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

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Abstract: The paper presents data on the total phenolic and flavonoid content in Deschampsia antarctica É. 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 (Esmaili 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 (Brakenchiel 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;
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
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 Na₂CO₃ 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).

<table>
<thead>
<tr>
<th>№</th>
<th>Clone</th>
<th>Locality, geographical coordinates and year of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>R30</td>
<td>Rasmussen Oasis, 65°14.819´ S, 64°5.156´ W, 2005</td>
</tr>
<tr>
<td>5</td>
<td>Y66</td>
<td>Great Yalour Island (the biggest of the Yalour Islands), 65°14.039´ S, 64°09.761´ W, 2005</td>
</tr>
<tr>
<td>6</td>
<td>Y67</td>
<td>Great Yalour Island, 65°14.039´ S, 64°09.761´ W, 2005</td>
</tr>
<tr>
<td>7</td>
<td>L57</td>
<td>Lahille Island, 65°33.167´ S, 64°23.249´ W, 2010</td>
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</table>
The appropriate volume of extract was adjusted to 1 mL with 96% ethanol. The resulting solution was mixed with 360 μL of 5% NaNO₂ and incubated for 5 minutes, then 600 μL of 2% AlCl₃ 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:

\[
C_f = C \times k / m,
\]

where \(C_f\) 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·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.
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.

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

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.
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).](image)
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 \textit{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 \textit{in vitro} plants contained 2.5 times more flavonoids than DAR13 \textit{in vitro} plants (the difference is significant at p<0.05). \textit{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 \textit{in vitro} plant (the difference is significant at p<0.05). Two samples of \textit{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).

\textbf{Qualitative analysis of phenolic compounds and flavonoids.} — We conducted qualitative analysis of phenolic compounds and flavonoids extracted from different \textit{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 \textit{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 \textit{D. antarctica} leaf tissue that were fractionated by HPLC and analysed at 318 nm.

![HPLC chromatogram of phenolic compounds from the leaf extracts of \textit{D. antarctica} (detection wavelength of 318 nm): 1,3–5 – unidentified substances, 2 –orientin.](image-url)
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.](image)

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
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 13C 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-metilorienten 2"-O-arabinopyranoside), isoswertijaponin ((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. (Srečković 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 (Esmaili 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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