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Original article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF SELECTED *SCORZONERA* SPECIES

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Abstract

Scorzonera species (Asteraceae) are mainly used as vegetables and medicinal plants in Europe and in Turkey. Current study is aimed to evaluate antioxidant capacities of Scorzonera species and characterize the phytochemical content of tested extracts to determine the responsible compounds. Superoxide anion scavenging method was used to determine antioxidant activities. Chemical composition of the tested extracts was also investigated by RP-HPLC method using phenolic acid and flavonoid standards. All extracts exhibited significant scavenger activity against superoxide anion radical. The highest inhibitory activity was observed with S. parviflora root extract with IC_{50}=2.25 mg/mL value. Hyperoside and rutin were found to be in the extracts from the aerial parts and chlorogenic acid was detected in all the extracts investigated.

Key words: *Asteraceae, Scorzonera, Antioxidant activity, Chlorogenic acid, Hyperoside*

Bazı *Scorzonera* Türlerinin Fitokimyasal Analizleri ve Antioksidan Etkileri

Scorzonera (Asteraceae) türleri başlıca sebze ve tıbbi bitki olarak Avrupa ve Türkiye'de kullanılmakta olan türlerdir. Bu çalışma Scorzonera türlerinin antioksidan kapasitelerini değerlendirmeyi ve etkiden sorumlu bileşiklerin belirlenebilmesi için ekstrelerin fitokimyasal içeriklerinin karakterize edilmesini amaçlamaktadır. Ekstrelerin antioksidan aktivitesini belirlemek için süperoksit anyon radikaline karşı süpürücü etki modeli kullanılmıştır. Ekstrelerin kimyasal bileşimi ters faz yüksek basınçlı sıvı kromatografisi (YBSK) ile fenolik asit ve flavonoit standartları kullanılarak analiz edilmiştir. En yüksek etki IC_{50}=2.25 mg/mL değeri ile S. parviflora kök ekstresinde gözlenmiştir. Hiperozit ve rutin toprak üstü kısımlarından hazırlanan ekstrelerde, klorojenik asit ise araştırılan tüm ekstrelerde tespit edilmiştir.

Anahtar kelimeler: *Asteraceae, Scorzonera, Antioksidan aktivite, Klorojenik asit, Hiperozit*

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INTRODUCTION

In all aerobic organisms, reactive oxygen species (ROS) occur as a part of normal metabolic processes, however they may increase dramatically from external sources such as exposure to X-rays, ozone, smoking, air pollutants, industrial chemicals and in the case of inflammation processes (1-3). ROS play a causative role in the pathogenesis of various diseases including heart diseases, aging, cancer, inflammation, diabetes and others by inducing oxidation of lipids, sugars, proteins as well as DNA and this may result in oxidative damage such as membrane dysfunction, protein modification, enzyme inactivation, breakage of DNA strands and modification of its bases (4-7). The human body has several mechanisms of defense against free radicals and other reactive oxygen species. One important line of defense is a system of enzymes, including glutathione peroxidase, superoxide dismutase and catalase. Some of small-molecular-weight compounds (glutathione, ubiquinol and uric acid) produced during normal metabolism in the body is also another line of defense. Furthermore there are other small-molecular-weight compounds, known as naturally occurring antioxidants (vitamin E, vitamin C, carotenoids, phenolic compounds) which are found in the diet. The body cannot manufacture these micronutrients, so they have to be supplied in the diet (3, 8, 9). Naturally occurring antioxidants belonging to various classes of compounds including flavonoids, phenolic acids, tannins, anthocyanins, carotenoids and others are primarily found in fruits, vegetables, spices and both edible and non-edible plants (5, 10-14).

Scorzonera species belonging to Asteraceae family mainly used as vegetables in Turkey as well as in Europe (15, 16). *Scorzonera hispanica*, growing naturally and widely in Europe, has been cultivated since the seventeenth century as a food from this genus. Roots of the plant consumed after cooked and the young leaves used in salads (17-19). *S. mollis* Bieb. (*goftigoda*), *S. suberosa* C. Koch (*wild carrot*), *S. cana* (*karakök, tekesakalı*) and *S. latifolia* (Fisch. and Mey.) DC. (*geniş yapraklı karakök* or *mesdek*) are some of the species mainly used as a vegetable in Turkey. Both their roots and green buds are edible (20, 21). This genus plants were also employed as medicinal herbs in Turkish additionally in European, Chinese and Mongolian folk medicines for different purposes (18-20, 22). In Turkish folk medicine *Scorzonera* species are used to treat arteriosclerosis, kidney diseases, hypertension, diabetes mellitus, rheumatism and for pain relief as well as wound healing (20, 23).

In our previous studies on *Scorzonera* genus antinocieptive, anti-inflammatory and wound healing activities of some species have been evaluated and promising results have been obtained for further studies (24-29).

Current study aims to evaluate antioxidant capacities of some *Scorzonera* species by using superoxide anion scavenging method. Furthermore content of the tested extracts was compared qualitatively and quantitatively by HPLC analyses.

EXPERIMENTAL

Plant material

Flowering plants of *Scorzonera* species were collected from different parts of Turkey. The taxonomic identification of these plants was confirmed by H. Duman, in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. Voucher specimens were kept in the herbarium of Ankara University, Faculty of Pharmacy (AEF) (Table 1).

Table 1. Locality of the plant materials

Plant Species	Locality	AEF No
S. cana (C.A. Meyer) Hoffm. var. *alpina* (Boiss.) Chamb. (SCVA)	Tokat, Akdelen	25893
S. cana (C.A. Meyer) Hoffm. var. *jacquiniana* (W. Koch) Chamb. (SCVJ)	Ankara, Çamlıdere	23834
S. cana (C.A. Meyer) Hoffm. var. *radicosa* (Boiss.) Chamb. (SCVR)	Erzurum, Kop Geçidi	25897
S. cinerea Boiss. (SC)	Sivas, Çetinkaya	23829
S. eriophora DC. (SE)	Ankara, Çubuk	23832
S. incisa DC. (SI)	Konya, Ermenek	23833
S. laciniata L. ssp. *laciniata* (SLSL)	Ankara, Çamlıdere	23835
S. parviflora Jocq. (SP)	Ankara, Gölbaşı	25894

Preparation of the extracts

Dried and powdered aerial parts and roots of the plant were extracted with 80% 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) using a rotary evaporator to give crude extracts. The yield of the extracts are determined as 29.61 % and 30.22 % for *S. cana* var. *alpina*; 40.51 % and 39.24 % for *S. cana* var. *jacquiniana*; 26.39 % and 41.34 % for *S. cana* var. *radicosa*; 22.78 % and 27.87 % for *S. cinerea*; 25.31 % and 43.50 % for *S. eriophora*; 31.85 % and 22.22 % for *S. incisa*; 33.73 % and 45.17 % for *S. laciniata* ssp. *laciniata*; 39.11 % and 45.14 % for *S. incisa* respectively for aerial parts and roots.

Chemicals

Ascorbic acid, xanthine, xanthine oxidase, cytochrome C, and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO).

Antioxidant and Radical Scavenging Properties

Superoxide radical scavenging assay

Enzymatic formation of superoxide anions was assayed by reduction of cytochrome C as described by McCord and Fridovich (30). The incubation mixture (1.0 mL, total volume) consisted of phosphate buffer (pH= 8.9, 0.1M), xanthine (50 mμ), cytocrome C (50 mμ), xanthine oxidase (0.32 units/mL) and 100 μL 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. IC_{50} values were determined from a calibration curve.

HPLC analysis

The HPLC analysis of *Scorzonera* species was carried out according to the Akkol et al. (27). As described previously this HPLC method was developed and validated to analyse phenolic acids including *p*-coumaric acid, ferulic acid, rosmarinic acid, caffeic acid, chlorogenic acid and flavonoids such as apigenin, luteolin, quercetin, rutin, hyperoside, hesperidin in the extracts from the roots and aerial parts of *Scorzonera* species. Agilent LC 1100 model chromatograph (Agilent Technologies, California, USA) was used and diode array detector (DAD) was set at wave length 254 nm. Separation was carried out using a Supelcosil (250 x 4.6 mm; 5μm) column. The mobile phase was made up of acetonitrile (A) and water (B) in gradient elution: initial 0 min, A-B (8:92, v/v), then 0-10 min, linear change from A-B (8:92, v/v) to A-B

(18:82), 10-20 min, there is also linear change from A-B (18:82) to A-B (20:80) and the linear gradient elution is from A-B (20:80) to A-B (30:70) with the range of 20-30 min. This was followed by A-B (30:70) from 30 min to 45 min. The flow rate was 0.5 ml/min and column temperature was maintained at 35 °C. The sample injection volume was 10 µL (27).

RESULTS AND DISCUSSION

In the current study, the extracts from the roots and the aerial parts of eight different *Scorzonera* species growing in Turkey were examined for their antioxidant activities using superoxide anion scavenging method. Table 2 shows the inhibitory effects of the aerial part and root extracts on superoxide anion radical respectively. The scavenging effects of the plant extracts were examined at three different concentrations (2.5, 5, and 10 mg/mL). All the tested extracts of *Scorzonera* species were found to possess significant antioxidant activities against superoxide anion radical. The extracts of *Scorzonera* aerial parts and roots displayed significant anti-superoxide anion formation with IC_{50} values ranged from 3.0 to 6.0 mg/mL and 2.25 to 9.0 mg/mL respectively. *S. parviflora* root extract was determined as the most active species with an IC_{50} value of 2.25 mg/mL among the *Scorzonera* species evaluated. The extracts of the aerial parts of *S. cinerea* and *S. incisa* also exhibited scavenging activity significantly with 3.0 mg/mL of IC_{50} value and these are followed by the extracts of *S. cana* var. *jacquiniana* and *S.eriophora* aerial parts with 3.3 and 3.5 mg/mL of IC_{50} value respectively.

Table 2. IC_{50} values of *Scorzonera* species aerial part and root extracts in superoxide anion radical scavenging assays

Plant material	Superoxide scavenging capacities (IC_{50} mg/mL)	
	Aerial Part	**Root**
SCVA	5.5 ± 0.3	4.5 ± 0.2
SCVJ	3.3 ± 0.4	3.8 ± 0.4
SCVR	5.0 ± 0.1	8.3 ± 0.5
SC	3.0 ± 0.2	6.25 ± 0.3
SE	3.5 ± 0.2	9.0 ± 0.3
SI	3.0 ± 0.5	7.5 ± 0.3
SLSL	6.0 ± 0.2	7.5 ± 0.3
SP	4.7 ± 0.6	2.25 ± 0.1
Vitamin E	0.37 ± 0.05	0.37 ± 0.05

The results of HPLC analysis have revealed that chlorogenic acid was determined in all tested aerial part and root extracts (Table 3). *S. incisa* aerial parts contain the highest amount of chlorogenic acid (569.19 ± 1.62 µg/mg) and this was followed by *S. eriophora* aerial parts (546.519 ± 0.812 µg/mg) and *S. parviflora* roots (509.96 ± 6.64 µg/mg). Among the tested flavonoids hyperoside, rutin as well as luteolin were detected in the extracts from the aerial parts of some *Scorzonera* species in varying amounts while the root extracts were found to be absent from all investigated flavonoids as shown in Table 3.

Table 3. Content of the standards in *Scorzonera* extracts (µg/mg)

Plant Species		Chlorogenic acid	Rutin	Hyperoside	Luteolin	Apigenin
SCVA	AE	443.864±2.788	104.692±4.961	-	-	-
	R	231.740±0.123	-	-	-	-
SCVJ	AE	340.548±1.347	24.510±3.088	30.722±1.624	-	tr
	R	331.028±2.835	-	-	-	-
SCVR	AE	58.716±0.304	-	-	37.965±0.057	tr
	R	72.747±0.233	-	-	-	-
SC	AE	266.51±1.51	-	124.22±0.56	-	-
	R	412.89±0.55	-	-	-	-
SE	AE	546.519±0.812	-	111.681±0.042	40.216±2.339	-
	R	277.508±0.233	-	-	-	-
SI	AE	569.19±1.62	198.81±0.18	-	-	-
	R	141.49±0.20	-	-	-	-
SLSL	AE	187.842±0.870	tr	-	-	-
	R	141.245±0.280	-	-	-	-
SP	AE	444.77±2.78	-	9.71±0.51	-	-
	R	509.96±6.64	-	-	-	-

tr: Trace amount

Epidemiological and experimental studies have revealed that there is a negative correlation between the increasing consumption of phenol-rich foods and beverages and decreasing the risk of various chronic diseases including, cardiovascular diseases, arthritis, cancer and chronic inflammation. Phenolic or polyphenolic compounds have importance for human health because of their antioxidant potency. Findings such as these have led to increase interest in the antioxidant as well as in the plants as potential sources of naturally occuring antioxidants (1,2, 31).

The present study is designed to evaluate antioxidant activities of some *Scorzonera* species of which genus plants used as vegetables as well as medicinal plants in Europe and in Turkey using *in vitro* assay; superoxide anion scavenging method. Results have revealed that the aerial parts exhibited higher scavenging activity against superoxide anion radical (Table 2), than root extracts except *S. parviflora*. *S. parviflora* possess strong scavenging capacity against superoxide anion radicals. Hence this extract can be considered as the most active extract amongst all. On the other hand, the results of the HPLC analysis showed that *S. parviflora* extract contains relatively high amount of chlorogenic acid (509.96 ± 6.64 µg/mg). Therefore, at this stage antioxidant activity of the *S. parviflora* could be due to its high chlorogenic acid content.

The antioxidant activity of plant materials is generally well correlated with their phenolic content (32). According to the literatures, the relation between amount of phenolic compounds and antioxidant activity have found to be highly correlated by some researchers while the others have found no direct correlation or only a very weak one since the other substances such as tocopherols and β-carotene raise the antioxidant activity (33). The results of the current study showed that *Scorzonera* species had the ability to act as a scavenger for superoxide anion radical significantly. Effects of plant extracts on superoxide anion formation could be attributed to their phenolic and/or flavonoid content. The root and aerial part extracts of *S. parviflora* as well as the aerial part extracts of *S. eriophora* and *S. incisa* found to contain chlorogenic acid in a high amount as indicated in HPLC results (Table 3), exhibited relatively stronger scavenging activity when compared to the other extracts. According to all these results it may be suggested that chlorogenic acid could be one of the contributing compound to radical scavenging activity

of the *Scorzonera* extracts but, clearly it is not directly responsible for the mentioned activity. Furthermore presence of hyperoside, rutin and luteolin among the tested flavonoids have been determined in aerial parts of investigated *Scorzonera* species in varying amounts. However it can be observed from these data, antioxidant activity also does not correlate with amount and/or type of flavonoid content.

Previously it has been reported that phenolic compounds such as dihydroisocoumarines (34-36), bibenzyl derivatives (20, 37, 38), flavonoids (39,40), lignans (40-42), stilbene derivatives (18,43) and chlorogenic acid derivatives (44) were isolated from *Scorzonera* species. Additionally, quinic acid derivatives; feruloylpodospermic acid A and B exhibited strong antioxidant activity in DPPH radical scavenging test model, have been isolated from the *S. divaricata* as responsible compounds from its antioxidant activity. Based on these results antioxidant activities of *Scorzonera* species could be attributed to their phenolic contents which could not been identified in the current study. On the other hand, *Scorzonera* species have significant analgesic, antiinflammatory and wound healing activities according to our previous findings (24-29). Triterpenes; taraxasteryl acetate, taraxasteryl myristate, motiol as well as β-sitosterol were isolated as analgesic compounds (24,25). It is considered that there is a relationship between antioxidant and analgesic activities. Free radicals play an important role in the pathogenesis of various diseases as well as inflammation and pain. Given their important role as mediators in provoking and/or sustaining of both acute and chronic inflammatory processes as well as pain causing tissue damage. 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 (45,46). There are also literatures that related to antioxidant activities of triterpenes (47,48). Therefore analgesic and anti-inflammatory activities of the *Scorzonera* species could be, at least partly, related to their antioxidant capacities.

CONCLUSION

In conclusion, the results of the present study suggest that *Scorzonera* species used as vegetables and medicinal plants possess promising antioxidant activities and their medicinal properties could be, at least partly, related to their antioxidant capacities. It is also suggested that chlorogenic acid is not directly responsible compound but it is obvious that this compound is one of the effective components of *Scorzonera* species for antioxidant activities. Therefore, further investigations are in progress in order to clarify bioactive principles which are responsible for the antioxidant activites of the *Scorzonera* species.

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