Jurnal Kedokteran Hewan September 2020, 14(3):73-79
P-ISSN : 1978-225X; E-ISSN : 2502-5600 DOI: https://doi.org/10.21157/j.ked.hewan.v14i3.17227

GROWTH INHIBITION AND INDUCTION OF APOPTOSIS IN MCF-7 AND T47D BREAST CANCER CELL LINES BY ETHANOL EXTRACT OF SEURAPOH (*Chromolaena odorata*) LEAVES

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ABSTRACT

This study aims to determine the growth inhibition and apoptosis induction of MCF-7 and T47D cancer cells by ethanol extract of *Chromolaena odorata* leaves. A post-test with control group design was used in this study. The extract was made by maceration with 80% ethanol and the tested concentrations used were 7.80 µg/mL-500 µg/mL with standard drug doxorubicin ranged from 1.56 µg/mL-100 µg/mL. The growth inhibition was determined by the MTT colorimetry method, apoptosis induction by double staining using acridine orange-ethidium bromide, and the existence of apoptosis was proven immunocytochemically through the expression of Bcl-2 proteins. The results showed that the growth inhibition of MCF-7 was 100.29%-28.19% and T47D was 100.37%-16.01%. The IC50 values of MCF-7 was 327.34 µg/mL and T47D was 135.16 µg/mL. The presence of apoptosis was marked by finding the morphological change of cells such as dead, necrosis, and chromatin condensation cells. This suggests that interventions with ethanol extract of *Chromolaena odorata* leaves can induce apoptosis that has been proven by reducing the expression of Bcl-2 proteins.

Key words: apoptosis, Bcl-2, *Chromolaena odorata*, extract, MCF-7, T47D

ABSTRAK

Penelitian ini bertujuan mengetahui hambatan pertumbuhan dan induksi apoptosis sel kanker MCF-7 dan T47D oleh ekstrak etanol daun Chromolaena odorata. Penelitian ini menggunakan post-test with control group design. Ekstrak dibuat secara maserasi dengan etanol 80% dengan konsentrasi uji 7,80 µg/ml-500 µg/ml, obat standar doxorubicin 1,56 µg/ml-100 µg/ml. Penghambatan pertumbuhan ditentukan dengan metode kolorimetri MTT, induksi apoptosis dengan pewarnaan ganda menggunakan acridine orange-ethidium bromide, dan keberadaan apoptosis dibuktikan secara immunositokimia melalui ekspresi protein Bcl-2. Hasil penelitian menunjukkan hambatan pertumbuhan sel MCF-7 adalah 100,29%-28,19% dan sel T47D 100,37%-16,01%. Konsentrasi ekstrak yang mampu menghambat pertumbuhan sel 50% (IC_{50}) MCF-7 adalah 327,34 µg/ml dan sel T47D 135,16 µg/ml. Adanya apoptosis ditandai dengan perubahan morfologi sel seperti sel mati, nekrosis, dan sel yang mengalami kondensasi kromatin. Studi ini membuktikan bahwa intervensi terhadap sel kanker MCF-7 dan T47D dengan ekstrak etanol daun Chromolaena odorata dapat menginduksi apoptosis dengan berkurangnya ekspresi protein Bcl-2.

Kata kunci: apoptosis, Bcl-2, Chromolaena odorata, MCF-7, T47D

INTRODUCTION

Breast cancer is a common cancer in women both in developed and developing countries. It is estimated, breast cancer is around 16% of all cancers that occur in women. Although it is considered a common cancer in developed countries, 69% of breast cancer deaths occur in developing countries (Jemal *et al*., 2011; Torre *et al.*, 2015; Siegel *et al.*, 2016). The main goal of cancer therapy is to inhibit the growth of cancer cells through several pathways including apoptosis. Some of the drugs used in cancer treatment work not selectively, affect normal cells and cause side effects that are difficult to tolerate so their use is limited. The emergence of toxicity and anticancer resistance has also been widely reported, so this condition requires fast efforts to find new anticancer with high effectiveness and, safe. Efforts to develop anticancer must be done through structured and systematic research to get more effective and safer anticancer. Anticancer development can start from the results of chemical synthesis, semisynthesis, medicinal plants that are popular in the community and proven to be efficacious including its products (Shoeb, 2006).

Several uses of medicinal plants in the community have been proven empirically, this has triggered researchers in various countries to prove their usefulness, as well as studying the mechanisms that work as scientific anticancer. Therefore, the discovery and development of new anticancer with specific mechanisms of action is very important to know as a definite target of drug action. Exploration of anticancer compounds is an alternative effort to increase the potential drug and reduce their side effects. One of medicinal plant that grows in Aceh Province, Indonesia is *Chromolaena odorata* in the family of Asteraceae. This weed also grows in plantation areas in Central America, South Asia and West Africa. *Chromolaena odorata* leaf extract has antiproliferative (Kouamé *et al.*, 2013), hemostatic (Pandith *et al.*, 2012), anti-inflammatory (Hanh *et al*., 2011), antidiabetic and anticataractic (Onkaramurthy *et al.*, 2013) and, also capable to activation of poly ADP ribose polymerase γ (Zhang *et al.*, 2012).

Other researchers state that *Chromolaena odorata* leaf extract can also be used for the treatment of dermato-mucosal infections (Suksamrarn *et al.*, 2004). The anticancer activity of leaf extracts of *Chromolaena odorata* on human cell lines and mouse

cell lines has been reported (Phan *et al.,* 2001; Vital and Rivera, 2009) and the ethyl acetate soluble fractions of the 70% ethanol extract of *Chromolaena odorata* leaf, showed significant cytotoxic activity in the inhibition of HL-60 cancer cell lines. The compounds in the leaves of this medicinal plant were identified as aromadendrin 4' methyl ether, eriodictyol 7, 4'-dimethy ether, 4' -methyl ether, isosakuranetin, quercetin 7, 4'-dimethy ether, kaempferide, acacetin, rhamnazin, quercetin 3-O-rutinoside, kaempferol 3-O-rutinoside, and kaempferol 3-O-glucopside (Hung *et al.,* 2011).

The inhibition of cancer cell growth after intervention with ethanol extract of *Chromolaena odorata* leaves was determined by the MTT colorimetry method (Mosmann,1983). This method is already commonly used in determining the presence or absence of anticancer activity based on its inhibitory growth. One mechanism of anticancer action is through apoptosis. Apoptosis is a process programmed from cell death that is genetically regulated for elimination of unwanted cells or death cells (Gherghi *et al.,* 2003). Apoptosis is an important process in the regulation of normal homeostasis and, this process produces a balance in the number of certain tissue cells through the elimination of damaged cells and physiological proliferation that will maintain the normal tissue function (Li-Weber, 2013).

Several methods have been carried out by researchers to identify the apoptosis induction in cancer cells *in vitro*. Fluorescent microscope is one of tool that can be used for detecting some characteristic morphological changes of cells undergoing apoptosis. This method can also accurately distinguish cells in different stages of apoptosis (Baskić *et al.,* 2006). The changes in cell morphology after anticancer intervention can be observed after double staining with acridine orange and ethidium bromide. The molecular mechanism underlying apoptosis is determined based on protein expression. In this study, observations of Bcl2 protein expression were carried out through immunocytochemical analysis, which aims to find out whether the extract has the ability to induce apoptosis through decreased protein expression (Endro *et al.,* 2012). This research is beneficial for the development of new anticancer in the future.

MATERIALS AND METHODS

Extraction of *Chromolaena odorata* Leaves

Briefly dried powder of *Chromolaena odorata* leaves was extracted by maceration using 80% ethanol (ratio 1 : 10) for 24 hours and stirring every 4 hours. After filtration, the residue was macerated for three times until a clear filtrate is obtained. Then, all filtrates are combined and concentrated with a vacum rotary evaporator until a concentrated extract obtained. The concentrated extract was heated at low temperature in oven to remove the solvent residue. Furthermore, the concentrated extract was weighed and stored in a tight dark bottle.

Test for Inhibition of Cancer Cell Growth by MTT Colorimetry Method

Extract of *Chromolaena odorata* leaves was made with the concentration of 500; 250; 125; 62.5; 31.25; 15.63 and 7.80 ug/mL. As a positive control is used doxorubicin in concentration of 100; 50; 25; 12.5; 6.25; 3.13; 1.56 ug/mL. Each concentration series was replicated 3 times. Cancer cell lines T47D and MCF-7 were suspended in media culture, then put into a plate containing 96 wells and incubated for 24 hours in 5% CO_2 incubator at 37° C. After 24 hours the media was discarded and washed with PBS. Furthermore, the tested extract and doxorubicin series concentrations were inserted into the well, then incubated for 24 hours. At the end of the incubation, the solution in the plate is removed and washed with PBS. Then 100 µL MTT reagent was added in each well and incubated for 4 hours until formazan salt is formed. After formazan salt is formed, 10% SDS stopper in 0.1 N HCl is added and then incubated in a dark place overnight. After that the reading is done by using a microplate Elisa Reader with a wavelength of 595 nm. Living cells will react with MTT to form a purple color. The absorbance was read from each well, and then the data converted to the percentage of viable cells. The percentage of viability cell was determined using the following formula:

$$\text{Percentage of Viable Cells} = [(B-C)/(A-C)] \times 100\%$$

A= Absorbance of control group
B= Absorbance of treatment group
C= Absorbance of medium

To determine the IC_{50} of the tested extract is calculated the percentage of viability cells by probit analysis. The potency of cancer activity is represented by IC_{50} value that represents a concentration of the extract that produce 50% cells death (CCRC, 2013)

Apoptotic Induction

The coverslip (Nunc) is planted into 24 well plates and cells are distributed on it with density used was 5 x 10^4 cells/well in 1000 µL of culture media. Incubation is carried out for 24 hours in a CO_2 incubator, up to 60-80% confluent. Then, the culture cells were given 100 µL of the extract at IC_{50} concentration in triplicate, including cells control. Furthermore, the culture was incubated in an incubator at temperature 37° C for 24 hours. At the end of incubation, the culture media was washed with PBS, and the coverslip was removed from the well and placed on a glass object and then dropped with acridin orange-ethidium bromide of 10 µL and allowed for 5 minutes. Furthermore, the coverslip is immediately observed under a fluorescence microscope (Zeiss MC 80) using a magnification of 10 x 40. Observations were made on all cells, normal and dead cells and undergoing changes such as membrane blebbing, nuclear condensation, and other features of

apoptosis also cytoplasmic and nuclear changes. The figure is documented with a digital camera (CCRC, 2013).

Immunocytochemistry Assay

Breast cancer cell lines (MCF-7 and T47D) in density 5 x 10^4 cells/well were seeded on coverslips in 24-well plates and incubated in 37° C incubator (5% CO_2) until confluent. Then, cells were incubated with ethanol extract of *Chromolaena odorata* leaves for 24 h. Culture medium were removed and cells were washed in PBS. Cells were fixed with cold methanol for 10 minutes and washed by PBS. Then, H_2O_2 as blocking solution were added on the fixed cells for 10 minutes, removed, and normal mouse serum was added for 10 minutes, removed, and incubated with monoclonal antibody anti-Bcl-2 (Dako) at 4° C overnight. Then, cells were washed with PBS and incubated with biotinylated universal secondary antibody for 10 minutes, removed, and washed with PBS. Cells were incubated with streptavidin-peroxidase complex reagent for 10 minutes, removed, and washed with PBS. Cells were stained with substrate solution Diamino Benzydine (DAB) (Sigma Aldrich, GmbH) for 10 minutes, removed, and wash with aquadest. Cells were stained with Mayer-Hematoxylin for 3 minutes, removed, and washed with aquadest. Coverslips were moved into object-glass and fixed with ethanol and xylol and added with mounting media, then covered by new coverslips. Protein expression was observed by light microscope (Nikon YS100). Cells that express a particular protein will provide the color brown, while the cells that do not give a specific protein will provide color dark blue or purple (CCRC, 2013).

RESULTS AND DISCUSSION

The Growth Inhibition of MCF-7 and T47D Cancer Cells

Research on in vitro inhibition of MCF-7 and T47D cancer cells growth is intended to understand the molecular mechanism of drug action that can help increase effectiveness and prevent undesirable effects in both experimental animals and humans. The growth inhibition of MCF-7 and T47D cancer cells after administration of ethanol extract of *Chromolaena odorata* leaves have been evaluated by using the MTT colorimetry method. This method will provide information about cell cytotoxicity, proliferation and viability. The main principle of MTT colorimetry method based on yellow tetrazolium of MTT compound that reduces the active metabolites of cells as the action of succinate dehydrogenase enzymes. Thus, the reduction occurs due to NADH and NADPH of survive cells, that produces intracellular purple formazan which is soluble and the absorbance can be measured with microplate ELISA readers (Berridge *et al.*, 2005). Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

The stronger intensity of purple color formed, the greater absorbance obtained, which showed more living cells react with tetrazolium salts. In the MTT method, the percentage of cell viability is calculated based on the difference in control absorbance with the absorbance of the sample divided by the absorbance of the control multiplied by 100%. The cell viability rates of ethanol extract of *Chromolaena odorata* leaves on MCF-7 were 100.29%-28.19% and T47D were 100.37%-16.01% at concentration 7.8 µg/mL-500 µg/mL. The results have showed that cell viability rate was significantly decreased depend on concentration increased ($P<0.0002$ and $P<0.0001$). This indicated that ethanol extract of *Chromolaena odorata* leaves showed more effective in screening breast anticancer activity using T47D than MCF-7 cell lines. New metabolits, Genkwanin 4-O- [aL-rhamnopyranosyl (1 → 2)-bD-glucopyranoside] compound, showed cytotoxicity to LLC and HL-60 cancer cells with IC50 values of 28.2 mM and 11.6 mM, respectively. Sakuranetin 4-O- [bD-glucopyranosyl (1 → 2)-b D-glucopyranoside] compounds showed cytotoxic activity against LLC with an IC50 value of 50 mM, while HL-60 cells with an IC50 value of 10.8 mM (Hung *et al.*, 2011).

Data of cell viability are processed using Probit analysis to obtain IC_{50} values. The IC_{50} values indicated the number of tested drugs that can inhibit cell growth by 50%. Based on probit analysis, the IC_{50} value of ethanol extract of *Chromolaena odorata* leaves on MCF-7 and T47D cells was 327.34 µg/mL and 135.16 µg/mL. Another study indicated that ethanol extract of *Chromolaena odorata* leaves showed significant inhibition of cell on both cell lines with IC_{50} of 60.18 µg/mL in HCT116 and 71.74 µg/mL in HeLa, respectively. The result also showed that percentage inhibition of cell by ethanol extract of *Chromolaena odorata* increases with increase in concentration of extract (Singh, 2012). The percentage of cell viability after administration of ethanol extract of *Chromolaena odorata* leaves on the growth of MCF-7 and T47D breast cancer cell lines are presented in Figure 1. The profile effect of administration ethanol extract of *Chromolaena odorata* leaves on MCF-7 and T47D cell lines have been shown in Figure 2 and Figure 3.

The Effect of Ethanol Extract of *Chromolaena odorata* Leaves on Apoptosis of MCF-7 and T47D Breast Cancer Cells

Michigan Cancer Foundation-7 (MCF-7) is one model of breast cancer cells that are often used in cancer research. These breast cancer cells can express estrogen receptors (ER +) and originate from pleural effusion breast adenocarcinoma in a 69-year-old Caucasian female patient, blood type O (Simstein *et al.*, 2003). The T47D cells are continuous cell lines isolated from breast ductal tumor tissue in a 54-year-old woman. This cells express constitutively high levels of progesterone receptor (PR) and they have become the major model to study the actions of progesterone and synthetic progestin in human cells. This cell lines are often used in cancer research in vitro because they are

easy to handle, have unlimited replication ability and, high homogenicity.

Study about apoptosis caused by ethanol extract of *Chromolaena odorata* leaves is by the double staining method. This method is able to detect cells undergoing morphological changes. Observations on apoptosis were carried out qualitatively by observing cell morphology under a fluorescence microscope, after administration of acridine orange-ethidium bromide. The profile of living cells will look fluorescent green, but reddish orange cells in the form of particles are dead cells. Cells undergoing early-stage apoptosis seen with chromatin condensation and bright green. Cells that undergo apoptosis in the late stage will fragment into smaller, orange-colored parts, while necrotic cells will be orange reddish with normal cell size. The particles are DNA fragmentation that occurs due to intercalation between ethidium bromide and DNA. Acridine orange is used to determine the number of cells undergoing apoptosis, but this compound cannot distinguish between living cells and dead cells, therefore ethidium bromide is used to clarify cells that are undergoing initial apoptosis but are still alive and cells that have died in the late stages of apoptosis.

Apoptosis can be identified in changes in cell morphology such as: 1). Cell shrinkage: cells are smaller, cytoplasmic solid, although the organelle is still normal but appears solid. 2). Chromatin condensation: chromatin condensation is the most typical feature of apoptosis. Chromatin undergoes peripheral aggregation under the lining of the core wall into a limited solid mass of various shapes and sizes. The nucleus can break apart to form two or more fragments. 3). Formation of cytoplasmic protrusions: apoptosis cells first show a broad surface "blebbing" then fragment into a number of apoptotic bodies that

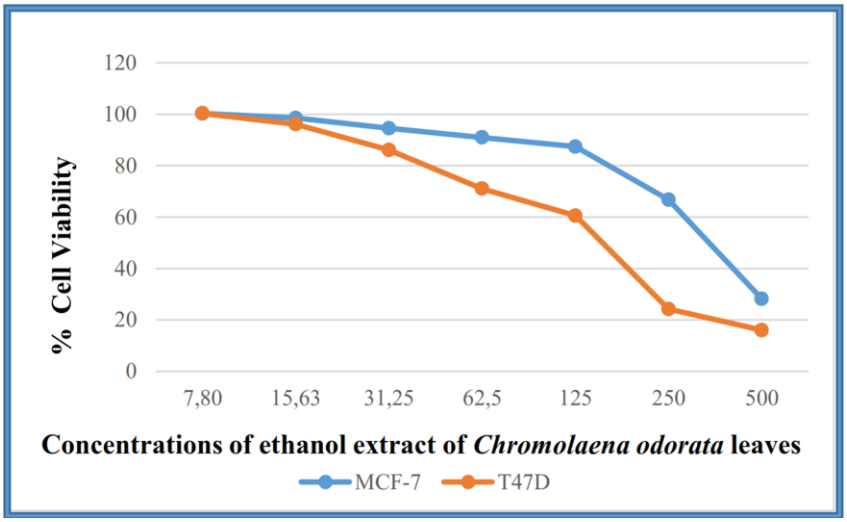


Figure 1. The percentage of cell viability of MCF-7 and T47D breast cancer cell lines after administration of ethanol extract of *Chromolaena odorata* leaves

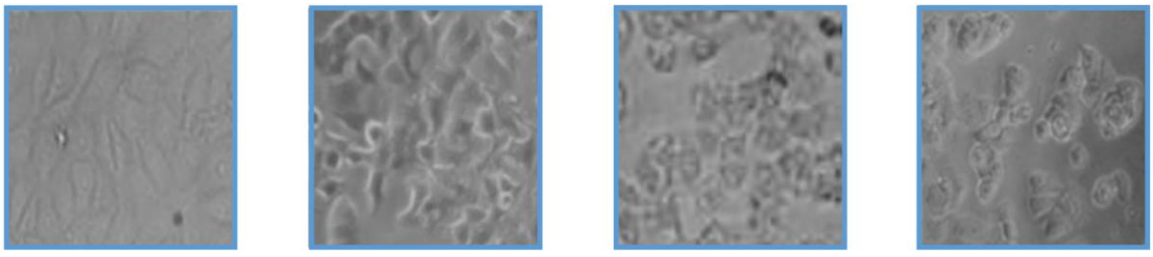


Figure 2. Effect of administration ethanol extract of *Chromolaena odorata* leaves on MCF-7 cancer cells (magnification 200x). MCF-7 cells without treatment (A); MCF-7 cells with extract 125 µg/mL (B); MCF-7 cells with extract 250 µg/mL (C); MCF-7 cells with extract 500 µg/mL (D)

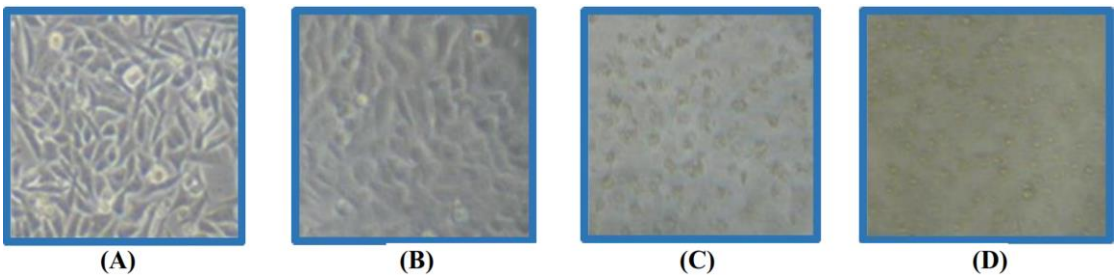


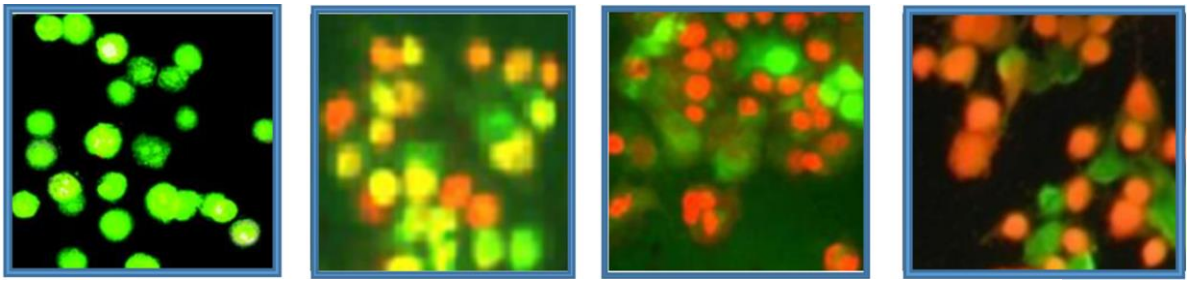
Figure 3. Effect of administration ethanol extract of *Chromolaena odorata* leaves on T47D cells (magnification 200x). T47D cells without treatment (A); T47D cells with extract 125 µg/mL (B); T47D cells with extract 250 µg/mL (C); T47D cells with extract 500 µg/mL (D)

bind to membranes composed by cytoplasm and dense organelles or without core fragments. 4). Phagocytosis of apoptotic bodies: this apoptotic body will be phagocytosed by phagocytic cells of neutrophils or macrophages. Apoptotic bodies can be degraded in the lysosomes and adjacent cells migrate or proliferate to replace the space previously filled with lost apoptotic cells. Apoptosis is a series of actions that control the removal of cells that play an important role in repair and homeostasis of adult tissue. In many cases, cancer occurs due to defects in apoptosis and dysregulation (Von and Vollmar, 2013).

Figure 4 and Figure 5 have been shown the effect of ethanol extract of *Chromolaena odorata* leaves on morphology MCF-7 and T47D cancer cell lines. The results showed the cells treated with this extract have orange colored fluorescence with fragmented DNA, which means the cells underwent apoptosis. This indicates that one mechanism of action of the extract on MCF-7 breast cancer cells is through the induction of apoptosis. However, to know the molecular mechanism that triggers apoptosis in MCF-7 and T47D cells is not yet clearly known.

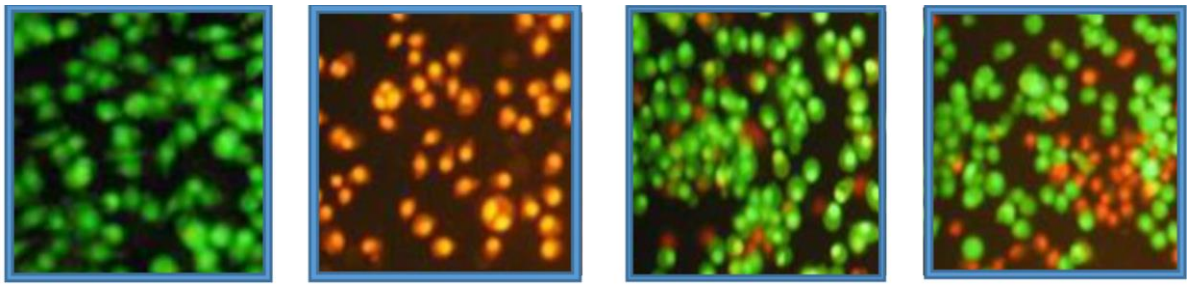
The Observation of Bcl-2 Proteins Expression in MCF-7 and T47D Breast Cancer Cells after Administration Ethanol Extract of *Chromolaena odorata* Leaves

The process of carcinogenesis is in principle related to changes in expression and regulation of genes that play a role in the cell cycle process. A deeper understanding of the cell cycle and the molecular mechanisms that accompany it can be used to explain the process of carcinogenesis as well as its use in controlling cancer. The molecular mechanism of apoptosis induction of ethanol extract of *Chromolaena odorata* leaves was confirmed using immunocytochemistry method. As we known the process of apoptosis is controlled by various cell signals, which originate from extrinsic or intrinsic triggers. Extrinsic trigger signals include hormones, growth factors, nitric oxide and cytokine. The response will arise if there are trigger signals that can penetrate the cell plasma membrane. Apoptotic process is also associated with genes that regulate cell cycle development, including p53, Bax, Rb, c-Myc, E1A, Bcl-2, Bcl-xl, kB-factor genes, activating caspases, activator proteins (AP-1) and, apoptosis inducing factor (Kumar, 2005). The primary mechanism by which chemotherapeutics destroy cancer cells is by apoptosis induction. High levels of apoptosis in cancer cells are strongly associated with chemotherapeutic sensitivity. Therefore, the main purpose of cancer treatment is to induce apoptosis. Multiple methods have been developed to detect apoptosis by monitoring changes in cell morphology and surface markers. The immunocytochemistry method is one way to explain the presence of apoptosis through protein expression that mediates apoptosis. The mechanism of apoptosis in this study was traced through the expression of Bcl-2 protein by immunocytochemical methods after administration of *Chromolaena odorata* ethanol leaf extract on MCF-7 and T47D breast cancer cells.



(A) **(B)** **(C)** **(D)**

Figure 4. The changes of morphology in MCF-7 cells after administration of ethanol extract of *Chromolaena odorata* leaves at IC_{50} concentrations for 24 hours (magnification 400x). Cells are distinguished by their ability to absorb acridine orange-ethidium bromide. Living cells (negative control) with green nuclei and regular structures (A); Dead cells (positive control, doxorubicin) with orange color that showed apoptosis (B); Early apoptotic cells have green nuclei, cells with perinuclear chromatin condensation or bright green fragments (C); Late apoptotic cells have orange nuclei (D)



(A) **(B)** **(C)** **(D)**

Figure 5. The changes of morphology in T47D cells after treatment with ethanol extract of *Chromolaena odorata* leaves at IC_{50} concentrations for 24 hours (magnification 400x). Cells are distinguished by their ability to absorb EO-EtBr dyes. Living cells (negative control) with green nuclei and regular structures (A); Dead cells (positive control, doxorubicin) with orange color that showed apoptosis (B); Early apoptotic cells have green nuclei, cells with perinuclear chromatin condensation or bright green fragments (C); Late apoptotic cells have orange nuclei (D)

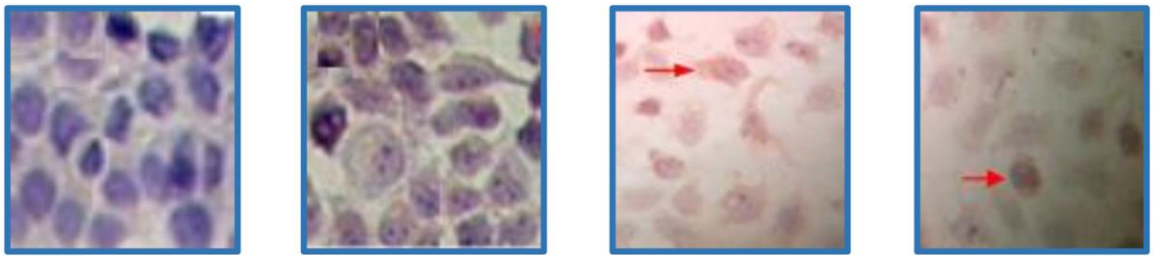
Bcl-2 protein is one type of anti-apoptotic protein involved in the process of apoptosis. Decreased or increased expression of Bcl-2 protein after intervention can explain the mechanism of action of the drug. Figure 6 and Figure 7 showed the expression marker of Bcl-2 protein in MCF-7 and T47D cell lines after administration of ethanol extract of *Chromolaena odorata* leaves.

The results showed that the possible mechanism of apoptotic triggering by the extract on MCF-7 and T47D cells was through inhibition of Bcl-2 protein expression. Bcl-2 is one type of anti-apoptotic protein which if Bcl-2 expression can be inhibited, then the process of apoptosis can occur. Ethanol extract of *Chromolaena odorata* leaves can inhibit the expression of Bcl-2 protein so that apoptosis can occur. In cancer cells there is an imbalance between proapoptotic protein (Bax) and anti-apoptosis (Bcl-2), when Bcl-2 levels are higher then the apoptotic process can be inhibited. Decreased expression of Bcl-2 will increase proapoptotic protein (Bax), so that the mitochondrial membrane permeability increases and cytochrome- C will come out and bind to the protein Apaf-1 (apoptosis activating factor-1) and activate caspase 9 then apoptosis happened (Martin, 2006).

The resuls show (Figure 6 and Figure 7) the cytoplasm of MCF-7 and T47D cells have a different color than the control cells with brown-specific Bcl-2 antibodies. This shows that there has been a decrease in Bcl-2 expression due to administration of the extract. Decreased expression of Bcl-2 protein happened with increasing concentrations of ethanol extract of *Chromolaena odorata* leaves that are given.

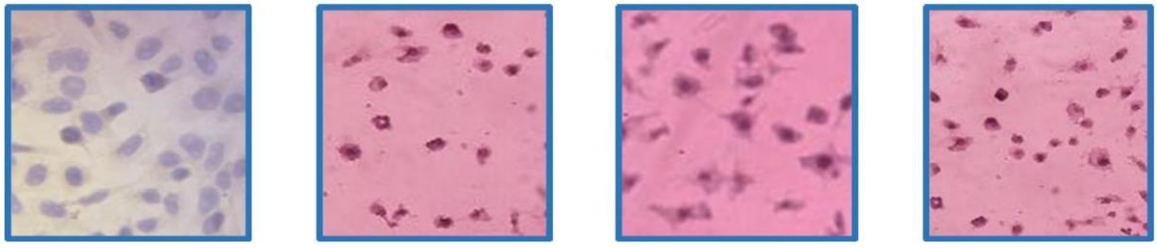
Anti-apoptotic protein Bcl-2 is expressed higher by T47D cells than MCF-7 cells. This is related to the very weak response of MCF-7 cells to chemotherapeutic agents. Based on data, the mechanism of ethanol extract of *Chromolaena odorata* leaves in inducing apoptosis in MCF-7 and T47D cells is estimated by inhibiting the expression of Bcl-2. The Bcl-2 expression was observed by the immunocytochemical method using Bcl-2 antibodies. Decreased expression of Bcl-2 was shown in extracts treatmens on T47D at concentration of 135.16 µg/mL. The differences in cell morphology als occurred in this concentration treatments, such as the different color intensity of the cell nucleus compared to the control and changes in the color of the cell plasma. Thus it can be assumed that tracing the mechanism of apoptosis can be correlated with the expression of several regulatory proteins, including Bcl-2. The results of this study are in accordance with those obtained by Ren *et al*. (2003), that assumed flavonoids are proven to be anticancer agents by inducing apoptosis through down regulation of Bcl-2 protein expression.

This is caused by the compounds contained in ethanol extract of *Chromolaena odorata* leaves such as 2'-hidroksi-4,4',5',6'-tetramethoxikalkon (Kouamé *et al.*, 2013). Another study state the acetone extract and ethyl acetate extract from this plant have strong cytotoxic activity against MCF-7 cells. The data shows that the apoptotic mechanism underlying this extract is due to autophagy and cell cycle arrest. In addition, it was also stated that the action target of the active compounds in the two extracts was different (Martin, 2006; Faizah *et al.*, 2012). The methyl ether narigenin which was isolated from the ethylacetate extract of *Chromolaena odorata* leaves has antiproliferation activity on L_{1210} (leukemia) cancer cells with an IC_{50} value of 1.515 bpj (Fitrah *et al.*, 2017). The ethyl



(A) **(B)** **(C)** **(D)**

Figure 6. Expression of Bcl-2 protein in MCF-7 cells with ethanol extract of *Chromolaena odorata* leaves after incubated for 24 hours. Observations were made under a light microscope at a magnification of 400x. Control cells without specific antibodies (A); Cells were incubated with Doxorubicin (B); Cells were incubated with extract concentrations of ½ IC_{50} (C); Cells were incubated with extract concentrations of IC_{50} (D)



(A) **(B)** **(C)** **(D)**

Figure 7. Expression of Bcl-2 protein in T47D cells with ethanol extract of *Chromolaena odorata* leaves after incubated for 24 hours. Observations were made under a light microscope at a magnification of 400x. Control cells without specific antibodies (A); Cells were incubated with Doxorubicin (B); Cells were incubated with extract concentrations of ½ IC_{50} (C); Cells were incubated with extract concentrations of IC_{50} (D)

acetate soluble fraction of 70% ethanol extract of *Chromolaena odorata* leaves produced thirteen pure compounds. The compounds that have been identified are aromadendrin 4'-methyl ether, eriodictyol 7, 4'-dimethyl ether, 4'-methyl ether, isosacuranetin, quercetin 7, 4'-dimethyl ether, kaempferide, acacetin, rhamnazin, quercetin 3-O-routineoside, isosacuranetin, quercetin 7, 4'-dimethyl ether, kaempferide, acacetin, rhamnazin, quercetin 3-O-routineoside, kaempferol 3-O-routineoside, and kaempferol 3-O-glucoside. The cytotoxicity of the ethanol extract of *Chromolaena odorata* leaves has also been carried out and the results show that significantly inhibit the growth of HL-6029 cancer cell lines (Yu *et al.*, 2017).

CONCLUSION

Ethanol extract of *Chromolaena odorata* leaves has the growth inhibitory activity on MCF-7 and T47D breast cancer cells with IC_{50} values 327.34 µg/mL and 135.16 µg/mL. The administration of this extract to MCF-7 and T47D breast cancer cells concentrations of IC_{50} can stimulate apoptosis. This research proves that the ethanol extract of *Chromolaena odorata* leaves can significantly reduce the expression of Bcl-2 anti-apoptotic protein.

ACKNOWLEDGEMENT

We thank you for the assistance of Mrs. Rumbiwati from the Parasitology Laboratory, Faculty of Medicine Public Health and Nursing, Gadjah Mada University Yogyakarta; Mr. Munawar from Faculty of Mathematics and Natural Sciences, Syiah Kuala University in terms of statistical analysis and Dr. Marhami Fahriani. M. Infect. Dis which has helped the interpretation of data.

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