Original Article

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Carbonic Anhydrase Isoenzymes I and II Inhibition Potentials of *Leiotulus dasyanthus* (K. Koch) Pimenov&Ostr. and *Ferulago pauciradiata* Boiss.&Heldr. (Apiaceae)

Leiotulus dasyanthus (K. Koch) Pimenov&Ostr. ve *Ferulago pauciradiata* Boiss.&Heldr.'nın (Apiaceae) Karbonikanhidraz İzoenzimleri I ve II İnhibisyon Potansiyelleri

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ABSTRACT

Objective: Members of the Apiaceae family have many biological and pharmacological effects like anticancer, antidiabetic, antioxidant, anti-inflammatory, anti-hypertensive, anticoagulant, antimicrobial, and anticholinesterase. Also, some of them are consumed as vegetables, pickles, and spices. Carbonic anhydrase has a remarkable role in diseases like osteoporosis, glaucoma, edema, cancer, epilepsy, and obesity. The inhibition effects of methanol extracts and sub-extracts (n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous residue) of *Leiotulus dasyanthus* and *Ferulago pauciradiata* roots on carbonic anhydrase I and II isoenzymes were investigated in this research.

Methods: *Leiotulus dasyanthus* and *Ferulago pauciradiata* were collected in 2017 from Erzurum and in 2013 from Nevşehir (Turkey), respectively. The roots of *L. dasyanthus* and *F. pauciradiata* were macerated with methanol, dispersed with methanol: distilled water (1:9), and fractionated with n-hexane, dichloromethane, ethyl acetate, and n-butanol, in turn. The hCA I and II isoenzymes were

ÖZ

Amaç: Apiaceae familyasının üyeleri antikanser, antidiyabetik, antioksidan, anti-enflamatuvar, antihipertansif, antikoagülan, antimikrobiyal ve antikolinesteraz gibi pek çok biyolojik ve farmakolojik etkiye sahiptir. Ayrıca, bu üyelerin bazıları sebze, turşu ve baharat olarak tüketilmektedir. Karbonik anhidraz osteoporoz, glokom, ödem, kanser, epilepsi ve obezite gibi hastalıklarda önemli bir role sahiptir. Bu çalışmada *Leiotulus dasyanthus* ve *Ferulago pauciradiata* köklerinden hazırlanan metanol ekstreleri ve alt ekstrelerinin (n-hekzan, diklorometan, etil asetat, n-butanol ve sulu artık) karbonik anhidraz I ve II izoenzimleri üzerindeki inhibisyon etkileri araştırılmıştır.

Yöntemler: *Leiotulus dasyanthus* ve *Ferulago pauciradiata* sırasıyla 2017 yılında Erzurum'dan ve 2013'te Nevşehir'den (Türkiye) toplanmıştır. Bitkilerin kökleri metanol ile masere edildi, metanol:su (1:9) ile çözüldükten sonra sırayla n-hekzan, diklorometan, etil asetat ve n-butanol ile fraksiyonlanmıştır. hCA I ve II izoenzimleri, taze insan kanının eritrositlerinden Sepharose-4B-L-Tirozin-

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isolated from erythrocytes of fresh human blood via Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. The analysis of hCA I and II isoenzymes was actualized by the Verpoorte method.

Results: The hCA I and II were influentially inhibited by the dichloromethane and ethyl acetate sub-extracts of *F. pauciradiata* root with IC_{50} values of 1.694 μg/mL towards hCA I and 1.677 μg/mL towards hCA II, respectively. Whereas dichloromethane sub-extract and methanol extract of *L. dasyanthus* root inhibited hCA I and II isoenzymes with IC_{50} values of 1.828 μg/mL towards hCA I and 1.852 μg/mL towards hCA II, respectively.

Conclusion: The hCA I and II were influentially inhibited by the dichloromethane and ethyl acetate sub-extracts of *Ferulago pauciradiata* root.

Keywords: Apiaceae, carbonic anhydrase, *Ferulago*, *Leiotulus,* hCA I, hCA II

sülfanilamid afinite kromatografisi yoluyla izole edilmiştir. hCA I ve II izoenzimlerinin analizi Verpoorte metodu ile gerçekleştirilmiştir.

Bulgular: hCA I ve II, *F. pauciradiata*'nın köklerinden hazırlanan diklorometan ve etil asetat alt ekstreleri tarafından IC_{50} değerleri hCA I'e karşı 1.694 μg/mL ve hCA II'ye karşı ise 1.677 μg/mL ile etkili bir şekilde inhibe edildi. *L. dasyanthus* köklerinden hazırlanan diklorometan alt ekstresi ve metanol ekstresi hCA I ve II izoenzimlerini hCA I'e karşı 1.828 μg/mL ve hCA II'ye karşı 1.852 μg/mL IC_{50} değerleriyle inhibe etmiştir.

Sonuç: hCA I ve II, *F. pauciradiata*'nın köklerinden hazırlanan diklorometan ve etil asetat alt ekstreleri tarafından etkili bir şekilde inhibe edilmiştir.

Anahtar Sözcükler: Apiaceae, karbonik anhidraz, *Ferulago*, *Leiotulus,* hCA I, hCA II

Introduction

Enzymes are biological molecules liable for a lot of metabolic processes that are synthesized through the living cells and speed up chemical reactions along with the metabolism in living organisms. Carbonic anhydrases (CAs) are systematized via five gene families such as α-, β-, γ-, δ-, and ζ-CA. The β-CA is found in herbs and several prokaryotes (1). CA is a family of metalloenzymes that are ubiquitous alive organisms. It has considerable pathological and physiological roles like gluconeogenesis, fluid balance, respiration, calcification, tumorigenicity, carbon dioxide (CO_2) and ion transport, carboxylation reactions, pH regulation, acid-base balance, lipogenesis, and many pathophysiological processes. The bicarbonate (HCO_3^-) and proton catalyzed the reversible conversion of CO_2 and water (H_2O) by CA. The clinical utilization of CA inhibitors has been identified for the treatment of glaucoma, neurological disorders such as epilepsy, osteoporosis, cancer, edema, gastric and duodenal ulceration, inflammatory illnesses, and obesity (2-5).

For ages, CA has been known to present in a lot of photosynthetic organisms, and a significant act is expected in their photosynthetic processes. Several organisms might possess various kinds of CAs in different cellular places, and each kind of CA might have a different act in photosynthetic processes. The evolution in our realization of CA function has been reported in a range of photosynthetic organisms, containing micro and macroalgae from marine and freshwater habitats, and cyanobacteria, terrestrial, and aquatic higher herbs (6).

In this study, the "*Leiotulus dasyanthus* (K. Koch) Pimenov & Ostr. and *Ferulago pauciradiata* Boiss. & Heldr. (Apiaceae)" were chosen as the resource of plant extracts. Apiaceae (Umbelliferae) is one of the biggest families containing about 450 genera and 3.700 species in the world. The taxons of Apiaceae are well-known such as medical, culinary, and vegetable herbs. Members of Apiaceae generally have a characteristically strong or aromatic fragrance due to the existence of essential oil or oleoresin. Plants of this family have varied components with many biological and pharmacological activities such as hepatoprotective, vaso-relaxant, cyclooxygenase inhibitory, antibacterial, and antitumor (7). A total of 104 genera and 486 species belonged to the Apiaceae family in Turkey (8). In 1994, *Malabaila dasyantha* has confirmed as a synonym of *L. dasyanthus* (K. Koch) Pimenov & Ostr. In Turkey, *M. dasyantha* is known as "dudakpatlatan" (9) and "mandak" (10). It is utilized in the Turkish conventional medicine for hemorrhoids, nail disorders, and stomachache treatment (11). *Malabaila* Hoffman. genus is represented by seven species in Turkey (9). Likewise, leaves and stems of these species are used for roasting, and making soup and pickle (12). *Ferulago* Koch. genus is represented by 34 species in Turkey (9). In Turkey *F. pauciradiata* Boiss. & Heldr. (Apiaceae) is known as "etekli kişniş"(9). *F. pauciradiata* was first identified in Diagn. Pl. Orient. ser. 1, 10: 37 (1849) (9). *Ferulago* species are traditionally utilized as an antiflatulent, digestive, aphrodisiac, sedative, vermicidal, and treatment against cephalalgia, ulceration, snake bite, hemorrhoid, and skin disorders (10,13).

This study aimed to assess the human CA isoenzymes I and II (hCA I and II isoenzymes) inhibition of roots extracts and sub-extracts of *L. dasyanthus* and *F. pauciradiata*.

Methods

Plant Materials

L. dasyanthus and *F. pauciradiata* were collected in 2017 from Erzurum and in 2013 from Nevsehir (Turkey), respectively and were qualified by Prof. Dr. Hayri Duman. Voucher specimens were stored at the Atatürk University, Pharmacy Faculty Herbarium (AUEF 1284) and Ankara University, Pharmacy Faculty Herbarium (AEF 26360).

Extraction and Fractionation

The roots of *L. dasyanthus* (80 g) and *F. pauciradiata* (80 g) were pulverized and macerated with methanol by a mechanical mixer at 150 rpm. Extracts of *L. dasyanthus* and *F. pauciradiata* roots were filtered and evaporated, which then dispersed with methanol and H_2O solution with 1:9 ratio and fractionated

with 150 mL of n-hexane, dichloromethane, ethyl acetate, and n-butanol, in turn. The combined n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous residue sub-extracts were evaporated, respectively, and then weighed. Sums of gained samples are exhibited in Table 1.

Purification and Inhibition Assays of Carbonic anhydrase Isoenzymes

The analysis of hCA I and II isoenzymes was actualized using the Verpoorte method with slight modifications. The isoenzymes hCA I and II were isolated from erythrocytes of fresh human blood via Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was utilized to check the purity of isoenzymes. (14). The enzyme unit of CA esterase effect is detected within one min as the hydrolysis of 1 μmol of *p*-nitrophenyl acetate to *p*-nitrophenol and acetate (15). Using the spectrophotometer, the *p*-nitrophenol content is determined at maximum absorbance at 348 nm (UV-1800 Shimadzu, Kyoto, Japan). Protein ingredient was estimated by the Bradford method at 595 nm (16). Bovine serum albumin was utilized as the reference protein. Acetazolamide (AZA) was used as a positive control for isoenzymes hCA I and II. IC_{50} values against extracts and sub-extracts parcel activity (%) were figured out. Three distinctive concentrations were utilized to attain Ki values. Lineweaver-Burk graphics were used for measurements (17).

Results

The methanol extracts of *L. dasyanthus* and *F. pauciradiata* roots were made to liquid-liquid partitioning with hexane, dichloromethane, ethyl acetate, butanol, and H_2O. The CA inhibitory activity of extracts and sub-extracts of *L. dasyanthus* and *F. pauciradiata* roots was carried out. Two physiologically relevant CA isoforms (hCA I and II) were utilized. Findings of enzymes inhibition values were demonstrated in Table 2. Findings attained from this study expressly display the efficacious inhibition activities of these samples toward cytosolic isoforms hCA I and II with low micromolar range.

Extracts and sub-extracts connect to hCA I in a micromolar range. IC_{50} values are ranging from 1.694±0.9828 to 2.783±0.9816 μg/mL for hCA I isoenzyme. Otherwise, AZA as a broad-specific

Table 1. Sums of gained samples from *Leiotulus dasyanthus* and *Ferulago pauciradiata* roots

Extracts/sub-extracts	*Leiotulus dasyanthus*	*Ferulago pauciradiata*
MeOH (g)	8.35	9.02
Hexane (g)	0.91	0.99
CH_2Cl_2 (g)	2.95	3.12
EtOAc (g)	0.88	0.97
BuOH (g)	1.37	1.53
Aqueous residue (g)	1.77	1.97

Table 2. Human carbonic anhydrase isoenzymes (hCA I and II) inhibition values of *Leiotulus dasyanthus* and *Ferulago pauciradiata* roots

Extracts and sub-extracts	IC_{50} (μg/mL for extracts and sub-extracts and μM for AZA)			
	hCA I	r^2	hCA II	r^2
MLR	2.287	0.9824	1.852	0.9817
HLR	2.510	0.9847	2.851	0.9775
DLR	1.828	0.9822	2.294	0.9715
ELR	2.112	0.9804	2.165	0.9755
BLR	1.838	0.9817	2.440	0.9862
WLR	2.483	0.9817	2.200	0.9780
MFR	2.158	0.9778	2.574	0.9802
HFR	2.242	0.9900	2.475	0.9851
DFR	1.694	0.9828	2.475	0.9722
EFR	2.132	0.9841	1.677	0.9833
BFR	2.783	0.9816	2.911	0.9851
WFR	2.179	0.9702	1.878	0.9872
AZA-	1.008	0.9935	0.222	0.9943

MLR: Methanol extract of *Leiotulus dasyanthus* root, HLR: hexane sub-extract of *L. dasyanthus* root, DLR: dichloromethane sub-extract of *L. dasyanthus* root, ELR: ethylacetate sub-extract of *L. dasyanthus* root, BLR: butanol sub-extract of *L. dasyanthus* root, WLR: H_2O sub-extract of *L. dasyanthus* root; MFR: methanol extract of *Ferulago pauciradiata* root, HFR: sub-extract extract of *F. pauciradiata* root, DFR: dichloromethane sub-extract of *F. pauciradiata* root, EFR: ethylacetate sub-extract of *F. pauciradiata* root, BFR: butanol sub-extract of *F. pauciradiata* root, and WFR: H_2O sub-extract of *F. pauciradiata* root.

CA inhibitor, displayed IC_{50} value of 1.008±0.9935 μg/mL toward hCA I due to its extended inhibition of CAs. Among these extracts and sub-extracts, dichloromethane sub-extract of *F. pauciradiata* root indicated the best inhibition toward hCA I with 1,694 μg/mL IC_{50} value. In addition, the butanol sub-extract of *F. pauciradiata* root indicated the lowest inhibition against hCA I with 2.783 μg/mL IC_{50} value. IC_{50} values range from 1.677±0.9833 to 2.911±0.9851 μg/mL for hCA II isoenzyme. Otherwise, AZA as a broad-specific CA inhibitor, displayed IC_{50} value of 0.222±0.9943 μM toward hCA II due to its extended inhibition of CAs. Among these extracts and sub-extracts, ethyl acetate sub-extract of *F. pauciradiata* root indicated the best inhibition against hCA II with 1.677 μg/mL IC_{50} value. Moreover, the butanol sub-extract of *F. pauciradiata* root indicated the lowest inhibition against hCA II with 2.911 μg/mL IC_{50} value. Inhibition effects of whole samples are strongly high when in proportion to the AZA. Furthermore, dichloromethane sub-extract and methanol extract of *L. dasyanthus* root inhibited hCA I and II isoenzymes with IC_{50} values of 1.828 μg/mL toward hCA I and 1.852 μg/mL toward hCA II, respectively.

Discussion

Whole CAs are zinc metalloenzymes that catalyze the conversion of CO_2 and HCO_3^-. Enzymes are omnipresent in nature and are an exemplification of convergent evolution, thus a great number of structurally and sequentially different families of CA were discovered. Plants possess three types of CA (α, β, and γ) (18).

Many natural and synthetic compounds could influence live metabolism via changing enzyme effects and influencing metabolic pathways at low concentrations. The inhibition activities of several antioxidant phenolics and polyphenolic compounds such as hydroquinone, catechol, quercetin, resorcinol, simple phenol, and pyrogallol have been investigated particularly, as well. Moreover, a series of active natural phenolic compounds containing curcumin, resveratrol, dobutamine, catechin, and silymarin were researched for the inhibition of whole catalytically active mammalian CA isoenzymes (1).

The main components of the Apiaceae family are coumarins and essential oils (19). Coumarins acted as classical CA inhibitors with resolved inhibition mechanisms (20). Coumarin compounds such as bergapten, pimpinellin, and umbelliferone were isolated from the roots of *L. dasyanthus* (21). In addition, other coumarin compounds such as prantschimgin and felamidin were isolated from the roots of *F. pauciradiata* (9). Thus, we estimate that CA activity is caused by coumarins in these plants. This paper is the first study on CA inhibition potentials of *L. dasyanthus* and *F. pauciradiata.*

Conclusion

Extracts and sub-extracts of *L. dasyanthus* and *F. pauciradiata* roots were carried out for their isoenzymes hCA I and II inhibition activity. The isoenzymes hCA I and II were influentially inhibited by the dichloromethane and ethyl acetate sub-extracts of *F. pauciradiata* root.

Ethics

Ethics Committee Approval: There is no need for an Ethics Committee Approval.

Informed Consent: The authors were informed about this research, and the informed consent form was signed.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.K., Z.B., İ.G., Design: S.K., İ.G., Data Collection or Processing: S.K., F.D., H.D., C.S.K., Analysis or Interpretation: S.K., Z.B., İ.G., Literature Search: S.K., İ.G., Writing: S.K., İ.G.

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