T.C.
KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMA PROJELERİ KOORDİNASYON BİRİMİ
PROJE YAYIN DİLEKÇESİ

Proje Adı		
Tribulus terrestris L. Bitkisinin Antioksidan Aktivitesinin Belirlenmesi, Asetilkolinesteraz, Bütirilkolinesteraz ve Karbonik Anhidraz Enzimleri Üzerinde İnhibisyon Etkilerinin İncelenmesi		
Proje No	**Başlama Tarihi**	**Bitiş Tarihi**
2021/1-35 M	25-03-2021	25-03-2024
Yayın Türü	**Yayın / Makele Başlığı**	
Makale	*Evaluation of antioxidant activities and inhibition effects of Tribulus terrestris L. extracts on some metabolic enzymes*	
Dergi ISSN	**DOI**	**Cilt / Sayfa / Yıl**
0254-6299	https://doi.org/10.1016/j.sajb.2024.05.031	170 / 156-162 / 2024
Yayınlandıgı Dergi Kısa Ad	**Yayınlandıgı Dergi**	
Belirtilmemiş	South African Journal of Botany	

İLGİLİ MAKAMA

Yukarıda bilgileri verilen ilgili otomasyona girilmiş yayın bilgileri içerisinde ; *"Söz konusu çalışma/yayın/sunum/poster/bildiri/* ***KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ Bilimsel Araştırma Projeleri birimi*** *tarafından 2021/1-35 M proje numaralı "Tribulus terrestris L. Bitkisinin Antioksidan Aktivitesinin Belirlenmesi, Asetilkolinesteraz, Bütirilkolinesteraz ve Karbonik Anhidraz Enzimleri Üzerinde İnhibisyon Etkilerinin İncelenmesi" konusu ile ilgili olup, ilgili birimce desteklenmiştir." ("This work is supported by the* ***Scientific Research Project Fund of KAHRAMANMARAŞ SÜTÇÜ İMAM ÜNİVERSİTESİ*** *under the project number 2021/1-35 M")* ifadesi yer almaktadır.

PROJE YÜRÜTÜCÜSÜNÜN

Ünvanı, Adı-Soyadı : Dr.Öğr.Üyesi Elife KAYA

Tarih : 08-06-2024

Bu belge, güvenli elektronik imza ile imzalanmıştır.

Belge Doğrulama Kodu :a1a493be43

Belge Takip Adresi : https://bapotomasyon.ksu.edu.tr/belgedogrula

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South African Journal of Botany 170 (2024) 156–162

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Evaluation of antioxidant activities and inhibition effects of *Tribulus terrestris* L. extracts on some metabolic enzymes

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ARTICLE INFO

Article History:
Received 29 March 2024
Revised 12 May 2024
Accepted 15 May 2024
Available online xxx

Edited by Prof U. Çakılcıoğlu

Keywords:
Tribulus terrestris
Antioxidant activity
Cholinesterase
Carbonic anhydrase
Inhibition

ABSTRACT

This research aimed to determine the antioxidant capacity of *Tribulus terrestris* extracts made using various solvents and their effects on some metabolic enzymes. Five different antioxidant methods were used to assess the antioxidant capacities of the water, ethanol, acetone, chloroform, and hexane extracts of *T. terrestris*. The inhibitory effects of the extracts on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), α-glycosidase enzymes and human carbonic anhydrase isoenzymes (hCA-I and hCA-II) were investigated. The study showed that acetone extract had the strongest inhibition effects in DPPH (IC_{50}:25.86 ± 0.010 μg/mL) and ABTS (IC_{50}:10.13 ± 0.023 μg/mL) radical scavenging activities. In CUPRAC/Fe^{3+}-Fe^{2+}/FRAP reduction tests, ethanol extract showed the highest reducing power. The ethanol extract exhibited the highest level of inhibition for the AChE/BChE and α-glycosidase enzymes (IC_{50}:12.13 ± 0.37 μg/mL, IC_{50}:22.33 ± 0.27 μg/mL and IC_{50}:25.98 ± 0.75 μg/mL), respectively. Chloroform extract showed the strongest inhibition effect on hCA-I/hCA-II isoenzymes (IC_{50}:21.82 ± 0.55 μg/mL, IC_{50}:20.97 ± 0.63 μg/mL), respectively.

1. Introduction

Tribulus terrestris L. is a perennial herb belonging to the Zygophyllaceae family that grows in the Mediterranean region, subtropics, and deserts around the world (Adaikan et al., 2001; Kostova and Dinchev, 2005). *T. terrestris* has been reported to be potentially therapeutic in treating liver, cardiovascular disorders and dizziness (Kapoor, 1990). It is utilized in the traditional medicine for its potential tonic, aphrodisiac, analgesic, anti-hypertensive, and diuretic properties (Hussain et al., 2009). The plant is commonly used in traditional herbal therapy (Gauthaman et al., 2002). *T. terrestris* L. is traditionally used in Türkiye, Karaisalı County (Adana) to treat kidney stones and stomach pain (Günes et al., 2017; Kawarty et al., 2020). It is also well-known as a sexual stimulant. According to research on its pharmacological properties, *T. terrestris* improves the reproductive systems of both sexes (Gauthaman et al., 2002). *T. terrestris* is a medicinal plant that contains steroidal glycosides and saponins. In this regard, certain steroidal saponins obtained from *T. terrestris* have been previously identified (Kostova et al., 2002; Kostova and Dinchev, 2005). There is widespread use of saponin-containing food supplements and pharmaceutical preparations to enhance libido, sexual and athletic performance in both males and females (Su et al., 2009; Ştefănescu et al., 2020). It also contains significant quantities of flavonoids, glycosides, tannins, alkaloids, and steroidal saponins. Flavonoids and steroidal saponins are biologically active compounds of considerable importance (Wu et al., 1999; Lv et al., 2008; Ştefănescu et al., 2020). Pharmacological investigations of plants have revealed that flavonoid constituents possess potent protective properties, including antioxidant, antibacterial, analgesic, and cardiovascular actions (Jiang et al., 2014; Akbaş et al., 2017; Tian et al., 2019; Kaya and Akbaş, 2023). Therefore a number of pharmaceutical products and dietary supplements have been produced using *T. terrestris*.

Several experimental studies have demonstrated the neuroprotective potential of natural products and bioactive compounds against neurodegenerative disorders. Numerous studies have demonstrated that saponins and flavonoids have strong neuroprotective activity in Parkinson's and Alzheimer's diseases (Dajas et al., 2003). Alzheimer's disease (AD) is an irreversible degenerative neurological condition characterized by gradual memory loss and abnormal behaviors (Parihar et al., 2004). As a neurodegenerative illness, AD primarily affects the elderly. Research indicates that around 10 % of individuals aged 65 and older and 32 % of those aged 85 and older, are affected by AD. The estimated occurrence rate of AD is roughly 6.48 % (Leng and Edison, 2021). Approximately 250 thousand people in Türkiye have been estimated to suffer from AD (Yabo Dambagi et al., 2020). Acetylcholine (ACh) levels in the hippocampus and cortices of the brain decrease in this disease. The acetylcholinesterase

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https://doi.org/10.1016/j.sajb.2024.05.031

(AChE) enzyme has been the subject of much research recently due to its association with AD (Tarawneh and Holtzman, 2012; Gulcin et al., 2019; Habtemariam, 2019). AChE inhibitors such as rivastigmine, donepezil, and galantamine are utilized to the manage symptoms of AD. These drugs, however, have been reported to have numerous side effects in addition to limited benefits for some patients. Similar to AChE inhibitors, butyrylcholinesterase (BChE) enzyme inhibitors have been found to have significant use in treating AD in recent years. A potent AChE inhibitor, tacrine is used in AD treatment due to its high efficiency in inhibiting both the BChE and AChE enzymes (Habtemariam, 2019).

Carbonic anhydrases are a set of isoenzymes that catalyze the transformation of carbon dioxide into bicarbonate (Adeva-Andany et al., 2015). Carbonic anhydrase inhibitors have been utilized as pharmacological agents to treat various diseases, including glaucoma, AD, cancer, osteoporosis, and epilepsy, and to prevent certain infectious diseases (Supuran, 2008). Current carbonic anhydrase (CA) inhibitors exhibit side effects. Hence, it is necessary to investigate new inhibitors that are effective and at the same time have no side effects. Medicinal plants can be safely used as new inhibitory agents.

Free radicals are defined as atoms or molecules with unpaired electrons. They are extremely reactive and highly unstable molecules. During various physiological and pathological processes, the human body produces free radicals that cause cell damage. Factors such as unhealthy eating habits, stress, prolonged exposure to sunlight and smoking can increase free radical production (Akyüz et al., 2022). Antioxidants are substances that impede the occurrence of free radical reactions and safeguard cells from harm. Crucial antioxidant compounds, including flavonoids, phenolic acids, and carotenoids, can be found in fruits, vegetables, and functional foods. These antioxidants can delay or prevent the onset of major degenerative diseases such as diabetes and AD (Gulcin et al.,2019). The primary objective in the managing diabetes is to suppress α-glycosidase, a crucial enzyme involved in the digestion of complex carbohydrates from the diet. α-glycosidase inhibitors (AGIs) restrict the enzymatic degradation of polysaccharides into individual monosaccharide molecules, hence impeding the absorption of mono sugar units in the gastrointestinal system. AGIs have the potential to treat diabetes mellitus (DM) and obesity (Oliveira et al., 2018; Bingol et al., 2021). Because of the negative side effects of α-glycosidase inhibitors used to treat hyperglycemia, like fatigue, sleeplessness, headaches, and gastrointestinal discomfort, the development of novel inhibitors with no adverse effects is becoming increasingly important (Oliveira et al., 2018). Chemical studies suggest that extracts from plants abundant in flavonoids often have efficacy against α-glycosidase inhibitors (AGIs) (Gholamhoseinian et al., 2009; Brahmachari, 2011). Therefore, it is essential to identify these natural compounds with potent activity against α-glycosidase but with minimal side effects.

The purpose of this study was to determine the antioxidant activities of *T. terrestris* extracts prepared with various organic solvents and their inhibition effects against some disease-related enzymes, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), human carbonic anhydrase (hCA-I and hCA-II) and α-glycosidase. In addition, since there are few studies on the inhibition of hCA-I and hCA-II isoenzymes of *T. terrestris*, the results of this study will contribute to the literature.

2. Materials and methods

2.1. Chemicals

All solvents and chemicals were purchased from Sigma-Aldrich (Germany) and were of analytical grade.

2.2. Plant material

Aerial parts of the *T. terrestris* plant were purchased commercially from a herbalist in Kahramanmaras province of Türkiye. The plant sample was identified and confirmed by Assist. Prof. Dr. Seyran PALABAS UZUN (Kahramanmaraş Sütçü İmam University, Türkiye). The samples were ground with a laboratory grinder (Waring Commercial) and stored in the laboratory protected from light and humidity until using in the experiment.

2.3. Preparation of T. terrestris plant extracts

The powdered plant was extracted using five different solvents (water, ethanol, acetone, chloroform, and hexane). The ground plant (20 g) was extracted with 150 mL of the appropriate solvent at room temperature for 12 h and filtered. After repeating the same process five times, the filtrates were combined and the ethanol, acetone, chloroform and hexane were removed from the extract by rotary evaporation. For the water extract, 150 mL of pure water was added to 20 g of the plant and heated using the decoction procedure. A few minutes after boiling, it was allowed to cool and filtered. The filtrate was frozen at −20 °C and lyophilized. The extracts obtained were stored at −20 °C until used.

2.4. Determination of CUPRAC reduction capacity

The cupric ion (Cu^{2+}) reducing the capacity of the extracts (water, ethanol, acetone, chloroform and hexane) was carried out according to the modified method used by Apak et al. (2004) and Gulcin et al. (2019). For this purpose, 0.01 M $CuCl_2$ solution, 10^{-3} M ethanolic neocuprin solution and 1 M ammonium acetate buffer were put into the test tubes and mixed. After mixing the solution, the absorbances of the extracts (water, ethanol, acetone, chloroform, and hexane) of the plant at different concentrations (10–30 μg/mL) were recorded with a spectrometer at 450 nm. BHT, BHA, Trolox and α-tocopherol were used as standard compounds. All assays were performed in triplicate.

2.5. Determination of FRAP reduction capacity

The $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ complex was reduced to the light blue $[Fe^{2+}\text{-}(TPTZ)_2]^{2+}$ complex in acidic media according to the FRAP technique (Bursal et al., 2019). Firstly, different concentrations of plant extracts (10–30 μg/mL) were added to the test tubes. A solution containing TPTZ (10 mM TPTZ in 40 mM HCl) was then prepared and added to acetate buffer (0.3 M), and $FeCl_3$ solution (20 mM). The total volume was brought to 5 mL, and the mixture was vortexed. All assays were performed in triplicate. Absorbance values were recorded spectrophotometrically at 593 nm after 30 min.

2.6. Determination of Fe^{3+}-Fe^{2+} reduction capacity

To determine the Fe^{3+} reduction ability of the plant extracts, the reduction method of Fe^{3+} $(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ was used (Bursal et al., 2019; Oyaizu, 1986). Stock solutions from the extracts were created for this purpose at a concentration of 1 mg/mL, and these stock solutions were transferred to test tubes at various concentrations. The final volume was completed to 1 mL with distilled water. Subsequently, a solution of 0.2 M phosphate buffer with a pH of 6.6 and 1 % potassium ferricyanide were introduced into each tube. The resulting mixture was then incubated at a temperature of 50 °C for a duration of 20 min. Following these steps, 10 % TCA, and 0.1 % $FeCl_3$ were added to the reaction mixture, and absorbance values were measured spectrophotometrically at 700 nm. All assays were performed in triplicate.

2.7. Determination of the DPPH free radical scavenging capacity

DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity of the extracts was determined by Blois's method (Blois,

1958). Certain amounts of extracts at different concentrations (10–30 μg/mL) were taken to test tubes and the volumes were made up to 3 mL. Then, 1 mL of DPPH solution (10^{-3} M) was added to each. Next, all the test tubes were vortexed thoroughly and incubated in the dark and at room temperature for 30 min. The absorbances were measured at 517 nm by a spectrophotometer. All assays were performed in triplicate. The DPPH radical cation scavenging activity was calculated by the following equation:

$$DPPH\ \ radical\ \ scavenging\ \ (\%) : \left(1 - A_{Sample}/A_{Control}\right) \times 100$$

Where $A_{Control}$ is the control's mean absorbance and A_{Sample} is the samples' mean absorbance.

2.8. Determination of $ABTS^{\cdot+}$ radical scavenging capacity

The $ABTS^{\bullet+}$ radical scavenging activity of extracts were determined using the method developed by Re et al. (1999). Firstly, $ABTS^{\bullet+}$ radical formation was obtained from the ABTS compound. 2.45 mM $K_2S_2O_8$ solution was mixed with 2 mM ABTS solution to produce an $ABTS^{\bullet+}$ cation radical. Then, the $ABTS^{\bullet+}$ cation radical solution was diluted with 0.1 M phosphate buffer (pH: 7.4) until the absorbance value at 734 nm was approximately 0.75 ± 0.02. Then, specific amounts and different concentrations of samples and standards solutions were taken into test tubes. After that, 1 mL of $ABTS^{\bullet+}$ radical solution was added to the extracts and control solution tubes. Afterwards, they were completed with phosphate buffer to make a total volume of 4 mL. The absorbances were measured at 734 nm with a spectrophotometer. All assays were performed in triplicate. The ABTS radical cation scavenging activity was calculated by the following equation:

$$ABTS\ \ radical\ \ scavenging\ \ (\%) : \left(1 - A_{Sample}/A_{Control}\right) \times 100$$

2.9. Cholinesterase inhibition studies

The Ellman method was utilized (Ellman et al., 1961; Topal and Gulcin, 2022) to evaluate the effects of plant extracts on the AChE and BChE enzymes. The AChE and BChE activities were measured using DTNB (5,5′-dithio-bis(2-nitro-benzoic)acid), acetylthiocholine iodide (AChI) and butyrylthiocholine iodide (BChI). Different concentrations of stock solutions of the extracts were prepared in ethanol. Firstly, (100 μL) of Tris/HCl buffer (1 M, pH 8.0), different concentration of extracts solutions and 50 μL of AChE/BChE enzyme solution were added into tubes, then AChI/BChI were added. The mixture was incubated at 25 °C for 5 min. Finally, 50 μL of 0.5 mM DTNB was added, the test tubes were vortexed, and the absorbances were mesasured at 412 nm by a spectrophotometry against their blanks. Tacrine was used as the positive control. All assays were performed in triplicate.

2.10. Human carbonic anhydrase (hCA-I and hCA-II) isoenzymes inhibition studies

The affinity technique used Sepharose-4B-l-tyrosine-sulfanilamide as an affinity matrix to selectively capture CA isoenzymes. The hCA-I and hCA-II enzymes activities were determined using spectrophotometry, following the method published by Verpoorte et al. (1967) and previously reported (Burmaoglu et al., 2019; Aydın et al., 2019). The isoenzymes utilized p-nitrophenylacetate as a substrate and enzymatically converted it into p-nitrophenolate ions. Absorbance is measured at a wavelength of 348 nm. All assays were performed in triplicate.

2.11. α-glycosidase inhibition studies

The α-glycosidase enzyme activity was determined by the procedure defined by Tao et al. (2013). p-nitrophenyl-D glycopyranoside (p-NPG) was used as a substrate for α-glycosidase enzyme. Firstly, 200 μL of phosphate buffer (pH 7.4), 20 μL of enzyme solution and 50 μL of different amounts of extracts were added to test tubes. Then, it was preincubated at 37 °C for 10 min before adding p-NPG to begin the reaction. After the incubation period, 50 μL of p-NPG was added to phosphate buffer (pH 7.4) and incubated at 37 °C for 30 min. The absorbances of sample and positive control were recorded spectrophotometrically at 405 nm. Acarbose was used as the positive control. All assays were performed in triplicate.

2.12. Kinetic studies

The impact of the extracts on the enzyme activities of α-glycosidase, AChE, BChE, hCA-I, and hCA-II was investigated, and IC_{50} values were calculated. For this objective, spectrophotometric measurements of enzyme activities at five different concentrations were performed on all extracts. The IC_{50} was obtained from enzyme activity (%) versus plant concentration plots.

2.13. Statistical analysis

The analyses were replicated three times, and the resulting data were evaluated using One-Way Analysis of Variance (One-way ANOVA/Duncan). A one-way ANOVA was performed to determine the significance of the difference. The values were expressed as mean ± standard deviation and analyzed by SPSS (standard version 20.).

3. Results and discussion

3.1. Reducing capacity and radical scavenging ability results

T. terrestris includes antioxidants and free radical scavengers, such as steroidal saponins and flavonoids, which can delay or prevent oxidative stress (Ştefănescu et al., 2020). For this purpose, the antioxidant capacities of different solvent (water, ethanol, acetone, chloroform, and hexane) extracts of *T. terrestris* were determined by five antioxidant methods (CUPRAC, Fe^{3+}-Fe^{2+} reduction, FRAP, ABTS, and DPPH). The extracts' radical scavenging ability and reducing capacity were determined at three concentrations (10, 20, and 30 μg/mL). The results were compared to standard antioxidant compounds (BHA, BHT, α-tocopherol, and trolox) and shown in the Tables 1 and 2.

Table 1 shows the cupric ion (Cu^{2+}) reducing activity of the extracts and standard antioxidant compounds at 30 μg/mL concentration. First, the Cu^{2+} reduction method was investigated. High absorbance indicates higher cupric ion (Cu^{2+}) reduction capacity. The study results showed that the extracts' reduction capacities (Cu^{2+}) increased in direct proportion with increased concentrations. At a concentration of 30 μg/mL, the Cu^{2+} ion reducing capacities of the extracts (water, ethanol, acetone, chloroform, hexane) and standard antioxidants were as follows: Trolox (2.648) > α-Tocopherol (2.607) > BHT (1.814) > BHA (1.393) > ethanol extract (0.699) > water extract (0.494) > acetone extract (0.443) > chloroform extract (0.401) > hexane extract (0.393). The results demonstrated that all extracts had lower Cu^{2+} reduction capacity compared to standard antioxidants (BHA, BHT, Trolox and α-Tocopherol). The ethanol extract exhibited the highest Cu^{2+}reducing power among all the extracts.

The plant extract's reduction capacity can be evaluated by the reduction of Fe^{3+} ions (Gulcin et al., 2006). This test was widely used

Table 1
CUPRAC, Fe^{3+}-Fe^{2+}, and Fe^{3+}-TPTZ reducing activities of *T.terrestris* plant extracts and standards (30 μg/mL).

Applications	CUPRAC reducing		Fe^{3+}-Fe^{2+} reducing		Fe^{3+}-TPTZ reducing	
	λ_{450}	r^2	λ_{700}	r^2	λ_{593}	r^2
BHA	1.393 ± 0.034^{c}	0.9424	2.169 ± 0.006^{a}	0.9238	1.774 ± 0.002^{b}	0.9618
BHT	1.814 ± 0.013^{b}	0.9903	2.074 ± 0.071^{b}	0.9837	1.966 ± 0.013^{a}	0.9569
α- Tocopherol	2.607 ± 0.060^{a}	0.9613	1.337 ± 0.012^{d}	0.9936	1.378 ± 0.090^{c}	0.9502
Trolox	2.648 ± 0.049^{a}	0.9748	1.696 ± 0.067^{c}	0.9953	1.033 ± 0.059^{d}	0.9601
Water extract	0.494 ± 0.007^{e}	0.9846	0.428 ± 0.005^{g}	0.9534	0.457 ± 0.007^{g}	0.9126
Ethanol extract	0.699 ± 0.002^{d}	0.9795	0.811 ± 0.004^{e}	0.9297	0.683 ± 0.004^{e}	0.9579
Acetone extract	0.443 ± 0.011^{f}	0.9480	0.465 ± 0.003^{f}	0.9295	0.595 ± 0.003^{f}	0.9436
Chloroform extract	0.401 ± 0.002^{g}	0.9661	0.299 ± 0.006^{i}	0.9617	0.314 ± 0.012^{h}	0.9940
Hexane extract	0.393 ± 0.007^{g}	0.9941	0.394 ± 0.001^{h}	0.9690	0.221 ± 0.006^{i}	0.9962

The values represent the mean ± standard deviation of n = 3 parallel measurements. The tables that are represented in different letters in the same column are different at the level of $p < 0.05$.

to measure the decrease of Fe^{3+} ions in the presence of plant components. At 30 μg/mL concentration, Fe^{3+}-Fe^{2+} reducing power of extracts and standards were as follows: BHA (2.169) > BHT (2.074) > Trolox (1.696) > α-Tocopherol (1.337) > ethanol extract (0.811) > acetone extract (0.465) > water extract (0.428) > hexane extract (0.394) > chloroform extract (0.299). The ethanol extract exhibited the strongest Fe^{3+} reduction ability among the extracts (Table 1). In addition, BHA exhibited the most effective reducing power activity among standard antioxidants.

The FRAP assay is a technique for determining the total reducing capacity of antioxidant molecules as well as plant extracts. The reducing capacity of organic molecules or plant extracts is based on spectrophotometric measurement of absorbance values. As can be seen in Table 1, the Fe^{3+}-TPTZ-Fe^{2+}-TPTZ reduction abilities of the plant extracts and positive controls at the same concentration (30 μg/mL) were as follows, respectively: BHT (1.966) > BHA (1.774) > α-Tocopherol (1.378) > Trolox (1.033) > ethanol extract (0.683) > acetone extract (0.595) > water extract (0.457) > chloroform extract (0.314) > hexane extract (0.221). According to these findings, ethanol extract had the highest absorbance among the extracts at the same concentration but had a lower reducing capacity than all of the positive controls. BHT demonstrated the greatest reduction capacity.

CUPRAC, Fe^{3+}-Fe^{2}, and FRAP reducing capacities of exhibited positive correlation. Thus, the ethanol extract had the highest reduction capability among all plant extracts, as determined by all three methods. The reduction capacity of ethanol extract was found to be significantly greater than that of other extracts. Furthermore, among all reduction methods, hexane extract had the lowest reduction power among the extracts.

The total antioxidant capacity of many food, medicinal, and pharmaceutical plants can be determined quickly and easily using this redox method (Polat Köse, 2021). *T. terrestris* extracts were tested for their ability to scavenge DPPH and $ABTS^{\cdot+}$ radicals. At the same time, the half-maximal concentrations (IC_{50}) were determined for all applications to compare their radical scavenging activities. A sample with a lower IC_{50} value has stronger DPPH and $ABTS^{\cdot+}$ scavenging potential. Table 2 shows the IC_{50} values of the extracts and standard antioxidants for DPPH and $ABTS^{\cdot+}$ scavenging activities.

Table 2
IC_{50} values of extracts and standard antioxidants for DPPH, $ABTS^{\cdot+}$ scavenging activities.

Applications	DPPH		ABTS	
	IC_{50} (μg/mL)	r^2	IC_{50} (μg/mL)	r^2
BHA	14.44 ± 0.082^{f}	0.9463	3.56 ± 0.001^{h}	0.8912
BHT	18.43 ± 0.138^{e}	0.9099	3.12 ± 0.011^{i}	0.8949
α- Tocopherol	10.24 ± 0.037^{g}	0.8640	5.84 ± 0.018^{f}	0.9723
Trolox	$9.48 \pm 0.025f^{h}$	0.9610	4.55 ± 0.029^{g}	0.9046
Water extract	39.60 ± 0.062^{b}	0.8830	11.02 ± 0.054^{c}	0.9893
Ethanol extract	30.13 ± 0.071^{c}	0.9228	10.86 ± 0.002^{d}	0.9784
Acetone extract	25.86 ± 0.010^{d}	0.9011	10.13 ± 0.023^{e}	0.9258
Chloroform extract	40.29 ± 0.028^{b}	0.9070	19.25 ± 0.080^{a}	0.9474
Hexane extract	51.33 ± 0.019^{a}	0.8957	14.50 ± 0.039^{b}	0.9193

The values represent the mean ± standard deviation of n = 3 parallel measurements. The tables that are represented in different letters in the same column are different at the level of $p < 0.05$.

The IC_{50} values for DPPH scavenging activity of the standard antioxidants and extracts were determined in the following order: Trolox (9.48 μg/mL) > α-Tocopherol (10.24 μg/mL) > BHA (14.44 μg/mL) > BHT (18.43 μg/mL) > acetone extract (25.86 μg/ mL) > ethanol extract (30.13 μg/mL) > water extract (39.60 μg/mL) > chloroform extract (40.29 μg/mL) > hexane extract (51.33 μg/mL) (Table 2). A comparison of IC_{50} values of all applications for DPPH revealed that acetone extract (IC_{50}=25.86 μg/mL) had the strongest radical scavenging effect (Table 2). Among the standard antioxidant substances, Trolox (IC_{50}=9.48 μg/mL) showed the highest DPPH radical scavenging activity.

The ABTS method is another widely used method to evaluate the radical scavenging capacity of antioxidant compounds (Liu et al., 2021), such as food extracts, natural and pure compounds (Hmidani et al., 2021; Akyüz, 2022). A lower IC_{50} value indicates greater antioxidant power. For the purpose of evaluating the ABTS scavenging activities of all extracts compare with BHA, BHT, Trolox, and α-Tocopherol, IC_{50} values were computed. Table 2 shows that standard antioxidant compounds were more effective than extracts. The IC_{50} values for ABTS scavenging activity of the standard antioxidants and extracts were determined in the following order: BHT (3.12 μg/mL) > BHA (3.56 μg/mL) > Trolox (4.55 μg/mL) > α-Tocopherol (5.84 μg/mL) > acetone extract (10.13 μg/mL) > ethanol extract (10.86 μg/mL) > water extract (11.02 μg/mL) > hexane extract (14.50 μg/mL) > chloroform extract (19.25 μg/mL). Acetone extract and BHT had the most potent ABTS radical scavenging activity with IC_{50} values of 10.13 μg/mL and 3.12 μg/mL respectively (Table 2).

The acetone extract exhibited the strongest DPPH and ABTS radical scavenging activities. However, hexane extract had a lesser effect scavenging DPPH while chloroform extract had the least effect to scavenging ABTS radicals. In addition it was found that acetone extract had the greatest effect in radical scavenging activity and ethanol extract had powerful reducing activity when all antioxidant tests compared. Based on these findings, it can be said that the antioxidant capacities of the extracts depend on the type of solvent. Different results have been determined in the literature regarding the antioxidant capacity of *T. terrestris*. Uysal et al. (2023) determined the antioxidant activities of ethyl acetate, methanol and water extracts of the *T. terrestris* using FRAP, CUPRAC, DPPH and ABTS methods. In this study, they emphasized that the water extract exhibited the highest DPPH, ABTS radical scavenging and iron reducing power (FRAP), while the ethyl acetate extract exhibited the highest activity in reducing copper ions (CUPRAC). Manonmani and Revathi (2023) investigated the antioxidant properties of different extracts of *T.*

Table 3
IC_{50} values (μg/mL) of the extracts on the AChE and BChE enzymes.

Applications	AChE		BChE	
	IC_{50}	r^2	IC_{50}	r^2
Water extract	16.48 ± 1.06	0.9726	68.65 ± 2.04	0.8713
Ethanol extract	12.13 ± 0.37	0.9633	22.33 ± 0.27	0.9429
Acetone extract	16.43 ± 0.77	0.9858	32.81 ± 1.01	0.9399
Chloroform extract	20.21 ± 0.55	0.9374	37.59 ± 0.42	0.9440
Hexane extract	25.46 ± 0.85	0.9474	50.10 ± 1.11	0.9481
Tacrine*	0.209 (nM)	0.9946	0.464 (nM)	0.9913

*It was used as a standard inhibitor for AChE and BChE enzymes.

terrestris at different concentrations (12.5–200 μg/mL) and reported that the ethanol extract had the highest activity in scavenging DPPH radicals.

3.2. Enzyme inhibition results

The effects of water, ethanol, acetone, chloroform, and hexane extracts of *T. terrestris* on AChE, BChE, hCA-I, hCA-II, and α-glycosidase enzyme activities were studied in this work and the results were given at Tables 3 and 4.

AD is treated with cholinesterase inhibitors. Inhibitors of the cholinesterase enzyme have several side effects and do not entirely achieve the desired effect, requiring the development or discovery of new inhibitors (Burmaoglu et al., 2019). From the past to the present, numerous natural inhibitors of acetylcholinesterase have been investigated (Hostettmann et al., 2006). It has been declared that some plant extracts with AChE enzyme inhibitory effects may be useful in treating neurodegenerative illnesses such as AD (Kiziltas, 2022). Studies in the literature found that extracts obtained from *T.terrestris* showed an inhibition effect on AChE and BChE enzymes (Orhan et al., 2004; Ghareeb et al., 2014; Uysal et al., 2023). The phenolic components and flavonoid content of medicinal plants are primarily responsible for inhibiting cholinergic enzymes. On the other hand, numerous herbal products have been identified that combine AChE inhibition with a multitude of neuroprotective mechanisms (Elufioye et al., 2017).

An assessment of the findings of cholinesterase enzyme inhibition revealed that the ethanol extract had the strongest inhibitory effect on the AChE enzyme among all extracts, with an IC_{50} value of 12.13 μg/mL (Table 3). IC_{50} values of other extracts were found as follows, respectively: Acetone extract (16.43 μg/mL), water extract (16.48 μg/mL), chloroform extract (20.21 μg/mL) and hexane extract (25.46 μg/mL). Hexane extract (IC_{50} =25.46 μg/mL) showed the weakest inhibitory effect against the AChE enzyme, while ethanol extract (IC_{50} =22.33 μg/mL) exhibited the strongest inhibitory effect against the BChE enzyme (Table 3). For the BChE enzyme, IC_{50} values of other extracts were found as follows, respectively: Acetone extract (32.81 μg/mL), chloroform extract (37.59 μg/mL), hexane extract (50.10 μg/mL) and water extract (68.65 μg/mL) (Table 3).

According to our findings (Table 3), hexane extract (IC_{50} =25.46 μg/mL) and water extract (IC_{50} =68.65 μg/mL) were determined as the weakest inhibitors against AChE and BChE enzymes, respectively. Furthermore, ethanol extract acted as the most effective inhibitor of both AChE and BChE enzymes.

Increased or decreased activity of enzymes involved in metabolism leads to various health problems in different metabolic pathways (Supuran, 2008). Some CA enzyme isoforms play an active role in physiological events (gluconeogenesis, lipogenesis, and electrolyte secretion) at a number of important human body. Therefore, numerous CA isozymes that are involved in these processes are important medicinal targets that need to be inhibited to treat a variety of diseases, such as glaucoma, cancer, and epilepsy. When hCA-I enzyme results were evaluated, it was observed that the chloroform extract had the most effective inhibitory impact on hCA-I enzyme activity, with an IC_{50} value of 21.82 μg/mL, among the other extracts (Table 4). The IC_{50} values of the other extracts against the hCA-I enzyme were found as follows, respectively: Hexane extract (37.80 μg/mL), ethanol extract (38.51 μg/mL), acetone extract (49.27 μg/mL) and water extract (94.1 μg/mL).

Considering the IC_{50} values of the extracts, it was determined that chloroform extract (IC_{50}:20.97 μg/mL) had the strongest inhibitory effect against the hCA-II enzyme. In addition, the IC_{50} values of the other extracts against the hCA-II enzyme were found as follows, respectively: Ethanol extract (26.76 μg/mL), acetone extract (32.3 μg/mL), hexane extract (38.09 μg/mL), and water extract (89.29 μg/mL) (Table 4). However, chloroform extract had the strongest inhibitory effect for both isoenzymes, while water extract had the weakest inhibitory effect. The extracts obtained from *T. terrestris* were found to have inhibitory effects on the hCA-I and hCA-II enzymes in the study's results.

The observed differences in the inhibitory effects between the extracts can be attributed to the chemical components present in the extracts. Different solvent systems used for extraction might selectively extract different classes of compounds from the plant material, leading to variations in bioactivity. For instance, the process of chloroform extraction can specifically enhance the concentration of lipophilic substances, which may have more potent inhibitory effects on hCA isoforms compared to hydrophilic chemicals extracted using water.

Diabetes mellitus (DM) is a major health issue that has a negative impact on patients' quality of life all over the world. The small intestinal cells release enzymes called α-amylase and α-glycosidase, which break down oligosaccharide and polysaccharide molecules into individual units of monosaccharides, such as glucose and fructose, by hydrolysis (Gulcin et al., 2018). In treating Type 2 Diabetes, the most typical approach is to minimize postprandial hyperglycemia by blocking the key digestion enzymes α-glycosidase and α-amylase (Yabo Dambagi et al., 2020). Therefore, we evaluated the inhibitory

Table 4
IC_{50} values (μg/mL) of the extracts on the hCA-I, hCA-II, and α-glycosidase enzymes.

Applications	hCA-I		hCA-II		α-glycosidase	
	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
Water extract	94.1 ± 1.96	0.9569	89.29 ± 2.91	0.9309	54.01 ± 0.88	0.9513
Ethanol extract	38.51 ± 0.92	0.9340	26.76 ± 0.47	0.9741	25.98 ± 0.75	0.9680
Acetone extract	49.27 ± 0.54	0.9944	32.3 ± 0.95	0.9546	45.30 ± 0.59	0.9385
Chloroform extract	21.82 ± 0.55	0.9823	20.97 ± 0.63	0.9746	37.21 ± 0.95	0.9826
Hexane extract	37.80 ± 0.43	0.9570	38.09 ± 0.76	0.9530	40.33 ± 1.52	0.9724
Standards	0.05 (μM)[a]	0.9638	0.05 (μM)[a]	0.9417	166.89[b] (μM)	0.9110

[a] Acetazolamide was used as a standard inhibitor for hCA I/ hCA II and was taken from reference (Aydın et al., 2022).
[b] Acarbose was used as a standard inhibitor for the α-glycosidase enzyme.

effects of different *T. terrestris* extracts on α- glycosidase enzyme activity to demonstrate their antidiabetic effects.

When the extracts were compared, ethanol extract (IC_{50}: 25.98 μg/mL) had the strongest while water extract (IC_{50}:54.01 μg/mL) had the weakest inhibitory effect against the α-glycosidase enzyme (Table 4). The IC_{50} values of the other extracts against the α-glycosidase enzyme were found as follows, respectively: Chloroform extract (37.21 μg/mL), hexane extract (40.33 μg/mL) and acetone extract (45.30 μg/mL) (Table 4). All extracts from *T. terrestris* showed an inhibitory effect on the α-glycosidase enzyme.

Other studies determined the strong inhibitory effect of *T. terrestris* extracts against α-glycosidase (Lamba et al., 2011; Alkhaldi et al., 2020; Ghareeb et al., 2014). In their studies, the authors suggested that the antidiabetic effect was due to flavonoids and polyphenols (Ghareeb et al., 2014). Many plant polyphenols have been demonstrated to inhibit carbohydrate hydrolyzing enzymes (Bothon et al., 2013). The nature of some extract components (phenolics, flavonoids, and their glycosides) is also in agreement with these studies showing that they are effective inhibitors of α-glycosidase. Furthermore, the α-glycosidase inhibitory effect of *T. terrestris* is considered to be due to its significant polyphenolic content.

4. Conclusions

This study measured the antioxidant properties of extracts (water, ethanol, acetone, chloroform, and hexane) obtained from *T. terrestris* using various antioxidant methods and researched their effects on some metabolic enzymes (AChE, BChE, hCA-I/hCA-II, and α-glycosidase). The findings indicated that all extracts had inhibitory effects on AChE, BChE, hCA-I/hCA-II isoenzymes, and α-glycosidase enzyme. In addition, ethanol extract showed a strong inhibitory effect on AChE/BChE and α-glycosidase enzymes, while chloroform extract on hCA-I/hCA-II isoenzymes. All extracts exhibited a significant antioxidant effect. Among all applications the acetone extract acted powerful role in eliminating DPPH and ABTS radicals and also the ethanol extract exhibited strong reducing activity. However, it was observed that the antioxidant activity of the extracts was relatively moderate against DPPH radicals but very powerful against ABTS radicals. These findings suggest that *T. terrestris* extracts have the potential to be used in the discovery of natural antioxidants and the development of enzyme inhibitors. It will also serve as a scientific basis for future research and development of novel, healthful, and natural herbal medicines for use in the food and pharmaceutical industries.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Elife Kaya: Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis. **Tuba Aydın:** Writing – review & editing, Methodology, Data curation. **Rüya Sağlamtaş:** Writing – review & editing, Methodology, Data curation.

Acknowledgements

Thanks to Assist. Prof. Dr. Seyran PALABAŞ UZUN for her contribution in plant identification. This research was supported by the Kahramanmaras Sutcu Imam University Scientific Research Projects Coordination Unit, Türkiye (Project Number: 2021/1-35M). The authors are grateful to Kahramanmaras Sutcu Imam University for the financial support of this research. We would also like to thank KSU-USKIM for laboratory support.

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