

## Effects of Inhibition Cell Cycle and Apoptosis of Poguntano leaves Ethylacetate Extract (*Picria fel-terrae* Lour.) on Breast Cancer Cells

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**Abstract:** Poguntano (*Picria fel - terrae* Lour.) is one of the medicinal plants in North Sumatra used to treat degenerative and metabolic diseases. Previous research has conducted tests on the cytotoxic effects of n-hexane extract of leaves poguntano with doxorubicin against breast cancer cells MCF-7 in vitro. Ethylacetate extract of leaves poguntano (EAELP), also has potent cytotoxic effect against breast cancer cells, but subsequent studies have not been conducted. This study aims to determine the mechanism of cell cycle inhibition and the mechanism of apoptosis. Preparation of extracts was done by graded with solvents on the level of polarity. The solvents were *n*-hexane, ethyl acetate and ethanol as well as performed phytochemical screening and characterization of simplex and extracts. Extracts were tested against MCF-7 cells with effects inhibition of cell cycle and apoptosis with flowcytometry method. Furthermore EEALP tested for cell cycle, the result is Inhibition of cell cycle on G<sub>0</sub>-G<sub>1</sub> phase with a percentage 49.46% and leads to apoptosis of 18.02 %.

**Keywords:** Poguntano leaves, *Picria fel-terrae* Lour, Breast Cancer Cells, Apoptosis, Inhibition Cell Cycle.

### Introduction

Research to obtain anticancer drugs, among others, performed by finding natural compounds derived from plants. This is due to the tendency of people to return to the higher nature using traditional medicine. Plant poguntano (*Picria fel - terrae* Lour.) In East and Southeast Asia have traditionally been used as a stimulant, diuretic, malaria drugs, hyperglycemia, fever, herpes infection, cancer and inflammation for over 200 years<sup>1</sup>. Poguntano in North Sumatra leaf commonly used as a remedy for hyperglycemia<sup>2</sup>. Research shows flavonoids contained in the extracts glucuronide poguntano butanol, are compounds apigenin 7-O-β-glucuronide, luteolin 7-O-β-glucuronide and apigenin 7-O-β-(2"-O-α-rhamnosyl) glucuronide<sup>3</sup>. Model of breast cancer cells are frequently used in the study were MCF-7 (Michigan Cancer Foundation-7). MCF-7 breast cancer cells that are expressing the estrogen receptor (ER+) and pleural effusion derived from a breast adenocarcinoma Caucasian female patient aged 69 years, blood group O<sup>4</sup>. These cells express estrogen receptors and have properties resistant to doxorubicin<sup>5</sup> and do not express caspase-3<sup>6</sup>. MCF-7 cells, P-glycoprotein expressed high, so the sensitivity to chemotherapeutic agents such as doxorubicin lower<sup>7</sup>. Breast cancer is a type of cancer that most often affects women and is the leading cause of death in women worldwide data from the World Health Organization in 2008, breast cancer is the commonest cancer with 1.38 million new cases and a cause of death in the world 458,000 per year<sup>8</sup>.

Test results cytotoxic leaf extract test solution poguntano against breast cancer cells MCF-7 IC<sub>50</sub> value 119.906 mg/mL to extract n-hexane, 119.990 mg/ mL for the ethyl acetate extract, and 307.719 mg/mL for the ethanol extract<sup>9</sup>.

Based on the above, further research needs to be done on the leaves poguntano is to determine the leaf ethyl acetate extract poguntano in the inhibition of cell cycle and apoptosis pacing.

## **Apparatus and Materials**

The apparatus were glasses, autoclave (Hirayama), blender (Philips), conical tube, eksikator, Elisa reader (Biorad BenMark), CO<sub>2</sub> 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, 96 - well plate, 6 - well plate and flowcytometry.

The materials used were poguntano leaves were taken from the village of Tiga Lingga, Dairi, North Sumatra. 96% ethanol, ethyl acetate and n-Hexane distilled, MCF-7 breast cancer cells is a collection of the Laboratory of Parasitology Faculty of Medicine Yogyakarta. 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 Poguntano Leave (*Picria fel-terrae* Lour.)**

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<sup>14</sup>.

### **Flowcytometry assay**

MCF-7 cell ( $5 \times 10^5$  cells/well) were seeded into 6-well plate and incubated for 24h. Then, the cells were treated by EAELP 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°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°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<sub>1</sub>, S and G<sub>2</sub>/M phases) were calculated using ModFit Lt.3.0.s.<sup>15</sup>.

## **Result and Discussion**

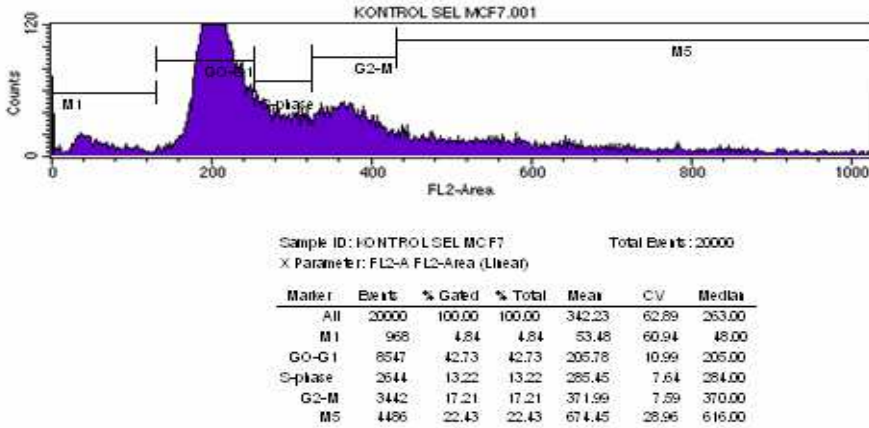
### **Inhibition of cell cycle**

The data was analyzed by flowcytometry cell quest program to see the distribution of cells in the phases of the cell cycle sub G<sub>1</sub>, S, G<sub>2</sub>/M and cells undergoing polyploidy. Flow-cytometry was performed with 488 nm light beam in medium speed (500 cells/sec).

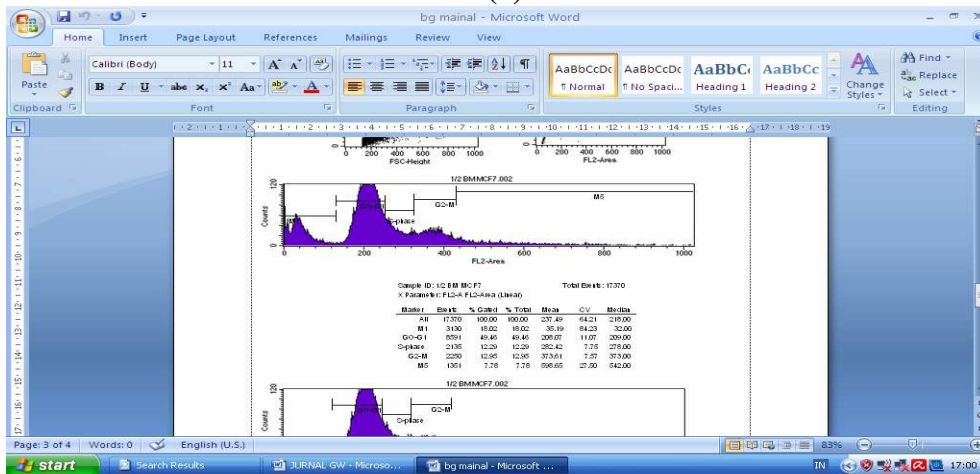
Accumulation of cells in the cell cycle was one of the main targets of anticancer agents. This study observed the cell cycle by flowcytometry method. With this method, we can see the distribution of cells in each of phase of the cell cycle after treatment, so it can be estimated EAELP inhibitory pathways cell cycle. Phases of the normal cell cycle have differences on the number of sets of chromosomes that G<sub>1</sub> phase is the number of sets of chromosomes 2n. Continuesly in S phase, the number of sets of chromosomes between 2n and 4n as a process of replication, whereas the G<sub>2</sub> and M phases, replication has been perfectly formed set of 4n chromosomes. With the fluorochrome that has the ability to be combined with bases such as propidium iodide DNA strands so each cell has a different number of sets of chromosomes to provide different fluorescence intensities. The more sets of chromosomes the greater fluorescence intensity will be. The tools that were used to

read the fluorescence intensity of each cell in this study were FACS (Fluorescence Activated Cell Sorting) or flowcytometer.

Testing of the cell cycle in MCF-7 cells with flowcytometry method was performed by a variety of treatments. Among them was the control shown in Figure 1 and EAELP on 1/2 IC50 concentration was 60 mg/mL (Figure 1). MCF-7 cell cycle profile after treatment was shown in Table 1 and shown below.



(a)



(b)

Figure 1: Overview of the cell cycle MCF-7 (a) control, (b) given EAELP 1/2 IC50

Table 1 Distribution of MCF-7 cells after treatment with IC50 concentrations EAELP (60 ug/ml)

Treatment	Concentration	Cell phase (%)				
		M1	G <sub>0</sub> -G <sub>1</sub>	S	G <sub>2</sub> -M	M5
Kontrol	0	4.84	42.73	13.22	17.21	22.43
EAELP	1/2 IC <sub>50</sub>	18.02	49.46	12.29	12.95	7.78

Data analysis was done descriptively, by comparing with the control treatment. Cell cycle analysis was performed on the phase of the cell cycle that the largest accumulation of the cells in each treatment was occurred.

Accumulation of cells in the treatment EAELP with concentration 60 ug/ml (1/2 IC<sub>50</sub>) was a G<sub>0</sub>-G<sub>1</sub> phase with value 49.46%. When compared with controls accumulation in G<sub>0</sub>-G<sub>1</sub> phase with value 42.73% and the effect of treatment with the extracts of G<sub>0</sub>-G<sub>1</sub> phase is much different, so it can be said that the concentration EAELP 1/2 IC<sub>50</sub> (60 ug/ml) showed an influence on the cycle MCF-7 cells.

Constraints on the cell cycle regulation of G<sub>0</sub>-G<sub>1</sub> cycle by EAELP 60 ug/ml was occurred by the decreasing of expression level of cyclin D so it was not activated of CDK4 and CDK6 which was cause the inhibition of PRB (retinoblastoma protein), which is not phosphorylated Rb binds with E2F transcription factors bind DNA and inhibit transcription of genes whose products are required for S phase of the cell cycle so that the

cells retained in the G1 phase or G1 arrest occurs. Inhibition of the cell cycle could be caused by the ability of the compounds contained EAELP increase expression of p21 and p27 proteins become a complex bond with Cyclin D and Cyclin Dependent Kinase 4/6 (CDK), so that would inhibit the phosphorylation PRB (retinoblastoma protein). Thus it was inactive E2F, that led to the cessation of the cell cycle. Cessation of the cell cycle at G0-G1 phase provides an opportunity for cells to repair damaged DNA if it could not be repaired further in to the apoptosis process. Accumulation of cells in the M1 phase was assumed as apoptosis ½ IC50 concentration (60 mg/ml) is equal to 18.02%. This activation is likely to cause inhibition of the protein expression of Bcl2 (anti- apoptotic protein).

## Conclusion

Tested for cell cycle, the result is Inhibition of cell cycle on G<sub>0</sub>-G<sub>1</sub> phase with a percentage 49.46% and leads to apoptosis of 18.02%.

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