Original Article

Antiproliferative activity of *Acalypha Wilkesiana* against human cervical cancer cell lines HeLa

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ABSTRACT

Cancer is still a problem and needs to be solved in all over the world. One of the most commen cancers which is experienced by women is cervical cancer. Therefore, alternative therapies derived from natural ingredients such as the sablo plant are needed that have the potential to be developed as an anti-cancer. This study evaluated the antiproliferative activity of the extract and fractions of sablo leaves to cervical cancer HeLa cells with Cell Counting Kit-8 (CCK-8).

The results showed that ethanol extract, n-hexane fraction, butanol fraction, water fraction, and ethyl acetate fraction of sablo leaves had antiproliferative activity against HeLa cells, with an IC50 of 604.14 µg/mL, 1054.11 µg/mL, 219.44 µg/mL, 578.33 µg/mL, and 531.72 µg/mL, respectively. The results showed that sablo leaves from ethyl acetate fraction were the best fraction in inhibiting the proliferation of cervical cancer cells HeLa and had the potential to be developed in cancer treatment.

Keywords: *Acalypha wilkesiana*, HeLa cell line, Antiproliferative, Cell counting Kit-8 (CCK-8)

Introduction

Cancer is a genetic desease considered a hyperproliferative disorder of cells in which cells in the body tissues grow abnormally [1, 2]. Annually, there are 14.1 million new cancer patients and 8.2 million deaths from cancer worldwide. In 2020, 1,806,590 new cancer cases and 606,520 cancer deaths are projected to occur in the United States [3-5].

In 2020, cervical cancer was the highly prevalent cancer among women with 604,127 new cases, which is closely related to HPV infection [6, 7]. Also, it is more prevalent in Africa, where more women suffer from cancer.

In 2018, throughout the world, there were approximately 569,800 deaths from cervical cancer that accounts for 13,1% of all female death from cancer. In less developed countries, about 9 out of 10 (87%) cervical cancer leads to death [8, 9]. In 2018, there were about 570,000 cases of cervical cancer and 311,000 deaths from it. Cervical cancer is the 4th most frequent cancer among females. It is the main reason for death from cancer in African women. In China, there were 106,000 cases and 48,000 deaths. There were 97,000 cases and 60,000 deaths in India. The mean age at the diagnosis of cervical cancer is 53 years (44-68) and the mean age at death from cervical cancer is 59 years (45-76) worldwide [1,10].

Many plants have anticarcinogenic and antimutagenic activity and it is evaluated that 60 % of the selection of safe anticancer therapies comes from nature and it has been estimated by the world health organization that 80% of the world is using traditional treatment methods [11-13]. In previous studies, acalypha wilkesiana had been tested regarding its toxicity against brine shrimp larvae (*Artemia salina*) and showed an LC50 value of 212 µg/mL [14], besides that it also had cytotoxic activity against MCF-7 breast cancer cells [15].

This study evaluated the antiproliferative activity of *Acalypha wilkesiana* fractions and extract against human cervical cancer HeLa cell line.

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Materials and Methods

Materials

The research materials include: Sablo (*Acalypha wilkesiana*) leaves from Lembang, West Java, Indonesia, ethanol, ethyl acetate, n-hexane, aquadest, DMSO (Dimethylsulfoxide) (Sigma-Aldrich, USA), RPMI-1640 medium (Sigma, MO, USA), penicillin and streptomycin (Merck), fetal bovine serum (FBS) (Invitrogen, USA), human cervical cancer cell lines (HeLa), HaCaT (ATCC, Manassas, VA, USA), and Cell Counting Kit-8/CCK-8 (Dojindo, Japan).

Extraction and fractionation

Sablo leaves were extracted by maceration with 96% ethanol for 3×24 h (72 h). The obtained extract was concentrated using an evaporator. Fractionation was carried out with liquid-liquid solvents using water, ethyl acetate, and n-hexane as solvents. The solvent from each fraction was evaporated by a rotary evaporator.

Cell culture

RPMI-1640 medium supplemented with FBS 10%, streptomycin 100 U/ml, and penicillin 100 U/ml was used to make HeLa and HaCaT cell cultures.

Antiproliferative assay

The antiproliferative test was carried out by exposing extracts and fractions to cell cultures using cell-counting kit-8.

Briefly, 100 µl/well cells were cultivated in 96-well plates. Then, different concentrations of Sablo leaves fraction were added and incubated for 1-4 h. Furthermore, the cell proliferation rate was determined using an Elisa plate reader (Becton Dickinson, NJ, USA) by measuring absorbance at 450 nm [15].

Calculate the inhibition of cell proliferation (CPI: Cell Proliferation Inhibition Rate) is determined by the following formula:

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

Results and Discussion

Sablo leaves extraction and fractionation

Ethanol extract of sablo leaves was obtained by 367.40 grams with a yield of 14.68%. While the results obtained from the fractionation of sablo leaves extract were 86.31 grams from n-hexane fraction, 92.13 grams from ethyl acetate fraction, and 101.12 grams from water fraction. The yields of each fraction were 23.49%, 25%, and 27.52%, respectively.

Antiproliferative activity

The percentage of inhibition of cell proliferation can be calculated by comparing the inhibition of proliferation between the Sablo leaf extract and fractions against cervical cancer cells HeLa. Concentration Proliferation Inhibition (CPI) of ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction of Sablo leaves on HeLa cell are shown in **Figures 1-4**, respectively.

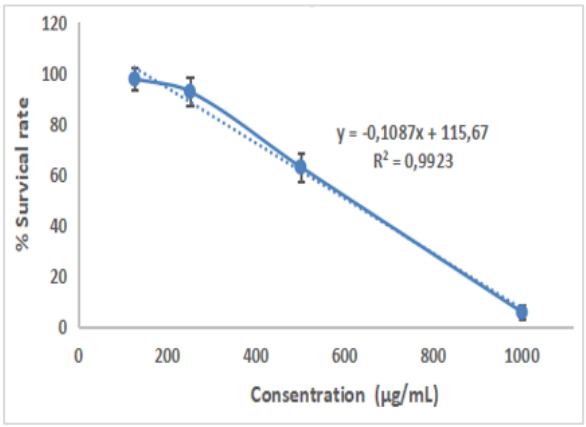

Figure 1. Cytotoxicity of the Ethanol Extract against Cervical Cancer HeLa Cells

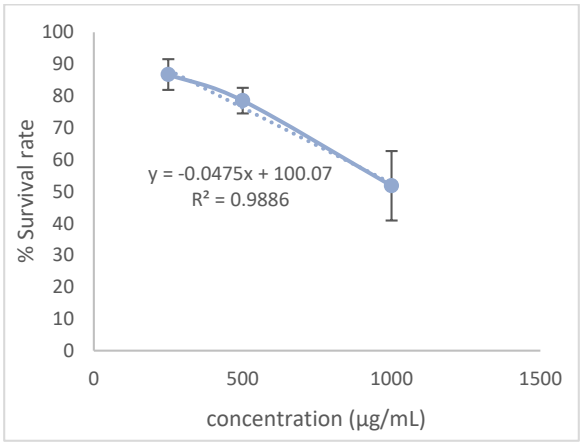

Figure 2. Cytotoxicity of the Water Fraction against Cervical Cancer HeLa Cells

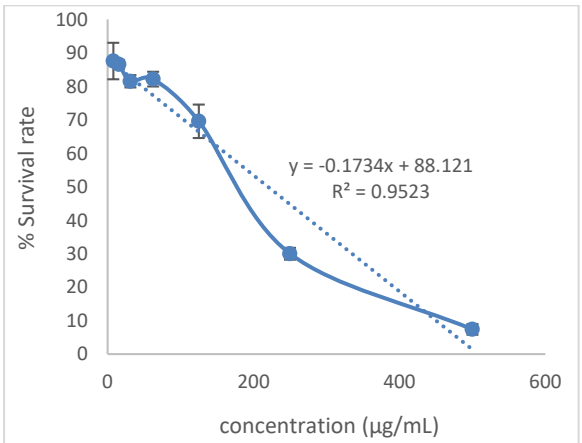

Figure 3. Cytotoxicity of the Ethyl Acetate Fraction against Cervical Cancer HeLa Cells

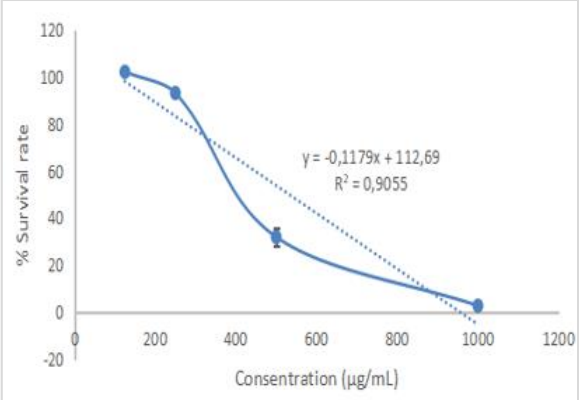

Figure 4. Cytotoxicity of the n-hexane Fraction against Cervical Cancer HeLa Cells

The IC50 value is obtained based on the linear regression equation.

Table 1 shows the IC50 of ethanol extract, water fraction, ethyl acetate, butanol fraction, and n-hexane fraction from Sablo leaves on cervical cancer HeLa cells.

The antiproliferative effect of sablo leaves extract and the fraction was determined using CCK-8, which is based on the metabolism of tetrazolium salt in the presence of living cells.

CCK-8 is a sensitive calorimetric measurement to determine the viability of cell growth and death. The tetrazolium salt in this product is very soluble in water and decreases by the dehydrogenase activity in cells, giving rise to a yellow color in the formazan, which is soluble in tissue culture media. The color intensity of the formazan formed is proportional to the number of living cells. CCK-8 has a higher sensitivity than other tetrazolium salts such as XTT, MTT, WST-1, or MTS. This measurement is the easiest method of measuring cell viability and proliferation that allows the quick and simultaneous measurement of many samples. Changes from MTS (CCK-8) into formazan can be seen in **Figure 5** [16-19].

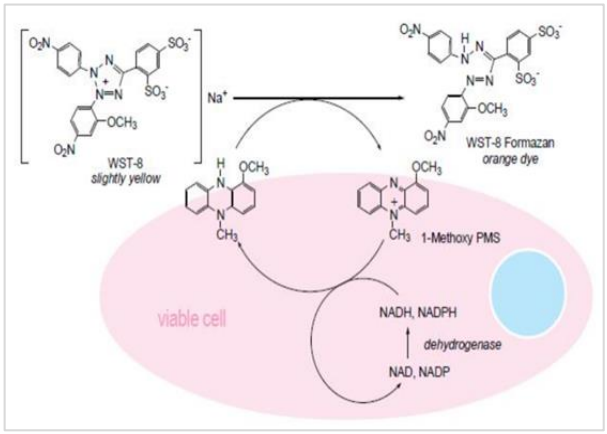

Figure 5. Mechanism of Detecting Cell Viability using CCK-8 [19].

As can be seen in **Figures 1-4**, evaluation 1-4 hours after treatment with the extract and fractions showed the inhibition of cervical cancer cell growth in a dose-dependent manner.

In this study, it was observed that the increase in extract and fraction concentrations was proportional to increased antiproliferative activity. According to these findings, n-hexane, butanol, ethyl acetate, and water fractions of Sablo leaves affected the viability of HeLa cells in a concentration manner.

Table 1. Antiproliferative Activity of the Sablo Leaf Extract and Fractions against Cervical Cancer Cells

Sample	IC50 Value (µg/mL)
Ethanol Extract	604.14
Water Fraction	1054.11
Ethyl Acetate Fraction	129.44
Butanol Fraction	578.33
N-hexane Fraction	531.72

In HeLa cells, as shown in **Table 1**, the IC50 value of ethanol extract was 604.14 µg/mL, while that of the n-hexane, butanol, ethyl acetate, and water fractions were 531.72, 578.33, 129.44, and 1054.11 µg/mL, respectively. Based on these results, HeLa cells demonstrated the highest and lowest sensitivity to ethyl acetate and water fractions, respectively.

The ethyl acetate fraction has the lowest IC50 value compared to the ethanol extract, water fraction, butanol fraction, and n-hexane fraction, respectively. Based on the above results, ethyl acetate fraction has stronger antiproliferative activity against cervical cancer HeLa cells when compared to the extract and other fractions. The IC50 value of sablo leaves extract and fractions showed considerable antiproliferative activity, but the ethyl acetate fraction had the best potential in inhibiting cancer.

Testing on normal cells was carried out by determining the antiproliferative influence of the Sablo leaf ethyl acetate fraction in normal cells. The normal cell used was HaCaT, which is obtained from immortal human keratinocytes and has a high ability to split [20].

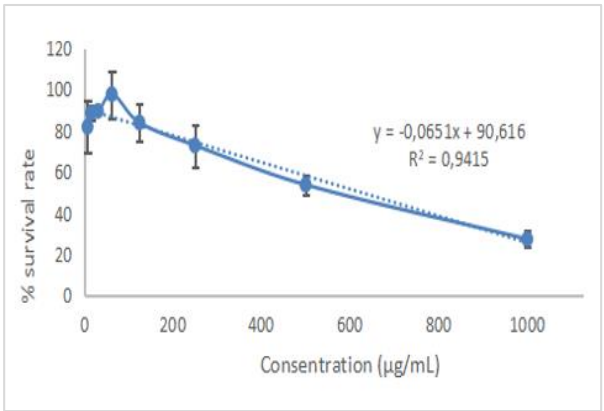

Figure 6. Cytotoxicity of Ethyl Acetate Fraction against Normal HaCaT Cells

As can be seen in **Figure 6**, the same treatment was carried out against normal cells by the same method performed on cervical cancer cells HeLa. In normal cells, the IC50 value of ethyl acetate fraction was 623,90 µg/Ml. Thus, it can be assumed that the ethyl acetate fraction of sablo leaves has a lower cytotoxic effect on normal cells.

This research is a preliminary study to develop Sablo leaves as an anticancer. Therefore, it is essential to do further research

on the molecular mechanism of this plant.

Conclusion

The ethanol extract, water fraction, ethyl acetate fraction, butanol fraction, and n-hexane fraction of sablo (*Acalypha wilkesiana*) have antiproliferative activity against cervical cancer cells HeLa with the strongest activity from ethyl acetate fraction. Based on the IC50 value, it can be concluded that the ethyl acetate fraction of sablo leaves has quite an active cytotoxicity against human cervical cancer cells and is relatively safe to normal cells.

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Conflict of interest: None

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Ethical statements: None

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