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Adres : Kahramanmaraş Sütçü İmam Üniversitesi Teknik Bilimler Meslek Yüksek Okulu Gıda İşleme  
Telefon : (344)300-2480  
e-Posta:elifekaya@ksu.edu.tr

Bilgi için: Elife KAYA  
Unvan: Dr.Öğr.Üyesi  
Telefon : (344)300-2480





# 7<sup>th</sup> ICANAS 2024

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# Acetylcholinesterase, Butyrylcholinesterase, and $\alpha$ -Amylase Enzymes Inhibitory Potentials of *Cichorium Pumilum* Extracts

E. Kaya<sup>1</sup>, Ö. Narlı<sup>2</sup> and E. Kök<sup>3</sup>

<sup>1</sup>Department of Food Processing, Technical Sciences Vocational School, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Türkiye, ORCID: 0000-0001-7213-3601

<sup>2,3</sup>Department of Bioengineering and Sciences, Institute of Natural and Applied Sciences, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Türkiye, <sup>2</sup>ORCID: 0000-0003-1218-019, <sup>3</sup>ORCID: 0009-0009-9339-5964

## Abstract

*Cichorium pumilum*, a member of the Astreacea family, is a perennial bushy plant species common to the Eastern Mediterranean region that produces blue or lavender blossoms. *Cichorium* species have many bioactive components. In this study, the in vitro inhibitory potentials of extracts of the aerial parts of *C. pumilum* prepared in solvents of different polarities (water, ethanol, and ethyl acetate) on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and  $\alpha$ -amylase enzymes were investigated. According to the results of the study, ethanol extract showed the highest inhibitory effect on both AChE (IC<sub>50</sub>:10.86  $\mu$ g/mL) and BChE enzymes (IC<sub>50</sub>:18.05  $\mu$ g/mL), respectively. Additionally, it was found that the water extract demonstrated the most potent inhibitory impact on  $\alpha$ -amylase, with an IC<sub>50</sub> value of 35.19  $\mu$ g/mL. These findings indicate that the extracts have anticholinergic and antidiabetic properties, emphasizing the need for further in vivo investigations for the treatment of diseases caused by associated enzymes.

**Keywords:** Enzyme inhibition, acetylcholinesterase, butyrylcholinesterase,  $\alpha$ -amylase

## Introduction

*Cichorium pumilum* Jacq. is a member of the Asteraceae family. The Asteraceae family has three subfamilies. These are the Barnadesioideae, Cichorioideae, and Asteroideae. *Cichorium* species plants belong to the subfamily Cichorioideae. This family includes *C. intybus*, *C. endivia*, *C. bottae*, *C. spinosum*, *C. calvum* and *C. pumilum* [1]. *C. pumilum* (often known as chicory) has lavender or blue-colored flowers. It is a bushy perennial plant occasionally referred to as dwarf chicory [2]. *Cichorium* species are widely used in traditional medicine.

The chemical composition of *Cichorium* species consists of phenolic and polyphenolic compounds, fatty and organic acids and essential oils. Furthermore, research on these species has shown that they possess different pharmacological activities such as

antioxidant, antiproliferative, anti-inflammatory, antibacterial, antihyperglycemic, antidiabetic, and hepatoprotective effects [3].

In recent years, chicory has also been widely used in the food industry. This plant has also long been consumed by people as a vegetable [4]. As an example, chicory roots have gained popularity as a coffee replacement, replacing traditional brews in many parts of the World [5]. One of the most important molecules found in chicory root, inulin, has been the focus of a great deal of research about the prebiotic effect it displays [6]. *C. pumilum* leaves are utilized in the Eastern Mediterranean regions for the treatment of several health conditions, including diabetes, bacterial infections, poisoning, and rheumatism [7].

Many chronic diseases, including diabetes and Alzheimer's disease, are linked to the types and quality of foods consumed. In particular, secondary metabolites such as phenolic structures of various plant-based products are reported to be beneficial against such chronic diseases [8]. It is important to investigate the biological activity of *Cichorium* Species consumed as food. Furthermore, phenolic compounds are known to influence biological processes in cells by inhibiting certain metabolic enzymes.

Alzheimer's disease (AD) is a complex, progressive, and multifaceted neurological illness. Cholinesterase inhibitor treatment for Alzheimer's disease patients improves cognitive, behavioral, and functional symptoms [9]. However, because the inhibitory drugs utilized have numerous negative effects, various natural inhibitors of the acetylcholinesterase enzyme have been investigated.

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to a lack of insulin or resistance. Herbal extracts and their ingredients have received wide attention in clinical settings as safe antioxidants and potential inhibitors of important metabolic enzymes. For example,  $\alpha$ -glycosidase and  $\alpha$ -amylase are critical digestive enzymes involved in carbohydrate metabolism. They play a significant role in decreasing high blood sugar levels (hyperglycemia) in diabetic patients [10]. Plants are traditionally used to treat diseases, including diabetes. The species *Cichorium endivia* [11] and *Cichorium intybus* [12,13], which belong to the *Cichorium* family, have been reported for having antidiabetic effects.

The main objective of this study was to determine the possible inhibitory effects of different polarity extracts (water, ethanol, and ethyl acetate) prepared from the aerial parts of *C. pumilum* against AChE, BChE, and  $\alpha$ -amylase enzymes. In the literature, it was observed that studies investigating *C. pumilum*'s inhibitory effect on enzymes associated with Alzheimer's disease were limited. The purpose of evaluating these inhibitory effects is to demonstrate the plant's potential antidiabetic and neuroprotective properties.

## Material and Methods

### Plant Materials

The *Cichorium pumilum* Jacq. plant used in this study was collected from the Hasanaga region of Kahramanmaraş in September 2022. The species identification of the plant was made by [Asst. Prof.](#) Alper UZUN (Kahramanmaraş Sutcu Imam University, Faculty of

Forestry, Türkiye). It was deposited at the Kahramanmaraş Sutcu İmam University Faculty of Forestry Herbarium (KASOF) with herbarium number KASOF-3903.

### **Extracts Preparation**

The plant, which were pulverized in a laboratory grinder, were taken at 10 grams each and mixed homogeneously with 200 mL of ultrapure water, ethanol, and ethyl acetate solvents in a magnetic stirrer for 24 hours. Then, all extracts were filtered in turn. The ethanol and ethyl acetate extracts were separated from their solvents using an evaporator. The plant's water extract was frozen at 20°C and then lyophilized.

### **Acetylcholinesterase/ Butyrylcholinesterase inhibition studies**

Water, ethanol, and ethyl acetate extracts of the aerial parts of *C. pumilum* were examined for their effects on acetylcholinesterase and butyrylcholinesterase enzymes using the Ellman method [14].

In the study, control tubes and sample tubes were prepared. Acetylthiocholine iodide/ butyrylthiocholine iodide, DTNB, Tris-HCl, purified water, AChE/BChE enzyme and extracts were added to the tubes and mixed. After the reaction started, the mixtures' absorbance was measured at 412 nm. The activity (%)-[extract] graph was drawn using the obtained data. IC<sub>50</sub> values were calculated using the graph.

### **α-amylase inhibition studies**

The antidiabetic potential of the plant was assessed by examining the inhibitory effects of water, ethanol, and ethyl acetate extracts on the α-amylase enzyme. α-amylase inhibition experiments were performed using previously reported methods [15].

Sodium phosphate buffer (10 mM, pH:6.9, 0.006 M NaCl) was prepared. The extracts were diluted in ethanol to make stock solutions containing different concentrations. α-amylase enzyme (1.5 µl enzyme) was prepared by dissolving in 10 ml sodium phosphate buffer. The test tubes were filled with buffer, sample, and enzyme solutions and incubated for 25 minutes at 37°C. Next, a 1% starch solution was added to test tubes and incubated at 37°C for 25 minutes. After that, DNS solution was added, and all test tubes were placed in a boiling water bath for 5 minutes. After the tubes were vortexed, their absorption was measured to at 540 nm. The results were calculated as IC<sub>50</sub> values.

### **Result and Discussion**

Many treatments used to relieve symptoms of disorders such as Alzheimer's, diabetes, and obesity include the inhibition of metabolic enzymes [16]. In this context, cholinesterases have been associated with several neurodegenerative diseases, such as Alzheimer's disease. There has also been a lot of research on the metabolic enzyme inhibition potential of pharmaceuticals and natural extracts.

This study was investigated the inhibitory effects of water, ethanol, and ethyl acetate extracts of *C. pumilum* on the enzymes acetylcholinesterase, butyrylcholinesterase, and α-amylase. The results are shown in Table 1 with IC<sub>50</sub> values.

**Table 1.** The enzyme inhibition results (IC<sub>50</sub> values) of the extracts on AChE, BChE and  $\alpha$ -amylase enzymes

Compounds	AChE( $\mu$ g/mL)		BChE( $\mu$ g/mL)		$\alpha$ -amylase( $\mu$ g/mL)	
	IC <sub>50</sub>	r <sup>2</sup>	IC <sub>50</sub>	r <sup>2</sup>	IC <sub>50</sub>	r <sup>2</sup>
Water extract	15.60	0.9654	30.15	0.9514	35.19	0.9923
Ethanol extract	10.86	0.9912	18.05	0.9346	44.32	0.9715
Ethyl acetate extract	29.26	0.9732	38.12	0.9596	59.55	0.9803

In this study, the ethanol extract shown significant inhibition on AChE/BChE, with IC<sub>50</sub> values of 10.86  $\mu$ g/mL (r<sup>2</sup>: 0.9912) and 18.05  $\mu$ g/mL (r<sup>2</sup>: 0.9346), respectively. Ethyl acetate extract showed the weakest effect on both the AChE and BChE enzymes (Table 1). Previous study has indicated that extracts obtained from the roots of *Cichorium intybus* L. exhibit a potent inhibitory effect on the AChE enzyme [17]. As can be seen in the study, extracts containing significant phytochemicals are thought to inhibit cholinesterase enzymes and may be useful in the treatment of neurodegenerative illnesses.

Inhibition of AChE/BChE and  $\alpha$ -amylase enzymes plays important roles in the treatment of Alzheimer's disease, hyperglycemia, and diabetes [18]. In this study (Table 1), it was found that the water extract of *C. pumilum* exhibited the most effective inhibitory effect against  $\alpha$ -amylase. However, ethyl acetate extract demonstrated the lowest inhibitory effect against  $\alpha$ -amylase. In another study, water extract of *Cichorium endivia* leaves also showed inhibitory effect on the  $\alpha$ -amylase enzyme [11]. The results of this study are in agreement with previous studies on the inhibition effects of different *cichorium* species on  $\alpha$ -amylase activities.

## Conclusions

The study found that extracts of *C. pumilum* at different polarity inhibited AChE, BChE, and  $\alpha$ -amylase enzymes. These findings indicate that *C. pumilum* has potential neuroprotective and antidiabetic properties. The results will contribute to a better knowledge of *C. pumilum*'s biological activities and will help in the search for promising natural therapeutic agents, particularly for the treatment of chronic illnesses like Alzheimer's disease and diabetes.

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