Original Article

Beta-sitosterol from sablo (*Acalypha wilkesiana* Muell. Arg.) leaves induce apoptosis in MCF-7 Breast cancer cell lines

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ABSTRACT

One of the most common malignancy breast cancer is highly prevalent among women. Based on the natural ingredients it contains, the plants of sablo have the potential to be employed as an anticancer therapeutic. Numerous studies have demonstrated that the chance of developing breast cancer rises with age. Additionally, the prevalence of breast cancer varies according to the different types of biased traditional Chinese medicine constitution, and the Qi-depressed constitution is highly concomitant and increases the prevalence of breast cancer. As a result, the Qi-depressed constitution is a separate risk factor for breast cancer. Previous studies have shown that the ethanol extract of Acalypha wilkesiana and its components have potential as anticancer agent against MCF-7. In the current study, using resazurin assays and flow cytometry, beta-sitosterol extracted from *Acalypha wilkesiana* was examined in MCF-7 human breast cancer cell lines. Resazurin experiments demonstrated its capacity to inhibit cell proliferation and trigger apoptosis, and flow cytometry was utilized to examine this capacity. Beta-sitosterol inhibits MCF-7 breast cancer cell proliferation and has an apoptotic activity that is proportional to the dose. The results of this study imply that beta-sitosterol may be used to treat cancer.

Keywords: *Acalypha wilkesiana*, Apoptosis, Beta-sitosterol, MCF-7, Breast cancer

Introduction

Particularly in poorer countries, breast cancer ranks as the fifth-leading cause of cancer-related fatalities and is one of the highest causes of mortality in the globe. The 20 regions of the world that the International Agency for Cancer Research (IARC) focuses on are geographically diverse. The International Agency for Research on Cancer reports that the highest cause of death from cancer among women is breast cancer [1]. In 2018, the cancer death rate was 9.6 million with 18.1 million new cases, implying that 1 in 5 men and 1 in 6 women worldwide had the disease. According to the statistics, with comparison 1 in 11 women died of cancer whereas in men 1 in 11. Breast cancer has the highest incidence rate among women of all types of cancer [2, 3]. Based on data from GLOBOCAN (IARC) in 2012, new cases of breast cancer have the highest percentage based on age control as well as the highest percentage of fatalities (after controlling for age), at 12.9%. Women are more likely than men to get breast cancer, with a mortality rate of 42,1 per 100,000 people [3-6]. Breast cancer in younger age groups has been found to have a more aggressive biological behavior and a worse clinical outcome than breast cancer in older age groups [7].

Anticancer chemical research has advanced during the past few years. Many researchers have examined plant-derived bioactive compounds' potential as prophylactic agents to reduce carcinogenesis. Many chemopreventive chemicals have been discovered based on their ability to regulate one or more specific chemical processes. The identification of potent herbs and the understanding of how they work could result in the development of complementary and alternative cancer prevention and treatment methods.

The family Euphorbiaceae, which contains 462 species and is frequently found in tropical and warm-weather settings, includes

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the genus Acalypha. Within the Acalyphoideae subfamily, Acalypha is the largest tribe. Acalypha species are frequently used in traditional medicine in Africa and Asia [8]. In West African countries, *A. wilkesiana* is traditionally a plant used to treat headaches, fever, and skin infections [9]. Based on research results, this plant also has antimicrobial, antioxidant, and cytotoxic activities [8-10], as well as an antibacterial, antifungal, and antimalarial effect [11-14], anti-inflammatory, analgesic, and anticancer effect [15, 16]. One of the Acalypha species that have the potential for use as an anticancer drug is *Acalypha wilkesiana*. According to earlier research, this plant extract, especially the ethyl acetate and n-hexane fractions, has a cytotoxic effect through the induction of DNA SSBs and DSBs, which cause apoptosis in U87MG brain cancer cells and A549 lung cancer cells [17]. The active fraction of *A. wilkesiana* leaves which has cytotoxic activity on breast cancer cells is the ethyl acetate fraction [18].

In previous studies, the major compound obtained from the active fraction of *A. wilkesiana* leaves which had been studied on breast cancer cells was beta-sitosterol.

Materials and Methods

Plant resources

A. wilkesiana Muell. Arg. plants were obtained from the Lembang region of West Java, Indonesia. The Department of Biology, Universitas Padjadjaran, identified the plant species.

Extraction and separation

A. wilkesiana leaves were first dried before being extracted three times in 70% ethanol for 24 hours. The resulting extract was evaporated at 50°C and then concentrated in a vacuum. The resulting ethanol extract was fractionated using water, n-hexane, and ethyl acetate as solvents. Utilizing a Wakogel C 200 chromatography column and the vacuum liquid chromatography method, which was based on increasing polarity, the active fraction was recovered (Wako Pure Chemical Industri, Ltd., Osaka, Japan)

Furthermore, the major component with the highest concentration was found after the most active fraction of *A. wilkesiana* was purified using silica G 60 and sulfuric acid ethanol (1:9). Finally, spectroscopic techniques and liquid chromatography-mass spectrometry were used to identify an active compound.

Culture cell and treatment

Cell line breast cancer MCF-7 obtained from Dainippon Pharmaceutical (Tokyo, Japan). The antibacterial drugs streptomycin and penicillin were purchased from Sigma, and the growth media for the cancer cell line was RPMI-1640 enriched with 10% fetal bovine serum (USA). Furthermore, beta-sitosterol with various concentrations was added to the cell culture media for 24 hours.

Sensitivity test

Cell proliferation was measured using a colorimetric resazurin assay at various beta-sitosterol concentrations. Resazurin is used to assess cell metabolism which is an active compound from Alamar Blue and acts as an indicator of oxidation-reduction (redox) reactions for a long time. Resazurin has a blue color non-fluorescent and reduced to color deep fluorescent pink resorption form. Changing color from blue (resazurin) to pink color (resorufin) represents an indicator of reduction by cells. In reaction to the chemical decrease of the growth media brought on by cell development, resazurin will transform from a nonfluorescent dye to a very red fluorescent dye called resorufin. While growth inhibition maintains an oxidized environment, sustained cell growth does the opposite. Due to the growth-related reduction, the redox indicator transforms from an oxidized (blue, nonfluorescent) to a reduced (red, fluorescent) state [19]. Furthermore, to detect reduced resazurin can be used fluorescence (590 nm) or absorbance at 570 nm. The sample's viable cell count is inversely correlated with the fluorescent or colorimetric signal.

Determination of apoptotic activity by flow cytometry

Reagent

1. FITC V Kit (no. 51-65874X)
2. Propidium Iodide (PI) (no. 51-66211E)
3. Annexin V binding buffer (no. 51-66121E)

Coloring

1. Cells were washed with cold PBS twice and then suspended in binding buffer with a concentration of 1 x 10^6 cells/ml
2. Then transferred 100 µl of solution (1 x 10^5 cells) to a 2 ml or 1.5 ml Eppendorf tube.
3. Furthermore, FITC Annexin V and PI were added as much as 5 µl each to all cells that had been treated with the test material and positive control.
4. In control cells are treated:
   a. Cells that were not stained with annexin and PI.
   b. Cells were stained with FITC Annexin V.
   c. Cells were stained with PI.
   d. FITC Annexin V and PI stained cells were then shaken gently and incubated at room temperature for 15 minutes in the dark.
5. Furthermore, 400 µl of binding buffer was added to each tube, and the results were analyzed within 1 hour using flow cytometry [20].

Results and Discussion

Beta-sitosterol isolation and identification

In this study, the most abundant chemical compound in *A. wilkesiana's* active fraction was shown to be beta-sitosterol. After purification from the active fraction of this plant, beta-sitosterol is obtained as a bioactive compound. The chemical structure of beta-sitosterol isolated from *A. wilkesiana* is shown below **(Figure 1)**.

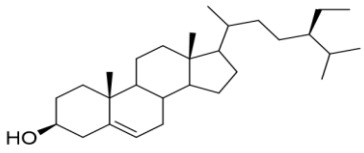

Figure 1. Structure of beta-sitosterol isolated from *A. wilkesiana*

Beta-sitosterol inhibits MCF-7 cells proliferation

Beta-sitosterol was evaluated for its antiproliferative activity against MCF-7 cells.The beta-sitosterol therapy of cancer (MCF-7) cell lines resulted in dose-dependent inhibition of cell growth, as shown by the resazurin assay. After exposure to beta- sitosterol for 48 hours in MCF-7 cells, inhibition of proliferation occurred in MCF-7 cells. The morphology of beta-sitosterol on MCF-7 cells was obtained as follows **(Figure 2)**.

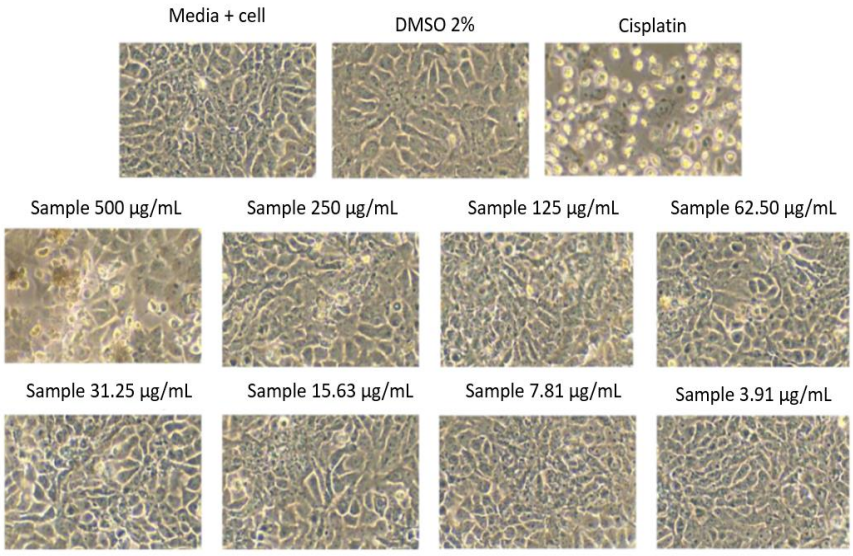

Figure 2. The morphology of beta-sitosterol on MCF-7 cells at various concentrations

Beta-sitosterol induces apoptosis on MCF-7

Annexin V acts as an anticoagulant that can bind phosphatidyl (PS) in the presence of calcium. In the early stages of apoptosis, PS will be translocated to the cell surface. Annexin V can be detected using flow cytometry because annexin V is conjugated with fluorescein isothiocyanate (FITC). Necrotic cells showed membrane permeabilization and would also bind to Annexin V-FITC, but propidium iodide was used to differentiate between surviving, early apoptotic cells and necrotic or late apoptotic cells. Propidium iodide was excreted from viable and early apoptotic cells. Late apoptotic cells will be stained with FITC and propidium iodide due to late necrotic-like cell disintegration.

Beta-sitosterol treatment of the MCF-7 cells stimulated apoptosis in MCF-7 cells after exposure for 24 hours [20].

Furthermore, the result of the beta-sitosterol test curve on MCF-7 cells was obtained as follows **(Figure 3)**:

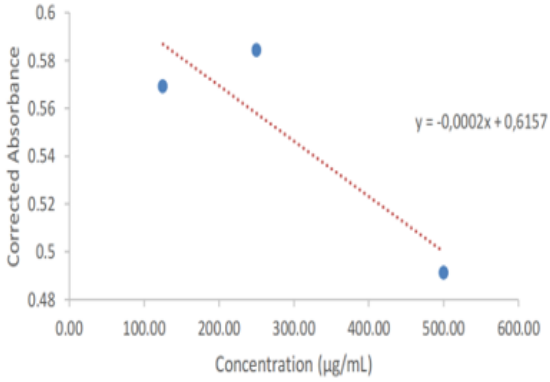

Figure 3. Shows the test result curve for beta-sitosterol on MCF-7 cells.

The result of testing the apoptotic activity of the beta-sitosterol of sablo leaves and identification of the type of cell death by flow cytometry using Annexin-Propidium Iodide (PI) is as follows **(Figure 4)**:

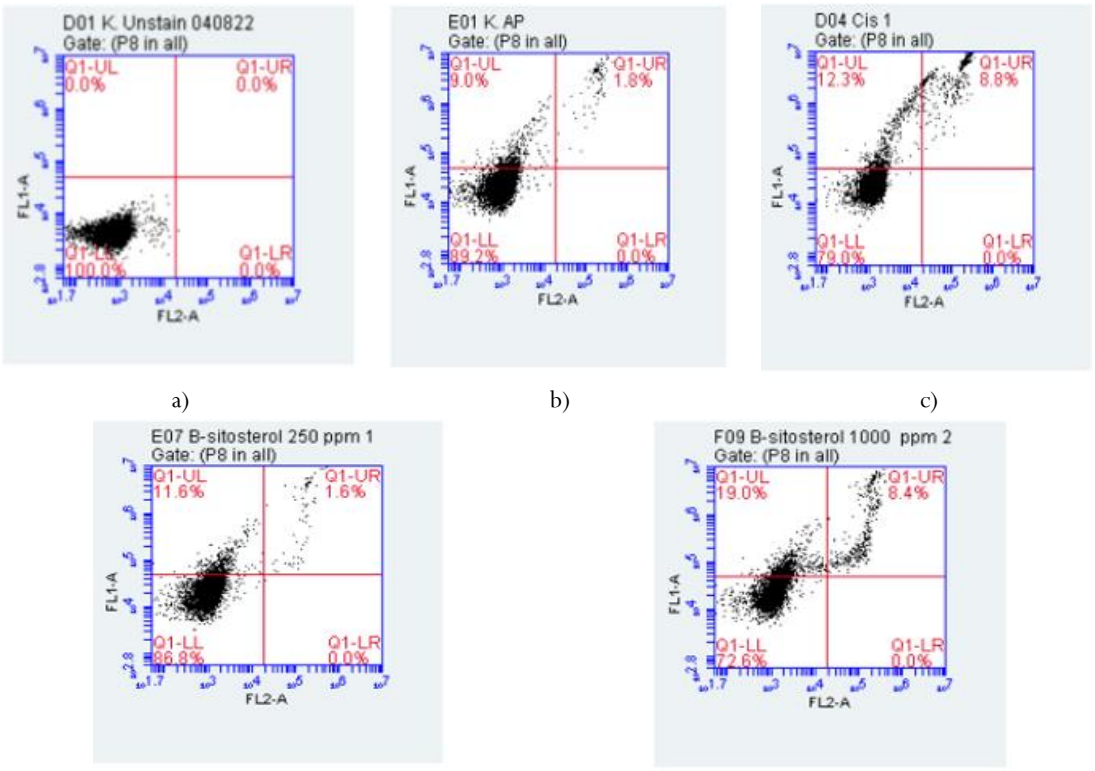

Figure 4. The result of testing the apoptotic activity of the beta-sitosterol of sablo leaves: a) control, b) cell control, c) cisplatin (2.85 ppm), d) beta-sitosterol (250 ppm), e) beta-sitosterol (1000 ppm).

Based on these results, it can be seen that beta-sitosterol 1000 ppm showed the highest apoptotic activity compared to 250 ppm. Compared with cisplatin (positive control), beta-sitosterol also increases apoptotic activity.

In the previous study, *A. wilkesiana* has been shown to have antioxidant, antimicrobial, and cytotoxic effects [8-10]; antibacterial and antifungal, and antimalarial [11-14]; anti-inflammatory, analgesic, and anticancer [15, 16].

Our previous research showed that extracts and fractions of *A. wilkesiana* in breast cancer cells had anticancer activity against MCF-7 breast cancer cells. The results showed that the extracts and fractions inhibited the proliferation of MCF-7 breast cancer cells [18].

In this study, beta-sitosterol inhibited the proliferation of MCF-7 cells according to the dose. In addition, research also shows that the anticancer properties of beta-sitosterol occur through the induction of apoptosis using flow cytometry.

Therefore, these results indicate that beta-sitosterol triggers cell death via the apoptotic pathway in MCF-7 breast cancer cells, so that this compound can be developed as an anticancer agent in the future.

Conclusion

Beta-sitosterol has activity inhibiting proliferation and inducing apoptosis in MCF-7 breast cancer cells.

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Conflict of interest: None

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Ethics statement: None

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