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Original research

Anti-tumor activity of *Teucrium polium* extracts in MCF-7 breast cancer cells: A comparison with Cisplatin

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ABSTRACT

Breast cancer is one of the most common and lethal cancers affecting women, with lung cancer being the only type with higher mortality rates. Among the challenges in treating breast cancer are tumor recurrence and resistance to existing therapies, highlighting the urgent need for novel therapeutic strategies that offer enhanced efficacy and reduced systemic toxicity. In this context, plant-derived compounds have attracted considerable attention for their roles in cancer prevention and treatment. This study focuses on the cytotoxic effects of *Teucrium polium* ethanolic extract on human breast cancer MCF-7 cells and the expression levels of key survival-related genes, including Bcl-2 and K-Ras. The MCF-7 cells were treated with varying concentrations of the extract for 48 hours, and cell viability was assessed via the MTT assay. The results demonstrated a significant decrease in cell viability, dropping to approximately 54%, 48%, and 35% after treatment with 2.5, 5, and 10 µg/mL of the extract, respectively. Moreover, the expression of Bcl-2 and K-Ras genes was reduced by approximately 37% and 13%, respectively, compared to the control group. In a comparative analysis, fibroblast viability in response to 20 µg/mL cisplatin was around 48%, while exposure to 20 µg/mL of *Teucrium polium* extract resulted in a viability of 76%. These findings suggest that *Teucrium polium* ethanolic extract exhibits substantial cytotoxic and anti-proliferative effects against MCF-7 cells, potentially through its impact on the expression of Bcl-2 and K-Ras, which are critical in regulating cancer cell proliferation and survival.

Keywords: Breast cancer; *Teucrium polium*; MCF-7 cells; Bcl-2; K-Ras.

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1. Introduction

Although there are advances in diagnosis and therapy of breast cancer, it is still the main cause of cancer-related death in women. A deeper understanding of the molecular mechanisms involved in the progression of breast cancer, and the identification of new active compounds may aid in the development of more effective treatments for this disease (Moo et al., 2018). There is no ideal anticancer drug, which is cytotoxic to cancer cells but has limited side effects on

Abbreviations: GC/MS: Gas Chromatography-Mass Spectrometry; HUVEC: Human umbilical vein endothelial cells; MTT: 4,5-dimethyl-thiazol-2-yl)2,5-diphenyl-tetrazolium bromide; TP: *Teucrium polium*.

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normal cells. Thus, the discovery of such compounds requires the reevaluation of previously known anticancer drugs. Furthermore, cancer cells that are genetically modified by the introduction of oncogenic molecules could be helpful in identification of new anticancer drugs (Oh et al., 2007).

Many studies on the pathophysiology of breast cancer indicate the relevance of several oncogenes and tumor suppressor genes for their carcinogenic potential. Bcl-2 is an anti-apoptotic, prosurvival gene that is an important prognostic marker for breast tumors. Aberrant regulation of Bcl-2 expression is also a component of the molecular assays for recurrence of breast cancer (Merino et al., 2016). In addition, the K-ras oncogene is frequently mutated in a variety of human cancers including breast cancer. Missense mutations occur at codons 12, 13, and 61, which encode a constitutively hyperactive form of Ras (Konish et al., 2007).

Phytochemicals, or their active constituents from plants have been widely investigated for discovery of anti-cancer agents with minimum or no side effects. In this regard, plants are known to possess important anti-cancer properties. Recently, there is a great interest in the usage of phytochemicals for cancer treatment (Emami Zeydi, 2016). We recently reported that ginger extracts can reduce the viability of MCF-7 breast cancer cells (Afshin et al.,2022). White tea and rosemary extracts show significant anticancer activity against MDA-MB-231 triple-negative breast cancer cells (Raad et al., 2024). The *Teucrium polium* (TP), as a wild growing flowering plant, is used in folk medicine for treating many diseases such as abdominal pain, indigestion, diabetes, and urogenital disease (Asadi-Samani et al., 2017). Previous studies reported that TP plant extract has strong anti-cancer activity against prostate cancer (Kandouz et al., 2010) and lung carcinoma cells (Haïdara et al., 2011). Furthermore, TP has shown remarkable anticancer activity in a preclinical rat model of hepatocellular carcinoma (Movahedi et al., 2014). Here we investigated the cytotoxic effects of TP extracts on breast cancer MCF-7 cells. Furthermore, the expression of Bcl-2 and K-Ras were evaluated in order to gain insight into the growth regulatory pathways impacted by TP extracts.

2. Material and Methods

2.1. Preparation of TP extracts

The dried aerial parts of TP were purchased from herbalists in Birjand and were authenticated by the herbarium of the Faculty of Agriculture, University of Zabol, Zabol, Iran. The dried aerial parts (200 g) of TP were grounded into fine powder, and extracted with 70% ethanol, acetone, and methanol by maceration at 55 °C for 48 h. Following incubation, the TP extracts were concentrated in a rotary evaporator under vacuum and lyophilized using a freeze drier. The lyophilized extracts were stored in the refrigerator at 4 °C to prevent any decomposition of the components. Suitable concentrations of the extracts (2.5, 5 and 10 µg/mL) were prepared by dilution of the stocks with dimethyl sulfoxide (DMSO).

2.2. Cell culture

A human breast cancer cell line (MCF-7) and normal fibroblasts were obtained from the Iranian Biological Resources Center (Tehran, Iran). Cells were cultured in RPMI-1640 culture medium containing 10% fetal bovine serum (FBS), penicillin (100U/mL) and streptomycin (100 µg/mL) (Sigma, Germany). Cells were grown at 37 ºC in a humidified atmosphere containing 5% CO_2.

2.3. Cytotoxicity assays

The cytotoxic activity of TP extracts on MCF-7 cells was measured using MTT assay (Behbahani, 2014). Briefly, cancer cells (5×10^3) were cultured in 96 well plates overnight. The next day, cells were incubated with various concentrations of TP extracts (2.5, 5 and 10 µg/mL) and cisplatin (20 µg/µL) as a chemotherapeutic drug and positive control. Untreated cells incubated with vehicle were used as the solvent control group. After incubations for 48 h, 100 µL of MTT solution (7 mg/mL) was added to each well. After incubation at 37 ºC for 2 h, the MTT solution was removed and formazan crystals were solubilized by addition of 100 µL of DMSO to each well. Thirty minutes after incubation at room temperature, absorbance at 570 nm was measured using a plate reader (Awareness Technology, USA).

2.4. Real time-PCR (qRT-PCR)

Expression levels of Bcl-2 and K-Ras were analyzed using RT-PCR assays as described previously (Bong et al., 2006; Suzuki et al., 1999). Total RNA was prepared from the untreated and treated MCF-7 cells using an RNX PLUS kit (Cinnagen, Iran) according to the manufacturer instructions. The RNA was then transcribed to cDNA using a reverse transcriptase kit (Promega, USA). RT-PCR was performed using the Corbett Rotor-Gene 6000 system (Corbett Life Science, Australia). The primers are shown in Table 1. Cycling conditions were as follow: initial denaturation step at 95 ºC for 3 min followed by 45 cycles at 95 ºC for 10 seconds, 58 ºC for 30 seconds, and 72 ºC for 20 seconds. A negative control was included in each run to access specificity of primers and possible contamination.

Table 1. The primers used in Real time-PCR assay.

Genes	Sequence of Primer (5´ to 3´)	Tm	GC%
K-Ras	F: TTCCTCAGGGCTCAAGAGAA	51.8	50
	R: ATTGGGCAGCAAAGAGATGT	49.7	45
Bcl-2	F: GTCTGGGAATCGATCTGGAA	51.8	50
	R: CATAAGGCAACGATCCCATC	51.8	50
HPRT	F: TATGGCGACCCGCAGCCCT	57.6	68
	R: CATCTCGAGCAAGACGTTCAG	54.5	52

2.5. GC/MS Analysis of TP extracts

Experiments were carried out using a Varian 3800 gas chromatograph (Walnut Creek, California, USA) equipped with a DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 µm) and a Saturn 2200 mass spectrometer. The injector temperature was set to 250 °C, and the oven temperature was programmed from 60 °C to 280 °C at a rate of 10 °C/min. Helium was used as the carrier gas with a flow rate of 1 mL/min. A sample volume of 2 µL was injected in split mode with a split ratio of 1:50. The sample components were identified by matching their mass spectra with those in the library or with pure standard components, and their identities were confirmed by their GC retention times.

2.6. Statistical Analysis

All data were expressed as mean ± SD. Data were analyzed by SPSS statistical software (version 21 SPSS Inc.). One-way analysis of variance (ANOVA) followed by a Tukey post hoc test was

performed to evaluate significant differences among different groups. Significant difference was set at $P < 0.05$.

3. Results and Discussion

3.1. Effects of TP extracts on cell viability

Cytotoxic effects of different ethanolic, acetone, and methanolic TP extracts on cell viability were assessed using an MTT assay. Fig. 1 shows that the TP ethanolic and methanolic extracts were more cytotoxic compared with TP acetonic extract after 48 h ($P < 0.05$).

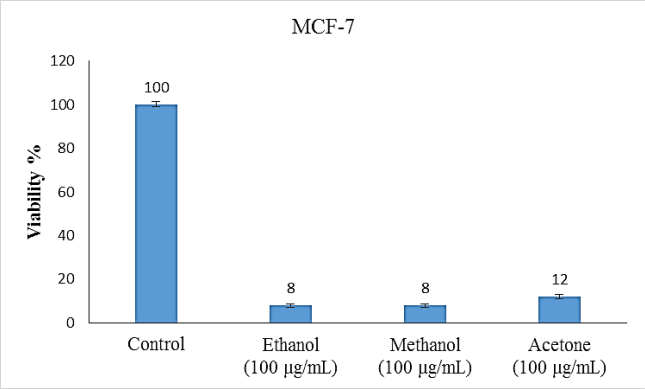

Fig 1. Effects of different TP ethanolic, methanolic and acetonic extracts on cell viability of MCF-7 cells after 48 h in MTT test (P < 0.05).

3.2. Effects of TP extract treatment on morphology of MCF7 cells

Fig. 2 shows the results of MCF-7 cell morphological analysis. These results revealed that incubation of MCF7 cells with TP ethanolic extract for 48 h caused significant morphological changes compared with untreated cells.

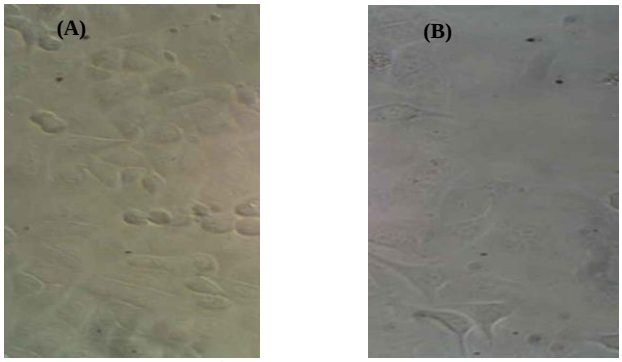

Fig 2. Cell morphology of MCF7 cell line with TP ethanolic extract treatment for 48 h (A) and without treatment (B).

3.3. Effects of different concentrations of TP ethanolic extract on cell viability

Different concentrations of TP ethanolic extract (2.5, 5 and 10 µg/mL) were tested for cytoxicity against MCF-7 cells. TP ethanolic extract showed dose-dependent cytotoxic activity on MCF-7 cells. A significant difference in cell viability between MCF-7 cells incubated with different concentrations of TP ethanolic extracts and untreated cells in MTT assays (Fig. 3; P < 0.05).

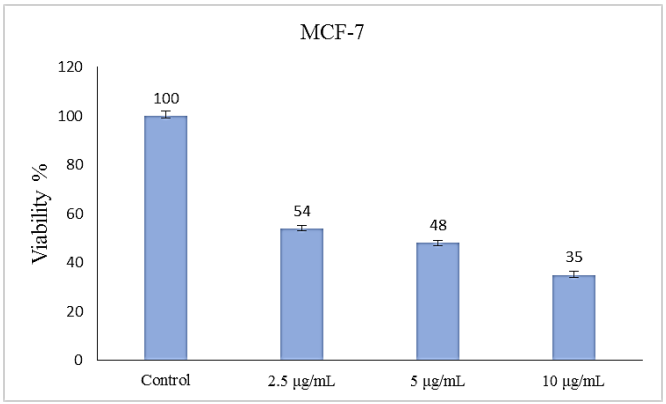

Fig 3. Effects of TP ethanolic extracts at different concentrations on cell viability of MCF7 cells after 48 h in MTT test ($P < 0.05$).

3.4. Comparison of the effects of TP ethanolic extract and cisplatin on cell viability

Fig. 4 indicates the cytotoxic effects of TP ethanolic extract (2.5, 5 and 10 µg/mL) and cisplatin (20 µg/mL) on MCF7 cells and normal fibroblasts in MTT test. TP ethanolic extract at different concentrations showed similar cytotoxic effects compared with cisplatin on normal fibroblasts (Fig. 4, P < 0.05).

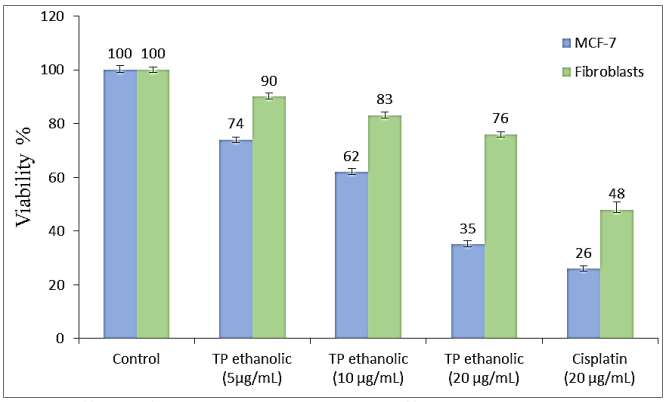

Fig 4. Effects of TP ethanolic extracts at different concentrations versus cisplatin (20 µg/mL) on cell viability of MCF7 cells and normal fibroblasts after 48 h in MTT test ($P < 0.05$).

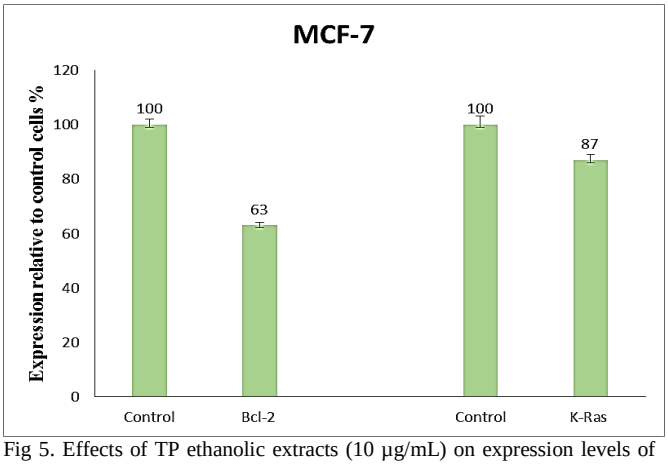

Fig 5. Effects of TP ethanolic extracts (10 µg/mL) on expression levels of Bcl-2 and K-Ras genes in MCF7 cells ($P < 0.05$).

Table 2. The chemical composition of TP ethanolic extract

No.	Compound	RI	percentage
1	α –Pinene	934	0.69
2	β-Pinene	977	22.37
3	β-Myrcene	989	5.41
4	Limonene	1030	2.48
5	β-Caryophyllene	1430	5.29
6	Farnesene	1445	2.57
7	α-Humulene	1460	3.34
8	Bicyclogermacrene	1500	50.80
9	β-Bisabolene	1509	1.35
10	*δ*-Cadinene	1524	2.69
11	(E)-γ- Bisabolene	1544	0.68
12	E Lemicine	1557	0.83
13	Germacrene B	1559	0.65
14	Spathulenol	1579	0.20
15	Guaiol	1585	0.33
16	α-Cadinol	1661	0.29
	Total		99.98

3.5. Effects of TP ethanolic extract on the expression of Bcl-2 and K-Ras

The expression levels of Bcl-2 and K-Ras genes in MCF7 cells incubated with TP ethanolic extract (10 µg/mL) were determined by Real-time PCR analysis. Fig. 5 shows that the expression level of these genes decreased in MCF-7 cells incubated with TP ethanolic extract for 48 h compared with control cells ($P < 0.05$).

3.6. Chemical composition of TP extract

The TP extract was analyzed and characterized using the GC/MS technique. The identification process was made by comparing their mass spectra retention indices with those given in the literature and authentic samples. Sixteen constituents were identified, and their qualitative and quantitative composition is presented in Table 2. The GC/MS analyses showed that the extract was mainly represented by β-pinene (22.37%), bicyclogermacrene (50.80%), β-myrcene (5.41%), and β-caryophyllene (5.29%).

3.7. Discussion

This study reports the cytotoxic effect of TP extracts, a medicinal plant, on MCF-7 cells for the first time. Nature is an enriched and reliable source for treatments of many diseases. More than half of the FDA-approved medications in the United States are derived by natural products, highlighting the significant role of plant extracts and their constituents in modern medicine for treating various diseases (Verma et al., 2024). The evolution of pharmacognosy has expanded the focus from solely plant-based products to include compounds from fungi and marine organisms (Badal et al., 2024). Natural products provide a diverse chemical structure that aids in the development of new therapeutic agents, particularly in cancer treatment (Chaachouay & Zidane, 2024). TP has been used for over 2000 years in folk medicine due to pharmacological properties including diuretic, diaphoretic, anti-pyretic and anti-spasmodic effects (Ljubuncic et al., 2006). TP contains flavonoids, terpenoids, and essential oils, which are responsible for its therapeutic properties (Aljaber et al., 2024).

Previous studies reported that TP extracts have strong anti-cancer activity. For example, aqueous extracts of TP significantly decreased cell invasion and metastasis of human prostate cancer cells (Kandouz et al., 2010). Another in vitro study indicated that TP plant extracts inhibit cell proliferation and induce a significant cell death in human lung carcinoma cells (Haïdara et al., 2011). The synergistic effects of TP extracts with other conventional chemotherapeutic drugs (vincristine, vinblastine, doxorubicin, and cisplatin) have been reported (Rajabalin, 2008). In addition to the cytotoxic effects of TP extract on different cancer cell lines (Khodaei et al. 2018, Emami Zeydi, 2016), TP has shown remarkable anticancer activity in a rat model of hepatocellular carcinogenesis (Movahedi et al., 2014). Thus, the cytotoxic effects of TP ethanolic extracts on MCF-7 cells observed in this study are in accordance with those previously reported anti-cancer effects of TP. These beneficial effects of TP could be explained by the significantly high level of flavonoid compounds and antioxidant competency of TP. The identification of the active compounds that play major role (s) in producing these effects of TP, and their possible molecular mechanisms of action are subject of future studies.

The expression of apoptosis-related genes has also been evaluated to elucidate the underlying mechanisms involved in the apoptotic properties of TP. Bcl-2 is an antiapoptotic, prosurvival gene that can inhibit cell death, and its expression is also recognized as a molecular marker for risk of breast cancer recurrence (Merino et al., 2016). In this study, we found that the expression level of Bcl-2 was decreased in cells incubated with TP ethanolic extract compared with untreated cells. The effect of TP ethanolic extract on reduced expression of Bcl-2 was recently reported as a molecular mechanism for reduced viability of human umbilical vein endothelial cells (HUVEC) incubated with TP (Sheikhbahaei et al., 2018).

K-Ras is one of the three members of the RAS oncogene family. Mutations in the Ras genes are some of the earliest and most frequent genetic events observed in tumor patients (Wang et al., 2012). The activation of the K-ras pathway has been well-documented in various tumor types, including lung cancer (Santos et al., 1984), pancreatic cancer (Aguirre et al., 2003), colon cancer (Janssen et al., 2006) and breast cancer (Forbes et al., 2006). In this study, we found that incubation of MCF-7 cells with TP ethanolic extract decreased K-Ras expression compared with untreated cells. This decrease in K-Ras expression could contribute to the molecular mechanism involved in the TP anti-cancer effects.

4. Conclusion

Collectively, these results show that TP ethanolic extracts induced cytotoxic effects on MCF-7 breast cancer cells in a dose-dependent manner. This effect is partly attributed to decreased expression of pro-survival genes including Bcl-2 and K-Ras. Further in vivo studies and future clinical trials will help further evaluating the efficacy of TP and/or its constituents as new therapeutic options against breast cancer.

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Authorship contribution statement

M. Bohlooli designed the research plane. S. Zahedi performed experiments. M. Bohlooli, A. Maghsoudi, R. Sheikhnejad, M. Khajeh, M. Ghaffari-Moghaddam, P. Hasanein, A. Khatibi and N. Sheibani analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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