JOURNAL of Horticulture, Forestry and Biotechnology
Volume 29(2), 2025, 229-237
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

Bioactive compound content and biological activity of blueberry leaves and fruits. A review

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Manuscript received: 03 October 2025; revised: 04 November 2025; accepted: 12 November 2025

Abstract
The blueberry is part of the *Ericaceae* family and is known for its expansion in different areas of the globe, due to the quality of the fruits and their beneficial properties on human health. In this work, in addition to the biological action (antimicrobial, antioxidant, therapeutic effects, possible toxic effects of the leaves), the differences in the content of metabolites from varieties, hybrids or wild plants from different regions are evaluated. The leaves are rich in flavonoids and polyphenols, especially chlorogenic acid, and isoquercetin. The fruits are rich in antimicrobial compounds, with anti-inflammatory and neuronal activity. It was found that there are differences in the level of phytochemical compounds depending on the tested variety. An important aspect that must be taken into account are the potential toxic compounds, especially if the aim is to use certain parts of the plant in food or as food supplements. The fruits have high antioxidant capacity, due to the content in biocompounds. The content of flavonoids and anthocyanins is very high. Some studies have shown that wild blueberries are characterized by a higher content of phenolic compounds, compared to cultivated blueberries. It is believed that the differences between hybrids or varieties are the result of climate, cultivation area and genotype.

Keywords: *Vaccinum* spp., anthocyanins, phenols, phytotherapy, antimicrobial activity, chlorogenic acid

Introduction

"You are what you eat" has become a slogan of our days. The connection between health and food has led modern man to focus on dietary, healthy, ecological products. Fruits, especially fresh, are an integral part of daily meals. Their consumption is recommended for several reasons: they have a varied chemical composition that can replace part of human nutritional needs, but also support and improve mental and physical health [43].

Among the fruits found in the diets of people in many areas of the world, we also mention blueberries. Blueberry fruits have a special taste and aroma. Blueberries have few calories and many benefits for human health. Human health is affected every day by free radicals, which have a negative effect on body cells and can cause serious diseases. The number of patients with various diseases has increased (metabolic disorders, cardiovascular disorders, nervous system diseases, allergies, tumors, etc.) [45]. Blueberries are recommended for consumption, both by healthy people and by those with various conditions, especially diabetics. Blueberries are considered the most powerful antioxidants, due to their high content in flavonoids

and anthocyanins. Both groups of compounds have a beneficial effect on health. They are consumed fresh and utilized in the form of alcoholic and non-alcoholic beverages, jams, in culinary recipes [65]. and in pastries and confectionery.

Research shows that blueberry juice is highly demanded by consumers, not only for its sensory qualities, but also for the fact that it retains most of its nutrients [7,76].

This means that blueberries can be consumed even after the harvest period [63]. The availability of blueberry fruits throughout the year is an advantage, especially given that many patients consume blueberries fresh or prepared in various forms.

Blueberry consumption has been increasing worldwide. Europe is the second largest importer of blueberries in the world. The first place is occupied by the USA [10, 89], which has a tradition in blueberry cultivation. Some studies claim that in the USA, fresh blueberry consumption is 58% [10]. From the south-eastern part of Europe, Romania and Serbia are the biggest producers of blueberries, on the consumer market in the EU [87]. In many European countries, blueberry is considered the most important shrub from an economic point of view [77].

The genus *Vaccinium* is part of the *Ericaceae* family [3] and includes around 450 species. Among these species, it is considered that *Vaccinum corymbosum* L. was the most cultivated species in the last century, and in the 21st century, it ranks second, in terms of productive yield [66,84].

In recent years, cultivars, hybrids and many varieties have been cultivated, in alternative and traditional systems. Cultivated varieties have a tall bush and are of American origin. Among the blueberries of American origin, we mention the northern blueberries (*Vaccinium corymbosum* L.), the rabbit-eyed blueberries (*Vaccinium ashei* Reade) and the common blueberries (*Vaccinium angustifolia* L.). *Vaccinium corymbosum* L. is considered to have origins in North America and East Asia [84]. This species has spread in Europe, due to its large fruits with special sensory qualities [79].

The tall southern blueberry has spread easily due to its plasticity [4,24]. The tall bush cultivars of interest are: Aurora, Bluecrop, Bluegold, Chandler, Duke, Liberty, Patriot, Toro and Spartan" [49]. The European flora includes the species: V. arctostaphylos, V. corymbosum L. (blueberry), *V. cylindraceum, V. microcarpum, V. macrocarpon, V. vitis-idaea, V. uliginosum, V. myrtillus, and V. oxycoccos* (cranberry).

Cultivation systems have been adapted according to the environmental conditions and economic factors in each country (in the field, in protected spaces, in containers, at high densities, etc.) [23]. The high consumption of blueberries and the increasing demand have led the producers to use the micropropagation method [16].

Among the spontaneous blueberry species, in Romania you can find the common blueberry or the black blueberry (*Vaccinium myrtillus* L.), blueberry (*Vaccinium uliginosum* L.) and cranberry (*Vaccinium vitis idaea* L.).

In Romania, blueberry was introduced into cultivation in Argeș County. The blueberry breeding process has been taking place since 1980 until now, at the Pitești-Mărăcineni Fruit Research Institute, Romania [28].

The common blueberry (*Vaccinum myrtillus* L., syn. with *Myrtillus niger* Gilib.) is spread across three continents, Europe, Asia and North America to Greenland. It grows freely in mountainous, wild areas, at altitudes of 2.000-2.500 meters or lower, pre-mountainous, with altitudes of 600-900 meters. It prefers special soil conditions. It grows in optimal conditions on soils with acidic pH. It is a perennial shrub. It appears as a richly branched bush, adorned with ovate and deciduous leaves and small fruits [85, 80]. Blueberry bushes are low in height (30–50 cm), and the flowers are single. The plants bloom in April-May. The fruit is a blue, juicy berry with a sweet and astringent taste. Propagation is by seeds and rhizomes [11,38,42].

Vaccinum myrtillus L. is rich in antioxidant and antimicrobial compounds. The antioxidant and antimicrobial capacity are determined by polyphenols and anthocyanins [6,27,44].

Due to its chemical properties, bilberry (both berries and fruits) is used in the therapy of some human diseases. Most often mentioned are diabetes, cardiac, ophthalmological and circulatory disorders. For medical conditions, bilberry is exploited in the form of dietary supplements [80].

The purpose of the work is to bring to the attention of the general public the latest news related to the antimicrobial properties, the potentiation of existing antibiotics in combination with, compounds from blueberry extracts, the phytochemical characteristics, the way to preserve the active compounds and the microbial balance from the products or fresh blueberries, the pharmacological properties, respectively the culinary uses and the cultivation systems of the black blueberry.

Phytochemical profile

Biochemical content and antioxidant capacity of blueberry leaves and fruits

Although many plant compounds have been extensively studied, interest in identifying new bioactive compounds has increased recently. Among the plants of interest are species of the genus *Vaccinum.* Berries

have a high content of phenolic compounds and organic acids (Fig. 1). Studies on biologically active compounds in leaves and stems are limited. Insufficient knowledge of the compounds in the aerial part and in blueberry fruits may limit our use of valuable polyphenols. Some authors have observed that in blueberry leaves the total content of phenolic compounds is higher (16–20 mg/g dry weight) than in blueberries (3.9–7.0), [68]. Brambilla et al. (2008) [3a] argue that the abundance of phenolic compounds in blueberry juice depends on the plant's ability to synthesize secondary metabolites, respectively on the juice extraction technology.

Czernicka et al. (2024) [18] studied the extracts obtained from the leaves of 25 varieties of V. corymbosum L. Biochemical analyzes proved that they have a high content of flavonoids and polyphenols. The total phenolic content ranged from 48.11 mg GAE/g to 177.31 GAE/g. The dominant biocompounds were chlorogenic acid, arbutin and isoquercetin. The chlorogenic acid content of the analyzed leaf extracts varied from one variety to another, being between 32.37 mg/g and 52.76 mg/g. Chlorogenic acid also dominates in blueberry fruits [54a]. A very high isoquercetin content was observed in three blueberry cultivars (Aurora, Ivanhoe and Toro). The concentration of isoquercetin was 21.57 mg/g to 28.40 mg/g. In most cases, the amount of arbutin did not exceed 10.55 mg/g, except for the Ivanhoe variety, where a concentration of 27.19 mg/g was found. In both leaf extracts and blueberry fruits, it was shown that there is a close correlation between the total phenolic content and antioxidant activity. Characteristic for the *Vaccinum* genus is the presence of p-coumaric acid [68].

Okan et al. (2018) [54a] demonstrated that the antioxidant potential and the total content of polyphenols, anthocyanins and flavonoids is higher in natural blueberries than in the fruits of the varieties [18,54a].

Many studies mention that the flavonols highlighted in blueberry fruits were: quercetin (which exceeds 50% of the total flavonoid content), myricetin, isorhamnetin, laricitrin and syringetin [58,47,86]. Kaempferol has been discovered in high amounts in blueberry leaves [15,29].

Some authors claim that blueberries contain high amounts of sugars. According to some results, these are between 240–600 mmol/kg. In addition, blueberries contain between 58–143 mmol/kg of organic acids [47.

According to Hera et al., (2023), [28a] blueberries have a balanced content of sugars and acids. Sucrose was highlighted in most blueberries analyzed by Okan et al., (2018), [54a]. Fructose and glucose in blueberries are in higher amounts, compared to sucrose. The authors observed that there are differences in antioxidant activity and phenolic acid content in blueberry fruits, depending on the region and the harvest year.

The European blueberry (*Vaccinum myrtillus* L.), is characterized by a high content of polyphenolic compounds [37,71]. Studies conducted by Tundis et al. (2021), [78] demonstrated that the content of bioactive compounds in *V. myrtíllus* L. extracts is 8% higher than that observed in *V. uliginósum* L. extracts.

The results obtained by Teleszko et al. (2015), [75a] proved that the antioxidant activity of *V. myrtillus* L. leaf extracts is twice as high as that of blueberries. Also, the content of flavonoids and phenolic acids is high [79a]. Among lignans, lioniside was discovered in the rhizomes and stems of V. myrtillus L. [74].

The toxic potential of some compounds in blueberry leaves

Czernicka et al. (2024) [18] recommended that before using blueberry leaves in the food industry, the presence of potentially toxic substances should also be investigated. They stated in their work that the bilberry leaves investigated have a low content of arbutin. This compound in bilberry leaves can be metabolized into hydroquinone, a compound that is carcinogenic and hematotoxic [21]. Kang et al., 2012, [35], discovered that metabolic activation of intestinal bacteria may play an essential role in arbutin-induced toxicity.

Cladis et al. (2020), [13a], studied possible negative, toxic effects of bilberry polyphenols on mice. Following blood and urine analyses, it was shown that there were differences between the samples subjected to treatments and the control, but all physiological parameters were within normal limits. Intestinal permeability was also increased at the highest dose, but no systemic changes were observed.

Blueberries are not toxic. Toxicity issues may arise from pesticide residues and heavy metals in soil, which may have adverse effects on consumer health [15a, 88]. There may also be rare cases of allergic reactions (an example of a case at *Vaccinium myrtillus* L.), [32]. Bog bilberry may cause adverse reactions in people with certain conditions.

Beneficial effects of blueberry leaves and fruits

Antimicrobial and phytopharmaceutical potential of blueberries

The pharmaceutical industry has reached an impasse, as the problem of antibiotic resistance of microorganisms is one of the acute problems of society and health professionals.

Antibiotic resistance occurs in both Gram positive and Gram negative bacteria. Among these bacterial species, we mention: *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium*, etc. [62,33a].

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In the genera *Staphylococcus* and *Streptococcus* there are species that cause skin infections [2]. Some species of the genera *Escherichia, Salmonella, Listeria, Staphylococcus* and *Vibrio* cause food poisoning [9]. Enterobacteria (*Escherichia coli, Klebsiella, Proteus*) and *Enterococcus faecalis* cause urinary infections [25].

Generally, antibiotics are used to treat these infections, but studies show that in the last 30 years, natural products have appeared on the market, because studies have shown that plant compounds have antimicrobial activity and can be useful in the medical field [5,50,73]. But the number of antimicrobial products is reduced, which is why finding pharmaceutical formulas with biological activity is a necessity of our days.

It is believed that if viable solutions are not found, by 2050 there will be no more effective antibiotics against microbial agents [8]. Therefore, the medical antimicrobial solutions [50] considered promising by many specialists are bioactive compounds from plants. Studies show that bilberry is also part of this group of plants.

Antibacterial compounds from *V. myrtillus* L. can be used as natural antibacterials (Fig. 1). Therefore, the fruits and leaves of this plant are natural sources of antioxidant substances of great importance. It was found that extracts from leaves and fruits of black bilberry (*V. myrtillus* L.) have a high content of phenolic compounds, a high antioxidant capacity and a moderate antimicrobial potential [82]. The authors observed that the alcoholic extract from the fruits, respectively the aqueous extract from the leaves of *V. myrtillus* L. have a high antioxidant capacity. Of the three bacteria studied (*Escherichia coli, Enterococcus faecalis* and *Proteus vulgaris*), the most sensitive was the Gram-positive bacterium, *Enterococcus faecalis*, to the alcoholic extract of *V. myrtillus* L. fruits. The study concludes that aqueous, alcoholic and ethyl acetate extracts of *V. myrtillus* L. inhibit bacterial growth and can prevent urinary tract infections. By comparing the antimicrobial effects of extracts obtained from fruits with those from *V. myrtillus* L. leaves, it was observed that other compounds in the extracts contribute to the antimicrobial effect, not only polyphenols. Most likely these compounds are organic acids. The variation in response to bioactive compounds in the extracts is due to the bacterial strain, the extraction solvent [48], and the concentration of the extract.

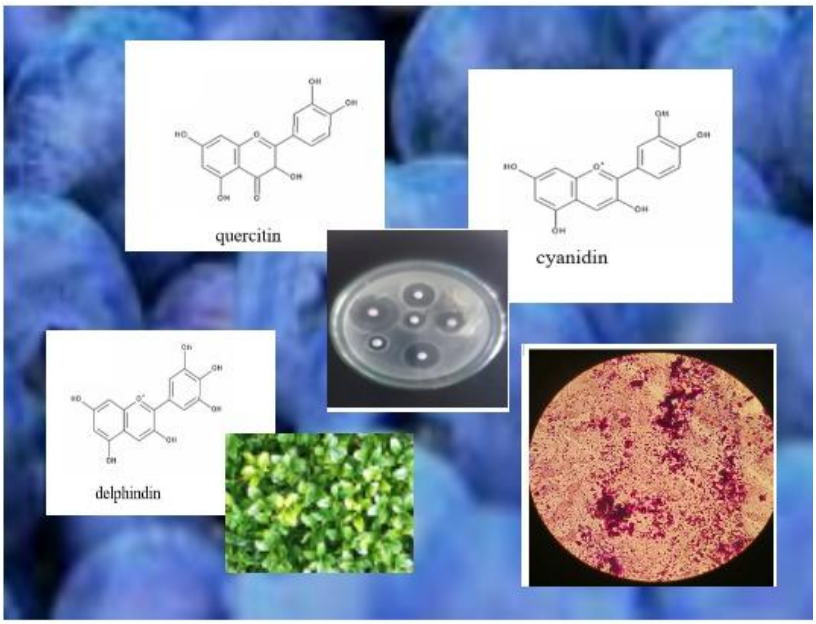

Figure 1. Biological activity of blueberries

Puupponen-Pimia et al. (2005), [56], have established with certainty that extracts of *V. myrtillus* L. have antibacterial effects against the bacteria *Salmonella enterica* and *Staphylococcus aureus.*

The compounds of *V. myrtillus* L. are also active against periodontal bacteria (example: *Porphyromonas gingivalis, Fusobacterium nucleatum, Prevotella intermedia, Streptococcus mutans*) [61]. According to the studies of these authors, the minimum inhibitory concentration against the bacterium *Streptococcus mutans* exceeded 62.5 µg/mL, for the other bacterial species, the minimum inhibitory concentration fell within the range of 26.0 ± 7.8 µg/mL - 59.0 ± 10.4 µg/mL.

Salaheen et al. (2017), [60], demonstrated that the use of blueberry (*Vaccinium corymbosum* L.) and blackberry (*Rubus fruticosus* L.) by-products in combination with antibiotics can be a good weapon against pathogens, such as *Staphylococcus aureus.*

Blueberry plants and fruits are used in traditional medicine from many countries [70]. Phenolic compounds from blueberry plants and fruits inhibit the growth of pathogenic microorganisms, reduce the risk of heart disease [14,51,54, 57,72,75], obesity, periodontal disease and have an antiaging effect [1,17]. Iridoid glycosides from blueberries have been shown to have antitumor, neuroprotective, hypolipidemic, hepatoprotective and anti-inflammatory effects [12,83].

Blueberry leaf teas are known for their beneficial effects on diabetics and urinary tract infections [31].

The fruits have a characteristic anthocyanin profile, which can be used for fingerprint analysis [30,58].

Mineral intake is a vital contribution to the human body [64], as it balances the body's functions.

Food and culinary uses

The demand for functional products with beneficial effects on health is increasing. The content of blueberries in fiber, polyphenols, vitamins, carotenoids, minerals and sugars, gives them nutritional and functional properties that can be useful in the pharmaceutical and food industries. *Vaccinium myrtillus* L. is known as a functional food, due to its high content in bioactive compounds associated with beneficial effects on human health.

Blueberries are consumed fresh or processed and used in the form of alcoholic and non-alcoholic beverages, jams, teas, pastries and confectionery and in culinary recipes [36,65,68]. 100 g of blueberries provide 10 mg of vitamin C, i.e. 1/3 of the required daily dose [46].

Blueberry pigments have industrial and pharmaceutical applications. Anthocyanins are natural sources of colorants and can replace synthetic dyes in foods and textiles [55].

To meet microbiological and sensory requirements, blueberry juice must undergo a filtration and pasteurization process [26,67].

It has also been fruit blanching of the fruit before grinding preserves the active biocompounds in blueberries [59].

Conclusions

All studies show that there is a positive correlation between the use of blueberries, respectively blueberry leaf extracts, and health benefits. The antioxidant, antimicrobial potential and the unlimited possibilities of exploitation of blueberries in areas of human interest (in various pharmaceutical formulas, functional foods, cosmetics, etc.) offer the consumer the opportunity to maintain the physiological balance of the body and have unlimited access to natural compounds. The existence of wild blueberries and the possibility of establishing blueberry crops through traditional or alternative agricultural systems, lead to the expansion of crops worldwide and the regular consumption of fruits in many regions of the world. Possible toxic effects may be related to the metabolism of arbutin into hydroquinone, if the former is present in large quantities, or to pesticide or metal residues from the environment. Allergic reactions are rare.

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