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The in vitro antioxidant activity of *Allium tuncelianum:* An endemic

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Abstract

This study was designated to evaluate in vitro antioxidant activity of MeOH extracts of Allium tuncelianum and to determined total phenolic content (TPC) of MeOH extract of this plant. The sample was subjected to screening for their possible antioxidant activity by using 2,2-diphenyl-1-picriylhydrazyl (DPPH) and β-carotene/linoleic acid assays. The MeOH extract was found to posses weak antioxidant activity. In the case of linoleic acid system, oxidation of the linoleic acid was moderately inhibited by the methanol extract (51.1 ± 5.5 %). Also, in the MeOH extract of A. tuncelianum, we have determined TPC in value of 4.8 ± 1.30 mg/g. Our results showed that MeOH extract of A. tuncelianum was exhibited more weak antioxidant activity than the synthetic antioxidant butylated hydroxytoluene (BHT), curcumin, and ascorbic acid.

Key words: Allium tuncelianum, antioxidant activity, total phenolic content, DPPH and β-carotene/linoleic acid.

INTRODUCTION

Free radicals may play an important role in the origin of life and biological evolution, implication their beneficial effects on the organisms. For example, oxygen radicals exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells. However, free radicals and other relative species could also cause the oxidation of biomolecules which leads to cell injury and death [1,2]. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Also, many fruits and vegetables are potentially useful for decreasing the risks of several chronic diseases, such as coronary heart disease and some cancers [3,4]. These protective effects have been paticularly attributed to various antioxidant compounds, such as vitamins C and E, β-carotene and polyphenolics [5]. The genus Allium is one of the major sources of dietary avonoids, which are a group of polyphenolics, in many countries [3,6].

The genus Allium comprises of 700 species of bulbous perennials and biennials that occur in temperate regions of the northern hemisphere [7] and 164 of which are available in the Turkish flora; 65 of them being endemic [8,9,10]. 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 [11,12]. This ability has mainly been attributed to a variety of sulphur-containing compounds and their precursors [4,13].

A. tuncelianum is originally named as A. macrochaetum Boiss and Haussk subsp. tuncelianum Kollmann [14]. Although, it is native to "Tunceli" province especially at Platos of Munzur Mountains in Ovacik district of Turkey, it naturally grows in the limited region located between Sivas and Erzurum provinces [15]. Due to its resemblance to common garlic, it is locally called as 'Tunceli garlic' or 'Ovacik garlic' in the region. A. tuncelianum usually forms single cloved white bulb, unlike garlic which has a multiple cloved bulb. The flower scape of A. tuncelianum coils early in its elongation, which is typical characteristic of some garlic types. While A. tuncelianum forms non-bulbiferous inflorescences with fertile flowers, all flowering garlic genotypes have bulbils formation in their inflorescences along with the flowers. Bulbil formation in the garlic inflorescence has been suggested as a cause of garlic sterility [16].

As far as our literature survey could ascertain, there is not any information about the in vitro antioxidative activity of A. tuncelianum. Therefore, the aim of this study was to investigate the in vitro antioxidant capacity of the methanolic extract of A. tuncelianum that it is an endemic for the flora of Turkey.

MATERIAL AND METHODS

Preparation of the MeOH extracts

The air-dried and finely ground bulbs of A. tuncelianum were extracted by using a method described elsewhere [17]. Briefly, weighing about 70 g of the sample was extracted in a Soxhlet apparatus with methanol (MeOH) at 60 ˚C for 6 h. The extract was then filltered and concentrated in vacuo at 45 ˚C, yielding a waxy material (11.42 %). Finally, the extracts were then kept in the dark at +4˚C until tested.

2,2-diphenyl-1-picriylhydrazyl (DPPH) assay

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured MeOH solution of stable free radical DPPH. This spectrophotometric assay uses stable DPPH as a reagent [18]. Fifty µl of various concentrations of the extracts in MeOH was added to 5 ml of a 0.004% MeOH 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 %= (Ablank– Asample / Ablank) x 100

Where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the test compound. Extract concentration providing 50% inhibit ion (IC50) was calculated form the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate.

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 [19]. A stock solution of β–carotene-linoleic acid mixture was prepared as following: 0.5 mg β–carotene was dissolved in 1 ml of chloroform, 25 µl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Than 100 ml distilled water saturated with oxygen (30 min 100 ml/min.) was added with a vigorous shaking. 2.5 ml of this reaction mixture was dispersed to test tubes and 350 µl portions of the extracts prepared at 2 g/l concentrations were added and emulsion system was incubated up to 48 hours at room temperature. Same procedure was repeated with positive control BHT and a blank. After this incubation period absorbance of the mixtures were measured at 490 nm. Antioxidant capacity of the extract was compared with those BHT and blank.

Determination of total phenolic content (TPC)

TPC was estimated colorimetrically using the modified Folin-Ciocalteau method [20]. An aliquot of 0.2 ml of free phenolic acid extract was added to 0.8 ml of water, 5 ml of 0.2 N Folin-Ciocalteau reagent, and 4 ml of saturated sodium carbonate solution (75 g/l) and mixed in a screw-top test tube. The absorbance was measured at 765 nm with a spectrophotometer (Cintra UV–VIS 202) after incubation for 2 h at room temperature. Quantification was based on the standard curve, established with 100, 200, 300, 400 and 500 mg/l of gallic acid. The results were expressed as gallic acid equivalent in microgram per gram dry weight. The results were the averages of triplicate analyses.

Statistical analysis

For statistical analyses, we chose the analysis of variance (ANOVA) in Statistical Analysis System (SPSS 11.0 for windows). The significance of differences between mean values were determined by a multiple range test (LSD; Least Significant Difference).

RESULTS AND DISCUSSION

The extract obtained by Soxhlet extraction was screened for A. tuncelianum possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and β-carotene/linoleic acid systems. Free radical-scavenging activity belonging to MeOH extract of A. tuncelianum was determined by DPPH assay and the results shown in Fig.1. According to the findings, extract of MeOH of A. tuncelianum (119.1±10.1 µg/mg) exhibited lower activity than BHT, curcumin, and ascorbic acid ($p<0.01$). In β-carotene/linoleic acid assay are shown in Figure 2. According to the finding, inhibition capasity of MeOH extract of A. tuncelianum was determined as 51.1±5.5 %. Additionally, antioxidant activities of BHT, ascorbic acid and curcumin were determined in parallel experiments.

Amount of total phenolic content was determined in value of 4.8±1.30 mg/g. Recently, the interest of finding natural antioxidants, especially those of plant origin, has increased greatly [21]. Natural antioxidants derived from plants, especially phenolics such as quercetin, carnosol, thymol, catechin, and morin are of considerable interest as dietary supplements or food preservatives [22]. Polyphenols, anthocyanins, flavonoids, quercetin, kaempferol and their glycosides have been reported in some Allium species [23,24]. In conclusion, we can say that low level of free radical-scavenging activity of the extract may be caused by this low amount of the phenolic components.

In DPPH assay, it was found that there is statistically a important difference between free radical-scavenging activities of the methanolic extract of A. tuncelianum bulbs and controls (BHT, curcumin, ascorbic acid) ($p<0.01$). Altogether, this difference is not mean of the extract has lower free radical-scavenging activity than controls as IC50. In this assay, a high value of IC50 is mean of low antioxidant activity (Figure 1). In β-carotene/linoleic acid assay, it was also found that there are statictially important differences between methanolic axtract of A. tuncelianum bulbs and controls ($p<0.01$) as inhibition % (I %). I % of linoleic acid oxidation of the extract was determined moderate rather than the controls, but low value of % inhibition is not mean of highest activity (Figure 2).

As far as our literature survey could ascertain, few

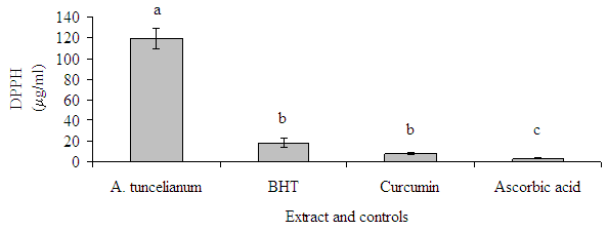

Figure 1. Antioxidant activities of methanolic extract from A. tuncelianum and positive control (BHT, Curcumin and Ascorbic acid) in DPPH. Bars expressed mean ± Standard deviation. Means with different letters are significantly different from one another according to LSD test ($p<0.01$).

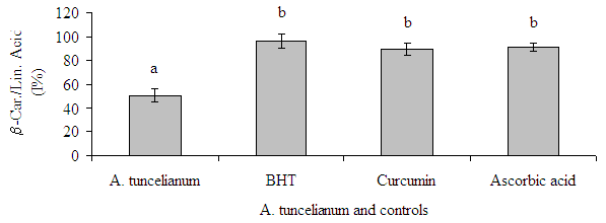

Figure 2. Antioxidant activities of methanolic extract from A. tuncelianum and positive control (BHT, Curcumin and Ascorbic acid)) in β-carotene/linoleic acid. Bars expressed mean ± standard deviation. Means with different letters are significantly different from one another according to LSD test ($p<0.01$).

Allium species had been taken into account for their possible antioxidant activities [25,26,27,28,29] and there is not report available on the antioxidant activities of the A. tuncelianum, an endemic from Turkey.

Tepe et al. [29] were shown that they determined possible antioxidant activities of MeOH extracts of A. sivasicum, A. dictyoprosum, A. scrodoprosum, A. atroviolaceum, A. nevsehirense. According to their study, polar subfractions of five Allium species were shown marked radical scavenging activities. However, some Allium members are considered to possess protective effects against cancer, owing to their organosulfur constituents, such as diallyl sulfides and dipropenyl sulfides [30,31]. Allicin is generally taken into account as being responsible for garlic's antioxidative properties [32], although its beneficial effects, as well as its action, have not been fully understood.

It can be concluded that the antioxidant activity of A. tuncelianum has been investigated therefore testing of its free radical-scavenging is of interest, primarily in order to find new promising sources for natural antioxidants, functional foods and pharmaceuticals. From this point of view, it may be taken into account as a new report based on antioxidant activity of A. tuncelianum growing wild in the flora of Turkey.

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