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ANTIOXIDANT CAPACITY AND THIN LAYER CHROMATOGRAPHY OF ETHANOL EXTRACTS OF *Allium ursinum* L. AND *Allium bulgaricum* L.

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Abstract

The radical scavenging capacity of 70 % ethanol extracts obtained from Allium ursinum L. and Allium bulgaricum L. was investigated in the present paper. The antioxidant capacity of the extracts was estimated with the use of ABTS, DPPH, FRAP, and CUPRAC assays and the total phenolic content was evaluated as well. The Allium bulgaricum extract appeared to possess a better antioxidant activity compared to the Allium ursinum extract, which was in accordance with the established higher content of total polyphenols for A. bulgaricum extract was 0.41 ± 0.09 mg GAE/g fresh plant weight. In comparison the polyphenols in the A. ursinum extract were found to be 0.40 ± 0.03 mg GAE/g fresh plant weight. The conducted simple TLC method for rapid determination of allicin and alliin in Allium spp. suggested a presence of those substances in both extracts considering the distinctive spots.

Keywords*: Allium spp., antioxidant capacity, TLC.*

INTRODUCTION

Garlic is well known across the centuries. It was used as a medicine by early civilizations (Rivlin, 2006; Thomson and Ali 2003). Moreover, garlic was mentioned as a medicine in some religions (Green and Polydoris 1993; Kahn 1996; Moyers 1996, Bergner 1996). Old Indians believed that it has a good effect for the treatment of joint infections, heart and digestive diseases which is well known nowadays (Woodward, 1996; Rivlin, 1998). As attention has been made more for the usage of plants in the early Renaissance, garlic has taken some importance as it was chosen to be grown for medical purposes (Moyers, 1996). Wild garlic (bear's garlic, wood garlic) grows in fens and river woods of Central Europe. The fresh leaves or dried herb is used in local cuisines of Europe. Since it has not been cultivated yet, it didn't gain any importance until several years ago where people started to look for this as it is natural.

Allium bulgaricum (samardala) is a glabrous plant, 50-100 (150) cm high. 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 found only in limited areas. It is more famous for the flowers in the gardens, than for its healing or flavouring properties. It is poorly known in the other countries as a medical plant or as a culinary spice. Data is missing even in the specialized guides for aromatic and medical plants (Cheshmedjiev I., 2002). There are studies, connecting phyto-nutrients in *Allium* spp. plants with the possibility to reduce the risk of a number of illnesses (Lanzotti, V., 2006): coronary heart disease (Gorinstein et al., 2007; Siegel et al., 2004), cancer (Sengupta et al., 2004), obesity, diabetes, disturbances of the gastrointestinal tract, hyper-cholesterolemia, and inflammatory diseases (Kalayarasan et al., 2009; Takahashi et al., 2008).

Many researchers studied traditional used plants (Alexieva, 2010) and evaluated their antioxidant effect of various plant extracts (Alexieva, 2012 a; Alexieva, 2013) but mainly in terms of essential oils (Alexieva, 2012 b).

Allium ursinum is a wild relative of Europe and Asia. The Latin name is due to the brown bear's taste for the bulbs and its habit of digging up the ground to get at them. Ramsons leaves are edible; they can be used as salad, spice, boiled. The bulbs and flowers are also very tasty. Ramsons leaves are easily mistaken for lily of the valley, sometimes also those of

Colchicum autumnale and *Arum maculatum*. All three are poisonous and possibly deadly. A good means of positively identifying ramsons is grinding the leaves between one's fingers, which should produce a garlic-like smell.

Alliin and isoalliin were the main cysteine sulfoxides found (Schmitt et al. 2005). If the plants are dried, many of the compounds are degraded so that the use of the fresh plant is recommended or alternatively it has to be lyophilised. In the fresh leaves of *A. ursinum* 0.005 % allicin and 0.07 % methyl-L-cysteinsulfoxid as well as E-glutamylpeptides such as E-glutamylallylcysteinsulfoxid have been found (Wagner and Sendl, 1990; Matsuura et al. 1996), E-glutamylallylcysteinsulfoxid reported to inhibit angiotensin-converting enzyme (Sendl et al. 1992; Rietz et al. 1993). Other components such as lectins and flavonoids have been found (Carotenuto et al. 1996; Smeets et al. 1997a, b). Flavonoids were described to be responsible for inhibition of platelets aggregation in humans (Carotenuto et al. 1996). As a property similar to other *Allium* species, it has a marked antioxidant activity because of the high content of carotenoids, chlorophylls, flavonoids and low toxic oxygen radicals (Stajner et al. 2003).

Many volatile compounds such as sulfides and disulfides have been identified in *Allium ursinum* (Schmitt et al., 2005).

The strong antioxidant properties of representatives of *Allium* spp. have caused considerable food technologist interest.

Moreover, there are phenolic compounds in *A. ursinum*. The bulbs and the leaves were found to contain 2.3 mg/g and 3.24 mg/g (dry weight) of total free phenolics, respectively, and the same amount of bound phenol forms (1.0 mg/g) (Djurdjevic et al., 2004). These phenolic compounds could be important because the phenolic compounds have antioxidant effects that are effective in prevention and treatment of different diseases (Stajner et al., 2003). The phenolic compounds in *A. ursinum* may be flavonoids. There are five flavonoids separated from *A. ursinum* (Carotenuto et al., 1996).

Thin layer chromatography (TLC) is a sensitive and effective analytical method, which can be performed easily and quickly. TLC enables to achieve precise separations of mixtures of high complexity, while only very small sample amounts in ranges of some milligrams are needed. This method is highly suitable for separations of plant extracts based on their complex constituents. Today, a large assortment exists enabling to detect nearly all natural products structures individually. Therefore, TLC is still a popular method widely used in research (Petkova & Denev, 2013; Petkova et al., 2013).

The aim of the present study was to determine the total phenolic content as well as the free radical scavenging activity of *Allium ursinum* and *Allium bulgaricum*. This study tried to clarify the existence of alliin/allicin in the tested samples. In addition, the objective of this exploration was also to try to fill in the blank in the research and apply these results not only in culinary technology but also in therapy treatment.

MATERIALS AND METHODS

Extract preparation

Allium bulgaricum and *Allium ursinum* plant material was subjected to a heat reflux extraction with 70 % ethanol (v/v) for 30 min. The extracts were then filtered and stored at 4 ºC without adding any preservatives.

Thin layer chromatography

In the present study thin layer chromatography was used to identify the chemical compounds of the *A. bulgaricum* and *A. ursinum* extracts. TLC was carried out on TLC sheets silica gel 60 with fluorescence indicator F254 20x20 cm (MERCK). The procedure was performed according to (Kanaki and Rajani 2005). For analysis of the alliins and allicins n-butanol: acetic acid: water 60:40:20 was chosen as solvent system. The amino acid Alanine was used as reference showing similar Rf according to alliin. Therefore 5 mg of alanine were dissolved in 1.5 ml methanol.

Detection was made by spraying with ninhydrine reagent. Spray reagent for detection of amino (-NH_2) groups: 200 mg of ninhydrine was dissolved in 100 ml water. After heating at 100 ºC for 5 min alanine and the alliins could be detected as red or pink spots, while allicins were visualized as orange spots in VIS.

Determination of total phenolics

A modified Kujala et al. (2010) method with Folin - Ciocalteu's reagent was used for the

determination of the total polyphenolic content (TPC). 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

ABTS radical scavenging assay

The radicals scavenging activity of the ethanol extract against radical caption ($ABTS^{\bullet+}$) was estimated according to Re et al. (1999) with some modifications. $ABTS^{\bullet+}$ 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 (20 - 22 °C) 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. Each sample (0.01 ml) was added to 1 ml of $ABTS^{\bullet+}$ solution and mixed vigorously. After reaction at 30 °C for 6 min, the absorbance at 734 nm was measured. The percentage of inhibition of $ABTS^{\bullet+}$ by the obtained extracts was calculated for each sample using the following formula:

% Inhibition = [(AB-AE)/AB] x100,

Where: A_B = absorbance of the control without sample; A_E = absorbance of the test sample with $ABTS^{\bullet+}$.

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 FW).

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of Brand-Williams, Cuvelier, and Berset (1995). Freshly prepared $4x10^{-4}$ M methanolic solution of DPPH was mixed with the samples and a standard solution in a ratio of 2:0.5 (v/v). The light absorption was measured at 515 nm and the percentage of inhibition of $DPPH^{\bullet}$ by the obtained extracts was calculated for each sample using the following formula:

$$\% \text{ Inhibition} = [(A_B-A_E)/A_B] \times 100$$

Where: A_B = absorbance of the control without sample; A_E = absorbance of the test sample with $DPPH^{\bullet}$.

The DPPH radical scavenging activity was presented as a function of the concentration of Trolox. The unit of Trolox equivalent antioxidant capacity (TEAC) was defined by the concentration of Trolox having equivalent antioxidant activity expressed as μM TE/g FW.

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. The absorbance of the reaction mixture was recorded at 593 nm. The results were expressed as μM TE/g FW.

CUPRAC assay

The CUPRAC assay was carried out according to the procedure of Ak and Gulcin, 2008. To a test tube were added 1 mL of $CuCl_2$ solution (1.0×10^{-2}M), 1 mL of neocuproine methanolic solution (7.5×10^{-3}M), and 1 mL NH_4Ac 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 extracts was expressed as μM TE/g FW.

Statistical analysis

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

RESULTS AND DISCUSSIONS

Thin layer chromatography

In the present study TLC for Bulgarian Allium and wild garlic was done. The aim of the experiment was to compare the leaf extracts of both plants in respect of their chemical composition in particular of alliins and allicins and their by-products (Figure 1.).

By analyzing the TLC plate it seems to be difficult to establish any visible difference between the two samples. However, the presence of alliin was conducted based on the reddish pink alliin spots which show a similar Rf range like the red spot of alanine. Other alliins are shown as red and pink spots above the alliin spot. The orange spots below alliin are considered to be allicin. The already mentioned results are in accordance with those reported by Sabha (2011). Based on the results we can assume lower concentration of alliin in wild garlic in comparison to Bulgarian Allium.

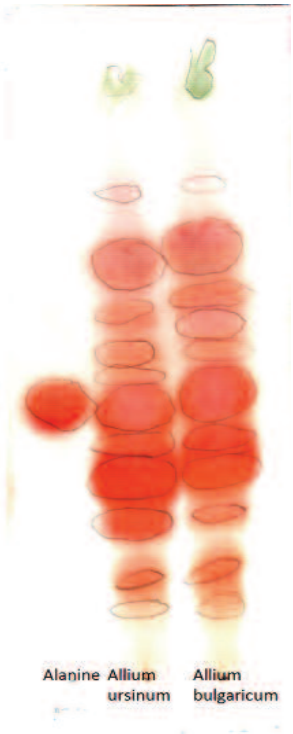

Figure. 1 TLC of Alanine and 20 µl extract of *A. ursinum* and *A. bulgaricum*, respectively.

Antioxidant activity

Total polyphenolic content

The total phenolic content was determined using Folin-Ciocalteu method, reported as gallic acid equivalents by reference to a standard curve. The total phenolics ranged from 0.40 ± 0.02 to 0.41 ± 0.08 mg GAE/g FW (Table 1). The values of polyphenolic content in the *A. bulgaricum* extract were established to be 0.41 mg GAE/g FW and those in the *A. ursinum* - 0.40 mg GAE/g FW. The results concerning the *Allium ursinum* correspond to several studies conducted by Stajner et al. in years 2003 and 2008.

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Van Acker et al., 1996). Due to the presence of those compounds in the studied extracts the antioxidant activity was also studied.

Table 1. Total phenol content (mg GAE/g FW) and *in vitro* antioxidant activity (µM TE/g FW) of *Allium spp.* ethanol extracts

Plant/Method	*Allium ursinum*	*Allium bulgaricum*
TPC	0.40 ± 0.02	0.41 ± 0.08
$TEAC_{DPPH}$	1.86 ± 0.22	4.77 ± 0.88
$TEAC_{ABTS}$	11.37 ± 1.96	5.79 ± 0.25
$TEAC_{FRAP}$	4.56 ± 0.04	7.16 ± 0.06
$TEAC_{CUPRAC}$	4.65 ± 0.08	7.69 ± 0.14

Antioxidant activity

The results from the DPPH, ABTS, FRAP and CUPRAC assays are presented in Table 1.

The DPPH assay is commonly used for fast evaluation of the antioxidant capacity due to the simplicity of the assay. Higher TEAC value indicates that a sample has stronger antioxidant activity. In accordance with the results of the TPC study the DPPH assay confirmed the higher values established by the *A. bulgaricum* ethanol extract – 4.77 ± 0.88 µM TE/g FW. Other previous conducted studies have stated the alcoholic extracts to possess better antioxidant activity compared to the aqueous ones (Sapundjieva et al, 2012).

The scavenging activity of the extracts toward ABTS radical was in favour of the *A. ursinum* extract – 11.37 ± 1.96 µM TE/g fresh plant weight. These results were contrary to the results in all other conducted assays – TPC, DPPH, FRAP, and CUPRAC. This is probably due to the different mechanism of contribution of each individual component to the total radical scavenging activity of the studied samples. The authors therefore strongly suggested that, when analyzing the AOA of samples, it is better to use at least two methods

due to the differences between the test systems (Ou et al., 2002).

The FRAP values of the *A. bulgaricum* extract were also higher than those of the *A. ursunum* – 7.16 ± 0.06 μM TE/g FW. The cupric ion (Cu^{2+}) reducing ability of ethanol extracts of *Allium spp.* leaves is shown in Table 1. Among the two investigated extracts the *Allium bulgaricum* leaves extract showed the higher CUPRAC value – 7.69 ± 0.14 μM TE/g FW. The results of this assay correspond well to the already mentioned results pursuant to the other methods.

CONCLUSIONS

The results obtained confirmed *A. ursinum* and *A. bulgaricum* phenolic compounds to be a contributor of the established antioxidant capacity of the ethanol leaves extracts. Furthermore, the simple TLC assay resulted in identifying the important antibacterial component- allicin in the investigated *Allium* spp. samples.

The outcomes of this study showed that there is a great potential of both *Allium ursinum* and *Allium bulgaricum* for the development of foods rich in compounds with antioxidant and antimicrobial properties.

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REFERENCES

Ak, T., Gülçin, I., 2008. Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 174, 27–37.

Alexieva I., 2010. Traditional Bulgarian Cuisine, BaSeFood Report.

Alexieva I., Mihailova, D., Popova, A., Baeva, M., 2012 a. Study on the Antioxidant Activity of Selected Local Bulgarian Culinary Spices, poster**,** Traditional Food International (TFI-2012), Traditional foods: from culture, ecology and diversity, to human health and potential for exploitation, Cesena, Italy, 4-6 October, 2012.

Alexieva I., Stoyanova, A., Merdzhanov, A., Popova, A., Baeva, M., 2012 b. Chemical Composition of Essential Oil of Some Local Bulgarian Culinary Spices, poster, Traditional Food International (TFI-2012), Traditional foods: from culture, ecology and diversity, to human health and potential for exploitation, Cesena, Italy, 4-6 October, 2012.

Alexieva, Mihaylova, Popova, 2013, Evaluation of the antioxidant capacity of aqueous extracts of fresh samardala (*Allium bulgaricum* l.) leaves, Scientific works, vol. LX, "Food science, engineering and technology",

Benzie I.F.F, J.J. Strain, 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry, v. 239, 70-76.

Bergner, P. (1996) The Healing Power of Garlic :3-26 Prima Publishing Rocklin, CA.

Bergner, P., 1996. The Healing Power of Garlic, Prima Publishing, Rocklin CA, 3-26.

Carotenuto, A., De Feo, V., Fattorusso, E., Lanzotti, V., Magno, S., Cicala, C, 1996. The flavonoids of *Allium ursinum*, Phytochemistry41, 531-36.

Cheshmedjiev, I., 2002. Plants of Bulgaria Flora with essential oils, Ub. Session, "120 Years Agrarian Science in Bulgaria", 21-22 May, Sadovo, Plovdiv, 24.

Djurdjevic, L., Dinic, A., Pavlovic, P., Mitrovic, M., Karadzic, B., Tesevic, V., 2004. Allelopathic potential of Allium ursinum L. Biochem Syst Ecol, 32,533–544.

Gorinstein, S., Jastrzebski, Z., Namiesnik, J., Leontowicz, H., Leontowicz, M., Trakhtenberg, S., 2007. The atherosclerotic heart disease and protecting properties of garlic: Contemporary data, Mol. Nutr. Food Res. 51, 1365-1381.

Green, O. C., III & Polydoris, N. G. (1993) The chemistry of garlic and onions. Garlic, Cancer and Heart Disease: Review and Recommendations :21-41 GN Communications Chicago, IL.

Green, O.C., Polydoris, N.G., 1993. Garlic, Cancer and Heart Disease: Review and Recommendations, GN Communications, Chicago IL, 21–41.

Kahn, G. (1996) History of garlic. Koch, H. P. Lawson, L. D. eds. Garlic: The Science and Therapeutic Application of Allium sativum L. and Related Species: 25-36 Williams and Wilkins New York, NY.

Kahn, G., 1996. History of garlic. In: Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species (Koch HP & Lawson LD,eds.), Williams and Wilkins, New York, NY, 25-36.

Kalayarasan, S., Prabhu, P.N., Manikandan, R., Arumugam, M., Sudhandiran, G., 2009. Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. Eur J Pharacol 606, 162-171.

Kanaki, N. S.,& Rajani, M. (2005). Development and Validation of a Thin-Layer Chromatography-Densitometric Method for the Quantitation of Alliin from Garlic (Allium sativum) and Its Formulations. Journal of AOAC International, 88(5),1568-1570.

Kujala, T.S., Loponen, J.M., Klika, K.D., Pihlaja K. 2000. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem. 48, 5388-5342.

Lanzotti, V., 2006. The analysis of onion and garlic. J. Chromatogr. A, 1112:3-22.
Matsuur, H., Inagaki, M., Maeshige, K., Ide, N., Kajimura, Y., Itakura, Y. 1996. Changes in Contents of gamma-Glutamyl Peptides and Fructan during Growth of Allium sativum, Planta Med62, 70-71.
Moyers, S. (1996) History of garlic. Garlic in Health, History and World Cuisine :1-36 Suncoast Press St. Petersburg, FL.
Moyers, S., 1996. Garlic in Health, History and World Cuisine, Suncoast Press, St. Petersburg FL, 1-36.
Ou, B.x., Hunag, D.j., haMPsCh- WooDill, M., Flanagan, j.a. & DeeMer, e.K., 2002. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. J. Agric. Food Chem., 50(11), 3122–3128.
Petkova, N., Denev, P., 2013. Evaluation of fructan content of the taproots of Lactuca serriola L. and Sonchus oleraceus L. Scientific Bulletin, Series F "Biotechnologies", Volume XVII, Bucharest, 2013, 117-122.
Petkova, N., Ehlimanov,E., Ivanov, I., Denev, P., 2013. Evaluation of Bulgarian medicinal plants as a potential source of inulin-type prebiotics, Proceeding book of International Scientific-Practical Conference, "Food, Technologies & Health", 2013, 142-146.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C.A. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med., 26, 1231-1237.
Rietz, B., Isensee, H., Strohbach, H., Makdessi, S., Jacob, R., 1993. Cardioprotective actions of wild garlic (*Allium ursinum*) in ischemia and reperfusion, Mol Cell Biochem119, 143-150.
Rivlin, R. S. (1998) Patient with hyperlipidemia who received garlic supplements. Lipid Management. Report from the Lipid Education Council 3:6-7.
Rivlin, R.S., 1998. Patients with hyperlipidemia who received garlic supplement lipid manegment; report from the lipid educational council, 3, 6-7.
Rivlin, R.S., 2006. Is garlic alternative medicine? J. Nutr, 136(3), 713-715.
Sabha D., 2011. Pharmaceutical and chemical analysis of the components carrying the antiplatelet activity of extracts from *Allium ursinum* and *Allium sativum.* Dissertation for acquiring educational and scientific degree "Doctor of Philosophy".
Sapundjieva T., Alexieva I. Stoyanova A, Merdzhanov A., Popova A., 2012. Antibacterial Activity of Some Local Bulgarian Culinary Spices, poster in Traditional Food International (TFI-2012), Traditional foods: from culture, ecology and diversity, to human health and potential for exploitation, Cesena, Italy, 4-6 October, 2012.
Sapunjieva T, Alexieva I, Mihaylova D, Popova A: Antimicrobial and antioxidant activity of extracts of Allium ursinum L. J BioSci Biotech 2012, 143–145.
Schmitt, B., Schulz, H, Storsberg, J., Keusgen, M., 2005. Chemical characterization of *Allium ursinum* L. depending on harvesting time. J. Agric. Food Chem. 53:7288–7294.
Sendl, A., Elbl, G., Steinke, B., Redl, K., Breu, W., Wagner, H., 1992. Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*, Planta Med 58, 1-7.
Sengupta, A., Ghosh, S, Bhattacharjee, S., 2004. *Allium* vegetables in cancer prevention: An overview. Asian Pac J Cancer Prev 5:237-245.
Siegel, G., Malmsten, M., Pietzsch, J., Schmidt, A., Buddecke, E., Michel, F., 2004. The effect of garlic on arteriosclerotic nanoplaque formation and size. Phytomedicine, 11, 24-35.
Smeets, K., Van Damme, E.J., Van Leuven, F., Peumans, W.J., 1997 a. Isolation, characterization and molecular cloning of a leaf-specific lectin from ramsons (*Allium ursinum* L.), Plant Mol Biol35, 531-35.
Smeets, K., Damme, E. J. M. V., Leuven, F. V. and Peumans, W. J. 1997 b. Isolation and characterization of lectins and lectin-alliinase complexes from bulbs of garlic (Allium sativum) and ramsons (Allium ursinum). Glycoconjugate Journal 14: 331-343
Stajner, D., Varga, I., 2003. An evaluation of the antioxidant abilities of *Allium species*, Acta Biologica Szegediensis, 47(1-4), 103-106.
Takahashi, M., Shibamoto, T., 2008. Chemical compositions and antioxidant/anti-inflammatory activities of steam distillate from freeze-dried onion (*Allium cepa* L.) sprout. J. Agr. Food Chem., 56, 10462-10467.
Thomson, M., Ali, M., 2003. Garlic [*Allium sativum*]: A Review of its Potential Use as an Anti-Cancer Agent, Curr Cancer Drug Targets(3) 1, 67-81.
Van Acker, S.A.B.E., Van Den Berg, D.J., Tromp, M.N.J.L., Griffioen, D.H., Van Bennekom, W.P., Van Der Vijgh, W.J.F., Bast, A., 1996. Structural aspects of antioxidant activity of flavonoids. *Free Rad. Bio. Med.,* 20(3), 331-342.
Wagner, H., Sendl, A., 1990. Baerlauch und Knoblauch. Vergleichende chemische und pharmazeutische Untersuchungen von Baerlauch- und Knoblauchextrakten, Dtsch Apoth Ztg130, 1809-1815.
Woodward, P. W. (1996) Changes in platelet function and susceptibility of lipoproteins to oxidation associated with administration of aged garlic extract. Garlic and Friends: The History, Growth and Use of Edible Alliums: 2-22 Hyland House Melbourne, Australia.
Woodward, P.W., 1996. Garlic and Friends: The History, Growth and Use of Edible Alliums, Hyland House, Melbourne, Australia, 2-22.