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Evaluation of The Antimicrobial, Antioxidant, and Other Biological Activities of *Tribulus terrestris* Plants Collected From Different Countries

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THE present investigation aimed to assess the antibacterial, antioxidant, anticholinesterase, and antiproliferative properties of *Tribulus terrestris* L. samples obtained from Iraq and Turkey. Furthermore, the total contents of flavonoids and phenols were ascertained. The above-ground parts of the plant samples were extracted with ethanol and Total antioxidant status (TAS), total oxidant status (TOS) as well as oxidative stress index (OSI) were determined. Antimicrobial activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii*, *Candida albicans, C. krusei and C. glabrata* was assessed. Also, the antiproliferative activity was tested against the lung cancer cell line. The results showed that plant extracts were effective against microorganisms at 50-200 µg/mL concentrations. TAS values of the plant samples collected from Iraq and Türkiye were determined as 7.703±0.246 mmol/L and 6.992±0.216 mmol/L, TOS values as 15.983±0.477 µmol/L and 10.595±0.253 µmol/L, and OSI values as 0.207±0.001 and 0.152±0.005, respectively. In addition, the anti-AChE values of the samples collected from Iraq and Türkiye were 51.99±1.78 µg/mL and 74.24±2.35 µg/mL, and the anti-BChE values were 40.85±2.66 µg/mL and 64.43±2.58 µg/mL, respectively. Plant extracts showed strong effects against the A549 cancer cell line in a concentration-dependent manner. In addition, the total phenolic contents of the samples collected from Iraq and Türkiye were determined to be 90.10±3.66 mg/g and 78.24±1.51 mg/g, and the total flavonoid contents were 112.17±3.12 mg/g and 119.50±2.52 mg/g, respectively. It can be concluded that *T. terrestris* plants have strong biological activities that enable them to be applied as food or feed additives as well as pharmaceutical supplements.

Keywords: medicinal plants, bioactivity, bacteria, fungi, free radicals.

Introduction

Nowadays, people around the world are seeking natural products either in food or in medication and many other aspects. Plants with their phytochemical components, flavor, fragrance, and health-promoting properties are an excellent source of bioactive compounds that may be utilized in the production of functional food [1]. Plant bioactive components also make them highly used for medical applications. Numerous plant substances, including carotenoids, flavonoids, and polyphenols, have strong anti-inflammatory and antioxidant properties. These substances shield cells from oxidative stress and inflammation-related damage by neutralizing dangerous free radicals and lowering inflammation. These conditions can lead to chronic illnesses including diabetes, cardiovascular disease, and neurological problems [2].

Concerns about the sustainability of human existence are growing, making the control of microorganisms' detrimental effects more and more important. Although many types of microbes live in biological harmony with the human body and its environs, their unrestrained and rapid expansion can

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lead to some extremely dangerous problems [3]. Antibiotic resistance has led to a change in focus towards biologically active components extracted from plant species that are utilized in herbal medicine. These components have the potential to generate new and powerful sources of antibacterial and antifungal activity [4].

Reactive oxygen species (ROS) and endogenous antioxidants are not balanced in humans, which results in oxidative stress and a chain reaction that damages proteins, DNA, and lipids. By acting as scavengers of reactive free radicals, antioxidants from plant extracts can prevent lipid peroxidation and other associated processes, shielding the body against the illnesses that come from them [5].

Cancer refers to a cell or collection of cells that have skipped the checkpoint and become unchecked, proliferating quickly and unabated. To provide patients with metastases with more effective therapies, it is imperative to find medications with a high selectivity toward cancer cells. Numerous plants have yielded beneficial medications for the management of several malignancies, such as lung cancer [6].

The neurodegenerative illness with the highest prevalence is Alzheimer's disease (AD). It has been proposed that oxidative damage is one of the main causes of AD. It has been recommended to use natural antioxidants instead of synthetic ones to avoid the risks involved. Natural antioxidants have been used as possible leads for the development of new medicines since they are an important part of health and may prevent or delay cell damage [7].

Many studies have reported that plants have many activities such as antiaging, antiallergic, anti-inflammatory, antioxidant, antimicrobial, antiproliferative, anticancer, hepatoprotective, and DNA protective activities [8].

Tribulus terrestris, a member of the Zygophyllaceae family, is also referred to as puncture vine, gokshur, or gokharu. İt is native to the Mediterranean region and has traditionally been used to cure a wide range of illnesses in Chinese and Indian medical systems [9]. In this context, determining the biological activities of such plants is very important for their medicinal use. Our study aimed to determine the antimicrobial, antioxidant, antiproliferative, and anticholinesterase activities of *Tribulus terrestris* L. plants collected from Iraq and Turkey. In addition, the total phenolic and flavonoid contents of the plant were also determined. The present work throws light on *T. terrestris* plant bioactivities which could be further employed in food or medicine.

Material and Methods

Plant collection and extraction

Plant samples were collected from Duhok (Iraq) and Gaziantep (Türkiye). The plant samples that were taken were air-dried and ground. Then thirty grams of each sample were extracted in a soxhlet apparatus at 50C° using 250 milliliters of ethanol (70%) for the extraction process. After extraction the excess solvent was eliminated using a rotary evaporator and the extracts were kept in the freezer until further used.

Antimicrobial analyzes

The antimicrobial activity of extracts prepared from plant samples against bacteria and fungi was determined using the agar dilution method. The tested bacterial and fungal stains are depicted in Table 1. Muller Hinton Broth (bacteria) and Roswell Park Memorial Institute (RPMI) 1640 Medium RPMI 1640 Broth (fungus) media were used as media. Plant extracts were tested for their antimicrobial effects by a range of concentrations (12.5-800 µg/mL). The lowest doses of extract that prevented microorganisms from growing were identified and given as µg/[10, 11].

Antioxidant tests

Antioxidant values of plant samples were measured using Rel Assay kits (MEGA TIP San. Tic. Ltd.Sti, Turkey) following the procedure provided with the kit. Trolox was used as a reference standard for total antioxidant status (TAS) tests and results were expressed as mmol Trolox equivalent/L. Hydrogen peroxide was used reference standard for total oxidant status (TOS) tests and the results were expressed as µmol hydrogen peroxide equivalent/L. Finally, the oxidative stress index (OSI) was determined as [(OSI = TOS/(TASx10)] [12].

Anticholinesterase activity test

Acetylcholinesterase and butyrylcholinesterase activities of plant samples were measured by the Ellman method [13]. The samples have been processed into solutions with concentrations ranging from 200 to 3.125 µg/mL. To prepare the microplate, 130 µL of 0.1 M pH=8 phosphate buffer, and 10 µL of stock solution were mixed with 20 µL of enzyme solution (AChE or BChE). Incubation was done for 10 minutes at 25 °C in the dark. Next, 20 µL of DTNB (5,5"-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL of the substrate (acetylcholine iodide or butyrylcholine iodide) were added and the absorbances were read at 412 nm. The IC50 values for the findings were computed and given in µg/mL.

Total Phenolic and Total Flavonoid Tests

Sample extracts (1mL) were mixed with Folin-Ciocalteu reagent (1mL) and Na_2CO_3 (0.75 mL) and incubated for 2 hours. Then, measurements were

made spectrophotometrically at 760 nm using a calibration curve of gallic acid standard solution, the total phenolic content was expressed in mg/g [14].

Total flavonoid content was determined by the aluminum chloride test [15]. 0.1 mL $Al(NO_3)_3$ (10%), 0.1 mL potassium acetate (1 M), 4.3 mL methanol, and 0.5 mL plant extract were mixed and incubated for 40 minutes. Then, absorbance was measured at 415 nm using Quercetin as reference standard, and total flavonoid content was expressed in mg/g.

Antiproliferative tests

The antiproliferative activity of plant extracts against the A549 lung cancer cell line was determined by the MTT test, a reduction assay that assesses cellular metabolic activity and is indicative of cell viability. Different concentrations of plant extracts were used 25, 50, 100, and 200 µg/mL and the plates were read at 570 nm [16].

Results and Discussion

Antioxidant properties of Tribulus terrestris

Oxidant substances are free radicals formed as a result of several metabolic processes. Their rise can cause cellular damage [17], and the antioxidant defense system helps to mitigate this impact [18]. Oxidative stress is caused by an imbalance between the antioxidant defense system and oxidant substances [19], which can lead to serious illnesses including cancer, cardiovascular problems, and neurological diseases. Supplemental antioxidants can reduce oxidative stress [20]. In our study, the antioxidant status of *T. terrestris* was determined and the results are shown in Table 2.

To the best of our knowledge, TAS, TOS, and OSI values of *Tribulus terrestris* have not been previously reported in the literature. The TAS value reflects the total amount of chemicals having antioxidant potential in natural products [28]. It was observed that the antioxidant potential of the *T. terrestris* samples collected from Iraq used in our study was higher than the samples collected from Türkiye. In addition, it has been observed that the plant has higher potential compared to other plants such as *Viola odorata, Alcea kurdica, Galium aparine, Silybum marianum,* and *Ferulago platycarpa* previously investigated using the same method. The TOS value represents the total amount of oxidant-active chemicals created by natural products as a result of environmental factors and metabolic activity [28]. In the present work, TOS value of *Tribulus terrestris* was found to be higher in the samples collected from Iraq than in the samples collected from Türkiye. In addition, the TOS value of *T. terrestris* samples collected from Iraq was determined to be higher than *V. odorata, A. kurdica, H. salicifolium, G. glabra, S. marianum* and *F. platycarpa*, and lower than *G. aparine*. Whereas, the TOS values of *Tribulus terrestris samples* collected from Türkiye were higher than *Viola odorata* and *Alcea kurdica*, and lower than *Helianthemum salicifolium, Glycyrrhiza glabra, Silybum marianum, Galium aparine* and *Ferulago platycarpa*. It could be noticed that as the regions where the plant samples were collected changed, the levels of oxidant compounds produced within it changed. Nevertheless, the fact that the plant generally had high TOS values. The OSI value shows the percentage of antioxidant compounds suppressing endogenous oxidant compounds [24]. The data indicated that *Tribulus terrestris* samples collected from Iraq had higher OSI value than compared to that from Türkiye. Also, OSI values of *Tribulus terrestris* samples from Iraq were higher than *Viola odorata, Helianthemum salicifolium, Glycyrrhiza glabra,* and lower than *Alcea kurdica, Galium aparine, Silybum marianum* and *Ferulago platycarpa*. While the OSI values of *Tribulus terrestris* from Türkiye were higher than *Viola odorata* and lower than *Alcea kurdica, Helianthemum salicifolium, Galium aparine, Glycyrrhiza glabra, Silybum marianum* and *Ferulago platycarpa*. In this context, *Tribulus terrestris* could be regarded as an important natural antioxidant source.

Total phenolic and total flavonoid contents

Plants have the potential to produce many biologically active compounds. It is known that these compounds with different properties have different effects. In our study, total phenolic and total flavonoid contents of *T. terrestris* were determined and depicted in Table 3. The total phenolic contents and total flavonoid contents of the *T. terrestris* samples collected from both Iraq and Türkiye used in our study were higher than the values previously reported by Patil et al. [29] for the ethanol extract of *T. terrestris* being 41.2 mg/g and 601.3 mg/g for total phenols and total flavonoid content; respectively. It is thought that this difference arises from the difference in the regions where the plants used are collected.

Anticholinesterase effect

Neurodegeneration has been established as the essential pathophysiological alteration in most brain-related illnesses. Despite contemporary science's ongoing efforts to provide a medicinal or surgical remedy, the results have been unfavorable. Most elderly adults continue to have clinical concerns about neurodegenerative illnesses such as Alzheimer's [30]. Natural products could offer a good supplement to inhibit cholinesterase and combat this illness. In our study, the anti-AChE and anti-BChE potentials of *T. terrestris* collected from Iraq and Türkiye were determined and the obtained IC_{50} values are shown in Table 4.

In our study, it was determined that the anti-AChE and anti-BChE potentials of *T. terrestris*

collected from Türkiye were higher than the samples collected from Iraq. In addition, it was determined that both samples exhibited higher activity than galantamine used as a control. The presence of enzymes that cause disease etiology and their suppression may be very beneficial in disease treatment [31]. It was determined that *T. terrestris* used in our study had anticholinesterase activity. It was also determined to have regionally varying effects.

Antimicrobial activity

In recent years, the treatment of many microbial diseases has become quite difficult. In particular, the emergence of resistant microorganisms has made it difficult to combat its effects [32]. The possible side effects of synthetic drugs and the insufficient effects of the antimicrobial drugs used have necessitated the discovery of new antimicrobial drugs (Sevindik et al., 2023). Thus, determining the potential antimicrobial activities of plants is very important for new drug designs [33]. In our study, the effects of *T. terrestris* samples collected from Iraq and Türkiye against bacterial and fungal strains were investigated and the results for the minimum extract concentration that inhibited microbial growth are shown in Table 5.

It has been reported in the literature that the methanol extract of *T. terrestris* collected from India is effective against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* [34]. In a different study conducted in India, it was reported that Tribulus terrestris had effects against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogens* [35]. In a study conducted in Iran, it was reported that the water extract of *T. terrestris* was effective against *Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis*, *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella flexneri, Salmonella typhimurium, Candida kruzei* and *Candida albicans* [36]. In our study, the tested extract showed different activity against different bacteria, it was determined that the samples collected from Türkiye generally showed a higher antimicrobial effect. In addition, it was determined that samples from both regions showed the highest effectiveness against *A. baumannii* among microorganisms. As a result, it was determined that *T. terrestris* has antimicrobial potential.

Antiproliferative activity

Cancer cases have been increasing in recent years. Different types of treatments come to the fore in cancer treatment. However, in addition to these treatments, supportive treatments are very beneficial in improving the health of patients. Herbal treatments are important supportive resources in cancer cases. In this context, determining the anticancer activities of plants is very important in terms of their potential use. In our study, the effects of *T. terrestris* against the A549 human lung adenocarcinoma cell line was determined and the findings are shown in Figure 1.

It has been reported in the literature that hexane, ethyl acetate, methanol, and aqueous extracts of *T. terrestris* are effective against the A549 human lung adenocarcinoma cell line [37]. In our study, the ethanol extract of Tribulus terrestris collected from Iraq and Türkiye was used and its effect against the A549 human lung adenocarcinoma cell line was investigated. As a result of the study, it was determined that the samples collected from Türkiye showed higher activity than the samples collected from Iraq. In addition, it was determined that the proliferation of the samples used in the study increased due to the increase in concentration. In this context, it was determined that *T. terrestris* could be a natural anticancer agent.

Conclusion

It could be concluded that *T. terrestris* exhibits robust and diverse biological activities including antibacterial, antioxidant, antiproliferative, and anticholinesterase characteristics. *T. terrestris* could be considered a natural antioxidant and antimicrobial agent that may be employed in food preservation or as a food/ feed additive. Also, the anticancer antiproliferative, and anticholinesterase activities of the plant make it a good candidate for pharmaceutical applications. Further studies could be addressed by extracting and purifying the bioactive phytochemicals of *T. terrestris* plant and identifying the mechanisms behind its bioactivities.

Author Contributions

Conceptualization, G.A.E., O.K., N.K., İ.U., S.R. and M.S.; methodology, G.A.E., O.K., N.K., İ.U., S.R. and M.S.; investigation, G.A.E., O.K., N.K., İ.U., S.R. and M.S.; data curation, G.A.E., O.K., N.K., İ.U., S.R. and M.S.; writing-original draft preparation, G.A.E., O.K., N.K., İ.U., S.R. and M.S.; writing-review and editing, G.A.E.; M.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical consideration

Not applicable for this work.

TABLE 1. Tested bacteria and fungi

Tested bacterial stains	Tested fungal strains
Staphylococcus aureus ATCC 29213	*Candida albicans* ATCC 10231
S. aureus MRSA ATCC 43300	*C. krusei* ATCC 34135
Enterococcus faecalis ATCC 29212	*C. glabrata* ATCC 90030
Escherichia coli ATCC 25922	
Pseudomonas aeruginosa ATCC 27853	
Acinetobacter baumannii ATCC 19606	

TABLE 2. TAS, TOS, OSI values of *Tribulus terrestris* extracts compared to previously studied plants

Plant	TAS (mmol trolox equivalent/L)	TOS (µmol hydrogen peroxide equivalent/L)	OSI (arbitrary unit)	References
***Tribulus terrestris* (Iraq)**	7.703±0.246	15.983±0.477	0.207±0.001	Present work
***Tribulus terrestris* (Türkiye)**	6.992±0.216	10.595±0.253	0.152±0.005	Present work
Viola odorata	6.752	7.886	0.117	[21]
Alcea kurdica	3.298	8.312	0.252	[22]
Helianthemum salicifolium	9.490	14.839	0.157	[23]
Galium aparine	5.147	18.679	0.346	[24]
Glycyrrhiza glabra	8.770	14.590	0.167	[25]
Silybum marianum	5.767	12.144	0.211	[26]
Ferulago platycarpa	5.688	15.552	0.273	[27]

TABLE 3. Total phenolic content and total flavonoids of *Tribulus terrestris*

Plant	TPC (mg gallic acid equivalent/g)	TFC (mg quercetin equivalent/g)
***Tribulus terrestris* (Iraq)**	90.10±3.66	112.17±3.12
***Tribulus terrestris* (Türkiye)**	78.24±1.51	119.50±2.52

TABLE 4. Anti-AChE and anti-BChE potentials of Tribulus terrestris

Samples	AChE (µg/mL)	BChE (µg/mL)
***Tribulus terrestris* (Iraq)**	51.99±1.78	40.85±2.66
***Tribulus terrestris* (Türkiye)**	74.24±2.35	64.43±2.58
Control (Galantamine cholinesterase inhibitor)	11.44±1.31	20.68±1.62

TABLE 5. Antimicrobial potential of *Tribulus terrestris*

Tested Microorganisms	Minium extract inhibitory concentration (µg/mL)	
	***Tribulus terrestris* (Iraq)**	***Tribulus terrestris* (Türkiye)**
S. aureus	100	100
***S. aureus* MRSA**	200	100
E. faecalis	200	200
E. coli	200	100
P. aeruginosa	100	100
A. baumannii	50	50
C. glabrata	100	100
C. albicans	200	100
C. krusei	200	100

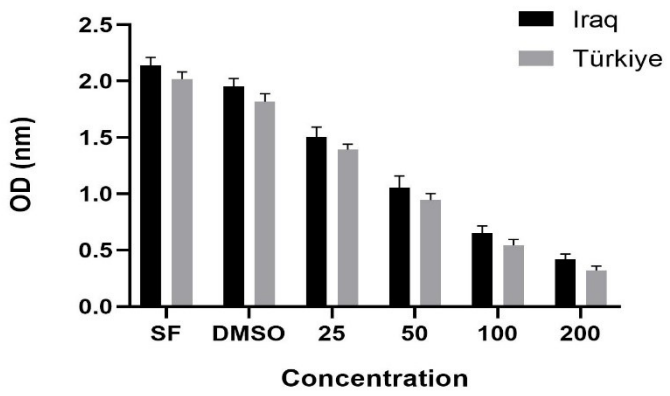

Fig. 1. Antiproliferative Effects of *Tribulus terrestris*
*25, 50, 100 and 200 extracts concentration

References

1. Maleš, I., Pedisić, S., Zorić, Z., Elez-Garofulić, I., Repajić, M., You, L., Vladimir-Knežević, S., Butorac, D. and Dragović-Uzelac, V. The medicinal and aromatic plants as ingredients in functional beverage production. *Journal of Functional Foods*, **96**, 105210(2022). https://doi.org/https://doi.org/10.1016/j.jff.2022.105210

2. Kowalczewski, P. Ł. and Zembrzuska, J. Advances in Biological Activities and Application of Plant Extracts. *Applied Sciences (Switzerland)*, **13**(16), 9324 (2023). https://doi.org/10.3390/app13169324

3. Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., RamaKrishna, S. and Berto, F. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. *Antioxidants (Basel, Switzerland)*, **9**(12) (2020). https://doi.org/10.3390/antiox9121309

4. Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., Kabli, S. A., Alsubki, R. A., Tawfik, A. M. and Marouf, S. A. Antimicrobial effect of different herbal plant extracts against different microbial population. *Saudi Journal of Biological Sciences*, **27**(12), 3221–3227 (2020). https://doi.org/10.1016/j.sjbs.2020.08.015

5. Joshi, T., Mandal, S. K., Puri, S., Asati, V., Deepa, P. R. and Sharma, P. K. Investigating the antioxidant activity enhancer effect of Cyamopsis tetragonoloba seed extract on phenolic phytochemicals. *Frontiers in Plant Science*, **14**(3), 1–11 (2023). https://doi.org/10.3389/fpls.2023.1131173

6. Albinhassan, T. H., Saleh, K. A., Barhoumi, Z., Alshehri, M. A. and Al-Ghazzawi1, A. M. Anticancer, anti-proliferative activity of Avicennia marina plant extracts. *Journal of Cancer Research and Therapeutics*, **17**(4), 879–886 (2021).

7. Nwidu, L. L., Elmorsy, E., Aprioku, J. S., Siminialayi, I. and Carter, W. G. In Vitro Anti-Cholinesterase and Antioxidant Activity of Extracts of Moringa oleifera Plants from Rivers State, Niger Delta, Nigeria. *Medicines (Basel, Switzerland)*, **5**(3) 3390(2018). https://doi.org/10.3390/medicines5030071

8. El-Chaghaby, G. A., Mohammed, F. S., Rashad, S., Uysal, I., Koçer, O., Lekesiz, Ö., Doğan, M., Şabik, A. E. and Sevindik, M. Genus Hypericum: General Properties, Chemical Contents and Biological Activities. *Egyptian Journal of Botany*, **64**(1), 1–26 (2024). https://doi.org/10.21608/ejbo.2023.217116.2378

9. Pokrywka, A., Morawin, B., Krzywański, J. and Zembroń-Lacny, A. 9 - An Overview on Tribulus terrestris in Sports Nutrition and Energy Regulation. In D. Bagchi (Ed.), *Sustained Energy for Enhanced Human Functions and Activity,* (pp. 155–165) (2017). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-805413-0.00009-0

10. Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**(4), 493–496 (1966).

11. Matuschek, E., Brown, D. F. J., Kahlmeter, G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, **20**(4), O255-66 (2014). https://doi.org/10.1111/1469-0691.12373

12. Sevindik, M. The novel biological tests on various extracts of Cerioporus varius. *Fresenius Environmental Bulletin*, **28**(5), 3713–3717 (2019).

13. Ellman, G. L., Courtney, K. D., Andres, V. J. and Feather-Stone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7**(88), 951–961(1961). https://doi.org/10.1016/0006-2952(61)90145-9

14. Bal, C., Sevindik, M. and Cem, E. E. Antıoxıdant, antımıcrobıal actıvıtıes, total phenolıc and element contents of wıld edıble mushroom bovısta nıgrescens. *Prospects in Pharmaceutical Sciences*, **21**(2), 37–41(2023).

15. Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. C. Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, **10**(3), 178–182 (2002). https://doi.org/10.38212/2224-6614.2748

16. Bal, C., Akgul, H., Sevindik, M., Akata, I. and Yumrutas, O. Determination of the Anti-Oxidative Activities of Six Mushrooms. *Fresenius Environmental Bulletin*, **26**(10), 6246–6252 (2017).

17. Mohammed, F. S., Kına, E., Uysal, İ. and Sevindik, M. Total Phenolic, Flavonoid Contents, Antioxidant and Antimicrobial Activities of Hesperis pendula. *Prospects in Pharmaceutical Sciences*, **21**(2), 57–61 (2023). https://doi.org/10.56782/pps.135

18. Krupodorova, T. and Sevindik, M. Antıoxıdant potentıal and some mıneral contents of wıld edıble mushroom *ramaria stricta. AgroLife Scientific Journal*, **9**(1), 186–191 (2020).

19. Sevindik, M. and Akata, I. Antioxidant, oxidant potentials and element content of edible wild mushroom Helvella leucopus. *Indian Journal of Natural Products and Resources*, **10**(4), 266–271 (2019).

20. Saridogan, B. G. O., Islek, C., Baba, H., Akata, I., and Sevindik, M. Antioxidant antimicrobial oxidant and elements contents ofxylaria polymorpha and x hypoxylon (xylariaceae). *Fresenius Environmental Bulletin*, **30**(5), 5400–5404(2021).

21. Doğan, M., Mohammed, F. S., Uysal, İ., Mencik, K., Eylem, K. I. N. A., Pehlivan, M. and Sevindik, M. Total antioxidant status, antimicrobial and antiproliferative potentials of Viola odorata (Fragrant Violet). *Journal of Faculty of Pharmacy of Ankara University,* **47**(3), 7-7 (2023).

22. Mohammed, F. S., Sevindik, M., Uysal, I., Sevindik, E. and Akgül, H. A natural material for suppressing the effects of oxidative stress: biological activities of Alcea kurdica. *Biology Bulletin*, **49**(2), S59-S66. (2022).

23. Mohammed, F. S., Kına, E., Sevindik, M., Doğan, M. and Pehlivan, M. Antioxidant and antimicrobial activities of ethanol extract of Helianthemum salicifolium (Cistaceae). *Indian Journal of Natural Products and Resources* (IJNPR), **12**(3), 459-462 (2021).

24. Korkmaz, N., Dayangaç, A. and Sevindik, M. Antioxidant, antimicrobial and antiproliferative activities of Galium aparine. *Journal of Faculty of Pharmacy of Ankara University,* **45**(3), 554-564 (2021).

25. Mohammed, F. S., Korkmaz, N., Doğan, M., Şabik, A. E. and Sevindik, M. Some medicinal properties of Glycyrrhiza glabra (Licorice). *Journal of Faculty of Pharmacy of Ankara University,* **45**(3), 524-534 (2021).

26. Mohammed, F. S., Pehlivan, M. and Sevindik, M. Antioxidant, antibacterial and antifungal activities of different extracts of Silybum marianum collected from Duhok (Iraq). *International Journal of Secondary Metabolite,* **6**(4), 317-322 (2019).

27. Mohammed, F. S., Günal, S., Pehlivan, M., Doğan, M., Sevindik, M. and Akgül, H. Phenolic content, antioxidant and antimicrobial potential of endemic Ferulago platycarpa. *Gazi University Journal of Science,* **33**(4), 670-677 (2020).

28. Gürgen, A. and Sevindik, M. Application of artificial neural network coupling multiobjective particle swarm optimization algorithm to optimize Pleurotus ostreatus extraction parameters. *Journal of Food Processing and Preservation*, **46**(11), e16949 (2022). https://doi.org/https://doi.org/10.1111/jfpp.16949

29. Patil, N. B., Adsul, V. B., Khatiwora, E., Kale, A. A., Tambe, S. P. and Deshpande, N. R. Spectroscopic determination of total phenolic and flavonoid contents of Tribulus terrestris fruits. *International Journal of ChemTech Research*, **4**(3), 899–902 (2012).

30. Lamptey, R. N. L., Chaulagain, B., Trivedi, R., Gothwal, A., Layek, B. and Singh, J. A Review of the Common Neurodegenerative Disorders: Current Therapeutic Approaches and the Potential Role of Nanotherapeutics. *International Journal of Molecular Sciences*, **23**(3), 23031851(2022). https://doi.org/10.3390/ijms23031851

31.Świątek, Ł., Sieniawska, E., Sinan, K. I., Maciejewska-Turska, M., Boguszewska, A., Polz-Dacewicz, M., Senkardes, I., Guler, G. O., Bibi Sadeer, N., Mahomoodally, M. F. and Zengin, G. LC-ESI-QTOF-MS/MS Analysis, Cytotoxic, Antiviral, Antioxidant, and Enzyme Inhibitory Properties of Four Extracts of Geranium pyrenaicum Burm. f.: A Good Gift from the Natural Treasure. *International Journal of Molecular Sciences*, **22**(14), 22147621 (2021). https://doi.org/10.3390/ijms22147621

32. Mohammed, F. S., Uysal, İ., and Sevindik, M. a Review on Antiviral Plants Effective Against Different Virus Types. *Prospects in Pharmaceutical Sciences*, **21**(2), 1–21 (2023). https://doi.org/10.56782/pps.128

33. . Eraslan, E. C., Altuntas, D., Baba, H., Bal, C., Akgül, H., Akata, I. and Sevindik, M. Some Biological Activities and Element Contents of Ethanol Extract of Wild Edible Mushroom Morchella Esculenta. *Sigma Journal of Engineering and Natural Sciences*, **39**(1), 24–28 (2021).

34. Baburao, B., Rajyalakshmi, G., Venkatesham, A., Kiran, G., Sunder, A. S. and Rao, B. G. Anti-Inflammatory and Antimicrobial Activities of Methanolic Extract of Tribulus Terrestris Linn Plant. *Int. J. Chem. Sci.*, **7**(3), 1867–1872 (2009).

35. Gopinath, V., MubarakAli, D., Priyadarshini, S., Priyadharsshini, N. M., Thajuddin, N. and Velusamy, P. Biosynthesis of silver nanoparticles from Tribulus terrestris and its antimicrobial activity: a novel biological approach. *Colloids and Surfaces. B, Biointerfaces*, **96**, 69–74 (2012). https://doi.org/10.1016/j.colsurfb.2012.03.023

36. Vala, M. H., Makhmor, M., Kobarfar, F., Kamalinejad, M., Heidary, M. and Khoshnood, S. Investigating of the antimicrobial effect of total extract of Tribulus terrestris against some gram positive and negative bacteria and candida spp. *Novelty in Biomedicine*, **2**(3), 85 (2014).

37. Alshabi, A. M., Alkahtani, S. A., Shaikh, I. A., Orabi, M. A. A., Abdel-Wahab, B. A., Walbi, I. A., Habeeb, M. S., Khateeb, M. M., Shettar, A. K. and Hoskeri, J. H. Tribulus terrestris Cytotoxicity against Breast Cancer MCF-7 and Lung Cancer A549 Cell Lines Is Mediated via Activation of Apoptosis, Caspase-3, DNA Degradation, and Suppressing Bcl-2 Activity. *Separations*, **9**(11) (2022). https://doi.org/10.3390/separations9110383

تقييم إمكانات مضادات الميكروبات، وخصائص مضادات الأكسدة، والأنشطة البيولوجية الأخرى لنباتات تريبولوس تيريستريس التي تم جمعها من بلدان مختلفة

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الملخص

يهدف البحث الحالي إلى تقييم الخصائص المضادة للبكتيريا، ومضادات الأكسدة، ومضادات الكولينستراز، والخصائص المضادة للتكاثر لعينات تريبولوس تيريستريس التي تم الحصول عليها من العراق وتركيا. وعلاوة على ذلك، تم التأكد من المحتوى الكلي للفلافونويدات والفينولات. تم استخلاص الأجزاء الموجودة فوق سطح الأرض من العينات النباتية باستخدام الإيثانول وتم تحديد حالة مضادات الأكسدة الكلية (TAS)، وحالة الأكسدة الكلية (TOS) وكذلك مؤشر الإجهاد التأكسدي (OSI). تم تقييم النشاط المضاد للميكروبات ضد المكورات العنقودية الذهبية، المكورات المعوية البرازية، الإشريكية القولونية، الزائفة الزنجارية، الراكدة البومانية، المبيضات البيضاء، C. krusei وC. glabrata. كما تم اختبار النشاط المضاد للتكاثر ضد خط خلايا سرطان الرئة. أظهرت النتائج أن المستخلصات النباتية كانت فعالة ضد الكائنات الحية الدقيقة بتركيزات 50-200 ميكروغرام/مل. تم تحديد قيم TAS للعينات النباتية التي تم جمعها من العراق وتركيا على أنها 7.703 ± 0.246 و6.992 ± 0.216، وقيم TOS على أنها 15.983 ± 0.477 و10.595 ± 0.253، وقيم OSI على أنها 0.207 ± 0.001 و 0.152 ± 0.005 على التوالي. بالإضافة إلى ذلك، كانت قيم مضادات AChE في العينات التي تم جمعها من العراق وتركيا 51.99 ± 1.78 و74.24 ± 2.35، وكانت قيم مضادات 2.66 ± 40.85 BChE و64.43 ± 2.58 على التوالي. أظهرت المستخلصات النباتية تأثيرات قوية ضد خط الخلايا السرطانية A549 بطريقة تعتمد على التركيز. بالإضافة إلى ذلك، تم تحديد إجمالي محتويات الفينول للعينات التي تم جمعها من العراق وتركيا لتكون 90.10 ± 3.66 و 78.24 ± 1.51، وكان إجمالي محتوى الفلافونويد 112.17 ± 3.12 و 119.50 ± 2.52 على التوالي. يمكن أن نستنتج أن نباتات تريبولوس تيريستريس لها أنشطة بيولوجية قوية تمكنها من استخدامها كإضافات غذائية أو علفية وكذلك كمكملات دوائية.

الكلمات الدالة: النباتات الطبية ، النشاط الحيوي ، بكتيريا ، الفطريات ، الشوارد الحرة.