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Antimicrobial Activities of Two *Scorzonera* Species Growing in Turkey

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The antimicrobial activities of the ethanolic extracts and petroleum ether, ethyl acetate and *n*-butanol fractions obtained from the subaerial parts of two *Scorzonera* species (*S. latifolia* and *S. veratrifolia*) (Asteraceae) have been tested against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi, Shigella flexneri* and *Candida albicans* ATCC 10231 using microbroth dilution technique. The ethyl acetate fractions of both *S. latifolia* and *S. veratrifolia* showed the highest antimicrobial activity against *Staphylococcus aureus* (MIC values 19.52 and 39.06 µg/mL, respectively).

Key Words: Antimicrobial activity, Asteraceae, *Scorzonera latifolia, S. veratrifolia*.

INTRODUCTION

The genus *Scorzonera* (Asteraceae) comprises about 170 species all over the world[1]. This genus is represented in Turkey by 39 species, including 17 endemics[2]. *Scorzonera* species have been widely utilized as vegetable. Several species of the genus have been used as traditional medicines, especially as analgesic, antirheumatic, anthelmintic, stomachic and diuretic, as well as for the treatment of wound, hypertension, infertility, lung eudema, diarrhea, poisonous ulcers, malignant stomach neoplasia[1,3,4]. Some *Scorzonera* species have been reported to contain dihydroisocoumarins[5,6], benzylphthalides[6], flavonoids[7,8], lignans[9,10], neolignans[11,12], dibenzyl derivatives[13-15], quinic acid derivatives[1,16], kava lactones[8], sesquiterpenes[17-19] and triterpenes[5,20,21].

For the genus *Scorzonera*, previous antimicrobial activity investigations have been carried out on *S. humilis* and *S. mollis*[15,22]. No antimicrobial activity was detected for *S. humilis*, whereas *S. mollis* were reported to exhibit antimicrobial activity.

In this work, the antimicrobial potential of the extracts obtained from the subaerial parts of *S. latifolia* and *S. veratrifolia* were tested against 8 pathogenic bacteria and one yeast using microbroth dilution technique.

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S. latifolia and *S. veratrifolia* are perennial herbaceous plants with yellow flowers. They are mainly distributed in east Anatolia. It has been reported that the latex obtained from the subaerial parts of *S. latifolia* is used in folk medicine for the treatment of wound[4].

EXPERIMENTAL

Scorzonera latifolia (Fisch. & Mey.) DC. (SL) was collected from Van, east Anatolia, Turkey at an altitude of 2173 m, in June 2003. *Scorzonera veratrifolia* Fenzl (syn.: *S. bella* Lipschitz) (SV) was collected from Bitlis, east Anatolia, at an altitude of 2500 m, in August 2004. Voucher specimens are deposited at the Herbarium, Faculty of Sciences and Letters, Yüzüncü Yıl University (SV: F 12446, SL: F 11851).

Preparation of the extracts: The air-dried, ground, subaerial parts of *S. latifolia* and *S. veratrifolia* (20 g, each) were repeatedly extracted with ethanol (4 × 50 mL) at room temperature, 4 d each. The extracts were filtered using Whatman filter paper no. 1 and the filtrates were then evaporated under reduced pressure to dryness using rotary evaporator at 45 ºC. The resulting ethanolic extracts were assayed for antimicrobial activity. The ethanolic extracts of *S. latifolia* and *S. veratrifolia* possessed antimicrobial activity. Therefore, a larger amount of materials (50 g) were extracted with ethanol as described above. The residues were dissolved in EtOH/H_2O 1:2 (50 mL) and then successively extracted with petroleum ether, ethyl acetate and *n*-butanol. The resulting fractions were filtered using Whatman filter paper no. 1. The filtrates were then evaporated under reduced pressure to dryness at 45 ºC. All the extracts and fractions were kept at -20 ºC until use. The yields obtained for each extract and fraction as percentages of the initial dry material were *S. latifolia* 23.2 % for ethanol (petroleum ether 17.7 %, ethyl acetate 16.6 %, *n*-butanol 18.2 %); *S. veratrifolia* 25.78 % for ethanol (petroleum ether 24.3 %, ethyl acetate 21.7 %, *n*-butanol 13.5 %).

Microorganisms: *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi*, *Shigella flexneri* and *Candida albicans* ATCC 10231 were used for testing the antimicrobial activity.

All the microorganisms except for *S. typhi*, *S. flexneri*, were obtained from American Type Culture Collection (ATCC), Rockville, Md., USA. *S. typhi* and *S. flexneri* were obtained from clinical specimens submitted to Clinical Microbiology Laboratories, Istanbul University, Istanbul Faculty of Medicine.

Test for antimicrobial activity: *In vitro* antimicrobial activities were tested by the microbroth dilutions technique using the Clinical and Laboratory Standards Institute (CLSI) 2000 and 2006 recommendations[23]. Mueller-Hinton broth for bacteria and RPMI-1640 medium for yeast strain were used as the test mediums. The extracts were dissolved in dimethyl sulfoxide (10 mg/mL) before the test for antimicrobial activity. Serial two-fold dilutions ranging from 5000 µg/mL to 4.9 µg/mL were prepared in medium. The inoculum was prepared using a 4-6 h broth culture of

each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 McFarland Standard, diluted in broth media to give a final concentrations of 5×10^5 colony-forming units (CFU)/mL for bacteria and 0.5×10^3 to 2.5×10^3 CFU/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35 ºC for 18-20 h and the trays containing RPMI-1640 medium were incubated at 35 ºC for 46-50 h.

Minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract or pure compound giving complete inhibition of visible growth.

Cefuroxime-Na and ceftazidime were used as positive control for the tested bacteria whereas clotrimazole was used as positive control for yeast.

All data represent at least three replicated experiments per microorganisms.

RESULTS AND DISCUSSION

All the MIC values of the ethanolic extracts and petroleum ether, ethyl acetate and *n*-butanol fractions of the ethanolic extracts obtained from the subaerial parts of *S. latifolia* and *S. veratrifolia* are listed in Table-1. The results revealed that the extracts and fractions were able to inhibit the growth of the selected microorganisms *in vitro*, showing MIC values between 2500 and 19.52 µg/mL. All the extracts and fractions of both *S. latifolia* and *S. veratrifolia* inhibited *S. aureus*, *S. flexneri* and *C. albicans* (MIC: ≤ 2500 µg/mL). Ethyl acetate fractions of *S. latifolia* and *S. veratrifolia* were found the most active fractions against *S. aureus* with the MIC

TABLE-1
MINIMUM INHIBITORY CONCENTRATIONS OF EXTRACTS AND FRACTIONS OF *S. latifolia* AND *S. veratrifolia* (µg/mL)

Species or reference compound	Extract[a]	Microorganism[b]								
		Sa	*Se*	*Kp*	*Pm*	*Ec*	*Pa*	*St*	*Sf*	*Ca*
S. latifolia	PE	625	-	-	-	-	-	-	2500	625
	EA	39.06	-	-	-	-	-	-	2500	625
	B	625	-	-	-	-	-	-	2500	1250
	E	625	-	-	-	-	-	-	2500	1250
S. veratrifolia	PE	625	625	-	-	-	-	1250	1250	625
	EA	19.52	625	-	-	-	-	1250	625	625
	B	625	-	-	-	-	-	-	2500	625
	E	156.2	625	-	-	-	-	-	1250	625
Cefuroxime-Na		1.2	9.8	4.9	2.4	4.9		2.4	4.9	
Ceftazidime							2.4			
Clotrimazole										4.9

[a]PE = Petroleum ether fraction; EA = Ethyl acetate fraction; B = *n*-Butanol fraction; E = Ethanolic extract; [b]*Sa* = *Staphylococcus aureus*; *Se* = *Staphylococcus epidermidis*; *Ec* = *Escherichia coli*; *Kp* = *Klebsiella pneumoniae*; *Pm* = *Proteus mirabilis*; *Pa* = *Pseudomonas aeruginosa*; *St* = *Salmonella typhi*; *Sf* = *Shigella flexneri*; *Ca* = *Candida albicans*. – = Not active.

values of 39.06 and 19.52 µg/mL, respectively. The petroleum ether, ethyl acetate fractions and ethanolic extract of *S. veratrifolia* showed the same antimicrobial effect against *S. epidermidis* (MIC: 625 µg/mL). Both petroleum ether and ethyl acetate fractions of *S. veratrifolia* showed antimicrobial effect against *S. typhi* (MIC: 1250 µg/mL). None of the extracts and fractions exhibited activity against *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa*.

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