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The cytotoxic effect of *Elephantopus scaber* Linn extract against breast cancer (T47D) cells

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Abstract. Breast cancer is one of the main cause of death. *Elephantopus scaber* Linn (ES) which has been used as a traditional medicine contains an antitumor compounds. This study aimed to expl the active fraction from ethanolic extract of ES as anticancer and to determine its inhibition effect on the cell proliferation cycle of breast cancer (T47D) cells. ES leaf was macerated with ethanol and then evaporated to get the concentrated extract. The extract was fractionated using petroleum ether, chloroform, and methanol respectively. The cytotoxic activity of each fraction was carried out with MTT method, and the inhibition of cell cycle test were observed by flowcytometry method. The result showed that ES and the fractions have cytotoxic activity against T47D cell lines with IC_{50} values of extract, petroleum ether, chloroform, and methanol fractions were 58.36±2.38, 132.17±9.69, 7.08±2.11, and 572.89±69.23 µg/mL. The inhibition effect of ethanol extract on the lifecycle of cells was occured in sub G1 phase. There was no prolonging of G1, S, G2/M and polyploidy phase of T47D cell lines. The chloroform fraction of ES is the most cytotoxic fraction against T47D cells without prolonging the cell lifecycle.

Keywords: *Elephantopus scaber*, cytotoxic, T47D, IC_{50}, cell cycle

1. Introduction

Breast cancer is the most common cancer among women and often causes death. The incidence of breast cancer is still high and difficult to be cured although the therapy has developed and progressed rapidly. The common treatment of breast cancer is consist of surgery, radiotherapy, chemotherapy and hormone therapy [1, 2].

Chemotherapy is a choise to stop the growth of cancer cell, but it is very toxic and has many side effects. The failure of chemotherapy can be associated with the failure of anticancer agents to induce programmed cell death (apoptosis). There are many reports of cancer cells resistance to chemotherapy. The resistance can be caused by overexpression of PGP in cells that lead to the presence of drug efflux out of the cell. Therefore, development of new cytotoxic agents for cancer therapy is urgently needed [3].

Many cytotoxic agents were provided in medicinal plants. *Elephantopus scaber* is a plant which has been reported as cytotoxic agent. The extract and fractions of *E. scaber* was proven to induce apoptosis against cervical cancer [4, 5]. *E. scaber* has been used traditionally to treat various deseases [6].

The anticancer potency of some chemothera was associated with the capability to inhibit the cells growth a ertain phase of cell lifecycle. Based on differences in DNA content, cells can be distributed in phases of the cell cycle i.e. sub G1, G1, S, G2 and M as well as the polyploidy cells [7, 8]. The objective of this research was to explore the potency of ES extract as cytotoxic agent against breast cancer cells and screening the active fraction from the extract. The active fraction was then assayed the potency for inhibiting cell cycle.

2. Materials and Methods

2.1. Plant material collection

The plant material was collected from Merapi Farma, Yogyakarta, Indonesia. It was dried and blended.

2.2. Extraction and fractionation

The 200 g of ES powder was macerated using 900 mL of ethanol. The extract was collected and evaporated to get the concentrated extract. The concentrated extract was subsequently dissolved in petroleum ether and shaked for 6 hours and allowed to equilibrium for 24 hours. The soluble fraction was separated as petroleum ether fraction and the non soluble fraction was then fractionated using chloroform, ethyl acetate and methanol respectively. All fractions were evaporated and the dried fractions were collected.

2.3. Cytotoxicity test

Cytotoxicity assay was carried out using MTT thod. The 100 µL suspension of T47D cells at a density of $1x10^4$ in 96 wells micro-plate were incubated for 24 h at 37°C (5% CO_2) with 100 µL RPMI medium and ES fraction with concentration series of test 2000; 1500; 1000; 800; 400; 200; 100; 50; 25; 12.5; 6:25; and 3.125 µg/mL.

After th media was discarded by inverting the plate slowly ad followed by adding 100 µL of MTT, n incubated for 4 hours at 37°C, 5% CO_2. After incubation, the mixture were added with 100 mL of SDS solution in 0.01 N HCl and incubated for over night at room temperature. The microplate were read by ELISA reader at 550 nm wavelength. The IC_{50} values were then calculated from percentage of living cells [9].

2.4. Flowcytometric analysis

he T47D cells was treated using ES extract with concentration equal to ½IC_{50} and IC_{50} and inc ated for 24 hours. The cells was then washed using PBS and subsequen was added with 100 µL of Annexin V-PI and 350 µL of PBS. Furthermore, the cells was given DNase free RNase (20 µg/mL) and incubated at 37°C for 10 m. The cell was analysed using FACS Calibour Flowcytometry.

3. Results and Discussion

Medicinal plants produce a lot of bioactive compounds and can be used in treatment of many deseases. The exploration of therapeutic potential of the plants has been ca ed out since several decades ago. One of them is addressed to find the potential anticancer. Cancer is one of the major health problems and ead to a high of death numbers. Breast cancer is the most common cancer among women. The great potential of plant-based compounds for the treatment and prevention of cancer is attributed to their safety, low cost, and oral bioavailability.

E. scaber have been used traditionally to treat various diseases as antiinflammation, diarrhea, hepatitis, arthritis. With the diverse traditional applications of *E. scaber*, United Nations Development Program has recommended *E. scaber* as a potential natural herb which should be further studied [6].

3.1. Cytotoxicity test

Fractionation process was conducted using petroleum ether, chloroform and methanol to separate the compounds with different polarity. It would be useful to get the fraction with the highest cytotoxicity. In this study, T47D cell lines were used as targetted cell in the cytotoxicity test of ES fractions. The T47D cell lines was derivated from breast cancer cells, so it can become the tested cells to find the potential activity of ES fraction against breast cancer cells. The citotoxic activity was evaluated by MTT method [10]. It was based on the activity of tetrazolium succinate reductase enzyme in viable

cells reducing tetrazolium salt *(3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromide)* to produce formazan crystal [9]. The concentration of dissolved formazan was measured by spectrophotometric method.

Figure 1 showed the percentage of T47D cell viability after ES fraction treatment. The viability of T47D cells were shown in dose dependent manner. The higher concentration resulted the more viability of the cells. The IC_{50} value of ES extract and each fraction were performed in Table 1.

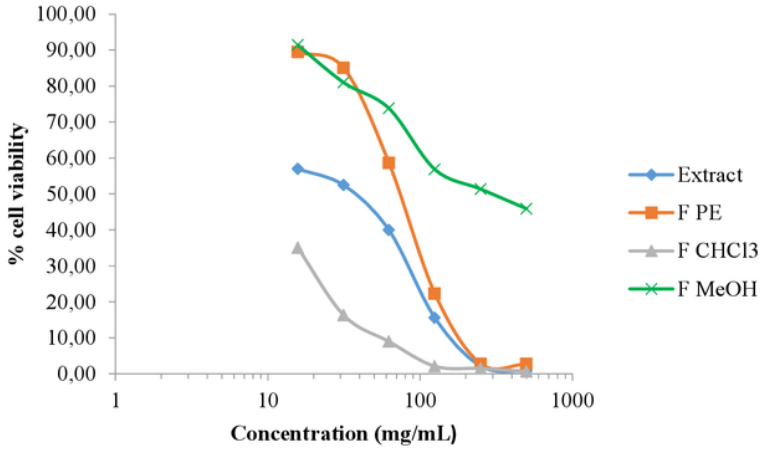

Figure 1. The cytotoxicity of ES extract and fractions against T47D cells.

Table 1. The cytotoxicity of ES extract and fraction against T47D cells presented as IC_{50}.

Fraction	IC_{50} (µg/mL)
Extract	58.36 ± 2.38
F PE	132.17 ± 9.69
F $CHCl_3$	7.08 ±2.11
F MeOH	572.89 ± 69.23

Based on the Table 1, it's revealed that chloroform fraction (F $CHCl_3$) treatment resulted the lowest IC_{50} indicated the high cytotoxicity against T47D cells. Some chemical constituent has been reported and identified as antitumor were deoxyelephantopin [11], scabertopinol, trans-caffeic acid, methyl 3,4-dicaffeoylquinate, luteolin-41-O-β-D-glucoside, trans-p-coumaric acid, indole-3-carbaldehyde, methyl trans-caffeate, luteolin-7-O- glucuronide 6"-methyl ester, and luteolin [12].

Figure 2 showed the cytotoxicity of the chloroform fraction. The morphology of cells in the control group (without ES fraction treatment) is very different from the treatment (using 1.5625 µg/mL of chloroform fraction) group. In the control group, the cells shape is tapering with nucleus looked clearly. On the other hand, the cells in the teatment seemed more little and circle with lower density than control.

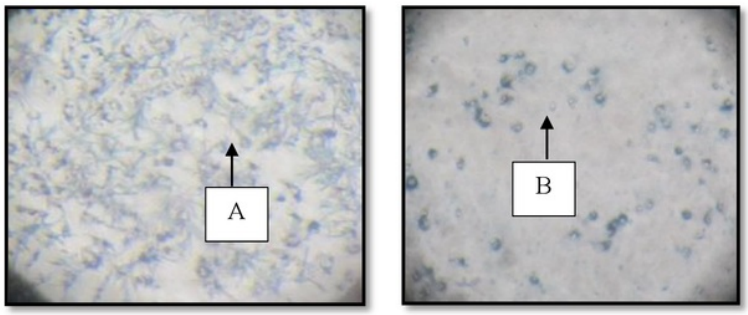

Figure 2. Microscopic performance of T47-D cells of control group (A) and group of treatment with 1.5625 µg/mL of chloroform fraction (B)

3.2. Flowcytometric analysis

The flowcytometric analyses was subject to study the effect of ES extract to the cell cycle. The flowcytometry can inform the cells distribution in phases of sub G1, G1, S, G2/M, and the polyploidy cells based on the amount of chromosome set. Effect of the extract can be known by comparing the effect between control and treatment. The concentrations of ES extract used in this study were equal to ½ IC_{50} and IC_{50}.

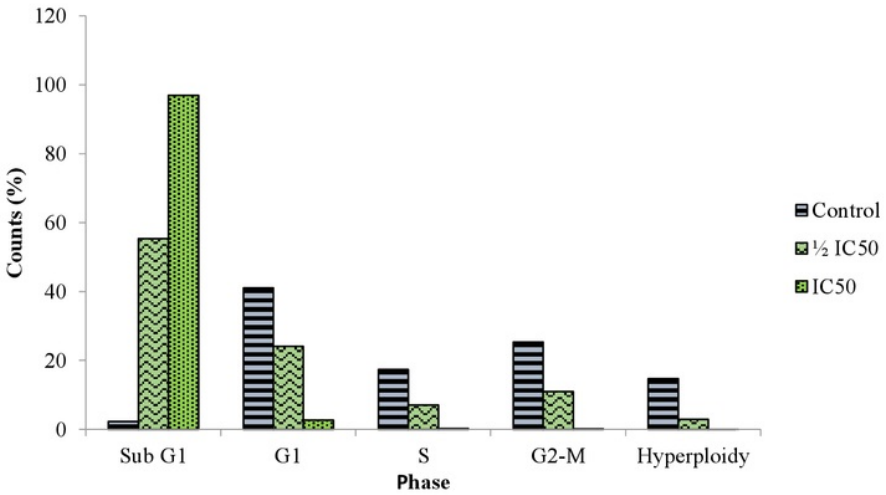

Figure 3. Comparation of T47D cell lines distribution in 5 cell phases among the control and the treatment with ½ IC_{50} as well as IC_{50} concentration of ES extract.

Figure 3 showed that in the control, the cells were distributed in all phases, mainly in G1 (41.07%), S (17.34%), and G2-M (25.34%) phases. The sub G1 phase only contained 2,27% of cells total. In the treatment of ½ IC_{50} of ES extract, the cells were most accumulated in Sub-G1 phase (55.34% of cells total). There were decending of cell concentration in phase of G1, S, G2-M and the polyploidy cells compared to the control. It means that this treatment resulted cell growth inhibition since the sub G1 phase or the cells were dead before growing to next stage. Nevertheles, the other cells still develop in the next steps. The same result occured when the cells were treated by ES extract with the concentration equal to IC_{50}. Almost all of cells (96.95% of cells total) were detected in sub G1 phase. This ES extract concentration arrested all cells in sub G1. Accumulation of cells in sub G1 phase may

consist of apoptotic and/or necrotic cells [13–15]. Many factors may contribute the apoptosis as well as necrosis process. Hence, this study can be use to observe what mechanism inducing cells death in the next study.

4. Conclusions

E. scaber extract exerted anti-cancer activity on breast cancer (T47D) by cell cycle arrest in sub G1 phase. The chloroform fraction of *E. scaber* ethanolic extract was the highest active fraction.

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