



CYTOTOXIC EFFECT OF SYNERGISM RELATIONSHIP OF OIL EXTRACT FROM *ORIGANUM MAJORANA* L. AND SILICON NANO PARTICLES ON MCF-7

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Abstract

The present study was conducted to investigate the cytotoxic activity of *Origanum majorana* L. extract on humane breast cancer cell line (MCF-7) on different exposure time *in vitro* by using five dilution series (ranging from 6.25- 100 μ g/ml). The results showed that the cytotoxicity effect of *Origanum majorana* L. depend on the amount of dose and exposure time. The concentration 100 μ g/ml gave higher inhibition rate. The cytotoxic activity of the oil extract, silicon nanoparticles and the synergism relationship of (SiNPs+Oil) against rat embryonic fibroblast cell line (REF) which is used as a control for comparing the extract effect on cancerous and non-cancerous cell lines was also conducted. The cytotoxicity effect on REF cells is very low at the same concentrations and same incubation period. The conclusions from this study suggest that marjoram extracts exhibit anti-proliferative effect and high antioxidant activity. For that it merits further investigation as a potential therapeutic agent.

Key words: cytotoxicity, breast cancer, *Origanum majorana* L., MCF-7, inhibition rate

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths; thus, research in this field is important to overcome both economical and psychological burden (Gunduz and Gunduz, 2011). In recent years it has become clear that breast cancer does not represent a single disease but rather a number of molecularly-distinct tumors arising from the epithelial cells of the breast (Done, 2011). The majority of breast cancer cases occur in women over the age of 50. Long before the advent of modern molecular profiling techniques, histopathologists recognized that breast cancer was heterogeneous through morphological observations. Classification was based on the following measures: histological type, tumor grade, lymph node status and the presence of predictive markers such as ER and more recently, human epidermal growth factor receptor 2 (HER2). The development of molecular profiling using DNA microarrays proved this heterogeneity, demonstrating through gene expression profiling and the immune histochemical expression of ER α , progesterone receptor (PR) and HER2 that breast cancer could be

classified into at least five subtypes: luminal A, luminal B, HER2, basal and normal (Perou, Jeffrey *et al.*, 1999). The available classic curatives for cancer therapy are radiotherapy and chemotherapy. Both have diverse side effects, such as: neurological effects, cardiac effects, renal and pulmonary toxicity effects that sorely affect the healthiness of the person. Thus, the demanded method for treatment includes the development of anticancer drug that is less toxic and more efficient in comparison to the already available drugs in the market. All these types of treatment are costly and carry a high risk of side effects and resistance, besides of their unavailability, resulting in high morbidity and mortality rates especially in poor countries (Hwu and Rosenberg, 1997) the researchers have developed anti-cancer strategies to overcome such fatal disease and accordingly novel pharmacological paradigms have been developed which quickly and efficiently moves prospective anti-cancer drugs from the discovery phase through pharmacology testing and therapeutic trial assessment. some of these developments are based on natural products (Gordaliza, 2007). There is a large and over expanding global population that refers the use of natural products in treating and preventing various medical complications (Gautam, Saklani *et al.*,

2007). *Origanum majorana* L. (Family Lamiaceae) is a frost tender perennial under shrub, native to Cyprus and naturalised in Mediterranean regions, particularly found in temperate regions of the Himalayas, *Origanum majorana* L. is one out of 200 genera in the family Lamiaceae (mint family) of 3500 species spread all over the world. Most of the species are aromatic. Marjoram was initially used by Hippocrates as an antiseptic agent, its well liked home remedy for chest infection, cough, sore throat, rheumatic pain, nervous disorders, cardio vascular diseases, epilepsy, skin care and stomach disorders. (Yazdanparast and Shahriyary, 2008). The current study aimed to evaluate the cytotoxic effect of *Origanum majorana* L. extract on human breast cancer (MCF-7). Nano silicon particles are a metalloid element with intermediated physical and chemical properties between those of metals and non-metals among the different metalloid. It is also considered somehow between an essential and non-essential element for the plant, as it is not required for the survival of the most plant, but plant benefit and are better adapted to different environmental stress conditions in the presence of silicon (Epstein, 1994 and Luyckx *et al.*, 2017). Nanoparticles may exhibit different properties due to their small size, greater surface, area to weight ratio and different shapes (Roduner, 2006). The comprehensive superiorities open

a new avenue to the application of Si nanoparticles (NPs) to an energy source, electronic, sensor, catalysis and biomedical purposes (Moore *et al.*, 2011; Astruc *et al.*, 2005).

Material and Method

Preparation of *Origanum majorana* extract

A known amount of dried leaves (150g) were taken from medicinal herbs store, then these dried leaves were identified by Ministry of Science and Technology/ Agriculture Research Directorate. 150g of dried leaves placed in a round-bottomed flask were subjected to hydro distillation using the Clevenger apparatus for 5 hour with (3 L) by heating to (60°C) (Baj, Sieniawska *et al.*, 2015). As shown in fig. 1, below and then the plant was filtered after cooling. Hexane was added to the collected liquid and shake for (30min.) as shown in fig. 2. The aqueous phase poured in separating funnel as shown in fig. 4, below which contained a Hexane, subsequently the aqueous phase was separated into two layers, the lower layer represented water was discard while the higher layer contained the oils retrieved and stored in a sealed marked glass vial in a refrigerator until required.

Maintenance cell culture preparation

Human breast cell line (MCF-7) was used in this study. MCF-7 was maintained in RPMI-1640

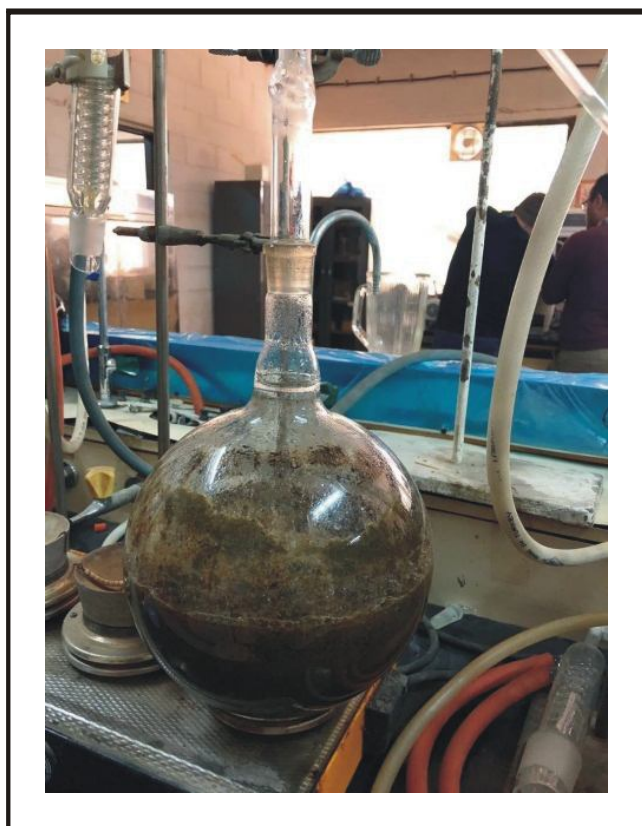


Fig. 1: *O. majorana* in Clevenger.



Fig. 2: Hexane with extracted liquid after shake.

supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week and incubated at 37°C (Sulaiman, Jabir *et al.*, 2018).

Cell growth preparation and cytotoxicity assay

To determine the cytotoxicity effect of *Origanum majorana* L. oil, silicon nanoparticles and synergism (Oil+SiNPs) consequently on breast cell line. The MTT cell viability assay was done using 96-well plates. Cell lines were seeded at 1×10^4 cells/well. After 24hrs. of a confluent monolayer was achieved, cells were treated with tested compounds at different concentration. Cell viability was measured after 72 hrs. of treated cells by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37°C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37°C incubation for 15 min with shaking (Al-Shammari, Salman *et al.*, 2016). The absorbency was determined on a micro plate reader at 492 nm (test wavelength); the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

$$\text{cytotoxicity} = \frac{A - B}{A} \times 100$$

Results and Discussion

Cytotoxic effect of the volatile oil extracted from *O. majorana* leaves was examined on the breast cell line (MCF-7) at different concentrations ranging from (12.5- 200 µg/ml) by five dilutions and all concentrations showed cytotoxicity against breast cell line.

Cytotoxic effect of *O. majorana* L. oil (µg/ml) on MCF-7cell line

The results showed in fig. 3, that the inhibitory effect after 72 hours of treatment was clear at the highest

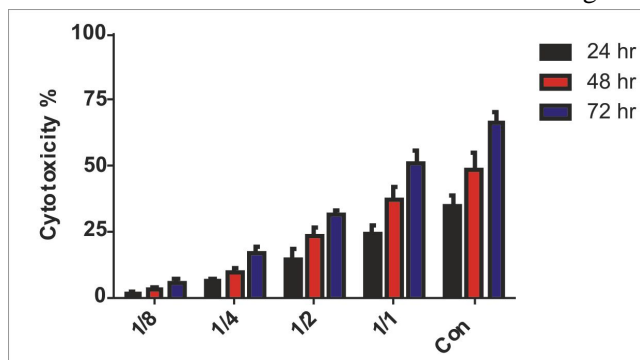


Fig. 3: Cytotoxic effect of *O. majorana* L. oil (µg/ml) on MCF-7 cell line at different incubation periods (24, 48 and 72 hours).

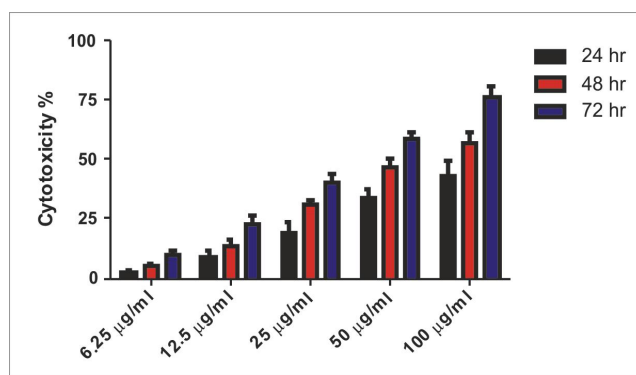


Fig. 4: Cytotoxic effect of Nano silicon particle (µg/ml) on MCF-7 cell line at different incubation periods (24, 48 and 72 hours).

concentrations 200µg/ml, gave the highest percentage of inhibitory rate (70%) (Viability was 30%) compared to control (11%), while the inhibitory rate decreases as the concentration and incubation time decrease.

However the concentrations between 12.5-100 µg/ml gave an inhibitory rate ranging from (7%-55%), compared with the control at these concentrations at the same incubation period (4%-7%).

After 48 hours of treatment with oil extract of *O. majorana* L. the highest inhibitory rate (54%) was observed when using the concentration 200µg/ml, compared to the control (8%). Followed by a gradual decrease with a decrease in concentration reached to (3%) 12.5µg/ml.

After 24 hours of treatment with oil extract the highest inhibitory rate (39%) was observed when using the concentration 200µg/ml, compared to the control (9%). Followed by a gradual decrease with a decrease in concentration reached to (1%) at 12.5 µg/ml.

From the above, it's obvious that the viability value recorded the highest percentage at the lowest concentration and minimum incubation period and begins with gradual decrease with the increase in concentrations and incubation periods.

Cytotoxic effect of Nano silicon particle (µg/ml) on MCF-7cell line

The results showed in fig. 4, that the highest cytotoxicity level of Nano silicon particles on MCF-7 was recorded at concentration 100µg/ml after 72hrs incubation time reaching 81%, which differ significantly from all treatments (50, 25, 12.5 and 6.25 µg/ml), while the cytotoxicity level of Nano silicon particles on REF at the same concentration and incubation time was recorded 13%. The less cytotoxicity level was recorded at SiNPs concentration 6.25 µg/ml after 24hr. incubation period giving 3%, while the cytotoxicity level of SiNPs on of

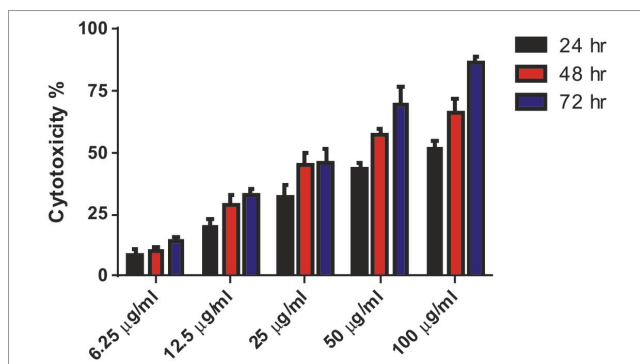


Fig. 5: Cytotoxic effect of synergism relationship (oil extract-silicon Nano particles) on MCF-7

REF at the same concentration and incubation time was recorded 1%.

Cytotoxic effect of synergism relationship (oil extract – silicon Nano particles) on MCF-7

The fig. 5, showed that the cytotoxicity level of the combination (oil+SiNPs) on breast cell line recorded the highest value at concentration 100µg/ml after 72 hrs. incubation time reaching 88%. While the cytotoxicity level of the combination (oil+SiNPs) on REF at the same concentration and incubation time was recorded 14%.

It was observed that the level of cytotoxicity began to decrease at 48 hours (72%) compared to the incubation period of 72 hours at the same concentration, while the effect of (Oil+SiNPs) on the control cells was 14%.

The lowest cytotoxicity percentage was recorded at concentration 6.25µg/ml after 24 hrs. incubation period giving 55% which differ significantly from all treatments, while the cytotoxicity level of oil extracts on of REF at the same concentration and incubation time was recorded 13%.

Discussion

The *Origanum L.* has been proven to be anticancer and antitumor activity, which the anticancer activity have been demonstrated in number of studies on the genus *Origanum L.* particularly by (Ramazan Erenler, 2016). Which showed that the extract exhibited significant antioxidant activities. Hence this plant has the potential to be a natural antioxidant in the food industry and an anticancer drug. However, (Roula M. Abdel-Massih, 2010). Who indicated that the marjoram extracts exhibit anti-proliferative effect and high antioxidant activity.

In this study, *Origanum majorana L.* extract inhibited human breast cell line. The cytotoxic effect of the *O.majorana* oil on the breast cell line (MCF-7) is due to the present of the terpinene-4-ol as the major component that inhibites the cell lines proleferation without affecting the normal cell.

These results agree with the feedback of (Hamedeyazdan *et al.*, 2012). Who found that the anti-proliferative activity of the extract of *M. persicum* showed that growth of MCF-7 cells was inhibited by the extract in a 1.dose and 2.time dependent manner, where a gradual increase of cytotoxicity effect has been achieved setting out on 200µg/mL concentration of the plant extract.

In our study we have demonstrate that the increasing in the cytotoxicity percent in the breast cell line due to the presence of silicon Nano particles (SiNPs) in which it works as a stimulation agent with the essential oil extract to inhibit the growth and development of cancer cell line. The (SiPNs) improve the oil efficiency in a safe way without affecting the normal cell.

A study done by (Bilia *et al.*, 2014) who approve that the EOs have promising potentials for maintaining and promoting health, as well as preventing and potentially treating some diseases and the Nanotechnology is an innovative approach that has potential applications in medicinal and health research. Indeed, nanoparticles are a very attractive tool and are able to solve the major inconvenience of EOs use increasing the chemical stability in the presence of air, light, moisture and, high temperatures, factors which can lead to the rapid evaporation and to the degradation of the active components.

Conclusions

In the light of analysis of the present study, the following points have been concluded:

1. The essential oil extract of *O.majorana* showed anticancer (cytotoxic) activity on breast cell line. The highest cytotoxicity obtained was 70% at 200µg of the extract concentration.
2. The cytotoxicity assay showed that the oil extract of *O.majorana* has a minimum activity on normal rat embryonic fibroblast cell line (REF) in comparison with breast and uterus cell line (11%) at 200µg/ml of the extract.
3. The synergisms of oil extract and silicon nano particles record the highest cytotoxicity level, which means, that the silicon nano particles worked as improvement agent for oil anti-proliferation activity.

Recommendations

Based on the results obtained from this study, it has been recommended the following:

1. Further studies are demanded in order to analyze and test each ingredient of the plant oil extract separately and evaluate their effects as cytotoxic compound.
2. Use Nano technology as a promising technology to

- improve the effect of plant extract on cancer cell line.
3. Additional work is demanded to produce a drug and to test it on a large scale *in vitro* and *in vivo*.
 4. Studying the mode of action of the plant extract on the cancerous cell lines at a molecular level.
 5. Studying the synergistic effect between crude plant extracts and different types of nano particles as cytotoxic compounds.
 6. Test the cytotoxicity of nano particles before work on higher concentrations to avoid the toxic accumulation at certain levels.

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References

- Al-Shammari, A. *et al.*, (2016). "In vitro synergistic enhancement of newcastle disease virus to 5-fluorouracil cytotoxicity against tumor cells." *Biomedicines.*, **4(1)**: 3.
- Abdel-Massih, R.M., R. Fares, S. Bazzi, N. El-Chami and E. Baydoun (2010). The apoptotic and anti-proliferative activity of *Origanum majorana* extracts on human leukemic cell line. *Leukemia research.*, **34(8)**: 1052-1056.
- Baj, T. *et al.*, (2015). "Effectiveness of the deryng and cleveger-type apparatus in isolation of various types of components of essential oil from the *Mutelina purpurea* Thell. flowers." *Acta. Pol. Pharm.*, **72**: 507-515.
- Bilia, A.R., C. Guccione, B. Isacchi, C. Righeschi, F. Firenzuoli and M.C. Bergonzi (2014). Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. *Evidence-Based Complementary and Alternative Medicine.*
- Done, S.J. (2011). "Breast Cancer-Recent Advances in Biology, Imaging and Therapeutics."
- Erenler, R., O. Sen, H. Aksit, I. Demirtas, A.S. Yaglioglu, M. Elmastas and I. Telci (2016). Isolation and identification of chemical constituents from *Origanum majorana* and investigation of antiproliferative and antioxidant activities. *Journal of the Science of Food and Agriculture.*, **96(3)**: 822-836.
- Gautam, R. *et al.*, (2007). "Indian medicinal plants as a source of antimycobacterial agents." *J. of ethnopharmacology.*, **110(2)**: 200-234.
- Gordaliza, M. (2007). "Natural products as leads to anticancer drugs." *Clinical and Translational Oncology.*, **9(12)**: 767-776.
- Gunduz, M. and E. Gunduz (2011). Breast Cancer-Carcinogenesis, Cell Growth and Signalling Pathways.
- Hamedeyazdan, S., F. Fathiazad, S. Sharifi and H. Nazemiyeh (2012). Antiproliferative activity of *Marrubium persicum* extract in the MCF-7 human breast cancer cell line. *Asian Pacific Journal of Cancer Prevention.*, **13**: 5843-5848.
- Hwu, P. and S. Rosenberg (1997). "Gene therapy of cancer." Cancer: principles and practice of oncology. Fifth edition. DeVita VT, Hellman S and Rosenberg SA (eds). Lippincott-Raven Publishers: Philadelphia: 3005-3022.
- Perou, C.M. *et al.*, (1999). "Distinctive gene expression patterns in human mammary epithelial cells and breast cancers." *Proceedings of the National Academy of Sciences.*, **96(16)**: 9212-9217.
- Sulaiman, G.M. *et al.*, (2018). "Nanoscale modification of chrysin for improved of therapeutic efficiency and cytotoxicity." *Artificial cells, nanomedicine and biotechnology.*, **46(1)**: 708-720.
- Yazdanparast, R. and L. Shahriyary (2008). "Comparative effects of *Artemisia dracunculoides*, *Satureja hortensis* and *Origanum majorana* on inhibition of blood platelet adhesion, aggregation and secretion." *Vascular pharmacology.*, **48(1)**: 32-37.