PHYTOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT POTENTIAL OF *VACCINIUM MYRTILLUS L.* A COMPARATIVE STUDY OF LEAVES AND FRUITS

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RESEARCH ARTICLE

Abstract

Bilberry (Vaccinium myrtillus L.) is a medicinally valuable shrub whose leaves (Myrtilli folium) and fruits (Myrtilli fructus) are rich sources of bioactive compounds. This study aimed to comparatively evaluate the phytochemical composition and antioxidant potential of ethanolic extracts obtained from leaves and fruits. Plant material was collected from spontaneous flora in Bihor County, Romania, and subjected to Soxhlet extraction. Total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively, while antioxidant activity was assessed via DPPH radical scavenging assay. The results indicated that leaf extracts contained a significantly higher total phenolic content (119.64 ± 1.014 mg GAE/g) and total flavonoid content (19.89 ± 0.195 mg QE/g) compared to fruit extracts (34.12 ± 0.895 mg GAE/g and 11.76 ± 0.180 mg QE/g, respectively). Correspondingly, DPPH radical scavenging activity was higher in leaves (76.56 ± 0.505%) than in fruits (61.74 ± 0.391%). These findings align with literature data and underscore the superior bioactive potential of leaves. The observed differences between organs reflect organ-specific metabolic allocation, with leaves likely accumulating phenolics for protection against environmental stress, while fruits prioritize compounds associated with reproductive functions. The study highlights Vaccinium myrtillus leaves as a promising source of natural antioxidants, with potential applications in nutraceuticals, functional foods, and therapeutic interventions targeting oxidative stress-related disorders.

Keywords: bilberry, ethanolic extract, polyphenol content, flavonoids, DPPH radical scavenging
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INTRODUCTION

Vaccinium myrtillus L. (*Ericaceae*), commonly known as bilberry, is a subfruticose shrub widely distributed in the mountainous regions of Europe, from the lower limits of coniferous forests up to alpine zones, where it forms dense clusters called bilberry patches. Both the leaves (*Myrtilli folium*) and the fruits (*Myrtilli fructus*) have been traditionally valued for their medicinal properties, including antidiabetic, hypolipidemic, antibacterial, antiviral, anticancer, and anti-inflammatory effects (Brasanac-Vukanovic et al., 2018; Chan and Tomlinson, 2020; Pires et al., 2020).

Previous studies have shown that the bioactivity of bilberry extracts is largely attributed to their polyphenolic content. In leaves, phenolic acids such as chlorogenic acid, caffeic and quinic acid derivatives, and feruloylquinic acid predominate, whereas fruits are especially rich in flavonoids, particularly anthocyanins, responsible for their red, blue, and violet pigmentation (Popović et al., 2016; Scalzo et al., 2015).

The antioxidant potential of bilberry extracts has been closely correlated with their phytochemical profile, with leaf extracts often demonstrating higher radical-scavenging activity than fruit extracts from the same species. This activity has been linked to protective effects against oxidative stress-related disorders, improvement of lipid profiles, and vascular health through endothelial stabilization and enhanced collagen and mucopolysaccharide biosynthesis in capillaries (Bljajić et al., 2017; Faleva et al., 2024).

The present study aims to comparatively evaluate the antioxidant potential of leaf and fruit extracts of *Vaccinium myrtillus L.*, using total phenolic and flavonoid content as key indicators of bioactivity, thereby providing a comprehensive insight into their functional relevance and therapeutic potential.

MATERIAL AND METHOD

PLANT MATERIAL

Specimens of *Vaccinium myrtillus L.* were collected from natural habitats in Ponoară village, Bratca commune, Bihor County,

Romania (46.891488, 22.682169), an unpolluted area. Leaves were harvested during dry weather in May 2022 using scissors, selecting only healthy, non-flowering branches, while fully ripened fruits were collected manually in July 2022. Only healthy and clean plant material was retained. A pressed voucher specimen is preserved in the Herbarium of the Faculty of Medicine and Pharmacy, Department of Pharmaceutical Botany (code UOP 05.710).

REAGENTS AND EQUIPMENT

Analytical grade reagents were employed throughout the study, including ethanol (Chimreactiv, Romania), distilled water, gallic acid (Silver Chemicals, Romania), quercetin (Silver Chemicals, Romania), DPPH (2, 2 – diphenyl – 1 - picrylhydrazyl, Merck, Germany), Folin–Ciocalteu reagent (Merck, Germany), sodium carbonate (Chimreactiv, Romania), sodium nitrite (Chimreactiv, Romania), aluminum chloride (Merck, Germany), and sodium hydroxide (Chimreactiv, Romania). Extraction and concentration procedures were performed using a rotary evaporator Hei-VAP Advantage (Heidolph, Germany), and absorbance measurements were conducted with a UV-VIS spectrophotometer PG Instruments T70+ (UK).

METHODS

Microscopy: Samples of stem, leaf, and fruit from *Vaccinium myrtillus L.* were examined using an optical microscope (Optika B-380 Series, Italy) with 10× and 40× objectives. Observations focused on the structural characteristics of each organ to document diagnostic features relevant for identification and comparative analysis.

Extraction: Dried leaf and fruit samples of *Vaccinium myrtillus L*. were finely ground to enhance the efficiency of bioactive compound extraction. Ten grams of *Myrtilli folium* were subjected to Soxhlet extraction with ethanol for 4 hours (10 cycles), while an equal amount of *Myrtilli fructus* underwent extraction for 3 hours (11 cycles). The resulting extracts were concentrated using a Hei-VAP Advantage rotary evaporator (Heidolph, Germany) at 40°C, 80 rpm, and 200 atm until a minimal residue remained. Each concentrated extract was subsequently reconstituted in 10 mL of ethanol for further analyses.

Total Phenolic Content: The total phenolic content of ethanolic extracts was measured using the Folin–Ciocalteu method. Extracts (0.3 mL) were mixed with 1.2 mL distilled water and 0.45 mL Folin–Ciocalteu reagent, left in the dark for 10 minutes, then 1.5 mL 20% sodium carbonate was added. After 60 minutes at room temperature, absorbance was recorded at 765 nm (Elferjane et al., 2024). A blank was prepared with ethanol, and all determinations were performed in triplicate. Quantification was based on a gallic acid calibration curve (10–50 mg/mL).

Total Flavonoid Content: Flavonoid content was assessed using the aluminum chloride colorimetric method. A volume of 1 mL extract was mixed with 4 mL distilled water and 0.3 mL 5% $NaNO_2$, allowed to react in the dark for 5 minutes, followed by the addition of 0.3 mL 10% $AlCl_3$ for 6 minutes. Subsequently, 2 mL 1 M NaOH was added, and the final volume adjusted to 10 mL with distilled water. Absorbance was recorded at 510 nm against a blank containing ethanol. Measurements were carried out in triplicate, and quercetin (2–10 mg/mL) was used for calibration (Altiok et al., 2022).

DPPH Radical Scavenging Assay: Antioxidant activity was assessed using a 0.1 mM DPPH solution. Extracts (0.5 mL) were mixed with 3 mL DPPH solution and incubated in the dark for 30 minutes. A blank containing ethanol instead of extract was used. Absorbance was recorded at 517 nm, and the percentage of radical scavenging was calculated. All determinations were performed in triplicate (Ciulca et al., 2021).

RESULTS AND DISCUSSIONS

Microscopic analysis of *Vaccinium myrtillus L.* stem sections reveals the organization of protective, vascular, and mechanical tissues, as illustrated in Figure 1. The stem is composed of the epidermis, cortical parenchyma, and central cylinder. The epidermis consists of a single layer of small, living cells with tightly appressed, slightly convex, cutinized walls. Beneath it, the cortical parenchyma comprises 5–6 layers of thin-walled cells, some containing chloroplasts. The central cylinder, or stele, includes the pericycle, collateral vascular bundles, and medullary parenchyma. Vascular bundles are closed and collateral, with xylem oriented inward and phloem outward, facilitating the transport of sap. Medullary parenchyma occupies the central region and consists of large, thin-walled cells arranged in a rosette.

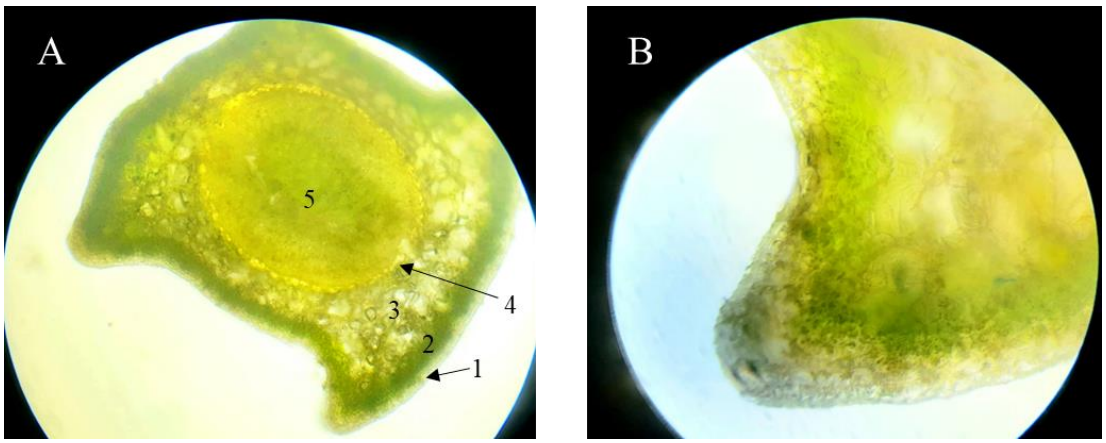

Figure 1. Transverse section of *Vaccinium myrtillus L*. stem.
A – 1-epidermis, 2-cortex, 3-parenchymatous tissue, 4-collateral vascular bundles accompanied by collenchyma arcs, 5-medullary parenchyma (10X); B– rib with collenchymatous tissue (40X)

Microscopic analysis of *Vaccinium myrtillus L.* leaves revealed a unistratified adaxial and abaxial epidermis and a bifacial mesophyll composed of palisade and spongy parenchyma. The palisade parenchyma contains tightly packed, chloroplast-rich cells, while the spongy parenchyma has loosely arranged cells with large intercellular spaces connected to substomatal chambers. The main vein includes a collateral vascular bundle surrounded by collenchyma, providing mechanical support and transport.

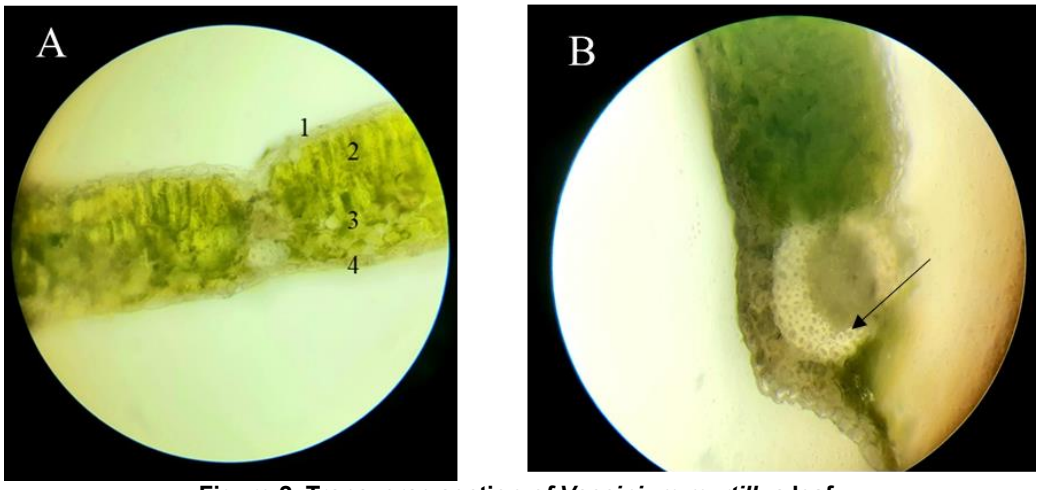

Figure 2. Transverse section *of Vaccinium myrtillus* leaf:
A – 1: adaxial (upper) epidermis, 2: palisade parenchyma, 3: spongy parenchyma, 4: abaxial (lower) epidermis (10×); B – collateral vascular bundle (40×)

Examination of *Vaccinium myrtillus L.* fruit sections highlights the diagnostic features of the pericarp and seed coat. The exocarp contains scattered sclereids with thick, lignified walls, contributing to the fruit's firm texture, while parenchyma cells are present to store water and nutrients. These anatomical details are illustrated in Figure 3.

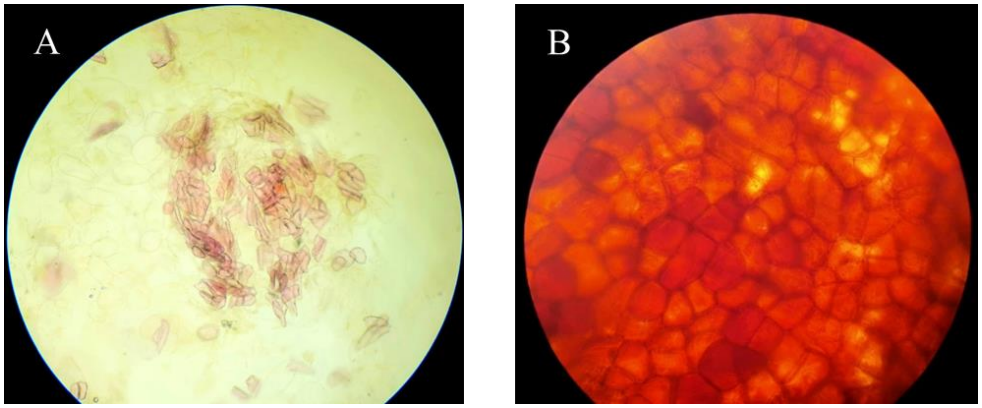

Figure 3. Microscopic examination of *Vaccinium myrtillus L*. fruits

The total phenolic content (TPC) of ethanolic extracts obtained from *Vaccinium myrtillus L.* leaves and fruits was determined using the Folin–Ciocalteu spectrophotometric method. The calibration curve for gallic acid showed excellent linearity across the tested concentration range, described by the regression equation Y = 0.007X + 0.0671 (R^2 = 0.9918). As presented in Table 1, the ethanolic extract of *Myrtilli folium* exhibited a mean total phenolic concentration of 119.64 ± 1.01 mg GAE/g dry extract, whereas the extract obtained from *Myrtilli fructus* recorded a mean value of 34.12 ± 0.89 mg GAE/g dry extract. When compared to literature data, the obtained results are in good agreement with previously reported ranges for *Vaccinium myrtillus* species, which indicate values between 107.79 and 173.12 mg GAE/g for leaf extracts and 20.79 to 41.32 mg GAE/g for fruit extract (Bujor et al, 2016; Ştefănescu et al, 2020; Tian et al, 2018).

Table 1

Total phenolic content in ethanolic extracts of *Myrtilli folium and fructus*

Sample	Sample absorbance measured at 765 nm	Sample concentration (mg GAE/g dry extract)	Mean concentration (mg GAE/g dry extract) ± SD
Ethanolic extract of *Myrtilli folium*	0,906	119,84	119,64 ± 1,014
	0,897	118,55	
	0,911	120,55	
Ethanolic extract of *Myrtilli fructus*	0,308	34,41	34,12 ± 0,895
	0,299	33,12	
	0,311	34,84	

Quantification of total flavonoids in the ethanolic extracts of *Vaccinium myrtillus L.* was achieved using the aluminum chloride colorimetric assay. The calibration curve for quercetin demonstrated good linearity within the tested range, expressed by the regression equation Y = 0.033X + 0.1418 (R^2 = 0.9873). According to the results summarized in Table 2, the leaf extract (*Myrtilli folium*) exhibited a mean flavonoid content of 19.89 ± 0.20 mg QE/g dry extract, while the fruit extract (*Myrtilli fructus*) contained 11.76 ± 0.18 mg QE/g dry extract. These values align with previously reported data for *Vaccinium myrtillus*, which indicate a concentration range of 12.05–25.92 mg QE/g for leaves and 3.88–14.64 mg QE/g for fruits (Neamtu et al., 2020; Ziemlewska et al., 2021).

Table 2

Total flavonoid content in ethanolic extracts of *Myrtilli folium and fructus*

Sample	Sample absorbance measured at 765 nm	Sample concentration (mg QE/1 g dry extract)	Mean concentration (mg QE/g dry extract) ± SD
Ethanolic extract of *Myrtilli folium*	0,798	19,88	19,89 ± 0,195
	0,805	20,09	
	0,792	19,70	
Ethanolic extract of *Myrtilli fructus*	0,530	11,76	11,76 ± 0,180
	0,524	11,58	
	0,536	11,94	

The DPPH radical scavenging activity of ethanolic extracts of *Myrtilli folium* and *Myrtilli fructus* was assessed, and the mean inhibition percentages are presented in Table 3. The ethanolic extract of *Myrtilli folium* demonstrated a high DPPH radical scavenging activity (76.56 ± 0.51%), consistent with previously reported ranges of 73.11–91.80% for leaf extracts. The fruit extract exhibited moderate activity (61.74 ± 0.39%), which aligns with literature values for bilberry fruits, ranging between 50.87–78.03% (Routray and Orsat, 2014).

Table 3

DPPH radical scavenging activity of ethanolic extracts of *Myrtilli folium and fructus*

Sample	Blank absorbance	Sample absorbance	Inhibition %	Mean inhibition % ± SD
Ethanolic extract of *Myrtilli folium*	0,895	0,210	76,53	76,56 ± 0,505
		0,205	77,09	
		0,214	76,08	
Ethanolic extract of *Myrtilli fructus*	0,895	0,346	61,34	61,74 ± 0,391
		0,339	62,12	
		0,342	61,78	

CONCLUSIONS

The evaluation of ethanolic extracts from *Myrtilli folium* and *Myrtilli fructus* demonstrates that both plant organs possess significant bioactive potential, with leaves exhibiting markedly higher concentrations of phenolic and flavonoid compounds, as well as superior antioxidant activity. Specifically, *Myrtilli folium*

displayed a total phenolic content of 119.64 ± 1.014 mg GAE/g dry plant material, a total flavonoid content of 19.89 ± 0.195 mg QE/g dry plant material, and DPPH radical scavenging activity of 76.56 ± 0.505%, all consistent with previously reported ranges in the literature. In contrast, *Myrtilli fructus* presented lower but nonetheless appreciable values: 34.12 ± 0.895 mg GAE/g total phenolics, 11.76 ± 0.180 mg QE/g total flavonoids, and 61.74 ± 0.391% DPPH inhibition, in line with prior studies.

Comparative analysis indicated that the leaf extract contained 71.48% more phenolics and 40.87% more flavonoids than the fruit extract, corresponding to a 19.5% higher antioxidant capacity. These results underscore the prominent potential of bilberry leaves as a rich source of natural antioxidants, supporting their prospective application in nutraceutical and functional food development, as well as in pharmacological interventions targeting oxidative stress-associated conditions.

Overall, the differential accumulation of bioactive compounds between leaves and fruits highlights the organ-specific metabolic allocation and ecological adaptation of *Vaccinium myrtillus L.* The elevated phenolic content in leaves likely reflects a protective strategy against environmental stressors, including ultraviolet radiation and pathogen attack, whereas the phytochemical profile of fruits primarily supports reproductive functions.

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