Study on the antioxidant activity of dried Allium bulgaricum leaves

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Abstract: *Allium bulgaricum L. is used as a culinary herb and it is a well- known flavouring principle. The antioxidant activity of dry leaves was determined as Trolox equivalent antioxidant capacity (TEAC) using different reliable methods- DPPH, ABTS, FRAP, and CUPRAC assays. Total phenolic contents was determined using a spectrophotometric technique, based on the Folin-Ciocalteau reagent, and calculated as gallic acid equivalents GAE/g DW. The total polyphenolics ranged from 9,01 ± 0,14 to 22,92 ± 0,30 mg GAE/g DW.*

Key words: *Allium bulgaricum L., antioxidant capacity, phenolic content*

INTRODUCTION

Free radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Oxidative damages caused by free radicals mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, acquired immunodeficiency syndrome diabetes, anemia and cardiovascular diseases (Agbor G 2007). Natural antioxidants have attracted a great deal of public and scientific interest because of anticarcinogenic potential and other health promoting effects. Plants and vegetables are good source of phenolic components, ascorbic acids, tocopherols, glutathione, vitamin C and E, carotenoids, flavonoids that may contribute to protection against oxidative damage [10].

Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables [7,12]. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity [6]. Phenolic compounds could be a major determinant of antioxidant potentials of foods [12], and could therefore be a natural source of antioxidants.

Allium bulgaricum (samardala) is a glabrous plant, 50-100 (150) cm high. In the soil it has an ovoid bulb, 18-30 mm long and 15-30 mm in diameter. The leaves are 30-50 cm long and 10-20 mm wide, thin, with a proeminent central nervure on the back, making it look triangular in section. The plant is characterized by a powerful and heavy specific smell. It is found only in limited areas.

Considering the very limited information concerning the antioxidant capacity of the dry leaves, the basic aim of the research was to determine the total phenolic content as well as the free radical scavenging activity of *dry* Bulgarian *Allium bulgaricum*.

MATERIALS AND METHODS

Plant material

Allium bulgaricum L. was collected in May 2013 from the Plovdiv region, Bulgaria, air dried and ground in an electric mill.

Extract preparation

Dried plant material of A. bulgaricum was subjected of four different types of extractions:

- decoction – extraction by boiling of the plant material for 30min with distilled water;
- infusion – extraction by boiling water and then pouring it over the plant material allowing it to steep in the liquid for 30min.
- microwave assisted extraction (MAE) – the experiments were performed with water as solvent in a domestic microwave oven (LG MB4047C) with frequency of the waves 2450 MHz and output power- 800W;
- heat reflux extraction - alcoholic extraction (70 % ethanol as solvent) for 30min;

The resulting extracts solutions were filtered before analyzed.

Determination of total phenolics

A modified Kujala et al. (2000) method with Folin- Ciocalteu's reagent was used for the determination of the total polyphenolic content (TPhC). Gallic acid was employed as a calibration standard and the results were expressed as mg gallic acid equivalents (mg GAE) per gram of plant fresh weight.

Determination of antioxidant activity

- *DPPH • radical scavenging assay*

Antioxidant activity is described as having activity against the stable form of the synthetic product DPPH • (2,2-diphenyl-1-pikrilhidrazil; Sigma-Aldrich, Steinheim, Germany) by the method of Brand-Williams et al. [1995] with slight modifications. Briefly, a freshly prepared 12.10-5 M solution of DPPH • (in methanol) is mixed with the sample in a ratio of 2:0.5. Light absorption is measured at a wavelength of 517 nm using a spectrophotometer (Spectrostar Nano, BMG Labtech). The absorption of samples (free sample) is measured with respect to the corresponding extractant for a certain period of time.

- *Ferric-reducing antioxidant power assay (FRAP)*

The FRAP assay was carried out according to the procedure of Benzie & Strain (1996) with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripyridyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. One hundred and fifty microliters of plant extracts were allowed to react with 2850 µl of the FRAP reagent solution for 4 min at 37 °C and the absorbance of the reaction mixture was recorded at 593 nm. The results were expressed as µM TE/g FW.

- *$ABTS^{\bullet+}$ radical scavenging assay*

The radicals scavenging activity of the investigated extracts against radical cation ($ABTS^{\bullet+}$) was estimated according to a previously reported procedure with some modifications [14]. $ABTS^{\bullet+}$ was produced by reacting 7 mM of $ABTS^{\bullet+}$ solution with 2.45 mM of potassium persulphate, and the mixture was kept in the dark at room temperature for 12-16 h. At the moment of use, the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30 °C. 1 ml of $ABTS^{\bullet+}$ solution was added to each sample (0.01 ml) was vigorously mixed. After reacting at 30 °C temperature for 6 min, the absorbance at 734 nm was measured. The results were presented as a function of the concentration of Trolox. The TEAC value was defined as the concentration of Trolox having equivalent antioxidant activity expressed as µM TE per gram fresh weight (µM TE/g PW).

- *CUPRAC assay*

The CUPRAC assay was carried out according to the procedure of Apak et al. (2004) with modifications. To a test tube were added 1 mL of CuCl2 solution (1.0×10^{-2}M), 1 mL of neocuproine methanolic solution (7.5×10^{-3}M), and 1 mL NH4Ac buffer solution (pH 7.0), and mixed; 0.1 mL of herbal extract (sample) followed by 1 mL of water were added (total volume = 4.1 mL), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as mM trolox equivalent.

Absorbance was measured at 450 nm against a reagent blank [2]. Increased absorbance of the reaction mixture indicates increased reduction capability.

Statistical analysis

All measurements were carried out in triplicates. The results were expressed as mean ± SD and statistically analyzed using MS-Excel software.

RESULTS AND DISCUSSION

Total phenolics

The phenolic compound family is huge and comprises a complex group of compounds (about 8000 identified compounds), varying from simple phenols to highly polymerized compounds such as tannins [5]. Phenolic compounds are found in almost every plant-derived food.

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalents. This is a very popular, convenient, simple and reproducible, and is commonly known as the total phenolic compounds assay [8]. The reagent, however, is non-specific, as it can also be reduced by other non-phenolic species [13, 16]. The values obtained for the concentration of total phenols were expressed as mg of GAE/g DW (Figue 1). The total phenolic contents in the examined extracts ranged from 9,01 ± 0,14 to 22,92 ± 0,30 mg GAE/g DW. The highest concentration of phenols was measured in the decoction extract.

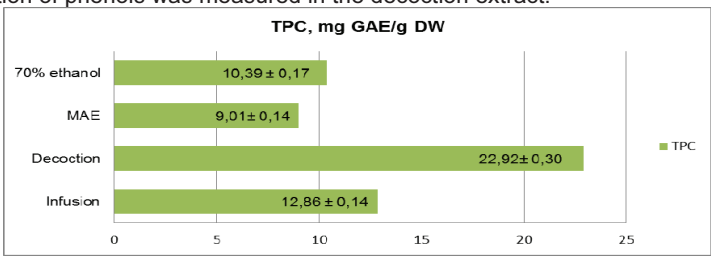

Fig. 1 Total polyphenolic content of different extracts of Allium bulgaricum

Antioxidant activity

An antioxidant is defined as a substance that inhibits free radicals and reactions promoted by oxygen [5]. The total antioxidant power can be measured by a wide range of assays [8, 11, 16], some of which are used in this study. The ABTS assay is frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. The FRAP assay is a simple, speedy, inexpensive, and robust method of measuring the radical scavenging ability of plants. The CUPRAC reagent is reasonably selective, stable, easily accessible, and more sensitive compared to the FRAP method. Figure 2 shows the CUPRAC, ABTS, DPPH, and FRAP assay results of different extracts of dry *Allium bulgaricum* leaves. The antioxidant capacities are given as Trolox equivalents, in the units of µlmol TE per gram dry plant weight.

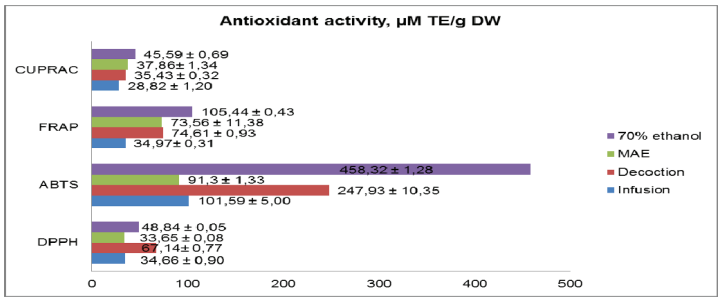

Fig. 2 Antioxidant activity of different extracts of *Allium bulgaricum* using four different complementary assays (CUPRAC, DPPH, ABTS, FRAP)

The ABTS reducing ability of the extracts was in range from 91.30 ± 1.33 μM TE/g DW to 458.32 ± 1.28 μM TE/g DW. Significant ABTS free radical scavenging activity was evident in the 70% ethanol extract of dry leaves (458.32 ± 1.28 μM TE/g DW), while the lowest was detected in the MAE (91.30 ± 1.33 μM TE/g DW). The antioxidant activity of the extracts by this assay implies that action may be by either inhibiting or scavenging the ABTS radicals since both inhibition and scavenging properties of antioxidants towards this radical have been reported in earlier studies [15]. The antioxidant potential of *A. bulgaricum* extracts was estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). Increasing absorbance indicates an increase in reductive ability. The FRAP values of the studied extracts were calculated and the results are presented in Figure 2. In accordance with the ABTS assay, the highest value in the FRAP method was obtained in the 70% ethanol extract of dry leaves – 105.44 ± 0.43 μM TE/g DW. The cupric ion (Cu2+) reducing ability of the extracts of A. bulgaricum leaves was also evaluated. Among the four extracts, the 70% ethanol extract of dry leaves showed the higher CUPRAC value – 45.59 ± 0.69 μM TE/g DW. The results of this assay correspond well to the already mentioned results pursuant to the ABTS, and FRAP methods. The DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the respective samples. The colour fades upon reaction with phenolic compounds in the test solution [8]. Contrary to all the other conducted studies the DPPH assay confirmed the higher values established by the decoction of dry leaves (67.14 ± 0.77μM TE/g DW).

CONCLUSION

The results of this study provided a better insight of the total polyphenolic content and antioxidant activity of dried Allium bulgaricum leaves. Since drying plays an important role in self preserving seasonal plants, these results may shape many culinary recipes to a more effective admeasuring of dry plants. The outcomes of this study show the great potential of *Allium bulgaricum* for the development of foods rich in compounds with antioxidant properties. Therefore, further investigation is needed to identify the antioxidant compounds present in the dry leaves.

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