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# The Effect of Bitter Melon Extract (Momordica Charantia) On CASPASE-9 and Bcl-2 Proteins Expression

**Desti Rahmasari[1], Titiek Sumarawati[2], Setyo Trisnadi[3]**

[1]Postgraduate student of Biomedical Sciences, Faculty of Medicine, Universitas Islam Sultan Agung, Jl Kaligawe KM 4 Semarang 50012

[2,3] Department of Biomedical Sciences, Faculty of Medicine, Universitas Islam Sultan Agung, Jl Kaligawe KM 4 Semarang 50012

**ABSTRACT:** Breast cancer has a significant morbidity and death rate worldwide, thus various therapeutic strategies, including the phytopharmaca approach, must be researched further. Bitter melon (*Momordica charantia*) extract is predicted to have anti-cancer properties due to its influence on *Caspase-9* and BcL2 proteins, which will be shown in this in vitro investigation utilizing MCF-7 cell culture. This study was an in vitro laboratory experimental study with a post-test-only control group design. The doses of *Momordica charantia* extract used were 125 µg/ml, 500 µg/ml, and 1,000 µg/ml. *Caspase-9* and BcL2 protein expression in MCF-7cell culture was measured by immunocytochemistry method, *Shapiro Wilk, Levene Test, One Way Anova,* and *Post Hoc* LSD. Administration of *Momordica charantia* extract at a dose of 500 µg/ml and 1,000 µg/ml significantly increased the average expression of *Caspase-9* protein ($p = 0.044$ and $p = 0.004$ respectively), but not at a dose of 125 µg/mL ($p = 0.125$). Administration of bitter melon extract at doses of 125 µg/ml, 500 µg/ml, and 1,000 µg/ml significantly reduced the average BcL2 protein expression ($p < 0.001$, $p < 0.001$, and $p = 0.001$ respectively). *Momordica charantia* extract has the potential to be employed as an anti-breast cancer agent due to its ability to increase the average expression of *Caspase-9* protein while reducing the average expression of BcL2 protein.

**KEYWORDS:** Breast cancer, CASPASE-9, BcL-2, bitter melon

## I. INTRODUCTION

Cancer is a degenerative disease that grows continuously without control and has the ability to metastasize to other organs. Breast cancer is a disease with a poor prognosis, often found at an advanced stage, and can lead to death.[1] In 2020, breast cancer patients in Indonesia amounted to 65,856 cases with the number of deaths due to this cancer of 22,430 cases.[2]

The high mortality of breast cancer encourages the need to investigate effective therapies, both anti-cancer drug therapy (chemotherapy) and phytopharmaceuticals.[3] One phytopharmaceutical method that is suspected to be effective for cancer treatment is bitter gourd extract.[4] Bitter gourd extract has anti-carcinogenic properties and has many benefits pharmacologically, Bitter gourd extract also contains various bioactive components.[4,5] Components include saponins, flavonoids, alkaloids, and triterpenoids that are toxic. Therefore, bitter gourd extract can be used as a phytopharmaceutical agent for breast cancer treatment.[5]

Bitter melon extract has cytotoxic potential and is carcinogenic. In previous studies, the IC50 value of 10.92 µg/ml in bitter gourd extract has the potential to fight breast cancer cells.[6] The half maximal inhibitory concentration or IC50 measures the potential of a substance to inhibit certain biological or biochemical functions. An IC50 value is a quantitative measure that indicates how much of a particular inhibitory substance (e.g. a drug) is required to inhibit a certain biological process or biological component by 50% in vitro. This value is commonly used as a measure of the potential of antagonistic drugs in pharmacological research.[7] Specialized in pharmacological research on breast cancer, Michigan Cancer Foundation-7 (MCF-7) cells are the most commonly used model of breast cancer cells for in vitro methods.

Michigan Cancer Foundation-7 (MCF-7) cells are monolayer-grown epithelial cells of the best shape compared to all types of human breast cancer cells.[8] This cell is also able to synthesize various proteins like cancer cells in humans, including CASPASE-9 and BcL-2 proteins. CASPASE-9 protein can accelerate the apoptosis process, and an increase in BcL-2 levels can inhibit the process of apoptosis.[9] In normal cells, apoptosis depends on the balance between proteins that act as activators and inhibitors of

apoptosis. Many signal transduction pathways play a role in the process of apoptosis, including intrinsic and extrinsic factors, through mitochondrial pathways and apoptosis receptors. Proteins such as p53, BcL-2, Bax, and CASPASE, greatly affect the process of apoptosis.[9]

Based on the description above, it can be seen that breast cancer is a health problem that is very important to pay attention to because it has a high morbidity and mortality rate. Various to treat this condition need to be further researched, including complementary therapy in phytopharmaceuticals. Bitter gourd extract is one of the plants reported to have a good toxic effect against breast cancer cells. However, previous studies have never been conducted to assess its effectiveness using CASPASE-9 and BcL-2 protein expression parameters in MCF-7 breast cancer cell cultures. This is what makes this research important to do.

## II. MATERIAL AND METHOD

### Material

This type of research is experimental. The research design that fits this problem is a post-test-only control group design of MCF-7 breast cancer cells. Samples were obtained from the culture storage area at the Parasitology Laboratory of Gajah Mada University.

### Methods

MCF-7 cells are divided into four treatments. The treatment consisted of a control group, the treatment group which was given bitter melon extract at a dose of 1/2 IC50, 1 IC50, and 2 IC50. The research was conducted in the Parasitology laboratory of the Faculty of Medicine, Gadjah Mada University, Yogyakarta. The study was conducted from February 13 to February 24, 2023

## III. RESULT

This study used MCF-7 cells as a research sample. MCF-7 cells were divided into 4 groups, cell control group, P1 MCF-7 treated with bitter melon extract dose 1/2 IC50 (250 µg/ml) P2 MCF-7 treated with bitter melon extract dose 1 IC50 (500 µg/ml), and P3 MCF-7 treated with bitter melon extract dose 2 IC50 (1000 µg/ml) The bitter melon extract used in this study was extracted by maceration method using 90% ethanol solvent for 72 hours.

**Table 1. Results of Average Analysis, Normality Test, Homogeneity Test on TNF-α and MCP-1 Expression**

| Variable | Group | | | | Sig. |
|---|---|---|---|---|---|
| | Control<br>KS | P1<br>Bitter Melon Extract ½ IC50 | P2<br>Bitter Melon Extract 1 IC50 | P3<br>Bitter Melon Extract 2 IC50 | |
| **CASPASE-9 Expression** | | | | | |
| Mean<br>Std. deviation | 3,98<br>2,05 | 7,66<br>0,89 | 9,15<br>1,73 | 12,91<br>1,27 | |
| *Shapiro Wilk* | 0,474 | 0,501 | 0,482 | 0,505 | >0,05* |
| *Levene test* | | | | | 0,405** |
| *One-way ANOVA* | | | | | 0,022*** |
| **BcL-2 Expression** | | | | | |
| Mean<br>Std. deviation | 13,66<br>0,33 | 4,16<br>1,39 | 2,56<br>1,59 | 2,38<br>1,48 | |
| *Shapiro Wilk* | 0,848 | 0,531 | 0,778 | 0,774 | >0,05* |
| *Levene test* | | | | | 0,324** |
| *One-way ANOVA* | | | | | 0,001*** |

*Saphiro Wilk test (p > 0,05 = normal)

** Levene's Test (p > 0,05 = homogeneous)

*** One Way Anova (p < 0,05 = difference)

### The Effect of Bitter Melon Extract (Momordica Charantia) On CASPASE-9 and Bcl-2 Proteins Expression

Based on the results of the study shown in Table 1. The average expression of BcL2 protein in the lowest P3 group was then followed by the average expression of BcL2 protein in group P2 and subsequently group P1. Shapiro Wilk test results obtained p>0.05 values and Levene's Test results with p=0.324 (p>0.05) values. The distribution and variance of the data on the expression of BcL-2 are normal and homogeneous, then the One Way Anova test produces a value of p=0.001 (p<0.05) so that it is stated that there is a significant difference in the mean expression of BcL-2 between the four groups. Significant One Way Anova test results followed by Post Hoc test.

**The Effect of Bitter Melon on CASPASE-9 Expression**

The Effect of Giving Bitter Melon Extract on CASPASE-9 Protein Expression The results showed that the average expression of CASPASE-9 protein in the P3 group was the highest, followed by the average expression of CASPASE-9 group P2 and subsequently group P1. The CASPASE-9 expression data of the four groups were all normally distributed, indicated by Shapiro Wilk's results obtained p>0.05 values and also had homogeneous data variants shown by the results of Levene's Test with a value of p=0.405 (p>0.05), then a One Way Anova test was carried out which obtained a value of p=0.022 (p<0.05) which means that there was a significant difference in the average expression of CASPASE-9 protein between the four groups. Post Hoc Results were conducted.

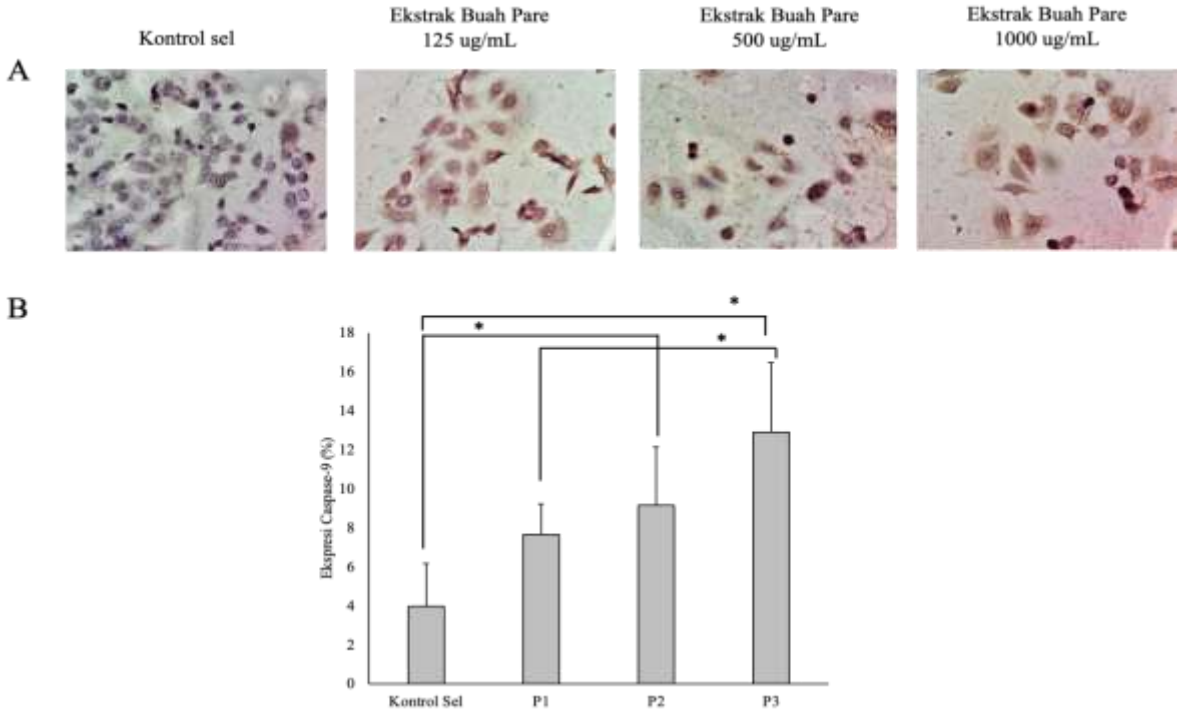


**Figure 1. Effect of bitter melon extract on Caspase-9 protein expression in MCF-7 cells. A. CASPASE-9 expression using ICC, B. the percentage graph of CASPASE-9 expression.**

The effect of bitter melon extract on CASPASE-9 protein expression in MCF-7 cells during 24-hour treatment (Figure 1). The visualization of CASPASE-9 expression was observed by the immunocytochemistry (ICC) method and the Percentage graph of CASPASE-9 expression after being treated with bitter gourd extract on MCF-7 cells for 24 hours. Protein expression profiles were presented from 5 experiments.

**Table 2. LSD Post Hoc test result on CASPASE-9 expression between group**

| Group | Comparison Group | Sig. |
|---|---|---|
| Control | P1 | 0,125 |
| | P2* | 0,044 |
| | P3* | 0,004 |
| P1 | P2 | 0,516 |
| | P3* | 0,046 |
| P2 | P3 | 0,132 |

* sign indicates a significantly different group.

### The Effect of Bitter Melon Extract (Momordica Charantia) On CASPASE-9 and Bcl-2 Proteins Expression

Based on the data in Table 2, it was found that the average cell control and P2 (p=0.044) were different meaning, that cell control with P3 (p =0.004) was also significantly different, while cell control and P1 (p =0.125) were not significantly different. CASPASE-9 protein expression between P1 and P3 differs significantly (p=0.046). Post Hoc LSD test results on CASPASE-9 protein expression showed that giving bitter gourd extract at doses of 1/2,1, and 2 IC50 can increase CASPASE-9 protein expression in MCF-7 breast cancer cells.

### The Effect of Bitter Melon Extract on BcL-2 Protein Expression

In this study, the BcL2 protein expression test was selected at 3 doses according to the IC50 results, namely 1/2 IC50, 1 IC50, and 2 IC50.

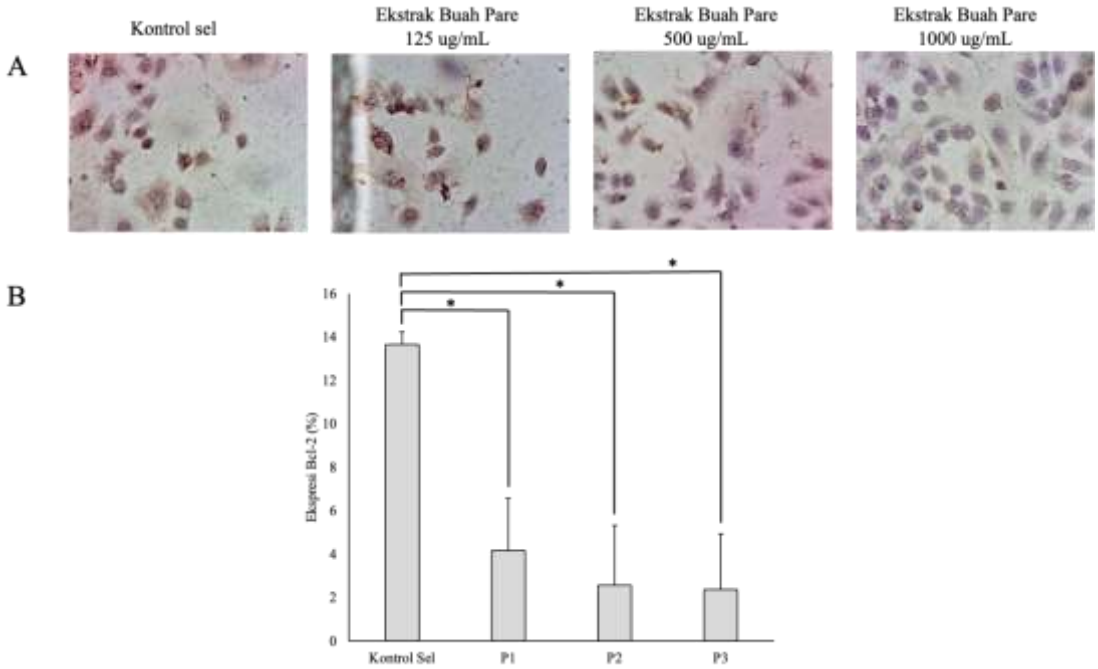


**Figure 2. Effect of bitter gourd extract on BcL2 protein expression in MCF- 7 cells. (A) BcL-2 expression images observed by the ICC method, (B) The percentage graph of BcL-2 expression after being treated with bitter gourd extract on MCF-7 cells.**

Figure 2 shows the effect of bitter melon extract on BcL-2 protein expression in MCF- 7 cells during 24 h treatment. The BcL-2 expression images were observed by the ICC method and the percentage graph of BcL-2 expression after being treated with bitter melon extract on MCF-7 cells for 24 hours. Protein expression profiles were presented from 5 experiments.

**Table 3. The Post Hoc LSD test result on BcL-2 expression between group**

| Group | Comparison Group | Sig. |
|---|---|---|
| Cell Control | P1* | 0,000 |
| | P2* | 0,000 |
| | P3* | 0,001 |
| P1 | P2 | 0,412 |
| | P3 | 0,363 |
| P2 | P3 | 0,925 |

* sign indicates a significantly different group

There was a significant difference between the cell control and P1 groups (p=0.000). Among the cell control group with the P2 group (p=0.000) and between the cell control group with the P3 group (p=0.001) there were meaningful differences with successive p values (p=0.000), (p=0.000), (p=0.001). Post Hoc LSD test results on BcL2 protein expression data showed that there were significant differences in the administration of bitter melon extract between the control group and the treatment group.

## IV. DISCUSSION

Breast cancer cells are the most common population of cancer cells found in the world with a therapeutic resistance incidence rate of up to 78 Breast cancer cells are the most common population of cancer cells found in the world with a therapeutic resistance incidence rate of up to 78%.[46] Induction of apoptosis is a strategy for killing cancer cells, including breast cancer cells. [47] Recent research reveals MCF-7 cancer cells have the ability to protect themselves from apoptosis programs by increasing the expression of anti-apoptosis proteins such as BcL-2 and suppressing the expression of pro-apoptosis proteins such as CASPASE-9. [48,49] The breast cancer cells in this study came from MCF-7 breast cancer cells. MCF-7 breast cancer cells have the characteristics of PgP overexpression, and BcL-2 overexpression, and do not express Caspase-3 so as to avoid apoptosis. [50] MCF-7 cell resistance to chemotherapy through the efflux pump mechanism on the cell membrane causing failure of apoptosis initiation. [48,51]

The concentration of each dose of bitter gourd extract used in the cytotoxic test was 7.8125 ug/mL, 15.625 ug/mL, 31.25 ug/mL, 62.5 ug/mL 125 ug/mL 250 ug/mL 500 ug/mL and 1000 ug/mL. The results showed that bitter gourd extract is cytotoxic with dose-dependent patterns (Figure 5.1). The pattern of cell growth will decrease with the increase in dose. The results of cytotoxic tests on MCF-7 cells obtained an IC50 value of 495.885 ug / mL. This value will be used in research on the effect of bitter gourd extract on the expression of Caspase-9 and BcL2 proteins at doses of 1/2 IC50, 1 IC50, and 2 IC50. These cytotoxic results illustrate that bitter gourd extract decreases the growth of MCF-7 cells. The mechanism of inhibition of MCF-7 cell growth can occur through the induction of apoptosis. These results are supported by previous studies that reported that bitter gourd extract has a cytotoxic effect on MCF-7 cells with an IC50 value of 10.98 ug/mL.[52] This value is lower because the type and place where the bitter gourd extract is used are different, causing the content of secondary metabolites to also be different. Several other studies have also reported that MDA-MB231 breast cancer cells also have the same phenomenon when treated with bitter gourd extract.

The cytotoxic effect of bitter gourd extract is due to the content of secondary metabolites such as flavonoids, saponins, alkaloids, and tannins in bitter gourd extract which can inhibit cell proliferation. Previous studies have also reported bitter gourd extract may inhibit through cell growth mechanisms inducing apoptosis, cell cycle, and metastasis. [53]The IC50 results in this study tend to be weakly cytotoxic with an IC50 value of > 100 ug / mL, this can be influenced by the content of active compounds in bitter gourd extract which is polar so that it is difficult to penetrate the MCF-7 cell membrane which is non-polar.

This study showed that the expression of Caspase-9 protein in the P2 and P3 groups experienced a significant increase compared to the cell control group. This shows that bitter gourd extract has an influence on the process of apoptosis of MCF-7 breast cancer cells through increased expression of Caspase-9 protein. This is thought to be due to the influence of secondary metabolite compounds of bitter gourd extract such as flavonoid compounds that can play a role in the apoptosis process through the induction of Caspase-9. Caspase-9 is a family of protease enzymes that play a central role in the process of intrinsic apoptosis that will induce caspase-3 to form DNA fragmentation and apoptotic bodies. The increase in Caspase-9 protein expression in this study is in line with previous studies that flavonoid compounds increase Caspase-9 protein expression which causes Caspase-3 induction so that apoptosis occurs. [54] Other studies have also reported that flavonoid compounds in bitter gourd extract induce cell death through the induction of Caspase-8 and Bad pathways that will induce cytochrome C thereby activating the release of Caspase-9. [55] *Bitter gourd extract has also been shown to induce cell death via the dependent caspase pathway.* [53]

In this study, it was also proven that bitter gourd extract decreased the expression of BcL2 protein. This is thought to be because the secondary metabolite compounds of bitter gourd extract inhibit the phosphorylation of protein kinase B (Akt), lower cell B 2 lymphoma (BcL2), and increase protein X related BcL2 (BAX) and the breakdown of caspase 3, which then triggers apoptosis of breast cancer cells. 56 It is also proved that bitter gourd extract in addition to affecting the dependent caspase pathway, can also trigger other independent pathways such as increasing the activity of cathepsin proteins, calpains, bax/bid pro-apoptosis proteins, serine proteases, and the BcL2 family. 52 Other studies have also reported that flavonoid compounds induce intrinsic or mitochondrial pathways of apoptosis.[54] This pathway begins with the release of cytochrome c into the mitochondria, which bind to apaf1, dATP, and proCaspase-9 to form apoptosomes. Then proCaspase-9 is split and effector caspases are activated. [57,58] Flavonoids also affect extrinsic or cytoplasmic apoptosis pathways by activating the binding of specific ligands to the corresponding cell surface death receptors. The death receptors are grouped and promote the recruitment of adapter proteins (e.g., FADD), which can interact with procaspase-8 to produce its active form. Then the downstream effector caspase is activated. Caspase-8 may also interact with the intrinsic apoptosis pathway by splitting the bid, which results in the release of cytochrome c. Antiapoptosis members of the BcL2 family (BcL-2 and Bcl-xL) can block apoptosis, but their proapoptosis members (Bax and Bid) can also regulate programmed cell death. [54,55]

## V. CONCLUSION

The administration of Administration of bitter gourd extract doses of 1/2 IC50, 1 IC50, and IC50 has been shown to significantly affect the increase in Caspase-9 protein expression in MCF-7 breast cancer cells. Administration of bitter gourd extract doses of

## The Effect of Bitter Melon Extract (Momordica Charantia) On CASPASE-9 and Bcl-2 Proteins Expression

1/2 IC50, 1 IC50, and IC50 has been shown to significantly affect the increase in Caspase-9 protein expression in MCF-7 breast cancer cells.

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