HYDROBIOLOGICAL RESEARCH

Hydrobiological Research 2(1), 1-6 (2024) https://www.doi.org/10.5281/zenodo.10547329

Research Article

Determination of the effect of marjoram and rhinoceros on UV damage in bacteria using microbiological and molecular techniques

Şükrü Önalan[a]*, Ekin Özköseoğlu[b], İsmail Işık[b]

[a] *Van Yüzüncü Yıl University, Faculty of Fisheries, 65100-Van, Türkiye*
[b] *Van Türk Telekom Science High School, 65100-Van, Türkiye*

Abstract: Nowadays, UV lights are a substance that has gained a place in many areas even if it is used in different conditions and jobs. At the same time, in today's technology, many technological products have been preferred with the use of UV at different intensities in terms of sterilization and cleaning. Chief among these are environmental sterilizers, and in laboratory areas, they are used for purposes such as environmental sterilizers, DNA imaging, sterile cabinets and PCR cabinets. In addition, its damages are also high. It can cause skin and skin cancers, as well as causing blindness in contact with the eyes. With this project, plant extracts, which are known to have many effects, were examined in this aspect in order to prevent possible damage from UV light. For this purpose, E. coli bacteria was chosen. Marjoram and rhinoceros extracts were obtained to positively affect UV application. Extracts were added to grown bacteria in broth and exposed to UV for 1 and 6 hours and compared with UV-damaged bacteria without added extract. The results were investigated by Real-Time PCR analysis of DNA and universal bacterial primers and re-isolation of bacteria in the medium. The results of the study showed that the morphological character of the bacteria used was same of *E. coli*'s morphological character. *E. coli* bacteria were grown in LB medium and were observed to grow in both solid and liquid media. The marjoram and rhinoceros extracts used in the study were obtained by the methanol method. Bacteria cultivated in liquid medium in 2.0 ml centrifuge tubes were divided into different groups and exposed to UV for 1 and 6 hours. No bacterial growth was observed in any group after 6 hours of UV. However, positive binding was observed in PCR. While there was no improvement in the medium in the 1-hour UV groups, the highest copy number was observed in the marjoram group compared to the control group in the PCR image. In light of these results, it was observed that the amount of amplifiable bacterial DNA in marjoram extract was higher than the control group. As a result, it was observed that marjoram extract absorbed UV at a higher rate than rhino herb. It has been shown that it can be fully effective if the UV exposure time is reduced.

Keywords: Bacteria, *E. coli*, UV, Real-Time PCR, Marjoram, Rhinoceros

Şükrü Önalan ORCID ID: 0000-0003-0058-5232
Ekin Özköseoğlu ORCID ID: 0009-0008-6114-6130
İsmail Işık ORCID ID: 0009-0003-1766-5738

*Corresponding author: sukruonalan@yyu.edu.tr
(Received: 7.11.2023, Accepted: 12.12.2023, Online Publication: 24.01.2024)

1. INTRODUCTION

DNA damage may occur for various internal and external reasons. The main ones are single and double strand breaks, oxidative damage, crossovers and mutations. (Fidan, 2000)

DNA damage has been linked to diseases such as cardiovascular disease, aging and cancer. (Ames & Shigenara, 1992). The increase in the number of free radicals formed in the body causes oxidative damage, which in turn causes DNA damage (Atmaca & Aksoy, 2009). In addition, ionizing radiation and chemicals that undergo autoxidation are the main causes of DNA damage. (Halliwell & Aruoma, 1991).

E. coli is one of the most used bacteria in microbial studies, and more than 1000 genes are involved in E. coli DNA damage (Khill & Othero, 2002). Living organisms create a response system against foreign agents that damage DNA. This response system is necessary for the repair of DNA damage (Khill & Othero, 2002).

One of the effective ways to prevent DNA damage is bioactive substance applications (Kaur et al., 2019). For example, studies on the protective role of flavonoids in DNA exposed to UV can be found in the literature (Koostra, 1994).

The marjoram plant (*Origanum majorana*) is a plant that grows in rocky areas and on slopes, usually on limestone (Davis, 1982). It is widespread in the southern and western parts of our country and blooms between April and August (Ietswaart, 1980). It is very rich in essential oils (Azcan, 1998). The leaves of the plant can be used whole, fresh or chopped. It is used among the public to relieve stomach and muscle pain and increase blood circulation. Marjoram is also known for its strong antioxidant properties (Aysel, 2008, Evren & Tekgüler, 2011). *Rhinoceros grass* (Prangos sp.) is a plant that grows in the mountainous areas of our region and is used by the local people for healing purposes for more than ten diseases. This plant, which is generally found and collected in mountainous areas in the spring, is not well known throughout our country.

In this study, the protective effects of certain doses of Marjoram and Rhinoceros herb plant extracts on the DNA of *E. coli* bacteria exposed to various doses of UV were examined.

2. MATERIAL AND METHODS

2.1. Bacteria Collection Supply

Bacteria were obtained from the clinical microbiology laboratory. The supplied bacteria were taken as O157:H7 strain. Re-isolation of the bacteria is essential to continue the study as a fresh culture. For this reason, bacteria taken from the liquid medium were replanted in the solid medium (Bearson et al., 2009).

2.2. Obtaining Plant Extracts

Weighed 2.5 mg of the plant extract. It was diluted by adding methanol at a ratio of 1:10 and was shredded with an ultrasonic shredder. After it was observed that it was completely disintegrated, it was centrifuged at 14000 rpm for 10 minutes. Then, the supernatant was taken and used as a plant extract (Correa & Evershed, 2014).

2.3. Medium Preparation

In the project, LB (solid) and LB (liquid) media were used for the cultivation and development of bacteria. 5 g of LB medium was weighed into 125 ml of pure water with a ratio of 40 g/L. The weighed medium was kept in the microwave oven at maximum temperature for 1 minute until it boiled (approximately 6 min), then the autoclave tape was applied and placed in the autoclave device. Sterilized at 121ºC for 15 min. The liquid medium was kept until it reached room temperature (approximately 35 min). Then, it was transferred to the types in the sterile cabin. The solid medium was waited until it reached the appropriate pouring temperature (approximately 20 min and 42ºC), then 20 ml was added to each petri dish in the sterile cabin. Petri dishes were allowed to dry by leaving them at room temperature for 15 min and incubated at 37ºC for 30 min (Lessard, 2013).

2.4. Bacterial Re-isolation (Solid Media)

The bacteria with a cell count of 0.6 OD at 600 nm ($1x10^7$ bacteria) in the LB liquid medium were taken with a 50 ul automatic pipette and added to the LB solid medium. It was spread by the loop drawing method and placed in the incubator at 37ºC for 24 h. The developing bacteria were stored at +4ºC to be planted in 1 ml liquid media prepared again for UV damage (Peters & Jagger, 1981; Önalan & Barlık, 2023).

2.5. Creating UV Damage

The bacteria were divided into different experimental groups in different tubes. These groups are as follows.

Table 1. The experimental setup used in the study

Group	Group name	Group description
1	Marjoram group	*E. coli +LB (1ml) + MKO Extract + 1 hour UV application*
2		*E. coli* +LB (1ml) + 1 hour UV application
3		*E. coli* +LB (1ml) + MKO Extract + 6 hours UV application
4		*E. coli* +LB (1ml) + 6 hours UV application
5	Rhinoceros group	*E. coli +LB (1ml) + GO Extract + 1 hour UV application*
6		*E. coli* +LB (1ml) + 1 hour UV application
7		*E. coli* +LB (1ml) + GO Extract + 6 hours UV application
8		*E. coli* +LB (1ml) + 6 hours UV application
9	Control group	*E. coli* +LB (1ml) + 6 hours UV application

The most commonly used UV-C light was used to cause damage. UV light exposure was applied for 0, 1 and 6 h. During the application, the UV glass light source was covered with a styrofoam box and a cardboard box and glued (Özbey & Dilek, 2022).

2.6. Determination of Bacterial Growth After UV

After UV application, the bacteria in the liquid medium in all centrifuge tubes were inoculated onto solid media using a loop. In order to examine the growth of the bacteria in the medium, the tubes were taken from the tubes with the help of a loop and re-cultivated (Lessard, 2013).

2.7. Real-Time PCR Application of Groups After UV

After UV application, bacterial DNA isolations from the tubes of all groups were performed with the QIACube Lt automatic isolation device (Qiagen). DNA amounts were measured with a nanospectrophotometer device (QIAxpert, Qiagen). Real-Time PCR analysis (Rotor Gene Q, Qiagen) was then performed using universal 27F-1492R primers. For this purpose, Real-Time PCR master mix (12.5 ul), Forward primer (27F-1.5 ul), Reverse Primer (1492R-1.5 ul), DNase RNase free water (5.5 ul) and isolated DNAs (4 µl) were used. In the PCR protocol, 45 cycles were completed: 15 min at 95ºC, then 30 s at 94ºC, 30 s at 60ºC, and 30 s at 72ºC. The PCR protocol was completed with the last extension stage at 72ºC for 7 min (Önalan, 2019).

3. RESULTS

3.1. Bacteria İsolation

The growth of the bacteria provided within the scope of the project in the medium was planned within 24 h at 37ºC. The transplanted *E. coli* bacteria completed their development within 24 h. It was observed that the developing bacteria showed the same characteristics as E. coli in terms of colony morphology. The developing bacterial colony morphology is given below.

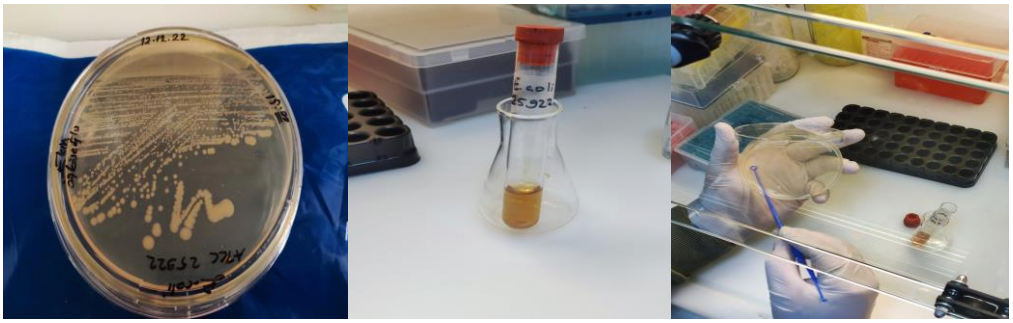

Figure 1. Isolation steps of bacteria in this study

3.2. DNA isolation after UV damage

After UV damage, total DNAs were isolated in an automatic isolation robot with a bacterial DNA isolation kit. DNA concentrations of isolated bacteria are given below.

Table 2. Concentration results of DNA isolated after from UV damage

Groups	Concentration (ng/ul)
Control 1 h	57,4
Control 6 h	38,5
Marjoram 1 h	108,4
Marjoram 6 h	90,3
Rhinoceros 1 h	95,4
Rhinoceros 6 h	72,0

In line with these results, it is understood that the total amount of amplifyable bacterial DNA was the least in the control group, therefore the control group suffered the most damage, and the least damage was observed in the marjoram extract.

3.3. Media Results After UV Damage

Bacteria cultivated in liquid medium in 2.0 ml centrifuge tubes were divided into different groups and exposed to UV for 1 and 6 h. No bacterial growth was observed in any group after 6 hours of UV. However, positive binding was observed in PCR. While there was no improvement in the medium in the 1-hour UV groups, the highest copy number was observed in the marjoram group compared to the control group in the PCR image.

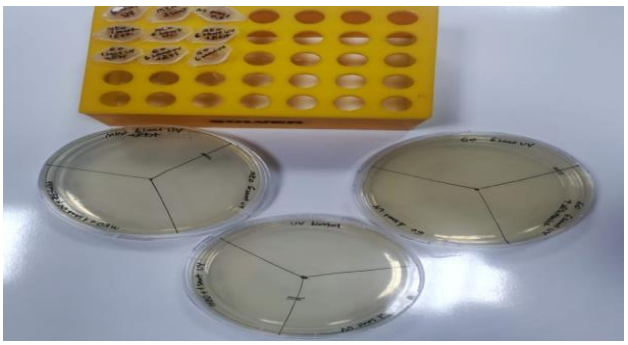

Figure 2. Media screen results from TSA after UV damage

3.4. Real-Time PCR Results After UV Damage

As a result of Real-Time PCR analysis, even if the bacteria lose their viability, primer binding with DNA gives an idea about the extent of the damage. So much so that in case of complete damage, denaturation due to DNA damage and binding of the primer in PCR cycles will not be observed. However, it is observed that PCR amplification occurs with DNAs that remain intact or completely intact when there is partial damage or quantitative damage. In line with these results, it was observed that the amount of amplifiable bacterial DNA in the marjoram extract was higher than the control group.

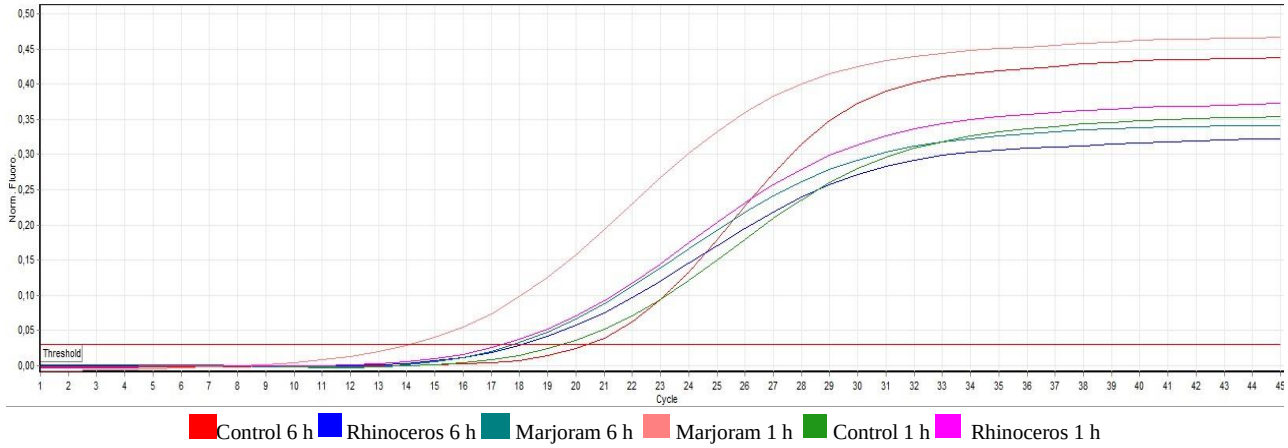

Figure 3. Real-Time PCR results screen (h: hour, Threshold: 0,03)

In order to verify the Real-Time PCR analysis and to show that the results did not give false peaks, it was confirmed by HRM analysis that the PCR amplicons were collected on the same peak.

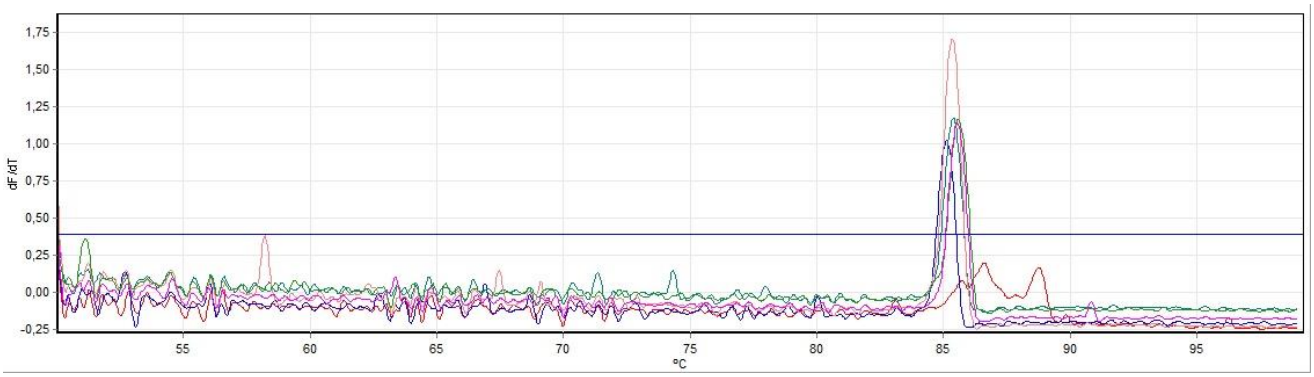

Figure 4. HRM analysis result obtained after Real-Time PCR analysis is given below.

Quantity measurements of Real-Time PCR products were carried out in real-time with concentration analysis on the Real-Time PCR device simultaneously with the PCR process.

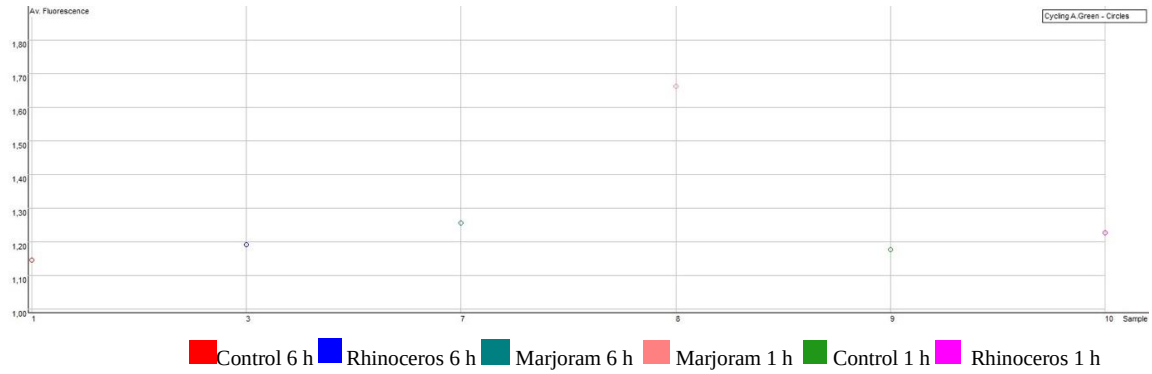

Figure 5. Concentration graph results (h:hour)

According to the results of Real-Time PCR analysis performed with post-UV DNAs, it is seen that the group with the highest concentration amount is the 1-h treatment group of the marjoram group.

4. DISCUSSION

The results of the study showed that the morphological character of the bacteria used was *E. coli*. The bacteria were grown in LB medium and were observed to grow in both solid and liquid media. The marjoram and rhinoceros extracts used in the study were obtained by the methanol method. Bacteria cultivated in liquid medium in 2.0 ml centrifuge tubes were divided into different groups and exposed to UV for 1 and 6 h. No bacterial growth was observed in any group after 6 h of UV. However, positive binding was observed in PCR. While there was no improvement in the medium in the 1-hour UV groups, the highest copy number was observed in the marjoram group compared to the control group in the PCR image. In light of these results, it was observed that the amount of amplifiable bacterial DNA in marjoram extract was higher than the control group. As a result, it was observed that marjoram extract absorbed UV at a higher rate than rhino herb. It has been shown that it can be fully effective if the UV exposure time is reduced. The transplanted E. coli bacteria completed their development within 24 h.

In their study in 2005, El-Ashmawy and colleagues investigated the protective effect of some compounds obtained from the marjoram (*Origanum majorana*) plant on toxicity in mice. In his study in 2019, Dinçoğlu investigated the antibacterial effect of marjoram plant on some food pathogens and determined that plant extracts were effective at different levels against 4 types of bacteria, but were not effective on bacteria of the Salmonella genus. In other study published in 2008, Kunduracı studied the antioxidant activity of marjoram plant essential oils and concluded that these essential oils showed higher antioxidant activity than carvacrol and similar substances. In another study conducted by Kaur and her colleagues in 2019, it was determined that extracts from various natural sources could stop or slow down DNA damage. In another related study in 2009, Kootstra investigated the role of flavonoids in preventing UV-B-induced DNA damage and used an *in vitro* transcription test for this purpose. The results obtained showed that flavonoids, especially obtained from apple peel, prevent DNA damage. In another study, the potential of 10 different phenolic acids to prevent DNA damage was investigated and it was determined that rosmarinic acid showed the highest activity in preventing DNA damage. In addition, the damage prevention potential of molecules such as beta carotene, ferulic acid and vanic acid was evaluated (Açar et al., 2014).

In line with this study results, it is understood that the total amount of amplifyable bacterial DNA was the least in the control group, therefore the control group suffered the most damage, and the least damage was observed in the marjoram extract. No bacterial growth was observed in any group after 6 hours of UV. However, positive binding was observed in PCR. While there was no improvement in the medium in the 1-h UV groups, the highest copy number was observed in the marjoram group compared to the control group in the PCR image. However, it is observed that PCR amplification occurs with DNAs that remain intact or completely intact when there is partial damage or quantitative damage. In light of these results, it was observed that the amount of amplifiable bacterial DNA in marjoram extract was higher than the control group. According to the results of Real-Time PCR analysis performed with post-UV DNAs, it is seen that the group with the highest concentration amount is the 1-hour treatment group of the marjoram group.

5. CONCLUSION

The determined plants in this study were tested again for DNA concentration and Real-Time PCR analysis after medium development, using a method that has not been used on these plants before. The expected point of difference here is the comparison of the amount of DNA that can be used in PCR, even if the UV-C light used causes damage. When all stages of this study are considered together, it is an unrealized study, so the full effect of the extracts used in this study will be seen when a much more grouped and costly study in which all time intervals of UV-C will be tested separately and the damage-free period is determined and a more capable result of the study is obtained. it is obvious.

Acknowledgments and Funding
I would like to thank Solver R&D company for providing laboratory facilities. No financial resources were used in this study.

Authors' Contributions
ŞÖ: Manuscript design, Field sampling, Draft checking, Writing, Draft checking, Reading, Editing, Laboratory experiments, Statistical analyses, and approved the final manuscript.
EÖ: Draft checking, Writing, Draft checking, Reading, Editing,
İI: Laboratory experiments, Statistical analyses, and approved the final manuscript.

Conflict of Interest
The authors declare that there is no conflict of interest.

Statement on the Welfare of Animals
Ethical approval: For this type of study, formal consent is not required.

Statement of Human Rights
Ethical approval: For this type of study, formal consent is not required.

Data Availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

Açar, K., Bektaş, S., & Sarıkürkçü, C. (2014). Antioxidant and DNA damage protection potentials of selected phenolic acids. *Food and Chemical Toxicology*, 77, 12-21.

Ames B. N., & Shigenara M. K. (1992). DNA damage by endogenous oxsidants and mithogenesis es eauses of aging and cancer. In: Scandalios, J. G., (Eds.), *Molecular Biology of Free Radical Scavenging Systems* (pp:1-21), Cold Spring Harbor Laboratuary Pres, Plainviev.

Atmaca, E., & Aksoy, A. (2009). Oksidatif DNA hasarı ve kromatografik yöntemlerle tespit edilmesi [Oxidative DNA damage and its chromatographic determination]. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, *20*(2), 79–83.

Aysel, M. B. (2008). Biberiye (*Rosmarinus officinalis* L.) ve mercanköşk (*Origanum Onites* L.) bitkilerindeki antioksidan aktivite potansiyellerinin araştırılması [Research on the potential antioxidant activity of rosemary (*Rosmarinus officinalis* L.) and origano (*Origanum onites* L.) plants]. MSc. Thesis, Ankara University.

Azcan, N. (1998). *Origanum onites* L. ve kekik siklon tozunun lipitleri ve kekik siklon tozunun değerlendirilmesi [Lipids of *Origanum onites* L. and origanum cyclene powder and utilization of origanum cylone powder]. Ph. D. Thesis, Osmangazi University, Türkiye.

Bearson, B. L., Lee, I. S., & Casey, T. A. (2009). *Escherichia coli* O157: H7 glutamate-and arginine-dependent acid-resistance systems protect against oxidative stress during extreme acid challenge. *Microbiology*, *155*(3), 805-812.

Correa-Ascencio, M., & Evershed, R. P. (2014). High throughput screening of organic residues in archaeological potsherds using direct acidified methanol extraction. *Analytical Methods*, *6*(5), 1330-1340.

Davis, P. H. (1982). *Flora of Turkey and East Aegean Island*. 7th ed. Edinburg University Pres, Edinburg.

Dinçoğlu, A.H., (2019). Mercanköşk (*Origanum Onites* L.) bitkisinin bazı gıda patojenleri üzerine antibakteriyel etkinliğinin saptanması [Detection of antibacterial effect of Oregano plant (*Origanum onites* L.) on some food pathogens]. *Mehmet Akif Ersoy University Journal of Health Sciences Institute*, *7*(2), 139-146.

El-Ashmawy, İ., El-Nahas, A. F., & Salama, O. M. (2005). Protective effect of volatile oil, alcoholic and aqueous extracts of *Origanum majorana* on lead acetate toxicity in mice. *Basic & Clinical Pharmacology & Toxicology*, 97(4), 238-243. https://doi.org/10.1111/j.1742-7843.2005.pto_136.x

Evren, M. & Tekgüler, B. (2011). Uçucu yağların antimikrobiyel özellikleri [Antimicrobial properties of essential oils]. *Elektronik Mikrobiyoloji Dergisi*, *9*(3), 28-40.

Fidan, F. (2000). DNA hasar tespitinde tek hücre jel elektroforezi [The single cell gel electrophoresis for determining the DNA damage]. *Afyon Kocatepe University Journal of Science*, *8*(1), 41-52.

Halliwell, B., & Aruoma, O. I. (1991). DNA damage by oxygenderived species. Its mechanism and measurement in mammalian systems. *FEBS Letters*, *281*, 9-19.

Ietswaart, J. H. (1980). A Taxonomic Revesion of the Genus Origanum (Labiatae). 1th ed. Leiden University Pres, The Hague, Boston, London

Kaur, P., Pureval, S. S., Snanhu, K. S., & Kaur, M. (2019). DNA damage protection: An excellent application of Bioavtive compunds. *Biosources and Bioprocessing*. *6*(2), 2-11.

Khil, P. P., & Othero, D. C. R. (2002). Over 1000 genes are involved in DNA damage response of *E. coli*. *Molecular Microbiology*, *4*(1), 89-105.

Kootstra, A., (1994). Protection from UV-B induced DNA damage by flavonoids. *Plant Molecular Biology*, *26*(2), 771-774.

Kunduracı, B. S. (2008). Farklı işlemlerle elde edilen mercanköşk (*Origanum onites* L.) ekstraktlarının ve uçucu yağının antioksidan aktivitesinin belirlenmesi [Determination of antioxidant activities of Origano (*Origanum onites* L.) essential oil and extracts obtained with different Methods]. MSc. Thesis, Ankara University.

Lessard, J. C. (2013). Growth media for *E. coli*. In: Lorsch, J., (Ed.), *Laboratory Methods in Enzymology: Cell, Lipid and Carbohydrate* (pp. 181-189), Academic Press London.

Önalan, Ş. (2019). Expression differences of stress and immunity genes in rainbow trout *(Oncorhynchus mykiss*, Walbaum 1792) with different bacterial fish diseases. *The Israeli Journal of Aquaculture-Bamidgeh*, *71*(1), 1-10.

Önalan, Ş., & Barlık, H. (2023). The expression levels of virulence and antibiotic resistance genes in *Lactococcus garvieae* exposed to different conditions. *Cellular and Molecular Biology*, *69*(12), 6-11. https://doi.org/10.14715/cmb/2023.69.12.2

Özbey, E., & Dilek, A. S. M. A. (2022). UV-C'nin, *Deinonoccus radiodurans* ve vitreoscilla hemoglobin (vgb) geni aktarılmış rekombinantlarında; SOD, KAT ve Karoten miktarı üzerine etkisi [In UV-C, *Deinonoccus radiodurans* and vitreoscilla hemoglobin (vgb) gene transferred recombinants; effect on SOD, KAT and Carotene amount. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, *25*(3): 476-485. https://doi.org/10.18016/ksutarimdoga.vi.916575

Peters, J., & Jagger, J. (1981). Inducible repair of near-UV radiation lethal damage in *E. coli*. *Nature*, *289*(5794), 194-195.