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Chromatographic and mass spectrometric analysis of secondary metabolites of *Deschampsia antarctica* from Galindez Island, Argentine Islands

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Abstract: The aim of this work was to study the polyphenolic composition of *Deschampsia antarctica* È. Desv. plants grown in natural conditions at different locations on the Galindez Island, Argentine Islands, the maritime Antarctic. The plants were collected during the summer season of the 26th Ukrainian Antarctic Expedition (2020–2022). The extracts of 21 plants were obtained and the composition of the extracts was analyzed by means of high-performance liquid chromatography and matrix-assisted laser desorption/ionization mass spectrometry. The antioxidant properties of the extracts were characterized using the DPPH (2,2-diphenyl-1-picrylhydrazyl) test. The extracts contained large amount of polyphenolic compounds, with flavonoids and phenolic acids, as well as their derivatives, being the most

common classes of the phenols. Using the HPLC data the content of various phenols in the plants was systematic studied. It has been found that in all plants the most abundant phenols were flavonoids/flavonoid derivatives (on average about 75% of total mass of phenols). Among the flavonoids, luteolin derivatives predominated (86–94% of the total mass of flavonoids), and, among luteolin derivatives, the main compounds were orientin, orientin 2"-*O*-*β*-arabinopyranoside and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside (67–83% of the total mass of luteolin derivatives). It has been also found that all the extracts had high activity in inhibition of DPPH radicals and that the antioxidant activity of the extracts correlated with total content of phenols in the samples. Thus, *Deschampsia antarctica* É. Desv. plants are a valuable source of natural phenolic antioxidants, and the most common antioxidants in the extracts are orientin, orientin 2"-*O*-*β*-arabinopyranoside and isoswertia-japonin 2"-*O*-*β*-arabinopyranoside.

Keywords: West Antarctic, antarctic hairgrass, polyphenolic antioxidants, flavonoids, luteolin derivatives.

Introduction

Plant secondary metabolites are widely used by mankind. Special attention is paid to the search for plant raw materials with a high content of biologically active compounds, including phenolic metabolites that possess antioxidant, anti-inflammatory, and antimicrobial properties. Natural complexes of biologically active compounds have a positive effect on physiological processes in the human body and on increase in its resistance. Typically, the content of bioactive compounds in most plants depends on their species, physiological state, stage of development, climate of the area, and cultivation technology (Kunakh 2005). The synthesis of polyphenols in plants is known to increase under stressful conditions, such as infection, damage, or UV irradiation (Ahmed *et al.* 2017). Therefore, there are reasons to consider Antarctic flowering plants growing in extreme environmental conditions and under high radiation levels as a promising source of polyphenolic compounds (Montiel *et al.* 1999; Zamora *et al.* 2013; Gidekel 2014; Köhler *et al.* 2017).

Interest in *Deschampsia antarctica* É. Desv. plants growing in the harsh Antarctic conditions led to the appearance of number of works devoted to the investigation of the biochemical composition of the plant extracts, their antioxidant properties and possible application areas. Thus, for instance, it has been shown (Pereira *et al.* 2009; Gidekel *et al.* 2014; Perez-Davó *et al.* 2019; Zamarrón *et al.* 2019) that the compounds extracted from *D. antarctica* plants demonstrate antioxidant activity and may be used as photoprotective and antiaging agents. *D. antarctica* plant extract was found to protect human skin fibroblasts from the action of aggressive oxidants and environmental pollutants (Ortiz-Espin *et al.* 2017; Fernández-Martos *et al.* 2021). The biological activity of extracts is associated, in particular, with the availability of substances with antioxidant properties, *e.g.*, polyphenols. It has been proven that the phenolic

metabolites of *D. antarctica* have antitumor activity and are able to inhibit the proliferation of melanoma cells (Gidekel *et al.* 2010; Poronnik *et al.* 2014), suppress the growth of colorectal carcinoma and liver metastases (Malvicini *et al.* 2018). Several studies were devoted to an assessment of the total content of phenolic compounds in *D. antarctica* plants, using the Folin-Ciocalteu method, and of the content of flavonoids, using a characteristic color reaction with aluminum chloride (Sequeida *et al.* 2012; Köhler *et al.* 2017; Twardovska *et al.* 2021). Nine main flavonoids (derivatives of luteolin, apigenin, and tricetin) were identified in the majority of *D. antarctica* plants (Webby and Markham 1994). A number of studies was devoted to the influence of plants growing conditions on the content of useful compounds (van de Staaij *et al.* 2002). Another direction of research is the introduction of the plant into *in vitro* culture and the investigation of the biochemical composition of the plants grown under such artificial conditions (Zahrychuk *et al.* 2011, 2012; Sequeida *et al.* 2012; Cortés-Antiquera *et al.* 2021). Cultivation of *D. antarctica in vitro* is expected to provide an opportunity to produce bioactive compounds under standard conditions and in the required quantity (Twardovska *et al.* 2021). Despite the growing volume of the studies on *D. antarctica*, the data on the biochemical composition of the plants are still incomplete. Also, there are no reliable quantitative data on the content of various polyphenols in the plants.

The aims of this work were to identify the main polyphenols available in *D. antarctica* growing in several locations of the Galindez Island (Argentine Islands), to evaluate the content of these polyphenolic compounds in the plants and to study the antioxidant properties of the plant extracts. Plant samples were collected in open and relatively sheltered (protected) areas from eleven different locations on Galindez Island during the summer season of the 26th Ukrainian Antarctic Expedition.

Materials and methods

The origin and basic characteristics of plant material. — For biochemical screening of the samples, plant material comprising of undamaged green parts of *D. antarctica* cushions, collected during the summer season of the 26th Ukrainian Antarctic Expedition (2020–2021), was used. Plants were collected in both relatively open and more protected areas of eleven populations of Galindez Island. The corresponding samples are marked with the letters O (open) and P (protected). Plants from the so-called "protected areas" are plants that did not grow on completely open surfaces but were partially sheltered from the influence of wind and sun, *e.g.*, these are plants in crevices, or those that look out from under stones. Despite the fact that the protection factor has a somewhat tentative character, we used this approach in an attempt to clarify the influence of plant growth conditions on the synthesis of useful metabolites. The places of plant

selection on Galindez Island are marked in Fig. 1, and the list of samples and the geographical coordinates of the sites are given in Table 1. The localities, where the samples were collected, also differed from each other by the presence or absence of penguins/flying birds activity in the vicinity of the sites. In general, this factor can significantly affect the plants growth.

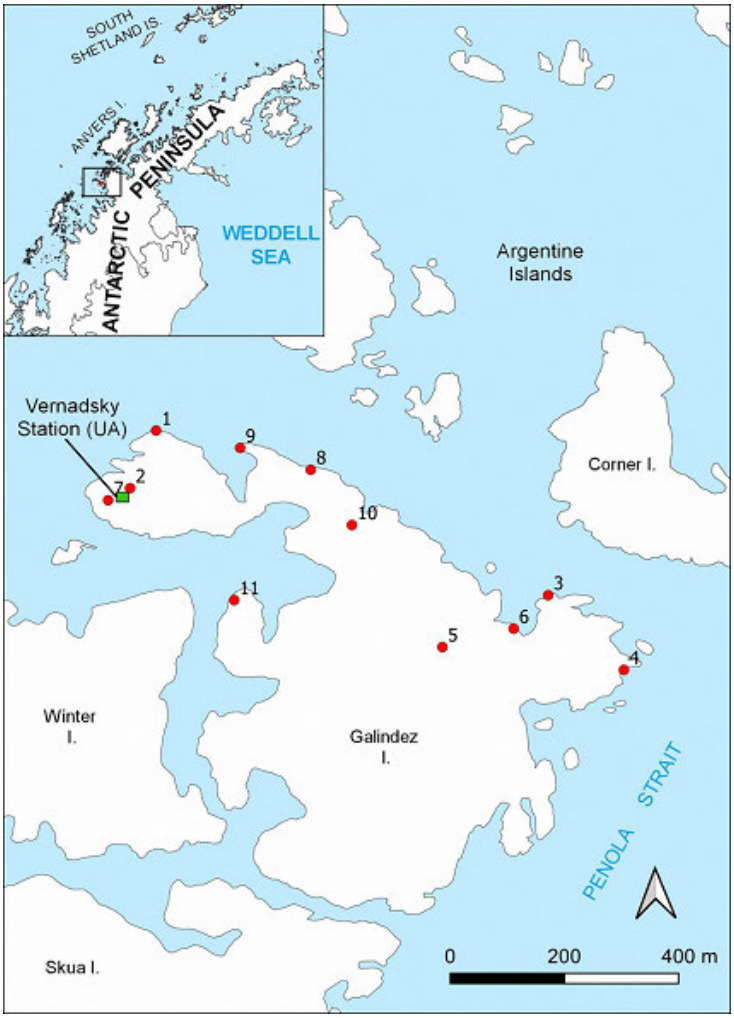

Fig. 1. Locations where *Deschampsia antarctica* É. Desv. plants were collected.

Preparation of plant extracts. — The extracts were prepared from dry plant leaves. Dry leaves were frozen to –20°C, then crushed and poured with methanol at the rate of 50 mL per 1 g of raw material. The extraction was carried out with sonication for 2 hours (4 times for 30 min with breaks of 15 min) at a temperature of 60°C. Upon completion of the ultrasound treatment, the samples were cooled and stored at –20°C.

Table 1.

Places of origin of *Deschampsia antarctica* plant samples form Galindez Island, Argentine Islands. A detailed description of permanent plots of the populations is described elsewhere (Miryuta *et al.* 2019).

Number of location (see Fig. 1)	Permanent plot of *Deschampsia antarctica* population / Sample name	Geographical Coordinates	Presence of ornithogenic influence: penguins or flying birds activity sites or nesting places
1	D1	65°14.686'S 64°15.348'W	+
2	D2	65°14.740'S 64° 15.409'W	–
3	D3	65°14.849'S 64°14.474'W	+
4	D4	65°14.921'S 64°14.307'W	+
5	D5	65°14.896'S 64°14.714'W	–
6	D6	65°14.880'S 64°14.553'W	+
7	D7	65°14.751'S 64°15.459'W	–
8	D9	65°14.726' S 64°15.002' W	–
9	D10	65°14.704'S 64°15.160'W	+
10	D11	65°14.779'S 64°14.912'W	–
11	D12	65°14.842'S 64°15.206' W	+

Analysis of the extracts composition by high-performance liquid chromatography. — Analysis and determination of classes of biologically active substances was carried out using an automatic four-channel liquid chromatograph Agilent 1100 with a diode-matrix detector. Separations were carried out on the chromatographic column Poroshell 120 EC-C18 (2.1x150 mm 2.7 μm). The following gradient composition used for each analysis: 0–2 min – 99% A + 1% B at the flow rate 0.2 mL min^{-1}, 8 min – 92% A + 8% B at the flow 0.2 mL min^{-1}, 28 min – 67% A + 33% B at the flow 0.2 mL min^{-1}, 38 min – 1% A + 99% B at the flow rate 0.2 mL min^{-1}, 48 min – 1% A + 99% B at the flow rate 0.2 mL min^{-1}, 50 min – 1% A + 99% B at the flow rate increased to 0.6 mL min^{-1}, 69 min – 1% A + 99% B at the flow rate 0.6 mL min^{-1}, 78 min – 99% A + 1% B at the flow rate decreased to 0.2 mL min^{-1}, where A is water (0.05 M H_3PO_4) and B is acetonitrile. The injection volume was 5 μL, column temperature was 20°C at 0 min and increased to 40°C at 45 min. Detection was performed at the wavelengths of 206 nm, 230 nm, 242 nm, 372 nm, and 550 nm.

The content of substances in raw materials was calculated according to the formula:

$$C = \frac{A \cdot 1000 \cdot V}{\varepsilon \cdot V_{inj} \cdot m_{rm}}$$

where C is the concentration of a substance, mg g^{-1}; A – the peak area for a substance in a chromatogram; ε – the proportionality factor, mg^{-1}; V_{inj} – a volume of the sample injected into the chromatograph, μL; m_{rm} - the row material mass, g; V - the total volume of an extract, mL.

The content of individual classes of substances was recalculated to that for substances belonging to this class or having a very similar structure: the content of hydroxycinnamic acids (OC) was recalculated to chlorogenic acid; derivatives of simple phenols and hydroxybenzoic acids (OB) – to gallic acid; apigenin derivatives (AD) – to vitexin; derivatives of luteolin (LD) and tricetin (TD) as well as of other flavonoids (FLO) – to orientin.

Analysis of the extracts composition by matrix-assisted laser desorption/ionization mass spectrometry. — Qualitative analysis of the extracts was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI MS). Mass spectra were recorded in positive ion registration mode using an Autoflex II mass spectrometer (Bruker Daltonics Inc., Germany) equipped with a nitrogen laser (337 nm). Sample preparation for the analysis was carried out as follows. 1 μL of the extract solution was applied to the steel target, and after drying an additional 1 μL of the matrix solution was applied. *α*-cyano-4-hydroxycinnamic acid (HCCA, a saturated solution in a mixture of acetonitrile, deionized water, and trifluoroacetic acid in a volume ratio of 70:30:0.1) was used as a matrix. After drying the samples were subjected to laser desorption/ionization in the pulse mode: the duration of the laser pulse was 3 ns, and the frequency was 20 Hz. Spectra were recorded in linear mode with an ion extraction delay of 10 ns and an accelerating voltage of 20 kV. The resulting spectra were the sum of 20 individual spectra obtained as a result of irradiation with 25 pulses at each the individual point with the deposited sample. The laser power was determined by the optimal signal-to-noise ratio and, where possible, kept the same for different samples.

Preliminary MALDI mass spectra processing (smoothing, baseline correction, and determination of major isotope peaks) was performed using FlexAnalysis software (Bruker Daltonics, Germany). Further processing of the mass spectra (in the form of a list of individual analytically important peaks (m/z – intensity) of the main isotopes) was carried out using the mMass program (http://www.mmass.org). Using this program, the peaks belonging solely to the analyte were located and the so-called "derivatized" MALDI mass spectra were plotted. Then, using the public databases on polyphenols, carotenoids, and other plant metabolites (http://phenol-explorer.eu/; http://carotenoiddb.jp/; http://meta-

bolomics.jp/wiki/Main_Page) as well as taking into account the results of HPLC analysis and the data from the literature (Webby and Markham 1994; Hillenkamp and Peter-Katalinic 2007; Suzuki *et al.* 2009), the most probable components of *D. antarctica* plant extracts were identified.

Study on the antioxidant properties of the extracts. — The reaction with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to evaluate the antiradical activity of the extracts (Brand-Williams *et al.* 1995). Usually, according to the standard procedure of the DPPH test, 1 mL of the studied extract is added to 2 mL of 70% ethanol and 2 mL of a 0.15 mM solution of DPPH in 70% ethanol. The mixture is stirred, and the concentration of stable radicals at different times after the start of the reaction is determined spectrophotometrically by the change in optical density at the absorption maximum of the DPPH solution at 520 nm. Since all the extracts under study were highly active in the reaction (an instant disappearance of color was observed when these extracts were added to the DPPH solution), they were 10-times diluted before testing. The $DPPH_{30}$ parameter (the percentage of radicals inhibited by antioxidants within 30 min) was used to characterize the properties of the extracts.

Results

High-performance liquid chromatography analysis of the composition of the extracts. — An example of chromatograms typical for the investigated extracts of *D. antarctica* plants from Galindez Island, Argentine Islands, is shown in Fig. 2. The obtained chromatograms indicate that the extracts contain such compounds as simple phenols and hydroxybenzoic acids and/or derivatives of simple phenols and hydroxybenzoic acids (phenolic compounds of group 1); hydroxycinnamic acids and/or derivatives of hydroxycinnamic acids (phenolic compounds of group 2); flavonoids and/or flavonoid derivatives (phenolic compounds of group 3); terpenoids and sterols; catabolites of chlorophylls; carotenoids. From the point of view of the antioxidant properties of the extracts, phenolic compounds are of the greatest interest among the identified substances. Chromatograms of the extracts show the presence of several representatives of each of the three specified classes of phenols (Fig. 2). Among the flavonoids, the most intense signals belong to three luteolin derivatives, designated as LD1–LD3, respectively; other luteolin derivatives are marked as LDO. Also, in the chromatograms there are signals of such flavonoids as apigenin derivatives (AD) and tricetin derivatives (TD). The data on the content of various phenols in plants from different open/protected areas of Galindez Island are shown in Tables 2 and 3.

The obtained results indicate a high content of polyphenols in the *D. antarctica* extracts, although the amount of compounds in the plants varies significantly. The total amount of phenolic compounds in the samples varies in the range of

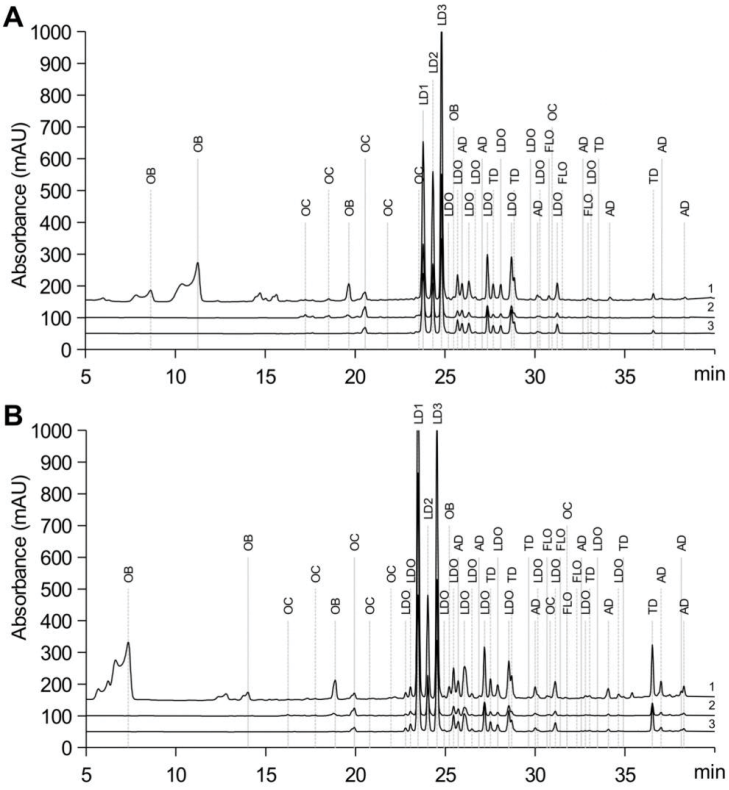

Fig. 2. Fragments of the chromatograms (1 – 206 nm, 2 – 300 nm, 3 – 350 nm) for the extracts of *Deschampsia antarctica* plants from relatively open (**A**) and from protected (**B**) places of D4 locality on Galindez Island, Argentine Islands. Peak designations: OB – derivatives of simple phenols and hydroxybenzoic acids; OC – derivatives of hydroxycinnamic acids; LD1 – luteolin derivative 1; LD2 – luteolin derivative 2; LD3 – luteolin derivative 3; LDO – other luteolin derivatives; AD – apigenin derivatives; TD – tricetin derivatives; FLO – other flavonoids.

4.0–26.3 mg g^{-1}, the amount of hydroxybenzoic, hydroxycinnamic acids and flavonoids – in the range of 1.5–4.1, 0.5–1.8 and 2.4–22.8 mg g^{-1}, respectively. Among the polyphenols, flavonoids prevail (the flavonoid fraction on average is 74% of the total content of polyphenols), and among flavonoids, luteolin derivatives predominate (86–94% of the total mass of flavonoids, Table 3). In most cases, plants from relatively protected areas have a higher phenolic content than plants from open areas, although the differences between plants from different localities can be more significant than between open and protected areas of the same locality (Fig. 3). At the same time, there is no clear effect of the presence of penguin or flying bird activity in the immediate vicinity of plant collection sites on the intensity of polyphenol synthesis in plants. Indeed, for one half of the samples (D3, D4, D12) collected near nesting sites, the content of phenols is higher than the average value for all the samples, and for the other half (D1, D6, D10) it is lower.

Table 2.

The content of compounds belonging to the main groups of phenols in *Deschampsia antarctica* plants from different localities on Galindez Island, Argentine Islands.

Sample	The content of compounds (mg g^{-1} of dry leaves)				R1 (%)
	OB	OC	Sum of flavonoids	Sum of polyphenols	
D1	2.7	0.5	9.2	12.4	74
D2, O/P	2.1/2.0	0.5/0.6	5.5/5.9	8.1/8.5	68/69
D3, O/P	2.6/4.1	1.1/1.1	10.7/14.5	14.4/19.7	74/74
D4, O/P	2.1/3.2	1.2/0.9	10.8/15.4	14.1/19.5	77/79
D5, O/P	1.5/1.5	0.9/0.7	8.3/8.6	10.7/10.8	78/80
D6, O/P	1.8/1.5	0.5/0.7	2.9/5.8	5.2/7.9	56/73
D7, O/P	2.0/2.7	0.7/1.8	10.4/12.3	13.0/16.8	80/73
D9, O/P	2.7/2.5	0.9/0.8	8.9/12.1	12.4/15.5	72/78
D10, O/P	1.3/2.1	0.3/0.6	2.4/6.2	4.0/8.9	60/70
D11, O/P	2.1/2.2	0.8/1.3	15.0/22.8	17.9/26.3	84/87
D12, O/P	2.6/3.5	1.0/1.4	11.3/15.2	14.8/20.1	76/76

O/P – open/protected location; R1 is a percentage of the total mass of flavonoids relative to the total mass of polyphenols.

Table 3.

The content of flavonoids / flavonoid derivatives in *Deschampsia antarctica* plants from different localities on Galindez Island, Argentine Islands.

Sample	Flavonoids content (mg g^{-1} of dry leaves)							R2 (%)	R3 (%)
	LD1	LD2	LD3	LDO	AD	TD	FL		
D1	2.5	1.2	2.7	2.0	0.4	0.4	9.2	91	76
D2, O/P	0.9/0.7	0.9/0.9	2.2/2.1	1.1/1.4	0.2/0.4	0.2/0.4	5.5/5.9	93/86	78/73
D3, O/P	3.0/3.7	1.3/2.0	2.9/4.9	2.5/3.1	0.4/0.4	0.6/0.5	10.7/14.5	91/94	74/77
D4, O/P	2.4/5.8	1.6/1.3	3.6/3.4	2.3/3.1	0.4/0.7	0.5/1.1	10.8/15.4	92/88	77/77
D5, O/P	2.1/2.5	1.3/1.1	3.0/2.3	1.4/2.1	0.2/0.3	0.2/0.3	8.3/8.6	94/93	82/73
D6, O/P	0.5/1.3	0.3/0.7	0.8/1.7	0.9/1.5	0.2/0.3	0.2/0.4	2.9/5.8	86/88	67/71
D7, O/P	2.6/2.7	1.3/1.8	3.4/3.5	2.1/3.0	0.4/0.6	0.6/0.7	10.4/12.3	90/89	78/73
D9, O/P	2.3/3.7	1.2/1.2	2.6/3.4	1.8/2.2	0.4/0.6	0.6/1.0	8.9/12.1	89/87	77/79
D10, O/P	0.4/1.3	0.3/0.9	1.0/2.7	0.5/1.1	0.1/0.2	0.1/0.1	2.4/6.2	92/95	77/82
D11, O/P	6.1/8.9	1.4/1.7	3.4/4.5	2.3/3.9	0.6/1.3	1.3/2.5	15.0/22.8	87/86	83/79
D12, O/P	3.5/4.5	1.1/2.0	3.5/4.1	1.9/3.0	0.5/0.7	0.7/0.9	11.3/15.2	89/90	81/78

LD – luteolin derivatives, AD – apigenin derivatives, TD – tricetin derivatives, FL – sum of flavonoids; R2 is a mass fraction (percentage) of luteolin derivatives relative to the total flavonoids; R3 is a mass fraction (percentage) of the three main luteolin derivatives relative to all its derivatives.

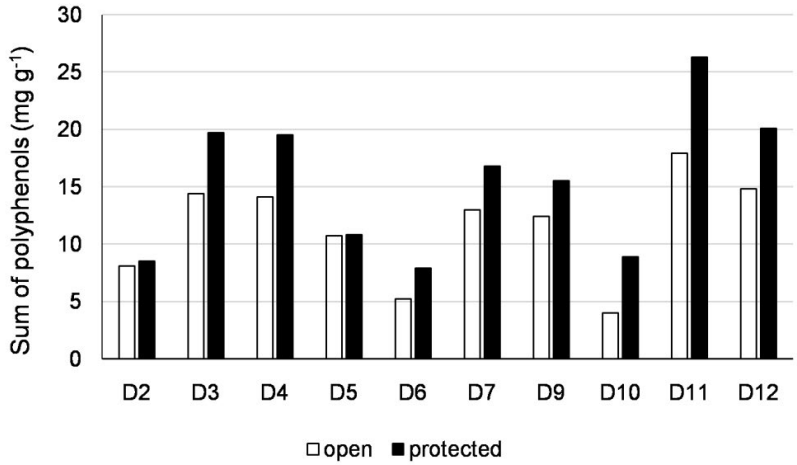

Fig. 3. Total amount of polyphenols in *Deschampsia antarctica* plants from Galindez Island, Argentine Islands, grown in open and protected areas.

Identification of biologically active compounds in the extracts by MALDI MS. — More careful identification of individual extracts constituents, in particular flavonoids, was performed by MALDI MS. An example of the derivatized MALDI mass spectra for extracts of *D. antarctica* plants from the locality D4 is shown on Fig. 4. In the mass spectra of the extracts from plants grown both in open and in protected areas, the main dominant signals are the peaks assigned to protonated molecular ions of the following flavonoids: orientin (m/z 449.1), orientin 2"-*O*-*β*-arabinopyranoside (m/z 581.2), and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside (m/z 595.2). These three compounds were shown to correspond to the three main flavonoids LD1–LD3 registered in the chromatograms of the extracts (Fig. 2). Indeed, after the chromatographic isolation of LD1, LD2, and LD3 fractions from D4 P extract, followed by their MALDI mass spectrometric analysis, it has been confirmed that the LD2 peak belongs to orientin, and it has been also revealed that LD1 corresponds to orientin 2"-*O*-*β*-arabinopyranoside, and LG3 – to isoswertiajaponin 2"-*O*-*β*-arabinopyranoside (Fig. 5).

In addition to luteolin derivatives, other flavonoids, which are characteristic of *D. antarctica* extracts (according to Webby and Markham 1994), were found in almost all the samples examined: tricin (tricetin derivative), isoswertisin (apigenin derivative), isoswertiajaponin (luteolin derivative), isosvertisin 2"-*O*-*β*-arabinoside (apigenin derivative), isoswertisin 2"-*O*-*β*-arabinoside acylated (apigenin derivative), and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside acylated (luteolin derivative). Intensities of the signals in the MALDI mass spectra, assigned to protonated molecular ions of the above nine flavonoids, as well as their molecular formulas, are given in Table 4.

Also, MALDI mass spectra contain peaks with m/z 147.1, 296.1, 493.0, assigned to protonated molecular ions of coumarin, radical cations of flavonoids

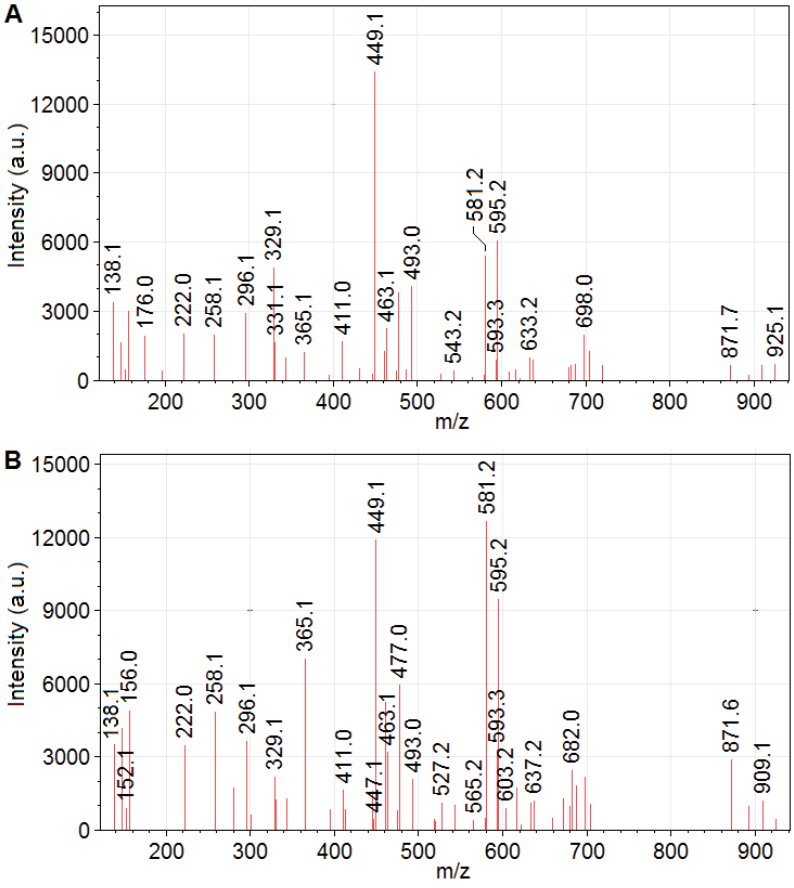

Fig. 4. Derivatized MALDI mass spectra of extracts for the *Deschampsia antarctica* plant samples from the locality D4 - open (**A**) and protected (**B**) on Galindez Island, Argentine Islands.

(derivatives of flavones/isoflavonoids) and sodium molecular cations-adducts of phenolic acids (valoneic acid dilactone), respectively. Other signals can be attributed to phenolic acids (m/z 138.1,152.1, 222.0, 343.1, 365.1), carboxylic acids (m/z 156.0, 176.0, 518.3, 520.3, 925.1), flavonoids (in addition to the nine above-mentioned typical ones, m/z 258.1, 329.1, 411.0, 413.2, 431.1, 461.1, 475.2, 477.0, 527.2, 543.2, 565.2, 603.2, 609.3, 633.2, 637.2, 679.2, 682.0, 698.0, 704.0, 720.0), carotenoids (m/z 975.6), chlorophylls and their derivatives (m/z 593.3, 871.6, 909.1).

Study on antioxidant properties of the extracts. — The high content of phenolic compounds in *D. antarctica* plants as well as the high percentage of such active antioxidants as luteolin and/or luteolin derivatives among the phenols allows one to expect significant antioxidant/antiradical properties of the corresponding extracts. The inhibition of the DPPH radicals by 10-fold diluted extracts is shown in Fig. 6 and the dependence of the $DPPH_{30}$ parameter on the total content of polyphenols in the samples is given in Fig. 7.

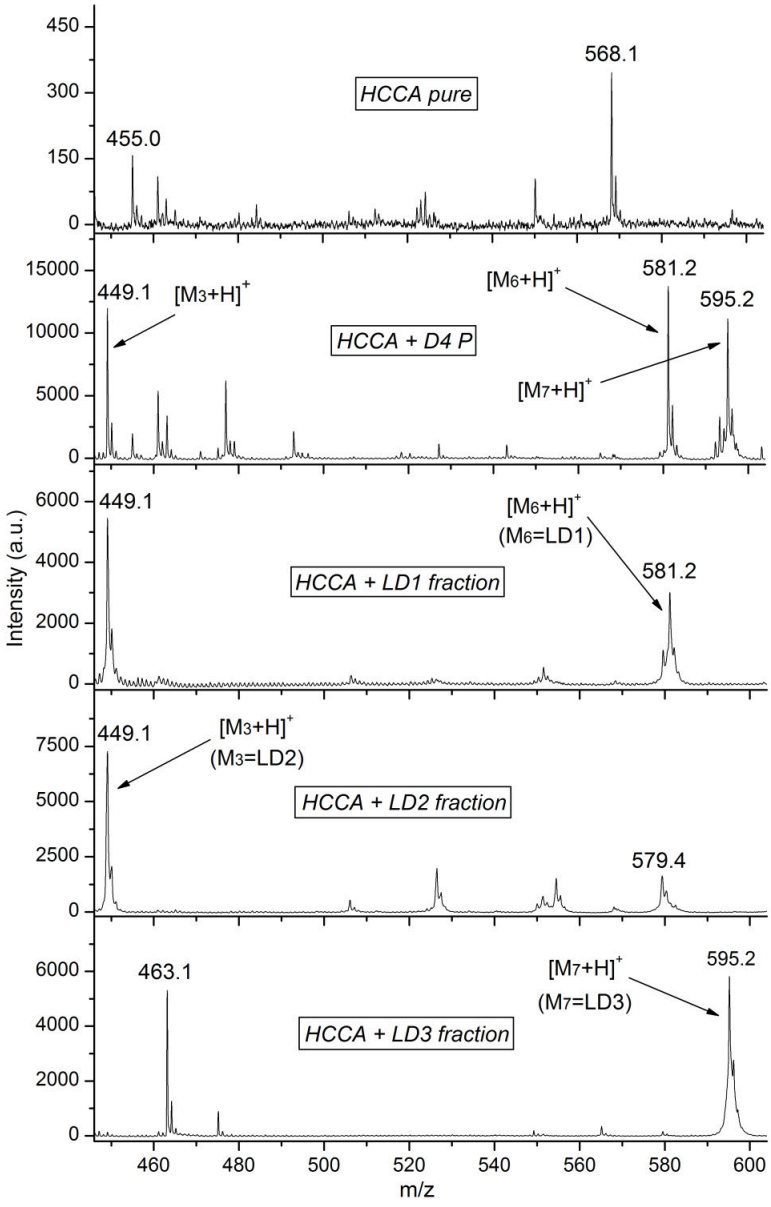

Fig. 5. The fragment of MALDI mass spectra of (upside down): pure HCCA, HCCA+D4 P extract, and HCCA+LD1, LD2 and LD3 fraction, respectively, after HPLC isolation from D4 P extract.

The obtained data indicate the high activity of all extracts in the reaction with DPPH radicals: even 10-fold diluted extracts inhibit up to 85% of radicals. The values of the $DPPH_{30}$ parameter increase with an increase in the total content of polyphenols in plants up to 10–12 mg g^{-1}, while a further increase in the polyphenols content does not lead to a corresponding change in the parameter

Table 4.

Absolute intensity (I) of peaks in MALDI mass spectra, corresponding to nine protonated molecular ions of the main flavonoids of *Deschampsia antarctica* plants (Webby and Markham 1994) from Galindez Island, Argentine Islands. The three most intense signals for each sample are highlighted in bold.

m/z (ion)	331.1 ($[M_1+H]^+$)	447.1 ($[M_2+H]^+$)	449.1 ($[M_3+H]^+$)	463.1 ($[M_4+H]^+$)	579.2 ($[M_5+H]^+$)	581.2 ($[M_6+H]^+$)	595.2 ($[M_7+H]^+$)	621.2 ($[M_8+H]^+$)	637.2 ($[M_9+H]^+$)
Sample	I, a.u.	I, a.u.	I, a.u.	I, a.u.	I, a.u.	I, a.u.	I, a.u.	I, a.u.	I, a.u.
D1	2180	502	**13180**	3848	703	**8802**	**9552**	205	1274
D2 O	856	452	**9390**	3479	579	**7734**	**9573**	—	1015
D2 P	1630	683	**11928**	5044	949	**6769**	**13223**	325	2487
D3 O	1527	251	**9600**	2385	291	**8710**	**5754**	195	1096
D3 P	1585	1202	**22266**	7933	1477	**18376**	**20480**	457	3567
D4 O	1680	302	**13456**	2268	280	**5434**	**6097**	132	924
D4 P	1272	478	**11939**	3209	499	**12715**	**9486**	233	1245
D5 O	3259	1135	**24874**	4654	1778	**2631**	**11675**	322	2013
D5 P	2823	1262	**22221**	6786	1327	**9687**	**14317**	381	3776
D6 O	2542	976	**10162**	4921	1482	**5729**	**11049**	538	3037
D6 P	1809	885	**12022**	4676	1184	**9070**	**11539**	340	3004
D7 O	3486	801	**18256**	4955	1132	**16845**	**15895**	279	2026
D7 P	3336	642	**16755**	4533	787	**22907**	**12344**	303	1812
D9 O	3885	767	**20912**	4979	889	**23131**	**17334**	307	2580

Table 4 - *continued.*

m/z (ion)	331.1 ($[M_1+H]^+$)	447.1 ($[M_2+H]^+$)	449.1 ($[M_3+H]^+$)	463.1 ($[M_4+H]^+$)	579.2 ($[M_5+H]^+$)	581.2 ($[M_6+H]^+$)	595.2 ($[M_7+H]^+$)	621.2 ($[M_8+H]^+$)	637.2 ($[M_9+H]^+$)
D9 P	5938	771	**23696**	5463	1045	**13756**	**19044**	258	2172
D10 O	1073	615	**7508**	3198	636	**8588**	**8593**	—	930
D10 P	779	660	**12390**	4914	734	**14981**	**16690**	—	1434
D11 O	8511	678	**34284**	5535	716	**18210**	**13840**	337	1327
D11 P	9392	707	**23458**	4186	881	**8461**	**9120**	273	1175
D12 O	1437	280	**10482**	2484	239	**7337**	**7409**	—	624
D12 P	3257	677	**20465**	3798	764	**23671**	**12797**	—	2022

M_1 – tricin ($C_{17}H_{14}O_7$), M_2 – isoswertisin ($C_{22}H_{22}O_{10}$), M_3 – orientin ($C_{21}H_{20}O_{11}$), M_4 – isoswertiajaponin ($C_{22}H_{22}O_{11}$), M_5 – isoswertisin 2"-*O*-*β*-arabinoside ($C_{27}H_{30}O_{14}$), M_6 – orientin 2"-*O*-*β*-arabinopyranoside ($C_{26}H_{28}O_{15}$), M_7 – isoswertiajaponin 2"-*O*-*β*-arabinopyranoside ($C_{27}H_{30}O_{15}$), M_8 –isoswertisin 2"-*O*-*β*-arabinoside acylated ($C_{29}H_{32}O_{15}$), M_9 – isoswertiajaponin 2"-*O*-*β*-arabinopyranoside acylated ($C_{29}H_{32}O_{16}$).

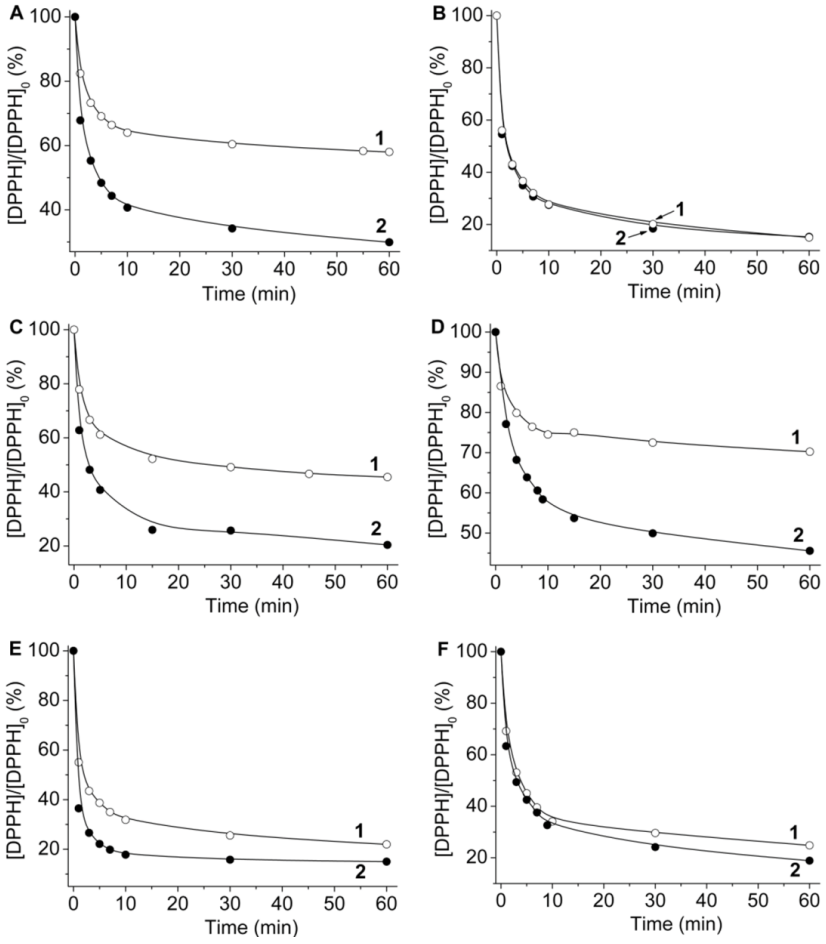

Fig. 6. Examples of the curves for inhibition of DPPH radicals by extracts from *Deschampsia antarctica* plants collected on open (curves 1) and protected (curves 2) areas of locations D2 (**A**), D3 (**B**), D4 (**C**), D6 (**D**), D11 (**E**), D12 (**F**) on Galindez Island, Argentine Islands. All extracts were 10-fold diluted before testing.

(apparently due to an excess of antioxidants even in diluted solutions). Thus, the antioxidant properties of the extracts correlate with the content of phenols in plants and, in most cases, antioxidant activity is higher for extracts from *D. antarctica* plants grown in protected areas.

Discussion

Although *D. antarctica* seems to be a promising raw material for the extraction of natural phenolic antioxidants, there are still no complete data on which phenols and in what quantities are synthesized in the plants. If one

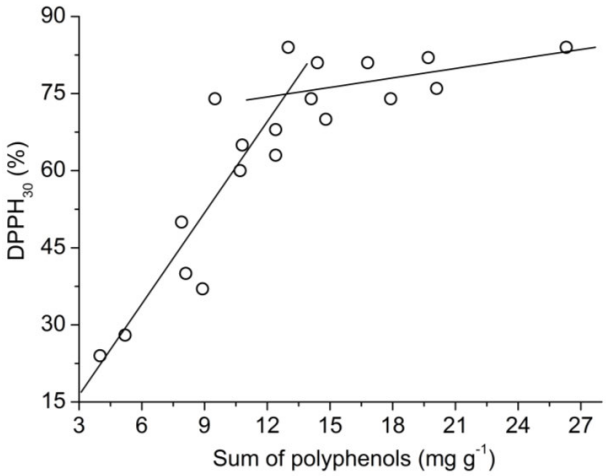

Fig. 7. Dependence of the $DPPH_{30}$ parameter for 10-fold diluted extracts on the total content of phenols in *Deschampsia antarctica* plants from Galindez Island, Argentine Islands.

considers plants as raw materials for obtaining antioxidants of natural origin, it is necessary to make a quantitative assessment of the content of these compounds in the plants. The use of Folin-Ciocolteu method and the characteristic color reaction with aluminum chloride to estimate the total content of phenols and to estimate the content of flavonoids, respectively, allows a qualitative comparison of the amount of active substances in different samples according to their activity in the corresponding reactions, but cannot be considered as a reliable characterization of the content of the compounds in a specific plant. Thus, for instance, the use of these methods gives contradictory results, when the total amount of phenolic compounds in the plant (~12.8–19.6 mg equivalent of ferulic acid per 1 g of dry leaves) turns out to be less than the mass of substances that belong to only one of the polyphenols classes – flavonoids (~16.6–25.3 mg equivalent of rutin per 1 g of dry leaves) (Twardovska *et al.* 2021). Such inconsistency seems to be a consequence of the fact that the aforementioned methods are not direct methods of determining the amount of compounds in a mixture, and the reactivity of a mixture of compounds of different structures depends not only on the amount of compounds in the solution, but also on a number of other factors. In our work, we used the HPLC method to systematically study the content of active compounds in extracts of *D. antarctica* and, accordingly, to find correlations between plant growing conditions, the content of individual components, and antioxidant properties of extracts. HPLC allows the assessment of the compound content in the extract by comparing the signal area of the corresponding compound with the signal area of the reference substance. Previously, HPLC was used to identify some components of extracts (van de Staaij *et al.* 2002), to determine the content of one of the metabolites - orientin (Twardovska *et al.* 2021), to compare the relative content of several

individual components (van de Staaij *et al.* 2002), but not for the general quantitative characterization of the composition of the extracts.

The content of bioactive compounds in plants growing in natural conditions can vary in a wide range. Thus Navrotska *et al.* (2018) determined the content of polyphenols as 51.1 to 105.4 mg g^{-1} (expressed in terms of gallic acid equivalent). At the same time Twardovska *et al.* (2021) estimated the content of phenolic compounds in plants from various locations on the Galindez Island, Great Yalour Island, Darboux Island, and the Rasmussen Point as 12.8–19.6 mg equivalent of ferulic acid per 1 g of dry leaves (from the data of Folin-Ciocolteu method). Ivannikov *et al.* (2021) registered a change in the polyphenol content in the range of 3.0–8.0 mg g^{-1} of dry leaves for plants from 6 locations on different islands of the maritime Antarctic. In this study, the content of polyphenols ranged from 4.0 to 26.3 mg g^{-1}.

The content of bioactive compounds depends on plant growth conditions, although, for example, van de Staaij *et al.* (2002) showed that such an important factor as increasing the intensity of radiation did not have a direct effect on the promotion of antioxidants synthesis. The degree of plant protection from the negative effects of weather conditions (*e.g.,* from cold wind) seems to be one of the factors that can affect the processes of biosynthesis in a plant. Comparison of our data on the content of polyphenols in plants from open and relatively protected areas of the same locations D2–D12 of Galindez Island shows that, in general, the mass of polyphenols per 1 g of biomass in plants from protected areas is greater than in plants from open areas (Fig. 3). At the same time, differences in the content of polyphenols between plants from different locations are more significant than between open and protected areas of the same location (Fig. 3).

Literature data on the composition of *D. antarctica* extracts indicate that the plants contain such flavonoids as derivatives of luteolin, apigenin, tricetin (Webby and Markham 1994; Gidekel *et al*. 2010; Patell *et al.* 2011), and in some cases – kaempferol and quercetin (Ruhland *et al.* 2005). Among phenolic acids, *p*-coumaric, caffeic, ferulic acids (Ruhland *et al.* 2005; Gidekel *et al.* 2010; Patell *et al.* 2011), and *o/m/p*-hydroxybenzoic acid are common (Gidekel *et al.* 2010; Patell *et al.* 2011). Coumarins, carotenoids, and chlorophyll catabolites were also found in *D. antarctica* extracts (Xiong and Day 2001; Ruhland *et al.* 2005; Pereira *et al.* 2009). Our mass spectrometric and chromatographic studies confirm that the extracts contain all the main flavonoids registered elsewhere (Webby and Markham 1994), as well as a number of other bioactive compounds (phenolic acids, coumarins, carotenoids, chlorophyll catabolites). The most common polyphenols in all the studied extracts are flavonoids, but not other classes of polyphenols (Table 2 and Fig. 8).

The dominance of flavonoids among the polyphenols of *D. antarctica* was typical only for the plants grown in natural conditions. Ivannikov *et al.* (2021) showed in a study devoted to the comparison of extracts from *D. antarctica*

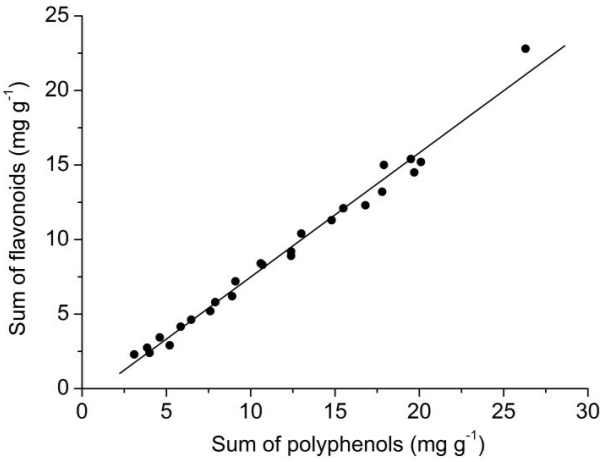

Fig. 8. The flavonoid content *vs.* the total polyphenol content for samples of *Deschampsia antarctica* plants from Galindez Island, Argentine Islands, studied in this and our previous works (Ivannikov *et al.* 2021).

plants grown *in situ* and *in vitro* that such extracts differ by the ratio of the compounds belonging to the three main registered groups of polyphenols (flavonoids and their derivatives; simple phenols/hydroxybenzoic acids and their derivatives; hydroxycinnamic acids and their derivatives). In extracts of plants grown *in vitro*, the amount of flavonoids decreased as compared to *in situ* plants, from ~75 to ~50% of the total weight of polyphenols, while the amount of phenolic acids increased, respectively. For *in situ* plant extracts studied previously (Ivannikov *et al*. 2021) and in this work, the mass fraction of flavonoids derivatives was on average ~75%. Data on the correlation between the content of phenols and content of flavonoids in the samples D1–D12 (Fig. 8) as well as in the samples studied earlier (Ivannikov *et al.* 2021) show a statistically significant linear relationship between these parameters with a correlation coefficient of 0.99. Thus, the fraction of flavonoids synthesized in plants *in situ* does not clearly depend on the conditions and place of plant growth and on the total amount of polyphenols in the plants, and is, on average, more than 70% of the total amount of polyphenols.

The study on the antioxidant properties of the extracts also shows a correlation between the content of phenols in the extracts and their activity in the test reaction with DPPH radicals (in the range of the total polyphenol content in plants of up to 10–12 mg g^{-1}). This is practically the first time when the data we obtained allowed us to talk about the existence of a correlation between the total content of polyphenols in plant extracts and their antioxidant properties. This is probably due to the similarity in the composition of extracts from all studied *D. antarctica* plants (all extracts have an almost uniform composition, containing about 70% flavonoids). Usually, due to differences in the

composition of the extracts (different content of the compounds of various classes) and owing to a significant difference in the reactivity of individual antioxidants, we did not observe a reliable correlation between the total amount of phenols and the antioxidant properties of the extracts.

A more detailed study on the flavonoids available in *D. antarctica* shows that the major flavonoid constituents are the luteolin derivatives (Table 3), and that among luteolin derivatives the main compounds are orientin, orientin 2"-*O*-*β*-arabinopyranoside, and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside (Tables 3, 4 and Fig. 5). According to HPLC data, in almost all the samples the total mass of orientin, orientin 2"-*O*-*β*-arabinopyranoside, and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside is more than 70% of the total mass of luteolin derivatives (Table 3). Although the ratios of different metabolites in the studied samples differ slightly from each other (Tables 2 and 3), the parameters R1 (percentage of the total flavonoids relative to the total mass of phenols), R2 (percentage of luteolin derivatives relative to the total mass of flavonoids) and R3 (percentage of the three main luteolin derivatives relative to the mass of all its derivatives) do not change significantly and are ~75, ~90 and ~75%, respectively (Tables 2, 3 and Fig. 9). Thus, *Deschampsia antarctica* plants are the valuable source of natural phenolic antioxidants, and the most common antioxidants among the components of the extracts are three luteolin derivatives (orientin, orientin 2"-*O*-*β*-arabinopyranoside and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside), the mass fraction of which in the extracts is ≥50% of the total content of polyphenols.

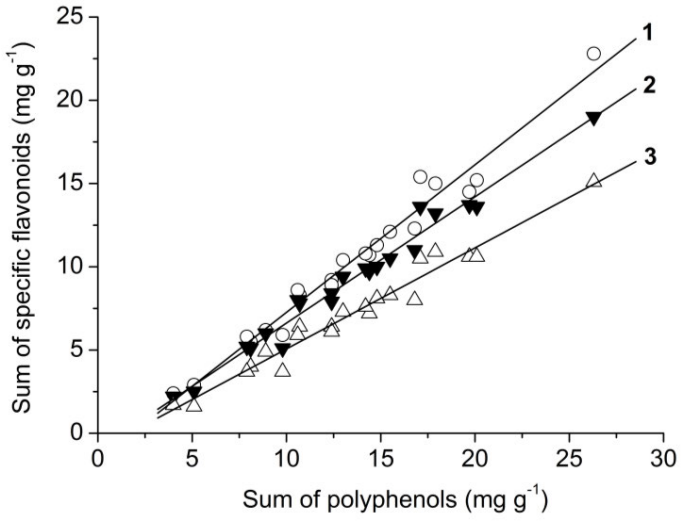

Fig. 9. The total mass of flavonoids (1), total mass of luteolin derivatives (2) and total mass of three main luteolin derivatives (3) *vs.* the total mass of polyphenols in different samples of *Deschampsia antarctica* plants from Galindez Island, Argentine Islands.

Conclusions

Using high-performance liquid chromatography, matrix-assisted laser desorption/ionization mass spectrometry and DPPH test, the extracts from 21 plants of *D. antarctica* from different locations on the Galindez Island, Argentine Islands, were investigated. The main secondary metabolites of *D. antarctica* plants were identified and the content of various polyphenolic antioxidants in the extracts was quantitatively estimated. The most abundant polyphenols in *D. antarctica* plants were flavonoids, and the most common flavonoids were three luteolin derivatives (orientin, orientin 2"-*O*-*β*-arabinopyranoside, and isoswertiajaponin 2"-*O*-*β*-arabinopyranoside). The total content of polyphenols in the plants was estimated to be in the range of 5–26 mg per 1 g of dry leaves, while the mass fraction of three main luteolin derivatives was ≥50% of the total content of polyphenols. All the extracts were found to be very active in the reaction with DPPH radicals, and the activity correlated with the content of polyphenols in the extracts. Thus, the results obtained show that *D. antarctica* plants may be considered as valuable source of natural polyphenols, primarily, luteolin derivatives, which can be used as antioxidants for various biomedical applications.

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