Oxidation Communications 37, No 1, 237–246 (2014)

Biological section – antioxidants, antagonists

POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF *Anthemis cretica* L. (ASTERACEAE)

N. STOJKOVIC[a], M. STOJKOVIC[a], M. MARINKOVIC[a], G. CHOPRA[b], D. KOSTIC[a], A. ZARUBICA[a]*

[a]*Faculty of Science and Mathematics, University of Nis, 18 000 Nis, Serbia*
E-mail: *zarubica2000@yahoo.com*
[b]*Manipal Institute of Technology, University of Manipal, 576 104 Manipal, India*

ABSTRACT

Flavonoids and phenols are considered to be natural antioxidants present in plant life. Although many of their functions in plant metabolism remain unknown, there is a positive connection between the amount of the 2 compounds in plant material and its antioxidant activity. In our paper, we have analysed root, leaves and flowers of *Anthemis cretica* L. (Asteraceae), collected in the mountain range of Stara Planina. Plant parts were extracted with 4 different solvents: methanol, ethanol, acetone and water. Total phenolic content (TPC) and total flavonoid content (TFC) were determined, and antioxidant activity of plant parts was assessed by 4 different methods: DPPH free radical-scavenging assay, ABTS radical-scavenging capacity assay, iron(III) to iron(II) reduction assay (IRA), and cupric ion reducing antioxidant capacity assay (CUPRAC). Root samples have demonstrated the highest TPC and TFC, as well as the highest antioxidant activity, when measured by DPPH, ABTS and IRA methods. When CUPRAC method was used, flowers demonstrated the highest antioxidant activity. There was a strong correlation between amount of phenols and flavonoids of a particular plant part and its antioxidant activity. Methanol was the best solvent for extraction of all plant parts.

Keywords: antioxidant, flavonoids, free radicals, phenolic compounds, plant extract.

AIMS AND BACKGROUND

Phenolic compounds are naturally present in plants. They are secondary plant metabolites, synthesised to counteract a number of environmental factors such as infection, physical damage and UV radiation[1]. Over 1000 of phenolic compounds have been identified, often in glycoside form[2]. Characteristic of phenolic compounds is an

* For correspondence.

aromatic ring with one or more hydroxylic groups (may be mutilated or esterified)[2]. Flavonoids are the largest group of phenolic compounds. They have a role as plant pigments and they protect the plant from insects, oxidative stress and extreme temperatures. Despite recent interest in flavonoids and phenols reflected in increased number of papers, many of their metabolic functions still remain unknown[3]. Due to their specific structure, phenolic compounds are considered as natural antioxidants. Phenolic compounds terminate free radical reactions by donating a hydrogen atom which deactivates free radicals. This means that the phenolic compound itself will become a free radical, but since it is stabilised by resonant effect, it remains inactive. By neutralising free radicals, phenolics decrease lipid peroxidation of cell membrane, may prevent oxidative degeneration of DNA and counteract the spread of tumors[4]. As antioxidants, phenolic compounds may influence the primary ageing process, can be efficient inhibitors of low-density lipoproteins (LDL) oxidation. Epidemiologic research shows an inverse relation between nutrition rich in phenolics and risk of cardiovascular disease. Increased intake of fruit and vegetables rich in nutritive components which demonstrate antioxidative properties can contribute to improvement of quality of life in consumers[4].

Anthemis cretica L. (Asteraceae) is a perennial that may be found in many mountain areas in southern and central Europe and southwest Asia. The plant is polymorphic, and many samples collected in Europe are probably local variations that can not be officially taxonomically recognised. In Turkey, 12 subspecies have been recognised[5,6], while in Mediterranean 23 subspecies have been reported[7]. There are few researches on phytochemical properties of *Anthemis cretica*[8]. Buruk et al.[9] have counted *Anthemis cretica* amoung the most bioactive plants in their study. There are no scientific papers concerning antioxidative properties of *Anthemis cretica* in spite of its use in traditional medicine in Eastern Serbia.

In order to assess possible health benefits and medicinal potential of *Anthemis cretica,* dried parts (flower, leaves and root) of the plant were extracted in different solvents (methanol, ethanol, acetone and water), and total phenolic and total flavonoid content was determined. Different antioxidant assays were performed: DPPH, ABTS, IRP and CUPRAC.

EXPERIMENTAL

Standards and chemicals. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate and catechin were obtained from Sigma-Aldrich (Steinheim, Germany), methanol, ethanol and acetone were purchased from J. T. Baker (Deventer, Holland). Gallic acid was obtained from Carl Roth (Karlsruhe, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 2,4,6-tris-2-pyridyl-1,3,5-triazine (TPTZ) were obtained from Acros Organics (Geel, Belgium). The Folin–Ciocalteu (FC) reagent, 2,9-dimethyl-1,10-phenanthroline (neocuproine), Na_2CO_3, $NaNO_2$, NaOH, HCl, Na_2HPO_4,

NaH_2PO_4, $K_3[Fe(CN)_6]$, trichloroacetic acid (TCA) and $AlCl_3$ were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade, and were used as received except that the solvents were distilled prior to use.

Plant material. Aerial and underground parts of *Anthemis cretica* L. (Asteraceae) were collected from the mountain range Stara planina (43.385° N, 22.660° E), eastern Serbia in the June 2012.

Preparation of the extracts for antioxidant assays. Leaves, flowers and roots were separately air-dried to constant weight and then crushed with an electrical stainless-steel grinder into a fine powder. The exact mass of around 0.25 g of dry powder was extracted with 25 ml of different solvents (water, acetone, ethanol and methanol) using an ultrasonic bath (Bandelin SONOREX® Digital 10 P, Sigma, USA) during 30 min. The extraction was repeated with several more 25-ml portions of the same solvent, the obtained extracts combined, concentrated in the stream of nitrogen, and finally made up to 50 ml and stored at 4°C until analyses. All extractions were done in triplicate.

Determination of total phenolic content (*TPC*). The total phenolic content of the extracts was determined using the Folin–Ciocalteau assay[10]. Briefly, 0.15 ml of the extracts were mixed with 2.0 ml of (20%, w/w) Na_2CO_3 solution and 0.5 ml of FC reagent and made up with deionised water to a final volume of 10.0 ml. The solution was mixed and, after ageing for 120 min at 25°C, the absorbance was measured at 760 nm, using an UV-vis. spectrophotometer (Agilent 8453, Agilent Technologies, USA). Results were expressed as mg of gallic acid equivalents per g of the dry sample.

Determination of total flavonoid content (*TFC*). The total flavonoid content of *A. cretica* solvent extracts was determined by a colorimetric method[11]. A known volume of the samples was mixed with 2 ml of distilled water and subsequently with 0.3 ml of a $NaNO_2$ solution (5%, w/w). After 5 min, 3 ml of $AlCl_3$ solution (1%, w/w) were added and the solution left for 5 min at room temperature. Then, 2 ml of NaOH solution (1 mol/l) were added to the mixture diluted with deionised water to the final volume of 10 ml. The mixture was thoroughly mixed and absorbance was immediately measured at 510 nm versus the previously prepared water blank. The results were expressed as mean values ± standard deviation of catechin equivalents (mg catechin/g dry weight).

DPPH free radical-scavenging assay. The antioxidant capacity of *Anthemis cretica* solvent extracts was studied through the evaluation of the extracts free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed earlier[12]. An aliquot (0.1 ml) of the different extracts was mixed with 2.5 ml of 100 μmol/l DPPH methanol solution. The mixture was thoroughly vortex-mixed, kept out of light for 30 min and absorption was measured at 515 nm. The absorption of the blank containing the same amount of methanol and

DPPH solution was prepared and measured daily. The radical scavenging activity was calculated using the following formula:

scavenging effect (%) = [1-(absorbance of the sample/absorbance of the blank)] × 100.

The results were expressed as mg of Trolox equivalents (TE) per 1 g of sample.

ABTS radical-scavenging capacity assay. The test described earlier[13] was used in this study. The ABTS radical cation ($ABTS^{\cdot+}$) solution was prepared by the reaction of solutions of 7 mmol/l ABTS and 2.45 mmol/l of potassium persulphate, at 23°C in the dark for 16 h. The $ABTS^{\cdot+}$ solution was then diluted with 80% (v/v) aqueous ethanol to obtain a solution with the absorbance of 0.700 ± 0.020 at 734 nm. The $ABTS^{\cdot+}$ solution (3.9 ml) was added to 0.1 ml of the test sample and mixed thoroughly. The reaction mixture was left to stand at 23°C for 6 min and then the absorbance was measured at 734 nm. The samples were diluted with 80% aqueous ethanol so as to give 20–80% reduction of the blank absorbance with 0.1 ml of the samples. The total antioxidant activity of *Anthemis cretica* extracts was expressed as mg of TE per g of dry weight.

Iron(*III*) *to iron*(*II*) *reduction assay* (*IRA*). Iron(III) to iron(II) reduction assay[14] was adopted. Different dilutions of the extracts (0.5 ml) were added to the mixture of 1.25 ml of the phosphate buffer (0.2 mol/dm^3, pH 6.6) and 1.25 ml of potassium ferricyanide (1%, w/w). The resultant solution was incubated at 50°C for 20 min. After that, trichloroacetic acid solution (1.25 ml, 10%, w/w) was added, diluted with 4.25 ml of water and 0.85 ml of ferric chloride solution (0.1%, w/w) were added. After 30 min, the absorbance was measured at 700 nm. IRA of the extracts was expressed as mg gallic acid equivalents/g.

Cupric ion reducing antioxidant capacity (*CUPRAC*). The CUPRAC method was applied as previously described[15]. A mixture comprised of 1 ml of 10 mmol/l copper(II) chloride, 1 ml of 1mol/l ammonium acetate buffer at pH 7.0, and 1 ml of 7.5 mmol/l neocuproine solution was prepared, x ml sample solution and $(1-x)$ ml distilled water were added, and well mixed (total volume: 4.0 ml). This final mixture in a stoppered test tube was left to stand at room temperature for 30 min. After that, the absorbance at 450 nm was measured against a blank. The standard curve was prepared using Trolox standard solution. The antioxidant activity of *Anthemis cretica* extracts was expressed as mg of TE per g of dry weight.

Statistical analysis. Results were expressed as the mean ± standard deviation. Statistically significant differences were determined by one-way analysis of variance (ANOVA) (Ref. 16) followed by Tukey *post hoc* test for multiple comparison[16] (Graph pad Prism version 5.03, San Diego, CA, USA). Probability values (p) less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phenolic compounds have strong antioxidant properties and are the most abundant antioxidants in plant materials. They are also considered the most important source of antioxidants in most diets[17,18]. Thus the amount of phenolic compounds present in a plant is a valuable factor for reflecting its value in terms of antioxidant activity. Flavonoids are a large group of phenolic compounds and are widespread in plants and fruits. They have many important roles in plants, such as acting as signal molecules and protecting the plant. They are responsible for colours of flowers, fruits, leaves, etc.[19] Flavonoids have demonstrated a high antioxidation potential and been known to act as scavengers of various oxidising species[20].

Total phenolic content (TPC) and total flavonoid content (TFC) of different plant parts (root, leaves and flowers) in methanol, ethanol, acetone and water are presented in Table 1 and Fig. 1. Highest values both of total phenolic and total flavonoid content are obtained when using methanol, similar to other authors[11,12]. The ranking of values for total phenolic content and total flavonoid content in different solvents was: methanol>ethanol>acetone>water, for all parts of the plant. The main antioxidant compounds in flowers are anthocyanins, which are responsible for colour of the flower[21]. They are equally soluble in methanol and ethanol, and thus the smaller difference in TPC for flowers in methanol and ethanol compared to root.

The highest TPC was found in the root, 61.61±0.9 mg GAE per g of dry weight in methanol, while TPC in leaves in the same solvent is 45.76±0.5 mg GAE per g of dry weight, which is significantly smaller ($p < 0.05$) (Table 1). This is in accordance with previous research which shows that root samples of plants have a higher antioxidant activity[22]. TPC of leaves in methanol is 33.01± 0.5 mg GAE per g of dry weight, which is almost half as that of the root (Table 1, Fig. 1). TFC follows the same trends as TPC, as it is highest when extraction is done with methanol, followed by ethanol, acetone and water. TFC values are ranked the same for different plant parts: root > flower > leaves.

Table 1. Total phenolic content, total flavonoid content, DPPH, ABTS, IRP and CUPRAC antioxidant activity for different parts of the plant, mean values and standard deviation

			Anthemis cretica					
			TPC[1]	TFC[2]	DPPH[3]	ABTS[3]	IRP[1]	CUPRAC[3]
1	methanol	root	61.61 ± 0.9	30.73 ± 0.5	35.24 ± 0.5	59.15 ± 0.8	48.64 ± 0.6	90.68 ± 1.0
2	ethanol	root	48.05 ± 0.8	23.33 ± 0.5	25.10 ± 0.5	44.52 ± 0.7	39.47 ± 0.5	68.94 ± 0.9
3	acetone	root	33.83 ± 0.5	22.39 ± 0.5	17.59 ± 0.5	30.19 ± 0.5	26.53 ± 0.5	65.32 ± 1.0
4	water	root	34.72 ± 0.5	21.72 ± 0.5	18.79 ± 0.5	32.62 ± 0.5	27.48 ± 0.5	63.10 ± 1.0
5	methanol	leaves	33.01 ± 0.5	26.89 ± 0.5	25.01 ± 0.5	38.33 ± 0.6	35.48 ± 0.5	69.89 ± 0.6
6	ethanol	leaves	29.46 ± 0.5	21.50 ± 0.5	21.92 ± 0.5	33.21 ± 0.5	30.68 ± 0.5	55.45 ± 0.5
7	acetone	leaves	23.02 ± 0.5	16.63 ± 0.5	14.47 ± 0.5	25.83 ± 0.5	23.48 ± 0.5	43.07 ± 0.5
8	water	leaves	23.90 ± 0.5	15.58 ± 0.5	14.80 ± 0.5	26.33 ± 0.5	24.89 ± 0.5	45.81 ± 0.5
9	methanol	flower	45.76 ± 0.5	25.26 ± 0.5	26.60 ± 0.5	54.35 ± 0.9	45.54 ± 0.6	96.25 ± 1.0
10	ethanol	flower	39.83 ± 0.5	22.53 ± 0.5	22.25 ± 0.5	47.55 ± 0.5	39.10 ± 0.5	84.06 ± 1.0
11	acetone	flower	30.94 ± 0.5	19.73 ± 0.5	16.65 ± 0.5	32.56 ± 0.5	27.56 ± 0.5	62.96 ± 0.8
12	water	flower	27.98 ± 0.5	18.34 ± 0.5	15.28 ± 0.5	31.39 ± 0.5	27.64 ± 0.5	65.47 ± 0.8

[1] mg GAE/g DW; [2] mg CE/g DW; [3] mg TE/g DW.

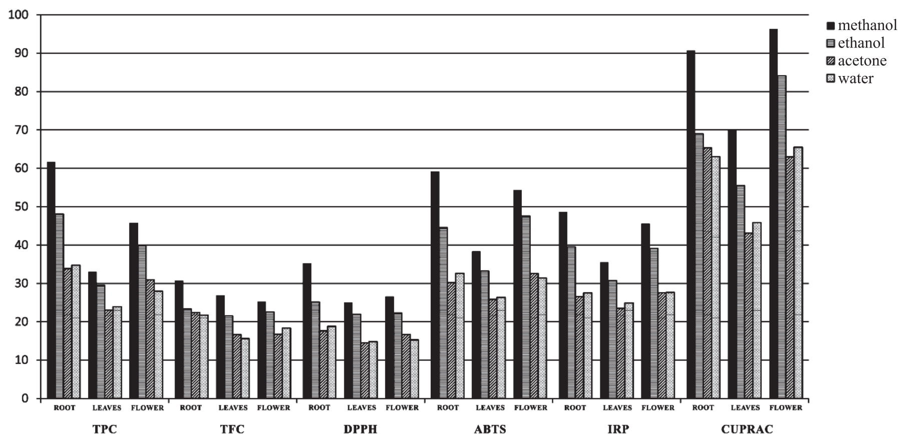

Fig. 1. Total phenolic content, total flavonoid content, DPPH, ABTS, IRP and CUPRAC antioxidant activity for different plant parts

Most plants contain a number of compounds that have different antioxidant activities. Therefore, determination of each compound for the sole reason of evaluating the value of the plant as a source of antioxidants would be ineffective and costly. A number of methods for determination of antioxidant activity of plants have been developed such as: DPPH, ABTS, IRA, CUPRAC, FRAP (Ref. 23), ORAC (Ref. 24), etc.). Antioxidant compounds can act through a number of mechanisms that reduce the potential damage of oxidants: free radical scavenging, breaking of radical chain reactions, oxygen quenching, etc.[17] Consequently, more than one method is required in order to fully determine the value of a plant or a food as an antioxidant[25]. In our work, we have used four methods for evaluating antioxidant activity of *Anthemis cretica*: DPPH, ABTS, IRA and CUPRAC method. Again, for each method of antioxidant activity determination and each plant part, plant material extracted in methanol demonstrated the highest values (Table 1, Fig. 1). Dependence of antioxidative behaviour of active components on solvent type and polarity was reported earlier[26]. However, it is also possible to ascribe the difference in antioxidant activity of the same plant part in a different solvent to the extraction power of the solvent. Methanol may simply extract more of the active compounds from dried plant material as reported[27], which can also explain higher values obtained when using it as a solvent.

CUPRAC method has shown distinctively higher TE per g of dry weight values compared to the other 2-electron transfer-based antioxidant assays, ABTS and DPPH. This is similar to previous researches[26,28,29] verifying that CUPRAC is the best method for assessment of antioxidant activity of plant extracts. Main advantage of CUPRAC and probably the reason for its highest TE per g of dry weight values is its sensitivity both to hydrophilic and lipophilic antioxidants[28].

Root extracts have demonstrated the highest values for DPPH, ABTS and IRA determination methods, for all the solvents used. When methanol was used as a solvent,

DPPH, ABTS, IRA and CUPRAC values for root extracts were: 35.24±0.5, 59.15±0.8, 48.64±0.6 and 90.68±1.0 mg TE per g of dry weight, respectively. The ranking of antioxidant activities for DPPH, ABTS and IRA was: root > flower > leaves. It is interesting that CUPRAC method shows higher value of mg TE per g of dry weight for flower extracts, followed by root and leaves.

Correlations among results derived from determining TPC, TFC and antioxidant activity assays (DPPH, ABTS, IRP and CUPRAC) are presented in Table 2. Only correlations between CUPRAC and TFC (0.777) and CUPRAC and DPPH (0.779) are significant at the level of $p<0.05$, while all of the other correlation are significant at higher level ($p < 0.01$).

Table 2. Correlation coefficients between TPC, TFC, DPPH, ABTS, IRP and CUPRAC methods

Method	TPC	TFC	DPPH	ABTS	IRP	CUPRAC
TPC	1					
TFC	0.838*	1				
DPPH	0.915*	0.935*	1			
ABTS	0.929*	0.823*	0.916*	1		
IRP	0.913*	0.845*	0.938*	0.990*	1	
CUPRAC	0.829*	0.777**	0.779**	0.927*	0.897*	1

* Correlation is significant at the level of $p<0.01$; ** correlation is significant at the level of $p<0.05$.

There is a very strong correlation between total phenolic content and antioxidant assays ($p < 0.01$) (Table 2). Similar correlation has been previously reported[11]. There are strong correlations ($p < 0.05$) among all of the different antioxidant assays used in our work.

CONCLUSIONS

Extracts of all parts of *Anthemis cretica* demonstrate certain antioxidant activity according to antioxidant assays used (DPPH, ABTS, IRP and FRAP). Strong correlation between total phenolic content and antioxidant assays points to phenolic compounds as main carriers of antioxidant activity in our plant samples. The highest phenolic and flavonoid content, as well as antioxidative activity (according to all assays used) was found in root samples, compared to other plant parts (flower and leaves), which is considered uncharacteristic for most plants. Methanol has proven to be the most effective solvent, since samples extracted with methanol have the highest values in all of the assays.

ACKNOWLEDGEMENTS

The authors wish to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project ON 172 016).

REFERENCES

1. S. KARAMAN, E. TUTEM, K. S. BASKAN, R. APAK: Comparison of Total Antioxidant Capacity and Phenolic Composition of Some Apple Juices with Combined HPLC–CUPRAC Assay. Food Chem, **120**, 1201 (2010).
2. L. SORIN, V. LAURIAN, G. SIMION, R. LUCIAN, F. CRISTINA: Determination of Phenolic Compounds from *Geranium sanguineum* by HPLC. J Liq Chrom Rel Technol, **28**, 3109 (2005).
3. Ø. ANDERSEN, K. MARKHAM: Flavonoids–Chemistry, Biochemistry and Applications. CRC Press Taylor & Francis Group, 2006.
4. B. HAVSTEEN: The Biochemistry and Medical Significance of the Flavonoids. Pharmacol Ther, **96**, 67 (2002).
5. A. J. C. GRIERSON: *Anthemis* L. [Perennial Species], Flora of Turkey and the East Aegean Islands. Edinburgh, 1975, 174–221.
6. R. FRANZEN: *Anthemis cretica* (Asteraceae) and Related Species in Greece. Willdenowia, **16** (1), 35 (1986).
7. W. GREUTER: A Critical Inventory of Vascular Plants of the Circum-Mediterranean Countries. Med-Checklist, **56,** 739 (2008).
8. V. VAJS, V. BULATOVIC, K. FODULOVIC-SAVIKIN, N. MENKOVIC, S. MACURA, N. JURANIC, S. MILOSAVLJEVIC: Highly Oxygenated Guaianolides from *Anthemis cretica* subsp. Cretica. Phytochemistry, **50**, 287 (1999).
9. K. BURUK, A. SOKMEN, F. AYDIN, M. ERTURK: Antimicrobial Activity of Some Endemic Plants Growing in the Eastern Black Sea Region, Turkey. Fitoterapia, **77**, 388 (2006).
10. V. SINGLETON, J. ROSSI: Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Amer J Enol Viticult, **16**, 144 (1965).
11. S. MITIC, M. STOJKOVIC, J. PAVLOVIC, M. MITIC, B. STOJANOVIC: Antioxidant Activity, Phenolic and Mineral Content of *Stachys germanica* L. (Lamiaceae). Oxid Commun, **35**, 1011 (2012).
12. D. KOSTIC, S. MITIC, M. MITIC, A. ZARUBICA, J. VELICKOVIC, A. DJORDJEVIC, S. RANDJELOVIC: Phenolic Contents, Antioxidant and Antimicrobial Activity of *Papaver rhoeas* L. extracts from Southeast Serbia. J Med Plants Res, **4**, 1727 (2010).
13. M. OZGEN, N. REESE, A. TULIO, J. SCHEERENS, R. MILLER: Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Methods. J Agr Food Chem, **54**, 1151 (2006).
14. D. DORMAN, A. PELTOKETO, R. HILTUNEN, M. TIKKANEN: Characterisation of the Antioxidant Properties of De-odourised Aqueous Extracts from Selected Lamiaceae herbs. Food Chem, **83,** 255 (2003).
15. R. APAK, K. GUCLU, M. OZYUREK, S. KARADEMIR: Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. J Agric Food Chem, **52**, 7970 (2004).
16. B. HIBBERT, J. GOODING: Data Analysis for Chemistry. Oxford University Press, 2006.
17. G. DUTHIE, S. DUTHIE, J. KYLE: Plant Polyphenols in Cancer and Heart Disease: Implications as Nutritional Antioxidants. Nutr Res Rev, **13**, 79 (2000).
18. JIANG-FEI MENG, YU-LIN FANG, MIN-YANG QIN, XI-FU ZHUANG, ZHEN-WEN ZHANG: Varietal Differences among the Phenolic Profiles and Antioxidant Properties of Four Cultivars of Spine Grape (*Vitis davidii* F o e x) in Chongyi County (China). Food Chem, **134**, 2049 (2012).
19. A. GHASEMZADEH, N. GHASEMZADEH: Flavonoids and Phenolic Acids: Role and Biochemical Activity in Plants and Human. J Med Plants Res, **5**, 6697 (2011).
20. J. HARBORNE, C. WILLIAMS: Advances in Flavonoid Research since 1992. Phytochemistry, **55**, 481 (2000).

21. K. PETRONI, C. TONELLI: Recent Advances on the Regulation of Anthocyanin Synthesis in Reproductive Organs. Plant Sci, **181**, 219 (2011).
22. A. MAJEWSKA, B. BALASINSKA, B. DABROWSKA: Antioxidant Properties of Leaf and Root Extract and Oil from Different Types of Horseradish (*Armoracia rusticana* G ae r t n.). Folia Horticulturae, **16** (1), 15 (2004).
23. S. GRIFFIN, R. BHAGOLI: Measuring Antioxidant Potential in Corals Using the FRAP Assay. J Exp Mar Biol Ecol, **302**, 201 (2004).
24. C. FOLCH-CANO, C. JULLIAN, H. SPEISKY, C. OLEA-AZAR: Antioxidant Activity of Inclusion Complexes of Tea Catechins with β-cyclodextrins by ORAC Assays. Food Res Int, **43**, 2039 (2010).
25. N. PELLEGRINI, M. SERAFINI, B. COLOMBI, D. del RIO, S. SALVATORE, M. BIANCHI, F. BRIGHENTI: Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different *in vitro* Assays. J Nutr, **133**, 2812 (2003).
26. S. E. CELIK, M. OZYUREK, K. GUCLU, R. APAK: Solvent Effects on the Antioxidant Capacity of Lipophilic and Hydrophilic Antioxidants Measured by CUPRAC, ABTS/persulphate and FRAP Methods. Talanta, **81,** 1300 (2010).
27. Z. U. REHMAN: Citrus Peel Extract – a Natural Source of Antioxidant. Food Chem, **99**, 450 (2006).
28. R. APAK, K. GUCLU, B. DEMIRATA, M. OZYUREK, S. E. CELIK, B. BEKTASOGLU, K. I. BERKER, D. OZYURT: Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. Molecules, **12**, 1496 (2007).
29. S. GORINSTEINA, Z. JASTRZEBINSKI, H. LEONTOWICZC, M. LEONTOWICZC, J. NAMIESNIK, K. NAJMANC, Y.-S. PARKE, B.-G. HEOF, J.-Y. CHOG, J.-H. BAE: Comparative Control of the Bioactivity of Some Frequently Consumed Vegetables Subjected to Different Processing Conditions. Food Control, **20,** 407 (2009).

Received 30 August 2013
Revised 19 October 2013