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Cytotoxicity of "Ekor naga" Leaf (*Rhaphidophora pinnata* (Lf) Schott) Chloroform Extract against T47D Cancer cells

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Abstract: Cancer is the second disease cause of mortality in USA, after cardiovascular diseases. Nowadays, the current research tend to develop anticancer agents from plants. One of the plants that can be used for anticancer therapy is the "ekor naga" leaves (*Rhaphidophora pinnata* (Lf) Schott), which is empirically used by some communities as breast cancer treatment.

The extraction of the dried leaf was done by soxletation with n-hexane, chloroform, ethyl acetate, and ethanol solvents. The cytotoxic activity was performed using T47D and Vero cell by MTT assay (tetrazolium salt { (3- (4,5-dimetiltiazol-2-yl) -2.5-diphenyl tetrazolium bromid}) method, and to determine the value of the Selectivity Index (SI).

The results of cytotoxicity test of n-hexane, chloroform, ethyl acetate, and ethanol extracts showed IC_{50} values of 422.921, 69.067, 475.448, 10185.896 μg/mL, respectively. The chloroform extract had IC_{50} values ≤ 100 μg/mL which had the anticancer activity on T47D cells. The result of extract IC_{50} selectivity index value of cancer cells againsted normal cells, showed that the chloroform extract had a high selectivity with value of the SI was 4.25, againsted T47D cancer cells.

Keyword: "ekor naga" leaf. T47D cell, Vero cell, *Rhaphidophora pinnata*.

Introduction

Indonesia is a rich country with diverse traditional medicinal plants that have anticancer activity. Among these plants is the leaves of ekor naga plant (*Rhaphidophora pinnata* (Lf) Schott), which has been used empirically by the Community for the treatment of several diseases, especially breast cancer. In the development of traditional medicine, it is needed a lot of researches to get accurate results of the efficacy of the plants.

The "Ekor naga" leaf is a plant that has been widely studied for its cytotoxic effects and can inhibit the growth of MCF-7 cancer cells. It contains chemical class of compounds such as saponin, tannin, alkaloids, steroid/triterpenoid, flavonoids, and glycoside, which play a role in the inhibition of cancer cells. Some studies of "ekor naga" leaf included: antibacterial activity of some extracts with different polarity against some bacteria (Kadriani[1] and Masfria[2] ,2009; 2013), the test of toxicity from ethanol extract (*crude*) and extracts (n-hexane, ethyl acetate and ethanol) by using *Brine Shrimp Lethality* Test (BST) method showed a cytotoxic result to *Artemia salina* Leach (Masfria[3], et al., 2011), and antioxidant activity test against DPPH (1,1-diphenyl-2-picrylhidrazyl) from n-hexane, ethyl acetate and ethanol extracts (Masfria[4], et al., 2012). It suggested that the cytotoxic test method using BST and the antioxidant properties positively correlated with anticancer activity (Silalahi[5], 2006).

Based on the above statement it is necessary to proceed cytotoxicity assay of the "ekor naga" leaf (*Rhaphidophora pinnata* (Lf) Schott) extracts with different polarity of solvent such as n-hexane, chloroform, ethyl acetate, ethanol and selectivity index, which can be developed as a chemopreventive drug against cell T47D breast cancer cell.

Methodology

Preparation of "Ekor Naga Leave Extracts

A total of 10 parts of dried powder leaves extracted by soxcletation. Dried powder leaves were inserted to the soxcletation tool using n-hexane, chloroform, ethyl acetate, and ethanol solvents. The extraction was stopped when the solvent in tubes had been clear. The obtained extract was evaporated to get a thick extract and dried using a freez dryer. (Ditjen POM[6], 1979).

Cytotoxic Test by MTT assay

The T47D cells were seeded in to 96 wells microplate in order to obtain the density of 1x 10^4 cells/well and incubated for 24 hours to get a good growth. After that, the medium was replaced with a new one and then 100 µL of test solution in series of concentrations of 250, 125, 62.5, 31.25, 15.625 ug/mL was added, and incubated for 24 hours again. At the end of incubation, the medium was discarded and the cells were washed with PBS solution, then each of the wells was added with 0.5% MTT (10 µL reagent 3- (4,5-dimetiltiazol-2-yl) -2.5-diphenyl tetrazolium bromide) in PBS solution. Incubation was continued for 4 hours at 37°C to form formazan. The living cells would converted MTT to the dark blue formazan. MTT reaction was stopped by 100 µL of the stopper reagent (Sodium dodecyl sulphate = SDS), and then incubated overnight at room temperature. MTT assay was performed according to Mossman[7] (1983) with the modification of stopper reagent.

Selectivity index

To determine the value of the Selectivity Index (SI), it was necessary to know the value of vero and T47D cells IC_{50} using MTT method.

Selectivity index was calculated using the following equation:

$$\text{Selectivity Index (SI)} = \frac{IC50 \ \text{Vero cell}}{IC50 \ \text{T47D cell}}$$

SI indicated the cytotoxic selectivity (security) of the extract against cancer cells *versus* normal cells, which was calculated by comparing IC_{50} of extract on normal cells and IC_{50} extract against cancer cells.

Analysis Data

The absorbance data obtained from the cytotoxic test was calculated by the following formula:

$$\% \text{ Living Cells} = \frac{Absorbance \ of \ treatment - Absorbance \ of \ control \ Media}{Absorbance \ of \ control \ cell - Absorbance \ of \ control \ Media} \ x \ 100 \ \%$$

IC_{50} concentrations were calculated by probit analysis using SPSS 17 program, from the linear correlation between log concentration and percentage of living cells. IC_{50} was the concentration that caused death of 50% of cell population.

Results and Discussion

Cytotoxic Activity

Cytotoxic activity test was conducted to determine the potential toxicity of extracts (n-hexane, chloroform, ethyl acetate, and ethanol) with IC_{50} parameter. The concentration of the test materials with the series of 15.625, 31.25, 62.5, 125, 250 µg/mL had a correlation between the concentration of the test solution with the resulting toxic effects.

The toxic effect increased with the increasing of concentration. The increase of toxic effects was characterized by the decrease of the living T47D cells percentage. The higher of the extract concentration, and the smaller the number of the living T47D cells (Figure 1 and 3). Cytotoxic activity test results are shown in Table 1.

Table 1. **Results of Cytotoxic Activity Against Multiple Extracts on T47D and Vero Cells**

No	Extracts	IC_{50} (µg/mL)	
		T47D cell	**Vero cell**
1	n Hexane Extract	422,921	3417,897
2	Chloroform Extract	69,067	293,410
3	Ethyl Acetate Extract	475,448	1180,121
4	Ethanol Extract	10185,896	-

Referring to Table 1, chloroform extract on T47D cells had IC_{50} values ≤ 100 µg/mL. It showed that chloroform extract had anticancer activity because it was thought to contain a chemical class of compounds which had the anticancer activity against T47D cells with IC_{50} values ≤ 100 µ/mL.
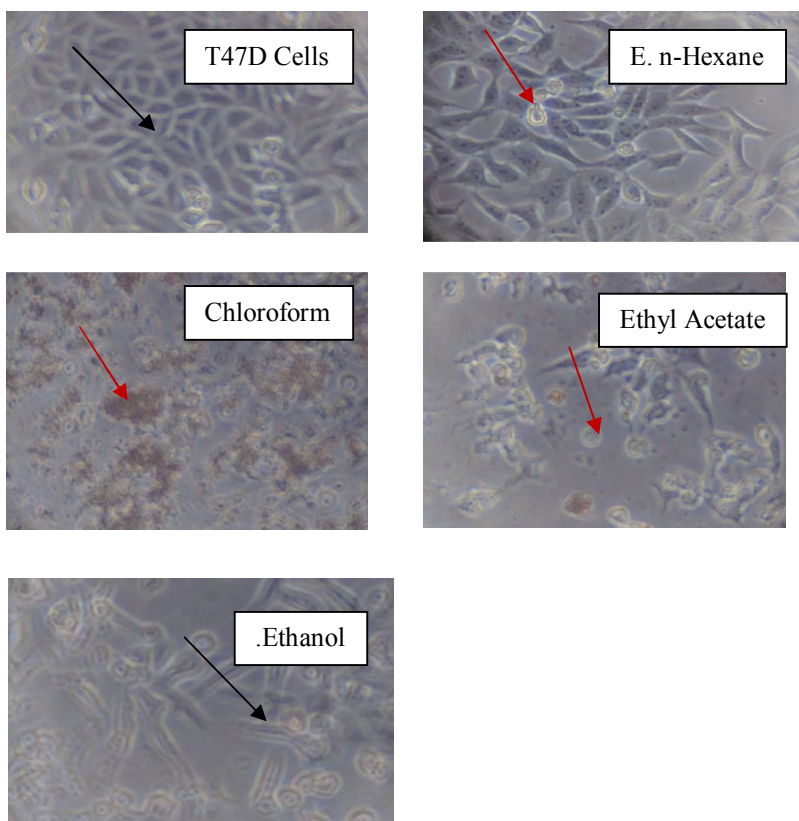

Figure 1. Morphology of T47D cells after incubated some extracts for 24 h (magnification 10 x 10). Living cells indicated by black arrows(⟶), while the dead cells was shown by red arrows (⟶)
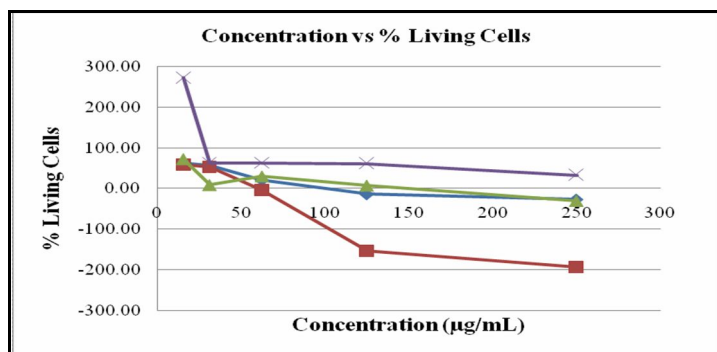

Figure 2. Graph of chloroform extracts concentration versus living cells

Treatment with some "ekor naga" leaf extracts on T47D cancer cells affected the morphology of the cell. Living cells appeared in the form of leaves and remained floating on the wells (Figure 1 and 3), whereas the death cell looked round and blackened. The chloroform, ethyl acetate and ethanol "ekor naga" leaf extracts

showed *dose-dependent* phenomenon where by percentage of living cells continued to decrease with the increasing of dose (Figure 2). It showed that the "ekor naga" leaf extract could induced cell death in T47D breast cancer cells.

The data showed that "ekor naga" chloroform extract had potential against T47D cells with IC_{50} value of 69.067 µg/mL. It gave a great hope to the treatment of ER-positive breast cancer by the representation of T47D cells.

Probit analysis results showed that all the extracts against vero cells had IC_{50} values $\geq$ 100 µg/mL. According to Ueda[9], *et al*., (2002), the IC_{50} value as an anticancer was potential if the $IC_{50} \leq$ 100 µg/mL and by Cho[10], (1998), IC_{50} value > 30 µg/mL = not active, so that all extracts were non toxic to normal cells, with the following criteria:

1. IC_{50} 5 µg/mL   = very active.
2. IC_{50} of 5-10 µg/mL   = active.
3. IC_{50} 11-30 µg/mL   = moderate.
4. $IC_{5}0$ > 30 µg/mL   = inactive.
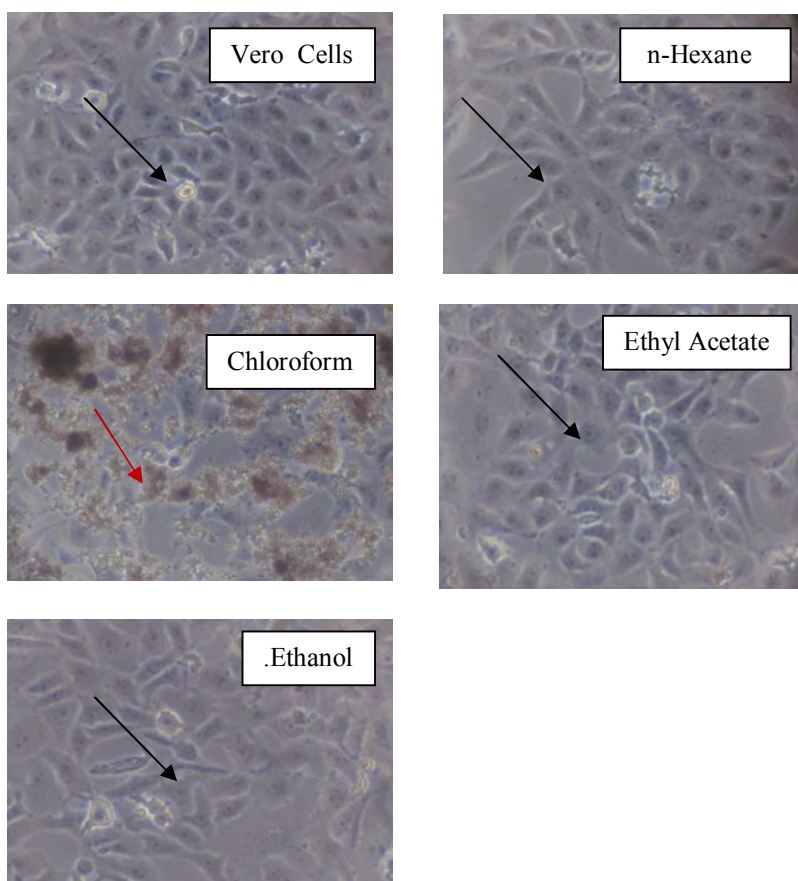
Gambar 2. Morphology of Vero cells after incubated with all extracts for 24 h (magnification 10 x 10). Living cells indicated by black arrows(⟶) , while the dead cells was shown by red arrows (⟶)

IC_{50} values can indicate the potential as a cytotoxic compound. Based on the IC_{50} values, the test materials were safe against Vero cells with IC_{50} values $\geq$ 30 µg / mL.

Selectivity Index

The ekor naga chloroform extract IC_{50} value against vero cells was 293.410 µg/mL and T47D cells was 69.067 µg/mL, therefore the value of the SI was 4.25. The extract had a high selectivity when the value of the SI > 3 (Machana[8], et. Al, 2011), Therefore the chloroform extract of "ekor naga" was selective against T47D cancer cells.

Conclusion

Based on the obtained results, it could be temporary concluded that the chloroform extract had a cytotoxic effect with IC$_{50}$ value of 69.067 mg/mL against T47D cells. All "ekor naga" extracts against vero cells (normal cells) were non toxic with IC$_{50} \geq 100$ µg/mL. electivity index value of chloroform extract that selective on T47D breast cancer cells was   > 3.

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