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Bioactive substances of *Colobanthus quitensis* (Kunth) Bartl. from the Darboux and Lagotellerie Islands, western coast of Antarctic Peninsula

Abstract. The study aimed to investigate a wide spectrum of biologically active substances of an aboriginal Antarctic plant (*Colobanthus quitensis*) from the central and southern parts of its Antarctic part of general spread collected in 2020–2022. For 17 plants from 2 populations, we obtained extracts and analyzed them using high-throughput chromatography (HPLC). This was the first biochemical screening of plants from previously not investigated parts of this species' range (Graham Coast and Marguerite Bay in the maritime Antarctic). The HPLC method characterized the overall metabolite pools and their separate components which could potentially have high biological activity. The most numerous groups of compounds included phenols and benzoic acids, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, apigenin glycosides, luteolin glycosides, tricin glycosides, flavonoid conjugates of the pearlwort's metabolites depended on the population, probably due to the differences in the microhabitats. Meanwhile, such variability offers a wide selection of possible targets for biochemical screening. The Antarctic pearlwort is richer in some conjugates (such as flavonoid conjugates with the hydroxybenzoic acids) than the other Antarctic aboriginal plant – Antarctic hairgrass (*Deschampsia antarctica*). The determined substances might potentially be of great practical significance.

Keywords: Antarctic pearlwort, high-performance liquid chromatography, metabolite content

1 Introduction

Antarctica's severity limits most harshly the diversity of living organisms able to survive in the extreme environment (Fernández-Marín et al., 2020). That two vascular plants grow there is probably due to a long period of adaptation by a number of adjustments. *Colobanthus quitensis* (Kunth) Bartl. is the only dicot found in Antarctica (Ruhland & Day, 2001).

In the process of adapting to severe habitats, plants have developed many efficient defence mechanisms which allow them to survive adverse conditions of lighting, temperature, and dehydration (Szymańska et al., 2017; Contreras & Zúñiga, 2018; Contreras et al., 2019; Clemente-Moreno et al., 2020). Secondary metabolite biosynthesis is one such mechanism. *C. quitensis* like other polar plants protecting themselves from the harsh polar environment also produces specific bioactive substances (Ruhland & Day, 2001; Pereira et al., 2009; Ozheredova et al., 2015).

Biosynthesis of the phenolic acids and flavonoids as a stress response is found in different plants (Chowdhary et al., 2022). The pearlwort's phenols are already being studied *in vitro* as a promising additive in post-harvesting processing of table grapes, to protect the yield from fungal decomposition by *Botrytis cinerea* Pers. (Contreras et al., 2022). Phenol compounds are natural secondary metabolites synthesized through the pentose phosphate, shikimate, and phenylpropanoid pathways (Cheynier et al., 2013).

The metabolites generated during plants' adaptations influence other organisms in various ways. Whether these substances act as antioxidants, imitate hormones, activate enzymes, inhibit DNA replication, destroy harmful bacteria or bind cell walls, they can potentially stall cancer (Chen et al., 2004; Rodríguez-García et al., 2019; Younas et al., 2018), be used as antiviral or antidiabetic agents (Tringali, 2001), treat inflammation, heart diseases or neurodegenerative disorders (Newman & Cragg, 2020). In particular, the phenols, hydroxycinnamic acids (Sova & Saso, 2020), and catabolites of chlorophyll (Dinkova-Kostova & Talalay, 2008; Gostner et al., 2013; Kräutler, 2016) are antioxidants with neuro-, cardio-, and hepatoprotective effects. Flavonoids and their glycosides (including glycosides of apigenin and luteolin) are antioxidants (Heim et al., 2002) and chelators of metals (Malesev & Kuntic, 2007), can have an impact on the signal systems of animal and human cells, such as induce the oncocells' apoptosis (Jacquemin et al., 2010), and have an antitoxic effect when binding to natural toxins (Poor et al., 2012). The terpenoids and sterols are antioxidants with anti-inflammatory, antimicrobial, and anticancer properties, and phytohormones (Kovaliov et al., 2000; Masyita et al., 2022). Wild plants have always been an important source of novel compounds with uncommon carbon skeletons, which are then modified into semisynthetic substances used, e.g., in medicine because of their biological activity (Newman & Cragg, 2020).

Some aspects of the secondary metabolites production of the Antarctic pearlwort have been published for the region of the South Shetland Islands, the northernmost and most favourable part of its range in the Antarctic, mainly in the context of studies of the physiological response of plants to the abiotic stresses, in particular the UV. The plant produces phenolic substances, flavonoids, carotenoids, luteolin glycosides, chlorophyll *b* and *a* and other compounds (Ruhland & Day, 2001; Lutz et al., 2008; Pereira et al., 2009; Cuba-Diaz et al., 2017; Contreras et al., 2019; 2022). Some data on the production of protective pigments in response to the UV were obtained during the study of the reaction of plants from Danco Coast (Xiong et al., 2002).

Meanwhile, its biochemical potential has not been studied extensively throughout its range; this is true for the whole genus in the Southern Hemisphere. Thus, *C. quitensis* plants from the more southerly populations can conceivably differ in the metabolite production.

So, we aimed to do a primary screening of *C. quiten-sis*'s metabolites using the plants from the central (Graham Coast) and southern (Marguerite Bay) parts of the western coast of Antarctic Peninsula region.

2. Materials and methods

2.1 Plant material

We used samples of the Antarctic pearlwort collected in seasons of 2021 and 2022 and dried using silica gel.



Figure 1. Studied populations of *Colobanthus quitensis* in the central and southern parts of the western coast of Antarctic Peninsula region: 1 - Lagotellerie Island, Marguerite Bay, 2 - Darboux Island, Graham Coast

They are described in Table 1, and the general map is given in Figure 1.

2.2 Analysis and identification of classes of bioactive substances in extracts using high-performance liquid chromatography (HPLC)

Table 2 lists the abbreviations used for different groups of organic substances.

Extraction

The material was put in pure methanol at 5 ml per 0.1 g raw material. The extraction was done by sonication at 60 °C in two 2 stages of 30 min. The primary extract was collected and fresh methanol was added for reextraction. The secondary extract was combined with the primary, and the methanol was evaporated using the rotary evaporator. The dry residue was reconstituted in methanol at 1 ml per 50 mg raw material and filtered through a PTFE filter with 0.2 mkm pores, then stored at -20 °C.

Studying the qualitative content and establishing the substances' qualitative composition in the plant extracts was done for the samples listed in Table 1 by the HPLC method (Harborne, 1984). The corresponding chromatograms are presented in the Appendix (Figures 1-17).

Analysis of secondary metabolites was done by reversed-phase HPLC. The samples were separated using the Agilent 1100 system with a 4-channel pump, vacuum degasser, autosampler, column thermostat, and a diode array detector. We used a 2-eluent set up (eluent A was 0.05 M aqueous solution of H₂PO₄; eluent B was acetonitrile; all eluents and additives were from Sigma-Aldrich, HPLC-grade) on the Poroshell 120 EC-C18 column with 2.7 µm particle size, 2.1×150 mm. Sample volume was 5 µl. The detection was done at 206, 254, 300, 350, and 450 nm. For all substances, the absorbance spectra were registered in the UV and visible light ranges to establish the nature of the secondary metabolites and to classify the chromatographic peaks as belonging to certain groups of substances.

The content of a substance in the dry material was calculated according to the formula by (Ivannikov et al., 2022):

$$c_0 = \frac{A \cdot 1000 \cdot V}{\varepsilon \cdot V_{_{\theta}} \cdot m},$$

where c_0 is a substance's concentration, mg/g dry matter; A is the peak area for the substance in a chromatogram; ε is the proportionality factor, mg⁻¹; V_g is the injected sample volume, μ l; *m* is the dry material mass, g; *V* is the total volume of the extract, ml.

The content of some substance groups was calculated indirectly, using their similarity to substances which belong to the same class or have a fairly close structure and for which we had the standards on hand: simple phenols, benzoic acids and the hydroxybenzoic acids were re-calculated to gallic acid content; apigenin glycosides were re-calculated to vitexin content; unidentified flavonoids, luteolin glycosides, tricin glycosides, and flavonoids conjugated to hydroxycinnamic acids were re-calculated to orientin content, and the catabolites of chlorophyll to feophorbid A.

Table 1. Sampled populations of *Colobanthus quitensis* from the central and southern parts of the western coast of Antarctic Peninsula region

Population	Samples	Habitat
1	LI1-LI10	Lagotellerie Island (Marguerite Bay), -67.883813°, -67.394355°, 7 masl. The plant grows together with <i>Deschampsia antarctica</i> É. Desv. and bryophytes on rock terraces
2	DI6150, DI6450, DI7150, DI7265, DI7495, DI7550, DI7650	Darboux Island (Graham Coast). Site DI6: 30 masl, -65.395394°, -64.215450°, slopes of a rocky grotto with bryophytes and <i>D. antarctica</i> in the vegetation cover. Site DI7: 30 masl, -64.215051°, -65.395253°, on rocky terrace with bryophytes and <i>D. antarctica</i> in the vegetation cover

We stress that classifying a substance based only on the UV-V is spectra is not final.

2.3 Statistical analysis

The difference between the studied populations' substances was determined using Mood's median test (Pollard, 2009). The test values were calculated from the equation:

 $\chi^2 = (experimental value - expected value)^2/(expected value).$

The results of pairwise comparisons are given in dimensionless units. The confirmed differences are presented in Table 4.

3 Results

All studied samples from the two populations of pearlwort contained polyphenols and chlorophyll catabolites. Among the phenolic compounds, the most common were flavonoids, hydroxycinnamic and hydroxybenzoic acids; the qualitative and quantitative content of phenolic substances probably depends on the habitat conditions. Table 3 shows the content of some substance groups in the analyzed natural samples of the Antarctic pearlwort.

There were quantitative differences between the populations in the similar groups of substances (Table 4). Statistical analysis confirmed the quantitative differences between the Darboux Island population and the Lagotellerie Island population in the hydroxycinnamic acids, flavonoids and their glycosides, and carotenoids. There were also found quantitative differences between the Darboux Island population and **Table 2.** List of abbreviations usedin analysis of the chromatograms of the studied*Colobanthus quitensis* extracts

N⁰	Abbreviation	Substance group					
1	В	Simple phenols and benzoic acids					
2	OB	Hydroxybenzoic acids					
3	OC	Hydroxycinnamic acids and their deriva- tives					
4	F	Flavonoids and their glycosides					
5	F-AG	Apigenin glycosides					
6	F-LG	Luteolin glycosides					
7	F-TG	Tricin glycosides					
8	F-OC	Flavonoids conjugated to hydroxycinna- mic acids					
9	TS	Monoterpenoids and sterins					
10	Х	Chlorophyll catabolites					
11	Y	Carotenoids					

the Lagotellerie Island population in flavonoid conjugates, hydroxycinnamic acids, and apigenin glycosides content. The Lagotellerie Island population has apigenin glycosides and luteolin glycosides in high concentrations; as for the Darboux Island population, the luteolin glycosides were in high concentration but the apigenin glycosides were in low (Table 4).

Thus, according to the data, the average content of phenols, benzoic acids, and hydroxybenzoic acids did not differ in the Darboux Island population and in the Lagotellerie Island one (Table 4). The content of tricin glycosides in all studied samples was approximately equal (Tables 3, 4). The apigenin glycosides content, on the other hand, was 7.8 times higher in the

Samula	Substance concentration, mg/g dry mass*										
Sample	В	OB	OC	F	F-OC	F-AG	F-LG	F-TG	Х	Y	TS
LI1	0.48	0.11	0.10	0.16	0.13	1.93	1.50	0.42	0.480	0.137	4.698
LI2	0.29	0.05	0.12	0.04	0.17	4.60	1.69	1.01	0.451	0.125	7.760
LI3	0.15	0.14	0.22	0.02	0.13	2.61	1.82	0.66	0.188	0.063	2.525
LI4	0.21	0.05	0.28	0.03	0.08	2.37	1.19	0.56	0.221	0.034	1.701
LI5	0.48	0.30	0.30	0.20	0.21	4.72	2.73	1.17	0.969	0.080	14.040
LI6	0.28	0.18	0.34	0.08	0.17	3.90	2.54	1.10	0.512	0.021	6.086
LI7	0.43	0.24	0.35	0.05	0.19	4.85	2.75	1.05	0.790	0.049	11.352
LI8	0.10	0.18	0.45	0.06	0.14	3.72	2.82	1.07	0.279	0.108	4.246
LI9	0.39	0.26	0.32	0.05	0.15	3.30	1.93	0.99	0.785	0.291	6.361
LI10	0.46	0.23	0.30	0.06	0.13	4.14	1.28	0.91	0.903	0.264	5.734
DI6150	0.63	0.03	0.08	0.04	0.82	0.42	4.19	1.24	1.124	0.492	11.647
DI6450	0.35	0.08	0.10	0.13	0.84	0.44	4.72	1.34	1.273	0.250	7.038
DI7150	0.56	0.08	0.12	0.17	1.16	0.57	4.65	1.37	1.467	0.460	10.389
DI7265	0.57	0.07	0.11	0.09	0.72	0.40	4.28	1.02	1.224	0.704	12.569
DI7495	0.36	0.08	0.08	0.14	0.81	0.40	4.13	1.10	0.606	0.374	7.436
DI7550	0.42	0.09	0.10	0.10	0.74	0.43	4.59	1.16	0.950	0.245	8.944
DI7650	0.19	0.05	0.10	0.11	0.72	0.39	3.67	1.08	1.237	0.362	6.944
	Sample LI1 LI2 LI3 LI4 LI5 LI6 LI7 LI8 LI9 LI10 DI6150 DI6450 DI7150 DI7265 DI7495 DI7550 DI7650	Sample B L11 0.48 L12 0.29 L13 0.15 L14 0.21 L15 0.48 L16 0.28 L17 0.43 L18 0.10 L19 0.39 L110 0.46 D16150 0.63 D16450 0.35 D17150 0.56 D17265 0.57 D17495 0.36 D17500 0.42 D17650 0.19	Sample B OB L11 0.48 0.11 L12 0.29 0.05 L13 0.15 0.14 L14 0.21 0.05 L15 0.48 0.30 L16 0.28 0.18 L17 0.43 0.24 L18 0.10 0.18 L19 0.39 0.26 L110 0.46 0.23 D16150 0.63 0.03 D16450 0.35 0.08 D17150 0.56 0.08 D17265 0.57 0.07 D17495 0.36 0.08 D17550 0.42 0.09 D17650 0.19 0.05	B OB OC L11 0.48 0.11 0.10 L12 0.29 0.05 0.12 L13 0.15 0.14 0.22 L14 0.21 0.05 0.28 L15 0.48 0.30 0.30 L16 0.28 0.18 0.34 L17 0.43 0.24 0.35 L18 0.10 0.18 0.45 L19 0.39 0.26 0.32 L110 0.46 0.23 0.30 DI6150 0.63 0.03 0.08 DI6450 0.35 0.08 0.10 DI7265 0.57 0.07 0.11 DI7265 0.57 0.07 0.11 DI7495 0.36 0.08 0.08 DI7550 0.42 0.09 0.10 DI7650 0.19 0.05 0.10	Sample Sub- B OB OC F L11 0.48 0.11 0.10 0.16 L12 0.29 0.05 0.12 0.04 L13 0.15 0.14 0.22 0.02 L14 0.21 0.05 0.28 0.03 L15 0.48 0.30 0.30 0.20 L16 0.28 0.18 0.34 0.08 L17 0.43 0.24 0.35 0.05 L18 0.10 0.18 0.45 0.06 L19 0.39 0.26 0.32 0.05 L10 0.46 0.23 0.30 0.06 D16150 0.63 0.03 0.08 0.04 D16450 0.35 0.08 0.10 0.13 D17150 0.56 0.08 0.12 0.17 D17265 0.57 0.07 0.11 0.09 D17495 0.36 0.08	Sample Substance cond B OB OC F F-OC L11 0.48 0.11 0.10 0.16 0.13 L12 0.29 0.05 0.12 0.04 0.17 L13 0.15 0.14 0.22 0.02 0.13 L14 0.21 0.05 0.28 0.03 0.08 L15 0.48 0.30 0.30 0.20 0.21 L16 0.28 0.18 0.34 0.08 0.17 L17 0.43 0.24 0.35 0.05 0.19 L18 0.10 0.18 0.45 0.06 0.14 L19 0.39 0.26 0.32 0.05 0.15 L110 0.46 0.23 0.30 0.06 0.13 DI6150 0.63 0.03 0.08 0.04 0.82 DI6450 0.35 0.08 0.10 0.13 0.84 DI7150 <t< td=""><td>SampleSubstance conventionBOBOCFF-OCF-AGL110.480.110.100.160.131.93L120.290.050.120.040.174.60L130.150.140.220.020.132.61L140.210.050.280.030.082.37L150.480.300.300.200.214.72L160.280.180.340.080.173.90L170.430.240.350.050.194.85L180.100.180.450.060.143.72L190.390.260.320.050.153.30L1100.460.230.300.060.134.14D161500.630.030.080.040.820.42D164500.350.080.100.130.840.44D171500.560.080.120.171.160.57D172650.570.070.110.090.720.40D175500.420.090.100.100.740.43D176500.190.050.100.110.720.39</td><td>Sample Image: Substance convertation, mg/g dry m B OB OC F F-OC F-AG F-LG LI1 0.48 0.11 0.10 0.16 0.13 1.93 1.50 LI2 0.29 0.05 0.12 0.04 0.17 4.60 1.69 LI3 0.15 0.14 0.22 0.02 0.13 2.61 1.82 LI4 0.21 0.05 0.28 0.03 0.08 2.37 1.19 LI5 0.48 0.30 0.30 0.20 0.21 4.72 2.73 LI6 0.28 0.18 0.34 0.08 0.17 3.90 2.54 LI7 0.43 0.24 0.35 0.05 0.19 4.85 2.75 LI8 0.10 0.18 0.45 0.06 0.14 3.72 2.82 LI9 0.39 0.26 0.32 0.05 0.15 3.30 1.93 L110</td><td>SumpleSumpleSubsurve council out out out out out out out out out out</td><td>$\begin{array}{ c c c c c c c c c c c c c c c c c c c$</td><td>Subset Subset S</td></t<>	SampleSubstance conventionBOBOCFF-OCF-AGL110.480.110.100.160.131.93L120.290.050.120.040.174.60L130.150.140.220.020.132.61L140.210.050.280.030.082.37L150.480.300.300.200.214.72L160.280.180.340.080.173.90L170.430.240.350.050.194.85L180.100.180.450.060.143.72L190.390.260.320.050.153.30L1100.460.230.300.060.134.14D161500.630.030.080.040.820.42D164500.350.080.100.130.840.44D171500.560.080.120.171.160.57D172650.570.070.110.090.720.40D175500.420.090.100.100.740.43D176500.190.050.100.110.720.39	Sample Image: Substance convertation, mg/g dry m B OB OC F F-OC F-AG F-LG LI1 0.48 0.11 0.10 0.16 0.13 1.93 1.50 LI2 0.29 0.05 0.12 0.04 0.17 4.60 1.69 LI3 0.15 0.14 0.22 0.02 0.13 2.61 1.82 LI4 0.21 0.05 0.28 0.03 0.08 2.37 1.19 LI5 0.48 0.30 0.30 0.20 0.21 4.72 2.73 LI6 0.28 0.18 0.34 0.08 0.17 3.90 2.54 LI7 0.43 0.24 0.35 0.05 0.19 4.85 2.75 LI8 0.10 0.18 0.45 0.06 0.14 3.72 2.82 LI9 0.39 0.26 0.32 0.05 0.15 3.30 1.93 L110	SumpleSumpleSubsurve council out	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	Subset S

Table 3. The content of biologically active substances in the extracts of Antarctic pearlwort populations from the central and southern parts of the western coast of Antarctic Peninsula region: Darboux Island (DI) and Lagotellerie Island (LI). The data are obtained based on measurements of HPLC with the equipment's error of $\leq 10\%$

Table 4. Differences between the determined biological compounds in plants of *Colobanthus quitensis* from the central and southern parts of the western coast of Antarctic Peninsula region: Darboux Island (DI) and Lagotellerie Island (LI)

Substance*	Average con	ntent, mg/g	Test volue**	Difference		
Substance*	LI	DI	lest value***			
В	0.32 ± 0.03	0.45 ± 0.05	0.5	LI <di 1.4="" in="" td="" times<=""></di>		
OB	0.18 ± 0.03	0.06 ± 0.001	3.68	LI>DI in 3 times		
OC	0.30 ± 0.03	0.120 ± 0.001	10.56	LI>DI in 2.5 times		
F	0.080 ± 0.001	0.120 ± 0.001	4.5	LI <di 1.5="" in="" td="" times<=""></di>		
F-OC	0.150 ± 0.001	0.81 ± 0.001	***	LI <di 5.4="" in="" td="" times<=""></di>		
F-AG	3.5 ± 0.3	0.450 ± 0.001	***	LI>DI in 7.8 times		
F-LG	3.86 ± 0.1	4.36 ± 0.1	0	LI <di 1.1="" in="" td="" times<=""></di>		
F-TG	1.0 ± 0.2	1.5 ± 0.03	0	LI <di 1.5="" in="" td="" times<=""></di>		
X	0.47 ± 0.2	1.21 ± 0.2	0	LI <di 2.6="" in="" td="" times<=""></di>		
Y	0.17 ± 0.04	0.52 ± 0.07	4.5	LI <di 3.1="" in="" td="" times<=""></di>		
TS	6.44 ± 0.85	9.09 ± 0.71	1.04	LI <di 1.4="" in="" td="" times<=""></di>		

Notes: * – the list of abbreviations see in Table 2; ** – the χ^2 -distribution tabular value for the limit p = 0.05 is 3.84 for comparing two distributions; *** – comparison does not require confirmation due to the difference in average sample values larger than 5–10; **bolded** are differences confirmed by the Mood's median test

Lagotellerie Island population than in the Darboux Island one.

The Darboux Island population practically did not differ from the Lagotellerie Island population by the content of luteolin glycosides; the highest luteolin glycosides content was found in samples DI6450, DI7150, and DI7550 from this island (Table 3). The ratio of apigenin glycosides to luteolin glycosides was important for understanding these results. The parameters of flavonoids and flavonoid conjugates with hydroxycinnamic acids in both studied groups of pearly pearly ($\geq 1 \text{ mg/g}$), yet the Darboux Island population had higher F-OC content (Table 4). The highest content of the group of compounds with oxycinnamic acids in the population was seen in sample DI7150 (Table 3). The average content of carotenoids differed between the studied populations, in contrast to the content of terpenoids, sterols, and chlorophyll catabolites (Table 4).

4 Discussion

Our results substantially enlarge and confirm the already accumulated data for *C. quitensis* (Lutz et al., 2008; Ruhland & Day, 2001; Pereira et al., 2009; Contreras et al., 2022), showing that the pearlwort produces numerous groups of compounds including phenols and benzoic acids, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, apigenin glycosides, luteolin glycosides, tricin glycosides, flavonoid conjugates of the hydroxycinnamic acids, chlorophyll catabolites, carotenoids, terpenoids, and sterols.

The variability presents a wide potential of plant material to screen for useful biochemicals. It is difficult for us to compare the content of biochemical compounds obtained in this research with the previous findings for the more northern Antarctic region – the South Shetlands Islands and the Danco Coast because of the differences in aims and methodology of these investigations. However, our comparison of plants from two quite distant from each other Antarctic populations allows to assume quantitative differences in the metabolites of plants from different populations. These differences are most likely explained by the individual variability of microhabitats of the studied plants. A similar individuality of the adaptive profile has been shown in a multi-year monitoring study of the Antarctic hairgrass in the maritime Antarctic (Parnikoza et al., 2015). Previous physiological studies of the pearlwort noted different modulation of oxidative stress response in different ecological conditions (Ozheredova et al., 2015). Our experiment shows that even plants from the same population can have different production potential.

How can microclimate modulate the response to external stresses? In the Antarctic, several kinds of stress act upon the plant. The low-temperature stress first induces in plant cells the biosynthesis of osmoprotectants and then stimulates the synthesis of antioxidant enzymes and compounds such as carotenoids, flavonoids, and phenolic substances. Under soil salinization, low temperatures, or UV irradiation the ensuing oxidative stress causes the appearance of reactive oxygen species. To protect themselves from this, plants produce, among other things, polyphenols, flavonoids, antocyanes, phenolic acids, and terpenes (Ruhland & Day, 2001; Pereira et al., 2009; Ozheredova et al., 2015).

Environmental factors such as high copper content (Cuba-Díaz et al., 2017) can induce in the pearlwort a significant reduction in chlorophyll *a* content and exhibited oxidative stress evaluated through an increase in malondialdehyde levels. Bertini et al. (2022) also show that *C. quitensis* finely regulates its metabolic activity and stress response capacity as a function of the environment.

What significance do these substances have for the polar plants? Contreras et al. (2019) assumed that the Antarctic pearlwort's survival mechanisms in the UV-B shock involve adaptation mediated especially by the flavonoid biosynthesis. Ruhland et al. (2005) analyzed the effect of the UV-B irradiation on the phenylpropanoids of *D. antarctica* and reported that *p*-coumaric, caffeic, and ferulic acids were the main hydroxycinnamic acids present, while luteolin derivatives were the main flavonoids in the insoluble and in the soluble leaf extracts. As for the underlying mechanisms, only tentative suggestions can be formed, based on the data for other species. The benzoic and hydroxybenzoic acids are known to participate in in-

ducing the protective reactions of plants like the tomato Lycopersicum esculentum L. cv Romano and Phaseolus vulgaris L. cv Brown Beauty (Senaratna et al., 2003). In wheat, their presence was connected to cold- and drought resistance (Yastreb et al., 2012). Studies of the hydroxycinnamic acids also link them to cold- or hot temperature stress; in grapevines, cold temperature stress induced an increase in the gallic, ferulic, and caffeic acids (Amarowicz et al., 2010), and in rapeseed, the hydroxycinnamic acids and flavonoid glycosides showed year-to-year variation depending on temperature and radiation (Groenbaek et al., 2019). In some plants, the overproduction of reactive oxygen species under stress is balanced by the production of phenolic compounds and flavonoids (Kumar et al., 2020). A complex of the pearlwort's polar secondary metabolites had a dose-dependent effect in an *in vitro* set-up as an antifungal agent (Contreras et al., 2022).

In our case, the studied populations differed in terms of levels and ratios of luteolin glycosides and apigenin glycosides. Markham et al. (1998) showed that the ratio of luteolin glycosides to apigenin glycosides increased (P < 0.05) under additional UV-B action in Marchantia polymorpha L. The UV-B absorbance spectra of the glycosides are the same. However, they differed in the levels of absorbing active forms of oxygen produced under UV irradiation. The ortho dihydroxyflavones of the B-ring (such as luteolin) are far more efficient absorbers of the free radicals (antioxidants) than the monohydroxyflavones of the B-ring (such as apigenin) (Montesinos et al., 1995). Luteolin glycosides can also be more efficient than apigenin glycosides in the scattering of the absorbed UV energy in a non-harmful way, providing protection as suggested previously (Smith & Markham, 1996). Thus, one can assume that difference among the glycosides in populations of Darboux Island and Lagotellerie Island can represent individual adaptation profiles for microhabitats, especially for the local UV lighting.

On the whole, the Lagotellerie Island plants LI1– LI10 possibly come from the habitats with a more favourable and balanced light regime. In general, the majority of plants from Darboux Island (DI6–DI7) have much higher content of carotenoids, terpenoids, sterines, and catabolites of chlorophyll. However, some plants from both groups have parameters outside of the general tendencies. A statistically significant difference between the studied populations is observed only in the carotenoids content which is greater in the Darboux Island population.

The previous metabolite screening of another Antarctic aboriginal plant - Antarctic hairgrass (D. antarctica) showed slight differences in the main groups of secondary metabolites compared to the Antarctic pearlwort (Ivannikov et al., 2021). The variety of flavonoid glycosides is greater in C. quitensis than in D. antarctica. In wild Antarctic hairgrass from different regions, luteolin glycosides predominate and apigenin glycosides are less abundant compared to luteolin glycosides. Also, its metabolites did not differ between different sites. In C. quitensis, depending on the population, the ratio was either in favor of luteolin glycosides or in favor of apigenin glycosides. The C. quitensis extracts contain more conjugates of compounds (e.g., apigenin glycosides conjugated with hydroxycinnamic acids) than the D. antarctica extracts (Ivannikov et al., 2022). This makes it a more promising source of novel substances than the Antarctic hairgrass.

Conclusions

We screened extracts of C. quitensis for metabolite substances, comparing, for the first time, the plants growing on Darboux Island (Graham Coast) and Lagotellerie Island (Marguerite Bay) from the central part and south of the western coast of Antarctic Peninsula region. Using high-performance liquid chromatography, we described pools of metabolites and characterized their constituents. Among the most numerous compounds, there were phenols and benzoic acids, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, glycosides of apigenin, glycosides of luteolin, glycosides of tricin, conjugates of flavonoids with hydroxycinnamic acids, and catabolites of chlorophyll; the findings agree with the literature. The quantitative content of the metabolites varied depending on the population, perhaps due to the differences in the microhabitats. Meanwhile, such variability presents a wide selection of possible sources of novel biochemicals to be obtained from the plant. Additionally, extracts of *C. quitensis* are biochemically richer in conjugated compounds (conjugates of flavonoids with hydroxycinnamic acids) than the previously studied *D. antarctica*.

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Біологічно активні сполуки *Colobanthus quitensis* (Kunth) Bartl. островів Дарбу та Лаготелеріє, західне узбережжя Антарктичного півострова

Реферат. Метою даної роботи було дослідження широкого спектру біологічно активних сполук однієї з аборигенних рослин Антарктики *Colobanthus quitensis* (Kunth) Bartl. з місцезростань центральної та південної частин морської Антарктики, зразки були зібрані у 2020–2022 роках. Отримано екстракти 17 рослин з 2 популяцій, а склад екстрактів проаналізовано методом високоефективної рідинної хроматографії (BEPX). Вперше проведено біохімічний скринінг рослин перлинниці антарктичної з раніше не досліджених частин цього виду з узбережжя Греяма та затоки Маргарита в морській Антарктиці. Методом BEPX встановлено пули метаболітів та їх окремі компоненти, які потенційно можуть мати високу біологічну активність. Серед виявлених найбільш чисельних груп хімічних сполук визначено феноли та бензойні кислоти, гідроксибензойні кислоти, гідроксикоричні кислоти, флавоноїди, глікозиди апігенину, глікозиди прицину, кон'югати флавоноїдів з гідроксикоричними кислотами, катаболіти хлорофілів, каротиноїди, терпеноїди та стерини. Кількісний склад метаболітів в рослинах перлинниці демонстрував відмінність в залежності від популяції, що, ймовірно, пов'язано з мікроумовами їх існування. Водночас, така мінливість демонструє багатий набір варіантів для біохімічного пошуку в досліджуваній рослині. Біохімічний склад перлинниці багатший за кон'югатами сполук, такими, як кон'югати флавоноїдів з гідроксибензойними кислотами, порівняно з рослинами щучника антарктичного. Визначені сполуки з рослин перлинниці антарктичної потенційно можуть мати велике практичне значення.

Ключові слова: високоефективна рідинна хроматографія, вміст метаболітів, перлинниця антарктична

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APPENDIX

Figure 1. Chromatogram of sample L11 of the extract of *Colobanthus quitensis* (Lagotellerie Island)



Figure 2. Chromatogram for sample LI2 of the extract of *Colobanthus quitensis* (Lagotellerie Island)



Figure 3. Chromatogram for sample LI3 of the extract of *Colobanthus quitensis* (Lagotellerie Island)



Figure 4. Chromatogram of sample LI4 of the extract of *Colobanthus quitensis* (Lagotellerie Island)



A, mAU



banthus quitensis (Lagotellerie Island)



Figure 7. Chromatogram for sample LI7 of the extract of *Colobanthus quitensis* (Lagotellerie Island)





F-TG F-TG F-TG

15202530t, minFigure 9. Chromatogram of sample LI9 of the extract of Colo-
banthus quitensis (Lagotellerie Island)



Figure 10. Chromatogram of sample L110 of the extract of *Colobanthus quitensis* (Lagotellerie Island)



Colobanthus quitensis (Darboux Island)

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Figure 12. Chromatogram for sample DI6450 of the extract of *Colobanthus quitensis* (Darboux Island)



Figure 13. Chromatogram for sample DI7150 of the extract of *Colobanthus quitensis* (Darboux Island)



Figure 14. Chromatogram for sample D17265 of the extract of *Colobanthus quitensis* (Darboux Island)





Figure 15. Chromatogram for sample DI7495 of the extract of *Colobanthus quitensis* (Darboux Island)



Figure 16. Chromatogram for sample DI7550 of the extract of *Colobanthus quitensis* (Darboux Island)



Figure 17. Chromatogram for sample DI7650 of the extract of *Colobanthus quitensis* (Darboux Island)