Variability of the antioxidant properties of *Berberis* fruits depending on the plant species and conditions of habitat

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Introduction

The introduction of fruit plants enriches the variety of the species composition of the regional flora and at the same time creates an opportunity to expand the vegetable raw material base to meet the nutritional needs of human health. To date, more than 400 species of fruit and berry plants have been introduced in different regions of Ukraine, but they have been introduced insufficiently in industrial gardens in the Steppe Dnieper due to the unfavorable climate. The anomalous weather conditions during the growing season of 2017 (snowfall in April followed by a drought in June) were accompanied by an earlier stage appearance of leaves, flowering and fruit ripening of all introduced plants, especially the Asian species *B. amurensis* and *B. koreana*, compared to the native species *B. vulgaris*. In accordance with the results obtained, fresh weight of the ripe fruits of *Berberis* species decreased in the order of *B. amurensis* > *B. vulgaris* > *B. canadensis* > *B. koreana* > *B. x declinata*. The highest total phenolics content, determined in the isopropanolic fruit extracts by Folin–Ciocalteau assay, was found in the fresh ripe fruits of *B. vulgaris* (1362 ± 66 mg GAE/100 g WW), followed by *B. x declinata* and *B. vulgaris* fruits (91% and 77% of the *B. koreana* phenolics content respectively). The highest total flavonoids content determined using the aluminum chloride method was revealed in the fruits of *B. koreana* (210 ± 6 mg RE/100 g FW) exceeding the content in fruits of other *Berberis* species by 1.1–2.1 times, while the lowest value (103 ± 4 mg RE/100 g FW) was found in the fruits of *B. amurensis*. The total reducing power, determined by RP assay, varied in the range from 5.0 to 9.6 mg AAE/100 g DW, and the highest levels were found in the fruits of *B. koreana* and *B. x declinata* (respectively, 9.6 ± 0.6 and 8.6 ± 0.5 mg AAE/100 g DW) exceeding the reducing capacity of other *Berberis* species by 1.7–1.9 times. In the fruits of genus *Berberis* species strong positive correlation was found between the total reducing power and the total content of phenols (r = 0.87), as well as between the reducing power and the total content of flavonoids (r = 0.84). High correlation coefficients confirm the significant contribution of the *Berberis* fruit phenolic compounds, including the flavonoids, to the antioxidant capacity. So, the study results showed that fruits of all examined *Berberis* species can be an easily accessible source of antioxidants, however, the antioxidant capacity of fruits decreased in order of *B. koreana* > *B. x declinata* > *B. vulgaris* > *B. amurensis* > *B. canadensis*.

Keywords: fruit plants; *Berberis*; antioxidants; phenols; flavonoids; total reducing power

### Key points

- The introduction of fruit plants enriches the variety of the species composition of the regional flora.
- More than 400 species of fruit and berry plants have been introduced in Ukraine.
- The climate in the Steppe Dnieper is unfavorable.
- The study found that the antioxidant capacity of *Berberis* fruits varies depending on the species and growing conditions.
- *B. vulgaris* fruits had the highest total phenolics content, while *B. amurensis* had the lowest.
- There was a strong positive correlation between the total reducing power and the content of phenols and flavonoids.

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In the recent decades, Alzheimer’s disease has become one of the most threatening diseases in the elderly, especially because of the lack of an effective therapeutic agent against this disease. According to the generally accepted cholinergic hypothesis of the origin of the disease, its appearance is associated with a deficiency of the neurotransmitters acetylcholine and butyrylcholine; consequently, the inhibition of the acetylcholinesterase and butyrylcholinesterase enzymes, that cleave acetylcholine and butyrylcholine, has become the common approach to the treatment of Alzheimer’s disease (Orhan, 2012). Since the necessary properties possess metabolites of plant origin, in this regard, the establishment of a wide range of pharmacological effects of the natural alkaloids has become of great importance. In the methanol extracts from roots and stems of different plants of the Berberis genus, four alkaloids were identified by HPLC, including berberine, jatroquine, berberine and palametin (Di et al., 2003). The most well-defined are the properties of isoquinoline alkaloid berberine, which is contained in plants of different species of the genus Berberis. The berberine effects include an inhibitory effect on acetylcholinesterase and butyrylcholinesterase, inhibition of monoamine oxidase, reduction of amloid-peptide levels and lower cholesterol levels, making berberine a promising agent against Alzheimer’s disease (Ji & Shen, 2011).

Among all the species of Berberis genus, the phytochemical composition of B. vulgaris is the most widely studied at present, including a large number of components, such as ascorbic acid, vitamin K, several triterpenoids, more than 10 phenolic compounds and more than 30 alkaloids. Thus, three phenolic compounds were identified in the extracts from root cortex of B. vulgaris, including N-(p-trans-Cumaroyl) tyramine, cannabidiol G and (+)-arysinstritol; of these, cannabinides exhibits high antioxidant activity (Tornoska et al., 2008). In addition to alkaloid berberine, the most important components of the roots, bark, leaves and fruits of B. vulgaris are isoquinoline alkaloids, beramines and palamatin. As a result, extracts from almost all parts of B. vulgaris can have anti-inflammatory, antioxidant, antibiotic, antibacterial, analgesic and hepatotoxic effects (Imenshahidi and Hosseinzadeh, 2008).

Recent studies have confirmed the known effects of B. vulgaris in traditional medicine and justified the use of fruits and other organs of B. vulgaris for the development of new drugs (Rahimi-Madisch et al., 2017). In particular, it has been shown that B. vulgaris extracts are safe and non-toxic and can induce the death of cancer cells due to their potent antioxidant activity (Abd El-Wahab et al., 2013; Hoshuy et al., 2016). In addition, based on clinical trials, the suitability of B. vulgaris extracts and berberine has been demonstrated as well for the treatment of tumors, diabetes, cardiovascular diseases, hyperlipidemia, inflammation, bacterial and viral infections, mental illnesses, Alzheimer’s disease, osteoporosis (Imenshahidi and Hosseinazadeh, 2016).

Other species of the Berberis genus require detailed phytochemical studies; however, data on the chemical composition of extracts from the roots of B. aemennis and B. libanotica indicate the possibility of using these plants as promising species for the treatment of Alzheimer’s disease (Boneli et al., 2013). The analysis of methanolic extracts from the bark of B. darwinii for inhibition of acetylcholine esterase in vitro has confirmed the therapeutic potential of the plants for the treatment of Alzheimer’s disease (Habtemariam, 2011).

The numerous studies of the plant’s physiologically active compounds have shown the dependence of their accumulation both on the plant properties and on the environment (Vagiri et al., 2013). For instance, in the B. asiatica plants in the western Himalayas, the berberine content was significantly higher in the populations growing at low altitudes and in all populations was higher in the roots than in the stems. In addition, moisture and potassium content of the soil significantly affected the berberine content (Andola et al., 2010). It was shown that the total content of alkaloids in the stems and roots of different plants of the Berberis genus depends on the origin of plants, their species and various organs (Di et al., 2003). Consequently, the content of physiologically active metabolites in fruits and other parts of the plant is determined genetically and simultaneously has a high dependence on the microclimatic and edaphic conditions in which the ontogenetic development of the fruit plants took place. In this paper, we aimed to study the implementation of the genetically determined antioxidant potential of fruits of the Berberis species during vegetation in the climatic conditions of the Steppe Dnieper.

Materials and methods

Study area. The research was conducted in 2017 in Dnipro city (steppe zone of Ukraine) in the Botanical Garden of Oles Honchar Dnipro National University (48°26’14” N, 35°02’35” E). The climate of the region has distinct continental features, including seasonal droughts with high temperatures and dry hot winds. The low average amount of precipitation (472 mm) decreases in arid years to 250 mm, and the total evaporation for a year exceeds the amount of precipitation by 2-3 times. The weather conditions during the period of research were characterized by abnormal features, in particular, the precipitation of 4 cm of snow with the simultaneous decrease in temperature to 3-6 °C from April 19 to 22, and thereafter the heat and droughts observed during June (Table 1).

| Period | Air temperature, °C | Temperature on the surface of the soil, °C | Relative humidity, % | Rainfall, mm |
|-------|---------------------|------------------------------------------|----------------------|-------------|
|       | average | norm | min | max | average | norm | min | max | average | norm | 10/20 | 11/20 | 12/20 | 13/20 | 14/20 |
| Decade | 20.1 | 19.1 | 6.3 | 57.0 | 23.0 | 52.0 | 0.3 | 14.0 |
| II decade | 19.0 | 19.1 | 8.8 | 58.2 | 28.6 | 64.1 | 17.1 | 27.0 |
| III decade | 23.2 | 20.6 | 8.8 | 60.3 | 27.0 | 60.3 | 19.7 | 18.0 |

Data collection. The study objects were the fruits of the Berberis plants from the collection of the Botanical Garden of DNU, among which there was a natural species B. vulgaris, as well as four introduced species from different geographic regions. The Berberis genus has up to 500 species of plants and belongs to the Barberry family (Berberidaceae). All plants of this genus are ornamental shrubs that are spectacular during flowering and fruiting, have smart leaves and spiny shoots. Their areas of natural growth are Transcaucasia, Southern and Eastern Europe, and Asia, where the plants prefer to settle in dry and light areas near forests, on mountain slopes, infertile soils.

B. vulgaris (European barberry) has a natural distribution within the Near East, Transcaucasia, Central, Eastern and Southern Europe, where it grows on the forest edges, slopes, lawns; in the mountains reaches up to 2000 m. Barberry prefers light and dry areas. It is also found on chalk outcrops and river gravels. This species has shoots of up to 2–3 m in height, blooms in the period from April to May; the fruits ripen during September – October. The main advantage over other fruit barberries is its high winter hardness; it is able to survive frosts down to –35 °C.

B. amurensis is common in the Far East, Korea, and China, where it grows on the fringes of forests and the banks of mountain streams, on stony ground. This is a thorny shrub with a sprawling crown up to 3.5 m in height. The shoots have a yellowish tint; they turn gray-yellow by the autumn. The color of the leaves also varies depending on the season: in summer they are bright green, and in autumn red or golden-red. The fruits of this species are red, shiny, and edible, ripen in October.

B. canadensis (American barberry) grows in the valleys and on the banks of the rivers of North America, where it also grows on mountain slopes. Brown and purple shoots of the bush reach a height of 2.0–2.5 m. The species blooms in June, the fruits have an elliptical shape, up to 1 cm long. This species blooms abundantly from mid-May to June, unpretentious, easily tolerates drought and winter frosts. This species has sufficient winter hardness and heat resistance, but suffers from dryness.

B. koreana grows on the Korean peninsula. This species has become known as a cultivar only relatively recently, at the beginning of the 20th century, and has not yet widely spread. The height of the shrub does not exceed 2 m, the leaves are larger, stiffer, almost leathery. This plant blooms from late May to the second decade of June, approximately in the course of two weeks. Fruits ripen in September and have an almost spherical shape. This species is characterized by high winter hardness; it prefers light, but it tolerates a partial shade, it is not exacting to the fertility of soils, it is drought-resistant. This species prefers alkaline soils, although it also grows on slightly acidic soils, but it does not survive the stagnant moistening and compaction of soils.
**B. x declinata** is a hybridogenic species, which is a spontaneous hybrid of *B. canadensis* and *B. vulgaris*. This is a shrub of up to 2 m in height with a densely curved crown, which has spines up to 1.5 cm in length. Plant leaves appear in April, flowering begins at the end of May and continues in June. Fruits up to 1 cm in length ripen during August—September. This species is one of the most winter-hardy species and can withstand frost up to −34 °C. The plants of this species do not require watering, however they need intensive lighting. The observations conducted during 2017 characterized the phenological peculiarities of the studied species of the genus *Berberis*, so the selection of fruits is carried out in accordance with the terms of their maturation for the different plant species (Table 2).

| Table 2 | Characteristics of the phenological differences of genus *Berberis* species in 2017 |
|------------------------|-----------------------------|
| **Species**               | **Introduction time** | **Source of the introduction** | **Phenological phases of plants** |
| *B. vulgaris* L.                | 1954 | M.M. Gryshko NBG, Ukraine, Kyiv | leaves appearance 10.04, flowering 27.04–07.05, ripe fruit appearance 28.09. |
| *B. amurensis* Rupr.              | 1956 | Finland, Helsinki | 03.04, 20.04–02.05, 16.09. |
| *B. canadensis* Mill.          | 1952 | Caruadu, Ottawa | 06.04, 25.04–05.05, 25.09. |
| *B. koreana* Palib.          | 1950 | Denmark, Copenhagen | 05.04, 21.04–02.05, 19.09. |
| *B. x declinata* Schrad.      | 1950 | State Nykytskyj Botanical Garden, Crimea, Yalta | 08.04, 25.04–04.05, 26.09. |

It should be noted that in April–2017 anomalous snowfall together with a sharp drop in temperature coincided with the flowering phase in the two studied species *B. amurensis* and *B. koreana*, and these species were the first to form ripe fruit. All other species started the flowering phase later, after the temperature had risen. The strong drought, which was observed in the first two decades of June, created unfavorable conditions for plants of all the species in the initial period of fruit formation.

**Data analysis.** The antioxidant compounds of fruits of all *Berberis* species were extracted using 80% isopropanol. The extracts intended to determine the total content of phenolic compounds and flavonoids were obtained by boiling 1 g of pulp of fresh fruits (without peel and seeds) in 10 ml of isopropanol for 1 h with a reflux condenser. After this, the crude extracts were cooled, filtered, and then the volume of isopropanol was adjusted to 10 ml. To determine the total antioxidant capacity (by FRAP assay) of the fruits, extracts obtained by holding the air-dried powdered vegetable material (200 mg) in 5 ml of isopropanol for 24 h at room temperature were used.

The total phenolic content of the fruit extracts was measured by the Folin – Ciocalteu method described by Singleton et al. (1999) in modification (Nwanna et al., 2013). Briefly, 0.2 ml of the plant extract diluted with 0.2 ml of distilled water was oxidized with 1 ml of 10% Folin – Ciocalteu reagent, and neutralized by 0.8 ml of 7.5% sodium carbonate solution in three minutes. Next, the reaction mixture was incubated for 40 minutes at 45 °C, and cooled, after which the optical density of the samples was measured at a wavelength of 720 nm. The total content of the phenolic compounds was calculated using a calibration graph prepared on the solutions of Gallic acid (GA) in the range 2.5–200.0 μg/ml. The study results were expressed as mg Gallic acid equivalents per 100 g of fruit wet weight (mg GAE/100 g WW).

Aluminum chloride spectrophotometric method in the modification of Pękal and Pyrzyńska (2014) was used for the measurement of the total flavonoids content in the *Berberis* fruits. In brief, to 2 ml of isopropanol extract from the fresh fruits, 1 ml of a 2% solution of aluminum chloride (AlCl3), and 1 ml of 1 M sodium acetate solution were added. The reaction mixture was maintained for 10 minutes at room temperature, filtered and the optical density measured at 425 nm. The quantitative content of flavonoids in the samples was calculated using a calibration graph prepared on the solutions of Gallic acid (GA) in the range 2.5–200.0 μg/ml. The study results were expressed as mg Gallic acid equivalents per 100 g of fruit wet weight (mg GAE/100 g WW).

A comparative analysis of fruit fresh weight of the introduced species with the weight of *B. vulgaris* fruits showed a significant (P < 0.05) difference in the characteristics. The greatest average weight was found for the fruits of *B. amurensis* (39.45 ± 2.211 g per 100 fruits), and the smallest weight was found for the fruits of the hybrid species *B. x declinata* (11.22 ± 1.076 g per 100 fruits).

The total phenolic compounds in the ripe fruits of the different *Berberis* species as determined by Folin – Ciocalteu assay, was expressed as gallic acid equivalents (GAE) by reference to a standard curve (y = 304.15x, and R2 = 0.99). The total flavonoids content of various species of *Berberis* was determined using aluminum chloride method, and is expressed as rutin equivalent (equation of regression y = 0.0139x, R2 = 0.999). The total reducing power (RP) of the fruits of *Berberis* different species was expressed as the equivalent of ascorbic acid (equation of linear regression y = 252.05x – 32.316, R2 = 0.993). Then, the study results (mean ± standard deviation) were statistically processed using the *B. vulgaris* indexes as a control (Table 4).

**Results**

The average mass of raw ripe fruits of the different species of *Berberis* measured at the time of ripe fruit selection varied considerably in the different species of the genus (Table 3).

| Table 3 | The weight of the fresh *Berberis* fruits (average of 100 fruits) collected in 2017 |
|------------------------|-----------------------------|
| **Species**               | **Fruit weight, g ± SD** | **P** |
| *B. vulgaris*               | 24.99 ± 8.374 | 0.0001 |
| *B. amurensis*             | 39.45 ± 6.050 | 0.0021 |
| *B. koreana*               | 16.58 ± 1.394 | 0.0007 |
| *B. x declinata*           | 11.66 ± 1.643 | 0.0001 |

**Discussion**

In accordance with the results obtained, average fruit weight of the different *Berberis* species decreased in the order of *B. amurensis* > *B. vulgaris* > *B. canadensis* > *B. koreana* > *B. x declinata*. The size and weight of the fruits is a specific feature of a certain genotype, as well as important attributes of fruit quality, which, along with other characteristics, help to correctly evaluate new or introduced species (McGhie et al., 2005; Kaldina et al., 2013). The weight of the *B. amurensis* fruits exceeded the weight of the fruits of all other *Berberis* species by 1.6–3.4.
times, which can be advantage of this species at the estimation of fruit harvest. In addition, Am. amurensis was the first of all the species to undergo the flowering and ripening of the fruits in less favorable conditions in 2017 (Table 2).

Table 4 Statistical estimate of total phenolic, total flavonoids content, and the reducing power of the Berberis fruits

| Species          | Index x ± SD | t-value | df | P   | F-ratio | P   |
|------------------|--------------|---------|----|-----|---------|-----|
|                  | Total phenolic content (mg GAE/100 g WW) |         |    |     |         |     |
| B. vulgaris      | 1052.3 ± 54.34 | –       | –  | –   | –       | –   |
| B. amurensis     | 923.2 ± 45.89  | 3.15    | 4  | 0.0347 | 1.40   | 0.833 |
| B. canadensis    | 899.2 ± 33.96  | 4.14    | 4  | 0.0044 | 2.56   | 0.562 |
| B. koreana       | 1362.8 ± 66.11 | –6.28   | 4  | 0.0033 | 1.48   | 0.806 |
| B. x declinata   | 1243.2 ± 27.43 | –5.43   | 4  | 0.0056 | 3.92   | 0.806 |

Total flavonoids content (mg Routine Extracts/100 g WW)

| Species          | Total reducing power (mg Ascorbic Acid Equivalents/g DW) |
|------------------|--------------------------------------------------------|
| B. vulgaris      | 3.3 ± 0.39                                             |
| B. amurensis     | 5.0 ± 0.54                                             |
| B. canadensis    | 6.0 ± 0.41                                             |
| B. koreana       | 7.3 ± 0.56                                             |

In our study, the total phenolic content of the crude extracts of the Berberis fruits varied in a relatively wide range, and the content of the phenols in the B. vulgaris was significantly (P < 0.05) different from those of all other species (Table 4). The study results showed that the highest phenolics concentration was revealed in the fresh ripe fruits of B. koreana (1362.8 ± 66.1 mg GAE/100 g WW), followed by B. x declinata (91% of the B. koreana phenolics content). The phenolics concentration in the fruits of the native species B. vulgaris (1052.3 ± 54.3 mg GAE/100 g WW) reached 77% of the highest content in B. koreana fruits. Similar content of the polyphenols was found by Pyrkosz-Biardzka et al. (2014) in the methanolic crude extracts of B. vulgaris fruits, where it reached 1024.3 ± 15.2 mg GAE/100 g DW. The reducing capacity of the extracts from the plants of Cyperus erectus in the range 5.6–20.0 mg AAE/g DW was estimated as high (Augustus et al., 2015). Therefore, the reducing power of the Berberis species defined in the range 5.0–9.6 mg AAE/100 g DW may be deemed sufficiently high. Since the flavonoids are the major contributors to the total reducing power in different fruit species (Borges et al., 2010), it is possible that antioxidant capacity of fruits of some Berberis species could be reduced due to the adverse effects of abnormal weather conditions during the growing season in 2017 (Table 2). This assumption is consistent, in particular, with the data of Bettea et al. (2011) on the dependence of the antioxidant level of cumin (Cuminum cyminum L.) on the power of stress in experimental drought conditions.

In our study, a strong positive correlation was found between the total reducing power and the total content of phenols in the fruit extracts of Berberis species (r = 0.87, P < 0.001), and the total content of flavonoids and the total reducing power as well (r = 0.84, P < 0.001). High correlation coefficients confirm the significant contribution of phenolic compounds, including flavonoids, to the antioxidant capacity of the fruits of all examined Berberis species, which can be an easily accessible source of antioxidants. The obtained results confirm the hypothesis that the antioxidant capacity and phenolic compounds content in fruits of B. vulgaris and B. croatica.

Conclusion

The study results confirmed the remarkable effect of genotype on the nature of phenology and accumulation of the phenolic compounds in the fruits of different Berberis species. Regional unfavorable weather...
conditions during the growing season stimulated the earlier stage appearance of leaves, flowering and fruit ripening of all introduced plants compared to the native species Berberis vulgaris. However, the most accelerated were the phenological rhythms of the Asian species Berberis amurensis and B. koreana, while the phenological phases of the northern species Berberis canadensis and the hybrid species B. x declinata were closer to B. vulgaris. The fruit weight of the different Berberis species decreased in the order of B. amurensis > B. vulgaris > B. canadensis > B. koreana > B. x declinata. Antioxidant capacity was determined as relatively high in the fruits of all Berberis species, with a significant predominance of B. koreana and B. x declinata. The total phenolic content of the fruits and also the total reducing power decreased in the order of B. koreana > B. x declinata > B. vulgaris > B. amurensis > B. canadensis, while the total flavonoids content – in the order of B. koreana > B. x declinata > B. vulgaris > B. amurensis > B. canadensis. The relatively low concentration of the antioxidants in the fruits of B. amurensis can be compensated for by the largest fruit weight of this species. Results showed that all the studied species of the genus Berberis are sufficiently rich sources of natural phenolic antioxidants. Species B. koreana and B. x declinata could potentially be the most promising in the unstable climatic conditions of the Steppe Dniester.

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