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Original article

Health attributes of ethnic vegetables consumed in the Eastern Anatolia region of Turkey: Antioxidant and enzyme-inhibitory properties

Abdullah Dalar [a, *], Yusuf Uzun [a], Musa Turker [b], Muzaffer Mukemre [c], Izabela Konczak [d, *]

[a] Department of Pharmaceutical Botany, Faculty of Pharmacy, Yuzuncu Yil University, Van, Turkey
[b] Department of Molecular Biology & Genetics, Faculty of Science, Yuzuncu Yil University, Van, Turkey
[c] Department of Biology, Faculty of Science, Yuzuncu Yil University, Van, Turkey
[d] Department of Food Science and Technology, School of Chemical Sciences and Engineering, New South Wales University, Sydney, NSW, Australia

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ABSTRACT

Background: Four ethnic vegetables from the Eastern Anatolia region of Turkey, *Malva neglecta* Wallr., *Plantago lanceolata* L., *Cichorium intybus* L. and *Eryngium bornmuelleri* Nab. are commonly used by the local population for food preparation. This study aimed at understanding their potential health attributes.

Methods: Hydrophilic extracts obtained from roots, stems, leaves and flowers were evaluated for their antioxidant capacities [total phenolics (TP), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) assays] and suppression of two isolated key enzymes relevant to metabolic syndrome: α-glucosidase and pancreatic lipase. Phytochemical composition of extracts was investigated by high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC-MS).

Results: The evaluated extracts exhibited pronounced antioxidant capacities, comparable to those of common spices and herbs, and effectively suppressed the activities of isolated α-glucosidase and pancreatic lipase enzymes. These activities correlated well with total phenolics contents. *Plantago lanceolata* was an effective inhibitor of α-glucosidase and *C. intybus* of pancreatic lipase enzyme. High performance liquid chromatography mass spectrometry analyses revealed the dominance of luteolin glycosides in *P. lanceolata*. The same compound was present in *C. intybus*, where it was accompanied by significant amounts of cichoric, chlorogenic and caftaric acid. *Malva neglecta* and *E. bornmuelleri* contained the lowest levels of phenolic compounds and exhibited the lowest antioxidant and enzyme inhibitory activities.

Conclusions: Among the investigated ethnic vegetables, *P. lanceolata* and *C. intybus* represent a valuable source of antioxidant phytochemicals of phenolic nature that modulated *in vitro* the activities of digestive enzymes. These ethnic food sources diversify diet and enhance health attributes of foods.

1. Introduction

Ethnic foods comprise all of the edible species that are available from local natural resources and prepared in accordance with the accepted patterns for their use within a particular culture [1]. Ethnic cuisine develops as the result of a fine interplay between two factors: (1) biodiversity of available food resources and (2) cultural inheritance and social changes. Ethnic foods accumulate the local knowledge and understanding of foods generated over centuries and are an essential part of cultural inheritance. Locally grown plants, including fruits, vegetables, herbs, and spices, are the basic ingredients of ethnic foods. Therefore, the local biodiversity directly affects the diversity of ethnic foods created in particular regions. Beside the available food sources, the local culture, history, and social changes equally importantly influence the development of ethnic foods.

* Corresponding authors. Abdullah Dalar, Department of Pharmaceutical Botany, Faculty of Pharmacy, Yuzuncu Yil University, Campus of Zeve, Van 65090, Turkey. Izabela Konczak, Department of Food Science and Technology, School of Chemical Sciences and Engineering, University of New South Wales, Sydney, NSW 2052, Australia.

E-mail addresses: dalar.abdullah@gmail.com, dalar.abdullah@yyu.edu.tr (A. Dalar), i.konczak@unsw.edu.au (I. Konczak).

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The ethnic Turkish cuisine is rich, based on numerous stews of vegetables and meat (predominantly lamb and beef) frequently accompanied by sourdough bread. A few ethnic foods from Turkey have become renowned across the world, with the Turkish kebab, dolma dishes, and borek being the most popular. Turkish kebab is made from meat roasted in pieces or slices on a skewer or as meatballs on a grill. Afyon kebab from Central Anatolia is prepared from boiled meat cubes, placed over bread and topped with vegetables and sauce (Figure 1). Dolma are vegetable dishes based on tomatoes, peppers, eggplants, and/or edible leaves (grape, cabbage, locally growing unconventional vegetables—*Malva* sp. and others) that are stuffed with or wrapped around rice or bulgar pilaf, ground meat, or spices (Figure 2). Borek is a pastry made of many thin layers of dough interspersed with cheese, spinach, and/or ground meat. It includes a sweet desert served on special occasions called baklava (Figure 3), which is made of pastry interspersed with nuts and immerged in honey-based syrup.

Savage plants and common vegetables, including olive oil-based dishes, represent the vast variety of ethnic foods in Turkey. These foods are frequently based on fried vegetables, fruit molasses, marmalades, sundried tomatoes, and pimento (allspice). Legumes and assorted vegetables are among the common local foods, with eggplants being the most commonly used and utilized in fried dishes, salads, and desserts. Food preparations and composition of the ingredients vary greatly by region and ethnicity. For example, the Black Sea region is noted for fish dishes, the eastern region for lamb and spicy vegetables dishes, while the southeastern region is known for Armenian pizza, thick corn Georgian bread, and corn soup.

Two important factors that have influenced the development of ethnic foods in Turkey are the exceptional biodiversity of the region and social changes. Over centuries, the country has served as a passageway between Europe, Asia, and Africa, where different cultures, traditions and experiences generated in distant lands have collided. A variety of flora, fauna, and cultures owe their geographical spread to this passageway. Their fusion played a decisive role in the development of the exceptionally rich Anatolian ethnic foods. Ethnic foods of Eastern Anatolia are predominantly based on spicy lamb dishes accompanied by a large variety of vegetable meals created from locally grown ethnic vegetables. This region, located in the Asian part of the country, represents the largest of geographical divisions with 14 provinces, bordering Iraq, Iran, Nakhichevan, Armenia, and Georgia and is covered with deciduous-mixed forests and deciduous tree steppes [2]. The area is surrounded by coastal mountain ranges, which protects it from the moderating effect of sea breezes. Subsequently, the winters are cold and long, with snow lasting for several months. They are followed by short and rainy springs, and hot and dry summers. The mountainous and strongly fragmented area offers numerous microclimatic and ecological zones, which provided suitable conditions for the development of diverse flora [3,4]. The Eastern Anatolia region is regarded as one of the richest areas of plant biodiversity with > 3,000 vascular plant taxa, of which, nearly 800 are endemic. The most important floral biodiversity centers in Eastern Anatolia are Munzur and Anti Taurus Mountains, Elbistan–Darende, Kemaliye, Kesis Mountain, and Harput and Hazar Lake [2]. Traditionally, the local people of the mountainous Eastern Anatolia were isolated from large cities and forced to rely exclusively on local food production, utilizing the locally grown ethnic vegetables (Figure 4). To date, these vegetables are especially important in rural areas, where opportunities to purchase commercially grown conventional vegetables are still limited. Locally grown *Plantago lanceolata* (giyamambel), *Malva neglecta* (tolk), *Eryngium bornmuelleri* (tûsi), and *Cichorium intybus* (kanej or tahlişk) (Figure 5) are the most common ethnic vegetables used in preparation of main meals in

Fig. 1. Ayfon kebab from Central Anatolia.

Fig. 2. Ethnic dish called "yaprac sarma" (a type of dolma) made of leaves (grape, *Malva neglecta* or other) stuffed with minced meat and rice prepared by the locals of Eastern Anatolia.

Fig. 3. Baclava – a sweet desert made of pastry, nuts and honey-based syrup.

Fig. 4. Preparation of *Malva neglecta* plants for cooking by the local people of Eastern Anatolia.

this region. During the summer, they are consumed fresh in salads, omelets, fried with onion, or steamed. During the winter, they are used in herbal teas (Figure 6). They are also used in common remedies to cure a range of health conditions such as diabetes, wound healing, inflammation, or cancer [5–8]. To date, research on these plants is limited. Previously, our research group investigated health attributes of their herbal infusions [9–11]. The current study is dedicated to their uses as traditional vegetables.

2. Materials and methods

2.1. Plant material

Fresh plants were collected during May 2014 to August 2014 and their identities confirmed at the Department of Biological Sciences, Faculty of Science, Yuzuncu Yil University, Van, Turkey according to the Flora of Turkey, Volume 2 [12], Volume 4 [13], Volume 5 [14], and Volume 7 [15], with voucher specimens stored in the university herbarium. *Malva neglecta* (Malvaceae) plants were harvested at Konalga village, Van City in the Eastern Anatolia Region of Turkey (37° 51′ 014″ N 043° 10′ 622″ E; 2,058 m; Herbarium code VANF-163745; Collector code MM173). *Plantago lanceolata* (Plantaginaceae) and *C. intybus* (Asteraceae) plants were collected from Campus of Zeve, Bardakçı village, Van City (38° 34′ 004″ N, 043° 17′ 067″ E; 1,693 m) and voucher specimens deposited as, respectively, Herbarium code VANF-163746; Collector code MM156 and Herbarium code VANF-163747; Collector code MM46. *Eryngium bornmuelleri* (Apiaceae) plants were harvested in Konalga (Êzdînan-Martanis) village, Çatak, Van city, (37° 50′ 597″ N 043° 09′ 844″; Herbarium code: VANF-162162; Collector code: MM96). The plants arrived in laboratory in sealed precleaned polythene bags within 2 hours after harvest. The roots, stems, leaves, and flowers were separated, dried at room temperature in the dark, ground into a fine powder (BarVista Coffee & Spice Grinder; Breville, Sydney, Australia), and stored at −20°C until analysis.

2.2. Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA) and were of analytical or high-performance liquid chromatography (HPLC) grade. Soluble starch, iodine reagent and coloring reagent (Glucose C2) were from Wako Pure Chemical Industries (Osaka, Japan). Sodium carbonate was from Ajax Chemicals (Sydney, Australia). Acetic acid and sodium hydroxide were from Ajax Finechem (Sydney, Australia). Folin–Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). α-Amylase from porcine, intestinal acetone powders from rat, porcine pancreatic lipase (type II), and orlistat and captopril were from Sigma–Aldrich (Sydney, Australia). Acarbose was purchased as “glucobay” from Bayer (Bayer Australia Ltd., Pymble, NSW, Australia).

2.3. Preparation of lyophilized extracts

The lyophilized extracts were prepared as described previously [10].

Fig. 5. Ethnic vegetables of Eastern Anatolia: (A) *Plantago lanceolata* L.; (B) *Malva neglecta* Wallr.; (C) *Eryngium bornmuelleri* Nab.; (D) *Cichorium intybus* L.

Fig. 6. *Plantago lanceolata* tea.

2.4. Antioxidant capacity

Total reducing capacities of extracts were evaluated using the Folin–Ciocalteu (total phenolics) and the ferric reducing antioxidant power (FRAP) assays, while oxygen radicals scavenging capacities were assessed in the oxygen radical absorbance capacity (ORAC) assay, as described previously [9].

2.5. Inhibitory activities towards selected enzymes

2.5.1. α-Glucosidase inhibitory activity

Inhibition of isolated α-glucosidase (obtained from intestinal acetone powders from rat) was determined according to Sakulnarmrat and Konczak [16], using sucrose (2 g sucrose in 100 mL maleic acid buffer) as a substrate. The relative α-glucosidase inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \{[(ACB - AC) - (ASB - AS)]/(ACB - AC)\} \times 100, \quad (1)$$

where AS and AC were the absorbance of sample and negative control, and where ASB and ACB were the absorbance of sample blank and control blank, respectively. The absorbance was measured at 505 nm using a Shimadzu 1601 spectrophotometer (Shimadzu Corporation, Tokyo, Japan).

2.5.2. Pancreatic lipase inhibitory activity

Lipase inhibitory activity was assayed according to Sakulnarmrat and Konczak [16], using 4-methylumbelliferyl oleate (0.1mM) as a substrate, with the exception of porcine pancreatic lipase (Sigma type II), which was prepared using a concentration of 0.085 g/mL. The relative lipase inhibition activity was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - (FS - FSB)/(FC - FCB)] \times 100, \quad (2)$$

where FS and FC were the values of samples and negative control measured fluorometrically (emission wavelength 460 nm; excitation wavelength 320 nm; slit width 5 nm; POLARstar Omega, BMG Labtech, Germany). FSB and FCB were the fluorescence readings of sample blank and control blank, respectively.

2.6. Identification of phenolic compounds by liquid chromatography-diode array–tandem mass spectrometry

Identification of phenolic compounds was performed on a Quantum triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an auto sampler, as previously described [10].

2.7. Quantification of phenolic compounds by HPLC-diode array detector

Quantification of phenolic compounds was performed on an HPLC system (Shimadzu Corporation) consisting of two LC-10ADVP pumps, SPD-M10ADVP diode array detector (DAD), CTO-1-ADVP column oven, DGU-12A degasser, SIL-10ADVP auto-injector, and SCL-10A system controller and equipped with an Atlantis column (dC_{18}, 4.6 mm internal diameter × 100 mm length, 5 μm particle size; Waters Associates, Chippendale, NSW, Australia), as described previously [10].

2.8. Statistical analysis

The mean values were calculated based on at least three independent evaluations. One way analysis of variance followed by the Bonferroni *post hoc* test were performed to assess differences between the samples at the level of $p < 0.05$. Statistical correlation analyses were performed using Graphpad Prism 5 (Graphpad Software, San Diego, CA, USA). Results for correlation analysis were considered statistically significant at $p < 0.05$. All 50% inhibitory concentration (IC50) values were calculated from the corresponding dose inhibition curve according to their best-fit shapes, based on at least four reaction points using Microsoft Excel.

3. Results and discussion

3.1. Yields, total phenolics, and antioxidant activities

Antioxidant capacities of lyophilized hydrophilic extracts obtained from leaf, flower, stem, and root of four indigenous vegetables from Eastern Anatolia were evaluated in reagent-based assays (Figure 7). Total reducing capacities were assessed by FRAP and Folin–Ciocalteu assays, which represent a single electron transfer antioxidant mechanism. Free-radical scavenging capacities were assessed by the ORAC assay, representing a hydrogen atom transfer mechanism. The yields, total phenolics, and antioxidant capacities of *M. neglecta*, *P. lanceolata*, *C. intybus*, and *E. bornmuelleri* extracts are shown in Table 1. The alcohol-based extraction applied in this study produced relatively high yields, which suggests presence at high levels of water/alcohol soluble compounds. The highest yield produced *P. lanceolata* leaf (29.5% dry weight; DW) and *E. bornmuelleri* root (28.9% DW), while the lowest yield gave *C. intybus* stem (8.6% DW).

Cichorium intybus and *P. lanceolata* leaf and flower extracts showed superior radical scavenging capacities (ORAC values) with a range of 3,343.9–3,869.1 μmol Trolox Eq./g DW. The ORAC values of *P. lanceolata* and *C. intybus* flower and leaf were similar to those of *Coffea arabica* (3,511 ± 57 μmol Trolox Eq./g DW) [17]. Among *E. bornmuelleri* extracts, leaf had superior ORAC value (3,021.4 μmol Trolox Eq./g DW), approximately two-fold that of the flower extract (1,729.7 μmol Trolox Eq./g DW). The lowest ORAC values exhibited *M. neglecta* extracts (491.1–1,638.4 μmol Trolox Eq./g DW) and *E. bornmuelleri* root (423.8 μmol Trolox Eq./g DW).

The highest reducing capacities (FRAP values) exhibited *P. lanceolata* leaf and flower (1,130.8–1,114.5 μmol Fe^{2+}/g DW,

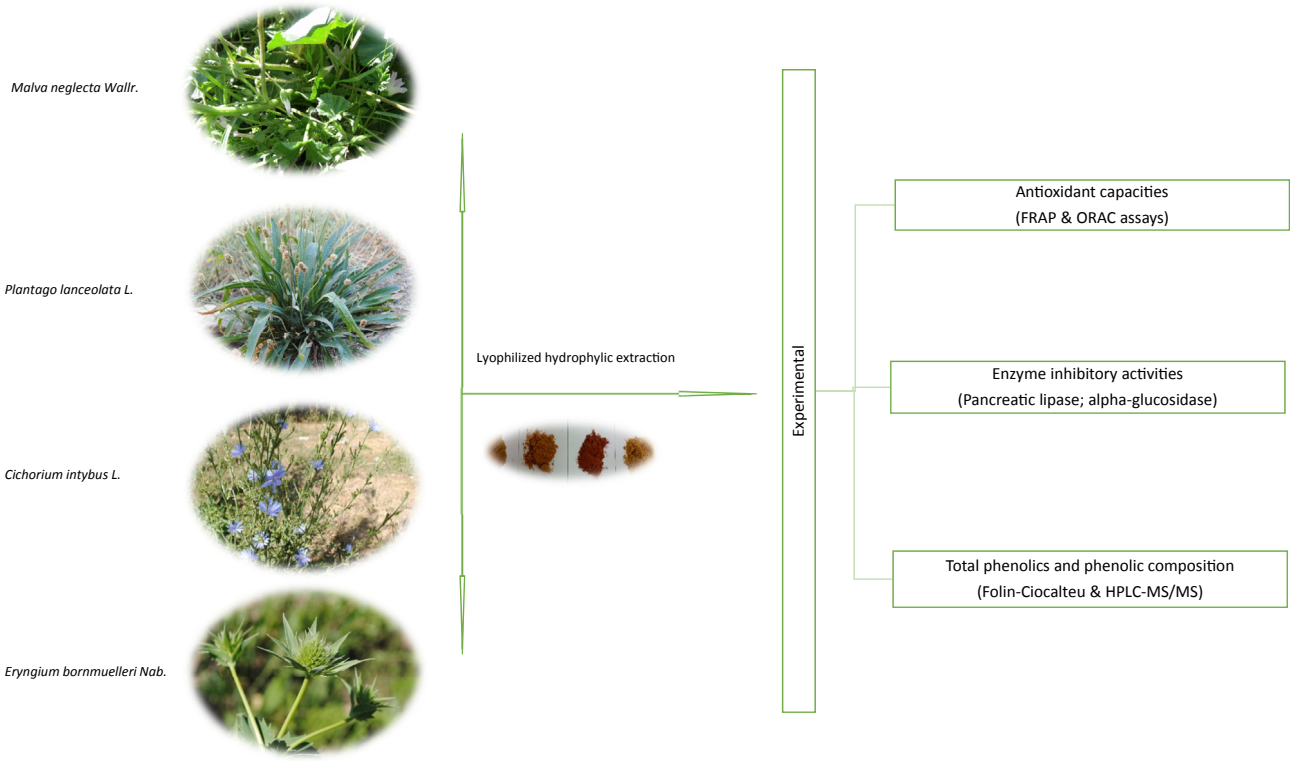

Fig. 7. Graphic presentation of experimental approach.

Table 1
Extraction yields, total phenolics and antioxidant capacities of ethnic vegetables from Eastern Anatolia.

	Plant part	*Malva neglecta*	*Plantago lanceolata*	*Cichorium intybus*	*Eryngium bornmuelleri*
Yield (%)	Root	16.7	21.4	13.1	28.9
	Stem	19.7	15.8	8.6	9.3
	Leaf	16.8	29.5	10.4	16.9
	Flower	14.5	12.2	13.6	18.5
ORAC*	Root	491.1 ± 32.1[c]	3,147.9 ± 21.2[a]	2,540.5 ± 138.2[b]	413.8 ± 4.0[c]
	Stem	517.7 ± 10.7[c]	2,775.4 ± 90.1[a]	2,490.2 ± 66.4[b]	2,303.2 ± 98.2[b]
	Leaf	1,638.4 ± 218.3[b]	3,343.9 ± 215.2[a]	3,620.3 ± 223.9[a]	3,021.4 ± 248.2[a]
	Flower	1,501.4 ± 11.9[d]	3,444.5 ± 36.2[b]	3,869.1 ± 81.6[a]	1,729.7 ± 57.1[c]
FRAP†	Root	91.7 ± 2.3[c]	717.6 ± 22.6[a]	513.9 ± 27.9[b]	54.6 ± 2.3[c]
	Stem	102.6 ± 1.4[c]	598.7 ± 15.6[a]	535.8 ± 12.8[a]	429.8 ± 27.1[b]
	Leaf	390.8 ± 13.5[d]	1,130.8 ± 15.6[a]	611.0 ± 15.1[c]	813.6 ± 39.9[b]
	Flower	318.3 ± 18.2[b]	1,114.5 ± 37.6[a]	1,059.0 ± 23.2[a]	159.4 ± 10.5[c]
F-C‡	Root	9.7 ± 0.2[b]	48.2 ± 2.2[a]	44.6 ± 0.7[a]	7.5 ± 0.3[b]
	Stem	11.0 ± 0.4[d]	44.2 ± 0.7[b]	50.0 ± 1.0[a]	33.5 ± 2.1[c]
	Leaf	27.9 ± 0.4[c]	80.4 ± 1.8[a]	61.6 ± 1.7[b]	63.5 ± 1.4[b]
	Flower	24.3 ± 2.0[b]	80.4 ± 4.3[a]	83.2 ± 2.2[a]	20.4 ± 0.8[b]

* Oxygen radical absorbance capacity – μmol Trolox E./g DW.
† Ferric reducing antioxidant power – μmol Fe^{2+}/g DW.
‡ Folin–Ciocalteu values – mg gallic acid equivalent/g DW,
[a-d] Means with different letters in the same row were significantly different at $p < 0.01$ ($n = 3$).

respectively), *C. intybus* flower (1,059.0 μmol Fe^{2+}/g DW) and *E. bornmuelleri* leaf (813.6 μmol Fe^{2+}/g DW). Among 51 edible wild flowers from China, *Rosa hybrida* and *Limonium sinuatum* showed the highest total reducing capacity of, respectively, 629.64 ± 24.61 μmol Fe^{2+}/g DW and 500.04 ± 70.78 μmol Fe^{2+}/g DW, respectively [18]. These values are two times lower than the FRAP values of the *P. lanceolata* and *C. intybus* flower extracts.

Leaf and flower of *P. lanceolata* and *C. intybus* exhibited the highest total phenolics (80.4–83.2 mg GAE./g DW), which correlated well with their high ORAC and FRAP values. The total phenolic levels of *P. lanceolata* extracts were superior to other *Plantago* species, including *Plantago argentea*, *Plantago holosteum*, *Plantago major*, *Plantago maritime*, and *Plantago media*, with TP values with a range of 38.43–70.97 mg GAE./g DW [19]. Total phenolics of *M. neglecta* leaf and flower were ~3.5 times lower than those of *P. lanceolata* and *C. intybus*, and correlated well with the lower antioxidant capacity of *M. neglecta* extracts.

The total phenolic levels of the evaluated indigenous vegetables are comparable to those of common spices and herbs, including basil (44.89 mg GAEq./g DW), coriander (18.5 mg GAEq./g DW), ginger (17.7 mg GAEq./g DW), nutmeg (17.6 mg GAEq./g DW),

oregano (82.3 mg GAEq./g DW), turmeric (25.9 mg GAEq./g DW), and thyme (23.24 mg GAEq./g DW) [20].

Leaf, flower, and stem of the evaluated traditional foods are frequently used in preparation of soups and omelets. This study suggests that the investigated indigenous vegetables, especially *P. lanceolata*, *C. intybus*, and *E. bornmuelleri*, represent a valuable source of antioxidant phytochemicals that diversify diet and could enrich health attributes of food.

3.2. Inhibitory activities towards isolated α-glucosidase and pancreatic lipase

α-Glucosidase is a key enzyme responsible for digestion of sugars and its inhibition reduces sugar uptake, contributing to lower postprandial hyperglycemic responses [16]. The α-glucosidase inhibitory capacities of the evaluated vegetables (expressed as IC50 and as an equivalent of a commercial drug acarbose) are presented in Table 2. *Plantago lanceolata* root, stem, leaf, and flower and *C. intybus* flower were the most efficient inhibitors, with *P. lanceolata* flower showing the strongest activity (IC50: 1.32 mg/mL). The extracts of *M. neglecta* were weak inhibitors (IC50 values > 10 mg/mL) and were closely followed by *E. bornmuelleri* leaf (IC50 7.4 mg/mL). The α-glucosidase inhibitory activities of *P. lanceolata* and *C. intybus* flower are comparable to those of cinnamon, a traditional antidiabetic remedy (IC50 0.42−2.96 mg/mL) [21]. In another study, *P. lanceolata* herbal tea exhibited a similar level of activity (IC50 1.4 mg/mL) [10]. The root, flower, and stem extracts of *E. bornmuelleri* and all parts of *M. neglecta* plant were the weakest inhibitors of α-glucosidase. These extracts also showed low antioxidant capacities (Tables 1 and 2).

Flower and leaf extracts of *P. lanceolata* and *C. intybus* also exhibited significant inhibitory properties against pancreatic lipase (IC50 3.0−4.53 mg/mL; Table 2), while the extracts of *E. bornmuelleri* and *M. neglecta* were not effective. The lipase inhibitory activities of *P. lanceolata* and *C. intybus* flower extracts were superior to those of traditional edible flowers from Thailand (IC50 range: 4.60−7.87 mg/mL) [22].

3.3. Correlation analysis

Correlation analysis for the relationship between phenolic compounds and antioxidant and enzyme-inhibitory activities of the evaluated extracts is presented in Table 3. A strong positive correlation was found between total phenolics and antioxidant capacities ($r^2 \leq 0.89$) as well as between total phenolics and enzyme inhibitory activities ($r^2 \leq 0.7$). A strong correlation was found between the FRAP and ORAC values ($r^2 = 0.82$) as well as antioxidant and enzyme inhibitory activities ($r^2 \leq 0.69$). A moderate correlation was detected between α-glucosidase and lipase inhibitory activities ($r^2 = 0.5487$). This analysis indicates that phenolic compounds are the major contributors to antioxidant and enzyme inhibitory activities of the evaluated extracts.

In this study hydrophilic extracts of *P. lanceolata* and *C. intybus* exhibited significantly higher activities across all applied assays than *E. bornmuelleri* and *M. neglecta* extracts. Their activities were concomitant with higher levels of total phenolics. Among *P. lanceolata* and *C. intybus* extracts, flower and leaf had superior levels of phenolics and exhibited the most pronounced activities. Subsequently, *P. lanceolata* and *C. intybus* represents valuable dietary sources of phytochemcials that may provide health benefits. They may effectively suppress the activities of α-glucosidase and pancreatic lipase, reducing the metabolic syndrome burden. To understand the sources of these activities of both plants, the composition of their extracts was evaluated.

3.4. Major phenolic compounds of P. lanceolata and C. intybus extracts

The levels and composition of phenolic compounds in lyophilized hydrophilic extracts of *P. lanceolata* and *C. intybus* root, stem, leaf, and flower, as assessed by the HPLC-DAD and HPLC-MS/MS (tandem mass spectrometry) are shown in Table 4. The HPLC-DAD analysis (280 nm, 326 nm, and 370 nm wavelength) showed that the majority of phenolic compounds were detected at 326 nm and 360 nm wavelength, indicating the presence of hydroxycinnamic acids and/or flavonoid glycosides. The levels of phenolics in *P. lanceolata* extracts detected at 326 nm were in agreement with the reported above total phenolic values (Folin−Ciocalteu assay). The phenolic levels in *C. intybus* extracts, as quantified by HPLC at 370 nm, were 1.5−2-fold those of estimated total phenolic values, which suggests that beside phenolic compounds, other redox-active compounds that interfered in the reaction were also present.

The identities of phenolic compounds were established through HPLC-MS/MS analysis, based on their UV spectrum, retention

Table 2
Enzyme-inhibitory properties of hydrophilic extracts obtained from ethnic vegetables from Eastern Anatolia.

		Malva neglecta	*Plantago lanceolata*	*Cichorium intybus*	*Eryngium bornmuelleri*
α-Glucosidase inhibitory activity	*IC50 (mg/mL)*				
	Root	16.66 ± 0.40^{c}	2.21 ± 0.14^{a}	5.44 ± 0.14^{b}	16.12 ± 0.34^{c}
	Stem	15.20 ± 0.30^{d}	2.70 ± 0.17^{a}	6.94 ± 0.79^{b}	11.69 ± 0.03^{c}
	Leaf	13.83 ± 0.17^{d}	1.43 ± 0.04^{a}	4.25 ± 0.08^{b}	7.42 ± 0.06^{c}
	Flower	11.89 ± 0.18^{b}	1.32 ± 0.08^{a}	1.63 ± 0.01^{a}	17.22 ± 0.40^{c}
	Acarbose Eq.(μmol/g DW)				
	Root	2.77 ± 0.07^{c}	20.87 ± 1.26^{a}	8.48 ± 0.22^{b}	2.86 ± 0.06^{c}
	Stem	3.03 ± 0.06^{b}	17.14 ± 1.09^{a}	6.70 ± 0.80^{b}	3.94 ± 0.01^{b}
	Leaf	3.33 ± 0.04^{d}	32.34 ± 0.96^{a}	10.85 ± 0.21^{b}	6.21 ± 0.05^{c}
	Flower	3.88 ± 0.06^{b}	34.93 ± 2.10^{a}	28.35 ± 0.22^{a}	2.68 ± 0.06^{b}
Pancreatic lipase inhibitory activity	*IC50 (mg/mL)*				
	Root	25.27 ± 0.83^{b}	6.76 ± 0.15^{a}	6.68 ± 0.10^{a}	22.94 ± 0.27^{b}
	Stem	30.87 ± 0.44^{c}	7.44 ± 0.09^{b}	4.74 ± 0.23^{a}	8.72 ± 0.08^{b}
	Leaf	10.21 ± 0.08^{c}	4.53 ± 0.08^{ab}	3.97 ± 0.20^{a}	5.44 ± 0.44^{b}
	Flower	9.66 ± 0.21^{b}	3.85 ± 0.06^{a}	3.00 ± 0.25^{a}	11.70 ± 0.20^{c}
	Orlistat Eq.(μmol/g DW)				
	Root	0.11 ± 0.00^{b}	0.41 ± 0.01^{a}	0.41 ± 0.01^{a}	0.12 ± 0.00^{b}
	Stem	0.09 ± 0.00^{c}	0.37 ± 0.00^{b}	0.58 ± 0.03^{a}	0.32 ± 0.00^{b}
	Leaf	0.27 ± 0.00^{c}	0.61 ± 0.01^{ab}	0.70 ± 0.04^{a}	0.51 ± 0.01^{b}
	Flower	0.29 ± 0.01^{b}	0.72 ± 0.01^{a}	0.92 ± 0.08^{a}	0.24 ± 0.00^{b}

IC50, 50% inhibitory concentration.
[a-d] Means with different letters in the same row were significantly different at $p < 0.01$ ($n = 3$).

Table 3
Correlation coefficients for relationship between phenolic compound levels, antioxidant capacity and enzyme inhibitory properties of hydrophilic extracts obtained from ethnic vegetables from Eastern Anatolia.

	TP (Folin–Ciocalteu values)	FRAP	ORAC	Lipase inhibition	α-Glucosidase inhibition
TP (Folin–Ciocalteu)	1	0.9584	0.8900	0.9007	0.6970
FRAP	0.9584	1	0.8290	0.7830	0.7882
ORAC	0.8900	0.8290	1	0.8356	0.5503
Lipase inhibition	0.9007	0.7830	0.8356	1	0.5487
α-Glucosidase inhibition	0.6970	0.7882	0.5503	0.5487	1

FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity; TP, total phenolics.

times, co-chromatography with commercial standards, when available, and mass fragmentation patterns. Table 4 presents the negatively and/or positively charged molecular ions ($[M-1]^-$/$[M-1]^+$) and MS^2 fragments of identified compounds. The major compounds were luteolin derivatives, caftaric acid, chlorogenic acid isomers, quercetin 3-*O*-glucuronide, and chicoric acid. Luteolin hexoside was the dominating compound across all evaluated extracts. In *P. lanceolata* extracts, it contributed 71% (root), 53% (stem), 53% (leaf), and 45% (flower) of total phenolics. This result supports earlier report that luteolin and luteolin hexoside are the main phenolic compounds of *Plantago* species [19].

Luteolin hexoside contributed 18%, 16%, 8%, and 7% of total phenolics in root, stem, leaf, and flower extracts of *C. intybus*, respectively. Quercetin 3-*O*-glucuronide, caftaric acid, and chicoric acid were also present. Chicoric acid contributed 41%, 14%, 17%, and 16% of total phenolics of root, stem, leaf, and flower extract, respectively (Table 4). The contribution of caftaric acid was low (< 7.2%) in all *C. intybus* extracts, which supports earlier reports [11,23]. Chicoric acid, a potent antioxidant [24], was the key component of *C. intybus* extracts investigated in this study (16–41% of total phenolics). In agreement, the same compound was present in numerous *Cichorium* species [23]. Chlorogenic acid contributed 23%, 12%, 18%, and 20% of total phenolics of *C. intybus* extract from root, stem, leaf, and flower, respectively. In *P. lanceolata* extracts, this compound was present only at traces levels (Table 4). Other phenolics detected in *C. intybus* at low levels were apigenin, hydroxybenzoic acid-*O*-hexoside, apigenin glucoside, and isoharmnetin. Quercetin hexoside and quercetin rutinoside were also found in both, *C. intybus* and *P. lanceolata* plant extracts (Table 4).

The dominant phenolic compound of *C. intybus* and *P. lanceolata* plants investigated in this study was luteolin hexoside. The characteristic feature of luteolin and its glycosides molecules is the "catechol" structure or *ortho*-dihydroxy substitution in Ring B, which is the structural requirement for antioxidant capacity [25]. Compounds with catechol structure exhibit oxygen and nitrogen free-radical scavenging capacities, chelate transition metals, inhibit pro-oxidant enzymes and induce antioxidant enzymes [26]. Our study demonstrated that both indigenous vegetables from Eastern Anatolia, *C. intybus* and *P. lanceolata*, are rich sources of luteolin and derivatives with the highest level identified in *C. intybus* flower. The same extract also exhibited pronounced α-glucosidase inhibitory activity (IC50 1.32 ± 0.08 mg/mL; Table 2). This suggests that luteolin hexoside is one of the major contributors towards α-glucosidase inhibitory properties of *C. intybus*.

Chlorogenic acid and its isomers, the esters of caffeic and quinic acid, are the key phenolic compounds of some dietary plants such

Table 4
Total phenolic content and phenolic composition of hydrophilic extracts of *Plantago lanceolata* and *Cichorium intybus*.

Quantification of phenolic compounds			*P. lanceolata*				*C. intybus*			
			Root	Stem	Leaf	Flower	Root	Stem	Leaf	Flower
Total phenolics at 280 nm (GA E./gDW)*			33.6 ± 0.5	26.2 ± 0.1	41.9 ± 1.4	57.7 ± 0.3	47.2 ± 0.7	57.6 ± 1.2	70.6 ± 2.4	81.8 ± 0.6
Total phenolics at 326 nm (CHA E./g DW)*			43.5 ± 0.2	38.7 ± 2.1	77.7 ± 8.9	109.5 ± 1.9	51.9 ± 0.4	79.7 ± 3.0	94.8 ± 3.6	111.7 ± 5.1
Total phenolics at 370 nm (R E./g DW)*			30.5 ± 1.0	31.1 ± 3.2	66.6 ± 0.7	72.9 ± 1.6	62.4 ± 8.2	99.8 ± 9.3	113.5 ± 1.5	145.4 ± 9.4
Identification of phenolic compounds	MS/MS $[M+1]^+$/$[M-1]^-$	Fragments (m/z) (+/−)								
Phenolic acids										
Hydroxybenzoic acid-O-hexoside[2]	−/299	−/137	ND	ND	ND	ND	T	T	T	T
Caftaric acid†,‡	−/311	−/179	ND	ND	ND	ND	T	T	6.2 ± 0.1	8.1 ± 0.1
Chlorogenic acid and isomers	−/353	−/191	T	T	T	T	12.0 ± 0.5	9.5 ± 0.0	17.0 ± 0.1	22.4 ± 0.1
Cichoric acid†,‡	−/473	−/311,179	ND	ND	ND	ND	21.3 ± 0.3	11.9 ± 0.1	16.2 ± 0.3	17.9 ± 0.1
Flavonoids										
Apigenin†	−/269	−/117	ND	ND	ND	ND	T	T	T	T
Isorhamnetin§	−/315	−/300	ND	ND	ND	ND	T	T	T	T
Apigenin glucoside§	−/431	−/269	ND	ND	ND	ND	T	T	T	T
Luteolin hexoside†,§	449/447	287/285	30.7 ± 0.6	20.5 ± 0.4	41.1 ± 0.3	49.4 ± 0.1	9.2 ± 0.3	13.1 ± 0.2	7.8 ± 0.2	8.0 ± 0.1
Quercetin/Hesperitin glucoside†,§	465/−	303/−	T	T	T	T	T	T	T	T
Quercetin 3-O-glucuronide§	−/477	−/301	ND	ND	ND	ND	ND	ND	ND	5.6 ± 0.0
Quercetin rutinoside (Rutin)†	611/609	303/301	T	T	T	T	T	T	T	T

All data represent the mean ± standard deviation of at least three independent experiments.
T, traces (concentration < 2%).
* GA E./g DW - gallic acid equivalent per gram dry weight of lyophilized extract; CHA E./g DW - chlorogenic acid equivalent per gram dry weight of lyophilized extract; R E./g DW - rutin equivalent per gram dry weight of lyophilized extract.
† Assignment confirmed with reference standard.
‡ Value is expressed as mg chlorogenic acid Eq./g DW.
§ Tentative assignment based on MS data only.

as coffee beans, and can be the primary source of antioxidant capacities [27,28]. They were also found in *C. intybus* extracts. Chlorogenic acid was identified as the main anti-diabetic and anti-obesity agents in other edible plants and traditional medicines [29,30].

In conclusion, two of the investigated indigenous vegetables commonly consumed in Eastern Anatolia, *P. lanceolata* and *C. intybus*, exhibited high antioxidant capacities, comparable to commonly used herbs, and pronounced inhibitory activities towards α-glucosidase and pancreatic lipase. The levels of phenolic compounds correlated well with their antioxidant and enzyme-inhibitory potential, indicating that phenolic compounds are the possible sources of these activities. Luteolin hexoside dominated in *P. lanceolata*. The same compound was present in *C. intybus*, where it was accompanied by significant amounts of cichoric, chlorogenic, and caftaric acid.

Conflicts of interest

All authors have no conflicts of interest to declare.

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