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

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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. marjorana) on the cancer cell lines AMN-3. This study shows that O.marjorana aqueous extract has anticancer potential and can be explored futher for active component isolation, identifaction and characterization.
Keywords: Origanum marjorana, 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 aliments 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 taping 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 200µ/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 compouned (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 compouned 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 soilution 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.

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

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.

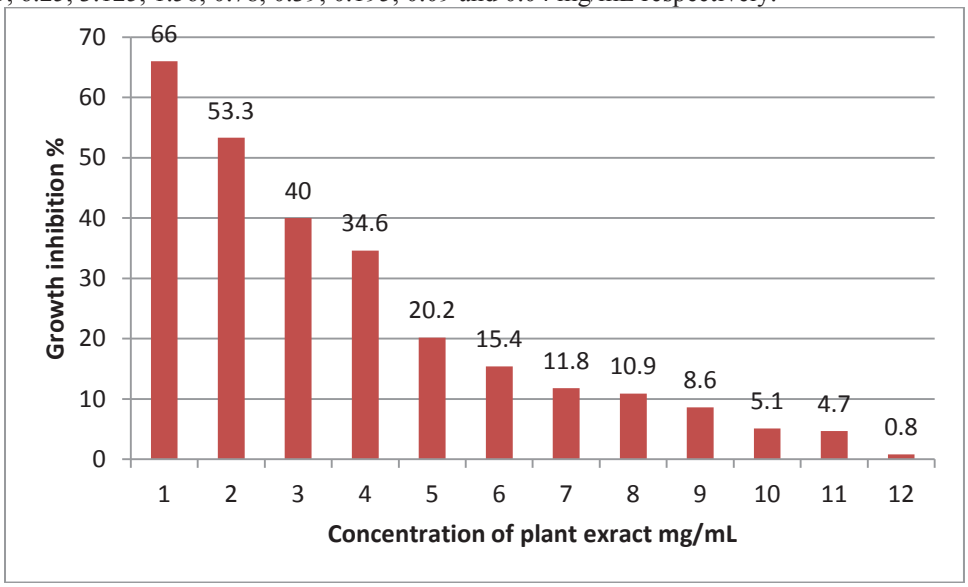

Figure (2): Cytotoxic effect of aqueous extract of *O. majorana* on AMN-3 cell line after an incubation period of 24 hours.

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 traditinal medicine for the treatment of various human aliments for many years. Extract*s* 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 roliferation 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 apotent source for isolation of a variety of bioactive molecules like terpenes, phenols, flavonoids, glycosides, tannins, sitosterol and essenital 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 evaluted 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 suggest that marjorana extracts exhibit anti prolifrative 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 activites 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 a gainst cancer cell. However, studies are needed to reach the main anticancer molecule or molecule of this crud extract.

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