Food Science and Applied Biotechnology, 2020, **3**(2), 149-156

UFT Academic Publishing House, Plovdiv

Food Science and Applied Biotechnology
e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
https://doi.org/10.30721/fsab2020.v3.i2.98

OPEN ACCESS

Research Article

Optimization of polyphenols extraction process with antioxidant properties from wild *Vaccinium myrtillus* L. (bilberry) and *Vaccinium vitis-idaea* L. (lingonberry) leaves

Radka Vrancheva[1], Ivan Ivanov[2]✉, Ilian Badjakov[3], Ivayla Dincheva[3], Vasil Georgiev[4], Atanas Pavlov[1,4]

[1] *Department of Analytical Chemistry and Physical Chemistry, Technological Faculty, University of Food Technologies. Plovdiv. Bulgaria*
[2] *Department of Organic Chemistry and Inorganic Chemistry, Technological Faculty, University of Food Technologies. Plovdiv. Bulgaria*
[3] *AgroBioInstitute, Agricultural Academy, Sofia. Bulgaria*
[4] *Laboratory of Cell Biosystems, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences. Plovdiv. Bulgaria*

Abstract

The aim of the current study was to optimize the extraction condition of polyphenol compounds with antioxidant properties from leaves of natural grown *Vaccinium myrtillus* L and *Vaccinium vitis-idaea* L. The extractions were carried out in ultrasonic bath at 40°C for 20 min with different solvents (water, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol and 96% ethanol) and different hydro module of samples and solvents used (1:50, 1:100 and 1:200). The highest total phenol content in the leaves of *V. myrtillus* L. and *V. vitis-idaea* L. was found when 40% ethanol extract was used (90.50 ± 0.05 mg GAE/g DW and 96.68 ± 0.05 mg GAE/g DW, respectively). The highest total flavonoid content of the leaves of both species was obtained with 80% ethanol as extraction solvent. The highest level of total proanthocyanidins were in the 60% ethanol extract of *V. myrtillus* L. and in the 80% ethanol extract of *V. vitis-idaea* L (13.12 ± 0.11 mg LE/g DW and 24.22 ± 0.21 mg LE/g DW, respectively). The highest ability to scavenge DPPH radicals possessed the 40% ethanol extracts from the leaves of both species (693.99 ± 4.05 mM TE/g DW for *V. myrtillus* L. and 1083.18 ± 8.48 mM TE/g DW for *V. vitis-idaea* L). Data analysis showed that the maximal amount of polyphenols was extracted at a hydro-module of 1: 100. HPLC analysis revealed that the dominant phenolic acid in the leaves of *V. myrtillus* L was chlorogenic acid (13.45 mg/g DW), while ferulic acid (49.48 mg/g DW) was present at the highest concentration in the leaves of *V. vitis-idaea* L.

Keywords: *Vaccinium myrtillus* L, *Vaccinium vitis-idaea* L, polyphenols, antioxidant activity

Abbreviations: DW – dry weight; RSM - Response Surface Methodology

✉*Corresponding author: Assoc. Prof. PhD Ivan Ivanov, Department of Organic Chemistry and Inorganic Chemistry, Technological Faculty, University of Food Technologies. 26 Maritza Blvd, 4000 Plovdiv, Bulgaria, E-mail: ivanov_ivan.1979@yahoo.com*

Article history:
Received 6 April 2020
Reviewed 4 June 2020
Accepted 4 July 2020
Available on-line 17 September 2020

https://doi.org/10.30721/fsab2020.v3.i2.98

Introduction

Bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) are flowering plants belonging to the large genus of *Vaccinium* Ericaceae (Hokkanen et al., 2009; Rohloff et al., 2015; Burdulis et al., 2009) These berries are native to Bulgarian forests (Dincheva and Badjakov, 2016). The investigated *Vaccinium* species are mainly noted for their high content of anthocyanin pigments (Dincheva and Badjakov, 2016; Rohloff et al., 2015). Indeed, *Vaccinium* berries such as bilberries and blueberries lingonberry contain high amounts of sugars and organic acids. Berry fruits are rich sources of polyphenols with different bioactive potential (Hokkanen et al., 2009). These compounds are mainly represented by flavonoids, phenolic acids, and tannins, which are known as natural antioxidants (Brasanac-Vukanovic et al., 2018). Many of the biological properties are closely associated with the antioxidant activity of anthocyanin pigments (Chu et al., 2011). Riihinen et al. (2008) reported that both bilberry and blueberry leaves contained high amounts of proanthocyanidins, flavonols and hydroxycinnamic acids. Also, bilberry and lingonberry are potential sources for triterpenoid and phytosterol compounds (Szakiel et al., 2012 a, b). The aim of the current study was to derive the most appropriate conditions for extraction of the highest yield of biologically active substances with high antioxidant potential from bilberry and lingonberry aerial part. The influence of different concentration ratio of solvent system (ethanol-water) and different hydromodules of samples and solvents on the concentration of biologically active substances have been studied.

Materials and Methods

Plant material. Aerial parts (leaves) from both *Vaccinium* species were collected from their natural habitats as follow: *Vaccinium myrtillus* L. from Gela village area (41°38'32.7"N 24°33'17.6"E); and *Vaccinium vitis-idaea* L. from Perelic mountaintop area (41°36'28.6"N 24°35'46.9"E) in the Rhodope mountain. The samples were finely ground using a laboratory homogenizer. The powder was used for further extraction of biologically active compounds.

Extraction procedure. One gram of dry ground leaves were placed in a flask together with 100 mL of ethanol-water mixture in different ratio (water, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol and 96% ethanol) was poured in it. Ultrasound-assisted extraction was performed in an ultrasonic bath SIEL UST 5.7-150 (Gabrovo, Bulgaria) with a frequency 35 kHz, power 240 W at a temperature of 40°C.

Analyses. Total polyphenols content. The total phenolics content was measured using the Folin-Ciocalteu assay as described by Ivanov et al. (2014): Folin-Ciocalteu reagent (1 mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL 7.5% Sodium carbonate. The reaction was 20 min at room temperature in darkness. After reaction the absorption was measured at 765 nm against a blank sample, prepared in the same way but without the extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), values derived from a calibration curve established in the concentration range of 0.02 - 0.10 mg gallic acid (Sigma) used as a standard.

Total flavonoids. Total flavonoids were determined spectrophotometrically following the method described by Ivanov et al. (2014). Each extract (1.0 mL) was added to test tubes containing 0.1 mL 10% aluminum nitrate (Sigma), 0.1 mL 1M potassium acetate (Sigma) and 3.8 mL ethanol (Merck). The reaction time was 40 min at ambient temperature. The absorbance was measured at 415 nm. The results were expressed in mg equivalent of quercetin per g dry weight (DW), derived from a calibration curve, linear in the range of 10-100 µg/mL quercetin as a standard.

Total proanthocyanidins assay. Acid butanol assay for proanthocyanidins, as described by Porter et al. (1985). The six milliliter of the acid butanol reagent mixture of 950 mL of n-butanol and 50 mL concentrated HCl), 0.5 mL aliquot of the fraction, and 0.1 mL of the iron reagent (2% ferric ammonium sulfate in 2 mol/L HCl were added to a 10 mL screw cap tube and then vortexed. The tube was capped loosely and put in a boiling water bath for 50 min. The absorbance of formed colored complex was read at 550 nm. Condensed tannins were analyzed as leucocyanidin equivalent (LE) (Hagerman, 2011).

Antioxidant activity. DPPH assay. DPPH assay was performed according to Ivanov et al. (2014). Each extract (0.15 mL) was mixed with 2.85 mL of a freshly prepared 0.1 mM solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, Sigma) in methanol (Merck). The reaction was performed at 37 °C in darkness and the absorptions at 517 nm were recorded after 15 min against methanol. The antioxidant activity was expressed as mM Trolox equivalents (TE) per g dry weight (DW) by using bration curve, established in the range of build by 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Fluka) dissolved in methanol (Sigma).

HPLC-DAD analysis of phenolic acids. Chromatographic analysis of the phenolic acids content was performed using a HPLC Elite Chrome Hitachi, coupled with diode-array detector (DAD) and ELITE LaCHrome software. The separation was performed on a reverse-phase column Supelco, Discovery® HS C18 (5 µm, 25 cm x 4.6 mm) operating at 30°C. Detection was done at wavelength 280 nm and 320 nm. Elution was performed with mobile phase A (2 % acetic acid) and mobile phase B (acetonitrile) in gradient mode described by Ivanov et al. (2014) at a flow rate 0.8 mL/min. The sample injection volume was 20 µL.

RSM (Response Surface Methodology) optimization method. In order to evaluate the effects of extraction parameters and to optimize conditions for various responses RSM optimization method was applied. Independent variables used in the experimental design were solvent concentration (water, 20% ethanol, 40% ethanol, 60% ethanol, 80% ethanol and 96% ethanol) and sample-solvent hydro modules (1:50, 1:100 and 1:200). The coded and uncoded independent variables used in the RSM design are presented in Table 1.

Ranges of ethanol (X_1) and hydro module (X_2) and the central points were selected based on literature data. Statistical analysis of experiment was performed using Statistical Software MINITAB 16. The response variables were fitted to the following second-order polynomial model (Eq. 1), which was able to describe the relationship between the dependent output variable and the independent variables:

$$Y = b_0 + \sum b_i \ x_i + \sum b_{ii} \ x_i^2 + \sum b_{ij} \ x_i x_j \quad (1),$$

where

Y represents response variable (total polyphenolic content and antioxidant activity – DPPH method); Xi and Xj are the independent variables (ethanol concentration and hydro module);

β0, βi, βii and βij are the regression coefficients for the intercept, linear, quadratic and interaction coefficient, respectively.

Table 1. Coded and uncoded levels of independent variables used in the response surface methodology.

Independent variables	Symbol	Level		
		Low (-1)	Middle (0)	High (+1)
Solvent concentration, %	X_1	0	40	96
Hydro module	X_2	1:50	1:100	1:200

Results and Discussion

There is an increasing interest for using phytochemicals from natural plants. The extracts obtained from different plant origin had different effects on human health. Extraction efficiency depends on a large number of parameters - extraction method, solvents, temperature, extraction time. Therefore, it is very important to find optimal extraction parameters for obtaining extracts with the highest content of biologically active compounds (Cvetanovic et al. 2015, Mašković et al., 2016). Response surface methodology is an effective technique for the optimization of complex processes, because it allows an efficient and easier interpretation of experimental data (Bezerra et al. 2008, Zekovic et al. 2014). Several investigators already used RSM for the optimization of extraction processes in order to maximize yield of various polyphenolic compounds from various sources (Radojković et al., 2012; Claus et al., 2015; Mašković et al., 2016). In the present study RSM was used to optimize the solid-liquid extraction ratio of compounds with improved antioxidant ability from *Vaccinium* species. The influence of solvent composition and hydro module on the

extraction yield of total polyphenols, total flavonoids, total proanthocyanidins and antioxidant activity was investigated. The experiments were set up according to RSM design, and the results are presented in Table 2 and Table 3. The effect of linear, quadratic or interaction coefficients on the response was tested for significance by analysis of variance. Experimental results from Table 4 were processed with multiple linear regressions using the second-order polynomial model – Eq. (1). The regression coefficients of the intercept, linear, cross product and quadratic terms are presented in the Table 4.

Suitability of the model was also analysed through the MINITAB 16. Calculated statistical parameters are presented in Table 4. According to the calculated p values and the F value for the suggested model was suitable for the investigated extraction system. Model equations for relationship between total phenol content and antioxidant activity and independent variables were obtained by applying multiple regression analysis (Table 4). By applying these equations, it is possible to predict values of each response. The values of R^2 for total polyphenol content, and antioxidant activity were 0.73, 0.94 and 0.56, respectively (Table 4). Therefore, it was suggested that the quadratic model fitted well with the experimental data.

The optimization procedures carried out using "Response optimizer" of MINITAB 16 software gave the following values of variable X_1 and X_2 for maximum yield of total polyphenolics, and antioxidant activities (DPPH methods) (Y) by *Vaccinium* species (Table 5). The deviation between the theoretically studied maximal amounts of total polyphenols and experimentally obtained for bilberry and lingonberry were 3.2 mg/g GAE DW and 5.9 mg/g GAE, respectively; and antioxidant activities (DPPH methods) (at 40% ethanol and 1:100 hydro module) under ultrasonic influence (Table 5). On this basis, we propose that 40% ethanol in water and hydro module 1:100 as optimal extraction conditions for highest yield of biologically actives substances from *Vaccinium* species leaves.

According to the obtained results, all investigated extracts of lingonberry contained a higher total polyphenol content and higher ability to scavenge DPPH radicals than the extracts of bilberry leaves, while a higher total flavonoid content was determined in the extracts of bilberry leaves.

After establishing the optimal parameters (40% ethanol and 1:100 hydro modules) for extraction of the highest amount of polyphenol components by ultrasonic irradiation, HPLC-DAD analysis of phenolic acids was carried out.

The results showed the presence of three phenolic acids in the bilberry leaves: chlorogenic acid – 13.45 mg/g DW, p-coumaric acid – 0.62 mg/g DW and sinapic acid – 1.85 mg/g DW. The main phenolic acid extracted from lingonberry leaves was ferulic acid – 49.48 mg/g DW, followed by sinapic acid (2.34 mg/g DW) and chlorogenic acid (2.55 mg/g DW) (Table 6). Brasanac-Vukanovic et al. (2018) investigated the phenolic compounds in different extracts obtained from bilberry leaves and obtained similar results – the main phenolic acid was chlorogenic acid (from 45.5 to 59.7 mg/g DW), followed by p-coumaric acid (from 1.26 to 2.08 mg/g DW).

Conclusions

The optimal conditions for the extraction of biologically active substances from *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. are as follow: 40% ethanol-water as solvent system and hydro module 1:100 in ultrasound bath with frequency 35 kHz. Under these condition the maximum amount of total polyphenols content and antioxidant activity was obtained. The main identified phenolic acid in the 40% ethanol-water extract in bilberry leaves was chlorogenic acid and for lingonberry extract was ferulic acid.

Acknowledgements

The authors are thankful for the financial support of Bulgarian Science Fund, Bulgarian Ministry of Education and Science by contract ДН 16/1 - 11.12.2017

Table 2. Experimental matrix and values of the observed responses of total polyphenolics, and antioxidant activities (DPPH methods) for bilberry.

Sample	Hydro-module	Total polyphenol contents, mg GAE/g DW	Total flavonoids, mg QE/g DW	Total proanthocyanidins, mg LE/g DW	Antioxidant ability, mM TE/g DW
Water	1:100	65.04±0.10	24.64±0.06	10.68±0.21	507.59±8.75
20% Ethanol	1:100	68.39±0.19	28.54±0.27	11.08±0.18	573.62±2.73
40% Ethanol	1:100	90.50±0.05	26.47±0.47	11.73±0.21	693.99±4.05
60% Ethanol	1:100	71.16±0.15	31.27±0.17	13.12±0.11	667.40±2.88
80% Ethanol	1:100	70.98±0.35	34.96±0.17	12.34±0.16	601.11±4.33
96% Ethanol	1:100	56.40±0.21	23.80±0.17	12.04±0.06	456.05±8.75
40% Ethanol	1:50	58.62±0.24	28.50±0.38	1.66±0.03	668.13±5.54
40% Ethanol	1:200	61.24±0.28	32.58±0.69	1.51±0.06	633.96±9.27

Table 3. Experimental matrix and values of the observed responses of total polyphenols, and antioxidant activities (DPPH methods) for lingonberry.

Sample	Hydro-module	Total polyphenol contents, mg GAE/g DW	Total flavonoids, mg QE/g DW	Total proanthocyanidins, mg LE/g DW	Antioxidant ability, mM TE/g DW
Water	1:100	79.91±1.31	15.16±0.16	7.78±0.25	544.16±8.09
20% Ethanol	1:100	84.13±0.83	16.82±0.10	10.93±1.18	858.46±4.09
40% Ethanol	1:100	96.68±0.68	13.62±0.27	13.05±0.60	1083.18±8.48
60% Ethanol	1:100	75.98±0.35	15.81±0.10	11.45±1.30	928.04±9.05
80% Ethanol	1:100	77.95±0.20	21.20±0.40	24.22±0.22	652.02±8.67
96% Ethanol	1:100	78.68±0.10	13.69±0.23	12.75±0.23	864.07±6.03
40% Ethanol	1:50	88.43±0.05	16.88±0.22	5.17±0.13	959.95±9.69
40% Ethanol	1:200	92.05±0.15	19.78±0.61	6.32±0.46	922.56±5.80

Table 4. Regression equation coefficients for the selected responses

Variable	Regression coefficient	F-value	p-value	Regression coefficient	F-value	p-value
Total polyphenols concentration						
		Bilberry			*Lingonberry*	
Intercept b_0	-0.69			89.48		
Linear		2.90	0.199		0.05	0.949
b_i	0.77	0.35	0.594	0.24	0.01	0.918
b_j	1.05	5.72	0.097	-0.14	0.10	0.770
Square (quadratic)		4.11	0.138		0.83	0.515
b_{ii}	-8.82×10^{-3}	5.58	0.099	-3.23×10^{-3}	0.76	0.448
b_{jj}	-4.10×10^{-3}	6.51	0.084	0.68×10^{-3}	0.18	0.699
R^{2a}	0.739			0.472		
Antioxidant activity						
		Bilberry			*Lingonberry*	
Intercept b_0	452.085			626.75		
Linear		3.53	0.163		0.94	0.482
b_i	8.22	7.07	0.076	13.95	1.83	0.269
b_j	0.74	0.16	0.720	-0.28	0.00	0.972
Square (quadratic)		22.23	0.016		1.84	0.302
b_{ii}	-87.56×10^{-3}	37.65	0.009	-0.13	2.83	0.191
b_{jj}	-3.87×10^{-3}	0.39	0.577	0.12×10^{-3}	0.00	0.997
R^{2a}	0.938			0.564		

Table 5. Comparison between theoretically calculated and experimentally obtained yields of total polyphenolics, and antioxidant activities of bilberry and lingonberry extracts.

	Theoretically calculated			Experimentally obtained			Deviation between
	X_1[1]	X_2[1]	$\bar{Y}$	X_1	X_2	Y	$\bar{Y}$ and Y
Total polyphenols							
Bilberry	48.0	1:125	83.3	40	1:100	90.5	7.9%
Lingonberry	34.0	1:200	92.0	40	1:100	96.7	4.8%
Antioxidant activity							
Bilberry	46.5	1:96	680.4	40	1:100	694.0	2.0%
Lingonberry	52.8	1:50	981.8	40	1:100	1083.2	9.3%

Table 6. HPLC analysis of phenolic acids in the 40% ethanol extracts (1:100 hydro modules) of *V. myrtillus and V. vitis-idaea* leaves

Compounds	Bilberry *V. myrtillus*, mg/g DW	Lingonberry *V. vitis-idaea*, mg/g DW
Chlorogenic acid	13.45	2.55
Caffeic acid	nd	0.36
Ferulic acid	nd	49.48
p-Coumaric acid	5.04	1.48
Sinapic acid	1.85	2.34

nd – not detected

References

Bezerra M. A., Santelli R. E., Oliveira E.P., Villar L.S., Escaleira L.A. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, 2008, 76(5): 965-977. https://doi.org/10.1016/j.talanta.2008.05.019

Brasanac-Vukanovic S., Mutic J., Stankovic D. M., Arsic I., Blagojevic N., Vukasinovic-Pesic V., Tadic V. M. Wild bilberry (*Vaccinium myrtillus* L., Ericaceae) from Montenegro as a source of antioxidants for use in the production of nutraceuticals, *Molecules*, 2018, 23(8): 1864. https://doi.org/10.3390/molecules23081864

Burdulis D., Sarkinas A., Jasutiené I., Stackevicené E., Nikolajevas L., Janulis V. Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (*Vaccinium myrtillus* L.) and blueberry (*Vaccinium corymbosum* L.) fruits. *Acta Poloniae Pharmaceutica – Drug Research*. 2009, 66(4): 399-408.

Chu W., Cheung S. C. M., Lau R. A.W., Benzie I. F. F. Bilberry (*Vaccinium myrtillus* L.) NCBI Bookshelf. In: *Herbal Medicine: Biomolecular and Clinical Aspects.* (2nd Edition), (I.F.F. Benzie, S. Wachtel-Galor Eds.). CRC Press, Taylor & Francis Group. 2011, pp. 55-72, ISBN-13: 978-1439807132, ISBN-10: 1439807132

Claus T., Palombini S.V., Carbonera F., Figueiredo I.L., Matsushita M., Visentainer J.V. Response surface methodology applied in the study of emulsion formulations in the presence of leaves of rosemary (*Rosmarinus officinalis* L.) as a source of natural antioxidants. *Journal of the Brazilian Chemical Society*, 2015, 26(10): 2097-2104. http://doi.org/10.5935/0103-5053.20150195

Cvetanović A., Švarc-Gajić J., Mašković P., Savić S., Nikolić L.J. Antioxidant and biological activity of chamomile extracts obtained by different techniques: perspective of using superheated water for isolation of biologically active compounds. *Industrial Crops and Products*, 2015, 65(3):582-591. https://doi.org/10.1016/j.indcrop.2014.09.044

Dincheva I., Badjakov I. Assessment of the anthocyanin variation in Bulgarian bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.). *International Journal of Medicine and Pharmaceutical Science*, 2016, 6(3): 39-50. Available at: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2838677

Hagerman, A., 2011. *The Tannin Handbook*. Available at: http://www.users.muohio.edu/hagermae

Hokkanen J., Mattila S., Jaakola L., Pirttila A. M., Tolonen A. Identification of phenolic compounds from lingonberry (*Vaccinium vitis-idaea* L.), bilberry (*Vaccinium myrtillus* L.) and hybrid bilberry (*Vaccinium* x *intermedium* Ruthe L.) leaves. *Journal of Agricultural and Food Chemistry*, 2009, 57(20): 9437-9447. https://doi.org/10.1021/jf9022542

Ivanov I., Vrancheva R., Marchev A., Petkova N., Aneva I., Denev P., Georgiev V., Pavlov A. Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. *International Journal of Current Microbiology and Applied Science*, 2014, 3(2): 296-306. Available at: https://pdfs.semanticscholar.org/3be9/df25ed8c217ed42ca21b00b7efbee45bbf14.pdf

Mašković P. Z., Diamanto L. D., Cvetanović A., Radojković M., Spasojević M. B., Zengin G. Optimization of the extraction process of antioxidants from orange using response surface methodology. *Food Analytical Methods*, 2016, 9(5): 1436-1443. https://doi.org/10.1007/s12161-015-0321-5

Porter L. J., Hrstich L. N., Chan B. C. The conversion of urocyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 1985, 25(1): 223-230. https://doi.org/10.1016/S0031-9422(00)94533-3

Radojković M., Zeković Z., Jokić S., Vidović S., Lepojević Z., Milošević S. Optimization of solid-liquid extraction of antioxidants from black mulberry leaves by response surface methodology. *Food Technology and Biotechnology*, 2012, 50(2): 167-176. Available at: https://hrcak.srce.hr/index.php?show=clanak&id_clanak_jezik=124077

Riihinen K., Jaakola L., Kärenlampi S., Hohtola A. Organ-specific distribution of phenolic compounds in bilberry (*Vaccinium myrtillus*) and 'north blue' blueberry (*Vaccinium corymbosum* x *V. angustifolium*). *Food Chemistry*, 2008, 110(1): 156-160. https://doi.org/10.1016/j.foodchem.2008.01.057

Rohloff J., Uleberg E., Nes A., Krogstad T., Nestby R., Martinussen I. Nutritional composition of bilberries (*Vaccinium myrtillus* L.) from forest fields in Norway - Effects of geographic origin, climate, fertilization and soil properties. *Journal of Applied Botany and Food Quality*, 2015, 88(2015): 274-287. https://doi.org/10.5073/JABFQ.2015.088.040

Szakiel A., Pączkowski C., Huttunen S. Triterpenoid content of berries and leaves of bilberry *Vaccinium myrtillus* from Finland and Poland. *Journal of Agricultural and Food Chemistry*, 2012a, 60(48): 11839-11849. https://doi.org/10.1021/jf3046895

Szakiel A., Paczowski C., Koivuniemi H., Huttunen S Comparison of the triterpenoids content of berries and leaves of lingonberry *Vaccinium litisidaea* from Finland and Poland. *Journal of Agricultural and Food Chemistry*, 2012b, 60(19): 4994-5002. https://doi.org/10.1021/jf300375b

Zeković Z., Cvetanović A., Pavlić B., Švarc-Gajić J., Radojković M. Optimization of the polyphenolics extraction from chamomile ligulate flowers using response surface methodology. *International Journal of Plant Research*, 2014, 4(2): 43-50. https://doi.org/10.5923/j.plant.20140402.01