Original Article

Avicenna Journal of Medical Biotechnology
Volume 17, Issue 2, 131-135
https://doi.org/10.18502/ajmb.v17i2.18564

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The Pro-Apoptosis Induction of *Teucrium persicum* Ethyl Acetate Extract on MCF-7 Cells: An *In Vitro* Study

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Received: 5 Sept 2024
Accepted: 30 Nov 2024

Abstract

Background: *Teucrium persicum* (*T. persicum*) is a well-known Iranian endemic plant that grows in the southern regions of Iran. It is used as a tea to treat abdominal pains, hyperlipidemia, and diabetes in traditional Iranian medicine. It has been previously found that the methanolic extract of *T. persicum* exerts significant cytotoxicity and inhibitory effects on different cancer cells. This study aimed to investigate the effects of ethyl acetate extract of *T. persicum* on MCF-7 cells.

Methods: The experiments included MTT, DAPI staining, and investigating the expression of *BAX* and *BCL2* genes. The extract had a significant cytotoxic effect on MCF-7 cells, with an IC_{50} value of 50 *µg/ml* for 48 *hr*.

Results: DAPI staining assays showed that the extract induced morphological changes, chromatin condensation, and nuclear fragmentation. Additionally, the ethyl acetate extract induced the expression of *BAX* and down-regulated *BCL2* genes.

Conclusion: These findings suggest that *T. persicum* has strong cytotoxic properties and warrants further investigation.

Keywords: Apoptosis, Breast cancer, Cytotoxicity, Gene expression, *Teucrium persicum*
To cite this article: Moeil M, Tafrihi M, Nazifi E, Radfar M. The Pro-Apoptosis Induction of *Teucrium persicum* Ethyl Acetate Extract on MCF-7 Cells: An *In Vitro* Study. Avicenna J Med Biotech 2025;17(2):131-135.

Introduction

Cancer is one of the leading causes of death worldwide, and epidemiological studies indicate that cancer incidence is expected to rise in the future [1]. For centuries, plants and plant-derived compounds have been used as therapeutic agents for various diseases, including cancers. However, the therapeutic importance of certain plant species has yet to be documented [2]. Today, over two-thirds of anti-cancer drugs, such as vinblastine, vincristine, and taxol, are derived from plants and have been used in their natural form or with slight chemical alterations [2,3].

The *Teucrium* genus, belonging to the Lamiaceae family, encompasses more than 300 species, 12 of which are endemic to Iran [4,5]. Numerous studies have highlighted *Teucrium* species' anti-diabetic, anti-inflammatory, antioxidant, *in vitro* and *in vivo* cytotoxic properties, [5-8]. *Teucrium persicum* (*T. persicum*) is an Iranian endemic plant that is found locally in the Fars province. In Iranian traditional medicine, it is commonly used to relieve headaches and abdominal pains [4-9]. Additionally, native people use it to treat conditions such as diabetes, hyperlipidemia, obesity, and inflammation [10]. Despite its potential, there have been limited *in vitro* and *in vivo* studies investigating the effects of *T. persicum* on biological systems including cancer cells.

Author's previous studies have demonstrated that treating various cancer cells with the methanolic extract of *T. persicum* resulted in decreased cell viability, inhibition of migration and invasion, and modulation of gene expression [9,10]. However, no study has been conducted on the effects on the MCF-7 cancer cell line as a model for breast cancer. Therefore, this study aims to evaluate the effect of the *T. persicum* ethyl acetate extract on the viability, invasion, and gene expression in the MCF-7 cancer cells.

Materials and Methods

Extract preparation

T. persicum plants were collected in April 2023 from the south of Iran. For extraction, 100 *g* of the powdered aerial parts of *T. persicum* were soaked in 300 *ml* of ethyl acetate and shaken for 24 *hr*. The supernatant was collected, and this step was repeated

three times. The obtained solution was collected and evaporated using a rotary device (Heidolph, Germany) at 40°*C*. The concentrated extract was dried by a freeze dryer (Christ, Germany) and stored at -20°*C* until use [11].

Cell culture

The MCF-7 cell line was purchased from the National Center for Genetic and Biological Resources (Tehran, Iran). After performing microbial and viral tests, the cells were cultured in DMEM containing 10% fetal bovine serum (Invitrogen), 100 *µg/ml* of streptomycin, and 100 *units/ml* of penicillin (Sigma) in a 5% CO_2 humidified incubator at 37°*C*.

MTT assay

The MTT assay was performed to evaluate cell viability. In brief, 8×10^3 cells were cultured in each well of a 96-well plate. The cells were then treated with 12.5, 25, 50, 100, 200, and 400 *µg/ml* of the extract for 48 *hr*. After removing the medium, 100 *µl* of Phosphate-Buffered Saline (PBS) containing 5 *µg/µl* MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) was added to each well. After 4 *hr*, the MTT solution was removed, and 100 *µl* of DMSO was added to each well. Once the dye was fully solubilized, the absorbance of light was measured at 590 *nm* using an ELISA reader (BioTek, USA).

DAPI staining

DAPI (4,6-diamidino2-phenylindole) staining was used to evaluate nuclear morphology. MCF-7 cells were cultured in 6-well plates and allowed to grow to 70% confluency. The cells were treated with 50 *µg/ml* (IC_{50}) and 100 *µg/ml* of the ethyl acetate extract for 48 *hr*, then fixed with 4% paraformaldehyde, followed by staining with 300 *nM* of DAPI dye (Sigma, USA), and finally checked by a fluorescence microscope (Novel, China).

Gene expression analysis

RNA was extracted from cells using the RNA extraction kit (Tehran Cavosh Clon), and the cDNA samples were synthesized using a cDNA synthesis kit (RNX- Plus, Tehran Cavosh Clon, Iran). The transcription of *BAX* and *BCL2* genes was measured using RealQ Plus 2× Master Mix Green (High Rox, Denmark). The primer sequences are listed as follows: *BCL2* (Forward: 5′-GAACTGGGGGAGGATTGTG-3′, Reverse: 5′-CGTACAGTTCCACAAAGGGA-3′), *BAX* (Forward: 5′-GGCCCACCAAGCTCTGAGCAG A-3′, Reverse: 5′-GCCACGTGGGCGGTCCCAAAG T-3′, *GAPDH* (Forward: 5′-GTCTCCYCTGACTTCA ACAGCG-3′, Reverse: 5′-ACCACCCTGTTGCTGCT GTAGCCAA-3′).

Statistical analysis

Data analysis was performed using SPSS (IBM Statistics 25.0). Each experiment was replicated three times. p-values <0.05 were considered statistically significant.

Results

Effect of the ethyl acetate extract on the viability of MCF-7 cells

To evaluate the inhibitory effect of the ethyl acetate extract of *T. persicum* on the survival of MCF-7 cancer cells, the cells were treated with different extract concentrations for 48 *hr*. Cell viability was then measured using the MTT test. As shown in figure 1, treatment of MCF-7 cells with 3.125, 6.25, and 12.5 *µg/ml* of the extract resulted in a slight reduction in cell viability. However, treatment of with 25 and 50 *µg/ml* of the extract led to a remarkable reduction in cell viability, and treatment with 100 *µg/ml* of the extract resulted in an 80% decrease in cell viability. The IC_{50} value of the ethyl acetate extract of *T. persicum* was calculated to be 50 *µg/ml* (Figure 1).

Effect of the extract on the nuclear status of MCF-7 cells

MCF-7 cells were treated with 50 (IC_{50}) and 100 *µg/ml* of the extract for 48 *hr* and subsequently stained with DAPI (1 *mg/ml*), followed by visualization with a fluorescence microscope. As shown in figure 2, no significant changes were observed in the nuclei of non-treated cells (control cells). However, cells treated with 50 and 100 *µg/ml* of the extract exhibited morphological changes associated with apoptosis, such as chromatin condensation, nuclear fragmentation, and margination of the nucleus.

Effect of the extract of T. persicum on the transcription of BAX and BCL2 genes

The previous results indicated that treatment of MCF-7 cells with the extract of *T. persicum* led to significant inhibitory effects on cell viability. To investigate whether these effects were associated with changes in the expression of certain genes controlling cell death, MCF-7 cells were treated with 50 *µg/ml* of the extract for 48 *hr*, and the transcription of *BAX* and *BCL-2* genes was measured. Compared to non-treated

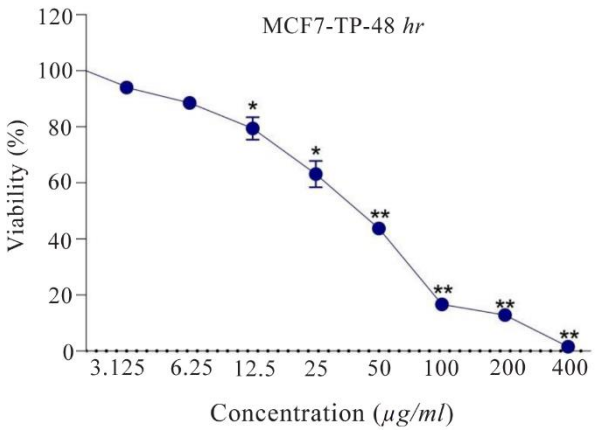

Figure 1. *T. persicum* extract reduces cancer cell viability. MCF-7 cells were treated with different concentrations of *T. persicum* extract for 48 *hr*, and then cell viability was measured by MTT test. The results are the mean±standard deviation of three separate experiments in which each treatment was repeated in 10 wells (*p<0.05; ** p<0.01; *vs*. control).

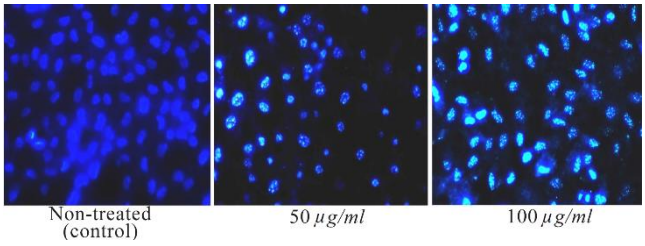

Figure 2. DAPI staining was performed on MCF-7 cells exposed to the ethyl acetate extract of *T. persicum* at concentrations of 50 and 100 *μg/ml*. MCF-7 cells treated with the extract showed apoptotic changes (condensed and fragmented nuclei). No such changes were observed in non-treated (control) cells (Magnification×40).

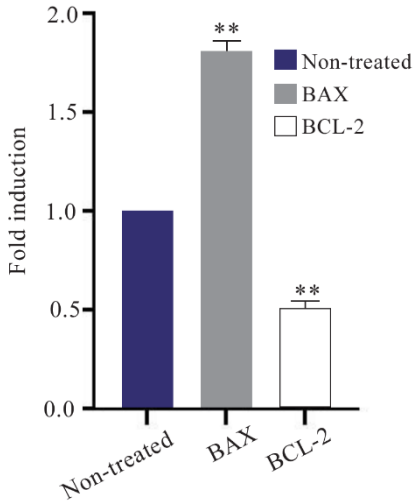

Figure 3. Real-time PCR experiments were conducted to measure the mRNA levels of the *BAX* and *BCL2* genes in the MCF-7 cells, which were treated with the ethyl acetate extract of *T. persicum*. MCF-7 cells were treated with 50 *μg/ml* of the extract for 48 *hr*. Total RNA was collected from different sets of cells and was used for real-time PCR experiments. Data are normalized to the average mRNA levels of GAPDH. The shown data are mean±SD of three independent experiments (*p<0.05 and **p<0.01 *vs*. control).

cells, treatment with 50 *μg/ml* of the extract resulted in an up-regulation of the *BAX* gene (1.8-fold increase) and a down-regulation of the *BCL2* gene (0.5-fold decrease) (Figure 3).

Discussion

Cancer is a complex disease that develops over a long period [12]. Consequently, treating it has always presented significant challenges. Thankfully, an increasing body of evidence suggests that certain plant-derived chemicals, known as phytochemicals, may possess anti-cancer properties. Some of these phytochemicals have already been used as effective anti-cancer drugs [13]. Epidemiological studies have also shown a negative correlation between consuming fruits and vegetables and the risk of cancer [14,15]. It has been established that approximately one-third of all human cancers could be prevented through dietary choices [16]. This study investigated the chemical profile, cytotoxicity, and inhibitory effects of the ethyl acetate extract of *T. persicum* on MCF-7 cells.

Based on previous studies, sesquiterpenes are typically the dominant group of compounds in essential oils of *Teucrium* species [17,18] Reviewing the literature reveals that some of these compounds have anti-inflammatory, antibacterial, antioxidant, and anti-cancer effects [17-19].

The results of MTT assays showed the significant cytotoxic effects of the extract on MCF-7 cells (IC_{50}= 50 *μg/ml*, $p \leq 0.01$). Compared to the cytotoxic potential of the ethyl acetate extract (IC_{50}=65.9 *μg/ml*, unpublished data) and the methanolic extract of *T. persicum* on PC-3 (IC_{50}=142 *μg/ml*) [4], the results of the current study showed that the ethyl acetate extract has greater cytotoxic potential on MCF-7 cells (Figure 1). Reviewing the literature revealed that the cytotoxicity potential of plant extracts may attributed to synergism of two or more compounds in the extract [20]. Phytochemical analysis has revealed that Teucrium species contain valuable amounts of phenolic and flavonoid compounds that may be the main cause of their biological activities [21,22]. Therefore, it can be concluded that the cytotoxic potential of the ethyl acetate extract *of T. persicum* may be due to the existence of phenolic and flavonoid compounds.

DAPI staining experiments indicated that lethal concentrations of the extract (50 *μg/ml* and 100 *μg/ml*) induced morphological changes associated with apoptosis, including chromatin condensation, nuclear fragmentation, shrinkage and margination of nuclei, and apoptotic bodies (Figure 2). In contrast, the nuclei of non-treated cells were large and normal, confirming the extract's apoptotic potential. The same results were observed in A549 human lung cancer cells treated with methanolic fraction of *S. grandiflora* [23], and in HFFF2 (human fibroblast foreskin) cell line treated with oleuropein [24].

Next, this research evaluated the effect of the extract on the expression of *BAX* and *BCL-2* genes, which are key regulators of apoptosis. Reviewing the literature indicated that targeting apoptosis is a hopeful strategy within cancer care practices [25]. The *BCL-2* gene prevents apoptosis, allowing cancer cells to survive, while the *BAX* gene promotes apoptosis and aids in the elimination of cancer cells [26]. The balance between these genes ultimately determines the susceptibility to apoptosis. Disruption of this balance occurs when the expression of *BCL-2* mRNA decreases and/or the expression of *BAX* mRNA increases, leading to apoptosis [26]. There are some reports indicating the apoptosis induction potentials of plant extracts in different cancer cells. In a recent study, Serala and colleagues reported that acetone leaf extract of *Momordica balsamina* induces apoptosis in MCF-7 breast cancer cells *via* modulating the expression of genes involving in apoptosis

regulation including *BAX* and *BCL-2* genes [27]. In another study, it has been reported that volatile oil of *T. alopecurus* induces apoptosis in colon cancer cells *via* TRAIL pathway and modulates the expression of some key apoptosis-regulating genes [28]. The effect of the ethyl acetate extract of *T. persicum* on the expression of these genes indicates its potential to inhibit cancer cells through the induction of apoptosis (Figure 2).

Conclusion

The potent cytotoxic and inhibitory effects we observed from the ethyl acetate extract of *T. persicum* on MCF-7 breast cancer cells seem to come from certain phytochemicals that may influence gene expression linked to cell death. Although the author's study provides a preliminary insight into the bioactivity of the extract, it is too early to draw any firm conclusions about its therapeutic value. Much more research is needed to pinpoint the active compounds, understand how they affect cell viability, and uncover the molecular pathways at work. These next steps will give us a better picture of how this extract might contribute to cancer treatment in the future.

Acknowledgement

We would like to thank the University of Mazandaran's research Department for its financial support.

Funding: This study was supported by the University of Mazandaran Research Department.

Conflict of Interest

The authors declare that they have no competing interests for this publication.

References

1. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health 2019; 9(4):217-22.
2. Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, Iurciuc S, et al. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. Molecules. 2021;26(14):1109.
3. Asma ST, Acaroz U, Imre K, Morar A, Shah SRA, Hussain SZ, et al. Natural products/bioactive compounds as a source of anticancer drugs. Cancers (Basel) 2022;14 (24):6203.
4. Tafrihi M, Toosi S, Minaei T, Gohari AR, Niknam V, Arab Najafi SM. Anticancer properties of Teucrium persicum in PC-3 prostate cancer cells. Asian Pac J Cancer Prev 2014;15(2):785-91.
5. Sadeghi Z, Yang J-L, Venditti A, Moridi Farimani M. A review of the phytochemistry, ethnopharmacology and biological activities of Teucrium genus (Germander). Nat Prod Res 2022;36(21):5647-64.
6. Sharifi-Rad M, Pohl P, Epifano F, Zengin G, Jaradat N, Messaoudi M. Teucrium polium (L): phytochemical screening and biological activities at different phenological stages. Molecules 2022;27(5):1561.
7. Monsef-Esfahani H, Miri A, Amini M, Amanzadeh Y, Hadjiakhoondi A, Hajiaghaee R, et al. Seasonal variation in the chemical composition, antioxidant activity and total phenolic content of Teucrium persicum Boiss. essential oils. Res J Biol Sci 2010;5(7):492-8.
8. Rajabalian S. Methanolic extract of Teucrium polium L potentiates the cytotoxic and apoptotic effects of anticancer drugs of vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. Exp Oncol 2008; 30(2):133-8.
9. Naeimi A, Tafrihi M, Mohadjerani M. Antioxidant and cytotoxic potentials of the methanolic extract of Teucrium persicum Boiss. in A-375 melanoma cells. Avicenna J Phytomed 2022;12(2):185-96.
10. Hajipour P, Eizadifard F, Tafrihi M. Chemical constituents, antioxidant and cytotoxic potential of chloroform and ethyl acetate extracts of Teucrium persicum. Jentashapir Journal of Cellular and Molecular Biology. 2022 Jun 30;13(2).
11. Tamokou DJD, Mpetga DJS, Lunga PK, Tene M, Tane P, Kuiate JR. Antioxidant and antimicrobial activities of ethyl acetate extract, fractions and compounds from stem bark of Albizia adianthifolia (Mimosoideae). BMC Complement Altern Med 2012;12:99.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. cell. 2011;144(5):646-74.
13. Rudzińska A, Juchaniuk P, Oberda J, Wiśniewska J, Wojdan W, Szklener K, et al. Phytochemicals in cancer treatment and cancer prevention—review on epidemiological data and clinical trials. Nutrients 2023;15(8): 1896.
14. Key T. Fruit and vegetables and cancer risk. Br J Cancer. 2011;104(1):6-11.
15. Turati F, Rossi M, Pelucchi C, Levi F, La Vecchia C. Fruit and vegetables and cancer risk: a review of southern European studies. Br J Nutr 2015;113 Suppl 2:S102-S10.
16. Surh Y-J. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003;3(10):768-80.
17. Henchiri H, Bodo B, Deville A, Dubost L, Zourgui L, Raies A, et al. Sesquiterpenoids from Teucrium ramosissimum. Phytochemistry 2009;70(11-12):1435-41.
18. Wu Z-K, Li H-Y, Zhu Y-L, Xiong M-Q, Zhong J-X. Neuroprotective and anti-inflammatory effects of eicosane on glutamate and NMDA-induced retinal ganglion cell injury. Int J Ophthalmol. 2024;17(4):638-45.
19. Chuah XQ, Okechukwu PN, Amini F, Teo SS. Eicosane, pentadecane and palmitic acid: The effects in: In vitro: Wound healing studies. Asian Pac J Trop Biomed 2018; 8(10):490-9.
20. Sajeev Wagle S, Anne Lee J, Vasantha Rupasinghe HP. Synergistic Cytotoxicity of Extracts of Chaga Mushroom and Microalgae against Mammalian Cancer Cells In Vitro. Oxidative Medicine and Cellular Longevity January 2024;2024(12):1-13.
21. Miri A, Sharifi-Rad J, Tabrizian K, Nasiri AK. Antinociceptive and Anti-Inflammatory Activities of Teucrium

persicum Boiss. Extract in Mice. Scientifica 2015; 2015: 972827.

22. Grujičić D, Marinković D, Milošević-Djordjević O. Genotoxic activity of secondary metabolites of Teucrium species. Teucrium species: biology and applications. 2020;2020:231-273.
23. Pajaniradje S, Mohankumar K, Pamidimukkala R, Subramanian S, Rajagopalan R. Antiproliferative and Apoptotic Effects of Sesbania grandiflora Leaves in Human Cancer Cells. Biomed Res Int 2014;2014:474953.
24. Asghariazar V, Vahidian F, Karimi A, Abbaspour-Ravasjani S, Mansoori B, Safarzadeh E. The Role of Oleuropein, Derived from Olives, in Human Skin Fibroblast Cells: Investigating the Underlying Molecular Mechanisms of Cytotoxicity and Antioxidant and Anti-Inflammatory Activities. Int J Clin Pract 2024;2024:8827501.
25. Pfeffer CM, Singh AT. Apoptosis: a target for anticancer therapy. Int J Mol Sci 2018;19(2):448.
26. Hata AN, Engelman JA, Faber AC. The BCL2 family: key mediators of the apoptotic response to targeted anticancer therapeutics. Cancer Discov 2015;5(5):475-87.
27. Serala K, Mmanoko Malemela K, Boshielo IT, Riedel S, Mampuru L, Mbazima V. Momordica balsamina acetone leaf extract induces apoptosis and inhibits the invasiveness and migration of MCF-7 breast cancer cells. S Afr J Bot 2024;165:257-263.
28. Guesmi F, Prasad S, Ben Ali M, Ismail IA, Landoulsi A. Thymus hirtus sp. algeriensis Boiss. and Reut. volatile oil enhances TRAIL/Apo2L induced apoptosis and inhibits colon carcinogenesis through upregulation of death receptor pathway. Aging 2021;13(18):21975-21990.