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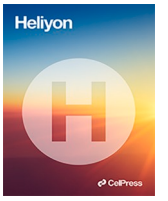

Research article

# Phenolic composition and bioactivity of *Ribes magellanicum* fruits from southern Patagonia

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ABSTRACT

Eight *Ribes magellanicum* collections from three different places in southern Patagonia were compared for content of different groups of phenolics, antioxidant capacity and inhibition of enzymes related to metabolic syndrome (α-amylase, α-glucosidase and pancreatic lipase). The sample with the highest antioxidant capacity was assessed for glutathione (GSH) synthesis stimulation in human gastric adenocarcinoma (AGS) cells. The chemical profile was determined by high performance liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS) and the main phenolics were quantified. The samples from Navarino Island and Reserva Nacional Magallanes showed higher content of anthocyanins and caffeoylquinic acid, with better activity towards α-glucosidase and antioxidant capacity. A sample from Omora (Navarino Island), significantly increased intracellular GSH content in AGS cells. Some 70 compounds were identified in the fruit extracts by HPLC-MS/MS. The glucoside and rutinoside from delphinidin and cyanidin and 3-caffeoylquinic acid were the main compounds. Different chemical profiles were found according to the collection places.

## 1. Introduction

The Grossulariaceae *Ribes magellanicum* Poir. is a shrub occurring in western Patagonia, including Tierra del Fuego and the austral archipelagos. The fruits are sweet, with a pleasant taste when ripe, and were gathered as food since prehistoric times [1]. The distribution range of this species extends from central Chile to the southernmost islands of South America. In southern Patagonia, indigenous people include Selknam or Ona in Tierra del Fuego, Aoniken or Tehuelches in eastern Patagonia and the sea nomads

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Kaweshkar or Alacalufs and Yamana or Yahgan in the fjords and archipelagoes of southwestern Patagonia [2,3]. Gathering of wild fruits was, and still is, part of their subsistence activities, and *R. magellanicum* was one of their core available resources. Indeed, Yahgan people appreciated its abundance and nutritious value and called this shrub "upush" [4]. On Navarino Island, they named Upushwaia; i.e., "the bay (=waia) of *R. magellanicum* (=upush)". Further north in Chile, the indigenous Huilliche people used the leaves of *R. magellanicum* to treat liver and intestinal diseases. The antimicrobial activity of the leaves was tested [5]. However, ethanol extracts showed only a weak effect against *Escherichia coli* EDL 933. At present, herbal tea is prepared from *R. magellanicum* leaves, and it is used as digestive.

In Chile, during the last decades, the production of *Ribes* fruits has increased based on cultivated *Ribes rubrum* and *R. nigrum,* both native to Europe [6]. Fruits of species native to Patagonia, including *R. magellanicum*, have been investigated and several bioactive compounds have been isolated and identified, comprising anthocyanins, phenylpropanoids and flavonoids [1,7–10].

The effect of colonic fermentation of phenolic-enriched extracts (PEE) from Chilean *Ribes* fruits has been investigated, as well as their activity on enzymes associated to metabolic syndrome [11]. The PEE of *R. magellanicum* and *R. punctatum* fruits displayed anti-inflammatory effect on Caco-2 cells after simulated gastrointestinal digestion [12]. In addition, the PEEs showed a positive effect on human fecal microbiota *in vitro* [13]. In a comparative study on *R. magellanicum* and *R. punctatum* fruits, the extracts showed strong changes in composition, antioxidant capacity and enzyme inhibition along with the digestive process [14]. The cytoprotective effect of Chilean native berries polyphenols (including *Ribes*) against free radical-induced damage in AGS cells [15] and the inhibitory effect of the fruit PEEs on advanced glycation products was described [16].

Strong differences in the content of the main constituents in the fruit extracts were highlighted [10] comparing *R. magellanicum* samples from the Eastern and Western Andean slopes of Chile and Argentina. The changes in composition were reflected in modifications in the antioxidant capacity and inhibition of enzymes associated to metabolic syndrome.

The samples investigated in the above-mentioned reports were collected in central-southern Chile and Argentina. At present, little is known about the composition and bioactivity of the constituents from fruits of *R. magellanicum* in their southernmost distribution range. The main goals of this study are (i) to characterize the composition and bioactivity of *R. magellanicum* fruits collected in the area of Punta Arenas in southern continental South America and on Navarino Island along the Beagle channel coasts, and (ii) to compare the composition and bioactivity with those reported in previous works based on fruits collected in central and southern Chile and Argentina.

## 2. Materials and methods

### 2.1. Reagents and chemicals

The following reagents and chemicals were from Sigma-Aldrich (St. Louis, MO, USA): AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride, 2,2'-azobis-(2-amidinopropane) (ABAP), 4-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl palmitate, sodium acetate, starch, quercetin, (+)-catechin, gallic acid, acarbose, L-glutamine, $AlCl_3$, 2′,7′-dichlorodihydrofluorescein diacetate, DPPH* (2,2-diphenyl-1-picrylhydrazyl radical), 2,4,6-tri(2-pyridyl)1,3,5-triazine (TPTZ), dinitrosalicylic acid, $NaHCO_3$, $Na_2CO_3$, Amberlite® XAD-7, α-amylase from porcine pancreas (A3176; EC 3.2.1.1), α-glucosidase from *Saccharomyces cerevisiae* (G5003; EC 3.2.1.20) and lipase from porcine pancreas type II (L-3126; EC 3.1.1.3). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), $FeCl_3.6H_2O$, Folin-Ciocalteu reagent, potassium sodium tartrate, potassium persulfate and HPLC-grade solvents, were from Merck (Darmstadt, Germany). Orlistat was obtained from Laboratorio Chile (Santiago, Chile). The standard compounds delphinidin glucoside and cyanidin rutinoside were from Cayman Chemical Co., USA. Delphinidin rutinoside, cyanidin 3-glucoside, 3-caffeoylquinic acid and 5-caffeoylquinic acid were from PhytoLab (Vestenbergsgreuth, Germany). All solvents used for HPLC analyses were HPLC grade solvents. Cell culture media and antibiotics were obtained from Gibco, Life Technologies (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Invitrogen Corp. (Grand Island, NY, USA). The ultrapure water used was obtained using a Barnsted EasyPure water filter (Thermo Scientific, Ohio, USA).

### 2.2. Plant material

In January–February 2022, samples of *R. magellanicum* fruits were collected in wild growing shrubs in two geographical areas. First, near the city of Punta Arenas, at Reserva Nacional Magallanes (RNM, 53°08′46″S 71°00′12″W), and Reserva Laguna Parrillar (RLP, 53°21′58″S 71°21′23″W). Second, near the city of Puerto Williams on Navarino Island at the Omora Ethnobotanical Park (54°56′08″S 67°36′17″W). Ripe fruits from *Ribes magellanicum* were collected at RNM from three individuals (RNM 1, RNM 2 and RNM 3) in January 2022, two samples were obtained from trees at the RLP (RLP 1 and RLP 2) in February 2022. Three samples were collected on Navarino Island in January 2022 as follows: Omora Park (OM 1 and OM 2) and adjacent areas of Upushwaia (UP). The strong wind, gradual ripening and consumption by birds made it difficult to collect large number of fruits from single shrubs.

For RNM, fruit samples were: RNM 1 (149.1 g), RNM 2 (54.9 g) and RNM 3 (195.1 g). For RLP samples, fresh weight of the fruits after removing stalk was 23.7 g for RLP 1 and 117.5 g for RLP 2, respectively. On Navarino Island, samples included OM1 (56.0 g), growing in forests at low elevations, and OM 2 (282.0 g) corresponding to a pool from individuals growing in the same area. The taste of the OM1 fruits was less sweet than other samples. The third sample was obtained from shrubs growing at Upushwaia (UP) (297.0 g). After collection, fruits were cleaned and separated from theirs stalks. Samples were kept refrigerated, and then transported in a cooler to the Universidad de Talca, where they were lyophilized for analyses.

### 2.3. Preparation of phenolic-enriched extracts (PEE)

Lyophilized fruits were powdered in a Sindelen LCM-18000GF blender (Santiago de Chile, Chile) and extracted four times with MeOH:formic acid (FA) 99:1 (v/v) in a 1:3 fruit:solvent ratio under sonication of 15 min each time. The solvent was removed after each extraction, filtered and the combined solutions were taken to dryness under reduced pressure and then lyophilized to afford the crude extracts. Treatment of crude extracts with activated Amberlite XAD resin after re-suspension in water, afforded the PEE. Prior to use, the Amberlite XAD-7 resin was activated by washing with 0.1 M NaOH, rinsed with distilled water, treated with 0.1 M HCl and washed with distilled water until pH 7.0 was reached.

### 2.4. Soluble solids

The soluble solid content in the fruits from samples collected on Navarino Island was estimated using a refractometer (Hanna HI96801, Hanna Instruments Inc., Santiago, Chile) calibrated with saccharose. The results are expressed as °Brix. The soluble solid content was determined separately in 10 berries from each sample and the result is presented as mean value ± SD.

### 2.5. Total phenolic, total flavonoid, total proanthocyanidin, and total anthocyanin content

Total phenolic (TP), total flavonoid (TF) and total proanthocyanidin (PAC) of the PEE was measured using the Folin-Ciocalteu reagent as described in Ref. [17]. Results are expressed as g gallic acid equivalent (GAE)/100 g of PEE. Total flavonoid (TF) content was determined by the aluminum trichloride method. Results are shown as g catechin equivalents (CE)/100 g of PEE. The 4-dimethylaminocinnamaldehyde (DMAC) methodology was used to determine the total proanthocyanidin content (TPA) [17]. Results are presented as g catechin equivalents (CE)/100 g of PEE. Total anthocyanin content (TA) was determined using the pH-differential method according to Ref. [18] and results are expressed as mg cyanidin equivalents (CyE/100 g of PEE).

### 2.6. Antioxidant capacity assays

The antioxidant capacity of the samples was investigated using four methods, as described in Refs. [9,17]. They included the discoloration of the 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid ($ABTS^{+}$*), the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) assay, the reduction of ferric cation (FRAP) and the ORAC assay. For the study, samples were diluted in stock solutions ranging from 5 to 300 μg/mL for individual experiments. The calibration curves for the FRAP, $ABTS^{+}$*, and ORAC assays were built using Trolox. The positive control in all assays were quercetin and catechin. For FRAP and ORAC, the results are expressed as μmol TE/g extract. The results for TEAC are given as μM TE/g extract and DPPH* as $SC_{50}$ (μg/mL).

### 2.7. Enzyme inhibition assays

The PEEs were assessed for the inhibition of α-amylase, α-glucosidase, and pancreatic lipase as described in Ref. [17].

#### 2.7.1. α-amylase inhibition assay

Samples were evaluated at final concentrations of 100 μg/mL. The dissolved samples (100 μL) were mixed with 0.02 M sodium phosphate buffer containing a 0.5 mg/mL α-amylase solution (100 μL). The mixture was pre-incubated at 37 °C for 10 min. Then, a 1% starch solution in sodium phosphate buffer (100 μL) was added. The mixture was further incubated at 37 °C for 20 min and the color reagent (200 μL) was added. The test tubes were boiled for 15 min. After boiling, 40 μL of the reaction were mixed with of water (210 μL). The absorbance was recorded at 550 nm in a microplate reader (TECAN Infinite M Nano+, Grödig, Austria). Acarbose was used as standard inhibitor. All determinations were carried out in triplicate. The results are presented in percentages of inhibition as mean values ± SD.

#### 2.7.2. α-glucosidase inhibition assay

The sample was dissolved in phosphate buffer (120 μL in 0.1 M sodium phosphate buffer pH 6.8) and mixed with the α-glucosidase solution (0.25 U/mL, in sodium phosphate buffer) (20 μL). After pre-incubation at 37 °C for 15 min, the substrate (5 mM *p*-nitrophenyl-α-D-glucopyranoside, in sodium phosphate buffer) was added (20 μL). The mixture was incubated again at 37 °C for 15 min. The reaction was stopped by adding 0.2 M sodium carbonate (80 μL) and the absorbance at 415 nm was recorded in a microplate reader. The standard inhibitor acarbose was used for comparison. The samples were evaluated at final concentrations of 0.1–100 μg/mL. All determinations were carried out in triplicate. The results are shown in percentages of inhibition or $IC_{50}$ (μg/mL) as mean values ± SD.

#### 2.7.3. Lipase inhibition assay

The porcine pancreatic lipase was prepared in ice-cold water at 20 mg/mL and the samples were tested at a final concentration of 50 μg/mL. The reaction mixture was prepared mixing 50 μL of the extract, 150 μL of enzyme solution, 450 μL of the substrate (*p*-nitrophenyl palmitate 0.08%, w/v), and 400 μL assay buffer (100 mM Tris, pH 8.2). The mixture was incubated at 37 °C during 2 h. Then, the absorbance of the solution was measured at 400 nm using a spectrophotometer (Genesys 10UV, Thermo Spectronic, Rochester, NY, USA). All determinations were carried out in quadruplicate and the results are presented in percentages as mean values ± SD. The reference compound was Orlistat®.

### 2.8. AGS cell culture

Human epithelial gastric cells AGS (ATCC CRL-1739) were grown as monolayers in Ham F-12 medium containing 1 mM L-glutamine and 1.5 g/L sodium bicarbonate. Medium was supplemented with 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin. Cells were grown in a humidified incubator with 5% $CO_2$ in air at 37 °C. Cells were plated at a density of $2.5 \times 10^4$ cells/mL for the subsequent experiments.

### 2.9. Cytotoxicity assay

The cytotoxicity values of the samples ($IC_{50}$, μg/mL) are needed as a reference to determine the working concentrations of the extracts in the intracellular reduced glutathione (GSH) content determination. To accomplish this, confluent cultures of AGS cells were treated during 24 h with medium containing the PEE at concentrations ranging from 0 up to 1000 μg/mL. The samples were dissolved in medium supplemented with 2% FBS. Cells treated with medium only were used as controls. At the end of the incubation, cell viability was determined by means of the MTT reduction assay [19]. Concentrations were assayed in sextuplicate, and experiments were repeated 2 times using different cell preparations.

### 2.10. Intracellular reduced glutathione (GSH) content

One day after confluence, AGS cells were treated overnight with different concentrations (0, 62.5, 125, 250, and 500 μg/mL) of the PEE from the OM sample. The extract was dissolved in medium supplemented with 2% FBS and antibiotics. At the end of the incubation, culture medium was removed by vacuum aspiration and replaced with culture medium only. Cells were detached with a cell scraper, re-suspended with cold MES buffer (50 mM, pH 6–7). Then, cells were lysed by sonication and kept on ice. The GSH content was determined using a colorimetric kit (BioAssays Systems, Hayward, CA, USA). The GSH synthesis stimulant *N*-acetyl-L-cysteine (750 μM) was used as positive control. Each concentration was tested in triplicate and experiments were repeated twice using different cell preparations. Results are expressed as nmol of soluble reduced sulfhydryl/$10^6$ cells.

### 2.11. HPLC-DAD

The chromatographic profile of the PEE from the different fruit samples was determined using Shimadzu HPLC equipment (Shimadzu Corporation, Kyoto, Japan) consisting of an LC-20AT pump, an SPD-M20A UV diode array detector, CTO-20 AC column oven, and LabSolution software. A Kinetex 5 μm EVO C18 100 Å column (Phenomenex Inc., California, USA) was used and the column oven was maintained at 25 °C. The HPLC solvent system consisted of water:formic acid 95:5 (v/v) (A) and MeOH:formic acid 95:5 (v/v) (B) eluted in a gradient as follows: 0–20 min, 15–35 % B; 20–30 min, 35–50 % B; 30–37 min, 50–100 % B1; 37–40 min, 100 % B; 40–43 min, 100-15 % B; 43–55 min, 15 % B. The flow rate was 0.6 mL/min. Each sample was dissolved in the mobile phase (1 mg/mL), filtered through 0.22 μm PVDF syringe filter (Agela technologies, DE, USA) and 20 μL was injected for analysis. The compounds were monitored at 330, 360, and 520 nm, and spectra from 200 to 650 nm were recorded for characterization. The HPLC traces were used to compare the occurrence of main compounds and for quantification. Anthocyanins were determined and quantified according to Ref. [20] with some modifications.

### 2.12. HPLC-DAD-MS/MS

The experiments were carried out on Thermo Fisher Scientific UHPLC system consisted of Accela 1250 quaternary UHPLC pump, Accela Open autosampler and Accela PDA detector (Thermo Fisher Scientific, San Jose, CA, USA) interfaced with a hybrid linear ion trap (LTQ) Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). LC-separation for DAD-ESI-MS/MS detection was performed on a CORTEX T3 column (2.1 × 150 mm, 2.7 μm, Waters Corporation, Milford, MA, USA), A flow rate of 0.3 mL/min was used for the analysis, The column was held at 45 °C and the sample tray was held at 4 °C. Samples were eluted with a gradient solvent system consisting of (A) 0.1% formic acid in water (v/v) and (B) 0.1% formic acid in acetonitrile (v/v), as follows: 0.0–40.0 min, 4–20%B; 40.0–55.0 min, 20–45%B; 55.0–56.0 min, 45–100%B; 56.0–60.0 min, 100%B; 60–60.5 min, 100-4%B; 60.5–70.0 min 4%B. Accela PDA was scanning the wavelength range from 200 to 650 nm with a scan bandwidth of 9 nm and 20 Hz scan rate.

The LTQ Orbitrap Velos Pro was equipped with an electrospray ionization (ESI) source and operated in the positive and negative ionization modes scanning ions from *m/z* 120 to 1200. The source voltage was 4.5 kV and heated capillary temperature was 270 °C. The nitrogen was used as the sheath gas at 30 units (arbitrary units). A data dependent mode of acquisition was applied during the complete chromatographic run. In this mode an accurate *m/z* survey scan is completed in FT cell. In parallel, a MS/MS based linear ion trap investigation of the top five most abundant precursor ions was also performed. By employing the automatic gain control of ion trapping, FT full-scan mass spectra were attained at 60,000 mass resolving power (*m/z* 400). Collision induced dissociation (CID) was achieved using helium as a target gas with a 2 Da isolation width and 30% of normalized collision energy. The precursor ions selected for CID was then dynamically excluded from further MS/MS analysis for 30 s. The resolving power for $MS^2$ scans was 7500. Raw data were processed using Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA).

### 2.13. Statistical analyses

Determinations were carried out in triplicate, quadruplicate or sextuplicate. Results are reported as the arithmetic means ± SD. Significant differences in the TP, TF, TPA, and TA contents and antioxidant capacity were detected by one-way analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$). In the GSH assay, statistical differences between different treatments and their respective control were determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test ($p < 0.05$). Statistical analyses were carried out using the software GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA).

## 3. Results and discussion

Eight samples from *R. magellanicum* fruits including five from southern continental Patagonia and three from Navarino Island were investigated. The moisture content in the RNM samples 1, 2 and 3 was 77.1, 76.5 and 75.2%, respectively. The percent extraction yields from the lyophilized MeOH:FA 99:1 extracts was 38.9, 31.3, and 37.6% and the yield of PEE 6.8, 10.1, and 5.8% from the corresponding extracts for RNM 1, RNM 2, and RNM 3, respectively. In RLP samples, the moisture for 1 and 2 was 70.5 and 77.8%, respectively. The w/w yield of the MeOH:FA 99:1 of the extracts obtained from the lyophilized samples was as follows: 1, 29.6 and 2, 29.9%, respectively. The w/w yield of the PEE obtained from the extracts was 5.7% and 18.5% for RLP 1 and RLP 2, respectively.

The moisture of the fruits from Navarino Island was 76.8, 74.5 and 76.1%, for OM 1, OM 2 and UP, respectively. The w/w yield of the MeOH:FA 99:1 extracts from the lyophilized samples was as follows: OM 1, 19.1; OM 2, 24.8; and UP, 28.7%, respectively. The PEE obtained from the extracts were 15.0, 9.8, and 9.3% for OM 1, OM 2, and UP, respectively.

### 3.1. Soluble solids

The soluble solid content for the OM 1, OM 2, and UP berries was 9.4 ± 1.2, 7.9 ± 1.2, and 11.7 ± 1.8 °Brix, respectively. The values are in accordance with the different maturity stages of the fruits, with higher content for the UP sample.

### 3.2. Total phenolic, total flavonoid, total procyanidin and total anthocyanin content

Large variations in the content of phenolics were observed. Highest total phenolic (TP) content was found in the Navarino Island

**Table 1**
Total phenolic (TP), total flavonoid (TF), total proanthocyanidin (TPA), total anthocyanidin (TA) content and antioxidant activity (DPPH*, FRAP, TEAC, ORAC) of the PEEs from Chilean *Ribes magellanicum* fruits.

| Sample | TP (g GAE/ 100 g PEE) | TF (g CE/ 100 g PEE) | TPA (g CE/ 100 g PEE | TA (g CyE/ 100 g PEE) | DPPH* ($SC_{50}$, μg/mL) | FRAP (μmol TE/g PEE) | TEAC (μM TE/ g PEE) | ORAC (μmol TE/ g PEE) |
|---|---|---|---|---|---|---|---|---|
| Reserva Nacional Magallanes | | | | | | | | |
| RNM 1 | 31.65 ± 0.65[a] | 17.43 ± 0.27[a] | 8.65 ± 1.22[a] | 10.78 ± 0.01[a] | 7.51 ± 0.14[a] | 3292.55 ± 112.18[a] | 1753.73 ± 16.20[a] | 910.23 ± 74.69[a] |
| RNM 2 | 30.01 ± 0.35[b] | 18.50 ± 0.30[b] | 7.30 ± 0.27[a, b] | 7.06 ± 0.03[b] | 7.35 ± 0.09[a] | 2769.78 ± 44.76[b] | 1444.30 ± 11.92[b] | 575.93 ± 43.19[b] |
| RNM 3 | 20.00 ± 0.30[c] | 11.66 ± 0.07[c] | 4.06 ± 0.05[c] | 7.00 ± 0.17[b] | 15.86 ± 0.12[b] | 1780.85 ± 33.76[c] | 657.62 ± 6.31[c] | 737.21 ± 62.57[c] |
| Reserva Laguna Parrillar | | | | | | | | |
| RLP 1 | 32.72 ± 0.27[a] | 16.57 ± 0.20[d] | 6.46 ± 0.18[b] | 13.59 ± 0.05[c] | 6.52 ± 0.14[c] | 3364.04 ± 54.60[a] | 2323.92 ± 20.36[d] | 746.90 ± 46.51[c] |
| RLP 2 | 16.89 ± 0.32[d] | 8.50 ± 0.15[e] | 4.73 ± 0.55[c] | 3.43 ± 0.00[d] | 23.50 ± 0.45[d] | 1401.06 ± 44.01[d] | 918.18 ± 8.03[e] | 213.43 ± 5.75[d] |
| Navarino Island | | | | | | | | |
| OM 1 | 37.67 ± 0.52[e] | 16.44 ± 0.32[d] | 13.61 ± 0.40[d] | 5.00 ± 0.06[e] | 3.47 ± 0.08[e] | 4385.74 ± 69.36[e] | 2997.19 ± 25.35[f] | 349.06 ± 18.77[e] |
| OM 2 | 37.52 ± 0.51[e] | 20.97 ± 0.13[f] | 12.01 ± 0.61[d] | 10.15 ± 0.05[f] | 4.06 ± 0.09[f] | 3961.27 ± 33.73[f] | 2348.10 ± 20.24[d] | 1113.29 ± 27.58[f] |
| UP | 40.65 ± 0.37[f] | 23.92 ± 0.34[g] | 12.06 ± 0.67[d] | 3.20 ± 0.01[g] | 6.54 ± 0.04[c] | 4049.14 ± 47.36f | 2023.06 ± 19.63[g] | 349.05 ± 0.29[e] |
| Catechin[#] | – | – | – | – | 11.11 ± 1.62 | 5380.15 ± 80.14 | – | 9328.16 ± 354.89 |
| Quercetin[#] | – | – | – | – | 8.01 ± 0.45 | 1000.32 ± 12.58 | 8220.15 ± 28.08 | 23374.06 ± 897.39 |

PEE: phenolic-enriched extract; RNM: Reserva Nacional Magallanes; OM: Parque Etnobotánico Omora; UP: Upushwaia; RLP: Reserva Laguna Parrillar; DPPH*: 2,2-diphenyl-1-picrylhydrazyl radical; FRAP: ferric reducing antioxidant power; TEAC: trolox equivalents antioxidant capacity; ORAC: oxygen radical antioxidant capacity. GAE: gallic acid equivalents; CE: catechin equivalents; CyE: cyanidin equivalents; $SC_{50}$: extract concentration scavenging 50% of the DPPH* radical. TE: Trolox equivalents. –: not determined. #: reference compounds. Results are expressed as the mean values ± SD of three independent experiments. Different superscript letters (a–g) in the same column show significant differences within each collection place, according to Tukey's test ($p < 0.05$).

samples (37.52–40.65 g GAE/100 g PEE). The same trend was observed for the total flavonoid (TF) (16.44–23.92 g CE/100 g PEE) and total proanthocyanidin (TPA) content (12.01–13.61 g CE/100 g PEE). The highest total anthocyanidin (TA) content was found in the RLP 1 sample, followed by RNM 1 (13.59 and 10.78 g CyE/100 g PEE), respectively. Contents in the range of 387.00–388.60 mg GAE/g PEE, 159.30–195.60 mg CAE/g PEE and 63.60–113.40 mg CAE/g PEE for TP, TF and TPA, respectively were reported in Patagonian *Ribes* fruit [9].

### 3.3. Antioxidant capacity

The antioxidant capacity of the samples was investigated using different and complementary methods (Table 1). The highest DPPH* and $ABTS^{+}$* radical scavenging capacity was observed in samples from OM 2 with $SC_{50}$ of 3.47–4.06 μg/mL and 2348–2997 μM TE/g PEE, respectively (Table 1). In the DPPH* assay, the samples from RNM and RLP were less active than those of Navarino Island, which showed $IC_{50}$ values ranging from 3.47 to 6.54 μg/mL. Using the same assay, a study based on fruits collected at Conguillío National Park (Región de la Araucanía, Chile) found an $IC_{50}$ of 4.7 μg/mL [19]. When the extract was partitioned in the anthocyanin-enriched and copigment-enriched fraction, the activity in the DPPH* assay decreased, suggesting a synergistic effect [19]. However, for the superoxide anion scavenging, higher effect was observed for the anthocyanin-enriched and copigment-enriched fractions. The ORAC value for the PEE was 1.9 mmol TE/g sample and decreased after fractionation [19]. Previous reports indicate higher ORAC values for *R. magellanicum* fruits, ranging from 1900 to 2008 μmol TE/g PEE [11,19]. However, the fruits collected for those studies were from northern populations of this species (Parque Nacional Conguillio), possibly explaining this variability [9].

The highest FRAP value was also found in the OM 2 sample, reaching 3961–4385 μmol TE/g PEE. These observations are in agreement with our previous work with *R. magellanicum* fruits from Argentina and Chile that also showed variations related to collection places and whose DPPH*, TEAC, and FRAP values were $SC_{50}$ 6.2–24 μg/mL, 1098–2856 μM TE/g PEE, and 816–4006 μmol TE/g PEE, respectively [10]. Regarding ORAC, the samples from OM 2, RNM 1, and RLP 1 showed the highest scavenging capacity against peroxyl radicals, reaching values of 746–1113 μmol TE/g PEE.

### 3.4. Enyme inhibition

The enzymes α-amylase, α-glucosidase, and lipase play a fundamental role in the hydrolytic breakdown of (poly-)oligosaccharides and triglycerides into monosaccharides and fatty acids previous absorption from the small intestine. The inhibition of these enzymes slows down the process, helping to prevent hyperglycemic and hyperlipidemic peaks [11]. The extracts assayed inhibited carbohydrate-related enzymes, α-amylase and α-glucosidase, with a higher activity towards the last.

Fruits from all the collection places exhibited intense inhibition towards α-glucosidase, with $IC_{50}$ values in the range of 0.06–0.29 μg/mL. The samples OM 2, RNM 1, and OM 1 showed the highest inhibitory activitity with $IC_{50}$ values of 0.06, 0.07, and 0.08 μg/mL, respectively. The samples from Navarino Island also exhibited the better effect against α-amylase, reducing the activity of the enzyme by 11.42–17.61% at 100 μg/mL. None of the samples inhibited lipase at 50 μg/mL (Table 2).

The activity of the PEE from our samples, collected at the southern end of South America, was higher than that reported for the *R. magellanicum* fruits from the Araucanía Region [11], with an $IC_{50}$ of 0.38 μg/mL. However, the effect of the sample from Araucanía was higher towards α-amylase, with an $IC_{50}$ of 21.65 μg/mL. Further work is required to disclose if the fruit extract can stimulate the secretion of insulin and/or decrease glucagon.

### 3.5. Cellular reduced glutathione (GSH) content

The OM 2 PEE was selected for the intracellular GSH content experiment because of its high TP, TF, and TA content, as well as best

**Table 2**
Inhibitory activity of the PEEs from *Ribes magellanicum* fruits towards α-glucosidase, α-amylase and pancreatic lipase.

| Sample | α-glucosidase ($IC_{50}$, μg/mL) | α-amylase (% at 100 μg/mL) | Lipase (%, at 50 μg/mL) |
|---|---|---|---|
| Reserva Nacional Magallanes | | | |
| RNM 1 | $0.06 \pm 0.00^{a}$ | $3.40 \pm 0.51^{a}$ | Inactive |
| RNM 2 | $0.21 \pm 0.00^{b}$ | $7.31 \pm 0.83^{b}$ | Inactive |
| RNM 3 | $0.29 \pm 0.01^{c}$ | Inactive | Inactive |
| Reserva Laguna Parrillar | | | |
| RLP 1 | $0.19 \pm 0.01^{d}$ | $12.41 \pm 0.92^{c}$ | Inactive |
| RLP 2 | $0.11 \pm 0.01^{e}$ | Inactive | Inactive |
| Navarino Island | | | |
| OM 1 | $0.08 \pm 0.00^{a,f}$ | $17.61 \pm 1.30^{d}$ | Inactive |
| OM 2 | $0.07 \pm 0.00^{a,f}$ | $12.32 \pm 0.82^{c}$ | Inactive |
| UP | $0.18 \pm 0.01^{d}$ | $11.42 \pm 1.10^{c}$ | Inactive |
| Acarbose[#] | 118.17 ± 2.06 μg/mL | 28.48 ± 0.29 μg/mL | – |
| Orlistat[#] | – | – | 0.04 ± 0.00 μg/mL |

PEE: phenolic-enriched extract; RNM: Reserva Nacional Magallanes; OM: Parque Etnobotánico Omora; UP: Upushwaia; RLP: Reserva Laguna Parrillar. # Reference compounds. Results are expressed as the mean values ± SD of three independent experiments. Different superscript letters (a–f) in the same column show significant differences within each collection place, according to Tukey's test ($p < 0.05$).

DPPH* and ORAC activity. This sample presented a very low cytotoxicity value towards human gastric mucosa cells (AGS), with an $IC_{50}$ > 600 μg/mL. A concentration of 500 μg/mL has been described to be close to the normal consumption and absorption of phenolics from berries [21]. Therefore, a maximum concentration of 500 μg/mL was chosen for the GSH determination experiment.

Significant increases in the intracellular GSH content of 13.08% and 7.62% were observed at concentrations of 500 and 250 μg/mL, respectively. Results are expressed as percent compared to untreated controls. The reference compound NAC, a known stimulant of GSH synthesis, increased intracellular GSH content by 18.51% ($p < 0.05$) (Fig. 1).

Interestingly [19], reported that the PEE from *R. magellanicum* and *R. cucullatum*, collected at the Parque Nacional Conguillio, did not significantly raise the GSH content in AGS cells. On the other hand [22], described that PEEs from three samples from the wild Chilean raspberry (*Rubus geoides*) stimulated in a significant way the synthesis of intracellular GSH in the same cell line, at concentrations ranging from 62.5 to 500 μg/mL.

### 3.6. HPLC-MS/MS analyses

Seventy compounds were tentatively identified in the PEE of the fruits, including phenylpropanoids, proanthocyanidins, anthocyanins, flavonoids and simple phenolics, among others. The proposed identification is based on the fragmentation patterns, molecular formula, literature, and database analyses, including www.foodb.ca. The tentative identification of the *Ribes* fruit phenolics is summarized in Tables 3 and 4. Representative HPLC-MS/MS traces are shown in Fig. 2 in the negative ion mode (Fig. 2A) and in the positive ion mode (Fig. 2B).

#### 3.6.1. Anthocyanins

Six anthocyanins were identified in the *R. magellanicum* fruits by HPLC-MS/MS in the positive ion mode detection. The compounds I, II and V shows the neutral loss of hexose, rutinose and coumaroyl hexose, leading to the base peak at *m/z* 303, in agreement with delphinidin. The compounds III, IV and VI also losses hexose, rutinose and coumaroyl hexose and show the base peak at *m/z* 287, supporting the presence of the cyanidin derivatives. Comparison with reference compounds confirmed the identity of the anthocyanins as delphinidin 3-glucoside (I), delphinidin 3-rutinoside (II), cyanidin 3-glucoside (III), cyanidin 3-rutinoside (IV), delphinidin coumaroyl hexoside (V) and cyanidin coumaroyl hexoside (VI), respectively. The identification agrees with previous work on this fruit [9, 10,14] (Table 3).

#### 3.6.2. Phenylpropanoids

Fifteen hexoside-, quinic- and shikimic acid derivatives from caffeic, ferulic and coumaric acid were identified in the samples. The UV spectrum of compound **5** agrees with a caffeic acid derivative. The pseudomolecular ion at *m/z* 341 and fragmentation of compound **5** and its isomer **10** show the loss of hexose (162 amu), yielding a caffeic acid ion at *m/z* 179 [9]. Compounds **5** and **10** were assigned as caffeoyl hexoside 1 and 2, respectively.

Compounds **2**, **3**, **12**, and **20** showed the molecular formula $C_{16}H_{17}O_9$ for $[M\text{-}H]^+$ and fragmentation according to caffeoylquinic acids [23]. Compound **2** eluted before the other isomers and did not match our reference compounds. Compound **3** agrees with neochlorogenic acid (3-caffeoylquinic acid following the IUPAC rules) while **12** and **20** matched the fragmentation pattern for cryptochlorogenic acid (4-caffeoylquinic acid), and chlorogenic acid (5-caffeoylquinic acid), respectively [23]. In Ref. [23], the elution

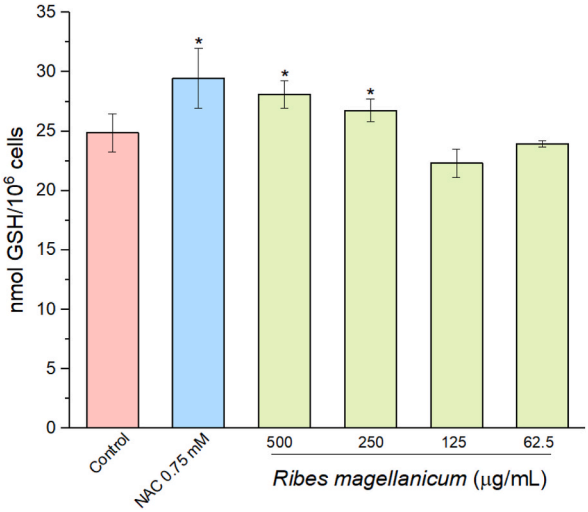



**Fig. 1.** Effect of *Ribes magellanicum* PEE on the GSH content in AGS cells.
Significant differences were determined by one way ANOVA followed by Dunnet's multiple comparison test. *$p < 0.05$ compared to untreated controls.

**Table 3**
Anthocyanins identified in the PEE extracts from *Ribes magellanicum* fruits through HPLC-MS/MS in positive ion mode.

| Compound | Rt (min) | $UV_{max}$ | $[M+H]^+$ | Calculated mass | Molecular formula | Error (ppm) | MS/MS fragments | Identification |
|---|---|---|---|---|---|---|---|---|
| I | 14.21 | 524, 280 | 465.1025 | 465.1033 | $C_{21}H_{21}O_{12}$ | 1.72 | 302.9639 (100) | delphinidin glucoside* |
| II | 16.07 | 516, 280 | 611.1609 | 611.1612 | $C_{27}H_{31}O_{16}$ | 0.49 | 302.9625 (100) | delphinidin rutinoside* |
| III | 17.03 | 515, 280 | 449.1078 | 449.1078 | $C_{21}H_{21}O_{11}$ | 0.02 | 287.0543 (100) | cyanidin 3-glucoside* |
| IV | 20.13 | 516, 280 | 595.1647 | 595.1657 | $C_{27}H_{31}O_{15}$ | 1.68 | 449.1067 (40), 287.0544 (100) | cyanidin rutinoside* |
| V | 37.34 | 524, 316, 274 | 611.1390 | 611.1401 | $C_{30}H_{27}O_{14}$ | 0.85 | 302.9561 (100) | delphinidin coumaroyl hexoside |
| VI | 40.60 | 522, 307, 282 | 595.1438 | 595.1446 | $C_{30}H_{27}O_{13}$ | 1.34 | 287.0544 (100) | cyanidin coumaroyl hexoside |

order of the monosubstituted chlorogenic acids was the following: 3-CQA, 4-CQA, and 5-CQA. Therefore, compounds **3**, **12**, and **20** were tentatively identified as 3-CQA, 4-CQA, and 5-CQA, respectively. For this work, we employed the standards neochlorogenic acid (94419) from Sigma-Aldrich, declared as 5-caffeoylquinic acid, and chlorogenic acid (89175) from Phytolab declared as 3-caffeoylquinic acid. However, most of the suppliers maintain the pre-IUPAC nomenclature. According to the IUPAC rules, the structure of chlorogenic acid corresponds to 5-CQA, and neochlorogenic acid should be 3-CQA [24]. The caffeoylquinic acids were reported among the major constituents of the fruits of several Patagonian *Ribes* species [9,10,14].

The compounds **14**, **17,** and **25** showed the pseudomolecular ion $[M-H]^+$ at *m/z* 367, differing in 15 mass units from caffeoylquinic acid and were identified as feruloylquinic acids (FQA) based on their molecular formula ($C_{17}H_{19}O_9$) and fragmentation pattern with characteristic ions at *m/z* 193 and 191. The occurrence of three isomers at different Rt suggest the presence of the corresponding 3-FQA, 4-FQA, and 5-FQA, respectively [23].

Compounds **7** and **23** show the pseudomolecular ion at *m/z* 337 and $MS^2$ fragments at *m/z* 191 and 163, indicating the presence of coumaroylquinic acids. The UV spectrum of **7** agrees with the coumaric acid moiety and supports the assignment. Thus, **7** and **23** were tentatively identified as 3- and 5-coumaroylquinic acids, respectively [23].

Three caffeoylshikimic acids (compounds **24**, **26**, and **29**) were identified based on the molecular formula $C_{16}H_{15}O_8$ for $[M-H]^+$ at *m/z* 335, differing in 18 amu from caffeoylquinic acid. These compounds showed a neutral loss of a dehydrated shikimic acid moiety (156 amu), yielding an $MS^2$ ion at *m/z* 179, compatible with caffeic acid [14]. The different $R_t$ and intensity of the daughter ions suggest differences in the placement of the caffeoyl moiety in the shikimic acid.

### *3.6.3. Proanthocyanidins*

Eleven compounds were assigned as proanthocyanidins, including the dimers **6**, **8**, **15**, **19**, and **41**, along with the trimers **27** and **32**.

The compounds **1** and **9**, with $[M-H]^+$ at *m/z* 305 and a molecular formula $C_{15}H_{13}O_7$ show characteristic fragments for (epi)-gallocatechin, while **11** and **22**, with $[M-H]^+$ at *m/z* 289 and a molecular formula $C_{15}H_{13}O_6$ agrees with catechin and (epi)-catechin, respectively [25]. The identity of catechin and (epi)-catechin was confirmed by the Rt of reference compounds.

The compounds **8**, **15**, and **19** show the same molecular formula $C_{30}H_{25}O_{12}$ for the $[M-H]^+$ ion at *m/z* 577, yielding a base peak at *m/z* 425 and further fragment to *m/z* 289, suggesting B-type proanthocyanidin dimers of (epi)-catechin [14,25]. The identity and placement of the monomers in the dimers remain to be established. The molecular formula of compound **6** differs in one oxygen atom from the previously described dimers, and the fragmentation with an ion at *m/z* 305 supports an (epi)-gallocatechin-(epi)-catechin dimer [14,25]. The compounds **27** and **32** with molecular formula $C_{45}H_{35}O_{18}$ for the $[M-H]^+$ ion at *m/z* 863 agree with (epi)-catechin trimer with one A-type bond, differing in the intensity of the MS/MS fragments, and they were tentatively assigned as (epi)-catechin trimer 1 and 2, respectively (Lin et al., 2014). Compound **41** showed a $[M-H]^+$ ion at *m/z* 575 and $MS^2$ fragments at *m/z* 423 and 289, suggesting an A-type proanthocyanidin dimer of catechin [25]. To the best of our knowledge, this is the first study reporting the occurrence of A-type proanthocyanidins in Patagonian *Ribes* species. B-type proanthocyanidins have been previously informed for *R. magellanicum*, *R. punctatum*, and *R. cucullatum* [9,14]. The proanthocyanidin content in *R. nigrum* cultivars was correlated with growth environmental conditions, including precipitation, humidity, temperature, and radiation exposure [26]. Therefore, the occurrence of these metabolites in Patagonian *Ribes* may be related to the different environmental conditions of the southernmost collection places of Chilean Patagonia.

### *3.6.4. Flavonoids*

Some 27 flavonoids were tentatively identified in the *R. magellanicum* fruits, including five kaempferol derivatives, quercetin and thirteen quercetin derivatives, four myricetin derivatives, four flavone/flavanone, and one chalcone.

Five kaempferol derivatives were tentatively assigned in the extracts. Compound **54** showed the neutral loss of one hexose (162 amu), while compounds **47**, **53**, **57**, and **58** lost rutinose (308 amu), acetyl hexose (204 amu), and malonyl hexose (248 amu), respectively, leading to the aglycone at *m/z* 285 [14].

Thirteen compounds were identified as quercetin derivatives by the neutral loss of sugars and/or acyl moieties, leading to the $MS^2$ base peak of quercetin at *m/z* 301. The mass spectra of compounds **28**, **33**, **37**, **38**, **40** showed the neutral loss of two hexoses and rhamnose (**28**), hexose and pentose (**33**), rhamnose and hexose (**37**, **38** and **40**), while for compounds **39**, **42**, **43**, **49**, **50**, **51**, **52** and

**Table 4**
Compounds tentatively identified in *Ribes magellanicum* PEE from different collections through HPLC-MS in negative ion mode.

| Peak | Rt (min) | UVmax | $[M-H]^-$ | Calculated mass | Molecular formula | Error (ppm) | MS/MS fragments | Tentative identification |
|---|---|---|---|---|---|---|---|---|
| 1 | 5.50 | | 305.0675 | 305.0667 | $C_{15}H_{13}O_7$ | −2.62 | 260.9627 (60), 218.9221 (95), 178.8747 (100) | (epi)-gallocatechin |
| 2 | 6.23 | 324, 295sh | 353.0887 | 353.0878 | $C_{16}H_{17}O_9$ | −2.54 | 190.9168(100), 178.8988 (50) | Caffeoylquinic acid |
| 3 | 7.19 | 324,295sh | 353.0886 | 353.0878 | $C_{16}H_{17}O_9$ | −2.26 | 190.9775 (100), 178.9718 (55) | 3-Caffeoylquinic acid |
| 4 | 7.40 | | 329.0886 | 329.0878 | $C_{14}H_{17}O_9$ | −2.43 | 166.9944 (100) | Vanillic acid hexoside 1 |
| 5 | 9.01 | 327, 302sh | 341.0887 | 341.0878 | $C_{15}H_{17}O_9$ | −2.63 | 178.9090 (100), 160.8846 (40) | Caffeoyl hexoside 1 |
| 6 | 10.19 | | 593.1315 | 593.1301 | $C_{30}H_{25}O_{13}$ | −2.50 | 467.0246 (35), 424.9667 (100), 305.0003 (25) | (epi)-gallocatechin-(epi)-catechin dimer |
| 7 | 10.64 | 310, 292sh | 337.0939 | 337.0929 | $C_{16}H_{17}O_8$ | −2.96 | 162.9064 (100) | 3-Coumaroylquinic acid |
| 8 | 10.70 | | 577.1371 | 577.1352 | $C_{30}H_{25}O_{12}$ | −3.29 | 424.9536 (100), 406.9898 (50), 288.9938 (20) | (epi)-catechin-(epi)-catechin dimer |
| 9 | 11.21 | 278 | 305.0677 | 305.0667 | $C_{15}H_{13}O_7$ | −3.27 | 261.0042 (60), 220.9342 (90), 178.9389 (100) | (epi)-gallocatechin |
| 10 | 11.23 | | 341.0885 | 341.0878 | $C_{15}H_{17}O_9$ | −2.05 | 178.9518 (100), 134.9598 (10) | Caffeoyl hexoside 2 |
| 11 | 11.27 | | 289.0727 | 289.0718 | $C_{15}H_{13}O_6$ | −3.13 | 245.0021 (100), 204.9901 (40) | catechin |
| 12 | 12.40–13.12 | | 353.0889 | 353.0878 | $C_{16}H_{17}O_9$ | −3.11 | 172.9503 (100), 178.9329 (60) | 4-Caffeoylquinic acid |
| 13 | 12.64 | | 325.0940 | 325.0929 | $C_{15}H_{17}O_8$ | −3.38 | 162.8929 (90), 144.9926 (100) | Coumaroyl hexoside |
| 14 | 13.59 | | 367.1042 | 367.1035 | $C_{17}H_{19}O_9$ | −1.90 | 192.9007 (100) | 3-Feruloylquinic acid |
| 15 | 14.60 | | 577.1370 | 577.1352 | $C_{30}H_{25}O_{12}$ | −3.11 | 450.9861 (30), 424.9871 (100), 288.9426 (30) | (epi)-catechin-(epi)-catechin dimer |
| 16 | 15.62 | | 341.1253 | 341.1242 | $C_{16}H_{21}O_8$ | −3.22 | 178.9248 (100) | Coniferyl hexoside 1 |
| 17 | 15.80 | | 367.1048 | 367.1035 | $C_{17}H_{19}O_9$ | −3.54 | 192.9132 (20), 160.8691 (100) | 4-Feruloylquinic acid |
| 18 | 15.59–15.84 | | 329.0890 | 329.0878 | $C_{14}H_{17}O_9$ | −3.65 | 166.9244 (100) | Vanillic acid hexoside 2 |
| 19 | 15.84 | | 577.1364 | 577.1352 | $C_{30}H_{25}O_{12}$ | −2.07 | 425.0286 (100), 288.9945 (30) | (epi)-catechin-(epi)-catechin dimer |
| 20 | 16.45 | | 353.0889 | 353.0878 | $C_{16}H_{17}O_9$ | −3.11 | 190.9185 (100), 178.9290 (5) | 5-Caffeoylquinic acid |
| 21 | 16.55 | | 387.1674 | 387.1661 | $C_{18}H_{27}O_9$ | −3.35 | 225.0626 (10), 206.9797 (100), 162.9967 (45) | Tuberonic acid hexoside |
| 22 | 17.54 | | 289.0729 | 289.0718 | $C_{15}H_{13}O_6$ | −3.80 | 245.0096 (100), 204.9734 (40) | (epi)-catechin |
| 23 | 17.61 | | 337.0941 | 337.0929 | $C_{16}H_{17}O_8$ | −3.56 | 190.9730 (100) | 5-Coumaroylquinic acid |
| 24 | 18.53 | | 335.0783 | 335.0772 | $C_{16}H_{15}O_8$ | −3.28 | 178.9949 (20), 160.9052 (100) | Caffeoylshikimic acid 1 |
| 25 | 20.78 | | 367.1046 | 367.1035 | $C_{17}H_{19}O_9$ | −2.99 | 192.9945 (10), 190.9523 (100) | 5-Feruloylquinic acid |
| 26 | 21.26 | | 335.0786 | 335.0772 | $C_{16}H_{15}O_8$ | −4.17 | 178.9560 (20), 160.9207 (100), 134.9433 (60) | Caffeoylshikimic acid 2 |
| 27 | 21.35 | | 863.1842 | 863.1829 | $C_{45}H_{35}O_{18}$ | −1.51 | 711.0045 (100), 451.1726 (20) | (epi)-catechin trimer 1 |
| 28 | 23.13 | | 771.2012 | 771.1989 | $C_{33}H_{39}O_{21}$ | −2.98 | 609.1127 (40), 299.9211 (100) | Quercetin rhamnoside dihexoside |
| 29 | 23.42 | | 335.0783 | 335.0772 | $C_{16}H_{15}O_8$ | −3.28 | 178.9863 (20), 160.9133 (100) | Caffeoylshikimic acid 3 |
| 30 | 23.64 | | 365.1824 | 365.1817 | $C_{16}H_{29}O_9$ | −1.91 | 203.0047 (100) | Menthane-tetrol hexoside |
| 31 | 24.85–25.22 | 349, 268 | 625.1428 | 625.1410 | $C_{27}H_{29}O_{17}$ | −2.87 | 315.8925 (100), 316.9262 (80) | Myricetin hexoside rhamnoside |
| 32 | 26.04 | | 863.1828 | 863.1829 | $C_{45}H_{35}O_{18}$ | 0.11 | 711.0868 (70), 575.0970 (100) | (epi)-catechin trimer 2 |
| 33 | 26.67 | | 595.1313 | 595.1305 | $C_{26}H_{27}O_{16}$ | −1.34 | 300.9417 (50), 299.9285 (100) | Quercetin hexoside pentoside |
| 34 | 26.78 | | 523.2195 | 523.2185 | $C_{26}H_{35}O_{11}$ | −1.91 | 361.0740 (100) MS3 346.0082 (100), 179.0105 (40), 165.0000 (70) | Secoisolariciresinol hexoside |
| 35 | 29.01 | | 447.0946 | 447.0933 | $C_{21}H_{19}O_{11}$ | −2.90 | 284.9260 (100) MS3 240.9709 (100) | Luteolin hexoside 1 |
| 36 | 29.08 | | 341.1252 | 341.1242 | $C_{16}H_{21}O_8$ | −2.93 | 178.9595 (100) | Coniferyl hexoside 2 |

(*continued on next page*)

**Table 4** (*continued*)

| Peak | Rt (min) | UVmax | $[M-H]^-$ | Calculated mass | Molecular formula | Error (ppm) | MS/MS fragments | Tentative identification |
|---|---|---|---|---|---|---|---|---|
| 37 | 29.14 | | 609.1478 | 609.1461 | $C_{27}H_{29}O_{16}$ | −2.79 | 300.9468 (100) | Quercetin hexoside rhamnoside 1 |
| 38 | 29.66 | | 609.1482 | 609.1461 | $C_{27}H_{29}O_{16}$ | −3.44 | 300.9664 (100) | Quercetin hexoside rhamnoside 2 |
| 39 | 30.28 | | 477.0691 | 477.0675 | $C_{21}H_{17}O_{13}$ | −3.35 | 300.9552 (100) | Quercetin glucuronide |
| 40 | 30.43 | 356, 265 | 609.1474 | 609.1461 | $C_{27}H_{29}O_{16}$ | −2.13 | 300.9485 (100) | Quercetin hexoside rhamnoside 3 |
| 41 | 30.66 | | 575.1208 | 575.1195 | $C_{30}H_{23}O_{12}$ | −2.26 | 422.9510 (100), 289.0659 (25) | A-type (epi)-catechin dimer |
| 42 | 30.75 | | 565.1213 | 565.1198 | $C_{25}H_{25}O_{15}$ | −2.65 | 299.9306 (100), 270.9115 (10) | Quercetin dipentoside |
| 43 | 30.78 | | 463.0888 | 463.0882 | $C_{21}H_{19}O_{12}$ | −1.29 | 300.9852 (100) | Quercetin hexoside |
| 44 | 31.86 | | 479.0845 | 479.0831 | $C_{21}H_{19}O_{13}$ | −2.92 | 316.9108 (100) | Myricetin hexoside |
| 45 | 31.87 | 349, 266 | 447.0945 | 447.0933 | $C_{21}H_{19}O_{11}$ | −2.68 | 284.9402 (100) | Luteolin hexoside 2 |
| 46 | 32.21 | | 597.1846 | 597.1824 | $C_{27}H_{33}O_{15}$ | −3.68 | 477.0134 (100), 417.0633 (30), 386.9921 (70), 356.9913 (90) | Phloretin-C-dihexoside |
| 47 | 32.43 | | 593.1528 | 593.1512 | $C_{27}H_{29}O_{15}$ | −2.69 | 284.9240 (100), 254.9236 (20) | Kaempferol rutinoside 1 |
| 48 | 33.26 | | 519.1884 | 519.1872 | $C_{26}H_{31}O_{11}$ | −2.31 | 357.0229 (100) $MS^3$ 311.0671 (20), 150.9125 (100), 135.8889 (50) | Pinoresinol hexoside |
| 49 | 33.55 | | 433.0791 | 433.0776 | $C_{20}H_{17}O_{11}$ | −3.46 | 300.9429 (100) | Quercetin pentoside |
| 50 | 33.68 | | 549.0899 | 549.0886 | $C_{24}H_{21}O_{15}$ | −2.36 | 505.0311 (100) $MS^3$ 462.9935 (20), 300.9135 (100) | Quercetin malonylhexoside |
| 51 | 33.93 | 357, 267 | 505.0998 | 505.0988 | $C_{23}H_{21}O_{13}$ | −1.97 | 462.9844 (65), 300.9307 (100) | Quercetin acetylhexoside |
| 52 | 34.62 | | 433.0786 | 433.0776 | $C_{20}H_{17}O_{11}$ | −2.31 | 300.9697 (100) | Quercetin pentoside |
| 53 | 34.93 | | 593.1522 | 593.1512 | $C_{27}H_{29}O_{15}$ | −1.68 | 284.9552 (100) | Kaempferol rutinoside 2 |
| 54 | 35.45 | | 447.0943 | 447.0933 | $C_{21}H_{19}O_{11}$ | −2.23 | 284.9992 (100) | Kaempferol hexoside |
| 55 | 36.55 | | 373.1881 | 373.1868 | $C_{18}H_{29}O_8$ | −3.48 | 211.0082 (100), 192.9650 (40) | Trinorguaiane-type sesquiterpene hexoside 1 |
| 56 | 36.57 | | 431.0997 | 431.0984 | $C_{21}H_{19}O_{10}$ | −3.01 | 268.9713 (100) | Apigenin hexoside |
| 57 | 37.68 | | 489.1050 | 489.1039 | $C_{23}H_{21}O_{12}$ | −2.24 | 284.9771 (100) | Kaempferol acetylhexoside |
| 58 | 39.64 | | 533.0952 | 533.0937 | $C_{24}H_{21}O_{14}$ | −2.81 | 488.9973 (100) | Kaempferol malonylhexoside |
| 59 | 41.08 | | 625.1215 | 625.1198 | $C_{30}H_{25}O_{15}$ | −2.71 | 478.9791 (100), 316.9240 (15) | Myricetin coumaroyl hexoside |
| 60 | 41.22 | | 373.1883 | 373.1868 | $C_{18}H_{29}O_8$ | −1.33 | 211.0450 (100), 192.9925 (10) | Trinorguaiane-type sesquiterpene hexoside 2 |
| 61 | 41.55 | | 433.1154 | 433.1140 | $C_{21}H_{21}O_{10}$ | −3.23 | 270.9736 (100) | Naringenin hexoside |
| 62 | 43.11 | | 625.1219 | 625.1198 | $C_{30}H_{25}O_{15}$ | −3.35 | 478.9245 (100), 316.8969 (20) | Myricetin coumaroyl hexoside |
| 63 | 44.89 | 368 | 301.0367 | 301.0354 | $C_{15}H_9O_7$ | −4.31 | 178.8420 (100), 150.8846 (80) | Quercetin |
| 64 | 45.24 | 348, 313 | 609.1264 | 609.1250 | $C_{30}H_{25}O_{14}$ | −2.29 | 462.9385 (100), 300.8918 (20) | Quercetin coumaroyl hexoside |

Error calculation (ppm): (Theoretical-experimental)/theoretical*$10^6$.

**64**, the neutral loss was of glucuronic acid (**39**), two pentoses (**42**), hexose (**43**), pentose (**49** and **52**), malonyl hexose (**50**), acetyl hexose (**51**) and coumaroyl hexose (**64**), respectively. The compound **63** was identified as quercetin by the UV and mass spectra and comparison with a reference compound.

Four myricetin derivatives were identified by the neutral loss of sugars and acyl esters leading to the aglycone at *m/z* 317. Compound **31** showed a loss of hexose (162 amu) and rhamnose (146 amu), while compound **44** lost a hexose (162 amu), **59** and **62** lost coumaroyl hexose (308 amu) leading to the aglycone. Therefore, they were tentatively assigned as myricetin hexoside rhamnoside (**31**), myricetin hexoside (**44**), and myricetin coumaroyl hexosides 1 (**59**) and 2 (**62**).

Luteolin hexosides **35** and **45**, apigenin hexoside **56**, and naringenin hexoside **61** were tentatively identified by the neutral loss of hexose (162 amu) and the $MS^2$ base peak at *m/z* 285, 269, and 271, respectively, supporting the proposed assignation. Compound **46** showed neutral losses of 120 and 60 amu, suggesting a C-dihexoside. The MS/MS fragments at *m/z* 477, 417, 387, and 357 matches the reported data for the chalcone phloretin C-dihexoside [27].

Previous studies informed a variety of flavonols, flavones, and flavanones derivatives for the Patagonian *R. magellanicum*, *R. puctatum*, *R. cucullatum*, and *R. trilobum* [8,9]. Quercetin, kaempferol, and myricetin derivatives are usually the most frequently found flavonoids besides anthocyanins; however, luteolin, apigenin, and hesperetin derivatives were also observed, in agreement with our results [11]. Regarding phloretin C-dihexoside (**46**), this chalcone is reported for the first time in *R. magellanicum* fruits.

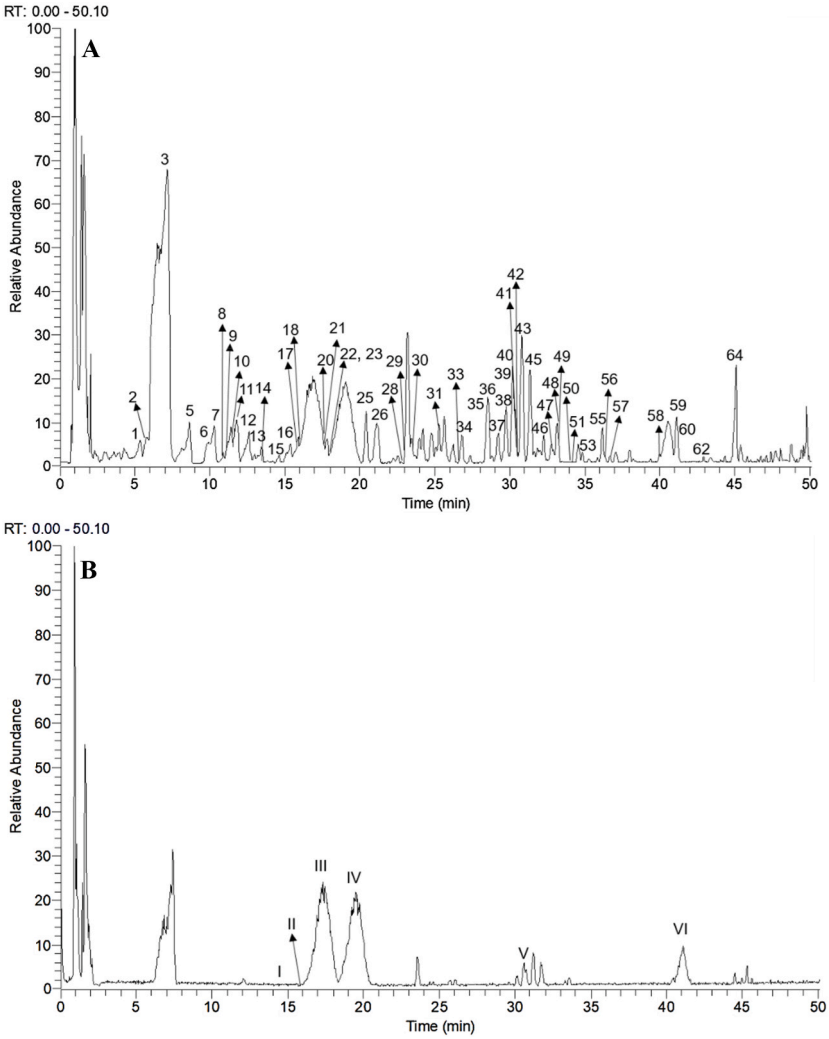



**Fig. 2.** Representative HPLC-MS/MS traces of the PEE from *R. magellanicum* fruits. The chromatograms in negative (2A) and positive (2B) ion mode are from the OM 2 sample. The identity of the compounds is shown in Tables 3 and 4.

### *3.6.5. Lignans*

Compounds **34** and **48** show the molecular formulas $C_{26}H_{35}O_{11}$ and $C_{26}H_{31}O_{11}$ for the $[M\text{-}H]^+$ ions at *m/z* 523 and 519, respectively. Both showed the neutral loss of hexose (162 amu), leading to the $MS^2$ base peak at *m/z* 361 and 357, respectively. The $MS^3$ spectrum of **34** showed the characteristic ions at *m/z* 346, 179, and 165 suggesting a secoisolariciresinol aglycon, while compound **48** yielded $MS^3$ ions at *m/z* 311, 151, and 136, compatible with pinoresinol [28]. Therefore, these compounds were tentatively assigned as secoisolariciresinol hexoside (**34**) and pinoresinol hexoside (**48**). However, related isomers differing in stereochemistry cannot be ruled out. This is the first time that lignans are described for *Ribes magellanicum*. Six lignans were isolated and fully characterized from the leaves of its Northern relative, *R. nigrum* [29].

### *3.6.6. Other compounds*

Two vanillic acid hexosides (compounds **4** and **18**) were detected in the fruits and show the neutral loss of hexose (162 amu) from the ion at *m/z* 329, leading to the $MS^2$ base peak at 167 amu, in agreement with vanillic acid [30]. The compounds were assigned as vanillic acid hexoside 1 and 2, respectively.

Coniferyl hexosides (compounds **16** and **36**) were identified by the neutral loss of hexose (162 amu) from the $[M\text{-}H]^+$ ion at *m/z* 341, leading to the $MS^2$ base peak of coniferyl alcohol at *m/z* 179, as described in literature [31].

The compound **21** show the neutral loss of hexose (162 amu) leading to the $MS^2$ base peak at *m/z* 207, supporting the occurrence of tuberonic acid hexoside [30,32].

The molecular formula of compound **30** according to the $[M\text{-}H]^+$ ion at *m/z* 365 is $C_{16}H_{29}O_9$, and shows the neutral loss of hexose (162 amu), leading to the $MS^2$ base peak at *m/z* 203. The molecular formula and fragmentation suggest the presence of a monoterpene

hexoside. Related compounds, including menthane glycosides, have been reported from caraway [33]. The compound was tentatively assigned as menthane-tetrol hexoside. Monoterpene hexosides were previously described in *R. nigrum* leaves [29].

Compounds **55** and **60** show the same molecular formula $C_{18}H_{29}O_8$, according to their $[M\text{-}H]^+$ ion at *m/z* 373, and differ in the Rt, suggesting different isomers. Both compounds lost one hexose (162 amu), leading to the $MS^2$ ions at *m/z* 211 and 193. The mass spectra of **55** and **60** are compatible with the trinorguaiane-type sesquiterpene dictaminoside N from *Dictamnus dasycarpus* [34]. Therefore, compounds **55** and **60** were tentatively identified as trinorguaiane-type sesquiterpene hexosides 1 and 2, respectively (Table 4).

### 3.7. Main phenolic content in the R. magellanicum samples

The content of the main anthocyanins (compounds **I-VI**) and phenylpropanoids (compounds **2**, **7**, **10** and **23**) was determined in the samples using calibration curves built with standard compounds. The anthocyanin and phenylpropanoid content are shown in Tables 5 and 6, respectively and the quality parameters including linear regression data, LOD and LOQ used in the quantification is summarized in Table S1. Representative HPLC traces for the extracts quantified are shown in Fig. 3, including detection at 520 nm for anthocyanins (Fig. 3A) and detection at 330 nm for phenylpropanoids (Fig. 3B).

Large variations in the anthocyanin content were observed, with compounds III (cyanidin 3-glucoside) and IV (cyanidin 3-rutinoside) as the main constituents in all samples. The content ranged from 20.17 to 132.62 and 10.75–95.25 mg/g extract for III and IV, respectively. Largest content was found for the RLP 1 collection. The differences can be explained due to different populations as well as the ripening degree of the fruits. For the phenylpropanoids, the main compound is 3-caffeoyquinic acid (6.73–130.25 mg/g extract) followed by 3-coumaroyl quinic acid (1.17.5.51 mg/g extract), respectively. The content for compound III was higher for the OM 2 and UP plants growing in more sunny places, compared with OM 1, growing in the shady forest. For the RNM samples, highest content of III was found for 2 and 3. Regarding phenylpropanoids, highest 3-caffeoylquinic content was also observed for the 2 and 3 samples from Reserva Nacional Magallanes and OM 2 and UP from Navarino Island. As *R. magellanicum* is not a domesticated species, further work is needed to select the high fruit yielding individuals with higher content of the anthocyanins and bioactive phenylpropanoids. Differences in the contents of the main anthocyanins and 3-CQA were observed previously for *R. magellanicum* fruits from samples collected at Reserva Nacional Malalcahuello and Parque Nacional Conguillio (Araucania Region) [10]. Importantly, the content of cyanidin 3-glucoside (47 mg/100 g PEE), cyanidin 3-rutinoside (20 mg/100 g PEE) and 3-caffeoylquinic acid (1703 mg/100 g PEE) in fruits from Araucanía Region [19], were much lower than in the samples examined in this work.

The content of cyanidin 3-glucoside, cyanidin 3-rutinoside and 3-caffeoylquinic acid were in the range of 270.56–515.90, 148.98–220.36 and 16.07–18.73 mg/100 g fresh fruits, respectively, for samples from Conguillío National Park (Araucanía Region) [9].

The HPLC traces obtained using HPLC-DAD show the main compounds detected and quantified and can serve as reference for further studies on the southern populations of *R. magellanicum* (Fig. 3A and B). The main phenolics are the anthocyanins cyanidin 3-glucoside and cyanidin rutinoside as well as the phenylpropanoid 3-caffeoylquinic acid. Flavonoids were also detected, including glycosides from quercetin and kaempferol, but due to the low content they were not quantified.

In previous works, the main anthocyanins identified in the fruits were the glucosides and rutinosides of cyanidin and delphinidin [9]. The main compound in *R. magellanicum* fruits was isolated and identified as 3-caffeoylquinic acid [9]. In the Patagonian samples examined in the present work, the main phenolic in the fruits was 3-caffeoylquinic acid. Flavonoids and hydroxycinnamic acids were also described in this fruit [8].

The variations observed in the antioxidant capacity and enzyme inhibition among the collection places, even between plant populations from the same National Reserve, can be explained by the different qualitative and quantitative compositions of phenolics among the samples (Tables 5, 6, and S2).

When the cyanidin 3-glucoside and cyanidin 3-rutinoside content are expressed as mg/100 g dry weight (DW), some of the

**Table 5**
Main anthocyanins content from *Ribes magellanicum* PEEs.

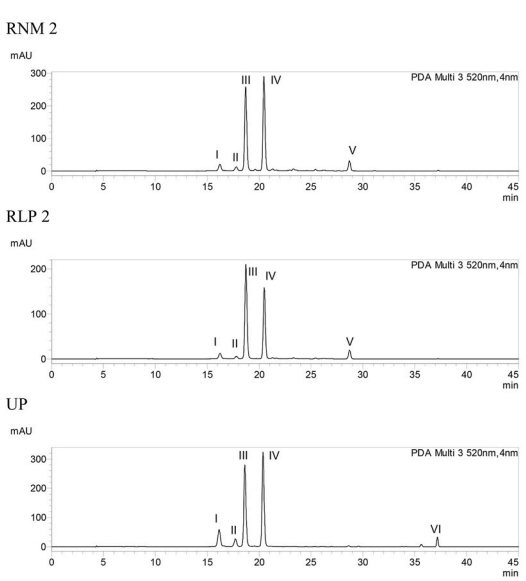
| Samples | I Rt: 16.14 | II Rt 17.72 | III Rt 18.62 | IV Rt 20.37 | V Rt 28.71 | VI Rt 37.19 |
|---|---|---|---|---|---|---|
| Reserva Nacional Magallanes | | | | | | |
| RNM 1 | 4.98 ± 0.21 | BQL | 83.11 ± 4.15 | 10.75 ± 0.50 | 4.85 ± 0.16 | BQL |
| RNM 2 | 3.33 ± 0.09 | 2.14 ± 0.16 | 27.07 ± 0.08 | 31.72 ± 0.94 | 6.69 ± 0.00 | BQL |
| RNM 3 | 3.73 ± 0.05 | 2.40 ± 0.02 | 39.67 ± 0.52 | 42.65 ± 0.63 | 10.87 ± 0.11 | BQL |
| Reserva Laguna Parrillar | | | | | | |
| RLP 1 | 34.72 ± 0.27 | 12.70 ± 0.09 | 132.62 ± 6.60 | 95.25 ± 4.27 | 1.68 ± 0.00 | BQL |
| RLP 2 | 1.68 ± 0.01 | BQL | 20.17 ± 0.10 | 15.19 ± 0.04 | 2.85 ± 0.09 | BQL |
| Navarino Island | | | | | | |
| OM 1 | 8.27 ± 0.51 | 2.12 ± 0.09 | 23.84 ± 1.50 | 12.17 ± 0.72 | BQL | BQL |
| OM 2 | 4.46 ± 0.09 | 1.79 ± 0.03 | 52.35 ± 1.29 | 38.48 ± 1.05 | BQL | 6.10 ± 0.11 |
| UP | 8.47 ± 0.12 | 3.44 ± 0.05 | 29.07 ± 0.31 | 32.32 ± 0.39 | BQL | 2.71 ± 0.03 |

PEE: phenolic-enriched extract; RNM: Reserva Nacional Magallanes; OM: Parque Etnobotánico Omora; UP: Upushwaia; RLP: Reserva Laguna Parrillar Compounds: I*: delphinidin glucoside; II*: delphinidin rutinoside; III*: cyanidin 3-glucoside; IV:* cyanidin 3-rutinoside; V: delphinidin coumaroyl hexoside; VI: cyanidin coumaroyl hexoside. BQL: below quantification limit; *confirmed by co-injection with standards. UV/Vis data: **I**: 525, 445 sh, 341 sh, 277; **II**: 528, 445 sh, 345 sh, 277; **III**: 517, 445 sh, 327 sh, 280; **IV**: 519, 448 sh, 329 sh, 280; **V**: 530, 330 sh; **VI**: 525, 312 sh. Results are presented as mean ± standard deviation (SD) mg of compound per gram of extract. Detection: 520 nm. Rt in minutes.

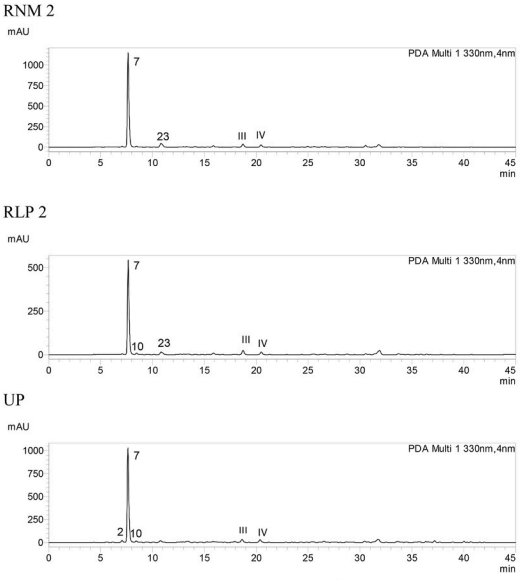
**Table 6**
Main phenylpropanoids content from *Ribes magellanicum* PEEs.

| Samples | CQA (Rt 7.0) | 3-CQA (Rt 7.50) | CH (Rt 8.45) | 3-CoQA (Rt 10.79) |
|---|---|---|---|---|
| | 2 | 7 | 10 | 23 |
| Reserva Nacional Magallanes | | | | |
| RNM 1 | 0.96 ± 0.07 | 72.94 ± 4.44 | 2.66 ± 0.15 | 1.21 ± 0.02 |
| RNM 2 | 1.88 ± 0.24 | 130.25 ± 1.73 | 0.94 ± 0.07 | 5.51 ± 0.13 |
| RNM 3 | 1.47 ± 0.03 | 113.56 ± 0.37 | BQL | 4.37 ± 0.19 |
| Reserva Laguna Parrillar | | | | |
| RLP 1 | BQL | 6.73 ± 0.06 | 2.20 ± 0.04 | 1.30 ± 0.06 |
| RLP 2 | BQL | 60.66 ± 0.68 | 0.96 ± 0.12 | 1.17 ± 0.11 |
| Navarino Island | | | | |
| OM 1 | 1.99 ± 0.06 | 28.80 ± 1.41 | 1.52 ± 0.05 | BQL |
| OM 2 | 2.71 ± 0.26 | 119.93 ± 0.80 | 4.83 ± 0.44 | 2.14 ± 0.18 |
| UP | 2.51 ± 0.03 | 119.78 ± 8.46 | 2.20 ± 0.17 | 1.96 ± 0.21 |

PEE: phenolic-enriched extract; RNM: Reserva Nacional Magallanes; OM: Parque Etnobotánico Omora; UP: Upushwaia; RLP: Reserva Laguna Parrillar CQA: caffeoylquinic acid; 3-CQA: 3-Caffeoylquinic acid; CH: Caffeoyl hexoside; 3-CoQA: 3-Coumaroylquinic acid; BQL: below quantification limit. Results are presented as mean ± standard deviation (SD) mg of compound per gram of extract. Detection: UV, 330 nm. Rt in minutes.




**A.** Compounds: I*: delphinidin glucoside; II: delphinidin rutinoside; III*: cyanidin 3-glucoside; IV*: cyanidin rutinoside; V: delphinidin coumaroyl hexoside; VI: cyanidin coumaroyl hexoside. *confirmed by co-injection with standards.




**B.** Compounds: **2**: Caffeoylquinic acid; **7**: 3-caffeoylquinic acid; **10**: caffeoyl hexoside; **23**: coumaroylquinic acid; **III**: cyanidin 3-glucoside, **IV**: cyanidin-3-rutinoside. Compounds number according to Table 4.

**Fig. 3.** HPLC-DAD profile of the PEE of *Ribes magellanicum* fruits. Collection places: RNM: Reserva Nacional Magallanes; RLP: Reserva Nacional Parrillar; UP: Upushwaia bay, Navarino Island. 3A: Detection at 520 nm for anthocyanins; 3B: Detection at 330 nm for phenylpropanoids.

southernmost samples from *R. magellanicum* showed cyanidin 3-glucoside values ranging from 565.15 to 755.93 mg/100 g DW for RNM 1 and RLP 1, respectively. The content of the cyanidin 3-rutinoside was 73.10 and 542.93 mg/100 g DW for RNM 1 and RLP 1, respectively. Compared with the anthocyanin content of the so-called superfruit açai (425–927 mg/100 g DW and 1255–2195 mg/100 g DW for cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively) [35], some of our *R. magellanicum* samples present a similar content of cyanidin 3-glucoside, but lower cyanidin 3-rutinoside. The total anthocyanin content of Chilean maqui berries (*Aristotelia chilensis*) (138 mg delphinidin 3-glucoside equivalents/100 g DW) and jaboticaba (280 mg cyanidin 3-glucoside/100 g DW and about 350 mg total anthocyanins/100 g DW) [35], is lower than that of some of the investigated samples from *R. magellanicum*.

### *3.8. Distribution of the compounds in the different samples*

The distribution of the compounds tentatively identified in the samples, including anthocyanins I-VI and compounds 1–64 is shown in Table S2. For the minor compounds, the comparison is only qualitative but can serve as reference for future work looking for the selection of plants to be taken into cultivation. The collection of *R. magellanicum* fruits for consumption, or to prepare preserves or liqueurs, is carried out at present only at a limited scale. The fruits are collected from wild growing trees or shrubs and their harvest depends on the strong winds from southern Patagonia, competition with birds and grazing of the plants by cattle. More favorable

conditions were observed for plants in the Aysén and Araucanía Regions, with warmer climate, resulting in larger fruits.

Several bioactivities have been reported for the main and minor constituents from the *R. magellanicum* fruits. A review on the existing literature about the present and past use of Patagonian berries, including *R. magellanicum* has been published [1]. The present work is focused on the southernmost populations of this native berry, and the higher number of fruit constituents identified in this work can be explained by the genetic background and edaphoclimatic conditions that influence the metabolism of the plant populations that grow in southern Patagonia [26]. The results show large variations in constituents as well as in the antioxidant capacity and enzyme inhibition assays. Our data support the presence of inhibitors of α-glucosidase in the fruit that might modulate sugar absorption lowering glycemia. The potential of *R. magellanicum* fruits to control glycaemia needs to be explored using animal experiments. The fruit extract of *Ribes stenocarpum* from the Tibetan Highlands showed hypoglycemic effect in alloxan-diabetic mice as well as in normoglycemic animals [36]. The extract also inhibited α-glucosidase and α-amylase with $IC_{50}$ values of 13 and 5 μg/mL, respectively [36]. The composition of the extracts from both species is different, with rutin, quercetin, and isorhamnetin glycosides as main phenolics in *R. stenocarpus* while *R. magellanicum* shows a more diverse array of constituents, with caffeoylquinic acids as main compounds. Assay-guided isolation of the bio actives is needed to associate the effect to single chemical entities or mixtures of compounds.

## 4. Conclusions

Eight *R. magellanicum* fruits samples from southernmost Patagonia were investigated. They included plants from continental Patagonia and Navarino Island. All samples contained variable amounts of phenolics, flavonoids, proanthocyanidins, and anthocyanins. High antioxidant capacity was found in most samples with best effect in the Navarino Island samples for DPPH*, FRAP and TEAC. A strong inhibition of α-glucosidase was found in the fruit extracts. The OM 2 PEE significantly increased intracellular GSH content in AGS cells. Some 70 compounds were identified in the fruit extracts by HPLC-MS/MS, including anthocyanins, phenylpropanoids, flavonoids, proanthocyanidins, among others.

The anthocyanins delphinidin and cyanidin glucoside and rutinoside, as well as caffeoylquinic acids were the main fruit compounds. Large variations were detected in the content of the main compounds allowing some distinction among the collection places.

The large differences in the content of the main compounds among samples, highlight the relevance of domestication efforts to obtain plants with higher fruit yields and content of health promoting phytochemicals.

## Chemical compounds studied in this article

Cyanidin 3-glucoside PubChem 12303220.
Cyanidin 3-rutinoside PubChem 441674.
Chlorogenic acid (3-caffeoylquinic acid) PubChem 1794427.
Neochlorogenic acid (5-caffeoylquinic acid) PubChem 5280633.
Delphinidin 3-glucoside PubChem 443650.
Delphinidin 3-rutinoside PubChem 192918.

## Data availability statement

Data will be made available upon request.

## Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because it did not involve animals neither human volunteer.

## CRediT authorship contribution statement

**Alberto Burgos-Edwards:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cristina Theoduloz:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sophia Miño:** Investigation, Data curation. **Debasish Ghosh:** Methodology, Investigation. **Vladimir Shulaev:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Crister Ramírez:** Investigation, Data curation. **Laura Sánchez-Jardón:** Investigation, Data curation. **Ricardo Rozzi:** Writing – review & editing, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Guillermo Schmeda-Hirschmann:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests.

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## Abbreviations

| | |
|---|---|
| AAPH | 2,2′-azobis(2-methylpropionamidine) dihydrochloride, 2,2'-azobis-(2- amidinopropane) |
| $ABTS^{+}$* | 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) |
| CE | Catequin equivalent |
| DPPH* | 2,2-diphenyl-1-picrylhydrazyl radical |
| DMAC | dimethylamino cinnamaldehyde |
| FRAP | ferric reducing antioxidant power |
| GAE | Gallic acid equivalent |
| GSH | reduced glutathione |
| HPLC-DAD-MS/MS | high performance liquid chromatography coupled with diode-array detection and tandem mass spectrometry |
| LOD | limit of detection |
| LOQ | limit of quantification |
| ORAC | oxygen radical absorbance capacity |
| PEE | polyphenol-enriched extract |
| TA | total anthocyanins |
| TEAC | Trolox equivalent antioxidant capacity |
| TE | Trolox equivalent |
| TF | total flavonoid |
| TP | total phenolic |
| TPA | total proanthocyanidin |
| TPTZ | 2,4,6-tri(2-pyridyl)1,3,5-triazine |

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25542.

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