STUDY ON THE POLYPHENOLS AND ANTOCYANINS CONTENT IN THE VACCINIUM MYRTILLUS L. FRUITS HARVESTED IN DIFFERENT REGIONS OF UKRAINE

¹Ochkur O., ²Del Bubba M., ²Orlandini S., ²Checchini L., ¹Kravchenko I., ¹Bohachik J., ¹Koshovyi O.

¹National University of Pharmacy, Kharkiv, Ukraine ²University of Florence, Florence, Italy

Bilberry (*Vaccinium myrtillus* L.) is a wild shrub native to mountain and forest areas of Northern and Central Europe, widely diffused also in Northern and Western Ukraine. Bilberry fruits has a large commercial importance due to its richness in nutritional and bioactive compounds, and are consumed as dietary supplements and pharmaceutical products for health benefits.

These fruits are well-known for their healthprotecting attributes, as anti-inflammatory, antihypertensive, anti-microbial and anti-cancer agents. Bilberry fruit extracts have been studied for the prevention and treatment of chronic pathologies, such as diabetes, cardiovascular disease and obesity. Many researches have confirmed the high antioxidant and antiradical activity of bilberry fruits [3, 6].

The interest in bilberries is due to the high content in phenolic compounds. The most abundant class of polyphenols in *V. myrtillus* berries are anthocyanins, such as glycosides of delphinidin, cyanidin, petunidin, peonidin and malvidin. Flavanols (i.e. catechin), phenolic acids (e.g. caffeic, p-coumaric, ferulic, chlorogenic acids) and flavonols (i.e. quercetin, myricetin) have also been determined in bilberries, however at lower concentrations [2, 4].

Significant differences in the relative abundance of phenolic compounds have been observed in berries harvested in different areas, due to the stage of growth, the environmental conditions and genetic factors. As a general rule, higher phenolic contents are favoured by northern latitudes, altitude and a sunny environment [1].

Many researches aiming at the characterization of phenolic compounds in *V. myrtillus* berries from different European countries have been published in recent years. However, papers focused on polyphenols in spontaneous bilberry grown in Ukraine, at a recent time have not been published. Considering the great importance of bilberries for the pharmaceutical and food industries and insufficient information about *V. myrtillus* berries harvested in Ukraine, to study the chemical composition of berries depending on the place of their harvesting is an actual task.

In our previous publication, we showed the effect of the drying method on the contents of total soluble polyphenols (TSP) and total monomeric anthocyanins (TMA) in *V. myrtillus* berries [5]. The aim of submitted study was to investigate the contents of TSP and TMA in *V. myrtillus* berries harvested in different regions of Ukraine. The objects of our research were fully ripe spontaneous *V. myrtillus* berries harvested in July-August 2018 in four regions of Ukraine with different environmental conditions (tab. 1).

Table 1

#	Collection zone	Geographical characteristic
1	Volyn region, Shatsk district	Wooded plain, northwest Ukraine
2	Ivano-Frankivsk region, Yaremche	Mountains, northeastern slopes of the Carpathians
3	Zacarpathian region, Tyachiv district	Mountains, southwestern slopes of the Carpathians
4	Chernihiv region, Semenivka district	Wooded plain, northeast Ukraine

Collection zones of V. myrtillus berries

СУЧАСНА ФАРМАЦІЯ: ІСТОРІЯ, РЕАЛІЇ ТА ПЕРСПЕКТИВИ РОЗВИТКУ

Research of chemical composition was carried out in the laboratory of analytical chemistry of Chemistry department of University of Florence. Bilberries were dried using the method of convective drying at a temperature about 60° C. Dried berries and extracts from them were stored in a refrigerator at a temperature -20° C. Bilberries powder was prepared by grinding of each sample with the hand blender in an ice bath. About 125 mg aliquots of dry weight (d.w.) raw material were extracted in an ultrasound water bath within 20 minutes with 5 ml of acetone-water (6:4) mixture. The extract was centrifuged and the supernatant was recovered. This procedure was repeated twice for each extract and the extracts were combined together.

TSP content was spectrophotometrically determined at $\lambda = 740$ nm according to the Folin-Ciocalteau method using (+)-catechin (CAT) as a reference standard. TMA were spectrophotometrically determined with the pH differential method using cyanidine-3-glucoside (CYD-3-GLU) as a reference standard.

Contents of TSP and TMA established by the results of our research are shown in Tab. 2.

Table 2

#	Content of TSP, mg	Contents of TMA, mg
	Cat. Equiv./100 g d.w.	CYD-3-GLU Equiv./100 g.
1	3053	1837
2	5576	2505
3	4803	1123
4	3942	2807

Contents of TSP and TMA in V. myrtillus berries

According to our research, the maximum TSP content was observed for sample 2 (Ivano-Frankivsk reg.), the minimum – for sample 1 (Volyn reg.). It is noteworthy that in samples collected in mountain regions polyphenol content is noticeably higher compared to the plains samples (on average by 32.6%). However, the maximum TSP content was observed for sample 4 (Chernihiv reg.), harvested in the easternmost of the represented regions. In the same sample, the largest ratio of anthocyanins relative to the content of TSP was found (71.2%). However, it should be noted that revealed TMA content may be underestimated due to the high thermolability of these compounds and not the most optimal method of drying.

Thus, in our study it is shown that higher polyphenol content is observed in bilberries growing in mountain areas. At the same time polyphenol compounds of *V. myrtillus* berries in the Ukrainian area requires further detailed study.

- 1. Akerström, A., Jaakola, L., Bång, U., & Jäderlund, A. (2010). Effects of latitude-related factors and geographical origin on anthocyanidin concentrations in fruits of Vaccinium myrtillus L. (bilberries). *Journal of Agricultural and Food Chemistry*, 58, 11939–11945.
- Ancillotti, C., Ciofi, L., Pucci, D., Sagona, E., Giordani, E., Biricolti, S., ... Del Bubba, M. (2016). Polyphenolic profiles and antioxidant and antiradical activity of Italian berries from *Vaccinium myrtillus* L. and *Vaccinium uliginosum* L. subsp. gaultherioides (Bigelow) S.B. Young. *Food Chemistry*, 204, 176–184.
- 3. Bujor, O., Le Bourvellec, C., Volf, I., Popa, V., & Dufour C. (2016). Seasonal variations of the phenolic constituents in bilberry (Vaccinium myrtillus L.) leaves, stems and fruits, and their antioxidant activity. *Food Chemistry*, 213, 58–68.

- 4. Koshovyi, O. M., Zagayko, A. L., Kolychev, I. O., Akhmedov, E. Yu., Komissarenko, A. N. (2016). Phytochemical study of the dry extract from bilberry leaves. *Azerbaijan Pharmaceutical and Pharmacotherapy Journal*, 16 (1), 18-23.
- 5. Ochkur, O., Koshovyi, O., Del Bubba, M., Checchini, L., Betsa, O. (2019) Study on the content of polyphenols and antocians in the bilberry fruits dried by different ways. *Medicines to People. Modern Problems of Pharmacotherapy and Prescription of medicines: III International Scientific and Practical Conference*, Kharkiv 14-15 March 2019, 2, 17.
- 6. Saponjac, V.; Canadanovic-Brunet, J.; Cetkovic, G. (May 2015). Dried bilberry (*Vaccinium myrtillus* L.) extract fractions as antioxidants and cancer cell growth inhibitors. *LWT Food Science and Technology*. 61 (2): 615–621.