Cytotoxic activity from ethanol extract and fractions sablo (*Acalypha Wilkesiana*) leaves on HeLa servical cancer cells

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Abstract
Cancer is still one of the main health problems in the world. Cervical cancer is a leading cause of cancer deaths in women worldwide. In parts of the developing world, cervical cancer is the major cause of death in women of reproductive age. Conventional therapies to treat cancer are still unsatisfied. Alternative therapy especially from natural materials is needed. One of the natural materials that has the potential to be developed as cancer drugs is sablo (Acalypha wilkesiana) leaf. This study was conducted to determine the cytotoxicity of extracts and fractions of sablo leaves against HeLa cervical cancer cells with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) Assay.

The results showed that the ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction of sablo leaves had cytotoxicity against HeLa cervical cancer cells with IC_{50} value of 98.91 µg/mL, 88.85 µg/mL, 79.84 µg/mL and 102.47 µg/mL respectively. The cytotoxicity on HaCaT normal cells showed that the ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction provided a cytotoxicity effect with IC_{50} values of 217 µg/mL, 115 µg/mL, 425 µg/mL and 617 µg/mL respectively. The results of this study indicate that sablo leaves of ethyl acetate fraction were quite effective to inhibit the proliferation of HeLa cervical cancer cells and might have potential for cancer therapy.

Keywords: Sablo (*Acalypha wilkesiana*), HeLa servical cancer cells, cytotoxicity, MTT assay.

Introduction

Cancer is a hyperproliferative disorders of cells in the abnormal growth of cells in the body's tissues[3]. There 14.1 million new cancer cases and 8.2 million cancer deaths worldwide. There are 529,000 new cases of cervical cancer in the world. Cervical cancer is the fourth most common cancer in women and the seventh overall with an estimated 528,000 new cases in 2012. Cervical cancer remains the most common cancer in women in Eastern and middle Africa. There were an estimated 266,000 deaths from cervical cancer worldwide in 2012 accounting for 7.5 0f all female cancer deaths. Almost nine out of ten (87%) cervical cancer deaths occur in the less developed regions.[8,13]

Approximately, 60% of the safe selection of anticancer therapy was derived from nature because many of plants have antimutagenic and anticarcinogenic activity.[4,10] Previous studies have shown that *acalypha wilkesiana* was tested for toxicity against brine shrimps larvae (*Artemia salina*) and showed LC50 values 212µg/mL[2] while against cytotoxic against MCF-7 breast cancer cells[7]. This study investigated the cytotoxicity of extracts and fraction of sablo (*Acalypha wilkesiana*) against HeLa cervical cancer cell line.

Material and Methods

Materials: Sablo (*Acalypha wilkesiana*) leaves from Manoco, Lembang, West Java, Indonesia; aquadest, ethanol, n-heksan, ethyl acetate, dimetil sulfoxside/DMSO (Sigma-Aldrich, USA} HeLa human cervical cancer cell lines, HaCaT (ATCC, Manassas, VA, USA). RPMI-1640 medium (Sigma, MO, USA), fetal bovine serum (Invitrogen, USA) and penicillin and streptomycin (Merck) MTT Reagent (Sigma, St. Louis MO, USA).

Collection and determination of test materials: Sablo (*Acalypha wilkesiana*) leaves were. identified and determined in Taxonomy Laboratory, Department of Biology, Faculty of Science, Padjadjaran University.

Sablo leaves extraction treatment: Sablo leaves were extracted by maceration method in ethanol 96% for 72 hours with a change of solvent every 24 hours. The concentrated extract obtained is then evaporated.

Sablo leaves fractionation treatment: Liquid-liquid extraction was conducted by using water, ethyl acetate and n-hexane. Solvent from each fraction is evaporated using a rotary evaporator and concentrated in waterbath (50 ^{0}C).

Cell Culture: HeLa and HaCaT cells were cultured in RPMI-1640 medium (Sigma, MO, USA) supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin).

Cytotoxicity Assay: Cytotoxic assay was performed with cells in the presence of extract and fractions by a colorimetric methyl thiazolyl tetrazolium (MTT) assay. Briefly, cells (2×104 in 50 µl/well) were plated in 96-well plates. After the initial cell seeding, different concentrations of Sablo leaves extracts and fraction were

added and incubated for 24 hours. After the addition of 100 µl/well of 1 N HCl, the cell proliferation rate was then determined by measuring the absorbance at a wavelength of 570 nm. The absorbance was read using a microtiter plate reader (Becton Dickinson, NJ, USA).

The inhibition of cell proliferation (CPI: Cell Proliferation Inhibition Rate) is calculated with the following formula:

$$(1- \frac{\text{Optical density of treated cells}}{\text{Optical density of control}}) \times 100$$

Results and Discussion

Sablo Leaves Extraction and Fractionation: Ethanol extract of sablo leaves was obtained by 351.47 grams with a yield of 18.15% while the results obtained from fractionation of sablo leaves extract are 10.21 grams n-hexane fraction, 43.44 grams of ethyl acetate fraction and 65.14 grams of water fraction. The yield of each fraction is respectively 3.46%, 14.72% and 22.08%.

Cytotoxicity Activity: The percentage of inhibition of cell proliferation was conducted to compare percentage inhibition of proliferation between extract and fraction from Sablo leaves in cervical cancer cells HeLa. Concentration Proliferation Inhibition (CPI) of ethanol extract, n-hexane fraction, ethyl acetate and water fraction from Sablo leaves on HeLa cervical as shown in fig. 1.

IC_{50} calculation was obtained using the linear regression equation. Table 1 and 2 show the IC50 of ethanol extract, n-hexane fraction, ethyl acetate and water fraction from Sablo leaves on HeLa cervical cancer cells and HaCaT cells as normal cells.

In this research, cytotoxic activity from extract and fractions of sablo leaves was conducted by MTT assay. The measurement method was based on the ability of living cells to metabolize tetrazolium salts. The assay is based on the reduction of MTT by succinate dehydrogenase enzyme resulting in formazan substances measured by spectrophotometry using ELISA plate reader. This measurement is one method of measuring cell viability and proliferation of the easiest and allows measurement of many samples quickly and simultaneously. Changes from MTT into formazan can be seen in fig. 2.[1,12]

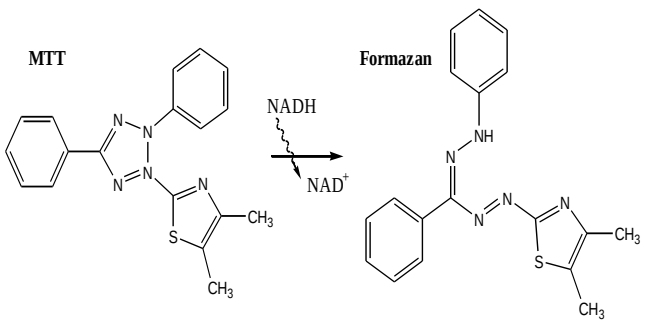

Figure 2: Changes from MTT into formazan[12]

As shown in figure 1, treatment with extract and its fractions resulted in the dose dependent inhibition of cervical cancer cell growth when assessed at 24 hours post treatment. The results showed the increase in the concentration of extract and fractions to give effect to the increase in cytotoxic activity. Based on these results, ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction of leaves against HeLa cervical cancer cells viability showed viability affected by its concentration.

Table 1
IC_{50} values of ethanol extract, n-hexane fraction, ethyl acetate and water fraction against cervical cancer cells HeLa

Sample	IC_{50} Value (µg/mL)
Ethanol Extract	98.91
N-hexane Fraction	88.85
Ethyl Acetate Fraction	79.84
Water Fraction	102.47

In HeLa cells, as shown in table 1, the IC50 value of ethanol extract was at concentration 98,91 µg/mL while n-hexane fraction, ethyl acetate fraction and water fraction were at concentration 88,85, 79,84 and 102,47 µg/mL respectively. Based on these results, HeLa cells demonstrated the highest sensitivity to treatment with water fraction while that with ethanol extract demonstrated the lowest sensitivity.

IC_{50} showed inhibitory concentration of cell growth by 50% of the total cell population. The smaller is the IC_{50} value, the sample is more toxic to cells whereas if the IC_{50} value is higher, then the compound is not toxic to cells. Fraction of ethyl acetate has the best IC_{50} value compared to the ethanol extract, n-hexane fraction and water fraction.

The results showed that ethyl acetate fraction has stronger cytotoxicity against cervical cancer cells HeLa when compared to the n-hexane fraction and water fraction. Based on the values of IC_{50}, cytotoxicity strength can be divided into several categories by the National Cancer Institute and Geran et al[9] namely $IC_{50} \leq 20$ µg/mL is very active; IC_{50} 21-200 µg/mL sufficiently active; IC_{50} 201-500 µg/mL weak and $IC_{50} > 501$ µg/mL active. IC_{50} value of sablo leaves extracts and fractions showed considerable cytotoxic activity but the fraction of ethyl acetate has the best potential in inhibiting cancer.

Tests on normal cells are also conducted to determine the cytotoxicity of sablo leaves extracts and fractions on normal cells. HaCaT used in normal cells is derived from human keratinocytes which is eternal (immortal) and has

the ability to split a high[5]. As shown in table 2, the same treatment was performed on normal cells as previously conducted in cervical cancer cells HeLa. The IC_{50} value of the extract, n-hexane fraction and ethyl acetate fraction can be categorized fairly active because it is still within the range of IC_{50} 21-200 µg/mL. IC_{50} value of the water fraction is considered not active because of its range of values > 501 µg/mL. Thus, it can be assumed the water fraction of sablo leaves is least cytotoxic on normal cells HaCaT.

Table 2
IC50 values of ethanol extract, n-hexane fraction, ethyl acetate and water fraction against normal cells HaCaT.

Sample	IC_{50} Value (µg/mL)
Ethanol Extract	217
N-hexane Fraction	115
Ethyl Acetate Fraction	425
Water Fraction	617

This research was preliminary investigation in development of anticancer drugs from sablo leaves. Therefore, it is necessary to conduct further research on the cytotoxic activity of isolates from the separation result of the most active fraction.

Conclusion

Ethanol extract, n-hexane, ethyl acetate fraction and water fraction of sablo (*Acalypha wilkesiana*) have cytotoxicity against cervical cancer cells HeLa and normal cells HaCaT with the strongest activity from ethyl acetate fraction. Based on the IC_{50} value it can be concluded that sablo leaves extracts and fractions have a fairly active cytotoxicity against cervical cancer cells HeLa and is relatively safe to normal cells HaCaT.

Acknowledgement

This research was supported by Riset Kompetensi Dosen Unpad.

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