KSÜ Tarım ve Doğa Derg 27 (1), 14-25, 2024
KSU J. Agric Nat 27 (1), 14-25, 2024
DOI:10.18016/ksutarimdoga.vi.1294720

Assessment of Inhibitory Ability Against Medicinally Important Enzymes with Invitro and In Silico Studies: Phenolic Content of Endemic *Centaurea cadmea* subsp. *pontica*

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ABSTRACT

Centaurea species has great potential as a traditional medicinal herb and *C. cadmea* subsp. *pontica* collected from rocky slope crevices of Küre Mountain is endemic to the flora of Türkiye. In the present work, to reveal the plant's pharmacological importance, its potency to inhibit various medicinal enzymes was investigated, supported by molecular docking studies. The half-maximal inhibitory concentration (IC_{50}) results for studied enzymes were quantified between 0.50-86.97 µg mL^{-1}, and the extract was efficient against HMG_CoA R, α-glucosidase, and α-amylase enzymes linked to diabetes and cholesterol. Nine phenolic compounds were identified in the *C. cadmea* subsp. *pontica* extract and the interactions of the most abundant phenolic compounds with the enzymes were examined with molecular docking studies. In conclusion, findings amassed from the present study inclined to support the opinion that *C. cadmea* subsp. *pontica* may be beneficial as an effective herb for formulating novel health-promoting ingredients.

Biochemistry

Research Article

Article History
Received : 09.05.2023
Accepted : 28.07.2023

Keywords
Centaurea cadmea
Enzyme inhibition
Molecular docking
Phenolic component

Tıbbi Açıdan Önemli Enzimlere Karşı İnhibisyon Yeteneğinin Invitro ve Insilico Çalışmalarla Değerlendirilmesi: Endemik *Centaurea cadmea* subsp. *pontica* Bitkisinin Fenolik İçeriği

ÖZET

Centaurea türleri geleneksel şifalı bitki olarak büyük bir potansiyele sahiptir. Küre Dağı'nın kayalık yamaç yarıklarından toplanan *C. cadmea* subsp. *pontica* Türkiye florasına endemiktir. Bu çalışmada, bitkinin farmakolojik önemini ortaya koymak için çeşitli tıbbi enzimleri inhibe etme pontansiyeli, moleküler yerleştirme çalışmaları ile desteklenerek araştırılmıştır. Çalışılan enzimler için yarı maksimum inhibitör konsantrasyon (IC_{50}) sonuçları 0.50-86.97 µg mL^{-1} arasında belirlendi. Bitki ekstraktı diyabet ve kolesterol ile bağlantılı olan HMG_CoA R, α-amilaz ve α -glukosidaz enzimlerine karşı etki gösterdi. *C. cadmea* subsp. *pontica* özütünde dokuz fenolik bileşik tanımlandı ve en fazla bulunan fenolik bileşiklerin enzimlerle etkileşimleri moleküler yerleştirme çalışmaları ile incelenmiştir. Sonuç olarak, mevcut çalışmadan elde edilen bulgular, bu endemik bitkinin sağlıklı yaşam kalitesinin geliştirilmesine yönelik yeni bileşenlerin formüle edilmesi için etkili bir bitki olarak yararlı olabileceğini göstermektedir.

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 09.05.2023
Kabul Tarihi : 28.07.2023

Anahtar Kelimeler
Centaurea cadmea
Enzim inhibisyonu
Moleküler kenetlenme
Fenolik içerik

To Cite: Kısa, D., Çelik, A., İmamoğlu, R (2024). Assessment of Inhibitory Ability Against Medicinally Important Enzymes with Invitro and In Silico Studies: Phenolic Content of Endemic *Centaurea cadmea* subsp. *pontica*. *KSU J. Agric Nat 27* (1), 14-25. DOI: 10.18016/ksutarimdoga.vi. 1294720.

Atıf İçin : Kısa, D., Çelik, A., İmamoğlu, R (2024). Tıbbi Açıdan Önemli Enzimlere Karşı İnhibisyon Yeteneğinin Invitro ve Insilico Çalışmalarla Değerlendirilmesi: Endemik *Centaurea cadmea* subsp. *pontica* Bitkisinin Fenolik İçeriği. *KSÜ Tarım ve Doğa Derg 27* (1), 14-25. DOI: 10.18016/ksutarimdoga.vi. 1294720.

INTRODUCTION

The genus *Centaurea* L. belongs to the Asteraceae family, Turkiye is the major distribution center of the plants, and it is the third-largest genus in Anatolia. The taxonomically problematic *Centaurea* genus is represented by over 600 species worldwide depending on the classification used, and there are 218 species in Türkiye, and the endemism rate of the taxon has

reached 64 %. *Centaurea cadmea* subsp. *pontica* spreads to Bartın and Zonguldak provinces in the northern Anatolia region (Türkiye). The plant is a chamaephyte that grows in limestone rock cracks and on rocky slopes far away from brookside. *C. cadmea* subsp. *pontica* was described by Köse et al. (2010) and considered as the least concern (LC) category. Flowering in the plant occurs in June-September, and fruiting occurs in July-September (Köse et al., 2010; Yaman et al., 2020; Özbek, 2021; Duman et al., 2021). Plants belonging to the genus *Centaurea* were reportedly used in traditional medicine as herbal remedies for digestive, antipyretic, antidiarrheal, expectorant, and tonic effects (Bancheva et al., 2023; Astari et al., 2014). As a continuation of research on the genus and secondary metabolites, we have investigated the phenolic components of the *Centaurea cadmea* subsp. *pontica* collected from its natural habitat. Since plants contain various phytochemical ingredients, their effects on enzymes of medical importance associated with various deficiencies are being screened.

Chronic and non-communicable diseases, including cardiovascular, cancer, diabetes, and neurodegenerative diseases (Alzheimer's), have long-term adverse effects on public health and cause 63% of deaths globally. Experimental and epidemiological studies show that some phytochemical molecules play an essential role in preventing these diseases. In fact, enzyme inhibitors are used to treat some diseases such as hypertension, metabolic disorders, neurodegenerative diseases, and some cancers (Gonçalves & Romano 2017). Bioactive molecules bind to the active site of proteins and function as organic compounds that decrease the bioactivity of enzymes. Many drug molecules are used as inhibitors or activators for enzymes. These compounds affect the catalysis rate of biochemical reactions leading to conformational changes on the active surface of the target protein. Some drugs used today are inhibitors of enzymes that are associated with regulation in the ailment process. According to the currently used therapy, the "inhibition of key enzymes" approach is an effective way to regulate pathologies. For example, HMG-CoA reductase and ACE (angiotensin-converting enzyme) inhibitors regulate cholesterol and blood pressure levels, respectively. Synthetic inhibitors lead to the research for novel, safe, and effective compounds, especially from natural resources, due to their comparatively low toxicity and side effects. Investigating the new biologically active agents from natural sources is one of the study strategies for medicinal drugs due to the easy accessibility of herbal phenolic substances (Guerrero et al., 2012; Baskaran et al., 2015; Zengin et al., 2016; Gonçalves & Romano 2018; Yin et al., 2019).

Phytochemical ingredients of plants vary according to the natural environment in which they grow, locality, and altitude. Studies on the *Centaurea* genus extensively focused on phytochemical contents, antidiarrheal, antimicrobial, antioxidant, and antipyretic activities (Astari, 2013; Grafakou, 2018; Alper & Güneş, 2019; Tugay 2020). Nonetheless, *Centaurea cadmea* subsp. *pontica* has been selected to reveal its medical importance as no enzyme inhibition has been performed on this species. As a novelty, enzyme inhibition, assisted with molecular docking studies in the broad spectrum, was investigated to gain further knowledge on the endemic plant.

MATERIALS and METHODS

Plant sample and extraction method

The samples (*Centaurea cadmea* subsp. *pontica*) were collected from the rocky slopes around Ulukaya waterfall at the foothills of Küre Mountain in Ulus-Bartın province, Türkiye, in July 2020. The collected samples were left to air dry at room conditions. The voucher specimen (BofHerb_495) has been authenticated by Prof. Z. Kaya and deposited in the Herbarium of the Department of Forest Engineering, Faculty of Forestry, Bartın University.

The aerial parts of dried plant samples were grounded in a mechanic grinder, and the powdered material was extracted in methanol at ambient conditions. The admixture was sonicated for 30 min at 37°C and centrifuged at 4500 x g for 12 min. The upper solvent of the filtrated extract was evaporated at an ambient temperature of less than 55 °C. The extract was kept at -20 °C for biochemical analysis.

Determination of the total phenolic compound (TPC) and total flavonoid contents (TFC)

The analysis of TPC and TFC in the *Centaurea cadmea* subsp. *pontica* extracts were performed by the Folin-Ciocalteu reagent and aluminum chloride ($AlCI_3$) colorimetric method, respectively described by the earlier studies. The quantities of them were expressed as mg of gallic acid and quercetin per gram of the extract (Singleton & Rosi, 1965; Pękal & Pyrzynska, 2014).

Determination of individual phenolic compositions

Phenolic compound profiles of the plant extract were quantified using a reverse-phase HPLC system coupled with an SPD-M20A detector and LC 20AT pump (Shimadzu Scientific Instruments, Japan). The extract solution (20 µL) was filled to the equipment set to 1 mL min^{-1} with automatic injection. The mobile phases, solvent A (methanol) and solvent B (acetic acid, 2% v/v), were formed, and the elution was given to the reverse phase C18 analytical column (GL Sciences, 5 µm, 4.6 mm x 250 mm,) and the

temperature was kept at 25°C. The flow rate was set to 1 mL min^{-1} for gradient elution. Then, the obtained chromatographic profiles belonging to the phenolic compound in the plant extract solutions were defined and quantified with the accompaniment of the UV spectra, retention times, and chromatograms with known standard phenolic compounds. The calculated results were specified as mg g^{-1} plant extract (Elmastaş et al., 2017).

Enzymes inhibition studies

The enzyme inhibitory potency of *C. cadmea* extract was evaluated using the 96-microwell plate spectrophotometric method referred to in the following sentences (Microplate Reader, Thermo Scientific). AChE and BChE activity were evaluated using the well-known Ellman's spectrophotometric method (Ellman et al., 1961). The inhibitory assay for Angiotensin-converting enzyme (ACE) was done in Tris-HCI buffer (50 mM, pH 7.5) with a referred research (Hou et al., 2003). The reaction components of wells contain 10 µL of ACE solution, 10 µL of plant extracts, and 150 µL of substrate FAPGG solution (0.88 mM). Captopril was used as a reference compound, and the reduced absorbance due to hydrolysis of the FAPGG was read at 340 nm at 37 °C for 5 min. The α-amylase inhibition method was achieved using the modified Caraway technique (Yang et al., 2012). Briefly, 30 µL of phosphate buffer, 20 µL of extract solution, and α-amylase (30 µL) were pipetted to wells, and the plate was preincubated for 10 min at 37 °C. After the 50 µL of the starch-substrate (1 %) was pipetted to the wells, the mixture was allowed to incubate for ten min at 25°C. Then, the reaction was aborted by adding 10 ml of 25 µL of 10 % HCl and was added 100 µl of iodine/potassium iodide solution. The absorbance of the colored wells d was recorded at 630 nm. The α-glucosidase inhibitory assay was done using p-nitrophenyl α-D-glucopyranoside (2 mM) as substrate (Tao et al., 2013). In each mixture of the well microplate, 120 µL of KH_2PO4, 10 µL of test solution, and 20 µL of α-glycosidase were added and incubated for 10 min at 37 °C. After the p-NPG (50 µL) was supplemented to each well, the absorbance of released p-nitrophenyl was measured at 405 nm. The inhibitory study of pancreatic lipase was carried out using substrate pnitrophenylpalmitate (p-NPP) with cited research (El-Korany et al., 2020). The wells comprised Tris-HCI (100 mM, pH 8.2), 20 µL of lipase solution and plant extract was incubated for 10 min at 37 °C and then the absorbance of liberated p-NP was monitored at 410 nm. The urease inhibitory property of plant extracts was determined using the indophenol method to quantify the amount of ammonia formed due to the enzymatic reaction (Ikram et al., 2017). In each well, microplates briefly containing 50 µL of urea prepared in KH_2PO4 buffer (pH 8.2, 100 mM), 25 µL of jack bean urease solution, and 20 µL of extract were incubated for 15 min at 37 °C. Then, then phenol reagent and alkali reagents were added to react with the released ammonia. The absorbance of colored complex was read at 630. For the tyrosinase inhibition assay; substrate L-DOPA, mushroom tyrosinase in phosphate buffer (pH 6.8, 100 mM), and plant extract were used according to the previously described spectrophotometric method (Masuda et al., 2005). The increased absorbance of the colored mixture caused by dopachrome was recorded at 475 nm. For the HMG-CoA reductase activity assay; an HMG-CoA reductase assay kit (BioVision Inc., USA) containing HMG-CoA reductase, NADPH, HMG-CoA, and assay buffer was used, and the inhibitory ability of the plant extract was assessed by reading the absorbance at 340 nm. The inhibitory ability of plant extract was performed using *Clostridium histolyticum* collagenase with a previously designed method (Thring et al., 2009). Briefly, collagenase in tricine buffer (pH 7.5) was incubated with the extract solutions at 25 °C for 15 min and substrate FALGPA was added to wells. The reduced absorbance due to hydrolysis of the FALGPA was read at 340 nm.

Antimicrobial test

The antibacterial and antifungal properties of *C. cadmea* subsp. *pontica* were determined according to the minimal inhibitory concentration (MIC) test. Fungal strains (*Cancida utilise* and *Cancida albicans*), Gram-positive (*E. faecalis* and *S. aureus*), and Gram-negative (*K. pneumonia* and *E. coli*) were used for this test. The frozen stock was taken from cells and inoculated. Cultures were adjusted to 0.5 McFarland Units, and the extract was added as serial dilutions to a concentration of 1 mg/mL in a 96-microwell plate. Microbial cells were incubated at 37°C for 24 hours, and MIC results were evaluated by reading in the spectrophotometer.

Computational study

Molecular docking is a widely used in silico method for learning protein-ligand interactions (Meng et al., 2012). In this context, to examine in silico enzyme inhibition ability, molecular docking-based computations were executed on AChE, ACE, BChE, α-glycosidase, α-amylase, lipase, collagenase, HMG_CoA R, urease, and tyrosinase by using Autodock vina 1.2.0 (Troot & Olson, 2010; Meng et al., 2012; Eberhardt et al., 2021). The chemical structure of the plant metabolites was retrieved from PubChem. Afterward, geometry optimization and energy minimization were performed to utilize Chimera software (Pettersen et al., 2004). The Auto- DockTools 1.5.7 package (Morris & Dallakyan, 2013) was utilized to generate entry files before molecular docking. Protein structures were

withdrawn from the protein data bank (PDB) and the structure of ligands (PubChem and Drugbank). Discovery Studio (DS) 2021 was also used to prepare the targets for docking studies. Water molecules were removed using the DS 2021 program, and polar hydrogen bonds were added to the structure. The grid of the studied complexes was portrayed with literature and via the subprotocol of DS 2021. The grid box of size 126 x 126 x126 represents X, Y, and Z coordinates, with 8 comprehensive values and 0.375 A °grid point space. The results were evaluated with the DS 2021 program, and images were taken.

RESULTS

Enzyme inhibitory activity of *C. cadmea* subsp. *pontica* extract

In the current study, the inhibitory potential of the plant extract was screened against ten different medicinal enzymes to reveal the herbal medicine potential. The IC_{50} values of the plant extract were shown in Fig 1 and Table 1. In this current screening study, the extract has inhibitory potential at different concentrations and the obtained IC_{50} values were 86.97±1.93 µg/mL, 75.60±1.87 µg/mL, 24.67±1.39 µg/mL, 0.509±0.00, 82.48±1.91 µg/mL, 66.20±1.82 µg/mL, 69.15±1.84 µg/mL, 72.05±1.5 µg/mL, 26.66±1.42 µg/mL, and 46.02±1.60 µg/mL for AChE, BChE, ACE, HMG_CoA R, α-glycosidase, α-amylase, lipase, collagenase, urease, and tyrosinase, respectively. The same extract was found to be effective against HMG_CoA R, amylase, and glycosidase, which is better than the IC_{50} values of the known standards. When the result of urease was examined, the extract was obtained from *C. cadmea subsp. pontica* was found to display a remarkable effect (IC_{50}: 26.66±1.42, r2: 0.999); it is slightly weaker than the IC_{50} of the thiourea.

Table 1. IC_{50} values of *C. cadmea* subsp. *pontica* extract on studied enzymes
Çizelge 1. Çalışılan enzimler üzerine C. cadmea subsp. *pontica ekstraktının* IC_{50} *değerleri*

Enzymes	AChE		BChE		ACE		α-Glucosidase		α-Amylase	
Values	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
Extract (µg mL⁻¹)	86.97±1.93	0.986	75.60±1.87	0.984	24.67±1.39	0.984	69.15±1.84	0.990	82.48±1.91	0.993
Standards (µg mL⁻¹)	23.36±1.36	0.985	24.84±1.39	0.992	21.36±1.33	0.988	174.3±2.24	0.993	131.2±2.11	0.995
Enzymes	HMG_CoA R		Collagenase		Lipase		Tyrosinase		Urease	
Values	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
Extract (µg mL⁻¹)	0.509±0.00	0.967	72.05±1.5	0.984	66.20±1.82	0.985	46.02±1.60	0.989	26.66±1.42	0.999
Standards (µg mL⁻¹)	8.78±0.94	0.997	2.52±0.40	0.998	35.5±2.58	0.991	3.49±0.54	0.999	20.36±1.30	0.989

Evaluation of phenolic compounds of methanolic extracts of *C. cadmea* subsp. *pontica*

The quantification of phenolic compositions was accomplished in the obtained extract *of C. cadmea* subsp. *pontica* and the constituents were given in Table 2. Chlorogenic acid, gentisic acid, rosmarinic acid, rutin, cinnamic acid, quercetin, eugenol, apigenin, and methyl chavicol were identified as the main phytochemical compounds. Rutin was determined as the richest metabolite identified in *C. cadmea* subsp. *pontica extract*, followed by methyl chavicol, the most abundant metabolite, calculated their values as 119.49 ±1.02 and 40,83±1.74 µg g^{-1}, respectively. The total phenol and flavonoid amount of the same extract were 36.93 ± 0.51 mg g^{-1} as gallic acid equivalent (GAE), 11.66 ± 0.41 mg g^{-1} as quercetin equivalents (QE), respectively (Table 3).

Antimicrobial assessment of *C. cadmea* subsp. *pontica* plant extracts

The MIC method was used to assess the antibacterial and antifungal activity of *C. cadmea* subsp. *pontica* against different bacteria strains such as *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and fungal strains *Candida albicans* and *Candida utilis*. As shown in Table 4, the MIC value of the plant extract ranged from 296.6 ± 2.472 to 645 ± 2.810 for microorganisms, and the extract exhibited higher inhibitory potential against *E. coli* than other microorganisms.

Molecular docking result of phenolic compounds of *C. cadmea* subsp. *pontica*

In the present work, the enzyme inhibition potency of three secondary compounds [methyl chavicol (1), gentisic acid (2), and rutin (3)] most detected in *C. cadmea* subsp. *pontica*, according to the data obtained from the HPLC study, was assessed with the assistance of computational methods. Although many in silico analyses were carried out in the literature, in this goal was to interrelate attachment points of substances to enzymes in which the quantity of secondary metabolites varies according to the plant species and even where the plant is grown. The three target enzymes with the best interaction and binding scores are HMG_CoA R, tyrosinase, and urease.

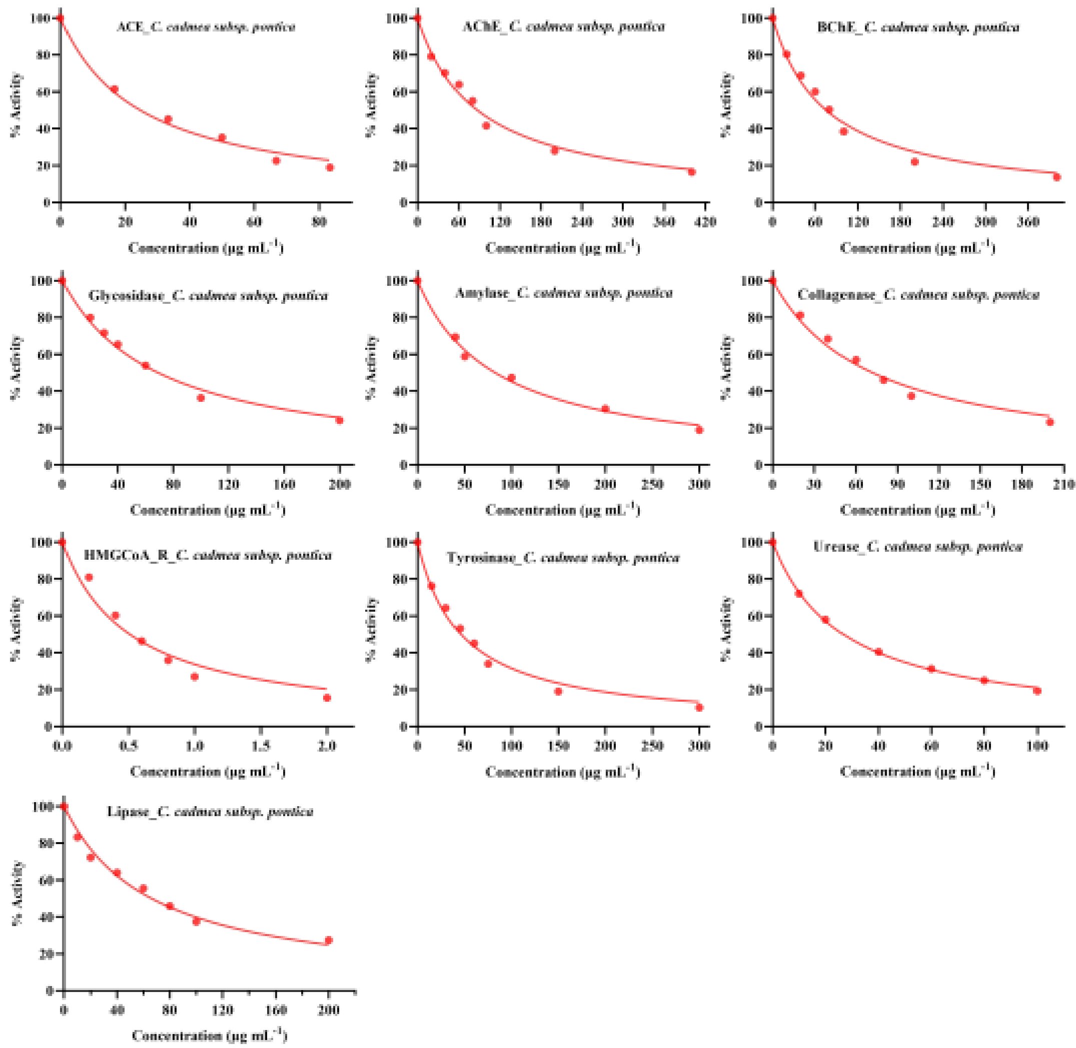

Figure 1. IC_{50} graphs for the studied enzymes of the *C. cadmea* subsp. *pontica* extracts
Şekil 1. C. cadmea subsp. *pontica ekstraktlarının incelenen enzimler için IC_{50} grafikleri*

Table 2. The main constituents of the aerial part extract of *C. cadmea* subsp. *pontica*
Çizelge 2. C. cadmea subsp. *pontica toprak üstü özütünün temel bileşenleri*

Phenolic Compounds	Retention time (min)	Amount (μg g^{-1})
Chlorogenic acid	11.348	19.06±0.19
Gentisic acid	12.099	26.45±0.21
Rutin	26.691	119.49±1.02
Rosmarinic acid	27.226	16.88±0.18
Cinnamic acid	38.622	16.89±0.17
Quercetin	42.209	10.01±0.61
Eugenol	44.646	5.92±0.01
Apigenin	47.434	22.22±0.23
Methyl chavicol	55.471	40.83±1.74

Table 3. TPC and TFC in the plant extract
Çizelge 3. Bitki özütünde TPC ve TFC

Extracts	TPC (mg g^{-1} DW)	TFC (mg g^{-1} DW)
C. cadmea subsp. pontica	36.93 ± 0.51	11.66 ± 0.41

Table 4. MIC results for selected bacterial and fungal strains
Çizelge 4. Seçilen bakteri ve mantar suşları için MİK sonuçları

Microorganisms	MIC Values of *C. cadmea* subsp. *pontica* (μg mL^{-1})
E. coli	296 ± 2.472
K. pneumoniae	645 ± 2.810
S. aureus	461 ± 2.664
E. faecalis	321 ± 2.507
C. albicans	347 ± 2.541
C. utilis	438 ± 2.641

Compound 3 exhibited the best results against target enzymes in the molecular insertion study, with a better binding tendency than control compounds. When the retrieved compounds' docking results were examined, it was concluded that the metabolites of the plant extract highly interacted with HMG_CoA R, tyrosinase, and urease, and the detailed interaction data of the selected compounds were provided in Table 5.

Table 5. The binding scores of the selected compounds (methyl chavicol_1, gentisic acid_2, and rutin_3) with the target proteins. *The known inhibitory substances are remarked for each target enzyme (BE: Binding energy).

*Çizelge 5. Seçili bileşiklerin (metil chavicol_1, gentisik asit_2 ve rutin_3) hedef proteinlere bağlanma skorları. *Bilinen inhibitör maddeler her bir hedef enzim için belirtilmiştir (BE: Bağlanma enerjisi).*

AChE	BE (kcal/mol)	HMG_CoA R	BE (kcal/mol)
1	-5.7	1	-6.2
2	-6.0	2	-7.4
3	-8.2	3	-10.3
Tacrine*	-7.6	Atorvastatin*	-7.7
BChE	**BE (kcal/mol)**	**α-Amylase**	**BE (kcal/mol)**
1	-6.0	1	-4.5
2	-6.1	2	-5.8
3	-8.3	3	-8.2
Tacrine*	-7.2	Acarbose*	-9.9
ACE	**BE (kcal/mol)**	**Collagenase**	**BE (kcal/mol)**
1	-5.2	1	-4.3
2	-5.6	2	-5.2
3	-9.5	3	-8.4
Captopril*	-4.3	EGCG*	-7.2
Urease	**BE (kcal/mol)**	**α-Glucosidase**	**BE (kcal/mol)**
1	-5.0	1	-5.2
2	-5.6	2	-6.7
3	-10.2	3	-8.8
Thiourea*	-3.5	Acarbose*	-9.5
Tyrosinase	**BE (kcal/mol)**	**Lipase**	**BE (kcal/mol)**
1	-5.6	1	-4.4
2	-6.3	2	-5.8
3	-9.7	3	-9.4
Kojic acid*	-5.5	Orlistat*	-7.2

Firstly, atorvastatin, the control compound atorvastatin for HMG-CoA R, formed a hydrogen bond with Ala110, and Ala187 residue, hydrophobic interaction with Lys108, and electrostatic interaction with Arg187 and Glu109. Compound 3 has a hydrogen bond (Thr152, Pro148, Gly149, and Gly198) and hydrophobic interaction (Phe236, Pro235, Leu239, Tyr205, Tyr307, Pro235, and Leu853) with the protein. Compound 2 has a hydrogen bond (Asn276, Gly198, and His233) and hydrophobic interaction (Pro235). Compound 1 showed only hydrophobic interaction (Pro148, Val196, Tyr307, and Val196). The interactions of the selected molecules with the control substance were shown in Fig. 2.

The control compound of tyrosinase, kojic acid, formed a hydrogen bond with Thr345 residue, hydrophobic interaction with Phe355, Ala295, Ala346, Val366 (Fig. 3). Compound 3 has a hydrogen bond (Ser291, Gln294, Asp344, Thr343, and Lys151) and hydrophobic interaction (Phe355) with the target enzyme. Second, compound 2 has a hydrogen bond (Gln37, Lys335, and Ser170) and hydrophobic interaction (Arg163 and Pro341). Compound 1 has a hydrogen bond (Thr345) and hydrophobic interaction (Phe355, Ala295, Ala346, and Val366). These locations are presented in Fig. 3.

Compound 3 displays the best numerical and visual results in possible complexes with the urease shown in Fig. 4. When the docking results are evaluated, compound 3 occurred in a hydrogen bond with Ala440, Asp633, Ala636, Asp633, and Pi-Sulfur interactions with His545 and Gly550 amino acid in the binding surface of the enzyme. Compound 2 has a hydrogen bond (Ser665, Asn668, Glu303, Asp664, and Asn668). Compound 1 has a hydrogen bond (The33) and hydrophobic interaction (Tyr32, Val36, Lys716, Phe712, and Val744). These situations are shown in Fig. 4.

DISCUSSION

Humankind has been compelled to use various natural substances by resorting to nature to manage their diseases, and the tendency to research the healing

aspects of medicinal plants has continued since ancient times. Nature has provided human beings with various opportunities, and medicinal plants have been used to cure and alleviate various diseases. Intercultural medicine essentially includes health practices, bridging indigenous medicine and modern medicine, both of which are considered complementary (Baydoun et al., 2015; Dutta et al., 2021; Tiwana et al., 2021). Interest in medicinal plants, including *Centaurea* species having several ethnopharmacological properties, is reemphasized, and they are being screened for pharmacological activities. Although various studies have been carried out on *Centaurea* species for their biological effect, the extract of *C. cadmea subsp. pontica* has not been evaluated for its potential to inhibit various enzymes and its phenolic compound content.

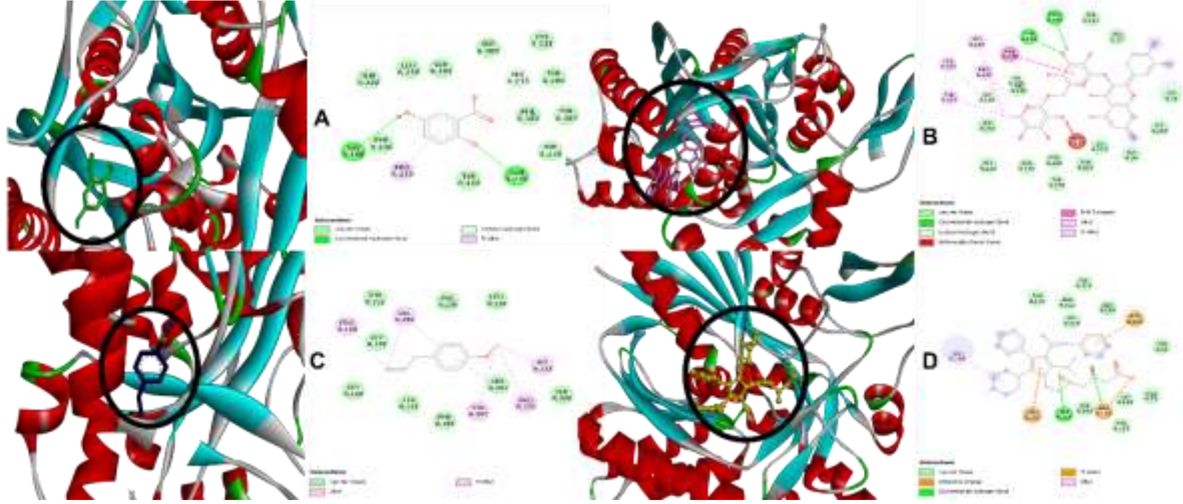

Figure 2. Demonstration of the interaction of methyl chavicol (A), gentisic acid (B), rutin (C), and atorvastatin (D) with HMG-CoA R, respectively.

Şekil 2. Metil chavicol (A), gentisik asit (B), rutin (C) ve atorvastatinin (D) sırasıyla HMG-CoA R ile etkileşiminin gösterilmesi

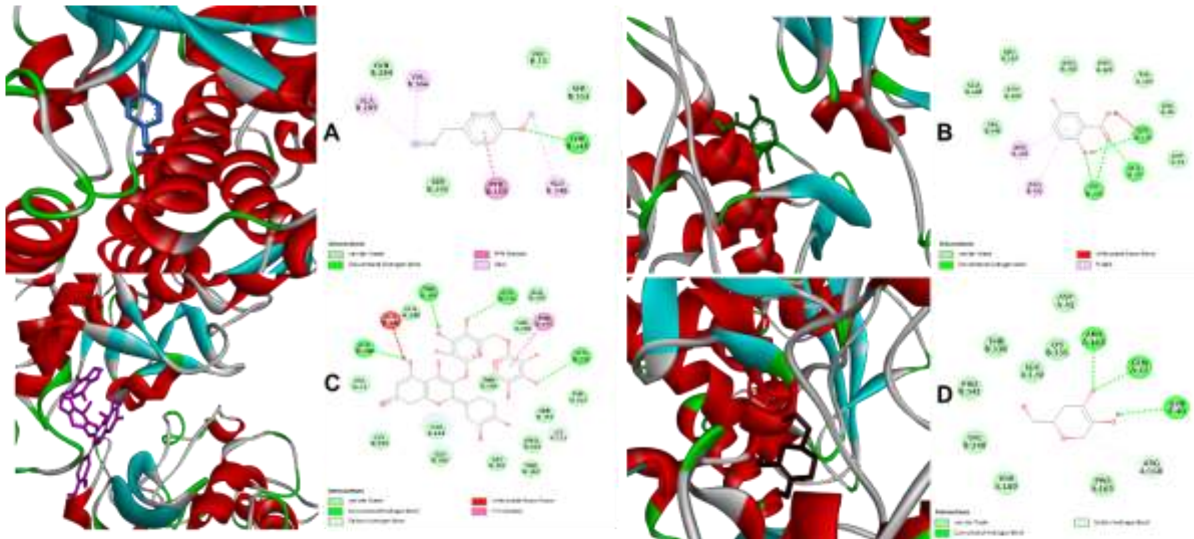

Figure 3. Demonstration of the interaction of methyl chavicol (A, blue color, stick form), gentisic acid (B, green color, stick form), rutin (C, purple color, stick form), and kojic acid (D, yellow color, stick form) with tyrosinase, respectively.

Şekil 3. Metil kavikol (A), gentisik asit (B), rutin (C) ve atorvastatinin (D) sırasıyla tirozinaz ile etkileşiminin gösterilmesi

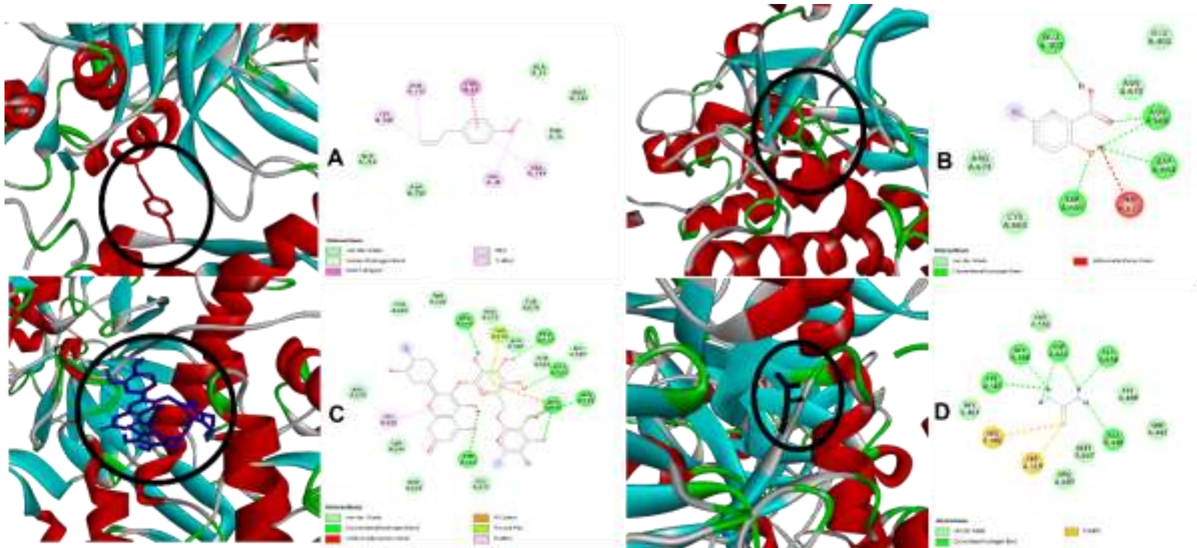

Figure 4. Demonstration of the interaction of methyl chavicol (A, red color, stick form), gentisic acid (B, green color, stick form), rutin (C, blue color, stick form), and kojic acid (D, black color, stick form) with urease, respectively.

Şekil 4. Metil chavicol (A), gentisik asit (B), rutin (C) ve atorvastatinin (D) sırasıyla üreaz ile etkileşiminin gösterilmesi

In this study, we screened the enzyme inhibition potency with molecular docking details to advance novelties related to herbal medicine. The evaluation of enzyme inhibitors such as plant-derived compounds, is one of the most important topics in pharmaceutical areas such as traditional medicine. The plant extract has lower IC_{50} values than the known standards of HMG-CoA R, α-amylase, and α-glucosidase, respectively. Although the plant extract inhibits studied enzymes (ACE, AChE, BChE, collagenase, lipase, tyrosinase), its inhibitory power is lower than known commercial inhibitors. Also, the obtained IC_{50} result of the study suggests that the plant extract may have an alternative potential to thiourea in terms of inhibiting the urease enzyme. The literature survey indicated an inadequacy of studies relevant to the enzyme inhibition effect of *C. cadmea subsp. pontica* extracts. In a study of them, Zengin (2016) reported different solvent extracts of *Centaurea* species, except *C. cadmea subsp. pontica* exhibited inhibitory activity against AChE, BChE, Try, α-Amyl, and α-Gly at the concentration of 2 mg/mL. They declared that inhibition ability changed to solvent types (ethyl acetate and chloroform) and plant taxa, and % inhibition value was between 43.94 ± 1.51 - 95.69 ± 0.06 for BChE, 60.04 ± 0.09 - 95.93 ± 0.07 for AChE, 0.91 ± 0.08 - 12.54 ± 0.08 for Try, 17.53 ± 0.08 - 59.54 ± 0.59 for α-Amyl, and 36.03 ± 0.24 - 60.31 ± 2.13 for α-Gly in chloroform extracts (Zengin et al., 2016). When the present study results are compared with the same enzymes in the aforementioned study, it is seen that *C. cadmea subsp. pontica* extracts have a higher inhibitory potential. Another study was conducted to screen for AChE inhibitory potency of *Centaurea* plants (*C. antalyensei, C. polypodiifolia,* and *C. pyrrhoblephara*) declared that methanol extracts had better ability against AChE than aqueus extracts at 2 mg mL^{-1}. Aqueous extracts of *C. antalyense* and *C. pyrrhoblephara* had no activity against AChE. The same methanolic extract, except for *C. pyrrhoblephara*, displayed inhibitory activity between 37.14 ± 8.17 - 45.50 ± 9.62 % at the concentration of 2 mg mL^{-1} (Aktumsek et al., 2013). In another work, it is stated that extracts of *C. depressa* Bieb., *C. balsamita* Lam., and *C. lycopifolia* Boiss collected from southeastern Türkiye did not show any inhibitory activity against AChE, while different solvent extracts showed moderate activity against BChE at 200 µg/mL (Boğa et al., 2016). Compared to the aforementioned studies, the current results show that *C. cadmea subsp. pontica* extracts are screened in a broader spectrum and have higher inhibitory ability. We assumed that the inhibitory ability of the plant extract could be connected to plant species and used solvents.

Secondary metabolites, including phenolic compounds, are thought to be the reason why plants have notable biological activities such as antioxidant, antimicrobial, and anti-carcinogenic. Some biological activities of plants, such as antidiabetic and anti-Alzheimer's properties, are linked with the inhibition of enzymes that play a role in the biochemical metabolic pathways. Therefore, the phenolic composition in the plant extract was investigated, as it is important to quantify their phenolic contents and to evaluate their contribution to enzyme inhibitory ability. Rutin (119.49 µg g^{-1}) is the most abundant flavonoid among the 11 metabolites calculated in the plant extract, while the amounts of chlorogenic acid, gentisic acid, and rosmarinic acid, cinnamic acid apigenin were found to be close to each other. TFC and TPC were

11.66 ± 0.41 mg g^{-1} and 36.93 ± 0.51 mg g^{-1} as QE and GAE, respectively. Different taxa of *Centaurea* have been investigated with respect to phytochemical ingredients. In one of them, Alper et al. (2021) reported that 15 phenolic compounds (caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, epicatechin, ellagic acid, ferulic acid, gallic acid, naringin, quercetin, rutin, vanillic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid) were identified in the ethanolic extracts of the both *Centaurea solstitialis*. They determined the TPC and TFC in the same extract as 52.31 mg g^{-1} GAEs and 30.10 mg g^{-1} QEs, respectively (Alper et al., 2021). In the study investigating the phytochemical ingredients in *Centaurea karduchorum* from Eastern Anatolia, apigenin glucuronide, chlorogenic acid, and luteolin derivatives were quantified in plant aerial parts by LC-MS spectroscopy (Dalar et al., 2015). A study conducted to the determination of the methanol extract of *C. gigantea* declared that chlorogenic acid and flavonoids (isoorientin, 2-(4-hydroxybenzoyl)-isoorientin, isoquercitrin, orientin, and cirsiliol studied with reversed-phase HPLC analysis (Shoeb et al., 2007). Acet (2021) investigated the phenolic composition and biological activity of *C. triumfetti* and explained caffeic acid, p-coumaric acid, chlorogenic acid, t-cinnamic acid ferulic acid, and syringic acid as phenolic components. She also stated that the plant extracts had α-glucosidase and α-amylase inhibitory activity, especially in the ethyl acetate extract of the plant stem (Acet, 2021). In a previous study, chlorogenic acid, scutellarin, and syringin were isolated from the aerial parts of *Centaurea cadmea*, and the structures of these compounds were elucidated by spectroscopic methods such as NMR and LC-MS/MS (Astari et al., 2013). In similar earliear studies, researchers also investigated the metabolite components and enzyme inhibitory activities of *Centaurea* species (*C. lycopifolia, C. drabifolia,* and *C. rupestris*). They have specified caffeic acid, apigenin, chlorogenic acid, luteolin, p-coumaric acid, and quercetin as plant phenolic ingredients and their enzyme inhibitory properties on AChE, try, α-amy, and α-gly (Ćurković-Perica et al., 2014; Zengin et al., 2018). When compared with the studies exemplified in the upper lines, it is seen that the plant has some similar phenolic substances but different phenolic compositions. We could propound that the content and quantity of metabolites vary according to the plant type, tissue, extraction method, and analysis equipment.

The antibacterial activity in the plant extract may be due to the presence of phenolic and flavonoid contents. It is stated in the literature that various plants, including *Centaurea* species, exhibit antibacterial and antifungal properties in vitro conditions, and this is related to medicinally important compounds synthesized by plants. MIC values were found in the concentration range of 0.0625 to 8 mg/mL against bacterial and fungal species in various antimicrobial studies (Karamenderes et al., 2006; Köse et al., 2016; Albayrak et al., 2017; Sönmez & Çakıloğlu, 2020; Naeim et al., 2020; Reda et al., 2021). It has been reported that the aerial parts of *C. cadmea* extracted in chloroform show strong activity on *Enterococcus faecalis* (8 µg/mL) and *Bacillus cereus* (16 µg/mL) (Astari et al., 2014). These values in such a wide range are due to factors such as the plant's collection time, light exposure time, soil type, the geography where it grows, and the extraction method of metabolites. These factors affect the plant's biological properties, causing a change in secondary metabolite content. From the results obtained according to the HPLC analysis of *C. cadmea subsp. pontica*, computational chemistry estimations were done to predict the biological activities of the main compounds found in the highest amount in the plant content against target enzymes and understand the interaction mechanisms.

CONCLUSION

In the treatment of some metabolic diseases, in the context of inhibition of key enzymes that play an important role in biochemical reactions, eleven different medically important enzymes associated with common diseases in the society were chosen as the target. In summary, this present study endeavors to highlight the phenolic contents of *C. cadmea* subsp. *pontica*, which is one of the endemic medicinal *Centaure* species in folk medicine in Anatolia. In order to assert natural sources as a substitute for synthetic enzyme inhibitor substances used for therapeutic purposes, the enzyme-inhibiting potentials of these compounds should be revealed. For this reason, enzyme inhibition capacity and phenolic components *of C. cadmea* subsp. *pontica* extracts were worked for the first time in addition to molecular docking details. Accordingly, among all inhibitory potential of the extracts, it came to the forefront in terms of higher inhibitory power against HMG_CoA R, α-glucosidase, and α-amylase. We suppose that the inhibitory ability might be linked to its phenolic components, or the synergic effects supported by molecular docking studies. Compared to previous studies on *Centaurea* taxa, the high bioactivity level of the plant might be related to the climatic conditions of the plant collected region. Taken together, in vitro findings emphasize the importance *of C. cadmea* subsp. *pontica* plant. However, further experimental studies (isolation of metabolites and in vivo animal studies) could be planned in the future to better understand the detailed pharmacological effect in light of present findings.

ACKNOWLEDGMENT

The present study was financially supported with a research grant coded 2021-FEN-A-004 by the Scientific Research Projects Unit of Bartın University, Türkiye.

We are very grateful to Prof. Dr. Zafer Kaya, who identified the plant and passed away from this world.

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

KAYNAKLAR

Acet, T. (2021). Determining the phenolic components by using HPLC and biological activity of *Centaurea triumfetti*. *Plant Biosyst*, *155*, 159-164. doi: 10.1080/11263504.2020.1722275.

Aktumsek, A., Zengin, G., Guler, G. O., Cakmak, Y. S. & Duran, A. (2013). Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* L. species. *Food Chem Toxicol*, *55*, 290-296. doi: 10.1016/j.fct.2013.01.018.

Albayrak, S., Atasagun, B. & Aksoy, A. (2017). Comparison of phenolic components and biological activities of two *Centaurea sp.* obtained by three extraction techniques. *Asian Pac J Trop Med. 10*(6), 599-606. doi: 10.1016/j.apjtm.2017.06.010.

Alper., M. & Güneş, H. (2019). The Anticancer and Anti-inflammatory Effects of *Centaurea solstitialis* Extract on Human Cancer Cell Lines. *Turk J Pharm Sci*, *16*(3), 273-281. doi: 10.4274/ tjps.galenos.2018.27146.

Alper, M., Özay, C., Güneş, H. & Mammadov, R. (2021). Assessment of Antioxidant and Cytotoxic Activities and Identification of Phenolic Compounds of *Centaurea solstitialis* and *Urospermum picroides* from Turkey. *Brazilian Arch Biol Technol*, *64*, 1-12. doi: 10.1590/1678-4324-2021190530.

Astari, K.. A., Erel, S. B., Bedir, E. & Karaalp, C. (2013). Secondary Metabolites of *Centaurea cadmea* Boiss. *Rec. Nat. Prod. 7*(3), 242-244.

Astari, K. A., Baykan, Erel, Ş., Aydin Köse F, Köksal Ç. & Karaalp C. (2014). Cytotoxic and Antibacterial Activities of *Centaurea cadmea* Boiss. *Turkish J Pharm Sci*, *11*, 101-106.

Bancheva, S., Badalamenti, N. & Bruna, M. (2023). The essential oil composition of the endemic plant species *Centaurea vandasii* and chemotaxonomy of section Phalolepis (Asteraceae). *Natural Product Research*, *37*(7), 1122-1129. https://doi.org/10.1080/ 14786419.2021.1992627.

Baskaran G, Salvamani S, Ahmad, S. A., Shaharuddin, N. A., Pattiram, P. D. & Shukor, M. Y. (2015). HMG-CoA reductase inhibitory activity and phytocomponent investigation of *Basella alba* leaf extract as a treatment for hypercholesterolemia. *Drug Des Devel Ther,* *9*, 509-517. doi: 10.2147/DDDT.S75056.

Baydoun, S., Chalak, L., Dalleh, H. & Arnold, N. (2015). Ethnopharmacological survey of medicinal plants used in traditional medicine by the communities of Mount Hermon, Lebanon. *J Ethnopharmacol*, *17*3, 139-156. doi: 10.1016/ j.jep.2015.06.052.

Boğa, M., Alkan, H., Ertaş, A., Oral, E.V., Yılmaz, M.A., Yeşil, Y., Gören, A.C., Temel, H. & Kolak, U. (2016). Phytochemical profile and some biological activities of three centaurea species from Turkey. *Trop J Pharm Res. 15*, 1865-1875. doi: 10.4314/ tjpr.v15i9.8.

Ćurković-Perica, M., Likić, S. & Rusak, G. (2014). Phenolic compounds in centaurea rupestris tissues and their antiphytoviral activity. *Croat Chem Acta*, *87*, 79-84. doi: 10.5562/cca2272.

Dalar, A., Uzun, Y., Mukemre, M., Turker, M. & Konczak, I. (2015). *Centaurea karduchorum* Boiss. from Eastern Anatolia: Phenolic composition, antioxidant and enzyme inhibitory activities. *J Herb Med. 5*, 211-216. doi: 10.1016/j.hermed. 2015.09.006.

Duman, H., Uzunhisarcıklı, M.E. & Bahadır, Y.N. (2021). A new species of Centaurea (Asteraceae) from northern Turkey. *Nordic Journal of Botany*, *39*(10), 1-8. doi: 10.1111/njb.03244

Dutta, T., Anand, U., Saha, S. C., Mane, A.B., Prasanth, D. A., Kandimalla, R., Proćków, J. & Dey, A. (2021). Advancing urban ethnopharmacology: A modern concept of sustainability, conservation and cross-cultural adaptations of medicinal plant lore in the urban environment. *Conserv Physiol, 9*, 1–20. doi: 10.1093/conphys/coab073.

Eberhardt, J., Santos-Martins, D., Tillack, A. F. & Forli, S. (2021). AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *Chem. Inf. Model. 61*(8), 3891-3898.

Ellman, G. L., Courtney, K. D., Andres, V. & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*, *7*, 88-95. doi:10.1016/0006-2952(61)90145-9.

Elmastaş, M., Demir, A., Genç, N., Dölek, Ü. & Güneş, M. (2017). Changes in flavonoid and phenolic acid contents in some Rosa species during ripening. *Food Chem*, *235*, 154-159. doi: 10.1016/j.foodchem. 2017.05.004.

Sönmez, P. & Çakıloğlu, U. (2020). Screening of Antimicrobial Effect against Microorganisms Threatening to Human Health of the Endemic Plant; *Centaurea saligna* (C. Koch) Wagenitz from Turkey*. Türk Doğa ve Fen Derg, 9*, 23-27 . doi: 10.46810/tdfd.732234.

Gonçalves, S. & Romano, A. (2017). Inhibitory Properties of Phenolic Compounds Against Enzymes Linked with Human Diseases. In: Soto-Hernández M (ed) Phenolic Compounds Biological Activity. IntechOpen, p 228.

Gonçalves, S. & Romano, A. (2018). Production of Plant Secondary Metabolites by Using Biotechnological Tools. In: Vijayakumar R (ed) Secondary Metabolites Sources and Applications. *IntechOpen*, p 138.

Grafakou, N. E., Djeddi, S., Tarek, H. & Skaltsa, H. (2018). Secondary metabolites from the aerial parts of *Centaurea papposa* (Coss.) Greuter. *Biochemical Systematics and Ecology*, *76*, 15-22. https://doi.org/10.1016/j.bse.2017.11.005.

Guerrero, L., Castillo, J., Quiñones, M., Garcia-Vallvé, S., Arola, L., Pujadas, G. & Muguerza, B. (2012.) Inhibition of Angiotensin-Converting Enzyme Activity by Flavonoids: Structure-Activity Relationship Studies. *PLoS One*, *7,* 1-11. doi: 10.1371/journal.pone.0049493.

Holdgate, G. A., Ward, W. H. J. & McTaggart, F. (2003). Molecular mechanism for inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase by rosuvastatin. *Biochem Soc Trans*, *31*, 528-531. doi: 10.1042/BST0310528.

Hou, W. C., Chen, H. J. & Lin, Y. H. (2003). Antioxidant peptides with angiotensin converting enzyme inhibitory activities and applications for angiotensin converting enzyme purification. *J Agric Food Chem*, *51*, 1706-1709. doi: 10.1021/jf0260242.

Ikram, M., Rehman, S., Subhan, F., Akhtar, M. N, & Sinnokrot, M. O. (2017). Synthesis, characterization, thermal degradation and urease inhibitory studies of the new hydrazide based Schiff base ligand 2-(2-hydroxyphenyl)-3-{[(E)-(2-hydroxyphenyl)methylidene]amino}-2,3-dihydroquinazolin-4(1H)-one. *Open Chem, 15*, 308-319. doi: 10.1515/chem-2017-0035.

Karamenderes, C., Khan, S., Tekwani, B. L., Jacob, M. R. & Khan, I. A. (2006). Antiprotozoal and antimicrobial activities of *Centaurea* species growing in Turkey. *Pharm Biol*, *44*(7), 534-539, doi: 10.1080/13880200600883080.

Karamenderes, C., Konyalioglu, S., Khan, S. & Khan, I. A. (2008). Inhibition of cholinesterase and amyloid aggregation by resveratrol oligomers from *Vitis amurensis*. *Phyther Res*, *22*, 544-549. https://doi.org/10.1002/ptr.2406

Köse, Y. B., İşcan, G., Göger, F., Akalın, G., Demirci, B. & Başer, K. H. C. (2016). Chemical Composition and Biological Activity of *Centaurea baseri*: New Species from Turkey. *Chem Biodivers*, *13*, 1369-1379. doi: 10.1002/CBDV.201600070.

Köse, Y. B., Ocak, A. & Erkara, I. P. (2010). *Centaurea cadmea subsp. pontica subsp. nov.* (Asteraceae) from northwest Anatolia, Turkey. *Nord J Bot*, *28*, 475-478. doi: 10.1111/j.1756-1051.2009.00585.x.

Masuda, T., Yamashita, D., Takeda, Y. & Yonemori, S. (2005). Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*. *Biosci Biotechnol Biochem*, *69*, 197-201. doi: 10.1271/bbb.69.197.

Meng X-Y, Zhang H-X, Mezei M, Cui M (2011) Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery. *Curr Comput Aided-Drug Des*. *7*(2), 146-157. doi: 10.2174/157340911795677602

Mohamed El-Korany S, Mohamed Helmy O, Mahmoud El-Halawany A, El-Mohammadi Ragab Y, Hafez Zedan H (2020) Kojic acid repurposing as a pancreatic lipase inhibitor and the optimization of its production from a local *Aspergillus oryzae* soil isolate. *BMC Biotechnol*, *20*, 1-16. doi: 10.1186/s12896-020-00644-9

Morris, G.M. & Dallakyan, S. (2013) AutoDock — AutoDock. 02-27.

Naeim, H., El-Hawiet, A., Abdel Rahman, R. A., Hussein, A., El Demellawy, M. A. & Embaby, A. M. (2020). Antibacterial activity of *Centaurea pumilio* L. Root and aerial part extracts against some multidrug resistant bacteria. *BMC Complement Med Ther*, *20*, 79. doi: 10.1186/s12906-020-2876-y.

Özbek, M.U. (2021). *Centaurea kirikkalensis* (Asteraceae), a new species from Turkey. *Nordic Journal of Botany*, *39*(7), 1-9. e03235, doi: 10.1111/njb.03235.

Pękal, A. & Pyrzynska, K. (2014). Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Anal Methods*, *7*, 1776–1782. doi: 10.1007/s12161-014-9814-x.

Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C. & Ferrin, T. E. (2004). UCSF Chimera - A visualization system for exploratory research and analysis. *J Comput Chem, 25*(13), 1605-1612 doi: 10.1002/jcc.20084

Reda, E. H., Abdel Shakour, Z. T., El-Halawany, A. M., El-Kashoury, E. S. A., Shams, K. A., Mohamed, T. A., Saleh, I., Elshamy, A. I., Atia, M. A. M., El-Beih, A. A., Abdel-Azim, N. S., El-Seedi, H. R. & Hegazy, M. E. F. (2021). Comparative study on the essential oils from five wild egyptian *Centaurea* species: Effective extraction techniques, antimicrobial activity and in-silico analyses. *Antibiotics*, *10*(3), 1-18. doi: 10.3390/antibiotics10030252

Shoeb, M., Jaspars, M., MacManus, S. M., Celik, S., Nahar, L., Kong-Thoo-Lin, P. & Sarker, S. D. (2007). Anti-colon cancer potential of phenolic compounds from the aerial parts of *Centaurea gigantea* (Asteraceae). *J Nat Med, 61*, 164-169. doi: 10.1007/s11418-006-0117-4

Singleton, V. L. & Rosi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am J Oenol Vitic, 16*, 144–158.

Tao, Y., Zhang, Y., Cheng, Y. & Wang, Y. (2013). Rapid screening and identification of α-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with

HPLC/MS and NMR. *Biomed Chromatogr*, *27*, 148-155. doi: 10.1002/bmc.2761.

Thring, T. S. A., Hili, P. & Naughton, D. P. (2009). Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complement Altern Med*, *9*, 1-11. doi: 10.1186/1472-6882-9-27.

Tiwana, G., Fua, J., Lu, L., Cheesman, M. J. & Cock, I. E. (2021). A review of the traditional uses, medicinal properties and phytochemistry of *Centaurea benedicta* l. *Pharmacogn J*, *13*, 798-812. doi: 10.5530/pj.2021.13.102.

Troot, O. & Olson, A. (2010). AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J Comput Chem, 31*, 455–461.

Tugay, O., Paşayeva, L., Demirpolat, E. & Şah,n, M. (2021). Comparative Evaluation of Cytotoxicity and Phytochemical Composition of *Centaurea iconiensis* (*Rhaponticoides iconiensis*). *Iran J Sci Technol Trans Sci, 45*, 65-7. https://doi.org/10.1007/s40995-020-01030-y.

Yaman, B., Kaya, Z., Tunçkol, B. & Özel, H. (2020). The endemic plants in Bartin, Turkey and their conservation status. *Biol Nyssana*, *11*, 23-29. doi: 10.5281/zenodo.4060285.

Yang, X. W., Huang, M. Z., Jin, Y. S., Sun, L. N., Song, Y. & Chen, H. S. (2012.) Phenolics from *Bidens bipinnata* and their amylase inhibitory properties. *Fitoterapia*, *83*, 1169-1175. doi: 10.1016/ j.fitote.2012.07.005.

Yin, B., Fang, D. M., Zhou, X.L. & Gao, F. (2019). Natural products as important tyrosine kinase inhibitors. *Eur J Med Chem, 182*, 111664. doi: 10.1016/j.ejmech.2019.111664.

Zengin, G., Locatelli, M., Carradori, S., Mocan, A. M. & Aktumsek, A. (2016). Total phenolics, flavonoids, condensed tannins content of eight *Centaurea* species and their broad inhibitory activities against cholinesterase, tyrosinase, α-amylase and α-glucosidase. *Not Bot Horti Agrobot Cluj-Napoca, 44*, 195–200. doi: 10.15835/nbha44110259.

Zengin, G., Zheleva-Dimitrova, D., Gevrenova, R., Nedialkov, P., Mocan, A., Ćirić, A., Glamočlija, J., Soković, M., Aktumsek, A. & Mahomoodally, M. F. (2018). Identification of phenolic components via LC–MS analysis and biological activities of two *Centaurea* species: *C. drabifolia subsp. drabifolia* and *C. lycopifolia*. *J Pharm Biomed Anal, 149*, 436-441. doi: 10.1016/j.jpba.2017.11.045.