

VACCINIUM VITIS-IDAEA L. (LINGONBERRY) AND ARCTOSTAPHYLOS UVA-URSI (L). SPRENG. (BEARBERRY): CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY

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ABSTRACT

The study aims to evaluate the total content of polyphenols and flavonoids (TPC and TFC) and the antioxidant properties of the methanolic extracts from the leaves of the medicinal plants *Vaccinium vitis-idaea* L. and *Arctostaphylos uva-ursi* (L). I agree, given the fact that in Romania, *Vitis idaeae folium* is used as a substitute for *Uvae ursi folium*, whose harvesting is prohibited in our country. TPC was comparable in the two species with a value of 46.89 ± 0.89 mg GAE/g in *Arctostaphylos uva-ursi* leaves and 46.26 ± 0.51 mg GAE/g in *Vaccinium vitis idaeae*. Bearberry leaves showed a higher value of TFC, 17.43 ± 0.57 mg RE/g, while lingonberry leaves had a lower value 14.51 ± 0.48 mg RE/g. This indicates a significant variation in the content of bioactive compounds depending on the species and habitat. Extracts of *Uvae ursi folium* and *Vitis idaeae folium* showed significant antioxidant properties. These results support the potential use of these species as a source of natural antioxidants, with the mention that the content may vary depending on the species and habitat. The study highlights the importance of evaluating phytochemical compounds to validate traditional uses and identify potential pharmaceutical applications.

INTRODUCTION

Herbal preparations have been used for thousands of years both in the prevention and treatment of many diseases. In recent years, the use of medicinal plants and the active compounds obtained from them has grown rapidly, becoming an important source for the development of new health products. Among the plant products used for their antiseptic action in the treatment of urinary infections, an important place is occupied by lingonberry leaves (*Vaccinium vitis idaeae*) and bearberry leaves (*Arctostaphylos uva ursi*) [1]. *Vaccinium vitis-idaea* L., called "red blueberry", "mountain cranberry", "mountain currant", is a species of plant in the genus *Vaccinium*, family *Ericaceae*, distributed in the forests of the Nordic countries, Central Europe, Europe West, Russia, Canada, East Asia, Northern Mongolia, North Korea. It is a 10-40 cm tall shrub that grows at high altitudes. In Romania it is widespread in the high areas of the Carpathian mountains, especially in Transylvania. The phytochemical profile of *Vaccinium vitis-idaea* leaves is characterized by the presence of hydroquinone glycosidic compounds, the most important structures of this type being arbutin (5-8%), methyl-arbutin, caffeoyl-arbutin. Arbutin can represent 31-50% of the total polyphenol content of the vegetable product. Flavonic O-glycosides represent 12-19% of the total polyphenols [2, 3, 11]. The most important flavonoids are derivatives of quercetol (hyperoside, isocverticoside, rutoside) and kaempferol (kaempferol-pentoside, kaempferol-hexoside). Hydroxycinnamic acids represent 6-14% of the total polyphenols. Caffeic acid derivatives predominate, caffeoyl-quinic acids, such as chlorogenic acid. There are variations in the content and profile of phenolic compounds depending on the region where the plant grows, soil type, temperature, cultivation method, maturity stage, weather conditions, genetic diversity and extraction methods. Other compounds that appear in the leaves of *Vaccinium vitis idaeae* are triterpenic saponins (ursolic, oleanolic acid), iridoids (monotropein), gallic tannins, heterosides of hydroxymethoxybenzoic acid, carbohydrates, waxes. Lingonberry leaves have antiseptic, antimicrobial, antifungal, astringent, diuretic, anti-inflammatory, cicatrizing, anthelmintic, hemostatic, anticancer activity. Pharmacological studies have confirmed its remarkable properties against urinary infections. There is scientific evidence suggesting that the plant product helps to destroy microorganisms (*Escherichia coli*, *Streptococci*) that cause urinary tract infections [3, 4, 6, 7].

Another representative of the *Ericaceae* family is the species *Arctostaphylos uva-ursi* (L.) Spreng., popularly known as bearberry, used for centuries in folk medicine as a natural source, abundant in secondary metabolites. This species is a small, creeping, evergreen shrub with a height of 20-100 cm, branched stem, white-pink flowers, 3-10 placed at the top of the branches, with red, later black fruits. This species is found at high and medium altitudes in Europe, Asia and North America [1]. In southern and southwestern Europe, the species grows in mountainous regions, and in Central Europe it occurs mainly in lowlands, in pine forests, on podsol

soils. In Romania, *Arctostaphylos uva-ursi* is protected by law, being declared a monument of nature in the reserves in Alba and Neamț counties. The leaves are oval, glossy, leathery, glabrous, dark green on the adaxial side and lighter green on the abaxial side. Their length is generally 7-30 mm and width 5-12 mm. The tips are rounded, narrow at the base and are arranged alternately on the stem. The taste of the vegetable product is bitter, astringent; it has no smell [8]. The plant product contains hydroquinone derivatives, especially in the form of glycosides: arbutoside (5-16%), methylarbutoside (4%) gailoyl- derivatives of arbutoside (0.05%). Free phenols (hydroquinone) and methylhydroquinone, gallic and catechin tannins (10-20%), phenolic acids (gallic, coumaric, syringic and also salicylic, ferulic, caffeic and lithospermic acid), flavonoids (hyperoside, quercetin, isoquercitrin, myricetin, kaempferol), triterpenes (0.4-0.8%), iridoid glycosides (monotropein), resins, volatile oil [4, 5, 9]. Arbutin (phenolic glycoside) is considered the main bioactive compound of this plant. Studies have reported antimicrobial activity through direct and specific action, causing increased aggregation of Gram-negative bacteria. Hydroquinone is the essential compound with antibacterial activity against some pathogens involved in the symptoms of an infectious nature of the urinary tract (*Escherichia coli*, *Neisseria gonorrhoeae*, *Proteus vulgaris*, *Actinobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*) [8].

MATERIAL AND METHODS

Plant Material

The leaves of *Vaccinium vitis-idaea* L. (lingonberry leaves) were purchased from the pharmacy, the product being marketed as "Lingonberry Tea" (*concisum*), used in Romania as a substitute for the species *Arctostaphylos uva-ursi* L. In the case of the species *Arctostaphylos uva-ursi* L., the plant material studied (leaves-product *in toto*) was imported, because in Romania the species is considered endangered and is protected by law, its harvesting for medicinal purposes being prohibited.

Identification of arbutin and derivatives

Arbutin and its derivatives (methylarbutin) are phenolic heterosides, which are formed from the aglycone hydroquinone or methylhydroquinone. Both heterosides and aglycones have reducing character through free phenolic -OH groups. The identification of the arbutin was carried out indirectly by identifying the hydroquinone obtained by the microsublimation method. Hydroquinone resulting from hydrolysis of arbutin with 10% HCl, by sublime heating in the form of acicular crystals, which in the presence of ferric chloride oxidizes to brown quinhydrone. The preparation was examined under a microscope. In the first stage, acicular crystals of hydroquinone are observed, which after adding FeCl₃ 10 g/L turn brown and change their orientation.

Total flavone content

The flavone content of the plant product (*Vitis idaeae folium* and *Uvae ursi folium*) was determined using a spectrophotometric method based on the complexation of flavones with aluminum chloride. The first step involved in the analysis is the preparation of extracts using 1.00 gram of the plant product (either *Vitis idaeae folium* or *Uvae ursi folium*). Extraction was performed using a Soxhlet apparatus with 100 mL of 50% methanol. Extraction continued until the plant product was exhausted. The extract was filtered, and the volume was adjusted to 100 mL by washing the residue with the same solvent. For each sample, 5.0 mL of the filtrate was mixed with 5 mL of sodium acetate (100 g/L) and 3 mL of aluminum chloride (25 g/L). The absorbance of the solution was measured at a wavelength of 430 nm. A compensation liquid was prepared using 5 mL of filtrate, 8 mL of water, and methanol (to a total volume of 25 mL). The samples were prepared in triplicate. A standard curve was established using a standard solution of rutoside (a flavonoid) in methanol at concentrations of 0.1 g/L. The standard solution was mixed with sodium acetate and aluminum chloride, similar to the sample preparation. Concentrations of the standard solution used were 1.0, 2.0, 3.0, and 4.0 mL. The concentration of flavonoids in the analyzed samples was calculated using the standard curve.

This method allows for the quantification of flavones in the plant extracts based on the formation of intensely yellow complexes with aluminum chloride. The spectrophotometric measurement at 430 nm provides information about the flavone content in the samples.

Total polyphenols contents

The determination of total polyphenols in the extracts from the two plants was conducted using the Folin-Ciocalteu spectrophotometric method. For the preparation of the sample to be analyzed, 0.500 g of the vegetable product was refluxed twice with 50 mL of 50% ethanol (v/v) for 30 minutes each time. The pooled solutions obtained from refluxing were filtered into a 100 mL volumetric flask. To 1 mL of the test solution, 5 mL of diluted Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate solution were added. The mixture was

allowed to stand for 60 minutes. After the incubation period, the absorbance of the analyzed solution was determined at 765 nm. The samples were prepared in triplicate. A standard curve was prepared using known amounts of gallic acid. Gallic acid stock standard solution was prepared at a concentration of 100 mg/mL by dissolving 0.110 ± 0.0001 g of gallic acid monohydrate in a 100 mL volumetric flask. Standard solutions with concentrations of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL were prepared by diluting volumes between 1-5 mL from the stock solution. To 1 mL of each diluted standard solution, 5.0 mL of diluted Folin-Ciâlteu reagent and 4.0 mL of 7.5% sodium carbonate solution were added. The standard solutions were left at rest for 60 minutes at room temperature, and the absorbances at $\lambda = 765$ nm were determined. The content of total polyphenols in the samples was determined by referencing the absorbance values to the standard curve. The content was expressed as gallic acid equivalents per gram of dry plant product (mg GA/g plant product).

This method provides a quantitative measure of the total polyphenolic content in the plant extracts, allowing for comparisons between different samples and the expression of results in a standardized unit (mg GA/g plant product).

Determination of antioxidant activity by the DPPH method

Antioxidants are substances that can reduce oxidative damage to biological processes by donating electrons to free radicals [9, 10]. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is commonly used to determine the antioxidant activity of substances. To prepare the methanolic solution of DPPH, accurately weigh 100 mg of DPPH and add it to a 200 mL graduated flask over which 100 mL of methanol is added, stirring until dissolved. The solution is made up to 200 mL with solvent, resulting in a methanolic solution of DPPH. 1 mL of the prepared extract was mixed with 5 mL methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) in 25 mL volumetric flasks and made up to the mark with methanol. The mixture was stirred and then stored in a dark space for 30 minutes. After the 30-minute incubation, the absorbance of the solution was measured at the wavelength $\lambda = 517$ nm. A blank was used as compensation liquid. The DPPH radical scavenging capacity, representing the antioxidant activity of the plant extract, was calculated using the formula:

$$\text{DPPH Scavenging Capacity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Blank}}\right) \times 100$$

This formula expresses the percentage of DPPH radicals scavenged by the plant extract. A higher percentage indicates a stronger antioxidant capacity.

This method allows for the quantitative assessment of the antioxidant activity of the plant extract by measuring its ability to scavenge DPPH radicals.

RESULTS AND DISCUSSION

Identification of hydroquinone on microsublimate

The presence of arbutin and its derivatives, phenolic heterosides specific to the plant products *Vitis idaeae folium* and *Uvae ursi folium*, was confirmed through the observation of hydroquinone crystals obtained after the hydrolysis of arbutin and its derivatives. The hydroquinone formed has the characteristic property of sublimating and crystallizing in an acicular (needle-like) form, as illustrated in figure 1. In the presence of ferric chloride, hydroquinone undergoes oxidation to quinhydrone, leading to a change in color and orientation, as depicted in figure 2.

This method of identification involves visualizing the crystal formations resulting from the hydrolysis process and observing the color change when exposed to ferric chloride. The specific characteristics of these observations help confirm the presence of arbutin and its derivatives in the plant products under investigation.



Figure 1-Crystals of hydroquinone

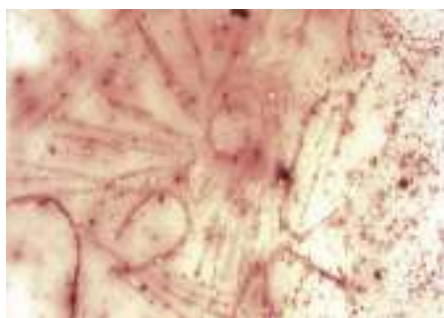


Figure 2 Quinhydrone crystals

Total flavones and polyphenols content

The obtained results emphasize the total flavonoid content (TFC) and the total phenolic content (TPC) of the extracts of *Vaccinium vitis-idaea* leaves and *Arctostaphylos uva-ursi* leaves.

The results are summarized in table 1.

The total flavonoid content (TFC) in *Vaccinium vitis-idaea* and *Arctostaphylos uva-ursi* leaf extracts was expressed as milligrams of rutin equivalents (RE) per gram of dry sample (mg/g). The total content of flavonoids is higher in the vegetable product *Uvae ursi folium* being 17.43 ± 0.57 mg RE/g, *Vitis idaeae folium* having a concentration of 14.51 ± 0.48 mg RE/g (according to Table 1). Total phenolic content (TPC) in *Vaccinium vitis-idaea* and *Arctostaphylos uva-ursi* leaf extracts was determined using the Folin–Ciocalteu method and gallic acid as standard. The method involved measuring absorbance at different concentrations of gallic acid to construct a calibration curve. The total phenolic content of the extracts was calculated using the calibration curve regression equation and expressed as milligrams of gallic acid equivalents (GAE) per gram of sample dry weight (mg/g). TPC values showed similar trends to those of TFC (total flavone content). The TPC values were higher in the leaves of *Arctostaphylos uva-ursi* 46.89 ± 0.89 mg GAE/g, the leaves of *Vaccinium vitis idaeae* having a fairly close content, 46.26 ± 0.51 mg GAE/g (Table 1).

Table 1- Total flavonoid content (TFC), Total phenolic content (TPC), Antioxidant activity (%)

Samples	TFC (mg RE/g)	TPC (mg GAE/g)	% of Antioxidant
<i>Vitis idaeae folium</i>	14.51 ± 0.48	46.89 ± 0.89	75.10
<i>Uvae ursi folium</i>	17.43 ± 0.57	46.26 ± 0.51	79.25

The free radical scavenging activity of the methanolic extracts was 79.75% for *Uvae ursi folium* and 75.10% for *Vitis idaeae folium* (according to Table 1). These results indicate high antioxidant activities of the methanolic extracts obtained from the two plant products. As a result, the action of free radical scavenging (DPPH) is closely related to the content of flavonoids and polyphenols in the plant extracts.

Based on qualitative and quantitative chemical analyses, as well as antioxidant activity testing, the following highlights emerge:

- The active principles characteristic of the *Uvae ursi folium* and *Vitis idaeae folium* plant products, namely arbutoside and its derivatives (methyларbutoside, caffeoyларbutoside), phenolic heterosides derived from the hydroquinone and methylhydroquinone aglycone, are compounds with important antioxidant properties.
- The high content of flavones and polyphenols in these two plant products, confirmed by quantitative determinations, especially flavonols, as well as catechin and epicatechin, are phenolic compounds characterized by exceptional antioxidant properties.
- The natural antioxidants present in plants are essential for inhibiting or preventing the harmful consequences of oxidative stress. The DPPH (stable free radical 2,2-diphenyl-1-picrylhydrazyl) test is mentioned as one of the convenient methods for determining the antioxidant potential of plants. Antioxidant substances such as flavonoids and phenols have the property to donate hydrogen, which leads to the reduction of the methanolic solution of DPPH due to the formation of non-radicals.
- In addition to antioxidant properties, flavonoids and other phenolic compounds may exhibit biological activities such as antimicrobial, antiviral, and anticancer.

These biological and pharmacological activities are often associated with their protein binding and free radical scavenging properties. Antioxidants, including plant polyphenols, are considered essential for protecting the body against damage induced by oxidative stress caused by free radicals. Polyphenols act as reducing agents and antioxidants due to their hydrogen donating property of hydroxyl groups.

These results highlight the conclusions of the study regarding the antioxidant compounds present in these plant extracts and their relationship with various pharmacological properties.

CONCLUSIONS

The conclusions of the study indicate an important content of total phenolic compounds, flavonoids and significant antioxidant properties for the leaves of *Vaccinium vitis-idaea* and *Arctostaphylos uva-ursi*. However, these parameters were higher in the extracts of *Uvae ursi folium* compared to those of *Vitis idaeae folium*, a fact that can also be reflected in the intensity of the pharmacological action. The differences in total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties observed could be attributed to both species and geographical variability. The results suggest that the two plants may represent powerful sources of natural antioxidants due to their high content of phenols and flavonoids, as well as their notable scavenging effects on free radicals as measured by the DPPH assay. Therefore, these plants may be important in the prevention of some human diseases. Future studies should focus on extended in vivo antioxidant activities, the relationship between individual phenolic compounds and antioxidants, and the isolation, screening, and characterization of individual compounds responsible for antioxidant properties. This would help to authenticate their probable uses as sources of natural antioxidants and to validate traditional uses in various medicinal practices.

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