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Anticancer Potential of Watermelon Seed Extracts Against Lung and Breast Cancer Cell Lines

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ABSTRACT

Objective: The aim of the study is to evaluate the anticancer potential of watermelon seed extracts against lung and breast cancer cell lines.

Methods: A549 lung cancer and MCF-7 breast cancer cell lines were used. The cells were treated with doses ranging from 0.1 to 1000 µg/mL of KI (inner part) and KD (outer part) extracts from watermelon seeds, starting when the cell density reached 80%. Viability was assessed using the MTT assay.

Results: For the A549 lung cell line, the KI extract demonstrated significant anticancer activity at doses of 10, 100, and 1000 µg/mL, with the 1000 µg/mL dose being the most effective against lung cancer cell line. Similarly, the KD extract showed efficacy across all doses tested, with the seed peel being effective at lower doses compared to the seed. In the MCF-7 breast cell line, both KI and KD extracts exhibited dose-dependent anticancer effects, with significant reductions in viability observed at all doses compared to the control group.

Conclusion: Interestingly, the seed and seed shell showed selective effectiveness against breast and lung cancer, indicating a dose-dependent and selective anticancer effect. Overall, these findings suggest the potential of watermelon seed extracts as promising anticancer agents with selective efficacy against different cancer types.

Keywords: Anticancer, A549, MCF-7, Watermelon

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Introduction

Cancer is defined as one of the significant diseases worldwide with a high mortality rate (Aydın and Köseoğlu, 2022). It is a disease that can affect any organ or tissue in our body and arises from the transformation of our body's own cells for various reasons. The most significant characteristic of cancer is the abnormal cells gaining the ability to divide rapidly and uncontrollably, invading nearby tissues and organs without restraint, and furthermore, spreading to distant organs and tissues through the bloodstream. The most common cause of death from cancer is metastasis. While there are many known causes of cancer, there are also many unknowns about cancer today. About 30% of cancers can be prevented with simple measures. Many types of cancer, such as lung, breast, colorectal, laryngeal, and stomach cancer, can be treated when diagnosed early (Özyiğit, 2017). In addition, cancer patients are in search of many new treatment options in addition to the current ones.

Breast cancer is one of the most common cancers in women worldwide and represents a major public health concern. The MCF-7 cell line is a human breast cancer cell line commonly used in breast cancer research. This cell line is used to evaluate the effects of cancer drugs in in vitro studies. The MCF-7 cell line is a breast cancer cell line with estrogen receptors and is widely used in breast cancer studies (Aybek et al., 2020). The A549 cell line is a cell line derived from human lung adenocarcinoma and is widely used in lung cancer research (Xu et al., 2022). Genetic factors, environmental factors, dietary habits, sedentary lifestyle, infections, and exposure to chemicals can be listed among the causes of cancer.

Foods provide essential substances for the organism's metabolic needs, such as proteins, and additionally contain components, like secondary metabolites, that have positive effects on our health (Arı et al., 2017).

Healthy eating is crucial for preventing diseases. In particular, antioxidant nutrition is important to combat the effects of free radicals. Antioxidant nutrients can be defined as substances that can neutralize some or all of the adverse effects caused by free oxygen radicals that occur in physiological conditions in humans (Yılmaz, 2010). A good antioxidant eliminates free oxygen radicals in a specific manner, chelates redox metals, triggers other antioxidants within the antioxidant network, and has a positive effect on gene expression (Arı et al., 2017). Antioxidants such as anthocyanins, and phenolic compounds play an important role in reducing the function of free radicals. It is necessary to have certain foods containing these elements in our daily diet. Recent studies have shown evidence supporting the appropriate use of particularly red and purple pigmented foods as anticarcinogenic (Dyshlovoy and Honecker, 2020). Examples of such foods include eggplant, damson plums, purple grapes, blackberries, blueberries, elderberries, purple onions, purple rice, lavender, and watermelon, (Aydın and Köseoğlu, 2022).

Watermelon (Citrullus lanatus [Thunb.] Matsum. & Nakai) is an annual creeping plant belonging to the Cucurbitaceae family. The fleshy part of the mature fruit (endocarp) can vary in color from yellow to red and contains numerous black seeds. Watermelon seeds contain approximately 30-40% protein and 45% edible oil. This oil is rich in linoleic acid, oleic acid, palmitic acid, and stearic acid (Baser, 2022). The coumarins isolated from watermelon seeds have been reported to be effective against the abnormal growth of various cancer cell lines (Mustafa et al., 2024). Also it is known that antioxidant compounds such as lycopene and tocopherol found in extracts from watermelon fruit have biological effects on lung cancer (Di Sano et al., 2022). Phytol isolated from watermelon sprouts inhibited the growth of human T-cell leukemia Jurkat cells and suppressed tumor progression in human lung adenocarcinoma epithelial cell line (Itoh et al., 2018).

In this study, the effect of the extract obtained from watermelon seeds on lung and breast cancer cell lines has been investigated.

Methods

Plant Material

Watermelon was brought from Şanlıurfa in September 2022. The outer part (KD, 11.128 g) and inner part (KI, 9.634 g) of watermelon seeds were used as the plant material.

Extraction

The specified amounts of plant materials were powdered to an appropriate size and transferred to 500 ml erlenmeyer flasks. 100 ml of absolute ethanol (Sigma-Aldrich) was added to each flask. They were left for maceration overnight and extracted for 3 hours at 40°C on a magnetic stirrer. This process was repeated twice. After the extraction process, the extracts were filtered, combined, and the solvents were evaporated using a rotary evaporator at 40°C and 120 rpm. As a result, 96 mg of extract was obtained from the outer parts of the

watermelon seeds, while 75 mg of extract was obtained from the inner parts.

A549 Lung and MCF-7 Breast Cancer Cells Culture Experiments

A549 lung and MCF-7 breast cancer cell lines were obtained for our research from the Department of Medical Pharmacology, Atatürk University, Erzurum, Türkiye. Briefly, the cell suspension was centrifuged at 1200 revolutions per minute for 5 minutes. Cells were suspended in a new medium, Dulbeco's modified Eagle's medium (DMEM HG), containing 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin, streptomycin and amphotericin B). The cells were then collected in a flask with a surface area of 25 cm2. The flask was placed in an incubator with a carbon dioxide concentration of 5% and a temperature of 37°C. Once 80% of the flask is covered with cells, they will be extracted using a trypsin-ethylenediaminetetetraacetic acid (EDTA) solution (0.25% trypsin - 0.02% EDTA) followed by centrifugation. The liquid portion will be removed and the cell mixture will be dispensed into 96-well tissue culture plates at a volume of 100 µl per well (containing 10,000 cells per well).

Experimental Protocol

When the cells in the plates reached 80% density, the experimental design was established by determining 0.1, 1, 10, 100 and 1000 µg/ml concentrations for watermelon extracts. A wide range of concentrations was studied to find the effective dose. 96-well flasks were inoculated with pre-seeded cells reaching 80% density. Cells were cultured in 5% CO2 at 37°C for 24 hours. 10 replicates were used for each concentration.

MTT Assay (Cytotoxicity Analysis)

Foods provide essential substances for the organism's metabolic needs, such as proteins, and additionally contain components, like secondary metabolites, that have positive effects on our health (Arı et al., 2017).

The assay was completed after 24 hours, at which point 10 µl 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well. The samples were incubated for 4 hours after the addition of MTT. Dimethyl sulfoxide (DMSO) in a volume of 100 µl per well will be applied to dissolve formazan crystals formed after MTT treatment. A spectrophotometer (µQuant, Bad Friedrichshall, Biotek) was used to measure absorbance at a wavelength of 570 nm.

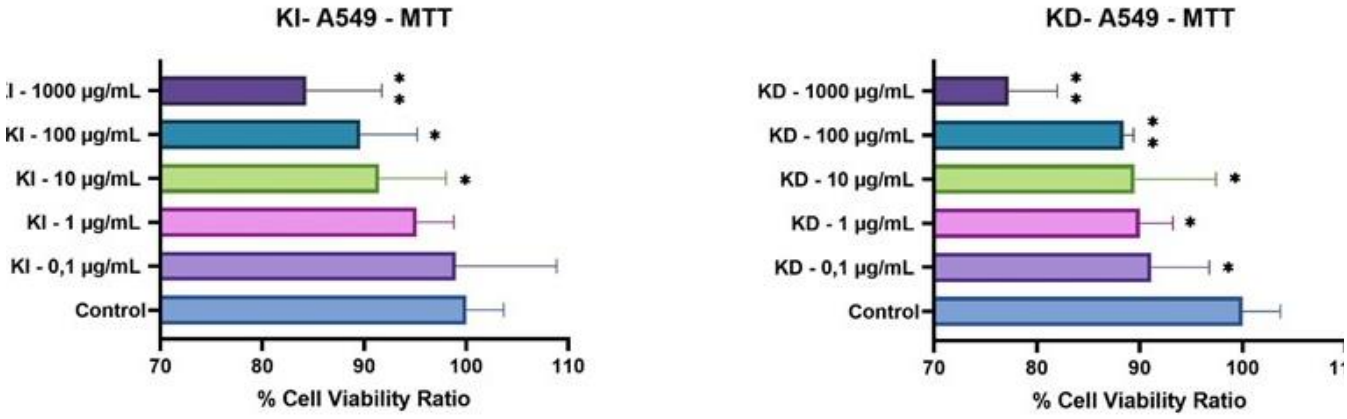

Figure 1. MTT results of KI and KD extracts in A549 lung cancer cell line (*p<0.05, **p<0.001 compared to control group)

Statistical Analysis

The data was analyzed using one-way analysis of variance (ANOVA) with post hoc Tukey's test (IBM SPSS 22.0). Statistical significance was determined at *p < 0.05 and **p < 0.001. The data were presented as the mean value plus or minus the standard deviation (SD).

Results

Experiments were performed with A549 lung cancer and MCF-7 breast cancer cell lines in cell culture. The experiment was initiated when the cell density in the wells reached 80%. Doses of 0.1, 1, 10, 100 and 1000 µg/mL of KI and KD extracts of watermelon seeds were prepared and A549 and MCF-7 cancer cell lines were treated for 24 hours. At the end of 24 hours, the cells were subjected to MTT assay and viability values were measured.

The MTT test results obtained by administering KI

extract to A549 lung cell line are presented in Figure 1. In the study, when the determined doses of the KI extract were compared with the control group, the viability rates in the 10, 100 and 1000 µg/mL groups were statistically significant. Cell viability levels were determined by proportioning the control groups to 100%. Watermelon seeds showed anti-cancer activity depending on the dose. 1000 µg/mL dose was found to be the most effective anticancer dose against lung adenocarcinoma type.

The MTT test results obtained by administering KD extract to A549 lung cell line are presented in Figure 1. all doses, the seed shell was found effective in lung cancer at all doses. In other words, there is a selective effect. At high doses, a killing rate approaching 25% in cancer cell numbers was obtained.

Discussion

Cancer is a pathological condition characterized by the uncontrolled proliferation and growth of cells in various organs or tissues of the body. Typically, cells undergo division and subsequent death in a specific order, but cancer cell lines deviate from this pattern (Ohshima and Morii, 2021). Cancer does not emerge only due to excessive and uncontrolled growth. In addition, the cell must exhibit additional malignant characteristics, including the ability to invade adjacent healthy tissues and migrate to neighboring healthy tissues via circulation, a process known as metastasis (Hanahan and Weinberg, 2000).

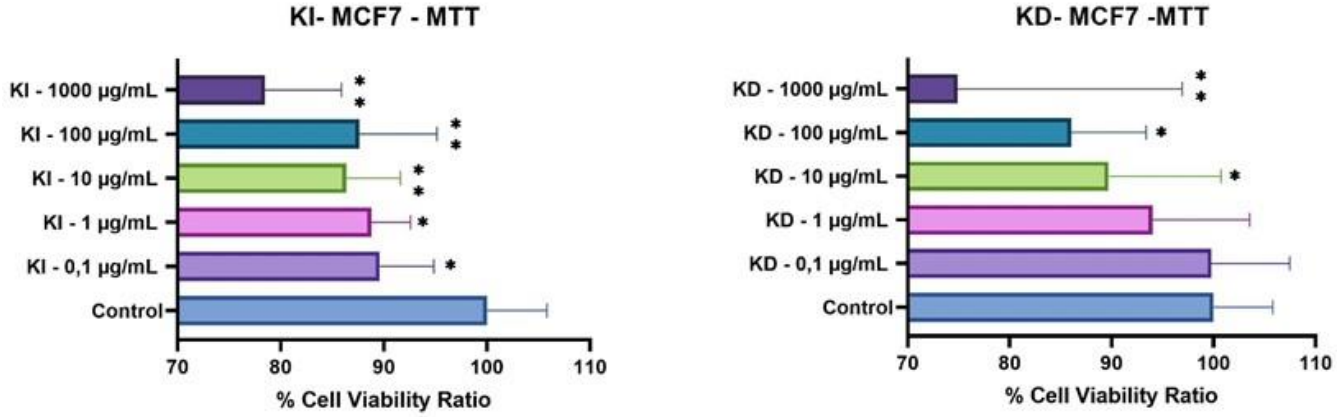

Figure 2. MTT results of KI and KD extracts in MCF-7 breast cancer cell line (*p<0.05, **p<0.001 compared to control group)

When the doses of KD extract were compared with the control group, the viability rates in all groups were found to be statistically different. It was an important result that watermelon seed peel was effective at the lowest 2 doses compared to the seed, while the effect could occur at the highest 3 doses tested in the seed. In addition, the dose-dependent increase in potency was similar for both watermelon seed extracts tested.

The MTT test results obtained by administering KI extract to MCF-7 breast cell line are presented in Figure 2. In the study, when the doses of the control group and KI extract were compared, the viability rates in all groups were statistically significant. The effect was dose dependent.

The MTT test results obtained by administering KD extract to MCF-7 cell line are presented in Figure 2. In the study, when the doses of KD extract were compared with the control group, the viability rates in the 10, 100 and 1000 µg/mL groups were statistically significant. Cancer killing rates were not significant at the lowest 2 doses.

While the seed was found effective in breast cancer at

The findings of this study demonstrate the potential anti-cancer properties of watermelon seed extracts, particularly the KI and KD extracts, against both A549 lung cancer and MCF-7 breast cancer cell lines. These results shed light on the dose-dependent efficacy and selective effects of the extracts, providing valuable insights into their mechanisms of action and potential applications in cancer treatment.

One of the key observations from this study is the significant decrease in cell viability rates observed in response to treatment with both KI and KD extracts across various doses. Particularly noteworthy is the substantial anti-cancer activity exhibited by the KI extract against A549 lung cancer cell lines, with statistically significant reductions in viability observed at doses of 10 µg/mL and above. This

indicates the potent cytotoxic effects of the KI extract on lung cancer cell lines, with the highest dose of 1000 µg/mL yielding the most pronounced anti-cancer activity.

Similarly, the KD extract also demonstrated notable efficacy against A549 lung cancer cell lines, with statistically significant reductions in viability observed across all doses tested. Interestingly, the KD extract showed effectiveness at lower doses compared to the KI extract, suggesting a potentially higher potency or different mechanism of action for the KD extract, possibly attributed to the presence of specific bioactive compounds concentrated in the seed peel.

In the case of MCF-7 breast cancer cell lines, both KI and KD extracts exhibited dose-dependent anticancer effects, with statistically significant reductions in viability observed across all doses tested. This indicates the broad-spectrum anticancer potential of watermelon seed extracts against different cancer cell lines, highlighting their versatility as potential therapeutic agents for breast cancer treatment.

Furthermore, the selective efficacy of watermelon seed extracts, with the seed shell demonstrating effectiveness against lung cancer cell lines and the seed against breast cancer cell lines, is particularly intriguing. This suggests the presence of differential molecular targets or cellular pathways in these cancer types that are selectively modulated by specific components of the extracts. Understanding the underlying mechanisms driving this selective effect could provide valuable insights into the development of targeted therapies for different cancer types.

Overall, the findings of this study underscore the promising anti-cancer properties of watermelon seed extracts and warrant further investigation into their therapeutic potential. Future studies focusing on elucidating the molecular mechanisms of action, identifying key bioactive compounds, and evaluating the efficacy of these extracts in in vivo models and clinical trials are warranted to fully harness their therapeutic benefits in cancer treatment.

Conclusion and Recommendations

This study demonstrates the selective and dose-dependent anticancer effects of watermelon seed extracts on A549 lung and MCF-7 breast cancer cell lines. Both KI and KD extracts exhibited dose-dependent efficacy against both lung and breast cancer cell lines. In conclusion, watermelon seed extracts hold promising potential as natural and effective anticancer agents. Further studies are needed to elucidate the mechanisms of action and in vivo efficacy.

Ethics Committee Approval: Since the cell line was studied in vitro, an ethics committee decision is not required.
Informed Consent: Since it is an in vitro study, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - FY, MEH, ANC, AD, OC; Design- FY, MEH, ANC, AD, OC; Supervision-OC; Resources- OC; Data Collection and/or Processing- OC; Analysis and/or Interpretation- FY, HÖ; Literature Search- FY, HÖ, MEH, ANC, AD; Writing Manuscript- FY, HÖ; Critical Review- FY, HÖ.
Conflict of Interest: The authors have no conflicts of interest to declare.
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