

Antibiofilm, Antioxidant, Antimutagenic Activities and Phenolic Compounds of *Allium orientale* BOISS.

Ozgun Ceylan* and Halime Alic

¹Mugla University – Apiculture; Ula Ali Kocman Vocational School Mugla Sitki Kocman University;, Mugla – Turkey. ²Mugla Sitki Kocman University – Biology; Mugla - Turkey

ABSTRACT

This is the first study to investigate the antibiofilm, antioxidant and antimutagenic activities and phenolic compounds of *Allium orientale*. Antimicrobial activity of ethanolic extracts of *A. orientale* was determined by a broth microdilution method. Antibiofilm activity was evaluated by microplate biofilm assay. The antioxidant activity was determined using three complementary assays; namely, DPPH scavenging, β -carotene-linoleic acid, and total phenolic compounds assays. Phenolic compounds were evaluated by reverse-phase high-performance liquid chromatography. The antimutagenic effect of extracts was analyzed by the Ames test. In RP-HPLC analysis, (+)-catechin, apigenin and caffeic acid were identified as major phenolic compounds in the aerial parts of *A. orientale*. The aerial parts extract possessed the highest total phenolic content (120.979 ± 1.05 mg gallic acid equivalent/g), which were in good correlation with its significant DPPH ($IC_{50} 42.18 \pm 1.68$ mg/mL) and lipid peroxidation ($89.98 \pm 0.69\%$ at 10 mg/mL concentration) capacities. *A. orientale* exhibited potent antimicrobial activity against the organisms tested with MICs ranging from 3.125 to 25 mg/mL. *Escherichia coli* biofilm formation was inhibited maximum by the aerial parts extract to an extent of 68.51%. The strongest antimutagenic activity was observed at 2.5 mg/plate concentration of aerial parts extract against *Salmonella typhimurium* TA98. These results suggested that the ethanolic extract of the aerial parts of *A. orientale* could become useful supplement for pharmaceutical products as a new antioxidant, antibiofilm and antimutagenic agent.

Key words: *Allium orientale*, Antibiofilm activity, Antimutagenic activity, Antioxidant activity, Phenolic compounds

INTRODUCTION

Depending on rapid urbanization, changes in the life styles and dietary habits cause many diseases (Tapsell et al. 2006). Due to their beneficial properties, a large number of medicinal plants and their purified constituents are used for the prevention and treatment of complex diseases, such as cancer, cardiovascular diseases and diabetes (Boffetta et al. 2010; Bernaert et al. 2012; Dziri et al. 2012). The biological effects of the plants are mainly due to the presence of various bioactive components, such as antioxidants,

including phenolics, vitamins, organo-sulfur compounds, volatile sulfur compounds, fatty acids and tocopherols (Ames et al. 1993; Corzo-Martinez et al. 2007; Bernaert et al. 2012; Rouis-Soussi et al. 2014). Because of potential health benefits and nutritional values, the *Allium* species, especially onion and garlic have been used in the daily diet by the majority of the world's population (Iciek et al. 2009; Dziri et al. 2012). There are several studies reporting the important roles of the members of the genus in the prevention and treatment of pathogenic infections, cancers and cardiovascular diseases (Lau 2006;

* Author for correspondence: ozgceylan@hotmail.com

Yeh and Yeh 2006; Islam et al. 2011; Dziri et al. 2012). Investigations dealing with *Allium* species have shown that their beneficial properties are associated with the chemical contents. In particular, bioactive constituents namely organo-sulfur compounds (alliin and γ -glutamylcysteine), volatile sulfur compounds (diallyl sulfide, diallyl disulfide etc.), proteins (lectins), prostaglandins, fructan, vitamins (B1, B2, B6, C and E), polyphenols, fatty acids, and essential oils have been identified in chemical investigations of *Allium* species (Corzo-Martinez et al. 2007; Dziri et al. 2012). Among the mentioned constituents, especially polyphenols have been subjected to many studies regarding to their antioxidant, antimicrobial, antifungal, cytotoxic and mutagenic activities (Redzic et al. 2008; Lazarevic et al. 2011; Karuppiah and Rajaram 2012; Simin et al. 2013; Timite et al. 2013; Rezgui et al. 2014; Rouis-Soussi et al. 2014). Besides their medicinal importance, they have an economic value, depending on their usage in perfume and cosmetic industries, and cultivation as an ornamental plant. The *Allium* genus (Liliaceae) is widespread in the northern hemisphere and has species diversity. In the northern hemisphere, 700 species have been described as belonging to *Allium* genus (Tepe et al. 2005) and 168 of these are available in Turkish flora (Eker and Koyuncu 2011). However, 65 species have been reported as endemic to Turkey (Davis 1984; Guner et al. 2000; Tepe et al. 2005). Studies on the genus have focused on well-known species, *A. cepa* (onion) and *A. sativum* (garlic). However, a few studies have assessed the chemical and biological properties of other species. *A. orientale* Boiss is bulbous perennial plant, native from the North Africa to West Asia. *A. orientale* with the other six taxon (*A. nigrum* L., *A. cyrilli* Ten. (three subspecies), *A. elmaliense* and *A. lycanicum*) have been reported in central and western Anatolia (Genc and Ozhatay 2014). In Turkey, *A. orientale* grows in limestone hills and slopes, rocky places, fields and vineyards between 600 and 1870 meters (Davis 1984). The flowers are hermaphrodite and pollinated by bees and insects. It prefers light (sandy), medium (loamy) and well-drained soils (PFAF). Investigations dealing with *A. orientale* are scarce. Recently, several studies have been done about the distribution areas of *A. orientale* in certain regions of Turkey. However, until now, there is no report describing the antibiofilm, antioxidant, antimutagenic activities and phenolic compounds

of *A. orientale*. Thus, the present study intended to: (a) assess the antimicrobial and antibiofilm activities against five microorganisms by using a broth microdilution method and microplate biofilm assay, respectively; (b) evaluate the antioxidant activities of ethanolic extracts by using three complementary *in vitro* assays: free radical scavenging activity (DPPH assay), β -carotene-linoleic acid method, and total phenolic compounds determination; (c) characterize the phenolic profiles of aerial parts and bulbs by reversed-phase high-performance liquid chromatography (RP-HPLC); (d) investigate the mutagenic and antimutagenic activities by Ames Salmonella/microsome mutagenicity test.

MATERIAL AND METHODS

Plant Material

The aerial parts and bulbs of *Allium orientale* BOISS, naturally growing plants belonging to the Liliaceae family were collected from Mugla, Turkey. The plant samples were identified by Mehtap Donmez Sahin and the voucher specimen has been deposited in the Herbarium of The Faculty of Education, University of Usak under acquisition number 1603. In the laboratory, the plant samples were air-dried at room temperature for later analysis.

Preparation of the ethanolic extract

The air-dried and powdered plant samples were extracted with ethanol (Merck) using the Soxhlet apparatus. The extract was evaporated and then extracted in ethanol/water (1:1, v/v), and then kept in small sterile opaque bottles under refrigerated conditions until used.

Microorganisms

The *in vitro* antimicrobial activity of the ethanol extracts of *A. orientale* was tested against a panel of laboratory control strains from the American Type Culture Collection, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633 (Gram-positive bacteria), *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative bacteria) and *Candida albicans* ATCC 10239. All the microorganisms were maintained at -20°C under appropriate conditions and regenerated two times before use. *Salmonella typhimurium* TA98 and *S. typhimurium* TA100 were used for the mutation

studies and antimutagenicity tests. The strains were analyzed for their histidine and biotin requirement, individually and in combination, rfa mutation, excision repair capability, presence of the plasmid pKM101, and spontaneous mutation rate according to Mortelmans and Zeiger (2000). Working cultures were prepared by inoculating nutrient broth with the frozen cultures, followed by an overnight incubation at 37°C with gentle agitation (Oh et al. 2008).

Determination of MICs

MICs for bacteria were determined by a broth microdilution method with Mueller-Hinton Broth (CLSI 2006). Sabouraud Dextrose Broth was used for *C. albicans*. The density of microorganisms was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (200 µL) were inoculated into the wells of 96-well microtitre plates (Nunc F96 MicroWell™ plates; Nunclon™ Δ, Denmark) in the presence of extracts with different final concentrations (0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 mg/mL). Gram-negative bacteria and *C. albicans* were incubated at 30°C for 18-24 h and 24-48 h, respectively. Other bacterial strains were incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of extract at which no visible growth was observed.

Effect on biofilm formation

The effect of *A. orientale* extract at concentrations including 1, ½, ¼ and 1/8 MIC on biofilm-forming ability of test microorganisms were tested with a microplate biofilm assay (Merritt et al. 2005). Briefly, 1% of overnight cultures of test pathogens were added into 200 µL of fresh TSB supplemented with 0.25% glucose and cultivated in the presence and absence of extracts without agitation at 37°C for 48 h. The wells containing only TSB+cells served as control. After incubation, the wells were washed with water to remove the planktonic bacteria. The remaining bacteria were subsequently stained with 0.1% crystal violet solution for 10 min at room temperature. Wells were washed once again to remove the crystal violet solution. Microplates inverted and vigorously tap on paper towels to remove any excess liquid and air-dried. Two hundred microliters of 95% ethanol and 33% glacial acetic acid (Sigma Chemical Co) were poured in Gram-negative bacterial/*C. albicans* wells and Gram-positive bacterial wells, respectively. After shaking and pipetting of the

wells, 125 µL of the solution from each well was transferred to a sterile tube and volume reached to 1.0 mL with distilled water. Finally, optical density (OD) of each tube was measured at 550 nm (Thermo Scientific Multiskan FC, Vantaa, Finland). Each strain was tested for biofilm production in duplicate and the assay was repeated three times. Replicate absorbance readings for each concentration tested were averaged and the average of the media control was subtracted. This value was then divided by the mean absorbance of the (cell+TSB) and multiplied by 100.

Antioxidant activity

DPPH assay

The free radical scavenging activity was performed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Yamasaki et al. 1994). For this, 5.0 µL of the extract {1.25, 2.5, 5.0 and 10 mg/mL in ethanol/water (1:1, v/v)} was added to 4995 µL DPPH solution (0.004%) in ethanol. After incubation at room temperature for 30 min, the absorbance of each solution was determined at 517 nm. Percentage of inhibitions and the concentrations of sample required for 50% scavenging of the DPPH free radical (IC₅₀) were determined. BHT and ascorbic acid were used as positive controls.

β-carotene-linoleic acid method

The total antioxidant activity of the extracts was evaluated by the β-carotene-linoleic acid model (Jayaprakasha and Jaganmohan Rao 2000). For this, 0.5 mg of the β-carotene in 1.0 mL of chloroform, 25 µL of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan monopalmitate) were mixed together. The chloroform was completely evaporated using a vacuum evaporator and the resulting solution was diluted with 100 mL of oxygenated water. A 2.5 mL aliquots of this mixture were transferred into different tubes containing 0.5 mL of samples at 3.15 mg/mL concentration in ethanol/water (1:1, v/v). The same procedure was repeated with the positive control BHT, ascorbic acid, and a blank. The emulsion system was incubated for up to 2 h at 50°C. Measurement of the absorbance was continued until the color of β-carotene disappeared in the control. After this incubation period, absorbance of the mixtures was measured at 490 nm. All the determinations were performed in triplicate.

The bleaching rate (R) of β -carotene was calculated using the following formula. $R = \ln(a/b)/t$ where, \ln = natural log, a = absorbance at time 0, b = absorbance at time t (120 min).

The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using the formula $AA = [(R_{\text{Control}} - R_{\text{Sample}})/R_{\text{Control}}] \times 100$. Antioxidative activity of the extracts was compared with those of BHT and ascorbic acid at 0.5 mg/mL.

Determination of total phenolic compounds

The phenolic constituent of the extracts were determined by the method involving the Folin-Ciocalteu reagent and gallic acid as a standard (Slinkard and Singleton 1977; Chandler and Dodds 1983). Two hundreds microliter of extract solution containing 0.1 mg extract was added to a test tube. Then, 100 μ L Folin-Ciocalteu reagent was added and tube was shaken vigorously. After 3 min, 2 mL solution of Na_2CO_3 (0.5%) was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. Content of phenolic compounds was determined as mg gallic acid equivalents per gram of extract (mg/g GAE extract) using the following linear equation based on the calibration curve: $A = 0.0257C$, $R^2 = 0.997$ where A was the absorbance and C gallic acid equivalents.

Quantification of phenolic compounds by RP-HPLC

Phenolic compounds were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Tokyo, Japan). Detection quantification was carried out with a LC-10ADvp pump, a Diode Array Detector, a CTO 10Avp column heater, SCL-10Avp system controller, DGU-14A degasser and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations were conducted at 30°C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm x 4.6 mm length, 5 μ m particle size). The eluates were detected at 278 nm. The mobile phases were A: 3.0% acetic acid in distilled water and B: methanol. For analysis, the samples were dissolved in ethanol: bidistilled water (1:1, v/v), and 20 μ L of this solution was injected into the column.

The elution gradient applied at a flow rate of 0.8 mL min^{-1} was: 93% A/7% B for 0.1 min, 72%A/28%B in 20 min, 75%A/25%B in 8 min,

70%A/30%B in 7 min and same gradient for 15 min, 67%A/33%B in 10 min, 58%A/42%B in 2 min, 50%A/50%B in 8 min, 30%A/70%B in 3 min, 20%A/80%B in 2 min 100%B in 5 min until the end of the run. Phenolic compositions of the extracts were determined by a modified method of Caponio et al. (1999). Protocatechuic acid, (+)-catechin, p-hydroxy benzoic acid, chlorogenic acid, caffeic acid, (-)-epicatechin, benzoic acid, rosmarinic acid, ferulic acid and apigenin were used as standard. Identification and quantitative analysis were done by comparison with the standards. The amount of each phenolic compound was expressed as mg per gram of extract.

Mutagenic and antimutagenic activity

Viability assays and determination of test concentrations

Cytotoxic doses of the extracts were determined by the method of Mortelmans and Zeiger (2000). The toxicity of the extract toward *S. typhimurium* TA98 and TA100 was determined as described by Santana-Rios et al. (2001) and Yu et al. (2001). These tests confirmed that there was normal growth of the background lawn, spontaneous colony numbers within the regular range, and no significant reduction in cell survival. Thus, for the concentrations and conditions reported here, no toxicity or other adverse effects were observed.

Mutagenicity and antimutagenicity tests

In this study, the plate incorporation method was used to assess the results of mutagenicity and antimutagenicity assays (Maron and Ames 1983). The 4-nitro-o-phenylenediamine (4-NPD) at 3.0 μ g/plate for *S. typhimurium* TA98 and sodium azide (NaN_3) (8 μ g/plate) for *S. typhimurium* TA100 were used as positive controls and ethanol/water (1:1, v/v) was used as a negative control in mutagenicity and antimutagenicity tests.

In the mutagenicity test performed with TA98 and TA100 strains of *S. typhimurium*, 100 μ L of the overnight bacterial culture, 100 μ L test compounds at different concentrations (2.5, 1.25 and 0.625 mg/plate), and 500 μ L phosphate buffer were added to 2.0 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on the plates after incubation at 37°C for 48 or 72 h. For the mutagenicity assays, the mutagenic index was calculated for each concentration, which was the average number of revertants per plate divided

by the average number of revertants per plate with the negative control

In the antimutagenicity test performed with the same strains, 100 μ L of the overnight bacterial culture, 100 μ L mutagen, 100 μ L test compounds at different concentrations (2.5, 1.25 and 0.625 mg/plate), and 500 μ L phosphate buffer were added to 2.0 mL of the top agar containing 0.5 mM histidine/biotin. The mixture was poured onto minimal glucose plates. Histidine independent revertant colonies and viable cells were scored on plates after incubation at 37°C for 48 or 72 h.

For the antimutagenicity assays, the inhibition of mutagenicity was calculated by using the following equation (M: number of revertants/plate induced by mutagen alone, S₀: number of spontaneous revertants, S₁: number of revertants/plate induced by the extract plus the mutagen): % Inhibition = [(M-S₁) - (M-S₀)] \times 100. 25-40% inhibition was defined as moderate antimutagenicity; 40% or more inhibition as strong antimutagenicity; and 25% or less inhibition as no antimutagenicity (Ikken et al. 1999; Negi et al. 2003; Evandri et al. 2005).

Statistical analysis

All of the experimental results were as mean \pm SD of three parallel measurements. The data was entered into a Microsoft Excel database and analyzed using SPSS. The IC₅₀ values were obtained by the linear regression analysis. Statistical analysis of the total antioxidant activity was performed using the Kruskal-Wallis test.

RESULTS AND DISCUSSION

Minimum inhibitory concentrations of *A. orientale* extracts were determined by a broth microdilution method. As shown in Table 1, MIC values of bulb ethanolic extract ranged between 6.25 and 25 mg/mL, whereas the aerial part extract showed the lowest MIC for both the microorganisms, namely, *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10239. The aerial parts extract showed the MIC values of 3.125 mg/mL against *B. subtilis* ATCC 6633 and *C. albicans* ATCC 10239 while bulb extract showed the MIC values of 12.5 and 6.25 mg/mL, respectively against the same reference strains.

Aerial part extracts exhibited MIC values against *S. aureus* ATCC 25923 (6.25 mg/mL) and both Gram-negative bacteria (*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922) (12.5 mg/mL). On the other hand, the bulb extract inhibited *S. aureus* ATCC 25923 with MIC value of 12.5 mg/mL and Gram-negative bacteria with MIC value of 25 mg/mL. MIC results showed that the aerial part extract had higher antimicrobial activity than the bulb's extract. Phenolic compounds possess a considerable antimicrobial capacity against a very large number of human pathogens (Tepe et al. 2005). The relatively higher antimicrobial activity of the aerial parts extract could be indicative for the presence of some phenolic compounds such as (+)-catechin, apigenin and caffeic acid.

Table 1 - Minimum inhibitory concentrations and antibiofilm activities of *A. orientale* ethanol extracts against pathogen bacteria.

Microorganism	Extract	Biofilm inhibitory activity (% inhibition)				
		Planktonic MIC (mg/mL)	MIC	MIC/2	MIC/4	MIC/8
<i>B. subtilis</i> ATCC 6633	Aerial parts	3.125	31.63 \pm 5.49	13.48 \pm 1.53	-	-
	Bulbs	12.5	55.24 \pm 3.31	35.91 \pm 1.66	26.51 \pm 1.32	12.98 \pm 1.94
<i>S. aureus</i> ATCC 25923	Aerial parts	6.25	13.81 \pm 0.49	7.83 \pm 0.7	3.29 \pm 0.22	-
	Bulbs	12.5	44.12 \pm 2.2	21.24 \pm 1.09	8.77 \pm 0.7	-
<i>P. aeruginosa</i> ATCC 27853	Aerial parts	12.5	45.63 \pm 7.23	32.13 \pm 2.58	8.73 \pm 1.25	-
	Bulbs	25	39.11 \pm 2.89	13.16 \pm 1.65	-	-
<i>E. coli</i> ATCC 25922	Aerial parts	12.5	68.51 \pm 7.94	37.24 \pm 6.26	8.78 \pm 3.1	-
	Bulbs	25	31.18 \pm 2.82	10.32 \pm 1.27	-	-
<i>C. albicans</i> ATCC 10239	Aerial parts	3.125	24.59 \pm 8.2	4.9 \pm 1.1	-	-
	Bulbs	6.25	24.04 \pm 2.3	6.01 \pm 0.75	-	-

Although antimicrobial activities of some *Allium* species have already been reported (Hannan et al. 2010; Najjaa et al. 2011; Ismail et al. 2013; Bakht

et al. 2014), little attention was paid to their antibiofilm potential (Nidadavolu et al. 2012; Ceylan 2014). In the antibiofilm quantification

assay, a concentration dependent decrease in biofilm formation was observed in the test microorganisms when treated with the ethanol extracts of *A. orientale*. *E. coli* ATCC 25922 was maximally inhibited by the aerial parts extract at a concentration of 12.5 mg/mL to an extent of 68.51%. This ratio was 31.18% on the same bacteria biofilm formation at MIC of bulb extract. At a concentration of 12.5 mg/mL, the bulb's extract (MIC), the mean biofilm formation value was equal to 55.24% for *B. subtilis* ATCC 6633. The antibiofilm activity of aerial parts extract on *B. subtilis* ATCC 6633 biofilm formation was observed lower than bulb extract. However, antibiofilm activity results showed that the bulb extract had less effect on biofilms compared with the aerial parts extract.

Antioxidant activities of *A. orientale* ethanol extracts, standard BHT and ascorbic acid are presented in Table 2. In this, lower IC₅₀ values indicated higher antioxidant activity. The extracts of the aerial parts and the extract of the bulbs reduced the stable free-radical DPPH with an IC₅₀ of 42.18 and 148.86 mg/mL whereas IC₅₀ of standard BHT and ascorbic acid were lower at 0.95 and 0.48 mg/mL, respectively. DPPH test results were in good agreement with previous

studies results, which showed that the aerial parts extract provided higher free radical scavenging than the bulb extract (Stajner et al. 2008; Nencini et al. 2011; Rouis-Soussi et al. 2014).

Table 2 - DPPH radical-scavenging activity of *A. orientale* extracts.

Sample	IC ₅₀ value ^a (mg/mL)
The extract of the aerial parts	42.18±1.68
The extract of the bulbs	148.86±0.62
BHT	0.95± 0.014
Ascorbic acid	0.48± 0.019

^aValues expressed are means ± S.D. of three parallel measurements. The IC₅₀ values were obtained by the linear regression analysis.

Table 3 shows β-carotene/linoleic acid assay of ethanol extracts of *A. orientale*. In β-carotene/linoleic acid assay, the aerial parts extract exhibited 71.29 ± 0.19% inhibition against lipid peroxidation at 0.5 mg/mL, while the bulb extract exhibited 52.47 ± 0.32% at the same concentration. Both the extracts showed weak lipid peroxidation compared with BHT, but the aerial part extract indicated higher lipid peroxidation than ascorbic acid.

Table 3 - Antioxidant activity (%) of the ethanolic extract of *A. orientale* in β-carotene-linoleic acid assay.

Sample	Concentration (mg/mL)			
	0.5	2.5	5	10
The extract of the aerial parts	71.29 ± 0.19 ^a	88.51 ± 0.79	88.86±0.76	89.98±0.69
The extract of the bulbs	52.47 ± 0.32	63.97±0.24	64.61±0.24	66.18±0.23
BHT	95.7 ± 0.1	-	-	-
Ascorbic acid	63.69 ± 3.24	-	-	-

^aValues expressed are means ± SD of three parallel measurements.

The total phenolic contents in the aerial parts extract using Folin-Ciocalteu's reagent was 120.979 ± 1.05 mg GAE/g. On the other hand, ethanol extract of *A. orientale* bulbs had less phenolic contents (35.664 ± 0.21 mg GAE/g). The quantitative analysis of the phenolic compounds found in *A. orientale* ethanol extracts was performed using RP-HPLC, which allowed the separation of ten components (Table 4).

Table 4 showed that seven compounds were in the ethanol extract of *A. orientale* aerial parts. (+)-catechin, apigenin and caffeic acid were the most abundant in this extract, with the content of 0.148 ± 0.0028, 0.136 ± 0.0032 and 0.124 ± 0.008 mg/g, respectively. In addition, protocatechuic acid

(0.096 ± 0.004 mg/g) and chlorogenic acid (0.092 ± 0.0064mg/g) were found in moderate amount. (-)-epicatechin and ferulic acid were found in low amounts (0.068 ± 0.002 and 0.044 ± 0.0028 mg/g respectively) whereas p-hydroxy benzoic acid, benzoic acid and rosmarinic acid were not detected.

Six phenolic compounds were identified in the bulb extract in which caffeic acid (0.176 ± 0.008 mg/g) was in highest amount whereas (-)-epicatechin (0.096 ± 0.002 mg/g), chlorogenic acid (0.076 ± 0.0064 mg/g), apigenin (0.044 ± 0.0032 mg/g) were found in a moderate amount. Ferulic acid and protocatechuic acid were found in low amounts (0.0016 ± 0.0008 and 0.0012 ± 0.0004

mg/g respectively) whereas (+)-catechin, p-hydroxy benzoic acid, benzoic acid and rosmarinic acid were not detected. Prakash et al. (2007) have reported gallic acid, ferulic acid, protocatechuic acid, quercetin and kaempferol in different varieties of *A. cepa* from India.

Table 4 - Phenolic compounds in *A. orientale* ethanolic extracts.

Phenolic compound	Concentrations (mg/g extract)	
	Aerial parts	Bulb
Protocatechuic acid	0.096 ± 0.004 ^a	0.012 ± 0.0004
(+)-Catechin	0.148 ± 0.0028	NT
p-hydroxy benzoic acid	NT	NT
Chlorogenic acid	0.092 ± 0.0064	0.076 ± 0.0064
Caffeic acid	0.124 ± 0.008	0.176 ± 0.008
(-)-Epicatechin	0.068 ± 0.002	0.096 ± 0.002
Ferulic acid	0.044 ± 0.0028	0.016 ± 0.00008
Benzoic acid	NT	NT
Rosmarinic acid	NT	NT
Apigenin	0.136 ± 0.0032	0.044 ± 0.0032

^a Values are expressed as mean ± standard deviation. NT: Not detected.

Among the identified components, (+)-catechin, apigenin and caffeic acid have been reported for their antioxidant activity (Gulcin 2006; Sharififar et al. 2009). The ethanolic extracts, which were

tested at three different concentrations, including 2.5, 1.25 and 0.625 mg/plate did not show any mutagenic effect in the mutagenicity assays performed with *S. typhimurium* TA98 and TA100 (data was not shown). Similar results have been previously reported indicating the mutagenic activity of onion and onion's organosulphur compounds (Shon et al. 2004; Llana-Ruiz-Cabello et al. 2015). The antimutagenic activity of *A. orientale* ethanol extracts against 4-NPD and NaN₃ was evaluated by the means of the Ames test using two strains of *S. typhimurium*, i.e TA98 and TA100. The results were evaluated by using standard plate incorporation method and summarized in Table 5. The extracts did not inhibit the mutagenicity of sodium azide in *S. typhimurium* TA100. The results of the present study showed that the extracts only exhibited antimutagenic activity against *S. typhimurium* TA98, which had a frameshift mutation. In the case of TA 98, a strong inhibitory effect was shown by aerial parts extract at 2.5 mg/plate only. The same extract showed moderate inhibition at 1.25 mg/plate and no inhibitory effect at 0.625 mg/plate. The bulb extract showed the moderate inhibition at 2.5 mg/plate and no inhibitory effects at 1.25 and 0.625 mg/plate. In addition, no extracts showed any antimutagenic activity against *S. typhimurium* TA100 at the tested concentrations.

Table 5 - The antimutagenicity assay results of the ethanolic extracts of *A. orientale*.

Test items	Concentration (mg/plate)	Number of revertants			
		TA98		TA100	
		Mean ± S. error	Inhibition %	Mean ± S. error	Inhibition %
Negative control		7.4 ± 2.30 ^b	-	15.75 ± 4.11	-
4-NPD ^a	3	401.6 ± 4.50	-	-	-
NaN ₃ ^a	8	-	-	430 ± 19.07	-
Extract of the aerial parts	2.5	134 ± 8.33	66.63	328.3 ± 30.85	23.64
	1.25	248.8 ± 46.69	38.05	374.3 ± 29.70	12.94
	0.625	338.25 ± 18.64	15.78	421 ± 2.64	2.09
Extract of the bulb	2.5	280 ± 24.43	30.29	338.5 ± 17.52	21.27
	1.25	334.83 ± 12.05	16.63	380.6 ± 8.38	11.47
	0.625	373 ± 19.65	7.13	396.3 ± 7.09	7.82

^a4-NPD and NaN₃ were used as positive controls for *S. typhimurium* TA98 and TA100 strains, respectively.

^bValues expressed are means ± S.D. of three parallel measurements. The regression analysis was carried out in Microsoft Excel between percent inhibition of mutagenicity and log values of concentrations of the plant extract.

CONCLUSION

The ethanolic extract of the aerial parts of *A. orientale* exhibited high antimicrobial and antibiofilm activities. Results clearly indicated that the aerial parts extract possessed high ability to

scavenge the DPPH and hydroxyl radicals and that the phytochemicals present in *A. orientale* aerial parts extract had potent antioxidant activity in positive correlation with total phenolic content. All the extracts of *A. orientale* could be considered

genotoxicity safe at the tested concentrations. The Ames test revealed that the aerial parts extract exhibited an antimutagenic activity against *S. typhimurium* TA98. Hence, potential therapeutic compounds isolated from the aerial parts of *A. orientale* could be preferred as a preventive medication against cancer and in the treatment of bacterial diseases.

ACKNOWLEDGEMENTS

Authors thank Ass. Prof. Dr. M.D. Sahin, Botanist, Usak University, Usak, Turkey, for providing plant taxonomic identification.

REFERENCES

- Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci. U.S.A.* 1993; 90: 7915-7922.
- Bakht J, Khan S, Shafi M. *In vitro* antimicrobial activity of *Allium cepa* (dry bulbs) against Gram positive and Gram negative bacteria and fungi. *Pak J Pharm Sci.* 2014; 27: 139-145.
- Bernaert N, De Paepe D, Bouten C, De Clercq H, Stewart D, Van Bockstaele E, et al. Antioxidant capacity, total phenolic and ascorbate content as a function of the genetic diversity of leek (*Allium ampeloprasum* var. *porrum*). *Food Chem.* 2012; 134: 669-677.
- Boffetta P, Couto E, Wichmann J, Ferrari P, Trichopoulos D, Bueno-de-Mesquita HB, et al. Fruit and vegetable intake and overall cancer risk in the European prospective investigation into cancer and nutrition (EPIC). *J Natl Cancer Inst.* 2010; 102: 529-537.
- Caponio F, Alloggio V, Gomes T. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem.* 1999; 64: 203-209.
- Ceylan O. Antibiofilm, mutagenic and antimutagenic activity of *Allium sphaerocephalon* L. *J Pure Appl Microbiol.* 2014; 8: 2879-2885.
- Chandler SF, Dodds JH. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum lacinitum*. *Plant Cell Rep.* 1983; 2: 105-108.
- CLSI-Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Sixteenth Informational Supplement. Document M100-S16, Wayne, Pa. 2006.
- Corzo-Martinez M, Corzo N, Villamiel M. Biological properties of onions and garlic. *Trends in Food Sci Tech.* 2007; 18: 609-625.
- Davis PH. Flora of Turkey and the East Aegean Islands, Vol. 8. Edinburgh, UK: Edinburgh University Press, 1984, 203-204
- Dziri S, Hassen I, Fatnassi S, Mrabet Y, Casabianca H, Hanchi B, et al. Phenolic constituents, antioxidant and antimicrobial activities of rosy garlic (*Allium roseum* var. *odoratissimum*). *J Func Foods.* 2012; 4: 423-432.
- Eker I, Koyuncu M. *Allium olivieri* Boiss. (Alliaceae), a new taxon to Turkey, with contributions to its taxonomy. *Acta Soc Bot Pol.* 2011; 80: 275-277.
- Evandri MG, Battinelli L, Daniele C, Mastrangelo S, Bolle P, Mazzanti G. The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. *Food Chem Toxicol.* 2005; 43: 1381-1387.
- Genc I, Ozhatay FN. *Allium efeae* (Amaryllidaceae), a new species from northwest Anatolia, Turkey. *Turk J Bot.* 2014; 38: 1022-1025.
- Gulcin I. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). *Toxicology.* 2006; 217: 213-220.
- Guner A, Ozhatay N, Ekim T, Baser KHC. Flora of Turkey and the East Aegean islands, Vol. 11. (Suppl 2)., Edinburgh, UK: Edinburgh University Press, 2000.
- Hannan A, Humayun T, Hussain MB, Yasir M, Sikandar S. *In vitro* antibacterial activity of onion (*Allium cepa*) against clinical isolates of *Vibrio cholerae*. *J Ayub Coll Abbottabad.* 2010; 22(2): 160-163.
- Iciek M, Kwiecieri I, Wlodek L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen.* 2009; 50: 247-265.
- Ikken Y, Morales P, Maetinez A, Marin ML, Haza AI, Cambero MI. Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evaluated by the Ames test. *J Agric Food Chem.* 1999; 47: 3257-3264.
- Islam MS, Kusumoto Y, Al-Mamun, MA. Cytotoxicity and Cancer (HeLa) Cell Killing Efficacy of Aqueous Garlic (*Allium sativum*) Extract. *J Sci Res.* 2011; 3(2): 375-382.
- Ismail S, Jalilian FA, Talebpour AH, Zargar M, Shameli K, Sekawi Z, et al. Chemical composition and antibacterial and cytotoxic activities of *Allium hirtifolium* Boiss. *Biomed Res Int.* 2013; ID 696835,1-8.
- Jayaprakasha GK, Jaganmohan Rao, L. Phenolic constituents from lichen *Parmotrema stuppeum* (Nyl.). Hale and their antioxidant activity. *Z Naturforsch C.* 2000; 55: 1018-1022.
- Karuppiyah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. *Asian Pac J Trop Biomed.* 2012; 2: 597-601.

- Lau BHS. Suppression of LDL oxidation by garlic compounds is a possible mechanism of cardiovascular health benefit. *J Nutr.* 2006; 136: 765-768.
- Lazarevic JS, Dordevic AS, Zlatkovic BK, Radulovic NS, Palic RM. Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. *sphaerocephalon* (Liliaceae) inflorescences. *J Sci Food Agric.* 2011; 91: 322-329.
- Llana-Ruiz-Cabello M, Maisanaba S, Gutierrez-Praena D, Prieto AI, Pichardo S, Jos A, et al. Cytotoxic and mutagenic *in vitro* assessment of two organosulfur compounds derived from onion to be used in the food industry. *Food Chem.* 2015; 166: 423-431.
- Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. *Mutat Res.* 1983; 113: 173-215.
- Merritt JH, Kadouri DE, O'Toole GA. Growing and analyzing static biofilms. *Curr Protoc Microbiol.* 2005; 1:1-3.
- Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. *Mutat Res.* 2000; 455: 29-60.
- Najjaa H, Zerria K, Fattouch S, Ammar E, Neffati M. Antioxidant and antimicrobial activities of *Allium roseum* L. "Lazoul" a wild edible endemic species in North Africa. *Int J Food Prop.* 2011; 14: 371-380.
- Negi PS, Jayaprakash GK, Jena BS. Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chem.* 2003; 80: 393-397.
- Nencini C, Menchiari A, Franchi Gian G, Micheli L. *In vitro* antioxidant activity of aged extracts of some Italian *Allium* species. *Plant Food Hum Nutr.* 2011; 66: 11-16.
- Nidadavolu P, Amor W, Tran PL, Dertien J, Colmer-Hamood JA, Hamood AM. Garlic ointment inhibits biofilm formation by bacterial pathogens from burn wounds. *J Med Microbiol.* 2012; 61: 662-671.
- Oh HT, Kim SH, Choi HJ, Chung MJ, Ham SS. Antioxidative and antimutagenic activities of 70% ethanol extract from masou salmon (*Oncorhynchus masou*). *Toxicol In Vitro.* 2008; 22: 1484-1488.
- PFAF, Plants For A Future. [Internet] England and Wales: c1996-2012 [cited 2014 Dec 26] Available from: <http://www.pfaf.org/user/Plant.aspx?LatinName=Allium+orientale>.
- Prakash D, Singh BN, Upadhyay G. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chem.* 2007; 102: 1389-1393.
- Redzic S, Pilipovic S, Pilav E. Comparative analysis of antimicrobial activity of fresh extracts of certain species of genus *Allium* L. (Alliaceae). *Planta Med.* 2008; 8: 74-91.
- Rezgui A, Mitaine-Offer AC, Paululat T, Delemasure S, Dutartre P, Lacaille-Dubois MA. Cytotoxic steroidal glycosides from *Allium flavum*. *Fitoterapia.* 2014; 93: 121-125.
- Rouis-Soussi LS, Boughelleb-M'Hamdi N, El Ayeb-Zakhama A, Flamini G, Jannet HB, Harzallah-Skhiri F. Phytochemicals, antioxidant and antifungal activities of *Allium roseum* var. *grandiflorum* subvar. *typicum* Regel. *S Afr J Bot.* 2014; 91: 63-70.
- Santana-Rios G, Orner GA, Amantana A, Prowost C, Wu SY, Dashwood, RH. Potent antimutagenic activity of white tea in the Salmonella assay. *Mutat Res.* 2001; 495: 61-74.
- Sharififar F, Dehghn-Nudeh G, Mirtajaldini M. Major flavanoids with antioxidant activity from *Teucrium polium* L. *Food Chem.* 2009; 112: 885-888.
- Shon MY, Choi SD, Kahng GG, Nam, SH, Sung NK. Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food Chem Toxicol.* 2004; 42: 659-666.
- Simin N, Orcic D, Cetojevic-Simin D, Mimika-Dukic N, Anackov G, Beara I, et al. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of small yellow onion (*Allium flavum* L. subsp. *flavum*, Alliaceae). *LWT-Food Sci Technol.* 2013; 54: 139-146.
- Slinkard K, Singleton VL. Total phenol analyses: automation and comparison with manual methods. *Am J Enol Viticult.* 1977; 28: 49-55.
- Stajner D, Popovic B, Čanadanovic-Brunet J, Stajner M. Antioxidant and scavenger activities of *Allium ursinum*. *Fitoterapia.* 2008; 79: 303-305.
- Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, et al. Health benefits of herbs and spices: the past, the present, the future. *Med J Aust.* 2006; 185: 4-24.
- Tepe B, Sokmen M, Akpulat HA, Sokmen A. *In vitro* antioxidant activities of the methanol extracts of five *Allium* species from Turkey. *Food Chem.* 2005; 92: 89-92.
- Timite G, Mitaine-Offer AC, Miyamoto T, Tanaka C, Mirjolet JF, Duchamp O, et al. Structure and cytotoxicity of steroidal glycosides from *Allium schoenoprasum*. *Phytochemistry.* 2013; 88: 61-66.
- Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. *Chem Pharm Bull.* 1994; 42: 1663- 1665.
- Yeh YY, Yeh SM. Homocysteine-lowering action is another potential cardiovascular protective factor of aged garlic extract. *J Nutr.* 2006; 136: 745-749.
- Yu Z, Xu M, Santana-Rios G, Shen R, Izquierdo-Pulido M, Williams DE, et al. A comparison of whole wheat, refined wheat and wheat bran as inhibitors of heterocyclic amines in the *Salmonella* mutagenicity assay and in the rat colonic aberrant crypt focus assay. *Food Chem Toxicol.* 2001; 39: 655-665.

Received: May 29, 2015;
Accepted: July 16, 2015.