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In vitro antioxidant activities of the methanol extracts of five *Allium* species from Turkey

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Abstract

This study was designed to examine the in vitro antioxidant activities of the methanol extracts of five *Allium* species, namely *Allium nevsehirense*, *A. sivasicum*, *A. dictyoprosum*, *A. scrodoprosum* subsp. *rotundum* and *A. atroviolaceum*; the former two are endemic for the Turkish flora. The extracts were screened for their possible antioxidant activities by two complementary tests; DPPH free radical-scavenging and β-carotene/linoleic acid assays. In the first case, non-polar subfractions of the extracts did not show any antioxidant potential, while the polar subfractions exhibited marked activity. Among the polar ones, the most active one was *A. atroviolaceum* with an IC_{50} of 79.0 ± 2.75 μg/ml. In the β-carotene/linoleic acid assay, the inhibition ratios of the oxidation of linoleic acid by *A. atroviolaceum* and *A. dictyoprosum* were too close to each other (71.2 ± 2.20% and 72.3 ± 1.20%, respectively), while that of the synthetic antioxidant, BHT, was 96%.

Keywords: *Allium*; Antioxidant activity; DPPH; β-Carotene/linoleic acid test

1. Introduction

Antioxidants are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases (Helen, Krishnakumar, Vijayammal, & Augusti, 2000). Many fruits and vegetables are potentially useful for decreasing the risks of several chronic diseases, such as coronary heart disease and some cancers (Block, Patterson, & Sapers, 1992; Hertog et al., 1995; Lampe, 1999). These protective effects have been paticularly attributed to various antioxidant compounds, such as vitamins C and E, β -carotene, and polyphenolics (Diplock et al., 1998). The genus *Allium* is one of the major sources of dietary flavonoids, which are a group of polyphenolics, in many countries (Hertog et al., 1995; Knekt, Jarvinen, Reunanen, & Maatela, 1996).

This genus comprises 700 species of bulbous perennials and biennials that occur in temperate regions of the northern hemisphere (Könemann, 1999) and 164 of which are available in Turkish flora; 65 of them being endemic (Davis, 1984; Davis, 1998; Guner, Ozhatay, Ekim, & Baser, 2000). As far as being beneficial to human health, *Allium* plants are already well known. For example, garlic (*A. sativum*), is of particular interest owing to its prophylactic and therapeutic actions. 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, Soc, & Prior, 1996; Gazzani, Papetti, Daglia, Berte, & Gregotti, 1998; Yin & Cheng, 1998), this ability has mainly been attributed to a variety of sulphur-containing compounds and their

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precursors (Kim, Kubota, & Kobayashi, 1997; Lampe, 1999). Reports claim that these chemicals have great potential in terms of decreasing lipid levels in experimental animals (Bobbi, Augusti, & Joseph, 1984; Bordia, Bansal, Arora, & Singh, 1975; Bordia, Verma, & Vyas, 1977).

Total antioxidant activities of vegetables cannot be evaluated by any single method, due to the complex nature of phytochemicals (Chu, Chang, & Hsu, 2000). Two or more methods should always be employed in order to evaluate the total antioxidative effects of vegetables (Nuutila, Puupponen-Pimia, Aarni, & Oksman-Caldentey, 2003). For the reason mentioned above, we applied two complementary test systems, namely β-carotene-linoleic acid and 2,2′-diphenylpicrylhydrazyl (DPPH), for evaluating the antioxidant capacities of the plants studied.

As far as our literature survey could ascertain, no information was available on the in vitro antioxidative activities of the *Allium* species given here. Therefore, the aim of this study was to investigate the in vitro antioxidant capacities of the methanolic extracts of five *Allium* species, two of which are endemic for the Turkish flora.

2. Materials and methods

2.1. Collection of plant material

Localities and collection periods of *Allium* species studied were given below,

1. *A. nevsehirense*: Taslidere-Domuzlukici district, Sivas-Turkey; 19th July, 2003
2. *A. sivasicum*: Celalli-Hafik road, Karayun, Sivas-Turkey; 10th July, 2003
3. *A. dictyoprosum*: Gurun turn off, Kangal-Sivas, Turkey; 19st July, 2003
4. *A. scrodoprosum* subsp. *rotundum*: Bolucan, Zara, Sivas-Turkey; 14th July, 2003
5. *A. atroviolaceum*: Celalli-Hafik road, Karayun, Sivas-Turkey; 10th July, 2003

The voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No. 1-AA3348; 2-AA3337; 3-AA3351; 4-AA3341; 5-AA3335, respectively).

2.2. Preparation of the methanol extracts

The air-dried and finely ground samples were extracted by using a method described elsewhere (Sokmen, Jones, & Erturk, 1999). Briefly, the sample, weighing about 100 g, was extracted in a Soxhlet apparatus with methanol (MeOH) at 60 °C for 6 h. The extract was then filtered and concentrated in vacuo at 45 °C, yielding a waxy material (14.36%, 10.49%, 11.22%, 12.08% and 11.39% w/w, respectively). Finally, the extracts were then lyophilised and kept in the dark at +4 °C until tested.

2.3. Antioxidant activity

2.3.1. DPPH assay

The hydrogen atoms or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable 2,2′-diphenylpicrylhydrazyl (DPPH) radical as a reagent (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). 50 μl of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent ($I\%$) was calculated in following way:

$$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100,$$

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. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. Tests were carried out in triplicate.

2.3.2. β-Carotene–linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), and 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (30 min 100 ml/min) was added with a vigorous shaking. 2500 μl of this reaction mixture were dispensed to test tubes and 350 μl portions of the extracts, prepared at 2 g l^{-1} concentrations were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with synthetic antioxidant, butylated hydroxytoluene (BHT), as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

3. Results and discussion

Methanolic extracts were individually assessed for their possible antioxidative capacities by employing two complementary tests: DPPH free radical-scavenging and β-carotene/linoleic acid assays. Free radical-scavenging capacities of the corresponding extracts were measured by DPPH assay and the results are shown in Fig. 1. Polar subfractions exhibited notable antioxidative potential, whereas non-polar ones remained almost inactive. In the former case, the most active polar subfraction was obtained from *A. atroviolaceum* with an IC_{50} at 79.0 ± 2.75 μg/ml, while that of *A. dictyoprosum* possessed weakest activity with an IC_{50} of 104 ± 1.76 μg/ml (Fig. 1). When compared to the synthetic antioxidant, BHT, polar subfractions of the all extracts showed marked radical-scavenging activities.

In the β-carotene/linoleic acid assay, the inhibition ratios of the oxidation of linoleic acid of *A. atroviolaceum* and *A. dictyoprosum* were too close to each other (71.2 ± 2.20% and 72.3 ± 1.20%, respectively), while that of synthetic antioxidant, BHT, was 96% (Fig. 2).

As far as our literature survey could ascertain, few *Allium* species had been taken into account for the evaluation of their possible biological activities, except for *A. sativum* (garlic) and *A. cepa* (onion) (Helen et al., 2000; Ide et al., 2002; Imai et al., 1994; Nuutila et al., 2003; Stajner, Milic, & Canadanovic-Brunet, 1999; Stajner, Milic-DeMarino, Canadanovic-Bruner, & Popovic, 2002; Yin & Cheng, 1998). However, no report was available on the biological activities of the *Allium* species, including the endemic ones given here.

Some *Allium* members are considered to possess protective effects against cancer, owing to their organosulfur constituents, such as diallyl sulfides and dipropenyl sulfides (Guyonett, Belloir, Suschetet, Siess, & Le Bon, 2001; Munday & Munday, 2001). Allicin is generally taken into account as being responsible for garlic's

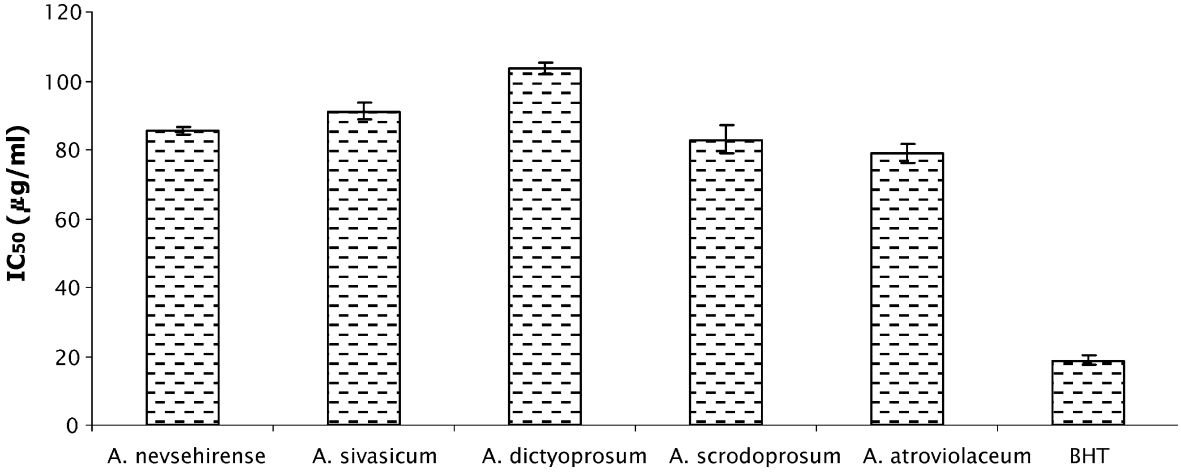

Fig. 1. Free radical scavenging capacities of the extracts measured in DPPH assay (results are means of three different experiments).

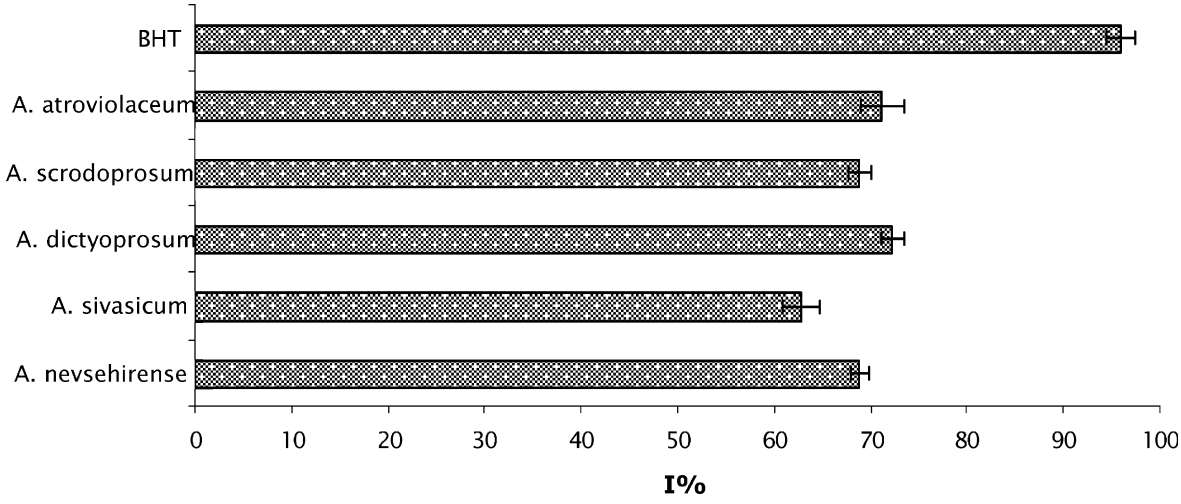

Fig. 2. Inhibition ratio of the linoleic acid oxidation by the extracts (results are means of three different experiments).

antioxidative properties (Lawson, 1998), although its beneficial effects, as well as its action, have not been fully understood.

Searching plant sources may bring new natural products into the food industry with safer and better antioxidants that provide good protection against the oxidative damage which occurs both in the body and our daily foods. 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 antioxidative potential of some *Allium* species growing wild in the Turkish flora and could be evaluated as a starting point for further investigations with the above-mentioned plants.

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