

The Effect of *Dracocephalum kotschy* Alcoholic Extract on the *BCL2* and *BAX* Expression in SKBR3 Cell Line



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ABSTRACT

Background: Breast cancer is the most prevalent malignancy among females worldwide, and its incidence is growing among Iranian women. Also, Iranian patients with breast cancer are younger than their Western counterparts. In this study, we aimed to investigate the effect of *Dracocephalum kotschy* alcoholic extract on cell proliferation and viability, as well as the expression level of *BCL2* and *BAX* genes in breast cancer cell lines. *Dracocephalum kotschy* belongs to the Labiatae family, a wildflower that has long been used in Iranian traditional medicine.

Materials and Methods: In this experimental research, the SKBR3 cell line was selected for treatment with the *Dracocephalum kotschy* alcoholic extract. Cell proliferation and cell viability were evaluated in the presence of different concentrations of the *Dracocephalum kotschy* alcoholic extract (10, 50, 100, and 500 µg/mL) using MTT-method. Additionally, the impact of the *Dracocephalum kotschy* alcoholic extract on the expression of *BCL2* and *BAX* genes was assessed using a quantitative real time-polymerase chain reaction.

Results: The obtained results indicated the anticancer characteristics of *Dracocephalum kotschy* alcoholic extract (10, 50, 100, and 500 µg/mL) on cell proliferation and cell viability. In the presence of *Dracocephalum kotschy* alcoholic extract, the half-maximal inhibitory concentration value of SKBR3 cells was 102.1 at 24 h. Moreover, *Dracocephalum kotschy* alcoholic extract decreased the expression of *BCL2* and increased the expression of *BAX*.

Conclusion: *Dracocephalum kotschy* holds promises for designing anticancer medicines and investigations on breast cancer.

Introduction

A

mong the non-communicable diseases, cancer is a leading cause of death next to cardiovascular diseases. Chemotherapy

is routinely used for cancer treatment. Plant-derived products are applied in several therapeutic methods for cancer. Plants have natural chemicals that may have chemoprotective properties against cancer; therefore, they have great potential to provide novel medications in

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this regard [1]. Breast cancer is highly prevalent among Iranian women. However, little is known about the epidemiological features of breast cancer among Iranian patients [2]. Breast cancer comprises almost one-third of all malignancies among females. Breast cancer is the second cause of cancer mortality after lung cancer; this cancer is the leading cause of death in American women aged 40-55 years [3].

The primary objective of breast cancer follow up is the diagnosis of potentially treatable relapses [4]. In Iran, breast cancer is among the main category of diagnosed cancers among women and is the fifth most common cause for death [5]. Despite advances in cancer treatment over the past decades, there are many deficiencies in this regard. Plant-derived compounds have been a significant source of clinically helpful anti-cancer agents [6]. *Dracocephalum kotschyi*, from Labiatae family, is an endemic plant in Iran; this is one of the pharmaceutical and aromatic herbs that can be found in the mountains [7, 8]. One hundred compounds, such as terpenoids, flavonoids, and alkaloids were extracted and identified from *Dracocephalum kotschyi*. Phenolic compounds, including caffeic acid, chlorogenic acid, phenylpropanoids, and flavonoids in *Dracocephalum* genus are probably accountable for its antioxidant activity.

Moreover, methoxylated flavones, such as apigenin, luteolin, isokaempferid, crisimaritin, penduletin, and xanthomicrol have anticancer effects. Luteolin has numerous biological properties, including anti-inflammatory, antioxidant, and anticancer activities [9]. Anticancer therapeutics act by activating the *BCL2*-regulated cell death pathway. *BCL2* requires *BAX*, or its close relative (*BAK1*) to block the death of mammalian cells [10]. The *BAX* gene has a vital role in regulating apoptosis, which is a member of the *Bcl-2* gene family; it encodes a 21-kDa protein related to *Bcl-2* [11]. Various *Bcl-2* family members are generally over- or under-expressed in human breast cancer. The *Bcl-2* expression has been related to accurate prognosis, while the reduced expression of *BAX* has been associated with poor clinical results in human breast cancer. The comprehensive role of *Bcl-2* family members is in regulating mammary epithelial cell survival is salient to affect the extension of the novel therapeutic outcome of this cancer [12].

The high expression of *Bcl-2* proto-oncogene is observed in 50%-70% of breast cancers. Such overexpression of *Bcl-2* leads to resistance to chemotherapy and radiation therapy [13]. Few studies have been conducted on *Dracocephalum kotschyi*, especially on the effect of this plant on *BCL2* and *BAX* expression in breast cancer.

Therefore, the current study investigated the prepared extractions of *Dracocephalum kotschyi* (0, 10, 50, 100, and 500 $\mu\text{g/mL}$) on *BCL2* and *BAX* expression in breast cancer cell line (SKBR3) and its cell proliferation and cell viability activity.

Materials and Methods

Preparation of *Dracocephalum kotschyi* extract

In this study, the leaves and aerial parts of *Dracocephalum kotschyi* were collected from Semirom City, Isfahan Province, in the central region of Iran. One hundred grams of dried and powdered desired parts of *Dracocephalum kotschyi* was chopped and soaked in 250 mL ethanol. Next, the *Dracocephalum kotschyi* extracts (10, 50, 100, and 500 $\mu\text{g/mL}$) were prepared, similar to our previous study [14].

Cell culture

Briefly, the human breast cancer cell line (SKBR3) was obtained from the Pasteur Institute of Cellular Bank of Iran (Tehran, Iran). The cells were cultured at 37°C, under 5% CO_2 in DMEM (Dulbecco's modified Eagle's medium (Gibco, USA) fully fitted based on the National Cell Bank of Iran (NCBI) suggestion. Cell line culture was treated with the different concentrations of *Dracocephalum kotschyi* extract (0, 10, 50, 100, and 500 $\mu\text{g/mL}$) for 24 h to investigate the effect of *Dracocephalum kotschyi* alcoholic extracts on the SKBR3 cell proliferation and viability as well as *BCL2* and *BAX* expression.

MTT assay

To investigate the impact of *Dracocephalum kotschyi* alcoholic extract on cell proliferation and cell viability of SKBR3, we used MTT assay. SKBR3 cell line was treated with the various concentrations of *Dracocephalum kotschyi* extract (0, 10, 50, 100, and 500 $\mu\text{g/mL}$) for 24 h. Next, MTT dye (Sigma-Aldrich, USA) (dissolved in DMSO) was added to the last concentration of 100 $\mu\text{g/mL}$. The combination was incubated for 4 h at 37°C in a CO_2 incubator. The violet crystal was dissolved in DMSO for 30 min. The absorbance was measured by a spectrophotometer at 540 nm (BioTek, Winooski, VT, USA). All experiments were repeated in three separate trials for 4 times. To determine the half maximal Inhibitory Concentration (IC₅₀), the following formula was applied: Cell proliferation inhibition percentage = mean absorption in extract wells/mean absorption in control wells [15].

RNA extraction and cDNA synthesis

The RNeasy Mini, RNA isolation kit (Qiagen) was used to extract the total RNA from the cells according to the above-mentioned protocol. RNA purity was calculated on the 260/280 nm absorbance ratio after measuring by a NanoDrop spectrometer (Thermo Scientific, Waltham, MA, USA). The synthesis of cDNA was performed by a QuantiTect Reverse Transcription Kit (Qiagen), based on the producer's instruction.

Real time polymerase chain reaction assay

The *BCL2* and *BAX* expression were evaluated using a real-time PCR assay with SYBR Premix Ex Taq II (Takara, Kusatsu, Shiga Prefecture, Japan). Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was selected to normalize mRNA expression levels. In a total volume of 10 μ L, cDNA products were added to a master mix comprising 5 μ L SYBR Premix Ex Taq II (Takara, Kusatsu, Shiga Prefecture, Japan), 0.5 μ L forward primer, 0.5 μ L reverse primer, and 3 μ L DEPC-treated water. The PCR was run at 95° C for 10 min followed by 40 cycles of 95° C for 15 s, 60° C for 10 s, and 72° C for 20 s. The mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method. In each PCR reaction, a negative control was run with no cDNA template. The procedure was repeated three times with cDNA template synthesized from the same RNA. Primers were designed using the National Center for Biotechnology Information (NCBI) Website and Gene Runner software. Table 1 lists the primer sequences applied for real-time PCR.

Statistical analysis

The obtained data were analyzed by Graph Pad Prism (Graph Pad, San Diego, USA). The Kolmogorov–Smirnov test was applied to measure the normality of data. One-way Analysis of Variance (ANOVA) was used to compare the achieved data. Statistical significance was set at the $P < 0.05$. The obtained data were presented as Mean \pm SD.

Results

Cell proliferation and cell viability analysis

The MTT assay was applied to investigate the impact of *Dracocephalum kotschy* alcoholic extract on cell proliferation and cell viability in the SKBR3 cell line after 24 h. In the presence of *Dracocephalum kotschy* alcoholic extract, the IC₅₀ value of SKBR3 cells was 102.1 at 24 h. The findings indicated a statistically significant difference between increasing the concentration of *Dracocephalum kotschy* alcoholic extract and cell proliferation and cell viability after 24 h.

Our data indicated that the *Dracocephalum kotschy* alcoholic extract significantly reduced cell proliferation and cell viability in a dose-dependent manner. By increasing the concentration of the *Dracocephalum kotschy* alcoholic extract (10, 50, 100, and 500 μ g/mL), the cell proliferation rate reduced, compared to the controls (Figure 1). Moreover, by increasing the concentration of the *Dracocephalum kotschy* alcoholic extract (50, 100, and 500 μ g/mL), the cell viability rate decreased. However, there was no significant difference in cell viability rate at the concentration of 10 μ g/mL. Eventually, the most reduction in cell viability was observed at 500 μ g/mL (Figure 2).

BCL2 expression analysis

To explore the effect of *Dracocephalum kotschy* alcoholic extract on *BCL2* gene expression, we investigated the expression level of the *BCL2* gene under the influence of the different concentrations of *Dracocephalum kotschy* alcoholic extract using real-time PCR. The obtained data demonstrated after treatment with the *Dracocephalum kotschy* alcoholic extract the expression of the *BCL2* gene reduced. However, the expression level of *BCL2* at the concentrations of 10 μ g/mL and 50 μ g/mL did not significantly decrease. In contrast, we observed that after treatment with the concentrations of 100 μ g/mL and 500 μ g/mL, the *BCL2* gene expression significantly reduced in a dose-dependent manner. The highest reduction in *BCL2* expression was observed after treatment with 500 μ g/mL of the *Dracocephalum kotschy* alcoholic extract (Figure 3).

Table 1. Primer sequences (5'-3')

Gene	Forward Primer	Reverse Primer	Size (bp)
<i>BCL2</i>	CGGTGGGGTCATGTGTGTG	CGGTTCAAGTACTCAGTCATCC	90
<i>BAX</i>	CGAGAGGTCTTTTCCGAGTG	GTGGGCGTCCCAAAGTAGG	242
<i>GAPDH</i>	CCACTCCTCCACCTTTGACG	CCACCACCTGTTGCTGTAG	107

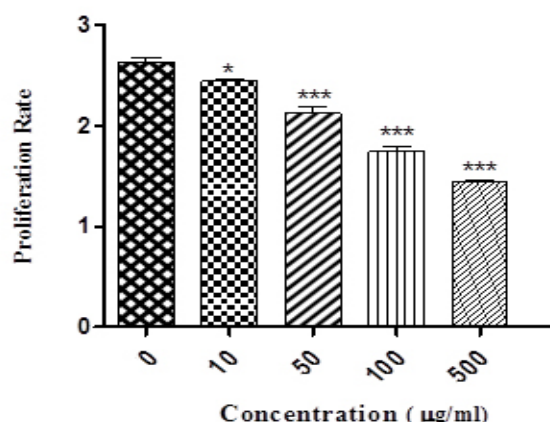


Figure 1. The rate of cell proliferation after treatment with various concentrations of *Dracocephalum kotschy* extract (0, 10, 50, 100, and 500 µg/mL) in SKBR3 cell line after 24 h. Results are presented as Mean±SD (*P<0.05,***P<0.0004)

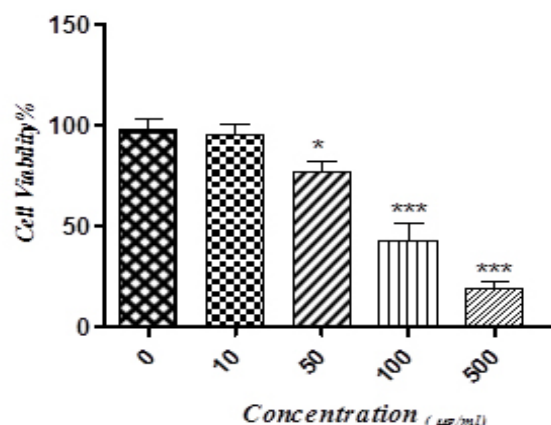


Figure 2. The rate of cell viability after treatment with various concentrations of *Dracocephalum kotschy* extract (0, 10, 50, 100, and 500 µg/mL) in SKBR3 cell line after 24 h. Results are presented as Mean±SD (*: P<0.03, ***: P<0.0005)

BAX expression analysis

The effects of the various concentration of *Dracocephalum kotschy* alcoholic extract on *BAX* expression was examined by real-time PCR method. We observed that *Dracocephalum kotschy* alcoholic extract increased the *BAX* expression in a dose-dependent manner; however, our findings indicated a higher expression in the concentration of 50 µg/mL, compared to the concentration of 100 µg/mL. Additionally, the highest increase in the expression level of *BAX* was observed at 500 µg/mL of the *Dracocephalum kotschy* alcoholic extract (Figure 4).

Discussion

In the current study, we examined the effect of *Dracocephalum kotschy* alcoholic extract at different concentrations (10, 50, 100, and 500 µg/mL) on cell proliferation, cell viability, as well as *BCL2* and *BAX* expression in breast cancer cell line. In this regard, the SKBR3 cell line was selected. This cell line was treated with different concentrations of *Dracocephalum kotschy* alcoholic extract (0, 10, 50, 100, and 500 µg/mL). Moreover, cell proliferation and cell viability were examined by MTT assay. The findings demonstrated a significant decrease in cell proliferation in a dose-dependent manner.

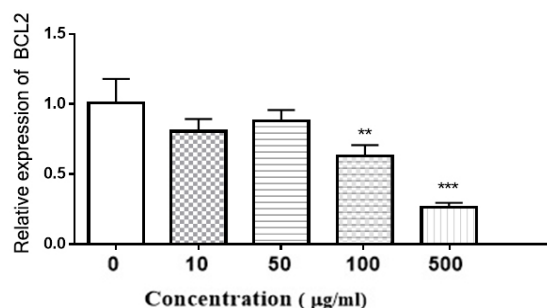


Figure 3. Reduction in *BCL2* expression after treatment with various concentrations of the *Dracocephalum kotschy* extract (0, 10, 50, 100, and 500 µg/mL) in the SKBR3 cell line compared to control (**P<0.004, ***P<0.0002); Results are presented as Mean±SD

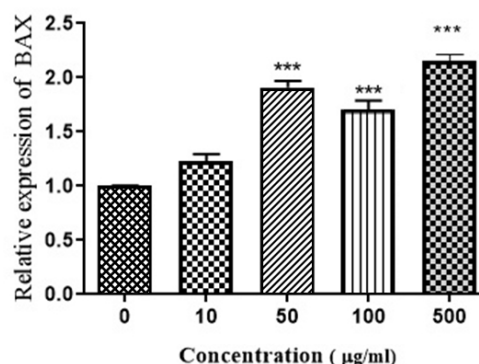


Figure 4. Increase in *BAX* expression after treatment with various concentrations of the *Dracocephalum kotschy* extract (0, 10, 50, 100, and 500 µg/mL) in the SKBR3 cell line compared to control ***P<0.0005; Results are presented as Mean±SD

We also observed a significant reduction in cell viability; however, there was no significant difference at the concentration of 10 µg/mL compared to the controls. Next, the impact of various concentrations of *Dracocephalum kotschy* alcoholic extract on *BCL2* and *BAX* expression in the SKBR3 cell line was evaluated. Our data demonstrated that the *BCL2* expression was reduced under the influence of *Dracocephalum kotschy* alcoholic extract; however, there was no statistically significant difference in the expression of the *BCL2* at the concentrations of 10 and 50 µg/mL.

In contrast, we observed an overexpression of *BAX* expression at the various concentrations of *Dracocephalum kotschy* alcoholic extract. However, the difference was not statistically significant at the concentration of 10 µg/mL. Additionally, the increased expression of *BAX* at the concentration of 50 µg/mL was higher than that of 100 µg/mL. The obtained results suggested that *Dracocephalum kotschy* is a promising candidate for future anticancer research; however, higher concentrations should also be considered, too.

The controlled liberation of medicines to the exact site of the disease using a nanocarrier vehicle increases the remedial efficiency of the drugs. Most cancer-induced deaths are because of drug resistance [16]. *Dracocephalum kotschy* inhibits the multiplication of tumor cells; therefore, it can be used as a new medicine against cancer. Iranian researchers have introduced an anticancer drug (Spinal-Z) extracted from *Dracocephalum kotschy* leaves and *Peganum harmala* seeds, which strongly affect leukemia [17]. Besides, *Dracocephalum kotschy* Boiss has a flavonoid, named xanthomicrol which is helpful in antiproliferative activity against cancer cells. Faghihinia et al. isolated 8 flavonoid aglycones from the aerial parts of *Dracocephalum kotschy*, acknowledged as luteolin, naringenin, apigenin, isokaempferide, cirsimaritin, penduletin, xanthomicrol, and calycopterin. The methoxylated hydroxyflavones (cirsimaritin, penduletin, xanthomicrol, and calycopterin) indicated anti-tumor properties [18].

Sonboli et al. have investigated the cytotoxicity and antioxidant activity of the essential oil of *Dracocephalum surmandinum* in several cell lines. K562, MCF-7, and PC12 cell lines were treated with the various concentrations of *Dracocephalum surmandinum* essential oil and its major constituents (perilla aldehyde and limonene). The cell viability decreased after 48 h by 50% at 16 µg/mL in K562, 14 µg/mL in MCF-7, and >100 µg/mL in PC12 cells [17]. It can separate the *Bcl2* family into two subgroups, including pro-death and anti-death members. Bak and *Bax* have been categorized between the

pro-death members, as the last gateway of cytochrome c release. In contrast, the anti-apoptotic *Bcl-2* members prevent mitochondrial protein liberation by interacting with Bak and *Bax* [19].

The existence of nuclear factor-κB, *Bax*, and *Bcl-2* factors in *Dracocephalum kotschy* is required in research as the associate agents in the infusion of cell cycle arrest and apoptosis using apigenin in human prostate carcinoma cells [20]. Investigating anti-cancerous plant compounds affecting the mechanisms and molecular interactions suggested that several phytochemicals with the anticancer potential from various plants led to *Bax* up-regulation and *Bcl-2* down-regulation [21]. Esmaeili et al. have examined the effect of a flavonoid compound isolated from *Dracocephalum kotschy* (calycopterin) in hepatoblastoma cancer cells. The western bolt analysis has suggested that this flavonoid leads to overexpression of the *Bax* but decreases the level of *Bcl-2* [19].

In conclusion *Dracocephalum kotschy* can be beneficial for preparing new anticancer medicines. The obtained results indicated the promising results of the alcoholic extract of *Dracocephalum kotschy* aerial parts on cell proliferation and cell viability in breast cancer cell line. Moreover, the alcoholic extracts of *Dracocephalum kotschy* down-regulates *BCL2* expression and up-regulates *BAX* expression. More investigations in this area with different types and concentrations of extraction could reveal the anticancer effects of *Dracocephalum kotschy* more.

Ethical Considerations

Compliance with ethical guidelines

There was no ethical considerations to be considered in this research.

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Authors contribution's

Contributing to study design: Mansoor Salehi and Nasrin Hadi; Contributing to all experimental work and molecular experiments, statistical analysis, and interpretation of data: Fatemeh Ketabchi, Faezeh Namazi, Nasrin Hadi, Farinaz Khosravian; Contribution to evaluate the findings: Mansoor Salehi; Mansoor Salehi, Nasrin Hadi, and Farinaz Khosravian; Approving the final manuscript: All authors.

Conflict of interest

The authors declared no conflicts of interest.

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References

- [1] Desai AG, Avni G, Ghulam N, Qazi GN, Ramesh K, Ganju RK, et al. Medicinal plants and cancer chemoprevention. *Cur Drug Metabol.* 2008; 9(7):581-91. [DOI:10.2174/138920008785821657] [PMID] [PMCID]
- [2] Mousavi SM, Montazeri A, Mohagheghi MA, Mousavi Jarrahi A, Harirchi I, Najafi M, et al. Breast cancer in Iran: An epidemiological review. *Breast J.* 2007; 13(4):383-91. [DOI:10.1111/j.1524-4741.2007.00446.x] [PMID]
- [3] Richie RC, Swanson JO. Breast cancer: A review of the literature. *J Insur Med.* 2003; 35(2):85-101.
- [4] Montgomery DA, Krupa K, Cooke TG. Follow-up in breast cancer: does routine clinical examination improve outcome? A systematic review of the literature. *Br J Cancer.* 2007; 97(12):1632-41. [DOI:10.1038/sj.bjc.6604065] [PMID] [PMCID]
- [5] Taghavi A, Fazeli Z, Vahedi M, Baghestani AR, Pourhoseingholi A, Barzegar F, Pourhoseingholi MA. Increased trend of breast cancer mortality in Iran. *Asian Pac J Cancer Prev.* 2012; 13(1):367-70. [DOI:10.7314/APJCP.2012.13.1.367] [PMID]
- [6] Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol.* 2005; 1-2(100):72-9. [DOI:10.1016/j.jep.2005.05.011] [PMID]
- [7] Sani TA, Mohammadpour E, Mohammadi A, Memariani T, Yazdi MV, Rezaee R, et al. Cytotoxic and apoptogenic properties of *Dracocephalum kotschyi* aerial part different fractions on calu-6 and mehr-80 lung cancer cell lines. *Farmacia.* 2017; 65(2):189-99.
- [8] Sonboli A, Mirzania F, Gholipour A. Essential oil composition of *Dracocephalum kotschyi* boiss, from Iran. *Nat prod Res.* 2019; 33(14):2095-8. [DOI:10.1080/14786419.2018.1482550] [PMID]
- [9] Kamali M, Khosroyar S, Kamali H, Ahmadzadeh Sani T, Mohammadi A. Phytochemical screening and evaluation of antioxidant activities of *Dracocephalum kotschyi* and determination of its luteolin content. *Avicenna J Phytomed.* 2016; 6(4):425-33.
- [10] Strasser A, Vaux DL. Viewing *BCL2* and cell death control from an evolutionary perspective. *Cell Death Differ.* 2018; 25(1):13-20. [DOI:10.1038/cdd.2017.145] [PMCID]
- [11] Apte SS, Mattei MG, Olsen BR. Mapping of the human *BAX* gene to chromosome 19q13.3-q13.4 and isolation of a novel alternatively spliced transcript, *BAXδ*. *Genomics.* 2005; 26(3):592-4. [DOI:10.1016/0888-7543(95)80180-T]
- [12] Schorr K, Li M, Krajewski S, Reed JC, Furth PA. *Bcl-2* gene family and related proteins in mammary gland involution and breast cancer. *J Mammary Gland Biol Neoplasia.* 1999; 4(2): 153-164. [DOI:10.1023/A:1018773123899] [PMID]
- [13] Reed JC, Cuddy M, Slabiak T, Croce CM, Nowell PC. Oncogenic potential of *bcl-2* demonstrated by gene transfer. *Nature.* 1988; 336(6196):259. [DOI:10.1038/336259a0] [PMID]
- [14] Moghimi M, Rashidian S, Khosravian F, Hadi N. Basil and *Dracocephalum kotschyi* alcoholic extracts affect *BCL2* expression and HepG2 cell proliferation. *Res Mol Med.* 2019; 7(1):32-42. [DOI:10.18502/rmm.v7i1.5257]
- [15] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol methods.* 1983; 65(1-2):55-63. [DOI:10.1016/0022-1759(83)90303-4]
- [16] Akar U, Chaves-Reyez A, Barria M, Tari A, Sanguino A, Kondo Y, et al. Silencing of *Bcl-2* expression by small interfering RNA induces autophagic cell death in MCF-7 breast cancer cells. *Autophagy.* 2008; 4(5):669-79. [DOI:10.4161/0.6083] [PMID]
- [17] Sonboli A, Esmaili MA, Gholipour A, Kanani MR. Composition, cytotoxicity and antioxidant activity of the essential oil of *Dracocephalum surmandinum* from Iran. *Nat Prod Communi.* 2010; 5(2):341-4. [DOI:10.1177/1934578X1000500234]
- [18] Faghini L, Monajjemi R, Ranjbar M. Cytotoxic and antioxidant effects of methanol, hexane, chloroform and aqueous extracts of *Dracocephalum kotschyi* aerial parts on MDA-MB-231 cell line. *J Biodivers Environ Sci.* 2015; 6(6):334-40.
- [19] Esmaili MA, Moridi Farimani M, Kiaei M. Anticancer effect of calycopterin via PI3K/Akt and MAPK signaling pathways, ROS-mediated pathway and mitochondrial dysfunction in hepatoblastoma cancer (HepG2) cells. *Mol Cell Biochem.* 2014; 397(1-2):17-31. [DOI:10.1007/s11010-014-2166-4] [PMID] [PMCID]
- [20] Heydari P, Yavari M, Adibi P, Asghari G, Ghanadian SM, Dida GO, et al. Medicinal Properties and Active Constituents of *Dracocephalum kotschyi* and Its Significance in Iran: A Systematic Review. *Evid Based Complement Alternat Med.* 2019; 19(2):1-14. [DOI:10.1155/2019/9465309] [PMID] [PMCID]
- [21] Shalini M, Taneja N, Jain S, Singh M. Anticancerous plant compounds affecting the power house of cancerous cells: a possible herbal mitocan. In: Akhtar M, Swamy M, editors. *Anticancer Plants: Mechanisms and Molecular Interactions.* Singapore: Springer; 2018. [DOI:10.1007/978-981-10-8417-1_10]