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RESEARCH ARTICLE

Phytochemical Analysis and Screening of Acetylcholinesterase and Carbonic Anhydrase I and II Isoenzymes Inhibitory Effect of *Heptaptera triquetra* (Vent.) Tutin Root

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SUMMARY

Alzheimer's disease (AD) is characterized by progressive memory loss, deterioration of other cognitive functions, and inability to perform activities of daily living. Inhibiting the acetylcholinesterase (AChE) enzyme causes Ach accumulation in cholinergic synapses. This situation is expected to increase cognitive functions. Carbonic anhydrase enzymes (CAs) are ubiquitous in all living organisms. They have crucial physiological and pathological roles. CA inhibitors (CAIs) bind to catalytic zinc ions in the active site of CA isoenzymes and block their activity. The clinical use of CAIs had been established as antiglaucoma, anticonvulsant agents, diuretics, and anti-obesity drugs, in managing mountain sickness, gastric and duodenal ulcers, neurological disorders, osteoporosis, and tumors. To evaluate the bioactive profile of Heptaptera triquetra root, isolation studies, AChE, and human carbonic anhydrase (hCA) I and II inhibitory activities were performed. According to isolation studies, one fatty acid, coniferyl palmitate (1); four sesquiterpene coumarins, umbelliprenin (2), badrakemin acetate (4), colladonin (5), karatavicinol (6); and two sterols, stigmasterol (3a), β-sitosterol (3b) were isolated. All isolated compounds showed high potency against all enzymes (except badrakemin acetate for AChE) compared to standards. Umbelliprenin (2) with an IC50 value of 31.500 nM against hCA I, colladonin (5) with an IC50 value of 36.473 nM against hCA II and stigmasterol (3a), and β-sitosterol (3b) mixture with an IC50 value 9.000 nM against AChE demonstrated the best activity.

Key Words: *Heptaptera triquetra, Apiaceae, enzyme inhibition, acetylcholinesterase, carbonic anhydrase, isolation*

Heptaptera triquetra (Vent.) Tutin Root'un Asetilkolinesteraz ve Karbonik Anhidraz I-II Enzimlerini İnhibe Edici Etkilerinin Taranması ve Fitokimyasal Analizi

ÖZ

Alzheimer hastalığı (AH), ilerleyen hafıza kaybı, diğer bilişsel işlevlerde bozulma ve günlük yaşam aktivitelerini yerine getirememe ile karakterizedir. Asetilkolinesteraz (AChE) enziminin inhibe edilmesi, kolinerjik sinapslarda Ach birikimine neden olur. Bu durumun bilişsel işlevleri artırması beklenir. Karbonik anhidraz enzimleri (CA'lar) tüm canlı organizmalarda bulunur. Çok önemli fizyolojik ve patolojik rolleri vardır. CA inhibitörleri, CA izoenzimlerinin aktif bölgesindeki katalitik çinko iyonuna bağlanır ve etkilerini inhibe eder. CA I'lerin klinik kullanımı, dağ hastalığı, mide ve duodenum ülserleri, nörolojik bozukluklar, osteoporoz ve tümörlerin tedavisinde, antiglokom, antikonvülsan, diüretik ve antiobezite ilaçları olarak belirlenmiştir. Heptaptera triquetra kökünden hazırlanan diklorometan ekstresinin biyoaktif profilini değerlendirmek için izolasyon çalışmaları, AChE, insan karbonik anhidraz (hCA) I ve II inhibitör aktivite tayinleri yapılmıştır. İzolasyon çalışmalarına göre, bir yağ asidi, koniferil palmitat (1); dört seskiterpen kumarin, umbelliprenin (2), badrakemin asetat (4), kolladonin (5), karatavisinol (6); ve iki sterol, stigmasterol (3a), β-sitosterol (3b) izole edilmiştir. İzole edilen tüm bileşikler, standartlarla karşılaştırıldığında (AChE için badrakemin asetat hariç) tüm enzimlere karşı yüksek etki göstermiştir. hCA I'e karşı 31.500 nM IC50 değerine sahip umbelliprenin (2), hCA II'ye karşı IC50 değeri 36.473 nM olan kolladonin (5) ve AChE'ye karşı IC50 değeri 9.000 nM olan stigmasterol (3a) ve β-sitosterol (3b) karışımı en iyi aktiviteyi göstermiştir.

Anahtar Kelimeler: *Heptaptera triquetra, Apiaceae, enzim inhibisyonu, asetilkolinesteraz, karbonik anhidraz, izolasyon*

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INTRODUCTION

The genus *Heptaptera*, which belongs to the Apiaceae family, is represented by ten species in the world and is naturally grown from Europe to the Middle East, including Italy, the Balkans, Turkey, Syria, and Palestine (IPNI, 2022). It is represented by four species in Turkey, named *H. anisoptera, H. anatolica, H. cilicica*, and *H. triquetra*, one of which (*H. cilicica*) is endemic (Güner et al., 2012). *Heptaptera* species are rich in sesquiterpene coumarins (Appendino et al., 1992a; 1992b; 1993). In previous studies it has been reported that several sterols and coumarins have exhibited potent inhibitory activity against acetylcholinesterase (AChE) and human carbonic anhydrase (hCA) enzymes (Aydın et al., 2019; Pereira et al., 2016; Sepheri et al., 2020; Supuran, 2020; Şenol et al., 2010).

Alzheimer's disease (AD) is a wide spread dementia disease that occurs due to the decrease of neurotransmitters in the brain, and the neurotransmitter that shows the most reduction in this condition is acetylcholine. AD has no definitive cure. Current treatments are aimed at eliminating the symptoms of the disease. For this purpose, AChE inhibitors such as tacrine, donepezil, and rivastigmine are used. Since these drugs cause side effects such as hepatotoxicity and gastrointestinal disorders, safe, effective, and especially natural AChE inhibitors have gained more importance recently (Göçer et al., 2013).

Carbonic anhydrase (CA) is a pH-regulating enzyme in all living elements. This enzyme is found in many living systems (Küçük and Gulcin, 2016). It plays a role in various pathologic and physiological effects, including neurological disorders, fluid balance, pH regulation, bone resorption, carboxylation reactions, glaucoma, calcification, osteoporosis, cancer, and tumor production (Gulcin and Beydemir, 2013; Nar et al., 2013). CA plays a critical role in long-term synaptic transformation and is related tomental retardation, AD. There is evidence that CAII is increased in the AD brain (Jang et al., 2010). hCA isoenzymes are critical therapeutic targets. Its inhibitors and activators are currently used as drugs. hCA I can be used as an indicator to differentiate autoimmune hemolytic anemia from other types of anemia (Akıncıoğlu et al., 2014; Çoban et al., 2009). hCA II, implicated as a biomarker of stromal tumors, is required to maintain ion transport in erythrocytes and its deficiency syndrome causes renal tubular acidosis and osteoporosis, a marble brain disease or Guibaud-Vainsel syndrome. hCAs are inhibited by two groups of compounds. The first group is metal complex-forming anions, and the second group is sulfonamides and their isosteres. The most potent organic inhibitors are aromatic and heteroaromatic sulfonamides ($R\text{-}SO_2\text{-}NH_2$). Acetazolamide is used to treat glaucoma, altitude sickness, and benign intracranial hypertension (Akbaba et al., 2014; Gökçen et al., 2017).

According to a clinical study, carbonic anhydrase-II levels in plasma and brain hippocampus of Alzheimer's patients increased 1.24 times compared to healthy individuals. It has been shown that there is a definite relationship between plasma carbonic anhydrase-II levels and AD (Jang et al., 2010). It is known that there are many factors in the pathophysiology of AD (Öztürk-Karan, 2009). Therefore, it is thought that new treatment targets will develop through multiple approaches (Akdağ et al., 2019). *Heptaptera triquetra* (Vent.) Tutin is known as the "Üçgen Çakşır" in Turkey (Güner et al., 2012). The plant is erect, perennial, and 65-120 cm tall (Davis et al., 1988). It is naturally grown in Bulgaria, and Turkey-in-Europe (POWO, 2022). According to previous studies, *H. triquetra* exhibited high antioxidant activity (Şenol et al., 2010) and contained coumarins (Simova et al., 1986). In our earlier study, coumarin derivatives isolated from the root of *Heptaptera* cilicica displayed potent activity against AChE (Özbek et al., 2018). Additionally, coumarins have intense AChE and CA enzymes inhibitory activity (Karakaya et al., 2020). In light of this information, we aimed to investigate the possible inhibition effects of *H. triquetra* root extract and its isolated compounds against AChE, hCA I and II

enzymes linked to AD, known as a prevalent disease.

MATERIALS AND METHODS

General Experimental Procedure

1D (^{1}H-NMR, ^{13}C-NMR, and DEPT) and 2D (COSY, HMQC, and HMBC) NMR spectra were derived using Varian Mercury Plus (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometers, with TMS as an internal standard. These instruments were in the Faculty of Science at Ataturk University. HRESIMS data were recorded using an Agilent 6530 Accurate-Mass apparatus and AB Sciex TripleTOF 4600 (Agilent Technologies Inc., California, USA) at the Ataturk University East Anatolia High Technology Application and Research Center (DAYTAM). Open column chromatography (CC) was carried out using silica gel 60 (0.063–0.2 mm) (Merck, Germany), and solvents were purchased from Sigma-Aldrich (USA). TLC was performed using precoated silica gel 60 F_{254} plates (Merck), and the spots were visualized by spraying with 1% vanillin solution in concentrated sulfuric acid, followed by heating at 110 °C. All commercially available reagents were purchased from Sigma-Aldrich (USA) were used for bioactivity assays.

Plant Material

The roots of *Heptaptera triquetra* were collected in Tekirdağ City, Turkey (A1, 12 km from Saray, right side of the road, under oak forest, 202 m). The plant material was authenticated by Assoc. Prof. Gülderen Yılmaz. A voucher specimen (No. AEF 23723) was deposited at Ankara University Faculty of Pharmacy Herbarium (AEF), Ankara, Turkey.

Extraction and Isolation

H. triquetra roots (463.30 g) were dried in the open air in a shaded location, powdered, and extracted in 1250 mL of dichloromethane for -three h at 40 °C using a mantle heater and reflux cooler. The filtered extracts were concentrated to dryness in a rotary evaporator at 40 °C and 120 rpm. The dichloromethane extract (16.66 g of total extract) was fractionated using silica gel CC (70–230 mesh, 75 g) with *n*-hexane:ethyl acetate (EtOAc) (100:0→0:100, v/v) to yield two fractions, *Fr. 1* and *Fr. 2*. *Fr. 1* (7.95 g) was further fractionated using silica gel CC with *n*-hexane:EtOAc (100:0→55:45, v/v) to obtain subfractions *Frs. 1.1–12*. Compound **1** (39 mg) was precipitated from *Fr. 1.4* (129.6 mg). Compound **2** (332 mg) was precipitated from *Fr. 1.6* (612 mg). Compound **3** (24 mg) was precipitated from *Fr. 1.7* (395 mg). *Fr. 1.11* (892.7 mg) was purified using a Sephadex LH-20 CC (50 g) with CH_2Cl_2:MeOH (25:75, v/v) to yield compound **4** (38.9 mg). *Fr. 2* (6.23 g) was further fractionated using silica gel CC with *n*-hexane:EtOAc (100:0→0:100, v/v) to obtain subfractions *Frs. 2.1–8*. Compound **5** (539 mg) was precipitated from *Fr. 2.4* (1.16 g). *Fr. 2.6* (448 mg) was purified using a Sephadex LH-20 CC (50 g) with CH_2Cl_2:MeOH (25:75, v/v) to obtain *Fr. 2.6.1*. *Fr. 2.6.1* (302.7 mg) was purified using a Sephadex LH-20 CC (50 g) with MeOH to obtain *Fr. 2.6.1.1*. *Fr. 2.6.1.1* (138.1 mg) was again purified using a Sephadex LH-20 CC (50 g) with water (H_2O):MeOH (10:10→0:20, v/v) to obtain *Fr. 2.6.1.1.1*. Compound **6** (13.8 mg) was precipitated from *Fr. 2.6.1.1.1* (71.6 mg).

Carbonic Anhydrase Activity Assay

Fresh human blood erythrocytes for carbonic anhydrase I and II isoenzymes were purified using the Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography technique (Gocer et al., 2016). CA activities of the isoenzymes were determined by spectrophotometric measurements at 348 nm (Verpoorte et al., 1967). Acetazolamide was used as positive control (Burmaoğlu et al., 2019; Hisar et al., 2005).

Anticholinergic Assay

The AChE inhibitory effect of dichloromethane extract and isolated compounds were determined by Ellman's method (1961) as given in previous studies (Eruygur et al., 2019; Polat Köse et al., 2015). AChE was commercially purchased and obtained from electric eel (*Electrophorus electricus*). 5,5′-Dithiobis(2-nitrobenzoic acid) and acetylthiocholine iodide (AChI) were used as a substrate for the cholinergic reaction. Tacrine was used as the positive control (Erdemir et

al., 2019; Lolak et al., 2020).

RESULTS AND DISCUSSION

The dichloromethane extract of the roots of *H. triquetra* afforded one fatty acid, coniferyl palmitate **(1)** (Lee et al., 2004); four sesquiterpene coumarins, umbelliprenin **(2)** (Appendino et al., 1994), badrakemin acetate **(4)** (Eshbakova et al., 2009), colladonin **(5)** (Appendino et al., 1992a), karatavicinol **(6)** (Ahmed, 1999); and two sterols, stigmasterol **(3a)** (Woldeyes et al., 2012), β-sitosterol **(3b)** (Güvenalp et al., 2009).

Coniferyl palmitate, umbelliprenin, badrakemin acetate, karatavicinol, stigmasterol, and β-sitosterol were firstly isolated from this species. Coniferyl palmitate, stigmasterol, and β-sitosterol were not reported in this genus before. Colladonin was previously reported in *H. triquetra* (syn.: *Colladonia triquetra*) (Simova et al., 1986). Chemical structures of all isolated compounds from *H. triquetra* are given in Figure 1, and NMR data arepresentedin Tables 1-3. The HRESIMS, 1D-, and 2D-NMR spectra of the compounds are available in Supporting Information.

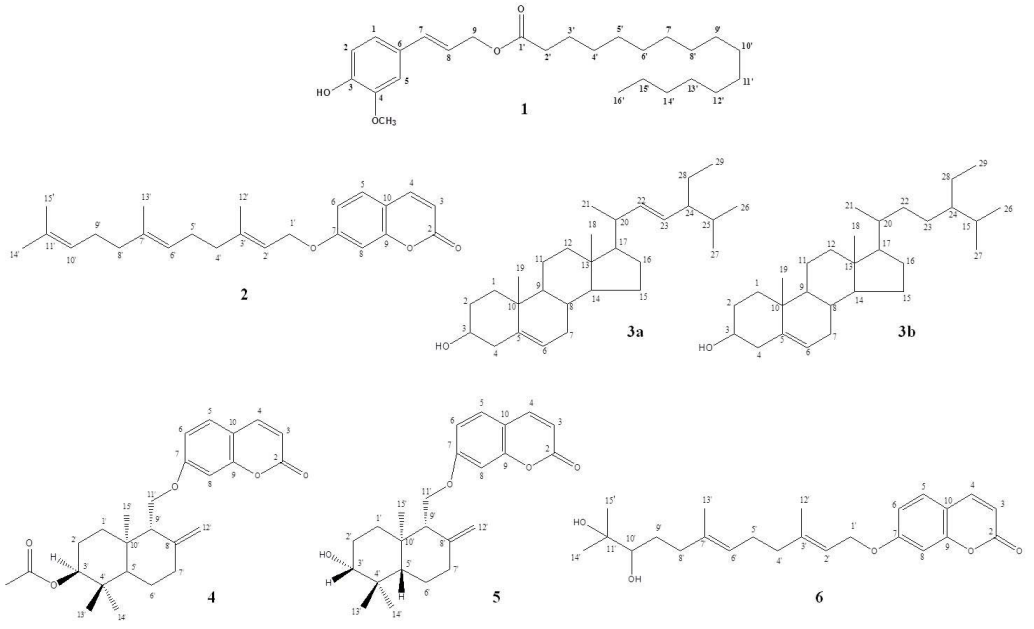

Figure 1. Chemical structures of isolated compounds (**1**-**6**)

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of coniferyl palmitate **(1)** in $CDCl_3$ (δ in ppm, *J* in Hz).

Position	Coniferyl palmitate (1)	
	δ_H	δ_C
1	6.92 (*d,* 2.3)	108.32
2	6.88 (*dd*, 8.0/2.3)	114.44
3		145.87
4		146.62
5	6.87 (*d*, 8.0)	120.63
6		128.81
7	6.56 (*d*, 15.8)	134.37
8	6.19 (*dt*, 15.8/6.6)	120.91
9	4.72 (*dd*, 6.56/0.8)	65.14
1'		173.83
2'	2.35 (*t*, 7.5)	34.39
3'	1.65 (*m*)	24.99
4'	1.22-1.36 (*m*)	29.18-29.71
5'	1.22-1.36 (*m*)	29.18-29.71
6'	1.22-1.36 (*m*)	29.18-29.71
7'	1.22-1.36 (*m*)	29.18-29.71
8'	1.22-1.36 (*m*)	29.18-29.71
9'	1.22-1.36 (*m*)	29.18-29.71
10'	1.22-1.36 (*m*)	29.18-29.71
11'	1.22-1.36 (*m*)	29.18-29.71
12'	1.22-1.36 (*m*)	29.18-29.71
13'	1.22-1.36 (*m*)	29.18-29.71
14'	1.22-1.36 (*m*)	31.94
15'	1.22-1.36 (*m*)	22.71
16'	0.90 (*t*, 6.6)	14.15
O-CH_3	3.91 (*s*)	55.88

Table 2. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of sesquiterpene coumarins in $CDCl_3$ (δ in ppm, *J* in Hz).

Position	Umbelliprenin (2)		Badrakemin acetate (4)		Colladonin (5)		Karatavicinol (6)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		161.32		161.24		161.26		161.39
3	6.24 (*d*, 9.5)	112.94	6.23 (*d*, 9.5)	113.01	6.23 (*d*, 9.5)	112.98	6.25 (*d*, 9.4)	112.95
4	7.63 (*d*, 9.5)	143.47	7.63 (*d*, 9.5)	143.46	7.63 (*d*, 9.5)	143.46	7.64 (*d*, 9.4)	143.52
5	7.36 (*d*, 8.5)	128.69	7.37 (*d*, 8.2)	128.77	7.36 (*d*, 8.9)	128.76	7.37 (*d*, 8.5)	128.71
6	6.85 (*dd*, 8.5/2.4)	113.22	6.83 (*dd*, 8.5/2.3)	113.14	6.83 (*dd*, 8.9/2.4)	113.10	6.85 (*dd*, 8.5/2.3)	113.36
7		162.16		162.13		162.22		162.12
8	6.82 (*d*, 2.4)	101.58	6.80 (*gs*)	101.27	6.82 (*gs*)	101.33	6.82 (*d*, 2.3)	101.55
9		155.87		155.90		155.90		155.86
10		112.42		112.48		112.47		112.46
1'	4.60 (*d*, 6.6)	65.48	1.53 (*dd*, 12.8/3.3) 1.78 (*m*)	36.83	1.45 (*m*) 1.79 (*m*)	37.19	4.61 (*d*, 6.4)	65.55
2'	5.47 (*td*, 6.6/1.1)	118.43	1.63 (*ddd*, 25/13.1/3.3) 1.76 (*m*)	24.10	1.65 (*ddd*, 24.8/12.9/3.3) 1.77 (*m*)	27.68	5.46 (*t*, 6.4)	118.69
3'		142.38	4.54 (*dd*, 11.50/4.2)	80.33	3.30 (*dd*, 11.6/4.3)	78.50		142.00
4'	2.12 (*m*)	39.52		38.05		38.81	2.15 (*m*) 2.11 (*m*)	39.38
5'	2.14 (*m*)	26.13	1.26 (*dd*, 12.4/2.5)	54.38	1.15 (*dd*, 12.4/2.6)	54.30	2.14 (*m*) 2.17 (*m*)	25.97
6'	5.09 (*m*)	123.49	1.43 (*ddd* 25.8/12.9/4.2) 1.74 (*m*)	23.27	1.47 (*m*) 1.75 (*m*)	23.45	5.18 (*t*, 6.4)	124.28
7'		135.58	2.11 (*dt* 4.2/2.1) 2.45 (*dq*, 13.1/2.2)	37.28	2.10 (*td*, 13.02/4.56) 2.46 (*ddd*, 13.1/4.1/2.3)	37.42		135.48
8'	1.97 (*m*)	39.67		146.11		146.28	2.29 (*m*) 2.06 (*m*)	36.75
9'	2.04 (*m*)	26.69	2.23 (*gt*, 5.24)	54.65	2.21 (*dd*, 6.3/4.9)	54.76	1.61 (*m*) 1.40 (*m*)	29.65
10'	5.08 (*m*)	124.30		38.66		39.18	3.35 (*dd*, 9.2/1.2)	78.13
11'		131.33	4.17 (*dd*, 9.7/6.7) 4.20 (*dd*, 9.7/4.2)	65.64	4.15 (*dd*, 9.6/6.8) 4.21 (*dd*, 9.6/4.2)	65.67		72.98
12'	1.77 (*s*)	16.78	4.91 (*s*) 4.52 (*gs*)	107.97	4.54 (*s*) 4.92 (*s*)	107.82	1.76 (*s*)	16.70
13'	1.60 (*s*)	16.04	0.90 (*s*)	28.29	1.03 (*s*)	28.34	1.62 (*s*)	15.91
14'	1.68 (*s*)	25.71	0.88 (*s*)	16.65	0.81 (*s*)	15.53	1.20 (*s*)	26.41
15'	1.59 (*s*)	17.69	0.86 (*s*)	15.39	0.85 (*s*)	15.35	1.16 (*s*)	23.29
C=O				170.97				
OCH_3			2.06 (*s*)	21.32				

Table 3. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of sterols in $CDCl_3$ (δ in ppm, *J* in Hz).

Position	Stigmasterol (3a)		*β*-sitosterol (3b)	
	δ_H	δ_C	δ_H	δ_C
1		37.26		37.26
2		26.07		31.66
3	3.55 *(m)*	71.81	3.55 (*m*)	71.81
4		42.30		42.30
5		140.76		140.76
6	5.36 (*gd,* 5.1)	121.72	5.36 (*gd*, 5.1)	121.72
7		31.66		31.90
8		31.91		31.90
9		50.15		50.13
10		36.51		36.51
11		24.31		21.22
12		39.68		39.78
13		42.32		42.22
14		56.77		56.87
15		25.42		24.31
16		29.83		28.25
17		55.95		56.06
18	0.70 (*s*)	12.26	0.71 (*s*)	12.05
19	1.01 (*s*)	19.40	1.01 (*s*)	19.40
20		40.51		36.15
21	1.03 (*s*)	21.09	0.94 (*d*, 6.6)	19.04
22	5.17 (*dd*, 15.1/8.6)	138.33		33.94
23	5.03 (*dd*, 15.1/8.6)	129.28		26.07
24		51.24		45.83
25		31.90		29.15
26	0.82 (*d*, 7.1)	18.99	0.82 (*d*, 7.1)	19.83
27	0.81 (*d*, 7.0)	19.04	0.81 (*d*, 7.0)	19.40
28		28.25		23.07
29	0.87 *(signal overlap)*	11.97	0.85 *(signal overlap)*	11.99

The dichloromethane extract and isolated compounds were evaluated to determine their human carbonic anhydrase I and II (hCA I and II), and acetylcholinesterase (AChE) enzyme inhibitory activities. Acetazolamide and tacrine were used as the positive controls, respectively (Gülçin et al., 2016). The dichloromethane extract exhibited activity with a 20.382 ng/µL IC_{50} value against hCA I, 21.656 ng/µL IC_{50} value against hCA II, and 5.634 ng/µL IC_{50} value against AChE.

Table 4. The inhibition effects of isolated compounds on human carbonic anhydrase I and II isoforms (hCA I and II) and acetylcholinesterase (AChE) enzyme.

Samples	IC_{50} (nM)						K_i (nM)		
	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
Coniferyl palmitate (1)	53.307	0.9606	40.500	0.9923	10.191	0.9901	59.412±10.86	117.343±19.41	4.538±0.59
Umbelliprenin (2)	31.500	0.9707	38.500	0.9832	11.550	0.9817	82.647±4.26	87.427±13.60	5.918±1.49
Stigmasterol and β-Sitosterol (3)	63.000	0.9734	59.300	0.9709	9.000	0.9770	83.183±8.87	74.560±8.88	3.806±0.27
Badrakemin acetate (4)	40.764	0.9634	69.300	0.9983	19.250	0.9840	32.963±4.49	93.333±11.06	6.631±1.11
Colladonin (5)	43.312	0.9629	36.473	0.9872	10.360	0.9840	60.560±10.61	38.571±5.72	2.771±0.95
Karatavicinol (6)	63.000	0.9836	53.412	0.9610	13.326	0.9847	65.155±9.86	49.026±6.37	3.156±0.92
Acetazolamide*	99.000	0.9959	87.954	0.9909	-	-	82.4079±10.45	159.597±9.05	-
Tacrine**	-	-	-	-	15.0652	0.9766	-	-	3.456±0.29

*Acetazolamide is a standard inhibitor of hCA I and II.

**Tacrine is a standard inhibitor for AChE.

For the hCA I isoenzyme, IC_{50} values were found as 53.307 nM for coniferyl palmitate, 31.500 nM for umbelliprenin, 63.000 nM for stigmasterol and β-sitosterol mixture, 40.764 nM for badrakemin acetate, 43.312 nM for colladonin, and 63.000 nM for karatavicinol. For the hCA II isoenzyme, IC_{50} values were found as 40.500 nM for coniferyl palmitate, 38.500 nM for umbelliprenin, 59.300 nM for stigmasterol and β-sitosterol mixture, 69.300 nM for badrakemin acetate, 36.473 nM for colladonin, and 53.412 nM for karatavicinol. The IC_{50} values of acetazolamide were determined as 99.000 nM against hCA I and 87.954 nM against hCA II. Regarding the K_i values of the isolated compounds (**1**-**7**) and the positive control (Table 4), remarkable activities against hCA I and hCA II in the ranges of 33.0-83.2 nM and 38.6-117.3 nM, respectively, were obtained.

The inhibitory effect of *H. triquetra* root against hCA I and II is reported here for the first time.As well as it is the first study for the *Heptaptera* genus. Coumarins are among the most isoform-selective CA inhibitors. They undergo hydrolysis of the lactone ring mediated by the esterase activity of CA. The resulting 2-hydroxy-cinnamic acids then bind to a particularpart of the enzyme's active site (Supuran, 2020). In a previous study, umbelliprenin was found to be apotent compound with a Ki value against CA XII of 5.8 nM when compared with acetazolamide and demonstrated high selectivity forthe CA I/II isoforms as in our study (Fois et al., 2020). According to our knowledge, it is the first study to evaluate hCA I and II enzymes inhibitory activity of other sesquiterpene coumarins such as badrakemin acetate, colladonin, and karatavicinol. As well as it is the first evaluation for coniferyl palmitate. In previous studies, β-Sitosterol has not inhibitedhCA I and II (Aydın, 2020; Saleem et al., 2019), while stigmasterol has displayed potent activity (Aydın et al., 2019). It shows that the activity is due to stigmasterol.

IC_{50} values of AChE were 10.191 nM for coniferyl palmitate, 11.550 nM for umbelliprenin, 9.000 nM for stigmasterol and β-sitosterol mixture, 19.250 nM for badrakemin acetate, 10.360 nM for colladonin, and 13.326 nM for karatavicinol. The IC_{50} value of tacrine was determined as 15.0652 nM against AChE (Table 4). In a study, the methanolic and ethyl acetate extracts prepared from *H. triquetra* aerial part and root were evaluated against AChE, and none of them showed inhibitory activity when compared with galantamine (98.88%) (Şenol et al., 2010). In our study, the isolated compounds from dichloromethane extract were effective against AChE. It shows that this effect is due to nonpolar compounds. In our previous study, umbelliprenin exhibited significantly high in-

hibitory potency against AChE (IC_{50} = 5.86 μM) as in our current study (Guvenalp et al., 2017). In another study, umbelliprenin, and colladonin were isolated from the roots of *Ferulago campestris* and showed moderate AChE inhibitory activity (Dall'Acqua et al., 2010). This is the first report on the AChE inhibitory activity of coniferyl palmitate. Additionally, as in our study, *β*-sitosterol isolated from acetone extract of the roots of *Salvia syriaca* exhibited high AChE activity with a 34.3 μg/mL IC_{50} value (Bahadori et al., 2016).

The evaluation of the bioactivity and determining the phytochemical content of *Heptaptera triquetra* had great importance. The dichloromethane extract of the root and isolated compounds were evaluated for their bioactivities on some metabolic enzyme's inhibitory properties related to several global diseases. The results indicated that all the isolated compounds were effective against hCA I, hCA II, and AChE. The main compounds, sesquiterpene coumarins, stigmasterol and *β*-sitosterol, were found as responsible for the inhibitory activities. Current studies show that enzyme inhibition is becoming a key target in the treatment or management of many common and global diseases. (Bayrak et al., 2019; Biçer et al., 2019). These findings suggest the potential of *H. triquetra* and its compounds as novel therapeutic candidates and herbal medicines for the treatment of glaucoma and AD.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

AUTHOR CONTRIBUTION STATEMENT

AÇK, HÖ, ZG: developing hypothesis; AÇK, HÖ, ZB, GY, CK: experimenting; AÇK, HY: preparing the study text; ZG, İG: reviewing the text; AÇK, HÖ, ZG, HY: analysis and interpretation of the data; AÇK, HÖ, HY, ZG: literature research.

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