RESEARCH ARTICLE

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Biological Activities of *Polygonum aviculare* Ethanol Extract

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ABSTRACT: *Polygonum aviculare* (*P. aviculare*) exhibits diverse biological activities thanks to its rich phenolic compounds and flavonoids. In this study, the antioxidant, anticholinesterase, and antiproliferative activities of *P. aviculare* were evaluated using ethanol extract. Plant material was prepared by Soxhlet extraction, and total antioxidant capacity (TAS), total oxidant level (TOS), and oxidative stress index (OSI) were measured using commercial Rel Assay kits. TAS value was found to be 5.436±0.097 mmol/L, TOS value was 10.830±0.103 µmol/L, and OSI value was 0.199±0.005, indicating that the extract has a strong antioxidant capacity. Anticholinesterase activity was assessed using the Ellman method, and IC_{50} values were determined as 85.773±3.551 µg/mL for AChE and 117.933±3.192 µg/mL for BChE. Although weaker activity was observed compared to the reference inhibitor galantamine, it was found to be a noteworthy natural inhibitor source. Furthermore, antiproliferative activity was investigated in the A549 lung cancer cell line using the MTT assay, and it was determined that the extract significantly reduced cell viability, especially at concentrations of 100 and 200 µg/mL. The findings demonstrate the multifaceted biological activities of *P. aviculare* and support its evaluation as a potential bioresource in natural therapeutic approaches.

Keywords: Antioxidant activity; cholinesterase inhibition; antiproliferative effect; natural products; phytotherapy.

INTRODUCTION

Throughout history, plants have held great importance for humanity not only as a source of nutrition but also for their therapeutic properties. Medicinal plants, thanks to the secondary metabolites they contain, are used in the prevention and treatment of many diseases and are currently at the center of research in pharmacology, biotechnology, and medicine. Plants are notable for their rich phytochemical content, such as alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, and essential oils (Sağlıker and Darici, 2005; Sağliker and Darici, 2007; Yaşar et al., 2009; Turkmen et al., 2023; Colak et al., 2025). The biological activities of plants are associated with the multifaceted effects of these phytochemical compounds, such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective (Mohammed et al., 2023). Antioxidant plant components play a significant role in reducing oxidative stress, particularly caused by free radicals. Phenolic and terpenoid compounds provide natural protection against bacterial and fungal pathogens (Akkaya et al., 2024; Yazar et al., 2024). Furthermore, compounds derived from some medicinal plants are used as lead molecules in the development of modern pharmaceutical products, thus establishing a strong bridge between traditional herbal medicine and modern medicine (Çömlekçioğlu et al., 2024). The increasing interest in natural products in recent years is directly related to the side effects and resistance problems of synthetic drugs. Therefore, systematic investigation of the biological activities of medicinal plants is of great importance both for the development of new therapeutic agents and for supporting existing treatment approaches (Uysal et al., 2023). Furthermore, these studies contribute to the preservation of cultural heritage by supporting ethnobotanical knowledge with scientific data. In this context, the study of the biological activities of medicinal plants has broad applications not only in health sciences but also in environmental biology, food technology, and sustainability (Dursun et al., 2017; El-Chaghaby et al., 2024). Therefore, multidisciplinary studies on medicinal plants play a strategic role in future drug discovery and protecting public health (Türkmen and Koçer, 2021).

Polygonum aviculare (commonly known as bird's bread) is an annual herbaceous plant that grows low to the ground and is widespread in temperate regions worldwide. It exhibits high morphological and genetic diversity (Mosaferi et al., 2015a; Mosaferi et al., 2015b; Benrahou et al., 2023). It has small, triangular seeds and white-pink flowers (Al-Newani et al., 2020; Mahmoudi et al., 2020). This species, rich in phytochemicals, contains numerous bioactive compounds, particularly flavonoids (quercetin, myricitrin, avicularin, kaempferol), phenolic acids (e.g., ferulic acid), and lignans (Granica, 2015; Petruk and Vysochina, 2019; Yu et al., 2022). Flavonol glucuronides and various phenolic compounds, in particular, are prominent components in the plant's pharmacological effects (Granica et al., 2013; Granica, 2015; Petruk and Vysochina, 2019). *P. aviculare*, which exhibits a wide range of pharmacological effects, is notable for its antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities. Thanks to its antioxidant properties, it plays an important role in scavenging free radicals and protecting DNA from oxidative damage (Hsu, 2006; Mahnashi et al., 2022). Its anti-inflammatory effect is mediated through activation of the Nrf2/HO-1 pathway and suppression of NF-κB (Granica et al., 2013; Jang et al., 2024). Its antimicrobial activity is potent against both Gram-negative and Gram-positive bacteria and has been found to be particularly effective against

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multidrug-resistant bacteria (Salama and Marraiki, 2010; Hanoun et al., 2025). Furthermore, it has antidiabetic potential due to its potent inhibition of the α-glucosidase enzyme (Cai et al., 2020; Mahnashi et al., 2022). In addition, diuretic and bronchodilator effects have also been reported by the researchers (Luo et al., 2018; Li, 2023). All these data suggest that *P. aviculare* exhibits versatile biological activities consistent with its rich phytochemical composition.

MATERIALS AND METHODS

Laboratory Studies

Plant samples were collected from Antalya, Turkey, and the herbarium material is preserved in the herbarium collection of the Biology Department of the Faculty of Science at Akdeniz University. The aerial parts were washed with distilled water to remove soil and other foreign particles and then dried under laboratory conditions at appropriate temperature and humidity. The dried plant material was ground to a fine powder in a mechanical grinder. 15 g of the prepared samples were weighed, and extraction was performed using a Soxhlet apparatus using n-hexane and dichloromethane solvents at approximately 50°C for six hours. After extraction, the solvents were removed using a rotary evaporator, and the resulting extracts were stored at +4°C until experimental studies were performed.

Antioxidant and Oxidant Tests

The total antioxidant capacity (TAS) and total oxidant level (TOS) of the ethanol extract used in this study were measured using commercial kits from Rel Assay Diagnostics. Analyses were conducted according to the protocol provided by the manufacturer. Trolox was used as the standard for TAS determination, while hydrogen peroxide was used as the reference for calibration in TOS measurements. The obtained TAS and TOS values were expressed in mmol/L and μmol/L, respectively (Erel, 2004; Erel, 2005). The oxidative stress index (OSI) was calculated by converting both parameters to the same units and dividing TOS by TAS (Sevindik, 2021).

Anticholinesterase Activity Tests

The anticholinesterase activity of the ethanolic extract was evaluated using the method described by Ellman et al. (1961). Inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were investigated, and galantamine was used as a reference inhibitor in comparative controls. Prior to experimental studies, stock solutions were prepared by diluting the extracts to a concentration range of 200-3.125 μg/mL. During the assay process, 130 μL of 0.1 M phosphate buffer (pH 8.0), 10 μL of extract solution, and 20 μL of enzyme solution (AChE or BChE) were added to 96-well microplates, respectively. The plates were incubated at 25 °C in the dark for 10 minutes. Following incubation, 20 μL of DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] solution and 20 μL of substrate (acetylcholine iodide or butyrylcholine iodide) were added to each well to initiate the reaction. Enzymatic activities were measured spectrophotometrically at 412 nm. Inhibitory effects of the extracts were calculated using IC_{50} (μg/mL) values, and all experiments were performed in triplicate.

Antiproliferative Activity Test

The antiproliferative effects of the ethanolic extracts of the plant were evaluated in the A549 lung cancer cell line using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] method. When the cells reached 70–80% density, they were detached from the culture surface with 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) and then transferred to appropriate plates. After cell seeding, they were incubated for 25 hours. Subsequently, extracts at concentrations of 25, 50, 100, and 200 μg/mL were applied to the cells, and the cells were incubated for another 25 hours. Medium without fetal calf serum (FCS) was used in the negative control groups. After a total of 48 hours of incubation, the culture supernatants were removed, and MTT solution (Sigma) prepared at a concentration of 1 mg/mL was added to each well. The cells were incubated at 37 °C until the formation of purple formazan crystals was observed. The supernatants were then removed, and the crystals were dissolved by the addition of dimethyl sulfoxide (DMSO, Sigma-Aldrich, MO, USA). The absorbance values of the obtained solutions were measured at a wavelength of 570 nm using an Epoch microplate spectrophotometer (BioTek Instruments, Winooska, VT, USA) (Ünal et al., 2025).

RESULTS AND DISCUSSION

Antioxidant Activity

Phenolic compounds, flavonoids, and other secondary metabolites found in plants exhibit strong antioxidant properties. By neutralizing free radicals, they protect cellular structures from oxidative stress and contribute to the prevention of many chronic

diseases (Sevindik et al., 2025). In this context, the antioxidant potential of *Polygonum aviculare* was determined in our study. The findings are shown in Table 1.

Table 1. *Antioxidant activity of ethanol extract of Polygonum aviculare*

Sample	TAS (mmol/L)	TOS (µmol/L)	OSI (TOS/(TASx10))
Polygonum aviculare	5.436±0.097	10.830±0.103	0.199±0.005

In our study, the ethanol extract of *Polygonum aviculare* was found to have antioxidant capacity. The measured TAS value (5.436±0.097 mmol/L) suggests that the plant may play a significant protective role against oxidative stress. In contrast, the low OSI value (0.199±0.005) indicates that the antioxidant effect is dominant in the face of oxidant load. In the literature, various researchers have confirmed the antioxidant properties of *P. aviculare* using various methods. Hsu (2006) reported that plant extracts exhibit free radical scavenging properties. Granica et al. (2013) emphasized that phenolic glycosides and flavonoids play a key role in this activity. In a separate study, Larijani and Aziziyan (2023) demonstrated significant antioxidant potential in plant extracts prepared with different solvents. Uçar (2024) supported the antioxidant activity with findings obtained in vitro analyses. The high TAS and low OSI values determined in our study are consistent with these results reported in the literature, demonstrating once again that *P. aviculare* is a potent natural antioxidant source. Furthermore, no previous findings regarding TAS, TOS, and OSI values have been found for *P. aviculare*. In studies conducted on different plants, TAS values for *Mentha longifolia, Hypericum spectabile, Anthemis cotula, Dittrichia graveolens, Equisetum ramosissimum*, and *Salvia absconditiflora* were reported as 6.094, 9.306, 7.625, 6.93, 4.802, and 7.350 mmol/L, respectively. TOS values were reported as 14.050, 13.065, 11.247, 12.53, 7.643, and 8.501 µmol/L, respectively. OSI values have been reported as 0.231, 0.140, 0.148, 0.18, 0.159 and 0.116, respectively (Akgul et al., 2020; Korkmaz et al., 2023; Gürgen et al., 2024; Korkmaz et al., 2024; Sabik et al., 2024; Sevindik et al., 2024). The TAS value is an indicator of the whole of the antioxidant compounds produced in natural products (Gürgen and Sevindik, 2022). The TOS value is an indicator of the whole of the oxidant compounds produced in natural products (Gürgen and Sevindik, 2022). The OSI value shows the percentage of suppression of oxidant compounds by antioxidant compounds (Gürgen and Sevindik, 2022). The TAS value of *P. aviculare* used in our study was determined to be higher than that of *Equisetum ramosissimum* but lower than that of *Hypericum spectabile, Anthemis cotula, Dittrichia graveolens,* and *Salvia absconditiflora.* The higher TAS value obtained in our study compared to *Equisetum ramosissimum* indicates that the *P. aviculare* extract has a stronger antioxidant potential compared to some species. However, the lower TAS value obtained compared to *Hypericum spectabile, Anthemis cotula, Dittrichia graveolens,* and *Salvia absconditiflora* suggests that antioxidant capacity can vary significantly among species. This can vary depending on many factors, such as the genetic structure of the plants, ecological conditions, growth environment, extraction method used, and solvent types. Therefore, it can be concluded that while the antioxidant activity of *P. aviculare* is strong, it may be limited compared to some species; nevertheless, it still exhibits significant biological activity from a pharmacological perspective.

The TOS value of *P. aviculare* used in our study was found to be higher than that of *Equisetum ramosissimum* and *Salvia absconditiflora*, but lower than that of *Mentha longifolia, Hypericum spectabile, Anthemis cotula*, and *Dittrichia graveolens*. The higher TOS value of the *P. aviculare* extract compared to *Equisetum ramosissimum* and *Salvia absconditiflora* suggests that this plant may have a relatively high oxidant load. However, the lower TOS value compared to *Mentha longifolia, Hypericum spectabile, Anthemis cotula,* and *Dittrichia graveolens* suggests that *P. aviculare* has a more advantageous profile than some species in terms of oxidant levels. These differences in TOS values are closely related to the diversity of phytochemical compounds contained in the plants, extraction conditions, environmental factors (soil structure, climate, growing conditions), and metabolic adaptations. These results demonstrate that the antioxidant capacity of *P. aviculare* is supported not only by high TAS values but also by low and moderate TOS results, thus demonstrating the plant's potential to maintain oxidative balance. The OSI value of P. aviculare used in our study was lower than that of *Mentha longifolia* but higher than that of *Hypericum spectabile, Anthemis cotula, Dittrichia graveolens, Equisetum ramosissimum*, and *Salvia absconditiflora*. According to the findings of our study, the OSI value of the *P. aviculare* extract was lower compared to *Mentha longifolia*. This suggests that the plant exhibits a more balanced antioxidant defense against oxidant load. Conversely, higher OSI values were recorded compared to *Hypericum spectabile, Anthemis cotula, Dittrichia graveolens, Equisetum ramosissimum*, and *Salvia absconditiflora*. The high OSI suggests that oxidative stress is relatively more pronounced in *P. aviculare* compared to these species. This variation among different plants can vary depending on numerous parameters, such as phytochemical content, antioxidant compound density, extraction method, growing environment, and ecological factors. However, the moderate OSI value of *P. aviculare* supports the plant's ability to maintain oxidative balance to a certain extent and, therefore, possesses significant pharmacological antioxidant potential. Therefore, *P. aviculare* can be considered a potential supportive agent in the prevention or treatment of chronic diseases associated with oxidative stress.

Anticholinesterase Activity

Anticholinesterase activity is associated with the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes and is the underlying mechanism of action of drugs used in the treatment of neurodegenerative diseases, particularly Alzheimer's. Natural compounds derived from plants may contribute to the maintenance of acetylcholine levels in synaptic clefts by inhibiting these enzymes (Ahmad et al., 2024; Sadeghi et al., 2024). In this context, the anticholinesterase potential of *Polygonum aviculare* was determined. The findings are shown in Table 2.

Table 2. *Anticholinesterase activity of ethanol extract of Polygonum aviculare*

Sample	AChE (µg/mL)	BChE (µg/mL)
Polygonum aviculare	85.773±3.551	117.933±3.192
Galantamine	6.800±0.269	15.730±0.337

In our study, the ethanol extract of *Polygonum aviculare* was found to exhibit significant inhibitory effects on AChE and BChE enzymes. The IC_{50} values obtained were 85.773±3.551 µg/mL for AChE and 117.933±3.192 µg/mL for BChE, indicating a stronger inhibitory effect on AChE. Although the extract appears to exhibit a weaker effect compared to the low IC_{50} values of galantamine, it has considerable potential as a natural inhibitory source. Findings in the literature support the results of our study. Yılmaz-Özden et al. (2021) reported the EC_{50} value for the ethanol extract of *P. aviculare* as 143.32 µg/mL, which was found to be higher than the AChE IC_{50} value obtained in our study. This difference may be due to the extraction method, solvent type, plant material used (leaf, stem, root), and geographical conditions. Similarly, Büyükyıldırım et al. (2025) reported the IC_{50} value for the BChE inhibitory effect of the aerial parts extract of the plant as 104.33 µg/mL. This value is close to the value of 117.933±3.192 µg/mL determined in our study, and minor differences may be attributable to methodological differences. In conclusion, the data obtained in our study indicate that P. aviculare has a moderate inhibitory effect on cholinesterase enzymes and is particularly prominent in AChE inhibition. Therefore, the plant has the potential to support the treatment of neurodegenerative diseases as a natural source of anticholinesterase.

Antiproliferative effect

Antiproliferative effect refers to the biological activity that suppresses cell proliferation and is particularly important in cancer research. Natural compounds derived from plants are being evaluated as potential therapeutic agents by inhibiting tumor cell growth (Elbozan and Korkmaz, 2025; Korkmaz, 2025). In this context, the antiproliferative potential of *Polygonum aviculare* was determined against the A549 lung cancer cell line. The findings are shown in Figure 1.

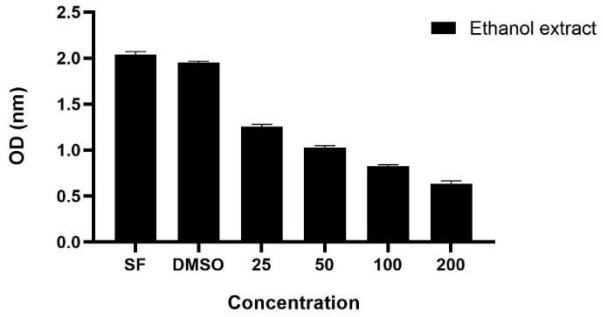

Figure 1*. Antiproliferative activity of ethanol extract of Polygonum aviculare*

In the literature, various researchers have reported that different extracts of *Polygonum aviculare* are effective against NRK-52E, HeLa, Hela-S cervical, and MCF-7 cancer cell lines (Büyükyıldırım et al., 2025). In our study, we determined that the ethanol extract of *Polygonum aviculare* exhibited a dose-dependent antiproliferative effect in A549 lung cancer cells. While high cell viability was observed in the control and DMSO groups, a significant decrease in cell viability occurred as the concentration increased in the extract-treated groups. The decrease in cell viability was particularly pronounced at concentrations of 100 and 200 µg/mL, indicating that the extract exhibits a potent cytotoxic effect at higher doses. Significant inhibition was observed even at low and moderate concentrations (25 and 50 µg/mL), supporting the plant's versatile antiproliferative capacity. Various researchers have reported in the literature that different *P. aviculare* extracts exhibit antiproliferative effects on NRK-52E, HeLa,

HeLa-S cervical, and MCF-7 breast cancer cell lines (Habibi et al., 2011; Mohammed et al., 2011; Büyükyıldırım et al., 2025). These results, when considered together with the findings for the A549 cell line obtained in our study, reveal that the plant has a broad-spectrum antiproliferative effect on different cancer cell types. The obtained data suggest that the flavonoid and phenolic compounds contained in *P. aviculare* may exert anticancer effects through mechanisms such as apoptosis induction, cell cycle arrest, and suppression of oxidative stress. In conclusion, both the results obtained in our study and previous research indicate that *P. aviculare* should be considered as a natural anticancer agent in cancer biology and provide a strong scientific basis for future in vivo and clinical studies.

CONCLUSION

The findings obtained in this study clearly demonstrate that the ethanol extract of Polygonum aviculare exhibits diverse biological activities. The high TAS value and low OSI ratio indicate that the plant possesses a strong antioxidant capacity. Although IC_{50} values in anticholinesterase assays were higher than those of the synthetic standard galantamine, the extract proved to be a noteworthy natural inhibitor source. Furthermore, the dose-dependent antiproliferative effect observed in the A549 cell line supports the plant's anticancer potential. These versatile biological activities suggest that P. aviculare could be an important candidate not only in traditional medicine but also in modern pharmaceutical research. Furthermore, additional studies supported by in vivo experiments and advanced pharmacological analyses will provide more comprehensive information regarding the plant's bioavailability, toxicological profile, and clinical applicability.

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None

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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