

## QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHENOLIC ACIDS IN *SCORZONERA TOMENTOSA* L.

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### Abstract

In the present study the qualitative and quantitative analysis of phenolic acids such as chlorogenic CA), caffeic (CFA), ferulic (FA), rosmarinic (RA) and p-coumaric acids (PCA), were performed by using high pressure liquid chromatography in the aerial part and the root extracts of *Scorzonera tomentosa* L. (Asteraceae) which were collected from five different regions of Anatolia. Chlorogenic acid was determined as major phenolic acid content of *Scorzonera tomentosa*. The calibration curve was established as  $Y=23.21X-21.83$  ( $r^2= 0.999$ ) for chlorogenic acid and the highest concentration was determined in the root extract of Yozgat sample with  $1360.090 \pm 6.732 \mu\text{g}$ .

**Key words:** *Scorzonera tomentosa*, Phenolic acid, Chlorogenic acid, HPLC

### *Scorzonera tomentosa* L. Bitkisinde Fenolik Asitlerin Kalitatif ve Kantitatif Analizi

Bu çalışmada Anadolu'nun beş farklı bölgesinden toplanan *Scorzonera tomentosa* L. (Asteraceae) bitkisinin toprak üstü kısımları ve köklerinin klorojenik, kafeik, ferulik, rosmarinik ve p-kumarik asit gibi fenolik asit içerikleri kalitatif ve kantitatif olarak yüksek basınçlı sıvı kromatografisi kullanılarak karşılaştırılmıştır. Tespit edilen major fenolik asit klorojenik asittir. Klorojenik asit için kalibrasyon denklemi  $Y=23.21X-21.83$  ( $r^2= 0.999$ ) olarak bulunmuş ve en yüksek klorojenik asit içeriğine  $1360.090 \pm 6.732 \mu\text{g}$  ile Yozgat'tan toplanan örneğin kök ekstresinin sahip olduğu belirlenmiştir

**Anahtar kelimeler:** *Scorzonera tomentosa*, Fenolik asit, Klorojenik asit, YBSK

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## INTRODUCTION

Phenolic acids are one of the important groups of phenolic compounds that are widely distributed in both edible and non edible plants (1-4). They are hydroxylated derivatives of benzoic and sinamic acids and the most commons are hydroxycinnamic acid derivatives such as chlorogenic, caffeic, *p*-coumaric, ferulic acids (1-2, 5-6). Phenolic acids have attracted considerable interest in the past few years due to their strong antioxidant activities against free radicals and other reactive oxygen species (ROS) which are the cause of many chronic diseases such as cancer, cardiovascular diseases, inflammation, brain dysfunction (4, 7). There are also many literatures related to the different pharmacological activities of phenolic acids including anticancer, antibacterial, antipyretic, antirheumatic activities (2, 4, 7-8).

The genus *Scorzonera* L. (Asteraceae) is widely distributed in the arid regions of Asia, Europe and in Northern Africa with more than 175 species (9-10). In Turkey *Scorzonera* genus contains 49 species with the addition of new species (10-13). Some species of *Scorzonera* have been widely used as a food as well as medicinal plants (14-16). Some species of this genus are known as yemlik in Anatolia and their roots as well as green buds are eaten freshly or after cooked. They have also some medicinal usage in Turkish folk medicine for the treatment of arteriosclerosis, kidney diseases, hypertension, diabetes mellitus and rheumatism as well as for pain treatment and wound healing (17-18). *Scorzonera tomentosa* L. is an endemic herb to Turkey. *S. tomentosa* roots contain latex, both of this latex and roots are used due to their wound healing activity in Turkish folk medicine (19).

Previous phytochemical studies on *Scorzonera* genus revealed that many compounds such as dihydroisocoumarines (20-21), bibenzyl derivatives (22-24), flavonoids (15, 25-27), lignans (24, 28-29), stilbene derivatives (16, 21, 30), quinic and caffeic acid derivatives (15, 27, 30) sesquiterpene, sesquiterpene lactones (15, 22, 28, 31) and triterpenes (16, 27, 32-33) have been isolated. In addition, stigmaterol 3 $\beta$ -glucoside,  $\beta$ -sitosterol, lupeol, lupeol acetate and  $\alpha$ -amyrine from the aerial parts (32) and hydrangenol, hydrangenol-4'-O-glucoside, hydramacrophyllol A and B as well as scorzotomentosin, scorzotomentosin-4'-O- $\beta$ -glucoside, scorzophthalide, scorzoerzincanin from the roots of *S. tomentosa* (21) hydrangenol and scorzotomentosin 4'-O- $\beta$ -glucopyranoside from the roots of *S. latifolia* (34) have been isolated previously.

In our previous studies we have been reported the analgesic activities of *S. latifolia*. Both plant root extracts and the isolated compounds; taraxasteryl myristate and taraxasteryl acetate showed promising antinociceptive activities (33, 35). This paper is a part of our on going studies on this genus (33-36). The aim of the current study was to give a general profile of the content of phenolic acid variation in *S. tomentosa* samples which were collected from different regions of Anatolia. Chlorogenic, caffeic, ferulic, *p*-coumaric and rosmarinic acid contents of *S. tomentosa* samples were investigated qualitatively and quantitatively by using RP-HPLC method.

## EXPERIMENTAL

### *Plant material*

Five *Scorzonera tomentosa* L. (Asteraceae) species were collected in different locations of Turkey. The species are listed in Table 1. Voucher specimens were deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF) and botanical authentication was confirmed by H. Duman a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University and A. Mine Gençler Özkan, Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, Ankara, Turkey.

### Chemicals

Chlorogenic acid (CA) (C3878-1G), caffeic acid (CFA) (095K1340) and *p*-coumaric acid (PCA) (C900856) were obtained from Sigma; ferulic acid (FA) (128708) and rosmarinic acid (RA) (536954) were purchased from Aldrich.

### General experimental procedures

Chromatography was performed on Agilent LC 1100 model chromatograph (Agilent Technologies, California, USA). The diode array detector (DAD) was set at wavelength 330 nm and peak areas were integrated automatically by computer using Agilent Software. The chromatograms were plotted and processed by using the above mentioned software. The column was Supelcosil (25 cm x 4 mm x 5 µm) (Hichrom). The column temperature was 25 °C and the mobile phase included water (containing 0.2% phosphoric acid, solvent A) and acetonitrile (solvent B) in the following gradient system: initial 6% B, linear gradient to 30% B in 20 min, hold at 30% for 5 min. The total running time was 25 min. The flow rate was 1.2 mL/min injection volume was 10 µL (37).

### Preparation of HPLC samples and standard solutions

HPLC samples were prepared from 0.25 g of plant material using stirrer at 50 °C for 60 min using 10 mL of methanol: water (80:20) mixture. After extraction, all samples were adjusted to 10 mL in volumetric flask and filtrated from 0.45 µm filters before HPLC analysis. All standard compounds were dissolved in ACN and water mixture for qualitative analysis. For quantitative analysis, CA (10 mg) was accurately weighed in 10 mL volumetric flask and dissolved in ACN and water mixture then diluted to the final volume separately. Seven different concentrations (0.0025 mg/mL, 0.005 mg/mL, 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL) were prepared by diluting the stock solutions. Triplicate 10 µL injections were performed for both standard solution and plant samples. Peak area of each solution was plotted against the concentration to obtain the calibration curve.

**Table 1.** Locality of *S. tomentosa* samples

Plant sample	Locality	AEF No	Collection Date
1	Yozgat-Akdağmadeni	23841	2005
2	Kayseri-Pınarbaşı	23838	2005
3	Sivas-Hafik	23840	2005
4	Erzincan	23839	2005
5	Erzurum-Kop Geçidi	25158	2005

### Limits of detection and quantification

Limits of detection (LOD) were established at a signal to noise ratio (S/N) of 3. Limits of quantification (LOQ) were established at a signal to noise ratio (S/N) of 10. LOQ were experimentally verified by 6 injections of CA at the LOD and LOQ concentrations. LOD and LOQ concentrations were calculated as 0.47 µg/mL, 1.56 µg/mL for CA respectively.

The precision of the method was performed by the evaluation of intra-day and inter-day variations of the same standard solution at the LOQ level. The intra-day precision was determined by analyzing the same samples six times in a single day and inter-day precision was determined three different days in triplicates of injections.

The precision of the method expressed as the RSD % at the LOQ level was for CA (Table 2).

**Table 2.** Precision of the method at the LOQ Level

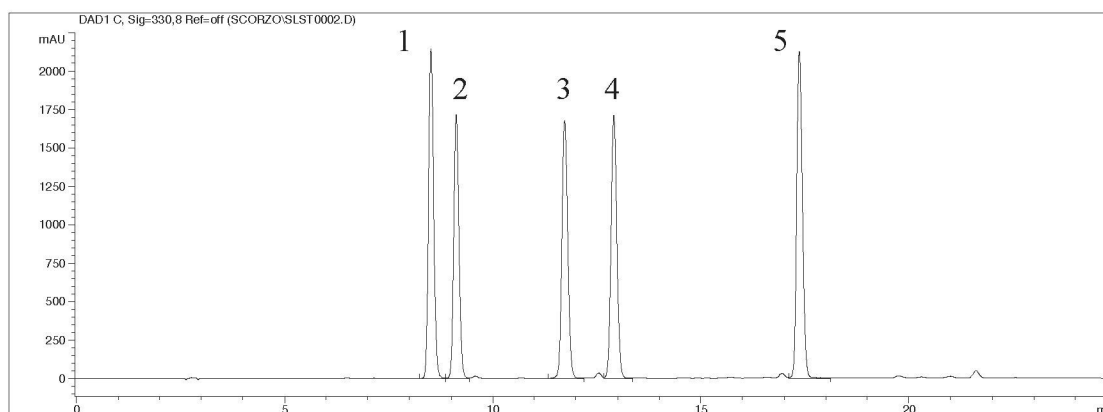
Standards	LOD ( $\mu\text{g/mL}$ )	LOQ ( $\mu\text{g/mL}$ )	Precision % RSD			
			Intra-day (n=6)		Inter-day (n=3)	
			$R_t$	$P_a$	$R_t$	$P_a$
CA	0.47	1.56	0.021	1.133	0.054	1.012

## RESULTS AND DISCUSSION

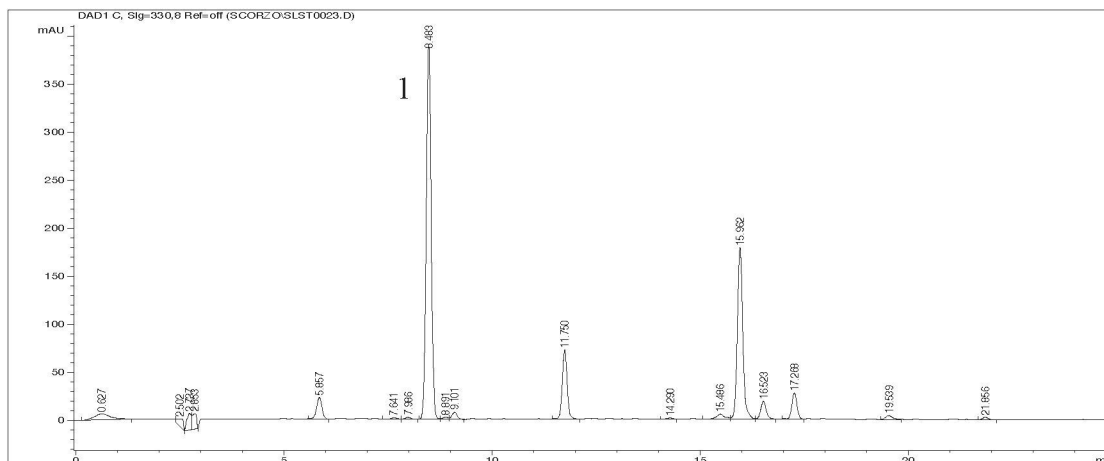
The purpose of the current study was to investigate the content of phenolic acids including CA, CFA, PCA, FA and RA (Fig 1) as well as to obtain a comparison of phenolic acid content amongst *S. tomentosa* samples from different geographical origins of Anatolia. HPLC results have revealed that CA was determined as the most abundant phenolic acid in both roots (Fig 2) and aerial parts (Fig 3) of *S. tomentosa*. HPLC chromatographic profiles of all *S. tomentosa* root and aerial part samples were found to be qualitatively similar among them. However there were significant differences in the level of CA content. *S. tomentosa* roots contain CA in higher percentage than aerial parts except Kayseri sample and the highest content of CA was determined in the roots of Yozgat-Akdağmadeni sample with  $1360.090 \pm 6.732 \mu\text{g/mg}$  as shown in Table 3.

**Table 3.** Chlorogenic acid contents of *S. tomentosa* samples

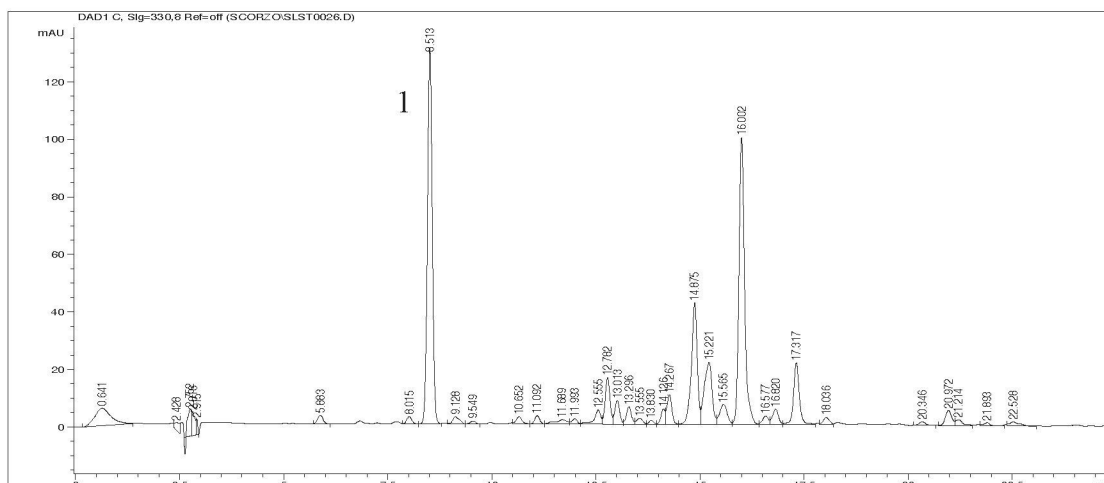
Plant sample	Aerial parts ( $\mu\text{g/mg}$ ) (n=3, mean $\pm$ SD)	Roots ( $\mu\text{g/mg}$ ) (n=3, mean $\pm$ SD)
1	$441.497 \pm 3.475$	$1360.090 \pm 6.732$
2	$764.203 \pm 0.657$	$336.557 \pm 1.195$
3	$191.300 \pm 1.573$	$1013.994 \pm 3.243$
4	$293.793 \pm 0.949$	$933.357 \pm 0.791$
5	$400.297 \pm 1.994$	$1048.403 \pm 4.398$



**Figure 1.** Chromatogram of the standart mixture, 1: Chlorogenic acid; 2: Caffeic acid; 3: *p*-Coumaric acid; 4: Ferulic acid; 5: Rosmarinic acid



**Figure 2.** Chromatogram of the root extract of Akdağmadeni sample



**Figure 3.** Chromatogram of the aerial part extract of Akdağmadeni sample

It has been reported that, several factors including genetic differences, climatic conditions, agricultural practices as well as geographical locations can affect the chemical composition of the plants (38). The results obtained in present study have also confirmed the variation in the phenolic acid content amongst samples from different geographical origin. Different growing conditions have probably influences on the content of CA.

CA that is one of the most abundant polyphenol in human diet is widely found in plant kingdom including fruits and vegetables. CA is known to have strong antioxidant activity. In addition, previous studies have revealed that CA has also many biological activities including anti-inflammatory (39-40), antinociceptive (39, 41) and cut effect on the glucose absorption (42). CA and derivatives have also been identified and isolated from some *Scorzonera* species (30, 36). Phenolic acids including CA, CFA, CPA, FA, RA and flavonoids such as apigenin, luteolin, kaempferol, luteolin-7-glucoside, rutin, hyperoside and hesperidin were investigated in *Scorzonera* species in our earlier study and CA was determined as one of the major constituent of *Scorzonera* species (36). It has been suggested that CA probably responsible for the biological activities of *Scorzonera* species in part.

In conclusion present study showed exhibited that *Scorzonera tomentosa* contains CA as one of the main component in significant amounts. Additionally amount of the CA was found to vary depending on the geographical locations of the plant samples.

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