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Original Article

Evaluation of Antioxidant, Anticholinesterase and Antiproliferative Activities of the Aerial Parts of *Dryopteris raddeana*

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Received: 21.05.2025 / Revised: 17.07.2025 Accepted: 06.08.2025 / Published online: 20.08.2025

ABSTRACT

This study aimed to evaluate the biological activities of the ethanol extract obtained from the aerial parts of *Dryopteris raddeana* (Fomin) Fomin. Total antioxidant capacity (TAS), total oxidant level (TOS) and oxidative stress index (OSI) values determined by Rel Assay kits using ethanol extract obtained from aerial parts of the plant were determined as 5.099±0.076 mmol/L, 7.354±0.107 µmol/L and 0.144±0.004, respectively. These results show that *D. raddeana* exhibits a balanced oxidative profile and has low oxidant load. In anticholinesterase activity analyses, the inhibitory effect of the extract against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes was determined as 58.99±1.48 µg/mL and 83.51±1.59 µg/mL, respectively. In the evaluation of antiproliferative effect, it was observed that extract concentrations in the range of 25–200 µg/mL applied to A549 human lung cancer cell line decreased cell viability in a dose-dependent manner. A significant decrease in cell viability was detected especially at concentrations of 100 and 200 µg/mL. In conclusion, in this study, it was determined that the plant could be an antioxidant, antimicrobial and antiproliferative source for these biological activities of *D. raddeana*.

KEYWORDS: *Dryopteris raddeana*; Antioxidant Activity; Anticholinesterase; Antiproliferative Effect; Phytotherapy.

1. Introduction

Plants have been at the forefront of natural resources that support human health throughout history; They have been widely used in traditional treatment systems with their medicinal, aromatic and nutritional properties [1]. Today, plants have become the focus of modern pharmaceutical research thanks to the wide biological activities of the secondary metabolites they contain [2]. Phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and saponins exhibit a wide range of biological effects, including antioxidant, antimicrobial, anticancer, anti-inflammatory, hepatoprotective, and antidiabetic activities. [3-9]. In recent years, interest in natural products has increased due to the side effects and resistance problems of synthetic drugs. In this context, determining the biological activities of extracts obtained from various plant species is of great importance both in terms of the discovery of new drug candidates and the development of natural treatment alternatives [10,11]. In addition, performing biological activity tests at in vitro and in vivo levels scientifically reveals the pharmacological potential of these extracts and contributes to the support of traditional knowledge with modern methods.

Dryopteris raddeana (Fomin) Fomin (Old name: *Dryopteris pallida*) is a fern species belonging to the Pteridaceae family, widespread in high mountainous regions. This species is known for growing in moist and shady habitats and has attracted attention in systematic botanical studies with its morphological differences [12]. (Recent studies have revealed that ferns are important not only in terms of taxonomy but also in terms of their chemical contents and potential biological activities. In this context, the chemical components of Dryopteris raddeana, especially phloroglucide derivatives, have been studied in detail by advanced semi-quantitative analysis methods. Phloroglucides are phenolic compounds generally known for their antioxidant, antimicrobial and cytotoxic effects [13]. The high levels of these compounds in *D. raddeana* were found to be remarkable in terms of the potential pharmacological effects of the species. In addition, it is thought that the secondary metabolites contained in this species may have protective effects in biological systems related to oxidative stress [14]. In this context, advanced biological activity tests on *D. raddeana* may contribute to new natural product-based treatment approaches. In this study, it was aimed to determine the antioxidant, antiproliferative and anticholinesterase activities of *D. raddeana*.

2. Materials and Methods

The *Dryopteris raddeana* samples used in this study were obtained from Fenk Yaylası, Osmaniye Province, Turkey. The aerial parts (leaves and stems) of the plant were dried in a laboratory environment at room temperature (~22–24 °C), in the shade, for 7 days to preserve phytochemical stability. During the extraction process, 10 grams of plant material was taken and extracted in a Soxhlet apparatus for approximately 6 hours with 250 mL of 96% ethanol at 50 °C. The obtained crude extract was separated from its solvent using a Buchi R100 rotary evaporator device operating at 40 °C. The resulting extracts were stored at +4 °C until experimental applications. All measurements were performed in triplicate and results are presented as mean ± standard deviation (StD). No statistical significance tests were applied.

2.1. Antioxidant tests

In this study, the total antioxidant capacity (TAS) and total oxidant level (TOS) of the extract obtained from the aerial parts of Dryopteris raddeana using ethanol were determined using commercial analysis kits belonging to Rel Assay company. The measurements were carried out in accordance with the analysis protocols provided by the manufacturer. While trolox was used as the standard substance in TAS analyses, calibration was performed with hydrogen peroxide for TOS analyses. TAS results were reported in mmol/L and TOS results in µmol/L [15,16]. In the calculation of the oxidative stress index (OSI), both parameters were converted to the same unit and the ratio obtained by dividing TOS by TAS was presented in percentage format. OSI was calculated using the formula: OSI (arbitrary unit) = TOS [µmol H_2O_2 equivalent/L] / (TAS [mmol Trolox equivalent/L]) × 10) [17].

2.2. Anticholinesterase tests

In this study, anticholinesterase activities of extracts of aerial parts of *D. raddeana* were evaluated based on the colorimetric method developed by Ellman et al. [18]. (1961). In inhibitory activity assays against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, galantamine was used as standard inhibitor. Stock solutions were prepared by diluting plant extracts in the concentration range of 200 to 3.125 µg/mL. In the experimental procedure, 130 µL (0.1 M, pH 8.0) phosphate buffer, 10 µL of test sample and 20 µL of enzyme solution (AChE or BChE) were added to each microwell, respectively; the mixture was incubated at 25 °C in the dark for 10 min. After incubation, 20 µL of DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] solution and then 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added to initiate the reaction. Enzymatic activity was measured spectrophotometrically at 412 nm wavelength. Each test was performed in triplicate, the inhibition percentages of the extracts were calculated and expressed as IC_{50} (µg/mL) values.

2.3. Antiproliferative activity tests

In this study, the cytotoxic effect of aerial parts of *D. raddeana* extract on A549 human lung carcinoma cell line was evaluated using MTT (methyl thiazolyl tetrazolium) cell viability test method. Before experimental applications, plant extracts were prepared at concentrations of 25, 50, 100 and 200 µg/mL to obtain stock solutions. After the cells reached approximately 70-80% density, the surface detachment was performed with 3.0 mL Trypsin-EDTA solution (Sigma-Aldrich, MO, USA). The separated cells were transferred to culture plates at appropriate density and incubated for 24 hours. Following incubation, plant extracts prepared at different doses were applied to the cells and a second 24-hour incubation period was started. At the end of the process, the supernatant medium was removed and MTT solution at a concentration of 1 mg/mL was added and the cells were incubated at 37 °C. After the formation of formazan crystals was observed, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was added to dissolve these crystals. Cell viability was measured at a wavelength of 570 nm using an Epoch brand spectrophotometer (BioTek Instruments, Winooski, VT, USA) [19].

3. Results and Discussion

3.1. Antioxidant Activity

Plants are exposed to various environmental stress factors throughout their growth and development processes, and these stresses result in excessive production of reactive oxygen species (ROS), causing oxidative stress [20]. The resulting oxidant compounds can cause damage such as lipid peroxidation, protein denaturation, and DNA damage in plant cells. In order to prevent this molecular damage, plants activate their antioxidant systems as a natural defense strategy [21,22]. These antioxidant components protect cell integrity by neutralizing the harmful effects of ROS and increase the resistance of the plant to stress conditions. Therefore, determining plant antioxidant capacity is of great importance both in terms of understanding the adaptation mechanisms against environmental stresses and in terms of biotechnological and pharmacological applications [23,24]. In this study, the antioxidant potentials of the aerial parts of *D. raddeana* were evaluated. The obtained data are presented in Table 1.

Table 1. TAS, TOS ve OSI values of *Dryopteris raddeana*

Plant	TAS mmol/L	TOS µmol/L	OSI
Dryopteris raddeana	5.099±0.076	7.354±0.107	0.144±0.004

* Values are presented as mean±SD

There is no direct study on the antioxidant activity of *D. raddeana* in the literature. However, there are various scientific findings on the antioxidant potential of different *Dryopteris* species [25-27]. In this context, our study was carried out to evaluate *D. raddeana* in terms of antioxidant properties. In our study, the total antioxidant capacity (TAS), total oxidant level (TOS) and oxidative stress index (OSI) values of the plant were measured using Rel Assay kits. Data on these parameters of different plant species have been widely reported in the literature. For example, TAS values of *Scorzonera papposa, Mentha longifolia, Anthemis cotula, Hypericum* *spectabile*, *Equisetum ramosissimum* and *Ferulago platycarpa* were determined as 6.328, 6.094, 7.625, 9.306, 4.802 and 5.688 mmol/L, respectively. TOS values were 11.525, 14.050, 11.247, 13.065, 7.643 and 15.552 µmol/L, respectively; OSI values were reported as 0.182, 0.231, 0.148, 0.140, 0.159 and 0.273 [28-33]. Compared to these studies, the TAS value of *D. raddeana* was higher than *E. ramosissimum* and lower than *S. papposa*, *M. longifolia*, *A. cotula*, *H. spectabile* and *F. platycarpa*. TAS is an important indicator reflecting the total antioxidant compounds in a natural product [34]. In this context, it can be said that *D. raddeana* has a moderate antioxidant capacity. Especially when compared to some species with lower TAS values, it is understood that *D. raddeana* has a remarkable potential in terms of antioxidant compound content. The TOS value represents the total oxidant load in the natural product [34]. In our study, the TOS value of *D. raddeana* was found to be lower than all compared species. This finding shows that the plant carries a low level of oxidant compounds and its potential to create oxidative stress is limited. In this respect, *D. raddeana* exhibits a positive profile, especially in terms of antioxidant/oxidant balance. In addition, this low oxidant load suggests that the plant may be a safe candidate for phytotherapeutic uses. The OSI value is a critical parameter reflecting the oxidative stress balance. The low OSI shows that antioxidants exhibit effective protection against oxidant compounds [34]. The OSI value of *D. raddeana* was found to be higher than only *H. spectabile* and lower than all other plant species. This indicates that the plant's antioxidant defense system exhibits an effective performance in combating oxidative load. When evaluated in general, the antioxidant profile determined in *D. raddeana* is supported by both low oxidant load and balanced OSI value. These properties of the plant, when supported by advanced biodirected studies, indicate that it is a valuable candidate for the discovery of new natural antioxidant sources. In addition, more detailed determination of the potential antioxidant compounds of this species with different extraction methods and fractionation studies may expand its pharmacological use areas.

3.2. Anticholinesterase activity

In recent years, studies on the anticholinergic activities of natural compounds of plant origin have gained great momentum. Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes has become an important pharmacological target, especially in the treatment of neurodegenerative disorders such as Alzheimer's disease [35]. Many medicinal and aromatic plant species have the potential to inhibit these enzymes through the alkaloids, flavonoids, terpenoids and phenolic compounds they contain. These compounds obtained from plant extracts can support synaptic transmission by increasing acetylcholine levels and slow down the progression of cognitive disorders. In this context, the discovery of natural products with anticholinergic effects is of great importance both in terms of developing safe alternative treatment strategies and revealing new drug candidates for the pharmaceutical industry [36,37]. In this study, the anticholinesterase activity of *D. raddeana* was investigated and the results are presented in Table 2.

Table 2. Anti-AChE and anti-BChE values of *Dryopteris raddeana*

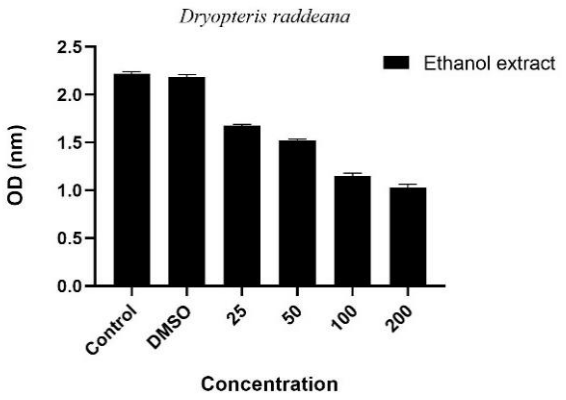
Sample	AChE µg/mL	BChE µg/mL
Dryopteris raddeana	58.99±1.48	83.51±1.59

* Values are presented as mean±SD

Literature reviews revealed that there is no study on the anticholinesterase activity of *D. raddeana*. However, there are various scientific data on the inhibitory effects of other species of the same genus on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes [25,38]. This situation increases the potential contribution of our study evaluating the anticholinesterase properties of *D. raddeana* to the literature. In our study, the inhibitory activity of aerial parts of *D. raddeana* against AChE and BChE enzymes was evaluated and galantamine was used as the reference standard. It was found that galantamine showed high levels of inhibition on both enzymes. This result, as expected, is consistent with galantamine being a clinically approved anticholinesterase agent. On the other hand, it was found that *D. raddeana* extract also showed a certain level of inhibitory effect on both enzymes. This finding indicates that the plant structure may contain natural compounds with anticholinesterase potential (e.g. phenolic compounds, flavonoids or terpenoids). However, the fact that the inhibitory activity is not as strong as galantamine may be due to the complex structure of the herbal extract and the presence of active compounds in diluted concentrations. Anticholinesterase activity is an important treatment target, especially in neurodegenerative disorders such as Alzheimer's disease [39]. Therefore, determining the AChE and BChE inhibitory capacity of *D. raddeana* is important for the preliminary evaluation of the pharmacological potential of the species. In addition, the obtained data may pave the way for the isolation of more specific and potent inhibitory compounds by biodirected fractionation studies of this plant. In conclusion, these first findings regarding the anticholinesterase activity of *D. raddeana* may form the basis for future in vivo and molecular level studies. Particularly purified extracts or association of specific phytochemical compounds with biomarker activities will enable the evaluation of this species as a potential natural resource in neuroprotective drug development studies.

3.3. Antiproliferative activity

Plants are among the important natural resources in anticancer drug research thanks to their rich structures in secondary metabolites. It has been scientifically proven that compounds such as flavonoids, alkaloids, terpenoids, phenolic acids and lignans, which are among these metabolites, have antiproliferative effects on many cancer cell lines [40,41]. Antiproliferative activity is usually achieved by mechanisms such as arresting the cell cycle at certain stages, triggering apoptotic pathways or mitochondrial dysfunction. These effects of plant extracts often occur both dose-dependent and cell type-specific. In this respect, plant sources offer valuable alternatives in terms of both efficacy and toxicity in the development of chemotherapeutic drugs [42,43]. In this study, the effects of *D. raddeana* on A549 lung cancer cells were investigated and the results are presented in Figure 1.



(Control: The group not treated with chemicals, only kept in the medium; DMSO: The group in which the medium and DMSO were applied; the group in which the extract was applied at 25, 50, 100 and 200 µg/mL concentrations, OD: Optical Density measured at 570 nm using MTT assay)

Fig 2. Antiproliferative effect of *D. raddeana* aerial part extract on A549 cells

In this study, the antiproliferative effects of *D. raddeana* ethanol extract on A549 human lung adenocarcinoma cell line were evaluated and the obtained findings are presented in Figure 1. Cell viability decreased significantly depending on the concentration of the applied extract. The significant decrease observed especially at 100 and 200 µg/mL concentrations reveals the dose-dependent cytotoxic effect of the extract. According to the data in Figure 1, no decrease in cell viability was observed in the DMSO group compared to the control group. This shows that DMSO did not show a toxic effect under experimental conditions and the observed cytotoxic effect was specific to *D. raddeana* extract. The decrease in OD values in the extract-applied groups reveals that cell proliferation was suppressed and the extract showed antiproliferative effects. Possible mechanisms of this effect may include induction of apoptosis, arrest of the cell cycle or increased cellular damage via oxidative stress. The absence of any findings in the literature regarding the effects of *D. raddeana* on the A549 cell line increases the originality and scientific contribution of this study. However, it has been reported that some *Dryopteris* species belonging to the same genus exhibit antiproliferative effects against A549 cells [44-46]. These previous studies show that *Dryopteris* species are rich in secondary metabolites and that these compounds may have anticancer properties. In this context, it is thought that *D. raddeana* may also contain similar structural components. The findings obtained reveal that *D. raddeana* may be a potential anticancer agent by reducing the viability of A549 cells at certain concentrations. The importance of plant sources is increasing especially in natural product research, and revealing the potential of species such as *D. raddeana* in this area may contribute to new therapeutic strategies. In future studies, biodirected fractionation of the extract, isolation of active compounds and elaboration of the mechanistic effects of these compounds at in vitro and in vivo levels are of great importance. In addition, analyses at the gene expression level will help to explain which molecular pathways this species affects to show antiproliferative effects.

4. Conclusions

This study is the first comprehensive study to evaluate the antioxidant, anticholinesterase and antiproliferative potential of aerial parts of *D. raddeana*. The results obtained showed that the total antioxidant capacity (TAS) of *D. raddeana* was significant, while the total oxidant level (TOS) was low and therefore the oxidative stress index (OSI) had a balanced structure. This situation reveals that the plant has a strong antioxidant profile and is a natural source that can be evaluated for pharmaceutical purposes. In addition, in the anticholinesterase analyses, it was determined that *D. raddeana* extract showed a certain level of inhibitory effect against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. It is thought that these effects may be related to the phenolic or flavonoid compounds in the structure of the plant. Although it did not exhibit as high an inhibitory power as galantamine, these results indicate that the plant can be evaluated among natural inhibitors for neurodegenerative diseases. In antiproliferative activity tests, it was determined that *D. raddeana* extract reduced cell viability in a dose-dependent manner on the A549 lung cancer cell line. The significant decrease observed especially at 100 and 200 µg/mL concentrations indicates that the plant can be considered as a potential anticancer agent. The absence of any study on this species in the literature specifically for the A549 cell line makes this study original and provides a new contribution to the literature. In conclusion, the antioxidant, anticholinesterase and antiproliferative activities of *D. raddeana* indicate that this plant should be evaluated among natural biological agents. In further studies, it is recommended that these findings be further deepened by performing biodirected fractionation of the plant extract, compound isolation and molecular level mechanism analyses. Thus, it can be demonstrated that *D. raddeana* may be a potential phytotherapeutic source for pharmaceutical, neuroprotective and oncological applications.

Author Contributions: This is a paragraph specifying the individual contributions to the article. The following statements should be used: Conceptualization, X.X. and Y.Y.; methodology, X.X.; validation, X.X., Y.Y. and Z.Z.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Nonr

Conflicts of Interest: The authors declare no conflict of interest.

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