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Research Article

In vitro Carbonic Anhydrase Inhibitory Effects of the Extracts of *Satureja cuneifolia*

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Abstract

Carbonic anhydrases catalyze the interconversion between carbon dioxide and bicarbonate. Carbonic anhydrase inhibition has therapeutic importance and there are many drugs which use this mechanism. Carbonic anhydrase inhibitors are used as diuretic, antiglaucoma, antitumor and antiepileptic agents. As an alternative for synthetic chemicals with strong side effects, natural products have gained popularity in the recent years. In this study, *Satureja cuneifolia*, a medicinal plant used as folk medicine, was investigated for its inhibition effects on carbonic anhydrase I and II enzymes. Four different extracts were obtained with maceration method and three different solvents were used. The results have shown that methanol extracts of *Satureja cuneifolia* has the strongest inhibition activity on the enzymes with the IC_{50} values of 31 µg/mL for hCA I and 12 µg/mL for hCA II. Further purification and analytical studies will be needed to obtain the active natural molecules and their potential for pharma and food industries.

Key words: Carbonic anhydrase, Inhibition, Extract*, Satureja cuneifolia.*

Satureja cuneifolia Ekstraktlarının *in vitro* Karbonik Anhidraz İnhibitör Etkileri

Öz

Karbonik anhidrazlar, karbon dioksit ve bikarbonat arasındaki karşılıklı dönüşümü katalize eder. Karbonik anhidraz inhibisyonunun terapötik önemi vardır ve bu mekanizmayı kullanan birçok ilaç vardır. Karbonik anhidraz inhibitörleri diüretik, antiglokom, antitümör ve antiepileptik ajanlar olarak kullanılmaktadır. Güçlü yan etkileri olan sentetik kimyasallara alternatif olarak son yıllarda doğal ürünler popülerlik kazanmıştır. Bu çalışmada, halk ilacı olarak kullanılan tıbbi bir bitki olan *Satureja cuneifolia*'nın karbonik anhidraz I ve II enzimleri üzerindeki inhibisyon etkileri araştırılmıştır. Maserasyon yöntemi ile dört farklı ekstrakt elde edilmiş ve üç farklı çözücü kullanılmıştır. Sonuçlar, *Satureja cuneifolia*'nın metanol ekstrelerinin, hCA I için 31 µg/mL ve hCA II için 12 µg/mL IC_{50} değerleri ile enzimler üzerinde en güçlü inhibisyon aktivitesine sahip olduğunu göstermiştir. Aktif doğal moleküllerin elde edilebilmesi ve bunların ilaç ve gıda endüstrileri için potansiyellerini değerlendirmek için daha fazla saflaştırma ve analitik çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Karbonik anhidraz, İnhibisyon, Ekstrakt, *Satureja cuneifolia*.

Introduction

Carbonic anhydrases (CAs; EC 4.2.1.1) are widespread metalloenzymes catalyzing carbon dioxide hydration to bicarbonate and protons (Supuran, 2008). The enzymes are expressed in organisms by eight gene families (α, β, γ, δ, ζ, η, ɵ and ι) (Güler et al., 2020). α-gene family secretes 16 CA isoforms with different locations (Nar et al., 2013; Orhan et al., 2016), molecular characteristics, kinetics to ligands and organization (Nocentini et al., 2016). hCA I, II, III, VII and XIII

isoenzymes are cytosolic isoforms, hCA IV, IX, XII, XIV and XV are membrane-bound forms, hCA VA and VB are present in mitochondria, hCA VI is secretory form (Almajan et al., 2008) and hCA VIII, X and XI are non-catalytic forms of the enzyme (Abdel-Aziz, et. al., 2014).

Activation and/or inhibition of carbonic anhydrases have provided unique approaches in the treatment/prevention of many diseases (Scozzafava et al. 2004). For instance, carbonic anhydrase inhibitors (CAIs) have been utilized as potential pharmaceuticals in Alzheimer, glaucoma, epilepsy, cancer, osteoporosis and prevention of some infectious diseases (Supuran, 2008). Several synthetic compounds have been used as CAIs up to date (Türkoğlu et al. 2017; Ucar et al., 2021). Production procedures of these compounds are complex, and these molecules may show possible side effects (Akkemik et al., 2019). However, natural products have been important candidates in pharmaceutical industry and acted as vital position for the discovery of novel drugs. These kinds of products may possess the ability for binding to specific protein-based biomolecules whose functions are crucial in the treatment of many diseases (Türkoğlu et al., 2019).

Satureja L. is a genus that belongs to Lamiaceae family (Oke et al., 2009), the tribe Mentheae within sub-family Nepetoideae. The genus includes over thirty species. Five of them are endemic, 15 different species of *Satureja* L. are located in Turkey (Kan and Uçan, 2006). Various members of the genus Satureja have medicinal and aromatic properties (Eminagaoglu et al., 2007). *Satureja* L. can be found in different areas of the world such as Mediterranean Region, North Africa, North Asia, South America, and Canary Islands (Taslimi et al., 2020).

S. cuneifolia is an aromatic plant which is found in Mediterranean area of Turkey. It has characteristic taste and it is used as spice and tea (Milos et al., 2001). The arial parts of the plant are used as traditional medicine. The tea prepared from *S. cuneifolia* is used for the treatment of infection, muscle pain, cramp, indigestion, diarrhea and nausea in folk medicine (Eminagaoglu et al., 2007).

In the view of therapeutic and medicinal potentials of CAs and natural products as CAIs, we aimed to determine the esterase activity of different extracts of *Satureja cuneifolia* on hCA I and II isoenzymes.

Materials and Methods

Plant material

Satureja cuneifolia was identified by Dr. İsmail Şenkardeş from Pharmaceutical Botany Department of Marmara University and deposited at the Herbarium of Marmara University with the number of 18815 for future reference.

Chemicals and instruments

Human carbonic anhydrase I and II isoenzymes, 4-nitrophenylacetate as substrate and trizma base were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Dimethyl sulfoxide was commercially supplied from Isolab (Turkey). Water for experimental purposes and buffer preparations was obtained Direct Q®3 UV water purification system (Millipore Corp., MA, USA). Ohaus PA224C (Ohaus Corp., USA) with the readability up to 0.1 mg was used to weigh all extracts and chemicals. Wtw - Inolab pH 730 pH-meter (WTW GmbH, Weilheim, Germany) was performed to arrange the pH values. For the mixing and stirring experimental processes, IKA RT10 magnetic stirrer (IKA-Werke GmbH & Co KG, Germany) and ZX3 Advanced Vortex Mixer (Velp Scientifica, Usmate, Italy) were operated. Bioactivities of plant extracts on the enzymes were performed by UV-1800 Spectrophotometer (Shimadzu, Kyoto, Japan) with the resolution of 1 nm.

Preparation of the extracts

As detailed in the previous study of one of the authors, the aerial parts of *S. cuneifolia* were dried at room temperature in the shade and then powdered. These powdered parts (50 g) were macerated with petroleum ether (SFPE), chloroform (SFC), methanol (SFM), respectively. In addition to this extraction process, another powdered parts of *S. cuneifolia* (20 g) were extracted directly with methanol and with the use of maceration method. This process was continued for the 24-hour cycles until colorless solution was obtained. Then filtration was performed for the extracts and these extracts were evaporated by rotary evaporator (Heidolph, Germany). The extracts were held in +4°C until the experimental use (Taşkın et al., 2020).

Esterase activity assay of human carbonic anhydrase I and II

The method described by Verpoorte et al. (1967) was used for the esterase activity assay hCA I and hCA II. Spectrophotometry-based detection in the conditions of 348 nm for 3 min. at 25°C was assayed in the absorbance changes of 4-nitrophenyl acetate (NPA) to 4-nitrophenylate ion (Ağgül et al., 2020). The reference analysis without enzyme was tested before kinetic analysis and then bioactivities of the extracts were carried out. The extracts for different concentration in the experiment were repeated in triplicate. Control

cuvette activity in the absence of inhibitor (extract) was acknowledged as 100%. Finally, the inhibition features for hCA I and II isoenzymes were determined from the activity (%)-[inhibitor] graph (Kuzu et al., 2016).

Result and Discussion

Natural products are chemical molecules produced by all organisms of the three domains of life and have many drug-like bioactivity (Sorokina and Steinbeck, 2020). Plants as naturally gifted organisms (Huie, 2002) consist of many bioactive natural products commonly used in pharma, food and cosmetic industries. Extraction processes have been commonly used to separate significant natural molecules/products for the first step of sample preparation of plant materials (Wang and Weller, 2006). Extraction is an important sample preparation step in the studies on the bioactive components of the plant, and the results of the study depend on the selection of the appropriate extraction technique (Azmir et al., 2013). The selection of suitable solvent is one of the vital issues in the extraction process. Therefore, polar solvents extract polar substances while non-polar solvents extract non-polar substances.

Solvent extraction is the most preferred extraction techniques for the preparation of extracts from plants (Gupta et al. 2012).

There are several *in vitro* bioactivity studies of plant extracts on carbonic anhydrase enzyme. Ahmad et al. (2019) reported bioactivity of *Cassia absus* L. seed extracts on CA. *In vitro* bioactivities of the fractions obtained from crude ethanol extract were investigated in the study. Ethanol extract of the plant exhibited the best potential inhibitory features (IC_{50}: 1875 ± 0.9 µg/mL) against CA.

Kaya et al. (2019) have focused on the bioactivities of the extracts of *Alcea rosea*, *Foeniculum vulgare*, *Elettaria cardamomum*, *Laurus azorica* and *Lavandula stoechas* on hCA I and hCA II. In this study, the methanol extract of *Elettaria cardamomum* has possessed the highest inhibition profile (0.032 mg/mL) for hCA I. The methanol extract of *Lavandula stoechas* demonstrated the highest inhibitory characteristics (0.054 mg/mL) on hCA II.

Another study was conducted on the inhibitory features of *Cucumis melo* L. seed extracts against hCA I and hCA II. In this study, oil and methanol extracts of *Cucumis melo* L. seeds showed different bioactivities for hCA I and hCA II. While these extracts activated the hCA I, other isoenzyme, hCA II, was inhibited by oil extract with the IC_{50} value of 0.497 ng/mL and also methanol extract with the IC_{50} value of 10.98 µg/mL (Akkemik et al., 2019).

Several bioactivity studies about the extracts of *Satureja cuneifolia* have been investigated in previous studies (Oke et al., 2009; Taslimi et al., 2020). To the best of our knowledge, the inhibitory features of the extracts of *Satureja cuneifolia* on hCA I and hCA II have been studied for the first time.

Table 1. Effect of *Satureja cuneifolia* extracts on hCA I and hCA II

Extracts of *S. cuneifolia*	hCA I		hCA II	
	IC_{50}	R^2	IC_{50}	R^2
Petroleum ether extract	44 µg/mL	0.9485	101 µg/mL	0.9569
Chloroform extract	161 µg/mL	0.9662	132 µg/mL	0.9799
Methanol extract	68 µg/mL	0.9511	12 µg/mL	0.9432
Direct methanol extract	31 µg/mL	0.9597	13.6 µg/mL	0.9261
Standard (acetazolamide)	6.07 nM (Taslimi et al., 2016)	0.9154 (Taslimi et al., 2016)	8.549 ng/mL	0.9891

Table 1 illustrates the effects of *Satureja cuneifolia* extracts on hCA I and II. According to the table, the direct methanol extract of *S. cuneifolia* has performed the best inhibitory characteristic among the evaluated extracts for hCA I isoenzyme with the IC_{50} value of 31 µg/mL. Direct methanol extract was followed by petroleum ether, methanol and chloroform extracts with the IC_{50} values 44 µg/mL, 68 µg/mL and 161 µg/mL, respectively. The methanol extract of *S. cuneifolia* possessed the highest inhibition profile with the IC_{50} value of 12 µg/mL for hCA II among all tested extracts. Direct methanol extract also showed the potent inhibitory characteristic with the IC_{50} value of 13,6 µg/mL against hCA II. Other extracts, petroleum ether and chloroform, showed the inhibition profiles against hCA II with the IC_{50} values of 101 µg/mL and 132 µg/mL, respectively.

Conclusion

The carbonic anhydrase inhibitory activities of *S. cuneifolia* have been investigated in this study and to best of our knowledge, it has been the first investigation performed on hCA I and II inhibitory features of *S. cuneifolia* extracts. In this study, direct methanol extract of *S. cuneifolia* has showed the best inhibitory potential against hCA I in all evaluated extracts. The extracts have potential inhibitory features for hCA II. However, methanol extract has performed the best inhibitory characteristics for hCA II in all studied extracts. According to the results, methanol and direct methanol extracts can be used as natural products/inhibitors because of their biological activities. Further purification and analytical studies for both extracts will be needed to obtain the active natural molecules and their potential for pharma and food industries.

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Conflict of Interest Statement: The authors declare that the study was performed in the absence of any commercial and/or financial relationships that could be perceived as a conflict of interest.

Contribution Rate Statement Summary: The authors declare that they have contributed equally to the article. The authors have verified that all data in the manuscript have not been published before and have given approval for the final version of manuscript.

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