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ANTIMICROBIAL STUDIES ON THREE ENDEMIC SPECIES OF *SIDERITIS* FROM TURKEY

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The antimicrobial activity of several extracts and fractions of some *Sideritis* species (*S. albiflora, S. brevibracteata* and *S. pisidica*) was investigated by disc diffusion and broth microdilution methods against *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* La 2971, *Micrococcus flavus* ATCC 14452, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Corynebacterium xerosis* CCM 7064, *Mycobacterium smegmatis* CCM 2067, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 9730, *Kluyveromyces fragilis* NRRL 2415 and *Rhodotorula rubra* CCY. The methanol extract and chloroform fraction of *S. pisidica*, as well as the methanol extracts, butanol and chloroform fractions of both *S. albiflora* and *S. brevibracteata* showed good antimicrobial activity against some bacteria and all yeasts tested, with growth inhibition area diameters in the range 10–20 mm, and MIC values between 0.03 and 0.38 µl/ml. The results of the study support the use of these species in traditional medicine.

Key words: *Sideritis*, antimicrobial, antifungal activity.

INTRODUCTION

The genus *Sideritis* (Labiatae) is widely distributed in subtropical and temperate regions (Davis, 1982). *Sideritis* species form a group of plants known as "mountain tea" in Turkey. Some species are used as tea, flavoring agents, and for medicinal purposes in several regions of Turkey (Ozcan et al., 2001). Infusions of aerial parts of a number of *Sideritis* species are used as tonics, carminatives, anti-inflammatory agents, antispasmodics, diuretics and digestives, and in the treatment of colds (Ezer et al., 1991; Koedam, 1986; Yesilada and Ezer, 1989). The *Sideritis* species used in this study (*S. albiflora* Hub.-Mor., *S. brevibracteata* P.H.Davis, *S. pisida* Boiss.&Heldr.) are endemic to Turkey. During our field studies it was determined that these plants have been used for ophthalmia, eczema, swelling and bruises. These experiments were intended to determine the antimicrobial effects of plant extracts obtained from three endemic *Sideritis* L. species.

MATERIAL AND METHODS

MATERIALS

Three species of *Sideritis* (*S. albiflora* Hub.-Mor., *S. brevibracteata* P.H.Davis and *S. pisida* Boiss.&Heldr.) were collected in Antalya province in Turkey during September-October 2001. Voucher specimens of the plants are deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey, with identifications by Dr. Ahmet Gonuz.

EXTRACT PREPARATION

The three plant species were dried in an oven at 40˚C and powdered. Methanol extracts were obtained by maceration of the plant material with methanol for 3 days at room temperature; the procedure was done three times. The extracts were filtered and dried under reduced pressure at temperature below 45˚C. Then the methanol extracts were separated between chloroform and water ($CHCl_3$, H_2O). Finally, the aqueous fraction was again subjected to separation between n-butanol and water (n-BuOH, H_2O). The yields obtained for each extract and fraction as percentages of the initial dry material were *S. albiflora* Hub.-Mor. 28.60% for MeOH ($CHCl_3$ 10.46%, BuOH 28.34%, aqueous 34.52%); *S. brevibracteata* P.H.Davis 34.78% for MeOH ($CHCl_3$ 14.89%, BuOH 28.75%, aqueous 46.24%); and *S. pisida* Boiss.&Heldr. 26.44% for MeOH ($CHCl_3$ 13.84%, BuOH 36.64%, aqueous 24.52%).

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TABLE 1. Antimicrobial activity of *Sideritis* methanol extracts as found by the disc diffusion method

Sample	Dose (mg/disc)	Diameter of inhibition (mm)														
		1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S. albiflora	1.25	12	-	14	-	-	-	10	12	-	14	16	14	14	10	12
MeOH extract	2.50	14	-	15	-	-	-	12	14	-	16	18	16	14	12	12
	3.75	14	-	15	-	-	-	12	16	-	16	18	16	14	12	12
S. brevibracteata	1.25	13	-	16	-	-	-	12	12	14	14	16	12	12	10	10
MeOH extract	2.50	15	-	16	-	-	-	12	12	14	14	16	12	12	10	12
	3.75	16	-	16	-	-	-	12	12	14	14	16	12	12	10	12
S. pisida	1.25	14	-	16	-	-	-	14	12	14	14	14	16	14	16	16
MeOH extract	2.50	15	-	14	-	-	-	14	14	14	14	16	16	14	18	16
	3.75	16	-	16	-	-	-	14	14	14	14	16	16	14	18	16
Choramphenicol	0.03	28	34	28	42	38	30	12	22	24	28	26	NT	NT	NT	NT
Amphotericin B	0.10	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	12	16	14	14

- – no inhibition zone; NT– Not tested; *1 – *Escherichia coli*; 2 – *Staphylococcus aureus*; 3 – *Klebsiella pneumoniae*; 4 – *Micrococcus luteus*; 5 – *Micrococcus flavus*; 6 – *Proteus vulgaris*; 7 – *Pseudomonas aeruginosa*; 8 – *Corynebacterium xerosis*; 9 – *Mycobacterium smegmatis*; 10 – *Bacillus cereus*; 11 – *Bacillus subtilis*; 12 – *Candida albicans*; 13 – *Saccharomyces cerevisiae*; 14 – *Kluyveromyces fragilis*; 15 – *Rhodotorula rubra*

Infusions were prepared with 100 g crude powder and 1000 ml water, and the volume was adjusted to a concentration of 1 g/ml under reduced pressure at 40˚C.

SAMPLE PREPARATION

In this study of antimicrobial activity, the different extracts and fractions were diluted in dimethylsulfoxide (DMSO). The corresponding concentrations are expressed as mg extract or fraction per ml solvent, with the exception of infusions, whose concentrations are expressed as mg initial dry material per ml. For each experiment, a disc containing only (DMSO) was used as a control.

BIOASSAYS

The antimicrobial tests employed the disc diffusion method (Bauer et al., 1966), and the minimum inhibitory concentration (MIC) was determined by microdilution according to the microplate method (Rabanal et al., 2002; Jones et al., 1987).

The microorganisms to be tested were inoculated into Brain Heart Infusion Agar (Oxoid) for the bacteria, and Sabouraud Dextrose Agar (Oxoid) for the yeasts. After 24 h incubation at 35˚C and 28˚C, respectively, three or four colonies isolated from the media were inoculated into 4 ml of Brain Heart Infusion Broth (Oxoid) for the bacteria, and Sabouraud Dextrose Broth (Oxoid) for the yeasts, and incubated for 2 h at 35˚C and 28˚C, respectively. The cultures were adjusted with sterile saline solution to turbidity comparable to that of McFarland 0.5 standard. Petri dishes containing Mueller-Hinton Agar (Oxoid) or Bacto Yeast Morphology Agar (Difco) were impregnated with these microbial suspensions for the bacteria and yeasts, respectively (Bauer et al., 1966; Rabanal et al., 2002).

MIC was estimated by the broth microdilution method in M24A microplates against the most sensitive microorganisms, using liquid media containing decreasing amounts of the test materials. From an initial solution extract of 375 mg/ml, double dilutions in the culture medium (Mueller-Hinton Broth for bacteria, Sabouraud Dextrose Broth for yeasts) were prepared and tested at concentrations ranging from 37.5 to 0.03 mg/ml. After mixing, 5 µl cell suspension (10^5 cells per µl) was added and mixed vigorously again. The microplates were then incubated at the appropriate temperature for 24 h in a humid atmosphere. Afterwards the microplates were centrifuged and examined for growth. All data represent at least three replicated experiments per microorganism.

MICROORGANISMS

Escherichia coli ATCC 11230, *Stapylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Micrococcus luteus* La 2971, *Micrococcus flavus* ATCC 14452, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Corynebacterium xerosis* CCM 7064, *Mycobacterium smegmatis* CCM 2067, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9730, *Kluyveromyces fragilis* NRRL 2415 and *Rhodotorula rubra* CCY were collections maintained in the Laboratory of Basic and Industrial Microbiology, Ege University.

RESULTS AND DISCUSSION

Table 1 summarizes the antimicrobial activity of *Sideritis albiflora*, *S. brevibracteata* and *S. pisida* meth-

TABLE 2. Antimicrobial activity of *Sideritis* fractions (aqueous, BuOH and $CHCl_3$) as found by the disc diffusion method

Sample	Dose (mg/disc)	Diameter of inhibiton (mm)									
		1*	2	3	4	5	6	7	8	9	10
S. albiflora											
Aqueous fraction	1.25	-	-	-	-	-	-	-	-	-	-
	2.50	-	-	-	-	-	-	-	-	-	-
	3.75	-	-	-	-	-	-	-	-	-	-
BuOH fraction	1.25	12	12	13	11	12	14	13	14	15	12
	2.50	10	13	12	12	10	13	15	12	15	12
	3.75	13	13	14	12	13	16	16	16	15	12
$CHCl_3$ fraction	1.25	14	13	13	14	13	16	14	15	15	13
	2.50	13	10	13	11	11	15	15	14	15	13
	3.75	15	14	13	11	11	20	16	16	15	13
S. brevibracteata											
Aqueous fraction	1.25	-	-	-	-	-	-	-	-	-	-
	2.50	-	-	-	-	-	-	-	-	-	-
	3.75	-	-	-	-	-	-	-	-	-	-
BuOH fraction	1.25	-	-	-	-	-	-	-	-	-	-
	2.50	-	-	-	-	-	-	-	-	-	-
	3.75	-	-	-	-	-	-	-	-	-	-
$CHCl_3$ fraction	1.25	11	10	13	12	12	14	12	14	12	13
	2.50	13	12	13	10	14	16	14	12	14	13
	3.75	13	12	13	13	13	16	13	12	14	13
S. pisida											
Aqueous fraction	1.25	-	-	-	-	-	-	-	-	-	-
	2.50	-	-	-	-	-	-	-	-	-	-
	3.75	-	-	-	-	-	-	-	-	-	-
BuOH fraction	1.25	12	12	12	14	13	16	14	14	14	14
	2.50	13	15	15	14	14	16	16	16	14	14
	3.75	14	16	15	15	14	16	15	15	14	13
$CHCl_3$ fraction	1.25	16	13	13	14	14	18	15	15	16	15
	2.50	14	13	13	12	14	17	16	15	15	15
	3.75	14	13	13	13	14	20	14	15	15	15

- – no inhibition zone; *1 – *Escherichia coli*; 2 – *Klebsiella pneumoniae*; 3 – *Pseudomonas aeruginosa*; 4 – *Corynebacterium xerosis*; 5 – *Bacillus cereus*; 6 – *Bacillus subtilis*; 7 – *Candida albicans*; 8 – *Saccharomyces cerevisiae*; 9 – *Kluyveromyces fragilis*; 10 – *Rhodotorula rubra*

anol extracts as tested by the disc diffusion method. No significant activity was found against *Staphylococcus aureus, Micrococcus flavus, Micrococcus luteus, Proteus vulgaris* or the acid-fast bacterium *Mycobacterium smegmatis*. The results were different in the tests against *Escherichia coli, Bacillus subtilis, Bacillus cereus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The diameters of the growth inhibition area ranged from 12 to 18 mm for the methanol extracts studied. The antimicrobial activity of the aqueous, butanol and chloroform fractions obtained from the methanol extracts of the three *Sideritis* species are presented in Table 2. The aqueous fraction of the three species and the butanol fraction of *S. brevibracteata* showed no antimicrobial activity at all. The diameters of the growth inhibition area ranged from 10 to 20 mm for the aqueous extracts. The extracts of *Sideritis* species showed the highest activity against *Bacillus subtilis, Escherichia coli,* and especially *Pseudomonas aeruginosa* (inhibition values close to that of Chloramphenicol) and all yeasts tested (inhibition values higher than that of Amphotericin B). It is worth noting that in the case of *S. brevibracteata* only the chloroform fraction conserved antimicrobial activity, suggesting that the active compounds were concentrated on it. The inhibition zone diameters around the control disc (containing only DMSO) were 0–0.5 mm.

Table 3 summarizes the MIC values of the active extracts and fractions. These values ranged from 0.03 to 0.38 μg/ml. Of the fractions assayed, the highest activity was observed for the chloroform fraction of *S. pisida,* with particularly low MIC values against *Bacillus subtilis* and yeasts.

TABLE 3. Minimum inhibitory concentration as found by microdilution method

Sample	MIC (mg/ml)									
	1*	2	3	4	5	6	7	8	9	10
S. albiflora										
MeOH extract	0.22	0.29	0.34	0.29	0.26	0.22	0.11	0.09	0.11	0.11
BuOH fraction	0.22	0.29	0.38	0.34	0.29	0.22	0.09	0.15	0.18	0.11
$CHCl_3$ fraction	0.15	0.22	0.29	0.22	0.18	0.18	0.05	0.03	0.09	0.07
S. brevibracteata										
MeOH extract	0.22	0.22	0.22	0.18	0.22	0.22	0.15	0.15	0.09	0.11
BuOH fraction	0.22	0.22	0.28	0.22	0.26	0.15	0.11	0.11	0.09	0.09
$CHCl_3$ fraction	0.18	0.18	0.18	0.15	0.18	0.11	0.09	0.05	0.03	0.05
S. pisida										
MeOH extract	0.07	0.15	0.18	0.18	0.09	0.03	0.09	0.03	0.09	0.05
BuOH fraction	0.11	0.11	0.22	0.26	0.16	0.05	0.18	0.09	0.11	0.07
$CHCl_3$ fraction	0.05	0.09	0.15	0.15	0.05	0.03	0.07	0.03	0.03	0.03
Chloramphenicol	0.002	0.002	0.008	0.001	0.0005	0.0005	NT	NT	NT	NT
Amphotericin B	NT	NT	NT	NT	NT	NT	0.008	0.002	0.006	0.006

*1 – *Escherichia coli*; 2 – *Klebsiella pneumoniae*; 3 – *Pseudomonas aeruginosa*; 4 – *Corynebacterium xerosis*; 5 – *Bacillus cereus*; 6 – *Bacillus subtilis*; 7 – *Candida albicans*; 8 – *Saccharomyces cerevisiae*; 9 – *Kluyveromyces fragilis*; 10 – *Rhodotorula rubra;* NT – not tested.

It would generally be expected that a much greater number of extracts would be active against Gram-positive than against Gram-negative bacteria. However, in this study the extracts were active against both Gram-positive and Gram-negative bacteria as well as yeasts. This action resembles the antimicrobial activity of broad-spectrum antibiotic compounds or general metabolic toxins.

The moderate activity of the extracts against *Bacillus subtilis* and opportunistic microorganisms supports the traditional use of *S. pisida* for treatment of eye infections. The observed antiyeast activity of extracts obtained from *Sideritis* species is also remarkable, although these are no reports related to this activity in traditional medicine. The extracts are being investigated further as potential antiyeast agents. *Sideritis* extracts demonstrating broad spectra of activity may help in developing new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health, and could provide biochemical tools for the study of infectious diseases.

In this study we found that *Sideritis* species showed antimicrobial activity against some Gram-positive and Gram-negative bacteria and yeasts. Notably, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were the bacteria most sensitive to them, and *Sideritis* species extracts exhibited high activity against yeasts. These promising results should encourage further investigations of *Sideritis* L. species.

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