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Antioxidant Properties and Enzyme Inhibitory Effects of Extracts of Three *Lathyrus* Species (Fabaceae) from Türkiye

Bekir Yildirim[1]

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ABSTRACT

Background: Phytotherapeutic approaches are becoming increasingly important for the treatment of various diseases and disorders. Therefore, the identification of plants with the desired bioactivity properties is important. The primary purpose of this study is to determine the phenolic composition of *Lathyrus annuus*, *L. hierosolymitanus* and *L. hirsutus* and reveal the bioactivities of extracts obtained using aerial parts of these species.

Methods: Twelve phenolic compounds were investigated and their amounts were determined by HPLC analysis. For the detection of the bioactivities of species, antioxidant ability tests (DPPH, ABTS, RP) and enzyme inhibition assays were carried out. Pancreatic lipase and amylase were selected as target enzymes for the inhibition assays.

Result: Eight of the investigated twelve phenolic compounds were detected in all species. The gallic acid and p-hydroxybenzoic acid contents of *L. hirsutus* were found to be in conspicuous amounts. The ethanol extracts of *L. hirsutus* and *L. annuus* and the water extract of *L. hierosolymitanus* showed the highest antioxidant activity in the DPPH, ABTS and RP assays, respectively. The lowest antioxidant property was shown by the acetone extract of *L. annuus* among all extracts. The acetone extract of *L. hierosolymitanus* and ethanol extract of *L. annuus* had the strongest inhibitory effects on amylase and lipase enzyme activities, respectively.

Key words: Antioxidant activity, Enzyme inhibitory, *Lathyrus*, Radical scavenging activity.

INTRODUCTION

Free radicals, which can cause significant health problems in humans, are highly reactive atoms or groups of atoms that try to capture electrons from other atoms because they have a missing electron. Since these radicals can form new radicals through electron transfer after they have been formed for the first time, they can cause reaction chains that can harm body tissues (Ceballos *et al.,* 2021). The most significant weapons that the human body can use against free radicals are antioxidants, which can scavenge these radicals and prevent cell damage (Karabulut and Gülay, 2016). Phenolic compounds, considered to be the most abundant antioxidants in the human diet, have been shown to be effective in the prevention of many diseases, including obesity, Alzheimer's disease, osteoporosis, cancer, diabetes mellitus, arthritis and cardiovascular and neurodegenerative diseases (Ha, 2016; Poovitha and Parani, 2016; Song *et al.,* 2017; Liu *et al.,* 2020; Papuc *et al.,* 2020; Kumar *et al.,* 2022; Singh and Khare, 2022). Antioxidants, which protect the body against oxidative damage, the main pathological disturbance in many chronic diseases, also have anti-allergic, anti-carcinogenic, anti-inflammatory, anti-microbial, antimutagenic and anti-viral activities (Kumar *et al.,* 2023; Wafa, 2024).

In addition to their antioxidant properties, phenolic compounds exhibit inhibitory activities on enzyme functions. Enzyme inhibitors, which have recently gained importance in the treatment of health problems, are synthetically produced as drugs. However, as these drugs may have side effects, there is a need to identify safer plant-derived natural compounds (Llorent-Martinez *et al.,* 2016). Controlled reduction in the activities of some enzymes shows promise in preventive and therapeutic practices against diseases caused by the overwork of these enzymes. Lipase and amylase, which are pancreatic secretions, have significant functions in the metabolism of fats and carbohydrates, respectively (Gil *et al.,* 2023; Sener *et al.,* 2023). In the digestive system, suppressing triglyceride uptake through lipase inhibition or delaying glucose uptake through amylase inhibition are important strategies for obesity and type 2 diabetes mellitus prevention or treatment (Marrelli *et al.,* 2013; Ci *et al.,* 2018).

Numerous studies have reported that the bioactivities of phenolic compounds are associated with the treatment or prevention of many diseases and disorders. For this

[1]Department of Plant and Animal Production, Burdur Food Agriculture and Livestock Vocational School, Burdur Mehmet Akif Ersoy University, Burdur-15030, Türkiye.

Corresponding Author: Bekir Yildirim, Department of Plant and Animal Production, Burdur Food Agriculture and Livestock Vocational School, Burdur Mehmet Akif Ersoy University, Burdur-15030, Türkiye. Email: bytr33@yahoo.com
ORCID: https://orcid.org/0000-0003-2378-7697

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reason, various studies have recently been carried out to investigate the phenolic contents, antioxidant properties and enzyme inhibitory effects of plants and the number of these studies is increasing day by day (Ranilla *et al.,* 2010; Arioua *et al.,* 2022; Tamfu *et al.,* 2022).

Legumes (Fabaceae) play a crucial role in nutrition, particularly in developing countries. Some members of *Lathyrus* L., a genus in this family, may be sources of strong antioxidant phenolics (Pastor-Cavada *et al.,* 2009). Recently, various studies have been carried out to investigate the bioactivities of the following *Lathyrus* taxa: *L. aureus* (Steven) Brandza*, L. pratensis* L. (Llorent-Martinez *et al.,* 2016), *L. cicera* L., *L. digitatus* (M. Bieb.) Fiori (Llorent-Martinez *et al.,* 2017a), *L. nissolia* L., *L. czeczottianus* Bässler (Llorent-Martinez *et al.,* 2017b), *L. czeczottianus* (Ceylan *et al.,* 2021), *L. brachypterus* Čelak var. *brachypterus*, *L. brachypterus* var. *haussknechtii* (Širj.) P.H. Davis, *L. nivalis* Hand.-Mazz. subsp. *sahinii* H.Genç and *L. tefennicus* H. Genç & A. Şahin (Yildirim *et al.,* 2023).

The aim of this study was to reveal the amount of phenolics, total phenolic/flavonoid contents, antioxidant properties and enzyme inhibitory effects of *L. annuus* L., *L. hierosolymitanus* Boiss. and *L. hirsutus* L. species and to determine whether there are differences between them.

MATERIALS AND METHODS

The study was conducted from 2023 to 2024 at Food Agriculture and Livestock Vocational School of Higher Education, Burdur Mehmet Akif Ersoy University.

Plant samples and extraction

Samples gathered from their natural distribution were identified using Flora of Turkey (Davis, 1970) as a reference. The investigated species and their localities are as follows:

***L. annuus*:** Around the ancient city of Stratonikeia, Yatağan, Muğla, Türkiye.

***L. hierosolymitanus*:** Around the Çamlıca neighborhood, Alanya, Antalya, Türkiye.

***L. hirsutus*:** Eğirdir-Aksu road, 5-6 km, roadside, Eğirdir, Isparta, Türkiye.

Whole aerial parts of the samples were separated and dried in a room without sunlight for ten days and then pulverized using a grinder. In the extractions, absolute acetone, absolute ethanol and water were used as solvents to prepare acetone extract, ethanol extract and water extract (abbreviated as AE, EE and WE later in the text, respectively) for each species. Mixtures formed via adding pulverized sample (1 g) to solvent (20 ml) were extracted by shaking for 6 h at 100 rpm at room temperature on an orbital shaker. Supernatants separated by centrifugation at 5000 x g for 10 minutes were filtered through filter paper and solvents were evaporated at 35°C using a vacuum rotary evaporator. Extracts were preserved at -20°C for subsequent analysis. In order not to affect bioactivities, the extracts were dissolved in DMSO (2%) for enzyme inhibitory activity tests and in ethanol (100%) for antioxidant activity tests.

HPLC analysis

In HPLC analyses, the method of Caponio *et al.* (1999) was applied with modification. The twelve phenolic compounds determined as targets were separated using a Shimadzu HPLC system, which had the following properties: Detector: SPD-M10 Avp DAD (λmax=278 nm), Autosampler: SIL-10 ADvp, System controller: SCL-10 Avp, Pump: LC-10 ADvp, Degasser: DGU-14A, Column oven: CTO-10Avp, Column: Agilent Eclipse XDB-C18 (250x4.60 mm) 5 μ, Mobile phase: 3% acetic acid (A), methanol (B), Flow rate: 0.8 ml/min, Column temperature: 30°C, Injection volume: 20 μl.

Total phenolic and flavonoid contents (TPCs, TFCs)

TPCs and TFCs of the extracts were calculated as defined by Lakka *et al.* (2020) and given as mg gallic acid equivalent/g dry weight (mgGAE/gdw) and mg quercetin equivalent/g dry weight (mgQE/gdw), respectively.

Antioxidant properties

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′ -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities of the extracts were performed according to the method of Sánchez-Moreno *et al.* (1998) and Re *et al.* (1999), respectively. The percentages of DPPH and ABTS scavenging activities were computed using Eq (1). $A_{Control}$ means the control sample's absorbance and A_{Sample} means the tested compound's absorbance. Results were expressed as IC_{50}.

$$\% \text{ Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad ....(1)$$

The reducing power (RP) of the extracts was determined using the method of Oyaizu (1986). The absorbance of the mixture was measured at 700 nm against a blank (ethanol). The inhibitory concentration (IC_{50}), expressed as the extract concentration producing 0.5 absorbance unit at λ700 nm, was used to define the RP (Oliveira *et al.,* 2009).

Enzymatic inhibitions

For the determination of amylase inhibitory activity, the method applied by Zengin *et al.* (2014) was exercised. Absorbance, *i.e.* abs 1, was then calculated at 630 nm. Amylase-free abs 2 (using buffer instead of amylase), abs 3 without a sample (ethanol replaced the sample) and abs 4 only for the starch solution were calculated using the same process. Amylase inhibitory assays were accomplished in 3 repetitions for all extracts and their mean % inhibition was calculated by Eq (2). The IC_{50} value, which expresses the inhibitory activity of the extract, is the concentration (as mg/ml) of the extract that inhibits the enzyme activity by 50%.

$$\% \text{ Inhibition} = \left[1 - \left(\frac{abs2 - abs1}{abs4 - abs3}\right)\right] \times 100 \quad ....(2)$$

The inhibitory activity of the lipase was accomplished by the technique suggested by Anigboro *et al.* (2021) with modification. Extract solutions (250 µL) with varying concentrations (0.5-5 mg dw/ml) were incubated with 500 µL of 1 mg/ml porcine pancreatic lipase solution in 80 mM Tris-HCl (pH 7.2), 1.0 ml of buffer solution and 250 µL of 5 µM para-nitrophenyl butyrate (pNPB) solution (in ethanol) at 37°C for 30 min. Blanks for each sample without enzyme are prepared through the addition of buffer instead of enzyme. Lipase activity was determined at 412 nm using a UV-Vis spectrophotometer. Lipase inhibition was computed according to Eq (3). $A_{Control}$ and A_{Sample} state the activity without inhibitor and with inhibitor, respectively. The extract concentrations that caused 50% inhibition of lipase activity (IC_{50}) were settled graphically.

$$\% \text{ Inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100 \quad ....(3)$$

RESULTS AND DISCUSSION

To the best of our knowledge, the amounts of the target phenolic compounds, total bioactive components and bioactivities of AEs, EEs and WEs of *L. annuus*, *L. hierosolymitanus* and *L. hirsutus* were revealed for the first time in this research.

Phenolic compounds

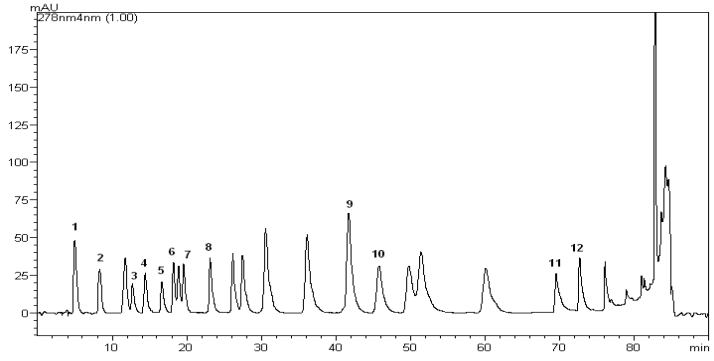
Phenolic compounds found everywhere in plants have a scavenger role against reactive oxygen/nitrogen species and some of them have various helpful effects on healthcare. It has been reported that more than 8000 phenolic compounds are naturally found in plants, half of which are flavonoids (Papuc *et al.,* 2020). Many phenolic compounds, such as gallic acid, quercetin, caffeic acid, protocatechuic acid, p-coumaric acid, luteolin and chlorogenic acid, have biological and pharmaceutical effects against many diseases and disorders (Kumar *et al.,* 2022).

In the current study, some phenolic compounds of the studied species were determined by HPLC analysis. Fig 1 reflects the standard chromatogram and retention times (Rt, min.). HPLC chromatograms of the studied species are also given in Fig 2.

In the quantitative screening, twelve compounds were assessed and the majority of them were defined in all species (Table 1). The highest gallic acid (1334.7), p-hydroxybenzoic acid (362.1), quercetin (64.0), caffeic acid (47.0) and p-coumaric acid (22.8) values (µg/g) were detected in *L. hirsutus*, protocatechuic acid (42.0), vanillin (20.0), luteolin (19.1), rutin (256.0) and epicatechin (178.0) values (µg/g) in *L. hierosolymitanus*, hesperidin (16.7) and chlorogenic acid (6.7) values (µg/g) in *L. annuus*. Compounds epicatechin in *L. annuus*, chlorogenic acid in *L. hierosolymitanus*, rutin, hesperidin and chlorogenic acid in *L. hirsutus* could not be detected.

In Ceylan *et al.* (2021), chlorogenic acid 2510 and 12090, epicatechin 5320 and 5710, p-hydroxybenzoic acid 430 and 960, gallic acid 40 and 650, quercetin 190 and 400, caffeic acid nd and 360, luteolin 250 and 290, protocatechuic acid 30 and 180 and hesperidin 30 and 30 µg/g were indicated in the ethyl acetate extracts and WEs of *L. czeczottianus* (aerial parts), respectively (nd: not determined). Vanillin, p-coumaric acid and rutin were not determined. In the extracts of aerial parts of *L. brachypterus* var. *brachypterus*, *L. brachypterus* var. *haussknechtii*, *L. nivalis* subsp. *sahinii* and *L. tefennicus*, respectively, the amounts of some phenolic compounds (µg/g) were specified as given in parentheses. Chlorogenic acid (3582, 2408, 15, 27), luteolin (412, 32, 11, 39), p-coumaric acid (121, 84, 28, 30), quercetin (1158, 230, nd, 8), protocatechuic acid (283, 93, 79, nd), hesperidin (139, 323, nd, 259), rutin (109, 282, nd, 240), vanillin (nd, 75, nd, 80), caffeic acid (27, nd, nd, nd) and gallic acid (10, nd, nd, nd). Epicatechin and p-hydroxybenzoic acid were not detected in any of the extracts (Yildirim *et al.,* 2023).

As seen in Table 1, the amounts of gallic acid and p-hydroxybenzoic acid in *L. hirsutus* and rutin in *L. hierosolymitanus* are remarkable. These plants have the potential to be used as a source of the aforementioned phenolic compounds,



1: Gallic acid (Rt: 4.9), 2: Protocatechuic acid (Rt: 8.3), 3: p-hydroxy benzoic acid (Rt: 12.7), 4: Chlorogenic acid (Rt: 14.4), 5: Caffeic acid (Rt: 16.6), 6: Epicatechin (Rt: 18.2), 7: Vanillin (Rt: 19.5), 8: p-coumaric acid (Rt: 23.0), 9: Rutin (Rt: 41.6), 10: hesperidin (Rt: 45.7), 11: Quercetin (Rt: 70.0), 12: Luteolin (Rt: 72.6).

Fig 1: Standard chromatogram.

as they contain relatively more of these compounds than the taxa reported in the literature.

TPCs, TFCs and antioxidant properties

The highest TPC and TFC values were determined in the EEs of *L. hirsutus* and *L. hierosolymitanus*, respectively, while the lowest values were determined in the WE of *L. hierosolymitanus* and the AE of *L. annuus*, respectively. Table 2 demonstrates the findings. Considering all species and extracts, calculated TPC and TFC values varied between 1.65-3.33 mgGAE/g dw and 1.37-3.82 mgQE/g dw, respectively.

The phenolic contents of widely used legumes vary from 0.325 to 6.378 mgGAE/g (Marathe *et al.,* 2011). The findings of this study regarding the TPC values are consistent with the mentioned study. Other summarized literature data are shown in Table 3. TPCs of the species investigated in our work were lower compared to the literature given in Table 3. Even extracts of the same species prepared using various solvents may give different findings. The reason why the TPC values are lower compared to the literature may be related to the species or solvents. TFC results have been expressed as mgRE/g in the literature. As in here, differences in the expression of results may not always allow for comparison with the other works.

It has been guessed that a cell has fallen into free radical harm thousands of times in 24 hours. Although most of these damages are restored, some are not and accumulate (Pruteanu *et al.,* 2023). Antioxidants can exert a protective effect by eliminating the adverse impacts of

Table 1: Amounts of phenolic compounds found in *Lathyrus* species (μg/g).

Analyte	*L. annuus*	*L. hierosolymitanus*	*L. hirsutus*
Gallic acid	10.9	13.6	1334.7
P-hydroxybenzoic acid	23.5	132.0	362.1
Quercetin	8.3	35.0	64.0
Caffeic acid	1.4	20.0	47.0
Protocatechuic acid	12.9	42.0	6.2
P-coumaric acid	2.9	10.4	22.8
Vanillin	4.0	20.0	6.6
Luteolin	4.3	19.1	12.5
Rutin	27.2	256.0	nd
Epicatechin	nd	178.0	24.7
Hesperidin	16.7	11.4	nd
Chlorogenic acid	6.7	nd	nd

Nd: Not detected.

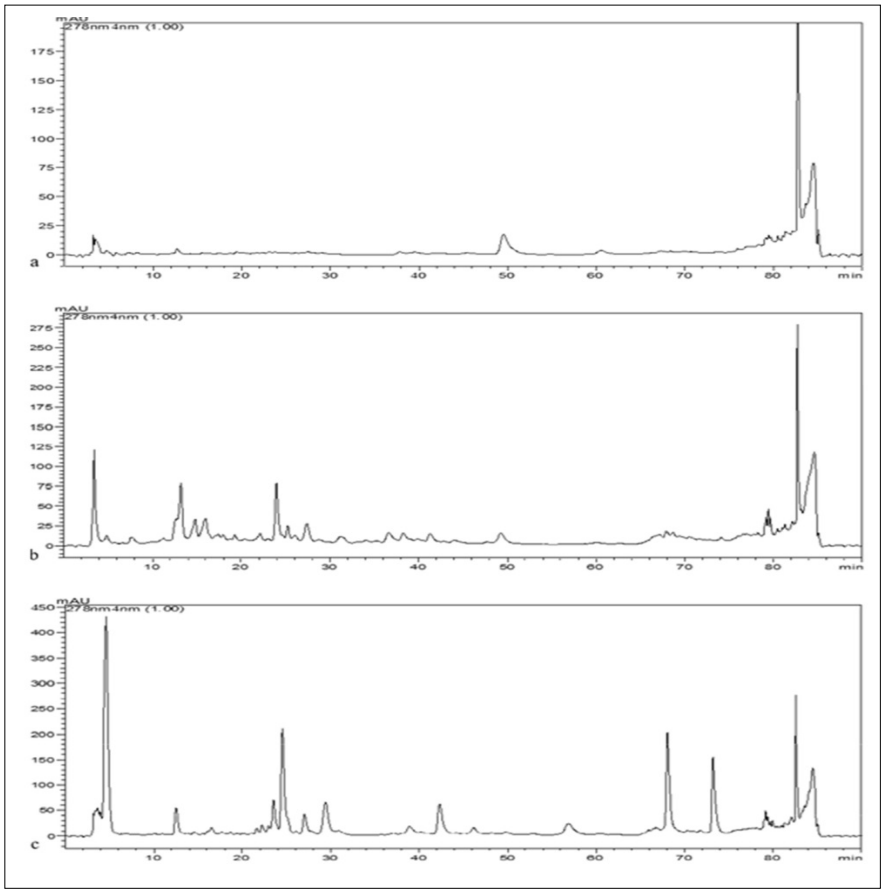


Fig 2: Chromatograms of *L. annuus* (a), *L. hierosolymitanus* (b) and *L. hirsutus* (c).

these radicals, which are toxic by-products of the normal metabolic processes of cells (Sen *et al.,* 2010). The physiological function of antioxidants is to inhibit harm to cells due to free radical-induced chemical reactions (Young and Woodside, 2001).

In this study, some commonly used tests (DPPH, ABTS and RP) were applied for the determination of antioxidant activities. As seen in Table 2, the highest DPPH scavenging activities were found in extracts of *L. hierosolymitanus*, *L. hirsutus* and *L. annuus* for acetone, ethanol and water, respectively. The EE of *L. hirsutus* had the best DPPH scavenging activity of all extracts. The lowest DPPH scavenging activities were shown by WEs for all species. Especially WE of *L. hirsutus* was the weakest. Considering the ABTS tests, the highest radical scavenging properties were found in extracts of *L. hierosolymitanus* (for AE and WE) and *L. annuus* (for EE). The weakest ABTS scavenging activities were shown by *L. annuus* (for WE) and *L. hirsutus* (for EE and AE). Another assay applied to find the antioxidant properties of the species was the RP test. In the RP tests, the most powerful effects were found in extracts of *L. hierosolymitanus* (for WE and EE) and *L. hirsutus* (for AE). AE of *L. annuus* had the lowest RP in all extracts. In the current study, the results of antioxidant tests were calculated as IC_{50} values (mg dw/ml). DPPH, ABTS and RP values of extracts of all studied species were in the range of 3.74-2.09, 2.09-1.81 and 5.21-1.87 mg dw/ml as IC_{50}, respectively.

Table 2: TPCs, TFCs and antioxidant properties of the extracts.

Species	Solvent	TPC (mgGAE/g dw)	TFC (mgQE/g dw)	DPPH-IC_{50} (mg dw/ml)	ABTS-IC_{50} (mg dw/ml)	RP-IC_{50} (mg dw/ml)
L. annuus	a.	2.86±0.04	1.37±0.01	2.73±0.04	1.96±0.05	5.21±0.07
	e.	2.81±0.02	2.70±0.02	2.43±0.03	1.81±0.04	3.54±0.07
	w.	1.73±0.02	2.02±0.02	3.53±0.04	2.09±0.03	3.38±0.02
L. hierosolymitanus	a.	3.06±0.06	3.69±0.06	2.12±0.05	1.94±0.04	4.52±0.20
	e.	2.83±0.03	3.82±0.03	2.38±0.02	1.83±0.07	3.17±0.06
	w.	1.65±0.02	2.12±0.02	3.63±0.08	1.89±0.02	1.87±0.04
L. hirsutus	a.	3.05±0.08	3.81±0.02	2.56±0.07	1.99±0.05	2.71±0.07
	e.	3.33±0.02	2.97±0.01	2.09±0.05	2.08±0.05	3.24±0.03
	w.	1.78±0.07	1.97±0.06	3.74±0.03	1.91±0.06	2.27±0.05

GAE: Gallic acid equivalent; QE: Quercetin equivalent; a.: Acetone; e.: Ethanol; w.: Water.

Table 3: TPCs, TFCs and antioxidant properties of some *Lathyrus* taxa in the literature.

Taxon	Solvent	TPC (mgGAE/g)	TFC (mgRE/g)	DPPH (mgTE/g)	ABTS (mgTE/g)	FRAP (mgTE/g)	CUPRAC (mgTE/g)	Literature
L. aureus	e.a.	21.34	0.89	6.92	3.39	14.89	37.47	(Llorent-Martinez
	m.	22.74	5.32	4.09	34.37	31.98	71.22	*et al.,* 2016)
	w.	19.42	15.77	9.77	67.38	39.22	54.17	
L. pratensis	e.a.	13.33	0.08	12.39	5.14	13.32	35.33	
	m.	40.54	26.16	126.68	67.85	82.02	117.37	
	w.	66.69	38.90	259.00	166.09	154.69	22.161	
L. cicera	m.	24.09	3.75	25.23	65.18	40.99	53.96	(Llorent-Martinez
L. digitatus		33.18	16.10	40.28	147.83	55.84	54.39	*et al.,* 2017a)
L. nissolia	m.	25.47	20.94	3.19*	>5*	1.38*	1.11*	(Llorent-Martinez
L. czeczottianus		63.16	14.16	1.42*	1.80*	0.51*	0.56*	*et al.,* 2017b)
L. czeczottianus	e. a.	18.42	12.44	4.57	9.69	51.50	23.41	(Ceylan *et al.,*
	w.	60.77	9.77	248.49	143.61	214.49	144.97	2021)
L. brachypterus var. *brachypterus*		44.31	41.84	41.74	75.87	77.32	114.94	
L. brachypterus var. *haussknechtii*	m.	34.99	42.98	36.59	67.61	56.67	85.52	(Yildirim *et al.,* 2023)
L. nivalis subsp. *sahinii*		27.25	25.78	19.43	65.23	45.37	62.32	
L. tefennicus		24.91	37.14	18.90	57.73	42.33	51.88	

GAE: Gallic acid equivalent; RE: Rutin equivalent; TE: Trolox equivalent; e. a.: Ethyl acetate; m.: Methanol; w.: Water; * as IC_{50} (mg/ml) for DPPH and ABTS and as EC50 (mg/ml) for FRAP and CUPRAC.

Table 4: Enzyme inhibitory activities of the extracts.

Species	Solvent	Inhibition of amylase activity-IC_{50} (mg/ml)	Inhibition of lipase activity-IC_{50} (mg/ml)
L. annuus	a.	4.85±0.01	5.01±0.06
	e.	4.92±0.05	3.29±0.10
	w.	7.17±0.20	7.55±0.01
L. hierosolymitanus	a.	4.46±0.06	7.35±0.10
	e.	5.32±0.03	4.80±0.04
	w.	7.41±0.10	9.39±0.10
L. hirsutus	a.	4.50±0.04	9.86±0.10
	e.	5.20±0.06	5.18±0.01
	w.	7.28±0.01	6.86±0.02

a.: Acetone, e.: Ethanol, w.: Water.

As seen in Table 3, the results have been generally expressed as mgTE/g in the literature. Comparisons are impossible due to differences in the expression of the results. According to the literature, DPPH and ABTS scavenging activity values are 3.19 and >5 for *L. nissolia* and 1.42 and 1.80 (mg/ml) for *L. czeczottianus*, respectively. While AEs and EEs of all species analyzed in this study showed better DPPH radical scavenging activity than *L. nissolia*, ABTS radical scavenging activity of all extracts of all three species was also found to be more successful than *L. nissolia*. The DPPH and ABTS radical scavenging activities of all extracts of the studied species were found to be close to or lower than those of *L. czeczottianus*. These results revealed that radical scavenging activities may vary depending on the species studied or the solvent used.

Enzyme inhibitory effects

Type 2 diabetes mellitus (T2DM) is a well-known risk factor for Alzheimer's disease (AD). T2DM and AD are significant health problems that have diverse symptoms but share a complex and linked mechanism. It is widely accepted that diabetic patients, especially those with T2DM, have a much greater risk of getting AD compared to normal people (Song *et al.,* 2017). It has been stated that amylase inhibitors can keep postprandial hyperglycemia under control and reduce the risk of developing diabetes by delaying the increase of blood glucose (Poovitha and Parani, 2016). Obesity, which is also associated with diabetes and is closely linked to the appearance of many chronic diseases, including cardiovascular diseases and even cancer, is caused by excessive intake and accumulation of fat in the body. Pancreatic lipase is an enzyme that breaks down triacylglycerols found in ingested fats and ensures their absorption in the intestine. Some clinical studies have shown that lipase inhibitors, which are important in the treatment of clinical obesity, can reduce obesity caused by a high-fat diet (Liu *et al.,* 2020).

It is known that the occurrence of some diseases and disorders is associated with the overactivity of various enzymes. Reducing the activity of these enzymes to normal levels or partially inhibiting them may prevent or delay the appearance of diseases. Therefore, inhibition tests were carried out to determine the enzyme inhibitory potentials of the extracts. Amylase and lipase were chosen as target enzymes. Table 4 reflects the findings of inhibition tests.

It was exhibited that AEs gave better results in the amylase inhibition test, while EEs were more successful in lipase inhibition tests. The WEs gave the most unsuccessful results in all enzyme inhibition tests except the lipase test of *L. hirsutus*. When all extracts and species were considered, the strongest and weakest amylase inhibitory effects were observed in the AE and WE of *L. hierosolymitanus*, respectively. The strongest and weakest lipase inhibitory effects were also monitored in the EE of *L. annuus* and in the AE of *L. hirsutus*, respectively.

Amylase inhibitory activities of the water, methanol and ethyl acetate extracts of *L. aureus* and *L. pratensis* are 0.17, 0.39, 0.55 and 0.13, 0.37, 0.39 mmol ACAE/g, respectively (Llorent-Martinez *et al.,* 2016). These values for the water and ethyl acetate extracts of *L. czeczottianus* are 0.14 and 0.34 mmol ACAE/g, respectively (Ceylan *et al.,* 2021). Amylase inhibitory activities of the methanolic extracts of *L. cicera, L. digitatus, L. tefennicus*, *L. brachypterus* var. *haussknechtii*, *L. brachypterus* var. *brachypterus* and *L. nivalis* subsp. *sahinii* have been found as 0.53, 0.56, 0.50, 0.53, 0.55 and 0.57 mmol ACAE/g, respectively (Llorent-Martinez *et al.,* 2017a; Yildirim *et al.,* 2023). In the studies above, enzyme inhibitory activity values have been determined as ACAE (Acarbose equivalent).

In another work (Llorent-Martinez *et al.,* 2017b), amylase inhibitory activities of the methanolic extracts of *L. nissolia* and *L. czeczottianus* have been calculated and the results expressed as 3.66 and 3.87 mg/ml (IC_{50}), respectively. Amylase inhibitory activities of extracts prepared using acetone, ethanol and water in the current work are relatively compatible with the values specified in the above-mentioned work. The results, especially with the AEs of the species (4.46, 4.50 and 4.86 for *L. hierosolymitanus*, *L. hirsutus* and *L. annuus*, respectively), are close to the literature data. The high amylase inhibitory activities of the AEs may be related to their high TPCs.

As far as we know, studies investigating the lipase inhibitory activities of *Lathyrus* species are not common. The lipase inhibitory activities (IC_{50}) of the WE and organic extract of *L. hierosolymitanus* collected from the ecological conditions of Palestine are 17.48 and 125.83 mg/ml, respectively (Jaradat *et al.,* 2017). In the current research, more promising results were obtained compared to the above-mentioned work. EEs of all species showed high inhibitory activity in lipase inhibition assays. Lipase inhibitory activity values were 3.29, 4.80 and 5.19 mg/ml for EEs of *L. annuus*, *L. hierosolymitanus* and *L. hirsutus*, respectively. The most effective extract, EE of *L. annuus*, showed a much better inhibitory effect on lipase activity than those reported in the literature.

CONCLUSION

The TPCs, TFCs and bioactivities of extracts may also vary depending on the plant species, method of extract preparation and the solvent, apart from ecological factors. In addition to their antioxidant effects, plants can also affect the work of some enzymes. Plants with high enzyme inhibitory activity can help lead a healthier life by limiting the overactivity of some enzymes, such as amylase and lipase. *L. annuus* and *L. hierosolymitanus* have the potential to be considered as sources of bioactive compounds due to their bioactivity.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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