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COMPOSITION AND RADICAL SCAVENGING ACTIVITY OF THE EXTRACTS FROM *DESCHAMPSIA ANTARCTICA* É. DESV. PLANTS GROWN *IN SITU* AND *IN VITRO*

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Abstract. The aim of the work was to study the compounds available in the *Deschampsia antarctica* plants from various sites on the Argentine Islands region, the maritime Antarctic, as well as to introduce the plants into *in vitro* culture and to compare the extracts from the plants grown *in situ* and *in vitro*. The composition of extracts was investigated using HPLC and MALDI MS methods while antiradical activity was tested in the reactions with DPPH$^{\bullet}$, NO$^{\bullet}$ and OH$^{\bullet}$ radicals. All the extracts were found to contain high amounts of phenols (up to 900 mg/L), with derivatives of luteolin and hydroxybenzoic acids being the main bioactive compounds in the extracts from the plants grown *in situ* and *in vitro*, respectively. All the extracts showed high antiradical activity, under standard tests conditions, 10-fold diluted extracts scavenged 50÷90% of DPPH radicals, 20÷40% of NO$^{\bullet}$ radicals and 40÷60% of OH$^{\bullet}$ radicals. Despite the differences in the composition, extracts from the plants grown *in vitro* were not inferior as radical scavengers to extracts from the plants grown *in situ*.

Keywords: phenolic acid, flavonoid, antiradical activity, plant extract, *Deschampsia antarctica*.

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Introduction

Polyphenolic compounds of plant origin are often used as the active non-toxic components of numerous herbal medicines and pharmaceutical preparations. Some natural phenols are of pharmacological value due to their anti-inflammatory and antimicrobial activity, while others are effective antioxidants and free radical scavengers [1]. Synthesis of polyphenols by plants is the basic mechanism of their adaptation to adverse environmental conditions; being extracted from plants, these compounds retain their bioactivity and may be used as protecting agents for other living organisms.

The synthesis of secondary metabolites, including polyphenols, in plant tissues is known to intensify under stressful conditions [2]. *Deschampsia antarctica* É. Desv. grows under the extremely hard environment of the Antarctic, and one can expect it to synthesize, in particular, effective antioxidants protecting this plant against intense UV irradiation [3-5]. Indeed, *D. antarctica* plants were found to contain high amounts of phenolic antioxidants such as luteolin, apigenin and their derivatives [6,7]. Also, UV irradiation of plants induces the synthesis of other flavonoids (scopoletin, rutin) as well as phenolic acids (*p*-coumaric, caffeic, ferulic, chlorogenic and gallic acids) [8,9]. Due to the increased

content of powerful antioxidants, the *D. antarctica* extract has been used as skin protector against solar irradiation, cold temperatures, dryness and oxidative stress [10]. The extract components can also act as antiaging and anticancer agents as well as crioprotectors [11-13]. The main drawback of the potential use of these plants as a raw material for antioxidants production is the complexity of their collection in the Antarctic region and the limited amount of *D. antarctica* plants in nature. For more effective use of the plant potential, it is desirable to introduce *D. antarctica* into *in vitro* culture. This appears to be a promising way to obtain the required amount of high-quality standardized raw material for antioxidants extraction.

The plants of *D. antarctica*, which grow in various localities on the Argentine Islands region, the maritime Antarctic, may differ from each other in their genotype and, therefore, in biochemical composition, and content of polyphenols [7,14]. When the plants are introduced into *in vitro* culture, the changes in growing conditions can influence the synthesis of secondary metabolites in the plant's tissues, thus affecting the availability and quantity of various bioactive compounds [15-17].

The aim of the work was to study the compounds available in *D. antarctica* plants gathered in six various localities on Argentine Islands, to introduce the plants into *in vitro* culture, and to compare the composition and antioxidant properties of the extracts from the plants grown *in situ* and *in vitro*. The subject of the study is of interest from the practical point of view to produce effective antioxidants of plant origin.

Experimental

Materials

All solvents, chemical compounds and reagents such as methanol, acetonitrile, ethanol, phosphate buffered saline, hydrogen peroxide, α-cyano-4-hydroxycinnamic, trifluoroacetic, gallic, salicylic, vanillic, protocatechuic, *p*-hydroxybenzoic, syringic, α-resorcylic, β-resorcylic, γ-resorcylic, cinnamic, *p*-coumaric, *m*-coumaric, *o*-coumaric, caffeic, ferulic, sinapic, feruloylquinic, chlorogenic and fertaric acids, apigenin, myricetin, quercetin, quercetin 3-*O*-glucoside, kaempferol, kaempferol 3-*O*-arabinoside, kaempferol 3-*O*-glucoside, vitexin, orientin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Greiss reagent, sodium nitroprusside, ferrous sulphate, brilliant green, were obtained from commercial sources (Merck, Germany) and used without further purification.

The samples of *Deschampsia antarctica* were collected during the season of the 25th Ukrainian Antarctic Expedition in 2020. The plant materials were gathered on the Darboux Island, on the Lahille Island, and on 4 sites of the Galindez Island (D1, D9, D11 and D12 localities), all in the vicinity of the Ukrainian research station "Academician Vernadsky". All the plants were introduced into *in vitro* culture; a sterile culture of *D. antarctica* was grown on solid agar medium based on Gamborg B5 medium [18]. The growing plants were exposed to artificial lighting of 3000-3500 lx for 16 hours per day at the temperature of 13-18°C and humidity of 65-70%. The places of origin of the plants used for preparation of the extracts are given in Table 1.

Methods

Extract preparation procedure

To prepare the extracts, the aboveground parts of *D. antarctica* plants grown *in situ* or *in vitro* were used. Plants were frozen to -20°C, then thoroughly ground and poured with methanol at a ratio of plant material to methanol of 1 g per 10 mL. The extraction was performed by maceration for 24 hours.

HPLC analysis

HPLC method was used for the identification and quantification of bioactive substances available in the extracts. The profiles of secondary metabolites were analysed using a reversed-phase HPLC column on the Agilent 1100 HPLC system with an autosampler, a diode array detector and the HP 3D Chem Station software. Spectral data were recorded from 200 to 900 nm during the whole run. A poroshell 120 C18 (2.1x150 mm, 2.7 um) column was used for separation.

Table 1

Places of origin of the samples of *Deschampsia antarctica* plant.

Place of origin	*Geographical Coordinates*
Galindez Island, locality D1	S65°14.687' W64°15.348'
Galindez Island, locality D9	S65°14.728' W64°14.992'
Galindez Island, locality D11	S65°14.770' W64°14.874'
Galindez Island, locality D12	S65°14.843' W64°15.205'
Darboux Island	S 65°23.730' W 64°13.031'
Lahille Island	S 65°33.119' W 64°23.512'

The mobile phase was composed of solvent (A) water (0.05 M H_3PO_4) and solvent (B) acetonitrile. The elution sequence was as follows: 1% B in 0÷2 min, 1÷20% B in 2÷8 min, 20÷33% B in 8÷28 min, 38÷99% B in 28÷38 min, and 99% B in 70 min. The flow rate was 0.2 mL/min in 0÷48 min, 0.2÷0.6 mL/min in the 48÷50 min, and 0.6 mL/min in 50÷70 min. The temperature was 20°C in 0÷45 min, 20÷40°C in the 45÷48 min, and 40°C in 48÷70 min. Volumes of injected sample were 5 µL. Samples and mobile phases were filtered through a 0.22 µm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Each fraction was analysed in duplicate.

Compounds were identified and quantified by comparing their retention time and UV-Vis spectral data to known previously injected standards (gallic, salicylic, vanillic, *p*-hydroxybenzoic, protocatechuic, syringic, *α*-resorcylic, *β*-resorcylic, *γ*-resorcylic, cinnamic, *p*-coumaric, *m*-coumaric, *o*-coumaric, caffeic, ferulic, sinapic, feruloylquinic, chlorogenic and fertaric acids, apigenin, myricetin, quercetin, quercetin 3-*O*-glucoside, kaempferol, kaempferol 3-*O*-arabinoside, kaempferol 3-*O*-glucoside, vitexin, orientin). The contents of hydroxybenzoic acids derivatives, hydroxycinnamic acids derivatives, and flavonoids derivatives were expressed in mg equivalents of gallic acid, chlorogenic acid, and rutin, respectively.

MALDI MS analysis

Qualitative analysis of extracts composition was also performed by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI MS). Mass spectra were recorded in positive-ion extraction mode, using an Autoflex II mass spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser (337 nm).

The sample preparation was as follows: 1 µL of the extract was pipetted on a steel target, followed by pipetting of 1 µL of a matrix (saturated solution of *α*-cyano-4-hydroxycinnamic acid (HCCA) in acetonitrile-water-trifluoroacetic acid (70:30:0.1) mixture).

The samples were ionized in the pulse mode: pulse length 3 ns, frequency 20 Hz. Spectra were recorded in the linear mode using a delayed extraction of 10 ns and an accelerating voltage of 20 keV. The resulting mass spectra were the sum of 20 individual spectra obtained as a result of irradiation with 25 laser pulses in each separate point on the target with the deposited sample. The mass spectra acquired were analysed using a FlexAnalisys software (Bruker Daltonics, Germany). Further derivatization of mass spectra to exclude characteristic ions of HCCA matrix was performed using mMass software [19]. Analyte ions identification and assignment to the most probable compounds were performed taking into account the HPLC results of the respective extracts analysis, as well as the previously published data [6,20].

Antiradical activity evaluation of the extracts

DPPH radical scavenging assay

The capacity of *D. antarctica* extracts to inhibit the DPPH free radicals was evaluated using the method described by Brand-Williams W. *et al.* [21]. Briefly, to test radical scavenging activity, 1 mL of original or diluted extract was pipetted into glass, and after that 2 mL of 70% ethanol and 2 mL of 0.15 mM DPPH solution were consecutively added to the glass. The solution was shaken at 25°C for 5÷120 min, and the change in concentration of stable radicals in reaction mixtures versus time of the reaction was determined from the change in absorption *A* at the maximum of 520 nm as compared to absorption A_0 for the control solution prepared by mixing 3 mL of 70% ethanol and 2 mL of 0.15 mM DPPH solution. Percentage of DPPH radicals inhibited during one hour of the reaction was calculated using the Eq.(1) [21].

$$\text{DPPH}^{\bullet}\text{ scavenging activity} = \frac{A_0 - A}{A_0} \cdot 100\% \quad (1)$$

where, *A*– absorbance of the mixture;
A_0– absorbance of the control.

$OH^{\bullet}$ radical scavenging assay

D. antarctica extracts were tested as $OH^{\bullet}$ radicals scavengers using the Fenton reaction [22]. To prepare the reaction mixture for the Fenton assay, 1 mL of 0.435 mM brilliant green solution was mixed with 2 mL of 0.5 mM $FeSO_4$ solution and 1.5 mL of 3.0% H_2O_2 solution. Then 1 mL of tested extract was added to the reaction mixture and incubated at room temperature for 20 min. The absorbance *A* of the mixture at 624 nm was measured and compared with the absorbance A_0 of control solution and with the absorbance A_B of blank solution. To prepare the blank solution, 1 mL of distilled water was added to the Fenton reaction mixture instead of the tested solution; then the mixture was incubated at room temperature for 20 min. To prepare control solution, 4.5 mL of distilled water were added to 1 mL of 0.435 mM brilliant green solution. The hydroxyl radical scavenging activity (%) was calculated using the Eq.(2) [15].

$$\text{OH}^{\bullet}\text{ scavenging activity} = \frac{A - A_0}{A_B - A_0} \cdot 100\% \qquad (2)$$

where, A– absorbance of the mixture;
A_0– absorbance of the control;
A_B– absorbance of the blank solution.

NO• radical scavenging assay

The capacity of the extracts to scavenger NO• radicals was tested using the Griess-Ilosvay reaction [23]. To prepare the reaction mixture, 0.5 mL of 5 mM sodium nitroprusside in phosphate buffered saline (pH 7.4) was mixed with 1 mL of tested extract, followed by the mixture incubation at 25°C for 2 hours. Then 1 mL of the Greiss reagent was added to the solution, and absorbance A of the mixture at 546 nm was measured. The same reaction mixture without the extract but with the equivalent amount of the solvent was used as control solution; the appropriate absorbance value A_0 was measured, as well. The NO• scavenging activity (%) was calculated using the Eq.(3) [23].

$$\text{NO}^{\bullet}\text{ scavenging activity} = \frac{A_0 - A}{A_0} \cdot 100\% \qquad (3)$$

where, A – absorbance of the mixture;
A_0 – absorbance of control.

UV/Vis spectra of solutions and reaction mixtures were recorded on a Lambda 35 UV/Vis spectrophotometer (Perkin Elmer, USA) at 25°C in the wavelength range of 200-800 nm.

Results and discussion

Extract composition

The chromatograms for the extracts obtained from the *D. antarctica* plants originated from Lahille Island and grown *in situ* and *in vitro* are presented in Figure 1. The designations for the compounds or the groups of the compounds identified in the extracts are given in Figure 1.

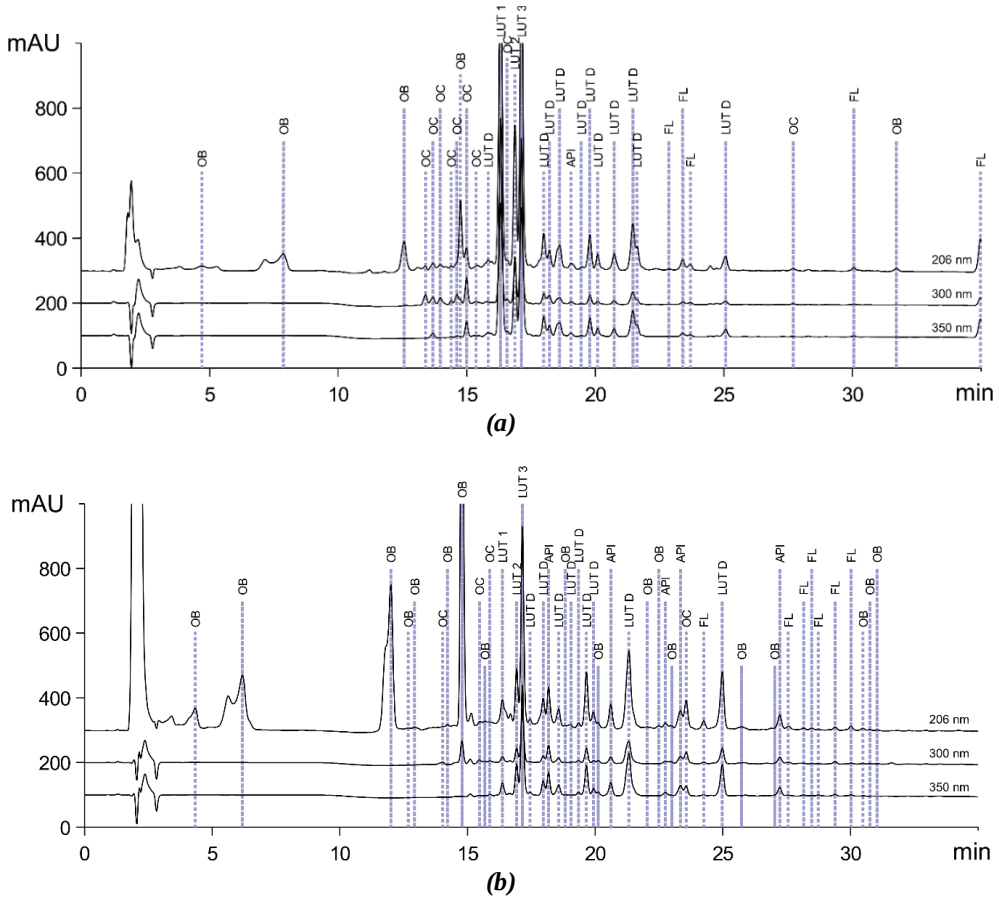


Figure 1. Fragments of chromatograms for the extracts obtained from *D. antarctica* plants grown *in situ (a)* and *in vitro (b)*. The plants originated from Lahille Island. Identified compounds: OB – hydroxybenzoic acids/simple phenols and their derivatives (group 1), OC – hydroxycinnamic acids and their derivatives (group 2), LUT 1-3 – luteolin glycosides 1-3, LUT D – other luteolin derivatives, API – apigenin derivatives, FL – other flavonoids (group 3).

The extracts from the plants growing *in situ* and *in vitro* are hereinafter referred to as "*in situ* extracts" and "*in vitro* extracts", respectively. Similar chromatograms differing from each other mainly by the intensities of the peaks corresponding to various groups of compounds were observed for all other extracts.

The data on the content of various phenols in all the investigated extracts are given in Figure 2. As one can see from the data, the main components of all the extracts belong to one of the following groups: simple phenols/hydroxybenzoic acids and their derivatives (group 1); hydroxycinnamic acids and their derivatives (group 2); flavonoids and their derivatives (group 3). The most intensive signals in the chromatograms of *D. antarctica* "*in situ* extracts" can be attributed to several luteolin glycosides, with three of them being the most abundant. These three luteolin glycosides are designated as Lut 1, Lut 2 and Lut 3, respectively, while the other minor luteolin-derived compounds are mentioned as other luteolin derivatives (Lut D). In all the *D. antarctica* "*in situ* extracts", the second most abounded flavonoid is apigenin glycoside; the chromatograms of "*in situ* extracts" also include quite intensive signals of compounds referred to the groups 1 and 2 of simple phenols/hydroxybenzoic acids (and their derivatives) and hydroxycinnamic acids (and their derivatives). In the chromatograms of *D. antarctica* "*in vitro* extracts" the most prominent peaks are those of simple phenols/hydroxybenzoic acids and their derivatives; among the flavonoids, the most abundant compounds are luteolin glycosides, as well. The main feature of "*in vitro* extracts" is the predominance of group 1 compounds and a lower amount of flavonoids. The changes in extracts composition depending on plants growing conditions were already reported elsewhere [24,25]. For example, we observed the essential increase in phenolic acids content in *Stevia* plants grown *in vitro* in contrast to higher flavonoids content in the plants grown *in situ* [25].

Figure 3 and Table 2 provide the results of extracts analysis by MALDI MS method. The data agree well with the above presented results of the chromatographic study and provide additional specification of the extracts' components. As one can see, the most intense peak in the mass spectrum of *D. antarctica* "*in situ* extract" (Figure 3*(a)*) corresponds to m/z 449 and, according to the results of comprehensive qualitative and structural analysis performed elsewhere [6], may be related to orientin (luteolin 8-*C*-glucoside). The spectrum also contains the signals at m/z 287, 329, 463, 595, 581, 637 that can be referred to luteolin and luteolin derivatives, such as luteolin trimethyl ether, isoswertiajaponin, isoswertiajaponin 2"-*O*-beta-arabinopyranoside, orientin 2"-*O*-beta-arabinopyranoside, isoswertiajaponin 2"-*O*-beta-arabinopyranoside acylated, respectively.

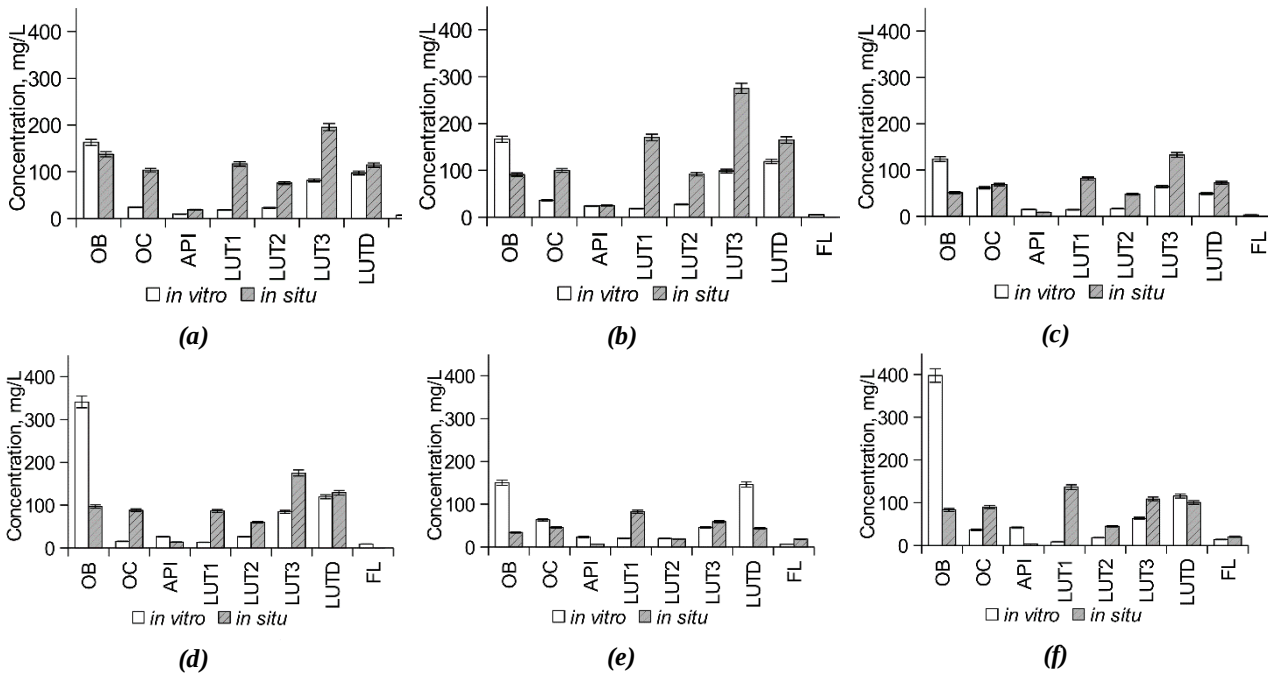


Figure 2. Content of various phenols in the extracts obtained from *D. antarctica* plants grown *in situ* and *in vitro*. The plants originated from D1, D9, D11, D12 localities of Galindez Island *(a-d)* and from Darboux Island *(e)* and Lahille Island *(f)*.

Table 2

Results of MALDI MS studies on the composition of the extracts of *D. antarctica* plants grown *in situ* and *in vitro*. The plants originated from Lahille Island.

Class of compounds	*m/z*	*Identified ion(s)*	*Assigned compound(s)*	*Relative intensity* (%) of the peak in the*	
				"in situ extract"	*"in vitro extract"*
1. Simple phenols / hydroxybenzoic acids and their derivatives	138	$C_7H_6O_3^{\bullet+}$	Hydroxybenzoic acids	8	100
2. Hydroxycinnamic acids and their derivatives	222	$C_{11}H_{10}O_5^{\bullet+}$	*p*-Coumaroyl glycolic acid	-	7
	296	$C_{13}H_{12}O_8^{\bullet+}$ $C_{13}H_{13}NO_7^{\bullet+}$	*p*-Coumaroyl tartaric acid Caffeoyl aspartic acid	-	7
	313	$[C_{13}H_{12}O_9+H]^+$	Caffeoyl tartaric acid	-	6
	343	$[C_{15}H_{18}O_9+H]^+$	Caffeic acid glucoside	8	6
3. Flavonoids and their derivatives	268	$C_{15}H_8O_5^{\bullet+}$	Coumestrol	-	27
	274	$C_{15}H_{14}O_5^{\bullet+}$	Tetrahydroxyflavan/ Tetrahydroxydihydroxychalcone	-	5
	287	$[C_{15}H_{10}O_6+H]^+$	Luteolin	5	-
	307	$[C_{20}H_{18}O_3+H]^+$	Lonchocarpin/Isolonchocarpin	11	-
	329	$[C_{18}H_{16}O_6+H]^+$	Luteolin trimethyl ether	45	8
	331	$[C_{17}H_{14}O_7+H]^+$	Tricin	31	10
	431	$[C_{21}H_{18}O_{10}+H]^+$	Chrysin 7-glucuronide/ Derhamnosylmaysin/ Coumestrin	6	-
	447	$[C_{22}H_{22}O_{10}+H]^+$	Isoswertisin	-	6
	449	$[C_{21}H_{20}O_{11}+H]^+$	Orientin	100	21
	463	$[C_{22}H_{22}O_{11}+H]^+$	Isoswertiajaponin	23	18
	492	$[C_{22}H_{20}O_{13}+H]^+$	Tetrahydroxy-methoxyflavone glucuronide	-	12
	579	$[C_{27}H_{30}O_{14}+H]^+$	Isoswertisin 2"-*O*-beta-arabinoside	6	8
	581	$[C_{26}H_{28}O_{15}+H]^+$	Orientin 2"-*O*-beta-arabinopyranoside	7	-
	595	$[C_{27}H_{30}O_{15}+H]^+$	Isoswertiajaponin 2"-*O*-beta-arabinopyranoside	38	31
	637	$[C_{29}H_{32}O_{16}+H]^+$	Isoswertiajaponin 2"-*O*-beta-arabinopyranoside acylated	6	10
	679	$[C_{32}H_{38}O_{16}+H]^+$ or $[C_{33}H_{42}O_{15}+H]^+$	8-Prenylkaempferol 3,7-diglucoside/ Linoside A or Wanepimedoside A	6	9
4. Other compounds	116	$C_6H_{12}O_2^{\bullet+}$	Fatty acids	25	-
	147	$[C_9H_6O_2+H]^+$	Coumarin	8	-
	156	$C_8H_{12}O_3^{\bullet+}$/ $C_9H_{16}O_2^{\bullet+}$	Fatty acids	-	10
	241	$[C_{12}H_{16}O_5+H]^+$/ $C_{12}H_{19}NO_4^{\bullet+}$	Fatty acids	-	6
	593	$[C_{35}H_{36}O_5N_4+H]^+$	Pheide *a*	21	-

**For the peaks with equal to or more than 5% intensity with respect to the most intensive analyte peak.*

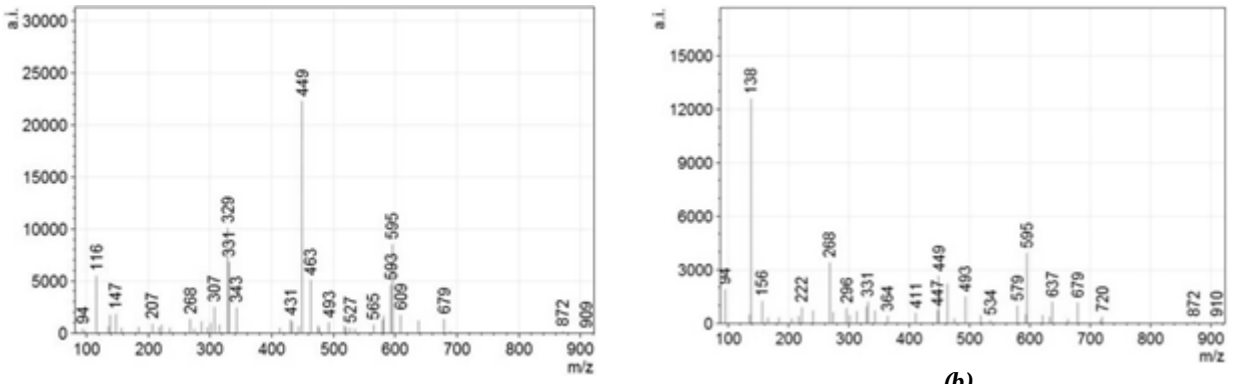
Thus, 7 of 17 signals can be referred to luteolin/luteolin derivatives, with three of those being among the most prominent in the mass spectrum. The spectrum also shows the presence of apigenin derivatives (m/z 579) as well as the other flavonoids/flavonoid derivatives (m/z 307, 331, 431, 679). The data also confirms the presence of hydroxybenzoic acids (m/z 138) and caffeic acid glucoside (m/z 343).

For the extract from the plants grown *in vitro* (Figure 3*(b)*), the main (100%) signal in the MALDI mass spectrum belongs to the ions referred to hydroxybenzoic acids (m/z 138). The spectrum also includes four peaks assigned to hydroxycinnamic acids derivatives (m/z 222, 296, 313, 343), six sufficiently intensive signals of luteolin derivatives (m/z 329, 449, 463, 595, 637) and two lines corresponding to apigenin

and apigenin derivative (m/z 447, 579). The data also show the presence of several other flavonoids/flavonoid derivatives (m/z 268, 274, 331, 492, 679). Both *D. antarctica* "*in situ* extract" and "*in vitro* extract" also contain the compounds attributed to fatty acids while "*in situ* extract" also includes coumarin (m/z 147).

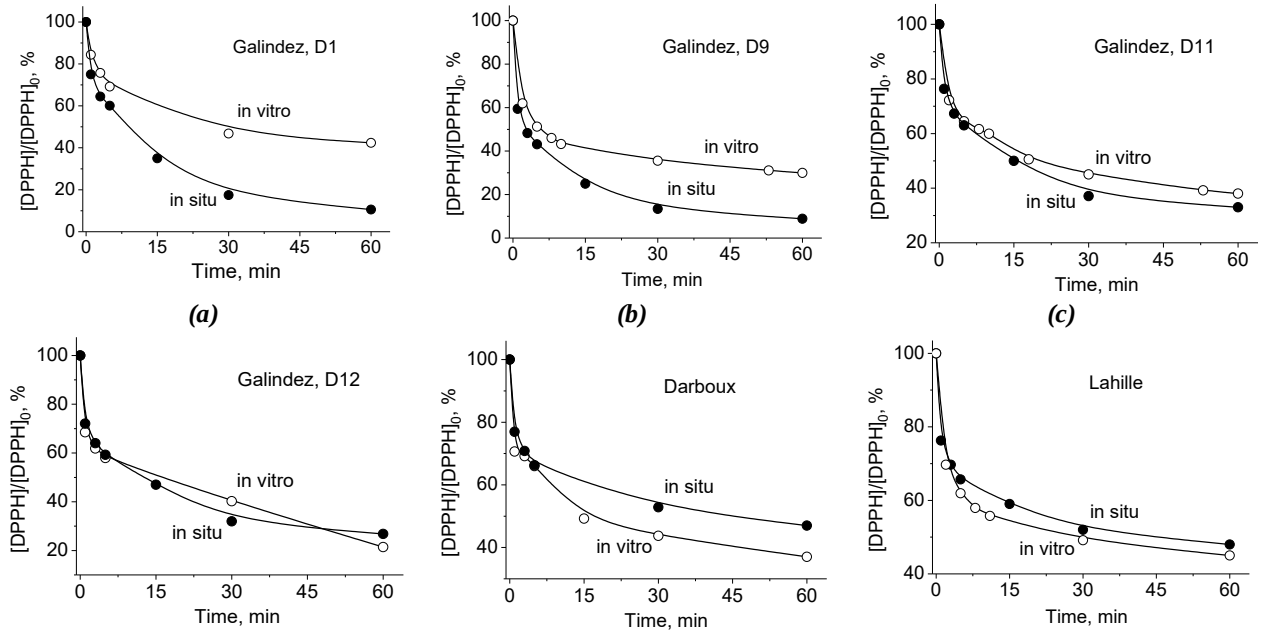
Antioxidant activity

Among the antioxidants of natural origin, phenolic compounds appear to have the best antioxidant/antiradical properties. The data obtained show a very high content of polyphenols in all the *D. antarctica* extracts under study (~300÷900 mg/L, Figure 2); therefore, the extracts can be expected to possess significant antioxidant activity. Indeed, all the extracts showed high activity in the test reaction with DPPH radicals. Under standard test conditions, when 1 mL of the extract reacted with 2 mL of 0.15 mM DPPH solution, instantaneous discoloration of the reaction mixture, indicating the full inhibition of the radicals, was observed. Thus, for a more detailed study of the reaction, the extracts were preliminary diluted by 10 times. Figure 4 shows that even in this case the diluted extracts inhibit 50÷90% of DPPH radicals during 30÷60 min. It is worth to note that the obtained data (Figure 2 and 4, and Table 3) do not reveal a distinct correlation between the total phenols content and the activity of the extracts in the reaction.



(a) ***(b)***

Figure 3. MALDI mass spectra of the extracts obtained from *D. antarctica* plants grown *in situ (a)* and *in vitro (b)*. The plants originated from Lahille Island.



(a) ***(b)*** ***(c)***

(d) ***(e)*** ***(f)***

Figure 4. Inhibition of DPPH radicals by the extracts obtained from *D. antarctica* plants grown *in situ* and *in vitro*. The plants originated from D1, D9, D11, D12 localities of Galindez Island *(a-d)* and from Darboux Island *(e)* and Lahille Island *(f)*.

Table 3

Overall content of phenols and antiradical activity for the extracts from *D. antarctica* plants grown *in situ* and *in vitro*.

Place of plants origin	*Growing conditions*	*Sum of phenols, mg/L*	*DPPH• scavenging activity, %*	*NO• scavenging activity, %*	*OH• scavenging activity, %*
Galindez D11	*in situ*	461	67	23	40
	in vitro	345	62	29	51
Galindez D12	*in situ*	647	75	30	46
	in vitro	635	78	31	54
Darboux	*in situ*	309	53	42	60
	in vitro	470	63	34	47
Lahille	*in situ*	585	52	40	57
	in vitro	710	55	39	51

For example, the "*in vitro* extract" of *D. antarctica* plants from Lahille Island has the highest phenol content but its activity in the DPPH test is one of the lowest. Perhaps, it is because of the presence of various phenols with different antiradical activity.

If we compare the data for *D. antarctica* extracts obtained from the same plants grown *in situ* and *in vitro*, we can conclude that in two cases "*in situ* extracts" have a higher free-radical scavenging activity than the respective "*in vitro* extracts" (Figure 4*(a)* and *(b)*). At the same time, for four plants, the "*in situ* extracts" and "*in vitro* extracts" reveal similar activity in DPPH radical inhibition (Figure 4*(c-f)*, Table 3). Further, to compare the antiradical activity of *D. antarctica* "*in situ* extracts" and *D. antarctica* "*in vitro* extracts", these four pairs of extracts were also tested in the reactions with NO• and OH• radicals. Hydroxyl and nitric oxide radicals are the representatives of the most common reactive oxygen species and reactive nitrogen species, respectively; these radicals are produced during normal cellular metabolism and play an important role in pathogenesis of several oxidative stress related diseases [26]. Thus, in contrast to DPPH test, OH• and NO• radicals scavenging assays appear to be a more suitable method to evaluate the antioxidant activity of the extracts in biological systems.

Table 3 shows the percentage of NO• and OH• radicals that were scavenged by 10-fold diluted *D. antarctica* "*in situ* extracts" and *D. antarctica* "*in vitro* extracts" under standard test conditions. Again, as in the case of the DPPH• test, the data does not reveal a clear correlation between the content of phenolic compounds in the extracts and their activity in the reactions with NO• and OH• radicals (Table 3). For instance, the "*in situ* extract" of *D. antarctica* plants from Darboux Island has the lowest phenol content and the highest hydroxyl and nitric oxide radicals scavenging activity. Also, the extracts showing high activity in DPPH test are not obviously the best in the reaction with NO• and OH• radicals (compare, for example, the data for the plants from Lahille Island and from D12 locality of Galindez Island). Nevertheless, within the selected pairs of samples, the properties of the extracts from the plant grown *in situ* and *in vitro* remain close to each other, as was also stated for the DPPH test. As it was shown above, the change in the environmental conditions of plants growing (from *in situ* to *in vitro*) leads to a decrease in flavonoids content in the plants and to an increase in phenolic acids content. On the other hand, these low-molecular-weight phenols may not be inferior to flavonoids as antioxidant/reducing agents. For example, in accordance with the previous experimental results and quantum-chemical calculations, the phenolic acids extracted from *Stevia* plants grown *in vitro* had an antioxidant/reducing potential similar to that for flavonoids extracted from *Stevia* plants grown *in situ* [25].

Thus, cultivating *D. antarctica* plants *in vitro* is a promising way to produce a sufficient amount of this raw material in the laboratory. The next step is to optimize the biotechnology procedure for growing the plants *in vitro*, aimed at further promoting the synthesis of antioxidants in *D. antarctica* plants.

Conclusions

In this paper, the composition and antioxidant properties of extracts of the *D. antarctica* plants is reported; the novelty of the work is a detailed investigation of original plants from the Antarctic region, and a comparison of the extracts from the plants grown in nature and under *in vitro* conditions.

The extracts were obtained from *D. antarctica* plants gathered in six various localities in the Argentine Islands region, the maritime Antarctic and from 6 corresponding plants cultivated *in vitro*. The extracts were found

to contain 300÷900 mg/L of phenolic compounds, with hydroxybenzoic acids, hydroxycinnamic acids and flavonoids, as well as the derivatives of above compounds, being the main groups of identified phenols (35÷400, 25÷100 and 120÷520 mg/L, respectively). Luteolin and luteolin glycosides were the most common compounds of flavonoids group, while hydroxybenzoic acids prevailed among phenolic low-molecular-weight compounds. The extracts from the same plants grown *in situ* and *in vitro* were found to have the similar composition, with the different concentrations of phenols of various groups being the main distinction between "*in situ* extracts" and "*in vitro* extracts". "*In situ* extracts" include more flavonoid (luteolin) derivatives while "*in vitro* extracts" contain higher amount of phenolic (hydroxybenzoic) acids.

Both *D. antarctica* "*in situ* extracts" and "*in vitro* extracts" showed high activity in the reactions with DPPH$^{\bullet}$, NO$^{\bullet}$ and OH$^{\bullet}$ radicals: under standard test conditions, 10-fold diluted extracts scavenged 50÷90% of DPPH radicals, 20÷40% of NO radicals and 40÷60% of OH radicals.

In general, despite the distinctions in the extracts' composition, "*in vitro* extracts" were not significantly inferior to "*in situ* extracts" as radical scavengers. Thus, *D. antarctica* plants are a valuable raw material for the extraction of phenolic compounds, which are effective radical scavengers of natural origin.

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