

RESEARCH ARTICLE

Anticancer Properties of *Teucrium persicum* in PC-3 Prostate Cancer Cells

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Abstract

Crude extracts or phytochemicals obtained from some plants have potential anti-cancer properties. *Teucrium persicum* is an Iranian endemic plant belonging to the *Lamiaceae* family which has traditionally been used to relieve abdominal pains. However, the anti-cancer properties of this species of the *Teucrium* genus have not been investigated previously. In this study, we have used a highly invasive prostate cancer cell line, PC-3, which is an appropriate cell system to study anti-tumor properties of plants. A methanolic extract obtained from *T. persicum* potently inhibited viability of PC-3 cells. The viability of SW480 colon and T47D breast cancer cells was also significantly decreased in the presence of the *T. persicum* extract. Flow cytometry suggested that the reduction of cell viability was due to induction of apoptosis. In addition, the results of wound healing and gelatin zymography experiments supported anti-cell invasion activity of *T. persicum*. Interestingly, sublethal concentrations of *T. persicum* extract induced an epithelial-like morphology in a subpopulation of cells with an increase in E-Cadherin and β -Catenin protein levels at the cell membrane. These results strongly suggest that *T. persicum* is a plant with very potent anti-tumor activity

Keywords: *Teucrium persicum* - PC-3 cells- epithelioid morphology - anti-tumor activity

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Introduction

Plant-derived phytochemicals have been used as therapeutic agents for hundreds of years (Cragg and Newman, 2005). Many such compounds with anti-cancer activities have been identified which some of them (e.g. vinblastine, vincristine, taxol and colchicine), either directly or after chemical modifications, have been used as anticancer drugs (Nirmala et al., 2011). Some of these agents compared to purely synthetic drugs have higher activity and lower toxicity (Fabricant and Fransworth, 2001).

Teucrium is a genus of plants belonging to the family of *Lamiaceae* and includes about 300 species (Moghtader, 2009; Monsef-Esfahani, 2010). The species are mainly distributed in central and southern America, southeast Asia, and Mediterranean region (Kandouz et al., 2010; Miri et al., 2012). The extract isolated from *Teucrium* species has been used for treatment of diabetes, obesity, inflammation, and hyperlipidemia (Twaij et al., 1987; Tariq et al., 1989; Miri et al., 2012). Also, there are reports on antioxidant, anticancer, and antibacterial activities of some *Teucrium* species (Kadifkova Panovska et al., 2005; Miri et al., 2012). For example, it has been shown that the organic extracts derived from *T. polium*, *T. chamaedrys*,

and *T. montanum* have remarkable antioxidant activity (Kadifkova Panovska et al., 2005). In addition, it has been reported that the ethanolic extract of *T. polium* has antibacterial effects on both gram positive and gram negative bacteria (Autore et al., 1984).

T. persicum is an Iranian endemic plant and grows in Fars province (south of Iran) (Miri et al., 2012). In Iran *T. persicum* has been used for years to relieve headache and abdominal pains (Miri et al., 2012). Several studies have been performed on chemical composition and antioxidant activity of the oils isolated from *Teucrium persicum* (Monsef-Esfahani et al., 2010; Miri et al., 2012). However, the anti-cancer properties of *Teucrium persicum* have not yet been investigated. Here, PC-3 prostate cancer cells have been used to study the biological properties of *T. persicum*. PC-3 cells are highly invasive and have a mesenchymal phenotype (Kandouz et al., 2010; Tai et al., 2011). Therefore, this cell line is an appropriate tool to study anti-cancer properties of plant extracts and their-derived compounds (Nyska et al., 2003). The results of this paper show that the methanolic extract of *T. persicum* potently inhibits viability of PC-3 cells. In addition, sublethal concentrations of *T. persicum* extract induce an epithelial-like morphology, leading to an increase in E-Cadherin and β -Catenin membrane localization.

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Prostate cancer is a leading cause of cancer death in western countries (Schulz et al., 2003) which is mainly seen as adenocarcinoma of epithelial cell-origin (Mazhar and Waxman, 2002). Epithelial tissue integrity is maintained via E-Cadherin-mediated cell adherens junction (Baum and Georgiou, 2011). Loss of E-Cadherin in epithelial cancers leads to cancer cell invasion and metastasis, a process called EMT (epithelial-mesenchymal transition) (Guarino, 2007). The ability of *T persicum* extract to restore E-Cadherin to the plasma membrane of PC-3 cells suggests that there are one or more compounds in the extract which can reverse EMT. The findings of this paper suggest that *T persicum* is a plant with potential anti-cancer properties.

Materials and Methods

Cell lines and cell culture

PC-3 and SW480 cell lines were purchased from National Cell Bank of Iran (NCBI, Pasteur institute, Tehran, Iran). T47D and NIH-3T3 cells were kindly provided by Dr. Nasrin Motamed (University of Tehran, Tehran-Iran) and Mr. Mehdi Mohaghegh (Tarbiat Modarres University, Tehran-Iran) respectively. All cell lines were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (Invitrogen), 100 µg/ml streptomycin, and 100 unit/ml penicillin.

Preparation of methanolic extract of *T persicum*

50g of the powder obtained from dried aerial parts of *Teucrium persicum* was soaked in 100 ml of methanol (MeOH) and shaken for 24 hours. The supernatant was collected and evaporated by using a rotary evaporator (Heidolph, Germany) at 37°C. The concentrated extract was dried by a freeze dryer (Christ, Germany) and the dried extract was dissolved in MeOH (20 mg/ml) and stored at -20°C until use.

MTT assay

Cell viability was examined by MTT assay. Briefly, 5.0×10^3 PC-3 cells (in 150 µl growth medium) were cultured in each well of a 96 well plate. 24 hours later, the cells were treated with different concentrations of *T persicum* extract for 48 hours. The medium of cells was removed and 100 µl of PBS containing 5 µg/µl MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) was added to each well. 3 hours later, the MTT solution was replaced by 100 µl of DMSO and light absorbency was measured at 590 nm by an Elisa reader (BioTek, USA).

Flow cytometry

Flow cytometry was performed by using an Annexin V/PI kit as described by the supplier (BD Bioscience, USA). Briefly, 7.5×10^4 PC-3 cells were plated in each well of 6-well plates and grown to 70% confluency. The cells were then treated with 50, 100 and 150 µg/ml of *T persicum* extract for 48 hours. 13.96 µg/ml of etoposide (Sigma, Barcelona, Spain) was used as a positive control for induction of apoptosis (Salido et al., 1999; Fukuta et al., 2008). The cells were then harvested and centrifuged

at 1500 rpm for 5 min at 4°C. The supernatant was discarded and the cell pellet was resuspended in 100 µl of binding buffer (100 mM HEPES, 140 mM NaCl, 2.5 mM CaCl₂, pH=7.4). 5 µl annexin V (2.5 µg/ml) and 1 µl propidium iodide (10 µg/ml) was added to each cell suspension followed by incubation at room temperature for 20 minutes. Finally the cells were analyzed by a flow cytometer (Partec, Germany) and the results were quantified by *Flow Jo* software.

DNA laddering

7.5×10^4 PC-3 cells were plated in each well of 6-well plates and grown to 60-70% confluency. The cells were then treated with 50, 100, 150 and 200 µg/ml of *T persicum* extract for 48 hours. The cells treated with 13.96 µg/ml of etoposide were used as a positive control (Salido et al., 1999; Fukuta et al., 2008). Genomic DNA was extracted by using a DNA extraction kit as described by the supplier (RCPN, Tabriz, Iran). DNA concentration was measured and then equal amount of DNA from each sample was electrophoresed in a 10% polyacrylamide gel. The gel was fixed twice (3 min, each) in a solution containing 10% v/v EtOH and 0.5% v/v glacial acetic acid (in water). The gel was then washed with water and stained with 0.1% w/v silver nitrate (in water) for 15 minutes. The gel was finally incubated in developing solution (1.5% w/v NaOH and 0.01% v/v formaldehyde) with gentle shaking until the bands appeared.

Light microscopy

4.0×10^4 PC-3 cells grown on 10% poly L lysine-coated coverslips were treated with 10 or 25 µg/ml of *T persicum* extract for 48 hours (the medium containing the extract was renewed after 24 hours). The cells were then rinsed with PBS (phosphate buffered saline) and fixed with a cold acetone-methanol solution (50:50 v/v) for 20 min at -20°C. The cells were then rinsed twice with PBS (5 min, each) and each coverslip was pressed down on top of a small drop of mounting medium placed on a clean slide and visualized with a light microscope (Zeiss, Germany).

Indirect immunofluorescence

PC-3 cells grown on coverslips were treated with the indicated concentrations of *T persicum* extract for 48 hours (the medium containing the extract was renewed after 24 hours). The cells were then rinsed with PBS and fixed with a cold acetone-methanol solution (50:50 v/v) for 20 min at -20°C. The cells were rinsed with PBS twice (5 min, each) and permeabilized in 0.2% Triton X-100 (in PBS) for 4 minutes. The cells were rinsed again with PBS, blocked with 3% BSA (in PBS), and were incubated with primary antibodies against E-Cadherin (DAKO, Denmark) or β-Catenin for 1 hour. The cells were washed three times with PBS, and then incubated with appropriate FITC-labeled secondary antibodies for 1 hour. The coverslips were finally washed with PBS three times (10 min, each) and then each one of them was pressed down on top of a small drop of mounting medium placed on a clean slide. The edges of coverslips were sealed with nail polish and the slides were visualized with a fluorescence microscope (Zeiss, Germany).

Cell scratch assay

1.0×10⁵ cells were plated in each well of a 6-well plate and allowed to grow to confluence. Then a scratch was generated in the monolayer with a sterile plastic yellow tip. The cells were washed with PBS three times to remove any damaged or floating cells and then were treated with the indicated concentrations of *T persicum* extract for 48 hours. The plates were photographed with an ordinary digital camera and the wound surface area was measured by *ImageJ* software.

Gelatin Zymography assay

7.5×10⁴ PC-3 cells were plated in each well of 6-well plates and grown to 60-70% confluency. The cells were then treated with the indicated concentrations of *T persicum* extract in a serum free medium for 48 hours. The medium from each well was collected and freeze dried. The dried medium was dissolved in 100 µl H₂O and protein concentration was measured (Sun et al., 2012). Equal amounts of protein from each sample were then electrophoresed in a 10% SDS-PAGE containing 0.1% (w/v) gelatin. The gel was first incubated in 2.5% Triton X-100 (in H₂O) at room temperature for 1 hour and then in activating buffer (50 mM Tris pH=7.4, 150 mM NaCl, 5 mM CaCl₂, 1 µM ZnCl₂) for 18 hours at 37°C. The gel was then stained with 0.5% commassie blue for 1 hour and destained with a solution containing 10% v/v glacial acetic acid and 30% v/v EtOH (in water) until the bands appeared. The gel was scanned and quantified by *Image J* software.

Results

T persicum extract reduces viability of cancer cells

To find out whether the extract obtained from *T persicum* had any effect on cell viability, PC-3 prostate cancer cells were exposed to different concentrations of the extract for 48 hours and then the viability of cells was measured by MTT assay. As shown in Figure 1, although treatment of cells with 10 and 25 µg/ml of *T persicum*

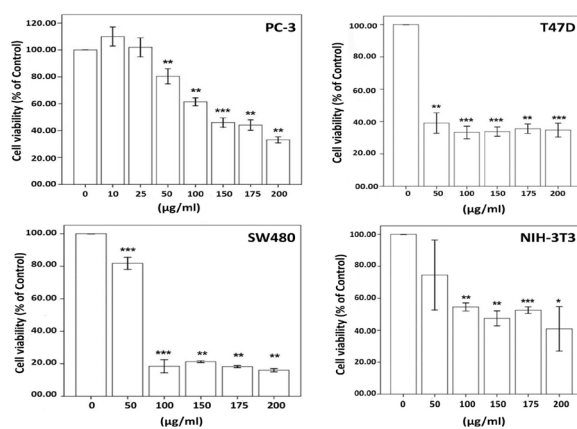


Figure 1. *T persicum* Extract Decreases Viability of Cancer Cells. The cells indicated in the figure have been treated with increasing concentrations of *T persicum* extract for 48 hours and then the viability of cells has been measured by MTT experiments. The data shown are mean±SD of three separate experiments in which each treatment was repeated in 10 wells (*p<0.05; **p<0.01; ***p<0.001 vs control)

extract resulted in a slight increase in the number of viable cells (up to 15%), 50 µg/ml or higher concentrations of the extract significantly reduced cell viability (Figure 1) in a concentration dependent manner. As shown in this Figure, the extract at concentration of 200 µg/ml led to more than 60% decrease in the number of viable cells. The IC₅₀ value of the extract for PC-3 cells was calculated to be 142 µg/ml. Staining of PC-3 cells with propidium iodide followed by flow cytometry analysis, further demonstrated that *T persicum* extract induces cell death (not shown).

Interestingly, the extract of *T persicum* also inhibited the viability of SW480 colon and T47D breast cancer cells, suggesting that *T persicum* probably inhibits viability of many cancer cells (Figure 1). The IC₅₀ values of *T persicum* extract for SW480 and T47D cells were estimated to be 79 and 50 respectively. These results indicate that the extract obtained from *T persicum* carries one or more compounds which have a strong negative effect on viability of cancer cells. *T persicum* extract also reduced the viability of mouse fibroblast NIH-3T3 cells with an IC₅₀ value of 143 µg/ml (Figure 1). Unfortunately, we did not have access to a normal human tissue cell line and therefore NIH-3T3 was used as a replacement.

We then used flow cytometry analysis to find out whether the reduction of PC-3 cell viability by *T persicum* extract was due to induction of apoptosis. Since it has been shown that etoposide induces apoptosis in PC-3 cells (Salido et al., 1999; Fukuta et al., 2008), we used this drug

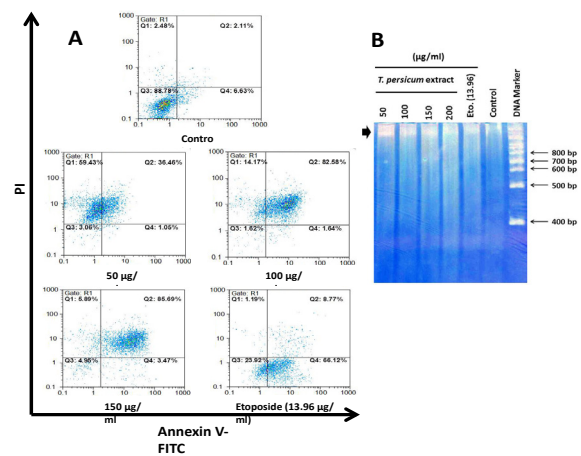


Figure 2. *T persicum* Extract Induces Apoptosis in PC-3 Cells. **A)** The cells were treated with 50, 100 and 150 µg/ml of *T persicum* extract for 48 hours and then used for flow cytometry analysis as described in the method section. Etoposide (13.96 µg/ml) was used as a positive control for apoptosis induction. In each chart, Q1, Q2, Q3, and Q4 respectively represent necrotic cells which have stained with propidium iodide, late apoptotic cells stained with PI and Annexin V, live cells which have not absorbed PI or Annexin V, and early apoptotic cells which have only absorbed Annexin V. **B)** *T persicum* extract induces DNA degradation. PC-3 cells were treated with 50, 100, 150 and 200 µg/ml of *T persicum* extract for 48 hours. Etoposide (13.96 µg/ml) was used as a positive control for apoptosis induction. The genomic DNA isolated from each sample was electrophoresed on a 10% polyacrylamide gel followed by silver nitrate staining. The thick arrow on top of the gel represents the main genomic DNA band. The control lane represents non-treated cells. Eto; Etoposide

as a positive control. We chose three lethal concentrations of the extract including 50, 100, and 150 $\mu\text{g}/\text{ml}$ (Figure 1). The extract concentrations greater than 150 $\mu\text{g}/\text{ml}$ were excluded because of a dramatic reduction in the number of treated cells. As shown in Figure 2A, while treatment of PC-3 cells with 50 $\mu\text{g}/\text{ml}$ of *T persicum* extract induced a mixture of necrosis and apoptosis, the cells exposed to 100 or 150 $\mu\text{g}/\text{ml}$ of the extract underwent late apoptosis (Figure 2A).

We also used DNA fragmentation experiments to test apoptosis. PC-3 cells were treated with 50, 100, 150 and 200 $\mu\text{g}/\text{ml}$ of *T persicum* extract and then genomic DNA was extracted from the cells (see the Methods). Equal amount of DNA from each sample was separated in a 10% polyacrylamide gel followed by silver staining. Despite modification of the assay, we never observed a typical DNA ladder even when etoposide, a known inducer of PC-3 cell apoptosis, was used for treatment of cells (Figure 2B). DNA degradation always appeared as a smear in the gel and the smear intensity increased with concentration of the extract, in expense of the main genomic DNA band on top of the gel (the arrow in Figure 2B). Collectively, the results of flow cytometry and DNA degradation assays suggest that *T persicum* induces apoptosis in PC-3 cells.

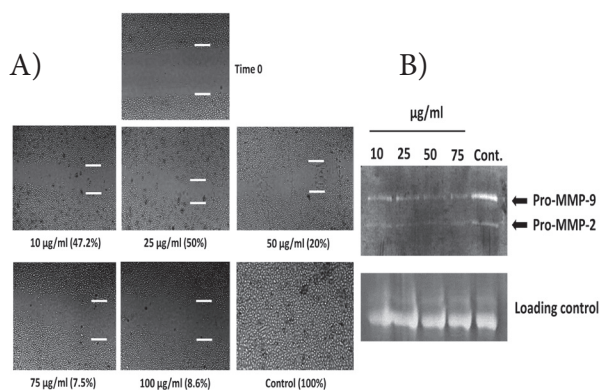


Figure 3. *T persicum* Extract Decreases Wound Healing of PC-3 Cell Monolayers. A) The cells were cultured to confluency and then a scratch was generated in the monolayer with a sterile plastic yellow tip. The cells were then treated with the indicated concentrations of *T persicum* extract for 48 hours. The value below each panel represents the percentage of wound healing. The short white lines represent the border of the wounds. B) *T persicum* extract decreases production and (or) secretion of pro-MMP-2 and pro-MMP-9 by PC-3 cells. A representative zymography gel electrophoresis has been shown in the figure (see the method section for details). The numbers on top of the gel represent the concentrations of *T persicum* extract which have been used for treatment of cells. The loading control is a section of a coomassie blue stained 10% SDS-PAGE loaded with equal amounts of protein from each sample

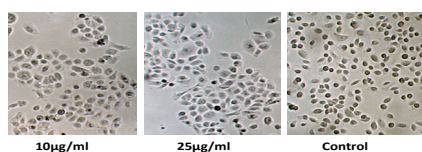


Figure 4. Morphology of PC-3 Cells Treated with *T persicum* Extract. The cells were remained untreated or treated with 10 and 25 $\mu\text{g}/\text{ml}$ of *T persicum* extract for 48 hours. The cells were then observed under a light microscope ($\times 400$) and photographed with an ordinary digital camera

T persicum inhibits invasion of PC-3 cells

First, we used wound healing assay to find out whether *T persicum* extract had any effect on invasion of PC-3 cells. The assay was performed as described in the Method section. As shown in Figure 3A, compared to that of non-treated cells (100%), the wound closure values for the cell sheets (monolayers) treated with 10 and 25 $\mu\text{g}/\text{ml}$ of *T persicum* extract were approximately 50% (Figure 3A). As expected, the wound repair values of the cell sheets treated with higher concentrations of the extract were much less compared to that of non-treated cells (20% and 7.5% for the cell treated with 50 and 75 $\mu\text{g}/\text{ml}$ of *T persicum* extract respectively) (Figure 3A).

The wound closure of a cell monolayer is dependent on both cell proliferation and cell invasion (Zahm et al., 1997). Since we observed a slight increase in viability of cells treated with 10 and 25 $\mu\text{g}/\text{ml}$ of *T persicum* extract (about 15%) (Figure 1), we conclude that the reduction observed in wound healing of the cell monolayers treated with these two concentrations of *T persicum* extract was probably due to a decrease in cell invasion.

We also tested the effect of *T persicum* extract on PC-3 cell invasion by using gelatin zymography assay. Matrix metalloproteinases are expressed by many invasive cancer cells and are the key proteins involved in cell invasion and metastasis (Stetler-Stevenson, 2001; Deryugina and Quigley, 2006). PC-3 cells are among the cells that express high levels of matrix metalloproteinases including MMP-2 and MMP-9 (Vijayababu et al., 2006) and therefore, gelatin zymography is an appropriate assay to measure the invasion ability of these cells (see the Methods). PC-3 cells were treated with several concentrations of *T persicum* extract for 48 hours and then the media of these cells were examined for the presence of matrix metalloproteinases as described in the Methods. As shown in Figure 3B, treatment of cells with *T persicum* extract significantly

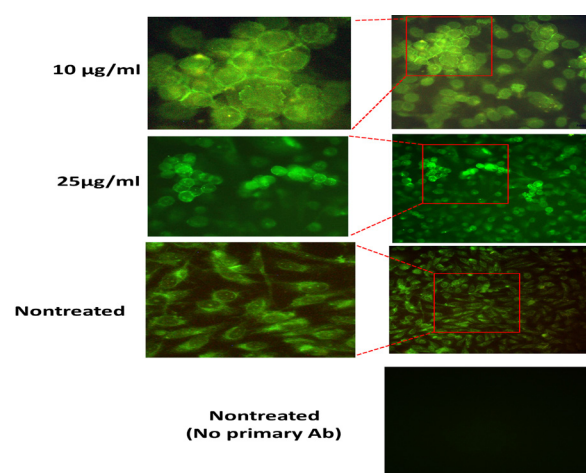


Figure 5. Immunofluorescence Staining of PC-3 Cells with the E-Cadherin Antibody ($\times 400$). PC-3 cells seeded on poly L-lysine coated coverslips were treated with *T persicum* extract (10 and 25 $\mu\text{g}/\text{ml}$) for 48 hours and then used for immunofluorescence microscopy experiments (see the methods). An enlarged section of the right panels is shown on the left. The non-treated panel have only been stained with the FITC-labelled secondary antibody

lowered (50-60%) the protein levels of MMP-2 and MMP-9 in the culture medium. These results indicate that production and (or) secretion of metalloproteinases decrease significantly in the presence of *T persicum* extract, supporting the idea that *T persicum* extract has a negative effect on invasion of PC-3 cells.

Sublethal concentrations of *T persicum* extract induce an epithelial-like phenotype in PC-3 cells

It was interesting to know whether *T persicum* extract had any biological activities at sublethal concentrations (10 and 25 $\mu\text{g/ml}$). First, we simply used a light microscope to compare the morphology of cells treated with either of these two concentrations of *T persicum* extract with that of non-treated cells. As shown in Figure 4, non-treated PC-3 cells have a mesenchymal phenotype and they look round and separated. Interestingly, treatment of cells with 10 or 25 $\mu\text{g/ml}$ of the extract partially led to a mesenchymal-epithelial transition (MET). The population of treated cells had fewer round cells and we could find patches of closely attached epithelial-like cells (Figure 4). This preliminary observation raised the possibility that *T persicum* extract has a negative effect on EMT (Epithelial-Mesenchymal Transition).

EMT is a feature of many advanced human carcinomas and also, is one of the causes of cancer cell invasion and metastasis (Behrens, 1993; Thiery, 2002; Guarino, 2007). E-Cadherin is a critical protein involved in cell adherens junction and maintenance of epithelial tissue integrity (Leckband and Prakasam, 2006; Van Roy and Berx, 2008). Lack of E-Cadherin expression has been reported in various invasive cancers (Stemmler, 2008). Although it has been reported that PC-3 cells are negative for E-Cadherin (Nakamura et al., 2003), the results of our immunofluorescence microscopy and western blotting

experiments showed that in fact, these cells express E-Cadherin, however, the protein does not appear to be localized at the cell membranes and it has a diffuse staining pattern inside the cells (Figure 5B and not shown). A similar observation has been reported by another research group (Kandouz et al., 2010). Therefore, we tested whether the extract of *T persicum* had any effect on E-Cadherin cellular localization. Treatment of PC-3 cells with 10 or 25 $\mu\text{g/ml}$ of *T persicum* extract led to staining of E-Cadherin protein at the cell membrane in a subpopulation of cells, appearing as patches of epithelial cells (Figure 5B). This observation is consistent with the results obtained from our cell invasion assays (Figures 3A and 3B), further suggesting that *T persicum* inhibits PC-3 cell invasion. It is important to emphasize that only a subpopulation of treated cells had epithelial-like phenotype and the rest of the cells appeared to be remained intact. This might be due to the low degree of homogeneity among cells in the monolayer.

β -Catenin is a major binding partner of E-Cadherin in cell-cell adherens junctions (Cavallaro and Christofori, 2004) and therefore, we hypothesized that the pattern of cell membrane localization of β -Catenin in the presence and absence of *T persicum* extract to be similar to that of E-Cadherin. The results of immunofluorescence microscopy supported this hypothesis and we found β -Catenin at cell membrane of the patches of epithelial cells formed in the presence of *T persicum* extract (Figure 6). In non-treated PC-3 cells, β -Catenin protein was mainly cytoplasmic and had a diffuse staining pattern (Figure 6).

Discussion

Human cancers, especially carcinomas are complex and multi-step diseases and therefore their treatment has always been a big challenge. Some plants contain powerful anti-cancer compounds which many of them have been purified and used as drugs for cancer prevention or treatment (Cragg and Newman, 2005). In this work, we studied the anticancer properties of *Teucrium persicum*, an endemic plant of Iran (Monsef-Esfahani et al., 2010). For the study, we chose PC-3 prostate cancer cells which are very invasive, poorly differentiated and have a mesenchymal phenotype (Kandouz et al., 2010; Tai et al. 2011). Therefore, this cell line is an excellent choice to study several anti-cancer features of a candidate compound.

The results of MTT experiments were very interesting to us. The methanolic extract of *Teucrium persicum* appeared to be very potent in decreasing the viability of PC-3 and a couple of other cancer cell lines including SW480 (colon) and T47D (breast) (Figure 1). When we compared the cytotoxic activity of *Teucrium persicum* with that of some known anti-cancer drugs, we found that the IC_{50} value of *Teucrium persicum* extract for PC-3 cells was much lower. The IC_{50} values reported for doxorubicin, imatinib and etoposide against PC-3 cells are 500, 5700 and 1730 $\mu\text{g/ml}$ respectively (Kubler et al., 2005; Dudley et al., 2008; Tsakalozou et al., 2012) which are much higher than that of *Teucrium persicum* extract (142 $\mu\text{g/ml}$).

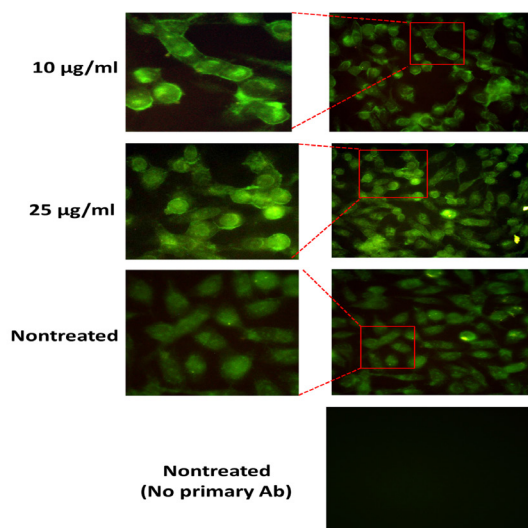


Figure 6. Immunofluorescence Staining of PC-3 Cells with the β -Catenin Antibody ($\times 400$). PC-3 cells were treated with *T persicum* extract (10 and 25 $\mu\text{g/ml}$) for 48 hours and then immunostained for β -Catenin protein as described in the methods. An enlarged section of the right panels is shown on the left. The non-treated cells in the bottom panel have only been stained with the FITC-labelled secondary antibody

There are at least two possibilities for this observation. Either there is a compound in *Teucrium persicum* extract which is very toxic to PC-3 cells, or there are two or more compounds in the extract which work synergistically to kill PC-3 cells. Laboratory results have supported both possibilities. For example, it has been reported that *Teucrium* species are rich in phenolic compounds like flavonoids (Cakir et al., 2006; Ljubuncic et al., 2006; Stankovic et al., 2011). Flavonoids have several anti-cancer activities including carcinogen inactivation, induction of apoptosis, antioxidation, and reversal of the drug resistance (Chahar et al., 2011). It has also been reported that some phytochemicals work synergistically to block cancer cell growth. For example it has been shown that quercetin (a dietary bioflavonoid) synergizes with epigallocatechin gallate (EGCG) to block prostate cancer stem cell invasion, migration and epithelial-mesenchymal transition (Tang et al., 2010). The synergistic effect is an important issue because it can minimize the toxicity and side effects of a single compound.

The results of flow cytometry and DNA laddering experiments showed that the reduction of viability of PC-3 cells by *T persicum* extract was due to induction of apoptosis. Reduction of cell viability and (or) induction of apoptosis started at concentration of 50 µg/ml of *T persicum* extract and continued in a dose dependent manner (Figure 1). Interestingly at two lower concentrations of the extract (10 and 25 µg/ml), not only cell viability was not reduced but also we observed a slight increase (up to 15%) in the number of viable cells. Although this increase was not statistically significant, it was reproducibly observed.

Although, sublethal concentrations of *T persicum* extract (10 and 25 µg/ml) did not reduce cell viability, treatment of PC-3 cells with these concentrations of the extract resulted in a significant decrease (about 50%) in wound healing of the cell sheets. This observation raised the possibility that *T persicum* extract reduces PC-3 cell invasion. This possibility was very well supported by the results of Gelatin zymography experiments which showed that treatment of PC-3 cells with *T persicum* extract significantly reduces the production and (or) secretion of at least two matrix metalloproteinases (MMPs), MMP-2 and MMP-9 (Figure 3). These enzymes are gelatinases which belong to matrix metalloproteinase family and are involved in ECM proteolytic hydrolysis, cell migration and cancer cell invasion (Roomi et al., 2009). It has been shown that blockade of MMP-2 and MMP-9 expression inhibits invasion of metastatic prostate cancer cells (Deryugina and Quigley, 2006). Collectively, our results show that *T persicum* extract has anti-invasion activity against PC-3 cancer cells.

The anti-cell invasion activity of *T persicum* extract was also supported by the results of immunofluorescence microscopy experiments with E-Cadherin and β-Catenin antibodies. *T persicum* extract induced an epithelial-like phenotype in PC-3 cells together with cell membrane localization of E-Cadherin and β-Catenin proteins (Figure 5 and Figure 6). PC-3 prostate cancer cells have a mesenchymal phenotype and are very invasive and produce tumors in laboratory animals (Zhou et al., 2009;

Kandouz et al., 2010; Zhang and Waxman, 2010; Tai et al., 2011). Our results showed that in these cells, both E-Cadherin and β-Catenin proteins were cytoplasmic and cells having either protein at the cell membrane were extremely rare (Figures 5 and 6). Treatment of PC-3 cells with 10 or 25 µg/ml of *T persicum* extract (sublethal concentrations) led to appearance of several patches of closely attached epithelial-like cells having E-Cadherin and β-Catenin at the cell membrane, an observation similar to a very important biological process called MET (mesenchymal-epithelial transition) (Thiery, 2002; Guarino, 2007). Epithelial-mesenchymal transition (EMT) is a feature of many advanced human carcinomas and results in cancer cell invasion and metastasis (Thiery, 2002; Guarino, 2007). Loss of epithelial tissue integrity due to downregulation of E-Cadherin is the main feature of EMT (Thiery, 2002; Guarino, 2007; Baum and Georgiou, 2011). Therefore, any therapeutic strategy to reverse EMT can be beneficial to cancer patients. The ability of *T persicum* extract to enhance membrane localization of E-Cadherin and β-Catenin represents a valuable anti-cancer activity of this plant. It is worth mentioning that *T persicum* extract led to membrane localization of E-Cadherin and β-Catenin only in a subpopulation of cells (less than 20%) and the rest of the cells had a phenotype similar to that of non-treated cells. More experiments should be performed to find the reason for this difference in cell response.

β-Catenin is a dual function protein that in addition to its role as a component of cell adherens junction, intracellular and intranuclear localization of this protein are oncogenic and may help tumor initiation and progression (Cavallaro and Christofori, 2004; Salmanian et al., 2010). The oncogenic role of β-Catenin, which is believed to be due to upregulation of the canonical Wnt signaling pathway, has been very well studied in colon cancer and some other human malignancies (Polakis, 2000; Salmanian et al., 2010).

Collectively the results of this paper present a new species of *Teucrium* genus with valuable anti-cancer activities. Definitely more experiments are needed to find out the molecular mechanisms of the biological properties of *T persicum*. In addition chemical analysis of *T persicum* extract is necessary to search for the active component(s).

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References

- Autore G, Capasso F, De Fusco R, et al (1984). Antipyretic and antibacterial actions of *Teucrium polium* (L). *Pharmacol Res Commun*, **16**, 21-9.
- Baum B, Georgiou M (2011). Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *J Cell Biol*, **192**, 907-17.
- Behrens J (1993). The role of cell adhesion molecules in

- cancer invasion and metastasis. *Breast Cancer Res Treat*, **24**, 175-84.
- Cakir A, Mavi A, Kazaz C, et al (2006). Antioxidant Activities of the Extracts and Components of *Teucrium orientale* L. var. *orientale*. *Turk J Chem*, **30**, 483-94.
- Cavallaro U, Christofori G (2004). Cell Adhesion and Signalling by Cadherins and IG-Cams in Cancer. *Nat Rev Cancer*, **4**, 118-32.
- Chahar MK, Sharma N, Dobhal MP, et al (2011). Flavonoids: A versatile source of anticancer drugs. *Pharmacogn Rev*, **5**, 1-12.
- Cragg GM, Newman DJ (2005). Plants as a source of anti-cancer agents. *J Ethnopharmacol*, **100**, 72-9.
- Deryugina E, Quigley JP (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev*, **25**, 9-34.
- Dudley AC, Shih S-C, Cliffe AR, et al (2008). Attenuated p53 activation in tumour-associated stromal cells accompanies decreased sensitivity to etoposide and vincristine. *Bri J Cancer*, **99**, 118-25.
- Fabricant DS, Fransworth NR (2001). The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect*, **109**, 69-75.
- Fukuta K, Kohri K, Fukuda H, et al (2008). Induction of multinucleated cells and apoptosis in the PC-3 prostate cancer cell line by low concentrations of polyethylene glycol 1000. *Cancer Sci*, **99**, 1055-62.
- Guarino M (2007). Epithelial-mesenchymal transition and tumour invasion. *Int J Biochemistry Cell Biol*, **39**, 2153-60.
- Kadifkova Panovska T, Kulevanova S, Stefova M (2005). In vitro antioxidant activity of some *Teucrium* species (*Lamiaceae*). *Acta Pharm*, **55**, 207-14.
- Kandouz M, Alachkar A, Zhang L, et al (2010). *Teucrium polium* plant extract inhibits cell invasion and motility of human prostate cancer cells via the restoration of the E-Cadherin/catenin complex. *J Ethnopharmacol*, **129**, 410-5.
- Kubler H, van Randenborgh H, Treiber U, et al (2005). *In vitro* cytotoxic effects of imatinib in combination with anticancer drugs in human prostate cancer cell lines. *The Prostate*, **63**, 385-94.
- Leckband D, Prakasam A (2006). Mechanism and dynamics of cadherin adhesion. *Annu Rev Biomed Eng*, **8**, 259-87.
- Mazhar D, Waxman J (2002). Prostate cancer. *Postgrad Med J*, **78**, 590-5.
- Miri A, Monsef-Esfahani HR, Amini M, et al (2012). Comparative chemical composition and antioxidant properties of the essential oils and aromatic water from *Teucrium persicum* boiss. *Iran J Pharm Res*, **11**, 573-81.
- Moghtader M (2009). Chemical composition of essential oil of *Teucrium polium* L. from Iran. *American-Eurasian J Agric Environ Sci*, **5**, 843-6.
- Monsef-Esfahani HR, Miri A, Amini M, et al (2010). Seasonal variation in the chemical composition, antioxidant activity and total phenolic content of *Teucrium persicum* Boiss. Essential oils. *Res J Biological Sciences*, **5**, 492-8.
- Nakamura T, Kato Y, Fuji H, et al (2003). E-Cadherin-dependent intercellular adhesion enhances chemoresistance. *Int J Mol Med*, **12**, 693-700.
- Nirmala MJ, Samundeeswari A, Deepa Sankar P, (2011). Natural plant resources in anti-cancer therapy-A review. *Res in Plant Biology*, **1**, 1-14.
- Nyska A, Suttie A, Bakshi S, et al (2003). Slowing tumorigenic progression in TRAMP mice and prostatic carcinoma cell lines using natural anti-oxidant from Spinach, NAO—A comparative study of three anti-oxidants. *Toxicol Pathol*, **31**, 39-51.
- Polakis P (2000). Wnt signaling and cancer. *Genes & Development*, **14**, 1837-51.
- Ljubuncic P, Dakwar S, Portnaya I, et al (2006). Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. *eCAM*, **3**, 329-38.
- Roomi MW, Monterrey JC, Kalinovskiy T, et al (2009). Patterns of MMP-2 and MMP-9 expression in human cancer cell lines. *Oncology Reports*, **21**, 1323-33.
- Salido M, Larran J, Lopez A, et al (1999). Etoposide sensitivity of human prostatic cancer cell lines PC-3, DU 145 and LNCaP. *Histol Histopathol*, **14**, 125-34.
- Salmanian S, Najafi SMA, Rafipour M, et al (2010). Regulation of GSK-3 β and β -Catenin by Gaq in HEK293T cells. *Biochem Biophys Res Commun*, **14**, 577-82.
- Schulz WA, Burchardt M, Cronauer MV (2003). Molecular biology of prostate cancer. *Mol Hum Reprod*, **9**, 437-48.
- Sharma G, Tyagi AK, Singh RP et al (2004). Synergistic anti-cancer effects of grape seed extract and conventional cytotoxic agent doxorubicin against human breast carcinoma cells. *Breast Cancer Res and Treatment*, **85**, 1-12.
- Stankovic MS, Curcic MG, Zizic JB, et al (2011). *Teucrium* plant species as natural sources of novel anticancer compounds: antiproliferative, proapoptotic and antioxidant properties. *Int J Mol Sci*, **12**, 4190-205.
- Stemmler M, (2008). Cadherins in development and cancer. *Molecular Biosystems*, **4**, 835-50.
- Stetler-Stevenson WG (2001). The role of matrix metalloproteinases in tumor invasion, metastasis, and angiogenesis. *Surg Oncol Clin N Am*, **10**, 383-92.
- Sun W, Guo MM, Han P, et al (2012). Id-1 and the p65 subunit of NF- κ B promote migration of nasopharyngeal carcinoma cells and are correlated with poor prognosis. *Carcinogenesis*, **33**, 810-7.
- Tai S, Sun Y, Squires JM, et al (2011). PC3 Is a Cell line characteristic of prostatic small cell carcinoma. *Prostate*, **71**, 1668-79.
- Tang SN, Singh C, Nall D, et al (2010). The dietary bioflavonoid quercetin synergizes with epigallocatechin gallate (EGCG) to inhibit prostate cancer stem cell characteristics, invasion, migration and epithelial-mesenchymal transition. *J Mol Signal*, **5**, 1-15.
- Tariq M, Ageel A, Al-Yahya MA, et al (1989). Anti-inflammatory activity of *Teucrium polium*. *Int J Tissue Reaction*, **11**, 185-8.
- Thiery JP (2002). Epithelial-mesenchymal transitions in tumour progression. *Nature Rev Cancer*, **2**, 442-54.
- Tsakalozou E, Eckman AM, Bae Y (2012). Combination effects of docetaxel and doxorubicin in hormone-refractory prostate cancer cells. *Biochemistry Res Int*, **2012**, 1-10.
- Twaij HAA, Albadr AA, Abul-Khail A (1987). Anti-ulcer activity of *Teucrium polium*. *Pharmaceutical Biology*, **25**, 125-8.
- Van Roy F, Berx G (2008). The cell-cell adhesion molecule E-Cadherin. *Cellular and Molecular Life Sciences*, **65**, 3756-88.
- Vijayababu MR, Arunkumar A, Kanagaraj P, et al (2006). Quercetin downregulates matrix metalloproteinases 2 and 9 proteins expression in prostate cancer cells (PC-3). *Molecular and Cellular Biochemistry*, **287**, 109-16.
- Zahm JM, Kaplan H, Herard AL, et al (1997). Cell migration and proliferation during the *in vitro* wound repair of the respiratory epithelium. *Cell Motil Cytoskeleton*, **37**, 33-43.
- Zhang K, Waxman DJ (2010). PC3 prostate tumor-initiating cells with molecular profile FAM65Bhigh/MF12low/LEF1low increase tumor angiogenesis. *Mol Cancer*, **9**, 1-13.
- Zhou Q, Yan B, Hu X, et al (2009). Luteolin inhibits invasion of prostate cancer PC3 cells through E-Cadherin. *Mol Cancer Ther*, **8**, 1684-91.