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SYMPOSIUM

An evaluation of the antioxidant abilities of *Allium* species+

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ABSTRACT In the present study we investigated antioxidative properties of leaves of different wild (*Allium flavum* L., *Allium sphaerocephalum* L., *Allium atroviolaceum* Bois., *Allium vienale* L., *Allium scorodoprasum* L.) and grown (*Allium nutans* L., *Allium fistulosum* L., *Allium vienale* L., *Allium pskemense* B. Fedtsch, *Allium schenoprasum* L., *Allium cepa* L., *Allium sativum* L.) *Allium* sorts were investigated. Activities of antioxidant enzymes (superoxide dismutase, catalase, peroxidase, glutathione peroxidase), quantities of malonyldialdehyde superoxide and hydroxyl radicals and reduced glutathione and also the content of total flavonoids, chlorophylls a and b, carotenoids, vitamin C and soluble proteins were determined. Our results indicate that leaves of grown *Allium sativum* L., *Allium cepa* L., *Allium vineale* L., *Allium fistulosum* L. and *Allium nutans* L. and wild *Allium flavum* L. and *Allium ursinum* L. exibited high antioxidant activites.
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KEY WORDS

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Oxygen free radicals – highly reactive species ($O^{\bullet}_2^-$, $^{\bullet}OH$, $HO^{\bullet}_2$) with one or more unapaired electrones, H_2O_2 and activated oxygen species ($^1\Delta g$, $^1\Sigma g$) are formed in almost every cell of the body at an astonishing rate during normal oxdative metabolism (Gey 1994). Environmental factors such as UV light, ozone, tobaco smoke, different xenobiotics, herbicides, pesticides, ionizing radiation etc. cause their formation in greater extent (Halliwel and Gutteridge 1984). They react voraciosly with almost every cellular component and contribute to many types of pathology. Antioxidant defense mechanism counteract free radicals formation and reactions. In free radicals caused pathology antioxidants neutralize free radicals and increasing levels of antioxidants should decrease pathology. Combinations of different natural antioxidants which could be find in different medicinal plants work better that separate antioxidants alone. Many epidemiological studies also support the idea that antioxidants are interdependent (Kery et al. 2001).

Onions are widely used in all parts of the world as a flavoring vegetable in various types of food. According to traditional medical literature they are source of many vitamins and are useful in fever, dropsy, catarrh and chronic bronchitis (Block 1985). Roasted or otherwise they are applied as a poultice to indolent boils, bruises, wounds, to relieve hot sensations and applied to the navel for dysentery and fever (Brewster and Rabinowitch 1990).

Today *Alliums* are used for their flavor, aroma and taste, beeing prepared domestically or forming raw material for a variety of food manufacturing processes (dehydration, freezing, canning and pickling). Also, dehydrated onion production is widely used, especially in the manufacture of other processed foods (Brewster and Rabinowitch 1990). On the other hand therapeutic and medicinal values of garlic and onions are the subjects of many researches. The different clinical studies have shown their benefit in the reduction of cardiovascular disease risk by inducing lowering of serum cholesterol and blood pressure (Steiner and Lin 1994). They have liver protective (Dion and Miler 1996), imune enhancement and anti-infection (Lau 1989), anti-stress and anti-fatigue (Kawashima 1986), anti-cancer and cancer preventive effects (Dion and Milner 1997; Pinto et al. 1997; Balasenthil et al. 2001), brain and neurotrophic (Moriguchi 1996) and other pharmacological effects (Yeh 1996). Many recent studies showed that *Alliums* have antioxidant effect what could be of the great importance for its use in prevention and treatment of different diseases (Lau 1989; Numagami 1996; Geng and Lau 1997) and contribute to its therapeutic physionomy (Kyo et al. 1998).

Our previous studies (Stajner et al. 1998a; 1988b; 1998c, 1999) showed that different *Allium* species posses well-defined antioxidant activity. Therefore the aim of this study was to perform the screening of different *Allium* species by determination activities of antioxidant enzymes superoxide dismutase (SOD), catalase (C-ase), peroxidase (P-ase), glutathione peroxidase (GP-ase), quantities of malonyldialdehide (MDA), superoxide ($O^{\bullet}_2^-$) and hydroxyl radicals ($^{\bullet}OH$), reduced glutathione (GSH) and contents of total flavonoids, chlorophylls a and b, carotenoids, vitamin C and soluble proteins. Our results could evaluate their antioxidant values and point to easy accessible sources of natural antioxi-

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Table 1. Enzyme activities in leaves of different *Alliums.*

Allium sort U/mg protein	SOD	C-ase	P-ase	GP-ase
Allium sativum L.	52.47±7.11	8.78±0.43	40.48±4,01	0.038±0.011
Allium vineale L. (grown)	39.95±2.34	23.77±15.92	8.54±0.40	0.035±0,010
Allium vinaele L. (wild)	3.63±0.52	7.32±4.54	22.51±2.26	0.020±0.006
Allium sphearocephalum L.	8.91±2.52	6.42±1.10	105.83±8.00	0.050±0.014
Allium scorodoprasum L.	45.54±23.88	7.32±11.96	130.10±10.00	0.977±0.052
Allium atroviolaceum Boiss.	7.46±1.51	8.81±6.63	17.06±1.18	0.048±0.004
Allium cepa L.	2.69±0.76	5.51±3.13	10.23±0.91	0.051±0,011
Allium pskemenese L.	4.08±0.84	3.16±1.25	4.87±0.04	0.065±0,049
Allium fistulosum L.	62.20±3.85	20.59±11.89	23.28±0.88	0.537±0.043
Allium schenoprasum L.	77.46±5.98	5.91±0.62	63.26±4.21	0.083±0.024
Allium nutans L.	20.27±3.27	10.01±5.11	10.46±0.45	0.018±0.002
Allium ursinum L.	2.51±1.21	11.48±6.90	8.85±0.19	0.052±0.007
Allium flavum L.	10.62±2.02	13.59±8.28	21.03±1.17	0.022±0.005

dants that could be used as a possible food supplements or in the cosmetic and pharmaceutical industries.

Materials and Methods

The leaves of next wild (*Allium flavum* L., *Allium sphaerocephalum* L., *Allium atroviolaceum* Bois., *Allium vienale* L., *Allium scorodoprasum* L.) and grown (*Allium nutans* L., *Allium fistulosum* L., *Allium vienale* L., *Allium pskemense* B.Fedtsch, *Allium schenoprasum* L., *Allium cepa* L., *Allium sativum* L.) *Allium* sorts were investigated.

One g of plant material was ground with quartz sand in a cold mortar. The ground material was suspended in 5 ml 1 mol/l K_2HPO_4 at pH 7.0. After a 10 min centrifugation at 4ºC and 15,000 g, the aliquots of the supernatant were used for SOD activity measurements. 20 µl of Tsuchihashi solution (chloroform:ethanol – 3:5) was added to the supernatant prior to measurement of the enzyme activity. The SOD activity was determined in aliquots by the method of Misra and Fridovich (1972) based on the inhibition of transformation of adrenaline to adrenochrome at pH 10.2 (Matkovics et al. 1977).

For the other antioxidant enzymes and biochemical determinations, the plant material was treated in the same way but the medium was 0.1 mol/l phospthate buffer (pH 7) with a plant material to medium ratio of 1:5, centrifuged for 10 min at 15.000 g. After the centrifugation the supernatant was evaluated for: P-ase activity, using guiacol as substrate (Matkovics et al. 1977); GP-ase activity using cumene hydroperoxide and GSH as substrates (Chiu et al. 1976); C-ase activity spectrophotometrically at 240 nm (Simon et al. 1974); lipid peroxidation by the thiobarbituric acid (TBA) method; values were given as equivalent amounts od MDA; the calibration curve was prepared with malonyldialdehyde bis-diacetal (Placer et al. 1968); superoxide radical was determined by adrenaline autooxidation (Misra and Fridovich 1972); hydroxyl radical by the inhibition of deoxyribose degradation (Cheesman et al. 1988).

The amount of GSH was determined with Ellman (Sedlak and Lindsay 1968) and protein by Folin reagents (Lowry et al. 1951).Total flavonoids were estimated according to Marckam (1989). Pigments were extracted with acetone and determined spectrophotometrically using molar extinction coefficients according to Wettstein (1957). The content of vitamin C was determined according to AOAC Official Methods of Analysis (1984).

Table 2. Quantities of ($O^{\bullet}_2^-$), $^{\cdot}OH$ and MDA in leaves of different *Alliums.*

Allium sort	($O^{\bullet}_2^-$)	$^{\cdot}OH$ (nmol/mg protein)	MDA
Allium sativum L.	508.8±218.1	2.10±0.29	24.55±1-02
Allium vineale L. (grown)	155.9±78.0	2.32±0.05	13.19±0.51
Allium vineale L. (wild)	608.8±189.5	2.56±0.03	30.67±0.47
Allium sphearocephalum L.	1341.8±372.1	1.29±0.11	50.83±0.73
Allium scorodoprasum L.	1587.6±357.8	25.52±1.209	130.19±1.24
Allium atroviolaceum Boiss.	1376.2±315.3	3.21±0.20	67.09±1.02
Allium cepa L.	473.5±147.4	0.82±0.16	7.61±0.36
Allium pskemenese L.	129.4±86.2	0.07±0.02	7.12±0.77
Allium fistulosum L.	153.4±124.3	0.20±0.02	4.98±0.34
Allium schenoprasum L.	623.4±150.4	4.06±0.24	36.85±0.60
Allium nutans L.	1611.9±619.6	0.30±0.04	12.40±0.16
Allium ursinum L.	108.0±70.7	0.38±0.03	7.27±0.21
Allium flavum L.	639.6±81.5	0.43±0.13	7.74±0.40

Table 3. Quantity of reduced glutathione and contents of flavonoids, vitamin C and soluble proteins in leaves of different *Alliums*

Allium sort	GSH nmol/mg	Flavonoids mg/g	Vitamin C mg/g	Soluble proteins mg/g
Allium sativum L.	0.215±0.009	333.87±12.22	0.036±0,003	2.22±0.09
Allium vineale L. (grown)	0.200±0,003	208.53±4.62	0.253±0.016	4.78±0.23
Allium vineale L. (wild)	0.108±0.001	259.20±0.00	0.066±0.010	4.72±0.12
Allium sphearocephalum L.	0.453±0.004	216.53±4.62	0.811±0.000	1.81±0.05
Allium scorodoprasum L.	2.182±0.016	344.53±9.24	0.142±0.000	0.25±0.03
Allium atroviolaceum Boiss.	0.399±0.011	59.20±13.86	0.157±0.005	3.42±0.05
Allium cepa L	0.127±0.001	496.53±12.22	0.005±0.000	4.78±0.15
Allium pskemenese L.	0.177±0.004	168.53±4.62	0.019±0.003	3.61±0.15
Allium fistulosum L.	0.297±0.008	365.87±9.24	0.061±0.000	7.30±0.15
Allium schenoprasum L.	0.669±0.009	432.53±4.62	0.122±0.000	1.86±0.12
Allium nutans L.	0.125±0.004	53.87±9.24	0.011±0.000	3.68±0.15
Allium ursinum L.	0.148±0.003	171.20±13.86	0.020±0.002	3.97±0.09
Allium flavum L.	0.146±0.002	37.87±9.26	0.344±0.000	4.51±0.04

All measurements were made in triplicate. The values are expressed as mean ± standard error.

Results and discussion

The results obtained from the study are presented in Tables 1, 2, 3. and 4. The SOD activity was detected in leaves of all investigated *Allium* sorts. It had values between 2.51 U/mg potein in *Allium ursinum* L. and 77.46 U/mg protein in *Allium schenoprasum* L.. C-ase activity ranged from 3.16 U/mg protein (*Allium pskemense* L.) to 23.77 U/mg protein (*Allium vineale* L. grown), P-ase activity from 4.87 U/mg protein (*Allium pskemense* L.) to 130.10 U/mg protein (*Allium scorodoprasum* L.) and GP-ase activity from 0.018 U/mg protein (*Allium nutans* L.) to 0.977 U/mg protein (*Allium scorodoprasum* L.; Table 1). Our results showed that leaves do to various enzyme activities had different susceptibility to the action of toxic oxygen species.

The results obtained from the study of oxygen radicals and MDA are presented in Table 2. The highest $O^{\bullet}_{2}^{-}$ quantity was obserwed in leaves of *Allium schorodoprasuu* L. (1587.6 nmol/mg protein) and lowest in leaves of *Allium ursinum* L. (108.0 nmol/mg protein). Quantity of $^{\bullet}OH$, was also highest in leaves of *Allium schorodoprasum* L. (25.52 nmol/mg protein) where the MDA quantity was the highest (130.19 nmol/mg protein) due to toxic oxygen radicals action. The quantity of $O^{\bullet}_{2}^{-}$ was lowest in leaves of *Allium psekemense* L. (0.07 nmol/mg protein) and MDA quantity in leaves of *Allium fistulosum* L. (4.98 nmol/mg protein).

The results obtained from the study of the nonenzymic antioxidants and proteins are presented in Table 3. Quantity of GSH ranged from 0.108 nmol/mg protein (*Allium vineale* L. wild) to 2.182 nmol/mg protein (*Allium scorodoprasum* L.). Content of flavonoids ranged from 37.87 mg/g (*Allim flavum* L.) to 496.53 mg/g (*Allium cepa* L.), vitamin C from 0.005 mg/g (*Allim cepa* L.) to 0.811 mg/g (*Allium sphaerocephalum* L.) and soluble proteins from 0.25 mg/g (*Allim scorodoprasum* L.) to 4.78 mg/g (*Allium vineale* L. grown and *Allium cepa* L.).

The content of pigments is presented in Table 4. In leaves of *Allium pskemense* L. the contents of all investigated pigments were the lowest: 0.39 mg/g for chlorophyll a, 0.11 mg/g for chlorophyll b and 0.66 mg/g for carotenoids. The highest content of investigated pigments was obserwed in leaves of *Allium ursinum* L.: 2.87 mg/g for chlorophyll a, 1.35 m/g for chlorophyll b and 9.99 mg/g for carotenoids (Table 4).

Table 4. Pigments content in leaves of different *Alliums.*

Allium sort	Chlorophyll a (mg/g)	Chlorophyll b	Carotenoids
Allium sativum L.	1.64±0.00	0.58±0.00	2.57±0.00
Allium vineale L. (grown)	1.91±0.013	0.94±0.05	3.04±0.01
Allium vineale L. (wild)	0.97±0.017	0.41±0.06	1.30±0.03
Allium sphearocephalum L.	1.08±0.003	0.57±0.06	1.91±0.02
Allium scorodoprasum L.	1.28±0.00	0.70±0.00	0.25±0.01
Allium atroviolaceum Boiss.	0.41±0.01	0.12±0.00	0.71±0.01
Allium cepa L.	1.23±0.05	0.67±0.02	1.92±0.01
Allium pskemanese L.	0.39±0.03	0.11±0.03	0.66±0.01
Allium fistulosum L.	1.14±0.03	0.58±0.03	0.87±0.03
Allium schenoprasum L.	1.48±0.01	0.44±0.03	2.14±0.01
Allium nutans L.	1.65±0.02	0.80±0.07	2.24±0.01
Allium ursinum L.	2.87±0.03	1.35±0.01	9.99±0.01
Allium flavum L.	0.70±0.03	0.33±0.06	1.10±0.01

Our results indicated that some of grown *Alliums* such as *Allium sativum* L., *Allium vineale* L., *Allium cepa* L., *Allium fistulosum* L. and *Allium nutans* L. possessed high antioxidant activities. They had small quantities of •OH and MDA. At the some time SOD, or P-ase activity were high and the content of flavonoids and carotenoids was also high what contribute to their antioxidant abilities.

Among the wild *Alliums*, *Allium flavum* L. and particulary *Allium ursinum* L. exibited high antioxidant activities. *Allium ursinum* L. had extraordinary antioxidant abilities inspite lowe SOD activity due to high C-ase activity high content of chlorophylls a and b and carotenoids. In leaves of *Allium ursinum* L. small quantities of $O^{\bullet}_{2}^{-}$ and •OH were detected what provoced small lipid peroxidation.

Our results indicates that leaves of menshioned grown and wild *Alliums* could be used as a source of natural untoxic antioxidants in food, cosmetic and pharmaceutical industries.

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