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RESEARCH ARTICLE

Evaluation of the Antioxidant Potential and Chlorogenic Acid Contents of Three Endemic *Sideritis* Taxa from Turkey

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SUMMARY

In the present study, the endemic Sideritis argyrea, S. congesta and S. erythrantha var. cedretorum methanol extracts from the dried aerial parts were evaluated for their antioxidant potential and total phenolic content. Also, one of the major constituents, chlorogenic acid was quantified using a previously validated HPLC method. The highest total phenolic content was determined in S. erythrantha var. cedretorum as 190.78±2.46 mg GAE/g; whereas, the highest antioxidant activity results were obtained for S. erythrantha var. cedretorum by using both DPPH and ABTS assays. Chlorogenic acid contents of the S. argyrea, S. congesta, and S. erythrantha var. cedretorum were determined as 0.196±0.01 g/100g dw, 0.289±0.007 g/100g dw, and 0.282±0.01 g/100g dw, respectively. Sideritis taxa with particular high total phenolic content and antioxidant potential can be addressed as a chlorogenic acid source.

Key Words: *Sideritis, DPPH, ABTS, Folin, Lamiaceae, HPLC,*

Türkiye'de Yetişen Üç Endemik Sideritis Taksonunun Antioksidan Potansiyelinin ve Klorojenik Asit İçeriklerinin Değerlendirilmesi

ÖZET

Bu çalışmada, Sideritis argyrea, S. congesta ve S. erythrantha var. cedretorum toprak üstü kısımlarının metanol ekstreleri, antioksidan özellikleri ve toplam fenolik içerik bakımından değerlendirilmiştir. Ayrıca ekstrelerin ana bileşenlerinden biri olan klorojenik asitin miktarı, daha önceden valide edilmiş bir YPSK yöntemi kullanılarak tespit edilmiştir. En yüksek total fenol miktarına 190,78±2,46 mg GAE/g değeri ile S. erythrantha var. cedretorum'da rastlanmıştır. Buna paralel olarak, DPPH ve ABTS analizlerinde en yüksek antioksidan aktivite sonuçları yine S. erythrantha var. cedretorum'dan elde edilmiştir. S. argyrea, S. congesta ve S. erythrantha var. cedretorum'un klorojenik asit içeriği sırasıyla 0,196±0,01 g/100g ka, 0,289±0,007 g/100g ka ve 0,282±0,01 g/100g ka olarak tespit edilmiştir. Önemli derecede toplam fenolik içeriğe ve antioksidan özelliğe sahip olan endemik Sideritis taksonlarının, ciddi birer klorojenik asit kaynağı olarak değerlendirilebileceği düşünülmektedir.

Anahtar Kelimeler: *Sideritis, DPPH, ABTS, Folin, Lamiaceae, YPSK*

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INTRODUCTION

The genus *Sideritis* includes approximately 150 species distributed mainly in the Mediterranean region and represented in the Flora of Turkey by 46 species and altogether 55 taxa, 42 of them being endemic. Plants belonging to *Sideritis* species are annual or perennial aromatic herbs or small shrubs (Huber-Morath, 1982, Tadić et al., 2012, Kılıç, 2014), and they have been used since ancient times in folk medicine due to their anti-inflammatory, antirheumatic and antimicrobial properties. They are widely known as "mountain tea" (local name "Dağçayı" or "Adaçayı") in Turkey and are used as herbal tea in folk medicine (Ayaz, 2008). In Spain, some *Sideritis* species are served in restaurants of tourist areas as a social beverage (like tea or coffee) and also used to aromatize homemade and commercial herb liqueurs (Łuczaj and Pieroni, 2016). The plants of *Sideritis* genus contain various types of plant secondary metabolites including predominantly terpenes, flavonoids, iridoids, coumarins, lignans and sterols which are supposed to be responsible for the various biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiulcer, antispasmodic, anticonvulsant and anti-osteoporotic (Gonzalez-Burgos et al., 2011).

The extracts of some *Sideritis* species of Turkey, including *S. argyrea,* have been shown to have antibacterial and anti-inflammatory activity against carrageenan-induced edema in mice. The essential oil of *S. argyrea* was previously reported to contain α-pinene and β-pinene as main constituents and exhibit antimicrobial activity (Kirimer et al., 2003). *S. congesta* which is native to Turkey, was previously found to have strikingly high antioxidant potency (Erkan et al., 2011).

The plant grows in the 1000 m altitude in Mediterranean region and its flowers, leaves and shoots are used in the form of infusion for constipation, stomachache, appetizing, pain relief, throat inflammation, neural appease and cold (Öztürk et al., 2016). *S. erythrantha* var. *cedretorum* is a perennial plant up to 50 cm height with yellow flowers and it grows only in Antalya province. It is locally known as "yayla çayı" and is used as herbal tea in Antalya-Alanya. Chemical composition, antimicrobial and antioxidant activities of the essential oil of *S. erythrantha* var. *cedretorum* have previously been reported in several studies, and the essential oil of the plant showed weak antioxidant activity but exhibited moderate antimicrobial activity against several Gram (+) bacteria (Tabanca et al., 2001, Köse et al., 2010).

Chlorogenic acid is an ester of caffeic acid with quinic acid, which is found naturally in Asteraceae, Solanaceae, Rubiaceae and Rosaceae, and common in various plants such as coffee beans, apples and blueberries. It has also been reported in a number of other angiosperm families (Kweon et al., 2001, Rønsted et al., 2002). Chlorogenic acid exhibits antimutagenic, carcinogenic and antioxidant activities *in vitro*, mainly by scavenging reactive oxygen species (Sato et al., 2011). *Sideritis* L. (Lamiaceae) species have an important place among medicinal and aromatic plants and are considered as a natural resource of phenolic acids including chlorogenic acid (Zengin et al., 2014).

The aim of this study was to evaluate the total phenolic contents along with their chlorogenic acid contents and *in vitro* antioxidant activities of methanolic extracts of the aerial parts of *S. argyrea, S. congesta* and *S. erythrantha* var. *cedretorum* which all are endemic.

MATERIALS AND METHODS

Plant material

Sideritis argyrea P.H.Davis, *Sideritis congesta* P.H.Davis & Hub.-Mor. and *Sideritis erythrantha* Boiss. & Heldr. var. *cedretorum* P.H.Davis samples were obtained from local market and identified by Prof. Dr. Hayri Duman.

Extraction

The dried and grounded aerial parts of the plants were accurately weighed (5g) and extracted by using methanol in an ultrasonic bath for 1 h. After filtration, methanol was completely evaporated and the crude extracts were obtained with the yields of 12.21% for *S. argyrea*, 25.22% for *S. congesta* and 12.30% for *S. erythrantha* var. *cedretorum*.

Total Phenolic Content

The total phenolic content of the extracts was determined by using a modified Folin Ciocalteu method (Singleton et al., 1999). The reduction of the reagent, which resulted in the generation of a blue color, was recorded at 765 nm. 100 µL of each sample (2 mg/mL) was mixed with 7.9 mL of distilled water. Folin Ciocalteu reagent (500 µL) was added and the contents of the tubes were shaken vigorously. After 8 min, 1.5 mL of 20% Na_2CO_3 was added. After 2 h incubation at room temperature, the absorbance was measured at 765 nm with a Shimadzu spectrometer. Gallic acid was used as standard. All measurements were performed in triplicate, and the average values were used to state the mg of gallic acid equivalents (GAE)/g dry extract.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity Assay

DPPH radical scavenging activity assay was

performed according to the modified method of Barros et al. (2007). Different concentrations of extracts (0.25 mL) were mixed with 2.75 mL of methanolic solution of DPPH radical (D9132, Aldrich). The mixture was shaken vigorously and left to stand for 10 min in the dark. The reduction of the DPPH radical was examined by measuring the absorption at 517 nm. The radical scavenging activity (Inh%) was calculated as a percentage of DPPH discoloration using the equation: $Inh\% = [(A_{DPPH} - A_s) / A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract was added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Extract concentrations were established in the range of 0.25-2 mg/mL. Trolox (238813, Aldrich) was used as standard.

ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] Assay

ABTS radical scavenging activity assay was performed using a modification of the method of Re et al. (1999). ABTS (A1888, Sigma) was dissolved in methanol and its concentration was adjusted to 7 mM. ABTS stock solution with 2.45 mM $K_2S_2O_8$ (as an oxidant for conversion of ABTS into a radical cation) was used to generate the ABTS radical cation. Blue-green resulting solution was formed. This radical solution was stored in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted with 96% ethanol to gain an absorbance of 0.70±0.02 at 734 nm. An aliquot of each sample (0.25 mL) was mixed with 2.75 mL of diluted ABTS radical cation solution. After waiting at room temperature for 6 min, the reduction in absorbance at 734 nm was measured. The radical scavenging activity (Inh%) was calculated as a percentage of ABTS inhibition using the equation: $Inh\% = [(A_{ABTS} - A_s) / A_{ABTS}] \times 100$, where A_S is the absorbance of the solution when the sample extract was added at a particular level, and A_{ABTS} is the absorbance of the ABTS radical cation solution. The extract concentration displaying 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Extract concentrations were established in the range of 0.125-1.5 mg/mL. Trolox was used as standard.

RP-HPLC Analysis

HPLC analyses of chlorogenic acid in three *Sideritis* methanol extracts were performed using a previously validated HPLC-DAD method (Gökbulut, 2015). Agilent 1260 Series HPLC system was used. Agilent Chemstation software was used for the data evaluation. The separation was made on ACE 5 µ C18 (250×4.60 mm) column. The mobile phase was a mixture of trifluoroacetic acid 0.1% in water (solution A), trifluoroacetic acid 0.1% in methanol (solution B), and trifluoroacetic acid 0.1% in acetonitrile (solution C). The composition of the gradient was (A:B:C), 80:12:8 at 0 min, 75:15:10 at 8 min, 70:18:12 at 16 min, 65:20:15 at 24 min, 50:35:15 at 32 min, 25:60:15 at 40 min and 80:12:8 at 45 min. The duration between runs was 2 min. The injection volume was 10 µL for each standard and sample solutions. Quantification was done by measuring at 330 nm for chlorogenic acid (C3878, Sigma) using a photo-diode array detector.

RESULTS AND DISCUSSION

The total phenolic amount of the *Sideritis* taxa ranged from 121.7±2.4 to 190.8±2.5 mg of gallic acid equivalents per gram of plant extracts dry weight. *S. erythrantha* var. *cedretorum* was found to be richer in total phenolics, and this finding is in accordance with the obtained radical scavenging activity results. The total phenolic content of the previously investigated *Sideritis* taxa was comparable to the results presented here (Tsibranska et al., 2011, Radojevic et al., 2012, Tadić et al., 2012, Kara et al., 2014).

Two types of radicals, DPPH and ABTS, were used to evaluate the free radical scavenging activities of the extracts. According to the DPPH and ABTS radical scavenging activity results, *S. erythrantha* var. *cedretorum* was determined as the most active taxon among the three with lower IC_{50} values as 0.36±0.15 and 0.20±0.01 mg/mL, respectively. Trolox had the IC_{50} values of 0.04 and 0.039 mg/mL for DPPH and ABTS assays, respectively. Knowing that the lower IC_{50} values mean higher antioxidant activity, we can mention that *S. argyrea* displayed lower radical scavenging activity with both methods compared to the other two *Sideritis* taxa.

One of the most abundant phenolic compounds found in the methanol extracts was chlorogenic acid. Its amount in the extracts was determined by using a previously validated HPLC method (Gökbulut, 2015), and ranged from 0.196±0.010 to 0.289±0.007g/100g dw (Table 1). The HPLC chromatograms of the *Sideritis* taxa were given in Figure 1-3. On the other hand, previous findings revealed that chlorogenic acid was determined in several *Sideritis* species and these findings supported our results regarding the striking amount in the extracts (Erkan et al., 2011, Tadić et al., 2012, Zengin et al., 2014, Tóth et al., 2015). Therefore, investigated three *Sideritis* taxa are considered as natural sources of this hydroxycinnamic acid derivative. Due to the fact that chlorogenic acid is one of the most effective radical scavengers, it may be responsible for the determined strong antioxidant activities of the endemic *Sideritis* methanol extracts together with the other phenolics.

Table 1. Total phenolic contents, radical scavenging activity results and chlorogenic acid amounts of *Sideritis* taxa.

	Total Phenolic Content (mg GAE/ g extract)	**DPPH IC_{50} (mg/mL)**	**ABTS IC_{50} (mg/mL)**	**Chlorogenic acid content (g/100g dw)**
S. argyrea	121.7 ± 2.4	0.80 ± 0.06	0.33 ± 0.05	0.196 ± 0.010
S. congesta	147.4 ± 9.6	0.41 ± 0.01	0.29 ± 0.01	0.289 ± 0.007
S. erythrantha* var. *cedretorum	190.8 ± 2.5	0.36 ± 0.15	0.20 ± 0.01	0.282 ± 0.010

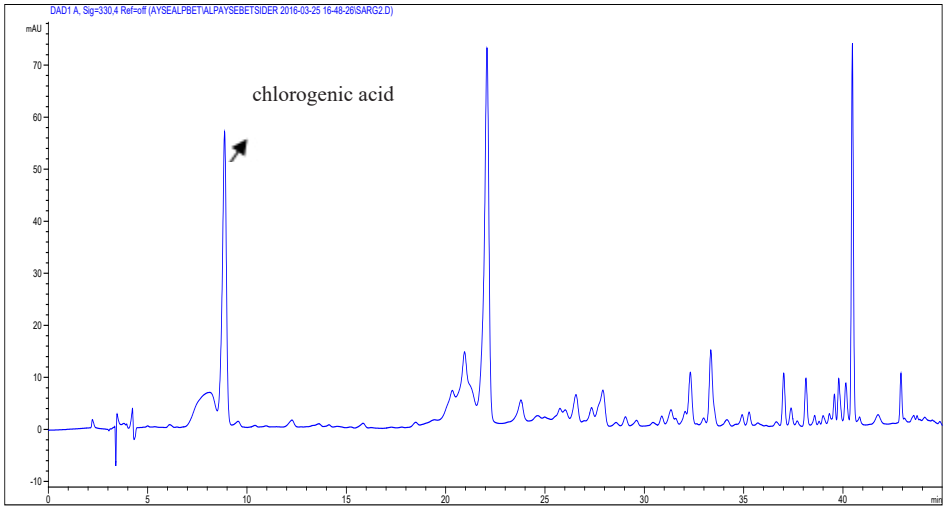

Figure 1. HPLC chromatogram of *S. argyrea*

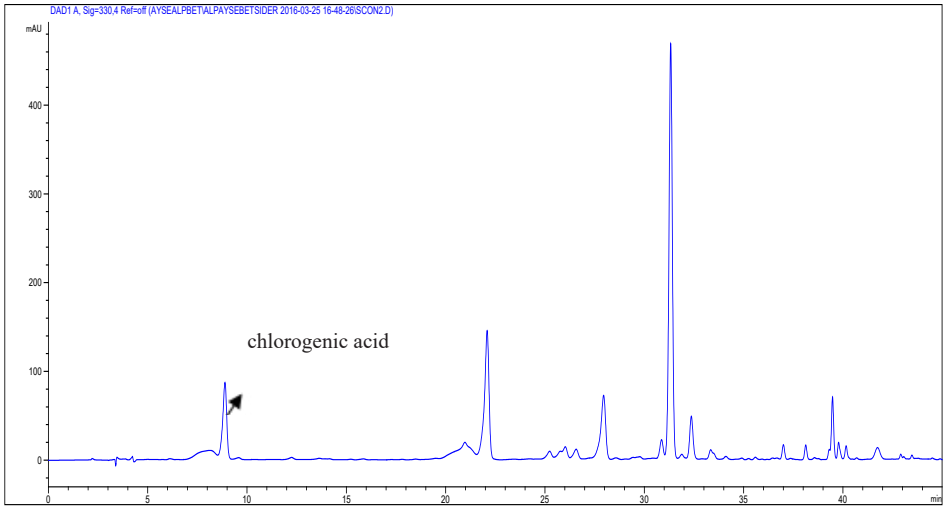

Figure 2. HPLC chromatogram of *S. congesta*

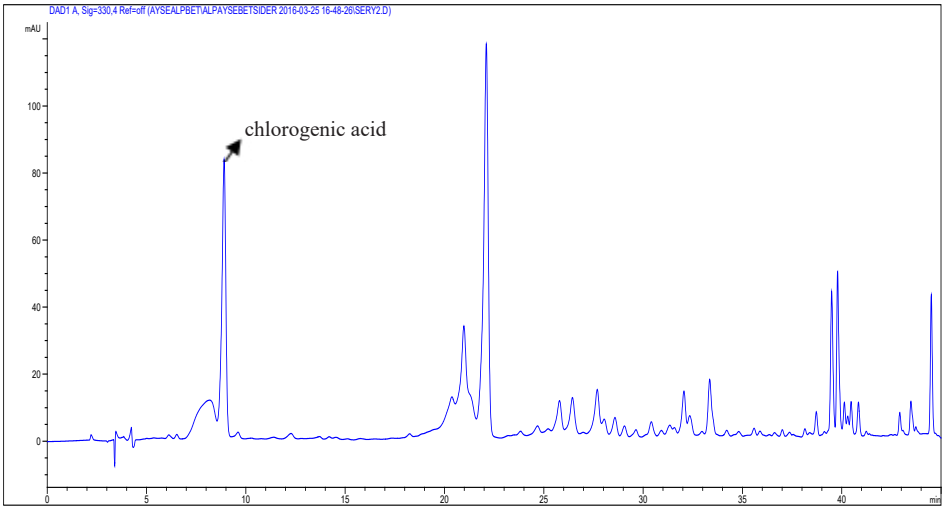

Figure 3. HPLC chromatogram of *S. erythrantha* var. *cedretorum*

The above mentioned data revealed that studied endemic *Sideritis* taxa herein have important biological activities and they are used commonly in various problems as folk remedies. Actually, we suggest that high total phenolic contents and chlorogenic acid levels of the investigated *Sideritis* taxa contribute to these biological activities especially to the antioxidant and antimicrobial potential.

In conclusion, *S. argyrea*, *S. congesta* and *S. erythrantha* var. *cedretorum* with remarkable total phenolic content and antioxidant potential should be evaluated as serious chlorogenic acid sources in various industries, also the standardization of the *Sideritis* extracts should be performed with this validated HPLC procedure in terms of chlorogenic acid. The obtained levels of the phenolic compounds and the chlorogenic acid support the importance of *Sideritis* taxa as good sources of natural antioxidants.

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