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# Cytotoxic activity of subfraction from ethyl acetate fraction of sablo (*Acalypha Wilkesiana*) leaves on HeLa servical cancer cells

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**Abstract**

Cervical cancer is one type of cancers in women which is the first rank in Indonesia. Medical treatment that is usualy used to treat cancer is chemotherapy. Yet, the side weakness of chemotherapy are the adverse effect and low of safety. Therefore, the discovery of safer agent is conducted continuously from plant to overcome the problem. The plant that has potential source of anticervical cancer agent is Acalypha wilkesiana Mull. Arg. This study aims to discover potential compound that has anticervical cancer activity from etyl acetate fraction of A. wilkesiana. A. wilkesiana leaves was extracted with ethanol using maceration method, then was fractionated using n-hexane and ethyl acetate. The ethyl acetate fraction then processed by chromatoography and resulted in four subfraction (SF1-4). The cytotoxic activities of four subfraction were assay on HeLa cervical cancer cells using 3-(4,5-dimethylthiazoyl-2-yl) 2,5 diphenyltetrazolium bromide (MTT). The result show that the $IC_{50}$ of subfraction 1-4 were 56.93, 151.52, 217.39 and 771.03 µg/ml, respectively. The strongest cytotoxicity shown by subfraction 1 (SF1). The results of this study indicate that A. wilkesiana leaves of SF1 and SF2 from ethyl acetate fraction are quite effective to inhibit the proliferation of HeLa cervical cancer cells.

**Keywords:,** A.wilkesiana Mull. Arg, Cervical cancer, MTT assay, subfraction

## INTRODUCTION

Cancer is a hyperproliferative disorders of cells in which the abnormal growth of cells in the body's tissues[1]. There have been 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.[2,3]

Approximately, 60% of the safe selection of anticancer therapy were derived from nature because many of plants have antimutagenic and anticarcinogenic activity [4,5].

Previous studies have shown that *A. wilkesiana* were tested for toxicity against MTT Assay in the n-hexane fraction, ethyl acetate and water and the result is the best $IC_{50}$ value in ethyl acetate fraction 79.84 µg / ml, so in this study investigated the cytotoxicity of subfraction from ethyl ascetate fraction of sablo (*Acalyphawilkesiana*) against HeLa cervical cancer cell line[6].

## 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 (ATCC, Manassas, VA, USA). RPMI-1640 medium (Sigma, MO, USA), fetal bovine serum (Invitrogen, USA) and penicillin & streptomycin (Merck) MTT Reagent (Sigma, St. Louis MO, USA).

**Methods :**

**Collection and determination of test materials:**

Sablo (*Acalypha wilkesiana*) leaves were. identified and determined in Taxonomy Laboratory, Department of Biology, Faculty of Science, Universitas Padjadjaran.

**Sablo leaves extraction, fractinasion and subfractination 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.

Liquid-liquid extraction was conducted by using water, ethyl acetate, and n-hexane. Solvents from each fraction is evaporated using a rotary evaporator and concentrated in waterbath (50 0C).

30 gr the ethyl acetate fractions was dissolved in amount of methanol and loaded on column using isocratic ($CH_2Cl_2$-MeOH, 1 : 1) elution which yielded 38 subfractions (SF). Then, these subfractions were intermingled with similar pattern resulting from thin layer chromatography using chloroform : methanol system. Afterward, a pilot study is carried out for all subfractions and finally, four subfractions (SF1, SF2, SF3, and SF4) were chosen and assessed for their impacts.

**Cell Culture:**

HeLa cells was 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 subfraction from ethyl acetate fraction 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 subfraction of ethyl acetate 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 by the following formula:

*1 −Optical density of treated cells Optical density of control) x100%*

## RESULTS AND DISCUSSION

In this research, cytotoxic activity from subfractionof sablo leaves was conducted by MTT assay. The measurement method was based on the ability of living cells to metabolize tetrazolium salts. The assay based on the reduction of MTT by succinate dehydrogenase enzyme resulting formazan substances which was 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 Figure1[7,8] .

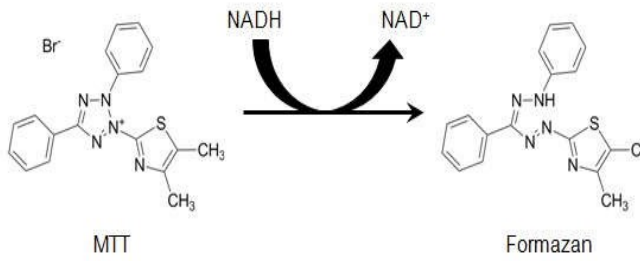


Figure 1. MTT structure and formazan colored products

The amounts of subfractions (SF1, SF2, SF3, and SF4) were 0.20, 2.20, 15.81, and 6.30 g, respectively. The yield of each subfraction (SF1-4) are 13.37%, 11.33% and 62.14%, respectively (Table 1).

Table 1: Rendemen of the Sablo Leaves Subfraction from the Ethyl Acetate Faction

| Sample Type | Weight (g) | Rendemen (%) |
|---|---|---|
| Subfaction1 (SF1) | 0,20 | 0,66 |
| Subfaction 2 (SF2) | 2,20 | 6,73 |
| Subfaction 3 (SF3) | 15,81 | 52,70 |
| Subfaction 4 (SF4) | 6,30 | 21,00 |

The percentage of inhibition of cell proliferation was conducted to compare percentage inhibition of proliferation subfraction1-4 from Sablo leaves in cervical cancer cells HeLa.

Percentage Inhibition of Cell Proliferation HeLa cervical cancer cells by subfraction of ethyl acetate fraction is shown in Table 2.

Table 2 Percentage of Proliferation Inhibition of HeLa cervical cancer cells by subfraction of ethyl acetate

| Fraction concentration (µg/mL) | Proliferation inhibition (%) | | | |
|---|---|---|---|---|
| | Subfraction1 | Subfraction 2 | Subfraction 3 | Subfraction 4 |
| 1000 | 95,23 | 88,19 | 76,10 | 55,39 |
| 500 | 94,66 | 72,34 | 51,03 | 40,70 |
| 250 | 93,69 | 63,08 | 50,37 | 40,22 |
| 100 | 63,85 | 32,50 | 44,61 | 6,46 |
| 50 | 33,93 | -2,11 | 37,14 | 5,01 |
| 25 | 28,94 | 10,09 | 15,85 | 0,73 |
| 10 | 23,31 | 8,77 | 8,71 | 18,58 |

$IC_{50}$ calculations obtained using the linear regression equation. $IC_{50}$ showed inhibitory concentration of cell growth by 50% of the total cell population. The smaller the $IC_{50}$ valueproves that the sample more toxic to cells, whereas if the $IC_{50}$ value is greater then the compound is not toxic to cells.

$IC_{50}$ calculation is obtained from the equation of each subfraction, then substitutes the number 50 on the y axis, then calculated and obtained the $IC_{50}$ value. $IC_{50}$ values indicate the concentration needed to inhibit cell growth by 50% of the total population. Based on the values of $IC_{50}$, cytotoxicity strength can be divided into several categories by the National Cancer Institute and Geran et al.[10]. The level of cytotoxicity of the extract can be divided into : $IC_{50} \leq 20$ 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 of inactivity.[10,11].

The results (Table 3) showed that $IC_{50}$ value of the subfraction of ethyl acetate fraction ofsablo leaves (SF1 and SF2) can be categorized fairly active because it is still within the range of $IC_{50}$ 21-200 µg/mL.While the $IC_{50}$ value SF3 considered weak and SF4 considered not activebecause its range of values>501 µg/mL.

Table 3 $IC_{50}$ Sub-fraction 1-4 of the ethyl acetate fraction of HeLa cervical cancer cells

| Sample | $IC_{50}$ (µg/mL) |
|---|---|
| Subfaction1 (SF1) | 56,93 |
| Subfaction2 (SF2) | 151,52 |
| Subfaction3 (SF3) | 217,39 |
| Subfaction 4 (SF4) | 771,03 |

Based on the results listed in Table 3 shows that the best $IC_{50}$ value is owned by SF1, with value 56.93 µg/mL.

## CONCLUSION

$IC_{50}$ values of subfraction of ethyl acetate fraction the sablo leaf (SF1-4) were 56.93, 151.52, 217.39 and 771,03 µg/ml respectively. Subfaction which has the smallest $IC_{50}$ value issubfraction 1 (SF1).

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