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Cytotoxic Activity of Methanol Fraction Hydroids *Aglaophenia cupressina* Lamoureoux Against HeLa Tumor Cells.

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ABSTRACT

Bioactive compounds from marine organisms widely explored in an effort to get raw material of anti tumor or cancer that until now still leading cause of death in the world. In medicine, basic materials drug search of antitumor from nature are generally focused on active compound that has ability to suppress tumor cell proliferation, have effect of cytotoxic and antimitotic, and has no side effects. Hydroids *Aglaophenia cupressina* Lamoureoux is a marine invertebrate animals that live attached to sponge, rich in bioactive compounds. Hydroid is one source of new compounds from marine organisms that have pharmacological activity. Previous studies have shown that hydroid has antimicrobial in some pathogenic bacteria and fungi. This study aims to determine cytotoxic activity of methanol fraction hydroid *Aglaophenia cupressina* Lamoureoux against HeLa tumor cells. Preliminary test cytotoxic against *Artemia salina* conducted using Brine Shrimp Lethality Test (BSLT), then proceed with MTT test 3-4,5-dimethylthiazol-2 yl-2,5 diphenyltetrazodium bromide assay on HeLa tumor cells. The test results of BSLT showed crude extract of hydroid *A. cupressina* L. has excellent early stage activity with LC_{50} = 19.70 mg/mL. MTT assay results also showed cytotoxic activity against HeLa cells with LC_{50} = 9.11 mg/mL, so it can be stated that methanol fraction of hydroid *A. cupressina* L. has strong antitumor effect.

Keywords: Cytotoxic, Hydroids *Aglaophenia cupressina* Lamoureoux, HeLa tumor cells.

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INTRODUCTION

Until now, cancer is still one of leading causes of death in the world. Various attempts were made to prevent and cure disease. One of intensive efforts being made is search antitumor compounds from natural materials. Basic materials drug search of antitumor from nature are generally focused on active compound that has ability to suppress tumor cell proliferation, have effect of cytotoxic, antimitotic or have ability to induce apoptosis in tumor cells. Hydroids (phylum *Coelenterata*) is marine invertebrate animals that live attached to sponge, rich in active compounds. According Sennet *et al* [14], environmental stresses such as competition space, light and other sources causing chemical diversity in variety of organisms including hydroid. The existence of bioactive metabolites in sessile organisms show their ecological adaptations that are formed as a means of self defense [13].

Results of isolation and characterization of secondary metabolites hydroid *Aglaophenia cupressina* Lamoureoux [7] found some bioactive compounds, among others: 1) a carboxylic acid group that is acid heksadekanoat 2) alkaloids that Aglao E. Unhas suspected of a new compound, two compounds showed toxicity against *Artemia salina* and also be antimicrobial against *Staphylococcus aureus* and *Salmonella typhi* against fungus *Candida albicans* and *Malassezia furfur*. Furthermore, Suada and Ni Wayan Suniti [16] proved that *Aglaophenia sp* crude extract (0.05%) were able to suppress growth of *F. oxysporum* f vanilla.

Johannes *et al* [8], state that compound β-sitosterol one isolate of hydroids have antimitotic to zygote cell division in sea urchins. Likewise, results of Johannes [9], proving some of active compounds of hydroid *A. cupressina* Lamoureoux is not toxic to mice, so it can be developed as a basic ingredient of antimicrobial and anticancer. This study aims to determine cytotoxic activity of methanol fraction hydroid *Aglaophenia cupressina* Lamoureoux against HeLa tumor cells.

MATERIALS AND METHODS

Sampling hydroids, Extraction and Fractionation (Hostettmann *et al*., 1995)

Samples of hydroid *A. cupressina* Lamoureoux obtained from island Samalona, South Sulawesi 1 kg cleaned then wind dried. Samples were dried chopped weighed 500 g and then macerated with methanol (1: 3) for 24 hours and repeated 3 times. The solution obtained is evaporated until solvent becomes dry. Water remaining in extract is dried with a freeze dryer at a temperature -43^{o}C to obtain crude extract in powder form. Fractionation is done by using methanol and ethyl acetate each performed three times with 100 mL of solvent volume. After second screened fraction is evaporated using nitrogen evaporator and dried using a freeze dryer.

Early Stage activity test against Artemia salina using Brine Shrimp Lethality method (Test BSLT) (McLaughlin & Rogers, 1998)

Activity test using *Artemia salina* larvae. *A. salina* cysts are hatched in artificial seawater (38 g NaCl in 1 L of water), then placed taken 40 watt fluorescent lamp. After 48 hours of cysts hatch into nauplius instar III/IV and ready to be tested. As many as 10 larvae of *A. salina* inserted into vial which already contains sample extract at dose of 10, 20, 30, 50, 70, 100 mg/mL (each dose consists of three vials so that number of *A. salina* each dose amounted to 30 individuals), Then artificial sea water is added until volume reaches 10 mL. As control use artificial seawater without sample extract. All vials were incubated at room temperature for 24 hours under a fluorescent lamp of 40 watts. Observations were made after 24 hours by counting number of dead *A. salina* on each treatment. *A. salina* larvae mortality was calculated using formula B-C/D X 100%. B is number of dead larvae, C is number of dead larvae on control, D is number of larvae that were tested [5]. Determination of LC_{50} done using probit analysis.

Cytotoxic Test against HeLa Tumor Cells (ATCC, 2001)

Cytotoxic test performed with MTT method 3-4,5-dimethylthiazol-2 yl-2,5 diphenyltetrazodium bromide. HeLa tumor cells obtained from Laboratory of Parasitology, Faculty of Medicine, Airlangga University Surabaya. Cells were cultured in medium Roswell Park Memorial Institute (RPMI) 1640, Fetal Bovine Serum (FBS) 10%, Fungison 0.5% and 2% penicillin-streptomycin. Extract is made with series of doses of 5, 10, 15, 20,

25, 30 mg/mL, with three replications. Extract solution was put into 96 microplate wells as much as 100 mg/L equivalent to 2 x 10^4 cells/100 mL. This test uses three types of control are control cells consisting of 100 µL cells + 100 µL media cell, 100 µL extract + 100 mL of media and media controls consisting of a 200 mL culture medium. Microplate was incubated for 24 hours at a temperature of 37^{o}C with a stream of CO_2 5 mL/min. After 24 hours as much as 5 mL MTT was added into each wells. Microplate incubated back on CO_2 incubator for 4 hours, then MTT reaction was stopped by adding 100 mL sodium dodesil sulfate (SDS) 10%. Microplate back incubated for 12 hours at room temperature. After 12 hours, absorbance of each of wells is read with spectrophotometer ELISA *microplate reader* at wavelength of 570 nm.

HeLa cell death is calculated based on amount of cell viability due to effect of extract. The higher the viability of cells, the cells that die are considered less and less. Determination of percentage that cell death is calculated based on the formula (A-B)/A x 100%, A is number of living cells (viable) in wells without extract treatment (control cells), B is number of living cells in test wells were given extract. Determining value of lethal concentration of cytotoxic test done using probit analysis.

RESULTS

BSLT results test from methanol extract of *A. cupressina* L. hydroid presented in Figure 1 that appears that with increasing doses of *A. salina* mortality rate also increased from 10 mg/mL to 70 mg/mL, showing all *A. salina* death. To determine level of toxicity extracts of *A. cupressina* L. be calculated LC_{50} value. Based on table 1, can be seen that methanol fraction LC_{50} values *A. cupressina* L. of 19.70 mg/mL had very high bioactivity. Table 2 shows percentage of deaths continues to rise to a concentration of 70 ug/mL showed all the tested *A. salina* death.

Table 1: Test results of BSLT methanol fraction *A. cupressina* L.

Dosage (µg)	Log Dose	% Mortality	Probit Value	Equation	LC_{50} (µg/ml)
10	4.00	24.82	3.78		
20	4.30	50.25	5.02		
30	4.48	65.70	5.45	Y= 4.312X – 13.518	19.70
50	4.70	97.40	6.91	R^2=0.959	
70	4.85	99.80	7.88		
100	5.00	99.60	7.66		

Table 2: Test Results Cytotoxic Fraction Methanol *A. Cupressina* L. against HeLa cells

Dosage (µg)	Log Dose	% Mortality	Probit Value	Equation	LC_{50} (µg/ml)
5	3.70	18.70	4.24		
10	4.00	58.50	5.24		
15	4.18	72.56	5.62	Y= 2.903X – 6.494	9.11
20	4.30	78.20	5.79	R^2=0.96	
25	4.40	85.90	6.10		
30	4.48	96.40	6.78		

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Fig. 1. Mortality *A. salina* after administration of ethanol fraction *A.cupressina* L.

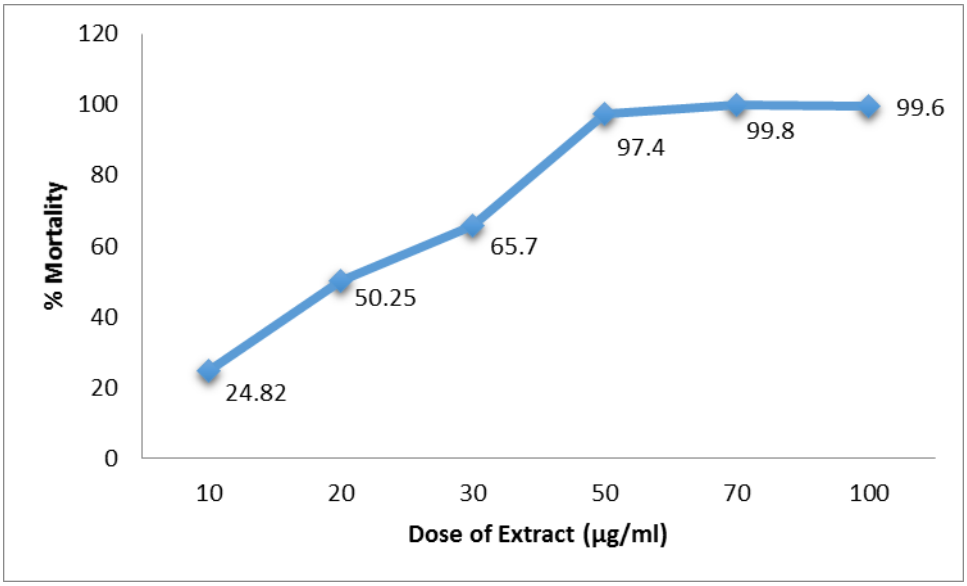


Fig. 2. Mortality of HeLa cells from MTT test results

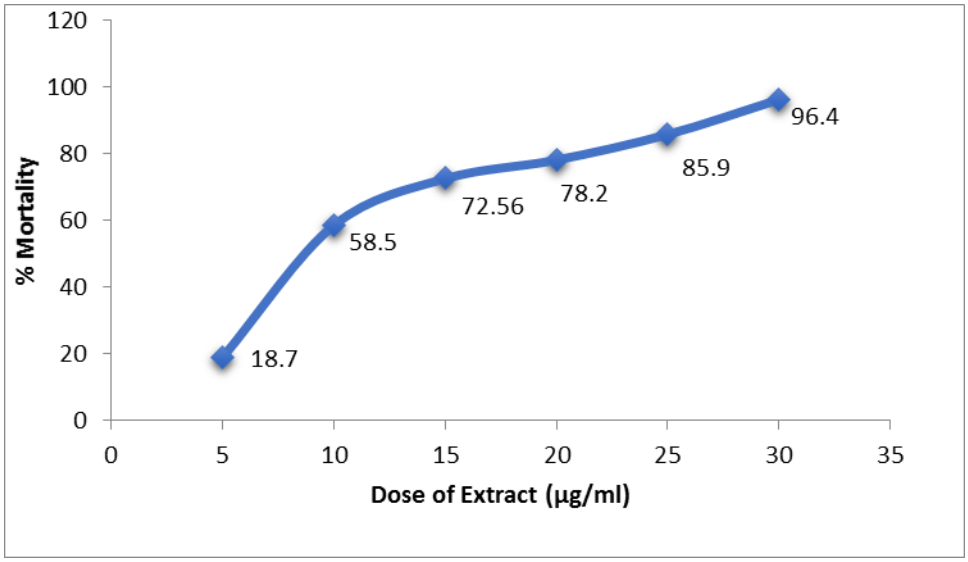
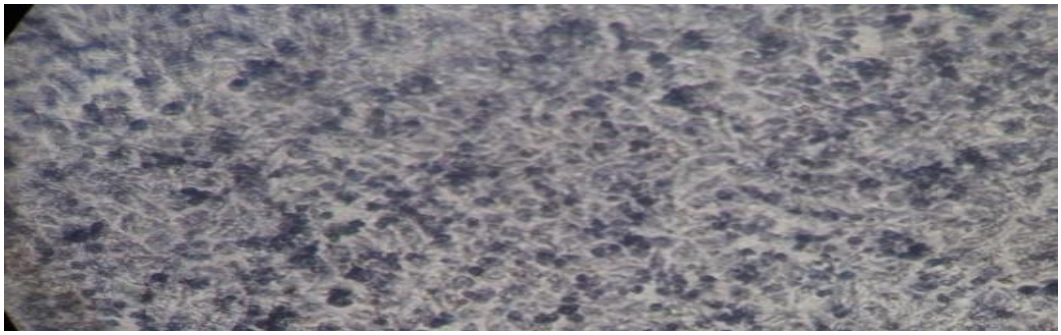


Fig. 3. Photo HeLa cells (A) without treatment/control, (B) HeLa cells treated with methanol fraction *A. cupressina* L. 5 ppm (1), 10 ppm (2), 15 ppm (3), 20 ppm (4), 25 ppm (5), 30 ppm (6)



(A)

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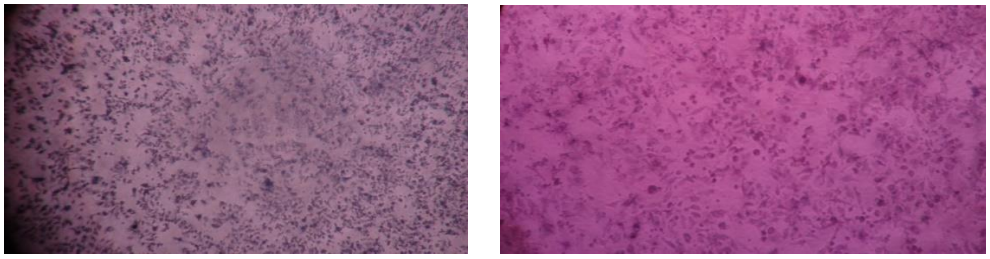
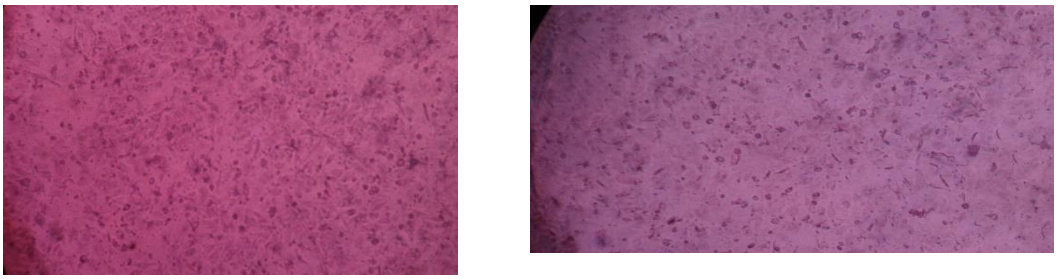
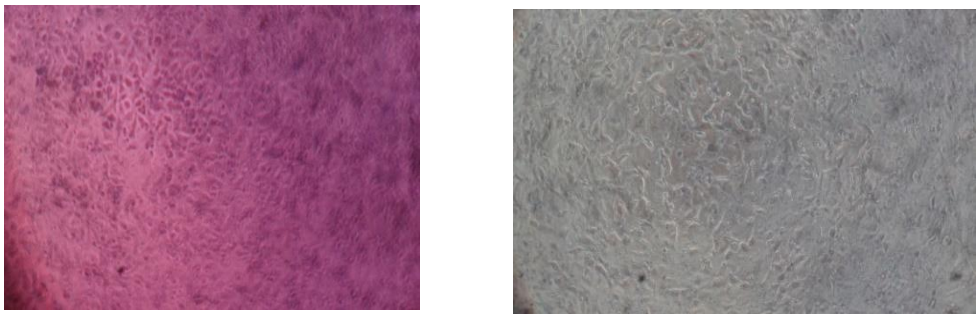


(1) (2)

(B)



(3) (4)



(5) (6)

From Figure 2 shows percent of HeLa cell death continues to increase with increasing number of doses ranging from 5 mg/mL, 10 mg/mL, 15 µg/mL, 20 mg/mL, 25 mg/mL, and 30 ug/mL. To determine level of toxicity extracts of *A. cupressina* L. then be calculated LC_{50} value.

DISCUSSION

According to McLaughlin & Rogers [10] in Widiastuti [17] an extract classified as very active if it has a LC50 value ≤ 30 ppm. Results of research Carballo *et al* [2] on advisability of using BSLT method for pharmacological activities of natural materials showed a positive correlation between BSLT and cytotoxic test revealed 50% of active species in BSLT also active in cytotoxic test. This shows Hydroid *A. cupressina* L. has potential bioactive interesting to explore further.

LC_{50} values *A. cupressina* L. methanol fraction of 9.11 µg/mL as very active cause extract is used not pure. According to Anderson (1994) in Sismindari *et al* [15] crude extract is considered to have strong antitumor effects when cytotoxic assay results have a LC_{50} less than 30 mg/mL. In general, active compound in pure form has a stronger biological activity [4]. MTT test is sensitive, quantitative and reliable [3]. MTT reaction is reduction reaction cell based on solving tetrazolium salt MTT yellow into blue formazan crystals purple [6]. The formazan crystals can be read absorbances by using Enzyme-linked Immunosorbent Assay (ELISA) reader [12]. Reaction involves pyridine nucleotide cofactors NADH and NADPH is only catalyzed by living cells, so that amount of formazan formed is proportional to number of living cells [18]. The more cells live, the acquired many formazan crystals are formed, the higher absorbance values obtained and indicated low mortality.

Results formazan crystal photo by HeLa cells by treatment with addition of methanol fraction 5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm showed a high bioactivity. It appears that extract bioactivity methanol fraction *A. cupressina* L. percentage HeLa cells were dead sharply about 75%. MTT breakdown occurs in mitochondria of living cells by enzyme succinate dehydrogenase [11].

CONCLUSION

Extract methanol fraction of hydroid *A.cupressina* Lamoureoux has bioactivity excellent early stage with LC_{50} = 19.70 mg/mL. In addition methanol fraction of *A.cupressina* L. has a strong cytotoxic activity against tumor cells Hela with LC_{50} = 9.11 mg / mL.

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