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Exploring the Antioxidant and Neuroprotective Potential of *Muscari armeniacum*: Phenolic Profiling and Enzyme Inhibition

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ABSTRACT

This study aimed to investigate the antioxidant and enzyme inhibitory properties of Muscari armeniacum, a plant native to Türkiye, which is known for its bioactive compound content. Ethanol extracts of Muscari armeniacum were prepared and analyzed using a variety of bioanalytical methods to assess its potential health-promoting properties. The antioxidant capacity was evaluated through Fe^{3+}-Fe^{2+} reduction capacity, CUPRAC, DPPH, and ABTS radical scavenging activities. Additionally, the total phenolic and flavonoid contents of the extracts were determined, as these compounds are critical indicators of antioxidant potential. The antioxidant activity of *Muscari armeniacum* was compared with five standard antioxidants commonly used for benchmarking: BHA, α-tocopherol, BHT, trolox, and ascorbic acid. The extract demonstrated a high flavonoid content (23.24 µg QE/mg extract) and a significant amount of phenolics (14.38 µg GAE/mg extract), with the flavonoid content being particularly noteworthy. In addition to its antioxidant properties, the enzymatic activity assays revealed that the extract significantly inhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, which are associated with neurodegenerative diseases such as Alzheimer's. The IC_{50} values were determined to be 54.14 mg/mL (R^2 = 0.9695) for AChE and 58.73 mg/mL (R^2 = 0.9609) for BChE, indicating moderate enzyme inhibition. These results suggest that *Muscari armeniacum* could serve as a valuable source of bioactive compounds with significant antioxidant and enzyme inhibitory activities. Its potential applications in the treatment of oxidative stress-related diseases and neurodegenerative disorders make it a promising candidate for further pharmacological studies. This research underscores the importance of exploring native plants for their medicinal and therapeutic potential, contributing to the development of alternative treatments for challenging health conditions.

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Introduction

Free iron is necessary for several physiological processes in living things, including DNA synthesis, oxygen transport, ATP synthesis, and chlorophyll synthesis. However, by producing reactive oxygen species (ROS), free iron can potentially present serious threats to cellular health

These ROS, in turn, can cause oxidative damage to critical biomolecules such as DNA, lipids, and proteins, often leading to lipid peroxidation and DNA damage (Topal et al., 2021). The accumulation of ROS in tissues further exacerbates this damage, negatively impacting lipids, proteins, carbohydrates, and DNA integrity. Certain environmental factors, such as excessive heating of organic materials, can accelerate ROS production, which explains the high levels of free radicals found in cigarette smoke (Özler et al., 2023).

Organisms that rely on oxygen for survival have developed intricate antioxidant defense systems to mitigate the detrimental effects of ROS. These systems repair damaged molecules and eliminate those that cannot be salvaged, thus reducing oxidative stress. Antioxidant enzymes are at the core of these systems, working in tandem to maintain cellular integrity. In the food industry, natural antioxidants, including specific enzymes, are increasingly employed to minimize ROS and prevent lipid peroxidation in food products. The relevance of antioxidants for human health lies in their chemical diversity, solubility, and availability from natural sources, underscoring the importance of encouraging their consumption through diet (Topal et al., 2021).

Natural antioxidants are organic compounds synthesized by plants, with phenolic compounds standing out for their pronounced health benefits. Phenolic compounds, when ingested through dietary sources, exert positive effects on the body by scavenging free radicals and reducing oxidative damage. In recent years, there has been a notable increase in the use of plant-derived antioxidants in the food industry, reflecting their potential to enhance food quality and safety. When antioxidant defense systems weaken, cellular damage escalates, potentially leading to programmed cell death. Antioxidants, by neutralizing free radicals, play a pivotal role in preventing such adverse outcomes, making their regular intake essential for maintaining health (Topal & Kocabaş, 2024).

Muscari armeniacum, commonly referred to as the Armenian grape hyacinth, is a plant species native to the Eastern Mediterranean. It is widely distributed across regions spanning Greece, Türkiye, the Caucasus, and Armenia (Bokov, 2019). Renowned for its vibrant blue blooms and unique aesthetic appeal, *Muscari armeniacum* is highly valued by plant enthusiasts and landscape designers alike. Beyond its ornamental value, the plant serves as an exceptional honey source, attracting bees, butterflies, and bumblebees with its pleasant fragrance (Bokov, 2019). The striking blue coloration of *Muscari armeniacum* flowers is attributed to the presence of anthocyanins, with p-coumaric acid identified as one of the acyl groups in muscarinin A, a complex anthocyanin structure (delphinidin-3-(6-p-coumaroylglucoside)-5-(4-rhamnosyl-6-malonylglucoside)) (Yoshida et al., 2002). Additionally, the plant's bulbs have been studied for their involvement in the hormonal regulation of gummosis, with a particular focus on the chemical composition of their polysaccharide-rich gums (Miyamoto et al., 2010).

Despite its aesthetic and ecological significance, research on *Muscari armeniacum* remains limited, particularly concerning its chemical composition and pharmacological potential. Given its widespread use and unique bioactive properties, there is a need for more comprehensive studies to explore its potential applications in medicine and industry. This study aims to fill this gap by investigating the biological activities of *Muscari armeniacum*, including its antioxidant and enzyme inhibitory properties, to provide insights into its possible medicinal significance. By enhancing our understanding of this species, the research seeks to highlight its potential as a valuable source of natural antioxidants and therapeutic agents.

Material and Methods

Material

Muscari armeniacum was collected during May-June 2022 from the valley of Ozanlar Village in Ağrı Province, at coordinates approximately 39.774153, 43.043444. The plant's flowers were sun-dried and cleaned of dust before undergoing analysis. For extraction, the dried flowers were crushed using a blender and mixed with 50 mL of ethanol. The mixture was left to stir overnight at room temperature on a magnetic stirrer. Following this, the solution was filtered through Whatman No.1 filter paper, and the ethanol was evaporated using a rotary evaporator to obtain the dry extract.

Methods

Cu^{2+}-Cu^{+} reduction capacity

A slightly modified version of the method described by Apak et al. (2006) was employed. In this procedure, 0.125 mL of $CuCl_2$ solution (0.01 M), 0.125 mL of ethanolic neocuproine solution (7.5×10^{-3} M), and 0.125 mL of CH_3COONH_4 buffer solution (1 M) were added to test tubes. Subsequently, ethanol extracts of *Muscari armeniacum* at various concentrations were introduced into the tubes. The final volume of each mixture was adjusted to 1 mL using distilled water. After a 30-minute incubation, the absorbance of the samples was measured at 450 nm against a blank solution prepared with distilled water.

Fe^{3+}-Fe^{2+} reduction capacity

The method was performed following the procedure described by Oyaizu (1986). Initially, a stock solution with a concentration of 1 mg/mL was prepared. Aliquots of this stock solution were transferred to test tubes at varying concentrations, and the volume was adjusted to 0.75 mL with distilled water. Next, 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] were added to each tube. The mixtures were then incubated at 50°C for 20 minutes. Subsequently, 1 mL of 10% trichloroacetic acid (TCA) and 0.25 mL of 0.1% $FeCl_3$ were added. The absorbance of the resulting solutions was measured at 700 nm, using distilled water as the blank.

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity was assessed following the Blois method (Blois, 1958). A 1 mM DPPH solution was used as the free radical source. A stock sample solution with a concentration of 1 mg/mL was prepared, and aliquots were transferred into test tubes to create solutions at final concentrations of 10, 20, and 30 µg/µL. The total volume of each solution was adjusted to 2 mL with ethanol. Subsequently, 0.5 mL of the DPPH stock solution was added to each tube. The mixtures were incubated in the dark at room temperature for 30 minutes, and their absorbance was measured at 517 nm, using ethanol as the blank reference.

ABTS Radical Scavenging Activity

The ABTS radical scavenging activity was evaluated following the method outlined by Re et al. (1999). A 7 mM ABTS solution was prepared, and ABTS radicals were generated by adding a 2.45 mM persulfate solution. The ABTS radical solution was allowed to stabilize, and its absorbance was adjusted to 0.700 ± 0.025 at 734 nm before use. Ethanol extracts at concentrations of 10, 20, and 30 µg/mL were mixed with 0.5 mL of the ABTS radical solution and incubated for 30 minutes. After incubation, the absorbance of each sample was measured at 734 nm, with ethanol used as the blank.

Determination of Total Phenolic Compound Amount

The total phenolic content was determined using gallic acid as the standard. A standard curve was constructed using gallic acid, and a stock solution of the plant extract was prepared. The sample was transferred into a volumetric flask, and the volume was adjusted to 23 mL with distilled water. Subsequently, Folin-Ciocalteu reagent and 2% Na_2CO_3 solution were added to the flask. The mixture was allowed to react at room temperature, and its absorbance was measured at 760 nm, with distilled water

serving as the blank. The gallic acid equivalents (GAE) of the sample were calculated based on the absorbance values using the equation obtained from the standard curve (Kalın et al., 2015).

Determination of Total Flavonoid Content

The total flavonoid content was determined following the method described by Kalın et al. (2015). A 750 µg sample of the extract was measured and transferred into a test tube. The extract was then diluted with an ethanol solution containing CH_3COOK and 10% $Al(NO_3)_3$ solutions. The mixture was vortexed thoroughly and incubated at room temperature. After incubation, the absorbance was measured at 415 nm. Quercetin was used as the standard to calculate the total flavonoid concentration based on the absorbance values.

Effect on Acetylcholinesterase (AChE) Enzyme

AChE catalyzes the hydrolysis of acetylcholine into thiocholine and acetate. During inhibition studies, the produced thiocholine reacts with DTNB (Ellman's reagent), leading to the formation of a yellow-colored compound, 5-thio-2-nitrobenzoic acid. This compound's presence is quantified by measuring its absorbance at 412 nm (Ellman et al., 1961). Absorbance readings for both control and sample cuvettes were taken at 412 nm, specifically at the 5th minute of the reaction. The inhibitory effects of the tested compounds on AChE were evaluated, and the IC_{50} value was determined based on the resulting data.

Effect on Butyrylcholinesterase (BChE) Enzyme

The inhibitory effects of the synthesized compounds on BChE were evaluated using a procedure similar to that employed for AChE. However, instead of acetylthiocholine iodide, which serves as the substrate for AChE, butyrylthiocholine iodide was used as the substrate for BChE. The reaction and analysis steps remained otherwise consistent.

Determination of Phenolic Compounds

The analyses were performed at the Central Research and Application Laboratory of Ağrı İbrahim Çeçen University using a liquid chromatography system (Spark Holland) integrated with a tandem mass spectrometry system (AB SCIEX 4000 QTRAP) (LC-MS/MS). Chromatographic separation was carried out using a C18 column (Inertsil ODS-3V, 250 mm × 4.6 mm, 5 µm). The mobile phases were 0.1% (v/v) formic acid solution (A) and methanol (B). The injection volume was set to 10 µL, with a flow rate of 0.700 mL/min, and the column temperature was maintained at 30°C. Qualitative and quantitative analyses were performed in Multiple Reaction Monitoring (MRM) mode, with electrospray ionization (ESI) used as the ionization method.

Results and Discussion

Alzheimer's disease, one of the illnesses caused by ROS, currently has no definitive cure, and existing treatments focus on slowing the progression of the disease. However, some of the medications used in treatment are associated with side effects. For instance, Tacrine has limited use due to its potential to cause liver damage. In this study, the antioxidant capacity of *Muscari armeniacum*, a plant rich in phenolic compounds, was investigated, and its potential application in Alzheimer's treatment was evaluated.

In the study, the antioxidant activities of *Muscari armeniacum* were evaluated using methods such as iron reduction capacity, CUPRAC, ABTS, and DPPH radical scavenging assays. Additionally, tests involving AChE and BChE enzymes were conducted to determine the types of inhibition based on IC_{50} and K_i values. The results suggest that *Muscari armeniacum* has potential as a candidate for the treatment of diseases linked to cellular damage, such as Alzheimer's.

Important factors, like reduction capacity, should be taken into account when assessing a compound's antioxidant potential, as was underlined in this section. Oxidation is the loss of electrons in chemical reactions, while reduction is the gain of electrons. It was mentioned that while reducible compounds frequently have antioxidant qualities, not all of them are.

To evaluate the antioxidant capacity of *Muscari armeniacum*, CUPRAC and Fe^{3+} reduction capacity methods were utilized. The results revealed that *Muscari armeniacum* exhibited a lower reduction capacity compared to standard antioxidants. In terms of Fe^{3+} reduction capacities, a comparison of absorbance values at 10 µg/mL showed the following order: BHA (1.441) > BHT (1.033) > Trolox (0.751) > Ascorbic acid (0.693) > α-Tocopherol (0.418) > *Muscari armeniacum* (0.108) (Table 1). Higher absorbance values indicate stronger reduction capacity. Iron is a critical element for metabolic processes in living organisms but can cause cellular damage when present in excessive amounts. Iron ions can increase the levels of reactive oxygen species (ROS) and free radicals in living organisms, leading to potential harm (Gulcin, 2020; Topal, 2020).

ABTS and DPPH radical scavenging activities are widely used spectrophotometric methods employed in this study. The DPPH radical, when interacting with an antioxidant compound, transitions from a purple color to a lighter shade, serving as an indicator of antioxidant activity. The ABTS radical, on the other hand, dissolves in both water and organic solvents, allowing measurements to be taken within approximately 30 minutes.

Table 1. Comparison of reducing capacities at 10 µg mL^{-1} concentration

Antioxidants	Fe^{3+} Reduction (700 nm)	CUPRAC Method (450 nm)
BHA	1.441	1.239
BHT	1.033	0.680
α-Tocopherol	0.418	0.169
Trolox	0.751	0.260
Ascorbic Acid	0.693	0.342
Muscari armeniacum	0.108	0.054

BHA: Butyl Hydroxy Anisole, BHT: Butyl Hydroxy Toluene, CUPRAC: CUPric Reducing Antioxidant Capacity

Table 2. Comparison of % removal activities at 10 µg mL^{-1} concentration

Antioxidants	$DPPH^{\cdot}$ Removal	$ABTS^{\cdot+}$ Removal
BHA	69.67	99.41
BHT	18.93	99.53
α-Tocopherol	20.70	67.64
Trolox	61.46	99.41
Ascorbic Acid	34.91	84.99
Muscari armeniacum	4.66	17.82

BHA: Butyl Hydroxy Anisole, BHT: Butyl Hydroxy Toluene

Table 3. The amount of total phenolic and total flavonoid compounds found in *Muscari armeniacum*

	Total Phenolic (µg GAE/mg extract)	R^2	Total Flavonoid (µg QE/mg extract)	R^2
Muscari armeniacum	14.38	0.9991	23.24	0.9963

GAE: Gallic acid equivalent, QE: Quercetin equivalent

Table 4. Comparison of *Muscari armeniacum* for AChE and BChE enzymes

	Muscari armeniacum IC_{50} (µg mL^{-1})	
Cholinesterases	IC_{50}	R^2
AChE	54.14	0.9695
BChE	58.73	0.9609

AChE: Acetylcholinesterase, BChE: Butyrylcholinesterase, IC_{50}: Half maximal inhibitory concentration

Additionally, the ABTS method offers the advantage of functioning effectively across a broad pH range (Muglu et al., 2024). At a concentration of 10 µg/mL, the %ABTS radical scavenging activity of *Muscari armeniacum* and standard antioxidants was ranked as follows: BHT > BHA > Trolox > Ascorbic Acid > α-Tocopherol > *Muscari armeniacum* (Table 2).

Copper (Cu) is an essential metal with vital importance for the human body. It is required for iron absorption, and copper deficiency can lead to iron deficiency anemia. Additionally, a correlation has been identified between copper imbalance and Alzheimer's disease. An increase in circulating free copper molecules can accelerate the progression of the disease. Human serum albumin plays a crucial role in the antioxidant defense system and is effective in regulating free copper levels (Durgun et al., 2024; İnan Ergün & Topal, 2023). Copper has been identified as an element that triggers ROS formation, and studies have shown increased copper levels in Alzheimer's disease (Lowell & Shulman, 2005; Topal et al., 2024). Therefore, in this study, the copper reduction capacity and antioxidant effects of *Muscari armeniacum* were evaluated in relation to findings associated with Alzheimer's disease. In conclusion, the antioxidant capacity and AChE and BChE inhibitory properties of *Muscari armeniacum* suggest its potential as an alternative approach in the treatment of Alzheimer's disease. This study aims to contribute to the development of novel pharmacological strategies for challenging diseases such as Alzheimer's, cancer, and Parkinson's.

In the present study, the %DPPH radical scavenging activity of *Muscari armeniacum* was calculated as 4.66% at a concentration of 10 µg/mL. In a study on the ABTS radical scavenging activity of *Sorbus subfusca* fruit, the IC_{50} values were reported as 36.47 µg/mL for the water extract and 33.00 µg/mL for the ethanol extract (Topal & Kocabaş, 2024). The ABTS radical scavenging activity of *Muscari armeniacum* was found to be lower compared to the standard antioxidants. The total phenolic content of the ethanol extract of *Muscari armeniacum* was determined using gallic acid as the standard phenolic compound. A standard curve was constructed, and the total phenolic content in the ethanol extract of *Muscari armeniacum* was calculated as gallic acid equivalents (GAE), amounting to 14.38 µg GAE/mg extract.

The total flavonoid content of the ethanol extract of *Muscari armeniacum* was determined using quercetin as the standard. Using the equation derived from the standard curve, the total flavonoid content was calculated as quercetin equivalents (QE), amounting to 23.24 µg QE/mg extract. The total flavonoid and phenolic contents in the extracts were calculated using the derived equations, and the results are presented in Table 3.

The effects of the ethanol extract of *Muscari armeniacum* on AChE and BChE enzymes were evaluated under saturated substrate concentrations. A graph illustrating the relationship between enzyme activity percentage and *Muscari armeniacum* extract concentration was plotted, and the IC_{50} value was calculated (Table 4). These findings suggest that *Muscari armeniacum* could serve as an alternative approach in the treatment of neurodegenerative diseases.

The phenolic compounds and their quantities of *Muscari armeniacum* were presented in Table 5. Among the phenolic compounds, *Muscari armeniacum* contains higher amounts of p-Coumaric acid (1853.3 ng/g), Quinic acid (1330.0), 4-Dihydroxybenzoic acid (1296.7 ng/g), and Salicilyc acid (960.3), respectively.

A literature review revealed no other studies on this topic, suggesting that the present study is the first to report on the detailed phenolic compounds in *M. armeniacum*. Due to this, we cannot compare the research. The studies are mostly focused on anthocyanins, homoisoflavonoids, odor-profile, and sugar profile. We could compare it with other plants. In a study conducted by Sarikurkcu et al. (2015), the methanol extract of *Phlomis armeniaca* was found to contain 0.77 mg/g catechin, 11.95 mg/g chlorogenic acid, and 0.85 mg/g rutin.

Table 5. Selected phenolics of *Muscari armeniacum* obtained from LC-MS/MS

Phenolic compounds	Retention time	Concentration (ng/g)
2,5-Dihydroxybenzoic acid	3.9	107.7
4-Dihydroxybenzoic acid	8.73	1296.7
Catechin	9.14	76.8
Chlorogenic acid	11.69	36.1
Epicatechin	12.63	29.8
Gallic acid	12.63	97.7
Hesperidin	13.17	nd
Myricetin	13.18	105.4
Naringenin	13.44	58.2
p-Coumaric acid	13.57	1853.3
Pyrogallol	14.11	54.2
Quercetin	14.65	130.3
Quinic acid	14.78	1330.0
Rosmarinic acid	14.91	60.4
Rutin	15.2	41.0
Salicilyc acid	15.21	960.3
Sinapinic acid	15.87	nd
Syringic acid	16.13	501.3
Vanilic acid	17.34	223.7
Vanilin	17.33	55.5

nd: Not detected

Conclusions

This study provides comprehensive insights into the antioxidant and enzyme inhibitory properties of *Muscari armeniacum*, highlighting its potential as a source of bioactive compounds with therapeutic applications. The ethanol extract of *Muscari armeniacum* demonstrated significant antioxidant activity through various assays, including Fe^{3+}-Fe^{2+} reduction capacity, CUPRAC, DPPH, and ABTS radical scavenging activities. Despite exhibiting lower reduction capacities and radical scavenging activities compared to standard antioxidants such as BHA, BHT, and trolox, the plant's flavonoid and phenolic content (23.24 µg QE/mg and 14.38 µg GAE/mg, respectively) underscores its antioxidant potential. The extract displayed moderate inhibition of AChE and BChE enzymes, with IC_{50} values of 54.14 µg/mL and 58.73 µg/mL, respectively. These findings suggest its relevance in the context of neurodegenerative diseases like Alzheimer's, where enzyme inhibition is a therapeutic target. LC-MS/MS analysis identified several phenolic compounds, including high levels of p-coumaric acid, quinic acid, and 4-dihydroxybenzoic acid, which likely contribute to the plant's bioactivity. The results indicate that *Muscari armeniacum* has potential applications in addressing oxidative stress-related conditions and neurodegenerative diseases. Its moderate antioxidant and enzyme inhibitory properties may make it a candidate for further pharmacological studies. As a plant native to Türkiye and a traditional ornamental species, *Muscari armeniacum* holds both ecological and medicinal value. This study underscores the importance of exploring native plants for developing novel therapeutic strategies. Further research is recommended to explore the mechanisms of action of *Muscari armeniacum*'s bioactive compounds and to evaluate its efficacy in in vivo models for oxidative stress and neurodegenerative diseases.

Declarations

CRediT authorship contribution statement

Fevzi Topal: Data curation; Formal analysis; Investigation; Methodology; Writing - review & editing. *Fırat Yılmaz:* Formal analysis; Writing - original draft. *Meryem Topal:* Formal Analysis; Investigation; Methodology; Writing - review & editing. Ahmed Menevşeoğlu: Conceptualization; Investigation; Resources; Writing - original draft; Writing - review & editing.

Declaration of competing interest

The authors declared that there is no conflict of interest.

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