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Full Length Research Paper

Antibacterial activity of the methanol extracts of two endemic *Sideritis* species of Turkey against plant pathogenic bacteria

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***Sideritis condensata* Boiss. & Heldr. (SC) and *Sideritis erythrantha* var. *erythrantha* Boiss. & Heldr (SE) are endemic species in Turkey. The extracts of the endemic species were investigated for antibacterial activity against thirteen plant pathogenic bacteria *in vitro* by using the agar diffusion method. Statistical differences within bacteria were important at $p < 0.05$. SC extract was more effective than the SE extract. 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. Total yields were found as 4.77±0.93 in SE extract and 6.47 ± 0.95 in SC extract. The SC extracts at 10% concentration were exhibited by the most effective antibacterial effects against *Xanthomonas axonopodis* pv. *vesicatoria*, *Xanthomonas campestris* pv. *campestris* and *Pseudomonas syringae* pv. *phaseolicola* and the less effective against *Ralstonia solanacearum*, *Pseudomonas savastanoi* pv. *savastanoi*, *Clavibacter michiganensis* subsp. *michiganensis*, *Rhizobium vitis* and *Rhizobium tumefaciens*. On the other hand, SE extracts at 10% concentration were showed less effective than SC extract. 10% concentration of SE extracts was shown to be more effective antibacterial activity against *R. vitis*. 1% concentration of SE was not shown antibacterial activity against *Pectobacterium* carotovorum pv. carotovorum, *Pseudomonas corrugata, Pseudomonas syringae pv. tomato, R. solanacearum and X. axonopodis pv. vesicatoria*.**

Key words: *Sideritis*, antibacterial activity, plant pathogenic bacteria.

INTRODUCTION

Sideritis species are widely used as herbal tea or mountain tea that grows in mountainous region between southern Europe and the eastern Mediterranean. About 100 species of *Sideritis* have been distinguished in the world. The genus *Sideritis* (Lamiaceae) is represented by 46 species and 53 taxa in Turkey (Ozhatay et al., 1998; Kırımer et al., 1999). The herbs of most *Sideritis* species including 24 endemics (and two endemic subspecies) were found to be collected at least locally in Turkey (Ozcelik, 2000). *Sideritis* species are widely used folk medicine in Turkey. These species are attributed to have anti-inflammatory, antispasmodic, carminative, analgesic, nervous system stimulant, sedative, antitussive, stomachic, anticonvulsant activities and antimicrobial properties (Ezer et al., 1991; Kırımer et al., 1999; Gonzales-Burgos et al., 2011; Brankovic et al., 2011). The genus *Sideritis* contains antimicrobial and antioxidant polyphenolics such as flavonoids. The freeze-dried extract of *Sideritis*, before and after hydrolysis was found to be rich in bound forms of phenolic compounds such as

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5, 8, 3'-trihydroxy-4'-methoxyflavone, 7-(6'''- O-acetylsophoroside) together with apigenin 7-(6''-p-coumaroylglucoside) and apigenin 7-(4''-p-coumaroylglucoside) (Venturella et al., 1995; Palomino et al., 1996; Gonzales-Burgos et al., 2011). In addition, the tea is pleasantly aromatic and the essential oils and 39 extracts of this aromatic herb have been shown to have potent antioxidant activity in 40 lipid substrates (Lemberkovics et al., 1998). Several active ingredients of *Sideritis* have also been synthesized for their antimicrobial properties. *Sideritis* also contains diterpenoids that interact with the eicosanoid system but do not interfere with tested leukocyte functions or with reactive oxygen species; these compounds are essentially non-toxic (Pang et al., 1996; Navarro et al., 1997; Navarro et al., 2001; Aligiannis et al., 2001).

Some endemic *Sideritis* has been described and analysed of main components. *S. phlomoides*, *S. vulcanica*, *S. vuralii* and *S. caesarea* are endemic species in Turkey. The main component of *S. vuralii* was found as β-pinene (35%), 1,8-cineole (15%) and α-pinene (15%). While β-caryophyllene (8%) and caryophyllene oxide (7%) were the major constituents in the oil of *S. caesarea.* The oils from *S. phlomoides* and *S. vulcanica* were found to contain β-caryophyllene (31 and 10%) as major constituents (Kırımer et al., 1999; Kose et al., 2010). Many spices and derivatives as antimicrobial compounds have been studied against important human, food and plant pathogenic bacteria (Deans and Svoboda, 1990; Dorman and Deans, 2000; Basım et al., 2000; Ozcan and Erkmen, 2001; Sagdıc et al., 2002; Sagdıc and Ozcan, 2003; Ozkan et al., 2003; Basım and Basım, 2003, 2004; Ozkan et al., 2005). But, there are no research reports on antimicrobial effect of *Sideritis condensata* and *Sideritis erythrantha* var. *erythrantha* extracts on plant pathogenic bacteria. This is the first report of antibacterial activity of Turkish endemic *Sideritis* extracts against economically important plant pathogenic bacteria in Turkey.

MATERIALS AND METHODS

Plant materials

The aerial parts of mountain teas of *S. condensata* Boiss. & Heldr. (SC) and *S. erythrantha* var. Boiss. & Heldr. *erythrantha* (SE) endemic in Turkey were collected from wild during the flowering stage from Isparta-Sutculer, the southwestern part of Turkey in August 2002 at altitudes of 1400 to 1600 m. The plants were identified by Dr. H. Ozcelik, Director of the Herbarium Section, Isparta. Voucher specimens were deposited at the Herbarium of University of Suleyman Demirel, Isparta, Turkey.

Preparation of the *Sideritis* 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 at 60°C for 6 h to obtain extract. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated at reduced pressure (Rotavator, T < 40°C) and the extract was further dried in a desiccator, under vacuum, to constant weight and then dissolved in methanol. The solution was stored at -18°C. Method was modified according to Gu and Weng (2001).

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).

Bacterial cultures

The thirteen plant pathogenic bacterial isolates given in Table 1 were obtained from the culture collection of Department of Plant Protection, University of Akdeniz, Turkey. The bacterial isolates were stored in Luria-Berthani medium (LB) plus 20% glycerol at -70°C.

Determination of antibacterial effect

Stock cultures of bacteria were grown in Nutrient Broth (Acumedia Manufacturers, Inc., Maryland, USA) at 26 to 27°C for 24 h in a shaker. All the test bacteria in Nutrient Broth were enumerated by using serial dilution method. Final cell concentrations were 10^7 to 10^8 cfu/ml. The agar diffusion method was used to detect the antimicrobial activity of *Sideritis* extracts. Four equidistant holes were made in the agar using sterile cork borers (Ø = 5 mm). 50 µl of a concentration of 1, 2.5, 5 and 10% volume of methanolic (Merck, Dramstadt, Germany) each extract solutions was added to the holes using a pipettor and absolute methanol was used as a control. The extracts were put on Nutrient agar inoculated with 100 µl of bacterial suspension adjusted to 10^7 to 10^8 cfu/ml. The plates were incubated at 27°C for 48 h. At the end of the period, inhibition zones formed on the medium were measured using Vernier calipers. All the tests were done in triplicate.

Statistical analyses

SPSS 10.0 was used to compare the data and all the tests were considered statistically significant at $p<0.05$ level (Ozdamar, 1999). Significance of differences between effects of various concentrations of extracts was determined by Kruskal-Wallis analysis of variance (Kruskal and Wallis, 1952).

RESULTS

Total yield (%) and total phenolics (mg GAE/g) of two *Sideritis* extracts are given in Table 2. The yields of SC and SE extracts were found ranged from 6.47 ± 0.95 to 4.77 ± 0.93, respectively. Statistical differences between two *Sideritis* (SC and SE) extracts were important for total phenolics. The amount of total phenolics extracted with the methanol were determined 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. SC extract

Table 1. Plant pathogenic bacterial species.

Bacteria	Host
Rhizobium tumefaciens	Sourcherry
Rhizobium vitis	Grape
Clavibacter michiganensis pv. *michiganensis*	Tomato
Erwinia amylovora	Pear
Pectobacterium carotovorum pv. *carotovorum*	Potato
Pseudomonas corrugata	Tomato
Pseudomonas savastanoi pv. *savastanoi*	Olive
Pseudomonas syringae pv. *syringae*	Citrus
Pseudomonas syringae pv. *phaseolicola*	Bean
Pseudomonas syringae pv. *tomato*	Tomato
Ralstonia solanacearum	Tomato
Xanthomonas campestris pv. *campestris*	Cabbage
Xanthomonas axonopodis pv. *vesicatoria*	Tomato and pepper

Table 2. Total yield (%) and total phenolics (mg GAE/g) of *Sideritis* extracts[a].

Extracts	Total yield	Total phenolics
SC	6.47 ± 0.95	247.62 ± 1.91
SE	4.77 ± 0.93	217.61 ± 0.95

[a] Values expressed are mean ± S.D. of three experiments.

had total phenolics more than SE extract. Zheng and Wang (2001) reported that total phenolic contents in extracts of 27 culinary herbs and 12 medicinal herbs were found ranging from 0.23 to 2.85 mg of gallic acid equivalents (GAE)/g of fresh weight. Our study showed that total phenolic contents in *Sideritis* extracts were higher than those of these results. The effects of extracts at 10, 5, 2.5 and 1% on the test bacteria are presented in Tables 3 and 4. Methanol (control) had no inhibitory effects on the 13 plant pathogenic bacteria tested, and the extracts at the lowest (2.5 and 1%) concentrations were also the least effect against all the tested bacteria. Antibacterial effect of different doses of SC was statistically different for *P. carotovorum* pv. *carotovorum*, *P. corrugata*, *P. syringae* pv *phaseolicola*, *P. syringae* pv. *tomato*, *R. solanacearum*, *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria* (Table 3, $p < 0.05$). Antibacterial effect of different doses of SE was statistically different for *R. vitis*, *P. carotovorum* pv. *carotovorum*, *P. corrugata*, *P. syringae* pv. *tomato*, *R. solanacearum*, *X. campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria* (Table 4, $p < 0.05$). SC extract at the same concentration exhibited as more effective than the SE extract (Tables 3 and 4). The SC extracts at 10% concentration were exhibited by the most effective antibacterial effects against *X. axonopodis* pv. *vesicatoria*, *X. campestris* pv. *campestris* and *P. syringae* pv. *phaseolicola* and the less effective against *R. solanacearum*, *P. savastanoi* pv. *savastanoi*, *C. michiganensis* pv. *michiganensis*, *R. vitis*, and *R. tumefaciens* ($X^2 = 29.702$; $p = 0.003$; $p < 0.05$).

On the other hand, SE extracts at 10% concentration were showed less effective than SC extract. Antibacterial effect of 10% concentration of SE extracts was not statistically different against the bacteria ($X^2 = 12.606$; $p = 0.398$; $p<0.05$). 1% concentration of SE was not shown antibacterial activity in *P. carotovorum* pv. *carotovorum*, *P. corrugata*, *P. syringae* pv. *tomato*, *R. solanacearum* and *X. axonopodis* pv. *vesicatoria*.

DISCUSSION

Rodriguez-Linde et al. (1994) reported that *Sideritis* essential oils had antimicrobial activity against six bacteria and three fungi. The extent of the antibacterial effects of the genus *Sideritis* containing the extracts may be attributed to their phenolic composition such as flavonoids and diterpenoids (Palomino et al., 1996). The freeze-dried extract of *Sideritis*, before and after hydrolysis was 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) (Venturella et al., 1995; Palomino et al., 1996). In addition, the tea is pleasantly aromatic and the essential oils and 39 extracts of this aromatic herb have been shown to have potent antioxidant activity in 40 lipid substrates (Lemberkovics et al., 1998). Several active ingredients of *Sideritis* have

Table 3. Zones of growth inhibition (mm) showing antibacterial activity of *Sideritis condensata* (SC) methanol extracts against plant pathogenic bacteria[a]; well diameter of 5.0 mm.

Bacteria	Concentrations (%)					
	10	5	2.5	1	X^2	p
R. tumefaciens	9.4±2.15	8.06±1.05	7.1±0.90	6.4±0.79	5. 777	0.123
R. vitis	9.1±2.95	8.9±0.84	8.5±1.56	6.6±1.42	4.128	0.248
C. michiganensis pv. *michiganensis*	9.1 ±1.64	8.7±0.36	8.2±1.72	7.1±1.70	2.277	0.517
E. amylovora	12.1±1.56	9.1±1.55	8.8±1.38	8.0±1.01	7.205	0.065
P. carotovorum pv. *carotovorum*	12.2±0.95	9.2±1.05	8.0±0.10	7.7±1.07	8.774	0.032
P. corrugata	12.1±0.91	8.5±0.84	8.1±1.68	7.1±0.97	8.435	0.037
P. savastanoi pv. *savastanoi*	9.2±1.06	9.0±0.20	8.3±1.60	7.3±0.66	4.970	0.174
P. syringae pv. *phaseolicola*	14.5±0.47	12.6±1.50	9.2±0.90	7.0±1.00	10.384	0.015
P. syringae pv. *syringae*	12.3±1.50	9.2±2.53	8.3±2.08	7.5±1.13	5.564	0.134
P. syringae pv. *tomato*	12.4±1.85	9.2±0.10	8.4±1.65	7.4±1.07	7.963	0.046
R. solanacearum	9.2±0.10	8.5±1.83	7.2±0.10	6.0±0.08	8.105	0.043
X. campestris pv. *campestris*	14.5±0.85	12.8±2.70	9.4±0.37	8.0±1.65	9.461	0.023
X. axonopodis pv. *vesicatoria*	15.2±1.68	9.4±1.80	7.1±0.10	6.0±0.10	10.384	0.015

[a]: Values expressed are mean standard deviation of three replicates. X^2: Chi Square.

Table 4. Zones of growth inhibition (mm) showing antibacterial activity of *Sideritis erythranta (SE)* methanol extracts against plant pathogenic bacteria[a]; well diameter of 5.0 mm.

Bacteria	Concentrations (%)					
	10	5	2.5	1	X^2	p
R. tumefaciens	9.3±0.87	7.1±0.22	6.6±0.70	6.3±0.65	7.452	0.058
R. vitis	12.5±1.76	9.4±0.71	7.6±0.76	6.7±1.30	9.666	0.021
C. michiganensis pv. *michiganensis*	9.3±1.20	9.2±2.39	8.6±0.70	7.3±0.59	6.033	0.110
E. amylovora	9.3±2.38	8.4±2.28	7.3±1.00	7.2±1.07	4.743	0.191
P. carotovorum pv. *carotovorum*	9.5±1.07	7.0±3.01	-	-	10.649	0.013
P. corrugata	8.5±0.66	7.6±2.42	6.1±0.10	-	10.115	0.017
P. savastanoi pv. *savastanoi*	8.1±0.10	7.6±0.65	7.2±1.15	6.1±0.10	5.629	0.131
P. syringae pv. *phaseolicola*	9.2±1.80	8.2±3.11	8.0±0.14	7.3±0.59	7.745	0.051
P. syringae pv. *syringae*	9.7±2.21	8.6±1.77	7.6±1.42	6.4±0.67	6.794	0.078
P. syringae pv. *tomato*	9.6±2.42	7.0±0.07	-	-	10.692	0.013
R. solanacearum	8.9±1.59	7.6±0.83	-	-	9.831	0.020
X. campestris pv. *campestris*	149.0±0.61	8.6±0.85	7.3±0.59	6.2±0.21	9.044	0.028
X. axonopodis pv. *vesicatoria*	8.5±1.01	8.1±0.10	6.6±0.78	-	9.251	0.026

[a]: Values expressed are mean standard deviation of three replicates, -: not detected. X^2: Chi Square.

also been synthesized for their antimicrobial properties (Sarac and Ugur, 2007). *Sideritis* also contains diterpenoids that are essentially non-toxic even above recommended-therapeutic-doses (Pang et al., 1996; Navarro et al., 1997; Navarro et al., 2001; Aligiannis et al., 2001). The bacterial pathogens tested in this study cause several kinds of diseases on so many different plants including vegetables and fruits. Although, losses due to the pathogen are difficult to estimate, it is known to cause economically significant yield losses (Hirano and Upper, 1983). Many different commercial copper mixtures and antibiotics were used to control these diseases. As known, EUREPGAP (European Good Agricultural Practice) protocol only allows tolerable residue limits of pesticides on vegetables and fruits. The increased awareness of the environmental and health risk problems concerning the chemical pesticides has led to the search for non-conventional chemicals of biological origin for management of these diseases. Plant originated-bactericides can be one approach to plant disease management because of their eco-friendly nature (Bolkan and Reinert, 1994). The plant originated-products are of greater advantage to the user, public and the environmentalists.

The SC and SE extracts do not possess toxic effect or impart unwanted taste/color to foods. Our laboratory screening of SC and SE extracts drunk as tea in Turkey has given encouraging results, indicating their potential use in the management of the diseases caused by specifically *X. axonopodis* pv. *vesicatoria*, *X. campestris* pv. *campestris* and *P. syringae* pv. *phaseolicola*. These three pathogens cause leaf spot disease on tomato and pepper, black vein disease on cabbage and halo blight on leaf and pod of bean. As these bacterial pathogens are known to be transmitted through seeds (Agrios, 1997), one important application of the plant extracts can be a seed protectant. SC appears to be promising in this respect. Our further work is underway to evaluate their potential efficacies in managing seed-borne bacterial diseases in different crops.

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