

Cytotoxicity of ethanol extract and its fractions from *Acalypha wilkesiana* against breast cancer cell MCF-7

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ABSTRACT

Cancer is a disease that is suffered by many people and is still a problem in the world. Breast cancer is one of the most common cancers with a high prevalence in women. Sablo plant is a plants that has the potential as an alternative therapy from natural ingredients that can be further developed as an anticancer. In this study, we evaluated the fraction of sablo (*Acalypha wilkesiana*) leaves and cytotoxic activity of extracts against breast cancer cells MCF-7 by colorimetric method using MTS assay.

According to the study's results, it was discovered that the ethanol extract, fraction of water, n-hexane fraction, butanol fraction, fraction of ethyl acetate, the fraction of butanol from sablo leaves and cisplatin had cytotoxic activity against MCF-7 breast cancer cells, with IC50 values of 143.73 µg/mL, 624.40 µg/mL, 81.97 µg/mL 463.19 µg/mL, 47.81 µg/mL, and 2.84 µg/mL, respectively. The results revealed that the ethyl acetate fraction from sablo leaves is the fraction that has the best cytotoxic activity against MCF-7 breast cancer cells which has the potential to be further developed as an alternative therapy for breast cancer.

Keywords: *Acalypha wilkesiana*, Breast cancer cell MCF-7, Cytotoxic, MTS assay

Introduction

The hyperproliferative disorder is considered to be cancer of cells in which cells in the body tissues grow abnormally [1-4]. Worldwide there are approximately 14.1 million new cancer patients and 8.2 million cancer deaths each year [5]. The number of new cancer cases and deaths estimated in 2021 in the United States shows that there is an estimation of 1.898.160 new cancer cases and 608.570 cancer deaths [6]. Based on data in 2015 in the United States, breast cancer occurs mostly in women with 231,840 new cases and 40,290 deaths from breast cancer [7]; In 2018, there will be an estimated 266.120 new cancer cases and 40.920 cancer deaths. In 2021, breast cancer was the highly

prevalent cancer among women with 281.550 new cases and 43.600 deaths. Based on these data, over time the prevalence of breast cancer continues to increase [6, 8, 9].

Natural products, including plants, have been widely used and form the basis of the treatment of human diseases and will remain one of the most important sources of future treatments and therapies [10]. Many of the various plants have anticancer and antimutagenic activity and have been evaluated and it shows that 60% of the selection of safe anticancer therapies comes from natural ingredients [11, 12]. The toxicity of the essential oil obtained from *Acalypha wilkesiana* leaves was previously tested on shrimp larvae (*Artemia salina*) and the results showed that the essential oil of the leaves of *Acalypha wilkesiana* had an LC50 value of 212 g/mL [13]. In addition, *Acalypha wilkesiana* leaves have cytotoxic activity against MCF-7 breast cancer cells using an acid phosphatase test; and against human brain & lung cancer cells [14, 15], in addition, it also induces the process of apoptosis [16].

In this opportunity, the extract and fractions of *Acalypha wilkesiana* leaves were investigated for MCF-7 human breast cancer cells on their cytotoxic activity.

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Materials and Methods

Materials

Materials : The Lembang area provide *Acalypha wilkesiana* leaves, Indonesia; n-hexane, ethanol, butanol, ethyl acetate, aquadest, DMSO (Dimethylsulfoxide) (Sigma-Aldrich, USA), penicillin and streptomycin (Merck), WST-8/MTS Kit (Dojindo, Japan), RPMI-1640 medium, human breast cancer cell lines (MCF-7) (Sigma, MO, USA) and fetal bovine serum (FBS) (Invitrogen, USA)

Fractionation and extraction

Extraction of sablo leaves was carried out by maceration using 96% ethanol solvent for 3x24 hours (72 hours), and the extract obtained was evaporated using an evaporator. Furthermore, liquid-liquid fractionation was carried out with n-hexane, ethyl acetate, and water as solvents. The obtained fractions were evaporated using a rotary evaporator.

Cell culture

The medium used to prepare MCF-7 cell cultures was RPMI-1640 supplemented with streptomycin 100 U/ml and FBS 10%, penicillin 100 U/ml

Cytotoxic assay

Cytotoxic activity was carried out by exposing the extract and fraction to the MCF-7 breast cancer cell culture by colorimetry using the MTS assay method.

In the 96-well plates, 100µl/well cells are cultivated. Furthermore, extracts and fractions of sablo leaves with different concentrations were added, then incubated for 1-4 hours. Then, the cytotoxic activity of the cells was measured by quantifying their absorbance at a wavelength of 450 nm using an Elisa plate reader (Becton Dickinson, NJ, USA) [17].

Furthermore, the formula used for determining the inhibition of cell proliferation (CPI: Cell Proliferation Inhibition) was using the following:

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

Results and Discussion

Fractionation and sablo leaves extraction

A total of 1000 grams of sablo leaf *Simplicia* was extracted by maceration using ethanol as a solvent, then evaporated to produce an extract of 140.50 grams with a yield of 14.05%. Furthermore, fractionation was performed and obtained 225.21 grams of n-hexane fraction, 245.13 grams of ethyl acetate fraction, and 255.42 grams of water fraction. The fraction of each yielded was 22.52%, 24.14%, and 25.54%, respectively.

Cytotoxic activity

The cytotoxic activity of the extract or fraction of sablo leaves can be determined from the percentage inhibition of cell proliferation which is calculated by comparing the inhibition of proliferation between the MCF-7 breast cancer cells and extract or its fraction against **Figures 1-5** show. The results of the Proliferation Inhibition Concentration (CPI) obtained from the ethanol extract, aqueous fraction, the fraction of ethyl acetate, butanol fraction, n-hexane fraction leaves of Sablo and cisplatin against MCF-7 breast cancer cells, respectively.

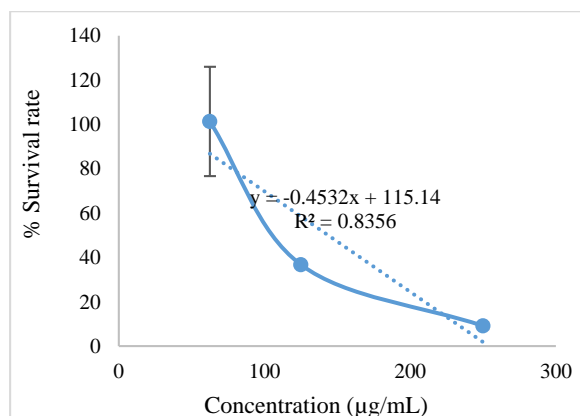


Figure 1. Breast Cancer MCF-7 Cells against Cytotoxicity of the Ethanol Extract

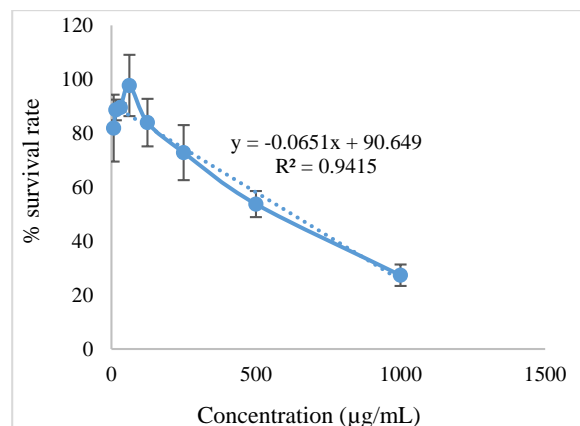


Figure 2. Breast Cancer MCF-7 Cells against Cytotoxicity of the Water Fraction

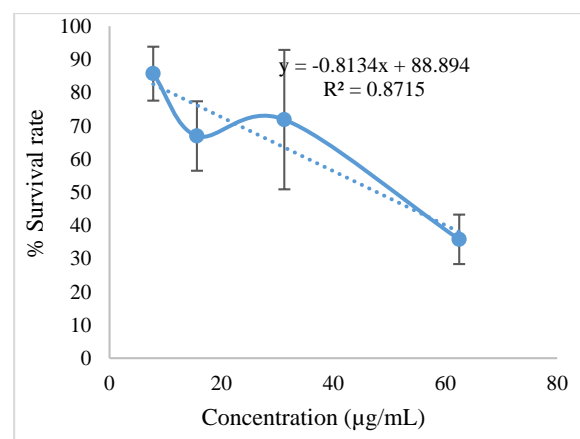


Figure 3. Breast Cancer MCF-7 Cells against Cytotoxicity of the Ethyl Acetate Fraction

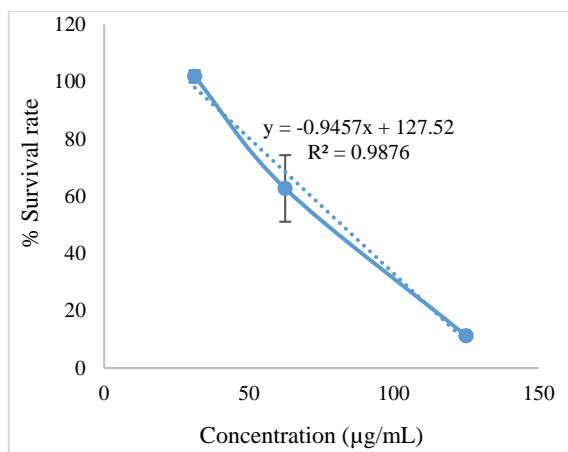


Figure 4. Breast Cancer MCF-7 Cells against Cytotoxicity of the n-hexane Fraction

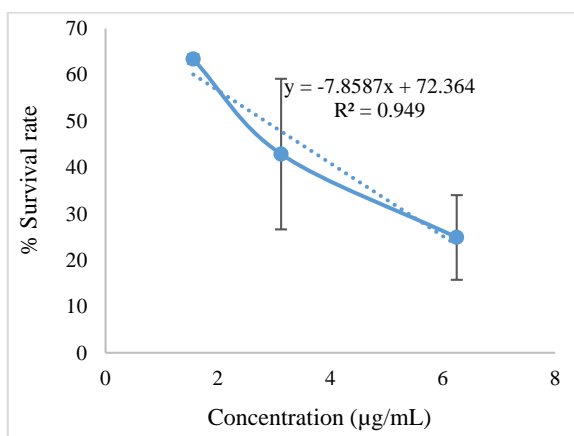


Figure 5. Breast Cancer MCF-7 Cells against Cytotoxicity of cisplatin

In this study, the IC₅₀ values of ethanol extract, the fraction of ethyl acetate, fraction of water, n-hexane fraction, and fraction of butanol from Sablo leaves on MCF-7 breast cancer cells evaluate the IC₅₀ value, the linear regression equation is utilized.

The cytotoxic activity of sablo leaves from extract and its fractions was determined based on the MTS assay method, which is based on the principle that the metabolism of tetrazolium salts occurs in living cells.

The MTS assay is a test used to determine the survival, cell growth and even cell death based on colorimetry

In this reagent kit, the presence of dehydrogenase enzyme activity in living cells will reduce the level of tetrazolium salt that is very soluble in water, so that it will cause a yellow color in formazan, which is soluble in culture media. Formazan color formation is proportional to the number of living cells. The sensitivity in this test is higher than other tetrazolium salts such as XTT, and MTT. This analysis method is a technique of determining cell feasibility and propagation that is easy, fast and simultaneous and allows the measurement of large numbers of samples. The reaction mechanism is in **Figure 6** [17-20].

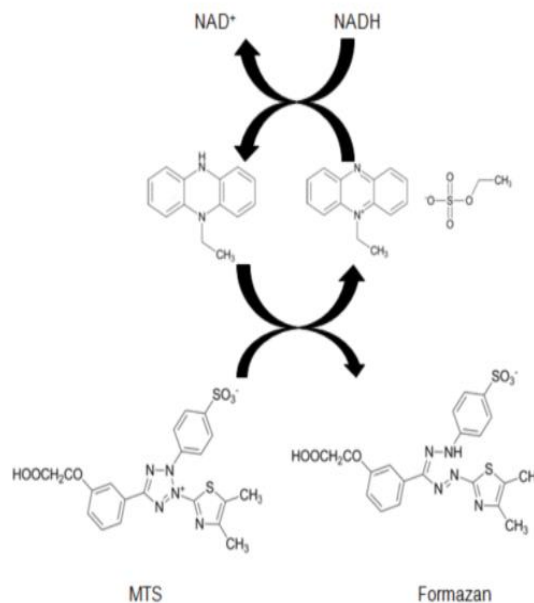


Figure 6. Mechanism of measuring cell viability using MTS assay [20].

As seen in **Figures 1-5**, the inhibition of breast cancer cell growth in a dose-dependent manner, evaluation 1-4 hours after therapy with the extract and fractions shown. In this study, it was observed that the increase extract and fraction concentrations was proportional to increased cytotoxic activity. According to these findings, extract of ethanol, butanol, water, n-hexane, and fraction of ethyl acetate of Sablo leaves affect the feasibility of concentration manner in MCF-7 breast cancer cells.

Table 1. Cytotoxic Activity of the Extract and Fraction of Sablo Leaves against Breast Cancer Cells

Sample	IC ₅₀ Value (µg/mL)
Ethanol Extract	143.73
Water Fraction	624.40
Ethyl Acetate Fraction	47.81
Butanol Fraction	463.19
N-hexane Fraction	81.97
Cisplatin	2.84

As shown in **Table 1**, in MCF-7 cells, the IC₅₀ value of ethanol extract was 143.73 µg/mL, while fractions were 624.40, 463.19, 47.81, 81.97, and 2.84 µg/mL, and that of the water, butanol, n-hexane, ethyl acetate and respectively. The fraction of ethyl acetate compared to the extract of ethanol has the lowest IC₅₀ value, fraction of water, fraction of butanol and fraction of n-hexane, respectively.

Based on the above results, the fraction of ethyl acetate has stronger cytotoxic activity against breast cancer MCF-7 cells when compared to the extract and other fractions.

The best in inhibiting breast cancer in the fraction of ethyl acetate and IC₅₀ value of sablo leaves extract and fractions showed considerable cytotoxic activity.

Conclusion

The extract of ethanol, fraction of butanol, n-hexane fraction, fraction of ethyl acetate, and the fraction of water sablo (*Acalypha wilkesiana*) leaves have cytotoxic activity against MCF-7. The cytotoxic activity seen from its IC50 value is the fraction that the best fraction of ethyl acetate.

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Conflict of interest: None

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Ethics statement: None

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