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Article

Evaluation of the Antioxidant and Anti-Lipoxygenase Activity of Berberis vulgaris L. Leaves, Fruits and Stem and Their LC MS/MS Polyphenolic Profile

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Abstract: *Berberis vulgaris* L. is currently widely studied for its antioxidant and chemopreventive properties, especially given the beneficial properties of its fruits in this regard. Although the bark and roots have been well known and used in traditional medicine since ancient times, little is known about the other parts of this plant. The aim of the research was to determine the antioxidant and LOX inhibitory activity effects of extracts obtained from leaves, fruits, and stems. Another aim of the work was to carry out the quantitative and qualitative analysis of phenolic acids, flavonoid aglycones, and flavonoid glycosides. Extracts were obtained with the use of ASE (accelerated solvent extraction). The total content of polyphenols was determined and was found to vary depending on the organ, with the highest amount of polyphenols found in the leaves extract. The free radical scavenging activity of the extracts was determined spectrophotometrically in relation to the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, with results ranging from 63.88 mgTE/g for leaves to 65.25 mgTE/g for the stem. Antioxidant activity was also assessed using the ABTS test. The lowest value was recorded for the barberry fruit (117.93 mg TE/g) and the highest level was found for the barberry leaves (140.49 mgTE/g). The oxygen radical absorption capacity test (ORAC) showed the lowest value for the stem (167.70 mgTE/g) and the highest level for the leaves (267.81 mgTE/g). The range of percentage inhibition of LOX was determined as well. The percentage inhibition of the enzyme was positively correlated with the sum of flavonoids, TPC, TFC, and the content of selected flavonoids. For the first time, phenolic acids, flavonoid aglycones, and flavonoid glycosides were determined qualitatively and quantitatively in individual parts of Berberis vulgaris L. The content of phenolic acids, flavonoid aglycones, and flavonoid glycosides was determined with the LC-MS/MS method. For the first time, the following phenolic acids were quantitatively and qualitatively identified in individual parts of Berberis vulgaris: 3-caffeoylquinic acid, protocatechuic acid, 5-caffeoylquinic acid, flavonoid glycosides: eleutheroside E, rutin, isoquercitin, luteoloside, nar-cissoside, naringenin-7-glucoside, isorhamnetin-3-glucoside, afzeline, and quercitrin. Flavonoid aglycones: catechin, luteolin, and eriodictyol were determined qualitatively and quantitatively for the first time as well.

Keywords: *Berberis vulgaris*; antioxidants; phenolic acids; flavonoid glycosides; flavonoid aglycones; anti-lipoxygenase

1. Introduction

Berberis vulgaris L. (Berberidaceae) is a medicinal plant of the genus *Berberis*. Despite the promising results obtained for selected barberry species, relatively few of them have been tested with respect to their chemical composition, chemopreventive potential, and nutraceutical use. The content of polyphenols and flavonoids and antioxidant properties

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have been determined only for selected *Berberis* L. species. For example, the high content of polyphenols in herb, roots, and fruits of *Berberis cretica* [1] and the aerial parts of *Berberis sibirica* [2], with the highest antiradical activity depending on the solvent used, was 40 μg/ml. The TPC value of 190 mgGAE/g was determined for *Berberis cretica* (with antiradical activity of IC50 = 60 μμg/ml) and the TPC value of 159 mgGAE/g was reported for *Berberis sibirica* [1;2].

In fresh *Berberis heteropoda* fruit, the TPC value was at the level of 68.55 mgGAE/g and the TFC value was 108.42 mgQE/g [3]. In turn, the evaluation of the potential benefits of *Berberis nummularia* and *Berberis atrocarpa* fruits showed that the content of TPC and TFC was relatively lower than in other barberry species, with the value of *Berberis nummularia* at the level of 2mg GAE/g and 2mg RE/g respectively. For *Berberis atrocarpa*, TPC was established at the level of 12mg GAE/g dry weight and TFC at the level of 9 mgRE/g [4]. The chemopreventive and nutraceutical potential of *Berberis orthobotrys* Bienert ex c.k. Schneider has also been demonstrated. The highest values were demonstrated for the extracts obtained with the use of 80% methanol: TPC 53.86 mgGAE/g dry extract and TFC 13.94 mgCE/G dry extract and 41.16 mm Trolox/100 G [5]. The valuable properties of *Berberis thunbergii* DCs have also been demonstrated, with the highest values for extracts obtained with 80%methanol, i.e. TPC 216 mg GAE/g 216 and TFC 46 mg RE/g. The antioxidant activity of *Berberis thunbergii* DC leaves was determined to be at the levels of DPPH 429mg TE/g and ABTS 450 mgTE/g [6].

Berberis vulgaris L. is currently becoming increasingly popular among scientists looking for new sources of antioxidants, chemopreventive agents, and nutraceuticals. Currently, the species is highly valued in the Middle East, mainly in Iran, which is the main producer of barberry fruit [7] for culinary purposes. In recent centuries, *Berberis vulgaris* L. has also been widely used in traditional medicine in Europe. It was used mainly for its content of berberine and its beneficial effects, e.g. in diseases of the liver and cardiovascular system. Unfortunately, for agricultural reasons, this species has been eradicated in Europe, as it is an intermediate host of stem rust - a parasite of cereals. Currently, research on *Berberis vulgaris* L. has generated renewed interest in this species, mainly in its valuable fruit, which is a nutrient and chemopreventive agent. Studies on total polyphenol content and antioxidant activity have so far focused on barberry fruit extracts, excluding the other parts of the plant [8;9]. However, the increasing popularity of *Berberis vulgaris* L. is also related to its anticancer effect [10-16].

Antioxidant activity of this plant has so far been examined only partially and, as already mentioned, literature data are focused on the fruits and shgow that the results largely depend on the extraction method, plant growing habitat, and varieties. Current data usually come from Iran and Turkey, and the review of these literature data has shown that the extraction with 80% methanol yields extracts with the highest antioxidant potential. So far, only some studies have been focused on the antioxidant potential of other plant organs. For example, Gorizpa et al. 2022 tested the usefulness of dried ethanol root and bark extracts of *Berberis vulgaris* subsp. *asperma* and *orientalis* as natural preservatives, showing high antioxidant activity [17]. El-Zahar et al. 2022 assessed the antioxidant activity of leaves and root extracts and determined the phenolic and flavonoid composition quantitatively and qualitatively [18].

However, the data on the quantity and quality of *Berberis vulgaris* secondary metabolites are incomplete and mostly refer to the fruit. It is known to be a very rich raw material, but it is still only partially known which secondary metabolites are responsible for antioxidant properties. The plant is phytochemically rich, and various groups of compounds may be responsible for the activity. Tannins, anthocyanins, stilben derivatives, and triterpenes are very important from this point of view. Because flavonoids and polyphenols are a very important group of relationships from this point of view, this work attempts to determine their quantitative and qualitative. The purpose of the study was to analyse the total content of polyphenols and flavonoids as well as the antioxidant and

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LOX inhibitory effects of extracts obtained from the bark, root, fruit, leaves, and stem of *Berberis vulgaris* L. (Fig. 1). We also analysed the qualitative and quantitative content of individual phenolic acids, active flavonoid aglycones, and flavonoid glycosides. In this article, the antioxidant activity of all parts of the plant was assessed using the DPPH, ABTS, and ORAC methods for the first time. Similarly, quantitative and qualitative analysis of the content of phenolic acids, flavonoid aglycones, and flavonoid glycosides in *Berberis vulgaris* was performed for the first time using the LC-MS method. Among flavonoid glycosides, we determined qualitatively and quantitatively eleutheroside E, rutin, luteoloside, isoquercetin, narcissoside, isorhamnetin-3-glucoside, quercitrin, naringenin 7-glucoside, and afzelin. We also evaluated the inhibitory activity of *Berberis vulgaris* L. against the inflammatory enzyme LOX and obtained interesting data, where barberry appears to have anti-inflammatory potential. Although the anti-inflammatory activity of specific secondary metabolites of barberry has already been demonstrated, the effect on the LOX enzyme has not been evaluated so far. The research was carried out on all organs of the plant growing in the Maria Curie-Sklodowska University Botanical Garden in Lublin and collected in September, which also gives information on the differences in the composition of secondary metabolites in relation to the specimens previously studied in Turkey or Iran.

Figure 1. Dried parts of plant material obtained for investigations: a)fruits, b)leaves, c)stem.

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2. Materials and Methods

2.1. Materials and Reagents

Plant material of *Berberis vulgaris* L. barberry stem, leaves and fruits, was obtained from the Maria Curie-Skłodowska University Botanical Garden in Lublin in September 2020 (Voucher specimen: AO2020091). The raw material was separated and dried at room temperature in the shade with ventilation. The raw material was weighed and ground in an electric mill, portioned, vacuum-packed, and stored in a closed package at -30 ° C until the start of the tests. DPPH•(2,2-diphenyl-1-picrylhydrazyl), trolox, gallic acid, 3-caffeoylquinic acid, protocatechuic acid, 5-caffeoylquinic acid, 4-caffeoylquinic acid, caffeic acid, catechin, luteolin, eriodictyol-7-glucopyranoside, quercetin, syringaresinol-di-o-glucoside, rutin, hyperoside, luteoloside, isoquercetin, narcissoside, isorhamnetin-3-glucoside, quercitrin naringenin 7-glucoside, afzelin, LC grade acetonitrile, trolox, 2,2′-azinobis-3 (ethylbenzthiazoline-6-sulfonic acid) (ABTS•+), Folin-Ciocalteu reagent, and 2,2′-azobis (2-methylpropionamide) dihydrochloride (AAPH) were purchased from Sigma – Aldrich (Stenheim, Germany); ascorbic acid was purchased from Stanlab (Poland); methanol and aluminium chloride hexahydrate of analytical grade were purchased in POCH (Gliwice, Poland).

2.2. Sample Extraction and Process

The amount of 2 g of powdered barberry stem, leaves and fruits from *Berberis vulgaris* L. was extracted by accelerated solvent extraction (ASE). Accelerated solvent extractions with an 80% methanol concentration (3 cycles for 10 minutes each at 80 °C) were performed on an ASE 150 system from Dionex Corporation (Sunnyvale, CA, USA). All extracts were prepared in triplicate. In all cases, the extracts obtained were evaporated to dryness under reduced pressure and lyophilised in a Free Zone 1 apparatus (Labconco, Kansas City, KS, USA). The residue was weighed and redissolved in the same solvent used for the extraction to obtain stock solutions with the appropriate concentration and stored in the refrigerator at -30 ° C until the start of the tests. Samples of plant extracts for testing were prepared immediately prior to analysis by dissolving in an ultrasonic bath. A weighed amount of the extracts after lyophilization was dissolved in a measuring volume of 80% methanol to obtain starting solutions with a concentration of 40 mg/ml. As required for the determinations, they were diluted with the same solvent to a specific concentration.

2.3. Determination of Total Phenolic, Total Tannins and Total Flavonoid Contents

The analysis of the total phenolic content (TPC) was carried out using the modified Folin-Ciocalteu method [19]. The TPC was determined using a standard curve prepared for gallic acid. The absorbance was read at 680 nm after 20-minute incubation. The results were expressed in mg of gallic acid per 1 g of dry weight of dry extract (mg GAE/g - gallic acid equivalent). The Total Flavonoid Content (TFC) was determined according to the method proposed by Lamaison and Carret (1990) with modifications. The absorbance was measured at 430 nm after 30-minute incubation against a blank containing methanol instead of the test sample. The results were expressed in mg of quercetin (Q) per 1 g of dry extract.

The Total Tannins (TTC) content was determined using the vanillin/HCl method [20]. The absorbance was measured at 500 nm after 20-minute incubation against a blank containing methanol instead of the tested sample. The results were expressed in mg of pyrogallol (P) per 1 g of dry extract. Measurements were determined using 96-well transparent µplates (Nunclon. Nunc. Roskilde, Denmark) and an Infinite Pro 200F µplate reader (Tecan Group Ltd., Männedorf, Switzerland).

2.4. Antiradical activity analysis

2.4.1. Determination of Antiradical Potential with the DPPH˚ Assay

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The antioxidant assay was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the method developed by Brand-Williams et al. with modifications [21;22]. Absorbance was measured after 60 min at 517 nm using an Infinite Pro 200F μplate reader (Tecan Group). The results were obtained from measurements made for each sample and expressed as Trolox equivalents [mgTE/g].

2.4.2. Determination of Antiradical Capacity with the ABTS•+ Assay

The antiradical activity was determined using the refined ABTS+• discolouration test with modifications [23;24].The absorbance was measured at 734 nm after 6-minute incubation. The ability of the extract to quench ABTS+• free radicals was determined using the following equation (1):

Capture % = [(AC − AA)/AC] x 100

where AC is the absorbance of the control and AA is the absorbance of the sample. The results were obtained from measurements made for each sample and expressed as Trolox equivalents [mgTE/g].

2.4.3. Oxygen Radical Absorbance Capacity (ORAC) Assay

The determination of the Oxygen Radical Absorption Capacity (ORAC) was carried out according to a method developed by Huang et al. (2002) [22;25]with modifications.

All assays were performed with an Infinite Pro 200F μplate reader in triplicate. The activity of the sample was expressed as Trolox equivalents [mgTE/g].

2.5. Lipoxygenase Inhibitor Screening Assay

The anti-lipoxygenase activity of *Berberis vulgaris* L. extracts was determined by spectrophotometric evaluation of inhibition of LOX enzyme activity using the method described by Baraniak and Szymanowska [26]. It was calculated from the absorbance measured immediately at the wavelength of 234 nm. The samples were measured in triplicate. The percent inhibition was calculated as follows:

% inhibition of LOX = (AK-AP)/AP x 100%

where AK means increased absorbance of the control and AP means increased absorbance of the sample.

2.6. Determination of Phenolic Acids, Flavonoid Glycosides, and Flavonoid Aglycones Using the LC-MS/MS method

The content of polyphenolic compounds was determined by liquid chromatography mass spectrometry (LC-MS) according to the method developed by Łyko et al. (2022) with modifications [27]. The experiments were carried out using an Agilent 1200 Series LC apparatus (Agilent Technologies, USA) connected to a triple quadrupole mass analyser (3200 QTRAP; Sciex, Redwood City, CA, USA). The electrospray ionization (ESI) interface worked in the following conditions: temperature 500 °C, curtain gas at 23 psi, source voltage in the nebulizer gas at 50 psi, negative ionisation mode 4500 V. The mass analyser was set to perform the analyses in the multiple reaction monitoring (MRM) mode. The separations were carried out on an Eclipse XDB-C18 column (4.6 × 150 mm, 5-μm bead diameter; Agilent Technologies, USA) at 20 °C using the same chromatographic conditions as those described by Łyko et al. 2022 [27]. Data were acquired and processed with Analyst 1.5 software (Sciex, Redwood City, CA, USA). Optimised LC-MS settings were determined experimentally for each compound and are given in Table S1 in the supplementary material. Quantitative analysis was also performed in the multiple reaction monitoring mode based on the peak area of the most intense MRM transition of every identified analyte and the results from the calibration curve prepared for its analytical standard. Standard curves were generated by three repeated injections of known concentrations of

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standard solutions. The optimised analytical parameters used for the quantitative determinations are given in Table S2 (supplemental material). The LOD (limit of detection) and LOQ (limit of quantification) values were established at a signal-to-noise ratio of 5:1 and 10:1, respectively. All experiments were performed in triplicate.

3. Results and Discussion

3.1. Polyphenol content in individual organs of Berberis vulgaris

In our study, the highest amount of polyphenols (58.52±0.01 mg/g) was found in the leaves extract, followed by the stem extract (57,72±0.00 mg/g), and the extract of the fruits (52,77±0,01 mg/g. The literature data show different levels of polyphenols depending on the organ but in general data are modest as regards different organs of *Berberis vulgaris*. El-Zahar et al. 2022 determined TPC in leaves of Berberis vulgaris at the level of 120.7±1.2 mgGAE/g [18]. In fruits according to the literature the content of polyphenols vary depending on the extract from the level of 92.75 mg GAE per g in the acetone extract, 49.92 mg GAE per g in ethanolic extract, 48.89 mg GAE per g in decoction and 39.37 mg GAE per g in infusion [8]. Mottaleb et al. showed TPC level in the fruit of Berberis vulgaris in the case of 80% methanol extract, where the content of polyphenols was 280 mg GAE per g of dry extract. Simultanously they compared the level in water extract which was at the level of 100 mg GAE per g of dry extract [28]. Dimitrijević et al. 2020 reported TPC in wild fruits at the level of 494±2 mg GAE per g of dry methanolic extract [29]. Eroğlu et al. 2020 described the TPC of barberry dry fruits, with the lowest level in water extract [148.0±30.3 mgGAE/g dry fruits] and highest content in ethanol extract [448.3±81.2 mgGAE/g dry fruits] [9]. Khromykh et al. 2018 determined TPC at the level of 10.52±54.34 mgGAE/g given on wet weight [30]. Okatan et al. 2018 described total phenol contents ranged from 11.98 to 26.17mg GAE/g given on fresh weight depending on the plant variety [31]. The values of TPC in the fruits analysed in our study are very similar in each investigated by us part of plant and rage between 52.77±0.01 mg of gallic acid per 1 g of dry extract for fruits and 58.52±0.01 mg of gallic acid per 1 g of dry extract for leaves is shown in **Table 1**. Tanins content in investigated plant parts was shown at the very similar level for stem and leaves (4.53±0.00 mgPE/g and 4.6±0.00mgPE/g respectively). Fruits occurred to posses higher level of tannins - 17.1±0.04 mgPE/g.

TFC in etanolic extracts determided by El-Zahar et al. 2022 was described at the level of 59.58±1.3 mg QE/g in leaves [18]. Khromykh et al. 2018 determined TFC at the level of 1.42±6.38 mg rutin equivalents/g wet weight in isopropanolic fruit extracts [30]. Okatan et al. 2018 described total flavonoid contents ranging from 2.62 to 965.97 mg CAT/g fresh fruit weight depending on the plant variety [31]. In our study the level of TFC determined rage between the literature data as regards fruits. The highest TFC levels we have determined in leaves - 15.32±0.08 mg of quercetin (Q) per 1 g of dry extract, while Dimitrijević et al. 2020 reported TFC for leaves at the level of 1745 μg rutin per mg dry extract [29]. The values of TFC in our study are shown in Table 1.

Table 1. Total content of polyphenols (TPC), Total taninns content (TTC) and the total flavonoid content (TFC) in the barberry extracts. TPC was calculated on the basis of the equation of the gallic acid standard curve and expressed in mg of gallic acid per 1 g of dry extract (GAE - equivalent gallic acid). TTC was calculated on the basis of the equation of the pyrogallol standard curve and expressed in mg of pyrogallol (PE) per 1 g of dry extract. TFC was expressed in mg of quercetin (Q) per 1 g of dry extract. All results were expressed as mean ± standard deviation (SD) of measurements in triplicate. Extraction efficiencies [EE] are expressed in % of raw plant material.

	EE	TPC	TTC	TFC
Stem	9.1	57.72±0.00	4.53±0.00	4.57±0.01
Leaves	20.46	**58.52±0.01**	4.6±0.00	**15.32±0.08**
Fruits	35.72	52.77±0.01	**17.1**±0.04	6.11±0.02

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3.2. Quantitative and qualitative analysis of phenolic acids and flavonoids in individual organs of Berberis vulgaris

In the next stage of the study, we performed LC-MS/MS quantitative and qualitative analysis of phenolic acids and flavonoids in barberry leaves, fruits, and stem (Figure 2).

Gallic acid was found only in the **stem** at the level of **10.48±0.2 ng/mg** (0.01±0.00 mg/g), while Eroğlu et al. described high concentrations of gallic acid in fruits from different harvesting locations; they ranged between 41.186 ppm in water extract and 330.407 ppm in ethanolic extract [9]. Gholizadeh-Moghadam et al. also reported that barberry fruits contained 334.82 mg/L gallic acid [32]. Our findings differ and confirm the influence of geographical conditions. **3-caffeoylquinic acid** was also found only in the **stem** at the level of **2.39±0.09ng/mg** (0.0±0.00 mg/g) and this secondary metabolite was not previously determined in *Berberis vulgaris.* For the first time in this species, we determined **protocatechuic acid** in the barberry **stem and fruits**, with the level of **120.50±6.366 ng/mg** (0.120±6.366 mg/g) in the fruits and the level of **54.99±1.107ng/mg** (0.05±1.107mg/g) in the stem.

5-caffeoylquinic acid was determined in each part of the plant with an exceptionally high level in the **leaves: 2556.44±47.653ng/mg** (2.56±47.653 mg/g) and **fruits 3049.24±17.874 ng/mg** (3.05±17.874 mg/g). **4-caffeoylquinic acid** was present in the **fruits** (**25.73±0.462 ng/mg** (0.02±0.462 mg/g)) and **leaves** (**25.10±0.387 ng/mg** (0.02±0.387 mg/g)). **Caffeic acid** was determined by Eroğlu et al. in fruits with the highest level of 152.225 ppm in water extract [9]. In turn, Gholizadeh-Moghadam et al. [32] reported the caffeic acid level of 51.78 mg/L in fruits. In our study, **caffeic acid** was present in each part of the plant**.** The determined values ranged between **105.35±3.923ng/mg** (0.10±3.923mg/g) in the **leaves** and **252.71±6.363ng/mg** (0.25±2.828 mg/g) in the **stem**..

In the study conducted by Eroğlu et al., high concentrations of syringic acid were observed [9]. In our study, fruits collected in Poland did not contain **syringic acid** or it was found in trace amount (concentration below LOQ – 732ng/ml), which indicates a relevant influence of climate conditions on plant metabolism and properties The phenolic acid content in individual organs is shown in **Table 2**.

The sum of phenolic acids determined in this study were 0.5 mg/g for stem, 3.4 mg/g for fruits and 2.7 mg/g for leaves. This values are lower than the determined tannis content and indicates that in this parts of *Berberis vulgaris* there are some additional phenolic acids, which we were not be able to determine using our standards. Tannin content determined in this study was 4.53±0.00 mgPE/g for stem, 4.6±0.00 mgPE/g leaves and 17.1±0.04mgPE/g for fruits (**Tab.1**). Values of TPC (57.72±0.00 for stem, **58.52±0.01 for leaves and** 52.77±0.01 for fruits) also indicate that despite phenolic acids there are some additional polyphenols not determined in this study but obtained data are consistent.

Figure 2. Extracted ion chromatograms of phenolic acids detected in the Berberis stem obtained in the multiple reaction monitoring (MRM) mode; 1- gallic acid; 2 - 3-caffeoylquinic acid; 3 - protocatechuic acid; 4 - 5-caffeoylquinic acid; 5 - 4-caffeoylquinic acid; 6 - caffeic acid

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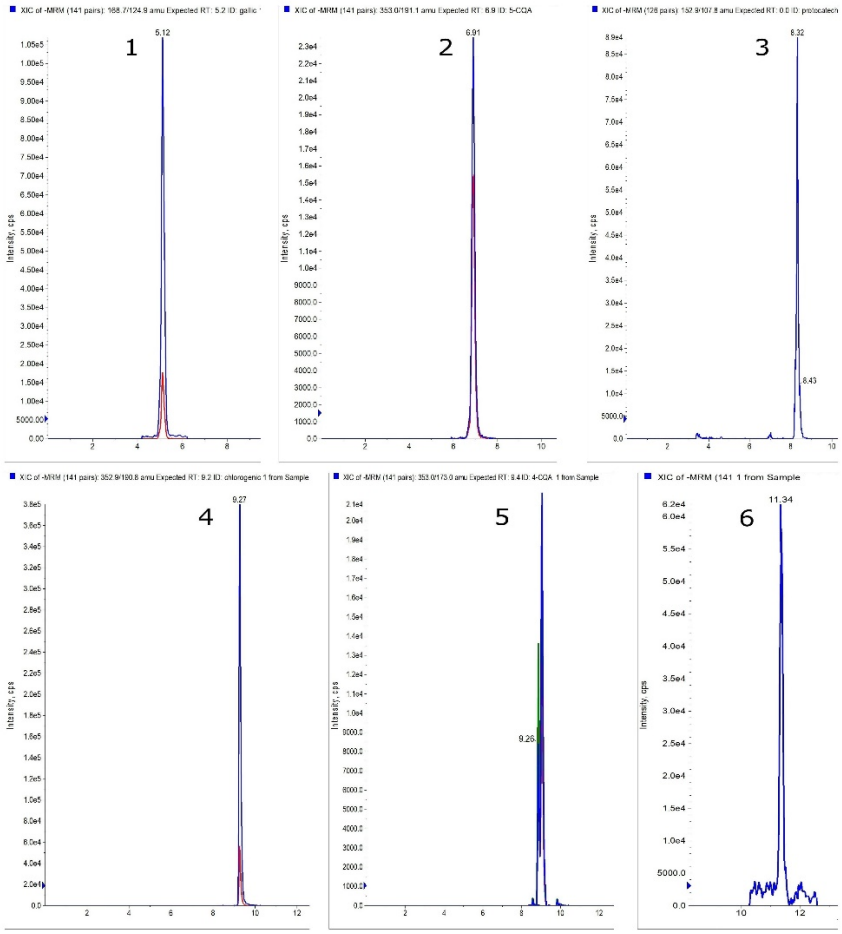

Flavonoid aglycones and flavonoid glycosides have not been quantitatively determined in *Berberis vulgaris* L. to date. We identified catechin, luteolin, and eriodictyol in the individual organs of *Berberis vulgaris* L. for the first time and confirmed the presence of quercetin in *Berberis vulgaris* L.. The fruits contained all four identified compounds and the highest level of catechin (**372.16±0.71 ng/mg** (0.37±0.00 mg/g)) and quercetin (**19.44±0.49 ng/mg** (0.02±0.00 mg/g)). The leaves contained the greatest amount of luteolin (**11.98±0.275 ng/mg** (0.01±0.00 mg/g)). The eriodictyol content ranged from **0.36±0.013 ng/mg** (0.00±0.00 mg/g) in the fruit to **3.97±0.092 ng/mg** (0.00±0.00 mg/g) in the stem. The content of flavonoid aglycones in the individual organs is shown in **Table 2**. Among flavonoid glycosides, we determined qualitatively and quantitatively eleutheroside E, rutin, luteoloside, isoquercetin, narcissoside, isorhamnetin-3-glucoside, quercitrin, naringenin 7-glucoside, and afzelin for the first time.

Eleutheroside E was present in the stem, with the content **of 116,203±1.86 ng/mg** (0.12±1.86 mg/g**)**. Rutin and isoquercitin were contained only in the leaves, but their content was relatively high **(263.37±8.46 ng/mg** (0.26±0.01mg/g) and **536.30±10.98 ng/mg** (0.54±0.01mg/g), respectively). Luteoloside was found only in the leaves and stem, with a higher level in the leaves (92.54±0.7 ng/mg (0.09±0.01 mg/g)) and **11.37±0.406 ng/mg** (0.01±0.00 mg/g), respectively). Narcissoside and naringenin 7-glucoside were identified in the leaves and fruits in small amounts. Isorhamnetin-3-glucoside and afzelin were determined only in the fruits in amounts **of 44.08±0.33 ng/mg** (0.04±00.00 mg/g) and **261.6±0.41 ng/mg** (0.26±0.00 mg/g), respectively. The fruits were also characterised by very high content of quercitrin (**1112.69±21.69 ng/mg** (1.11±0.02 mg/g)), which was also present

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in the leaves and stem, but in much lower concentrations (**9.48±0.25 ng/mg** (0.00±0.00 mg/g) and **8.89±0.22 ng/mg** (0.00±0.00 mg/g), respectively). We confirmed the presence of hyperoside in the leaves and fruits [31-34]. Additionally, this compound was detected in the stem. The fruit and leaves, however, contained relatively high amounts of hyperoside (**282.91±6.71 ng/mg** (0.28±0.01 mg/g)) and **1942.24±47.39 ng/mg** (1.94±0.05 mg/g), respectively) compared to the stem, which contained **38.86±1.07 ng/mg** of the compound (0.04±0.00 mg/g). The content of flavonoid glycosides in the individual organs is shown in **Table 2. Figure 3** shows PCA analysis of chemical composition of leaves, fruits and stem of *Berberis vulgaris* L.

Table 2. Phenolic acids, flavonoid aglycones, and flavonoid glycosides in the barberry extracts. Metabolite content expressed in ng/mg of dry extract. Mean values of three replicate assays with standard deviation. Abbreviations: < LOQ - below the quantification level – the metabolite was detected, but its concentration could not be determined; nd – not detected.

Sample	***Berberis* stem**	***Berberis* fruits**	*Berberis* **leaves**
	Phenolic acids		
Gallic acid	10.48±0.19	< LOQ	nd
3-caffeoylquinic acid	2.39±0.09	nd	<LOQ
Protocatechuic acid	54.99±1.11	120.5±6.37	nd
5-caffeoylquinic acid	**175.56±5.57**	**3049.24±17.87**	**2556.43±47.65**
4-caffeoylquinic acid	0.44±0.01	25.73±0.46	25.09±0.39
Caffeic acid	**252.71±6.36**	**237.45±8.99**	**105.35±3.92**
Sum of phenolic acids	**496.57**	**3432.93**	**2686.88**
	Flavonoid aglycones		
Catechin	46.75±1.43	**372.16±0.71**	nd
Luteolin	2.96±0.21	0.33±0.01	11.98±0.27
Quercetin	< LOQ	19.44±0.5	1.57±0.06
Eriodictyol	3.97±0.09	0.36±0.01	1.64±0.04
	Flavonoid glycosides		
Eleutheroside E	**116.20±1,86**	nd	nd
Eriodictyol-7-glucopyranoside	3.97±0.09	0.36±0.01	1.63±0.037
Rutin	< LOQ	< LOQ	**263.37±8.46**
Hyperoside	38.86±1.07	**282.91±6.71**	**1942.24±47.39**
Luteoloside	11.37±0.40	nd	92.53±0.7
Isoquercetin	<LOQ	<LOQ	**536.30±10.98**
Narcissoside	nd	46.33±0.33	17.77±0.36
Isorhamnetin-3-glucoside	nd	44.08±0.18	<LOQ
Quercitrin	8.89±0.22	**1112.69±21.69**	9.48±0.25
Naringenin 7-glucoside	nd	3.81±0.15	4.54±0.08
Afzelin	< LOQ	261,6±0,41	< LOQ
Sum of flavonoids	229.01	2143.70	2881.43

Fig. 3 Principal Component Analysis of chemical component of investigated Phenolic acids, flavonoid aglycones, and flavonoid glycosides in the barberry extracts. Analysis was performed using Statistica 6.0.

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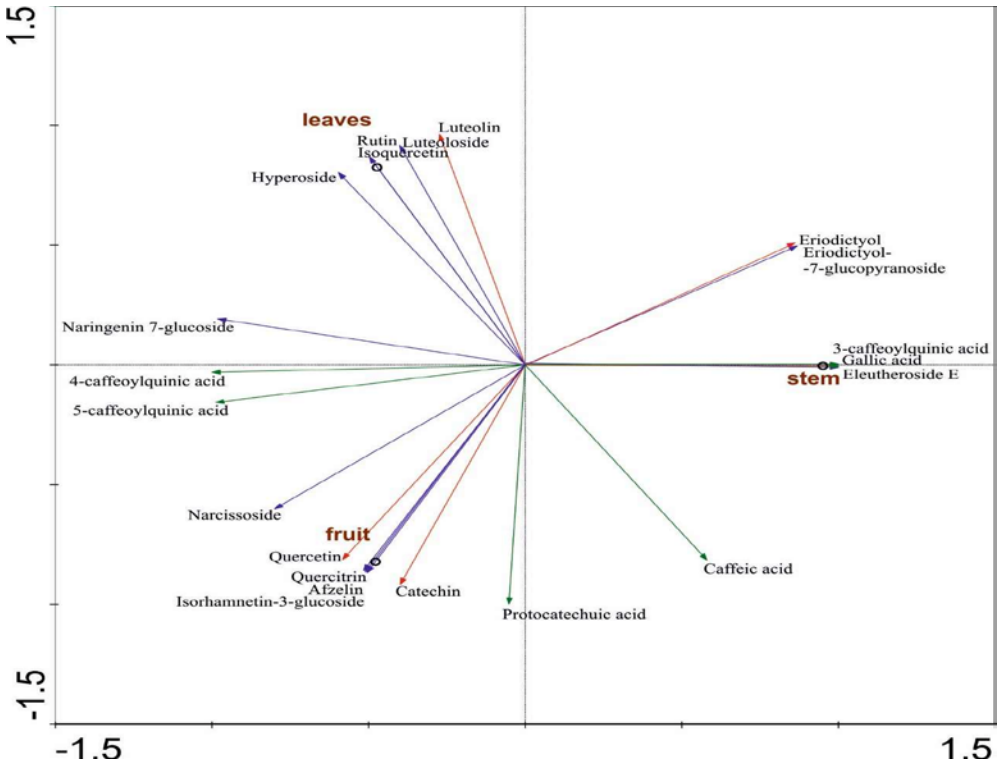

3.3. Antiradical activity of individual organs of Berberis vulgaris

The work evaluated the antioxidant activity of extracts obtained from fruit, leaves and stem. The DPPH analysis showed that the raw material extracts from the individual plant parts were characterised by very similar values ranging from 63.88 mgTe/g for the stem to 65.25 mgTe/g for the leaves extract. The ORAC results showed that the leaves had the highest antioxidant activity, i.e. 267.81 mgTE/g, followed by the fruits (215.25 mgTE/g), and stems (167.7 mgTE/g). The ABTS results also showed that the leaves had the highest antioxidant activity (140.49 mgTE/g). The other values determined with the ABTS method were 122.96 mgTE/g for the stem and 117.93 mgTE/g for the fruit. The high antioxidant activity in the leaf and fruit extracts may be related to the highest content of polyphenols in these extracts compared to other samples.

Aliakbarlu et al. 2018 described a significantly strong scavenging effect of barberry fruits acetone extract, while ethanol extract showed the lowest scavenging activity [8]. Eroğlu et al. 2020 reported the DPPH radical scavenging activity of barberry fruits in the range of 11.92%-40.44% [9], while Motalleb et al. determined the DPPH scavenging activity of barberry fruits in the range of 82.52±0.64% and 73.62±1.87 for water and ethanol extracts %, respectively [28]. Gholizadeh-Moghadam et al. reported the antioxidant activity of *Berberis vulgaris* fruit extract at a level of 56.84% [32]. These authors generally highlight the higher antioxidant potential of water extracts [14]. Although fruits have attracted attention so far, Nova-Baza et al. (2022) showed that leaves are the most valuable part of *Berberis vulgaris* in terms of antioxidant and nutritional properties [35], what is also determined in our study. Other data from the literature indicate a weaker antioxidant potential of various extracts of the root or herb of *Berberis vulgaris*, e.g. Movahedi et al. 2018 determined the EC50 for *Berberis vulgaris* herb at the level of 31.2±0.5 μg/ml [37]. El Khalki et al. 2018 reported an EC50 value of 69.65 μg/ml for the root methanol extract and 77.75 μg/ml for the acetone extract [11]. The results obtained from the antiradical activity analysis are presented in **Table 3**.

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Table 3. Antioxidant activity of *Berberis vulgaris* extracts. Antioxidant activity expressed as the DPPH• scavenging assay [expressed as mg Trolox (Trolox equivalents)/g of dry extract], antiradical capacity (ABTS+•) [expressed as mg Trolox (Trolox equivalents)/g of dry extract], and Oxygen Radical Absorbance Capacity (ORAC) [expressed as mg Trolox (Trolox equivalents)/g of dry extract]. Values are presented with the mean ± standard deviation of triplicate measurements.

Sample	ABTS [mgTE/g]	ORAC [mgTE/g]	DPPH [mgTE/g]
Stem	122.96±001	167.70±0.04	63.88±0.001
Leaves	**140.49±0.04**	**267.81±0.03**	**65.25±0.005**
Fruits	117.93±0.01	215.25±0.02	64.73±0.008

3.4. Lipoxygenase inhibitory activity of individual organs of Berberis vulgaris

In traditional Iranian medicine, *Berberis vulgaris* is a known antiinflammatory agent. In addition, traditional European medicine used *Berberis* bark to treat inflammation, especially in diseases of the digestive system. Recent studies have shown that the main anti-inflammatory mechanisms involve changes in the cell immune response to Th2, Treg induction, inhibition of inflammatory cytokines (IL-1, TNF and IFN-γ), and stimulation of IL-4 and IL-10 [38]. However, the anti-inflammatory effect of individual parts of the plant has not been evaluated so far. In order to study the anti-lipoxygenase properties of *Berberis vulgaris*, an experiment with one of the enzymes involved in the development of inflammation was carried out. Therefore, the direct ability of the extracts to inhibit lipoxygenase (LOX) activity was investigated. **Table 4** shows the percentage of LOX inhibition for the concentrations of 1 mg dry extract/ml reaction mixture. The stem and leaves extracts were shown to have higher LOX inhibitory activity (83.56% and 79.78% LOX inhibition, respectively). The weakest effect (45.24% of inhibition) was exerted by the fruit extract.

Table 4. Inhibition of lipoxygenase (LOX) by *Berberis vulgaris* samples tested at concentrations of 1 mg of dry extract/ml of the reaction mixture. Mean values of three replicate assays with standard deviation. The subscript letter (a–c) indicates significant differences at $p < 0.05$.

Sample	% LOX inhibition
Stem	**83.57±0.13**^{a}
Leaves	79.78±2.19^{a}
Fruits	45.24±2.45^{b}

As shown in **Table 5**, there were negative correlations between antioxidant activity and the content of phenolic acids. The total content of polyphenols as well as the chromatographically determined sum of phenolic acids negatively correlated with the antioxidant activity of the tested extracts. ABTS, DPPH and ORAC was negatively correlated with TPC, the sum of polyphenols and selected chromatographically phenolic acids. On the other hand the total content of flavonoids as well as the chromatographically determined sum of flavonoids correlated with the antioxidant activity of the tested extracts. ABTS, DPPH and ORAC was positively correlated with TFC and the sum of flavonoids. in addition, there were positive correlations with most of most of flavonoids: luteolin, eriodictyol, hyperoside, luteoloside, naringenin 7-glucoside, narcissoside and quercitrin. *Berberis vulgaris* is a chemically very rich raw material. Its anti-inflammatory activity has not yet been studied in sufficient detail. In our study, we only assessed inhibitory activity against an inflammation-related enzyme, and this should be considered a preliminary study facilitating further research. The correlations determined in the present study show that TPC, TFC, and the sum of flavonoids affect the level of enzyme inhibition in

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direct proportion, and phenolic acids seem not to be responsible for this effect. Results of our study indicate that compounds belonging to other chemical groups may affect the ability of barberry extracts to inhibit the LOX enzyme.

Table 5. Pearson's correlation coefficients between biological activities and concentrations in extracts of *Berberis vulgaris* L.

	ABTS	ORAC	DPPH	% LOX Inhibition
TPC	1	1	1	1
TFC	1	1	1	1
Sum of phenolic acids	-1	-1	-1	-1
Sum of Flavonoids	1	1	1	1
Protocatechuic acid	-1	-1	-1	-1
5-caffeoylquinic acid	-1	-1	-1	-1
4-caffeoylquinic acid	-1	-1	-1	-1
Caffeic acid	-1	-1	-1	-1
Catechin	-1	-1	-1	-1
Luteolin	1	1	1	1
Eriodictyol	1	1	1	1
Quercetin	-1	-1	-1	-1
Hyperoside	1	1	1	1
Luteoloside	1	1	1	1
Naringenin 7-glucoside	1	1	1	1
Narcissoside	1	1	1	1
Quercitrin	1	1	1	1

4. Conclusions

Berberis vulgaris L. is a species with high antioxidant potential. All three methods for determination of antioxidants indicate that the genus has high and similar antioxidant activity. This study confirms the high antioxidant potential of barberry fruit, but the tests used have shown that the leaves are more valuable part than fruits and stem in this respect. The content of phenolic acids, flavonoid glycosides, and flavonoid aglycones in *Berberis vulgaris* varies depending on the organ, with the high amount of polyphenols present in the leaves and fruit extract. Leaves occurred also be the richest source of flavonoids. Chemically rich and so far underestimated leaves turn out to be, next to popular fruits, a valuable food and chemopreventive raw material. The present results indicate also that some other chemicals may be responsible for the antioxidant activity of *Berberis vulgaris* and that there is a great need to continue research on the content and influence of other bioactive compounds, including alkaloids, tannins and terpene derivatives, on the antioxidant activity of *Berberis*. The percentage of LOX inhibition indicates *Berberis vulgaris* L. as the valuable plant material in this respect. It is dependent on the chemical composition of the part of plant and needs further research. The present results provide new and important knowledge on the chemical composition of *Berberis vulgaris* L., which is undoubtedly a valuable and noteworthy raw material with high antioxidant and chemopreventive potential.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Summary of optimized QTRAP parameters for the LC-MS analysis of phenolic acids and flavonoid compounds. Abbreviations: Q1/Q3 – m/z values for precursor and fragment ion detected in Q1 and Q3 quadrupole, respectively (tracked MRM transitions);

declustering potential (DP); entrance potential (EP); collision cell exit potential (CXP); collision energy (CE).; Table S2: Analytical parameters used for quantitative determination of phenolic acids and flavonoids detected in samples.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, A.O. and R.N.; methodology, A.O. and R.N.; software, A.O.,M.C.,A.C.; validation, A.O., R.N. and M.O.; formal analysis, A.O.; investigation, A.O.;S.K.,M.C. and K.B; resources, R.N.; data curation, A.O.; writing—original draft preparation, A.O.; writing—review and editing, A.O.; visualization, A.O.;A.C.; supervision, R.N.; project administration, A.O.; funding acquisition, R.N.

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