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In vitro Antimicrobial and Antioxidant Activity Evaluation of *Melampyrum arvense* L. var. *elatius* Boiss. and *Sedum spurium* M. Bieb. Extracts

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ABSTRACT

Sedum spurium M. Bieb. (Crassulaceae) is a common ornamental plant, whereas, *Melampyrum arvense* L. var. *elatius* Boiss. (Orobanchaceae) is a semi-parasitic plant and grows naturally in the fields. In this study, the dichloromethane and ethyl acetate extracts of *M. arvense* and *S. spurium* were evaluated for their *in vitro* antioxidant and antimicrobial activities. The antioxidant activity was evaluated by DPPH$^{\cdot}$-ABTS$^{\cdot}$ methods. The antimicrobial activity of *S. spurium* and *M. arvense* extracts was determined using the *in vitro* broth microdilution assay against following human pathogenic strains; *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* NRLL B-3008, *Helicobacter pylori* ATCC 43504, *Mycobacterium smegmatis* ATCC 25291, *Mycobacterium avium* ssp. *avium* and *Pseudomonas aeruginosa* ATCC 10145.

The extracts showed weak antimicrobial activity against Gram-negative/positive bacteria, having the MIC values of 500-1000 µg/mL. Antibacterial activity was not observed against *Mycobacteria* at 2000 µg/mL. In addition, antioxidant activity of *M. arvense* ethyl acetate extract was higher than those of the other extracts.

Keywords: *Melampyrum arvense*, *Sedum spurium,* Antibacterial, Antioxidant, *Mycobacteria*

INTRODUCTION

Melampyrum L. genus is an annual and semi-parasitic plant group. It is represented by two species, *M. arvense* and *M. pratense* in the Flora of Turkey[1]. Iridoid glycosides were the major bioactive secondary metabolites of

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Melampyrum species[2]. The previous *in vitro* studies showed that *Melampyrum* extracts have antioxidant, protein kinase C inhibitory, antimalarial, cytotoxic and antiprotozoal activities[3-5] and it is used as animal fodder traditionally[6]. *Sedum* L. is represented by 43 species in Turkey[7-8]. It is reported that several *Sedum* species have wound healing properties and were used as diuretic and laxative and as well as for the treatment of various diseases such as hemorrhoids in folk medicine [9-11]. The major components of *Sedum* species have been described as alkaloids and flavonoids in previous studies [12-16].

The aim of the present study was evaluation of the antimicrobial and antioxidant activities of *M. arvense* and *S. spurium* extracts. The phenolic compound composition of the extracts was analyzed by High Performance-Liquid Chromatography (HPLC).

METHODOLOGY

Plant Material and Extraction

M. arvense and *S. spurium* were collected in the vicinity of Trabzon-Tonya and Trabzon-Hamsiköy, respectively. Plants were identified by Prof. M. Vural and voucher specimens have been deposited at Herbarium of the Depertment of Pharmacognosy, School of Pharmacy, Istanbul Medipol University, Istanbul, Turkey. (Voucher specimens no. IMEF: 1055 and IMEF: 1142 resp.) The air-dried and coarsely ground aerial parts of plant material were macerated with 70% ethanol. The extract was filtered and evaporated to dryness *in vacuo* (Heidolph, Germany), and then dissolved in a water-ethanol (90:10) mixture and extracted with dichloromethane and ethyl acetate, respectively.

Antioxidant Activity

DPPH$^{\cdot}$ and ABTS$^{\cdot}$ Scavenging Assay

The antioxidant capacity was determined in terms of hydrogen donating or radical scavenging ability using DPPH$^{\cdot}$ by its capability to bleach the stable radical[17]. The reaction mix contained 100 µM DPPH$^{\cdot}$ in methanol and dichloromethane or ethyl acetate extracts. After 30 min, absorbance was read at 517 nm by using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 25 ± 2°C and the radical scavenging activity (RSA) was determined as the percentage of radical reduction as follows:

$$\text{DPPH}^{\cdot}\ \text{RSA}\ \% = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test sample}}) / \text{Absorbance}_{\text{control}})] \times 100$$

The total antioxidant activity of the samples was measured using the ABTS radical cation decolorization assay[18]. ABTS$^{\cdot}$ was produced by reacting 7 mM

aqueous ABTS· with 2.45 mM potassium persulfate. The reaction mixture was left at room temperature overnight (12–16 h) in the dark. The resulting intensely colored ABTS radical cation was diluted with ethanol. Absorbance was measured at 734 nm at room temperature. The assay was performed in triplicate. Negative controls in which 990 µL ethanol was substituted for ABTS· were used. The assay was carried out on Trolox as a positive control[19]. The results were expressed as IC_{50} as follows:

$$ABTS^{\cdot} \text{ RSA } \% = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test sample}})/\text{Absorbance}_{\text{control}})] \times 100$$

Each experiment was performed in triplicate. The IC_{50} value of the extracts was calculated from a calibration graph. Test results are presented as mean ± standard deviation (SD). Statistical analysis of antioxidant test results was completed using one-way ANOVA with the SPSS 23.0 software. A difference in the mean values of $P<0.05$ was considered to be statistically significant.

Antimicrobial Activity

The antimicrobial activity of the extracts was determined using the broth microdilution assay[20] to determine the minimum inhibitory concentrations (MIC). *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* NRLL B-3008, and *Pseudomonas aeruginosa* ATCC 10145 strains were grown in Mueller Hinton Broth (MHB) at 37°C in aerobic conditions for 24 h. All microorganisms were standardized to McFarland No: 0.5.

Helicobacter pylori ATCC 43504 strain was grown for 24 hours in Brucella broth containing 5% (v/v) horse blood and 10% (h/h) fetal bovine serum at 37°C in an anaerobic incubator (5% CO_2). After incubation at 37°C, 100 µL *H. pylori* ($2x10^7$ CFU/mL) strain was transferred to the microplate evaluation[21, 22]. Diluted bacterial suspensions were added to each well and then allowed to incubate at 37 °C for further 24 h.

Mycobacteria strains were inoculated in Middlebrook 7H11 agar and incubated in aerobic conditions at 37 °C for 4-5 days. The microorganism was transferred to media and incubated for a further five days. Diluted bacterial suspensions (10^6 CFU/mL) were added to each well and then allowed to incubate at 37 °C for 5 days [23-25].

Test samples stock solution was prepared in dimethyl sulfoxide and serial dilutions were prepared for each sample. The minimum non-reproductive concentration was reported as minimum inhibitory concentration (MIC). The MIC was calculated as the mean of three repetitions.

HPLC Analysis

The HPLC analyses studied on an Agilent (1200 LC) and UV-Vis detector (G1314A). HPLC was run on an Agilent C18 column (4.6 x 250 mm x 5 µm) and its temperature was maintained at 40°C. The mobile phases were Solvent A: Acetonitrile: Water (10:90, v/v) and Solvent B: Acetonitrile: Water (90:10, v/v). The composition of solvent B was increased from 15% to 100% in 35 min, and at a flow rate of 0.6 mL/min. The injection volume is 10 µL[26]. Phenolic compounds were identified by matching their retention times against those of the standards analyzed under the same conditions (Figure 1).

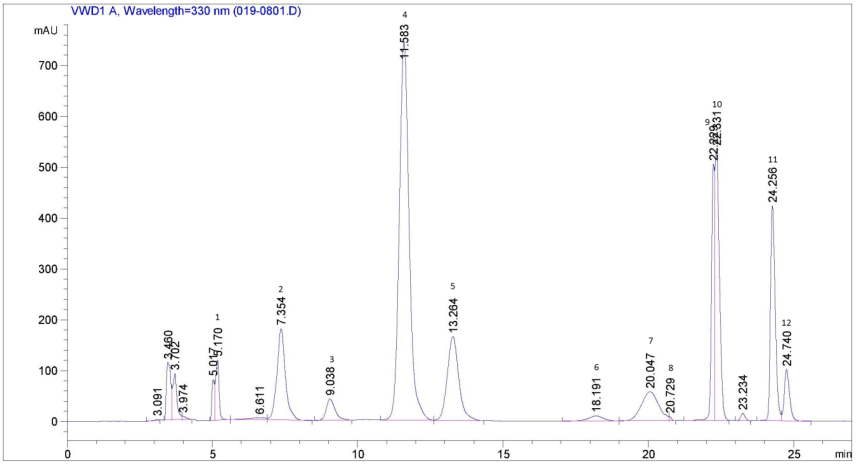

Figure 1. HPLC Chromatogram of References

1, Chlorogenic acid (RT: 5.1); 2, Caffeic acid (RT: 7.3); 3, Luteolin-O-Glycoside (RT: 9.03); 4, Coumaric acid (RT: 11.5); 5, Ferulic acid (RT: 13.2); 6, Rosmarinic acid (RT: 18.1); 7, Myrcetin (RT: 20.04); 8, Eriodictyol (RT: 20.7); 9, Luteolin (RT: 22.2); 10, Quercetin (RT: 22.3); 11, Apigenin (RT: 24.2); 12, Gallic acid (RT: 24.7)

RESULTS AND DISCUSSION

Antioxidant Activity

Antioxidant activities of the ethyl acetate and dichloromethane extracts of *M. arvense* and *S. spurium* were measured by the ability to scavenge DPPH free radicals and ABTS radical scavenging method, by comparing with Ascorbic acid and Trolox, respectively. Antioxidant capacities were expressed by IC_{50} values, indicating the extracts concentrations scavenge 50% of ABTS radical. It was observed that ethyl acetate extract of *M. arvense* has higher antioxidant capacity than those of the other extracts. The results were shown in Table

1. Although there is no detailed study of antioxidant activity on *M. arvense*, the results of previous studies on antioxidant activities other *Melampyrum* species were similar to those of the current study results[3, 27]. As shown in a previous study[28], phenolic compounds found in the *M. barbatum* extract may be responsible for the antioxidant activity. To the best of our knowledge, this is the first report on the antioxidant capacity of *M. arvense* extract.

Table 1. ABTS and DPPH radical scavenging activities of extracts

ABTS and DPPH radical scavenging activities + [IC_{50} ± SD (mg/mL)]					
	1	**2**	**3**	**4**	**References**
ABTS•	0,19 ± 0,04	1,43 ± 0,03	1.54 ± 0,04	2.01 ± 0,03	0,015 ± 0,001 (Trolox)
DPPH•	0,16 ± 0,03	1,13 ± 0,04	1.41 ± 0,03	1,97 ± 0,04	0,002 ± 0,001 (Ascorbic acid)

1: M. arvense ethyl acetate extract; 2: M. arvense dichloromethane extract; 3: S. spurium ethyl acetate extract; 4: S. spurium dichloromethane extract

Antimicrobial Activity

Antimicrobial activities of *M. arvense* and *S. spurium* extract were evaluated according to their MIC values against various strains. Table 2 shows antimicrobial activities of *M. arvense* and *S. spurium* extracts against bacterial strains. The results revealed that the extracts have weak antimicrobial activity against Gram-negative/positive bacteria with the MIC values in the range to 500-1000 μg/mL. Antibacterial activity was not observed against *Mycobacterias* at 2000 μg/mL. In a previous study, antimicrobial activity of *S. spurium* essential oil was evaluated[29] but this is the first report on antimicrobial activity evaluation of *S. spurium* extracts. Also, the results obtained by Tosun and co-workers in a previous study of different *Mycobacteria* strains on the *S. spurium* extract were similar to the results of the current study[30].

Table 2. Antimicrobial activity of extracts (MICs in µg/mL).

Bacteria / Sample	E. a.	S. a.	P. a.	E. f.	H. p.	M. a.	M. s.
1	>2000	1000	500	500	>2000	>2000	>2000
2	>2000	1000	500	500	>2000	>2000	>2000
3	>2000	>2000	>2000	1000	>2000	>2000	>2000
4	>1000	>2000	>2000	1000	1000	>2000	>2000
Chloramphenicol	8	8	>32	16	16	-	-
Tetracycline	16	0.25	>16	0.025	0.025	-	-
Amikacin	-	-	-	-	-	250	250

1: *M. arvense* ethyl acetate extract; 2: *M. arvense* dichloromethane extract; 3: *S. spurium* ethyl acetate extract; 4: *S. spurium* dichloromethane extract

E.a.: Escherichia coli; S.a.: Staphylococcus aureus; P.a.: Pseudomonas aeruginosa; E.f.: Enterococcus faecalis; H.p.: Helicobacter pylori; M.a.: Mycobacterium avium; M.s.: Mycobacterium smegmatis

HPLC Analysis

The phytochemical constituents of the extracts were analyzed using HPLC technique. The phenolic compounds of *M. arvense* ethyl acetate extract was characterized as chlorogenic acid, caffeic acid, luteolin-7-O-glycoside, coumaric acid, ferulic acid, and quercetin (Figure 2). The high antioxidant capacity of *M. arvense* ethyl acetate extract may be due to the aforementioned phenolic compounds. It was reported that the phenolic compounds were responsible for the antioxidant activity in the previous studies on *Melampyrum* species[3]. In addition, eriodictyol, luteolin and quercetin were detected in *S. spurium* ethyl acetate extract by HPLC (Figure 3).

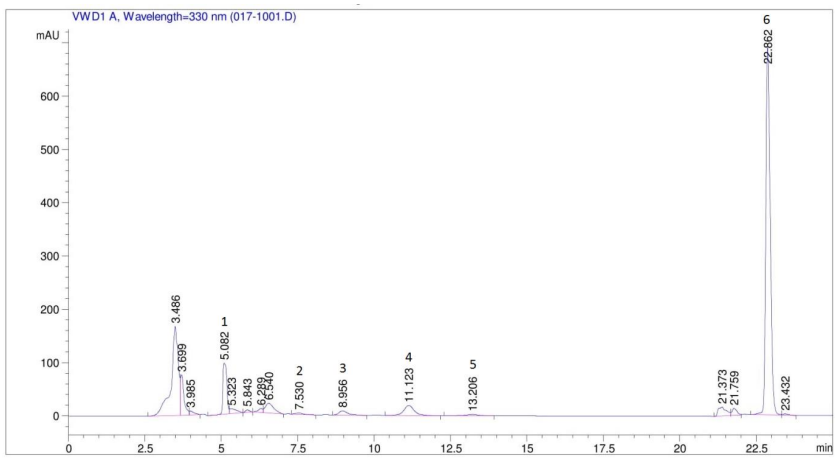

Figure 2. HPLC Chromatogram of *M. arvense* methanol extract

1, Chlorogenic acid (RT: 5.082); 2, Caffeic acid (RT: 7.5); 3, Luteolin-O-Glycoside (RT: 9.03); 4, Coumaric acid (RT: 11.5); 5, Ferulic acid (RT: 13.206); 6, Quercetin (RT: 22.3)

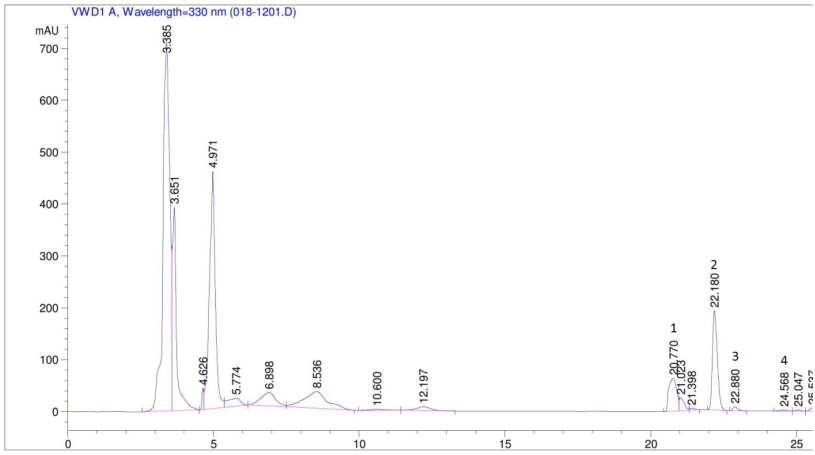

Figure 3: HPLC Chromatogram of *S. spurium* metanol extract

1, Eriodictyol (RT: 20.7); 2, Luteolin (RT: 22.2); 3, Quercetin (RT: 22.3)

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