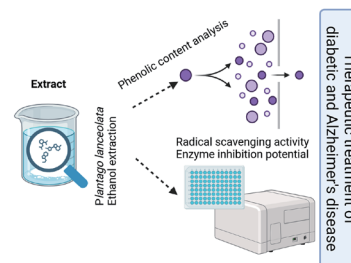


Phytochemical Compositions and Bioactive Properties of *Plantago lanceolata* L.: Anti-oxidant, Anti-diabetic, and Anti-cholinergic Potentials

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Abstract: In this study, the phenolic compound profile, antioxidant capacity, and inhibition potential on enzymes associated with diseases such as Alzheimer's disease (AD) and diabetes of *Plantago lanceolata* L. (PL) extract were investigated in detail. Quantitative analysis of phenolic compounds was performed by advanced liquid chromatography-tandem mass spectrometry (LC-MS/MS), and the highest concentration of the phenolic was observed in fumaric acid (4486.80 µg/L). Vanillic acid, kaempferol, resveratrol and caffeic acid were also detected in significant amounts. The antioxidant activities of PL ethanol extract were determined by DPPH, ABTS, FRAP, and CUPRAC methods and revealed moderate radical scavenging and metal-reducing capacities. The PL extract exhibited notable free radical scavenging activity, with inhibition rates of $37.86 \pm 2.42\%$ in the DPPH assay and $34.72 \pm 2.86\%$ in the ABTS assay. These results revealed that the extract showed an activity close to standard antioxidants such as α -tocopherol. The extract exhibited inhibitory activity against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α -glucosidase enzymes, with IC_{50} values of 8.77 ± 0.52 , 7.37 ± 0.53 , and 3.63 ± 0.27 µg mL⁻¹, respectively. These findings may indicate that PL extract may have therapeutic potential for disorders such as Alzheimer's disease and diabetes.



Key words: phenolic composition, enzyme inhibition, antidiabetic, antioxidant, *Plantago lanceolata* L.

1 Introduction

Excessive production of reactive oxygen species in living organisms and inadequate defense mechanisms against the harmful effects of these species are called oxidative stress. Oxidative stress causes damage to the cellular structure and plays an important role in the development of various health problems such as Alzheimer's, Parkinson's, cardiovascular diseases, diabetes and cancer. Antioxidants can effectively neutralize free radicals at low concentrations and prevent or delay oxidative damage to biomolecules through their radical scavenging and metal-reducing properties. Phenolic compounds such as flavonoids and phenolic acids, which are commonly found in medicinal aromatic plants, play critical roles in the cellular defense system as potent exogenous antioxidants¹⁻³.

Alzheimer's disease (AD) is a neurodegenerative disorder, the most common form of dementia, affecting more than

55 million people worldwide. One of the main pathologies of this disease is cognitive dysfunction, which can be caused by a decrease in acetylcholine (ACh) levels in the brain and damage to cholinergic pathways. Approaches used in the treatment of AD include cholinesterase inhibitors and inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes to stabilize ACh levels and thus contribute to the alleviation of cognitive symptoms^{4,5}. Currently, tacrine and donepezil and galantamine are among the drugs approved for the treatment of AD, but central and peripheral side effects are observed that limit the use of these drugs^{6,7}.

Inhibition of enzymes involved in carbohydrate digestion, such as α -amylase and α -glucosidase, is one of the commonly used methods in the treatment of diabetes to slow down postprandial blood glucose elevation. The enzyme α -glucosidase (EC 3.2.1.20) is a hydrolase that

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cleaves 1,4- α -glycosidic bonds to release monosaccharides from carbohydrates^{8–10}. Therefore, α -glucosidase inhibitors offer an effective approach to control postprandial blood glucose spikes in Type 2 Diabetes Mellitus (T2DM) patients by delaying the digestion and absorption of carbohydrates in the gastrointestinal tract^{11, 12}. Long used in clinical practice, α -glucosidase inhibitors include acarbose and voglibose, which are prescribed alone or in combination with other antidiabetic drugs. However, their use has been associated with a variety of gastrointestinal side effects, including diarrhea, gas formation, abdominal bloating and abdominal discomfort^{13, 14}.

Medicinal plants have been used throughout history to treat various diseases thanks to their biologically active compounds. According to the World Health Organization (WHO), approximately 4 billion people worldwide regularly use medicinal plants. Therefore, medicinal plants have a wide potential for use in the field of health, and it is thought that about half of the current pharmaceutical drugs are derived from these plants^{15–17}. In order to understand the therapeutic effects of medicinal plants, their chemical composition, biological activities, and safety profiles should be thoroughly investigated¹⁸.

The genus *Plantago*, represented by 21 species in Turkey, has an important place in traditional medicine. Antiviral, antibacterial, antifungal, anti-inflammatory, analgesic, hepatoprotective and cytotoxic effects of *Plantago* species have been demonstrated in various studies^{19, 20}. Especially *P. major* and *P. lanceolata* leaves and *P. psyllium* seeds are widely used in conditions such as wound treatment, cancer, urinary infections, constipation and diabetes. *Plantago* species are rich in iridoids, iridoid glycosides, polyphenols, flavonoids, polysaccharides and triterpene acids, and these compounds are reported to be responsible for antioxidant, hepatoprotective and antidiabetic effects^{1, 20–23}.

In this study, PL extract was investigated in detail for its phenolic compound profile, antioxidant capacity and enzyme inhibition potential associated with diseases such as AD and diabetes. Quantitative analysis of phenolic compounds was performed using an advanced LC-MS/MS method. The antioxidant activities of PL extracts were determined by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), Ferric Reducing Power Assay (FRAP) and Cupric Reducing Antioxidant Capacity (CUPRAC) methods. The inhibitory effects of the extracts on AChE, BChE and α -glucosidase enzymes were also evaluated.

2 Materials and Methods

2.1 Chemicals

All reagents employed in this study were of analytical

grade with purities exceeding 99%, thereby minimizing experimental variability and ensuring the reliability and reproducibility of the analytical results. The chemical compounds included gallic acid, quercetin, 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, tris (hydroxymethyl) aminomethane (Tris), ethylenediaminetetraacetic acid (EDTA), and sodium citrate. All chemicals were supplied by Sigma-Aldrich, an internationally accredited provider of high-purity reagents widely used in biochemical and analytical research.

2.2 Preparation of the extract

The PLs belonging to plant species identified within the scope of a floristic study conducted in the Giresun Mountains were collected from the Doğankent district of Giresun Province, located in northeastern Turkey, in May 2021²⁴. The collected PL leaves were subjected to drying in a well-ventilated environment protected from direct sunlight to avoid the degradation of bioactive compounds. The extraction process was based on the method described by Atmani et al. (2009) with some modifications^{25, 26}. Ethanol was preferred as a solvent to extract high levels of phenolic and flavonoid compounds. The dried plant material was mixed with ethanol at a ratio of 1:10 (g/mL) and extracted on a shaker at room temperature for 16 hours. The filtrate was filtered through Whatman No.1 filter paper and the ethanol was removed by evaporation. The remaining dry extract was stored in sealed containers at 4°C until the time of analysis.

2.3 Phenolic profile of MLs extract with LC-MS/MS analysis

The characterization of the phenolic constituents in the PL extract was carried out through liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). This technique enabled the quantitative determination of 21 individual phenolic compounds. Prior to sample analysis, method conditions were optimized using certified standard phenolics to ensure accurate identification and quantification. The analytical procedure employed for phenolic profiling was adapted from the validated method proposed by Yilmaz^{27, 28}, and aligns with protocols commonly reported in previous studies. Chromatographic separation was performed using a reverse-phase Inertsil ODS-4 C18 column (3.0 mm × 100 mm, 2 μ m particle size), with the column temperature precisely regulated at 40°C to enhance compound resolution and compatibility with the mobile phase. The binary mobile phase system consisted of solvent A (water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid), where formic acid improved analyte ionization and helped maintain ion source stability. A gradient elution strategy was applied to effectively resolve complex mixtures of phenolics by modulating the proportion of each phase over time. The sample injection

volume was set at 4 μ L, and a constant flow rate of 0.5 mL/min was maintained to ensure consistent analytical performance. All measurements were conducted in triplicate, and mean values were used to report the quantitative results, thereby ensuring precision and reproducibility of the findings.

2.4 Antioxidant activity assays

2.4.1 Free radical scavenging activity (DPPH and ABTS assay)

Free radical scavenging capacities of the PL extracts were evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging methods. Within the scope of the DPPH method, 0.1 mM DPPH solution prepared in methanol was mixed with different concentrations of extract samples (10–40 μ g/mL), incubated in the dark for 30 minutes and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer^{29, 30}. 2.45 mmol/L potassium persulfate and 2 mmol/L ABTS solution were mixed and incubated at room temperature for 14 hours in the dark. The ABTS method is based on the absorbance decrease at 734 nm of ABTS⁺ radical activated with potassium persulfate, and the radical scavenging capacity of ABTS⁺ solution mixed with the PL extract was determined spectrophotometrically^{3, 31}. The data obtained in both methods were calculated as percentage radical scavenging ratios reflecting the antioxidant activity of the extracts, and comparative analyses were performed.

2.4.2 Ferric reducing power assay

The metal ion reduction capacity of ethanol extracts of the PLs was determined based on the method described by Elmastas et al. (2006) with some modifications. This analysis was carried out to evaluate the electron-donating properties of the extracts and to determine their ability to reduce ferric ions to ferrous form. As part of the experimental procedure, extract samples prepared at different concentrations (10, 20, 30 and 40 μ g mL⁻¹) were mixed with 1% potassium ferricyanide [K₃Fe(CN)₆] solution and 0.2 M phosphate buffer solution adjusted to pH 6.6 in appropriate proportions. The prepared mixtures were incubated at 50°C for 20 min. After this period, 10% trichloroacetic acid (TCA) and 0.1% iron (III) chloride (FeCl₃) solutions were added to the reaction mixtures, and the mixtures were centrifuged at 3,000 rpm for 10 minutes. The absorbance values of the supernatants were measured spectrophotometrically at a wavelength of 700 nm^{32, 33}. The measured absorbance increase was evaluated as a quantitative indicator of the ferric ion-reducing capacity of the samples. The data obtained were compared and interpreted with standard antioxidant compounds, and the antioxidant potentials of the extracts were revealed in the context of redox properties.

2.4.3 Cupric reducing antioxidant capacity

Using the Cupric Reducing Antioxidant Capacity (CUPRAC) method developed by Apak et al. (2006), the reduction capacity of Cu(II)-Nc complex to Cu(I)-Nc form was investigated. To each assay tube, 1 mL of 2,9-dimethyl-1,10-phenanthroline (neocuprin), 1 mL of 0.01 M CuCl₂ and 1 mL of buffer solution (ammonium acetate) were added, then the PL extracts at different concentrations were added, and the total volume was completed to 4 mL with deionized water^{34, 35}. The mixtures were incubated at room temperature for 30 minutes, and the absorbance values were measured at 450 nm.

2.5 Cholinesterase inhibition assay

The cholinesterase inhibitory potential of the PL extract was determined through a modified version of the Ellman colorimetric assay, which remains a standard approach for evaluating AChE and BChE activity^{2, 36}. For each assay, the reaction system was composed of Tris-HCl buffer (pH 8.0, 50 mM), DTNB as the chromogenic reagent, the respective enzyme (AChE or BChE), and their corresponding substrates—acetylthiocholine iodide for AChE or butyrylthiocholine iodide for BChE. The extract was introduced into the reaction at a range of concentrations to assess its inhibitory efficiency in a concentration-dependent manner. Following incubation at 25°C, the enzymatic hydrolysis of the substrate resulted in the release of a yellow-colored thiolate anion, whose absorbance was measured at 412 nm via a UV-Vis spectrophotometer. Dose-response data were used to derive IC₅₀ values, indicating the concentration required to achieve 50% inhibition of enzyme activity. Each experimental condition was replicated three times to ensure precision and consistency of the findings.

2.6 α -Glucosidase inhibition assay

The α -glucosidase inhibitory activity of the PL extract was determined through a colorimetric assay utilizing *p*-nitrophenyl-D-glucopyranoside (*p*-NPG) as the chromogenic substrate. In this method, the enzyme was incubated with various concentrations of the extract in a buffer solution (phosphate buffer, pH 6.8), followed by the addition of *p*-NPG. The enzymatic hydrolysis of the substrate was monitored by measuring the release of *p*-nitrophenol at 405 nm using a UV-visible spectrophotometer^{12, 37}. Enzyme inhibition was quantified by comparing the absorbance of treated samples to that of a control lacking the extract. The IC₅₀ value, indicating the concentration required to inhibit 50% of the enzyme's activity, was extrapolated from the plotted dose-response curves.

2.7 Statistical analysis

To ensure data accuracy and minimize experimental variability, all assays were independently repeated three times under identical conditions. The outcomes of these

measurements are presented as mean values accompanied by their respective standard deviations (mean $\pm$ SD), providing a quantitative assessment of data dispersion. Statistical evaluations including one-way ANOVA were performed using GraphPad Prism software (version 8), a validated platform widely utilized for advanced data analysis, graphical representation, and inferential statistical testing in research. In biological activities experiments, measurements were performed in 3 independent replicates. Differences among the data sets were deemed statistically significant when the associated p -value was <0.05 .

3 Results and Discussion

3.1 Phenolic profile of PL extract with LC-MS/MS analysis

The phenolic compound profile of PL ethanol extract was determined by LC-MS/MS method and presented in Table 1. According to the results obtained, the highest concentration of phenolic compounds was observed in fumaric acid ($4486.80 \mu\text{g L}^{-1}$), Vanillic acid ($2727.95 \mu\text{g L}^{-1}$), kaempferol ($244.79 \mu\text{g L}^{-1}$), resveratrol ($218.70 \mu\text{g L}^{-1}$) and caffeic acid ($133.85 \mu\text{g L}^{-1}$) were also detected in significant amounts. However, lower concentrations of quercetin (3.62

$\mu\text{g L}^{-1}$), naringenin ($4.75 \mu\text{g L}^{-1}$) and butein ($13.25 \mu\text{g L}^{-1}$) were found. Catechin hydrate, gallic acid, phloridzin dihydrate, oleuropein, ellagic acid, myricetin, protocatechuic acid, luteolin, alizarin and 4-hydroxybenzoic acid were not detected in the analysis. The extraction efficiency and analytical reliability were supported by recovery rates approaching 100%. Moreover, all compounds exhibited high determination coefficients ($R^2 \geq 0.996$), confirming the precision and linearity of the calibration curves. The validation data for phenolic compounds, analyzed via LC-MS/MS, are detailed in Table S1 and illustrated in Fig. S1 (Supplementary file). Collectively, these results indicate that the PL extract possesses a high concentration of phenolic constituents, which are likely responsible for its notable biological activities.

In a study, bis(2-ethylhexyl) phthalate, 1-methoxy-3-(2-hydroxyethyl)nonane and various cyclic siloxane derivatives were reported as the main components of *Plantago lanceolata* L. and *Plantago major* L. leaf extracts³⁸). The compounds detected in the study are generally volatile and low polarity compounds, and it is expected that there will be differences between the profile obtained due to the differences in the analysis methods used in the study (GC-MS) and solvent differences (generally organic low po-

Table 1 Phenolic composition of the ethanolic extract of *Plantago lanceolata* L.

Standard compounds	Recovery (%)	^a RT	Concentration ($\mu\text{g L}^{-1}$)
Quercetin	1.001	3.874	3.62
Acetohydroxamic acid	1.000	0.399	143.71
Catechin hydrate	—	—	ND
Vanillic acid	1.001	3.510	2727.95
Resveratrol	0.998	3.190	218.70
Fumaric acid	0.997	0.574	4486.80
Gallic acid	—	—	ND
Caffeic acid	1.009	2.750	133.85
Phloridzin dihydrate	—	—	ND
Oleuropein	—	—	ND
Ellagic acid	—	—	ND
Myricetin	—	—	ND
Protocatechuic acid	—	—	ND
Butein	0.096	4.571	13.25
Naringenin	0.998	4.150	4.75
Luteolin	—	—	ND
Kaempferol	0.999	3.812	244.79
Alizarin	—	—	ND
4-Hydroxybenzoic acid	—	—	ND

^a RT: Retention time. ND: Not Detected.

larity solvents) and the high polarity phenolic compound profiles in our study. It is also known that the plant part used, harvest time, and extraction methods have significant effects on the chemical content. In another study, many metabolites from *P. major* and *P. lanceolata* species were investigated. It was reported that methanol extracts contained secondary active metabolites including polysaccharides, lipids, alkaloids, flavonoids and phenolic compounds, vanillic acid, tannins, kaempferol, caffeic acid, fatty acids and vitamins^{1, 39}. In particular, the detection of vanillic acid, kaempferol, and caffeic acid in high concentrations in our study coincides with the findings of this study in terms of phenolic content.

3.2 Antioxidant and inhibition activities of PL extract against metabolic enzymes

Iron and copper ion reduction potentials of PL ethanol extract were evaluated spectrophotometrically. The increasing absorbance values obtained showed that the extract had high reducing capacity for both metal ions. The high absorbance values support that the extract is an effective reducing agent. It is seen in Fig. 1 that the iron and copper reducing capacity of PL extract is lower than the reference compound trolox in the range of 10-30 $\mu\text{g mL}^{-1}$, but close to the other reference compound α -tocopherol.

Free radical scavenging capacities of PL ethanolic extract were determined by DPPH and ABTS methods. DPPH radical scavenging activity of the extract was $37.86 \pm 2.42\%$, while ABTS radical scavenging activity was $34.72 \pm 2.86\%$ (Table 2). When compared with the standard antioxidants α -tocopherol and trolox, α -tocopherol showed a scavenging activity of $42.86 \pm 3.75\%$ and $58.33 \pm 4.37\%$ on DPPH and ABTS, respectively, while Trolox showed higher activity of $63.08 \pm 5.24\%$ and $96.53 \pm 7.68\%$ (Table 2). These results reveal that PL extract has a free radical scavenging capacity close to the reference compound α -tocopherol.

In a study, the radical scavenging activities of water,

methanol and ethyl acetate extracts of *P. lanceolata* were evaluated, and it was determined that the extracts exhibited approximately 28%, 51% and 24% DPPH radical scavenging potential at 200 $\mu\text{g/mL}$, respectively¹. In another study, it was determined that the aqueous *P. lagopus* extract showed dose-dependent DPPH radical scavenging ability and this activity was approximately 40% at a concentration of 100 $\mu\text{g/mL}$ ¹⁹. In this study, the DPPH radical scavenging activity of the PL ethanolic extract was found to be $37.86 \pm 2.42\%$ at 10 $\mu\text{g mL}^{-1}$, and it has a higher radical scavenging potential than both studies. These differences may be due to many factors, such as the type of extraction solvent, the method of preparation of the extract, the plant material used from different species or varieties, the environmental conditions in which the plant grows, and differences in extract concentration. It is known that solvents with medium polarity, especially ethanol, may be more effective in the extraction of antioxidant components such as phenolic compounds and flavonoids. This may be one of the main reasons for the high antioxidant capacity observed in our study. In conclusion, it appears that the ethanolic extract of PL has a strong free radical scavenging potential even at low concentrations and may be a promising source of natural antioxidants, especially when compared to known antioxidants such as α -tocopherol.

Inhibitory effects of PL extract on AChE and BChE enzymes associated with AD and on α -glucosidase enzymes associated with diabetes were determined. The ethanol extract of PLs exhibited concentration-dependent inhibitory effects on BChE, AChE and α -glucosidase enzymes, with enzyme activities progressively declining as the extract concentration increased. The inhibitory potential of PL extract was calculated as IC_{50} value of $8.77 \pm 0.52 \mu\text{g mL}^{-1}$ for AChE, $7.37 \pm 0.53 \mu\text{g mL}^{-1}$ for BChE and $3.63 \pm 0.27 \mu\text{g mL}^{-1}$ for α -glucosidase. These results revealed that PL extract showed a remarkable effect, especially in the inhibition of α -glucosidase enzyme, compared to acarbose

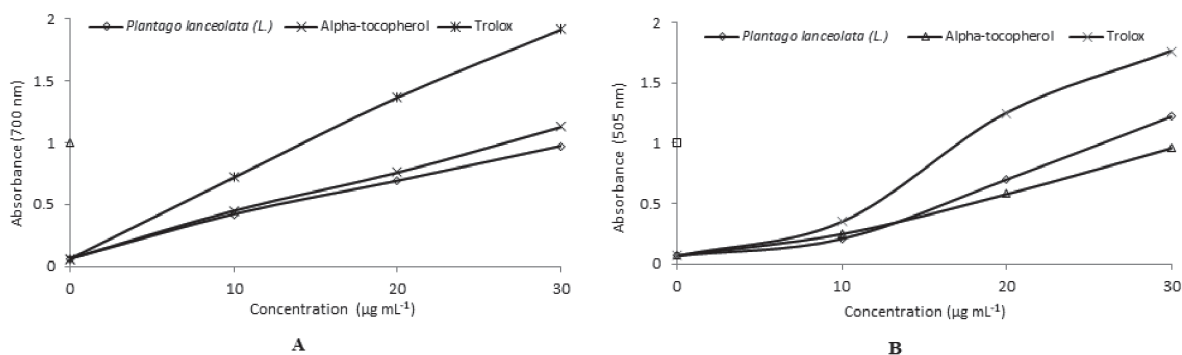


Fig. 1 Iron and copper reduction potential of *Plantago lanceolata* (L.) ethanol extract. Values are expressed as absorbance. High absorbance indicates high metal reduction capacity (A indicates iron reduction and B indicates copper reduction).

Table 2 Inhibition effect on AChE, BChE and α -glucosidase and radical scavenging activity of *Plantago lanceolata* L.

Inhibitors	^a DPPH (10 $\mu\text{g mL}^{-1}$)	^a ABTS (10 $\mu\text{g mL}^{-1}$)	^b AChE IC ₅₀ ($\mu\text{g mL}^{-1}$)	R ²	^c BChE IC ₅₀ ($\mu\text{g mL}^{-1}$)	R ²	α -glucosidase IC ₅₀ ($\mu\text{g mL}^{-1}$)	R ²
<i>Plantago lanceolata</i>	37.86 $\pm$ 2.42	34.72 $\pm$ 2.86	8.77 $\pm$ 0.52	0.923	7.37 $\pm$ 0.53	0.952	3.63 $\pm$ 0.27	0.982
^d Tacrine	—	—	4.36 $\pm$ 0.34	0.972	4.28 $\pm$ 0.28	0.987	—	—
^d Acarbose	—	—	—	—	—	—	3.87 $\pm$ 0.18	0.985
^e α -tocopherol	42.86 $\pm$ 3.75	58.33 $\pm$ 4.37	—	—	—	—	—	—
^e Trolox	63.08 $\pm$ 5.24	96.53 $\pm$ 7.68	—	—	—	—	—	—

^a Values are expressed as percent radical scavenging activity. ^bAcetylcholinesterase. ^cbutyrylcholinesterase. ^dStandards compounds used for positive control as enzyme inhibitors. ^eStandard antioxidants (α -tocopherol and trolox) used for positive control.

used as a reference inhibitor. The IC₅₀ value of acarbose on α -glucosidase was determined as 3.87 $\pm$ 0.18 $\mu\text{g mL}^{-1}$ and PL extract was found to have an inhibitory potential very close to this value. This suggests that PL extract may be a potential natural inhibitor candidate, especially in diabetes management. Tacrine showed a stronger inhibitory effect against AChE and BChE enzymes with IC₅₀ values of 4.36 $\pm$ 0.34 $\mu\text{g mL}^{-1}$ and 4.28 $\pm$ 0.28 $\mu\text{g mL}^{-1}$, respectively (Table 2). Although PL extract exhibited a lower inhibitory effect on these enzymes, it can be considered as an alternative or complementary agent, considering that it is a compound of natural origin, its toxicity potential may be low and side effects may be reduced.

In a study, the inhibitory effects of *Plantago lanceolata* leaf extracts (hexane, ethanol, water) against cholinesterase enzymes such as AChE and BChE were investigated. Hexane, ethanol and water extracts had inhibitory potential on AChE with IC₅₀ values of >200, 195 and 192 $\mu\text{g mL}^{-1}$, respectively, and showed inhibitory effect on BChE with IC₅₀ values of 30, 88 and 43 $\mu\text{g mL}^{-1}$, respectively⁴⁰⁾. In another study, the inhibitory effects of phenolic compounds isolated from *Plantago subulata* were tested against BChE, AChE and α -glucosidase enzymes. It was determined that these compounds exhibited high inhibitory potential with K_i values of 0.043 $\pm$ 0.01–0.154 $\pm$ 0.02 μM against AChE, 3.92 $\pm$ 1.08–11.93 $\pm$ 4.45 μM against BChE and 6.93 $\pm$ 2.74–17.17 $\pm$ 6.70 μM against α -glucosidase²³⁾. In this study, it is thought that the inhibitory potential of these PL extracts from the same family against these metabolic enzymes may be due to the phenolic compounds they contain, which is supported by the results of the literature.

4 Conclusion

This study revealed that PL leaf extracts may exhibit various biological effects in terms of phenolic compound content, antioxidant capacity and enzyme inhibition potential. Especially the presence of phenolic compounds such as fumaric acid, vanillic acid, kaempferol, resveratrol and caffeic acid suggests that these extracts may contribute to

the reduction of oxidative stress and support neuroprotective effects. The findings suggest that PL extracts may help in the management of diabetes and neurodegenerative diseases by showing inhibitory effects on AChE, BChE and α -glucosidase enzymes. However, further in vivo and clinical studies are needed to confirm this potential. Overall, this study indicates that PL leaves may be a promising source of natural bioactive compounds and may offer new opportunities for therapeutic and industrial applications.

Author Contributions

Mesut Işık: Methodology, conceptualization, investigation, project administration, writing - original draft, writing - review & editing. Emrullah Sümer: Methodology, investigation, writing - original draft.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess25104

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