Research Article

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Exploring the enzyme inhibitory properties of Antarctic algal extracts

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Abstract

Objectives: Marine organisms obtained from Antarctica are prominent sources for many important activities. Algae are known for adapting to various adverse environmental conditions and for producing secondary metabolites with various biological activities. This study examined the enzyme inhibitory properties of six different Antarctic algal extracts.

Methods: We investigated the activity of specific enzymes, including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carbonic anhydrase (CA I/II), glutathione reductase (GR), and α-glucosidase (AG), as these enzymes have potential therapeutic applications such as in Alzheimer's disease, malaria, cancer, and diabetes mellitus.

Results: The results of the study found that the algal extracts had potent inhibitory effects on these enzymes, with IC_{50} values ranging from 0.60 to 48.85 µg/mL, indicating that these extracts could be source of potential new drugs. *Monostroma harioti* and *Cystosphaera jacquinotii* extracts demonstrated highest AChE and CA I enzymes inhibiton. *M. harioti* and *Desmarestia antarctica* extracts presented highest GR enzyme inhibiton, *C. jacquinotii* and *D. antarctica* extracts presented highest inhibitory activity against BChE, CA II and α-glucosidase enzymes.

Conclusions: Extracts of algae samples taken from Antarctica have high enzyme inhibitory activity, and further studies are needed to find out which compounds may be responsible for the effect.

Keywords: cholinesterases; Antarctic algae extracts; enzyme inhibition; glutathione reductase; carbonic anhydrases; α-glucosidase

Introduction

Algae are a heterogeneous group of marine organisms commonly divided into microalgae and macroalgae, based on size. Macroalgae, also known as seaweeds, have a complex and dynamic taxonomy [1].

Climate extremes and biogeographic isolation from other continents by distance, high depths, and the Antarctic Circumpolar Current characterize Antarctica. Even under these harsh conditions, macroalgae thrive in different coastal ecosystems contributing to primary production and serving as habitat and food for a variety of species of marine fauna. Due to the extreme environmental conditions, Antarctic marine organisms can show unique properties.

In the Antarctic, the Desmarestiales are a primary constituent of the benthic marine algal flora. Desmarestia species are generally abundant on all Antarctic coasts where algae occur. *Desmarestia antarctica,* a brown algae, was first described in 1989 [2]. In a study on the sterol content of algae in Antarctica, B-sitosterol, fucosterol, ergosterol, cholesterol, campesterol, and brassicasterol were determined in *D. antarctica* [3].

In the chemical analysis study conducted on *D. antarctica*, fatty acids, esters, terpenes, and sterols were the most common substances in the crude extract. Antifouling activity has been reported in previous studies with these species [4]. The antiplasmodial and cytotoxic activity was also observed in activity studies on *D. antarctica* fractions [5]. In another study, the antibiotic activities of different algae found in Antarctica were examined, and *D. antarctica* was the second species that inhibited the most bacterial strains among these algae species. Lipophilic and hydrophilic extracts were

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obtained with ether and butanol. The lipophilic extract of *D. antarctica* was found to be effective on a greater number of bacterial strains compared to the hydrophilic extract [6].

Researchers studying *Monostroma hariotii,* a green algae, have seen that fungi isolated from this algae species can produce compounds with antifungal, trypanocidal, and antiviral effects [7–9]. Studies on *Gigartina skottsbergii,* a red algae, have focused on the antiviral, anticoagulant, and antiherpetic activities of sulfated polysaccharides and especially various carrageenans isolated from this alga [10–13]. In studies on *Palmaria decipiens*, it has been found that red algae contain different types of sterols such as fucosterol, stigmasterol, brassicasterol, and also microsporin-like amino acids, which have UV protective effects [14, 15]. It has been demonstrated that hydroquinone derivative compounds isolated from *Desmarestia menziesii,* a brown algae, show antinematode activity, and the aqueous extract of the algae also showed effective results as a reducing and stabilizing agent in nanoparticles [16–18].

CAs (EC 4.2.1.1) are metal-containing enzymes that catalyze the conversion of CO_2 to HCO_3^- and H^+. These enzymes are present in living organisms and are encoded by six gene families across different species [19]. Fifteen forms of the enzyme CA, belonging to the alpha-CA gene family, have been identified in humans. Among the human CA (hCA) isoenzymes, hCA I/II are cytosolic isoforms common in all tissues. Studying how CA activity can be inhibited or enhanced is crucial for developing treatments for many clinically significant diseases [19, 20]. Inhibitors of specific forms of the enzyme CAs (e.g., CA I/II) have been used to create new drugs for conditions such as epilepsy, edema, and glaucoma. As a result, new inhibitors of CA isoenzymes need to be developed because of their potential to be used as therapeutic agents [20–22].

Enzyme inhibitors have a significant impact on the treatment of various diseases. AChE (EC 3.1.1.7) is significant, as it plays a crucial role in ending the signaling process within the cholinergic system by breaking down acetylcholine (ACh), a neurotransmitter that plays a significant role in memory formation and the functioning of motor neurons [23].

AChE, located in the postsynaptic membrane, breaks down ACh to stop neuron signal transduction. BChE (EC 3.1.1.8) is primarily produced in the liver and found in various body areas, such as blood plasma and in the central and peripheral nervous systems [24]. Clinical trials have shown that ACE inhibitors of AChE can boost ACh in cholinergic synapses and enhance cholinergic activity [25]. Usually, ACh is broken down primarily by AChE compared to BChE. While BChE is believed to have only a minor impact on regulating ACh levels in the brain, it has been found to play a crucial role in drug metabolism and removing toxins from the body [26]. Specific inhibitors can be utilized to treat motor neuron diseases like dementia, myasthenia gravis, and Alzheimer's by decreasing the activity of AChE/BChE [23].

Approximately 90 % of all diabetes cases are type 2, and the number of cases has been increasing steadily over time [27]. Type 2 diabetes is characterized by a resistance to insulin and dysfunction in beta cells, which leads to decreased glucose uptake and high blood sugar levels after eating. This condition is becoming increasingly prevalent, particularly among younger adults, due to the increasing obesity epidemic and lack of physical activity [27–29]. Studies have shown that consuming a diet high in carbohydrates and foods with a high glycemic index can contribute to increased insulin and glucose levels and a higher risk of type 2 diabetes [30, 31]. In a clinical study, low-carbohydrate ketogenic diets were effective in improving markers of diabetes such as fasting insulin, glycated hemoglobin, body weight, and fasting glucose leading to a reduction or discontinuation of diabetes medications in 95.2 % of participants [32]. Elevated blood glucose levels can cause damage to various body systems, including blood vessels and nerves. One approach to reducing postprandial hyperglycemia is to inhibit the hydrolyzing enzymes that break down carbohydrates, such as alpha-glucosidase (EC 3.2.1.20), thereby reducing glucose absorption [33].

GR (EC 1.8.1.7) performs a vital role in the maintenance of the high GSH/GSSG ratio. It also plays a vital role by ensuring intracellular signal transmission, neutralizing free radicals and reactive oxygen species, and maintaining the redox balance within the cell. Under normal conditions, glutathione primarily exists in its reduced form (GSH), but can quickly be oxidized to GSSG in response to oxidative stress, thus protecting cellular components. GR, however, reduces GSSG back to GSH using NADPH, ensuring that the GSH/GSSG ratio remains at or above 99 % [34].

The vital role of GSH in various cellular processes is reflected in its correlation with numerous human diseases such as cardiovascular disease, cancer, AIDS, diabetes, and Alzheimer's disease. Moreover, GSH plays a crucial role in detoxifying heme, and an increase in intracellular GSH levels has been linked to the development of chloroquine resistance. Inhibitors of GR have been demonstrated to exhibit antimalarial and anticancer properties [35, 36].

In this study, some enzyme inhibitory properties of six different Antarctic algal extracts were examined.

Materials and methods

Chemicals and laboratory

All enzymes and chemicals used for experiments were bought from Sigma Aldrich (Germany). Extract preparation and enzyme inhibiton

studies were carried out in Central Research Laboratory of Agri İbrahim Cecen University.

Sample collection

Antarctic macroalgae samples were taken from the marine environment of Antarctica's Robert Island, King George Island and Nansen Island during the TAE-II (Turkish Antarctic Science Expedition 2018, -7 March-26 April 2018) expedition. With the SCUBA diving method, samples were collected from different depths and identified by Bülent Gözcelioğlu. The samples were brought to the laboratories in Turkey in −40 °C containers. Voucher specimens for collected algaes are available at Dr. Gozcelioğlu's publicly available collection.

Preparation of algal extracts

The wet algae were dried at room temperature and subjected to extraction. The coarsely ground algal material were extracted with 100 % methanol at room temperature. The extraction process was repeated three times, and the algaes were extracted with a magnetic stirrer for 12 h by adding 300 mL of 100 % methanol each time. The methanolic phases obtained at the end of the extraction were combined and the methanol was evaporated with a rotavapor and the crude extract for each algae species were obtained. Dry mass of extracts for *Cystosphaera jacquinotii, G. skottsbergii, P. decipiens, Desmarestia menziesi, M. hariotii* and *D. antarctica* numbered **1** to **6**, respectively.

Glutathione reductase inhibition

GR enzyme activity was measured using the Beutler method. The assay mixture included 0.2 mM NADPH, 100 mM (pH 7.4) phosphate buffer (PB), and 1 mM GSSG. The decrease in NADPH absorbance at 340 nm was monitored using spectrophotometry at 37 °C. The assay was conducted in triplicate [37, 38].

Carbonic anhydrase I/II inhibition

The activity of these isoenzymes was measured using spectrophotometry by observing the change in absorbance at 348 nm as 4-nitrophenyl acetate (NPA) was converted to 4-nitrophenolate (NP) over 3 min at 25 °C. The reaction mixture consisted of 1.4 mL of 50 mM Tris-SO4 buffer at pH 7.4, 1 mL of 3 mM NPA, 0.5 mL of water, and 0.1 mL of enzyme solution, for a total volume of 3.0 mL [39].

Cholinesterase enzymes inhibition

The assay system employed comprised a sample of the inhibitor, with a volume ranging from 5 to 60 µL, in conjunction with 200 µL of buffer, specifically 1 M Tris-HCl buffer for the assay of AChE and PB for the assay of BChE, both at a pH of 8.0. Additionally, 50 µL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.5 mM) and 50 mL of acetylthiocholine iodide/S-butrylthiocholine chloride (10 mM) were incorporated, as well as 10 µL of the enzyme, with a concentration of 0.28 U/mL for the AChE assay and 0.32 U/mL for the BChE assay [40]. The reaction was started by adding the enzyme, and the absorbance at 412 nm was followed at room temperature, measuring every two minutes for a total of 6 min. A control consisting of all the chemicals specified except the inhibitor was also performed [41].

Alpha-glucosidase inhibition

The inhibitory effects on the AG enzyme were determined using AG from Bacillus stearothermophilus and p-nitrophenyl-alpha-D-glucopyranoside (pNPG). Acarbose was added to the enzyme solution at a final concentration of 0.5–5.0 mg/mL by mixing 100 µL of 2–20 mg/mL acarbose with 50 µL of AG (1 U/mL) prepared in 100 mM PB (pH 6.9) and 250 µL of 100 mM PB. The mixture was pre-incubated at 37 °C for 20 min. Then, 10 µL of 10 mM pNPG prepared in 100 mM PB (pH 6.9) was added to the mixture and incubated at 37 °C for an additional 30 min. The reaction was stopped by adding 650 µL of 1 M Na_2CO_3, and the absorbance was measured at 405 nm using a spectrophotometer [42].

General enzyme inhibition studies

Inhibition activities of Antarctic algal extracts (1–6) on CA I/II, GR, AChE, BChE, and AG were defined using the spectrophotometric methods In this analysis, acetazolamide for CA I/II, galantamine for AChE/BChE, N,N-bis(2-chloroethyl)-N nitrosourea for GR, and acarbose for AG enzyme were used as reference molecules.

Stock solutions of the extracts under investigation were meticulously prepared by dissolving them in dimethyl sulfoxide to achieve a concentration of 1 mg per mL. The resulting stock solution was then meticulously diluted one thousandfold with distilled water. To evaluate the inhibitory activity of these extracts on the enzymes under examination, measurements were conducted at seven distinct concentrations. The methodology employed in this study has been previously described in detail in prior studies [22, 43, 44].

Statistical analysis of data

SPSS program was used for the analysis of the experimental data. Standard deviation for IC_{50} values were calculated and shown in Table 1.

Results

Among the six extracts tested against AChE enzyme, extract **2** had the weakest inhibitory effect with an IC_{50} value of 4.4 µg/mL. However, extracts **1** and **5** (with IC_{50} values of 0.8 and 0.6 µg/mL respectively) had a more potent inhibitory profile compared to the other four extracts (Figure 1). In addition, galantamine (IC_{50}: 0.4 µg/mL) was used as the reference molecule for the AChE enzyme in this study. When the IC_{50} values (in the range of 0.6–4.4 µg/mL) obtained for the whole extracts were compared with the results obtained for galatamine, it was determined that all algae extracts we used in this study were effective at the same rates as the reference molecule. In a study conducted by Imran et al. in 2020, they discovered that the IC_{50} values for the AChE

Table 1: IC50 values (μg/mL) of extracts against tested enzymes.

	AChE	BChE	CA I	CA II	GR	AG
Extract 1 *Cystosphaera jacquinotii*	0.80 ± 0.02	1.02 ± 0.01	0.48 ± 0.01	0.025 ± 0.001	27.23 ± 0.11	23.5 ± 0.1
Extract 2 *Gigartina skottsbergii*	4.40 ± 0.05	4.16 ± 0.05	0.93 ± 0.02	0.043 ± 0.001	25.17 ± 0.10	32.7 ± 0.1
Extract 3 *Palmaria decipiens*	2.65 ± 0.03	4.58 ± 0.05	0.87 ± 0.02	0.041 ± 0.001	35.24 ± 0.12	35.4 ± 0.1
Extract 4 *Desmarestia menziesi*	3.00 ± 0.03	4.60 ± 0.05	1.05 ± 0.02	0.042 ± 0.001	48.85 ± 0.15	36.1 ± 0.1
Extract 5 *Monostroma hariotii*	0.60 ± 0.01	3.09 ± 0.03	0.37 ± 0.01	0.038 ± 0.001	12.64 ± 0.09	27.4 ± 0.1
Extract 6 *Desmarestia antarctica*	1.83 ± 0.02	2.20 ± 0.02	0.76 ± 0.02	0.021 ± 0.001	21.34 ± 0.10	18.6 ± 0.1
Galantamine[a]	0.40 ± 0.10	2.20 ± 0.30	–	–	–	–
Acetazolamide	–	–	1.652 ± 0.03	0.016 ± 0.001	–	–
N,N-bis(2-chloroethyl)-N-nitrosourea	–	–	–	–	138.0 ± 0.2	–
Acarbose	–	–	–	–	–	26.4 ± 0.3

[a][45].

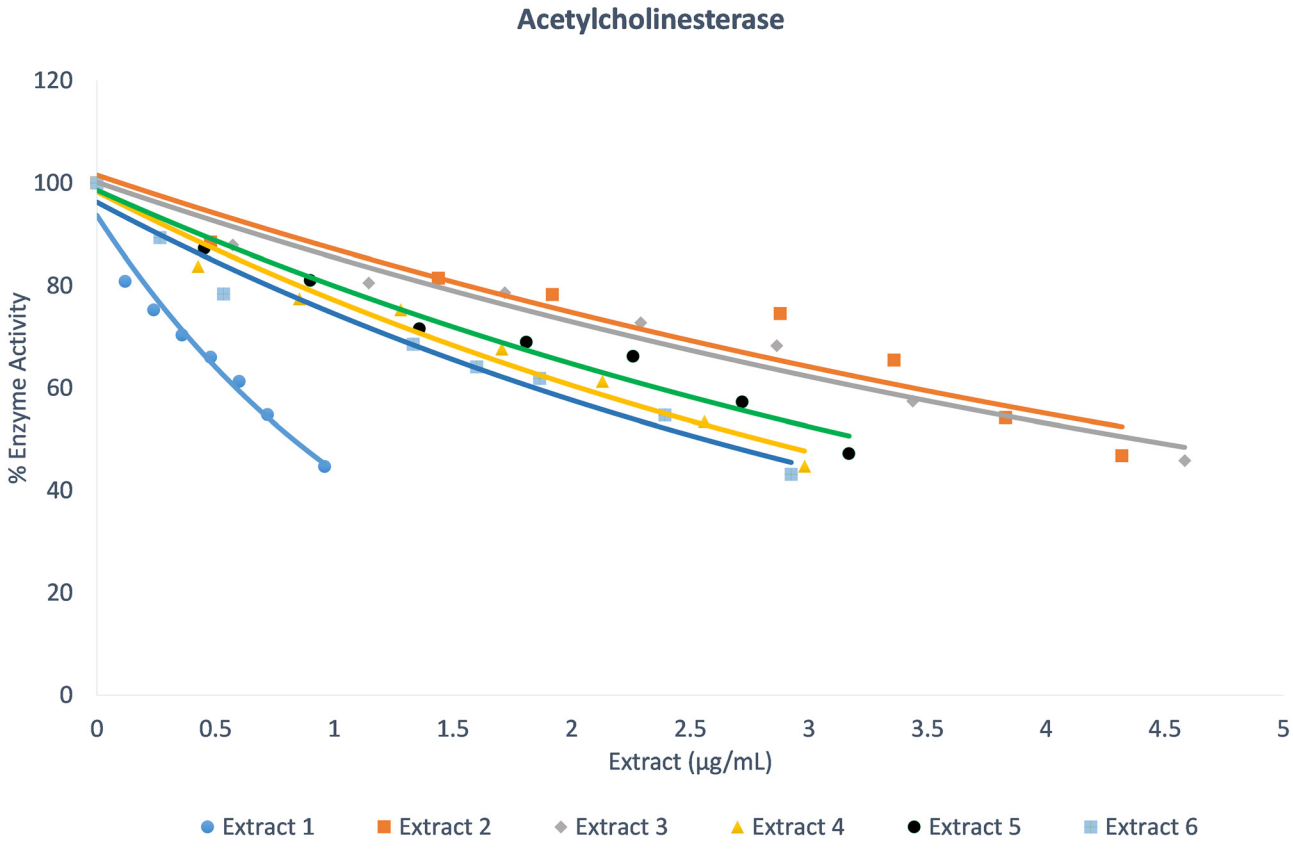


Figure 1: AChE enzyme inhibition of extracts.

enzyme were 75.23 μg/mL for the ethanol extract and 99.55 μg/mL for the ethyl acetate extract of *Oxalis corniculata* L [46]. When we compare our study's findings on the AChE enzyme with the results mentioned in the previous literature, it becomes evident that all algal extracts exhibit a higher level of effectiveness on the AChE enzyme.

In testing the inhibitory effects on BChE enzyme, extract 4 was found to have the least inhibitory effect, with an IC_{50} value of 4.60 μg/mL. Among the algal extracts tested (1–6), extract 1 was the most effective BChE inhibitor, with an IC_{50} value of 1.02 μg/mL (Figure 2). In addition, galantamine (IC_{50}: 2.2 μg/mL) was used as a reference molecule for the BChE enzyme in this study. When the IC_{50} values obtained for all extracts (in the range of 1.02–4.60 μg/mL) were compared with the results obtained for galatamine, we determined that all algae extracts we used in this study were effective at almost the same rates. In a study conducted by Imran and colleagues in 2020, they reported the IC_{50} values of *O. corniculata* L.

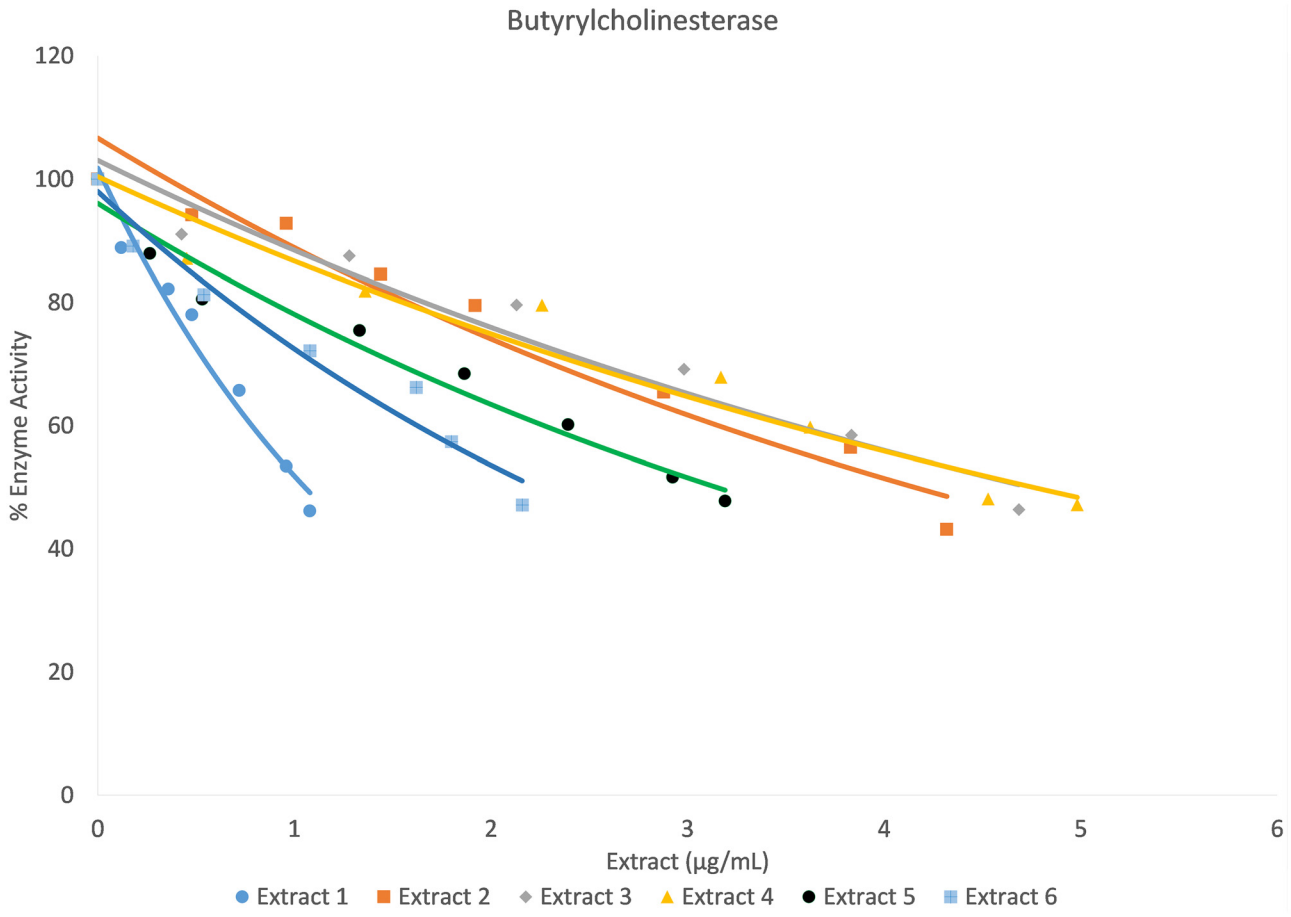


Figure 2: BChE enzyme inhibition of extracts.

ethanol and ethyl acetate extracts on the BChE enzyme as 72.05 µg/mL and 97.07 µg/mL, respectively [46]. Upon comparing the results obtained for the BChE enzyme in our study with the findings mentioned in the literature above, it becomes apparent that all algal extracts demonstrate a higher level of effectiveness on the BChE enzyme.

Among the extracts tested with the CA I isoenzyme, extract 4 had the weakest inhibitory effect with an IC_{50} value of 1.05 µg/mL. Even this value was a better score than the reference molecule acetazolamide. However, extracts 1 and **5** (with IC_{50} values of 0.37 and 0.48 µg/mL, respectively) had a much stronger inhibitory profile than the other four extracts (Figure 3). In addition, acetazolamide (IC_{50}: 1.652 µg/mL) was used as the reference molecule for the CA I enzyme in this study. When the IC_{50} values obtained for all extracts (in the range of 0.37–1.05 µg/mL) were compared with the results obtained for acetazolamide, we determined that all algae extracts we used in this study were even more effective. Ethanol, methanol and water extracts of *Alcea rosea, Foeniculum vulgare, Elettaria cardamomum, Laurus azorica* and *Lavandula stoechas* plants and IC_{50} values for CA I enzyme were determined. The IC_{50} values obtained for these plant extracts with CA I were found to be in the range of 32–991 µg/mL [47]. When the results of our study on the CA I enzyme were compared with the literature mentioned above, it was determined that all algae extracts showed better inhibition effects.

Among the extracts tested with the CA II isoenzyme, extract 2 had the weakest inhibitory effect with an IC_{50} value of 0.043 µg/mL. Even this value was a better score than the reference molecule acetazolamide. However, extracts 1 and 6 (with IC_{50} values of 0.025 and 0.021 µg/mL, respectively) had a much stronger inhibitory profile than the other four extracts (Figure 4). In addition, acetazolamide (IC_{50}: 0.016 µg/mL) was used as the reference molecule for the CA II enzyme in this study. When the IC_{50} values obtained for all extracts (in the range of 0.021–0.043 µg/mL) were compared with the results obtained for acetazolamide, we determined that all algae extracts we used in this study were almost equally effective. Ethanol, methanol and water extracts of *A. rosea, F. vulgare, E. cardamomum, L. azorica* and *L. stoechas* plants and IC_{50} values for CA II enzyme were determined. The IC_{50}

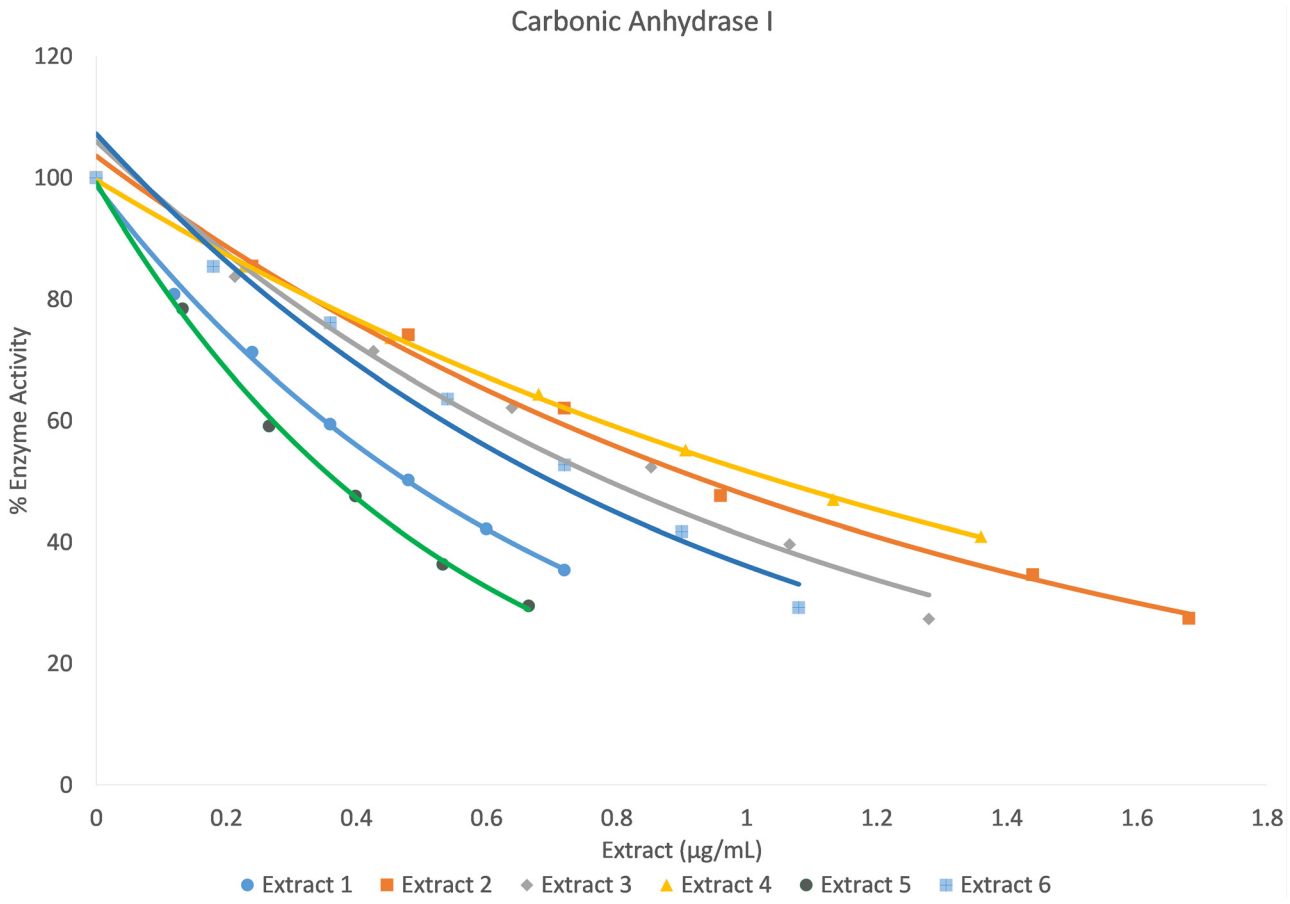


Figure 3: Carbonic anhydrase I enzyme inhibition of extracts.

values obtained for these plant extracts with CA II were found to be in the range of 54–1,400 µg/mL [47]. When the results of our study on the CA II enzyme were compared with the literature mentioned above, it was determined that all algae extracts showed better inhibition effects.

The IC_{50} values (12.64–48.85) obtained for six Antarctic algal extracts tested with the GR enzyme were higher when compared to the reference molecule N, N-bis(2-chloroethyl)-N nitrosourea (IC_{50}: 138 µg/mL). They were found to be effective inhibitors. Extract 4 had the weakest inhibitory effect with an IC_{50} value of 48.85 µg/mL. However, extract 5 had the highest inhibitory effect (12.64 µg/mL) (Figure 5). Kıvanç and Türkoğlu chromatographed the n-hexane extract of *Arum rupicola* Boiss var rupicola plant in their GR enzyme research. This process yielded seven different fractions labeled R1 to R7 using the hexane-ethyl acetate system. IC_{50} values could only be determined for the R4 and R6 fractions, 193 µg/mL for R4 and 3.98 µg/mL for R6 [48]. When the results of our study on the GR enzyme were compared with the literature mentioned above, it was determined that all algae extracts showed better inhibition effects.

It was determined that the IC_{50} values (18.6–36.1) obtained for six Antarctic algal extracts tested with the AG enzyme showed a closer inhibitory effect when compared to the reference molecule acarbose (IC_{50}: 26.4 µg/mL). Extract 4 had the weakest inhibitory effect with an IC_{50} value of 36.1 µg/mL. However, extract 6 (18.6 µg/mL) showed the best inhibitory profile (Figure 6). The IC_{50} value obtained for the AG enzyme with the ethyl acetate extract of *Hyptis monticola Mart ex. Benth.* flowers by Lianza et al. was found to be 15.2 mg/mL [45]. When the results of our study on the AG enzyme were compared with the literature mentioned above, it was determined that all algae extracts showed better inhibition effects.

Discussion

The present study elucidates the significant inhibitory potential of Antarctic algal extracts against a spectrum of key enzymes, shedding light on their promising bioactivity and therapeutic implications. Notably, the evaluation of AChE

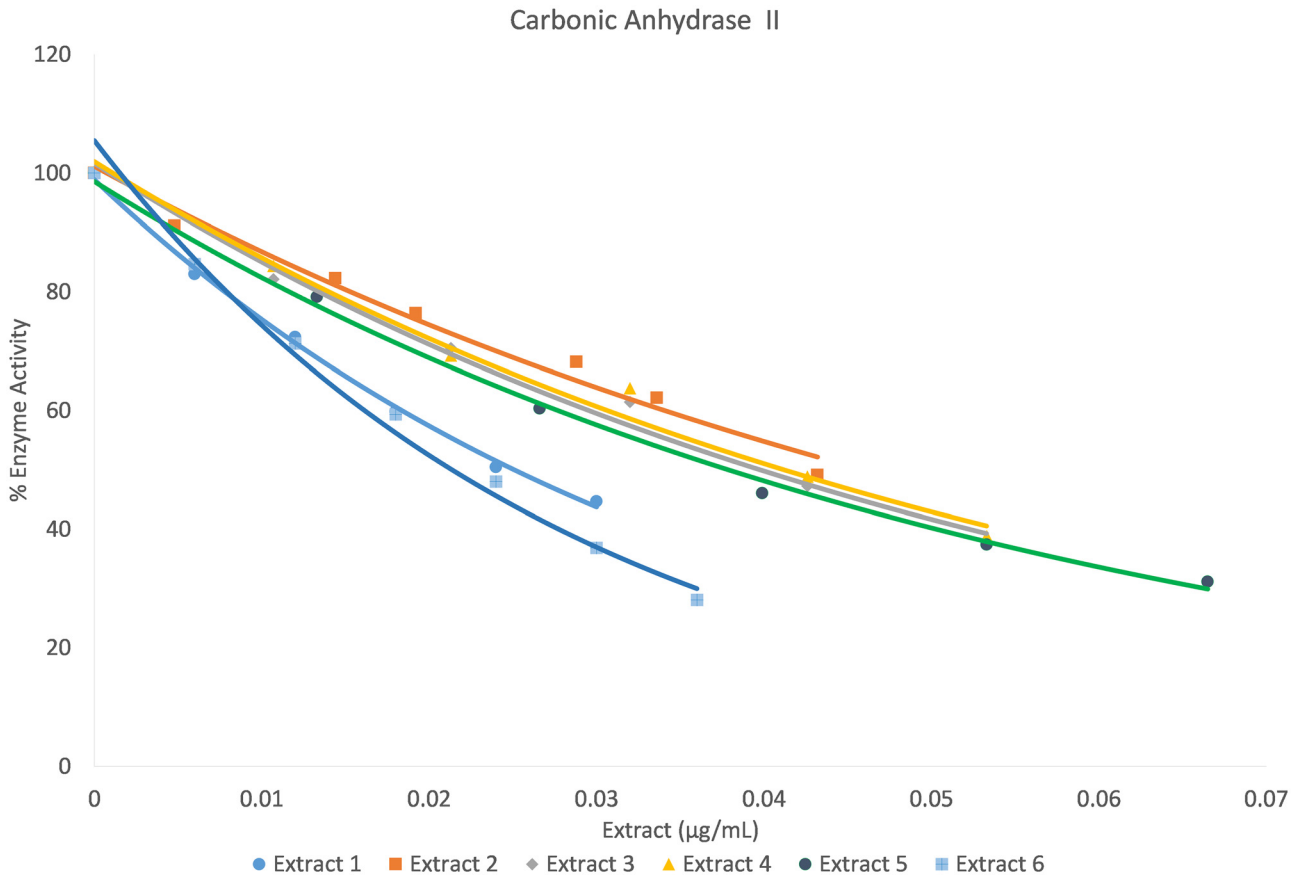


Figure 4: Carbonic anhydrase II enzyme inhibition of extracts.

inhibition revealed intriguing trends in extract effectiveness. While extract 2 exhibited the weakest inhibition, extracts 1 and 5 displayed substantially potent inhibitory profiles, surpassing the inhibitory capacity of galantamine, a recognized reference molecule. These findings suggest that these algal extracts possess notable AChE inhibitory properties, rendering them potential candidates for further investigation in neurodegenerative disorders characterized by cholinergic dysfunction. Comparing our results with a previous study by Imran et al., which reported lower IC_{50} values for the AChE enzyme inhibition using different plant extracts, underscores the superior efficacy of the algal extracts in our study. This highlights the potential of Antarctic algal resources as an alternative source for more effective cholinesterase inhibition.

Turning our attention to BChE inhibition, extract 1 emerged as the most potent inhibitor, surpassing the efficacy of galantamine. This finding, along with the consistency in inhibition profiles across all extracts, supports the notion that these algal extracts possess substantial BChE inhibitory potential. In contrast to the comparative study by Imran and colleagues, where the plant extracts exhibited lower inhibitory effects, our findings indicate a heightened level of BChE inhibition for algal extracts, possibly attributing this variation to the distinct bioactive constituents and environmental factors of the algal species.

Suganthy et al. studied the inhibitory effects of eight different seaweed extracts on choline esterase enzymes. They showed that *Hypnea valentiae* and *Ulva reticulata* exhibit mixed-type AChE and BChE inhibition. They determined that reticulata showed a strong inhibitory effect for both ChE enzymes and could act as a good neuroprotectant. *H. valentiae*, *Padina gymnospora*, *U. reticulata*, and *Gracilaria edulis* from eight seaweeds screened in this study showed inhibitory activity against AChE with IC_{50} values of 2.6, 3.5, 10, and 3 mg/mL, respectively. *H. valentiae*, *Enteromorpha intestinalis*, *Dictyota dichotoma*, and *U. reticulata* showed 50 % inhibition against BChE at concentrations of 3.9, 7, 6.5, and 10 mg/mL, respectively [49, 50]. In our study, it was observed that Antarctic algae extracts were more effective on AChE and BChE enzymes (Table 1).

Investigation of CA I and CA II enzyme inhibition further underscores the potential of these Antarctic algal extracts. The extracts' superior inhibitory effects, even in comparison with established reference molecules, point to their ability to

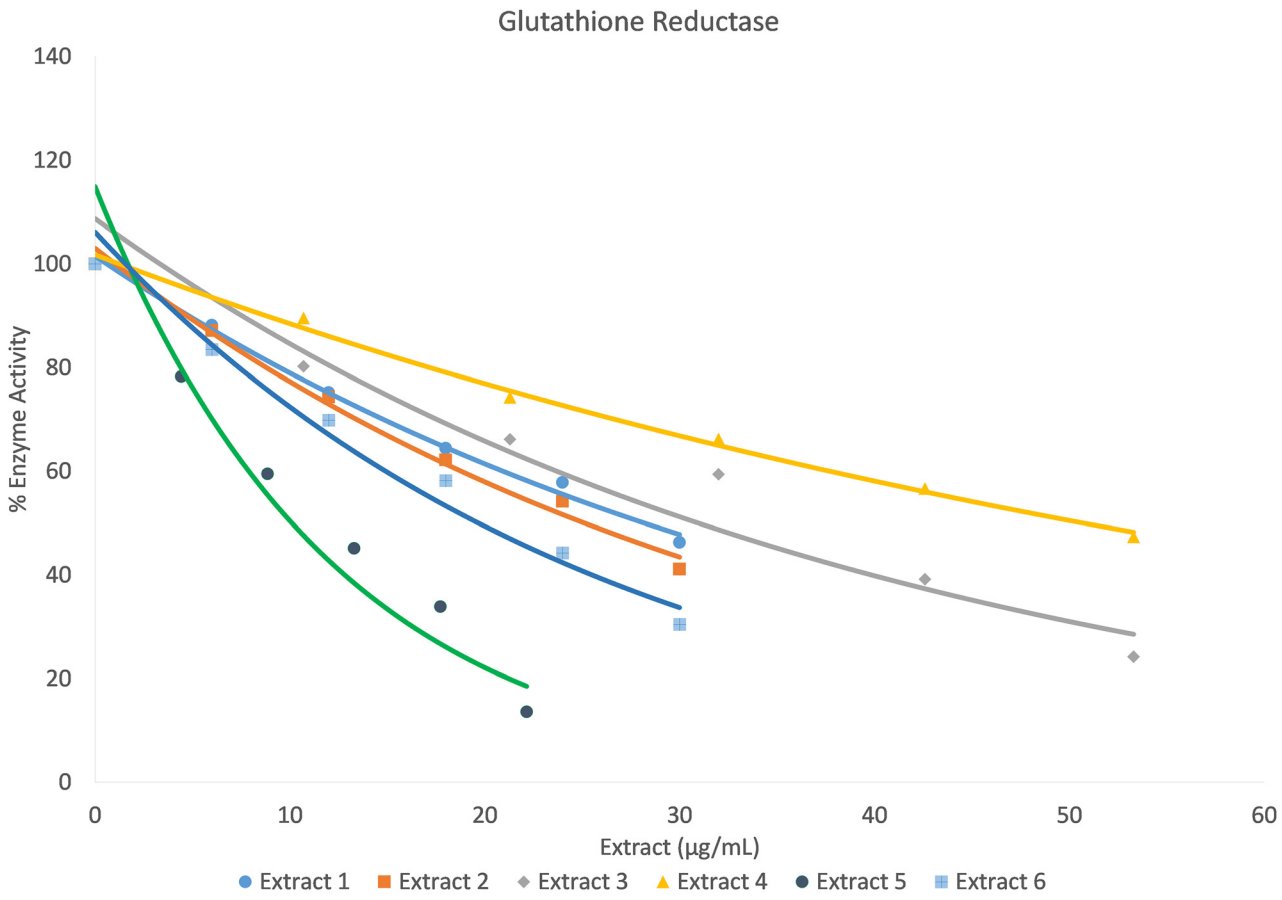


Figure 5: Glutathione reductase enzyme inhibition of extracts.

effectively modulate carbonic anhydrase activity. These observations are consistent with studies on plant extracts, with algal extracts demonstrating notably enhanced inhibitory effects. This demonstrates the unique and robust bioactivity of these algal extracts, which could be attributed to the diverse array of secondary metabolites and compounds present in the Antarctic algae.

Kaya et al. investigated the inhibition of water, ethanol, and methanol extracts of *A. rosea*, *F. vulgare*, *E. cardamomum*, *L. azorica*, and *L. stoechas* against hCA I, hCA II and hPON1. As a result, even the most effective extracts were found to be effective at the level of mg/mL [44]. Our study determined that Antarctic algae extracts were effective on CA I and CA II isoenzymes at microgram/mL level (Table 1).

The inhibition of GR and AG enzymes by algal extracts presents a novel perspective on their multifaceted bioactivity. Since all extracts (1–6) are effective at lower doses compared to the N,N-bis(2-chloroethyl)-N-nitrosourea substance used as a reference for the GR enzyme, they have the potential to be used in the treatment of both malaria and some types of cancer [51]. Compared to the acarbose substance used as a reference for the AG enzyme, extracts 1 and 6 were effective at lower doses. However, other extracts were found to be effective at doses close to the reference molecules. According to these results, all the extracts we tested have the potential to be used in the treatment of diabetes [52]. The comparison of our results with the chromatographic study by Kıvanç and Türkoğlu on *A. rupicola* Boiss var rupicola plant extracts highlights the distinct inhibitory potential of Antarctic algal extracts on GR enzyme activity, hinting at their diverse bioactive components that warrant further exploration [48].

In the literature review, the first study on the GR enzyme with algal extras is presented in this study. Pandithurai et al. investigated the *in vitro* inhibition effects of methanolic extract of Brown algae *Spatoglossum asperum* against α-amylase and α-glucosidase enzymes. The methanolic extract showed α-glucosidase enzyme inhibitory activity at a concentration of IC_{50}=61 µg/mL [53]. The results obtained in our experiments are much more promising than this value (Table 1).

In conclusion, the comprehensive evaluation of the inhibitory effects of Antarctic algal extracts against various

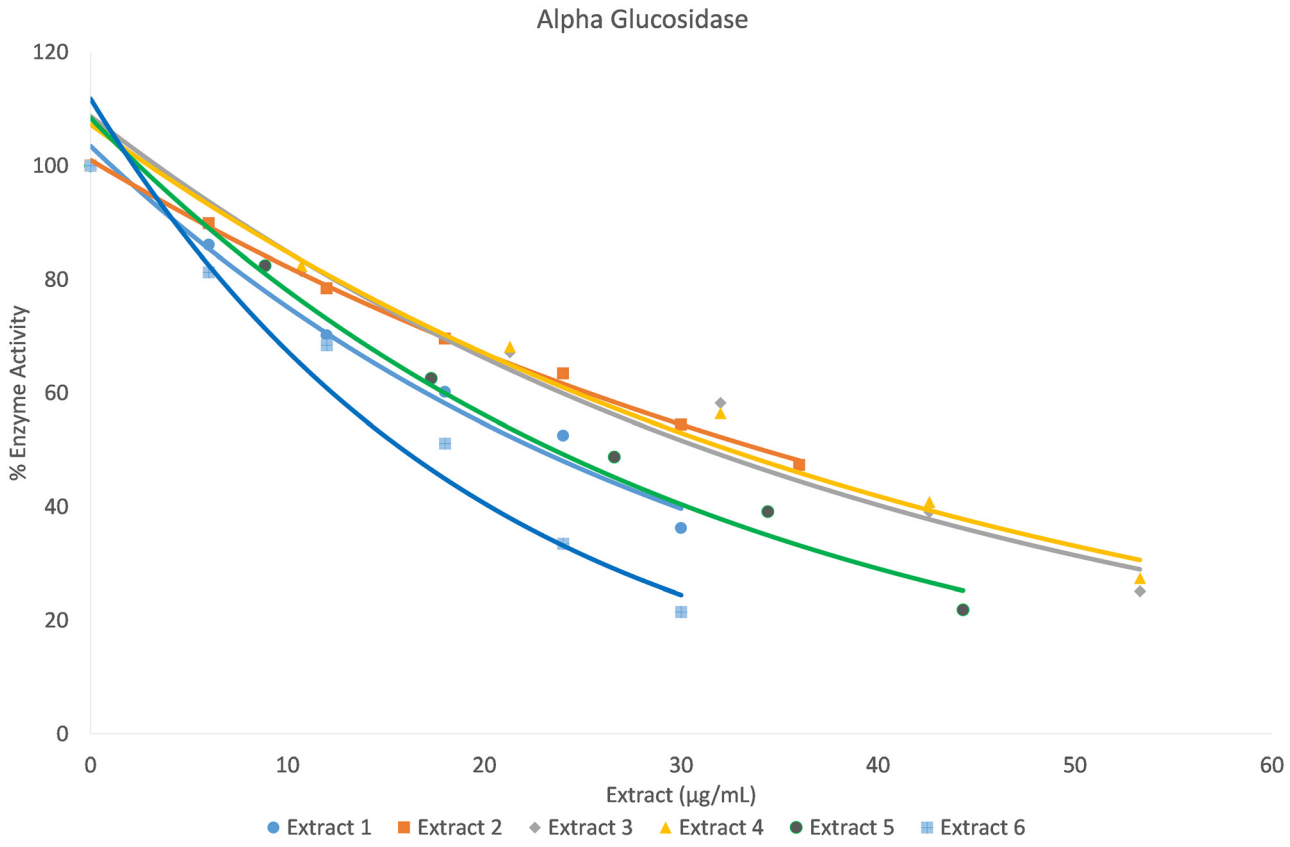


Figure 6: α-Glucosidase enzyme inhibition of extracts.

key enzymes, including AChE, BChE, CA I, CA II, GR, and AG, has provided profound insights into their potential therapeutic applications. Extracts 1 and 5 exhibited remarkable inhibitory potency against AChE, BChE, and CA I enzymes, surpassing the activity of the reference molecules. These findings, coupled with the comparative analysis of IC_{50} values between the algal extracts and established reference compounds, underscore the substantial effectiveness of the algal extracts in enzyme inhibition. Furthermore, the observed inhibition effects against the GR and AG enzymes, albeit with relatively higher IC_{50} values, further highlight the multifaceted bioactivity of these algal extracts. In the context of previous research on plant extracts and enzyme inhibition, the demonstrated superior inhibitory effects of the algal extracts across diverse enzymes not only emphasize their potential as promising candidates for pharmaceutical development but also contribute to our understanding of natural sources with significant therapeutic potential. This study thus contributes to the expanding body of knowledge in the field of enzymology and offers a solid foundation for future investigations aimed at harnessing the unique bioactive properties of Antarctic algal extracts for novel therapeutic interventions.

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Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission. Conceptualization: Belma KONUKLUGİL; Investigation: Bülent Gözcelıoğlu; Formal analysis and investigation: Murat Şentürk, Ibrahim Seyda Uras; Writing – original draft preparation: Murat Şentürk, Ibrahim Seyda Uras; Writing – review and editing: Bülent Gözcelıoğlu; Supervision: Belma Konuklugıl.
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Data availability: The raw data can be obtained on request from the corresponding author.

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