Acta fytotechnica et zootechnica 1
Nitra, Slovaca Universitas Agriculturae Nitriae, 2011, s. 13–16

ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF FRACTIONS FROM SELECTED BULGARIAN MEDICINAL PLANTS

ANTIOXIDAČNÁ AKTIVITA A CELKOVÝ OBSAH FRAKCIÍ FENOLOV VO VYBRANÝCH LIEČIVÝCH RASTLINÁCH BULHARSKA

Yoana KISELOVA,[1] Diana IVANOVA,[1] Antoaneta TRENDAFILOVA,[2] Stefka MARINOVA,[1]
Yanka ZAPRYANOVA,[1] Milka TODOROVA[2]

Medical University Varna, Bulgaria[1]
Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Bulgaria[2]

The antioxidant activities (AOA) of crude methanolic extracts (CME) and its ethyl acetate (EAF), buthanol (BF), petroleum ether (PEF), chloroform (CF) and water fractions (WF) of eight Bulgarian medicinal plants were analyzed using the ABTS cation radical decolorizaton assay. The presence of compounds possessing antioxidant activity was identified in three fractions: in EAF of *Fragaria vesca, Rheum oficinale* and *Melissa officinalis*, in BF of *Cydonia vulgaris, Hypericum perforatum* and *Origanum vulgare* and in CME of *Arctostaphylos uva-ursi*. Total phenolic content (TPC) was also determined for each extract/fractions. EAF contained the highest TPC as compared to the other fractions for all species followed by the BF for *Arctostaphylos uva -ursi, Fragaria vesca, Hypericum perforatum, Melissa officinalis* and *Origanum vulgare* and by CME for *Alchemilla vulgaris, Cydonia vulgaris* and *Rheum officinale*. AOA correlated positively to TPC of CME, BF and EAF, all of them showing a potential value as a source of natural antioxidants.

Key words: Bulgarian medicinal plants, antioxidant activity, total polyphenol content, fractions

Since ancient times plants have been used to prepare teas and beverages, and spices and herbs have been added to different type of food to improve quality, taste and flavor. Recently many plants and plant products have been recognized for their antioxidant properties (Kähkönen et al., 1999; Choi et al., 2002; Cai et al., 2004; Djeridane et al., 2006; Pourmorad et al., 2006; Wong et al., 2006). Plant phenolic compounds (flavonoids, phenolic acids and tannins) are the substances thought to contribute to a great extend to the antioxidant potential of plants (Nijveldt et al., 2001; Higdon et al., 2003; Scalbertet al., 2005). The antioxidant activity (AOA) and total phenolic content (TPC) of aquous and aquous-alcoholic extracts of over 50 Bulgarian medicinal plants have been extensively studied (Ivanov 2007; Ivanova et al.. 2005, Ivanova et al., 2009, Kiselova et al., 2004; Kiselova et al., 2006), and plants with high TPC correlating to their antioxidant potential were identified. However, there are no relevant studies on the fractions containing highest amount of antioxidants from these plants. The aim of this work was to examine phenolic compounds of extracts of eight Bulgarian plants and their fractions and to evaluate their AOA.

Material and methods

Plant material

Plant material was collected in different regions in Bulgaria. The species were identified and voucher specimens were deposited at the Department of Biology and Pharmaceutical Sciences, Faculty of Pharmacy, Medical University of Varna.

Extraction and fractionation

5 g powdered dry material was extracted for 30 min with 100 ml methanol at room temperature in ultrasound chamber. The extract was filtered and the plant material was extracted another two times using the same procedure. All filtrates were combined and the crude methanolic extract was evaporated to

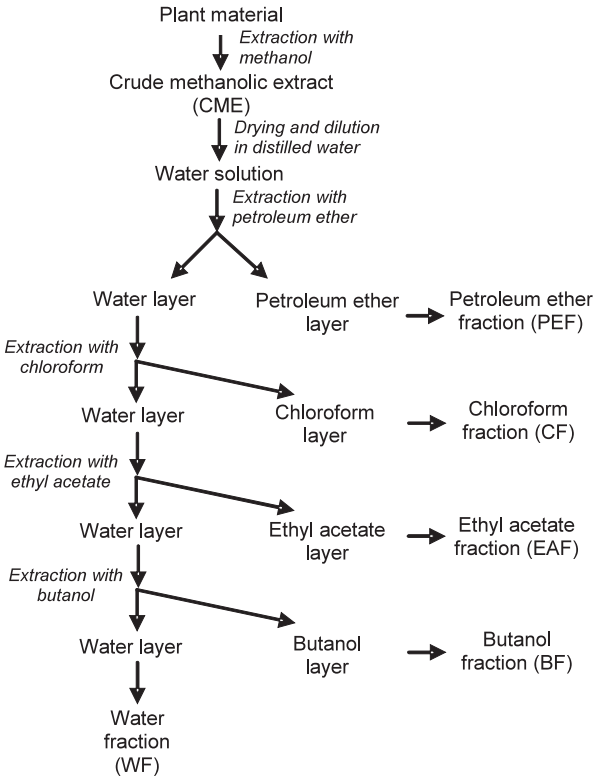

Figure 1 Extraction and fractionation procedure
Obrázok 1 Proces extrakcie a frakcionácie

dryness under vacuum. The residual was dissolved in 50 ml distilled water and the solution was further consecutively extracted with petroleum ether, chloroform, ethyl acetate and butanol (fig. 1).

For measuring the AOA and the TPC of fractions 10 mg fractionated dry material was dissolved in 1 ml of appropriate solvent: chloroform for petroleum ether (PEF) and chloroform (CF) fractions; absolute ethanol for crude methanolic extract (CME), ethyl acetate (EAF) and butanol (BF) fractions and distilled water for water fraction (WF).

Antioxidant activity was measured using the ABTS (2,2`-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) cations radical decolourization assay (Re et al., 1999). The method is based on the consumption of preformed in the presence of potassium persulfate ABTS radical (ABTS+). Addition of antioxidants to ABTS+ reduces it to ABTS. Absorption was measured at 734 nm. Uric acid was used as a standard. The antioxidant activity is presented as mmol/L Uric Acid Equivalents (UAE). The results are presented as means ± S.D. Each measurement was performed at least in triplicate on Synergy 2 plate reader.

Total phenolic content was measured using the Folin-Ciocalteu reagent as described by Singleton and Rossi (1965). Absorption was measured at 760 nm. TPC was expressed as mmol/L Quercetin Equivalents (QE). Results are presented as means ± S.D. Each measurement was performed at least in triplicate on Synergy 2 plate reader.

Statistical analysis

All results are presented as means±standard deviation of three determinations and all were averaged. Statistical analysis was performed by employing GraphPad Prizm 3.0 statistical software. TPC was plotted against AOA and the correlations were analyzed by calculating the r correlation coefficient.

Results and discussion

Eight Bulgarian medicinal plants (*Alchemilla vulgaris, Arctostaphylos uva-ursi, Cydonia vulgaris, Fragaria vescŕ, Hypericum perforatum, Melissa officinalis, Origanum vulgare* and *Rheum officinale*) were selected for fractioning based on their AOA and TPC of water and water-alcoholic extracts AOA and TPC of each fraction from all eight plants were measured (fig. 2).

The highest AOA for the CME was measured for Arctostaphyllos uva-ursi (63.23±2.97 mmol/L), for the PEF – for *Cydonia vulgaris* (23.75±0.1 mmol/L), for CF – for *Hypericum perforatum* (6.51±0.01 mmol/L) and *Arctostaphyllos uva-ursi* (6.02±0.4 mmol/L), for EAF – for *Fragaria vesca* (98.42±7.43 mmol/L), for BF – for *Fragaria vesca* (60.27±0.93 mmol/L) and *Arctostaphyllos uva-ursi* (59.91±1.23 mmol/L) and for WF – for *Fragaria vesca* (22.49±0.15 mmol/L).

Highest TPC for the CME was determined for *Alchemilla vulgaris* (12.26±0.29 mmol/L), for the PEF and CF – for *Rheum officinale* (2.38±0.05 mmol/L and 2.91±0.04 mmol/L, respectively), for EAF – for *Fragaria vesca* (16.78±0.47 mmol/L), for BF – for *Hypericum perforatum* (9,76±0,13 mmol/L) and for the WF – for *Melissa officinalis* (6.3±0.11 mmol/L), *Fragaria vesca* (5.63±0.22 mmol/L) and *Origanum vulgare* (5.6±0.31 mmol/L).

With the aim of establishing a quantitative relationship between the AOA and the content of phenolic compounds correlation study was carried out (Fig. 3). A high positive correlation ($r = 0.87$) was established for the EAF; ($r = 0.83$) for BF and for the CME ($r = 0.71$). The other fractions did not exhibit this quantitative relationship – the r values or the PEF CF and WF were $r = 0.19$, $r = -0.13$ and $r = 0.21$, respectively. These results indicate that the phenolic compounds were extracted

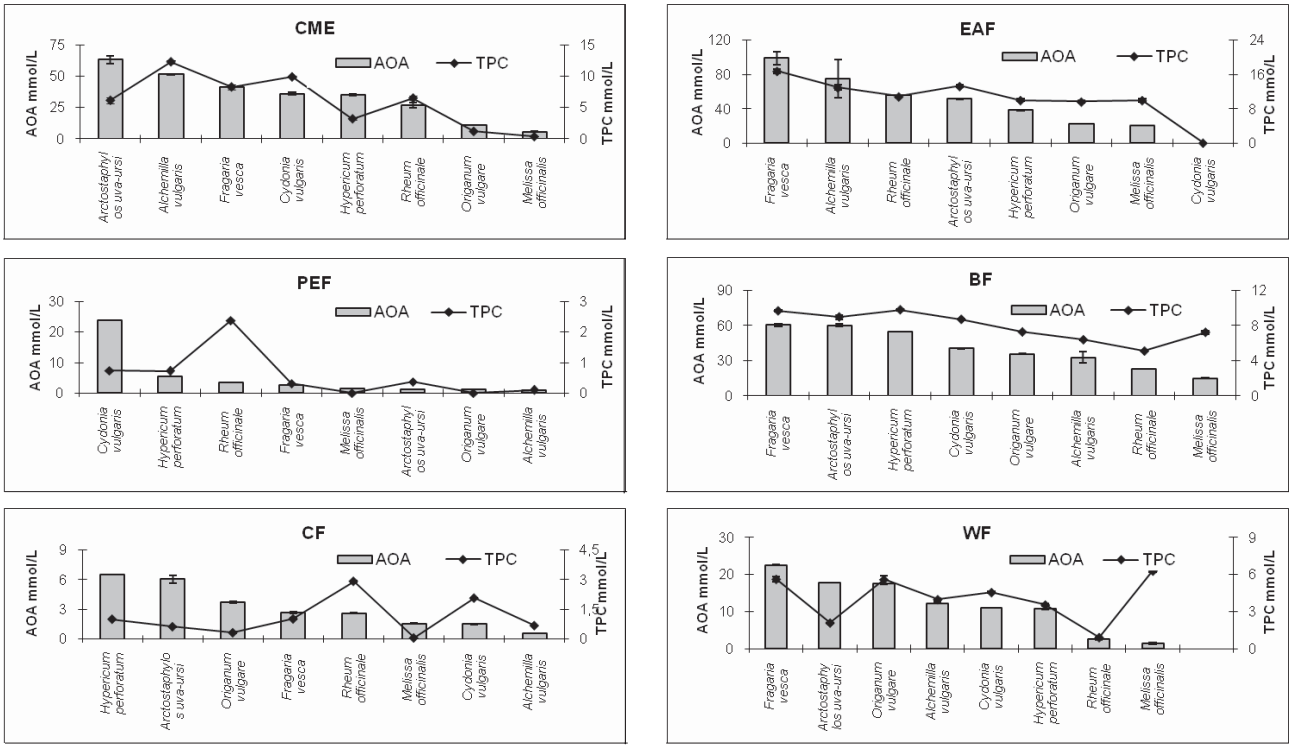

Figure 2 Antioxidants activity and total phenolic content of extracts/fractions of selected Bulgarian medicinal plants
Obrázok 2 Antioxidačná aktivita a celkový obsah fenolov v extraktoch/frakciách vybraných bulharských liečivých rastlín

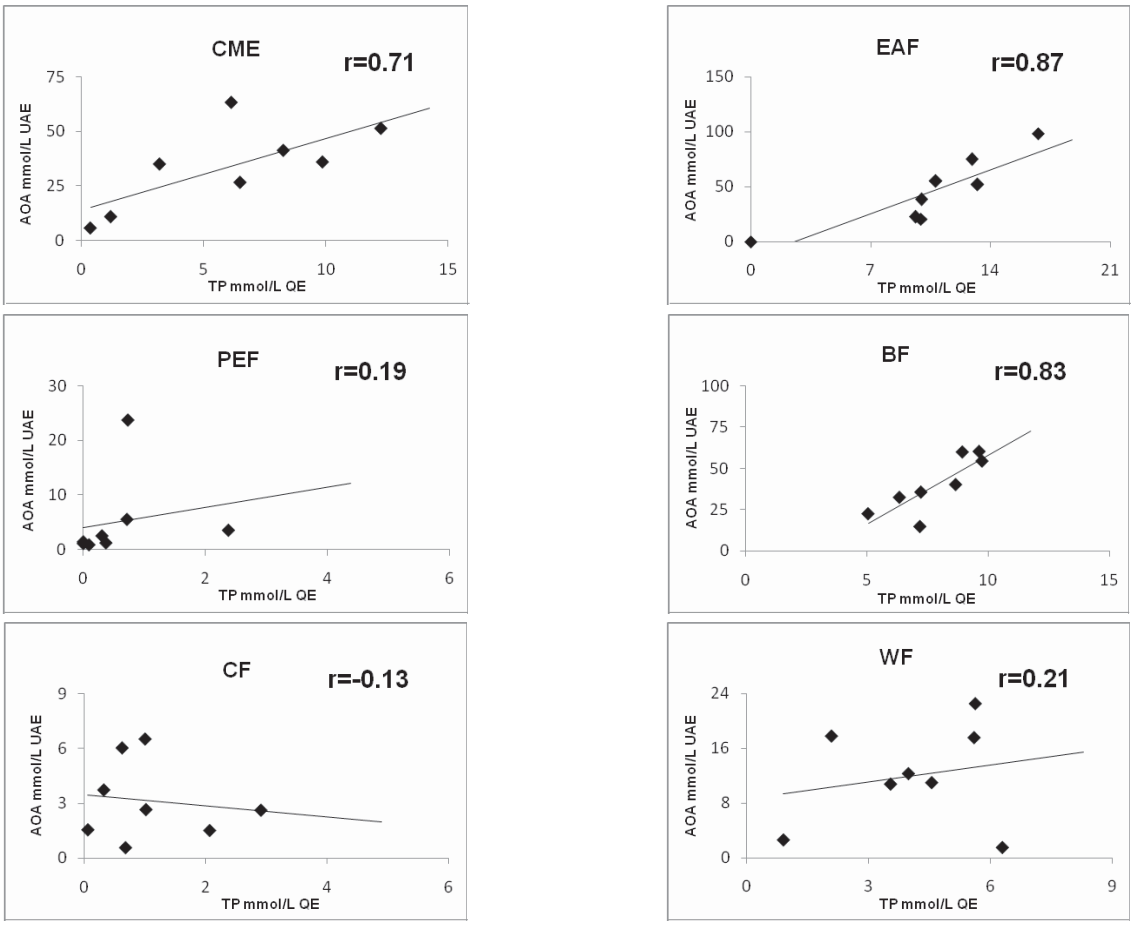

Figure 3 Correlation between antioxidant activity and total phenolic content in extracts/fractions
Obrázok 3 Korelácia medzi antioxidačnou aktivitou a celkovým obsahom fenolov v extraktoch/frakciách

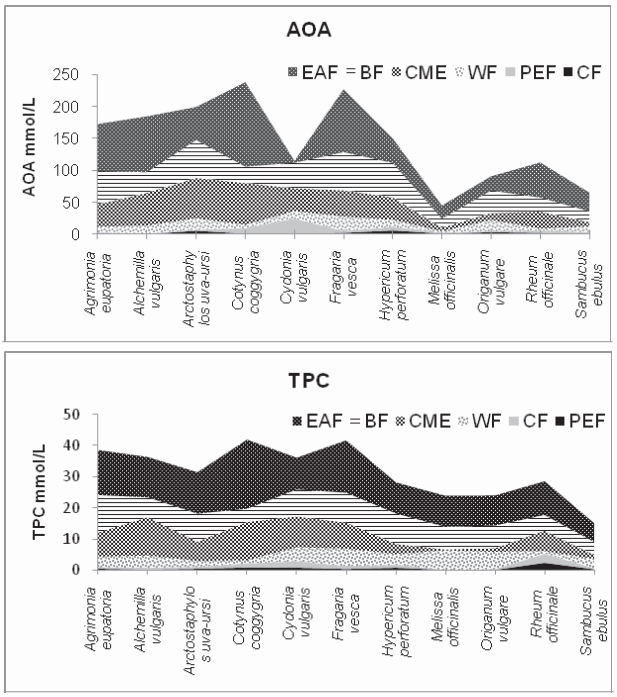

Figure 4 Quantitative comparison between antioxidant activity and total phenolic content in extracts/fractions
Obrázok 4 Kvantitatívne porovnanie antioxidačnej aktivity a obsahu celkových fenolov v extraktoch/frakciách

predominantly into the CME and in the EAF and BF fractions. The absence of significant correlation indicates that the low but still present AOA measured in PEF, CF and WF could be contributed to other extracted compounds different from phenolics. A correlation between AOA and TPC has been reported for methanolic extracts from different plants (Jeetendra et al., 2011; Alali et al., 2007).

AOA analyses of plant fractions indicated that *Alchemilla vulgaris, Fragaria vesca, Rheum oficinale* and *Melissa officinalis* exhibited highest AOA of their EAF; *Cydonia vulgaris, Hypericum perforatum* and *Origanum vulgare* – of their BF. Only *Arctostaphyllos uva-ursi* had most active CME against the pre-formed ABTS radical.

Highest TPC was measured for all species in the EAF followed by the BF for *Arctostaphyllos uva-ursi, Fragaria vesca, Hypericum perforatum, Melissa officinalis* and *Origanum vulgare* and further by CME for *Alchemilla vulgaris, Cotinus coggygria, Cydonia vulgaris* and *Rheum officinale*.

Comparison between the extract/fractions indicated that EAF had generally highest AOA and concentration of total polyphenols followed by the BF and CME, while the WF, PEF and CF exhibited low AOA and total phenolics concentration (fig. 4).

Conclusions

This investigation identified the presence of compounds possessing antioxidant activity in three fractions: in EAF of F. vesca, R. oficinale, and M. officinalis, in BF of *C. vulgaris, H. perforatum* and *O. vulgare* and in CME of *A. uva-ursi.*

The TPC of EAF was highest as compared to the other fractions for all species followed by the BF for *A. uva-ursi, F. vesca, H. perforatum, M. officinalis* and *O. vulgare* and by CME for *A. vulgaris, C. vulgaris* and *R. officinale*. AOA correlated positively to TPC of CME, BF and EAF, all of them showing a potential value as a source of natural antioxidants. These results represent a good basis for further analyses of selected plants to the discovery of new natural food additives.

Súhrn

Antioxidačná aktivita (AOA) hrubého metanolového extraktu (CME) a jeho etyl acetátu (EAF), butanolu (BF), benzénu (PEF), chloroformu (CF) a vodnej frakcie (WF) deviatich liečivých rastlín Bulharska bola analyzovaná použitím ABTS katiónovej radikálovej dekolorizačnej analýzy. Prítomnosť zlúčenín s antioxidačnou aktivitou bola identifikovaná v troch frakciách: v EAF pri *Fragaria vesca, Rheum oficinale* a *Melissa officinalis,* v BF frakcii pri *Cydonia vulgaris, Hypericum perforatum* a *Origanum vulgare* a v CME frakcii pri *Arctostaphylos uva-ursi.* Pre každý extrakt/frakciu bol zisťovaný celkový obsah fenolov (TPC). Najvyšší obsah TPC, v porovnaní s ostatnými frakciami, obsahovala frakcia EAF, potom nasledovala frakcia BF pri *Arctostaphylos uva-ursi, Fragaria vesca, Hypericum perforatum, Melissa officinalis* a *Origanum vulgare* potom frakcia CME pri *Alchemilla vulgaris, Cydonia vulgaris* a *Rheum officinale*. AOA mala pozitívnu koreláciu s TPC pri CME, BF a EAF. Všetky uvedené frakcie majú potenciálnu hodnotu ako zdroje prírodných antioxidantov.

Kľúčové slová: liečivé rastliny Bulharska, antioxidačná aktivita, celkový obsah polyfenolov, frakcie

Acknowledgements

We acknowledge the general support of Bulgarian Ministry of Education, Youth and Science, Grant #VU-L-320/07.

References

ALALI, F. – TAWAHA, K. – EL-ELIMAT, T. – SYOUF, M. – EL-FAYAD, M. – ABULAILA, K. – NIELSEN, S. – WHEATON, W. – FALKINHAM, J. – OBERLIES, N. 2007. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. In: Natural Products Research, vol. 21, 2007, no. 12, p. 1121 – 1131.

CAI, Y. – LUO, Q. – SUN, M. – CORKE, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. In: Life Sciences, vol. 74, 2004, no. 17, p. 2157 – 2184.

CHOI, C. – KIM, S. – HWANG, S. – CHOI, B. – HYE, A. – LEE, M. – PARK, S. – KIM, S. 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. In: Plant Science, vol. 163, 2002, no. 6, p. 1161 – 1168.

DJERIDANE, P. – VIDAL, N. 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. In: Food Chemistry, vol. 97, 2006, no. 4, p. 654 – 660.

HIGDON, J. – FREI, B. 2003. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. In: Critical Reviews in Food Science and Nutrition, vol. 43, 2006, p. 89–143.

IVANOVA, D. – KISELOVA, Y. – IVANOVA, D. 2007. Cetraria islandica as a natural source of antioxidants. „Ovidius" University Annals of Medical Science – Pharmacy, vol. 5, 2007, no. 1, p. 80 – 83.

IVANOVA, D. – GEROVA, D. – CHERVENKOV, T. – YANKOVA, T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. In: Journal of Ethnopharmacology, vol. 96, 2005, p. 145 – 150.

IVANOVA, D. – KISELOVA, Y. – IVANOV, D. 2009. Ŕntioxidative potential of medicinal herbs from the northern wet black sea coastal zones. In: Scripta Scientifica Medica, vol. 41, 2009, no. 1, p. 7 – 10.

JEETENDRA, N. – MANISH, B. 2011. International Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, 2011, no. 2, p. 48 – 52.

KÄHKÖNEN, M. – HOPIA, A. – VUORELA, H. – RAUHA, J. – PIHLAJA, K. – KUJALA, T. – HEINONEN, M. 1999. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds, vol. 47, 1999, no. 10, p. 3954 – 3962.

KISELOVA, Y. – GALUNSKA, B. – IVANOVA, D. – YANKOVA, T. 2004. Total antioxidant capacity and polyphenol content correlation in aqueous-alcoholic plant extracts used in phytotherapy. In: Scripta Scientifica Medica, vol. 36, 2004, p. 11 – 13.

KISELOVA, Y. – IVANOVA, D. – CHERVENKOV, T. – GEROVA, D. – GALUNSKA, B. – YANKOVA, T. 2006. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. In: Phytotherapy Research, vol. 20, 2006, no. 11, p. 961 – 965.

NIJVELDT, R. – VAN NOOD, E. – VAN HOORN, D. – BOELENS, P. – VAN NORREN, K. – VAN LEEUWEN, P. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. In: American Journal of Clinical Nutrition, vol. 74, 2001, p. 418 – 425.

POURMORAD, F. – HOSSEINIMEHR, S. – SHAHABIMAJD, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. In: African Journal of Biotechnology, vol. 5, 2006, no. 11, p. 1142 – 1145.

RE, R. – PELLEGRINI, N. – PROTEGGENTE, A. – PANNALA, A. – YANG, M. – RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. In: Free Radical Biology & Medicine, 1999, no. 26, p. 1231 – 1237.

SCALBERT, A. – JOHNSON, I. – SALTMARSH, M. 2005. Polyphenols: antioxidants and beyond. In: American Journal of Clinical Nutrition, vol. 81, 2005, suppl., p. 215S – 217S.

SINGLETON, V. – ROSSI, J. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. In: American Journal of Enology and Viticulture, vol. 16, 1965, p. 144 – 158.

WONG, C. – LI, H. – CHENG, K. – CHEN, F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. In: Food Chemistry, vol. 97, 2006, no. 4, p. 705 – 711.

Contact address:
Yoana Kiselova, Laboratory of Nutrigenomics, Functional Foods and Nutraceuticals, Department of Biochemistry, Molecular Medicine and Nutrigenomics, Medical University – Varna, 55 Marin Drinov Str., 9002 Varna, Bulgaria, e-mail: ykisselova@abv.bg