Antioxidant and Antibacterial Activities of Two Endemic *Allium* L. Taxa from Turkey

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***Abstract*—** In this study, antioxidant and antibacterial activities of various solvent extracts (methanol, ethanol, acetone and petroleum benzine) obtained from bulbs and leaves of *Allium deciduum* subsp. *deciduum* and subsp. *retrorsum* were investigated. Antioxidant activity of the extracts was determined by DPPH radical scavenging and β-carotene/linoleic acid assays. In addition, total phenolic contents in all the extracts were determined as gallic acid equivalents (GAEs). *A.deciduum* subsp. *deciduum* methanol bulbs extract showed the highest phenolic content (68.36 mg/g GAEs.).Bulbs extracts of two endemic taxa exhibited higher antioxidant activity than leaf extracts with all the types of solvent used. A positive correlation was observed between antioxidant activity and amount of phenolic contents of the extracts. Antibacterial activity of methanol and ethanol extracts was examined against two bacteria *Staphylococcus aureus* and *Eschericha coli* by agar well diffusion method and bulb methanolic extracts of *A.deciduum* subsp. *deciduum* showed more antibacterial activity against S. aureus than E.coli.

***Keywords*—** *Allium deciduum,* antibacterial activity, DPPH, β-caroten-linoleic acid assay.

I. Introduction

ANTIOXIDANTS are of great importance in terms of oxidative stress prevention, which may result from several degenerative diseases [1]. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [2]. Research has shown that many plant species have some levels of antioxidant activities. Currently, there are many researches on the use of herbs to reduce the damage caused by oxidant agents [3]. The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, b-carotene, and a-tocopherol are known to possess antioxidant potential [4-6]. A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported [7]. *Allium* is the largest genus consisting of about 600 species, widespread throughout the world in the Alliaceae family [8]. Plants belonging to *Allium* genus are rich in organosulfur compounds and flavonoids showing antioxidant and antibacterial activities. These plants have proved useful in the prevention of some chronic diseases [9]. *Allium* species are used as a food or as medicinal herbs since ancient times. Many recent studies have suggested that certain *Allium* spp. may prevent a number of diseases such as carcinogenesis, atherosclerosis, pulmonary damages, liver necrosis, etc [10].

The genus *Allium* (Alliaceae) contains important vegetables like onions and chive [11]. *Allium* species have been used for food and medicine for thousands of years, especially *Allium sativum* (garlic) and *A. cepa* (onion) recently interest in other species has been increasing [12]. The antioxidant activity of *Allium* species has been attributed mainly to a variety of sulphur-containing compounds (alliin, γ-glutamylcysteine, diallyl sulfide, diallyl disulfide etc.) and proteins (lectins) their precursors [13], but it is also related to other bioactive compounds: dietary fibres, microelements and polyphenols [14]. The *Allium* genus is one of the major sources of polyphenolic compounds and the antioxidative activity of some *Allium'*s species has been reported and has been mainly attributed to a variety of organo-sulfurous compounds as well as their precursors [15-16].

Allium deciduum subsp. *deciduum* and subsp.*retrorsum* taxa are Turkey endemic taxon. The aim of the present study was to assess the antioxidant and antibacterial activity of different solvent extracts of endemic *A. deciduum* taxa bulbs and leaves.

II. Materials And Methods

A. Plant Materials

Different parts (leaves and bulbs) of *Allium deciduum* subsp. *deciduum* and subsp.*retrorsum* taxa (Fam:Alliaceae) were collected in the spring from Sandras Mountain, Köyceğiz district, near Muğla province, Turkey in July 2011. Its bulbs and leaves were dried, chopped up with a blender and prepared for the experiment.

B.Preparation of the extract

Leaves and bulbs of plant materials were dried in shade at room temperature and cut into small pieces with a blender. Extractions were prepared using different solvents (methanol, ethanol, acetone and benzine). For extractions 10 g of the powered plant materials and 100 mL of solvent were used for each sample. The mixture was extracted after being heated in a shaker water bath at 55ºC for 6 h. The extract obtained was filtered through filter paper (Whatman No: 1), and the solvents were evaporated in a rotary evaporator at 48–49°C. The water in each extract was frozen in Freeze-drying machine and all the extracts were stored at -20ºC.

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C.Determination of total phenolic content

The total phenolic content of extracts was determined using to the Folin-Ciocalteu method as gallic acid equivalents (GAE) [17]. Briefly, 0.75 mL of Folin-Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and 100 mL of sample (5 mg/mL) were put into a test tube. The mixture was mixed and allowed to stand for 5 min at room temperature. Then 0.75 mL of 6 % (w/v) Na_2CO_3 was added to the mixture and then mixed gently. The mixture was homogenized and allowed to stand at room temperature for 90 min and its absorbance was measured at 750 nm against a methanol blank. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g plant extract.

D. Determination of antioxidant activity by β –Carotene-linoleic acid assay

Antioxidant activity of plant extracts were measured according to the method described by Amin and Tan, 2002 [18]. One mL of β-carotene solution (0.2 mg/ mL chloroform) was pipetted into a round-bottom flask (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of 100% Tween 20. The mixture was then evaporated at 40 ºC for 10 min by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 mL of distilled water. The distilled water was added slowly to the mixture and agitated vigorously to form an emulsion. 4.8 mL of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 of the extract in them. For control, 0.2 mL of solvent (methanol, ethanol, acetone and benzine) was placed in test tubes instead of the extract. As soon as the emulsion was added into the test tubes, initial absorbance was measured with a spectrophotometer (TU-1880 Double Beam UV-VIS) to be at 470 nm. The measurement was carried out at 0.5 h intervals for 2 h. All samples were assayed in triplicate. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation. The measurements were made using the equation below:

$$AA: (1- (A0-At / A0^{o} - At^{o}) \times 100$$

Where AA is the total antioxidant activity, A0 is the initial absorbance of the sample, At is the initial absorbance of the control, $A0^{o}$ is the sample's absorbance after 120 min, and At^{o} is the control's absorbance after 120 min.

E. Determination of free radical scavenging activity by DPPH assay

Free radical scavenging activity of the extracts was determined using the free radical DPPH [19]. 4 ml of the DPPH's 0.004% metanolic solution was mixed with 1 ml (1.0 mg/ml) of the extracts, and their absorbances were measured to be at 517 nm after incubation for 30 min at room temperature the absorbance value of the samples were evaluated against empty control group (where all determinants except the test compound were present). Every test was treated three times and the averages as determined. Free radical scavenging activity was measured using the equation below:

$$\text{Scavenging activity} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

F. Antibacterial activity

Antibacterial activity of the extracts *A.deciduum* subsp. *deciduum* and subsp. *retrorsum* was assessed using the agar well diffusion method [20]. Antibacterial activity of extracts was determined against the following pathogen bacteria: *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 11230). The bacterial strains were cultivated in Mueller Hinton Broth (MHB) ve Mueller Hinton Agar (MHA) at 37°C for 24 h. The dry extracts were resuspended in DMSO solvent at a concentration of 1 mg/mL. On the preparation of bacteria, 1 mg/ml of concentration was prepared from the extracts obtained from the bulbs and leaves of the *Allium* taxa extracts methanol and ethanol. The extracts were saturated into paper discs 8 mm in diameter. The petri dishes that had bacteria were incubated at 37°C for 24 h. After incubation, all zones of growth inhibition and diameters of zones were measured in milimetres.

III. Results And Discussion

A. Antioxidant activity

The results of total phenolic contents obtained for of different solvent (ethanol, methanol, acetone and benzine) extracts from the bulbs and leaves are given in Table 1. The effects of the solvents tested on the extraction yield was significant in all extraction. In the present study total phenolic content was highest in the methanol extract and lowest in the benzine extract. Total phenolic content in the extracts ranged from 39.87 to 68.36 mg/g GAE (Table 1). The phenolic contents in different extracts varied significantly in all extractions. The methanolic extract bulbs of *A.deciduum* subsp. *deciduum* had the highest phenolic content, the benzine extracts leaves of *A.deciduum* subsp.*retrorsum* had the lowest phenolic content.

Antioxidant activity of methanol, ethanol, acetone and benzine extracts increased in the bulbs extracts. The extracts showed 41.87%-80.21% antioxidant activity in β–carotene-linoleic acid assay. The antioxidant capacity of extracts, measured by the β-carotene– linoleic acid model system, is presented in Table. 2. In the present study, among different solvents of *A.deciduum* subsp. *deciduum* and subsp.*retrorsum*, the methanolic bulb extracts were highly antioxidant activity (80.21%) followed by ethanolic bulb extracts (77.87%). In the present study the methanol extract of *A. deciduum* taxa had the highest phenolic content, as well as the highest DPPH free radical scavenging activity. In the present study the methanol extract had the highest antioxidant activity while the benzine extract had the lowest antioxidant activity (Table 2). DPPH assay shows that the highest free radical scavenging activity demonstrated *A.deciduum* subsp. *deciduum* bulbs methanol extracts.

TABLE I
PHENOLIC CONTENTS OF THE EXTRACTS OF *A.DECIDUUM* SUBSP.*DECIDUUM* AND *A.DECIDUUM* SUBSP.*RETRORSUM*

	Total phenolic contents (mg/g GAE)			
Plant extracts	**Ethanol**	**Methanol**	**Acetone**	**Benzine**
***A.deciduum* subsp. *deciduum* bulbs**	48.21	68.36	46.40	30.35
***A.deciduum* subsp. *deciduum* leaves**	40.36	49.25	31.83	29.03
***A.deciduum* subsp.*retrorsum* bulbs**	43.69	62.63	34.79	30.08
***A.deciduum* subsp.*retrorsum* leaves**	45.69	49.87	30.73	28.75

TABLE II
ANTIOXIDANT ACTIVITIES AND DPPH FREE RADICAL SCAVENGING ACTIVITY OF THE *A.DECIDUUM* SUBSP. *DECIDUUM* AND SUBSP. *RETRORSUM.*

Extracts	***A.deciduum* subsp. *deciduum***			
	β –Carotene asaays (%)		**DPPH-RSC (%)**	
	Bulbs	**Leaves**	**Bulbs**	**Leaves**
Ethanol	77.27	73.70	55.86	52.36
Methanol	80.21	76.85	65.46	60.58
Acetone	49.62	45.76	42.58	40.16
Benzine	44.12	42.78	44.56	38.54
	A.deciduum* subsp. *retrorsum			
	β –Carotene asaays (%)		**DPPH-RSC (%)**	
Extracts	**Bulbs**	**Leaves**	**Bulbs**	**Leaves**
Ethanol	76.85	71.73	52.48	50.58
Methanol	78.54	73.65	56.45	54.36
Acetone	47.74	43.77	40.86	38.54
Benzine	43.22	41.87	42.67	36.25

B. Antibacterial activity

The antibacterial activity levels of the extracts of *A.deciduum* subsp. *deciduum* and subsp. *retrorsum*, evaluated by the agar well diffusion method are reported in Table 3. In the agar well diffusion method, the maximal inhibition zones ranged between 1.4 and 6.3 mm. The methanol extracts showed antibacterial activity against Gram (+) bacteria except *S. aureus*. The ethanol extract had no effect on the entire tested Gram (-) bacteria except *E.coli.* As clearly seen in the Table 3, the extract of bulbs was effective against tested against bacteria. As far as our literature survey could ascertain, there is no study about antimicrobial activity of *A. deciduum* extract.
Several studies have been carried out to determine the antimicrobial activity of extracts and compounds isolated from various *Allium* species. Many researchers later found that oils of alliums [21-23] and their constituting sulfides have significant antimicrobial effects and are much more antifungal than antibacterial.

TABLE III
ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF *A.DECIDUUM* SUBSP. *DECIDUUM* AND SUBSP. *RETRORSUM*

	Inhibition zone(mm)			
Strains	***A.deciduum* subsp. *deciduum***			
	Ethanol		**Methanol**	
	Bulbs	**Leave**	**Bulbs**	**Leave**
***Escherichia coli* ATCC 11230 Gr (-)**	4.3	3.0	5.7	5.6
***Staphylococcus aureus* ATCC 29213 Gr (+)**	4.8	3.5	6.3	5.2
Strains	***A.deciduum* subsp. *retrorsum***			
	Ethanol		**Methanol**	
	Bulbs	**Leave**	**Bulbs**	**Leave**
***Escherichia coli* ATCC 11230 Gr (-)**	3.4	2.0	1.8	1.4
***Staphylococcus aureus* ATCC 29213 Gr (+)**	3.0	2.5	3.2	2.7

Extracts of the white shaft and green leaves of 30 *Allium ampeloprasum* var. *porrum* cultivars were investigated for their antioxidant properties, total phenolic (TP) and L-ascorbic acid (AA) content. The measured antioxidant properties included free radical scavenging activities against peroxyl (ORAC) and 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) and their Fe3+ reducing capacity (FRAP). The results from this study suggest that the green leek leaves generally have significantly stronger antioxidant properties than the white shaft [24]. Previous study on the antioxidant activity of five *Allium* methanolic extracts species (*Allium nevsehirense*, *Allium sivasicum*, *Allium dictyoprosum*, *Allium scrodoprosum* subsp. *rotundum* and *Allium atroviolaceum*), measured by DPPH, showed an IC_{50} ranged between 79 and 104 μg/ml with an efficiency of 3.95 (IC_{50} extract/IC_{50} BHT) [25].

A four-factor and three-level BoxBehnken design was used to optimise the extraction parameters for polysaccharides from *Allium macrostemon* Bunge (AMBP). As a result, the optimal conditions for AMBP extraction were determined. During in vitro antioxidant assay, AMBP40 exhibited relative stronger scavenging activities on hydroxyl radical and superoxide radical and metal chelating activity than AMBP60 and AMBP80 [26].

The results of the present study show that the methanol extract of *A. deciduum* subsp. *deciduum* and subsp. *retrorsum* contained a high total phenolics level, and is a good source of antioxidant as well as antibacterial agents; therefore, it can be considered potentially useful for medicinal application.

REFERENCES

[1] S. Zouari, M. Ketata, N. Boudhrioua and E. Ammar, '*Allium roseum* L. volatile compounds profile and antioxydant activity for chemotype discrimination – Case study of the wild plant of Sfax (Tunisia)', *Industrial Crops and Products*, vol.41, pp.172-178, 2013.
[2] AS. Sharif, N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava and A. Sahu, 'Indian Medicinal Herbs As Sources Of Antioxidants', *Food Research International*, vol.41, pp.1-15, 2008.

[3] RG. Finch, 'Antibiotic Resistance',*J.Antimicrobial Chemotherapy*, vol.42, pp.125-128, 1998.
[4] PH Davis, '*Flora of Turkey and the East Aegeon Islands',* Edinburgh Univ., Press, Edinburgh. Vol. , 1984
[5] F Aqıl, I Ahmad and Z. Mehmood, 'Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants', *Turk J Biol., vol.* 30, pp**.** 177-183, 2006.
[6] RL.Prior, 'Fruit and vegetables in the prevention of cellular oxidative damage' *Am J Clin Nutr*, vol.78, pp. 570-578, 2003.
[7] Y Cai, Q Luo, M Sun and H. Corke, 'Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer', *Life Sciences*, vol. 74, pp**.** 2157-2184, 2004.
[8] WT. Stearn, 'How many species of *Allium* are known?' *Kew Magazine* vol.**9**, pp.180–181, 1992.
[9] N. Benkeblia, 'Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*A. sativum*)'. *LWT-Food Sci. Technol*, vol. 37, pp**.** 263-268, 2004.
[10] D. Stajner, N. Milic-DeMarino, J. Canadanovic-Brunet, M. Stajner and B.M. Popovic, 'Screening for antioxidant properties of *Allium giganteum',* Fitoterapia, vol. 77, pp. 268-270, 2006.
[11] T. Fossen, R. Slimestad and M. Andersen, 'Anthocyanins with 40-glucosidation from red onion, *Allium cepa', Phytochemistry, vol.* 64, pp. 1367–1374, 2003.
[12] T. Bahorun, A. Luximon-Ramma, A. Crozier and OI. Aruoma, 'Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables', *Journal of the Science of Food and Agriculture, vol*. 84(12), pp. 1553–1561, 2004.
[13] M. Corzo-Martinez, N. Corzo and M. Villamiel, 'Biological properties of onions and garlic', *Trends İn Food Science & Technology*, vol.18, pp**.** 609-625, 2007.
[14] M. Parvu, A. Toui, L. Vlase and EA. Parvu, 'Determination of some polyphenolic compounds from *Allium* species by HPLC-UV-MS' *Natural Product Research*, vol. 24(14), pp**.** 1318–1324, 2010.
[15] SM. Kim, K. Kubota and A. Kobayashi, 'Antioxidative activity of sulfur-containing flavor compounds in garlic'. *Bioscience Biotechnology and Biochemistry*, vol. 61, pp. 1482–1485, 1997.
[16] J. Auger, W. Yang, I. Arnault, F. Pannıer and M. Potin-Gautıer, 'High-performance liquid chromatographic–inductively coupled plasma mass spectrometric evidence for Se-alliins in garlic and onion grown in Se-rich soil', *Journal of Chromatography*, vol. 1032, pp.103–107, 2004
[17] Y. Tekeli, 'Chemical and Biological Characterization Some of Species *Centaurea* in Konya', PhD, Selcuk University Institute of Science Chemistry Department, 2008.
[18] I. Amin and SH. Tan, 'Antioxidant activity of selected commercial seaweeds', *Malays. J. Nutr*. Vol. 8, pp. 167-177, 2002.
[19] C. Wu, F. Chen, X. Wang, H.J. Kim, GQ. He, V. HaleyZitlin, and G. Huang, 'Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification', *Food Chem*. Vol. 96, pp. 220–227, 2006.
[20] Y. Baravalıa, M. Kanerıa, Y. Vaghasıya, J. Parekh and S. Chanda, 'Antioxidant and Antibacterial Activity of *Diospyros ebenum* Roxb. Leaf Extracts', *Turk J Biol*., vol.**3, pp.** 159-164, 2009.
[21] N. Bernaert, D. De Paepe, C. Bouten, H. De Clercq, D. Stewart, E. Van Bockstaele, M. De Loose and B. Van Droogenbroeck, 'Antioxidant capacity, total phenolic and ascorbate content as a function of the genetic diversity of leek (*Allium ampeloprasum* var. *porrum*)', *Food Chemistry*, vol. 134, pp. 669-677, 2012.
[22] B. Tepe, M. Sokmen, A. Akpulat and A. Sökmen, 'In Vitro Antioxidant Activities of the Methanol Extracts of Five *Allium* Species from Turkey', *Food Chemistry*, vol. 92, pp. 89-92, 2005.
[23] ZJ. Zhang, FH. Wang, MC. Wang, L. Ma and X. Zeng, 'Extraction optimisation and antioxidant activities in vitro of polysaccharides from *Allium macrostemon* Bunge', *Internatıonal Journal Of Food Scıence And Technology, vol.*47, pp. 723-730, 2012.
[24] MG. Curcic, MS. Stankovic, ID. Radojevic, OD. Stefanovic, LR. Comic, MD. Topuzovic, DS. Markovic SD 'Biological Effects, Total Phenolic Content and Flavonoid Concentrations of Fragrant Yellow Onion (*Allium flavum* L.)' *Medicinal Chemistry*, vol.8, pp. 46-51, 2012.
[25] B. Tepe, M. Sokmen, A. Akpulat and A. Sokmen, 'In Vitro Antioxidant Activities of the Methanol Extracts of Five *Allium* Species from Turkey', *Food Chem*, vol. 92, pp.89, 2005.
[26] ZJ. Zhang, FH. Wang, MC. Wang, LP. Ma and XX. Zeng, 'Extraction optimisation and antioxidant activities in vitro of polysaccharides from *Allium macrostemon* Bunge', *Internatıonal Journal Of Food Scıence And Technology*, vol.47, pp. 723-730, 2012.