Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 613 - 614 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.296
Short communication

Quality assessment of Macedonian wild bilberries (*Vaccinium myrtillus* L.) based on anthocyanin content and HPLC fingerprint

Jovan Madevski*, Filip Todorov, Veronika Stoilkovska Gjorgievska, Iskra Davkova, Ana Trajkovska Matevska, Ivana Cvetkovikj Karanfilova, Marija Karapandzova, Gjoshe Stefkov

Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Teresa 47, 1000 Skopje, North Macedonia

Introduction

Berries have become increasingly important in the global fruit market due to their sensory properties and reported health benefits. Bilberries (*Vaccinium myrtillus*, fam. Ericaceae) are particularly valued for their bioactive polyphenolic compounds, especially anthocyanins. These components contribute to beneficial biological effects, including improved blood glucose levels, cardioprotective and neuroprotective properties, underlining the high market demands.

Bilberries thrive on heathlands and meadows and pastures on acidic silicate soils, covering vast areas in Europe, especially in Scandinavian and Balkan countries. North Macedonia has substantial native bilberry resources contributing to the domestic market trade but also exports to international markets (Stefkov et al., 2025).

The quality assessment of bilberries (Vaccinium myrtillus L.) is mainly based on the determination of anthocyanin content and profile, as well as chromatographic fingerprinting for authenticity and adulteration control. HPLC and spectrophotometric methods are commonly applied for the identification and quantification of anthocyanins and anthocyanidins. The European Pharmacopoeia establishes specific requirements for bilberry fruits and standardized extracts, including minimum limits for total anthocyanins expressed as cyanidin-3-glucoside, limits for anthocyanidins as indicators of degradation, and characteristic chromatographic profiles to confirm identity and quality of the raw material and extracts.

The aim of this study was to evaluate the total anthocyanin content and anthocyanin composition of fresh wild bilberry fruits collected from different mountain regions throughout North Macedonia, thereby contributing to the quality characterization of this important non-timber forest product for the Macedonian local mountain economy.

Materials and methods

Plant material

Fresh bilberry fruits were collected from seven mountain ranges - main sources which supply the bilberry market in North Macedonia: National Park Shar Planina (NPSP), Karadzica (Ka), National Park Pelister (NPPe), Osogovo (Os), Ograzden (Og), Belasica (Be) and Kozuf (Ko). Approximately two kilograms of fresh bilberry fruits were collected per location and were further processed.

*madevski.j@gmail.com

Determination of anthocyanin content in fresh bilberry fruits according to European Pharmacopeia

Fresh bilberry fruits were extracted and analysed for total anthocyanins according to the procedure described in the monograph for Fresh Bilberry Fruit (*Myrtilli fructus recens,* 01/2019:1602) (European Pharmacopoeia, 2025). Varian Cary 50 UV-Vis Spectrophotometer was used and the anthocyanin content was expressed as cyanidin-3-*O*-glucoside chloride equivalents (%).

Identification of anthocyanins in bilberry fruits extract

Anthocyanin profile of the extract was determined according to the liquid chromatography procedure in Fresh Bilberry Fruit Dry Extract monography 04/2019:2394 (European Pharmacopoeia, 2025). To obtain a dry bilberry extract, bilberry paste was extracted twice with methanol (0,25% HCl, 1:25 ratio) using ultrasound at <30 °C for 20 minutes. The extracts were evaporated to dry extract under reduced pressure. The extracts were analysed on Agilent 1200 model HPLC system with DAD detector.

Results and discussion

The total anthocyanin content of fresh bilberry fruits collected from seven mountain ranges in North Macedonia ranged from 0.42% to 0.73%. The highest contents were determined in samples from Ko (0.73%) and NPPe (0.70%), followed by Ka (0.62%), NPSP (0.56%), and Og (0.52%), while lower values were observed for Be (0.45%) and Os (0.42%). Since all analysed samples exceeded the minimum requirement of 0.30% total anthocyanins prescribed by the European Pharmacopoeia monograph, the investigated bilberry fruits can be considered compliant with pharmacopoeial quality criteria and suitable as high-quality raw material for different applications.

Qualitative analysis performed according to the European Pharmacopoeia monograph for Fresh Bilberry Fruit Dry Extract revealed the presence of all 20 characteristic anthocyanins in every analysed extract. Dominant anthocyanins were delphinidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside. This finding confirms the authenticity of the investigated bilberry samples and demonstrates a phytochemical profile fully consistent with the pharmacopoeial requirements. The complete anthocyanin fingerprint further indicates that the fruits originate from genuine bilberry populations and have not undergone adulteration or substitution with other Vaccinium species.

The obtained results are in agreement with previous investigations of North Macedonian bilberries. Stanoeva et al. (2017) reported anthocyanins as the dominant phenolic group in bilberry fruits, accounting for approximately 72% of the total phenolic composition. In the same study, thirteen anthocyanins were identified, with a total anthocyanin content of 507 mg/100 g fresh weight, and petunidin-3-O-glucoside was found to be the predominant anthocyanin, while peonidin derivatives were present in relatively low amounts.

Conclusion

All studied wild bilberries from North Macedonia complied with European Pharmacopoeia requirements, exhibiting high anthocyanin contents and a complete pharmacopoeial anthocyanin profile. These findings reveal their quality thus highlighting the significant potential of Macedonian wild bilberries for nutritional, commercial, and pharmaceutical applications.

References

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