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Research Article

Phytochemical composition and biological activity of bilberry fruit juice (*Vaccinium myrtillus* L.) from Bulgaria*

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Abstract

This study examined the polyphenol composition, antioxidant properties, and biological activity of bilberry (*Vaccinium myrtillus* L.) fruit juice from Bulgaria. Antioxidant potential was analyzed using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) methods. Total polyphenol, flavonoid, and anthocyanin contents were measured using colorimetric methods. Specific anthocyanin content was measured by UPLC-MS/MS. Cytoprotective and superoxide anion radical-scavenging activities were analyzed in *in vitro Saccharomyces cerevisiae* experiments. UPLC-MS/MS analysis revealed a total anthocyanin content of 338.5 mg/100 g fresh fruits, and the dominant compounds were malvidins (38.1%), delphinidins (33.2%), petunidins (18.6%), and cyanidins (10.1%). Total polyphenol content was 8.40 ± 0.29 mg GAE/100 mL, and total flavonoid content was 7.26 ± 0.11 mg QE/100 mL. Antioxidant potential was as follows: ABTS (3.6 ± 0.08 mM UAE), DPPH (89 ± 2.1%), and FRAP (2.4 ± 0.09 mM Fe(II)/L). Cell-based assays demonstrated significant cytoprotective activity and reduced methyl methanesulfonate-induced superoxide anions. These results demonstrate the value of Bulgarian bilberry juice as a source of bioactive compounds and as a potential raw material for the pharmaceutical and nutritional industries.

Keywords

antioxidants, anthocyanins, bilberry, cytoprotective, polyphenols, *Vaccinium myrtillus*

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PENSOFT

Introduction

The European blueberry (*Vaccinium myrtillus* L.) of the *Ericaceae* family is a medicinal and honey plant, and the fruits are used for food. It is a small shrub with a height of up to 40 cm. Its stem is strongly branched, with greenish-brown bark. Leaves are alternate, nearly sessile or shortly petiolate to 1 mm, ovate, gradually acuminate, 1–3 cm long and 0.6–1.2 cm wide, glabrous, acutely toothed, and with a pointed tip. The flowers are arranged singly on 2.5–4.0 mm-long pedicels in the axils of the upper leaves. The fruit is a spherical, dark blue, juicy berry with a dark red fleshy part, 0.5–0.7 cm in diameter. It is found in stony and rocky meadows, in sparse coniferous forests, and less often in broad-leaved forests, as well as in mountainous areas. It is widespread in Europe, Asia Minor, the Caucasus, and Siberia.

Blueberries have been used for centuries in traditional medicine in Bulgaria and many countries worldwide. They exhibit astringent, anti-inflammatory, and hypoglycemic effects. The stems, leaves, and fruits of the blueberry are used. The stalks are collected in the summer from massifs where the plants do not bear fruit, in dry weather, during flowering, when the leaves are well developed and preserved. The leaves are picked from the collected and partially dried stalks. The fruits are gathered by hand in the summer (July–September) when they are fully ripe. An infusion of the fruits or leaves is used as an effective remedy for gastrointestinal diseases, including gastritis, ulcers, colitis, chronic diarrhea, and hemorrhoids, as well as for inflammatory processes in the oral cavity, such as gingivitis, aphthae, sore throats, and pharyngitis. The fruits are recommended for improving eyesight due to their high anthocyanin content (Kubov et al. 2024).

Bilberries contain a high concentration of trace elements, vitamins, organic acids, fiber, sugars, and phenolic compounds, primarily anthocyanins, which confer functional and nutritional properties (Sarma and Sharma 1999). Bilberry is one of the richest natural sources of anthocyanins—polyphenolic components responsible for the fruit's color. They are potent antioxidants that neutralize radicals and chelate metal ions and are responsible for the plant's health effects. Several reviews summarize and classify the health-beneficial effects of anthocyanins (Kalt et al. 2020; Martău et al. 2023; Negruşier et al. 2024).

Colantuoni et al. (1991) established their beneficial effect on blood vessels and circulation experimentally in hamsters. Denev et al. (2014) studied the effect of bilberry and five other polyphenol-rich fruits on experimental myeloid tumors in hamsters.

However, the data of other authors (Georgieva et al. 2018, 2020) show that the amount of these beneficial ingredients in bilberries can vary significantly by region. They found that the content of total anthocyanins in Bulgarian bilberry fruits from Velingrad was 217 mg/100 g, which was higher than that in those from Troyan – 162.4 mg/100 g. The content of total polyphenols in bilberry from Velingrad was 940.3 mg/100 g, which was also higher than that in bilberries from Troyan – 893.1 mg/100 g. These differences in the content of anthocyanins and polyphenols account for the higher antioxidant capacity of bilberries from Velingrad compared to those from Troyan. The content of individual anthocyanins (delphinidin 3-glucoside, delphinidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-glucoside, cyanidin 3-galactoside, peonidin 3-galactoside, peonidin 3-glucoside, malvidin 3-glucoside, malvidin 3-galactoside, malvidin 3-arabinoside, petunidin 3-galactoside, and petunidin 3-arabinoside) in bilberry fruits from Velingrad was also higher than that in those from Troyan. Other studies demonstrate that environmental conditions influence the anthocyanin content and antioxidant properties of Bulgarian bilberry fruits (Georgieva et al. 2018; Georgieva et al. 2020).

The successful use of this plant in the treatment and prevention of diabetes is also due to the high content of anthocyanins, which are effective in improving glycemic control. Due to the hypoglycemic effect of bilberries, they are considered a plant remedy that helps control or prevent type 2 diabetes. Asgary et al. (2016) found experimentally that dietary supplementation with bilberry fruit can protect against glucose and lipid metabolism impairment in diabetes mellitus. Adding bilberries to the diet of experimental animals resulted in a significant reduction in blood glucose compared to treatment with glibenclamide. It also increased insulin and decreased cholesterol levels (total, LDL, and VLDL) and triglycerides. In addition, bilberries are often used to improve vision, but they also have anti-inflammatory and lipid-lowering effects, as well as lower oxidative stress. Therefore, they are used in the treatment or prevention of inflammation, dyslipidemia, hyperglycemia, cardiovascular disease, cancer, diabetes, dementia, and other age-related diseases, as well as for the treatment of gallstones, bladder stones, cough, scurvy, and pulmonary tuberculosis. Bilberry also exhibits antimicrobial activity, as well as other beneficial properties, including anticancer, anti-inflammatory, cardioprotective, neuroprotective, and antioxidant effects, primarily due to its rich content of bioactive anthocyanins, particularly delphinidins and cyanidins. It is a reliable natural remedy to combat chronic and infectious diseases in the aging population (Chu et al. 2011; Chehri et al. 2022).

The present work aimed to study the antioxidant content of Bulgarian bilberry fruits collected in 2024 in the Belmeken region of the Rila Mountain and to perform spectral analysis of their properties.

Materials and methods

Plant material and crude extract preparation

Fresh bilberry fruits (*Vaccinium myrtillus* L.) were collected in 2024 in the region of Belmeken in the Rila Mountain in Bulgaria. For the determination of total polyphenols, total flavonoids, and antioxidant potential, fresh bilberry fruits were squeezed, and the juice was filtered and frozen for further analyses.

Total polyphenol content measurement

The total polyphenol content was measured using the Folin-Ciocalteu reagent-based method as follows: 7 µL of the sample was added to 1 mL of Folin-Ciocalteu reagent, followed by the addition of 800 µL of Na_2CO_3 and incubation at 50 °C for 10 min. Absorbance was measured at 760 nm (Camspec M501, Leeds, UK). Measurements were performed at least in triplicate. The total polyphenol content was expressed as mg gallic acid equivalents (GAE)/100 mL ± standard deviation (SD).

Total flavonoid content measurement

Total flavonoid content was determined with the aluminum chloride colorimetric assay. A 10 µL sample was added to 1 mL of H_2O, followed by 75 µL of 5% $NaNO_2$. The mixture was then incubated for 5 min, after which 75 µL of 10% $AlCl_3$ was added, and the mixture was incubated for an additional 6 min. Finally, 500 µL of 1 M NaOH was added, and absorbance was measured at 510 nm (Synergy 3, BioTek, Winooski, VT, USA) (Chang et al. 2002; Koleva-Valkova et al. 2025). Measurements were performed at least in triplicate. The total flavonoid content was expressed as mg quercetin equivalents (QE)/100 mL ± standard deviation (SD).

Antioxidant potential measurement

Radical scavenging activity

Radical scavenging activity was determined using two methods: the ABTS cation radical decolorization assay and the DPPH decolorization assay (Ivanova et al. 2014).

For the ABTS assay, 10 µL of the sample was added to 1 mL of the ABTS radical solution, and absorbance was measured at the time points 0 min and 6 min at 734 nm (Camspec M501, Leeds, UK). Absorbance was calculated as follows:

$$A = (A_{0\ min} - A_{6\ min})\text{-}A_{blank}, \text{ where}$$

$A_{0\ min}$ is the absorbance of the radical solution at time point 0 min;

$A_{6\ min}$ is the absorbance of the radical solution after the addition of the sample at time point 6 min;

A_{blank} is the absorbance ($A_{0\ min} - A_{6\ min}$) of PBS.

Measurements were performed at least in triplicate. ABTS radical scavenging activity was expressed as mM uric acid equivalents (UAE).

For the DPPH assay, 10 µL of the sample was added to 1 mL of 50 µM DPPH radical solution in ethanol. The mixture was incubated in the dark for 30 min, and absorbance was measured at 517 nm using a Camspec M501 spectrophotometer (UK). Measurements were performed at least in triplicate, and DPPH radical scavenging activity was expressed as follows:

$$\%\text{DPPH scavenging} = [(A_{control}\text{-}A_{sample})/A_{control}]\times 100$$

where:

A_{sample} is the absorbance of DPPH solution with the added sample;

$A_{control}$ is the absorbance of the DPPH solution without sample.

Ferric reducing antioxidant potential (FRAP)

For ferric reducing antioxidant potential (FRAP) measurement, 2 µL of the sample was added to 1 mL of FRAP reagent, and after 4 min of incubation at 37 °C, absorbance was measured at 593 nm (Synergy 3, BioTek, Winooski, VT, USA). FRAP activity was expressed as mM Fe(II)/L ± SD. Measurements were performed at least in triplicate (Ivanova et al. 2014).

Cell-based quantitative determination of superoxide anions in live cells

The cytoprotective and antioxidant potential of bilberry juice was tested on the model organism *Saccharomyces cerevisiae*. Briefly, cell suspensions in the exponential phase of growth were treated for 30 min with two concentrations of the juice, 1% and 10% (diluted in sterile deionized water), to evaluate the potential cytotoxic or pro-oxidant activity. Methyl methanesulfonate (MMS) at a concentration of 16 mM was used as a positive control. Furthermore, combined treatment with the two concentrations of the juice and MMS was performed to determine the cytoprotective and antioxidant properties of bilberry juice (Valentova et al. 2006; Ciulca et al. 2021). The cytotoxic or cytoprotective effect was determined based on the cell survival percentage. The pro-oxidant or antioxidant potential was established by measuring the quantity of physiologically active superoxide anions in live cells (Stamenova et al. 2008; Todorova et al. 2025). Briefly, the methodology is related to the ability of XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) (Thermo Fisher Scientific, Waltham, MA, USA) to enter living cells only and to be reduced by superoxide anion radical ($O_2\bullet-$) to water-soluble, orange-colored formazans.

Anthocyanin extraction

An Ultimate 3000 UPLC system and a Hypersil GOLD™ chromatographic column (2.1 × 200 mm, 1.9 µm) (Thermo Scientific, Waltham, MA, USA) were used to separate different forms of anthocyanins. A solid-phase extraction (SPE) column, SOLA HRP 10 mg (Thermo Scientific, Waltham, MA, USA), was used to purify the samples before UPLC-MS analysis for MS/MS detection of different forms of anthocyanins. The LTQ Orbitrap XL (Thermo Scientific, Waltham, MA, USA) was used for this purpose.

LC-MS-grade water, acetonitrile, and formic acid were purchased from Thermo Fisher Scientific, Waltham, MA, USA. The anthocyanins were purchased from Sigma-Aldrich (St. Louis, MO, USA).

To extract anthocyanins, 5 g of fruit was mixed with 10 mL of 80% methanol in five samples. After homog-

enization, the mixture was sonicated for 20 min on an ultrasonic instrument, Elmasonic S30H (Elma, Germany). After centrifugation at 10,000 × *g* for 5 min, the supernatant was decanted, and the extraction was repeated with 10 mL of 80% methanol. The combined supernatants were evaporated. The residue was dissolved in 5% methanol containing 1% formic acid, and the volume was adjusted to 5 mL.

On the activated SPE cartridge, 0.5 mL of the sample was applied. The SPE cartridge was rinsed twice with 1 mL of water. The anthocyanins were eluted with 0.5 mL of methanol containing 0.1% formic acid. After evaporation at 40 °C under a nitrogen flow, the residue was dissolved in 1 mL of 5% methanol containing 0.1% formic acid. After filtration through a 0.22 µm polyvinylidene fluoride filter, 2 µL was loaded onto the UPLC-MS/MS.

UPLC conditions

Eluent A was 0.1% formic acid in water, and eluent B was 0.1% formic acid in acetonitrile. The gradient elution mode was as follows: eluent B: 5% (0 min), 5% (2 min), 20% (15 min), 40% (20 min), 80% (22 min), 80% (25 min), 5% (27 min), and equilibration for 10 min.

MS/MS conditions

Electrospray ionization was employed in positive ion detection mode with multiple reaction monitoring. The temperature of the ion source was 300 °C, and the ion transfer capillary temperature was 350 °C. Ion fragmentation was performed by high-energy collision dissociation (HCD) at 45 V with nitrogen as the collision gas.

Statistical analysis

Statistical analyses were performed using GraphPad 6 software. In the cell-based data, the results are from three independent experiments, and the mean value ± SEM is presented. Student's *t*-test was used to compare treatment groups in *in vitro* experiments.

Results

Polyphenol contents

Results on the concentration of polyphenols and flavonoids in bilberry fruit extract are presented in Table 1.

Table 1. Total polyphenol, total flavonoid, and total anthocyanin contents in *V. myrtillus* fruit juice.

Analysis	Concentration (mean ± SD, $n = 3$)
Total polyphenols [mg GAE/100 mL]	8.40 ± 0.29
Total flavonoids [mg QE/100 mL]	7.26 ± 0.11
Total anthocyanins [mg/L]	338.54 ± 4.52

The total polyphenol content of bilberry juice was 8.40 ± 0.29 mg GAE/100 mL, and the total flavonoid content was 7.26 ± 0.11 mg QE/100 mL (mean ± SD, $n = 3$). The average total content of measured anthocyanins in the bilberry extract was 338.54 mg/L of fruit juice.

Antioxidant potential

Data from triplicate measurements of the antioxidant potential of bilberry fruit juice are presented in Table 2.

The bilberry fruit juice demonstrated strong antioxidant potential, as evidenced by high ABTS and DPPH radical-scavenging activities, as well as FRAP potential.

Table 2. Antioxidant potential of Bulgarian bilberry fruit juice.

Analysis	Activity (mean ± SD, $n = 3$)
ABTS [mM UAE]	3.6 ± 0.08
DPPH [% scavenging]	89.0 ± 2.1
FRAP [mM Fe(II)/L]	2.4 ± 0.09

Anthocyanin analysis

Table 3 presents MS/MS transitions for various anthocyanins analyzed for their presence in Bulgarian bilberry extract.

Among the different forms of anthocyanins, malvidins (38.08%) and delphinidins (33.15%) dominated compared to petunidins and cyanidins (Table 4). Most anthocyanins contained galactoside groups, followed by those with glucosidic groups.

The total anthocyanin content (338.54 mg/100 g fresh fruits) was calculated as the sum of individual anthocyanin groups quantified by UPLC-MS/MS, confirming malvidins and delphinidins as the dominant compounds in Bulgarian bilberries.

Table 3. MS/MS transitions for anthocyanins.

Compound	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)
Malvidin-3-glu/gal	493.2	331.1
Malvidin-3-ara	463.2	331.1
Delphinidin-3-glu/gal	465.2	303.1
Delphinidin-3-ara	435.2	303.1
Petunidin-3-glu/gal	479.2	317.1
Petunidin -3-ara	449.2	317.1
Cyanidin-3-glu/gal	449.2	287.1
Cyanidin-3-ara	419.2	287.1

Abbreviations: glu = glucoside; gal = galactoside; ara = arabinoside.

Table 4. Concentration of anthocyanin groups in bilberry based on five different measurements.

Anthocyanins	Concentration [mg/100 g]
Malvidins	128.9 ± 6.4
Delphinidins	112.2 ± 5.6
Petunidins	63.0 ± 3.1
Cyanidins	34.4 ± 1.7

Cell survival and quantity of physiologically active superoxide anions (O_2-)

For safety reasons, the first step in this research was to evaluate the potential toxic effects of bilberry juice. Treatment with 1% and 10% bilberry juice did not reduce the cell survival of the *Saccharomyces cerevisiae* model system (Fig. 1A). The survival was comparable to that of the control—untreated cells. Based on this, it could be suggested that the juice, together with its constituents, lacks cytotoxicity in this experimental design. Furthermore, both concentrations demonstrated significant cytoprotection against the standard mutagen methyl methanesulfonate (MMS), resulting in a 1.5-fold increase in cell survival ($P < 0.0001$). The effect of both treatments was similar to the control levels. No statistically significant difference was calculated between the treatments.

Regarding the levels of physiologically active superoxide anions, none of the bilberry juice concentrations resulted in the induction of reactive oxygen species (ROS). All levels were comparable with the control—untreated cells. Conversely, a similar strong positive effect was observed when combined treatment with MMS was performed. Both concentrations of bilberry juice (1% and 10%) showed potent antioxidant potential and were able to reduce MMS-induced superoxide anion generation by approximately 5-fold. Thus, superoxide anions generated in bilberry juice-treated groups were indistinguishable from the control group levels (Fig. 1B). The procedure for the quantification of superoxide anions (O_2-) is described in Todorova et al. (2025). No effect of the concentration was observed.

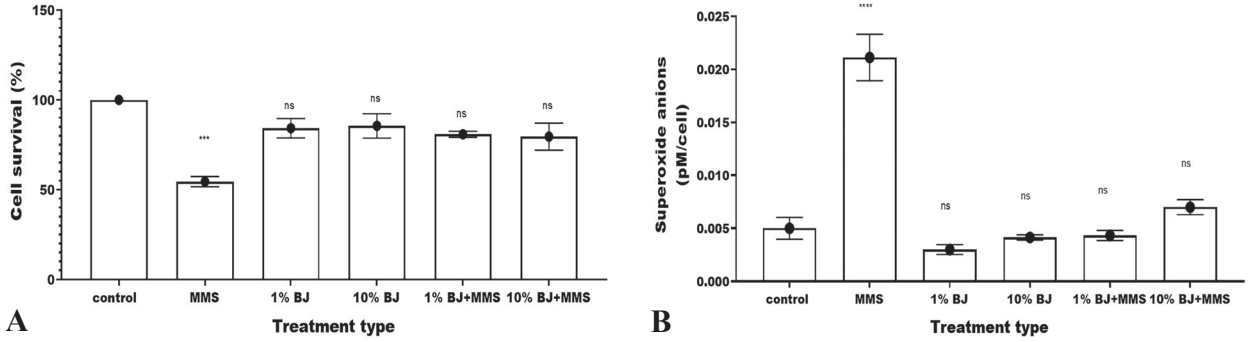


Figure 1. Cell survival (**A**) and levels of superoxide anions (**B**), measured after the treatment of *Saccharomyces cerevisiae* strain 551 with 1% and 10% bilberry juice, as well as combined treatment with each concentration together with methyl methanesulfonate (MMS). Treatment was as follows: control – untreated cells; MMS – treatment with MMS; 1% BJ – treatment with 1% bilberry juice; 10% BJ – treatment with 10% bilberry juice; 1% BJ + MMS – treatment with 1% bilberry juice and MMS; 10% BJ + MMS – treatment with 10% bilberry juice and MMS. The statistical significance of differences was evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. Asterisks represent statistical significance (ns $P > 0.05$; *** $P < 0.001$; **** $P < 0.0001$). Where no error bars are evident, they are equal to or smaller than the values. Abbreviations: BJ = bilberry juice; MMS = methyl methanesulfonate.

Discussion

In this study, *V. myrtillus* fruit extract was analyzed for its antioxidant content, with an emphasis on anthocyanins. Fruits were collected from the Belmeken region in the Rila Mountain in Bulgaria. The analyses revealed a total polyphenol content of 8.40 mg GAE/100 mL and a total flavonoid content of 7.26 mg QE/100 mL. Among the different forms of anthocyanins, malvidins (38.08%) and delphinidins (33.15%) dominated compared to petunidins and cyanidins (Table 4). The concentration of malvidins was similar to that reported in other studies, while the delphinidin results were slightly higher than those in other studies (Ciulca et al. 2021). Most anthocyanins contained galactoside groups, followed by those with glucosidic groups, consistent with other studies (Ștefănescu et al. 2020). The total anthocyanin content in this sample was higher (338.54 mg/100 g) than in other sources, e.g., 143.9 mg/100 g for commercially available bilberry juices (Diaconeasa et al. 2015) and 297.4 mg/100 g (Calò and Marabini 2014). *V. myrtillus* from Finland has been established to contain 206.18–867.52 mg/100 g anthocyanins (Colak et al. 2017). The content of anthocyanins in bilberry fruits tested in this study was higher than previously established by other authors (Georgieva et al. 2018; Georgieva et al. 2020) in different regions of Bulgaria. Accordingly, the amount of these beneficial ingredients can vary significantly by region. Regional and climatic factors in different years are probably crucial for this. These results confirm that bilberries are an excellent source of polyphenols, flavonoids, and anthocyanins. Water and methanolic extracts from bilberry fruits have been previously found to be rich in polyphenols and to exert antioxidant activity in the ABTS assay (Kiselova et al. 2005). Bilberry secondary metabolites have been proven as potent antioxidants capable of neutralizing ROS in intestinal cells, as confirmed by radical-scavenging assays (Novi et al. 2025). Spectrometric analyses demonstrated strong radical-scavenging activity of an anthocyanin-rich *Vaccinium myrtillus* fruit extract, confirmed by DPPH assays identifying delphinidin, petunidin, and cyanidin gly-

cosides as the most active constituents. In the same study, anthocyanins have been suggested as major contributors to the antioxidant capacity of the extracts (Fraisse et al. 2020). Anthocyanins are well-known plant-derived antioxidants. Numerous studies have demonstrated their ability to cope with oxidative stress. The high anthocyanin content in bilberries contributes to their use as functional foods against oxidative stress. Some authors have experimentally demonstrated, both *in vitro* and *in vivo*, that bilberry extracts protect cells from oxidative damage (Valentova et al. 2006; Bao et al. 2010). However, no significant changes in biomarkers of antioxidant status or oxidative stress have been reported in humans after the ingestion of bilberry juice (Karlsen et al. 2007). This is explained by the fact that many bilberry extracts used in trials were not characterized or standardized for anthocyanin content. Chu et al. (2011) pointed out that the positive effect of antioxidant phytochemicals may be independent of or indirectly related to antioxidant activity. It can vary depending on the anthocyanin concentration or may only appear in other bioactive substances or under specific redox conditions.

Chronic inflammation increases the reactive oxygen species (ROS) burden and is a significant risk factor for cancer development (Aggarwal et al. 2009). Anthocyanins from berries exhibit preventive and anticancer properties through their antioxidant action, as well as anti-inflammatory, anti-proliferative, anti-angiogenic, apoptotic, and other cytoprotective effects (Lala et al. 2006; Zafra-Stone et al. 2007; Wang and Stoner 2008; Seeram 2009; Matsunaga et al. 2010). The cytoprotective and antioxidant activity of anthocyanin-rich bilberry juice was confirmed in these experiments. Direct interaction of anthocyanins with DNA is another mechanism of genoprotection. Natural anthocyanins have been reported to bind to DNA, forming a DNA copigmentation complex. Thus, anthocyanins may regulate gene expression and protect DNA against oxidative damage (Sarma and Sharma 1999; Mas et al. 2000). Anthocyanins have been shown to protect DNA against oxidative stress induced by various agents (UV irradiation, hydrogen peroxide, tert-butyl hydroperoxide) *in vitro* (Seeram 2008; Svobodová et al. 2009). However, it should be noted that the doses used (100 μM or higher) in *in vitro* studies are significantly higher than those reached in plasma through dietary intake of anthocyanins. The chemopreventive and therapeutic effects of anthocyanins and berries against cancer have been tested in various oral experimental animal models. Although conclusive evidence from human studies is lacking, there is sufficient evidence from *in vitro* and animal experiments with anthocyanins demonstrating their chemopreventive effects against cancer (Choi et al. 2007; Seeram 2008; Thomasset et al. 2009). Bao et al. (2008) experimentally demonstrated the beneficial health effects of bilberry extract due to its antioxidant activity. Its hepatoprotective effect was demonstrated after 5 days of oral administration of bilberry extract containing 42.04% anthocyanins to mice. This extract protects against stress-induced liver damage by neutralizing free radicals and inhibiting lipid peroxidation. Faria et al. (2005) determined the content of polyphenols, flavonoids, and anthocyanins in bilberry extract for the first time. They found that these substances protect membranes against peroxide radicals and the extracts' antiradical properties, thereby reducing the radicals' ability to cause damage. However, anthocyanin pigments are easily degraded during industrial processing, which affects bilberries' color and nutritional and pharmaceutical properties. It is necessary to apply technological strategies to provide active ingredients with excellent stability and solubility (Antonella et al. 2014).

Bilberry anthocyanin-rich extracts prevented lipid peroxidation in UVB-irradiated keratinocytes and reduced UVA-triggered genotoxicity (Calò and Marabini 2014). In neuronal cells, *V. myrtillus* extracts increased cell viability, reduced the products of oxidative damage, and increased the activity of antioxidant enzymes in the H_2O_2 treatment model (Cásedas et al. 2018). In rats, *V. myrtillus* anthocyanins have been shown to have neuroprotective effects mediated by their antioxidant and anti-apoptotic properties (Ozdemir et al. 2023).

Conclusion

The average total content of anthocyanins in bilberry fruits (*Vaccinium myrtillus* L.), collected in 2024 in Bulgaria and determined by chromatographic methods, was 338.54 mg/100 g fresh fruit. This defines bilberry as an excellent natural source of anthocyanins. Among the different anthocyanins, malvidins (38.08%) and delphinidins (33.15%) dominated compared to petunidins and cyanidins. *V. myrtillus* juice demonstrated radical-scavenging and reducing potential, representing strong antioxidant activity. These results, together with the data on cytoprotective properties and prevention of radical formation in yeast cell culture, demonstrate the nutritional value of Bulgarian bilberry juice as a functional food.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
No AI tools were used in the preparation of this manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.