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Nutrients content and composition in different morphological parts of Cornelian cherry (*Cornus mas* L.)

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The aim of this study, focused on the nutritional value of different morphological parts: leaves, flowers, fruits and seeds of *Cornus mas* L., was to determine the contents of essential nutrients, fatty acid and amino acid profiles, and the content of selected elements. The most importantly, it was concluded that the contents of studied nutrients differed significantly ($p < 0.05$) depending on the morphological part of plant. Protein content (2.27–10.58%) was generally higher than in many wild or cultivated fruits, while flowers were distinguished by the highest content (10.58%), as compared with other studied parts of this plant. *Cornus mas* contained a substancial amount of lipids (3.34–5.23%, except fruits). Leaves proved to be extremely rich in carotenoids, mainly β-carotene (88.5 $mg.kg^{-1}$) compared with the rest of the plant. The application of GC enabled detecting 14 fatty acids in lipid fraction extracted from *Cornus mas* samples, belonging to all groups. SFAs were represented by palmitic acid (C16:0) 10.87–32.20 $g.100\ g^{-1}$ of oil. Among MUFAs oleic acid (C18:1 *9c*) 3.25–19.61 $g.100\ g^{-1}$ of oil dominated, with the highest content in fruits. Lipid fraction was extremely rich in PUFAs: 32.52–61.86 $g.100\ g^{-1}$ of oil. The major PUFA was linoleic acid (C18:2 *9c12c*): 7.93 (in leaves) – 60.18 $g.100\ g^{-1}$ of oil (in seeds); second most abundant was α-linolenic acid (C18:3 *9c12c15c*), especially in leaves and flowers (24.59–30.22 $g.100\ g^{-1}$ of oil, respectively). *Cornus mas* samples may represent a novel dietary source of valuable α-linolenic acid from n-3 PUFAs family. In total, 18 amino acids were detected in *Cornus mas* samples, while 9 of them belonged to essential ones. Flowers were distinguished by the highest content of amino acids with the highest amounts of leucine ($6.1\ g.kg^{-1}$), lysine ($6.2\ g.kg^{-1}$), aspartic ($10.7\ g.kg^{-1}$) and glutamic ($10.7\ g.kg^{-1}$) acids. Calcium was the most abundant element in all samples (2647–25687 $mg.kg^{-1}$ of dry weight, except fruits. Nutrients content and composition suggest that *Cornus mas* may become an inexpensive novel plant source of functional foods, and as new ingredient in human diet, especially with regard to underappreciated leaves and flowers.

Keywords: *Cornus mas*, fruits, leaves, seeds, flowers, fatty acids, amino acids, elements

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Introduction

Recently, nutrition gained much attention due to an extremely important role in prevention of many diseases. Thus, healthy diet and lifestyle as well as usage of underutilized and little-known edible medicinal plants has become popular (Monka et al., 2014; Ivanišová et al., 2017; Klymenko et al., 2017, 2019; Grygorieva et al., 2018a, 2018b; Horčinová Sedláčková et al., 2018; Bayram and Ozturkcan, 2020). A good example may be noticeable increased interest in the *Cornus mas*, which can be explained by an abundance of many bioactive components (flavonoids, phenolic acids, anthocyanins, iridoids, tannins), carbohydrates, fatty acids, vitamins (vitamin C) and minerals.

The Cornelian cherry (*Cornus mas* L.), also known as dogwood, belongs to Cornaceae family. Plants take a form of a large shrub or small tree mostly grown in natural conditions in Balkan and Apennine peninsulas, central Europe, and southwest Asia (Blagojević et al., 2021; Dinda et al., 2016; Szczepaniak et al., 2019;), while is cultivated in many countries like Ukraine, Slovakia, Poland, Georgia, Armenia, Czech Republic, Turkey, Serbia, and Austria (Kucharska et al., 2015). *Cornus mas* is best-known for its attractive red-purple or, less frequently, yellow or pink fruits, possessing astringent taste (Szczepaniak et al., 2019; Kucharska et al., 2015). Regarding its traditional use and prospective uses in food and beverage production (Blagojević et al., 2021; Dinda et al., 2016), fruits are recognized food ingredient in traditional cusines of several countries, such as Poland, Ukraine, Serbia, Romania, Torkey, Iran, and Czech Republic. Fruits may be consumed fresh or processed, in form of jam, gel, marmalade, compote, soup with rice, fruit yoghurt, puree, tea, sweets, jelly, marinated, candied fruits, syrup, juice, alcoholic beverages – liquolr, wine, beer (Blagojević et al., 2021; Kucharska et al., 2015; Brindza et al., 2007; Stankovic et al., 2014; Klymenko et al., 2019). Generally, Cornelian cherry presents a very promising functional fruit due to its powerful biological potential.

Cornus mas represents valuable source of vitamin C and phenolic compounds possessing strong activity. The *Cornus mas* fruits are rich in ascorbic acid 101–193 mg.100 g^{-1} of fresh fruits, anthocyanins 223–292 mg cyanidin-3-O-glucoside equivalent.100 g^{-1}, and phenolics 281–704 mg GAE.100 g^{-1}. Interestingly, leaves of *C. mas* contain a lot of phenolics (11.7%) compared with fruits (9.1%). Moreover, anthocyanins are not detectable in leaves, unlike in fruits – anthocyanins are important and responsible for unique colour and properties of fruits (Dinda et al., 2016; Milenkovic-Andjelkovic et al., 2015). Flowers are also rich in phenolics and flavonoids (42–188 mg GAE.100 g^{-1} and 50–56 mg Routine.g^{-1}, respectively) (Stankovic et al., 2014). One of the most valuable sources of both iridoids and anthocyanins are *C. mas* fruits. The presence of iridoids (monoterpenoid compound group), mainly loganic acid, cornuside, loganin, sweroside, are characteristic for *C. mas* fruits because they rarely occur in fruits (can be found in some berries species, exotic fruits, like noni (Yamabe et al., 2007; Kucharska et al., 2015; Kucharska et al., 2017; Klymenko et al., 2021). In addition to iridoids and anthocyanins, *Cornus mas* fruits contain also other flavonoids (e.g., flavonols), phenolic acids, terpenoids (ursolic acid), carotenoids and organic acids (Danielewski et al., 2021). Significant amounts of flavonoids, anthocyanins, and iridoids were identified in fruits and leaves of *C. mas*. These compounds are linked with strong radical scavenging potential and antitumour properties.

It should be highlighted that bioactive compounds which possess beneficial properties are not only located in fruits, but also in other morphological parts of the plant like leaves, flowers, seeds, or bark. Interestingly, most parts of *Cornus mas* plant have been used in folk medicine since ancient times (Klymenko et al., 2021; Kazimierski et al., 2019). Previous studies have shown that *Cornus mas* therapeutic effects include: antioxidant, antimicrobial, antidiabetic, antiatherosclerosis, antiobesity, antiglaucoma; cytoprotective, neuroprotective, cardioprotective, liverprotective, renalprotective; hypolipidemia and hypotensive properties (Bayram and Ozturkcan, 2020).

The spectrophotometric analysis of different plant parts (leaf, flower, and fruit) of cornelian cherry indicated that all plant parts of *C. mas* are extrimely rich in phenolic compounds and flavonoids. While antioxidant activity of extracts assayed by DPPH test and expressed as IC_{50} values range from 518.47 to 11.06 µg.ml^{-1} (Stankovic et al., 2014). Furthermore, studies of Celep et al. (2013) on *in vitro* and *in vivo* antioxidant activity properties of cornelian cherry leaves clearly demonstrated that the consumption of *C. mas* leaves, rich in antioxidant polyphenolics, might increase total antioxidant capacity of the body. These results evidenced the use of plant parts of *Cornus mas* in agriculture, food industry and pharmacy, as well as can be regarded as promising natural plant sources of high antioxidant value.

Although the composition and properties of *Cornus mas* fruits has been well-studied, the nutritional value of different morphological parts of plant: leaves, flowers, fruits, and seeds remain to be thoroughly studied. Thus,

the aim of this study was to determine the contents of most essential nutrients, profiles of fatty and amino acids, content of selected elements, and the content of β-carotene, vitamin A and E of leaves, flowers, fruits, and seeds of *Cornus mas*.

Material and methodology

Sampling

Fruits, leaves, flowers, and seeds of *Cornus mas* (Figure 1) were collected in 2020 from the trees growing in the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv, Ukraine).

Chemicals and reagents

All the chemicals and reagents were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany) and CentralChem (Slovakia).

Analysis of proximate composition

Dry matter, ash, and protein content were determined according to CSN-EN 12145 procedures (1997). Total lipid content was determined according to ISO 659 (1998).

Analysis of sugars

Sample preparation: cornelian cherry samples of 1 g with 10 mL of water/ethanol (4 : 1) were vigorously mixed (vertical shake table; GFL, Germany). After 60 min of extraction, the mixture was centrifuged at 6000 rpm for 4 min (EBA 21, Hettich, Germany). The supernatant was filtered through the filter paper with 0.45 mm pore size (Labicom, Czech Republic) and filled up to 50 mL in a volumetric flask with water.

An HPLC analysis of sugars (fructose, maltose, sucrose, lactose) was performed using a Agilent 1260 Infinity instrument (Agilent Technologies, Santa Clara, USA) coupled to a evaporative light scattering (ELSD) detector. Separation of sugars was conducted on a Prevail Carbohydrates ES column (250 × 4.6 mm). Acetonitrile/water (75 : 25, v/v) was used as the mobile phase. The identification of sugars was made by comparison the relative retention times of sample peaks with standards (Sigma-Aldrich, Steinheim, Germany). The contents of sugars were expressed as $g.kg^{-1}$ of dry sample.

β-carotene content

β-carotene from *Cornus mas* samples was extracted by the Sarker and Oba (2019). Briefly, 1 g of dry sample was ground in a mortar and pestle with 10 mL of 80% acetone. After removing the supernatant in a volumetric flask, the extract was centrifuged at 10000 × g for 4 min. The final volume was brought up to 20 mL. The absorbance was measured spectrophotometrically at 480 and 510 nm (UV-VIS spectrophotometer, Jenway Model 6405, England). The content of β-carotene was computed from the following equation: β-carotene = 7.6 (Abs. at 480) – 1.49(Abs. at 510) × final volume/1000. The results were expressed as mg $β\text{-carotene.kg}^{-1}$ of dry sample.

Analysis of fatty acid composition

Lipid fraction extracted from each morphological part of *Cornus mas* was determined as follows: the samples were prepared according to official methods Ce 2-66 (1997) to convert triacylglycerols into methyl esters of fatty acid (FAMEs). The FAMEs were analyzed by gas chromatography using an Agilet 6890N instrument (Agilent Technologies, Santa Clara, USA) equipped with flame ionization detector (FID; 250 °C; constant flow, hydrogen 40 $mL.min^{-1}$, air 450 $mL.min^{-1}$), a capillary column DB-23 (60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Santa Clara, CA, USA). A detailed description of the chromatography

Figure 1 *Cornus mas* L.
1 – leave; 2 – flower; 3 – fruits; 4 – seed

conditions is presented in the work Szabóová et al. (2020). Standards of a C4-C24 FAME mixture (Supelco, Bellefonte, PA, USA) were applied to identify FAME peaks. The evaluation was carried out by the ChemStation 10.1 software. The contents of FAs were expressed as g.100 g^{-1} of lipids.

Analysis of amino acid profile

Amino acid (AA) profile was determined by ion-exchange chromatography using an AAA-400 Amino Acid Analyzer (Ingos, Czech Republic) and post-column derivatization with ninhydrin and a VIS detector, as described elsewhere (Zhurba et al., 2021). Separation was provided on a glass column (length 350 mm, inner diameter 3.7 mm) filled with a strong cation exchanger in the LG ANB sodium cycle (Laboratory of Spolchemie) with average particles size of 12 µM and 8% porosity. The column was heated within the range of 35–95 °C, with the elution of AAs at 74 °C. A double-channel VIS detector with the inner cell volume of 5 µL was set to 440 and 570 nm. A solution of ninhydrin was prepared in 75% v/v methyl cellosolve and in 2% v/v 4 M acetic buffer (pH 5.5). $SnCl_2$ was used as a reducing agent. Solution of ninhydrin was stored in an inert atmosphere (N_2) without access of light at 4 °C. The flow rate was 0.25 mL.min^{-1}, and the reactor temperature was 120 °C. Individual amino acids values were expressed as g.kg^{-1} of dry sample.

Elemental analysis

The contents of macroelements, microelements and trace metals were determined by the inductively coupled plasma optical emission spectroscopy (ICP-OES) according to Divis et al. (2015) by using an ICP-OES instrument (Ultima 2, Horiba Scientific, France). Samples were prepared for analysis after microwave digestion (Milestone 1200, Milestone, Italy), 0.25 g of sample was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha Ltd, Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha Ltd, Czech Republic). After the decomposition sample was filtered through filter paper (0.45 mm pore size) and filled up to 25 mL in a volumetric flask with pure water.

Statistic analysis

The results were subjected to one-way ANOVA followed by Tukey-Kramer test, when the differences between mean values were considered significant at $p < 0.05$. The variability of all parameters was evaluated by descriptive statistics. The results were presented as means with standard error (SE). The PAST 2.17 software was used.

Results and discussion

The results revealed a significant ($p < 0.05$) variation in the nutrient contents of different morphological parts of cornelian cherry (Table 1). The protein content of *Cornus mas* (2.27–10.58%) was generally higher than in many wild or cultivated fruits (e.g., strawberry, cherry), up to 1% (de Souza et al., 2014) and higher than in seedless red fleshy part of berries (red arils) of *Taxus baccata* L., 1.79–3.80% (Tabaszewska et al., 2021). It should be pointed out that *Cornus mas* flowers were distinguished by the highest content of

Table 1 Proximate composition of *Cornus mas* L. (means ± SE)

Component	Leaves	Flowers	Fruits	Seeds
Proteins (%)	8.66 ±0.10	10.58 ±0.05	4.50 ±0.02	2.27 ±0.04
Lipids (%)	3.38 ±0.08	5.23 ±0.02	1.49 ±0.02	3.34 ±0.03
SFAs (g.100 g^{-1} oil)	46.10 ±0.12	26.33 ±1.50	36.78 ±1.68	13.56 ±0.09
MUFAs (g.100 g^{-1} oil)	10.22 ±0.09	4.87 ±0.09	15.21 ±1.02	22.13 ±1.07
PUFAs (g.100 g^{-1} oil)	27.80 ±1.10	48.45 ±1.65	32.12 ±1.40	62.19 ±1.55
Fructose (g.kg^{-1})	33.28 ±1.33	26.60 ±0.89	68.33 ±1.78	3.21 ±0.04
Maltose (g.kg^{-1})	<0.5	<0.5	<0.5	<0.5
Sucrose (g.kg^{-1})	<0.5	4.12 ±0.04	<0.5	<0.5
Lactose (g.kg^{-1})	<0.5	<0.5	<0.5	<0.5
Dry matter (%)	92.10 ±2.18	91.87 ±2.23	84.38 ±1.89	90.86 ±2.09
Ash (%)	8.86 ±0.15	5.21 ±0.01	2.11 ±0.02	0.98 ±0.01
Vitamin A (retinyl acetate) (mg.kg^{-1})	<0.1	<0.1	<0.1	<0.1
β-carotene (mg.kg^{-1})	88.45 ±1.30	25.68 ±0.76	4.87 ±0.05	2.08 ±0.02
Vitamin E (α-tocopherol) (mg.kg^{-1})	228.0 ±2.58	198 ±1.68	49.43 ±1.12	22.19 ±0.78

proteins (10.58%), as compared with other studied parts of this plant. *Cornus mas* contained a substancial amount of lipids, reaching from 3.34 to 5.23% (except fruits). Surprisingly, flowers had also the highest amount of lipids (5.23%) in relation to other parts of plant. Obtained results are in agreement with previous studies, which indicated that fruits and leaves may be regarded as important source of lipids (Tabaszewska et al., 2021; Zhurba et al., 2021).

Among sugars, *Cornus mas* plant were characterized by the highest content of fructose. As expected, fruits were the most abundant in simple sugars, mainly fructose (68.33 g.kg^{-1}), followed by leaves and flowers. Perova et al. (2014) and Bijelic et al. (2011) stated that in fruits glucose (2.5–7.0%) is present as major carbohydrate component, followed by fructose and sucrose. Studies of Wind et al. (2010) proved that monosaccharides may accumulate in leaves of plants that grow under unfavorable and stress conditions. Contrary, seeds contained only about 3.21 g.kg^{-1}. Literature data indicated that fructose and glucose were predominant among reducing sugars in *Cornus mas*, reaching 3.69 and 5.39%, respectively (Szczepaniak et al., 2019). Generally, other sugars (maltose, sucrose, and lactose) were detected in trace amounts (<0.5 g.kg^{-1}). The moisture content has an impact on physical properties of fruits. *Cornus mas* samples had rather low moisture content (7.9–9.14%), except for fruits – 15.62%.

Cornus mas leaves proved to be extremely rich in carotenoids, mainly β-carotene (88.5 mg.kg^{-1}) compared with the rest of the plant (Table 1). For comparison, carrots which are known for high amount of β-carotene, contained about 140 mg.kg^{-1} (Anjum et al., 2008). For example, pawpaw (*Asimina triloba*) contains β-carotene in seeds, pulp, and peel at the levels of 4.8, 6.6 and 12.7 mg.kg^{-1}, respectively. Results obtained by the other authors, clearly shows that β-carotene accumulated in peel 2-fold more than in pulp and 3-fold more than in seeds (Grygorieva et al., 2021).

The application of GC (gas chromatography) enabled detecting 14 FAs in lipid fraction extracted from *Cornus mas* samples: leaves, flowers, fruits and seeds, belonging to all groups (Table 1 and 2). The results of FA composition, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) of the studied samples are shown in Table 2. It should be highlighted that the contents of many of them differed significantly ($p < 0.05$) depending on the morphological part of plant.

SFAs were represented mainly by palmitic acid (C16:0) 10.87–32.20 g.100 g^{-1} of oil and stearic acid (C18:0) 1.48–4.12 g.100 g^{-1} of oil. The content of other SFAs can be regarded as low. *Cornus mas* leaves and fruits

Table 2 Fatty acid composition (g.100 g^{-1} of oil) of lipids of *Cornus mas* L. (means ±SE)

Fatty acid	Leaves	Flowers	Fruits	Seeds
SFAs	**41.33**	**27.51**	**35.98**	**17.30**
C12:0	0.27 ±0.02	0.20 ±0.03	0.48 ±0.03	<0.1
C14:0	2.22±0.05	1.48 ±0.02	2.26 ±0.03	0.10 ±0.02
C16:0	32.20 ±1.34	19.87 ±1.12	29.07 ±1.18	10.87 ±0.33
C17:0	0.39 ±0.02	0.26 ±0.02	0.38 ±0.03	0.12 ±0.03
C18:0	4.12 ±0.07	1.86 ±0.06	1.48 ±0.03	3.74 ±0.08
C20:0	1.33 ±0.02	1.83 ±0.06	0.50 ±0.02	1.34 ±0.03
C22:0	0.44 ±0.02	1.06 ±0.02	0.94 ±0.01	0.65 ±0.01
C24:0	0.36 ±0.01	0.95 ±0.02	0.87 ±0.02	0.38 ±0.01
MUFAs	**12.29**	**4.61**	**20.18**	**12.31**
C16:1	0.84 ±0.04	0.18 ±0.003	<0.1	2.37 ±0.04
C18:1	10.57 ±1.01	3.25 ±0.06	19.61 ±1.37	9.28 ±0.78
C20:1	0.78 ±0.03	0.86 ±0.02	0.37 ±0.03	0.56 ±0.02
C22:1	<0.1	0.32 ±0.01	<0.1	<0.1
PUFAs	**32.52**	**50.30**	**39.48**	**61.86**
C18:2	7.93 ±0.56	20.08 ±1.29	32.27 ±1.87	60.18 ±1.48
C18:3	24.59 ±1.05	30.22 ±1.65	7.21 ±1.06	1.68 ±0.07

Note: Saturated fatty acids – SFAs; monounsaturated fatty acids – MUFAs; polyunsaturated fatty acids – PUFAs

were distinguished by the highest amounts of C16:0. Red arils of *Taxus baccata* L. also contained substantial amounts of C16:0 acid (an average 22.5 g.100 g^{-1} of FA) (Tabaszewska et al., 2021). Studies of Zhurba et al. (2021) on FA profile of *Schisandra chinensis* leaves proved that its also characterized by the high content of C16:0 (about 44.60 g.100 g^{-1} of oil).

Among MUFAs oleic acid (C18:1 *9c*) 3.25–19.61 g.100 g^{-1} of oil undoubtedly dominated in *Cornus mas* samples, with the highest content in fruits. It shoud be noted that flowers contained only 3.25 g.100 g^{-1} of oil. The difference between the contents of MUFAs in leaves and seeds was irrelevant. Generally, other MUFAs were identified in rather small quantities in all studied samples.

The total content of PUFAs in *Cornus mas* lipids varied from 32.52–61.86 g.100 g^{-1} of oil. It should be highlighted that the lipid fraction of *Cornus mas* was extremely rich in PUFAs, even though only two acids from this group were identified. The major PUFA was linoleic acid from n-6 family (C18:2 *9c12c*) which content ranged from 7.93 (in leaves) up to 60.18 g.100 g^{-1} of oil (in seeds), it was the most abundant in the lipid fraction of seeds. In studies of Brindza et al. (2009) also linoleic acid was the most abundant FA in seeds (and barks) of *C. mas*. It should be noted that FAs composition of seeds of *Asimina triloba* (L.) Dunal, with regard to the linoleic acid content, was similarly high. However, with the substantial presence of oleic acid – 40.13 g.100 g^{-1} of oil (Grygorieva et al., 2021). The α-linolenic acid (C18:3 *9c12c15c*) was the second important PUFA, especially in leaves and flowers samples (24.59–30.22 g.100 g^{-1} of oil respectively). The *Cornus mas* may be perceived as a valuable source of α-linolenic acid belonging to the n-3 family (Rutkowska et al., 2012). Also, worth mentioning is that the differences in FAs contents resulted from the lipid content (Table 1 and 2).

In studies of Krivoruchko (2014) seven FAs of high content are reported for fruits and leaves of *Cornus mas*. In the fruits, the most abundant was linoleic (2.8 g.kg^{-1}), followed by oleic acid (2.47 g.kg^{-1}). Contrary, in leaves, the most abundant was 2,4-heptadienoic (2.38 g.kg^{-1}), and palmitic acid (2.24 g.kg^{-1}).

It is well-known that edible plant oils are the primary sources of essential unsaturated FAs form n-3 and n-6 families. Recently, was observed an increase in the number of studies aimed to support the specific health benefits of plant-derived omega-3 fatty acids, mainly α-linolenic, and the importance of searching for its new sustainable plant sources. The α-linolenic acid plays a significant role in the prevention and treatment of a variety of cardiovascular disorders, including heart attacks and stroke, as well as to have a positive impact on both function and behavior of the central nervous system (Mikołajczak et al., 2020; Tabaszewska et al., 2021).

Based on obtained results of FA composition, *Cornus mas* may represent relevant plant dietary source of valuable n-3 PUFAs (α-linolenic acid) in human diet.

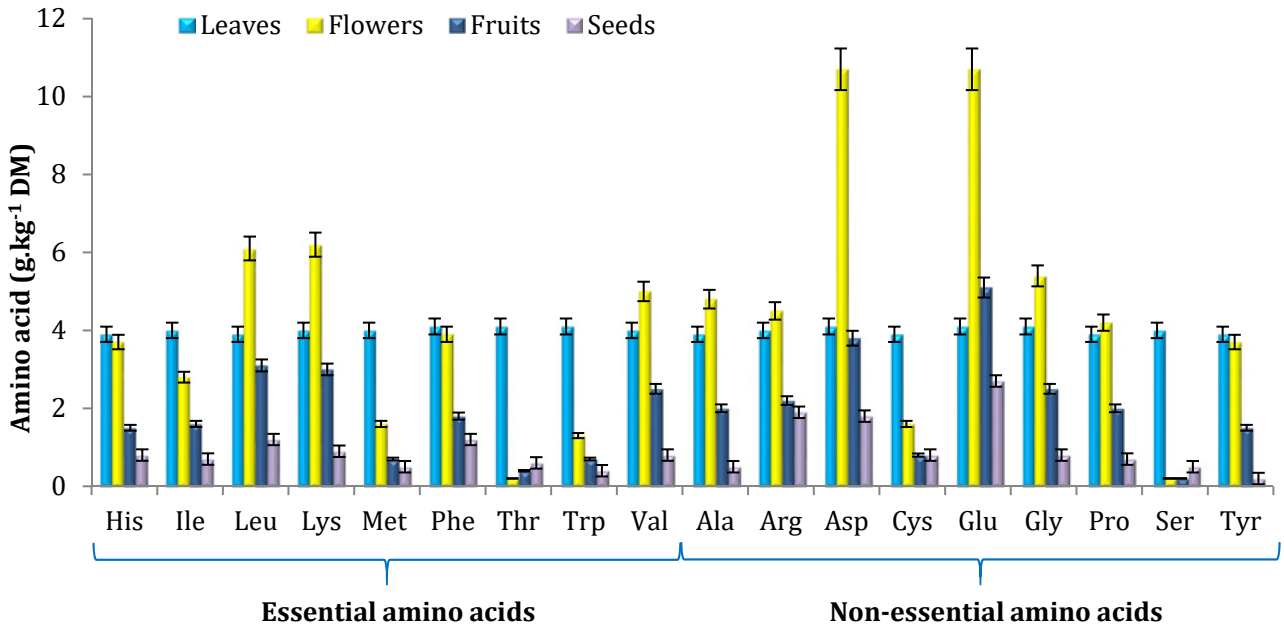

Figure 2 Amino acid composition (g.kg^{-1} of dry matter; DM) of *Cornus mas* L. leaves, flowers, fruits, and seeds

For nutritional reasons, it is important to search for long-chain PUFAs, especially those from the n-3 family.

In total eighteen amino acids were detected in *Cornus mas* samples, nine of them belonged to essential amino acids and also nine to non-essential ones (Figure 2). It should be highlighted that the contents of most of them differed significantly ($p < 0.05$) depending on the morphological part of plant.

The content of amino acids in leaves, flowers, fruits, and seeds was at the level of 72.0, 76.6, 35.4, and 17.0 $g.kg^{-1}$ of dry matter, respectively; while content of total essential amino acids was 36.1, 30.8, 15.3, and 7.1 $g.kg^{-1}$ of dry matter (amounted 50.1, 40.2, 43.2 and 41.8%, respectively) and 35.9, 45.8, 20.1 and 9.9 $g.kg^{-1}$ of dry matter (49.9, 59.8, 56.8 and 58.2%, respectively) for total non-essential amino acids.

It is important to emphasise that flowers of *Cornus mas* were distinguished by the highest content of amino acids in total. Furthermore, flowers contained the highest amounts for some amino acids, such as: leucine (6.1 $g.kg^{-1}$) and lysine (6.2), aspartic (10.7) and glutamic (10.7) acids (Figure 2). Predominating shares of lysine and leucine (from essential amino acids) were also found in other fruits, plants (Hegazy et al., 2019), as well as for glutamic acid (Guo et al., 2015). *Cornus mas* seeds has been shown to have the lowest content of amino acids compared with the other morphological parts of plant.

In the study of Zhurba et al. (2021) on the composition of *Schisandra chinensis* (Turcz.) Baill. leaves glutamic acid resulted to be the major component of non-essential amino acids (25 $g.kg^{-1}$ of dry matter), followed by aspartic acid (16.2 $g.kg^{-1}$) and leucine (14.2 $g.kg^{-1}$). This proves that *Cornus mas* leaves are not abundant in amino acids.

The contents of macroelements: K, P, Ca, S, Mg, Na, microelements: Zn, Fe, Cu, Mn, Cr, Se, and metals: Al, As, Cd, Ni, Hg, Pb in studied *Cornus mas* samples are presented in Table 3. It should be marked that the contents of most of them differed significantly ($p < 0.05$) depending on the morphological part of plant.

Calcium (Ca) was the most abundant element in *Cornus mas* samples (2647–25687 $mg.kg^{-1}$ of dry weight, except fruits (1644 $mg.kg^{-1}$ of dry weight), followed by K, P, Mg and S. Interestingly, *Cornus mas* fruits were rich in potassium (9545 $mg.kg^{-1}$ of dry weight)

Table 3 Elements composition of *Cornus mas* L. leaves, flowers, fruits, and seeds ($mg.kg^{-1}$ of dry weight; mean ± SE)

Element	Leaves	Flowers	Fruits	Seeds
Macroelements				
K	9190 ±366	10351 ±398	9545 ±289	844 ±68
P	3615 ±265	2390 ±199	929 ±54	977 ±62
Ca	25687 ±878	18856 ±812	1644 ±112	2647 ±217
S	2245 ±189	1917 ±128	1080 ±118	462 ±56
Mg	3004 ±212	1613 ±131	395 ±46	432 ±49
Na	7.0 ±0.8	40 ±1.7	8.0 ±0.8	9 ±0.6
Microelements				
Zn	30.0 ±1.3	37 ±1.1	5 ±0.2	24 ±1.1
Fe	54.0 ±1.2	29 ±0.9	42 ±1.2	82 ±1.5
Cu	5.0 ±0.3	13 ±0.9	6 ±0.1	6 ±0.1
Mn	12.5 ±1.1	7.9 ±1.3	2.9 ±0.05	2.3 ±0.04
Cr	0.68 ±0.08	0.38 ±0.02	0.40 ±0.01	0.47 ±0.02
Se	<0.2	0.48 ±0.02	<0.2	<0.2
Metals				
Al	14.6 ±1.5	16.1 ±1.2	6.1 ±0.1	2.6 ±0.02
As	<0.3	<0.3	<0.3	<0.3
Cd	0.013 ±0.002	0.073 ±0.001	<0.01	<0.01
Ni	0.60 ±0.04	0.29 ±0.02	0.23 ±0.01	0.39 ±0.91
Hg	0.026 ±0.001	0.013 ±0.001	0.007 ±0.0001	0.004 ±0.0001
Pb	0.25 ±0.016	1.64 ±0.04	0.27 ±0.012	1.51 ±0.02

at the level similar to other two studied samples – leaves and flowers. The content of calcium in leaves or flowers was much higher than in other cultivated fruits; like different types of berries and cherries, and also significantly higher than in wild yew berries (de Souza et al., 2014; Tabaszewska et al., 2021). Thus, not only fruits, but other parts of *Cornus mas* plant may be regarded as a valuable source of important elements in human diet.

Accordingly, literature data fruits of *Cornus mas* cultivars are rich in mineral content (Dinda et al., 2016). The concentration of minerals highly depends on cultivar, growing conditions, genotype, usig method, and technical treatments (Bayram and Ozturkcan, 2020). The mineral content in fruits from different countries are reported. For example, fruits: from Czech Republic contain K 3798–3411 mg.kg^{-1} of dry fruits, Ca 656–301 mg.kg^{-1}, Mg 290–241 mg.kg^{-1} and P 412–313 mg.kg^{-1} (Dokoupil and Reznicek, 2012); from Serbia K 5609–1845 mg.kg^{-1}, Ca 466–27 mg.kg^{-1} (Bijelic et al., 2011); from Greece K 1320–880 mg.kg^{-1}, Ca 30–20 mg.kg^{-1} (Sotiropoulos et al., 2011).

With regard to the presence of metals, the content of aluminium (2.6–16.1 mg.kg^{-1} of dry weight) strongly dominated among all detected metals in *Cornus mas* samples. The lead (Pb) was the second most abundant metal (0.25–1.64 mg.kg^{-1} of dry weight). The presence of trace metals in plants may resulted not only from the natural heavy metals occurrence in the soil but also originated from the environment contamination. From food safety reasons, control of the content and the tendency to accumulate toxic trace elements in fruits and other edible parts of plants seems to be of high importance.

Conclusions

This study provided new and valuable information regarding the nutrients content and composition in different morphological parts of *Cornus mas* from Ukraine. It was concluded that the contents of nutrients differed significantly ($p < 0.05$) depending on the morphological part of plant. Protein content varied from 2.27 up to 10.58% (in flowers). Authors determined substancial share of lipids (3.34–5.23%, except fruits). As expected, fruits were the most abundant in fructose (68.33 g.kg^{-1}), followed by leaves and flowers. Leaves were extremely rich in carotenoids, mainly β-carotene (88.5 mg.kg^{-1}) compared with other parts of plant. The fatty acid profile was represented by palmitic C16:0 (10.87–32.20 g.100 g^{-1} of oil), linoleic C18:2 *9c12c* (7.93–60.18 g.100 g^{-1} of oil), and α-linolenic C18:3 *9c12c15c* (1.68–30.22 g.100 g^{-1} of oil) acids. Nine out of 18 amino acids were essential amino acids (7.1–36.1 g.kg^{-1} of dry weight), with significant share of leucine, lysine, aspartic and glutamic acids in flowers. *Cornus mas* leaves and flowers may be regarded as an important source especially of calcium (18856–25687 mg.kg^{-1} of dry weight), and other minerals such as K, P, S and Mg. The obtained results clearly suggest that *Cornus mas* may become an inexpensive novel plant source of functional foods and as new ingredient in human diet, especially with regard to underappreciated leaves and flowers. Accordingly, to the profile of bioactive components presented in literature data (Bayram and Ozturkcan, 2020) and findings presented in this study regarding nutrients, *Cornus mas* may contribute to a healthy diet as a "super food".

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Lipid peroxidation and total antioxidant capacity in the muscle tissue of atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) after *in vitro* treatment by extracts derived from various species of *Dracaena* genus (Asparagaceae Juss.)

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Water extracts from selected *Dracaena* plants cultivated in greenhouse conditions were evaluated for antioxidant properties by *in vitro* methods using the muscle tissue of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill). The level of 2-thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC) in the muscle tissue of Atlantic sturgeon after treatment *in vitro* by extracts derived from leaves of selected *Dracaena* plants (in final concentration 10 mg.mL^{-1}) were assessed. When muscle tissue was incubated with leaf extracts of various species belonging to the *Dracaena* genus, the TBARS level was significantly increased for the sixteen extracts studied. Moreover, all extracts (except *D. singularis* extract) increase the formation of TBARS in the extracts-treated muscle tissue, and these results were statistically significant. Treatment of muscle tissue by extracts derived from various species from the *Dracaena* genus revealed also increase the TAC level. When homogenates were incubated with leaf extracts derived from various species from the *Dracaena* genus, the TAC level was significantly increased for the fifteen extracts studied. Moreover, all extracts (except *D. hyacinthoides* and *D. roxburghiana* extracts) induced the TAC increase in the extracts-treated muscle tissue of Atlantic sturgeon, and these increases were statistically significant. It can be supposed that secondary plant metabolites, i.e. polyphenolic compounds, alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, etc., in extracts derived from the leaves of various species belonging to the *Dracaena* genus, may contribute to their antioxidant activity. Further detailed studies on the effect of extracts derived from leaves of selected *Dracaena* plants on long time intervals, antioxidant, and molecular aspects are necessary to understand the mechanism of action of extracts in other fish and animals.

Keywords: 2-thiobarbituric acid reactive substances, total antioxidant capacity, Atlantic sturgeon, muscle tissue, *in vitro*

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Fish and fish products are the most valuable agricultural commodity traded internationally. Their annual sales are nearly US$ 80 billion and increasing each year (FAO-FishStat, 2006). The diseases in aquaculture are the most serious constraint that causes damage to the livelihood of farmers, loss of jobs, reduced incomes, and food insecurity (Assefa and Abunna, 2018). In recent years, to develop alternative practices for disease management in aquaculture, attention was diverted to finding the use of medicinal plant products as potential therapeutic measures for modulating the immune response and, specifically, the use of herbs to prevent and control fish diseases (Galina et al., 2009). Plant product application in aquaculture to combat microbial and parasitic diseases is considered an alternative approach for sustainable aquaculture, which is one of the promising alternatives to antibiotics (Dawood et al., 2021; Su et al., 2021).

The use of herbal therapy within animal production, as well as in the diet of commercial fish has shown promise, in that it is natural and biodegradable and has antimicrobial activity against various pathogens, including those relating to fish (Valladão et al., 2015). The herbals having the characteristics of immunostimulants have been able to increase survival and reduce the pathogenic load against pathogenic challenges by improving the immune system in fish (Anusha et al., 2014). However, applying a new component as a therapeutic drug in the fish diet requires more research on the effects on the physiological condition, biochemical changes in the cells, as well as health welfare of animals. Certainly, a healthy diet and safe feed are important factors in the prevention of widespread various diseases in aquaculture. Therefore, the study of diet components such as dietary supplements, particularly drugs, is an essential approach in aquaculture and fishery (Banaee et al., 2011).

In this study, attention was focused on *Dracaena*, a genus with diverse ethnobotanical uses in its geographical distribution range, which occupies an important place among plant genera applied for the treatment of a broad spectrum of diseases and disorders (Khalumba et al., 2005; Staples and Herbst, 2005; Kiringe, 2006; Owuor and Kisangau, 2006; Takawira-Nyenya et al., 2014). This is a historically recognized genus of flowering plants, now included in the genus *Dracaena* on the basis of molecular phylogenetic studies (Archibald et al., 2015).

The genus *Dracaena* consists of more than 100 accepted species which are mainly distributed in the tropics and subtropics, especially in Africa, Australia, and Southern Asia (Thu et al., 2020, 2021). They are mainly succulent shrubs and trees, and a few are commonly grown as shrubby houseplants, especially the variegated forms (Thu et al., 2020). Leaves and rhizomes of these plants are used in folk medicine for treating asthma, cough, sexual weakness, hypertension, diarrhea, hemorrhoids, abdominal pains, colics, eczema, piles, edema, jaundice, anuria, palpitations, viral hepatitis, malaria, snake- and insect bites, etc. (Andhare et al., 2012; Kpodar et al., 2016; Giovannini and Howes, 2017; Thu et al., 2021). Besides the medicinal aspects, several *Dracaena* species have great horticultural importance and are commercialized for use in landscaping and as indoor ornamental plants (Lekawatana and Suwannamek, 2017). Moreover, it has been reported that *Dracaena* species can be used as bioindicators for the control of increasing air pollution in urban cities (Saxena and Ghosh, 2013).

Our previous study (Buyun et al., 2016, Tkachenko et al., 2017a) has highlighted the antibacterial capacity of ten species of *Sansevieria* genus against *Staphylococcus aureus*. These plants have been screened in order to validate scientifically the inhibitory activity for microbial growth attributed to their popular use and to propose new sources of antimicrobial agents. Our results proved that the zones of inhibition ranged from 16 to 34 mm. Extracts from the leaves of *S. fischeri* and *S. francisii* were particularly active against the tested organisms (inhibition zones comprise up to 34 mm in diameter). This was followed by the activities of extracts from the *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, *S. metallica* leaves (diameters of inhibition zones ranged from 25 to 31 mm). The ethanolic extracts of *S. canaliculata* and *S. trifasciata* showed less antimicrobial activity (diameters of inhibition zones ranged between 16 and 16.5 mm). The results proved that the ethanolic extracts from *S. fischeri*, *S. francisii*, *S. parva*, *S. kirkii*, *S. aethiopica*, *S. caulescens*, *S. metallica* exhibit a favorable antibacterial activity against *S. aureus* (Buyun et al., 2016; Tkachenko et al., 2017a).

Although antimicrobial and antioxidant activities of extracts derived from leaves of various species belonging to the *Dracaena* genus were investigated (Al-Fatimi et al., 2007; Buyun et al., 2016, 2017; Maryniuk et al., 2017, 2018, 2019; Tkachenko et al., 2017a, 2017b, 2018, 2019a, 2019b), studies regarding their protective effects against free radical-induced oxidative stress in the muscle tissue of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) have not yet been undertaken.

Therefore, the aim of the current study was to evaluate *in vitro* the effect of buffer extracts derived from leaves of selected species belonging to the *Dracaena* genus (in final concentration 10 mg.mL^{-1}) against lipid peroxidation and protein damage using 2-thiobarbituric acid reactive substances and total antioxidant capacity.

Materials and methodology

Collection of plant materials and preparation of plant extracts

The leaves of plants, cultivated at glasshouse conditions, were sampled at M.M. Gryshko National Botanical Garden (NBG), National Academy of Science of Ukraine. The leaves of *Dracaena aethiopica* (Thunb.) Byng & Christenh., *D. canaliculata* (Carrière) Byng & Christenh, *D. caulescens* (N.E.Br.) Byng & Christenh, *D. angolensis* (Welw. ex Carrière) Byng & Christenh, *D. dooneri* (N.E.Br.) Byng & Christenh, *D. singularis* (N.E.Br.) Byng & Christenh, *D. francisii* (Chahin.) Byng & Christenh, *D. forscaliana* (Schult. & Schult. f.) Byng & Christenh, *D. serpenta* Byng & Christenh, *D. hyacinthoides* (L.) Mabb., *D. volkensii* (Gürke) Byng & Christenh, *D. pethera* Byng & Christenh, *D. zebra* Byng & Christenh, *D. parva* (N.E.Br.) Byng & Christenh, *D. roxburghiana* (Schult. Schult.f.) Byng & Christenh, *D. suffruticosa* (N.E.Br.) Byng & Christenh, *D. trifasciata* (Prain) Mabb. were sampled for study. Freshly collected leaves were washed, weighed, crushed, and homogenized in 0.1 M sterile phosphate buffer solution (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extracts were filtered and investigated for their antioxidant activity.

Tissue samples and experimental design

Clinically healthy Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus* Mitchill) with a mean body mass of 450–500 g were used in the experiments obtained from the Department of Salmonid Research, Stanislaw Sakowicz Inland Fisheries Institute (Rutki, Poland). The muscle tissue samples derived from fish were homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer immersed in an ice water bath. Homogenates were centrifuged at 3000 rpm for 15 min at 4 °C. After centrifugation, the supernatants were collected and frozen at -25 °C until analyzed. All enzymatic assays were carried out at 21 ±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate. The reactions were started by adding the tissue supernatants.

The supernatant of the muscle tissue was used to incubate with extracts of various species of *Dracaena* genus (in a ratio of 9 : 1) at room temperature. The control group (muscle tissue) was incubated with 100 mM Tris-HCl buffer (pH 7.2) (in a ratio of 9 : 1). The incubation time was 2 h. Biomarkers of lipid peroxidation and total antioxidant capacity, were studied in the incubated homogenates (control group and in samples with extracts of various species of *Dracaena* genus).

Determination of 2-thiobarbituric acid reactive substances (TBARS)

The level of lipid peroxidation was determined by quantifying the concentration of TBARS by Kamyshnikov (2004) for determining the malonic dialdehyde (MDA) concentration. Briefly, 2.1 mL of sample homogenate was added to 1 mL of 20% of trichloroacetic acid (TCA), and 1 mL of 0.8% of 2-thiobarbituric acid (TBA). The mixture was heated in a boiling water bath for 10 min. After cooling, the mixture was centrifuged at 3,000 rpm for 10 min. The absorbance of the supernatant was measured at 540 nm. The concentration of MDA (nmol. mg^{-1} of protein) was calculated using $1.56.10^5$ mM^{-1}. cm^{-1} as the extinction coefficient.

Measurement of total antioxidant capacity (TAC)

The TAC level in the samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). Sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The absorbance of the obtained solution was measured at 532 nm. The absorbance of the blank was defined as 100%. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank samples.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). All statistical calculation was performed on separate data from each individual with STATISTICA 8.0 software (StatSoft Polska Sp. z o.o., Krakow, Poland).

Results and discussion

A progressive accumulation of oxidative-induced damage to important cellular molecules resulting in oxidative stress and lipid peroxidation and protein damage is involved in various and numerous physiological and pathological states, i.e. senescence, inflammation, atherosclerosis, neurodegenerative diseases, cancer, etc. (Praticò, 2002; Guéraud et al., 2010). Oxidative damage occurs when free radicals produced within an organism are not completely destroyed by the appropriate endogenous defense systems. Because lipids are a major component of living organisms and probably the first easy target of free radicals once they are produced, lipid peroxidation might play an important role in initiating and/or mediating some aspects of the pathological processes (Praticò, 2002).

The first stage of our study was the assessment of 2-thiobarbituric acid reactive substances in the muscle tissue of Atlantic sturgeon after treatment *in vitro* by extracts derived from leaves of selected *Dracaena* plants.

When muscle tissue was incubated with leaf extracts of various species belonging to the *Dracaena* genus, the TBARS level was significantly increased ($p < 0.05$) for sixteen extracts studied (Figure 1). Moreover, all extracts (except *D. singularis* extract) increase the formation of TBARS in the extracts-treated muscle tissue, and these results were statistically significant. The most potent effect was demonstrated for the extracts derived from *D. angolensis* (TBARS increased by 154.9%, $p < 0.05$), *D. francisii* (by 143.7%, $p < 0.05$), *D. hyacinthoides* (by 136.2%, $p < 0.05$), *D. canaliculata* (by 128.7%, $p < 0.05$), *D. roxburghiana* (by 116.6%, $p < 0.05$), *D. aethiopica* (by 109.1%, $p < 0.05$). Also, extracts derived from *D. caulescens* (by 48.9%, $p < 0.05$), *D. suffruticosa* (by 30.1%, $p < 0.05$), *D. zebra* (by 81.3%, $p < 0.05$), *D. pethera* (by 63.9%, $p < 0.05$), *D. trifasciata* (by 45.9%, $p < 0.05$), *D. forscaliana* (by 49.7%, $p < 0.05$), *D. singularis* (by 10.6%, $p < 0.05$), *D. dooneri* (by 80.5%, $p < 0.05$), *D. volkensii* (by 54.2%, $p < 0.05$), *D. serpenta* (by 45.2%, $p < 0.05$), and *D. parva* (by 96.3%, $p < 0.05$) exhibited increase in the TBARS level in the muscle

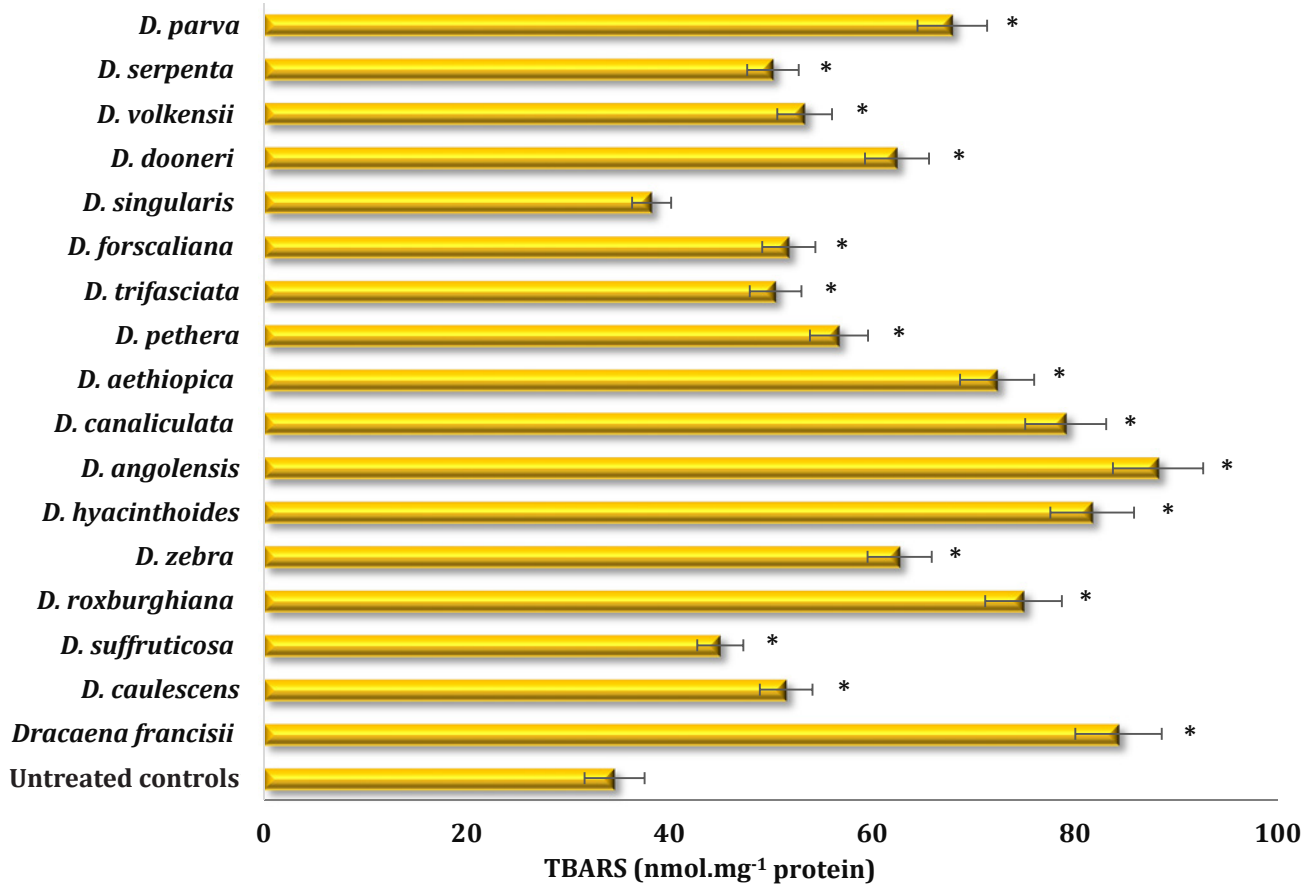

Figure 1 The level of 2-thiobarbituric acid reactive substances (TBARS) in the muscle tissue of Atlantic sturgeon after treatment *in vitro* by extracts derived from leaves of selected *Dracaena* plants (M ±m, n = 6)
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* the changes are statistically significant ($p < 0.05$) compared to the untreated control group

tissue compared to phosphate buffer as a control samples (Figure 1).

One of the strategies most commonly used to assess a free radical-antioxidant balance in chemical and biological systems is the determination of the total antioxidant capacity (Fraga et al., 2014). The measure of antioxidant capacity considers the cumulative action of all antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance *in vivo* between oxidants and antioxidants (Ghiselli et al., 2000). Thus, the next step of our study was the evaluation of the total antioxidant capacity in the muscle tissue of Atlantic sturgeon after treatment *in vitro* by extracts derived from leaves of selected *Dracaena* plants.

Treatment of muscle tissue by extracts derived from various species from the *Dracaena* genus revealed also increase the TAC level. When homogenates were incubated with leaf extracts derived from various species from the *Dracaena* genus, the TAC level was significantly increased ($p < 0.05$) for fifteen extracts studied. Moreover, all extracts (except *D. hyacinthoides* and *D. roxburghiana* extracts) induced the TAC increase in the extracts-treated muscle tissue of Atlantic sturgeon, and these increases were statistically significant (Figure 2).

The most potent effects were demonstrated for leaf extracts derived from various species belonging to the *Dracaena* genus, i.e. *D. singularis* (by 78%, $p < 0.05$), *D. serpenta* (by 78%, $p < 0.05$), *D. zebra* (by 71%, $p < 0.05$), *D. volkensii* (by 68%, $p < 0.05$), *D. caulescens* (by 69.5%, $p < 0.05$), *D. forscaliana* (by 61.1%, $p < 0.05$), *D. trifasciata* (by 58.8%, $p < 0.05$), *D. pethera* (by 57.3%, $p < 0.05$), and *D. suffruticosa* (by 53.4%, $p < 0.05$) compared to the control samples. Also, leaf extracts of *D. francisii* (by 41.1%, $p < 0.05$), *D. dooneri* (by 39.6%, $p < 0.05$), *D. angolensis* (by 30.4%, $p < 0.05$), *D. aethiopica* (by 18.9%, $p < 0.05$), *D. canaliculata* (by

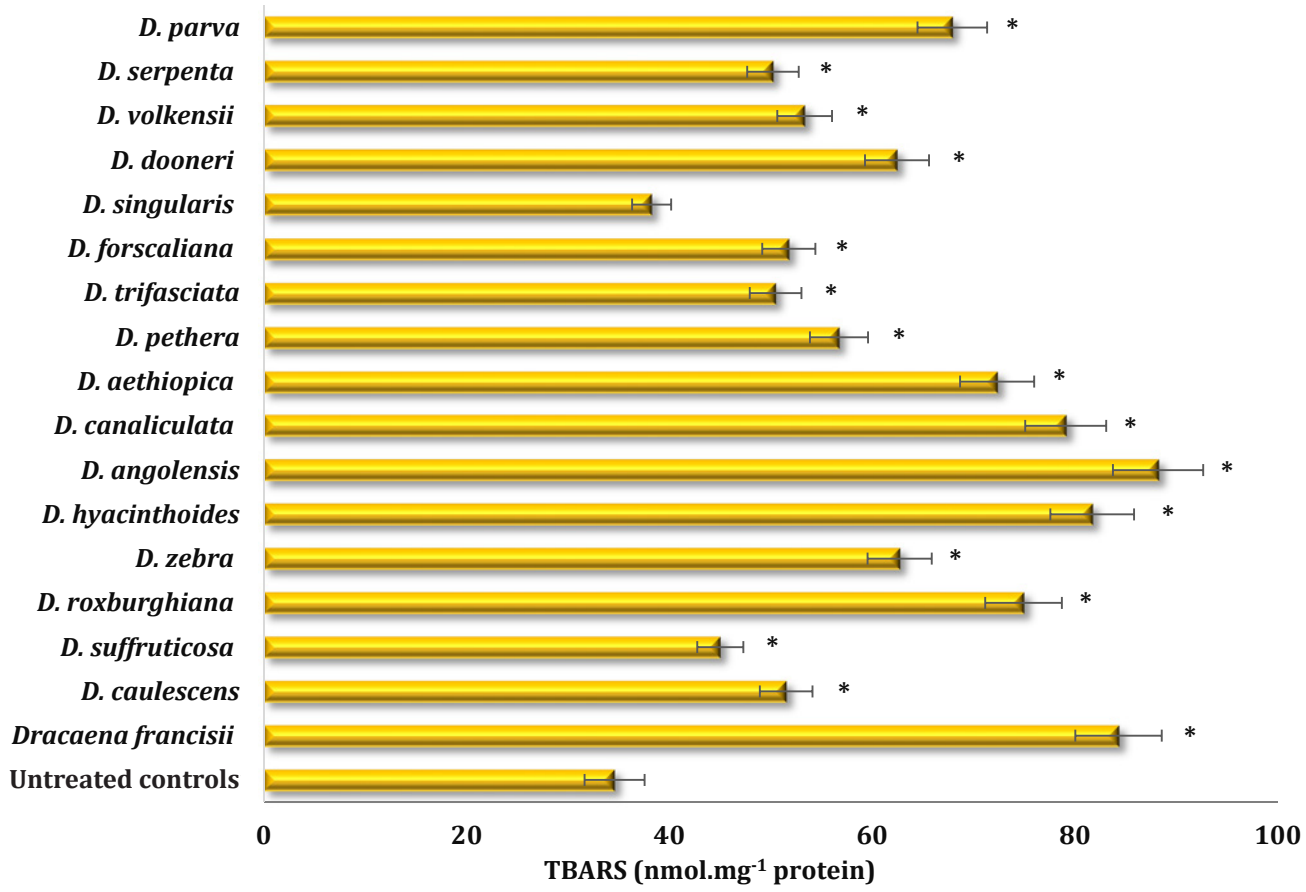

Figure 2 The level of total antioxidant capacity (TAC) in the muscle tissue of Atlantic sturgeon after treatment *in vitro* by extracts derived from leaves of selected *Dracaena* plants (M ±m, n = 6)
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* the changes are statistically significant ($p < 0.05$) compared to the untreated control group

14.3%, p <0.05), *D. roxburghiana* (by 13.5%, p <0.05), and *D. hyacinthoides* (by 12.8%, p <0.05) also increase the TAC level in the muscle tissue of Atlantic sturgeon compared to control samples (Figure 2).

In our previous study, we also studied the antioxidant activity of extracts obtained from leaves of selected species from *Sansevieria* species against oxidative stress using equine erythrocyte suspension (Tkachenko et al., 2017b). When erythrocytes were incubated with leaf extracts of various species from the *Sansevieria* genus, the aldehydic derivatives level was significantly reduced by 13.6% (p <0.05) for *S. forskaliana* extract. Moreover, all extracts (except *S. francisii* extract) reduced the formation of intracellular aldehydic derivatives of oxidatively modified proteins in the extracts-treated erythrocytes, but these results were non-significant. Treatment by extracts of various *Sansevieria* species reduced the concentration of ketonic derivatives of OMP when compared to untreated erythrocytes. The most potent effect was demonstrated by the *S. canaliculata*, *S. forskaliana*, *S. aethiopica*, *S. cylindrica*, *S. metallica*, *S. hyacinthoides*, and *S. kirkii* compared to control samples (phosphate buffer) (16.1%, 14.7%, 13.4%, 12.9%, 12.9%, 12.7%, 12.1%, respectively). However, there were no significant changes in other extracts. The experimental evidence obtained in our previous study indicated that various species of *Sansevieria* genus are a rich source of compounds that manifest antioxidant activity and can effectively protect erythrocytes against oxidative-induced damage. Thus, *S. canaliculata*, *S. forskaliana*, *S. aethiopica*, *S. cylindrica*, *S. metallica*, *S. hyacinthoides*, and *S. kirkii* may be a valuable source of natural antioxidants that may potentially be recommended for applications in medicine and veterinary practice. According to the above-mentioned antioxidant mechanisms, extracts of various species from the *Sansevieria* genus may inhibit the formation of protein carbonyl by scavenging free radicals formed *in vitro*. According to many supporting documents, it can be assumed that secondary plant metabolites, i.e. polyphenolic compounds in extracts of various species from *Sansevieria* genus extract may contribute to their antioxidant activity (Tkachenko et al., 2017b).

Really, the study of *D. roxburghiana* and *D. trifasciata* has revealed the presence of important compounds which were separated by thin layer chromatography (Kingsley et al., 2013). Preliminary phytochemical screening of the extracts of *D. trifasciata* plant showed the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins and carbohydrates (Anbu et al., 2009). Additionally, the methanolic extract of the whole plant of *D. trifasciata* has yielded 12 steroidal saponins, 10 of which are new constituents (Mimaki et al., 1996). Phytochemical analysis of the whole plant of *D. trifasciata* has resulted in the isolation of four new pregnane glycosides (Mimaki et al., 1997). Gas chromatographic analysis of the leaves revealed the presence of alkaloids, allicins, glycosides, and saponins (Ikewuchi et al., 2011). *Dracaena* and *Sansevieria* species are rich sources of steroidal saponins and have intriguing structures and interesting biological properties, including high cell antiproliferative/cytotoxic and anti-inflammatory activities. Several bioactive saponins from *Dracaena* and *Sansevieria* have the potential to become lead compounds for the development of anticancer therapeutic agents (Thu et al., 2021).

We suggested that the high TAC value in the muscle tissue of Atlantic sturgeon after treatment *in vitro* by extracts derived from leaves of selected *Dracaena* plants is the result of the high content of by-products, i.e. alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins, carbohydrates, etc. in the plant extracts. Thu et al. (2021) have reviewed the literature of about 180 steroidal saponins, isolated from *Dracaena* and *Sansevieria* species, as a basis for further studies. Saponins are among the most characteristic metabolites isolated from the two genera. They show a great variety in structural motifs and a wide range of biological activities, including anti-inflammatory, anti-microbial, anti-proliferative effects and, in most cases, remarkable cytotoxic properties (Thu et al., 2021). Saponins, an important group of bioactive plant natural products, are glycosides of triterpenoid or steroidal aglycones. Accumulated evidence suggests that saponins have significant neuroprotective effects on attenuation of central nervous system disorders, such as stroke, Alzheimer‘s disease, Parkinson‘s disease, and Huntington‘s disease. The proposed mechanisms of their neuroprotective function include antioxidant, modulation of neurotransmitters, anti-apoptosis, anti-inflammation, attenuating Ca^{2+} influx, modulating neurotrophic factors, inhibiting tau phosphorylation, and regeneration of neural networks (Sun et al., 2015).

The chemical structures of flavonoids support their capacity to scavenge free radicals and chelate redox-active metals. The thermodynamic analysis predicts that both, scavenging of oxygen-derived radicals and the sequestration of redox-active metals are energetically favored. Lipid-flavonoid and protein-flavonoid interactions can indirectly mediate a decrease in oxidant (free radical) production and/or oxidative damage to both cell and extracellular

components (Galleano et al., 2010). Also, biologically active isoquinoline alkaloids exhibit a broad range of bioactivities, including antitumor, antidiabetic and its complications, antibacterial, antifungal, antiviral, antiparasitic, insecticidal, anti-inflammatory, antioxidant, neuroprotective, and other activities (Shang et al., 2020). Moreover, many sesquiterpene's biological activities (anti-inflammatory, antiparasitic and anti-carcinogenic activities) are based on antioxidant or pro-oxidant actions of sesquiterpenes. Structure, concentration, metabolism as well as the type of cells determine if sesquiterpene acts as an antioxidant or prooxidant (Bartikova et al., 2014). On the other hand, the natural flavones, as well as some of their synthetic derivatives, have been shown to exhibit several biological activities, including antioxidant, anti-inflammatory, antitumor, anti-allergic, neuroprotective, cardioprotective, and antimicrobial (Catarino et al., 2015). Also, flavonoids are found to influence several mammalian enzymes like protein kinases that regulate multiple cells signaling pathways and alterations in multiple cellular signaling pathways are frequently found in many diseases (Singh et al., 2014). Some flavones interfere in distinct oxidative-stress related events by directly reducing the levels of intracellular free radicals (hydroxyl, superoxide, and nitric oxide) and/or of reactive species (e.g. hydrogen peroxide, peroxynitrite, and hypochlorous acid) thus preventing their amplification and the consequent damage of other biomolecules such as lipids, proteins, and DNA (Catarino et al., 2015). Flavones and flavonols re-establish the redox regulation of proteins, transcription factors and signaling cascades that are otherwise inhibited by elevated oxidative stress (Dajas et al., 2013). Flavones can also hinder the activity of central free radical-producing enzymes, such as xanthine oxidase and nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) or inducible nitric oxide synthase (iNOS) and can even modulate the intracellular levels of pro-oxidant and/or antioxidant enzymes (Catarino et al., 2015).

Conclusions

The present findings revealed that the extracts derived from various species belonging to the *Dracaena* genus have exhibited remarkable antioxidant potential in increasing the total antioxidant capacity in the muscle tissue of Atlantic sturgeon after *in vitro* treatment by extracts in a final concentration of 10 $mg.mL^{-1}$. On the other hand, according to the above-mentioned antioxidant mechanisms, extracts of various species from the *Dracaena* genus resulted in increasing the formation of lipid peroxidation using the measurement of TBARS as biomarkers of these processes. Thus, future studies are needed to assess the dose- and time-dependent changes in the antioxidant capacity in the different cell models after treatment by extracts derived from various species belonging to the *Dracaena* genus. It can be supposed that secondary plant metabolites, i.e. polyphenolic compounds, alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, etc., in extracts derived from the leaves of various species belonging to the *Dracaena* genus, may contribute to their antioxidant activity.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Research Article

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Risk elements, antioxidant activity and polyphenols in pseudocereal grains

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Pseudocereals, with their irreplaceable nutritional composition and amounts of bioactive substances with a positive effect on human health, are becoming a trend in human nutrition. In this work, we compared the safety of individual types of pseudocereals, namely buckwheat (*Fagopyrum esculentum*, var. Zita), quinoa (*Chenopodium quinoa*, var. Carmen), amaranth (*Amaranthus cruentus*, var. Pribina), and sorghum (*Sorghum bicolor*, var. Ruzrok) in terms of the content of hazardous metals. We assessed the ability of individual species of pseudocereals to accumulate hazardous metals from the soil in the consumable parts of the plant. The ability of heavy metals to accumulate was calculated using a bioaccumulation factor. We also evaluated the influence of the content of selected hazardous metals on the antioxidant capacity of grains of individual types of pseudocereals. We determined the total polyphenol content and the total antioxidant content using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical spectrophotometrically. We determined the content of hazardous metals by the AAS method (Atomic Absorption Spectrometry). In the soil from all plots with cultivated species of pseudocereals, we recorded an increased content of Cd, Pb, Co in comparison with the limit value set by Law no. 220/2004. The content of risk elements was not exceeded in the consumption parts of plants and the content of Cd and Pb was below the detection limit. From a safety point of view, it is possible to prefer the *Chenopodium quinoa*, which had the lowest content of heavy metals in the grains. Buckwheat follow, and at about the same level are amaranth and sorghum bicolor. The safest or the most resistant plant species with the lowest ability to accumulate hazardous metals from soil to grains, from the group of crops we monitor, is the *Chenopodium quinoa*.

Keywords: pseudocereals, DPPH, polyphenols, risk elements

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Introduction

The definition of pseudocereals is that they are like the fruits or seeds of non-digestive species that are consumed in a very similar way to cereals (Das, 2016). From a botanical point of view, they belong to dicotyledonous plants, therefore they do not belong to cereals that are monocotyledonous, but since they produce starch-rich seeds, they are grown and used similarly as cereals. According to most researchers, they are termed "pseudocereals", due to their related properties to cereal grains: starch content, texture, palatability, and method of preparation (Ciudad-Mulero et al., 2019).

Native Americans already fed pseudocereals, which were able to improve their endurance and mental development, thanks to their positive qualities. The Mayans and Incas considered these grains sacred (Rollán et al., 2019). The origin of the amaranth and the quinoa is in Latin America. Buckwheat originally spread from Asia (Cai et al., 2016). Hermuth et al. (2012) state that sorghum is one of the longest-grown crops currently grown on all continents. Its origin is from Egypt, where it was used as a crop. He returned to Europe in the 15th and 16th centuries through the Arabs.

Pseudocereals are a relatively common ingredient in human nutrition. They are a staple food as they are the primary suppliers of carbohydrates and proteins to the world's population. They also provide significant amounts of energy and trace elements for human nutrition (Margitanová et al., 2009).

Pseudocereals are important gluten-free crops, including buckwheat, amaranth, quinoa, and sorghum. Their nutritional properties and suitability for the preparation of gluten-free foods predetermine their use as functional foods (Martínez-Villaluenga et al., 2020). They are known to have good nutritional value, thanks to proteins with high biological value. Amaranth, quinoa, and buckwheat have a much higher protein content than cereals, and also the quality of the proteins is better because the amount of lysine found in cereals is limited (Kocková and Valík, 2011; Das, 2016).

Pseudocereals are promising crops of the future due to their high genetic variability, which can adapt to different climatic conditions, from tropical to temperate climates (Joshi et al., 2018). Pseudocereal grains have a high content of starch, fiber, and protein with a quality, balanced composition of essential sulfur-rich amino acids. They are also a good source of minerals (Ca, Fe, and Zn), vitamins, and phytochemicals such as saponins, polyphenols, phytosterols, phytosteroids, and betalains with potential health benefits (Martínez-Villaluenga et al., 2020).

Material and methodology

Biological material

We took samples of individual pseudocereals in the full ripening phase: amaranth (*Amaranthus cruentus*, var. Pribina), quinoa (*Chenopodium quinoa*, var. Carmen), buckwheat (*Fagopyrum esculentum*, var. Zita), and sorghum (*Sorghum bicolor*, var. Ruzrok). Subsequently, the treatment of the samples was continued by mechanical cleaning, water jet cleaning, and drying to constant weight. Finally, the individual samples were ground. The ground samples were stored in paper bags for further analysis. The pseudocereal seeds were homogenized with a mixer to a final fine powder. Subsequently, we prepared extracts. We weighed 10 g of the homogenized sample on analytical balances, added it to the extraction cartridges, and extracted it in 100 mL of 80% methanol for 8 h in Twisselmann. Upon completion, the resulting extract was filtered into 50 mL centrifuge tubes using filter paper.

Determination of soil exchange reaction in KCl (pH/KCl)

We poured 20 g of fine soil I. over with KCl solution (c = 1 mol.dm^{-3}) and left for 24 h at room temperature. The prepared suspension was shaken by Heidolph Promax 1020 shaker at a frequency of 180 oscillations per minute for 10 minutes. After shaking and gradually settling the suspension and subsequent filtration through FILTRAK 390 filter paper, pH/KCL in the filtrate was measured.

Determination of total heavy metal content in the soil

1 g fine soil II. Was weighed into a boiling flask and added 2–3 cm^3 of distilled water, 2.5 cm^3 of concentrated HNO_3 and 7.5 cm^3 of concentrated HCl. The suspension was left over night. The suspension was extracted at reflux for 2 h. Suspension was filtered through FILTRAK 390 filter paper into a dry volumetric flask (V = 100 cm^3). Before the filtration the filter was moistened by 10% HNO_3. We determine the content of heavy metals by the VARIAN AA 240FS using the AAS method.

Soil extraction with NH_4NO_3 solution

20 g of fine soil I. was weighed into containers (100 cm^3). Subsequently, we added 50 cm^3 of NH_4NO_3 (c = 1 mol.dm^{-3}). Content was mixed and closed into the container. Extract the suspension on a Heidolph

Promax 1020 was shaken for 2 h at 180 oscillations per minute. We used FILTRAK 390 filter paper. The first part of the filtrate was discarded. We also performed a blank experiment with the samples. Later, 0.5 cm^3 of concentrated HNO_3 was added to the filtrate. The content of hazardous metals was determined by the AAS method using the VARIAN 240 FS instrument.

Determination of the content of macroelements in the grains of the examined pseudocereals

2 g of the homogenized sample poured with 10 cm^3 of HNO_3 and 5 cm^3 of concentrated $HClO_4$. Let stand for 24 hours. The sample was mineralized in a sand bath to form white $HClO_4$ vapors, then filtered into a volumetric flask (100 cm^3), which we made up to the mark with distilled water. We pipetted 2 cm^3 from the extract into a volumetric flask (50 cm^3) and made up to the mark with distilled water. We determined the content of macroelements on a Varian AA 240 FS instrument by atomic absorption spectrometry. In the determination of P, we diluted 1 cm^3 of the extract into a 50 cm^3 volumetric flask, while we also added 8 cm^3 of the mixed solution. Subsequently, we replenished the bank with distilled water to the mark. The sample prepared in this way was allowed to stain for 2 hours and the intensity of the staining was measured at a wavelength of 666 nm on a Shimadzu UV/VIS-1800 spectrophotometer.

Determination of heavy metal content in pseudocereal grains

1 g of homogenized sample was poured into 5 cm^3 of HNO_3 and 5 cm^3 of redistilled water in a mineralization cartridge. We performed the mineralization on a MARS X-press. After the mineralization was completed, the minerals were filtered into a 50 cm^3 volumetric flask. We filled the contents of the bank to the mark with distilled water. We determined the content of risk elements in the seeds of individual pseudocereals by the AAS method using the VARIAN 240 FS device.

Transport of heavy metals from soil to grains

To determine the ability to absorb hazardous metals from the soil and accumulate them in the seeds of the monitored pseudocereals, we calculated a bioaccumulation factor:

$$BAF = c\ (plant)/c\ (soil)$$

We calculated bioaccumulation factors (BAF) based on the values of pseudo total risk metal content determined in soil leachate with aqua regia (AR) and heavy metal content in grains (BAF_{AR}), as well as based on values of bioavailable forms determined in soil leachate with ammonium nitrate (NH_4NO_3) and heavy metal content in pseudocereal grains (BAF_{AN}).

Determination of the total content of polyphenols in the grains of the examined pseudocereals

To determine the total content of polyphenols (TPC) in the consumables of the examined pseudocereals, we applied the commonly used spectrophotometric method according to Lachman (2003) using a Folin-Ciocalteu probe. We prepared suspension from 10 g of sample and 100 mL of 80% methanol extract. After extraction, the suspension is filtered using FILTRAK 390 filter paper. Dilute 0.1 mL of the filtrate with distilled water in a 50 mL volumetric flask. We added 2.5 mL of Folin-Ciocalteu tube to the diluted sample. After 3 minutes, we added 5 mL of 20% aqueous Na_2CO_3 solution. The sample prepared in this way was made up to the mark with distilled water with a total volume of 50 mL and the flask was mixed thoroughly. After two hours of standing, a color complex formed. The absorbance of the blue-colored solutions was measured on a Shimadzu UV/VIS-1800 spectrophotometer at a wavelength of 765 nm. Based on the equation of the calibration curve, we obtained the values of the total content of polyphenols in pseudocereals. The obtained results were recalculated and expressed as mg of gallic acid per kg of dry material (mg $GAE.kg^{-1}$/d.m.).

Spectrophotometric determination of total antioxidant activity

We determined the total antioxidant activity by the method of Brand-Williams (1995). The method is based on the use of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reactions. In the free radical scavenging activity assay, 3900 µL of the DPPH solution was added to 4000 µL tubes, followed by 100 µL of the extract or its dilution. The solutions were mixed and incubated in the dark at room temperature. We measured the absorbance value that corresponds to the initial concentration at time A0. We measured dependeces after 10 minutes (A10). Absorbance was measured using a Shimadzu UV-VIS 1800 spectrophotometer at a wavelength of 515.6 nm.

Statistical analysis

We applied a one-way analysis of variance (ANOVA) to evaluate the results mathematically and statistically. We determined statistically significant differences using the LSD contrast test with a 95% confidence level,

with a P-value <0.05. We used the STATHATGRAPHICS centurion XVI program for statistical processing of results. We evaluated the content of heavy metals in the soil with descriptive statistics.

Results and discussion

Land from the territory of Piešťany, from plots on which individual types of pseudocereals were grown, belongs to sandy-clayey to clayey soils. We evaluated the measured values based on the Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic no. 338/2005. The soil reaction was neutral to alkaline. The pH of the soil significantly affects the balance and availability of macroelements for plants. All species studied have the best yields when grown on soil with a neutral, alkaline pH, which corresponds to our soil (Jaroszewska et al., 2019; Farooq et al., 2016; Jäger, 2016).

Macronutrients and pH of soil

Our values for the content of macroelements in the soil (Table 1), where amaranth was grown, had on average higher values than reported by Jimoh et al. (2020). In their study, they have the results of analyses of soil macronutrients and pH/KCl in the soil before and after planting. We compared our values with the measured values after planting, because our soil sample was also taken after planting amaranth. Jimoh et al. (2020) claim that some macroelement concentrations in soil have decreased after planting, while others have increased. The concentration of P and K decreased after planting, on the contrary, the content of Ca and Mg increased almost threefold compared to the original concentration. For this reason, the amaranth could serve as a phytoremediator, especially in areas with reduced macroelements. In comparison with the content of K and P in the soil of quinoa, reported by Haseeb et al. (2018) is significantly lower than ours. Janovská et al. (2008) argue that buckwheat does not use nutrients evenly from the soil during its growth. Its requirements for the amount of P received are usually higher during the flowering period and the production of sutures. Kováč (2011) states that sorghum as a crop is relatively demanding on the content of macronutrients occurring in the soil.

Risk elements in the soil

In Table 2, we present the values of the determined content of hazardous metals in the soil extract of the aqua regia, the so-called pseudo total content. It represents all forms of metal present in the soil except for silicate forms. We compared the recorded values with the limit amount set by Law no. 220/2004.

Exceedance of the limit value of hazardous metals in soil was recorded for two risk elements, namely Co and Cd. The exceeding amounts of these risk elements in soil extracts were measured in all monitored pseudocereal species (Table 2).

Acidic soil pH is said to be considered the most important factor influencing the increased uptake of heavy metals by plants. In alkaline soils with pH (7.1–8.1) the risk of heavy metal leaching and their bioavailability to plants is lower and the presence of organic substances may also inhibit the absorption of metals from the soil solution. By altering these

Table 1 Characteristics of macronutrient content (mg.kg^{-1}) and pH in soil from Piešťany

Species	K	Ca	Mg	P	pH/KCl
Amaranthus cruentus	199.60	4372.30	534.60	45.40	7.42
Sorghum bicolor	188.70	4628.30	398.80	59.90	7.34
Chenopodium quinoa	222.30	4780.90	434.50	51.00	7.32
Fagopyrum esculentum	192.60	4429.90	387.40	65.90	7.25

Table 2 Pseudo total content of risk elements in the soil extract of aqua regia (mg.kg^{-1})

Species	Zn	Cu	Co	Ni	Cr	Pb	Cd	Hg
Amaranthus cruentus	55.10	22.70	15.60	46.90	34.30	37.60	1.14	0.067
Sorghum bicolor	55.60	22.30	15.70	44.30	30.50	35.80	1.02	0.046
Chenopodium quinoa	52.20	21.70	17.00	49.40	31.20	37.40	1.06	0.044
Fagopyrum esculentum	54.90	21.70	16.50	46.50	29.80	37.30	1.09	0.018
Limit value AR	150	60	15	50	70	70	0.70	0.5

Notes: AR – aqua regia

soil properties, which determine the solubility of metals in the soil, it is possible to immobilize heavy metals in the solid phase. The mobility of metals and their bioavailability can be affected by the addition of organic and inorganic substances. The basic treatment limiting the mobility of metals is deacidification of the soil by liming (Paltseva et al., 2018; Zwolak et al., 2019). Table 3 shows the content of bioacceptable forms of hazardous metals in the soil extract with ammonium nitrate.

The content of Cd and Pb was exceeded in soil extracts with ammonium nitrate in all monitored pseudocereals. Vollmannová et al. (2013) also reported an increased Pb content in the soil ammonium nitrate leachate. Their measured value was 2.3 times higher, while the our measurement was 4.5 times higher than the critical value.

Amari et al. (2017) report that cadmium and lead are very common pollutants in the environment with a long biological half-life. Muszyńska and Labudda (2019) argue that in temperate soils the half-life of selected heavy metals ranges from 75 to 380 years for Cd and from 1000 to 3000 years for Cu, Ni, Pb, Zn, and Se. For this reason, all these elements are considered non-biodegradable and persistent. However, they can be partially removed from the place where they accumulate by the natural ability of the plant species. Certain plant species can absorb heavy metals through penetration into roots or leaves. Radovanovic et al. (2020) report that cadmium is a highly toxic metal for humans and plants even in very low concentrations. The toxic effects of cadmium on plants can manifest themselves as a stress factor causing various physiological disorders. Jeddou et al. (2017) report that cadmium is found in elevated concentrations (0.1–1 $mg.kg^{-1}$) in soils worldwide. Harangozo (2018) states that lead is insoluble in neutral, weakly alkaline, or weakly alkaline soils with a higher content of organic matter and humus. Its content in the soil depends on the organic low molecular weight substances present in the soil. Demková et al. (2017) argue that heavy metals accumulated in the soil usually migrate into the vegetation and subsequently through the food chain into the human body.

Macroelements in pseudocereal grains

In comparison with the results of Gordillo-Bastidas et al. (2016) and Nowak et al. (2016), we can state that our measured values (Table 4) in quinoa are comparable in the content of K, P, Ca, Na. The value of Mg measured by us was lower than stated by the authors. Angeli et al. (2020) report comparably higher contents of P, Ca, Mg and similar contents K. Rodríguez et al. (2020) report the same P content in quinoa, higher Ca content, and lower K and Na content. In contrast, Palombini et al. (2013) in their study report lower values of P, Na, and K content and higher amounts of Ca and Mg in quinoa. He also states in his work significantly higher measured values of K, P, Mg, Na content in amaranth. On the contrary, the P content is halved. Comparable K and Na contents in amaranth are reported by Coelho et al. (2018) and Rodríguez et al. (2020), but they have a higher P and Ca content, but report a lower Mg content. Abdelhalim et al. (2019) report a comparable P content in sorghum, but the Ca content is several times higher than our value. Pontieri et al. (2014)

Table 3 Content of bioacceptable forms of risk elements in soil leachate NH_4NO_3

Species	Zn	Cu	Ni	Pb	Cd	Fe	Mn	Co
Amaranthus cruentus	0.08	0.09	0.28	0.46	0.12	0.25	0.33	0.25
Sorghum bicolor	0.10	0.08	0.32	0.45	0.13	0.22	0.22	0.27
Chenopodium quinoa	0.10	0.09	0.33	0.40	0.13	0.25	0.32	0.83
Fagopyrum esculentum	0.08	0.09	0.31	0.49	0.13	0.29	0.20	0.28
CV NH_4NO_3	2.0	1.0	1.5	0.1	0,1	–	–	–

Notes: CV – critical value NH_4NO_3 (c = 1 $mol.dm^{-3}$) set by Law no. 220/2004

Table 4 Content of macroelements in pseudocereal grains

Species	K ($mg.kg^{-1}$)	Na ($mg.kg^{-1}$)	Ca ($mg.kg^{-1}$)	Mg ($mg.kg^{-1}$)	P ($mg.kg^{-1}$)
Amaranthus cruentus	4470.5 ±189.6^{a}	43.6±1.85^{a}	751.6±30.68^{c}	1225.1±64.05^{a}	1301.1±59.56^{a}
Sorghum bicolor	4576.0 ±186.81^{a}	85.3 ±4.46^{c}	191.6±7.82^{a}	1159.8±60.64^{a}	1461.3±118.05^{b}
Chenopodium quinoa	9759.1 ±510.2^{c}	145.6±7.61^{d}	857.10±44.81^{d}	1534.4±62.64^{b}	2100.0±109.79^{c}
Fagopyrum esculentum	5642.8 ±295.01^{b}	63.80±2.60^{b}	239.5 ±9.78^{b}	1137.4±59.46^{a}	2891.7±68.02^{d}

Notes: LSD test, mean ± standard deviation (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, p <0.05

report a comparable K content, several times higher Na content, higher Mg, Ca, and P content in sorghum. Al-Snafi (2017) and Joshi et al. (2019) and Zhang and Xu (2017) report higher values of Ca, Mg, P content in edible buckwheat, but lower values of K content. In comparison with the results of macroelements reported by Rodríguez et al. (2020) in edible buckwheat, the Ca content coincides with ours, the amount of P is slightly higher, K and Na are lower.

Microelements in pseudocereal grains

Table 5 shows the values of the determined content of microelements in the investigated species of pseudocereals.

In comparison with the measured concentrations in amaranth and quinoa according to Bratovcic and Saric (2019), we can state that in the copper content our results were comparable in both crops. As for the amount of zinc, our measured values were lower; in quinoa, the value was half, and in amaranth 3 times lower. Our values also differed in the amount of iron. In quinoa and amaranth, our value was halved. Angeli et al. (2020), in turn, report comparably higher iron and zinc contents in quinoa.

The correlating Fe and Zn content in quinoa is reported by Nowak et al. (2016), but the amount of measured copper is higher in comparison of our results. In contrast, Rodríguez et al. (2020) report corresponding copper and manganese contents in quinoa, but have lower iron and zinc contents. Martinez-Lopez et al. (2019) and Rodríguez et al. (2020) state in their publication the amount of iron and copper in amaranth is comparable to our results, but the content of manganese and zinc is higher, even zinc 2.3 times. Coelho et al. (2018) report similar iron and cobalt contents, but zinc and manganese contents are higher than our measurements. Al-Snafi (2017) states a comparable content of zinc in buckwheat, a higher content of manganese, copper compared to our measured values, but on the contrary a lower content of iron. Joshi et al. (2019) and Zhang and Xu (2017) report a comparable content of iron and copper in buckwheat, but a higher content of manganese. In contrast, Rodríguez et al. (2020) in their study states that the content of manganese in buckwheat is identical to our values, the registered content of copper and zinc is higher, on the contrary, iron indicates less. Pontieri et al. (2014) report a similar amount of iron in sorghum, but higher manganese, nickel, and 2.3 times higher zinc.

Heavy metals in pseudocereal grains

Table 6 has shown the values in the heavy metal content of the monitored pseudocereal species. From the point of view of safety, based on the results, we can state that the content of Cd and Pb in the grains of the monitored pseudocereal species was below the detection limit. In this respect, the monitored pseudocereals are safe for the consumer.

Table 6 Heavy metal content in pseudocereal grains

Species	Hg (mg.kg^{-1})	Cd	Pb
Amaranthus cruentus	0.0099 ±0.0004^{c}	ND	ND
Sorghum bicolor	0.0065 ±0.0002^{b}	ND	ND
Chenopodium quinoa	0.0040 ±0.0001^{a}	ND	ND
Fagopyrum esculentum	0.0416 ±0.0017^{d}	ND	ND
Limit value (FC SR)	0.05	0.1	0.2

Notes: LSD test, mean ± standard deviation (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, p <0.05; FC SR – Food Code of the Slovak Republic; ND – not detected

The basis for increasing the safety of the food chain is a correct understanding of the mechanisms, regulation of storage, and distribution of heavy metals in plants (Aprille and Bellis, 2020). Zhang and Xu (2017) state that plant-derived products can often be influenced by environmental and geological factors such as soil type and pH, climatic conditions, and others. Elemental analysis is usually considered an effective tool because plants can absorb macro-and micro-elements as well

Table 5 Content of microelements in pseudocereal grains

Species	Cu (mg.kg^{-1})	Zn (mg.kg^{-1})	Mn (mg.kg^{-1})	Fe (mg.kg^{-1})	Cr (mg.kg^{-1})	Ni (mg.kg^{-1})	Co (mg.kg^{-1})
Amaranthus cruentus	5.5 ±0.04^{c}	11.5 ±0.60^{c}	19.9 ±0.81^{c}	91.9 ±3.75^{c}	0.9 ±0.037^{b}	0.3 ±0.01^{c}	0.3 ±0.01^{b}
Sorghum bicolor	3.8 ±0.05^{a}	9.8 ±0.51^{b}	10.8 ±0.56^{a}	53.1 ±2.78^{a}	3.60 ±0.20^{c}	0.3 ±0.02^{c}	0.6 ±0.03^{c}
Chenopodium quinoa	4.0 ±0.20^{a}	10.0 ±0.52^{b}	16.20 ±0.65^{b}	60.3 ±2.46^{b}	0.5 ±0.03^{a}	0.1 ±0.004^{a}	0.3 ±0.012^{b}
Fagopyrum esculentum	4.6 ±0.24^{b}	7.3 ±0.38^{a}	11.5 ±0.47^{a}	56.3 ±2.28$^{a, b}$	0.9 ±0.05^{b}	0.2 ±0.01^{b}	0.1 ±0.005^{a}
FC SR	10	50	–	–	4	3	–

Notes: LSD test, mean ± standard deviation (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, p <0.05; FC SR – the hygienic limit set by the Food Code of the Slovak Republic

as heavy metals from the soil, and therefore there is a link between the content of elements in the soil and the degree of their accumulation in crops.

Transport of heavy metals from soil to grains

The resulting values are given in Tables 7 and 8. As the content of lead and cadmium in the NH_4NO_3 soil extract was exceeded in all monitored pseudocereal species and their amount was below the detection limit in grains, we can state that the investigated plant species have a low ability to absorb these hazardous metals from the soil as well as a low ability to subsequently accumulate in grains.

Ogunkunle et al. (2015) report bioaccumulation factor values in amaranth in zinc and cadmium levels higher than 1. They argue that amaranth has the potential to accumulate these hazardous metals from the soil. In comparison with the reported values of Memoli et al. (2017) determined bioaccumulation factor values in *Sorghum bicolor* for chromium content higher, but for nickel and copper content comparable to our BAF_{AR} values. Bhargava et al. (2008) report bioaccumulation factor values in quinoa comparable for chromium and nickel content, but lower for copper and zinc content compared to our BAF_{AR} values. The bioaccumulation factor values according to Fu et al. (2015) in zinc and chrome buckwheat are compatible with our BAF_{AN} values. The pseudocereals we research are characterized by the significant formation of above-ground biomass, which can accumulate hazardous metals. It is plants that can absorb heavy metals from the soil and accumulate them in large volumes of biomass, which are considered to be hyperaccumulators of heavy metals and are suitable as phytoremediation crops.

Total polyphenol content and antioxidant activity of grains

Roccheti et al. (2017, 2019) measured the total content of polyphenols in amaranth (570 mg GAE.kg^{-1}), which is much lower than us (1251.1 mg GAE.kg^{-1}). Roccheti et al. (2019) and Liu et al. (2019) state the total content of polyphenols in buckwheat (2750–5320 mg GAE. kg^{-1}), in this range is also our measured value (3560 mg GAE.kg^{-1}). Han et al. (2019) measured the total content of polyphenols in quinoa (1672–3083 mg GAE.kg^{-1}), we can say that our contents correlate with each other. Our results for TPC in *Sorghum bicolor* are much higher, as reported by Roccheti et al. (2019). Shen et al. (2018) in their study recorded the total content of polyphenols in different cultivars of *Sorghum bicolor*

Table 7 BAF_{AR} values in pseudocereal seeds

Species	Cu	Zn	Cr	Ni	Co	Pb	Cd
Amaranthus cruentus	0.209	0.242	0.019	0.006	0.026	0.145	–
Sorghum bicolor	0.177	0.170	0.045	0.007	0.118	0.137	–
Chenopodium quinoa	0.192	0.184	0.018	0.002	0.016	0.089	–
Fagopyrum esculentum	0.133	0.212	0.006	0.004	0.030	2.271	–

Table 8 BAF_{AN} values in pseudocereal seeds

Species	Cu	Zn	Cr	Ni	Co	Pb	Cd
Amaranthus cruentus	61.11	143.75	9.0	1.07	1.20	–	–
Sorghum bicolor	47.50	98.0	32.73	0.93	2.59	–	–
Chenopodium quinoa	44.45	100.0	4.55	0.30	0.36	–	–
Fagopyrum esculentum	51.11	91.25	6.92	0.65	0.35	–	–

Table 9 Total polyphenol content (TPC) and antioxidant activity values (TAC) in the grains of the examined pseudocereals

Species	TPC (mg GAE.kg^{-1})	TAC (%)	TAC (µmol TE.g^{-1})*
Amaranthus cruentus	1251.1^{a}	11.175^{a}	1.184^{a}
Sorghum bicolor	11495.8^{d}	77.35^{d}	8.196^{d}
Chenopodium quinoa	2952.5^{b}	33.05^{b}	3.502^{b}
Fagopyrum esculentum	3560.3^{c}	74.0^{c}	7.840^{c}

Notes: LSD test, mean (n = 4), the coefficients (a, b, c, d) show a statistically significant difference, $p < 0.05$; * for a better comparison of the results, we also stated the values of µmol TE.g^{-1}

(1744–12388.3 mg GAE.kg^{-1}). Based on the knowledge from the literature, it can be stated that the values of TPC in *Sorghum bicolor* are in a wide range.

Škrovánková et al. (2020) state in their research the value of antioxidant activity in buckwheat (167–280 mg TE.100 g^{-1}), which is after recalculation (6.68–11.2 µmol TE.g^{-1}); it can be stated that our values are comparable with each other (7.840 µmol TE.g^{-1}). A similar value of antioxidant activity in buckwheat (6.2 µmol TE.g^{-1}) is published by Aleksenko (2013). In contrast, Salehi et al. (2018) report values of antioxidant activity in buckwheat (2.68–6.270 mg.g^{-1}), which is calculated (10.72–25.08 2 µmol TE.g^{-1}), that is higher compared to our values. Škrovánková et al. (2020) also measured the values of antioxidant activity in amaranth (26.4 mg.100 g^{-1}), which is calculated (1.056 µmol TE.g^{-1}), which corresponds to our results (1.184 mol TE.g^{-1}). Comparable values of the antioxidant activity of amaranth (43.2–110.7 mg.100 g^{-1}) are also reported by Park et al. (2020), which is calculated (1.728–4.428 µmol TE.g^{-1}). Antioxidant activity in quinoa in a study by Škrovánková et al. (2020) was in the range of 97.4–100.6 mg.100 g^{-1}, which is 3.896–4.024 µmol TE.g^{-1} after conversion. It can be stated that these results are consistent with our values (3.502 µmol TE.g^{-1}). Also, Valencia et al. (2018) present values of antioxidant activity in various varieties of quinoa in the range of 1.95–6.18 µmol TE.g^{-1}, which also corresponds to our values. Similar values of antioxidant activity in quinoa (4.4–4.8 µmol TE.g^{-1}) were reported by Tang et al. (2015), while its results correspond to ours (3.502 µmol TE.g^{-1}). Shen et al. (2018) determined the values of antioxidant activity in different varieties of sorghum, with an interval of 0.92–19.05 mg TE.g^{-1}, which, after recalculation (3.68–7.8 µmol TE.g^{-1}), which can be considered as comparable results with our values (8.196 µmol TE.g^{-1}).

Conclusion

We compared the soil contents of risk metals from the plots where the said crops were grown, in the aqua regia and ammonium nitrate leachate, with the limit values set by Law no. 220/2004. Exceeding the hygienic limits was recorded for three risk elements, namely Co, Cd, and Pb. Subsequently, we compared the safety of individual types of pseudocereals in terms of the content of hazardous metals, based on the maximum permissible amounts of elements given in the Food Code of the Slovak Republic (Decree No. 2/1994 of the Ministry of Health of the Slovak Republic). The content of none of the microelements exceeded the set hygienic limit in any of the monitored species of pseudocereals. The content of Cd and Pb in the grains of the monitored pseudocereal species was below the detection limit. In this respect, the monitored pseudocereals are safe for the consumer. The safest or most resistant plant species with the lowest ability to accumulate hazardous metals from soil to grains, from the group of crops which we monitored, is quinoa. BAF_{AR} values have also shown that quinoa is the safest or most durable with the lowest ability to accumulate hazardous metals. The total content of polyphenols (TPC) in our pseudocereal species ranged from 1 251.1 to 11 495.8 mg GAE.kg^{-1}. The results of our research showed that the total antioxidant activity (TAC) in the monitored pseudocereal species ranged from 1.184 to 8.196 µmol TE.g^{-1}. We found that amaranth showed the lowest values of antioxidant activity and, conversely, we recorded the highest antioxidant activity in sorghum.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Oxidative stress biomarkers in the blood of rainbow trout (*Oncorhynchus mykiss* Walbaum) and equine plasma after incubation with hemp oil "Annabis BIO"

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Industrial hemp is a multi-use crop that has been widely cultivated to produce fibers and nutrients, such as protein, dietary fiber, minerals, and unsaturated fatty acids, which make them a good fortifying component in food production. The antioxidant capability of hemp oils has been reported. In the current study, for evaluating the antioxidant activity of commercial hemp oil "Annabis BIO" derived from certified industrial hemp seeds without the psychoactive substance THC (Olomouc, Czech Republic), biomarkers of oxidative stress [2-thiobarbituric acid reactive substances (TBARS), oxidatively modified proteins (OMP), total antioxidant capacity (TAC)] were used in models of the blood collected from adult healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma. A volume of 0.1 mL of the hemp oil was added to 1.9 mL of fish blood or equine plasma. After incubation of the mixture for 60 min with continuous stirring, biomarkers of oxidative stress were studied in samples. After *in vitro* incubation of hemp oil with the blood of clinically healthy rainbow trout, we noted a statistically significant decrease in biomarkers of lipid peroxidation by 55.6% ($p < 0.05$). The highest increase in TBARS level was observed after *in vitro* incubation of hemp oil with the blood of UDN-affected rainbow trout. *In vitro* incubation of hemp oil with equine plasma resulted in a statistically significant increase in the level of ketonic derivatives (by 29%, $p < 0.05$) and aldehydic derivatives of OMP (by 33.1%, $p < 0.05$). Incubation of hemp oil with the blood of UDN-affected trout resulted in a decrease of the ketonic derivative of OMP (by 43.3%, $p < 0.05$). Incubation of hemp oil with equine plasma, we observed a statistically significant decrease in TAC level by 56.6% ($p < 0.05$). Similarly, after incubation hemp oil with blood samples of UDN-affected trout, a statistically significant decrease in total antioxidant capacity (by 59.3%, $p < 0.05$) was observed. The results suggest that the investigated hemp oil have shown varied antioxidant capacities. Accordingly, this study proposes that the therapeutic benefit of this hemp oil can be, at least in part, attributed to using different biological materials (blood, plasma) used *in vitro* in the current study.

Keywords: hemp oil, oxidative stress biomarkers, rainbow trout, equine plasma, *in vitro*

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Introduction

Oxidative stress (OS), defined as disturbances in the pro- and antioxidant balance, is harmful to cells due to the excessive generation of highly reactive oxygen (ROS) and nitrogen (RNS) species (Filomeni et al., 2015). When the balance is not disturbed, OS has a role in physiological adaptations and signal transduction (Apel and Hirt, 2004; Finkel, 2011). However, an excessive amount of ROS and RNS results in the oxidation of biological molecules such as lipids, proteins, and DNA (Juan et al., 2021). Oxidative stress has been reported in many diseases, due to both antioxidant depletions as well as increased ROS production (Forman and Zhang, 2021). For example, the kidney is a highly metabolic organ, rich in oxidation reactions in mitochondria, which makes it vulnerable to damage caused by OS, and several studies have shown that OS can accelerate kidney disease progression (Sies, 2015). On the other hand, oxidative stress is important in the pathophysiology of altering regulatory factors of mitochondrial activity, modifying the concentration of inflammation mediators associated with a large number and size of adipocytes, promoting lipogenesis, stimulating differentiation of preadipocytes to mature adipocytes, and regulating the energy balance in hypothalamic neurons that control appetite (Jones, 2008).

In the last decades, a lot of attention has been paid to the compounds present in medicinal *Cannabis sativa* L., such as Δ^9-tetrahydrocannabinol (Δ^9THC) and cannabidiol (CBD), and their effects on inflammation and other disorders (Pellati et al., 2018). *Cannabis sativa* L. is a dioicous plant of the Cannabaceae family and it is widely distributed all over the world (Pellati et al., 2018). It has been used as a psychoactive drug, as a folk medicine ingredient, and as a source of textile fibre since ancient times. *Cannabis* is thought to have originated from central Asia and has been domesticated for over 5,000 years (Irakli et al., 2019; Farinon et al., 2020). *Cannabis* varieties that are low in psychoactive cannabinoids are used to produce fiber and oilseed. However, the most valuable cannabis product today is the terpene- and cannabinoid-rich resin with its various psychoactive and medicinal properties. The resin is produced and accumulates in glandular trichomes that densely cover the surfaces of female (pistillate) inflorescences and, to a lesser degree, the foliage of male and female plants. In total, more than 150 different terpenes and approximately 100 different cannabinoids (House et al., 2010; VanDolah et al., 2019). *C. sativa* is characterized by a complex chemical composition, including terpenes, carbohydrates, fatty acids and their esters, amides, amines, phytosterols, phenolic compounds, and the specific compounds of this plant, namely, the cannabinoids. In the ambit of nonpsychoactive compounds, cannabichromene (CBD) represents the most valuable one from the pharmaceutical point of view, since it has been found to possess a high antioxidant and anti-inflammatory activity, together with antibiotical, neuroprotective, anxiolytic, and anticonvulsant properties. Cannabidiolic acid (CBDA) has antimicrobial and antinausea properties, while cannabigerol (CBG) has anti-inflammatory, antimicrobial, and analgesic activities. Thanks to its lack of psychoactivity, CBD is one of the most interesting compounds, with many reported pharmacological effects in various models of pathologies, from inflammatory and neurodegenerative diseases to epilepsy, autoimmune disorders like multiple sclerosis, arthritis, schizophrenia, and cancer (Sommano et al., 2020). Concerning other phenolics present in *C. sativa*, several flavonoids have been identified, belonging mainly to flavones and flavonols, together with cannflavins A and B, which are *C. sativa* typical methylated isoprenoid flavones. *Cannabis* flavonoids exert several biological effects, including properties possessed also by cannabinoids and terpenes. Anti-inflammatory, neuroprotective, and anti-cancer activities have been described for these compounds (Rupasinghe et al., 2020).

Hemp essential oil can inhibit or reduce bacterial growth, also exerting antioxidant activity, and therefore it can find an advantageous application in the food processing field (Pellegrini et al., 2021). Hemp essential oil can inhibit or reduce bacterial proliferation and it can be a valid support to reduce microorganism contamination, especially in the food processing field (Iseppi et al., 2019). Hemp inflorescences can be used as a source of natural antioxidants in vegetable oils and lipid products to retard their oxidation, especially those characterized by a high degree of unsaturation (Cantele et al., 2020). Hempseed and hempseed oil can safely be utilized as feed ingredients for laying hens to produce table eggs that are enriched in essential fatty acids. Additionally, the eggs procured from these hens had a similar aroma and flavor compared to eggs from hens that did not feed any hemp. The greater the dietary hemp inclusion, the more pigmented the resulting yolks became in terms of darkness, redness, and yellowness (Goldberg et al., 2012). Also, hemp seed products could be recommended as a feed ingredient for enhancing the essential fatty acid contents of fish which in turn can have a good impact on consumer health (Afridi et al., 2019). Protein content and amino

acids profile of hempseed and hempseed derivatives (cake, meal, and oil) make these products suitable for inclusion in ruminant diets. In addition, the fatty acid composition of hemp oil allows to transfer of the PUFA and in particular, n-3 fatty acid to the milk of dairy ruminants (Bailoni et al., 2021).

The oil extracted from hemp seeds has significant biological properties through the unique composition of polyunsaturated fatty acids, chemical elements, and various antioxidant compounds (Vitorović et al., 2021). The potential of this oil for the prevention of oxidative stress and for the treatment of oxidative-stress-induced ailments is of increasing interest (Vitorović et al., 2021).

In the current study, for evaluating the antioxidant activity of commercial hemp oil "Annabis BIO" derived from certified industrial hemp seeds without the psychoactive substance THC (Olomouc, Czech Republic), oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), oxidatively modified proteins (OMP), total antioxidant capacity (TAC)] were used in models of the blood collected from adult healthy rainbow trout (*Oncorhynchus mykiss* Walbaum), the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma.

Material and methodology

Hemp oil

Cold-pressed commercial hemp oil "Annabis BIO" 250 ml (Olomouc, Czech Republic) from certified industrial hemp seeds, without the psychoactive substance THC, was used for the current study. The greatest treasure of hemp oil is the unsaturated fatty acids Omega-3 and Omega-6, which are about 75%, including linoleic, alpha-linolenic, gamma-linolenic, and oleic acids. The ratio of these acids is 3 : 1 (Omega-6 to Omega-3). Hemp oil is a source of valuable vitamins (i.e. A, B_1, B_2, B_3, B_6, C, D, E) and minerals (calcium, magnesium, sulfur, potassium, iron, zinc, and phosphorus). In addition, "Annabis BIO" hemp oil is rich in vitamin K, essential for the synthesis of important proteins and enzymes, which has anti-hemorrhagic, antifungal, antibacterial, anti-inflammatory, and analgesic properties. Vitamin K also plays an important role in calcium metabolism. "Annabis BIO" hemp oil also contains health-promoting phytosterols and phospholipids, contained in cell membranes in all living organisms. It contains 20 essential amino acids, including 9 essential amino acids that cannot be synthesized by the body itself, so must be provided through the diet. The green color of the oil is caused by the presence of a large amount of chlorophyll, which acts as a strong antioxidant, possessing antiseptic, astringent, and regenerating properties. It also improves the blood supply to the skin, therefore it contributes to its oxygenation and nourishment. Hemp oil is credited with improving the functioning of the cardiovascular system, i.e. supporting the heart and blood vessels, regulating blood pressure and cholesterol levels. In addition, it supports the immune system and thus contributes to the building of the body's immunity (https://dobrekonopie.pl/product/olej-konopny-bio-250ml/).

Fish and collection of blood samples

Clinically healthy rainbow trout (*Oncorhynchus mykiss* Walbaum) with a mean body mass of 300–350 g were used in the experiments. The study was carried out in the Department of Salmonid Research, Stanisław Sakowicz Inland Fisheries Institute (Rutki, Poland). The experiments were performed in water at 14.5 ±0.5 °C and pH 7.2–7.4. The fish were fed a commercial pelleted diet. Adult fish, 3–5 years of age, were collected from the site on the Słupia River, Słupsk, the central part of northern Poland. The blood sampling for analysis from males and females of trout affected by ulcerative dermal necrosis (UDN) syndrome was collected directly after the catch. After catching, microbiological tests were also performed. These tests revealed that *Aeromonas* spp. complex caused the UDN syndrome. Blood was drawn from the efferent branchial arteries of the rainbow trout. Blood was stored in tubes with sodium citrate as the anticoagulant and held on the ice. A volume of 0.1 mL of the hemp oil was added to 1.9 mL of blood samples. For positive control, 4 mM phosphate buffer (pH 7.4) was used. After incubation of the mixture at 25 °C for 60 min with continuous stirring, biomarkers of oxidative stress were studied in samples.

Horses and collection of blood samples

Eighteen healthy adult horses from the central Pomeranian region in Poland (Strzelinko, N 54º 30' 48.0" E 16º 57' 44.9"), aged 8.9 ±1.3 years old, including 6 Hucul pony, 5 Thoroughbred horses, 2 Anglo-Arabian horses, and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical, and vital parameters that were in the reference ranges. The females were non-

pregnant. Blood was drawn from the jugular vein of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10:00 AM). Blood samples were stored in tubes with 3.8% sodium citrate as the anticoagulant and held on the ice until centrifugation at 3000 rpm for 5 min to remove plasma. The pellet of blood was re-suspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 mL of the hemp oil was added to 1.9 mL of plasma. For positive control, 4 mM phosphate buffer (pH 7.4) was used. After incubation the mixture at 37 °C for 60 min with continuous stirring, biomarkers of oxidative stress in samples were studied. Plasma aliquots were used in the study.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with TBA under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56.10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives content of protein oxidative modification (OMP) assay

To evaluate the protective effects of hemp oil against free radical-induced protein damage in equine plasma and fish blood, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the erythrocyte suspension and plasma was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in the samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). Sample inhibits the Fe^{2+}/ascorbate-induced oxidation of Tween 80, resulting in a decrease in the TBARS level. The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Mann-Whitney *U* test (Zar, 1999). All statistical calculation was performed on separate data from each individual with Statistica 13.3 software (StatSoft, Krakow, Poland).

Results and discussion

The batch spectrophotometric and spectrofluorometric 2-thiobarbituric acid (TBA)-based methods are the most commonly used assays to measure lipid peroxidation (Tsikas, 2017). Level of lipid peroxidation determined by the concentration of TBARS in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of UDN, and equine plasma after incubation with commercial hemp oil "Annabis BIO" (Olomouc, Czech Republic) was presented in Figure 1.

Analyzing data presented in Figure 1, we obtained statistically significant changes in TBARS content in the blood and plasma samples after *in vitro* incubation with hemp oil. After *in vitro* incubation of hemp oil with the blood of clinically healthy rainbow trout, we noted a statistically significant decrease in biomarkers of lipid peroxidation by 55.6% ($p < 0.05$), i.e. TBARS content was (6.15 ±0.46 nmol. mL^{-1}) compared to the control samples (13.85 ±0.85 $nmol.mL^{-1}$). The contrary tendency was observed after incubation of hemp oil with blood samples of rainbow trout with clinical symptoms of UDN, where we recorded a statistically significant increase in TBARS content by 594.2% ($p < 0.05$) compared to the values in control untreated samples (79.49 ±5.1 nmol. mL^{-1} *vs.* 11.45 ±1.13 $nmol.mL^{-1}$). Also, when hemp oil was exposed to the equine plasma, we observed a statistically significant increase in the concentration of lipid peroxidation end products by 64.5% ($p < 0.05$),

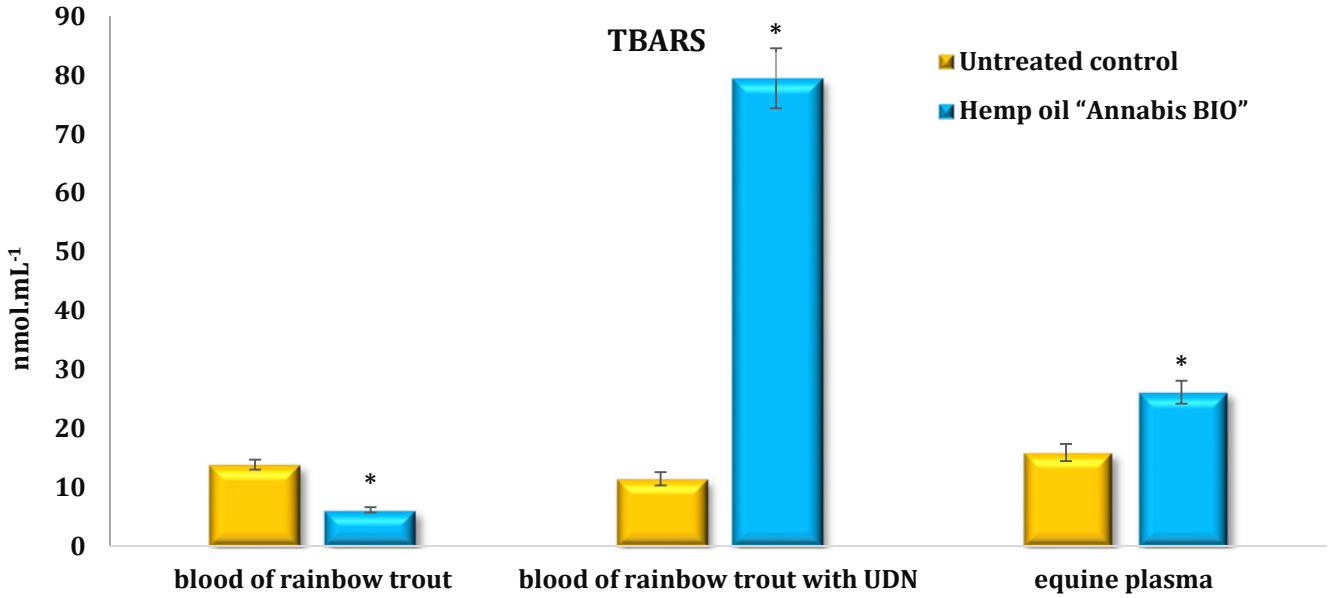

Figure 1 Level of lipid peroxidation determined by the concentration of 2-thiobarbituric acid reactive substances in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

i.e. (26.15 ±1.94 nmol.mL^{-1}) compared to untreated controls (15.9 ±1.45 nmol.mL^{-1}) (Figure 1).

Level of aldehydic derivatives of OMP in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of UDN, and equine plasma after incubation with commercial hemp oil "Annabis BIO" was presented in Figure 2. After *in vitro* incubation of hemp oil with equine plasma, we observed a statistically significant increase in the level of aldehydic derivatives of oxidatively modified proteins by 33.1% ($p < 0.05$), i.e. (19.45 ±0.89 nmol.mL^{-1})

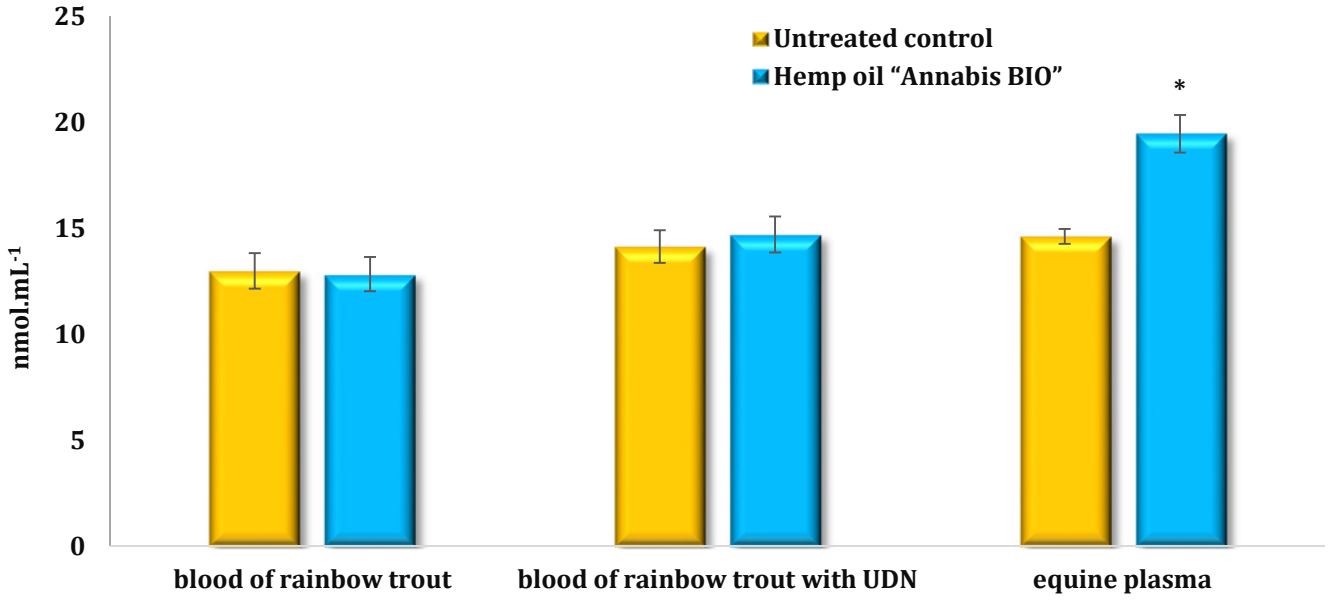

Figure 2 Level of aldehydic derivatives of oxidatively modified proteins in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

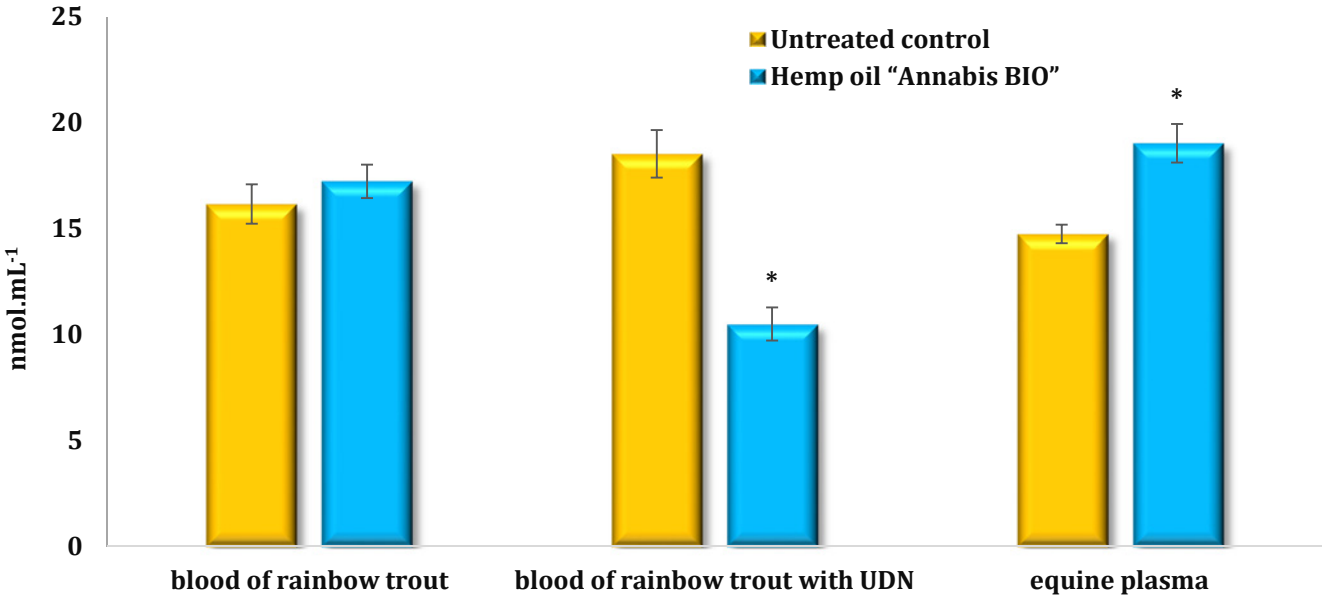

Figure 3 Level of ketonic derivatives of oxidatively modified proteins in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

compared to untreated controls (14.61 ±0.35 nmol.mL^{-1}). On the other hand, after incubation of hemp oil with the blood of UDN-affected rainbow trout, we also observed an increase in the level of aldehydic derivatives of OMP (14.7 ±0.85 nmol.mL^{-1}), but this increase was statistically not significantly (by 4%, $p > 0.05$) compared to the untreated samples (14.13 ±0.77 nmol.mL^{-1}). The contrary tendency was observed after incubation of hemp oil with the blood of clinically healthy rainbow trout, where the level of aldehydic derivatives of OMP was statistically non-significant decreased (by 1.2%, $p > 0.05$) compared to untreated controls (12.83 ±0.81 nmol.mL^{-1} *vs.* 12.98 ±0.84 nmol.mL^{-1}) (Figure 2.).

Level of ketonic derivatives of OMP in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis, and equine plasma after incubation with commercial hemp oil "Annabis BIO" was presented in Figure 3. Different trends were observed when we examined the concentration of ketonic derivatives of oxidatively modified proteins in the blood of rainbow trout and equine plasma samples. *In vitro* incubation of hemp oil with equine plasma resulted in a statistically significant increase (by 29%, $p < 0.05$) in level of ketonic derivatives of OMP, i.e. (19.03 ±0.91 nmol.mL^{-1}) compared to untreated controls (14.75 ±0.44 nmol.mL^{-1}). On the contrary, after incubation commercial hemp oil "Annabis BIO" with blood sampled from UDN-affected rainbow trout, we recorded a statistically significant decrease in the level of ketonic derivatives of oxidatively modified proteins (by 43.3%, $p < 0.05$) compared to untreated controls, i.e. (10.5 ±0.48 nmol.mL^{-1}) *vs.* (18.53 ±1.12 nmol.mL^{-1}). When commercial hemp oil "Annabis BIO" was incubated with the blood of clinically healthy rainbow trout, a non-statistically significant increase (by 6.6%, $p > 0.05$) in the concentration of ketonic derivatives of OMP was observed, i.e. (17.23 ±0.79 nmol.mL^{-1}) compared to untreated controls (16.16 ±0.93 nmol.mL^{-1}) (Figure 3).

Level of total antioxidant capacity in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis, and equine plasma after incubation with commercial hemp oil "Annabis BIO" was presented in Figure 4. When evaluating the total antioxidant capacity after *in vitro* incubation of hemp oil with equine plasma, we observed a statistically significant decrease in TAC level by 56.6% ($p < 0.05$), i.e. (33.34 ±5.19%) compared to the untreated controls (76.77 ±6.54%). Similarly, after incubation hemp oil with blood samples of UDN-affected rainbow trout, a statistically significant decrease in total antioxidant capacity (by 59.3%, $p < 0.05$) was observed (30.66 ±8.54% *vs.* 75.39 ±10.13%). In contrast, after *in vitro* incubation of hemp oil with blood samples of clinically healthy rainbow trout, we recorded a non-statistically

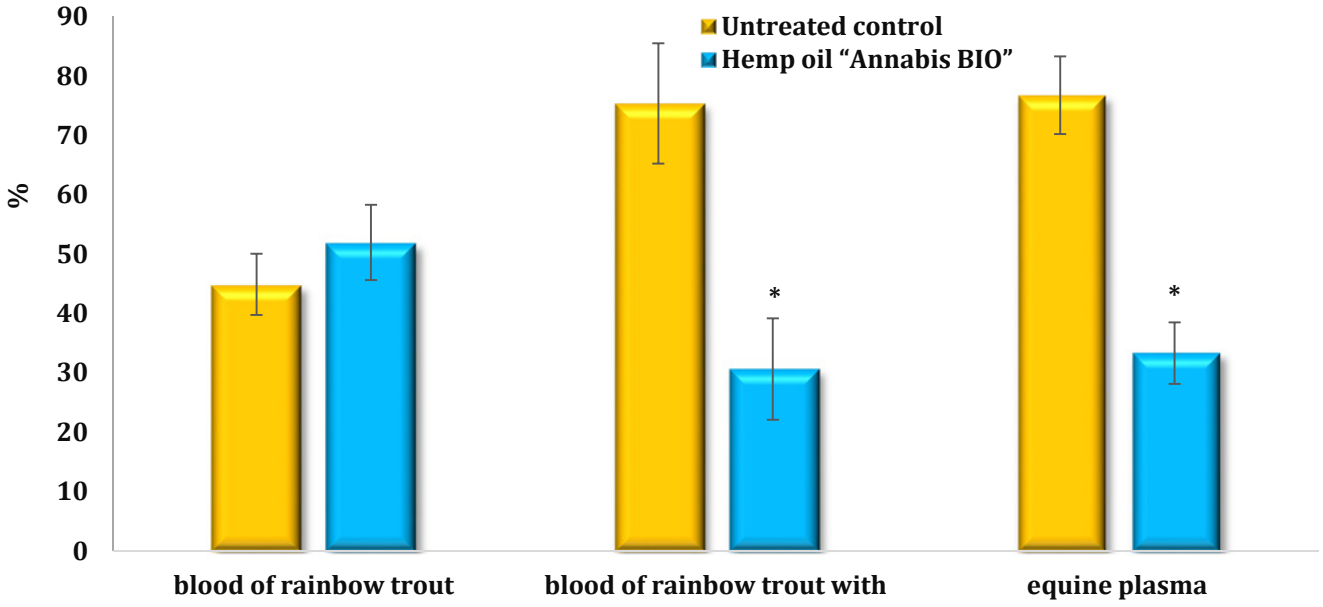

Figure 4 Level of total antioxidant capacity in the blood of clinically healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis (UDN), and equine plasma after incubation with commercial hemp oil "Annabis BIO"
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
* changes were statistically significant ($p < 0.05$) compared to the untreated controls

significant increase in TAC levels (by 15.7%, $p > 0.05$), i.e. (51.98 ±6.34%) compared to the untreated controls (44.92 ±5.15%) (Figure 4).

Thus, the incubation of hemp oil "Annabis BIO" resulted in different changes in the concentration of TBARS as biomarkers of lipid peroxidation, aldehydic and ketonic derivatives of oxidatively modified proteins, and total antioxidant capacity in the blood of healthy rainbow trout, the blood of rainbow trout with clinical symptoms of ulcerative dermal necrosis, and equine plasma. Incubation of hemp oil with the blood of healthy rainbow trout caused a decrease in TBARS level without statistically significant changes in the level of aldehydic and ketonic derivatives of OMP and TAC. When the blood of UDN-affected rainbow trout was incubated with hemp oil, the content of ketonic derivatives of OMP was significantly decreased, while TBARS as biomarkers of lipid peroxidation was increased with simultaneously decreased TAC level. When equine plasma was incubated with hemp oil, a statistically significant increase of biomarkers of oxidative stress with simultaneously decreased TAC level (Figure 1–4).

Recent investigations have associated plants belonging to the *Cannabis* genus with antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective properties that may impact human health (Ford et al., 2017). For example, *Cannabis* whole extracts acted on both phases of lipid oxidation in copper-challenged LDL. Those effects were just partially related to the content of cannabinoids and partially recapitulated by isolated pure cannabinoids (Musetti et al., 2020). In the current study, the antioxidant activity of hemp oil *in vitro* was detected using the blood of healthy rainbow trout, and this finding is in line with literature data suggesting that this species possesses antioxidant properties (Hacke et al., 2019).

Hemp seed oil is effective for reducing oxidative stress at the cellular level. The results obtained by Vitorović et al. (2021) point to the potential of hemp seed oil for the prevention and treatment of conditions caused by the action of reactive oxygen species. These authors have evaluated the hypothesis that hemp seed oil at different concentrations improves the oxidative state of *Drosophila melanogaster* under non-stress as well as hydrogen-peroxide-induced stress. These authors have analyzed the effects of hemp seed oil on oxidative stress markers and on the life cycle of *D. melanogaster* under non-stress and hydrogen-peroxide-induced stress conditions. The results revealed that under non-stress conditions, oil concentrations up to 62.5 $\mu L.mL^{-1}$ did not induce negative effects on the life cycle of *D. melanogaster* and maintained the redox status of the larval cells at similar levels to the control level. Under oxidative stress conditions, biochemical parameters were significantly affected and only two oil concentrations, 18.7 and 31.2 $\mu L.mL^{-1}$

provided protection against hydrogen peroxide stress effects. A higher oil concentration (125 µL.mL^{-1}) exerted negative effects on the oxidative status and increased larval mortality. The tested oil was shown to contain polyunsaturated fatty acid triglycerides and low levels of tocopherols. The high levels of linoleic and linolenic acids in the oil are suggested to be responsible for the observed *in vivo* antioxidant effects (Vitorović et al., 2021).

The significant antioxidant properties shown by hemp seed oil might generally depend on the phenolic compounds, especially flavonoids, such as flavanones, flavonols, and isoflavones. Smeriglio et al. (2016) have characterized the polyphenolic compounds and antioxidant activity of cold-pressed seed oil from Finola cultivar of industrial hemp. Several methodologies have been employed to evaluate the *in vitro* antioxidant activity of Finola hempseed oil (FHSO) and both lipophilic (LF) and hydrophilic fractions (HF). From the results is evident that FHSO has high antioxidative activity, as measured by DPPH radical (146.76 mmol of TE.100 g^{-1} oil), inhibited β-carotene bleaching, quenched chemically generated peroxyl radicals *in vitro*, and showed high ferrous ion chelating activity. Reactivity towards 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation and ferric-reducing antioxidant power values were 695.2 µmol of TE.100 g^{-1} oil and 3690.6 µmol of TE.100 g^{-1} oil respectively. FHSO contains a significant amount of phenolic compounds of which 2780.4 mg of QE.100 g^{-1} of total flavonoids. The whole oil showed higher antioxidant activity compared with LF and HF (Smeriglio et al., 2016).

Afridi et al. (2019) examined if the inclusion of dietary hempseed (HS) and hempseed oil (HO) in the diet of the fish could revert the copper-induced toxic effects on the muscle fatty acid profile of rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). Fingerlings of both species were exposed to a sub-lethal concentration of copper i.e., 20% of LC_{50} (1.34 ppm for rohu and 1.52 ppm for mrigal) for 96 h for 30 days. Following exposure, fish were maintained on graded levels of HO (1, 2, and 3%) or on HS (5, 10, and 15%) for 50 days. Copper exposure showed a significant effect on the fatty acid composition of both species; increased their saturated (SFA) to unsaturated (USFA) and altered their omega-3/omega-6 (ω-3/ω-6) ratios. However, feeding graded levels of hempseed products reverted the toxic effects of copper on the fatty acid profile of both the species, significantly increased muscle total fatty acid contents, improved ω-3/ω-6 ratios, and decreased SFA/USFA ratio in % inclusion dependent manner. Furthermore, hemp seed products showed a species-specific effect on USFA. The ω-3/ω-6 ratios decreased in the muscle of *C. mrigala* whereas an increasing trend with an increase in hempseed product % inclusion was observed in *L. rohita* (Afridi et al., 2019).

Conclusions

Our studies have shown that commercial hemp oil has an antioxidant effect only after incubation *in vitro* with blood samples of clinically healthy rainbow trout, as the values of the oxidative stress biomarkers (TBARS and OMP) have decreased with simultaneously increasing the total antioxidant capacity. When the blood of UDN-affected rainbow trout was incubated with hemp oil, the content of ketonic derivatives of OMP was significantly decreased, while TBARS as biomarkers of lipid peroxidation was increased with simultaneously decreased TAC level. When equine plasma was incubated with hemp oil, a statistically significant increase of biomarkers of oxidative stress with simultaneously decreased TAC level. These results may prompt veterinarians and biologists to carry out further studies to elucidate the dose-dependent antioxidative effects of hemp oil.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Biochemical composition of *Vigna* spp. genotypes

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Plants from Fabaceae Lindl. are widely distributed in the world as economically important crops due to their high content of useful nutrients. Species of the *Vigna* L. genus are used in many countries of the world because of the high content of protein in the seeds. However, no less important is to study the biochemical composition of above-ground parts of plants that can be used as fodder, medicinal or energetic. It was a study of six genotypes of *Vigna* spp. (f. 1 – *Vigna aconitifolius* Jacq., f. 2 – *V. umbellata* (Thunb.) Ohwi & H. Ohashi, f. 3 – *V. unguiculata* (L.) Walp., f. 4 – *V. unguiculata* (L.) Walp., f. 5 – *V. unguiculata* (L.) Walp., f. 6 – *V. mungo* (L.) Hepper.) to determine selected biochemical parameters. The content of dry matter for six genotypes was from 17.92 to 34.25%, total sugar content from 7.03 to 15.65%, the total content of ascorbic acid was from 62.96 (f. 2) to 115.66 (f. 1) mg%, β-carotene content for six *Vigna* genotypes was from 0.23 (f. 2) to 1.74 (f. 5) mg%, the content of tannins was 1.51–3.10%, lipids 1.78–4.22%, titrable acidity was 2.50–7.85%, content of ash in our study was from 6.58 (f. 2) to 10.75 (f. 3) %, calcium from 1.27 (f. 2) to 3.75 (f. 3) %, and phosphorus from 0.71 (f. 3) to 1.18 (f. 5) % depending on genotypes. The correlation analysis showed a very strong relations between total ash content and total calcium content ($r = 0.971$, $p \leq 0.01$), between titrable acidity and total tannin content ($r = 0.913$, $p \leq 0.01$), between carotene content and total tannin content ($r = 0.863$, $p \leq 0.01$), carotene content and titrable acidity ($r = 0.845$, $p \leq 0.01$). Thus, this study demonstrated that different genotypes of *Vigna* spp. are a good source of nutrients such as vitamins, dry matter, selected mineral components, etc. The research of the chemical composition of plants of six *Vigna* genotypes allowed to detect maximal content of dry matter for f. 2, titrable acidity, the content of tannins, β-carotene, and phosphorus for f. 5. The highest content of lipids, ash, and calcium was determined in raw f. 3. The ascorbic acid content was maximal in raw f. 1 plants.

Keywords: *Vigna*, genotype, dry matter, sugars, vitamins, macroelements

Introduction

Fabaceae Lindl. is one of the largest families known from ancient times as a food and medicinal plant group. Different representatives of Fabaceae exhibited numerous biological activities such as antioxidant, antimicrobial, antifungal, and anticancer, used for the treatment of liver disorders, hypertension, arthritis, hemorrhoids, etc. (Obistioiu et al., 2021).

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Representatives of this plant group are widespread in the different geographic areas due to various adaptive strategies and chemical evolution (Benjamim et al., 2020).

Plants of *Vigna* L. belong to the Fabaceae family, which is cultivated widely in the world and consists of more than 200 species. The main countries of *Vigna* production are Asia, Australia, subtropical Africa, etc. (Narayana and Agamuthu, 2021).

The name of this genus got from the name of the Italian botanist of the 17th century Dominico *Vigna*. More than 15 species of *Vigna* are commonly used in the world and have an important economic value due to protein content. The plant raw of *Vigna* spp. exhibited the antioxidant activity and used to treat numerous diseases: diabetes, cancer, rheumatism, etc. (Pandey, 2019).

The biochemical composition of plant raw *Vigna* spp. is glycosides, tannins, alkaloids, terpenoids, saponins, sterols, amino acids, etc. (Pandey, 2019). According to Aziagba et al. (2017), different organs of seven genotypes of *V. unguiculata* (L.) Walp. showed that minimal content of flavonoids was determined in seed extracts whereas the maximal content found depended on genotypes in the leaves, stems, or roots.

V. mungo (L.) Hepper is extensively cultivated in India and its raw is rich in flavonoids, isoflavonoids, phenolic acids, enzymes, lectins, saponins, and tocopherols. The raw of this species possess anti-inflammatory, antioxidant, and antimicrobial activity. Roots possess narcotic action and leaves demonstrated analgesic, ulcerogenic, and anti-inflammatory activities (Khan et al., 2021). As reported Solanki and Jain (2010), *V. mungo* is used in Indian traditional medicine and possesses immunomodulatory activity.

V. radiata (L.) R. Wilczek in some studies exhibited antidiabetic activity (Ayenewu, 2017). Leaf extract of *V. unguiculata* demonstrated antifungal activity against some phytopathogens that characterized them as the source of fungicide activity but it still has some diseases that fail to have maximal yield (Masangwa et al., 2013). According to Nderity et al. (2017), the antiobesity activity of methanol extracts *V. unguiculata* was determined.

V. unguiculata varieties can be recommended as fodder plants due to their chemical composition and yield of dry matter (Mohatla et al., 2016). Seeds of these plants contain 25.07–28.60% of protein, 5.13–6.22% of total lipids, 54.81–58.13% of carbohydrates, 4.25–6.84% of crude fiber, 4.97–6.72% of ash (Zia-Ul-Haq et al., 2014). However, seeds of *Vigna* spp. can be infected by *Colletotrichum destructum* O'Gara in storage that is reduced the values and content of nutrients (Amadioha and Nwazuo, 2019).

The biochemical composition of seeds of some *Vigna* species is amino acids where prevails glutamic and aspartic acids, fatty acids were to prevail in linoleic, palmitic, and oleic acids, and macroelements with high content of potassium (Zaheer et al., 2021).

The study of the genetic variation of *V. unguiculata* allowed to classify it into five cultivar-groups such biflora, melanophthalmus, sesquipedalis, textilis, and unguiculata (Pasquet, 1999).

The numerous existing reviews concerning *Vigna* spp. relate to the biochemical and nutritive composition of seeds of these species because of wide use for human food (Katoch, 2013) and fewer data about the above-ground part of these crops. This study was aimed to determine the biochemical composition of above-ground parts of raw *Vigna* genotypes in the M.M. Gryshko National Botanical Garden to evaluate the nutritional potential of these plants.

Biological material

Plant raw material was collected from a collection of Cultural Flora Department of M.M. Gryshko National Botanical Garden (NBG) of the National Academy of Sciences of Ukraine at the stage of flowering. For the biochemical analyses was used fresh raw of six genotypes of *Vigna* L. spp.: f. 1 – *Vigna aconitifolius* Jacq., f. 2 – *V. umbellata* (Thunb.) Ohwi & H. Ohashi, f. 3 – *V. unguiculata* (L.) Walp., f. 4 – *V. unguiculata* (L.) Walp., f. 5 – *V. unguiculata* (L.) Walp., f. 6 – *V. mungo* (L.) Hepper. The biochemical analysis was done at the laboratory of the Cultural Flora Department of NBG.

Biochemical analysis

Dry matter determination

Plant samples were dried in a drying oven at 105 °C till constant weight in aluminum boxes. Results are given in percentages (Hrytsajenko et al., 2003).

The total content of sugars determination

The total content of sugars was investigated by Bertrand's method in water extracts. 4 g of fresh mass was mixed and homogenized with distilled water (approximately 50 mL) in the 100 mL test-tubes and heated in the water bath at 70 °C during 15–20 min. After cooling in the obtained mixtures added 1 mL of the phosphate-oxalate mixture. After this was added

1.5 mL of lead acetate. The obtained mixture brings to the mark (100 mL) with water. After filtration from obtained solution took 50 mL and mixed with 8 mL of 20% HCl (at 70 °C in a water bath for 5 min), after cooling was neutralized by 12% NaOH and brought to the mark by distilled water (100 mL). 3 mL of obtained solution mixed with 6 mL of Fehling's solution reagent (6 min boiling in the water bath). Obtained mixture was analyzed for the total content of sugars. Results are given by percentages (Hrytsajenko et al., 2003).

The total content of ascorbic acid

Determination of ascorbic acid content conducted by method offered by K. Murri. 2 g of fresh mass mixed with 50 mL of 2% oxalic acid. Obtained mixture put into the dark for 20 min. The content of ascorbic acid in obtained extracts was determined by a 2,6-dichlorophenol-indophenol method based on the reduction properties of ascorbic acid. Obtained results are expressed in the mg% DW (Hrytsajenko et al., 2003).

The total content of carotene

The concentration of total carotene is determined according to Pleshkov (1985) using extraction with rubber solvent (petrol). 1 g of absolutely dried raw mixed with 20 mL of Kalosha petrol for 2 hours. After this obtained filtrate was measured spectrophotometrically at the wavelength 440 nm at the Unico Spectrophotometer. Obtained results expressed in mg% DW.

The total content of tannins

The content of tannins was determined with indigo carmine as an indicator (Yermakov et al., 1972). 5 g of fresh mass mixed with distilled water (approximately 50 mL) in 100 mL taste-tubes. Obtained mixture heated in the water bath at 70 °C for 2 hours. After cooling, adding water to the 100 mL, and following filtration 10 mL of filtrate was used to determine the total content of tannins. This procedure used 700 mL distilled water and 25 mL of 1% solvent of indigo carmine. Obtained results are expressed in %.

The total content of organic acids

The total content of organic acids is determined with phenolphthalein and results are calculated with a malic acid coefficient (Krishchenko, 1983). 10 mL of filtrate (the same procedure described for the determination of total content of tannins) titrated with 1 N solvent of NaOH in presence of phenolphthalein. Obtained results are expressed in percentages.

The total content of lipids

The total content of lipids is determined in the Soxhlet apparatus (Yermakov et al., 1972). The low-boiling petroleum ether (40 °C) was used as an extractor. The difference in masses before and after the extraction process is used to calculate the total lipid content.

Statistical analysis

Data were analyzed with ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$). The variability of all these parameters was evaluated using descriptive statistics.

Results and discussion

The value of Fabaceae plants as a rich source of nutrients depends on many factors such as agro-climatic conditions and genetic characteristics that also relate to *Vigna* spp. (Gonçalves et al., 2016). The biological (Tang et al., 2014) and pharmacological activities of these plants are manifested through biochemical composition (Pandey, 2019; Udeh et al., 2020).

The content of dry matter for six genotypes of *Vigna* was from 17.92 to 34.25% (Figure 1). The growth of plant organisms depends on many factors one of which is the regulatory functions of sugars. Accumulation of sugars in the plant tissues may be caused by environmental factors such as cold or drought stress, phosphorus deficiency, pathogens, and peculiarities of development such as increased sugar demand in apical meristems, buds, and seeds, etc. (Ciereszko, 2018). During gene expression, sugars translate the nutrient status in growth and developing modulation (Stephen et al., 2021). The total sugar content of investigated genotypes was from 7.03 to 15.65%. As shown in Figure 1, the maximum content of dry matter found for plants of f. 2 and minimum for plants of f. 5. The total content of sugars was highest in raw of f. 6 plants and lowest in raw of f. 4.

The content of carbohydrates of *Trifolium* spp., according to Gounden et al. (2018), was from 26.7 to 47.0% which is higher than in our samples. Another fodder crop, *Bunias orientalis* L., and its genotypes accumulated sugars from 5.07 to 8.86% which was less than in represented study (Vergun et al., 2021a). The above-ground part of legume plants of *Cicer arietinum* L. genotypes accumulated total content of sugars from 7.39 to 12.81% (Vergun et al., 2021b).

One of the most important metabolites in plant organisms is ascorbic acid which plays a significant

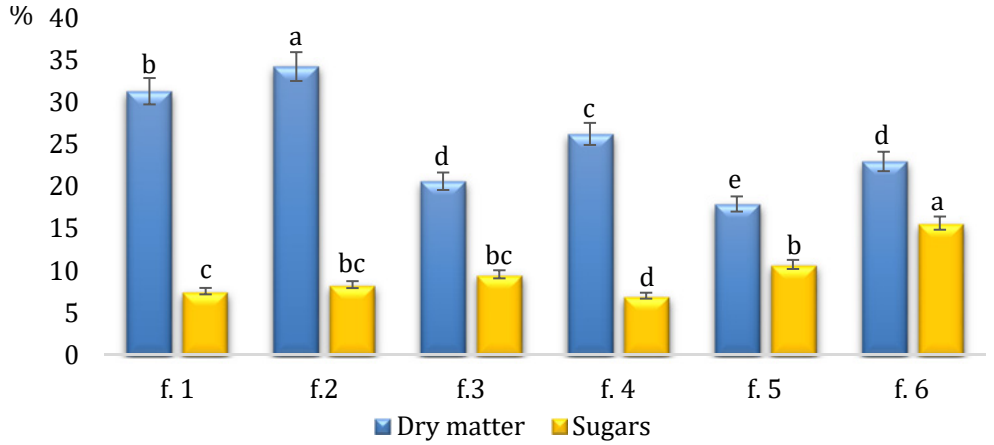

Figure 1 Content of dry matter and sugars in raw of *Vigna* spp. genotypes at the flowering stage (%)
f. 1 – *Vigna aconitifolius* Jacq.; f. 2 – *V. umbellata* (Thunb.) Ohwi & H. Ohashi; f. 3 – *V. unguiculata* (L.) Walp.; f. 4 – *V. unguiculata* (L.) Walp.; f. 5 – *V. unguiculata* (L.) Walp.; f. 6 – *V. mungo* (L.) Hepper; different superscripts in each column indicate the significant differences in the mean at p <0.05

role as an antioxidant, a cofactor for some hydroxylase enzymes, etc. (Smirnoff, 1996). The total content of ascorbic acid was from 62.96 (f. 2) to 115.66 (f. 1) mg% (Figure 2). The β-carotene content for six *Vigna* genotypes was from 0.23 (f. 2) to 1.74 (f. 5) mg%. As reported Ahenkora et al. (1998), the content of ascorbic acid in the leaves of *V. unguiculata* was 33.5–148.0 mg%. According to Wawire et al. (2011), plants of *Vigna* are a rich source of ascorbic acid, however, leaves of 1–2-months plants are prone to ascorbic acid losses. As reported Lu et al. (2019), the content of ascorbic acids in the seeds of *V. radiata* after flowering was 16.92–19.91 mg%. Comparing ascorbic acid content with other representatives of Fabaceae showed that plants from *Astragalus* L. and *Galega* L. genera accumulated 102.44–398.45 and 63.64–592.12 mg%, respectively (Vergun et al., 2012; Rakhmetov et al., 2018).

Tannins are important natural bioactive compounds, the source of which is mainly barks, leaves, seeds, and stems (Das et al., 2020). The positive and negative effect of tannins on an animal organism depends on its quantity and biological activity of it (Schofield et al., 2001). Tannins distributed in Fabaceae are mostly catechin type (Wink, 2013). In Figure 3 represented the content of tannins, lipids, and titrable acidity concentration and it was 1.51–3.10%, 1.78–4.22%, and 2.50–7.85%, respectively. Minimal titrable acidity was noticed in extracts of plants of f. 2, tannin, and lipids content in plants of f. 4. Maximal titrable acidity and tannin content was determined in plants of f. 5, and lipids in plants of f. 3.

According to Tresina et al. (2014), the content of tannins of *V. radiata* varieties was 0.50–0.59% which is 3–5 times less than in our research. The content of lipids in that study was from 3.78 to 4.34%. As reported

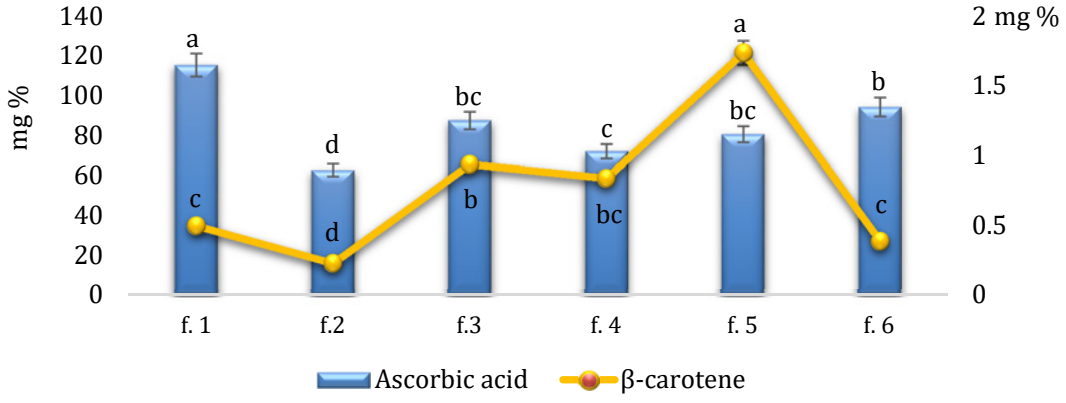

Figure 2 Content of ascorbic acid and β-carotene in raw of *Vigna* spp. genotypes at the stage of flowering (mg%)
f. 1 – *Vigna aconitifolius* Jacq.; f. 2 – *V. umbellata* (Thunb.) Ohwi & H. Ohashi; f. 3 – *V. unguiculata* (L.) Walp.; f. 4 – *V. unguiculata* (L.) Walp.; f. 5 – *V. unguiculata* (L.) Walp.; f. 6 – *V. mungo* (L.) Hepper; different superscripts in each column indicate the significant differences in the mean at p <0.05

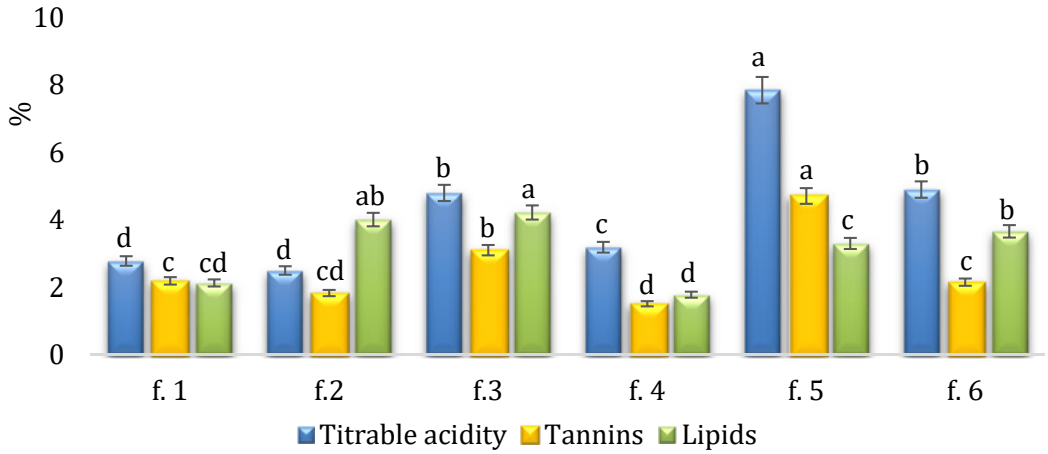

Figure 3 Content of tannins, lipids, and titrable acidity in raw *Vigna* spp. genotypes at the stage of flowering (%)
f. 1 – *Vigna aconitifolius* Jacq.; f. 2 – *V. umbellata* (Thunb.) Ohwi & H. Ohashi; f. 3 – *V. unguiculata* (L.) Walp.; f. 4 – *V. unguiculata* (L.) Walp.; f. 5 – *V. unguiculata* (L.) Walp.; f. 6 – *V. mungo* (L.) Hepper; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

Harris et al. (2018), the main component of tannins of V. subterranea was chlorogenic acid. The content of tannins and titrable acidity in raw *Cicer arietinum* was 1.04–1.55% and 2.32–3.19%, respectively (Vergun et al., 2021b).

The mineral element accumulation in plants depends on ecological, edaphic factors and the ability of the plant to accumulate one or the other element. According to Juknevičius and Sabienė (2007), the accumulation of calcium in the legume plant was higher than in grasses. Also, calcium is an essential plant nutrient that is necessary for a plant's natural habitat with a concentration of 0.1–5%, however content of it depends on different factors (White and Broadley, 2003). Phosphorus is an equally essential element in plant life as calcium and its accumulation in plant tissues depends on the level of P in the soil (Ham et al., 2018). Phosphorus is poorly available and plays an important role in plant growth. In solubilization of phosphor participates different mechanisms including microorganisms (Gupta and Sahu, 2017).

The content of ash in our study was from 6.58 (f. 2) to 10.75 (f. 3) %, calcium from 1.27 (f. 2) to 3.75 (f. 3) %, and phosphorus from 0.71 (f. 3) to 1.18 (f. 5) % depending on genotypes. According to Tresina et al. (2014), the content of ash in *V. radiata* raw was 4.05–4.59% which is less than in our study. Udeh et al. (2020) reviewed 0.7% of ash in raw of *V. subterranean*.

Commercial *V. unguiculata* had ash content in the wet season (Nigeria) higher than in the dry season 1.32 times (Anele et al., 2011). As reported Antova et al. (2014), ash content of *V. unguiculata* was 3.2–3.7%. According to Enyiukwu et al. (2018), leaves of *V. unguiculata* contain 11.5% of ash, 5.42% of lipids, 1.61% of calcium, and 0.55% of phosphorus.

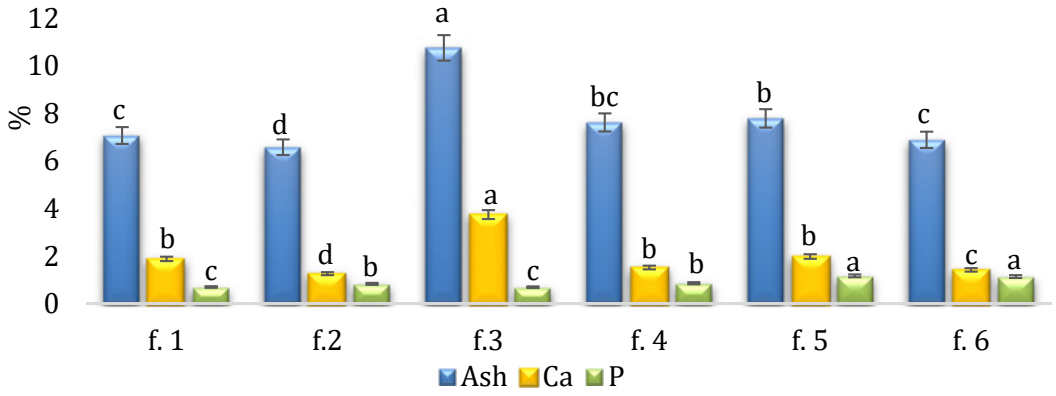

Figure 4 Content of ash, calcium, and phosphorus in raw *Vigna* spp. genotypes at the stage of flowering (%)
f. 1 – *Vigna aconitifolius* Jacq.; f. 2 – *V. umbellata* (Thunb.) Ohwi & H. Ohashi; f. 3 – *V. unguiculata* (L.) Walp.; f. 4 – *V. unguiculata* (L.) Walp.; f. 5 – *V. unguiculata* (L.) Walp.; f. 6 – *V. mungo* (L.) Hepper; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

Table 1 Correlation between studied parameters in raw of *Vigna* spp. genotypes

Parameters	DM	TSC	AA	CC	TA	TTC	TLC	TAC	TCC
TSC	-0.486*	1							
AA	-0.052	0.172	1						
CC	-0.785**	-0.018	-0.089	1					
TA	-0.893**	0.500*	-0.030	0.845**	1				
TTC	-0.738**	0.232	0.061	0.863**	0.913**	1			
TLC	-0.222	0.483*	-0.277	-0.055	0.286	0.317	1		
TAC	-0.564	-0.088	0.045	0.406*	0.289	0.370	0.343	1	
TCC	-0.500	-0.079	0.217	0.361*	0.275	0.417*	0.373*	0.971**	1
TPC	-0.518	0.711**	-0.179	0.391*	0.700**	0.435*	0.129	-0.376	-0.433

Note: DM – dry matter content; TSC – total sugar content; AA – total ascorbic acid content; CC – total β-carotene content; TA – titrable acidity; TTC – total tannin content; TLC – total lipid content; TAC – total ash content; TCC – total calcium content; TPC – total phosphorus content; ** correlation is significant at p ≤0.01; * correlation is significant at p ≤0.05

The conducting of correlation analysis between studied biochemical parameters gives the possibility to evaluate levels depending on one other and the opposite. Pearson's coefficient is often used to describe the correlation between different biochemical parameters (Ngamdee et al., 2016). The relation of accumulation of different nutrient and non-nutrient components in raw depends on species, genotypes, conditions of growth, stage of growth, and other (Maharjan et al., 2019; Vergun et al., 2021; Yu et al., 2021). The correlation analysis showed a very strong relations between total ash content and total calcium content (r = 0.971, p ≤0.01), between titrable acidity and total tannin content (r = 0.913, p ≤0.01), between carotene content and total tannin content (r = 0.863, p ≤0.01), carotene content and titrable acidity (r = 0.845, p ≤0.01) (Table 1). A strong correlation was found between titrable acidity and total phosphorus content (r = 0.700, p ≤0.01). A very strong negative correlation was determined between dry matter content and titrable acidity (r = -0.893) of investigated raw. Strong negative relations were found between dry matter content and carotene content (r = -0.785), dry matter content, and total tannin content (r = -0.738).

According to Mohatla et al. (2016), dry matter yield of *V. unguiculata* varieties positively correlated with crude protein content (r = 0.85) and a negative correlation was found between protein content and tannins (r = -0.99).

Conclusion

Thus, this study demonstrated that different genotypes of *Vigna* spp. are a good source of nutrients such as vitamins, dry matter, selected mineral components, etc. The research of the chemical composition of plants of six *Vigna* genotypes allowed to detect maximal content of dry matter for f. 2, titrable acidity, the content of tannins, β-carotene, and phosphorus for f. 5. The highest content of lipids, ash, and calcium was determined in raw f. 3. The ascorbic acid content was maximal in raw f. 1 plants. Considering the wide use of seed raw plants of the *Vigna* genus, the results of this study about the biochemical composition of above-ground parts of these plants could be used in further biochemical and pharmacological studies as potential forage, energetic, and medicine plants.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Dose-dependent changes in the levels of oxidative stress biomarkers in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after *in vitro* treatment by extracts derived from stalks and roots of great celandine (*Chelidonium majus* L.)

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Consistent with our previous studies, we continue to evaluate the antioxidant potential of representatives belonging to the Papaveraceae family collected from the northern part of Poland using a muscle tissue model of rainbow trout (*Oncorhynchus mykiss* Walbaum). Therefore, in the present study, oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), protein oxidative modification carbonyl derivative content, total antioxidant capacity (TAC)] were used to evaluate the antioxidant activity of stalk and root extracts of *Chelidonium majus* L. (CM) at doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, and 0.63 mg.mL^{-1}. Muscle tissue homogenates of rainbow trout were used in this study. Phosphate buffer was used as a positive control. After incubation of the mixture at 25 °C for 2 h with continuous mixing, samples were used for biochemical studies. Results of our study revealed that a dose of CM extracts of 0.63 mg.mL^{-1} showed the highest antioxidant activity in the muscle tissue of rainbow trout. The extracts derived mainly from the roots of CM collected from rural areas were effective in reducing the levels of oxidative stress biomarkers by reducing lipid peroxidation markers, which may suggest that the active substances such as alkaloids (chelidonine, sanguinarine, berberine), flavonoids, phenols in these plants can effectively protect the membrane structures in muscle cells of salmonids. We also observed statistically significant reductions in levels of both aldehydic and ketonic derivatives of oxidatively modified proteins in muscle tissue of rainbow trout after incubation with CM extracts at this dose compared to the controls. The comparison of these results shows that CM extracts can effectively inhibit protein damage by scavenging free radicals and/or activation of antioxidant defenses. The secondary metabolites of CM, i.e. polyphenols and alkaloids, are most likely responsible for this effect. Using doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} of both root and stalk extracts *in vitro* study, statistically significant increases in levels of TBARS and OMP were observed. Screening of species belonging to the family Papaveraceae for other biological activities, including antioxidant activity, is essential and may be effective in the search for preventive measures in the pathogenesis of diseases caused by oxidative stress in human and veterinary medicine.

Keywords: *Chelidonium majus*, rainbow trout, total antioxidant capacity, lipid peroxidation, oxidatively modified proteins, muscle tissue, dose-dependent changes

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Introduction

Nature gifts medicinal plants with the untapped and boundless treasure of active chemical constituents with significant therapeutic potential that makes these plants a beneficial source in the phytomedicines (Yurdakok-Dikmen et al., 2018). Phytomedicines are believed to have benefits over conventional drugs and are regaining interest in current research (Li et al., 2021). The development of health products of phytomedicine has often stemmed from traditional or historical use, or from long-term evidence that consumption of phytomedicine is associated with better health outcomes. Phytomedicine is a collection of therapeutic knowledge that is deeply rooted in culture and forms the basis for an early version of pharmacopeias based largely on natural products of predominantly plant origin as sources of antioxidants used in veterinary and human medicine (Cheng et al., 2016).

Oxidative stress plays a key role in the onset of many diseases in humans, but also in animals. Reactive oxygen species and reactive nitrogen species are continuously produced in the body through oxidative metabolism, mitochondrial bioenergetics, and immune function (Tan et al., 2018). They can be bound to nucleic acids, enzymes, membrane lipids, proteins, and other small molecules lowering their biological potential (Poljsak et al., 2013; Tan et al., 2018). Antioxidants can act as chain breakers, scavenging chain initiating radicals like hydroxyl, alkoxyl, or peroxyl, quenching singlet oxygen, decomposing hydroperoxides, and chelating prooxidative metal ions (Pisoschi and Pop, 2015). Epidemiological studies confirm that the incidence of oxidative stress-related conditions is lowered by using herbs rich in compounds possessing high antioxidant activity (Pisoschi et al., 2016; Carocho and Ferreira, 2013).

Recent studies have reported on the antioxidant properties of plants belonging to the Papaveraceae family (Krošlák et al., 2017; Zhang et al., 2020; Nile et al., 2021). *Chelidonium majus* L. (CM, Papaveraceae), or greater celandine, is an important plant in western phytotherapy and in traditional Chinese medicine (Zielińska et al., 2018). Crude extracts of CM, as well as purified compounds derived from it, exhibit a broad spectrum of biological activities (antiinflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective and antioxidant) that support some of the traditional uses of CM plants (Colombo and Bosisio, 1996; Nawrot, 2017; Zielińska et al., 2018; Huang et al., 2019; Popovic et al., 2021; Krzyżek et al., 2021). However, herbal medicine also claims that this plant has several important properties which have not yet been scientifically studied (Colombo and Bosisio, 1996; Gilca et al., 2010; Zielińska et al., 2018). The results of CM studies offered new insights into the preliminary steps regarding the development of a high-value product for phytomedicine applications through promising metabolic variations with antioxidant and anticancer potentials (Nile et al., 2021).

In our previous study, we assessed if the oxidative stress biomarkers [2-thiobarbituric acid reactive substances (TBARS), carbonyl derivatives content of protein oxidative modification (OMP), total antioxidant capacity (TAC)] and also activities of antioxidant enzymes (catalase, ceruloplasmin) in the equine plasma after treatment by extracts derived from roots and stalks of CM collected from rural and urban agglomerations. Our results demonstrated that statistically significant reductions in lipid peroxidation biomarkers were noted after incubation with extracts derived from roots of CM collected from both urban (by 35%, $p < 0.05$) and rural (by 34%, $p < 0.05$) agglomerations compared to the untreated samples. Stalk extracts derived from CM also reduced TBARS levels, but only extracts derived from CM collected from the rural areas; a statistically significant decrease (by 21%, $p < 0.05$) was observed compared to the control untreated samples. The lowest values in the content of the aldehydic derivatives of OMP were observed after incubation with extracts derived from roots of CM collected from both rural and urban areas. On the other hand, levels of ketonic derivatives of OMP were significantly increased after incubation with extracts derived from stalks of CM collected from both rural and urban areas compared to the control samples, in contrast to extracts derived from roots of CM collected from urban areas, where there was a statistically significant reduction in ketonic derivatives of OMP (by 15%, $p < 0.05$) compared to the control sample. A significant increase in the TAC levels was observed after incubation with root and stalk extracts of CM collected from both urban and rural areas, but the highest values were observed after incubation with extracts derived from roots of CM collected from rural areas (by 66.7%, $p < 0.05$) compared to the control samples. Stalk extracts of CM collected from urban agglomerations were found to be most effective in increasing catalase activity (by 115%, $p < 0.05$). Both root and stalk extracts of CM collected from rural areas caused a statistically significant reduction in ceruloplasmin levels. These *in vitro* studies indicate that extracts from this plant are

a significant source of natural antioxidants that could prevent the progression of various disorders caused by oxidative stress (Stefanowski et al., 2021).

The current study is a continuation of our study according to the assessment of antioxidant effects of extracts derived from roots and stalks of CM collected from rural and urban agglomerations in the northern part of Poland (Kartuzy district, Pomeranian voivodeship). Thus, the aim of our study was evaluation the oxidative stress biomarkers, i.e. 2-thiobarbituric acid reactive substances, carbonyl derivatives of protein oxidative modification, total antioxidant capacity in the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after treatment by extracts in different doses derived from roots and stalks of CM collected from rural and urban agglomerations for estimation the optimal doses of extracts exhibiting the antioxidant activity.

Material and methodology

Collection of plant material

Plant materials (Figure 1B) were harvested from natural habitats on the territory of the Kartuzy district (54° 20′ N 18° 12′ E) in the Pomeranian province (northern part of Poland) (Figure 1A). Kartuzy is located about 32 kilometers (20 miles) west of Gdańsk and 35 km (22 miles) south-east of the town of Lębork on a plateau at an altitude of approximately 200 meters (656 feet) above sea level on average. The plateau, which is divided by the Radaune lake, comprises the highest parts of the Baltic Sea Plate (http://www.kartuzy.pl/). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.

Preparation of plant extracts

The collected roots and stalks were brought into the laboratory for biochemical studies. Freshly washed samples of plants were weighed, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. The extract was stored at -20 °C until use.

Experimental fish and muscle tissue samples

Clinically healthy rainbow trout with a mean body mass of 80–120 g were used in the experiments. The muscle tissue samples were homogenized in ice-cold buffer (100 mM Tris-HCl, pH 7.2) using a glass homogenizer immersed in an ice water bath. Homogenates were centrifuged at 3,000 rpm for 15 min at 4 °C. After centrifugation, the supernatant was collected and frozen at −20 °C until analyzed. All enzymatic assays were carried out at 22 ±0.5 °C using a Specol 11 spectrophotometer (Carl Zeiss Jena, Germany) in duplicate. The reactions were started by adding the tissue supernatant.

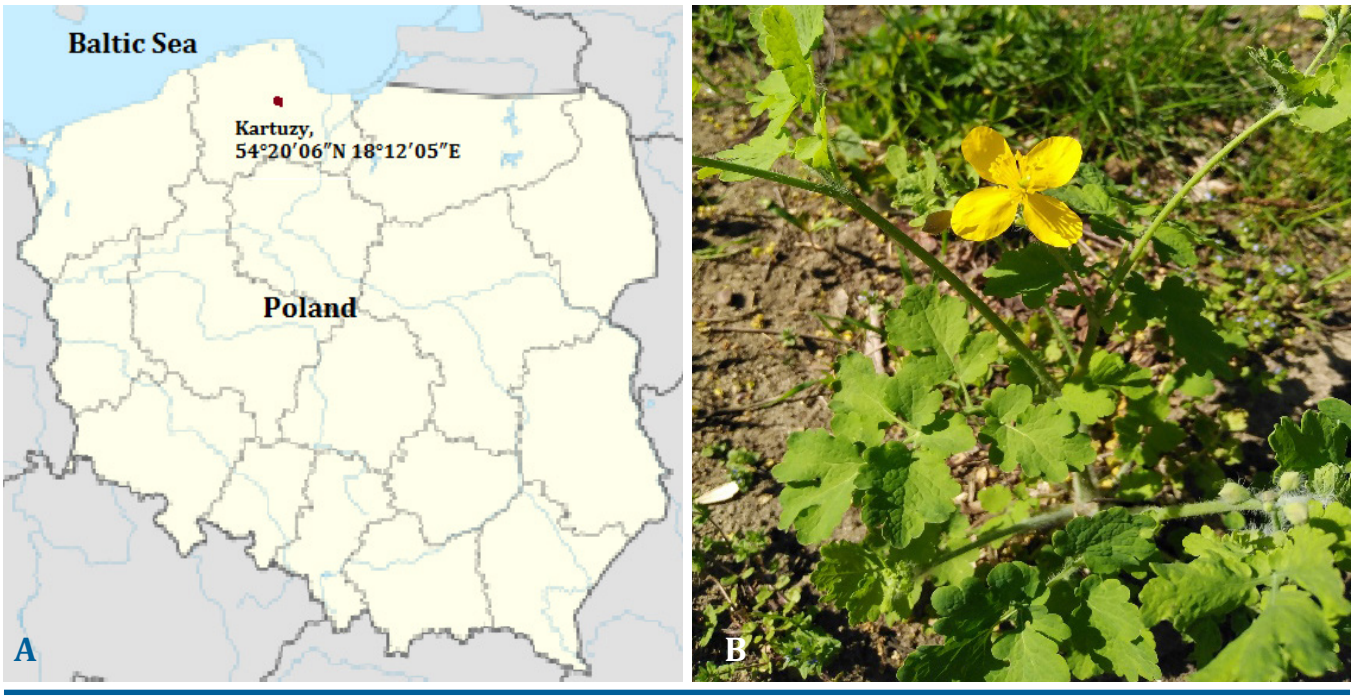

Figure 1 Location of Kartuzy in the map of Poland (A), where the greater celandine (B) was collected

Experimental design

The supernatant of the muscle tissue was used to incubate with extracts derived from stalks and roots of CM at four final concentrations, i.e. 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1} and 0.63 mg.mL^{-1}, respectively, at room temperature. The control samples (muscle tissue) were incubated with 100 mM Tris-HCl buffer (pH 7.2). The incubation time was 2 h. Biomarkers of oxidative stress were studied in the incubated homogenates (control samples and in samples with extracts derived from stalks and roots of CM). Tissue homogenates were used for the determination of the levels of 2-Thiobarbituric acid reactive substances (TBARS), oxidative modification of proteins (OMP), antioxidant defense enzymes, and total antioxidant capacity (TAC). The method described by Bradford (1976) with bovine serum albumin as a standard was used for the quantification of proteins. Absorbance was recorded at 595 nm.

Assay of 2-thiobarbituric acid reactive substances (TBARS)

Lipid oxidation was evaluated by TBARS according to the method described by Kamyshnikov (2004) with some modifications. TBARS were calculated as nmoles of malonic dialdehyde (MDA) per mg of protein.

The content of carbonyl derivatives of protein oxidative modification (OMP)

To evaluate the protective effects of the extracts derived from stalks and roots of CM against free radical-induced protein damage in muscle samples, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in samples was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining carbonyl content in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity (TAC)

The TAC level in the samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998). The level of TAC in the sample (%) was calculated with respect to the absorbance of the blank sample.

Statistical analysis

Statistical analysis of the data obtained was performed by employing the mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Kruskal-Wallis one-way analysis of variance. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica software, version 8.0 (StatSoft, Poland) (Zar, 1999).

Results and discussion

The content of 2-thiobarbituric acid reactive substances as a biomarker of lipid peroxidation in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses (5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, 0.63 mg.mL^{-1}) derived from stalks and roots of CM collected from urban and rural agglomerations was presented in Figure 2. The highest significant increase in TBARS levels after incubation with CM extracts at dose 5 mg.mL^{-1} was observed for root extracts of CM collected from urban areas (232.56 ±2.64 nmol.mg^{-1} protein) compared to the untreated samples (196.72 ±1.34 nmol.mg^{-1} protein). An increase of TBARS level was 18.2% ($p < 0.05$). Similar results were obtained for TBARS level in the muscle tissue of rainbow trout after incubation with root extracts of CM (5 mg.mL^{-1}) collected from rural areas (223.97 ±1.45 nmol.mg^{-1} protein) compared to the untreated samples, where it was statistically significantly increased by 13.9% ($p < 0.05$). Similar results were observed for TBARS level in the muscle tissue of rainbow trout after incubation with stalk extracts of CM collected from both urban (223.79 ±2.3 nmol.mg^{-1} protein) and rural areas (223.71 ±0.84 nmol.mg^{-1} protein) compared to the untreated samples. There was a statistically significant increase in lipid peroxidation biomarkers by 13.8 and 13.7% ($p < 0.05$) for stalk extracts of CM collected from both urban and rural agglomerations, respectively (Figure 2).

According to CM extracts at a dose of 2.50 mg.mL^{-1}, statistically significantly increase in TBARS levels compared to the untreated samples were observed for all extracts. After *in vitro* incubation of muscle tissue with root extracts of CM collected from urban (221.33 ±0.89 nmol.mg^{-1} protein) and rural areas

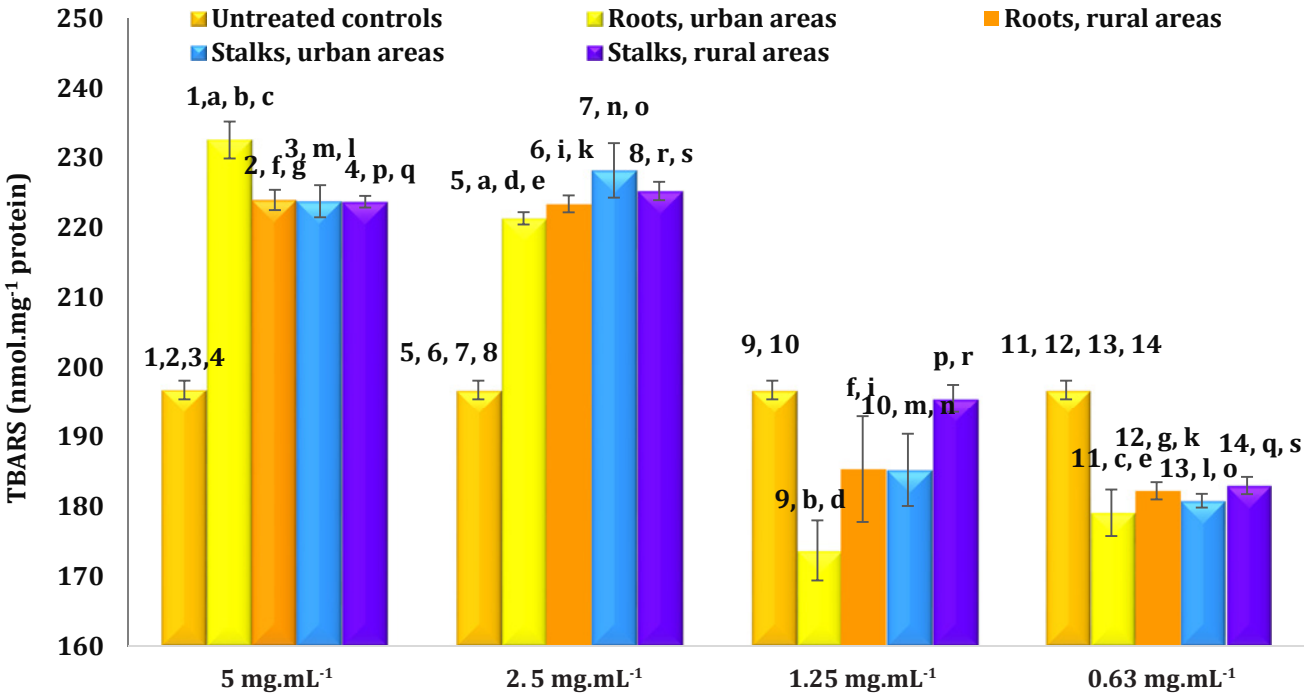

Figure 2 The content of 2-thiobarbituric acid reactive substances as a biomarker of lipid peroxidation in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of *Chelidonium majus* collected from urban and rural agglomerations (n = 8)
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
Changes are statistically significant ($p < 0.05$) in relations: 1 – untreated controls *vs.* extracts derived from roots collected in urban areas (5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from roots collected in rural areas (5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from stalks collected in urban areas (5 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from stalks collected in rural areas (5 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from roots collected in urban areas 2.5 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}); 8 – untreated controls *vs.* extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}); 9 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 10 – untreated controls *vs.* extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}); 11 – untreated controls *vs.* extracts derived from roots collected in urban areas (0.63 mg.mL^{-1}); 12 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 13 – untreated controls *vs.* extracts derived from stalks collected in urban areas (0.63 mg.mL^{-1}); 14 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); b – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); c – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from roots collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); e – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); f – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); g – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); i – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); k – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); m – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); l – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); n – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); o – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); p – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); q – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); r – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); s – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

(223.4 ±1.21 nmol.mg^{-1} protein), a statistically significant increase in TBARS levels compared to untreated samples (196.72 ±1.34 nmol.mg^{-1} protein) was noted. Based on the results, there was an increase in TBARS levels by 12.5% ($p < 0.05$) and 13.6% ($p < 0.05$) for root extracts collected both in urban and rural areas, respectively. Similar results were obtained after using stalk extracts of CM collected from both urban (228.2 ±3.91 nmol.mg^{-1} protein) and rural areas (225.25 ±1.32 nmol.mg^{-1} protein), where we observed a statistically significant elevation in TBARS levels compared to the untreated samples (196.72 ±1.34 nmol.mg^{-1} protein); the increase in TBARS levels was by 16% ($p < 0.05$) and 14.5% ($p < 0.05$) for stalk extracts of CM collected from both urban areas and rural areas.

By reducing the dose of CM extracts sequentially to 1.25 mg.mL^{-1}, we obtained remarkably different results compared to the previous cases. After incubating

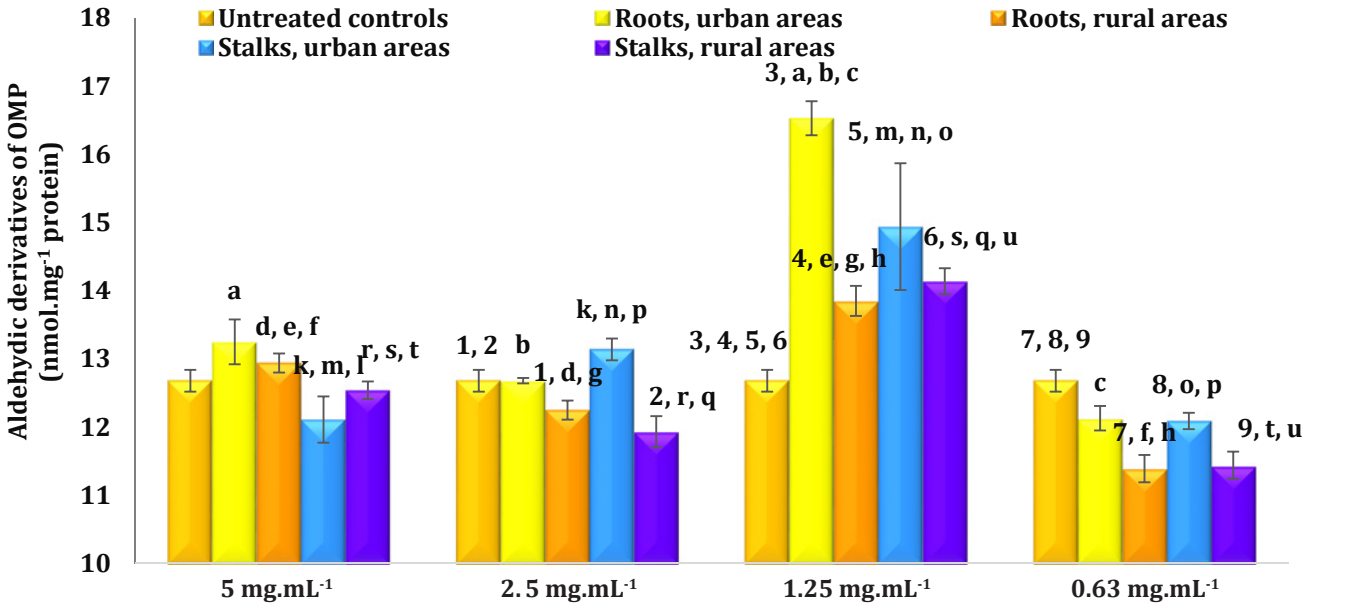

Figure 3 The content of aldehydic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of *Chelidonium majus* collected from urban and rural agglomerations (n = 8)

Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)

Changes are statistically significant ($p < 0.05$) in relations: 1 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from roots collected in rural areas (1.25 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 8 – untreated controls *vs.* extracts derived from stalks collected in urban areas (0.63 mg.mL^{-1}); 9 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); b – extracts derived from roots collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); c – extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); e – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); f – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); g – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); h – extracts derived from roots of CM collected in rural areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); k – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); m – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); l – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); n – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); o – extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); p – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); q – extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); r – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); s – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); t – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); u – extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

extracts from both roots and stalk extracts of CM collected only in the urban area, we obtained statistically significant changes [(173.74 ±4.31 nmol.mg^{-1} protein) and (185.28 ±5.18 nmol.mg^{-1} protein)] compared to the untreated samples (196.72 ±1.34 nmol.mg^{-1} protein). We recorded statistically significant reductions in TBARS level by 11.7% and 5.8% ($p < 0.05$) for both root and stalk extracts of CM collected in urban areas.

After undergoing dose reduction of CM extracts to 0.63 mg.mL^{-1}, we have obtained even more satisfactory results. After incubation of muscle tissue with root extracts of CM collected from urban areas (179.13 ±3.33 nmol.mg^{-1} protein), we obtained statistically significantly the lowest TBARS levels compared to untreated controls (196.72 ±1.34 nmol.mg^{-1} protein), i.e. by 8.9% ($p < 0.05$). Similar results were noted also for stalk extracts of CM collected from urban areas (180.87 ±0.99 nmol.mg^{-1} protein), where we also obtained a statistically significant reduction in TBARS levels (8.1%, $p < 0.05$) compared to the untreated controls (196.72 ±1.34 nmol.mg^{-1} protein). A statistically significantly reduced in the TBARS level was demonstrated after incubation of muscle tissues with root (182.29 ±1.23 nmol.mg^{-1} protein) and stalk extracts (183.04 ±1.24 nmol.mg^{-1} protein) of CM collected from rural agglomerations. Statistically

significant reduced levels of lipid peroxidation biomarkers were 7.3% ($p < 0.05$) and 7% ($p < 0.05$) for root and stalk extracts compared to controls (Figure 2).

The content of aldehydic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM collected from urban and rural agglomerations was presented in Figure 3.

After *in vitro* incubation of muscle tissue with root and stalk extracts at a final dose of 5 mg.mL^{-1}, we did not note statistically significant changes in the level of aldehydic derivatives of OMP compared to the untreated controls. Another situation was observed after reducing the extract dose to 2.5 mg.mL^{-1}. After incubation with root extracts of CM collected from rural areas, we noted a statistically significant decrease in the level of aldehydic derivatives of OMP (12.25 ±0.14 nmol.mg^{-1} protein) compared to untreated controls (12.68 ±0.16 nmol.mg^{-1} protein); a decrease was by 3.4% ($p < 0.05$). A similar trend was observed after incubation of muscle tissue homogenate with stalk extracts of CM collected from rural areas, where we obtained a statistically significant decrease in the level of aldehydic derivatives of OMP (11.93 ±0.23 nmol.mg^{-1} protein) compared to untreated control (by 5.9%, $p < 0.05$).

By reducing the extract dose to 1.25 mg.mL^{-1}, incubation of muscle tissue homogenates with root extracts of CM collected from both urban and rural agglomerations, we obtained a statistically significant increase in the level of aldehydic derivatives of OMP [(16.53 ±0.25 nmol.mg^{-1} protein) and (13.85 ±0.22 nmol.mg^{-1} protein), respectively] compared to the untreated samples (12.68 ±0.16 nmol.mg^{-1} protein); increase was by 30.4% ($p < 0.05$) and 9.2% ($p < 0.05$) for root extracts of CM collected from both urban areas and rural areas, respectively. Statistically significant changes were also obtained after incubation of muscle tissue with stalk extracts of CM collected from both urban (14.94 ±0.93 nmol.mg^{-1} protein) and rural areas (14.14 ±0.19 nmol.mg^{-1} protein), where there was an increase in the level of aldehydic derivatives of OMP in relation to the untreated samples by 17.8% ($p < 0.05$) and by 11.5% ($p < 0.05$) for stalk extracts of CM collected from both urban and rural agglomerations, respectively.

Using extracts at a dose of 0.63 mg.mL^{-1}, there was a statistically significant reduction in levels of aldehydic derivatives of OMP after incubation of muscle tissue homogenate with root extracts of CM collected from rural areas (11.39 ±0.2 nmol.mg^{-1} protein) and stalk extracts of CM collected from both urban (12.09 ±0.12 nmol.mg^{-1} protein) and rural agglomerations (11.4 ±0.2 nmol.mg^{-1} protein) compared to controls (12.68 ±0.16 nmol.mg^{-1} protein). There was a statistically significant decrease in levels of aldehydic derivatives of OMP by 10.2% ($p < 0.05$) for root extracts of CM collected from rural areas and by 4.7% ($p < 0.05$) and 9.8% ($p < 0.05$) for stalk extracts of CM collected from urban and rural agglomerations, respectively (Figure 3).

Comparing values obtained after using root extracts of CM collected from urban areas, we noted a statistically significant reduction in levels of aldehydic derivatives of OMP by 19.8% ($p < 0.05$) compared to the values obtained after using a dose of 1.25 mg.mL^{-1}. A statistically significant increase in level of aldehydic derivatives of OMP by 30.4% ($p < 0.05$) was observed after using the above-mentioned extract at the dose of 1.25 mg.mL^{-1} compared to those obtained at the dose of 2.5 mg.mL^{-1}. A comparison of the values obtained after using root extracts of CM collected from urban areas at a dose of 1.25 mg.mL^{-1} *vs.* those obtained at the dose of 0.63 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 36.3% ($p < 0.05$) (Figure 3).

The use of root extracts of CM in a dose of 2.5 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 5.3% ($p < 0.05$) compared to those values using extracts at the dose of 5 mg.mL^{-1}, which also showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 6.6% ($p < 0.05$) compared to values obtained after incubating with extracts at a dose of 1.25 mg.mL^{-1}. A comparison of values obtained from the above-mentioned extract using *in vitro* at the dose of 5 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 13.6% ($p < 0.05$) compared to results obtained using a dose of 0.63 mg.mL^{-1}. A comparison of the values obtained after using root extracts of CM at a dose of 1.25 mg.mL^{-1} *vs.* those obtained at the dose of 2.5 mg.mL^{-1} and 0.63 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 13.1% ($p < 0.05$) and 21.6% ($p < 0.05$). The use of stalk extracts of CM collected from urban areas at a dose of 5 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 7.8% ($p < 0.05$) compared to those values using extracts in the dose of 2.5 mg.mL^{-1}, which also showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 18.9% ($p < 0.05$) compared to values obtained after incubating

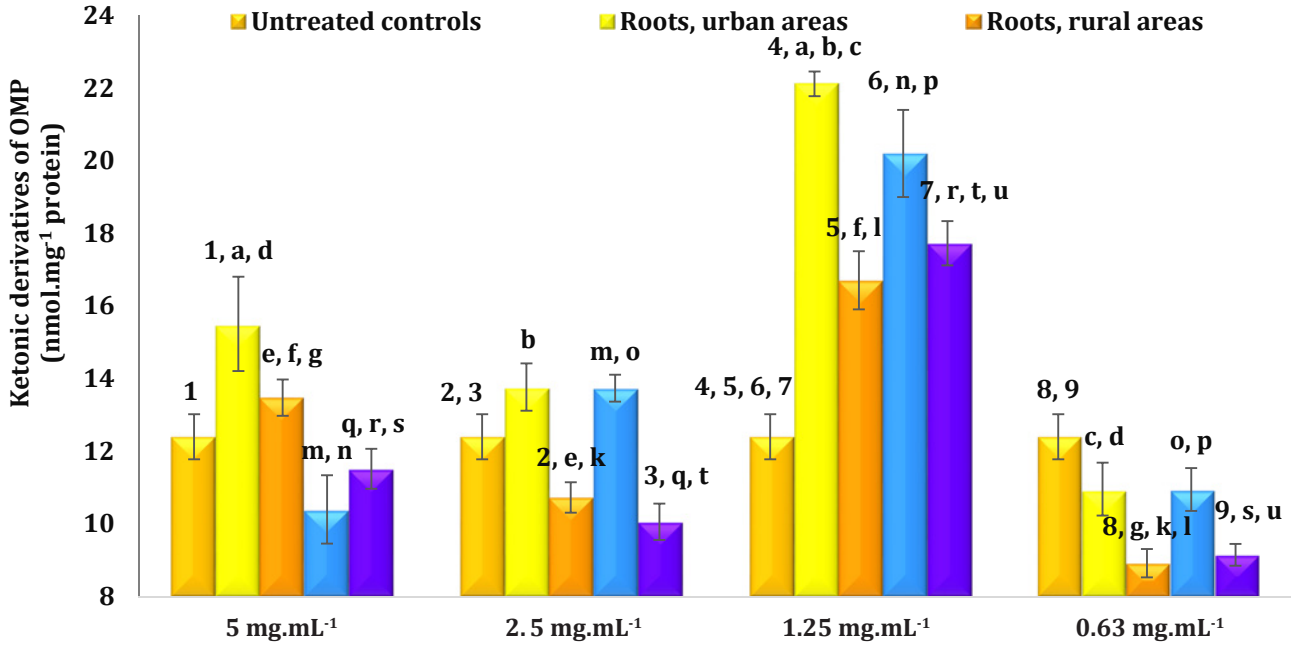

Figure 4 The content of ketonic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM collected from urban and rural agglomerations (n = 8)

Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)

Changes are statistically significant ($p < 0.05$) in relations: 1– untreated controls *vs.* extracts derived from roots collected in urban areas (5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from roots collected in rural areas (1.25 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}); 8 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 9 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); b – extracts derived from roots collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); c – extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); e – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); f – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); g – extracts derived from roots collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); k – extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); m – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (2.5 mg.mL^{-1}); n – extracts derived from stalks collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); o – extracts derived from stalks collected in urban areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); p – extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); q – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); r – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); s – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); t – extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); u – extracts derived from stalks collected in rural areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

with extracts at a dose of 1.25 mg.mL^{-1}. The use of stalk extracts of CM collected from urban areas at a dose of 1.25 mg.mL^{-1} showed a statistically significant increase in levels of aldehydic derivatives of OMP by 23.6% ($p < 0.05$) compared to those values using extracts in the dose of 0.63 mg.mL^{-1}, which also showed a statistically significant increase in levels of aldehydic derivatives of OMP by 13.7% ($p < 0.05$) compared to values obtained after incubating with extracts at a dose of 2.5 mg.mL^{-1}. A comparison of the values obtained after using stalk extracts of CM collected from rural areas at a dose of 2.5 mg.mL^{-1} *vs.* those obtained at the dose of 5 mg.mL^{-1} and 1.25 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 4.9% ($p < 0.05$) and 15.6% ($p < 0.05$), respectively. A stalk extract of CM collected from rural areas at the dose of 0.63 mg.mL^{-1} showed a statistically significant decrease in levels of aldehydic derivatives of OMP by 19.1% ($p < 0.05$) compared to those obtained at a dose of 1.25 mg.mL^{-1}, while compared to the dose of 2.5 mg.mL^{-1} showed an decrease in levels of aldehydic derivatives of OMP by 4.1% ($p < 0.05$) (Figure 3).

The content of ketonic derivatives as a biomarker of oxidatively modified proteins in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM

collected from urban and rural agglomerations was presented in Figure 4.

Focusing on a dose of 5 mg.mL^{-1} of CM extracts after *in vitro* incubation with the muscle tissue of rainbow trout, we observed a statistically significant increase in ketonic derivatives after incubation with root extracts of CM collected from urban areas (15.51 ±1.3 nmol. mg^{-1} protein) compared to untreated samples (12.4 ±0.62 nmol.mg^{-1} protein); the increase was 25.1% ($p < 0.05$). Another situation was observed after the analysis of the dose of 2.5 mg.mL^{-1}, where there was a statistically significant reduction in ketonic derivatives of OMP by 13.5% ($p < 0.05$) for root extracts of CM collected from a rural agglomeration was observed compared to the controls [(10.73 ±0.42 nmol.mg^{-1} protein) *vs.* (12.4 ±0.62 nmol.mg^{-1} protein)]. For the stalk extract of CM collected from rural areas, we recorded the lowest statistically significant value of ketonic derivatives by 18.9% ($p < 0.05$) compared to the controls [(10.06 ±0.5 nmol.mg^{-1} protein) *vs.* (12.4 ±0.62 nmol.mg^{-1} protein)] (Figure 4).

In subsequent steps, decreasing the dose to 1.25 mg. mL^{-1}, we obtained a statistically significant increase in ketonic derivatives of CM after incubation of muscle tissue homogenates with root extracts of CM collected from both urban (22.12 ±0.34 nmol.mg^{-1} protein) and rural areas (16.71 ±0.8 nmol.mg^{-1} protein) compared to the untreated samples (12.4 ±0.62 nmol.mg^{-1} protein); increase was 78.4 and 34.8% ($p < 0.05$), respectively. Similarly, after incubation of muscle tissue with stalk extracts of CM collected from both urban (20.2 ±1.2 nmol.mg^{-1} protein) and rural agglomerations (17.73 ±0.61 nmol.mg^{-1} protein), we observed a statistically significant increase in levels of ketonic derivatives of OMP by 62.9 and 43% ($p < 0.05$), respectively, compared to the control samples (12.4 ±0.62 nmol.mg^{-1} protein). Analyzing the dose of 0.63 mg.mL^{-1}, we recorded a statistically significant decrease in levels of ketonic derivatives of OMP (by 28.1%, $p < 0.05$) in the muscle tissue after treatment with root extracts of CM collected from rural areas (8.92 ±0.39 nmol.mg^{-1} protein) compared to untreated samples (12.4 ±0.64 nmol.mg^{-1} protein) (Figure 4).

Comparing the dose of 5 mg.mL^{-1}, we observed a statistically significant decrease in ketonic derivatives of OMP after incubation of muscle tissue with root extracts of CM collected from urban areas by 29.9% ($p < 0.05$), increase by 12.6% ($p < 0.05$) and increase by 41.5% ($p < 0.05$) compared to those using a dose of 1.25 mg.mL^{-1}, 2.5 mg.mL^{-1} and 0.63 mg.mL^{-1}, respectively. Root extracts at a dose of 0.63 mg.mL^{-1} after incubation of muscle tissue homogenates showed a statistically significant decrease in ketonic derivatives of OMP (by 29.3%, $p < 0.05$) compared to the values obtained using a dose of 5 mg.mL^{-1}. When comparing values obtained to root extracts of CM collected from rural areas at a dose of 5 mg.mL^{-1}, we recorded a statistically significant increase in ketonic derivatives of OMP by 25.6% ($p < 0.05$) compared to those obtained using a dose of 2.5 mg.mL^{-1}, while the dose of 1.25 mg. mL^{-1} showed a statistically significant decrease in ketonic derivatives of OMP by 19.3% ($p < 0.05$). On the other hand, using the dose of 5 mg.mL^{-1} of the root extracts of CM collected from the rural areas showed a statistically significant increase in ketonic derivatives of OMP by 51.1% ($p < 0.05$) compared to the dose of 0.63 mg.mL^{-1}, while a statistically significant increase in ketonic derivatives of OMP by 25.6% ($p < 0.05$) compared to the dose of 2.5 mg.mL^{-1} was observed. The stalk extracts of CM collected from urban areas at a dose of 5 mg.mL^{-1} showed a statistically significant decrease in ketonic derivatives of OMP by 24.3% ($p < 0.05$) compared to those obtained at a dose of 2.5 mg.mL^{-1}, while a statistically significant decrease in ketonic derivatives of OMP by 48.5% ($p < 0.05$) was noted compared to those obtained at a dose of 1.25 mg.mL^{-1} (Figure 4).

Using the dose of 2.5 mg.mL^{-1} of the stalk extracts of CM collected from urban areas showed a statistically significant increase in ketonic derivatives of OMP by 25.5% ($p < 0.05$) compared to the dose of 0.63 mg.mL^{-1}. On the other hand, a dose of 0.63 mg.mL^{-1} of the above CM extracts showed a statistically significant decrease in levels of ketonic derivatives of OMP by 45.8% ($p < 0.05$) compared to those values obtained using a dose of 1.25 mg.mL^{-1}. We also observed that a dose of 0.63 mg.mL^{-1} of the stalks extracts of CM collected from rural agglomerations showed a statistically significant decrease in the levels of ketonic derivatives of OMP by 48.4% ($p < 0.05$) compared to those values obtained using a dose of 1.25 mg.mL^{-1}, while statistically significant decrease in the levels of ketonic derivatives of OMP (by 20.6%, $p < 0.05$) was observed compared to a dose of 5 mg.mL^{-1}. A dose of 1.25 mg.mL^{-1} of stalk extracts from CM collected from rural agglomerations statistically significantly increased the levels of ketonic derivatives of OMP (by 76.2%, $p < 0.05$) compared to values obtained at a dose of 2.5 mg.mL^{-1}, while a statistically significant increase in the levels of ketonic derivatives of OMP (by 53.9%, $p < 0.05$) was observed compared to values obtained at a dose of 5 mg.mL^{-1} (Figure 4).

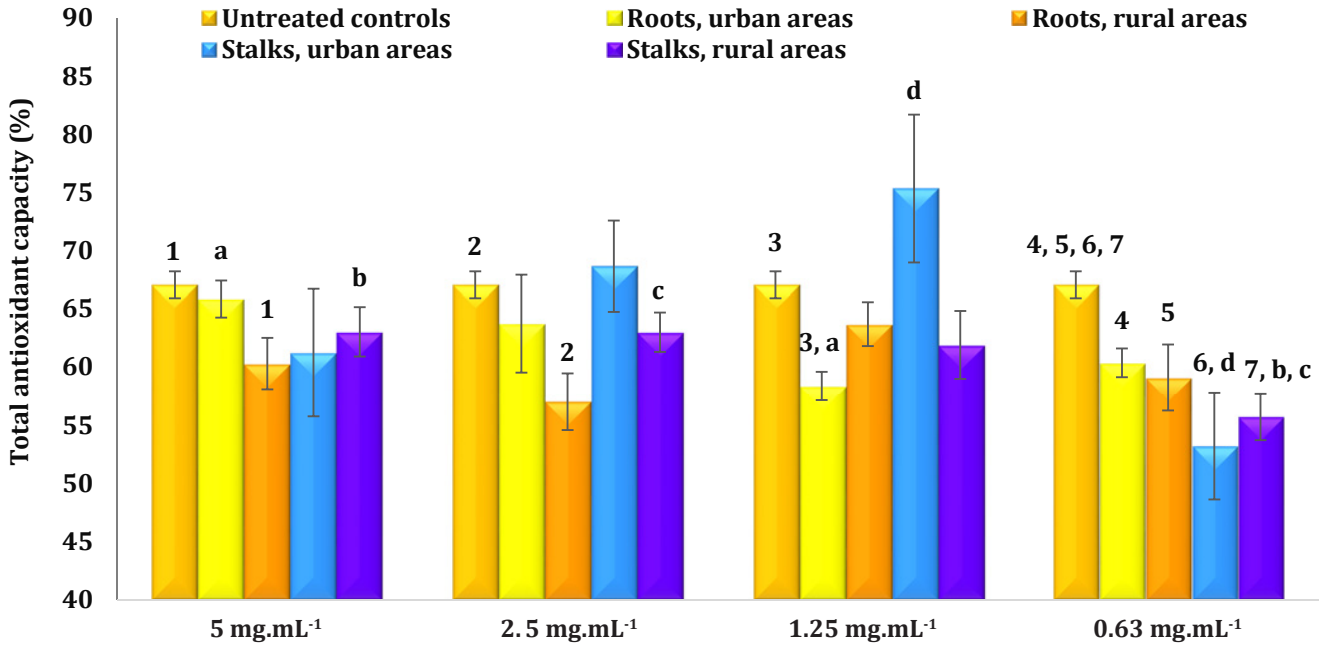

Figure 5 The total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses (5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, 0.63 mg.mL^{-1}) derived stalks and roots of *Chelidonium majus* collected from urban and rural agglomerations (n = 8)
Results are presented as the mean (M) ± the standard error of the mean (S.E.M.)
Changes are statistically significant (p <0.05) in relations: 1 – untreated controls *vs.* extracts derived from roots collected in rural areas (5 mg.mL^{-1}); 2 – untreated controls *vs.* extracts derived from roots collected in rural areas (2.5 mg.mL^{-1}); 3 – untreated controls *vs.* extracts derived from roots collected in urban areas (1.25 mg.mL^{-1}); 4 – untreated controls *vs.* extracts derived from roots collected in urban areas (0.63 mg.mL^{-1}); 5 – untreated controls *vs.* extracts derived from roots collected in rural areas (0.63 mg.mL^{-1}); 6 – untreated controls *vs.* extracts derived from stalks collected in urban areas (0.63 mg.mL^{-1}); 7 – untreated controls *vs.* extracts derived from stalks collected in rural areas (0.63 mg.mL^{-1}); a – extracts derived from roots collected in urban areas (5 mg.mL^{-1}) *vs.* those ones (1.25 mg.mL^{-1}); b – extracts derived from stalks collected in rural areas (5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); c – extracts derived from stalks collected in rural areas (2.5 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1}); d – extracts derived from stalks collected in urban areas (1.25 mg.mL^{-1}) *vs.* those ones (0.63 mg.mL^{-1})

The total antioxidant capacity in the muscle tissue of rainbow trout after *in vitro* incubation with extracts in different doses derived from stalks and roots of CM collected from urban and rural agglomerations was presented in Figure 5.

After incubation of muscle tissue with root extracts of CM collected from rural areas at a dose of 5 mg.mL^{-1} we observed a statistically significant decrease by 10.1% (p <0.05) in total antioxidant capacity (60.3 ±1.6%) compared to the untreated controls (67.07 ±1.16%). We obtained similar results after incubation with the same extracts at a dose of 2.5 mg.mL^{-1}, i.e. (57.03 ±2.43%) compared to the untreated controls (67.07 ±1.16%), where a statistically significant decrease in TAC was 14.97% (p <0.05). A statistically significant change occurred only for root extracts of CM collected from urban agglomerations at a dose of 1.25 mg.mL^{-1} after incubation with muscle tissue homogenates, where we also observed a statistically significant decrease in TAC levels (58.39 ±1.21%) compared to the untreated controls (67.07 ±1.16%) by 5.04% (p <0.05).

By reducing the dose to 0.63 mg.mL^{-1}, we observed a statistically significant decrease in TAC levels after incubation with both root (60.37 ±1.24%) and stalk (53.21 ±4.58%) extracts from CM collected from urban agglomerations compared to the control samples (67.07 ±1.16%) by 9.99% (p <0.05) for root and by 20.66% (p <0.05) for stalk extracts. Similarly, after incubation of muscle tissue at the above dose with root and stalk extracts from CM collected from rural areas, we recorded a statistically significant decrease in total antioxidant capacity [(59.11 ±2.83) and (55.72 ±1.99%), respectively compared to the untreated controls (67.07 ±1.16%)] by 11.87 and 16.92% (p <0.05), respectively (Figure 5).

Comparing a dose of 5 mg.mL^{-1} of root extracts of CM collected from urban areas to a dose of these extracts of 1.25 mg.mL^{-1}, we observed a statistically significant increase in TAC level by 12.8% (p <0.05). A dose of 5 mg.mL^{-1} of stalk extracts of CM collected from rural areas statistically significantly increased the TAC level by 13.1% (p <0.05) compared to a dose of 0.63 mg.

mL^{-1}, while a statistically significant increase in the total antioxidant capacity by 0.05% ($p < 0.05$) was observed compared to a dose of 2.5 $mg.mL^{-1}$. On the other hand, stalk extracts of CM collected from urban agglomerations at a dose of 1.25 $mg.mL^{-1}$ resulted in statistically significantly increased TAC levels (by 41.6%, $p < 0.05$) compared to values obtained using a dose of 0.63 $mg.mL^{-1}$ (Figure 5).

Conclusions

The purpose of our study was evaluation the oxidative stress biomarkers, i.e. 2-thiobarbituric acid reactive substances, carbonyl derivatives content of protein oxidative modification, total antioxidant capacity in the muscle tissue of rainbow trout after treatment by extracts in different doses derived from roots and stalks of CM collected from rural and urban agglomerations for estimation the optimal doses of extracts exhibiting the antioxidant activity. From our study, a dose of CM extracts of 0.63 $mg.mL^{-1}$ showed the highest antioxidant activity in the muscle tissue of rainbow trout. The extracts derived mainly from the roots of CM collected from rural areas were effective in reducing the levels of oxidative stress biomarkers by reducing lipid peroxidation markers, which may suggest that the active substances such as alkaloids (chelidonine, sanguinarine, berberine), flavonoids, phenols in these plants can effectively protect the membrane structures in muscle cells of salmonids. We also observed statistically significant reductions in levels of both aldehydic and ketonic derivatives of oxidatively modified proteins in muscle tissue of rainbow trout after incubation with CM extracts at this dose compared to the controls. The comparison of these results shows that CM extracts can effectively inhibit protein damage by scavenging free radicals and/or activation of antioxidant defenses. The secondary metabolites of CM, i.e. polyphenols and alkaloids, are most likely responsible for this effect. Using doses of 5 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ of both root and stalk extracts *in vitro* study, statistically significant increases in levels of TBARS and OMP were observed. This phenomenon may explain the use of destructive effects of CM on the membrane structures of cancer cells, due to the presence of a wide range of active compounds and other secondary metabolites.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Total polyphenol content and antioxidant activity of *Solanum lycopersicum* L.

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Tomato, the edible berry of the plant tomatoes (*Solanum lycopersicum* L.) is currently one of the most widely used crops worldwide. Tomatoes are considered to be one of the most popular vegetables, although, from a botanical point of view, it is a fruit. It is a rich source of numerous important bioactive substances that have a positive effect on human health. Antioxidant compounds of tomatoes, with their protective properties, have a significant effect on oxidative stress. Seven tomato cultivars, namely Perun, Tornado, Bejbino F1 60S, Darinka F1, Glazier, Tramezzino, and Brioso were analysed in this study, that aimed to determine the total polyphenol content and antioxidant activity of tomatoes. The total polyphenol content has been determined by the Folin-Ciocalteu assay, using an UV-VIS spectrophotometer. The values have ranged from 231.48 mg.kg^{-1} FW to 559.81 mg.kg^{-1} FW. Statistically highest TPC was determined in the cultivar Bejbino F1 60S. Statistically lowest TPC was determined in the cultivar Brioso. The total antioxidant activity (AA) has been determined by the DPPH radical scavenging assay, using an UV-VIS spectrophotometer. The values have ranged from 0.261 to 0.554 mmol TE.kg^{-1} FW. Statistically highest AA was determined in the cultivar Bejbino F1 60S. Statistically lowest AA was determined in the cultivar Darinka F1. Statistical evaluation of the results showed a weak correlation between total polyphenol content and antioxidant activity of tomatoes. These results indicate that content of polyphenols and antioxidant activity of tomatoes are influenced by cultivar.

Keywords: Tomato, polyphenols, antioxidant activity

Introduction

Solanum L., the genus of the family Solanaceae, includes about 1250 to 1700 species. *Solanum* species are present in all temperate, subtropical to tropical zones and are remarkable for their morphological and ecological diversity. *Solanum* is possibly the most important genus in economic terms. Tomatoes (*Solanum lycopersicum* L.) come from America, specifically from the Andean Mountain region, where it was domesticated by the indigenous people (Bergougnoux, 2014). They have easily spread around the world due to the great diversity of their usability

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and its adaptation. As a result, there are currently hundreds of varieties of tomatoes. Current varieties were created by genetic breeding of native, wild species (Nuez and Díez, 2013). Tomato is one of the most important horticultural crops worldwide, with more than 186 million tonnes produced in 2020 (FAOSTAT, 2020). It does not contain toxic substances, is palatable uncooked, can be easily processed into various products, and is even used to make pharmaceuticals. The plants have a high yield of fruit, they are easily grown under containment, indoors, in greenhouses, and in fields (Tzfira, 2007). Tomato is consumed fresh or as a processed product, i.e. ketchup, puree, paste, canned tomatoes, juice, and pasta sauces (Li et al., 2018).

Tomatoes are considered to be one of the most popular vegetables, although, from a botanical point of view, it is a fruit. This species is a good source of various phytochemicals, mainly carotenoids and polyphenols. Tomatoes also contain a large number of other bioactive compounds, such as vitamins, terpenoids, glycoalkaloids, and many others that accumulate in their fruits (Mechlouch et al., 2012; Hafeznia et al., 2014; Perveen et al., 2015; Martí et al., 2016). Tomatoes are the major source of lycopene and a number of other carotenoids, such as α-, β-, and γ-carotene, phytoene, phytofluene, and neurosporene (Perveen et al., 2015). Consumption of tomatoes has been linked to many health benefits, such as prevention of oxidative stress-related diseases, and prevention of cardiovascular diseases (Szabo et al., 2019). Tomatoes are generally recognized for their outstanding antioxidant, anticancer, and antidiabetic properties (Faizan et al., 2021).

Even though phenolic compounds are secondary metabolites, they play a significant role in plant existence. Besides being involved in defense against herbivores and pathogens, in allelopathy processes, mechanical support, and attraction of pollinators and fruit dispersers, they also absorb damaging ultraviolet radiation (Taiz and Zeiger, 2006). Because of this, they tend to accumulate in the dermal tissue of the plant body (Peng et al., 2008). Polyphenol content in plants depends on various factors such as plant genetics and type of cultivar, growing conditions, soil composition, maturity state and post-harvest conditions, and others (Faller and Fialho, 2010).

Therefore, the purpose of this work was to evaluate the antioxidant activity and total polyphenol content in different tomato cultivars and determine the influence of cultivar on these parameters. These results could establish the basis for future research into the elaboration of tomatoes as a natural source of antioxidants and polyphenols.

Material and methodology

Plant material

The researched cultivars of tomatoes (Perun, Tornado, Bejbino F1 60S, Darinka F1, Glacier, Tramezzino, Brioso) were obtained from various locations in Slovakia. Samples have been harvested at the state of complete ripeness.

Extract preparation

25 g of homogenized tomatoes were extracted in 50 mL of 80% methanol by horizontal shaker (Unimax 2010, Heidolph Instrument GmbH, Germany) for 12 h and filtered through Munktell no. 390 filtrating paper (Munktell & Filtrac, Germany).

Total polyphenol content

Total polyphenol content was determined by Folin-Ciocalteau colorimetric method (Lachman et al., 2003). Folin-Ciocalteu phenol reagent (Merck, Germany), 20% Na_2CO_3 (Sigma Aldrich, USA), and distilled water were used. 0.1 mL of extract was pipetted into a 50 mL volumetric flask. 0.85 mL of Folin-Ciocalteau reagent was added, and after 3 minutes, 5 mL of 20% Na_2CO_3 was added. The mixture was stirred, and the flask was filled with distilled water to the mark. Flasks were left for 2 h at laboratory temperature and then measured against blank solution at 765 nm, using a Shimadzu UV-VIS scanning spectrophotometer (Shimadzu, Japan). Total polyphenol content was expressed as mg of gallic acid equivalent (GAE) in 1 kg of fresh weight (FW), based on the calibration curve (R^2 = 0.996).

Antioxidant activity

Antioxidant activity (AA) was measured by DPPH radical scavenging assay (Brand-Williams et al., 1995). DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) (Sigma Aldrich, USA) and methanol (Sigma Aldrich, USA) were used to produce a working DPPH solution. 1 mL of extract was pipetted into 3.9 mL of working DPPH solution, stirred, and left in dark. After 10 minutes, the solution was measured against blank solution at nm, using a Shimadzu UV-VIS scanning spectrophotometer (Shimadzu, Japan). Antioxidant activity was expressed as mmol of Trolox equivalent (TE) in 1 kg of fresh weight (FW), based on the calibration curve (R^2 = 0.994).

Statistical analysis

Statistical analysis was performed using RStudio (2020) software package. A nonparametric Kruskal-Wallis test was performed to find statistically significant information about differences among the tested samples ($p < 0.05$). 7 samples, with 4 measurement replications each were analyzed.

Results and discussion

Regular intake of tomatoes has been linked to decreased risk of chronic diseases. Epidemiological findings confirm the observed health effects are due to the presence of different antioxidant molecules such as phenol compounds, carotenoids, and vitamin C (Frusciante et al., 2007). Antioxidants are important in the prevention of both animal and plant diseases, as they could delay or inhibit oxidation (Martínez-Valverde et al., 2002).

The total polyphenol content and antioxidant activity of tomato cultivars are given in Table 1.

Total polyphenol content

Polyphenols are the most abundant antioxidants in the human diet. Recent data support the role of polyphenols in the prevention of cancer, cardiovascular diseases, and osteoporosis, and implies a contribution to the prevention of diabetes mellitus and neurodegenerative diseases (Abbas et al., 2017).

The total polyphenol content (TPC) in analysed tomato cultivars ranged from 231.48 to 559.08 mg GAE.kg^{-1} FW. Highest TPC was determined in the cultivar Bejbino F1 60S, while the lowest TPC was determined in the cultivar Brioso. According to the results, the order for tomato cultivars based on their TPC could be as follows: Brioso< Darinka F1< Tornado< Perun< Glazier< Tramezzino< Bejbino F1 60S.

Similar values were determined by other authors. Tamasi et al. (2019) reported TPC in tomato cultivars in the range 253.3–508.7 mg GAE.kg^{-1} FW. Carrilo-López and Yahia (2013) reported TPC in Mexican tomatoes in the range of 227–437 mg GAE.kg^{-1} FW. Minutolo et al. (2013) reported TPC in tomato cultivars in the range of 260–421 mg GAE.kg^{-1} FW. García- Valverde et al. (2013) reported TPC in tomato cultivars in the range of 186.92–558.63 mg GAE.kg^{-1} FW. Chandra and Ramalingam (2011) reported TPC in commercially important Indian tomato cultivars in the range of 188.4–266.0 mg GAE.kg^{-1} FW. Chang et al. (2006) reported TPC in tomato cultivars in the range of 340–380 mg GAE.kg^{-1} FW. Slimestad and Verheul (2015) reported 215 mg GAE.kg^{-1} FW in cherry tomato cultivar Jennita. Pék et al. (2010) reported 294 mg GAE.kg^{-1} FW in tomato cultivar Lemance F1. Minoggio et al. (2003) reported lower TPC in different tomato cultivars in the range of 44.3–258.4 mg GAE.kg^{-1} FW. Asensio et al. (2019) reported lower TPC in Spanish traditional tomatoes in the range of 66.71–175.42 mg GAE.kg^{-1} FW. Jacob et al. (2010) reported 23.0 mg GAE.kg^{-1} FW in the tomato cultivar Pera.

Antioxidant activity

Antioxidants acquired from diet play an important part in helping endogenous antioxidants in the neutralization of oxidative stress. The nutrient antioxidant deficiency could be the cause of several chronic diseases (Pham-Huy and Pham-Huy, 2008).

The antioxidant activity (AA) in analysed tomato cultivars ranged from 0.261 to 0.554 mmol TE.kg^{-1} FW (10.95 to 22.40%). The highest AA was determined in the cultivar Bejbino F1 60S, while the lowest AA was determined in the cultivar Darinka F1. According to the results, the order for tomato cultivars based on their AA could be as follows: Darinka F1< Brioso< Glazier< Tramezzino< Tornado< Perun< Bejbino F1 60S.

Table 1 The total polyphenol content and antioxidant activity of tomato cultivars

Cultivar	TPC (mg GAE.kg^{-1} FW ±SD)	AA (mmol TE.kg^{-1} FW ±SD)	AA (%)
Perun	307.69 ±8.50^{d}	0.545 ±0.005^{e}	22.40 ±0.64
Tornado	262.81 ±4.99^{e}	0.508 ±0.008^{d}	20.90 ±0.76
Bejbino F1 60S	559.08 ±5.92^{c}	0.554 ±0.001^{e}	22.75 ±0.47
Darinka F1	247.29 ±6.52^{b}	0.261 ±0.023^{a}	10.95 ±1.37
Glazier	361.69 ±9.54^{e}	0.339 ±0.001^{b}	14.11 ±0.45
Tramezzino	380.47 ±9.69^{f}	0.435 ±0.013^{c}	17.98 ±0.97
Brioso	231.48 ±8.30^{a}	0.274 ±0.023^{a}	11.48 ±1.35

Notes: Results are expressed as a mean of 4 measurement replications ± standard deviation (SD). Different letters indicate significant differences ($p < 0.05$); TPC – total polyphenol content; AA – antioxidant activity; FW – fresh weight; GAE – gallic acid equivalent; TE – Trolox equivalent

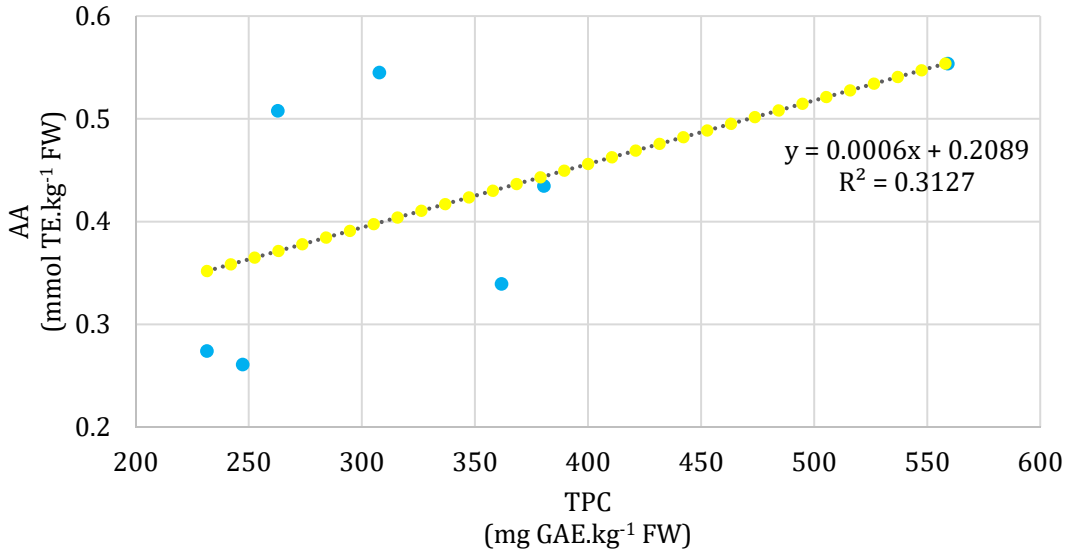

Figure 1 Relationship between total polyphenol content (TPC) and antioxidant activity (AA) of tomato cultivars

Similar values were determined by other authors. Erge and Karadeniz (2011) reported AA in Turkish tomatoes in the range of 0.42–0.58 mmol TE.kg^{-1} FW. Odriozola-Serrano et al. (2008) reported AA in Spanish tomato cultivars in the range of 9.8–26.6%. Gougoulias et al. (2012) reported AA in hydroponically cultured tomato Sandin F1 in the range of 0.07–0.84 mmol TE.kg^{-1} FW. Borguini et al. (2013) determined AA in various tomato extracts in the range of 11.33–72.34%. Tommonaro et al. (2021) determined AA in tomato cultivars in the range of 10.32–49.83%. Tamasi et al. (2019) reported higher AA in tomato cultivars in the range of 0.823–1.780 mmol TE.kg^{-1} FW. Nour et al. (2013) reported higher AA in Romanian tomatoes in the range of 0.81–1.74 mmol TE.kg^{-1} FW. Shahzad et al. (2014) reported higher AA in tomato cultivars in the range of 35.80–37.60%. Chandra and Ramalingam (2011) reported higher AA in commercially important Indian tomato cultivars in the range of 40.6–55.3%. Borguini et al. (2013) determined AA in various tomato extracts in the range of 11.33–72.34%. Tommonaro et al. (2021) determined AA in tomato cultivars in the range of 10.32–49.83%. Kaur et al. (2013) determined 2.5–4.61 mmol TE.kg^{-1} FW of hydrophilic antioxidant activity and 0.17–0.24 mmol TE.kg^{-1} FW of lipophilic antioxidant activity in selected Indian tomatoes.

Statistical evaluation of the results showed a weak positive correlation between total polyphenol content and antioxidant activity of tomatoes (R^2 = 0.3127). Gougoulias et al. (2012) also determined a weak correlation between TPC and AA of tomatoes. De Souza et al. (2021) determined a moderate correlation between TPC and AA of tomatoes. Nour et al. (2013) and Patanè et al. (2019) determined a strong positive correlation between TPC and AA of tomatoes. On the other hand, Fidrianny et al. (2015) determined a strong negative correlation between TPC and AA of various tomato cultivars.

Conclusions

Tomatoes contain a number of health-promoting substances that have a beneficial effect on human health. They are a source of important bioactive substances characterized by antioxidant activity, among other positive effects. Based on this fact, it is important to include frequent consumption of tomatoes into our diet. The total polyphenol content and antioxidant activity of seven tomato cultivars were analysed in this study. Based on the results, we can state that tomatoes are a natural source of antioxidants and polyphenols. Statistical evaluation of the results confirmed significant differences among TPC and AA of individual cultivars. Linear regression revealed a weak positive correlation between TPC and AA of tomatoes.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

In vitro antibacterial effect of various berries on *Listeria monocytogenes* as food borne patogen

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Listeria monocytogenes is a food borne pathogen and causes illnesses with a high mortality rate in susceptible populations. It is often incriminated in outbreaks of human listeriosis. Increasing interest in the health benefits of various berries has led to investigation of their antibacterial activity. Causative agent can multiply at refrigerator temperatures, is resistant to disinfectants, and adheres to various surfaces. Native berries were assessed for their ability to inhibit the growth of bacteria *L. monocytogenes*. Extracts and powder berries – sea buckthorn (*Hippophae rhamnoides* L.), rosehip (*Rosa canina* L.), black chokeberry (*Aronia melanocarpa* (Michx.) Elliott), grape marc (*Vitis vinifera* L.) and hawthorn (*Crataegus oxyacantha* L.) were used. All plant materials come from the Rudi-Arionești Natural Complex in the Republic of Moldova in 2017–2019. In previous studies it has been found that sea buckthorn, rosehip, black chokeberry, and hawthorn have antimicrobial effects on pathogenic microorganisms responsible for food alteration. Bacteria showed varying susceptibilities to the berry fruits. Antimicrobial properties were evaluated using well diffusion method and broth dilution method. According to the results obtained, sea buckthorn was found to have the most pronounced effect on *Listeria monocytogenes*, the diameter of the growth inhibition zone being 32 mm, followed by rosehip samples 26 mm. The minimum inhibition concentration (MIC) and the minimum bactericidal (MBC) were determined.

Keywords: chemical compositions, leaves, antioxidants, antibacterial activity

Introduction

Food borne illness is a common, costly, sometimes life-threatening disease, but largely preventable and are public health problem. Many disease-causing agents can contaminate aliments, causing food poisoning. Researchers have identified more than 250 food borne illnesses. Most of them are infections, caused by a variety of bacteria, viruses, and parasites (Lakshmi and Rajendran, 2013). It is estimated that 48 million people get a food borne illness (or food borne infection) each year, 128,000 are hospitalised, and 3000 die. According to the WHO, an estimated 600 million people get sick each year, or almost one in 10 people on the planet, from food contaminated with microorganisms or chemicals, and 420000 die, resulting in the loss of 33 million years of healthy life (WHO, 2015).

Listeriosis is a life-threatening disease, especially for immunocompromised people and pregnant women.

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In 2019, 2621 confirmed invasive human cases were reported. The population groups that suffered the most from listeriosis were those over 64 years of age. The most common foods associated with listeriosis are ready-to-eat products, being rich in protein and low microflora and moderate water activity.

Listeria monocytogenes is a member of the *Listeria* genus, which also includes other species: *L. ivanovii*, *L. seeligeri*. *L. monocytogenes* is a Gram-positive non-spore-forming rod. Optimum temperature of bacteria is range from 30–37 °C. Microorganism is one of the leading foodborne pathogens and the causative agent of the disease listeriosis. The organism can withstand freezing, but it is inactivated by heating at 60 °C for 30 min (Batt, 2014). Causative agent can multiply at refrigerator temperatures, is resistant to disinfectants, and adheres to various surfaces. Once introduced into the processing plants, it is able to survive and remain for a long period under adverse conditions. *L. monocytogenes* is able to form biofilm which can act as a potential source of contamination and this property is dangerous for food industry (Batt, 2014; Jamshidi and Zeinali, 2019). Bacteria is well known for its tolerance of low pH, high salt conditions (10% NaCl), low temperature (-1 °C), and acid tolerance response, which contribute to its common contamination of food. *Listeria* contamination is habitually reported in dairy products, ready-to-cook fish, and meat products such as smoked salmon and sausage; therefore, they are considered as high-risk foods. Inspite of the conditions of food storage and processing, such as high salt and low temperature, infectious agent can survive and multiply because of its halotolerance and psychrotolerance ability (Jamshidi and Zeinali, 2019; Yap et al., 2021). A sustainable future requires control of antimicrobial resistance (Jeong et al., 2010; Akinduti et al., 2019; Wu et al., 2022).

Inspection of *L. monocytogenes* in food production remains henceforward important not only for producers, but also for consumers. Therefore, efficient antimicrobial approaches for preventing food contamination or the occurrence of listeriosis are urgently needed. Today, consumers desire to choose more natural, healthy, and safe food because inappropriate use of antimicrobial agents in food production might result in undesirable residues in food and the emergence of antimicrobial-resistant microorganisms (Wu et al., 2022). Excessive use of antibiotics has become a modern epidemic. These drugs have destroyed our natural immunity. They killed the beneficial bacteria in our gut and led to the creation of super bacteria that proved to be resistant to almost any form of prescription drugs. Consuming synthetic food preservatives may cause health concerns, including potential side effects and increased cancer risks. Thereby, the use of natural antimicrobials in food preservation has attracted increasing attention from scientists, food manufacturers, and consumers (Wu et al., 2022). Increasing interest of natural foods and their antibacterial activity, our research focused of various forms of native berries and ability to inhibit the growth of bacteria *L. monocytogenes*.

Material and methodology

Extracts and powder berries

The objects of research were the berries of sea buckthorn (*Hippophae rhamnoides* L.), wild rosehip (*Rosa canina* L.), black chokeberry (*Aronia melanocarpa* (Michx.) Elliott) and hawthorn (*Crataegus oxyacantha* L.), as well as grape marc (*Vitis vinifera* L.). These berries contain biologically active compounds (carotenoids, polyphenols) and antioxidant activity, which are presented in many fields of research. All plant materials come from Rudi-Arioneşti Natural Complex in the Republic of Moldova. To obtain the extracts, the berries have been frozen at -18 °C, dried at room temperature (20.0 ±2.0 °C), and at a temperature of thermal agent 65.0 ±1.0 °C. Dry vegetable matter have moisture of 8.0 ±1.0%. For the experiments, the powder was obtained from berries (sea buckthorn, hawthorn, rosehip, black chokeberry), dried at a temperature of 55 ±1 °C to a final humidity of 6.8 ±0.5%. For extraction, dry matter has been ground and sieved in powder. To obtain the extracts, a different amount of solvent was dosed depending on the raw material according to the following ratio: for sea buckthorn powder – 1 (solid): 12 (solvent); rosehip – 1 : 15; hawthorn – 1 : 20, grape pomace – 1 : 8. The hydroethanolic solution (EtOH, 50% v/v) was used as solvent. The extraction process was performed by two methods: agitation and ultrasound, respecting two temperature regimes: 20.0 ±1.0 °C and 45.0 ±1.0 °C and 3 time periods: 0.5 h, 1.0 h and 1.5 h.

Microbial strain

The antimicrobial properties of plant extracts were tested against Gram-positive *Listeria monocytogenes* Strain EGDe, overnight culture standardised according to Mc Farland 0.5 (105) standard. Culture media – Triptone Soya Broth (Oxoid) 6%, TSA – Tryptone Soy Agar (Oxoid).

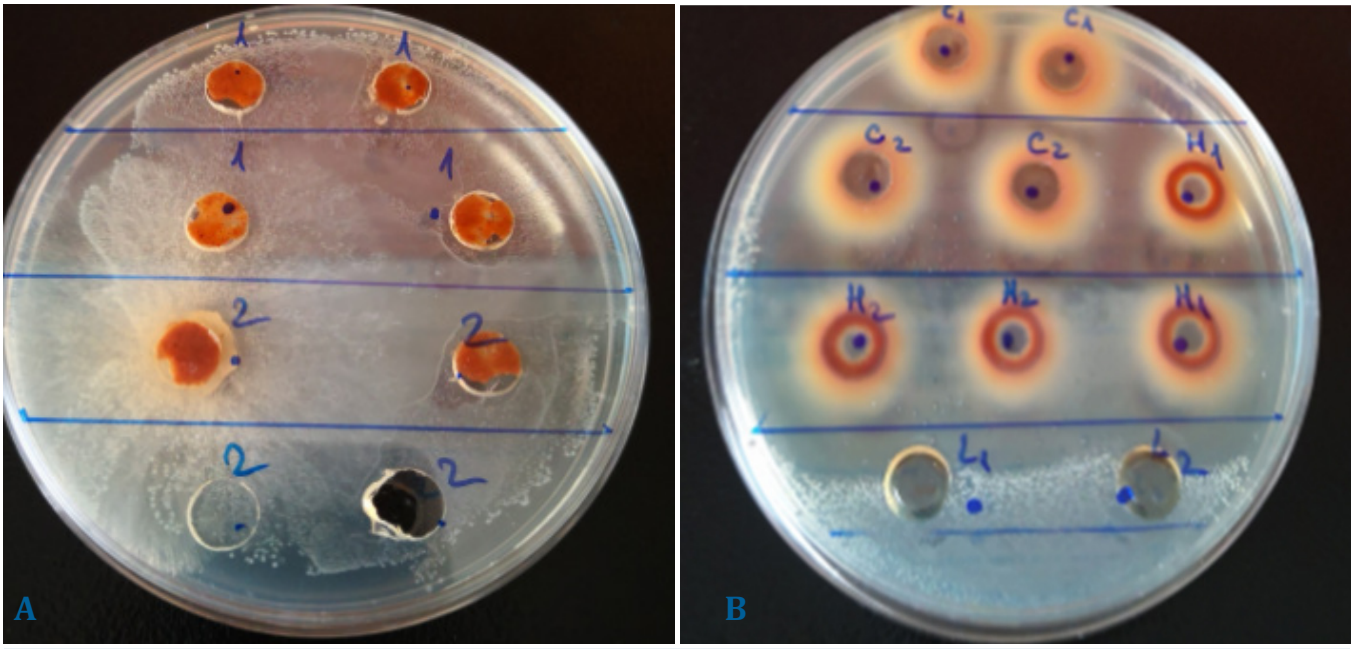

Figure 1 Well diffusion method
A – activity of *C. oxyacantha* extract on *L. monocytogenes*; B – activity of *H. rhamnoides* extract on *L. monocytogenes*

Antibacterial activity. Well diffusion method

\sAgar well diffusion method was done to evaluate antibacterial activity of berry extract and powder as describe by Bauer et al. (1966), Rovná et al. (2015), Olejar et al. (2019), Zhang et al. (2020), and Stefanowski et al. (2021a, b). The strains were grown on nutrient agar slants at 37 °C for 24 h. After the incubation, the cells were washed off the surface of agar and suspended in sterile physiological solution. One mL of fresh bacterial culture was pipetted in the centre of sterile Petri dish with optimum nutrition medium. Then wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums. Then, 100 µL of each extract was added to respective wells. The plates were placed in the refrigerator for 30 min to let the extracts diffusion well into the agar. Then, the plates were incubated at 37 °C for 18–20 h. Antimicrobial activity was detected by measuring the zone of inhibition (including the wells diameter) appeared after the incubation period (Figure 1).

Determination of minimum inhibitory concentrations (MIC)

Almost all tested extracts exhibited antimicrobial activity. The basic 18 h culture suspension of *L. monocytogenes* was repared. One colony was dissolved in 10 mL of 0.6% TSB (Tryptic Soy Broth) + YE (broth culture medium). Initially the optical density corresponding to 108 was determined, two dilutions were made to obtain 105. For determination of minimum inhibitory concentration, 50 µL of culture (10^5) was inoculated into each well of the microtiter plate. To each well was added 50 µL TSB + YE 0.6% (nutrition medium for *Listeria*) and then 50 µL of each test extract (each of a certain concentration) was added. Binary dilutions were made from the basic solution of each extract: rosehip – 66.7 $mg.mL^{-1}$, sea buckthorn – 83 $mg.mL^{-1}$, grape marc – 125.0 $mg.mL^{-1}$, black chokeberry 55.5 $mg.mL^{-1}$. The microtiter plates thus prepared were incubated on a thermostat at 37 °C/overnight (18 h). Then the MIC was determined using a spectrophotometer, the OD (Optical Density) measured at λ = 600 nm. Anything above 0.1 at OD is considered microbial growth. The "Tecon" spectrophotometer was used.

Statistical analysis

All the experiments were repeated three times and the data were calculated as means ±SD. One-way ANOVA was used to determine the differences in yields of different extracts.

Results and discussion

Over the last few years, there has been an increasing global trend toward the use of natural antioxidants present in fruits and green leafy vegetables due to fact that consumers are more concerned regarding the safety of using synthetic compounds in convenient food products (Arora et al., 2012; Criste et al., 2020; Vågsholm et al., 2020). For this reason, probably, the

diseases caused by psychotrophic bacteria such as *L. monocytogenes* increased. So, all possible strategies to prevent the proliferation of the pathogen in food, especially those using natural bioactive compounds, may contribute to the maintenance of human health (Puupponen-Pimiä et al., 2005). The antimicrobial activity of plant extracts depends on the type and amount of phenolics present in the plant tissue and the pathogen's inherent resistance (Romha et al., 2018; Arora et al., 2012).

According to presence or absence of inhibition zones and zone diameters we determined *in vitro* antimicrobial activity of sea buckthorn extracts and powders against the tested *L. monocytogenes*. In previous studies it has been found that sea buckthorn, rosehip, black chokeberry, and hawthorn have antimicrobial effects on pathogenic microorganisms (Efenberger-Szmechtyk et al., 2020; Sandulachi, et al., 2020; Shah et al., 2020; Efenberger-Szmechtyk et al., 2021). According to the data obtained (Table 1), we observed that *H. rahmnoides* has the most pronounced effect on *L. monocytogenes*, especially concentrated extract 1 and 2 followed by *R. canina* and grape marc of *V. vinifera*. The diameter of inhibition zone for sea buckthorn has shown 30 mm and 32 mm. *A. melanocarpa* indicate weak activity on *Listeria*. The *C. oxyacantha* does not show any activity on the tested bacteria. *Listeria* is resistant to all types of hawthorn preparations (water-soluble, fat-soluble concentrates and powders).

Above mentioned diffusion tests are widely used to determine the susceptibility of bacteria but have their limitations as result of "susceptible and resistant". This method is considered qualitative method. But a precise assessment is to determine the minimum inhibitory concentration (MIC) of the natural product or antibiotic against the organisms concerned. Dilution methods are used to determine the minimal concentration of antimicrobial that can inhibit or kill the microorganism. This can be achieved by dilution of natural product or antimicrobial agent in either agar or broth media by making serial dilutions (usually in two folds) (Tiwari et al., 2009). MIC was reported as the lowest concentration of the extract causing complete inhibition of the growth of the bacteria. The Figure 2 and 3 below shows the MIC determination.

According to the data presented above (Figure 2, 3), we can see that sea buckthorn has a more pronounced action on *L. monocytogenes*, followed by rosehips. *H. rhamnoides* concentrated extract 1 and aqueous preparations 1 are very active. Values of minimum inhibitory concentration are 2.6 ±1.45 and 5.2 ±1.22 mg.mL^{-1}. All *H. rhamnoides* extract had shown marked antibacterial activity against *L. monocytogenes* (Upadhyay et al., 2010). *A. melanocarpa* and *V. vinifera* have a lower effect on *Listeria*. For *A. melanocarpa* MIC is 7.8 ±0.88 mg.mL^{-1} for concentrated extracts. Tescovina has a lower effect activity, the minimum amount of inhibition is 15.6 ±5.22 mg.mL^{-1} for concentrated extracts (undiluted).

Previous research (Ghendov-Moşanu et al., 2018) analysed rosehip and hawthorn powder and their minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and bactericidal effect against *S. aureus* ATCC 25923 (3.91 ±0.15 – MIC, 7.81 ±0.21 – MBC and 41.66 ±1.35 – MIC,

Table 1 Determination of antibacterial activity by well diffusion method

Liquid extracts	*Listeria monocytogenes* Strain EGDe				
	zone inhibition (mm)				
	Hippophae rhamnoides	***Rosa canina***	***Vitis vinifera***	***Aronia melanocarpa***	***Crataegus oxyacantha***
C1	32	20	20	12	R
C2	30	21.5	20	9.5	R
H1	29	21	–	15.5	R
H2	30	22	–	16	R
P1	27	26	–	–	R
P2	27	25	–	–	R
L1	R	R	–	R	R
L2	R	R	–	R	R
P1'	–	–	–	R	R
P2'	–	–	–	R	R

Notes: C – concentrated extract 1 and 2; H – hydroalcoholic extract – 1 and 2; L – liposolubile extract – 1 and 2; P – powder 1 and 2, 1'and 2'; "–" no samples, R – resistant

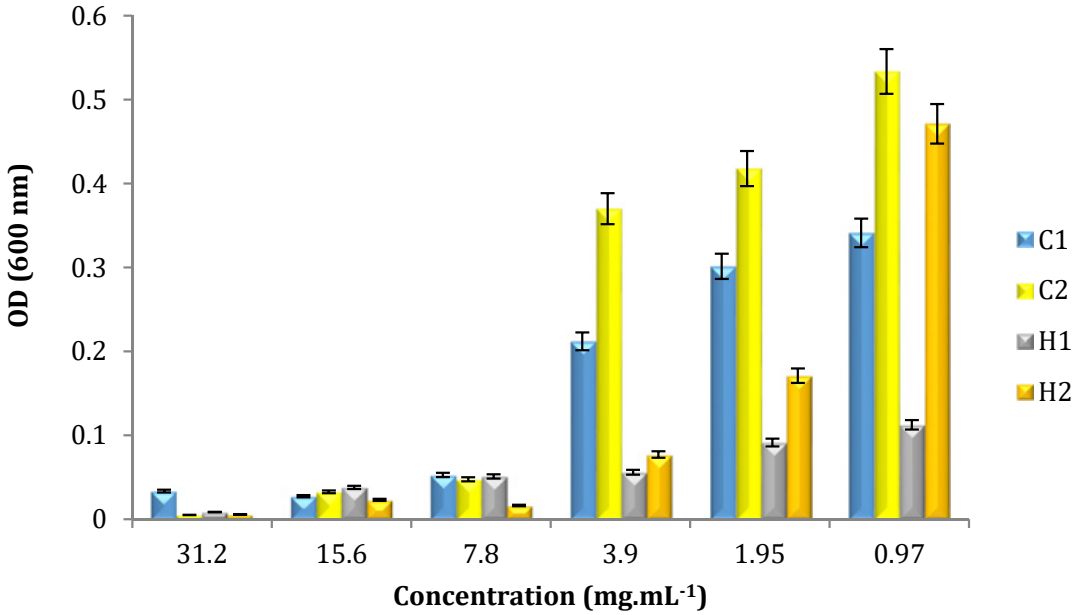

Figure 2 Determination of minimum inhibitory concentration *Aronia melanocarpa* (Michx.) Elliott (mg.mL^{-1})
C – concentrated extract 1 and 2; H – hydroalcoholic extract 1 and 2; 1, 2 – powders 1 and 2

83.33 ±2.47 – MBC, respectively), *E. coli* ATCC 25922 (31.25 ±0.98 –MIC, 62.5 ±1.8 – MBC and 62.5 ±2.2 – MIC, 125 ±5.0 – MBC, respectively) and *K. pneumoniae* ATCC 13883 (62.5 +2.1 – MIC, 125 ±5.0 – MBC for rosehip).

Negi et al. (2005) determined antibacterial activity of sea buckthorn seeds. The MIC values, with respect to MeOH extract for *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Listeria monocytogenes*, *Yersinia enterocolitica*, were found to be 200, 300, 300, 300, and 350 ppm, respectively. These results indicated the possibility of using sea buckthorn seeds for medicinal uses and food preservation.

Kim et al. (2018) showed results with antibacterial activity of various concentration of *Aronia melanocarpa* powder against *Bacillus cereus* ATCC10876,

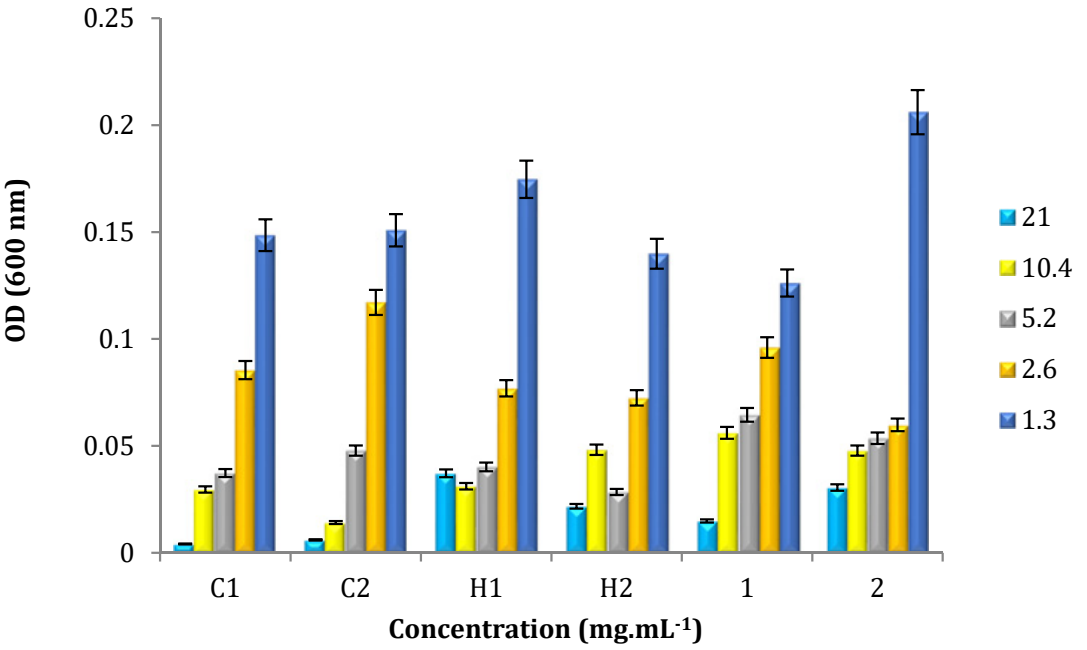

Figure 3 Determination of minimum inhibitory concentration *Hippophae rhamnoides* L. (mg.mL^{-1})
C – concentrated extract 1 and 2; H – hydroalcoholic extract 1 and 2; 1, 2 – powders 1 and 2

Staphylococcus aureus ATCC6538, *Cronobacter sakazakii* KCTC2949, and *Salmonella enteritidis* 110 tested by the spot-on-lawn assay with some modifications (Cadirci and Citak, 2005). The culture broth was diluted using MHB to 0.5 McF and spread onto Mueller-Hinton agar (MHA; Difco) and incubated at 37 °C ±0.5 for one day. This study demonstrated the potential of *A. melanocarpa* to inhibit the growth of *Bacillus cereus* and *S. aureus* as antimicrobial activity, except for *C. sakazakii* and *S. enteritidis*.

In another work, researchers analysed the rose fruits (*Rosa rugosa*) aqueous extract and determined the highest inhibitory activity against 5 strains of Gram-positive bacteria (*B. cereus* ATCC 11778, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *L. innocua* SGGW) and 5 strains of Gram-negative bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATCC13883, *P. mirabilis* ATCC 35659, *P. aeruginosa* ATCC 27853 *S. enteritidis* ATCC 13076) bacterial strains tested (Cendrowski et al., 2020). The reduction in the number of bacterial cells in matrices imitating protein food depended on the concentration of the extract used. The obtained test results confirm the possibility of using rose extracts to extend the microbiological stability of food.

Radulescu et al. (2020) found favourable antagonistic activities against the tested common bacteria strains which were exhibited by the hydroalcoholic extracts from the grapes (seeds) of the organic varieties, respectively the skin of the tested conventional varieties.

Conclusions

Due to the current fast pace of life, and the profit-oriented industries which minimize production and distribution costs by using preservatives, additives, antibiotics, hormones, and people eat a lot of fast food and quick meals, their health is often severely affected, and the general population immunity is much more weakened. The trend of organic food has been growing more and more over the past decades. In this context, coming up with reliable natural alternatives to the synthetic compounds is imperative. With a future hope to decrease the use of synthetic additives and antibiotics as preservatives. In conclusion, the antibacterial activity, give a scientific support to the modern studies which reported the positive influence of berries extracts. Our experiments determined *in vitro* antimicrobial activity of extracts and powders of berries against the tested *L. monocytogenes* food pathogen. The well diffusion method determined that the most pronounced effect on *L. monocytogenes* has *Hippophae rhamnoides*, with a diameter of 32 mm inhibition zone, for alcoholic *H. rhamnoides* extracts (C1, C2), followed by *Rosa canina*. *L. monocytogenes* is resistant to *Crataegus oxyacantha* action. MIC (minimum inhibitory concentration) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested. The smallest concentration of *H. rhamnoides* that can inhibit the growth and multiplication of tested *Listeria* is 2.6 $mg.mL^{-1}$. From the results we had concluded that the most active substances we can found in *H. rhamnoides* species.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Alhagi kirghisorum Schrenk: technological aspects of its thick extract for the pharmaceutical application

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The elaboration of medicinal substances with high pharmacological activity is the primary task of the pharmaceutical industry. In this regard, medicinal plant raw materials as a source of valuable biologically active substances are of great interest. The Kyrgyz camelthorn (*Alhagi kirghisorum* Schrenk) is a plant representing the genus of plants of the Fabaceae family growing in the deserts. The therapeutic properties of the desert plant are characterized by hemostatic, wound-healing, choleretic and astringent effect; bactericidal effect on staphylococci, streptococci and dysentery bacillus is manifested. The monograph on the herb of *Alhagi kirghisorum* is included in the State Pharmacopoeia of the Republic of Kazakhstan. The article presents the results of the research on the substantiation and development of the technology of a thick extract of *Alhagi kirghisorum*, the study of the chemical composition of the obtained thick extract of camel thorn and presents the results of studying the antimicrobial properties of this thick extract. The choice of 70% ethanol as an extractant has been experimentally substantiated, the dynamics of the extraction process was studied and the amount of extractant necessary for complete depletion of the raw materials in the extraction process was established as 1 : 5 (raw material : extractant). The presence of flavonoids was confirmed by common color reactions. The assay of the sum of flavonoids was performed by spectrophotometry. It has been found that the thick extract obtained by extraction with 70% ethanol has a higher quantitative content of flavonoid substances compared to the aqueous one. The microbiological studies (method of "wells") of *Alhagi kirghisorum* thick extract obtained by the extraction with 70% ethanol compared to ethanolic solution of chlorophyllipt showed that our extract had a moderate activity against of *Staphylococcus aureus* (zone diameters were 21.2 ±0.6 and 20.6 ±0.5 mm, respectively) and more pronounced antimicrobial activity against *Bacillus subtilis* (20.0 ±0.6 and 13.6 ±0.5 mm). Relative to the gram-negative culture of *Escherichia coli*, the activity of the thick extract of *Alhagi kirghisorum* was 21.6 ±0.5 mm while ethanolic solution of chlorophyllipt was not active. Therefore, the thick extract *Alhagi kirghisorum* is a prospective active substance for the development of herbal antibacterial preparations.

Keywords: Kyrgyz camelthorn, flavonoids, extraction dynamics, antimicrobial activity

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Introduction

The creation of highly effective and available medicines is the most important task of the pharmaceutical industry. Herbal preparations possess different biological properties, since they contain complexes of chemical compounds that simultaneously exhibit a combined therapeutic effect. Currently, wild medicinal plants are an integral part of natural wealth. Medicinal herbal raw materials are a powerful source of active substances for the manufacture of herbal medicinal products with high therapeutic activity. One of the well-known plants that have long been used in folk medicine in Kazakhstan is the wild plant *Alhagi kirghisorum*. The therapeutic properties of camelthorn are mentioned in the Canon of Medicinal Science of the famous Abu Ali Ibn Sina, better known as Avicenna (Sokolov and Shestakov, 2015; Varshochi and Asadollahi, 2015).

Kyrgyz camelthorn (*Alhagi kirghisorum* Schrenk) is a plant from the Fabaceae family, in folk medicine it is called "jantak" or "yantak". In folk medicine, the aerial parts (herb) of the *Alhagi kirghisorum*, less often the fruits and roots are used. Infusions and decoctions from *Alhagi kirghisorum* herb are used as a diuretic and sweating agent. Sometimes they are taken to soften the cough in the case of cold. Infusions, decoctions or fresh juice are taken for the treatment of gastrointestinal diseases, mainly chronic diarrhea and dysentery. Extracts from the aerial parts of camelthorn have an antimicrobial effect, and they exert a pronounced bactericidal effect on streptococci, staphylococci, and dysentery rods (Ahmad et al., 2010; Neamah, 2012; Asghari et al., 2016; Tavassoli et al., 2020). Decoctions are successfully used in the form of throat rinses in acute tonsillitis. Sometimes, *Alhagi kirghisorum* decoction is used in folk medicine for the treatment of hemorrhoids (baths, rinsing), for the external treatment of eczema, abscesses, putrefactive wounds and ulcers (washing, compresses). The decoction is used to treat patients with colitis, dysentery, gastric ulceration and gastritis, liver diseases, as a choleretic (Awaad Amani et al., 2006; Marashdah and Al-Hazimi 2010; Burasheva et al., 2012).

Our research was aimed at substantiating the technology of liquid extract in order to ensure the maximum extraction of biologically active substances from the raw material is relevant.

Material and methodology

Plant material

The aerial parts of the *Alhagi kirghisorum* (herb), which were harvested in the end of July to mid-August, was used for this research. Harvesting was carried out in the Almaty region, plant identification was performed according to the Pharmacopoeia monograph (2014). The upper, non-wooden part of the shoots was cut together with leaves and flowers, making a cut at a height of at least 8–10 cm from the ground. The raw materials were dried in the shade, spread in a layer 2–3 cm thick. Dried to a characteristic crack of the raw material at break. Damaged, blackened and browned parts of *Alhagi kirghisorum* were removed. The raw materials were crushed using a grass cutter; fractionated using a set of sieves, a fraction of raw materials with a size of 0.5–5.0 mm was used in the work.

Method for determining extractible substances

About 1 g of the crushed raw material (accurate weight) sieved through a sieve with holes of 1 mm diameter placed in a 200–250 mL conical flask. Later 50 mL of the extractant were added. The flask was closed with a stopper, weighed, and left for 1 h. The flask is then connected to a reflux condenser, heated maintaining a low boil for 2 h. After cooling, the flask with the content is closed again with a stopper, weighed and the loss in mass is replenished with the extractant. The content of the flask is thoroughly shaken and filtered through a dry paper filter into a dry 150–200 mL conical flask. 25 mL of the filtrate is pipetted onto a cup and the dry residue is determined according to the method of the State Pharmacopeia of Ukraine (SPU, 2015) (2.8.16). The percentage content of extractables (X) in terms of absolutely dry raw material is calculated by the formula:

$$X = \frac{m \times 200 \times 100}{m_1 (100 - W)}$$

where: m – the weight of the dry residue (g); m_1 – filtrate weight (g); W – weight loss during raw material drying (%)

Method for obtaining liquid extractions from the medicinal raw materials

Liquid extracts were obtained by the percolation method. The method includes three consecutive stages: wetting of the raw material (swelling of the raw material), infusion and actually percolation. The

essence of the method is that the raw material is loaded into the percolator and poured with the extractant until the formation of a "mirror", after which the infusion takes place during the 1st day. For the extraction process, we selected a fraction of camelthorn herb of 0.5–5.0 mm. After infusion, liquid extracts are drained (tempered) and the extractant is simultaneously fed at the same rate to create a high concentration difference in the raw material and in the extraction medium, which is the driving force of the extraction process. The percolation rate in our case was 1 drop per 1 second. In the process of percolation, consecutive drains were collected in an amount equal to the weight of the raw material (100 mL), in a ratio of 1 : 1 (weight-volume ratio).

The determination of the dry residue content in liquid extracts (drains) was carried out using the express moisture analyzer "SARTORIUS MA-150". The test was performed at a temperature of 105 °C in accordance with SPU 2.8.16.

Study of the dynamics of biologically active substances extraction from the plant raw material

The content of dry residue (An, g) in individual portions of liquid extracts V_n obtained with a corresponding extractant at an appropriate "raw material : extract" ratio was calculated by the formula:

$$A = \frac{\varpi_n \times V_n}{100}$$

where: V_n – the volume of a separately collected portion of the liquid extract obtained by the corresponding extractant with a step of the "raw material : extract" ratio of 1 : 1 (mL); ω_n – dry residue in a separately collected portion of liquid extract n (%)

Determination of the content of dry residue (B_n, g) in the total extracts V_{n+1} obtained by the corresponding extractant at the appropriate ratio of "raw material : extract" obtained at the stage was carried out by the formula:

$$B_n = \sum_{n=1}^{n} A_n$$

where: A_n – the dry residue in a separately collected portion of the extract V_n (g)

Determination of the dry residue (C_n, %) in the total extracts V_{n+1} obtained by the corresponding extractant at the appropriate ratio "raw material : extract" at the stage was carried out by the formula:

$$C = \frac{B_n}{V_{n+1}} \times 100$$

where: V_{n+1} – the volume of the total extract at the stage (mL); B_n – the content of dry residue in total extracts V_{n+1} (g)

Determination of the yield of extractives (absolutely dry extract) (D_n, %) from the extracted raw materials at each of the extraction stages with the appropriate extractant at the appropriate ratio "raw material : extract", was carried out by the formula:

$$D_n = \frac{B_n}{m_c} \times 100$$

where: m_c – the weight of the raw material loaded into the extractor (g); B_n – the content of dry residue in total extracts V_{n+1} (g)

Method for assay of the number of flavonoids in thick extracts of *Alhagi kirghisorum* herb

The assay of the sum of flavonoids was performed according to the method of SPU 2.1-2.2.25.

The quantitative content of the sum of flavonoids in the thick extract of camelthorn was determined by absorption spectrophotometry in the ultraviolet and visible region after the complexation reaction with aluminum chloride in terms of rutin. The test samples for spectrophotometric studies were prepared according to the following procedure.

Stock solution

Place 1.0 g (exact weight) of thick camelthorn extract in a 50 mL volumetric flask, dissolve in 25 mL of alcohol (70%, v/v) R, adjust the volume to the mark with the same solvent, stir. Filter through the "blue tape" filter.

Test solution

Place 2.0 mL of the stock solution into a 25 mL volumetric flask, add 1.0 mL of aluminum chloride R and adjust the volume to the mark with 5% (v/v) of glacial acetic acid R in ethanol R (70%), stir.

Compensation solution

Place 2.0 mL of the test solution into a 25 mL volumetric flask and adjust the volume to the mark with 5% (v/v) of glacial acetic acid R in ethanol R (70%), stir.

Comparison solutions

Were prepared in similar way, the initial sample (exact sample) of which was: for rutin – 0.0243 g, for quercetin – 0.0501 g, for gallic acid – 0.0398 g. The absorbance of the test solutions is measured 30 minutes after preparation for the compensation solution on Evolution 60 S (USA) spectrophotometer in a cuvette with a layer thickness of 1 cm at a wavelength of 410 nm. The content of the sum of flavonoids (in terms of rutin) in 1 g of thick extract is calculated by the formula:

$$X = \frac{D \times 50 \times 25 \times m_{st} \times 2 \times 100}{D_{st} \times m_n \times 2 \times 50 \times 25}$$

where: D – the absorbance of the test solution; D_{st} – the absorbance of the standard sample solution; m_n – the weight of the dense extract sample (g); m_{st} – the weight of the standard sample (g)

Chromatographic studies were carried out using Sorbfil plates (Russia). The test samples together with the comparison samples were applied to the start line of chromatographic plates in an amount of 5 µg. The plate with the applied samples was dried in air, then placed in a chromatographic chamber containing the solvent system glacial acetic acid:water:ethyl acetate (20 : 20 : 60) and then chromatographed in an ascending manner. Once the eluent front reached the edge of the plate, it was taken out and dried in air.

Microbiological methods of research of *Alhagi kirghisorum* thick extract

The antimicrobial activity of the test samples of the thick extract was studied *in vitro* by the method of diffusion into agar ("wells" method). This method is based on the ability of the active substances to diffuse into the agar previously cropped with cultures of microorganisms. As test cultures, pure cultures from the American Test Culture Collection (ATCC) were used: gram-positive microorganisms – *Staphylococcus aureus* ATCC 25293 and spore *Bacillus subtilis* ATCC 6633, gram-negative culture *Escherichia coli* ATCC 25922. Antifungal activity was determined in relation to yeast-like fungi *Candida ablicans* ATCC 885-653. An indicator of antimicrobial activity is the size of the inhibition zone that is formed in the nutrient medium around the wells in Petri dishes.

Statistical analysis

The calculation of metrological characteristics of analytical methods was performed in accordance with the requirements of the SPU monograph "*Statistical analysis of the results of chemical experiment N*" (2018). Statistical assessment of microbiological data are reported as mean ± SEM and were analyzed using STATISTICA 6 software with one-way ANOVA. *P* values less than 0.05 was assumed statistically significant.

Results and discussion

The scientific interest in *Alhagi* genus plants is due on the one hand to the rich composition of pharmacologically active substances with a wide range of therapeutic activity, and on the other – an extensive raw material base, as plants of this genus are wild and adapted to adverse growth conditions (Awaad et al., 2011).

According to numerous publications, among the biologically active substances in *Alhagi* plants, phenols, flavonoids, alkaloids, terpenoids, polysaccharides and fatty acids have been identified (Laghari et al., 2010; Laghari et al., 2012; Muhammad et al., 2015). The publications highlight the results of the study of antioxidant, anti-inflammatory, hepatoprotective, antibacterial, antidiarrheal and urolithic activity of *Alhagi* plant extracts with the content of total biologically active substances and some isolated individual substances (Muhammad et al., 2015; Nishanbaev et al., 2019). Analysis of the data shows that the most studied are *A. maurorum* and *A. pseudalhagi* both in terms of chemical composition and pharmacological action (Nishanbaev et al., 2019).

The works of Burasheva et al. (2012) are devoted to photochemical research of *A. kirgisorum*, according to which *A. kirgisorum* contains amino acids, condensed tannins, flavonoids, carbohydrates, carotene. Khalmatov (1960) found that the leaf of *A. kirgisorum* contains a significant amount of ascorbic acid – 1088.57 mg% in terms of dry matter.

The dependence of the chemical composition of the obtained plant extracts and their pharmacological activity on the used extractant is obvious, as the substances have different solubilities in polar and non-polar solvents (Marashdah and Al-Hazimi, 2010; Nishanbaev et al., 2019).

Thus, when determining the content of extractables in the raw material, a water-alcohol solution of various concentrations (40%, 50%, 60%, 70%, 80%) and purified water were used as the extractants. Ethyl alcohol is the most used extractant due to its high ability to dissolve biologically active substances. We have studied the extracting ability of hydroalcoholic

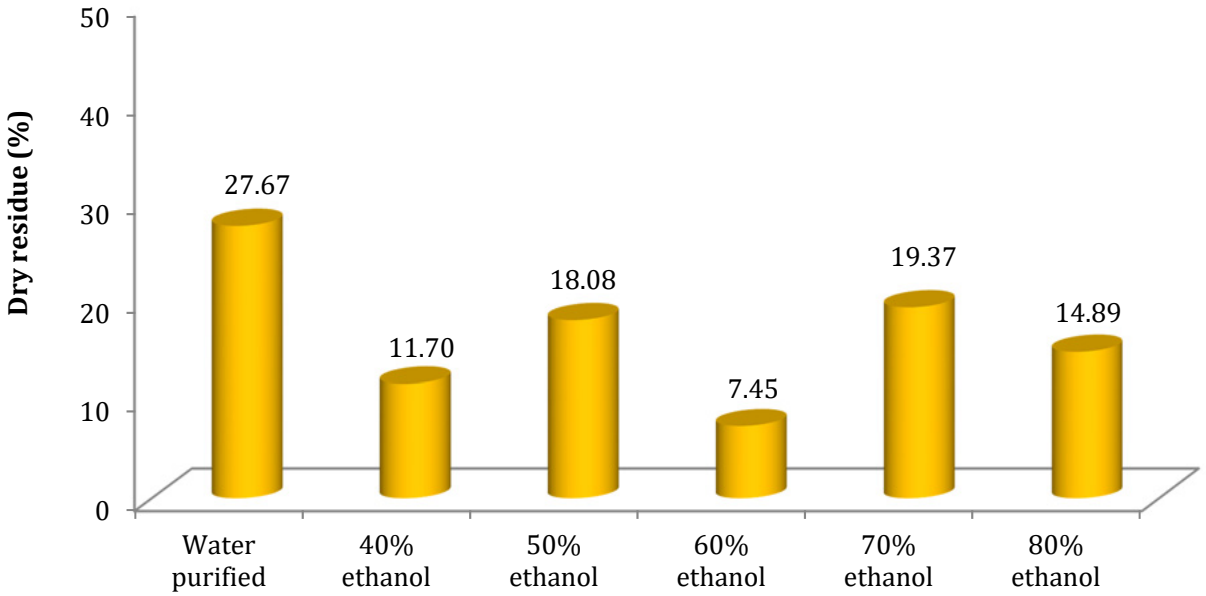

Figure 1 Amount of extractives depending on the solvent

solutions of various concentrations and purified water, the results are shown in Figure 1.

According to the above data, the maximum extraction of extractives (27.67%) is achieved with the use of purified water. Water-alcohol solutions extract from camelthorn herb from 7.45 to 19.37% of extractables.

In view of the fact that biologically active substances have different solubility, it was decided to use both purified water and 70% aqueous-alcoholic solution for further research. The extraction with 70% ethanol was performed at room temperature, with purified water at two temperature modes: room temperature and at 90 ±0.5 °C. The extraction of biologically active substances was carried out by the method of percolation. The efficiency of percolation as an extraction method is based on the high difference in the concentration of biologically active substances in the raw material and in the extractant, which is the motive force of the extraction process (Jones and Kinghorn, 2012; Blicharski and Oniszczuk, 2017). The advantage of this method is also that the pharmaceutical enterprises

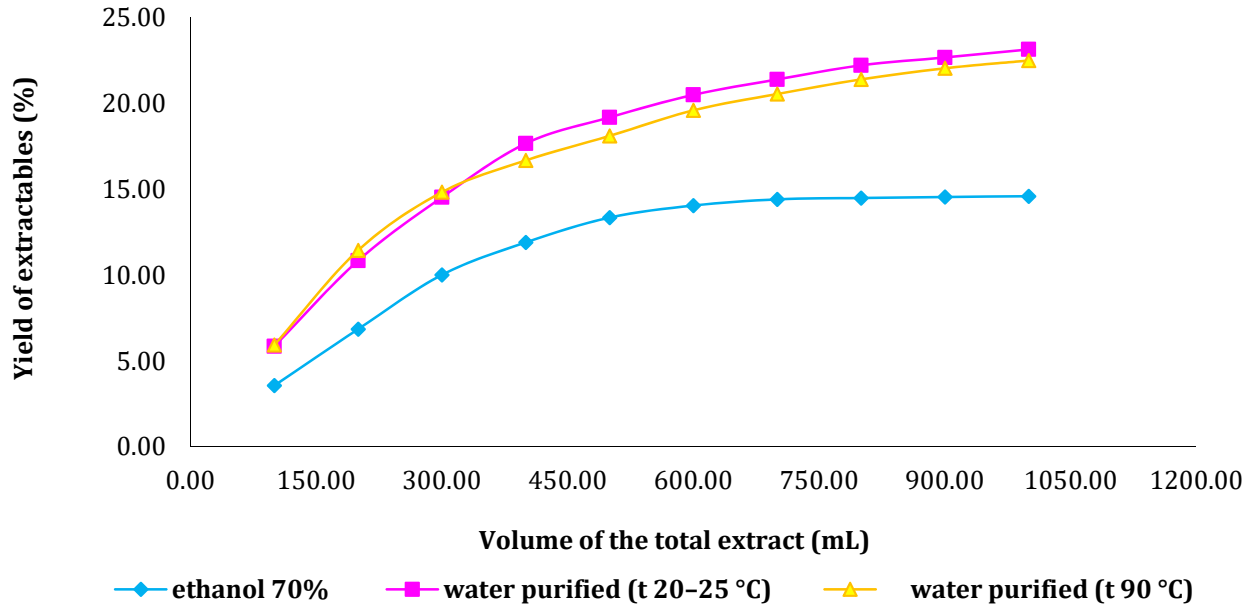

Figure 2 Dynamics of the yield of extractives in liquid extracts

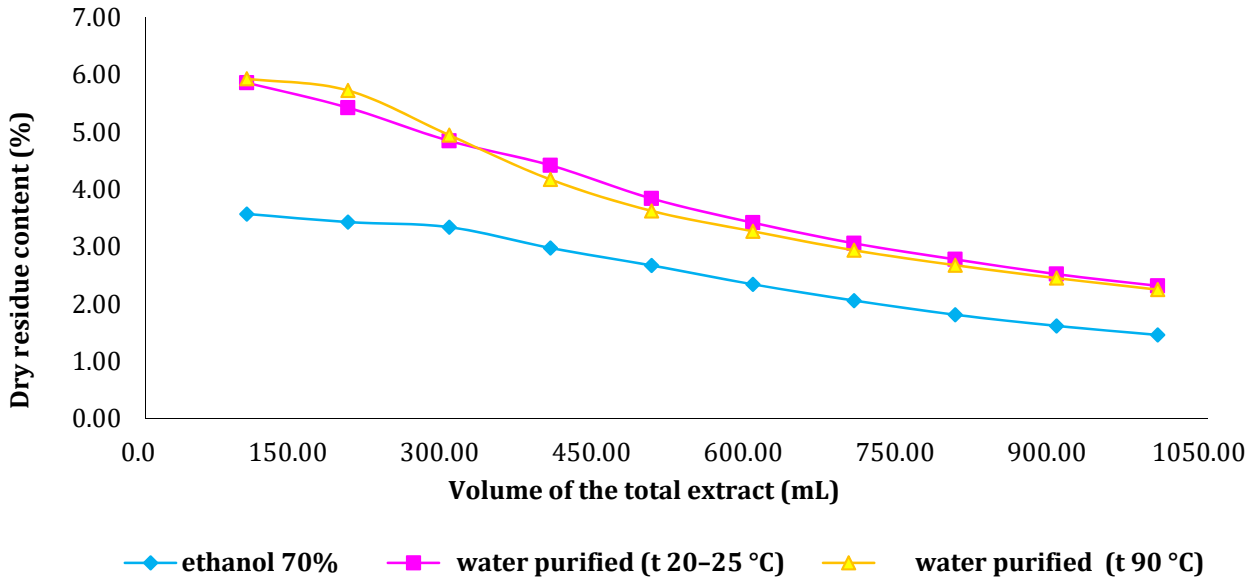

Figure 3 Dynamics of reduction of dry residue in liquid extracts

of Kazakhstan are well equipped with an appropriate equipment for the implementation of technology into serial production.

The study of the dynamics of the extraction process by percolation method was performed in accordance with the standard algorithm and the following criteria were calculated that characterize the extraction process: the content of dry residue in separately collected volumes of liquid extract (drains) and in the total extracts collected at each subsequent extraction stage (C_n, %); the content of extractives in the total extracts (D_n, %). The results of the study of the extraction process dynamics are shown in Figure 2 and 3.

Based on the data presented in Figure 2 and 3, it can be seen that at the extraction multiplicity from 1 to 5 at each extraction stage, there is a significant increase in the amount of extractable. A further increase in the extraction multiplicity slightly increases the yield of biologically active substances. Thus, it is rational to use the raw material : extractant ratio as 1 : 5 in percolation.

Thick extracts were obtained by condensing the resulting liquid extracts on a laboratory vacuum evaporator. The evaporation was carried out at a temperature of 45–50 °C, vacuum – 0.06 MPa. Under such conditions, the maximum possible preservation of biologically active substances at condensation is achieved (Blicharski and Oniszczuk, 2017).

The pharmacopoeial monograph for *A. kirgisorum* herb (State Pharmacopoeia of the Republic of Kazakhstan, 2014) provides data on its standardization by the content of tannins, which should be not less than 2.0%. However, numerous publications on phytochemical studies of plants of *Alhagi* genus cover data on the content of flavonoid substances and pharmacological action related to these groups of substances (Laghari et al., 2010; Laghari et al., 2012; Olas et al., 2015; Nishanbaev et al., 2019; Nishanbaev et al., 2020).

Flavonoids have a wide range of pharmacological activity, and show capillary-stabilizing, choleretic, diuretic, hepatoprotective, sedative, anti-inflammatory, anti-ulcer, hemostatic, bactericidal, hypotensive, hypoglycemic and antioxidant effects (Kukhtenko, 2016; Hudz et al., 2017a, b). Therefore, the paper is focused on the qualitative and quantitative analysis of flavonoids in the thick extract. Qualitative and quantitative analysis are important components of comprehensive research in the development of herbal medicines.

In the process of chemical analysis of thick extracts, pharmacopoeial methods were used to study the qualitative composition of the obtained thick extracts. For the study, aqueous and alcoholic (70%) solutions of thick extracts were diluted to a ratio of 1 : 10, which were subjected to qualitative analysis for the presence of flavonoids. The presence of biologically active substances of flavonoid structure was confirmed by generally accepted color reactions with the following reagents: concentrated hydrochloric acid (cyanidin test), 10% sodium hydroxide solution, 10% iron (III) chloride solution, 10% lead acetate solution. The results of experimental studies are presented in Table 1.

Table 1 Qualitative reactions to flavonoid structure substances

Reaction	Staining in reactions			Conclusion
	thick extract (aqueous) with water	thick extract (aqueous) with ethanol	thick extract (ethanol) with ethanol	
Cyanidin test	pink	pink	pink	red to raspberry coloring flavonols
KOH (without heating)	brown	green with sediment	green with sediment	thick extract (aqueous): chalcones and aurons thick extract (ethanol): flavones, flavonols, flavanones, flavanonols
KOH (after heating)	yellow with brown-red precipitate	brown	brown	
$FeCl_3$	black-green	black-green	brown-black	flavones, flavonols, flavanones, flavanonols, chalcones, aurons, catechins
Lead acetate	clear solution with loose precipitate	yellow coloring with sediment	yellow with sediment	flavones, chalcones, aurons containing a free o-hydroxyl group in ring B

As can be seen from the above data, thick extracts obtained by extraction with both aqueous (at room temperature and at a temperature of 90 °C) and alcoholic extracts contain both flavane and flavone derivatives. The qualitative reactions confirmed the presence of flavonoids in the extracts.

To compare the qualitative composition of thick extracts, we used the method of thin-layer chromatography according to SPU (2015) 2.1–2.2.26. Determination of the presence of phenolic compounds (phenolic acids) and flavonoids (flavonols) was carried out using comparison samples: rutin, quercetin and gallic acid.

The results of the analysis of chromatograms in daylight are presented in Figure 4 and indicate that the analyzed alcoholic extract contains substances of flavonoid structure similar to quercetin and rutin, since the chromatogram shows spots that correspond in color and location to the spots in the chromatogram of the mixture of rutin and quercetin. The TLC method did not reveal flavonoid nature substances in the aqueous extract in this concentration.

When studying the absorption spectra of the analyzed extracts obtained after interaction with aluminum chloride solution (Figure 5) it was found that in the thick extract obtained by extraction with water, the substances of the flavonoid structure are contained, but in a small amount, whereas in an alcoholic extract the amount of such substances is much higher 2.9%. Such an amount of flavonoid substances correlates with the data of Burasheva et al. (2012) and Nishanbaev et al. (2019).

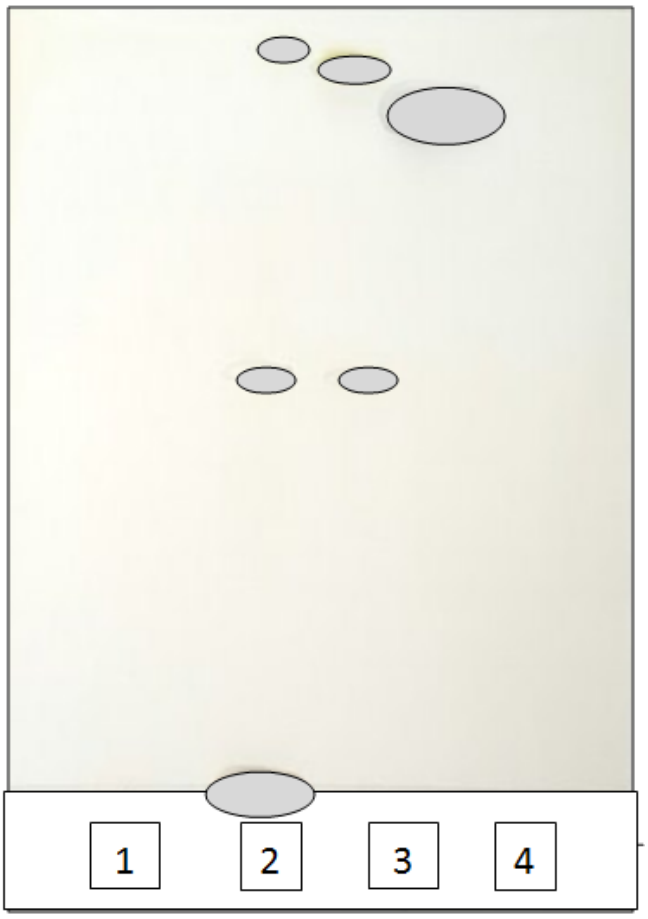

Figure 4 Scheme of the TLC chromatogram of the analysis of thick extracts of *Alhagi kirghisorum* Schrenk
1 – obtained by extraction with purified water; 2 – obtained by extraction with 70% ethanol; 3 – a solution of rutin with quercetin (1 : 1)

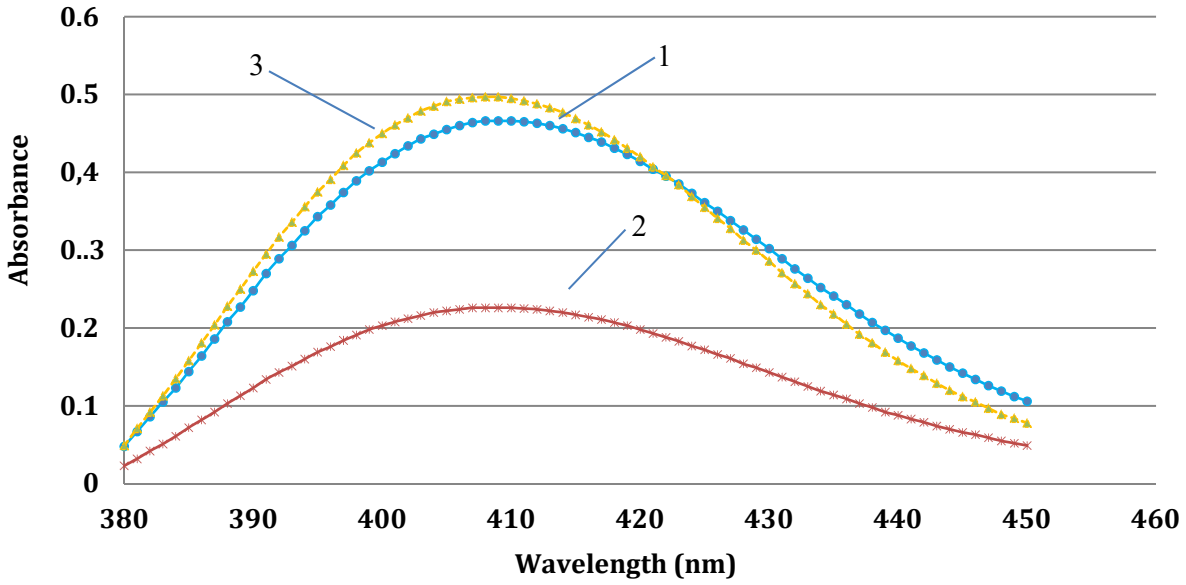

Figure 5 Absorption spectra of the sum of flavonoids in the thick extracts of *Alhagi kirghisorum* Schrenk obtained by extraction with 70% ethanol (1), purified water (2), and a standard sample of rutin (3) after reaction with aluminum chloride solution

It was found that extracts of *Alhagi* genus plants, obtained using ethanol or methanol with subsequent fractionation (*n*-hexane, chloroform, ethyl acetate, n-butanol) have antibacterial properties (Bakht et al., 2014; Ahmad et al., 2015). According to Orynkul et al. (2016) studies of antibacterial activity of the composition of camel thorn water extract with biopolymers polyhexamethylene guanidine hydrochloride (metacide) and β-C1 have shown fungicidal activity against crops pathogen *Puccinia recondita*.

Therefore, the next step was to investigate the antimicrobial properties of the thick extract of *Alhagi kirghisorum* obtained by extraction with 70% ethanol. The research results are given in Table 2.

The results obtained indicate that the test samples of thick extract of *Alhagi kirghisorum* compared to the alcohol solution of chlorophyllipt 10 mg.mL^{-1} have a moderate (culture of *Staphylococcus aureus* (growth retardation zone diameters 21.2 ±0.6 mm and 20.6 ±0.5 mm, respectively) and more pronounced (culture of *Bacillus subtilis* – 20.0 ±0.6 mm and 13.6 ±0.5 mm) antimicrobial activity; relative to the gram-negative bacterium *Escherichia coli* the activity of the thick extract of camelthorn amounted to 21.6 ±0.5 mm, while the alcohol solution of chlorophyllipt showed no activity relative to this microorganism.

The data of studying the antimicrobial activity of *Alhagi kirghisorum* thick extract obtained by extracting with 70% of ethanol, indicate the prospect of the elaboration of herbal medicinal products for the treatment of infectious diseases of the oral cavity or wounds, for the treatment of human diseases caused by bacterial infection.

Table 2 Antimicrobial activity of the test samples

Samples	Test cultures of microorganisms			
	S. aureus	*B. subtilis*	*E. coli*	*C. ablicans*
	diameters of the microbial growth inhibition zones (mm)			
Thick camelthorn extract	21.2 ±0.7	20.0 ±0.6	21.6 ±0.5	–
Ethyl alcohol (control)	–	–	–	–
Alcohol solution of chlorophyllipt 10 mg.mL^{-1} (reference drug)	20.6 ±0.5	13.6 ±0.5	–	–

Note: "–" – no zone of microbial growth inhibition.

Conclusions

Thus, the effect of the extractant nature on the yield of biologically active substances from the medicinal plant raw material at each step of extraction was studied. The dynamics of the extraction process were studied and the amount of the extractant required for the complete depletion of the raw material during the extraction process was set as 1 : 5 (raw material to the extractant). The use of 70% ethanol as an extractant was experimentally justified. The reaction on flavonoids confirmed their presence. The microbiological studies indicated that the test samples of thick extract of *Alhagi kirghisorum* compared to the alcohol solution of chlorophyllipt 10 $mg.mL^{-1}$ had a moderate activity against *Staphylococcus aureus* and more pronounced activity against *Bacillus subtilis*. The thick extract of camelthorn was active against *Escherichia coli* while ethanolic solution of chlorophyllipt was not active.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Genetic diversity of selected *Gladiolus* (*Gladiolus* × *gandavensis* Van Houtte) cultivars assesed by microRNA-based markers

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Gladiolus as economically important flowering plants reflect the wide range of morphological variability of the inflorescences. The aim of current gladiolus breeders is to create interesting new genotypes by different breeding techniques, carrying a variety of colour, size, texture and shapes of flowers and inflorescences. Despite the well-established morphological characterization of gladiolus germplasm, genomic screening by functional markers can provide useful information for breeders. The miRNA-based assay was conducted on 9 gladiolus (*Gladiolus* × *gandavensis* Van Houtte) hybrids by 9 individual markers of following families: miR156, miR160, miR398, miR408, miRr414 and miR482. A total of 291 loci were amplified, of which loci of marker miR408 were the most abundant (25%), with the following representation of other marker types: 21% – miR414; 19% miR156; 16% – miR160; 12% – miR482 and 7% – miR398. Genetic diversity of selected gladiolus cultivars was assessed by two markers, lus-miR408 and hyp-miR414, which provided genotype-specific marker profiling in the form of molecular fingerprinting. Stress-sensitive marker miR398 specifically amplified loci in genotype Správna Susane, which is the most susceptible to water deficit of all analysed genotypes. Marker miR141 was able to distinguish cultivars Athos and Dandy among themselves, but also from other cultivars.

Keywords: DNA fingerprinting, microRNA markers, *Gladiolus* × *gandavensis*

Introduction

Gladiolus is bulbous flowering plant, economically important, cultivated throughout the world for its colourful spikes. The genus *Gladiolus* L. consists of approximately 265 species and is one of the most widespread genera of the family Iridaceae Juss. The center of diversity for the genus *Gladiolus* is considered the Cape of Good Hope, located in the Republic of South Africa. It is widespread throughout the region of tropical Africa, Madagascar, Europe, the Mediterranean, the Arabian Peninsula, Asia, including Afghanistan and Iran (Kumar et al., 2016; Singh et al., 2016). The most widespread is the hybrid gladiolus *Gladiolus* × *gandavensis* Van Houtte). *Gladiolus* varieties exhibit a huge rang of variability in colour, size, texture and shape of flowers, growth habit and flowering behaviour (Singh et al., 2017).

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The aim of the current gladiolus breeders is to create, by different breeding techniques, interesting new genotypes bearing a variety of colours, sizes, textures, as well as a variety of sizes and shapes of flowers (Chaudhary et al., 2018). *Gladiolus* are alien-pollinating species showing diverse pollination mechanisms (Singh et al., 2018). Polyploidy and hybridization have played a significant part in evolution of gladioles. The genus has a basic chromosome set of 15 (n = 15), but overall, the species range from diploids (2n = 30) to hypododecaploid (2n = 180) (Chaudhary et al., 2018; Singh et al., 2018). *Gladiolus* have the smallest genome size of a number of ornamental species of bulbous and tuberous plants. Their genome reaches the size of 1100 Mbp (mega base pairs) for the haploid genome (n).

Gladiolus have a high coefficient, both vegetative using coral – brut, (capable of producing over 500 corals during one vegetative period) and generative reproduction (capable of producing over 500 seeds from a single plant during vegetation) (Pathania and Misra, 2003). Most of the commercial varieties of gladiolus over the past decades have been created using the hybrid crossing technique. Although the mutation through physical or chemical mutagens has been an effective means used to obtain new varieties. The aim of the induced mutagenesis was to create new commercially interesting varieties with fragrance, more complex colouring of petals, full bloom, distinctive spotted drawing of floral petals, more pronounced border at the edges of petals and veining of the leaves (Pathania and Misra, 2003; Kasumi, 2005).

Although there are not many theoretical foundations regarding the genetic characteristics of gladiolus, genomic analyses of this species could be useful to breeders. Molecular markers are an important tool for the analysis of plant genomes and genetic variability.

The most applied molecular markers used for the evaluation of genetic diversity and population structure of gladioulus are ISSR (Inter Simple Sequence Repeats), DAMD (Directed Amplification of Minisatellites) and RAPD-derived SCARS (Random Amplified Polymorphic DNA-derived Sequence-Characterised Amplified Region) markers (Kumar et al., 2016; Singh et al., 2016, 2017a, 2017b, 2018; Chaudhary et al., 2018).

RNA Based Markers – RBMs represent functional type of molecular markers used in genetic technologies (Bežo et al., 2015). An ideal molecular marker is highly polymorphic, occurring in different forms that are different from each other (Gálová et al., 2018). Molecular markers developed based on miRNA molecules represent a new, low cost, protocol transferable, reproducible, stable, and highly efficient process of genotyping and assessing the genetic potential of plants (Fu et al., 2013; Mondal and Ganie, 2014; Yadav et al., 2014). MicroRNAs are 21 to 24 nucleotide long RNA sequences derived from single-stranded RNA precursors that can form intra-molecular complementary hairpin structures. These molecules have significant regulatory potential of gene expression at the genetic and epigenetic level. They play a key role in plant genome response to (a)biotic stress factors. Especially, deeply conserved miRNA families are integral components of developmental processes in plant organism (Xie et al., 2010; Bej and Basak, 2014).

The objectives of this study were to verify the applicability and species transferability of miRNA-based markers and to identify genotype-specific polymorphism profiles for genetic diversity characterization of selected gladiolus genotypes.

Material and methodology

Biological material

Nine cultivars of hybrids gladiolus (*Gladiolus* × *gandavensis* Van Houtte) of interesting flower colours and shapes were analysed (Figure 1). All cultivars, except ‚Dandy' which comes from locality Kotešová (Slovak Republic), come from the town district Levice, locality Géňa (Slovak Republic). Detailed cultivars characterization is described in Table 1 and 2.

Genomic DNA was isolated from leaves (pooled sample of 5 randomly collected plants in the stage of flowering) by CTAB extraction procedure (Rogers and Bendich, 1994) and quantify by nanophotometer P360 (Implen). After spectrophotometric quantification was DNA diluted to 100 ng.μL^{-1}. The sequences of microRNA-based primers are shown in Table 3. The miRNA-based assay including results analyses was conducted based on Ražná et al. (2015).

Figure 1 Flowers morphological diversity of *Gladiolus* × *gandavensis* Van Houtte cultivars
A – Dandy; B – Falling Snow; C – Athos; D – Petra; E – Pulchritude; F – Fidorka; G – Správna Susane; H – Rajathos; I – Ráj Srdce. (Photo by M. Majtán)

Table 1 Origin characterization of gladiolus genotypes

Code	Cultivar	Introduction	Breeder	Pedigree
445	Správna Susan	2005	Belička	Gay Festival × seedling
341	Rajathos	2011	Belička	Raj Srdce × Athos
373	Pulchritude	1992	Klutey	Regency × Sabre × Apollo × Powder Puff
500	Falling Snow	1992	Mackenzie	Cliffs Of Dover × Incomparable
327	Petra	2001	Šaran	Elen × Red Alert
401	Ráj Srdce	2006	Mimránek	Pulchritude × Cream De Mint
401	Athos	1985	Hajduček	Darienka × Orient × Shell Pink
377	Dandy	2010	Rýpar	Regency × Sestra Štěst
471	Fidorka	2008	Belička	Super High Brow × seedling

Notes: Description provided by the breeder Majtán (2022)
code – code of North American Gladiolus Council

Table 2 Morphological description of flowers of gladiolus cultivars

Cultivars	Description
Správna Susan	base colour – medium salmon pink, dark red eye with attractive yellow frosted border; attractive unusual flower colouring, contrast of colour and petal shape – star-shaped flowers; flower diameter 11–14 cm, large-flowered, weak average flower set averaging 16 buds; medium maturity, 80–84 days; medium shrivelling; medium waxiness; unique cultivar due to the colour and shape of the flowers, but most susceptible of all to water stress – ear wilt
Rajathos	base colour – salmon pink, distinctive red-orange tongues with white edging; flower diameter 9.0–11.4 cm – medium flower size; medium maturity, 80–84 days; medium shrivelled with a star-shaped hint; strong waxiness; exceptional cultivar, regularly wins exhibitions in Slovakia, beautiful double ear row, average flower set of 26 flowers (can produce 34 flowers), one of the best flower sets ever – can have up to 12 flowers in bloom at a time, i.e. once as many as usual
Pulchritude	base colour – light lavender-olive; Dark olivaceous tongues on the lower three petals, only a subtle hint on the upper three; Flower diameter 9.0–11.4 cm – medium flower size; medium early, 75–79 days; weak shrinkage; medium waxiness; genotype bearing exceptional show parameters, excellent ear structure, average flower set of 22–26 buds, regular double row of flowers in the tall ear, in history often used for breeding new genotypes
Falling Snow	basic colour – white; flower diameter 9.0–11.4 cm – medium flower size; medium maturity, 80–84 days; medium shrivelling; medium waxiness; classic white cultivar, bearing medium characteristics, average flower set of 17 buds; mainly used for market purposes
Petra	basic colour – red; attractive unusual shading of flowers; flower diameter 9.0–11.4 cm – medium flower size; medium late, 85–90 days; strong shrinkage; strong waxiness; genotype with exceptional exhibition parameters, excellent ear structure, average flower set of 22–26 buds, often used in breeding because of its excellent exhibition parameters, regular arrangement of flowers in the ear, massive ear resistant to transport
Ráj Srdce	basic colour – white with a distinctive orange-red eye; unusual flower colouring, colour contrast; flower diameter 11–14 cm, large-flowered; medium-late, 85–90 days; medium shrivelling; high waxiness; cultivar used in breeding for its solid and multiple inflorescences (22–24 buds on average)
Athos	basic colour – white; orange-red strong colouring of the lower 3 petals; flower diameter 11–14 cm – large-flowered; medium early cultivar flowers in 80–84 days; medium shrivelling; strong waxiness; cultivar used in breeding for its interesting colour – distinctive orange-red colour of the petal tongues; weaker flower set with an average of 18 buds
Dandy	basic colour – lavender-purple to purple; flower colour – a combination of dark purple and creamy yellow; flower diameter 9.0–11.4 cm – medium flower size; medium late, 85–90 days; medium shrivelling; high waxiness; cultivar with exceptional show parameters, excellent ear structure, average flower set of 24 buds, not susceptible to transport due to its high wax content, good marketability
Fidorka	base colour – pale lavender-lavender; unusual flower colour, distinctive lilac tongue with vanilla edging; flower diameter 11–14 cm, large-flowered; medium maturity, 80–84 days; strong shrinkage with star-shaped petals; medium waxiness; genotype bearing above average show parameters, spikelet structure is more susceptible to irrigation conditions, if not regular may cause inflorescence curvature; average flower set of 22 buds

Note: Description provided by the breeder Majtán (2022)

Table 3 Sequences of applied miRNA-based markers

Primer	Sequences 5'–3'	Sequence origin
cca_miR156 – forward	TGA CAG AAG AGA GTG AGC AC	*Cynara cardunculus* L.
cca_miR156_reverse	GTG CTC ACT CTC TTC TGT CA	
ghr_miR156 – forward	AGG GAG GTG ACA GAA GAG AGT	*Gossypium hirsutum* L.
ghr_miR156_reverse	TGA GCA CGC AGA GCT TCA A	
hyp_miR156 – forward	TTG AGA GGG AGA GGG AAT TT	*Hypericum perforatum* L
hyp_miR156_reverse	TTG AAG GTG ATG ACA GAA GC	
ghr_miR160 – forward	TGG CTC CCT GTA TGC CAT TT	*Gossypium hirsutum* L.
ghr_miR160_reverse	TGG CTC CTC ATA CGC CAT TC	
mdo_miR160 – forward	TGC CTG GCT CCC TGT ATG CCA	*Malus domestica* Borkh.
mdo_miR160_reverse	TGG CAT ACA GGG AGC CAG GCA	
mdo_miR398 – forward	TGT GTT CTC AGG TCA GGG GTT	
mdo_miR398_reverse	AAC CCC TGA CCT GAG AAC ACA	
lus_miR408 – forward	GGC TGG GAA CAG ACA GAG CAT GGA	*Linum usitatissimum* L.
lus_miR408_reverse	GGG AAA AAG GCC AGG GAA GAG	
hyp_miR414 – forward	AGA GTA CAG GGA AAT GGA GGA	*Hypericum perforatum* L.,
hyp_miR414_reverse	CAC AGC GAA ACC CAC GAG	
gb_miR482 – forward	TGG GTT GTA GTC TTC AGG AGT GGG	*Ginkgo biloba* L.,
gb_miR482_reverse	GAA GGC AAT AGG AAT GGG AGG ATC	

Results and discussion

Phenotypic variability among the *Gladiolus* cultivars is extensive and is caused by genetic, environmental, and physiological factors (Singh et al., 2017b). Due to high conservation of miRNA sequences was developed an effective type of molecular markers useful not only for genetic diversity studies but also for functional polymorphism analysis. The fundamental potential of miRNA-based markers relies on the primers design based on the mature miRNAs sequences as a part of their step-loop structure. The advantages of this marker system include reproducibility and transferability across species (Fu et al., 2013). The high level of transferability demonstrates the usability of miRNA-based markers for comparative genome mapping and phylogenetic studies (Yadav et al., 2014).

Due to the unavailability of sequences of a given species or family, we used different types of miRNA-based markers whose amplification efficiency and species transferability have been verified in our previous studies. As these markers are derived from conserved miRNA sequences, a high degree of universality between genera is expected (Yadav et al., 2014). Here we applied 9 types of miRNA-base markers derived from sequences of conservative microRNA families (miR156, miR160, miR398, miR408, miRr414 and miR482) of following species: *Cynara cardunculus* L., *Ginkgo biloba* L., *Gossypium hirsutum* L., *Hypericum perforatum* L., *Linum usitatissimum* L. and *Malus domestica* Borkh.

Out of nine miRNA-based markers, only marker hyp-miR156, was not amplified and two markers, ghr-miR156 and ghr-miR160 amplified one monomorphic DNA fragment. Due to the standardization of DNA quantity and amplification conditions, different intensities of amplified miRNA locus can reflect the developmentally specific activity of these two types of markers (Figure 2). The family miR156 is one of the most stable and highly expressed miRNA families in plants (Xie et al., 2012), participating in regulation of plant growth and development and flowering time (Rubio-Somoza and Weigel, 2011). Stonger amplification of ghr-miR156 loci was observed in genotypes Athos, Pulchritude, Rajathos and Ráj Srdce. In terms of ancestry, these genotypes are strongly linked (Table 1). MiR160 family participates in meristematic tissue formation, differentiation, cell division and hormonal control (Vionnet, 2009). They also play roles in plant responses to diverse environmental factors (Shen et al., 2015). These miRNA-based loci did not show the same level of amplification as the previous ones, which may be related to the developmental phase of growth, as genotypes were sampled at the time of flowering, where differentiation and elongation were

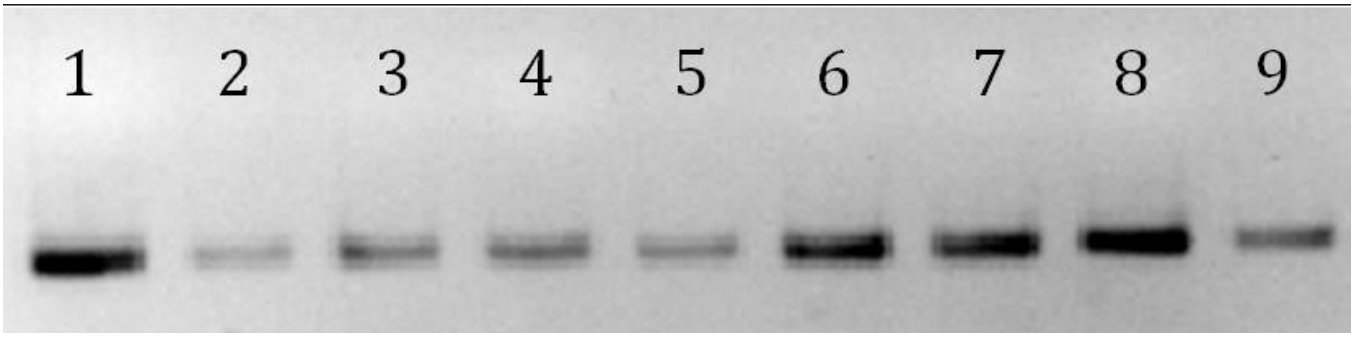

Figure 2 Amplification of ghr-miR156 locus in tested gladiolus cultivars
1 – Athos; 2 – Dandy; 3 – Falling Snow; 4 – Fidorka; 5 – Petra; 6 – Pulchritude; 7 – Rajathos; 8 – Ráj Srdce; 9 – Správna Suzanne

finalized. The role of miR156 in plant growth processes has also been confirmed by Hlavačková et al. (2016), where the proportion of the miR156 loci gradually increased depending on the stage development of the flax plants.

Monomorphic miRNA loci were amplified also by marker, mdo-miR398, where polymorphism was observed only in genotype Správna Suzane (picture not shown). This is a unique genotype due to the colour and shape of the flowers, but it is the most susceptible to water deficit, manifested by the conduct of the flower spikes, of all analysed cultivars. MiRNA398 is considered as stress-responsive miRNA involved in plant stress regulation mechanism (Zhu et al., 2011) and has been reported to be associated with various stress conditions as oxidative stress (Sunkar et al., 2006), water deficit (Trindade et al., 2010), salt stress and abscisic acid stress (Jia et al., 2009). Therefore, observed miR398 polymorphism in this gladiolus genotype may reflect the genome susceptibility to this abiotic stress.

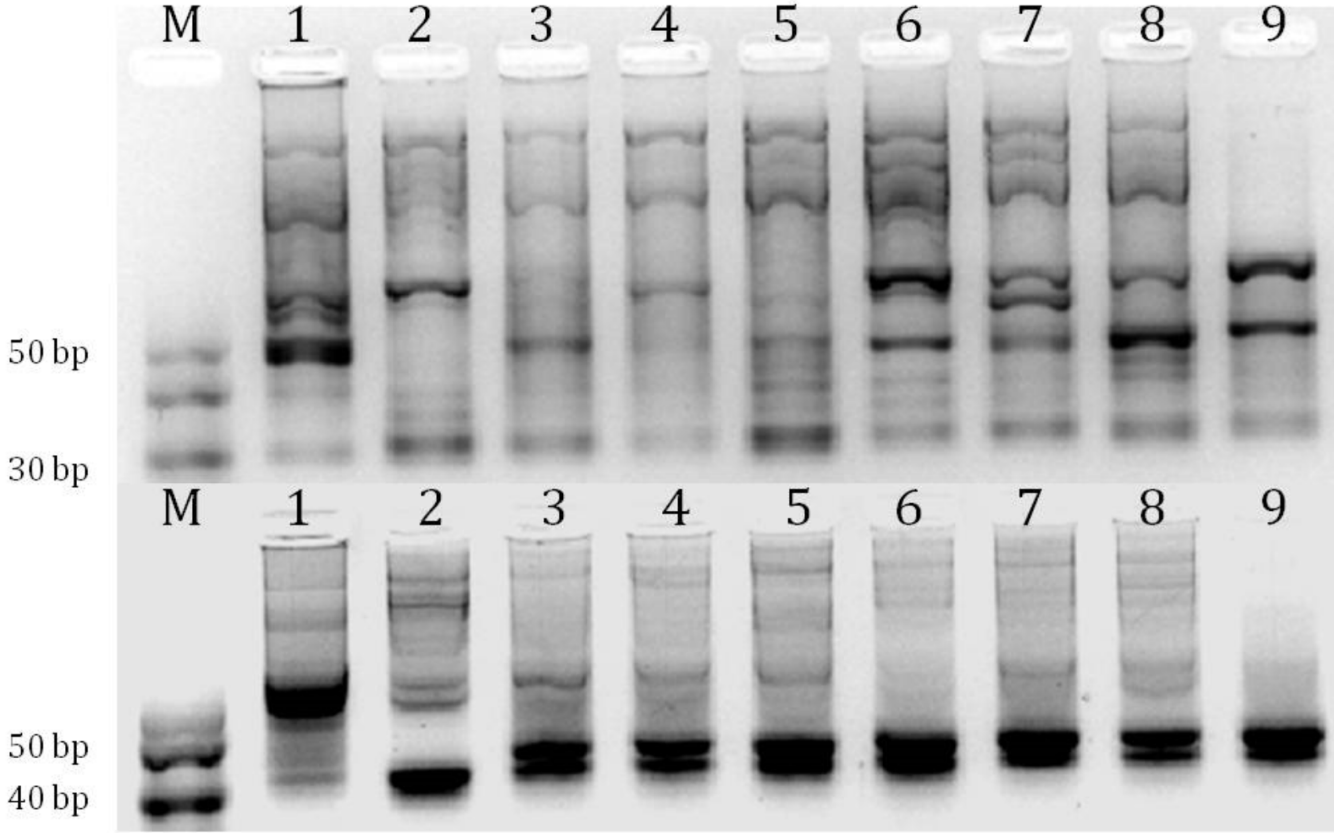

Figure 3 Amplification profile of lus-miR408 (upper) and hyp414 (lower) loci in tested gladiolus cultivars
1 – Athos; 2 – Dandy; 3 – Falling Snow; 4 – Fidorka; 5 – Petra; 6 – Pulchritude; 7 – Rajathos; 8 – Ráj Srdce; 9 – Správna Suzanne

In terms of amplification activity, we can sort the markers in descending order as follows, lus-miR408 (72 miRNA-based loci), hyp-miR414 (62 miRNA-based loci), cca-miR156 (54 miRNA-based loci), mdo-miR160 (47 miRNA-based loci), gb-miR482 (36 miRNA-based loci) and mdo-miR398 (20 miRNA-based loci).

Genotype-specific amplification profiles were observed by markers lus-miR408 and hyp-miR414 (Figure 3). These markers provided the highest number of amplified loci, with the average number of loci per genotype, 8 (lus-miR408) or 7 (hyp-miR414) respectively. Based on miRNA-based DNA fingerprinting is possible to distinguished individual genotypes profiles (Figure 4). Activity of miR408 underlies higher tolerance to salinity, cold, oxidative stress, drought, and osmotic stress (Ma et al., 2015). Constitutive expression of miR408 affects various stages of development and promotes intense plant growth and seed yield by increasing the efficiency of photosynthesis. Therefore, miR408 is likely to have a pleiotropic effect on plant growth and development (Pan et al., 2018).

Family miR414 play an essential function in plant growth, development, physiological and morphological changes, metabolism, and plant defense responses (Guleria and Yadav, 2011). In the genome of most analysed gladiolus cultivars (Falling Snow, Fidorka, Petra, Pulchritude, Rajathos, Ráj Srdce and Správna Susane) were amplified among others, two distinctive loci within the size interval from 40 bp up to 50 bp (Figure 3). In two genotypes were these loci amplified out of this size range, in genotype Anthos was the locus length approximately 70 bp and in genotype Dandy 40 bp.

The loci of marker cca-miR156 were amplified but without the observed polymorphism (picture not shown), so it is not a suitable marker for gladioulus diversity recording purposes. In the cultivar Fallin Snow, was not recorded the gb-miR482 loci amplification even after repeated amplification. MiR482 is involved in defence's mechanism of plants genome (Wang et al., 2015). Additional analyses would be needed to clarify the reasons for the absence of this locus; at this stage, the explanation would be speculative.

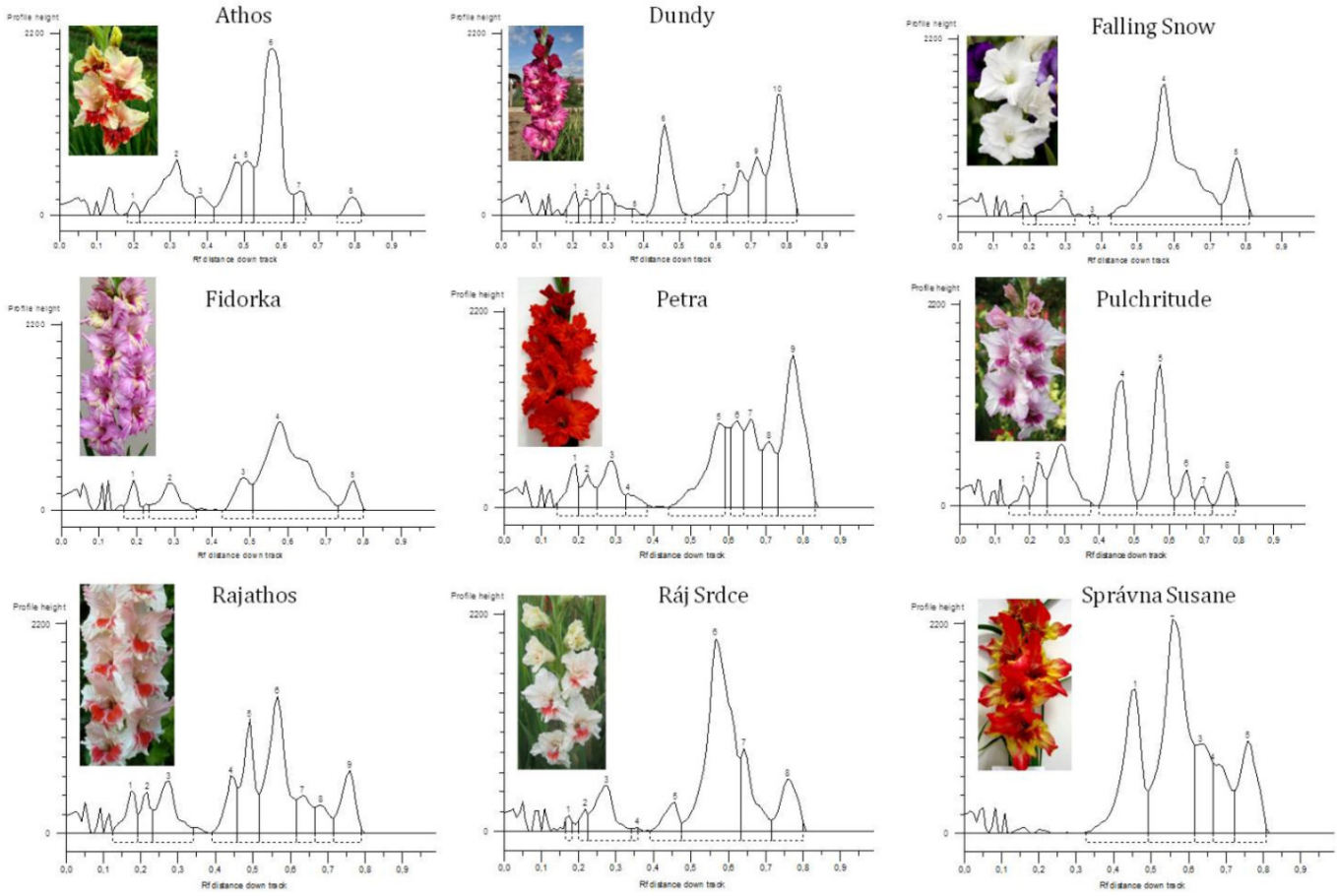

Figure 4 Elecrophoretic miRNA loci profile of lus-miR408 in tested gladiolus cultivars
1 – Athos; 2 – Dandy; 3 – Falling Snow; 4 – Fidorka; 5 – Petra; 6 – Pulchritude; 7 – Rajathos; 8 – Ráj Srdce; 9 – Správna Suzanne
(Photo of gladiolus by M. Majtán)

The evaluation of diversity in gladiolus genotypes based on phenotypic variability has its well-established platform (Momin et al., 2017; Patil et al., 2017; Ramzan et al., 2016) and irreplaceable place given the economic importance of this species. The purpose of several genetic studies was to characterize and identify the genetic diversity of the *Gladiolus* species by morphological and physiological markers (Singh et al., 2017a), minisatellite markers (Singh et al., 2017a; Singh et al., 2018) ISSR (Kumar et al., 2016; Chaudhary et al., 2018; Singh et al., 2017b; Singh et al., 2018), RAPD (Pragya et al., 2010), RAPD-derived SCAR markers (Singh et al., 2017b) and AFLP fingerprinting (Kutlunina et al., 2017). Singh et al. (2017) conducted a study focused on the analysis of the nucleotide diversity of gladiol, phylogeny of varieties and molecular systematic of the family Iridaceae using chloroplast DNA (cpDNA) regions.

One approach to assessing genetic potential is not only the application of DNA markers, but also the search for new types of functional molecular markers. Molecular markers based on miRNA molecules represent such type of functional markers (Fu et al., 2013; Mondal and Ganie, 2014; Yadav et al., 2014). Functional markers applied in this study correspond to miRNA sequences, particularly to precursor stem-loop regions, which are relatively highly conserved within closely related species (Fu et al., 2013; Yadav et al., 2014). The specificity of the molecular miRNA markers and reproducibility are improved by using higher annealing temperatures (primer binding), above 55 °C, during amplification in the "touchdown" PCR reaction.

For the genetic diversity analysis of gladiolus genotypes (*Gladiolus* × *gandavensis*), we applied markers which microRNA sequences are integrated in regulation of different processes in plants. Molecule's miRNAs are responsible for regulation of several developmental processes, including leaf morphology and plant polarity, root formation, processes of transition from embryogenic phase into vegetative, flowering time, formation of flower organs and reproduction and defense mechanisms (Xie et al., 2010; Cuperus et al., 2011; Chen et al., 2013; Hong and Jakson, 2015). Among others, the abundance of mature miRNAs, linked to the expression of MIRNA genes, varies greatly depending on miRNA family, tissue types or developmental stages.

Conclusions

Using molecular markers for genomic characterization of gladiolus cultivars provides a tool for selection of elite genetic resources for breeding process. Our study verified the suitability and species transferability of miRNA-based markers for genetic diversity characterization of selected gladiolus genotypes and identify genotype-specific functional markers originated from sequences of regulating microRNA molecules. Besides that, the application of functional miRNA-based markers will contribute to the deepening of knowledge about genomic polymorphism background of gladiolus.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Influence of application forms of alginite on phytomass formation, bioactive contents and antioxidant activity of extracts from plants *Rosmarinus officinalis* L.

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In this study, we compared the effect of soil-climatic factors and application forms of alginite on the weight of vegetable drug, total content of polyphenols, flavonoids, phenolic acids and antioxidant activity of extracts from *Rosmarinus officinalis* L. The experiment was based on a site in Tornal (SR), in 2020. The plot was divided into three equal (1 m^2) parts (variants), on which 9 plants were planted and alginite was applied in two forms. On variant (V_1) alginite was incorporated in powder form, (V_2) alginite in the form of a top dressing (1 : 10) and control (V_K) without application. When the effect of alginite application on plant weight was evaluated, the highest increase (508.8 g) was observed in variant V_1, followed by V_2 (469.6 g) and the lowest value (251.2 g) in V_K. The highest total amount of flavonoids expressed as quercetin equivalent (QE) was determined in acetone extracts of *Rosmarinus officinalis* samples in variant V_1 (0.3225 mg QE.100 g^{-1} DW). In the extract of the drug from variant V_2 we determined the total amount of flavonoids to be 0.2922 mg QE.100 g^{-1} DW and in the control V_K – 0.2235 mg QE.100 g^{-1} DW. In the aqueous extracts of *Rosmarinus officinalis* samples, we determined the highest total polyphenol content converted to caffeic acid equivalent (CAE) per 100 g dry drug in variant V_1 (27.69 mg CAE.100 g^{-1} DW), followed by variant V_2 (26.13 mg CAE.100 g^{-1} DW) and in the control V_K it was 16.42 mg CAE.100 g^{-1} DW. In the ethanolic extracts of *Rosmarinus officinalis* samples, we determined the total phenolic acid content converted to caffeic acid equivalent (CAE) per 100 g of dried drug in variant V_1 (34.61 mg CAE.100 g^{-1} DW) >V_2 (33.41 mg CAE.100 g^{-1} DW) >V_K (29.32 mg CAE.100 g^{-1} DW). The antioxidant activity of methanol extracts of samples from variants V_1, V_2, V_K was expressed as % inhibition of DPPH radical. The highest percentage of inhibition (37.76%) was observed in the drug methanol extract V_1, followed by variant V_2 (30.77%) and control V_K (15.38%). The results showed that alginite as compared to control significantly affects the growth, weight of *Rosmarinus officinalis* and increases the content of total polyphenols, flavonoids, phenolic acids and antioxidant activity, which is crucial in terms of production of medicinal plants as well as their pharmaceutical uses.

Keywords: *Rosmarinus officinalis*, rosmarinic acid, alginite, antioxidant activity

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Introduction

Rosmarinus officinalis L. belongs to the order family Lamiaceae Martinov (URL1, 2022). The Lamiaceae are represented by species that have essential oils (aetheroleum) stored in the essential oil cells, secretory ducts, glandular trichomes, representing a mixture of heterogeneous, lipophilic, easily volatilized substances with a distinct fragrance (Košťálová et al., 2012; Suchý et al., 2013; Petruzzello, 2021). Essential oils have antibacterial, anti-inflammatory, carminative, antispasmodic, hyperacidifying, antiviral, antitussive, diuretic, sedative, granulating and deodorizing effects (Košťálová et al., 2012). Rosmarinic acid is antiviral, antibacterial, antifungal, and anti-inflammatory (Petersen and Simmonds, 2003). It eliminates inflammation in osteoarthritis, pancreatitis, hepatitis, colitis, atopic dermatitis, asthma, and other inflammatory diseases (Chunxu et. al., 2020). It has nociceptive, neuroprotective and neuroregenerative effects on the CNS (Ghasemzadeh and Rahbardar, 2020). It inhibits the aggregation of β-amyloid plaques and reduces the amount of reactive oxygen species, which may be used in the treatment of Alzheimer's disease (Mahboubi, 2019).

Rosmarinus officinalis requires stony soils, slopes, sunny, dry positions, is sensitive to frost and does not overwinter in the climatic conditions of the Slovak Republic (Bednářová, 2017). Medicinal plants for drug production and pharmaceutical use must have optimal conditions for growth and development to exploit their genetic potential. They must have optimum climatic, soil conditions, optimum doses of inorganic, organic fertilizers, organic-mineral substance (alginite). Alginite is an algal rock, formed by the process of decomposition of plants, has the properties of soil sorbent (water, heavy metals – Si) but is a suitable alternative to organic fertilizers (Kúšik et al., 2017, Brindza et al., 2021a; Elisovetcaia et al., 2021; Kovár et al., 2021). Natural organic fertilizers include alginite, which contains algae. Clay, humus (40%), mineral nutrients (aragonite, dolomite, smectite, quartz, siderite, and others). Smectite can retain water and provides long-term hydration. Of the biogenic elements, Ca and Mg are the most abundant, with less N, Fe, Zn, Cu, Mn, Ti, Cr, Li, Co, Ni. It does not contain any chemical additives and phytotoxins (Kulich-Valko-Obernauer, 2001; Brindza et al., 2021b).

The aim of the experiment was to evaluate the effect of soil-climatic factors and alginite application on the yield and content of *Rosmarinus officinalis* bioactive substances involved in antioxidant effects.

Material and methodology

Samples of the drug *Rosmarinus officinalis* folium were used in the experiment. Plants were planted on 20. 5. 2020 on the experimental plot in Tornaľa – part of Stárňa, region Gemer, SR. Brown soils are typical for the area (Tolmáči and Gajdoš, 2011). The soil is mechanically treated before planting to create suitable conditions for plant rooting. The area was not fertilized with organic or inorganic fertilizers in order not to affect the metabolism and synthesis of plant contents. The plot was divided into three equal 1 m^2 sections (variants), on which 9 plants were planted and alginite was applied in two forms. The first variant (V_1) – plants with incorporation of powdered form of alginite 1 $kg.m^{-2}$, the second variant (V_2) – plants with alginite in the form of 1 : 10 drench, the third variant (V_K) = control variant – plants without alginite application. The irrigation was in the form of rainwater sprinkling applied once a day in the morning hours. Harvesting of plants was divided on 27. 7. and 2. 9. 2020. The ratio of fresh sample to dried sample was 8 : 1.

Morphometric characteristics

When the aerial part of *Rosmarinus officinalis* the height of planted individuals was measured on V_1, V_2 and V_K variants. Then the above-ground part of each variant was harvested, and the mass was weighed. Leaves were then separated from the stems and their length measured. The vegetative drug from each variant was dried and then the dry weight of the drug was weighed. All the evaluated data were recorded in a table and standard deviations were calculated.

Total polyphenol content

The total polyphenol content in the aqueous plant extracts was measured using Folin-Ciocalteu reagent by the spectrophotometric method according to Singleton and Rossi (1965) modified by Suchý et al. (2013). We prepared a 250 mL aqueous extract of 0.5 g of homogenized dry drug. The extract was filtered, 5 mL of the filtrate was diluted with water to 25 mL. From the solution, we pipetted 2 mL for determination, added 1.0 mL of Foulin-Chicoulet reagent and 17.0 mL of 20% sodium carbonate Na_2CO_3 solution. We mixed the samples and after 2 min, we measured the absorbance at 750 nm against the blank. was measured using the spectrophotometer Jenway (6405 UV/Vis, England). The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. The total polyphenol content in the aqueous extracts (as caffeic acid equivalent) was calculated from the caffeic acid

calibration curve (10–100 µg caffeic acid equivalents (CAE)/L; R^2 = 0.9991) and is expressed in mg CAE.100 g^{-1} DW.

Total flavonoid content

The total contetnt of flavonoids expressed as qvercetin was determined by the spectrophotometric method with aluminium chloride according to European Pharmacopoeia 7 (04/2013:1174). The total flavonoid content expressed as quercetin was determined by the spectrophotometric method with aluminium chloride, according to European Pharmacopoeia 7 (Article 04/2013:1174). Absorbance was measured with a Jenway spectrophotometer (6405 UV/Vis, England) at a wavelength of 425 nm. The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. The total flavonoid content of the acetone extracts (as quercetin equivalent) was calculated from the quercetin calibration curve (2–100 µg.l^{-1} quercetin equivalent (QE)/L; (R^2 = 0.9997) and expressed in mg CAE.100 g^{-1} DW.

Total phenolic acids content

Total phenolic acid content in the ethanol plant extracts was carried out using a method of Farmakopea Polska (1999). Briefly, 0.5 mL of extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL of Arnova reagent, 0.5 mL of 1 M sodium hydroxide (w/v), and 0.5 mL of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. Caffeic acid was used as a standard for the calibration curve (0.01–1.0 mg caffeic acid equivalents (CAE)/L, R^2 = 0.9997) and the results are expressed in mg CAE.100 g^{-1} DW.

Antoxidant activity

The radical scavenging activity in the methanol plant extracts of the samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Ahmad et al., 2014). The extracts (0.5 mL) were mixed with 3.6 mL of DPPH medium (0.025 g of DPPH in 100 mL of ethanol). Methanol was used as blank. The absorbance of the sample extract was determined sing the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm (Varényi, Chrenová, Lukáč 2019). The absorbance was measured in triplicate and the mean value of the samples, and their standard deviation were determined. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (10–100 mg Trolox/L; R^2 = 0.9997) was used as the standard for the calibration and the results were expressed in mg Trolox equivalent antioxidant capacity (TEAC).100 g^{-1} DW.

Statistical analysis

Data were analyzed with ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$). The variability of all these parameters was evaluated using descriptive statistics.

Results and discussion

Plants of the Lamiacae chaeliad are nowadays intensively investigated and studied mainly for analgesic, antiphlogistic, antioxidant, antimicrobial, gastroprotective, hypoglycemic, anticancer, and hypolipidemic effects (Costea et al., 2020). Investigating the polyphenolic profile of natural *Rosmarinus officinalis* populations may reveal essential compounds that have biological activities (Elansary et al., 2020).

Morphometric characteristics of *Rosmarinus officinalis*

Cultivation of medicinal plants on arable land requires knowledge of the requirements of individual species for soil, climatic conditions, cultivation method, fertilization, protection with respect to the environment (Eftimová and Habán, 2012).

Effect of soil-climatic factors and application forms of alginite on plant height, leaf length as well as weight

Table 1 Comparison of the effect of applied alginite in different forms (V_1 and V_2) on the evaluated traits on plants *Rosmarinus officinalis* L. in comparison with the control variant (V_K)

Variants	Traits evaluated		
	height (cm)	leaf length (cm)	total dry weight of the drug (g)
V_K	52.4 ±0.34^b	1.8 ±0.18^b	95.6 ±0.42^c
V_1	63.4 ±0.33^a	2.3 ±0.16^a	182.4 ±0.81^a
V_2	57.8 ±0.21^b	2.1 ±0.16^a	162.8 ±0.78^b

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

of the plant drug *Rosmarinus officinalis* is shown in Table 1.

Table 1 shows that the best growth of *Rosmarinus officinalis* was recorded in V_1. The height of the plants was 63.4 cm, and the leaf length was 2.3 cm. The total dry weight of all two harvests was 182.4 g. In V_2, plant height was 57.8 cm, leaf length 2.1 cm and total dry weight 162.8 g. In V_K, plant height was 52.4 cm, leaf length 1.8 cm and total dry weight 95.6 g. In V_K, plant height was 52.4 cm, leaf length 1.8 cm and total dry weight 95.6 g.

Total polyphenol content

Among the constituents present in *Rosmarinus officinalis* are essential oils (1,8-cineole, α-pinene, camphor, borneol, bornyl acetate, camphene, α-terpinol and (+)-verbenone), diterpenes (carnosol, carnosic acid, rosin, rosadiol, rosadiol), triterpenes (betulin, betulinic acid, ursolic acid, oleanoic acid), flavonoids (diosmin, hesperidin, luteolin glycosides, cirsimaritin), and cinnamic acid derivatives with rosmarinic acid being the most prominent (Nagy, Mučaji, and Grančai 2017). Major polyphenols confirmed in *Rosmarinus officinalis* leaf extracts, are flavonoids: apigenin, luteolin, nepetin, nepitrin and phenolic acids: rosmarinic acid (c.a. 23%), chlorogenic acid, caffeic acid, and other organic acids like: ursolicacid, betulinic acid, carnosic acid, and carnosol (Begum et al., 2013). Polyphenols as secondary metabolites contain one or more hydroxyl groups bound to one or more aromatic rings (Stagos, 2020). Bourhia et al. (2019) confirmed that polyphenols, carnosol and carnosic acid are responsible for the antioxidant activity of ethanolic extracts of *R. officinalis* leaves from Morocco. The contents of total polyphenols in the aqueous extract of the *Rosmarinus officinalis* treated by the differet aplication of alginite are presented in Table 2.

After fitting the measured absorbance values of the samples to the calibration equation, we calculated the total polyphenol content as caffeic acid equivalent. Table 2 shows that the highest polyphenol content was observed in V_1 (27.69 mg CAE.100 g^{-1} DW). Slightly lower values were exhibited by V_2 (26.13 mg CAE.100 g^{-1} DW). And the lowest values were recorded in V_K (16.42 mg CAE.100 g^{-1} DW).

Total flavonoid content

Flavonoids are among the most widely consumed phenolic compounds. Many studies refer to their strong antioxidant activity, which is manifested by their ability to destroy free radicals and thus eliminate their adverse effect on DNA damage and lipid peroxidation. The number of hydroxyl OH-groups and their position in the molecule are important for the antioxidant activity of flavonoids. One of the most studied flavonoids is quercetin, which we used as a standard in our determinations (Stratil and Kuban, 2018). Michalak et al. (2021) reported that extracts of *Rosmarinus officinalis* contain polyphenols mainly flavonoids, which are involved in antioxidant activity and recommend them in free radical scavenging.

From Table 3 showed that the highest flavonoid content reached V_1 (0.3255 mg QE.100 g^{-1} DW). The lowest flavonoid content was found in V_2 (0.2922 mg QE.100 g^{-1} DW). V_K reached the lowest flavonoid content of 0.2235 mg QE.100 g^{-1} DW. Based on the measured values of flavonoid content, we can conclude that the best effect is shown by powdered

Table 2 Content of total polyphenols in the samples of plants of *Rosmarinus officinalis* L. treated by the different application of alginite (V_1, V_2) in comparison of control variant (V_K)

Variants	Average absorbance (nm)	Total polyphenols (mg CAE.100 g^{-1} DW
V_K	0.159 ±0.00115^b	16.42 ±0.112129^c
V_1	0.275 ±0.01159^a	27.69 ±0.1125483^a
V_2	0.259 ±0.00379^a	26.13 ±0.367638^b

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

Table 3 Content of total flavonoid in the samples of plants of *Rosmarinus officinalis* L. treated by the different application of alginite (V_1, V_2) in comparison of control variant (V_K)

Variants	Average absorbance (nm)	Total flavonoid content (mg QE.100 g^{-1} DW)
V_K	0.123 ±0.0098^b	0.22 ±0.0192^b
V_1	0.175 ±0.0005^a	0.32 ±0.0011^a
V_2	0.158 ±0.0020^a	0.29 ±0.0040^b

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

alginite. This is followed by alginite in the form of top dressing. This confirms the positive effect of alginite on the growth and the amount of content in *Rosmarinus officinalis*. Citrjáková (2019) confirmed that the application of alginite increases the content of total flavonoids in *Origanum vulgare* L. Her results showed a twofold increase in flavonoid content in samples grown with alginite.

Total phenolic acids content

Phenolic acids are a group of substances derived from cinnamic acid. The best known are caffeic acid and p-coumaric acid. It also includes depsides – rosmarinic acid. It is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid. The synthesis is based on two amino acids: L-PHE and L-TYR (Blažević et al., 2021). Rosmarinic acid was the major polyphenol in both of *R. officinalis*. *R. officinalis* methanolic leaf extracts contained other phenols such as gentisic acid (Elansary et al., 2020). Many studies have confirmed that rosmarinic acid alone has potent antioxidant activities (Swisłocka et al., 2019). Gentisic acid, exhibited antioxidant activity and have as antioxidant agents in the food industry (Elsary et al., 2020).

The results of the determination of the total phenolic acid content expressed as caffeic acid equivalents showed that alginite was positive. After substituting the measured absorbance values of the samples into the calibration equation, the total phenolic acid content was determined for each sample. From Table 4, it can be seen that V_1 showed the highest content of 34.61 mg CAE.100 g^{-1} DW. V_2 showed the lowest content of 33.4 mg CAE.100 g^{-1} DW. The lowest content was observed in V_K (29.32 mg CAE.100 g^{-1} DW). Rosmarinic acid is responsible for the higher levels of phenolic acids. Rosmarinic acid encompasses a wide range of effects that are still the subject of research today. It is antiviral, antibacterial, antifungal, anti-inflammatory (Petersen and Simmonds, 2003). In the *Rosmarinus officinalis* methanolic leaf extract, the HPLC-DAD qualitative and quantitative analyses of selected phenolic compounds confirmed very high amount of rosmarinic acid (4040.00 mg.100 g^{-1} DW) (Elansary et al., 2020).

With its antioxidant effect, it shows high potential in the treatment of cancer. In the food industry, the antioxidant activity of rosemary is used as a preservative (Bednářová, 2017). Phenolic acids inhibit ACE, thereby exerting an antihypertensive effect. Caffeic acid contributes to this effect by inhibiting the enzyme renin. The antiphlogistic effect is provided by inhibition of COX-2, PGE-2, NF-κB and decrease in NO production. They have strong antioxidant effects, thereby scavenging free radicals. P-coumaric acid has anxiolytic effect at a dose of 30 mg.kg^{-1} similar to diazepam at a dose of 3 mg.kg^{-1} (Nagy et al., 2015).

Antoxidant activity

Clinical studies investigating the effects of free radicals and oxidative stress have confirmed the effect of these irritants in the development of age-related degenerative diseases including atherosclerosis, cancer, asthma, heart attack, stroke and others (Packer and Weber, 2001). Free radicals can be characterized as unstable, highly reactive and energy-rich molecules capable of independent existence. Peroxidative enzyme systems, inflammation in the body, smoking, lipid oxidation, and others have been implicated in their formation (Stief, 2003). Studies have confirmed that polyphenols, rosmarinic acid, hesperidin and rosmanol are responsible for the antioxidant activity of *R. officinalis* leaf extracts (Nieto et al., 2018). Elansary et al. (2020) investigated the content of total polyphenols, phenolic acids, and antioxidant activity of two natural populations of *R. officinalis* in northern Riyadh using HPLC-DAD assays. They found that higher antioxidant activity was associated with higher rosmarinic acid content in the leaf extracts. For the determination of antiradical or antioxidant activity we chose the DPPH method. The antiradical activity determined as % reduction of DPPH was determined in the methane extract of *Rosmarinus officinalis* sample presented in Figure 1.

Table 4 Content of phenolic acids in the samples of plants of *Rosmarinus officinalis* L. treated by the different applications of alginite (V_1, V_2) in comparison of control variant (V_K)

Variants	Average absorbance (nm)	Total phenolic acids content (mg CAE.100 g^{-1} DW)
V_K	0.510 ±0.001^b	29.32 ±0.066^b
V_1	0.602 ±0.033^a	34.61 ±1.892^a
V_2	0.581 ±0.010^b	33.41 ±0.607^a

Notes: different superscripts in each row indicate the significant differences in the mean at $p < 0.05$

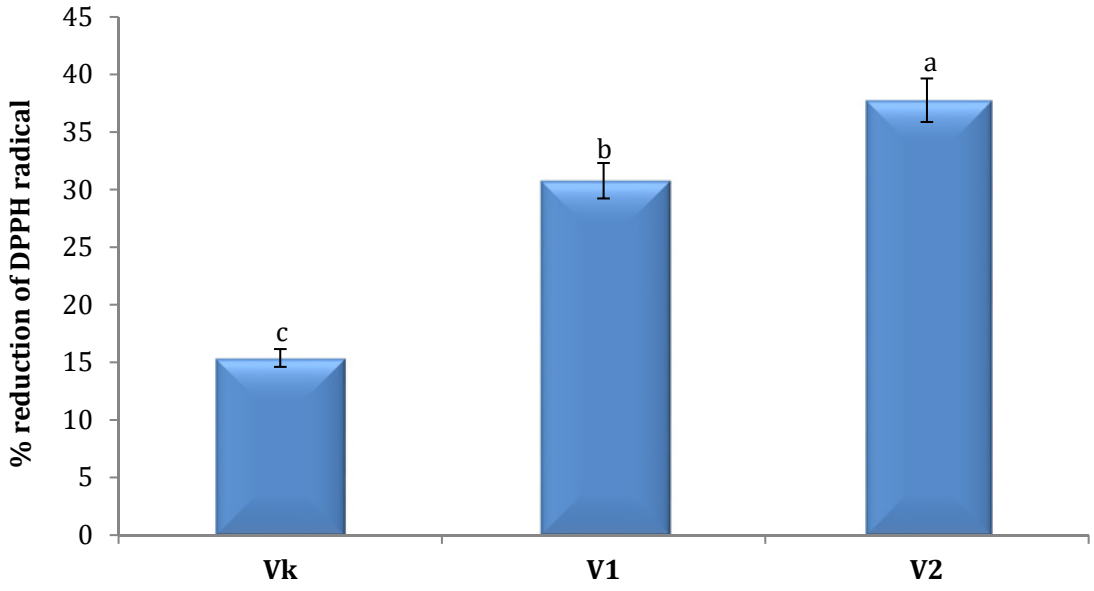

Figure 1 Comparison of the effect of applied alginite in different forms (V_1 and V_2) on antiradical activity determined as % reduction of DPPH in samples from plants of *Rosmarinus officinalis* L. in comparison with the control variant (V_K)
The different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

V_1 showed the highest antioxidant activity of 37.76 ±1.47%. In V_2 variant, 30.77 ±1.69% inhibition of DPPH radical was observed, and the lowest inhibition was exhibited by V_K (15.38 ±0.31%). The antioxidant activity for 25-fold diluted extract corresponds to 0.04 g of dry sample.

Gnipova (2021) and pointed out the beneficial effect of alginite on the antioxidant activity of *Thymus serpyllum* L. The highest antiradical activity as % reduction of DPPH radical 39.29% was determined in diluted (1 : 19) methanolic extract of the sample grown on soil treated with powdered form of fertilizer (V_3) The antiradical activity was 411.2 mg TEAC. kg^{-1} DW. The methanolic extract of diluted (1 : 19) sample grown with alginite in suspension form (V_2) showed antiradical activity of 32.86% and that of diluted (1 : 19) control without alginite (V_1) 25%. The antiradical activity converted to Trolox (TEAC) in the sample extract V_2 was 341.78 mg TEAC.kg^{-1} and in the control was 256.92 mg TEAC.kg^{-1} DW.

The results of the experiment confirmed that alginite increased the content of bioactive compounds in *Rosmarinus officinalis*, which exhibit antioxidant activity and can be used in the prevention and adjunctive therapy of oxidative stress-induced diseases. Based on the results obtained from the experiment, we can conclude that both application forms of alginite had a beneficial effect on the growth, leaf size as well as weight of the vegetative drug *Rosmarinus officinalis* compared to the control. They also positively influenced the total polyphenols, flavonoids, phenolic acids, and antiradical activity of the extracts. When *Rosmarinus officinalis* is grown, incorporation of powdered form of alginite into the soil before ploughing may be recommended as the most suitable form. Application by drench in the form of alginite dissolved in water is also suitable. Both applied forms achieved significantly higher favourable results compared to the control.

The effect of alginite on the contents contained in Lamiaceae plants was investigated by Plichta (2016), Horný (2017), Citrjáková (2019), Eftimova et al. (2021), Gnipova (2021), Horčinová Sedláčková et al. (2021) and Janovská (2021). Their results confirm our conclusions that alginite is beneficial for plants, which is manifested by an increase in aboveground mass and an increase in the contents of the plant.

Conclusion

Alginite is generally recommended for use on soils to maintain and efficiently use water and improve soil quality and fertility. Alginite as a natural bituminous rock contains more than 10% fossil organic matter, all macroelements (except nitrogen) and a significant content of microelements and other known and unknown organic components. For this reason, the application of alginite in the cultivation of individual plant species effect as a specific natural stimulator in the formation of phytomass and increases the content of some biologically active components. In our experiment, it was confirmed that the application

of alginite significantly increased the plant height, leaf length, total dry weight and simultaneously the content of polyphenols, flavonoids, phenolic acids and antioxidant activity in *Rosmarinus officinalis* plants. The increase in the given morphological and biochemical traits significantly improved the economic value of the raw material for pharmaceutical and agronomic use. It follows that the use of the right dose and form of alginite allows farmers and producers of medicinal plants to improve their socio-economic conditions.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

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Morphological and biochemical characteristics of plant parts *Mahonia aquifolium* (Pursh) Nutt. and some physical indicators of its extracts in activated water

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The research focused on determining the economic value of a selected collection of 20 shrubs from a wild-growing population of *Mahonia aquifolium* (Pursh) Nutt. from Arboretum Mlyňany and Nitra region. By morphological analysis, we determined weight of fruits 0.18–0.50 g, the height of fruits 5.57–13.22 mm, the width of fruits 0.98–11.00 mm, and the number of seeds 1.67–5.30 pcs. The content of macro- and microelements was found in the fruits and leaves. *M. aquifolium* samples are a very valuable source of potassium as the main mineral element contained in leaves (10.437 mg.kg^{-1}) and fruits (9.763 mg.kg^{-1}). Microelements such as manganese and iron prevailed in leaves (80.1 mg.kg^{-1} of Mn and 35.0 mg.kg^{-1} of Fe), fruits (29.7 mg.kg^{-1} of Mn and 25.0 mg.kg^{-1} of Fe), and heavy metals (Al, As, Cd, Ni, Pb, Hg) are present only in the small amounts with the most abundant aluminium (17.6 mg.kg^{-1} of Al in leaves and 3.6 mg.kg^{-1} of Al in fruits) content and can be used as indicator suggesting the environmental pollution status in the region. We determined the antioxidant activity by the Trolox method in methanol extracts (76.2 and 101.2 mg TE.g^{-1} DW), in ethanol extracts (54.3 and 47.4 mg TE.g^{-1} DW), in acetone extracts (63.4 and 51.9 mg TE.g^{-1} DW) and water extracts (35.5 and 60.3 mg TE.g^{-1} DW) for fruits and leaves, respectively. Extraction of whole fruit (A1-WF), mashed fruit (A1-MF), and fruitless clusters (A1-CT) in structured (activated) water obtained by Kalyxx for 5 days determined a significant reduction trend pH in mashed fruits (A1-MF). The electrolytic conductivity and total dissolved solids of the extracts decreased significantly from the third day of extraction in variants A1-MF and A1-WF. Significant stability of pH, electrolytic conductivity and total dissolved solids during the experimental period was determined for the fruitless clusters' extracts (A1-CT). The results show that *Mahonia aquifolium* has a multifunctional practical use even in the conditions of the Slovak Republic.

Keywords: *Mahonia aquifolium*, activated water, morphometry analysis, fruit, leaves, macro- and microelements, antioxidant activity

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Introduction

Mahonia Nutt. is the second-largest genus included in the family Berberidaceae Juss., next to the genus *Berberis* L., mostly headquartered in moderate areas in the middle temperate zones. *Mahonia* is distributed mainly in East and Southeast Asia, also in western North America, Central America, and western South America, including 31 species in China (Ying, 2001; Wu et al., 2009).

A variety of ethnomedical usages of *Mahonia* have been recorded in ancient Chinese books and references (He and Mu, 2015). The phytochemical research of this genus has resulted in the identification of more than 150 chemical constituents, among which alkaloids are predominant in all parts (root, leaves, stem, flowers, seeds). Investigation of *Mahonia aquifolium* (Pursh) Nutt. identified berberine, jatrorrhizine, palmatine, oxyacanthine, berbamine, isotetrandrine, isocorydine, corydine, isoboldine, aromoline, obamegine and oxyberberine (Košťálová et al., 1986) in roots, and isotetrandine besides of the other above-mentioned isolated from seeds (Košťálová et al., 1986), while berberine (Košťálová et al., 1981), corytuberine, magnoflorine, isocorydine, corydine, isoboldine, and berbamine were isolated from leaves and magnoflorine like principal alkaloid of the above-ground bark together with isocorydine, berbamine, corytuberine, scoulerine and columbamine were found in other plant parts (Slavík et al., 1985). Isocorydine and berbamine were found in fresh flowers of this species.

M. aquifolium is a good source of phenols, flavonoids, anthocyanins, and antioxidants and other isolated compounds and crude extracts have been shown to exhibit a wide spectrum of *in vitro* and *in vivo* pharmacological effects, including antioxidant (Pyrkosz-Biardzka et al., 2014; Andreicuţ et al., 2018), antimicrobial and antibacterial (Slobodníková et al., 2004), anticancer (Damjanović et al., 2020), anti-mutagenic (Čerňáková et al., 2002), antimalarial activities (Iwasa et al., 1998), antitumoral, immunomodulatory and anti-inflammatory effects (Andreicuţ et al., 2018, 2019), skin disorders like psoriasis (Gieler et al., 2009) and atopic dermatitis (Donsky and Clarke, 2007).

In addition, it has been used as a medicinal plant, and as a dye and food source in North America and Europe (Abrams, 1950; Auge and Brandl, 1997). The berries of *M. aquifolium* have a long tradition of use as an edible fruit, especially in Indigenous American communities. Today, the berberine content dissuades many from using these berries as food (Baumann, 2008).

For our experiments with various plant parts of *M. aquifolium* was important water, mainly activated (structured) water. Water is a complex subject of study, and its properties depend on a great number of factors. Currently, considerable attention is being focused on the study of the structural properties of water and the possibility of data transfer through water and memory of water (Johansson, 2009). This principle is based on quantum electrodynamics (Del Giudice et al., 1988). This follows that liquid water should be a multiphase, non-equilibrium, and, therefore, the active complex system. That alone makes water a complex dynamic system with much richer behaviour than that of any homogenous matter. For example, under certain conditions, it should change its state in response to the weak resonance signals, and for a long time maintain such a condition. This property is known as "structured water" (Clark et al., 2010, Korotkov and Orlov, 2010).

Water has over 50 anomalous chemical-physical properties; no other substance behaves like this. These properties have important implications for engineering, chemistry, biology, and medicine. Yet so far water research is full of contradictory results. There are many water scientists, who have crossed the line into the science of the capacity of water as a unique molecule to hold and transfer information. Most of them have described structured water as having a unique arrangement of molecules that makes it biologically active. That is, structured water has a life-affirming effect on all living species. These findings led most of these scientists to support the long tradition of using homeopathy because structured water holds the energy of the specific ingredient (as opposed to the scientific idea that water holds only physical or chemical forms) and when a homeopathic formulation is delivered in structured water, it transmits the energy of that ingredient to cells. All living things are based on energy to function, and it is the strength of cellular energy that determines its capacity for life (Chaplin, 2000; Voeikov and Del Giudice, 2009; Pollack, 2013; Voeikov and Korotkov, 2017; Korotkov, 2019).

There is a lot of evidence that drinking structured water is beneficial for plant growth Souza et al., 2006; Abdul Qados and Hozayn, 2010), the effect on productivity (Hozayn and Abdul Qados, 2010; Kumar Gora et al., 2018), human health (Ling, 2006; Ho et al., 2019; Korotkov et al., 2019).

Our study aimed to determine some morphological characteristics of fruits and seeds, analysed the elementary profile of dried fruits and leaves, their macro- and microelements, and at last evaluation of

structured (once-activated) various water extracts with fruits and clusters to observe a change in pH, electrolytic conductivity, and total dissolved solids content in examined variants.

Material and methodology

Biological material

For experimental purposes, we used genotypes *M. aquifolium* from Arboretum Mlyňany and Nitra region (Slovak Republic). Fruits with peduncles were taken from shrubs in September and October 2018 in the full ripening stage and analysed in the morphometric laboratory at the Institute of Plant and Environmental Sciences in Nitra (Slovak Republic).

Morphometrical analysis

Samples were marked as MA and the appropriate number (MA-01 – MA-20). The total number of evaluated genotypes was 20. They have evaluated the following characters:

a) fruits – 30 fruits were evaluated from each genotype (n = 30), weight of fruit (g), height of fruit (mm), width of fruit (mm);
b) seeds – 30 seeds were evaluated from each genotype (n = 30) number of seeds in one berry.

The weights were determined by a digital scale (Kern ADB-A01S05, Germany; KERN DS – type D-72336, Kern and Sohn GmbH, Germany), accurate to 0.01 g. Fruits were measured by a digital calliper (METRICA 111 – 012, Czech Republic) accurate to 0.02 mm.

Image analysis

a) fruit: the shape of the fruit, the shape of the basal part of the fruit (at the stalk), cross- and longitudinal section, basic colour of the skin at the full maturity, the colour of the pulp of ripe fruit;
b) seeds: the shape of seeds.

Images were obtained using the stereomicroscope ZEISS SteREO Discovery.V20 (Microlmaging GmbH 37081 Göttingen, Germany), and Fuji FinePix S 7000 and Panasonic DMC FZ50 digital cameras.

Determination of dry matter, ash, and protein content

Total dry matter, ash, and protein content were determined according to the EN method (CSN EN 12145, 1997). Total lipid content was determined according to methods specified in the ISO method (ISO 659:1998).

Determination of carotenoid

Total carotenoid content expressed as β-carotene was analyzed at a wavelength of 445 nm spectrophotometrically (VIS spectrophotometer UV Jenway Model 6405 UV/VIS). Sample (1 g) was disrupted with sea sand and extracted with acetone until complete discolouration. Petroleum-ether was added and then water, the purpose for the separation of phases. After the separation, the petroleum ether-carotenoid phase was obtained and the absorbance was measured (ČSN 560053, 1986).

Determination of mineral contents

A sample for elemental analysis was prepared using the wet ashing method in a microwave oven (Milestone 1200, Milestone, Italy). A total of 0.25 g sample matrix was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha Ltd, Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha Ltd, Czech Republic). After the decomposition sample was filtered using a filter with 0.45 mm pore size and filled up to 25 mL in a volumetric flask with ultrapure water. Elemental analysis was performed using ICP-OES (Ultima 2, Horiba Scientific, France) according to the procedure described by Divis et al. (2015).

Determination of antioxidant activity

The antioxidant activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH): the ethanol (1 mL), methanol (1 mL), acetone (1 mL), and distilled water extracts were mixed with 4 mL of DPPH solution (0.025 g of radical in 100 mL of solvent). The absorbance of the sample extract was determined using the spectrophotometer at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 10–100 $mg.L^{-1}$ ($R^2 = 0.983$) was used as a standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents (TE).

Determination of physicochemical characteristics in plant extracts with activated water

To detect changes in pH, electrolytic conductivity (EC), and total dissolved solids (TDS) from fruit extracts and clusters, we performed a special experiment using activated water. We activated the water with a prototype of the Kalyxx equipment. Water activation is ensured by pouring water through the Kalyxx, in which the galvanic effect is realized. In the experiments, we used control variants (C) and once-activated water variants (A1).

In the experiment, we evaluated three different products of mahonia to determine the pH of the obtained extracts: (a) whole fruit, (b) mashed fruit, (c) fruitless cluster. We put the fruits and clusters into the activated water variant on Monday, December 9, 2018, and we ended the experiment on Friday, December 13, 2018, at 2:00 p.m. We ensured the pH measurement with a pH meter for 5 days at 8:00 a.m. and 2:00 p.m. every day.

The physicochemical analyses were performed by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990). The parameters were measured by EUTECH instrument conductometer (set 2041138) – pH, EC – electrolytic conductivity (μS.cm^{-1}), and total dissolved solids (TDS) (mg.L^{-1}).

The electrolytic conductivity of extracts is defined as that of a 20% weight in volume solution in water at 20 °C, where the 20% refers to dry matter. This was the temperature at which all subsequent measurements were made, the uniform temperature being necessary since the conductivity of electrolytes varies with the temperature (Heald, 1902). The result is expressed in micro-Siemens per metre (μS.m^{-1}). The electrolytic conductivity of a solution of 20 g dry matter of plant parts in 100 mL solvent is measured using an electrolytic conductivity cell. The determination of the electrolytic conductivity is based on the measurement of the electrical resistance, of which the EC is the reciprocal (Vorwohl, 1964a, b).

Statistical analysis

It evaluated the variability of the test files in each character using descriptive statistics. For the characteristics of the files, it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, and the coefficient of variation (%). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (Stehlíková, 1998). Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$) in the program STATISTICA 1.10. The variability of all these parameters was evaluated using descriptive statistics.

Results and discussion

Morphometry analyses of fruits and seeds

When evaluating wild-growing genotypes of *Mahonia aquifolium*, we determined the average weight of fruits in the range of 0.18 g (MA-16) – 0.50 g (MA-14). The coefficients of variation determined in the range of 12.16–49.35% document that the character shows a medium to a very high degree of variability.

Sorokopudov and Chlebnikov (2007) found an average fruit weight in the range of 0.15 to 0.30 g in a study of the mahonia genetic resources collection. Gunduz

Table 1 Variability of fruit traits of selected wild-growing genotypes of *Mahonia aquifolium* (Pursh) Nutt.

Weight of fruits (g)							**Number of seeds**						
Parameters/ Samples	**n**	**min**	**max**	**x̄**	**V**	**H**	**Parameters/ Samples**	**n**	**min**	**max**	**x̄**	**V**	**H**
Genotypes with low values													
MA-20	30	0.10	0.26	0.18	27.50	i	**MA-12**	30	1.00	3.00	1.67	60.00	h
MA-18	30	0.13	0.37	0.19	36.19	i	**MA-18**	30	0.00	4.00	1.90	63.01	h
Genotypes with high values													
MA-15	30	0.38	0.58	0.47	15.67	ab	**MA-07**	30	3.00	7.00	4.70	28.46	ab
MA-14	30	0.20	0.62	0.50	26.40	a	**MA-14**	30	4.00	8.00	5.30	23.62	a
Height of fruits (mm)							**Width of fruits (mm)**						
Genotypes with low values													
MA-12	30	6.12	9.70	5.57	12.62	g	**MA-18**	30	4.98	6.45	5.75	8.25	l
MA-18	30	6.90	8.03	7.75	4.71	h	**MA-16**	30	5.30	7.04	5.99	8.67	kl
Genotypes with high values													
MA-15	30	9.57	11.82	10.64	6.46	b	**MA-15**	30	7.23	9.37	8.51	7.39	b
MA-01	30	12.32	13.79	13.22	4.02	a	**MA-01**	30	10.33	11.79	11.00	4.38	a

Notes: n – the number of measurements; min, max – minimal and maximal measured values; x̄ – arithmetic mean; V – coefficient of variation (%); H – LSD homogeneity test at $P_{0.05}$

(2013) studied morphological properties of *Mahonia aquifolium* from Turkey with fruit weight and fruit seeds weight between 2.9 and 7.3 g and 0.4 and 1.2 g, respectively.

The average height of fruits in the collection of wild-growing genotypes was in the range of 5.57 (MA-12) – 13.22 (MA-01) mm. The coefficients of variation confirm the low 4.02% (MA-01) to medium 14.05% (MA-04) degree of variability of the character. We determined the average width of fruits in the interval from 0.98 (MA-01) to 11.00 (MA-02) mm. The coefficients of variation determined in the range of 4.38% (MA-01) – 25.03% (MA-12) document that the character shows a medium to a high degree of variability.

Gunduz (2013) determined average fruit width and length in the range of 7.0 ±0.3–9.9 ±0.4 mm and 8.0± 0.3 and 11.9 ±0.3 mm, respectively. Fruits are berries that reached 4–6 mm in diameter at the maturity stage according to Şofletea and Curtu (2007).

The average number of seeds in the fruits was determined in the range of 1.67 (MA-12) – 5.30 (MA-14) pieces. The coefficients of variation determined in the range of 15.19% (MA-01) – 66.67 (MA-03) document that the character shows a medium to a very high degree of variability. In comparison with other authors, this trait in the interval 3.0 ±0.3–4.6 ±0.7 (Gunduz, 2013) shows a small difference.

The results from the analysis of variance (ANOVA) of the evaluated traits (Table 2) confirm the statistically significant differences between and within the evaluated genotypes.

Sorokopudov and Chlebnikov (2007) characterize the berries in the ripe state as red-blue to blue, with more or less intact grey coating. Şofletea and Curtu (2007), who studied *Mahonia aquifolium* in Romania determined fruits of black colour with a bluish tinge and abundantly pruinose.

By evaluating the collection of genotypes in our study, we confirm the findings of these authors. The fruits of all evaluated genotypes were characterized by blue colour and grey film on their surface. This is documented in the photo documentation in Figure 1.

The differences in fruit shape are also evident in Figure 1 on the left (A). In general, egg-shaped modifications predominate. Figure 1 on the right (B) presents the shape in the basal part of the fruits of the evaluated genotypes. We determined the differences between the genotypes mainly in the size of the stem hole, which is related to the thickness of the fruit stem.

The seeds of the mahonia are about 5 mm in size, according to Mikula and Vanke (1989), and the following authors present the mahonia seeds as triangular seeds. As we can see in Figure 2(A), the seeds are also triangular, but most of them are oval. Figure

Table 2 Analysis of variance of evaluated fruit traits of genotypes of *Mahonia aquifolium* (Pursh) Nutt.

Factors	f	S	MS	F	H	LSD	
Weight of fruit (g)							
Between genotypes	19	1.96	0.10	9.71	0.000	0.05	0.15
Within genotypes	178	1.89	0.01			0.01	0.17
Total	197	3.86					
Number of seeds							
Between genotypes	19	222.68	11.72	8.79	0.000	0.05	1.64
Within genotypes	178	238.79	1.33			0.01	1.89
Total	198	461.46					
Height of fruits (mm)							
Between genotypes	19	465.12	24.48	23.96	0.000	0.05	1.44
Within genotypes	178	181.83	1.02			0.01	1.66
Total	197	646.95					
Width of fruit (mm)							
Between genotypes	19	666.51	35.08	39.73	0.000	0.05	1.33
Within genotypes	179	158.06	0.88			0.01	1.54
Total	198	824.56					

Notes: f – number of degrees of freedom; S – the sum of squares; MS – average square; F – Fischer test value; P – statistical significance by Fischer test; H – homogeneity; LSD – a least significant difference

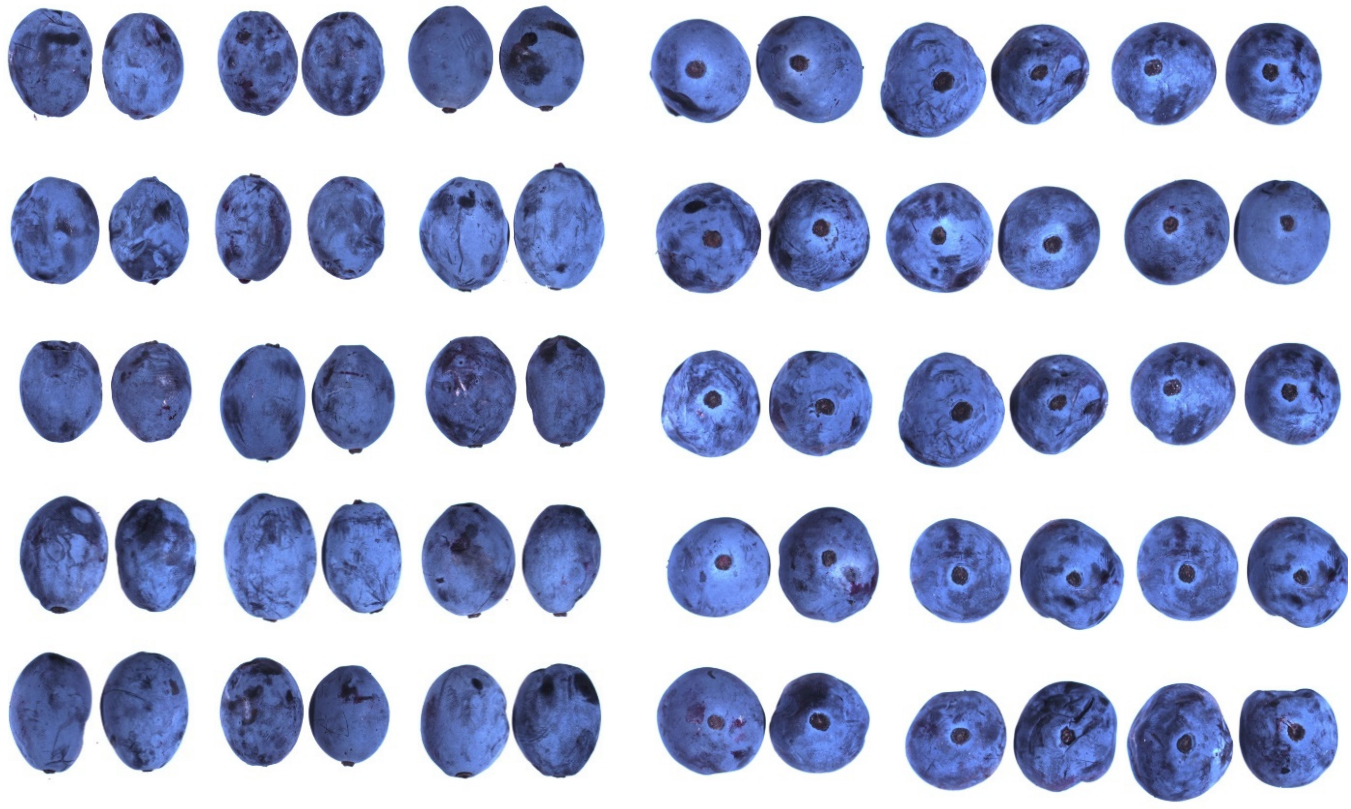


Figure 1 Variability in the shape and the colour of fruits of evaluated genotypes of *Mahonia aquifolium* (Pursh) Nutt.

2(B) showed genotype differences in colour, size, and number of clusters on the stem.

Elementary components

In this study, we determined elementary components of dried fruits *M. aquifolium* in comparison to other two species *Hippophae rhamnoides* L. and *Morus nigra* L. Dry matter and ash were similar in all three species. The content of proteins was following 10.89%, 15.16%, and 7.22% for *M. aquifolium*, *H. rhamnoides*, and *M. nigra*, respectively. Interesting differences were recorded in total fat, where the highest content was in *H. rhamnoides* (14.96%), followed *M. aquifolium* (9.76%), and the lowest in *M. nigra* (2.37%). Saturated

Figure 2 Comparison of shape and colour of seeds (A) and detail of clusters (B) of *Mahonia aquifolium* (Pursh) Nutt.

Table 3 Comparison of elementary components in evaluated dried fruits of *Mahonia aquifolium* (Pursh) Nutt. with other species

Component	SI	*Mahonia aquifolium* (mean ±SE)	*Hippophae rhamnoides* (mean ±SE)	*Morus nigra* (mean ±SE)
Dry matter	%	82.70 ±2.03	87.88 ±2.12	86.36 ±1.67
Proteins	%	10.89 ±0.12	15.16 ±0.18	7.22 ±0.08
Ash	%	3.20 ±0.07	3.68 ±0.04	2.70 ±0.01
Fats	%	9.76 ±0.10	14.96 ±0.17	2.37 ±0.07
β-carotene	mg.kg^{-1}	14.20 ±0.12	55.40 ±1.18	2.80 ±0.04
Saturated fatty acids	g. 100 g^{-1} fat	11.70 ±0.13	41.90 ±1.22	20.50 ±0.76
Monounsaturated fatty acids	g. 100 g^{-1} fat	18.00 ±0.21	21.90 ±0.19	31.40 ±1.05
Polyunsaturated fatty acids	g. 100 g^{-1} fat	59.20 ±2.37	33.00 ±1.78	43.40 ±2.08

fatty acids were predominant in *H. rhamnoides* in the amount of 41.90 g.100 g^{-1} DW of total fat content, while polyunsaturated fatty acids were the predominant in *M. aquifolium* and *M. nigra*, accounting for 59.20 and 43.40 g.100 g^{-1} DW of total fat content. Regarding the β-carotene, the most represented was in *H. rhamnoides* (55.40 mg.kg^{-1}), while in *M. aquifolium* and *M. nigra* samples were the following amounts 14.20 and 2.80 mg.kg^{-1}, respectively (Table 3).

The present study, bioaccumulation, and biosorption of minerals and heavy metal concentration (K, P, Ca, Mg, Na, S, Fe, Mn, Zn, Al, Cu, Ni, Cd, As, Sn, Hg, Se, Pb) were observed in leaves and fruits of *M. aquifolium* (Table 4).

Macroelements (K, Ca, P, Mg, S) are the most represented group, dominated by potassium contained in leaves (10437 ±95 mg.kg^{-1}), and fruits (9763 ±79 mg.kg^{-1}). Potassium is the main mineral element with an average of one-third of the total. Potassium, mostly as a cation (K^+), together with calcium (Ca^{2+}) are the most abundant inorganic elements in plant cellular tissues. Many studies have reported on the role of K^+ in several physiological functions, including controlling cellular growth and wood formation, xylem-phloem water content and movement, nutrient and metabolite transport, and stress response (Sardans and Peñuelas, 2021).

Calcium (Ca) is third in abundance and very close to P in abundance in plant tissue. The highest amounts of Ca are found in mitochondria. It is involved in cell division and cell elongation (Helper, 1994). It is a messenger in several developmental and environmental changes (Heintz, 1960; Sanders et al., 2002). It is responsible for cell integrity (Zhang et al., 2018) and therefore plant survival.

In our experiment calcium was the prominent element in the leaf's samples in the amount of 5152 ±111 mg.kg^{-1}. A high amount of phosphorus was found in leaves (2306 ±68 mg.kg^{-1}) and fruit samples (2389 ±77 mg.kg^{-1}). We can compare the percentages of individual components, which show that potassium was the most represented element in the leaves (48%), as well as fruits (63%), and phosphorus was the second most abundant element in the leaves (24%) of the total minerals, while calcium was represented in the fruits (15%) like the second abundant element.

Microelements (Mn, Fe, Zn, Cu, Al, Se, and Ni) are the second represented group of biogenic elements, where the content of manganese, iron, and zinc prevailed in leaves and fruit samples. The content of manganese and iron predominate in leaves (80.1 ±3.12 mg.kg^{-1} of Mn and 35.0 ±2.65 mg.kg^{-1} of Fe) and fruits (29.7 ±mg.kg^{-1} of Mn and 25.0 ±1.56 mg.kg^{-1} of Fe) following by zinc in the both samples (27.0 ±1.38 mg.kg^{-1} in leaves and 18.0 ±0.8 mg.kg^{-1} in fruits). Microelements have a specific function in the plant tissues. Fe and Zn are essential for the synthesis of chlorophyll, Fe and Mn in photosynthesis, Fe, Mn, and Zn as electron transport mechanisms, and other several enzymes' systems (Voss, 1998). Heavy metals (Hg, As, Cd, Cr, Pb) are present with the most abundant Cr (0.91 ±0.08 mg.kg^{-1} in leaves) and Pb (0.28 ±0.02 mg.kg^{-1} in leaves) content and others only in the trace level (<0.20 mg.kg^{-1}).

Sorokopudov et al. (2017) determined a variety of mineral composition of fruits of *Mahonia aquifolium* collected in the Belgorod (Russia) region between years 2009–2011 (Pb 0.40–0.63 mg.kg^{-1}, Zn 2.00–3.44 mg.kg^{-1}, Cu 0.12–1.45 mg.kg^{-1}, Ca 0.034–0.068%, P 0.040–0.056%, K 0.24–0.41%, Fe 5.3–12.8 mg.kg^{-1}, Mn 4.36–8.36 mg.kg^{-1}).

Samecka-Cymerman and Kempers (1999) reported a study on the concentration of macroelements (N, P, K,

Table 4 Composition of macro- and microelements of leaves and fruits of selected wild-growing genotypes of *Mahonia aquifolium* (Pursh) Nutt.

Element	Leaves (mean ±SE)	Fruits (mean ±SE)
Macroelements (mg.kg^{-1})		
K	10437 ±95	9763 ±79
P	2306 ±68	2389 ±77
Ca	5152 ±111	1126 ±65
S	1465 ±99	1290 ±69
Mg	2126 ±110	1015 ±65
Na	6.0 ±0.7	15.0 ±0.8
Microelements (mg.kg^{-1})		
Zn	27.0 ±1.12	18.0 ±0.8
Fe	35.0 ±2.65	25.0 ±1.56
Cu	10.0 ±0.9	10.0 ±0.9
Mn	80.1 ±3.12	29.7 ±1.38
Cr	0.91 ±0.08	<0.2
Se	0.33 ±0.02	<0.2
Metals (mg.kg^{-1})		
Al	17.6 ±0.4	3.6 ±0.2
As	<0.3	<0.3
Cd	<0.01	0.013 ±0.001
Ni	<0.2	0.98 ±0.08
Hg	0.013 ±0.001	0.007 ±0.0001
Pb	0.28 ±0.02	0.16 ±0.01

Ca, Mg, S, and Fe) and heavy metals (Ni, Cr, Co, V, Zn, Mn, Pb, Cd, Cu, Hg, Ba, and Sr) in the soil and three species of the evergreen plant *Ilex aquifolium*, *Mahonia aquifolium* and *Rhododendron catawbiense* collected in various places in Poland and Netherland after urban pollution (one place is exposed to atmospheric exhausts of heavy traffic, chemical factories, metal smelters and a heat and power plant partly alimented with lignite and coals, two others are unpolluted). Especially pollution with Hg via soil is supported by a significant positive correlation between Hg content in soil and in all the examined species of which *Ilex aquifolium* seemed to be the best monitor of soil pollution with this element.

Heavy metals such as Cd, Pb, Cr, and Ni in plant tissues can be used as indicators suggesting the environmental pollution status in the region (Porrini et al., 2003; Wang and Li, 2011).

Antioxidant activity

The highest antioxidant activity is shown in methanol extracts by the DPPH method in fruits at 76.2 mg TE.g^{-1} DW and leaves at 101.2 mg TE.g^{-1} DW of *M. aquifolium* samples (Figure 3). The lowest antioxidant activity was determined in water extract for fruits at 35.5 mg TE.g^{-1} DW and leaves in ethanol extract at 47.4 mg TE.g^{-1} DW. The analysis showed the presence and comparable values of antioxidants not only in the fruits but also in the leaves of *M. aquifolium* species. Various solvents demonstrated different values of antioxidant activity for fruits and leaves in our experiments.

Other study (Coklar and Akbulut, 2017) demonstrated antioxidant activity of fresh berries *M. aquifolium* determined by DPPH method in various solvents with following values: methanol (35.26 ±1.88 mmol TE.kg^{-1} FW), ethanol (27.36 ±1.47 mmol TE.kg^{-1} FW), acetone (14.41 ±0.68 mmol TE.kg^{-1} FW), water (13.03 ±0.25 mmol TE.kg^{-1} FW), ethyl acetate (4.36 ±0.17 mmol TE.kg^{-1} FW), chloroform (0.36 ±0.04 mmol TE.kg^{-1} FW). According to Gunduz (2013), fruits of *M. aquifolium* showed antioxidant activity by Trolox equivalent antioxidant capacity (TEAC) assay in the range from 4.1 ±0.1 to 21.1 ±0.1 µmol TE.g^{-1} FW, total phenolics ranged from 5009.3 ±176.3 to 6646.8 ±332.1 µg GAE.g^{-1} FW and total monomeric anthocyanins capacities ranged from 52.8 ±4.6 to 361.0 ±15.8 µg cy-3-glu.g^{-1} FW.

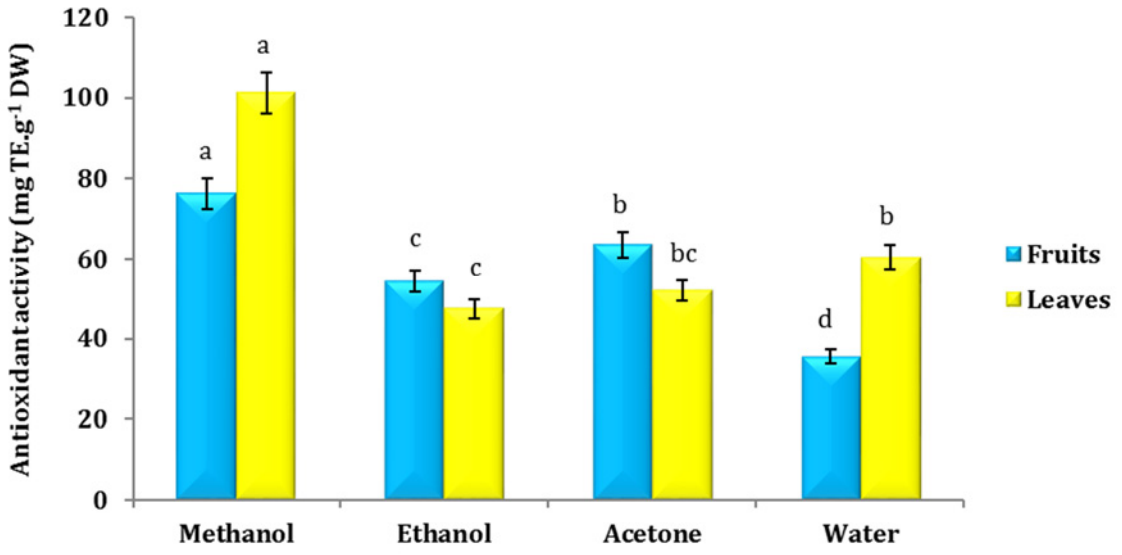


Figure 3 Antioxidant activity of plant parts of *Mahonia aquifolium* (Pursh) Nutt. in various extracts. Different superscripts in each column indicate the significant differences in the mean at p <0.05

Physicochemical parameters

Table 5 show that the pH in the water was determined in the range of 6.97–7.52 (C-TW) during the tested period, after the addition of whole fruits to the water the pH decreased from 7.74 to 3.41 (C-WF) and after adding the mashed fruits to the water, the pH decreased from 5.83 to 3.48 (C-MF). After adding cluster to water (C-CT), the pH stabilized in the range of 7.03 to 7.17.

In the control variant (C-TW), the dynamics of EC increased from 851 to 2430 µS.cm^{-1} during the time period. After adding the fruits to the water (C-WF), the dynamics of EC decreased significantly from 634 to 881 µS.cm^{-1}. After adding the mashed fruits to the water (C-MF), the dynamics of EC decreased significantly from 640 to 887 µS.cm^{-1}. After adding clusters (C-CT) to the water, the EC dynamics stabilized in the range of 637 to 672 µS.cm^{-1}.

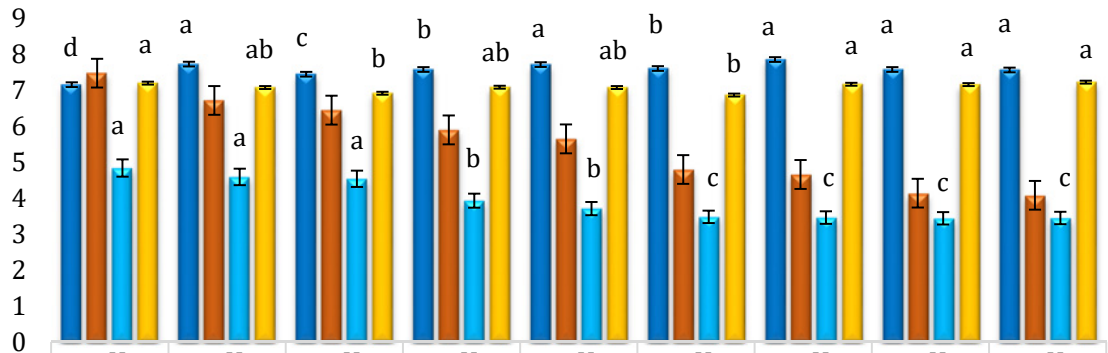
The investigation of TDS presented the following results. The dynamics in the control variant increased from 425 to 1220 mg.L^{-1} during the time period. After adding the whole fruits to the water (C-WF), the dynamics of TDS decreased significantly from 317 to 439 mg.L^{-1}. After adding the mashed fruits to the water (C-MF), the dynamics of TDS decreased significantly from 320 to 443 mg.L^{-1}. After the addition of clusters (C-CT) to the water, the dynamics of TDS stabilized significantly in the range 318 to 335 mg.L^{-1}.

Structured (once-activated) water with plant samples changed properties in all variants and evaluated traits as follows. Figure 4 presents a comparison of variants in once-activated tap water (A1) in pH changes

Table 5 Comparison of control variants of water and extracts (without activation) in the evaluated parameters (ph, EC and TDS) during the tested time period

Variants	C-TW	C-WF	C-MF	C-CT	C-TW	C-WF	C-MF	C-CT	C-TW	C-WF	C-MF	C-CT
Date/Parameters	**pH**				**electrolytic conductivity (µS.cm^{-1})**				**total dissolved solids (mg.L^{-1})**			
09.12.2:00 PM	6.97	7.74	5.83	7.03	851	634	640	637	425	317	320	318
10.12.8:00 AM	7.28	6.78	5.18	6.91	1577	647	672	642	792	323	335	320
10.12.2:00 PM	7.03	6.44	5.12	6.76	1319	676	669	639	662	338	335	319
11.12.8:00 AM	7.46	5.10	3.99	6.94	1536	709	719	647	770	353	360	323
11.12.2:00 PM	7.40	4.68	3.81	6.96	1751	702	749	641	879	351	374	320
12.12.8:00 AM	7.04	3.79	3.47	6.78	1986	777	825	655	993	388	412	328
12.12.2:00 PM	7.52	3.74	3.45	6.52	2380	803	857	698	1190	401	428	336
13.12.8:00 AM	7.19	3.43	3.43	6.95	2350	879	867	666	1180	439	434	333
13.12.2:00 PM	6.98	3.41	3.48	7.17	2430	881	887	672	1220	439	443	335

Notes: C-TW – sample of tap water; C-WF – extract of whole fruit in water; C-MF – extract of mashed fruit in water; C-CT – extract of cluster in water

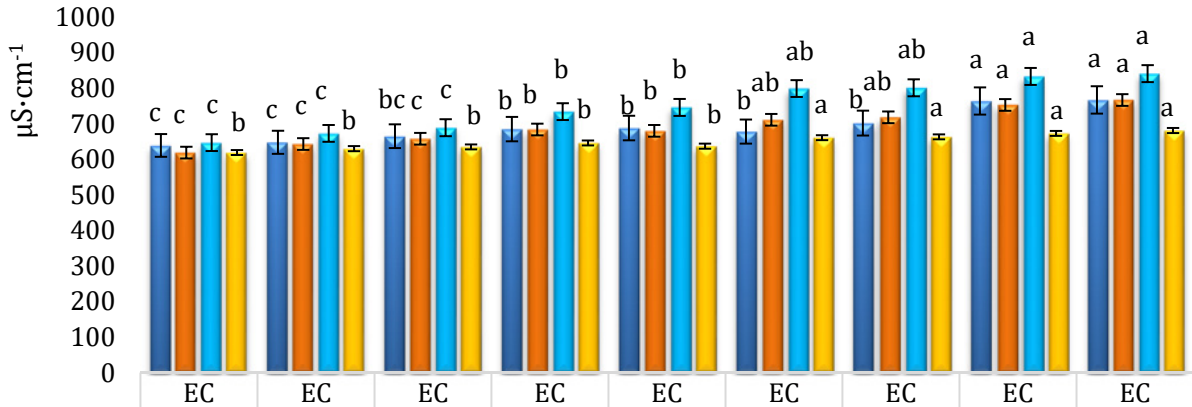


	pH 14:00 9.12	pH 8:00 10.12	pH 14:00 10.12	pH 8:00 11.12	pH 14:00 11.12	pH 8:00 12.12	pH 14:00 12.12	pH 8:00 13.12	pH 14:00 13.12
A1-TW	7.16	7.73	7.45	7.58	7.72	7.61	7.86	7.58	7.57
A1-WF	7.48	6.72	6.45	5.9	5.65	4.80	4.66	4.14	4.08
A1-MF	4.84	4.59	4.54	3.93	3.71	3.48	3.46	3.44	3.45
A1-CT	7.20	7.08	6.92	7.09	7.08	6.87	7.17	7.16	7.23

Figure 4 Comparison of variants with once-activated water (A1) at pH during the experimental period
A1-TW – sample of activated tap water; A1-WF – extract of whole fruit in once-activated water; A1-MF – extract of mashed fruit in once-activated water; A1-CT – extract of the cluster in once-activated water. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

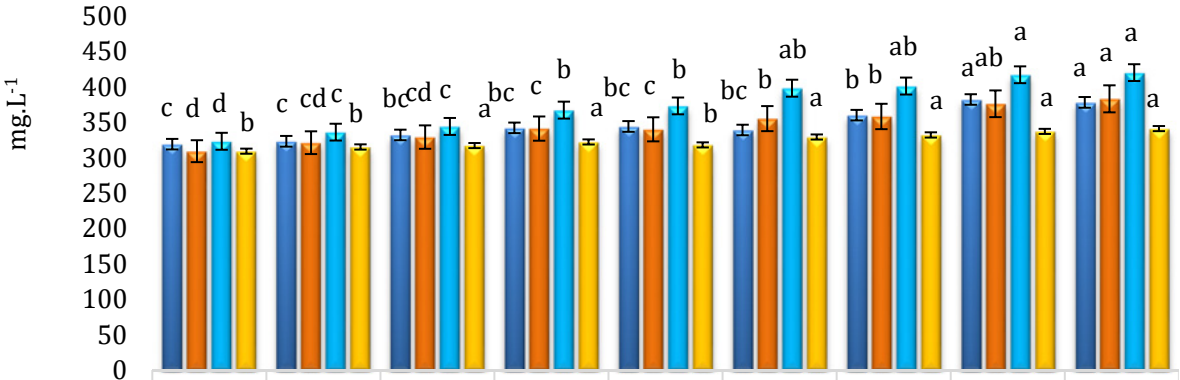
throughout the experiment. The trend of decreasing pH was evident in all variants. Activation of tap water (A1-TW) conditioned minimal pH changes. We also determined similar changes in the extraction of the cluster (A1-CT) in once-activated water. During the extraction of whole fruits (A1-WF) and mashed fruits (A1-MF), we recorded a decrease in pH from the beginning of the experiment, while a more significant decrease was determined for the variants mashed fruits (A1-MF).

On the first day of the experiment, there were no differences in electrolytic conductivity (EC) between



	EC 14:00 9.12	EC 8:00 10.12	EC 14:00 10.12	EC 8:00 11.12	EC 14:00 11.12	EC 8:00 12.12	EC 14:00 12.12	EC 8:00 13.12	EC 14:00 13.12
A1-TW	639	648	665	685	688	678	702	764	767
A1-WF	619	643	658	684	680	711	718	753	767
A1-MF	647	673	689	734	746	799	801	833	841
A1-CT	619	630	635	646	637	661	663	673	681

Figure 5 Comparison of variants with once-activated water (A1) at electrolytic conductivity (EC) during the experimental period ($\mu S.cm^{-1}$)
A1-TW – sample of activated tap water; A1-WF – extract of whole fruit in once-activated water; A1-MF – extract of mashed fruit in once-activated water; A1-CT – extract of the cluster in once-activated water. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$



	TDS 14:00 9.12	TDS 8:00 10.12	TDS 14:00 10.12	TDS 8:00 11.12	TDS 14:00 11.12	TDS 8:00 12.12	TDS 14:00 12.12	TDS 8:00 13.12	TDS 14:00 13.12
A1-TW	319	323	332	342	344	339	360	382	378
A1-WF	309	321	329	341	340	355	358	376	383
A1-MF	323	336	344	367	373	398	401	417	420
A1-CT	309	315	317	322	318	329	332	337	341

Figure 6 Comparison of variants with once-activated water (A1) at total dissolved solids (TDS) during the experimental period (mg.L^{-1})
A1-TW – sample of activated tap water; A1-WF – extract of whole fruit in once-activated water; A1-MF – extract of mashed fruit in once-activated water; A1-CT – extract of the cluster in once-activated water. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

the variants. The change was seen on the third day, where the highest conductivity was achieved by the mashed fruits variant (A1-MF), which continued until day 5 (Figure 5) in the interval from 647 (1st day) to 841 µS.cm^{-1} (5th day). The lowest EC can see in the clusters variant (A1-CT) in the interval from 619 (1st day) to 681 µS.cm^{-1} (5th day).

Activation of water (A1-TW) and addition of whole fruits (A1-WF), mashed fruits (A1-MF) and clusters (A1-CT) to activated water in generally resulted in a reduction and stabilization of EC values during the experiment. It follows that water activation significantly improves the quality of water for human use.

Various types of water have specific conductivity. According to Bendlin (1995) in Gordalla's study (Gordalla et al., 2007) we known ultrapure water (0.055 µS.cm^{-1}), demineralized (0.1–1.0 µS.cm^{-1}), drinking (30–2000 µS.cm^{-1}), brackish (20000–1000000 µS.cm^{-1}), lake constance water (322 µS.cm^{-1}), groundwater, Munich (537 µS.cm^{-1}), bank filtrate, river Rhine nearby Düsseldorf (702 µS.cm^{-1}).

On the first day of the experiment, there were no differences in total dissolved solids (TDS) between the variants. The change was seen on the third day, where the highest content of TDS was achieved by the mashed fruits variant (A1-MF), which continued until day 5 (Figure 6) in the interval from 323 (1st day) to 420 mg.L^{-1} (5th day). The lowest content of TDS can see in clusters variant (A1-CT) in the interval from 309 (1st day) to 341 mg.L^{-1} (5th day).

Activation of water (A1-TW) and addition of whole fruits (A1-WF), mashed fruits (A1-MF) and clusters (A1-CT) to activated water in generally resulted in a reduction and stabilization of TDS values during the experiment. Water activation significantly improves water quality.

Differences in the correlation coefficient values are significant between the tested samples of variants with once-activated water. A very strong relationship is maintained between electrolytic conductivity and total dissolved solids (r = 0.999). This trend was described in our previous studies (Horčinová Sedláčková et al., 2019a, b). Other values of the correlation coefficients between pH and electrolytic conductivity samples and pH and the total dissolved solids of the tested samples are very low (r = 0.195–0.359). A very strong negative correlation was determined between pH and electrolytic conductivity (r = -0.961), pH, and total dissolved solids (r = -0.962) in the investigated variant of mashed fruits (A1-MF).

Conclusions

Our study was the evaluation of genotypes of *Mahonia aquifolium* based on the morphological analysis of leaves, fruits, and seeds, determination of macro- and microelements of leaves and fruits, and new original experiments with activated water of various combinations of fruits and clusters. Results confirmed variability of morphological traits such as weight of fruits, the height of fruits, and the width of fruits. When comparing the determined variation ranges for all evaluated traits, we found a significant degree of agreement. Based on micronutrient content leaves and fruits may be regarded as an important source, especially of potassium, calcium, and other minerals such as K, P, S and Mg. The highest antioxidant activity was determined in methanol extracts by the DPPH method in the dried fruits and leaves. Experiments with activated water most changed the character of pH, electrolytic conductivity, and content of total dissolved solids mashed fruits variants until the 5th day of the experiment. Results document that the study of the extraction of plant parts in activated water opens up new possibilities for recognizing the influence of water activation on the change in the properties of the extracts. Knowledge of the field can significantly affect water quality improvement, technological processes in the production of beverages and the food industry. Activation of water, and thus also the activation of extracts of plant parts does not have to result in a change in the chemical composition of the products, but at the same time, it can have a significant impact on the improvement of water or beverages quality, and thereby also improving the health of consumers.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Statement

This article does not contain any studies that would require an ethical statement.

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