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Research Article

Anti-Cancer Effects of Water Extract of *Thymbra Capitata* (L.) Cav. Plant on MCF-7 and SH-SY5Y Cell Lines

Canan Yılmaz Kapancık

Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey; https://orcid.org/0000-0003-1432-4471

*** Corresponding author:** cananyilmazkapancik@gmail.com

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Abstract: Traditional medical practices have emerged as the sum of experiential accumulations from the past to the present and are increasingly used today for the purpose of protection from and treatment of diseases. The therapeutic effects of herbal medicines, which form the basis of traditional medical practices, in the treatment of many diseases, especially cancer, have been known since ancient times. Herbal medicines, since they are obtained from natural sources, are considered reliable and healthy, and recently, the interest in herbal medicines has increased even more. *Thymbra capitata* (L.) Cav. is a plant that grows in the Mediterranean region, is edible, belongs to the class of medicinal plants and has therapeutic properties against many diseases. *T. capitata* plant has high antioxidant capacity and antimicrobial properties. In our study, the possible anti-cancer effects of the water extract of *T. capitata* plant on MCF-7 and SH-SY5Y cancer cell lines were determined by the MTT method. As a result of the experimental data obtained, it was determined that the water extract of *T. capitata* (plant caused a decrease in the percentage of viability of MCF-7 and SH-SY5Y cancer cells, and thus, the water extract of *T. capitata* plant had anti-cancer effects on MCF-7 and SH-SY5Y cells.

Keywords: Anti-cancer, Cytotoxicity, Plant Medicines, Traditional Medicine, *Thymbra capitata* (L.) Cav.

1. Introduction

Traditional medical practices have emerged among the public based on the information obtained through experiences. Traditional medical practices are aimed at protecting human health and treating diseases. Today, traditional medical practices are used intensively in the treatment of diseases in many parts of the world due to the high level of trust in traditional medical methods (Che et al., 2024).

The basis of traditional medical practices is plant-based medicines. The therapeutic effects of plants on human health have been known since the past and an accumulation of experience-based knowledge has been created among people on this subject. These traditional herbal medicines have been used and continue to be used in many cultures to treat many different diseases. These medicines are considered reliable and healthy because they are obtained from natural sources. Recently, interest in herbal medicines has increased even more. Therefore, it has become important to investigate whether herbal medicines are reliable on human health and their quality. In addition, plants have been used in the studies of the modern pharmaceutical industry to determine and use active ingredients with known mechanisms of action, and effective drugs have

been produced for the treatment of diseases by purifying active ingredients from herbal products. However, in complex diseases, especially cancer, which occur as a result of the contribution of many mechanisms, these drugs containing active ingredients targeting a single target have not been able to achieve the desired therapeutic results. Therefore, research on herbal medicines, which may have the potential to affect many targets, will make a significant contribution to the treatment of complex diseases (van Wyk and Prinsloo, 2020; Li and Weng, 2017; Houghton, 1995).

Thymbra capitata (L.) Cav. is a plant that has therapeutic properties against many diseases such as cancer, cough, asthma, bronchitis, diarrhea, etc., through its organic extracts, volatile and herbal oils belonging to the medical plant class. Studies have determined that the plant has a high antioxidant capacity and antimicrobial properties. *T. capitata.* is a perennial plant that is native to the Mediterranean region and belongs to the Lamiaceae family. It is consumed as a foodstuff as herbal tea and spice. The herbal oil obtained from the plant is used in salads and soups (Saoulajan et al., 2022; Pasa, 2022). In the study, in order to evaluate the possible therapeutic effects of the *T. capitata.* plant on cancer through the phytochemicals it contains, we aimed to reveal the anti-cancer effects of exposing MCF-7 and SH-SY5Y cell lines to the water extract of the plant, through cell viability.

2. Materials and Methods

2.1. Preparation of plant extracts

The plant was finely ground and divided into 10 g pieces. The extract was prepared at the end of 2 hours in water at 90 °C in a soxhlet column and then the extract was filtered (Kapancık et al., 2024). After evaporation of the solvent, the extract was stored in the dark at 4 °C.

2.2. Preparation of cell culture

Of the cell lines, MCF-7 cells were grown in DMEM (Dulbecco's Modified Eagle Medium) and SH-SY5Y cells were grown in RPMI 1640. Medium was prepared by adding 10% FBS (Fetal Bovine Serum) and 1% Penicillin-Streptomycin to DMEM and RPMI 1640. Cells were grown in this prepared medium at 37 °C in 5% CO_2 (Tüzün, 2025).

2.3. MTT method

The % cell viability values and IC_{50} values of MCF-7 and SH-SY5Y cells after exposure to plant extract at 24, 48 and 72 hours were determined using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) method. For this purpose, cells were seeded in 96-well plates in 100 µl volume and 1 x 10^5 cells per ml in medium and then cell viability analysis was performed using MTT (Akkaya et al., 2024). The obtained data were analyzed using the GraphPad Prism method, % cell viability graphs were drawn and IC_{50} values were calculated. Finally, microscope images of MCF-7 and SH-SY5Y cell lines exposed to water extract of *T. capitata* (plant at a concentration of 10 µg/ml for 24 hours were recorded.

2.4. Statistical analysis

In order to reveal the anti-cancer effects in MCF-7 and SH-SY5Y cell lines at 24, 48 and 72 hours, analyses were performed in triplicate. Results are given as mean±SEM. Statistical analysis was performed using the GraphPad Prism program (*$p<0.05$, **$p<0.01$ and ***$p<0.001$).

3. Results

3.1. MTT assay

MCF-7 and SH-SY5Y cell lines were exposed to 1000µg/ml, 500µg/ml, 250µg/ml, 100µg/ml, 50µg/ml and 10µg/ml water extract for 24, 48, and 72h to determine IC_{50} effective doses (Figures 1-3.).

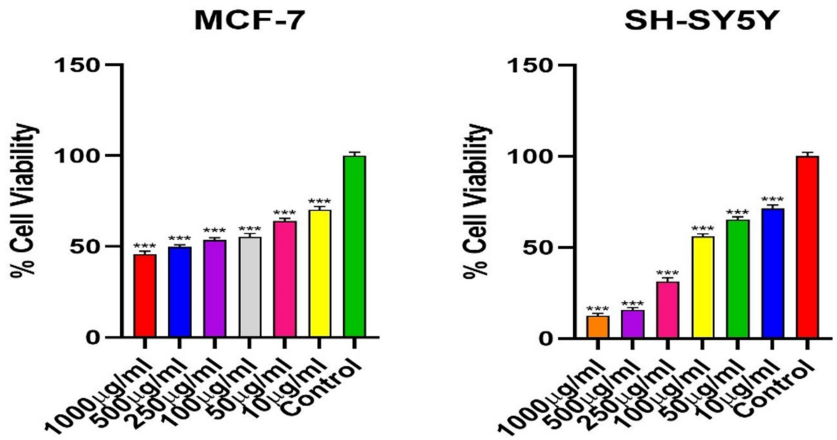


Figure 1. Viability percentages of MCF-7 and SH-SY5Y cells after 24 h exposure (***P < 0.001, **P < 0.01, *P < 0.05).

As the 24-hour exposure dose of water extract of *Thymbra capitata* plant on MCF-7 and SH-SY5Y cancer cells increased, there was a general decrease in cell viability rates. It was determined that the highest cytotoxic effect on both cancer cells was at the concentration of 1000µg/ml. Additionally, in MCF-7 cell lines, the IC_{50} effective doses of water extracts water extract was 439,10 µg/ml for the 24th h incubation. For the SH-SY5Y cell line, it was 88.19 µg/ml (Figure 1.).

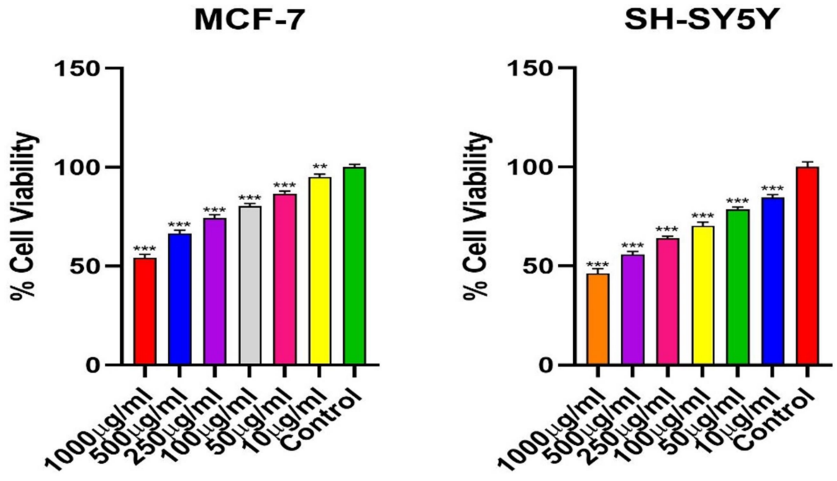


Figure 2. Viability percentages of MCF-7 and SH-SY5Y cells after 48 h exposure (***P < 0.001, **P < 0.01, *P < 0.05).

It was determined that the increase in the applied dose concentrations in MCF-7 and SH-SY5Y cells exposed to the water extract of *T. capitata* plant for 48 hours also mediated an increase in the anti-cancer effects of the plant extract. Additionally, the greatest anti-cancer effect was at a concentration of 1000 µg/ml. However, when the cell viability data at 48 hours were compared with the cell viability data at 24 hours, it was determined that there was a decrease in the cytotoxic effects of the plant extract on MCF-7 and SH-SY5Y cells at 48 hours. The IC50 effective dose for the 48th hour exposure was 1488.00 µg/ml. For the SH-SY5Y cell line, these were 823.40 (Figure 2.).

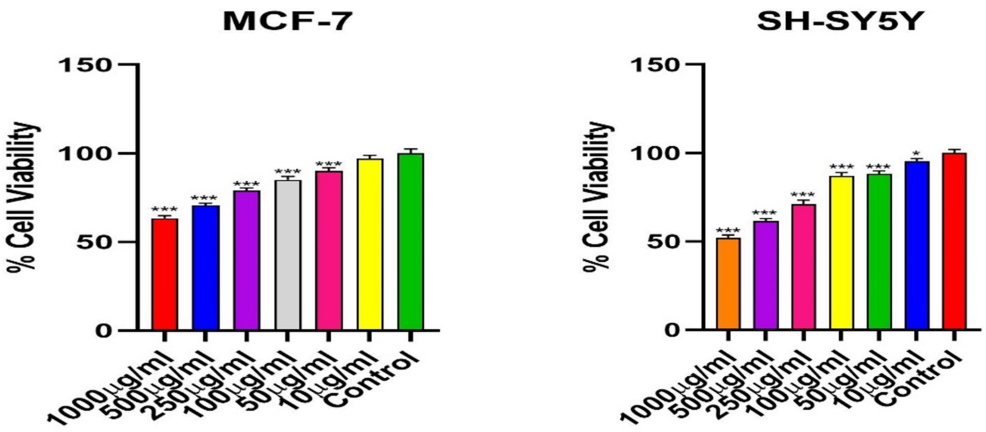
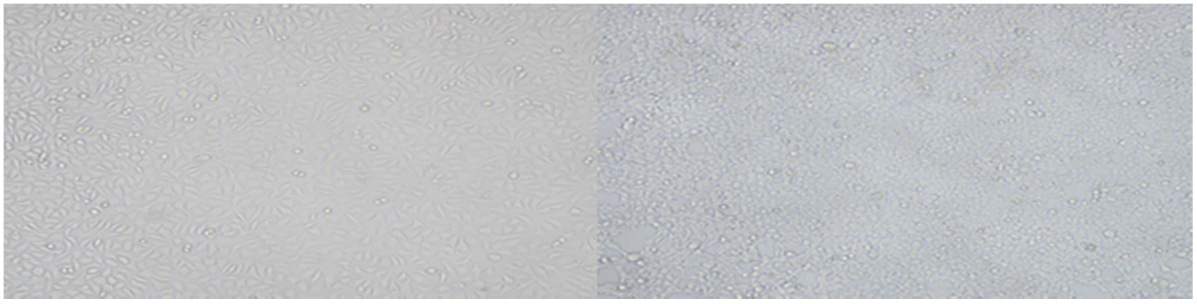


Figure 3. Viability percentages of MCF-7 and SH-SY5Y after 72 h exposure (***P < 0.001, **P < 0.01, *P < 0.05).

At 72nd hour, similar to the data from the other two hours, it was determined that the increase in the application concentrations of the water extract of the *T. capitata* plant increased the anti-cancer effect. The highest anti-cancer activity of the plant extract was determined to be 1000µg/ml. At 72nd hour, the cytotoxic effect of the plant extract on MCF-7 and SH-SY5Y was approximately similar to the cytotoxic effect at 48 hours. Also, IC_{50} effective doses after 72h was 2569,00 µg/ml for MCF-7. For HT-29, the IC_{50} effective doses was 1053,00 µg/ml (Fig. 3.).

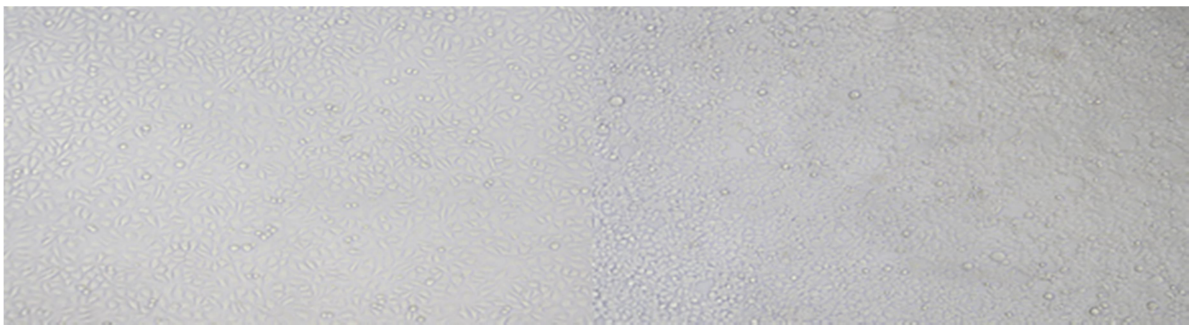
3.2. Microscope images

Microscope images of MCF-7 and SH-SY5Y cell lines exposed to *T. capitata* water extract at a concentration of 10 µg/ml for 24 hours and MCF-7 and SH-SY5Y cell lines in the control group were recorded (Figures 4,5).



(a) **(b)**

Figure 4. Microscope images of (a) MCF-7 and (b) SH-SY5Y cell lines exposed to *T. capitata* water extract for 24h.



(a) **(b)**

Figure 5. Microscope images of control group (a) MCF-7 and (b) SH-SY5Y cell lines at 24h.

4. Discussion

Thymbra capitata (L.) Cav. medical plant is a plant that grows in the Mediterranean region and is widely used among the public for the treatment of cough, asthma, diabetes, cold, skin diseases and digestive system diseases. The main components found in the *T. capitata* plant were determined to be taxifolin-di-O-glucoside and thymusin, and it also contained high levels of rosmarinic acid. In addition, it was determined that it has a high content of flavonoids, terpenoids and phenolic acids. Through the components identified in the phytochemical characterization of *T. capitata* plant, it has been shown that it has high antioxidant content, high antibacterial and antifungal activity, in addition to having antiviral and antiparasitic effects, wound healing, anti-aging, showing a protective effect on the liver and causing anti-cancer effects (Bouyahya et al., 2020; Saija et al., 2016; Hepokur et al., 2020; Alves-Silva et al., 2023).

It has been reported that essential oils obtained from *T. capitata* by hydrodistillation suppressed proliferation in human colorectal cancer cell lines RKO and breast cancer cell lines MCF-7, and that the essential oils of the plant exhibited antitumor activity in this respect (Poças et al., 2014). In a study examining the antioxidant properties and antiproliferative properties of essential oils obtained from the *T. capitata* plant grown in Portugal, it was determined that radicals causing oxidative stress were strongly captured by the plant essential oils, and that the plant essential oils had a strong antioxidant capacity in this respect. In addition, it was determined that exposing THP-1 leukemia cancer cells to *T. capitata* essential oils mediated cytotoxicity in these cells and caused anti-proliferative effects (Miguel et al., 2015). In addition, it has been reported that the essential oils obtained from the fruits and flowers of the *T. capitata* plant have high antioxidant content and also induce cell death in the human epithelioid cervix carcinoma cell line HeLa and histiocytic leukemia cell line U937, and therefore the essential oils of the plant may be a drug source for the treatment of cancer (Delgado-Adámez et al., 2017). Again, in another study on essential oils obtained from *Thymus capitatus*, it was reported that exposure of the MCF-7 cell line and the human colorectal carcinoma cell line HCT-116 to the essential oils of the plant had a strong anticancer effect, and therefore may be a potential candidate for drug development studies (Imtara et al., 2025).

In study investigating the antioxidant, antimicrobial, wound healing and anti-cancer effects of the ethanol extract of the *T. capitata* plant, it was reported that cytotoxicity occurred in human osteosarcoma cell lines MG63 and MCF-7 exposed to the plant extract, and that *T. capitata* species may be a new pharmaceutical compound with therapeutic properties in cancer (Hepokur et al., 2020 In addition, it has been reported that the methanol extract of *T. capitata* has inhibitory effects on the butyrylcholinesterase enzyme, and the plant extracts have inflammation-suppressing properties by mediating a decrease in COX-2 and IL-6 gene expression. Due to these properties, it has been predicted that they can be used in the protection of health and the prevention of disease (Llorent-Martínez et al. 2022). In our study, similar to the information in the literature, it was determined that *T. capitata* plant water extract had a cytotoxic effect on MCF-7 and SH-SY5Y cancer cell lines.

5. Conclusions

Based on the data of our study, we can say that *Thymbra capitata* (L.) Cav. water extract has an anticancer effect on MCF-7 and SH-SY5Y cancer cell lines.

Conflicts of Interests
Authors declare that there is no conflict of interests

Financial Disclosure
Author declare no financial support.

Statement contribution of the authors
This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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