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FULL ARTICLE

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Argentine Patagonia barberry chemical composition and evaluation of its antioxidant capacity

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Abstract

An important portion of vitamins, minerals and polyphenols components in human diet are captured from fruit consumption. Argentinean Patagonia *Berberis microphylla* was characterized with the phenolic content, the proximate composition and the identification and quantification of anthocyanins, not-anthocyanins and proteins. The antioxidant capacity of berberis ethanolic extracts (EB) was determined by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. EB was used to reduce production of reactive substances species (ROS) in zebrafish. EB presented a total polyphenols content of 1,035.03 mg GAE/100 g fresh weight (FW). EB presented an ABTS value of 116.25 ± 17 μmol TE/g FW. EB presented a DPPH value of 137.80 ± 1.90 μmol TE/g FW. EB was able of reducing the ROS in zebrafish. Berberies Protein Isolate (BPI) presented proteins with bands from 15 to 62 kDa. BPI presented an ABTS value of 593.11 ± 8.60 μmol TE/g. The BPI duodenal digest presented a value of 641.07 ± 12.60 μmol TE/g digests.

Practical applications

The practical applications of the present study are to increase scientific knowledge for consumers about the quality and benefits of the consumption of the native fruit (*Berberis microphylla*) from the Patagonia region of Argentine. This work describes the protein profile of berberies, their digestibility and their antioxidant activity. This study allows to better understand the phytonutrients that make up this fruit. Future studies may identify the peptides present in hydrolyzates. The bio-compounds of this fruit could be used as functional ingredients by the food industry for different purposes.

KEYWORDS

antioxidant activity, *Berberis microphylla*, calafate barberry, total polyphenols content, zebrafish

1 | INTRODUCTION

Polyphenols bioactive compounds are phytochemical components that have been widely studied for their benefits on human health. Many epidemiological studies have been described in the literature on the protective effect of diets based on components of plant origin (Speisky, López-Alarcón, Gómez, Fuentes, & Sandoval-Acuña, 2012). These diets are rich in polyphenol and prevention of diseases such as cancer, coronary heart, oxidative-stress-related diseases, and osteoporosis (Kampa, Nifli, Notas, & Castanas, 2007;

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Pennington & Fisher, 2010). The results of these studies have raised interest in the polyphenols research and its antioxidant capacity to scavenge reactive oxygen species (ROS). Studies have reported that ROS may be related to different degenerative diseases such as cancer, respiratory diseases, Alzheimer. Antioxidants as a nutritional supplement have shown being essential for ROS neutralization of associated oxidative damages (Liu et al., 2018). In recent years, Patagonia fruits have been the subject of several investigations describing their antioxidant potential and their potential benefits on human health (Afrin et al., 2016; Fuentes, Figueroa, Valdenegro, & Vinet, 2019). Blueberries among other fruits have gained popularity and significant attention. They have been denominated "superfruits" as a term to promote the health benefits of these fruits. The superfruits are considered "super'' by nutrition scientists when fruits have extremely high levels of antioxidants, fibers, vitamins, minerals, and other nutrients that improve health (Chang, Alasalvar, & Shahidi, 2019).

Calafate barberry (*Berberis microphylla* G. Forst, sin.) is an endemic species of the Patagonian Andes of Argentina and Chile, with a potential use for agroindustry. Although the phytochemical and ethnopharmacological analysis of the medicinal Argentine flora has been described in the literature (Barboza, Cantero, Núñez, Ariza-Espinar, & Pacciaroni, 2009), native berries such as calafate have not been deeply studied. The *B. microphylla* fruits produce small dark berries with great edible value and are considered superfruits due to their vitamin C high content and their polyphenols levels. The polyphenols levels are higher than levels registered in other berries (López de Dicastillo, López-Carballo, Gavara, Muriel Galet, Guarda, & Galotto, 2019; Ramirez, Zambrano, Sepulveda, Kennelly, & Simirgiotis, 2015; Rothwell et al., 2016). The fruits have a high antioxidant capacity demonstrated by different traditional quantitative in vitro methods (Ruiz et al., 2010, 2013, 2014). Environmental conditions during fruit growth influence the synthesis of fruit polyphenols compounds and the antioxidant capacity (Arena, Postemsky, & Curvetto, 2017. Roussos, Denaxa, & Damvakaris, 2009). The main objective of this research was to evaluate the chemical composition of the *B. microphylla* fruit pulp and its seeds and, finally, to evaluate the antioxidant capacity of polyphenols components and proteins.

2 | MATERIALS AND METHODS

2.1 | Reactive

Folin–Ciocalteu reactive, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid standard, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox reactive), 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH), Dichloro-dihydro-fluorescein diacetate (DCFH-DA) were obtained from Sigma Chemical Co. (St Louis, MO, USA).

2.2 | Standards of non-anthocyanins and anthocyanins compounds

The next standards were used to identify and quantify the phenolic compounds:

Gallic acid, 3-hydroxytyrosol, (–) gallocatechin, (–) gallocatechin gallate, (–) epicatechin gallate, procyanidin B1, (+) catechin, procyanidin B2, (–) epicatechin, caffeic acid, syringic acid, coumaric acid, ferulic acid, *trans*-resveratrol, quercetin hydrate, kaempferol-3-glucoside, and malvidin- 3-O-glucoside chloride with values of purity between 90% and 99.5%.

All compounds were obtained of Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). The standard of 2-(4-hydroxyphenyl) ethanol (tyrosol) with 99.5% of purity was obtained from the company Fluka (Buchs, Switzerland).

2.3 | Samples

Berberis fruits (*B. microphylla*) from 20 different trees were harvested in February 2017 and February 2018 in the extra-Andean Patagonia (41°19′16.33″ S, 70°07′52.87″ W) (Figure 1). Ripe fruits were quickly processed to facilitate the seeds manual separation. The pulp was frozen at −20°C until further analysis.

2.4 | Polyphenol extraction from *B. microphylla* fruit

The extracts from berberis (EB) were obtained by the solid–liquid extraction method. Briefly, 10 g of berberis pulp was mixed with a volume of the extraction solvent (ethanol: water, 50:50 v/v) at a ratio of 25:1, solvent: sample. EB was isolated during 2 hr at 60°C. EB was lyophilized and kept at −20°C. EB dry content was calculated by lyophilization in triplicate. Yields were expressed as g of EB/100 g of berberis pulp (DW).

2.5 | Nutritional characterization of fresh fruit pulp

The proximate analysis of berberis was made using the standard protocols of the Association of Official Analytical Chemists (AOAC, 1990). Total polysaccharides were determined using the phenol sulfuric acid method. The content of total soluble reduced sugars was calculated by the Somogyi-Nelson method. The results calculated were expressed as g of glucose/100 g (DW).

2.6 | Total polyphenols content (TPC)

The EB total soluble polyphenols components was determined by the Folin–Ciocalteu (FC) method using the gallic acid standard. The assays were performed in triplicate, and the date calculated were

FIGURE 1 Patagonia Argentina Berries. Calafate barberry (*Berberis microphylla* G. Forst, sin.). The top photograph is a Calafate bush. Size ratio (cm) of the whole fruit (A) of *B. microphylla* compared to the seeds, seen from above (B) and from the side (C)

presented as mg gallic acid equivalent (GAE)/g of EB. The standard curve obtained was ($Y = 0.197x + 0.0442$, $R^2 = 0.994$).

2.7 | Determination polyphenols components

Non-anthocyanins and anthocyanins components were analyzed by the HPLC-DAD technique according to Fontana, Antoniolli, Fernández, and Bottini (2017). HPLC separation was made using a Dionex Ultimate 3000 HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany) and a Kinetex C18 column (3.0 × 100 mm, 2.6 mm) (Phenomenex, Torrance, CA, USA). The conditions of analysis to non-anthocyanins component was as solvent A (Milli-Q- water + 0.1% of formic acid) and as solvent B (acetonitrile). The analysis was made with a gradient of 0–14 min with 95% of solvent B; 14–15.5 min with 95% of solvent B; 15.5–17 min with 5% of solvent B. The measurements were made at wavelengths of 254, 280, 320 and 370 nm. The conditions of analysis of anthocyanins components were as solvent A (Milli-Q water: formic acid: acetonitrile, 87:10:3, *v:v:v*), as solvent B (Milli-Q water: formic acid: acetonitrile, 40:10:50, *v:v:v*). The analysis was made using a gradient of 0–15 min, 100% of solvent B; 15–17 min, 10% of solvent B. The measurement was taken at wavelengths of 520 nm.

2.8 | In vitro antioxidant activity

EB antioxidant capacity was calculated by the DPPH and ABTS assays. Trolox reactive was used as standard with concentrations of 0–0.95 mM of calibration curve ($Y = 0.2808x + 0.3552$; $R^2 = 0.998$). Antioxidant capacity was calculated as % of activity = (Absorbance of control − Absorbance of sample/Absorbance of control) × 100. Also, the samples IC50 was calculated and expressed as the concentration of the sample necessary to inhibit 50% of free radicals (Piñuel, Vilcacundo, et al., 2019).

2.9 | In vivo antioxidant activity assay with the zebrafish embryos model

2.9.1 | Exposure of embryos of zebrafish to phenolic extracts and AAPH

Zebrafish embryos of 4–6-hr post-fecundation (hpf) ($n = 8$) were put in wells of 24-well sterile plates in 500 µl of media with 0.1% DMSO. The EB sample was added to the wells at concentrations of 0, 5 and 10 µg/ml with 0.1% DMSO. Zebrafish embryos were incubated with the samples for 2 hr. After incubation 500 µl of 25 mM of AAPH solution was added in each well and was incubated for 4 hr at 28°C.

Then, zebrafish embryos were rinsed using fresh embryo media until 24 hpf were completed. The assay was performed in triplicate (Hu et al., 2017; Vilcacundo et al., 2018).

2.10 | Measurement of stress-induced oxidative intracellular ROS generation

Zebrafish embryos model was used to evaluate the formation of ROS in the cells. The DCFH-DA fluorescent assay was used to quantify ROS in zebrafish embryos. Zebrafish embryos of one day post-fecundation (dpf) were treated with a DCFH-DA reactive (2.0 µl/ml) for 1 hr at 28.5 ± 1°C, protected from light. Then, zebrafish embryos were rinsed by fresh embryo media, dechorionated, anesthetized before observation, and photographed under the microscope (Leica DM1000 LED, Wetzlar, Germany) with a camera Moticam 2000 (Taiwan, China). ROS generation percentage of zebrafish embryos was calculated using the image J program (Piñuel, Boeri, et al., 2019; Yang et al., 2012).

2.11 | Nutritional characterization of seeds fruit

For the proximal analysis the determination of the moisture content, total proteins, lipids and ashes of both pulp and seeds was carried out with the protocol recommended by the Association of Official Analytical Chemists (AOAC, 1990).

2.12 | Fresh weight and dry weight

To determine the moisture content, 20 g of seed flour were disposed. The humidity was determined by weight difference before and after drying the sample at 75°C, until constant weight was reached.

2.13 | Ashes

The ashes were obtained by mineralization of the dehydrated samples, at a temperature of 550°C for 12 hr in muffle (TecnoEdu S.A).

2.14 | Total protein

Calafate seeds protein content was calculated by the Kjeldhal method (AOAC, 1990) in duplicate. The protein calculation was made from the nitrogen data obtained from the sample, using the 6.25 conversion factor.

2.15 | Lipids

Lipid extraction was performed from 5 g of seed and 5 g of pulp, with two repetitions of each. The Soxhlet method was made with sulfuric ether as solvent. The extraction of the fats was made for 2 hr. The percentage of lipids was obtained by the difference between the final and initial weight of the ball (Lanzi et al., 2018).

2.16 | Berberies protein isolate (BPI)

The seeds were used to obtain flour. The seeds were dried for 24 hr at 28°C. An alkaline extraction followed using the isoelectric precipitation method was made according to Acosta, Carpio, Vilcacundo, and Carrillo (2016) with some modifications. About 3 g of flour were mixed with 30 ml of distilled water and pH was adjusted at 9.0. Then, the sample was centrifuged at 5,000 rpm, and the pellet was discarded. The pH of the supernatant was adjusted to pH 5.0. Finally, the sample was centrifuged at 5,000 rpm and the precipitate was adjusted at pH 7.0. The aqueous extract obtained was lyophilized to obtain peptides and to carry out the in vitro analysis of the antioxidant activity.

2.17 | Gastrointestinal hydrolysis

The hydrolyzates were obtained from the BPI by simulating the digestive process with proteases, for which the proteins were hydrolyzed by simulating the gastric and duodenal digestive process as described by Vilcacundo et al. (2018).

2.17.1 | Gastric digestion

10 mg of the lyophilized BPI was used and 1 ml of (pepsin 2,000 U/ml, at pH 3.0) was added. The sample was incubated for 120 min at 37°C. The hydrolytic activity of the enzymes was stopped by raising the pH with 100 µl of 1 M NaOH and with a bath at 80°C for 10 min.

2.17.2 | Duodenal digestion

1 ml of gastric hydrolysis was mixed with 1 ml of pancreatin solution (100 U/ml at pH 7.0) and incubated for 120 min at 37°C. Then, the reaction was terminated with a bath at 80°C for 10 min. The pH was adjusted to pH 7.0.

2.18 | Physicochemical characterization of BPI and digests

For the characterization of the protein isolate, a 12% SDS-PAGE gel according to Quinteros, Vilcacundo, Carpio, and Carrillo (2016) was used. The polypeptide profile of the protein hydrolyzate was characterized by a 16% Tricine-SDS-PAGE gel.

2.19 | Analysis of the in vitro antioxidant activity

The ability to capture free radicals from the protein isolate, protein hydrolyzate and its fractions was determined. For the measurement of this activity, the DPPH and ABTS methods were used (Piñuel, Boeri, et al., 2019). The reference standard used was 1 mM of Trolox (0–0.95 mM of calibration curve). The standard curve obtained was ($Y = 0.4939x + 1.2438$, $R^2 = 0.995$). The IC50 value was calculated. This value was defined as the concentration of the sample necessary to inhibit 50% of the free radicals for the ABTS method.

2.20 | Cytotoxicity test of BPI in zebrafish eggs

The wild type zebrafish colony was established in the laboratory of the Universidad Nacional de Rio Negro (UNRN) Viedma, Argentina, in an environmental growth or glass aquarium, provided with a system of filtration and an aerator activated carbon for water oxygenation. Adult zebrafish were kept on 16 hr light and 8 hr dark cycles. Zebrafish embryos were obtained by photo-induced spawning over green plants, and then, cultured at 28°C in a fishbowl. Early zebrafish eggs were maintained according to Vilcacundo et al. (2017). Zebrafish eggs post-fecundation were used to the assays. Zebrafish eggs (30 eggs/well) were incubated in 24-well plates. BPI digests were added at different concentrations (0 and 0.25 mg/ml) in 200 µl of water. The effect of BPI on zebrafish embryos were analyzed for 4, 12 and 24 hr. At the end of the incubation time, zebrafish eggs mortality and morphologic changes were observed, determining the percentage of dead eggs. Stereoscopic microscope images were taken to obtain a registration of the morphological effects of BPI on the embryos anatomy and to compare them to controls.

2.21 | Statistical analysis

The results obtained in this work were presented as the means ± standard deviation. To determine the statistical differences, the data were evaluated with one-way ANOVA. Differences in $p < .05$ were considered statistically significant. Probit values were calculated using a probit table. The IC50 of each sample was calculated, using the probit value.

3 | RESULTS AND DISCUSSION

3.1 | Nutritional composition of *B. microphylla* pulp and seeds fruit

Table 1 shows the results of the berberis pulp proximate analysis. There is a lack of research studies that characterize *B. microphylla* nutritionally in the North Patagonia. Southern Chile fruits were analyzed by Ruiz et al. (2010). Our results compared to similar studies obtained previously, show no significant differences. The main nutrient found in pulp were sugars. The soluble-sugar content in pulp were 74.40 ± 0.90 mg glucose equivalents/g of FW. Total reduced sugars were 3.10 ± 0.10 mg equivalents of glucose/g FW, which comprises up to 0.3%. The glucose and fructose concentration in calafate have been described varying from 0.3% to 5% of fresh weight (Ruiz et al., 2010). Agronomic and ecological studies described that there is a strong relationship between the soluble sugar content and a possible tolerance to stress states. The plant metabolism is redirected toward synthesis of cryoprotectant molecules such as sugar (Boeri, Piñuel, Sharry, Tombari, & Barrio, 2017; Rosa et al., 2009). The accumulation of carbohydrates biomolecules on *Berberis buxifolia* fruits relate to fruit quality characteristics such as texture, flavor and color (Arena, Zuleta, Dyner, Constenla, & Curvetto, 2013). Table 1 shows the proximal analysis of berberies seeds. The studies about. *B. microphylla* are focused in the analysis of pulp components and there are only few studies of seeds berberies. The total content of carbohydrates is higher with a value of 65.24 ± 1.14 followed by the content of fat with values of 18.90 ± 0.65 and a higher content of protein with values of 13.65 ± 0.01. This value was higher when compared to the content of protein of pulp berberies (Table 1).

TABLE 1 Proximate composition of the fresh edible portion of *B. microphylla* fruit

Parameters	Pulp	Seeds
Moisture	93.32 ± 0.08[a]	N.D
Ash	3.65 ± 0.08[a]	2.21 ± 0.01[a]
Protein	1.33 ± 0.09[a]	13.65 ± 0.01[a]
Fat	1.70 ± 0.04[a]	18.90 ± 0.65[a]
Available carbohydrates	77.50 ± 0.90[b]	65.24 ± 1.14[b]
Total soluble sugar	74.40 ± 0.90[b]	
Total reducing sugar	3.10 ± 0.10[b]	

Notes: Results are represented as mean ± standard deviation ($n = 3$) of three independent experiments. a = (g/100 g FW). b = (mg equivalents of glucose/g FW).

3.2 | Extraction yield and total polyphenols content (TPC)

The TPC extraction yield was of 38.60 g lyophilized EB/100 g fruit (DW). Ethanol is the most valued solvent in the extraction of possible antioxidant agents present in natural extracts. Ethanol has characteristics that make it the best option to develop nutritional supplements such as its low economic cost and its nontoxic effect. The TPC values determined by the FC method was in accordance to the ones reported by Mariangel, Reyes-Diaz, Lobos, Bensch, Schalchli, and Ibarra (2013) under the same extraction conditions. The result of this study was higher values when compared to the values described by Ruiz et al. (2014) in different berberis species with a TPC value of

60.85 umoL GAE/g, FW. *B. microphylla* previous studies have shown that production of secondary metabolites depends on growth environmental conditions (Mariangel et al., 2013; Ruiz et al., 2010, 2014). Environmental and nutrient stresses have an impact on the phenolic compounds levels in plants and can affect the metabolic routes involved for the formation and accumulation of these compounds (Akula & Ravishankar, 2011). *B. microphylla* fruits analyzed in this work, were collected from the Eastern Patagonia region with semi-arid climates. This could explain the TPC higher content determined in this work with respect to Ruiz et al. (2010, 2014) who used samples from hyper humid to humid climates (south of Chile). To be considered as a superfruit, TPC is expressed as mg GAE/100 g FW, with values between 113.02 and 1,620.00 mg GAE/100 g FW (Chang et al., 2019). Our results show that the calafate could be considered a superfruit due to a TPC of 1,035.03 mg GAE/100 g FW.

3.3 | Determination of polyphenols components in *B. microphylla* pulp fruit

The polyphenolic components of berberis fruits were analyzed using the HPLC-DAD method. In this study the content of EB anthocyanins was higher than the content of flavanols and flavon-3-ols (Table 2). The main anthocyanins identified were the group of 3-glucoside conjugates, delphinidin-3-glucoside with a concentration of 23.61 μmol/g, followed by petunidin-3-glucoside with a concentration of 7.70 μmol/g and Cyanidin 3- O-p-coumaroyl-glucoside with a concentration of 3.8 μmol/g. The concentration of anthocyanins in berberis was 40 μmol/g of fresh weight, 1.5 times higher that values reported for this specie by Ulloa-Inostroza et al. (2017). The main anthocyanin family was the glucose derivatives of delphinidin, petunidin, and cyanidin components. Analysis of anthocyanidin components indicate that delphinidin-3-glucoside was the major anthocyanin of *B. microphylla*, with a percentage of 57.50% of total anthocyanins, 1.7 times higher than the percentage determined on "maqui" (*Aristotelia chilensis*) (Schreckinger, Lotton, Lila, & De Mejía, 2010).

TABLE 2 Quantification of phenolic compounds (non-anthocyanin and anthocyanin) in the hydroethanolic extracts of fruit by HPLC-DAD

Analyte	Concentration
Non-anthocyanin phenolic compounds (μg/g EE)	
Gallic acid	48.17 ± 6.84
OH-Tyrosol	155.00 ± 10.58
Syringic acid	368.55 ± 4.41
Caffeic acid	1,134.54 ± 77.06
p-coumaric acid	86.46 ± 4.83
trans-resveratrol	5.08 ± 0.76
Quercetin-3-glucoside	4.97 ± 0.75
Quercetin-3-galactoside	5.87 ± 0.88
Quercetin	1,092.75 ± 25.25
Anthocyanin phenolic compounds (mg/g EE)	
Delphinidin 3- O-glucoside	11.10 ± 0.04
Cyanidin 3- O-p-coumaroylglucoside	2.23 ± 0.11
Petunidin 3- O-glucoside	3.69 ± 0.05
Peonidin 3- O-glucoside	0.69 ± 0.04
Malvidin 3-O-glucoside	1.61 ± 0.05

Note: Results are expressed as mean ± standard deviation (n = 3).

Nine non-anthocyanins components in fruits of berberis were identified and quantified (Table 2). A chromatogram of chromatographic profile of a sample is presented in Figure 2. The analyzed compounds were successfully separated and identified by comparing their elution times and chromatogram with standards used. Table 2 presents the concentration of individual non-anthocyanins. The most interesting result was the identification of caffeic acid and quercetin, showing a concentration of 6.30 and 3.60 μmol/g extract respectively. Caffeic acid and quercetin are the components that have been most identified and quantified in plant tissues. These polyphenols are present in many food sources, including blueberries. Currently, there is a high research interest in phenolic compounds for their biological properties such as the antioxidant activity, antibacterial activity, antiviral activity and anti-inflammatory activity. (Magnani, Isaac, Correa, & Salgado, 2014; Wang et al., 2016). The quercetin molecule and its main derivatives have been reported as the most abundant flavonoid of calafate fruits. Many of the compounds identified by HPLC, were the same compounds determined by Ruiz et al. (2010) except for the caffeic acid. However, most of the concentrations obtained by these groups of research were low compared to the value determined in this work. Due to its potential health benefits of quercetin and caffeic acid, the calafate berry could be used as a nutraceutical ingredient in the food and pharmaceutical industries. To the best of our knowledge, this is the first report of two important compounds in terms of potential application of the fruit as a bioactive compounds source: trans-resveratrol and OH-tyrosol. These compounds have been reported in different matrixes and have been associated to several health promoting effects. The OH-tyrosol was found at similar levels than levels found in grape derived products such as wine. Its high antioxidant capacity has been described using in vitro models. Its eventual preventive capacity in certain pathologies has also been described. The results of the present study in could have a potential future use for these therapeutic purposes. With respect to trans-resveratrol, these levels were relatively low. However, the fact of being present could be helpful to understand possible interactions effects between the extracts phenolics composition and its antioxidant activity.

3.4 | In vitro antioxidant activity

Research on antioxidant activity may be a key issue in the medical and food industries due to the protection of ROS cells. Previous research has described the positive linear correlation between plant bioactive compounds with their antioxidant abilities (Karimi, Oskoueian, Hendra, Oskoueian, & Jaafar, 2012). The antioxidant activity EB was calculated using the DPPH and ABTS assays. The results obtained

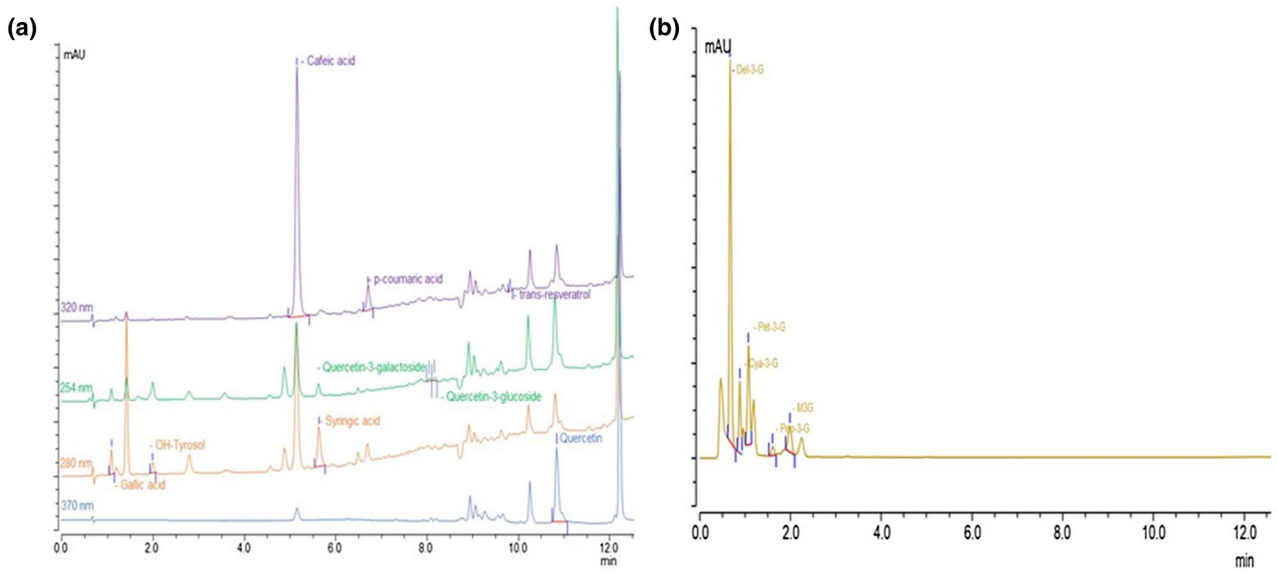

FIGURE 2 Chromatogram obtained for (a) Non-anthocyanin phenolic compounds and (b) Anthocyanin phenolic compounds quantified in this work

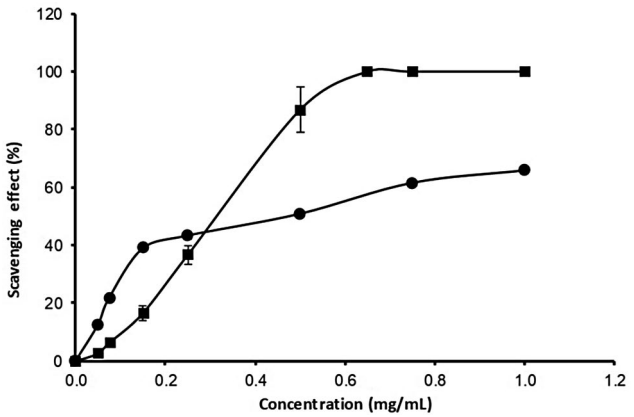

FIGURE 3 Scavenging effect on radicals ABTS+ (■) and DPPH (●) of EB lyophilized from *B. microphylla* at different concentrations (0–1 mg/ml). Bars represent the standard deviation of the mean

were 116.25 ± 17.01 and 137.8 ± 1.90 μmol TE/g FW respectively. These antioxidant activity values are in line with values reported by Ruiz et al. (2014). Calafate fruit antioxidant activity was around four times higher than the maqui berry, which is considered a superfruit for its high antioxidant activity (Genskowsky et al., 2016). EB was found to be effective in scavenging the ABTS and DPPH radical (Figure 3). The inhibition percentage of these radicals was concentration dependent. The IC50, concentration necessary to inhibit 50%, using ABTS was of IC50 = 0.26 mg/ml, and IC50 = 0.38 mg/ml when using the DPPH method. The scavenging of the $ABTS^{+}$ radical by the EB in this study was found to be as high as 100% up to a concentration of 0.65 mg EB/ml. However, the scavenging of the DPPH radical by EB was unable to get to 100% up to a concentration of 1 mg EB/ml. This shows that *B. microphylla* fruits extract presents a good ability to scavenge the ABTS radical.

The antioxidant tests used to evaluate the antioxidant activity of different molecules have different mechanisms of action. The tests can produce different trends of antioxidant activity. The ABTS antioxidant assay is generally indicated to evaluate the antioxidant activity of the hydrophilic compounds primarily. The DPPH antioxidant assay is used to analyze aqueous-organic extracts with hydrophilic and lipophilic compounds (Moo-Huchin et al., 2015). The ability of *B. microphylla* extracts to inhibit free radicals in different assays indicates that they could be useful as therapeutic agents to study pathological damage related to radicals.

3.5 | Protective effect of polyphenols against AAPH-induced oxidative stress in the in vivo zebrafish embryo model

In recent years, the interest in the evaluation of the antioxidant capacity of natural extracts is increasing due to the importance of ROS in the aging processes and its indication in different pathologies (Prior, 2003). Among the methods used to evaluate and quantify the generation of ROS at cellular level, we find the DCFH-DA fluorescent assay. This test was carried out with in vitro models (cell lines) and in vivo models (zebrafish). The exposure of free radical cells causes lipid oxidation and protein fragmentation (Peyrat-Maillard, Cuvelier, & Berset, 2003). Zebrafish embryos were used to assess the reduction of oxidative stress induced by AAPH. The exposure and reduction of intracellular ROS was quantified by a decrease in green fluorescence. Figure 4a shows the zebrafish embryos after the DFCH-DA test. It was observed that the intensity of the fluorescence of 5 and 10 μg EB/ml was lower when compared to the intensity of the group of embryos treated with AAPH. The nontreated group basal control presented the lowest fluorescence intensity.

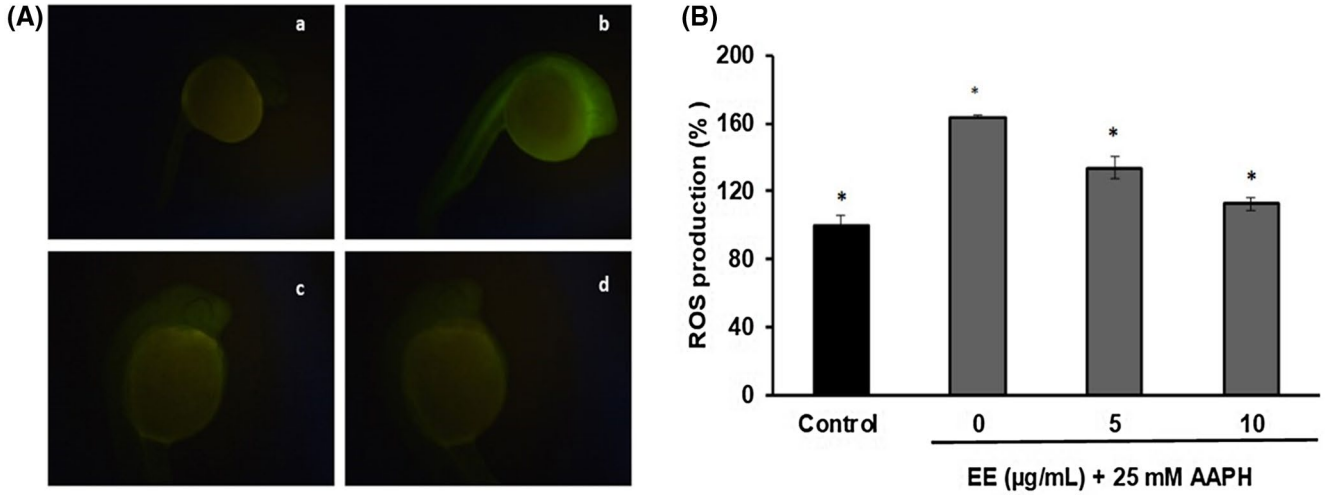

FIGURE 4 (A) Micrographs of reduction of ROS in zebrafish embryos of EB from B. microphylla (a) water (control without AAPH) (b) 25 mM of AAPH (positive control), (c) 5 µg EE/ml + 25 mM AAPH, and (d) 10 µg EE/ml + 25 mM of AAPH (n = 5). (B) Protective effect of EB on AAPH-treated reactive oxygen species (ROS) production in zebrafish. ROS levels were measured by Image J. Experiments were performed in triplicate and data are mean ± *SD*. *p < .05

In the DCFH-DA test the fluorescence green intensity increases with ROS generation in the cells (Walker et al., 2012). In this study, the ROS level was 164% in AAPH-treated zebrafish compared to the control group (zebrafish treated with water). In contrast, zebrafish embryos treated with AAPH and EB at different concentrations (5 and 10 EE µg/ml) showed significantly reduced levels of ROS generation in cells. No significant differences with the control (134% and 112%) (Figure 4b) were observed. Natural extracts obtained of plants with antioxidant capacity have different compounds, which can function as antioxidants or exert synergistic effects between them (Dapkevicius, Venakutonis, van Beek, Linssen, 1998). The zebrafish model allowed detecting in vivo, the antioxidant properties of the calafate berberis fruits. Our results show that the *B. microphylla* fruits have excellent antioxidant properties evaluated using in vitro (ABTS/DPPH) and in vivo (zebrafish) models.

3.6 | Characterization of proteins from berberies seeds

Studies on berberies fruits and its antioxidant activity have focused mainly on the identification and quantification of their polyphenol component and their relationship with antioxidant ability. Little has been studied about the protein components present in the fruit and their possible biological activities. This study has proposed the characterization of Calafate seed proteins and evaluation of their antioxidant capacity.

The BPI protein profiles and their gastro- duodenal digests were analyzed using the SDS-PAGE method. BPI present a simple profile of proteins with four abundant bands with molecular weights of 15, 24, 45 and 62 kDa (Figure 5a). BPI was subject to simulation of gastric and duodenal digestion in vitro using pepsin and pancreatin respectively. Gastric digest of BPI present only two bands with molecular weight of 3 and 25 kDa. BPI duodenal digest present a protein profile of four bands with molecular weights of 3, 12, 15 and 25 kDa (Figure 5b). The bands of 3 kDa correspond to small peptides produced during the hydrolysis process. The BPI cytotoxicity was tested in the zebrafish eggs in vivo model. BPI demonstrated a toxic effect for 50% of the zebrafish eggs in the concentration of 0.09 mg/ml within the first 24 hpf (Figure 6).

3.7 | Antioxidant activity of proteins from berberies seeds

BPI, duodenal digest and fractions (3 and 10 kDa) were used to evaluate their antioxidant capacity by the ABTS and DPPH tests. BPI present a value of ABTS of 593.11 ± 8.50 µmol TE/g. BPI duodenal digest present a value of 641.07 ± 12.60 µmol TE/g digests, the 3 kDa fraction present a value of 45.92 ± 2.60 µmol TE/g fraction and the 10 kDa fraction present a value of 361.02 ± 20.30 µmol TE/g fraction. The IC50 value was evaluated for BPI and duodenal digest samples. It was found that BPI present a value of 2.95 mg/ml and duodenal digests present a value IC50 of 0.80 mg/ml. Duodenal digest present lower values than BPI. When the sample was evaluated using the DPPH method it was found that BPI present a value of 128.67 ± 3.10 µmol TE/g BPI and duodenal digests present a value of 61.91 ± 7.60 µmoL TE/g of digest. BPI present a value of IC50 of 16.50 mg/ml (Table 3). Protein isolates obtained from quinoa and amaranth have been described for their antioxidant capacity and their ability to inhibit lipid peroxidation in zebrafish. These protein isolates were hydrolyzed using pepsin and pancreatin. Their antioxidant capacity in vitro and in vivo was also demonstrated. (Vilcacundo et al., 2017, 2018). Piñuel, Boeri, et al. (2019) reported proteins isolate from white, black and red quinoa (*Chenopodium quinoa* Wild variety Real). Quinoa protein isolates (QPI) were obtained

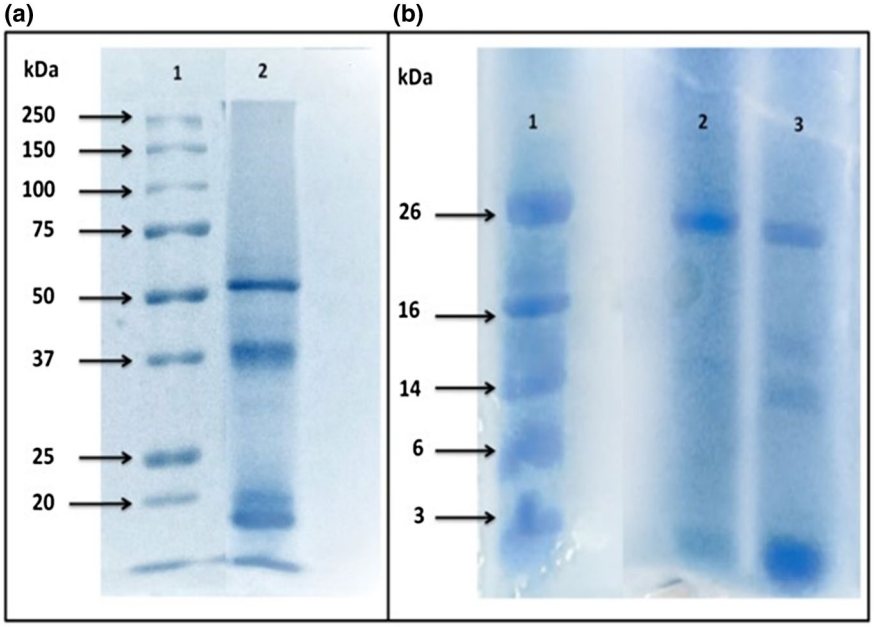

FIGURE 5 (a) Protein profile obtained through a 12% SDS-PAGE gel. Lane 1: molecular marker. Street 2: isolated protein. (b) peptide profile obtained by a 16% Tricine-SDS-PAGE gel. Lane 1: molecular marker. Lane 2: gastric digestion. Lane 3: duodenal digestion

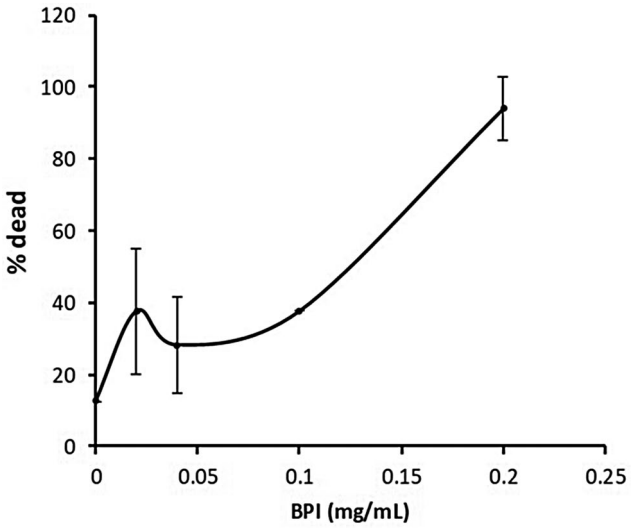

FIGURE 6 Cytotoxicity analysis of BPI in zebrafish eggs

for isoelectric precipitation from germinated quinoa. QPI underwent simulation gastro-intestinal model in vitro. The hydrolyzates present high antioxidant activity by the ORAC, DPPH and ABTS assays. Piñuel, Vilcacundo, et al. (2019) reported proteins with antioxidant activity obtained from the *Phaseolus vulgaris* variety Pinto. Protein concentrates present antioxidant activity by ORAC, DPPH and ABTS tests. Bean proteins present inhibition of peroxidation lipidic in the zebrafish model. The bean protein concentrate was hydrolyzed with pepsin and pancreatin enzymes. These hydrolyzates were fractionated by the ultrafiltration membrane technique. The fractions also presented antioxidant activity. For all the above, vegetable proteins obtained from different matrices can have antioxidant capacity. This activity can be evaluated using different analytical methods.

TABLE 3 Antioxidant activity of the BPI and duodenal digest determined by ABTS and DPPH

	Sample	IC50 mg/ml	Antioxidant activity (µmol TE/g)
	ABTS	ABTS	DPPH
BPI	2.90^{b}	593.11 ± 8.50^{d}	128.67 ± 3.11^{b}
DD	0.74^{a}	641.07 ± 12.60^{c}	61.91 ± 7.60^{b}
10 KDa fraction	n-d	361.02 ± 20.30^{b}	19.50 ± 8.32^{a}
3 KDa fraction	n-d	45.92 ± 2.60^{a}	22.8 ± 4.40^{a}

Note: Different letters indicate significant differences between the means of same column ($p < .05$).

Food quality can be impaired by the accumulation of oxidative reactions that affect the taste, aroma, texture and color of the food. To avoid this, there must be a balance between oxidizing reactions and antioxidant factors (Ali, Ahsan, Zia, Siddiqui, & Khan, 2020). The molecules that are involved in the oxidation reactions of food can be lipids and proteins molecules. In lipids, these reactions result in lipid peroxidation. During this process, carbonyl compounds are produced resulting in the rancid aroma of lipids. Carbonyls compounds can be associated with proteins and affect their functionality (Elias, Kellerby, & Decker, 2008). However, it is known that many proteins have antioxidant capacity and can be used to inhibit oxidation reactions and lipid peroxidation. Blood proteins have from 10% to 50% ability to inhibit peroxyl radicals present in the blood plasma (Frei, Stocker, & Ames, 1998). These proteins with antioxidant activity can be used to produce hydrolyzate using different methods such as enzymatic hydrolysis, fermentation and simulated gastrointestinal digestion (gastric and duodenal simulation). These hydrolyzates

content small peptides with capacity to inhibit free radical and inhibit lipid peroxidation. Enzymatic hydrolysis is the most widely used method to produce hydrolyzates of proteins and bioactive peptides. Enzymatic hydrolysis of food proteins is generally recognized as an efficient, safe, fast and relatively inexpensive procedure for the biotechnology and food industry (Nwachukwu & Aluko, 2019).

4 | CONCLUSIONS

Calafate berberis (*B. microphylla*) fruits cultivated in the arid Argentinian Patagonia region present anthocyanins high concentration with high antioxidant capacity. Anthocyanins components could be responsible for the EB antioxidant activity. The polyphenols compounds and their antioxidant activity increase berberis fruit value and quality. For the first time, the proteins present in seeds of Calafate berberis fruit were described. The proteins from seeds of *B. microphylla* and their gastric and duodenal digests present antioxidant activity. In the future, the proteins and peptides can be identified using the mass spectrometric analysis. The characterization of the components of Calafate fruit can increase the interest of the consumers and can be used as a functional ingredient or simply as an ingredient in foods supplements.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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