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Research Article

ORIGANUM LAEVIGATUM BOISS GROWING IN KAHRAMANMARAŞ INVESTIGATION OF ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF PLANT IN VITRO

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ABSTRACT. In this study, *Origanum laevigatum* Boiss. Plant extracts were obtained by using methanol from the sun-facing parts of the plant (flower, leaf, and branch), and the antimicrobial and anti-cancer properties of these extracts were investigated.

In the study, the antimicrobial properties of the extracts on a total of 6 bacterial strains, three-gram negative (*Escherichia coli* (ATCC 25922), *Klebsiella pneumonia, Salmonella infantis*) and three gram-positive (*Enterococcus faecalis* (ATCC 29212), *Listeria innocua, Staphylococcus aureus* (ATCC 25923)) were investigated via agar well diffusion method.

In order to determine the anti-cancer activity, extracts of varying concentrations were applied to MCF-7 (Michigan Cancer Foundation-7 breast cancer) and HUVEC (human umbilical vein endothelial cells) cell lines, and cell viability was determined by the MTS method. Through the calculations made with the GraphPad Prism program, the IC50 values of the relevant methanol extracts on the cells were determined.

As a result, *Origanum laevigatum* Boiss. plant appeared to be highly effective only on *Staphylococcus aureus* (ATCC 25923) bacterial strain, while it has been observed to be less effective on the development of *Enterococcus faecalis, Salmonella infantis, Klebsiella pneumonia, Escherichia coli* bacteria. In addition, the IC50 of the extract on MCF7 cells was determined as 0.1279 (mg mL^{-1}), while the IC50 value calculated for HUVEC was calculated as 1.670 (mg mL^{-1}), and the difference between these two values was statistically significant ($p<0.05$). Judging by these findings, We can say that the antimicrobial activity of the methanol extract of *Origanum laevigatum* Boiss. may be quite limited, but promising results have been obtained regarding its anti-cancer properties.

Keywords: *HUVEC, MCF-7, MTS*

INTRODUCTION

Turkey is a country that is rich in plant diversity due to its location in the world and its climatic characteristics. Studies have shown that Turkey hosts 3000 endemic species, and its endemism rate is approximately 32% [1]. *Origanum laevigatum* is a flowering plant in the Lamiaceae family, native to Cyprus, Syria, and Turkey. The Lamiaceae family is represented in Turkey with 46 genera, 586 species, and 755 taxa (246 subspecies and 23 hybrids) (Saran, B..& Karahan, Z.C. 2010). *Origanum laevigatum* is used as an ornamental and culinary plant. It is popularly known as *kırmercanı* in Turkish.

Cancer is a complex disease charaterized by the rapid and irregular proliferation of cells and their invasion of healthy tissues through metastasis. Among the causes of cancer formation bacteria, viruses, environmental conditions, chemicals, bad habits and genetic predisposition can be counted [3]. Breast cancer is the most common type of cancer, especially in women, and its incidence is increasing rapidly in the late years [4]. Approximately 2.3 million new breast cancer cases were reported in 2020, indicating that 1 in 8 cancers diagnosed is breast cancer. Again in 2020, breast cancer caused 685,000 mortalities, and it was placed as the 5th deadliest cancer worldwide [5]. As in many types of cancer, chemotherapy and radiotherapy are used in breast cancer treatment. However, patients have to face the adverse side effects of these treatments, such as nausea, fatigue, and hair loss [6]. Scientists are looking for natural and side-effect-free therapies for cancer patients and generally investigate plants for this purpose. Plants and their extracts are essential for human health with their antimicrobial, antioxidant, anti-inflammatory, anti-biofilm, and anti-cancer potentials [3,6]. Besides, plants with antimicrobial activity have been shown to be more reliable than synthetically synthesized substances [7].

Today, the high side effects of synthetic drugs have led many people to consider alternative treatments with herbal medicines. Research on the use of herbs as medicine is attracting attention daily because of the absence of side effects of herbal drugs and their high effectiveness. Recently, the number of the studies investigating biological activities of extracts obtained from many plants is increasing. However, measuring the potential of all plants still presents a challenge especially in regions with rich biodiversities, such as Turkey.

In order to close this gap in the literature, in this study, methanol extracts were prepared from the sun-facing parts of *Origanum laevigatum* grown in Kahramanmaraş Region of Turkey. These extracts were applied to various bacterial species such as Escherichia coli (ATCC 25922), *Klebsiella pneumonia, Salmonella infantis, Enterococcus faecalis* (ATCC29212), *Listeria innocua, Staphylococcus aureus* (ATCC 25923) and its effect on their viabilities was investigated. Moreover, the cytotoxicity of the extracts on a cancerous (MCF-7 "breast cancer") and a healthy (HUVEC "human umbilical vein endothelial cells") human cell population was also examined.

MATERIALS AND METHODS

Plant Material

Origanum laevigatum Boiss. plant used in the study was collected and identified by Yusuf Ziya KOCABAŞ. The information about the plant material can be seen in Table 1 and Table 2.

Table 1. *Plant sample locality*

Taxon Name	Herbarium number	Locality	Collection Date
Origanum laevigatum	YZK-719	Kahramanmaraş, Başkonuş, Suluyayla road, 1550m.	12/08/2019

Table 2. *The local name of the plants used in the study, the parts used in the studies*

Plant Name	**Family**	**Local Name**	**Parts Used**
Origanum laevigatum Boiss.	Lamiaceae /Ballibabagiller	Kırmercanı	flower, leaf and branch

Preparation of Plants

The *Origanum laevigatum* Boiss. plant was collected and was left to dry for ten days at room temperature in laboratory conditions. Once the targeted parts dried, they were ground in a commercial blender and 10 mL of the extraction solvent methanol (Merck) was added to 1 g of the sample. the extraction process was carried out in a soxhlet device for 6-8 hours. At the end of the method, it was accepted that the extract was prepared at a concentration of 1g/10mL. The final concentrations were reached by mixing the prepared extract with the medium in the relevant study.

Antimicrobial Activity

Test microorganisms to be treated with plant concentrations were incubated in nutrient broth for 24 hours at 37ºC in an incubator to regenerate. A turbidity test was performed on test microorganisms in a 0.5 cell mL^{-1} McFarland device. These organisms were then seeded on Mueller Hilton agar (MHA) with a sterile swab under aseptic conditions. DMSO is used as a negative control whereas Penisilin is used as a positive control in the study. After seeding, the petri dishes were left in the 37ºC incubator for 18-24 hours at the end of which the zone diameters were measured by a ruler. The study was performed in triplicate and the standard deviations of each condition were calculated.

Anticancer Activity

The breast cancer cell line (MCF-7) and human umbilical cord vein endothelial cells (HUVEC) used in the study were purchased from ATCC (USA). The method used here is the modified version of the method presented by Comertpay and Helvacı (2018). Briefly; cells were grown in DMEM (Dulbecco's Modification on Eagle'sMedium) medium containing 1% (v/v) PEN (penicillin + Streptomycin) and 10% (v/v) FBS (Fetal bovine serum) for the experiment. When they reached a sufficient confluence, the cells were counted and seeded into 96 wells in 4 replicates with 5000 cells per well. It was left overnight to allow the cells to adhere. The next day, the cell medium was aspirated, and 100µL of the medium mixed with the plant extract (0.01mg/mL, 0.1mg/mL, and 1mg/mL) was added to each well. After being treated with the extract in an incubator with 5% CO_2 at 37ºC for 24 hours, , 10µL of MTS agent was added and waited for 3 more hours. Later, the absorbance of the wells was measured at 490nmand these data were used to determine cell viability.

Since untreated cells were the control group here, their measured absorbance was assumed to represent 100% cell viability. Each treatment's percentage of cell viability was found by proportioning the absorbance in the treated cells to the untreated ones. Wells without cells were also measured for all groups. These values were subtracted from the cell wells, and the calculation was carried out using only living cells' absorbance.

Statistical Analysis

Statistical evaluations were made using the GraphPadPrism program. IC_{50} values of the extract on human cells were calculated using the software's EC50 shift function after cell viability versus concentration was entered in XY format. Two-Way ANOVA was used to compare cell types, and if a p-value less than (or equal to) 0.05 was observed in the "interaction" panel, the difference was considered statistically significant.

RESULTS AND DISCUSSION

Antimicrobial Activity

A total of 6 bacterial strains, three gram-positive (*Enterococcus durans, Staphylococcus aureus, Bacillus subtilis*) and three gram-negative (*Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella typhimurium*), were used in the study. The study was carried out in three repetitions, and the Table 3 was created by taking the arithmetic average of the results obtained with standard deviations.

Table 3. *Table representation of antimicrobial activity results*

Microorganisms	**20 mg mL^{-1}**	**10 mg mL^{-1}**	**5 mg mL^{-1}**	**Positive Control (PENICILLIN P-10)**	**Negative Control (DMSO)**
***Escherichia coli* (ATCC 25922)**	9±0.21	7±0.04	--	19±0.418	--
Klebsiella pneumoniae	11±0.42	8±0.124	--	30±0.205	--
Salmonella infantis	12±0.408	--	--	23±0.309	--
***Enterococcus faecalis* (ATCC 29212)**	10±0.339	9±0.899	--	--	--
Listeria innocua	--	--	--	29±0.124	--
***Staphylococcus aureus* (ATCC 25923)**	16±0.899	15±1.08	12±0.188	--	--

The diameter of the well was not subtracted from the zone diameters shown in Table 1. The zone diameter of the well is 6mm. (--): no inhibition zone diameter.

As a result, it was observed that *Staphylococcus aureus* bacteria were forming a zone diameter of 16 mm. while *Enterococcus faecalis*, *Salmonella infantis*, *Klebsiella pneumonia, Escherichia coli were forming the zones of* 9-12 mm inhibition against the extract. It did not show any effect on the bacterial strain of *Listeria innocua*.

Aligned with our study, Sarıboga and Korkmaz (2009) [8] also investigated the in vitro antimicrobial activities of *Thymus leucotrichus* and *Origanum laevigatum* leaves and extracts in a study they conducted using fungi and bacteria selected by the disc diffusion method. *Bacillus subtilis* KUEN16 II D-75, *Candida albicans* KUEN 1475, *Escherichia coli* W 3110, *Proteus vulgaris* KUEN 1329, *Pseudomonas aureginosa* ATCC 28753, *Staphylococcus aureus* ATCC 46300, *Streptococcus pyogenes* KUEN 719 test

microorganisms were used in theirstudy. Antimicrobial activity of the ethanol extract of *O. laevigatum* was observed on *S.pyogenes, S. aureus*, and *P.vulgaris*. The extracts of *O. laevigatum*. showed strong inhibition against *S. aureus*as it was seen in our study as well.

Anticancer Activity

In the study, the cytotoxic effects of different concentrations of plant extraction (0.01mg mL^{-1}, 0.1mg mL^{-1}, and 1mg mL^{-1}) on MCF-7 (human breast cancer cells) and HUVEC (human umbilical vein endothelial cells) cells were determined by MTS. The cytotoxicty of the obtained extracts is summarized in Figure 1.

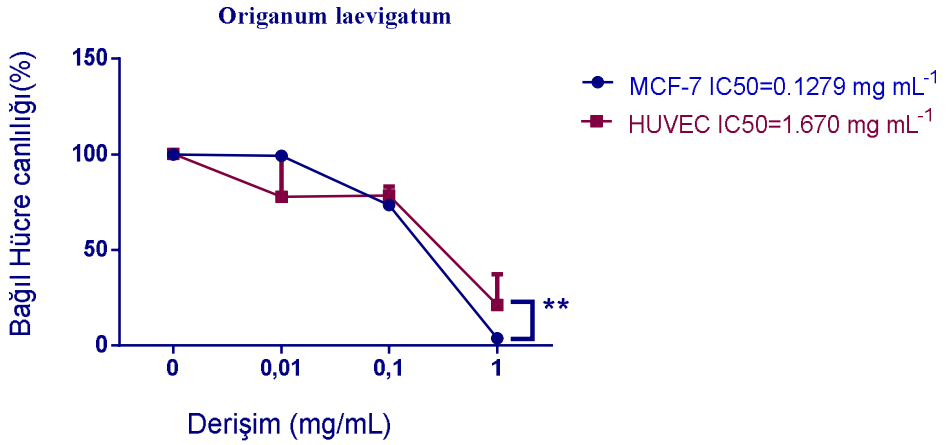

Fig. 1. *Cytotoxic effect of Origanum laevigatum on MCF-7 and HUVEC*

The cytotoxic effects of *Origanum laevigatum* plant extract on MCF-7 and HUVEC cell lines were investigated. The IC_{50} value of the MCF-7 cell line was determined as 0.1279 mg mL^{-1} while the IC_{50} of the HUVEC cell line was 1.670 mg mL^{-1}. In the Two-Way ANOVA analysis, it was concluded that the responses of MCF-7 and HUVEC cells to the extract were different (**). HUVEC cell survival rate was statistically significantly higher ($p<0.05$).

For this reason, it can be thought that the extract will remove cancerous cells without harming healthy cells when it is used at appropriate concentrations. Therefore, we concluded that methanol extract of *Origanum laevigatum* Boiss. may have the potential for use in cancer treatment.

Somehow similarly to our study, Shahneh et al and ark [9] tested the methanol-extracted extract of the *Salvia officinalis L* plant, which is in the same family as the plant species used in this study, on MCF-7 and HUVEC cell lines. While their extract showed a cytotoxic effect on the MCF-7 cell line, it did not significantly affect the healthy cell line HUVEC. Considering the results, in our study, while the MCF-7 cell line died and doses that did not affect the HUVEC cell were observed. Our findings, Shahneh et al., parallels the results of his study. However, In our study alone, Shahneh et al and ark [9] the MTS method was preferred instead of MTT.

CONCLUSION

As a result of the antimicrobial activity test of *Origanum laevigatum* Boiss., it was observed that *Staphylococcus aureus* formed the largest zone diameter. It was also observed that the extract was less effective in the growth of *Enterococcus faecalis*, *Salmonella infantis*, *Klebsiella pneumonia,* and *Escherichia coli* bacteria while no effect of the plant extract was determined on *Listeria innocua* bacteria. *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae*, and *Salmonella infantis* bacteria were found to be moderately susceptible when the extract was compared with the antibiotic used as a positive control. In the anti-cancer study, the cytotoxic effects of different concentrations of the plant extraction on MCF-7 (human breast cancer cells) and HUVEC (human umbilical vein endothelial cells) cell lines were determined by MTS. The difference between the IC_{50}'s of the extract on these cell lines suggested that, when used at the right concentration, the extract might kill cancerous cells without destroying the healthy cell population.

The plant extracts used in the study may have potential in health industries, food, and alternative medicine, but much more comprehensive studies will be needed to reveal this. On the other hand, our study is the first to examine the anti-cancer properties of methanol extract from the *Origanum laevigatum* plant.

REFERENCES

[1] Akkurt, A. (2011): Bazı centaurea L. türlerinin uçucu yağlarının kimyasal bileşimleri ve antimikrobiyal aktivitelerinin incelenmesi. Kırıkkale Üniversitesi, Fen Bilimleri Enstitüsü, Kırıkkale.

[2] Saran, B. & Karahan, Z. C. (2010): Antimikrobiyal ajanlara genel bakış. Turk Urol Sem, 1: 216-20.

[3] Çelik, E. & Çelik, G. Y. (2007): Bitki uçucu yağlarının antimikrobiyal özellikleri. Orlab On-Line Mikrobiyoloji Dergisi, 5(2): 1-6.

[4] Sarıova, A. N. (2019): Meme kanseri (mcf-7) hücre hattında Arbutinin etkileri. Afyon Kocatepe Üniversitesi, Fen Bilimleri Enstitüsü, Kimya Ana Bilim Dalı, Biyokimya Bilim Dalı, Yüksek Lisans Tezi, Afyonkarahisar.

[5] Özdoğan, M. (2020): dunya-kanser-istatistikleri. https://www.drozdogan.com/dunya-kanser-istatistikleri-2020-her-5-kisiden-biri-kanserle-karsilasiyor/

[6] Duran, E. T. (2011): Kanser tedavisinin yan etkilerine yönelik alternatif uygulamalar. SDÜ Tıp Fakültesi Dergisi, 18(2): 72-77.

[7] Onaran, D. İ. & Şeker, S. (2016): Meme Kanseri Hücre Hattı Mcf-7 Üzerinde Taksan Sınıfı Bileşiklerin Ultra Seyreltilmiş Konsantrasyonlarının Sitotoksik Etkilerinin Değerlendirilmesi. İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Biyoloji Ana Bilim Dalı, Yüksek Lisans Tezi, İstanbul.

[8] Sariboga, B., & Korkmaz, H. (2009): Antimicrobial activity of Thymus leucotrichus and Origanum laevigatum. Asian Journal of Chemistry, 21(4): 2777.

[9] Shahneh, F. Z., Baradaran, B., Orangi, M. & Zamani, F. (2013): In vitro cytotoxic activity of four plants used in Persian traditional medicine. Advanced Pharmaceutical Bulletin, 3(2): 453.

[10] Sarıboğa, B. (2002): Bazı tıbbi bitkilerin antimikrobiyal aktivitelerinin incelenmesi. Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü, Kimya Ana Bilim Dalı, Yüksek Lisans Tezi, Samsun.

[11] Ersoy, H. (2005). EDTU Herbaryumu'nda bulunan Lamiaceae (ballıbabagiller) familyası'nın revizyonu. Trakya Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Ana Bilim Dalı, Yüksek Lisans Tezi, Edirne.
[12] Faydaoğlu, E. & Sürücüoğlu, M. (2013): Tibbi ve aromatik bitkilerin antimikrobiyal, antioksidan aktiviteleri ve kullanim olanaklari. Erzincan University Journal of Science and Technology, 6(2): 233-265.
[13] Frost, K. J. (2007): An Overview of Antibiotic Therapy. Nursing Standard, 22(9): 51-57.
[14] Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M. T. (2012): Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, 350 s., 341.
[15] İlçim, A., Dıgrak, M., & Bagcı, E. (1998): Bazı bitki ekstarktlarının antimikrobiyal etkilerinin araştırılması. Turkish Journal of Biology, 22(1): 119-125.
[16] Top, R., Erden, Y. & Tekin, S. (2019): Bazı önemli tıbbi bitkilerin antioksidan ve antikanser etkilerinin araştırılması. Bitlis Eren Üniversitesi Fen Bilimleri Dergisi, 8(2): 435-442.
[17] Saraç, H., Daştan, T., Demirbaş, A., Daştan, S. D., Karaköy, T. & Durukan, H. (2018): Madımak (Polygonum cognatum Meissn.) Bitki Özütlerinin Besin Elementleri ve In Vitro Antikanserojen Aktiviteleri Yönünden Değerlendirilmesi. Ziraat Fakültesi Dergisi, 340-347.
[18] Skoula, M. & Harborne, J. B. (2002). The taxonomy and chemistry of Origanum. Oregano: the genera Origanum and Lippia, 67.
[19] Top, R. (2018): Bazı önemli tıbbi bitkilerin antioksidan, antimikrobiyal ve antikanser etkilerinin araştırılması. Bartın Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Ana Bilim Dalı, Yüksek Lisans Tezi, Bartın.
[20] Uçar, S. (2015): A549 küçük hücre dışı akciğer kanseri hücre hattında daha önce etkinliği tanımlanmamış Türkiye endemiği olan 3 bitkinin antitümöral aktiviteleri açısından değerlendirilmesi. Akdeniz Üniversitesi, Sağlık Bilimleri Enstitüsü, İç Hastalıkları Ana Bilim Dalı, Yüksek Lisans Tezi, Antalya.
[21] Ulcay, S. & Şenel, G. (2018): Lamiaceae Familyasına Ait Lycopus europaeus Türü Üzerinde Anatomik Bir Araştırma. Sinop Üniversitesi Fen Bilimleri Dergisi, 3(1): 45-52.
[22] Baser, K. H. C. (2002): The turkish origanum species. Oregano. The Genera Origanum and Lippia, 109.
[23] Bayar, F. U. & Çınar, O. (2020): Kültür koşullarında yetiştirilen farklı origanum spp. Türlerinin bazı verim ve kalite parametreleri. Derim, 37(1): 10-17.