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Biological activity potential of Thalictrum orientale: antioxidant, anticholinesterase, and antiproliferative effects

Thalictrum orientale'nin biyolojik aktivite potansiyeli: antioksidan, antikolinerjik ve antiproliferatif etkiler

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Abstract

Plants are among the important natural resources in terms of biological activity. In this study, the biological activity of *Thalictrum orientale* species was evaluated comprehensively. For this purpose, ethanol extract of the plant was obtained by Soxhlet extraction method. To determine the antioxidant capacity, total antioxidant status (TAS) and total oxidant status (TOS) were analyzed using Rel Assay kits. Anticholinesterase activity was determined by measuring acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme activities. In addition, antiproliferative activity was evaluated on A549 lung cancer cell line. As a result of the analyzes, TAS value of *Thalictrum orientale* was determined as 5.413±0.149 mmol/L, TOS value as 8.300±0.056 µmol/L and oxidative stress index (OSI) as 0.154±0.004. Within the scope of anticholinesterase activity, AChE inhibition value was determined as 35.41±1.88 µg/mL and BChE inhibition value was determined as 58.63±1.24 µg/mL. It was observed that the antiproliferative effect increased in direct proportion to the increase in concentration. The findings show that *Thalictrum orientale* has high biological potential in terms of antioxidant, antiproliferative and anticholinesterase activities.

Özet

Bitkiler, biyolojik aktivite açısından önemli doğal kaynaklar arasında yer almaktadır. Bu çalışmada, *Thalictrum orientale* türünün biyolojik aktivitesi kapsamlı bir şekilde değerlendirilmiştir. Bu amaçla, bitkinin etanol özütü Soxhlet ekstraksiyon yöntemiyle elde edilmiştir. Antioksidan kapasitesinin belirlenmesi için toplam antioksidan statusu (TAS) ve toplam oksidan statusu (TOS) Rel Assay kitleri kullanılarak analiz edilmiştir. Antikolinesteraz aktivitesi, asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) enzim aktiviteleri ölçülerek belirlenmiştir. Ayrıca, antiproliferatif aktivite, *A549* akciğer kanser hücre hattı üzerinde değerlendirilmiştir. Yapılan analizler sonucunda *Thalictrum orientale*'in TAS değeri 5.413±0.149 mmol/L, TOS değeri 8.300±0.056 µmol/L ve oksidatif stres indeksi (OSI) 0.154±0.004 olarak belirlenmiştir. Antikolinesteraz aktivitesi kapsamında AChE inhibisyon değeri 35.41±1.88 µg/mL, BChE inhibisyon değeri ise 58.63±1.24 µg/mL olarak saptanmıştır. Antiproliferatif etkinin, konsantrasyon artışı ile doğru orantılı olarak arttığı gözlemlenmiştir. Elde edilen bulgular, *Thalictrum orientale*'in antioksidan, antiproliferatif ve antikolinesteraz aktiviteleri açısından yüksek biyolojik potansiyele sahip olduğunu göstermektedir.

INTRODUCTION

Studies on the biological activities of plant components play an important role in revealing the therapeutic potential of natural resources. Plants have a wide spectrum of pharmacological effects thanks to the phenolic compounds, flavonoids, alkaloids and other bioactive molecules they contain (Savchenko et al. 2022, Yazar et al. 2024). The antioxidant, anti-inflammatory, antimicrobial and anticancer properties of these compounds hold promise in the prevention and treatment of diseases associated with oxidative stress. In particular, phenolic compounds reduce cellular damage by neutralizing free radicals and thus show anti-aging effects (Nik Salleh et al. 2020, Turkmen et al. 2023, Sevindik et al. 2023, El-Chaghaby et al. 2024, Mohammed et al. 2024). In addition, some herbal components have been reported to have hepatoprotective and DNA-protective properties, which increases the potential of plants to be used in clinical applications (Mohammed et al. 2023). Therefore, analyzing the biological activities of herbal extracts and examining the mechanisms of these compounds in more detail provides a scientific basis for modern medicine and drug development processes. In this study, *Thalictrum orientale* Boiss. plant was used as material.

Thalictrum orientale is a small-structured plant belonging to the Ranunculaceae family and usually grows in cracks on rocky slopes (Singh et al. 2023). Ethanolic extracts obtained from the underground organs of this species allowed the isolation of various alkaloid compounds. The main alkaloids isolated include fangchinoline, fuzitine and berberine. Fangchinoline is an alkaloid reported for the first time in the *Thalictrum* genus and is the compound found in the highest amount in the underground parts of the plant. Fuzitine is an aporphine derivative alkaloid, which was first identified in the genus *Thalictrum*. In addition, a new phenolic compound, thalictricoside, and a known cyanoglycoside, lithospermoside, were also isolated from the plant (Erdemgil et al. 2001, Erdemgil et al. 2003). The aim of this study was to determine the antioxidant, antiproliferative and anticholinesterase activities of *Thalictrum orientale*.

MATERIALS AND METHODS

Raw Materials

Plant samples of *Thalictrum orientale* included in the study were procured from the Adana province of Türkiye. The aerial components of the plant were desiccated under suitable laboratory conditions and subsequently readied for examination. The plant identification was made Dr. Mustafa SEVİNDİK. Herbarium Samples (Number: M.S -1515) of the plant are kept in Osmaniye Korkut Ata University, Pharmacy Services Laboratory.

Extraction

Ten grams of the plant sample were utilized for the extraction process using the Soxhlet extraction method. Extraction was conducted utilizing 250 mL of ethanol at 50 °C for roughly 6 hours. The crude extracts were concentrated using a Buchi R100 Rotary Evaporator at 40 °C, and the solvent was eliminated. The extracts were preserved at +4 °C until the experimental analyses were finalized.

Antioxidant Tests

In this study, total antioxidant capacity (TAS) and total oxidant level (TOS) of ethanolic extract obtained from aerial parts of *Thalictrum orientale* plant were evaluated by special kits provided by Rel Assay. Analyses were carried out in accordance with the protocol recommended by the kit manufacturer. Trolox was used as a calibrator substance for TAS tests and hydrogen peroxide was used as a calibrator for TOS tests. The obtained TAS and TOS values were expressed in mmol/L and µmol/L units, respectively (Erel 2004, 2005). When calculating the Oxidative Stress Index (OSI), TOS and TAS values were converted to equal units and a percentage value was calculated by taking the ratio of TOS to TAS (Sevindik 2019).

Anticholinesterase Activity Tests

This experiment examined the anticholinesterase activity of plant extracts from *Thalictrum orientale* using the Ellman method (Ellman et al. 1961). Galantamine functioned as a reference inhibitor during the assessment of the inhibitory effects on the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Stock solutions were prepared by diluting plant extracts within the concentration range of 200–3.125 µg/mL. The experiments entailed the addition of 20 µL of enzyme (AChE or BChE), 10 µL of stock extract solution, and 130 µL of 0.1 M phosphate buffer at pH 8 to the microplate wells, respectively. The microplate wells were thereafter incubated for 10 minutes at 25 °C in darkness. Subsequent to the incubation period, the reaction commenced with the addition of 20 µL of DTNB (5.5''-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL of substrate (either butyrylcholine iodide or acetylcholine iodide). Enzyme activity was determined by spectrophotometric measurement at a wavelength of 412 nm. The percentage inhibition of the extracts, or IC50 values (µg/mL), evaluated in three distinct assays, was utilized to indicate the enzyme inhibition capacity.

Antiproliferative Activity Test

The MTT (Cell Viability Test) method was used in this work to examine the cytotoxic effects of *Thalictrum orientale* plant extracts on the A549 lung cancer cell line. Initially, stock solutions with concentrations of 25, 50, 100, and 200 µg/mL were made. Prior to the experiment, 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) was used to separate the cells from the surface after they had reached 70–80% confluence. After being separated, the cells were placed on culture plates and left for a full day. Plant extracts at varying amounts were added to the cells after the incubation time, and the cells were then incubated for an additional twenty-four hours. Following the procedure, the cells were incubated at 37 °C after the supernatant was discarded and 1 mg/mL MTT solution was added. When the reaction was finished, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was added to dissolve the purple precipitate that had formed with the MTT solution. Lastly, an Epoch spectrophotometer (BioTek Instruments, Winooski, VT, USA) was used to evaluate the cells' vitality at a wavelength of 570 nm (Bal et al. 2017).

RESULTS AND DISCUSSION

Antioxidant Activity

Oxidative stress is a multifaceted chemical and physiological state that arises in plants due to biotic and abiotic stressors, disrupting cellular equilibrium. This condition inflicts cellular damage due to the overproduction and buildup of reactive oxygen species (ROS) (Sevindik et al. 2018). While reactive oxygen species (ROS) are inherently generated during plant metabolic processes, their production markedly escalates under environmental stressors such as excessive light, dehydration, and elevated temperatures. Plants establish many antioxidant defense mechanisms to safeguard themselves from the detrimental impacts of oxidative stress (Gürgen et al. 2020). The primary mechanisms include enzymes like superoxide dismutase (SOD) and glutathione reductase (Akgul et al. 2020). These enzymes inhibit the buildup of reactive oxygen species (ROS) in cells, enhance plant tolerance to oxidative stress, and safeguard cellular structures. Consequently, plants enhance their likelihood of survival under environmental stress circumstances (Mohammed et al. 2021). This study assessed the antioxidant capability of the aerial portions of *Thalictrum orientale*. The acquired data are displayed in Table 1.

Table 1. TAS, TOS ve OSI values of *Thalictrum orientale*

Plant	TAS mmol/L	TOS µmol/L	OSI
Thalictrum orientale	5.413±0.149	8.300±0.056	0.154±0.004

* Values are presented as mean±SD

No previous findings have been reported regarding the TAS, TOS, and OSI values of *Thalictrum orientale*. Literature has documented the TAS, TOS, and OSI values for various plant species. The TAS values for *Alcea kurdica*, *Mentha longifolia*, *Anthemis cotula*, *Origanum laevigatum*, and *Hypericum spectabile* are reported as 3.298, 6.094, 8.201, 8.609, and 9.306 mmol/L, respectively. The reported values for TOS are 8.312, 14.050, 10.870, 10.685, and 13.065. Reported OSI values include 0.252, 0.231, 0.133, 0.124, and 0.140 (Mohammed et al. 2022, Gürgen et al. 2024, Sabik et al. 2024, Sevindik et al. 2024, Uysal et al. 2024). The TAS value serves as an indicator of the total antioxidant compounds present in natural products (Gürgen and Sevindik 2022). The TAS value of *Thalictrum orientale* in our study was found to be greater than that of *Alcea kurdica*, but less than that of *Mentha longifolia*, *Anthemis cotula*, *Origanum laevigatum*, and *Hypericum spectabile*. TOS values serve as an indicator of the total oxidant compounds generated in natural products (Gürgen and Sevindik 2022). The TOS value of *Thalictrum orientale* in our study was found to be lower than that of *Alcea kurdica*, *Mentha longifolia*, *Anthemis cotula*, *Origanum laevigatum*, and Extraction. The OSI value indicates the percentage reduction of oxidant compounds by antioxidant compounds (Gürgen and Sevindik 2022). The OSI value of *Thalictrum orientale* in our study was found to be lower than that of *Alcea kurdica* and *Mentha longifolia*, while higher than *Anthemis cotula*, *Origanum laevigatum*, and *Hypericum spectabile*.

The results obtained show that the antioxidant capacity of *Thalictrum orientale* is at a moderate level compared to some plants in the literature. The fact that the TAS value is lower than certain plants indicates that there may be differences in the amount and composition of phenolic compounds, flavonoids or other redox active metabolites found in this plant. It is known that phenolic compounds and flavonoids directly contribute to the total antioxidant capacity (Gu et al. 2019). Therefore, the chemical profile of *Thalictrum orientale* should be examined in detail and the relationship between the phenolic compound content and antioxidant capacity should be revealed. The low TOS level indicates that the oxidant production of the plant is minimal and thus the oxidative stress is limited. This situation can be associated with the effectiveness of the antioxidant defense mechanisms of the plant.

The fact that the OSI value is lower for some plants and greater for others suggests that metabolic differences across species may be a significant factor and that the antioxidant-oxidant balance may vary depending on the environmental conditions. Numerous factors, including soil structure, climatic conditions, genetic variety, and the ecological settings in which the plant thrives, may contribute to this variability. Actually, according to reports in the literature, different environmental stressors have different effects on plants' antioxidant defense mechanisms (Mishra et al. 2023). In conclusion, a more thorough examination of *Thalictrum orientale*'s oxidative stress dynamics and antioxidant capability will help determine the plant's therapeutic potential. In this regard, it is advised to evaluate the activities of antioxidant enzymes, examine the expression levels of genes linked to oxidative stress, and conduct a thorough examination of the plant's phenolic and flavonoid profiles.

Anticholinesterase Activity

Anticholinesterase activity is an important biological mechanism that plays a role in the regulation of nerve conduction by inhibiting acetylcholinesterase and butyrylcholinesterase enzymes (Cichon et al. 2024). This mechanism, which is especially effective in the treatment of neurodegenerative diseases, has increased the interest in the research of natural compounds. Flavonoids, alkaloids, terpenoids and phenolic compounds obtained from various plants may exhibit anticholinesterase effects. Due to the positive effects of these compounds on the nervous system, the pharmacological potential of herbal extracts is increasingly being investigated (Jamal et al. 2023). In this study, the anticholinesterase activity of *Thalictrum orientale* was investigated and the results obtained are presented in Table 2.

Table 2. Anti-AChE and anti-BChE values of *Thalictrum orientale*

Sample	AChE µg/mL	BChE µg/mL
Thalictrum orientale	35.41±1.88	58.63±1.24
Galantamine	6.37±0.17	16.41±0.24

* The values were studied with 3 repetitions and the std values are given

In our study, the inhibitory effect of *Thalictrum orientale* plant on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes was evaluated and the obtained results are presented in Table 2. These results show that the anticholinerase activity of *Thalictrum orientale* is quite weak compared to galantamine. No study on the anticholinerase activity of *Thalictrum orientale* was found in the literature. However, it was reported that the AChE inhibition values of ethanol and methanol extracts of *Thalictrum minus*, a different species of the same genus, were in the range of 58.90-65.11 µg/mL and the BChE inhibition values were in the range of 72.25-85.79 µg/mL (Sevindik et al. 2025). Compared to *Thalictrum minus*, the inhibitory effect of *Thalictrum orientale* on AChE and BChE is significantly lower. This difference suggests that the phytochemical profiles of plants may vary at the species level and that anticholinersterase activity may exhibit significant differences among species. The findings suggest that although the anticholinersterase potential of *Thalictrum orientale* is low, detailed characterization of the compounds it contains should be performed. In particular, the presence and concentrations of certain phytochemical groups (alkaloids, flavonoids, terpenoids, etc.) may be important factors affecting the activity. Future studies on bioguided fractionation and isolated compounds may contribute to a better understanding of the pharmacological potential of this species.

Antiproliferative Activity

Antiproliferative activity plays an important role in the treatment of diseases characterized by uncontrolled cell growth, such as cancer, by suppressing cell division and proliferation (Sevindik et al. 2020). Plants constitute a large area of research as natural antiproliferative agents thanks to the alkaloids, flavonoids, terpenoids, phenolic compounds and other secondary metabolites they contain (Oran et al. 2022). These compounds can inhibit the growth of cancer cells through various molecular mechanisms, stop the cell cycle and show therapeutic effects by inducing apoptosis (programmed cell death). Therefore, the investigation of the antiproliferative effects of herbal extracts and natural compounds has significant potential for the discovery of new and effective chemotherapeutic agents (Alzahrani et al. 2024). In this study, the effects of *Thalictrum orientale* on A549 lung cancer cells were examined and the results obtained are presented in Figure 1.

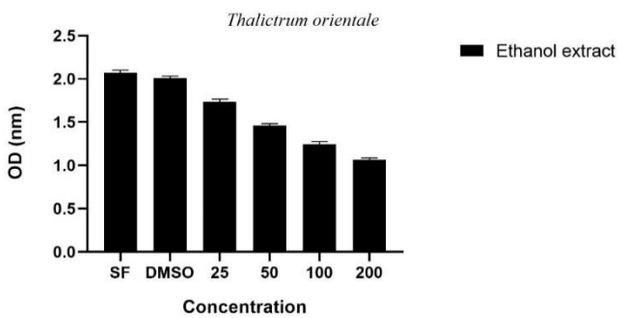


Figure 1. Antiproliferative activity of *Thalictrum orientale*

In our study, the effect of ethanol extract of *Thalictrum orientale* on cell proliferation at different concentrations was determined. OD (optical density) values reflect cell viability rate and lower OD values indicate higher antiproliferative activity. According to the findings, the highest OD values were observed in SF (serum-free, negative control) and DMSO (solvent control) groups, and this determined that the cells proliferated healthily. It was determined that *Thalictrum orientale* ethanol extract applied at different concentrations reduced cell proliferation in a dose-dependent manner. Especially, the lowest OD value was observed at the concentration of 200 µg/mL, and it was determined that cell viability decreased significantly. These results reveal that *Thalictrum orientale* ethanol extract may exhibit antiproliferative activity and inhibit cell growth. No study was found in the literature on the effect of *Thalictrum orientale* against A549 lung cancer cell line. However, it has been reported that *Thalictrum atriplex, Thalictrum fortunei, Thalictrum omeiense* and *Thalictrum minus* species belonging to the same genus have antiproliferative effects against A549 lung cancer cell line (Jiang et al. 2017, Meng et al. 2021, Chen et al. 2024, Sevindik et al. 2025). This suggests that some species of the *Thalictrum* genus have anticancer potential and that biological activity may vary among species. The findings reveal that *Thalictrum orientale* can inhibit cell proliferation in a certain dose range. However, additional studies are necessary to better understand the mechanism of the antiproliferative effect. In particular, cell cycle analyses, examination of apoptosis markers and determination of bioactive compounds contained in the extract will contribute to a better understanding of the anticancer potential of this species.

CONCLUSION

This study evaluated the antioxidant, anticholinesterase, and antiproliferative activities of *Thalictrum orientale* species and revealed its biological potential. The findings show that *Thalictrum orientale* has high antioxidant capacity and may be a potential natural resource in the management of diseases associated with oxidative stress. In addition, the acetylcholinesterase and butyrylcholinesterase inhibition effects of the plant emphasize its pharmacological importance in terms of neurodegenerative diseases. Antiproliferative activity analyses revealed the inhibitory effect of *Thalictrum orientale* on lung cancer cell line, suggesting that the plant may be a candidate plant in the discovery of new compounds for cancer treatment. In the future, it is recommended to expand studies on the biological activity of *Thalictrum orientale*. In particular, the isolation and chemical characterization of the active components of the plant are important in terms of elucidating the mechanism of its activity. In addition, the pharmacokinetic and pharmacodynamic properties of

this species should be evaluated by focusing on in vivo studies. Further research supported by preclinical and clinical studies will reveal the therapeutic potential of *Thalictrum orientale* more clearly.

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