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Cytotoxic effect of hydroalcoholic extract from *Thymus daenesis* Celak on MCF-7 cancer cells line

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ABSTRACT

Background & Aim: Breast cancer is the second most common cancer in women after lung cancer. Given that the herbal ingredients are used for centuries to treat cancer, The aim of this study was to determine the cytotoxic effect of hydroalcoholic extract from *Thymus daenesis* Celak on MCF-7 cancer cells line.

Experimental: Breast cancer cells MCF-7 and natural fibroblast cells were cultured in DMEM medium containing fetal bovine serum and antibiotics. The cells were exposed to different doses of hydroalcoholic extract of *Thymus daenesis* Celak (0.156, 0.312, 0.625, 1.25, 2.5mg/ml) and incubated for 24, 48 and 72 hours, respectively. After incubation, the modified MTT colorimetric test was used to determine cytotoxicity.

Results: The results of MTT test showed that hydroalcoholic extract of *Thymus daenesis* Celak has dose- and time-dependent anti-cancer effect on MCF-7 cancer cells, so that by increasing the concentration and 72h incubation, the most cell death was observed ($P<0.05$). Plant extract did not show significant cytotoxicity on natural fibroblast cells. Then, it seems that its compounds can be used in treating cancer through more future research.

Recommended applications/industries: With regard to the increasing use of herbal medicines to treat many diseases, hydroalcoholic extract of *Thymus daenesis* Celak can be used to treat cancer with drug therapy due to having antioxidant properties.

1. Introduction

Cancer is one of the non-communicable chronic diseases which involves broad group of diseases. Like other chronic diseases, this disease is occurred in any person, age groups and all races and is considered as a major hygiene problem which is effective on the heath of society. Cancer is the second most common cause of death after cardiovascular diseases and accidents in less developed countries (Siegel *et al.*, 2011).

Breast cancer is the second most common cancer after lung cancer and the most common type of cancer among women around the world (Hamta & Parvini, 2011). This cancer is responsible for 33 percent of all cancers in women and 20 percent of deaths from cancer, incidence of breast cancer is increasing in developing countries and in many parts of the world, it

has become the most common malignancy disease among women (O'Hara *et al.*,1998).

Complementary therapies such as surgery and drug therapy, hormonal, chemotherapy and radio therapy are among the methods of treating breast cancer. These treatments have many limitations and side effects for cancer patients. Today, using herbal drugs is considered in relation to chemical ones duo to their less side effects (Gordanian *et al.*, 2014). Many herbs and spices contain some factors to prevent cancer which can enforce their effects in various steps of growth of cancer cells (Abdullaev, 2001).

The main objective in the prevention of cancer by natural or chemical materials is to slow or inhibit the carcinogenic process. This approach focuses on abnormal intracellular pathways that lead to abnormal cell functions (Aggarwal *et al.*, 2007).

Thymus daenesis with the scientific name *Thymus daenesis* Celakis from mint family Lamiaceae (Golparvar *et al.*, 2012). It is an herbaceous and perennial plant with multiple and thick whose height is up to 25-30cm. Leaves are mutual and small, oval or ovate, sharp and up to1 cm in length. Flowers are purplish- white or violet and integrated along the leaves, a flower bowl is in pipe or Bluebell form whose teeth are about 0.5 mm in the upper part. The plant is growing in some areas of the Chaharmahal & Bakhtiari, Fars, Hamedan, Ilam, Central, Kohkilooye and Boyer Ahmad and Kurdistan (Karimi *et al.*, 2010).

Thymus daenesis Celak contains tannins, flavonoids, glycosides, caffeic acid and rosemaric acid compounds (Ghasemi Pirbalouti *et al.*, 2013). Based on the results obtained by some researchers, thymol, Paracemenu, Gamatripinin, carvacrol and beta-cariofilin are the major compounds of this plant (Barazandeh & Bagherzadeh, 2007).

Brewed and decoction of this plant is used as taster, anti-cough, anti-spasm, mucus, carminative, anti-microbial and anti-fungal. In Iran and other countries, it is used for treating the common cold (Karimi *et al.*, 2010). In several studies, its antimicrobial properties against *Candida albicans* (Ghasemi Pirbalouti *et al.*, 2009), *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* has been confirmed (Ghasemi Pirbalouti *et al.*, 2010). What has been recently considered by researchers is anti-cancer and anti-carcinogenic properties of thyme plant (Hamta & Ghazaghi, 2014).

Cell culture is one of the modern methods of research and study and its traces are found in almost all scientific disciplines. One of the objectives of cell culture is to study the cells in terms of growth, food needs and cause of stop in their growth. So studying the cell cycle, development of cancer cell growth methods and gene expression modulation require the culture of these cells *in vivo*. (Forouzandeh *et al.*, 2014).

MCF-7 is human breast cancer cell line which first time was separated in 1970 from a malignant cancerous tissue of a 69 year old Caucasian woman who had breast cancer metastasis and thereafter, this cell line is used as useful model for studying cancer (Osborne *et al.*, 1987).

The antioxidant properties *Thymus daenesis* Celak have been demonstrated in previous studies (Sefidkon & Ahmadi, 2000). In this study the cytotoxic effect of hydroalcoholic extract of *Thymus daenesis* Celak on MCF-7 cancer cells line was evaluated.

2. Materials and Methods

2.1. Extraction of hydroalcoholic extract of Thymus daenesis Celak

Aerial organs including leaves and stem of *Thymus daenensis* Celak were collected from Chahrmahal & Bakhtiari and extraction of its hydroalcoholic extract was performed by Rotary. For extraction, the leaves and stems of plants were first dried and powdered by mechanical grinding, then the intended powder was poured into cylindrical casting and solvent was poured on it, the solvent was ethanol 90 % mixed with water, this hydro alcoholic solvent was used to the extent that the plant powder is completely covered. The resulted solution was placed in the oven that was set to 50°C, After 72 hours remaining in the oven, solution was exited of the machine and passed through filter paper, then the filtrated solution was placed in stearic rotary apparatus little by little to concentrate. The resulted extract was used for providing extract in various dosses.

2.2. Cell culture

MCF-7 Cell line of breast cancer and fibroblast cell line were provided from National Center for Genetic Resources of Iran. For culturing MCF-7 cells and fibroblast cells, the culture medium DMEM (Dulbecco's Modified Eagle Medium) Containing 10%

FBS (Fetal bovine serum) and 1% Penicillin-Streptomycin was used. And they were cultured under standard incubation conditions (temperature of 37°Cand 5% $CO_2$and humidity 95%). After three passages, the cells were used for later processing, cell count and the number of living cells were performed by using hemocytometer using Trypan blue.

2.3. MTT (Methyl Tetrazolium) test

To measure the cytotoxic effect of hydroalcoholic extract of *Thymus daenesis* Celak, MTT test was used. In this method, the methyl thiazolyltetrazolium bromide salt or MTT is converted to insoluble and purple formazan through mitochondrial dehydrogenase enzymes of active cells. Light absorption of this compound after dissolving in DMSO (Dimethyl sulfoxide) can be measured by using Eliza reader and in wavelength 492-630 nm (Mosmann, 1983).

2.4. Study the toxicity of hydroalcoholic extract of Thymus daenesis Celak*, by using MTT*

After covering the flask bed with cell, cell layer sticky to flask bottom was separated in enzyme method and by using Trypsin and was centrifuged in 1200 rpm in 5 min after transferring to sterile test tubes. Then cells were suspended by using Pasteur pipette in new culture medium and cell suspension was provided from them. After counting, cells were poured in smooth-floor 96-well plates as 10^4 cells and plates were incubated at 37°Cfor 24 hours. After the required time, the supernatant was removed slowly and carefully and new medium and hydroalcoholic extract of *Thymus daenesis* Celak in concentrations 0.156, 0.312, 0.625, 1.25 and 2.5mg/ml were added to all wells. Serum containing medium without extract was added to control wells. Plates were incubated for 24, 48 and 72 h. After the incubation period, plates were removed from the incubator, supernatant of each well was completely removed by sampler, cells were washed with 100 ml PBS (Phosphate – buffered saline) and then 80 microliter medium and 20 ml yellow MTT solution was added and the plates were incubated for 3 hours, after the required time ,first the supernatant was completely removed and each well was washed with 100 ml PBS and 100ml DMSO was added to dissolve formazan crystals, then the resulted color change was read by device Eliza reader at a wavelength of 492-630 nm.

In order to convert the amount of light absorption (OD) the percentage of live cells, the following formula was used and life percent of cells after 24, 48 and 72 h was computed.

Biological ability percent = OD Control / OD Test ×100

A concentration of tested compound which halved the cell viability was considered as IC_{50} (The half maximal inhibitory concentration).

2.5. Statistical analysis

Data were analyzed using SPSS software via one way ANOVA method and mean comparison was done through Tukey method. The p-values less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Effect of different concentrations of hydroalcoholic extract of Thymus daenesis Celak on bio-viability of MCF-7 cancer cells at different times by using MTT

Statistical analysis showed that in 24-hour incubation time, viability was reduced by increasing the dose of hydroalcoholic extract of *Thymus daenesis*CelakinMCF-7cell line. So that the viability percentage was reduced from 96.79% to in 0.156 mg/ml concentration to 17.39% in concentration 2.5 mg/ml, it was statistically significant ($p<0.05$).

In 48-hour incubation, dose-dependent reduction of viability was observed. So that the percentage of viability was decreased from 90.29% in 0.156 mg/ml concentration to 13.92% in concentration 2.5 mg/ml, it was statistically significant ($p<0.05$).

In 72-hour incubation, the reduction of viability percent was also observed from 79.71 % in concentration 0.156 mg/ml to 11.45% in 2.5 mg, it was statistically significant ($p <0.05$).

Statistical analysis by using ANOVA test and Tukey showed significant difference at all concentration and in all three time in this cell line ($p <0.05$). The most toxicity effect was observed in concentration 2.5 mg/ml and 72 h incubation (Figure 1). 50% cell growth inhibitory concentration (IC_{50}) of *Thymus daenesis* Celak hydroalcoholic extract for MCF-7 cancer cells was 0.625 mg/ml.

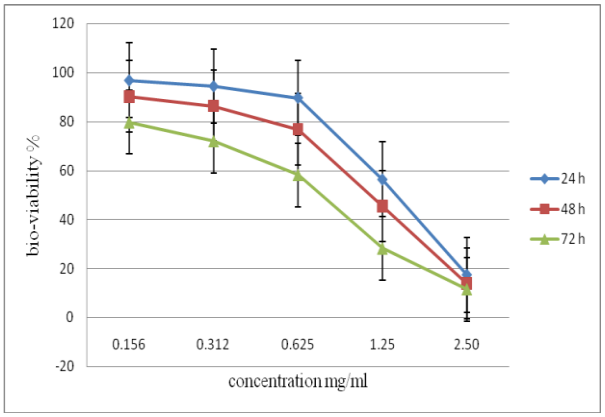

Fig. 1. The changes in bio-viability (%) of hydroalcoholic extract of *Thymus daenesis* Celak on MCF-7 cancer cells in different concentrations (mg/ml) and times by using MTT.

3.2. Effect of different concentrations of hydroalcoholic extract of Thymus daenesis Celak on the bio-viability of natural fibroblast cells at different times by using MTT

Natural fibroblast cells with various concentrations of hydroalcoholic extract of *Thymus daenesis* Celak (0.156, 0.312, 0.625, 1.25, 2.5 mg/ml) were treated for 24, 48 and 72 hours. The results of MTT assay showed that hydroalcoholic extract of *Thymus daenesis* Celak had little effect on fibroblast cells (Figure 2).

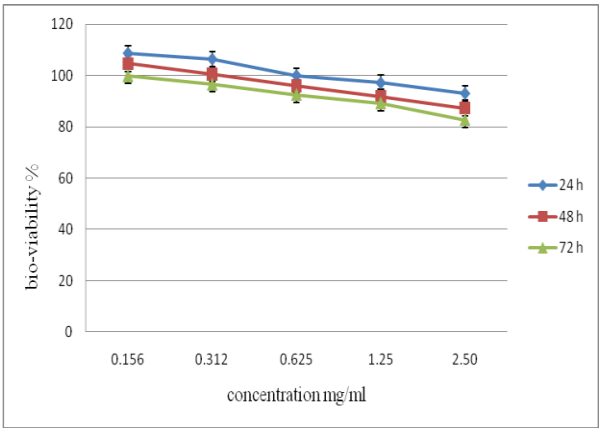

Fig. 2. The changes in bio-viability (%) of hydroalcoholic extract of *Thymus daenesis* Celak on fibroblast cells in different concentrations (mg/ml) and times by using MTT.

MCF-7 cancer cell growth after 72 hours of treatment with hydroalcoholic extract of *Thymus daenesis* Celak at a concentration of 2.5 mg/ml (Figure 3. B) was prevented in comparison to the control group (no treatment with hydroalcoholic extract of *Thymus daenesis* Celak) (Figure 3. A).After exposure to a concentration of 2.5 mg/ml of extract, cells were distorted and their morphology was changed, showing toxicity effect of hydroalcoholic extract of *Thymus daenesis* Celak on these cells.

In the present study, cytotoxic effect of hydroalcoholic extract of *Thymus daenesis* Celak on MCF-7 cancer cell line and natural fibroblast cells was examined. The results of this study showed that the effects of hydroalcoholic extract of *Thymus daenesis* Celak results in death of MCF-7 cancer cells. While it doesn't have any significant toxic effect on natural fibroblast cells.

Many herbs and spices have pharmacological and biochemical properties including antioxidant, anti-inflammatory and anti-cancer property that appears to be involved in anti-mutagenic activity of the cell. Given that tumor progression is closely related with inflammation and oxidative stress, antioxidant compound that has anti-inflammatory properties can be an anti-cancer agent (Afshari *et al.*, 2011).

Thymus daenesis Celakis one of the plants that not only has many applications in traditional medicine, but also has confirmed anti-microbial activity against fungal and bacterial isolates and antioxidant property due to phenolic compounds such as thymol and carvacrol (Sefidkon& Ahmadi, 2000).

Based on the results of various studies, traces of oxidative stress and free radical production in the transformation of the growth and differentiation and carcinogenesis have been demonstrated (Keramati *et al.*, 2011).

Studies have shown that plant extracts rich in phenolic compounds result in protective effect of cells through reducing oxidative stress. Phenolic compounds are a group of aromatic plant secondary metabolites that are widely distributed throughout the plant and have various biological effects including antioxidant and antibacterial activity (Kumaran & Karunakaran, 2006).

The antioxidant activity of phenolic compounds in plants is mostly resulted from their regenerative power and chemical structure, allowing them to neutralize free radicals, form complexes with metal ions and turn off the single and triple oxygen molecules. Phenolic compounds inhibit oxidation reactions through

donating electron to free radicals (Shun *et al.,* 2003). Therefore, it is likely that phenolic compounds such as thymol and carvacrol in hydroalcoholic extract of *Thymus daenesis* Celak reduce oxidative stress through scavenging free radicals.

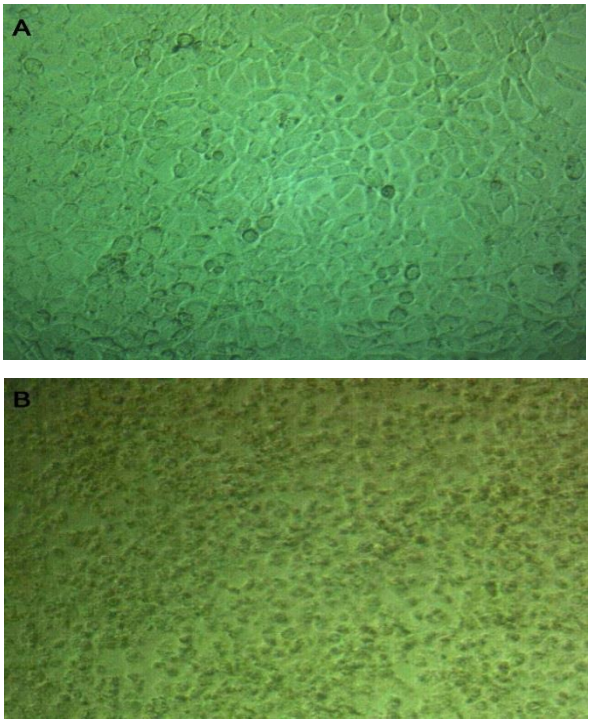

Fig. 3. (A) MCF-7 cancer cells in the control group without treatment with hydroalcoholic extract of *Thymus daenesis* Celak(B) MCF-7 cancer cells after 72 h treatment with hydroalcoholic extract of *Thymus daenesis* Celak at a concentration of 2.5 mg/ml.

Studies have shown that extract of thyme improves the antioxidant potential and thus help preventing from oxidative stress (Rana & Soni, 2008). The findings of some researchers showed that antioxidant compounds such as phenolic compounds can be considered as effective factors on cytotoxic capacity of *A. Campesteris*, phenolic compounds protect cells against reactive oxygen species (ROS) (Angel *et al.*,2009) .

Thymol and carvacrol are important compounds in *Thymus daenesis* Celak to which different biological effects can be attributed. Studies have shown that the thymol and carvacrol function in reducing oxidative stress on the one hand and inhibiting the cyclooxygenase enzymes on the other hand. However, investigations have shown that cyclooxygenase enzymes play a very important role in carcinogenesis mechanism (Keramati *et al.,* 2011).

Cyclooxygenase enzymes can inhibit aromatase enzyme through producing prostaglandins type E2, this enzyme can convert androgen to estrogen (Diaz-Cruz *et al.,* 2005). Since estrogen promotes tumor growth, it is likely that the amounts of estrogen and tumor growth are reduced through inhibiting the enzyme aromatase by inhibiting cyclooxygenase enzymes (Dixon, 2008). So it is likely that phenolic compounds such as thymol and carvacrol of hydroalcoholic extract of *Thymus daenesis* Celak reduce the estrogen level and in turn less tumor growth by inhibiting the aromatase enzyme through inhibiting cyclooxygenase enzymes.

Therapeutic and prophylactic effects of hydroalcoholic extract of Thymus vulgaris on precancerous lesions and carcinoma of the prostate gland cobblestone cells of Wistar albino rats were seen which were related tothymol and carvacrol (Singh & Lucci, 2002).

In a study by Hamta *et al*.(2013) compounds in hydroalcoholic extract of Thymus vulgaris were responsible for inducing apoptosis in cancer cellslines4T1 and anti-cancer and cytotoxic properties of hydroalcoholic extract can be resulted from such compounds as thymol and carvacrol (Hamta & Ghazaghi, 2014). In the present study, cytotoxic effects of hydroalcoholic extract of *Thymus daenesis* Celak can be attributed to phenolic compounds such as thymol and carvacrol, agreeing with results of Hamta *et al*.(2013) study.

Studies show a significant difference at three treatment times (24, 48 and 72h) so that as the time and concentration of hydroalcoholic extract of *Thymus daenesis* Celak is increased, cytotoxicity on MCF-7 cancer cells is also increased. While it doesn't have significant toxicity on natural fibroblast cells. The results of this study are consistent with the findings of Mahdian and co-workers (2015), who studied the effect of hydroalcoholic extract of Brassica *Olerace (Red cabbge)* on inhibiting the growth of breast cancer cells (MCF-7) and normal fibroblast cells (HFF). The results showed that hydroalcoholic extract of *Brassica Olerace (Red cabbge)* cancer inhibited dose-dependent and time-dependent growth of cancer cells, while hydroalcoholic extract of the red cabbage didn't have any toxicity at any time (Mahdian *et al.,* 2015).

The results of this study are also in agreement with the findings of Rezaei and colleagus (2014), who examined cytotoxic effect of hydroalcoholic extract of green fruit and ripe *Cornus mas L*on three cell lines MCF-7 (breast cancer), HepG2 (liver cancer) and CHO (hamster normal cells) by MTT. The results showed that hydroalcoholic extract of *Cornus mas L. fruit* has significant dose-dependent and time-dependent toxicity effect on cancer cells, while it doesn't have any significant toxicity on normal cells (Rezaei *et al.*, 2014).

These results could be a first step in examining and identifying anticancer compounds, however, studies have shown that plant compounds and their derivatives can be considered as part of the standard protocols for cancer treatment and effective weapon to prevent and treat cancer. Due to wide variety of plants, researchers face a long way to research in this field.

4. Conclusion

The results of this study show that hydroalcoholic extract of Thymus *daenesis* Celak has anti-cancer effect which can inhibit the growth of these cells through dose- dependent and time-dependent effect on MCF-7 cancer cells. So that as time spends and in higher doses, growth of cancer cells was more inhibited. Hydroalcoholic extract of *Thymus daenesis* Celak didn't have any significant effect on natural fibroblast cells.

5. References

Abdullaev, F. 2001. Plant-derived agents cancer,*Journal of Pharmacology*. 15(3): 345-354.

Afshari, J., Brook, A., and Moheghi, N. 2011. The cytotoxic effect of *zingiberafficinale* in breast cancer(MCF7) cell line.*Ofogh-e-Danesh*, 17(4): 28-33.

Aggarwal, B.B., Sundaram, C., Malani, N., and Ichikawa, H .2007. Curcumin: the Indian solid gold. *Advances in Experimental Medicine and Biology*, 595: 1-75.

Angel, S., Morana, A., Salvatore, A., Zappia, V., and Galletti, P. 2009. Protective effect of polyphenols from Glycyrrhizaglabra against oxidative stress in Caco-2 cells. *Journal of Medicinal Food*, 12; 1326–1333.

Barazandeh, M., and Bagherzadeh, K. 2007. Investigation on the Chemical Composition of the Essential of *Thymus daenensis* Celak from Four Different Regions of Isfahan Province. *Journal of Medicinal Plant*, 3 (23): 15-19.

Diaz-Cruz, E.S., Shapiro, C.L., and Brueggemeier, R.W.2005. Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. *Journal of Clincal Endocrinology & Metabolism*, 90(5): 2563-2570.

Dixon, M.J. Role of ErbB2 in selection for adjuvant tamoxifen or aromatase inhibitors.2008.*Womens Health*, 14(3): 229-231.

Forouzandeh, F., Salimi, S., Naghsh, N., Zamani, N., and Jahani, S.2014.Evaluation of anti-cancer effect of *Peganumharmala* L hydroalcoholic extract on human cervical carcinoma epithelial cell line. *Journal of Shahrekord University of Medical Sciences*, 16(4): 1-8.

Ghasemi Pirbalouti, A., Bahmani, M., and Avijgan, M.2009. Anti-*candida* activity of Iranian medicinal plants. *Electronic Journal of Biology*, 5: 85-88.

Ghasemi Pirbalouti, A., Hashemi, M., and Ghahfarokhi, F.T. 2013. Essential oil and chemical compositions of wild and cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L. *Industrial Crops and Products*, 48: 43-48.

Ghasemi Pirbalouti, A., Malekpoor, F., Enteshari, S., Yousefi, M., Momtaz, H., and Hamedi, B. 2010. Antibacterial activity of some folklore medicinal plants used by Bakhtiari tribal in Southwest. *International Journal of Biology*, 2: 55-63.

Golparvar, A., Ghasemipirbalouti, A., Zinaly, H.,and Hadipanah, A. 2012. Effect OF Harvest Times on Quantity (Morphological) and Quality Characteristics of *Thymus daenensis* Celak. In Isfaha. *Journal of Herbal Drugs*, 2(4): 245-254.

Gordanian, B., Behbahani, M., Carapetian, J., and Fazilati, M. 2014. In vitro evaluation of cytotoxic activity of flower, leaf, stem and root extracts of five *Artemisia* species. *Research in Pharmaceutical Sciences*, 9:91-96.

Hamta, A., and Ghazaghi, S. 2014. The study of *Thymus vulgaris* Cytotoxicity effects on breast cancer cell's line. *Quarterly Journal of Sabzevar University of Medical Sciences*, 21(1):122-130.

Hamta, A., and Parvini, P. 2011. Study of cytotoxic effects of Taxol and Rosemary extracts on

cancerous cells derived from DMB Ainduced breast cancer in SD rats. *Journal of Cell & Tissue*, 2: 117-126.

Karimi, A., Ghasemi pirbalouti, A., Malekpoor, F., Yousefi, M,, and Golparvar, A. 2010. Evaluation of Ecotype and Chemotype Diversity of *Thymus Daenensis* Celak on Isfahan and Chaharmahal va Bakhtiari Provinces. *Journal of Herbal Drugs*, 1(3): 1 – 10.

Keramati, k., Sanaie, k., Babakhani, A,. Rakhshan, M., Vaezi, G., and Hareri, A. Effect of *Thymus Vulgaris* hydro- alcoholic extraction on DMBA induced prostate cancer Wistar rat. 2011. *Research in Medicine*, 35(3): 135-140.

Kumaran, A., and Karunakaran, R.J. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry*,97: 109-114.

Mahdian, D., Hosseini, A., Mousavi, H., Bihamta, M., and Vahedi, M. 2015. The Evalution of the Effects of Hydro-Alcolic Extract of *Brassica Olerace* (Red cabbge) on Growth inhibition and Apoptosis indution in Breast cancer cell line MCF-7. *Iranian Journal of Obstetrics Gynecology and Infertility*, 18 (151):1-11.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 1983; 65: 55-63.

O'Hara, M., Kiefer, D., Farrell, K., and Kemper, K. 1998. A review of 12 commonly used medicinal herbs. *Archives of Family Medicine*, 7(7): 523-536.

Osborne, C.K, Hobbs, K., and Trent, J.M. 1987. Biological differences among MCF-7 human breast cancer cell lines from different laboratories.*Breast Cancer Research and Treatment*, 9(12):111-121.

Rana, P., and Soni, G.2008.Antioxidantpotential of thyme extract: alleviation of Nnitrosodiethylamine - in duced oxidative stress.*Human & Experimental Toxicology*. 27(3): 215- 221.

Rezaei, F., Shokrzadeh, M., Majd, A., andNezhadsattari, T. 2014.Cytotoxic Effect of Hydroalcoholic Extract of *Cornus mas* Lfruit on MCF7, HepG2 and CHO cell line by MTTAssay. *Journal of Mazandaran University Medical Sciences*, 24(113):130-138.

Sefidkon, F., and Ahmadi, S.h. 2000. Essential oil of *Saturejakhuzistanica*Jamzad. *Journal of Essential Oil Research*, 12: 427-428.

Shun, Y.M., Wen, Y.H., Yong, C.Y., and Jian, G.S. Two Benzyl Dihydroflavones from Phellinus Igniarius. 2003. *Chinese Chemical Letters*, 14(8): 810-813.

Siegel, R., Ward, E., Brawley, O., and Jemal, A. 2011. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths, *Cancer Journal for Clinicians.* 61(4): 212-236.

Singh, B., andLucci, A. 2002. Role of cycloxygenase-2 in breast cancer,*Journal of Surgical Research*. 108: 173-179.