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Investigation of the Cytotoxic Activity of *Elettaria Cardamomum* Extract with Different Cancer Drugs Combination on Breast Cancer Cell Lines

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Abstract

Cancer is a fatal disease that has been going on from the past to the present. Breast cancer is one of the most common types of cancer. Breast cancer occurs when healthy cells in the breast tissue proliferate uncontrollably as a result of exposure to various factors. There are many studies on treatment approaches against breast cancer. However, while existing treatment methods kill cancer cells, they also damage healthy cells. Due to these harmful effects of current treatment methods, herbal treatment approaches are gaining importance for cancer therapies day by day. It is stated in the literature that Diindolylmethane (DIM) and indole-3-carbinol (I3C) molecules found in *Elettaria cardamomum* (*E. cardamomum*) extract have a cytotoxic effect by affecting the hormone pathway. In this study, the effect of *E. cardamomum* and drug combinations on MDA-MB-231, MCF-7 and J774 was investigated. The study aimed to compare the data in the literature by using both hormone-positive (MCF-7) and triple-negative (MDA-MB-231) breast cancer cell lines. As a result, it was determined that extract had higher cytotoxicity on MCF-7 compared to the MDA-MB-231, due to the presence of I3C and DIM molecules in *E. cardamomum* extract. In drug combinations, the highest cytotoxicity data was obtained in the Tamoxifen-*E.cardamomum* extract combination.

Keywords: *E. cardamomum*, MDA-MB-231, MCF-7, J774, Breast cancer, Cytotoxicity

Introduction

Cancer is a disease that occurs as a result of uncontrolled division of cells. According to statistics, it is seen that cancer-related deaths have a large share of deaths. Breast cancer is a type of cancer that occurs in the breast tissue. Factors such as menopause (1), menstrual cycle, late pregnancy, breastfeeding (2), radiation (3), alcohol consumption (1), smoking, and weight gain (4) may be risk factors in the development of breast cancer. Treatment approaches in breast cancer are examined in 4 classes. These include surgical treatment, radiotherapy, drug therapy, and alternative treatment approaches. Surgical treatment is an invasive option used for breast cancer treatment. In radiotherapy, radiation therapy is applied to the patient to get rid of cancerous tissue. However, since cancer cells can be resistant to X-rays, the survival of cancer cells continues and can later lead to metastases.

In the breast cancer drug treatment approach, Docetaxel (5), doxorubicin (6), paclitaxel (7), epirubicin (8) Drugs such as trastuzumab (9), tamoxifen, aromatase inhibitors, leuprolide, goserelin (10) are used. Although these synthetic drugs have many advantages, their use alone is not sufficient due to their toxic effects and effectiveness. In recent years, studies using combinations of anticancer agents with herbal agents have shown a decrease in the toxicity of anticancer agents and an increase in their therapeutic effects, and it is stated that this is of great importance in cancer treatments.

Plants used with medicines can also be used alone to form alternative treatment approaches against breast cancer. There are many plants used in the treatment of breast cancer in alternative treatment approaches. Echinacea (11) on BT-549 cell line, garlic (12), curcumin (13), ginger (14) on breast cancer cell lines the cytotoxic activity of ginseng (15) has been reported in the literature.

E. cardamomum properties such as antispasmodic, antiseptic, diuretic, immunomodulatory, antimicrobial, and anti-inflammatory are important for its use in treatment approaches. In addition to these features, *E. cardamomum* also has anticancer properties. Primary and secondary metabolites such as DIM and I3C are important phytochemicals obtained from plants. These phytochemicals can affect cancer pathways and regulate hormone activities in breast cancer. I3C is found in *E. cardamomum*; It reduces metastasis, inflammation, tumorigenesis, and immunomodulation (16). I3C-DIM triggers apoptotic pathways for cancer cells. In a study, it was stated that I3C stimulated the apoptotic pathway in the MCF-7 (17). Additionally, the antiestrogenic properties of I3C are also involved in preventing breast cancer development, as shown in Figure 1. Due to these effects of the *E. cardamomum* plant, research shows that *E. cardamomum* can reduce the size of tumors and that its use for therapeutic purposes will be effective in the treatment of breast cancer.

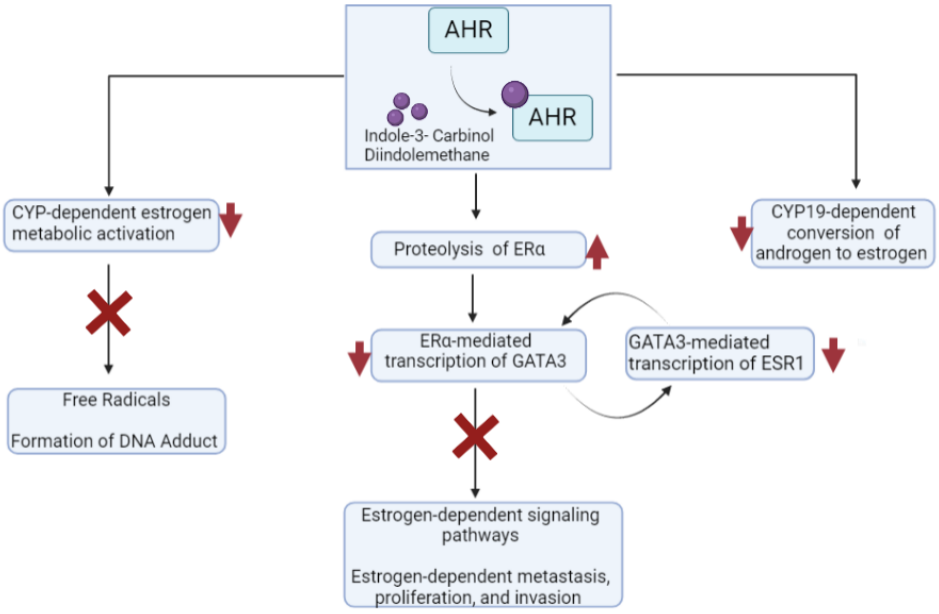

Figure 1. The anti-estrogenic activity of I3C and DIM

In this study, it was aimed to apply *E. cardamomum* extract and drug combinations on different cell lines. In this way, it is aimed at preventing the development of breast cancer. At the same time, this study aims to increase patient comfort and life expectancy by using plant extract to induce apoptosis in breast cancer cells and to make breast cancer treatment non-invasive and non-chemical.

Material and Method

Preparation of *E. cardamomum* Extract

This The *E. cardamomum* shells were peeled and the seeds were collected by crushing them in a mortar. 8 g of *E. cardamomum* seeds were weighed and methanol was added. It was incubated for 6 days in a dark environment. The extract obtained at the end of the 6th day was filtered through the Whatman paper. It was then placed in a beaker and the methanol was allowed to evaporate through airflow and temperature (18,19). The amount of *E. cardamomum* extract was determined by precise weighing.

Cell Culture

In the study, MCF-7, MDA-MB-231, J774 mouse macrophage cell lines in the cryobank of our laboratory were used. Cell culture was performed in 75cm2 (Polystyrene surface, NEST) flasks. Stock media were prepared by adding 1% penicillin-streptomycin and L-glutamine. For cell proliferation, MCF-7 culture was carried out in DMEM medium containing 10% FBS, and J774 mouse macrophage culture and MDA-MB-231 culture were carried out in RPMI-1640 medium containing 10% FBS (20).

Cell culture was carried out under incubation conditions of 37oC, 95% humidity and 5% CO2. MCF-7 and MDA-MB-231 cells were collected enzymatically, and J774 cell lines were collected physically, and cell counts were performed on the thoma slide. Cells were planted at 1x105 cells/mL per well in 96-well plates and incubated for 24 hours (21,22).

Cytotoxicity Analysis

For cell viability analysis, MTT was applied to the cells incubated for 48 hours at 37oC temperature, 95% humidity and 5% CO2 incubation conditions. Cell viability rates were evaluated with MTT containing 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltatrazilium bromide. 10 µl of MTT solution was added to each well on the 96-well plate. Cells in 96-well plates were incubated in the dark at 37°C for 3 hours. After incubation, liquids containing MTT solution were removed from the environment by aspiration. After 100 µl dimethylsulfoxide (DMSO) was added to each well, the well plates were kept in a dark environment for 30 minutes. With the complete dissolution of formazan crystals, cell viability analysis was performed by taking measurements at a wavelength of 570 nm (23,24). Each experimental group was repeated three times and averaged. Cell viability analysis data were obtained using equation 1 and data graphs were created.

$$\text{Cell viability (\%)} = \frac{\text{OD values in experimental group}}{\text{OD value in control group}} x100 \quad (1)$$

Statistical analysis

The data obtained from the study were analyzed in the IBM SPSS 25.0 (IBM Corporation, Armonk, NY, USA) package program. Comparisons between groups were made with a one-way analysis of variance One-Way ANOVA test. The results are given as mean±standard deviation

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(Mean±SD) and statistical significance was accepted as $p<0.05$.

Result and Discussion

It has been proven in the literature that many active compounds found in plants suppress cancer pathways. Secondary metabolites in plants are phenolic acids, flavonoids, tannins, quinones, anthocyanins, etc. It can be effective in preventing cancer (25). Considering the studies in the literature; The cytotoxic effect of the extract prepared from the *E. cardamomum* plant using the maceration method, alone and in combination with various breast cancer drugs, was examined on the J774 macrophage cell line to determine its usability in the treatment of breast cancer. In addition, its killing effects on breast cancer cells were also examined. As a result of this study, the most suitable extract-drug (tamoxifen, methotrexate, proleukin, altuzan) formulation for the treatment of breast cancer was determined to be used in in vivo studies. For this purpose, firstly the most suitable extract concentrations were determined and drug formulations were tested with the determined extract concentrations.

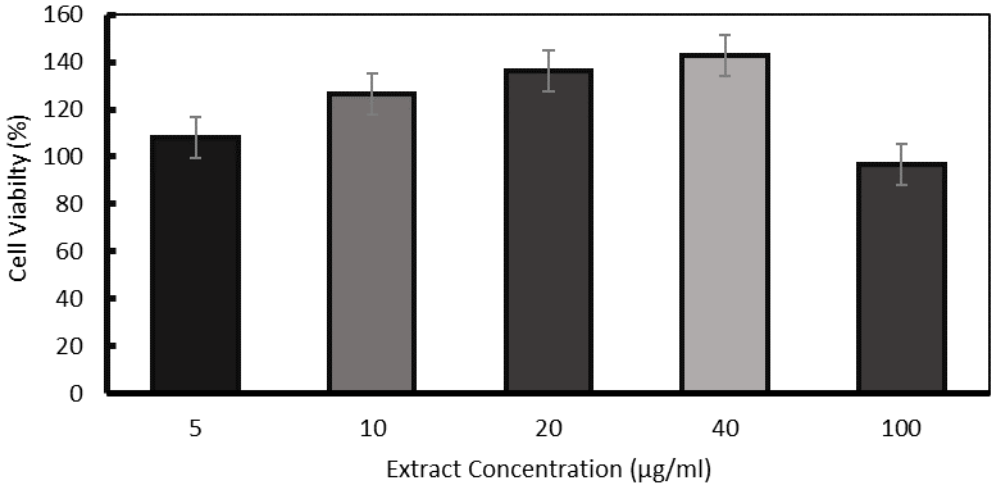

Figure 1. Cytotoxic effect of the *E. Cardamomum* extract on the J774 macrophage cell line

Viability analyses based on cytotoxicity of the J774 cells treated with the extract are shown in Figure 1. The highest killing efficiency of the extract obtained from the *E. cardamomum* is at a concentration of 100 µg/ml. However, when this concentration is applied to macrophage and fibroblast cells, it appears to be unsuitable for use as it has a serious cytotoxic effect on the cells. *E. cardamomum* extract showed the highest cytotoxic activity at the concentration of 40 µg/ml after 100 µg/ml. In the study, 142.76% viability was detected in macrophages in the cytotoxicity analysis alone of *E. cardamomum* extract at a concentration of 40 µg/ml ($p<0.05$).

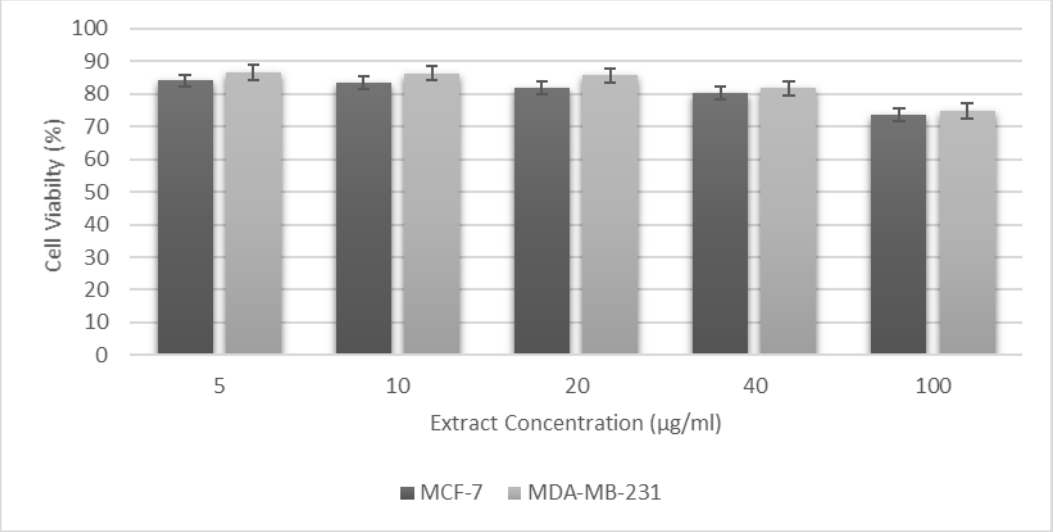

Figure 2. Cytotoxicity analysis of extract-treated breast cancer cell lines

A study indicates that the phytochemicals contained in *E. cardamomum* play a role in regulating hormone activities in breast cancer. I3C found in *E. cardamomum* regulates the expression of cyclin-dependent kinase (CDK), which stops G1 cell cycle activation and results in the death of cancer cells (16). Viability analyses of breast cancer cells treated with the extract are shown in Figure 2. In this study, there were decreases in cell viability as a result of the application of extract to breast cancer cells. When *E. cardamomum* extract was applied to the cells at a concentration of 40 µg/ml, 80.28% viability was detected in the MCF-7 breast cancer cells and 81.80% viability in the MDA-MB-231.

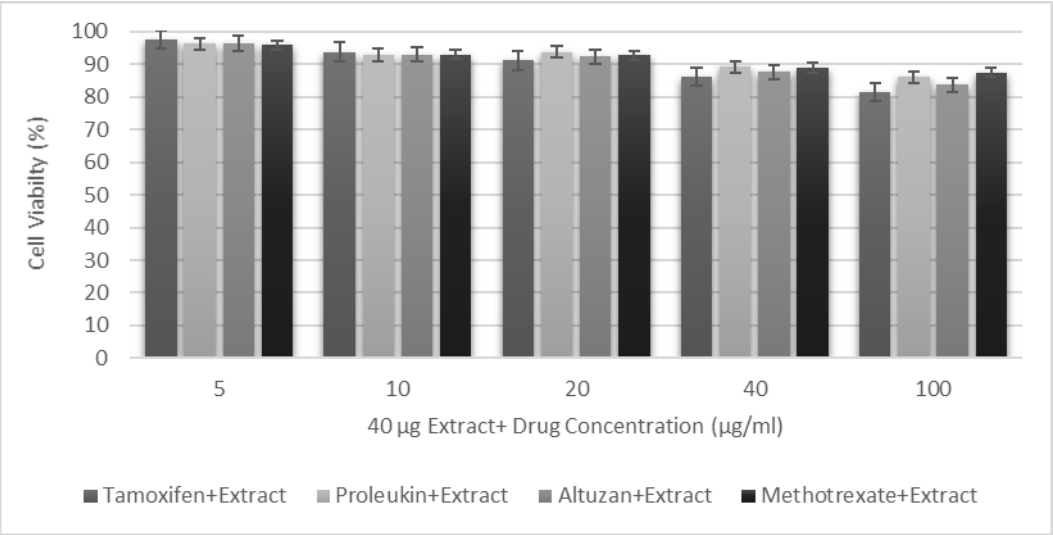

Figure 3. Cytotoxicity activity analysis in the J774 macrophage cell line treated with 40 µg/ml extract and drug combinations

Cytotoxic analysis of combinations of *E. cardamomum* extract with drugs in the macrophage cells is shown in Figure 3. In drug combinations in which 40 µg/ml *E. cardamomum* extract is used, the highest cytotoxic activity is observed in combinations in which 40 µg/ml tamoxifen drug is used.

86.25% viability was detected in the treatment of 40 µg/ml extract + 40 µg/ml tamoxifen drug with macrophages ($p<0.05$).

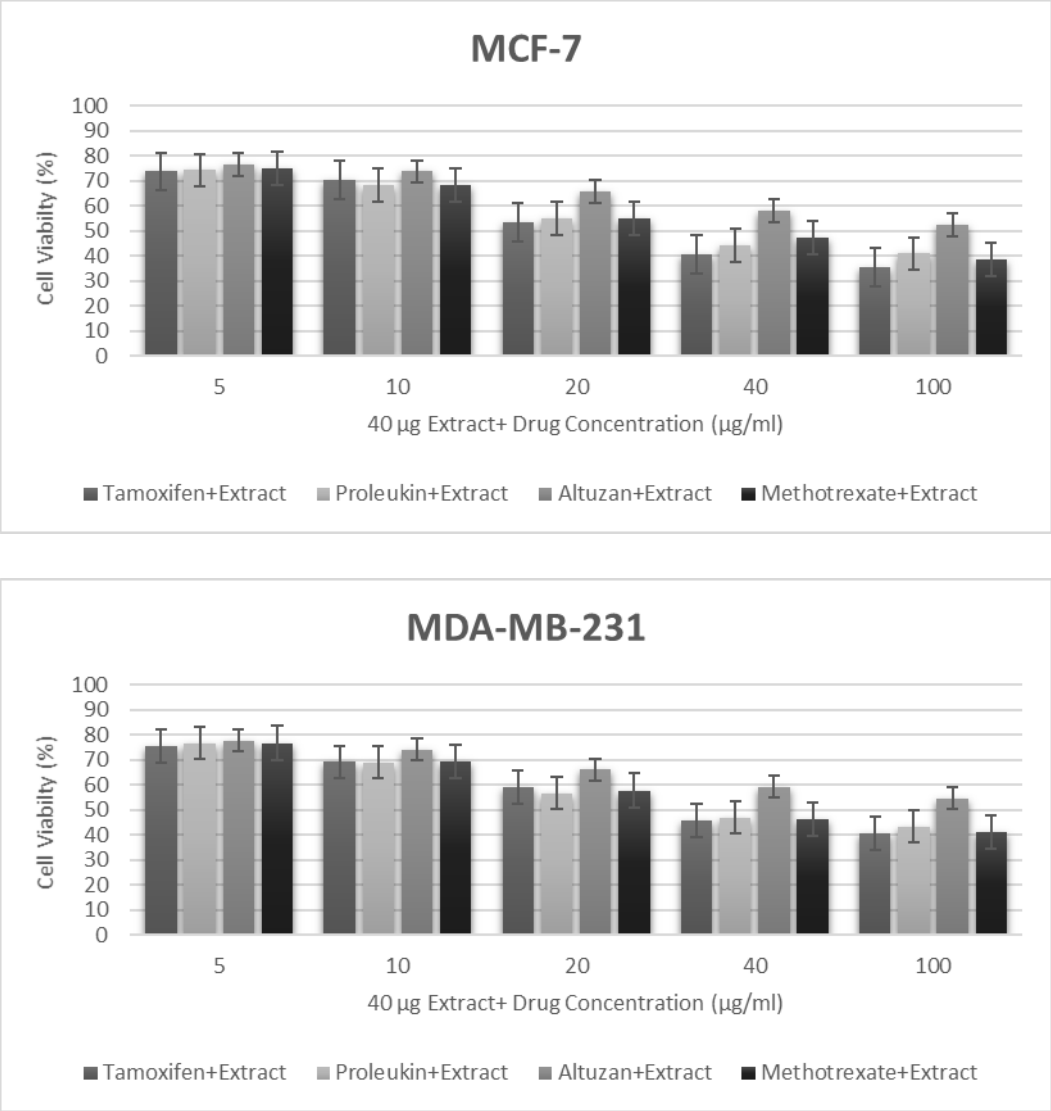

Figure 4. Cytotoxicity analyses of breast cancer cell lines treated with 40µg/ml extract and drug combinations

Although synthetic drugs used in breast cancer treatment have many advantages, they have disadvantages due to their cytotoxic effects when used alone and their insufficient effectiveness. Recent studies show that combinations of anticancer agents with herbal agents are of great importance in reducing the toxicity of anticancer agents and increasing their therapeutic effects (26). The results obtained in the study support this information. Figure 4 shows the cytotoxic effects of extract and drug combinations on breast cancer cell lines. According to these results, in drug combinations using 40 µg/ml *E. cardamomum* plant extracts, the combination using 40 µg/ml tamoxifen drug showed the highest killing effect on 2 breast cancer cell lines. In the cytotoxicity analysis of the combination using 40 µg/ml extract + 40 µg/ml tamoxifen drug,

40.81% viability was detected in the MCF-7 and 45.63% viability in the MDA-MB-231.

Many methods are used in cancer treatment. Methods such as chemotherapy and radiotherapy currently used in the clinic have a significant lethal effect on cancer cells. At the same time, cancer inhibitors are also effective in cancer treatment (27). However, while these treatment methods kill cancer cells, they also damage healthy body cells. Complementary medicine or alternative medicine methods have become a popular research topic day by day because they do not have a cytotoxic effect on healthy cells. Many active compounds such as secondary metabolites, phenolic acids, flavonoids and tannins obtained from plants such as ginger, green tea, garlic, curcumin, and *E. cardamomum* are used in cancer studies. In recent studies, herbal agents have gained great importance as they increase the therapeutic effects of anticancer agents when used with anticancer agents. Effective results have also been obtained in studies where herbal agents were used alone. Primary and secondary metabolites such as DIM and I3C obtained from the *E. cardamomum* plant are of great importance in cancer treatments as they enable the regulation of hormone activities. These two metabolites induce apoptosis in breast cancer cells by affecting the hormone pathway. The presence of these metabolites in *E. cardamomum* extract forms the basis of this study.

The extract obtained in the study was applied to three different cell lines. It was determined that *E. cardamomum* extract had no cytotoxic effect in cytotoxicity studies conducted on J774 macrophage cells. When *E. cardamomum* extract was applied to healthy cell lines at a concentration of 5, 10, 20 40 µg/ml, it was observed that the viability of the cell lines increased. Its cytotoxic effect on the cell was detected at a concentration of 100 µg/ml. In the results obtained, it was observed that extract alone had a killing effect on breast cancer cells at a concentration of 40 µg/ml, but this rate was low. As a result of the use of *E. cardamomum* extract alone, it was observed that MCF-7 had higher cytotoxicity than the MDA-MB-231. The reason for this is thought to be the presence of DIM and I3C metabolites found in *E. cardamomum* extract, as previously stated in the literature. It has also been determined that *E. cardamomum* has cytotoxic activity on many cancers such as gastric cancer. In the study e. It has been determined that extract alone has cytotoxic activity on gastric cancer cells. to. It was determined that the combination of extract and proleukin drug had high lethality on AGS gastric cancer cells (18).

In this study, the combination of *E. cardamomum* extract with four different drugs used clinically for breast cancer treatment was investigated. Combinations of Tamoxifen, Methotrexate, Proleukin, and Altuzan have been used. It has been determined that extract and drug combinations have a high killing effect when used together. It was determined that the extract and drug combinations showed the highest cytotoxic effect on breast cancer cells in the combination of 40 µg/ml plant extracts and 40 µg/ml tamoxifen drug. Among the four drugs used in the study, the highest cytotoxic effect was detected in the MCF-7 in the cytotoxicity analysis using the combination of 40 µg/ml tamoxifen and 40 µg/ml extract. This is because I3C-DIM molecules in *E. cardamomum* extract suppress the estrogen signaling pathway, leading the cell to apoptosis. Due to the effect of I3C-DIM on estrogen metabolism, it blocks the growth and proliferation of hormone-positive cells and induces apoptosis (28).

Conclusion

In the study, *E. cardamomum* extract showed the highest cytotoxic effect on the hormone-positive breast cancer cell line due to the metabolites it contains. It also has a cytotoxic effect on hormone-negative breast cancer cell lines. In addition, *E. cardamomum* extract did not show toxic effects on healthy cell lines. In light of this information, it has been observed that although *E. cardamomum* extract, which has anti-cancer properties and does not have a toxic effect, does not give effective results in the treatment of breast cancer when used alone, it gives effective results when used with drug combinations. Therefore, it is thought that extract-drug combination approaches will give effective results in the treatment of breast cancer and can be used as a new drug formulation in the treatment of breast cancer by moving on to in vivo studies.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The author declares that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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