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IN VITRO ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *ALLIUM SCORODOPRASUM*

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Abstract

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The antioxidant properties of methanol extract of *Allium scorodoprasum* bulb and seed were evaluated, through determination of total phenolics and flavonoids content, as well as DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and ferric thiocyanate methods. Bulb extract showed greater reducing power (AEAC value per 1 mg of dry extract for bulb was 6.00 ± 1.03 mg) and ability to scavenge DPPH radical, as well as total phenolic (36.692 ± 4.475 μg GA/mg of dried bulb extract) and flavonoid content. Total phenolic and flavonoid content assay were correlated with ferric reducing power and "scavenging" radical capacity test, and correlation among them was significant. Our research showed that *Allium scorodoprasum* can be considered as plant with antioxidant compounds.

Key words: *Allium scorodoprasum*, antioxidant activity, phenolic compounds, correlation

Abbreviations: ROS-reactive oxygen species; SOD- enzymes superoxide dismutase; C-ase- catalase; P-ase-peroxidase; GP-ase-glutathione peroxidase; MDA –malonyldialdehide; $O^{\bullet 2-}$ -superoxide; •OH- hydroxyl radicals; GSH -reduced glutathione; DPPH - 2,2-diphenyl-1-picrylhydrazyl; BHT –butylhydroxytoluene; Trolox - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; G.A. -gallic acid; A.A. -ascorbic acid; GAE -gallic acid equivalent; RE -rutin equivalent; AEAC –ascorbate equivalent antioxidant capacity

Introduction

Vegetable is a source of essential nutrients such as vitamins and minerals and it is important source of potent natural antioxidants. Natural antioxidants are differing widely in terms of chemical structure and biological properties and they could be used as potential agents for preventing and treating oxidative stress-related diseases. In recent years, there has been a worldwide trend towards the use of the natural antioxidants present in herbs, fruits and vegetables. The most important group of plant antioxidants are polyphenols, which are recognized as beneficial to human health, mostly due to their ability to neutralize reactive oxygen species (ROS) and to have antioxidant activity (Shi et al., 2001). Antioxidant activity is associated with a polyphenol ability to scavenge free radicals and up-regulate certain metal chelation reactions. Polyphenols are the most abundant antioxidants in our diet and they are constituents of fruits, vegetables, cereals, olive, chocolate, tea, coffee and wine (Scalbert et al., 2005). Epidemiological studies show that consumption of fruits and vegetables with high phenolic and flavonoid contents are correlated with reduced cardiovascular, cancer mortality, inflammation and some other disease rates.

Polyphenols have been reported to display a variety of biological actions, so they protect human body against free radicals that may cause pathological conditions. Endogenous and exogenous antioxidants act interactively to maintain or re-establish redox homeostasis, which is critical in maintaining a healthy biological system (Bouayed and Bohn, 2010). It was found that synthetic antioxidants cause negative health and toxic effects (Ndhlala et al., 2010). Antioxidant activities of many fruits, vegetables, spices, medicinal plants and microalgae have been evaluated, and the results showed that some of them could be rich sources of natural antioxidants

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(Robards et al., 1999), so the search for raw materials containing potent natural antioxidants continues to attract the attention of researchers. Also, the antioxidant effectiveness of fruits, vegetables, spices, medicinal plants are suggested as a superior alternative for the single phenolic compounds, both natural and synthetic, due to the synergistic action of a wide range of active molecules existing in plant products.

Allium species have long history of use as culinary herbs and spices because their flavor, aroma and taste. At the same time, large number of species of the genus *Allium* is frequently used in folk medicine. Recent researches indicate that these species have a high antioxidant power and ability to prevent many diseases (Lau, 1994; Numagami, 1996; Geng and Lau, 1997; Reuter, 1995; Štajner et al., 1998, 2003, 2008; Tepe et al., 2005). It's a real superfood that helps in the treatment of many diseases, including some types of cancer (Dion and Milner, 1997; Pinto et al., 1997), brain and neurotrophic diseases (Moriguchi, 1996), type 2 diabetes, cataract, diseases of the heart and blood vessels (Steiner and Lin, 1994). Biochemical investigations showed that *Allium* species contain proteins, amino acids, traces of essential mineral elements and also different antioxidant (Haim et al. 2004; Rose et al., 2005; Štajner et al., 2003). Species of the genus Allium are rich source of thiosulfinates and other organosulfur compounds, because their active compounds are cysteine, sulphoxides and alliin flavones, tannins etc (Lanzotti, 2006; Krest et al., 2000; Bonaccorsi et al., 2008). In Štajner et al., (2003) *Allium scorodoprasum* was one of the *Allium* species that were screening 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•2-) and hydroxyl radicals •OH), reduced glutathione (GSH). In fact, as far as we know, there are no data for non-enzymic antioxidants in methanol extract of *Allium scorodoprasum* except the data for DPPH free radical scavenging activity of methanol extracts of wild garlics published by Božin et al. (2008). In our study, the antioxidant status of methanol extract of *Allium scorodoprasum* was assessed as content of phenols and flavonoids and their ferric reducing power and DPPH free radical scavenging activity.

Materials and Methods

Chemicals

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), $FeCl_3$, and gallic acid, were purchased from Sigma Co. St. Louis, Missouri, USA.

All other chemicals and reagents used $K_3[Fe(CN)_6]$, phosphate buffer (NaH_2PO_4-Na_2HPO_4), CCl_3COOH, $AlCl_3$, ascorbic acid, butylhydroxytoluene (BHT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin, quercetin, CH_3COONa, Na_2CO_3, $K_2S_2O_8$, methanol were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

Apparatus

All spectrophotometric measurements of antioxidative potential of selected plant extracts were performed on a spectrophotometar Perkin Elmer lambda 15.

Plant material

Bulb and seed of *Allium scorodoprasum* were investigated. The plant material was collected in Stara Planina (Vrelo locality) in July 2010, and the plant was determined by Bojan Zlatković, Department of Biology and Ecology, Faculty of Science and Mathematics, University of Nis. The voucher specimen of *Allium scorodoprasum* was confirmed and deposited in Herbarium at the Department of Biology and Ecology, Faculty of Science and Mathematics, University of Nis. The collected bulb and seed of *Allium scorodoprasum* were airdried in darkness at room temperature (20°C).

Preparation of the methanol extracts

The prepared plant material (10 g) was coarsely crushed in small pieces of 2-6 mm by using the cylindrical crusher and extracted two times for 30 min with methanol in ultrasonic bath. The temperature was maintained at 20°C. Ratio of plant material and solvent was 1:10. The extract was filtered through a paper filter (Whatman, No. 1) and evaporated under reduced pressure by the rotary evaporator. The obtained extracts were stored in dark glass bottles for further processing.

Methods

***Determination of total phenols*:** Total phenolic content was determined using Folin-Ciocalteu colorimetric method. An aliquot of different concentrations (0, 1 to 1 μg/ml) of extract, 0.625 mL of Folin-Ciocalteu reagent and 2.5 mL of sodium carbonate (20% v/v) were added to volumetric flask and diluted with water to a total volume of 10 ml. Prepared solutions were left in the dark for 30 min and then the absorbance was measured at 750 nm. Total phenolic content was determined using gallic acid calibration curve (50-500 μg/ml), and the results were expressed as gallic acid equivalents (GAE) averaged from three measurements.

***Determination of total flavonoid*:** Total flavonoid content was determined by spectrophotometric method with $AlCl_3$ reagent, based on measuring the absorbance of complexes

of flavonoids and aluminum. The reaction mixture were prepared in following way: appropriate amounts of extracts (concentrations 0.1 to 1 mg/ml) were diluted with solvent mixture ($MeOH/H_2O/CH_3COOH$ = 14:5:1) to a volume of 2.5 ml, and $AlCl_3$ solution was added to a total volume of 10 ml. Reaction mixture was left for 5 minutes and then absorbance of reaction mixtures was measured at a wavelength of 430 nm. Total flavonoid content was calculated based on the calibration curve and expressed as rutin equivalent, averaged from three measurements.

Determination of ferric reducing power: The reducing power was determined according to the method of Oyaizu (1986). Different concentrations of reaction mixture were prepared (0.1 to 1 mg/ml). Mixtures of appropriate amounts of the extract, 2.5 ml of 1% $K_3[Fe(CN)_6]$ solution and 3 ml Na_2HPO_4-NaH_2PO_4 buffer, were incubated at 20°C for 30 min. After cooling 2.5 ml of 10% trichloroacetic acid, 1.5 ml of $FeCl_3$ were added and diluted with water to a total volume of 10 ml. Absorbance of obtained mixtures was measured at a wavelength of 700 nm. Increased absorbance of reaction mixture indicates higher reducing capacity. Phosphate buffer (pH 6.6) was used as blank solution. Methanolic solutions of pure compounds (gallic acid, BHT, Trolox, ascorbic acid, rutin and quercetin) were tested too at different concentrations. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. Total reducing power of extracts was calculated using the following equation:

$$AEAC = \frac{c_A \cdot A_s}{A_A} \quad (1)$$

c_A - final concentration of ascorbic acid (μg/ml)
A_S - apsorbance of samples
A_A - apsorbance of ascorbic acid

"Scavenging" radical capacity of samples was determined using DPPH radical (Hatano et al., 1988). Reaction mixtures (0.001 to 1 mg/ml) of samples were prepared by mixing appropriate amounts of extract, 2.5 ml of DPPH and methanol to a total volume of 10 ml. Prepared solutions were left in the dark for 60 minutes and then the absorbance was measured at 515 nm. All determinations were performed in triplicate. Methanol was used to zero spectrophotometer. Methanolic solutions of pure compounds (gallic acid, BHT, Trolox, ascorbic acid, rutin and quercetin) were tested too at different concentrations. DPPH radical "scavenging" capacity was expressed by applying the following equation:

$$\text{DPPH} - \text{RSC}(\%) = 100 \times \left(\frac{A_0 - A_1}{A_0} \right) \quad (2)$$

A_0 - absorbance of blank solution
A_1 - absorbance of solution in the presence of active components

Statistical analyses

All results are expressed as mean of three determinations. All linear regression and correlation between antioxidant activities and total phenolic and flavonoid contents in this paper are analyzed by NCSS 2007 software.

Results and Discussion

There are many methods to determine antioxidant capacity. These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods particular antioxidants have varying contributions to total antioxidant potential (Katalinić et al., 2006).

Phenol compounds are high level antioxidants because they have the ability to absorb and neutralize free radicals. The mechanism of action of phenol compounds as antioxidants is based on scavenging and chelating process. The levels of total phenols in methanol extract of *A. scorodoprasum* determined according to the Folin–Ciocalteu method are not absolute measurements of the amounts of phenolic materials but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of gallic acid (Katalinić et al., 2006). Data presented in Figure 1 are average of three measurements. The total phenolic contents of bulb and seed samples increased as concentration of extracts increases. Test has shown that bulb extracts have higher content of polyphenolic compounds from seed extracts of *A. scorodoprasum*. The total phenolic content in the methanol extracts of *A. scorodoprasum*, determined from regression quotation of calibration curve, expressed as microgram of GAE per milligrams of dried extract, was 20.995 ± 1.569 μg GA/mg of dried seed extract, and 36.692 ± 4.475 μg GA/mg of dried bulb extract (Figure 1).

Flavonoids are the most common group of plant polyphenols. Different studies have shown that these compounds are used for prevention and cure of many diseases. Flavonoids transfer hydrogen atom to free radicals, leading to interruption of free-radical reactions. The content of flavonoids is presented in Figure 2. Standard solution of rutin is used for calibration curve construction. The lowest flavonoid content is shown in 0.1 mg/ml seed extract (0.055 mg/ml), while 1 mg/ml bulb extract has the highest flavonoid content (0.142 mg/ml). This method has also showed increases of antioxidant activity with concentration increasing. Flavonoid content was also expressed as rutin equivalent per mass of dry extract. Bulb extracts showed higher flavonoid content (0.230 ± 0.019 mg rutin/mg of dried extract) than seed extract (0.264±0.018 mg rutin/mg of dried extract).

In this study we used determination of ferric reducing power assay and DPPH radical scavenging activity assay to determine total antioxidant potential, because they are quick and simple to perform, reproducible and they have linearly related to the molar concentration of the antioxidant.

The reducing power of an extract may serve as a significant indicator of its potential antioxidant activity. Compounds with reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Jayanthi and Lalitha, 2011). The reducing power ability of the methanol extracts was determined using ascorbic acid as standard. Presence of reducers causes the conversion of the Fe^{3+}/ferricyanide complex used in this method to the ferrous form. Indeed, the reducing power property of a compound indicates that it is electron donor, and can reduce the oxidized intermediates of lipid peroxidation processes and convert them to more stable products and conesquently terminate radical chain reactions (Yen and Chen, 1995). Measurements of reducing power, based on measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of Fe^{3+} ion in presence of extract. The results are shown in Figure 3.

It can be seen that the reducing power values of all extracts were concentration related and increased with the increase in sample concentration in the range of the tested concentrations. The highest reducing power was detected in 1 mg/ml bulb extract (4.491 mg/ml), and the lowest in 0.1 mg/ml seed extract (4.08 mg/ml). Significant difference can be seen in reducing capacity between higher concentrations of bulb and seed extracts. Total reducing power of extracts was also expressed as ascorbic acid equivalent per mass of dry extract. Bulb methanol extract showed higher reducing ability than seed extract. AEAC value per 1 mg of dry extract for bulb was 6.00 ± 1.03 mg, while for seed value was 4.29 ± 0.58 mg. All of the tested synthetic antioxidants (BHT, ascorbic acid A.A., rutin, gallic acid G.A., Trolox and quercetin) showed higher reducing power than bulb and seed extracts (Figure 4).

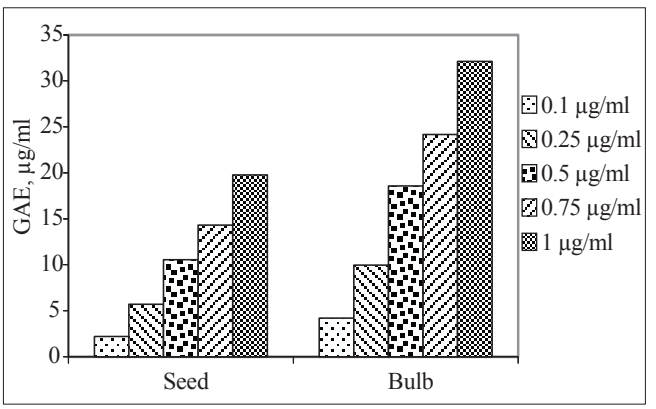

Fig. 1. Total phenolic content of methanol bulb and seed extracts of *A. scorodoprasum*

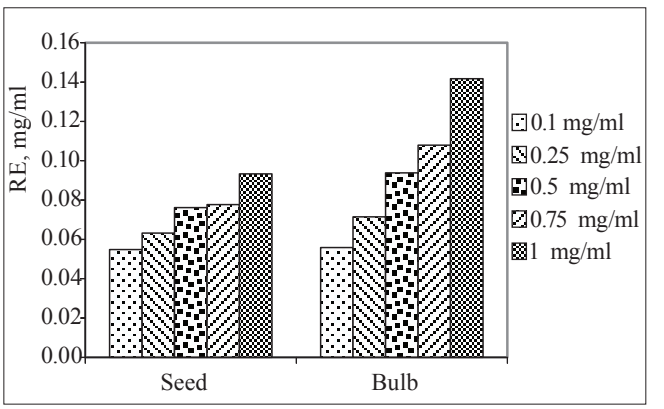

Fig. 2. Total flavonoid content of methanol bulb and seed extracts of *A. scorodoprasum*

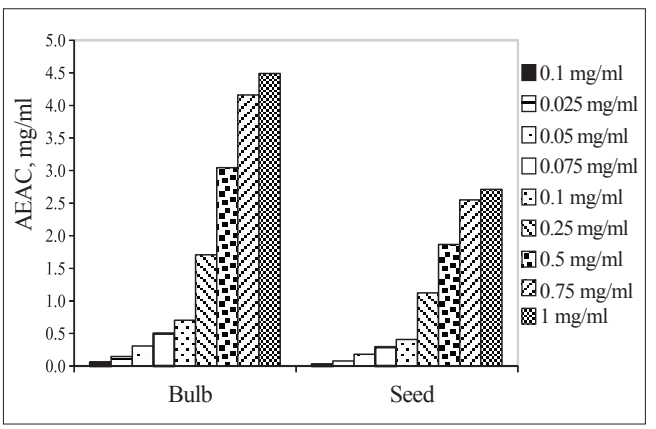

Fig. 3. Reducing power of methanol bulb and seed extracts of *A. scorodoprasum*

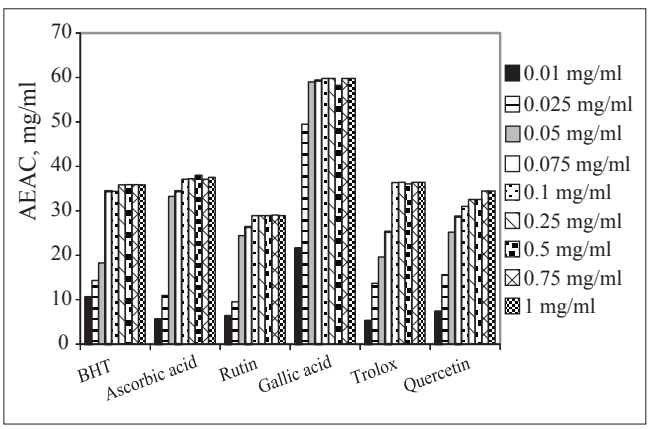

Fig. 4. Reducing power of BHT, A.A., rutin, G.A., Trolox and quercetin

In the group of tested synthetic antioxidants, the highest reducing power is measured in the gallic acid solution.

DPPH method has been used to examine antioxidative activity in complex biological systems because this assay is sensitive, requiring only small amount of samples and allows testing of both lipophilic and hydrophobic substances (Kulišić et al., 2004). DPPH is a stable purple chromogen radical and accepts an electron or hydrogen; it can be reduced and become a stable diamagnetic molecule. In fact, the scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution. The DPPH radical scavenging activity of methanol extracts of *A. scorodoprasum* is given in Figure 5.

DPPH inhibition percentage values were dose dependent, whereby they increased in the range of the tested concentrations. Results obtained by DPPH method showed deviation from results of other three methods. At lower concentrations (0.01 to 0.05 mg/ml) seed extracts showed higher radical scavenging ability, while at higher concentrations bulb extracts showed better ability to "capture" free radicals. Also, the increasing in the percentage of inhibition as concentration increase up to a certain value, and then percentage of inhibition value is decreasing. Maximum value for percentage of inhibition of bulb extracts is at 0.25 mg/ml, and for seed extracts at 0.5 mg/ml. The same phenomen was observed for well-known synthetic antioxidants (BHT, ascorbic acid A.A., rutin, gallic acid G.A., Trolox and quercetin) (Figure 6). The best activity was recorded for trolox and rutin. All of synthetic antioxidants showed maximum activity at highest concetrations. In comparison to radical scavenging capacity values of methanol bulb and seed extracts of *A. scorodoprasum* all testeted synthetic antioxidants manifest the strongest capacity to neutralize DPPH radicals.

Furthermore, quantitative analysis was also used for investigating the correlation between antioxidant activities and phenolic contents in different methanol extracts of *A. scorodoprasum*. To correlate the results obtained with the different methods, a regression analysis was performed (correlation coefficient (r), Tables 1 and 2).

Significant correlations were found among various methods. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds (Shimada et al., 1992) which is also related to the presence of reductant agents (Duh et al., 1998). Correlation analysis carried out showed a positive correlation between phenolic compounds and reducing power. Antioxidant properties of phenolic compounds are directly linked to their structure. Indeed, phenolics are composed of one (or more) aromatic rings bearing one or more hydroxyl groups and are therefore potentially able to quench free radicals by forming resonance-stabilized phenoxyl radicals (Rice-Evans et al., 1996; Sanchez-Moreno, 2002). Correlation analysis indicates significant contribution of phenolics

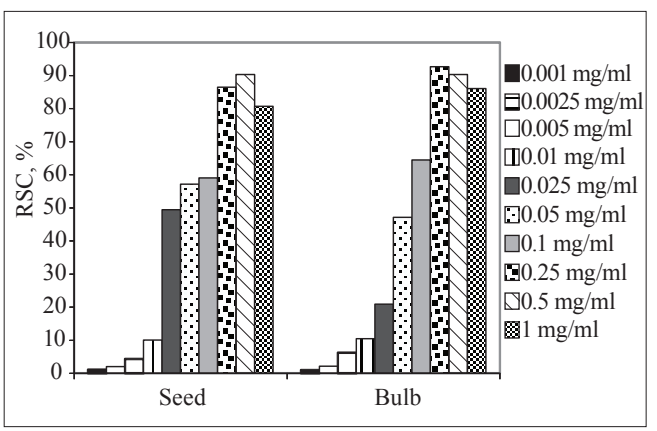

Fig. 5. DPPH scavenging activity of methanol bulb and seed extracts of *A. scorodoprasum*

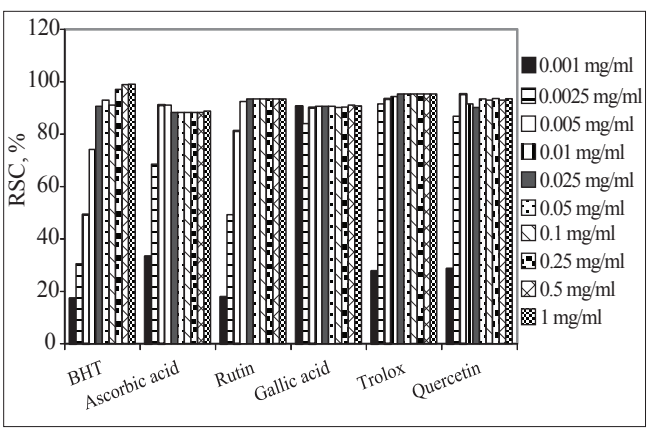

Fig. 6. DPPH scavenging activity of BHT, A.A., rutin, G.A., Trolox and quercetin

Table 1
Correlation coefficient (r) between the studied variables on bulb extracts

	DPPH	AEAC
Total flavonoids content	r = 0.996	r = 0.952
Folin-Ciocalteu	r = 0.996	r = 0.982

Table 2
Correlation coefficient (r) between the studied variables on seed extracts

	DPPH	AEAC
Total flavonoids content	r = 0.934	r = 0.946
Folin-Ciocalteu	r = 0.926	r = 0.970

to this antioxidant assay. Bulb extracts showed stronger correlation among different methods, especially between total flavonoids content and DPPH assay (r = 0.996) and DPPH and Folin-Ciocalteu assay (r = 0.996). The lowest correlations were found between DPPH and Folin-Ciocalteu assay (r = 0.926) for seed extract. The relationship between DPPH radical scavenging activity of samples and the amount of total polyphenols and flavonoids was investigated, and a positive correlation was observed for both of them.

Conclusion

Results of our study showed that methanol bulb extract of *A. scorodoprasum* have higher antioxidant capacity compared to the methanol seed extract, according to three of four used methods. In DPPH assay we found that at low concentrations (0.01 to 0.05 mg/ml) methanol seed extracts have stronger antioxidant activity than bulb extract. A higher content of polyphenols and flavonoids were detected in bulb extracts. Antioxidant activity of all the extracts increased with increase in concentration. Antioxidant activity of *A. scorodoprasum* may be attributed to high phenolic and flavonoid content in each extract. Correlation analysis showed positive correlations between amount of total phenolic and flavonoid content and reducing power and antioxidative activities. Compared to analysed well known natural and synthetic antioxidants, total antioxidant capacity determined as reducing power of methanol extract of *A. scorodoprasum* is very promising.

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References

Bonaccorsi, P., C. Carisiti, C. Gargiulli, and U. Leuzzi, 2008. Flavonol glucosides in *Allium* species: A comparative study by means of HPLC-DAD-ESI-MS-MS. *Food Chemistry,* **107** (4): 1668-1673.

Bouayed, J. and T. Bohn, 2010. Exogenous antioxidants–double-edged swords in cellular redox state. Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxidative Medicine and Cellular Longevit,* **3:** 228–237.

Božin, B., N. Mimica-Dukić, G. Anackov, B. Zlatković, and R. Igić, 2008. Antioxidant properties of methanol extracts of wild garlics (genus *Allium* L., sect. *Allium*). *Planta Medica,* **74:** 969–969.

Dion, M. E. and J. A. Milner, 1997. Garlic inhibits cytochrome P 450 2E1 mediated chlorzoxazone metabolism. *FASEB J* 1(3), **A 370**: 2144-2150.

Duh, P. D., W. J. Yen, P. C. Du, and G. C. Yen, 1997. Antioxidant activity of Mung Bean Hull. *Journal of American Oil Chemist Society,* **74** (9): 1059-1063.

Geng, Z., and B. H. S. Lau, 1997. Aged garlic extract modulates glutathione redox cycle and superoxide dismutase activity in vascular endothelial cells *Phytotherapy Research,* **11** (1): 54–56.

Haim, T., D. M. Townsend, D. Kenneth, and K. D. Tew, 2004. Organosulfur compounds from *Alliaceae* in the prevention of human pathologies. *Biomedicine & Pharmacotherapy,* **58**: 183–193.

Hatano, T., H. Kagawa, T. Yasuhara, and T. Okuda, 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin,* **36**: 2090–2097.

Jayanthi, P. and P. Lalitha, 2011. Reducing power of the solvent extracts of *eichhornia crassipes* (mart.) Solms. *International Journal of Pharmacy and Pharmaceutical Sciences,* **3:** 126-128.

Katalinić, V., M. Miloš, T. Kulisić, and M. Jukić, 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry* **94**: 550–557.

Krest, I., J. Glodek, and M. Keusgen, 2000. Cysteine Sulfoxides and Alliinase Activity of Some *Allium* Species. *Journal of Agricultural and Food Chemistry,* **48** (8): 3753–3760.

Kulišić, T., A. Radonić, V. Katalinić and M. Miloš, 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry,* **83**: 633–640.

Lanzotti, V., 2006. The Analysis of Onion and Garlic. *Journal of Chromatography,* **1112** (1-2): 3-22.

Lau, B. H. S., 1989. Detoxifying, radio-protective and phagocyte-enhancing effects of garlic. *International Clinical Nutrition Review,* **9**: 27-31.

Moriguchi, T., 1996. Aged garlic extract prolongs longevity and improves special memory deficit in senescence-accelerated mouse. *Biological & Pharmaceutical Bulletin,* **19**: 305-307.

Ndhlala, A. R., M. Moyo and J. Van Staden, 2010. Natural antioxidants: fascinating or mythical biomolecules? *Molecules,* **15**: 6905–6930.

Numagami, Y., 1996. Attenuation of rat ischemic brain damage by aged garlic extracts: a possible protecting mechanism as antioxidants. *Neurochemistry International,* **29**: 135-143.

Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition,* **44**: 307–315.

Pinto, J., C. Quiao, J. Xing, B. Suffoletto, R. S. Rivlin and W. Heston, 1997. Garlic constituents modify expression of biomarkers for human prostatic carcinoma cells. *FASEB J*, **22** (3), **A 439:** 2541-2545.

Reuter, H. D., 1995. *Allium sativum* and *Allium ursinum*: Part 2.Pharmacology and medicinal application. *Phytomedicine* **2**: 73–91.

Rice-Evans, C. A., N. J. Miller and G. Paganga, 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acid. *Free Radical Biology and Medicine,* **20**: 933–956.

Robards, K., P. D. Prenzler, G. Tucker, P. Swatsitang and W. Glover, 1999. Phenolic compounds and their roles in oxidative process in fruits. *Food Chemistry,* **66**: 401-436.

Rose, P., M. Whiteman, P. K. Moore and Y. Z. Zhu, 2005. Bioactives salk(en)yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. *Natural Product Reports,* **22:** 351-368.

Sanchez-Moreno, C., 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International,* **8**: 121-137.

Scalbert, A, I. T. Johnson and M. Saltmarsh, 2005. Polyphenols: antioxidants and beyond. *The American Journal of Clinical Nutrition,* **81**: 215-217.

Shi, H., N. Noguchi and E. Niki, 2001. Introducing natural antioxidants, In: Antioxidants in food, Practical applications. Pokorny J., Yanishlieva N., Gordon M., Woodhead Publishing Limited, EDS., Cambridge, England, pp. 22-70.

Shimada, K., K. Fujikawa, K. Yahara, and T. Nakamura, 1992. Antioxidative properties of xanthan on the autoxidation of soyabean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry,* **40**: 945-948.

Steiner, M. and R. I. Lin, 1994. Cardiovascular and lipid changes in response to aged garlic extract ingestion. *Journal of the American College of Nutrition,* **13**: 542-548.

Štajner, D., N. Milić, N. Mimica-Dukić, B. Lazić and R. Igić, 1998. Antioxidant abilities of cultivated and wild species of garlic. *Phytotherapy Research,* **12**: 13–14.

Štajner, D. and I. Varga, 2003. An evaluation of the antioxidant abilities of *Allium* species. *Acta Biologica Szegediensis*, **47** (1-4): 103-106.

Štajner, D., R. Igić, B. M. Popović., and Đ. Malenšić, 2008. Comparative Study of Antioxidant Properties of Wild Growing and Cultivated *Allium* species. *Phytotherapy Research,* **22**: 113–117.

Tepe, B., M. Sokmen, H.A. Akpulat, and A. Sokmen, 2005. *In vitro* antioxidant activities of the methanol extracts of five *Allium* species from Turkey. *Food Chemistry,* **92** (1): 89-92.

Yen G. C., and H. Y. Chen, 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry,* **43** (1): 27-32.

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