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Cytotoxic and antioxidant effects of methanol, hexane, chloroform and aqueous extracts of *Dracocephalum kotschyi* aerial parts on MDA-MB-231 cell line

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Abstract

Breast cancer is the most general cancer in women all over the world and constitutes one third of all cancer cases in women. *Dracocephalum kotschyi* is a wild-growing flowering plant belonging to the family *Labiatae* and found abundantly in Iran. This plant has been used in Iran folk medicine as analgesic. In this study, we evaluated the *in vitro* cytotoxic of hexane, aqueous, chloroform and methanolic extracts of *Dracocephalum kotschyi* aerial parts on MDA-MB-231 cell line as a model of breast cancer. Aerial parts of *Dracocephalum kotschyi* collected from Isfahan province and extraction using maceration method. MDA-MB-231 cells were cultured and treated with concentrations of different extracts (100, 250, 500, 1000 µg/ml). Cytotoxicity of *Dracocephalum kotschyi* extracts against MDA-MB-231 cells was estimated by the MTT test method. The absorbance was measured using an ELISA plate reader at 540 nm. Methanolic extract showed the highest cytotoxic effect whereas aqueous extract were least cytotoxic effect among the extracts. Aqueous and methanol extracts showed the antioxidant effect with the IC_{50}=0.0899 and 2.3 µg/ml on MDA-MB-231 cell line. Both extracts exhibited a cytotoxic and antioxidant effect on MDA-MB-231 cell line. Considering the cytotoxic and antioxidant activities of different extracts of *Dracocephalum kotschyi* aerial parts on MDA-MB-231cells, this plant can be considered as a potential candidate for further studies on breast cancer.

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Introduction

Plants have a long history of use in the treatment of diseases. Plants contain a various group of phenolic compounds including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. All the phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants (Reische *et al.,* 1998). Factors inducing the antioxidant activity of plant phenolics include position and degree of hydroxylation, stability of the phenol to food processing operations, polarity, solubility, reducing potential, and stability of the phenolic radical (Decker, 1998).

Nature has showed many effective anticancer agents in common use, some derived from microorganisms such as dactinomycin and doxorubicin, or from plants in the case of vinblastine, irinotecan, topotecan, vincristine, taxanes, etc. (Cordell *et al.,* 1991; Popoca *et al.,* 1998). Uncontrolled proliferation is a general property of tumor cells. Investigation of the cellular growth control mechanism has contributed to the comprehension of carcinogenesis and to the identification of compounds with specific antitumoral activity (Kang *et al.,* 2000).

Breast cancer is the most general malignancy in women, as more than 80% of patients death with breast cancer have skeletal metastases. These skeletal metastases cause harmful complications including indocile bone pain, pathological breaks and hypercalcemia. Nevertheless, the mechanism by which breast cancer preferentially extentions to bone remains unknown (Yoneda *et al.,* 2001).

Dracocephalum is a genus belonging to the Labiatae family and this plant is widely distributed in Iran. These herbaceous plants have been used in traditional medicine for stomach and liver disorders, headache and congestion (Mirheydar, 1995).

One of the important endemic species, *Dracocephalum kotschyi* Boiss., is found in many parts of the Alborz Mountains. Its oil has been used in folk medicine as an antispasmodic agent (Zargari, 2000). There are a few reports on the chemical composition of the oil of *D. kotschyi*, which document remarkable amounts of oxygenated monoterpenes (Golshani *et al*., 2004; Saeidnia *et al.,* 2004).

Saeidnia *et al.,* 2004 in research on collected *Dracocephalum kotschyi* from Tochal Mountain, north of Tehran, Iran, showed that this plant have limonen, geranial, neral, b-sitosterol, oleanolic acid, ursolic acid, *p*-mentha-8-en-1,2-diol, colosolic acid, and limonen.

Thus, this plant in combination with *Peganum harmala* L. as a treatment for many forms of human cancer especially leukemia and gastrointestinal malignancies (Sobhani *et al.,* 2002). In other research, the antitumor activity of *D. kotschyi* extract was determined in an *In vivo* model. Also, a flavonoid identified as xanthomicrol was isolated as an antiproliferative constituent present in its extract with excellent activity towards malignant cells (Jahaniani *et al.,* 2005). Xanthomicrol has been reported to exist in *D. kotschyi* together with a number of other flavonoid compounds (Gohari *et al.,* 2003; Faham *et al.,* 2008) for only two of which, i.e. apigenin and luteolin, significant data exist supporting their antiproliferative activities against various cancer cell lines (Takahashi *et al.,* 1998; Kawaii *et al.,* 1999) .

Considering that few studies have been conducted on *Dracocephalum kotschyi* so far, especially no cytotoxicity and antioxidant activity studies, thus, the object of current study is investigation of different prepared fractions of *Dracocephalum kotschyi* on cell line MDA-MB-231 and their antioxidant activity .

Materials and methods

Plant material and preparation of extract

The aerial parts of *Dracocephalum kotschyi* were collected in summer, 2014 from Isfahan province in

Iran. The plants were identified by Research Institue of Agriculture, Isfahan, Iran. The samples were air-dried in shade at room temperature and then grounded. Extraction was done with 20g of plant dry powder using 200ml 80% Hexane solvent for 48 hours. The mixtures were filtered with whatman filter paper. The residue was further extracted with chloroform, methanol, and water sequentially in a serial manner. Finally, each extract was weighed and the yield was calculated (Yusri *et al.,* 2012). The extracts were stored in the dark kept at 4˚C for further studies.

In vitro cytotoxic activity assay

Cell line MDA-MB-231 was purchased from Pasteur Institute of Iran and transported to cell culture laboratory of Islamic Azad University of Falavarjan. Cells were cultured in RPMI-1640 (PAA-Austria) medium enriched with 10% fetal bovine serum (FBS), (Gibco-Scotland) and Streptomycin (100 µg/ml) and penicillin (100 IU/ml) antibiotics, in an incubator with 5% CO_2, 37°C and humidity 95% (Meshkini and Yazdanparast, 2007). After 5 or 6 times passages, cells went to logarithmic phase.

In order to investigate the aerial parts extract effect of *Dracocephalum kotschyi* on cell death rate, methyl thiazol tetrazolium (MTT) assay was used which is based on breaking tetrazolium yellow salt into purple insoluble crystals in pramosone water via metabolically mitochondrial activity of living cells (Meshkini and Yazdanparast, 2007).

To this purpose, 100 µl cell suspension contained 3×10^4 cells was poured into 96 wells of a microplate and incubated 24 h in mentioned conditions. Afterwards, 20 µl of extract different concentrations (100, 250, 500, 1000 µg/ml) were added to wells. The microplate was then incubated for 48 h and after this time, 180 µl culture medium of each well was exchanged with new medium and 20 µl MTT (Sigma-Germany) was added to each well and incubated for 3 h. Next, 200 µl of the old culture medium contained MTT was exchanged with 150 µl DMSO to dissolve pramosone crystals and absorbance was read in 540 nm by ELISA plate reader (Meshkini and Yazdanparast, 2007). Each concentration of sample was repeated in 3 separate experiments and in each experiment 4 replicates was used. The growth rate in negative control samples considered 100% and doxorubicin was used as positive control. The IC_{50} value reported for each extract was the concentration of the extract that resulted in a 50% inhibition of cell growth.

Determination of antioxidant activity

The antioxidant activity of aerial parts extracts of *Dracocephalum kotschyi* were evaluated by the DPPH method as described in the literature (Shirzad *et al.,* 2011). The stable 1,1-diphenyl-2-picryl hydrazyt radical (DPPH) was used for determination of free radical scavenging activity of the extracts. Different concentrations of each extract was added, at an equal volume, to methanolic solution of DPPH (100 µM). After 30 min at room temperature, the absorbance was recorded at 517nm. The experiment was repeated for three times. BHT were used as standard control.

Statistical analysis

Statistical analysis was carried out by SPSS-20 software and ANOVA test were carried out. Charts were drawn by Microsoft Excel 2007.

Results

Comparative studies, cytotoxic effects of hexane, chloroform, methanol and aqueous extracts showed that all 4 extracts have cytotoxic effect on MDA-MB-231 cells ($p<0.05$)(Fig. 1). Methanolic extract showed the most killed effective on the cells (Fig. 1).

According to figure 1, methanol extract had the highest cytotoxic effect among the other extracts and aqueous extract had the lowest effect, and hexane and chloroform extracts after methanol extract had the highest effects respectively.

Figure 2 shows the amount of each extract needed for 50% inhibition (IC_{50}) free radicles. IC_{50} of the

standard compounds, BHT were 0.384 µg/ml. The highest radical scavenging activity was showed by methanolic extract with IC_{50}=0.0899 µg/ml.

Discussion

We have found that four of the extract (hexane, methanol, chloroform, aqueous) assayed present different cytotoxic activity against the breast cancer line MDA-MB - 231, in the in vitro screening (Figure 1). The four of different extract may have of antitumoral activity. Nevertheless, methanolic extract had the greatest activity, this value being within the concentration limit required for further purification (Zee-Cheng, 1997). Flavonoids are known to be present in *Dracocephalum kotschyi* (Moghaddam *et al.* 2012). The breast carcinoma is considered to be one of the most common malignant tumors, Therefore, the search for new drugs is imperative and the results of our investigation call for future isolation and characterization of the active constituents by bioguided assay. The our investigations showed that *Dracocephalum kotschyi* aerial parts have antioxidant effects and methanol extract of this plant showed highest antioxidant activity (Figure 2).

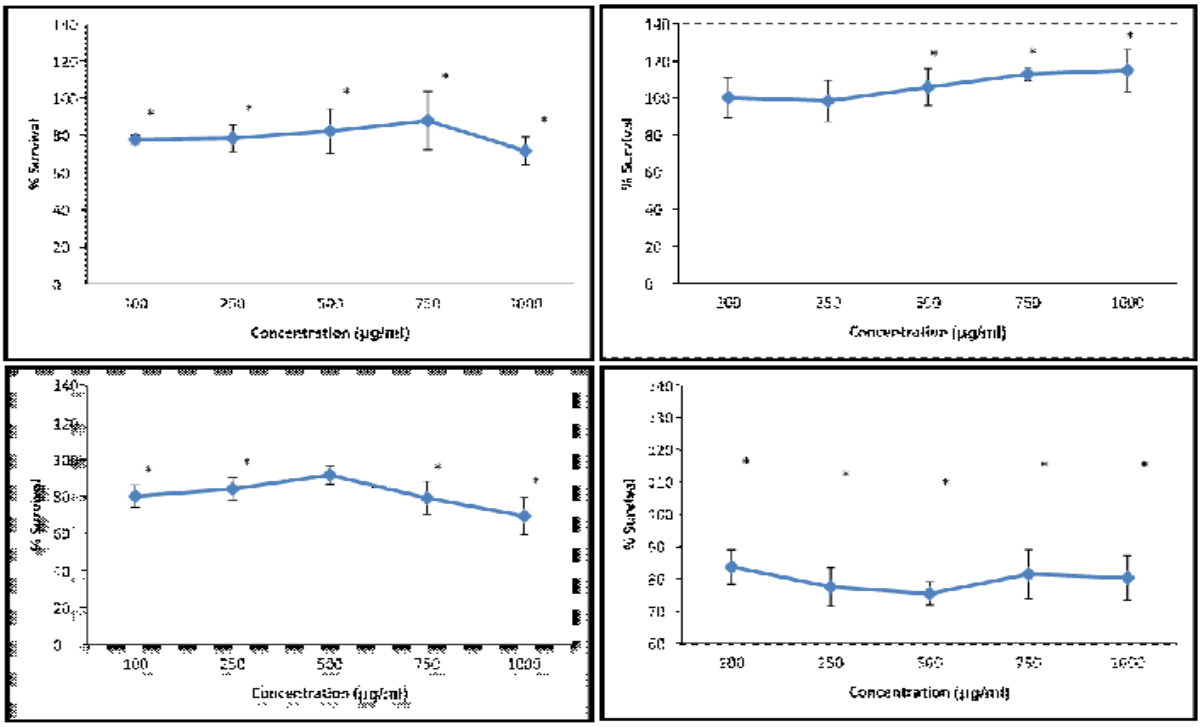

Fig. 1. The cytotoxic effects of Dracocephalum kotschyi aerial parts hexane (A), chloroform (B), methanol (C) and aqueous (D) extracts on MDA-MB-231 cell line. N=12,
*Bars are least significant differences where $p < 0.05$.

Multiple biologic activity of flavonoids and other phenolic compounds including their antioxidant effects, has been presented in many studies. It is also approved that the source of many pharmaceutical and medical materials is due to secondary metabolism in plants that phenolic compounds and flavonoids with antioxidant and pharmaceutical properties are of these metabolites (Maghsoudlou *et al.,* 2012). Flavonoids can cause apoptosis in cancer cells by different methods such as topoisomerase I and II inhibition, cascade caspases activation, inhibiting effective enzymes in cell proliferation like cyclooxygenase, lipooxigenase, xaninoxidase and ornithine decarboxylase. There are different instruments that show a correlation between increasing flavonoid level and decreasing cancer risk (Ren *et al.,* 2003). Increasing the concentration of phenolic compounds straight increases the ability of

various extracts to inhibit free radicals (Ren *et al.* 2003).

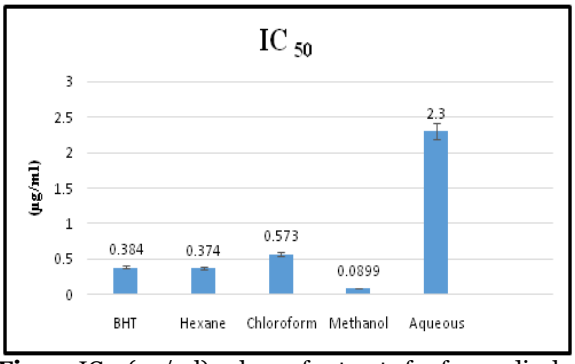

Fig. 2. IC_{50} (µg/ml) values of extracts for free radical scavenging activity by DPPH radical. Lower IC_{50} value indicates higher antioxidant activity

Moghaddam *et al.* 2012 showed that *Dracocephalum kotschyi* Boiss. have a flavonoid named xanthomicrol contributes to its preferential antiproliferative activity against malignant cells. In this research, eight flavonoid aglycones were isolated from the aerial parts of the plant and their identities were confirmed as luteolin, naringenin, apigenin, isokaempferide, cirsimaritin, penduletin, xanthomicrol and calycopterin. The hydroxyflavones (luteolin, apigenin and isokaempferide) exerted comparable antiproliferative activities against malignant and normal cells, while the methoxylated hydroxyflavones (cirsimaritin, penduletin, xanthomicrol and calycopterin) showed preferential activities against tumor cells. In study of Moghadam *et al.* 2012, luteolin has been showed to effective antiproliferative properties against various cancer cell lines including human HL-60 leukemia (Takahashi *et al.,* 1998), AGS human gastric adeno-carcinoma (Wu *et al.,* 2008), MDA-MB-435 and MCF-7 breast cancer, HT-29 colon carcinoma, DU-145 prostate cancer, SK-MEL5 melanoma and DMS-114 lung cancer cell lines (Manthey and Guthrie, 2002). The observed antiproliferative activity of luteolin in this study was consistent with the previously reported results on AGS (Wu *et al.,* 2008), HT-29 (Manthey and Guthrie, 2002) and HL-60 (Takahashi *et al.,* 1998; Manthey and Guthrie, 2002) cell lines. Thus, penduletin showed effective antiproliferative activities against all the malignant cell lines.

Jahaniani *et al.* 2005 demonstrated that flavonoid of xanthomicrol was isolated from *D. kotschyi* and it had preferential antiproliferative activity against a number of cancerous cell lines compared with normal cells (Jahaniani *et al.,* 2005).

Researchers had reported the antiproliferative activity of naringenin and epigenin compounds against HT-29 (Manthey and Guthrie, 2002) and HL-60 (Takahashi *et al.,* 1998) cell lines.

Conclusion

Results of this research showed the relatively strong cytotoxic effects of methanol extract of *Dracocephalum kotschyi* aerial parts and the high content of phenols and flavonoids in these extracts. Based on these results, *Dracocephalum kotschyi* can be a suitable choice for designing and manufacturing anticancer drugs.

Considering the researches has formerly been carried out on this plant and in order to improve researching in this field, some performance is essential such as extracting different types of secondary metabolites such as flavonoids from this plant species and examining the effects of different plant parts extracted compounds on kind cancers and understanding the cell death molecular methods induced by them.

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