

In Vitro Cytotoxic Effect of Aqueous Extract of *Origanum Marjoram* on AMN-3 Cell Line

Fayhaa M. Khaleel¹ Areej SH. Hameed¹ Taghreed U. Mohammd²

¹ Chemistry Department, College of Science for Women, Baghdad University, Iraq

² Chemistry Department, College of Education for Pure Science, Ibn Al-Haitam, Baghdad University, Iraq

Abstract

The genus *Origanum* consist of different aromatic and medicinal plants some of which are used in folk medicine and as food additives since ancient time. This study amid to evaluate the potential anticancer activity of *Origanum majorana* (marjoram) (*O. majorana*) on the cancer cell lines AMN-3. This study shows that *O. majorana* aqueous extract has anticancer potential and can be explored futher for active component isolation, identifaction and characterization.

Keywords: *Origanum majorana*, Anticancer, Cytotoxic.

Introduction

Cancer is one of the leading causes of death in the world. Several chemotherapeutic, cytotoxic and immunomodulating agents are available in western medicine to treat cancer [1]. Medicinal plants are frequently used by traditional healers to treat a variety of ailments and symptoms including diabetes and cancer. Over 50% of drugs in clinical trials for anticancer activity were isolated from natural plants sources [2].

The genus *Origanum* (*Oregano*), an Important genus of the Lamiaceae family, is widespread throughout the world, comprosing about 900 species [3]. (Figure1).



Figure 1: Aerial parts of *Origanum majorana* L.[4]

Origanum majorana commonly known as marjoram, it is utilized as a spice and flavoring agent, and in traditional medicine as well for the treatment of chest, infection, cough, sore throat, rheumatic pain, nervous disorder, stomach disorders, cardiovascular diseases, and skin care. There is increasing evidence that *O. majorana* possess extensive range of biological activity, including antioxidant, antimicrobial, anti-inflammatory and hepatoprotective activities [5-9].

Recently, we have shown that *Origanum majorana* suppresses the growth of the triple negative MDA-MB-231 breast cancer cell by causing cell cycle arrest and apoptpsis [10]. However, its effect against tumor invasion and metastasis is largely unknown. In this study, we investigated the antitumor effects of *O. majorana* on AMN-3 cell line in *vitro*.

Materials and Methods

Collection of the plant material

The herbal parts of *O. majroana* were collected from Iraqi market in Baghdad on 2015.

Preparation of *O. majroana* extract

Extracts of air -dried plant materials were prepared by using water as solvent. Aportion (25 g) of dried plant material from *O. majroana* was extracted with (250mL) deionized water in a soxhlet apparatus during 6 hr. After

this period, the solvent –extract mixture was filtrated and concentrated using a rotary evaporator at low temperature and pressure. The crude extracts were weighed and stored at -20 °C until use.

Method of Cytotoxicity assay

Single cell suspension was prepared by treating 25 cm² flask of tissue culture at passage 13 with 2 ml 25% trypsin solution incubated for 2 min at 37°C in an incubator supplemented with (5%) CO₂ after detachment of the cells from the flask surface. Single cell suspension gently tapping of the flask. This was followed by the addition of 20 mL of growth medium supplemented with 10% fetal calf serum. Then, the viability test of the cells was made by using trypan blue dye which stains the dead cells. Cell suspension was well mixed followed by transferring 200µl/well of the 96 well flat bottom micro titer plate using automatic micropipette containing (1x10⁵ cell/ well). Plate were incubated at 37°C for 24 hrs in an incubator supplemented with (5%) CO₂ until 60-70% confluence of the internal surface area of the well for AMN-3 cell line. The cells were then exposed to different concentration of new compound (0.04, 0.09, 0.195, 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100) mg/mL respectively, each compound was added to the cells in triplicate from of with culture media represented the negative control, then the 96 well cell culture plate incubated at 37°C in an incubated supplemented with (5%) CO₂ for 24 hrs. After elapsing the incubation period, 50 µl/well of neutral red dye freshly prepared were added to each well and incubated again for 2 hrs, viable cells will uptake the dye and the dead cells will not. The plates washed by PBS to remove the excess dye, then 100µl/well of eluent solution were added each well to draw out the dye from the viable cells. Optical density of each well was measured by using ELISA reader at a transmitting wave length on 492 nm.

$$\text{Absorbance of negative control} - \text{Absorbance of test}$$

$$\text{Inhibition rate \%} = \frac{\text{Absorbance of negative control} - \text{Absorbance of test}}{\text{Absorbance of negative control}} \times 100$$

Results and Discussion

Cytotoxic effect of aqueous extract of *O. majorana* on AMN-3 cell line after an incubation period of 24 hour:

The percentage of plant extract growth inhibition values represented in figure (2) appeared that after 24 hours incubation growth inhibition of AMN-3 cell line was increased with the increased of plant extract of *O. majorana* concentration when compared with the negative control. Aqueous extract of plant has significant differences of cytotoxic effect on AMN-3 cell line ($P < 0.05$), 66%, 53.3%, 40%, 34.6%, 20.2%, 15.4%, 11.8%, 10.9%, 8.6%, 5.1%, 4.7%, 0.8% these percentage of growth inhibition rate were showed at concentration 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.09 and 0.04 mg/mL respectively.

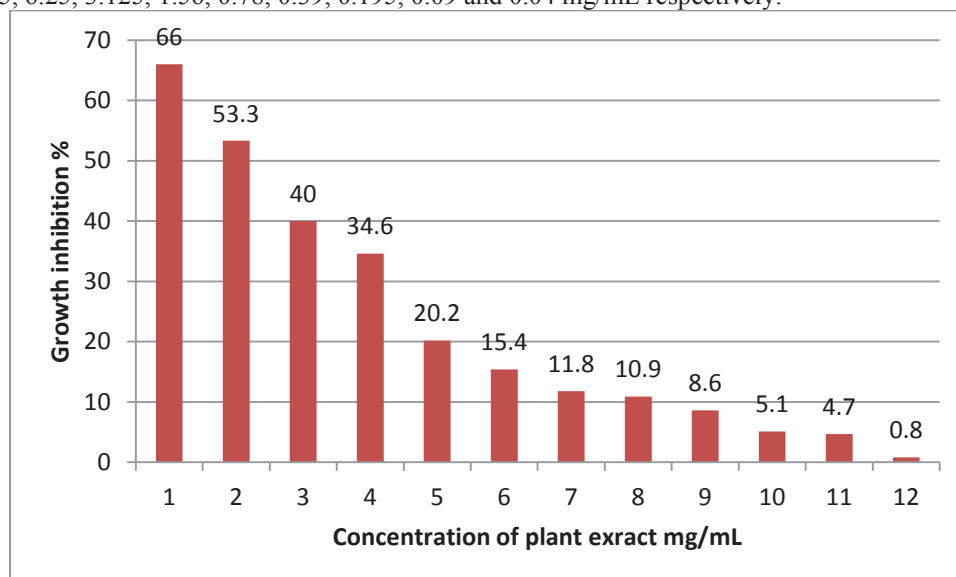


Figure (2): Cytotoxic effect of aqueous extract of *O. majorana* on AMN-3 cell line after an incubation period of 24 hours.

Tumors, besides being very difficult to treat because of its enormous complexity and variability, are widespread and very serious disease. It has been demonstrated antitumor activity on some species of *Origanum*, and furthermore, they have cytotoxic activity against several tumor cell lines[11].

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world [12,13]. Approximately, 60% of the anticancer drugs currently used have been

isolated from natural products from the plants. At this time, more than 3000 plants world wide have been reported to possess anticancer properties. The parts of *O. majorana* have been commonly used in traditional medicine for the treatment of various human ailments for many years. Extracts of this medicinal plant are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and/or therapeutic properties. On a whole our goal was to determine whether the extracts of these plants exerted an inhibitory effect on cancer cell proliferation and caused cell death [14].

Current studies have shown that the essential oil, as well as their active principles possess several pharmacological properties like antimutagenic, angiogenic, antiparasitic, antiplatelet, antielastase, antihepatotoxic ones [15].

The species studied by various workers indicate that the genus *Origanum* is a potent source for isolation of a variety of bioactive molecules like terpenes, phenols, flavonoids, glycosides, tannins, sitosterol and essential oil, etc. Thereby, this genus has important biological activities and acts against different types of diseases and is being used for culinary and economic uses [16,17]. Roula evaluated that the potential anticancer effect of *O. majorana* aqueous extract on human leukemic cell line Jurkat.

They saw antiproliferative activity of plant extracts from *majorana hortensis* and concluded that this study suggests that *majorana* extracts exhibit anti-proliferative effect and high antioxidant activity [18]. The results of our studies suggest that aqueous extract of *O. majorana* possess the strongest cytotoxic effect on AMN-3 cell lines.

In conclusion, it is worthwhile screening the commonly used plants from the local flora for different biological activities because some of them might be a source of new bioactive substance. The results of the present study showed that natural *O. majorana* extract may constitute a potential antitumor compound against cancer cells. However, studies are needed to reach the main anticancer molecule or molecules of this crude extract.

References

1. Roa GV, Kumar S, Islam M, Mansour SE. (2008) Folk medicines for anticancer therapy- a current status. *Cancer Ther*, 6:913-922.
2. Shirisha R, Bibechana T, Varalakshmi K.N. (2014) Evaluation of the anticancer potentials of *Origanum majorana* on fibrosarcoma (HT_1080) cell line. *Asian Pac J Trop Dis*, 4(Suppl 1): S389-S394.
3. Tuncer E, Unver S, Tepe B, Karadayi S, Ozar H, Sen M, *et al.* (2013) Antitumor effect of *Origanum acutidens* Extracts on human breast cancer. *J BUON*, 18(1):77-85.
4. Perna and Neeru Vasudeva. (2015). *Origanum majorana* L. _Phyto_pharmacological review. *Indian J of Natural Products and Resources*, 6(4):261-267.
5. Al-Dhaheeri Y, Samir A, Kholoud A, Synan A, Jean V, Alaaeldin S, *et al.* (2013). Anti-Metastatic and Anti-Tumor Growth Effect of *Origanum majorana* on Highly Metastatic Human Breast Cancer Cells: Inhibition of NF κ B Signaling and Reduction of Nitric Oxide Production. *PLOS ONE*, 8(7):e68808-
6. Vagi E, Rapavi E, Hadolin M, Vasarhelyine Peredi K, Balazs A *et al.* (2005) Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. *Agric Food Chem*, 53:17-21.
7. Al-Harbi NO. (2011) Effect of marjoram extract treatment on the cytological and biochemical changes induced by cyclophosphamide in mice. *J Med Plants Res*, 5(23): 5479-5485.
8. Miron TL, Plaza M, Bahrim G, Ibariez E, Herrero M, (2011) Chemical composition of bioactive pressurized extracts of Romanian aromatic plants. *J Chromatogr A*. 1218:4918-4927.
9. Leeja L, Thoppil JE (2007) Antimicrobial activity of methanol extract of *Origanum majorana* L. (Sweet marjoram). *J Environ Biol*, 28(1):145-146.
10. Al-Dhaheeri Y, Eid A, AbuQamar S, Attoub S, Khasawneh M *et al.* (2013) Mitotic Arrest and Apoptosis in Breast Cancer Cells Induced by *Origanum majorana* Extract: Upregulation of TNF- α and Downregulation of Survivin and Mutant p53. *PLOS ONE*, 8(2): e56649.
11. Jose Maria G.B and Maria E. (2016) Properties and Application of Plants of *Origanum* Sp. Genus. *SM of Biol*, 2(1):1006.
12. Metha R.G, Murillo G, Naithani R, Peng X.(2010) Cancer chemoprevention by natural products: how far have we come? *Pharma.Res*, 27:950-61.
13. Soobratteeancer. M.A, Bahorun T, Aruoma O.I. (2006) Chemopreventive actions of polyphenolic compounds in cancer. *Biofactors*, 27:19-35.
14. Dai J and Mumper R.J. (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15:7313-52.
15. Khakdan F and Piri K. (2013) *In vitro* Cytotoxic Activity of Aqueous Root Extract of *Althea Kurdica* against Endothelial Human Bone Marrow Cells(line k562)and Human Lymphocytes. *Bull. Env. Pharmacol. Life Sci*,2(6):23-29.
16. Shayista Ch, Zahoor A, Kaloo and Phalestine S. (2013) Medicinal importance of genus *Origanum*: A

-
- review. *J of Pharmacognosy and Phytotherapy*, 5(10):170-177.
17. Naif O Al-Harbi. (2011) Effect of marjoram extract treatment on the cytological and biochemical changes induced by cyclophosphamide in mice. *J of Medicinal plants Research* ,5(23):5479-5485.
 18. Dipali S, Shiv K.J, Kamashi S, Kratika N. (2016) *Origanum Majorana* : A Potential Herbe For Functional Food. *EJPMR*, 3(2):321-325.