KSÜ Tarım ve Doğa Derg 27 (Ek Sayı 1), 1-15, 2024
KSU J. Agric Nat 27 (Suppl. 1), 1-15, 2024
DOI:10.18016/ksutarimdoga.vi.1472403

# Determination of *in vitro* Antioxidant, Anticholinergic, and Antiepileptic Activities of some Medicinal and Aromatic Plant Extracts

Bayram YURT[1], Rüya SAĞLAMTAŞ[2], Yeliz DEMİR[3], Ebubekir İZOL[4]✉, Halit DİRİL[5], Cüneyt ÇAĞLAYAN[6]
[1]Department of Food Engineering, Faculty of Engineering and Architecture, Bingöl University, Bingöl, Türkiye, [2] Vocational School of Health Services, Department of Medical Services and Technology, Agri Ibrahim Cecen University, Agri, Türkiye , [3] Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, Ardahan, Türkiye , [4] Bee and Natural Products R&D and P&D Application and Research Center, Bingöl University, Bingöl, Türkiye, [5] Medical Biochemistry Laboratory, Dursun Odabaş Medical Center, Van Yüzüncü Yıl University, Van, Türkiye, [6] Department of Medical Biochemistry, Faculty of Medicine, Bilecik Şehy Edebali University, Bilecik, Türkiye
[1]https://orcid.org/0000-0001-5447-1586, [2]https://orcid.org/0000-0002-4400-2302, [3]https://orcid.org/0000-0003-3216-1098
[4]https://orcid.org/0000-0003-0788-4999, [5]https://orcid.org/0000-0003-4409-8268, [6]https://orcid.org/0000-0001-5608-554X
✉: eizol@bingol.edu.tr

## ABSTRACT

Medicinal and aromatic plants such as *Crocus cancellatus*, and *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* have many different biological activities. While antioxidants are significant in preventing many diseases, inhibition of metabolic enzymes is also effective in preventing many diseases. In this study, antioxidant activities of water, ethanol, and dichloromethane extracts of four different medicinal and aromatic plant species were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and 2,20-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS•+) radical scavenging and $Cu^{2+}$, $Fe^{+3}$, and $Fe^{3+}$-2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) reducing assays. Enzyme inhibition studies were performed with metabolic enzymes acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase I and II isoenzymes. The ethanol extract of *A. nemorosa* showed the highest activity in DPPH and ABTS assays ($IC_{50}$: 17.36 µg $mL^{-1}$, $IC_{50}$: 7.02 µg $mL^{-1}$). In the $Fe^{3+}$ reducing assay, the dichloromethane extract of *A. nemorosa* showed the highest activity (1.96±0.060 µg $mL^{-1}$). In the $Cu^{2+}$ reducing assay, the dichloromethane extract of *J. oxycedrus* showed the highest activity (1.773±0.066 µg $mL^{-1}$). In the $Fe^{3+}$-TPTZ reducing assay, the ethanol extract of *S. siberica* showed the highest activity (1.256±0.011 µg $mL^{-1}$). In the enzyme inhibition results, it was determined that all plants and all extracts inhibited the enzymes studied. As a result of this study, it was determined that these four medicinal and aromatic plants have high biological activities.

**Biochemistry**

**Research Article**

**Article History**
Received : 23.04.2024
Accepted : 09.08.2024

**Keywords**
*Crocus cancellatus,*
*Scilla siberica*
*Juniperus oxycedrus*
*Anthriscus nemorosa*
Antioxidant and enzyme inhibition

# Bazı Tıbbi ve Aromatik Bitki Ekstraktlarının *in vitro* Antioksidan, Antikolinerjik ve Antiepileptik Aktivitelerinin Belirlenmesi

## ÖZET

*Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa gibi* tıbbi ve aromatik bitkiler birçok farklı biyolojik aktiviteye sahiptir. Antioksidanlar birçok hastalığın önlenmesinde önemli rol oynarken, metabolik enzimlerin inhibisyonu da birçok hastalığın önlenmesinde etkilidir. Bu çalışmada, dört farklı tıbbi ve aromatik bitki türünün su, etanol ve diklorometan ekstraktlarının antioksidan aktiviteleri 1,1-difenil-2-pikrilhidrazil (DPPH•) ve 2,20-azino-bis-3-etilbenzthiazoline-6-sülfonik asit (ABTS•+) radikal giderme ve $Cu^{2+}$, $Fe^{3+}$ ve $Fe^{3+}$-TPTZ indirgeme deneyleri ile belirlenmiştir. Enzim inhibisyon çalışmaları metabolik enzimler olan asetilkolinesteraz, bütirilkolinesteraz, karbonik anhidraz I ve II izoenzimleri ile gerçekleştirilmiştir. *A. nemorosa*'nın etanol ekstresi DPPH ve ABTS deneylerinde en yüksek aktiviteyi göstermiştir ($IC_{50}$: 17.36 µg $mL^{-1}$, $IC_{50}$: 7.02 µg $mL^{-1}$). $Fe^{3+}$ indirgeme deneyinde, *A. nemorosa*'nın

**Biyokimya**

**Araştırma Makalesi**

**Makale Tarihçesi**
Geliş Tarihi : 23.04.2024
Kabul Tarihi : 09.08.2024

**Anahtar Kelimeler**
*Crocus cancellatus,*
*Scilla siberica*
*Juniperus oxycedrus*
*Anthriscus nemorosa*
Antioksidan ve enzim inhibisyonu

diklorometan ekstresi en yüksek aktiviteyi göstermiştir (1.96±0.060 µg $mL^{-1}$). $Cu^{2+}$ indirgeme deneyinde, *J. oxycedrus*'un diklorometan ekstresi en yüksek aktiviteyi göstermiştir (1.773±0.066 µg $mL^{-1}$). $Fe^{3+}$-TPTZ indirgeme deneyinde, *S. siberica*'nın etanol ekstraktı en yüksek aktiviteyi göstermiştir (1.256±0.011 µg $mL^{-1}$). Enzim inhibisyon sonuçlarında, tüm bitkilerin çalışılan enzimleri inhibe ettiği belirlenmiştir. Bu çalışma sonucunda tıbbi ve aromatik bitkilerden olan bu dört bitkinin yüksek biyolojik aktiviteye sahip olduğu belirlenmiştir.

**Atıf İçin**: Yurt, B., Sağlamtas, R., demir, Y., İzol, E., Diril, H., & Çağlayan, C (2024) Bazı Aromatik Bitki Ekstraktlarının in vitro Antioksidan, Antikolinerjik ve Antiepileptik Aktivitelerinin Belirlenmesi. *KSÜ Tarım ve Doğa Derg 27* (Ek Sayı 1), 1-15. DOI: 10.18016/ksutarimdoga.vi.1472403.

**To Cite**: Yurt, B., Sağlamtas, R., demir, Y., İzol, E., Diril, H., & Çağlayan, C (2024). Determination of *in vitro* Antioxidant, Anticholinergic and Antiepileptic Activities of Some Aromatic Plant Extracts. *KSU J. Agric Nat 27* (Suppl 1), 1-15. DOI: 10.18016/ksutarimdoga.vi.1472403.

## INTRODUCTION

Medicinal and aromatic plants are used in many different fields and are the subject of scientific studies. Especially the biological activities they show with their phytochemical contents are very valuable. They act as pioneers in the treatment of many diseases and are the source of drug-active ingredients (Yilmaz et al., 2024; İzol et al., 2023; Yapıcı and İzol, 2023). In this study, the biological activities of four important medicinal and aromatic plants were investigated.

The *Crocus* has flowers in different colors (Ahouran et al., 2012). *Crocus* is a significant traditional medicinal herb (Abdullaev et al., 2003; Dimitra G et al., 2007; Fatehi et al., 2003). From Iran, Türkiye, and Jordan, the plant *Crocus cancellatus* is pretty common. Türkiye is a rich country regarding the Crocus species (Kandemir, 2010). *Crocus cancellatus* is called "Çiğdem," and grows on rocky slopes at an altitude of 50-2400 in the southeastern regions of Türkiye (Mammadov & Sahranc, 2003; Öntaş et al., 2020). This species' corms are available in local markets and consumed both cooked and raw (Ahouran et al., 2012).

The geographical range of *Scilla Siberica subsp. armena*, an Iranian-Turanian species, includes Georgia and Türkiye (Aydın et al., 2023). *Scilla Siberica* subsp. *armena*, (Grossh.) Mordak, known as "camışkıran" in Türkiye (Guner et al., 2012). The bulbs of S. siberica subsp. *armena* are sold in Turkish markets and are used mainly as a garden herb (Aydın et al., 2023; Özdemir et al., 2016). This plant grows in rocky slopes at an altitude of 50-2400 in the southeastern regions of Türkiye (Özdemir & Yildirim 2016).

*Juniperus oxycedrus* subsp. *oxycedrus* is a variable species, particularly in the distribution range's western and central parts (Klimko et al., 2007). Folk medicine uses of *Juniperus* (Cupressaceae) species are widespread in developing nations (Orhan et al., 2012). It is growing on a variety of rocky sites from sea level up to 1600 m altitude (Orhan et al., 2011). In Türkiye, *J. oxycedrus* subsp. *oxycedrus* L. leaf decoction is used to reduce blood sugar levels (Orhan et al., 2012).

*Anthriscus*, a member of the Apiaceae family and one of the fragrant herbs, is used therapeutically throughout the world in traditional medicine (Karakaya et al., 2019). *Anthriscus nemorosa* is called as 'gimigimi, peçek' in Türkiye. Fruits from the *A. nemorosa* plant have been used to treat inflammation, gastrointestinal disorders, and rheumatism (Bagci et al., 2016; Karakaya et al., 2019; Menemen, 2012). It grows in groves, rocky slopes, and watery meadows at an altitude of 500-3200 in all regions of Türkiye (Kiliç, 2017).

Alzheimer's disease (AD) is a progressive neurological condition marked by abnormal patient behavior and cognitive deficits (Güleç et al., 2022; Yaşar et al., 2021; Inci et al., 2023). Reactive oxygen species (ROS) have reportedly been linked to neuronal damage and cellular aging. Antioxidants may thereby slow the development of AD and prevent neuronal damage (Karageçili et al., 2023a; Demir et al., 2023; Osmaniye et al., 2022; Çelik et al., 2024). The ability of an antioxidant meal to suppress the main enzymes involved in the pathogenesis of AD, butyrylcholinesterase (BChE) and acetylcholinesterase (AChE), is advantageous (Izol et al., 2021; Oztaskin et al., 2022; İzol, 2024; Bursal et al., 2021).

Carbonic anhydrases (CAs) are metalloenzymes that help a variety of biological systems produce bicarbonate and proton from carbon dioxide through a very straightforward hydration reaction (Karageçili et al., 2023b; Kaya et al., 2022). They control several pathological and physiological processes, including the transfer of $CO_2$ and bicarbonate ions between tissues involved in metabolism and the lungs, which helps keep the blood's pH and homeostasis in check (Ağgül et al., 2020; Buza et al., 2023; Yılmaz et al., 2023). Also, they are essential for the release of electrolytes from different tissues, bone resorption, and a few other biosynthetic processes like ureagenesis, lipogenesis, and gluconeogenesis (Bayindir et al., 2019; Caglayan

& Gulcin 2018; Taslimi et al., 2017). The inhibition of CA isozymes may be responsible for several important physiological advantages against osteoporosis, epilepsy, hypertension, oedema, obesity, glaucoma, and cardiac hypertrophy (Ağgül et al., 2020; Anil et al., 2022; Ozer et al., 2022).

Oxygen is an oxidizing agent that is highly reactive and non-metal and easily forms oxides, unlike other compounds. It exists in the atmosphere as a more stable biradical ($^3O_2$) and undergoes a gradual reduction process (Leyla & Gülçin, 2024; Gulcin, 2020). ROS are short-lived, active structures that contain oxygen atoms. Among them, singlet oxygen ($^1O_2$), superoxide anion radicals ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$), hypochlorite ions ($ClO^-$), and hydroperoxyl radicals ($HOO^{\cdot}$) are the most abundant. These molecules are natural byproducts of the known methanolysis of oxygen and significantly affect the transmission of cell signals and homeostasis (Apak et al., 2022; Gulcin, 2020). These molecules have different half-lives. They are formed as radicals, molecules, and ions in various biological and chemical processes, including photosynthesis and the electron transport chain. ROS and free radicals are formed not only during metabolism but also due to the effects of various environmental sources such as exercise, exposure to chemicals, and sunlight (Durmaz et al., 2022; Kiziltas et al., 2021). Excessive levels of ROS in tissues and cells cause various disorders known as oxidative stress, including neurological and cardiovascular diseases, cancer, and lung diseases (Erdoğan et al., 2021; Polat Kose & Gulcin 2021). Antioxidants play a vital role in the human body and food systems, reducing ROS harmful effects and oxidative processes (Çakmakçı et al., 2015; Gulcin, 2020). Aerobic organisms have defense systems, including antioxidant compounds and enzymes to remove and repair damaged molecules. Cells are protected against oxidative stress by antioxidant enzyme networks (Davies, 1995; Gulcin, 2020).

The biological research done on plant extracts supports most species' traditional applications, but it falls short of fully supporting rational phytotherapy. Because there are so many species, physiotherapists continue looking for fresh sources of biologically active substances and assessing their pharmacological activity profiles, primarily based on *in vivo* and/or *in vitro* studies. All these studies must be performed in conjunction with a multicomponent pattern analysis of the extracts to evaluate the primary components. So, in the present study, tri extracts prepared from the leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *sprengel* were investigated for their AChE, BChE, hCA I, hCA II, and antioxidant potential.

## MATERIAL and METHOD

### Chemicals

Butyrylthiocholine, acetylthiocholine, ethanol, dichloromethane, BHT, BHA, DPPH, ABTS, trolox, and α-tocopherol were commercially obtained from Sigma-Aldrich. The other chemicals were used as analytical grades.

### Plant Material

In this research, four plants (*Crocus cancellatus, Scilla Siberica subsp. armena, Juniperus oxycedrus subsp. Oxycedrus, and Anthriscus nemorosa* (M.bieb.) *sprenge*) were obtained from Bingöl region, Türkiye. The collected plants were identified in the Herbarium laboratory of Bingöl University, Department of Molecular Biology and Genetics, and added to the herbarium library.

### Preparation of Extracts

Three different extracts from dried and ground plants were prepared separately. Extracts were prepared using dried herbs (2.5 g) and solvent (50 mL). The water extract was prepared using the boiling method, and the other extracts were prepared using the maceration method. Volatiles were extracted with a rotary evaporator and stored in the refrigerator until the study was carried out.

### Enzyme Inhibition Assay

AChE and BChE enzyme inhibition studies were performed using Ellman's colorimetric method (Ellman et al., 1961). Based on this method, cholinesterases catalyze the breakdown reaction of ACh or BCh to thiocholine and acetate or butyrate. DTNB, which is used during inhibition studies, is formed as a result of the reaction with thiocholine, which is one of these degradation products, as a yellow compound, 5-thio-2-nitrobenzoic acid. An inhibition study was performed by measuring the color intensity of the colored compound formed at 412 nm.

The study purified both hCA I and II isoenzymes by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. Here, Sepharose-4B-L-Tyrosine-sulfanilamide is used as an affinity matrix for hCA isoenzymes. The activity of these isoenzymes is determined spectrophotometrically as in previous studies (Caglayan & Gulcin 2018; Gocer & Gulcin 2013). CA isoenzymes are considered to be the units in which they convert PNP from 348 nm PNA over 3 minutes at 25 °C (Verpoorte et al., 1967).

$IC_{50}$ values were calculated by examining the three extracts (water WE, ethanol EE, and dichloromethane DME) prepared leaves of *Crocus cancellatus*, *Scilla Siberica subsp. armena*, *Juniperus oxycedrus subsp. oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *sprengel* were investigated for their AChE, BChE, hCA I, hCA

II enzyme activities. For this purpose, enzyme activities at five different concentrations were measured spectrophotometrically for all extracts. The obtained data was drawn using the % activity [extract] graph. $IC_{50}$ values were calculated using the graph.

### Antioxidant Activities Assays

DPPH solution was prepared daily and kept in a glass bottle in the dark (4°C). Plant extracts (1.5 mL) were dissolved in ethanol and transferred to fresh 500 µL of DPPH· solution (0.1 M). These mixtures were mixed vigorously and incubated in the dark for 30 minutes. Then, their absorbance was recorded spectrophotometrically at 517 nm (Aras et al., 2016; Köksal et al., 2009). $ABTS^{+}$ was obtained by reacting ABTS (7.0 mM) with $K_2S_2O_8$ (2.5 mM). $ABTS^{+}$ scavenging ability of extracts prepared using different solvents was determined according to the previously described spectroscopic method (Erdoğan et al., 2021).

$Fe^{3+}$-reducing effects of plant extracts were done in accordance with Oyaizu's method (Oyaizu, 1986). $Cu^{2+}$-reducing effects of plant extracts were measured according to a minor modification of Apak et al. (2006) method (Bursal et al., 2019). The last reduction method we used, the FRAP method, is based on the complicated degradation of $Fe^{3+}$-TPTZ. The increased absorbance of $Fe^{2+}$-TPTZ was measured spectrometrically at 593 nm as described in previous studies (Gulcin et al., 2019; Polat Kose et al., 2020).

### Statistical Analyses

Statistical analyses were performed with SPSS, and p-values less than 0.05 were considered statistically significant at a 95% confidence interval. P values for differences were obtained as a result of a two-way (2x2) ANOVA analysis. Post-hoc Tukey test was used for pairwise comparisons.

## RESULTS and DISCUSSION

### Enzyme Inhibition Studies

Memory loss and other cognitive impairments are the earliest symptoms of AD, which are thought to be linked to acetylcholine (ACh) depletion, inflammation, and oxidative stress. Hence, consumption of antioxidant-rich vegetables can halt the onset of AD and neurodegeneration. Inhibiting AChE and BChE can be significant because it is a cutting-edge therapeutic strategy for treating neurodegenerative diseases. In the current study, the activities of the three extracts (water WE, ethanol EE, and dichloromethane DME) were prepared from *C. cancellatus* and *S. Siberica* Subsp. *Armena*, *J. oxycedrus* subsp. *oxycedrus* and *A. nemorosa* were investigated against AChE and BChE. All extracts inhibited BChE and AChE in a dose-dependent manner. $IC_{50}$ values, which represent the inhibition effect of the tested extracts, were determined and are shown in Table 1.

Çizelge 1. *Crocus cancellatus, Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) *spreng* yapraklarının su, etanol ve diklorometan ekstraktlarının AChE ve BChE için $IC_{50}$ değerleri

*Table 1. $IC_{50}$ values of water, ethanol, and dichloromethane extracts leaves of Crocus cancellatus, Scilla Siberica subsp. Armena, Juniperus oxycedrus subsp. oxycedrus and Anthriscus nemorosa (M.bieb.) spreng for AChE and BChE*

| Samples | $IC_{50}$ (mg $mL^{-1}$) | | | |
|---|---|---|---|---|
| | BChE | $r^2$ | AChE | $r^2$ |
| ***C. cancellatus*** **DME** | 63.58 | 0.9633 | 18.05 | 0.9957 |
| ***C. cancellatus*** **EE** | 74.52 | 0.9852 | 64.77 | 0.9544 |
| ***C. cancellatus*** **WE** | 51.72 | 0.9911 | 83.49 | 0.9815 |
| ***S. Siberica*** **subsp.** ***armena*** **DME** | 54.14 | 0.9392 | 97.61 | 0.9679 |
| ***S. Siberica*** **subsp.** ***armena*** **EE** | 41.5 | 0.9314 | 60.26 | 0.9839 |
| ***S. Siberica*** **subsp.** ***armena*** **WE** | 40.53 | 0.9601 | 91.18 | 0.9282 |
| ***J. oxycedrus*** **subsp.** ***oxycedrus*** **DME** | 66.63 | 0.9715 | 62.43 | 0.9950 |
| ***J. oxycedrus*** **subsp.** ***oxycedrus*** **EE** | 72.19 | 0.9943 | 60.79 | 0.9884 |
| ***J. oxycedrus*** **subsp.** ***oxycedrus*** **WE** | 31.5 | 0.9315 | 72.95 | 0.9819 |
| ***A. nemorosa (M.bieb.) spreng*** **DME** | 35.72 | 0.9649 | 17.46 | 0.9900 |
| ***A. nemorosa (M.bieb.) spreng*** **EE** | 15.4 | 0.9888 | 7.71 | 0.9139 |
| ***A. nemorosa (M.bieb.) spreng*** **WE** | 47.47 | 0.9719 | 38.72 | 0.9303 |

The studied plant extract demonstrated concentration-dependent inhibition of AChE, with activity ranging from 15.40 mg $mL^{-1}$ to 74.52 mg $mL^{-1}$. The AChE-inhibitory capacity of studied plant extracts is in the following order: *A. nemorosa* (M.bieb.) *Spreng*. EE ($IC_{50}$, 15.40 mg $mL^{-1}$, $r^2$: 0.9888)> *J. oxycedrus* subsp. *oxycedrus* WE ($IC_{50}$, 31.50 mg $mL^{-1}$, $r^2$: 0.9315) > *A. nemorosa* (M.bieb.) *Spreng*. DME ($IC_{50}$, 35.72 mg $mL^{-1}$, $r^2$: 0.9649) > *S. Siberica* Subsp. *armena* WE ($IC_{50}$, 40.53 mg $mL^{-1}$, $r^2$: 0.9601) > *S. Siberica* Subsp. *armena* EE ($IC_{50}$, 41.50 mg $mL^{-1}$, $r^2$: 0.9314) > *A. nemorosa* (M.bieb.) *Spreng* WE ($IC_{50}$, 47.47 mg $mL^{-1}$, $r^2$: 0.9719)

> *C. cancellatus* WE ($IC_{50}$, 51.72 mg $mL^{-1}$, $r^2$: 0.9911) > *S. Siberica* Subsp. *armena* DME ($IC_{50}$, 54.14 mg $mL^{-1}$, $r^2$: 0.9392) > *C. cancellatus* DME ($IC_{50}$, 63.58 mg $mL^{-1}$, $r^2$: 0.9633) > *J. oxycedrus* subsp. *oxycedrus* DME ($IC_{50}$, 66.63 mg $mL^{-1}$, $r^2$: 0.9715) > *J. oxycedrus* subsp. *oxycedrus* EE ($IC_{50}$, 72.19 mg $mL^{-1}$, $r^2$: 0.9943) > *C. cancellatus* EE ($IC_{50}$, 74.52 mg $mL^{-1}$, $r^2$: 0.9852).

The concentration-dependent AChE inhibition effect of the WE of *C. cancellatus*, *S. Siberica* Subsp. *Armena*, and *J. oxycedrus* subsp. *oxycedrus* leaves were shown, to be higher than that of EE and DME. On the other hand, the AChE inhibition effect of the EE *A. nemorosa* (M.bieb.) *Spreng*. was shown, to be higher than that of WE and DME. The EE and WE AChE inhibition effects of *S. Siberica* Subsp. *Armena* plants were almost close to each other.

The studied plant extract demonstrated concentration-dependent inhibition of BChE, with activity ranging from 7.71 mg $mL^{-1}$ to 97.61 mg $mL^{-1}$. The BChE-inhibitory capacity of studied plant extracts is in the following order: *A. nemorosa* (M.bieb.) *Spreng*. EE ($IC_{50}$, 7.71 mg $mL^{-1}$, $r^2$: 0.9139) > *A. nemorosa* (M.bieb.) *Spreng*. DME ($IC_{50}$, 17.46 mg $mL^{-1}$, $r^2$: 0.9900) > *C. cancellatus* DME ($IC_{50}$, 18.05 mg $mL^{-1}$, $r^2$: 0.9957) > *A. nemorosa* (M.bieb.) *Spreng*. WE ($IC_{50}$, 38.72 mg $mL^{-1}$, $r^2$: 0.9303) > *S. Siberica* Subsp. *armena* EE ($IC_{50}$, 60.26 mg $mL^{-1}$, $r^2$: 0.9839) = *J. oxycedrus* subsp. *oxycedrus* EE ($IC_{50}$, 60.79 mg $mL^{-1}$, $r^2$: 0.9884) > *J. oxycedrus* subsp. *oxycedrus* DME ($IC_{50}$, 62.43 mg $mL^{-1}$, $r^2$: 0.9950) > *C. cancellatus* EE ($IC_{50}$, 64.77 mg $mL^{-1}$, $r^2$: 0.9544) > *J. oxycedrus* subsp. *oxycedrus* WE ($IC_{50}$, 72.95 mg $mL^{-1}$, $r^2$: 0.9819) > *C. cancellatus* WE ($IC_{50}$, 83.49 mg $mL^{-1}$, $r^2$: 0.9815) > *S. Siberica* Subsp. *armena* WE ($IC_{50}$, 91.18 mg $mL^{-1}$, $r^2$: 0.9282) > *S. Siberica* Subsp. *armena* DME ($IC_{50}$, 97.61 mg $mL^{-1}$, $r^2$: 0.9679). The concentration-dependent BChE inhibition effect of the EE of studied all plant leaves was shown, to be higher than that of WE and DME. When the results of this study were compared, *C. cancellatus* DME inhibited BChE enzyme 3.52 times more than AChE enzyme and *A. nemorosa* (M.bieb.) *Spreng*. DME inhibited 2.04 more. *A. nemorosa* (M.bieb.) *Spreng*. EE showed the best inhibition effect on the activity of both AChE and BChE enzymes. *J. oxycedrus* subsp. *oxycedrus* DME inhibited the two cholinesterase enzymes studied at close values.

As this is the first study to examine four plants using AChE and BChE enzymes, the data provided here cannot be compared to the literature currently in use. Studies on other species of these plants for AChE and BChE enzymes are available in the literature. For instance, Menghini et al. (2018) studied the effect of *C. sativus* L. Stigmas extract on AChE and BChE enzymes. This plant inhibited AChE and BChE enzymes with 2.51 ± 0.18 for AChE and 3.44 ± 0.13 galantamine equivalents $g^{-1}$ extract for BChE. Linardaki et al. (2017) investigated the neurotoxic effects of aflatoxin B1 and the preventive effects of *C. sativus*. They tested the activity of AChE and BChE in the liver, cerebellum, and whole brain. They showed that pretreatment of aflatoxin B1-exposed mice with *C. sativus* infusion resulted in even lower activity in brain, cerebellar and liver AChE, while higher activity in brain BChE enzyme compared to aflatoxin B1-exposed mice. *A. nemorosa* essential oil was tested by Bagci et al. (2016) on rats given scopolamine to see how it affected their memory functions, anxiety levels, and depressive-like behaviours. Öztürk et al. (2011) looked at AChE and BChE enzyme inhibition effects by preparing acetone methanol and hexane extract of *J. oxycedrus* subsp. *oxycedrus* plant. It was found to be the hexane extract of this plant, having 81.40% inhibition at 200 mg $mL^{-1}$ against AChE. Hexane extract of this plant showed 95.75% inhibition against BChE.

One of the most popular and effective mechanisms for regulating pH in all biological systems is the CAs. (Aktaş et al., 2022). These enzymes are involved in numerous other biochemical and physiological processes; therefore, they are not just pH regulators. In clinical practice or as pharmacological tools, most CA inhibitors and activators are synthetic derivatives developed over time through conventional drug design campaigns from synthetic lead molecules (Hamide et al., 2022; Zengin et al., 2023). Yet, research into several natural items' CA inhibitory effects has also begun over the past ten years. This has resulted in substantial advancements in the field (Tugrak et al., 2021). All extracts inhibited hCA I and hCA II in a dose-dependent manner. $IC_{50}$ values, which represent the inhibition effect of the tested extracts, were determined and are shown in Table 2.

In this study, the plant extract demonstrated concentration-dependent inhibition of hCA I, with activity ranging from 14.59 mg $mL^{-1}$ to 68.61 mg $mL^{-1}$, The hCA I inhibitory capacity of studied plant extracts in the following order: *C. cancellatus* DME ($IC_{50}$, 14.59 mg $mL^{-1}$, $r^2$: 0.9752)> *J. oxycedrus* subsp. *oxycedrus* DME ($IC_{50}$, 22.79 mg $mL^{-1}$, $r^2$: 0.9671) > *A. nemorosa* (M.bieb.) *Spreng*. EE ($IC_{50}$, 26.55 mg $mL^{-1}$, $r^2$:0.9304) = *C. cancellatus* EE ($IC_{50}$, 26.55 mg $mL^{-1}$, $r^2$: 0.9808)> *A. nemorosa* (M.bieb.) *Spreng*. WE ($IC_{50}$, 27.50 mg $mL^{-1}$, $r^2$: 0.9698)> *S. Siberica* subsp. *armena* DME ($IC_{50}$, 34.85 mg $mL^{-1}$, $r^2$: 0.9543) > *S. Siberica* subsp. *armena* WE ($IC_{50}$, 36.09 mg $mL^{-1}$, $r^2$: 0.9420) > *S. Siberica* subsp. *armena* EE ($IC_{50}$, 41.75 mg $mL^{-1}$, $r^2$: 0.9343) > *J. oxycedrus* subsp. *oxycedrus* EE ($IC_{50}$, 52.90 mg $mL^{-1}$, $r^2$: 0.9247) > *A. nemorosa* (M.bieb.) *Spreng*. DME ($IC_{50}$, 53.31 mg $mL^{-1}$, $r^2$: 0.9443)> *J. oxycedrus* subsp. *oxycedrus* WE ($IC_{50}$, 61.87 mg $mL^{-1}$, $r^2$: 0.9244) > *C. cancellatus* WE ($IC_{50}$, 68.61 mg $mL^{-1}$, $r^2$: 0.9609). The concentration-dependent hCA I inhibition effect of the DME of *C. cancellatus*, *S. Siberica* subsp. *armena* and *J. oxycedrus* subsp. *oxycedrus* leaves were shown, to be higher than that of WE and EE. On the other hand, the order of inhibition in the leaves of *A. nemorosa* (M.bieb.) spreng. Is in the form of EE >WE > DME. DME extract of *C. cancellatus* inhibited hCA I enzyme 4.7 times more than WE. *C. cancellatus* and *A. nemorosa* EE inhibited the hCA I enzyme at the same rate. DME extract *J. oxycedrus* subsp. *oxycedrus* inhibited hCA I enzyme 2.71 times more than WE.

In the current study, the studied plant extract demonstrated concentration-dependent inhibition of hCA II, with activity ranging from 8.14 mg $mL^{-1}$ to 48.80 mg $mL^{-1}$. The HCA II inhibitory effect of studied plant extracts in the following order: *S. Siberica* Subsp. *armena* WE ($IC_{50}$, 8.14 mg $mL^{-1}$, $r^2$: 0.9557) > *S. Siberica* Subsp. *armena* EE ($IC_{50}$, 12.63 mg $mL^{-1}$, $r^2$: 0.9215) > *C. cancellatus* WE ($IC_{50}$, 14.23 mg $mL^{-1}$, $r^2$: 0.9468) > *S. Siberica* Subsp. *armena* DME ($IC_{50}$, 14.53 mg $mL^{-1}$, $r^2$: 0.9716) > *J. oxycedrus* subsp. *oxycedrus* EE ($IC_{50}$, 14.78 mg $mL^{-1}$, $r^2$: 0.9631) > *C. cancellatus* EE ($IC_{50}$, 18.53 mg $mL^{-1}$, $r^2$: 0.9936) > *J. oxycedrus* subsp. *oxycedrus* DME ($IC_{50}$, 19.41 mg $mL^{-1}$, $r^2$: 0.9708)> *A. nemorosa* (M.bieb.) *Spreng*. EE ($IC_{50}$, 27.18 mg $mL^{-1}$, $r^2$:0.9341)> *A. nemorosa* (M.bieb.) *Spreng*. WE ($IC_{50}$, 30.26 mg $mL^{-1}$, $r^2$: 0.9859)> *C. cancellatus* DME ($IC_{50}$, 30.80 mg $mL^{-1}$, $r^2$: 0.9346)> *A. nemorosa* (M.bieb.) *Spreng*. DME ($IC_{50}$, 35.18 mg $mL^{-1}$, $r^2$: 0.9887)> *J. oxycedrus* subsp. *oxycedrus* WE ($IC_{50}$, 48.80 mg $mL^{-1}$, $r^2$: 0.9139).

Çizelge 2. *Crocus cancellatus, Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) spreng yapraklarının su, etanol ve diklorometan ekstraktlarının hCA I ve hCA II için $IC_{50}$ değerleri

*Table 2. $IC_{50}$ values of water, ethanol, and dichloromethane extracts leaves of Crocus cancellatus, Scilla siberica subsp. armena, Juniperus oxycedrus subsp. oxycedrus and Anthriscus nemorosa (M.bieb.) spreng for hCA I and hCA II*

| Samples | $IC_{50}$ (mg $mL^{-1}$) | | | |
|---|---|---|---|---|
| | hCA I | $r^2$ | hCA II | $r^2$ |
| *C. cancellatus* DME | 14.59 | 0.9752 | 30.8 | 0.9346 |
| *C. cancellatus* EE | 26.55 | 0.9808 | 18.53 | 0.9936 |
| *C. cancellatus* WE | 68.61 | 0.9609 | 14.23 | 0.9468 |
| *S. Siberica* subsp. *armena* DME | 34.85 | 0.9543 | 14.53 | 0.9716 |
| *S. Siberica* subsp. *armena* EE | 41.75 | 0.9343 | 12.63 | 0.9215 |
| *S. Siberica* subsp. *armena* WE | 36.09 | 0.9420 | 8.14 | 0.9557 |
| *J. oxycedrus* subsp. *oxycedrus* DME | 22.79 | 0.9671 | 19.41 | 0.9708 |
| *J. oxycedrus* subsp. *oxycedrus* EE | 52.90 | 0.9247 | 14.78 | 0.9631 |
| *J. oxycedrus* subsp. *oxycedrus* WE | 61.87 | 0.9244 | 48.80 | 0.9139 |
| *A. nemorosa (M.bieb.) spreng* DME | 53.31 | 0.9443 | 35.18 | 0.9887 |
| *A. nemorosa (M.bieb.) spreng* EE | 26.55 | 0.9304 | 27.18 | 0.9341 |
| *A. nemorosa (M.bieb.) spreng* WE | 27.5 | 0.9698 | 30.26 | 0.9859 |

Some studies in the literature include the effects of the plants studied in this study on the activities of other enzymes. Loizzo et al. (2016) studied *C. cancellatus* subsp. damascenus extract inhibited α-glycosidase and α-amylase. The $IC_{50}$ values of 68.6 for α-glycosidase and 57.1 μg/mL for α-amylase were determined. Another study examined the α-glycosidase and α-amylase inhibitory effect *of Scilla siberica* subsp. *armena* corm, flower, and leaf methanolic extracts. The flower extract displayed no inhibition against α-amylase as well as α-glycosidase inhibitory effect with an $IC_{50}$ value of 5239 μg $mL^{-1}$. Blue pollen, leaf, and Corm extracts showed no inhibition against α-glycosidase and α-amylase enzymes (Aydın et al., 2023).

## Antioxidant Results

In this section, water, ethanol, and dichloromethane extracts of the leaves of *Crocus cancellatus*, *Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *Sprengel* was prepared, and studies to determine the antioxidant capacities of these extracts were included. The ABTS•+ and DPPH• methods were used to determine how antioxidant plant extracts work and measure their ability to eliminate free radicals. In addition, the reduction capacity of copper ions ($Cu^{2+}$) to copper ions ($Cu^{+}$), the reduction capacity of ferric ions ($Fe^{3+}$) to iron ions ($Fe^{2+}$), and the reduction capacity of $Fe^{3+}$-TPTZ by the FRAP method were determined by different methods. Comparisons were made with synthetic and standard antioxidants such as BHA, BHT, α-tocopherol, and the α-tocopherol analogue Trolox.

## Determination of Radical Scavenging Effects

The ABTS•+ and DPPH• scavenging procedures are remarkable due to their rapidity, simplicity, sensitivity, and reproducibility (Aras et al 2016; Gulcin 2020). The DPPH• method is based on the DPPH• scavenging percentage of antioxidants in the plant extract. DPPH• has a dark blue colour and is a longlived nitrogen radical species capable of dimerization (Gulcin, 2020). This method was first reported as a decolourization assay by Blois (1958). Today, DPPH• is generally known as a reagent used to determine antioxidants' free radical scavenging activity. This molecule shows maximum absorbance at 517 nm (Bursal et al., 2020; Gulcin, 2020; Türkan et al., 2020). The difference between control values and different concentrations (10-30 μg $mL^{-1}$) of plant extracts was found to be statistically significant ($p < 0.01$). The $IC_{50}$ values of the extracts were between 19.86 and 38.93 μg $mL^{-1}$, and the values of the water extracts were higher than the others. The $IC_{50}$ values of the standards were calculated as 7.3 ($r^2$: 0.9733) for Trolox and 8.35 ($r^2$: 0.9823) for α-tocopherol (Table 3, Figure 1). The DPPH radical scavenging capacities of the studied plant extracts are in the following order: *A. nemorosa (M.bieb.) Spreng EE* ($IC_{50}$:17.36, $r^2$:0.9513)> *S. Siberica* Subsp. *armena* DME ($IC_{50}$:19.86, $r^2$:0.9555) > *A. nemorosa* (M.bieb.) *Spreng* DME ($IC_{50}$:20.2, $r^2$:0.9053)> *J. oxycedrus* subsp. *Oxycedrus* DME ($IC_{50}$:27.18, $r^2$:0.9247) > *S. Siberica* Subsp. *armena* E*E* ($IC_{50}$:28.17, $r^2$:0.9107) > *S. Siberica* Subsp. *armena* W*E* ($IC_{50}$:29.12, $r^2$:0.9549)> *C. cancellatus EE* ($IC_{50}$:30.13, $r^2$:0.9417) > *J. oxycedrus* subsp. *Oxycedrus EE* ($IC_{50}$:32.54, $r^2$:0.9420) > *C. cancellatus DME* ($EC_{50}$:33.31, $r^2$:0.9552)~ *C. cancellatus WE* ($IC_{50}$:33.97, $r^2$:0.9631) > *A. nemorosa* (M.bieb.) *spreng WE* ($IC_{50}$:34.48, $r^2$:0.9366)> *J. oxycedrus* subsp. *Oxycedrus WE* ($IC_{50}$:38.93, $r^2$:0.9556). The current study determined that extracts obtained from *C. cancellatus* had relatively higher $IC_{50}$ values than other plants.

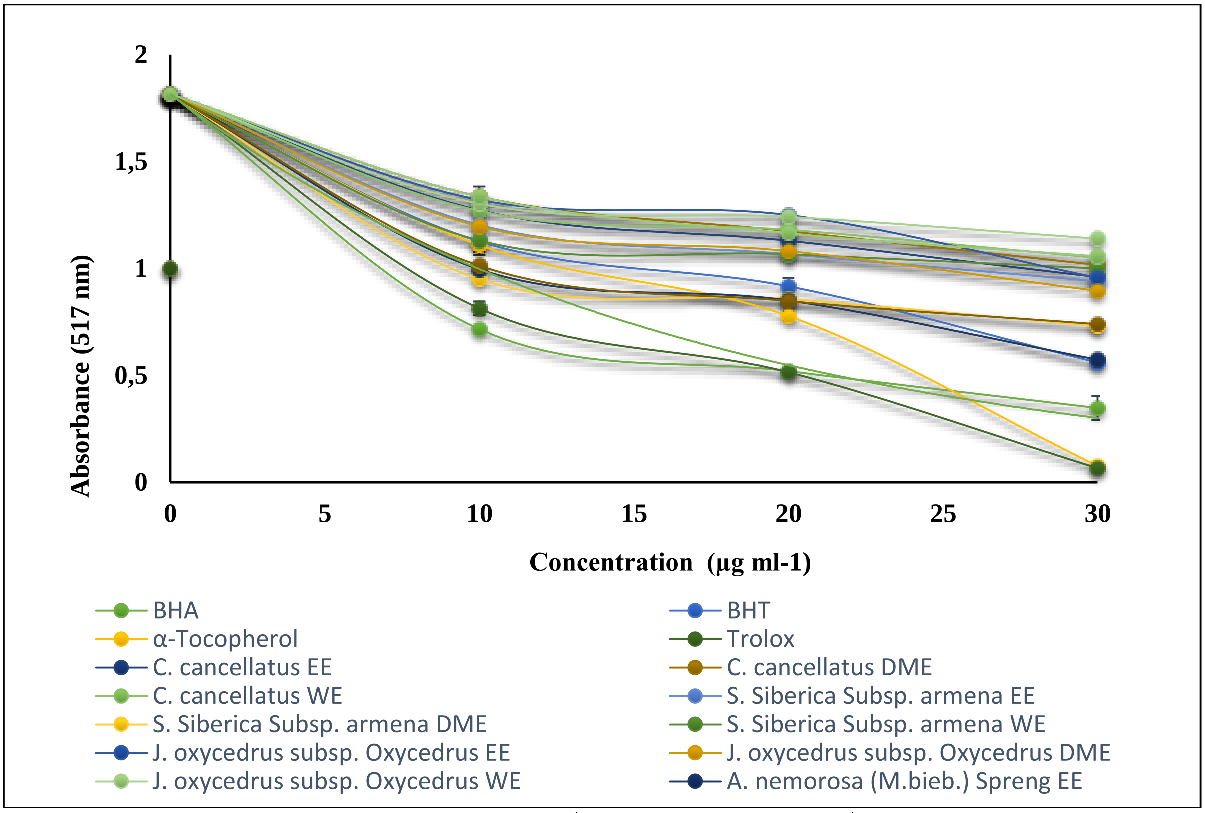
ABTS radical scavenging method is one of the different radical scavenging methods used to measure the antioxidant activities of extracts, pure substances, and food products (Gulcin, 2020). It can be easily applied as a spectrophotometric analysis method. It facilitates its use for routine screening and detection. $ABTS^{\bullet+}$ is generally obtained by the oxidation of ABTS with $K_2S_2O_8$ (Gülçin, 2012). The $ABTS^{\bullet+}$ radical can react rapidly with antioxidants and is easily used to determine the antioxidant effects of various food products and plant extracts, where it is effective over a wide pH range (Gulcin, 2020; Güven et al., 2023). For the ABTS radical scavenging method, control values and different concentrations of plant extracts (10-30 μg $mL^{-1}$) were studied, and the difference between the plant extracts was found to be statistically significant ($p<0.01$). The $IC_{50}$ values of the extracts were between 7.02-84.51 μg $mL^{-1}$, and the values of the water extracts were higher than the other extracts in this method, as in the DPPH radical scavenging method. The $IC_{50}$ values of the standards were calculated as 7.06 ($r^2$: 0.9420) for Trolox, 9.62 ($r^2$: 0.9683) for α-tocopherol, 5.2 ($r^2$: 0.9869) for BHA, and 9.68 ($r^2$: 0.9465) for BHT (Table 3, Figure 2).

Çizelge 3. *Crocus cancellatus, Scilla Siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* ve *Anthriscus nemorosa* (M.bieb.) *spreng* yapraklarının su, etanol ve diklorometan ekstraktlarının $DPPH^{\cdot}$, $ABTS^{\cdot+}$ süpürme aktiviteleri ve standart antioksidanlar için $IC_{50}$ (μg $mL^{-1}$) değerleri.

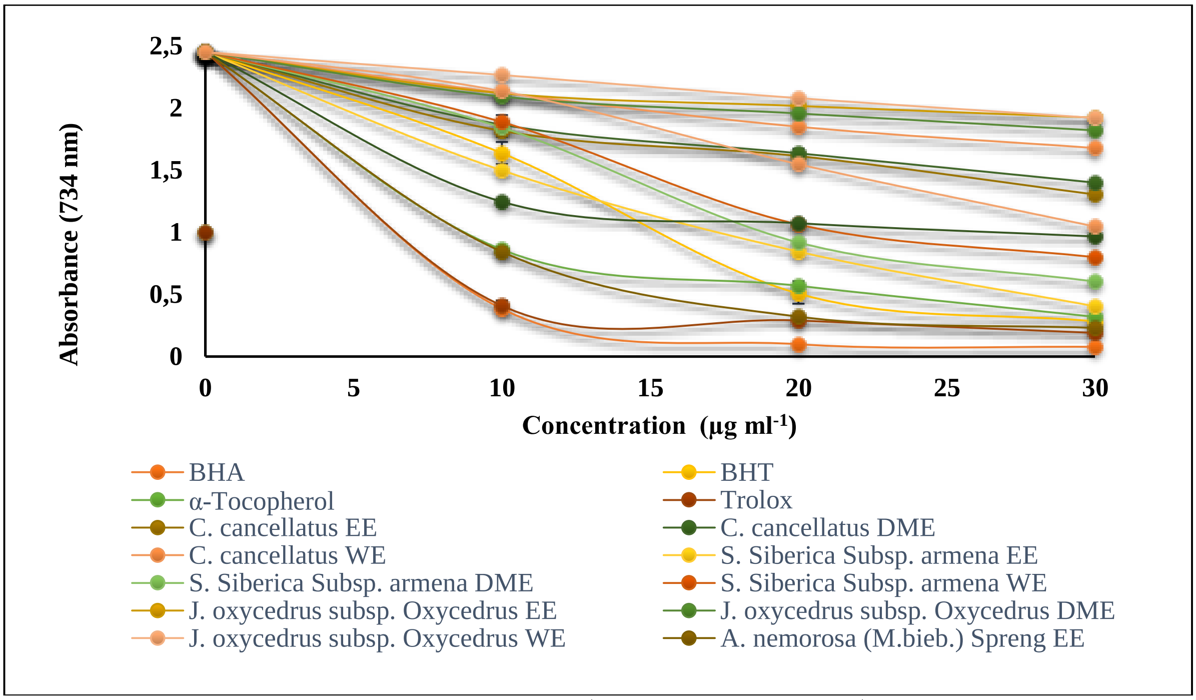
*Table 3. $IC_{50}$ (μg $mL^{-1}$) values for DPPH•, $ABTS^{\bullet+}$ scavenging activities of water, ethanol, and dichloromethane extracts leaves of Crocus cancellatus, Scilla siberica subsp. armena, Juniperus oxycedrus subsp. oxycedrus and Anthriscus nemorosa (M.bieb.) spring and of standard antioxidants.*

| Antioxidants and Samples | $DPPH^{\cdot}$ Scavenging | | $ABTS^{\cdot+}$ Scavenging | |
|---|---|---|---|---|
| | $IC_{50}$ | $r^2$ | $IC_{50}$ | $r^2$ |
| BHA | 11.57 | 0.9517 | 5.2 | 0.9869 |
| BHT | 18.05 | 0.9804 | 9.68 | 0.9645 |
| Trolox | 7.3 | 0.9733 | 9.62 | 0.9683 |
| α-Tocopherol | 8.35 | 0.9823 | 7.06 | 0.9420 |
| *C. cancellatus* EE | 30.13 | 0.9417 | 31.94 | 0.9707 |
| *C. cancellatus* DME | 33.31 | 0.9552 | 35.18 | 0.9710 |
| *C. cancellatus* WE | 33.97 | 0.9631 | 52.5 | 0.9939 |
| *S. Siberica* subsp. *armena* EE | 28.17 | 0.9107 | 12.07 | 0.9942 |
| *S. Siberica* subsp. *armena* DME | 19.86 | 0.9555 | 15 | 0.9650 |
| *S. Siberica* subsp. *armena* WE | 29.12 | 0.9549 | 18.33 | 0.9742 |
| *J. oxycedrus* subsp. *oxycedrus* EE | 32.54 | 0.9420 | 77 | 0.9170 |
| *J. oxycedrus* subsp. *oxycedrus* DME | 27.18 | 0.9247 | 64.17 | 0.9530 |
| *J. oxycedrus* subsp. *oxycedrus* WE | 38.93 | 0.9556 | 84.51 | 0:9997 |
| *A. nemorosa* (M.bieb.) *spreng* EE | 17.36 | 0.9513 | 7.02 | 0.9898 |
| *A. nemorosa* (M.bieb.) *spreng* DME | 20.2 | 0.9053 | 18.93 | 0.9671 |
| *A. nemorosa* (M.bieb.) *spreng* WE | 34.48 | 0.9366 | 26.86 | 0.9543 |

The results show significant differences ($p<0.05$) in post-hoc comparisons between different groups.




Şekil 1. Ekstraktların radikal giderici etkileri (DPPH giderici etkileri)
*Figure 1. Radical scavenging effects of extracts (DPPH˙ scavenging effects)*




Şekil 2. Ekstraktların radikal giderici etkileri (ABTS giderici etkileri)
*Figure 2. Radical scavenging effects of extracts (ABTS˙ scavenging effects)*

The ABTS radical scavenging capacities of the studied plant extracts are in the following order: *A. nemorosa* (M.bieb.) *spring*

EE ($IC_{50}$:7.02, $r^2$:0,9898)> *S. Siberica* subsp. *armena* EE ($IC_{50}$:12.07, $r^2$:0.9942)> *S. Siberica* subsp. *armena* DME ($IC_{50}$:15.0, $r^2$:0.9650) > *S. Siberica* subsp. *armena* WE ($IC_{50}$:18.33, $r^2$:0.9742)~ *A. nemorosa* (M.bieb.) *spreng* DME ($IC_{50}$:18.93, $r^2$:0.9671)> *A. nemorosa* (M.bieb.) *spreng* WE ($IC_{50}$:26.86, $r^2$:0.9543)> *C. cancellatus* EE ($IC_{50}$:31.94, $r^2$:0.9707)> *C. cancellatus* DME ($IC_{50}$:35.18, $r^2$:0.9710)> *C. cancellatus* WE

($IC_{50}$:52.5, $r^2$:0.9939)> *J. oxycedrus* subsp. *oxycedrus* DME ($IC_{50}$:64.17, $r^2$:0.9530)> *J. oxycedrus* subsp. *oxycedrus* EE ($IC_{50}$:77, $r^2$:0.9170)> *J. oxycedrus* subsp. *oxycedrus* WE ($IC_{50}$:84.51, $r^2$:0.9997). The current study determined that extracts obtained from *J. oxycedrus subsp. Oxycedrus* had higher $IC_{50}$ values than extracts from other plants. In the ABTS radical scavenging method, as in the DPPH method, it was determined that the extracts of the *A. nemorosa* (M.bieb.) *spring* plant had the lowest $IC_{50}$ value.

A previous study reported that the root $CH_2Cl_2$ fraction of *Anthriscus nemorosa*, root essential oil, and the main compound α-pinene found in the root essential oil have antioxidant capacity (Karakaya et al., 2019). Another study on the branches and fruits of different Juniperus species reported that different extracts had DPPH and ABTS radical scavenging properties, and their total antioxidant capacity was relatively high (Gök et al., 2021). Taviano et al. (2011) examined the antioxidant potential of water and methanol extracts of branches of Juniperus species (*J. oxycedrus subsp. macrocarpa, J. communis var. communis, J. drupacea, J. communis var. saxatilis* and *J. oxycedrus subsp. oxycedrus*). They reported that *J. oxycedrus subsp.* Extracts had high DPHH scavenging activity.

## Determination of Reducing Capacities

The reducing activity of water, ethanol, and dichloromethane extracts leaves of *Crocus cancellatus, Scilla siberica* subsp. *armena, Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *spring* was evaluated by measuring their ability to reduce $Fe^{3+}$ to $Fe^{2+}$. Compounds with functional groups such as -OH, -SH, and -COOH, essential electron donor groups found in plant extracts, are greatly important in reducing capacity. $Fe^{3+}$ reduction abilities prepared with different solvents were determined. Plant extracts made with various solvents have been shown to have beneficial reducing effects by lowering ferric ions when categorized using standard criteria like BHT, Trolox, BHA, and α-Tocopherol. As seen in Table 4 and Figure 3, the $Fe^{3+}$ reducing the ability of the extracts at 30μg/mL concentration showed absorbance in the range of 0.863-1.960 at 700 nm. Compared to standard antioxidants, the results obtained from this test showed that *A. nemorosa* (M.bieb.) *spring* DME, *A. nemorosa* (M.bieb.) *spring* EE*, and J. oxycedrus* subsp. *oxycedrus* DME*, S. Siberica* subsp. *armena* EE*, A. nemorosa* (M.bieb.) *spreng* WE, *J. oxycedrus* subsp. *oxycedrus* EE was found to have a very effective $Fe^{3+}$-reducing ability, and other extracts were found to have a close to moderate effect on α-Tocopherol and Trolox values.

The copper ions ($Cu^{2+}$) reducing capacity (CUPRAC) method was first developed and used by Apak's working group (Apak et al., 2006), and this CUPRAC reagent is stable and easily accessible compared to chromogenic radical reagents. The method has been applied to various matrices containing both hydrophilic and lipophilic antioxidants, and positive results have been obtained. The method is based on the reduction of $Cu^{2+}$ to $Cu^{+}$ or neocuproine (2,9-dimethyl-1,10-phenanthroline) via polyphenols in the aqueous ethanolic medium (Gulcin, 2020). In this method, the copper-reducing capacities of water, ethanol, and dichloromethane extracts of the leaves of *Crocus cancellatus, Scilla Siberica* subsp*. armena, Juniperus oxycedrus* subsp. *oxycedrus and Anthriscus nemorosa* (M.bieb.) *spring* were determined to be between 1.02 and 1.773 absorbances at 30 μg $mL^{-1}$ concentration. CUPRAC of the examined plant extracts and standard antioxidants are as follows: BHA ($\lambda_{450}$: 2.459±0.027, $r^2$:0.9904)>BHT ($\lambda_{450}$: 1.975±0.091, $r^2$:0.9422)> *J. oxycedrus* subsp. *oxycedrus* DME ($\lambda_{450}$: 1.773±0.066, $r^2$:0.9084)> *S. Siberica* subsp. *armena* DME ($\lambda_{450}$: 1.74±0.003, $r^2$:0.9354)> *J. oxycedrus* subsp. *oxycedrus* EE ($\lambda_{450}$: 1.598±0.095, $r^2$:0.9221)> *C. cancellatus* DME ($\lambda_{450}$: 1.481±0.016, $r^2$:0.9188) > *A. nemorosa* (M.bieb.) *spreng* EE ($\lambda_{450}$:1.436±0.032, $r^2$:0.9457)> *A. nemorosa* (M.bieb.) *spreng* DME ($\lambda_{450}$:1.31±0.036, $r^2$:0.9706)> *S. siberica* subsp*. armena* EE ($\lambda_{450}$:1.303±0.055, $r^2$:9962)> *A. nemorosa (M.bieb.) spreng* WE ($\lambda_{450}$:1.133±0.028, $r^2$:0.9543)> *S. siberica* subsp. *armena* WE ($\lambda_{450}$:1.204±0.020, $r^2$:0.9739)> *C. cancellatus* EE ($\lambda_{450}$:1.195±0.015, $r^2$:0.9245) > *J. oxycedrus* subsp. *oxycedrus* WE ($\lambda_{450}$:1.037±0.003, $r^2$:0.9653)~ *C. cancellatus* WE ($\lambda_{450}$:1.020±0.03, $r^2$:0.9479) > *α*-Tocopherol ($\lambda_{450}$:1.014±0.054, $r^2$:0.9287)> Trolox($\lambda_{450}$:0.987±0.007, $r^2$:0.9663). When the results were examined, it was determined that plant extracts had better results than standard antioxidants, α-Tocopherol and Trolox.

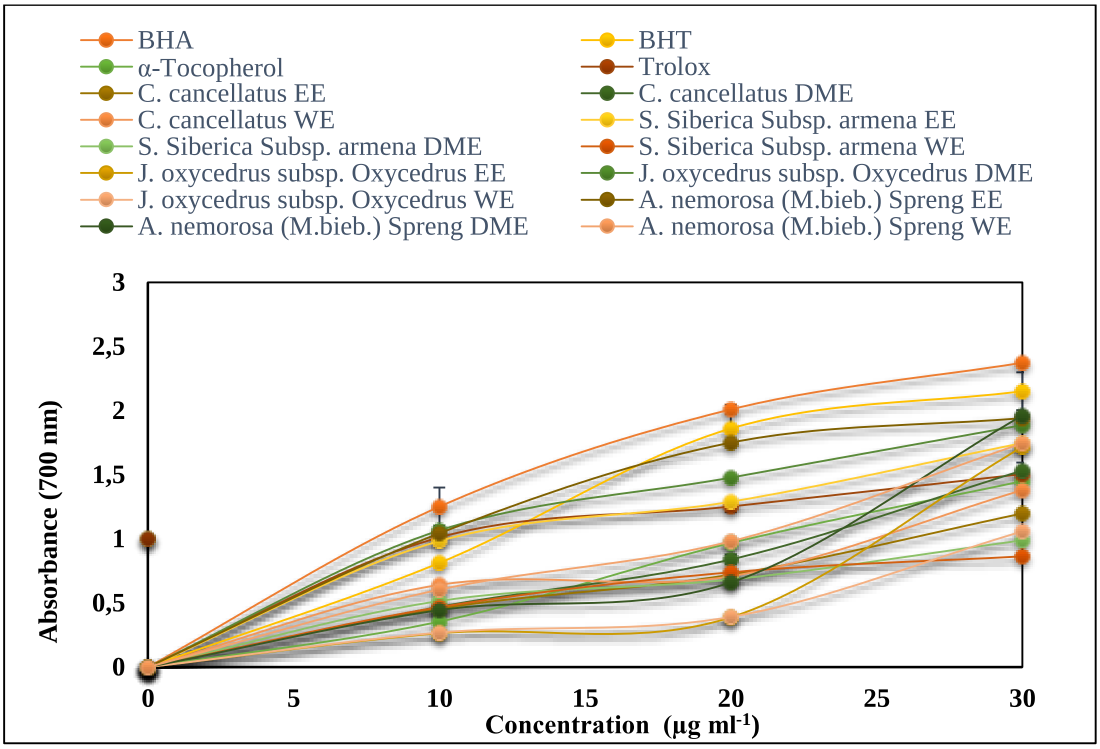
Ferric reducing antioxidant power (FRAP assay) is known as a method based on measuring the reduction of ferric ions ($Fe^{3+}$)-ligand complex by antioxidants in an acidic environment to intense, blue-coloured iron ions ($Fe^{2+}$) complex (Gulcin, 2020). This method was first used to analyze plasma assays and then began to be used in various places, including various biological fluids, plant extracts, foods, and beverages (Elmastas et al., 2006; Gülçin, 2012). In this method, Ferric reduced antioxidant power capacities (FRAP) of water, ethanol, and dichloromethane extracts of the leaves of *Crocus cancellatus, Scilla Siberica* subsp*. armena, Juniperus oxycedrus* subsp*. oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *Sprengel* was determined to be between 0.529 and 1.256 absorbances at 30 μg $mL^{-1}$ concentration (Table 4, Figure 5). FRAP of the examined plant extracts and standard antioxidants are as follows: BHA ($\lambda_{593}$: 1.635±0.038, $r^2$:0.9227) > Trolox ($\lambda_{593}$:1.443±0.020, $r^2$:0.9603) ~ BHT ($\lambda_{593}$:1.441±0.006, $r^2$:0.9202) > *α*-Tocopherol ($\lambda_{593}$:1.380±0.072, $r^2$:0.9784)> *S. Siberica* subsp. *armena* EE ($\lambda_{593}$:1.256±0.011, $r^2$:0.9554)> *J. oxycedrus* subsp. *oxycedrus* DME ($\lambda_{593}$:1.217±0.030, $r^2$:0.9623) > *A. nemorosa (M.bieb.) spreng* DME ($\lambda_{593}$:1.185±0.012, $r^2$:0.9884)> *A. nemorosa* (M.bieb.) *spreng* EE ($\lambda_{593}$:1.163±0.015, $r^2$:0.9912)> *S. siberica* subsp. *armena* DME ($\lambda_{593}$:1.040±0.014, $r^2$:0.9059)> *J. oxycedrus* subsp. *oxycedrus* EE ($\lambda_{593}$:0.956±0.041, $r^2$:0.9533)> *S. Siberica Subsp. armena* WE

Çizelge 4. Crocus cancellatus, Scilla siberica subsp. armena, Juniperus oxycedrus subsp. oxycedrus ve Anthriscus nemorosa (M.bieb.) spreng yapraklarının su, etanol ve diklorometan ekstraktlarının ve standart antioksidanların (30 µg mL$^{-1}$) $Fe^{3+}$, $Cu^{2+}$ indirgeme ve FRAP aktiviteleri

*Table 4. $Fe^{3+}$, $Cu^{2+}$-reducing, and FRAP activities of water, ethanol, and dichloromethane extracts leaves of Crocus cancellatus, Scilla siberica subsp. armena, Juniperus oxycedrus subsp. oxycedrus and Anthriscus nemorosa (M.bieb.) spring and of standard antioxidants (30 µg mL$^{-1}$)*

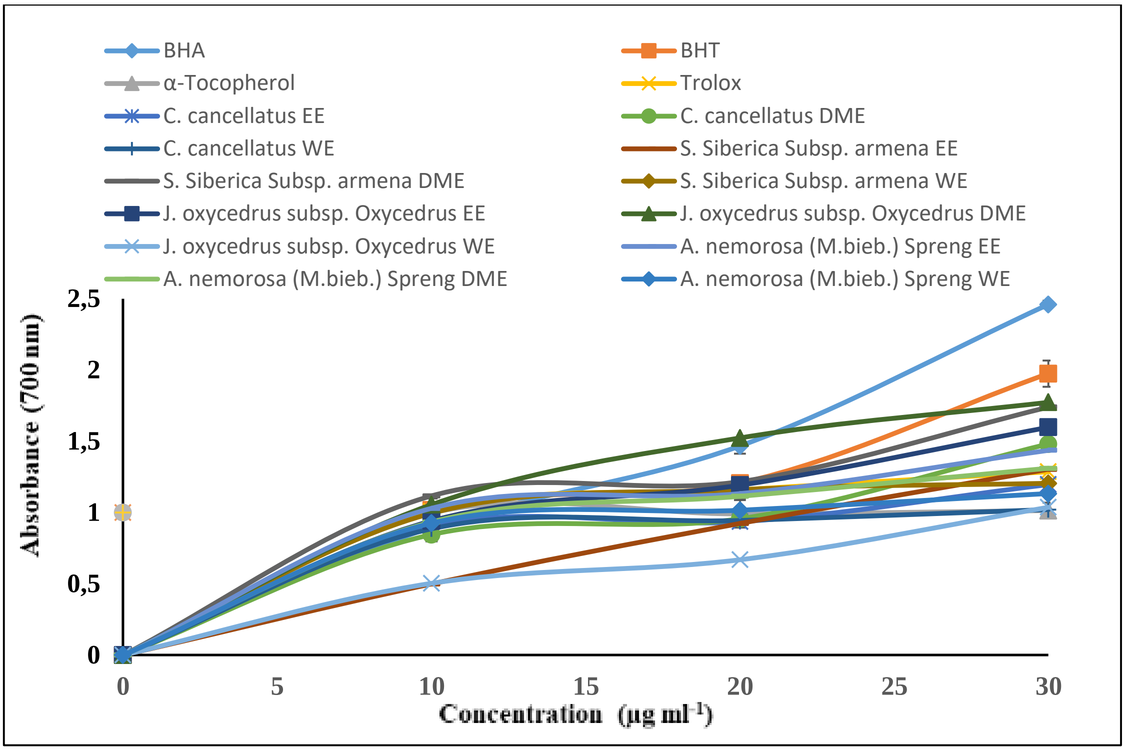
| Antioxidants and Samples | $Fe^{3+}$ reducing | | $Cu^{2+}$ reducing | | $Fe^{3+}$-TPTZ reducing | |
|---|---|---|---|---|---|---|
| | $\lambda_{700}$ | $r^2$ | $\lambda_{450}$ | $r^2$ | $\lambda_{593}$ | $r^2$ |
| BHA | 2.372±0.020 | 0.9403 | 2.459±0.027 | 0.9904 | 1.635±0.038 | 0.9227 |
| BHT | 2.151±0.147 | 0.96 | 1.975±0.091 | 0.9422 | 1.441±0.006 | 0.9202 |
| Trolox | 1.449±0.047 | 0.9907 | 1.014±0.054 | 0.9287 | 1.380±0.072 | 0.9784 |
| α-Tocopherol | 1.504±0.016 | 0.9766 | 0.987±0.007 | 0.9663 | 1.443±0.020 | 0.9603 |
| *C. cancellatus* EE | 1.197±0.017 | 0.9891 | 1.195±0.015 | 0.9245 | 0.622±0.005 | 0.9333 |
| *C. cancellatus* DME | 1.532±0.060 | 0.9829 | 1.481±0.016 | 0.9188 | 0.564±0.022 | 0.9607 |
| *C. cancellatus* WE | 1.379±0.046 | 0.927 | 1.02±0.03 | 0.9479 | 0.529±0.018 | 0.9567 |
| *S. Siberica* subsp. *armena* EE | 1.747±0.039 | 0.9371 | 1.303±0.055 | 0.9962 | 1.256±0.011 | 0.9554 |
| *S. Siberica* subsp. *armena* DME | 0.988±0.017 | 0.9556 | 1.74±0.003 | 0.9354 | 1.040±0.014 | 0.9059 |
| *S. Siberica* subsp. *armena* WE | 0.863±0.015 | 0.9329 | 1.204±0.020 | 0.9739 | 0.915±0.005 | 0.9525 |
| *J. oxycedrus* subsp. *oxycedrus* EE | 1.716±0.028 | 0.9488 | 1.598±0.095 | 0.9221 | 0.956±0.041 | 0.9533 |
| *J. oxycedrus* subsp. *oxycedrus* DME | 1.886±0.015 | 0.9347 | 1.773±0.066 | 0.9084 | 1.217±0.030 | 0.9623 |
| *J. oxycedrus* subsp. *oxycedrus* WE | 1.058±0.040 | 0.9605 | 1.037±0.003 | 0.9653 | 0.854±0.033 | 0.9693 |
| *A. nemorosa* (M.bieb.) *spreng* EE | 1.94±0.062 | 0.9211 | 1.436±0.032 | 0.9457 | 1.163±0.015 | 0.9912 |
| *A. nemorosa* (M.bieb.) *spreng* DME | 1.96±0.060 | 0.9596 | 1.31±0.036 | 0.9706 | 1.185±0.012 | 0.9884 |
| *A. nemorosa* (M.bieb.) *spreng* WE | 1.744±0.040 | 0.9842 | 1.133±0.028 | 0.9543 | 0.867±0.028 | 0.95 |

The results show significant differences ($p<0.05$) in post-hoc comparisons between different groups.

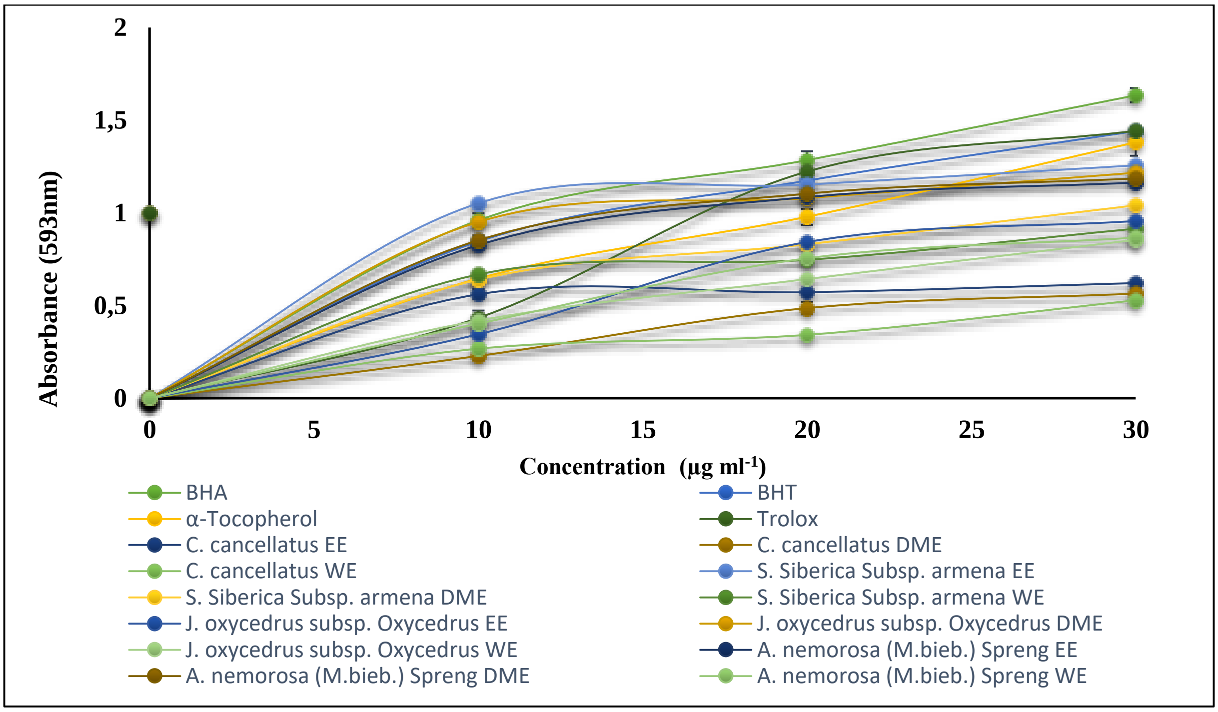



Şekil 3. Ekstraktların antioksidan aktiviteleri ($Fe^{3+}$ indirgeme aktivitesi)

*Figure 3. Antioxidant activities of extracts ($Fe^{3+}$ reducing activity)*




Şekil 4. Ekstraktların antioksidan aktiviteleri Ekstraktların antioksidan aktiviteleri ($Cu^{2+}$ indirgeme aktivitesi)
*Figure 4. Antioxidant activities of extracts ($Cu^{2+}$ reducing activity)*




Şekil 5. Ekstraktların antioksidan aktiviteleri ($Fe^{3+}$-TPTZ indirgeyici)
*Figure 5. Antioxidant activities of extracts ($Fe^{3+}$-TPTZ reducing)*

($\lambda_{593}$:0.915±0.005, $r^2$:0.9525)> *A. nemorosa (M.bieb.) spreng* WE ($\lambda_{593}$:0.867±0.028, $r^2$:0.9500)> *J. oxycedrus* subsp. *oxycedrus* WE ($\lambda_{593}$:0.854±0.033, $r^2$:0.9693)> *C. cancellatus* EE ($\lambda_{593}$:0.622±0.005, $r^2$:0.9333)> *C. cancellatus* DME ($\lambda_{593}$:0.564±0.022, $r^2$:0.9607)> *C. cancellatus* WE ($\lambda_{593}$:0.529±0.018, $r^2$:0.95).

## CONCLUSION

This study was designed to reveal the health benefits of plants. In the study, four plants (*Crocus cancellatus*, *Scilla siberica* subsp. *armena*, *Juniperus oxycedrus* subsp. *oxycedrus* and *Anthriscus nemorosa* (M.bieb.) *sprenge)* used in different ways in our country and many regions were used, and water, ethanol, and dichloromethane extracts of these plants were prepared. It was decided that the phenolics and flavonoids in the ethanol and dichloromethane extracts were what made them so good at reducing and scavenging radicals. It was also observed that the prepared extracts exhibited significant biological effects on critical metabolic enzymes, and in general, DME and EE fractions had significant inhibitory effects on enzyme activity. However, further research is needed to identify the phenolic active constituents that are among the main drivers of antioxidant activity and to evaluate their mechanisms of action *in vivo*.

## Author's Contributions

**Bayram Yurt,** investigation, methodology **Rüya Sağlamtaş,** methodology, data curation, formal analysis, writing – original draft. **Yeliz Demir,** investigation, formal analysis, writing - review & editing, supervision. **Cuneyt Caglayan,** investigation, writing - review & editing, supervision. **Halit Diril,** methodology, writing – original draft. **Ebubekir İzol,** investigation, writing - review & editing, supervision. All authors have read and agreed to the published version of the manuscript.

## Statement of Conflict of Interest

The authors declare no conflict of interest.

## REFERENCES

Abdullaev, F. I., Riveron-Negrete, L., Caballero-Ortega, H., Hernández, J. M., Perez-Lopez, I., Pereda-Miranda, R., & Espinosa-Aguirre, J. J. (2003). Use of in vitro assays to assess the potential antigenotoxic and cytotoxic effects of saffron (Crocus sativus L.). *Toxicology in vitro*, *17*(5-6), 731-736.

Aggül, A. G., Müslüm, K., Kandemir, F. M., Küçükler, S., & Çağlayan, C. (2020). Alterations in enzyme activity of carbonic anhydrase, 6-phosphogluconate dehydrogenase and thioredoxin reductase in rats exposed to doxorubicin and morin. *Clinical and Experimental Health Sciences*, *10*(3), 228-234.

Ahouran, M., Hosseini, R., & Zarghami, R. (2012). Corms as a source of explants for the successful clonal propagation of Crocus cancellatus. *Journal of crop science and biotechnology*, *15*, 47-51.

Aktaş, A., Yakalı, G., Demir, Y., Gülçin, İ., Aygün, M., & Gök, Y. (2022). The palladium-based complexes bearing 1, 3-dibenzylbenzimidazolium with morpholine, triphenylphosphine, and pyridine derivate ligands: Synthesis, characterization, structure and enzyme inhibitions. *Heliyon*, *8*(9), e10625

Anil, D. A., Aydin, B. O., Demir, Y., & Turkmenoglu, B. (2022). Design, synthesis, biological evaluation and molecular docking studies of novel 1H-1, 2, 3-Triazole derivatives as potent inhibitors of carbonic anhydrase, acetylcholinesterase and aldose reductase. *Journal of Molecular Structure*, *1257*, 132613.

Apak, R., Calokerinos, A., Gorinstein, S., Segundo, M. A., Hibbert, D. B., Gülçin, İ., ... & Arancibia-Avila, P. (2022). Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (IUPAC Technical Report). *Pure and Applied Chemistry*, *94*(1), 87-144.

Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S., & Erçağ, E. (2006). The cupric ion reduces antioxidant capacity and polyphenolic content of some herbal teas. *International journal of food sciences and nutrition*, *57*(5-6), 292-304.

Aras, A., Dogru, M., & Bursal, E. (2016). Determination of antioxidant potential of Nepeta nuda subsp. Lydia. *Analytical Chemistry Letters*, *6*(6), 758-765.

Aydın, B., Yuca, H., Karakaya, S., Bona, G. E., Göger, G., Tekman, E., ... & Guvenalp, Z. (2023). The anatomical, morphological features, and biological activity of Scilla siberica subsp. armena (Grossh.) Mordak (Asparagaceae). *Protoplasma*, *260*(2), 371-389.

Bagci, E., Aydin, E., Ungureanu, E., & Hritcu, L. (2016). Anthriscus nemorosa essential oil inhalation prevents memory impairment, anxiety and depression in scopolamine-treated rats. *Biomedicine & Pharmacotherapy*, *84*, 1313-1320.

Bayindir, S., Caglayan, C., Karaman, M., & Gülcin, İ. (2019). The green synthesis and molecular docking of novel N-substituted rhodanines as effective inhibitors for carbonic anhydrase and acetylcholinesterase enzymes. *Bioorganic Chemistry*, *90*, 103096.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Bursal, E., Aras, A., Kılıç, Ö., Taslimi, P., Gören, A. C., & Gülçin, İ. (2019). Phytochemical content, antioxidant activity, and enzyme inhibition effect of Salvia eriophora Boiss. & Kotschy against acetylcholinesterase, α-amylase, butyrylcholinesterase, and α-glycosidase enzymes. *Journal of food biochemistry*, *43*(3), e12776.

Bursal, E., Taslimi, P., Gören, A. C., & Gülçin, İ. (2020). Assessments of anticholinergic, antidiabetic, antioxidant activities and phenolic content of Stachys annua. *Biocatalysis and agricultural biotechnology*, *28*, 101711.

Bursal, E., Yılmaz, M. A., Izol, E., Türkan, F., Atalar, M. N., Murahari, M., ... & Ahmad, M. (2021). Enzyme inhibitory function and phytochemical profile of Inula discoidea using in vitro and in silico methods. *Biophysical Chemistry*, *277*, 106629.

Buza, A., Türkeş, C., Arslan, M., Demir, Y., Dincer, B., Nixha, A. R., & Beydemir, Ş. (2023). Discovery of novel benzenesulfonamides incorporating 1, 2, 3-triazole scaffold as carbonic anhydrase I, II, IX, and XII inhibitors. *International Journal of Biological Macromolecules*, *239*, 124232.

Caglayan, C., & Gulcin, İ. (2018). The toxicological effects of some avermectins on goat liver carbonic anhydrase enzyme. *Journal of biochemical and molecular toxicology*, *32*(1), e22010.

Çakmakçı, S., Topdaş, E. F., Kalın, P., Han, H., Şekerci, P., P. Köse, L., & Gülçin, İ. (2015). Antioxidant capacity and functionality of oleaster (E laeagnus angustifolia L.) flour and crust in a new kind of fruity ice cream. *International Journal of Food Science & Technology*, *50*(2), 472-481.

Çelik, Ş., Dervişoglu, G., İzol, E., Sęczyk, Ł., Özdemir, F. A., Yilmaz, M. E., Yilmaz, M. A., Gülçin, İ., Al-Anazi, K. M., Farah, M. A., Zafar, M., Makhkamov, T., & Khan, M. A. (2024). Comprehensive phytochemical analysis of Salvia hispanica L. callus extracts using LC–MS/MS. *Biomedical Chromatography, e5975.*

Davies, K. J. (1995). Oxidative stress: the paradox of aerobic life. In *Biochemical Society Symposia* (Vol. 61, pp. 1-31). Portland Press Limited.

Demir, Y., Türkeş, C., Çavuş, M. S., Erdoğan, M., Muğlu, H., Yakan, H., & Beydemir, Ş. (2023). Enzyme inhibition, molecular docking, and density functional theory studies of new thiosemicarbazones incorporating the 4-hydroxy-3, 5-dimethoxy benzaldehyde motif. *Archiv der Pharmazie*, *356*(4), 2200554.

Chryssanthi, D. G., Lamari, F. N., Iatrou, G., Pylara, A., Karamanos, N. K., & Cordopatis, P. (2007). Inhibition of breast cancer cell proliferation by style constituents of different Crocus species. *Anticancer research*, *27*(1A), 357-362.

Durmaz, L., Erturk, A., Akyüz, M., Polat Kose, L., Uc, E. M., Bingol, Z., ... & Gulcin, İ. (2022). Screening of carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzyme inhibition effects and antioxidant activity of coumestrol. *Molecules*, *27*(10), 3091.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95.

Elmastas, M., Turkekul, I., Ozturk, L., Gulcin, I., Isildak, O., & Aboul-Enein, H. Y. (2006). Antioxidant activity of two wild edible mushrooms (Morchella vulgaris and Morchella esculanta) from North Turkey. *Combinatorial Chemistry & High Throughput Screening*, *9*(6), 443-448.

Erdoğan, M., Polat Köse, L., Eşsiz, S., & Gülçin, İ. (2021). Synthesis and biological evaluation of some 1-naphthol derivatives as antioxidants, acetylcholinesterase, and carbonic anhydrase inhibitors. *Archiv der Pharmazie*, *354*(8), 2100113.

Fatehi, M., Rashidabady, T., & Fatehi-Hassanabad, Z. (2003). Effects of Crocus sativus petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. *Journal of ethnopharmacology*, *84*(2-3), 199-203.

Gocer, H., & Gulcin, I. (2013). Caffeic acid phenethyl ester (CAPE): a potent carbonic anhydrase isoenzymes inhibitor. *International Journal of Academic Research, 5*(4A), 150-155

Gök, H. N., Orhan, N., Özüpek, B., Pekacar, S., Selvi, Ş. N., & Orhan, D. D. (2021). Standardization of Juniperus macrocarpa Sibt. & Sm. and Juniperus excelsa M. Bieb. extracts with carbohydrate digestive enzyme inhibitory and antioxidant activities. *Iranian journal of pharmaceutical research: IJPR*, *20*(3), 441.

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3), 651-715.

Gulcin, I., Kaya, R., Goren, A. C., Akincioglu, H., Topal, M., Bingol, Z., ... & Alwasel, S. (2019). Anticholinergic, antidiabetic and antioxidant activities of cinnamon (Cinnamomum verum) bark extracts: polyphenol contents analysis by LC-MS/MS. *International Journal of Food Properties*, *22*(1), 1511-1526.

Guner, A., Aslan, S., Ekim, T., Vural, M., Babac, M. (2012). Turkey Plant List (Vascular Plants). Nezahat Gokyigit Botanical Garden and Flora Research Association Publication. İstanbul.

Gülcin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of toxicology*, *86*, 345-391.

Güleç, Ö., Türkeş, C., Arslan, M., Demir, Y., Yeni, Y., Hacımüftüoğlu, A., ... & Beydemir, Ş. (2022). Cytotoxic effect, enzyme inhibition, and in silico studies of some novel N-substituted sulfonyl amides incorporating 1, 3, 4-oxadiazol structural motif. *Molecular Diversity*, *26*(5), 2825-2845.

Güven, L., & Gülçin, İ., (2024). Determination of metabolic profiling by LC-MS/MS, evaluation of antioxidantactivities, and enzyme inhibition effects of Helichrysum plicatum subsp. pseudopliacatum. *KSU Journal of Agriculture and Nature, 27*(3), 501-514.

Güven, L., Erturk, A., Miloğlu, F. D., Alwasel, S., & Gulcin, İ. (2023). Screening of antiglaucoma, antidiabetic, anti-alzheimer, and antioxidant activities of Astragalus alopecurus pall—analysis of phenolics profiles by LC-MS/MS. *Pharmaceuticals*, *16*(5), 659.

Hamide, M., Gök, Y., Demir, Y., Sevinçek, R., Taskin-Tok, T., Tezcan, B., ... & Güzel, B. (2022). Benzimidazolium Salts Containing Trifluoromethoxybenzyl: Synthesis, Characterization, Crystal Structure, Molecular

Docking Studies and Enzymes Inhibitory Properties. *Chemistry & Biodiversity*, *19*(12), e202200257.

Izol, E., Temel, H., Yilmaz, M. A., Yener, I., Olmez, O. T., Kaplaner, E., ... & Ertas, A. (2021). A detailed chemical and biological investigation of twelve Allium species from Eastern Anatolia with chemometric studies. *Chemistry & Biodiversity*, *18*(1), e2000560.

Inci, H., Izol, E., Yilmaz, M. A., Ilkaya, M., Bingöl, Z., & Gülçin, I. (2023). Comprehensive Phytochemical Content by LC/MS/MS and Anticholinergic, Antiglaucoma, Antiepilepsy, and Antioxidant Activity of Apilarnil (Drone Larvae). *Chemistry & Biodiversity*, *20*(10), e202300654.

İzol, E. (2024). Determination of Naphthalene Concentration in Honey a New Method using by HS-GC/MS (Headspace-Gas Chromatography/Mass Spectrometry). *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *27*(5), 1095-1104.

İzol, E., Çiçek, İ., Behçet, L., Kaya, E. & Tarhan, A. (2023). Trace Element Analysis of some Medicinal and Aromatic Plant Species by ICP-MS. *Türk Doğa ve Fen Dergisi, 12* (1), 21-29.

Kandemir, N. (2010). A morphological and anatomical investigation about two rare and endemic Crocus taxa (Iridaceae) from Southern Anatolia. *EurAsian Journal of BioSciences*, *4*.

Karageçili, H., İzol, E., Kireçci, E., & Gülçin, İ. (2023a). Antioxidant, antidiabetic, antiglaucoma, and anticholinergic effects of Tayfi grape (Vitis vinifera): A phytochemical screening by LC-MS/MS analysis. *Open Chemistry*, *21*(1), 20230120.

Karagecili, H., İzol, E., Kirecci, E., & Gulcin, İ. (2023b). Determination of antioxidant, anti-alzheimer, antidiabetic, antiglaucoma and antimicrobial effects of zivzik pomegranate (punica granatum)—a chemical profiling by LC-MS/MS. *Life*, *13*(3), 735.

Karakaya, S., Yılmaz, S. V., Koca, M., Demirci, B., & Sytar, O. (2019). Screening of non-alkaloid acetylcholinesterase inhibitors from extracts and essential oils of Anthriscus nemorosa (M. Bieb.) Spreng.(Apiaceae). *South African journal of botany*, *125*, 261-269.

Kaya, Y., Erçağ, A., Zorlu, Y., Demir, Y., & Gülçin, İ. (2022). New Pd (II) complexes of the bisthiocarbohydrazones derived from isatin and disubstituted salicylaldehydes: Synthesis, characterization, crystal structures and inhibitory properties against some metabolic enzymes. *JBIC Journal of Biological Inorganic Chemistry*, *27*(2), 271-281.

Kiliç, Ö. (2017). Essential oil composition of aerial parts of two Anthriscus Pers. species from Turkey. *Journal of Essential Oil Bearing Plants*, *20*(2), 591-596.

Kızıltaş, H., Bingol, Z., Gören, A. C., Kose, L. P., Durmaz, L., Topal, F., ... & Gulcin, İ. (2021). LC-HRMS profiling and antidiabetic, anticholinergic, and antioxidant activities of aerial parts of kınkor (Ferulago stellata). *Molecules*, *26*(9), 2469.

Klimko, M., Boratyńska, K., Montserrat, J. M., Didukh, Y., Romo, A., Gómez, D., ... & Boratyński, A. (2007). Morphological variation of Juniperus oxycedrus subsp. oxycedrus (Cupressaceae) in the Mediterranean region. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *202*(2), 133-147.

Köksal, E., Gülçin, I., Beyza, S., Sarikaya, O., & Bursal, E. (2009). In vitro antioxidant activity of silymarin. *Journal of enzyme inhibition and medicinal chemistry*, *24*(2), 395-405.

Linardaki, Z.I., Lamari, F.N., Margarity, M. (2017). Saffron (Crocus sativus L.) Tea Intake Prevents Learning/Memory Defects and Neurobiochemical Alterations Induced by Aflatoxin B1 Exposure in Adult Mice. *Neurochemical Research, 42*(10), 2743-2754.

Loizzo, M. R., Marrelli, M., Pugliese, A., Conforti, F., Nadjafi, F., Menichini, F., & Tundis, R. (2016). Crocus cancellatus subsp. damascenus stigmas: chemical profile, and inhibition of α-amylase, α-glucosidase and lipase, key enzymes related to type 2 diabetes and obesity. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *31*(2), 212-218.

Mammadov, R., & Sahranc, B. (2003). Mugla Il merkezinde sonbaharda tespit edilen Bazi geofitler. *Ekoloji çevre dergisi*, 12(48), 13-18.

Menemen, Y. (2012). Anthriscus Pers. Türkiye Bitkileri Listesi (Damarlı Bitkiler); Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, MT, Eds, 62-64.

Menghini, L., Leporini, L., Vecchiotti, G., Locatelli, M., Carradori, S., Ferrante, C., ... & Orlando, G. (2018). Crocus sativus L. stigmas and byproducts: Qualitative fingerprint, antioxidant potentials and enzyme inhibitory activities. *Food research international*, *109*, 91-98.

Orhan, N., Aslan, M., Pekcan, M., Orhan, D. D., Bedir, E., & Ergun, F. (2012). Identification of hypoglycaemic compounds from berries of Juniperus oxycedrus subsp. oxycedrus through bioactivity guided isolation technique. *Journal of ethnopharmacology*, *139*(1), 110-118.

Orhan, N., Orhan, I. E., & Ergun, F. (2011). Insights into cholinesterase inhibitory and antioxidant activities of five Juniperus species. *Food and Chemical Toxicology*, *49*(9), 2305-2312.

Osmaniye, D., Türkeş, C., Demir, Y., Özkay, Y., Beydemir, Ş., & Kaplancıklı, Z. A. (2022). Design, synthesis, and biological activity of novel dithiocarbamate-methylsulfonyl hybrids as carbonic anhydrase inhibitors. *Archiv der Pharmazie*, *355*(8), 2200132.

Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*, *44*(6), 307-315.

Ozer, E. B., Caglayan, C., & Bayindir, S. (2022). The solvent-controlled regioselective synthesis of 3-amino-5-aryl-rhodanines as novel inhibitors of human carbonic anhydrase enzymes. *Tetrahedron*, *120*, 132896.

Oztaskin, N., Goksu, S., Demir, Y., Maras, A., & Gulcin, İ. (2022). Synthesis of Novel Bromophenol with Diaryl Methanes—Determination of Their Inhibition Effects on Carbonic Anhydrase and Acetylcholinesterase. *Molecules*, *27*(21), 7426.

Öntaş, C., Uluköy, G., Esin, B. A. B. A., & Mammadov, R. (2020). Crocus cancellatus subsp. mazziaricus (Herbert) mathew bitki ekstraktının avrupa deniz levrek balığı (Dicentrarchus labrax, L. 1758) doğal bağışıklık sistemi üzerine etkisi. *Acta Aquatica Turcica*, *16*(1), 148-157.

Özdemir, F., Yildirim, M. (2016). In vitro multiple shoot regeneration from Scilla siberica subsp. armena petiole [Scilla siberica subsp. armena yaprak sapindan in vitro çoklu sürgün rejenerasyonu].

Özdemir, F.A., Yıldırım, M.U., Kahrız, M.P., Kılıç, Ö. (2016). In vitro bulblet regeneration from Scilla Siberica Haw. subsp. armena (Grossh.) mordak peduncle.

Öztürk, M., Tümen, İ., Uğur, A., Aydoğmuş-Öztürk, F., & Topçu, G. (2011). Evaluation of fruit extracts of six Turkish Juniperus species for their antioxidant, anticholinesterase and antimicrobial activities. *Journal of the Science of Food and Agriculture*, *91*(5), 867-876.

Polat Kose, L., Bingol, Z., Kaya, R., Goren, A. C., Akincioglu, H., Durmaz, L., ... & Gülçin, İ. (2020). Anticholinergic and antioxidant activities of avocado (Folium perseae) leaves–phytochemical content by LC-MS/MS analysis. *International Journal of Food Properties*, *23*(1), 878-893.

Polat Kose, L., & Gulcin, İ. (2021). Evaluation of the antioxidant and antiradical properties of some phyto and mammalian lignans. *Molecules*, *26*(23), 7099.

Taslimi, P., Caglayan, C., & Gulcin, İ. (2017). The impact of some natural phenolic compounds on carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes: An antidiabetic, anticholinergic, and antiepileptic study. *Journal of biochemical and molecular toxicology*, *31*(12), e21995.

Taviano, M. F., Marino, A., Trovato, A., Bellinghieri, V., La Barbera, T. M., Güvenç, A., ... & Miceli, N. (2011). Antioxidant and antimicrobial activities of branches extracts of five Juniperus species from Turkey. *Pharmaceutical Biology*, *49*(10), 1014-1022.

Tugrak, M., Gul, H. I., Demir, Y., & Gulcin, I. (2021). Synthesis of benzamide derivatives with thiourea-substituted benzenesulfonamides as carbonic anhydrase inhibitors. *Archiv Der Pharmazie*, *354*(2), 2000230.

Türkan, F., Atalar, M. N., Aras, A., Gülçin, İ., & Bursal, E. (2020). ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of Achillea schischkinii Sosn. *Bioorganic chemistry*, *94*, 103333.

Verpoorte, J. A., Mehta, S., & Edsall, J. T. (1967). Esterase activities of human carbonic anhydrases B and C. *Journal of biological chemistry*, *242*(18), 4221-4229.

Yapıcı, İ., İzol, E. (2023). "Phytochemicals and their Bioavailability", In Medicinal, Aromatıc Plants and Phytochemicals, ed. İzol E., Yılmaz M. A., Haspolat Y. K., *Orient Publications,* 43-60.

Yaşar, Ü., Gönül, İ., Türkeş, C., Demir, Y., & Beydemir, Ş. (2021). Transition-metal complexes of bidentate Schiff-base ligands: in vitro and in silico evaluation as non-classical carbonic anhydrase and potential acetylcholinesterase inhibitors. *Chemistry Select 6*(29), 7278-7284.

Yilmaz, M. A., Cakir, O., Izol, E., Tarhan, A., Behcet, L., & Zengin, G. (2023). Detailed Phytochemical Evaluation of a Locally Endemic Species (Campanula baskilensis) by LC-MS/MS and Its In-Depth Antioxidant and Enzyme Inhibitory Activities. *Chemistry & Biodiversity*, *20*(12), e202301182.

Yilmaz, M. A., Cakir, O., Zengin, G., Izol, E., & Behcet, L. (2024). The Uprisal of a Lost Endemic Edible Species, Micromeria cymuligera: Comprehensive Elucidation of its Biological Activities and Phytochemical Composition. *Food Bioscience*, 104690.

Zengin, R., Gök, Y., Demir, Y., Şen, B., Taskin-Tok, T., Aktaş, A., ... & Aygün, M. (2023). Fluorinated benzimidazolium salts: Synthesis, characterization, molecular docking studies and inhibitory properties against some metabolic enzymes. *Journal of Fluorine Chemistry*, *267*, 110094.