Research Article

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INVESTIGATION OF THE ANTIEPILEPTIC EFFECTS OF WATER AND ETHANOL EXTRACTS OF *ARTEMISIA ABSINTHIUM* L.

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Keywords

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ABSTRACT

Epilepsy is a neurological condition that affects millions of people worldwide, regardless of social class, age, or race discrimination. Current medications used in the treatment of epilepsy are unable to provide a curative effect on more than twenty percent of patients, necessitating the need for additional treatment methods. Artemisia absinthium L., traditionally used for its insecticidal, antiseptic, antispasmodic, liver-inflammatory, and fever-reducing properties, is also employed in Turkish folk medicine as an antipyretic, antihelminthic, diuretic, tonic, and for relieving stomach pain. Carbonic anhydrase (CA) is a transmembrane metalloenzyme found in the structure of living organisms from the simplest to the most advanced, catalyzing the conversion of carbon dioxide to bicarbonate and containing a Zn2+ ion in its structure. CA inhibitors are used clinically as diuretics, antiglaucoma agents, and anti-epileptics. In our study, the effects of water and ethanol extracts of Artemisia absinthium L., collected in April, on carbonic anhydrase isoenzymes I and II were investigated spectrofotometrically. It was observed that both water and ethyl alcohol extracts did not significantly affect the enzyme activity. The ethyl alcohol extract of A. absinthium exhibited a higher inhibition potential compared to the water extract. However, when the in vitro enzyme activity test results were examined, it was found that these extracts did not have a significant impact on enzyme activities. This result, which is inconsistent with the literature, is thought to be due to the fact that the plant was not collected during its flowering period.

INTRODUCTION

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Epilepsy is a neurological condition that affects millions of people worldwide without regard to social class, age, or race. Three-quarters of these individuals live in areas where access to healthcare and treatment is limited[1]. Many people with this condition fear stigmatization in society, leading them to keep their symptoms hidden from others[2]. As a result, the data obtained from epidemiological studies on the prevalence of the disease can be insufficient in some cases. Seizures and the physical and psychological consequences they bring can have a negative impact on patients. However, treatment with antiepileptic drugs, the first-line therapy for epilepsy, has reduced the presence of these seizures by up to seventy percent[3]. Nevertheless, current medications used in epilepsy treatment are unable to provide a curative effect for more than twenty percent of patients, necessitating the need for additional treatment methods[4].

Artemisia genus is one of the most widespread and diverse genera in the Asteraceae family, containing numerous aromatic and medicinal species. This genus is predominantly found in temperate regions of Asia, Europe, and North America and comprises more than 500 species. The species we used in our

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Artemisia absinthium L., is commonly known as "Wormwood" or "Pelin otu" in local parlance. It is a perennial shrub found in Asia, the Middle East, Europe, and North Africa[5]. Its flowering period occurs between June and September. Traditionally, it has been used for its insecticidal, antiseptic, antispasmodic, liver-inflammatory, and fever-reducing properties. In Turkish folk medicine, it is also employed as an antipyretic, antihelminthic, diuretic, tonic, and for relieving stomach pain[6-8].

Carbonic anhydrase (CA), a transmembrane metalloenzyme found in the structure of living organisms ranging from the simplest to the most advanced, catalyzes the conversion of carbon dioxide to bicarbonate and contains a Zn^{2}+ ion in its structure. Carbonic anhydrase plays crucial roles in mechanical, physical, and biochemical aspects. CA enzyme is known to function in maintaining acid-base balance and regulating pH in various tissues and metabolic processes. Additionally, it is involved in physiological and pathological events such as ureagenesis, gluconeogenesis, lipogenesis, electrolyte secretion, bone resorption, and tumorigenesis[9].

CA inhibitors are commonly used in clinical practice as diuretics, anti-glaucoma agents, and anti-epileptics. However, recently, new applications have been reported for these inhibitors in the treatment of cancer, neuropathic pain, sleep apnea, migraine, reducing intracranial pressure, and treating cerebral ischemia. This indicates that CA inhibitors are being explored for their potential therapeutic effects in a broader range of medical conditions, and their use is being investigated in various medical fields. These developments suggest that this class of drugs may have a role in addressing a wider range of medical issues in the future[10].

The aim of this study is to investigate the effects of water and ethanol extracts of the aerial parts of *Artemisia absinthium* L., collected in April, on carbonic anhydrase isoenzymes I and II spectrophotometrically.

Collection of Plant Materials and Preparation of Plant Extracts

Samples of *Artemisia absinthium* L. were collected in April from Hatay province, at an altitude of 50 meters, with coordinates of 36° 7' 16" North and 35° 55' 39" East. The collected plant species were identified Assist. Prof. Dr. Hulya Ozpinar. After collection and drying, the plant samples were initially washed with tap water and then with distilled water. Subsequently, they were dried. Next, the samples were ground in a grinder, and 100 grams of the ground material were taken and mixed with ethanol. After being left in a shaker for 48 hours, the samples were filtered through filter paper, and the filtrates were collected. The liquid portion was then completely evaporated using an evaporator (equipped with a Vacuum Pump V300 and Control Unit I-300, Buchi R-100). The same method was used for preparing the water extract.

Investigation of the Effects of Plant Extracts on Carbonic Anhydrase (CA) Isoenzymes Using the Esterase Activity Method

The method used to spectrophotometrically detect the antiepileptic, antiglaucoma, and anti-diuretic effects of the obtained extracts *in vitro* is the esterase activity method. This method relies on the esterase activity of carbonic anhydrase for the hydrolysis of *p*-nitrophenyl acetate as a substrate, which is part of the reaction mechanism involving *p*-nitrophenol or *p*-nitrophenolate. Both *p*-nitrophenol and *p*-nitrophenolate exhibit the same absorbance at 348 nm.

In this method, first, the data were prepared as described in the section for preparing solutions in Table 1. Subsequently, the spectrophotometer device at 348 nm was zeroed using the cuvette content prepared in accordance with the instructions. Then, a cuvette content containing the enzyme, differing from the blank cuvette, was prepared according to Table 1 and placed into the spectrophotometer device. Absorbance was measured at the first minute and the third minute at 348 nm. The difference in absorbance between these measurements represented the enzyme activity, i.e., the control activity. Then, plant extracts at different concentrations were added, and absorbances were measured again at 0 and 3 minutes, and the differences were calculated. The absorbance differences obtained from different concentrations of plant extracts were compared to the control activity. If the plants yielded higher absorbance compared to the control, it means they increased enzyme activity, whereas if they gave a lower absorbance difference, it means they decreased enzyme activity. To better understand this, the obtained enzyme activity values were converted to percentage activity, and graphs were plotted in Excel based on different concentrations of plant extracts (Figures 3 and 4). The IC_{50} value,

which is the concentration of plant extract that reduces enzyme activity by half, was determined. (In this case, since the plant extracts increased enzyme activity, the IC_{50} values, which double the enzyme activity, were interpreted.)[12-14].

Table 1. *Composition of 1 mL cuvette content used in esterase activity studies for hCA isoenzymes.*

Substances Used in the Experiment	Content of Control Tube (Blank) (µL)	Content of Sample Tube
Tris-SO4 (0.5 M) pH 7.4	400	400
p-Nitrophenyl acetate	360	360
Distilled water	240	220
Enzyme solution	-	20
Total final volume	1000	1000

Effects of Artemisia absinthium L. Water and Ethanol Extracts on Carbonic Anhydrase Enzyme Activity

The effects of *Artemisia absinthium* L. extracts on carbonic anhydrase enzyme activity were assessed using the esterase activity method. The data from the study are presented in Figure 1, Figure 2, and Table 2.

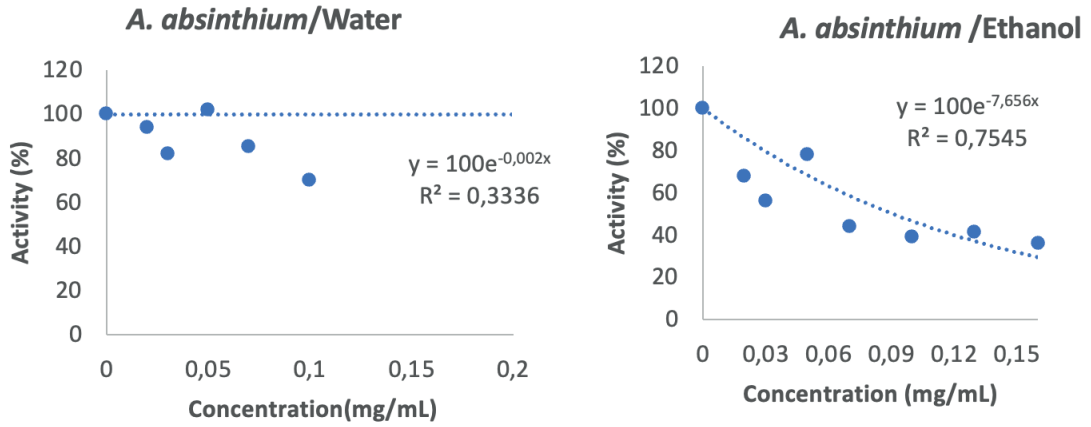

Figure 1. *Inhibition Graphs of Artemisia absinthium L. Extracts Against Carbonic Anhydrase I Enzymes.*

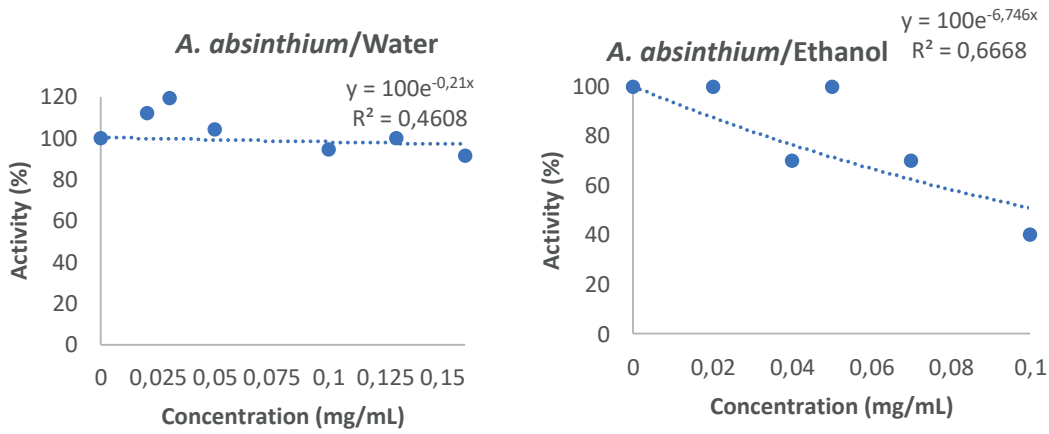

Figure 2. *Inhibition Graphs of Artemisia absinthium L. Extracts Against Carbonic Anhydrase II Enzymes.*

Table 2. *Summarized Inhibition Parameters of Artemisia absinthium L. Plant Extracts Against Human Carbonic Anhydrase I and II Isoforms.*

Comp. #	IC50 (mg/mL)			
	hCA I	r^2	hCA II	r^2
A. absinthium /Water	-	-	-	-
A. absinthium /Ethanol	0.090	0.624	0.102	0.675

Artemisia absinthium L. plant extracts were investigated spectrophotometrically for their effects on carbonic anhydrase isoenzyme I and II. The data obtained were used to plot the % Activity - Plant Extract Concentration graph (Figure 1 and 2). Based on this, it can be stated that both water and alcohol extracts of the plants did not have a significant effect on enzyme activity. It can be noted that the ethanol extract of *A. absinthium* exhibited a higher inhibition potential compared to the water extract.

CONCLUSION

In this study, the effects of *Artemisia absinthium* L. ethyl alcohol and water extracts on the carbonic anhydrase enzyme, which plays a role in various physiological and pathological processes in our body, primarily pH regulation, ureagenesis, gluconeogenesis, lipogenesis, electrolyte secretion, bone resorption, and tumorigenesis, were investigated. When the results of the in vitro enzyme activity experiments were examined, it was observed that these extracts did not have a significant impact on enzyme activities.

DISCUSSION

Plants are natural sources containing a variety of compounds with many different biological effects[15]. They play a significant role in the fight against numerous diseases such as cardiovascular diseases, diabetes, hypertension, Alzheimer's disease, atherosclerosis, and cerebral disorders[16]. The use of plants by humans dates back to the dawn of human history. Plants in both global and our country's flora are widely used not only in areas such as food, dye, resin, flavoring, and the cosmetic industry but also for therapeutic purposes. The World Health Organization (WHO) indicates that there are approximately 21,000 medicinal plants used for therapeutic purposes[17].

Oxidative stress, neuroinflammation, and excitotoxicity contribute to seizure-mediated neuronal damage and apoptosis, leading to potential effects such as epileptogenesis and cognitive impairment. There are numerous studies on the neuroprotective and antiepileptic potentials of certain species within the *Artemisia* genus, particularly the species we are working with, *Artemisia absinthium* L. This effect has been associated with antioxidant phenolic compounds that are effective against ROS-induced oxidative stress[18,19].

The reason for not being able to conclusively prove the antiepileptic potential of *Artemisia absinthium* L. in our study can be attributed to the timing of the species' collection. In the literature, this species is reported to have its flowering period in June and July in many studies where its antioxidant, antidepressant, and antiepileptic effects are observed[18,19].
The fact that there were no flower

parts in the above-ground portion of the samples collected in April may lead to differences in the study results due to its potential impact on the quantity and types of active substances in the extracts.

Conflict of interest statement

The authors declare that they have no conflicts of interests.

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