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Research Article

# EFFECTS OF HYDROID *Aglaophenia cupresina* LAMOUROUX EXTRACT AGAINST CYTOTOXICITY ON HELA TUMOR CELLS

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**ABSTRACT**

Hydroid *Aglaophenia cupressina* Lamouroux is a marine invertebrate animal from living *Phylum coelenterata* attached to a sponge, rich in chemical compounds such as alkaloids, steroids, terpenoids, histamine which can be used as medicinal ingredients. The aim of this study was to find hydroid extract *Aglaophenia cupressina* Lamouroux which has cytotoxic properties against Hela tumor cells. The hydroid extract of *Aglaophenia cupressina* Lamouroux was obtained from multilevel maceration results in accordance with the polar gradient, to obtain chloroform extract, and ethyl acetate extract, and methanol extract. Cytotoxic testing for the three extracts using the MTT ({3- (4,5-dimethylthiazol-2yl) -2,5diphenyltetrazodium bromide} method assayed against Hela tumor cells with a concentration of 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL. From the results of this study found 3 extracts of hydroids that have $IC_{50}$ values that differ in toxicity to Hela tumor cells. Chloroform extract has $IC_{50}$ value = 12.79 µg/mL ethyl acetate extract with $IC_{50}$ value = 10.52 µg/mL and methanol extract has $IC_{50}$ value = 14.09 µg/mL, from the three extracts showed very high toxicity to cells. Although ethyl acetate extract showed the highest toxicity among the two other extracts, the $IC_{50}$ value of the three extracts was ≤ 20 µg/mL in the category of very toxic. Based on these results, it was concluded that extracts from *Aglaophenia cupressina* Lamouroux hydroids had cytotoxic effects on Hela tumors so that they had the potential to be developed as basic ingredients for anticancer drugs.

**Keywords:** *Aglaophenia cupressina* Lamouroux, cytotoxic, HeLa tumor cell, hydroid, $IC_{50}$, solvent

## INTRODUCTION

Cervical cancer is the cause of death in women in the third place in the world. The most common types of cancer in women are breast cancer (43.3%) and cervical cancer (14%)[22]. According to the American Cancer Society in 2014 an estimated 4,020 deaths from cervical cancer. As an effort to treat the disease various therapeutic treatment strategies have been carried out using surgical therapy, radiotherapy, and chemotherapy as well as a combination of the three[15], but until now there has been no type of drug that gives satisfactory results without adverse side effects. Some cancer treatment therapies are effective at certain periods of time and are damaging to all cells including normal cells. Alternative cancer treatments can be done by utilizing compounds contained in natural ingredients. The search for cancer drugs is now increasingly interesting with the higher movement back to nature, through the use of phytopharmaca / biopharmaca, by exploring the potential chemical constituents of living organisms to be used as basic ingredients of drugs[14].

Biodiversity of Indonesian marine waters provide an opportunity to utilize marine biota in the search for new bioactive compounds, one of which is *Aglaophenia cupressina* Lamouroux hydroid. Hydroid is a marine invertebrate animal from the *Phylum coelenterata*, has a unique shape like ferns, and is widely distributed around the Spermonde Islands. Hydroid *Aglaophenia cupressina* Lamouroux lives attached to the sponge, is rich in chemical compounds such as alkaloids, steroids, terpenoids, histamine which can be used as medicinal ingredients[17]. The results of Johannes et al[9] study showed that *Aglaophenia cupressina* Lamouroux hydroid had a very high bioactivity with $IC_{50}$ value = 29.54 µg/mL, through a toxicity test against *Artemia salina* using the BSLT (*Brine Shrimp Lethality Test*) method which was the initial test or introduction to knowing the potential of an extract or compound can be used as an anticancer drug. Based on this, further research was conducted by Johannes et al[10] on the toxicity of methanol extract from hydroids on Hela cells which showed very active results with $LC_{50}$ values = 13.91 ppm, meaning that it could cause mortality of 50% of the population of tumor cells in concentration ≤ 30 ppm, so it has the opportunity to do further research as an alternative material that can be developed as a candidate for anticancer drugs.

This study used 3 solvents namely methanol, chloroform, and ethyl acetate, through multilevel maceration tested on Hela tumor cells. The use of HeLa cells as a model for cervical cancer cells because HeLa Cells are continuous cell lines infected by Human Papilloma Virus (HPV), which have the p53 and p105Rb genes in the wild type. Excessive proliferation activity in HeLa cells is caused by the bond between the E6 protein that binds to the p53 gene so that it accelerates the degradation of p53 and stimulation of the activity of the telomerase enzyme. E7 protein plays a role in increasing cell proliferation activity through hyperphosphorylation of p105Rb[20,21].

## MATERIALS AND METHODS

The material used in this study was Hydroid *Aglaophenia cupressina* Lamouroux obtained from the island of Samalona, South Sulawesi. Maceration and extraction of samples using experimental methods. The subjects used in this study were HeLa cervical cancer cells obtained from the Center for Development and Research of Stem Cell Airlangga University Surabaya using the MTT ({3- (4,5-dimethylthiazol-2yl) -2,5diphenyltetrazodium bromide}) method.

### Preparation of Methanol, Ethylacetate and Chloroform Extracts

Hydroid is washed and cleaned from the dirt, then dried and cut into pieces, weighed as much as 3 kg, macerated with chloroform (1: 2) for 24 hours at room temperature, repeated 3 times. The chloroform extract obtained was evaporated using a rotary evaporator, obtained as much thick matter (42.7 g). The remaining samples were then dried and re-macerated using ethylacetate (1: 2) solvent for 24 hours at room temperature, then carried out in the same way as with chloroform solvents. Ethyl acetate extract was obtained (37.8 g). The remaining samples are dried and then re-macerated with methanol (1: 2) for 24 hours at room temperature. Then carried out in the same way as with chloroform and ethyl acetate solvents. Obtained as thick as 60.6 g.

### Preparation of Extract Concentration Series

Each extract of chloroform, ethylacetate, and methanol, weighed 5.0 mg and dissolved in Dimethyl sulfoxide (DMSO) as much as 100 µL, to obtain a solution with a concentration of 50,000 µg/mL, then diluted with RPMI medium into six concentrations (5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL).

### Cytotoxic test with the (MTT) [3-(4,5dimethylthiazol-2-il) -2,5-dipheniltetrazolium bromide] method

Using 96 microplate wells. Each well was filled with 100 µL RPMI 1640 medium, 0.5% FBS containing HeLa cells with a density of 2-4 x 103 cell/ well, then incubated for 24 hours. The next day the media was discarded and replaced with a series of sample doses (0.24 to 500) µg / mL in RPMI 1640 media, 10% FBS. Each dose is made triplicate. The cell culture was incubated for 24 hours at 37 °C, 5% $CO_2$. After 24 hours MTT solution was added (5 mg/ml PBS) (10ηL / well). Cell culture was incubated for 4 hours at 37 °C, 5% $CO_2$. A solution stop (100 mL/well) was added to the cells incubated overnight at 37 °C, % $CO_2$, then optical density was measured at λ 540nm with ELISA plate reader.

### Percentage calculation of cell death

To find out the percentage of inhibition of Hela cell proliferation, it is calculated using the following formula:

$$\% \text{ cell viability} = \frac{A-B}{C-B} \times 100\%$$

Information
A = Absorbance of cells with treatment
B = Absorbance of media controls
C = Absorbance of cell control
The percentage of inhibition cell proliferation was processed using linear regression analysis to obtain $IC_{50}$ values. An extract is declared active or has the potential as an anti-cancer if the $IC_{50}$ value is ≤ 20 µg/mL[3].

### Data analysis

Data obtained in the form of absorbance of each well converted into percent of living cells and analyzed statistically, using a correlation test method followed by a significance test to determine the significance of differences between the control group and the treatment group. Then the $IC_{50}$ concentration was calculated using the log probit method to obtain linearity between the log of concentration and the percent of living cells.

## RESULTS AND DISCUSSION

According to Norlia[16] types of solvents affect the effectiveness of the extraction process. It can be seen from the average cell death in Table 1. It shows the percentage of different deaths between chloroform solvents which are non polar, ethylacetate solvents which are semipolar and methanol which are also semipolar but the polarity is higher than ethyl acetate. In general, the higher the concentration given, the higher the percentage of cell death.

**Table 1: Mean percentage of cell death after 24 hours incubation from chloroform, ethyl acetate, and methanol extracts**

| Average of % cell deaths | | | |
|---|---|---|---|
| Concentration (µg/mL) | Chloroform Extract | Ethylacetate Extract | Methanol Extract |
| **5** | **14,26** | **29,98** | **8,43** |
| **10** | **39,16** | **41,37** | **31,24** |
| **15** | **51,26** | **52,93** | **48,57** |
| **20** | **74,58** | **67,81** | **67,38** |
| **25** | **81,87** | **93,14** | **74,61** |
| **30** | **94,35** | **96,44** | **85,66** |

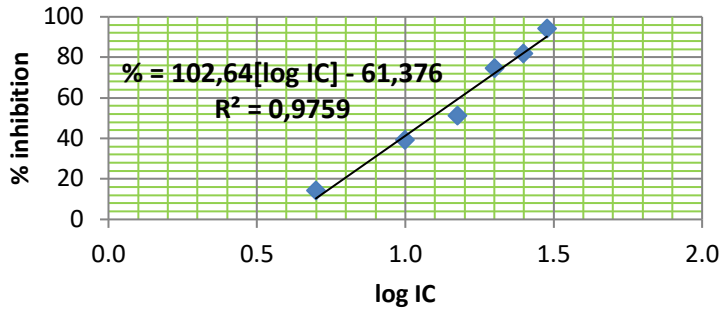


**Figure 1: Regression analysis of the relationship between the mean percentage of inhibition of the logarithm of inhibitory concentration (log IC) of chloroform extract, correlation coefficient R² = 0.9759, IC50 value = 12.79 µg / mL.**

From the $IC_{50}$ results showed chloroform solvents have very high cytotoxic effects on Hela cells, according to the statement of Benzivin et al[3] the requirements of a compound as anticancer have an $IC_{50}$ value <20 µg/mL. Chloroform is a non-polar solvent that can attract non-polar compounds. The selection of solvents in the maceration process will provide high effectiveness on the solubility of natural ingredients in the solvent[1,7]. The selection of solvents in the maceration process will provide high effectiveness on the solubility of natural ingredients in the solvent[1,7]. This is in accordance with Bahrami et al[3], to free compounds that are non-polar fat, terpene, chlorophyll, xanthophyll and others by extraction using hexane or chloroform. This is in accordance with Bahrami et al[2] to free compounds that are non-polar fat, terpene, chlorophyll, xanthophyll and others by extraction using hexane or chloroform. Johannes[9] states that hexadecanoic acid is a group of carboxylic acids which have very high toxicity ($LC_{50}$) = 29.54 µg / mL in *Artemia salina*, Ravi Lokesh and Kannabiran Krishnan[18] states that fatty acid derivatives such as hexadecanoic acid act as anticancer compounds.

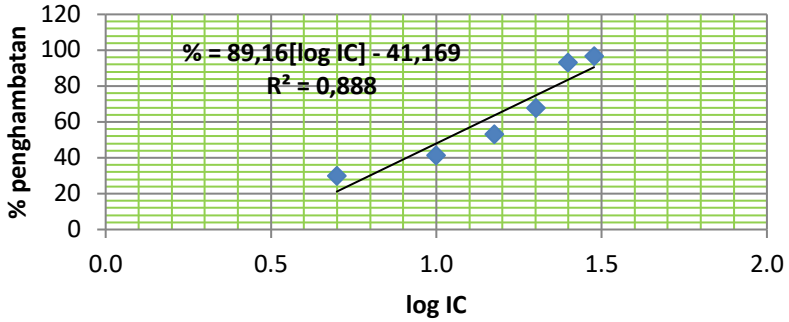


**Figure 2: Regression analysis relates the mean percentage inhibition to the logarithm of inhibitory concentration (IC log) of ethylacetate extract. The correlation coefficient R² = 0.888, IC50 value = 10.52 µg / mL.**

This data shows that ethyl acetate extract is cytotoxic to Hela cells. Ethyl acetate solvents are semipolar solvents which can attract polar and non-polar compounds. The compounds found in the ethyl acetate extract were able to inhibit 50% of Hela cells so as to provide strong results in inhibiting death in Hela cells. According to Kristanti Handriani[11] one of the semipolar compounds is alkaloid, because it has a nitrogen base in its cyclic chain and contains various substituents, so it is suspected that the compound is in ethyl acetate extract. In accordance with Johannes et al[9], hydroid *Aglaophenia cupressina* Lamouroux contains alkaloids. According to Isah Tasiu[8] alkaloids are used as antitumor drugs because they are able to induce apoptosis through their association with DNA. Although the polarity level is lower than methanol.

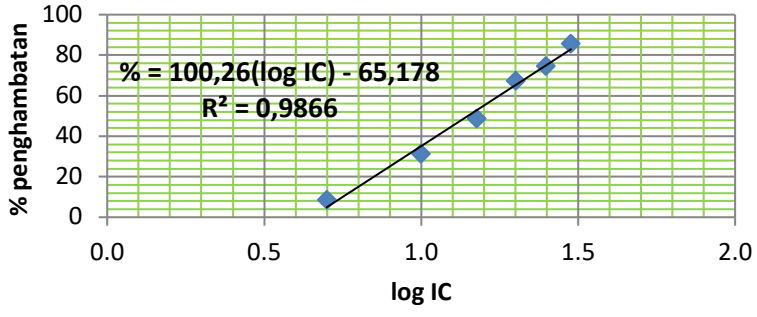


**Figure 3: Regression analysis relates the average inhibition percentage to the logarithm of inhibitory concentration (IC log) of methanol Extract. correlation coefficient R² = 0.9866, $IC_{50}$ value = 14.09 µg / mL.**

Data of $IC_{50}$ methanol extract = 14.09 µg/mL showed active properties in inhibiting Hela tumor cells or cytotoxic to Hela cells. Methanol is a solvent that can dissolve almost all organic compounds both polar and non polar because methanol has a polar group (-OH) and nonpolar group (-CH3). Methanol contains active compounds that can inhibit Hela tumor cells. The results of Johannes et al[10] showed that methanol extract from *Aglaophenia cupressina* Lamouroux had cytotoxic activity against *Artemia salina* cells and Hela tumor cells with an $LC_{50}$ value of 19.70 ppm and 13.91 ppm, which shows methanol extract can kill 50% of line hela cells.

**Cytotoxic Test on Hela Tumor Cells by [3-(4,5dimethylthiazol-2-il)-2,5-dipheniltetrazolium bromide] (MTT) method**

The cytotoxic parameters used are the ability to convert MTT substrates to purple formazan by succinate dehydrogenase enzymes in living cells. Testing of cytotoxic effects carried out with each concentration was tested in three wells to avoid refractive in this study. Cytotoxic properties are a major step in efforts to discover new anticancer drugs from natural ingredients[4]. According to the National Cancer Institute (NCI) criteria a substance is said to be cytotoxic if it has an $IC_{50}$ value of less than 20 µg/mL[3]. Thus, chloroform extract ($IC_{50}$ = 12.79 µg/mL), ethyl acetate extract ($IC_{50}$ = 10.52 µg/mL), and methanol extract ($IC_{50}$ = 14.09 µg/mL) has cytotoxic ability against Hela tumor cells. In Figure 4, A = cell control, it appears that many living cells are marked with a dense purple color, whereas in Figure B the treatment control shows the higher the concentration of extracts of the test compound (30 µg/mL) the less the number of living cells marked by not a little purple is formed.

The MTT method involves the pyridine nucleotide of the NADH co-factor which is only catalyzed by living cells, the more cells living, the more formazan crystals formed [6,19]. The formation of color in the cell is caused by the ability of mitochondrial reductase enzyme to reduce salt / methylthiazol tetrazolium. When metabolism progresses, living cells will produce mitochondrial reductase enzymes. This enzyme reacts with MTT (3- (4,5-dimethylthiazol-2-yl) 2,5diphenyl-tetrazolium bromide) and forms purple cryztal formazan[13]. Morphological changes in cells as a result of exposure to active compounds or certain chemotherapy agents is a reflection of biochemical conditions that can cause apoptosis or necrosis[5,12].

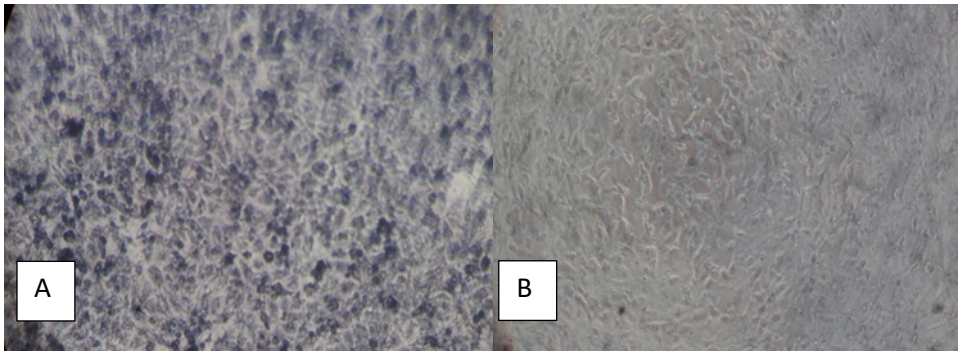


**Figure 4: HeLa cell morphology was observed under a microscope 100x magnification. images taken with Canon Lexus 100 camera. (A) Morphology of HeLa cells in the control group after 24 hours (B) Morphology of HeLa cells in the treatment group 30 µg/mL.**

**Recapitulation of $IC_{50}$ analysis and calculation results**

Ethyl acetate extract has the smallest $IC_{50}$ value, which means that this extract is potentially the most toxic than the other two types of extract. This indicates that the compounds contained in the ethylacetate extract are more active in inhibiting Hela tumor cell growth compared to chloroform and methanol extracts.

**Table 2: Comparison of $IC_{50}$ values of chloroform extract, ethylacetate extract and methanol extract**

| Extract | $IC_{50}$ µg/mL |
|---|---|
| Methanol | 14,09 |
| Chloroform | 12,79 |
| Ethylacetate | 10,52 |

Table 2 shows that the three extracts: chloroform, ethylacetate, and methanol have active properties in inhibiting 50% of Hela tumor cells. From the $IC_{50}$ value, ethyl acetate extract is more active compared to chloroform and methanol extract.

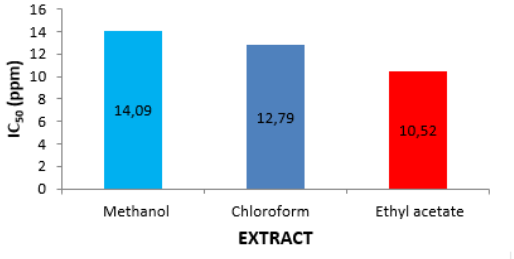


**Figure 5: Comparison of $IC_{50}$ values from methanol, chloroform, and ethyl acetate extracts**

## CONCLUSION

Chloroform, ethylacetate, and methanol extracts from *Aglophenia cupressina* Lamouroux hydroid have active cytotoxic effect on Hela tumor cells and the most toxic is ethyl acetate extract.

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