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ORIGINAL ARTICLE / ÖZGÜN MAKALE

VALIDATED HPLC METHOD TO ANALYZE PHYTOCHEMICAL STRUCTURE OF *SCORZONERA* SPECIES GROWN IN TÜRKİYE

TÜRKİYE'DE YETİŞEN SCORZONERA TÜRLERİNİN FİTOKİMYASAL ANALİZİ İÇİN VALİDE EDİLMİŞ YPSK YÖNTEMİ

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ABSTRACT

Objective: *The current study evaluated Scorzonera L. (Asteraceae) species, which are used as vegetables and medicinal plants in different countries where they grow naturally, such as Türkiye, Europe, Mongolia, and China, for their phenolic composition.*
Material and Method: *The twenty-five members of the Scorzonera genus, collected from different parts of Turkey, were investigated using a newly developed and validated High-Performance Liquid Chromatography (HPLC) method using some standard compounds, including chlorogenic acid, hyperoside, isoorientin, orientin, 7-O-methyl-isoorientin, isoquercetin, luteolin-7-O-β-glycoside, rutin, swertisin, and vitexin. The limit of detection and quantification levels were determined for each standard compound.*
Result and Discussion: *This study has revealed that the aerial parts are rich in phenolic compounds, with significantly higher amounts than the roots. Chlorogenic acid was detected in aerial parts and roots of all tested species and Scorzonera kotschyi aerial parts contained the highest amount (1787.26±32.88 µg/g). Most of the tested species contained varying amounts of hyperoside, isoorientin, isoquercetin, and orientin. Scorzonera aucheriana (572.93±0.04 µg/g), Scorzonera laciniata ssp. laciniata (524.07±5.06 µg/g), Scorzonera tomentosa (892.00±4.58 µg/g) and Scorzonera cana var. jacquiniana (309.23±1.69 µg/g) aerial parts contain these compounds respectively in higher amount. In contrast, vitexin, rutin and luteolin-7-O-β-glycoside were detected in a relatively small number of the tested species.*
Keywords: *Asteraceae, flavonoid, phytochemical analysis, Scorzonera, validation*

ÖZ

Amaç: *Bu çalışmada, Türkiye, Avrupa, Moğolistan ve Çin gibi yetiştiği ülkelerde sebze ve tıbbi bitki olarak kullanılan Scorzonera L. (Asteraceae) türleri fenolik bileşikleri açısından değerlendirilmiştir.*
Gereç ve Yöntem: *Türkiye'nin farklı bölgelerinden toplanan Scorzonera cinsine ait yirmi-beş örnek, klorojenik asit, hiperozit, izoorientin, orientin, 7-metil izoorientin, izokersetin, luteolin-7-O-β-glikozit, rutin, swertisin, viteksin gibi bazı standart bileşikler kullanılarak yeni geliştirilen ve valide edilen bir Yüksek Performanslı Sıvı Kromatografisi (YPSK) yöntemi ile incelendi. Her standart bileşik için tespit limiti ve tayin limiti hesaplandı.*
Sonuç ve Tartışma: *Toprak üstü kısımların köklere kıyasla fenolik bileşikler açısından çok daha*

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zengin olduğu ortaya konmuştur. Test edilen tüm türlerin toprak üstü kısımları ve köklerinde klorojenik asit tespit edilirken en yüksek miktarda ise Scorzonera kotschyi'nin toprak üstü kısımlarında (1787.26±32.88 µg/g) bulunmuştur. Test edilen türlerin çoğunda değişen miktarlarda hiperozit, izoorientin, izokersetin ve orientin saptanmıştır. Sırasıyla, Scorzonera aucheriana (572.93±0.04 µg/g), Scorzonera laciniata ssp. laciniata (524.07±5.06 µg/g), Scorzonera tomentosa (892.00±4.58 µg/g) ve Scorzonera cana var. jacquiniana (309.23±1.69 µg/g)'nın toprak üstü kısımları bu bileşikleri yüksek miktarlarda içerirken, viteksin, rutin and luteolin-7-O-β-glikozit test edilen türlerde daha düşük oranda tespit edilmiştir.

Anahtar Kelimeler: *Asteraceae, fitokimyasal analiz, flavonoit, Scorzonera, validasyon*

INTRODUCTION

Scorzonera L. is a genus of plants that belongs to the Asteraceae family, which is widely distributed across diverse regions of Eurasia and Northern Africa. With around 160 species, *Scorzonera* is a diverse and widely distributed genus. This genus is particularly interesting in Türkiye due to its remarkable 52 species, 31 of which are endemic to the region [1]. *S. hispanica* L. is a European plant grown as a vegetable in some selected European countries. In Türkiye, various species such as *S. cana* (C.A. Mey.) Hoffm., *S. latifolia* (Fisch. and Mey.) DC., *S. mollis* Bieb., and *S. suberosa* C. Koch are also used as vegetables at the beginning of spring as their fresh twigs and roots [2,3]. Various species of *Scorzonera* have been used for medicinal purposes in traditional medicines worldwide. In European traditional medicine, various species of *Scorzonera* have been utilized for their therapeutic properties in treating respiratory diseases, colds, wounds, and other ailments. In addition to these medicinal benefits, *Scorzonera* is also renowned for its stomachic, diuretic, galactagogue, antipyretic, and appetizing effects. Similarly, traditional Mongolian medicine has been used to treat diarrhoea, lung oedema, parasitic diseases, and fever caused by bacterial and viral infections. The members of *Scorzonera* species have also been used in Libyan, Chinese, Tibetan, and Turkish traditional medicine to relieve hepatic pains and treat breast inflammation, abscesses, rheumatism, arteriosclerosis, hypertension, kidney diseases, and diabetes due to their anti-inflammatory and antipyretic properties [2,4]. Several in vitro and preclinical studies have demonstrated a diverse range of biological activities associated with the *Scorzonera* genus. Among these activities are wound healing effects, anti-inflammatory, antioxidant, and analgesic properties. Various phytochemicals such as dihydroisocoumarins [5-9], bibenzyl derivatives [10-12], flavonoids [6,13-16], lignans [12,17,18], stilbene derivatives [9,19,20], quinic and caffeic acid derivatives [6,13,16,19] sesquiterpene and sesquiterpene lactones [16,18,21-23] and triterpenes [13,24-27] have been isolated from the *Scorzonera* species. In the current study, twenty-five species of *Scorzonera* collected from different parts of Türkiye [28] were investigated for their phenolic content, particularly for their flavonoid composition by newly developed and validated HPLC method using some standard compounds, including chlorogenic acid, hyperoside, isoorientin, orientin, 7-methylisoorientin, isoquercetin, luteolin-7-glycoside, swertisin, rutin and vitexin. Limit of detection (LOD) and limit of quantification (LOQ) levels were determined for each standard compound.

MATERIAL AND METHOD

Preparation of Standard Solutions and Calibration

Ten different stock solutions were prepared, each with a concentration of 1 mg/ml, for the following compounds: chlorogenic acid, hyperoside, isoorientin, orientin, 7-methylisoorientin, isoquercetin, luteolin-7-glycoside, swertisin, rutin, and vitexin. Each stock solution was diluted to obtain six different concentration levels (0.01 mg/ml, 0.02 mg/ml, 0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, and 0.5 mg/ml). To establish the calibration curves, a total of three 10 µl injections were carried out for each standard solution. The resulting curves were constructed by plotting the peak area of each solution against its corresponding concentration. The HPLC chromatogram of the standard compounds was given in Figure 1.

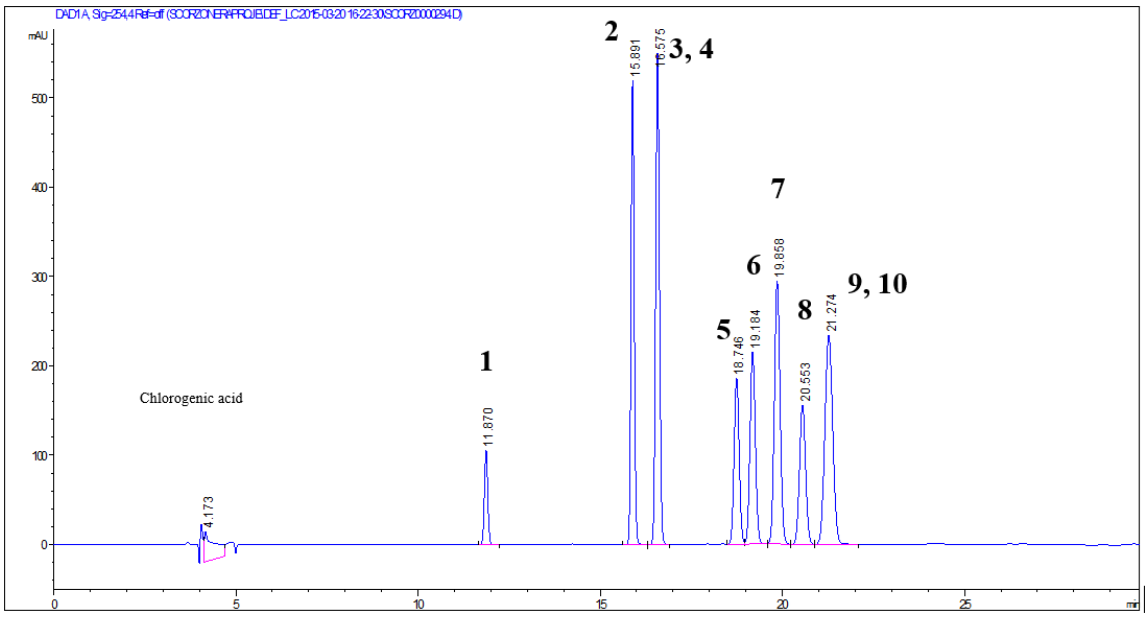

Figure 1. HPLC chromatogram of standard compounds. **1:** Chlorogenic acid; **2:** Isoorientin; **3:** Orientin; **4:** 7-*O*-methylisoorientin; **5:** Isoquercetin; **6:** Rutin; **7:** Hyperoside; **8:** Vitexin; **9:** Luteolin-7-*O*-glucoside; **10:** Swertisin. When standard substances were given as a mixture, the presence of 8 phenolic compounds was detected in the spectrum. The compounds, which were not separated but appeared as a single phenolic compound, were separated under ultraviolet light

Validation Procedure

Limit of Detection and Quantification

Achieving optimal levels of sensitivity and accuracy is crucial in any analytical method. With this in mind, it's important to note that limit of detection (LOD) and limit of quantification (LOQ) were established at Signal/Noise ratios of 3 and 10, respectively [29]. Table 1 displays the concentrations of each standard compound LOD and LOQ. Six injections of each standard compound experimentally verified LOD and LOQ concentrations.

Table 1. LOD and LOQ values for each standard compound

Standard compound	LOD concentration (µg/ml)	LOQ concentration (µg/ml)
Hyperosid	0.59	1.96
Isoquercetin	0.58	1.92
Isoorientin	0.13	0.42
Chlorogenic acid	0.21	0.71
Luteolin-7-*O*-*β*-glucoside	0.17	0.58
7-*O*-methylisoorientin	1.78	5.92
Orientin	0.32	1.06
Rutin	0.13	0.45
Swertisin	0.25	0.85
Vitexin	1.26	4.19
Hyperoside	0.59	1.96
Isoquercetin	0.58	1.92
Isoorientin	0.13	0.42

Preparation of the Extracts

To extract the plant material, 0.5 g of powdered aerial parts and roots were mixed with methanol: water (80:20) mixture (10 ml) in an ultrasonic bath for 30 minutes [30]. After extraction, each extract was filtered through filter paper and adjusted to 10 ml with the same solvent in a volumetric flask. Before injection, each extract was filtered again through a 0.22µ membrane filter.

HPLC Analysis

The HPLC analyses were expertly conducted on an Agilent LC 1100 model chromatograph, manufactured by the highly reputable Agilent Technologies based in California, USA. The diode array detector (DAD) was set to 254 nm wavelength. The peak areas were automatically integrated via Agilent software, and the resulting chromatograms were processed and plotted using the same software. The Supelcosil column (250 mm x 4.6 mm; 5 µm) was skillfully employed for separation, with acetonitrile (A) and water (B) expertly used as the mobile phase for gradient elution. The initial phase was A-B (8:92, v/v), which was followed by a linear change from A-B (8:92, v/v) to A-B (18:82) for 10 minutes. From 10 to 20 minutes, there was an isocratic flow of A-B (18:82), and the linear gradient elution was from A-B (18:82) to A-B (22:78) with a range of 20-45 minutes. From 45 to 55 minutes, A-B (22:78) was expertly changed and the flow rate was set to 0.7 ml/min. The column temperature was maintained at a precise 40°C and the sample injection volume was a carefully measured 10 µl.

RESULT AND DISCUSSION

The investigation aimed to examine the phenolic compounds present in *Scorzonera* species, utilizing both well-known flavonoids and pre-existing flavonoids extracted from plants within this genus and chlorogenic acid. The research revealed that the aerial parts of the plant exhibit a greater abundance of phenolic substances than the roots. Chlorogenic acid was detected in the aerial parts and roots of all the tested *Scorzonera* species and *S. kotschyi* Boiss. aerial parts were detected to contain the highest amount of this compound (1787.26±32.88 µg/g). Various tested species contained derivatives of quercetin such as hyperoside, isoorientin, isoquercetin, and orientin (namely the luteolin-C-glycoside). *S. aucheriana* Boiss. (572.93±0.04 µg/g), S. *laciniata* L. ssp. *laciniata* (524.07±5.06 µg/g), *S. tomentosa* L. (892.00±4.58 µg/g) and *S. cana* var. *jacquiniana* (W. Koch) Chamberlain (309.23±1.69 µg/g) aerial parts contain these mentioned compounds respectively at higher amounts compared to other tested species of *Scorzonera* genus. Vitexin, rutin and luteolin-7-*O*-*β*-glycoside were detected in a relatively small number of the tested *Scorzonera* species (Table 2).

The literature suggests that flavonoids can serve as taxonomic markers for Asteraceae. These compounds exhibit a broad range of structural diversity and have been isolated from many Asteraceae plants. Due to their wide structural diversity and abundance in Asteraceae species, flavonoids can serve as taxonomic markers at lower hierarchical levels [31]. However, the latest studies about flavonoids and their roles in the point of chemotaxonomy in Asteraceae, more specifically in Cichoridae which involves the *Scorzonera* genus, have reported that flavonoids are not very useful as markers on a higher level since rare compounds are found throughout the plant kingdom at various levels. However, flavonoids are the natural product class most widely employed for chemosystematic investigations. Flavonoids are frequently used in chemosystematics because they are easily separated and detected using simple techniques such as paper and thin-layer chromatography with UV-shift or spraying reagents [32].

Studies on the chemical composition of *Scorzonera* species have shown that they contain flavonoids and phenolic compounds. This study presents a simple and validated method for qualitative and quantitative analysis of some frequently isolated phenolic compounds from *Scorzonera* species. This study is of immense importance as it provides valuable insights into the phytochemical properties of *Scorzonera* species. By revealing their chemical compositions, this research can help develop new medicines and treatments that can significantly impact human health. The findings of this study can be used as a foundation for future research in this field.

Table 2. The amounts of the standard compounds in plant materials

Species		Standard compounds (µg/g plant material)									
		Chlorogenic acid	**Isoorientin**	**Orientin**	**7-*O*-methyl isoorientin**	**Isoquercetin**	**Rutin**	**Hyperoside**	**Vitexin**	**Luteolin-7-*O*-*β*-glucoside**	**Swertisin**
S. acuminata	AE	412.51±33.89	49.89±0.46	41.15±3.15		9.79±5.14	442.69±24.33				13.065±7.71
	R	327.16±30.34					0.23±2.79				
S. argyria	AE	756.65±28.97	127.08±0.93		110.81±1.93	13.521±0.11	107.22±0.47				57.51±2.44
	R	676.47±14.20									
S. aucherana	AE	551.01±3.99				170.77±0.05		572.93±0.05			
	R	244.68±4.03									
S. boissieri	AE	186.58±3.44	25.93±0.78	23.63±0.53		67.19±0.68	37.56±0.90	32.89±0.01		166.81±0.99	
	R	217.34±5.33									
S. cana var. *alpina*	AE	465.26±17.48	94.03±0.29	26.62±0.49					72.45±2.59		
	R	243.34±1.94									
S. cana var. *jacquiana*	AE	473.44±17.20	111.81±0.83	309.23±1.69					58.32±0.27	7.46±1.10	
	R	188.03±3.79									
S. cana var. *radicosa*	AE	334.52±56.79	263.11±3.19	71.69±7.74		18.51±3.81				116.29±2.47	130.92±2.74
	R	179.90±1.47									
S. cinerea	AE	218.75±5.36	34.92±0.12		61.79±0.20	53.11±0.34		153.99±0.58			28.38±0.11
	R	422.43±5.89									
S. ekimii	AE	732.29±35.26	38.91±3.23		260.64±1.43	48.31±2.06		136.03±1.75			175.75±2.13
	R	219.17±5.51									
S. elata	AE	201.22±3.33	154.40±0.86	70.91±0.24		0.74±0.08	1.39±0.18		12.79±0.03	3.52±0.11	
	R	150.34±2.18									
S. eriophora	AE	135.04±5.20	77.54±0.70			20.58±0.59	23.30±0.07	28.32±0.71			24.24±0.89
	R	306.45±3.92									
S. gokcheoglui	AE	363.06±14.38				50.69±0.28	31.78±0.41	117.19±0.42			
	R	361.26±5.88									
S. incisa	AE	653.06±14.59	137.04±11.24	76.26±20.71		25.58±0.31	182.44±0.03				
	R	122.66±25.77									
S. kotschyi	AE	1787.26±32.88				123.68±0.42	43.47±0.21	213.27±0.53			
	R	249.26± 0.96									
S. lacera	AE	1160.27±52.59				280.59±1.34		445.47±1.67		10.64±0.39	
	R	398.42±5.06						26.15±0.03			
S. laciniata ssp. *laciniata*	AE	333.72±11.91	524.07±5.06	130.58±0.42		2.92±0.04			17.91±0.03		
	R	98.83±0.76	42.71±0.07	130.58±0.16							
S. latifolia	AE	432.67±18.16	43.84±0.01		94.82±0.04	23.58±0.17	8.700±0.02	255.81±0.26			56.34±0.02
	R	1015.51±17.06									
S. mollis ssp. *szowitzii*	AE	1097.47±30.09	207.67±1.77	51.09±1.36		41.92±3.11	53.46 ±1.59	83.21±1.92		71.36±3.38	
	R	173.87±0.02									
S. parviflora	AE	438.49±8.38		38.29±2.23		74.58±0.32		30.72±0.14	19.2±0.13		
	R	549.01±9.08									
S. pisidica	AE	702.11±18.78	69.97±0.77			15.81±0.11	60.04±0.29	63.30±0.15			6.45±0.02
	R	752.10±11.59									

Table 2 (*continue*). The amounts of the standard compounds in plant materials

Species		Standard compounds (µg/g plant material)									
		Chlorogenic acid	**Isoorientin**	**Orientin**	**7-*O*-methyl isoorientin**	**Isoquercetin**	**Rutin**	**Hyperoside**	**Vitexin**	**Luteolin-7-*O*-*β*-glucoside**	**Swertisin**
S. pseudolanata	AE	379.37±12.84	125.63±0.27		123.07±1.49	23.26±0.71					61.76±0.25
	R	21.11±0.60									
S. sericea	AE	103.84±1.57						55.34±0.07			
	R	81.67±0.27									
S. suberosa ssp. *cariensis*	AE	612.53±21.47	38.33±0.15								
	R	500.71±13.47				122.76±0.44					
S. sublanata	AE	1207.77±37.56	339.99±6.21	382.88±7.65							
	R	391.89±0.45									
S. tomentosa	AE	365.73±4.89	49.68±1.24		71.06±0.91	213.89±1.23		892.00±4.58			32.41±0.01
	R	617.33±14.22									

AE: Aerial parts, R: Root

AUTHOR CONTRIBUTIONS

Concept: Ö.B.A.; Design: Ö.B.A.; Control: E.K.; Sources: Ö.B.A.; Materials: Ö.B.A.; Data Collection and/or Processing: S.E.; Analysis and/or Interpretation: S.E., E.K., Ö.B.A.; Literature Review: S.E., Ö.Y., Ö.B.A.; Manuscript Writing: E.K., Ö.Y., Ö.B.A.; Critical Review: S.E., E.K., Ö.Y., Ö.B.A.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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