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The Apoptosis Effects of Ethylacetate Extract of *Eleutherine bulbosa* (Mill.) Urb. Against T47D Cells

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Abstract: Eleutherine bulbosa (Mill.) Urb. containing compounds and derivatives such as elecanacine naftakuinon, eleutherine, eleutherol and eleuthernone. Naftakuinon have biological activity as antimicrobial, antiviral, anti-inflammatory, antipyretic, anti-fungal, anti-proliferative and cytotoxic effects against colon cancer and cervical cancer. This research was Aimed to Determine the apoptotic effects of ethylacetate extract of Eleutherine bulbosa (mill.) urb.) Against T47D cells.
Extracts preparation was done using the stepwise method maceration using solvents based on polarity level, the which were n-hexane, ethylacetate and ethanol. Apoptotic effects of ethyl acetate extract of Eleutherine bulbosa (mill.) urb.) were tested using flowcytometry method. The result of Eleutherine bulbosa ethylacetate extract at a dose of ½ and ¼ IC50 showed late apoptosis and necrosis mechanisms.
Key words: Eleutherine bulbosa, T47D, cancer, apoptosis, flowcytometri.

Introduction

The cancer cells arise from normal cells in your body that is being transformed into malignant, due to spontaneous mutations or induced carcinogens (materials/agents trigger cancer). Cell transformation was due to mutations in genes that regulate the growth and differentiation of cells, proto-oncogenes and suppressor genes or (anti-oncogenes). Exposure to carcinogens include various types of viruses, chemicals, radiation and ultraviolet. Most of these carcinogens have the same biological properties that may result in damage to the DNA. One characteristic of cancer cells are able to evade apoptosis mechanisms so that these cells can undergo proliferation is high, due to the absence of a balance between apoptosis and proliferasi[1].The study Showed that the Eleutherine bulbosa (Mill.) Urb. contains naphtoquinonens and derivatives such as elecanacine, eleutherine, eleutherol, eleuthernone[2]. Naphtoquinones has biological activity as antimicrobial, antiviral, anti-inflammatory, antipyretic, antifungal, antiproliferative and cytotoxic effects against colon has and cervical cancer [3,4].

The test results of the test solution T47D cells cytotoxic IC50 value 265 023 ug / ml for n-hexane extract Eleutherine bulbosa (Mill.) Urb., 147 124 ug/ml for ethyl acetate extract and 3782.29 mg/ml for the ethanol extract. Otherwise potent extract if you have IC 50 values less than 500 ug /ml 5. From the test results and calculation of IC50 extracts against T47D cells, IC50 Obtained results below 500 ug / ml for n - hexane and ethyl acetate extract . The Data Showed that the ethyl acetate extract Eleutherine bulbosa (Mill.) Urb . potent against T47D cells with IC50 value of the extract on T47D cells µg/mL 147.124 [6].

This research was Aimed to Determine the triggering apoptotic effects of ethylacetate extract of Eleutherine bulbosa (mill.) urb .) Against T47D cells

Apparatus and Materials

The aparatus were glasses, autoclave (Hirayama), blender (Philips), conical tube, eksikator, Elisa reader (Biorad BenMark), CO2 incubator (Heraceus), an inverted microscope (Olympus), porcelain crucible, laminar air flow (Labconco), micropipette, a rough balance (Home Line), electrical balance (Vibra AJ), oven (Memmert), water bath (Yenaco), rotary evaporator (Haake D1), sentrifugator, a set of water content determination, set of tools distillation, flatbed porcelain cup, porcelain crucible with a lid, desiccator furnace, vortex, 6 - well plate and flowcytometry.

The materials used were aged sabrang onion bulbs ± 4 months (harvest time) were taken from the village of Simalingkar B, Medan, North Sumatra. 96% ethanol, ethyl acetate and n-Hexana distilled, T47D breast cancer cells is a collection of the Laboratory of Parasitology Faculty of Medicine Yogjakarta. Media grower Roswell Park Memorial Institute (RPMI), Media M 199-serum, Fetal Bovine Serum (FBS) 10% (v/v) (Gibco), penicillin- streptomycin 2% (v/v) (Gibco), and Fungizone (amphotericin B) 0.5%. In addition to the above materials are also used 0.25% Trypsin-EDTA (Gibco), Fetal Bovine Serum (FBS), MTT [3-(4,5-dimetiltiazol-2-yl)-2,5 difeniltetrazolium bromide] (Sigma), at a concentration of 5 mg / mL and propidium iodide. Stopper used was sodium dodecyl sulfate in 0.01 N HCl.

Method

Preparation of Extract onion Bulbs Sabrang (Eleutherine bulbosa (Mill.) Urb.)

A total of 10 parts of simplicia was inserted into a vessel, with n-hexane 75 parts, then closed and left for 5 days protected from light, with frequent stirring. After 5 days it was filtered diserkai juice, pulp squeezed and washed with solvents to obtain 100 parts. Maserates moved into a closed vessel, left in a cool area protected from light for 2 days. It was then filtered, evaporated by rotary evaporator and freeze dried. The pulp was dried and macerated again with ethyl acetate and ethanol[7].

Apoptosis With Flowcytometry Assay

T47D cell ($5x10^5$ cells/well) were seeded into 6-well plate and incubated for 24h. Then, the cells were treated by EAEOS and control, and then incubated for 24 h. both floating and adherent cells were collected using 0,025% tripsin and transferred into 1,5 mL tube. The cells were washed twice cold PBS and centrifuged. The supernatant was discharge, while the pellet was collected and fixed gently in cold 70% ethanol in PBS at -20^{o}C for 1 h. the fixed cell were then washed twice with cold PBS and resuspenden in PBS containing PI (40 ug/ml), RNAse (100ug/mL) and triton-100 at 37^{o}C for 30 min. The samples were then analysed using FACScan flowcytometer. Based on DNA contents, percentage of cell in each stage of cell cycle (G_1, S and G_2/M phases) were calculated using ModFit Lt.3.0.s.

Result and Discusion

Apoptosis effect

Observation of apoptosis was conducted using flowsitometri to count living cells , cell necrosis and apoptosis rapidly. In the current study used an Annexin V protein that can specifically bind to phosphatidylserine found on the plasma membrane of cells during the process of apoptosis. DNA in damaged cells both necrosis and apoptosis will be colored by propidium iodide (PI) which produces orange to red fluorescence. As it passes through the laser beam, the cell will be excited and scatter light to produce light fluorescence [8.9].

Testing is done with a variety of treatments apoptosis. Among them is the control shown in Figure 1, the ethyl acetate extract at a concentration of Eleutherine bulbosa ½ IC50 is 73.5 mg / mL is shown in Figure 2 , namely IC50 ¼ 36.75 mg / mL is shown in Figure 3 , In T47D cells were given a test solution ½ IC50 seen the percentage of cells undergoing early apoptosis 0.00 % ; IC50 ¼ 0.20 % ; 4.93 % of control . The percentage of cells undergoing apoptosis and necrosis beginning at the end of the administration of various concentrations very much . In granting ½ IC50 58.21 % ; IC50 ¼ 33.62 % ; whereas 1.71 % in the control cells . On treatment with EEABS combination with doxorubicin and spur apoptosis because of ethylacetate Ekstral Eleutherine bulbosa possibility to suppress the expression of Bcl - 2 and bax protein boost that induces apoptosis pathway . Decreased expression of Bcl - 2 causes increased permeability of the mitochondrial membrane, some proteins can activate the caspase cascade . One of these proteins is cytochrom - c required for the process of respiration

in motokondria . In the cytosol , cytochrom c binds to the protein Apaf - 1 (apoptosis activating factor - 1) and activates caspase - 9 and there was cell death (apoptosis)[10].

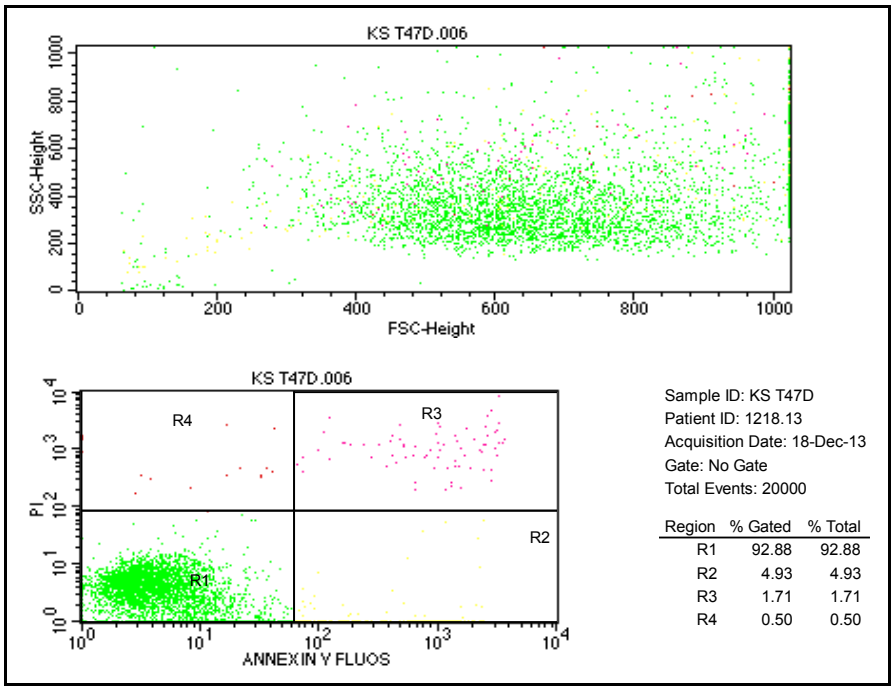

Figure 1 Overview percentage of control T47D cell conditions

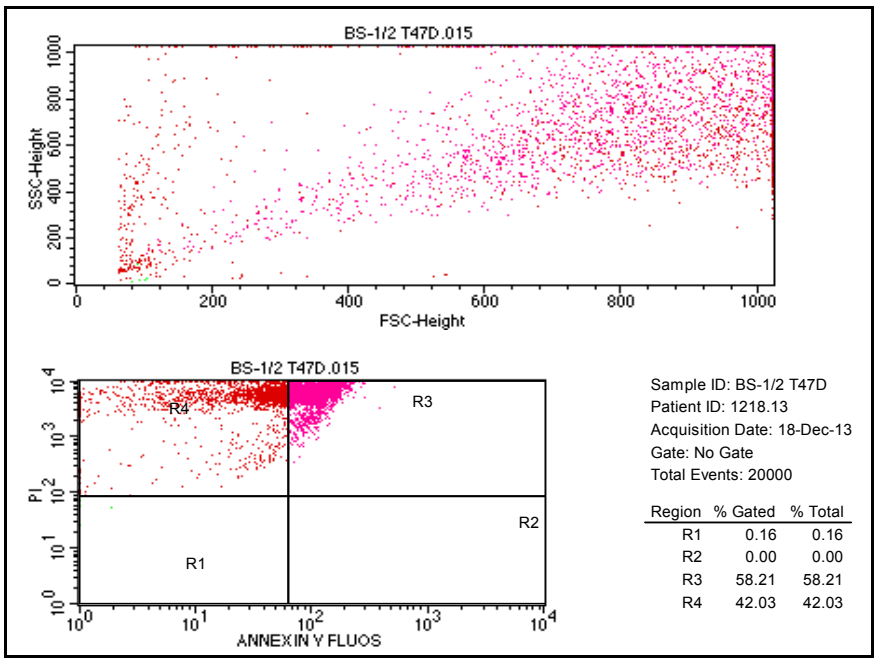

Figure 2 Preview percentage T47D cell conditions are given ½ IC50

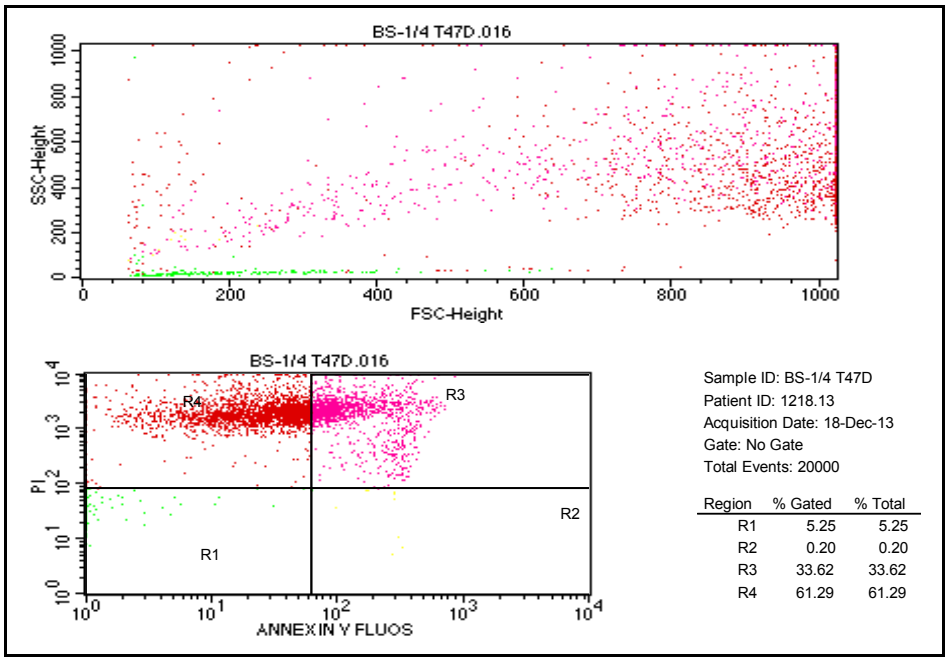

Figure 3 percentage description given conditions T47D cells IC50 ¼ EEABS

Information
The top image shows a picture of the overall cell
The bottom image is the image of the cell after grouped and dipersentase.

The mechanism of apoptosis is also due to the flavonoid content of ethylacetate Ekstral Eleutherine bulbosa, Flavonoids inhibit the expression of the enzyme topoisomerase I and topoisomerase II plays a role in catalysis screening and DNA relaxation. Topoisomerase enzyme inhibitor complex will stabilize and cause DNA topoisomerase clipped and damaged so continue to the process of apoptosis[11].

Late cells undergoing necrosis are also very much . In T47D cells were given Ekstral ethylacetate Eleutherine bulbosa ½ IC50 of 42.03 %; IC50 ¼ 61.29 %; 0.50% whereas in control. The number of living cells in the control of 92.88%; on treatment with ½ IC50 0.16 %; IC50 ¼ 5.25 %. This may be due to exposure to cells for too long. According to the study of apoptosis is a time dependent process . The low number of living cells on treatment with ethyl acetate Ekstral of Eleutherine bulbosa can be caused by too long exposure given that the number of cells that die will be more and more[12].

Tabel 1 The test results of apoptosis Ekstral ethylacetate Eleutherine bulbosa in T47D cells with various concentrations

Treatment	Concentration µg/mL	R_1 (%)	R_2(%)	R_3(%)	R_4(%)
Kontrol	0	92,88	4,93	1,71	0,50
EEABS $^1/_2$ IC_{50}	73,5	0,16	0,00	58,21	42,03
EEABS $^1/_4$ IC_{50}	36,75	5,25	0,20	33,62	61,29

Description: R1 = live cells, R2 = cells undergoing early apoptosis, R3 = cells undergoing apoptosis and necrosis early end, R4 = cells undergoing late necrosis

Conclusion

Ethyl acetate extract of Eleutherine bulbosa showed late apoptosis and necrosis mechanisms at ½ and ¼ IC50 concentrations.

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