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Phenolics and enzyme inhibitors from the Patagonian *Empetrum rubrum* berries

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ABSTRACT

The small fruits from *Empetrum rubrum* were consumed by Native Americans in southern Patagonia. Two samples collected close to the Magellan Street and the Beagle channel in Tierra del Fuego were investigated. Three different extracts from the berries were analyzed, namely: methanol, phenolic-enriched (PEE) and ethyl acetate (EtOAc). The total phenolic, flavonoid, procyanidin and anthocyanin content of the extracts was determined. The effect of the extracts towards the metabolic syndrome-associated enzymes α-glucosidase, α-amylase and pancreatic lipase was assessed. In addition, the antioxidant capacity was measured using DPPH, FRAP, TEAC and ORAC assays. The composition of the extracts was determined by HPLC-MS/MS. Seventy-one compounds were identified for the first time in the berries, including 10 anthocyanins, 35 flavonoids, 11 phenylpropanoids and 13 procyanidins. The main compounds were quantified by HPLC-DAD. The extracts displayed strong activity as α-glucosidase inhibitors, with IC_{50} values ranging from 0.10 to 0.19 μg/mL for the PEE and 0.82–1.95 μg/mL for the EtOAc extracts, respectively. The results show high chemical diversity and differences with the boreal species *E. nigrum*.

1. Introduction

Native berries were a relevant food source for hunters and gatherers in the circumpolar areas of the world. The consumption of the small fruits from *Empetrum rubrum* Vahl ex Willd. (synonym: *Empetrum nigrum* var. andinum A.DC.) by the Kawashkar, Yámana and Selknam in southern South America, was reported by botanists and anthropologists (Domínguez Díaz, 2010; Gusinde, 1917, 1982; Ladio and Lozada, 2004; Martínez Crovetto, 1968, 1982; Mösbach, 1992; Rapoport and Ladio, 1999). The genus *Empetrum* (Empetraceae) comprises *E. nigrum* L. (crowberry) and *E. nigrum* ssp. *hermaphroditum* (Hagerup) Böcher in the northern hemisphere. In the South American Patagonia, *E. rubrum* is the single representative of this circumpolar genus (Bezverkhniaia et al., 2021; https://www.worldfloraonline.org/).

The use of Patagonian berries as food and medicine shows common traits associated with an optimal use of available resources in southern South America (Schmeda-Hirschmann, Jiménez-Aspee, Theoduloz, & Ladio, 2019). The anthocyanin composition of Patagonian berries was described by Ruiz et al. (2013) as well as the flavonoids and hydroxycinnamic acids (Ruiz et al., 2015) but *E. rubrum* was not included.

Studies on the effect of edible wild berries from Alaska have been carried out with the phenolic-enriched extracts (PEE) which were further partitioned into anthocyanin-enriched and proanthocyanidin-enriched extracts (Kellogg et al., 2010). The PEE reduced the lipid accumulation in 3T3-L1 adipocytes and showed hypoglycaemic effect in the acute T2DM model (Kellogg et al., 2010). The procyanidins from

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E. nigrum fruits comprised different dimers, trimers and a tetramer based on (epi)catechin and (epi)gallocatechin. In crowberry, Ogawa et al. (2008) described 13 anthocyanins, with cyanidin, delphinidin and peonidin monoglycosides, occurring in samples from Alaska and Korea. Koskela et al. (2010) reported delphinidin, cyanidin, petunidin, peonidin and malvidin hexosides and pentosides from different accessions in Finland. From a sample of *Empetrum nigrum* var. japonicum, Park, Lee, Han, Lee, and Lee (2012) identified quercetin and kaempferol as the main flavonoid aglycones. The extract showed antioxidant effects in the pulmonary artery endothelial cells model under $H_2O_2^-$-induced oxidative damage.

Most studies on the chemistry and bioactive compounds in *Empetrum* fruits have been carried out with *E. nigrum* as well as with subspecies sometimes recognized as different taxa (Bezverkhniaia et al., 2021). The flavonoids occurring in crowberry and the reported activities when tested as single chemical entities were summarized in a review by Jurikova et al. (2016). The effects of crowberry fruits on healthy human volunteers were described (Park et al., 2012). After a daily intake of 2 g powdered fruit for four weeks, several parameters improved, including an increase in total antioxidant status, superoxide dismutase, and lipid profiles. From the acetone soluble part of the chloroform extract from the aerial part of *E. nigrum*, Krasnov et al. (2000) described chalcones, as well as 6,8-dimethylpinocembrine. A study on the phenolics from *E. hermaphroditum* fruits showed delphinidin 3-galactoside as the main anthocyanin The 3-galactoside, 3-glucoside and 3-arabinoside of delphinidin, cyanidin, peonidin and malvidin were also reported (Lavola, Salonen, Virjamo, & Julkunen-Tiitto, 2017).

The antibacterial activity of extracts from leaves and fruits of *E. nigrum* and other berries from Finland was described (Tian et al., 2018). Separation of the extracts using Sephadex LH-20 allowed to relate the activities with groups of compounds but the single constituents responsible for the ORAC effect and antibacterial effect were not identified.

The association of obesity, hyperlipidemia, hypertension, and insulin resistance is known as metabolic syndrome and precedes the onset of type-2 diabetes and an increase in the risk of cardiovascular diseases. The enzymes α-glucosidase and α-amylase are key biocatalysts for the hydrolysis of (poly-)oligosaccharides into monosaccharides. Lipase hydrolyses triglycerides and fatty acids derivatives before absorption from the small intestine. When these enzymes are inhibited, the hydrolysis of polysaccharides and fats is reduced, preventing hyperglycaemic and hyperlipidemic peaks (McDougal, Kulkarni & Stewart, 2009; Burgos-Edwards, Jiménez-Aspee, Theoduloz, & Schmeda-Hirschmann, 2018; Burgos-Edwards et al., 2023).

In this study, we investigated the composition and effect of *E. rubrum* fruit extracts towards enzymes associated to metabolic syndrome, including α-glucosidase, α-amylase, and pancreatic lipase.

2. Materials and methods

2.1. Reagents and chemicals

The source of the chemicals and reagents was as follows. Sigma-Aldrich (St. Louis, MO, USA): 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), lipase from porcine pancreas type II (L-3126; EC 3.1.1.3), AAPH (2,2′-azobis (2-methylpropionamidine) dihydrochloride, ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), 4-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl palmitate, sodium acetate, starch, quercetin, (+)-catechin, gallic acid, acarbose, L-glutamine, AlCl3, 2′,7′-dichlorodihydrofluorescein diacetate, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), 2,4,6-tri (2-pyridyl)1,3,5-triazine (TPTZ), dinitro salicylic acid, NaHCO3, Na2CO3. Merck (Darmstadt, Germany): HPLC-grade solvents, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), FeCl3.6H2O, Folin-Ciocalteu reagent, potassium sodium tartrate, potassium persulfate. PhytoLab (Vestenbergsgreuth, Germany): Delphinidin rutinoside, cyanidin 3-glucoside, 3-caffeoylquinic acid and 5-caffeoylquinic acid. Cayman Chemical Co., USA: delphinidin glucoside and cyanidin rutinoside. Laboratorio Chile (Santiago, Chile): Orlistat. Ultrapure water was obtained using a Barnsted EasyPure water filter (Thermo Scientific, Ohio, USA).

2.2. Berry samples

Samples from *Empetrum rubrum* fruits were collected at Reserva Nacional Magallanes (RNM) (January 23, 2022) and Cerro Bandera, Navarino Island (CB) (January 26, 2022). The fruits from RNM were from plants growing on sandy soil, well drained and exposed to sunlight. The collection from CB was from the top of the hill, growing on rock cracks, exposed to strong wind. The fruits were taken to the laboratory, cleaned, weighed and frozen until extraction.

2.2.1. Extraction and purification

The fruits were powdered in a blender and extracted four times with MeOH:formic acid (99:1 v/v) in a fruit-to-solvent ratio of 1:3, sonicating for 15 min each time. Then, the fruits were extracted three times with 0.5 L of ethyl acetate (EtOAc). After removing the solvent under reduced pressure, the MeOH extract was enriched in phenolics by adsorption on activated Amberlite XAD-7 as described previously (Burgos-Edwards et al., 2018, 2023; Burgos-Edwards, Jiménez-Aspee, Thomas-Valdés, Schmeda-Hirschmann, & Theoduloz, 2017). The resin was previously 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. Briefly, the combined dry crude MeOH extract was resuspended in distilled water (2 L) and stirred with activated Amberlite XAD-7 (300 mL of the slurry) for 2 h at room temperature. After filtration of the water-soluble, the resin was washed two times with distilled water and the adsorbed compounds were desorbed by MeOH:formic acid 99:1. Concentration of the desorbed compounds and lyophilization afforded the phenolic-enriched extract (PEE) from the fruits. The EtOAc soluble were partitioned with $CHCl_3$. The $CHCl_3$-insoluble portion of the EtOAc extract was redissolved in hot MeOH and was left at −20 °C overnight to precipitate non-polar constituents. The crude MeOH extract, the PEE and the EtOAc extract were evaluated in the parameters described below. All organic solvents were removed from the extracts under reduced pressure and then the remaining solid were lyophilized before analyses.

2.3. Chemical analyses

2.3.1. Total phenolic, flavonoid, procyanidin and anthocyanin content

The total phenolic (TP), total flavonoid (TF) and total procyanidin (TPC) of the different fruit extracts was determined using the Folin-Ciocalteu reagent (Nina et al., 2023). The results are presented as gallic acid equivalents (GAE)/100 g of extract. The total flavonoid (TF) content was measured by the aluminum trichloride method, and the results are shown as g catechin equivalents (CE)/100 g of extract. The total procyanidin content (TPC) was measured using the 4-dimethylaminocinnamaldehyde (DMAC) method (Nina et al., 2023) and results are shown as g catechin equivalents (CE)/100 g of extract. For total anthocyanin content (TA), the pH-differential method was used (Lee, Durst, Wrolstad, & Collaborators, 2005). The results are presented as mg cyanidin equivalents (CyE)/100 g of extract. All quantifications were carried out in triplicate.

2.3.2. Antioxidant capacity assays

Four different and complementary methods were used to assess the antioxidant capacity of the samples, as described in Jiménez-Aspee et al. (2016) and Nina et al. (2023). The assays comprised the discoloration of the 2,2′-azinobis (3-ethylbenzothiazoline)-6- sulfonic acid radical cation (ABTS·+), the reduction of ferric cation (FRAP), the 2,

2-diphenyl-1-picrylhydrazyl radical (DPPH·) assay, and the ORAC assay. The samples were diluted in stock solutions ranging from 5 to 300 μg/mL for individual experiments, according to the different protocols. Trolox was used to build the calibration curves for the FRAP, ABTS, and ORAC assays. Quercetin and catechin were used as positive controls. The results are expressed as μmol TE/g extract for FRAP and ORAC. The TEAC results are presented as μmol TE/g extract and DPPH as SC_{50} (μg/mL).

2.4. Enzyme inhibition assays

The different extracts were evaluated for the inhibition of α-glucosidase, α-amylase, and pancreatic lipase according to (Nina et al., 2023).

2.4.1. α-glucosidase inhibition assay

The samples, dissolved in phosphate buffer (120 μL in 0.1 mol/L sodium phosphate buffer pH 6.8), were mixed with 20 μL of the α-glucosidase solution (0.25 U/mL, in sodium phosphate buffer). After pre-incubation for 15 min at 37 °C, the substrate (5 mmol/L *p*-nitrophenyl-α-D-glucopyranoside) dissolved in sodium phosphate buffer was added (20 μL). After mixing, the solution was incubated again for 15 min at 37 °C. Then, the reaction was stopped adding 80 μL of 0.2 mol/L sodium carbonate. The absorbance was recorded at 415 nm in a microplate reader (Tecan Infinite M Nano+, Maennedorf, Switzerland). Acarbose, a standard inhibitor, was used for comparison. Samples were assessed at final concentrations of 0.1–100 μg/mL in triplicate. The results are presented as percent inhibition or IC_{50} (μg/mL) as mean values ± SD.

2.4.2. α-amylase inhibition assay

For the α-amylase inhibition assay, the samples were assessed at final concentrations of 100 μg/mL. Some 100 μL of the dissolved samples were mixed with 0.02 mol/L sodium phosphate buffer containing a 0.5 mg/mL α-amylase solution (100 μL). Then, the mixture was pre-incubated for 10 min at 37 °C and a 1 g/100 mL starch solution in sodium phosphate buffer (100 μL) was added. The mixture was further incubated for 20 min at 37 °C and then the color reagent (200 μL) was added. After mixing, the test tubes were boiled for 15 min and then, 40 μL of the reaction were mixed with 210 μL of water. Absorbance was recorded in a microplate reader at 550 nm. The standard inhibitor Acarbose was used as reference. All determinations were carried out in triplicate and the results are shown in percentages of inhibition as mean values ± SD.

2.4.3. Lipase inhibition assay

Porcine pancreatic lipase was prepared at 20 mg/mL in ice-cold ultrapure water. The samples were assessed at a final concentration of 50 μg/mL. For the assay, 50 μL of the extract, 150 μL of enzyme solution, 450 μL of the substrate (*p*-nitrophenyl palmitate, 80 mg/100 mL), and 400 μL assay buffer (100 mmol/L Tris, pH 8.2) were mixed. After incubation at 37 °C during 2 h, the absorbance of the reaction mixture was determined at 400 nm with a spectrophotometer (Genesys 10UV, Thermo Spectronic, Rochester, NY, USA). The determinations were carried out in quadruplicate. The results are shown in percentages as mean values ± SD. Orlistat® was used as a reference compound.

2.5. Liquid chromatography

2.5.1. HPLC-DAD

The chemical profile of the polar extracts was analyzed using a Shimadzu HPLC equipment (Shimadzu Corporation, Kyoto, Japan). The instrument consisted of an LC-20AT pump, an SPD-M20A UV diode array detector and a CTO-20 AC column oven. The software was LabSolution. The column used was a Kinetex 5 μm EVO C18 100 Å column (Phenomenex Inc., California, USA) and the column oven was kept at 25 °C. For anthocyanins, 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 % B; 37–40 min, 100 % B; 40–43 min, 100%-15 % B; 43–55 min, 15 % B. The flow rate was 0.6 mL/min.

For hydroxycinnamic acid derivatives and flavonoids, the HPLC solvent system was 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient was: 0–25 min, 5%–25% B; 25–50 min, 25%–58% B; 50–55 min, 58%–100% B; 55–60 min: 100% B; 60–65 min: 100%–60% B; 65–75 min: 60%–5% B. The flow rate was 0.8 mL/min.

The samples were dissolved in the mobile phase (1 mg/mL) and filtered using a 0.22 μm PVDF syringe filter (Agela technologies, DE, USA). For analysis, 20 μL was injected and the compounds were monitored at 330 nm and 360 nm for flavonoids and hydroxycinnamic acid derivatives, and 520 nm to detect anthocyanins. For characterization, spectra were recorded from 200 to 650 nm. The anthocyanins were identified and quantified according to (Gras, Carle, & Schweiggert, 2015) with slight modifications. The HPLC traces were used for quantification of the main compounds and to compare the different samples. All quantifications were carried out in triplicate.

2.5.2. UHPLC-DAD-MS/MS

The equipment used was a 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). The 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) held at 45°C. For the analysis, a flow rate of 0.3 mL/min was used. 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.

The 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 operating in the negative and positive ionization modes. Ions were scanned from *m/z* 120 to 1200. The source voltage was 4.5 kV and heated capillary temperature was 270 °C. Nitrogen was used as the sheath gas at 30 arbitrary units. During the complete chromatographic run, a data dependent mode of acquisition was applied to complete an accurate *m/z* survey scan in the FT cell. A MS/MS based linear ion trap investigation of the top five most abundant precursor ions was performed in parallel. Using the automatic gain control of ion trapping, FT full-scan mass spectra were attained at 60,000 mass resolving power (*m/z* 400). Helium as a target gas with a 2 Da isolation width and 30% of normalized collision energy was used to achieve collision induced dissociation (CID). The precursor ions selected for CID were dynamically excluded from further MS/MS analysis for 30 s. The resolving power for MS^2 scans was 7500. The raw data were processed using the Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA).

2.6. ^1H NMR analysis

The composition of the lipophilic fruit constituents was examined by ^1H NMR using a Bruker 400 NMR spectrometer operating at 400 MHz. Samples were dissolved in MeOH-d4 with a few drops of $CDCl_3$ and lock was performed using residual MeOH.

2.7. Assay-guided isolation of α-glucosidase inhibitors

The MeOH extract from the RNM sample and the EtOAc extract from CB were separately fractionated to get an insight into the identity of α-glucosidase inhibitors from the fruits. The PEE of the RNM collection

(1.2 g) was permeated in a Sephadex LH-20 column (column length: 68 cm, internal diameter 3.5 cm, filled with 29 cm Sephadex), eluting with MeO:H_2O:formic acid (FA) 80:20:0.5 v/v/v. The void volume was 115 mL. Thin layer chromatography (TLC) was performed on silica gel plates, eluting with EtOAc:acetic acid:water 10:2:1.5 v/v/v. The chromatograms were visualized under UV light before and after spraying with diphenylboric acid β-ethylamino ester) (NPR) (Wagner & Bladt, 1996). After TLC comparison, fractions were pooled according to the TLC patterns as follows. Fraction 1 (29 mL 20.3 mg); 2–4 (62 mL; 186.3 mg); 5–6 (18 mL; 69.4 mg); 7–9 (29 mL; 95.9 mg); 10–11 (38 mL; 45.6 mg); 12–13 (34 mL; 21.3 mg); 14–15 (40 mL; 26.7 mg); 16–17 (40 mL; 34.7 mg); 18 (21 mL; 28.3 mg); 19 (31 mL; 45.2 mg); 20 (30 mL; 29.3 mg), 21 (72 mL; 50.3 mg); 22 (80 mL; 30.7 mg); 23 (175 mL; 21.3 mg); 24 (220 mL; 156.1 mg); 25 (118 mL; 15.2 mg); 26 (82 mL; 64.7 mg); 27 (210 mL; 79 mg) and 28 (112 mL; 61.2 mg).

The defatted EtOAc extract from CB (2.83 g) was permeated in Sephadex LH-20 using MeOH:H_2O 9:1 as eluent. Some 26 fractions were collected as follows. Dead volume 151 mL; fractions 1–2 (38 mL; 198.1 mg); 3 (15 mL; mg); 4–5 (24 mL; 534.5 mg); 6–7 (23 mL; 114.2 mg); 8–9 (80 mL; 29.6 mg); 10–11 (45 mL; 40.7 mg); 12–14 (69 mL; 34 mg); 15–18 (78 mL; 28.7 mg); 19–20 (45 mL; 12.1 mg); 21–23 (74 mL; 12.6 mg); 24–25 (101 mL; 18.9 mg); 26 (250 mL; 34.3 mg).

2.8. Statistical analyses

Determinations were carried out in triplicate or quadruplicate. Results are reported as the arithmetic means ± SD. Significant differences in the TP, TF, TPC, TA contents, inhibition of enzymes and antioxidant capacity were detected by one-way analysis of variance (ANOVA) followed by Tukey's 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

3.1. Extraction yields

Two Patagonian samples from *E. rubrum* berries were investigated for inhibition of enzymes related to metabolic syndrome, composition, and antioxidant capacity. The w/w extraction yields for the MeOH extract and Amberlite XAD-7 PEE from the fresh berries was as follows. RNM: 5.6% and 0.52%; CB: 6.29 and 0.29%, respectively. The CB extraction yield with EtOAc was 0.69%.

3.2. Phenolics and antioxidant capacity

Determinations were performed with the total MeOH (crude) extract of the fruits as well as with the extracts enriched in phenolics (PEE). The EtOAc-soluble from the fruit residue after MeOH extraction contained less polar compounds not extracted with MeOH. Higher TP and TF content was found in the CB sample (44.25 g GAE/100 g PEE and 30.21 g CE/100 g PEE) while higher TPA and TA was for the RNM fruits (20.74 g CE/100 g PEE and 1.83 g CyE/100 g PEE). The content of the different groups of phenolics was higher in the PEE since sugars, salts and other polar compounds were removed. The antioxidant capacity of the PEE was moderate, with SC_{50} values of 8.93 and 11.89 μg/mL for the CB and RNM fruits, respectively. However, the CB PEE was more active in the ORAC assay, with values of 3139.67 and 1560.33 μmol TE/g PEE for the CB and RNM fruit PEE, respectively. In the DPPH assay, the EtOAc soluble and the total MeOH extracts were less active than the PEE. The EtOAc solubles were the less active in the ORAC values due to the lower phenolic content and composition (Table 1). The FRAP and TEAC values increased after enrichment of phenolics, with higher values for the CB sample.

3.3. Effect on metabolic syndrome-related enzymes

Best effect was found for α-glucosidase, with IC_{50} values of 0.10 and 0.19 μg/mL for the CB and RNM sample, respectively. The EtOAc solubles were less active, with IC_{50} values of 1.95 (CB) and 0.82 μg/mL (RNM), respectively (Table 2). At 100 μg/mL, the extracts were devoid of activity towards α-amylase. Only the sample from Cerro Bandera showed some inhibitory activity on lipase at 50 μg/mL, reducing the effect of the enzyme by 14.06 ± 0.96 %.

3.4. Compounds identification by HPLC-MS/MS analyses

Seventy-one compounds were identified in the fruit extract. The identification proposed is supported on the molecular formula, fragmentation patterns, database analyses, including www.foodb.ca and literature. The identification of the *E. rubum* fruit phenolics is summarized in Table 3 (anthocyanins) and Table 4. The HPLC-MS/MS traces

Table 1
Total phenolic (TP), total flavonoid (TF), total procyanidin (TPC), total anthocyanidin (TA) content and antioxidant capacity (DPPH, FRAP, TEAC, ORAC) of the crude MeOH, PEE and EtOAc extracts from Chilean *Empetrum rubrum* fruits.

Sample	TP (g GAE/100 g extract)	TF (g CE/100 g extract)	TPC (g CE/100 g extract)	TA (g CyE/100 g extract)	DPPH (% at 100 μg/mL or SC_{50} μg/mL)	FRAP (μmol TE/g extract)	TEAC (μmol TE/g extract)	ORAC (μmol TE/g extract)
Crude								
CB	7.04 ± 0.05^{a}	5.19 ± 0.04^{a}	2.56 ± 0.08^{a}	0.16 ± 0.00^{a}	*40.10 ± 2.21%*^{a}	745.44 ± 14.70^{a}	324.61 ± 4.85^{a}	1213.06 ± 47.98^{a}
RNM	5.20 ± 0.08^{a,d}	3.16 ± 0.03^{b}	1.42 ± 0.01^{a,d}	0.15 ± 0.00^{a}	*50.23 ± 1.34%*^{b}	507.07 ± 11.69^{b}	284.69 ± 4.33^{a}	611.09 ± 43.24^{b}
PEE								
CB	44.25 ± 1.56^{b}	30.21 ± 0.34^{c}	18.02 ± 0.86^{b}	0.64 ± 0.01^{b}	8.93 ± 0.22^{c}	3645.53 ± 91.82^{c}	2504.46 ± 30.17^{b}	3139.67 ± 126.63^{c}
RNM	40.28 ± 0.65^{c}	26.79 ± 0.32^{d}	20.74 ± 0.88^{c}	1.83 ± 0.02^{c}	11.89 ± 0.08^{d}	3331.27 ± 47.07^{d}	2029.41 ± 20.89^{c}	1560.33 ± 112.60^{d}
EtOAc								
CB	5.43 ± 0.06^{a,d}	5.49 ± 0.10^{a}	1.50 ± 0.08^{a,d}	0.02 ± 0.00^{d}	*33.71 ± 0.44%*^{e}	458.97 ± 16.02^{e,b}	548.50 ± 13.70^{d}	374.57 ± 3.23^{e}
RNM	4.00 ± 0.14^{d}	3.78 ± 0.07^{e}	0.76 ± 0.02^{d}	0.03 ± 0.00^{d}	*30.72 ± 1.33%*^{e}	381.87 ± 9.80^{e}	377.21 ± 7.69^{e}	166.63 ± 3.28^{f}
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

CB.: Cerro Bandera; RNM: Reserva Nacional Magallanes; 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. Percent DPPH scavenging at 100 μg/mL in italics TE: Trolox equivalents. –: not determined. #: reference compounds. Results are 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 ($p < 0.05$).

Table 2
Inhibitory activity of the *Empetrum rubrum* fruit extracts towards α-glucosidase and assay-guided isolation of α-glucosidase inhibitors from the EtOAc (CB) and MeOH (RNM collection) extract of *E. rubrum* fruits.

Cerro Bandera (CB)	α-glucosidase (IC_{50}, μg/mL)	Reserva Nacional Magallanes (RNM)	α-glucosidase (IC_{50}, μg/mL)
MeOH	0.10 ± 0.01[a]	MeOH	0.19 ± 0.01[a]
EtOAc	1.95 ± 0.06[b]	EtOAc	0.82 ± 0.04[c]
LH-20 fraction, CB	α-glucosidase (IC_{50}, μg/mL)	LH-20 fraction, RNM	α-glucosidase (IC_{50}, μg/mL)
1–2	20.22 ± 1.02	1	5.14 ± 0.59
3	35.59 ± 1.97	2–4	4.26 ± 0.42
4–5	10.76 ± 0.61	7–9	0.79 ± 0.10
6–7	12.85 ± 0.13	10–11	0.20 ± 0.00
8–9	2.84 ± 0.17	12–13	0.30 ± 0.01
10–11	2.56 ± 0.04	14–15	0.39 ± 0.09
12–14	0.49 ± 0.07	16/17	0.61 ± 0.05
15–18	0.46 ± 0.02	18	0.77 ± 0.14
19–20	0.51 ± 0.02	19	0.95 ± 0.00
21–23	0.35 ± 0.09	20	0.46 ± 0.00
24–25	0.27 ± 0.04	21	0.93 ± 0.00
26	0.26 ± 0.05	22	0.91 ± 0.01
		23	0.74 ± 0.08
Acarbose[#]	118.17 ± 2.06	24	0.18 ± 0.02
		25	0.21 ± 0.05
		26	0.15 ± 0.00
		27	0.26 ± 0.02

IC_{50}: concentration that inhibits 50% of the enzyme activity; #: reference compound. Results are the mean values ± SD of three independent experiments. Different superscript letters (a-c) show significant differences within each collection place, according to Tukey's test ($p < 0.05$).

with the compounds identified are shown in Figs. 1 and 2.

3.4.1. Anthocyanins

Ten anthocyanins were identified in the fruits by the visible/UV spectra and the fragmentation patterns in MS (Table 3). The compounds II, IV and IX were cyanidin derivatives based on the base peak at *m/z* 287 and the loss of 162, 132 and 190 amu from the $[M+H]^+$ ion. The compounds were the hexoside (II) and pentoside (IV) of cyanidin while (IX) was assigned as cyanidin derivative. The compounds V and VIII loss 162 and 132 amu leading to the base peak at *m/z* 301, in agreement with peonidin hexoside (V) and pentoside (VIII), respectively. Delphinidin 3-glucoside (I) was identified based on the $[M+H]^+$ ion at *m/z* 465 and the loss of 162 amu. The identity was confirmed by co-chromatography with a standard. Two compounds (III and VI) were identified as petunidin glycosides based on the neutral loss of hexose and pentose from the pseudomolecular ion, leading to the base peak at *m/z* 317. Compounds III and VI were assigned as petunidin hexoside and pentoside, respectively. The malvidin derivatives VII and X were identified by the loss of hexose (VII) and pentose (X), leading to the base peak at *m/z* 331 (De la Cruz et al., 2012; Jiménez-Aspee et al., 2016).

3.4.2. Phenylpropanoids

Compounds **1**, **7** and **13** were identified as 3-caffeoyl, 4-caffeoyl and 5-caffoylquinic acids, respectively, according to (Clifford, Johnston, Knight, & Kuhnert, 2003) and co-injection with standards. The related compounds **3**, **9** and **15**, with a $[M-H]^+$ ion at *m/z* 337 shows the loss of 174 amu leading to the base peak at *m/z* 163, in agreement with coumaroylquinic acids. According to the elution sequence and fragmentation, the compounds were assigned as 3-coumaroyl-, 4-coumaroyl- and 5-coumaroyl quinic acid, respectively. The compound **8**, with a $[M-H]^+$ of 367 show the neutral loss of 174 amu leading to the base peak at *m/z* 193, in agreement with feruloylquinic acid. The coumaric acid hexoside **6** and the shikimic acid derivatives **22**, **25** and **30** were assigned based on the neutral loss of shikimic acid, leading to the base peak of coumaric acid (Karaköse, Jaiswal, & Kuhnert, 2011).

3.4.3. Flavonols

The glycosides **19**, **21** and **28** were identified as myricetin pentosides (**19** and **28**) and hexoside (**21**), based on the neutral loss of pentose and hexose, leading to the base peak of myricetin at *m/z* 317. The compounds **29**, **34**, **35**, **36**, **40** and **45** were assigned as methyl myricetin glycosides due to the loss of pentose (**36**, **40** and **45**), hexose (**29** and **34**) or rutinose (**35**), leading to the base peak of the aglycon at *m/z* 331. The related compounds **46** and **51** showed the neutral loss of hexose and pentose, respectively, leading to the base peak at *m/z* 345, in agreement with dimethyl myricetin. The compounds were assigned as dimethyl myricetin hexoside **46** and dimethyl myricetin pentoside **51** (Gordon, Jungfer, da Silva, Maia, & Marx, 2011). Two coumaroyl hexosides from myricetin (compounds **47** and **52**) and two benzoyl hexosides (compounds **48** and **53**) were identified based on the neutral loss of coumaric acid and hexose or benzoic acid and hexose, respectively. The compounds were assigned as coumaroyl hexoside 1 and 2 and benzoyl-hexoside 1 and 2, respectively. The different R_t suggest either different placement of the aromatic moiety or differences in the sugar identity. Ten quercetin glycosides (compounds **31**, **33**, **37**, **41**, **54**, **55**, **56**, **57**, **58** and **60**) were assigned, based on the neutral loss of rutinose (**31**), hexose (**33**), pentose (**37** and **41**), coumaroyl hexose (**54** and **56**), feruloyl hexose (**55**), benzoyl hexose (**57** and **58**) or cinnamoyl hexoside (**60**), leading to the base peak of the aglycone quercetin. Two rhamnetin/i-sorhamnetin glycosides, including the rutinoside **44** and the pentoside **50** were detected, in agreement with the loss of rutinose and pentose from the pseudomolecular ion, leading to the base peak at *m/z* 315. The compound **42** loss hexose and show the base peak at *m/z* 285, as required for kaempferol hexoside. The flavanones occurring in the fruits comprises the naringenin hexosides **27**, **39** and **49**, with the neutral loss of hexose, and the base peak at m/z 271, in agreement with the assignation proposed. The related compounds **38** and **43** showed the loss of hexose and a base peak at *m/z* 287, compatible with dihydrokaempferol

Table 3
Anthocyanins tentatively identified in *Empetrum rubrum* phenolic enriched extracts (PEE) from Reserva Magallanes (RM) and Cerro Bandera (CB) trough LC-MS in positive ion mode.

Peak	Rt (min)	UV_{max}	$[M+H]^+$	Theoretical mass	Molecular formula	Error (ppm)	MS/MS fragments	Tentative identification	Distribution	
									RM	CB
I	13.55	523, 280	465.1022	465.1033	$C_{21}H_{21}O_{12}$	2.36	302.9706 (100)	Delphinidin 3-glucoside*	X	X
II	15.88	515, 280	449.1073	449.1078	$C_{21}H_{21}O_{11}$	1.11	286.9659 (100)	Cyanidin hexoside	X	X
III	18.13	528, 280	479.1181	479.1190	$C_{22}H_{23}O_{12}$	1.87	316.9938 (100)	Petunidin hexoside	X	
IV	18.78	515, 282	419.0969	419.0973	$C_{20}H_{19}O_{10}$	0.95	286.9235 (100)	Cyanidin pentoside	X	X
V	20.15	521, 276	463.1233	463.1240	$C_{22}H_{23}O_{11}$	1.51	300.9818 (100)	Peonidin hexoside	X	X
VI	20.82	516, 278	449.1070	449.1079	$C_{21}H_{21}O_{11}$	2.00	316.9882 (100)	Petunidin pentoside	X	
VII	21.91	536, 280	493.1339	493.1346	$C_{23}H_{25}O_{12}$	1.41	330.9818 (100)	Malvidin hexoside	X	X
VIII	23.13	515, 281	433.1125	433.1135	$C_{21}H_{21}O_{10}$	2.31	300.9699 (100)	Peonidin pentoside	X	X
IX	23.74	–	477.1027	477.1033	$C_{22}H_{21}O_{12}$	1.25	286.9526 (100)	Cyanidin derivative	X	X
X	24.39	536, 280	463.1230	463.1235	$C_{22}H_{23}O_{11}$	1.07	331.0185 (100)	Malvidin pentoside	X	

Table 4
Compounds tentatively identified in the phenolic-enriched extracts (PEEs) of *Empetrum rubrum* from Reserva Magallanes (RM) and Cerro Bandera (CB) trough LC-MS in negative ion mode.

Peak	Rt (min)	UVmax	$[M-H]^-$	Theoretical mass	Molecular formula	Error (ppm)	MS/MS fragments	Tentative identification	Occurrence	
									RM	CB
1	7.23	324, 298sh	353.0884	353.0878	$C_{16}H_{17}O_9$	−1.69	190.9255 (100), 178.9066 (55)	3-Caffeoylquinic acid*	X	X
2	8.46		593.1302	593.1301	$C_{30}H_{25}O_{13}$	−1.68	424.9945 (100), 289.0034 (20)	(epi)-catechin-(epi)-gallocatechin dimer	X	X
3	9.8	309, 295sh	337.0936	337.0929	$C_{16}H_{17}O_8$	−2.07	190.9754 (10), 162.9601 (100)	3-Coumaroylquinic acid	X	X
4	10.97	278	305.0674	305.0667	$C_{15}H_{13}O_7$	−2.29	220.9363 (100), 219.0095 (85), 178.9241 (95)	(epi)-gallocatechin	X	X
5	11.74	280	289.0726	289.0718	$C_{15}H_{13}O_6$	−2.76	244.9741(100), 204.9626 (40)	Catechin	X	X
6	11.74		325.0937	325.0929	$C_{15}H_{17}O_8$	−2.46	162.9299 (100)	Coumaroyl hexoside	X	X
7	11.98	320, 290sh	353.0886	353.0878	$C_{16}H_{17}O_9$	−2.26	190.9686 (100), 178.9746 (10)	Caffeoylquinic acid	X	X
8	12.36	321, 285sh	367.1039	367.1035	$C_{17}H_{19}O_9$	−1.08	192.9188 (100)	Feruloylquinic acid	X	X
9	14.36		337.0936	337.0929	$C_{16}H_{17}O_8$	−2.07	162.9386 (100)	4-Coumaroylquinic acid	X	X
10	15.51		577.1359	577.1352	$C_{30}H_{25}O_{12}$	−1.21	425.0043 (100), 288.9947 (20)	(epi)-catechin-(epi)-catechin dimer	X	X
11	16.05		865.2002	865.1985	$C_{45}H_{37}O_{18}$	−1.96	739.0664 (80), 695.0172 (100), 577.0236 (80)	(epi)catechin-(epi) catechin-(epi) catechin isomer 1	X	X
12	16.18		609.1264	609.1250	$C_{30}H_{25}O_{14}$	−2.29	440.9727 (100), 304.9927 (30)	(epi)gallocatechin-(epi) gallocatechin dimer	X	
13	16.27	327, 290sh	353.0885	353.0878	$C_{16}H_{17}O_9$	−1.98	190.9648 (100), 178.9777 (5)	5-Caffeoylquinic acid	X	X
14	17.62	280	289.0726	289.0718	$C_{15}H_{13}O_6$	−2.76	245.0011 (100), 204.9823 (40)	(epi)-catechin	X	X
15	17.62	310, 295sh	337.0937	337.0929	$C_{16}H_{17}O_8$	−2.37	190.9964 (100), 162.9606 (10)	5-Coumaroylquinic acid	X	X
16	19.12		863.1842	863.1829	$C_{45}H_{35}O_{18}$	−1.50	711.0038 (100), 572.9567 (30), 450.9428 (30), 410.9485 (40)	(epi)catechin-(epi)catechin-A-(epi) catechin trimer 1	X	X
17	22.01		865.1993	865.1985	$C_{45}H_{37}O_{18}$	−0.92	739.07 (50), 713.04 (70), 695.04 (100)	(epi)catechin-(epi)catechin-(epi) catechin isomer 2	X	X
18	22.79		1151.2485	1151.2463	$C_{60}H_{47}O_{24}$	−1.91	981.0498 (100), 861.0515 (70), 739.0503 (85), 577.0765 (55), 411.0207 (75)	(epi)catechin-A-(epi)catechin-(epi) catechin-(epi)catechin tetramer	X	X
19	24.49		449.0733	449.0726	$C_{20}H_{17}O_{12}$	−1.55	316.9273 (100)	Myricetin pentoside	X	X
20	25.07		575.1208	575.1195	$C_{30}H_{23}O_{12}$	−2.26	448.9992 (100), 288.9933 (40)	Proanthocyanidin A	X	
21	25.29		479.0844	479.0831	$C_{21}H_{19}O_{13}$	−2.71	315.9094 (100), 316.8994 (80)	Myricetin hexoside	X	X
22	25.11		319.0835	319.0823	$C_{16}H_{15}O_7$	−3.76	162.9946 (20), 144.9118 (100), 118.9387 (90)	Coumaroyl shikimic acid 1	X	X
23	26.36		863.1843	863.1829	$C_{45}H_{35}O_{18}$	−1.62	710.98 (90), 574.99 (100), 558.96 (35)	(epi)catechin-(epi)catechin-A-(epi) catechin trimer 2	X	X
24	26.36		509.1316	509.1300	$C_{23}H_{25}O_{13}$	−3.14	346.9538 (50) 328.9668 (100)	dihydrosyringetin hexoside	X	
25	27.09		319.0835	319.0823	$C_{16}H_{15}O_7$	−3.76	162.98 (20), 144.95 (100), 118.98 (65)	Coumaroyl shikimic acid 2	X	X
26	27.09		567.2099	567.2083	$C_{27}H_{35}O_{13}$	−2.82	521.1005 (30), 358.9852 (60), 341.0135 (100), 329.0609 (90)	$[M + HCOOH]^-$(iso)lariciresinol hexoside	X	X
27	27.86		433.1152	433.1140	$C_{21}H_{21}O_{10}$	−2.77	270.9236 (100)	Naringenin hexoside isomer 1	X	X
28	28.75		449.0736	449.0726	$C_{20}H_{17}O_{12}$	−2.22	316.9039 (100)	Myricetin pentoside	X	X
29	29.45		493.0998	493.0988	$C_{22}H_{21}O_{13}$	−2.02	330.9084 (100), 315.8931 (20)	Methyl myricetin hexoside isomer 1	X	X
30	29.53		319.0834	319.0823	$C_{16}H_{15}O_7$	−3.44	162.8808 (100), 154.8986 (20), 119.0080 (18)	Coumaroylshikimic acid 3	X	X
31	30.17	353, 266	609.1479	609.1461	$C_{27}H_{29}O_{16}$	−2.95	300.9411 (100)	Quercetin rutinoside	X	X
32	30.77	278	575.1205	575.1195	$C_{30}H_{23}O_{12}$	−1.73	422.9957 (100), 289.0241 (22)	Proanthocyanidin A 2	X	X
33	30.96	353, 255	463.0894	463.0882	$C_{21}H_{19}O_{12}$	−2.59	300.9827 (100)	Quercetin hexoside	X	X
34	31.42		493.1002	493.0988	$C_{22}H_{21}O_{13}$	−2.83	330.9416 (100)	Methyl myricetin hexoside isomer 2	X	X
35	31.99		639.1585	639.1566	$C_{28}H_{31}O_{17}$	−2.97	330.9249 (100)	Methyl myricetin rutinoside	X	
36	32.75		463.0893	463.0882	$C_{21}H_{19}O_{12}$	−2.37	330.9440 (85), 329.9714 (100), 315.9594 (22)	Methyl myricetin pentoside isomer 1	X	X
37	33.20		433.0785	433.0776	$C_{20}H_{17}O_{11}$	−2.07	299.9250 (100), 300.9088 (95)	Quercetin pentoside	X	X
38	33.27		449.1099	449.1089	$C_{21}H_{21}O_{11}$	−2.22	286.9491 (100)	Dihydrokaempferol hexoside	X	X
39	34.08		433.1153	433.1140	$C_{21}H_{21}O_{10}$	−3.00	270.9195 (100)	Naringenin hexoside isomer 2	X	X
40	34.60		463.0890	463.0882	$C_{21}H_{19}O_{12}$	−1.72	330.9718 (100), 315.9662 (18)	Methylmyricetin pentoside isomer 2	X	X
41	34.64	352, 255	433.0788	433.0776	$C_{20}H_{17}O_{11}$	−2.77	300.9189 (100)	Quercetin pentoside	X	X
42	35.64	346, 265	447.0944	447.0933	$C_{21}H_{19}O_{11}$	−2.46	284.9497 (100)	Kaempferol hexoside	X	X
43	36.10		449.1102	449.1089	$C_{21}H_{21}O_{11}$	−2.89	286.9320 (100)	Dihydrokaempferol hexoside	X	X
44	36.26		623.1633	623.1618	$C_{28}H_{31}O_{16}$	−2.40	314.9312 (100), 299.8745 (25)	Isorhamnetin rutinoside		X

(*continued on next page*)

Table 4 (*continued*)

Peak	Rt (min)	UVmax	[M-H]⁻	Theoretical mass	Molecular formula	Error (ppm)	MS/MS fragments	Tentative identification	Occurrence	
									RM	CB
45	36.81		463.0892	463.0882	$C_{21}H_{19}O_{12}$	−2.15	330.9466 (100)	Methylmyricetin pentoside isomer 3	X	X
46	37.68		507.1158	507.1144	$C_{23}H_{23}O_{13}$	−2.76	344.0027 (100), 345.0053 (50)	Dimethylmyricetin hexoside	X	
47	38.47		625.1215	625.1198	$C_{30}H_{25}O_{15}$	−2.71	478.9926 (100), 316.9444 (30)	Myricetin coumaroyl hexoside isomer 1	X	X
48	40.18		583.1103	583.1093	$C_{28}H_{23}O_{14}$	−1.71	460.9494 (20), 316.9317 (60), 315.9219 (100)	Myricetin benzoyl hexoside isomer 1	X	X
49	41.13		433.1148	433.1140	$C_{21}H_{21}O_{10}$	−1.84	270.9775 (100)	Naringenin hexoside isomer 3	X	X
50	41.43		447.0942	447.0932	$C_{21}H_{19}O_{11}$	−2.23	315.0817 (100)	Isorhamnetin pentoside	X	X
51	41.84		477.1048	477.1038	$C_{22}H_{21}O_{12}$	−2.09	344.9678 (100), 328.9370 (60)	Dimethylmyricetin pentoside		X
52	42.88		625.1215	625.1198	$C_{30}H_{25}O_{15}$	−2.71	479.0155 (100), 315.9275 (20)	Myricetin coumaroyl hexoside isomer 2	X	
53	45.36		583.1109	583.1093	$C_{28}H_{23}O_{14}$	−2.74	316.9152 (30), 315.9469 (100)	Myricetin benzoylhexoside isomer 2	X	X
54	45.47		609.1264	609.1250	$C_{30}H_{25}O_{14}$	−2.29	462.99 (100), 300.92 (20)	Quercetin coumaroyl hexoside isomer 1	X	X
55	45.87		639.1369	639.1355	$C_{31}H_{27}O_{15}$	−2.19	462.9649 (100), 300.9303 (30)	Quercetin feruloyl hexoside	X	X
56	46.94		609.1263	609.1250	$C_{30}H_{25}O_{14}$	−2.13	462.9944 (100), 300.9190 (20)	Quercetin coumaroyl hexoside isomer 2	X	X
57	46.94		567.1157	567.1144	$C_{28}H_{23}O_{13}$	−2.29	445.0172 (20), 300.9536 (100)	Quercetin benzoylhexoside isomer 1	X	X
58	47.65	350, 254	567.1153	567.1144	$C_{28}H_{23}O_{13}$	−1.58	445.0101 (20), 300.9412 (100)	Quercetin benzoylhexoside isomer 2	X	X
59	49.81		327.2180	327.2177	$C_{18}H_{31}O_5$	−0.91	291.1151 (30), 229.0343 (30), 170.9787 (100)	Dihydroxy-oxo-octadecenoic acid	X	X
60	50.35		593.1313	593.1300	$C_{30}H_{25}O_{13}$	−2.19	300.8959 (92), 299.8952 (100)	Quercetin cinnamoyl hexoside	X	X
61	51.54		329.2340	329.2333	$C_{18}H_{33}O_5$	−2.12	311.0980 (40), 293.0343 (30), 228.9990 (100), 210.9913 (70)	Trihydroxy-octadecenoic acid	X	X

hexosides. The compound **24** loss hexose and present two main daughter ions at *m/z* 347 and 329, compatible with the flavanonol dihydro syringetin hexoside (De Rosso, Panighel, Dalla Vedova, & Flamini, 2020).

3.4.4. Procyanidins

Compounds **5** and **14**, with the same molecular formula and pseudomolecular ion at *m/z* 289, were identified as catechin and epicatechin by comparison with standards. The compound **4** with [M-H]⁻ at *m/z* 305 was compatible with (epi)gallocatechin (Lin, Sun, Chen, Monagas, & Harnly, 2014). Ten compounds were identified as procyanidins, including the dimers **2**, **10**, **12**, the trimers **11**, **16**, **17**, **23**, the tetramer **18** and the procyanidin A isomers **20** and **32**. The dimers were based on (epi)catechin and (epi)gallocatechin monomers and included (epi) catechin (epi)gallocatechin **2**, (epi)catechin (epi)catechin **10** and (epi) gallocatechin (epi)gallocatechin **12**. The trimers **11** and **17**, with the same $[M-H]^+$ ion at *m/z* 865, show fragmentations compatible with (epi)gallocatechin-(epi)gallocatechin)-(epi)catechin isomers and differ in the intensity of the daughter ions. The compounds were assigned as (epi)gallocatechin-(epi)gallocatechin-(epi)catechin isomer 1 (**11**) and isomer 2 (**17**), respectively. The related trimers **16** and **23** show a $[M-H]^+$ ion at *m/z* 863 and fragments assignable to (epi)catechin trimers with one A-type bond and were assigned as the corresponding isomers 1 and 2, respectively (Lin et al., 2014). The tetramer **18** is in agreement with (epi)catechin-(epi)catechin-(epi)catechin-(epi)catechin tetramer with one A-type bond (Lin et al., 2014). The compounds **20** and **32** showed a [M-H]+ ion at *m/z* 575 and fragments compatible with (epi) catechin dimers with A-type bond (proanthocyanidin A). However, the relative ratio of the fragments and the Rt differs, being assigned as procyanidin A **20** and procyanidin A isomer **32**, respectively.

3.4.5. Other compounds

The mass spectrum of compound **26** shows a $[M + HCOOH]^+$ at *m/z* 567 and fragments to *m/z* 521, 359, 341 and 329 amu, in agreement with the formic adduct of (iso)lariciresinol hexoside (Rodríguez-Pérez, Quirantes-Piné, Fernández-Gutiérrez, & Segura-Carretero, 2013). The mass spectra of compounds **59** and **61** shows a molecular formula of $C_{18}H_{31}O_5$ and $C_{18}H_{33}O_5$ for the $[M-H]^+$ ion at 327 and 329, respectively. The characteristic fragments at *m/z* 311, 293, 229, and 171 suggest the occurrence of oxidized long chain fatty acids derivatives (Liu, Porter, Schneider, Brash, & Yin, 2011). These compounds were tentatively identified as dihydroxy-oxo-octadecenoic acid (**59**) and trihydroxyoctadiecadecenoic acid (**61**). Additional studies and isolation of the compounds is needed for unambiguous identification.

3.5. ^1H NMR analysis of the lipophilic fruit constituents

The ^1H NMR spectrum of the lipophilic compounds from the EtOAc-soluble fraction from *E. rubrum* (Fig. 3) showed characteristic signals for triterpenes with a double bond at C-12, two exomethylene protons and the dd for 3-hydroxy, supporting the presence of oleanolic acid in mixture with other pentacyclic triterpenes, including taraxasterol and ursolic acid.

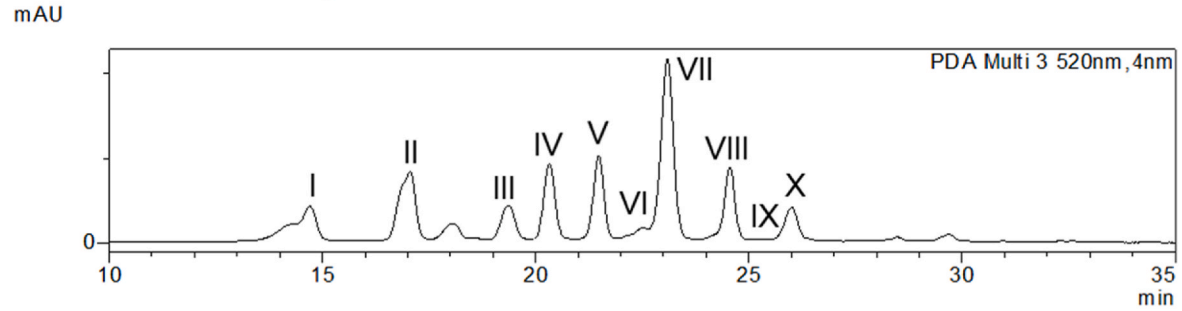
3.6. Main compounds content in the fruits

The main anthocyanins in *E. rubrum* fruits were petunidin pentoside for the RNM sample, followed by cyanidin hexoside, cyanidin pentoside, peonidin hexoside and delphinidin hexoside. In the CB sample, the petunidin pentoside content was below the quantification level. The main anthocyanidins were cyanidin hexoside, peonidin hexoside and cyanidin pentoside. The main hydroxycinnamic acids were coumaroyl- and caffeoylquinic acids, with higher content for the RNM sample (Table 5).

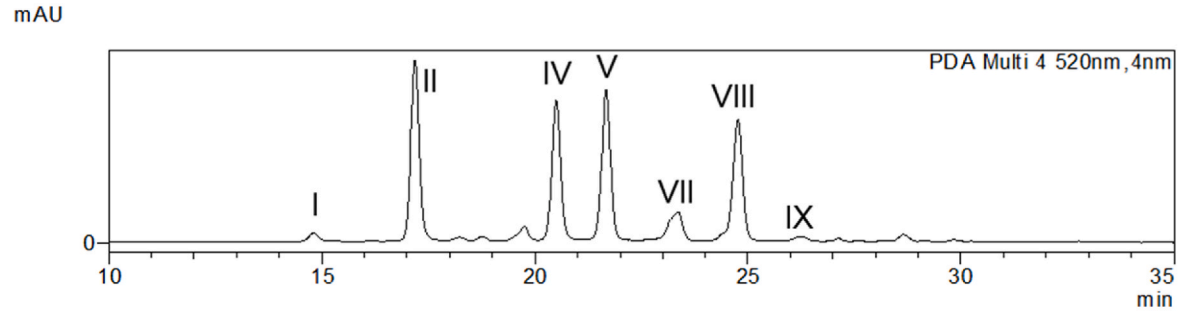
3.7. Assay-guided isolation of α-glucosidase inhibitors

The fractions obtained after Sephadex permeation were assessed for composition by thin layer chromatography (TLC) (Wagner & Bladt, 1996) (Fig. 4) and for α-glucosidase inhibition (Table 2). The most active fractions of the RNM sample (fractions 24, 25 and 26; IC_{50}: 0.18, 0.21 and 0.15 μg/mL, respectively) showed in TLC compounds with R_f values > 0.90), revealing as yellow spots after spraying with diphenylboric acid ethanolamine complex. The elution order in Sephadex, high R_f values,

A: Reserva Nacional Magallanes

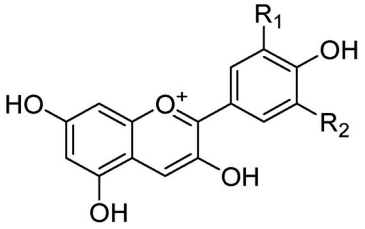


B: Cerro Bandera



Compounds: I: Delphinidin 3-glucoside*; II: Cyanidin hexoside; III: Petunidin hexoside; IV: Cyanidin pentoside; V: Peonidin hexoside; VI: Petunidin pentoside; VII: Malvidin hexoside; VIII: Peonidin pentoside; IX: Cyanidin derivative; X: Malvidin pentoside.

C: Anthocyanidins identified in the fruits



R_1	R_2	
H	OH	Cyanidin
H	OCH_3	Peonidin
OH	OH	Delphinidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

Fig. 1. HPLC-MS/MS chromatogram in the positive ion mode of the PEE of *E. rubrum* fruits from (A) Reserva Nacional Magallanes and (B) Cerro Bandera (B). Detection: 520 nm. For the identification of the compounds see Table 3. (C) Structure of the anthocyanidins identified in the fruits.

and color reaction support the occurrence of flavonol aglycones. TLC comparison with standards and HPLC-DAD showed three main products with Rt 33.0, 35.1 and 40.6 min, the second compound identical to quercetin. Other compounds were flavonols with free OH at C-3 and UV maxima at 382 and 370 nm. The fraction pools 10–11, 12–13 and 14–15 (IC_{50}: 0.20, 0.30 and 0.39 μg/mL, respectively) showed a more complex pattern with compounds compatible with mono- and diglycosides as well as caffeoyl/coumaroyl quinic acids. The main compounds revealed as yellow (Rf 0.60), deep purple (Rf 0.51), fluorescent blue (Rf 0.35) and deep purple (Rf 0.20). HPLC analyses at 520 nm showed five main anthocyanins and at 330 nm, the main products were caffeoylquinic and coumaroylquinic acids. In the EtOAc extract from the CB fruits, separation of the constituents by Sephadex allowed to obtain fractions with higher enzyme inhibition than the starting mixture. Best effect was observed for the last fractions eluted (24–25 and 26), as well as from 15 to 16 and 19–20 (Table 2). The HPLC-DAD profile of the fraction 26 showed quercetin as main compound. Fractions 15–16 contain three flavonol glycosides, identified as rutin and quercetin 3-*O*-glucoside by co-injection with standards as well as other minor products. The fraction pool 19–20 shows two flavonoid glycosides, a main phenolic eluting at Rt 40.82 min and UV maxima at 362 sh, 314, 266 sh and 246 nm. The fraction 12 of CB contained 5-caffeoylquinic acid, a coumaroyl derivative and a flavonol eluting at Rt 29.8 min with UV_{max} at 360 and 329 sh. Fraction pool 13–14 contains quercetin 3-*O*-glucoside as main compound and a more polar constituent with Rt 19.9 min and UV_{max} at 342, 311 sh, 275 and 262 sh nm, compatible with a glycosyl flavone. Fractions 17–18 were a mixture of three glycosides, compatible with quercetin derivatives.

4. Discussion

The antioxidant capacity of *Empetrum* berries was lower than that of *Ribes* spp. (Burgos-Edwards et al., 2017, 2018; Jiménez-Aspee et al., 2016), *Gaultheria* (Mieres-Castro et al., 2022) and other Patagonian wild berries (Schmeda-Hirschmann et al., 2019). However, the inhibition of α-glucosidase was higher than *Ribes magellanicum* and *R. punctatum* (21.7 and 20.1 μg/mL) (Burgos-Edwards et al., 2017), *Gaultheria phillyreifolia* (0.7 μg/mL), *G. poeppigii* with pink fruits (3.1 μg/mL) and

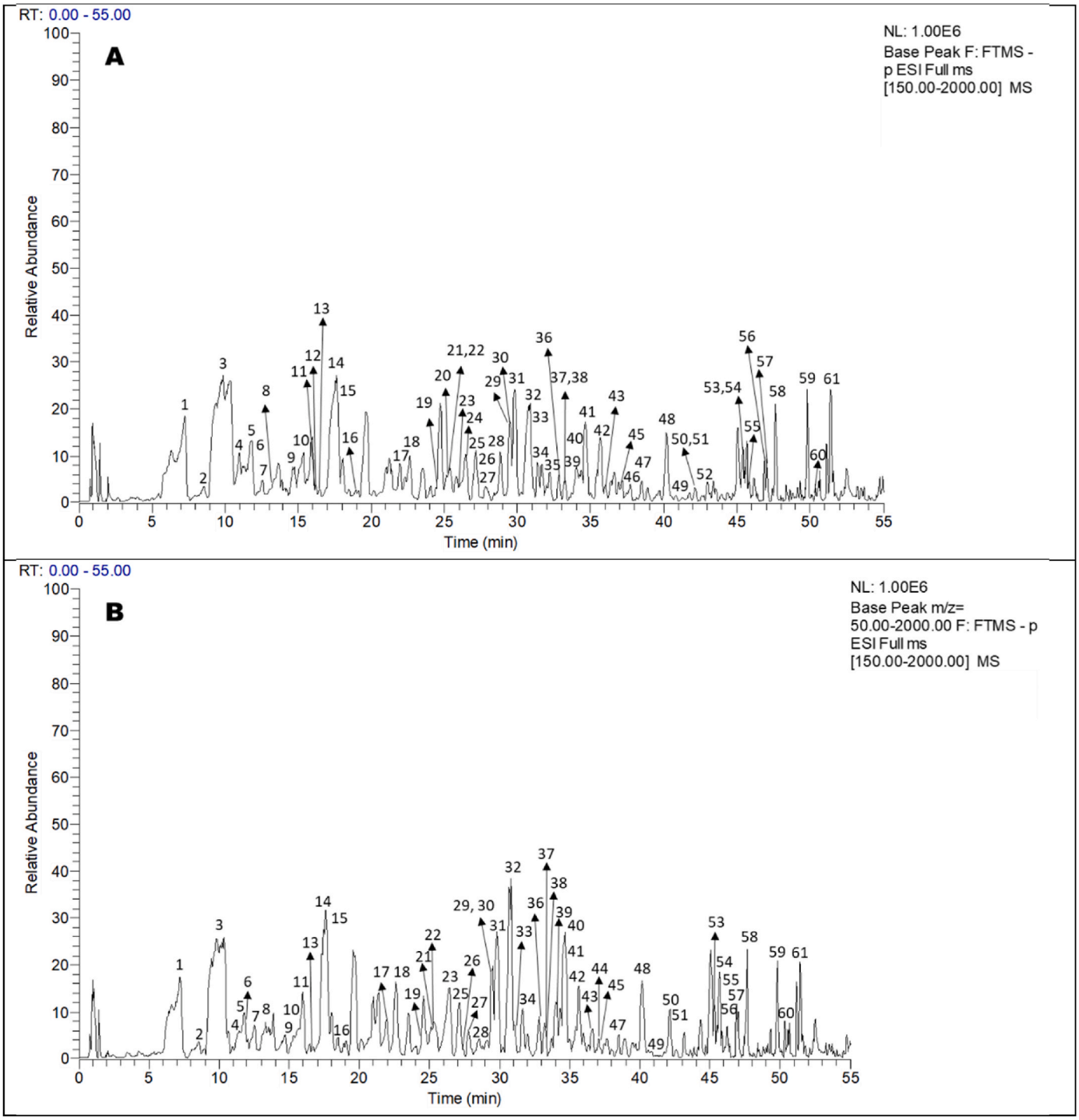


Fig. 2. HPLC-MS/MS chromatogram in negative ion mode of the PEE of *E. rubrum* fruits from Reserva Nacional Magallanes (A) and Cerro Bandera (B). The number corresponds to the compounds listed in Table 4.

G. poeppigii with white fruits (Mieres-Castro et al., 2022). The PEE from a Navarino Island sample of *R. magellanicum* was more active against α-glucosidase, with IC_{50} values in the range of 0.06–0.08 μg/mL (Burgos-Edwards et al., 2023).

In the present work, 10 anthocyanins were identified in *E. rubrum*. The *E. nigrum* fruits afforded 15 anthocyanins, main compounds were delphinidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside and a mixture of malvidin-3-*O*-galactoside and peonidin 3-*O*-glucoside, with 1100, 1100 and 1200 mg/kg of berry, respectively (Laaksonen, Sandell, Järvinen, & Kallio, 2011). The main anthocyanins in *E. hermaphroditum* fruits are malvidin 3-galactoside, delphinidin 3-galactoside and cyanidin 3-galactoside, accounting for 135.0, 117.9 and 111.1 mg/100 g fresh fruits, respectively (Lavola et al., 2017). The differences in the anthocyanin composition relate with the color of the fruits.

Coumaroyl quinic acids are the main phenylpropanoids in *E. rubrum* fruits, followed by caffeoyquinic acids. In *E. nigrum* fruits, Laaksonen et al. (2011) found as main flavonoid myricetin 3-*O*-galactoside, laricitrin 3-*O*-galactoside and quercetin 3-*O*-galactoside. In *E. hermaphroditum*, *p*-coumaric acid was reported but the content was lower than that of the main anthocyanins (Lavola et al., 2017). A

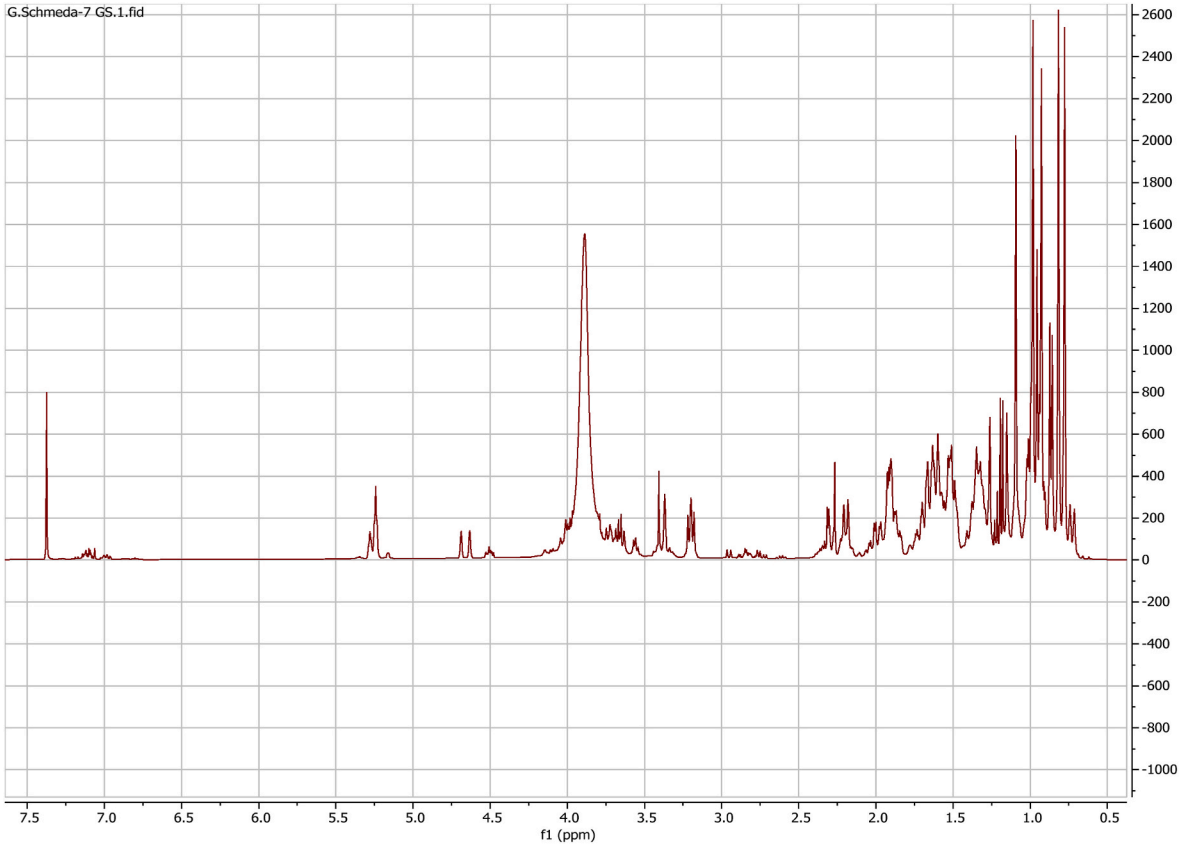


Fig. 3. ^{1}H NMR spectra of the constituents from the EtOAc-soluble fraction from *E. rubrum* fruits.

Table 5
Main anthocyanins and hydroxycinnamic acids content from *Empetrum rubrum* PEEs from Chilean Patagonia.

Compound	R_t (min)	Reserva Nacional Magallanes	Cerro Bandera
Anthocyanins			
Delphinidin-hexoside	14.7–14.8	2.75 ± 0.08	BQL
Cyanidin hexoside	17.0–17.3	3.68 ± 0.11	2.35 ± 0.06
Petunidin hexoside	19.3–19.5	1.68 ± 0.05	BQL
Cyanidin pentoside	20.33	2.92 ± 0.09	1.88 ± 0.05
Peonidin hexoside	21.69	2.93 ± 0.09	1.98 ± 0.07
Petunidin pentoside	23.09–23.26	7.12 ± 0.24	BQL
Malvidin hexoside	24.56–24.73	2.56 ± 0.08	1.72 ± 0.05
Malvidin pentoside	26.03	1.55 ± 0.04	BQL
Hydroxycinnamic acid derivatives			
5-Caffeoylquinic acid (neochlorogenic acid)	7.63	13.70 ± 0.32	10.44 ± 0.11
Coumaroyl quinic acid	9.58	1.13 ± 0.02	0.97 ± 0.02
Coumaroyl quinic acid 1	10.10	7.28 ± 0.17	4.99 ± 0.06
Coumaroyl quinic acid 2	10.78	22.73 ± 0.55	17.06 ± 0.21
3-Caffeoyl quinic acid (chlorogenic acid)	13.47	1.81 ± 0.36	2.04 ± 0.03
Coumaroyl quinic acid 3	19.33	1.19 ± 0.01	1.06 ± 0.07

Results are presented as mean ± SD in mg of compound per gram of extract. BQL: below quantification limit.

relevant difference with the tetraploid subspecies *E. hermaphroditum*, is higher phenolic in this subspecies compared to the diploid *E. nigrum* (Lavola et al., 2017). Caffeic acid, *p*-coumaric acid conjugates and *p*-coumaric acid were described in *E. nigrum* fruits (Laaksonen et al., 2011). In *E. nigrum* leaves, Muravnik and Shavarda (2012) reported dihydrocinnamic alcohol and cinnamic alcohol, phenolic acids, bibenzyls, chalcones, flavonols and flavanones as phenolic constituents, among other compounds.

The fruits of *E. rubrum* are rich in procyanidins, including dimer, trimer and tetramers based on catechin and epicatechin. The flavan-3-ols catechin and epicatechin were reported in the trichomes of *E. nigrum* (Muravnik & Shavarda, 2012), but no information was available on higher molecular weight procyanidins in *Empetrum* fruits.

Flavonoids in *E. nigrum* fruits include a series of quercetin, myricetin, isorhamnetin, laricitrin and syringetin glycosides, identified as the 3-*O*-glycosides by Laaksonen et al. (2011). In *E. rubrum*, the flavonoids were glycosides of quercetin, rhamnetin/isorhamnetin, myricetin, methyl- and dimethylmyricetin and flavanones, including naringenin. Some of the flavonoid glycosides were coumaroyl or benzoyl hexosides.

Quercetin glycosides occur in our samples of *E. rubrum* and the aglycone, quercetin, was described from *E. nigrum* trichomes (Muravnik & Shavarda, 2012).

The occurrence of olenolic acid and ursolic acid as well as other triterpenes in *E. rubrum* agrees with the report of ursolic acid, dehydroursolic acid, oleanolic acid, α-amyrin, uvaol and β-sitosterol from the glandular trichomes of *E. nigrum* leaves (Muravnik & Shavarda, 2012).

The complexity and the strong α-glucosidase inhibition observed for *E. rubrum* fruit fractions points out to combinations of components with different activities. Isolation of the single constituents for structural elucidation and further testing is needed to get a better picture on the α-glucosidase inhibitors, including synergistic effects. Several of the main compounds identified in *E. rubrum* fruits have been reported as

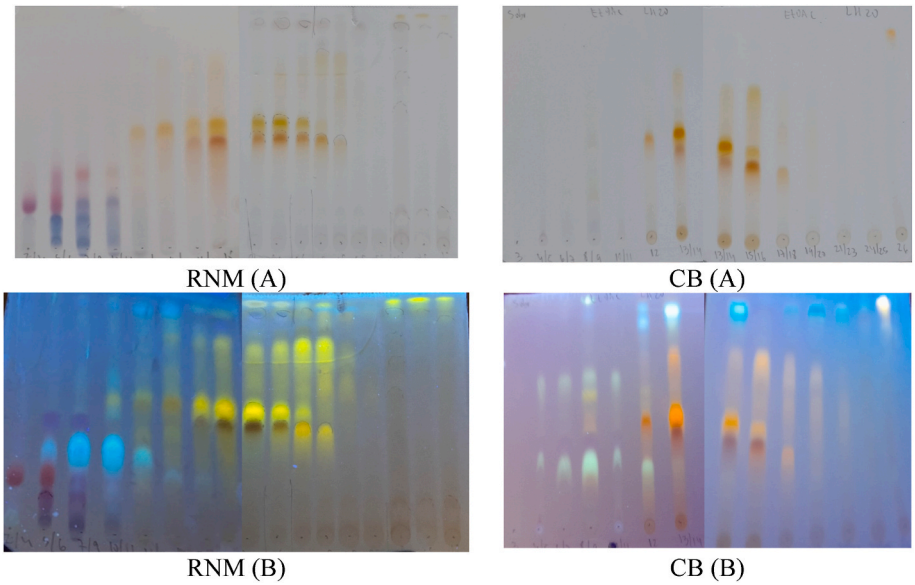


Fig. 4. TLC analyses of the Sephadex LH-20 fractions from the MeOH extract (RNM) and EtOAc extract (CB) of *E. rubrum* fruits (Silica gel; EtOAc:Acetic acid:water 10:1.5:1 v/v/v, revealed with NPR). A: under normal light; B: UV, 365 nm.

hypoglycemic, including caffeic acid and derivatives (Akhlaghipoura, Shada, Askari, Maharatia, & Rahimi, 2023) and oleanolic acid (Errichiello et al., 2023). Extracts from *E. nigrum* shows α-glucosidase inhibition and hypoglycemic effect both in vitro and with human volunteers (Bezverkhniaia et al., 2021; Törrönen et al., 2012). The anthocyanins occurring in the *E. rubrum* fruits, previously reported from *Ribes* species, showed cytoprotective effect in AGS cells (Jiménez-Aspee et al., 2016).

5. Conclusions

An HPLC-DAD and HPLC-DAD-MS/MS^n method was developed to get a first insight into the composition of the *E. rubrum* fruit phenolics, associated with the effect of the extract on α-glucosidase. *E. rubrum* from southern Chilean Patagonia showed strong inhibition towards α-glucosidase and differ from other Patagonian berries in the identity and composition of phenolics. Some 71 compounds, including anthocyanins, flavonoids, phenylpropanoids and procyanidins were identified for the first time in the fruits. The results encourage further studies, including comparison of populations under different environmental conditions and in a latitudinal gradient. Our findings provide useful information on the composition and in vitro enzyme inhibitory potential of the only South American species belonging to genus *Empetrum* opening the possibility for new studies on the nutraceutical potential of this genus with circumpolar distribution.

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CRediT authorship contribution statement

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

Declaration of competing interest

The authors declare that they have no competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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