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ORIGINAL ARTICLE

Prospective neurobiological effects of the aerial and root extracts and some pure compounds of randomly selected *Scorzonera* species

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Abstract

Context: *Scorzonera* L. species (Asteraceae) are edible and as medicinal plants are used for various purposed in folk medicine.
Objective: The methanol extracts of the aerial parts and roots from 27 *Scorzonera* taxa were investigated for their possible neurobiological effects.
Materials and methods: Inhibitory potential of the *Scorzonera* species was tested against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYRO) at 100 μg mL^{-1} using ELISA microtiter assay. Antioxidant activity of the extracts was tested with radical scavenging activity, metal-chelation capacity, ferric- (FRAP), and phosphomolibdenum-reducing antioxidant power (PRAP) assays. Chlorogenic acid, hyperoside, rutin, and scorzotomentosin-4-*O*-β-glucoside were also screened in the same manner. Total phenol and flavonoid quantification in the extracts were determined spectrophotometrically.
Results: The aerial parts of *Scorzonera pisidica* (40.25 ± 0.74%) and chlorogenic acid (46.97 ± 0.82%) displayed the highest TYRO inhibition, while the remaining samples showed only trivial inhibition against cholinesterases (2.08 ± 1.35%–25.32 ± 1.37%). The same extract of *S. pisidica* was revealed to be the most potent in scavenging of all three radicals and FRAP assay.
Discussion and conclusion: Out of 27 taxa, *S. pisidica*, in particular, may deserve further investigation for its neuroprotective potential.

Keywords

Antioxidant activity, Asteraceae, cholinesterase, neuroprotection, *Scorzonera*, tyrosinase

History

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Introduction

Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) usually affect the aging population. Many factors, including oxidative stress, mitochondrial DNA mutations, formation of toxic proteins, etc., have been shown to contribute invariably to neurodegeneration (Sorce et al., 2012). AD is mainly characterized by memory deficits and abnormal behaviors in a progressive manner and deficiency in acetylcholine/butyrylcholine levels as revealed in the brains of AD patients (Giacobini, 2004). In contrast, PD, which is often accompanied by AD, is another neurodegenerative disorder indicated by tremors as well as rigidity in body movement and coordination, muscle atrophy, cognitive problems, and shortage in dopamine (Aarslan & Kurz, 2010). Both these diseases have been shown to develop intense and steady escalation in terms of incidence and prevalence after the age of 50 (Van der Schyf & Geldenhuys, 2011). At this point, no medication is able to cure AD and PD, although the current drugs are only capable of symptomatic treatment of these diseases. Cholinesterase inhibitors are presently the most prescribed drug class for AD treatment (Orhan et al., 2009), while dopamine agonists and cholinesterase inhibitors are also used in the treatment of PD (Lang, 2009). Enzymatic activity of tyrosinase (TYRO) is known to create dopamine–quinones and some other oxidizing compounds that finally lead to neuromelanin formation and dopamine neurotoxicity in PD (Greggio et al., 2005). In this regard, inhibition of TYRO (also known as polyphenol oxidase) could be considered as a beneficial approach to PD treatment. Hence, intense research is being conducted to discover novel drug candidates of synthetic or natural origins that can inhibit cholinesterases and TYRO.

Scorzonera L. species (Asteraceae) are represented by approximately 175 species, which are widely distributed in arid regions of Eurasia and Africa. Many species of this genus are consumed as vegetable in Turkey. *Scorzonera mollis* (goftigoda), *Scorzonera suberosa* (wild carrot), *Scorzonera cana* (karakök, tekesakali), and *Scorzonera latifolia* (*geniş*

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yapraklı karakök or *mesdek*) are some examples of the edible species in this region. Besides, the roots are cooked, while the young leaves are used in salads in European cuisine (Tsevegsuren et al., 2007). In addition to edible properties of the genus *Scorzonera*, some of the species have been recorded to be used in traditional medicine for various purposes such as wound healing, stomachic, diuretics, antipyretic, and appetizing effects as well as for the treatment of diarrhea, lung edema, parasitic diseases, and fever in China, Mongolia, Turkey, and European countries (Baytop, 1999; Tsevegsuren et al., 2007; Wang et al., 2009).

In our ongoing research on finding new cholinesterase and TYRO inhibitors of herbal origin, we have now targeted the *in vitro* neuroprotective potential of the methanol extracts prepared from the aerial parts and roots of 27 *Scorzonera* taxa growing in Turkey through their inhibitory effects against acetylcholinesterase (AChE), butyrylcholinesterase, and TYRO at $100\,\mu g\,mL^{-1}$ using ELISA microtiter assays. Since oxidative damage is one of the prompting factors in neurodegeneration, the extracts were subjected to several antioxidant assay systems using 2,2-diphenyl-1-picrylhydrazyl (DPPH), *N*,*N*-dimethyl-*p*-phenylendiamine (DMPD), and nitric oxide free radical scavenging, metal-chelation capacity, ferric- (FRAP), and phosphomolibdenum-reducing antioxidant power (PRAP) assays. Besides, four compounds (namely chlorogenic acid, hyperoside, rutin, scorzotomentosin-4-*O*-β-glucoside), which were previously detected or isolated from several *Scorzonera* species by our group, were also subjected to the same enzyme inhibitory and antioxidant assays. Total phenol and flavonoid quantification in the extracts were achieved using a spectrophotometric method.

Materials and methods

Plant materials

The samples of 27 *Scorzonera* taxa, namely *S. acuminata* Boiss., *S. argyria* Boiss., *S. aucherana* DC., *S. boissieri* Lipschitz, *S. cana* (C.A. Meyer) Hoffm. var. *alpina* (Boiss.) Chamberlain, *S. cana* (C.A. Meyer) Hoffm. var. *jacquiniana* (W. Koch) Chamberlain, *S. cana* (C.A. Meyer) Hoffm. var. *radicosa* (Boiss.) Chamberlain, *S. sericea* DC., *S. cinerea* Boiss., *S. elata* Boiss., *S. ekimi* A. Duran, *S. eriophora* DC., *S. gokcheoglui* O.Ünal & R.S. Göktürk, *S. incisa* DC., *S. kotschyi* Boiss., *S. lacera* Boiss. & Bal., *S. laciniata* L. subsp. *laciniata*, *S. latifolia* (Fisch. & Mey.) DC., *S. mirabilis* Lipschitz, *S. mollis* Bieb. subsp. *szowitsii* (DC.) Chamberlain, *S. parviflora* Jacq., *S. pisidica* Hub-Mor., *S. pseudolanata* Grossh., *S. suberosa* C. Koch subsp. *suberosa*, *S. suberosa* C. Koch subsp. *cariensis* (Boiss.) Chamberlain, *S. sublanata* Lipschitz, and *S. tomentosa* L. were collected throughout Turkey. Details of collection, i.e., locality, year, and herbarium numbers are listed in Table 1. Taxonomic identification of the plants was confirmed by Prof. Dr. H. Duman and Dr. U. Özbek from Department of Biological Sciences, Faculty of Art and Sciences, Gazi University, Ankara (Turkey) as well as Assist. Prof. Dr. Fevzi Ozgökce from Department of Biological Sciences, Faculty of Art and Sciences, Yuzuncu Yıl University, Van (Turkey). Voucher specimens are preserved in the Herbarium of Faculty of Pharmacy, Ankara University (AEF) and Yuzuncu Yil University (F) (Table 1).

Extraction of plant materials

The aerial parts and roots of each species were separately dried, powdered, and weighed accurately. Then, each plant sample was extracted with 80% aqueous methanol (100 mL) at room temperature for 24 h by continuous stirring separately three times, which was later filtered and concentrated to dryness under reduced pressure and low temperature (40–50 °C) on a rotary evaporator to give the crude methanol extracts. Yields (w/w) of each extract are given in Table 2.

Enzyme inhibition assays

Cholinesterase inhibition assays

AChE and BChE inhibitory activities of the samples were determined by a modified spectrophotometric method of Ellman et al. (1961). As the enzyme sources, electric eel AChE (Type-VI-S, EC3.1.1.7, Sigma, St. Louis, MO) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO) were employed, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO) were used as substrates of the reaction. 5,5-Dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO) was used for the measurement of the cholinesterase activity. In brief, 140 µL of 0.1 mM sodium phosphate buffer (pH 8.0), DTNB, the sample solutions, and AChE/BChE solution (20 µL for each) were added by multichannel automatic pipette (Gilson Pipetman, Villiers-le-Bel, France) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 10 µL of acetylthiocholine iodide/butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing ELISA microplate reader (VersaMax, Molecular Devices, Sunnyvale, CA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software (Union City, CA). Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH 8) using the formula $(E-S)/E \times 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine, the anticholinesterase drug isolated firstly from the bulbs of snowdrop (*Galanthus* sp.), was purchased from Sigma (St. Louis, MO) and employed as the reference.

TYRO inhibition assay

Inhibition of TYRO (EC 1.14.1.8.1; 30 U, mushroom tyrosinase, Sigma, St. Louis, MO) was determined using the modified dopachrome method with L-DOPA as a substrate (Masuda et al., 2005). The assays were conducted in a 96-well microplate using ELISA microplate reader (VersaMax Molecular Devices, Sunnyvale, CA) to measure absorbance at 475 nm. An aliquot of the extracts dissolved in DMSO with

Table 1. Locality and year of collection and herbarium numbers of the screened *Scorzonera* species.

No.	Plant species	Locality and year of collection	Herbarium no
1	*S. acuminata* Boiss.	Yumakli village, Cankiri, 2010	AEF 25938
2	*S. argyria* Boiss.	Yilanli mountain, Mugla	AEF 25956
3	*S. aucherana* DC.	Hafik town, Sivas	AEF 26030
4	*S. boissieri* Lipschitz	Yalak vicinity, Kayseri	AEF 26027
5	*S. cana* (C.A. Meyer) Hoffm. var. *alpina* (Boiss.) Chamberlain	Akdelen town, Tokat, 2008	AEF 25893
6	*S. cana* (C.A. Meyer) Hoffm. var. *jacquiniana* (W. Koch) Chamberlain	Camlidere town, Ankara, 2008	AEF 23834
7	*S. cana* (C.A. Meyer) Hoffm. var. *radicosa* (Boiss.) Chamberlain	Aladaglar, Nigde, 2005	AEF 26028
8	*S. sericea* DC.	Kop passage, Bayburt	AEF 26026
9	*S. cinerea* Boiss.	Çetinkaya town, Sivas, 2005	AEF 23829
10	*S. elata* Boiss.	Yilanli mountain, Mugla	AEF 25955
11	*S. ekimi* A. Duran	Kazankaya town, Yozgat	AEF 26031
12	*S. eriophora* DC.	Cubuk town, Ankara, 2007	AEF 23832
13	*S. gokcheoglui* O.Ünal & R.S. Göktürk	Alanya town, Antalya	AEF 26025
14	*S. incisa* DC.	Ermenek town, Konya, 2005	AEF 23833
15	*S. kotschyi* Boiss.	Ermenek town, Konya	AEF 25954
16	*S. lacera* Boiss. & Bal.	Erdemli town, Mersin	AEF 25962
17	*S. laciniata* L. subsp. *laciniata*	Camlidere town, Ankara, 2008	AEF 23835
18	*S. latifolia* (Fisch. & Mey.) DC.	Arpacay town, Kars	AEF 23830
19	*S. mirabilis* Lipschitz	Vicinity of Van province	F 18386
20	*S. mollis* Bieb. subsp. *szowitsii* (DC.) Chamberlain	Kizilcahamam town, Ankara, 2006	AEF 23844
21	*S. parviflora* Jacq.	Gölbasi town, Ankara, 2008	AEF 25894
22	*S. pisidica* Hub-Mor.	Dirmil town, Burdur	AEF 26029
23	*S. pseudolanata* Grossh.	Vicinity of Aksaray province	AEF 25958
24	*S. suberosa* C. Koch subsp. *suberosa*	Pinarbasi town, Kayseri, 2006	AEF 23843
25	*S. suberosa* C. Koch subsp. *cariensis* (Boiss.) Chamberlain	Yilanli mountain, Mugla	AEF 25953
26	*S. sublanata* Lipschitz	Kizilcahamam town, Ankara, 2010	AEF 25937
27	*S. tomentosa* L.	Akdagmadeni town, Yozgat, 2005	AEF 23841

80 μL of phosphate buffer (pH 6.8), 40 μL of tyrosinase, and 40 μL of L-DOPA were put in each well. Results were compared with control (DMSO). α-Kojic acid (Sigma, St. Louis, MO) was used as the reference. The percentage tyrosinase inhibition (I%) was calculated as follows:

$$I\% = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{control}} \times 100$$

Antioxidant assays

DPPH radical scavenging activity

The stable DPPH radical scavenging activity was determined by the method of Blois (1958). The samples (30 μL) dissolved in ethanol (75%) were mixed with 2700 μL of DPPH solution (1.5×10^{-4} M). Remaining DPPH amount was measured at 520 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). Gallic acid (Sigma, St. Louis, MO) was employed as the reference.

DMPD radical scavenging activity

The assay is based on the reduction of the purple-colored radical $DMPD^+$ (*N*,*N*-dimethyl-*p*-phenylendiamine). According to the method of Schlesier et al. (2002), a reagent comprising of 100 mM DMPD, 0.1 M acetate buffer (pH = 5.25), and 0.05 M ferric chloride solution, which led to the formation of DMPD radical, was freshly prepared and the reagent was equilibrated to an absorbance of 0.900 ± 0.100 at 505 nm. Then, the reagent was mixed up with 50 μL of the extract dilutions and absorbance was taken at 505 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). Quercetin (Sigma, St. Louis, MO) was employed as the reference.

NO radical scavenging activity

The scavenging activity of the samples against NO was assessed by the method of Marcocci et al. (1994). Briefly, the sample dilutions were mixed with 5 mM sodium nitroprusside and left to incubation for 2 h at 29 °C. An aliquot of the solution was removed and diluted with Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylene-diamine dihydrochloride). Absorbance of the chromophore occurred was measured at 550 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). Quercetin (Sigma, St. Louis, MO) was the reference in this test.

Metal-chelation capacity by Fe^{+2}-ferrozine test system

The ferrous ion-chelating capacity of the samples was estimated by the method of Chua et al. (2008). Briefly, dilutions of the samples were incubated with 2 mM $FeCl_2$ solution. The reaction was initiated by the addition of 5 mM ferrozine into the mixture and left standing at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). The reference was employed as ethylenediamine tetraacetic acid (EDTA) (Sigma, St. Louis, MO) in this assay.

Ferric-reducing antioxidant power (FRAP)

FRAP of the samples was tested using the assay of Oyaizu (1986). Different concentrations of the samples were mixed with 2500 μL of phosphate buffer (pH 6.6) and 2500 μL of potassium ferricyanide. Later, the mixture was incubated at 50 °C for 20 min and, then, trichloroacetic acid (10%) was added. After the mixture was shaken vigorously, this solution

Table 2. Yield percentages and AChE, BChE, and TYRO inhibitory activity of the methanol extracts of *Scorzonera* species.

			Inhibition (% ± SEM[a]) at 100 μg mL^{-1}		
Extracts	Plant part	Yield% (w/w)	AChE	BChE	TYRO
S. acuminata	Aerial	25.26	$12.17 \pm 1.34^{****}$	–[b]	$10.68 \pm 2.65^{****}$
	Root	40.21	$14.90 \pm 0.95^{****}$	–	$6.55 \pm 1.93^{****}$
S. argyrea	Aerial	21.21	–	–	$20.01 \pm 2.63^{****}$
	Root	38.16	$8.92 \pm 0.28^{****}$	–	$9.38 \pm 0.90^{****}$
S. aucheriana	Aerial	19.09	–	–	$11.47 \pm 2.35^{****}$
	Root	18.75	–	–	$7.47 \pm 2.90^{****}$
S. boissieri	Aerial	16.05	–	–	$12.95 \pm 2.98^{****}$
	Root	26.59	–	$2.08 \pm 1.35^{****}$	$2.90 \pm 0.32^{****}$
S. cana var. *alpina*	Aerial	29.61	–	–	$12.95 \pm 2.53^{****}$
	Root	30.22	–	–	$8.33 \pm 1.07^{****}$
S. cana var. *jacquiniana*	Aerial	40.51	–	–	$7.66 \pm 0.14^{****}$
	Root	39.24	–	–	$7.52 \pm 4.14^{****}$
S. cana var. *radicosa*	Aerial	26.39	–	–	$8.63 \pm 0.42^{****}$
	Root	41.34	–	–	$6.75 \pm 2.37^{****}$
S. cericea	Aerial	9.83	–	–	$10.35 \pm 1.45^{****}$
	Root	16.54	–	$6.42 \pm 2.13^{****}$	$4.39 \pm 0.53^{****}$
S. cinerea	Aerial	22.78	–	$4.98 \pm 0.31^{****}$	$14.24 \pm 0.43^{****}$
	Root	27.87	–	$8.06 \pm 0.79^{****}$	$5.61 \pm 0.67^{****}$
S. elata	Aerial	21.20	$5.05 \pm 2.38^{****}$	–	$12.77 \pm 0.91^{****}$
	Root	31.64	$15.97 \pm 0.43^{****}$	–	$3.67 \pm 1.88^{****}$
S. ekimii	Aerial	33.28	–	$5.30 \pm 0.88^{****}$	$17.96 \pm 1.34^{****}$
	Root	27.09	$9.38 \pm 2.75^{****}$	$10.56 \pm 2.51^{****}$	$10.22 \pm 0.09^{****}$
S. eriophora	Aerial	25.31	–	–	$9.19 \pm 1.09^{****}$
	Root	43.50	–	$5.18 \pm 1.96^{****}$	$3.76 \pm 1.77^{****}$
S. gokceoglui	Aerial	15.99	–	$5.69 \pm 0.37^{****}$	$17.56 \pm 2.58^{****}$
	Root	26.25	–	–	$5.01 \pm 2.37^{****}$
S. incisa	Aerial	31.58	–	–	$8.64 \pm 1.05^{****}$
	Root	22.22	–	$2.74 \pm 0.60^{****}$	$5.25 \pm 1.05^{****}$
S. kotschyi	Aerial	37.58	–	–	$27.30 \pm 1.69^{***}$
	Root	28.20	$11.04 \pm 0.09^{****}$	–	$9.47 \pm 2.60^{****}$
S. lacera	Aerial	27.46	–	–	$15.43 \pm 0.73^{****}$
	Root	39.95	$8.98 \pm 0.10^{****}$	$20.78 \pm 2.95^{****}$	$9.97 \pm 1.69^{****}$
S. laciniata subsp. *laciniata*	Aerial	33.73	–	–	$11.33 \pm 0.29^{****}$
	Root	45.17	$14.66 \pm 3.55^{****}$	–	$2.74 \pm 1.45^{****}$
S. latifolia	Aerial	32.09	–	$10.48 \pm 2.92^{****}$	$12.07 \pm 0.95^{****}$
	Root	43.48	–	$11.23 \pm 2.90^{****}$	$9.01 \pm 0.79^{****}$
S. mirabilis	Aerial	14.21	$3.75 \pm 0.71^{****}$	–	$10.07 \pm 1.80^{****}$
	Root	26.03	–	–	$5.57 \pm 1.21^{****}$
S. mollis subsp. *szowitsii*	Aerial	36.64	–	–	$22.25 \pm 1.37^{****}$
	Root	37.66	–	$4.42 \pm 0.72^{****}$	$4.12 \pm 0.23^{****}$
S. parviflora	Aerial	39.11	–	–	$12.50 \pm 2.15^{****}$
	Root	45.14	–	–	$10.96 \pm 1.12^{****}$
S. pisidica	Aerial	25.53	–	–	$40.25 \pm 0.74^{***}$
	Root	42.93	$8.26 \pm 1.15^{****}$	$4.15 \pm 1.10^{****}$	$9.28 \pm 1.55^{****}$
S. pseudolanata	Aerial	21.95	–	–	$22.93 \pm 3.53^{****}$
	Root	31.92	$10.11 \pm 3.80^{****}$	–	$3.67 \pm 0.49^{****}$
S. suberosa	Aerial	29.02	–	–	$8.73 \pm 1.12^{****}$
	Root	31.31	–	–	–
S. suberosa subsp. *cariensis*	Aerial	21.43	$2.87 \pm 0.01^{****}$	$10.26 \pm 0.91^{****}$	$17.46 \pm 1.19^{****}$
	Root	30.24	$11.90 \pm 3.29^{****}$	$25.32 \pm 1.37^{***}$	$13.54 \pm 0.21^{****}$
S. sublanata	Aerial	26.46	$6.46 \pm 1.53^{****}$	$4.70 \pm 1.34^{****}$	$12.88 \pm 2.07^{****}$
	Root	38.14	$13.52 \pm 2.92^{****}$	$10.57 \pm 2.57^{****}$	$11.37 \pm 1.32^{****}$
S. tomentosa	Aerial	17.45	–	$11.19 \pm 2.50^{****}$	$9.70 \pm 3.61^{****}$
	Root	45.35	–	$9.36 \pm 1.64^{****}$	$8.12 \pm 1.98^{****}$
Compounds					
Chlorogenic acid			–	–	$46.97 \pm 0.82^{****}$
Hyperoside			–	$3.19 \pm 1.49^{****}$	$5.26 \pm 0.99^{****}$
Scorzotomentosin-4-glucoside			$18.49 \pm 1.97^{****}$	$12.35 \pm 2.96^{****}$	$3.35 \pm 1.58^{****}$
Rutin			–	–	$1.79 \pm 0.54^{****}$
References					
Galanthamine (reference for AChE & BChE)			92.72 ± 0.15	67.72 ± 1.70	
α-Kojic acid (reference for TYRO)					78.89 ± 0.09

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.
[a]Standard error mean ($n = 3$).
[b]No inhibitory activity.

was mixed with distilled water and ferric chloride (0.1%). After 30 min of incubation, absorbance was read at 700 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). Experiments were achieved in triplicate. Increased absorbance of the reaction meant increased reducing power and compared to that of chlorogenic acid (Sigma, St. Louis, MO) used as the reference.

Phosphomolybdenum-reducing antioxidant power (PRAP)

In order to perform PRAP assays on the samples, each dilution was mixed with 10% phosphomolybdic acid solution in ethanol (w/v) (Falcioni et al., 2002). The solution was subsequently subjected to incubation at 80 °C for 30 min and the absorbance was read at 600 nm using a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ) and compared to that of quercetin as the reference.

Data processing for antioxidant activity assays

Inhibition of DPPH, DMPD, and NO radicals and metal-chelation capacity was calculated using the same equation as given below and the results were expressed as percent inhibition ($I\%$): $I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} is the absorbance of the extracts. Analyses were run in triplicate and the results were expressed as average values with standard error of the mean (SEM).

The FRAP and PRAP assays were also applied in triplicate and increased absorbance of the reaction meant increased reducing power in both assays.

Statistical analysis of data

Data obtained from *in vitro* enzyme inhibition and antioxidant experiments were expressed as the mean standard error (±SEM). Statistical differences between the reference and the sample groups were evaluated by ANOVA (one way). Dunnett's multiple comparison tests were used as post hoc tests. $p < 0.05$ was considered to be significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$).

Spectrophotometric determination of total phenol and flavonoid contents in the extracts

Phenolic content of the extracts was determined in accordance with Folin–Ciocalteau's method (Singleton & Rossi, 1965). In brief, a number of dilutions of chlorogenic acid dissolved in ethanol (75%) were obtained to prepare a calibration curve. The extracts and chlorogenic acid dilutions were mixed with 750 µL of Folin–Ciocalteau's reagent and 600 µL of sodium carbonate in test tubes. The tubes were then vortexed and incubated at 40 °C for 30 min. Afterward, absorption was measured at 760 nm at a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). Total flavonoid content of the extracts was calculated by the aluminum chloride colorimetric method (Woisky & Salatino, 1998). To sum up, a number of dilutions of rutin dissolved in ethanol (75%) were obtained to prepare a calibration curve. Then, the extracts and rutin dilutions were mixed with 95% ethanol, aluminum chloride reagent, 100 µL of sodium acetate as well as distilled water. Following incubation for 30 min at room temperature, absorbance of the reaction mixtures was measured at wavelength of 415 nm with a Unico 4802 UV-visible double beam spectrophotometer (Dayton, NJ). The total phenol and flavonoid contents of the extracts were expressed as chlorogenic acid and rutin equivalents (mg g^{-1} extract), respectively.

Tested compounds isolated/detected in *Scorzonera* species

Scorzotomentosin-4-*O*-β-glucoside was isolated from the root methanol extract of *S. latifolia*, purified by successive chromatographic methods, and, then, identified as a dihydroisocoumarin derivative, which was described in detail in our earlier publication (Citoglu et al., 2010). Chlorogenic acid, hyperoside, and rutin tested in this study were previously purchased from Sigma (St. Louis, MO) for HPLC analysis, which was performed by our group in order to detect their presence in *Scorzonera* species (Küpeli Akkol et al., 2011).

Results and discussion

The methanol extracts obtained from the aerial parts and roots of 27 *Scorzonera* species were screened against AChE, BChE, and TYRO at 100 µg mL^{-1}. As tabulated in Table 2, the extracts exhibited either no or low inhibition against AChE (3.75 ± 0.71–15.97 ± 0.43%) and BChE (2.08 ± 1.35–25.32 ± 1.37%), whereas they were more capable of inhibiting TYRO at the same concentration. The highest TYRO-inhibitory effect was caused by the aerial parts of *S. pisidica* (40.25 ± 0.74%) as well as chlorogenic acid (46.97 ± 0.82%). The tested compounds (chlorogenic acid, hyperoside, rutin, and scorzotomentosin-4-glucoside) showed either no or ignorable cholinesterase and TYRO inhibitory activity (except chlorogenic acid for TYRO) (Table 2).

All the extracts and compounds tested were evaluated in six antioxidant assay systems at 1000 µg mL^{-1}. As listed in Table 3, the radical scavenging effect was revealed to be generally below 50% against all three radicals (DPPH, DMPD, and NO) in most of the extracts and compounds. However, the aerial parts of some *Scorzonera* species exerted notable antiradical activity towards DPPH, whose potency order over 50% was as follows: *S. pisidica* (84.55 ± 2.55%) > *S. kotschyi* (61.53 ± 3.71%) > *S. suberosa* subsp. *cariensis* (58.93 ± 2.17%) > *S. lacera* (50.61 ± 2.18%) > *S. aucheriana* (50.53 ± 2.83%).

DPPH radical scavenging effect (over 85%) was observed by three of the compounds (chlorogenic acid, hyperoside, and rutin) comparable to that of the reference (quercetin, 90.13 ± 0.31%) (Table 3), while the extracts and compounds were determined with low DMPD scavenging effect, which was below 20%. In contrast, the extracts displayed remarkable scavenging properties toward NO radical, in which the aerial parts of *S. pisidica* (67.56 ± 0.75%), followed by *S. pseudolanata* (67.39 ± 1.88%), *S. argyrea* (66.42 ± 0.63%), and *S. kotschyi* (65.88 ± 1.23%) had the strongest effect (Table 3). Nevertheless, hyperoside (72.13 ± 2.71%) and rutin (70.13 ± 1.95%) exhibited higher NO scavenging effect than those of the extracts.

Table 3. Scavenging activity of the methanol extracts of *Scorzonera* species against DPPH, DMPD, and NO radicals at 1000 μg mL^{-1}.

Extracts	Plant parts	Radical scavenging activity (% ± SEM[a]) DPPH	DMPD	NO
S. acuminata	Aerial	43.08 ± 0.58****	12.85 ± 0.36****	59.12 ± 1.99****
	Root	11.56 ± 0.86****	6.81 ± 2.01****	47.40 ± 1.05****
S. argyrea	Aerial	47.85 ± 2.11****	12.08 ± 2.18****	66.42 ± 0.63****
	Root	16.79 ± 2.56****	7.71 ± 0.73****	37.64 ± 3.58****
S. aucheriana	Aerial	50.53 ± 2.83****	12.72 ± 1.27****	64.94 ± 0.34****
	Root	22.62 ± 1.94****	6.17 ± 0.36****	37.03 ± 3.25****
S. boissieri	Aerial	36.08 ± 2.16****	10.15 ± 0.55****	62.43 ± 0.38****
	Root	21.82 ± 2.91****	9.38 ± 2.36****	45.41 ± 2.07****
S. cana var. *alpina*	Aerial	36.01 ± 0.52****	14.01 ± 0.18****	54.56 ± 2.12****
	Root	26.33 ± 1.42****	17.87 ± 1.64****	48.55 ± 1.55****
S. cana var. *jacquiniana*	Aerial	11.25 ± 1.65****	15.30 ± 1.27****	63.60 ± 2.05****
	Root	24.45 ± 1.54****	8.23 ± 0.36****	53.26 ± 1.09****
S. cana var. *radicosa*	Aerial	28.01 ± 1.18****	10.80 ± 1.45****	46.30 ± 1.97****
	Root	12.01 ± 3.06****	6.43 ± 2.54****	40.23 ± 1.78****
S. cericea	Aerial	35.33 ± 1.44****	6.17 ± 1.09****	48.84 ± 0.52****
	Root	14.99 ± 3.03****	–[b]	34.37 ± 3.36****
S. cinerea	Aerial	37.32 ± 3.15****	11.05 ± 3.27****	59.02 ± 0.33****
	Root	15.84 ± 3.81****	2.19 ± 0.18****	39.87 ± 2.89****
S. elata	Aerial	37.04 ± 2.40****	12.47 ± 1.64****	61.18 ± 0.09****
	Root	17.91 ± 1.17****	6.81 ± 018****	33.88 ± 3.24****
S. ekimii	Aerial	48.96 ± 3.63****	10.78 ± 2.29****	60.91 ± 2.39****
	Root	24.56 ± 1.11****	6.74 ± 1.52****	46.91 ± 1.46****
S. eriophora	Aerial	34.21 ± 3.77****	10.28 ± 2.54****	64.10 ± 0.94****
	Root	9.10 ± 0.94****	3.34 ± 0.18****	29.89 ± 3.65****
S. gokceoglui	Aerial	37.25 ± 0.84****	8.23 ± 1.45****	57.56 ± 0.37****
	Root	26.42 ± 3.08****	4.50 ± 1.27****	44.02 ± 1.92****
S. incisa	Aerial	27.69 ± 2.54****	9.38 ± 0.91****	58.30 ± 2.77****
	Root	10.93 ± 2.11****	2.96 ± 1.27****	46.43 ± 1.14****
S. kotschyi	Aerial	61.53 ± 3.71****	16.32 ± 0.55****	65.88 ± 1.23****
	Root	19.66 ± 0.94****	8.74 ± 0.73****	53.04 ± 1.28****
S. lacera	Aerial	50.61 ± 2.18****	12.40 ± 1.52****	53.22 ± 4.32****
	Root	31.78 ± 1.39****	7.68 ± 1.33****	45.44 ± 3.02****
S. laciniata subsp. *laciniata*	Aerial	39.28 ± 3.32****	13.62 ± 0.36****	65.26 ± 1.41****
	Root	19.44 ± 0.19****	15.42 ± 0.73****	32.89 ± 3.59****
S. latifolia	Aerial	20.12 ± 3.13****	13.88 ± 0.36****	60.96 ± 1.61****
	Root	28.57 ± 3.35****	11.70 ± 0.55****	55.55 ± 0.80****
S. mirabilis	Aerial	37.38 ± 0.53****	8.35 ± 0.55****	60.06 ± 1.88****
	Root	14.39 ± 2.19****	5.66 ± 0.36****	31.86 ± 5.85****
S. mollis subsp. *szowitsii*	Aerial	36.38 ± 3.16****	11.57 ± 1.09****	54.98 ± 2.12****
	Root	11.70 ± 1.19****	2.83 ± 0.73****	39.87 ± 1.52****
S. parviflora	Aerial	28.49 ± 3.23****	11.32 ± 0.76****	49.14 ± 3.90****
	Root	26.45 ± 3.01****	9.30 ± 1.33****	52.16 ± 0.82****
S. pisidica	Aerial	84.55 ± 2.55****	16.98 ± 2.29****	67.56 ± 0.75****
	Root	20.29 ± 0.60****	7.68 ± 0.19****	47.48 ± 1.39****
S. pseudolanata	Aerial	44.64 ± 1.28****	11.70 ± 3.09****	67.39 ± 1.88****
	Root	6.45 ± 2.08****	6.68 ± 1.09****	45.49 ± 0.68****
S. suberosa	Aerial	29.25 ± 1.85****	14.14 ± 0.73****	60.56 ± 2.19****
	Root	4.55 ± 1.51****	3.73 ± 0.91****	44.96 ± 1.17****
S. suberosa subsp. *cariensis*	Aerial	58.93 ± 2.17****	11.31 ± 0.73****	61.09 ± 1.09****
	Root	47.26 ± 2.61****	13.37 ± 1.82****	52.95 ± 2.84****
S. sublanata	Aerial	40.65 ± 2.54****	11.95 ± 0.55****	57.08 ± 1.94****
	Root	29.49 ± 2.84****	5.40 ± 1.09****	44.45 ± 1.74****
S. tomentosa	Aerial	24.35 ± 0.33****	11.44 ± 2.73****	60.12 ± 1.71****
	Root	19.72 ± 4.10****	9.77 ± 2.18****	27.49 ± 3.84****
Compounds				
Chlorogenic acid		91.38 ± 0.37**	30.05 ± 0.19****	59.06 ± 2.58****
Hyperoside		85.77 ± 1.71	–	72.13 ± 2.71****
Scorzotomentosin-4-glucoside		2.47 ± 0.80****	–	26.83 ± 0.90****
Rutin		87.69 ± 0.59	17.39 ± 0.57****	70.13 ± 1.95***
References				
Quercetin (reference for DPPH-1000 μg mL^{-1})		90.13 ± 0.31		
Quercetin (reference for DMPD-2000 μg mL^{-1})			68.32 ± 0.67	
Gallic acid (quercetin, reference for NO-1000 μg mL^{-1})				93.12 ± 3.11

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.
[a]Standard error mean ($n = 3$).
[b]No scavenging activity.

The extracts and compounds tested were revealed to possess either no or low to moderate activity in metal-chelation capacity (4.51 ± 2.22–$33.28 \pm 3.67\%$), PRAP, and FRAP assays (Table 4). In fact, chlorogenic acid and hyperoside displayed higher FRAP values than the reference (quercetin).

Total phenol content of the methanol extracts was calculated according to the equation ($y = 1.4974\times \pm 0.1013$, $r^2 = 0.9996$) as chlorogenic acid equivalent (CAE, mg g^{-1} extract), while their total flavonoid contents were determined in accordance with the equation ($y = 2.3853\times \pm 0.0852$, $r^2 = 0.9993$) obtained by calibration curves as rutin equivalent (QUE, mg/g extract). The aerial parts of the screened *Scorzonera* species were found to contain higher total phenol and flavonoid amounts than those of the roots (Figures 1 and 2). *S. pisidica* was the richest extract in terms of total phenol content (376.12 ± 11.81 mg g^{-1}), followed by *S. kotschyi* (278.95 ± 5.67 mg g^{-1}) and *S. suberosa* subsp. *cariensis* (278.62 ± 2.36 mg g^{-1}). As rutin equivalent, the extracts appeared to have lower total flavonoid amounts as compared to their total phenol amounts. The extracts of *S. pseudolanata* (88.37 ± 5.93 mg g^{-1}) and *S. pisidica* (85.44 ± 4.08 mg g^{-1}) were revealed to be the richest ones in terms of total flavonoid content.

In our previous HPLC study on *Scorzenara* species (Küpeli Akkol et al., 2011), chlorogenic acid was detected in *S. acuminata, S. cinerea, S. cana* var. *alpina, S. cana* var. *jacquiniana, S. cana* var. *radicosa, S. incisa, S. laciniata* subsp. *laciniata, S. latifolia, S. mollis* subsp. *szowitsii, S. sublanata, S. parviflora*, and *S. tomentosa,* while hyperoside was found in *S. cana* var. *jacquiniana, S. cinerea, S. incisa*, *S. latifolia*, *S. mollis* subsp. *szowitsii*, *S. parviflora, S. sublanata*, and *S. tomentosa*, while the presence of rutin was shown in *S. acuminata, S. cana* var. *alpina, S. cana* var. *jacquiniana*, *S. incisa,* and *S. mollis* subsp. *szowitsii*.

Various *Scorzonera* species are used as food plants in addition to their utilization in traditional medicine. Nevertheless, our literature survey pointed out the fact that studies relevant to neuroprotective action of *Scorzonera* species are quite limited, which, therefore, directed us to perform the current study in order to understand probable effects of the selected *Scorzonera* species in central nervous system. Our results indicated that the extracts and compounds screened showed low to moderate inhibition of cholinesterase enzymes, whereas they afforded more promising outcomes in TYRO inhibitory assays, which could be beneficial in prevention of dopamine neurotoxicity in PD. Inhibition of TYRO is not only important for dopamine neurotoxicity but also imperative for enzymatic browning in foods as well as hyperpigmentation (Kim & Uyama, 2005). Since polyphenolic compounds have been shown to be more effective to inhibit TYRO, the marked inhibitory activity of the extracts against this enzyme could be presumably associated with their phenolic content. In fact, the number of hydroxyl groups in the phenolic compounds, which can make a hydrogen bond with the enzyme active site and cause lesser enzymatic activity, has been suggested to play critical role in inhibition of TYRO (Alam et al., 2011). In connection with these data, rutin and hyperoside were reported to have no inhibitory action on TYRO in some former studies (Kubo et al., 2000; Xue et al., 2011), which are in accordance with our data (Table 2). Nevertheless, no data have been available up to date on cholinesterase or TYRO inhibitory activity of scorzotomentosin-4-glucoside, a phenolic compound previously found in several *Scorzonera* species.

In fact, chlorogenic acid has been recently proposed to be a substrate for TYRO, rather than being an inhibitor (Kuijpers et al., 2012). Conversely, the chlorogenic acid-rich fractions of *Etlingera elatior* (Zingiberaceae) were demonstrated with a high anti-TYRO and antioxidant activity (Chan et al., 2011). In accordance with Chan et al. (2011), we herein disclosed a finding that the richest extracts in chlorogenic acid-equivalent total phenol amounts such as *S. pisidica*, *S. kotschyi*, and *S. pseudolanata* exerted higher TYRO-inhibiting effects. As we showed that chlorogenic acid has a marked ability ($40.25 \pm 0.82\%$) to inhibit TYRO, it could be conceivably contributing to anti-TYRO activity of the *Scorzonera* extracts tested herein.

Many studies have persuasively reported a strong connection between antioxidant activity and phenolic contents of the plants (Duan et al., 2007). In relation with this statement, the highest antioxidant activity was observed to occur in our extracts which are the richest in terms of total phenol amount such as *S. pisidica*, the richest extract in total phenol amount. Relevantly, it exerted the best radical scavenging activity against three radicals (DPPH, DMPD, and NO) (Table 3) as well as FRAP assay. *S. kotschyi*, the second richest extract in total phenol content, was also the most effective one against DPPH and DMPD radicals after *S. pisidica*. Only a few reports have described antioxidant activity of *Scorzonera* species. Among them, new quinic acid derivatives (feruloylpodospermic acids A and B) isolated from the aerial parts of *S. divaricata* were stated to have potent antiradical effect against DPPH (Tsevegsuren et al., 2007). In another study (Wang et al., 2009), scorzodihydrostilbenes A–E isolated from *S. radiata* were shown to exhibit stronger antioxidant activity than resveratrol in the DPPH assay. Similar results were also obtained with a number of quinic acid derivatives from *S. divaricata* roots with a marked antioxidant activity (Yang et al., 2013). Actually, in an earlier study of our group (Bahadir et al., 2010), antiradical activity of some *Scorzonera* species growing in Turkey was evaluated against DPPH and super oxide, in which *S. parviflora* was revealed to have higher activity than *S. suberosa* subsp. *suberosa* and *S. cinerea*. However, the extracts of *S. parviflora* had a moderate level of activity in our antioxidant assays. This could be due to obviously phytochemical differences in these plant samples collected from different localities.

According to present results, the root extracts possessed lesser activity in anti-TYRO and antioxidant assays than those of the aerial parts (Tables 2–4). Without any exception, the root extracts also contained no or lower amounts of total phenol and flavonoid (Figure 2), which might be a reason for their lesser activity in the mentioned assays.

Among the tested 54 extracts obtained from the aerial parts and roots of 27 *Scorzonera* species, the methanol extract of the aerial parts of *S. pisidica* particularly outshined with higher anti-TYRO and antioxidant activity.

Table 4. Metal-chelation capacity, phosphomolibdenum-reducing antioxidant power (PRAP) and ferric-reducing antioxidant power (FRAP) results of the methanol extracts of *Scorzonera* species tested at 1000 μg mL^{-1}.

Extracts	Plant parts	Metal-chelation capacity (chelation% ± SEM[a])	PRAP[b]	FRAP[c]
S. acuminata	Aerial	16.86 ± 0.76****	0.143 ± 0.001****	0.528 ± 0.011****
	Root	11.38 ± 2.90****	0.140 ± 0.001****	0.243 ± 0.009****
S. argyrea	Aerial	20.61 ± 1.06****	0.136 ± 0.003****	0.544 ± 0.010****
	Root	10.73 ± 3.58****	0.135 ± 0.014****	0.285 ± 0.028****
S. aucheriana	Aerial	6.16 ± 2.60****	0.139 ± 0.001****	0.586 ± 0.017****
	Root	–[d]	0.125 ± 0.007****	0.317 ± 0.088****
S. boissieri	Aerial	23.65 ± 2.67****	0.172 ± 0.020****	0.376 ± 0.037****
	Root	11.27 ± 0.51****	0.119 ± 0.004****	0.259 ± 0.009****
S. cana var. *alpina*	Aerial	30.76 ± 0.45****	0.135 ± 0.006****	0.390 ± 0.019****
	Root	21.79 ± 2.88****	0.146 ± 0.002****	0.295 ± 0.037****
S. cana var. *jacquiniana*	Aerial	–	0.142 ± 0.001****	0.491 ± 0.010****
	Root	–	0.126 ± 0.004****	0.341 ± 0.041****
S. cana var. *radicosa*	Aerial	24.46 ± 1.59****	0.141 ± 0.001****	0.342 ± 0.014****
	Root	11.94 ± 2.42****	0.149 ± 0.008****	0.212 ± 0.013****
S. cericea	Aerial	17.52 ± 2.25****	0.224 ± 0.009****	0.337 ± 0.026****
	Root	12.82 ± 2.60****	0.117 ± 0.011****	0.173 ± 0.015****
S. cinerea	Aerial	18.46 ± 2.19****	0.133 ± 0.008****	0.421 ± 0.037****
	Root	14.71 ± 3.05****	0.144 ± 0.004****	0.237 ± 0.009****
S. elata	Aerial	12.64 ± 1.78****	0.148 ± 0.003****	0.421 ± 0.002****
	Root	6.49 ± 1.67****	0.130 ± 0.001****	0.280 ± 0.003****
S. ekimii	Aerial	25.20 ± 2.77****	0.135 ± 0.008****	0.498 ± 0.020****
	Root	19.63 ± 0.24****	0.123 ± 0.003****	0.314 ± 0.008****
S. eriophora	Aerial	7.05 ± 1.08****	0.141 ± 0.001****	0.485 ± 0.009****
	Root	6.69 ± 2.93****	0.122 ± 0.001****	0.209 ± 0.008****
S. gokceoglui	Aerial	7.48 ± 0.80****	0.136 ± 0.002****	0.522 ± 0.012****
	Root	12.12 ± 2.40****	0.123 ± 0.013****	0.295 ± 0.002****
S. incisa	Aerial	12.08 ± 3.63****	0.127 ± 0.003****	0.427 ± 0.010****
	Root	4.53 ± 1.75****	0.140 ± 0.006****	0.217 ± 0.002****
S. kotschyi	Aerial	10.89 ± 2.95****	0.136 ± 0.010****	0.749 ± 0.035****
	Root	7.16 ± 2.98****	0.128 ± 0.006****	0.296 ± 0.009****
S. lacera	Aerial	33.28 ± 3.67****	0.131 ± 0.006****	0.570 ± 0.045****
	Root	14.26 ± 2.15****	0.130 ± 0.004****	0.347 ± 0.045****
S. laciniata subsp. *laciniata*	Aerial	12.55 ± 3.89****	0.142 ± 0.003****	0.436 ± 0.012****
	Root	7.35 ± 3.16****	0.122 ± 0.002****	0.290 ± 0.021****
S. latifolia	Aerial	–	0.154 ± 0.009****	0.596 ± 0.024****
	Root	12.33 ± 0.47****	0.130 ± 0.006****	0.454 ± 0.003****
S. mirabilis	Aerial	–	0.139 ± 0.001****	0.435 ± 0.012****
	Root	8.99 ± 2.87****	0.118 ± 0.001****	0.274 ± 0.003****
S. mollis subsp. *szowitsii*	Aerial	19.41 ± 2.96****	0.135 ± 0.007****	0.507 ± 0.015****
	Root	10.39 ± 0.14****	0.111 ± 0.001****	0.210 ± 0.006****
S. parviflora	Aerial	24.02 ± 3.05****	0.139 ± 0.003****	0.459 ± 0.122****
	Root	16.85 ± 1.75****	0.131 ± 0.001****	0.322 ± 0.008****
S. pisidica	Aerial	17.88 ± 3.08****	0.131 ± 0.009****	0.790 ± 0.012****
	Root	16.86 ± 1.94****	0.110 ± 0.003****	0.276 ± 0.023****
S. pseudolanata	Aerial	10.63 ± 1.20****	0.133 ± 0.003****	0.560 ± 0.015****
	Root	9.89 ± 3.20****	0.135 ± 0.001****	0.185 ± 0.006****
S. suberosa	Aerial	21.47 ± 5.32****	0.133 ± 0.002****	0.463 ± 0.002****
	Root	5.07 ± 0.55****	0.108 ± 0.001****	0.185 ± 0.029****
S. suberosa subsp. *cariensis*	Aerial	14.56 ± 2.77****	0.162 ± 0.002****	0.552 ± 0.032****
	Root	14.15 ± 3.35****	0.147 ± 0.008****	0.439 ± 0.055****
S. sublanata	Aerial	17.71 ± 1.60****	0.125 ± 0.001****	0.455 ± 0.012****
	Root	4.51 ± 2.22****	0.128 ± 0.001****	0.243 ± 0.024****
S. tomentosa	Aerial	16.37 ± 0.22****	0.140 ± 0.004****	0.515 ± 0.022****
	Root	–	0.132 ± 0.001****	0.365 ± 0.008****
Compounds				
Chlorogenic acid		17.55 ± 3.33****	0.114 ± 0.006****	1.768 ± 0.030
Hyperoside		8.06 ± 2.32****	0.188 ± 0.004****	1.716 ± 0.001
Scorzotomentosin-4-glucoside		13.65 ± 0.10****	0.095 ± 0.001****	0.120 ± 0.004****
Rutin		16.39 ± 1.65****	0.112 ± 0.004****	1.735 ± 0.022
References				
EDTA (reference for metal-chelation-2000 μg mL^{-1})		96.21 ± 0.13		
Trolox (reference for PRAP-2000 μg mL^{-1})			1.871 ± 0.012	
Quercetin (reference for FRAP-1000 μg mL^{-1})				1.491 ± 0.041

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.
[a]Standard error mean ($n = 3$).
[b]Absorbance at 600 nm (higher absorbance indicates higher antioxidant power).
[c]Absorbance at 700 nm (higher absorbance indicates higher antioxidant power).
[d]No activity.

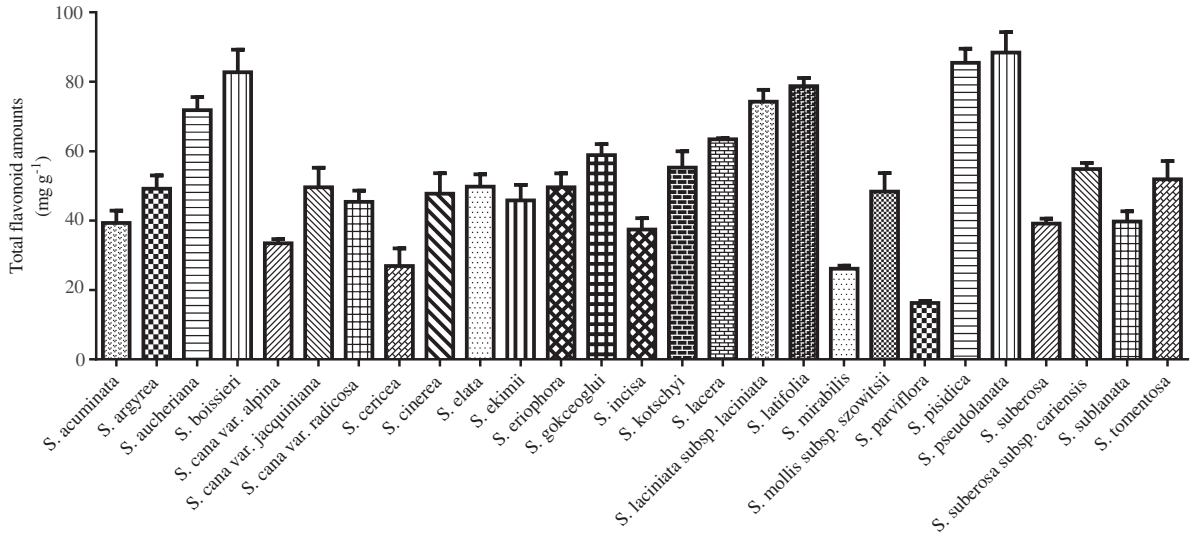

Figure 1. Total flavonoid amounts in the methanol extracts of the aerial parts of *Scorzonera* species as rutin equivalent (mg g^{-1} extract).

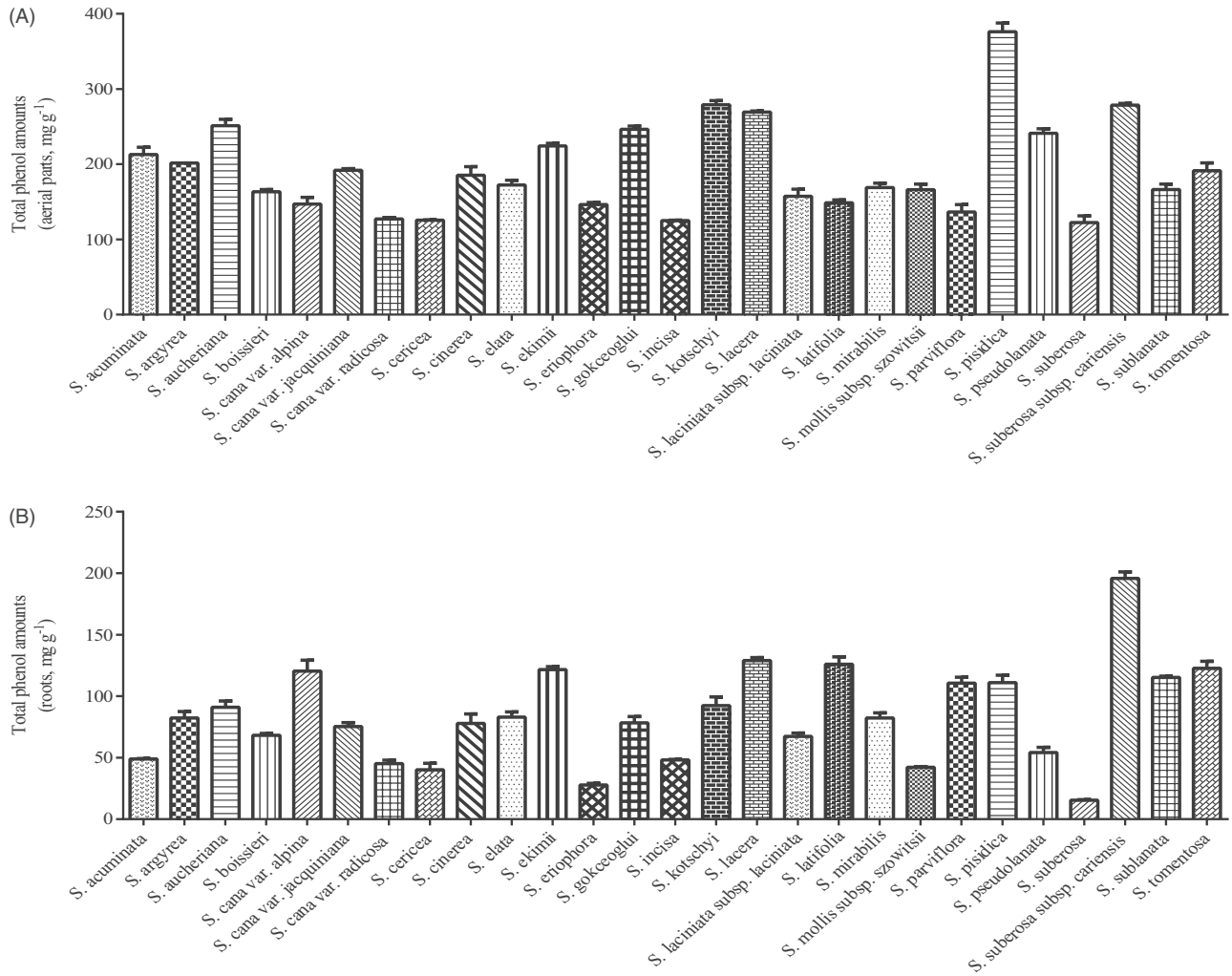

Figure 2. Total phenol amounts in the methanol extracts of the aerial parts (A) and roots (B) of *Scorzonera* species as chlorogenic acid equivalent (mg g^{-1} extract).

Among the tested compounds, chlorogenic acid, hyperoside, and rutin could be suggested to contribute antioxidant and TYRO inhibitory activity of the extracts screened. To the best of our knowledge, we herein disclose the first study on anticholinesterase, anti-TYRO, and antioxidant activity (except DPPH radical scavenging assay) of the aforementioned *Scorzonera* species.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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