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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF TWO ENDEMIC PLANTS FROM AKSARAY IN TURKEY

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Abstract

This study was designed to examine the *in vitro* antimicrobial and antioxidant activities of the methanol, ethanol, water, n-hexane and dicholoromethane extracts of two *Allium* species (*Allium scabriflorum* and *Allium tchihatschewii*) which are endemic for the flora of Turkey. The antimicrobial efficiency of the plant was evaluated according to disc diffusion and microdilution broth methods. The antimicrobial test results showed that the extracts of *A. scabriflorum* and *A. tchihatschewii* showed varying degrees of antimicrobial activity on the tested microorganisms. The extracts were screened for their possible antioxidant activities by three complementary tests; DPPH free radical-scavenging, scavenging of hydrogen peroxide and metal chelating activity assays. All the extracts of *A. scabriflorum* and *A. tchihatschewii* exhibited lower DPPH free radical scavenging activity but higher metal chelating activity when compared to standards. The values of scavenging of hydrogen peroxide of the extracts were higher than the standards that of α-tocopherol, BHA, BHT and trolox, but close to that of ascorbic acid. In addition to the antioxidant activity of these plants, the total phenolic compounds and flavonoids were also measured in the extracts. The results presented here may suggest that the extracts of *A. scabriflorum* and *A. tchihatschewii* possess antimicrobial and antioxidant properties, and therefore, they can be used as a natural preservative ingredient in food and/or pharmaceutical industry.

Key words: *Allium scabriflorum*, *Allium tchihatschewii*, antimicrobial activity, antioxidant activity, phenolic

Introduction

The spoilage and poisoning of foods by microorganisms are problems that have not yet been brought under adequate control despite the range of robust preservation techniques available. Raw and/or processed foods are open to contamination during the production, sale and distribution of them (Deak and Beuchat, 1996). Thus, at present, it is a necessity to use the chemical preservatives to prevent the growth of food spoiling microbes in the food industry (Sağdıç and Özcan, 2003). Consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve sufficiently long shelf life of foods and a high degree of safety with respect to foodborne pathogenic microorganisms (Rauha et al., 2000).

Synthetic antioxidants and antimicrobials in use have been shown to have harmful side effects (Gao et al., 1999; Williams et al., 1999; Osawa and Namiki, 1981), therefore, there is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources. Recently, there are a lot of reports on the antioxidant and antimicrobial activities of plant-based extract, which has a potential application both in the food and in medicinal industries (Zeng et al., 2011).

Since ancient times, several species of the genus *Allium* have been used as foods, spices or herbal remedies. The genus *Allium* L. is a member of the family Liliaceae and probably the largest genus of the petaloid monocotyledons, comprising some 780 species (Friesen et al., 2006). *Allium* is naturally distributed in the northern hemisphere and South Africa, mainly in seasonally dry regions. The genus *Allium* comprises 168 species (188 taxa) in the Flora of Turkey (Davis et al., 1988; Kollmann, 1984; Özhatay and Tzanoudakis, 2000). 73 of them are endemic (Özhatay and Kültür, 2006; Özhatay et al., 2009; Özhatay et al., 2011). Generally, all plant parts of *Allium* may be consumed by humans (except perhaps the seeds), and many wild species are exploited by the local inhabitants (Fritsch and Friesen, 2002). Anectodal evidence supports the important roles of the members of this genus in the prevention and treatment of pathogenic infections, tumors and cardiovascular diseases. Antioxidative activity of some *Allium* species has been reported elsewhere (Cao et al., 1996; Gazzani et al., 1998; Yin and Cheng, 1998).

As far as our literature survey could ascertain, *A. scabriflorum* Boiss. and *A. tchihatschewii* Boiss., the endemic plants in Aksaray-Turkey, have not been chemically or biologically investigated. Therefore, the aims of the present study were to estimate phenolic and flavonoid contents and to evaluate the antibacterial and antioxidant activities of these plants collected from Aksaray.

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Materials and Methods

Plant material and extraction

Flowering samples of *A. scabriflorum* (voucher No. Teksen 2571 & Karaman) and *A. tchihatschewii* (voucher No. Teksen 2614 & Karaman) were collected during June-July in 2010 from Genç Osman Village (an altitude of ca. 1050 m) and Aksaray University Campus Area, Aksaray, Turkey (altitude of ca. 950m), respectively. They were dried at room temperature. The plants were identified and deposited at the herbarium of Biology Department, Faculty of Science and Art, Aksaray University, Turkey.

The air-dried and powdered plant materials (15 g of each) were extracted seperately with ethanol, methanol, water, n-hexane and dicholoromethane (DCM) by Soxhlet extraction for 24 hours. The extracts were filtered and evaporated by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany) and stored in the dark at 4ºC until used within a maximum period of one week.

Determination of antimicrobial activity

Test organisms

Different extracts of various plant parts of *A. scabriflorum* and *A. tchihatschewii* were individually tested against a set of 13 microorganisms. The following microorganisms were used in the screening of antimicrobial activity: Seven gram negative bacteria, namely *Escherichia coli* ATCC 11229, *Escherichia coli* ATCC 35218, *Escherichia coli* O157:H7, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* Mu:57, *Yersinia enterocolitica* NCTC 11175, four gram positive bacteria namely, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863, *Micrococcus luteus* NRRL B-4375 and two yeasts, *Saccharomyces cerevisiae* and *Candida albicans* ATCC 10231. Bacterial strains were cultured overnight at 37ºC in Nutrient Agar (NA) and Tryptic Soy Agar (TSA) and yeasts were cultured for 48 h at 30ºC in YPD Agar medium.

Antimicrobial assay

The disc diffusion assay was used to determine the antimicrobial potential of the investigated extracts (Murray et al., 1995). The culture suspensions were adjusted by comparing with 0.5 McFarland. One hundred microlitres of suspension of the test microorganisms were spread on solid media plates. Sterile filter paper discs of 6 mm diameter were impregnated with 10 µL of the extract solution. The plates were kept for 2 hours in refrigerator to enable prediffusion of the extracts into the agar. Then the inoculated plates were incubated at 37 °C for 24 h for bacterial strains and 30 °C for 48 h for yeasts. Ampicillin (Amp, 10 µg/disc), Gentamicin (CN, 10 µg/disc) and Amikacin (AK, 30 µg/disc) were used as positive controls. Negative controls were performed with paper discs loaded with 10 µL of solvents (ethanol, methanol, water and dimethyl sulfoxide (DMSO)). At the end of the incubation period the antimicobial activity was evaluated by measuring the inhibition zones (the diameter of inhibition zone plus the diameter of the disc).

Broth microdilusion assay for minimum inhibitory concentrations (MICs)

The antimicrobial activity of the extracts was also evaluated through the determination of the MIC by the microdilution method in culture broth with some modifications (Koneman et al., 1997). The minimal inhibitory concentration (MIC) values were studied for the microorganisms, being sensitive to the extracts in the disc diffusion assay. Tubes were prepared by dispensing 95 µL of growth medium, 100 µL of the extract and 5 µL of the inoculum. The serial dilutions of the extracts were done in a concentration range from 1.41 to 180 µg/µL in the tubes. The inoculum of microorganisms was prepared using 12 h cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The final volume in each tube was 200 µL. A positive control (containing 5 µL inoculum and 195 µL growth medium) and a negative control (containing 100 µL of extract, 100 µL growth medium without inoculum) were included on each microtube. The contents of the tubes were mixed by pipetting, and they were incubated at appropriate temperatures for 24 h. Then, 5 µL samples from clear tubes were plated on solid growth medium to confirm microbial growth because the plant extracts tested in this study were colored (Şahin et al., 2003). The MIC was defined as the lowest concentration of the compounds needed to inhibit the growth of microorganisms. Each test was repeated at least triplicate.

Antioxidant capacity

DPPH radical scavenging activity

The radical scavenging activity was determined by a spectrophotometric method based on the reduction of ethanol solution of DPPH (Blois, 1958). The extracts were dissolved in ethanol (1 mg/mL), and various concentrations (5, 25, 50 and 75 µg/mL) of each extract were used. Similar concentrations of α-tocopherol, BHA, BHT and ascorbic acid were used as positive control. The prepared extracts were transferred in tubes and then total volume was adjusted to 1.5 mL with ethanol. 0.5 mL of 0.1 mM solution of DPPH radical in ethanol DPPH radical was added to each test tube, and the mixture was shaken vigorously. After 30 min incubation at room temperature in the dark, the decrease in absorbance was measured at 517 nm. The radical scavenging activity was calculated from the equation [1]:

% of radical scavenging activity = [$(Abs_{control} - Abs_{sample})/Abs_{control}$]× 100

wherein $Abs_{control}$ is the absorbance of the control reaction, and Abs_{sample} is the absorbance in the presence of the test compound.

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Metal chelating activity

The chelating of ferrous ions by the extracts and standards was estimated according to the method of Dinis et al. (1994) with some modifications. Briefly, 30 µg/mL concentration of the each extract in ethanol was added to a solution of 2 mM $FeCl_2$ (25 µL). Then, total volume was adjusted to 1.9 mL with ethanol. The reaction was initiated by the addition of 5 mM ferrozine (0.1 mL), and the mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. α-tocopherol, BHA, BHT, ascorbic acid, trolox and EDTA were used as positive controls. The percentage of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the equation [1].

Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (43 mM) was prepared in 0.1 M phosphate buffer (pH 7.4). 30 µg/mL concentration of the each extract in ethanol was added to a solution of 43 mM H_2O_2 (130 µL). Then, total volume was adjusted to 1.0 mL with 0.1 M phosphate buffer (840 µL). The absorbance of hydrogen peroxide at 230 nm was determined after 30 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. α-tocopherol, BHA, BHT, ascorbic acid and trolox were used as positive controls. The percentage of scavenging activity of hydrogen peroxide was calculated using the equation [1].

Determination of the amount of phenolic compounds and flavonoids

The total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965) with some modification as described below, using gallic acid as a standard phenolic compound. Briefly, 1 mL solution of extracts containing 1mg extract in ethanol was mixed with 22 mL distilled water. 0.5 mL of Folin-Ciocalteu reagent was added, and the content of the flask mixed thoroughly. After 3 min, 1.5 mL of 2% Na_2CO_3 solution was added to the mixture. After incubation for 2 h at room temperature, the absorbance against blank was measured at 760 nm with an UV-Vis Spectrophotometer (HITACHI U-2000). Gallic acid was used as a standard for calibration curve. The total phenolic content was expressed as µg gallic acid equivalents (GAE)/mg dry extracts. All samples were analyzed in triplicates.

The measurement of total flavonoid content was based on the method described by Park et al. (1997) with slight modifications. 1 mL of the extract solution containing 1 mg extract was added to a test tube containing 0.1 mL of aluminum nitrate (10%), 0.1 mL of aqueous potassium acetate (1 M), and 4.1 mL of ethanol. After incubation for 40 min at room temperature, the absorbance was read spectrophotometrically at 415 nm. A calibration curve of quercetin was prepared, and flavonoid contents were determined from the linear regression equation of the calibration curve. The total flavonoid content was expressed as µg quercetin equivalents (QE)/mg of dry extracts. All extracts were analyzed in triplicate.

Results and Discussion

Antimicrobial activity

The antimicrobial activities of *A. scabriflorum* and *A. tchihatschewii* extracts were employed against the microorganisms and their activity potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, zone diameters and MIC values. The results showed that the *A. scabriflorum* and *A. tchihatschewii* extracts those with except water had great potential as antimicrobial agents against the tested microorganisms. The antimicrobial activities of the plants extracted in different solvents varied greatly because there are many factors that influence the active principle present in the plant (Parekh and Chanda, 2006). Here the polarity of the extracting solvents was different, and it greatly influenced the antimicrobial property (Table 1). According to the results given in Table 1, the aqueous extracts of *A. scabriflorum* and *A. tchihatschewii* did not exhibit any activity against all the tested microorganisms. Similarly, several researchers (Martin, 1995; Paz et al., 1995; Vlientinck et al., 1995) have generally reported that water extracts of plants do not have much activity against bacteria. Durmaz et al. (2006) reported that water extract of *A. vineale* from Bitlis, Turkey had no antibacterial activity against any of nine tested bacteria. DCM extracts of the two species, however, showed antimicrobial activity against 10 out of 13 microorganisms. DCM extracts had the highest inhibitory activities against *B. cereus* RSKK 863 (30.24 mm) and *S. sonnei* Mu:57 (13.17 mm) for *A. scabriflorum* and *A. tchihatschewii*, respectively. The results obtained from the disc diffusion method, indicated that n-hexane extract of *A. tchihatschewii* exhibited a stronger antimicrobial activity in comparison with the *A. scabriflorum* n-hexane extract (Table 1). However, all *A. scabriflorum* extracts could not inhibit the growth of *E. coli* O157:H7 and *S. enteritidis* ATCC 13076. *P. aeruginosa* ATCC 27853 was the most sensitive organism tested to the both plants extracts. Najjaa et al. (2007) reported antimicrobial potential of a wild endemic *Allium* species growing in the arid region of Tunisia. They found that *A. roseum* essential oil had a high effect against gram negative *P. aeruginosa*, which is a similar result with our study. Especially greater and remarkable antimicrobial activities of dicholoromethane extract were recorded (Najjaa et al., 2011). The results obtained from the controls indicated that the solvents had no effect on the microorganisms.

Some of these plants extracts were more effective than traditional antibiotics (Ampicillin, Amikacin and Gentamicin) to combat the pathogenic microorganisms and some reference strains studied (Table 1). Some *A. scabriflorum* DCM extracts, for example, have shown better antibacterial activity against 4 out of 11 bacteria when compared with standard Gentamicin and Amikacin (Table 1). An *A. scabriflorum* ethanol extract exhibited a stronger antibacterial activity in

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Table 1: Antimicrobial activity of *Allium scabriflorum* and *Allium tchihatschewii* extracts

Org.	Diameter[a] of zone of inhibition (mm)										MIC (µg/µL)										Diameter[a] of zone of inhibition (mm)		
	Allium scabriflorum					*Allium tchihatschewii*					*Allium scabriflorum*					*Allium tchihatschewii*					Antibiotics		
	M-E	E-E	W-E	H-E	DCM-E	M-E	E-E	W-E	H-E	DCM-E	M-E	E-E	W-E	H-E	DCM-E	M-E	E-E	W-E	H-E	DCM-E	Amp	AK	CN
1	-	-	-	10.03± 0.52	13.10± 0.03	-	-	-	10.36± 0.31	-	-	-	-	45.00	22.50	-	-	-	11.25	-	25.11 ±0.01	15.60 ±0.01	10.46 ±0.02
2	-	16.97± 1.85	-	-	7.72± 1.53	-	-	-	-	6.96± 0.08	-	22.50	-	-	22.50	-	-	-	-	11.25	25.01 ±0.02	14.68 ±0.02	10.19 ±0.02
3	-	15.58± 3.76	-	15.96± 0.03	28.26± 5.52	9.76± 1.94	11.13± 1.35	-	9.67± 0.86	-	-	11.25	-	5.63	11.25	11.25	22.50	-	22.50	-	36.34 ±0.02	17.34 ±0.01	13.05 ±0.02
4	-	-	-	-	12.32± 0.15	9.55± 0.71	11.18± 0.28	-	9.58± 0.24	8.84± 0.02	-	-	-	-	45.00	45.00	45.00	-	22.50	45.00	30.67 ±0.02	19.50 ±0.01	19.38 ±0.02
5	-	-	-	-	-	-	-	-	9.89± 0.65	-	-	-	-	-	-	-	-	-	45.00	-	30.02 ±0.02	18.61 ±0.02	14.07 ±0.01
6	10.16± 1.17	16.42± 0.12	-	-	30.24± 0.01	8.68± 2.18	10.47± 0.92	-	-	7.48± 0.03	22.50	90.00	-	-	45.00	90.00	45.00	-	-	11.25	36.86 ±0.01	19.35 ±0.01	13.87 ±0.02
7	11.70± 0.63	13.56± 2.77	-	9.15± 0.01	13.96± 0.07	9.07± 0.59	10.73± 1.40	-	9.59± 0.06	6.76± 0.09	11.25	5.63	-	45.00	11.25	22.50	22.50	-	45.00	11.25	-	18.88 ±0.01	16.31 ±0.02
8	9.96± 0.28	-	-	-	29.32± 1.19	9.25± 0.35	-	-	11.06± 0.13	13.17± 1.45	5.63	-	-	-	5.63	11.25	-	-	11.25	22.50	34.50 ±0.02	13.28 ±0.02	10.93 ±0.01
9	-	10.08± 2.21	-	7.63± 0.64	18.26± 2.12	-	-	-	11.16± 0.14	6.96± 0.08	-	11.25	-	22.50	45.00	-	-	-	11.25	5.63	34.05 ±0.02	13.99 ±0.02	11.51 ±0.01
10	-	15.65± 2.08	-	9.59± 1.68	11.76± 3.22	10.92± 1.44	12.98± 1.99	-	10.68± 0.54	8.45± 0.09	-	22.50	-	22.50	5.63	11.25	45.00	-	22.50	22.50	12.32 ±0.02	23.04 ±0.02	19.92 ±0.01
11	9.06± 0.88	-	-	10.98± 0.13	12.12± 0.51	-	-	-	10.43± 0.36	8.23± 0.11	22.50	-	-	22.50	11.25	-	-	-	22.50	22.50	-	-	-
12	20.37± 0.75	22.50± 2.59	-	-	-	11.48± 1.95	16.71± 1.01	-	11.13± 0.43	6.36± 0.02	11.25	11.25	-	-	-	22.50	22.50	-	22.50	11.25	-	-	-
13	-	-	-	-	-	10.08± 0.43	9.87± 1.34	-	9.87± 0.38	8.91± 0.05	-	-	-	-	-	11.25	22.50	-	22.50	11.25	25.41 ±0.02	13.87 ±0.01	10.51 ±0.02

1: *E. coli* ATCC 11229, 2: *E.coli* ATCC 35218, 3: *S. aureus* ATCC 25923, 4: *L. monocytogenes* ATCC 7644, 5: *E. coli* O157:H7, 6: *B.cereus* RSKK 863, 7: *P. aeruginosa* ATCC 27853, 8: *M. luteus* NRRL B-4375, 9: *S. sonnei* Mu:57, 10: *Y. enterocolitica* NCTC 11175, 11: *C. albicans* ATCC 10231, 12: *S.cerevisiae* (grape isolate), 13: *S. enteritidis* ATCC 13076

-: no zone of inhibition

[a]diameter of the inhibition zone including disc diameter. Values are means ± SD of three seperate experiments done in triplicate.

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Table 2: Scavenging activity (%) on DPPH radicals of different *Allium scabriflorum* and *Allium tchihatschewii* extracts and standart antioxidants at different concentrations

Concentration	DPPH Scavenging Activity[a] (%)													
	Allium scabriflorum					*Allium tchihatschewii*					Standards			
	ME	EE	WE	HE	DCM-E	ME	EE	WE	HE	DCM-E	α-Tocopherol	BHA	BHT	Vitamin C
5 µg/mL	18.15 ±0.15	17.44 ±0.09	12.10 ±0.16	14.59 ±0.06	13.88 ±0.05	11.74 ±0.16	12.81 ±0.02	12.81 ±0.08	12.46 ±0.09	1.07 ±0.12	89.01 ±0.04	86.01 ±0.06	56.04 ±0.05	92.31 ±0.06
25 µg/mL	23.49 ±0.03	27.40 ±0.24	12.81 ±0.12	17.08 ±0.02	22.06 ±0.09	27.76 ±0.06	14.60 ±0.03	14.60 ±0.16	16.73 ±0.08	1.78 ±0.17	94.14 ±0.05	94.14 ±0.07	93.41 ±0.03	94.51 ±0.08
50 µg/mL	28.11 ±0.06	37.01 ±0.11	15.30 ±0.15	20.29 ±0.04	57.65 ±0.13	39.50 ±0.07	15.30 ±0.14	18.51 ±0.07	19.57 ±0.11	2.49 ±0.03	95.60 ±0.08	95.60 ±0.12	97.44 ±0.06	96.34 ±0.07
75 µg/mL	32.38 ±0.20	43.06 ±0.16	17.08 ±0.09	22.42 ±0.08	61.57 ±0.12	46.26 ±0.11	16.01 ±0.12	19.22 ±0.09	28.47 ±0.03	3.20 ±0.08	97.07 ±0.04	97.07 ±0.11	99.64 ±0.03	99.63 ±0.04

[a]each value represents the mean of triplicate studies.

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Table 3: Percentage of metal chelating and hydrogen peroxide scavenging activities of different *Allium scabriflorum* and *Allium tchihatschewii* extracts and standart antioxidants

% Activity[a]	*Allium scabriflorum*					*Allium tchihatschewii*					Standards					
	ME	EE	WE	HE	DCM-E	ME	EE	WE	HE	DCM-E	α-Tocopherol	BHA	BHT	Vitamin C	Trolox	EDTA
Metal chelating (15 µg/mL)	98.96 ±0.23	99.19 ±0.13	98.92 ±0.06	99.42 ±0.25	99.69 ±0.11	97.64 ±0.09	99.73 ±0.08	99.00 ±0.14	99.42 ±0.17	99.69 ±0.08	8.33 ±0.10	40.91 ±0.07	30.30 ±0.04	60.61 ±0.08	11.36 ±0.02	84.09 ±0.09
H_2O_2 scavenging (30 µg/mL)	82.61 ±0.27	77.36 ±0.09	83.58 ±0.43	80.53 ±0.12	67.43 ±0.14	82.61 ±0.04	84.66 ±0.09	84.81 ±0.25	79.44 ±0.24	82.26 ±0.12	42.85 ±0.08	0.73 ±0.11	37.60 0±0.05	86.17 ±0.03	1.55 ±0.03	Ns[b]

[a]each value represents the mean of triplicate studies; [b]Not studied

Table 4: Total phenolic and flavonoid contents of different *Allium scabriflorum* and *Allium tchihatschewii* extracts

Antioxidant Components	*Allium scabriflorum*					*Allium tchihatschewii*				
	ME	EE	WE	HE	DCM-E	ME	EE	WE	HE	DCM-E
Phenolics[a] (µgGAE/mg exract)	28.25 ±0.01	30.20 ±0.02	28.25 ±0.01	27.42± 0.09	33.25± 0.03	29.36± 0.08	27.69± 0.01	29.64± 0.01	46.86± 0.02	27.42± 0.11
Flavonoids[a] (µgQE/mg exract)	1.42± 0.01	11.23± 0.06	1.79± 0.01	8.02± 0	44.06± 0.03	9.15± 0.02	0.85± 0	0.66± 0	17.83± 0.14	12.74± 0.07

[a]each value represents the mean of triplicate studies.

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comparison with Ampicillin. Only the DCM extract of *A. tchihatschewii* has shown better antibacterial activity against *M. luteus* NRRL B-4375 when compared with standard Gentamicin. It is interesting to note that the extracts are not pure compounds and in spite of it, good results were obtained, which only suggests the potential of these extracts. Hence, some *A. scabriflorum* and *A. tchihatschewii* extracts could be used as new natural antimicrobial ingredients for the food industry.
Minimum inhibitory concentration (MIC) is a quantitative endpoint measurement which is most commonly used for evaluating the antimicrobial effect of antibiotics or extracts. MIC values for microorganisms which were sensitive to extracts of *A. scabriflorum* and *A. tchihatschewii* were determined. MIC values of *A. scabriflorum* and *A. tchihatschewii* extracts were in the range of 5.63-45.00 µg/µL and 11.25-90.00 µg/µL, respectively. The both extracts (methanol and DCM) of *A. scabriflorum* which were sensitive to *M. luteus* NRRL B-4375 had the lowest MIC (5.63 µg/µL) values. Two other extracts (n-hexane and DCM) of the species showed the same MIC value. The methanol extract of *A. tchihatschewii*, which had low inhibitory activity in the disc diffusion method, had the highest MIC (90.00 µg/µL) values. Conclusively, as can be seen from the Table 1, *A. scabriflorum* and *A. tchihatschewii* extracts showed various degrees of antimicrobial activity depending on the tested microorganisms, solvents and plant species.

Previous studies showed high antibacterial activity of sulfur and other numerous phenolic compounds found in *Allium* plants (Rivlin, 2001; Griffiths et al., 2002). Aroma components of *Allium* species are physiologically active and exhibit a widespread possibility of application. There is ample evidence that many sulfur compounds found in *Allium* tissue preparations are responsible for various biological effects such as antimicrobial (antibacterial, antifungal, antiviral), antiprotozoal, antioxidant, antitumour, antihypertensive, hypolipidaemic, hepatoprotective andantithrombotic activities (Rose et al., 2005; Tapiero et al., 2004).

Antioxidant capacity

In this research, three most widely used complementary test systems, namely DPPH free radical scavenging activity, metal chelating and hydrogen peroxide scavenging assays, were applied to evaluate the antioxidant capacities of *A. scabriflorum* and *A. tchihatschewii.* The results were given in Table 2 and 3. In the DPPH test, the extracts obtained using different solvents from *A. scabriflorum* and *A. tchihatschewii* were all able to inhibit the DPPH (at concentrations ranging from 5 to 75 µg/mL) (Table 2). The antioxidant potential varied widely and ranged from 17.08- 61.57% and from 3.20-46.26% at 75 µg/mL concentration of *A. scabriflorum* and *A. tchihatschewii*, respectively. The radical scavenging of BHT and BHA, synthetic antioxidants used in food industry, was higher than the extracts of *A. scabriflorum* and *A. tchihatschewii*, 97.07% and 99.64%, respectively. When compared to all extracts, the natural antioxidants such as ascorbic acid and α-tocopherol showed better radical scavenging activities as with synthetic antioxidants. Free radicals cause auto oxidation of unsaturated lipids in food (Kaur and Perkins, 1991), and the radical scavenging system of the extracts could be attributed to their hydrogen donating ability. Tepe et al. (2005) found that, of the methanol extracts of five *Allium* species, namely *A. nevsehirense Koyuncu et Kollmann*, *A. sivasicum N. Özhatay et Kollmann*, *A. dictyoprasum Ledep*, *A. scorodoprasum* L. subsp. *rotundum* (L.) Stearn and *A. atroviolaceum Boiss.*; the first two are endemic for the Turkish flora, showed a strong radical scavenging activity against DPPH when compared to synthetic antioxidant BHT. The radical scavenging values, however, depended on plant species, locality and polarity of extraction solvents.

The ferrous ion chelating activities of α-tocopherol, BHA, BHT, Vitamin C, trolox, EDTA and extracts of *A. scabriflorum* and *A. tchihatschewii* are shown in Table 3. It has been well recognized that transition metal ions such as those of iron and copper are important catalysts for the generation of the first few free radicals to initiate the radical chain reaction or the radical mediates lipid peroxidation (Nawar, 1996). Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. The chelating activity of the plants was evaluated against Fe^{2+} to estimate the potential antioxidant activities of the two plant extracts and standards. The metal scavenging capacities of 15 µg/mL doses of all tested extracts of *A. scabriflorum* and *A. tchihatschewii* were found to be marked higher when comparing to all standards at the same concentration. Table 3 reveals that all the extracts of *A. scabriflorum* and *A. tchihatschewii* demonstrate a marked capacity for iron binding, suggesting their action as lipid peroxidation protector.

The scavenging abilities of various extracts of *A. scabriflorum* and *A. tchihatschewii* on hydrogen peroxide is shown in Table 3 and compared with that of α-tocopherol, BHA, BHT, ascorbic acid and trolox as standards. The extracts (30 µg/mL) of *A. scabriflorum* exhibited 83.58 (water), 82.61 (methanol), 80.53 (n-hexane), 77.36 (ethanol) and 67.43 (DCM) % scavenging activities on hydrogen peroxide. On the other hand, *A. tchihatschewii* extracts showed 84.81 (water), 84.66 (ethanol), 82.61 (methanol), 82.26 (DCM) and 79.44 (n-hexane) % hydrogen peroxide scavenging activities at the same dose. The results show that both *A. scabriflorum* and *A. tchihatschewii* extracts have stronger hydrogen peroxide scavenging activities. Those values are higher than those of α-tocopherol, BHA, BHT and trolox, but close to that of ascorbic acid. Hydrogen peroxide itself is not very reactive, but it is sometimes is toxic to cells because it may give rise to hydroxyl radical in the cells (Halliwell, 1991). Therefore, removal of H_2O_2 is very important for antioxidant defence in cell or food systems. Dietary polyphenols have also been shown to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide, especially compounds with the orthodihydroxy phenolic structures such as quercetin, catechin, gallic acid ester, caffeic acid ester (Nakayama, 1994; Nakayama et al., 1993).

Total phenolics and flavonoids

Total phenolics (µg GAE/mg extracts) and flavanoids (µg QE/mg extracts) of *A. scabriflorum* and *A. tchihatschewii* extracts were shown in Table 4.

As shown in Table 4, there are large variations in the total phenolic content of the plant species investigated, ranging from 27.42 to 33.25 and from 27.69 to 46.86 µg GAE/mg dry extracts for those of *A. scabriflorum* and *A. tchihatschewii* extracts, respectively. Mladevonić et al. (2011) reported that the total phenolic was found as 45.39 mg GAE/g

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in *A. porrum* L. dry extract. According to our findings, total phenolics of *A. scabriflorum* and *A. tchihatschewii* extracts, except *A. tchihatschewii* n-hexane extract (46.86 µg GAE/mg extract), were lower than that of the *A. porrum* L. leaf extract.

The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Komali et al., 1999; Moller et al., 1999). Phenols are important components of plants. They were reported to eliminate radicals due to their hydroxyl groups (Hatano et al., 1989), and they contribute directly to antioxidant effect of system (Duh et al., 1999). Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Cakir et al., 2003; Wagner et al., 1992). The phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999).

It has been reported that compounds such as the flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants (Das and Pereira, 1990). The mechanisms of action of the flavonoids are through scavenging or chelating process (Cook and Samman, 1996). According to our findings, flavonoid contents of *A. scabriflorum* extracts are higher than those of *A. tchihatschewii.* The total flavonoid contents of *A. scabriflorum* and *A. tchihatschewii* varied from 1.42 to 44.06 and from 0.66 to 17.83 µgQE/mg, respectively (Table 4).

Conclusion

Antioxidant and antimicrobial properties of extracts from many plants are of great interest in the food industry, since their potential use as natural additives can fulfill a growing market for replacements of synthetic substances with natural sources. Therefore, new plant species, as natural sources, could be introduced for this purpose. From this point of view, our study may be considered as a new report based on antimicrobial and antioxidative potentials of *A. scabriflorum* and *A. tchihatschewii* growing wild in the Turkish flora and they could be evaluated as a starting point for further investigations.

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