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Phytochemical profiling, therapeutic target enzymes inhibitory, antioxidant, and cytotoxic potential of *Cardopatium corymbosum*

Gozde Hasbal-Celikok[a], Nermin Çakmak[b], Yasin Celikok[c], Servet Duranay[d], Bahar Gürdal[e], Ebru Özdemir Nath[f], Tugba Yilmaz-Ozden[a,*]

[a] Department of Biochemistry, Faculty of Pharmacy, Istanbul University, Fatih, Istanbul 34116, Türkiye
[b] Graduate School of Health Sciences, Istanbul University, Fatih, Istanbul 34126, Türkiye
[c] Department of Biophysics, Faculty of Medicine, İstanbul Yeni Yüzyıl University, Türkiye
[d] Department of Biochemistry, Faculty of Medicine, İstanbul Yeni Yüzyıl University, Türkiye
[e] Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, Fatih, Istanbul 34116, Türkiye
[f] Department of Pharmaceutical Botany, Faculty of Pharmacy, Altınbaş University, Bakırköy, Istanbul 34147, Türkiye

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ABSTRACT

Cardopatium corymbosum (L.) Pers. (Asteraceae) has been used ethnomedicinally against intestinal worms, for skin diseases, and in wound treatment. This study investigated the phytochemical profile and biological activities of ethanol (CCE), methanol (CCM), and water (CCW) extracts of *C. corymbosum*. LC-MS/MS analysis revealed the presence of various metabolites. Antioxidant activities of the extracts were determined by measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activities and ferric-reducing power (FRAP). The cytotoxicity against human breast cancer (MCF-7) and human umbilical vein endothelial (HUVEC) cell lines were determined using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. Enzyme inhibitory activities against α-amylase, α-glucosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), thioredoxin reductase (TrxR), and elastase were measured to assess potential antidiabetic, anti-Alzheimer's, anticancer, and antiaging/wound healing effects. CCM exhibited the highest total phenolic and flavonoid contents and antioxidant activity. Fumaric acid, chlorogenic acid, vanillic acid, and rutin were identified as major components in CCM. *C. corymbosum* extracts, particularly those from CCE and CCM, displayed significant cytotoxicity on MCF-7 cells while exhibiting lower toxicity towards HUVEC cells. Notably, CCM demonstrated potent α-glucosidase inhibitory activity, while no α-amylase inhibition was observed. All extracts showed weak inhibition of AChE, BChE, and elastase. Conversely, the extracts displayed moderate TrxR inhibitory activity. These findings suggest that *C. corymbosum* possesses significant antioxidant, enzyme inhibitory, and cytotoxic potential, highlighting its promise as a source of novel bioactive molecules.

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1. Introduction

Enzyme inhibition, a fundamental approach in pharmacology, involves the interaction of chemicals or biosynthetic compounds with enzyme active sites (Sharma, 2012). This approach has been widely used in developing treatments for a wide range of human diseases, including inflammatory conditions, diabetes, neurological disorders, and cancer (Rengasamy et al., 2014). α-Amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) are digestive enzymes responsible for breaking down dietary carbohydrates into absorbable monosaccharides. By inhibiting these enzymes, the absorption of monosaccharides is delayed, leading to reduced postprandial glucose levels (Durmaz et al., 2022). Several commercially available anti-diabetic drugs, such as acarbose, sitagliptin, and gliclazide, are employed in the treatment of Type 2 diabetes mellitus (T2DM) by inhibiting

Abbreviations: T2DM, type 2 diabetes mellitus; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; TrxR, thioredoxin reductase; Trx, thioredoxin; DPPH, 2,2′-diphenyl-1-picrylhydrazyl; DMSO, dimethyl sulfoxide; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); STANA, N-succinyl-AlaAla-Ala-p-nitroanilite; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; UHPLC, ultra-high performance liquid chromatography; MS, mass spectrometer; ESI, electrospray ionization; GAE, gallic acid equivalents; CE, catechin equivalents; HUVEC, human umbilical vein endothelial cells; ATCC, American Type Culture Collection; DMEM/F12, Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; FBS, fetal bovine serum; TNB, 5-thio-2-nitrobenzoic acid; ACh, acetylcholine; CCE, ethanol extract of *C. corymbosum*; CCM, methanol extract of *C. corymbosum*; CCW, water extract of *C. corymbosum*

* Corresponding author.
E-mail address: tugbay@istanbul.edu.tr (T. Yilmaz-Ozden).

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α-amylase and α-glucosidase (Awosika and Aluko, 2019; Gong et al., 2020). Acetylcholinesterase (AChE) (EC 3.1.1.7) and butyrylcholinesterase (BChE) (E.C 3.1.1.8) are enzymes responsible for hydrolyzing acetylcholine (ACh) within the synaptic cleft, thereby regulating cholinergic neurotransmission (Yusufzai et al., 2018; Durmaz et al., 2022). Cholinesterase inhibitors prevent this breakdown, enhancing cholinergic function and ameliorating cognitive decline associated with Alzheimer's disease (Yusufzai et al., 2018; Durmaz et al., 2022). Currently, three FDA-approved cholinesterase inhibitors (donepezil, galantamine, and rivastigmine) are used for the symptomatic treatment of Alzheimer's disease (Ahmed et al., 2021). Thioredoxin reductase (EC 1.8.1.9) (TrxR) is a flavoenzyme containing selenocysteine, an important member of the thioredoxin (Trx) system. TrxR catalyzes the NADPH-induced reduction of oxidized Trxs (Zhang et al., 2019). The Trx system regulates DNA and protein synthesis/repair. It also maintains intracellular redox balance, thus controlling cell growth, differentiation, and cell death (Cai et al., 2012). Recent studies have consistently demonstrated a correlation between the hyperactivation of TrxR and tumorigenesis (Bian et al., 2019). Therefore, TrxR inhibition is a promising anticancer strategy. Elastase (EC 3.4.4.7), a protease, is responsible for elastin breakdown and also catalyzes the degradation of collagen, fibronectin, and other extracellular matrix proteins (Azmi et al., 2014). Elastase inhibitors have potential applications in skin care, anti-aging, and wound healing by reducing excessive tissue degradation (Boran, 2018; Chiocchio et al., 2018; Tian et al., 2022). The discovery and development of novel, specific enzyme inhibitors with therapeutic potential remains an active area of research. The increasing prevalence of chronic diseases underscores the urgent need for new, effective, and safe treatment options, particularly from natural sources.

The Asteraceae family is a large and diverse family of flowering plants, with an estimated distribution of about 1700 genera and 33,000 species worldwide (POWO, 2024). These plants are primarily found in the Mediterranean, Eastern Europe, and Asia (Bessada et al., 2015). *Cardopatium* genus, which is in the Asteraceae family, is represented in the world by two species, *C. corymbosum* (L.) Pers. and *C. amethystinum* Spach. *C. corymbosum* is distributed in Italy, Macedonia, Greece, Crete, Lodosa, Türkiye, Lebanon, Algeria, Syria, and Cyprus (Arık and Okmen, 2018; POWO, 2024). Also, synonym of *C. corymbosum* is *Echinops corymbosus* L. (POWO, 2024). Numerous Echinops species have been traditionally employed as diuretics, nerve tonics, and in the management of diabetes, blood circulation disorders, indigestion, and uterine tumors (Mahmoud and Gairola, 2013; Bouzabata et al., 2018; Bitew and Hymete, 2019). *C. corymbosum*, which is also known as "wormy thorn", "purple thorn", "meadow thistle", "wormy herb" and "çoban çırası", is used in traditional medicine to treat intestinal worms, skin diseases and wounds (Bulut et al., 2017; Nath and Kültür, 2017; Güneş, 2018; Satıl et al., 2019). It is traditionally used in gastrointestinal diseases in Cyprus, and the roots of the plant are also used in topical applications as an antiseptic on wounds (González-Tejero et al., 2008). Additionally, the plant is utilised in Türkiye to treat mastitis in cattle and sheep (Elif et al., 2022). In the literature search, there are few biological studies on the *C. corymbosum*. Previous studies have reported its DPPH and ABTS radical scavenging, as well as antibacterial and antimicrobial properties (Arık and Okmen, 2018; Okmen et al., 2019). Also, the essential oil content of *C. corymbosum* leaves was determined by GC–MS analysis (Jeribi et al., 2016). Despite its traditional use in various ailments, there is a significant gap in scientific research on the cytotoxic, antidiabetic, anti-Alzheimer, anticancer, and antiaging/wound healing properties of *C. corymbosum*.

Based on this information, ethanol, methanol, and water, extracts were prepared from *C. corymbosum*. The total phenol and flavonoid contents, the phytochemical profile, the antioxidant and cytotoxic effects of the extracts were determined. Also, the extracts were evaluated for their inhibitory activity against some therapeutic enzymes; α-amylase, α-glucosidase, AChE, BChE, TrxR, and elastase. This study aims to address this knowledge gap by investigating the plant's phytochemical profile and evaluating its *in vitro* some bioactivities. The novelty of this study lies in providing the first comprehensive investigation of *C. corymbosum* as a potential source of bioactive compounds with therapeutic implications. However, it is important to note that this research represents a preliminary exploration, and further studies are required to elucidate the underlying mechanisms of action and to assess the plant's efficacy and safety *in vivo*.

2. Material and methods

2.1. Chemicals

α-Amylase, α-glucosidase, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), acarbose, AChE, BChE, catechin, curcumin, elastase, Folin-Ciocalteu reagent, galantamine, gallic acid, N-succinyl-AlaAla-Ala-p-nitroanilite (STANA), quercetin, thioredoxin reductase (TrxR) assay kit, and, ursolic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 3-(4,5-dimethylthiazol-2-yl)–2,5-diphenyltetrazolium bromide (MTT; Glentham Life Sciences) were purchased from Glentham Life Sciences (Corsham, UK). All other chemicals were analytical grade.

2.2. Plant material and extraction

Cardopatium corymbosum (L.) Pers. was collected from Yamanlar Mountain (Izmir, Türkiye) in July 2019 and botanically identified by Assoc. Prof. Bahar Gürdal and Asst. Prof. Ebru Özdemir Nath. The voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE- 116,888).

The aerial parts of *C. corymbosum* were dried in the shade, then ground into powder using a grinder (IKA, Staufen, Germany). For the preparation of the water extract (CCW), 15 g of the ground plant was boiled in 150 mL of distilled water. After cooling, it was filtered and frozen at −80 °C for 24 h. The frozen sample was placed in a freeze-dryer (Labconco, Kansas City, USA) for three days until it was completely dried. For the preparation of the ethanol (CCE) and methanol (CCM) extracts, the aerial parts (15 g) were extracted in a Soxhlet apparatus using ethanol or methanol. The solvents were evaporated to dryness in a rotary evaporator (Buchi, Switzerland) under reduced pressure. After complete removal of solvents, the resulting dry extracts were stored at −20 °C and each extract were dissolved in dimethyl sulfoxide (DMSO) before use in biochemical assays.

2.3. Phytochemical profile by LC-MS/MS analysis

LC-MS/MS analysis was performed by the Drug Application and Research Centre, Bezmialem Vakif University. An ultra-high performance liquid chromatography (UHPLC) system (UltiMate 3000, Thermo) coupled to a Thermo ORBITRAP Q-EXACTIVE mass spectrometer (MS) equipped with an electrospray ionization (ESI) source was employed to determine the phytochemical profile of the *C. corymbosum* extracts. Extracts were dissolved in 1.5 mL methanol, centrifuged for 5 min, and the supernatant transferred to a fresh tube. An internal standard solution (100 mg/L) was added to achieve a final concentration of 3 ppm. Following filtration through a 0.45 μm pore size filter, the sample was injected into the LC-MS/MS system (Gülçin et al., 2010). Chromatographic separation was performed on a Fortis C18 column (150 × 3.0 mm, 3 μm; Fortis Technologies Ltd., UK) maintained at 25 °C. A 1 μL injection volume was employed. Mobile phases consisted of (A) 1 % formic acid in water and (B) 1 % formic acid in methanol, delivered at a flow rate of 0.35 mL/min. A linear gradient was applied, starting at 50 % B for 1 min, increasing to 100 % B over the next 2 min, held at 100 % B for 3 min, returned to 50 % B in

1 min, and equilibrated for 3 min before the next injection. Mass spectrometric detection was conducted in both positive and negative ESI modes, scanning a mass range of 85–1500 *m/z*. Optimal instrument parameters were as follows: sheath gas flow rate, 45 mL/min; auxiliary gas flow rate, 10 mL/min; spray voltage, 3.80 kV; capillary temperature, 320 °C; auxiliary gas heater temperature, 320 °C; and S-lens RF level, 50.0. Quantification was achieved using the external standard method with calibration curves generated through linear regression analysis of repeated measurements.

2.4. Total phenolic and flavonoid compounds

Total phenolic content in the plant extracts was determined using the modified Folin-Ciocalteu method (Slinkard and Singleton, 1977). Total phenolic content was expressed as mg gallic acid equivalents (GAE)/g extract using a pre-established calibration curve (y=0.7768x +0.0093, R^2=0.9991) prepared with standard gallic acid solutions. Total flavonoid content in the extracts was determined using the modified aluminum chloride method (Sakanaka et al., 2005). Total flavonoid content was expressed as mg catechin equivalents (CE)/g extract using a pre-established calibration curve (y=1.8501x+0.0141, R^2=0.9995) prepared with standard catechin solutions (Yilmaz-Ozden et al., 2021).

2.5. The antioxidant activity

2.5.1. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the extracts was determined with some modifications according to the method developed by Brand-Williams et al. (1995). Quercetin was used as a standard antioxidant (Yilmaz-Ozden et al., 2021).

2.5.2. Ferric (III) reducing antioxidant power (FRAP)

The reducing powers of the extracts were quantified by the modified FRAP assay (Benzie and Strain, 1996). Quercetin was used as a standard antioxidant. Reducing power of the extracts was expressed as FRAP value (mM Fe^{2+} equivalents/mg) using the equation of a standard regression curve (y=0.6908x-0.0321, R^2=0.9998) prepared with $FeSO_4{\cdot}7H_2O$ solutions (Yilmaz-Ozden et al., 2021).

2.6. The cytotoxic activity

Human breast cancer (MCF-7; ATCC HTB-22) and human umbilical vein endothelial (HUVEC; ATCC CRL-1730) cells were obtained from the American Type Culture Collection (ATCC). The cytotoxic effect of the extracts on these cell lines was determined using the MTT method. The cells were maintained in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12; Hyclone) supplemented with 10 % fetal bovine serum (FBS; Biosera FB-1101), 100 U/mL penicillin and 100 μg/mL streptomycin (Biosera LM-A4118) and cultured at 37 °C in a humidified atmosphere.

90 μL of cell suspension (1×10^4 cell/well) was seeded into 96-well microplates and incubated for 24 h in a 37 °C incubator supplemented with 5 % CO_2. Then, 10 μL of the extract was added to the wells and incubated for 72 h under the same conditions. At the end of the incubation, 20 μL of MTT solution was added to each well and incubated for another 3 h. The liquids in the wells were then aspirated and discarded. 100 μL of sterile DMSO was added to the wells, and the microplates were incubated for another 15 min to dissolve the formazan crystals. Absorbances were measured at 590 nm (versus 670 nm reference wavelength). The control was prepared by adding the medium instead of the extract on the cell lines. Cytotoxic activity (% inhibition) was calculated. Also, the results were expressed as the IC_{50} value (the concentration of the extracts that inhibited the growth of cells by 50 %).

2.7. The enzyme inhibitory acitivities

2.7.1. α-Amylase inhibitory activity

The α-amylase inhibitory activities of the extracts were determined with some modifications according to the method of Ali et al. (2006). Acarbose was used as a standard (Hasbal et al., 2020).

2.7.2. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activities of the extracts were determined with some modifications according to the method of Bothon et al. (2013). Acarbose was used as a standard (Hasbal et al., 2020).

2.7.3. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities

The AChE and BChE inhibitory activities of the extracts were determined with some modifications according to the method of Ellman et al. (1961). Galantamine was used as standard (Hasbal et al., 2020).

2.7.4. Thioredoxin reductase (TrxR) inhibitory activity

The TrxR inhibitory activities of the extracts were determined using the TrxR assay kit (Sigma CS0170), which measures the yellow color at 412 nm resulting from the reduction of NADPH and DTNB to 5-thio-2-nitrobenzoic acid (TNB). Briefly, 5 μL of TrxR solution, 5 μL of the buffer (1X; 100 mM potassium phosphate containing 10 mM EDTA, pH 7), 4 μL of extract, 180 μL of working buffer with NADPH and 6 μL of DTNB solution were mixed. The change in absorbance was measured at 412 nm for 60 min. TrxR inhibitor solution provided by the kit and curcumin were used as standards. A control was prepared containing the solvent instead of amount of extract/standard. TrxR inhibitory activity (%) was calculated according to the formula below. Results were expressed as the IC_{50} value (the concentration of extract or standard that inhibited the TrxR by 50 %).

$$\%\ \text{inhibition} = 1 - \left(\frac{\text{Reaction rate of sample at 412 nm}}{\text{Reaction rate of control at 412 nm}}\right) \times 100$$

2.7.5. Elastase inhibitory activity

The elastase inhibitory activities of *C. corymbosum* extracts were determined according to the modified method of Moon et al. (2010), using 10 μL of extract, 100 μL of elastase solution (0.045 U/mL), and 15 μL of Tris–HCl buffer (0.2 M pH 7.8) mixed and incubated for 10 min at 25 °C. Then, 75 μL of STANA solution (1 mM) was added, and the mixture was incubated at 37 °C for 30 min. The absorbance of the released p-nitroaniline was measured at 410 nm at 37 °C for 20 min. Ursolic acid was used as standard. A control was prepared containing the solvent instead of amount of extract/standard. Elastase inhibitory activity (%) was calculated according to the formula below. Results were expressed as the IC_{50} value (the concentration of extract or standard that inhibited the elastase by 50 %).

$$\%\ \text{inhibition} = 1 - \left(\frac{\text{Reaction rate of sample at 410 nm}}{\text{Reaction rate of control at 410 nm}}\right) \times 100$$

3. Results and discussion

3.1. Total phenolic and flavonoid compounds

Phenolic compounds are a diverse group of plant chemicals with potential health benefits. They are found in a variety of plant sources including fruits, vegetables, legumes, tea, and wine (Alara et al., 2021). The positive effects on human health of most phytochemicals obtained from plants, such as phenolic compounds, have been reported in studies (Azwanida, 2015). This study determined the total phenolic and flavonoid content of *C. corymbosum* extracts. Total phenolic and flavonoid compound amounts of the extracts were given as

Table 1
Total phenolic and flavonoid contents of the extracts from *C. corymbosum*.

	Phenolic (mg GAE/g)	Flavonoid (mg CE/g)
CCE	30.72±1.04	29.82±0.76
CCM	42.58±0.60	38.46±0.77
CCW	35.59±0.87	31.04±0.41

Values are the means of three replicates±SD.
Total phenolic contents were defined as mg gallic acid equivalents (GAE) per g extract.
Total flavonoid contents were defined as mg catechin equivalents (CE) per g extract.

"mg GAE/g extract" and "mg CE/g extract", respectively. The results showed that the CCM had the highest content of both phenolics and flavonoids, followed by the CCW. The CCE exhibited the lowest content (Table 1). *Cardopatium* Juss, which includes *C. corymbosum* spp. genus is included in subtribus Carlininae in Flora of Turkey. Other genera in subtribus Carlininae are *Carlina* L., *Atractylis* L., *Xeranthemum* L., *Chardinia* Desf., and *Siebera* J. Gay. There are a limited number of studies in the literature with different species belonging to subtribus Carlininae. Triterpene content of *Carlina* species (Strzemski et al., 2016), cyanogenic compounds of *Xeranthemum annuum* L. (Orhan et al., 2016), phenolic and tannin content of *Atractylis gummifera* L. (Bouabid et al., 2020) were shown in the studies.

3.2. Phytochemical profile

In the present study, the identified phytochemicals in all *C. corymbosum* extracts, as determined by LC-MS/MS analysis, are presented in Table 2. The chromatograms obtained from the analysis of the extracts were shown in Fig. 1.

The major compound in the CCE was chlorogenic acid, followed by rutin, quercitrin, and fumaric acid, respectively. Fumaric acid and chlorogenic acid were most abundant in the CCM and CCW, with these extracts also being rich in rutin and quercitrin content. All extracts shared a high content of chlorogenic acid and rutin.

Table 2
Amounts of the phytochemicals present in the extracts of *C. corymbosum*.

Compounds	Amount (μg/g extract)			U%*
	CCE	CCM	CCW	
Ascorbic acid	134.02	422.42	314.49	11.07
Chlorogenic acid	1496.12	2882.64	1894.49	11.14
Fumaric acid	448.90	3611.15	3592.44	11.14
Orientin	<LOD	6.04	1.97	11.47
Caffeic acid	223.38	189.83	128.97	11.07
Vanilic acid	<LOD	1007.55	<LOD	11.61
Rutin	722.15	595.03	536.58	11.79
Hyperoside	211.64	209.50	95.90	11.50
Dihydrokaempferol	32.05	2.58	0.73	11.35
Quercitrin	674.89	499.20	250.30	11.69
Quercetin	27.08	3.15	1.71	11.42
Salicylic acid	152.74	108.04	55.56	11.40
Naringenin	11.55	<LOD	<LOD	11.04
Kaempferol	184.20	36.68	<LOD	11.90
Hispidulin	0.09	<LOD	<LOD	11.23
Chrysin	3.88	3.64	3.89	11.09
Acacetin	3.38	3.15	2.44	11.36
Sarsasapogenin	172.47	203.29	<LOD	10.96

* Uncertainty (at 95 % confidence level, k=2).
LOD; limit of detection.

Chlorogenic acid, the ester of caffeic acid and quinic acid, exhibits various pharmacological effects, including antimutagenic, anticarcinogenic, and antioxidant properties (Sato et al., 2011; Yao et al., 2019). Fumaric acid is a naturally occurring organic acid involved in the citric acid cycle, first isolated from *Fumaria officinalis* L. (Ilica et al., 2019). All three extracts contained significant amounts of ascorbic acid, caffeic acid, and hyperoside. While vanillic acid was undetectable in the CCE and CCW, the CCM contained a high amount.

This study is the first to report the phytochemical profile of aerial parts from *C. corymbosum*. Previous literature focused on the essential oil content of *C. corymbosum* leaves, with GC–MS analysis revealing 105 compounds, with methyl-5-phenylpentanoate as the major component (25.87 %), followed by undecanal (14.07 %), (E)–3.7-dimethyl-2,6-octadienyl (E)–2-methyl 2-butenoate (7.11 %), α-acorenol (5.17 %), and 2-butyltetrahydrofuran (3.58 %) (Jeribi et al., 2016).

Our findings revealed chlorogenic acid, fumaric acid, and rutin as the major compounds in all three extracts (CCE, CCM, and CCW). Similar results were reported for *Achillea* species. A study on aerial part and root extracts of *A. coarctata* Poir. and *A. monocephala* Boiss. and Balansa also showed significant amounts of chlorogenic acid, fumaric acid, and rutin (Yilmaz et al., 2018). Likewise, studies on *Centaurea* species within the Asteraceae family demonstrated that chlorogenic acid is a major component in *C. triumfettii* All. (Acet, 2021), *C. aksoyi* Hamzaoğlu and Budak (Albayrak et al., 2017), *C. balsamita* Lam., and *C. lycopifolia* Boiss. and Kotschy (Boğa et al., 2016).

In the present study, when the total phenolic and flavonoid contents of *C. corymbosum* were measured, it was observed that these amounts changed according to the solvent used in the extraction and methanol was the best solvent to obtain the phenolic components of *C. corymbosum*. This observation aligns with findings from other species in subtribus Carlininae. For instance, a study by Bouabid et al. (2020) showed that the methanol extract of *Atractylis gummifera* had the highest total phenolic and flavonoid content. Conversely, in *Xeranthemum annuum*, Orhan et al. (2016) reported a higher phenolic content in the water extract compared to those obtained using organic solvents. These contrasting results highlight that the optimal solvent for extracting phenolic compounds can vary depending on the plant species.

3.3. The antioxidant activity

The IC_{50} value, representing the concentration required to scavenge 50 % of the DPPH radical, was used to assess antioxidant activity (Table 3). Lower IC_{50} values indicate stronger antioxidant activity. The concentration-dependent DPPH radical scavenging activities of the extracts are graphically represented in Fig. 2. Consistent with the observed phenolic and flavonoid content, the CCM exhibited the lowest IC_{50} value, indicating the strongest antioxidant activity.

Previous studies have investigated the antioxidant activity of *C. corymbosum* using DPPH (Arık and Okmen, 2018) and ABTS (Okmen et al., 2019) assays. Similar to our findings, Arık and Okmen (2018) reported that the methanol extract of *C. corymbosum* demonstrated a higher DPPH radical scavenging activity compared to the ethanol and water extracts. They found that the methanol extract exhibited 82.1 % inhibition at a concentration of 300 mg/mL. Okmen et al. (2019) evaluated the ABTS radical scavenging activity of the extracts at 300 mg/mL, finding values of 5 %, 13.5 %, and 12 % for the ethanol, methanol, and water extracts, respectively. These findings collectively suggest that, like in our study, the CCM possesses the strongest free radical scavenging effect and, consequently, the highest antioxidant activity among the tested extracts.

LC-MS/MS analysis revealed that chlorogenic acid is one of the most abundant phytochemicals in the CCM. Yao et al. (2019) reported that chlorogenic acid exhibits stronger DPPH and ABTS scavenging

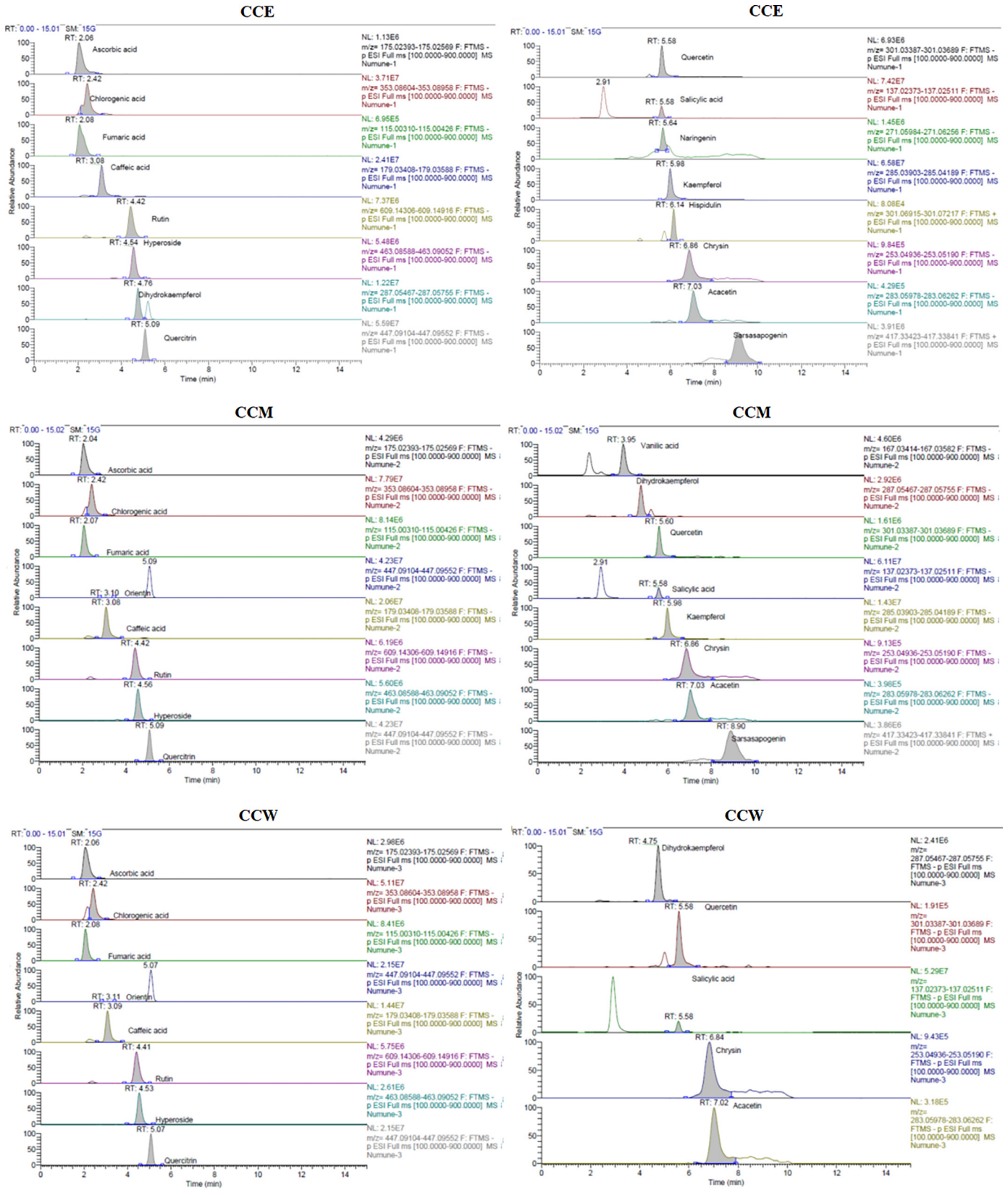


Fig. 1. Quantification chromatograms of LC-MS/MS analysis.

activities compared to butylated hydroxytoluene. The higher radical scavenging activity observed in the CCM compared to the other extracts might be explained by its greater content of chlorogenic acid.

The FRAP assay was employed to assess the reducing power of the extracts, with a higher FRAP value indicating stronger reducing potential. Consistent with the observed phenolic and flavonoid content, the CCM exhibited the highest FRAP value (Table 3). The

Table 3
Antioxidant activities of the extracts from *C. corymbosum*.

	DPPH (IC_{50}) (mg/mL)	FRAP (mM Fe^{+2}/mg)
CCE	0.17719±0.00411	44.36595±0.52647
CCM	0.11357±0.00286	59.69118±0.30396
CCW	0.11639±0.00167	56.02393±0.94714
Quercetin	0.00515±0.00001	1700.73345±15.19785

Values are the means of three replicates±SD.
IC_{50} is the effective concentration which is required to scavenge 50 % of DPPH radical.
The IC_{50} values were calculated by linear regression analysis from dose-response curves.
The reducing power of the extracts was expressed as FRAP value (mM Fe^{+2} equivalents/mg sample).
Concentrations represent final concentrations in the reaction medium.

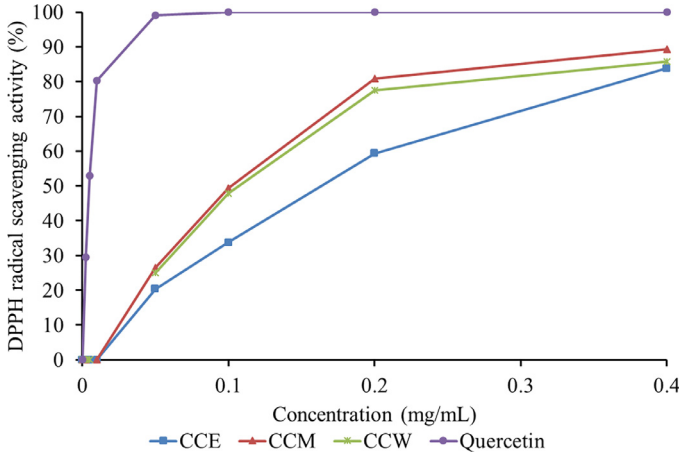


Fig. 2. DPPH radical scavenging activities of the extracts from *C. corymbosum*. Concentrations represent final concentrations in the reaction medium.

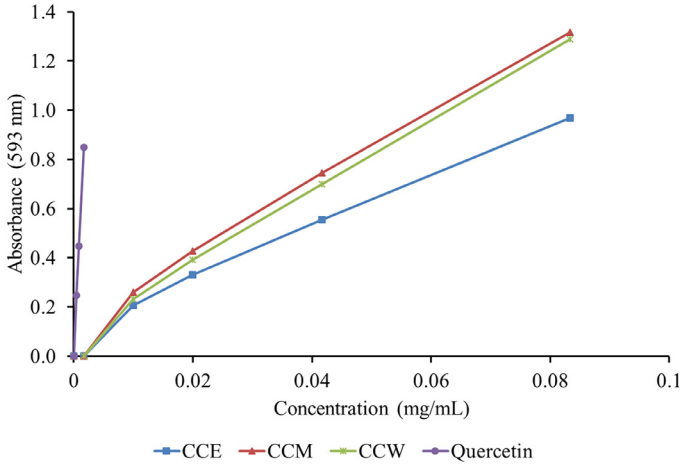


Fig. 3. Ferric reducing powers of the extracts from *C. corymbosum*.Concentrations represent final concentrations in the reaction medium.

absorbances, which indicate the ferric reducing powers of the extracts, are also depicted graphically in Fig. 3. There is currently no published data on the reducing power of *C. corymbosum*. In studies on the reducing powers of other species in subtribus Carlininae, it has been shown that aqueous macerated extract of *Atractylis gummifera* has a higher FRAP value than extracts prepared with organic solvents (102.5±1.66 mg ET/gE) (Bouabid et al., 2020). In another study in which the FRAP values of the aerial parts and root hydromethanolic extracts of two *Carlina* species (*C. acaulis* and *C. acanthifolia*) were measured, it was shown that the aerial parts extract of *C. acaulis* had the highest FRAP value (0.592±0.001 μmoL Fe^{2+}/mg dry weight) (Đorđević et al., 2012).

3.4. The cytotoxic activity

As shown in Table 4, *C. corymbosum* extracts, particularly those prepared with ethanol and methanol, exhibited selective cytotoxicity against MCF-7 breast cancer cells, with minimal inhibition of HUVEC cell proliferation. The CCE demonstrated the strongest cytotoxic effect based on IC_{50} values. Notably, no prior studies have reported on the cytotoxic activity of *C. corymbosum*.

LC-MS/MS analysis revealed higher concentrations of hyperoside, salicylic acid, and sarsasapogenin in the CCM and CCE compared to the CCW. Qiu et al. (2019) demonstrated that hyperoside exhibits concentration-dependent inhibition of MCF-7 cell viability. These findings suggest that the observed cytotoxicity of the extracts, particularly the CCE, might be attributed to the presence of these specific compounds.

Studies on other *Centaurea* species have shown promising antiproliferative activity against MCF-7 breast cancer cells. For instance, Nasr et al. (2020) reported that the ethyl acetate fraction from the methanol extract of *Centaurea bruguierana* (DC.) Hand.-Mazz. exhibited the strongest activity, with an IC_{50} value of 10 μg/mL. Similarly, Sen et al. (2015) found that the chloroform extract of *Centaurea kilaea* Boiss. aerial parts displayed high antiproliferative activity against MCF-7 cells (IC_{50}=68.64 μg/mL). Bakr et al. (2016) also observed cytotoxic activity against MCF-7 cells in the methanol extract of *Centaurea aegyptiaca* L. (IC_{50}=30.9 μg/mL). The observed cytotoxic activity of the extracts may be attributed, at least in part, to the presence of specific bioactive compounds. Chlorogenic acid, a major component of the extracts, has been previously reported to exhibit cytotoxic activity against MCF-7 cells with an IC_{50} value of 52.5±4.72 μg/mL (Deka et al., 2017). Furthermore, fumaric acid, another constituent identified in the extracts, has been shown in numerous *in vivo* studies to possess inhibitory effects on carcinogenesis (Das et al., 2016). These findings suggest that the combined effects of these and potentially other bioactive compounds within the extracts may contribute to the observed cytotoxicity against MCF-7 cells.

3.5. The enzyme inhibitory activities

T2DM represents a chronic metabolic disorder defined by insulin resistance and/or relative insulin deficiency. Current therapeutic approaches for T2DM, including metformin, acarbose, and voglibose, target α-amylase and α-glucosidase enzymes to achieve glycemic control. However, these synthetic drugs can cause side effects, prompting research into natural alternatives with enzyme inhibitory properties (Senthil et al., 2019). Therefore, there is an increasing interest in research aimed at measuring the enzyme inhibitory effects of natural compounds and their potential applications in diabetes treatment. This study investigated the potential antidiabetic properties of *C. corymbosum* by measuring its α-glucosidase and α-amylase inhibitory activity. Here, we report the first demonstration of *C. corymbosum*'s antidiabetic potential *in vitro*.

The Fig. 4 illustrates the concentration-dependent α-glucosidase inhibitory effects of the extracts and the positive control, acarbose. As shown in the figure, the CCM exhibited the strongest α-glucosidase inhibitory activity at a concentration of 1.25 mg/mL. The IC_{50} values were 1.1106±0.0359 mg/mL for the CCM and 0.1367±0.0052 mg/mL for acarbose. CCE and CCW IC_{50} values could not be determined for α-glucosidase inhibition. The α-glucosidase inhibitory activity of *C. corymbosum* extracts, particularly CCM, may be attributed to the presence of phenolic acids like chlorogenic acid and caffeic acid. These compounds have been shown to inhibit α-glucosidase and

Table 4
The cytotoxic activities of *C. corymbosum* extracts on MCF-7 and HUVEC cell lines.

	MCF-7 Inhibiton %				HUVEC Inhibition %			
Extracts	400 μg/mL	200 μg/mL	100 μg/mL	IC_{50} (μg/mL)	400 μg/mL	200 μg/mL	100 μg/mL	IC_{50} (μg/mL)
CCE	76.50±1.08	51.07±0.45	21.48±1.20	235.10±0.89	47.61±0.51	34.86±0.98	29.16±0.41	>400
CCM	65.38±0.86	31.46±1.80	14.96±1.77	309.09±2.84	35.19±0.65	29.52±0.88	–	–
CCW	26.81±1.17	–	–	–	10.34±1.05	–	–	–

The cytotoxicity of the extracts was assayed under continuous exposure for 72 h.
Concentrations represent final concentrations in the reaction medium.

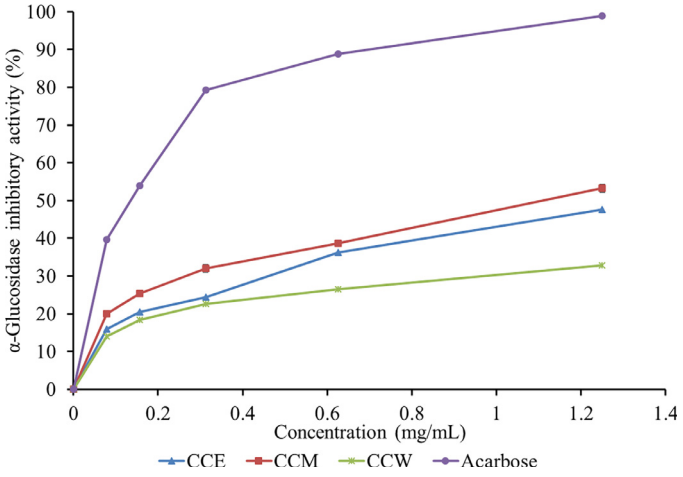


Fig. 4. α-Glucosidase inhibitory activity of the extracts from *C. corymbosum*. Concentrations represent final concentrations in the reaction medium.

α-amylase enzymes in previous studies (Oboh et al., 2015; Wang et al., 2022). Interestingly, none of the extracts (CCE, CCM or CCW) displayed any inhibitory activity against α-amylase. In a related study on *Carlina curetum* Heldr. (belonging to subtribus Carlininae), Jaradat et al. (2019) found that the methanol fraction possessed the strongest α-glucosidase inhibitory activity (IC_{50}=27.54±4.28 μg/mL) compared to hexane, acetone, and water fractions. Notably, this activity even surpassed the standard acarbose (IC_{50}=37.15±0.33 μg/mL). The authors suggested that the plant's antidiabetic potential might be linked to its phenolic, flavonoid, and tannin content.

Alzheimer's disease is characterized by the loss of cholinergic neurons and decreased ACh levels in the brain. One therapeutic approach involves inhibiting AChE and BChE, enzymes that break down ACh. Donepezil, rivastigmine, galantamine, and tacrine are examples of cholinesterase inhibitors used in Alzheimer's treatment (Sharma, 2019). We evaluated the potential of *C. corymbosum* extracts to inhibit AChE and BChE activity. As shown in Fig. 5, all extracts exhibited less than 50 % inhibition at the maximum tested concentration (0.8 mg/mL), preventing the calculation of IC_{50} values. Notably, the extracts displayed higher inhibitory activity against BChE compared to AChE. The CCM exhibited the strongest inhibition among the extracts tested. However, galantamine, the reference standard, showed significantly greater inhibitory activity against both AChE (85.30 % inhibition at 0.004 mg/mL) and BChE (84.26 % inhibition at 0.004 mg/mL).

There is no prior research on the AChE and BChE inhibitory activity of *C. corymbosum*. In a study on *Atractylis cancellata* L. (another species belonging to subtribus Carlininae), Badaoui et al. (2021) reported that the plant extracts exhibited weak cholinesterase inhibitory activity. However, a purified alkaloid (pyrroloquinolone A) isolated from the plant demonstrated potent cholinesterase inhibition. Interestingly, Orhan et al. (2016) found that *Xeranthemum annuum* extracts lacked AChE inhibitory activity but displayed strong BChE inhibition (87.62±3.01 % - 97.85±0.94 %).

TrxR is an enzyme with multifaceted roles in cellular processes, including DNA synthesis, cell proliferation, and angiogenesis. Due to its involvement in these pathways, TrxR inhibitors are being investigated as potential anticancer agents (Biaglow and Miller, 2004). Notably, this study represents the first investigation of TrxR inhibitory activity in Asteraceae family plants. The TrxR inhibitory potential of *C. corymbosum* extracts was evaluated and quantified using IC_{50} values (lower IC_{50} indicates stronger inhibition). The ranking of extracts based on IC_{50} was CCW (0.1107±0.0059 mg/mL) > CCM (0.0079± 0.0009 mg/mL) > CCE (0.0077±0.0005 mg/mL). Interestingly, CCE and CCM extracts displayed promising TrxR inhibitory activity comparable to curcumin (IC_{50} 0.0052±0.0004 mg/mL), a standard reference compound. The Fig. 6 illustrates the concentration-dependent TrxR inhibitory effects of the extracts and the positive control, curcumin.

The observed TrxR inhibition by the extracts might be attributed to their phytochemical content. Studies have demonstrated the potent TrxR inhibitory activity of flavonoids such as quercetin (Lu et al., 2006), naringenin and chlorogenic acid (Türkoğlu et al., 2019), with IC_{50} values of 0.97 mM, 46.7 μM, and 75.8 μM, respectively. LC-MS/MS analysis confirmed the presence of these compounds, particularly chlorogenic acid, in *C. corymbosum* extracts. Furthermore, the TrxR inhibitory activity of fumaric acid (another phytochemical identified in the extracts) was measured in this study and it inhibited TrxR by 55.16±1.49 % at 0.01 mg/mL.

Elastase is an enzyme associated with skin aging by degrading elastin, a component of the extracellular matrix. To investigate the potential anti-aging/wound healing effects of the extracts, their elastase inhibitory activities were determined. The CCE (14.74±1.16%)

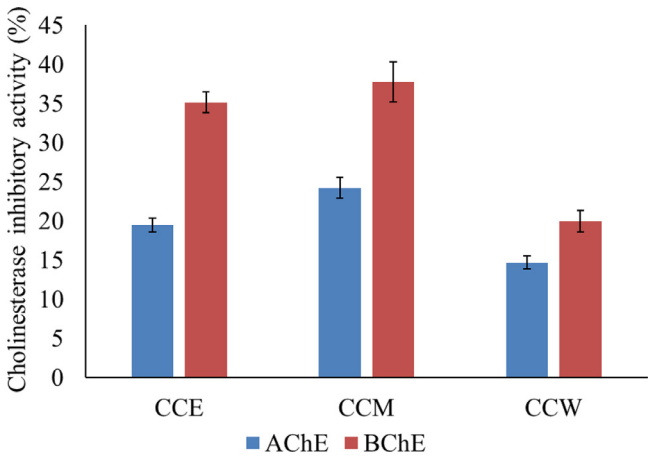


Fig. 5. Cholinesterase inhibitory activity of the extracts from *C. corymbosum* at 0.8 mg/mL.Concentration represents final concentrations in the reaction medium.

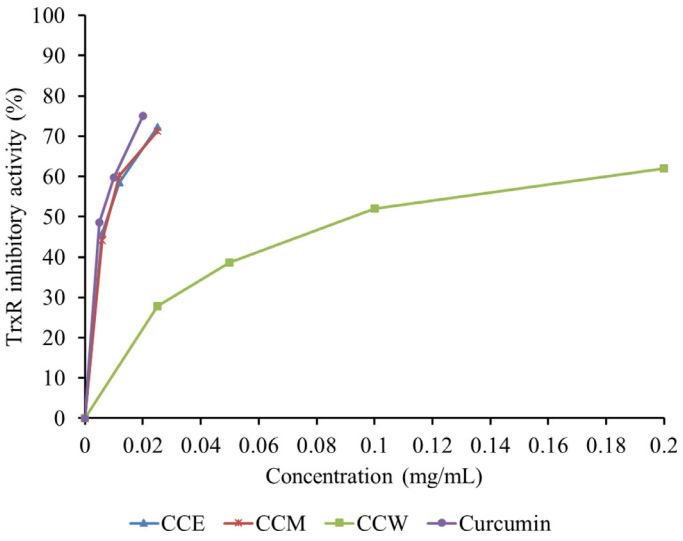


Fig. 6. TrxR inhibitory activity of the extracts from *C. corymbosum*.Concentrations represent final concentrations in the reaction medium.

and CCM (13.15±1.40%) displayed minimal elastase inhibition at 0.3 mg/mL, and IC_{50} values could not be determined. In contrast, the positive control, ursolic acid, exhibited a potent inhibitory effect with an IC_{50} value of 0.0182±0.0004 mg/mL. The CCW lacked any elastase inhibitory activity.

There is no prior research on *C. corymbosum*'s *in vitro* elastase inhibitory activity. However, Azwanida et al. (2020) reported significant elastase inhibition by the leaf water extract of *Cosmos caudatus* Kunth, another member of the Asteraceae family. Kanashiro et al. (2007) demonstrated that the presence of a catechol group in the B ring of certain flavonoids, such as kaempferol, quercetin, galangin, and myricetin, contributes to their elastase inhibitory activity. While further investigation is needed, these findings suggest that the lack of elastase inhibition in *C. corymbosum* extracts might be due to the absence of these specific flavonoid compounds.

4. Conclusion

This study aimed to investigate the phytochemical profile and therapeutic target enzymes inhibitory, antioxidant, and cytotoxic activities of *Cardopatium corymbosum*. Given the limited research on this plant, a broad screening approach was adopted to explore its therapeutic potential. The findings have revealed the presence of various bioactive compounds, including chlorogenic acid, fumaric acid, and potentially others, in *C. corymbosum* extracts, and suggesting that this plant could be a promising source of natural health-promoting agents. Notably, the extracts demonstrated strong inhibitory effects on α-glucosidase and TxrR, enzymes implicated in diabetes and cancer, respectively. The overall results suggest that *C. corymbosum* may be a suitable candidate for further studies on diabetes and cancer treatment. Future research should prioritize the isolation and characterization of bioactive compounds, elucidating their mechanisms of action, and conducting *in vivo* studies to assess their efficacy and safety.

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

Gozde Hasbal-Celikok: Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Nermin Çakmak:** Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Yasin Celikok:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Servet Duranay:** Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Bahar Gürdal:** Resources, Methodology, Investigation, Formal analysis, Data curation. **Ebru Özdemir Nath:** Resources, Methodology, Investigation, Formal analysis, Data curation. **Tugba Yilmaz-Ozden:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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