Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(1); 59-65

ISSN: 0975-4873

Research Article

Assessment of Anti-Inflammatory and Free Radical Scavenger Activities of Selected Scorzonera Species and Determination of Active Components

Özlem Bahadır-Acıkara[1], *Esra Küpeli-Akkol[2], İpek Süntar[2], Burçin Ergene[1], Gülçin Saltan-Çitoğlu[1], Tülay Çoban[3]

[1]*Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara, Turkey*
[2]*Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Etiler 06330, Ankara, Turkey*
[3]*Ankara University, Faculty of Pharmacy, Department of Toxicology, 06100 Ankara, Turkey*

Available online: 15th February 2014

ABSTRACT

The aim of the present study is to evaluate anti-inflammatory and antioxidant activities of the S. latifolia, S. mollis ssp. szowitsii, S. suberosa, S. tomentosa and yakı sakızı. Carrageenan, PGE2 and serotonin- induced hind paw edema and 12-O-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema models were used to evaluate anti-inflammatory activity. Antioxidant capacities were measured using superoxide anion and DPPH radical scavenging methods. Chemical composition of the tested extracts was investigated qualitatively and quantitatively by using RP-HPLC method. S. latifolia, S. mollis ssp. szowitsii, and S. tomentosa exhibited notable inhibition in carrageenan-induced hind paw edema model. S. latifolia and S. tomentosa also showed potent activity against PGE2-induced hind paw edema model as well as in (TPA)-induced mouse ear edema model. All extracts were found to have scavenging activity against DPPH and superoxide anion radicals. Chlorogenic acid was detected as major compounds in yakı sakızı and all the species investigated. Hyperoside was determined as major constituents of aerial part extracts.

Keywords: anti-inflammatory activity; antioxidant activity; carrageenan; prostaglandin; Scorzonera; TPA

INTRODUCTION

Medicinal plants which have been utilized as medicines by humans to treat various diseases for thousand of years have gained an increasing popularity in recent years. Among the many methods used in attempts to discover new drugs, natural sources and especially medicinal plants represent a practically unexplored reservoir of potentially active metabolites not only as drugs, but also as lead structures. In Turkey, numerous plant species have been known for their therapeutic properties and have been used in traditional Turkish folk medicine to treat a wide range of diseases [1]. Among them, Scorzonera genus, which is mainly used as a vegetable in Turkey and in some European countries, has also ethnomedicinal importance in Turkish as well as in European, Chinese, Mongolian and Libyan folk medicines [2, 3]. In Turkish folk medicine members of this genus are used to treat a variety of illnesses, including arteriosclerosis, kidney diseases, hypertension, diabetes mellitus and rheumatism, as well as for pain relief and healing of different injuries [4, 5]. Antioxidant, analgesic, anti-inflammatory and wound healing activities of some Scorzonera species have been reported previously [2, 3, 6].

The present study is aimed to investigate the possible anti-inflammatory effects of both aerial parts and roots of S. latifolia, S. mollis ssp. szowitsii, S. suberosa, S. tomentosa which have not yet been investigated for their anti-inflammatory and antioxidant activities, collected from different regions of Anatolia and yakı sakızı, a traditional mastic prepared from the roots of S. latifolia, using in vivo experimental models, i.e., carrageenan-induced hind paw edema, PGE2-induced hind paw edema, serotonin-induced hind paw edema and 12-O-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema models to clarify the traditional usage of Scorzonera species in Turkish folk medicine. Moreover 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and superoxide anion scavenging methods were used for the evaluation of antioxidant capacities of plant samples. In order to determine the responsible compounds of extracts for mentioned activities, phytochemical screening was performed by validated HPLC method, that was developed and described previously, using some phenolic acid and flavonoid standards qualitatively as well as quantitatively [6]. According to the literature survey, no information exist for the mentioned activities of S. latifolia, S. mollis ssp. szowitsii, S. suberosa, S. tomentosa and yakı sakızı. Hence in current study we report anti-inflammatory and radical scavenging activities of stated Scorzonera species.

MATERIALS AND METHODS

*Author for correspondence: E-Mail: esrak@gazi.edu.tr

Table 1: Effect of the extracts from *Scorzonera* species aerial parts and roots on carrageenan-induced paw edema model in mice

Material	Parts used	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)±SEM (Inhibition %)			
			90 min	180 min	270 min	360 min
Control			45.8 ±3.4	55.2±3.2	56.1±3.0	60.9±3.7
S. latifolia	AE	100	38.7±2.4 (15.5)	41.0±3.9 (25.7)*	39.6±2.8 (29.4)**	44.1±2.7 (27.6)**
	R	100	48.3±3.5	56.2±3.4	59.2±2.9	61.3±2.6
S. mollis ssp. *szowitsii*	AE	100	44.6±3.5 (2.6)	53.2±3.9 (3.6)	42.4±3.2 (24.4)*	51.2±2.9 (15.9)
	R	100	42.8±3.3 (6.6)	56.2±3.8	51.1±3.9 (8.9)	59.0±3.3 (3.1)
S. suberosa ssp. suberosa	AE	100	40.8±3.9 (10.9)	48.1±3.1 (12.9)	50.1±3.6 (10.7)	53.8±3.1 (11.7)
	R	100	41.2±3.5 (10.0)	55.3±4.1	55.0±3.6 (1.9)	66.8±4.2
S. tomentosa	AE	100	39.3±3.7 (14.2)	52.4±2.7 (5.1)	41.6±2.6 (25.8)*	53.1±2.5 (12.8)
	R	100	40.9±4.2 (10.7)	45.3±2.9 (17.9)	46.4±3.5 (17.3)	52.9±2.8 (13.1)
Yakı sakızı		100	41.6±3.0 (9.2)	48.7±2.6 (11.8)	50.6±3.3 (9.8)	55.4±2.5 (9.03)
Indomethacin		10	33.3±2.2 (27.3)*	39.2±2.0 (28.9)**	35.1±1.9 (37.4)**	35.3±2.1 (42.0)***

*AE: Aerial part, R: Root, S.E.M.: Standard error meaning * p<0.05. **p<0.01. *** p<0.001 significant from the control*

Plant material: S. latifolia (Fisch. & Mey.) DC. (AEF 23830), S. mollis Bieb. ssp. szowitzii (DC.) Chamb. (AEF 23844), S. suberosa C. Koch ssp. suberosa (AEF 23843) and S. tomentosa L. (AEF 23841) were collected from Kars (Arpaçay), Ankara (Kızılcahamam), Kayseri (Pınarbaşı) and Yozgat (Akdağmadeni) respectively. Taxonomic identification of the plants were confirmed by H. Duman, A.M. Özkan Gençler and M. Koyuncu. Voucher specimens were kept in the Herbarium of Ankara University, Faculty of Pharmacy. Yakı sakızı was obtained from Van, local market.

Extraction of plant material: Dried and powdered aerial parts and roots of the plant were extracted with 20% aqueous methanol (100 ml) at room temperature for 3 h by continuous stirring separately. Each extract was filtered and concentrated to dryness under reduced pressure and low temperature (40-50 °C) on a rotary evaporator to give crude extracts. The yields of the plant materials were as follows (w/w): S. latifolia (AE) 32.09%, (R) 43.48%; S. tomentosa (AE) 17.45%, (R) 45.35%; S. mollis ssp. szowitzii (AE) 36.64%, (R) 37.66% and S. suberosa ssp. suberosa (AE) 36.64%, (R) 31.31%.

HPLC analysis: The HPLC analysis was carried out according to the method of Küpeli Akkol et al. [6].

Animals: Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratory of Saki Yenilli (Ankara, Turkey). The animals left for two days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but free access to water was allowed. A minimum of six animals was used in each group. The study was permitted by the Institutional Animal Ethics Committee (Gazi University Ethical Council Project Number: G.U.ET-11.025) and was performed according to the international rules considering the animal experiments and biodiversity right.

Preparation of test samples for bioassay: All extracts were administered in 100 mg/kg doses after suspending in 0.5 % sodium carboxymethylcellulose (CMC) suspension in distilled water. The control group animals received the same experimental handling as those of the test groups except the drug treatment was replaced with appropriate volumes of dosing vehicle. Indomethacin (10 mg/kg and 0.5 mg/ear) in 0.5 % CMC was used as reference drug.

Carrageenan-induced hind paw edema model: After 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of carrageenan (0.5 mg/25 µl) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 µl saline solutions were injected into that of the left hind paw. Paw edema was then measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with those of a control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug [7].

PGE2-induced hind paw edema model: PGE2-induced hind paw edema model which was described by Kasahara et al. [8] was used. As given 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of PGE2 (5 µg/5 µl) in Tyrode's solution into subplantar tissue of the right hind paw. As the control, 5 µl Tyrode's

Table 2: Effect of the extracts from *Scorzonera* species aerial parts and roots on PGE_2-induced paw edema in mice

Material	Parts used	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)± SEM (Inhibition)						Ratio of ulceration
			0 min	15 min	30 min	45 min	60 min	75 min	
Control			2.3±0.7	13.5±1.1	19.7±1.9	15.6±1.7	10.6±2.3	8.8±1.5	0/6
S. latifolia	AE	100	2.1±0.9 (8.7)	12.1±1.4 (10.4)	13.5±1.3 (31.5)**	10.4±1.2 (33.3)**	9.1±1.9 (14.2)	7.6±1.9 (13.6)	0/6
	R	100	2.2±0.6 (4.4)	11.5±1.7 (14.8)	17.7±1.5 (10.2)	14.9±1.4 (4.5)	9.8±2.1 (7.6)	7.9±1.4 (10.2)	0/6
S. mollis ssp. *szowitsii*	AE	100	1.9±0.8 (17.4)	10.9±1.5 (19.3)	15.6±1.8 (20.8)	14.4±1.9 (7.7)	9.8±1.7 (7.5)	9.0±1.8	0/6
	R	100	2.0±0.8 (13.0)	11.9±1.5 (11.9)	15.2±1.1 (22.8)	12.9±1.2 (17.3)	9.2±2.4 (13.2)	8.0±1.3 (9.1)	0/6
S. suberosa ssp. suberosa	AE	100	2.5±0.8 -	13.2±1.9 (2.2)	18.6±2.3 (5.6)	15.7±1.8 -	11.8±2.5 -	9.9±1.6 -	0/6
	R	100	2.7±0.7 -	13.3±1.6 (1.5)	19.8±2.0 -	15.9±2.2 -	12.5±2.8 -	8.9±1.9 -	0/6
S. tomentosa	AE	100	2.1±0.6 (8.7)	10.2±1.3 (24.4)	14.7±1.2 (25.4)*	1.2±1.0 (28.2)**	8.1±2.1 (23.6)*	7.9±1.4 (10.2)	0/6
	R	100	3.1±1.1 -	14.0±1.9 -	20.4±2.2 -	16.3±1.8 -	12.2±2.7 -	9.1±1.3 -	0/6
Yakı sakızı		100	2.2±1.5 (4.3)	13.8±1.5 -	19.9±1.7 -	17.0±2.1 -	10.7±2.8 -	10.6±1.7 -	0/6
Indomethacin		10	1.9±0.4 (17.4)	11.0±1.0 (18.5)	11.58±1.1 (41.2)***	8.6±0.9 (44.9)***	7.2±1.6 (32.1)**	7.8±0.9 (11.4)	3/6

*AE: Aerial part, R: Root, S.E.M.: Standard error meaning, * p<0.05. **p<0.01. *** p<0.001 significant from the control*

Table 3: Effect of the extracts from *Scorzonera* species aerial parts and roots on serotonin-induced paw edema in mice

Material	Part used	Dose mg/kg	Swelling thickness (x 10^{-2}mm)± S.E.M. (Inhibition%)					
			0 dk	6 dk	12 dk	18 dk	24 dk	30 dk
Control			3.9±0.5	7.4±1.3	11.5±1.1	18.5±0.8	21.6±1.2	22.9±1.8
S. latifolia	AE	100	4.5±0.5	8.4±1.5 -	10.2±1.8 (11.3)	17.8±1.5 (3.8)	18.4±1.9 (14.8)	20.3±1.6 (11.4)
	R	100	4.2±0.9	7.9±1.2	11.9±1.4	19.2±1.1	20.4± 0.9 (5.6)	25.5±1.4
S. mollis ssp. *szowitsii*	AE	100	3.7±0.3 (5.1)	7.6±1.9 -	13.4±1.9 -	17.0±1.8 (8.1)	18.6±1.6 (13.9)	19.6±1.4 (14.4)
	R	100	5.1±0.8	7.7±1.8	11.6±1.3	17.9±1.5 (3.2)	25.4± 1.1	26.1±1.9
S. suberosa	AE	100	3.8±0.7 (2.6)	7.3±0.5 (1.4)	10.5±1.5 8.7	15.5±1.6 (16.2)	17.6±1.1 (18.5)	19.2±1,5 (16.2)
	R	100	4.5±0.7	7.5±1.4	11.8±1.5	23.0±1.3	21.7± 1.5	23.2±1.6
S. tomentosa	AE	100	3.6±0.6 (7.7)	7.9±1.6 -	11.7±1.7 -	16.7±1.9 (9.7)	19.8±1.5 (8.3)	18.4±1.2 (19.7)
	R	100	4.9±0.6	8.1±1.3	11.9±1.6	21.0±1.0	20.1± 1.4 (6.9)	22.2±2.1 (3.1)
Yakı sakızı		100	3.5±0.9 (10.3)	8.5±1.7 -	9.5±1,6 (17.4)	16.4±1.7 (11.4)	20.7±1.8 (4.2)	23.6±1.3
Indomethacin		10	4.0±0.7 -	6.8±0.8 (8.1)	10.1±1.8 (12.2)	13.1±0,6 (29.2)*	14.9±1.7 (31.0)**	15.3±1.4 (33.2)**

*AE: Aerial part, R: Root, S.E.M.: Standard error meaning, * p<0.05. **p<0.01. *** p<0.001 significant from the control*

solution was injected into that of the left hind paw. Paw edema was measured in every 15 min during a period of 75 min after the induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and statistically analyzed. Indomethacin (10 mg/kg) was used as reference drug.

Table 4: Effect of the extracts from *Scorzonera* species aerial parts and roots against TPA-induced ear edema in mice as measurement swelling thickness and weight measurement of edema

Test samples	Part used	Dose (mg/ear)	Swelling thickness (μm) ± SEM	Inhibition %	Weight edema (mg) ± SEM	Inhibition %
Control			291.4 ± 35.4		25.3 ± 4.0	
S. latifolia	AE	0.5	136.1 ± 19.5	53.3***	16.1 ± 2.6	36.4*
	R	0.5	286.7± 29.8	1.6	27.0 ± 5.2	-
S. mollis ssp. *szowitsii*	AE	0.5	156.0 ±19.6	46.5**	18.2 ± 1.2	28.1
	R	0.5	304.8± 37.9	-	29.6 ± 4.8	-
S. suberosa	AE	0.5	285.2 ± 21.7	2.1	23.2 ± 1.8	8.3
	R	0.5	292.6± 27.5	-	30.4 ± 5.7	-
S. tomentosa	AE	0.5	142.8 ± 17.6	50.9***	14.6 ± 1.8	42.3**
	R	0.5	277.4± 28.3	4.8	26.3 ± 3.6	-
Yakı sakızı		0.5	285.6 ± 28.4	-	24.3 ± 2.2	3.9
Indomethacin		0.5	73.4 ± 5.2	74.8***	10.5 ± 0.7	58.5***

AE: Aerial part, R: Root, S.E.M.: Standard error meaning
**: p<0.05, **: p<0.01, ***: p<0.001 significant from control*

Serotonin- induced hind paw edema model: The method of Kasahara et al. [8] was used. Sixty minutes after the oral administration of test sample or dosing vehicle each mouse was injected with serotonin (serotonin creatinin sulfate, Merck, Art. 7768) in Tyrode's solution (0.5 g/5 l) into subplantar tissue of the right hind paw and 5 l of Tyrode's solution into that of the left as secondary control. Measurements were done and evaluated as described above in every 6 min during 30 min.

TPA-induced mouse ear edema: Each mouse received 2.5 g of TPA (12-O-tetradecanoylphorbol 13-acetate) dissolved in 20 l of EtOH 70 % [9]. This was applied by an automatic pipette in 20 l volumes to both anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of solvent (EtOH 70%), simultaneously with TPA. Indomethacin (0.5 mg/ear) was used as reference drug. For the evaluation of the activity, two different measurements were taken as given below.

The thickness of each ear was measured 4 h after induction of inflammation using a gauge calipers (Ozaki Co., Tokyo, Japan). The edema was expressed as the difference between the right and left ears due to TPA application and consequently inhibition percentage was expressed as a reduction thickness with respect to the control group. After 4 h of the administration the animals were killed under deep ether anesthesia. Discs of 6 mm diameter were removed from each ear and weighed in balance. The swelling was estimated as the difference in weight between the punches from right and left ears and expressed as an increase in the ear thickness.

Acute toxicity: Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded, if happens, for each group at the end of observation period.

Gastric-ulcerogenic effect: After the employment of PGE2-induced hind paw edema model, mice were killed under deep ether anesthesia and the stomachs of each mouse were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleedings.

STATISTICAL ANALYSIS OF DATA

Data obtained from animal experiments were expressed as the mean standard error (±SEM). Statistical differences between the treated and the control groups were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. $p<0.05$ was considered to be significant (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Chemicals antioxidant and radical scavenging properties: Ascorbic acid, xanthine, xanthine oxidase, cytochrome c, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene, and a-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO).

Superoxide radical scavenging assay: Enzymatic formation of superoxide anions was assayed by reduction of cytochrome C as described by McCord and Fridovich [10]. The incubation mixture (1.0 ml, total volume) consisted of phosphate buffer (pH= 8.9, 0.1M), xanthine (50 mm), cytocrome C (50 mm), xanthine oxidase (0.32 units/ml) and 100 ml test samples. The reaction was started by addition of xanthine oxidase and was conducted at 30 °C in a heating block. The absorbance was measured spectrophotometrically at 550 nm for cytochrome c reduction. IC50 values were determined from a calibration curve.

DPPH radical scavenging assay: DPPH assays were performed using test compounds purified isolates as previously described by Blois [11]. Test samples were dissolved in DMSO and mixed with methanol solutions of DPPH (100 mM) in 96-well micro titer plates, following incubation at 37°C for 30 min. DPPH reduction was estimated at 517 nm. For each test sample, different concentrations were tested. Final concentrations of test materials were typically in a range from 31.25 to 250 µM. Percentage inhibition by the sample treatment was determined by comparison with a DMSO-treated control group. All experiments were carried out in triplicate. The antioxidant activity of each test compound was expressed as an IC50 value ± SD, i.e. the concentration in µM that inhibits DPPH absorption by 50%, and was calculated by linear regression analysis. BHT was used as a positive control and its IC50 value was found to be 95 ± 3.5µM.

The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Radical scavenging activity (%) = ((A0 – A1 / A0) ×100)

Where A0 is the absorbance of the control (blank, without compound) and A1 is the absorbance of the compound.

RESULTS AND DISCUSSION

Aerial part extracts of S. latifolia and S. tomentosa provided remarkable anti-inflammatory activity ranging between 15.5-29.4%, and 5.1-25.8%, respectively, against carrageenan-induced hind paw edema model at the dose of 100 mg/kg and the results were quite comparable to indomethacin (27.3-42.0% inhibition) (Table 1). Moreover, aerial parts of S. mollis ssp. szowitsii showed significant anti-inflammatory effect (2.6-24.4%). However, the extract of S. suberosa, yakı sakızı and all of the root extracts did not show any anti-inflammatory activity in this model.

The carrageenan induced paw edema is a biphasic event, involves several chemical mediators such as histamine, serotonin, bradykinin, and prostaglandins. In this inflammatory reaction mast cell amines play a minor role [12]. The early phase (90-180 min) of the inflammation is due to the release of histamine, serotonin and similar substances. The later phase (270-360 min) is associated with the activation of kinin-like substances, i.e. prostaglandins, proteases and lysosome [13]. In order to test the effect of the extracts on the prostaglandin synthesis, they were also studied using prostaglandin E2-induced hind paw edema model. S. latifolia (8.7-33.3%) and S. tomentosa (8.7-28.2%) aerial part extracts displayed remarkable activity against this model at dose of 100 mg/kg (Table 2). Serotonin-induced hind paw edema model was also employed. As shown in Table 3 test samples did not show any remarkable effect.

Mouse ear edema induced with TPA is an acute inflammation animal model, closely related to the infiltration of macrophages and neutrophils, the induction of TNF-α and IL-1 as well as the generation of ROS. Hence, can be very useful short-term test to detect the agents which have anti-arthritic potential [14]. The mechanism of TPA-induced inflammation is suggested to be dependent mainly on leukotrienes (LT), which are synthesized by the lipoxygenases [15]. According to Furstenberger et al. [16] TPA strongly increases the epidermal content of the cysteinyl LTs, LTC4, LTD4, and LTE4 in mouse skin. This model was used to test the effect of extracts on lipoxygenase metabolites. A similar activity pattern was observed in TPA-induced ear edema model, the aqueous methanolic extract of aerial part from S. latifolia (53.3% and 36.4%) , S. mollis ssp. szowitsii (46.5% and 28.1%) and S. tomentosa (50.9% and 42.3%) displayed potent anti-inflammatory activity as measurement of edema weight as well as swelling thickness, compared to indomethacin (74.8% and 58.5%), respectively (Table 4).

As shown in Table 5, all the tested extracts of Scorzonera were found to possess antioxidant activities both in DPPH and superoxide anion radical scavenging methods. Roots extract of S. latifolia exhibited scavenging activity significantly against superoxide anion with an IC50 value of 2.5 mg/ml and this is followed by S. tomentosa root extract with 5.25 mg/ml of IC50 value. On the other hand in DPPH radical scavenging activity test, S. latifolia roots extract displayed the highest scavenging activity (IC50=35 µg/ml) (Table 5). S. latifolia aerial part extract was also found to have the highest antioxidant capacity against DPPH and superoxide anion radicals by 68 µg/ml and 3.5 mg/ml IC50 respectively (Table 5).

Results of the HPLC analysis have revealed that all the species tested contain chlorogenic acid and the highest amount was determined in S. latifolia roots (1246.78±3.20 µg/mg). Aerial parts of the S. latifolia, S. tomentosa and S. mollis ssp. szowitsii were found to have hyperoside in varying amounts. Rutin was detected only in the aerial parts of S. mollis ssp. szowitsii. S. suberosa ssp. suberosa aerial parts and yakı sakızı as well as all the root samples were found to be absent from whole investigated flavonoids [6].

Cyclooxygenase and lipoxygenase are the inflammatory mediators, involved in the release of arachidonic acid, which is a starting point of inflammatory response. It has been demonstrated that flavonoids are able to inhibit both the cyclooxygenase and 5-lipoxygenase pathways [17, 18]. This inhibition reduces the release of arachidonic acid and the formation of these inflammatory metabolites [19, 20]. Prostaglandins and nitric oxide biosynthesis is also involved in inflammation. In vitro studies have confirmed that the flavonoids inhibit nitric oxide production and the expression of iNOS [21]. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase [22]. Another anti-inflammatory feature of flavonoids is to inhibit neutrophil degranulation, which is a direct way to decrease the release of arachidonic acid by neutrophils and other immune cells [23, 24].

Anti-inflammatory activity is also related, in part, to antioxidant activity. It is well known that inflammation is characterized largely by the synthesis and release of large amounts of reactive species through the activation of phagocytes. Although the production of reactive oxygen species (ROS) occurs in all aerobic organisms as part of normal metabolic processes, it may increase dramatically as a result of chemical ionization, UV radiation, or enzymatically, in the case of inflammation processes [25]. The overproduction of reactive species causes lipid peroxidation at membranes and tissue injury by damaging cellular biomolecules such as nucleic acids, proteins and carbohydrates. ROS and reactive nitrogen species (RNS) also stimulate inflammation through the release of cytokines, which induce the recruitment of additional neutrophils and macrophages [25, 26, 27]. Recent studies have revealed that the usage of suitable antioxidants reduced the adverse effects of pain and excessive inflammation either by preventing the formation of oxygen free radicals or by scavenging them before they react with sites such as unsaturated lipids in the cell membrane [28, 29]. Thus, compounds that are able to scavenge these radicals and/or suppress lipid peroxidation may be expected to have therapeutic potential in treating various

Table 5. IC_{50} values of *Scorzonera* species aerial part and root extracts in DPPH and superoxide anion radical scavenging

Test samples	Part used	DPPH scavenging activity (IC 50 µg/ml)	Superoxide scavenging capacities (IC50 mg/ml)
S. latifololia	AE	68 ± 7	3.5 ± 0.5
	R	35 ± 0.5	2.5 ± 0.2
S. mollis ssp. *szowitsii*	AE	80 ±5	3.8 ± 0.3
	R	138 ± 2.5	7.0 ± 0.5
S. suberosa	AE	88 ±4	4.0 ± 0.4
	R	154 ± 2.0	6.9 ± 0.5
S. tomentosa	AE	99 ±5	3.7 ± 0.4
	R	58 ± 1.0	5.25 ± 0.4
Vitamin E		13 ± 0.5	0.37 ± 0.05

AE: Aerial part, R: Root

diseases including inflammatory diseases. The beneficial effects of antioxidants for human health maintenance and illness prevention have been revealed by epidemiological and experimental studies. Antioxidant compounds belong to various classes of compounds such as flavonoids, carotenoids, phenolic acids and other polyphenols are found primarily in fruits, vegetables, spices as well as both edible and non-edible plants. Therefore there is a great deal of interest to antioxidant as well as anti-inflammatory potential of plant[25, 26, 27].

S. latifolia, S. mollis ssp. szowitsii and S. tomentosa aerial part extracts displayed significant anti-inflammatory activity in mice at 100 mg/kg dose without inducing any gastric damage. Direct correlation couldn't find between the antioxidant capacities and the anti-inflammatory activities of Scorzonera species. However it could be suggested that anti-inflammatory activities of Scorzonera species, at least partly, related to their antioxidant activities.

In conclusion, this study confirms the anti-inflammatory activity of S. latifolia, S. mollis ssp. szowitsii and S. tomentosa that might be attributed, at least in part, to the presence of phenolic compounds and especially flavonoids which are potent radical scavengers. In current study, as we mentioned in our earlier study [6], anti-inflammatory activity of the S. latifolia, S. mollis ssp. szowitsii and S. tomentosa appears to be related with their flavonoid content. Discrepancies in the anti-inflammatory as well as antioxidant potential observed for these Scorzonera species could be due to the different chemical composition of the plants. However, further studies are in progress in order to clarify the bioactive principles responsible for these activities.

Conflict of Interest: The authors declared that there is no conflict of interest.

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