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Phenolic and flavonoid contents in *Deschampsia antarctica* plants growing in nature and cultured *in vitro*

Maryana TWARDOVSKA*[1], Iryna KONVALYUK[1], Kateryna LYSTVAN[2], Igor ANDREEV[1], Ivan PARNIKOZA[1,3] and Viktor KUNAKH[1]

[1] *Department of Cell Population Genetics, Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine,150 Acad. Zabolotnogo Str., 03143, Kyiv, Ukraine*

[2] *Department of Genetic Engineering, Institute of Cell Biology and Genetic, Engineering of the National Academy of Sciences of Ukraine, 148 Acad. Zabolotnogo Str., 03143, Kyiv, Ukraine*

[3] *State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, 16 Shevchenko Ave., 01601, Kyiv, Ukraine*

**corresponding author <maryana.tvardovska@gmail.com>*

Abstract: The paper presents data on the total phenolic and flavonoid content in *Deschampsia antarctica* È. Desv. plants collected from natural habitats, plants cultured *in vitro*, regenerated plants, and plants cultivated in a growth chamber. It was found that the shoots (aerial parts) had higher phenolic and flavonoid contents compared to the roots. The largest amount of these substances was found in wild plants from Great Yalour Island. The content of phenolics and flavonoids in plants cultured *in vitro* was generally comparable to that in plants collected from natural habitats except for some clones. HPLC analysis revealed five main metabolites present in similar ratios in the studied samples of wild and *in vitro* plants. Minor substances varied slightly in different extracts, however their presence did not depend on plant growth conditions. No significant qualitative differences in HPLC profiles were found between the wild and *in vitro* plants. The studied samples did not contain quercetin, kaempferol and luteolin, whereas orientin was found in all studied samples of *D. antarctica.*

Key words: Antarctic, phenolic compounds, flavonoids, HPLC analysis.

Introduction

Plants are a source of natural biologically active substances (BAS), in particular, with antimicrobial and antioxidant properties. The most important groups of biologically active secondary metabolites are phenolic compounds, flavonoids, vitamins (C, E), carotene, etc. Phenolic compounds of plant origin have high therapeutic efficacy and low toxicity to humans. Phenolics have been reported to possess a wide spectrum of biochemical activities, such as antioxidant, antimutagenic, antimicrobial, anticarcinogenic, have direct constrictive action on capillaries, as well as ability to chelate free radicals and to modify gene expression (Marinova *et al.* 2005; Tanase *et al.* 2019). Phenolics can also act as an intracellular antioxidant through inhibition of free radical generating enzymes (Esmailli *et al*. 2019).

Flavonoids are secondary metabolites belonging to polyphenols and widely distributed in many plant species (Oliveira *et al*. 2016; Yadav and Malpathak 2016). They are known to play an important role in the human body as antioxidants, free radical scavengers, carbohydrate metabolism promoters, and stimulators of the immune system (Gidekel *et al.* 2011). They have anti-inflammatory (Araujo and Leon 2001), cytotoxic (Murakami *et al*. 2014), and antispasmodic action (Ammon and Wahl 1991), anticancer, antihyperglycemic, and hepatoprotective activities (Venkatesan *et al*. 2017; Bello *et al*. 2019), can inhibit mitochondrial adhesion and activity of protein kinases (Bhat *et al*. 2004), as well as protect against age-related degeneration of cellular components. It has been reported that flavonoids possess anti-inflammatory activity in both proliferative and exudative phases of inflammation via inhibition of various enzymes such as xanthine oxidase, aldose reductase, phosphodiesterase, LOX, Ca^{2+}-ATPase, and COX (Ibrahim *et al*. 2018). The antiproliferative effect of flavonoids is currently compared with the action of modern anticancer drugs (Brakenchielm and Cao 2001; Ren *et al*. 2003).

In plants growing in extreme conditions, abiotic stresses affect biosynthesis and accumulation of secondary metabolites in the tissues and organs, in particular, stress response is often associated with activation of flavonoid synthesis (Kunakh 2005). The biochemical characteristics of plants, especially the content of secondary metabolites, such as phenolics and flavonoids, may also play an important role in adaptation to extreme conditions. Their biosynthesis increases in response to stress (infection, drought, injury or ultraviolet (UV) radiation) (Ahmed *et al*. 2017), so they can be endogenous regulators of physiological processes under the influence of adverse environmental factors. It is known that intense radiation and cold stress lead to increased accumulation of flavonoids in plants (Kreps *et al*. 2002; Tattini *et al*. 2005). Flavonoids act as photoreceptors and antioxidants, thus protecting plants from oxidative stress and damage caused by free radicals (reactive oxygen species and reactive nitrogen species – ROS and RNS), have antimicrobial action, etc. (Cotelle 2001;

Ververidis *et al.* 2007). Phenolic compounds are also involved in many physiological processes and can be used as phenotype markers (Quattorocchio *et al*. 2006).

Deschampsia antarctica È. Desv. is an extremophile plant species that has successfully adapted to the harshest environmental conditions of Antarctica and can be used as a source of BAS. It is known that under low temperatures and high-level UV radiation, *D. antarctica* is capable to produce significant amounts of BAS, in particular phenolic compounds (Ruhland *et al*. 2005; Poronnik *et al*. 2014). The high antioxidant capacity of the secondary metabolites may be a determinant for *D. antarctica* survival in the Antarctic environment (Perez--Torres *et al*. 2004). The concentration of flavonoids, in particular orientin, luteolin and isoswertiajaponin (7-O-methylorientin) 2"-O-beta-arabinopyranoside in Antarctic hair grass increases under UV irradiation (Webby and Markham 1994; Day *et al.* 2001; Ruhland *et al.* 2005). *D. antarctica* is a natural source of antioxidants that can be used in the pharmaceutical and food industries, as well as in cosmetology (Gidekel *et al*. 2010). Phenolic compounds isolated from this plant species have been demonstrated to inhibit melanoma cell proliferation (Gidekel *et al*. 2010), to induce antitumor immunity against colorectal carcinoma and its metastasis to the liver, and can be used to develop new drugs for the treatment of colorectal carcinoma (Malvicini *et al*. 2018).

Due to the difficulties of *D. antarctica* research in natural habitats and impossibility of collecting sufficient amounts of plant raw material of *D. antarctica*, we have introduced this species into *in vitro* culture (Zahrychuk *et al*. 2011/ 2012; Konvalyuk *et al*. 2019). Development of protocols for obtaining aseptic plants of *D. antarctica* made it possible to produce plant biomass in required amounts.

In this paper, we provide the data of comparative study of total phenolic and flavonoid contents in extracts from *D. antarctica* plants growing in nature, cultured *in vitro*, regenerated plants and plants grown in a growth chamber.

Material and methods

Plant material. — Micropropagated plants grown *in vitro* or in soil in growth chamber as well as regenerated plants obtained from callus as described in detail in the work (Konvalyuk *et al*. 2019) were used for the biochemical analysis along with the wild plants collected from the nearby areas of the Ukrainian Antarctic Station *Academician Vernadsky* during the XXIV Ukrainian Antarctic Expedition in 2019. Information about the origin of the cultured plants is provided in Table 1.

The plants grown *in vitro* were cultured on Gamborg's B5 medium (Gamborg and Eveleigh 1968) supplemented with 0.1 mg/L of 1-naphthylacetic acid. To obtain plants in growth chamber, *in vitro* plants were transferred into pots

Table 1

Location and years of collecting of *D. antarctica* seeds used to obtain cultured plants

№	Clone	Locality, geographical coordinates and year of collection
1	G/D12-2a	Galindez Island, 65°14.845′ S, 64°15.156′ W, 2007
2	R30	Rasmussen Oasis, 65°14.819′ S, 64°5.156′ W, 2005
3	DAR12	Darboux Island, 65°23.707′ S, 64°12.905′ W, 2007
4	DAR13	Darboux Island, 65°23.707′ S, 64°12.905′ W, 2007
5	Y66	Great Yalour Island (the biggest of the Yalour Islands), 65°14.039′ S, 64°09.761′ W, 2005
6	Y67	Great Yalour Island, 65°14.039′ S, 64°09.761′ W, 2005
7	L57	Lahille Island, 65°33.167′ S, 64°23.249′ W, 2010

containing a mix of soil : vermiculite : peat (3 : 1 : 1). The plants *in vitro* and in soil were cultured at 16–18 °C on a 16 h light/8 h dark photoperiod at a light intensity of 6500 lux and relative humidity of 55–65%.

Extract preparation. — Lyophilized plant material was used to prepare extracts; leaf and root tissues were analysed separately, for wild plants, – only leaf tissue was analysed. Three plants of each clone were used for biochemical analysis.

Ethanolic extracts were prepared as follows: 20 mg of the sample was ground to a powder using a ball mill and extracted with 2 mL of 96% ethanol at 27° C for 18–20 h, then subjected to 30-minute ultrasonic extraction in an ultrasonic bath. The extracts were centrifuged, and the supernatant was collected for the analysis.

Determination of total phenolic content. — The total phenolic content of the extracts was determined using the Folin and Ciocalteu assay (Singleton and Rossi 1965), which is based on colorimetric reaction between phenolic compounds and the Folin-Ciocalteu reagent (phosphomolybdic-tungstic acid) resulting in the production of molybdenum–tungsten blue concentration of which was measured spectrophotometrically at 765 nm.

The appropriate volume of extract was adjusted to 100 µL with 96% ethanol. To the resulting solution, 200 µL of 10% (v/v) aqueous solution of Folin-Ciocalteu reagent was added and stirred for 20–30 seconds. Then, 800 µL of 7.5% aqueous Na_2CO_3 solution was added to create an alkaline environment optimal for the reaction. The resulting solutions were left for 2 h at room temperature before the measurement of the absorbance using a spectrofluorometer Fluorat®-02-Panorama in the spectrophotometer mode.

The calibration curve was constructed using standard solutions of ferulic acid. Data were expressed as mg of ferulic acid per 1 g of dry weight.

Determination of the total flavonoid content. — Total flavonoids expressed as rutin equivalents were quantified using spectrophotometric assay. This technique is based on the ability of flavonoids to form a coloured complex with aluminium (Pękal and Pyrzynska 2014).

The appropriate volume of extract was adjusted to 1 mL with 96% ethanol. The resulting solution was mixed with 360 µL of 5% $NaNO_2$ and incubated for 5 minutes, then 600 µL of 2% $AlCl_3$ was added, mixed thoroughly, and left for 6 minutes. Then 600 µL of 1 M NaOH solution was added and incubated for 10 minutes. The reaction mixture changed colour to pink; the absorption of the formed complex was measured at 510 nm using a spectrofluorometer Fluorat®-02-Panorama in the spectrophotometer mode.

The calibration curve was constructed using standard solutions of rutin. Data were expressed as mg of rutin per 1 g of dry weight.

The content of phenolics or flavonoids was calculated by the formula:

$$Cf = C \times k/m,$$

where Cf is the content of phenolics / flavonoids, mg equivalent of ferulic acid/rutin per 1 g of dry weight; C – the content of phenolics/flavonoids on the calibration curve, mg/mL; k – the dilution factor; m – the weight of the extracted plant material (g).

Qualitative analysis of phenolic compounds and flavonoids. — Profiling of phenolic and flavonoid content in *D. antarctica* leaves was performed using high performance liquid chromatography (HPLC). HPLC analysis was performed on a Shimadzu HPLC10Avp system (Japan) using a Zorbax Eclipse column (XDB-C18, 6x250 mm, 5 µm, Agilent) with a Waters Symmetry C8 pre-column. Chromatographic conditions: mobile phases were acetonitrile (B) and deionized water + 1% formic acid (A); gradient: increase from 10% B to 40% B in 22 min; the total run time: 30 min. Column temperature: 40° C, flow rate: 0.8 $mL \cdot min^{-1}$, injection volume: 20 µL, UV detection: at 318 nm.

Standards (luteolin, apigenin, orientin, rutin, quercetin and kaempferol) were dissolved in 96% ethanol.

Extracts of the aerial parts of plants were used for analysis without further processing; root extracts were vacuum-concentrated 10 times using the Thermo Scientific Savant SpeedVac system before analysis. The processing and visualisation of the chromatogram and absorption spectra were performed using Shimadzu LabSolution software.

Statistical analysis. — Descriptive statistics methods were used to analyse the data. The significance of differences in phenolic and flavonoid content between genotypes was evaluated using Student's *t*-test at a confidence level of $p<0.05$.

Results

Quantification of phenolic compounds and flavonoids. — Quantification of the content of phenolic compounds and flavonoids in alcoholic extracts of *D. antarctica* was performed by spectrophotometric method. The results obtained are presented in Table 2.

Table 2

Quantitative content of phenolic compounds and flavonoids in alcoholic extracts of *D. antarctica* leaf and root tissues under different growth conditions

№	Origin/Clone (growth conditions)	Total content, mg/g of dry weight			
		phenolic compounds		flavonoids	
		leaves	roots	leaves	roots
	Natural habitats				
1	Galindez Island	16.3 ± 1.4	—	24.3 ± 1.0	—
2	Rasmussen Oasis	15.9 ± 2.1	—	19.5 ± 3.9	—
3	Darboux Island	12.8 ± 1.6	—	16.6 ± 3.8	—
4	Great Yalour Island	19.6 ± 3.2	—	25.3 ± 6.4	—
	In vitro grown plants				
5	G/D12-2a	7.3 ± 3.5	7.4 ± 0.4	9.5 ± 2.6	6.1 ± 0.4
6	R30	12.1 ± 0.7	14.1 ± 3.1	14.6 ± 1.0	17.4 ± 5.1
7	DAR12	16.5 ± 3.0	7.2 ± 1.3	21.3 ± 3.0	5.4 ± 0.9
8	DAR13	15.9 ± 3.6	11.2 ± 1.4	8.6 ± 0.8	8.5 ± 1.4
9	Y67	16.0 ± 1.4	4.8 ± 1.3	16.9 ± 2.3	5.5 ± 1.1
10	Y66	12.1 ± 1.2	4.8 ± 0.3	13.6 ± 0.7	2.9 ± 0.2
11	L57	9.0 ± 2.2	2.1 ± 1.5	2.3 ± 0.7	1.9 ± 0.3
12	G/D12-2a (regenerated plant)	15.6 ± 0.8	—	19.5 ± 2.4	—
13	DAR12 (regenerated plant)	16.2 ± 0.8	3.2 ± 1.4	19.1 ± 1.5	2.6 ± 1.3
	Growth chamber				
14	Y66	19.9 ± 2.2	6.3 ± 1.3	5.2 ± 0.1	1.1 ± 0.4
15	DAR12 (regenerated plant)	12.8 ± 0.6	3.4 ± 1.2	3.2 ± 0.4	1.9 ± 0.9

The total content of phenolic compounds was 7.3–16.5 mg/g in leaves and 2.1–14.1 mg/g in roots *D. antarctica* grown *in vitro*. The total content of flavonoids was 2.3–21.3 mg/g and 1.9–17.4 mg/g, respectively. In general, the total content of these secondary metabolites was higher in leaves of *in vitro* plants compared to roots (Fig. 1). The content of the substances in leaf and root tissues was comparable only in certain *in vitro* plants.

The largest amount of phenolic compounds was found in wild plants from Great Yalour Island and in the Y66 plants with a hypotriploid karyotype grown in a growth chamber (19.6 and 19.9 mg/g, respectively). However, in the Y66 plants grown *in vitro*, the total content of phenolics was lower (12.1 mg/g) (Fig. 2A). When comparing the myxoploid Y67 and hypotriploid Y66 *in vitro* plants originated from Great Yalour Island, the total content of phenolic compounds in the Y67 plants was slightly larger (the difference is not statistically significant

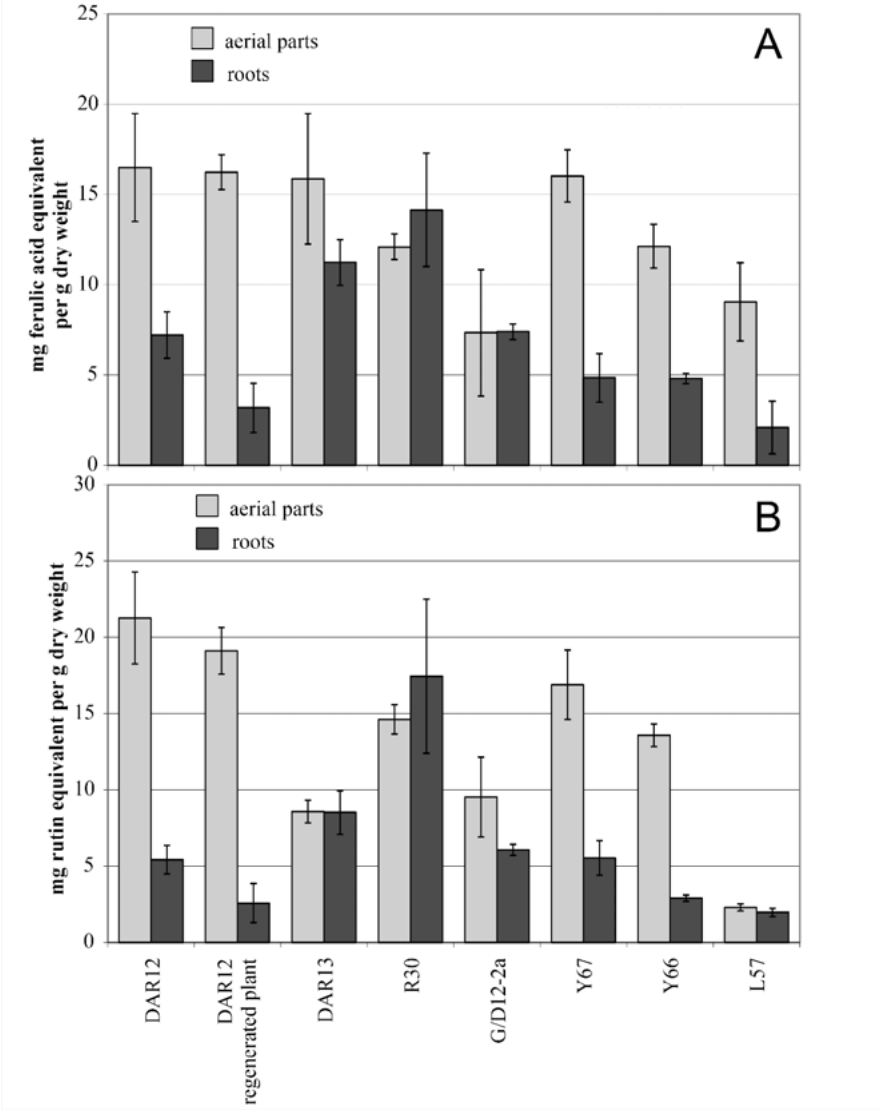

Fig. 1. The total content of phenolic compounds (**A**) and flavonoids (**B**) in *D. antarctica* plants grown *in vitro*. The data expressed as the mean of three independent measurements ± standard error.

at $p<0.05$). The lowest amount of phenolic compounds was found in the G/D12-2a and the L57 *in vitro* plants, 7.3 and 9.0 mg/g, respectively. In the remaining clones, the content of these compounds ranged from 12.1 to 16.5 mg/g. Comparison of plants of different clones originated from Darboux Island (DAR12 and DAR13 *in vitro* plants, and DAR12 regenerated plants grown *in vitro*) showed that they contained almost the same amounts of phenolics. The wild plants from this locality and DAR12 plants grown in a growth chamber contained a slightly lower amount of these substances (12.8 mg/g).

Since it was shown that the aerial part of plants had a higher content of phenolic compounds and flavonoids compared to the roots, further comparative analysis included only data on the content of these substances in the aerial part of plants.

Fig. 2 shows the total content of phenolic compounds and flavonoids in the aerial part of *D. antarctica* plants grown in various conditions.

It should be noted that the amount of flavonoids was greater in the leaves (in some genotypes 6 times) than in the roots (Fig. 2B).

The flavonoid content ranged from 16.6 to 25.3 mg/g in the analysed samples of *D. antarctica* wild plants and from 2.3 to 21.3 mg/g in the plants grown *in vitro,* that was slightly smaller than in the wild plants.

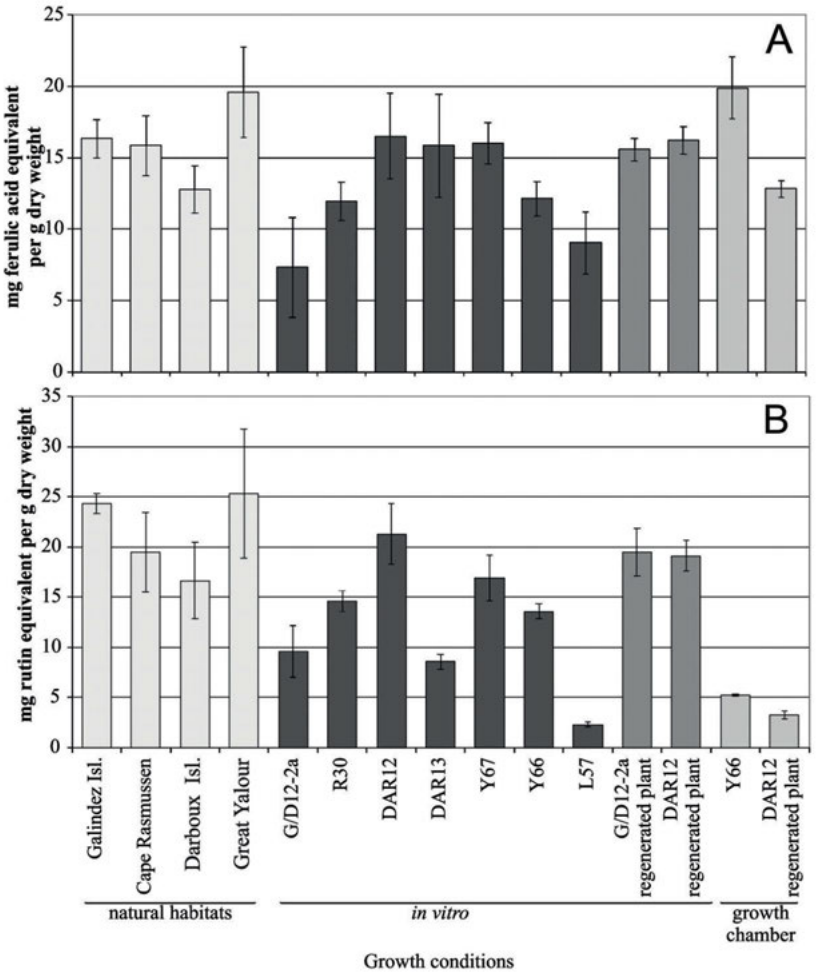

Fig. 2. The total content of phenolic compounds (**A**) and flavonoids (**B**) in *D. antarctica* plants from different growth conditions: natural habitats (white shading), *in vitro* (dark grey shading), and growth chamber (light grey shading).

The cultured plants of different clones significantly differed in the content of flavonoids (Fig. 2B). The highest content of these secondary metabolites was in DAR12 *in vitro* plants and regenerated plants, as well as in G/D12-2a regenerated plants (19.1–21.3 mg/g). Similar to fenolics, the smallest amount of flavonoids was found in L57 plants (2.3 mg/g). When comparing two clones originated from Darboux Island, DAR12 *in vitro* plants contained 2.5 times more flavonoids than DAR13 *in vitro* plants (the difference is significant at $p<0.05$). *In vitro* plant and regenerated plant of the DAR12 clone had very similar content of flavonoids. In contrast, the flavonoids content in G/D12-2a regenerated plant was almost twice as much as that in G/D12-2a *in vitro* plant (the difference is significant at $p<0.05$). Two samples of *in vitro* plants originated from Great Yalour Island (Y66 and Y67) did not differ significantly in flavonoid content. However, Y66 plants grown in a growth chamber contained small amounts of these metabolites (only about 5 mg/g).

Qualitative analysis of phenolic compounds and flavonoids. — We conducted qualitative analysis of phenolic compounds and flavonoids extracted from different *D. antarctica* samples using HPLC to assess the potential effects of growing conditions on metabolites profiles. The HPLC profiles of phenolic compounds and flavonoids extracted from the leaves of wild-grown and *in vitro* cultured plants were found to be very similar in the vast majority of samples. Fig. 3 shows a typical profile of phenolics and flavonoids in alcoholic extract of *D. antarctica* leaf tissue that were fractionated by HPLC and analysed at 318 nm.

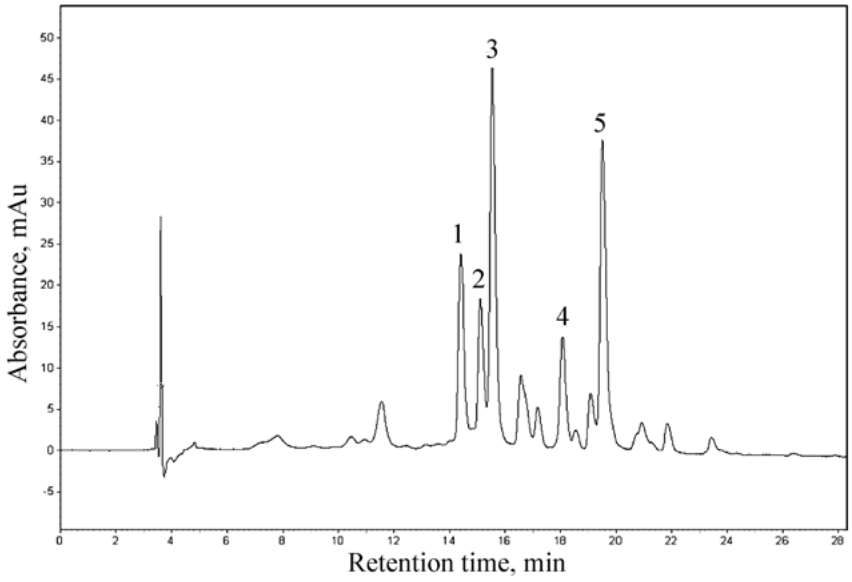

Fig. 3. HPLC chromatogram of phenolic compounds from the leaf extracts of *D. antarctica* (detection wavelength of 318 nm): 1,3–5 – unidentified substances, 2 –orientin.

There were detected five most abundant metabolites, that were present in similar ratios in the samples of wild and *in vitro* grown plants. The most abundant was currently unidentified substance 3 corresponding to the peak which had the largest area in all analysed samples. Minor peaks may vary slightly in different extracts, but no dependence of their presence on plant growth conditions was found.

Furthermore, to identify individual substances we compared their chromatographic characteristics (retention time) and specific features of UV-Vis absorption spectra with some standard substances. It was found that the extracts did not contain quercetin, kaempferol and luteolin. Residual amounts of rutin and small amounts of chlorogenic acid were detected in some extracts, however, their presence did not depend on plant growth conditions.

One of the five most abundant substances from the extracts was identified as orientin or luteolin-8-C-glucoside (peak 2 in Fig. 3), the chemical structure of which is shown in Fig. 4.

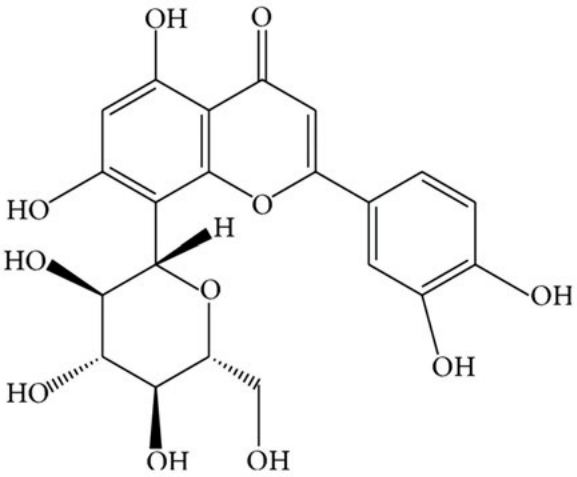

Fig. 4. Chemical structure of orientin.

This flavonoid was found in all of the samples of wild and *in vitro* grown plants of *D. antarctica*. The results of the quantitative content analysis of orientin in *D. antarctica* plants are shown in Fig. 5.

The content of orientin in the leaf tissue of *D. antarctica* plants ranged from 2.0 to 4.8 mg/g. Wild-grown plants from different localities differ significantly in the content of this substance: plants collected from Rasmussen Oasis and Great Yalour Island contained twice as much orientin as plants from Galindez and

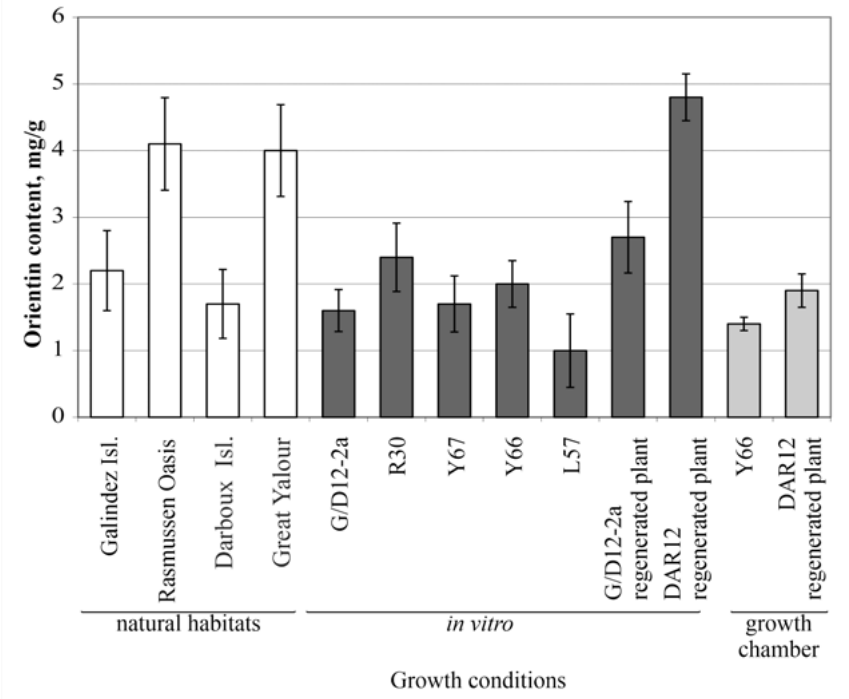

Fig. 5. The content of orientin in the leaf tissue of *D. antarctica* plants grown in different conditions: natural habitats (white shading), *in vitro* (dark grey shading), and growth chamber (light grey shading).

Darboux Islands (the difference is significant at $p<0.05$). Among plants grown *in vitro*, the content of orientin was highest in regenerated plants (2.7 and 4.8 mg/g), while in others it ranged from 1.0 to 2.5 mg/g.

Discussion

The total content of phenolic compounds and flavonoids in *D. antarctica* plants grown in various conditions, including natural habitats and *in vitro* plant culture was determined. We found that amount and content of phenolic compounds in *in vitro* cultured plants and regenerated plants were similar to those in wild plants that gives grounds to consider *in vitro* cultured *D. antarctica* plants as a potential source for obtaining the corresponding BAS. HPLC analysis revealed five main substances in extracts of *D. antarctica* plants, one of which was orientin that was previously found in the samples of this species by other researchers (Webby and Markham 1994). Slight amounts of rutin as well as chlorogenic acid were also found in some of the samples.

HPLC analysis of phenolic compounds and flavonoids from *D. antarctica* extracts have been also performed by other researchers. A first assay of

flavonoids for this plant was conducted by Webby and Markham (1994), who extracted dried leaves and separated flavonoids using 2-D paper chromatography. Spots analysed by ^{13}C NMR revealed mainly different luteolin glycosides, such as orientin and isoswertiajaponin, as well as lower amounts of tricin and isoswertisin (Webby and Markham 1994). It has been found that the increased UV radiation may increase the synthesis of some flavonoids (Montiel *et al.* 1999), as well as accumulation of orientin, luteolin, and isoswertiajaponin 2"-O-beta-arabinopyranoside in *D. antarctica* plants (Webby and Markham 1994; Day *et al.* 2001; Ruhland *et al.* 2005).

A study of the content of flavonoids in two species of vascular plants in Antarctica – *D. antarctica* and *Colobanthus quitensis* (Kunth) Bartl. showed that the species differ in their flavonoid composition (Lutz *et al.* 2008). The detected peaks were compared with apigenin, kaempferol, luteolin, myricetin and quercetin, as common in European plants (Grace 2005), but any of these substances were found. The authors assume that some of the peaks may be phenylpropanes, the rest may be derivatives of apigenin and luteolin (Lutz *et al.* 2008). Apigenin is produced under the action of UV radiation and is a cofactor of auxin oxidase. Increased flavonoid content has been suggested to play a role in the antioxidant system and pathogen suppression and thus contribute to the survival of *D. antarctica* and *C. quitensis* in the harsh conditions of Antarctica (Lutz *et al.* 2008).

Previously, extracts from *D. antarctica* plants growing in natural habitats and *in vitro* were studied using HPLC (Gidekel *et al.* 2011). HPLC-mass spectrometry analysis identified two peaks in the extracts from wild-grown plants: one at 260 nm and another at 350 nm, which were virtually absent in plants cultured *in vitro*. The first peak corresponds to isoswertiajaponin ((7-*O*-methylorientin) 2"-O-beta-arabinopyranoside) and the second is orientin 2"-beta-arabinopyranoside. Pure orientin has not been found in *D. antarctica* extracts. Subsequently, the same researchers identified six new compounds in *D. antarctica* extracts: 2-O-beta galactopyranosylorientin, 2"-O-beta arabinopyranoside orientin, orientin, isowetiajaponin (7-O-metilorientin 2"-O-arabinopyranoside), isowertijaponin ((7-O-metilorientine 2"-)-arabinopyranoside) and luteolin. It was assumed that luteolin with different degrees of glycosylation and substitution of glycosides through C–C bonds is the molecule that is largely present in extracts and causing the biological activity. These compounds were found in extracts from *D. antarctica* wild-grown plants or plants subjected to 4° C for 72 hours, but they were not detected in plants grown *in vitro* at 13°C. This indicates that these compounds are inducible at low temperatures or other types of stress experienced by plants in wild (Gidekel *et al*. 2011).

Using HPLC analysis, the flavonoids orientin and luteolin were identified in *D. antarctica* and orientin in *D. borealis* (Trautv.) Roshev. (Staaij *et al*. 2002). The amount of flavonoids did not differ in the two *Deschampsia* species grown in different conditions, including environments with increased exposure to UV-B

radiation. The scientists suggest that the constitutive levels of flavonoids in these species are adequately high to protect them against ambient and elevated levels of solar UV-B radiation (Staaij *et al*. 2002).

The interest in luteolin and luteolin derivatives, which usually were found in some plant species is argued in the recent years (Pirvu *et al*. 2020). The advantages offered by luteolin derivatives as opposed to other flavonoid subclasses are related to their chemical structures: they are more resistant to the (auto)oxidation process and resistant to acidic hydrolysis (Pirvu *et al*. 2014), as well as more stable and safer, and able to pass through the cell membranes more easily. Luteolin inhibits the angiogenesis process induced by the vascular endothelial growth factor, which reduces the chance of colonization of metastatic targets, a key step in the neovascularization and tumour invasion process, inhibits the secretion of interleukin (IL)-8 and IL-6, acting as an amplifying signal of cancer cell growth and invasion, promotes cell cycle arrest in breast cancer, acts as an insulin-like growth factor-mediated proliferation antagonist (Sun *et al*. 2015; Lin *et al*. 2017; Pirvu *et al*. 2020). Ten flavonoid derivatives of luteolin and apigenin were isolated from the flowers of *Trollius europaeus* L. and identified. The investigation into the antioxidant activity revealed that orientin 2″-O-α--arabinopyranoside and orientin 2″-O-β-glucopyranoside had a significant antioxidant effect (Witkowska-Banaszczak 2018).

Orientin belongs to the class of C-glycosides whereas the majority of plant flavonoids are O-glycosides. In flavonoid C-glycosides the sugar moiety is linked with the flavonoid skeleton through a C-C bond. Flavonoid C-glycosides fulfil the role of siderophores in plant tissues (Pirvu *et al*. 2020) and possess various biological activities such as antidiabetic, anti-inflammatory, and antiaging (Xiao *et al*. 2016). This class of flavonoids is not widely present in plants (Yang *et al*. 2018). The main of them (orientin, isoorientin, vitexin, isovitexin) were found in some other plant species, including buckwheat (Kim *et al*. 2008; Salehi *et al*. 2019; Borovaya and Klykov 2020), *Lythrum salicaria* L. (Srećković *et al*. 2020), *Morinda citrifolia* L. (Zhu *et al*. 2020), *Vitis berlandieri* Planch. (Kedrina--Okutan *et al*. 2019), *Sophora viciifolia* Hance flowers (Lin *et al*. 2019), *Vitex grandifolia* Gürke (Bello *et al*. 2019), *Clinacanthus nutans* (Brum. F.) Lindau (Esmailli *et al*. 2019), *Passiflora quadrangularis* L. (Echeverry *et al*. 2018), *Cyperus rotundus* L. (Ibrahim *et al*. 2018). The known source of orientin is buckwheat sprouts and it is believed that this and others flavonoid presence increase their value as functional food. It emphasizes the potential value of orientin-rich *Deschampsia* leaves. It is worth to note that the content or orientin in *Deschampsia* aerial part is higher than in mentioned flavonoids-rich sprouts: the *Deschampsia* leaves contain about 2–5 mg/g of orientin whereas buckwheat sprouts contain only 0.02-0.8 mg/g DW (Krahl *et al*. 2008).

Phenolics content may depend on environmental conditions such as photoperiod, altitude, level of UV radiation, humidity, and temperature (Day *et al*. 2001; Ruhland *et al*. 2005; Jaakola and Hohtola 2010). It was found that

the concentration of flavonoids, such as orientin and luteolin increased in *D. antarctica* plants that is influenced by UV-B radiation (Day *et al*. 2001; Sequeida *et al.* 2012). It should be noted that, in our study, all plants were cultivated under standard conditions and were not exposed to any external influences that could alter the accumulation of secondary metabolites. Therefore, it can be assumed that the differences in the content of phenolic compounds and flavonoids, as well as in some other phenotypic characteristics (including leaf length) (Poronnik *et al*. 2017; Navrotska *et al.* 2018), between *D. antarctica* plants cultured *in vitro*, are largely due to the environmental conditions in the wild habitats from which the plant originated from and adaptation to which may cause genetic and epigenetic changes in some way.

The study involved different chromosomal forms of *D. antarctica*, including diploids with the typical karyotype of 26 chromosomes (DAR13, R30, G/D12-2a, L57 – 2n = 26), diploid with B chromosomes (DAR12 – 2n = 26+0–3B), hypotriploid with Robertsonian translocation (Y66 – 2n = 36–38) and a myxoploid (Y67 – 2n = 26, 39) (Navrotska *et al*. 2014, 2018; Amosova *et al*. 2015). Biochemical analysis did not reveal significant differences between different chromosomal forms in the content of phenolic compounds and flavonoids. It should only be noted that the hypotriploid (Y66) grown in a growth chamber had the highest content of the phenolic compounds, while the content of flavonoids in this sample was the lowest. The absence of differences in the content of phenolics and flavonoids in different chromosome forms of *D. antarctica* was also shown by Navrotska *et al*. (2014).

Previous study showed that the total content of flavonoids in the plants of *D. antarctica* from different localities of the Argentine Islands ranged from 1.22 mg/g to 4.67 mg/g (Navrotska *et al*. 2018). The flavonoids content for diploid genotypes ranged from 1.62 to 4.67 mg/g. The DAR12 plants with B chromosomes had the lowest amount of flavonoids (1.22 mg/g). In the hypotriploid (Y66) the total flavonoids content was 2.23 mg/g, which falls within the range observed for the diploid genotypes. Furthermore, the chromatographic profiles of the flavonoids from the leaf extracts were almost similar for different *D. antarctica* clones. Slight quantitative differences were observed only for individual peaks in the profiles of the hypotriploid plants compared to the diploid ones (Navrotska *et al*. 2018). We assume that the discrepancies between our data on flavonoid content in the studied samples of *D. antarctica* with the results obtained by previous researchers may be due to differences in growing conditions (including lighting), duration of *in vitro* culturing, and methods of drying plant material for biochemical analysis. Furthermore, there have been reported that the content of flavonoids, in particular orientin, may vary depending on extraction conditions. This may be due to differences in the solvents employed and the extraction method (Echeverry *et al*. 2018). Extraction of *Passiflora quadrangularis* leaves with 25% aqueous ethanol resulted in a slight increase in the content of orientin-2″-O-glucoside and orientin-2″-O-xyloside, as well as in

a significant decrease in the content of vitexin-derived flavonoids as compared to higher ethanol concentrations (Echeverry *et al*. 2018). This fact may be related to the greater affinity of the solvent used (higher proportion of water in the mixture) for orientin derivatives, as its luteolin nucleus has a higher number of phenolic hydroxyls groups than vitexin derivatives with apigenin nucleus. Similar findings have been previously reported for other species (Wang *et al*. 2014).

In addition, it is known that the contents of polyphenols, flavonoids, and alkaloids are different in extracts from different parts of the plant (flowers, leaves, and fruits). The total content of flavonoids in *Sophora viciifolia* was shown to be the highest in the leaf (15.4 mg/g of rutin) (Lin *et al*. 2019). The authors explain this may be due to prolonged exposure to environment factors, oxidative stress, photosynthesis, and other conditions that contribute to the synthesis of more flavonoids in the leaves. Total flavonoids in flowers measured only 1.35 mg/g DW, probably due to a short flowering period, thus a shorter time exposed to sunlight than leaves (Lin *et al*. 2019). Carvalho *et al*. (2010) showed that a longer photoperiod promotes the synthesis of flavonoids in the leaves of sweet potatoes (*Ipomoea batatas* L.). It has been shown that treatment with low temperature and light could significantly improve the expression of flavonoid synthesis in the pulp of grape berries (*Vitis vinifera* L.) (Azuma *et al*. 2012).

Our data demonstrated that *D. antarctica* plants grown in a growth chamber contained increased amounts of phenolic compounds and reduced amounts of flavonoids as compared to the plants of the same clones cultured *in vitro*. A similar difference in the content of phenolic compounds and flavonoids between aseptic plants and plants grown in a growth chamber was also observed for other plant species, such as *Fittonia albivenis* (Lindl. ex Veitch) Brummitt. (Belokurova *et al*. 2019).

It is known that the biochemical properties of plants producing BAS may depend on both the conditions of growth (cultivation) and genetic characteristics of the plants. Our results indicate the variation in the amounts of phenolic compounds and flavonoids contained in *D. antarctica* wild and *in vitro* grown plants of different genotypes that may be a manifestation of genetic variation at the species level, thus necessitating further biochemical studies and careful selection of plants for the use as a possible source of BAS.

Conclusions

The results of the biochemical analysis demonstrated for the first time that the aerial part of *D. antarctica* plants has a higher content of these compounds compared to the roots. The largest amount of phenolic compounds and flavonoids was found in wild plants from Great Yalour Island. The highest content of phenolic compounds was also found in the hypotriploid plant Y66 grown in a growth chamber, which originated from the same island. The content of

phenolic compounds and flavonoids in *in vitro* plants is generally comparable to that in wild-grown plants with the exception of individual genotypes. No significant difference was found in the amount of phenolic compounds and flavonoids between plants differing in chromosome number. HPLC analysis revealed five main substances present in similar ratios in the studied samples of wild and *in vitro* plants. The main substances have not been identified so far except for orientin, which was found in all studied samples of *D. antarctica*. Minor substances differed slightly in different extracts, however no dependence of their presence on plant growth conditions was found. No significant qualitative changes in HPLC-profiles (appearance of new peaks or disappearance of existing ones) of the studied plant samples were detected *in vitro* in comparison to the wild plants.

The high content of phenolic compounds in *in vitro* plants and regenerated plants gives grounds to consider the *in vitro* culture of *D. antarctica* as a potential source for obtaining appropriate BAS, promising for use for therapeutic and prophylactic purposes.

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References

AHMED E., ARSHAD M., KHAN M.Z., AMJAD M.S., SADAF H.M., RIAZ I., SABIR S., AHMAD N. and SABAOON. 2017. Secondary metabolites and their multidimensional prospective in plant life. *Journal of Pharmacognosy and Phytochemistry* 6: 205–214.

AMMON H. P.T. and WAHL M.A. 1991. Pharmacology of *Curcuma longa. Planta medica* 57: 1–7.

AMOSOVA A.V., BOLSHEVA N.L., SAMATADZE T.E., TWARDOVSKA M.O., ZOSHCHUK S.A., ANDREEV I.O., BADAEVA E.D., KUNAKH V.A. and MURAVENKO O.V. 2015. Molecular Cytogenetic Analysis of *Deschampsia antarctica* Desv. (*Poaceae*), Maritime Antarctic. *PLoS ONE* 10: e0138878.

ARAÚJO C.A.C. and LEON L.L. 2001. Biological activities of *Curcuma longa* L. *Memórias do Instituto Oswaldo Cruz* 96: 723–728.

AZUMA A., YAKUSHIJI H., KOSHITA Y. and KOBAYASHI S. 2012. Flavonoid biosynthesis-related genes in grape skin are differentially regulated by temperature and light conditions. *Planta* 236: 1067–1080.

BELLO O.M., OGBESEJANA A.B., ADETUNJI C.O. and OGUNTOYE S.O. 2019. Flavonoids isolated from *Vitex grandifolia*, an underutilized vegetable, exert monoamine A & B inhibitory and anti-inflammatory effects and their structure activity relationship. *Turkish Journal of Pharmaceutical Sciences* 16: 437–443.

BELOKUROVA V., LYSTVAN K., VOLGA D., VASYLENKO M. and KUCHUK M. 2019. *In vitro* culture and some biochemical characteristics of *Fittonia albivenis* (Lindl. ex Veitch) Brummitt. *Agrobiodiversity for improving nutrition, health and life quality* 3: 186–194.

BHAT S.V., NAGASAMPAGI B.A. and SIVAKUMAR M. 2005. *Chemistry of natural products*. New Delhi, Narosa Publishing House.

BOROVAYA S.A. and KLYKOV A.G. 2020. Some aspects of flavonoid biosynthesis and accumulation in buckwheat plants. *Plant Biotechnology Reports* 14: 213–225.

BRAKENCHIELM E.R. and CAO Y. 2001. Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. *FASEB Journal* 15: 1798–1800.

CARVALHO I.S., CAVACO T., CARVALHO L.M. and DUQUE P. 2010. Effect of photoperiod on flavonoid pathway activity in sweet potato (*Ipomoea batatas* (L.) Lam.) leaves. *Food Chemistry* 118: 384–390.

COTELLE N. 2001. Role of flavonoids in oxidative stress. *Current Topics in Medicinal Chemistry* 1: 569–590.

DAY T.A., RUHLAND C.T. and XIONG F.S. 2001. Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4–year field study. *Journal of Photochemistry and Photobiology B: Biology* 62: 78–87.

ECHEVERRY S.M., MEDINA H.I., COSTA G.M. and ARAGÓN D.M. 2018. Optimization of flavonoid extraction from *Passiflora quadrangularis* leaves with sedative activity and evaluation of its stability under stress conditions. *Revista Brasileira de Farmacognosia* 28: 610–617.

ESMAILLI K., SHAFAEI A., AISHA A.F.A., AL-SUEDE F.S.R., MAJID A.M.S.A. and ISMAIL Z. 2019. Evaluation of *in vitro* cytotoxicity effect of *Clinacanthus nutans* (Brum. F.) Lindau standardized leaf extracts. *Tropical Journal of Pharmaceutical Research* 18: 2341–2348.

GAMBORG O.L. and EVELEIGH D.E. 1968. Culture methods and detection of glucanases in cultures of wheat and barley. *Canadian Journal of Biochemistry* 46: 417–421.

GIDEGEL M., WEBER H., CABRERA G., GUTIERRES A., OSORIO J., BECERA J., PODHAJCER O., CAFFERATA E., SUNKEL C. and MIHOVILOVIC I. 2010. Extracts of *Deschampsia antarctica* Desv. with antineoplastic activity. Pat. US 2010/0310686 A1, Appl. No. 12/734,597.

GIDEGEL M., WEBER H., CABRERA G., GUTIERRES A., OSORIO J., BECERA J., PODHAJCER O., CAFFERATA E., SUNKEL C. and MIHOVILOVIC I. 2011. Extracts of *Deschampsia antarctica* Desv. with antineoplastic activity. Pat. US 2011/0177178 A1, Appl. No. 12/928,317.

GRACE S.C. 2005. Phenolics as antioxidants. *In*: E. Smirnoff (ed.) *Antioxidants and Reactive Oxygen Species in Plants*. Blackwell Publishing: 141–168.

IBRAHIM S.R.M., MOHAMED G.A., ALSHALI K.Z., HAIDARI R.A.A., EL-KHOLY A.A. and ZAYED M.F. 2018. Lipoxygenase inhibitors flavonoids from *Cyperus rotundus* aerial parts. *Revista Brasileira de Farmacognosia* 28: 320–324.

JAAKOLA L. and HOHTOLA A. 2010. Effect of latitude on flavonoid biosynthesis in plants. *Plant, Cell & Environment* 33: 1239–1247.

KEDRINA-OKUTAN O., NOVELLO V., HOFFMANN T., HADERSDORFER J., SCHNEIDER A., SCHWAB W. and FERRANDINO A. 2019. Polyphenolic diversity in *Vitis* sp. leaves. *Scientia Horticulturae* 256: 108569.

KIM S.J., ZAIDUL I.S.M., SUZUKI T., MUKASA Y., HASHIMOTO N., TAKIGAWA S., NODA T., MATSUURA-ENDO C. and YAMAUCHI H. 2008. Comparison of phenolic compositions between common and tartary buckwheat (*Fagopyrum*) sprouts. *Food Chemistry* 110: 814–820.

KONVALYUK I.I., MOZHYLEVS'KA L.P. and KUNAKH V.A. 2019. Callus initiation and organogenesis *in vitro* in *Deschampsia antarctica* E. Desv. *Visnik ukrains'kogo tovaristva genetikiv i selekcioneriv* 17: 8–15 (in Ukrainian).

KRAHL M., BACK W., ZARNKOW M. and KREISZ S. 2008. Determination of optimised malting conditions for the enrichment of rutin, vitexin and orientin in common buckwheat (*Fagopyrum esculentum* Moench). *Journal of the Institute of Brewing* 114: 294–299.

KREPS J.A., WU Y., CHANG H., ZHU T., WANG X. and HARPER J.F. 2002. Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. *Plant Physiology* 130: 2129–2141.

KUNAKH V.A. 2005. *Biotechnology of medicinal plants. Genetic, physiological and biochemical basis*. Kyiv, Logos (in Ukrainian).

LIN B.B., LIU X., WU S.Q., ZHENG H.X., HUO K.K., QI S.S. and CHEN C. 2019. Phytochemicals content, antioxidant and antibacterial activities of *Sophora viciifolia*. *Chemistry and Biodiversity* 16: e1900080.

LIN D., KUANG G., WAN J., ZHANG X., LI H., GONG X. and LI H. 2017. Luteolin suppresses the metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition via downregulation of β-catenin expression. *Oncology Reports* 37: 895–902.

LUTZ C., BLASSNIGG M. and REMIAS D. 2008. Different flavonoid patterns in *Deschampsia antarctica* and *Colobanthus quitensis* from the marine Antarctic. *Berichte zur Polar- und Meeresforschung* 571: 192–199.

MALVICINI M., GUTIERREZ-MORAGA A., RODRIGUEZ M.M., GOMEZ-BUSTILLO S., SALAZAR L., SUNKEL C., NOZAL L., SALGADO A., HIDALGO M., LOPEZ-CASAS P.P., NOVELLA J.L., VAQUERO J.J., ALVAREZ-BUILLA J., MORA A., GIDEKEL M. and MAZZOLINI G. 2018. A tricin derivative from *Deschampsia antarctica* Desv. inhibits colorectal carcinoma growth and liver metastasis through the induction of a specific immune response. *Molecular Cancer Therapeutics* 17: 966–976.

MARINOVA D., RIBAROVA F. and ATANASSOVA M. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy* 40: 255–260.

MONTIEL P., SMITH A. and KEILLER D. 1999. Photosynthetic responses of selected Antarctic plants to solar radiation in the southern maritime Antarctic. *Polar Research* 18: 229–235.

MURAKAMI S., NAKATA R., ABOSHI T., YOSHINAGA N., TERAISHI M., OKUMOTO Y., ISHIHARA A., MORISAKA H., HUFFAKER A., SCHMELZ E. and MORI N. 2014. Insect--induced daidzein, formononetin and their conjugates in soybean leaves. *Metabolites* 4: 532–546.

NAVROTSKA D.O., ANDREEV I.O., BETEKHTIN A.A., ROJEK M., PARNIKOZA I.YU., MYRYUTA G.YU., PORONNIK O.O., MIRYUTA N.YU., SZYMANOWSKA-PUŁKA J., GRAKHOV V.V., IVANNIKOV R., HASTEROK R. and KUNAKH V.A. 2018. Assessment of the molecular cytogenetic, morphometric and biochemical parameters of *Deschampsia antarctica* from its southern range limit in maritime Antarctic. *Polish Polar Research* 39: 525–548.

NAVROTSKA D.O., TWARDOVSKA M.O., ANDREEV I.O., PARNIKOZA I.YU., BETEKHTIN A.A., ZAHRYCHUK O.M., DROBYK N.M., HASTEROK R. and KUNAKH V.A. 2014. New forms of chromosome polymorphism in *Deschampsia antarctica* Desv. from the Argentine Islands of the Maritime Antarctic region. *Ukrainian Antarctic Journal* 13: 185–191.

OLIVEIRA F.G.S., GOMES DE LIMA-SARAIVA S.R., OLIVEIRA A.P., RABÊLO S.V., ROLIM L.A. and SILVA ALMEIDA J.R.G. 2016. Influence of the extractive method on the recovery of phenolic compounds in different parts of *Hymenaea martiana* Hayne. *Pharmacognosy Research* 8: 270–275.

PĘKAL A. and PYRZYNSKA K. 2014. Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods* 7: 1776–1782.

PEREZ-TORRES E., GARCÍA A., DINAMARCA J., ALBERDI M., GUTIÉRREZ A., GIDEKEL M., IVANOV A.G., HUNER N.P.A., CORCUERA L.J. and BRAVO L.A. 2004. The role of photochemical quenching and antioxidants in photoprotection of *Deschampsia antarctica*. *Functional Plant Biology* 31: 731–741.

PIRVU L., HLEVCA C., NICU I. and BUBUEANU C. 2014. Comparative analytical, antioxidant and antimicrobial activity studies on a series of vegetal extracts prepared from eight plant species growing in Romania. *Journal of Planar Chromatography* 27: 346–356.

PIRVU L., NEAGU G., TERCHESCU I., ALBU B. and STEFANIU A. 2020. Comparative studies of two vegetal extracts from *Stokesia laevis* and *Geranium pratense*: polyphenol profile, cytotoxic effect and antiproliferative activity. *Open Chemistry* 18: 488–502.

PORONNIK O.O., KUZMENKO A.V., VOLOVYK A.V., SHVACHKO L.V., VOYTSEHIVSKA O.V., MYRYUTA G.U., RUBAN T.A., PARNIKOZA I.J. and KUNAKH V.A. 2014. Plant clones of *Deschampsia* as a source of phenolic compounds with antitumor properties. *Visnik ukrains'kogo tovaristva genetikiv i selekcioneriv* 12: 200–204 (in Ukrainian).

PORONNIK O.O., PARNIKOZA I.YU., MIRYUTA N.YU., MYRYUTA G.YU., GRAHOV V.P., NAVROTSKA D.O. and KUNAKH V.A. 2017. *Deschampsia antarctica* E. Desv. plants with different chromosome number cultivated *in vitro*. Plants length and flavonoids in *in vitro* culture and in nature. *Factors in experimental evolution of organisms* 20: 310–313 (in Ukrainian).

QUATTOROCCHIO F., BAUDRY A., LEPINIEC L. and GROTEWOLD E. 2006. The regulation of flavonoid biosynthesis. *In*: E. Grotewold (ed.) *The science of flavonoids*. Springer, New York: 97–122.

REN W., QIAO Z., WANG H., ZHU L. and ZHANG L. 2003. Flavonoids: promising anticancer agents. *Medical Research Reviews* 23: 519–534.

RUHLAND C., XIONG F., CLARK W. and DAY T.A. 2005. The influence of ultraviolet-B radiation on growth, hydroxycinnamic acids and flavonoids of *Deschampsia antarctica* during springtime ozone depletion in Antarctica. *Photochemistry and Photobiology* 81: 1086–1093.

SALEHI A., FALLAH S., ZITTERL-EGLSEER K., KAUL H.-P., SURKI A.A. and MEHDI B. 2019. Effect of organic fertilizers on antioxidant activity and bioactive compounds of fenugreek seeds in intercropped systems with buckwheat. *Agronomy* 9: 367.

SEQUEIDA A., TAPIA E. and ORTEGA M. 2012. Production of phenolic metabolites by *Deschampsia antarctica* shoots using UV-B treatments during cultivation in a photobioreactor. *Electronic Journal of Biotechnology* 15: 1–8.

SINGLETON V.L. and ROSSI J.A. 1965. Colorimetry of total phenolics with phosphomolybdic--phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144–158.

SREĆKOVIĆ N., KATANIĆ STANKOVIĆ J.S., MATIĆ S., MIHAILOVIĆ N.R., IMBIMBO P., MONTI D.M. and MIHAILOVIĆ V. 2020. *Lythrum salicaria* L. (Lythraceae) as a promising source of phenolic compounds in the modulation of oxidative stress: Comparison between aerial parts and root extracts. *Industrial Crops and Products* 155: 112781.

STAAIJ J., DE BAKKER N.V.J., OOSTHOEK A., BROEKMAN R., VAN BEEM A., STROETENGA M., RIEN A. and ROZEMA J. 2002. Flavonoid concentrations in three grass species and a sedge grown in the field and under controlled environmental conditions in response to UV–B radiation. *Journal of Photochemistry and Photobiology B: Biology* 66: 21–29.

SUN D.W., ZHANG H.D., MAO L., MAO C.F., CHEN W., CUI M., MA R., CAO H.X., JING C.W., WANG Z., WU J.Z. and TANG J.H. 2015. Luteolin inhibits breast cancer development and progression *in vitro* and *in vivo* by suppressing notch signaling and regulating miRNAs. *Cellular Physiology and Biochemistry* 37:1693–1711.

TANASE C., COSARCĂ S. and MUNTEAN D.L. 2019. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules* 24: 1182.

TATTINI M., GUIDI L., MORASSI-BONZI L., PINELLI P., REMORINI D., INNOCENTI E., GIORDANO C., MASSAI R. and AGATI G. 2005. On the role of flavonoids in the integrated mechanisms of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation. *New Phytologist* 167: 457–470.

VENKATESAN A., KATHIRVEL A., PRAKASH S. and SUJATHA V. 2017. Antioxidant, antibacterial activities and identification of bioactive compounds from *Terminalia chebula* bark extracts. *Free Radicals and Antioxidants* 7: 43–49.

VERVERIDIS F., TRANTAS E., DOUGLAS C., VOLLMER G., KRETZSCHMAR G. and PANOPOULOS N. 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part II: Reconstruction of multienzyme pathways in plant and microbes. *Biotechnology Journal* 2: 1235–1249.

WANG H., YANG L., ZU Y. and ZHAO X. 2014. Microwave-assisted simultaneous extraction of luteolin and apigenin from tree peony pod and evaluation of its antioxidant activity. *The Scientific World Journal* 2014: 506971.

WEBBY R. and MARKHAM K. 1994. Isoswertiajaponin 2”-O-β-arabinopyranoside and other flavone-C-glycosides from the Antarctic grass *Deschampsia antarctica*. *Phytochemistry* 36: 1323–1326.

WITKOWSKA-BANASZCZAK E. 2018. Flavonoids from *Trollius europaeus* flowers and evaluation of their biological activity. *Journal of Pharmacy and Pharmacology* 70: 550–558.

XIAO J., CAPANOGLU E., JASSBI A.R. and MIRON A. 2016. Advance on the Flavonoid C-glycosides and Health Benefits. *Critical reviews in food science and nutrition* 56: 29–45.

YADAV P. and MALPATHAK N. 2016. Estimation of antioxidant activity and total phenol, flavonoid content among natural populations of caper (*Capparis moonii*, Wight) from Western Ghats region. *Indian Journal of Pharmaceutical Education and Research* 50: 495–501.

YANG B., LIU H., YANG J., GUPTA V.K. and JIANG Y. 2018. New insights on bioactivities and biosynthesis of flavonoid glycosides. *Trends in food science and technology* 79: 116–124.

ZAHRYCHUK O.M., DROBYK N.M., KOZERETSKA I.A., PARNIKOZA I.YU. and KUNAKH V.A. 2011/2012. Introduction in culture *in vitro* of *Deschampsia antarctica* Desv. (Poaceae) from two regions of Maritime Antarctica. *Ukrainian Antarctic Journal* 10/11: 289–295 (in Ukrainian).

ZHU H., ZHANG J., LI C., LIU S. and WANG L. 2020. *Morinda citrifolia* L. leaves extracts obtained by traditional and eco-friendly extraction solvents: relation between phenolic compositions and biological properties by multivariate analysis. *Industrial Crops and Products* 153: 112586.

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