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PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN IRONWORT (*Sideritis syriaca* L.) FROM STRANDZHA MOUNTAIN

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Abstract

The current investigation aimed to evaluate total carotenoids, chlorophylls, phenols, flavonoids, as well as the individual phenolic compounds and antioxidant potential in ironwort (Sideritis syriaca L.) collected from Strandzha mountain in Bulgaria and Turkey. Five phenolic acids (chlorogenic, caffeic, p-coumaric, ferulic and sinapic acids) and ten flavonoids (lavandulifolioside, verbascoside, forsythoside A, isoscutellarein 7-O-allosyl(1→2)glucoside, apigenin 7-O-allosyl(1→2)glucoside, isoscutellarein 7-O-allosyl-(1→2)-[6''-O-acetyl]-glucoside, hypolaetin 7-O-allosyl-(1→2)-[6''-O-acetyl]-glucoside 3'-O-Methylhypolaetin 7-O-[6'''-O-acetyl]-allosyl(1→2) glucoside, 4'-O-methylhypolaetin 7-O-[6'''-O-acetyl]-allosyl(1→2)glucoside, apigenin 7-(6''-p-coumaroylglucoside) and apigenin 7-4''-p-coumaroylglucoside) were detected. The samples collected from the Bulgarian part of Strandzha mountain showed 1.5 to 2.5 times higher values for individual phenolic compounds and twice higher antioxidant activity and total carotenoids content in comparison to Turkish samples. Ferulic acid (8173 µg/g dw) and isoscutellarein 7-O-allosyl-(1→2)-[6''-O-acetyl]-glucoside (4452 µg/g dw) were detected in the highest content in Bulgarian ironwort.

***Key words**: Sideritis syriaca L., antioxidant activity, phenolic acids, flavonoids.*

INTRODUCTION

Sideritis syriaca L. (syn.: *Sideritis imbrex* auct. bulg., non Juz.; *Sideritis taurica* auct. bulg.; *Sideritis catillaris* Juz.; *S. taurica* Steph) known also as Strandzhanski chaj, Strandzhanski bilkov chay, Siriiski mirizliv buren, Crimean tea is a perennial herb, 10-50 cm tall that grows on dry calcareous, stony terrains, strongly eroded, in places with an outcropping of the bedrock from 0 to 400 m. It is more common on the karst rocky areas and is massively harvested (Stoyanov et al., 2022) https://www.tuns.eu/flora/; Prodanov, 2019). Strandzhanski bilkov chay (*S. syriaca*) is a protected species and is listed in the Red Book of Bulgaria, volume 1 - category "Endangered". It blooms in yellow during May-June, and its seeds ripen at the end of July-August. In Strandzha it is distributed to the south of Malko Tarnovo in the Turkish part of the mountain (Prodanov, 2019; Veli et al., 2023). Under the synonym *S. taurica* Stephan ex Willd, this species grows in Bulgaria, Turkey, Crimea and Asia (Fraga Citation, 2012; Prodanov, 2019). *Sideritis syriaca* (known also as Cretan mountain tea, Μαλοτήρας Τσαι του βουνού) grows also in Crete, Lebanon, Syria, Turkey (Aneva et al., 2019) and Italy (Menghini et al, 2005). Crimean tea is sold as dried flowers on the market in Kirklareli https://www.tuns.eu/flora/. Strandzha tea reduces the risk of a number of cancers, stroke and heart disease. It has a beneficial effect on high blood pressure, protects against atherosclerosis, strengthens the immune system, has anti-aging properties and has an invigorating effect. Strandja tea is endemic - a type that in Bulgaria is found only in Strandja. It is resistant to mountain climates and does not need care such as digging or spraying, besides it has a natural resistance to cold, frost and ice. (Prodanov, 2019; https://www.tuns.eu/flora/).

Sideritis syriaca has been used as an anti-inflammatory, antimicrobial (espcially significant activity against *S. aureus*, *E. coli, E. faecalis*), antioxidant and analgesic agent in folk medicine (Menghini et al, 2005,

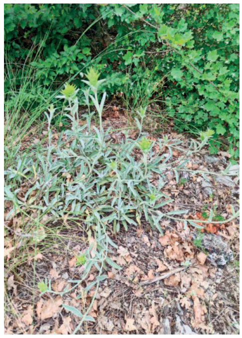
Kostadinova et al., 2008, Goulas et al. 2014, Aneva et al.,2019; Veli et al., 2023). It possesed also antiviral (against paramyxovirus) (Sattar et al., 1995) and anti- analgesic effects (Menghini et al., 2005). It is used as a tonic (Menghini et al., 2005), in Greece it is applied for the treatment of dyspepsia, in Turkey for the treatment of chesty cough and especially in western part of Anatolia as diuretics (Hanlidou et al., 2004; Çarıkçı et al., 2023).
Previous reports on *S. syriaca* were mainly focused on decoction, 95% ethanol, methanol, ethyl acetate, hexane, petroleum ether, butanol, diethyl ether and dichloromethane extract as well as its essential oil (Koleva et al., 2003; Armata et al, 2008; Goulas et al., 2014; Veli et al., 2023). In most of the papers DPPH was used as method to evaluate the antioxidant potential (Armata et al., 2008).
However, even the numerous studies about Balkan endemic *S. syriaca* there also missing gaps about its phytochemical compounds and antioxidant potential especially in plants growing wildly in the protected and unprotected area of Strandzhda mountains. The current research aimed to evaluate total carotenoids, chlorophylls, phenols, flavonoids, as well as the individual phenolic compounds and antioxidant potential in ironwort (*Sideritis syriaca* L.) collected from Strandzha mountain in Bulgaria and Turkey.

MATERIALS AND METHODS

Plant material

The samples of ironwort (*Sideritis syriaca* L.) were collected in summer 2023 in the flowering phase of the plants from natural habitats in the Strandzha mountains in Bulgaria (Strandzha Nature Park, Pic.1; Permit № 977/26.07.2023 from the Ministry of Environment and Water, Republic of Bulgaria) and Turkey (Kozulu, Pic. 2). The localites of the *Sideritis* population in Strandzha Nature Park are the same as reported by Aneva et al. (2012).
The fresh plant material was air-dried at 25°C and finely ground using laboratory homogernizer BN1200AL (Gorenje). The ground material was kept in tightly closed plastic containers for further analysis. The moisture of ironwort (%) was analyzed on moisture analyzers balance Kern DAB 100-3 (Germany).

Picture1. *Sideritis syriaca* L. (Bulgaria)



Picture 2. *Sideritis syriaca* L. (Turkey)

Photo credits: Pl. Zorovski

Ultrasound-assisted extraction

The samples were weighted in a centrifuge tube of 50 ml and extracted with 95% ethanol in solid to liquid ratio 1:20 (w/v) for dry samples. The extraction was performed in an ultrasonic bath (IsoLab 621.05.001, Germany), operating with ultrasonic frequency 40 kHz and ultrasonic power 60W for 20 mins, at 75°C. The obtained extracts were filtered, and the residues were extracted once again under the above mentioned conditions. The both extracts were combined and used for further analysis.

Total chlorophylls and carotenoids

Total chlorophylls and carotenoids were evaluated in 95% ethanol extracts *Sideritis syriaca* using spectrophotometer using the equations described by Lichtenthaler and Wellburn (1983). The concentrations of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll and total carotenoids were calculated and presented as μg/g (dry weight).

Total phenols and flavonoids

The total phenolic content in ironwort (*Sideritis syriaca* L.) extracts was evaluated using the Folin-Ciocalteu reagent (Stintzing et al., 2005), as the ironwort extract (0.2 mL) was mixed with 1 mL Folin-Ciocalteu reagent diluted 1:4 and then 0.8 mL 7.5% Na_2CO_3 was added then measured after 20 min at 765 nm against a blank sample. The results were expressed in mg equivalent of gallic acid (GAE) per g sample (Ivanov et al., 2014).

The total flavonoid content was determined by $Al(NO_3)_3$ reagent (Kivrak et al., 2009) with small modification (Ivanov et al., 2014). The results were reported as mg equivalents quercetin (QE)/g dry sample.

Antioxidant activity

The DPPH radical-scavenging ability, ABTS assay, ferric reducing antioxidant power (FRAP) assay and Cupric reducing antioxidant capacity (CUPRAC) assay of 95% ethanol extracts from *Sideritis syriaca* were performed as previously described (Ivanov et al., 2014). Antioxidant activity was expresse as mM Trolox equivaelt (TE)/g dry weight.

HPLC analysis of phenolic compounds

Individual phenolic acids and flavonoids were analyzed on a HPLC system equipped with Waters 1525 Binary Pump (Waters, Milford, MA, USA), Waters 2484 Dual Absorbance Detector (Waters, Milford, MA, USA), and a C18 column (Supelco Discovery HS, 5 μm, 25 cm × 4.6 mm), and Breeze 3.30 software. For flavonoids, separation gradient mode was used with a mobile phase composed of 2.0% (v/v) acetic acid (solvent A) and methanol (solvent B). The injected volume was 20 μL (Vrancheva et al., 2021). The results were calculated according to calibration curves.

Statistical analysis

Statistical analysis was performed using MS Excel 2010. The data were presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSIONS

Moisture content

The moisture content was 10.74±0.36% for Bulgarian representatives of *Sideritis syriaca collected* from Strandzha mountain. The Turkish polulaton showed slightly higher moisture content 11.18±0.12%. The results were needed for further presentation of the other results to dry weight basis.

Total chlorophylls and carotenoids

The results for presence of chlorophylls and carotenoids were summerizedin Table 1. This the first detailed study about photosynthetic pigments in *Sideritis syriaca* L. collected from Strandzha mountain Bulgaria. The results showed that total chloropylls (230.05±0.1 μg/g dry weight) and total carotenoids (54.96±0.52 μg/g dry weight) dominated in samples collected from Strandzha mountain Bulgaria. Their content was almost twice higher than in the Turkish samples. In all samples chl a dominated among chlorphyll b, as the ratio between Chla/Chlb is 1:7 or 1:8. Usually, Chla/Chlb varies in the range of 1.5-3.0, where lower values indicate adaptation to stress by activating Chlb, while higher values are maintained under normal conditions, as previously decribed for lavender samples growing in organic and conventional farming (Dobreva et al., 2024), Total carotenoids was almost four times lower than values of total chlorophylls. It is well known that foliar Chl content is a good indicator of variousbiotic and abiotic stresses (Li et al., 2017), that could explain the differences in chlorophyll content in both samples.

Phenolic compounds

The results for total phenolic content and total flavonoids were summarized in Table 2. It was obvious that samples collected from location Propada Bulgaria, showed more than 1.5 times higher values than the samples collected from Turkey. It was found that *Sideritis syriaca* from Strandzha mountain, Bulgaria contained total phenols 11.87±0.25mg GAE/g dw and total flavonoids 5.17±0.15 mg QE/g. The levels of total flavonoids were comparable with a previous report for cultivated *Sideritis scardica* from Bulgaria (Yanchev et al., 2022). However, in the current study the values of total phenols were twice lower in comparison with prevous data (Yanchev et al., 2022). Our data for total phenols in *Sideritis syriaca* from Strandzha mountain, Bulgaria were comparable with those reported by Goulas et al. (2014) values for a phenolic content in infusions prepared from Greek *Sideritis syriaca* (Epirus, Greece) equivalent to 1863 mg of GAE per 100 g of dry material. Prodanov (2019) reported for close to our results for cultivated *Syderitis syriaca* samples from Malko Tarnovo total flavonoids 5.73 and 5.65 mg/g, and 6.07 mg/g for cultivated samples on limestone, however the results for wild free growing samples from Golyam Valog and Dokuzak (2.79 and 3.35 mg

RE/g) were more than twice time lower than ours results for samples collected from Propada and comparable with samples from Turkish part of Strandzha. The results for total phenols from these places (Golyam Valog and Dokuzak) were comparable with our results (18.39 and 16.65 mg CAE/g).

Table 1. Natural pigments in the aerial parts of *Sideritis syriaca* L. collected from Bulgaria and Turkey

Sample	Chla, μg/g dry weight	Chlb, μg/g dry weight	Total chloropylls, μg/g dry weight	Total carotenoids, μg/g dry weight	Cha/Chlb
Sideritis syriaca from Strandzha mountains, Bulgaria	149.07±0.15	80.98±0.20	230.05±0.15	54.96±0.52	1.84
Sideritis syriaca from Turkey	92.28±0.14	50.02±0.25	145.29±0.20	27.16±0.26	1.74

Table 2. Total phenols, flavonoids, antioxidant potential of *Sideritis syriaca* L. collected from Bulgaria and Turkey

Sample	Total phenolic content GAE/g	Total flavnoids, mg QE/g	DPPH	ABTS	FRAP	CUPRAC
			mM TE/g dw			
Sideritis syriaca from Strandzha mountains, Bulgaria	11.87±0.25	5.17±0.15	163.09±2.11	226.18±2.62	45.27±0.21	224.55±2.20
Sideritis syriaca from Turkey	7.67±0.12	3.04±0.05	83.07±1.18	73.98±3.15	24.33±0.10	132.87±0.95

Table 3. Content of individual phenolic acids and flavonoids in the aerial parts of *Sideritis syriaca* L., μg/g dry weight

Compounds	*Sideritis syriaca* from Strandzha mountains, Bulgaria	*Sideritis syriaca* from Turkey
Phenolic acids		
Chlorogenic acid	509.8±1.2	201.6±0.9
Caffeic acid	61.1±0.5	29.0±1.1
p-Coumaric acid	447.4±1.6	222.4±0.6
Ferulic acid	8172.5±2.6	7634.7±2.1
Sinapic acid	379.9±0.5	257.9±0.8
Flavonoids		
Lavandulifolioside	281.2	114.6
Verbascoside	201.5	53.9
Forsythoside A	143.5	68.8
Isoscutellarein 7-O-allosyl(1→2)glucoside	2180.3	689.8
Apigenin 7-O-allosyl(1→2)glucoside	471.1	658.4
Isoscutellarein 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside	4452.4	2450.7
Hypolaetin 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside	2343.2	1300.6
3'-O-Methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2)glucoside	3985.3	3749.5
4'-O-Methylhypolaetin 7-O-[6′′′-O-acetyl]-allosyl(1→2)glucoside	246.2	219.5
Apigenin 7-(6"-p-coumaroylglucoside)	411.7	289.9
Apigenin 7-(4"-p-coumaroylglucoside)	485.1	343.2

The detailed content of individual phenolic acids and flavonoids were in Table 3. Five phenolic acids (chlorogenic, caffeic, p-coumaric, ferulic and sinapic acids) were detected in all *Sideritis syriaca* sampes. Ferulic acid was the dominating phenolic acid, found in the highest amount 8172.5 and 7634.7μg/g. Caffeic acid was detected in the lowest values. From flavonoids ten representives were detected, as follows: lavandulifolioside, verbascoside, forsythoside A, isoscutellarein 7-O-allosyl(1→2)glucoside, apigenin 7-O-allosyl(1→2)glucoside, isoscutellarein 7-O-allosyl-(1→2)-[6′′-O-acetyl]-glucoside, hypolaetin 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside 3'-O-Methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2) glucoside, 4'-O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside, apigenin 7-(6"-p-coumaroylglucoside) and apigenin 7-4"-p-coumaroylglucoside). Isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside were dominating compounds in samples collected from Bulgarian part of Strandzha mountain 4452.4 µg/g, followed by 3'-O-Methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2) glucoside with content 3985.3 µg/g. 3'-O-Methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside was the dominating compound in samples collected from Turkish part of Strandzha (3749.5 mg/g dw) and the second in the highest amount in samples collected from Bulgarian part of the mountain. Isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside, hypolaetin 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside, 3'-O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside, apigenin 7-(6"-p-coumaroylglucoside), forsythoside A and lavandulifolioside in *S. syriaca* collected from Bulgarian part of Strandzha mountain was with values almost twice time higher than their content in Turkish samples collected from this mountain. This is first detailed study about individual phenolic and flavonoids compounds in endemitic species samples collected from Strandzha mountains. A recent study reported that in the aerial parts of *Sideritis syriaca* ssp. *syriaca* endemic species in Crete were identified 1-rhamnosyl, 1-coumaroyl, dihydrocaffeoyl, protocatechuic tetraester of quinic acid, as well as chlorogenic acid, apigenin 7-O-glucoside, apigenin, 4′-O-methylisoscutellarein 7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1→2)-β-d-glucopyranoside], isoscutellarein 7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1→2)-β-d-glucopyranoside], 4′-O-methylisoscutellarein 7-O[β-d-allopyranosyl-(1→2)-β-d-glucopyranoside] and 4′-O-methylisoscutellarein 7-O-[β-d-allopyranosyl-(1→2)-6″-O-acetyl-β-d-glucopyranoside] (Armata et al, 2008). Moreover, in extracts from Bulgarian representatives of *S. syriaca* and *S. montana* were identified the following compounds: verbascoside, hypolaetin-4 -methylether-7-O-[6-O-acetyl-β-Dallopyranosyl-(1 →2)-β-D-glucopyranoside] and hypolaetin-4 -methylether-7-O-[6-O-acetylβ-D-allopyranosyl-(1 →2)-β-D-6-O-acetyl-β-Dglucopyranoside] (Koleva et al., 2003). It was reported that hypoelatin and isoscutellarein diglucosides, chlorogenic acid, and three phenylpropanoids were the major constituents of the decoction of *S. syriaca* collected from Greece as isoscutellarein-7-O-[6’’0-O-acetyl-β-D-allopyranosyl-(1 → 2)-β-D-glucopyranoside was detected in the highest amount 184.2 ± 16.6 mg/100 g dw) (Goulas et al., 2014). The detected values or this compound were twice lower than found in our current research. Isoscutellarein-7-O-[6‴-O-acetyl-β-D-allopyranosyl-(1→2)-β-D-glucopyranoside and 4′-Methylhypolaetin-7-O-[6‴-O-acetyl- *β*-D-allopyranosyl-(1→2)-b-D-glucopyranoside were the dominating compounds that bring about almost 50% of antioxidant potential of decotion from Greek *S. syriaca* (Goulas et al., 2014). The main compounds were found in *S. perfoliata* were verbascoside, chlorogenic acid and apigenin 7-glucoside (Çarıkçı, et al.,2023). In our case 4'-O-Methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside and isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside were both dominating compounds in both *S. syriaca* samples collected from Bulgarian and Turkish part of Strandzha mountain.

Antioxidant potential

The antioxidant activity of *Sideritis syriaca* from Bulgarian samples was faintly studied. There are a research which states that the methanolic extracts of its aerial parts were equal antioxidants to rosmarinic acid, when antioxidant power was measured with the DPPH radical dot•test (Koleva et al., 2003). This authors state that the inhibitory effect on β-carotene bleaching of the polar extracts and rosmarinic acid was much lower than that of BHT. Goulas et al., (2014) used DPPH, FRAP and phosphmolybdenum assay to evaluate the antioxidant potential of decoction prepared with Greek *Sideritis syriaca.* Veli et al., 2023 used DPPH method to evaluate antioxidant potential of the extracts prepared with cultivated *Sideritis syriaca* from village Varovnik, Sredets Municipality, Bulgaria. All this showed the gap in analysis of antioxidant potential of wild growing plants from *Sideritis syriaca* from their natural habitat Strandzha mountain. In the current study the results for antioxidant potential of 95% extracts prepared with samples from Bulgarian and Turkish part of the mountain were listed in table 2. Four methods (DPPH, ABTS, FRAP and CUPRAC) based on different mechanism were used to evaluate the antioxidant potential of *Sideritis syriaca.* CUPRAC method that is based on single electron transfer demonstrated the highest andtioxiant potential from both samples (224.55±2.20 and 132.87±0.95 MTE/g dw). ABTS with a mixed mechanism showed the highest results for samples collected from Bulgaria. The lowest results were found for FRAP assay, that is based on single electron transfere and metal ferric reducing properties - 45.27 and 24.33 mMTE/g dw. In general, samples collected from Bulgarian part of Strandzha demonstrated almost twice higher antioxidant potential than Turkish samples (Table 2). This could be explained with the levels of total flavonoids and phenols in these samples.

Veli et al. (2023) reported that methanol and ethanol extracts from cultivated Bulgarian *Sideritis syriaca* demonstrated - 579.3 and 524.3 μM EVCAA/100 μg extract by DPPH method, while Goulas et al. (2014) reported FRAP antioxidant activity for *Sideritis syriaca* decoction (13.4 mmoL AsA/100 g DM). In our case 95% ethanol extracts especially form Propada Bulgaria gave the highest values 163.09±2.11 mMTE/g (DPPH assay) and 45.27±0.21 mM TE/g (FRAP assay). This study demonstrayed that *Sideritis syriaca* showed highest cupper reducing properties and good radical scavenging abitily, than ferric reducing capacity.

CONCLUSIONS

The current study contributes to the better understanding and utilization of the bioactive compounds found in *Sideritis syriaca* L. growing in Strandzha mountain on territry of Bulgaria and Turkey. To the best of our knowledge this is the first detailed study about natural pigments, phenolic acids profile and flavonoid content, antioxidant potential in representatives collected from this protected aria in Strandzha mountain (Bulgaria). Hypolaetin 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside, O-methylhypolaetin 7-O-[6‴-O-acetyl]-allosyl(1→2)glucoside and isoscutellarein 7-O-allosyl-(1→2)-[6″-O-acetyl]-glucoside were there dominating compounds in both *S. syriaca* samples collected from Bulgarian and Turkish part of Strandzha mountain. The detected phenolic acids and flavonoids bring about and enlarge the knowledge about healthy properties of this plant and engourge their cultivation and further utilization in food and cosmetics, due to its antioxidant potential and rich flavonoids profile.

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