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Submission date: 01-Mar-2023 03:33AM (UTC+0700)
Submission ID: 2025537900
File name: JPM.docx (788.11K)
Word count: 2732
Character count: 15277

Jurnal Profesi Medika : Jurnal Kedokteran dan Kesehatan
ISSN 0216-3438 (Print). ISSN 2621-1122 (Online)

Decrease of BCL-2 Expression by Ethanol Extract of *Ocimum Basilicum* L. Leaves in Breast Cancer Cells

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ABSTRACT

Ocimum basilicum L has been known ~~proven~~ to have in vitro cytotoxic activity against breast cancer cells. Pathways that cause cell death mightean involve one of the proteins~~,~~ 2~~which is~~such as BCL-2. This study aim~~ed~~s to determine the decrease of BCL-2 protein expressions in breast cancer cells (T47D and MCF-7) ~~tat are~~ treated with the ethanol extract of *Ocimum basilicum* L leaves.

The leaves of *Ocimum basilicum* L. was extracted using ~~the~~ maceration method with 70% ethanol solvent. The ~~concentration of ethanol~~ extract ~~of *Ocimum basilicum* L.~~was used to see the expression of BCL-2 protein in T47D and MCF-7 cells ~~was~~at concentrations of 199 µg/ml and 388 µg~~/~~mL, respectively. The observation of BCL-2 protein expression is using ~~-~~immunocytochemical methods of T47D and MCF-7 cancer cells.

The results showed that the ethanol extract of *Ocimum basilicum* L ~~could~~ reduced BCL-2 protein expression in breast cancer cells (T47D and MCF-7) at concentrations of 199 µg/ml and 388 µg/ml, respectively.

Keywords: *Ocimum basilicum* L; BCL-2; T47D; MCF-7

INTRODUCTION

Breast cancer is one of ~~a~~ the cancers that still occurs ~~quite~~ relatively high in the world (1). In 2018, the prevalence of breast cancer in Indonesia was relatively~~quite~~ high; there were 58,256 new cases and 22,692 deaths (2). Various cancer treatments have been carried out, including surgery, chemotherapy, radiotherapy, and ~~also~~ therapy with monoclonal antibodies. However, ~~O~~one ~~treatment uses a widely used~~of the widely used treatment as a chemotherapy agent, such as doxorubicin, ~~but the use of doxorubicin~~ tends to have toxic side effects on normal tissues and cancer cell resistance (3). Efforts in treating cancer are now ~~constantly~~ continually being developed~~,~~ because there is no specific drugs ~~that are~~ capable of~~in~~ killing cancer cells. Current treatments still show side effects on normal cells (3). Plants are considered to have lower side effects~~,~~ so that natural drug-based medications are always being developed.

Ocimum basilicum L. is a plant that has anticancerous activity in breast cancer cells (4). *Ocimum basilicum* contains an active ursolic acid compound~~s~~ that is able to inhibit the proliferation of cells (5). *Ocimum basilicum* has proved to ~~be an~~contain active flavonoids, saponins, essential oils and tannins (6). Flavonoids and saponins are known to inhibit BCL-2 expression (7).

An anti-apoptotic protein, which is BCL-2, is expected to bring a decrea10 to stimulate apoptosis. BCL-2 protein is a protein that play11 role in the regulation of apoptosis (8). Based on the background above, it is necessary to develop research to identify potential natural materials as anti-cancer by testing them into one of the mechanisms that block the BCL-2 protein inhibition that can ~~assist in~~ trigger~~ing~~ the

apoptosis of the cell. ThereforeSo, it is important to know the decline of the BCL-2 expression by the ethanol extract of *Ocimum basilicum* L. on breast cancer (T47D and MCF-7).

MATERIAL AND METHODS

The tools used in this study were dryer cabinets, moisture balance, a set of maceration tools, a scale and a pigetic blower (Heildolph), a flow cabinet (LAF), a CO_2 incubator (Heraceus), a microscope, a 6-well plate (Nunc), and a Hemositometer (Neubauer).

The materials used in this study were *Ocimum basilicum* L. leaves (from Bandungan, Semarang district), 70% ethanol (70%), T47D and MCF-7 cell lines (Parasitology Laboratory of FK UGM), DMEM (Gibso) media, DMSO (Merck), primary antibodies against BCL-2. (Dako), Streptavidin (Novocacastra), Secondary IgG biooncology antibodies (Novocacastra), Hematoxylin (Dako), 3.3 Chromosome-diaminobenzidine (DAB) (Novocastra).

Plants' determination of *Ocimum basilicum* L. was conducted at the Faculty of Mathematics and Natural Sciences, Diponegoro University. The determination was done by matching plant morphology with reference books. Number of determination 1b, 2b, 3b, 4b, 6b, 7b, 9b, 10b, 11b, 12b, 13b, 14b, 16a, gol 10. 239b, 243b, 244b, 248b, 249b, 250b, 266b, 267a, 268b, 271b, Family 110: Labiatae. 1a, 2b, 4b, 6b,7b, 8. Genus: Ocinum. Species: *Ocinum basilicum* L (Selasih, Telasih, Kemangi).

Immunocytochemical test concentration: The basis for using the concentration used is the IC50 value of the ethanol extract of *Ocimum basilicum* L. against T47D and MCF-7 cells. The IC50 value is a concentration that can inhibit 50% of cancer cells in the cytotoxic test. The IC50 values obtained from previous studies on T47D and MCF-7 cells were 399.86 µg/mL and 387.76 µg/mL, respectively (4). Where in the immunocytochemical test, the concentration of the ethanol extract of Ocimum basilicum L. used was ½ IC50 (199 µg/mL on T47D cells) and IC50 (388 µg/mL on MCF-7 cells).

T47D and MCF-7 cell preparation. The cells in the cryotube were taken from the liquid nitrogen tank followed by thawing at 37°C and sprayed with alcohol. The cells were transferred to a sterile conical tube in which DMEM media was present. The cells were then centrifuged for 10 minutes until supernatant and pellets emerged. The supernatant was discarded, while the pellets were added 10 mL of growth medium (containing 10% FBS). After becoming homogeneous, divide it into two2 tissue culture dishes (TCD) at 37°C in a CO2 incubator until the . cCells were grown to confluent so that they can be used for research.

Harvesting of T47D and MCF-7 cells: The cells which were confluent and sufficient for the study were discarded and then washed with PBS. Cells attached to TCD were removed by adding trypsin-EDTA. If the cells have been removed,, followed by adding the DMEM media. Then, the suspension was centrifuged, and the supernatant was discarded. Centrifugate the suspension and discard the supernatant. ThenFurthermore, add culture media to the pellets and count the number of cells using a hemocytometer under an inverted microscope. The cell suspension was transferred into a sterile conical tube according to the number of cells to be used for immunocytochemical testing.

Immunocytochemical test: testing was performed using T47D cell density (1 x 10^5 cells/well) and MCF-7 cells (5 x 10^4 cells/well). Treat different cells using different 6-well plates. Each well was planted with 1000 µL cells. Wait until the cells are attached to the coverslip, and add 100 µL of culture media, and then incubate for 24 hours in a CO2 incubator. After 24 hoursincubation, the media was takentake the media out, and the cells was washed it with PBS, and [illegible] 388 µg/mL and 199 µg/mL for MCF-7 and T47D cells, respectively. [illegible] Furthermore, the cells were re-incubated for

Commented [1]: Please mention the source of all materials. For example: DMSO from Merck (Darmstadt, Hesse, Germany).

Commented [2]: Please provide voucher number of plant specimens and the name of herbarium where the specimens were deposited.

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24hours.Preparate Microscope slide was prepared was followed by fixation and incubated in a freezer -4 ° C for 10 minutes. The cells in the coverslip were removed and placed on a 6 cm dish and washed with distilled water, then dripped with hydrogen peroxidase blocking solution (10 minutes) at room temperature, discard. Preparate Microscope slide was iIncubated with prediluted blocking serum (10 minutes), discard. Then dripped the slide with anti-Bcl-2 Monoclonal Primary Antibody (1:50 dilution) on T47D/ MCF-7 cells for 24 hours at 4ºC, and. 24 hours later, washed with PBS. Furthermore, Tthe microscope slides were incubated in a biotinylated universal secondary antibody (10 minutes). The microscope slides were incubated, followed by incubating in the streptavidin-peroxidase complex reagent (10 minutes),. The microscope slides were incubated in DAB substrate solution (2-10 minutes), washed, and soaked in Mayer Haematoxylin (1-3 minutes) for further counterstain washing. Finally, DThe slide was dipped the xylol, dripped with mounting media, and covered with a microscope slide cover. Protein expression was observed under a light microscope with a magnification of 100-1000x and control cells were treated similarly (9).

Analysis

BCL-2 protein expression was observed by immunocytochemical staining using BCL-2 antibody. Observations were made qualitatively using a light microscope. Cells with positive expressions will have brown cytoplasm, while cells with negative expressions will have purple or blue cytoplasm. A positive control is given primary antibodies so that the brown cytoplasm appeared (Fitriasih, et al, 2019).

RESULT

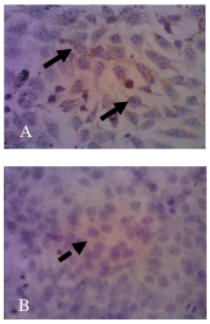
Plant determination is the initial step that must be carried out if a study uses natural ingredients. The determination aims to find out that the plant identity used is correct by comparing the plant morphology with reference books. The results showed the keys of determination and stated that the plant used is *Ocimum basilicum* L.

The leaves of *Ocimum basilicum* L. were transformed from simplicia form to leaf powder to expand the contact surface with the solvent, thereby facilitating the solubility of the compound with the solvent (16). The ethanol extract of *Ocimum basilicum* L. has cytotoxic activity against T47D and MCF-7 cells with IC50 values of 399.86 µg/mL and 387.76 µg/mL, respectively (4). Another study related to the cytotoxic test of the methanol extract of *Ocimum basilicum* L. In MCF-7 cancer cells showed an IC_{50} of 98.51 µg/mL. This showed that the difference is possible because the solvent used is different. Methanol solvent can attract higher levels of flavonoids than ethanol solvent (14). However, from a safety point of view, ethanol solvent is less toxic than methanol. The concentration reference used was the study of the ethanol extract of *Ocimum basilicum* L cells T47D and MCF-7 with IC_{50} values of 399.86 µg/mL and 387.76 µg/mL (4), because the solvent and materials used came from the same place.

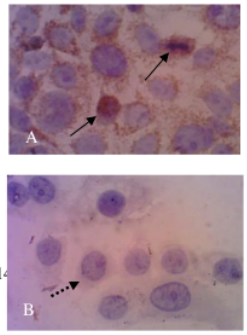
The immunocytochemical test is a test to see the expression of the BCL-2 protein. It has been proven that the effect of *Ocimum basilicum* L. ethanol extract has anticancer activity against T47D and MCF-7 cells (4). The methanol extract of *Ocimum basilicum* L. was shown to have cytotoxic activity against MCF-7 cells (10). Similar research on the methanol extract of *Ocimum basilicum* L. stated that the extract was able to stimulate the death of MCF-7 cells (5). Therefore, it is necessary to investigate whether cell death (apoptosis) due to the treatment of *Ocimum basilicum* L. ethanol extract can affect and through a decrease in the antiapoptotic protein, namely BCL-2.

Immunocytochemical testing was carried out at the IC50 value obtained in the previous cytotoxic test, which is a concentration that can kill 50% of cells. According to Fitriasih et al, (2019), the concentrations that can be used in immunocytochemical testing are 1/4 IC50, 1/2 IC50, and IC50. However, in this study,

we only wanted to see which concentration began to show a decrease in BCl-2 expression. So, the concentration of the ethanol extract of *Ocimum basilicum* L. used was ½ IC_{50} (199 µg/mL on T47D cells) and IC_{50} (388 µg/mL on MCF-7 cells). Immunocytochemical testing was performed on MCF-7 cells because these types of cells had BCL-2 overexpression characteristics (11). BCL-2 protein is one of the anti-apoptotic proteins, meaning that the increase in this protein inhibits apoptosis or increases cell survival (1, 12). Immunocytochemical test results for the ethanol extract of *Ocimum basilicum* L. (EEOB) on T47D cells (Figure 1) and MCF-7 cells (Figure 2).



Picture 1. Effects of *Ocimum basilicum* L ethanol extract treatment on BCL-2 expression in T47D cells. Positive control of cells with anti-BCL-2 (A) primary antibody staining, EEOB concentration of 199 µg/mL (B), → positive BCL-2 expression, ----> negative BCL-2 expression.



Picture 2. Effects of *Ocimum basilicum* L ethanol extract treatment on BCL-2 expression in MCF-7 cells. Positive control of cells with anti BCL-2 (A) primary antibody staining, EEOB concentration 388 µg/mL (B), → positive BCL-2 expression, ---> negative BCL-2 expression

DISCUSSION

The observation of the BCL-2 protein expressions was done by the immunocytochemical method, in which the principle uses specific antibody binding. Cells showed a brown color when expressing protein and purple when expressing no protein. Picture 1B and 2B showed that compared to 1A and 2A, they showed the expression of BCL-2 that experienced a decrease after treatment of ethanol *Ocimum basilicum* L extract. The decreased expression of BCL-2 after EEOB treatment was visible because it showed blue cell cytoplasm compared to the controls that looked browner. This suggested that the ethanol extract of *Ocimum basilicum* could reduce BCL-2 protein expression in (T47D and MCF-7) breast cancer cells.

Ocimum basilicum had been proven to have active flavonoids, saponins, essential oils, and tannins (6). Flavonoids were reported to be able to stimulate apoptosis by several mechanisms, including inhibition of DNA topoisomerase I / II, decreased BCL-2 and BCL-XL, and increased expression of Bax and Bak genes (13). BCL-2 protein was one of the proteins involved in the apoptosis process (8). The suppression of BCL-2 protein expression could induce apoptosis induction through the release of cytochrome c by entering the outer mitochondrial membrane that could then activate the caspase pathway. Mitochondrial cytochrome

c together with Apaf-1 and protease 9 formed the apoptosome complex. The formation of apoptosomes would result in an auto-activation of caspase 9 and would activate caspase 3. This was what could cause cancer cells to experience apoptosis (18). This research is still in qualitative form, therefore, further quantitative research is needed to see how much BCL-2 inhibition is caused by giving Ocimum basilicum L. ethanol extract and confirms the pathway that is considered to play a role in increasing apoptosis in T47D and MCF-7 cells.

CONCLUSION

Studies have shown that ethanol extract of *Ocinum basilicum* L was able to reduce the expressions of BCL-2 protein in breast cancer cells (T47D and MCF-7) at concentrations of 199 μg/ml and 388 μg/ml, respectively.

REFERENCES

[1]. Pusat Data dan Informasi Kemenkes. (2015). Stop Kanker. Jakarta: Departemen Kesehatan

[2]. Globogan. (2018). International Agency or Research on Cancer. http://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf. diakses tanggal 27 Agustus 2020

[3]. Xu, F., Wang, F., Yang, T., Sheng, Y., Zhong, T., and Chen, Y. (2014). Differential Drug Resistance Acquisition to Doxorubicin and Paclitaxel in Breast Cancer Cells, Cancer Cell Internasional, 14(142), 1-13. DOI

[4]. Hidayati DN, Arifin I, Zahroh F, Wahyuni L. (2019). Efek Sitotoksik Ekstrak Etanolik *Ocimum basilicum* L. Pada Sel Kanker Payudara. Jurnal Pharmascience. 6(2):74-79. http://dx.doi.org/10.20527/jps.v6i2.7353

[5]. Qamar KA, Dar A, Siddiqui BS, Kabir N, Aslam H, Ahmed S, Erum S, Habib S, and Begum S, (2010). Anticancer Activity of *Ocimum basilicum* and the Effect of Ursolic Acid on the Cytoskeleton of MCF-7 Human Breast Cancer Cells. Letters in Drug Design and Discovery. 7:726-736. 10.2174/157018081100701072 6

[6]. Depkes RI. (2001). Inventaris Tanaman Obat Indonesia (1) jilid 2. Jakarta: Departemen Kesehatan dan Kesejahteraan Sosial Republik Indonesia

[7]. Raju and Rao. (2004). Diosgenin a Steroid Saponin of *Trigonella Foenum Graceum* (Fenugreek) Inhibits Azoxymethane Induced Aberrant Crypt Foci Fomation in F344 Rats and Induces Apoptosis in HT-29 Human Colon Cancer Cells. Cancer Epidemiology Biomarker and Prevention. 13: 1392. PMID: 15298963

[8]. Simstein R, Burow M, Parker A, Weldon C, and Beckman B. (2003) Apoptosis, Chemoresistance, and Breast Cancer: Insights from The MCF7 Cell Model System. Exp Biol Med. 228:995–1003. DOI: 10.1177/153537020322800903

[9]. Cancer Chemoprevention Research Center. (2009). In Vitro Test Protocol. Available at http://ccrc.farmasi.ugm.ac.id. (12 Agustus 2016).

[10]. Al-Ali KH, Beshbishy HSE, Badry AAE, and Alkhalaf M. (2013). Cytotoxic Activity of Methanolic Extract of Mentha Longifolia and *Ocimum basillicum* Against Human Breast Cancer. Pakistan Journal of Biological Science. 10:1028-8880. DOI: 10.3923/pjbs.2013.1744.1750

[11]. Weigert A, Tziepy N, Knethen A, Johann A, Schmidt H, Geisslinger G, and Brune B. (2007). Tumor Cell Apoptosis Polarizes Macrophages—Role of Sphingosine-1-Phosphate. Molecular Biology of the Cell. 18(10): 3810–3819. doi: 10.1091/mbc.E06-12-1096

[12]. Marleen FS, Syahruddin E, Hudoyo A, Endarjo S, Hidayat H, and Suzanna E. (2008). Ekspresi protein Bcl-2 pada sediaan blok parafin jaringan kanker paru. Jakarta: Departemen Pulmonologi & Ilmu Kedokteran Respirasi FKUI

[13]. Ren W, Qiao Z, Wang H, Zhu L, and Zhang L. (2003). Flavonoids: Promicing

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Anticancer Agents. Med Res Rev. 23 (4):519-534. DOI: 10.1002/med.10033

[14]. Zulharmitta, Elrika D, dan Rivai H. (2010). Penentuan Pengaruh Jenis Pelarut Ekstraksi Terhadap Perolehan Kadar Senyawa Fenolat dan Daya Antioksidan dari Herba Meniran (Phyllanthus niruri L.,). 2 (1): 37-45.

[15]. Depkes RI. (2000). Parameter Standar Umum Ekstrak tumbuhan Obat. Jakarta: Departemen Kesehatan Republik Indonesia

[16]. Voight R. (1994). Buku Pelajaran Teknologi Farmasi. Diterjemahkan oleh Soendani Noerono Soewandhi. Yogyakarta: Gadjah Mada University Press

[17]. Fitriasih, Komariyah, S.M., Sandra, Ferry, Pratiwi, N., Hidayati, D.N. (2019). Mangifera indica L. Leaves Extract Induced Intrinsic Apoptotic Pathway in MCF-7 Cells by Decreasing Bcl-2 Expression and Inducing Bax Expression. Indonesian Journal of Cancer Chemoprevention. 10 (1): 1-6. DOI: http://dx.doi.org/10.14499/indonesianjcanchemoprev10iss1pp1-6.

[18]. Wickenden, J.A., Clarke, M.C.H., Rossi, A.G., Rahman, I., Faux, S.P., Donaldson, K., and MacNee, W., 2003, Cigarette Smoke Prevents apoptosis trough Inhibition of Caspasa Activation dan Induce Necrosis, Am. J. Respir. Cell Mol. Biol., 29:567-570. DOI: 10.1165/rcmb.2002-0235OC

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6