µg/mL for column fractions; 25, 50, 100 µg/mL for doxorubicin and 62.5; 125; 250 µg/mL for KLTP fraction with incubation time 24, 48 and 72 hours. This test is performed with MTT method. Doubling time test results can be seen in Figure 2. It appears that after administration of fraction and incubated for 24 hours a decline in the percentage of living cells. The highest decline seen in fraction 5, which is at a concentration of 300 µg/mL. At 48 hours of incubation, the concentration fraction granting 600 and 150 mg / mL showed a decrease in the percentage of live cells. At 72 hours of incubation, the increase percentage of WiDr cancer cells. This is presumably because of the time needed by cancer cells WiDr cells to complete one cycle is very short is 15 hours. So at 72 hours of incubation, the number of population is very high WiDr cells. Giving fraction sponge *Cinachyrella* sp. and doxorubicin can not inhibit the growth of cancer cells WiDr. One of the characteristics of WiDr cells is the expression cyclooxygenation-2 (COX-2) is a high spur WiDr cell proliferation (Palozza *et al*., 2005).

Differences in the ability of marine sponge fraction activity *Cinachyrella* sp. against cancer cells WiDr because fractions have different compound components (Jenei *et al*., 2009). Fraction 5 has a value of Rf 0.125; 12:25 and 0437 and is a group of terpenes and alkaloids. Bioactive compounds found in marine sponges *Cinachyrella* sp. among others cinachyramine (Shimagawa *et al*., 2006), enigmasole A (Oku *et al*., 2010). Sponge secondary metabolites are influenced by water conditions, and the type of sponge symbiont organisms (Larghi *et al*., ;2008). Positive control used is doxorubicin. Toxicity of doxorubicin because intercalation with DNA, forming a tripartite complex with topoisomerase II inhibition and DNA causing cell cycle and stopped at the G1 and G2 phases (Gewirtz, 1999).

CONCLUSION

The fraction number 5 exhibited proliferation inhibitory activity to cell line T47D. The isolate fractions preparative number 5 exhibited proliferation inhibitory activity against cell line T47D. Fraction which determined by cerium sulfate and results was expressed terpene and alkaloid.

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