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# A HERBAL TREATMENT METHOD FOR BREAST CANCER: ANTITUMOR EFFECT OF MOMORDICA CHARANTIA

## MEME KANSERİNE BİTKİSEL TEDAVİ YÖNTEMİ: MOMORDİCA CHARANTİA'NIN ANTİTÜMÖR ETKİSİ

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## Abstract

Using plant-based treatment approaches and herbal medicines is getting attention these days to avoid the side effects of the current treatment methods for cancer. According to the literature, bitter melon extract exhibits cytotoxic effects against cancer by affecting the hormonal pathways. Therefore, in this research, the effects of the bitter melon extract with its combination of different concentrations of drugs were investigated against different cell lines (MCF-7, MDA-MB-231, RAW 264.7, J774). Both hormone-positive and negative breast cancer cell lines were used and the obtained data were compared with the literature. As a result, when exposed to bitter melon extract, the hormone-positive breast cancer cell line MCF-7 exhibits more cytotoxic effects compared to the triple hormone-negative breast cancer cell line MDA-MBA-231. For the combination of drugs along with the bitter melon extract; the highest cytotoxicity was observed with Tamoxifen.

**Keywords:** Breast cancer, cancer treatment, Momordica charantia, phytotherapy.

## Öz

Kanserde mevcut tedavi yöntemlerinin yan etkilerinden korunmak için bitki bazlı tedavi yaklaşımları ve bitkisel ilaçların kullanımı günümüzde dikkat çekmektedir. Literatüre göre acı kavun ekstraktı hormonal yolları etkileyerek kansere karşı sitotoksik etki göstermektedir. Bu nedenle bu araştırmada acı kavun ekstraktının farklı konsantrasyonlarda ilaç kombinasyonu ile farklı hücre hatlarına (MCF-7, MDA-MB-231, RAW 264.7, J774) karşı etkileri araştırıldı. Hem hormon pozitif hem de negatif meme kanseri hücre hatları kullanıldı ve elde edilen veriler literatürle karşılaştırıldı. Sonuç olarak; Acı kavun ekstraktına maruz bırakıldığında, hormon pozitif meme kanseri hücre dizisi MCF-7, üçlü hormon negatif meme kanseri hücre dizisi MDA-MBA-231 ile karşılaştırıldığında daha fazla sitotoksik etki sergiler. Kudret narı ekstraktı ile birlikte kullanılan ilaçların kombinasyonu için en yüksek sitotoksisite Tamoksifen ile gözlendi.

**Anahtar Kelimeler**: Fitoterapi, kanser tedavisi, meme kanseri, Momordica charantia.

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# 1. INTRODUCTION

Cancer; it can be caused by external factors such as uncontrolled proliferation of cells, carcinogens, radiation, and chemicals, or internal factors such as genetic mutations and DNA damage (Ihlamur et al.,2024a; Ihlamur et al.,2024b). Breast cancer is one of the most common cancer types among women worldwide and can cause a significant amount of deaths per year. However, in the early stages of breast cancer, approximately 70% of the patients can be cured with proper treatment. Chemotherapy and radiotherapy can significantly damage and kill cancer cells. Due to the side effects of the therapies used today, it is crucial to develop new treatment strategies that can be both effective and safe at the same time (Ihlamur et al.,2022; Zengin et al.,2022).

Since natural plants do not show any side effects, their use has gained importance in the development of new alternative treatments against cancer (Alkabban et al. 2022). To date, it has been observed that plant extracts such as *Taraxacum officinale* (Mir et al.,2013), *Uncaria tomentosa* (De Paula et al.,2015), *Astragalus membranaceus* (Zhou et al.,2018), *Ocimum sanctum* (Flegkas et al.,2018), *Allium sativum* (Shareef et al.,2016)., *Punica granatum* (Kelleci,2023), ginger (Ibrahim et al.,2022), green tea (Cheng et al.,2020), and bitter melon (Weng et al.,2013) have been the subject of research for the treatment of breast cancer. These plants have various active components such as secondary metabolites, phenolic acids, flavonoids, tannins, anthocyanin, etc. that can be used in the treatment of cancer (Shobha et al.,2015). These active agents obtained from plants can exhibit anticancer activity. In addition, recent studies also show that using plant-based active agents with a combination of drugs against cancer can increase the efficiency of anticancer drugs.

It has been reported that dandelion (*Taraxacum officinale*) extract affects the growth of breast cancer cells dependent on extracellular receptor kinases on the MCF-7 / AZ cell line (Sigstedt et al.,2008). Another study proved that the anti-carcinogenic effect of Taraxacum officinale was due to the presence of compounds such as phenyl acetyl, 4-hydroxyphenylacetyl, 4-hydroxyphenylglyoxyl (Mir et al.,2013). Cat's claw (*Uncaria tomentosa*) bark extract has been reported to affect the growth of MCF-7 cell line through anti-proliferative effect (Riva et al.,2001) Cat's claw has been reported to cause apoptosis because it contains anti-proliferative alkaloids such as pteropodine, uncarine, oxindole and isopterpodine (De Paula et al.,2015; Pilarski et al.,2010). They also reported that the extract has anti-proliferative, anti-inflammatory and anti-mutagenic properties due to the activation of caspase-3, which also shows a cytostatic effect (De Martino et al.,2006). Goat thistle (*Astragalus membranaceus*) contains toxic components such as trihydroxyoctahydroindolizidine, a lysosomal inhibitor of α-mannosidase, which is used to treat breast cancer and suppress tumor growth (Zhou et al.,2018). It induces cell proliferation and apoptosis of *Astragalus membranaceus* (Wang et al.,2015). A study reported that Astragalus membranaceus extract inhibited cell proliferation of MCF-7, MDA-MB-231 and SK-BR-3 cell lines and reduced p-PI3K, p-Akt, p-GSK-3β and p-mTOR enzyme levels has been reported (Zhou et al.,2018). Holy basil (*Ocimum sanctum*) has been reported to inhibit MDA-MB-435 breast cancer cell migration by targeting vascular endothelial growth factors. (Flegkas et al.,2018). Garlic (*Allium sativum*) exhibits anticancer properties by stimulating macrophages and lymphocytes, as it contains high amounts of polysulphide and sulphite. It is also known that it contains a sulfur-binding substance called Ajoene, which delays the progression of cancer (Shareef et al.,2016).

*Momordica charantia*, also known as bitter melon or bitter gourd, is a vegetable that can be easily found in the tropics, Asia, South America and Africa. It has been used as a traditional herbal medicine among the public for decades due to its beneficial effects on human health. *Momordica charantia* is known to have antidiabetic, anticarcinogenic, antiviral and anti-inflammatory properties thanks to its bioactive components (steroids, alkaloids, flavonoids, proteins, etc.) (Bortolotti et al. 2019). These metabolites induce apoptosis in breast cancer cells by affecting

related hormonal pathways. The most important factor in exhibiting antimutagen and antioxidant properties of bitter melon is that it has rich phenolic compounds, especially gallic acid (Budrat and Shotipruk, 2008). One study reported that a bioactive triterpene isolated from Momordica charantia extract induced apoptotic death in breast cancer cell lines (MCF-7 and MDA-MB-231) (Weng et al.,2013). It is known to be effective against breast cancer thanks to the Cucurbitan type triterpenoids it contains (Sur et al. 2020; Güneş et al. 2019). In a study, these triterpenes were isolated and proved to have cytotoxic effects against different cell lines of breast cancer (MDA-MB-231 and MCF-7). Also kuguaoside A; momordicosit I, F1 and K; It has been reported that goyaglycoside-b and goyaglycoside-b reduce cell viability and proliferation, while eleostearic acid, RNase MC2 and MAP30 inhibit cell growth and cause apoptosis (Raina et al. 2016). Another study investigated the efficacy of MCE treatment in MCF-7 and MDA-MB-231 breast cancer cells and primary human breast epithelial cells. As a result, it was reported that cell apoptosis increased and a serious decrease in cell proliferation occurred (Ray et al.,2010). In a study investigating the cytotoxicity of bitter melon extract against cervical cancer, it was determined that it was 98% at 120 µg/ml concentration and 0% at 80 µg/ml concentration for HeLa cells. In SiHa cells, it was reported to be 0% at 140µg/ml and 70% at 180µg/ml (Fongmoon, 2013). It has been reported that bitter melon fruit and its extract exhibit a high antiproliferative activity in HeLa cells (Deshmukh et al., 2014). In another study, it was reported that the concomitant use of MCE with doxorubicin (DOX) increased the proliferation effect in colon cancer, HT-29 cells, and MDCK cells and decreased the expression of multidrug resistance (Kwatra et al.,2013). All these studies show that MCE increases the effectiveness of chemical drugs used in cancer treatment, positively affects bioavailability, and exhibits anticancer effects.

It is expected that the use of natural plant extracts in addition to existing anticancer drugs in cancer treatment will have beneficial effects. Therefore in this study, bitter melon extract, which is known to be rich in flavonoids was combined with two different anticancer drugs (Tamoxifen and Proleukin) to investigate its anticarcinogenic activity on breast cancer cell lines (hormone positive: MCF-7, hormone negative: MDA-MBA-231). RAW 264.7 macrophage cells and L929 fibroblast cells were used to detect non-toxic concentrations of bitter melon extract. Proleukin and Tamoxifen are drugs used for immunotherapy and are approved for use against cancer (Debela et al. 2021; Amaria et al. 2015). The effects of the extract alone and in combination with drugs have been observed through *in vitro* experiments to prove our claim.

## 2. MATERIALS AND METHODS

### 2.1. The Extractıon of *M. Charantıa*

For the extraction, *M. charantia* plant was taken and washed under distilled water. The seeds were removed, and the microwave was used for 20 minutes to remove the excess water. The 8 grams of remaining dusted bitter melon and 50 ml methanol solution were mixed. The mixture was left for incubation for 6 days in the dark. After that, filtration paper (Whatman No.1) was used, as a result, a mixture of extract and methanol was obtained. To separate the extract from the mixture, methanol was evaporated. Therefore, the bitter melon extract was ready to be used for further experimentation (Sultana et al. 2009).

### 2.2. Preparation of Cell Culture

Human breast cancer cells (MCF-7 and MDA-MB-231) and macrophage cell lines (J774 and RAW 264.7) were obtained from the cryobank at Yildiz Technical University Cell Culture Laboratory (İstanbul, Turkey). Breast cancer cells were cultured in DMEM/F12 medium and other cell lines were cultured with RPMI-1640 (Gibco) medium. Additionally, Fetal Bovine Serum

(FBS) (Sigma), 1% L-glutamine, and 1% penicillin-streptomycin were added to the medium. After that, cells were incubated in the incubator that contained 5% $CO_2$, at under 37°C. The cell growth was observed each day under the inverted microscope. The number of passages of the cell cultures that were used in the experiment is between 10-15.

Once the cells reached the necessary growth, MCF-7 and MDA-MBA-231 cell lines were gathered in an enzymatic way while J774 and RAW 264 cell lines were centrifuged for 5 minutes at 1000 rpm and 25°C in a physical way. After that, cells were seeded using a 96-well plate. Each well had $1x10^5$ cell/ml and they were supplemented with colorless DMEM/F12 medium with 10% FBS solution. The plates were incubated for 24 hours (Gallet et al. 2004).

### 2.3. Cell Viability Analysis

To determine the cytotoxic effects of the bitter melon extract and its combination with drugs, a 3-(4.5-dimetiltiazol-2-il)-2.5-difeniltatrazilyum bromideas (MTT) assay was conducted. Extract (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml ), drug (10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml ) and the combination of extract-drug (40 µg/ml extract ve 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 100 µg/ml drug) were added to the plate wells where cells were seeded with the colorless medium. Each well had $1x10^5$ cells inside. Afterward, the plates were left for incubation for 48 hours inside the incubator with 5% $CO_2$ and 37°C. Then, 10 µl MTT (10 mg/ml) solution was added to each well. After incubation for 3 hours under the same conditions, 100 µl DMSO (Dimethyl sulfoxide) was added to each well, and plates were kept in the dark for 30 minutes so that the reaction could occur. The cell viability was measured under a UV-Vis Spectrometer under 570 nm wavelength (Ghasemi et al. 2021). Every experiment group was tested 3 times and the mean of it was calculated. The cell viability was measured by using equation 1 given below and the graphics were obtained.

Cell viability (%) = (sample absorbance/control absorbance)*100 (1)

### 2.4. Statistical Analysis

All experimental data were presented as the mean ± standard deviation and each experiment was performed at least three times. The statistical analyses were performed by one-way ANOVA using SPSS version 25.0 (SPSS, IL, USA). Values of $p < 0,05$ were considered statistically significant.

## 3. RESULTS AND DISCUSSION

It is approved many previous studies in the literature that plants have active components that can suppress cancer pathways. The secondary metabolites, phenolic acids, tannins, anthocyanin, etc. can be used in the design of new treatments against cancer. Based on this knowledge in the literature, in this research, bitter melon extract and its combination with different cancer drugs in various concentrations were used to determine its effects against breast cancer. The extract was prepared by using the maceration method. To determine the cytotoxic effects of bitter melon extract, J774, and RAW 264.7 macrophage cell lines were used. To observe its killing activity against breast cancer cells, MCF-7 and MDA-MB-231 breast cancer cell lines were used.

In addition, the most suitable and efficient combination of bitter melon extract-drug (Tamoxifen, Proleukin) formulations were determined which can be used for *in vivo* studies for the treatment of breast cancer. For this purpose, first of all, the most suitable extract concentration was determined and then its combination of different concentrations of drugs was investigated to determine the most efficient one.

The percentage of cell viability is given in the figures. For each figure, the percentage of cell viability is taken as 100% and the rate of cell viability of other groups was determined according to the positive control group. After the incubation for 48 hours, MTT cell viability analysis was conducted, and the groups were compared with each other by their cell viability percentage. The cell viability analysis of the J774 and RAW 264.7 when they are exposed to the different concentrations of the extract is given in Figure 1.

The highest killing activity is observed in the group where 100 µg/ml bitter melon concentration is used. Therefore, this concentration was not evaluated. The results of the experiment are consistent with the literature. For the 40 µg/ml concentration of bitter melon extract, 108,02 % cell viability is observed for the J774 cell line while the percentage slightly increased to 109,44 % for the RAW 264,7 cell line according to the MTT test which was conducted 24$^{th}$ hours after the incubation.

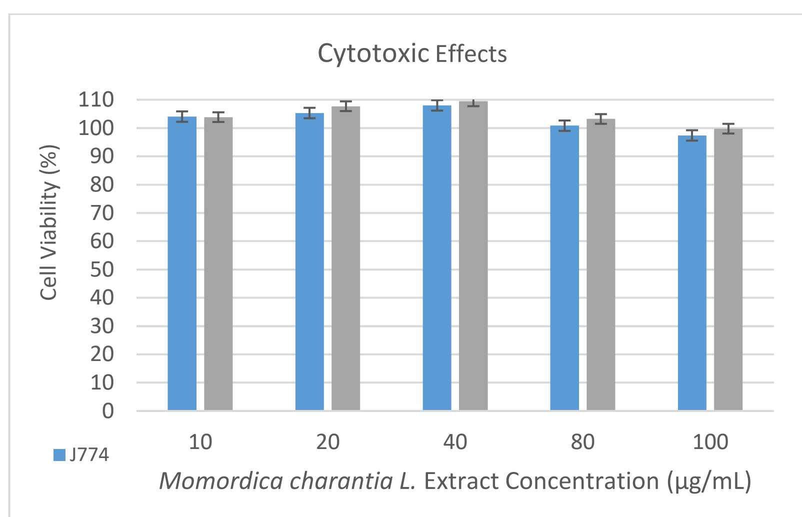


Figure 1. The Cytotoxic Effects of The Bitter Melon Extract Against J774 and RAW 264,7 Cell Lines

The viability of the breast cancer cells when they are exposed to the bitter melon extract is given in Figure 2. As can be seen in the figure given above, the cancer cell viability is decreased as the concentration of the extract decreases. When 40 µg/ml concentration of the bitter melon extract is used against MCF-7 breast cancer cell lines, after incubation for 48 hours, it is determined that the percentage of cell viability is 80,26%. When the same concentration of bitter melon extract is applied to the MDA-MB-231 breast cancer cell line, the cell viability is observed as 81,06% after the 48$^{th}$ hour.

The cell viability analysis at the 48$^{th}$ hour of J774 and RAW 264,7 macrophage cell lines is given in Figure 3. For the 40 µg/ml concentration of Tamoxifen, 79,89% cell viability is observed for the J774 cell line while the percentage is 80,57% for the RAW 264,7 cell line. For the 40 µg/ml concentration of Proleukin, 81,69% cell viability is observed for the J774 cell line while the percentage is 82,31% for the RAW 264.7 cell line.

The results of the cell viability analysis of the MCF-7 breast cancer cell line at the 48$^{th}$ hour, when exposed to Tamoxifen and Proleukin, are given in Figure 4. For the 40 µg/ml concentration of Tamoxifen, 62,03% cell viability is observed while the 40 µg/ml concentration of Proleukin exhibited 65,18% viability. The results of the cell viability analysis of the MDA-MB-231 breast cancer cell line at the 48$^{th}$ hour, when exposed to Tamoxifen and Proleukin, are given in Figure 4.

For the 40 µg/ml concentration of Tamoxifen, 65.96% cell viability is observed while the 40 µg/ml concentration of Proleukin exhibited 68,13% viability.

In this research, the highest cytotoxicity is observed with a 40 µg/ml concentration of the extract. Therefore, the drug combinations were applied with this concentration. The cell viability of J774 and RAW 264,7 cell lines, when exposed to the Tamoxifen and Proleukin drugs at the 48$^{th}$ hour, is given in Figure 5. For the 40 µg/ml bitter melon extract-40 µg/ml concentration of Tamoxifen, 81,85% cell viability is observed in the J774 cell line while the RAW 264.7 cell line showed 82,03% viability.

For the 40 µg/ml bitter melon extract-40 µg/ml concentration of Proleukin, 83,4 7% cell viability is observed in the J774 cell line while RAW 264,7 showed 84% cell viability. The drugs used for the treatment of breast cancer are used to kill cancer cells. However, they have some disadvantages such as; when the drugs are applied alone, they show cytotoxic effects and they are not efficient enough. Therefore, studies show that combining drugs with plant extracts can increase the efficiency of the anti-cancer effects of the drugs while decreasing their cytotoxicity. The results of this research also support this hypothesis.

The results of the cell viability analysis of the MCF-7 breast cancer cell line at the 48$^{th}$ hour, when exposed to Tamoxifen and Proleukin, are given in Figure 6. For the 40 µg/ml bitter melon extract-40 µg/ml concentration of Tamoxifen, 44,03% cell viability is observed while 40 µg/ml bitter melon extract-40 µg/ml Proleukin, showed 48,02% cell viability at 48$^{th}$ hour. The results of the cell viability analysis of the MDA-MB-231 breast cancer cell line at the 48$^{th}$ hour, when exposed to Tamoxifen and Proleukin, are given in Figure 6. For the 40 µg/ml bitter melon extract-40 µg/ml concentration of Tamoxifen, 47,36% cell viability is observed while 40 µg/ml bitter melon extract-40 µg/ml Proleukin, showed 52,96% cell viability.

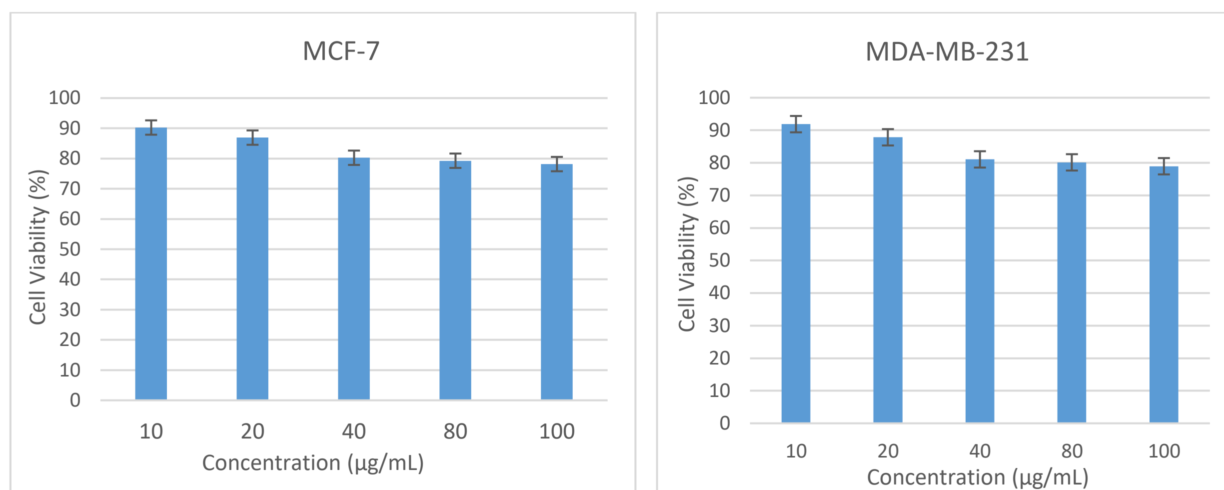


Figure 2. The Cytotoxicity Analysis of Bitter Melon Extract Against Breast Cancer Cells

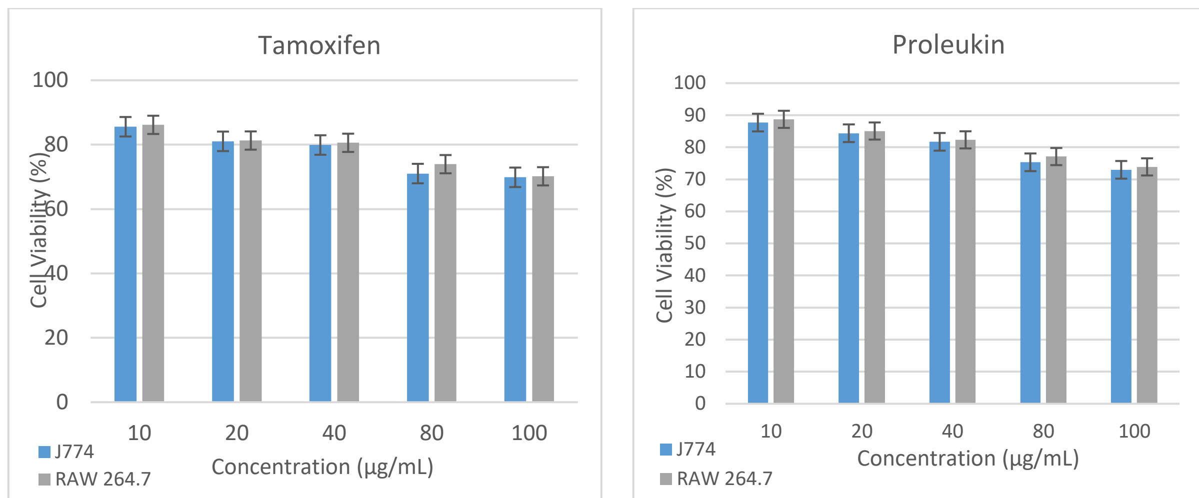


Figure 3. The Cytotoxicity of Tamoxifen and Proleukin Against J774 and RAW 264,7 Cell Lines

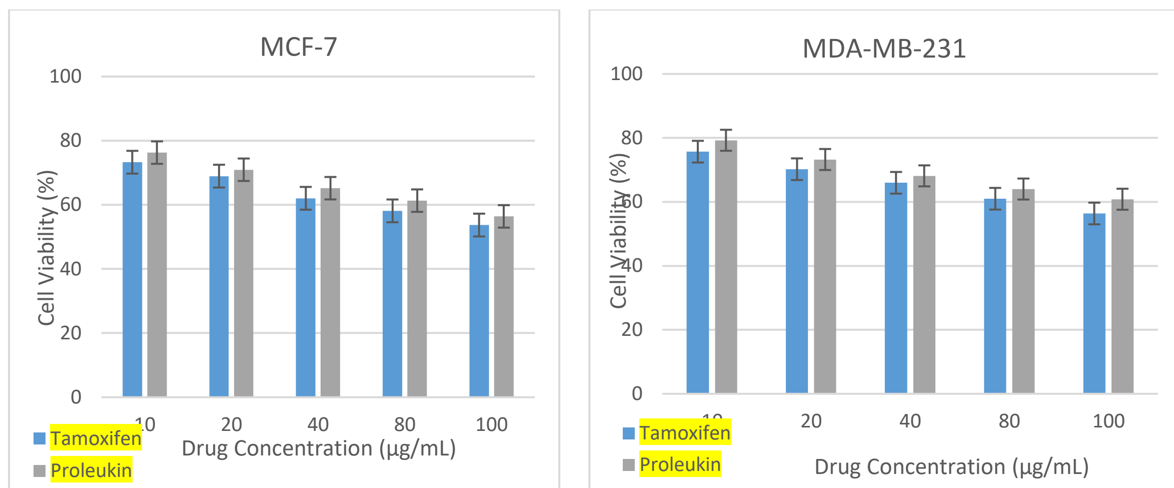


Figure 4. The Cytotoxic Effect of Tamoxifen and Proleukin Cancer Drugs Against MCF-7 and MDA-MB-231 Breast Cancer Cell Line

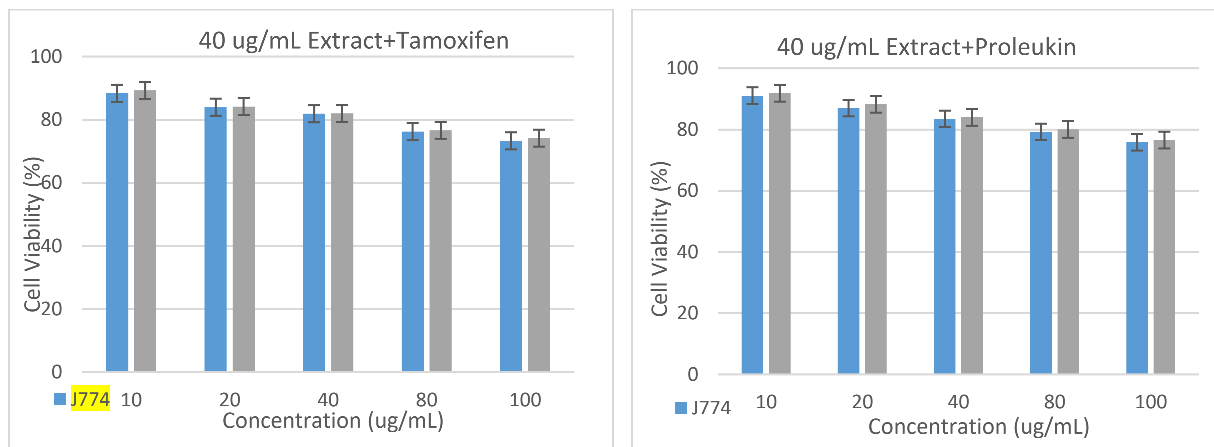


Figure 5. The Cytotoxic Effect of Tamoxifen And Proleukin Cancer Drugs Against J774 and RAW 264,7 Cell Line

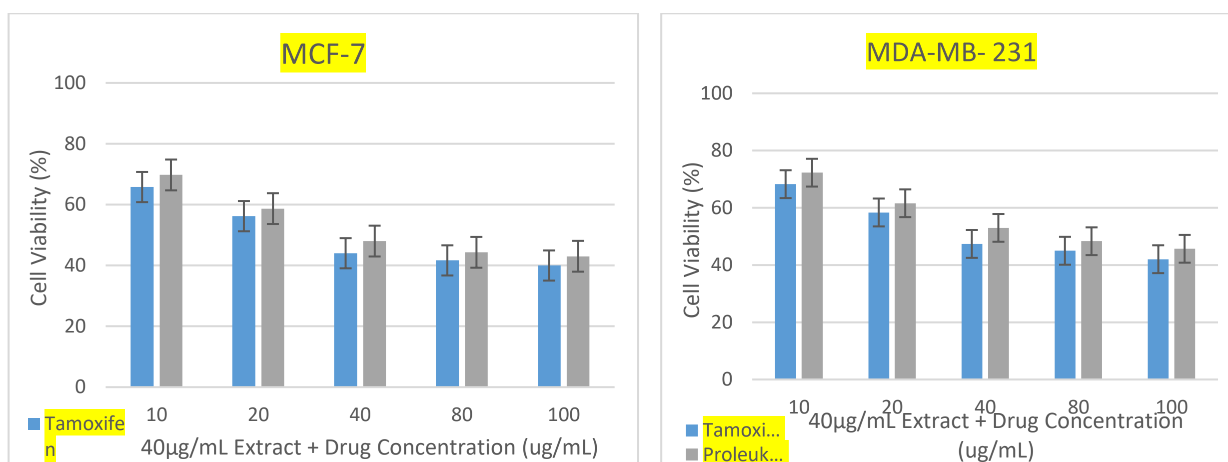


**Figure 6**. The Cytotoxic Effect of Tamoxifen and Proleukin Cancer Drugs Against MCF-7 and MDA-MB- 231 Cancer Cell Line.

In this research, the bitter melon extract was obtained by the maceration method. The extract was applied to 4 different cell lines. As a result of the cytotoxicity analysis, the bitter melon extract did not exhibit any cytotoxic effects against J774 and RAW 264.7 macrophage cell lines. Cell viability has increased when exposed to bitter melon extract with 10, 20, 40, and 80 µg/ml concentrations. However, for the 100 µg/ml concentration of bitter melon, the extract showed cytotoxic effects.

MCF-7 and MDA-MB-231 breast cancer cell lines were exposed to 40 µg/ml concentration of bitter melon extract alone and it was observed that the extract can kill cancer cells, however, this rate was not high. In addition, the cytotoxic effects of the extract were higher against hormone receptor-positive cell line MCF-7 when compared to the triple-negative MDA-MD-231 cell line. The metabolites in bitter melon could affect the MCF-7 cell line more and this could be responsible for this difference.

Additionally, the effects of the combination of a bitter melon extract with breast cancer drugs, Tamoxifen and Proleukin, were investigated. High mortality rates in cancer cells were observed when exposed to the combination of bitter melon extract and anti-cancer drugs. The combination of 40 µg/ml extract and 40 µg Tamoxifen exhibits the highest cytotoxic effect against MCF-7 and MDA-MB-231 breast cancer cell lines.

When Tamoxifen and Proleukin were compared, 40 µg Tamoxifen with 40 µg/ml extract had the highest cytotoxic effect against MCF-7 cancer cell lines. The reason for this is due to the specific molecules inside bitter melon that can cause apoptosis by inhibiting the estrogen signaling pathway. As a result, it was observed that the combination of extract and Tamoxifen can induce apoptosis, cell growth, and proliferation.

## 4. CONCLUSION

In this research, the highest cytotoxic effect was observed against positive breast cancer cell lines due to the bitter melon metabolites. In addition, the bitter melon extract also exhibits cytotoxic effects against triple-negative cancer cell lines. However, the extract did not show any toxic effects on healthy cells. Therefore, it is clear that the bitter melon extract can be an effective new agent when combined with drugs against breast cancer due to its anticancer effects. even though the extract on its own was not sufficient enough for treatment. However, it was observed that; when combined with drugs, bitter melon extract can increase the effectiveness of the drugs. As a result,

it is promising to use bitter melon extract-drug combinations to design new treatment approaches with new drug formulations against breast cancer.

Further studies are needed to know the exact cause of the increased cell inhibition. Bioactive compounds, especially phenols, in the extract, should be identified with high-tech devices such as HPLC and MS. Identified compounds should be retested in MCF-7 as well as MDA-MB-231 breast cancer cell lines to determine their cytotoxicity. Developed formulations must be adapted on a large scale. It is also essential to determine the therapeutic index and detect toxic symptoms by performing animal experiments.

**Contribution of The Authors**

Kübra KELLECİ and Murat IHLAMUR contributed to the concept, design, and to the writing of the manuscript. Melisa ÖZKAN and Murat IHLAMUR contributed to the conduct of the experiments. Murat IHLAMUR, Melisa ÖZKAN and Kübra KELLECİ contributed to the literature search and preparation of figures. Emrah Şefik ABAMOR is a supervisor. The article has been read and approved by all authors.

**Declaration of Interest statement**

The authors declare no conflict of interest.

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**Statement of Research and Publication Ethics**

Research and publication ethics were complied with in the study.

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