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In Vitro Evaluation of the Effects of *Pteridium aquilinum* Ethanol Extract on Antioxidant, Anticholinesterase, and A549 Cell Viability

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ABSTRACT: This study investigated the antioxidant properties of *Pteridium aquilinum* ethanol extract, its inhibitory effect on cholinesterase enzymes, and its effect on the viability of A549 human lung cancer cells. Plant samples collected from Antalya province were dried and ground, then extracted using ethanol via the Soxhlet method. The oxidant-antioxidant profile of the extract was determined by total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) analyses. Anticholinesterase activity was examined against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes using the Ellman method, with galantamine considered as the reference inhibitor. The effect on the viability of A549 cells was analyzed using the MTT method in the concentration range of 25–200 µg/mL. The TAS, TOS, and OSI values of *P. aquilinum* extract were determined as 3.177±0.024 mmol/L, 8.364±0.022 µmol/L, and 0.263±0.002, respectively. The IC_{50} values of the extract against AChE and BChE enzymes were found to be 162.10±0.12 and 184.93±1.47 µg/mL, respectively, while these values were measured as 7.52±0.19 and 17.48±0.11 µg/mL for galantamine. Although the inhibitory effect of the extract on AChE was higher than on BChE, its activity against both enzymes remained lower than that of galantamine. MTT results showed that as the extract concentration increased, the metabolic activity of A549 cells decreased, with the most pronounced effect observed at 100 and 200 µg/mL applications. The findings revealed that *P. aquilinum* ethanol extract possesses measurable antioxidant and anticholinesterase properties and can reduce A549 cell viability in a concentration-dependent manner. However, due to the toxic components of the species, the observed biological activities need to be evaluated in conjunction with safety and selectivity analyses.

Keywords: *Pteridium aquilinum*, antioxidant activity, cholinesterase inhibition, A549 cells, MTT assay

INTRODUCTION

Plants are among the natural materials frequently studied in biological activity research due to the large number of primary and secondary metabolites they contain (Mohammed et al., 2023). In particular, polyphenols, terpenoids, alkaloids, saponins, and volatile compounds play a role in the adaptation of plants to environmental conditions and can also exhibit various biological effects on human health (Zengin et al., 2008; Sevindik et al., 2023). Antioxidant, antimicrobial, anti-inflammatory, cytotoxic, and enzyme inhibitor activities determined in plant extracts are closely related to the quantity and chemical structure of these metabolites (Sevindik et al., 2025; Sağlıker et al., 2025). However, even within the same species, growing region, climate, harvest time, plant part used, and extraction conditions can alter the level of biological activity (Uygun et al., 2025; Khassanov et al., 2026). Therefore, evaluating the traditional uses of plants together with their chemical composition and experimental biological activity results contributes to more reliably determining their potential uses (Mohammed et al., 2024; Selamoğlu et al., 2026). The aim of this study is to evaluate the biological activity potential of *Pteridium aquilinum* extract by determining its antioxidant, anticholinesterase, and antiproliferative activities under in vitro conditions.

Different extracts and fractions of *P. aquilinum* have been reported to exhibit antioxidant, antimicrobial, immunomodulatory, anti-inflammatory, insecticidal, antidiabetic, and cytotoxic activities. It has been determined that polysaccharides isolated from the species scavenge DPPH and ABTS radicals, induce an immunomodulatory response in macrophages, and that antioxidant activity and α-glucosidase inhibition are increased after modification with selenium (Zhao et al., 2022; Li et al., 2025). Leaf and frond extracts have been reported to have antioxidant capacity; this activity can vary depending on the extraction solvent, phenolic compound content, and the plant part used (Kardong et al., 2013; Panneerselvam et al., 2015; Baonda et al., 2025). Extracts prepared with different solvents have been reported to show inhibitory effects against various bacterial and fungal species, and the essential oil has been reported to exhibit significant antibacterial activity against some phytopathogenic bacteria (Bouchekouk et al., 2019; Adebayo et al., 2025). However, since ptaquiloside, present in *P. aquilinum*, is associated with immunosuppressive and carcinogenic effects, its toxicological properties should also be considered when evaluating the biological activity potential of the species (Vetter, 2009; Latorre et al., 2009; Kato-Noguchi, 2026). This study aimed to

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determine the antioxidant, anticholinesterase, and antiproliferative properties of *P. aquilinum* extract. The goal was to experimentally demonstrate the potential of this species in various biological activity areas, considering both its biological effects and toxicological properties.

MATERIAL AND METHODS

The *P. aquilinum* samples used in this study were collected from Antalya province in Türkiye. The plant material brought to the laboratory was dried under controlled conditions to reduce its moisture content and then ground to a homogenous consistency. For the extraction process, 30 g of dried plant sample was taken and extracted with 250 mL of ethanol using a Soxhlet apparatus. The process was carried out at 50 °C for approximately 6 hours. After extraction, the solvent was removed at 40 °C using a Büchi R-100 rotary evaporator, and the concentrated crude extract was obtained. The prepared *P. aquilinum* extract was stored at +4 °C until biological activity analyses were performed.

Antioxidant Activity Tests

The oxidant and antioxidant properties of *P. aquilinum* extract were evaluated spectrophotometrically based on total antioxidant status, total oxidant status, and oxidative stress index. A TAS kit was used to determine the total antioxidant status. The analysis is based on measuring the change in absorbance resulting from the reduction of the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation by the antioxidant components in the extract. The obtained TAS values were expressed as mmol Trolox equivalent/L (Erel, 2004).

The total oxidant status of *P. aquilinum* extract was determined using a TOS kit. The method is based on the oxidation of ferrous ions to ferric ions by the oxidant components in the extract, and the formation of a colored complex of ferric ions with xylenol orange. The change in absorbance due to complex formation was measured spectrophotometrically, and the results were given as μmol H_2O_2 equivalent/L (Erel, 2005). To evaluate the balance between the oxidant load and antioxidant capacity of the extract, the oxidative stress index (OSI) was calculated. After unit conversion, the OSI value was obtained by dividing the TOS value by the TAS value and multiplying the result by 100, and this result was expressed as a percentage (Sevindik, 2025).

Anticholinesterase Activity Tests

The anticholinesterase activity of *P. aquilinum* ethanol extract was determined by adapting the colorimetric method developed by Ellman et al. (1961) to a 96-well microplate format. The inhibitory effects of the extract on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were investigated separately, with galantamine used as the reference inhibitor. The extract was dissolved in dimethyl sulfoxide, and serial dilutions were prepared to achieve final concentrations of 200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL in the reaction medium. 130 μL of 0.1 M sodium phosphate buffer (pH 8.0), 10 μL of extract solution, and 20 μL of AChE or BChE enzyme solution were added to each well. The mixture was pre-incubated in the dark at 25 °C for 10 minutes. Then, 20 μL of 0.5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and 20 μL of substrate solution were added to bring the total reaction volume to 200 μL. Acetylthiocholine iodide was used as the substrate for AChE analysis, and butyrylthiocholine iodide was used for BChE analysis. The color change resulting from the enzymatic reaction was measured at 412 nm using a microplate reader. Enzyme inhibition rates were calculated considering control absorbances, and the concentrations at which the extract inhibited AChE and BChE activities by 50% were given as IC_{50} values in μg/mL. All analyses were performed in triplicate.

Antiproliferative Activity Tests

The effect of *P. aquilinum* ethanol extract on the viability of A549 human lung cancer cells was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method. Extract solutions were prepared to achieve final concentrations of 25, 50, 100, and 200 μg/mL in the culture medium. A549 cells, reaching approximately 70–80% confluence, were detached from the culture surface using Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) and transferred to 96-well microplates. After 24 hours of incubation to allow cell adhesion to the surface, P. aquilinum extract at the determined concentrations was added to the wells, and the application was continued for another 24 hours. At the end of this period, the culture medium was removed, and MTT solution was added to determine the metabolic activity of the cells. Formazan crystals formed in live cells after incubation were dissolved in DMSO (Sigma-Aldrich, MO, USA). Absorbance values were recorded at 570 nm using an Epoch microplate reader (BioTek Instruments, Winooski, VT, USA). Cell viability was calculated by assuming the absorbance of the control group without extract application was 100%, and the results were expressed as percentage viability compared to the control group (Korkmaz et al., 2025).

RESULTS AND DISCUSSION

Antioxidant Activity

The shift in the balance between the production of reactive oxygen species and cellular antioxidant defense mechanisms in favor of oxidants gives rise to the oxidative stress process, which can cause damage to proteins, lipids, and nucleic acids (Mushtaq et al., 2020). Plants synthesize phenolic compounds, flavonoids, and various secondary metabolites during their metabolic responses to environmental conditions (Kına et al., 2021). These compounds can exhibit antioxidant activity through mechanisms such as neutralizing free radicals, binding transition metals, and limiting chain reactions caused by reactive oxygen species (Mohammed et al., 2021). In this context, investigating the oxidant and antioxidant properties of plant extracts using different methods contributes to the evaluation of components responsible for biological activity (Pehlivan et al., 2018). *P. aquilinum*, a perennial fern species with a wide distribution area, is the subject of biological activity research due to its diverse phytochemical components. In this study, the antioxidant properties of *P. aquilinum* ethanol extract were determined using TAS, TOS and OSI methods, and the findings are presented in Table 1.

Table 1. TAS, TOS and OSI values of Pteridium aquilinum extract

Sample	TAS (mmol/L)	TOS (μmol/L)	OSI ((TOS)/(TASx10))
Pteridium aquilinum	3.177±0.024	8.364±0.022	0.263±0.002

Note: Values are presented as mean±SD

The TAS value indicates the total capacity of antioxidant compounds found in natural products, while the TOS value expresses the overall level of compounds with oxidant properties. The OSI value, calculated from TAS and TOS data, reveals the ratio of oxidant load to antioxidant capacity and is used, for example, in evaluating the oxidative equilibrium state (Gürgen and Sevindik, 2022). In this study, the TAS value of *P. aquilinum* ethanol extract was determined as 3.177±0.024 mmol/L, the TOS value as 8.364±0.022 μmol/L, and the OSI value as 0.263±0.002. The obtained TAS value shows that the extract has a measurable total antioxidant capacity, while the TOS value revealed that the extract also contains components that can exhibit oxidant properties. The calculation of the OSI value as 0.263 indicates that the ratio of the existing oxidant load to the antioxidant capacity is relatively high. The antioxidant capacity determined in *P. aquilinum* extract is thought to be related to the phenolic compounds, flavonoids, tannins, and other redox-active secondary metabolites contained within the species. However, since TAS analysis reflects the total reducing capacity of the compounds in the extract, the results cannot be attributed to a single group of compounds. Similarly, the TOS value does not directly indicate that the extract is toxic or harmful; it only provides information about the total level of components exhibiting oxidant properties under analytical conditions. Therefore, evaluating TAS and TOS results together with the OSI value, rather than separately, provides a more appropriate approach. In the literature, TAS, TOS, and OSI values for *Salvia absconditiflora* have been reported as 7.350 mmol/L, 8.501 μmol/L, and 0.116, respectively (Akgül et al., 2020). The TAS value of *Mentha longifolia* was determined as 6.094 mmol/L, the TOS value as 14.050 μmol/L, and the OSI value as 0.231 (Sevindik et al., 2024). For *Hypericum spectabile*, TAS values of 9.306 mmol/L, TOS values of 13.065 μmol/L, and OSI values of 0.140 were reported (Gürgen et al., 2024). The TAS, TOS, and OSI values of *Anthemis cotula* were found to be 7.625 mmol/L, 11.247 μmol/L, and 0.148, respectively (Sabik et al., 2024). While these values were reported as 6.93 mmol/L, 12.53 μmol/L and 0.18 for *Dittrichia graveolens* (Korkmaz et al., 2023), 5.350 mmol/L TAS, 11.608 μmol/L TOS and 0.217 OSI values were determined for *Equisetum ramosissimum* (Korkmaz et al., 2024).

Among the compared plants, the TAS value of *P. aquilinum* extract was found to be the lowest. Although the TOS value was lower than the values reported for other species except *S. absconditiflora*, the OSI value was determined to be higher than all compared species due to the low antioxidant capacity. This result suggests that the amount of oxidant components in *P. aquilinum* extract alone is not high, but the existing antioxidant capacity may be more limited in terms of balancing this oxidant load compared to the compared plants. However, the differences between the plants; The metabolic characteristics of the species may be related to growing conditions, the plant organ used, harvest time, drying process, extraction solvent, and the amount of extract used in the analysis. Therefore, it is not appropriate to make a direct assessment in terms of species superiority or weakness. In addition, TAS, TOS, and OSI analyses reveal the general oxidant-antioxidant profile of the extract, but do not directly provide information about the identity and quantity of compounds contributing to activity. In conclusion, *P. aquilinum* was found to have antioxidant potential.

Anticholinesterase Activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), involved in the regulation of cholinergic transmission, are enzymes responsible for the hydrolysis of acetylcholine and related choline esters. Inhibition of these enzymes is considered a fundamental approach in studies related to cholinergic dysfunction, as it contributes to maintaining acetylcholine levels in the

synaptic cleft (Lai Shi Min et al., 2022). It has been reported that alkaloids, flavonoids, phenolic acids, and other secondary metabolites found in plant sources can interact with cholinesterase enzymes and suppress enzyme activity at varying levels (Santos et al., 2018; Murray et al., 2024). Therefore, determining the inhibitory effects of natural extracts on AChE and BChE is important for the investigation of new anticholinesterase compounds. In this study, the inhibitory capacity of *P. aquilinum* ethanol extract against AChE and BChE enzymes was evaluated, and the results obtained are presented in Table 2.

Table 2. Anti-AChE and anti-BChE activities of Pteridium aquilinum

Sample	AChE (µg/mL)	BChE (µg/mL)
Pteridium aquilinum	162.10±0.12	184.93±1.47
Galantamine	7.52±0.19	17.48±0.11

Note: Values are presented as mean±SD

In our study, the cholinesterase inhibitory effect of *P. aquilinum* ethanol extract was evaluated using IC_{50} values. The lower IC_{50} value for AChE indicates that the inhibitory effect of the extract on this enzyme is slightly higher compared to BChE. However, the limited difference between the two values suggests that the extract has a similar level of effect on both cholinesterase enzymes. The IC_{50} values for the reference inhibitor galantamine were determined as 7.52±0.19 and 17.48±0.11 µg/mL for AChE and BChE, respectively. Accordingly, the inhibitory effect of *P. aquilinum* extract remained at a lower level than galantamine. This situation can be explained by the fact that galantamine is a pure inhibitor, while the plant extract exhibits a complex structure containing numerous active and inactive compounds. Previous studies on the anticholinesterase activity of *P. aquilinum* have shown that some pterosin derivatives present in the species exhibit different levels of inhibition. The compound (−)-pteroside N isolated from the species has been reported to show high inhibitory activity on both AChE and BChE, while the compound pterosinone exhibits lower activity (Choi et al., 2018). Similarly, the pterosinone compound isolated from the ethanol extract of *P. aquilinum* has been reported to have a low inhibitory effect on AChE and BChE (Arya et al., 2021). These findings suggest that the anticholinesterase potential of the species may vary depending on the type and amount of compounds present in the extract. In the present study, the weaker inhibition shown by the crude ethanol extract compared to galantamine may be related to the low amount of compounds with strong activity in the extract, or to the fact that compounds with low activity contribute more to the total activity. The results obtained reveal that the *P. aquilinum* ethanol extract has measurable but limited inhibitory activity on both AChE and BChE. The relatively lower IC_{50} value of the extract against AChE suggests that the components may exhibit a partial preference for this enzyme; however, calculation of the selectivity index and further analyses with pure compounds are necessary to confirm enzyme selectivity. The co-occurrence of compounds such as (−)-pteroside N, previously reported to have high activity in this species, and pterosinone, which has low activity, indicates that the overall effect of the crude extract cannot be attributed to a single compound. Therefore, fractionation of the *P. aquilinum* extract, determination of the compound profile, and separate evaluation of the isolated components are necessary to elucidate the chemical source of the observed anticholinesterase activity.

Antiproliferative Activity

The proliferation of cancer cells independently of regulatory mechanisms is one of the decisive processes in tumor development and progression (Sofi and Tabassum, 2023). Therefore, the investigation of natural products that can reduce cell viability and suppress proliferation is among the main topics of plant-derived biological activity studies. Phenolic compounds, flavonoids, alkaloids, and other secondary metabolites found in plant extracts can cause loss of viability in different cancer cell lines by affecting cellular metabolism, oxidative balance, and proliferation mechanisms (Yuan et al., 2022; Situmorang et al., 2024). However, the level of these effects varies depending on the plant species, extraction method, applied concentration, and cell line characteristics (Unal et al., 2026). In this study, the effect of *P. aquilinum* ethanol extract on the viability of A549 human lung cancer cells was investigated using the MTT method, and the results regarding the antiproliferative/cytotoxic potential of the extract are shown in Figure 1.

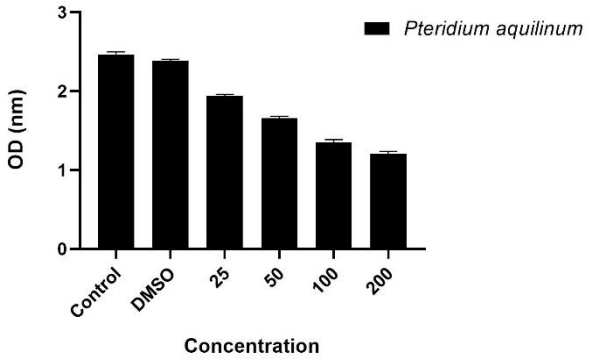

Figure 1. Concentration-dependent antiproliferative effect of Pteridium aquilinum

In our study, when the effect of *P. aquilinum* ethanol extract on A549 lung cancer cells was examined, it was observed that the optical density values decreased with increasing extract concentration. The fact that the control and DMSO groups showed similar values suggests that the decrease in cellular metabolic activity is related more to the extract application than to the solvent. A gradual decrease in OD values was determined as the extract concentration increased from 25 µg/mL to 200 µg/mL, and the lowest value was obtained at the 200 µg/mL application. These results show that *P. aquilinum* extract reduces the metabolic activity of A549 cells in a concentration-dependent manner and has antiproliferative/cytotoxic potential. However, the fact that the decrease between the 100 and 200 µg/mL groups was more limited compared to the previous concentration ranges suggests that the effect may approach a plateau tendency at higher doses. The literature reports that pteroside Z, isolated from P. aquilinum, reduces cell viability, suppresses intracellular LDH activity, and induces mitochondrial-mediated apoptosis in the A549 cell line (Li et al., 2020). This finding is consistent with the concentration-dependent decrease in viability determined with the crude ethanol extract in the present study and suggests that pteroside derivatives may contribute to the observed cellular effect. However, since a crude extract containing multiple compounds was used instead of pure pteroside Z in the present study, it is not possible to attribute the effect solely to this compound. The tendency of the response to plateau, especially at high concentrations, may be related to the saturation of the active components at their cellular targets, the limitation of the entry of extract components into the cell, or the emergence of synergistic and antagonistic interactions between compounds within the same extract (Caesar and Cech, 2019; Rønneberg et al., 2021). While the MTT test reveals changes in metabolic activity, it does not directly explain the mechanism of cell death. Therefore, to determine whether the observed effect is related to apoptosis, cell cycle arrest, mitochondrial dysfunction, or membrane damage, it needs to be supported by analyses of LDH release, caspase activity, mitochondrial membrane potential, and cell cycle data.

CONCLUSION

This study evaluated the oxidant-antioxidant profile of *Pteridium aquilinum* ethanol extract, its inhibitory effects on AChE and BChE enzymes, and its activity on A549 cell viability. The extract was found to have a measurable total antioxidant capacity, but the OSI value indicated a relatively high oxidant load relative to the antioxidant capacity. In cholinesterase analyses, the extract inhibited both enzymes, showing higher activity against AChE than BChE. However, compared to galantamine, the anticholinesterase effect remained limited. The concentration-dependent decrease in metabolic activity observed in A549 cells suggests that the extract possesses antiproliferative or cytotoxic potential. The results indicate that *P. aquilinum* contains compounds that may contribute to different biological activities. However, analyses performed with the crude extract do not explain which compounds are responsible for the observed effects or the mechanism by which the decrease in cell viability occurs. Given the potential toxic components that may be present in the species, these findings should not be interpreted as direct evidence of therapeutic use or safety. Further investigation is needed to determine the chemical profile of the extract, separate the active fractions, measure the ptaquiloside level, perform selectivity analyses in normal cell lines, and investigate the mechanisms of cell death.

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None

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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