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Research Article

Evaluation of Antioxidant, Antiproliferative and Anticholinesterase Activities of *Iberis carnosa* Willd

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ABSTRACT

This study investigated the bioactivity of *Iberis carnosa* Willd, a plant known for its diverse natural compound. Utilizing Soxhlet extraction, an ethanol extract was prepared and subsequently analyzed for antioxidant, antiproliferative, and anticholinesterase activities. Antioxidant capacity was quantified using Rel Assay kits, yielding a total antioxidant status (TAS) of 4.489±0.095 mmol/L, a total oxidant status (TOS) of 11.325±0.130 µmol/L, and an oxidative stress index (OSI) of 0.252±0.003. Antiproliferative activity was assessed against the A549 lung cancer cell line, demonstrating a dose-dependent inhibition of cell growth. Anticholinesterase activity was evaluated by measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition, revealing values of 76.52±1.93 µg/mL for AChE and 119.84±1.50 µg/mL for BChE, respectively. These findings suggest that *I. carnosa* possesses significant biological activities and warrants further investigation for potential therapeutic applications, particularly in neurodegenerative diseases and cancer.

Keywords: *Iberis carnosa*, antioxidant activity, antiproliferative effect, anticholinesterase activity, A549 cell line, biological activity.

1. INTRODUCTION

Herbal components are compounds containing various phytochemicals found in nature and have a wide range of biological activities.[1] Through comprehensive chemical analysis, the constituents were shown to contain a complex profile of active metabolites, characterized by the presence of phenolic compounds, flavonoids, alkaloids, terpenoids, and saponins. The antioxidant mechanisms of phenolic compounds and flavonoids, mediated through free radical neutralization, are critical in protecting cells from oxidative damage. Additionally, their anti-inflammatory actions suggest a potential role in modulating inflammatory pathways associated with chronic disease development.[2-7] Alkaloids are generally known for their neuroactive properties, and some may have analgesic or antimicrobial properties. Terpenoids and saponins attract attention with their immune system supporting, anticancer and antimicrobial activities.[8] Phytochemical constituents demonstrate diverse applications within the pharmaceutical and food industries, and they are pivotal in the development of naturally derived therapeutic agents.[9] Contemporary research endeavors are directed towards optimizing the salutary effects of herbal extract constituents on human physiology through the development of methodologies designed to enhance their bioavailability.[10] Therefore, studies on the biological activities of herbal components are of great importance for both the scientific world and the health sector. In this study, *Iberis carnosa* Willd. plant was used as material.

The perennial plant *I. carnosa*, classified within the Brassicaceae family, demonstrates a strong ecological association with coastal habitats. It is important to acknowledge the taxonomic synonymy of this species, previously designated as *I. attica*. It is naturally distributed in the Mediterranean basin, especially in the coastal areas of Spain, Portugal and North Africa. This

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plant is notable for its ability to adapt to salty and arid soils and is mostly found in rocky areas, limestone grounds or coastal dunes.[11] Having fleshy and thick leaves increases its resistance to drought, while its flowers, which vary from white to pink, make an aesthetic contribution to natural habitats by blooming in spring and summer. Its flowers are arranged in small, dense clusters and are an important nectar source for bees and butterflies. *I. carnosa* is also evaluated in landscape studies due to its ecological functionality and soil erosion prevention effect.[12] However, like many species belonging to the Brassicaceae family, it has the potential to carry some medicinal and phytochemical properties. However, scientific studies on the use of this plant are limited. *I. carnosa*, which is also preferred as an ornamental plant thanks to its resistance to arid and marine areas, plays an important role in natural and semi-natural ecosystems.[13]

2. EXPERIMENTAL

2.1. Materials

Plant samples of *I. carnosa* were procured from the Mersin province of Türkiye. Following collection, the aerial portions of the plants were dried under standardized laboratory conditions to ensure consistent moisture content. A 10 g aliquot of the dried plant material was subjected to Soxhlet extraction using 250 mL of ethanol as the solvent, maintained at 50 °C for a period of 6 hours. The resulting crude extract was then concentrated by rotary evaporation on a Buchi R100 apparatus at 40 °C. The concentrated extracts were stored at +4 °C to preserve their integrity until analytical procedures were conducted.

2.2. Methods

2.2.1. Antioxidant Tests

The total antioxidant status (TAS) and total oxidant status (TOS) of an ethanolic extract derived from the aerial parts of *I. carnosa* were quantified using commercially available assay kits (Rel Assay). Procedures adhered to the manufacturer's protocol. TAS was determined using Trolox as a standard, and TOS was calibrated against hydrogen peroxide. Results are presented in mmol/L for TAS and µmol/L for TOS.[14-15] To quantify oxidative stress, the Oxidative Stress Index (OSI) was calculated. This index represents the ratio of total oxidant status (TOS) to total antioxidant status (TAS), with both TOS and TAS values normalized to equivalent units to allow for direct comparison.[16]

2.2.2. Anticholinesterase Activity Tests

Anticholinesterase activity was assessed using the Ellman method.[17] The inhibitory potential against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was quantified, with galantamine serving as a positive control. Stock solutions of plant extracts, ranging from 3.125 to 200 µg/mL, were prepared. Reaction mixtures, comprising 130 µL of 0.1 M phosphate buffer (pH 8), 10 µL of extract solution, and 20 µL of enzyme (AChE or BChE), were incubated at 25 °C for 10 minutes. Following incubation, 20 µL of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added. Enzyme activity was determined spectrophotometrically at 412 nm. Inhibition was calculated as a percentage, and IC50 values (µg/mL) were derived from triplicate measurements.

2.2.3. Antiproliferative Activity Test

The cytotoxic effects of *I. carnosa* plant extracts on the A549 lung cancer cell line were quantified using the MTT assay. A549 cells, cultured to 70-80% confluence, were detached using trypsin-EDTA, seeded into culture plates, and incubated for 24 hours. Subsequently, cells were exposed to *I. carnosa* extracts at concentrations of 25, 50, 100, and 200 µg/mL for 24 hours. Following incubation, cell viability was determined by adding MTT solution (1 mg/mL), dissolving the resulting formazan crystals with DMSO, and measuring absorbance at 570 nm using a spectrophotometer.[18]

3. RESULTS and DISCUSSION

3 .1. Antioxidant Activity

A condition of oxidative stress is defined by the imbalance between the production and scavenging of free radicals and reactive oxygen species (ROS), leading to their excessive accumulation within cellular environments. This state exerts profound biological consequences across a broad range of biological systems, including those of plants.[19] Plants response to environmental stressors, including ultraviolet radiation, heavy metal exposure, drought, salinity, and pathogen infection, these processes entail the induction of cellular antioxidant defense systems to mitigate oxidative stress. Cellular protection against oxidative stress is achieved through a dual antioxidant system. Effective cellular protection against oxidative stress is achieved through a coordinated system of enzymatic antioxidants (superoxide dismutase, catalase, peroxidase) and non-enzymatic antioxidants (phenolic compounds, flavonoids, ascorbic acid, tocopherols). These components act synergistically to neutralize free radicals, thereby minimizing cellular damage.[20] Antioxidant compounds enhance plant stress tolerance and are crucial for human health, as dietary intake of these substances mitigates oxidative stress-induced damage.[21] Consequently, plant-derived antioxidants exhibit broad applicability across pharmacological, food, and cosmetic sectors. The identification and characterization of novel natural antioxidant sources

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remain a significant area of investigation within the scientific community.[22] In this study, the antioxidant potential of *I. carnosa* aerial parts was evaluated. The obtained data are presented in Table 1.

Table 1. TAS, TOS and OSI values of *Iberis carnosa.*

Plant	TAS mmol/L	TOS µmol/L	OSI (TOS/(TAS*10))
Iberis carnosa	4.489±0.095	11.325±0.130	0.252±0.003

* Values are presented as mean±SD

In our study, antioxidant and oxidant levels and thus oxidative stress index of *I. carnosa* were determined. OSI, TOS and TAS values of *I. carnosa* have not been reported in many studies. In the literature, antioxidant activity of *Iberis amara*, a different *Iberis* species, has been reported using different methods.[23-24] TAS, TOS and OSI values of different plant species have been reported. In this context, TAS values of *Mentha longifolia*, *Rumex scutatus*, *Origanum laevigatum* and *Hypericum spectabile* were reported as 6.094, 8.656, 8.609 and 9.306 mmol/L. TOS values were reported as 14.050, 4.951, 10.685 and 13.065. OSI values have been reported as 0.231, 0.057, 0.124 and 0.140.[25-28]

Compared to this study, the TAS value of *I. carnosa* used in our study was determined to be lower than *M. longifolia*, *R. scutatus*, *O. laevigatum* and *H. spectabile*. TAS values provide a comprehensive assessment of the collective antioxidant compounds inherent in natural sources.[29] The TOS serves as a quantitative measure of the aggregate concentration of oxidant compounds present within natural product matrices.[29] The TOS value of *I. carnosa* was found to be higher than *R. scutatus* and *O. laevigatum*, but lower than *M. longifolia* and *H. spectabile*. The OSI value shows how much oxidant compounds are suppressed by antioxidant compounds.[29] The OSI value of *I. carnosa* used in our study was found to be higher than *M. longifolia*, *R. scutatus*, *O. laevigatum* and *H. spectabile*.

The presented data, detailing the antioxidant and oxidant profiles of *I. carnosa*, significantly contributes to the elucidation of natural product bioactivity related to oxidative stress modulation. In particular, the TAS value of *I. carnosa* was found to be lower compared to some other plants, suggesting that the amount of antioxidant compounds contained in the plant is limited compared to plants with stronger antioxidant capacity. However, the higher TOS value than some plants indicates that *I. carnosa* is rich in oxidant compounds and that these compounds can potentially cause cellular oxidative damage. The oxidative stress index (OSI) value reveals how effectively the oxidant compounds of the plant are controlled by antioxidants.[30] The higher OSI value of *I. carnosa* than other plants suggests that this plant has a greater oxidative stress-increasing effect and that its antioxidant activity is not at a sufficient level. However, these findings reveal that antioxidant and oxidant activities should be carefully balanced when evaluating the potential pharmacological uses of the plant. It can be said that *I. carnosa* is a species that requires further research, and that larger-scale studies are needed to better understand its potential therapeutic effects, especially on oxidative stress and related diseases.

3.2. Anticholinesterase Activity

The inhibition of cholinesterase enzymes, a strategy termed anticholinesterase activity, represents a significant therapeutic target in neurological disease management. These enzymes mediate the hydrolysis of neurotransmitters, notably acetylcholine. Consequently, pharmacological inhibition of cholinesterase may ameliorate neuropathological conditions, including Alzheimer's and Parkinson's diseases, by enhancing synaptic neurotransmission.[31] Plants provide rich sources of natural compounds that inhibit this activity. In particular, compounds such as alkaloids, flavonoids, terpenoids, and polyphenols found in many plant species can function as cholinesterase inhibitors. Therefore, a more in-depth examination of the anticholinesterase activity of plants may contribute to the development of new strategies in the treatment of neurological diseases.[32-33] In this study, *I. carnosa* was examined for anti-ACHE and anti-BChE activities, the results obtained are presented in Table 2.

Table 2. Anti-AChE and anti-BChE values of *Iberis carnosa.*

Sample	AChE µg/mL	BChE µg/mL
Iberis carnosa	76.52±1.93	119.84±1.50
Galantamine	6.54±0.24	15.31±0.29

* Studies were performed in triplicates and standard deviation values are given.
* Galantamine was used as a control.

The existing literature lacks evidence supporting the anticholinesterase activity of *I. carnosa*. In our study, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of *I. carnosa* were presented in comparison with galantamine. According to the obtained data, the AChE and BChE inhibitory activities of *I. carnosa* were determined as 76.52±1.93 µg/mL and 119.84±1.50 µg/mL, respectively. These values indicate that the plant has an inhibitory effect on both cholinesterase enzymes. However, when compared to galantamine, the inhibitory effect of *I. carnosa* remains at lower levels. Pharmacological intervention targeting cholinesterase enzyme activity, specifically inhibition, is a key therapeutic strategy employed in the management of Alzheimer's disease and other neurological disorders characterized by cholinergic deficits. Phytochemicals derived from various plant species represent a promising source of natural cholinesterase inhibitors.[34] Our investigation revealed that *I. carnosa* exhibits anticholinesterase activity, albeit significantly lower than that of galantamine. This observation suggests a potential, though limited, therapeutic role in

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neurological disease management. While preliminary findings suggest an interaction between *I. carnosa* compounds and the target enzymes, a more rigorous and detailed examination is necessary to fully characterize the nature and extent of this interaction. The contribution of the compounds contained in the plant, especially to AChE and BChE inhibitory activities, may be an important resource in the development of neurological treatment strategies. In addition, these results indicate that *I. carnosa* is effective not only on AChE but also on BChE, indicating that this plant may have broad-spectrum cholinesterase inhibitory activity.

3.3. Antiproliferative Activity

Antiproliferative activity, the capacity to inhibit cellular proliferation, is a critical biological property with implications for disease prevention and the therapeutic management of neoplastic disorders. This activity refers to the effect of natural compounds to prevent the growth and spread of cancer cells.[35] Plants can show this activity thanks to the various phenolic compounds, alkaloids, flavonoids and terpenes they contain. Natural resources offer potential antiproliferative agents that have been used in traditional medicine for centuries and have been proven effective in modern research.[36] In particular, the effects of some plant compounds on cancer cells are associated with mechanisms that regulate the cell cycle, and many of these compounds show anticancer activity through various means such as reducing oxidative stress, inducing apoptosis and arresting the cell cycle.[37] Therefore, compounds obtained from plant sources hold great promise as alternative or adjunctive treatment methods in the treatment of cancer and other diseases. In this study, the effects of *I. carnosa* on A549 lung cancer cells were examined and the results are presented in Figure 1.

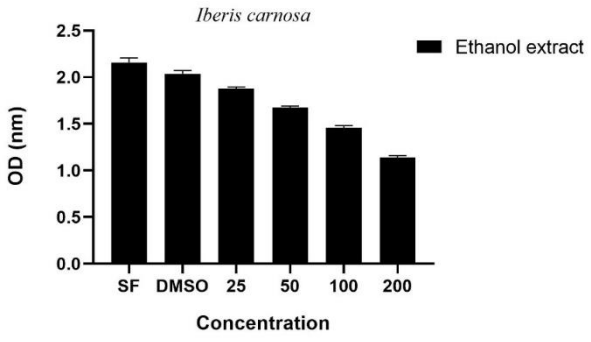

Figure 1. Antiproliferative activity of *Iberis carnosa*.

The existing scientific literature lacks evidence substantiating the antiproliferative activity of *I. carnosa*. To determine the influence of *I. carnosa* ethanol extract on cell proliferation, cells were exposed to a concentration gradient of the extract. Subsequently, optical density (OD) measurements were employed to quantify cell viability and proliferation, thereby enabling a quantitative analysis of the extract's inhibitory potential on cell growth. The data obtained reveal that *I. carnosa* ethanol extract may exhibit antiproliferative properties. SF (serum-free) and DMSO, which were used as control groups, were the groups in which cells exhibited the highest proliferation rates. Since DMSO was used as the solvent of the extract, it shows that it does not have a significant effect on cell viability on its own. In contrast, the application of ethanol extract at concentrations of 25, 50, 100 and 200 µg/mL resulted in a gradually increasing inhibition of cell proliferation. A significant decrease was observed especially at the concentration of 200 µg/mL, suggesting that the extract exhibits a dose-dependent effect. These results suggest that *I. carnosa* may contain bioactive components that inhibit cell growth. Further studies are required to determine how the extract inhibits cell growth. The observed effects may be mediated through mechanisms such as cell cycle arrest, apoptosis induction, and increased oxidative stress.

4.CONCLUSION

This study evaluated the biological activity of *I. carnosa* plant and revealed its antioxidant, antiproliferative and anticholinesterase effects. The obtained data show that the plant has high antioxidant capacity and may play a potential protective role in biological processes related to oxidative stress. Experimental investigations utilizing the A549 lung carcinoma cell line demonstrated that the ethanol extract of *I. carnosa* elicited a concentration-dependent reduction in cell viability and exhibited antiproliferative activity. Anticholinesterase activity analyses showed that the plant can inhibit acetylcholinesterase and butyrylcholinesterase enzymes, which may have therapeutic potential in neurodegenerative diseases. In the light of these findings, the pharmacological potential of *I. carnosa* is quite important and should be supported by future studies. First of all, the active components in the ethanol extract of the plant should be isolated and characterized to determine which phytochemicals contribute to these biological activities. In addition, investigation of its antiproliferative effect on different cancer cell lines will be useful in revealing the effectiveness of the plant against specific cell types. In addition, toxicity and bioavailability analyses in in vivo models are critical to determine the safe use range of *I. carnosa*. Pharmacokinetic and pharmacodynamic studies are required to confirm its potential, especially for the treatment of neurodegenerative diseases and cancer. In conclusion, this study reveals that *I. carnosa* is a promising plant for pharmaceutical and biotechnological applications. However, it is not possible to make definitive judgments about its use as a therapeutic agent without more comprehensive evaluations with preclinical and clinical studies. Future studies will elucidate the medicinal and commercial potential of the plant in more detail.

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Conflict of Interest

Author declares that there is no potential conflict of interest in this paper.

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