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THERAPEUTIC EFFECTS OF ANTHOCYANINS FROM *VACCINIUM* GENUS L

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Keywords: *Vaccinium spp., anthocyanins, therapeutic effects.*

Abstract

The purpose of this review is to examine the botanical characteristics of four species of *Vaccinium* genus: *Vaccinium vitis-idaea* L. (Lingonberry), *Vaccinium myrtillus* L. (Bilberry), *Vaccinium uliginosum* L. (Bog bilberry) and *Vaccinium arctostaphylos* L. (Caucasian whortleberry) as well as to consider the chemical composition and therapeutic effects of anthocyanins isolated from these species.

The following therapeutic properties have been found in the extracts rich in anthocyanins and obtained by fruits or leaves of *Vaccinium* spp.: vasoprotective, anti-inflammatory, anti-diabetic, antimicrobial, anticancer, genoprotective and antioxidant.

Introduction

Genus*Vaccinium* L. includes around 450 species found mostly in the northern hemisphere of our planet. These are predominantly shrubs or vines and belong to the *Ericaceae* family (heather). The fruits and leaves of these *Vaccinium* species produce a wide range of compounds: flavonoids such as anthocyanins, flavonols, flavanols (catechins), phenolic acids (benzoic and cinnamic acid derivatives), chromones, coumarins, lignans, iridoids, sterols, and triterpenoids[1-3]. The principal components are flavonoids (anthocyanins)[3]. Over 116 anthocyanins and flavonoid compounds have been isolated and identified within the *Vaccinium* genus[3]. Extracts of anthocyanins from *Vaccinium* fruits and leaves demonstrate various pharmacological effects such as antidiabetic[4], anti-inflammatory[5,6], vasoprotective[7], antimicrobial[8], antitumor[9,10], genoprotective[11], and antioxidative[12-24].

Botanical characteristics and distribution of genus *Vaccinium* L. in Bulgaria

The *Ericaceae* family (heather) includes 140 genera and 3500 species. Seven genera and twelve species have been found in Bulgaria. The distribution is cosmopolitan, especially in temperate and cool areas. They are not found in steppes and deserts. The habitus of the species consists of evergreen or deciduous shrubs and semi-shrubs, sometimes small trees[25].

There are four species found in natural habitats throughout Bulgaria: *Vaccinium vitis-idaea* (Lingonberry), *Vaccinium myrtillus* (Bilberry), *Vaccinium uliginosum* (Bog Bilberry) and *Vaccinium arctostaphylos* (Caucasian whortleberry). The latter is protected under the Biological Diversity Act[26]. These are deciduous or evergreen shrubs, reaching a height of up to 25 cm (*V. vitis-idaea*), 40 cm (*V. myrtillus*), 70 cm (*V. uliginosum*), and 3 m (*V. arctostaphylos*). The leaves are arranged consecutively and have short stems or almost sessile ones; the edge of the leaf lamina is entire or serrated. The blossoms are 4- or 5-sectional, collected in axil bunches. The corolla is bulb-tobell-shaped, thestamen are 8-10 in number, the ovary is positioned low with 4-5 carpels. The fruit is a juicy blue, blue-black or red berry with small seeds[26] (Figure 1).

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***Vaccinium vitis-idaea* L.**

***Vaccinium myrtillus* L.**

***Vaccinium uliginosum* L.**

***Vaccinium arctostaphylos* L.**

Figure 1. Images of Vaccinium species, distributed in Bulgaria (www.plantarium.ru)

The parts useful for pharmacological purposes are the leaves and fruits. The fruits are produced between July and September.

In Bulgaria, Caucasian whortleberry is only found in the Strandzha Mountains at an altitude of 150-300 m in humid shaded areas. The other *Vaccinium* species are wide-spread in stony or grassy locations, in coniferous and more rarely broad-leaf forests in almost every Bulgarian mountains. These are also found at altitudes of 700-2000 m for Lingonberry, 900- 2200 m for Bilberry, and 1700-2500 m for the Bog Bilberry[26].

Main components of the polyphenol composition responsible for the pharmacological activity

According to the *European Pharmacopoeia* 8[27] fresh *V. myrtillus* fruits are classified according to anthocyanin content, defined as cyanidin-3-O-glucoside chloride (minimum 0.3%), and dried *V. myrtillus* fruits - according to tannin content, defined as pyrogallol (minimum 1%).

The main isolated components of *Vaccinium myrtillus* fruit, responsible for the biological activity are flavonol-O-glycosides such as quercetin-3-rhamnoside (quercitrin), quercetin-3-glucoside (isoquercitrin), quercetin-3-

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galactoside (hyperoside), and kaempferol-3-glucoside (astragalin), myricetin glycosides, as well as over 15 anthocyanins (total quantity of approximately 0.5%), which have been identified as 3-arabinosides, 3-glucosides, and 3-galactosides of five anthocyanidins: cyanidin, delphinidin, malvidin, peonidin, and petunidin (Figure 2). The quantity of glycosides of cyanidin and delphinidin (Figure 3) makes up to 64% of the total anthocyanins content[7,28]. The flavonoids quercetin and quercetin-3-rutinoside (rutin), along with 11 other anthocyanins have been isolated from the fruit of *Vaccinium uliginosum*; with the most abundant being petunidin-3-glucoside and malvidin-3-glucoside[29]. In the fruit of *Vaccinium vitis-idaea,* evidence has been found only of the presence of quercetin glycosides and glycosides of cyanidin (3-arabinosides, 3-glucosides, and 3-galactosides)[28]. Nine anthocyanins have been found in *Vaccinium arctostaphylos*, with the most characteristic being cyanidin-3-O-xyloside, delphinidin-3-O-xyloside, malvidin-3-O-xyloside, and petunidin-3-O-xyloside[30,31].

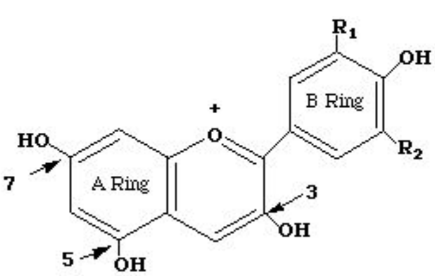
The content of organic acids in *Vaccinium myrtillus*is 3-7% (predominantly citric and malic acid). The fruit also contain up to 10% tannins[7].

The phenolic acids *p*-coumaric, caffeic, ferulic, chlorogenic, and ellagic have been isolated in genus *Vaccinium*[1,2]. According to data by Kader *et al.* (1996) chlorogenic acid is predominant in Bog Bilberry, and according to Chen *et al.* (2001) benzoic acid is present in the largest quantity in Lingonberry[32,33].

The health benefits of the fruits of these species include maintaining good eyesight, prevention of socially significant disease such as cardiovascular disease and cancer, diabetes, rheumatoid arthritis, Parkinson's and Alzheimer's disease[2]. It is considered that the main components responsible for this protective action are the phenolic compounds they contain, such as anthocyanins, flavones, flavonols, and phenolic acids[2].

Phenolic compounds are synthesized by plants in order to protect against adverse environmental conditions, such as drought, UV light, insects, viruses, bacteria, or physical damage[2,34]. Phenolic compounds contain one or more aromatic rings in their molecules with one or more hydroxyl groups as substitutes. Phenolic compounds are mostly found in the form of glycosides with predominant sugar residues of glucose, xylose, arabinose, rhamnose, often bound to organic acids, lipids, and amines.

Anthocyanins are a large group of water soluble pigments, which give the fruits their red, violet, and blue color. Anthocyanins are glycoside-bound polyphenols, and though rarely, are found in free form as aglycones, called anthocyanidins. Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin are the six anthocyanidins most frequently found in the fruits[29]. These are indicated in Figure 2.



Anthocyanidin	R_1	R_2
Cyanidin	-OH	-H
Delphinidin	-OH	-OH
Malvidin	$-OCH_3$	$-OCH_3$
Pelargonidin	-H	-H
Peonidin	$-OCH_3$	-H
Petunidin	-OH	$-OCH_3$

Figure 2. Chemical structure of anthocyanidins.

Anthocyanins are usually found as mono-, di- or triglycosides, where sugar residues are bound at C_3, and more rarely at C_5 or C_7. The most common sugar residues are glucose, galactose, rhamnose, arabinose, rutinose, and

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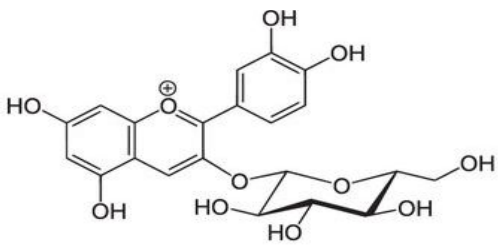
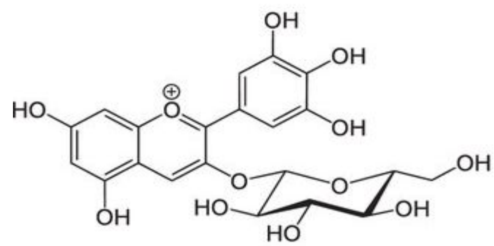
sophorose, which are usually acilated with acids such as *p*-coumaric, caffeic, ferulic, and more rarely bnezoic or acetic acid[35].



(1)



(2)

Figure 3. Chemical structure of cyanidin-3-O-glucoside (1) and delphinidin-3-O-glucoside (2).

Around 35 glycosides (anthocyanins) have been isolated and identified within the *Vaccinium* genus[3]. Most of these, contained in the four species *V. myrtillus, V. uliginosum, V. vitis-idaea, V. arctostaphylos* are specified in Table 1.

Table 1. Isolated and identified anthocyanidins and anthocyanins from Vaccinium species, distributed in Bulgaria.

Anthocyanidins and anthocyanins	Species of *Vaccinium* genus			
	V. myrtillus (fruits)	*V. uliginosum* (fruits)	*V. vitis-idaea* (fruits)	*V. arctostaphylos* (fruits)
Cyanidin	+ [36, 37] (leaves and fruits)	-	-	-
Delphinidin	+ [37]	-	-	-
Cyanidin-3-O-glucoside	+ [36, 38]	+ [31]	+ [39]	-
Cyanidin-5-O-glucoside	+ [40]	-	-	-
Cyanidin-3,5-O-diclucoside	+ [40]	-	-	-
Cyanidin-3-O-arabinoside	+ [36, 37]	+ [31]	+ [39, 41]	-
Cyanidin-3-O-galactoside	+ [5, 38]	+ [31]	+ [39]	-
Cyanidin-3-O-xyloside	+ [37]	-	-	+ [31]
Delphinidin-3-O-arabinoside	+ [36, 38]	+ [31]	+ [42, 43, 44]	-
Delphinidin-3-O-galactoside	+ [36, 38]	+ [31]	+ [42, 43, 44]	-

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Delphinidin-3-O-glucoside	+ [36, 38]	+ [31, 45]	+ [39]	+ [31]
Delphinidin-3-O-xyloside	-	-	-	+ [31]
Malvidin-3-O-arabinoside	+ [44]	+ [31, 45]	-	-
Malvidin-3-O-galactoside	+ [36, 38]	+ [45]	+ [42, 43, 44]	+ [31]
Malvidin-3-O-glucoside	+ [36, 38]	+ [31, 45]	+ [42, 43, 44]	+ [31]
Malvidin-3-O-xyloside	-	-	-	+ [30]
Peonidin-3-O-arabinoside	+ [36, 38]	+ [31]	+ [42, 43, 44]	-
Peonidin-3-O-glucoside	+ [36, 38]	+ [31]	+ [42, 43, 44]	-
Peonidin-3-O-galactoside	+ [36, 38]	+ [31]	+ [42, 43, 44]	-
Petunidine-3-O-galactoside	+ [36, 38]	+ [45]	+ [42, 43, 44]	+ [31]
Petunidine-3-O-glucoside	+ [36, 38]	+ [31, 45]	+ [42, 43]	+ [31]
Petunidine-3-O-xyloside	-	-	-	+ [30]

Therapeutic effects

Antidiabetic activity

The polyphenols contained in genus *Vaccinium* exhibit antidiabetic properties, inhibiting glucohydrolase enzimes *α*-amylase (EC 3.2.1.1), *α*-glucosidase (EC 3.2.1.20), and *β*-glucosidase (EC 3.2.1.21)[46,47]. Wang*et al.* (2012) found that the fruit shells demonstrate about 4 times greater inhibitory activity compared to the fruit core[48]. This is probably due to the fact that the external layers of such shells are rich in anthocyanins[48]. There are two presumed possible inhibition mechanisms of anthocyanin enzymes. One is competitive inhibition, resulting from the structural similarity between the enzyme substrate and the glycoside groups of the anthocyanins. The second - polar groups present on the surfaces of the enzymes interact with the hydroxyl groups of the anthocyanins. As a result, the molecular configuration of the enzyme changes at a 3D level, its hydrophilic and hydrophobic behavior alter enzyme activity[4].

The antidiabetic potential of the anthocyanins includes a reduction of blood glucose, glycosuria, and glycated hemoglobin. They prevent the production of free radicals, protect pancreatic *β*-cells, increase insulin secretion, improve insulin resistance, and reduce the absorption of sugars in the small intestines[4].

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Low doses of cyanidin-3-O-galactoside show synergistic action in combination with acarbose with regard to the inhibitory activity on intestinal α-glucosidase[49]. The inhibitory potential of cyanidin-3-O-glucoside is greater than that of cyanidin and cyanidin-3-O-galactoside[50].

Anti-inflammatory activity

The pretreatment of THP-1 macrophages with cyanidin-3-O-β-glucoside, typical for genus *Vaccinium*, over 12 hours can improve the expression and the transcription activity of the nucleus receptor γ, activated by peroxisome proliferator (PPARγ) and a liver X receptor α (LXRα). In addition, pretreatment of these cells causes dose-dependent inhibition of lipopolysaccharide (LPS) induced nitrous oxide synthase and cyclooxygenase-2 (COX-2) (EC 1.14.99.1), along with a reduction of the nitrous oxide (NO) and prostaglandin E2 (PGE2)[6].

Karlsen *et al.* (2010) report reduced concentration of inflammatory biomarkers in the plasma within a controlled trial performed with 31 patients, consuming bilberry juice for 4 weeks. Significant reduction is noted in plasma levels of highly sensitive C-reactive protein (hsCRP) and proinflammatory cytokine interleukin 6 (IL-6)[5].

Vasoprotective activity

An extract of Bilberry fruit, containing 25% anthocyanidins, has vasoprotective and anti-inflammatory activity in test animals. In rabbits, chloroform induced skin capillary permeability is reduced after intraperitoneal administration of the extract at a dose of 25-100 mg/kg of body weight or intragastric administration of anthocyanins at a dose of 200-400 mg/kg of body weight. The anti-inflammatory effect of the extract persists longer when compared to that of mepyramine. Anthocyanins at a dose of 25-100 mg/kg of body weight and intragastric administration showed effectiveness both in the capillary permeability test and the vascular resistance in animals subjected to a diet deficient in vitamin P[7].

Antimicrobial activity

The antimicrobial activity of *V. vitis-idaea* has been evaluated with regard to the two pathogenic microorganisms in the oral cavity - *Streptococcus mutans* and *Fusobacterium nucleatum*. Minimum inhibitory concentrations of 125 and 250 µg/ml were established against the growth of *F. nucleatum* and *S. mutans*. In order to achieve these concentrations, the necessary flavonoids may be acquired from one tea spoon of fresh lingonberries (3g), containing approximately 400 µg flavonoid glycosides, 4000 µg anthocyanins, and 750 µg flavan-3-ols. Fractions, enriched with anthocyanins, flavan-3-ols, and procyanidins inhibit the growth of *F. nucleatum* at a concentration of 63-125 µg/ml and the growth of plankton *S. mutans* cells at varying MIC 125-250 µg/ml[8].

Antitumor activity

The fruits of genus *Vaccinium* have demonstrated their ability to inhibit enzymes such as matrix metalloproteinases (MMPs), which play an important role in the metastasis of cancer cells. Enriched anthocyanin fractions of *Vaccinium* fruit extracts regulate the activity of matrix metalloproteinases in human prostate cancer[10].

Katsube *et al.* (2003) compare ethanol extracts of 10 different berry species tested for the induction of apoptosis in human tumor cells HL60 and HCT116. The extract from *V. myrtillus* has shown the greatest efficacy. For the aglycones delphinidin and malvidin, inhibitory activity has been found on HL60 cells, while for delphinidin and its glycosides, the activity is expressed only for the HCT116 cells[9].

Genoprotective and anxiolytic activity

Barros *et al.* (2006) experimentally determine the possible effects of lyophilized fruit extracts from the genus *Vaccinium* on the cognitive functions of rats after a 30-day feeding period. Certain tests are used, including a labyrinth task, also monitoring the possibilities for DNA damage to the hippocampus and the cortex. The study indicates that the extract improves significantly long-term memory and an anxiolytic effect is reported for the labyrinth task. What is more, the extract reduces oxidative damage to brain tissue DNA[11].

Antioxidant activity

Fresh *Vaccinium* fruit are a rich source of anthocyanins - 1210 mg/100 g for *Vaccinium myrtillus*[19], but their bio availability is low, from 1.7% to 3.3%[17]. Despite this, unmodified anthocyanins and their metabolites may be found in the blood, bile, liver, kidneys, heart, brain, urine, testes, prostate, and the lung of rats and/or mice[15,17,20,21,23]. Fruit extracts have been shown to have an intracellular antioxidative activity[51].

The antioxadant potential is determined *in vitro* through several basic methods based on scavenging free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC), and lipid peroxidation inhibition assay[16,18]. Following solid-phase extraction in order to obtain anthocyanin-rich extracts from five species of berries, the extract from *Vaccinium* fruits demonstrated the highest capacity for preventing the formation of hydroxyl radicals (HORAC) - 1293 µmol GAE/g[13].

Many polyphenols demonstrate antioxidative properties by actively participating in oxidation reduction processes within cells. These can neutralize free radicals by donating an electron or a hydrogen atom (H^+). The highly conjugated system in their molecule and the presence of certain hydroxyl groups, such as the 3-hydroxy group in flavonols, are considered to be crucial for their antioxidative activity. Polyphenols suppress the formation of free radicals by reducing the rate of oxidation by inhibiting the formation or deactivating ROS (reactive oxygen species). It has been found that polyphenols usually do not act alone but as co-antioxidants or participate in the regeneration of cofactors and prosthetic groups of enzymes[24]. Another possible mechanism of antioxidant action of polyphenols is by inhibition of xanthine oxidase (EC 1.1.3.22) and induction of secretion of endogenous antioxidant enzymes such as glutathione peroxidase (EC 1.11.1.9), catalase (EC 1.11.1.6), and superoxide dismutase (EC 1.15.1.1)[14]. Wang *et al*. (1997) report higher values of antioxidative activity of cyanidin-3-O-glycoside and cyanidin-3-O-rhamnoglucoside compared to Trolox[52].

Numerous observations indicate that anthocyanins are active for cardioprotection and neuroprotection as well as antitumor, normolipidemic and normoglycemic agents[12,22]. The common mechanism at the core of these different effects may be related to the antioxidative properties of phenolic compounds, including anthocyanins.

Conclusion

Scientific research has contributed significantly to the fact that anthocyanins have become not only food products but also therapeutic agents. The selected literature data on anthocyanins raise the hope for their wide-spread use in prevention and therapy of many diseases.

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REFERENCES

1. *Häkkinena, S., Törrönena, R. Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. Food Res Int, 2000, 33(6): 517-524.*
2. *Meskin M. S. et al. In: Phytochemicals in Nutrition and health (Ed. M. E. Camire). CRC Press, Boca Raton London New York Washington, 2002, p.19.*
3. *Zushang, Su. Anthocyanins and Flavonoids of Vaccinium L. Pharm Crops, 2012, 3:7-37.*
4. *Sancho, R. A. et al. Evaluation of the effects of anthocyanins in type 2 diabetes. Food Res Int,2012, 46(1), 378-386.*
5. *Karlsen A. et al. Bilberry juice modulates plasma concentration of NF-κB related inflammatory markers in subjects at increased risk of CVD. Eur J Nutr, 2010, 49: 345-55.*
6. *Wang, Q. et al. Cyanidin-3-O-β-glucoside inhibits iNOS and COX-2 expression by inducing liver X receptor alpha activation in THP-1 macrophages. Life Sci, 2008, 83: 176-184.*

7. *World Health Organization. WHO Monographs on Selected Medicinal Plants, Vol. 4. WHO Press, Geneva, 2009 p. 210-225.*
8. *Kaisu, R. et al. The antibiofilm activity of lingonberry flavonoids against oral pathogens is a case connected to residual complexity. Fitoterapia, 2014, 97: 78-86.*
9. *Katsube N. et al. Induction of apoptosis in cancer cells by bilberry (Vaccinium myrtillus) and the anthocyanins. J Agric Food Chem, 2003, 51: 68-75.*
10. *Samuel-Peterson, N. Cultural Competence in the Prevention and Treatment of Cancer: The Case of Blueberries in North America.Adv in Anthrop, 2013,3(2): 65-70.*
11. *Barros, D.et al. Behavioral and genoprotective effects of Vaccinium berries intake in mice. Pharmacol Biochem Behav,2006, 84(2): 229-234.*
12. *Chong et al. Fruit polyphenols and CVD risk: A review of human intervention studies.Br J Nutr Suppl. 3, 2010, 28-39.*
13. *Denev, P. et al. Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties, Food Chem., 2010, 123: 1055-1061.*
14. *Du, Q., Jerz, G. Winterhalter, P. Isolation of two anthocyanin sambubiosides from bilberry (Vaccinium myrtillus) by high-speed counter-current chromatography. J Chromatogr A, 2004, 1045: 59-63.*
15. *Felgines, C. et al. Radiolabelled cyanidin 3-O-glucoside is poorly absorbed in the mouse.Br J Nutr, 2010, 103: 1738-1745.*
16. *Giovanelli,G., Buratti, S.Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties, Food Chem, 2009, 112: 903-908.*
17. *Marczylo,T. H. et al. Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice.Cancer Chemother Pharmacol, 2009, 64:1261-1268.*
18. *Mazza, G. et al. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. JAgricFood Chem,2002, 50: 7731-7737.*
19. *Moze et al.Phenolics in Slovenian bilberries (Vaccinium myrtillus L.) and blueberries (Vaccinium corymbosum L.).JAgricFood Chem, 2011, 59: 6998-7004.*
20. *Passamonti, S. et al.The stomach as a site for anthocyanins absorption from food.FEBS Lett, 2003, 544: 210-213.*
21. *Passamonti, S. et al. Fast access of some grape pigments to the brain. JAgricFood Chem, 2005, 53: 7029-7034.*
22. *Tarozzi, A. et al. Neuroprotective effects of cyanidin 3-O-glucopyranoside on amyloid beta (25–35) oligomer-induced toxicity. Neurosci Lett, 2010, 473: 72-76.*
23. *Vanzo, A. et al. Uptake of grape anthocyanins into the rat kidney and the involvement of bilitranslocase.Mol Nutr Food Res, 2008, 52: 1106-1116.*
24. *Zhou, B. et al. Evidence for alpha-tocopherol regeneration reaction of green tea polyphenols in SDS micelles. Free Radic. Biol. Med. 2005, 38: 78-84.*
25. *Systematics of plants, Ed M. Popova, Plovdiv, 2012, p. 193-194.*
26. *Flora of Bulgaria,Vol 10, Eds S. Kozhuharov and B. Kuzmanov,AI "Prof. M. Drinov",Sofia, 1995, p. 294-297.*
27. *European Pharmacopoeia Commission. European Pharmacopoeia 8th ed. Bilberry fruit, fresh (Myrtilli fructus recens), Strasbourg: Council of Europe, European Directorate for the Quality of Medicines and Health Care, 2014, p 1173.*
28. *Lätti, A. K. et al. Phenolic compounds in berries and flowers of a natural hybrid between bilberry and lingonberry (Vaccinium x intermedium Ruthe).Phytochemistry, 2011, 72: 810-815.*
29. *Rui, Li et al. Anthocyanin composition and content of the Vaccinium uliginosum berry. Food Chem, 2011, 125: 116-120.*
30. *Latti, A.K.et al. Characterization of anthocyanins in caucasian blueberries (Vaccinium arctostaphylos L.) native to Turkey. J. Agric. Food Chem, 2009, 57: 5244-5249.*
31. *Nickavar, B. et al. Anthocyanins from Vaccinium arctostaphylos Berries. Pharm. Biol, 2004, 42: 289- 291.*
32. *Kader, F. et al. Fractionation and identification of the phenolic compounds of Highbush blueberries (Vaccinium corymbosum, L.). Food Chem, 1996, 55(1) 35-40.*

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33. Chen H. et al. Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. J Chromatogr A, 2001, 913(1-2): 387-395.
34. Heleno, S. A. et al. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. Food Chem, 2015, 173: 501-513.
35. Szajdek, A., Borowska, E. J. Bioactive Compounds and Health-Promoting Properties of Berry Fruits: A Review. Plant Food Hum Nutr, 2008, 63(4): 147-156.
36. Laaksonen, O., Sandell, M., Kallio, H. Chemical factors contributing to orosensory profiles of bilberry (Vaccinium myrtillus) fractions. Eur Food Res Technol, 2010, 231: 271-285.
37. Spela,M et al.Phenolics in Slovenian Bilberries (Vaccinium myrtillus L.) and Blueberries (Vaccinium corymbosum L.). J AgricFood Chem, 2011, 59: 6998-7004.
38. Lietti, A. et al. Studies on Vaccinium myrtillus anthocyanosides. I. Vasoprotective and antiinflammatory activity. Arzneim.-Forsch, 1976, 26: 829-832.
39. Andersen, O.M. Chromatographic separation of anthocyanins in cowberry (lingonberry) Vaccinium vites-idaea L. J Food Sci, 1985,50: 1230-1232.
40. Suomalainen, H., Keranen, A.J. A.The first anthocyanins appearing during the ripening of blueberries. Nature, 1961, 191: 498-499.
41. Ek, S.et al. Characterization of phenolic compounds from lingonberry (Vaccinium vitis-idaea). J Agric Food Chem, 2006, 54: 9834-9842.
42. Hokkanen, J. et al. A. Identification of phenolic compounds from lingonberry (Vaccinium vitis-idaea L.), bilberry (Vaccinium myrtillus L.) and hybrid bilberry (Vaccinium intermedium Ruthe L.) leaves. J. Agric. Food Chem, 2009, 57: 9437-9447.
43. Laetti, A. K. et al. Phenolic compounds in berries and flowers of a natural hybrid between bilberry and lingonberry (Vaccinium intermedium Ruthe). Phytochemistry, 2011, 72: 810-815.
44. Pan, Y.F. et al. Qualitative and quantitative analysis of flavonoid aglycones from fruit residue of Vaccinium vitis-idaea L. by HPLC. Nat Prod Res Develop, 2005, 17: 642- 644, 641.
45. Li, R. et al. Anthocyanin composition and content of the Vaccinium uliginosum berry. Food Chem, 2011, 125: 116-120.
46. McDougall, G. J. et al. Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. JAgricFood Chem, 2005, 53: 2760-2766.
47. McDougall, G. J., D. Stewart, The inhibitory effects of berry polyphenols on digestive enzymes. BioFactors, 2005, 23: 189-195.
48. Wang, S. I. et al.Antioxidant capacity and a-glucosidase inhibitory activity in peel and flesh of blueberry (Vaccinium spp.) cultivars.Food Chem,2012, 132: 1759-1768.
49. Adisakwattana, S., Charoenlertkul, P.,Anun, S.α-Glucosidase inhibitory activity of cyanidin-3-galactoside and synergistic effect with acarbose. JEnzyme Inhib Med Chem, 2009, 24: 65-69.
50. Xiao, J. B.,Högger, P.Dietary polyphenols and type 2 diabetes: current insights and future perspectives.Curr Med Chem, 2015, 22: 23-38.
51. Wolfe, K. L., Liu, R. H. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. J AgricFood Chem, 2007, 55: 8896-8907.
52. Wang, H. et al. Oxygen Radical Absorbing Capacity of Anthocyanins.J AgricFood Chem, 1997, 45: 304-309.