ANTIPROLIFERATIVE EFFECTS OF *Origanum syriacum* L. AND *Myrtus communis* L. ON HUMAN COLON CANCER CELL LINE

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In this study, we aimed to investigate the composition and antiproliferative efficacy of *Origanum syriacum* L. and *Myrtus communis* L. essential oils on the HCT cell line. Also, it was aimed to search the synergistic activities of *Origanum syriacum* L. and *Myrtus communis* essential oils with MTX (Methotrexate) on HCT 116 cells. The main essential oil components of *Origanum syriacum* L. were Thymol (42,18%), Carvacrol (33,95%), Cymene (8,87%), γ -Terpinene (8,21%) and for *Myrtus communis* L. the main essential oil components were Eucalyptol (45,41%), α -Pinene (20,86%), Linalool (9,71%). In this study, HCT 116 human colon cancer cell line and Vero cell line were used. To determine the non-cytotoxic concentration of *Origanum syriacum* L. and *Myrtus communis* L. essential oils Vero cell line was selected. The cytotoxic activity of the essential oils was measured using the MTT method and the results were evaluated as IC₅₀. The Inhibition concentrations of the essential oils of *Origanum syriacum* L. and *Myrtus communis* L. essential oils against cancer HCT 116 and Vero cells were evaluated. It has been found that the essential oils of the *Origanum syriacum* L. and *Myrtus communis* have an important antiproliferative effect on human colon cancer cells. In our study findings, we think that these two plant essential oils may be a potent drug active compound for cancer treatment.

Keywords: Candida, *Origanum syriacum* L., essential oils, antifungal, resistance

INTRODUCTION

Colorectal cancers are the third leading cause of death among all cancer types. Colorectal cancers have only been reported to cause about 600 deaths per year in the Americas. It is known that the incidence of colorectal cancers is increasing every year. Today, the drugs used for the treatment of cancer are limited. Because of the large number of side effects of cancer drugs and the lack of high efficacy of drugs, more effective cancer drug researches continue rapidly (Garcia *et al.*, 2007; Yabroff *et al.*, 2008; Vogelaar *et al.*, 2006).

Today, it is known that the most important active ingredient researches concentrate on natural products. Among these natural products, *Origanum syriacum* L. and *Myrtus communis* L., which have very rich bioactive components, are important plants. These two plants contain significant terpenoid flavonoids in their essential oil contents (Al-Kalaldeh *et al.*, 2010; Aidi *et al.*, 2009; Aidi *et al.*, 2010).

In this study, we aimed to investigate the antiproliferative efficacy of *Origanum syriacum* L. and *Myrtus communis* L. essential oils on the HCT cell line. In addition, it was aimed to search the synergistic activities of *Origanum syriacum* L. and *Myrtus communis* L. essential oils with MTX (Methotrexate) on HCT 116 cells.

MATERIALS AND METHODS

Plant Materials

Origanum syriacum L. and *Myrtus communis* L. plants were harvested during the full bloom period, which the amount of active substance was most intense and after being dried in the shade at room temperature.

Preparation of Plant Extracts

Plant material was weighted and placed in a round bottom flask with a volume of distilled water as extraction solvent; the herba-water mixture was refluxed about 2 h, during which the oil was collected in the side arm of the system. The installation was allowed to stand for about half an hour to prevent the oil to reach room temperature. The oil was dried over anhydrous sodium sulphate and then stored in dark color glass bottles and keep to refrigerator (about 4 °C) until use for analysis.

GC/MS Analysis

Analysis of essential oil was performed using the Thermo Scientific Focus gas chromatograph equipped with a DSQ II single quadrupole mass spectrometer, Triplus autosampler and fused-silica capillary column TR-5MS (5% phenyl-polysilphenylene-siloxane, 30 m×0.25 mm inner diameter, film thickness 0.25 µm). The injection volume was 2 µL. The samples were injected with a split ratio of 250:1 by using helium (99.99 %) as carrier gas, at a flow rate of 1 mL/min; ionization energy was 70 eV. The transfer line temperature of the mass spectrometer was 220 °C, while the temperature of orifice injection was of 220 °C. The temperature of oven was programmed in the range 50–220 °C at a rate of 3 °C/min. Data acquisition was made in the scanning mode. Identification was done on full scan mode in the m/z range of 50–650 a.m.u.

Determination of Non-Toxic DMSO Concentration

In order to dissolve the essential oils of *Origanum syriacum* L. and *Myrtus communis* L., DMSO (dimethyl sulfoxide) was used. To determine the non-toxic concentration of DMSO on Vero cell line, 1×10^6 cells were inoculated into each well of flat bottomed plates. RPMI-1640 was selected as a growth medium. Plates were incubated 96 hours in the presence of decreasing amounts of DMSO (8%, 4%, 2%, 1%, 0.5%).

To determine the non-cytotoxic concentration of *Origanum syriacum* L. and *Myrtus communis* L. essential oils Vero cell line was selected. The cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum 1% (w/v). Cells were incubated in a humidified atmosphere at 37 °C in 5% CO₂.

Cytotoxicity Tests

In this study, HCT 116 human colon cancer cell line was used. Vero cell line were used as healthy cell line. Then, non-toxic concentrations of *Origanum syriacum* L. and *Myrtus communis* L. essential oils were determined on Vero cells. Cytotoxicity of these two essential oils on the cells was determined by MTT (3- (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The effects of different concentrations (1.25,

2.5 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml) of *Origanum syriacum* L. and *Myrtus communis* L. essential oils on human colon cancer cells were investigated. After incubation of the cells treated with *Origanum syriacum* L. and *Myrtus communis* L. essential oils, firstly, morphological evaluation was made by inverted microscope. And, the cells were collected and cell viability was determined by trypan blue exclusion method.

Statistical Analysis

Statistical analyses were performed using Student t-test. The p value <0.05 was considered significant. All statistics in the present study were done using SPSS program.

RESULTS

GS/MS analysis results of *Origanum syriacum* L. and *Myrtus communis* L. essential oils and their main components were given in Table 1,2 and Figure 1,2.

Figure 1. GC/MS chromatogram of *Origanum syriacum* L.

Table 1. The main essential oil components of *Origanum syriacum* L.

RT	Compound Name	SI	RSI	Cas #	%
6,78	α -Pinene	942	969	80-56-8	1,02
10,19	α -Terpinene	962	979	99-86-5	1,40
11,49	γ -Terpinene	988	992	99-85-4	8,21
13,07	Cymene	984	995	25155-15-1	8,87
39,35	Thymol	970	982	89-83-8	42,18
40,48	Carvacrol	966	966	499-75-2	33,95

Figure 2. GC/MS chromatogram of *Myrtus communis* L.

Table 2. The main essential oil components of *Myrtus communis* L.

RT	Compound Name	SI	RSI	Cas #	%
6,86	α -Pinene	987	990	80-56-8	20,86
10,38	Limonene	984	990	138-86-3	5,88
12,47	Eucalyptol	988	988	470-82-6	45,41
21,12	Linalool	994	994	78-70-6	9,71
27,51	α -Terpineol	983	984	98-55-5	6,11

The main essential oil components of *Origanum syriacum* L. were Thymol (42,18 %), Carvacrol (33,95 %), Cymene (8,87 %), γ -Terpinene (8,21 %) and for *Myrtus communis* L. the main essential oil components were Eucalyptol (45,41 %), α -Pinene (20,86 %), Linalool (9,71 %).

The inhibition concentrations of the essential oils of *Origanum syriacum* L. and *Myrtus communis* L. essential oils against cancer HCT 116 and Vero cells were evaluated. The IC_{50} values of *Myrtus communis* L. were determined as 25 μ g/ml and 15 μ g/ml for HCT 116 and Vero cells, respectively (figure 3). The IC_{50} values of *Origanum syriacum* L. were determined as 15 μ g/ml and 10 μ g/ml for HCT 116 and Vero cells, respectively (Figure 3).

The IC_{50} value for MTX against MCF-7 cells was determined to be 5 μ g/ml. This value was calculated for the essential oils of *Origanum syriacum* L. plus MTX as 2.5 μ g/ml. The IC_{50} value for MTX against MCF-7 cells was determined to be 2.5 μ g/ml. This value was calculated for the essential oils of *Myrtus communis* L. plus MTX as 1.25 μ g/ml. It had been found that the essential oils of *Origanum syriacum* L. and *Myrtus communis* L. essential oils enhanced synergistically the effect of MTX against HCT 116 cells (Figure 4, 5).

Figure 3. Effects of the essential oils of *Origanum syriacum* L. and *Myrtus communis* L. on the proliferation of HCT 116 cell line

Figure 4. Comparison of MIC values of Methotrexate with the essential oils of *Origanum syriacum* L. and *Myrtus communis* L. on the proliferation of HCT 116 cell line

Figure 5. Comparison of the combined use of *Origanum syriacum* L. and *Myrtus communis* L. essential oils with the combined use of these essential oils with Methotrexate on HCT 116 cells

DISCUSSION AND CONCLUSION

This work is a preliminary work on the essential oils of *Origanum Syriacum* and *Myrtus Communis*. In this study, it has been found that the essential oils of the *Origanum syriacum* and *Myrtus communis* have an important antiproliferative effect on human colon cancer cells. As in our country, cancer is the second most important cause of death worldwide. The availability of a new effective and potent anti-cancer drug will be crucial for humanity. In our study findings, we think that these two plant essential oils may be a potent drug active compound for cancer treatment. In addition, further *in-vivo* studies are needed.

REFERENCES

- Aidi Wannes, W., Mhamdi, B., Marzouk, B. (2009), "Variations in essential oil and fatty acid composition during *Myrtus communis* var. *italica* fruit maturation", *Food Chem*, 112, 621–626, <https://doi.org/10.1016/j.foodchem.2008.06.018>.
- Aidi Wannes, W., Mhamdi, B., Sriti, J. (2010), "Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower", *Food Chem Toxicol*, 48, 1362–1370, <https://doi.org/10.1016/j.fct.2010.03.002>.
- Al-Kalaldeh, J.Z., Abu Dahab, R., Afifi, F.U. (2010), "Volatile oil composition and antiproliferative activity of *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells", *Nutr Res*, 30, 271–278, <https://doi.org/10.1016/j.nutres.2010.04.001>.
- Garcia, M. et al. (2007), *Global Cancer Facts and Figures*. Atlanta, GA: American Cancer Society, 200.
- Vogelaar, I., van Ballegooijen, M. and Schrag, D. (2006), "How much can current interventions reduce colorectal cancer mortality in the U.S.? Mortality projections for scenarios of risk-factor modification, screening, and treatment", *Cancer*, 107(7), 1624–1633, <https://doi.org/10.1002/cncr.22115>.
- Yabroff, K.R., Bradley, C.J., Mariotto, A.B. (2008), "Estimates and projections of value of life lost from cancer deaths in the United States", *J Natl Cancer Inst*, 100(24), 1755–1762, <https://doi.org/10.1093/jnci/djn383>.