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In vitro antioxidant activity and carbonic anhydrase inhibitory features of *Ferula communis* extracts

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Abstract

Carbonic anhydrases (CAs; EC 4.2.1.1) are essential family of metalloenzymes which catalyze the interconversion between carbon dioxide (CO_2) and bicarbonate (HCO_3^-) in all organisms of three-domains of life. Huge amounts of attempts related to catalytic activity of CAs have been widely expanded to treat many clinical diseases. This study aimed to determine *in-vitro* antioxidant activities and human CA I (hCA I) and II (hCA II) inhibitory properties of *Ferula communis* extracts. Among all extracts of *F. communis*, the hexane extract has showed the best inhibitory profile on hCA I and II with IC_{50} values 8.68 µg/mL and 28 µg/mL and K_i values 2.026 µg/mL and 11.6 µg/mL, respectively. All extracts showed mild to moderate antioxidant activity. According to the results of DPPH assay, ethanol-water extract showed the highest activity with IC_{50}: 0.1128±0.0066 mg/mL value. Chloroform extract showed the highest activity on CUPRAC assay with the value of 1.305±0.037 mM Trolox equivalent/mg extract. However, further analytical, *in-vivo* and clinical studies are needed to confirm the activities of *F. communis*.

Keywords: Antioxidant activity, Carbonic anhydrase, *Ferula communis*, Inhibition

Introduction

Belonging to Apiaceae family, genus *Ferula* consist of 180-185 species, which makes it the third largest genus in the family. Plants from genus *Ferula* possess plenty of compounds which are biologically active. These include disulfide compounds coumarin derivatives, sesquiterpene compounds, aromatic lactones, daucane esters (Salehi et al., 2019). Plants belonging to this genus are also used in various diseases for therapeutic purposes such as antipyretic, smooth-muscles relaxant, contraceptive and aphrodisiac. In addition to this, plant compounds obtained from this genus are used as folk medicine for the treatment of numerous problems such as headache, digestive and neurological disorders, arthritis, rheumatism and dysentery (Iranshahi et al., 2018).

Ferula communis has been used to treat hysteria, skin infections and dysentery in folk medicine and has a long history of medical use in practice. Additionally, it has been used as analgesic, diuretic and anti-helmintic. *Ferula communis* has two chemotypes, poisonous and non-poisonous ones. Non-poisonous chemotype possesses sesquiterpene daucane esters while poisonous chemotype has mostly prenylated coumarins (Akaberi et al., 2015). These two chemotypes cannot be separated according to their morphological features. *Ferula communis* has been traditionally used as phytohormone and reported as a potential source of phytoestrogens. This effect is associated with isoflavones and ferutinin which is an aromatic ester of a daucane alcohol (Arnoldi et al., 2004; Nguir et al., 2016).

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Free radicals are highly reactive molecules and produced in the body as part of the physiological process. Free radicals cause oxidative stress, resulting in cell damage or even cell death (Amir Aslani and Ghobadi 2016). Diseases like cancer, atherosclerosis and diabetes mellitus are caused by oxidative stress (Ceylan et al., 2019). Plant extracts have been reported to show biological activities and are known to have phenolic compounds. Phenolic compounds delay lipid peroxidation caused by free radicals. Antioxidant activities of plant extracts have been widely studied and various extract and their compounds have been determined as antioxidants. Therefore, plant extracts and their components have been investigated for their antioxidant activities thoroughly (Kähkönen et al., 1999; Pernin et al., 2018).

Enzymes which are protein-based biomolecules regulate many metabolically important reactions in all living beings (Nagao et al., 2014; Çetinkaya et al., 2014). Carbonic anhydrases (CAs; EC 4.2.1.1) are a superb family of metalloenzymes catalyzing the interconversion between carbon dioxide (CO_2) and bicarbonate (HCO_3^-) (Aggarwal et al., 2013; Mete et al., 2016; Özensoy Güler et al., 2016). They are expressed in all organisms of three-domains of life (Uygun et al., 2014) with encoded eight unrelated gene families (α, β, γ, δ, ζ, η, ɵ ve ι) (Ozensoy Güler et al., 2020). In mammalian organisms, α-gene family expresses 16 isoforms of the enzyme which differ from each other in terms of their location (Nar et al., 2013; Orhan et al., 2016) their molecular characteristics, organization, and kinetics towards inhibitors/ligands (Nocentini et al., 2016). Some isoenzymes are present in cytosol (hCA I, II, III, VII and XIII), five isoenzymes are in the form of membrane-bound (hCA IV, IX, XII, XIV and XV), hCA VA and VB are found in mitochondria, and hCA VI is secretory isoform (Almajan et al., 2008). The other three isoforms (hCA VIII, X and XI) are described as non-catalytic protein forms (Abdel-Aziz et al., 2014; Ekinci et al., 2010).

Common practices related to catalytic activity of CAs have been largely expanded to treat many clinical diseases (Akbaba et al., 2013). Modulation of the catalytic activity of CAs with carbonic anhydrase activators (CAAs) or carbonic anhydrase inhibitors (CAIs) are crucial for prevention and/or treatment of many diseases/syndromes (Scozzafava et al., 2004). CAIs which are classified according to their affinities and inhibitory characteristics for the CA isoforms (Maslanka, 2015) have been used in essential clinical applications as pharmaceutical agents for ophthalmology, obesity, cancer, osteoporosis and neurological disorders (Supuran, 2008). Up to date, heavy metal ions (Kuzu et al., 2018), anions (De Simone and Supuran, 2012), bromophenols (Balaydın et al., 2012; Taslimi et al., 2016), chalcones (Gençer et al., 2013), coumarins (Karatas et al., 2014), pyrazoles (Mert et al., 2016), sulfonamides (Ceruso et al., 2014; Le Darz et al., 2015; Arslan et al., 2016; Carta et al., 2016), thiosemicarbazone and its metal complexes (Ucar et al., 2020), thiourea derivatives (Korkmaz et al., 2015), uracil derivatives (Güney et al., 2015; Türkoğlu et al., 2017) have been used as CAIs. These types of compounds have been synthesized in complex procedures and showed considerable side effects. However, natural products have gained popularity and also performed in the inhibition studies of CAs (Akkemik et al., 2019).

Keeping in view of the medical and therapeutic importance of the enzyme and natural product, this study was designed to evaluate the extracts of *F. communis* for total phenolic content and antioxidant features and also investigate the inhibitory characteristics on hCA I and II for the first time.

Materials and Methods

Plant material

Ferula comminus was obtained from Zeytinburnu Medicinal Plant Botanic Gardens in İstanbul, Turkey in 2020 and authenticated by Dr. Zeki Severoğlu from Biology Department of Marmara University with the number of 5087 for future reference.

Chemicals and instruments

Human carbonic anhydrase I and II enzymes, trizma base and 4-nitrophenylacetate as substrate were commercially obtained from Sigma-Aldrich (Saint Louis, MO, USA). N-hexane, chloroform, ethanol, acetone, sulfuric acid and dimethyl sulfoxide were purchased from Isolab (Turkey). The laboratory blender (8011 EG, Waring Commercial, USA) was used for fine powder. Water for buffers and experimental processes was supplied from Direct Q®3 UV water purification system (Millipore Corp., France). ZX3 Advanced Vortex Mixer (Velp Scientifica, Usmate, Italy) and IKA RT10 magnetic stirrer (IKA-Werke GmbH & Co KG, Germany) were used for stirring and mixing purposes. Accurate weighing measurements of all chemicals and extracts were obtained from Ohaus PA224C (Ohaus Corp., USA) with the readability up to 0.1 mg. pH values were analyzed with Mettler Toledo Seven Compact pH-meter (Greifensee, Zürich, Switzerland). Organic solvents were evaporated by Hei-VAP Core rotary evaporator (Heidolph Instruments, Germany). Bioactivities of plant extract on the enzymes were examined by V-730 UV–Visible Spectrophotometer (JASCO International Co., Tokyo, Japan) with the resolution of 1 nm. All liquid in the experiment was transferred with Eppendorf Research Plus single channel pipettes (Eppendorf AG, Hamburg, Germany).

Preparation of the extracts

The aerial parts of *Ferula communis* were dried in the shade and powdered. The powdered aerial parts (40 g) were macerated with 200 mL of hexane, chloroform, ethanol, ethanol:water (50:50), respectively. Procedure was repeated at a 24-hour cycle until colorless solvent was obtained. The extracts were filtered and evaporated with a rotary evaporator. Extracts were kept at +4°C until the experiment day.

Determination of total phenolic contents

Extracts were prepared at the concentration of 5

mg/mL. 0.1 mL of extract were taken into a tube then 4.5 mL water was added. Diluted with distilled water (1/3 ratio with distilled water), 0,1 mL Folin-Ciocalteu reagent was taken and added to the mixture. After adding 0.3 mL of 2% sodium carbonate solution, mixture was kept at room temperature for 2 hours. Absorbance was measured at 760 nm against the reference. The total phenolic contents in the extracts were given as mg gallic acid equivalents/mg extract (Taşkın et al., 2018).

***In vitro* evaluation of antioxidant assays**

2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

DPPH• solution was prepared as 0.10 mM using MeOH. 3.90 mL of the solution was added to 0.1 mL of the extracts which were prepared at different concentrations (0.5-5 mg/mL). Absorbance was measured at 517 nm 30 min later. IC_{50} values were determined by percentage inhibition-concentration curve. Ascorbic acid was used as reference (Fu et al., 2010).

Cupric reducing antioxidant capacity (CUPRAC) assay

60 µL ethanolic solution of neocuproine ($7.3.10^{-3}$ M), 1 M NH_4Ac buffer solution (pH 7) and Cu(II) (1.10^{-2} M), were mixed in 96-well microplate. Afterwards, 10 µL ethanol was added to the mixture. Lastly, extracts were prepared in the concentration of 1 mg/mL and 60 µL of the extracts were added to the plate. Mixture was kept at room temperatures for 1 h and then absorbance was measured at 450 nm against the blank. CUPRAC values of the plant extracts were given as mM trolox/mg extract (Taşkın et al., 2019).

Esterase activity assay of human carbonic anhydrase isoenzymes

Esterase activity assay of hCA I and hCA II was assayed with the method of (Verpoorte et al. 1967). Absorbance changes of 4-nitrophenyl acetate (NPA) to 4-nitrophenylate ion under certain conditions (348 nm, 3 min, 25°C) was recorded in this spectrophotometry-based detection (Ağgül et al., 2020; Balaydin et al., 2012). The reference measurement without enzymatic solution was investigated before the kinetic studies and then the effects of extracts were tested. The extract for each concentration in the assay were examined in triplicate. Activity of control cuvette in case of no inhibitor was accepted as 100%. First hCA I and II, inhibition (%) studies were performed with all extracts. Then activity (%) - [Inhibitor] graph was drawn for the extract which possesses best inhibition (%) profile for the hCA isoenzymes. K_i values of the extract was calculated from Cheng–Prusoff equation (Kuzu et al., 2016).

Results and Discussion

Total phenolic content was analyzed with Folin-Ciocalteu method and the results are shown in Table 1. In the results, the highest phenolic content was observed in ethanol:water (50:50) extract (0.031±0.001 mg gallic acid equivalent/mg extract).

Hexane, chloroform, ethanol and ethanol:water (50:50) extracts have been tested for their 2,2-diphenyl-1-picryl-hydrazyl scavenging activity and cupric reducing antioxidant capacity. The results are given in Table 2. According to the results of the experiments, all extracts have mild to moderate antioxidant properties. DPPH scavenging activity of the extracts from highest to lowest was in the following order: Ethanol:water (IC_{50}: 0.1128±0.0066 mg/mL), hexane (IC_{50}: 0.2058±0.0114 mg/mL), ethanol (IC_{50}: 0.2121±0.0170 mg/mL), chloroform (IC_{50}: 1.0451±0.0550 mg/mL). None of the extracts showed stronger activity than ascorbic acid (IC_{50}: 0.0028±0.0004 mg/mL) which was used as standard.

Cupric reducing antioxidant capacity of the extracts obtained from *Ferula communis* was evaluated. According to the results, the highest activity was determined in chloroform extract (1.305±0.037 mM Trolox equivalent/mg extract). It was followed by ethanol:water extract (1.127±0.030 mM Trolox equivalent/mg extract), ethanol extract (0.712±0.028 mM Trolox equivalent/mg extract) and hexane extract (0.702±0.024 mM Trolox equivalent/mg extract). Extracts showed lower activity than ascorbic acid (5.700±0.020 mM Trolox equivalent/mg extract).

There are some studies related to the antioxidant activities of *Ferula communis* in the literature. Aerial parts of *Ferula communis* were tested for its antioxidant properties. In the study, methanol extracts of different parts of the plant (stem, fruit and flower) were evaluated separately. According to the results, highest DPPH radical scavenging activity was obtained from flower extract (IC_{50}: 24.07 µg/mL) (Rahali et al., 2018). In another study, essential oil from *F. communis* was evaluated for its antioxidant properties. The essential oil was obtained from four different organs as roots, stems, flowers and leaves. Essential oil from stems exhibited the best results on DPPH assay (IC_{50}: 0.03±0.001 mg/mL) (Nguir et al., 2016).

In vitro activity of seed extracts of *Cassia absus* L. on carbonic anhydrase enzyme have been reported by Ahmad et al. (2019). In this study crude ethanol extract and the fractions of *n*-butanol, chloroform, *n*-hexane and water obtained from ethanol extract were examined as *in vitro* carbonic anhydrase bioactivity. In this study, ethanol extract of *Cassia absus* L. showed the best potent inhibitory activity (inhibition (%): 54.1±0.06; IC_{50}: 1875 ± 0.9 µg/mL) on carbonic anhydrase enzyme.

Table 1. Total phenolic contents of the extracts of *Ferula communis*

Extract	Total Phenolic Content (mg gallic acid equivalent/mg extract)
Hexane	0.030±0.003
Chloroform	0.027±0.001
Ethanol	0.025±0.001
Ethanol:water (50:50)	0.031±0.001

Table 2. *In vitro* antioxidant activity assay of the extracts of *Ferula communis*

Extract	DPPH (IC_{50} mg/mL)	CUPRAC (mM Trolox equivalent/mg extract)
Hexane	0.2058±0.0114	0.702±0.024
Chloroform	1.0451±0.0550	1.305±0.037
Ethanol	0.2121±0.0170	0.712±0.028
Ethanol:water (50:50)	0.1128±0.0066	1.127±0.030
Ascorbic acid*	0.0028±0.0004	5.700±0.020

* : Standard

Another study has focused on the effects of the extracts of five different plants (*Alcea rosea*, *Foeniculum vulgare*, *Elettaria cardamomum*, *Laurus azorica* and *Lavandula stoechas*) on hCA I and II. According to the results, the methanol extract of *Elettaria cardamomum* has showed the highest inhibitory potential (0.032 mg/mL) on hCA I and the highest inhibition for hCA II (0.054 mg/mL) has been obtained from the methanol extract of *Lavandula stoechas* (Kaya et al., 2019).

Akkemik et al. (2019) has investigated the inhibition characteristics of *Cucumis melo* L. seed extracts on hCA I and II. While oil and methanol extracts of the seeds activates the hCA I isoenzyme activity, these extracts have also inhibited the hCA II isoenzyme. IC_{50} and K_i values of oil extract on hCA II are 0.497 ng/mL and 0.369±0.166 ng/mL, respectively and 10.98 µg/mL and 7.25±0.400 µg/mL for hCA II have been obtained from the methanol extracts.

To the best of our knowledge, the inhibitory potential of the extracts of *Ferula communis* on hCA I and II have been investigated for the first time. The hexane extract has showed the best inhibitory feature on hCA I. It was followed by chloroform extract which exhibits good inhibition for hCA I. However, other two extracts, ethanol and ethanol-water (50:50), have demonstrated weak inhibition for hCA I. The inhibition studies on hCA II isoenzyme have been performed with the extracts of *Ferula communis*. The hexane extract has demonstrated the best inhibitory effect on hCA II and other three extracts have had no meaningful inhibition on the isoenzyme. Inhibition (%) studies of the extracts of *Ferula communis* on hCA I and hCA II are illustrated in Table 3. According to inhibition (%) studies, hexane extract of *Ferula communis* has showed the most potent inhibition against hCA I and hCA II isoenzymes.

The hexane extract has exhibited the highest inhibitory activity on hCA I and hCA II among all tested extracts. Therefore, the hexane extract has been determined as the best inhibitor of the evaluated extracts for hCA I and II with IC_{50} values 8.68 µg/mL and 28 µg/mL and K_i values 2.026 µg/mL and 11.6 µg/mL, respectively (Table 4).

Table 3. Inhibition (%) studies of the extracts of *Ferula communis* on hCA I and II

Extract	Inhibition (%) for hCA I	Inhibition (%) for hCA II
Hexane	61.236±1.685	18.595±1.894
Chloroform	59.551±2.919	2.479±0.716
Ethanol	25.281±0.973	-
Ethanol-water (50:50)	16.854±0.973	-

- : No activity

Table 4. Effect of *Ferula communis* n-hexane extract on hCA I and II

	n-hexane extract of *F. communis*		Standard (acetazolamide)	
Sample Enzyme	hCA I	hCA II	hCA I (Taslimi et al., 2016)	hCA II
IC_{50}	8.68 µg/mL	28 µg/mL	6.07 nM	8.549 ng/mL
R^2	0.9251	0.9510	0.9154	0.9891
K_i	2.026 µg/mL	11.6 µg/mL	6.76±2.55 nM	5.813 ng/mL

The antioxidant and carbonic anhydrase inhibitory activities of *F. communis* have been reported in this study and to best of our knowledge, it has been the first study conducted on hCAI and hCAII inhibitory effects of *Ferula communis* extracts. These discoveries about the bioactivities of natural products are essential for pharmaceutical and nutritional sciences. According to the results, the study can be advanced by further analytical studies. Thus, novel active natural compounds might be illuminated by determining the extract content.

Conclusion

Carbonic anhydrase activity of hexane extract of *Ferula communis* has showed the most inhibitory potential among the other extracts. Inhibition profiles of the hexane extract of *Ferula communis* on hCA I and II have been determined as µg/mL. IC_{50} and K_i values of acetazolamide were determined in the range of ng/mL and nM. Further purification studies for the extracts and clinical studies will be

needed to determine the active natural products and their potential for pharmaceutical and nutritional sciences.

Compliance with Ethical Standards

Conflict of interest

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.

Author contribution

F.G.A. obtained the bioactivity measurements, analyzed the data of antioxidant activity and wrote the manuscript. Z.A.K. obtained the enzymatic activity and performed in all laboratory tasks. E.A.T. operated methodology, collaboration, analysis, validation, supervision and writing of the study. M.K. has contributed on the analysis and validation of the bioactivity data for the study. Z.S. identified the plant material. 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.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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References

Abdel-Aziz, A. A.-M., El-Azab, A. S., Ceruso, M., Supuran, C. T. (2014). Carbonic anhydrase inhibitory activity of sulfonamides and carboxylic acids incorporating cyclic imide scaffolds. Bioorganic & Medicinal Chemistry Letters, 24(22), 5185-5189. Doi: https://doi.org/10.1016/j.bmcl.2014.09.076

Aggarwal, M., Boone, C. D., Kondeti, B., McKenna, R. (2013). Structural annotation of human carbonic anhydrases. Journal of Enzyme Inhibition and Medicinal Chemistry, 28(2), 267-277. Doi: https://doi.org/10.3109/14756366.2012.737323

Ağgül, A., Kuzu, M., Kandemir, F., Küçükler, S., Çağlayan, C. (2020). Alterations in enzyme activity of carbonic anhydrase, 6-phosphogluconate dehydrogenase and thioredoxin reductase in rats exposed to doxorubicin and morin. Clinical and Experimental Health Sciences, 10(3), 228-234. Retrieved from https://dergipark.org.tr/en/pub/clinexphealthsci/issue/56955/632320

Ahmad, S., Hassan, A., Rehman, T., Basit, A., Tahir, A., Adeel Arshad, M. (2019). In vitro bioactivity of extracts from seeds of *Cassia absus* l. growing in Pakistan. Journal of Herbal Medicine, 100258. Doi: https://doi.org/10.1016/j.hermed.2019.100258

Akaberi, M., Iranshahy, M., Iranshahi, M. (2015). Review of the traditional uses, phytochemistry, pharmacology and toxicology of giant fennel (*Ferula communis* L. subsp. *communis*). Iranian Journal of Basic Medical Sciences, 18(11), 1050-1062. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4764105/

Akbaba, Y., Balaydın, H. T., Menzek, A., Göksu, S., Şahin, E., Ekinci, D. (2013). Synthesis and Biological Evaluation of Novel Bromophenol Derivatives as Carbonic Anhydrase Inhibitors. Archiv Der Pharmazie, 346(6), 447-454. Doi: https://doi.org/10.1002/ardp.201300054

Akkemik, E., Aybek, A., Felek, I. (2019). Effects of cefan melon *(Cucumıs melo* l.*)* seed extracts on human erythrocyte carbonic anhydrase I-II enzymes, Applied Ecology and Environmental Research, 17(6), 14699-14713. Doi: https://doi.org/10.15666/aeer/1706_1469914713

Almajan, G. L., Barbuceanu, S.-F., innocenti, A., Scozzafava, A., Supuran, C. T. (2008). Carbonic anhydrase inhibitors. Inhibition of the cytosolic and tumor-associated carbonic anhydrase isozymes I, II and IX with some 1,3,4-oxadiazole- and 1,2,4-triazole-thiols. Journal of Enzyme Inhibition and Medicinal Chemistry, 23(1), 101-07. Doi: https://doi.org/10.1080/14756360701342417

Amir Aslani, B., Ghobadi, S. (2016). Studies on oxidants and antioxidants with a brief glance at their relevance to the immune system. Life Sciences, 146, 163-173. Doi: https://doi.org/10.1016/j.lfs.2016.01.014

Arnoldi, L., Ballero, M., Fuzzati, N., Maxia, A., Mercalli, E., Pagni, L. (2004). HPLC-DAD-MS identification of bioactive secondary metabolites from *Ferula communis* roots. Fitoterapia, 75(3-4), 342-354. Doi: https://doi.org/10.1016/j.fitote.2004.03.001

Arslan, T., Türkoğlu, E. A., Şentürk, M., Supuran, C. T. (2016). Synthesis and carbonic anhydrase inhibitory properties of novel chalcone substituted benzenesulfonamides. Bioorganic & Medicinal Chemistry Letters, 26(24), 5867-5870. Doi: https://doi.org/10.1016/j.bmcl.2016.11.017

Balaydın, H. T., Şentürk, M., Göksu, S., Menzek, A. (2012). Synthesis and carbonic anhydrase inhibitory properties of novel bromophenols and their derivatives including natural products: Vidalol B. European Journal of Medicinal Chemistry, 54, 423-428. Doi: https://doi.org/10.1016/j.ejmech.2012.05.025

Balaydin, H. T., Durdağı, S., Ekinci, D., Şentürk, M., Göksu, S., Menzek, A. (2012). Inhibition of human carbonic anhydrase isozymes I, II and VI with a series of bisphenol, methoxy and bromophenol compounds. Journal of Enzyme Inhibition and Medicinal Chemistry, 27(4), 467-475. Doi: https://doi.org/10.3109/14756366.2011.596836

Carta, F., Ferraroni, M., Scozzafava, A., Supuran, C. T. (2016). Fluorescent sulfonamide carbonic anhydrase inhibitors incorporating 1,2,3-triazole moieties: Kinetic and X-ray crystallographic studies. Bioorganic & Medicinal Chemistry, 24(2), 104-112. Doi: https://doi.org/10.1016/j.bmc.2015.11.031

Ceruso, M., Antel, S., Vullo, D., Scozzafava, A., Supuran, C. T. (2014). Inhibition studies of new ureido-substituted sulfonamides incorporating a GABA moiety against human carbonic anhydrase isoforms I-XIV. Bioorganic & Medicinal Chemistry, 22(24), 6768-6775. Doi: https://doi.org/10.1016/j.bmc.2014.10.041

Ceylan, S., Cetin, S., Camadan, Y., Saral, O., Ozsen, O., Tutus, A. (2019). Antibacterial and antioxidant activities of traditional medicinal plants from the Erzurum region of Turkey. Irish Journal of Medical Science, 188(4), 1303-1309. Doi: https://doi.org/10.1007/s11845-019-01993-x

Çetinkaya, Y., Göçer, H., Gülçin, İ., Menzek, A. (2014). *Synthesis and carbonic anhydrase isoenzymes ınhibitory effects of brominated diphenylmethanone and ıts derivatives.* Archiv Der Pharmazie, 347(5), 354-359. Doi: https://doi.org/10.1002/ardp.201300349

De Simone, G., Supuran, C. T. (2012). (In)organic anions as carbonic anhydrase inhibitors. Journal of Inorganic Biochemistry, 111, 117-129. Doi: https://doi.org/10.1016/j.jinorgbio.2011.11.017

Ekinci, D., Çavdar, H., Talaz, O., Şentürk, M., Supuran, C. T. (2010). NO-releasing esters show carbonic anhydrase inhibitory action against human isoforms I and II. Bioorganic & Medicinal Chemistry, 18(10), 3559-3563. Doi: https://doi.org/10.1016/j.bmc.2010.03.082

Fu, W., Chen, J., Cai, Y., Lei, Y., Chen, L., Pei, L., Zhou, D., Liang, X., Ruan, J. (2010). Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. Journal of Ethnopharmacology, 130(3), 521–528. Doi: https://doi.org/10.1016/j.jep.2010.05.039

Gençer, N., Bilen, Ç., Demir, D., Atahan, A., Ceylan, M., Küçükislamoğlu, M. (2013). *In vitro* inhibition effect of some chalcones on erythrocyte carbonic anhydrase I and II. Artificial Cells, Nanomedicine, and Biotechnology, 41(6), 384-388. Doi: https://doi.org/10.3109/21691401.2012.761226

Güney, M., Çavdar, H., Şentürk, M., Ekinci, D. (2015). Synthesis and carbonic anhydrase inhibitory properties of novel uracil derivatives. Bioorganic & Medicinal Chemistry Letters, 25(16), 3261-3263. Doi: https://doi.org/10.1016/j.bmcl.2015.05.073

Iranshahi, M., Rezaee, R., Najaf Najafi, M., Haghbin, A., Kasaian, J. (2018). Cytotoxic activity of the genus *Ferula* (Apiaceae) and its bioactive constituents. Avicenna Journal of Phytomedicine, *8*(4), 296-312. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6204145/

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., Heinonen, M. (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agricultural and Food Chemistry, 47(10), 3954-3962. Doi: https://doi.org/10.1021/jf990146l

Karataş, M. O., Alici, B., Çakir, Ü., Çetinkaya, E., Demir, D., Ergün, A., Gençer, N., Arslan, O. (2014). New coumarin derivatives as carbonic anhydrase inhibitors. Artificial Cells, Nanomedicine, and Biotechnology, 42(3), 192-198. Doi: https://doi.org/10.3109/21691401.2013.794352

Kaya, E., Erğun, B., Demir, Y., Alım, Z., Beydemir, Ş. (2019). The in vitro impacts of some plant extracts on carbonic anhydrase I, II and paraoxonase-1. Hacettepe Journal of Biology and Chemistry, 47(1), 51-59. Retrieved from https://dergipark.org.tr/en/pub/hjbc/issue/43015/553934

Korkmaz, N., Obaidi, O. A., Senturk, M., Astley, D., Ekinci, D., Supuran, C. T. (2015). Synthesis and biological activity of novel thiourea derivatives as carbonic anhydrase inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry, 30(1), 75-80. Doi: https://doi.org/10.3109/14756366.2013.879656

Kuzu, M., Aslan, A., Ahmed, I., Comakli, V., Demirdag, R., Uzun, N. (2016). Purification of glucose-6-phosphate dehydrogenase and glutathione reductase enzymes from the gill tissue of Lake Van fish and analyzing the effects of some chalcone derivatives on enzyme activities. Fish Physiology and Biochemistry, 42(2), 483-491. Doi: https://doi.org/10.1007/s10695-015-0153-7

Kuzu, M., Çomaklı, V., Akkemik, E., Çiftci, M., Küfrevioğlu, Ö. İ. (2018). Inhibitory properties of some heavy metals on carbonic anhydrase I and II isozymes activities purified from Van Lake fish *(Chalcalburnus tarichi)* gill. Fish Physiology and Biochemistry, 44(4), 1119-1125. Doi: https://doi.org/10.1007/s10695-018-0499-8

Le Darz, A., Mingot, A., Bouazza, F., Castelli, U., Karam, O., Tanc, M., Supuran, C. T., Thibaudeau, S. (2015). Fluorinated pyrrolidines and piperidines incorporating tertiary benzenesulfonamide moieties are selective carbonic anhydrase II inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry, 30(5), 737-745. Doi: https://doi.org/10.3109/14756366.2014.963072

Maślanka, T. (2015). A review of the pharmacology of carbonic anhydrase inhibitors for the treatment of glaucoma in dogs and cats. The Veterinary Journal, 203(3), 278-284. Doi: https://doi.org/10.1016/j.tvjl.2014.12.017

Mert, S., Alım, Z., İşgör, M. M., Beydemir, Ş., Kasımoğulları, R. (2016). The synthesis of novel pyrazole-3,4-dicarboxamides bearing 5-amino-1,3,4-thiadiazole-2-sulfonamide moiety with effective inhibitory activity against the isoforms of human cytosolic carbonic anhydrase I and II. Bioorganic Chemistry, 68, 64-71. Doi: https://doi.org/10.1016/j.bioorg.2016.07.006

Mete, E., Comez, B., Inci Gul, H., Gulcin, I., Supuran, C. T. (2016). Synthesis and carbonic anhydrase inhibitory activities of new thienyl-substituted pyrazoline benzenesulfonamides. Journal of Enzyme Inhibition and

Medicinal Chemistry, 31, 1-5. Doi: https://doi.org/10.1080/14756366.2016.1181627

Nagao, C., Nagano, N., Mizuguchi, K. (2014). Prediction of detailed enzyme functions and identification of specificity determining residues by random forests. PLoS ONE, 9(1), e84623. Doi: https://doi.org/10.1371/journal.pone.0084623

Nar, M., Çetinkaya, Y., Gülçin, İ., Menzek, A. (2013). (3,4-Dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives as carbonic anhydrase isoenzymes inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry, 28(2), 402-406. Doi: https://doi.org/10.3109/14756366.2012.670807

Nguir, A., Mabrouk, H., Douki, W., Ben Ismail, M., Ben Jannet, H., Flamini, G., Hamza, M. A. (2016). Chemical composition and bioactivities of the essential oil from different organs of *Ferula communis* L. growing in Tunisia. Medicinal Chemistry Research, 25(3), 515-525. Doi: https://doi.org/10.1007/s00044-016-1506-1

Nocentini, A., Vullo, D., Bartolucci, G., Supuran, C. T. (2016). N-Nitrosulfonamides: A new chemotype for carbonic anhydrase inhibition. Bioorganic & Medicinal Chemistry, 24(16), 3612-3617. Doi: https://doi.org/10.1016/j.bmc.2016.05.072

Orhan, F., Şentürk, M., Supuran, C. T. (2016). Interaction of anions with a newly characterized alpha carbonic anhydrase from *Halomonas* sp. Journal of Enzyme Inhibition and Medicinal Chemistry, 31(6), 1119-1123. Doi: https://doi.org/10.3109/14756366.2015.1100177

Ozensoy Guler, O., Capasso, C., Supuran, C. T. (2016). A magnificent enzyme superfamily: carbonic anhydrases, their purification and characterization. Journal of Enzyme Inhibition and Medicinal Chemistry, 1-6. Doi: https://doi.org/10.3109/14756366.2015.1059333

Ozensoy Guler, O., Supuran, C. T., Capasso, C. (2020). Carbonic anhydrase IX as a novel candidate in liquid biopsy. Journal of Enzyme Inhibition and Medicinal Chemistry, 35(1), 255-260. Doi: https://doi.org/10.1080/14756366.2019.1697251

Pernin, A., Dubois-Brissonnet, F., Roux, S., Masson, M., Bosc, V., Maillard, M. N. (2018). Phenolic compounds can delay the oxidation of polyunsaturated fatty acids and the growth of Listeria monocytogenes: structure-activity relationships. Journal of the Science of Food and Agriculture,98(14), 5401-5408. Doi: https://doi.org/10.1002/jsfa.9082

Rahali, F. Z., Kefi, S., Bettaieb Rebey, I., Hamdaoui, G., Tabart, J., Kevers, C., Franck, T., Mouithys-Mickalad, A., Hamrouni Sellami, I. (2018). Phytochemical composition and antioxidant activities of different aerial parts extracts of *Ferula communis* L. Plant Biosystems-An International Journal Dealing with All Aspects of Plant Biology, 1–9. Doi: https://doi.org/10.1080/11263504.2018.1461696

Salehi, M., Naghavi, M. R., Bahmankar, M. (2019). A review of *Ferula* species: Biochemical characteristics, pharmaceutical and industrial applications, and suggestions for biotechnologists. Industrial Crops and Products, 139, 111511. Doi: https://doi.org/10.1016/j.indcrop.2019.111511

Scozzafava, A., Mastrolorenzo, A., Supuran, C. T. (2004). Modulation of carbonic anhydrase activity and its applications in therapy. Expert Opinion on Therapeutic Patents, 14(5), 667-702. Doi: https://doi.org/10.1517/13543776.14.5.667

Supuran, C. T. (2008). Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nature Reviews Drug Discovery, 7(2), 168-181. Doi: https://doi.org/10.1038/nrd2467

Taskin, T., Levent, M., Sahin, T. (2019). Investigation of the effects of biological activity and steeping time of four different black teas commercially valuable in Turkey. Clinical and Experimental Health Sciences, 9 (4), 283-287. Doi: https://doi.org/10.33808/clinexphealthsci.516099

Taslimi, P., Gülçin, İ., Öztaşkın, N., Çetinkaya, Y., Göksu, S., Alwasel, S. H., Supuran, C. T. (2016). The effects of some bromophenols on human carbonic anhydrase isoenzymes. Journal of Enzyme Inhibition and Medicinal Chemistry, 31(4), 603-607. Doi: https://doi.org/10.3109/14756366.2015.1054820

Taşkın, D., Taşkın, T., Rayaman, E. (2018). Phenolic composition and biological properties of *Achillea nobilis* L. subsp. *neilreichii* (Kerner*)* Formanek. Industrial Crops and Products, 111, 555-562. Doi: https://doi.org/10.1016/j.indcrop.2017.11.022

Türkoğlu, E. A., Şentürk, M., Supuran, C. T., Ekinci, D. (2017). Carbonic anhydrase inhibitory properties of some uracil derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry, 32(1), 74-77. Doi: https://doi.org/10.1080/14756366.2016.1235043

Ucar, A., Findik, M., Kuzu, M., Pehlivanoglu, S., Sayin, U., Sayin, Z., Akgemci, E. G. (2020). Cytotoxic effects, microbiological analysis and inhibitory properties on carbonic anhydrase isozyme activities of 2-hydroxy-5-methoxyacetophenone thiosemicarbazone and its Cu(II), Co(II), Zn(II) and Mn(II) complexes. Research on Chemical Intermediates, 47, 533-550. Doi: https://doi.org/10.1007/s11164-020-04284-8

Uygun, M., Karagözler, A. A., Denizli, A. (2014). Molecularly imprinted cryogels for carbonic anhydrase purification from bovine erythrocyte. Artificial Cells, Nanomedicine, and Biotechnology, 42(2), 128-137. Doi: https://doi.org/10.3109/21691401.2013.864663

Verpoorte, J.A., Mehta, S., Edsall, J.T., (1967). Esterase activities of human carbonic anhydrases B and C. Journal of Biological Chemistry, 242; 4221-4229. Doi: https://doi.org/10.1016/S0021-9258(18)95800-X