Bulgarian Chemical Communications, Volume 50, Special Issue C (pp. 90 – 97) 2018

Flavonoid content and antioxidant activity of *Betonica bulgarica* Degen et Neič

M. T. Tzanova[1*], N. H. Grozeva[1], M. A. Gerdzhikova[1], M. D. Argirova[2], D. H. Pavlov[1] and S. R. Terzieva[1]

[1]*Faculty of Agriculture, Trakia University, Studentski grad Str., 6000 Stara Zagora, Bulgaria*
[2]*Faculty of Pharmacy, Medical University of Plovdiv, 15A Vassil Aprilov Str.4002 Plovdiv, Bulgaria*

Received September 4, 2017; Revised November 2, 2017

The Bulgarian endemic *Betonica bulgarica* Degen et Neič (syn. *Stachys bulgarica* Hayek) is a protected plant by the Biological Diversity Act and it is included in the Red Data Book of Bulgaria under the category "endangered". The aim of this study was to determine the flavonoid content and antioxidant activity of different plant organs of this species (leaves, flowers, roots, stems and seeds), from four populations. Three flavonoids were found in significant amounts: rutin, quercetin and hispidulin. Rutin was in the largest quantity, followed by quercetin and hispidulin. The largest total flavonoid content was measured in leaves, followed by roots and flowers. The antioxidant activity of methanol extracts was tested by DPPH-method. The total polyphenol was also assayed. The correlation between flavonoid content and antioxidant activity of the studied plant organs was established.

Key words: Flavonoids; Polyphenols; Antioxidant activity; *Betonica bulgarica*

INTRODUCTION

The Bulgarian endemic *Betonica bulgarica* Degen et Neič (syn. *Stachys bulgarica* Hayek) is a protected plant by the Biological Diversity Act (2002) [1], and it is included in the Red Data Book of Bulgaria under the category "endangered" [2]. *Betonica* and *Stachys* species are widely used in folk medicine as anti-inflammatory [3, 4], antibacterial [5, 6], anti-cancer [7, 8] and antioxidant agents [9-11]. Recently, they were officially applied in homeopathic medicine [12, 13]. Previous studies of *B. officinalis* showed presence of bioactive compounds with proven antioxidant activity like phenolic compounds, flavonoids and essential oils [14-20]. Nevertheless, the literature data about antioxidant activities of Bulgarian endemic species are missing and little is known about chemical components with antioxidant activity, like flavonoids and polyphenols. The quantification of three major flavonoids: rutin (RU), quercetin (QU) and hispidulin (HI) and total polyphenols, their distribution in different plant parts (leaves, flowers, roots, stems and seeds) of these endemic species from four populations was the aim of this work, in order to study the natural variability of *B. bulgarica*. The relation of this content to the antioxidant activity was also investigated.

* To whom all correspondence should be sent:
E-mail: rmilenatzanova@hotmail.com

MATERIALS AND METHODS

Plant material and extract preparation

Aerial parts of *Betonica bulgarica* were harvested from July to September 2016 in four locations from naturally growing populations in Bulgaria (Table 1). The roots were collected at the end of the vegetative period. The voucher specimens from the studied populations are kept in the herbarium of the Agricultural university in Plovdiv (SOA). Plant material was airdried in shade at room temperature and ground in a mechanical grinder (final powder size less than 400 μm). The samples were stored in dark and cool rooms at 16 – 18 ° C prior to the analysis.

The target compounds were extracted by ultrasonication of 1 g of powdered plant material in 10 ml of methanol for 30 min at 40 ° C in triplicate. Ultrasonic extraction is convenient and straightforward and was selected because of the high rate of extraction of flavonoids and polar bioactive compounds [21].

Flavonoids determination

The flavonoids levels in methanolic extracts were determined by HPLC analysis developed and validated by Ashokkumar *et al*. [22]. The extract of each sample was filtered through a 0.45 μm membrane and the volume was adjusted to 25 ml with methanol. The solutions were stored overnight at -12 ° C prior to the HPLC analysis. A small quantity of each extract was transferred into a screw-capped vial and placed in the HPLC system autosampler.

Table 1. Basic characteristics of the populations from where the plant materials of *Betonica bulgarica* were collected

Population No	Location, voucher number	North	East	Elev. m a.s.l.	Ecological conditions
1	Balkan Foothill Region, Lovnidol village, Pashova Livada area (SOA 062252)	42°59.079’	25°15.846’	368	Soil type – Cambisols (WRBSR, 2006). Herbaceous community dominated by *Festuca pratensis.* The terrain is slightly sloped (4 ° – 5 °), non-eroded, facing south-west.
2	Balkan Foothill Region, Lovnidol village, Above Avdjiiski trap area (SOA 062253)	43°01.327’	25°15.154’	503	Soil type – Cambisols (WRBSR, 2006). Herbaceous community dominated by *Trifolium pratense* L. The terrain is very slightly sloped (2 ° – 3 °), non-eroded, facing north-east.
3	Eastern Stara planina (the Balkan), Sinite kamani Natural; park, Karandilska poliana area (SOA 062254)	42°71.688’	26°36.872’	972	Open meadow of the clifs northwest. Herbaceous community dominated by *Betonica bulgarica.* The terrain is slightly sloped (4 ° – 5 °), non-eroded, facing north-east.
4	Eastern Stara planina (the Balkan), Sinite kamani Natural; park, Ablanovo area (SOA 062255)	42°42.638’	26°17.262’	540	Soil type – Chromic Luvisols (WRBSR, 2006); Open meadow on the edge of a mixed deciduous forest comprising *Carpinus betulus L., Quercus robur L., Ulmus minor Mill., Fraxinus ornus L. and Crataegus monogyna Jacq.* The herbaceous community is dominated by *B. bulgarica*. The terrain is very slightly sloped (3 ° – 4 °), non-eroded, facing south-east.

Analytical HPLC was performed with a C18 column Hypersil Gold (5 μm; 150 mm × 4.6 mm) on a Thermo system composed of a Surveyor LC Pump Plus, Surveyor Autosampler Plus, and Surveyor photodiode array detector PDA Plus. Quantitative analysis was performed in a 6-min run, isocratic mode, with methanol/acetonitrile/ water/acetic acid (40+20+39+1, v/v/v/v) at a flow rate of 0.8 ml.min^{-1}. The flavonoids were simultaneously identified using UV absorbance at 350 nm for hispidulin (HI), and 254 nm for rutin (RU) and quercetin (QU). The external calibration was carried out using five concentration levels (0.05, 0.5, 1.0, 2.0 and 5.0 mg.l^{-1}) of reference materials - rutin hydrate (min 94 %, HPLC), quercetin (min 98 %, HPLC) and hispidulin (min 98 %, HPLC), purchased from Sigma-Aldrich (St. Louis, MO). Each calibration standard was run in triplicate. The squared correlation coefficients (r^2) obtained by linear regression (0.9990 for RU, 1.000 for QU and 0.9995 for HI) demonstrated an excellent relationship between peak area and concentration according to the International Conference on Harmonization (ICH) guidelines [23]. Figure 1 illustrates a typical chromatogram of a standard solution containing 1 mg.l^{-1} rutin, quercetin and hispidulin. The retention times were ca 2.2 min for RU, ca 3.3 min for QU, and ca 4.9 min for HI.

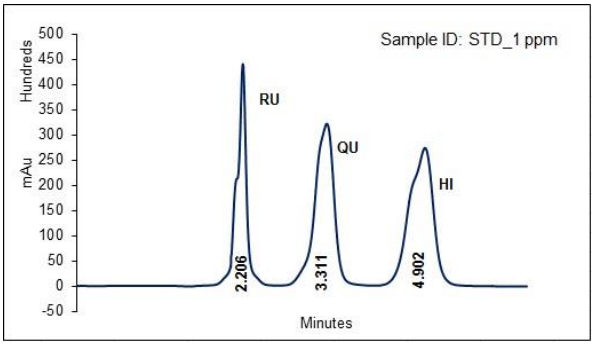

Figure 1. Typical chromatogram of a standard solution containing 1 mg.l^{-1} rutin, quercetin and hispidulin

Polyphenol determination

The collected methanol extracts were concentrated to a final volume of ca 7 ml by a rotary evaporator under vacuum at 30 ° C and transferred to a 10-ml volumetric flask. The dry matter of these methanol extracts was determined gravimetrically by drying 1 ml of each extract at 120 ° C for 6 hours. The experimental procedure described by Anesini *et al.* [24] was applied for determination of total polyphenol content (TPC). Briefly, 1 ml of the methanol plant extract with concentration of 0.2

mg.ml^{-1} or 1 ml of standard solution were mixed in separate tubes with 5.0 ml of Folin-Ciocalteu's reagent (1/10 dilution with water of the commercial reagent). Then, 4 ml of Na_2CO_3 in water (7.5 % w/v) was added and the tubes were left at room temperature for one hour. The absorbance at 765 nm was measured against water. Each sample was analyzed in triplicate. Gallic acid (Sigma-Aldrich, St. Louis, MO) solutions in methanol ranging from 0.1 to 10 μg.ml^{-1} were used for the calibration curve (R^2 = 0.998). TPC of each sample was expressed as mmol GAE in 1 kg dm of starting plant material.

Determination of radical scavenging activity by DPPH method

1,1'-diphenyl-2-picrylhydrazyl-radical (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO). This substance has a single electron on the nitrogen atom and its solution in methanol has an absorption maximum at λ = 517 nm. The mechanism of the DPPH-method is based on the reaction between the test compound and DPPH-radical, wherein the potential free radical scavengers reduce the DPPH-radical (violet solution) to a yellow colored 1,1'-diphenyl-2-(2,4,6-trinitrorhenyl) hydrazine by donating a hydrogen atom.

The method described by Serpen *et al.* [25] was applied to measure the radical-scavenging potential of methanolic extracts obtained from different plant parts of *B. bulgarica*. Briefly, to 2 ml of a 60 μM solution of DPPH in methanol was added 20 μl of methanol extract (1 mg.ml^{-1}). Two parallel samples of each extract were analyzed. Absorption at 517 nm was measured 30 min later. Since the composition of the extracts is complex, the results for their radical-binding capacity were compared with that of Trolox (water-soluble analogue of Vitamin E) and calculated by regression analysis from the linear dependence between concentration of Trolox and absorption at 517 nm. The results were expressed as μmol Trolox equivalent in 1 kg dm of plant material.

Statistical data analysis

All analytical assays were carried out in duplicate or triplicate as specified above and the data are mean values ± standard deviation (SD). The Pearson correlation coefficients were determined using SPSS Statistics for Windows, Version 17.0 SPSS Inc. 2008, Chicago.

RESULTS AND DISCUSSION

A number of research teams, reviewed by Tundis *et al.* [26], have studied the polyphenol incl. flavonoid profile of *Stachys* species in relation to chemotaxonomy. Looking for new structures, they forgot the main and well-known ones, like rutin, quercetin, and hispidulin, which are widely spread in the plant world, including species from the genus *Stachys* [27]. These antioxidants were found in the object of the present study, Bulgarian endemic *B. bulgarica*. The identification and quantification of RU, QU and HI were carried out by HPLC-PDA method, described in the section Materials and Methods, by comparison with external standards of reference materials.

Five different plant parts from four populations of *B. bulgarica* were analyzed. The flavonoid content differences between organs in the populations were significant due to the specific growth conditions. In all of them, the rutin was in the largest quantity. The second one was quercetin except in the leaves, were the amount of hispidulin was higher than that of quercetin (Table 2). The obtained results clearly show significant differences in the flavonoid content between organs and locations.

The largest total content of RU, QU and HI in the tested plant was found in the leaves of *B. bulgarica* from two populations: Pashova livada and Above Avdjiiski trap, followed by seeds and roots, whose content was 22% and 15%, respectively, less than that in the leaves. The flavonoid content in the leaves of the other two populations (Karandilska poliana and Ablanovo) was 20% and 25%, respectively, and was lower than that measured in leaves from the first two populations. The highest flavonoid content was found in the endemic species from the population Above Avdjiiski trap, followed by Pashova Livada, Karandilska poliana and Ablanovo. Hajdari *et al.* [18] established the total flavonoid content in leaves and roots of *Betonica officinalis* L. from Kosovo. They found no significant total flavonoids differences between the localities for both organs and an about 3-fold higher content of flavonoids and polyphenols in leaves than in roots.

Table 2. Flavonoid content in different plant parts of *Betonica bulgarica* Degen et Neič from four studied populations (n=3 for organs in populations; n=20 for organs average)

Population No	Organ	Content, mean ± SD*, mg.kg^{-1} dm		
		Rutin	Quercetin	Hispidulin
1 Pashova Livada	Leaves	3789.3 ± 274.8	94.5 ± 7.6	202.5 ±14.1
	Flowers	1165.8 ± 90.4	431.0 ± 32.7	15.5 ±1.2
	Seeds	2638.4 ± 164.6	261.7 ± 19.8	255.7 ± 17.4
	Stems	209.9 ± 18.9	291.9 ± 23.6	143.9 ± 9.5
	Roots	3478.6 ± 306.4	36.2 ± 3.6	8.9 ± 0.7
2 Above Avdjiiski trap	Leaves	4941.7 ± 345.1	279.1 ± 21.2	412.7 ± 30.8
	Flowers	2324.2 ± 139.4	376.2 ± 26.9	42.9 ± 3.9
	Seeds	3702.6 ± 201.5	291.6 ± 19.5	304.0 ± 19.6
	Stems	277.6 ± 20.7	334.9 ± 24.3	158.4 ± 9.7
	Roots	4576.1 ± 364.3	104.7 ± 8.1	21.5 ± 1.7
3 Karandilska poliana	Leaves	1842.1 ± 128.3	174.3 ± 13.2	428.5 ± 34.9
	Flowers	981.9 ± 78.5	472.9 ± 32.4	22.4 ± 2.1
	Seeds	1322.4 ± 95.3	237.6 ± 14.8	408.8 ± 20.6
	Stems	123.0 ± 10.2	133.9 ± 9.3	104.6 ± 4.5
	Roots	1693.5 ± 98.8	67.3 ± 5.1	28.9 ± 1.8
4 Ablanovo	Leaves	1602.9 ± 109.4	103.2 ± 8.1	355.8 ± 24.0
	Flowers	713.6 ±59.2	328.8 ±23.4	14.1 ± 0.9
	Seeds	1168.3 ± 84.3	154.3 ± 11.3	319.5 ± 19.2
	Stems	104.6 ± 8.4	198.0 ± 15.5	112.1 ± 4.7
	Roots	1492.8 ± 119.7	40.2 ± 3.0	7.9 ± 0.7

*SD- Standard Deviation

Table 3. Factor influence on distribution of *Betonica bulgarica* Degen et Neič populations

Population	Factor 1	Factor 2	Factor 3
1 Pashova Livada	-0.03692	0.387644	-0.02283
2 Above Avdjiiski trap	-2.36332	-0.10301	0.016024
3 Karandilska poljana	0.82401	-0.29183	-0.03496
4 Ablanovo	1.57623	0.007197	0.041765

Principle component analyses for distributions of *B. bulgarica* population depending on the content of rutin, quercetin and hispidulin by plant parts demonstrate that three main factors can be defined (Figure 2). Eigen value for these factors was F1- 2.917; F2 - 0.082: F3 - 0.001. Factor 1 has the greatest influence describing 97.22 % of the variations, while Factor 2 describes 2.73 % and Factor 3 – 0.042%. Distribution of the population shows that *B. bulgarica* originated from Karandilska poliana is positive for F1 describing 97.22 % of the variations, but is negative by F 2 (Table 3). Population from Ablanovo is positive by the two factors, while population above Avdjiiski trap is negative by the two factors (Figure 2).

The methanolic extracts of *B. bulgarica* were also tested for total phenolic content (TPC) and radical scavenging activity by the DPPH method. The results obtained are shown in Table 4. The GAE-equivalents between organs and localities were significant different. The antioxidative activity of root extracts (Trolox equivalents) did not show any significant difference between the localities.

The highest total phenolic content was found in leaves, followed by flowers, seeds, roots and stems. This distribution was valid for all populations. The largest TPC was found in the species of *B. bulgarica* from the population Above Avdjiiski trap, followed by Pashova livada, Karandilska

poliana and Ablanovo, the same population ranking as for flavonoid content.

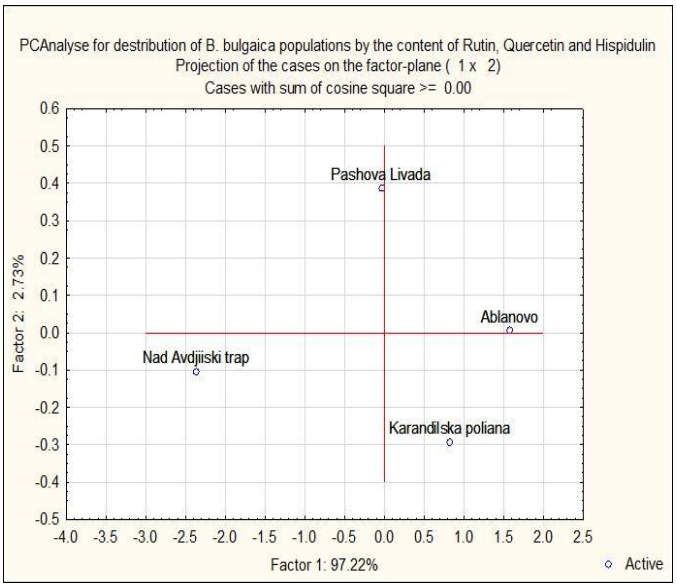

Figure 2. Component analysis for distribution of the four studied populations of *Betonica bulgarica* Degen et Neič

The radical scavenging activity of the *B. bulgarica* from the population Above Avdjiiski trap was the highest one, followed by Pashova livada, Karandilska polyana and Ablanovo. The most potent radical scavenging capacity had the methanolic extracts obtained from leaves followed by seeds, flowers, roots and stems. Within one and the same population the results for antioxidant potential of the seeds, flowers, and roots were similar.

Table 4. TPC and antioxidant activity in different organs of *Betonica bulgarica* Degen et Neič from four populations

Population	Organ	mmol GAE eq. kg^{-1} dm	μmol Trolox eq. kg^{-1} dm
1	Leaves	80.64 ± 5.23	52.73 ± 2.80
Pashova	Flowers	71.02 ±4.12	37.28 ± 1.53
Livada	Seeds	62.92 ± 4.25	43.41 ± 2.09
	Stems	28.21 ± 1.91	8.16 ± 0.35
	Roots	59.13 ± 3.55	38.49 ± 1.97
2	Leaves	122.43 ± 6.77	94.19 ± 5.92
Above	Flowers	91.32 ± 5.49	72.45 ± 3.61
Avdjiiski	Seeds	83.03 ± 3.74	60.64 ±3.07
trap	Stems	32.22 ± 1.87	20.76 ± 1.05
	Roots	72.69 ± 3.58	51.44 ± 2.49
3	Leaves	91.83 ± 4.33	63.25 ± 3.06
Karandilska	Flowers	87.02 ± 3.89	52.29 ± 2.21
poliana	Seeds	72.17 ± 4.06	60.64 ± 2.97
	Stems	25.06 ± 1.66	9.16 ± 0.44
	Roots	79.91 ± 4.01	50.26 ± 2.69
4	Leaves	90.84 ± 4.63	52.26 ± 2.76
Ablanovo	Flowers	68.64 ± 3.47	55.71 ± 3.11
	Seeds	69.39 ± 3.75	39.76 ± 2.18
	Stems	26.87 ± 1.37	12.66 ± 0.76
	Roots	44.81 ± 2.22	26.12 ± 1.83

All indicators were normally distributed by the One-Sample Kolmogorov-Smirnov test. The methanolic extracts of *B. bulgarica* showed a similar TPC and DPPH radical scavenging capacity compared with other species of the *Lamiaceae*, such as the leaves of *Stachys sylvatica* and leaves of *Betonica officinalis* [18, 28].

Consistent with most polyphenolic antioxidants, both the configuration and the total number of hydroxyl groups in flavonoids structure substantially influence their antioxidant activity. Free radical scavenging capacity is primarily attributed to the high reactivity of phenol group that participates in the following reaction:

$$\text{Flav-OH} + \text{R}^{\cdot} \longrightarrow \text{Flav-O}^{\cdot} + \text{RH}$$

Single electron delocalization makes this reaction thermodynamically favorable and the free radical formed may further react with a second radical; a reaction that turns the phenolic group into a stable quinone structure.

Correlation between TPC and antioxidant activity, tested by the DPPH method, of *Stachys* species was found by a number of research teams [17, 18, 26]. Several possible mechanisms of the demonstrated antioxidant properties of flavonoids have been proposed [29]; among them are direct scavenging of reactive oxygen species and metal chelating properties. In our *in vitro* study a good Pearson correlation between radical scavenging activity and TPC at significance level $p \leq 0.01$ was found (Figure 3) underlying the importance of polyphenol moiety in flavonoid structure for radical scavenging potency.

Comparison between mmol gallic acid equivalents and μmol Trolox equivalents in kg dry plant material, and concentration of the three quantified flavonoids in different plant parts on the other hand also showed good correlations (Figure 4, panel A and panel B). The Pearson correlation was established and showed positive dependence.

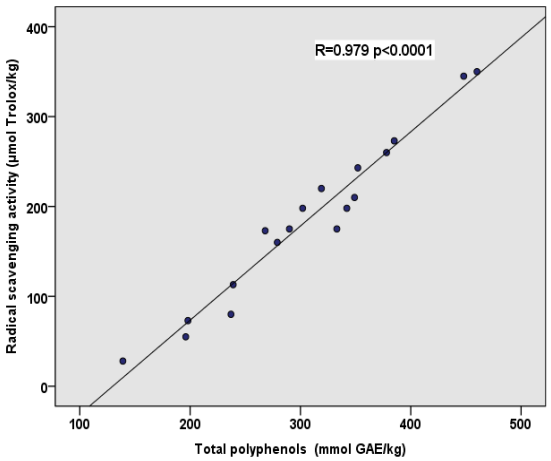

Figure 3. Pearson correlation between TPC and antioxidant activity by significant level, $P \leq 0.01$ (2-tailed)

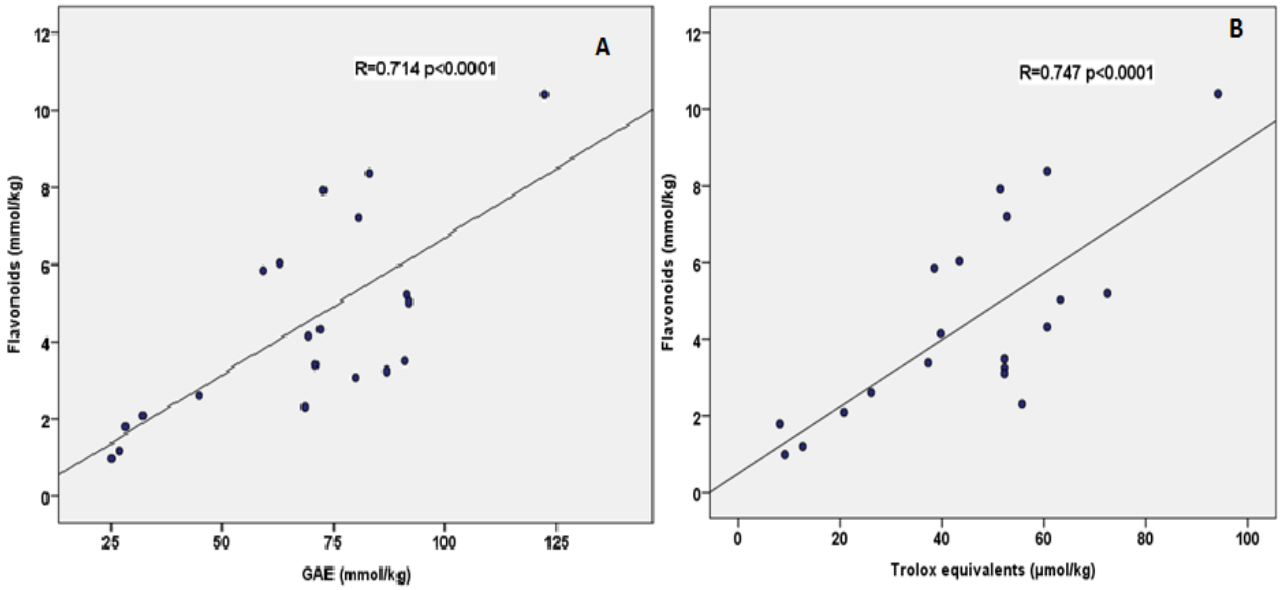

Figure 4. Pearson correlation between flavonoid content and TPC (panel A) and flavonoid content and antioxidant activity (panel B), $P \leq 0.01$ (2-tailed)

The correlation coefficients were lower: 0.714 and 0.746, respectively. Most likely the phenolic compounds including flavonoids present in the leaves are responsible for the high antioxidative capacity of these parts of plants. In roots and seeds, antioxidant capacities assayed by the two methods were not so well correlated with flavonoid content indicating that different substances aside from flavonoids might be responsible for the specific antioxidant effects.

Based on the results obtained, the main conclusions that can be drawn are:

- The Bulgarian endemic *B. bulgarica* contains three major flavonoids: rutin, quercetin and hispidulin, in good quantities;
- *B. bulgarica* has lower antioxidant activity than the other studied *Stachys* species [11, 17, 18, 30];
- The correlation between flavonoid content and TPC and flavonoid content and antioxidant activity is very high.
- The highest flavonoid and polyphenol concentrations were found in the leaves;

Acknowledgements: *This work is part of the Project No 3AF16 "Determination of the chemical composition (organic, mineral, biological active substances), and the nutritional value of cultivated and wild plants", financially supported by the Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria*.

REFERENCES

1. Biological diversity act, Republic of Bulgaria, National Assembly, Promulgated, State Gazette No 77/9.08.2002.
2. E. Genova, Betonica bulgarica Deg. et Neic. in: Plants & Fungi, Red Data Book of the Republic of Bulgaria, Peev D (eds), vol. 1, 2011.
3. M. Sharifzadeh, K. Sharifzadeh, M. Khanavi, A. Hadjiakhoondi, A. Shafiee, *Int. J. Pharmacol.*, **1**, 132 (2005).
4. J. Kukik, S. Dobric, S. Petrovic S., *Pharm. Biol.*, **45**, 560 (2007).
5. S. Grujic-Jovanovic, H. D. Skaltsa, P. Marin, M. Sokovic, *Flavour Frag. J.*, **19,** 139 (2004).
6. A. Sonboli, P. Salehi, S. N. Ebrahimi, *Chem. Nat. Comp.*, **41**,171 (2005).
7. Z. Amirghofran, M. Bahmani, A. Azadmehr, K. Javidnia, *Neoplasma*, **53,** 428 (2006).
8. Z. Amirghofran, M. Bahmani, A. Azadmehr, K. Javidnia, *Med. Sci. Monit.* **13**, 145 (2007).
9. A. Aydin, B. Sener, I. Cakici, N. N. Turan, N. Erdemoglu, *Phytother. Res.*, **20**, 91 (2006).
10. 10. J. Kukik, S. Petrovic, M. Niketic,*Biol. Pharm. Bull.*,**29**, 725 (2006).
11. A. Matkowski, M. Piotrowska, *Fitoterapia*, **77**, 346 (2006).
12. M. Wiesenauer, Homöopathie für Apotheker und Ärzte, Deutscher Apotheker Verlag, Stuttgart, 2000, p. 184.
13. W. Blaschek, S. Ebel, E. Hackenthal, U. Holzgrabe, K. Keller, J. Reichling, V. Schulz, Hagers Enzyklopädie der Arzneistoffe und Drogen, Wissenschaftliche Verlagsgesellschaft GmbH, Stuttgart, 2007, p. 829
14. V. Bankova, J. Koeva-Todorovska, T. Stambolijska, M. D. Ignatova-Groceva, D. Todorova, S. Popov, *Z. Naturforsch.*, **54c**, 876 (1999).
15. I. Dimitrova-Dyulgerova, P. Merdzhanov, K. Todorov, D. Seymenska, P. Stoyanov, R. Mladenov, A. Stoyanova, *Comptes rendus de l'Academie bulgare des Sciences*, **68(8)**, 991 (2015).
16. V. Bilušić-Vundać, A. H. Brantner, M. Plazibat, *Food Chem.*, **104** 1277 (2007).
17. M. Khanavi, M. Hajimahmoodi, M. Cheraghi-Niroomand, Z. Kargar, Y. Ajani, A. Hadjiakhoondi, M. R. Oveisi, *African J. Biotech.*, **8(6)**, 1143 (2009).
18. A. Hajdari, B. Mustafa, Ch. Franz, J. Novak, Total Flavonoids, *Acta Horticult.*, **860**, 75 (2010).
19. I. Sliumpaitea, R. P. Venskutonisa, M. Murkovicb, O. Ragazinskiene, *Ind. Crops and Products*, **50**, 715 (2013).
20. K. Bączek, O. Kosakowska, J. L. Przybył, Z. Węglarz, *Herba Polonica*, **62(2)**, 7 (2016).
21. H. Hajimehdipoor, B. M. Kondori, G. R. Amin, N. Adib, H. Rastegar, M. Shekarchi, *J. Pharm. Sci.*, **20**, 57 (2012).
22. K. Ashokkumar, K. Selvaraj, S. K. M. Devi, *J. Med. Plants Res.*, **7(47)**, 3393 (2013).
23. Validation of Analytical Procedures: Methodology. ICH, Q2B, FDA, Federal Register, 1997.
24. C. Anesini, G. E. Ferraro, R. Filip, *J. Agr. Food Chem.*, **56(19)**, 9225 (2008).
25. A. Serpen, E. Capuano, V. Fogliano, V. Gökmen, *J. Agr. Food Chem*., **55(19)**, 7676 (2007).
26. R. Tundis, L. Peruzzi, F. Menichini, *Phytochemistry*, **102**, 7 (2014).
27. V. Vundać, Z. Maleš, M. Plazibat, P. Golja, B. Cetina-Čižmek, *J. Planer Chromatography*, **18**, 104 (2007).
28. A. Hajdari, B. Mustafa, A. Kaçiku, X. Mala, B. Lukas, A. Ibraliu, G. Stefkov, J. Novak, Chemical Composition of the Essential Oil, Total Phenolics, *Rec. Nat. Prod.*, **10(6)**, 750 (2016).
29. D. Procházková, I. Boušová, N. Wilhelmová, *Fitoterapia*. **82(4)**: 513 (2011).
30. G. Miliauskas, P. R. Venskutonis, T. A. Van Beek, *Food Chem.*, **85**, 231 (2004).

СЪДЪРЖАНИЕ НА ФЛАВОНОИДИ И АНТИОКСИДАНТНА АКТИВНОСТ НА *BETONICA BULGARICA* DEGEN ET NEIČ

М. Т. Цанова[1*], Н.Х. Грозева[1], М.А. Герджикова[1], М.Д. Аргирова[2], Д.Х. Павлов[1], С.Р. Терзиева[1]

[1]*Агрономически факултет, Тракийски университет, ул. Студентски град, 6000 Стара Загора, България*
[2]*Фармацевтичен факултет, Пловдивски медицински университет, ул. В. Априлов 15А, 4002 Пловдив, България*

Постъпила на 4 септември, 2017 г.; коригирана на 2 ноември, 2017 г.

(Резюме)

Българският ендемит *Betonica bulgarica* Degen et Neič е защитен вид от Закона за биологичното разнообразие и е включен в Червената книга на България под категорията "застрашени". Целта на това изследване е да се определи съдържанието на флавоноиди, както и антиоксидантната активност на различни органи на растението (листа, цветове, корени, стебла и семена) от четири популации. В значителни количества са определени три флавоноида: рутин, кверцетин и хиспидулин. В най-голямо количество е рутинът, последван от кверцетина и хиспидулина. С най-високо флавоноидно съдържание се отличават листата, след което се нареждат корените и цветовете. Атиоксидантната активност е тествана чрез DPPH-метод. Определено е също така и общото полифенолно съдържание. Установена е положителна корелация между флавоноидното съдържание и антиоксидантната активност на изследваните органи на растението.