Cytotoxic Activity of Ethyl Acetate Fraction Of Ethanolic Extract Green Algae (Ulva LACTUCA Linn.) On Mcf-7 Cells

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ABSTRACT

Background: Based on data from the GLOBOCAN International Agency for Research on Cancer (IARC), in 2012 there were ± 14 million new cases of cancer and ± 8 million deaths from cancer worldwide. Various treatments were taken to overcome this type of cancer. On of the treatment for breast cancer is herbal medicine. The herbal medicine that are currently being discussed are plants that come from the sea (marine biota), one of which is green algae (Ulva lactuca Linn.). It is not yet known whether the ethanolic extract form and extract fraction also has antioxidant and cytotoxic abilities.

Objectives: Conducted a cytotoxic test of the ethyl acetate fraction of green algae extract (Ulva lactuca Linn.)

Methods: The method used is MTT Assay

Results: IC_{50} value of Ethyl acetate fraction of ethanolic Extracts of Green Algae is1489,3 μg/ml and Ascorbic acid is 13,99 μg/ml

Conclusions: The Ethyl Acetate Fraction of Green Algae Extract (Ulva lactuca Linn.) Have an IC50 value greater than the standard (Doxorubicin).

Keywords: *Green Algae, Cytotoxic Test, MTT Assay*

INTRODUCTION

Based on data from the GLOBOCAN International Agency for Cancer Research (IARC), in 2012 there were ±14 million new cases of cancer and ± 8 million cancer deaths worldwide. Breast cancer is one type of new cases with a high percentage of 43.3% and is the highest cause of death from cancer (1). Various kinds of treatment are done to overcome this type of cancer. On of the treatment for breast cancer is herbal medicine. The herbal medicine that are currently being discussed are plants that come from the sea (marine biota), one of which is green algae (Ulva lactuca Linn.). Plants that are currently being discussed are plants that come from the sea (marine biota), one of which is green algae (Ulva lactuca Linn.). Research conducted by Thanh et al (2) shows that green algae water extract has anti-cancer activity. From the results of cytotoxic tests on hepatocellular cell carcinoma (IC50 29.67 ± 2.87 μg / ml), human breast cancer (IC50 25.09 ± 1.36 μg / ml) and cervical cancer (IC50 36.33 ± 3.84 μg / ml) (3). It is not yet known whether the ethanol extract form and extract fraction also have antioxidant and cytotoxic abilities.

MATERIALS AND METHODS

This research is a Quasi Pre-Post experimental research type experimental with Control Group. The material used in this study is green algae from the Special Region of Yogyakarta, MTT reagent, reagent for preliminary test. The equipment used are maceration vessel, rotary evaporator, buchner funnel, electric balance, porcelain cup, separating funnel, filter paper, oven, flakon, glassware, UV-Vis spectrophotometer, wellplate.

1. **Preparation of Extract**

   Extraction Of Green algae (Ulva lactuca Linn.)

   Extraction is done by immersion method. 250 grams of green algae powder was added with 96% ethanol as much as 1 L, stirring for 3 hours. Furthermore, soaking is continued for up to 24 hours. After that the maserat is evaporated using a rotary evaporator at 40°C until a thick ethanol extract is obtained. Maceration process is repeated 2 times.

   Fraksinasi and Purifikasi Ekstrak etanolik ganggang Hijau

   Ethanolic extract obtained from the results of evaporation, then carried out fractionation and purification. The method used follows the research conducted by Srijanto et al. (4) by the liquid-liquid extraction technique. Ethyl acetate with a certain volume is put into the erlenmeyer flask and then heated at 35°C. A total of 500 ml of ethanolic extract of green algae is taken and put into an erlenmeyer flask containing ethyl acetate for purification. Extraction is done 3 times. In this process, 100 ml of mineral-free water is added to clarify the two-phase separation process. After that the separation is done by using a separating funnel. The ethyl acetate phase obtained was then evaporated until a thick extract was obtained which was purified extract.

2. **Kultur Sel and Sitotoksik test**

   The cells that will be used in this study are MCF-7 cells. MCF-7 cells were cultured on DMEM high glucose (Gibco) or RPMI-1640 culture media containing 2mM L-glutamine, 1.5 g / L sodium bicarbonate, 4.5 g / L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate and Fetal Bovine Serum supplement 10%. Medium MCF-7 was added with 0.01 mg / ml bovine insulin. Cell subculture carried out for 3-5 days was incubated at 37°C, 5% CO2 and 100% humidity.

   Cytotoxic tests were carried out by the MTT 3- (4,5-dimethylthiazol-2-il) method -2,5-diphenyltetrazolium bromide (Sigma Aldrich, USA). Cell culture was added 10 μL MTT remand in PBS in each well. MCF-7 cells were incubated for 4 hours at 37°C (5% CO2 flow), then 50 μL SDS (Sodium Dodecyl Sulphate) solution was added in 0.01 N HCl and incubated for 1 night at room temperature. Then the absorbance reading on the ELISA reader [1; 2 0]. To determine the presence or absence of cytotoxic effects, a linear regression analysis was performed between the concentrations of the test compounds with% viability with Microsoft Excel to obtain IC50 values.

$$\%\ viability\ Cells = \frac{Absorbance\ of\ Sample - Absorbance\ of\ Medium\ Control}{Absorbance\ of\ Sel\ Control - Absorbance\ of\ Medium\ Control} x\ 100\%$$

RESULTS AND DISCUSSION

Cytotoxic tests were performed using the MTT-Assay method. MTT (3- [4,5-dimethylthiazol-2-yl] -2,5 diphenyl tetrazolium bromide) is the method of calculating cell viability by inserting it into a well (96-well plates) without the need for complex cell counts. The principle of the MTT test is based on the

conversion of MTT into formazan crystals in living cells that determine mitochondrial activity detected by Optical Density (OD) using a plate reader at 540 and 720 nm (5). MTT assay is suitable for measuring drug sensitivity in cell lines and primary cells. The decrease in cell count reflects the inhibition of cell growth and drug sensitivity which is usually determined as the concentration of the drug needed to achieve 50% growth inhibition compared to the control (IC_{50}) (5).

In this study sample treatments were given for 3-5 days then the MTT reagent was added. After the addition of the MTT reagent, it will form formazan crystals. Then the stopper reagent is added in the form of SDS. SDS functions to stop and degrade enzymes. After that the absorbance is measured using an ELISA reader. The principle of reading an ELISA (Enzyme-linked Immunosorbent Assay) reader is an analysis of interactions between antigens and antibodies that are passively adsorbed using conjugate antibodies or antigens assisted by enzymes. This enzyme will react with the substrate and produce color. The color that arises can be determined qualitatively by eye view or quantitatively by reading the absorbance value on the ELISA plate reader. The more formazan crystals that are formed, the more living cells (figure 2).

Table 1. Cytotoxic test results of ethyl acetate fraction of green algae extract compared with standards (doxorubicin)

Doxorubicin			Ethyl Acetate Fraction of Green Algae			
Concentration (µg/ml)	Viability of Cells (%)	IC_{50}	No.	Concentration (µg/ml)	Viability of Cells (%)	IC_{50}
200	0,70	13,99 µg/ml	1	2000	42,23	1489,3 µg/ml
100	2,09		2	1600	39,21	
50	18,51		3	1200	62,41	
25	46,17		4	800	73,55	
12,5	58,71		5	400	79,58	
6,25	68,47					
3,125	73,59					

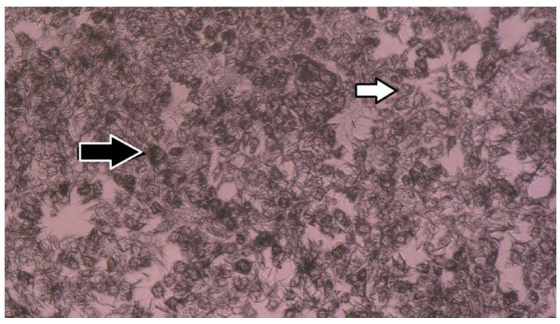
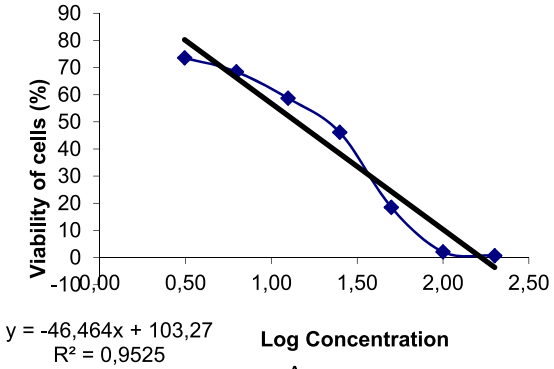


Figure 2. Formazan crystal formation in mcf-7 cancer cells

Cell is Dead

Cell is Live



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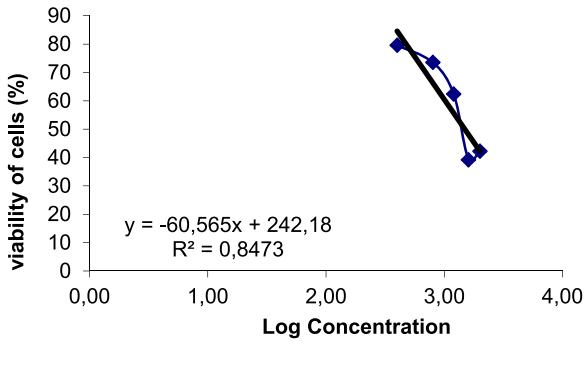

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Figure 3. Graphic of viability cells of mcf-7 (a) standard and (b) ethyl acetate fraction of ethanolic extract of green algae (ulva lactuca linn.)

Cytotoxic tests were carried out on MCF-7 cells. In the cytotoxic test the parameters used are IC50. IC50 or 50% inhibitory concentration is a parameter to determine the 50% inhibitory concentration of living cell populations. The concentration used in this study is 200; 100; 50; 25; 12.5; 6.25; 3,125 μg / mL. (Table 1).

From table 1 and figure 1 it can be seen that with an increase in the concentration of fractions and standards there is a decrease in the percentage of the viability of MCF-7 cells. Percentage of MCF-7 cell viability is the number of living cells in the treatment divided by the total number of cells (number of living cells plus the number of dead cells). The IC50 value of the ethanol extract of green algae (Ulva lactuca Linn) Was 1489.3 μg / ml and doxorubicin 13.99 μg / ml. According to Ueda et al. (6) a compound is said to be potential as an anticancer if the IC50 value is $\leq$ 100 μg / ml. From these results it can be said that the ethyl acetate fraction of green algae extract has no better activity compared to doxorubicin. This can be made possible by the use of concentrations of fractions that are too small so that the effect produced is also small. It is also possible that compounds which have anticancer activity are not present in the ethyl acetate fraction of ethanolic extracts of green algae (Ulva lactuca Linn.).

CONCLUSION AND RECOMMENDATION

The Ethyl Acetate Fraction of Green Algae Extract (Ulva lactuca Linn.) Has an IC50 value greater than the standard (Doxorubicin). Further research needs to be done on the cytotoxic test of ethyl acetate fraction of ethanolic extract of green algae (Ulva lactuca Linn.) By using higher concentrations and can also with other types of fractions.

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