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Biological Activities of *Elaeagnus umbellata* Methanol Extract

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ABSTRACT

Elaeagnus umbellata, known as Autumn olive and growing widely in Asia and Southern Europe, is a shrub tree used in the traditional treatment of many diseases, including cancer. In this study, it was aimed to investigate the several biological effects of methanol extract of *E. umbellate*. The activity of the extract on 11 microorganisms was determined by the agar well diffusion technique. The anti-quorum sensing activity of the extract was tested using *Chromobacterium violaceum* ATCC 12472. The anti-biofilm and anti-swarming activities were tested using *Pseudomonas aeruginosa* PAO1. Cytotoxic effect of the extract against pancreatic tumoral cell line (AR42J), breast cancer cell line (MDA-MB-231), lung adenocarcinoma cell line (A549), and normal epithelial cell line (Vero), and antiviral effect of the extract against herpes simplex virus type 1 was analyzed using the MTT method. It was determined that the extract was moderately effective against 6/11 microorganisms and showed anti-quorum sensing activity. While the extract did not have a cytotoxic effect on cancer cell lines, it was found to have a cytotoxic effect on Vero cells at concentrations of 100 µg/mL and above. However, no anti-biofilm, anti-swarming, and antiviral activity of the extract was observed. The study shows that *E. umbellata* fruit has limited biological activity.

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Elaeagnus umbellata Metanol Ekstraktının Biyolojik Aktivitesi

ÖZET

Asya ve Güney Avrupa'da yaygın olarak yetişen ve Sonbahar zeytini olarak bilinen *Elaeagnus umbellata*, kanser dahil olmak üzere birçok hastalığın geleneksel tedavisinde kullanılan bir çalı ağacıdır. Bu çalışmanın amacı *E. umbellate* ekstraktının çeşitli biyolojik etkilerinin belirlenmesidir. Ekstraktın 11 mikroorganizma üzerindeki aktivitesi agar kuyucuk difüzyon tekniği ile belirlendi. Ekstraktın anti-quorum sensing aktivitesi *Chromobacterium violaceum* ATCC 12472, anti-biyofilm ve anti-swarming aktiviteleri ise *Pseudomonas aeruginosa* PAO1 kullanılarak test edildi. Ekstraktın pankreatik tümöral hücre hattı (AR42J), meme kanseri hücre hattı (MDA-MB-231), akciğer adenokarsinoma hücre hattı (A549) ve normal epitel hücre hattı (Vero)'nasitotoksik etkisi ve herpes simpleks virüs tip-1'e karşı antiviral etkisi MTT yöntemi ile araştırıldı. Ekstraktın 6/11 mikroorganizmaya karşı orta düzeyde etkili olduğu ve anti-quorum sensing aktivite gösterdiği belirlendi. Ekstraktın kanser hücre hatlarında sitotoksk etkisi görülmezken, Vero hücrelerine 100 µg/mL ve üzeri konsantrasyonlarda sitotoksik etkisi olduğu tespit edildi. Bununla birlikte ekstraktın anti-biyofilm, anti-swarming ve antiviral aktivitesi görülmedi. Yapılan çalışma *E. umbellata* meyvesinin sınırlı biyolojik aktivitesinin olduğunu göstermektedir.

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INTRODUCTION

Plants (especially medicinal ones), have been used in many different civilizations from past years to the present. it has been serving the field of medicine since ancient times (Temel et al., 2018; Saraç and Özpınar, 2024). They have been included in the human diet not only for their nutritional value but also for use as prophylactic and therapeutic agents in the treatment of different diseases. (Ak et al., 2022) *E. umbellata* is found at an altitude of 1200–2100 m above sea level and grows at temperatures ranging from 43 to 55 °C and a pH range of 5.5–9.5 (Bhat et al., 2023).

Elaeagnus umbellata Thunb., Fl. Jap. (Thunberg) 66, t. 14 (1784) is belonging to the Elaeagnaceae family. *E. umbellata* fruit is rich in vitamins A, C, and E, minerals, flavonoids, and fatty acids (Wu et al., 2011; Patel et al., 2015). *Elaeagnus* berries have a lot of bioactive compounds such as lutein, β-carotene, β-cryptoxanthin, and α-cryptoxanthin. (Patel et al., 2015).

Elaeagnus species have traditionally been used as antioxidant, anticancer, antinociceptive, anti-inflammatory, antimutagenic, antiulcerogenic, antimicrobial, antidiabetic, and neuroprotective agents (Nazir et al., 2020).

It is known to be effective on many types of cancer as well as many different diseases. It has also been reported to be used as an antipyretic (Ahmad et al., 2005). It is reported that *E. umbellata* fruits are potentially effective in bacterial infections and complications related to type 2 diabetes (Nazir et al., 2018; Nazir et al., 2021). The flowers and seeds of *E. umbellata* are very useful as they are used as a tonic to cure cough. It is known that the oil of *E. umbellata* seeds is preferred in the treatment of infection. At the same time, its essential oil has antioxidant anticholinesterase and antidiabetic activity (Nazir et al., 2021).

In the study, anti-quorum sensing, anti-microbial, antiviral, and anti-cancer activities of *E. umbellata* methanol extract were investigated.

MATERIAL and METHOD

Collection of autumn olive fruits and preparation of their extracts

E. umbellata fruits were obtained from the high regions of Rize and dried in a Pasteur oven at 55°C overnight. Methanol extracts were obtained using the Macerate. Briefly, 10 to 20 g of fruit were weighed, ground into powder in a mortar, placed in a conical flask, and 100 mL of methanol was added. Incubated overnight on a magnetic stirrer at room temperature. Then, it was filtered through filter paper and evaporated in the evaporator at 40 °C, followed by the extracts dissolved in DMSO (Dimethyl sulfoxide) to 50 to 100 mg/mL. These extracts were stored at -20 °C until use (Eksi et al., 2020).

Antimicrobial activity

Bacillus cereus (ATCC 14579), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Mycobacterium smegmatis* (ATCC 607), *Chromobacterium violaceum* (ATCC 12472), *Klebsiella pneumoniae* (ATCC 13883), *Salmonella thymurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter haemolyticus* (ATCC 19002), *Candida albicans* (ATCC 10231) and *Candida parapsilosis* (ATCC 22019) strains were used.

The antimicrobial activities were investigated by agar diffusion method. Minimal Inhibitory Concentration (MIC) was calculated using Mueller Hinton broth-II (Woods et al., 2003). The well located before the well where growth started was determined as the MIC well and the value in this well was written as the MIC value. To determine the Minimum Bactericidal Concentration (MBC) value, 50 µL was taken from the MIC well and the three wells before it and planted on agar medium. Incubated overnight at 37 °C. Plantings from the MIC well and other wells were evaluated and the lowest value at which no growth was observed was determined as MBC (Gür, 2016).

Anti-quorum sensing activity

To determine the violacein suppression activities of the extract, firstly the Sub-MIC value was determined in *C. violaceum* ATCC 12472 strain. *C. violaceum* 12472 strain was cultured in 5 mL of Luria Bertani (LB) broth for 8 hours in a 175 RPM shaking incubator. Then, 50 µL of *C. violaceum* 12472, which was left for shaking incubation, was taken and added to 5 mL of soft LB agar and poured into LB agar petri dishes. Wells was opened on the dried petri dish. 50 µL of the Sub-Mic concentration was added to the well and incubated overnight. The formation of a transparent zone with growth in the petri dish but no violacein pigment was determined as positive (Eksi et al., 2020).

Anti-swarming activity

First, the MIC of the extract in *P. aeruginosa* PAO1 strain was determined. Values below the MIC were used in the study. Concentrations below the MIC value were added to tubes containing 5 mL LB soft agar, poured onto petri dishes containing LB agar, and allowed to solidify. A colony from the fresh culture of *P. aeruginosa* PAO1 was picked with a sterile toothpick placed in the middle of the prepared LB plates and incubated overnight at 37 °C (Rashid and Kornberg 2000). The activity was determined by measuring the colony spread of bacteria growing from the point where *P. aeruginosa* was inoculated. *P. aeruginosa* PAO1 without added extract was used as a

control and the measured zones were evaluated by comparing them with this zone.

Anti-biofilm activity

To test the biofilm inhibition of the extract, *P. aeruginosa* PAO1 strain was adjusted to 0.5 McFarland. Extract and *P. aeruginosa* PAO1 were added to microplates containing LB medium. After 24 hours of incubation, microplates were washed with distilled water and 0.3% crystal violet was added to each well. Finally, the microplates were washed with distilled water and treated with ethanol for 15 minutes. Absorbances were measured at 570 nm in spectrophotometer (Truchado et al., 2009; Üreyen Esertaş et al., 2022).

Cell culture, virus, and standard drug

Breast cancer cell line (MDA-MB-231), pancreatic tumoral cell line (AR42J), lung adenocarcinoma cell line (A549), and normal epithelial cell line (Vero) from Karadeniz Technical University, Medical Microbiology Department culture collection, originally obtained from American Type Culture Collection (ATCC, USA) were used in the study. AR42J, A549, and Vero cell lines were maintained in Dulbecco's Modified Eagles Medium (DMEM), MDA-MB-231 cell line was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin solution. Cultures were incubated at 37 °C with 5% CO_2.

The HSV-1 Wal strain was o originally obtained from the University of Sheffield (England).

Cytotoxicity of the extract

The cytotoxic effect of the extract on pancreatic tumoral cell line (AR42J), breast cancer cell line (MDA-MB-231), lung adenocarcinoma cell line (A549) and normal epithelial cell line (Vero) was examined by3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as previously described (Cora et al., 2023). Concentrations of 3.12-400 µg/mL of the extract were placed on the cells in a 96-well plate containing $1x10^4$ cells in each well, with three wells of each concentration. After the incubation for 72 h at 37 ˚C with 5% CO_2, the MTT assay was performed. Wells contained untreated cells were used as negative control. The results were evaluated in Microsoft Excel with reference to the control wells.

Antiviral activity of the extract

Concentrations of 50 µg/mL and below, which are not cytotoxic to Vero cells, were added to the cells infected with the virus at a concentration of 1TCID50. After three days incubation at 37 ˚C with 5% CO_2 the MTT assay was performed. Acyclovir was used as a positive control, wells containing Vero cells infected with the virus were used as a negative control, and wells containing Vero cells were used as reproductive control. The data were evaluated using the Microsoft Excel program and the viability of the cells in the wells was calculated as a percentage compared to the growth control (Cora et al., 2023).

Statistical analysis

Statistical analysis was conducted with the SPSS 15.0 software. Mean ± standard error was employed to depict continuous variables conforming to a normal distribution. In cases where a normal distribution was not evident, the median value was utilized.

RESULTS and DISCUSSION

The use of plants and herbal products in treatment by humans has been of great importance since ancient times. *E. umbellata*, is one of the medicinal plants widely used among the public for various ailments (Bhat et al., 2023). Today, with the developing technology, the chemical content of *E. umbellata* has been investigated and it has been understood that the plant contains different secondary metabolites and accordingly exhibits different pharmacological properties such as antiviral, anticancer and antioxidant activity (Bhat et al., 2023).

Preparation of the extract

E. umbellata fruits were kept in a Pasteur oven overnight to remove water. Then, the extraction process of the dried fruits was carried out (Figure 1). Methanol extraction is a method used to isolate compounds from plant materials, such as essential oils, pigments, or other chemical substances (Parekh et. al., 2005). It's one of several solvent extraction techniques, each with its own applications, benefits, and drawbacks (Methanol extraction is highly effective for certain laboratory applications and non-consumable products, but for consumable goods, safer solvents like ethanol or mechanical/physical methods like CO_2 extraction are preferred due to their lower toxicity and environmental impact (Nauman and Arshad, 2011).

Antimicrobial activity results

Antimicrobial activity results of *E.umbellata* extract show that it has activity against six microorganisms (Table 1) . When the zone diameters and MIC values of these microorganisms are examined, it is seen that the antimicrobial activity of the extract is at a medium level.

Although different methods and solvents were used, anti-bacterial properties of *E. umbellata* against *K. pneumoniae*, *S. aureus*, *P. aeruginosa*, *E. coli*, *Propionibacterium acnes*, and *Proteus mirabilis* were demonstrated (Kang et al., 2020; Zulfikar et al., 2022). Uddin & Rauf (2012) investigated the antimicrobial

activity of extracts prepared from *E. umbellata* using different solvents and found that MeOH extract was only effective against Gram-positive bacteria (Uddin & Rauf 2012). In this study, the extract was found to be effective against Gram-positive bacteria as well as Gram-negative bacteria such as *K. pneumoniae*, *S. thymurium*, *C. violaceum*, *P. aeruginosa*.

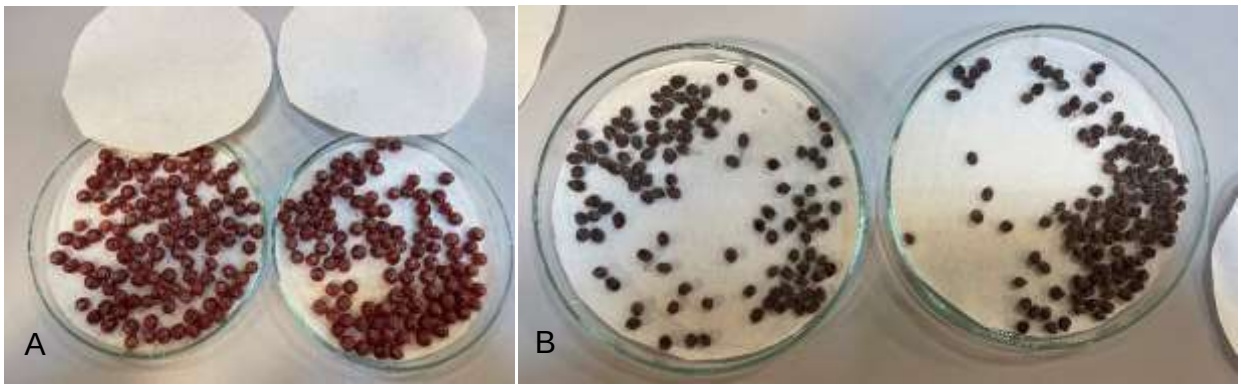

Figure 1. Images of *E.umbellata* before and after incubation A; Before, B; After
Şekil 1. E.umbellata'nın inkübasyon öncesi ve sonrası görüntüleri A; Önce B; Sonra.

Table 1. Antimicrobial activity results of *E. umbellate* MeOH extract
Çizelge 1. E. umbellate MeOH ekstraktının antimikrobiyal aktivite sonuçları

Microorganisms	*K. pneumoniae*	*S. thymurium*	*C. violaceum*	*B. subtilis*	*P.aeruginosa*	*B. cereus*
Zone (mm)	10.33±2.08	10.0±1.0	14.66±0.57	12.66±0.57	13.33±2.08	14±0.0
MIC/MBC (ug/mL)	500/1000	500/1000	250/500	500/1000	500/1000	500/1000

Anti-quorum sensing activity results

It has been determined that *E. umbellata* extract suppresses violacein pigment, one of the quorums sensing steps. (Table 2, Figure 2).

Figure 2. Anti-violacein activity assay result of the extract
Şekil 2. Ekstraktın anti-violasin aktivite deneyi sonucu

Anti-biofilm activity

Considering the biofilm suppression results it was determined that the *E. umbellata* extract had a low level of anti-biofilm activity (Figure 3).

Table 2. Quorum sensing activity assay result of the extract
Çizelge 2. Ekstraktın Quorum sensing aktivite deney sonucu

	C. violaceum ATCC 12472
E. umbellata	+
Positive control (Vanilin)	+

While studies to examine its antimicrobial activity began in 2007 (Sabir et al., 2007), studies involving quorum sensing activity are still lacking today. Antibiofilm activity literature data show that *Elaeagnus angustifolia* plant extract has been studied and that the plant has anti-biofilm activity. Anti-biofilm literature with *E. umbellate* is not yet available. The study is among the first scans in this regard. Likewise, while it was determined that *E. angustifolia* extracts were studied in the literature in terms of quorum sensing scans and the activity was low compared to other plants, *E. umbellate* data will be added to this study (Erdonmezve et al., 2016). The results show that there is activity, albeit at a low level. This is promising for further studies.

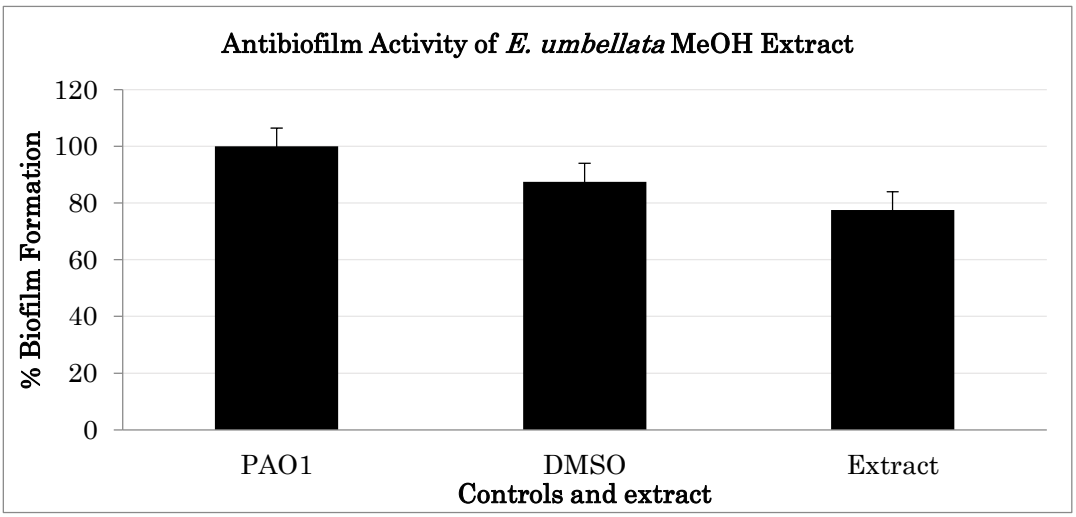

Figure 3. Anti-biofilm activity of *E. umbellate* MeOH extract
Şekil 3.E. umbellate MeOH özütünün anti-biofilm aktivitesi

Results of cytotoxicity assay

It was determined that *E. umbellata* MeOH had mild cytotoxic activity against Vero cells at concentrations of 100 µg/mL and above. On the other hand, it was determined that the extract had no cytotoxic effect on AR42J cells, MDA-MB-231 cells, and A549 cells. The results of the experiment were summarized in Figure 4.

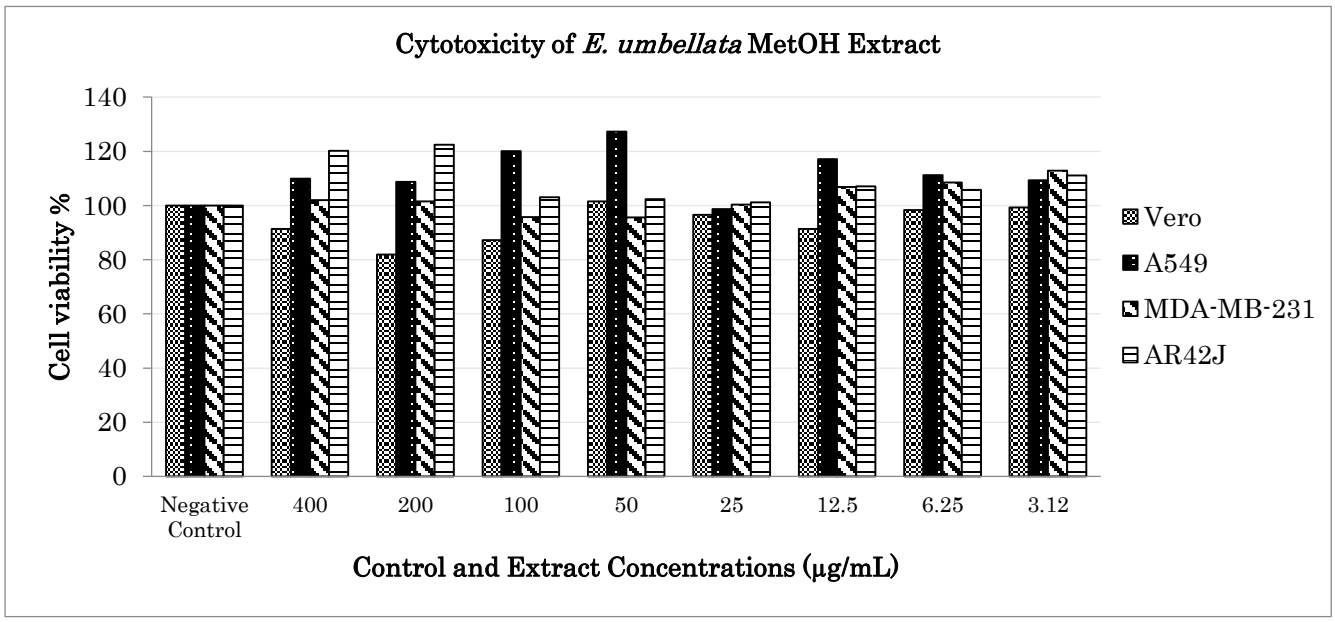

Figure 4. The cytotoxic effect of the extract on different cell lines
Şekil 4. Ekstraktın farklı hücre dizileri üzerindeki sitotoksik etkisi

Wang et al. (2007) investigated the antiproliferative properties of six different genotypes of *E. umbellata* and found that all six genotypes inhibited proliferation of human leukemia cancer cells and human lung epithelial cancer. Aziz et al. (2015) investigated the activity of fruit and leaf parts of *E.umbellata* on human cervical cancer cell line (HeLa) and human colorectal adenocarcinoma cell line (HT29) cells and determined that leaves were more effective than fruits. In current study, the effect of the extract prepared only from the fruit of *E. umbellata* plant on AR42J, MDA-MB-231, A549, and Vero cell lines was investigated, and it was observed that the extract had no cytotoxic effect on the cancer cell lines studied. However, it was found that the extract had a dose-dependent effect on Vero cells.

Antiviral activity assay results

It was observed that the extract included in the study

did not show antiviral activity against HSV-1. The results of the experiment were shown in Figure 5.

There are very few studies on other members of the genus in terms of antiviral studies. A study involving the *Elaeagnus rhamnoides* species reported that the antiviral potential of the genus may be high (Olas and Skalski et al., 2022).

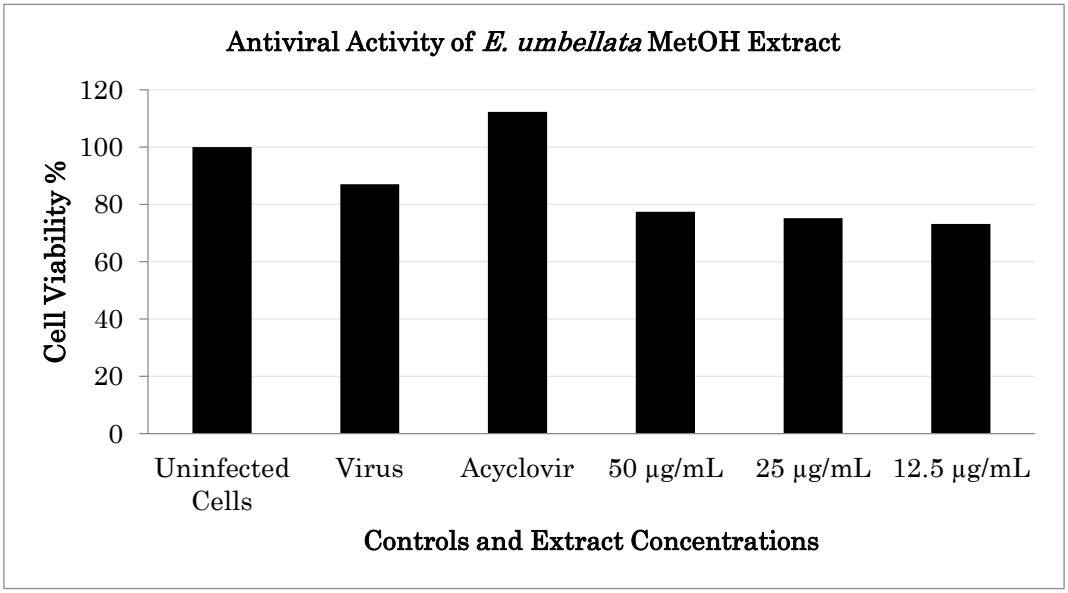

Figure 5. The antiviral activity of the extract on HSV-1. Uninfected cells; uninfected Vero cells, virus; Vero cells infected with virus, acyclovir; positive control

Şekil 5. Ekstraktın HSV-1 ile enfekte olmamış hücreler üzerindeki antiviral aktivitesi; enfekte olmamış Vero hücreleri, virüs; Virüs, asiklovir ile enfekte olmuş Vero hücreleri; pozitif kontrol.

CONCLUSION

Although *E. umbellata* methanol extract had no antiproliferative activity on the cell lines studied and no antiviral activity against HSV-1, it is valuable that it showed antimicrobial activity. In addition, it is noteworthy that small activity was observed in the biofilm study. In line with the data obtained, it suggests that the plant can be evaluated in terms of active substance and is worthy of research.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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REFERENCES

Ahmad, S.D., Sabir, M.S., Juma, M., Asad, H.S. (2005). Morphological and biochemical variations in *Elaeagnus umbellata* Thunb. from mountains of Pakistan. *Acta Botanica Croatica, 64*(1), 121–128.

Ak, B. E., Fidancı, A., & Hatipoglu, I. H. (2022). Schefflera arbicola (Hayata) Merr.Bitkisinin Çelikle Çoğaltılmasında Farklı Yetiştirme Ortamları ile Oksinlerin ve Konsantrasyonların Etkisi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *25*(5), 1023-1027.

Aziz, S., Rehman, H., Andleeb, S. (2015). Biological screening of *Elaeagnus umbellata* Thunb. *Pakistan Journal of Pharmaceutical Sciences, 28*(1),65–70.

Bhat, M. A.,Mishra, A.K., Kamal, M.A., Rahman, S., & Jan, A.T. (2023). Elaeagnus umbellata: A miraculous shrub with potent health-promoting benefits from Northwest Himalaya. *Saudi Journal of Biological Sciences*, *30*(6), 103662.

Cora, M., Buruk, C.K., Unsal, S., Kaklikkaya, N., Kolayli, S. (2023). Chemical Analysis and in Vitro Antiviral Effects of Northeast Türkiye Propolis Samples against HSV-1. *Chemistry & Biodiversity,20*(8), e202300669.

Ekşi, S., Esertaş, Ü.Z.Ü., Kilic, A.O., Ejder, N., Uzunok, B. (2020). Determination of the antimicrobial and anti-biofilm effects and 'quorum sensing' inhibition potentials of Castanea sativa mill. extracts. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48*(1),66–78.

Erdonmez, D., Kenar, N. & Turkmen, K.E. (2016). Evaluation of anti-quorum sensing activity of

Elaeagnus angustifolia L. extracts from Central Anatolia. *Journal of Biotechnology,* 231-234.

Gür, D. (2016). Antibiyotik Duyarlılık Testleri, EUCAST: Uygulama, Yorum ve Uzman Kurallar. *Türk Mikrobiyoloji Cemiyeti Dergisi ,46,* 1-10.

Kang, H.R., Jung, J.K., Park, S.H., Lee, J.Y., Yang, S.A. (2020). Antioxidant, anti-inflammatory, and anti-bacterial effects of extracts from Elaeagnus umbellate leaves obtained using different extract conditions. *Korean Journal Food Preservation, 27*(3),374-384.

Nauman, K., & Arshad, M. (2011). Screening of aqueous methanol plant extracts for their antibacterial activity. In *Science And Technology Against Microbial Pathogens: Research, Development and Evaluation,* 123-127.

Nazir, N., Zahoor, M., Nisar, M., Khan, I., Karim, N., Abdel-Halim, H., Ali, A. (2018). Phytochemical analysis and antidiabetic potential of Elaeagnus umbellata (Thunb.) in streptozotocin-induced diabetic rats: Pharmacological and computational approach. BMC *Complementary and alternative Medicine, 18*, 1-16.

Nazir, N., Zahoor, M., Nisar, M., Khan, I., Ullah, R., Alotaibi, A. (2021). Antioxidants Isolated from Elaeagnus umbellata (Thunb.) Protect against Bacterial Infections and Diabetes in Streptozotocin-Induced Diabetic Rat Model. *Molecules, 26*(15), 4464.

Nazir, N., Zahoor, M., Nisar, M., Karim, N., Latif, A., Ahmad, S., Uddin, Z. (2020). Evaluation of neuroprotective and anti-amnesic effects of Elaeagnus umbellata Thunb. On scopolamine-induced memory impairment in mice. BMC *Complementary Medicine and Therapies, 20*, 1-17.

Olas, B., Skalski, B. (2022). Preparations from Various Organs of Sea Buckthorn (Elaeagnus rhamnoides (L.) A. Nelson) as Important Regulators of Hemostasis and Their Role in the Treatment and Prevention of Cardiovascular Diseases. *Nutrients, 14*(5), 991.

Patel, S. (2015). Plant genus Elaeagnus: underutilized lycopene and linoleic acid reserve with permaculture potential. *Fruits, 70*(4),191–199.

Parekh, J., Jadeja, D., & Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology*, *29*(4), 203-210.

Rashid, M.H., Kornberg, A. (2000). Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of Pseudomonas aeruginosa. *Proceedins of the National Academy of Science, 97*(9),4885-90.

Sabir, M.S., Ahmad, D.S., Imtiaz, H., Tahir, K.M. (2007). Antibacterial activity of Elaeagnus umbellata (Thunb.) a medicinal plant from Pakistan. *Saudi Medical Journal, 28* (2),259.

Saraç, H., & Özpınar, H. (2024). The Effect of Nettle (Urtica dioica L.), Carob (Ceratonia siliqua L.), and Chaste (Vitex agnus-castus L.) Plants on Fertility in Caenorhabditis elegans. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *27*(4), 758-766.

Temel, M., Tinmaz, A. B., Öztürk, M., Gündüz, O. (2018). Dünyada ve Türkiye'de Tıbbi -Aromatik Bitkilerin Üretimi ve Ticareti. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *21*,198-214

Truchado, P., Gil-Izqierdo, A., Tomas-Barberan, F., Allenda, A. (2009). Inhibition by chestnut honey of N-Acyl-L-homoserinelactones and biofilm formation in Erwinia carotovora, Yersinia enterocolitica and Aeromonas hydrophila. *Journal of Agricultural and Food Chemistry*, *57*(23),11186-11193.

Uddin, G. & Rauf, A. (2012). Phytochemical screening and biological activity of the aerial parts of Elaeagnus umbellate. *Scientific Research and Essays 7*(43), 3690–3694.

Üreyen Esertaş, Ü. Z., Kara, Y., Kiliç, A. O., & Kolayli, S. (2022). A comparative study of antimicrobial, anti-quorum sensing, anti-biofilm, anti-swarming, and antioxidant activities in flower extracts of pecan (Carya illinoinensis) and chestnut (Castanea sativa). *Archives of Microbiology, 204*(9), 589.

Wang, S.Y., Bowman, L., Ding, M. (2007). Variations in Free Radical Scavenging Capacity and Antiproliferative Activity Among Different Genotypes of Autumn Olive (Elaeagnus umbellata). *Planta Medica, 73*(5), 468-477.

Woods, G.L., Brown-Elliott, B.A., Desmond, E.P., Hall, G.S., Heifets, L. (2003). Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; *Approved Standard. NCCLS document M24-A, 23* (18).

Wu, M.C., Hu, H.T., Yang, L. (2011). Proteomic analysis of up-accumulated proteins associated with fruit quality during autumn olive (Elaeagnus umbellata) fruit ripening. *Journal of Agricultural Food Chemistry,59*(2),577–83.

Zulfiqar, H., Amjad, M.S., Mehmood, A., Mustafa, G.; Binish, Z.; Khan, S.; Arshad, H.; Proćków, J.; Pérez de la Lastra, J.M. (2022). Antibacterial, Antioxidant, and Phytotoxic Potential of Phytosynthesized Silver Nanoparticles Using Elaeagnus umbellata Fruit Extract. *Molecules, 27*(18),5847.