

Antioxidant and antibacterial activities of Turkish endemic *Sideritis* extracts

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RESUMEN

Actividades antibacteriana y antioxidante de extractos de especies endémicas turcas de *Sideritis*.

Las especies de *Sideritis* se usan tradicionalmente para la elaboración del té, como flavorizantes y con fines médicos en Turquía. En este estudio, se han determinado las actividades antimicrobiana y antioxidante de extractos de especies endémicas de la Familia Lamiaceae, como son *Sideritis condensata* Boiss. & Heldr. (SC) y *Sideritis erythrantha* var. *erythrantha* Boiss. & Heldr. (SE). La actividad antibacteriana fue determinada mediante el método de difusión en agar con 15 especies de bacterias: *Aeromonas hydrophila*, *Bacillus cereus*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus* y *Yersinia enterocolitica*. Se consideraron diferencias estadísticamente significativas cuando $p < 0,05$. El extracto de SC tuvo mayor actividad antimicrobiana que el extracto de SE. La bacteria más sensible fue *P. aeruginosa*, mientras que las más resistentes fueron *E. faecalis* para el extracto de SC, y *E. faecalis* y *S. aureus* para el extracto de SE. También se determinó en los extractos el contenido de polifenoles, la capacidad de secuestrar radicales libres mediante el método del 1,1-difenil-2-picrilhidrazilo (DPPH), y la capacidad antioxidante mediante el método de formación del complejo fosfomolibdeno. Los compuestos fenólicos totales fueron 247,62±1,91 mg de equivalentes de ácido gálico (GAE)/g de extracto seco en SC, y 217,61±0,95 mg GAE/g de extracto seco en SE. A la concentración de 100 ppm, se observó una actividad secuestradora de radicales libres de 72,01±1,93% y 71,48±1,95% para los extractos de SC y SE, respectivamente. La actividad antioxidante mediante el método del fosfomolibdeno fue de 279,37±3,61 y 146,11±3,11 mg/g de extracto seco de SC y SE, respectivamente.

PALABRAS-CLAVE: Actividad antimicrobiana - Actividad antiradicales - Antioxidante - Extractos de *Sideritis*

SUMMARY

Antioxidant and antibacterial activities of turkish endemic *Sideritis* extracts.

Sideritis species are traditionally used as teas, flavoring agents and for medicinal purposes in Turkey. In this study, the antioxidant and antimicrobial activities of *Sideritis condensata* Boiss. & Heldr. (SC) and *Sideritis erythrantha* var. *erythrantha* Boiss. & Heldr. (SE) endemic species' extracts of Lamiaceae were determined. These extracts were investigated for antibacterial

activity by using the agar diffusion method against 15 species of bacteria: *Aeromonas hydrophila*, *Bacillus cereus*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica*. Statistical differences within bacteria were significant at $p < 0,05$. SC extract was more effective than the other. At all concentrations, although the most sensitive bacteria was *P. aeruginosa*, the most resistant bacteria were *E. faecalis* for SC extract and *E. faecalis*, *S. aureus* for SE extract. Total phenolics, radical scavenging activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method and the antioxidant capacities by the formation of phosphomolybdenum complex method of the extracts were determined. The total phenolics were found as 247.62±1.91 mg gallic acid equivalent (GAE)/g in SC extract and 217.61±0.95 mg GAE/g in SE extract of dried weight. At 100 ppm concentration, the free radical scavenging activities of SC and SE extracts were found as 72.01±1.93 and 71.48±1.95%, respectively. The antioxidant activities by phosphomolybdenum method of SC and SE extracts were 279.37±3.61 and 146.11±3.11 mg/g dried extract.

KEY-WORDS: Antibacterial activity - Antioxidant - Antiradical activity - *Sideritis* extracts

1. INTRODUCTION

Sideritis condensata Boiss. & Heldr. (SC) and *Sideritis erythrantha* Boiss. & Heldr. var. *erythrantha* (SE) taxa, also known as mountain tea, are perennial herbs that grow in the mountainous regions between southern Europe and the eastern Mediterranean. About 100 species of *Sideritis* have been distinguished in the world. In Turkey, the herbs of most *Sideritis* species (Özhatay, et al., 1998), including 24 endemics (and two endemic subspecies) were found to be collected at least locally (Özçelik, 2000).

The Genus *Sideritis* contains antimicrobial and antioxidant 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) (Palomino et al., 1996; Venturella et al., 1995; Gomez-Serranillos et al., 1998).

Mountain tea contains a fragrant oil and other aromatic substances that are widely used as tonics, carminatives, diuretics and digestives, and in the treatment of colds (Baytop, 1984; Koedam, 1986; Yesilada and Ezer, 1989; Ezer et al., 1991). Some studies on the essential oil composition of mountain tea have been carried out and results have been shown in literature (Kirimer et al., 1996; Baser et al., 1997; Özcan et al., 2001). In addition to this, the tea is pleasantly aromatic and the essential oils and extracts of this aromatic herb have been shown to have potent antioxidant activity in lipid substrates (Lemberkovics et al., 1998). Several active ingredients of *Sideritis* have also been synthesized for their antimicrobial properties. The *in vitro* antimicrobial activity of the essential oils against six bacteria and three fungi has also been reported (Aligiannis et al., 2001). *Sideritis* medicinal decoctions have been shown to exert their anti-inflammatory effects through two different mechanisms. *Sideritis* also contains diterpenoids, especially andalusol previously described (Navarro et al., 1996).

Although, as antimicrobial compounds, many species and their derivatives have been studied in detail against important human pathogenic bacteria (Sagdiç et al., 2002; Sagdiç and Özcan, 2003; Özkan et al., 2003), there are no research reports on the antimicrobial and antioxidant effect of *Sideritis condensata* and *S. erythrantha* var. *erythrantha* extracts. In this study, it was aimed to determine the antioxidant and antimicrobial activity of some endemic *Sideritis* extracts.

2. MATERIALS AND METHODS

2.1. Plant materials

The aerial parts of mountain teas of *Sideritis condensata* (SC) and *S. erythrantha* var. *erythrantha* (SE), endemics for Turkey, were collected during the flowering stage from Isparta, Sütçüler city, the southwestern part of Turkey in August, 2002 at altitudes of 1400–1600 m. The plants were identified by Dr. H. Özçelik, Director of the Herbarium Section, Isparta. Voucher specimens were deposited at the Herbarium of the University of Süleyman Demirel, Isparta, Turkey.

2.2. Preparation of the herb extracts

Dried herb at room temperature was ground to fine powder with a grinder. Then the powdered plant material (10 g) was extracted in a Soxhlet extractor

with 100 ml methanol (60 °C for 6 h) to obtain an extract. The sample was then filtered through Whatman No 1 paper in a Buchner funnel and the filtered solution was evaporated at reduced pressure (Rotavator, T<40 °C) to obtain crude extract. They were kept in a desiccator until use (Gu and Weng, 2001). The extractions were performed in duplicate.

2.3. Determination of total phenolics

The concentration of phenolic compounds in the extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolics were expressed as gallic acid equivalents (mg GAE/g extract).

2.4. Antiradical activity determination

A 1.0 ml methanolic solution of the extracts and butylated hydroxytoluen (BHT) at 100 ppm (methanol for the control) was placed in a test tube, and 2.0 ml of a α , α -diphenyl- β -picrylhydrazyl (DPPH) methanolic solution (10 ppm) was added. The absorbance was measured at 517 nm after 5 min of reaction. The percentage of DPPH decoloration of the samples was calculated according to the formula (Lee et al., 1998):

$$\text{Antiradical activity} = 100 \times (1 - \text{absorbance of sample} / \text{absorbance of control})$$

2.5. Evaluation of antioxidant activity

The antioxidant activity of the samples was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). An aliquot of 0.4 ml of sample solution (100 ppm in methanol) was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

2.6. Bacterial cultures

The fifteen bacteria were used as test organisms: *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* FMC 19, *Enterobacter aerogenes* CCM 2531, *Enterococcus faecalis* ATCC 15753, *Escherichia coli* DM, *Escherichia coli* O157:H7 KUEN 1461, *Klebsiella pneumoniae* FMC 5, *Mycobacterium smegmatis* RUT, *Proteus vulgaris* FMC 1, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens*

EU, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus* Cowan 1 and *Yersinia enterocolitica* EU. These bacteria were supplied by the Department of Biology, Sütçü Imam University, Kahramanmaraş-Turkey.

2.7. Determination of antibacterial effect

P. vulgaris, *P. aeruginosa*, *P. fluorescens*, and *Y. enterocolitica* were grown in nutrient broth (Acumedia Manufacturers, Inc., Maryland) at 25 °C for 22h. The other bacteria were grown in the same medium at 35 °C for 22 h (Ilçim et al., 1998). All test bacteria in nutrient broth were enumerated by using the serial dilution method. Final cell concentrations were 10^6 - 10^7 cfu/ml. 250 µl of each bacteria was added into a flask containing 25 ml sterile nutrient agar at 45 °C and poured into Petri dishes (8 cm diameter). Then the agar was allowed to solidify at 4 °C for 1 h. The agar diffusion method was used to detect the antibacterial activity of the extracts. Four equidistant holes were made in the agar using sterile cork borers ($\varnothing = 4$ mm). 50 µl of 1, 2.5, 5, and 10% volume of methanolic (Merck-Darmstadt, Germany) extract solutions was added to the holes using a pipettor and absolute methanol was used as control. The *P. vulgaris*, *P. aeruginosa*, *P. fluorescens*, and *Y. enterocolitica* bacteria test plates were refrigerated at 8 °C for 1 h and then incubated at 25 °C for 18-24 h in the inverted position. The other bacteria were grown in the same media at 35 °C for 18-24 h. At the end of the period, inhibition zones which formed on the medium were measured in millimetres (mm). All the tests were performed in triplicate.

2.8. Statistical analyses

SPSS 10.0 was used to compare the data and all the tests were considered statistically significant at $p < 0.05$ (Özdamar, 1999).

3. RESULTS AND DISCUSSION

In this study, total yields (%), total phenolics (mg GAE/g), antiradical activities (%), antioxidant activities (mg/g) and antibacterial effects of two *Sideritis* extracts were determined. The percent yields of the extracts were determined as 4.77 ± 0.93 for SE and 6.47 ± 0.95 for SC.

The amounts of total phenolics extracted with methanol were determined as 217.61 ± 0.95 mg GAE/g in dried weight of SE extract and 247.62 ± 1.91 mg GAE/g in SC extract. Statistically, differences between total phenolics of the two extracts were significant. SC extract had total phenolics higher than SE extract.

At 100 ppm concentration, the free radical scavenging activity of SC and SE extracts were found as 72.01 ± 1.93 and $71.48 \pm 1.95\%$, respectively. It was found that BHT (as control) antiradical activity was as $39.02 \pm 1.02\%$ at the same concentration. Statistically no differences were found between the two extracts for antiradical activity. The activity of the extracts is attributed to their hydrogen donating ability (Shimada et al., 1992). It is well known that free radicals cause auto oxidation of unsaturated lipids in food (Kaur and Perkins, 1991). In comparison with BHT, these data obtained reveal that the extracts are free radical inhibitors and primary antioxidants that react with the radicals. Koleva et al. (2003) found ranges from 90 to 94% for *S. scardica*, *S. montana* and *S. syriaca* methanolic extracts, respectively.

The antioxidant activities of SC and SE extracts showed 279.37 ± 3.61 and 146.11 ± 3.11 mg/g dried extract (equivalent to ascorbic acid), respectively. Koleva et al. (2003) found that antioxidant activity (β -carotene bleaching test method) of *Sideritis scardica*, *S. montana* and *S. syriaca* methanolic extracts changed from 48.2 to 72.5%. Statistical differences between the two extracts were important for antioxidant activity analyses. The method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex with maximum absorption at 695 nm (Prieto et al., 1999). The reducing properties are generally associated with the presence of reductones and the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom (Gordon, 1990; Pin-Der Duh, 1998). According to Jayaprakasha et al. (2003), the antioxidant activity of different extracts may depend on the presence of polyphenols which may act as reductones.

The effects of extracts at 10, 5, 2.5 and 1% on the test bacteria are presented in Table I. Methanol (control) had no inhibitory effects on the fifteen bacteria tested, and the extracts at the lowest (2.5 and 1%) concentrations were also the least effective. Statistical differences within bacteria were important. While, the SC extract at 10 and 5% concentration exhibited antibacterial effects against all the bacteria except *E. faecalis*, the SE extract at the same concentration exhibited antibacterial effects against all the bacteria except *S. aureus* and *E. faecalis*. SC extract was more effective than SE. At all concentrations, the most sensitive bacterium was *P. aeruginosa*, the most resistant bacteria were *E. faecalis* to SC extract and *E. faecalis*, *S. aureus* to SE extract. Similarly, Kiliç et al. (2003) reported that *Sideritis aethiops*, *Sideritis trojana*, *Sideritis dichotoma*, *Sideritis spilyea*, and *Sideritis argyrea* essential oils were active against *E. coli*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. faecalis*. On the contrary,

Table I
Antibacterial activity of two *Sideritis* extracts (inhibition zones, mm)

Bacteria	Concentrations (%)							
	SC extract				SE extract			
	10	5	2.5	1	10	5	2.5	1
<i>A. hydrophila</i>	17±2.93	13±2.12	11±5.00	7±2.14	11±2.83	8±2.42	6±3.06	-
<i>B. cereus</i>	15±1.86	14±2.41	8±2.14	7±2.41	12±1.77	10±2.46	9±3.04	-
<i>E. aerogenes</i>	13±3.22	8±1.88	8±2.45	-	9±1.78	8±2.10	7±2.15	-
<i>E. faecalis</i>	-	-	-	-	-	-	-	-
<i>E. coli</i>	15±1.42	13±3.11	11±0.65	7±2.56	11±3.14	8±1.29	7±2.18	6±2.77
<i>E. coli</i> O157:H7	13±1.21	12±2.18	10±0.41	7±2.44	8±2.48	7±0.48	7±2.21	-
<i>K. pneumoniae</i>	12±0.99	12±2.13	10±0.76	6±1.18	12±1.00	10±1.29	7±0.45	-
<i>M. smegmatis</i>	14±1.26	12±2.13	12±1.21	7±1.74	10±0.78	8±2.35	8±1.64	6±2.41
<i>P. vulgaris</i>	16±2.03	9±1.16	8±2.14	6±2.85	10±1.46	8±2.11	7±1.64	6±2.63
<i>P. aeruginosa</i>	17±2.00	14±0.98	12±1.89	9±3.00	19±1.48	17±2.78	11±3.01	7±2.01
<i>P. fluorescens</i>	14±3.01	12±0.42	9±0.78	6±3.47	16±1.19	12±2.99	10±3.18	-
<i>S. enteritidis</i>	17±1.78	13±2.99	10±1.32	6±0.98	8±0.74	8±1.99	7±2.58	-
<i>S. typhimurium</i>	14±1.66	12±2.16	11±1.56	6±1.19	13±1.12	12±0.96	8±2.75	-
<i>S. aureus</i>	7±2.46	6±3.03	6±0.87	-	-	-	-	-
<i>Y. enterocolitica</i>	8±2.11	7±2.41	-	-	7±1.45	-	-	-

^a Values expressed are mean standard deviation of three experiments (p)
- : not detected

Hernandez-Perez and Rabanal Gallego (2002 a,b) found no relevant antimicrobial activity of the antimicrobial activities of crude ethanol extracts of *Sideritis lotsyi* var. *mascaensis* and *Sideritis canariensis* var. *pannosa* against the microorganisms tested such as *S. aureus*, *B. cereus* var. *mycoides*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*.

Generally, the extent of the antioxidant and antibacterial effects of the genus *Sideritis* contained in the extracts could be attributed to their phenolic composition such as flavonoids (Palomino et al., 1996; Navarro et al., 1996; Venturella et al., 1995).

Our results suggest that the *Sideritis* extracts drunk as tea in Turkey may be used as antibacterial agents to prevent the deterioration of stored foods by bacteria, if any organoleptic effects are acceptable. In addition, *Sideritis* extracts (and/or their components) may serve as natural antioxidative additives which do not produce toxic effects or impart unpleasant taste/color to foods. After these screening experiments, further work will be performed to describe the antioxidative and antimicrobial activities in more detail.

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