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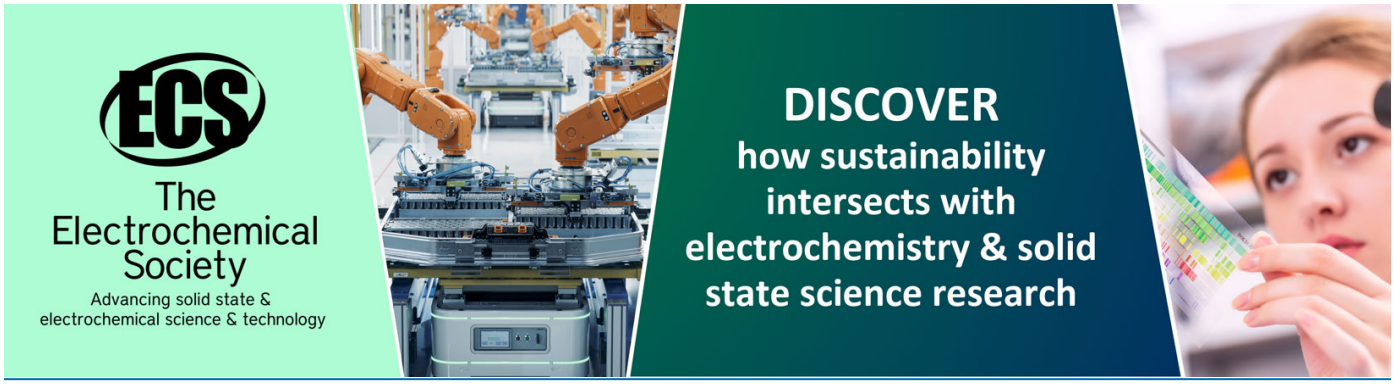

Cytotoxicity effect of nonpolar extract from parijoto (*medinilla speciosa reinw. ex. bl*) fruit against hela and widr cell line

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Abstract. Cisplatin is a chemotherapy agent in the treatment of cervical cancer. However, due to drug resistance and its side effects are much needed an agent that can be combined with cisplatin. Parijoto fruit (*Medinilla speciosa Reinw.ex.Bl*) have potential cytotoxic effect derived from natural ingredients. The study aims to determine the potential cytotoxic effects of ethyl acetate and n-hexane extract from Parijoto fruit calculated from the value of IC_{50} and the profile of these extract on normal cell. The determination of the cytotoxic effect of Parijoto fruit extract using MTT assay that the results can be read absorption by using ELISA reader. Data analysis is calculated by linear regression methods by using Microsoft Excel software. Results showed that ethyl acetate and n-hexane of parijoto fruit performed cytotoxic effect on HeLa cell line with IC_{50} respectively, 352,9 µg/mL; 904,7 µg/mL while the value of ethyl acetate and n-hexane of parijoto fruit performed cytotoxic effect on WiDr cell line with IC_{50} respectively, 554,9 µg/mL; 434,4 µg/mL. Data analysis showed that the cytotoxicity effect of nonpolar extract from parijoto fruit is include in the moderate cytotoxic category.

1. Introduction

Cancer is a disease with a high prevalence that is characterized by abnormal cell growth that is not controlled [1]. Cervix cancer and colon cancer are two types of cancer that are often suffered and become the cause of death worldwide. Based on data from the World Health Organization (2018) [2], the number of cases of colon cancer in the world occurs as many as 1.8 million cases (10, 8% of total cancer cases. A total of 30.017 new cases of colon cancer occurred in Indonesia. GLOBOCAN (IARC) (2013) [3], estimates that cervical cancer has the second highest percentage of new cases experienced by women in the world by 14%. Treatment methods such as surgery, radiotherapy, chemotherapy and the use of drugs have been carried out to treat cancer. Chemotherapy has quite serious side effects including nausea, vomiting, hair loss, swelling, sores in the mouth and throat, drastic weight loss and memory disorders. Radiotherapy and surgery have local work effects that work only on the affected part of the cancer, but not all sufferers can take the treatment because the costs are greater. One of the chemotherapy agents is cisplatin. However, most colon cancer patients experience a relapse and resistance to cisplatin [4].

One plant that has potential as a chemopreventive agent is parijoto (*Medinilla speciosa*). Based on research conducted by [5], ethanol extract of parijoto fruit showed moderate cytotoxicity to T47D breast cancer cells with IC_{50} values of 614.50 µg/mL. Parijoto fruit contains chemical compounds such as flavonoids, saponins, tannins and glycosides. There are no studies that show the cytotoxic activity of parijoto against colon cancer cells and cervical cancer. Therefore this study will examine the cytotoxic potential of nonpolar extracts of parijoto fruit against cervical cancer and colon cancer using the HeLa and WiDr cell lines with the MTT assay method.

2. Experimental

2.1 Sampel preparation

Parijoto (Medinilla speciosa) plants were taken from Colo Village, Dawe District, Kudus Regency, Central Java. The collected parijoto is sorted and washed. Thus fresh parijoto fruit is weighed as much as 500 grams, blended and each soaked in 500 mL ethyl acetate and n-hexane solvents. Maceration is done for 24 hours. Separate maserat by filtration. Perform remaceration using the same solvent of 250 mL each. All maserates that have been collected are evaporated using a rotary evaporator until a thick extract is obtained. Parijoto fruit extracts dissolved in DMSO made a series of concentrations ranging from 30-240 µg/mL for cytotoxic testing of HeLa and WiDr cells.

2.2 Cell culture and MTT assay

WiDr cells and Hela cells are taken from liquid nitrogen tanks, then immediately thawed and grown on a tissue culture dish. Both of cells (10^4 / well) were cultured to 96well-plates in the RPMI MK media, then incubated at 37 ^oC for 24 hours. Confluent cells are transferred to a new sterile conical tube. Cells were counted by haemocytometer and cell counter then cell suspension was made with a cell density of $1x10^4$ per 100 µL. Then the extract and cisplatin were added in DMSO, incubated in a 5% CO_2 incubator for 24 hours. MTT 0.5 mg/mL in 100 µL of culture media was added to each well, incubated for 4 hours at 37 °C. A 10% SDS stopper in 0.01 N HCl was added and incubated at room temperature for 24 hours and protected by light. Next, absorbance measurements with ELISA reader at a wavelength of 595 nm.

2.3. Statistical analysis

The results of cell viability versus sample concentration are made linear regression equation graphs so that the obtained equation function $y = bx + a$ then the IC_{50} value calculation is obtained. It was analyzed statistically by using Microsoft Excell and statistical significance was estimated by using ANOVA test. Statistical significance was placed at $p<0.05$.

3 Result and Discussion

3.1 Effect of Nonpolar Extract from Parijoto (Medinilla speciosa Reinw. ex. Bl) Fruit Against Hela Cells growth

The cytotoxic effect test on HeLa cells is a preliminary test using culture of HeLa cells in 96-well plates with the MTT assay method whose absorption results will give a purple color and read the absorbance using the ELISA reader. The absorbance results are used to calculate the IC_{50} (Inhibition Concentration) value, which is the ability of a compound that can cause growth inhibition in 50% of the cell population. IC_{50} values obtained in the treatment of ethyl acetate extract and n hexane parijoto extract were 352.9 µg/mL and 904.7 µg/mL, respectively. While the IC_{50} value of cisplatin in HeLa cells was 12.08 µg/mL. The% viability results of extracted cells and are presented in Figure 1.

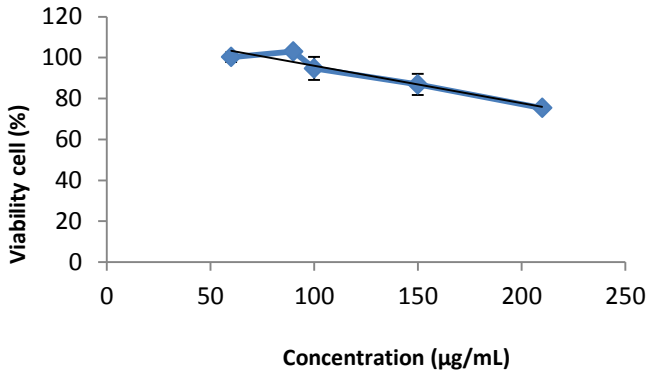

Figure 1. Linear regression equation between extract concentration versus % cell viability treatment of ethyl acetate extract of parijoto fruit with a concentration series of 60; 90; 120; 150; 210 µg/mL with respect to HeLa cell.

Based on the linear regression graph the function equation is obtained: y = -0,1856x + 115.41 with the value R^2 amounted to 0.92. IC_{50} value which is obtained at 353.5 µg/mL. Cytotoxic effects of the treatment of parijoto ethyl acetate extract are categorized as cytotoxic moderate so that it can be used as a chemopreventive agent [6]. The morphological profile of HeLa cell growth in the treatment of parijoto ethyl acetate extract is shown in Figure 2.

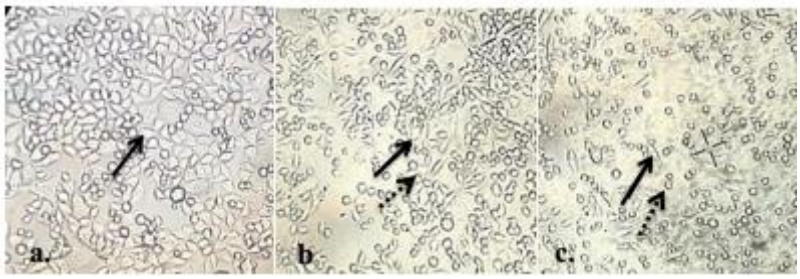

Figure 2. Effect of treatment of parijoto ethyl acetate extract on HeLa cells (1 x 104 cell / well) after 24 hours with 100x magnification, using RPMI media in 96well-plate shows decreased viability of HeLa cells which is directly proportional to increase extract concentration. Note: a) cell control b) parijoto ethyl acetate extract concentration of 150 µg/mL c) parijoto ethyl acetate extract concentration of 210 µg/mL. ⟶ living cell, ⋯⋯> dead cell.

Meanwhile, the treatment of hexane parijoto extract obtained a percentage relationship graph Cell viability versus extract concentration is shown in Figure 3.

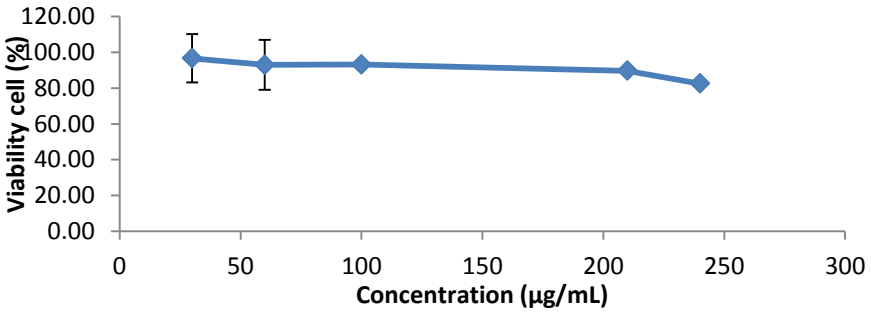

Figure 3. Linear regression equation between extract concentration versus % cell viability hexane parijoto extract treatment with a concentration series of 30; 60; 120; 210; 240 µg/mL with respect to HeLa cell.

Parijoto n-hexane extract treatment on HeLa cells obtained by the function equation y = -0.0528x + 97.975 with the value of R^2 amounting to 0.8125. Meanwhile, the IC_{50} value obtained at 908.6 µg/mL with effect moderate cytotoxics. The morphological profile HeLa cells after heksana extract treatment are shown in Figure 4

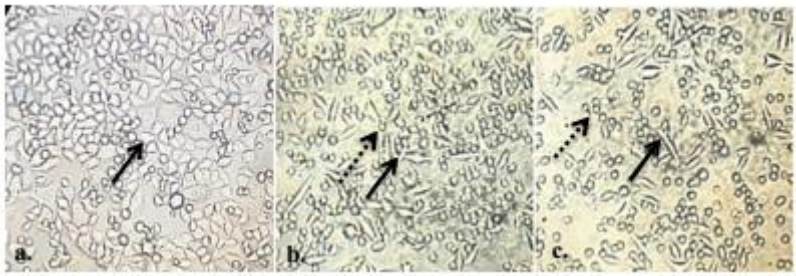

Figure 4. Effect of treatment of hexane parijoto extract on HeLa cells (1 $x10^4$ cell / well) after 24 hours with 100x magnification using RPMI media in 96well-plate shows decreased viability of HeLa cells which is directly proportional to increase extract concentration. Information: a) cell control b) hexane extract parijoto concentration 210 µg/mL c) hexane parijoto extract concentration of 240 µg/mL. → living cell, ⇢ dead cell

3.2. Effect of Nonpolar Extract from Parijoto (Medinilla speciosa Reinw. ex. Bl) Fruit Against WiDr Cells growth

WiDr cells are human colon cancer cells isolated from the colon of a 78-year-old woman. WiDr cells are other colon cancer cells derived from HT-29 cells [7]. WiDr cells produce carcinoembrionic antigens and require a span of about 15 hours to complete 1 cell cycle. One of the characteristics of these WiDr cells is the high expression of cyclooxygenase-2 (COX-2) which stimulates the proliferation of WiDr cells [8]. WiDr is a cell that has a low sensitivity to treatment with 5-fluorouracil (5-FU), an antimetabolite class of chemotherapy agents. Transfection of WiDr with normal p53 did not cause an increase in sensitivity to 5-FU [9]. WiDr cell resistance to 5-FU is mediated by an increase in the expression of the thymidylate synthetase enzyme which is the main target of inhibition of 5-FU [10]. P-glycoprotein (Pgp) in WiDr cells is not expressed so high that there may be other mechanisms that mediate WiDr resistance to 5-FU [11]. The administration of n-hexane and ethyl acetate extracts showed changes in cell morphology that could be observed in the results of the morphology of WiDr cells (Figure 5).

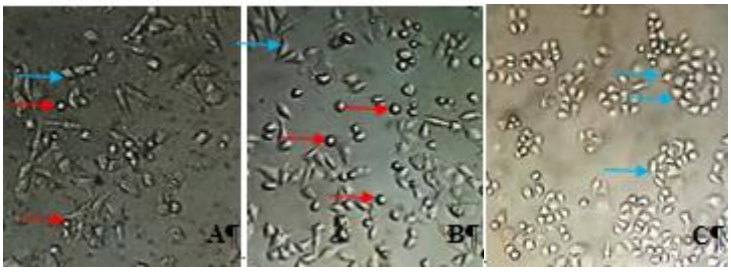

Figure 5. The morphology of WiDr cells was observed under an inverted microxcope at 100x magnification. Note: (A) Concentration of ethyl acetate extract 350 µg/mL (B) Concentration of n-hexane extract 400 µg/mL; (C) Cell control → living cell → dead cell

The principle of the MTT method is the reduction of yellow tetrazolium MTT salt (3- (4,5-dimethyltiazol-2-il) -2,5-diphenyl tetrazolium bromide) by the reductase system. Mitochondrial reductase enzymes in cells react with MTT to form purple formazan crystals. The intensity of the purple color that is formed is proportional to the number of living cells, so the more the number of living cells, the greater the intensity of the purple color and when the number of living cells is less

then the intensity of the purple color fades. Formazan crystals that form are impermeable to the cell membrane and insoluble in water. Changes in the stopper reagent will dissolve formazan crystals so that absorbance can be measured using an ELISA reader at a wavelength of 490 to 570 nm. The absorbance value indicates the level of cell proliferation as a manifestation of the level of cell immunity [12]. The results obtained from the cytotoxicity test in the form of IC_{50} value which shows the concentration value of a compound that results in inhibition of cell proliferation by 50% and also the potential for the oxidation of a compound to cells (Figure 6)

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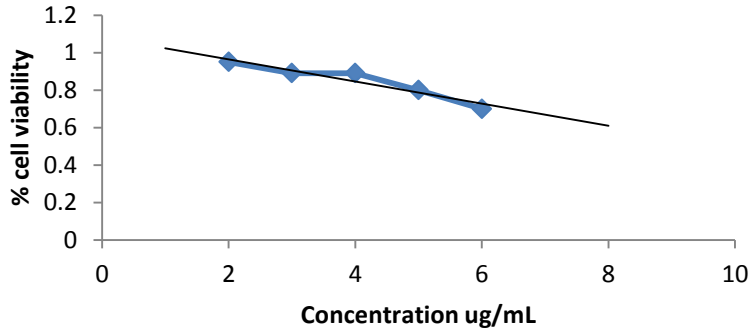

Figure 6. Linear regression equation between extract concentration versus % cell viability treatment of ethyl acetate extract of parijoto fruit with a concentration series of 60; 90; 120; 150; 210 µg/mL with respect to WiDr cell line.

The data used are in the form of 5 concentration series which are then performed linear regression calculations so that a linear equation y = -0.0995x + 105.49 is obtained with a value of r = 0.9602. IC_{50} value calculations are determined using linear regression (y = -0.0995x + 105.49). Based on the results of the linear regression, the IC_{50} value of the parijoto methanol extract was 554.9 µg/mL.

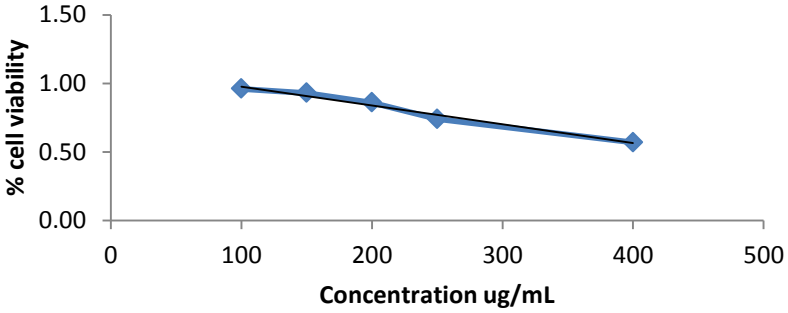

Figure 7. Linear regression equation between extract concentration versus % cell viability hexane parijoto extract treatment with a concentration series of 30; 60; 120; 210; 240 µg/mL with respect to WiDr cell line

The data used are in the form of 5 concentration series which are then performed linear regression calculations to obtain a linear equation y = -0.1372x + 111.38 with a value of r = 0.9788. IC_{50} value calculations are determined using linear regression (y = -0.1372x + 111.38). Based on the results of linear regression, the IC_{50} value of parijoto methanol extract was 434.43 µg/mL. The greater the IC_{50} value of a compound, the more toxic it is because it requires a large concentration in order to inhibit cell growth, conversely the smaller the IC_{50} value, the greater the potential for oxidation to cells because the required concentration is small. Cytotoxicity tests can provide information about the concentration of a compound that still allows cells to survive [13]. The results of a single cytotoxic test of the three parijoto fruit extracts showed that both extracts had moderate cytotoxic effects on

HeLa cells and WiDr cells. A compound that has an IC_{50} value <100 μg/mL is a compound with a potential cytotoxic effect, a compound that has an IC_{50} value of 100-1000 μg/mL can be said to be a compound with a moderate cytotoxic effect. The compound has no cytotoxic effect if the IC_{50} value> 1000 μg/mL [14].

4 Conclusion

Based on a single cytotoxic test IC_{50} values were obtained that ethyl acetate and n-hexane of parijoto fruit performed cytotoxic effect on HeLa cell line with IC_{50} respectively, 352.9 μg/mL; 904.7 μg/mL while the value of ethyl acetate and n-hexane of parijoto fruit performed cytotoxic effect on WiDr cell line with IC_{50} respectively, 554.9 μg/mL; 434.4 μg/mL. Data analysis showed that the cytotoxicity effect of nonpolar extract from parijoto fruit is include in the moderate cytotoxic category on Hela and WiDr cell line.

Acknowledgment

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