



Determination of Total Flavonoid Content in the Fruits and Leaves of *Viburnum opulus* L. (Common Viburnum)

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Abstract

This article describes methods for analyzing the flavonoid content of *Viburnum opulus* L. (common guelder-rose) fruits and leaves. The results are presented in tables and diagrams. High-performance liquid chromatography was used to determine the vitamin content. The results are presented in tables and diagrams. A study of the flavonoid composition revealed that the fruits and leaves of this plant contain high amounts of rutin and gallic acid.

Keywords: viburnum, apigenin, rabinin, rutin, hypolaetin, isorhamnetin, gallic acid, hyperazide, isoquercetin.

1 Introduction

In Uzbekistan, numerous textbooks and manuals have been developed on the study of medicinal plant resources, their botanical and geographical zoning, the establishment of cultivated plantations, and the development of processing technologies. These publications provide detailed information on the cultivation technologies of medicinal plants, their ecology, and the principles of rational resource utilization [1].

In recent years, increasing attention has been devoted to the investigation of the chemical composition of medicinal plants due to their potential application in the development of pharmaceuticals and functional food products containing natural bioactive compounds. Among these plants, *Viburnum opulus* L. (common viburnum) occupies a prominent place in traditional medicine owing to its cardioprotective, anti-inflammatory, and immunostimulatory properties.

The fruits and leaves of *Viburnum opulus* L. contain a wide range of biologically active substances, including phenolic compounds, flavonoids, vitamins, and essential microelements. Consequently, a comprehensive study of their chemical composition is of both scientific and practical significance. The objective of the present study was to analyze the chemical composition of *Viburnum opulus* L. based on published scientific sources and to evaluate the importance of its bioactive constituents [2].

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2 Research Object and Methods

Although *Viburnum opulus* L. is not a common species in the natural flora of Uzbekistan, its medicinal value has been documented in various textbooks and methodological manuals on medicinal plant resources and pharmacognosy. These sources describe the therapeutic properties of viburnum fruits and leaves, the chemical and pharmacological

characteristics of their bioactive compounds, and present practical approaches to the harvesting, characterization, processing, and standardization of viburnum-derived medicinal raw materials.

Viburnum is a genus of woody flowering plants belonging to the family Viburnaceae. The genus comprises approximately 200 species, which are distributed predominantly throughout the Northern Hemisphere.

Members of the genus are represented by deciduous and evergreen shrubs or small trees. The fruit is a drupe that is typically red or black, and less frequently yellow, containing a single seed that is usually laterally compressed. The endosperm is fleshy and may occasionally exhibit a folded structure.

The fruits of several *Viburnum* species are used as food products and serve as raw materials for the production of syrups and other processed foods. In addition, the bark and fruits of certain species are utilized in both conventional and traditional medicine due to their medicinal properties. Many *Viburnum* species are also cultivated as ornamental plants because of their attractive flowers and decorative appearance.

Leaves are opposite, rarely whorled. Winter buds are either naked or protected by scales. The leaves may be deciduous, biennial, or persistent, and are simple, entire or lobed, with margins that are either entire or serrate. They are borne on petioles and may possess stipules, which are occasionally modified into glandular structures, although stipules may be absent in some species.

The inflorescences are terminal, either simple or compound, and arranged in umbel-like or corymbose-umbellate clusters. The flowers are white or pinkish, predominantly regular (actinomorphic), although the marginal flowers may occasionally be zygomorphic. Flowers are generally bisexual, while the marginal flowers may sometimes be sterile.

The calyx is fused with the ovary and bears five small teeth. The corolla may be rotate, campanulate, or possess a more or less elongated and narrow tube. The androecium consists of five stamens, and the gynoecium is represented by a single pistil. The ovary is trilocular, although only one locule develops fully, while the other two remain undeveloped; it contains a single pendulous ovule.

The fruits of *Viburnum opulus* L. are characterized by their bright red coloration and slightly bitter

flavor. They constitute a valuable source of bioactive compounds, including vitamins C, K, PP (niacin), and A, together with important mineral elements such as iron, phosphorus, magnesium, and zinc. Furthermore, the fruits are rich in antioxidant constituents that contribute to their biological activity. Exposure to low winter temperatures reduces bitterness and enhances sweetness, thereby improving their organoleptic properties.

Traditionally, viburnum fruits have been employed as natural remedies for enhancing immune function, relieving cold-related symptoms, supporting cardiovascular health through blood pressure regulation, and promoting digestive well-being. Various processed products derived from the fruits, including juices, jams, and herbal infusions, have demonstrated anti-inflammatory, hemostatic, and restorative effects, making them valuable components of both traditional medicine and functional nutrition [3].

Among the biologically active constituents of *Viburnum opulus* L., flavonoids represent an important group of polyphenolic compounds predominantly found in the fruits and bark. Major flavonoids identified in the plant include kaempferol derivatives, anthocyanins, and proanthocyanidins. These compounds exhibit strong antioxidant and anti-inflammatory activities, as well as vitamin P-like properties that contribute to vascular protection. By scavenging reactive oxygen species and reducing oxidative stress, flavonoids help maintain cellular integrity, support immune function, and improve capillary resistance. Their biological activity has also been associated with the regulation of blood pressure and the prevention of cardiovascular disorders.

Phytochemical investigations have revealed that the flowers of *Viburnum opulus* L. are a source of flavonoid glycosides, predominantly kaempferol-3-glucoside and kaempferol-3,7-diglucoside. The fruits are characterized by a high content of anthocyanins, responsible for their red pigmentation, and proanthocyanidins, both of which possess potent antioxidant properties. In combination with vitamin C, these bioactive compounds play an important role in neutralizing reactive oxygen species and protecting cellular components from oxidative damage. Furthermore, the bark contains significant amounts of catechins and leucoanthocyanins, which contribute to the antioxidant and therapeutic potential of *Viburnum opulus* L.

Flavonoids found in *Viburnum opulus* L. possess a wide range of biological activities that contribute to the medicinal value of the plant. These compounds promote vascular health by improving blood vessel elasticity and assisting in the regulation of elevated blood pressure. Owing to their potent antioxidant properties, flavonoids inhibit free radical-induced oxidation, thereby protecting cellular structures from oxidative stress and reducing the risk of age-related diseases. In addition, they exhibit immunomodulatory effects by enhancing the body's resistance to viral and bacterial pathogens. Their anti-inflammatory activity further supports their use in the treatment of inflammatory conditions, including respiratory infections and gastrointestinal disorders such as ulcers and colitis [4].

3 Results

To investigate the flavonoid composition of *Viburnum opulus* L., dried vegetative plant materials were ground to a particle size of 0.1–1.5 mm. An accurately weighed sample (1.0000 g) was extracted with 99 mL of 70% ethanol at 50–60 °C for 2 h under continuous stirring in a flat-bottom flask equipped with a reflux condenser.

The resulting solution was subsequently transferred to an ultrasonic bath, where extraction was performed twice for 15 min with 10 min intervals at 35 °C. After extraction, the sample was cooled to room temperature.

The extract was first filtered through an ashless paper filter (blue ribbon grade). Subsequently, 2 mL of the filtrate was passed through a 0.2 µm membrane filter. An aliquot of 100 µL of the purified solution was then diluted to a final volume of 1 mL using the eluent.

The analysis was carried out by high-performance liquid chromatography (HPLC) using an isocratic elution mode coupled with a diode-array detector (DAD). Acetonitrile and a buffer solution were employed as the mobile phase components. Spectral data were recorded within the wavelength range of 200–400 nm.

The chromatographic analysis was performed using an Agilent Technologies 1260 HPLC system. Separation was achieved on an Eclipse XDB-C18 column (4.6 × 250 mm, 5.0 µm particle size).

The mobile phase consisted of acetonitrile and buffer solution (30 : 70, v/v) with a pH of 2.92. Isocratic elution was applied. The flow rate of the mobile phase was maintained at 0.75 mL · min⁻¹. The injection

volume was 5 µL, and the total analysis time was 20 min.

The detector used was a diode-array detector (DAD), with detection performed at wavelengths of 254, 320, and 381 nm. The obtained results are presented in the table below.

The results presented in Table 1 indicate that the fruits of *Viburnum opulus* L. contain higher levels of rhamnin, rutin, and gallic acid. Among the identified flavonoids, the contents of hypolaetin, isorhamnetin, hyperoside, and isoquercetin were relatively low. Apigenin was not detected in the fruits of the plant.

The leaves of *Viburnum opulus* L. also exhibited higher levels of rhamnin, rutin, and gallic acid. In contrast, the contents of hypolaetin, isorhamnetin, hyperoside, and isoquercetin were comparatively low, while apigenin was not. Rhamnin is a natural flavonoid compound (flavonol glycoside) that is used in pharmacognosy as a reference standard in the analysis of medicinal plant composition.

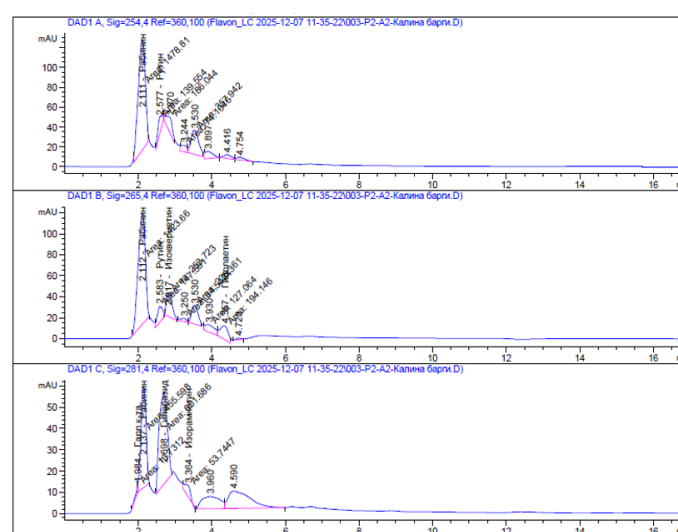


Figure 1. Representative HPLC-DAD chromatogram showing the flavonoid profile of the leaves of *Viburnum opulus* L.

It is frequently mentioned in studies of choleric herbal preparations. In addition, rhamnin is applied in high-performance liquid chromatography (HPLC) studies for the quantitative determination of flavonoid content in plant raw materials [5].

Rutin (vitamin P, rutoside) is a bioflavonoid belonging to the class of quercetin glycosides, characterized by strong antioxidant activity and the ability to strengthen capillary walls. It reduces vascular fragility, improves blood circulation, and exerts anti-inflammatory effects. The main dietary sources of rutin include citrus fruits,

Table 1. Total Flavonoid Content in the Fruits and Leaves of *Viburnum opulus* L.

№	Plant sample	Flavonoid content (mg/100 g)						
		Rhamnin	Rutin	Hypolaetin	Isorhamnetin	Gallic acid	Hyperoside	Isoquercetin
1	Fruits	10.326	16.564	2.787	0.684	33.656	6.054	4.844
2	Leaves	6.654	15.494	2.654	0.584	31.264	6.258	4.745

buckwheat, tea, black chokeberry, blueberries, and apples [6].

Rutin decreases vascular permeability, enhances capillary elasticity, and acts as an antioxidant, thereby protecting cells from oxidative damage. It is widely used in the treatment of varicose veins and hemorrhoids, as well as for immune system support. Its biological effects are most pronounced when used in combination with vitamin C, which enhances its absorption and efficacy.

Gallic acid (3,4,5-trihydroxybenzoic acid) is a naturally occurring phenolic compound belonging to the class of polyphenols and hydrolysable tannins. Although it is often found together with flavonoids and exhibits similar antioxidant properties, it is not technically classified as a flavonoid. It is present in tea, oak bark, nuts, and various medicinal plants [7].

This is a low-molecular-weight phenolic acid (a trihydroxyphenolic compound), not a flavonoid, whose structure is based on a diphenylpropane skeleton. It is a potent antioxidant and exhibits anti-inflammatory, antiviral, and radioprotective properties.

Hyperoside (quercetin-3-O-galactoside) is a biologically active flavonoid glycoside found in hawthorn, St. John's wort, and other medicinal plants. It demonstrates pronounced antioxidant, anti-inflammatory, neuroprotective, and cardioprotective activities. Due to its ability to reduce oxidative stress, hyperoside is widely used in pharmacological research and applications [8]. It improves coronary and cerebral circulation, reduces excitability of the central nervous system and cardiac muscle, and counteracts oxidative stress induced by reactive oxygen species. Additionally, it has been reported to inhibit tumor cell proliferation and induce apoptosis.

4 Conclusion

The high content of rhamnin, rutin, gallic acid, and hyperoside in *Viburnum opulus* L., compared with other flavonoids, indicates that this plant is a valuable

natural source of these bioactive compounds.

Like many flavonoids, rhamnin may exhibit biological activities typical of this class of compounds, including antioxidant and capillary-strengthening effects similar to those of rutin and quercetin. Rutin is widely used in pharmaceutical preparations and dietary supplements (e.g., Ascorutin) for strengthening vascular walls. Gallic acid is utilized in pharmaceutical science for the development of antioxidant formulations and in analytical chemistry for the determination of total polyphenol content [9].

Although gallic acid is not a flavonoid, it is often analyzed together with flavonoids as part of the phenolic compound group due to their co-occurrence in plant extracts. Hyperoside is an important bioactive constituent contributing to capillary protection and cellular defense against oxidative damage.

Overall, *Viburnum opulus* L. represents a valuable raw material for the development of pharmaceutical preparations and functional food products and requires further in-depth experimental and clinical investigations in the future.

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