Pharmaceutical Biology, 2011; 49(8): 800–804

ISSN 1388-0209 print/ISSN 1744-5116 online
DOI: 10.3109/13880209.2010.550052

informa
healthcare

RESEARCH ARTICLE

Iron(III) reducing and antiradical activities of three *Sideritis* from Turkey

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Abstract

Context: *Sideritis* species (Lamiaceae) are widely used as herbal tea and have been used in folk medicine for their anti-inflammatory, anti-rheumatic, digestive, and antimicrobial activities in Turkey. *Sideritis dichotoma* Huter., *Sideritis erythrantha* Boiss. var. *cedrotorum*, and *Sideritis vuralii* H. Duman et Başer are available as commercial products in Turkey.

Objective: The antiradical activities of the various solvent extracts of *Sideritis* species are investigated here for the first time.

Materials and methods: Plant samples were sequentially extracted with *n*-hexane, dichloromethane, methanol, and aqueous methanol (50%, v/v) in Soxhlet apparatus. The extracts of *Camellia sinensis* (L.) Kuntze (Theaceae) were also prepared for use as a positive control. Total phenolics, iron(III) reductive effects, and 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical scavenging activities of the all extracts were measured colorimetrically.

Results: The aqueous MeOH and MeOH extracts contained the highest amount of total phenols, whereas the *n*-hexane extract contained the lowest amounts. The polar extracts of *C. sinensis* showed higher antiradical activity and also iron(III) reductive effects than the *Sideritis* species; however, the non-polar extracts of *Sideritis* species were found to be more active than those from *C. sinensis* in the iron(III) reductive assay and in the $DPPH^{\cdot}$ assay as well. But none of the extracts was found to be as active as with positive controls, viz., ascorbic acid, butylated hydroxyanisole (BHA), and Trolox.

Discussion and conclusion: These results can be shown to have antioxidant activities of these *Sideritis* species and support the ethnopharmacological use of these *Sideritis* plants.

Keywords: *Sideritis dichotoma*, *Sideritis erythrantha* var. *cedretorum*, *Sideritis vuralii*, antiradical activity, iron(III) reduction activity, total phenol content, lamiaceae

Introduction

The Lamiaceae family is comprised of 200 to 250 genera and between 3200 and 6500 species (Anon, 2003a, b). In the Mediterranean region, it is particularly well-represented, for example, in Turkey 556 species and 741 taxa are associated with this family (Davis, 1982). The taxa appear to possess a variety of exploitable beneficial properties, including anti-inflammatory and antioxidant activities among others (Ismaili et al., 2002; Dorman et al., 2004). The aerial parts may be added to foodstuffs to improve their organoleptic properties and are often consumed as teas for their restorative properties or as ingredients in folk medicines for the treatment of various ailments.

One genus of Lamiaceae, *Sideritis*, is represented in Turkey by 52 taxa belonging to ~44 species, of which 34 are endemic (Başer, 2002). The high rate of endemism is due to the fact that Turkey is one of the two main gene centers of the genus, the other being the Iberic peninsula (Başer, 2002). *Sideritis* species are widely used as herbal

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(Received 16 September 2010; revised 25 November 2010; accepted 18 December 2010)

teas and have been used in folk medicine for their anti-inflammatory, anti-rheumatic, digestive, and antimicrobial activities in Turkey (Kirimer et al., 1996). Despite many publications reporting the volatile oil composition of *Sideritis* species, reports of their nonvolatile chemistry or potential beneficial properties are underrepresented in the scientific literature (Koleva et al., 2003; Tunalier et al., 2004; Gabrieli et al., 2005). Thus, we report the results of a total phenolic content analysis, the iron(III) reductive and antiradical activities of three *Sideritis* (*Sideritis dichotoma* Huter., *Sideritis erythrantha* Boiss. var. *cedrotorum*, and *Sideritis vuralii* H. Duman et Başer) species harvested in Turkey. The results are compared with the activity of fermented tea [*Camellia sinensis* (L.) O. Kuntze (Theaceae)] and standard antioxidant compounds, viz., ascorbic acid, butylated hydroxyanisole (BHA), and Trolox.

Materials and methods

Plant material and reagents

Dried aerial parts of three *Sideritis* and *C. sinensis* samples were obtained from commercial sources. Ultrapure water (18.2 MΩcm) was used throughout and was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., Danver, MA). All standards and reagents were of the highest purity available and obtained from the Sigma Chemical Co. (St. Louis, MO).

Preparation of the extracts

Air-dried *Sideritis* and tea herb material (100 g) was powdered and sequentially extracted with hexane, dichloromethane (CH_2Cl_2), methanol (MeOH), and 50% (aqueous) MeOH using a Soxhlet apparatus for 8 h. Afterward, the extract was filtered (Whatman No. 4) and evaporated to dryness *in vacuo* at (40°C) and freeze-dried. All the extracts were stored at –20°C until required for analysis.

Total phenolic content

The total phenol content was estimated as gallic acid equivalents (GAE), expressed as mg of gallic acid/g extract (Singleton et al., 1999). To ~6.0 mL of H_2O, 100 µL of sample was transferred into a 10.0 mL volumetric flask, to which 500 µL undiluted Folin-Ciocalteu reagent was subsequently added. After 1 min, 1.5 mL 20% (w/v) Na_2CO_3 solution was added and the volume was made up to 10.0 mL with H_2O. After 2 h incubation at 25°C, the absorbance was measured at 760 nm and compared with a gallic acid calibration curve. The data are presented as the average of triplicate analyses.

Iron(III) to iron(II) reduction activity

The ability of the extracts to reduce iron(III) was assessed by the method of Oyaizu (1986). Each extract (1 mL), dissolved in H_2O, was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) potassium hexacyanoferrate solution. After 30-min incubation at 50°C, 2.5 mL 10% (w/v) trichloroacetic acid was added and the mixture was centrifuged for 10 min. Finally, 2.5 m of the upper layer was mixed with 2.5 mL H_2O and 0.5 mL 0.1% (w/v) $FeCl_3$ solution and the absorbance was recorded at 700 nm. The reductive activities of the extracts are estimated as ascorbic acid equivalents (AscAE) that is expressed as mmol ascorbic acid/g sample (Dorman et al., 2003). The larger the AscAE value, the greater the reducing power of the sample. The data are presented as the average value of quadruplicate analyses.

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

The ability of the extracts to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radicals was determined by the method of Gyamfi et al. (1999). A 50 µL aliquot of each extract, in 50 mM Tris–HCl buffer (pH 7.4), was mixed with 450 µL of Tris–HCl buffer and 1.0 mL of 0.1 mM DPPH• in MeOH. After 30-min incubation in the dark and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using Equation (1). The estimated IC_{50} values are presented as the average of quadruplicate analyses.

$$\text{Percentage inhibition} = [(\text{Abs}_{\text{control}} \quad \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100 \qquad (1)$$

Statistical analyses

All statistical analyses were carried out using Minitab Release 10.5 Xtra for Windows (Minitab Inc., State College, PA). Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $P < 0.05$.

Results

Sideritis and fermented tea herbs were sequentially extracted with hexane, CH_2Cl_2, MeOH, and aqueous MeOH (50% v/v) using a Soxhlet apparatus. The results for the fraction yields, total phenols, iron(III) reduction, and DPPH• radical scavenging activity of the extracts are presented in Table 1. According to the data presented in Table 1, the aqueous MeOH and MeOH extracts contained the highest amount of total phenols, whereas the hexane extract contained the lowest amounts. The highest yields were also obtained from the MeOH extracts.

The hierarchy of iron(III) reductive capacity for the hexane-soluble extracts was: *S. dichotoma* > *S. vuralii* > *S. erythrantha* var. *cedrotorum* > *C. sinensis*; for the CH_2Cl_2-soluble extracts: *S. erythrantha* var. *cedrotorum* > *S. vuralii* > *S. dichotoma* > *C. sinensis*; for the MeOH-soluble extracts: *C. sinensis* > *S. vuralii* > *S. erythrantha* var. *cedrotorum* > *S. dichotoma*; and for the aqueous MeOH-soluble extracts: *C. sinensis* > *S. dichotoma* > *S. vuralii* > *S. erythrantha* var. *cedrotorum* (Table 1). None of the *Sideritis* extracts were as effective as the positive

Table 1. Fraction yields, total phenols, iron(III) reduction, and DPPH radical scavenging activity data for the *Sideritis* extracts and positive controls.

Standards/extracts	EY[a]	TP[b]	AscAE[c]	DPPH•[d]
Ascorbic acid	—	—	5678.7 ± 143.4	0.08 ± 0.00
BHA	—	—	2507.0 ± 35.5	0.06 ± 0.00
Trolox	—	—	1780.4 ± 11.1	0.09 ± 0.00
C. sinensis				
n-Hexane	8.7	20.9 ± 3.1	13.6 ± 1.5	nd[e]
CH_2Cl_2	2.0	35.3 ± 1.5	75.4 ± 9.9	nd
CH_3OH	236.3	261.0 ± 7.1	765.6 ± 10.4	0.30 ± 0.01
CH_3OH:H_2O	112.3	400.5 ± 26.7	801.6 ± 48.0	0.20 ± 0.02
Σ	**359.3**	**717.7 ± 27.8**	**1656.2 ± 50.1**	**0.5 ± 0.02**
S. dichotoma				
n-Hexane	17.7	29.5 ± 1.2	58.4 ± 6.5	nd
CH_2Cl_2	4.3	57.1 ± 2.5	104.5 ± 12.1	6.25 ± 0.10
CH_3OH	114.9	137.5 ± 3.7	282.5 ± 11.5	1.32 ± 0.07
CH_3OH:H_2O	27.7	114.0 ± 3.0	363.2 ± 18.3	0.59 ± 0.01
Σ	**164.6**	**227.1 ± 5.5**	**808.6 ± 25.6**	**8.16 ± 0.10**
S. erythrantha var. *cedrotorum*				
n-Hexane	42.4	23.6 ± 0.4	27.8 ± 1.1	nd
CH_2Cl_2	5.9	57.4 ± 3.5	115.3 ± 7.4	5.90 ± 0.19
CH_3OH	161.8	195.8 ± 6.5	312.1 ± 16.1	0.78 ± 0.02
CH_3OH:H_2O	63.8	132.80 ± 3.1	179.7 ± 2.7	1.84 ± 0.14
Σ	**273.9**	**220.3 ± 8.0**	**634.9 ± 18. 0**	**8.52 ± 0.24**
S. vuralii				
n-Hexane	11.5	23.3 ± 0.6	32.4 ± 0.8	nd
CH_2Cl_2	4.2	53.6 ± 1.0	111.8 ± 7.4	7.09 ± 1.98
CH_3OH	150.3	119.8 ± 2.7	333.9 ± 25.6	0.82 ± 0.02
CH_3OH:H_2O	64.0	147.96 ± 3.3	221.6 ± 17.7	1.30 ± 0.19
Σ	**230**	**343.86 ± 4.4**	**699.7 ± 32. 0**	**9.21 ± 1.99**

Data are mean values ± 95% confidence limits.
[a]EY, extract yield (mg of extract per gram of plant material).
[b]TP, total phenols (mg gallic acid per gram of extract).
[c]AscAE, ascorbic acid equivalents (μmol ascorbic acid per gram of extract).
[d]DPPH radical scavenging (IC_{50} concentration, mg of extract per mL).
[e]nd: not determined.

control substances. However, when compared with the fermented tea control, all the *Sideritis* hexane-soluble extracts and the *S. dichotoma*, *S. erythrantha* var. *cedrotorum*, and *S. vuralii* CH_2Cl_2-soluble extracts were significantly ($P < 0.05$) better reducers of iron(III) than the corresponding tea extracts. Despite this, the MeOH and aqueous MeOH-soluble *C. sinensis* extracts were significantly ($P < 0.05$) more potent than the corresponding *Sideritis* extracts.

The hierarchy of DPPH• radical scavenging activity for the CH_2Cl_2-soluble extracts was: *S. erythrantha* var. *cedrotorum* > *S. dichotoma* > *S. vuralii* > *C. sinensis*; for the MeOH-soluble extracts: *C. sinensis* > *S. erythrantha* var. *cedrotorum* > *S. vuralii* > *S. dichotoma*; and for the aqueous MeOH-soluble extracts: *C. sinensis* > *S. dichotoma* > *S. vuralii* > *S. erythrantha* var. *cedrotorum* (Table 1). None of the *Sideritis* extracts were as effective as the positive control substances. The hexane extracts of the *Sideritis* and tea samples were not determined in this assay, because of their solubility. MeOH and aqueous MeOH-soluble *C. sinensis* extracts were significantly ($P < 0.05$) more potent than the corresponding *Sideritis* extracts.

Discussion

There were no records found in the literature about the antioxidant properties of the extracts of *Sideritis* species with which we worked in this study. Only essential oil-based research has been carried out on these specific species (Kirimer et al., 1992; Tabanca et al., 2001). Therefore, this is the first study on the iron(III) reductive and antiradical activities of *S. dichotoma*, *S. erythrantha* var. *cedretorum*, and *S. vuralii*. *Sideritis* species are used as herbal tea throughout the world and especially in Turkey. Furthermore, *Sideritis* species are used in the food and drug industries as they are considered to play an important role in preventing some chronic diseases. Perhaps, this is due to the fact that these species are known to be rich in flavonoids and hydroxycinnamic acids more than other types of phenolic compounds (Triantaphyllou et al., 2001).

The ability of a sample to reduce iron(III) is considered to represent its ability to donate electrons (Yildirim et al., 2000). The reduction is a very important mechanism in the termination of deleterious free radical chain reactions (Yildirim et al., 2000). The literature suggests that there is a high correlation between iron(III) to iron(II) reduction activity by aqueous plant extracts and antioxidant activity (Dorman et al., 2003, 2004; Kosar et al., 2005); however, this may not always be the case (Yildirim et al., 2000). In the iron(III) reduction assay, the ability of an extract to participate in redox reactions can be assessed and ranked according to its AscAE value and expressed as μmol ascorbic acid/g extract (Oomah & Mazza, 1996; Singleton et al., 1999; Dorman et al., 2003). The ability of all the extracts to reduce ferric iron to ferrous iron was investigated and the results are shown in Table 1 as AscAE values. Accordingly, none of the fractions was as effective as the positive controls ascorbic acid, BHA, and Trolox. The ability of the aqueous MeOH extracts on the reduction of iron(III) to iron(II) were found higher for *C. sinensis* and *S. dichotoma*; however, the MeOH extracts of *S. erythrantha* var. *cedretorum* and *S. vuralii* were higher than the others. The hierarchy of total reductive activity of the plants was *C. sinensis* > *S. dichotoma* > *S. vuralii* > *S. erythrantha* var. *cedretorum*. Phenolic acids and flavonoids are well-known as natural antioxidants. Phenolic acids especially hydroxycinnamates and flavonoids show their antioxidant activities principally by a hydrogen-donating mechanism (Rice-Evans et al., 1996; Gu & Weng, 2001). Both phenolic acids and flavonoids are soluble in polar solvents and show strong activity in polar test systems. Both iron(III) reduction and DPPH• radical scavenging activities are performed in a polar media.

DPPH• is a stable free radical used to estimate the antiradical activities of the plant extracts especially those rich in polar compounds. Polar phenolic compounds used for their antioxidant properties can donate an electron to the DPPH radical that can be monitored colorimetrically, viz., the purple color of radical changes to yellow. This difference can be quantified spectrophotometrically at 517 nm to calculate the antiradical activity of the samples (Charami et al., 2008; Kosar et al., 2008). This interaction indicates its radical scavenging ability in an iron-free system. In cases where the structure of the electron donor is not known (e.g. as in plant extracts), this method can afford data on the reduction potential of the sample, and hence can be helpful in comparing the reduction potential of unknown materials (Charami et al., 2008). In this study, either MeOH or aqueous MeOH extracts of *Sideritis* and fermented tea showed the most reductive activity on the iron(III) or radical scavenging activity. In the literature, same results reported for the polar extracts of different *Sideritis* species contained flavonoid aglycones and glycosides (Charami et al., 2008).

All Lamiaceae plants have hydroxycinnamic acids and flavonoids as the main phenolic compounds (Triantaphyllou et al., 2001). According to the literature, cinnamic acid derivatives were found to have been more active than the benzoates, because cinnamates may occur due to the presence of the conjugated unsaturation that facilitates the delocalization of the resulting free radicals. Cinnamic acids and caffeic acid derivatives had better activity than ferulic and coumaric acids (Ho et al., 2000; Kosar et al., 2008). Flavonoids have relatively weak DPPH• radical scavenging activity and glycosylation has been reported to have decreased radical scavenging activity (Lu & Foo, 2001; Kosar et al., 2008). Luteolin derivatives are more active than apigenin derivatives in DPPH• assay. This activity depends on hydroxylation, especially *ortho*-dihydroxylation, on the phenol ring (Lu and Foo, 2001). Most of the works carried out are based on the use of different solvents such as *n*-hexane, diethyl ether, ethyl acetate, acetonitrile, MeOH, and water. Polar extracts were found to be richer in polar phenolic compounds, therefore more effective in scavenging of free radical than the non-polar extracts such as *n*-hexane and ethyl acetate (Charami et al., 2008). In this study, the MeOH and aqueous MeOH extracts were also found to be more active in radical scavenging activity assay than the hexane and CH_2Cl_2 extracts.

The genus *Sideritis* contains polyphenolics such as flavonoids, especially flavones and flavonol glycosides. The freeze-dried extract of *Sideritis*, before and after hydrolysis, were found to be rich in bound forms of phenolic compounds such as 5,8,3'-trihydroxy-4'-methoxyflavone, 7-(6″-*O*-acetylsophoroside) together with apigenin 7-(6″-*p*-coumaroylglucoside), and apigenin 7-(4″-*p*-coumaroylglucoside) (Özkan et al., 2005). Petreska et al. (2011) detected the hydroxycinnamic acids, such as 3-caffeoylquinic acid, 5-caffeoylquinic acid, and feruloylquinic acid, five flavonoid 7-*O*-diglycosides of apigenin, hypolaetin, 3'-*O*-methylhypolaetin, isoscutellarein, and 4'-*O*-methylisoscutellarein, and 11 acetylated flavonoid 7-*O*-diglycosides in some *Sideritis* species from Macedonia. These phenolic compounds were tested for antioxidant and antibacterial properties.

Conclusions

All the *Sideritis* species assessed in this study possessed the ability to reduce iron(III) ions to iron(II) and thus can be described as containing compounds capable of participating in redox reactions through a mechanism of electron transfer. The more polar the extract, the better the iron(III) reducing power appeared to be. A calculation of each species overall reductive activity showed that the most potent *Sideritis* species was *S. dichotoma* (808.6 ± 25.6 AscAE) followed by *S. vuralii* (699.7 ± 32.0 AscAE), and *S. erythrantha* var. *cedrotorum* (634.9 ± 18.0 AscAE); however, the *Sideritis* species demonstrated a lower efficacy than the fermented tea sample (1656.2 ± 15.9 AscAE). All the extracts contained varying amounts of phenolic compounds, as estimated by the Folin-Ciocalteu reagent method. As with the iron(III) reductive ability, the total phenolic content appeared to have increased with increasing extract polarity. In fact,

there was a strong overall linear association between these two antioxidant indices ($r^2 = 0.8287$, $P < 0.0001$). The antiradical activities of the same *Sideritis* species and extracts were investigated against the 1,1-diphenylpicryl-2-hydrazyl free radical using decolorization assay. The total scavenging activities of the extracts were calculated as IC_{50} values. The IC_{50} order of the samples were *S. dichotoma* (8.16 ± 0.10 mg/mL) > *S. erythrantha* var. *cedretorum* (8.52 ± 0.24 mg/mL) > *S. vuralii* (9.21 ± 1.99 mg/mL); however, the *Sideritis* species demonstrated a lower scavenging activity than the fermented tea sample (0.50 ± 0.02 mg/mL). The present findings would be useful for leading to further work on the development of antiradical products to protect against certain diseases.

Declaration of interest

The authors acknowledge the financial support of the Paulig Group Ltd., Finland.

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