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

Çiğdem Aydın, Cennet Özyay and Ramazan Mammadov

Abstract—In this study, antioxidant and antibacterial activities of various solvent extracts (methanol, ethanol, acetone and petroleum benzene) obtained from bulbs and leaves of Allium deciduum subsp. deciduum and Allium 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). Allium 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 Allium deciduum subsp. deciduum showed more antibacterial activity against S. aureus than E. coli.

Keywords— Allium deciduum, antioxidant activity, DPPH, β-carotene-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, β-carotene, and α-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 Allium 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 Allium deciduum bulbs and leaves.

II. MATERIALS AND METHODS

A. Plant Materials

Different parts (leaves and bulbs) of Allium deciduum subsp. deciduum and Allium 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 55°C 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 -20°C.
C. Determination of total phenolic content

The total phenolic content of extracts was determined using 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) Na₂CO₃ 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°C 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:

\[
AA: (1 - (A_0-At / A_0º - Atº) \times 100
\]

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 millimetres.

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. In the present study total phenolic content of the 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.
no study about antimicrobial activity of the extract of bulbs was effective against tested against A. deciduum. The antibacterial activity levels of the extracts of leaves subsp. A. deciduum bulbs subsp. A. deciduum leaves showed antibacterial activity against Gram (+) bacteria except S. aureus. The ethanol extract had no effect on the entire tested strains of S. aureus and 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.

### B. Antimicrobial 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 I

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

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Benzine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. deciduum subsp. deciduum bulbs</td>
<td>48.21</td>
<td>68.36</td>
<td>46.40</td>
<td>30.35</td>
</tr>
<tr>
<td>A. deciduum subsp. deciduum leaves</td>
<td>40.36</td>
<td>49.25</td>
<td>31.83</td>
<td>29.03</td>
</tr>
<tr>
<td>A. deciduum subsp. retrorsum bulbs</td>
<td>43.69</td>
<td>62.63</td>
<td>34.79</td>
<td>30.08</td>
</tr>
<tr>
<td>A. deciduum subsp. retrorsum leaves</td>
<td>45.69</td>
<td>49.87</td>
<td>30.73</td>
<td>28.75</td>
</tr>
</tbody>
</table>

### Table II

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>A. deciduum subsp. deciduum</th>
<th>DPPH-RSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β - Carotene assays (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bulbs</td>
<td>Leaves</td>
</tr>
<tr>
<td>Ethanol</td>
<td>77.27</td>
<td>73.70</td>
</tr>
<tr>
<td>Methanol</td>
<td>80.21</td>
<td>76.85</td>
</tr>
<tr>
<td>Acetone</td>
<td>49.62</td>
<td>45.76</td>
</tr>
<tr>
<td>Benzine</td>
<td>44.12</td>
<td>42.78</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Extracts</th>
<th>A. deciduum subsp. retrorsum</th>
<th>DPPH-RSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β - Carotene assays (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bulbs</td>
<td>Leaves</td>
</tr>
<tr>
<td>Ethanol</td>
<td>76.85</td>
<td>71.73</td>
</tr>
<tr>
<td>Methanol</td>
<td>78.54</td>
<td>73.65</td>
</tr>
<tr>
<td>Acetone</td>
<td>47.74</td>
<td>43.77</td>
</tr>
<tr>
<td>Benzine</td>
<td>43.22</td>
<td>41.87</td>
</tr>
</tbody>
</table>

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 nevshihirese, Allium sivasicum, Allium dictyoprosopum, Allium scrodonporus subsp. rotundum and Allium atrovilaceum), measured by DPPH, showed an IC50 range between 79 and 104 µg/ml with an efficiency of 3.95 (IC50 extract/IC50 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


[17] Y. Tekeli, ‘Chemical and Biological Characterization Some of Species Centaurea in Konya’, PhD, Selcuk University Institute of Science Chemistry Department, 2008.


