Original Article

# Effect of Bitter Melon *(Momordica charantia)* on Caspase - 3 Expression and the number of Apoptotic Cells in T47D Cancer Cell Culture

Chodidjah[1], Titiek Sumarawati[1], Eni Widayati[1]


## ABSTRACT

### Background

Cancer is reason death number two in the world. Bitter melon has component bioactive that shows potency activity anti-cancer. Extract bitter melon fruit is known as anti- cancer in human breast cancer cells, MCF-7 and MDA-MB-231, ***Objectives:*** Objective study in vitro, knowing caspase 3 expression and amount cells undergoing apoptosis in the given T47 D cell culture extract bitter melon.

### Methodology

Study experimental laboratory with post-test only randomized control group design.T47 D cell culture samples group control and group treatment each as many as 3 wel with density 5x10 $^4$ cells /100 µl, each well filled with T47 D cell culture density cells 5x10 $^4$ cells /100 µl, given extract ethanol bitter melon fruit dose ½ IC 50. IC50 and 2 IC50, incubation for 24 hours. After 24 hours, the Coverslip is removed and carried out painting immuno cytochemistry for see expression caspase -3 and Fluocytometry for see the number of cells undergoing apoptosis Data on caspase 3 expression were tested with One-way anova test and Post hoc test. Fluocytometry data, served in a way descriptive.

### Result

The IC50 dose resulted in a value of 269.4 µg/mL. The average expression of caspase-3 in T47D cell cultures was 15.23+2.05 in the control group, 21.17+1.44 in the half IC50 dose group, and the highest in the IC50 dose group was 23.28+2.25. The lowest expression of caspase-3 in T47D cells was found in the group with a dosage of 2 IC50, which was 7.58+0.68. The amount of apoptotic cells with Fluocytometry was increased expression . The highest amount of apoptotic cells was found in the 2 IC50 dose group, whereas it was the lowest in the 1/2 IC50 dose group.

### Conclusion

Ethanol extrac of bitter melon fruit has anti cancer activity.



## Keywords

Cancer cells; T47D; Bitter Melon; Caspase 3; apoptosis


## INTRODUCTION

Breast cancer is a type of malignancy that originates in the breast tissue. In Indonesia, In Indonesia, the 10 types of cancers, ranked the most common are breast (36.1%), cervical (17.3%), nasopharyngeal (8.2%), lung (7.4%), rectal (6.9%), leukemia (6.7%), ovarian (6.3%), lymphoma (5.3%), colon (4.0%), and prostate (2.0%).

In Central Java, the most common cancer amongst 6,714 patients is breast cancer (29%), followed by the cervix (16%), leukemia (10%), nasopharynx (9%), ovarium (9%), lymphoma (8%), rectal (7%), lungs (7%), colon (4%), and prostate cancer (1%).[1]

Despite various treatments such as surgery, radiotherapy, and chemotherapy, there is still a lack of success in finding a good cure. However, empirical evidence suggests that herbal plants can be effective in treating various diseases, including cancer, and one such plant is bitter melon[2,3] T47 D cell line is one of breast cancer cell line classification[4]

Study breast cancer cell in MCF-7 and MDA-MB-231culture , and epithelium human cells an in vitro model for see effect of bitter melon (*Momordica charantia* ) extract (BME) as agent


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anticancer . Recent studies have shown the effects of bitter melon extract (BME) on human breast cancer cells (MCF-7 and MDA-MB-231) and human primary breast epithelial cells as an in vitro model. The results indicate that the treatment with BME significantly decreased the proliferation of cancer cells and increased the rate of cell death through apoptosis. These findings suggest that BME has an anti-tumor effect by hindering tumor growth and increasing apoptotic activity[5]

Prostate cancer cells culture. treated with BME will happen increased Bax protein which is a pro-apoptotic protein[6]

Bitter melon plant extract include its leaves, fruit, and roots, which contain a high phenol content of 49.42+1.28 mg. The cytotoxic effect of bitter melon plants was tested by LDH (lactate dehydrogenase) assay in cell culture of cervical cancer (Hela cells) at concentrations of 80, 100, and 120 µg/ml, resulting in 0.51 and 98% cell death in Hela cells. At concentrations of 140, 160, and 180 µg/ml, the cytotoxic effect on Siha cells was 0.3 and 70%. These findings suggest that BEM (bitter melon plant extract) has a powerful antioxidant effect with a moderate cytotoxic effect on Hela and Siha cell cultures[7]

A recent study shows that bitter melon extract has a cytotoxic effect on cancer cells in human cell cultures, including nasopharynx, gastric, colon, and lung cancer. The study found that BEM shows cytotoxic activity at IC50 levels ranging from 0.25 to 0.35 mg/ml after 24 hours. The researchers also measured apoptosis using the DAPI staining method and DNA fragmentation with *Agarose gel Electroporese*, which showed an increase in Bax protein (anti-apoptotic protein) and a decrease in BCl2 protein (anti-apoptotic protein.[8]

Further research is required to explore the potential of bitter melon plants that have proven characteristics of cytotoxicity against cancer cells. This can be achieved by conducting in vitro studies using T47 D cell culture. A recent study show that bitter melon fruit etanol extract was found to increase Bax protein expression and decrease BCl2 protein expression, which were measured with Immunohistochemistry[9] However, the current quantity of data on the death consequences of cancer is still high. Therefore, more studies are needed to determine the efficacy of bitter melon extract in preventing cancer cells from proliferating. It is important to note that this research was conducted with the approval of the ethics committee.

## METHOD

This study is an experimentals laboratory , with *posttest only randomized control group design,* Study done at the Integrated Biomedical Laboratory Sultan Agung Islamic University Semarang. and at the Parasitology laboratory of the Faculty of Medicine, Gadjah Mada University, Yogyakarta.

T47 D cell culture samples each control group and treatment group are 3 well with density 5x10 $^{4}$ cells /100 µl, each well filled with T47 D cell culture density with 5x10$^{4}$ cells /100 µl then given extract ethanol bitter melon fruit dose ½ IC 50. IC50 and 2 IC50, incubated for 24 hours in an incubator with temperature 37 $^{0}$ C. After 24 hours, the Coverslip was taken and carried out painting *immuno cytochemistry* for see expression caspase - 3 and also measured apoptosis using Flowcytometry.

### Preparation of Ethanol Extract of Bitter Melon Fruit

To prepare the ethanol extract of bitter melon fruit, take 5 kg of the fruit and wash it thoroughly with running water. Then, slice the fruit and separate the seeds. Allow it to dry in the air, without exposing it to direct sunlight, until it becomes simplicia dry. After this, pound the dried fruit until it becomes smooth. Next, take 200 g of the powder of dried bitter melon leaves and extract them using a Soxhlet tool. Use 2 L of 90% ethanol solvent in a ratio of 1:10. The yield extract obtained should be weighed, and the result should be 50 g. _Measuring of cytotoxic, result IC50 dose of bitter melon fruit 269.4 µg/ml

### Caspase -3 expression with immunocytochemistry technique. (11)

T47D cells were seeded at 5x10$^{45}$ cells/well on coverslips in 12 -well plate until 80% confluent (24 h incubation). The medium was then repl a c e d by fresh medium containing ethanol extract of bitter melon dose ½ IC 50, one IC 50 and two IC50.. The cells was then incubated in a humidifien incubator (37oC and 5% CO2) O2 for 24 h. After incubation, the medium was discharded and the cells were washed with PBS and then fixed with cold methanol for 10 min at -4oC. Afterward, the cells were washed with PBS and blocked in hydrogen peroxide blocking solution for 10 min at room temperature. The cells were incubated with primary antibody of caspase – 3 for 1 h at room temperature. The cells were washed three times with PBS, then incubated with secondary antibody for 10

min. After washing with PBS, the cells were incubated in 3,3 diaminobenzidine solution

for 10 min and then washed with aquadest. After this step, the cells were counterstained with Mayer-Haematoxylin for 3 min. After incubation, the coverslips were taken and the stained cells were washed with aquadest, and then immersed with xylol and alcohol. The expression of caspase - 3 proteins were observed using a light microscope (Nikon, Japan) and photographed using a digital camera (Canon, Japan). Positive and negative expressions of protein were represented by a dark brown and purple color in a the cells cytoplasm, respectively.

### Flowcitometry technique

Collect cell suspension into 12×75 mm Falcon FACS tube and centrifuge 5 min, 1100 rpm at room temperature (RT). Resuspend cell pellet in 1–2 mL of Annexin V Binding Buffer (AVBB) and centrifuge as in step 1.Discard supernatant and add 100 µL of PI staining mix in AVBB. Add 2–4 µl of Annexin V-FITC or -APC conjugate. Incubate 15 min at RT. Add 500 µL of AVBB and keep samples on ice. Analyze samples on a flow cytometer. Use 488 nm excitation line (Argon-ion laser or solid state laser) and emission collected at 530 nm (green, FITC) and 575–610 nm (orange, PI). Alternatively use flow cytometer with 488 nm excitation for PI (emission collected at 530 nm) and 633 nm excitation for Annexin V-APC conjugate (emission collected at 660 nm). Carefully adjust the logarithmic amplification scale and compensation between green and orange channels. No compensation between PI and APC conjugate is needed. Distinguish between viable cells (Annexin $V^-$ / $PI^-$), early apoptotic cells (Annexin $V^+$ / $PI^-$), late apoptotic/necrotic cells (Annexin $V^+$ / $PI^+$) and late necrotic cells (Annexin $V^-$ / $PI^+$)

### Data Analysis:

The number of cells expressing caspase -3 and the number of cells that underwent apoptosis during a 48-hour incubation period are presented in image form. Data calculated using the hot spot and Statistical analysis with One-way ANOVA test.

### Ethical Clearance

The study protocol was approved by the Commission of Heath Research Ethics, Faculty of Medicine Sultan Agung Islamic University, Semarang

## RESULTS

**Table 1.** shows the mean and standard deviation of caspase -3 expression in T47D cell cultures, along with the results of *Fluocytometry* analysis of T47D cells undergoing apoptosis.

| Dosage/ Group | Caspase 3 | Fluocytometry | | | |
|---|---|---|---|---|---|
| | | Living cells | Early Apoptosis | Late Apoptosis | Necrosis |
| Control | 15.23 + 2.05 | 97.2 | 1 | 0.4 | 1.5 |
| ½ IC50 | 21.17 + 1.44 | 90.3 | 0.9 | 1.8 | 7.9 |
| IC50 | 23.28 +2.25 | 82.3 | 0.8 | 2.7 | 16.4 |
| 2 IC 50 | 7.50 +0.68 | 10.9 | 0.2 | 16 | 75 |

Caspase-3 data were tested with normality and homogeneity tests the result normal and homogeneous data distribution Then continued with the One Way Anova Test the results were $p<0.005$ then tested with the Post Hoc LSD Test, the results seen in table 2.

Post hoc statistical test results for LSD expression of Caspase - 3 T47D cells

The data related to Caspase-3 were subjected to normality and homogeneity tests, and the results showed a normal and uniform distribution. The One-Way ANOVA test was then conducted, which yielded a result of $p<0.005$. This was followed by a Post Hoc LSD Test, and the results are presented in Table 2.

**Table 2.** The post hoc statistical test results for the LSD expression of Caspase-3 in T47D cells can be found in

| Group | Control | ½ IC50 | 1 IC50 | 2 IC50 |
|---|---|---|---|---|
| Control | | 0.27 | 0.006 | 0.004 |
| 1/2 IC50 | | 0,27 | 0.4 | 0,000 |
| One IC50 | | 0,000 | 0,4 | 0.000 |
| two IC 50 | | 0,000 | 0,000 | 0,000 |

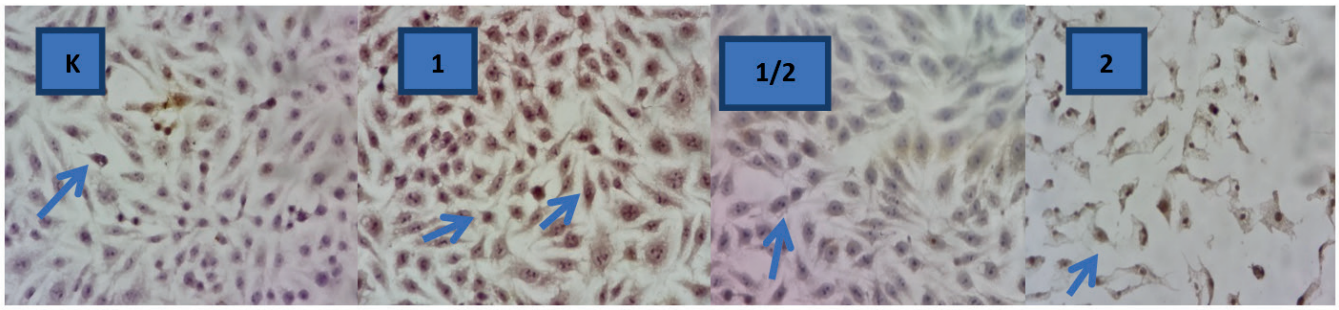
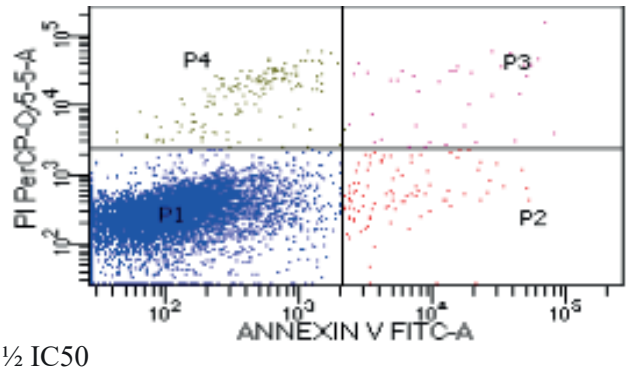
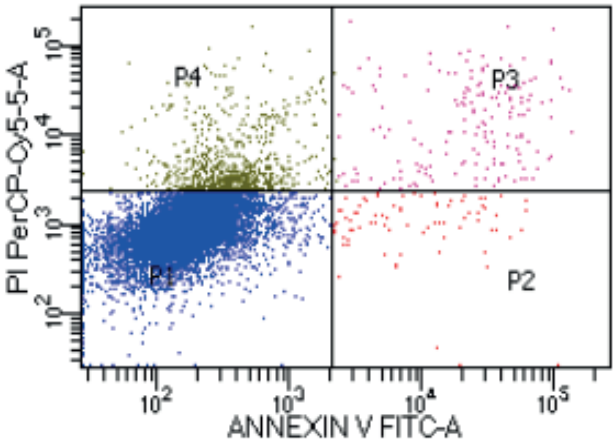



**Figure 1.** Expression of caspase -3 in group T47D cells control dose 1 IC 50, dose ½ IC 50 and dose 2 IC50. caspase -3: color brown in cytoplasm

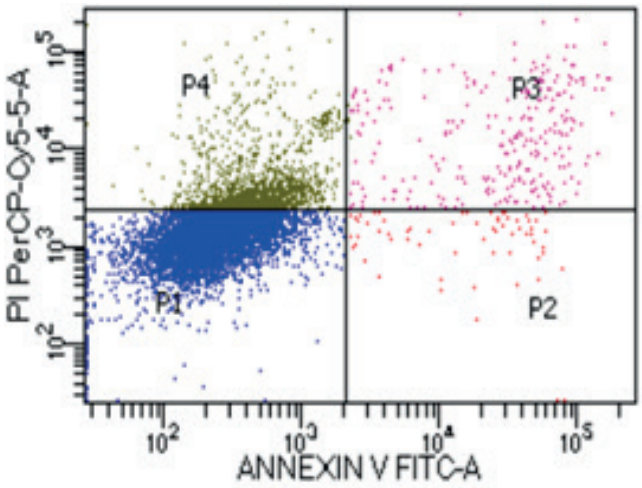
Control




½ IC50




IC50




2 IC50

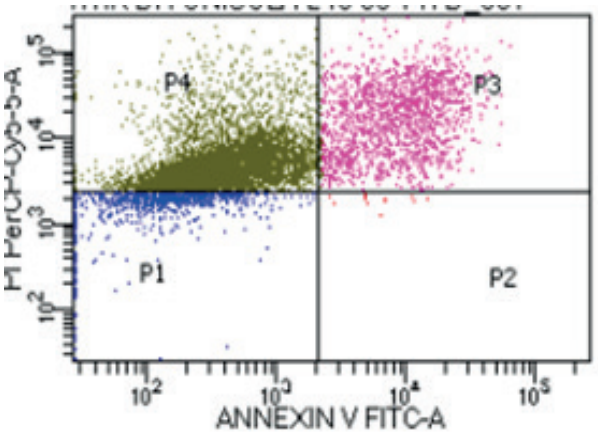



**Figure 2.** *Fluocytometry* results on Control cell ,one IC50, ½ IC50 and two IC50

Bottom left : cell life (P1), Right bottom : early apoptosis (P2), Right top : final apoptosis (P3), and top left (P4): necrosis

Table 1 show the expresion the Caspase - 3 expression of cultured T47 D cells. The control group had an expression of 15.23+2.05 , with increased to 21.17+1.44 in the dose half IC50 and reached the highest expression of 23.28+2.25, in the dose one IC50, the lowest expression of caspase - 3 in T47 D cells was seen in the dose two IC50 with was 7.58+0.68 (figure 1)

The result of the_ *Fluocytometry* ,the highest amount of T47D cells underwent apoptosis in the two IC50 dose (16.2 %), IC50 dose (3,5 %) , and the lowest amount was seen at the ½ IC 50 dose

(2,7 %) .The results of the *Fluocytometry* test are shown in Figure 2. The test was performed on control cells, as well as cells treated with IC50, half IC50, and 2 IC50. The bottom left of the figure shows the cell life (P1), the bottom right shows early apoptosis (P2), the top right shows final apoptosis (P3), and the top left shows necrosis.

## DISCUSSION

Caspase - 3 expression and quantity cells undergoing apoptosis experience increase in IC 50 dose. With LSD test, in dose ½ IC 50 caspase - 3 expression is the same with control dose , that mean this extract has not effect to apoptosis in ½ IC 50 dose. IC50 extract bitter melon in this research as big as amounting to 269.4 µg/ml.

Extract ethanol the IC50 dose of bitter melon fruit (269.4 µg/ml) is capable increase right amount cells undergoing apoptosis (23.28+2.25) compared with group control (15,23+ 2,05) this is potential activity antioxidant extract bitter melon fruit against T47 cells are shown from apoptotic ability . The IC50 is the concentration of an antioxidant-containing substance required to scavenge 50% of the initial DPPH radicals. The lower the IC50 value, the more potent is the substance at scavenging DPPH and this implies a higher antioxidant activity The IC50 value of a compound determines how active it is. For example, an IC50 value of 5µg/mL is categorized as very active, IC50 values ranging from 5-10 µg/mL are categorized as active, IC50 values ranging from 11-30 µg/mL are categorized as medium, and IC50 values above 30 µg/mL are categorized as no active.

In this particular study, the IC50 value of bitter melon was found to be 269.4 µg/ml, which is categorized as no active. However, it is still capable of having an apoptotic effect on cells.

Apoptosis is a process in which cells undergo programmed cell death. During this process, cells experience an increase in two IC50 dosage. Interestingly, the quantity of caspase -3 expression remains the same at both dose ½ IC50 and dose IC50 ( table 2). In a research study, it was found that bitter melon extract has an IC50 value of 269.4 µg/ml. This means that the IC50 dose of bitter melon fruit (269.4 µg/ml) can increase the number of cells undergoing apoptosis as compared to the control group. The potential antioxidant activity of bitter melon fruit extract against T47 cells is shown in its apoptotic ability. [9]

Apoptosis is programmed cells death consequence various internal stimulation or external The process of apoptosis exists two tract that is intrinsic pathway where exists involvement mitochondria extrinsic pathways (death receptor pathway) . Caspase proteins play a central role in the apoptosis process. Cascade regulation that occurs caused active caspase -3. Caspase -3 functions for breaks down of cellular proteins so that cell become apoptosis. Researchers have found that extracts from bitter melon (*Momordica charant*) can increase caspase - 3 activity in various cancer cell cultures namely Hone 1, AGS, HCT-116, and CL1-0.

Bitter melon fruit contains inhibitory compounds that hinder cell proliferation in different cancer cell cultures, including breast cancer (MDA-MB-231, MCF-7), colon cancer (HT-29, SW480), and pancreatic cancer (BxPC-3, MiaPaCa-2, AsPC-1). [10] Bitter melon extract with water solvent ethanol or methanol has anti- cancer activity in hinder metastasis cancer without effect toxic to normal cells . Apoptosis is a process relate with ROS, activation from enzyme that hinder stem cell population in cancer, cycle cells invasion metastasis and induces apoptosis and autophagy as well induce system immune. [13]

Crude extract of bitter melon can through various apoptotic pathways in various cell cultures in the woman system reproduction .[14] Apoptotic stimuli can increase regulation of the BH3 protein which activates the BAX and BAK proteins. The BAX protein is regulated by the tumor suppressor gene namely P53. Activation of the BAX and BAK proteins results increasing mitochondrial membrane permeability so that the proteins cytochrome c, SMAC (second mitochondria-derived activator of caspase (SMAC) and Omi come out from mitochondria. Cytochrome c with APAF-1 (apoptotic protease-activating factor-1), dATP and procaspase-9 forms apoptosome inside apoptosomal procaspase -9 is altered becomes caspase -9 and has an effect activates caspase -3 and caspase -7 which damage DNA.[15] A bitter melon extract has been ability induction of apoptosis of something compound very expected especially for agent anti-cancer Extract bitter melon fruit has effect antiproliferation strong

through induction of caspase dependent apoptosis in breasts cancer cells [8]

The ability to induce apoptosis is important in the development of anticancer agents. Bitter melon extract has a strong antiproliferative effect due to its ability to induce caspase-dependent apoptosis in breast cancer cells. Additionally, apoptosis is a sign of inhibition of cell proliferation, making it a desirable outcome for cancer treatment.

Caspase dependent is the apoptotic pathway consists from track extrinsic and intrinsic .

The extrinsic track through bond ligands and receptors death of the cell membrane while the

intrinsic track involves mitochondria.

Receptor death lies on the surface cell is all over family tumor necrosis factor (TNF) receptor CD95 (Fas), and TNF related apoptosis inducing ligand (TRAIL)-R1 and R2. [15,16] IC 50 water extract of bitter melon fruit from China and India in A549 cell culture ( cancer lungs humans cells culture ) of 28.1 µg/mL and 26.7 µg/mL able increasing apoptosis through caspase -3 and caspse -7 activities as well through mitochondrial ROS track .[17] Extracts Inducing Apoptosis in Human Lung Cancer Cell Line A549 via ROS-Mediated Mitochodria Injury. Evidence-based Complement Altern Med. 2019;2019. We can differentiate IC50 from Indonesia, China and India caused by differences geography weather and elements land Activity Bitter melon fruit ethanol extract can be increase Bax protein expression and decreased BCL2 protein expression at doses of ½ IC 50, IC 50 and 2 IC 50 (IC 50 269.4 µg/mL).[18]Bitter melon extract is known to increase the accumulation of p62 protein, induce autophagy and apoptosis in mice with tumors. The p62 protein plays a role in autophagy and correlates with caspase in the apoptotic pathway in MCF7 and MDAMB-231 cell cultures *Momordia methanol extract charantina (Pare))* has effectincreases Bax protein and decreases BCl2 protein expression in cell culture carcinoma nasopharyngeal (Hone -1), AGS cell culture ( Gastric Adenocarcinoma Cells ), HCT-116 ( colorectal cancer ) at a dose of 0.25 to 0.35 mg/mL at 24 Hours [8)]Flavonoids from other plant as immunomodulator can induce apoptosis. Mangosteen peel (*Garcinia mangostana Linn*.) contains xanthan known as an antioxidant. Administration of 12% dose ethyl acetate extract of mangosteen peel cream is proven to significantly lower, TNF-α levels and Caspase-3 levels in the epidermal tissues of guinea pig skin exposed to ultraviolet B light.[19] Pinpinella Alpina treatment with 100-150 mg daily dose for 15 days capable of improving oxidative stress marked by increase in GPx activity and decrease in XO activity and inhibit apoptosis characterized by decrease in the expression of Bax and caspase3 mRNA. There were strong negative correlations between antioxidant activity and apoptosis on Sprague male rats after UVB irradiation.[20] the administration of *Typhonium flagelliforme* ethanoic extract at the dose of 200 mg / kg BW increase apoptotic cells with TUNEL staining from adenocarcinoma mamma mice C3H but can not increased level of TNFα.[21]

Exposure to UV light on epidermal keratinocytes can cause apoptosis, some inflammatory mediators are involved in the activation of apoptosis pathways such as TNF-α, Mangosteen peel (*Garcinia mangostana Linn*.)contains xanthon known is an antioxidant. Administration of 12% dose ethyl acetate extract of mangosteen peel cream is proven to significantly lower TNF-α levels and Caspase-3 levels in the epidermal tissues of guinea pig skin exposed to ultraviolet B light.[22] Other antioxidant, Silk fibroin (SF) is obtained as a biomaterial from the silkworm. SF is used in regenerative medicine. SF also has anti inflammatory and antioxidant that can exhibits protective effects by preventing apoptosis and tissue damage its anti-apoptotic and anti-inflammatory.[23]

The lowest caspase 3 in this study was seen at the 2 IC 50 this can be caused by Sirtuin protein activity. Sirtuin1 (SIRT1) is a histone deacetilase enzyme class III (HDAC III) that plays an important role in substrate deacetylation. Deacetylation in various transcription factors will activate anti apoptotic genes and inhibit pro-apoptotic genes[24]

In this study the cells undergoing apoptosis is hight (16,2%) in 2 IC 50 dose whereas the caspase 3 expression is lowest, this apoptotic pathways can via caspase independent pathways . Apoptotic stimulus causes translocation of the pro-apoptotic protein, Bax, from cytoplasm to mitochondrion. This translocation is caused by the activation of proteases, calpains and cathepsins. It is the lysosomes that release cathepsins and calpains, which are triggered, following the influx of Ca2+ in the cell activated by stressed endoplasmic reticular ER.[25]

## CONCLUSION

Ethanol extract of Bitter Melon (*Momordica charantia*) fruit can increased Caspase - 3 expression and apoptotic cells in T47D cancer cell culture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### Conflict of interest

All authors declare that there are no conflict of interest in the present study.

### Contribution of Authors

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Data gathering : Eva Lutfiyana, Rumbi

Writing and submission of manuscript : Chodidjah,Rina, Putri R Ayuningtyas

Editing and approval of final draft : Putri R Ayuningtyas, Chodidjah

## REFERENCES


1. Jayalie1 FV, Kotambunan1 C, Apriantoni1 R, Manuain1 DA , Hawariy S, Ben Prajogi1 G. Epidemiology of 10 Cancer Types in Indonesia: A Multicenter Study . *Beranda* 2023;**14** (1):
2. Saeed F, Afzaal M, Niaz B, Arshad MU, Tufail T, Hussain MB, et al. Bitter melon (Momordica charantia): A natural healthy vegetable. *Int J Food Prop [Internet*]. 2018;**21**(1):1270–90. Available from: https://doi.org/10.1080/10942912.2018.1446023
3. Yung MMH, Ross FA, Hardie DG, Leung THY, Zhan J, Ngan HYS, et al. Bitter Melon (Momordica charantia) Extract Inhibits Tumorigenicity and Overcomes Cisplatin-Resistance in Ovarian Cancer Cells Through Targeting AMPK Signaling Cascade. *Integr Cancer Ther.* 2016;**15**(3):376–89.
4. Dai X, Cheng H, Bai Z, Li J. Breast cancer cell line classification and Its relevance with breast tumor subtyping. *J Cancer.* 2017;**8**(16):3131–41.
5. Ray RB; Raychoudhuri A; Steele R; Nerurkar P. Bitter melon (Momordica charantia) extract inhibits breast cancer cell proliferation by modulating cell cycle regulatory genes and promotes apoptosis. *Cancer Res*., 2010;**70**:1925–1931.
6. Ru P, Steele R, Nerurkar P V., Phillips N, Ray RB. Bitter melon extract impairs prostate cancer cell-cycle progression and delays prostatic intraepithelial neoplasia in TRAMP
7. Fongmoon D, Lalitwongsa S, Keyoonwong W, Nakong M, Iamsaard S. Antioxidant activity and cytotoxicity of bitter melon (Momordica charantia L.) extract cultured in Lampang Thailand. *NU Sci J [Internet].* 2013;**10**(2):18–25. Available from: http://www.thaiscience.info/journals/Article/NUSJ/10970158.pdf)
8. Li CJ, Tsang SF, Tsai CH, Tsai HY, Chyuan JH, Hsu HY. Momordica charantia extract induces apoptosis in human cancer cells through caspase-and mitochondria-dependent pathways. *Evidence-based Complement Altern Med.* 2012;2012.
9. Rahmasari D , Sumarawati]. T , Trisnadi · The Effect of Bitter Melon Extract (Momordica Charantia) On CASPASE-9 and Bcl-2 Proteins Expression.International *Journal of Multidisciplinary Reseacrh and Analysis*, September 2023 ; **06**(09): Page No. 4256-4263
10. Marti GE, Stetler-Stevenson M, Bleesing JJH, Fleisher TA. *Introduction to flow cytometry*. 2001;**38**: Seminars in Hematology.. 93–99 p.
11. Putra PN, Wulandari S, NugrohoAE, Fakhrudin N, Astuti P, Sudarsono.
12. Tylophorine Abrogates G2/M Arrest Induced by Doxorubicine and Promotes Increased Apoptosis in T47D Breast Cancer Cells . *Asian Pac J Cancer Prev,* **19** (11): 3065-3069)
13. Fang EF, Froetscher L, Scheibye-Knudsen M, Bohr VA, Wong JH, Ng TB. Emerging Antitumor Activities of the Bitter Melon (Momordica charantia). *Curr Protein Pept Sci.* 2018;**20**(3):296–301.
14. Sur S, Ray RB. Bitter melon (Momordica charantia), a nutraceutical approach for cancer prevention and therapy. *Cancers (Basel).* 2020;**12**(8):1–22.
15. . Psilopatis I, Vrettou K, Giaginis C, Theocharis S. The Role of Bitter Melon in Breast and Gynecological Cancer Prevention and Therapy. *Int J Mol Sci.* 2023;**24**(10)

16. Pfeffer CM, Singh ATK. Apoptosis: A target for anticancer therapy. *Int J Mol Sci*. 2018;**19**(2):

17. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 2018;**25**(3):486–541.

18. Thiagarajan S, Arapoc DJ, Husna Shafie N, Keong YY, Bahari H, Adam Z, et al. Momordica charantia (Indian and Chinese Bitter Melon)Extracts Inducing Apoptosis in Human Lung Cancer Cell Line A549 via ROS-Mediated Mitochodria Injury. *Evidence-based Complement Altern Med.* 2019;2019.

19. Anggrahenny D, Sumarawati T, Chodidjah C. The Effect of Pare Ethanol Extract on Bax and Bcl-2 Protein Expressions (In vitro study on T47D Breast Cancer Cell Culture). *Int J Multidiscip. Res Anal.* 2023;**06**(03):1056–61

20. Harlisa P, Sentono HK, Purwanto B, Dirgahayu P,Soetrisno. The Ethyl Acetate Extract of MangosteenPeel Cream Attenuates Ultraviolet B Radiation-Induced Apoptotic Cell Death Via Antioxidant EffectBy Regulation TNF-A and Caspase 3 in Guinea PigSkin. *Bangladesh J Med Sci.* 2022;**21**(3):512–20. doi/10.329/bjms.V2i3.59563

21. Widayati. E, Nasihun T, Treatment of Pimpinella Alpina Molk Improve Oxidative stress and Inhibit Liver Cellular Apoptosis in Rats Following UVBIrradiation: Is there Any Correlation? *BangladeshJournal of Medical Science.* Jan2018; **17**(01):118-128 doi/10.329/bjms.V17i1.35292

22. Chodidjah, .-., Dharmana, E. ., Susanto, H. ., & Ekawuyung, P. The effect of the ethanol extract of Typhonium flagelliforme on apoptosis adenocarcinoma mamma cells in C3H mice. *Bangladesh Journal of Medical Science*, 2023;**22**(2): 329–335. https://doi.org/10.3329/bjms.v22i2.64991

23. Harlisa, P. ., Sentono, H. K. ., Purwanto, B. ., Dirgahayu, P. ., & Soetrisno, .-. The Ethyl Acetate Extract of Mangosteen Peel Cream Attenuates Ultraviolet B Radiation-Induced Apoptotic Cell Death Via Antioxidant Effect By Regulation TNF-A and Caspase 3 in Guinea Pig Skin. *Bangladesh Journal of Medical Science*, 2022;**21**(3): 512–520. https://doi.org/10.3329/bjms.v21i3.59563

24. Şehirli, A. Özer ., Aykaç, A. ., Özkayalar, H. ., Savtekin, G. ., Sayıner, S. ., & Sayıner, S. Ameliorative effect of Silk Fibroin against 5-Fluorouracil (5-FU)-induced gastrointestinal damage in rats. *Bangladesh Journal of Medical Science*, 2023;**22**(4): 907–915. https://doi.org/10.3329/bjms.v22i4.68679

25. Rahman, S., and Islam, R. Mammalian Sirt1: insights on its biological functions. Cell Commun Signal. 2011; 9(11): 1-8. in Ahsani, D. N., Susilowati, R., & Sumiwi, Y. A. A. Sirtuin 1 (SIRT1) and activated caspase 3 expression on rat spinal cord in acute phase after Sciatic nerve injury. *Bangladesh Journal of Medical Science*, 2018; **18**(1): 50–56. https://doi.org/10.3329/bjms.v18i1.39548

26. Torres, A.C.M.; Ruiz, A.R.; Londoño, M.B.; Molina, M.A.F.; Padilla, C.R. IMMUNEPOTENT CRP induces cell cycle arrest and caspase-independent regulated cell death in HeLa cells through reactive oxygen species production. *BMC Cancer* 2018, **18**, 13 in Kakali Bhadha.