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ORIGINAL ARTICLE / ÖZGÜN MAKALE

TOTAL ANTIOXIDANT STATUS, ANTIMICROBIAL AND ANTIPROLIFERATIVE POTENTIALS OF *VIOLA ODORATA* (FRAGRANT VIOLET)

VIOLA ODORATA'NIN (KOKULU MENEKŞE) TOPLAM ANTİOKSİDAN DURUMU, ANTİMİKROBİYAL VE ANTİPROLİFERATİF POTANSİYELLERİ

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ABSTRACT

Objective: *Plants are preferred for biological effect. It is a natural resource used in the field of alternative medicine due to its biological effect. In our study, the total oxidant status (TOS) and oxidative stress index (OSI) and total antioxidant status (TAS) of Viola odorata L. species were detected. In addition, antimicrobial and antiproliferative effect of species was detected.*
Material and Method: *The some parts of the species were used with the help of a soxhlet equipment, and ethanol was preferred as a solvent. TOS, OSI and TAS capacitiy were detected using Rel Assay kits. Agar dilution method was preferred to determine antimicrobial effect against bacteria and fungi. Lung cancer cell line (A549) was used to find out the antiproliferative effect by MTT assay.*

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Result and Discussion: *Consequently, the studies, the TAS capacitiy of V. odorata extract was detected as 6.752±0.139, the TOS capacitiy as 7.886±0.224 and the OSI capacitiy as 0.117±0.001. V. odorata extracts were determined to be influential against standard bacteria at 25-100 µg/ml intensiy and against fungi at 100-200 µg/ml intensiy. It was detected that the antiproliferative effect of V. odorata extract increased depending on the extract intensiy and showed strong effects. Consequently, it has been detected that V. odorata has important biological effects and in the pharmaceutical industry, it can be preferred after certain stages.*
Keywords: *Antioxidant, fragrant violet, medicinal plants, oxidant, Viola odorata*

ÖZ

Amaç: *Bitkiler birçok biyolojik aktiviteden sorumludur. Bu kapsamda tamamlayıcı tıpta önemli doğal materyallerdir. Bu çalışmada Viola odorata L. bitkisinin toplam antioksidan durumu (TAS) ve toplam oksidan durumu (TOS) ve oksidatif stress indeksi (OSI) belirlenmiştir. Ayrıca bitkinin antimikrobiyal ve antiproliferatif aktivitesi tespit edilmiştir.*
Gereç ve Yöntem: *Bitkinin toprak üstü kısımlarının etanol ile soxhlet cihazından ekstraksiyon işlemi yapılmıştır. TAS, TOS ve OSI değerleri Rel Assay kitleri kullanılarak belirlendi. Antimikrobiyal aktivite agar dilisyon metodu ile bakteri ve fungus suşlarına karşı test edilmiştir. Antiproliferatif aktivite A549 akciger kanser hücre hattına karşı MTT testi ile test edilmiştir.*
Sonuç ve Tartışma: *Yapılan çalışmalar sonucunda bitki ekstraktının TAS değeri 6.752±0.139, TOS değeri 7.886±0.224 ve OSI değeri 0.117±0.001 olarak belirlenmiştir. Bitki özütleri standart bakterilere karşı 25-100 µg/ml, funguslara karşı 100-200 µg/ml konsantrasyonlarda etkili olduğu görülmüştür. Bitki özütünün antiproliferative aktivitesi özüt konsantrasyonuna bağlı olarak arttığı ve güçlü etkiler gösterdiği belirlenmiştir. Sonuç olarak V. odorata'nın önemli biyolojik aktivitelere sahip olduğu bu kapsamda farmakolojik ilaç dizaynlarında doğal kaynak olarak kullanılabileceği belirlenmiştir.*
Anahtar Kelimeler: *Antioksidan, kokulu menekşe, oksidan, şifalı bitkiler, Viola odorata*

INTRODUCTION

People have used many natural materials for different purposes since ancient times. The environment we live in is the habitat of fungi, plants and animals that contain beneficial compounds for humans. These living organisms, which have an important place in the ecosystem, contain nutrients and metabolic products that are very important in human health [1]. Plants are the living groups that contain the largest number of organisms among these organisms. They have been used to meet basic human needs such as shelter, medicine and food [2]. Medicinal features of plants with important nutritional features have been emphasized by many researchers [3]. Some trials have detected that some plant species preferred in experiments have biological effect such as antiaging, anticancer, antitumor, antiproliferative, DNA damage protective, antimicrobial, antioxidant, antialergic, hepatoprotective and anti-inflammatory [4-7]. Consequently, it is very important to research plants in the discovery of new effects and new natural products.

V. odorata L. is a dwarf, herbaceous and perennial flowering plant of European and Asian origin in the Violaceae family. Common names include tree violet, English violet, garden violet, sweet violet and common violet. It has been widely used in perfumes and cosmetics because of the sweet scent of the flowers of *V. odorata* [8]. *V. odorata*, which is used for many purposes in different region of the world, was used by the French to make violet syrup, in the USA it was used to make syrup, donuts and confectionery. In addition to these features, it is used against respiratory disorders, insomnia and skin disorders [9]. The antimicrobial, antioxidant, oxidant, and antiproliferative effects of the plant used in our study were analyzed.

MATERIAL AND METHOD

V. odorata used in the study was obtained from Duhok (Iraq). Soil and soil-like materials in the sample we used were cleaned. *V. odorata* was ground into powder with the help of some grinding materials. After pulverization, approximately 30 g was weighed. This powdered sample was treated at 50°C for approximately 6 hours. Crude extracts were obtained from the extracts formed consequently

the process with the support of a rotary evaporator device.

Total Oxidant and Antioxidant Tests

The extract kits obtained from the plant were celebrated and their antioxidant and oxidant capacitiy were determined. Total oxidant was detected with TOS kits. Trolox and hydrogen peroxide were prefered as calibrators for TAS and TOS tests. The manufacturer's protocol was followed for the tests [10,11]. The oxidative stress index is formed by the ratio of total oxidant to total antioxidant [12].

Antimicrobial Test

Antimicrobial effect with ethanol extract of *V. odorata* was detected by the agar dilution method. Bacteria used in the experiment were cultured using Mueller Hinton Broth medium. The fungi used in the experiment were added to the culture medium using RPMI 1640 Broth medium. All planted plates were evaluated after they were kept in an oven at 35°C for 16-20 hours for bacteria and 48 hours for fungi.

Test bacteria: *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* MRSA ATCC 43300, *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212

Test fungi: *Candida krusei* ATCC 34135, *Candida glabrata* ATCC 90030 and *Candida albicans* ATCC 10231

All extracts were tested at concentrations of 800-12.5 µg/ml and all dilutions were made with distilled water. Fluconazole, amphotericin B (Fungi) and amikacin, ampicillin and ciprofloxacin (Bacteria) were used as reference drugs. The lowest intensiy that inhibited the position of fungi and bacterial strains was detected. The data obtained were expressed as µg/ml [12-15].

MTT Test

Consequently, the extract obtained from *V. odorata* on the lung cancer cell (A549) were examined using the MTT test. These preferred cells were separated from each other using 3.0 ml of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) after 70-80% confluence. These separated structures were then incubated for approximately 24 hours. Controls were cultured in growth medium without FCS treatment. After 48 hours of incubation, the supernatants were thawed in growth medium. MTT (Sigma) (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) was added to the supernatant at a intensiy of 1 mg/ml. Incubation was carried out at 37°C until a purple precipitate formed was observed and then removed. Dimethyl sulfoxide (DMSO) was added to cell-penetrating MTT (Sigma-Aldrich, MO, USA) to dissolve it. After these procedures, the results were read at 570 nm with the help of Epoch spectrophotometer (BioTek Instruments, Winooska, VT) [16].

RESULT AND DISCUSSION

Total Antioxidant and Oxidant Effect

Plants are natural products responsible for many biological effects. These natural products attract attention especially with their antioxidant features [17]. Antioxidants are attached to combat reactive oxygens. And it usually suppresses [18]. In cases where antioxidant compounds are insufficient to suppress oxidant compounds, oxidative stress occurs. In addition, antioxidants used are important in both reducing and suppressing oxidative stress [19]. Consequently, examination of antioxidant status in plants is important for new studies. In this study, antioxidant and oxidant results of *V. odorata* were found. The results are presented in Table 1.

Table 1. Antioxidant and oxidant effect of *V. odorata* extract

Sample	TAS (mmol/l)	TOS (µmol/l)	OSI
V. odorata	6.752±0.139	7.886±0.224	0.117±0.001

Capacitiy are presented as mean±S.D.

No data on TAS, TOS and OSI capacitiy of *V. odorata* have been found in the literature before. It has been indicated that V. odorata has antioxidant potential using different methods [20-23]. In our study, it was observed that the antioxidant status of *V. odorata* was high. The data we obtained Consequently are in agreement with the literature data. In our study, the total antioxidant capacitiy of *V. odorata* were detected for the first time. In studies on different plant species using this method, TAS capacitiy as 3.628, TOS capacitiy as 4.046 and OSI capacitiy as 0.112 of *Mentha longifolia* ssp. *longifolia*, TAS capacitiy as 7.342, TOS capacitiy as 5.170 and OSI capacitiy as 0.071 of *Rhus coriaria* var. *zebaria*, TAS capacitiy as 5.853, TOS capacitiy as 16.288 and OSI capacitiy as 0.278 of *Allium calocephalum*, TAS capacitiy as 6.328, TOS capacitiy as 11.525 and OSI capacitiy as 0.182 of *Scorzonera papposa*, TAS capacitiy as 8.656, TOS capacitiy as 4.951 and OSI capacitiy as 0.057 of *Rumex scutatus*, TAS capacitiy as 9.490, TOS capacitiy as 14.839 and OSI capacitiy as 0.157 of *Helianthemum salicifolium*, TAS capacitiy as 6.831, TOS capacitiy as 3.712 and OSI capacitiy as 0.054 of *Gundellia tournefortii* have been indicated [24-30]. The TAS capacitiy of *V. odorata* was detected to be higher than *M. longifolia* ssp. *longifolia*, *A. calocephalum*, *S. papposa*, and lower than *R. coriaria* var. *zebaria*, *R. scutatus*, *H. salicifolium* and *G. tournefortii*. Plants contain phenolic compounds with antioxidant effect and electron source feature in many different features and structures. In addition, it is abundance in antioxidant vitamins A, C and E. With these features, they have potentially powerful antioxidant characters [31,32]. TAS capacitiy is an indicator of antioxidant compounds used to reduce the effect of oxidant compounds for living things [33]. Consequently, the determination of TAS capacitiy of plants is important for the detection of new antioxidant sources. In our study, TAS capacitiy of *V. odorata* were detected and it was detected that it has an important antioxidant potential.

The TOS capacitiy of *V. odorata* was detected to be higher than *M. longifolia* ssp. *longifolia*, *R. coriaria* var. *zebaria*, *R. scutatus* and *G. tournefortii*, and lower than *A. calocephalum*, *S. papposa* and *H. salicifolium*. TOS value is an indicator of all oxidant compounds produced in living things [33]. In the literature, the differences in TOS capacitiy attract attention in the studies of different researchers on different plant species. It is thought that the main reason for this is the differences in the regions where the plants are collected, the differences in the plant species and the potential to produce and accumulate oxidant compounds consequently metabolic processes. The OSI capacitiy of *V. odorata* was detected to be higher than *M. longifolia* ssp. *longifolia*, *R. coriaria* var. *zebaria*, *R. scutatus* and *G. tournefortii*, and lower than *A. calocephalum*, *S. papposa* and *H. salicifolium*. The OSI value shows how much the oxidant compounds produced in living organisms are suppressed by endogenous antioxidant compounds [33]. The TOS capacitiy of *V. odorata* detected in our study resulted in lower OSI capacitiy consequently the plant's total antioxidant system being more potent and influential. Consequently, oxidative stress triggered by oxidant molecules could be prevented by being eliminated by antioxidant compounds, which are a reflection of enzymatic and nonenzymatic systems, and consequently, OSI capacitiy were detected at low levels.

Antimicrobial Effect

The study and use of herbal medicine has gained importance in last period. The number of diseases caused by microorganisms has been rising in last years [34]. In the fight against diseases caused by microorganisms, drugs of synthetic origin take the lead. Due to the possible side effects of synthetic drugs and unconsciously used antibiotics, the number of resistant microorganisms has been increasing in last years [35]. Consequently, the research of timely antimicrobial sources has become inevitable in the fight against microbial diseases. The antimicrobial agent status of *V. odorata* ethanol extract against some bacterial and fungal strains was investigated. The analyzed findings are presented in Table 2.

It has been indicated that petroleum ether, dichloromethane, ethyl acetate and aqueous extracts of *V. odorata* are influential against *Klebsiella pneumoniae* and *Escherichia coli* at different intensiy [36]. In another study, it was indicated that petroleum ether, acetone, methanol and aqueous extracts of *V. odorata* had effects against *Haemophilus influenzae*, *Staphylococcus aureus*, *S. pyogenes*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* at different intensiy [37]. It has been indicated that the aqueous extract of *V. odorata* is influential against *Shigella flexneri*, *K. pneumoniae*, *Salmonella typhi*, *P. aeruginosa*, *E. coli* and *S. aureus* [38]. In our research, the ethanol extract of *V. odorata* was preferred. It was detected that the plant extract was influential against *A. baumannii* as 25 µg/ml extract intensiy.

The plant extract was influential against *P. aeruginosa* and *E. faecalis* as 50 µg/ml, against *S. aureus*, *E. coli*, *S. aureus* MRSA, *C. krusei* and *C. glabrata* as 100 µg/ml, against *C. albicans* as 200 µg/ml extract intensiy. Consequently, it has been detected that the antimicrobial effect of the plant extract is high. Consequently, it is thought that the plant can be used as antimicrobial agent.

Table 2. MIC values of *V. odorata* extract

Sample	A	B	C	D	E	F	G	H	J
***V. odorata* Extract (µg/ml)**	100	100	50	100	50	25	100	200	100
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloxacin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Fluconazole	-	-	-	-	-	-	3.12	3.12	-
Amphotericin B	-	-	-	-	-	-	3.12	3.12	3.12

(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei*.

Antiproliferative Effect Against Lung Cancer Cell

In last years, efforts to minimize the possible side effects of many drugs used in cancer treatments, to prevent tissue damage, to increase drug efficacy and to design new drugs have been increasing [39]. Many methods are used in cancer treatments. In addition, different methods can be used together in complexity. These methods often have serious side effects [40]. Many supplements are used to cope with these side effects and accelerate the healing process [41]. Consequently, the discovery of new natural resources to be used in cancer treatments is inevitable. In this study, the antiproliferative effect of the plant species we used against the condition known as the lung cancer cell (A549) was examined. The data found are expressed in Figure 1.

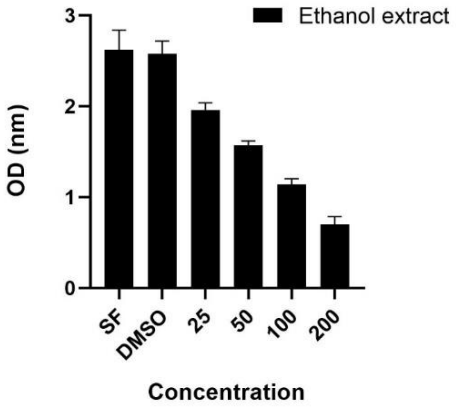

Figure 1. Antiproliferative effect of *V. odorata* extract

In our study, it was detected that the effects of ethanol extract of *V. odorota* against Lung Carcinoma cell (A549) increased with increasing intensiy. Strong cytotoxic effects were observed at 200 µg/ml test intensiy. It has been previously indicated that *V. odorota* has significant effects against prostate (PC-3), breast (MDA-MB-231), and ovarian cancer cell (OVCAR3) [42]. In another study, it was indicated that *V. odorota* was influential at different intensiy against B16F10 murine melanoma cells [43]. In addition to these studies, in our study, it was detected that *V. odorota* has strong effects against Lung Carcinoma cell (A549).

In our research, the antioxidant, antimicrobial and antiproliferative effects of the used parts of *V.*

odorota in the extract formed with the thanks to ethanol, which is preferred as a solvent, were investigated. According to the datas obtained, it was detected that *V. odorota* extract can be used as anticancer agent, antioxidant and antimicrobial.

AUTHOR CONTRIBUTIONS

Concept: M.D., F.S.M., I.U., E.K., M.S.; Design: M.D., F.S.M., I.U., M.P., M.S.; Control: M.D., F.S.M., I.U., M., M.S.; Sources: F.S.M.; Materials: M.D., F.S.M., I.U., K.M., M.S.; Data Collection and/or Processing: M.D., F.S.M., I.U., K.M., M.S.; Analysis and/or Interpretation: M.D., F.S.M., I.U., K.M., E.K., M.P., M.S.; Literature Review: M.D., F.S.M., I.U., K.M., E.K., M.P., M.S.; Manuscript Writing: M.D., F.S.M., I.U., K.M., E.K., M.P., M.S.; Critical Review: M.D., F.S.M., I.U., K.M., E.K., M.P., M.S.; Other: M.D., F.S.M., I.U., K.M., E.K., M.P., M.S.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Wu, W., Chen, W., Liu, S., Wu, J., Zhu, Y., Qin, L., Zhu, B. (2021). Beneficial relationships between endophytic bacteria and medicinal plants. Frontiers in Plant Science, 12, 646146. [CrossRef]
2. Mohammed, F.S., Uysal, I., Sevindik, M. (2023). A review on antiviral plants effective against different virus types. Prospects in Pharmaceutical Sciences, 21(2), 1-21. [CrossRef]
3. Mohammed, F.S., Korkmaz, N., Doğan, M., Şabik, A.E., Sevindik, M. (2021). Some medicinal properties of *Glycyrrhiza glabra* (Licorice). Journal of Faculty of Pharmacy of Ankara University, 45(3), 524-534. [CrossRef]
4. Khan, U.M., Sevindik, M., Zarrabi, A., Nami, M., Ozdemir, B., Kaplan, D.N., Selamoğlu, Z., Hasan, M., Kumar, M., Alshehri, M.M., Sharifi-Rad, J. (2021). Lycopene: Food sources, biological activities, and human health benefits. Oxidative Medicine and Cellular Longevity, 2021, 2713511. [CrossRef]
5. Rehman, M.F.U., Akhter, S., Batool, A.I., Selamoglu, Z., Sevindik, M., Eman, R., Mustaqeem, M., Akram, M.S., Kanwal, F., Lu, C., Aslam, M. (2021). Effectiveness of natural antioxidants against SARS-CoV-2? Insights from the *in-silico* world. Antibiotics, 10(8), 1011. [CrossRef]
6. Manzione, M.G., Herrera-Bravo, J., Sharifi-Rad, J., Kregiel, D., Sevindik, M., Sevindik, E., Selamoglu, Z., Zam, W., Vitalini, S., Hano, C., Kukula-Koch, W., Koch, W., Pezzani, R. (2022). *Desmodium adscendens* (Sw.) DC.: A magnificent plant with biological and pharmacological properties. Food Frontiers, 2022, 1-12. [CrossRef]
7. Korkmaz, N., Dayangaç, A., Sevindik, M. (2021). Antioxidant, antimicrobial and antiproliferative activities of *Galium aparine*. Journal of Faculty of Pharmacy of Ankara University, 45(3), 554-564. [CrossRef]
8. Mittal, P., Gupta, V., Goswami, M., Thakur, N., Bansal, P. (2015). Phytochemical and pharmacological potential of *Viola odorata*. International Journal of Pharmacognosy, 4, 693. [CrossRef]
9. Janbaz, K.H., Khan, W.U., Saqib, F., Khalid, M. (2015). Pharmacological basis for the medicinal use of *Viola odorata* in diarrhea, bronchial asthma and hypertension. Bangladesh Journal of Pharmacology, 10(4), 836-843. [CrossRef]
10. Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry, 37(4), 277-285. [CrossRef]
11. Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry, 38(12), 1103-1111. [CrossRef]
12. Sevindik, M. (2019). The novel biological tests on various extracts of *Cerioporus varius*. Fresenius Environmental Bulletin, 28(5), 3713-3717.
13. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45, 493-496. [CrossRef]
14. Hindler, J., Hochstein, L., Howell, A. (1992). Preparation of routine media and reagents used in antimicrobial susceptibility testing. Part 1. McFarland Standards, In: H.D. Isenberg (ed) Clinical

Microbiology Procedures Handbook, vol. 1. American Society for Microbiology, Washington, D.C. p. 5.19.1-5.19.6.
15. Matuschek, E., Brown, D.F., Kahlmeter, G. (2014). Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection, 20, 255-266. [CrossRef]
16. Bal, C., Akgul, H., Sevindik, M., Akata, I., Yumrutas, O. (2017). Determination of the anti-oxidative activities of six mushrooms. Fresenius Environmental Bulletin, 26(10), 6246-6252.
17. Abubakar, A.R., Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy & Bioallied Sciences, 12(1), 1. [CrossRef]
18. Soares, C., Carvalho, M.E., Azevedo, R.A., Fidalgo, F. (2019). Plants facing oxidative challenges-A little help from the antioxidant networks. Environmental and Experimental Botany, 161, 4-25. [CrossRef]
19. Selamoglu, Z., Sevindik, M., Bal, C., Ozaltun, B., Sen, İ., Pasdaran, A. (2020). Antioxidant, antimicrobial and DNA protection activities of phenolic content of *Tricholoma virgatum* (Fr.) P. Kumm. Biointerface Research in Applied Chemistry, 10(3), 5500-5506. [CrossRef]
20. Akhbari, M., Batooli, H., Kashi, F.J. (2012). Composition of essential oil and biological activity of extracts of *Viola odorata* L. from central Iran. Natural Product Research, 26(9), 802-809. [CrossRef]
21. Katoch, M., Singh, A., Singh, G., Wazir, P., Kumar, R. (2017). Phylogeny, antimicrobial, antioxidant and enzyme-producing potential of fungal endophytes found in *Viola odorata*. Annals of Microbiology, 67(8), 529-540. [CrossRef]
22. Jamshed, H., Siddiqi, H.S., Gilani, A.U.H., Arslan, J., Qasim, M., Gul, B. (2019). Studies on antioxidant, hepatoprotective and vasculoprotective potential of *Viola odorata* and *Wrightia tinctoria*. Phytotherapy Research, 33(9), 2310-2318. [CrossRef]
23. Yousefi, M., Khanniri, E., Shadnoush, M., Khorshidian, N., Mortazavian, A.M. (2020). Development, characterization and *in vitro* antioxidant activity of chitosan-coated alginate microcapsules entrapping *Viola odorata* Linn. extract. International Journal of Biological Macromolecules, 163, 44-54. [CrossRef]
24. Sevindik, M., Akgul, H., Pehlivan, M., Selamoglu, Z. (2017). Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresenius Environmental Bulletin, 26(7), 4757-4763.
25. Mohammed, F.S., Akgul, H., Sevindik, M., Khaled, B.M.T. (2018). Phenolic content and biological activities of *Rhus coriaria* var. *zebaria*. Fresenius Environmental Bulletin, 27(8), 5694-5702.
26. Mohammed, F.S., Karakaş, M., Akgül, H., Sevindik, M. (2019). Medicinal properties of *Allium calocephalum* collected from Gara Mountain (Iraq). Fresenius Environmental Bulletin, 28(10), 7419-7426.
27. Saraç, H., Demirbaş, A., Daştan, S.D., Ataş, M., Çevik, Ö., Eruygur, N. (2019). Evaluation of nutrients and biological activities of Kenger (*Gundellia tournefortii* L.) seeds cultivated in Sivas province. Turkish Journal of Agriculture-Food Science and Technology, 7(sp2), 52-58. [CrossRef]
28. Mohammed, F.S., Günal, S., Şabik, A.E., Akgül, H., Sevindik, M. (2020). Antioxidant and antimicrobial activity of *Scorzonera papposa* collected from Iraq and Turkey. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23(5), 1114-1118. [CrossRef]
29. Mohammed, F.S., Kına, E., Sevindik, M., Doğan, M., Pehlivan, M. (2021). Antioxidant and antimicrobial activities of ethanol extract of *Helianthemum salicifolium* (Cistaceae). Indian Journal of Natural Products and Resources, 12(3), 459-462. [CrossRef]
30. Unal, O., Eraslan, E.C., Uysal, I., Mohammed, F.S., Sevindik, M., Akgul, H. (2022). Biological activities and phenolic contents of *Rumex scutatus* collected from Turkey. Fresenius Environmental Bulletin, 31(07), 7341-7346.
31. Horváth, M., Babinszky, L. (2018). Impact of selected antioxidant vitamins (Vitamin A, E and C) and micro minerals (Zn, Se) on the antioxidant status and performance under high environmental temperature in poultry. A review. Acta Agriculturae Scandinavica, Section A-Animal Science, 68(3), 152-160. [CrossRef]
32. Milisav, I., Ribarič, S., Poljsak, B. (2018). Antioxidant vitamins and ageing. Biochemistry and Cell Biology of Ageing: Part I Biomedical Science, 1-23. [CrossRef]
33. Sevindik, M. (2020). Antioxidant and antimicrobial capacity of *Lactifluus rugatus* and its antiproliferative activity on A549 cells. Indian Journal of Traditional Knowledge, 19(2), 423-427. [CrossRef]
34. González-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M.S., Malouin, F., Bouarab, K. (2009). Plant antimicrobial agents and their effects on plant and human pathogens. International Journal of Molecular Sciences, 10(8), 3400-3419. [CrossRef]
35. Uysal, I., Koçer, O., Mohammed, F.S., Lekesiz, Ö., Doğan, M., Şabik, A.E., Sevindik, E., Gerçeker, F.Ö., Sevindik, M. (2023). Pharmacological and nutritional properties: Genus Salvia. Advances in Pharmacology and Pharmacy, 11(2), 140-155. [CrossRef]

36. Ziad, D., Elias, A., Roula, A.M. (2011). Antibacterial activity of *Rheum rhaponticum*, *Olea europaea*, and *Viola odorata* on ESBL producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. International Journal of Pharmaceutical Sciences and Research, 2(7), 1669.
37. Gautam, S.S., Kumar, S. (2012). The antibacterial and phytochemical aspects of *Viola odorata* Linn. extracts against respiratory tract pathogens. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 82(4), 567-572. [CrossRef]
38. Arora, D.S., Kaur, G.J. (2007). Antibacterial activity of some Indian medicinal plants. Journal of Natural Medicines, 61(3), 313-317. [CrossRef]
39. Sevindik, M., Bal, C. (2021). Antioxidant, antimicrobial, and antiproliferative activities of wild mushroom, *Laeticutis cristata* (Agaricomycetes) from Turkey. International Journal of Medicinal Mushrooms, 23(11), 85-90. [CrossRef]
40. Sevindik, M. (2021). Anticancer, antimicrobial, antioxidant and DNA protective potential of mushroom *Leucopaxillus gentianeus* (Quél.) Kotl. Indian Journal of Experimental Biology, 59(05), 310-315. [CrossRef]
41. Iqbal, J., Abbasi, B.A., Mahmood, T., Kanwal, S., Ali, B., Shah, S.A., Khalil, A.T. (2017). Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine, 7(12), 1129-1150. [CrossRef]
42. Parsley, N.C., Kirkpatrick, C.L., Crittenden, C.M., Rad, J.G., Hoskin, D.W., Brodbelt, J.S., Hicks, L.M. (2018). PepSAVI-MS reveals anticancer and antifungal cycloviolacins in *Viola odorata*. Phytochemistry, 152, 61-70. [CrossRef]
43. Rahimi, V.B., Askari, V.R., Emami, S.A., Tayarani-Najaran, Z. (2017). Anti-melanogenic activity of *Viola odorata* different extracts on B16F10 murine melanoma cells. Iranian Journal of Basic Medical Sciences, 20(3), 242. [CrossRef]